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**In Focus**

The cover picture shows *Xenorhina wiegankorum* sp. nov Günther & Richards

See paper of **Günther R, Richards S:** Description of six new species of *Xenorhina* Peters, 1863 from southern Papua New Guinea (Amphibia, Anura, Microhylidae)

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# Molecular diagnostic based on 18S rDNA and supplemental taxonomic data of the cnidarian coelozoic *Ceratomyxa* (Cnidaria, Myxosporea) and comments on the intraspecific morphological variation

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## Abstract

*Ceratomyxa amazonensis* is a cnidarian myxosporean originally described with strongly arcuate crescent-shaped myxospores, absence of vegetative stages and infecting *Symphysodon discus*, an important Amazonian ornamental fish in the aquarium industry. As part of a long-term investigation concerning myxosporeans that infect discus fish *Symphysodon* spp. from different rivers of the Amazon Basin, thirty specimens of *S. discus* collected from Unini River were examined. Plasmodial vegetative stages therefrom were found freely floating in the bile of gall bladders from eighteen fish. Mature myxospores were slightly crescent-shaped, measuring 4.72 ± 0.1 (4.52–4.81) µm in length, 24.2 ± 0.4 (23.9–25.3) µm in thickness with polar capsules 2.31 ± 0.1 (2.29–2.33) µm in length and 2.15 ± 0.1 (2.13–2.17) µm in width. Strong morphological differences were observed between the newly isolated myxospores obtained and the previously described *C. amazonensis*; however, molecular assessment, based on 18S rDNA, revealed a high similarity (99.91%), with only a single nucleotide base change. This study provides new data, expanding the original description of the species with a discussion on differences in myxospore-morphology in the context of intraspecific morphological plasticity.

## Key Words

Brazil, ceratomyxid, morphological plasticity, ornamental fish, parasitic cnidarian

## Introduction

The global aquarium market moves millions of ornamental fish worldwide and is the primary mode for international transport of cnidarian myxosporean parasites (Hallett et al. 2015). As such, there is a fundamental need for constant monitoring to enable diagnosis and timely control of infections by this parasite group in aquarium fish (Mathews et al. 2018). Although the Amazon Basin is amongst the most important sources of wild-caught ornamental fishes in the international aquarium industry (Moreau and Coomes 2007), there are few surveys concerning cnidarian myxosporean infections in Amazonian ornamental fish (Mathews et al. 2015, 2017, 2020a, b). The three recognised species of the discus genus *Symphysodon* Heckel, 1840, in the family Cichlidae, are popular, expensive and widely exploited ornamental fish (Bleher et al. 2007). These neotropical freshwater

cichlids are endemic to the Amazon Basin and restricted to areas where seasonal flooding occurs (Bleher et al. 2007). The red discus *Symphysodon discus* Heckel, 1840 inhabits lentic aquatic environments, such as floodplains and flooded forests in the lower Rio Negro, upper Uatumã, Unini, Nhamundá, Trombetas and Abacaxis Rivers in Brazil (Amado et al. 2011).

Myxosporeans are endoparasitic microscopic cnidarians with worldwide distributions (Atkinson et al. 2018). With over 2,400 species recorded from aquatic and terrestrial hosts, there is evidence of extensive diversification in and dispersion of this group of parasitic cnidarians (Atkinson et al. 2018). Annelids are the definitive hosts, releasing infective actinospores into the aquatic environment (Fiala et al. 2015). Although virtually all vertebrate groups can be infected, fish comprise the largest number of known secondary hosts (Fiala et al. 2015). Amongst the myxosporeans, species of the genus *Ceratomyxa* Thélohan, 1892 are mostly highly host-specific coelozoic parasites with approximately 300 species that mainly parasitise the gall bladders of a wide range of fish species (Eiras et al. 2018), with some species reportedly generating pathologies in their hosts (Alama-Bermejo et al. 2011, Barreiro et al. 2017). Despite the enormous diversity of fish species in the Amazon Basin, only seven *Ceratomyxa* species have been reported (Eiras et al. 2018, Da Silva et al. 2020). *Ceratomyxa amazonensis* Mathews, Naldoni, Maia & Adriano, 2016 was described as parasitising *S. discus* from the Rio Negro River, Amazonas State, Brazil, with the first published nucleotide sequence of a *Ceratomyxa* species from a strictly freshwater environment (Mathews et al. 2016).

As part of a long-term investigation concerning myxosporeans that infect discus fish *Symphysodon* spp. from different rivers of the Amazon Basin, specimens of *S. discus* collected from the Unini River were examined. This study supplements the original description of the cnidarian myxosporean *C. amazonensis*, providing new data on the stages of and morphological variation in myxospores, thereby extending the original description of the species. Furthermore, differences in myxospore morphology are discussed in the context of intraspecific morphological plasticity.

## Materials and methods

In August 2019, thirty specimens of *S. discus* (ranging from 10.3 ± 1.2 cm in total length and 23.4 ± 4.2 g in weight) were collected from the Unini River, near Barcelos Municipality (0°58'30"S, 62°55'26"W), Amazonas State, Brazil. The fishes were sampled under a collection licence issued by the Brazilian Ministry of the Environment (SISBIO Process No. 73241-2). The euthanasia procedure was approved by the Federal University of Amazonas Ethics Committee for Scientific Use of Animals (CEUA-UFAM No. 025/2019). After necropsy, gall bladders were carefully removed and placed in small Petri dishes for further examination under stereo and optical microscopes. Samples of the bile were collected by puncturing the gall bladder using a pointed glass pipette; a drop of bile was then pipetted on to a microscope slide, covered with a cover slip and observed under an Olympus BX53 light microscope at 400× magnification.

Morphological and morphometric analyses were performed on 30 randomly selected mature myxospores using a computer, equipped with Axivision 4.1 image capture software coupled to an Axioplan 2 Zeiss microscope. Following the criteria outlined by Lom and Arthur (1989) and Heiniger et al. (2008), measurements taken for each myxospore included spore length (SL), spore thickness (ST), polar capsule length (PCL) and polar capsule width (PCW) in micrometres (μm) and posterior angle (PA) in degrees (°). The myxospore dimensions were expressed as mean and standard deviation, followed by the range in parentheses. Smears containing free myxospores were air-dried, fixed with methanol and stained with Giemsa to mount on permanent slides. Slides with stained myxospores and vials containing formalin-fixed plasmodia were deposited in the cnidarian collection of the Zoology Museum at the University of São Paulo – USP, São Paulo, Brazil (MZUSP).

For transmission electron microscopy, infected gall bladders were fixed for two days in 2.5% glutaraldehyde, diluted in 0.1 M sodium cacodylate buffer (pH 7.4), washed in a glucose-saline solution for 2 h and post-fixed in 2% osmium tetroxide ($OsO_4$) for 4 to 5 h. After dehydration in an ascending concentration series of ethanol, the samples were embedded in EMbed 812 resin (Electron Microscopy Sciences, Hatfield, PA, USA) (Mathews et al. 2020c). Ultra-thin sections, double stained with uranyl acetate and lead citrate, were examined under a LEO 906 electron microscope operating at 60 kV in the Center for Electronic Microscopy (CEME) at the Federal University of São Paulo.

Genomic DNA (gDNA) was extracted from infected bile of a fish sample and preserved in absolute ethanol. The sample was pelleted through centrifugation at 8,000 rpm for 12 min and the ethanol removed. The gDNA was extracted from the pellet using a DNeasy Blood & Tissue Kit (animal tissue protocol) (Qiagen Inc., California, USA), in accordance with the manufacturer's instructions. The gDNA concentration was quantified in a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, USA) at 260 nm. Polymerase chain reaction (PCR) was performed in accordance with Mathews et al. (2016) with a final reaction volume of 25 μl, which comprised 1 μl of DNA (10–50 ng), 0.5 μl of each specific primer (0.2 μM), 12.5 μl of Dream Taq Green PCR Master Mix (Thermo Scientific) and 10.5 μl of nuclease-free water. Partial 18S rDNA sequences were amplified using the universal eukaryotic primer pair ERIB1 (forward: ACCTGGTTGATCCTGCCAG) and ERIB10 (reverse: CTTCCGCAGGTTCACCTACGG) (Barta et al. 1997).

The amplification of the partial 18S rDNA was performed on a Mastercycler nexus (Eppendorf, Hamburg, Germany) and the PCR cycle consisted of an initial

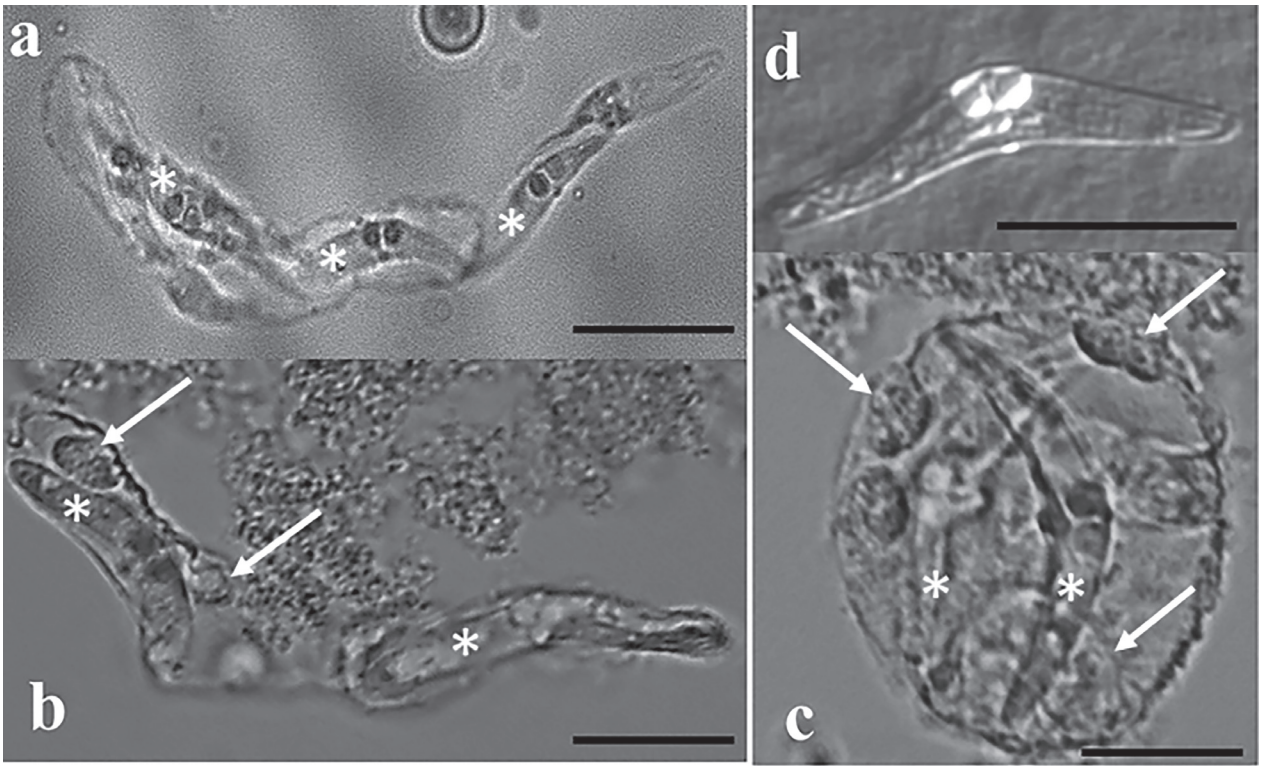


**Figure 1.** Light photomicrographs of *Ceratomyxa amazonensis* plasmodia. **a, b.** Slightly elongated plasmodia showing mature myxospores (white asterisks) and few early sporogonic stages (arrows); **c.** Spherical plasmodium with two slightly crescent-shaped mature myxospores (ms) and containing early sporogonic stages (arrows); **d.** Differential interference contrast microscopy snapshot of a slightly crescent-shaped mature myxospore. Scale bars: 10 μm.

denaturation step at 95 °C for 5 min, followed by 35 denaturation cycles at 95 °C for 1 min, annealing at 60 °C for 1 min and extension at 72 °C for 2 min, with a terminal extension at 72 °C for 5 min. A control reaction was processed in order to check for possible contamination. The amplified PCR product was subjected to electrophoresis on 1.0% agarose gel (BioAmerica, California, USA) in a TAE buffer (Tris–Acetate EDTA: Tris 40 mM, acetic acid 20 mM, EDTA 1 mM), stained with Sybr Safe DNA gel stain (Invitrogen by Life Technologies, California, USA) and then analysed with a Stratagene 2020E trans-illuminator. For sizing and approximate quantification of PCR product, 1 Kb Plus DNA Ladder (Invitrogen by Life Technologies, USA) was used. The PCR product was purified on a USB ExoSap-IT (Thermo Fisher Scientific, Massachusetts, USA) in accordance with the manufacturer's instructions and sequenced using the PCR primer pair, as well as the additional primer pair MC5, CCTGAGAAACGGCTACCACATCCA and MC3, GATTAGCCTGACAGATC ACTCCACGA (Molnár 2002). This additional primer pair was used in the sequencing to connect the overlapping fragments. Sequencing was performed with a BigDye Terminator v.3.1 cycle sequencing kit (Applied Biosystems Inc., California, USA) on an ABI 3730 DNA sequencing analyser. The sequences obtained were visualised, assembled and edited in BioEdit 7.1.3.0 (Hall 1999) to produce a consensus sequence. A basic local alignment search (BLASTn) was performed to evaluate the similarity of our sequence with other myxosporean sequences available in the NCBI database (Altschul et al. 1997). The newly-acquired 18S rDNA gene sequence was aligned with all available Amazonian *Ceratomyxa* spp. sequences, in order to evaluate pairwise genetic distance using the p-distance model in MEGA 6.0 (Tamura et al. 2013).

## Results

Freely-floating plasmodia were found in the gall bladder bile of 18 (60%) out of the 30 *S. discus* specimens collected in the Unini River. After rupturing the plasmodia, slightly crescent-shaped mature myxospores were observed with sub-spherical polar capsules. These capsules were located close to the myxospore suture line in a plane perpendicular to it, at the anterior myxospore pole, thus defining classification within the genus *Ceratomyxa*. No signs of infection were observed in the parasitised organs.

### Description

Plasmodia were asymmetric and slightly elongated, with mean length 62.3 (range 58.4–64.2) μm and mean width 7.8 (range 6.6–8.9) μm; they contained both mature myxospores and early sporogonic stages (Fig. 1A, B). Some

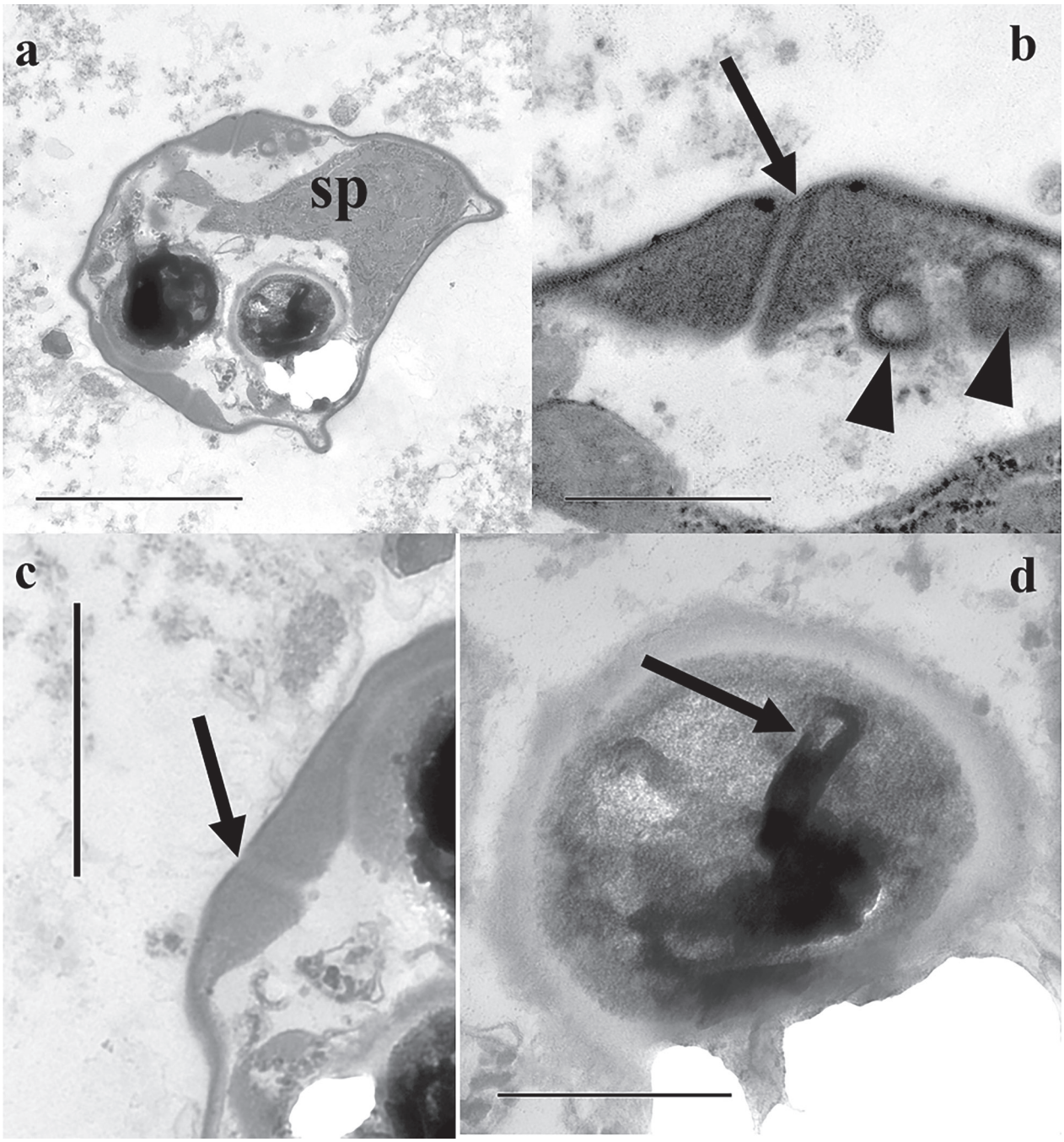


**Figure 2.** Transmission electron microscopy images of *Ceratomyxa amazonensis* isolated of *Symphysodon discus* from the Unini River, Amazonas State, Brazil. **a**. Myxospore showing two sub-spherical polar capsules and sporoplasm (sp) occupying most of the myxospore volume; **b.** Detail of the apical suture (black arrow) and sporoplasmosomes (arrowheads); **c.** Detail of lateral suture (black arrow); **d.** Polar capsule displaying still uncoiled internal polar tubule (black arrow). Scale bars: 2 µm (**a**); 1 µm (**c**); 500 nm (**b**, **d**).

plasmodia were spherical, mean diameter 30 (range 27–32) µm, n = 11, containing both mature myxospores and visible sporogonic stages (Fig. 1C). Mature myxospores were slightly crescent-shaped, measuring 4.72 ± 0.1 (4.52–4.81) µm in length and 24.2 ± 0.4 (23.9–25.3) µm in thickness (Fig. 1D). Shell valves were approximately equal in size with rounded extremities (Fig. 1D). Apical and lateral sutures were noticeable at the junction of the valves with fine material between them (Fig. 2B, C). The posterior angle was 154° (153°–156°). Polar capsules were equal in size, sub-spherical, measuring 2.31 ± 0.1 (2.29–2.33) µm in length and 2.15 ± 0.1 (2.13–2.17) µm in width (Figs 1D and 2A) and displayed a still uncoiled internal polar tubule (2D). Sporoplasm occupied most of the myxospore volume (Figs 1D and 2A) with sporoplasmosomes present (Fig. 2B).

**Host:** *Symphysodon discus* Heckel, 1840 (Perciformes: Cichlidae).

**Type locality:** Unini River, near Barcelos Municipality (0°58'30"S, 62°55'26"W), Amazonas State, Brazil.

**Table 1.** Pairwise genetic distance of 18S rDNA sequences from *Ceratomyxa* species described from strictly Amazonian fish hosts. The upper triangular matrix shows the number of different nucleotide positions; the lower triangular matrix shows the percentage of differing nucleotide positions.

| Species (GenBank ID) | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 1. Unini River isolates (this study) (MN064752) | · | 77 | 48 | 32 | 1 |
| 2. *Ceratomyxa gracillima* (KY934184.1) | 7 | · | 92 | 109 | 109 |
| 3. *Ceratomyxa vermiformis* (KX278420.1) | 4 | 5.2 | · | 82 | 74 |
| 4. *Ceratomyxa brasiliensis* (KU978813.1) | 3 | 6.8 | 5.1 | · | 38 |
| 5. *Ceratomyxa amazonensis* (KX236169.1) | 0.1 | 6.9 | 4.7 | 2.4 | · |

**Sites of infection:** Within gall bladder (plasmodia floating free in the bile).

**Material deposited:** The partial 18S rDNA gene sequence was deposited in GenBank (accession number MN064752). Slides with stained myxospores and vials containing formalin-fixed plasmodia were deposited in the cnidarian collection of the Zoology Museum at the University of São Paulo – USP, São Paulo, Brazil (MZUSP 8469).

### Molecular Analysis

The BLAST search revealed a high similarity between the newly-obtained 18S rDNA gene sequence and a previously-published sequence of *C. amazonensis* (query cover 100%, maximum identity 99.91%), a parasite of *S. discus* from Rio Negro River. The pairwise comparison between the new isolate from the Unini River and a previously deposited 18S rDNA gene sequence of *C. amazonensis* found an overall genetic divergence of 0.1% with just a single nucleotide base change between the two sequences (Table 1).

## Discussion

As pointed out by Lom and Arthur (1989) in their guidelines for the description of myxosporean cnidarians, it is indispensable to provide as much information as possible about the plasmodial stage, such as the site of infection, structure, shape and size. In contrast to the previous description of *C. amazonensis* from *S. discus* from the Rio Negro River, where only free myxospores were reported (Mathews et al. 2016), in our study, plasmodia containing mature myxospores and early stages were found floating freely in the bile within host gall bladders. Here, we provide new data on the plasmodial stage, extending and thereby improving the original description of *C. amazonensis*.

Morphological plasticity in myxospores are acknowledged to be one of the main factors responsible for the difficulties encountered in myxosporean taxonomy and species identification, resulting in taxonomic dilemmas (Zhai et al. 2016, Guo et al. 2018, Xi et al. 2019). It is widely recognised that *Ceratomyxa* spp. myxospores can display a high degree of morphological plasticity (Atkinson et al. 2015, Bartošová-Sojková et al. 2018); thus, classifications, based strictly on morphology, can result in ambiguous descriptions, especially considering that there is a high level of natural morphological and morphometric variation in myxospores both within and between hosts (Atkinson et al. 2015). One reason for this variation is that myxosporean infections typically feature asynchronous myxospore development, so that changes in shape and size during maturation result in a range of myxospore morphologies (Atkinson et al. 2015). A further problem in using myxospore morphology alone to distinguish species concerns the fixative methods used, because it is known that fixatives can affect the morphological dimensions of myxospores relative to fresh samples (Parker and Warner 1970, Zhai et al. 2016). In our study, no changes were observed in the dimensions of formalin-fixed myxospores compared to fresh samples; this could be explained by the type of fixative used, which is reported to have little, if any, effect on myxospore dimensions (Parker and Warner 1970). These observations are consistent with those reported for *Myxobolus drjagini* Akhmerov, 1954, where myxospores, fixed in 10% formalin since the 1980s, showed little shrinkage compared with fresh myxospores (Xi et al. 2019). However, morphological variations in some *Ceratomyxa* spp. have been described as created by deformations of their presumably thin-walled shell valves (Morrison et al. 1996), thus limiting the use of myxospore features as a sole approach to taxonomic classification. Under this scenario, in order to accurately identify new myxosporeans species, it is highly recommended to use a combination of morphological and biological traits, factors related to host ecology and molecular characteristics, particularly within genera with high intraspecific variation in myxospores, with *Ceratomyxa* being a remarkable example (Atkinson et al. 2015).

In our study, the morphological comparison between the new myxospore isolate from Unini River, *S. discus* and previously described *C. amazonensis* myxospores found in specimens from the Rio Negro River, shows some dissimilar characteristics (Table 2). Unini River myxospores were slightly crescent-shaped, shorter in length and significantly thicker compared to the Rio Negro River myxospores which were strongly arcuate shaped, comparatively longer and less thick (Mathews et al. 2016). Although we noticed some discordance in myxospore morphology between the new isolate obtained and previously described *C. amazonensis* from Rio Negro River, the molecular analysis revealed a high similarity (99.91%) in 18S rDNA sequence data, with only a single

**Table 2.** Comparative morphometric data for the newly-isolated myxospores and other *Ceratomyxa* parasites of Amazonian fish. T: thickness; L: length; PCL: length of polar capsule; PCW: width of polar capsule; PA°: Polar angle; –: no data. All measurements expressed as mean ± SD.

| Species | T | L | PCL | PCW | PA° | Source |
|---|---|---|---|---|---|---|
| New isolate | 24.2 ± 0.4 | 4.7 ± 0.1 | 2.31 ± 0.1 | 2.15 ± 0.1 | 154 | This study |
| *C. amazonensis* | 15.8 ± 0.4 | 7.0 ± 0.3 | 3.2 ± 0.3 | 2.6 ± 0.2 | 103.7 | Mathews et al. (2016) |
| *C. fonsecai* | 28.9 ± 2.7 | 2.6 ± 0.1 | 1.9 ± 0.3 | 1.7 ± 0.2 | 164.8 | Silva et al. (2020) |
| *C. mylei* | 24.6 ± 08 | 5.1 ± 0.2 | 2.1 ± 0.3 | - | - | Azevedo et al. (2011) |
| *C. brasiliensis* | 41.2 ± 2.9 | 6.3 ± 0.6 | 2.6 ± 0.3 | 2.5 ± 0.4 | 147 | Zatti et al. (2017) |
| *C. gracillima* | 28.0 ± 3.4 | 4.4 ± 1.1 | 1.9 ± 0.4 | 1.9 ± 0.4 | 36.6 | Zatti et al. (2018) |
| *C. microlepis* | 35.5 ± 0.9 | 5.2 ± 0.4 | 2.2 ± 0.3 | 2.2 ± 0.3 | | Azevedo et al. (2013) |
| *C. vermiformis* | 8.4 ± 0.4 | 4.5 ± 0.2 | 2.7 ± 0.1 | 2.7 ± 0.1 | 30.2 | Adriano and Okamura (2017) |

nucleotide base change. Previous studies in several geographic regions have reported different values for 18S rDNA intraspecific divergence in *Ceratomyxa* (Sanil et al. 2017, Bartošová-Sojková et al. 2018) and there is no universal criterion regarding what constitutes a sufficient level of sequence variation to represent distinct species within the genus *Ceratomyxa* (Bartošová-Sojková et al. 2018). However, the low 18S rDNA genetic difference (0.1%) observed between the isolate obtained in the present study and the previously available *C. amazonensis* sequence is not sufficient to designate them as different species, taking into account that 18S rDNA intraspecific sequence variation of < 1% is common (Zhao et al. 2013, Atkinson et al. 2015, Wang et al. 2019). The low genetic divergence observed between isolates from these two widely-separated localities (511 km apart), indicates that geography has had limited impact in terms of genetic differentiation. Our finding is consistent with previous studies where samples of the same myxosporeans species, collected in distant geographic areas, had zero or very low genetic divergence in their 18S rDNA sequences (Urawa et al. 2011, Adriano et al. 2012, Wang et al. 2019). According to Whipps and Kent (2006), host distribution and migration can be equally important factors in maintaining parasite gene flow over broad geographic areas. The high genetic similarity between the Unini River isolate and the Rio Negro River *C. amazonensis* is likely a result of adaptation of the host, *S. discus*, to floodwater habitats of the Amazon Region, ensuring that populations share a continuous habitat across a large geographical area, despite dry periods between the floods. This adaptation to a specific ecological niche may lead to high parasite gene flow over broad geographic areas. For instance, Zatti et al. (2018) observed an absence of genetic variation in the typically more variable ITS-1 region in widely separated *Ceratomyxa gracillima* samples infecting the gall bladder of the Amazonian catfish *Brachyplatystoma rousseauxii* Castelnau, 1855. They suggest high gene flow as a result of panmixia in the parasite populations due to migratory behaviour of the fish host.

Based on the discussion above, we infer that the myxospores, newly isolated from Unini River *S. discus*, should be regarded as belonging to the previously described species *C. amazonensis*. Furthermore, the observations made during this study highlight that classifications, based strictly on morphology, can result in ambiguous descriptions and reinforce the importance of molecular methods (DNA sequencing) for identifying and distinguishing between *Ceratomyxa* species.

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# Uncovering the herpetological diversity of small forest fragments in south-eastern Madagascar (Haute Matsiatra)

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## Abstract

Madagascar has historically suffered from high fragmentation of forested habitats, often leading to biodiversity loss. Neverthless, forest fragments still retain high levels of biological diversity. The Haute Matsiatra Region (south-eastern Madagascar) hosts the renowned Andringitra National Park and several surrounding isolated forest fragments embedded in a matrix of human-dominated landscape. During a herpetological survey conducted in the Region, we visited a total of 25 sites. We applied a molecular taxonomic approach to identify the collected material and generate new reference sequences to improve the molecular identification of Malagasy herpetofauna. We identified a total of 28 amphibian and 38 squamate taxa and provided a systematic account for each one of them. Nine of the identified taxa are candidate species, amongst which one was newly identified. We extended the known distributional range of 21 taxa (nine amphibians and 12 squamates). Although the largest forest fragments hold a higher number of species, we also detected a relatively high herpetological diversity in small patches. Our results highlight the importance of investigating small forest fragments to contribute to a better understanding of the patterns of diversity and distribution of the amphibians and reptiles of Madagascar.

## Key Words

herpetofauna, forest patches, Andringitra, barcoding, 16S, COI, microendemic, rapid assessment

## Introduction

Ranked as one of the top megadiversity hotspots on Earth, Madagascar hosts exceptional and highly threatened fauna and flora (Myers et al. 2000; Wilmé et al. 2006). The proportion of native endemic vertebrate fauna is remarkable, with families, subfamilies and several genera being entirely limited to Madagascar. At the species level, 92% of native non-marine "reptiles" (intended as all Sauropsida, excluding birds) and 100% of native amphibians are endemic to the Island (Glaw and Vences 2007; AmphibiaWeb 2021; Uetz et al. 2021). Yet, some recent anthropogenic introductions are currently known (e.g. *Duttaphrynus melanostictus*, Licata et al. 2020;

*Hoplobatrachus tigerinus*, Mohanty et al. 2021; *Agama agama*, Wagner et al. 2009; *Indotyphlops braminus*, Uetz et al. 2021). The great environmental and bioclimatic heterogeneity of the Island has played a major role in the diversification of the rich Malagasy biota (Vences et al. 2009; Ganzhorn et al. 2014). Following Cornet (1974) and Schatz (2000), five major biomes are recognised in Madagascar: the eastern rainforest, the western dry deciduous forest, the sub-humid forest of the central highlands, the southern sub-arid spiny forest and the montane thickets. The main biodiversity distributional patterns largely follow this bioclimatic subdivision, with the highest abundance of both amphibian and reptile species found along the eastern rainforest belt (Goodman and Benstead 2003; Wollenberg et al. 2008; Crottini et al. 2012a; Brown et al. 2014, 2016).

Amphibians and reptiles are particularly diverse, with ca. 369 and 440 currently recognised species, respectively (Glaw and Vences 2007; AmphibiaWeb 2021; Uetz et al. 2021). Nevertheless, the level of undescribed diversity is high, as reported by Nagy et al. (2012) for reptiles and Perl et al. (2014) for amphibians. Field research efforts over the last three decades, the widespread use of large-scale species inventories and the application of an integrative taxonomic approach employing molecular, morphological and bioacoustic (for amphibians) identification, coupled with voucher collection (Yoder et al. 2005; Padial et al. 2010), enabled impressive progress in uncovering this hidden diversity (e.g. Rosa et al. 2012; Cocca et al. 2018). In times of major biodiversity loss, field research is fundamental to catalogue world biodiversity and represents a fundamental step for its conservation (Dijkstra 2016). As in several other places of the world (Böhm et al. 2013), a large portion of Malagasy herpetofauna is at high risk of extinction (e.g. Irwin et al. 2010; Jenkins et al. 2014), with 46.2% of the assessed amphibians and 37.7% of the assessed reptiles currently listed as threatened according to the IUCN Red List categories (Vulnerable, Endangered and Critically Endangered) (IUCN 2020).

With most species being forest dwellers, the major threat to Malagasy amphibians and reptiles is forest loss, degradation and fragmentation (e.g. Irwin et al. 2010; Böhm et al. 2013; Jenkins et al. 2014; Riemann et al. 2017). The extent of deforestation that Malagasy biota has historically undergone is dramatic (Hornac 1943; Jarosz 1993; Vieilledent et al. 2018). In the first 30 years of French colonisation (1895–1925), the amount of primary forest that was destroyed is estimated to be 70% of the vegetation present in the pre-colonial period (Hornac 1943; Jarosz 1993), while a further 44% of the remaining forest was estimated to be lost in the period of 1953–2014 (Vieilledent et al. 2018). Deforestation not only results in an overall decrease in the surface of forested areas, but is also responsible for the heavy fragmentation of remaining forests. In fact, Vieilledent et al. (2018) estimated that, in 2014, 46% of Malagasy remaining forests were less than 100 m distant from forest edges. Species richness tends to decrease with fragment size and species composition is also affected by fragmentation, with forest specialists disappearing rapidly with increasing degradation and fragmentation (e.g. Vallan 2002; Lehtinen and Ramanamanjato 2006; Riemann et al. 2015; Nopper et al. 2018). Nevertheless, the diversity and endemism in small forest fragments remain notable (e.g. Rosa et al. 2012). These small patches, embedded in a mosaic landscape, can act as refugia to local herpetofauna (Crottini et al. 2011a; Durkin et al. 2011; Riemann et al. 2015). The description of several new species, microendemic to these tiny forest fragments, has further increased the awareness on their conservation value (e.g. Gehring et al. 2010; Rosa et al. 2014; Jenkins et al. 2014; Crottini et al. 2015; Prötzel et al. 2018).

The Region of Andringitra is located in south-eastern Madagascar (Haute Matsiatra Region). This area is dominated by the Andringitra Massif, protected by Andringitra National Park (Fig. 1). Most of the herpetological research conducted in this Region has been focused on the protected area. The Andringitra Massif was first surveyed over 90 years ago and the first species inventories took place during the 1970s (see Paulian et al. 1971; Nicoll and Langrand 1989; Blommers-Schlösser and Blanc 1993; Goodman 1996; Raselimanana 1999; Goodman and Razafindratsita 2001). In 1993, Goodman (1996) carried out a thorough herpetological assessment within the framework of a multidisciplinary inventory of the eastern slopes of the massif. Several amphibian and reptile species were collected, providing a fundamental contribution to the knowledge of the local herpetofauna. The most updated species list of Andringitra National Park includes 50 amphibians and 40 reptiles (of which five are locally endemic to the Andringitra Massif ), resulting from several biological inventories (both published and unpublished) and observations from the area (Goodman et al. 2018).

In the area surrounding the Andringitra Massif (especially the western side), several small patches of forest are the remains of a much more extensive forest cover (Fig. 1). This area is dominated by a human-modified landscape made of pastures, villages and rice fields (Crottini et al. 2012b; Gould and Andrianomena 2015). Knowledge of the herpetofauna that inhabits these small forest patches is scarce (see Glaw and Vences 2007) and most of the information comes from Anja Community Reserve (e.g. Crottini et al. 2011b, 2012b, 2015), a tiny forest fragment managed by the local community (Fig. 1A). Three reptile species were recently described from Anja and, to date, they are reliably known only from this Reserve (or from a few scattered isolated rock boulders close by): *Brookesia brunoi* Crottini, Miralles, Glaw, Harris, Lima & Vences, 2012; *Paragehyra felicitae* Crottini, Harris, Miralles, Glaw, Jenkins, Randrianantoandro, Bauer & Vences, 2014 and *Phelsuma gouldi* Crottini, Gehring, Glaw, Harris, Lima & Vences, 2011. These exceptional findings stress the conservation value of this small forest fragment and point to the importance and

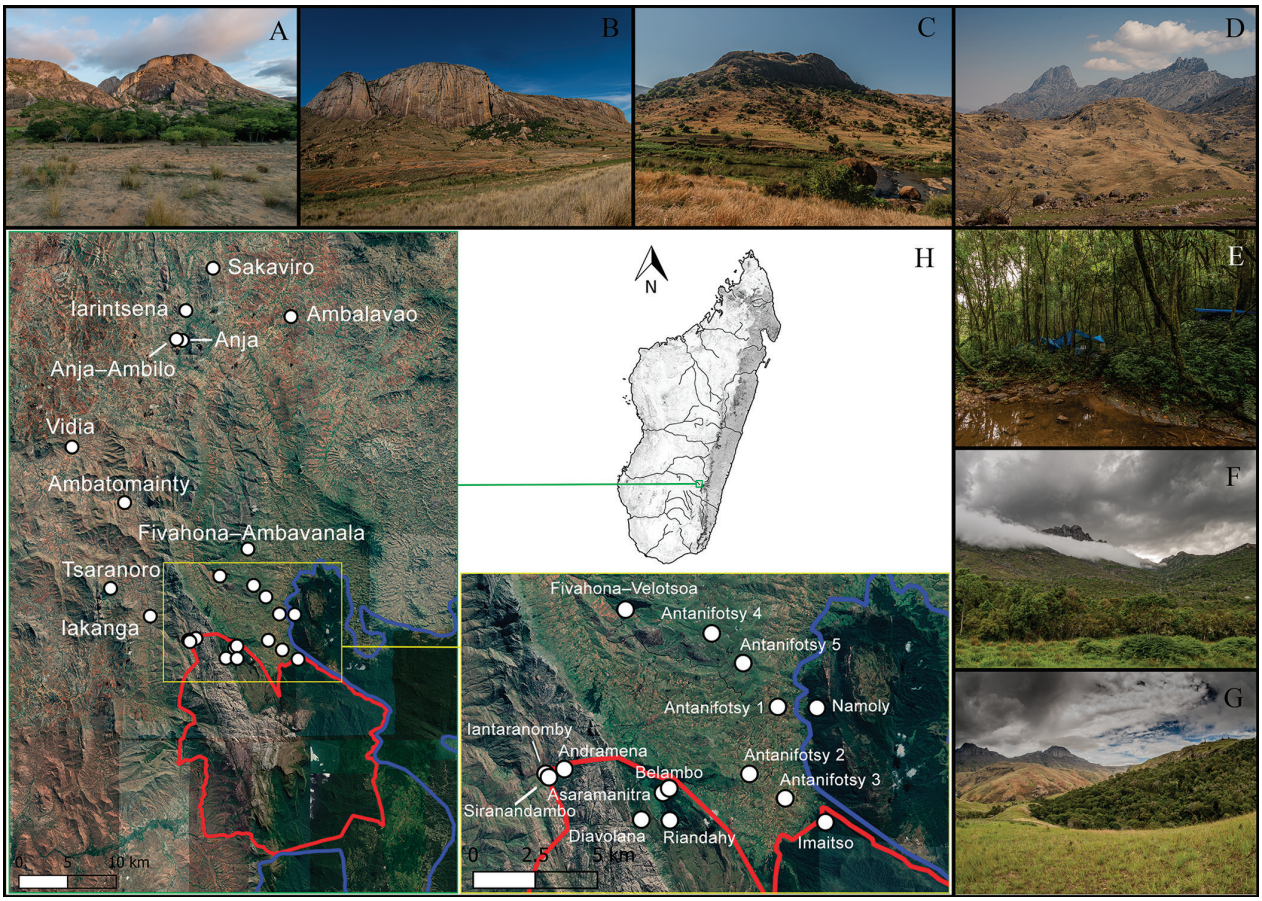


**Figure 1.** Map of the study area and sampling sites. The borders of Andringitra National Park (red) and Paysage Harmonieux Protégé du Corridor Forestier Ambositra–Vondrozo (Ambositra–Vondrozo Forest Corridor) (blue) are shown. See Suppl. material 1: Table S1 for more details on the sampled localities. Map data ©2015 Google (QGIS Development Team 2020). **ANP ES** – Andringitra National Park Eastern Slopes; **ANP WS** – Andringitra National Park Western Slopes. **A.** Anja; **B.** Sakaviro; **C.** Ambatomainty; **D.** Western slopes of the Andringitra Massif from Iantaranomby (ANP WS); **E.** Imaitso (ANP ES); **F.** Belambo (ANP ES); **G.** Fivahona–Velotsoa; **H.** Map with sampling sites. Photographs by Javier Lobón-Rovira.

potential herpetological interest of all other nearby fragments of the Region.

We surveyed the Region of Andringitra, collected tissue samples and specimens, and performed a species-level identification of the sampled amphibians and reptiles. Despite visiting some localities within the borders of Andringitra National Park, we focused our efforts on the several forest fragments surrounding the Massif, to fill the knowledge gap of the herpetological diversity of these poorly explored areas. Here, we provide a first barcoding reference database for the surveyed areas.

# Methods

## Study area

The study area is in the administrative region of Haute Matsiatra, encompassing a portion of the Andringitra Massif and the areas in the immediate surroundings (Fig. 1). This mountain chain dominates the area and is composed of several granitic peaks, amongst which Pic Boby soars as the highest of the region (2,658 m a.s.l.) and as the second highest in the whole country (Nicoll and Langrand 1989; Goodman 1996). The Massif is located at the south-eastern limit between the eastern escarpment and the central high plateau, which determines a sharp bioclimatic gradient with humid conditions in the eastern part and drier weather in the western (Goodman 1996). The regional climate can be defined as cold and humid with marked seasonality (Vidal Romani et al. 2002). Between May and October, the weather is cold and dry, with extreme temperatures that can drop below 0 °C at night, whereas the following season, from November to April, it is warm and wet with heavy rainfalls that represent 80% of the yearly precipitations (Vidal Romani et al. 2002). The strong elevational and climatic variability is responsible for the great diversity of habitats, amongst which there are lowland rainforest and dry forest in the eastern and western parts of the Region, respectively, and, at higher elevations, montane meadows, heathlands and rocky outcrops (Goodman 1996; Goodman et al. 2018).

Most of the Massif is included in Andringitra National Park, which protects an overall area of 31,160 ha (Nicoll

and Langrand 1989). Paysage Harmonieux Protégé du Corridor Forestier Ambositra–Vondrozo (thereafter "Ambositra–Vondrozo Forest Corridor") is eastwards of Andringitra National Park and protects the ca. 200 km of low-elevation forest corridor connecting Vondrozo, Andringitra and Pic d'Ivohibe Special Reserve (ca. 10 km further south than Andringitra National Park) with Ranomafana National Park and Ambositra further north (Fig. 1) (Goodman et al. 2018). Besides these protected areas, three private Reserves are present in the surroundings of the Andringitra Massif: Anja Community Reserve, Sakaviro Community Reserve and Tsaranoro Valley Forest (Fig. 1A and B). They are characterised by fragments of semi-arid deciduous forest located at ca. 950 m a.s.l. at the base of low-elevation granitic mountains rising a few hundred metres relative to the ground level. These forest fragments are particularly small: 36 ha (Anja), 14 ha (Sakaviro) and 46 ha (Tsaranoro) (Crottini et al. 2012b; Gould and Andrianomena 2015). Several other small forest fragments are scattered throughout the area, especially in the western part of the Region, but no legal protection is known for these sites. We sampled in 25 localities, eight of which are within Andringitra National Park, one locality within Ambositra–Vondrozo Forest Corridor and 16 sites are located in the surroundings of the Andringitra Massif (Fig. 1; Suppl. material 1: Table S1). In the present study, we refer to Andringitra as the overall study area comprising the eponymous Massif and the forest fragments in the surrounding areas that were investigated, irrespective of the limits of the protected areas present in the region (Fig. 1).

## Sampling

The samples included in this study were collected between 2009 and 2018, although most of the sampling effort was deployed between the 13th of November and the 18th of December 2018. This period matches the onset of the rainy season and the peak of activity of reptiles and amphibians. We spent a minimum of two days at each sampling locality (defined as where campsites were established). Continuous opportunistic searches took place while moving amongst different sites (Suppl. material 1: Table S1). Animals were opportunistically sought in all microhabitats during both day and night searches, visually detected and caught by hand. Each individual was photographed and the geographic location was recorded with a GPS receiver. One tissue sample was taken from each collected individual. Samples were stored in 96% ethanol and the caught animals were released upon sampling. We identified each individual, based on the morphological descriptions provided in Glaw and Vences (2007) and subsequent descriptions and, in the case of putative candidate species, a limited number of specimens across different localities were collected as vouchers. These individuals were anesthetised and subsequently euthanised with an overdose of MS222 either by immersion in a saturated solution (amphibians) or through intracoelomic injection (reptiles) of the same solution. All voucher specimens were fixed in 96% ethanol and placed in 70% ethanol for long-term storage.

## Laboratory procedures

Total genomic DNA was extracted from tissue samples following the protocol described in Bruford et al. (1992), consisting of a high-salt extraction using proteinase K digestion (10 mg/ml concentration). We amplified a fragment of ca. 550 bp of the 3' terminus of the mitochondrial 16S rRNA gene (16S) for all amphibian samples (Palumbi et al. 1991) and a fragment of ca. 650 bp of the cytochrome oxidase I gene (COI) for reptiles (Nagy et al. 2012), both of which have been widely used for molecular taxonomic identification of Malagasy herpetofauna (Vences et al. 2005); Nagy et al. 2012. Whenever the amplification of the two markers was either unsuccessful or not informative to provide an accurate species identification, we amplified additional markers, namely the 5' terminus of the 16S fragment (16S 5') for amphibians and 16S, NADH dehydrogenase subunit 1 (ND1), NADH dehydrogenase subunit 2 (ND2) or Cytochrome b (Cytb) for reptiles (see Suppl. material 2: Table S2 for primers and PCR conditions). Successfully amplified samples were sequenced with an ABI 3730XL automated sequencer at Macrogen Inc. (Spain). Chromatograms were manually checked and edited, when necessary, with BIOEDIT 7.2.6 (Hall 1999).

## Molecular species identification

Newly-generated sequences were aligned with the Clustal W algorithm implemented in BIOEDIT 7.2.6 (Thompson et al. 1994; Hall 1999). Neighbor joining trees, based on Kimura 2–parameter model distances, were computed for each investigated gene with MEGA X 10.0.5 (Kumar et al. 2018) to roughly divide the samples into genetically uniform groups (16S for amphibians and COI for reptiles). Each group was compared to the molecular database available in GenBank through the BLAST algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Altschul et al. 1997), using nblast default parameters, to retrieve the most similar homologous sequences available in the online database. Species identification was based on the inter-specific thresholds suggested by Vieites et al. (2009) for the 16S fragment in amphibians (3%) and the different thresholds for the different Malagasy reptile groups suggested by Nagy et al. (2012) for the COI fragment. Molecular identification was confirmed by the analysis of the photographic records. In a few instances where molecular data were not available, sample identification was based on the photographic records or the morphological examination of the collected specimens. We used MEGA X 10.0.5 (Kumar et al. 2018) to compute the average uncor-

rected pairwise genetic distances (uncorrected *p*–distances) for each identified taxon. Whenever it was not possible to assign a sample to a formally described species, we used the definitions of different categories of candidate species proposed in Vieites et al. (2009). The working names for the candidate species follow the nomenclature proposed in Vieites et al. (2009) and following publications (e.g. Zimkus et al. 2017) or, in case of first identification, we used the species epithet of the morphologically most similar species, which was prefixed with "sp. aff." and followed by the locality name in quotation marks.

To verify if each taxon was already reported from the Region, we retrieved information from Glaw and Vences (2007), Brown et al. (2014), the IUCN Red List of Threatened Species (IUCN 2020), Goodman et al. (2018) and taxon-specific publications. Species records that were found to be outside the known species distribution were considered range extensions. Information on species identification, distribution, microhabitat preferences and intra-specific genetic variability of each identified taxon are reported in the section Species accounts.

# Results

We generated a total of 520 sequences (308 of amphibians and 212 of reptiles; see Suppl. material 3: Table S3 and Suppl. material 4: Table S4 for GenBank accession numbers (MZ285088–MZ285597) and sampling information and identification of each analysed sample; see Suppl. material 9: Table S7 for locality records of all identified species). Thirteen records (four for amphibians and nine for reptiles) were based on the inspection of photographic material or the morphological examination of the preserved specimens. Neighbor joining trees of the 16S (amphibians) and COI (reptiles) gene fragments are provided in Suppl. material 7: Fig. S1 and Suppl. material 8: Fig. S2.

We sampled a total of 28 amphibian taxa. Most of them belong to the Mantellidae family, of which were 11 Boophinae, 10 Mantellinae and two Laliostominae. We also recorded two hyperoliids and *Ptychadena mascareniensis* (Ptychadenidae). Only two microhylids, both belonging to the subfamily Scaphiophryninae, were sampled. We collected four candidate species (*Boophis* sp. Ca33, *Mantidactylus* sp. Ca14, *Mantidactylus* sp. Ca48 and *Ptychadena* sp. aff. *mascareniensis* "OTU1"). We contributed to extending the current species range for nine taxa (*Heterixalus luteostriatus*, *Scaphiophryne* (*Pseudohemisus*) *calcarata*, *Boophis* (*Sahona*) *doulioti*, *Boophis* (*Boophis*) *boppa*, *Boophis* (*Boophis*) *occidentalis*, *Boophis* (*Boophis*) *rhodoscelis*, *Gephyromantis* (*Phylacomantis*) *corvus*, *Mantella betsileo* and *Mantidactylus* (*Brygoomantis*) sp. Ca14). Amongst the collected material, two taxa are microendemic to the area: *Boophis laurenti* and *Mantidactylus bourgati*.

We identified a total of 38 squamate reptiles. Geckos and chameleons are the most represented groups in our sampling, with 10 taxa each. They are followed by pseudoxyrhophiid snakes (six), skinks (four), gerrhosaurids (three), oplurids (two), sanziniid snakes (two) and one psammophiid snake. We collected five candidate species (*Lygodactylus* sp. aff. *pictus* Ca01 "Isalo", *Paragehyra* sp. aff. *felicitae* "Tsaranoro", *Paroedura* sp. aff. *bastardi* Lineage D, *Trachylepis* sp. aff. *vato* and *Pseudoxyrhopus* sp. Ca2). Amongst these, *Paragehyra* sp. aff. *felicitae* "Tsaranoro" was unknown to science and identified in the present study for the first time. We contributed to extending the known distribution area for 12 taxa (*Furcifer nicosiai*, *Furcifer willsii*, *Lygodactylus* sp. aff. *pictus* Ca01 "Isalo", *Paragehyra felicitae*, *Paroedura rennerae*, *Paroedura* sp. aff. *bastardi* Lineage D, *Phelsuma gouldi*, *Phelsuma lineata elanthana*, *Zonosaurus laticaudatus*, *Trachylepis* sp. aff. *vato*, *Leioheterodon modestus* and *Pseudoxyrhopus* sp. Ca2). Amongst the collected material, five taxa are microendemic to the surveyed region (*Brookesia brunoi*, *Paragehyra felicitae*, *Paragehyra* sp. aff. *felicitae* "Tsaranoro", *Paroedura* sp. aff. *bastardi* Lineage D and *Phelsuma gouldi*).

## Species accounts

**Amphibians**
**Family Hyperoliidae**
**Subfamily Hyperoliinae**

***Heterixalus betsileo* (Grandidier, 1872)**
Fig. 2L

*Heterixalus betsileo* occurs in Madagascar's central highlands, where it can be found in swamps and rice fields (Glaw and Vences 2007). Reported within Andringitra National Park, which is close to the southern limit of its distributional range (Glaw and Vences 2007; Goodman et al. 2018), the species was sampled at night within the forests of Anja and Sakaviro, in the western part of the surveyed region, between ca. 930 and 1,020 m a.s.l. (Suppl. material 3: Table S3). The species resulted in being common in both fragments. The two sampled individuals are genetically almost identical to each other (Suppl. material 5: Table S5) and to published sequences of this taxon sampled in Andringitra (16S: EF646668, JQ346497; 16S 5': EF646633) and Andasibe (e.g. 16S: EF646661).

***Heterixalus luteostriatus* (Andersson, 1910)**
Fig. 2M

*Heterixalus luteostriatus* has a wide and discontinuous distribution across north-western and western Madagascar, including the Isalo Massif. This species can commonly be found in swamps and rice fields (Glaw and Vences 2007; Mercurio et al. 2008) and the record reported here constitutes an extension of the known species distributional range by ca. 180 km to the east from the Isalo Massif (Glaw and Vences 2007). Our study found this taxon at Anja, Anja–Ambilo, Iarintsena,

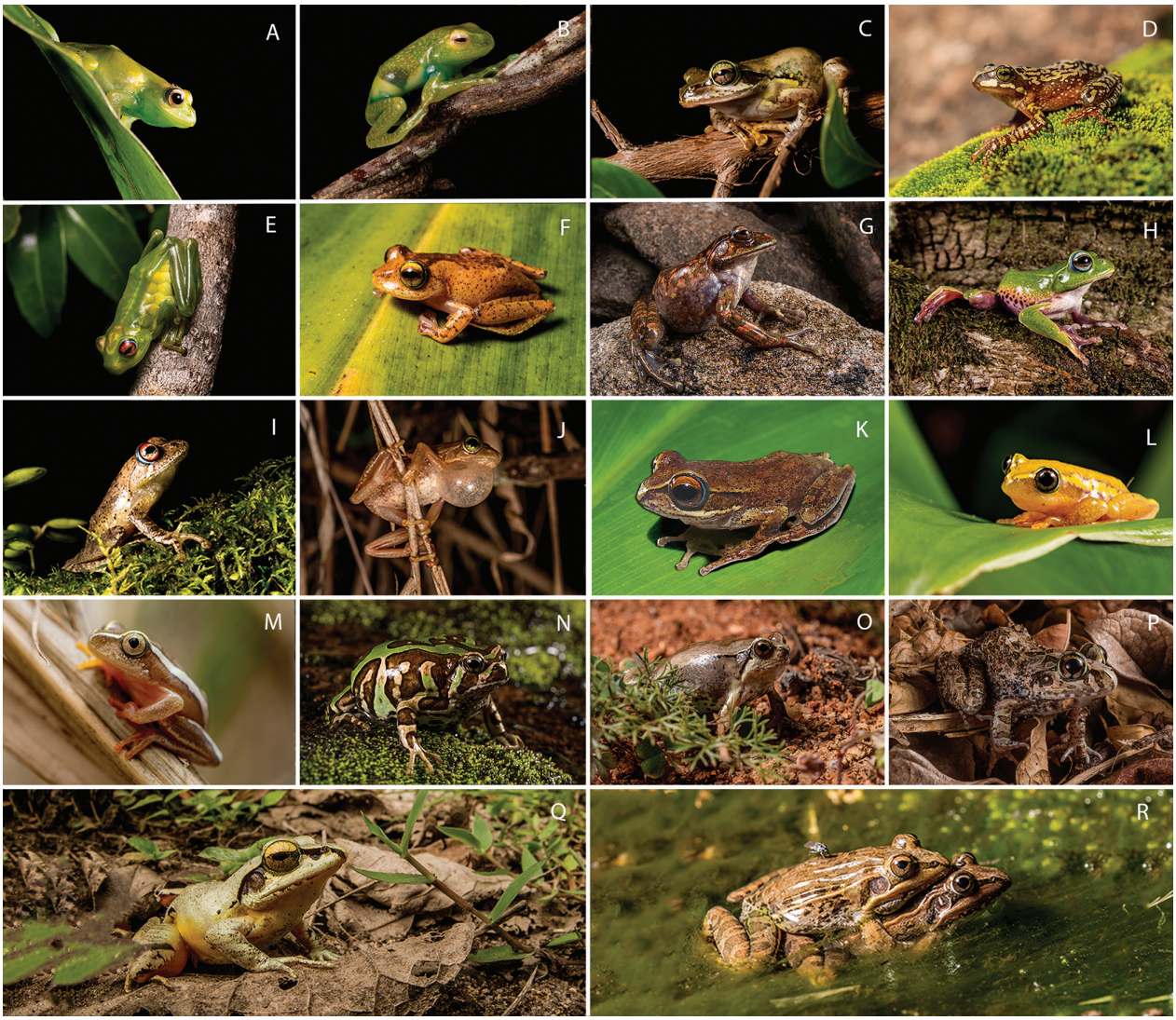


**Figure 2.** Hyperoliid, microhylid, ptychadenid and mantellid (subfamilies Boophinae and Laliostominae) species identified in this study. Sampling localities for each photographed individual are provided. **ANP ES** – Andringitra National Park Eastern Slopes; **ANP WS** – Andringitra National Park Western Slopes (Fig. 1; Suppl. material 1: Table S1). **A.** *Boophis* (*Boophis*) *ankaratra* from Imaitso (ANP ES); **B.** *Boophis* (*Boophis*) *boppa* from Iantaranomby (ANP WS); **C.** *Boophis* (*Sahona*) *doulioti* from Ambalavao; **D.** *Boophis* (*Boophis*) *laurenti* from Iantaranomby (ANP WS); **E.** *Boophis* (*Boophis*) *luteus* from Fivahona–Velotsoa; **F.** *Boophis* (*Boophis*) *majori* from Asaramanitra (ANP ES); **G.** *Boophis* (*Boophis*) *obscurus* from Imaitso (ANP ES); **H.** *Boophis* (*Boophis*) *occidentalis* from Andramena (ANP WS); **I.** *Boophis* (*Boophis*) *popi* from Imaitso (ANP ES); **J.** *Boophis* (*Boophis*) sp. Ca33 from Asaramanitra (ANP ES); **K.** *Boophis* (*Boophis*) *rhodoscelis* from Fivahona–Ambavanala; **L.** *Heterixalus betsileo* from Sakaviro; **M.** *Heterixalus luteostriatus* from Anja; **N.** *Scaphiophryne* (*Scaphiophryne*) *madagascariensis* from Andramena (ANP WS); **O.** *Scaphiophryne* (*Pseudohemisus*) *calcarata* from Ambalavao; **P.** *Laliostoma labrosum* from Anja; **Q.** *Aglyptodactylus madagascariensis* from Namoly; **R.** *Ptychadena* sp. aff. *mascareniensis* "OTU1" from Iantaranomby (ANP WS). Photographs by Javier Lobón-Rovira (A–J, L–R) and Francesco Belluardo (K).

Sakaviro and Tsaranoro (between ca. 940 and 1,020 m a.s.l.) (Suppl. material 3: Table S3), sometimes in syntopy with *H. betsileo*. This species seemed abundant and was found at both day (sleeping on leaves) and night on small shrubs in areas with degraded and open vegetation outside forests. The 16S sequences of the individual sampled during our survey are identical to each other (Suppl. material 5: Table S5) and individuals from Isalo (e.g. KX066672). The genetic distance between the individuals of *H. luteostriatus* and *H. betsileo* sampled in this study is 3.33%.

**Family Microhylidae**
**Subfamily Scaphiophryninae**

***Scaphiophryne* (*Pseudohemisus*) *calcarata* (Mocquard, 1895)**
Fig. 2O

*Scaphiophryne calcarata* belongs to the subgenus *Pseudohemisus*. A recent study assigned the lectotype of five nomina (including *Calophrynus calcaratus* Mocquard, 1895) (Scherz et al. 2021). This taxonomic

proposal, together with the analysis of newly-collected material, enabled the restriction of the lineage of *Scaphiophryne calcarata* to the southern and south-western portion of the Island. The samples analysed in this study, collected at night in Ambalavao and Sakaviro (at ca. 1,000–1,010 m a.s.l.), are assigned to this lineage. They were included in Scherz et al. (2021) contributing to extend the known distributional range by ca. 180 km in a northern direction. This species was rare and only two individuals were found active at night: one in the garden of a hotel and the second on the ground in an open environment next to a small forest patch surrounded by pastures (Suppl. material 3: Table S3). The two analysed samples are identical to each other (Suppl. material 5: Table S5) and to individuals from Isalo (e.g. MH063283), and are almost identical to the other published sequences of this species (e.g. Berenty, AY834192; Tolagnaro AY834193). The genetic distance from individuals of *S. obscura* and the north-west lineage is ca. 3.3% (e.g. Kirindy, KU937802; Isalo, KX066692) and ca. 4% (Ampijoroa, KU937797), respectively.

***Scaphiophryne* (*Scaphiophryne*) *madagascariensis* (Boulenger, 1882)**
Fig. 2N

This species is distributed in the central highlands, between the Ankaratra Massif in the north and the Andringitra Massif, in the south. This frog generally inhabits high elevation sites, both above and below the tree line (Glaw and Vences 2007; Goodman et al. 2018). *Scaphiophryne madagascariensis* was sampled at Andramena, Asaramanitra and Belambo, on both the eastern and western slopes of the Massif and between ca. 1,580 and 1,740 m a.s.l. The species was frequently encountered during our surveys. The individuals were often spotted in clearings next to a forest (Suppl. material 3: Table S3), both during day and night. We observed no genetic difference amongst the analysed samples (Suppl. material 5: Table S5). When comparing the samples analysed here with the sequences available in Genbank, we found no difference between our samples and individuals collected in the same area (Andohariana Plateau, DQ787110) and they are less than 1% divergent from individuals from Ankaratra (KC180053).

**Family Ptychadenidae**

***Ptychadena* sp. aff. *mascareniensis* "OTU1" UCS**
Fig. 2R

*Ptychadena mascareniensis* (Duméril & Bibron, 1841) is the most widespread amphibian in Madagascar. It is usually found next to any pond, swamp and rice field, often outside the forest. Until a few years ago, Malagasy populations were considered conspecific to the populations from mainland Africa and it was thought that the species had been recently introduced to the Island (Glaw and Vences 2007). A recent study showed that *P. mascareniensis* is a species complex. Malagasy populations are not conspecific with the populations of the African continent and the populations assigned to *P. mascareniensis* from Madagascar belong to three operational taxonomic units (OTUs) in need of taxonomic revision (Zimkus et al. 2017). All the samples analysed in this study belong to *P.* sp. aff. *mascareniensis* "OTU1" (sensu Zimkus et al. 2017). This species was amongst the most frequent and abundant frogs we recorded, found both during day and night, despite being more commonly active during the day. It was normally found in rice fields or close to temporary water bodies, between ca. 870 and 1,650 m a.s.l. (Suppl. material 3: Table S3). We sampled this taxon at Andramena, Asaramanitra, Iantaranomby, Imaitso, Ambalavao, Ambatomainty, Antanifotsy 1, 3, 5, Anja, Fivahona–Ambavanala, Fivahona–Velotsoa, Namoly and Tsaranoro. The individuals, analysed for this study, are genetically uniform amongst each other (Suppl. material 5: Table S5) and are almost identical to other individuals collected in the area (Andohariana: AY517587 and AY517588) and across Madagascar (e.g. Toliara, KX836419; Ranomafana, KX836413; Bemaraha, KX836392; Andohahela, KX836390; Isalo, KX066671).

**Family Mantellidae**
**Subfamily Boophinae**

***Boophis* (*Sahona*) *doulioti* (Angel, 1934)**
Fig. 2C

Distributed in western and southern Madagascar, this taxon is mostly found in open areas and secondary vegetation (Glaw and Vences 2007). *Boophis doulioti* had not been reported to this area before and our finding represents an extension of the species distributional range by ca. 150 km towards the east. Individuals were spotted active at night in Anja and Iarintsena (on the western side of the Andringitra Massif), perching on trees and shrubs within semi-arid deciduous forest, in open environments next to villages, close to rice fields and within the town of Ambalavao at an elevation of ca. 950–1,030 m a.s.l (Suppl. material 3: Table S3). The analysed samples are all identical to each other (Suppl. material 5: Table S5) and samples collected at Isalo (KX066561), Tranomaro (MK132751) and Ranomafana (AY848515; this record being reported as *Boophis tephraeomystax* (Duméril, 1853)).

***Boophis* (*Boophis*) *ankaratra* Andreone, 1993**
Fig. 2A

*Boophis ankaratra* is commonly found in the central highlands at high-elevation locations, both in rainforest and degraded gallery vegetation (Glaw and Vences 2007; IUCN SSC Amphibian Specialist Group 2016a). We collected a single individual during a night search at Imaitso, within an area of dense rainforest (ca. 1,670 m a.s.l.), perching on riverine vegetation surrounding a small stream (Suppl. material 3: Table S3). This sample

is identical to other available sequences from the Andringitra Region (Andringitra: AF411611; Imaitso Forest: DQ068396, DQ068397, DQ068398). The individual analysed in this study is molecularly almost identical to the population from Ranomafana (difference: 0.30%; GU974475).

***Boophis* (*Boophis*) *boppa* Hutter, Lambert, Cobb, Andriampenomanana & Vences, 2015**
Fig. 2B

*Boophis boppa* has been recently described from Ranomafana National Park and Antoetra (Andreone et al. 2007; Hutter et al. 2015). This record represents a range extension for the species by ca. 80 km towards the south. Individuals were locally abundant and were collected at Asaramanitra (along the eastern slope of the Massif) and Iantaranomby (on the western slope), at an elevation of ca. 1,580–1,600 m a.s.l. Sampled individuals were perching at night on riverine vegetation along large streams in both forested areas and open environments with scattered trees (Suppl. material 3: Table S3). The analysed samples collected in this study are identical (Suppl. material 5: Table S5). They are also identical to individuals from the type locality (Ranomafana: KT588038) and very similar (99.80% similarity) to individuals from Antoetra (e.g. AY848438).

***Boophis* (*Boophis*) *laurenti* (Guibé, 1947)**
Fig. 2D

This species is currently known only from Andringitra National Park, where it can be found in montane heathlands above 1,500 m a.s.l. (IUCN SSC Amphibian Specialist Group 2016b; Goodman et al. 2018). We sampled *Boophis laurenti* either on mossy rocks in streams or perching on dense vegetation and scattered trees close to running water. The species was detected during day and night (although more frequently active at night) and seemed common along both the western (Andramena, Iantaranomby) and the eastern (Diavolana) slopes of the Massif (between ca. 1,580 and 1,740 m a.s.l.) (Suppl. material 3: Table S3). Our samples are genetically uniform to each other (Suppl. material 5: Tables S5) and in comparison with previously available sequences (Andohariana: AY848599, AY659964; Andringitra: AY659963; Cuvette Boby: AY848575).

***Boophis* (*Boophis*) *luteus* (Boulenger, 1882)**
Fig. 2E

This species can be found along streams in rainforest and secondary vegetation in several localities of eastern Madagascar (including Andringitra National Park), but also in Isalo in the south-west and Ambohitantely in the central highlands (Glaw and Vences 2007; Cocca et al. 2018; Goodman et al. 2018). The species was rare and only two individuals were spotted at Fivahona–Velotsoa, in the eastern part of the surveyed region, at ca. 1,270 m a.s.l. (Suppl. material 3: Table S3). Both individuals were found in roosting positions on trees near a stream at night. They show limited genetic differentiation and are almost identical to individuals collected at Ranomafana (e.g. FJ559330) and Isalo (FJ559354) (Suppl. material 5: Table S5).

***Boophis* (*Boophis*) *majori* (Boulenger, 1896)**
Fig. 2F

*Boophis majori* is distributed in eastern Madagascar within a restricted region comprised of Antoetra, Ranomafana, Andringitra National Park (Imaitso Forest) (Brown et al. 2014) and Ivohibe, where it can be found on trees along rainforest streams (Glaw and Vences 2007; IUCN SSC Amphibian Specialist Group 2016c; Goodman et al. 2018). *Boophis majori* was a common species. During a night search, we collected three samples perching on trees along a large stream within rainforest habitat in Asaramanitra, on the eastern slope of the Massif, at ca. 1,590 m a.s.l. (Suppl. material 3: Table S3). These samples are identical to each other (Suppl. material 5: Table S5) and have 0.50% genetic distance with samples from Ranomafana (e.g. AY848586).

***Boophis* (*Boophis*) *obscurus* (Boettger, 1913)**
Fig. 2G

*Boophis obscurus* was recently resurrected from the synonymy with *Boophis goudotii* Tschudi, 1838 (Glaw et al. 2010). The species is distributed in south-eastern Madagascar between Ranomafana, Isalo and Andringitra National Park (Andohariana Plateau) (Glaw et al. 2010; IUCN SSC Amphibian Specialist Group 2016d; Goodman et al. 2018). *Boophis obsurus* was common and abundant across the Region and was sampled in Andramena, Asaramanitra, Belambo, Iantaranomby, Imaitso, Riandahy, Siranandambo, Fivahona–Ambavanala, Fivahona–Velotsoa, Namoly and Tsaranoro (Suppl. material 3: Table S3). *Boophis obscurus* was collected between ca. 950 and 1,740 m a.s.l., normally found on rocks and boulders along water streams. It was sampled during both diurnal and nocturnal searches, despite being more frequent at night. The samples analysed in this study are molecularly similar to each other (Suppl. material 5: Table S5) and another sample collected from the same area (AY848568). They show only limited genetic difference to samples from other localities (e.g. Ranomafana: GU975058; Isalo: KX066565).

***Boophis* (*Boophis*) *occidentalis* Glaw & Vences, 1994**
Fig. 2H

*Boophis occidentalis* has a discontinuous distribution in western (Tsingy de Bemaraha) and central Madagascar (between Isalo, Zazafotsy and Antoetra), where it is found in dry deciduous forest (Vences et al. 2003; Glaw

and Vences 2007). This species was previously not reported from our study area and this record extends the species distributional range by ca. 50 km towards the east. This species was collected in the eastern and western part of the Region (Andramena, Iantaranomby, Ambatomainty, Fivahona–Velotsoa and Tsaranoro; Suppl. material 3: Table S3) between ca. 920 and 1,740 m a.s.l., where it was often found on high trees close to streams. *Boophis occidentalis* was particularly common in Tsaranoro, whereas, in the other sampling sites, it was rarer. The individuals were all spotted at night. The collected samples are identical (Suppl. material 5: Table S5) and show high genetic affinity with individuals from Isalo (e.g. KX066570) and Antoetra (AY341720), with less than 1% genetic distance.

***Boophis* (*Boophis*) *popi* Köhler, Glaw, Rosa, Gehring, Pabijan, Andreone & Vences, 2011**
Fig. 2I

*Boophis popi* is distributed in central-eastern Madagascar between Tsinjoarivo and Andringitra. This species can be found along streams in montane rainforest in a narrow elevational range of 1,000–1,500 m a.s.l. (Andreone et al. 2007; Köhler et al. 2011; Goodman et al. 2018). The samples included in the present study were collected on the eastern slopes of the Massif at Imaitso (where other individuals were previously sampled) at higher elevation (ca. 1,520–1,690 m a.s.l.), slightly above the currently known range for the species (Köhler et al. 2011) (Suppl. material 3: Table S3). The species was particularly abundant in this site. The collected individuals were found along small slow-flowing streams within rainforest, perching on the riverine vegetation. They were mostly spotted at night, despite a single individual being found during the day. The analysed samples are almost identical to each other (Suppl. material 5: Table S5) and to previously available sequences (Andringitra: e.g. JN679879; Antoetra: e.g. AY848551).

***Boophis* (*Boophis*) *rhodoscelis* (Boulenger, 1882)**
Fig. 2K

This species is distributed between Ranomafana and Antoetra, where it can be found in swampy areas. Previous records from Ambohitantely have recently been assigned to *Boophis andrangoloaka* (Ahl, 1928) (Glaw et al. 2010). *Boophis rhodoscelis* was not yet reported from the study area and this record represents a range extension by ca. 70 km to the south. The species was rare. Two calling males were sampled at Fivahona–Ambavanala, in the eastern part of the surveyed region. The individuals were found during the night at ca. 1,480 m a.s.l. perching on trees next to a fast-flowing stream within rainforest (Suppl. material 3: Table S3). These two samples are identical to each other (Suppl. material 5: Table S5) and to samples collected at Antoetra (AY848616) and Ranomafana (e.g. AY848619).

***Boophis* (*Boophis*) sp. Ca33 UCS**
Fig. 2J

This unconfirmed candidate species is part of the species group comprising *Boophis microtympanum* (Boettger, 1881) and *B. laurenti*. *Boophis* sp. Ca33 (following the terminology as in Vieites et al. (2009) and reported in Glaw and Vences (2007) as *B.* sp. aff. *microtympanum*) is morphologically similar to *B. microtympanum* from which it is distinguished by a more uniform dorsal colouration rather than the vermiculated pattern described in the nominal species. Vocalisations of the two taxa are very similar and present only some quantitative difference in note duration (Glaw and Vences 2007; Vieites et al. 2009). Glaw and Vences (2007) report the presence of *Boophis* sp. Ca33 in the surveyed area at Cirque Namoly and Vieites et al. (2009) states that this taxon was found to live in sympatry with *B. microtympanum*, an observation that might support the distinction of these taxa (sensu Miralles et al. 2021), although the genetic differentiation at the 16S marker is below the standard threshold of 3% (Vieites et al. 2009). The species was not abundant. We sampled three individuals of *Boophis* sp. Ca33 at Asaramanitra, on the eastern slopes of the Massif. These individuals were found at night perching on branches next to a large fast-flowing stream at ca. 1,590 m a.s.l. of elevation (Suppl. material 3: Table S3). The samples analysed here are identical to each other (Suppl. material 5: Table S5), to other available sequences from the area (AY848597) and Mahahira (AY848604; in the Ranomafana area). They are also almost identical to samples from Itremo (JF903885). Individuals from Ankaratra (HM769929), Col des Tapias (AJ315918), Ambohitantely (HM769928) and Andasibe (AY848598) attributed to *B. microtympanum* are ca. 1.60% different.

**Subfamily Laliostominae**

***Aglyptodactylus madagascariensis* (Duméril, 1853)**
Fig. 2Q

This species inhabits rainforest in northern and eastern Madagascar. The Andringitra Massif represents the southernmost limit of its distributional range and the highest elevational record (Imaitso forest, 1,509 m a.s.l.) for the species (Köhler et al. 2015; Goodman et al. 2018). It is an explosive breeder reproducing in temporary ponds, but it can often be found on the forest floor outside the breeding season (Glaw and Vences 2007). The four analysed individuals were found on the floor of the rainforest at Imaitso and Namoly, in the eastern part of the Region at an elevation range between ca. 1,550 and 1,650 m a.s.l. (Suppl. material 3: Table S3). They were recorded during both night and day, although more frequently spotted at night. The collected samples are genetically identical to each other (Suppl. material 5: Table S5) and to other samples from the Region (e.g. KT159884). They are slightly different (ca. 0.40% ge-

netic distance) from individuals collected in Ranomafana (e.g. AY847991).

***Laliostoma labrosum* (Cope, 1868)**
Fig. 2P

This species is widely distributed in dry habitats of western, northern and southern Madagascar (Glaw and Vences 2007). Within the Region of Andringitra, this species was already reported from the town of Ambalavao (e.g. AY848009). *Laliostoma labrosum* was not frequently encountered during our surveys. Individuals were spotted at night in the western portion of the Region in Iantaranomby and Anja. The encounters took place on the floor of semi-arid deciduous forest, close to large streams in open environments and within the town of Ambalavao, in a hotel garden, at an elevation between ca. 980 and 1,560 m a.s.l. (Suppl. Material 3: Table S3). The analysed samples are genetically identical to each other (Suppl. material 5: Table S5) and to other populations from different parts of Madagascar (e.g. Toliara: KR337974; Ankarafantsika: KR337954; Kirindy: KR337951; Isalo: KX066667; Tsaratanana: KR337858).

**Subfamily Mantellinae**

***Gephyromantis* (*Phylacomantis*) *corvus* (Glaw & Vences, 1994)**
Fig. 3H

The samples of this taxon, collected at Anja, Sakaviro and Tsaranoro (in the western portion of the surveyed area; Suppl. material 3: Table S3), have been included in a recent taxonomic study (Cocca et al. 2020) where the authors synonymised *Gephyromantis azzurrae* Mercurio & Andreone, 2007 with *G. corvus* and described the second *Phylacomantis* lineage inhabiting Isalo as *Gephyromantis kintana* Cocca, Andreone, Belluardo, Rosa, Randrianirina, Glaw & Crottini, 2020. Previously known only from the Isalo Massif, where it inhabits large and deep canyons with fast-flowing water and gallery forests (Glaw and Vences 2007; Mercurio and Andreone 2007), these records represent an important range extension for the species by ca. 180 km towards east (Cocca et al. 2020). This species was rare. The individuals were found during night searches at an elevational range of ca. 950–1,020 m a.s.l. along small canyon-like streams in banks, ravines and crevices both within semi-arid deciduous forest and in open habitats. The analysed samples are identical (Suppl. material 5: Table S5) and have a limited genetic differentiation (ca. 0.60%) from the individuals of Isalo (KX066651).

***Gephyromantis* (*Gephyromantis*) *blanci* Guibé, 1974**
Fig. 3G

Males of *Gephyromantis blanci* are easily found in rainforest and secondary vegetation while calling on the forest floor or low branches. Ambalamarovandana, located in the eastern slopes of the Andringitra Massif, is the type locality of the species (Glaw and Vences 2007; IUCN SSC Amphibian Specialist Group 2016e; Goodman et al. 2018). *Gephyromantis blanci* was locally abundant and was sampled at Imaitso and Namoly, in the eastern part of the Region, at an elevation range of ca. 1,540–1,690 m a.s.l. (Suppl. material 3: Table S3). All collected individuals were males calling from the forest floor within dense rainforest, spotted both during day and night-time. The analysed samples are genetically identical to each other and to sequences previously available from the area (e.g. AY848324) (Suppl. material 5: Table S5).

***Mantella betsileo* (Grandidier, 1872)**
Fig. 3I

This species is discontinuously reported from multiple localities in western Madagascar between the Isalo Massif, at the south, and Bemaraha, at the north. Within Isalo National Park, this species can be observed around both temporary and permanent water bodies, generally outside of the canyons (Glaw and Vences 2007; Mercurio et al. 2008). Although not yet confirmed from the surveyed area, the species was described from individuals likely collected in the Betsileo Region, which includes the Region of Andringitra. Sightings of *Mantella betsileo* were rare in our survey. We found this taxon during the night in the western portion of the Region at Ambatomainty, where two individuals were spotted active within a humid pit in the ground at ca. 930 m a.s.l. of elevation. Another individual was found in Anja, in an open area close to a granitic boulder. These records extend the known distributional range of the species by ca. 180 km towards the east (Suppl. material 3: Table S3). The analysed samples are identical (Suppl. material 5: Table S5) and very similar to individuals from Isalo (e.g. EF674841) and Morondava (AF215288).

***Mantidactylus* (*Brygoomantis*) *betsileanus* (Boulenger, 1882)**
Fig. 3B

This species is distributed in central-eastern Madagascar where it is often found along slow-flowing streams within rainforest, but it can also be found in degraded vegetation and human-dominated areas (e.g. rice fields) (Glaw and Vences 2007). We sampled *M. betsileanus* at Fivahona–Ambavanala, Fivahona–Velotsoa, Namoly and Tsaranoro, both in the eastern and western sides of the surveyed area (between ca. 930 and 1,650 m a.s.l.) (Suppl. material 3: Table S3). The species seemed abundant, especially in Fivahona–Velotsoa and Tsaranoro. The individuals were spotted both during day and night-time along the banks of slow-flowing streams within forest. Molecularly, the individuals analysed here are uniform (Suppl. material 5: Table S5) and are almost identical to individuals collected at Itremo (JF903887), Ranomafana (AY848275), Andasibe


**Figure 3.** Amphibians of the mantellid subfamily Mantellinae identified in this study. Sampling localities for each photographed individual are provided. **ANP ES** – Andringitra National Park Eastern Slopes; **ANP WS** – Andringitra National Park Western Slopes (Fig. 1; Suppl. material 1: Table S1). **A.** *Mantidactylus* (*Bryogoomantis*) *bourgati* from Namoly; **B.** *Mantidactylus* (*Bryogoomantis*) *betsileanus* from Namoly; **C.** *Mantidactylus* (*Chonomantis*) *delormei* from Imaitso (ANP ES); **D.** *Mantidactylus* (*Ochthomantis*) *femoralis* from Asaramanitra (ANP ES); **E.** *Mantidactylus* (*Bryogoomantis*) sp. Ca14 from Tsaranoro; **F.** *Mantidactylus* (*Hylobatrachus*) sp. Ca48 from Fivahona–Velotsoa; **G.** *Gephyromantis* (*Gephyromantis*) *blanci* from Imaitso (ANP ES); **H.** *Gephyromantis* (*Phylacomantis*) *corvus* from Sakaviro; **I.** *Mantella betsileo* from Ambatomainty; **J.** Subadult and tadpoles of *Spinomantis elegans* from Imaitso (ANP ES). Photographs by Javier Lobón-Rovira.

(e.g. FJ559234), Fierenana (e.g. EF606877) and Mandraka (AY848238).

### *Mantidactylus* (*Brygoomantis*) *bourgati* Guibé, 1974

Fig. 3A

Endemic to the Andringitra Massif (already reported from Imaitso Forest and Andohariana Plateau), the species is known to live along streams within forest above the tree line (Glaw and Vences 2007). Ambalamarovandana, located in the eastern slopes of the Massif, is the type locality of the species. We sampled *Mantidactylus bourgati* across a large portion of the surveyed sites (Andramena, Asaramanitra, Belambo, Iantaranomby, Imaitso, Riandahy, Siranandambo, Fivahona–Ambavanala, Fivahona–Velotsoa, Namoly and Tsaranoro; Suppl. material 3: Table S3), both on the western and eastern portion of the Region at a wide elevational range (between ca. 930 and 1,740 m a.s.l.). In all sites, *M. bourgati* was abundant and the individuals were spotted during both day and night along the banks of streams within forest. Analysed individuals are genetically almost identical (Suppl. material 5: Table S5).

***Mantidactylus* (*Brygoomantis*) sp. Ca14 UCS**
Fig. 3E

*Mantidactylus* sp. Ca14 is closely related to *Mantidactylus alutus* (Peracca, 1893) and morphologically similar to *Mantidactylus ulcerosus* (Boettger, 1880), relative to which it exhibits significant bioacoustic differences, slight morphological dissimilarities and substantial mitochondrial divergence (Vieites et al. 2009). This taxon is already known from Isalo and Tsingy de Bemaraha where it is typically encountered along slow-flowing streams in forested areas (Glaw and Vences 2007; Cocca et al. 2018). This record extends the known distributional range of this taxon by ca. 180 km towards the east from the Isalo Massif. The species was rare, with only two individuals sampled at night in Tsaranoro (at ca. 910 m a.s.l.), in the western part of the Region (Suppl. material 3: Table S3). They were spotted in a small pond below a large boulder. These two samples show no genetic differentiation to each other (Suppl. material 5: Table S5) and with samples from Isalo (e.g. KX066586).

***Mantidactylus* (*Chonomantis) delormei* Angel, 1938**
Fig. 3C

This species is typically found along streams in montane forests between Ranomafana and the Andringitra Massif, which is also its type locality (Glaw and Vences 2007; Goodman et al. 2018). We sampled *Mantidactylus delormei* at Asaramanitra, Belambo and Imaitso, along the eastern slopes of the Massif between ca. 1,570 and 1,710 m a.s.l. Although a leaf litter-dwelling species, invididuals were always in proximity to streams within rainforest (Suppl. material 3: Table S3). The animals were sampled during both day and night searches, although they were more frequent at night. The analysed individuals are almost identical to each other (Suppl. material 5: Table S5) and to a previously available sequence from Andringitra (AY848148) and 1.1% distant from individuals from Ranomafana (e.g. GU975171).

***Mantidactylus* (*Ochthomantis*) *femoralis* (Boulenger, 1882)**
Fig. 3D

The type locality of this taxon is "East Betsileo", which roughly corresponds to the surveyed area and the lineage currently assigned to this name is known from Andringitra and the Isalo Massifs (Glaw and Vences 2007; Cocca et al. 2018; Goodman et al. 2018). This locally abundant species was recorded at Asaramanitra, Iantaranomby, Imaitso, Riandahy, Siranandambo, Anja, Fivahona–Velotsoa and Tsaranoro, at an elevation range between ca. 930 and 1,730 m a.s.l., both in the eastern and western portions of the study area, showing a similar distribution to *M. bourgati* (Suppl. material 3: Table S3). The sampled individuals were observed along streams banks within forest and were collected during both day and night-time, despite being more frequent at night. They are genetically identical to each other (Suppl. material 5: Table S5) and almost identical with previously available sequences (e.g. Andringitra: HQ610918; Isalo: AY324813).

***Mantidactylus* (*Hylobatrachus*) sp. Ca48 UCS**
Fig. 3F

*Mantidactylus* sp. Ca48 is morphologically similar to both *Mantidactylus lugubris* (Duméril, 1853) and *Mantidactylus cowanii* (Boulenger, 1882). This undescribed lineage is widely distributed amongst Isalo, Itremo, Antoetra (in sympatry with *M. cowanii*), Ranomafana, Ambohitsara, Vondrozo and Manombo (Cocca et al. 2018; Scherz et al. 2019). Goodman et al. (2018) report the possible presence of *M. lugubris* in the species list of Andringitra National Park although the record is considered as doubtful. We found this taxon at Asaramanitra, Iantaranomby and Fivahona–Velotsoa, in both the eastern and western parts of the Region (between ca. 930 and 1,650 m a.s.l.) (Suppl. material 3: Table S3). The species seemed common in Fivahona–Velotsoa, whereas, in the other sites, it was rarer. Individuals were found at night on rocks along streams. The analysed samples are genetically identical (Suppl. material 5: Table S5) and ca. 100% identical to individuals from Ranomafana (e.g. MK447667), Ambohitsara (e.g. MK447637), Ambatolahy (MK447645), Valohoaka (MK447661), Miranony (MK447658) and Manombo (AY848186).

***Spinomantis elegans* (Guibé, 1974)**
Fig. 3J

*Spinomantis elegans* is distributed in south-eastern Madagascar between Ranomafana to the north and Andohahela to the south, including the area of the Andringitra Massif, which is close to the type locality (Ivohibe). This species lives at high elevations and is often found within small caves, between outcrops or hiding below rocks, both within forest and above the tree line (Glaw and Vences 2007). *Spinomantis elegans* was rarely encountered in our surveyes. We sampled a tadpole in a small pond at Imaitso and a subadult next to a large permanent stream at Asaramanitra, on the eastern slopes of the Massif at ca. 1,540–1,600 m a.s.l. of elevation (Suppl. material 3: Table S3). Both individuals were found at night. The two analysed samples are identical to each other (Suppl. material 5: Table S5) and previously available sequences (e.g. Cuvette Boby: AY659960; Ranomafana: AY848405).

**Reptiles**
**Family Chamaeleonidae**
**Subfamily Brookesiinae**

***Brookesia brunoi* Crottini, Miralles, Glaw, Harris, Lima & Vences, 2012**
Fig. 4A

This species is currently known only from Anja Community Reserve where it is typically encountered on

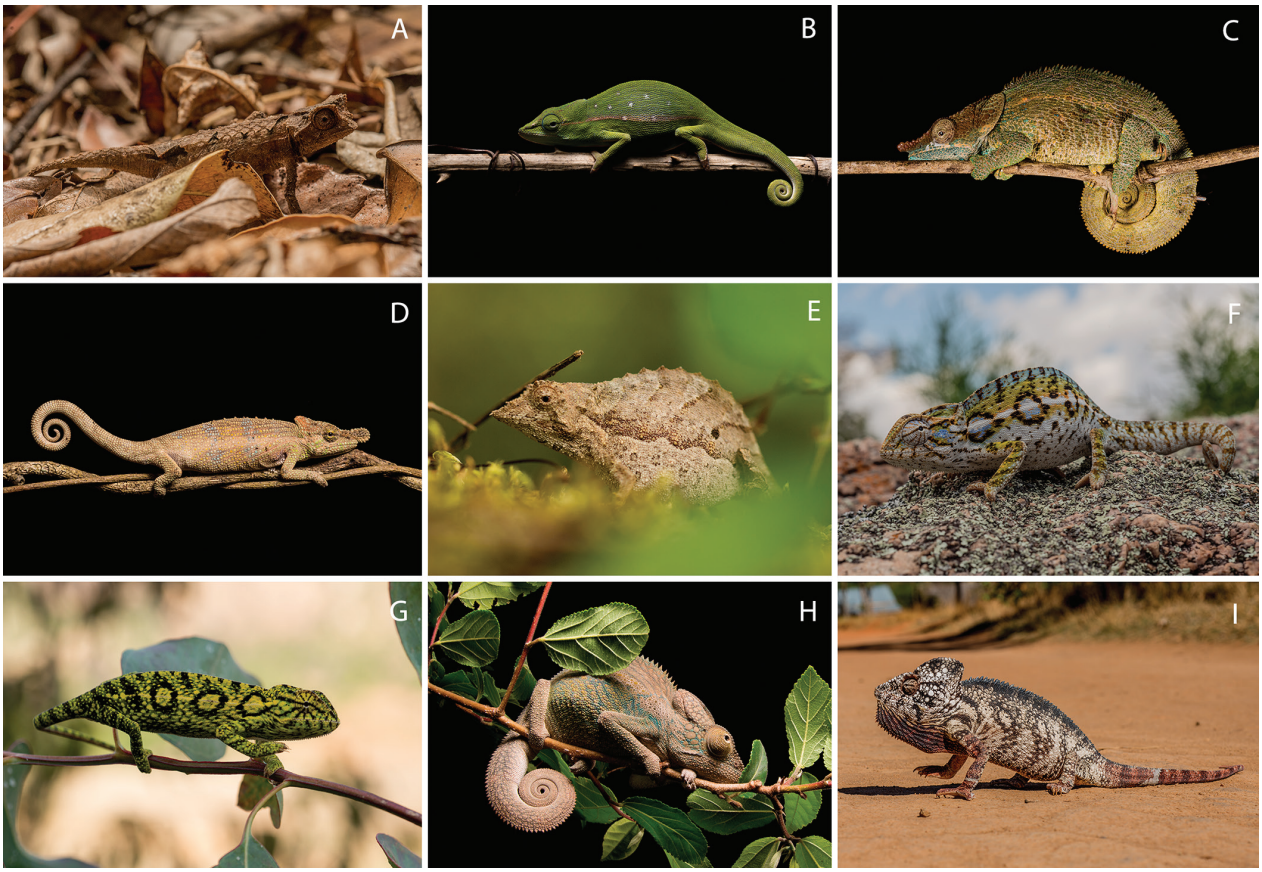


**Figure 4.** Chameleonid species identified in this study. The picture of the individual identified as *Furcifer willsii* from Fivahona–Velotsoa, found within the stomach content of a *Mimophis mahfalensis*, is not shown. Sampling localities for each photographed individual are provided. **ANP ES** – Andringitra National Park Eastern Slopes; **ANP WS** – Andringitra National Park Western Slopes (Fig. 1; Suppl. material 1: Table S1). **A.** *Brookesia brunoi* from Anja; **B.** *Calumma andringitraense* from Imaitso (ANP ES); **C.** *Calumma crypticum* from Imaitso (ANP ES); **D.** *Calumma fallax* from Asaramanitra (ANP ES); **E.** *Palleon nasus* from Namoly; **F.** *Furcifer lateralis* from Iantaranomby (ANP WS); **G.** *Furcifer major* from Anja; **H.** *Furcifer nicosiai* from Tsaranoro; **I.** *Furcifer oustaleti* from Anja. Photographs by Javier Lobón-Rovira.

the leaf litter during the day or roosting at a few centimetres height after the sunset, within the semi-arid deciduous forest patches of the Reserve (Crottini et al. 2012b). *Brookesia brunoi* was found only at Anja, at an elevation of ca. 970–980 m a.s.l. (Suppl. material 4: Table S4). The species seemed common in this forest fragment. All sampled individuals were found during the day moving on the leaf litter. Their sequences are molecularly uniform (Suppl. material 6: Table S6, COI) and show a maximum genetic distance of ca. 1.50% from previously available sequences (e.g. ND2: JX101752).

***Palleon nasus* (Boulenger, 1887)**
Fig. 4E

*Palleon nasus* has originally been described from "Ekongo", which probably refers to Ikongo, ca. 55 km north-east of the Andringitra Massif. This species is distributed in south-eastern Madagascar between Ranomafana and Andringitra to the north and Tolagnaro to the south-east. *Palleon nasus* has been subdivided into two subspecies, based on morphological differences: *P. n. nasus* (Boulenger, 1887) and *P. n. pauliani* (Brygoo, Blanc & Domergue, 1972), the latter being described from Manjarivolo (in the Andringitra Massif), where it was collected at an elevation of 1,620–1,650 m a.s.l. Based on morphological examination, Goodman (1996) identified *P. n. nasus* (specimens collected at an elevation of 720–1,630 m a.s.l.) during a herpetological assessment on the eastern slopes of the Massif. We collected six individuals of *P. nasus* in the eastern parts of the Region (Imaitso and Namoly), at an elevation range between ca. 1,580 and 1,640 m a.s.l. (Suppl. material 4: Table S4). The species was common. The animals were all found at night within rainforest while roosting on branches (one individual close to the floor and the other at ca. 2 m from the ground) and they were not active. The analysed samples are genetically uniform to each other (Suppl. material 6: Table S6) and they show 10.5% genetic distance to sequences obtained from individuals of Andohahela (COI: JQ909283) and are ca. 4% distant to an individual from Ranomafana (16S: HQ130509). Morphological examination of the collected individuals suggests a closer affinity with *P. n. nasus*.

**Subfamily Chamaeleoninae**

***Calumma andringitraense* (Brygoo, Blanc & Domergue, 1972)**
Fig. 4B

This species is known from Andringitra, which represents the type locality, and the Andohahela Massif (Glaw and Vences 2007), where it inhabits montane rainforest at an elevation range of 1,550–1,680 m a.s.l. (Goodman 1996; Goodman et al. 2018). We collected samples of this species at Imaitso and Fivahona–Ambavanala, in the eastern part of the Region, while roosting on branches in dense rainforest at an elevation between ca. 1,480 and 1,560 m a.s.l. (Suppl. material 4: Table S4). They were all spotted at night in a sleeping position. The species was abundant in Imaitso, while rarer in Fivahona–Ambavanala. The analysed individuals are genetically uniform to each other (Suppl. material 6: Table S6) and they have ca. 5.5% genetic distance to individuals from Andohahela (COI: JQ909303).

***Calumma crypticum* Raxworthy & Nussbaum, 2006**
Fig. 4C

*Calumma crypticum* has a scattered distribution including the Tsaratanana Massif in the north and several localities in the central highlands, Ranomafana and Andohahela. It inhabits montane forests at an elevation between ca. 1,050 and 1,850 m a.s.l. (Boumans et al. 2007; Glaw and Vences 2007; Randrianantoandro et al. 2010). Boumans et al. (2007) showed that the species is composed of several intra-specific lineages characterised by a certain degree of mitochondrial differentiation at the 16S gene. This species was the most common chameleon we encountered during our sampling. We collected the species at Asaramanitra, Belambo, Imaitso, Fivahona–Velotsoa and Namoly, in the eastern portion of the Region (Suppl. material 4: Table S4). The sampled individuals were found both during the day and at night (sleeping) while roosting on branches in dense rainforest. They are genetically quite similar to each other (Suppl. material 6: Table S6) and show limited genetic differentiation compared to other samples collected in Ranomafana (COI: ca. 3%, JQ909308; 16S: ca. 0.50%, EF210643).

***Calumma fallax* (Mocquard, 1900)**
Fig. 4D

Following its new definition, *Calumma fallax* is distributed in eastern Madagascar from Andohahela, to the south, to Mandraka to the north, where it can be found in rainforest at low and middle elevations (Glaw and Vences 2007; Gehring et al. 2011, 2012; Prötzel et al. 2020). The type locality of this taxon is Ikongo, which is located ca. 55 km north-east of the Andringitra Massif. We sampled this species at Asaramanitra, Fivahona–Ambavanala, Fivahona–Velotsoa and Namoly in the rainforest of the eastern part of the surveyed area at an elevation range between ca. 1,490 and 1,670 m a.s.l. (Suppl. material 4: Table S4). The species was common in Namoly, while in the other sites, it was rarer. The animals were spotted at night on tree branches while sleeping. The analysed individuals are molecularly uniform across the different sampling localities (Suppl. material 6: Table S6) and show a 4% genetic distance from individuals of the same species from Ranomafana (ND2: JQ734064).

***Furcifer lateralis* (Gray, 1831)**
Fig. 4F

Florio et al. (2012) revised the taxonomy of the *F. lateralis* complex, assigning the populations of southern and north-western Madagascar to *Furcifer major* (Brygoo, 1971) and *Furcifer viridis* Florio, Ingram, Rakotondravony, Louis Jr. & Raxworthy, 2012, respectively. The distribution of *F. lateralis* was restricted to eastern Madagascar, where it can be found within rainforest, at forest edges, in shrubby grasslands and more degraded vegetational formations (Raselimanana and Rakotomalala 2003; Glaw and Vences 2007). The species is reported from Andringitra (Goodman et al. 2018) and was sampled on the western slopes of the Massif (Iakanga and Iantaranomby) where it was encountered with low frequency. Only two individuals were collected during the day, actively perching in open grassland with scattered trees at an elevation between ca. 900 and 1,560 m a.s.l. (Suppl. material 4: Table S4). The analysed samples are molecularly identical to each other (Suppl. material 6: Table S6) and show limited genetic differentiation (16S) from samples collected at Cirque Namoly (EF210582; within the study area), Vondrozo (EF210589) and Tampina forest (EF210593).

***Furcifer major* (Brygoo, 1971)**
Fig. 4G

Distributed in southern Madagascar, *F. major* inhabits almost any arid habitat including human-disturbed environments (Raselimanana and Rakotomalala 2003; Florio et al. 2012). We sampled this species at Ambatomainty, Anja, Anja–Ambilo and Sakaviro (Suppl. material 4: Table S4), on the western part of the Region at only ca. 10 km from the localities where we collected *F. lateralis*. Individuals were spotted during both day and night (sleeping), perching on tree branches. Despite present in multiple sites, the species was locally rare to encounter. Relative to *F. lateralis*, *F. major* was found at a lower elevation (between ca. 930 and 1,030 m a.s.l.) and in semi-arid deciduous forest. The analysed samples are genetically identical to each other (Suppl. material 6: Table S6) and show 2.6% genetic distance (COI) from conspecific populations from Isalo (e.g. MH063344).

***Furcifer nicosiai* Jesu, Mattioli & Schimmenti, 1999**
Fig. 4H

This chameleon was first described from Tsingy de Bemaraha, in western Madagascar, where it is mostly found within dense sub-humid and dry forests. This species has been later reported from Paysage Harmonieux Protégé de Beanka, Paysage Harmonieux Protégé du Complexe Tsimembo Manambolomaty, Paysage Harmonieux Protégé du Complexe Lac–Forêt Ambondrombe, Paysage Harmonieux Protégé de Menabe Antimena and Réserve Spéciale d'Andranomena (Goodman et al. 2018). We sampled *Furcifer nicosiai* at Tsaranoro, in the western part of the region, at ca. 960–970 m a.s.l. (Suppl. material 4: Table S4). This record was included in Belluardo et al. (2021), along with other new records from central and western Madagascar, significantly expanding the known distribution of this species by ca. 300 km towards the south-east. *F. nicosiai* did not seem abundant. We sampled three individuals both during night (sleeping) and day while roosting on branches within semi-arid deciduous forest. The analysed individuals are genetically identical to each other (Suppl. material 6: Table S6) and they show 4% (COI: JQ909373) and 3% (16S: HF57045) genetic distance with individuals from the type locality (Tsingy de Bemaraha).

***Furcifer oustaleti* (Mocquard, 1894) Clade D**
Fig. 4I

This species is widely distributed in Madagascar. It can inhabit a wide variety of habitats, including degraded vegetation and human-dominated environments (Glaw and Vences 2007; Florio and Raxworthy 2016). We identified this species at Ambalavao, Anja, Anja–Ambilo and Sakaviro (in the western part of the Region) at an elevation range of ca. 980–1,010 m a.s.l. (Suppl. material 4: Table S4). The species seemed quite common. The individuals were found during the day, both within semi-arid deciduous forest and in human-associated environments, actively perching on tree braches. The analysed animals are molecularly identical to each other (Suppl. material 6: Table S6) and to individuals from Isalo (COI: MH063345; 16S: MH063288) attributed to Clade D (sensu Florio and Raxworthy 2016), which is distributed in southern, central and north-western Madagascar.

***Furcifer willsii* (Günther, 1890)**

This chameleon is distributed in central-eastern Madagascar and in the north, in the area of Tsaratanana, while records from western Madagascar require verification (Glaw and Vences 2007). *Furcifer willsii* can be found in rainforest where it is usually spotted roosting high above the ground. The only recorded individual was sampled in Fivahona–Velotsoa, in the eastern part of the study area (ca. 1,270 m a.s.l.) and it was recovered in the stomach of a specimen of *Mimophis mahfalensis* (Grandidier, 1867) (ACZC11133) (Suppl. material 4: Table S4) (Lobón-Rovira et al. 2020). The previous southernmost record of this chameleon (Ikongo) is at ca. 55 km north-east from Fivahona–Velotsoa. The sample was included in Lobón-Rovira et al. (2020), contributing to extend the species distributional range further south. The sample shows 7.2% genetic distance from a conspecific individual collected at Ranomafana (COI: JQ909382).

**Family Gekkonidae**
**Subfamily Gekkoninae**

***Hemidactylus mercatorius* Gray, 1842**
Fig. 5A

This species is widely distributed in Madagascar, commonly found in human-dominated areas (Glaw and Vences 2007). *Hemidactylus mercatorius* was found at Iantaranomby, Ambalavao, Ambatomainty, Anja–Ambilo, Iarintsena, Sakaviro and Tsaranoro (Suppl. material 4: Table S4), in the western part of the Region (between ca. 930 and 1,580 m a.s.l.), mostly in human settlements and sometimes also within semi-arid deciduous forest. The individuals were normally found in nocturnal activity on rocks, despite one individual being sampled during the day in Ambalavao. The species was common in Tsaranoro. The analysed samples show limited genetic differentiation (Suppl. material 6: Table S6). To the best of our knowledge, the population from Isalo is genetically the closest to the samples analysed here (ca. 2.70%, MH063351).

***Lygodactylus pictus* (Peters, 1883)**
Fig. 5B

This diurnal gecko is known from south-eastern Madagascar and a few other localities in the central highlands. It is commonly found in degraded and secondary forest patches and in human-dominated environments. The Andringitra Massif and the surrounding areas are at the southern limit of the distributional range of the species (Puente et al. 2005; Glaw and Vences 2007). We sampled *Lygodactylus pictus* in degraded and secondary forest at Asaramanitra, Belambo, Fivahona–Ambavanala and Tsaranoro, both in the eastern and western parts of the surveyed region and in the town of Ambalavao at an elevational range between ca. 970 and 1,610 m a.s.l. (Suppl. material 4: Table S4). This gecko was mostly found in human-associated environments (e.g. roofs, houses) and only rarely on tree trunks. The individuals were mostly found active during the day, despite two being spotted at night, probably sleeping. The species was common in Belambo, while it seemed rarer in the other sites. The analysed samples are genetically homogeneous (COI, Suppl. material 6: Table S6) and almost identical to an individual from Ambositra (JQ909452, COI). There is some genetic difference (in 16S) between the individuals collected in Tsaranoro and Fivahona–Ambavanala. The sample from Tsaranoro (ACZC10950) is ca. 1% distant (16S) from an

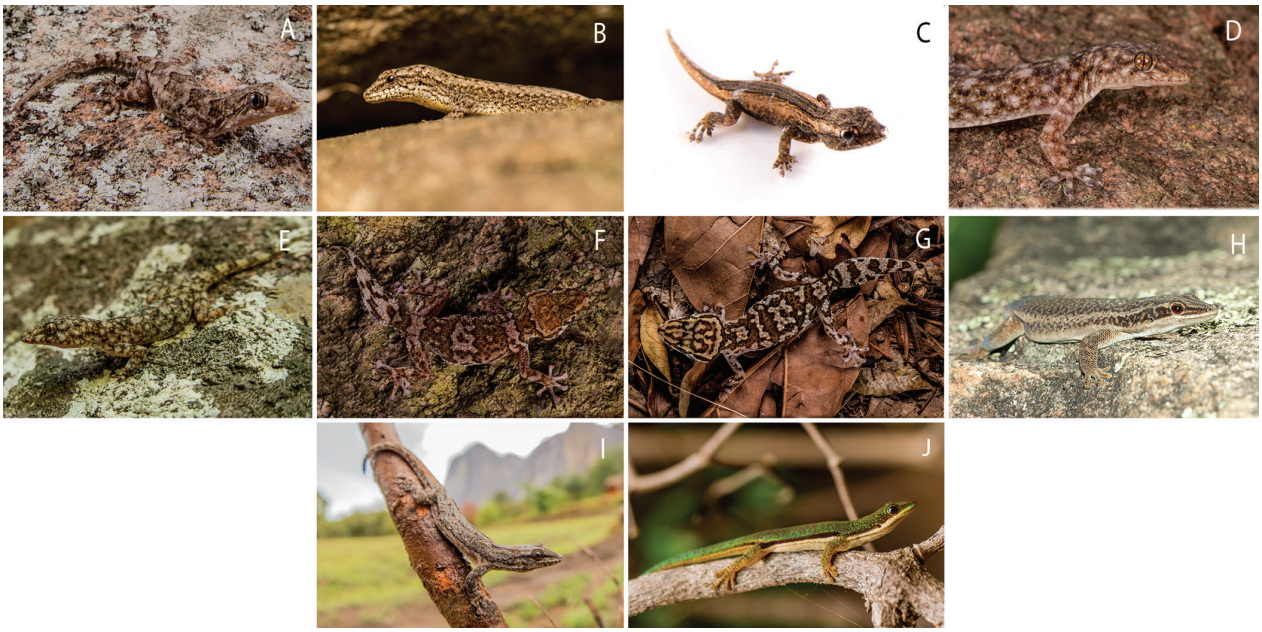


**Figure 5.** Geckos species identified in this study. Sampling localities for each photographed individual are indicated. **ANP ES** – Andringitra National Park Eastern Slopes; **ANP WS** – Andringitra National Park Western Slopes (Fig. 1; Suppl. material 1: Table S1). **A.** *Hemidactylus mercatorius* from Tsaranoro; **B.** *Lygodactylus pictus* from Belambo (ANP ES); **C.** *Lygodactylus* sp. aff. *pictus* Ca01 "Isalo" from Ambatomainty; **D.** *Paragehyra felicitae* from Anja; **E.** *Paragehyra* sp. aff. *felicitae* "Tsaranoro" from Tsaranoro; **F.** *Paroedura rennerae* from Anja; **G.** *Paroedura* sp. aff. *bastardi* Lineage D from Anja; **H.** *Phelsuma barbouri* from Belambo (ANP ES); **I.** *Phelsuma gouldi* from Tsaranoro; **J.** *Phelsuma lineata elanthana* from Fivahona–Velotsoa. Photographs by Javier Lobón-Rovira (A–G, I–J) and Gonçalo M. Rosa (H).

individual from Ambositra (AY653269), while the sample from Fivahona–Ambavanala (ACZC11175) is identical (16S) with two individuals of *L. pictus* from Ambositra (GU593455) and Sendrisoa (AY653270), a locality only ca. 6 km away from Fivahona–Ambavanala.

### *Lygodactylus* sp. aff. *pictus* Ca01 "Isalo" UCS
Fig. 5C

We sampled a juvenile within the semi-arid deciduous forest of Ambatomainty (ca. 970 m a.s.l.) (Suppl. material 4: Table S4). The collected individual was found at night on a rock along a trail. This sample shows limited genetic distance from an individual collected at Analalava forest in Isalo (16S: 2.7%, AY653238; COI: 4.50%, JQ909445). The new record significantly extends the known distributional range of this taxon by ca. 180 km towards the east from the Isalo Massif.

### *Paragehyra felicitae* Crottini, Harris, Miralles, Glaw, Jenkins, Randrianantoandro, Bauer & Vences, 2015
Fig. 5D

This gecko was known only from Anja Community Reserve and from a site a few km away from Anja. This species can be observed on granitic boulders associated with both semi-arid deciduous forest and grasslands. Even though the other geckos of the genus *Paragehyra* are nocturnal, these animals can also be spotted during the day (Crottini et al. 2015). We sampled this species in Anja and Sakaviro, the latter record representing a distributional range extension (ca. 8 km). The collected samples were active at night on granitic boulders and rocks within semi-arid deciduous forest, sometimes in clearings within the forest, at ca. 950–990 m a.s.l. (Suppl. material 4: Table S4). The species was present in high densities in both sites. Collected samples show some genetic differentiation at the COI fragment (Suppl. material 6: Table S6) and they are almost identical (16S) to individuals from Anja (e.g. KP025816).

### *Paragehyra* sp. aff. *felicitae* "Tsaranoro" CCS
Fig. 5E

This taxon was collected at Iantaranomby, Ambatomainty and Tsaranoro, all localities in the western part of the surveyed region (between ca. 910 and 1,610 m a.s.l.) (Suppl. material 4: Table S4). Animals were found active at night on granitic boulders both in open habitats and semi-arid deciduous forest. The species was particularly abundant in Tsaranoro and, to a less extent, in Ambatomainty, whereas only one individual was sampled in Iantaranomby. The analysed samples show limited genetic differentiation amongst sampled localities (Suppl. material 6: Table S6). The genetic distance between this taxon and *P. felicitae* sampled in Anja is ca. 15% (COI:

ACZC10432) and 7.6% (16S: KP025811), while it has 17% genetic difference with *Paragehyra petiti* Angel, 1929 (COI: JQ909497). This taxon is morphologically similar to *P. felicitae*. However, the analysis of the collected specimens highlighted the presence of a distinct number of longitudinal rows of enlarged turbercles on the dorsolateral surfaces of the body relative to the sister species *P. felicitae* (which is a morphological diagnostic character of this species), confirming its distinction also at the morphological level and determining the status of confirmed candidate species. The description of this candidate species is currently in progress.

***Paroedura rennerae*** **Miralles, Bruy, Crottini, Rakotoarison, Ratsoavina, Scherz, Schmidt, Köhler, Glaw & Vences, 2021**
Fig. 5F

The *Paroedura bastardi* (Mocquard, 1900) species complex has been recently revised (Miralles et al. 2021). This work identified at least three evolutionary lineages within this species group. Following this finding, the authors propose a new definition for *P. bastardi* sensu stricto. They resurrected the binomen *Paroedura guibeae* Dixon & Kroll, 1974 and formally described a third lineage as *P. rennerae*. The latter species is currently known from Miandrivazo, Kirindy, Marofandilia, Anja and Isalo. We sampled this gecko at Ambatomainty, Anja, Sakaviro and Tsaranoro, all sites in the western part of the surveyed region (at ca. 930–990 m a.s.l.) (Suppl. material 4: Table S4), extending the known distribution of this species by ca. 25 km towards the south from Anja. This species was abundant in all visited sites. The animals were always found active at night on boulders, mostly within semi-arid deciduous forest, but sometimes also at the edge of the forest and in human settlements. The collected samples are molecularly uniform (Suppl. material 6: Table S6) and almost identical to an individual collected from Anja (COI: MG734947). They are slightly differentiated from conspecific populations from other localities (COI: 2.60%, MG734948, Kirindy; 16S: 1.4%, GU128989, Miandrivazo; 1.8%, GU129005, Marofandilia).

***Paroedura*** **sp. aff.** ***bastardi*** **Lineage D UCS**
Fig. 5G

Individuals belonging to this lineage of the *P. bastardi* species complex were previously known only from Anja (Miralles et al. 2021). We collected this taxon at Anja and Tsaranoro, in the western part of the Region (at ca. 930–970 m a.s.l.), extending the distributional range by ca. 25 km towards the south (Suppl. material 4: Table S4). In both sites, this lineage was found in syntopy with *Paroedura rennerae*, which is very similar in morphology, and in high densities The individuals were found active at night on boulders within semi-arid deciduous forest and show a limited degree of genetic differentiation (Suppl. material 6: Table S6). The collected samples are almost 100% identical to ZCMV 12790 (COI: MW311368), which is the only individual molecularly characterised for this taxon before our study (Miralles et al. 2021).

***Phelsuma barbouri*** **Loveridge, 1942**
Fig. 5H

This diurnal gecko is known from a few montane areas on the central highlands and in south-eastern Madagascar. The area of Andringitra represents the southern limit of its distributional range. Unlike most *Phelsuma* geckos, which are arboreal, this species is normally found in montane rocky habitats on the ground and boulders (Glaw and Vences 2007; Goodman et al. 2018). We sampled a single individual of *Phelsuma barbouri* active during the day on a small wooden bridge at Belambo in the eastern slopes of the Massif, at ca. 1,570 m a.s.l. (Suppl. material 4: Table S4). This sample is 96% similar to a conspecific individual from Tsiafajavona (Ankaratra) (COI: JQ909518).

***Phelsuma gouldi*** **Crottini, Gehring, Glaw, Harris, Lima & Vences, 2011**
Fig. 5I

This species was reliably known only from Anja, where a single individual (the holotype) was spotted on a trunk within the forest patch of the Reserve (Crottini et al. 2011b). The species is confirmed to be quite rare. Two individuals were sampled at night on lianas in the semi-arid deciduous forest of Tsaranoro, in the western part of the Region, at an elevation range of ca. 910–950 m a.s.l. (Suppl. material 4: Table S4). This finding extends the known distributional range of the species by ca. 25 km to the south. The two analysed samples are identical to each other (Suppl. material 6: Table S6) and identical to the holotype (16S: JF810252).

***Phelsuma lineata elanthana*** **Krüger, 1996**
Fig. 5J

*Phelsuma lineata elanthana* is distributed in the central highlands and the northern part of central-eastern Madagascar (Boumans et al. 2007; Gehring et al. 2013). It inhabits a great variety of habitats, from the rainforest to bushes in more arid areas and it is also commonly found in human settlements (Glaw and Vences 2007). This gecko was rare. We sampled *P. lineata* only in Fivahona–Velotsoa, in the eastern part of the Region (at ca. 1,290 m a.s.l.), where the animals were found active during the day on *Pandanus* trees within the rainforest (Suppl. material 4: Table S4). This record represents a range extension for this taxon by ca. 350 km towards the south. The analysed samples show a 3% genetic distance (16S) from samples from Andasibe (EF210615) and Ambohitantely (EF210617).

**Family Gerrhosauridae**
**Subfamily Zonosaurinae**

***Zonosaurus aeneus* (Grandidier, 1872)**
Fig. 6E

This species is distributed at a wide latitudinal range between the central highlands and the eastern and south-eastern escarpment. It is typically found on the edges of rainforest or in open areas within forest (Glaw and Vences 2007). *Zonosaurus aeneus* was previously reported from Andringitra (Goodman et al. 2018) and was found at Fivahona–Ambavanala and Namoly, in the eastern part of the studied region (between ca. 1,480 and 1,650 m a.s.l.). *Zonosaurus aeneus* was sampled during the day. The animals were active on the ground in grassy clearings inside rainforest and in open areas immediately next to rainforest patches. One individual was found next to a ricefield (Suppl. material 4: Table S4). The analysed individuals are identical (Suppl. material 6: Table S6) and they show a 3.3% genetic distance from a sequenced individual from Ranomafana (COI: JQ909624) and a 1.5% genetic distance from individuals from Torotorofotsy (16S: KC515131).

***Zonosaurus laticaudatus* (Grandidier, 1869)**
Fig. 6C

This large plated lizard lives over a wide latitudinal range throughout western Madagascar (Glaw and Vences 2007). This species is reported from dry forest, rocky open areas, degraded and human-disturbed habitats (Glaw and Vences 2007; Recknagel et al. 2013). This taxon was not previously reported from the area of Andringitra and was collected at Ambatomainty, Anja and Tsaranoro, all localities in the western part of the Region (between ca. 870 and 960 m a.s.l.) (Suppl. material 4: Table S4). This record extends the known distributional range of the species by ca. 180 km to the East. The individuals were found both in open areas on rocky substrate and on large boulders next to semi-arid deciduous forest patches. They were active during the day. Analysed samples show 2.2% genetic distance from the population of Isalo (e.g. COI: MH063372) and 1% genetic distance from the population of Hazofotsy, close to Tolagnaro (16S: AY167372).

***Zonosaurus ornatus* (Gray, 1831)**
Fig. 6D

This species is found in central-eastern Madagascar where it inhabits open habitats and forest edges at a wide elevational range. In Ankaratra and Andringitra, this species is found in montane savannah and heathlands above the tree line (Glaw and Vences 2007; Goodman et al. 2018). We sampled *Zonosaurus ornatus* at Antanifotsy 2, Fivahona–Ambavanala and Namoly, in the eastern part of the Region (between ca. 1,450 and 1,650 m a.s.l.) in open environments next to rainforest, often in human-disturbed areas (Suppl. material 4: Table S4). One of the individuals was spotted next to a ricefield. The animals were all active during the day. The analysed samples are identical (Suppl. material 6: Table S6) and show a 2.3% genetic distance from individuals from Ambatolahy (e.g. COI: JQ909633). However, the population from Ambatolahy is almost identical at the 16S marker (e.g. KC515145).

**Family Opluridae**

***Oplurus grandidieri* Mocquard, 1900**
Fig. 6A

This species is distributed in the south-central part of the central highlands where it is often found in rocky environments (Glaw and Vences 2007). We found this species at Andramena, Iantaranomby, Siranandambo, Ambatomainty, Anja, Sakaviro and Tsaranoro in the western portion of the studied area (Suppl. material 4: Table S4). The animals were normally found in high densities on boulders and outcrops at an elevation range between ca. 930 and 1,740 m a.s.l. They were all active during the day. The analysed samples show a limited degree of genetic differentiation (Suppl. material 6: Table S6) and low genetic distance to the population from Isalo (COI: 3.5%, MH063380; 16S: 3.2%, MH063315). In Iantaranomby, two adult males were observed displaying an aggressive interaction on a sunny boulder (Lobón-Rovira et al. 2019).

***Oplurus quadrimaculatus* Duméril & Bibron, 1851**
Fig. 6B

*Oplurus quadrimaculatus* is distributed in the south and the south-eastern part of the central highlands, including the area of Andringitra (Glaw and Vences 2007; Münchenberg et al. 2008). It was also recently identified at Isalo (Cocca et al. 2018). *Oplurus quadrimaculatus* is a saxicolous species, normally observed on large boulders within arid environments and, in some cases, also next to forest patches. We found this species at Andramena, Asaramanitra, Imaitso, Ambatomainty, Anja, Fivahona–Velotsoa, Sakaviro and Tsaranoro in both the eastern and western parts of the surveyed area (Suppl. material 4: Table S4). The animals were present in high densities and active during the day on large boulders and outcrops both in open and forested areas at an elevation between ca. 870 and 1,740 m a.s.l. Within forest, they were normally spotted in rocky clearings or next to large streams delimited by outcrops. *Oplurus quadrimaculatus* was sometimes found in syntopy with *O. grandidieri* and, in some cases, the two species shared the same rocks. Analysed samples show limited genetic differentiation among them (Suppl. material 6: Table S6) and in relation to other individuals collected in the Andringitra Region (e.g. 16S: EU099752). They show a 4.4% genetic distance from the population from Andohahela (COI: JQ909486) and no differentiation from the population from Antoetra (e.g. 16S: EU099737) and Ambositra (16S: EU099742).

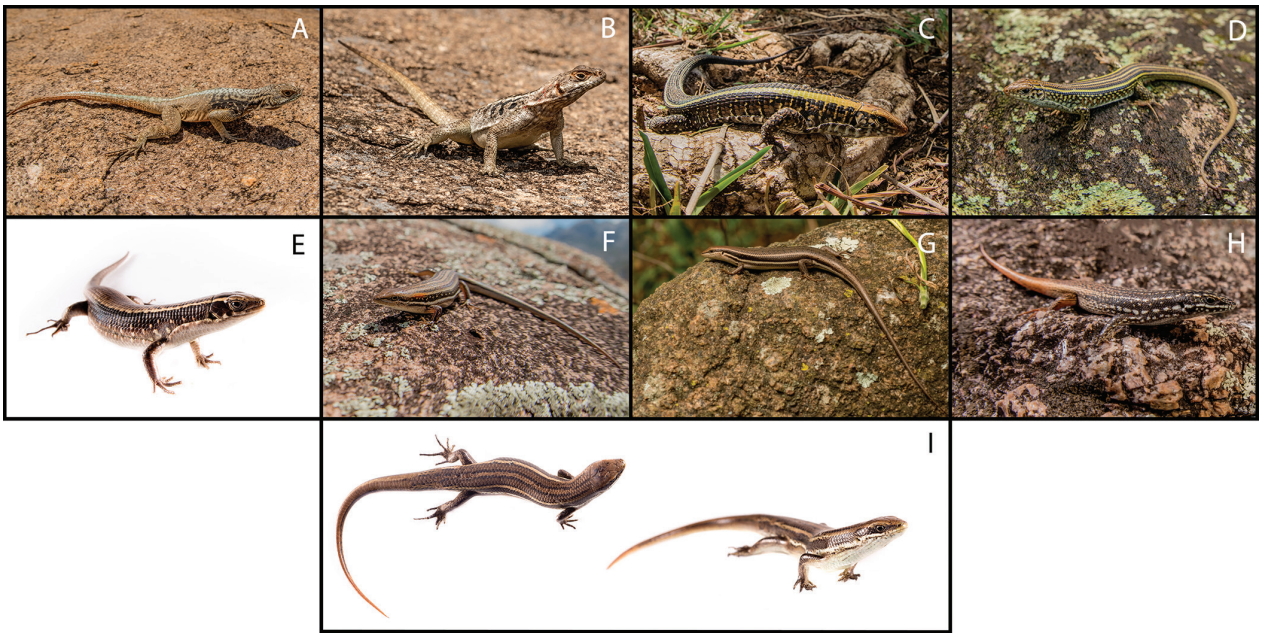


**Figure 6.** Scincid, gerrhosaurid and oplurid species identified in this study. Sampling localities for each photographed individual are provided. **ANP ES** – Andringitra National Park Eastern Slopes; **ANP WS** – Andringitra National Park Western Slopes (Fig. 1; Suppl. material 1: Table S1). **A.** *Oplurus grandidieri* from Anja; **B.** *Oplurus quadrimaculatus* from Anja; **C.** *Zonosaurus laticaudatus* from Anja; **D.** *Zonosaurus ornatus* from Antanifotsy 2; **E.** *Zonosaurus aeneus* from Namoly; **F.** *Trachylepis elegans* from Iantaranomby (ANP WS); **G.** *Trachylepis gravenhorstii* from Anja; **H.** *Trachylepis* sp. aff. *vato* from Asaramanitra (ANP ES); **I.** *Trachylepis boettgeri* from Antanifotsy 3. Photographs by Javier Lobón-Rovira.

**Family Scincidae**
**Subfamily Mabuyinae**

***Trachylepis boettgeri* (Boulenger, 1887)**
Fig. 6I

*Trachylepis boettgeri* is found in central-eastern Madagascar where it inhabits the open habitats of the central highlands, including the Region of Andringitra (Goodman et al. 2018). We sampled one individual at Antanifotsy 3, in an area dominated by grasslands and rice fields in the eastern part of the Region at ca. 1,440 m a.s.l. (Suppl. material 4: Table S4). The animal was active on the ground during the day. The analysed sample is genetically almost identical to a population from Ankaratra (COI: JQ909591; 16S: DQ238879) and Ambatolampy (16S: AY070355).

***Trachylepis elegans* (Peters, 1854) Lineage A**
Fig. 6F

This skink is distributed throughout Madagascar, except for the eastern escarpment (Glaw and Vences 2007). It mostly inhabits open habitats in both dry and humid environments and can be found in cities and other human-dominated environments (Glaw and Vences 2007; Vences et al. 2014). We found this taxon in high densities at Iantaranomby, Ambalavao, Ambatomainty, Anja–Ambilo and Fivahona–Velotsoa (in the western part of the surveyed region) and in Antanifotsy 4 (in the eastern) between ca. 830 and 1,670 m a.s.l. (Suppl. material 4: Table S4). All individuals were found in grassy open areas, sometimes next to small boulders, either close to forest patches or in human-dominated areas. They were found active during the day. The analysed samples show a limited degree of genetic differentiation (Suppl. material 6: Table S6) and they show a 2% genetic distance from the population of Isalo (e.g. COI: KF250670) attributed to lineage A (sensu Vences et al. 2014), which is the most widespread lineage in Madagascar.

***Trachylepis gravenhorstii* (Duméril & Bibron, 1839) Lineage 4A**
Fig. 6G

*Trachylepis gravenhorstii* is found almost everywhere in Madagascar up to 1,400 m a.s.l. (Glaw and Vences 2007; Vences et al. 2014; Goodman et al. 2018). We sampled this taxon at Belambo, Iantaranomby, Riandahy and Anja, in both the eastern and western part of the Region at an elevation between ca. 960 and 1,640 m a.s.l. (Suppl. material 4: Table S4). *Trachylepis gravenhorstii* was often found in syntopy with *T. elegans*, although less common than the latter species. Where *T. elegans* was not present, *T. gravenhorstii* was found in less human-disturbed habitats. The individuals were sampled during the day, both in grassy open areas with presence of small boulders and within forest active on the ground. The analysed samples show some degree of intra-populational variability

(Suppl. material 6: Table S6) and are identical to a sample collected at Ranomafana (COI: KF250703) attributed to lineage 4A (sensu Vences et al. 2014) distributed in central-eastern Madagascar. Previously available data of this species from the area (Ambalavao) belong to the lineage 4B (COI: KF250708). As previously reported for Isalo (Cocca et al. 2018), it seems that these two lineages occur sympatrically in the surveyed area.

***Trachylepis* sp. aff. *vato* UCS**
Fig. 6H

*Trachylepis vato* (Nussbaum & Raxworthy, 1994) is distributed in central and southern Madagascar where it inhabits arid environments with boulders and rocks (Glaw and Vences 2007). It was described from a locality close to Andohahela (Type locality: Mananara River between Bevia and Hazofotsy, 24°51.00'S, 46°31.00'E), in south-eastern Madagascar (Nussbaum and Raxworthy 1994). We collected this lineage at Asaramanitra, Iantaranomby, Anja, Fivahona–Velotsoa, Iarintsena and Sakaviro, both in the eastern and western parts of the surveyed region (between ca. 990 and 1,660 m a.s.l.), mostly on boulders, both in open environments and within forest (Suppl. material 4: Table S4). The animals were all active during the day. This skink was not common in the sampling sites we visited. These records represent a slight range extension within the area of Andringitra. Lima et al. (2013) assigned individuals from Ambalavao (16S: KC345435; ND1: KC345095), Andringitra (16S: KC345394; ND1: KC345053) and Col des Tapias (16S: KC345432; ND1: KC345092) to a candidate species *T.* cf. *vato*. Our samples show some sign of genetic differentiation (Suppl. material 6: Table S6) and the genetic distance between our samples and the samples of *T.* cf. *vato* included in Lima et al. (2013) is between 1% and 2.3% (ND1: KC345092, KC345053, KC345095). Our samples are also almost identical to individuals from Ibity (16S: AY159097), close to Col des Tapias.

**Family Psammophiidae**

***Mimophis mahfalensis* (Grandidier, 1867)**
Fig. 7G

This snake is widely distributed across almost all of the southern half of Madagascar, where it can be found in rainforest, dry forest, arid spiny thornbush savannah and human-dominated areas (Glaw and Vences 2007; Ruane et al. 2017). We sampled this species at Iakanga, Anja, Fivahona–Velotsoa and Sakaviro, both in the western and eastern parts of the surveyed area, at an elevation between ca. 900 and 1,270 m a.s.l. (Suppl. material 4: Table S4). The animals were found during the day active on the ground in a wide variety of habitats: rainforest, semi-arid deciduous forest and grassland. The analysed samples are molecularly similar to each other (Suppl. material 6: Table S6) and 0.8% distant (COI) to the population from Ibity (JQ909481) and Isalo (MH063403). The individual collected at Fivahona–Velotsoa was found with a *Furcifer willsii* chameleon (ACZC11200) in its stomach (Lobón-Rovira et al. 2020).

**Family Pseudoxyrhophiidae**

***Compsophis infralineatus* (Günther, 1882)**
Fig. 7A

This semi-arboreal snake is distributed in eastern and south-eastern Madagascar where it is normally found along ponds and small streams (Glaw and Vences 2007). *Compsophis infralineatus* was already reported from Andringitra (Goodman et al. 2018). Two individuals were found at night in Fivahona–Velotsoa and Namoly, in the eastern part of the Region, within the rainforest at an elevational range of ca. 1,270–1,640 m a.s.l. (Suppl. material 4: Table S4). In Fivahona–Velotsoa, this snake was found active on the ground along the banks of a small stream. The analysed individuals are genetically identical amongst each other (Suppl. material 6: Table S6) and they are ca. 1% distant (COI) from the population from Manjakatompo (e.g. JQ909355).

***Leioheterodon modestus* (Günther, 1863)**
Fig. 7B

This snake has a patchy distribution in central, western and southern Madagascar, where it is generally found in dry areas, both inside and outside the forest and in anthropogenic environments (Glaw and Vences 2007). This species has not yet been reported from the study area and we found it at Antanifotsy 1, Fivahona–Ambavanala and Fivahona–Velotsoa in the eastern part of the Region, contributing to the extension of its known distributional range by ca. 200 km towards the east. Two individuals were spotted during the day active on the ground close to rice fields and villages near forest patches at an elevational range between ca. 1,280 and 1,460 m a.s.l. (Suppl. material 4: Table S4). The analysed samples are genetically identical to each other (Suppl. material 6: Table S6) and 0.60 % distant to an individual collected at Zazafotsy (COI: MH063415).

***Liophidium torquatum* (Boulenger, 1888)**
Fig. 7F

This terrestrial snake is distributed in eastern and northern Madagascar where it mostly inhabits rainforest, despite being also found in dry deciduous forest (Glaw and Vences 2007). This species is reported from Andringitra (Goodman et al. 2018) and we sampled a single individual inside the semi-arid deciduous forest fragment of Anja, in the western part of the surveyed region (at ca. 990 m a.s.l.) (Suppl. material 4: Table S4). The animal was active during the day. This sample is almost 100% identical to the population of *L. torquatum* from Ranomafana (Cytb: DQ979984).


**Figure 7.** Psammophiid, pseudoxyrhophiid and sanziniid snakes identified in this study. Sampling localities for each photographed individual are provided. **ANP ES** – Andringitra National Park Eastern Slopes; **ANP WS** – Andringitra National Park Western Slopes (Fig. 1; Suppl. material 1: Table S1). **A.** *Compsophis infralineatus* from Namoly; **B.** *Leioheterodon modestus* from Antanifotsy 1; **C.** *Madagascarophis meridionalis* from Anja; **D.** *Thamnosophis lateralis* from Anja; **E.** *Pseudoxyrhopus* sp. Ca2 from Ambatomainty; **F.** *Liophidium torquatum* from Anja **G.** *Mimophis mahfalensis* from Sakaviro; **H.** *Sanzinia* cf. *volontany* from Anja; **I.** *Acrantophis dumerili* from Sakaviro. Photographs by Javier Lobón-Rovira (A–E, G, I), Gonçalo M. Rosa (F) and Franco Andreone (H).

***Madagascarophis meridionalis* Domergue, 1987**
Fig. 7C

This species inhabits arid environments in southern and south-western Madagascar (Glaw and Vences 2007). *Madagascarophis meridionalis* is known to inhabit the Andringitra Region (Nagy et al. 2007) and was sampled at Ambatomainty, Anja, Iantaranomby and Tsaranoro, in the western part of the Region at an elevation range between ca. 930 and 1,580 m a.s.l. (Suppl. material 4: Table S4). The animals were mostly found active on the ground in open areas next to semi-arid deciduous forest, sometimes along streams and in human-disturbed environments. They were mostly spotted at night, although one individual was found during the day. The analysed samples are genetically uniform (Suppl. material 6: Table S6). They are identical to a published sequence from the area (16S: AY586213) and almost identical to the population from Antoetra (16S: AY586212). They are 1% distant (COI) from the population from Andranovorivato (KU925345).

***Pseudoxyrhopus* sp. Ca2 UCS**
Fig. 7E

This taxon was sampled in Ambatomainty, in the western part of the Region (at ca. 960 m a.s.l.) (Suppl. material 4: Table S4). The only collected individual was moving on the ground at night in an open area with a few scattered trees next to a small fragment of semi-arid deciduous forest. This specimen is genetically very similar (2.7% distance at COI) to a candidate species collected at Zombitse–Vohibasia National Park (RAN 43545–UMMZ 203648, Burbrink et al. 2019). Our finding extends the known distribution of this taxon by ca. 230 km to the north-east.

***Thamnosophis lateralis* (Duméril, Bibron & Duméril, 1854)**
Fig. 7D

This is one of Madagascar's most common snakes (Glaw and Vences 2007), being mostly found outside dense forest and often in degraded areas (Vences 2011). We

sampled this species at Antanifotsy 3, Anja, Tsaranoro, Fivahona–Ambavanala and Fivahona–Velotsoa in both the western and eastern parts of the surveyed region at an elevation between ca. 930 and 1,480 m a.s.l. (Suppl. material 4: Table S4). We found this species active on the ground during the day, both within forest and in anthropogenic environments (next to ricefields). Analysed samples are genetically uniform (Suppl. material 6: Table S6) and show 1% distance from the population from Isalo (COI: MH063410).

**Family Sanziniidae**

***Acrantophis dumerili* Jan, 1860**
Fig. 7I

*Acrantophis dumerili* is distributed in central and southern Madagascar where it inhabits dry forest, savannah, as well as open and cultivated areas (Glaw and Vences 2007). *Acrantophis dumerili* is reported from Ambalavao (Glaw and Vences 2007) and was sampled at Sakaviro and Vidia, in the western part of the surveyed region (at ca. 850–990 m a.s.l.) (Suppl. material 4: Table S4). While the specimen sampled in Sakaviro was found after dusk active on the ground at the edge of semi-arid deciduous forest, the other was found dead on the Route Nationale 7. The samples are identical to each other (Suppl. material 6: Table S6) and to an individual from an imprecise sampling locality (COI: JQ909244, "300 km from Tana") and the population from Ambositra (16S: AY336072). They are also almost identical to the population from Isalo (16S: EU419793).

***Sanzinia* cf. *volontany* Vences & Glaw, 2004**
Fig. 7H

Following a recent taxonomic revision, the genus *Sanzinia* comprises two species, *Sanzinia volontany* and *S. madagascariensis* (Duméril & Bibron, 1844) (Reynolds et al. 2014). The two species are genetically divergent and show some degree of morphological differentiation in colouration and pholidosis (Vences and Glaw 2003; Orozco-terWengel et al. 2008; Reynolds et al. 2014). They are divergently distributed, with *S. madagascariensis* occurring in eastern Madagascar and *S. volontany* throughout the west. An individual of *Sanzinia* sp. was photographed at night in Anja while moving on the florest floor, in the western part of the Region, but no tissues were collected from that specimen (Suppl. material 4: Table S4). The analysis of the photographic material would lead to assign the individual to *S. volontany*, although the record requires further confirmation. *Sanzinia madagascariensis* is known from Ivohibe (Glaw and Vences 2007), whereas *S. volontany* from Isalo (Orozco-terWengel et al. 2008). Given the geographic proximity of these records and the presence of both rain and dry forests in the Region, there is the possibility that both species inhabit this area.

## Discussion

We provided the first list and barcoding reference database for 28 amphibians and 38 reptiles of the area surrounding the Andringitra Massif and extended the known distributional range of nine amphibians and twelve reptiles. Species composition is probably influenced by the environmental diversity of the Region (Goodman 1996; Goodman et al. 2018). Besides the several microendemics (*Boophis laurenti*, *Mantidactylus bourgati*, *Brookesia brunoi*, *Paragehyra felicitae*, *Paragehyra* sp. aff. *felicitae* "Tsaranoro", *Paroedura* sp. aff. *bastardi* Lineage D and *Phelsuma gouldi*), many taxa are distributed only in the eastern part dominated by rainforest (e.g. *Boophis ankaratra*, *B. boppa*, *B. majori*, *B. popi*, *B. rhodoscelis*, *Aglyptodactylus madagascariensis*, *Gephyromantis blanci*, *Mantidactylus delormei*, *Spinomantis elegans, Calumma crypticum*, *Phelsuma lineata*, *Zonosaurus ornatus* and *Compsophis infralineatus*), while others are only present in the western dry habitats (e.g. *Laliostoma labrosum*, *Mantella betsileo*, *Scaphiophryne calcarata*, *Heterixalus luteostriatus, Furcifer major* and *Madagascarophis meridionalis*) (Glaw and Vences 2007).

Surveyed sites are highly fragmented and embedded within a matrix of anthropogenically-modified landscape. Despite the likely loss in species richness and the alteration of species composition, forest fragments retain high levels of diversity in Madagascar (Crottini et al. 2011a; Durkin et al. 2011; Jenkins et al. 2014; Riemann et al. 2015). Such diversity can be irreplaceable when it includes microendemics. These species are reliably known only from a few geographically close localities, therefore fragments alteration and destruction may lead to their extinction. As already reported by Jenkins et al. (2014), Anja Community Reserve stands out at the national level for its multiple microendemic taxa and, in our sampling, Anja resulted as the most species-rich (24 species) amongst the surveyed fragments (Fig. 1A; Suppl. material 9: Table S7). Meaningful comparisons of the number of recorded species amongst sampling sites are hampered by the non-standardised sampling effort and the limited time spent in each locality, which was probably not enough to sample the actual total diversity. However, we can still notice an expected positive relationship between fragment area and species richness. We sampled 21 and 19 species in Tsaranoro and Fivahona–Velotsoa (Fig. 1G), respectively, the two largest fragments along with Anja. In the smallest patches, we found a lower number of species. In both Sakaviro and Ambatomainty (Fig. 1B and C), we found 14 species and, in Fivahona–Ambavanala, we recorded 12 species. Beyond species numbers, it is interesting to note that even the smallest fragments could host taxa that were

not detected in any other locality, some of which represent relevant range extensions. Ambatomainty is probably the most interesting example in this sense. In this highly degraded forest of only two hectares (Fig. 1C), we recorded two candidate new species: *Lygodactylus* sp. aff. *pictus* Ca01 "Isalo" and *Pseudoxyrhopus* sp. Ca2, to date reliably known only from a few other sites some hundreds of kilometres away (i.e. Isalo and Zombitse–Vohibasia National Parks, respectively).

Deforestation and habitat fragmentation are more pronounced in the western part of the surveyed region where, even within the National Park borders, the forest cover is reduced (Goodman 1996). The taxa that inhabit these fragments may not find large forests at a similar elevation within the Park and may consequently lack available legally protected habitat. These small forest fragments can thus play a fundamental role as refugia to the local herpetofauna. Their conservation should, therefore, be prioritised for the long-term survival of their unique herpetological diversity and, more in general, for the conservation of the biodiversity of the entire Region. Finally, the improved knowledge on the species distribution of the candidate taxa, identified in this study, will now likely enable their formal description (e.g. in the case of *Paragehyra* sp. aff. *felicitae* "Tsaranoro" and *Paroedura* sp. aff. *bastardi* Lineage D).

## Conclusions

In a country plagued by centuries of forest loss and fragmentation (Hornac 1943; Jarosz 1993; Vieilledent et al. 2018), species inventories of remnant forest fragments are of paramount importance to achieve a better understanding of Malagasy biodiversity. We highlighted the herpetological significance of the small forest patches surrounding the Andringitra Massif, where we identified several taxa that were previously unknown from this area and, in several instances, we contributed to the extension of their known distributional ranges by hundreds of kilometres (e.g. Belluardo et al. 2021). Many of these taxa are candidate new species and the newly-collected specimens will enable future taxonomic evaluations and descriptions. We also identified one candidate species previously unknown to science and provided a better characterisation of the distribution of several microendemic species that inhabit the study area. We generated a first barcoding reference database for this area that will facilitate future systematic research, both at the regional and country level. These results emphasise the relevance of the Region of Andringitra in terms of microendemic diversity hosted in highly altered habitats. Apart from three private reserves managed by local communities, and despite their herpetological value, the other investigated fragments are not officially protected. Granting some legal protection to these sites is highly desirable to warrant the conservation of this unique biodiversity.

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## Supplementary material 1

### Table S1. Sampling localities visited in this study, with associated coordinates and elevation

Authors: Francesco Belluardo, Darwin Díaz Quirós, Javier Lobón-Rovira, Gonçalo M. Rosa, Malalatiana Rasoazanany, Franco Andreone, Angelica Crottini

Data type: sampling sites coordinates



Link: https://doi.org/10.3897/zse.97.63936.suppl1

## Supplementary material 2

### Table S2. Amplified genes, primers and PCR conditions used in this study

Authors: Francesco Belluardo, Darwin Díaz Quirós, Javier Lobón-Rovira, Gonçalo M. Rosa, Malalatiana Rasoazanany, Franco Andreone, Angelica Crottini

Data type: Primers and PCR conditions



Link: https://doi.org/10.3897/zse.97.63936.suppl2

## Supplementary material 3

### Table S3. Amphibian samples identified in this study

Authors: Francesco Belluardo, Darwin Díaz Quirós, Javier Lobón-Rovira, Gonçalo M. Rosa, Malalatiana Rasoazanany, Franco Andreone, Angelica Crottini

Data type: occurrences and species identification



Link: https://doi.org/10.3897/zse.97.63936.suppl3

## Supplementary material 4

### Table S4. Reptile samples identified in this study

Authors: Francesco Belluardo, Darwin Díaz Quirós, Javier Lobón-Rovira, Gonçalo M. Rosa, Malalatiana Rasoazanany, Franco Andreone, Angelica Crottini

Data type: occurrences and species identification



Link: https://doi.org/10.3897/zse.97.63936.suppl4

## Supplementary material 5

### Table S5. Within taxa uncorrected *p*–distances (16S) of amphibian taxa identified in this study

Authors: Francesco Belluardo, Darwin Díaz Quirós, Javier Lobón-Rovira, Gonçalo M. Rosa, Malalatiana Rasoazanany, Franco Andreone, Angelica Crottini

Data type: genetic distances



Link: https://doi.org/10.3897/zse.97.63936.suppl5

## Supplementary material 6

### Table S6. Within taxa uncorrected *p*–distances (COI) of reptile taxa identified in this study

Authors: Francesco Belluardo, Darwin Díaz Quirós, Javier Lobón-Rovira, Gonçalo M. Rosa, Malalatiana Rasoazanany, Franco Andreone, Angelica Crottini

Data type: genetic distances



Link: https://doi.org/10.3897/zse.97.63936.suppl6

## Supplementary material 7

### Figure S1. Amphibians Neighbor joining tree of the 16S rRNA gene 3' terminus

Authors: Francesco Belluardo, Darwin Díaz Quirós, Javier Lobón-Rovira, Gonçalo M. Rosa, Malalatiana Rasoazanany, Franco Andreone, Angelica Crottini

Data type: phylogenetic tree

Explanation note: The tree was computed with MEGA X 10.0.5 (Kumar et al. 2018) setting 1000 bootstrap replicates. The evolutionary distances were computed with the Kimura 2–parameter method, and ambiguous positions were removed with the pairwise deletion option.



Link: https://doi.org/10.3897/zse.97.63936.suppl7

## Supplementary material 8

### Reptiles Neighbor joining tree of the cytochrome oxidase I gene

Authors: Francesco Belluardo, Darwin Díaz Quirós, Javier Lobón-Rovira, Gonçalo M. Rosa, Malalatiana Rasoazanany, Franco Andreone, Angelica Crottini

Data type: phylogenetic tree

Explanation note: The tree was computed with MEGA X 10.0.5 (Kumar et al. 2018) setting 1000 bootstrap replicates. The evolutionary distances were computed with the Kimura 2–parameter method, and ambiguous positions were removed with the pairwise deletion option.



Link: https://doi.org/10.3897/zse.97.63936.suppl8

## Supplementary material 9

### Table S7. Locality records of amphibian and reptile species identified in this study

Authors: Francesco Belluardo, Darwin Díaz Quirós, Javier Lobón-Rovira, Gonçalo M. Rosa, Malalatiana Rasoazanany, Franco Andreone, Angelica Crottini

Data type: occurrences

Explanation note: Species records from this study are marked with '+'. Records from previous publications are labelled with '*', and the source is reported in the column 'Reference'. Localities coordinates are available in Suppl. material 1: Table S1.



Link: https://doi.org/10.3897/zse.97.63936.suppl9

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PENSOFT

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# *Idiomysis bumbumiensis* sp. nov., a new mysid species (Mysida, Mysidae, Anisomysini) from Southeast Asia

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*http://zoobank.org/C21D141B-0CDF-427F-8EE3-1FCABAD668C6*

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## Abstract

We provide a detailed description, including illustrations, of a new species of mysid belonging to the genus *Idiomysis* W. M. Tattersall, 1922 from Pulau Bum Bum, Sabah, Malaysia. The presence of two segments of antennal scale, a shorter endopod of uropod than the exopod and a pair of minute spines at the apex of the telson distinguishes *Idiomysis bumbumiensis* sp. nov. from all other species in the genus. The present species is the seventh member of the genus *Idiomysis* and it is the first described in Southeast Asia. It is also the third species of tribe Anisomysini discovered in Malaysian waters. We include an updated dichotomous key of all *Idiomysis* species.

## Key Words

Pulau Bum Bum, *Idiomysis*, Malaysia, new species, Sabah

## Introduction

Mysids are considered as one of the most abundant and widely distributed crustaceans in the world, are known to inhabit all aquatic areas, but they are predominantly found in marine environments (Gan et al. 2010). Wittmann et al. (2014) established the tribe Anisomysini (former Mysini) for the first time, encompassing seven genera; *Anisomysis* Hansen, 1910; *Carnegieomysis* W. M. Tattersall, 1943; *Halemysis* Băcescu & Udrescu, 1984; *Idiomysis* W. M. Tattersall, 1922; *Javanisomysis* Băcescu, 1992, *Mysidium* Dana, 1852 and *Paramesopodopsis* Fenton, 1985. Today, six species of *Idiomysis* have been recorded from various locations. They include *Idiomysis diadema* Wittmann, 2016 from the coast of Dahab, Red Sea; *Idiomysis inermis* W. M. Tattersall, 1922 from Kilakarai, Gulf of Manaar, India; *Idiomysis japonica* Murano, 1978 from Nomo, Nagasaki, Japan; *Idiomysis mozambicus* Deprez, Wooldridge & Mees, 2001 from Nacala Bay, Mozambique, South Africa; *Idiomysis robusta* Connell, 2008 from the east coast of South Africa; and *Idiomysis tsurnamali* Băcescu, 1973 from Gulf of Elat, Red Sea. Some species (*Idiomysis japonica*, *I. mozambica* and *I. robusta*) are free-living by nature (Murano 1978; Deprez et al. 2001; Connell 2008), while some others (*Idiomysis diadema*, *I. inermis* and *I. tsurnamali*) associate with other organisms, such as sea anemones and sea urchins (Băcescu 1973; Greenwood and Hadley 1982; W. M. Tattersall 1922; Bhaduri and Crowther 2016; Wittmann 2016). Numerous species of mysids from the tribe Anisomysini have been discovered in Southeast Asian waters (Sawamoto 2014). To date, only two species, namely *Anisomysis* (*Anisomysis*) *aikawai* Ii, 1964 and *A.* (*Paranisomysis*) *ohtsukai* Murano, 1994, have been identified from Malaysian waters (Gan et al. 2010; Tan et al. 2014; Moriya 2016; Tan and Azman 2018) and there was no record of any mysid of the genus *Idiomysis*.

Pulau Bum Bum is situated in the Semporna District of southeast Sabah, an East Malaysian State. It is a con-

stituent of the Sulu Sulawesi Marine Ecoregion (SSME) and Coral Triangle Initiative (CTI), making it one of the richest marine biodiversity territories in the world (Ho and Kassem 2009). The most recent discovery was the newly-described *Cerapus bumbumiensis* Nurshazwan, Ahmad-Zaki & Azman, 2020, collected from Pulau Bum Bum (Nurshazwan et al. 2020). Even though this location is well-known for its extraordinary marine life diversity, there is little information on reef-associated crustacean fauna, including mysids. The present study described and identified *Idiomysis bumbumiensis* sp. nov. as a new species from Pulau Bum Bum, Sabah, Malaysia.

## Materials and methods

The specimens were collected using SCUBA diving equipment on a silty substrate near a large coral ledge of ND Divers House Reef, Pulau Bum Bum in Semporna, Sabah of East Malaysia (Fig. 1). Specimens were initially fixed with a 4% formaldehyde-seawater solution and subsequently preserved with 85% ethyl alcohol after sorting in the laboratory. The body length of the mysids was measured in the laboratory from the tip of the rostrum to the end of the telson, excluding apical spines. Appendages were dissected using a stereomicroscope (Olympus SZX9) and mounted on a temporary slide with a glycerol-ethanol mixed solution for illustrative purposes. An optical microscope (Olympus BX43), equipped with a camera lucida, visualised the images. They were then pencil-drawn and digitised in Adobe Illustrator CS6 following guidelines by Coleman (2003). The terminology used was according to Wittmann et al. (2014). All specimens were deposited in the Universiti Kebangsaan Malaysia Muzium Zoologi (UKMMZ), Bangi, Malaysia.

## Results

### Systematics

***Idiomysis bumbumiensis* sp. nov.**

http://zoobank.org/BA9ADF20-A7DB-4C50-B301-D3036F77925B

Figs 2–6

**Type material.** Holotype, adult male (BL. 2.3 mm, UKMMZ-1611); Allotype, ovigerous female (BL. 3.2 mm, UKMMZ-1612); Paratypes, two males (BL. 2.0 and 2.2 mm), one female (BL. 2.2 mm) (UKMMZ-1613); two females (BL. 2.6 and 3.1 mm, UKMMZ-1614), ND Divers House reef, Pulau Bum Bum, Semporna, Sabah, Malaysia, 4°26'43.2"N, 118°39'08.1"E, SCUBA diving, 29 November 2018, 10.5 m depth, collectors: Abu-Bakar A.Z., Azman B.A.R. and Dendy A.O.

**Diagnosis.** Antennal scale 2-segmented, with short apical segment, scale without any spine; rostrum subtriangular with broad rounded apex; thoracic exopod 1–8 with 7–9 segments; thoracic endopod 1–2 robust, thoracic endopod 3–8 elongate; all pleopods longer than wide; fourth male pleopod with distinct exopod and endopod not separated by sutures at the base, exopod terminally with 1 large barbed seta (armed with a few setules); en-

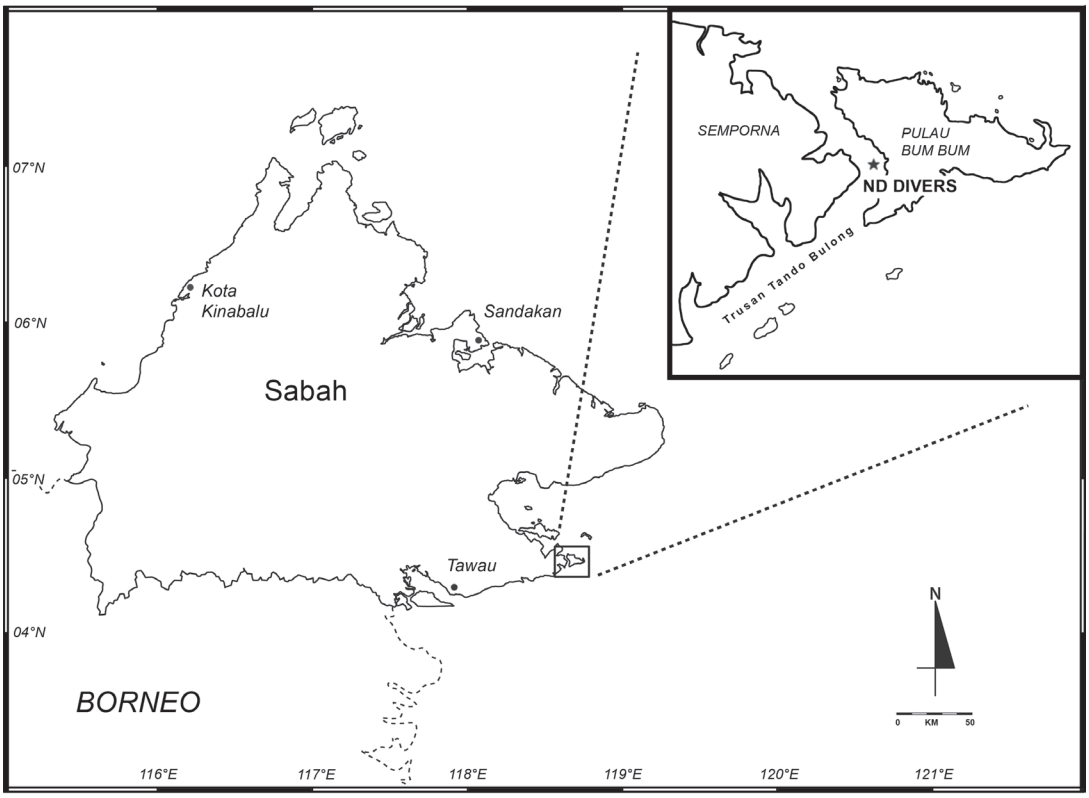


**Figure 1.** Map of Pulau Bum Bum, Semporna, Sabah, Malaysia

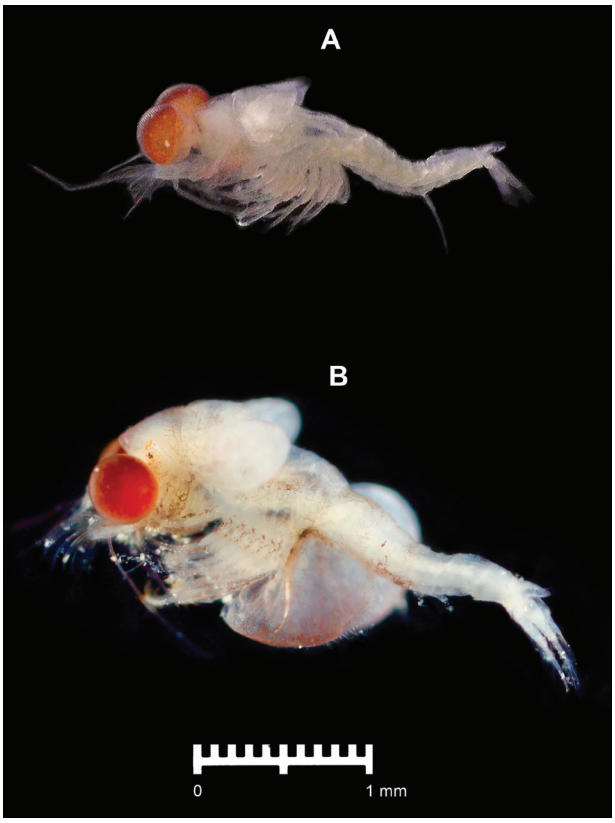


**Figure 2.** *Idiomysis bumbumiensis* sp. nov. (freshly fixed), **A.** Lateral view of the holotype (BL. 2.3 mm, UKMMZ-1611); **B.** Lateral view of allotype (BL. 3.2 mm, UKMMZ-1612).

dopod of uropod shorter than exopod; telson with a pair of minute spines on terminal margin; telson length ratio to sixth abdominal somite is 0.8.

**Description for male. Head and cephalic appendage.** A pale-white and brownish body part (Fig. 2A, B). Orange to the yellowish-red cornea (Fig. 2A, B). Stout and bulky body (Figs 2A, 3A) due to slightly double-flexed pleon antero-dorsally; short carapace, exposed last three thoracic somites, upwards-pointed trapezoid-shaped wing-like extension (Fig. 3A); subtriangular shaped rostrum (Fig. 3B) with broad rounded apex (subtriangular and bluntly pointed) extending between eyes reaching a middle basal segment of antennule peduncle; very large eyes (Fig. 3B), globular; the cornea is wider than eyestalk, covering almost all of the eye surface.

Antennule peduncle (Fig. 3D) with three segments; the basal segment is the longest with a ventral short lobe on subterminal position with three setae; the median segment is the shortest with a ventral short lobe on subterminal position with three setae; the terminal segment is almost 0.5 times as long as the first/basal segment, with eight setae and one plumose seta, hirsute appendix masculina present; inner flagellum with four segments; outer flagellum with 9–10 segments; aesthetascs present. Antennal peduncle (Fig. 3E) is very short and stout, with three segments; antennal scale is extending beyond antennule peduncle, long, robust and broad; suture present at 11–14% from apex; terminal segment with five plumose setae; proximal outer margin without plumose setae from the base of antennal scale is 64%, while proximal inner margin without plumose setae is 27%.

Mandible (Fig. 3F) with incisor and molar process; well-developed lacinia mobilis; the molar process is present; palp with three segments; small basal segment without setae; median segment with eight setae along the outer (lateral) margin and three setae along inner (mesial) margin; terminal segment with six normal setae and four plumose setae. Normal maxilla (Fig. 3G) for the genus; exopod bearing five apical setae; two-segmented endopod, the sub-ellipsoidal shaped terminal segment with seven setae including two normal setae. Normal maxillula (Fig. 3H); basal lobe with nine large spines; precoxal lobe with two long setae and two small setae.

**Thoracopods.** A round basal plate of thoracic exopod at both distal corners with 7–9 segments with the last 3–4 segments bearing 1–2 plumose setae; robust thoracopods 1–2, slender and elongated thoracopods 3–8; carpopropodus of thoracic endopod 1–8 with 2, 2, 2, 1–2, 3, 1–2, 1, 1 segments, but some segmental borders are not well distinct in thoracopods 3–8; smaller dactylus of thoracopods 3–8 than thoracopods 1–2; nail of thoracopods 3–8 is more slender compared to thoracopods 1–2. The first thoracopod epipod (Fig. 4A) is linguiform-subtriangular without seta; seven-segmented exopod, first four segments without seta, the fifth segment with one plumose seta, the sixth and seventh segments with two plumose setae; normal and robust endopod, densely setose along both lateral margins of the ischium to dactylus, each segment bearing 1–2 plumose setae; nail with a swollen base. Second thoracic exopod (Fig. 4B) with eight segments, last three segments with 1–2 plumose setae; robust thoracic endopod similar to the first thoracopod, but armed with lesser setae, from basis to dactylus bearing 1, 0, 2, 2, 6, 7 setae, respectively.

Third thoracic exopod (Fig. 4C, D) with nine segments, the last four segments with 1–2 plumose setae; thoracic endopod is more slender and elongate instead of robust, two-segmented carpopropodus, all segments are armed with setae, dactylus is smaller than in thoracopods 1–2, the nail is more slender than first and second thoracopods. The fourth thoracic endopod (Fig. 4E) is similar to the third thoracopod, carpopropodus, with 1–2 segments. The fifth thoracic endopod (Fig. 4F) is slightly longer than in the fourth thoracopod, merus nearly equal in length to the preceding segment, three-segmented carpopropodus, elongated and slender nail. Eighth thoracic exopod (Fig. 4G) with seven segments; thoracic endopod is smaller and more slender than other thoracopods, merus is longer than the preceding segment, separate carpopropodus, short and small dactylus, the nail is shorter than other thoracopods.

**Pleopods.** Pleopods 1, 2, 3 and 5 (Fig. 5A, C, E) reduce to simple separate plates, each with 4–6 setae of different lengths, longer than width; length of pleopod is more than twice its width. Male pleopod 4 (Fig. 5D) has distinct endopod and exopod, both undivided and basal-

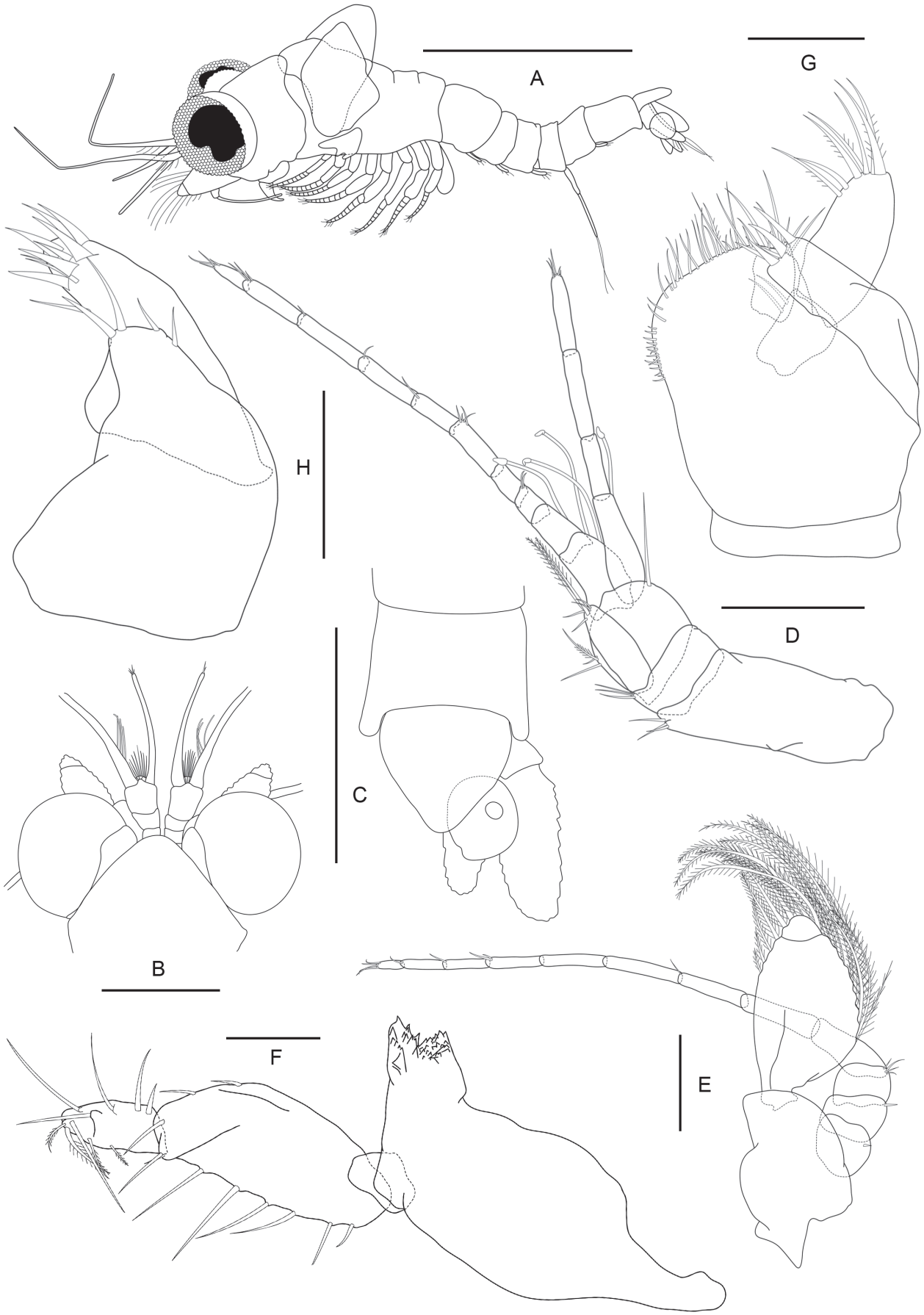


**Figure 3.** *Idiomysis bumbumiensis* sp. nov., holotype (BL. 2.3 mm, UKMMZ-1611). **A.** Habitus; **B.** Anterior body, dorsal view; **C.** Posterior body, dorsal view; **D.** Antennule, obliques dorso-lateral view; **E.** Antennal; **F.** Mandible; **G.** Maxilla; **H.** Maxillula. Scale bars equal 0.1 mm for D–E; 1 mm for A; 0.4 mm B–C; 0.05 mm for F–H.

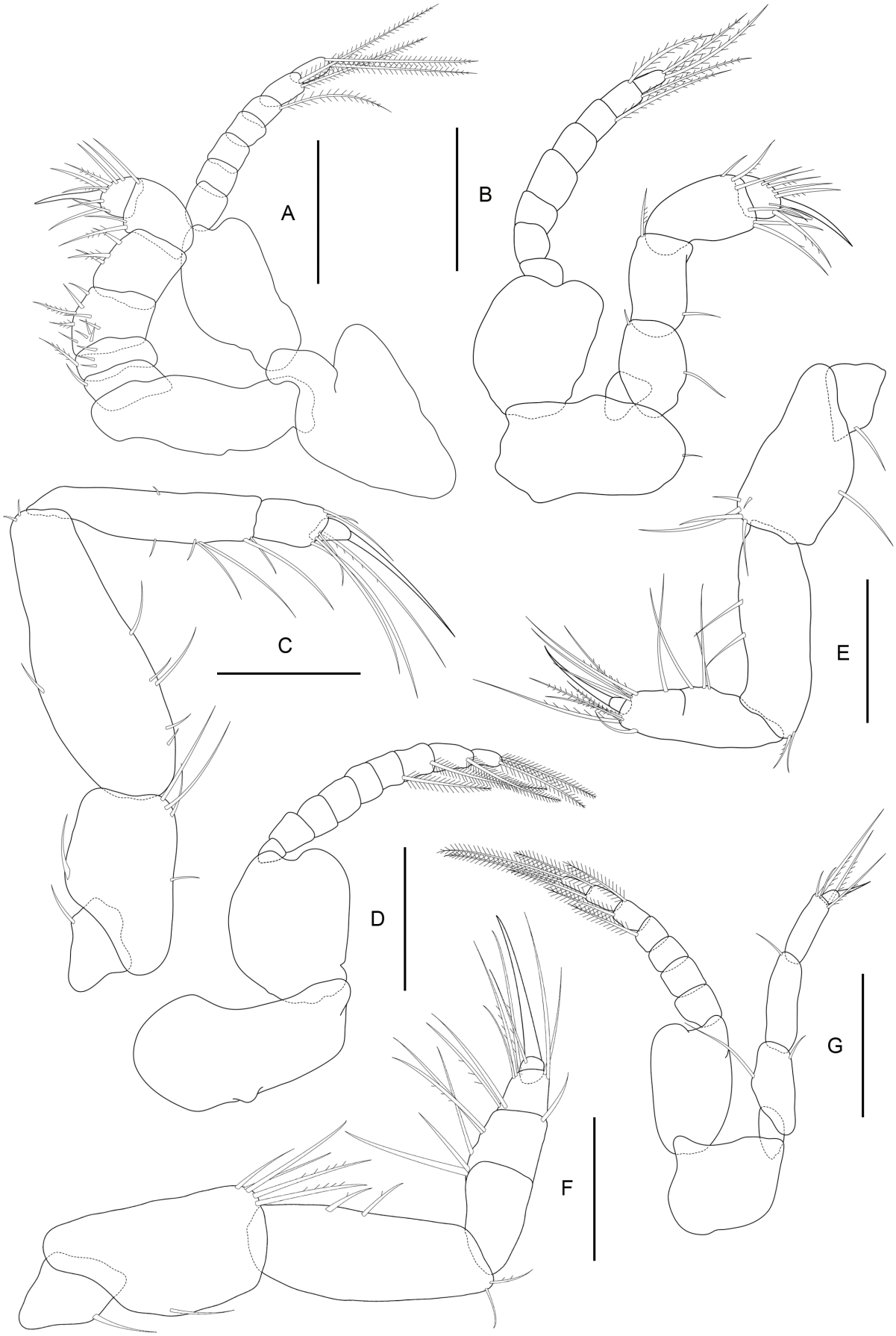


**Figure 4.** *Idiomysis bumbumiensis* sp. nov., holotype (BL. 2.3 mm, UKMMZ-1611). **A.** First thoracopod; **B.** Second thoracopod; **C.** Third thoracic endopod; **D.** Third thoracic exopod; **E.** Fourth thoracic endopod; **F.** Fifth thoracic endopod; **G.** Eighth thoracopod. Scale bars equal 0.1 mm for A–G.

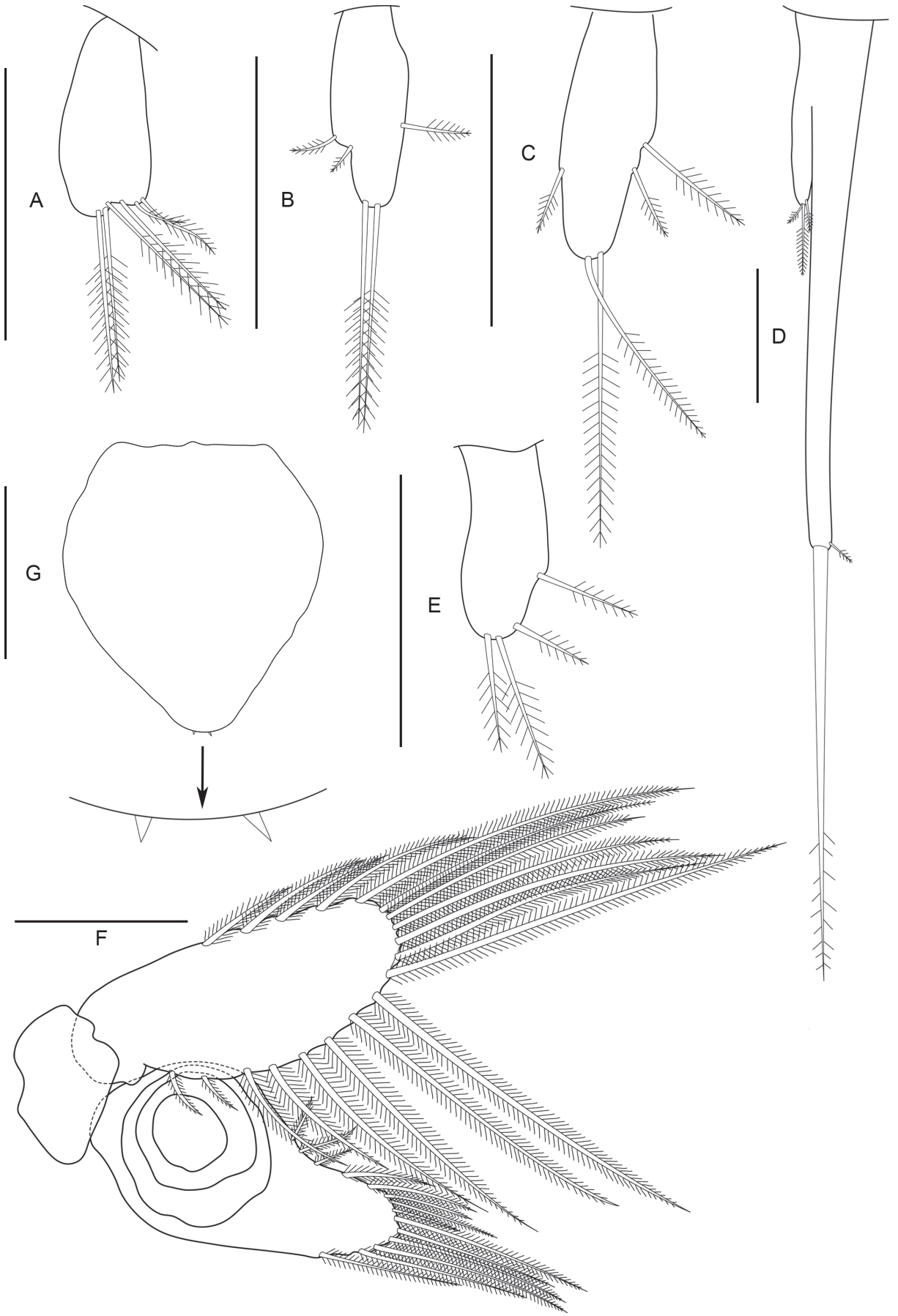


**Figure 5.** *Idiomysis bumbumiensis* sp. nov., holotype (BL. 2.3 mm, UKMMZ-1611). **A.** Pleopod 1; **B.** Pleopod 2; **C.** Pleopod 3; **D.** Pleopod 4; **E.** Pleopod 5; **F.** Uropod; **G.** Telson. Scale bars equal 0.1 mm for A–G.

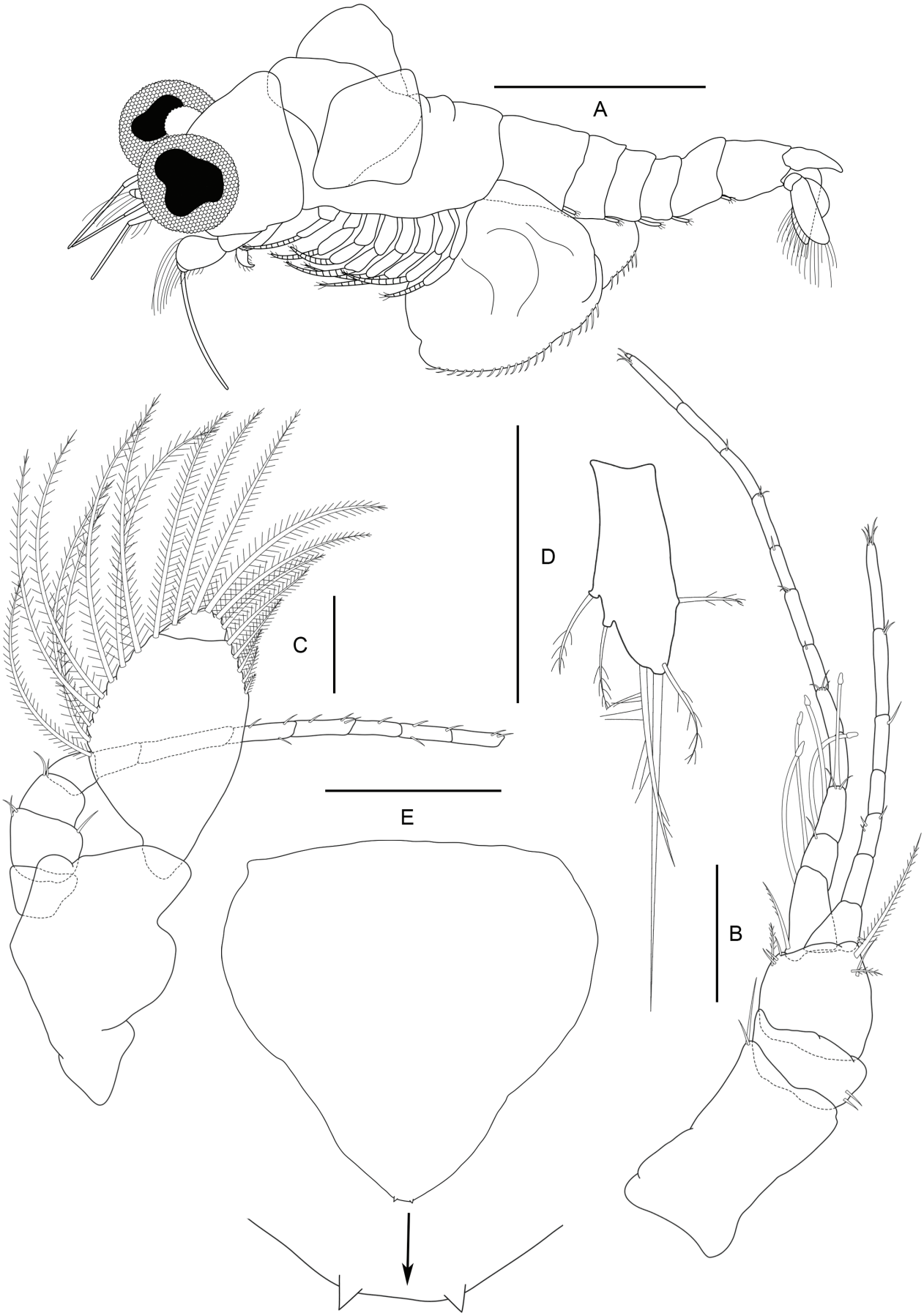


**Figure 6.** *Idiomysis bumbumiensis* sp. nov., allotype (BL. 3.2 mm, UKMMZ-1612). **A.** Habitus; **B.** Antennule, oblique dorso-lateral view; **C.** Antennal; **D.** Pleopod 4; **E.** Telson. Scale bars equal 0.1 mm for B–E; 1 mm for A.

ly not separated by sutures; endopod with three plumose setae; exopod has one small terminal seta and a large-barbed seta (armed with a few setules).

**Uropod and telson.** Uropod (Figs 3C, 5F) has a shorter endopod than exopod; both endopod and exopod have plumose setae all around, without setae on both margins of the proximal part of endopod and exopod; endopod with 14 plumose setae; exopod with 19 plumose setae; large statocyst (there are circular borders between ambitus versus tegmen and ambitus versus fundus). Telson (Figs 3C, 5G) is approximately 1.12 times longer than the width and 0.8 times longer than the sixth abdominal somite; short, subtriangular with rounded tip; extending halfway across statocyst of endopod; smooth margin, except for apex with a pair of minute spines.

**Female.** Similar to male, except for the following differences: stouter and bulkier body (Fig. 6A) than male due to marsupium; marsupium of female on the eighth thoracopod is larger than seventh thoracopod, large with short setae along the distal margin. Antennule (Fig. 6B); inner flagellum with seven segments; outer flagellum with 12 segments; aesthetascs is present. Antennal scale (Fig. 6C) with suture present at 10%–14% from apex; from the base of antennal scale, 70% of the proximal outer margin is without plumose setae while 45% of the proximal inner margin is without plumose setae. Pleopod 4 (Fig. 6D) is similar to male pleopods 1–3, 5; longer than its width with six setae. Telson (Fig. 6E) is approximately 1.03 wider than its length; apex with a pair of minute spines.

**Etymology.** The specific epithet refers to the type locality; Pulau Bum Bum, Sabah, Borneo, Malaysia.

**Colouration in freshly fixed specimens** (based on UKMMZ-1611, BL. 2.3 mm; UKMMZ-1612, BL. 3.2 mm; Fig. 2A, B). Zesty orange eyes. Antennal scale, carapace including thoracic and translucent abdominal somites with a combination of small orange, yellow and black patches.

**Remarks.** The present species is the seventh member of the genus *Idiomysis*, but it is the first species of this genus to be described in Southeast Asia. It is also the third species of the tribe Anisomysini found in Malaysian waters besides *Anisomysis* (*Anisomysis*) *aikawai* Ii, 1964 and *A.* (*Paranisomysis*) *ohtsukai* Murano, 1994 (Moriya 2016; Tan and Azman 2018). The genus *Idiomysis* can be easily classified into two groups, based on the antennal scale; (i) entire or (ii) 2 segments. *Idiomysis inermis*, *I. mozambica*, *I. robusta* and *I. tsurnamali* are in the group of an entire antennal scale, while *I. diadema* and *I. japonica* are in the group of two-segmented antennal scale. The presence of the spine, which is exclusively in *I. robusta*, distinguishes the group with the entire antennal scale. The present new species, *Idiomysis bumbumiensis* sp. nov., has two antennal scale segments, similar to *I. diadema* and *I. japonica*. However, they can be differentiated by observing the apex of the telson. Both *I. diadema* and *I. bumbumiensis* sp. nov have a pair of minute apical spines exclusive to these two species; on the other hand, *I. japonica* has a bluntly rounded telson apex. The endopodal uropod in *I. diadema* shows a clear extension beyond the exopod, but this structure is shorter than the exopod in *I. bumbumiensis*.

## Key to species of the genus *Idiomysis* (Based on males)

| | | |
|---|---|---|
| 1 | Not segmented antennal scale | 2 |
| – | Antennal scale with two segments | 5 |
| 2 | Broadly rounded rostrum | 3 |
| – | Triangular or subtriangular rostrum | 4 |
| 3 | Not segmented antennal scale, with spine | *I. robusta* |
| – | Not segmented antennal scale, without spine | *I. inermis* |
| 4 | Endopodal uropod is subequal to exopod | *I. mozambica* |
| – | Endopodal uropod is clearly shorter than exopod | *I. tsurnamali* |
| 5 | Bluntly rounded telson apex | *I. japonica* |
| – | Telson apex with a pair of minute spines | 6 |
| 6 | Endopodal uropod clearly extends beyond exopod | *I. diadema* |
| – | Endopodal uropod is shorter than exopod | *I. bumbumiensis* sp. nov. |

# Discussion

*Idiomysis bumbumiensis* sp. nov. is the sole representative of this genus in Southeast Asian waters. *Idiomysis bumbumiensis* sp. nov. was relatively abundant and easily found in the shallow water of lower than 15 m during night-sampling sessions (28 November 2018 and 29 November 2018). As they were directly collected using SCUBA diving equipment, supplementary information on their natural habitat and body colour is available. The recently described *Cerapus bumbumiensis* Nurshazwan, Ahmad-Zaki & Azman, 2020 was also observed in the accompanying fauna. Although one species of *Idiomysis* was categorically described in this paper, fellow macro-photographers discovered further evidence of at least two other distinctive species of *Idiomysis* in the vicinity.

*Idiomysis* lives either in a symbiotic relationship (*Idiomysis diadema*, *I. inermis* and *I. tsurnamali*) or

**Table 1.** Morphological variation of seven species of the genus *Idiomysis*, including the new species.

| Characters | *I. diadema* Wittmann, 2016 | *I. inermis* W. M. Tattersall, 1922 | *I. japonica* Murano, 1978 | *I. mozambica* Deprez, Wooldridge & Mees, 2001 | *I. robusta* Connell, 2008 | *I. tsurnamali* Băcescu, 1973 | *I. bumbumiensis* sp. nov. (Present study) |
|---|---|---|---|---|---|---|---|
| Body length | Male: 2.2–2.3 | Male: 3.4–4.4 | Male: 3.3 | Male: 2.9–3.9 | Male: 4.9–6.0 | Male: 4.2–4.5 | Male: 2.0–2.3 |
| | Female: 2.2–3.3 | Female: 4.0–5.0 | Female: 3.7–3.9 | Female: 2.6–2.9 | Female: 4.8–5.4 | Female: 4.2–4.5 | Female: 2.2–3.2 |
| Rostrum | Broadly rounded | Broadly rounded | Subtriangular (bluntly pointed) | Subtriangular (bluntly pointed) | Broadly rounded | Triangular (pointed) | Subtriangular (bluntly pointed) |
| Antennal scale | Two segments (no spine) | Entire (no spine) | Two segments (no spine) | Entire (no spine) | Entire (spine) | Entire (no spine) | Two segments (no spine) |
| Segments of thoracic exopod 1–8 | 6–8 | 7–10 | 7–8 | 7–9 | 7–8 | 5–8 | 7–9 |
| Male pleopod 4 exopod | Single segment | Single segment | Single segment | Two segments | Single segment | Single segment | Single segment |
| Endopodal uropod | Clearly extend beyond exopod | Subequal to exopod | Subequal to exopod | Subequal to exopod | Shorter than exopod | Clearly shorter than exopod | Shorter than exopod |
| Telson apex | A pair of minute spines | Bluntly rounded | Bluntly rounded | Bluntly rounded | Bluntly rounded | Bluntly rounded | A pair of minute spines |
| Length ratio of fifth to sixth abdominal somite | 0.5 | 0.5 | 0.5 | 0.4 | 0.4 | 0.5 | 0.6 |
| Length ratio of telson to last abdominal somite | 1.0 | 0.8–0.9 | 0.8 | 0.4 | 0.8 | 0.8 | 0.8 |
| Distribution | Coast of Sinai at Dahab, Red Sea | Kilakarai, Gulf of Manaar & Moreton Bay, Australia | Nagasaki, Japan | Nacala Bay, Mozambique | Park Rynie, East Coast of South Africa | Gulf of Eilat, Red Sea | Pulau Bum Bum, East Malaysia |
| Occurrence | Swarms between spines of sea urchin | Amongst weeds, sea anemone | Near rocky bottom | Near uneven rock and patches of sand | Near sandy substrate, amongst rocks and low-profile reef | Hovering over medusa or sea anemone | Near coral ledge; silty substrate |
| Depth range | 1–8 m | 1–4 m | 1–5 m | 4 m | 2–38 m | 1–20 m | 10–11 m |

free-living (*I. japonica*, *I. mozambica* and *I. robusta*). *Idiomysis bumbumiensis* is a free-living mysid that was found swimming in a swarm on the silty substrate. By comparing the body lengths of all the species of the genus *Idiomysis*, this new species is one of the smallest species, besides *I. diadema*. Another feature that distinguishes species within the genus *Idiomysis* is the length ratio between the telson and the last abdominal somite. As this feature has not been described for the six known species, the ratios are calculated, based on the original-drawn figures describing each species. The ratio is mostly 0.8–1.0, except for *I. mozambicus*, which has a ratio of 0.4. The ratio of the present species is 0.8. Thus, the telson of most *Idiomysis* species is estimated to be more than 4/5 times as long as the last abdominal somite, while *I. mozambicus* is 2/5 times as long as the last somite. As shown in Table 1, *I. bumbumiensis* sp. nov. can be distinguished from *I. inermis* and *I. tsurnamali* by several morphological features: two segments of antennal scale and a pair of minute spines on the apex of the telson. Table 1 shows a brief morphological variation from each species of the genus *Idiomysis*. More research would be required to uncover more underwater macrolife, particularly in this area known as the heart of the Coral Triangle. More unique and unidentified marine life would undoubtedly be discovered with the overwhelming support of local underwater photographers.

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PENSOFT

für Natur MUSEUM FÜR NATURKUNDE BERLIN

# Description of six new species of *Xenorhina* Peters, 1863 from southern Papua New Guinea (Amphibia, Anura, Microhylidae)

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## Abstract

We describe six new species of the microhylid frog genus *Xenorhina* from the southern slopes of Papua New Guinea's central cordillera and adjacent lowlands, based on a combination of morphological (including osteology) and bioacoustic features. All of the new species are fossorial or terrestrial inhabitants of tropical rainforest habitats and belong to a group of *Xenorhina* having a single, enlarged odontoid spike on each vomeropalatine bone. Advertisement calls and habitat preferences are described for each species, one of which is amongst the smallest hitherto members of the genus. Description of these six species brings the total number of *Xenorhina* known to 40 and emphasises the importance of the high-rainfall belt that extends along the southern flanks of New Guinea's central cordillera as a hotspot of Melanesian amphibian diversity.

## Key Words

acoustics, Asterophryinae, central cordillera, fossorial frogs, morphology, odontoid spike, rainforest, taxonomy

## Introduction

Asterophryine microhylid frogs occur from mainland and insular southeast Asia eastwards through New Guinea to New Britain Island and northern Australia (Clulow and Swan 2018, Suwannapoom et al. 2018). The group reaches its greatest diversity in the New Guinea region, where many genera are endemic or near-endemic to the New Guinea mainland (Menzies 2006). *Xenorhina* Peters, 1863 is a moderately speciose genus of asterophryine microhylid frogs that currently contains 34 named species distributed across New Guinea and some nearby islands (Zweifel 1972, Blum and Menzies 1989, Günther and Richards 2005, Menzies 2006, Kraus 2011, Günther et al. 2020, Frost 2021). With the exception of three arboreal species, *X. arboricola* Allison & Kraus, 2000, *X. macrodisca* Günther & Richards, 2005 and *X. varia* Günther & Richards, 2005, other members of the genus are fossorial or inhabit litter or subterranean burrows and have squat bodies, small, pointed heads, small eyes and short, robust limbs (Menzies and Tyler 1977, Blum and Menzies 1989). The genus *Xenorhina* includes two main groups: 1) species having one or two spikes on each vomeropalatine and 2) species lacking vomeropalatine spikes. The former group was previously recognised as a separate genus, *Xenobatrachus* Peters & Doria, 1878 (e.g. Blum and Menzies 1989, Menzies 2006), but Kraus and Allison (2002) noted the lack of synapomorphies distinguishing *Xenorhina* from *Xenobatrachus* and suggested that the two genera may need to be combined. Frost et al. (2006) subsequently synonymised *Xenobatrachus* with *Xenorhina* (the older available name), based on molecular evidence, a move supported by Köhler and Günther (2008).

The monophyly of *Xenorhina* + *Xenobatrachus* is now well supported (de Sa et al. 2012, Peloso et al. 2015, Rivera et al. 2017), but the relationships of *Xenorhina* to other asterophryine genera remain poorly resolved. For example, molecular studies by Köhler and Günther (2008) and Pyron and Wiens (2011) concluded that *Xenorhina* is closely related to *Asterophrys* and some *Callulops*, while

Rivera et al. (2017) found that *Xenorhina* is the sister taxon to *Callulops*, *Mantophryne* and *Hylophorbus*.

In this paper, we describe six new *Xenorhina* that belong to the group of species with enlarged vomeropalatine spikes. They were collected from localities within and adjacent to the high-rainfall belt extending along the southern flanks of New Guinea's central cordillera (McAlpine et al. 1983), a region that has previously been reported to support a diverse amphibian fauna (Hyndman and Menzies 1990, Richards and Günther 2019). Description of these frogs brings to 40 the number of recognised *Xenorhina*, making it the third most speciose microhylid genus in the New Guinea region after *Oreophryne* (61 species) and *Cophixalus* (48 species) (Richards and Günther 2019, Frost 2020). It also reinforces the importance of the extensively forested southern flanks of Papua New Guinea's central cordillera as a hotspot of Melanesian amphibian diversity.

## Material and methods

Fieldwork was conducted in tropical rainforest habitats along the southern flanks of Papua New Guinea's central cordillera. Most frogs were located at night by their advertisement calls. Representative specimens were photographed in life and voucher animals were euthanised in an aqueous chlorobutanol solution (Gamble 2014) and subsequently fixed in 10% formalin. All specimens were transferred to 70% ethanol within two days of fixation. Descriptions follow a template developed for taxonomic treatments of New Guinea microhylid frogs, including *Xenorhina* (e.g. Günther et al. 2014, Günther et al. 2020). We adopt the biological species concept of E. Mayr (1963 and elsewhere), placing emphasis on reproductive isolation and we treat morphological, osteological and ethological (acoustic) differences as expressions of genetic differences that are large enough to prevent exchange of genes between the populations concerned. Our approach follows that of other taxonomic studies of this genus (Zweifel 1972, Blum and Menzies 1989, Kraus and Allison 2002, Günther et al. 2014).

The following measurements were taken with a digital calliper (> 10 mm) or with a binocular dissecting microscope, fitted with an ocular micrometer (< 10 mm) to the nearest 0.1 mm from preserved specimens using protocols for microhylid frogs adopted previously (e.g. Günther et al. 2014): **SUL** – snout-urostyle length from tip of snout to posterior tip of urostyle (SUL is sufficiently similar to SVL that, where relevant, we compare our SUL measurements with SVLs presented for members of the genus in some papers); **TL** – tibia length: external distance between knee and tibio-tarsal articulation (referred to herein also as "shank"); **TaL** – length of tarsus: external distance between tibio-tarsal and tarsal-metatarsal joints when held at right angles; **T4L** – length of 4$^{th}$ toe: from tip of toe to proximal end of inner metatarsal tubercle; **T4D** – transverse diameter of disc of 4$^{th}$ toe; **T1D** – transverse diameter of disc of first toe; **F3L** – length of 3$^{rd}$ finger: from tip of 3$^{rd}$ finger to proximal edge of palm; **F3D** – transverse diameter of disc of 3$^{rd}$ finger; **F1D** – transverse diameter of disc of 1$^{st}$ finger; **HL** – head length, from tip of snout to posterior margin of tympanum; **HW** – head width, taken across the tympana; **SL** – snout length, from an imaginary line connecting the centres of the eyes to tip of the snout; **EST** – distance from anterior corner of orbital opening to tip of snout; **END** – distance from anterior corner of orbital opening to centre of naris; **IND** – internarial distance between centres of nares; **ED** – eye diameter, from anterior to posterior corner of orbital opening; **TyD** – horizontal diameter of tympanum. Measurements are presented as arithmetic means ± standard deviation and range. Statistical calculations were done with the programme Statgraphics Centurion Version 15.2.14 (Statpoint Technologies, Inc., Warrenton, Virginia, USA). All p-values are calculated by the non-parametric Mann-Whitney (Wilcoxon) Test for comparison of medians. Osteological features were determined by superficial dissection.

Sex was determined mainly by observations of calling in the field and/or the presence of vocal slits or testes (males) or absence of vocal slits and/or presence of eggs (females). Advertisement calls were recorded under natural conditions with a Sony WM-D6C Professional Walkman tape recorder, a Marantz PMD-661 or an Edirol R09 digital recorder and a Sennheiser ME66 shotgun microphone and analysed with the sound-analysis package Avisoft-SAS Lab Pro. Air temperatures adjacent to calling males were recorded using a rapid-reading digital thermometer. Terminology and acoustic analysis procedures mostly follow Köhler et al. (2017). All of the species described here produce calls in groups, which are separated from other groups by periods of silence that are much longer than the inter-call intervals and within which calls are repeated at regular intervals. As such, they meet the definition of a "call series" from Köhler et al. (2017). For all species, each call within a series comprises a single unpulsed note (so call = note); we use the term "call" in preference to "note" throughout to provide consistency. Measurements of call parameters are presented predominantly as range and mean ± standard deviation.

Colour of animals in life was described from digital photographs and of preserved specimens from direct observations. Most colours were determined according to a colour matching system that is created and administrated by the German RAL GmbH (RAL non-profit LLC). It should be stressed, however, that in many cases it was impossible to find an exact match between observed colours and RAL colour numbers. In those cases, the most similar RAL number was chosen.

Specimens are stored in the South Australian Museum, Adelaide, Australia (**SAMA**) and the Museum für Naturkunde, Berlin, Germany (**ZMB**). Paratypes for most species will also be repatriated to the Papua New Guinea National Museum, Port Moresby, Papua New

Guinea (**PNGNM**). Abbreviations for other institutions mentioned are: American Museum of Natural History, New York, U.S.A. (**AMNH**); Bernice P. Bishop Museum, Hawaii, U.S.A. (**BPBM**); Institut Royal des Sciences Naturales de Belgique, Brussels (**IRSNB**); Museo Civico di Storia Naturale di Genova, Genoa, Italy (**MSNG**); Museum of Comparative Zoology, Harvard, U.S.A. (**MCZ**); Museum Zoologicum Bogoriense, Cibinong, Indonesia (**MZB**); National Museum of Natural History, now Naturalis Biodiversity Center, Leiden, The Netherlands (**RMNH**); University of Papua New Guinea, Port Moresby (**UP**); Zoological Museum Amsterdam, now Naturalis Biodiversity Center, Leiden, The Netherlands (**ZMA**). SJR refers to the original field collection tag of Stephen Richards.

Specimens examined for comparative purposes are listed in Appendix 1. Additional morphometric and other data were extracted from original species descriptions and/or recompiled treatises, particularly Zweifel (1972), Blum and Menzies (1989), Kraus and Allison (2002) and Menzies (2006).

## Systematics

Specimens were assigned to the genus *Xenorhina* on the basis of the following combination of features: jaw symphygnathine; clavicles and procoracoids absent; each vomeropalatine bone with elongated odontoid spike; body squat, head small, triangular, with small eyes; cutaneous tubercles present dorsolaterally, absent on eyelids; tips of toes 2–5 expanded, with circum-marginal grooves; life style subterrestrial.

### *Xenorhina lacrimosa* sp. nov.

http://zoobank.org/D78F9340-1032-4D34-976A-B91C7001CC1C

**Holotype.** SAMA R71648 (SJR 14203), adult male, from Rentoul River, Western Province, Papua New Guinea (6.4355°S, 142.5615°E; 380 m a.s.l.), collected on 10-08-2014 by S.J. Richards.

**Paratypes.** SAMA R71647 (SJR10389), female with ripe eggs, ZMB 91129 (SJR10417) male, Camp 2, upper Strickland River basin, Western Province, Papua New Guinea (5.9018°S, 142.4360°E; 950 m a.s.l.), collected by S.J. Richards on 18-02-2008 and 20-02-2008, respectively; ZMB 91130 (SJR10466) male, Camp 1, upper Strickland River basin, Western Province, Papua New Guinea (5.8078°S, 142.3083°E; 215 m a.s.l.), collected by S.J. Richards on 26-03-2008; SAMA R65069 (SJR10902) and R65070 (SJR10949), males, R65071 (SJR10963), (subadult?) female with scarcely developed eggs and R65072 (SJR10985), juvenile, Gugusu Camp, Muller Range, Western Province (5.7290°S, 142.2630°E; 515 m a.s.l.), all collected by S.J. Richards and C. Dahl between 7–9-09-2009.

**Referred specimens.** SAMA R71649, R71650 (SJR2577, 2582), PNGNM (SJR2571), adult males, Herowana, Eastern Highlands Province, Papua New Guinea (6.6220°S, 145.1962°E; 1,400 m a.s.l.), collected by S.J. Richards between 20 and 24-11-2001.

**Diagnosis.** This species of *Xenorhina* is characterised by the unique combination of: medium size (SUL of five males 34.5–41.0 mm); vomeropalatines each with one long and acuminate spike; legs moderately long (TL/SUL 0.42–0.46); all fingers without and all toes with expanded discs; eye-naris distance greater than internarial distance (END/IND 1.18–1.48); tympanum diameter smaller than or equal to that of eye (TyD/ED 0.75–1.00); dorsal surfaces in life different tones of brown or blue or a mixture of these colours; ventral surfaces different tones of orange with irregular whitish spots or mouse grey (RAL 7005) with whitish spots and reticulations; advertisement calls uttered in series containing 7–12 single, mournful "hoots" separated by long intervals of about five seconds.

**Description of the holotype.** Measurements are summarised in Table 1, a dorsolateral view in life is shown in Fig. 1a and ventral surfaces in life in Fig. 1b. Head broader than long (HL/HW 0.74); snout acuminate from above and below, distinctly protruding in profile; tongue very broad, only its lateral and posterior edges free; prepharyngeal ridge with five roundish denticles; left vomerine spike very well developed, right spike present, but malformed; loreal region oblique, no canthus rostralis; nostrils near tip of snout, directed more lateral than dorsal, visible from above, but not from below; eye-naris distance greater than internarial distance (END/IND 1.18); tympanum visible in life and preservative, its diameter slightly less than that of eye (TyD/ED 0.87); supratympanic fold weakly expressed, extending from behind eye to insertion of fore leg; shank short (TL/SUL 0.42); fingers moderately short, not webbed; tips of all fingers with circum-marginal grooves, all not wider than penultimate phalanges; relative lengths of fingers 3 > 4 = 1 = 2 (Fig. 1c); all toe tips with circum-marginal grooves and wider than penultimate phalanges; toes not webbed, relative lengths 4 > 3 > 5 > 2 > 1 (Fig. 1d); plantar and palmar tubercles (with exception of prominent, oval inner metatarsal tubercle; Fig. 1d), as well as subarticular tubercles scarcely visible. Body laterally with numerous distinct tubercles in life, less prominent in preservative; dorsal surfaces of limbs and middle of dorsum with fewer tubercles, all ventral surfaces smooth; tip of snout with several tiny elevations (especially on underside).

In life, dorsal surfaces of head and anterior portion of body and fore limbs, uniform bluish-brown; remaining dorsal surfaces and flanks a mixture of saffron-yellow (RAL 1017) and blue-grey; tubercles with brown bases and whitish apices concentrated on flanks; body dorsally with light yellow mid-dorsal line that continues on to hind legs; lumbar region with light yellow semi-circular spot (Fig. 1a); vent and adjacent region enclosed within

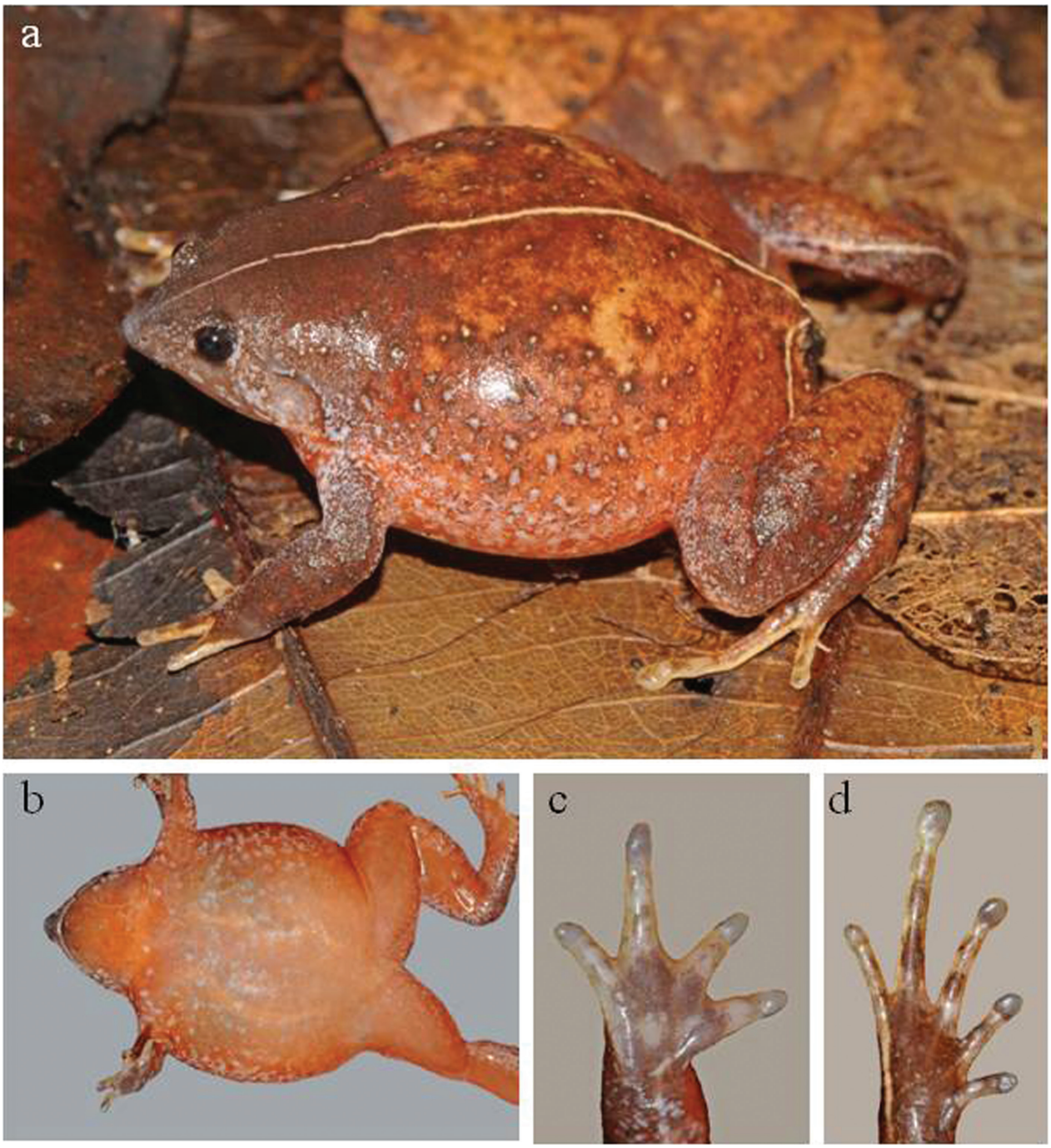


**Figure 1.** Holotype (SAMA R71648) of *Xenorhina lacrimosa* sp. nov. in life: (**a**) Dorsolateral view; (**b**) Ventral view; (**c**) Volar view of right hand; (**d**) Thenar view of right foot.

dark brown triangular patch; iris blackish with barely visible golden reticulation; plantar and ventral surfaces of toes predominantly brown, palms and ventral surfaces of fingers predominantly grey and cream; throat, chest, abdomen and ventral surfaces of extremities deep orange (RAL 2011), with some whitish spots (Fig. 1b).

In preservative, dorsal surfaces of head, anterior back and fore limbs signal brown (RAL 8002); other dorsal surfaces ivory with diffuse brownish smears, tubercles with terra brown (RAL 8028) bases and whitish apices; ventral surfaces light ivory (RAL 1015); ivory lumbar spot on left side more clearly pronounced than on right.

**Morphological variation.** Measurements and proportions of most paratypes show limited variation (Table 1). An exception is a juvenile (SAMA R65072) measuring 16.6 mm SUL that exhibits some major deviations in proportions from the remainder of the type series. As these differences are almost certainly due to allometry, measurements of this specimen are disregarded in Table 1. Males and females have the same body size, although some ratios of the adult female (SAMA R71647 differ to a negligible degree (Table 1). The smallest specimen in the series is the just-mentioned adult female with an SUL of "only" 34.3 mm that contains ripe ovarian eggs;

the largest specimen in the series is a male (ZMB 91130) with an SUL of 41.0 mm.

Colour of paratypes in life varies considerably. Dorsal surfaces may be uniform blue-brown (SAMA R65070, Fig. 2a), uniform light red-orange similar to RAL 2008 (ZMB 91129, Fig. 2b), bluish on head and lower flanks, but reddish-brown on back and dorsal extremities (ZMB 91130, Fig. 2c) or dark brown with bluish hue on head, body and thighs, but beige on fore limbs, shanks and tarsi (SAMA R71647, Fig. 2d). Colour of ventral surfaces is also highly variable. Some specimens are uniform deep orange or traffic orange (RAL 2009) interspersed with scattered irregular whitish spots (SAMA R65070, Fig. 2e); others are more extensively spotted (ZMB 91129, Fig. 2f) or exhibit a mixture of whitish, orange and brown spots, but with throat and thighs more or less uniform traffic orange (SAMA R65071, Fig. 2g); others exhibit grey-brown ground colour with many irregular whitish spots, some of them interspersed with small irregular red patches (SAMA R71647, Fig. 2h) .

In preservative dorsal surfaces of three specimens predominantly violet, of two specimens copper brown, of one specimen beige and of the juvenile specimen beige-brown; ventral surfaces of three specimens almost completely light ivory, of the four other specimens a light ivory ground colour with a brown-beige pattern of various extent. All paratypes, except SAMA R71647, have a light ivory mid-dorsal line and all specimens including the juvenile have a greyish snout tip. None of the paratypes has a clearly pronounced lumbar spot in life or in preservative.

**Distribution and ecological notes.** Most records of *Xenorhina lacrimosa* sp. nov. are from lowland and foothill forest in south-central Papua New Guinea (Fig. 8), where this species appears to have a broad distribution at altitudes ranging from near sea level around Kopi to at least 950 m a.s.l. We also refer several specimens from Herowana Village at 1,400 m a.s.l. (the most easterly location in Fig. 8) to this species pending confirmation of genetic relationships. Males called at night, normally after rain, either from within the leaf litter on the forest floor or down to several centimetres depth in the humus layer beneath the litter.

**Table 1.** Body measurements and body ratios of the type series of *Xenorhina lacrimosa* sp. nov. SAMA R71648 is the male holotype, others are paratypes. All measurements in mm; for explanation of abbreviations see "Material and methods"; M = male, F = female, sa = subadult.

| Reg.-No. | SAMA R71647 | ZMB 91129 | ZMB 91130 | SAMA R71648 | SAMA R65069 | SAMA R65070 | SAMA R65071 | Mean ± SD |
|---|---|---|---|---|---|---|---|---|
| Sex | F | M | M | M | M | M | sa F | |
| SUL | 34.3 | 36.9 | 41.0 | 36.2 | 37.3 | 34.5 | 35.1 | 36.47 ± 2.30 |
| TL | 15.0 | 17.0 | 18.6 | 15.3 | 15.8 | 15.3 | 15.4 | 16.06 ± 1.30 |
| TaL | 10.0 | 11.8 | 12.6 | 10.2 | 11.0 | 10.3 | 9.9 | 10.83 ± 1.03 |
| T4L | 16.9 | 17.6 | 19.1 | 15.2 | 16.9 | 15.1 | 15.5 | 16.61 ± 1.46 |
| T4D | 1.3 | 1.6 | 1.5 | 1.3 | 1.5 | 1.4 | 1.3 | 1.41 ± 0.12 |
| T1D | 0.8 | 1.0 | 1.0 | 0.9 | 1.0 | 0.9 | 0.9 | 0.93 ± 0.076 |
| F3L | 6.3 | 8.1 | 9.1 | 7.0 | 7.8 | 6.7 | 7.2 | 7.46 ± 0.82 |
| F3D | 0.8 | 1.0 | 1.1 | 0.8 | 0.8 | 0.9 | 0.9 | 0.93 ± 0.076 |
| F1D | 0.7 | 0.9 | 1.0 | 0.6 | 0.7 | 0.8 | 0.7 | 0.77 ± 0.14 |
| HL | 9.5 | 10.4 | 12.0 | 9.7 | 11.2 | 10.1 | 10.3 | 10.46 ± 0.87 |
| HW | 11.9 | 14.6 | 16.0 | 13.1 | 12.7 | 11.4 | 13.1 | 13.26 ± 1.58 |
| END | 2.5 | 3.0 | 3.6 | 2.6 | 3.5 | 3.4 | 3.0 | 3.09 ± 0.43 |
| IND | 1.7 | 2.3 | 2.6 | 2.2 | 2.4 | 2.3 | 2.2 | 2.24 ± 0.28 |
| SL | 4.0 | 4.3 | 5.1 | 4.2 | 5.0 | 4.7 | 4.7 | 4.57 ± 0.42 |
| EST | 3.6 | 4.1 | 5.0 | 3.5 | 4.6 | 4.7 | 4.2 | 4.24 ± 0.56 |
| ED | 2.4 | 2.7 | 2.8 | 2.3 | 2.4 | 2.2 | 2.1 | 2.41 ± 0.25 |
| TyD | 2.4 | 2.5 | 2.1 | 2.0 | 2.1 | 1.8 | 1.9 | 2.11 ± 0.25 |
| TL/SUL | 0.44 | 0.46 | 0.45 | 0.42 | 0.42 | 0.44 | 0.44 | 0.44 ± 0.015 |
| TaL/SUL | 0.29 | 0.32 | 0.31 | 0.28 | 0.29 | 0.30 | 0.28 | 0.30 ± 0.015 |
| T4L/SUL | 0.49 | 0.48 | 0.47 | 0.42 | 0.45 | 0.44 | 0.44 | 0.46 ± 0.025 |
| T4D/SUL | 0.038 | 0.043 | 0.037 | 0.036 | 0.040 | 0.041 | 0.037 | 0.039 ± 0.003 |
| T1D/SUL | 0.023 | 0.027 | 0.024 | 0.025 | 0.027 | 0.026 | 0.026 | 0.025 ± 0.002 |
| F3L/SUL | 0.184 | 0.220 | 0.222 | 0.193 | 0.209 | 0.194 | 0.205 | 0.204 ± 0.014 |
| F3D/SUL | 0.023 | 0.027 | 0.027 | 0.022 | 0.021 | 0.026 | 0.026 | 0.025 ± 0.003 |
| F1D/SUL | 0.020 | 0.024 | 0.024 | 0.017 | 0.019 | 0.023 | 0.020 | 0.021 ± 0.003 |
| T4D/F3D | 1.63 | 1.60 | 1.36 | 1.63 | 1.88 | 1.56 | 1.44 | 1.59 ± 0.165 |
| T1D/F1D | 1.14 | 1.11 | 1.00 | 1.50 | 1.43 | 1.13 | 1.29 | 1.23 ± 0.183 |
| HL/SUL | 0.28 | 0.28 | 0.29 | 0.27 | 0.30 | 0.29 | 0.29 | 0.29 ± 0.009 |
| HW/SUL | 0.35 | 0.40 | 0.39 | 0.36 | 0.34 | 0.33 | 0.37 | 0.36 ± 0.026 |
| HL/HW | 0.80 | 0.71 | 0.75 | 0.74 | 0.88 | 0.89 | 0.79 | 0.79 ± 0.069 |
| END/SUL | 0.073 | 0.081 | 0.088 | 0.072 | 0.094 | 0.099 | 0.085 | 0.085 ± 0.010 |
| IND/SUL | 0.050 | 0.062 | 0.063 | 0.061 | 0.064 | 0.067 | 0.063 | 0.061 ± 0.005 |
| END/IND | 1.47 | 1.30 | 1.38 | 1.18 | 1.46 | 1.48 | 1.36 | 1.38 ± 0.109 |
| ED/SUL | 0.070 | 0.073 | 0.068 | 0.064 | 0.064 | 0.064 | 0.060 | 0.066 ± 0.004 |
| TyD/SUL | 0.073 | 0.068 | 0.051 | 0.055 | 0.056 | 0.052 | 0.054 | 0.058 ± 0.009 |
| TyD/ED | 1.00 | 0.93 | 0.75 | 0.87 | 0.88 | 0.82 | 0.90 | 0.88 ± 0.079 |
| SL/SUL | 0.117 | 0.117 | 0.124 | 0.116 | 0.134 | 0.136 | 0.134 | 0.125 ± 0.009 |
| EST/SUL | 0.105 | 0.111 | 0.122 | 0.097 | 0.123 | 0.136 | 0.120 | 0.116 ± 0.013 |


**Figure 2.** *Xenorhina lacrimosa* sp. nov. paratypes in life. Dorsolateral views: (**a**) SAMA R65070; (**b**) ZMB 91129; (**c**) ZMB 91130; (**d**) SAMA R71647. Ventral views: (**e**) SAMA R65070S; (**f**) ZMB 91129; (**g**) SAMA R65071; (**h**) SAMA R71647.

**Vocalisation.** One call series from SAMA R71648 (holotype), one from ZMB 91129 (paratype), one from SAMA R65069 (paratype) and four from ZMB 91130 (paratype) recorded at air temperatures of 21.2–25.5 °C were analysed. Each call is a single, unpulsed mournful note that is always produced in a series, during which both volume and pitch increase gradually between first and last call (Fig. 3a). Despite some variation in recording temperature, all recorded calls are extremely similar, so they were pooled for analyses. Call series last 26.4–60.4 s (mean 40.0 ± 11.8 s, n = 7), with 7–12 calls/series (mean 9.0 ± 2.2, n = 7) produced at a rate of 0.20–0.27 calls/s (mean 0.23 ± 0.02). Call length is 141–231 ms (mean 193.5 ± 19.1 ms, n = 63) and first and last call in a series are often the shortest; inter-call interval length is 2.8–8.0 s. (mean 4.8 ± 1.0 s, n = 56). Calls start abruptly at high amplitude that rises quickly to a maximum, then decreases gradually until termination of call (Fig. 3b). All calls have 5–7 harmonics (Fig. 3c and 3d)). Dominant frequency may be carried by a second harmonic (i.e. first two calls of series from holotype, with peak at 1.2 kHz) or by first harmonic (all other calls, with peaks increasing from 0.7 kHz in third call to 0.8 kHz in last call in the series. Frequency declines at end of each call in second half of series (Fig. 3c).

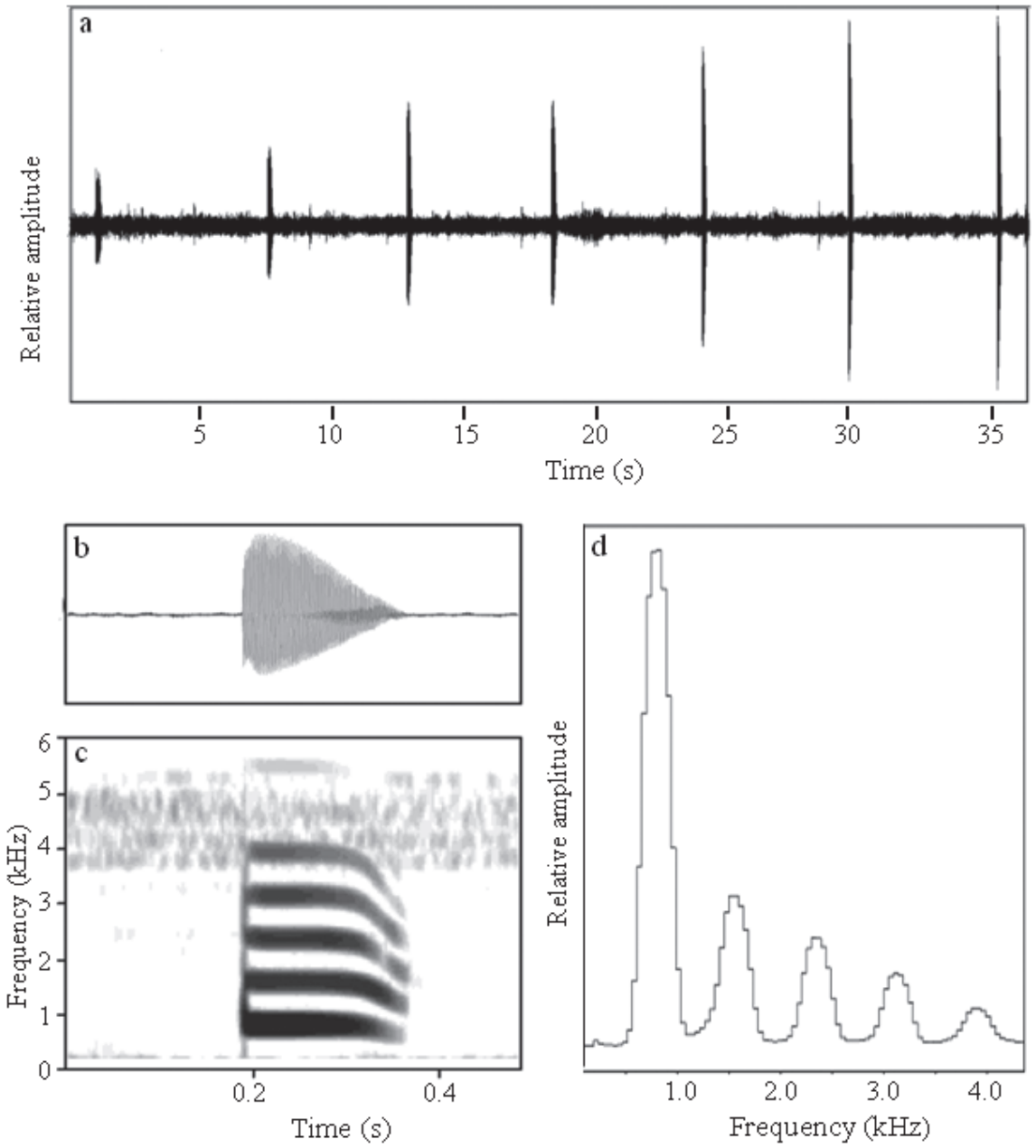


**Figure 3.** (**a**) Oscillogram of a complete advertisement call series with seven calls from the holotype of *Xenorhina lacrimosa* sp. nov.; (**b**) oscillogram and (**c**) spectrogram of the penultimate call of the call series shown on Fig. 3a; (**d**) amplitude spectrum of the call on (**b.**) and (**c.**).

**Etymology.** The specific epithet *lacrimosa* is a Latin adjective in female gender; translated literally it means "tearful", but it is also translated as "lamentable voice" and refers to the mournful sounding advertisement call of the new species.

**Comparisons with other species.** We compare *Xenorhina lacrimosa* sp. nov. with all congeners of a similar size (SUL 30–43 mm) that have a single spike on each vomeropalatine bone.

*Xenorhina fuscigula* (Blum & Menzies, 1989) has hind legs shorter (TL/SVL < 0.40 vs. > 0.40 in *Xenorhina lacrimosa* sp. nov.), eye-naris distance shorter (END/SVL 0.064–0.074 vs. 0.072–0.099), inner metatarsal tubercle absent (vs. present), ventral surfaces black (vs. orange-red or grey-brown) and call consisting of a single long note (vs. a series of 7–12 notes = calls).

*Xenorhina huon* (Blum & Menzies, 1989) is smaller (SUL to 32 mm vs. 34.3–41.0 mm), with hind legs shorter (TL/SUL < 0.40 vs. > 0.40), internarial distance greater (0.064–0.081 vs. 0.050–0.067), eyes larger (ED/SVL 0.070–0.091 vs. 0.060–0.073) and ventral surfaces with dark flecking (vs. ventral surfaces with no or sparse brownish reticulation).

*Xenorhina subcrocea* (Menzies & Tyler, 1977) is smaller (SUL 30.5–33.3 mm vs. 34.3–41.0 mm), with hind legs longer (TL/SVL > 0.46 vs. < 0.46 in *Xenorhina lacrimosa* sp. nov.) and ventral surfaces with dark reticulation (vs. without dark reticulation); call length is shorter 64–69 ms (vs. 141–231 ms), with inter-call interval also much shorter (154–285 ms vs. 2.8–8.0 s).

*Xenorhina zweifeli* (Kraus & Allison, 2002) is about the same size and has similar body ratios. It differs by having a conspicuous dark brown supratympanic stripe (vs. absent in *Xenorhina lacrimosa* sp. nov.) and in several aspects of its advertisement calls. *Xenorhina zweifeli* utters single calls at irregular intervals, with two or three calls sometimes produced in quick succession (Kraus and Allison 2002), during both day and early evening. In contrast, *Xenorhina lacrimosa* sp. nov. always produces calls in discrete series of at least seven relatively evenly spaced calls of increasing pitch and volume; calls are never produced in quick succession and males always call at night. Other differences include: mean length of calls produced by holotype of *X. zweifeli* is 310 ms (Kraus and Allison 2002) (vs. mean length of calls from *Xenorhina lacrimosa* sp. nov. 194 ms); the fundamental frequency of *zweifeli* calls is at 610 Hz and dominant frequency at 1910 Hz (third harmonic), (vs. fundamental and dominant frequency of *Xenorhina lacrimosa* sp. nov., both at 800 Hz); amplitude of *X. zweifeli* calls rises more slowly than that of *lacrimosa* calls and all harmonics are frequency modulated, with pitch decreasing during entire length of call (vs. frequency modulation only occurring at end of harmonics in *Xenorhina lacrimosa* sp. nov. calls). Moreover, *X. zweifeli* occurs only on two mountain ranges in northern Papua New Guinea, while *Xenorhina lacrimosa* sp. nov. lives predominantly in the lowlands and foothills of southern Papua New Guinea. Therefore, the known distributions of the two species are separated by a major biogeographic barrier, New Guinea's central cordillera.

### *Xenorhina perexigua* sp. nov.

http://zoobank.org/96AFDF65-2D60-4245-AB15-BDA2728A6A88

**Holotype.** SAMA R71645 (SJR 10418), adult male, from Camp 2, upper Strickland River basin, Western Province, Papua New Guinea (5.9018°S, 142.4360°E; 950 m a.s.l.), collected by S.J. Richards on 20-02-2008

**Diagnosis.** This species of *Xenorhina* is characterised by the unique combination of: very small body size (SUL of the only adult male 16.7 mm); vomeropalatines each with a single triangular spike; legs moderately long (TL/SUL 0.46); all fingers and first toe without and toes 2–5 with expanded discs; eye-naris distance greater than internarial distance (END/IND 1.27); tympanum smaller than eye (TyD/ED 0.77); dorsal surfaces in life beige brown (RAL 8024) with darker areas on upper flanks, in middle of back and on neck; lower flanks with whitish spots and reticulations and some irregular dark brown flecks; supratympanic area with dark brown fleck; ventral surfaces off-white with extensive blackish-brown reticulation. Advertisement calls in series containing about 30 soft "popping" calls of 30–40 ms duration, produced at a rate of 6.8–6.9 calls/s.

**Description of the holotype.** Measurements and ratios are presented in Table 2. Body squat (Fig. 4a and b), head broader than long (HL/HW 0.84); snout strongly acuminate from above and below and protruding in profile; tongue broad, only its lateral and posterior edges free; prepharyngeal ridge without denticles; a single tri-

**Table 2.** Body measurements and body ratios of the male holotype (SAMA R71645) of *Xenorhina perexigua* sp. nov.. All measurements in mm; for explanation of abbreviations see "Material and methods".

| Reg.-No. | SAMA R71645 | Reg.-No. | SAMA R71645 |
|---|---|---|---|
| SUL | 16.7 | TL/SUL | 0.46 |
| TL | 7.6 | TaL/SUL | 0.30 |
| TaL | 5.0 | T4L/SUL | 0.45 |
| T4L | 7.5 | T4D/SUL | 0.036 |
| T4D | 0.6 | T1D/SUL | 0.018 |
| T1D | 0.3 | F3L/SUL | 0.186 |
| F3L | 3.1 | F3D/SUL | 0.021 |
| F3D | 0.35 | F1D/SUL | 0.012 |
| F1D | 0.2 | T4D/F3D | 1.71 |
| HL | 4.6 | T1D/F1D | 1.50 |
| HW | 5.5 | HL/SUL | 0.28 |
| END | 1.4 | HW/SUL | 0.33 |
| IND | 1.1 | HL/HW | 0.84 |
| SL | 2.2 | END/SUL | 0.084 |
| EST | 2.0 | IND/SUL | 0.066 |
| ED | 1.3 | END/IND | 1.27 |
| TyD | 1.0 | ED/SUL | 0.078 |
| | | TyD/SUL | 0.060 |
| | | TyD/ED | 0.77 |
| | | SL/SUL | 0.132 |
| | | EST/SUL | 0.120 |


**Figure 4.** Holotype (SAMA R71645) of *Xenorhina perexigua* sp. nov. in life: (**a**) Dorsolateral view; (**b**) Ventral view; (**c**) Volar view of right hand; (**d**) Thenar view of right foot.

angular spike of moderate size on each vomeropalatine; loreal region oblique, no canthus rostralis; nostrils near tip of snout, directed dorsolaterally, visible from above, but not from below; eye-naris distance greater than internarial distance (END/IND 1.27); greater part of tympanum visible in life and preservative, its diameter smaller than that of eye (TyD/ED 0.77); supratympanic fold short, not contacting posterior edge of eye and not reaching insertion of fore leg; shank of moderate length (TL/SUL 0.46); fingers moderately short, not webbed; tips of fingers not wider than penultimate phalanges, all with circum-marginal grooves that extend along entire length of digits, relative lengths of fingers 3 > 4 > 2 = 1 (Fig. 4c); all toe tips with circum-marginal grooves, all tips, except that of toe 1 wider than penultimate phalanges; toes not webbed, relative lengths 4 > 3 > 5 > 2 > 1 (Fig. 4d); plantar and palmar tubercles (with exception of small, but prominent inner metatarsal tubercle), as well as subarticular tubercles scarcely visible. Dorsal surfaces with only a few tubercles and a raised mid-dorsal ridge, ventral surfaces smooth (Fig. 4b).

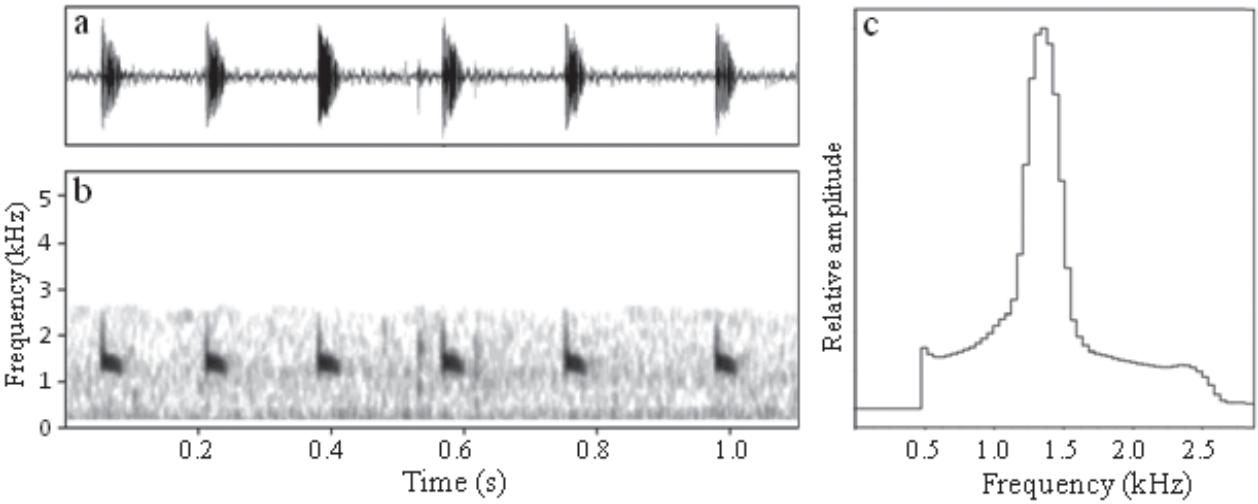


**Figure 5.** (**a**) Oscillogram and (**b**) Spectrogram of the last six calls of a call series containing 31 calls from the holotype of *Xenorhina perexigua* sp. nov.; (**c**) Amplitude spectrum of an advertisement call from the holotype of *X. perexigua* sp. nov.

In life, dorsal surfaces beige brown with darker areas on upper flanks, in middle of back and in scapular region (Fig. 4a); lower flanks with whitish spots and whitish reticulations and three (left side of body) or four (right side of body) irregular dark brown flecks which merge with dark brown reticulum of abdomen; supratympanic area with conspicuous dark brown fleck; all ventral surfaces light grey with dense dark brown reticulations (Fig. 4b); lumbar spot absent; vent and adjacent areas of thighs enclosed in large, triangular dark brown patch, borders of which disintegrate ventrally; outer margin of iris blackish, inner margin golden.

In preservative, dorsal surfaces reddish-brown, flanks with dark irregular spots and supratympanic region with large, dark brown fleck; ventral surfaces ivory-white with brown beige (RAL 1011) reticulum; large ivory-white area between eye and insertion of fore-leg present (not evident in life).

**Distribution and ecological notes.** *Xenorhina perexigua* sp. nov. is known only from one locality, in hill forest at an altitude of 950 m a.s.l. in the upper Strickland River basin of south-western Papua New Guinea (Fig. 8). The holotype was calling from within leaf litter on the forest floor at night during rain.

**Vocalisation.** Two call series, produced by the holotype (SAMA R71645) at an air temperature of 21.2 °C, were analysed. Each call is a single soft, unpulsed "pop" note uttered in rapid succession (Fig. 5a). The two series lasted 4.1 s and 4.5 s and contained 28 and 31 calls produced at a rate of 6.8 and 6.9 calls/s. Call length 29–42 ms (mean 34.6 ± 3.6 ms, n = 59) and inter-call interval 101–195 ms (mean 115.0 ± 17.1 ms, n = 57). While calls are of approximately equal length throughout each series, inter-call intervals are slightly longer at the end of call series than at the beginning. Volume of calls increases during course of series, as is typical for many *Xenorhina* species. Calls start abruptly at high amplitude, which then decreases at an irregular rate until the end of each call (Fig. 5a). The start of each call also has a broad frequency range that drops rapidly to a more narrowly defined, frequency-modulated band (Fig. 5b). Fundamental and dominant frequency peak at 1.4 kHz (Fig. 5c).

**Etymology.** The specific epithet *perexigua* is a Latin adjective of feminine gender, meaning very small (translation of perexiguus, -a, -um in the Dictionarium latino-germanicum means "sehr klein") and refers to the diminutive size of the new species.

**Comparisons with other species.** Although this species is represented by only a single specimen, it is an adult male of very small size (16.7 SUL mm) and, given knowledge about the size ranges of congeners, its SUL is unlikely to exceed 25 mm. We, therefore, compare *Xenorhina perexigua* sp. nov. with all congeners of a similar size (SUL 15–25 mm) that have a single spike on each vomeropalatine.

*Xenorhina anorbis* (Blum & Menzies, 1989) is larger (holotype is an adult male with SVL of 21.3 mm [range of type series 21.3–23.4 mm but sex of other specimens not specified] vs. SUL 16.7 mm in one male), has hind legs shorter (TL/SVL < 0.38 vs. > 0.38) and discs of fingers and toes not wider than penultimate phalanges (vs. discs on toes 2–5 clearly wider than penultimate phalanges in *Xenorhina perexigua* sp. nov.).

*Xenorhina brachyrhyncha* Kraus, 2011 appears to be larger (two adult females with SVL 21.2 and 22.8 mm vs. SUL 16.7 mm in one male), with snout blunt in dorsal and ventral view (vs. strongly acuminate), head wider and longer (HW/SVL 0.35–0.38 vs. 0.32 and HL/SVL 0.30–0.32 vs. 0.28) with much lower ratio of eye-naris distance to internarial distance (END/IND 1.06–1.13 in *X. brachyrhyncha* vs. 1.27 in *Xenorhina perexigua* sp. nov.); differences in colour include lack of a dark supratympanic spot in *X. brachyrhyncha* (vs. present in *Xenorhina perexigua* sp. nov.) and less pronounced dark reticulation on all ventral surfaces.

*Xenorhina lanthanites* is larger SUL 21.3–22.4 mm vs. SUL 16.7 mm), with tips of toes wider than penultimate phalanges only on 4th toe (vs. toes 2–5 with expanded terminal discs); ratio of END/IND lower (0.94–1.20 vs. 1.27); and advertisement call series much longer, lasting up to more than one minute (vs. < 5 s) with average call length of 121 ms (vs. 35 ms in *Xenorhina perexigua* sp. nov.), dominant frequency of about 1.0 kHz (vs. 1.4 kHz) and call repetition rate of 1–2 calls/s (vs. 6.8–6.9 calls/s).

Although it is known from just one specimen, it is an adult male suggesting that *Xenorhina perexigua* sp. nov. is amongst the smallest known members of the genus. Only one other species, *X. bouwensi*, may be smaller than *Xenorhina perexigua* sp. nov., but it can be immediately distinguished from the new species by its lacking odontoid spikes on the vomeropalatines.

### *Xenorhina pohleorum* sp. nov.

http://zoobank.org/91F9054A-3CF0-4672-8A86-F64F6A2BA7AA

**Holotype.** SAMA R71644 (SJR 14202), adult male, from Rentoul River, Western Province, Papua New Guinea (6.4355°S, 142.5615°E; 380 m a.s.l.), collected on 11-08-2014 by S.J. Richards and K. Aplin.

**Paratype.** SAMA R60217 (SJR 3223), adult male, Darai Plateau, Gulf Province, Papua New Guinea (7.1295°S, 143.6134°E; 435 m a.s.l.), collected on 1-08-2003 by S.J. Richards.

**Diagnosis.** This species of *Xenorhina* is characterised by the unique combination of: small size (SUL of two adult males 20.3 and 21.2 mm); vomeropalatines each with a single moderately developed triangular vomerine spike; legs of medium length (TL/SUL 0.44 in both specimens); all fingers without and all toes with, expanded terminal discs; tips of all fingers and toes with circum-marginal grooves, all grooves extending at least partly along digits; head short (HL/SUL 0.26 in both specimens); eye-naris distance greater than internarial distance (END/IND 1.33 in both specimens); dorsal surfaces in life brown-beige (RAL 1011) or grey-brown; ventral surfaces ivory-white with extensive pale brown (RAL 8025) reticulation; mid-dorsal line and lumbar spots absent; advertisement calls uttered in series lasting 4–9 s, containing 10–30 "piping" calls, each 56–93 ms duration with repetition rate of 2.5–3.6 calls/s.

**Description of the holotype.** Measurements are summarised in Table 3. Body squat (Fig. 6a and b), head broader than long (HL/HW 0.83); snout short (HL/SUL 0.26), strongly acuminate from above and below, protruding in profile; tongue broad, only its lateral edges and posterior lobes free; prepharyngeal ridge with few tiny denticles; vomerine spikes triangular and of moderate size; loreal region oblique, canthus rostralis absent; nostrils near tip of snout, directed more laterally than dorsally, visible from above, but not from below; eye-naris distance significantly greater than internarial distance (END/IND 1.33); tympanum nearly as large as eye (TyD/ED 0.92); supratympanic fold weakly expressed, not reaching eye or insertion of fore leg; shank moderately long (TL/SUL 0.44); fingers moderately short, not webbed, all fingers without and all toes with expanded terminal discs; circum-marginal grooves on all fingers and all toes, extending at least partly along most digits; head short (HL/SUL 0.26); eye-naris distance greater than internarial distance (END/IND 1.33); tympanum slightly larger than half the size of eye (TyD/ED 0.59); relative lengths of fingers 3 > 4 = 2 = 1 (Fig. 6c); toes not webbed, relative lengths 4 > 3 > 5 > 2 > 1 (Fig. 6d); plantar and palmar tubercles, as well as subarticular tubercles, not clearly demarcated, with the exception of small, but prominently raised inner metatarsal tubercle (Fig. 6d). Dorsolateral surfaces of body and dorsal surfaces of shanks with some tubercles, more conspicuous in life than in preservative; ventral surfaces smooth; tip of snout lighter than surrounding skin, with some tiny depressions.

**Table 3.** Body measurements and body ratios of the male holotype of *Xenorhina pohleorum* sp. nov. (SAMA R71644) and the male paratype (SAMA R60217). All measurements in mm; for explanation of abbreviations see "Material and methods".

| Reg.-No. | SAMA R71644 | SAMA R60217 | Mean |
|---|---|---|---|
| SUL | 20.3 | 21.2 | 20.75 |
| TL | 9.0 | 9.4 | 9.20 |
| TaL | 5.9 | 5.7 | 5.80 |
| T4L | 9.0 | 9.7 | 9.35 |
| T4D | 0.7 | 0.7 | 0.70 |
| T1D | 0.4 | 0.4 | 0.40 |
| F3L | 3.6 | 3.8 | 3.70 |
| F3D | 0.4 | 0.4 | 0.40 |
| F1D | 0.3 | 0.3 | 0.30 |
| HL | 5.3 | 5.5 | 5.40 |
| HW | 6.4 | 6.5 | 6.45 |
| END | 1.6 | 1.7 | 1.65 |
| IND | 1.2 | 1.3 | 1.25 |
| SL | 2.6 | 3.0 | 2.80 |
| EST | 2.2 | 2.5 | 2.40 |
| ED | 1.3 | 1.2 | 1.25 |
| TyD | 1.2 | 0.9 | 1.05 |
| TL/SUL | 0.44 | 0.44 | 0.44 |
| TaL/SUL | 0.29 | 0.27 | 0.28 |
| T4L/SUL | 0.44 | 0.46 | 0.45 |
| T4D/SUL | 0.035 | 0.033 | 0.034 |
| T1D/SUL | 0.020 | 0.019 | 0.020 |
| F3L/SUL | 0.178 | 0.179 | 0.179 |
| F3D/SUL | 0.020 | 0.019 | 0.020 |
| F1D/SUL | 0.015 | 0.017 | 0.016 |
| T4D/F3D | 1.75 | 1.75 | 1.75 |
| T1D/F1D | 1.33 | 1.33 | 1.33 |
| HL/SUL | 0.26 | 0.26 | 0.26 |
| HW/SUL | 0.32 | 0.31 | 0.32 |
| HL/HW | 0.83 | 0.85 | 0.84 |
| END/SUL | 0.079 | 0.080 | 0.080 |
| IND/SUL | 0.059 | 0.061 | 0.060 |
| END/IND | 1.33 | 1.31 | 1.32 |
| ED/SUL | 0.064 | 0.057 | 0.061 |
| TyD/SUL | 0.059 | 0.042 | 0.051 |
| TyD/ED | 0.92 | 0.75 | 0.84 |
| SL/SUL | 0.129 | 0.142 | 0.136 |
| EST/SUL | 0.109 | 0.118 | 0.114 |

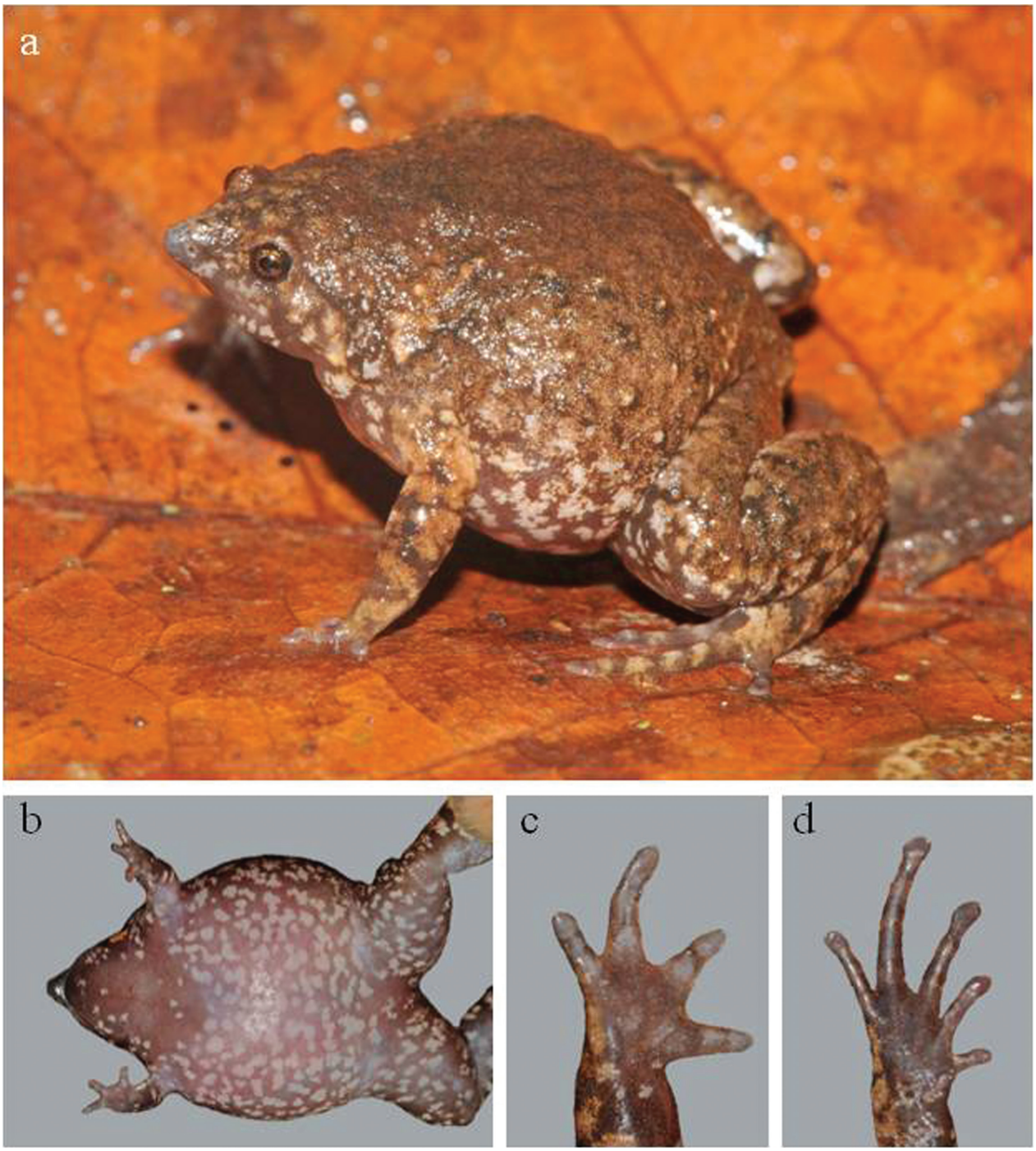


**Figure 6.** Holotype (SAMA R71644) of *X. pohleorum* sp. nov. in life: (**a**) Dorsolateral view; (**b**) Ventral view; (**c**) Volar view of right hand; (**d**) Thenar view of right foot.

In life, dorsal surfaces brown beige (RAL 1011); lumbar spots and mid-dorsal line absent; tubercles with whitish apices concentrated on upper flanks; lower flanks, lateral surfaces of head and anterior hind limbs off-white with conspicuous fawn (RAL 8007) reticulum; snout tip window grey (RAL 7040); iris blackish with few golden specks (Fig. 6a); ventral surfaces pearl-white (RAL 1013) with dusky pink (RAL 3014) reticulum and irregular pearl-white spots; throat dusky pink with only a few whitish spots (Fig. 6b).

In preservative, ground colour of dorsal surfaces of head, back and hind limbs fawn brown (RAL 8007) with some inconspicuous darker areas; head less densely pigmented than adjacent neck; ground colour of dorsal surfaces of fore limbs and anterior hind limbs beige (RAL 1001) with conspicuous terra-brown strikes and reticula; rear of thighs predominantly terra-brown with a few whitish spots below and small blackish area around vent; ventral surfaces fawn-brown with conspicuous pearl-white spots; throat and middle of chest least spotted.

**Morphological variation.** Measurements and body ratios of paratype are similar to holotype (Table 3). Dorsal

surfaces more tubercular in life (Fig. 7), but in preservative, lateral surfaces with fewer tubercles; colour of dorsal surfaces in life a mixture of indistinct lighter and darker grey-brown flecks, lower lateral surfaces of body and upper arms beige-brown (RAL 8024) with off-white spots and ventral surfaces beige-brown with off-white spots. Dorsal surfaces in preservative beige with signal brown (RAL 8002) spots, stripes and reticula; ventral surfaces in preservative paler than holotype, light ivory (RAL 1015) with scarcely visible brownish network.

**Figure 7.** Paratype SAMA R60217 of *X. pohleorum* sp. nov. in life.

**Distribution and ecological notes.** *Xenorhina pohleorum* sp. nov. is known from two localities approximately 140 km apart in the lowland rainforests of Gulf and Western Provinces in south-central Papua New Guinea (Fig. 8), where males called from under the litter or within the humus layer, at night during rain.

**Vocalisation.** Advertisement call is a single short, unpulsed and melodic "piping" note and is always uttered in series. Call length and inter-call interval are variable, but call intervals are always much shorter than the interval between call series. Due to some differences in call features, we analysed five call series from the holotype (SAMA R71644) recorded at an air temperature of 24 °C separately from seven call series produced by the paratype (SAMA R60217) at an air temperature of 22 °C. Call series produced by the holotype last 3.6–8.8 s (mean 5.8 ± 1.8 s) and contain 13–28 calls (mean 18.2 ± 5.6) produced at a rate of 2.55–3.61 calls/s (mean 3.22 ± 0.41, n = 5). Call length is 56–93 ms (mean 74.5 ± 8.5 ms, n = 91) and length of call intervals is 139–528 ms (mean 253.4 ± 71.7 ms, n = 86). Calls start abruptly at maximum or almost maximum amplitude which then decreases at an irregular rate until end of call (Fig. 9a). Fundamental and dominant frequencies are at 1.5 kHz and the only upper harmonic (at 3.0 kHz) has much less energy (Fig. 9b and

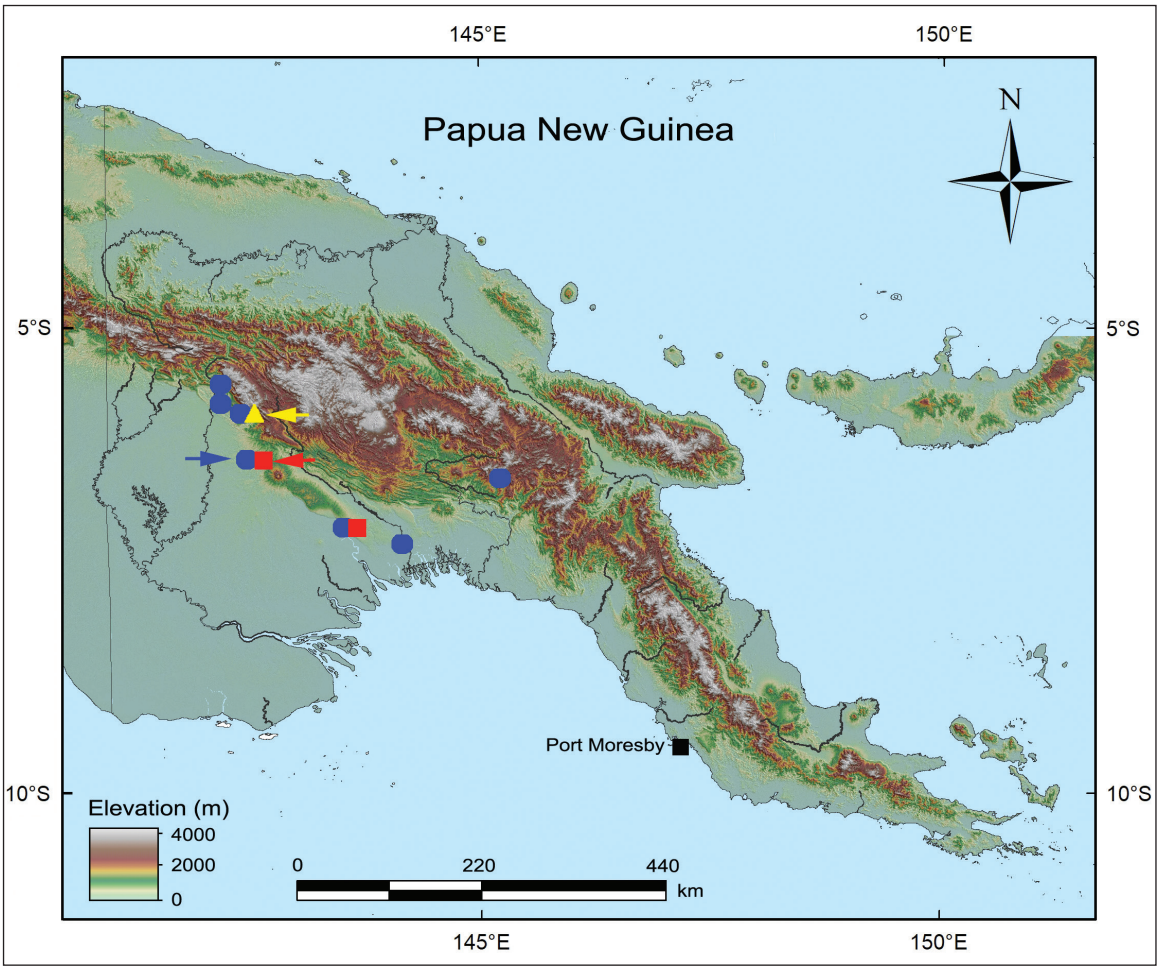


**Figure 8.** Map of Papua New Guinea showing the known distributions of *X. lacrimosa* sp. nov. (blue circles), *X. perexigua* sp. nov. (yellow triangle) and *X. pohleorum* sp. nov. (red squares). Arrows indicate the type localities.

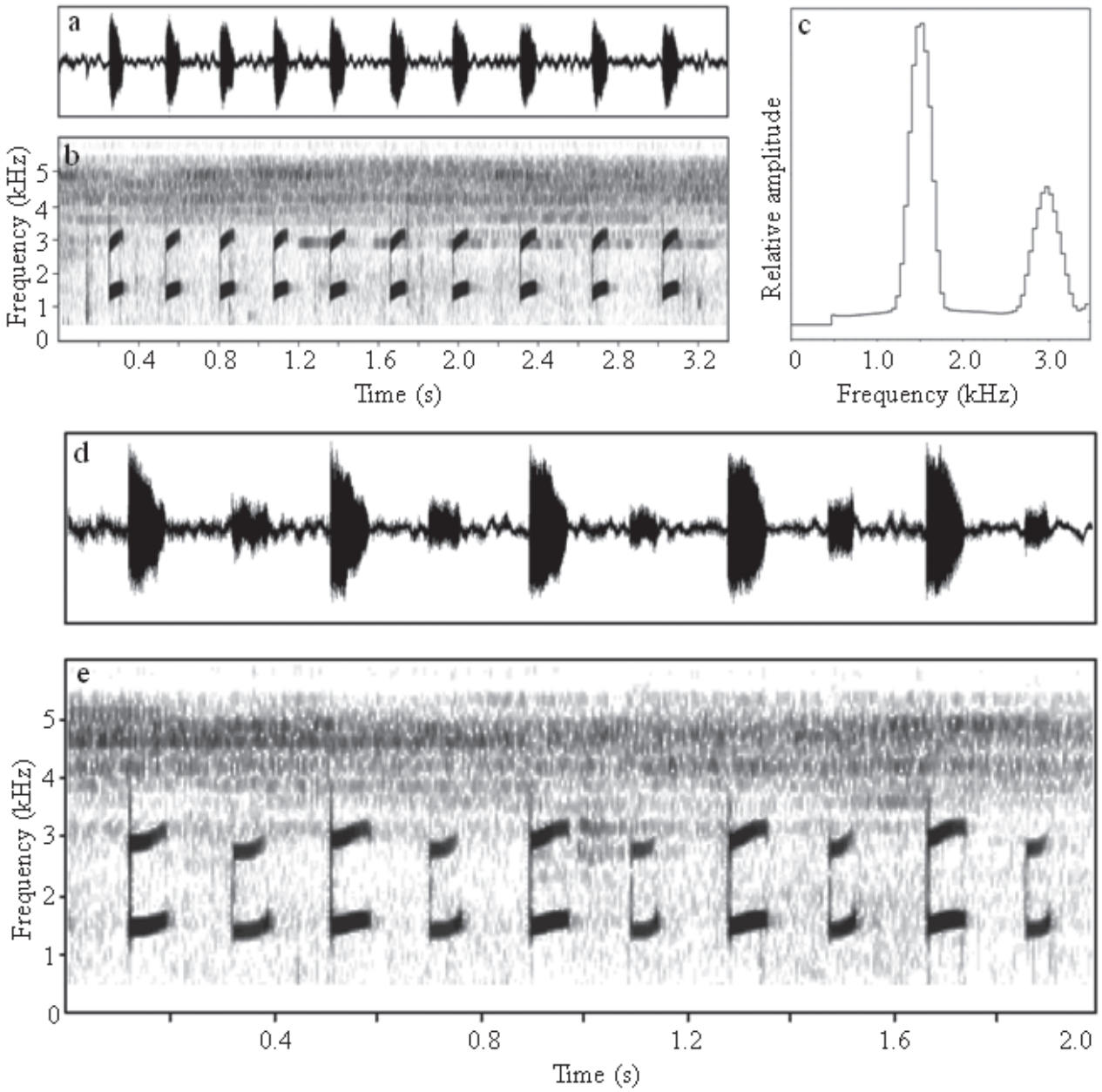


**Figure 9.** (**a**) Oscillogram; (**b**) Spectrogram and (**c**) Amplitude spectrum of 10 consecutive advertisement calls from a longer series produced by the holotype of *Xenorhina pohleorum* sp. nov; (**d**) Oscillogram and (**e**) Spectrogram of six advertisement calls from the holotype of *X. pohleorum* sp. nov. (higher volume) are answered in exact antiphony by an unvouchered male (lower volume).

9c). Frequency of calls is weakly modulated with a slight increase over the duration of the call. A number of calls were uttered in exact antiphony with calls from an unvouchered specimen (Fig. 9d and 9e).

Calls of the paratype (SAMA R60217) are similar to those of the holotype, but call series generally contain fewer calls (10–15, mean 12.9 ± 1.77, n = 7, vs. 13–28 mean 18.2; see above) and so are shorter (3.4–5.4 s, mean 4.6 ± 0.72 s, n = 7 vs. 3.6–8.8 s, mean 5.8 ± 1.8 s), although there is some overlap. Calls of the paratype are also slightly longer (66–98 ms, mean 88.4 ± 4.8 ms, n = 89 vs. 56–93 ms, mean 74.5). Other structural parameters of calls from the paratype fall within the range produced by the holotype: inter-call intervals 234–408 ms (mean 290.0 ± 31.1 ms, n = 83) and mean repetition rate 2.73–3.0 calls/s (mean 2.83 ± 0.10, range, n = 7). Calls of the holotype do not show the typical increase in volume and pitch that is typical of the series produced by the paratype. However, the holotype was calling within a group of closely adjacent males and exhibited antiphonal calling behaviour (Fig. 9d and 9e). It cannot be discounted that the slight differences noted between calls of holotype and paratype were a result of their different calling situations (alone vs. within a chorus).

**Etymology.** The specific epithet *pohleorum* is the Latinised patronymic adjective in genitive plural derived from the family name Pohle. It is to recognise a very

long-lasting friendship of the senior author with Sybille and Claus Pohle from Berlin.

**Comparisons with other species.** We compare *Xenorhina pohleorum* sp. nov. with all congeners of a similar size (SUL 18–25 mm) that have a single spike on each vomeropalatine.

*Xenorhina anorbis* has hind legs shorter (TL/SVL < 0.38 vs. > 0.38) and fingers and toes without expanded terminal discs (vs. enlarged discs on all toes in *Xenorhina pohleorum* sp. nov.).

*Xenorhina brachyrhyncha* has legs longer (TL/SVL 0.46–0.49 vs. twice 0.44), head longer (HL/SVL 0.30–0.32 vs. twice 0.26) and broader (HW/SVL 0.35–0.38 vs. 0.31–0.32), with END/IND ratio lower (1.06–1.13 vs. 1.31–1.33).

*Xenorhina lanthanites* has expanded disc only on 4$^{th}$ toe (vs. on all toes), head broader (HW/SVL 0.35–0.37 vs. 0.31–0.32), eyes larger (ED/SUL 0.071–0.081 vs. 0.057–0.064), END/IND ratio lower (0.94–1.20 vs. 1.31–1.33) and advertisement call series much longer (up to more than one minute vs. less than 10 seconds).

*Xenorhina mehelyi* appears to be much larger (SVL 20.7–35.2 mm vs. 20.3–21.2 mm); although the sex (or state of maturity) of previously reported specimens is unknown, with a male SUL of 20.3–21.2 mm, it is unlikely that *Xenorhina pohleorum* sp. nov. of either sex will approach the upper size limit reported for *X. mehelyi*. *Xenorhina mehelyi* also has eyes larger (ED/SVL 0.067–0.079 vs. 0.057–0.064) and different advertisement calls. Mean call interval 1.5 s, (vs. 0.25 s) and mean call rate 0.60 calls/s (vs. 3.2 calls/s); calls are also longer (mean 140 ms vs. 74.5 ms) and have a much lower dominant frequency (0.88 kHz vs. 1.5 kHz) (Blum and Menzies 1989).

*Xenorhina perexigua* is smaller than *Xenorhina pohleorum* sp. nov. (males 16.7 mm vs. 20.3–21.2 mm SUL). Some body ratios also differ (Tables 2 and 3), but sample sizes are too small for robust comparisons. However, substantial differences in advertisement calls support recognition of *Xenorhina pohleorum* sp. nov. as a distinct species: calls of *Xenorhina perexigua* sp. nov. are shorter (29–42 ms vs. 56–93 ms), there are more calls/series (28–31 vs. 10-28 calls) and inter-call intervals are shorter (101–195 ms vs. 139–528 ms), so the call rate is twice as fast in *Xenorhina perexigua* sp. nov. (6.8–6.9 calls/s vs. 2.6–3.6 calls/s). The substantially greater call rate of *Xenorhina perexigua* sp. nov. (double that of *Xenorhina pohleorum* sp. nov.) cannot be attributed to differences in temperature because the recording temperature for the former was lower than that of latter, which should reduce, not increase, the call rate.

*Xenorhina schiefenhoeveli* (Blum & Menzies, 1989) is larger (SVL 26.7–30.7 mm vs. 20.3–21.2 mm) and its call series lasts more than 100 s (vs. not more than 10 s in *Xenorhina pohleorum* sp. nov.), with call intervals of more than 700 ms (vs. < 528 ms).

*Xenorhina tumulus* (Blum & Menzies, 1989) is larger (male SVL more than 26.0 mm vs. less than 22.0 mm), has ventral surfaces of toes with striped pattern (vs. absent) and abdomen partly pink or red (vs. pearl-white with dusky pink reticulum and irregular pearl-white spots); and supratympanic ridge is absent (vs. present). Advertisement calls of *X. tumulus* differ in, amongst other characters, having a much lower dominant frequency (0.9 kHz vs. 1.5 kHz). *Xenorhina tumulus* is known only from an elevation of about 1500 m a.s.l. in the Adelbert Range, an isolated mountain range near the north coast of Papua New Guinea, while *Xenorhina pohleorum* sp. nov. is known only from altitudes of around 400 m on the southern side of New Guinea's central cordillera.

### *Xenorhina thiekeorum* sp. nov.

http://zoobank.org/772E7466-6C63-48E7-8CAE-96A1BE5F78F5

**Holotype.** SAMA R71651 (SJR 209047), adult male, from Ok Menga near Tabubil, Western Province, Papua New Guinea (5.3205°S, 141.3049°E; 620 m a.s.l.), collected by S.J. Richards, M. Cunningham and A. Dennis on 14-11-1994.

**Paratypes.** ZMB 91131 (SJR 209051), PNGNM (SJR209052), SAMA R71652 (SJR209053), same details as for holotype.

**Diagnosis.** This species of *Xenorhina* is characterised by the unique combination of: moderately small size ( males 20.7–23.5 mm SUL); vomeropalatines each with one moderately developed triangular vomerine spike; legs moderately short (TL/SUL 0.40–0.44); all fingers and first toe without and toes 2–5 with, expanded terminal discs; tips of all fingers and toes with circum-marginal grooves that extend, at least partially, along most digits; head short (HL/SUL 0.26–0.28), eye-naris distance much greater than internarial distance (END/IND 1.36–1.54); tympanum approximately 2/3 size of eye (TyD/ED 0.63–0.69). Dorsal surfaces in life reddish-brown, covered extensively with small, white-tipped tubercles, lower flanks with larger off-white spots; back with faint yellowish mid-dorsal line. Advertisement calls uttered in series containing less than 10 short, extremely soft "piping" calls of 133–162 ms duration, produced at a rate of 2.5–3.0 calls/s.

**Description of the holotype.** Measurements are summarised in Table 4, a dorsolateral view in life is shown in Fig. 10a and ventral surfaces in life in Fig. 10b. Head broader than long (HL/HW 0.81); snout short (HL/SUL 0.27), strongly acuminate from above and below, protruding in profile; tongue long, broad, only its lateral and posterior edges free; prepharyngeal ridge with eight small denticles; vomerine spikes triangular and of moderate size; loreal region oblique, no canthus rostralis; nostrils near tip of snout, directed dorsolaterally, visible from above, but not from below; eye-naris distance significantly greater than internarial distance (END/IND 1.46); borders of tympanum poorly defined in life and preservative, its diameter 2/3 that of eye (TyD/ED 0.67); supratympanic

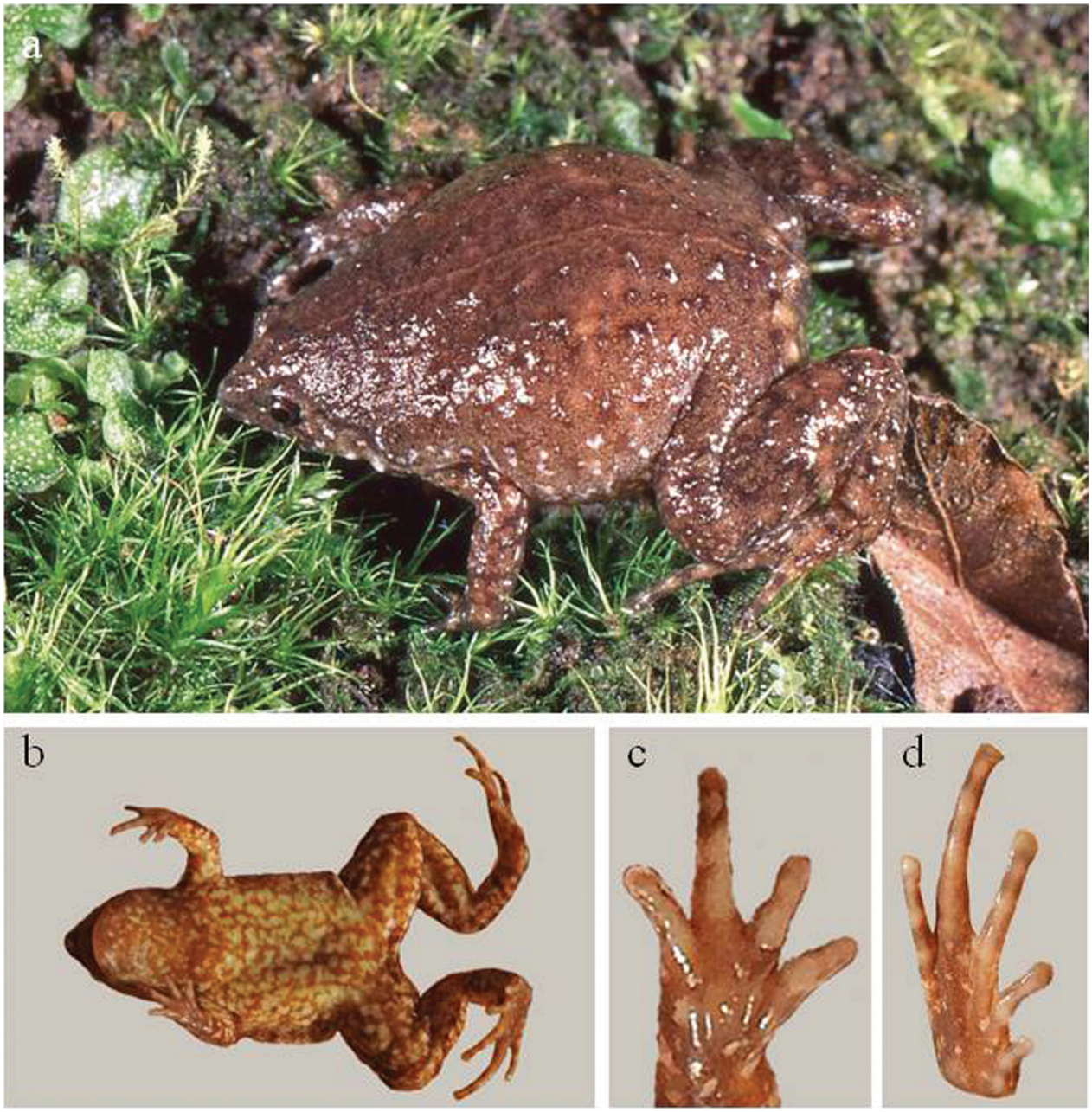


**Figure 10.** Holotype (SAMA R71651) of *Xenorhina thiekeorum* sp. nov. (**a**) Dorsolateral view in life; (**b**) Ventral view, (**c**) Volar view of right hand and (**d**) Thenar view of right foot of preserved specimen.

fold not reaching posterior edge of eye or insertion of fore-leg; shank short (TL/SUL 0.40); fingers moderately short, not webbed; all fingers and first toe without and toes 2–5 with, expanded terminal discs; circum-marginal grooves on tips of all fingers and toes, extending at least partly along most digits; relative lengths of fingers 3 > 4 = 2 = 1 (Fig. 10c); toes not webbed, relative lengths 4 > 3 > 5 > 2 > 1 (Fig. 10d); plantar and palmar tubercles as well as subarticular tubercles poorly defined. Dorsal surfaces with scattered low tubercles and a slightly raised yellowish mid-dorsal ridge, ventral surfaces smooth.

In life, dorsal surfaces brown beige (RAL 1011) with irregularly shaped, indistinct lighter markings in lumbar region and narrow, pale mid-dorsal line; dorsum with numerous small, white-tipped tubercles; lower flanks and anterior and posterior of tympana with whitish spots; dorsal surfaces of limbs and dorsal edge of tympana with few dark brown spots and/or streaks; iris blackish with scarcely visible golden veins and solid golden inner margin. Colour of ventral surfaces in life was not documented.

In preservative, ground colour of dorsal surfaces reddish-brown; dorsolateral surfaces with conspicuous blackish-brown spots, mostly associated with white-tipped tubercles; extremities and anterior back with lighter brown flecks than those on dorsolateral surfaces; solid reddish-brown areas of back merge on lower flanks into ivory-white ground colour of ventral surfaces, which are covered by a dense orange-brown reticulum.

**Morphological variation.** Measurements of the type series are summarised in Table 4. Ground colour of dorsal surfaces in preservative is the same in all types (including holotype), except SAMA R71652 which is slightly paler; number and intensity of brown dorsal and lateral spots varies slightly. Ventral surfaces and rear of thighs in all frogs show a more or less dense orange brown reticulum on ivory-white ground.

**Table 4.** Body measurements and body ratios of the type series of *Xenorhina thiekeorum* sp. nov. SAMA R71651 is the male holotype. ZMB 91131, PNGNM (SJR 209052) and SAMA R71652 are male paratypes. All measurements in mm; for explanation of abbreviations see "Material and methods".

| Reg.-No. | SAMA R71651 | ZMB 91131 | PNGNM | SAMA R71652 | Mean ± SD |
|---|---|---|---|---|---|
| SUL | 23.0 | 20.7 | 23.5 | 22.6 | 22.45 ± 1.22 |
| TL | 9.2 | 9.2 | 10.1 | 9.7 | 9.55 ± 0.44 |
| TaL | 6.4 | 6.5 | 7.3 | 6.5 | 6.68 ± 0.42 |
| T4L | 9.2 | 9.7 | 10.3 | 10.1 | 9.83 ± 0.49 |
| T4D | 0.7 | 0.7 | 0.8 | 0.7 | 0.73 ± 0.05 |
| T1D | 0.4 | 0.4 | 0.5 | 0.5 | 0.45 ± 0.06 |
| F3L | 4.3 | 4.1 | 5.0 | 4.5 | 4.48 ± 0.39 |
| F3D | 0.5 | 0.5 | 0.6 | 0.6 | 0.55 ± 0.06 |
| F1D | 0.3 | 0.3 | 0.5 | 0.4 | 0.38 ± 0.09 |
| HL | 6.1 | 5.8 | 6.4 | 5.9 | 6.05 ± 0.26 |
| HW | 7.5 | 6.9 | 7.7 | 7.3 | 7.35 ± 0.34 |
| END | 1.9 | 1.7 | 2.0 | 1.9 | 1.88 ± 0.13 |
| IND | 1.3 | 1.2 | 1.3 | 1.4 | 1.30 ± 0.08 |
| SL | 2.8 | 2.5 | 2.7 | 2.6 | 2.65 ± 0.13 |
| EST | 2.5 | 2.4 | 2.6 | 2.3 | 2.45 ± 0.13 |
| ED | 1.8 | 1.6 | 1.6 | 1.7 | 1.68 ± 0.09 |
| TyD | 1.2 | 1.0 | 1.1 | 1.1 | 1.10 ± 0.08 |
| TL/SUL | 0.40 | 0.44 | 0.43 | 0.43 | 0.43 ± 0.017 |
| TaL/SUL | 0.28 | 0.31 | 0.31 | 0.29 | 0.30 ± 0.015 |
| T4L/SUL | 0.40 | 0.47 | 0.44 | 0.45 | 0.44 ± 0.029 |
| T4D/SUL | 0.030 | 0.034 | 0.034 | 0.031 | 0.032 ± 0.002 |
| T1D/SUL | 0.017 | 0.019 | 0.021 | 0.022 | 0.020 ± 0.002 |
| F3L/SUL | 0.187 | 0.198 | 0.212 | 0.199 | 0.199 ± 0.010 |
| F3D/SUL | 0.022 | 0.024 | 0.026 | 0.027 | 0.025 ± 0.002 |
| F1D/SUL | 0.013 | 0.014 | 0.017 | 0.018 | 0.016 ± 0.002 |
| T4D/F3D | 1.40 | 1.40 | 1.33 | 1.17 | 1.33 ± 0.108 |
| T1D/F1D | 1.33 | 1.33 | 1.00 | 1.25 | 1.23 ± 0.156 |
| HL/SUL | 0.27 | 0.28 | 0.27 | 0.26 | 0.27 ± 0.008 |
| HW/SUL | 0.33 | 0.33 | 0.33 | 0.32 | 0.33 ± 0.005 |
| HL/HW | 0.81 | 0.84 | 0.83 | 0.81 | 0.82 ± 0.015 |
| END/SUL | 0.083 | 0.082 | 0.085 | 0.084 | 0.084 ± 0.001 |
| IND/SUL | 0.057 | 0.058 | 0.055 | 0.062 | 0.058 ± 0.003 |
| END/IND | 1.46 | 1.42 | 1.54 | 1.36 | 1.45 ± 0.075 |
| ED/SUL | 0.078 | 0.077 | 0.068 | 0.075 | 0.075 ± 0.005 |
| TyD/SUL | 0.052 | 0.048 | 0.047 | 0.049 | 0.049 ± 0.002 |
| TyD/ED | 0.67 | 0.63 | 0.69 | 0.65 | 0.66 ± 0.026 |
| SL/SUL | 0.122 | 0.121 | 0.115 | 0.115 | 0.118 ± 0.004 |
| EST/SUL | 0.109 | 0.116 | 0.111 | 0.102 | 0.110 ± 0.006 |

**Distribution and ecological notes.** *Xenorhina thiekeorum* sp. nov. is known only from the type locality adjacent to the Ok Menga ("Ok" = River in the local Min language), at an altitude of 620 m a.s.l. in the foothills of the Hindenburg Range, Ok Tedi headwaters in Western Province, Papua New Guinea (Fig. 16). The frogs were calling from 1–3 cm beneath the soil surface at the base of ginger plants after rain at night. Unlike many *Xenorhina* species, the distribution of calling males was "clumped"; all four frogs were detected by their calls within an area of approximately 4 $m^2$ of wet hill forest, while none was heard calling in apparently suitable adjacent forest.

**Vocalisation.** Three call series from the holotype (SAMA R71651) recorded at an air temperature of 22.5 °C were analysed, but due to poor recording quality, the lengths of calls and length of call intervals could not be measured for one of these series. Calls are a single, unpulsed "piping" notes produced in short series. Calls are extremely soft and were barely audible to the human ear. Call series contain 6–8 calls produced at a rate of 2.5–3.0 calls/s and last 2.0–2.9 s (mean 2.3 s) (Fig. 11a and b). Call length is 133–162 ms (mean 143.4 ± 8.8 ms, n = 14) and length of call intervals is 168–376 ms (mean 250.6 ± 51.8 ms, n = 12). There are four harmonics with frequency peaks at 1.1, 2.2, 3.3 and 4.4 kHz; the third harmonic carries the dominant frequency (Fig. 11c). Volume and pitch of calls both increase marginally during the course of call series.

**Etymology.** The specific epithet *thiekeorum* is the Latinised patronymic adjective in genitive plural of the family name Thieke. It is given to recognise a very long-lasting friendship of the senior author with Heidi and Ulrich (Uli) Thieke from Berlin.

**Comparisons with other species.** We compare *Xenorhina thiekeorum* sp. nov. with all congeners of a similar size (males with SUL ~ 18–25 mm) that have a single spike on each vomeropalatine.

*Xenorhina anorbis* has hind legs shorter (TL/SVL < 0.38 vs. > 0.38), digital discs on toes absent (vs. expanded discs present on toes 2–5) and END/IND ratio lower (1.26–1.32 vs. 1.36–1.54).

*Xenorhina brachyrhyncha* has legs longer (TL/SVL 0.46–0.49 vs. 0.40–0.44), head longer (HL/SVL 0.30–0.32 vs. 0.26–0.28) and broader (HW/SVL 0.35–0.38 vs. 0.32–0.33) and END/IND ratio much lower (1.06–1.13 vs. 1.36–1.54).

*Xenorhina lanthanites* has legs longer (TL/SUL 0.44–0.46 vs. 0.40–0.44), dilated disc only on 4$^{th}$ toe (vs. dilated discs on toes 2–5), T4D/F3D ratio higher (1.50–2.0 vs. 1.17–1.40), END/IND ratio lower (0.94–1.20 vs. 1.36–1.54) and advertisement call series much longer (up to more than one minute vs. a few seconds), with call intervals longer (397–896 ms vs. 168–376 ms) and repetition rate lower (1.2–1.8 vs. 2.5–3.0 calls/s).

*Xenorhina mehelyi* is probably much larger (SVL to > 35 mm vs. males 20.7–23.5 mm), internarial distance greater (IND/SVL 0.061–0.077 vs. 0.055–0.062) and has different advertisement calls: call series of *X. mehelyi* contain > 10 calls produced at a rate of 0.60 calls/s (vs. < 10 calls produced at a rate of 2.75 calls/s in *Xenorhina thiekeorum* sp. nov.); and dominant frequency is 0.88 kHz in *X. mehelyi* (vs. 3.3 kHz in *X. thiekeorum*).

*Xenorhina perexigua* is smaller (16.7 mm vs. 20.7–23.5 mm SUL) and many body ratios differ from

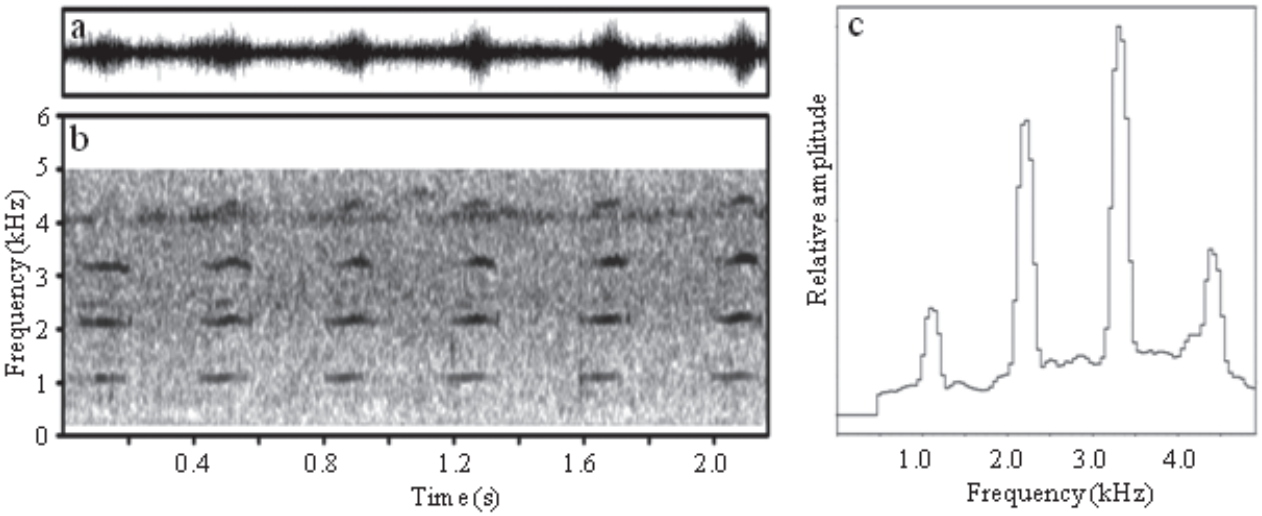


**Figure 11.** (**a**) Oscillogram; (**b**) Spectrogram and (**c**) amplitude spectrum of a series of six calls from the holotype of *Xenorhina thiekeorum* sp. nov.

*Xenorhina thiekeorum* sp. nov. (Tables 2 and 4), but small sample sizes preclude robust comparisons of body ratios. Advertisement calls differ as follows: *Xenorhina perexigua* sp. nov. utters calls in series lasting more than 4 s, containing about 30 calls produced at rate of 6.8–6.9 calls/s (vs. call series lasting 2–3 s containing just 6–8 calls produced at rate of 2.5–3.0 calls/s); call length of *Xenorhina perexigua* sp. nov. is also much shorter (29–42 ms vs. 133–162 ms in *Xenorhina thiekeorum* sp. nov.).

*Xenorhina pohleorum* has fingers shorter (F3L/SUL 0.178–0.179 vs. 0.187–0.212), disc on third finger smaller (F3D/SUL 0.019–0.020 vs. 0.022–0.027), T4D/F3D ratio higher (1.75 vs. 1.17–1.40), END/IND ratio lower (1.31–1.33 vs. 1.36–1.54), eyes smaller (ED/SUL 0.057–0.064 vs. 0.068–0.078) and TyD/ED ratio higher (0.75–0.92 vs. 0.63–0.69). Moreover, call length of *Xenorhina pohleorum* sp. nov. is much shorter (~ 70–90 ms vs. 130–150 ms).

*Xenorhina schiefenhoeveli* is larger (SVL 26.7–30.7 mm vs. 20.7–23.5 mm), with ratio of END/IND lower (1.04–1.33 vs. 1.36–1.54) and different calls; call series last > 100 s (vs. 2–3 s in *Xenorhina thiekeorum* sp. nov.), with call intervals > 700 ms (vs. less than 400 ms).

*Xenorhina tumulus* is larger (SVL > 26.0 mm vs. < 24.0 mm), with internarial distance relatively longer (IND/SVL 0.063–0.069 vs. 0.055–0.062), distance between eye and naris relatively shorter (END/SVL 0.073–0.081 vs. 0.082–0.085), END/IND ratio lower (1.11–1.28 vs. 1.36–1.54) and call length shorter (60–70 ms vs. 133–162 ms).

### *Xenorhina wiegankorum* sp. nov.

http://zoobank.org/DD757B96-EAFD-427B-9844-1BD80D13544C

**Holotype.** SAMA R71653 (SJR 10372), adult male, from Baia River, Western Province, Papua New Guinea (6.0205°S, 142.5473°E; 330 m a.s.l.), collected by S.J. Richards on 15-02-2008.

**Paratypes.** PNGNM (FN SJR10373), adult male, same details as for holotype; SAMA R71654 (FN SJR10400), adult male, from Camp 2, upper Strickland River basin, Western Province, Papua New Guinea (5.9018°S, 142.4360°E; 950 m a.s.l.), collected by S.J. Richards on 19-02-2008; ZMB 91132 (FN SJR14220), adult male, Rentoul River, Western Province, Papua New Guinea (6.4355°S, 142.5615°E; 380 m a.s.l.), collected on 14-08-2014 by S.J. Richards; SAMA R65073 (FN SJR10948), adult male, Gugusu Camp, Muller Range, Western Province (5.7290°S, 142.2630°E; 515 m a.s.l.), collected by S.J. Richards and C. Dahl on 8-09-2009.

**Diagnosis.** This species of *Xenorhina* is characterised by the unique combination of: medium size (males 32.0–35.7 mm SUL); vomeropalatines each with one strongly developed triangular spike; legs moderately long (TL/SUL 0.44–0.47); all fingers tips without and all toe tips with expanded discs; eye-naris distance greater than internarial distance (END/IND 1.19–1.37); tympanum same size as, or slightly smaller than, eye (TyD/ED 0.80–1.00). Dorsal surfaces in life different shades of grey or brown; ventral surfaces different shades of red or yellow, throat and chest with some darker flecks. Advertisement calls uttered in series lasting 10–20 s and containing 20–40 calls; length of calls 60–100 ms, dominant frequency at 0.5 kHz.

**Description of the holotype.** Measurements are summarised in Table 5, a dorsolateral view in life is shown in Fig. 12a and ventral surfaces in life in Fig. 12b. Head broader than long (HL/HW 0.84); snout acuminate from above and below and distinctly protruding in profile; vomerine spikes strongly developed; prepharyngeal ridge clearly expressed with about 14 denticles; tongue long, broad, not bilobed posteriorly; loreal region oblique, no canthus rostralis; nostrils near tip of snout, positioned dorsolaterally, visible from above, but not from below;


**Figure 12.** Holotype (SAMA R71653) of *Xenorhina wiegankorum* sp. nov. in life: (**a**) Dorsolateral view; (**b**) Ventral view; (**c**) Volar view of left hand; (**d**) Thenar view of left foot.

eye-naris distance greater than internarial distance (END/IND 1.37); tympanic annulus more strongly defined in preservative than in life, its diameter smaller than that of eye (TyD/ED 0.80); well defined supratympanic fold extends from marginally behind eye to insertion of fore leg; shank moderately short (TL/SUL 0.44); fingers moderately short, not webbed, tips of all fingers not wider than penultimate phalanges, but with circum-marginal grooves, relative lengths of fingers 3 > 4 > 2 = 1 (Fig. 12c); all toe tips acuminate, but wider than penultimate phalanges, with circum-marginal grooves; toes not webbed, relative lengths 4 > 3 > 5 > 2 > 1 (Fig. 12d); plantar, palmar and subarticular tubercles barely defined. Body laterally and dorsum of legs partly, with scattered small tubercles in life and in preservative; all ventral surfaces smooth; tip of snout (especially ventrally) with several tiny elevations.

In life, all dorsal surfaces almost uniformly light olive-brown (RAL 8008); lumbar spot absent; back with yellowish mid-dorsal line that continues along hind legs on to tarsus; tubercles with whitish apices concentrated mainly on lateral surfaces of body; large dark triangular spot on posterior of thighs around vent absent; iris blackish with golden speckles; ventral surfaces of toes predominantly signal-grey (RAL 7004), plantar surfaces

**Table 5.** Body measurements and body ratios of the type series of *Xenorhina wiegankorum* sp. nov. SAMA R71653 is the male holotype; all others are male paratypes. All measurements in mm; for explanation of abbreviations see "Material and methods".

| Reg.-No. | SAMA R71653 | PNGNM (SJR10373) | ZMB 91132 | SAMA R71654 | SAMA R65073 | Mean ± SD |
|---|---|---|---|---|---|---|
| SUL | 32.4 | 32.0 | 34.9 | 33.1 | 35.7 | 33.62 ± 1.61 |
| TL | 14.4 | 14.9 | 16.0 | 15.5 | 15.6 | 15.38 ± 0.63 |
| TaL | 9.5 | 10.0 | 10.7 | 10.4 | 10.2 | 10.16 ± 0.45 |
| T4L | 14.5 | 15.1 | 16.3 | 15.6 | 16.8 | 15.66 ± 0.92 |
| T4D | 1.2 | 1.3 | 1.2 | 1.3 | 1.4 | 1.28 ± 0.08 |
| T1D | 0.9 | 0.9 | 0.8 | 1.0 | 0.9 | 0.90 ± 0.07 |
| F3L | 6.1 | 6.8 | 6.7 | 7.0 | 7.1 | 6.74 ± 0.39 |
| F3D | 0.8 | 0.9 | 0.7 | 0.8 | 0.8 | 0.76 ± 0.09 |
| F1D | 0.7 | 0.8 | 0.7 | 0.7 | 0.7 | 0.72 ± 0.05 |
| HL | 9.0 | 9.5 | 8.6 | 9.1 | 9.7 | 9.18 ± 0.43 |
| HW | 10.7 | 11.3 | 11.9 | 11.5 | 11.4 | 11.36 ± 0.43 |
| END | 2.6 | 2.7 | 2.7 | 2.5 | 2.5 | 2.60 ± 0.10 |
| IND | 1.9 | 2.1 | 2.2 | 2.1 | 2.0 | 2.06 ± 0.11 |
| SL | 4.1 | 4.2 | 4.5 | 4.7 | 4.5 | 4.40 ± 0.24 |
| EST | 3.9 | 3.8 | 3.9 | 4.0 | 4.1 | 3.94 ± 0.11 |
| ED | 2.0 | 2.1 | 2.2 | 2.2 | 2.2 | 2.14 ± 0.09 |
| TyD | 1.6 | 2.0 | 1.9 | 2.1 | 2.2 | 1.96 ± 0.23 |
| TL/SUL | 0.44 | 0.47 | 0.46 | 0.47 | 0.44 | 0.46 ± 0.015 |
| TaL/SUL | 0.29 | 0.31 | 0.31 | 0.31 | 0.29 | 0.30 ± 0.011 |
| T4L/SUL | 0.45 | 0.47 | 0.47 | 0.47 | 0.47 | 0.47 ± 0.009 |
| T4D/SUL | 0.037 | 0.041 | 0.034 | 0.039 | 0.039 | 0.038 ± 0.003 |
| T1D/SUL | 0.028 | 0.028 | 0.023 | 0.030 | 0.025 | 0.027 ± 0.003 |
| F3L/SUL | 0.188 | 0.213 | 0.192 | 0.211 | 0.199 | 0.201 ± 0.011 |
| F3D/SUL | 0.025 | 0.028 | 0.020 | 0.024 | 0.022 | 0.024 ± 0.003 |
| F1D/SUL | 0.022 | 0.025 | 0.020 | 0.021 | 0.020 | 0.022 ± 0.002 |
| T4D/F3D | 1.50 | 1.44 | 1.71 | 1.63 | 1.75 | 1.61 ± 0.133 |
| T1D/F1D | 1.29 | 1.13 | 1.14 | 1.43 | 1.29 | 1.26 ± 0.124 |
| HL/SUL | 0.28 | 0.30 | 0.25 | 0.27 | 0.27 | 0.27 ± 0.018 |
| HW/SUL | 0.33 | 0.35 | 0.34 | 0.35 | 0.32 | 0.34 ± 0.013 |
| HL/HW | 0.84 | 0.84 | 0.72 | 0.79 | 0.85 | 0.81 ± 0.054 |
| END/SUL | 0.080 | 0.084 | 0.077 | 0.076 | 0.070 | 0.078 ± 0.005 |
| IND/SUL | 0.059 | 0.066 | 0.063 | 0.069 | 0.056 | 0.063 ± 0.005 |
| END/IND | 1.37 | 1.24 | 1.23 | 1.19 | 1.25 | 1.26 ± 0.068 |
| ED/SUL | 0.062 | 0.066 | 0.063 | 0.066 | 0.062 | 0.064 ± 0.002 |
| TyD/SUL | 0.049 | 0.063 | 0.054 | 0.063 | 0.062 | 0.058 ± 0.006 |
| TyD/ED | 0.80 | 0.95 | 0.86 | 0.95 | 1.00 | 0.91 ± 0.080 |
| SL/SUL | 0.127 | 0.131 | 0.129 | 0.142 | 0.126 | 0.131 ± 0.006 |
| EST/SUL | 0.120 | 0.119 | 0.112 | 0.121 | 0.115 | 0.117 ± 0.004 |

brown-grey; ventral surfaces of fingers and palms predominantly signal-grey; abdomen and ventral surfaces of thighs, shanks and arms melon-yellow (similar to RAL 1028) with inconspicuous whitish spots; ground colour of throat and chest also melon-yellow, but overlain with dense pattern of beige-grey and off-white spots.

In preservative, all dorsal surfaces pastel-violet (RAL 4009), with only few darker areas and inconspicuous whitish tubercle apices. Melon-yellow ventral surfaces faded to ivory colour in preservative and pattern on chest and throat changed from beige-grey to brown-beige (RAL 1011).

**Morphological variation.** Morphometric data for all paratypes are similar (Table 5). Colour pattern of ZMB 91132 (and probably of PNGNM [SJR 10373]) in life is similar to holotype. Dorsal surfaces of SAMA R71654 are telegrey (RAL 7045) with small whitish spots (Fig. 13) and ventral surfaces predominantly broom-yellow (RAL 1032). Dorsal surfaces of SAMA R65073 are a mixture of stone-grey (RAL 7030) and brown-grey (RAL 7013)

**Figure 13.** *Xenorhina wiegankorum* sp. nov. paratype SAMA R71654 in dorsolateral view.

reticula interspersed with whitish spots (mainly on lower flanks) and ventral surfaces predominantly zinc-yellow (RAL 1018).

In preservative, ground colour of dorsal surfaces of head and back of all specimens is dark shades of pastel-violet (RAL 4009), with dorsal surfaces of extremities light brown with dark brown stripes and spots. Two paratypes with and two without, light mid-dorsal line. Snout tip grey in all specimens. Part of chest, entire abdomen and ventral surfaces of thighs light ivory; throat and part of chest light ivory overlain by more or less expanded brown-beige areas. Rear of thighs in all type specimens predominantly brown, only a small area around vent blackish.

**Distribution and ecological notes.** *Xenorhina wiegankorum* sp. nov. has a known distribution limited to altitudes of 330–950 m a.s.l. in the foothills of the upper Strickland River catchment in Western Province, south-western Papua New Guinea (Fig. 16). Males called at night from under the litter on the forest floor or from slightly beneath the soil surface, during or immediately after heavy rain.

**Vocalisation.** We analysed one call series from the holotype (SAMA R71653) recorded at an air temperature of 23.7 °C, two call series from paratype SJR 10400 recorded at 21.0 °C and one call series of paratype ZMB 91132 recorded at 25.0 °C. Calls are rather deep, unpulsed "popping" notes that, as is typical for many *Xenorhina* species, increase in volume during the course of the call series. Pitch of calls also increases slightly during the course of each series. Although there is some variation in call length and inter-call interval amongst calls of the three animals recorded, there is high overlap in all call parameters and we have no doubt that all represent the same species. We, therefore, combined the calls for analysis

Calls are of approximately equal length, but inter-call intervals are somewhat variable. A call starts abruptly at high amplitude, which then decreases gradually until end of call (Fig. 14a). There are 2–7 harmonics, though the second is often missing (Fig. 14b and c); fundamental and dominant frequencies are at 0.55 kHz (Fig. 14c). Length

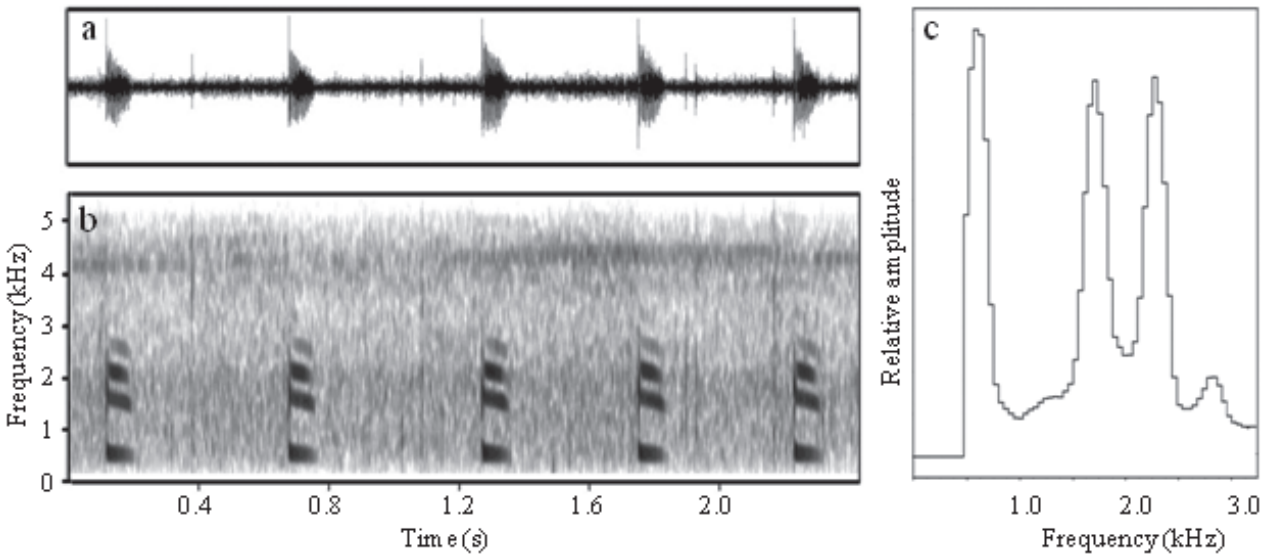


**Figure 14.** (**a**) Oscillogram; (**b**) Spectrogram and (**c**) Amplitude spectrum of the last five calls from a call series containing 29 calls, produced by paratype ZMB 91132 of *Xenorhina wiegankorum* sp. nov.

of call series is 13.8–18.1 s (mean 15.3 s, n = 4); with 22–39 calls per series (mean 28.8, n = 4); call length is 60–104 ms (mean 87.1 ± 6.7 ms, n = 115); intercall interval length is 286–1073 ms (mean 459.6 ± 137.6 ms, n = 111) with call repetition rate of 1.71–2.15 calls/s (mean 1.86 calls/s).

**Etymology.** The specific epithet *wiegankorum* is the Latinised patronymic adjective in genitive plural of the family name Wiegank. It is given to recognise a very long-lasting friendship of the senior author with Ulla and Friedrich-Manfred (Conny) Wiegank from Potsdam.

**Comparisons with other species.** We compare *Xenorhina wiegankorum* sp. nov. with all congeners of a similar size (SUL ~ 28–38 mm) that have a single spike on each vomeropalatine bone.

*Xenorhina fuscigula* has hind legs shorter (TL/SVL < 0.40 vs. > 0.40), eye-naris distance shorter (END/SVL 0.064–0.074 vs. 0.070–0.084) and fourth toe shorter (T4L/SVL 0.34–0.41 vs. 0.45–0.47); advertisement calls of *X. fuscigula* are produced singly (vs. in a long series containing up to 39 calls).

*Xenorhina huon* (Blum & Menzies, 1989) has hind legs shorter (TL/SVL < 0.40 vs. > 0.40), eyes larger (ED/SVL 0.070–0.091 vs. 0.062–0.066) and ventral surfaces with dark flecking (vs. ventral surfaces without dark flecking). *Xenorhina huon* is also known only from mountainous regions 1800–2000 m a.s.l. on the Huon Peninsula, near the north coast of Papua New Guinea (vs. lowlands south of the central cordillera).

*Xenorhina lacrimosa* exhibits considerable overlap in many morphometric characters, but displays extensive variation in dorsal colouration (vs. predominantly brown or grey); vent enclosed in dark brown patch (vs. patch absent) and ventral surfaces deep orange or occasionally grey-brown, with white spots (vs. ventral surfaces at least partially yellow) (Figs 1–2 vs. 12–13); dorsal surfaces also appear less rugose in life (Figs 1–2 vs. 12–13). Advertisement calls are very different: call series of *X. lacrimosa* much longer (26–60 s vs. 12–18 s), with fewer calls (7–12 vs. 22–39), repetition rate much slower (0.20–0.27 vs. 1.70–2.15 calls/s), call length longer (141–231 ms vs. 60 to 104 ms) and call interval longer (2.8–8.0 s vs. 286–1073 ms).

*Xenorhina subcrocea* (Menzies & Tyler, 1977) is smaller (SVL 30.5–33.3 vs. 32.0–35.7), with hind legs longer (TL/SVL > 0.46 vs. < 0.47), ventral surfaces with dark reticulation in preservative (vs. without dark reticulation), call intervals within series shorter (154–285 ms vs. 286–1073 ms), produced at rate of about 4 calls/s (vs. 1.7–2.2 calls/s).

*Xenorhina zweifeli* has similar body size and ratios. It differs from *Xenorhina wiegankorum* sp. nov. by having a conspicuous dark brown supratympanic stripe (vs. absent) and greatly different advertisement calls: *X. zweifeli* utters single calls at long and irregular intervals (Kraus and Allison 2002), with 2–3 calls sometimes uttered in quick succession, during the day and early evening (Kraus and Allison 2002); in contrast, *Xenorhina wiegankorum* sp. nov. produces calls in discrete series with 22–39 calls produced in rapid succession, only at night.

### *Xenorhina woxvoldi* sp. nov.

http://zoobank.org/2F2CA28A-5E2F-485C-911B-E3FD35AF7E27

**Holotype.** SAMA R71646 (SJR10249), adult male, from southern edge of Karius Range, Hela Province, Papua New Guinea (5.9911°S, 142.6707°E; 1,368 m a.s.l.), collected on 07-02-2008 by S.J. Richards.

**Paratype.** ZMB 91133 (SJR 10311), adult male, same collection details as for holotype.

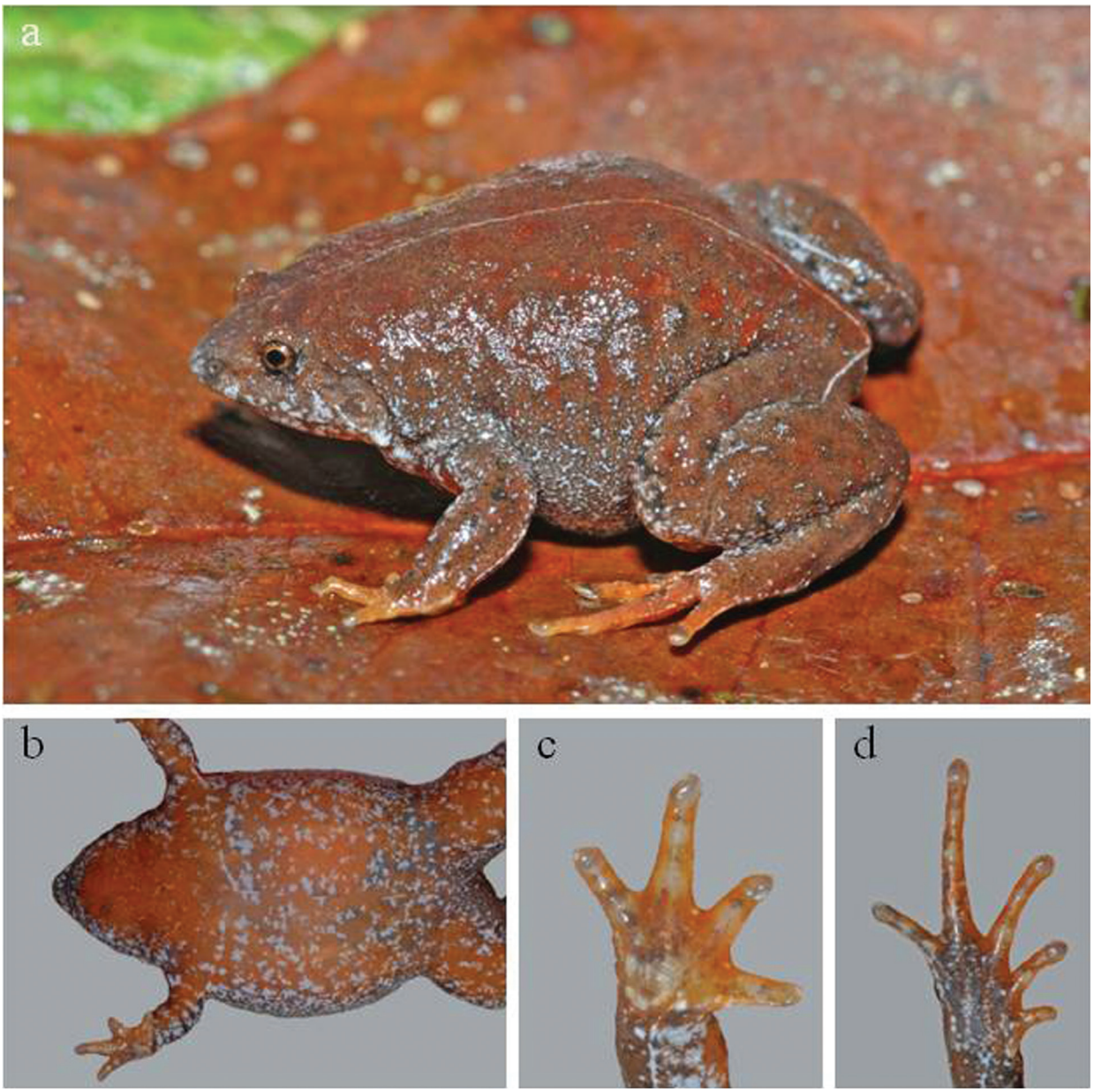


**Figure 15.** Holotype (SAMA R71646) of *X. woxvoldi* sp. nov. in life: (**a**) Dorsolateral view; (**b**) Ventral view; (**c**) Volar view of right hand; (**d**) Thenar view of right foot.

**Diagnosis.** This species of *Xenorhina* is characterised by the unique combination of: small to medium-size (males 28.7–30.1 mm SUL); vomeropalatines each with one moderate-sized vomerine spike; legs short (TL/SUL 0.36 in two specimens); all fingers and toe 1 without expanded discs, toes 2–5 with weakly expanded discs (T4D/SUL 0.038–0.040); eye-naris distance smaller than internarial distance (END/IND 0.80–0.91); tympanum slightly larger than eye (TyD/ED 1.11 in two specimens). Dorsal surfaces bluish-brown in life, ventral surfaces dark orange with irregular whitish and greyish spots. Advertisement calls uttered in series lasting 3–5 s, calls per series 13–19, call length 37–84 ms, repetition rate 4.0–4.5 calls/s.

**Description of the holotype.** Measurements are summarised in Table 6, a dorsolateral view in life is shown in Fig. 15a and ventral surfaces in life in Fig. 15b. Head broader than long (HL/HW 0.75); snout acuminate from above, protruding in profile; loreal region oblique, no canthus rostralis; nostrils near tip of snout, directed more laterally than dorsally, visible from above, but not from below; eye-naris distance less than internarial distance (END/IND 0.90); tympanum visible in life and preservative, its diameter slightly larger than eye (TyD/ED 1.11); tongue very broad; vomerine spikes triangular, moderately large; prepharyngeal ridge narrow with four denticles; supratympanic fold well-developed, not reaching eye or insertion of fore leg (Fig. 15a); shank short (TL/SUL 0.36); fingers moderately short, not webbed; tips of all fingers with circum-marginal grooves, not or only marginally wider than penultimate phalanges, relative lengths of fingers 3 > 4 > 2 > 1 (Fig. 15c); all toe tips

**Table 6.** Body measurements and body ratios of the type series of *Xenorhina woxvoldi* sp. nov. SAMA R71646 is the male holotype; ZMB 91133 is a male paratype. All measurements in mm; for explanation of abbreviations see "Material and methods".

| Reg.-No. | SAMA R71646 | ZMB 91133 | Mean |
|---|---|---|---|
| SUL | 30.1 | 28.7 | 29.40 |
| TL | 10.8 | 10.4 | 10.60 |
| TaL | 7.8 | 7.0 | 7.40 |
| T4L | 12.2 | 12.0 | 12.10 |
| T4D | 1.2 | 1.1 | 1.15 |
| T1D | 0.7 | 0.6 | 0.65 |
| F3L | 5.7 | 5.0 | 5.35 |
| F3D | 0.8 | 0.7 | 0.75 |
| F1D | 0.6 | 0.6 | 0.60 |
| HL | 7.6 | 7.1 | 7.35 |
| HW | 10.2 | 8.7 | 9.45 |
| END | 1.8 | 1.6 | 1.70 |
| IND | 2.0 | 2.0 | 2.00 |
| SL | 3.3 | 3.1 | 3.20 |
| EST | 3.0 | 2.8 | 2.90 |
| ED | 1.8 | 1.9 | 1.85 |
| TyD | 2.0 | 2.1 | 2.05 |
| TL/SUL | 0.36 | 0.36 | 0.36 |
| TaL/SUL | 0.26 | 0.24 | 0.25 |
| T4L/SUL | 0.41 | 0.42 | 0.415 |
| T4D/SUL | 0.040 | 0.038 | 0.39 |
| T1D/SUL | 0.023 | 0.021 | 0.22 |
| F3L/SUL | 0.189 | 0.174 | 0.182 |
| F3D/SUL | 0.027 | 0.024 | 0.026 |
| F1D/SUL | 0.020 | 0.021 | 0.021 |
| T4D/F3D | 1.50 | 1.57 | 1.54 |
| T1D/F1D | 1.16 | 1.00 | 1.08 |
| HL/SUL | 0.25 | 0.25 | 0.25 |
| HW/SUL | 0.34 | 0.30 | 0.32 |
| HL/HW | 0.75 | 0.82 | 0.79 |
| END/SUL | 0.060 | 0.056 | 0.058 |
| IND/SUL | 0.066 | 0.070 | 0.068 |
| END/IND | 0.90 | 0.80 | 0.85 |
| ED/SUL | 0.060 | 0.066 | 0.063 |
| TyD/SUL | 0.066 | 0.073 | 0.070 |
| TyD/ED | 1.11 | 1.11 | 1.11 |
| SL/SUL | 0.110 | 0.108 | 0.109 |
| EST/SUL | 0.100 | 0.098 | 0.099 |

with circum-marginal grooves, those on toes 2–4 clearly wider than penultimate phalanges, those on toe 1 and toe 5 scarcely wider than penultimate phalanges; toes not webbed, relative lengths 4 > 3 > 5 > 2 > 1 (Fig. 15d); plantar, palmar and subarticular tubercles barely visible; body laterally with some distinct tubercles in life, barely visible in preservative; dorsal surfaces of extremities, middle of dorsum and all ventral surfaces smooth; tip of snout with several tiny pimples.

In life, dorsal surface of head, body and extremities a mixture of grey-brown and copper-brown (RAL 8004) (Fig. 15a); lower flanks uniform greyish with bluish hue and off-white dots and streaks; semicircular lumbar spot present, but only vaguely defined; a distinct whitish mid-dorsal line extends on to rear of thighs and on shanks and tarsi, then as broken line on to abdomen and chest; dorsal and ventral surfaces of fingers and toes and palmar surfaces orange; plantar surfaces a mixture of irregular light grey, dark grey and orange spots. Ventral surfaces of throat, chest, abdomen and extremities orange-brown (RAL 8023) with irregular light grey spots (Fig. 15b); most tubercles on flanks and extremities with whitish tips; snout tip light grey with tiny dark grey spots; outer margin of iris blackish and inner margin gold-orange, with some integration of colours at their margins.

In preservative, dorsal surfaces changed from copper-brown to mahogany-brown (RAL 8016), that of ventral surfaces from orange-brown to ivory (RAL 1014). Dorsal surfaces of fingers and toes also become ivory coloured. Lumbar spots no longer visible.

**Morphological variation.** All body measurements and body ratios of holotype and paratype are similar (Table 6). In life, dorsal surfaces of paratype a mixture of lighter and darker brown and reddish areas, with reddish components more restricted than in holotype. Colours of flanks and dorsal surfaces of fingers and toes and colour and extent of mid-dorsal line (extending on to hind limbs and abdomen) as for holotype. Ventral surfaces more yellow and light grey spotting more extensive, in paratype. Dorsal surfaces in preservative slightly paler than holotype, ventral surface with more extensive pale brown reticulation.

**Distribution and ecological notes.** *Xenorhina woxvoldi* sp. nov. is known only from one location at an altitude of 1,368 m a.s.l. on the southern fringe of the Karius Range in Hela Province, Papua New Guinea (Fig. 16), where males called from within the humus layer in lower montane rainforest during late afternoon and early evening.

**Vocalisation.** Two call series from the holotype (SAMA R71646) and one from the paratype (ZMB 91133), recorded at air temperatures of 18–19.5 °C, were analysed. Call is a single unpulsed, piping note produced in discrete series. Call series last 2.9–4.8 s (mean 3.7 s, n = 3) and contain 13–19 calls (mean 5.7 calls, n = 3) produced at a rate of 4.0–4.5 calls/s (mean 4.3 calls/s, n = 3). Call length is 37–84 ms (mean 75.1 ± 8.3 ms, n = 47) and call intervals last 137–250 ms (mean 172.4 ± 28.4 ms, n = 44). Calls are of approximately equal length throughout a series (first call may be shorter) with approximately equal intervals (intervals between first two and last two calls of a series may be slightly longer). Volume of each call increases during course of call series, but rise in pitch is marginal. Calls start abruptly at maximum amplitude, which then decreases gradually until end of call (Fig. 17a). All calls have harmonic structure with 4–5 harmonics between 0.7 and 3.2 kHz (Fig. 17b). First harmonic clearly dominant, with peak at 0.8 kHz (Fig. 17c). Third harmonic often with more energy than second. Frequency of calls weakly modulated with slight reduction during course of call.

**Etymology.** The specific epithet *woxvoldi* is the Latinised patronymic adjective in genitive singular derived from the family name Woxvold. It is in gratitude of the junior author to Iain Woxvold for the many years of friendship, camaraderie and shared adventures in remotest New Guinea.

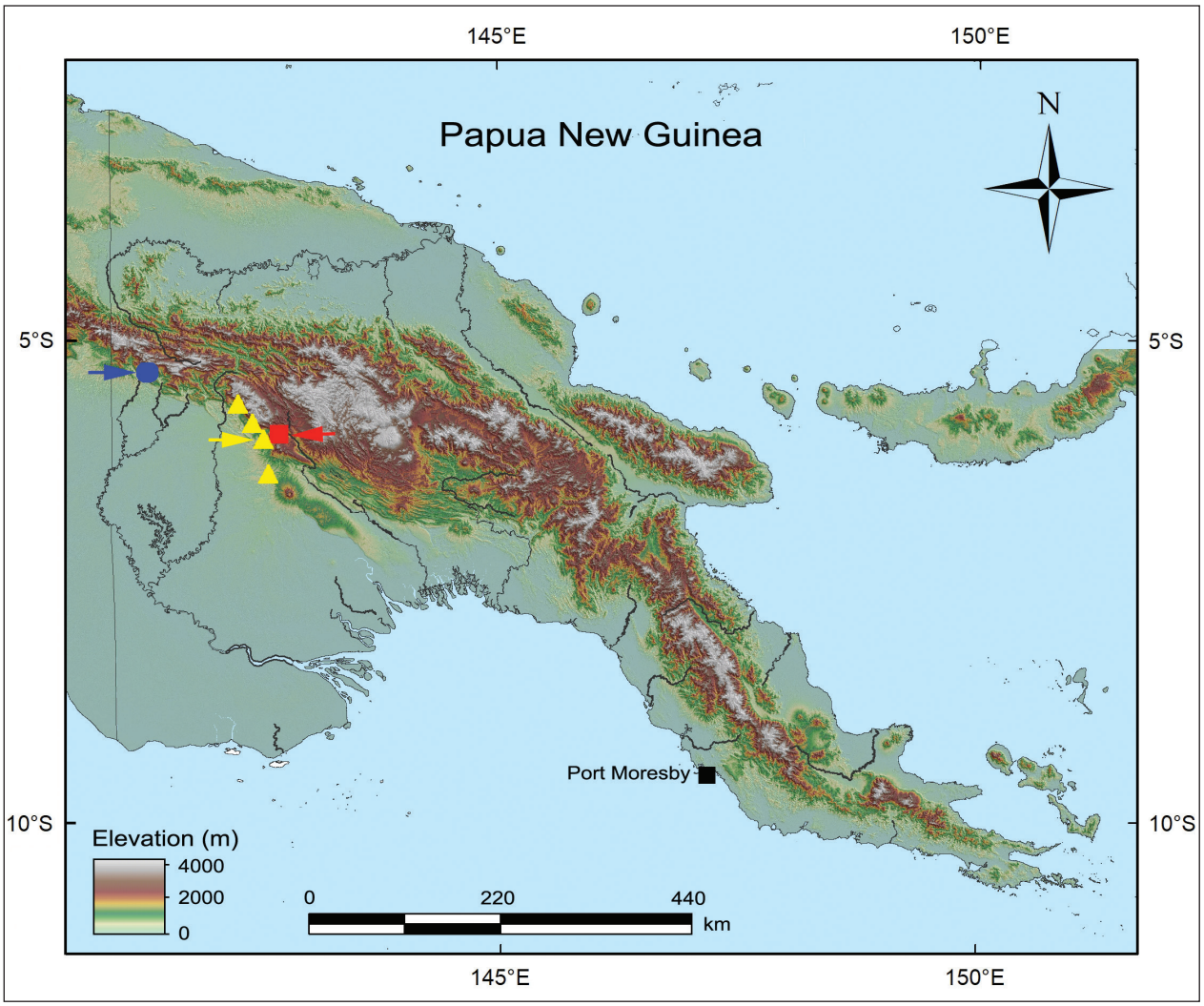


**Figure 16.** Map of Papua New Guinea showing the known distributions of *X. thiekeorum* sp. nov. (blue circle), *X. wiegankorum* sp. nov. (yellow triangles) and *X. woxvoldi* sp. nov. (red square). Arrows indicate the type localities.

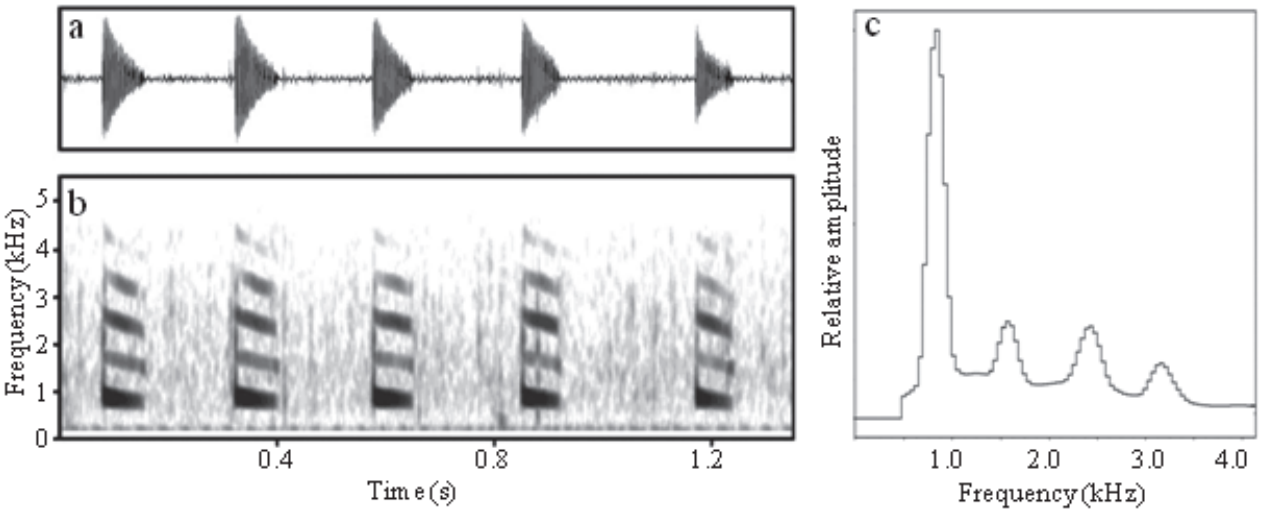


**Figure 17.** (**a**) Oscillogram, (**b**) Spectrogram and (**c**) Amplitude spectrum of the last five calls of a series containing 15 calls from the holotype of *Xenorhina woxvoldi* sp. nov.

**Comparisons with other species.** We compare *Xenorhina woxvoldi* sp. nov. with all congeners of a similar size (SUL ~ 25–35 mm) that have a single spike on each vomeropalatine.

*Xenorhina fuscigula* differs from *Xenorhina woxvoldi* sp. nov. by having an internarial distance shorter (IND/SVL 0.054–0.064 vs. 0.066–0.070), eye-naris distance greater (END/SVL 0.064–0.074 vs. 0.056–0.060), END/

IND ratio higher (1.00–1.36 vs. 0.80–0.90), ventral surfaces pale with dark reticulation (vs. orange with light grey spots) and calls produced singly (vs. produced in rapid series of 13–19 calls).

*Xenorhina huon* has eye-naris distance greater (0.073–0.103 vs. 0.056–0.060), END/IND ratio higher (1.00–1.27 vs. 0.80–0.90), eyes larger (ED/SVL 0.070–0.091 vs. 0.060–0.066), head wider (HW/SVL 0.35–0.47 vs. 0.30–0.34) and ventral surfaces with dark flecking (vs. ventral surfaces with light flecking in life and pale brown reticulation in preservative).

*Xenorhina lacrimosa* is larger (SUL 34.3–41.0 mm vs. 28.7–30.1 mm), has shanks longer (TL/SUL 0.42–0.46 vs. 0.36 in both known *Xenorhina woxvoldi* sp. nov.), fourth toe longer (T4L/SUL 0.42–0.49 vs. 0.41–0.42), head longer (HL/SUL 0.27–0.30 vs. 0.25 in both known *Xenorhina woxvoldi* sp. nov.), eye-naris distance greater (END/SUL 0.073–0.099 vs. 0.056–0.060) and advertisement calls longer (141–231 ms vs. 37–84 ms) with lower repetition rate (0.20–0.27 vs. 4.0–4.5 calls/s).

*Xenorhina mehelyi* has hind legs longer (TL/SVL > 0.42 vs. 0.36), eye-naris distance greater (END/SVL 0.076–0.096 vs. 0.056–0.060), END/IND ratio higher (1.12–1.50 vs. 0.80–0.90), eyes larger (ED/SVL 0.067–0.079 vs. 0.060–0.066), ventral surfaces with dark mottling (vs. no dark mottling) and calls longer (on average140 ms vs. 75 ms) with inter-call intervals also longer (on average 1500 ms vs. 172 ms).

*Xenorhina schiefenhoeveli* has eye-naris distance greater (END/SVL 0.077 vs. 0.056–0.060), END/IND ratio higher (1.16–1.21 vs. 0.80–0.90), eyes larger (ED/SVL 0.071–0.081 vs. 0.060–0.066), ventrum cream, with reticulated brown (vs. orange-red with whitish flecking); calls longer (~ 100 ms vs. mean of 75 ms), uttered in very long series of more than 100 calls (vs. 13–19 calls) with repetition rate about 2 calls/s (vs. 4.0–4.5 calls/s).

*Xenorhina subcrocea* has hind legs longer (TL/SVL > 0.46 vs. < 0.40), ratio of END/IND much larger (1.26–1.41 vs. 0.80–0.90), ventral surfaces with dark reticulation (vs. with whitish flecking) and mid-dorsal line absent (vs. distinct dorsal line present).

*Xenorhina tumulus* has eye-naris distance greater (0.073–0.081 vs. 0.056–0.060), END/IND ratio higher (1.11–1.28 vs. 0.80–0.90), ventral surfaces in life pinkish, mottled with brown (vs. orange-brown with no brown mottling) and call intervals within series 300–400 ms (vs. 137–250 ms).

*Xenorhina wiegankorum* appears to be larger (five males 32.0–35.7 mm vs. two males 28.7–30.1 mm SUL), has hind legs much longer (TL/SUL 0.44–0.47 vs. 0.36 in two specimens), has toes longer (T4L/SUL 0.45–0.47 vs. 0.41–0.42), fingers longer (F3L/SUL 0.188–0.213 vs. 0.174–0.189), END/IND ratio higher (1.19–1.37 vs. 0.80–0.90) and a different advertisement call (see description of *X. wiegankorum*, this paper).

*Xenorhina zweifeli* is larger (SVL 33.2–38.0 vs. 28.7–30.1), with internarial distance smaller (IND/SVL 0.052–0.063 vs. 0.066–0.070), eye-naris distance larger (END/SVL 0.071–0.085 vs.0.056–0.060), END/IND ratio higher (1.17–1.47 vs. 0.80–0.90); ventral colour pattern of dark brown flecks on a cream ground in preservative (vs. pale brown flecks on ivory-coloured ground) and call consisting of a single note (vs. 13–19 calls produced in distinct series.

# Discussion

Recent assessments of anuran faunas on the large tropical islands of Sri Lanka (Meegaskumbura et al. 2002, Pethiyagoda et al. (2014) and Madagascar (Vieites et al. 2009) have revealed vastly underestimated levels of diversity. A similar pattern is emerging for New Guinea, the world's largest and highest tropical island. New Guinea has the most diverse insular anuran fauna globally, with more than 400 species currently recognised (Frost 2021). Furthermore, field-based species inventories across the Island during the past 2–3 decades have rapidly increased the rate of species discovery and description, a trend that shows no sign of approaching an asymptote (Allison 2014). This rapid advance in taxonomic knowledge of the amphibian fauna has been generated substantially by studies of morphological and bioacoustic variation (e.g. Günther 2001, Richards and Günther 2018, Kraus 2019), while molecular assessments of New Guinea anuran diversity remain relatively rare (Oliver et al. 2013).

Anuran advertisement calls are useful for taxonomic studies because they are mate recognition signals that are generally species-specific, exhibit limited variation amongst individuals and populations (although some features can be influenced in partially predictable ways by environmental factors, such as temperature) and likely have a genetic basis (Hoskin 2005, Köhler et al. 2017, Emmrich et al. 2020). We, therefore, consider the unique bioacoustics traits of each new species described here to be a strong indicator of species level divergence. Known calls of *Xenorhina* species reflect the acoustic constraints imposed by a fossorial existence. They comprise short, precise and melodious "hooting" or "piping" notes with a low fundamental frequency and well-defined harmonics that are normally produced in regular call series (Menzies and Tyler 1977, Blum and Menzies 1989). The six new species described here each produce advertisement calls of this type, but each is distinct from the known calls of congeners and these differences are concordant with the patterns of morphological variation documented. These calls meet the criteria for Call Guild A: "non-frequency modulated, non-pulsed simple call," or Call Guild B: "frequency modulated, non-pulsed simple call" of Emmrich et al. (2020), depending on the extent of modulation exhibited amongst species (defined as "with significant change" vs. "without significant change" by Emmrich et al. (2020).

The description of *Xenorhina perexigua* sp. nov. on the basis of a single specimen reflects the difficulty of detecting and capturing small, nocturnal, fossorial frogs in an inaccessible terrain, that furthermore call most frequently during torrential rain. Thus, we are unable to determine whether this species is genuinely rare or merely difficult to detect. However, it is notable that the holotype was the only individual encountered during nearly one week of survey effort at the type locality. Numerous species

of microhylid frogs have been described from the New Guinea region on the basis of "singletons" (Allison and Kraus 2000, Günther et al. 2016). Lim et al. (2012) noted that "rare" species are common in taxonomic treatises and that additional sampling often leads only to singletons becoming "doubles," accompanied by detection of additional new species, based on singletons.

The high-rainfall belt that extends across the southern slopes and adjacent lowlands of Papua New Guinea's Central Cordillera (McAlpine et al. 1983) is proving to be a hotspot of anuran diversity (Günther and Richards 2016, 2017, 2018, 2019, 2020, Richards and Günther 2019) and our documentation of six previously undescribed frogs in the genus *Xenorhina* adds substantially to this already exceptional known diversity. Further studies are required to better document the distributions of these *Xenorhina* species. Several of them are known from only one or a handful of locations, but it is unclear whether they are genuinely range-restricted or whether their apparent rarity reflects the difficulty of conducting surveys throughout much of the region's remote and rugged landscape.

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# Appendix 1.

**Table A1.** Specimens examined.

| Species | Location | Registration numbers |
|---|---|---|
| *Xenorhina adisca* Kraus & Allison, 2003 | Indonesia: Papua Province: Tembagapura | MZB Amph.8403 (holotype) |
| *Xenorhina arboricola* Allison & Kraus, 2000 | Papua New Guinea: West Sepik Province: Mt Menawa | BPBM 13747 (paratype) |
| *Xenorhina arboricola* Allison & Kraus, 2000 | Papua New Guinea: West Sepik Province: Mt Hunstein | BPBM 13745 (paratype) |
| *Xenorhina arndti* Günther, 2010 | Indonesia: Papua Province: Bomberai Peninsula | ZMB 74629–31 (type series) |
| *Xenorhina bidens* van Kampen, 1909 | Indonesia: Papua Province: "Digul-Fluss" | ZMA 5705 (holotype) |
| *Xenorhina bouwensi* (De Witte, 1930) | Indonesia: West Papua Province: Arfak Mountains | IRSNB 1019 (holotype), plus several specimens collected by R. Günther between 1998–2008 and stored in the ZMB collection |
| *Xenorhina eiponis* Blum & Menzies, 1989 | Indonesia: Papua Province: Eipomek Valley | AMNH 128234 (paratype) |
| *Xenorhina gigantea* van Kampen, 1915 | Indonesia: Papua Province: Snow Mountains | ZMA 5702 (lectotype), ZMA 5703 (paralectotype) |
| *Xenorhina lanthanites* (Günther & Knop, 2006) | Indonesia: Papua Province: Yapen Island | ZMB 69557–61 (type series) |
| *Xenorhina macrodisca* | Indonesia: Papua Province: Wapoga River Headwaters | MZB Amph.10916 (holotype) |
| *Xenorhina macrops* van Kampen, 1913 | Indonesia: Papua Province: Hellwig Mountains | ZMA 5725 (lectotype), ZMA 5726–5728 (paralectotypes) |
| *Xenorhina mehelyi* (Boulenger, 1898) | Papua New Guinea: Central Province: "Vikaiku", Angabunga River | MSNG 29112 (holotype) |
| *Xenorhina minima* (Parker, 1934) | Indonesia: Papua Province: Went Mountains | ZMA 5818 (holotype), ZMA 5817 (paratype) |
| *Xenorhina ocellata* van Kampen, 1913 | Indonesia: Papua Province: Hellwig Mountains | ZMA 5815–16 (syntypes) |
| *Xenorhina ophiodon* (Peters & Doria, 1878) | Indonesia: Papua Province: Hatam, Arfak Mountains | MSNG 29129 (lectotype) |
| *Xenorhina oxycephala* Schlegel, 1858 | Indonesia: Papua Province: Triton Bay | RMNH 2280A and 2280B (syntypes) (plus several specimens collected by R. Günther between 1998–2008 and stored in the ZMB collection) |
| *Xenorhina parkerorum* Zweifel, 1972 | Papua New Guinea: Western Province: Imigabip | MCZ 81678 (holotype), |
| *Xenorhina parkerorum* Zweifel, 1972 | Indonesia: Papua Province: Tenmasigin, Star Mountains | RMNH 16619 (paratype) |
| *Xenorhina salawati* Günther, Richards, Tjaturadi & Krey, 2020 | Indonesia: West Papua Province: Salawati Island | MZB Amph.12121–22, 12124–26, 12132, 12134, (type series) |
| *Xenorhina tillacki* Günther, Richards & Dahl, 2014 | Papua New Guinea: Western Province: Muller Range | SAMA R65067–68, ZMB 79532 (type series) |
| *Xenorhina varia* | Indonesia: Papua Province: Yapen Island | ZMB 65133–37 (type series) |
| *Xenorhina waigeo* Günther, Richards, Tjaturadi & Krey, 2020 | Indonesia: Papua Province: Waigeo Island | MZB Amph. 12119–20, 12123, 12127-31, 12133, 12155 (type series) |

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PENSOFT

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# Three new species of the spider genus *Nopsma* (Araneae, Caponiidae, Nopinae) from Colombia

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## Abstract

Three new Colombian species of the spider genus *Nopsma* Sánchez-Ruiz, Brescovit & Bonaldo, 2020 are described and illustrated: *Nopsma leticia* **sp. nov.** (male) from Amazonas department, *Nopsma macagual* **sp. nov.** (male) from Caquetá department and *Nopsma paya* **sp. nov.** (male and female) from Putumayo department. The collection data of the holotype of *Nopsma florencia* Sánchez-Ruiz, Brescovit & Bonaldo are corrected. Additionally, an updated identification key for all species of the genus and a distribution map for the Colombian species are included.

## Key Words

Arachnida, neotropical region, synspermiata, taxonomy

## Introduction

The two-eyed spider genus *Nopsma* was recently proposed by Sánchez-Ruiz et al. (2020) for four species occurring in Colombia, Ecuador, Peru and Nicaragua. The Ecuadorian species *Nopsma juchuy* (Dupérré, 2014) was transferred from *Nyetnops* Platnick & Lise, 2007 and was elected as the type species. Members of this genus can be distinguished from other two-eyed Nopinae (except *Nyetnops*), by lacking a crista and an arolium on anterior metatarsi, and from *Nyetnops* by the presence of a gladius. Additionally, *Nopsma* species are characterized by having an elongated, prolaterally protruded embolus, unique among nopines, and by the shape of endites, without projected outer sides on anterior margin (Sánchez-Ruiz et al. 2020).

Members of *Nopsma* are poorly known in Colombia, being represented by only one species, *N. florencia* Sánchez-Ruiz, Brescovit & Bonaldo, 2020. The species was described from male specimens revised and photographed prior to the publication by the second author of this paper (L.M.), who unfortunately confused the type´s data label with those of other Colombian *Nopsma* specimens from Macagual in Caquetá department, while the actual locality of *N. florencia* is in Chocó department. The collection data of this Colombian species is corrected below. On the bright side, this mislabeling event led to the discovery of a new *Nopsma* species, since the specimens from Macagual belong to an undescribed species. This finding also triggered the opportunity to review caponiids from three Colombian collections, leading to the discovery of two additional undescribed *Nopsma* species. These three species are herein described, along with the correction of the type locality of *N. florencia*. Photos of the habitus, male palpal morphology and female genital organs, line drawings of the male copulatory bulbs and schematic drawings of the female internal genitalia are provided. Additionally, a distribution map for all Colombian species and an updated identification key for all species of the genus are presented.

## Materials and methods

The specimens examined in this study were supplied by the following collections (acronym and curator in parentheses): Instituto Alexander Von Humboldt, Bogotá, Colombia (IAvH-I, J.C. Neita) Instituto de Ciencias Naturales of the Universidad Nacional, Bogotá, Colombia (ICN-Ar, E. Flórez) and Pontificia Universidad Javeriana, Bogotá (MPUJ-ENT, D. Forero).

Morphological observations and illustrations were made using a Leica MC125 stereomicroscope with a camera lucida. Multifocal images were taken with the Leica MC-190 HD and Leica MC-170 HD digital cameras attached to Leica S8AP0 and Leica MC125 stereomicroscopes respectively with extended focal range. All multifocal images were assembled using Helicon Focus Pro ver. 5.3.14. The measurements are in millimeters (mm) and were made using an ocular micrometer. Descriptions and measurements follow Sánchez-Ruiz and Brescovit (2018). Coloration patterns were described based on specimens preserved in 70–80% ethanol. The internal female genitalia was dissected with fine forceps and their soft tissues were digested for 24 hours with Ultrazyme enzymatic eye lens cleaner, diluted with distilled water at the proportion of 1 tablet/5 ml. After cleaning, samples were immersed in clove oil for visualization of internal structures. The terminology for copulatory structures follows Sánchez-Ruiz et al. (2015). All digital photos were edited using Adobe Photoshop CS ver. 12.0 and the distribution map was prepared in QGIS (QGIS 2021). Plates were edited with Corel Draw X7 ver. 17.1. Geographic coordinates were extracted from original labels. Locality elevations refer to meters above sea level.

The following abbreviations are used in the text and figures: ap = anterior plate, as = anterior tracheal spiracles, dmr = distal margin of receptaculum, e = embolus, ess = external sclerotization around spiracles, go = genital opening (gonopore), mk = membranous keel on embolus, re = receptaculun, pmr = proximal margin of receptaculum, pp = posterior plate, ps = posterior tracheal spiracles, t = tegulum.

## Taxonomy

### Caponiidae Simon, 1890

### Genus *Nopsma* Sánchez-Ruiz, Brescovit & Bonaldo, 2020

### Updated key to the species of *Nopsma* (males only)

1 Large tegulum, reaching or exceeding the palpal tibia length (Sánchez-Ruiz et al. 2020: figs 11B, 17B) ..... 2
– Small tegulum, not reaching the palpal tibia length (Sánchez-Ruiz et al. 2020: figs 15B, 18B) ..... 3
2 Elongated palpal tibia, two times the patella length (Sánchez-Ruiz et al. 2020: fig. 11B), embolus projecting from the prolateral distal surface of the tegulum with a keel bordering the tip (Sánchez-Ruiz et al. 2020: figs 11B, C, 14A, D–F) ..... *N. enriquei*
– Short palpal tibia, just a little longer than patella length (Sánchez-Ruiz et al. 2020: fig. 17B), embolus projecting from the prolateral median surface of the tegulum with three very thin, long projections on the tip (Sánchez-Ruiz et al. 2020: fig. 17B, C, F) ..... *N. armandoi*
3 Embolus posteriorly directed (Figs 1C, D, 2C, D, 4E, H) ..... 4
– Embolus anteriorly directed (Fig. 3C, D) ..... *N. macagual* sp. nov.
4 Tegulum one-third of the cymbium length (Figs 2C, D, 3C, D, 4E, F) ..... 5
– Tegulum conspicuously small, only one-fifth the cymbium length (Fig. 1C, D) ..... *N. florencia*
5 Embolus with a membranous keel at the opening, extended proximally towards the embolus shaft (Fig. 6E, F) ..... 6
– Embolus with membranous keel restricted to the opening of embolus tip (Fig. 6G, H) ..... *N. paya* sp. nov.
6 Membranous keel long, reaching more than one-third of the embolus shaft (Fig. 6F) ..... *N. leticia* sp. nov.
– Membranous keel short, reaching only one-fourth or less of the embolus shaft (Sánchez-Ruiz et al. 2020: fig. 16B–E) ... ..... *N. juchuy*

#### *Nopsma florencia* Sánchez-Ruiz, Brescovit & Bonaldo, 2020

Figures 1A–D, 5A, B, 6A, B, 8

*Nopsma florencia* Sánchez-Ruiz, Brescovit & Bonaldo, 2020: 483, fig. 18A–F.

**Type material.** Holotype ♂, COLOMBIA: Chocó department, Jardín Botánico El Darién, Capurgana, Acandí, Camino a los Ríos; 8°37'53.95"N, 77°21'23.43"W; 260 m; 14 April 2008; C. Peña leg; pitfall trap; MPUJ-ENT 61986; examined, type locality corrected.

**Remark.** The type locality of this species is here corrected. The data labels of the holotype and paratype reported in the original description are actually those belonging to *Nopsma macagual* sp. nov.

**Diagnosis.** Males of *Nopsma florencia* resemble those of *Nopsma leticia* sp. nov. by the similarly shaped membranous keel on embolus tip (Fig. 6A, B, E, F), but can be distinguished by the conspicuous small oval tegulum, with only one-fifth the cymbium length (Fig. 1C, D), (one-third in *N. leticia* sp. nov., Fig. 2C, D) and by the enlarged embolus (Fig. 1C, D) (shorter in *N. leticia* sp. nov., Fig. 2C, D).

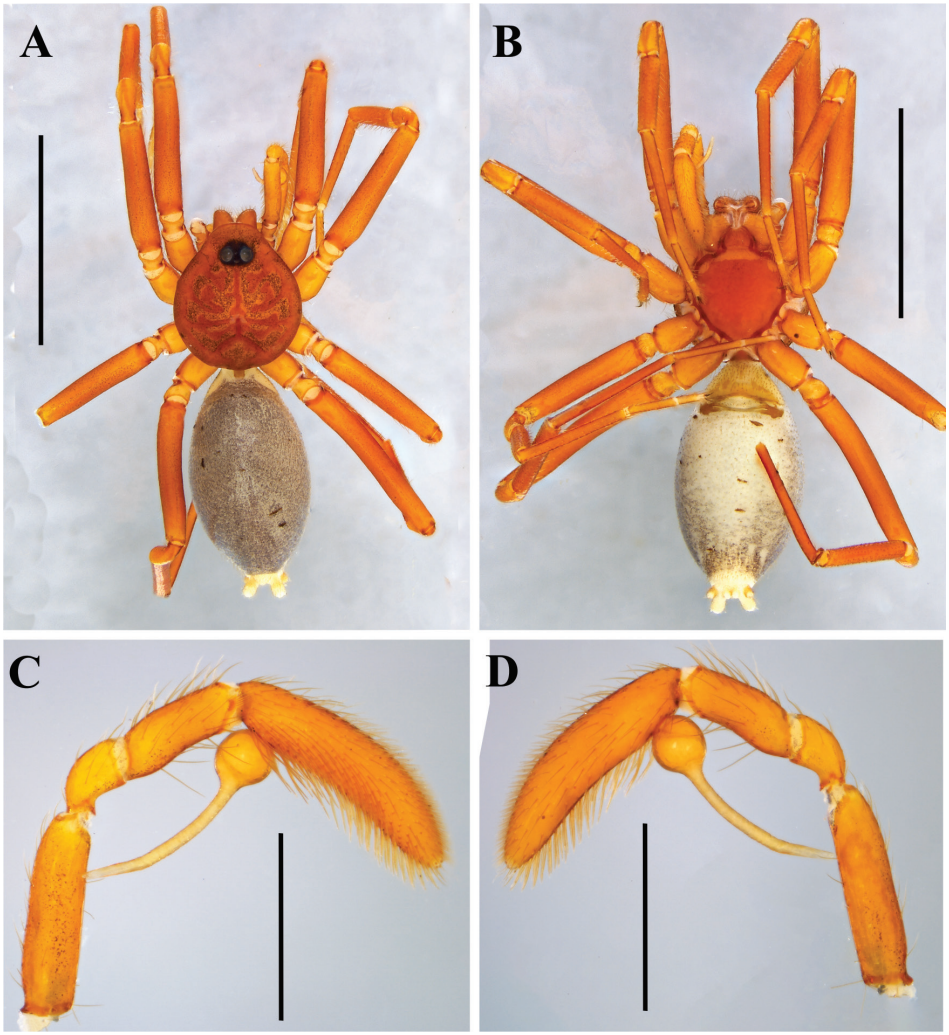


**Figure 1.** *Nopsma florencia* Sánchez-Ruiz, Brescovit & Bonaldo, male (holotype). **A.** Habitus, dorsal view. **B.** Habitus, ventral view. **C.** Left palp, retrolateral view. **D.** Left palp, prolateral view. Scale bars: **A, B:** 1.5 mm, **C, D:** 0.7 mm.

**Description.** Male described by Sánchez-Ruiz et al. (2020). Female unknown.

**Distribution.** Known only from the type locality in Chocó, Colombia (Fig. 8).

**Preservation status.** Preserved in 70% ethanol. Male holotype in good condition, left palp dissected in a separate microvial.

### *Nopsma leticia* sp. nov.

http://zoobank.org/D736BA90-2C7F-4339-8629-10D88C9EAC7A

Figures 2A–D, 5E, F, 6E, F, 8

**Type material.** Holotype ♂, COLOMBIA: Amazonas department, Leticia, Comunidad Indígena Monifue Amena, km 9,8 Vía Leticia-Tarapacá; 4°8'30"S, 69°55'23.72"W; 70 m; 12 Oct. 2003; Ospina leg; MPUJ-ENT 70460. Paratypes: 2 ♂; same collection data as for holotype; 13 Oct. 2003; MPUJ-ENT 70411.

**Additional material examined.** COLOMBIA • 1 ♂; Amazonas department, Leticia, Reserva Forestal del Río Calderón, Estación Biológica El Zafire; 4°00'21"S, 69°53'55"W; 150 m; 2–13 Dec. 2007; L. Franco & S. Flórez leg; IAvH-I 3784 • 1 ♂; same data as for preceding; IAvH-I 3785.

**Diagnosis.** Males of *Nopsma leticia* sp. nov. resemble those of *Nopsma florencia* by having a similarly shaped membranous keel on embolus tip (Fig. 6A, B, E, F), but can be distinguished by having a larger tegulum, one-third of the cymbium length (Fig. 2C, D), and by the thicker embolus and membranous keel (Fig. 6E, F).

**Description. Male** (holotype): Total length 3.92. Carapace 1.67 long, 1.42 wide. Sternum 1.02 long, 0.92 wide. Leg measurements: I: 4.37; II: 4.54; III: 4.31; IV: 5.52. Carapace orange-brown with remarkable dorsal pattern of dark brown stains (Fig. 2A). Chelicerae, palps, sternum, endites, labium and legs orangish brown (Fig. 2B). Abdomen dorsally gray with tenuous light stripes (Fig. 2A), ventrally yellowish (Fig. 2B). Anal tubercle and spinnerets pale yellowish. Palp with rounded tegulum and a posteriorly directed embolus (Fig. 2C, D), with a long membranous keel, reaching more than one-third of the embolus tip (Fig. 6F). **Female:** unknown.

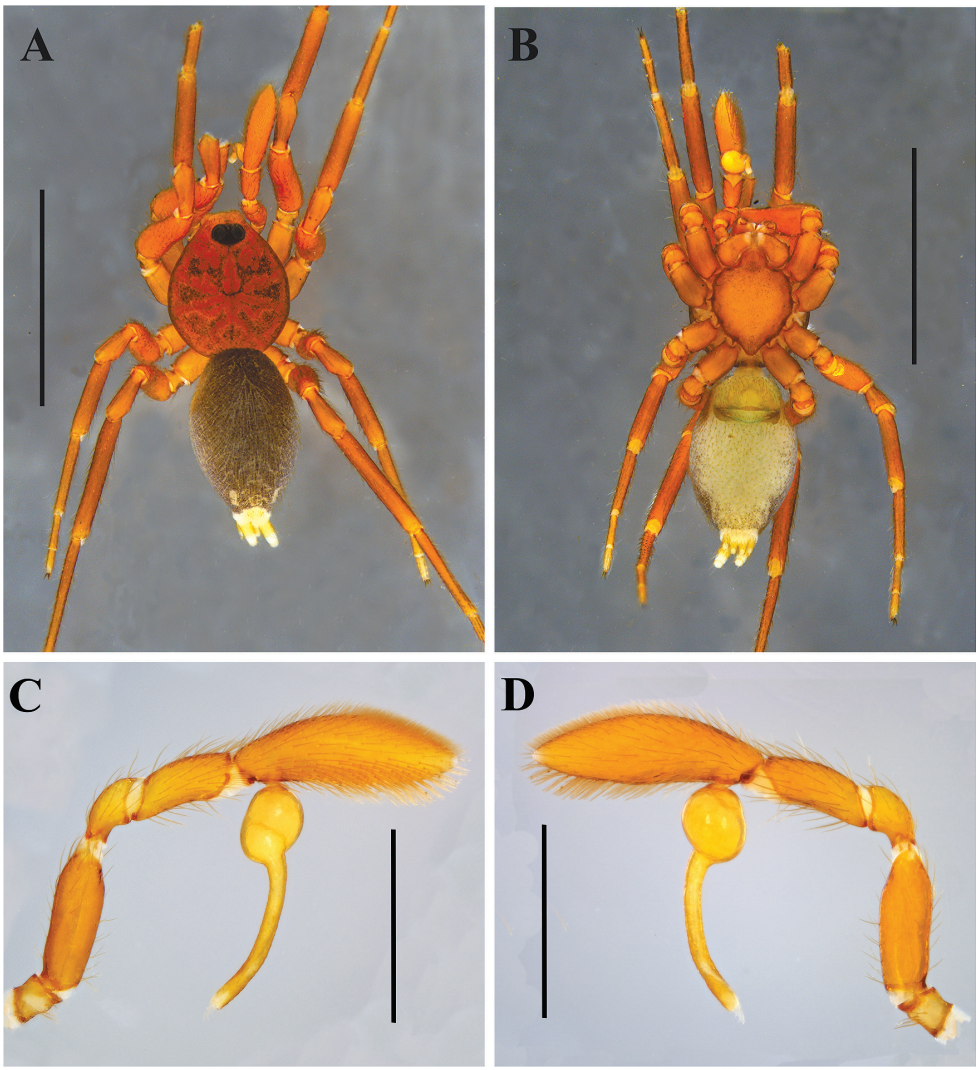


**Figure 2.** *Nopsma leticia* sp. nov., male (MPUJ-ENT 0070411). **A.** Habitus, dorsal view. **B.** Habitus, ventral view. **C.** Left palp, retrolateral view. **D.** Left palp, prolateral view. Scale bars: **A, B:** 1.5 mm, **C, D:** 0.7 mm.

**Etymology.** The specific name is a noun in apposition taken from the type locality.

**Variation.** Males (n=5): total length: 2.68–3.99; carapace length: 1.54–1.71.

**Distribution.** Known from two localities in Amazonas department (Fig. 8).

**Preservation status.** Preserved in 70% ethanol. Male holotype in good condition, left palp dissected in a separate microvial.

### *Nopsma macagual* sp. nov.

http://zoobank.org/939217FE-49BE-4E54-B6F0-AD6D58207A67

Figures 3A, D, 5C, D, 6C, D, 8

**Type material.** Holotype ♂, COLOMBIA: Caquetá department, Florencia, Centro de Investigaciones de la Universidad de la Amazonia Macagual; 1°30'5.364"N, 75°39'46.26"W; 250 m; 29 Mar.–04 Apr. 2017, E. Flórez leg; ICN-Ar 10354.

**Diagnosis.** *Nopsma macagual* sp. nov. can be distinguished from all other members of *Nopsma* by having the embolus anteriorly directed (Figs 3C, D, 6C, D).

**Description. Male** (holotype): Total length 2.67. Carapace 1.51 long, 1.25 wide. Sternum 1.05 long, 0.86 wide. Leg measurements: I: 4.83; II: 4.44; III: 4.42; IV: 5.67. Carapace orange-brown with remarkable dorsal pattern of dark brown stains (Fig. 3A). Chelicerae, palps, and sternum orange-brown. Labium, endites and legs light orange, excepting coxae and tarsi pale orange (Fig. 3A, B). Abdomen dorsally dark gray, without stripes (Fig. 3A), ventrally light gray (Fig. 3B). Anal tubercle and spinnerets pale light brown. Palp with large, pear-shaped tegulum, one-third of the cymbium length (Fig. 3C, D, with retrolateral torsion (Fig. 6C, D), embolus long, anteriorly directed (Fig. 3C, D), with a membranous keel at the opening and surrounding the embolus tip (Fig. 6C, D). **Female:** unknown.

**Etymology.** The specific name is a noun in apposition taken from the type locality.

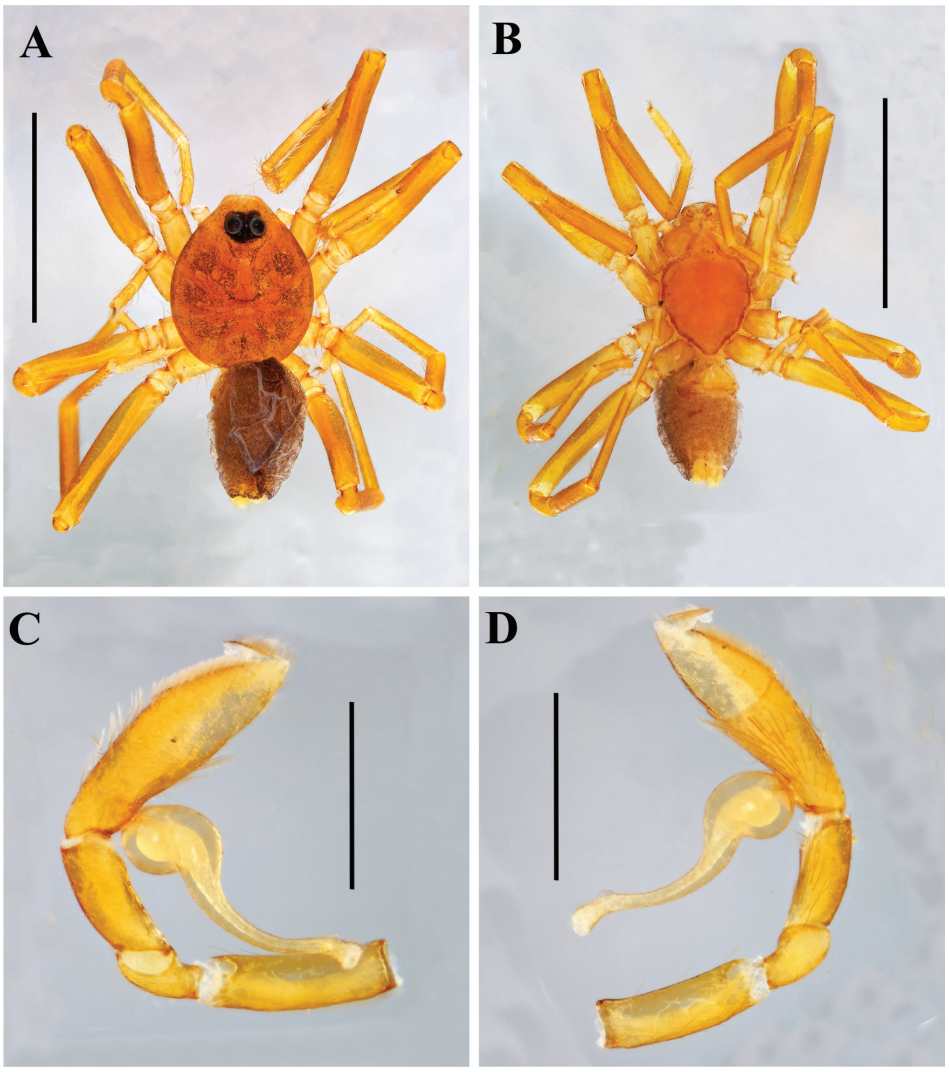


**Figure 3.** *Nopsma macagual* sp. nov., male (holotype). **A.** Habitus, dorsal view. **B.** Habitus, ventral view. **C.** Left palp, retrolateral view. **D.** Left palp, prolateral view. Scale bars: **A, B:** 1.5 mm, **C, D:** 0.7 mm.

**Distribution.** Known only from the type locality (Fig. 8).

**Preservation status.** Preserved in 80% ethanol. Male holotype in good condition, but abdomen is dry and shriveled (Fig 3A). Both palps of holotype dissected in a separate microvial with partial damage on left cymbium tip (Fig 3D).

### *Nopsma paya* sp. nov.

http://zoobank.org/41F19726-8256-446E-98DF-43404AB773D9

Figures 4A–G, 5G, H, 6G, H, 7B, 8

**Type material.** Holotype ♂, COLOMBIA: Putumayo, Parque Natural Nacional La Paya, Cabaña Viviano Cocha; 0°7'S, 74°56'W; 320 m; 15–20 Jul. 2003; R. Cobete leg; IAvH-I 3786. Paratypes: 1♀; same data as for holotype; IAvH-I 3796 • 1♀; same data as for holotype; IAvH-I 3806.

**Diagnosis.** Males of *N. paya* sp. nov. resemble those of *N. leticia* sp. nov. by having an oval tegulum reaching one-third of the cymbium length (Figs 2C, D, 4E, F), but can be distinguished by having a straighter embolus with membranous keel restricted to the opening tip (Fig. 6G, H) (curved, with membranous keel surrounding the embolus in *N. leticia*; Fig. 6E, F). Females differ from those of *N. juchuy*, the only other *Nopsma* species known by females, by the external genitalia strongly sclerotized around spiracles, with V-shaped margin on posterior plate (Fig. 4G) (concave margin on posterior plate, weak sclerotization around spiracles in *N. juchuy*; Sánchez-Ruiz et al. 2020: fig. 15I); internally with median concavity on distal margin of receptaculum (Figs 4H, 7B) (straight distal margin in *N. juchuy*; Fig. 7A, Sánchez-Ruiz et al. 2020: fig. 16G–J).

**Description. Male** (holotype): Total length (approximately) 3.04. Carapace 1.60 long, 1.29 wide. Sternum 0.94 long, 0.81 wide. Leg measurements: I: 5.09; II: 4.84; III: 4.58; IV: 6.14. Carapace orange-brown with disperse dorsal pattern of dark brown stains (Fig. 4A). Chelicerae, palps, sternum, labium, endites and legs

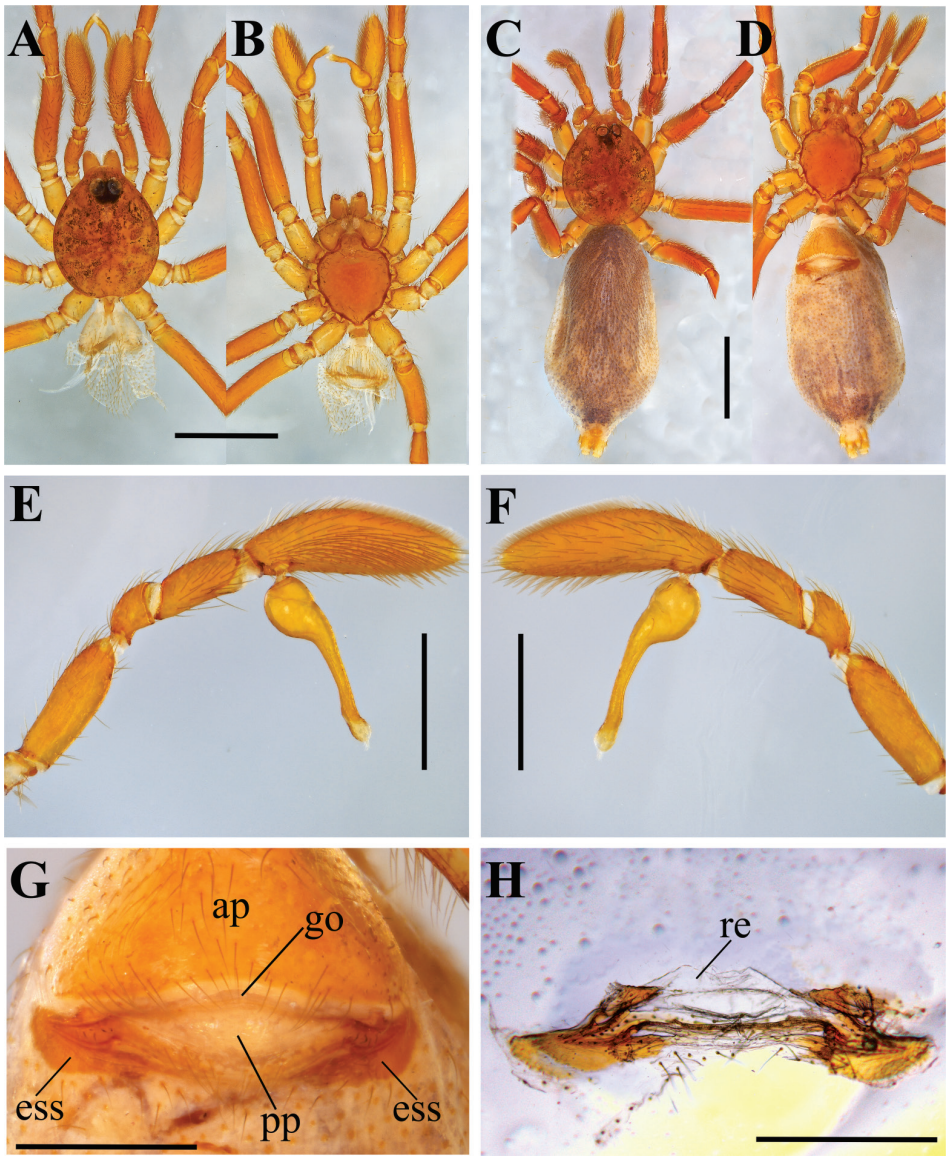


**Figure 4.** *Nopsma paya* sp. nov.; **A, B, E, F.** male holotype; **C, D, G.** female paratype (IAvH-I 3796). **A.** Habitus, dorsal view. **B** Habitus, ventral view. **C.** Habitus, dorsal view. **D.** Habitus, ventral view. **E.** Left palp, retrolateral view. **F.** Left palp, prolateral view. **G.** External genitalia, ventral view. **H.** Internal genitalia, dorsal view. Scale bars: **A–D:** 1.5 mm, **E, F:** 0.7 mm, **G, H:** 0.5 mm. Abbreviations: ap = anterior plate, ess = external sclerotization around spiracles, go = genital opening, pp = posterior plate, re = receptaculum.

light orange-brown, except coxae and tarsi pale orange (Fig. 4A, B). Abdomen dorsally dark gray with dark patches, but not forming a pattern, ventrally light gray. Anal tubercle and spinnerets pale light brown. Palp with oval, pear-shaped tegulum with retrolateral torsion (Fig. 6G, H) and a straight embolus with membranous keel only at the opening tip (Fig. 6G, H). **Female** (paratype, IAvH-I 3796): Total length 5.24. Carapace 1.80 long, 1.55 wide. Sternum 1.07 long, 0.99 wide. Leg measurements: I: 5.21; II: 5.01; III: 4.58; IV: 6.37. Coloration as in male. External genitalia strongly sclerotized around spiracles and in the anterior plate, with V-shaped margin on posterior plate (Fig. 4G); internal genitalia with median concavity or invagination on distal margin of receptaculum, slightly sloping on sides (Figs 4H, 7B).

**Etymology.** The specific name is a noun in apposition taken from the type locality.

**Variation.** Females (n=2): total length: 4.97–5.24; carapace length: 1.64–1.80.

**Distribution.** Known only from the type locality (Fig. 8).

**Preservation status.** Preserved in 80% ethanol. Male holotype with only a half of the abdomen, left palp dissected in a separate microvial. Female paratype IAvH-I 3796 in good condition, genitalia dissected in a separate microvial.

## Discussion

The three new Colombian species of *Nopsma* described here increase to seven the number of known species of this genus. These new species are probably endemic, being currently known from only one or two localities

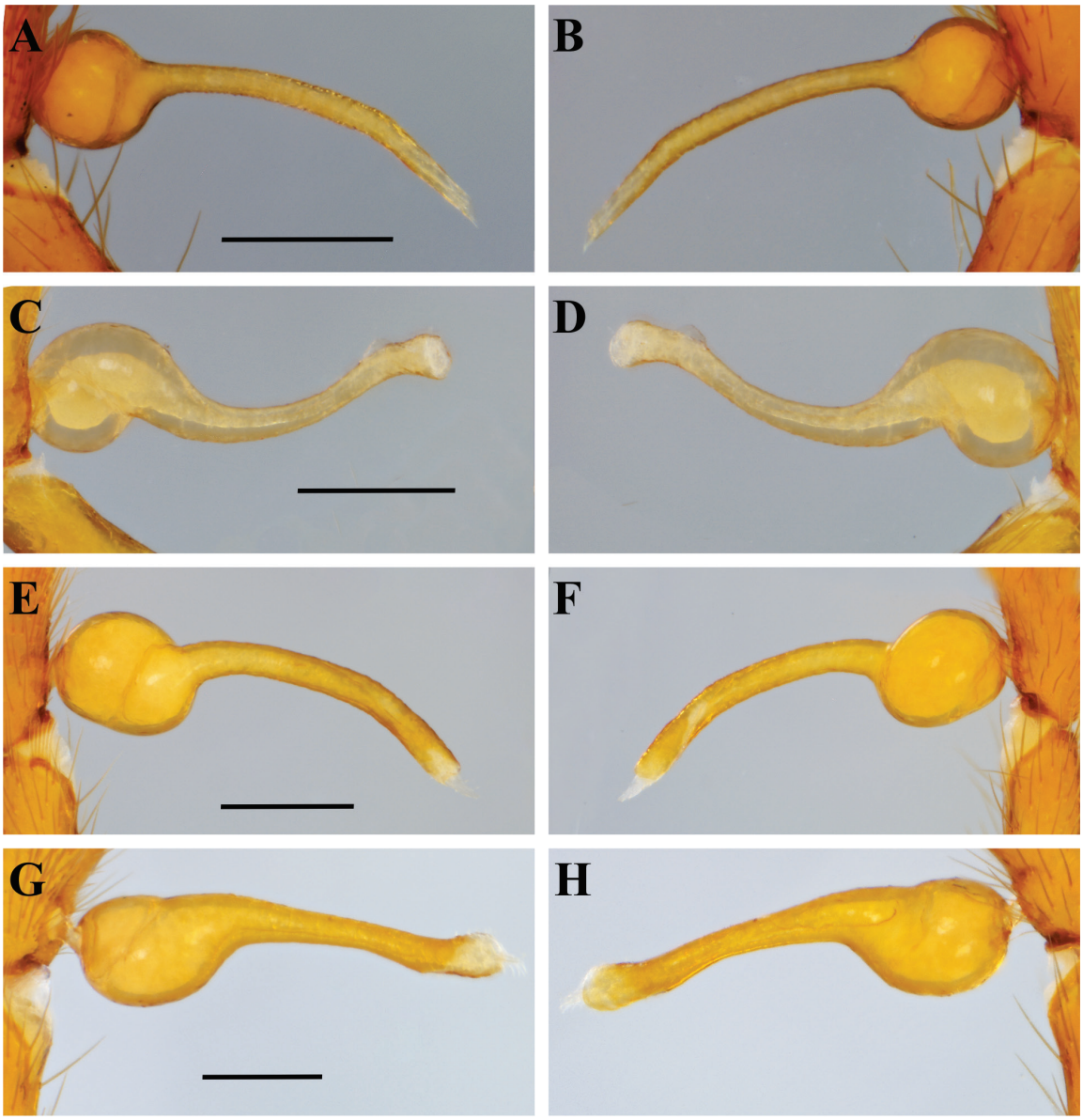


**Figure 5.** Male copulatory bulbs of Colombian *Nopsma* species. **A, B.** *Nopsma florencia* Sánchez-Ruiz, Brescovit & Bonaldo. **C, D.** *Nopsma macagual* sp. nov. E, F *Nopsma leticia* sp. nov. **G, H.** *Nopsma paya* sp. nov. **A, C, E, G.** Retrolateral view. **B, D, F, H.** Prolateral view. Scale bars: 0.7 mm.

within the country. Until now, only the males of *Nopsma* are well known since the few known female specimens belong to the type species, *N. juchuy* (Dupérré), which was internally studied by (Sánchez-Ruiz et al. 2020). In this paper we present the internal genitalia of *Nopsma paya* sp. nov., the second species represented by females within the genus. The internal genitalia of the Nopinae females are weakly sclerotized (Sánchez-Ruiz and Brescovit 2018) and sometimes it is necessary to dissect several females in order to resolve the internal morphology. Generally, internal female genitalia of Nopinae genera tend to be very similar among its species and only differences in the morphology of the receptaculum were observed in previous studies on these genera (see Platnick 1995; Sánchez-Ruiz et al. 2010, 2020; Sánchez-Ruiz and Brescovit 2017, 2018). The genus *Nopsma* seems to be no exception, since the female genitalia of *N. juchuy* and *N. paya* are very similar internally, with only few differences in the distal margin of the receptaculum (Fig. 7A, B). The straight distal margin of the receptaculum in *N. juchuy* (Fig. 7A, Sánchez-Ruiz et al. 2020: fig. 16G–J) appears to be diagnostic, while *N. paya* sp. nov. shows an invagination on distal margin of receptaculum with both sides slightly sloping (Fig. 7B). Furthermore, comparing the external genital area of both species we found also some discrete differences that are diagnostic for each of these species (see diagnosis of *N. paya* sp. nov.).

Members of *Nopsma* lack *crista* and *arolium* but retain the gladius on the anterior legs. Sánchez-Ruiz et al. (2020) suggested that this genus could be phylogenetically related to *Nyetnops* Platnick & Lise and *Cubanops* Sánchez-Ruiz, Platnick & Dupérré, since these three genera have several common characteristics in the shape and stain patterns of the cephalothorax, sharing a

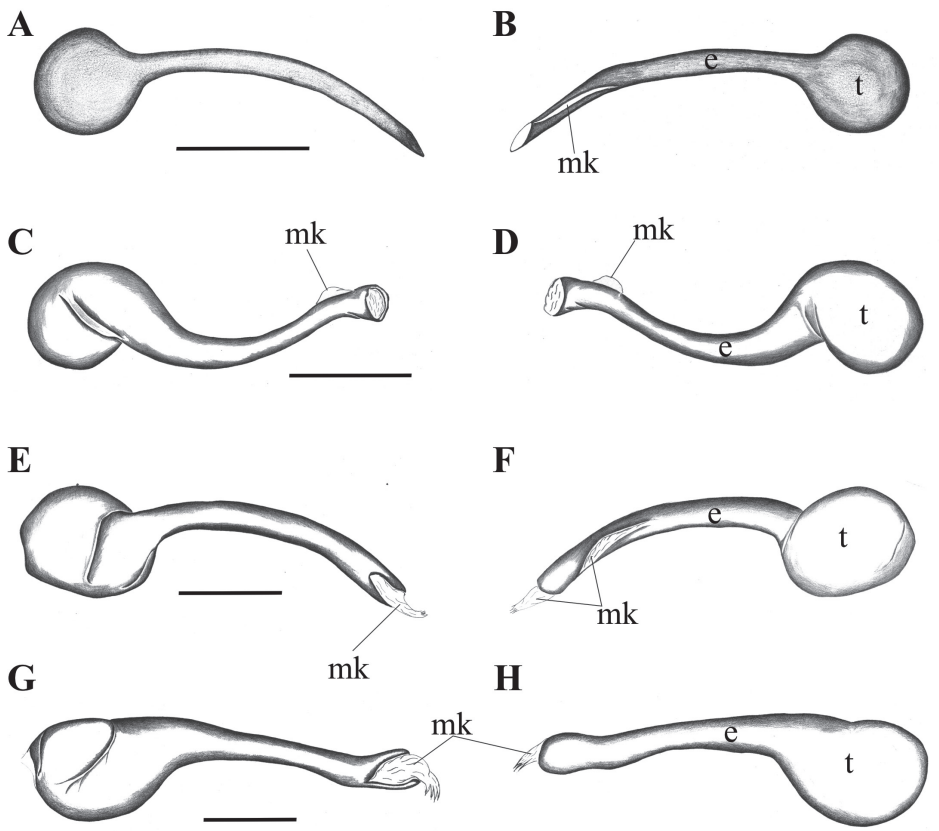


**Figure 6.** Drawings of male copulatory bulbs of Colombian *Nopsma* species. **A, B.** *Nopsma florencia* Sánchez-Ruiz, Brescovit & Bonaldo. **C, D.** *Nopsma macagual* sp. nov. **E, F.** *Nopsma leticia* sp. nov. **G, H.** *Nopsma paya* sp. nov. **A, C, E, G.** Retrolateral view. **B, D, F, H.** Prolateral view. Scale bars: 0.7 mm. Abbreviations: e = embolus, mk = membranous keel, t = tegulum.

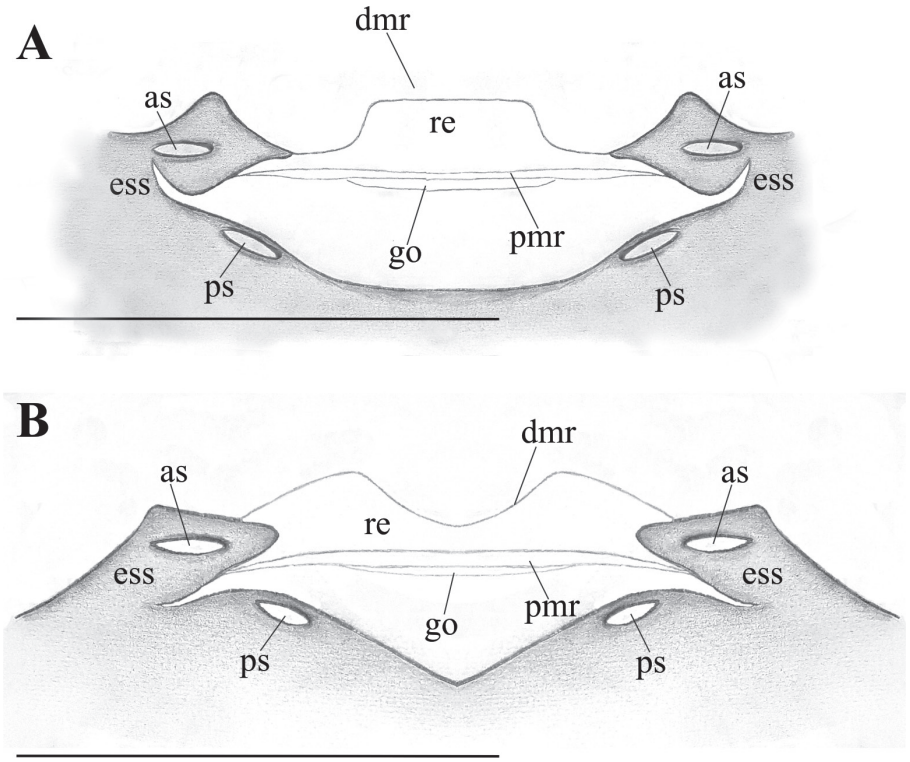


**Figure 7.** Schematic drawing of internal female genitalia in two *Nopsma* species. **A.** *Nopsma juchuy* (Dupérré). **B.** *Nopsma paya* sp. nov. Scale bars: **A, B:** 0.5mm. Abbreviations: as = anterior tracheal spiracles, dmr = distal margin of receptaculum, ess = external sclerotization around spiracles, go = genital opening, pmr = proximal margin of receptaculum, pp = posterior plate, ps = posterior tracheal spiracles, re = receptaculum.

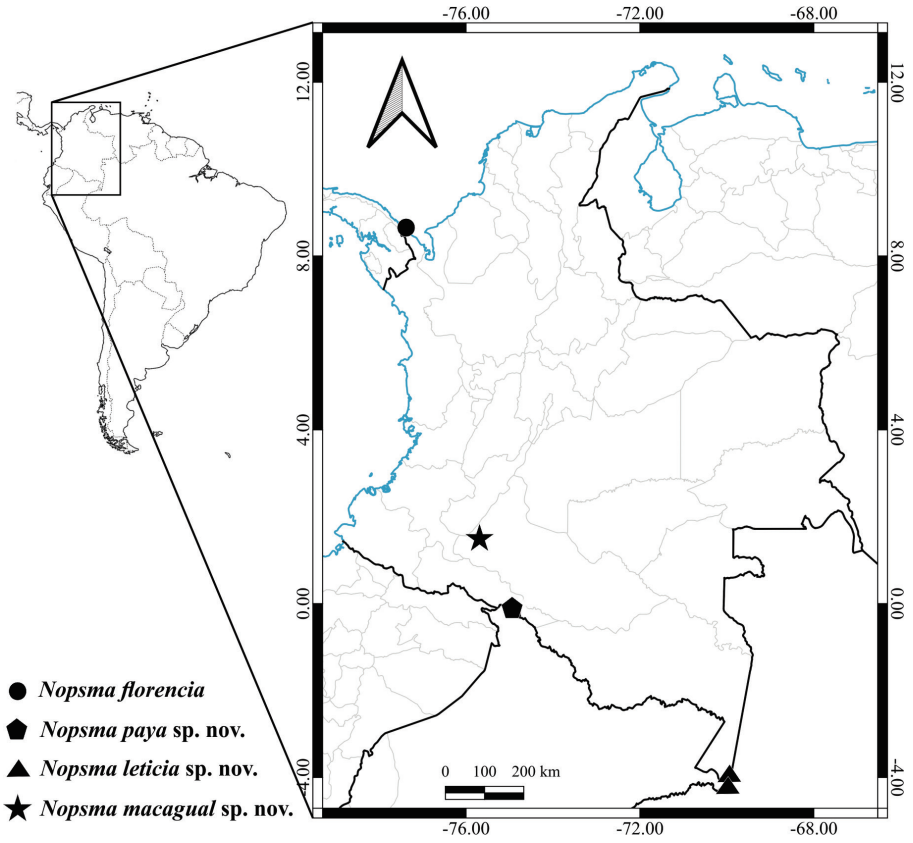


**Figure 8.** Distribution map of Colombian *Nopsma* species. *Nopsma florencia* Sánchez-Ruiz, Brescovit & Bonaldo (circle), *Nopsma leticia* sp. nov. (triangles), Nopsma macagual sp. nov. (star) and Nopsma paya sp. nov. (polygon).

distinct sub-circular, broad carapace shape, with narrow pars cephalica on dorsal view. Besides, the pars thoracica is elevated near the middle and slops abruptly posteriorly on lateral view. The only known species of *Nopsma* that diverges from those patterns is *Nopsma armandoi* Sánchez-Ruiz, Brescovit & Bonaldo (see Sánchez-Ruiz et al. 2020: fig. 17A). This species, however, exhibits the *Nopsma* diagnostic characteristics mentioned by Sánchez-Ruiz et al. (2020). Thus, although lacking an *arolium* on the pretarsi, *N. armandoi* has the cephalothorax similar to that presented by genera of the clade with *arolium* (*Nops* MacLeay, *Medionops* Sánchez-Ruiz & Brescovit and *Nopsides* Chamberlin). The absence of a membranous keel on the embolus tip is another important characteristic that set apart *N. armandoi* from other members of *Nopsma*. All presently known *Nopsma* species have this membranous keel, however *N. armandoi* has three, very thin, long projections at the embolus tip (see Sánchez-Ruiz et al. 2020: fig. 17B, C, F). These projections at the embolus tip again relate this species to those representatives from the *arolium* clade. Therefore, this species must be better studied, preferably with additional specimens, including the females which are currently unknown. An updated phylogeny of Nopinae that includes *Nopsma*, must address the hypothesis that this species would not belong to the genus, but instead to a hitherto undescribed lineage of Nopinae in which the loss of *arolium* has occurred independently.

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PENSOFT

für Natur MUSEUM FÜR NATURKUNDE BERLIN

# Review of the Australian and New Zealand orb-weaving spider genus *Novakiella* (Araneae, Araneidae)

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## Abstract

The orb-weaving spider genus *Novakiella* Court & Forster, 1993 (family Araneidae Clerck, 1757) is reviewed to include two species, *N. trituberculosa* (Roewer, 1942) (type species, Australia and New Zealand) and *N. boletus* **sp. nov.** (Australia). *Novakiella* belongs to the informal, largely Australian 'backobourkiine' clade and shares with the other genera of the clade a single macroseta on the male pedipalp patella and a median apophysis of the male pedipalp that forms an arch over the radix. The proposed genus synapomorphies are the presence of a large basal conductor lobe expanding apically over the radix and the shape of the median apophysis, which extends into a basally directed, pointy projection. Males have an apico-prolateral spur on the tibia of the second leg that carries a distinct spine. Females have an epigyne with triangular base plate bearing transverse ridges and an elongate triangular scape, which is almost always broken off. The humeral humps of the abdomen are distinct. *Novakiella trituberculosa* build characteristic dome-shaped webs; however, the foraging behaviour and web-shape of *N. boletus* **sp. nov**., currently only known from museum specimens, are not known.

## Key Words

dome-shaped orb-web, new species, systematics, taxonomy

## Introduction

A recent multi-gene molecular phylogenetic analysis of the orb-weaving spider family Araneidae Clerck, 1757 recovered a well-supported Australasian clade informally termed 'backobourkiines' (Scharff et al. 2020). This clade includes species that would previously have been considered members of the Araneinae Clerck, 1757 (see Scharff and Coddington 1997), specifically those in the genera *Acroaspis* Karsch, 1878; *Backobourkia* Framenau, Dupérré, Blackledge & Vink, 2010; *Carepalxis* L. Koch, 1872; *Novakiella* Court & Forster, 1993 and *Plebs* Joseph & Framenau, 2012; but also *Singa* C. L. Koch, 1836, which has an almost global biogeographical distribution. In addition, the backobourkiines harbour Australian species apparently wrongly placed at the genus level, including some species currently listed in *Araneus* Clerck, 1757, *Eriophora* Simon, 1864 and *Parawixia* F. O. Pickard-Cambridge, 1904. All these genera were polyphyletic in Scharff et al.'s (2020) analysis, with Australian species placed in clades outside

those that included the respective type-species or assumed close relatives from the same biogeographic region.

Morphologically, the backobourkiines are still poorly circumscribed, although a single macroseta on the male pedipalp patella and the median apophysis forming an arch over the radix seem to morphologically unite all genera against many clades previously considered part of the traditional Araneinae (Scharff and Coddington 1997; Scharff et al. 2020). An ongoing revision of Australian Araneidae suggests that the backobourkiines include many more described – generally misplaced at genus level – and undescribed species.

The orb-weaving spider genus *Novakiella* was not diagnosed in detail when initially described (sub *Novakia* Court & Forster, 1988). However, the original description of the genus pointed to a peculiar genital morphology and an unusual horizontal orb-web "*drawn up into a cone by threads attached to the hub*" built by the type species, *N. trituberculosa* (Roewer, 1942) (Court and Forster 1988, p. 123, figs 563–566). Therefore, *Novakiella* is easily distinguished from other backobourkiine spiders by their male pedipalp morphology with a distinct enlarged conductor lobe and the curved and basally projected median apophysis (Court and Forster 1988, figs 559, 560). Female epigynes have a subtriangular base plate with transverse ridges. The scape is wrinkled, elongate triangular and extends posteriorly past the base plate (Court and Forster 1988, figs 554, 555). The genus currently comprises the type species only, originally described from New Zealand but also known from Australia (Court and Forster 1988). The recent discovery of a second species from Australia allows a more comprehensive generic diagnosis of the genus in comparison to other backbourkiines. Therefore, we herein review *Novakiella*, provide a modern diagnosis against other backobourkiine orb-weavers and (re)describe its two species.

## Materials and methods

Descriptions and terminology follow recent publications on Australian orb-weaving spiders (e.g., Framenau et al. 2010; Joseph and Framenau 2012; Castanheira et al. 2019). Colour patterns are described based on specimens preserved in 75% ethanol. Male pedipalps were expanded by alternatively submerging it for 10 min in 10% KOH and distilled water until fully expanded. Female genitalia of *N. trituberculosa* were dissected and cleared with lactic acid. Measurements are given in millimetres.

Images of specimens were taken in different focal planes with a Nikon D300 digital SLR camera attached via a C-mount adapter from LM-Scope (http://www.lmscope.com) to a Leica M16A stereomicroscope and combined with Auto Montage (vers. 5.02) software from Syncroscopy to increase depth of field. We used 2 Nikon R1C1 wireless speedlights instead of fibre optics to illuminate the exposures. The latter were used as guide-light for focusing. Microscopic images of cleared epigynes were taken in different focal planes (ca. 20–30 images) on a Leica DMC4500 digital camera mounted to a Leica M205C stereomicroscope and combined using the Leica Application Suite X, v. 3.6.0.20104. Images of expanded pedipalps of *N. trituberculosa* were taken with a BK Plus Laboratory System from Visionary Digital (Palmyra, PA, USA) equipped with a Canon EOS 7D camera. All photos were edited and mounted with Photoshop CC 2020.

Maps were compiled in the software package QGis v. 2.14.0 Girona (https://qgis.org/en/site/; accessed 21 January 2020). Geographic coordinates were extracted directly from original labels or the registration data as provided by the museums. When no detailed geographic information was available, localities were estimated based on Google Earth v. 9.1.39.3 (https://earth.google.com/web/; accessed 21 January 2021).

### Abbreviations

*Collections*

| | |
|---|---|
| **AM** | Australian Museum, Sydney, Australia; |
| **CMNZ** | Canterbury Museum, Christchurch, New Zealand; |
| **LUNZ** | Lincoln University Entomology Research Collection, New Zealand; |
| **MV** | Museums Victoria, Melbourne, Australia; |
| **NZAC** | New Zealand Arthropod Collection, Auckland, New Zealand; |
| **QM** | Queensland Museum, Brisbane, Australia; |
| **QVM** | Queen Victoria Museum and Art Gallery, Launceston, Australia; |
| **SAM** | South Australian Museum, Adelaide, Australia; |
| **WAM** | Western Australian Museum, Perth, Australia; |
| **NHMD** | Natural History Museum of Denmark, Zoological Museum, University of Copenhagen, Denmark. |

*Morphology*

Males: C, conductor; CL, conductor lobe; E, embolus; MA, median apophysis; P, paracymbium; Ra, radix; TA, terminal apophysis. Females: CO, copulatory openings; S, scape; Sp, spermatheca.

## Taxonomy

### Class Arachnida Cuvier, 1812
### Order Araneae Clerck, 1757
### Family Araneidae Clerck, 1757

**Genus *Novakiella* Court & Forster, 1993, in Platnick (1993)**

*Novakia* Court & Forster, 1988: junior homonym of *Novakia* Strobl, 1893 (Diptera) and *Novakia* Tolmachoff, 1926 (Mollusca) is another junior homonym.

**Type-species.** *Epeira tri-tuberculata* Urquhart, 1887.

**Diagnosis.** The informal clade of the backobourkiines is well supported by the molecular phylogeny of Scharff et al. (2020), but the taxonomy and systematics of the species and genera within this clade are poorly resolved. Only three genera within the clade have been revised using modern taxonomic methods: *Plebs*, *Backobourkia* and *Lariniophora* Framenau, 2011. The genera *Carepalxis* and *Acroaspis* have not been revised and their putative synapomorphies remain unknown. It is therefore difficult to diagnose *Novakiella* against these genera. Other Australian backobourkiines included in Scharff et al. (2020) represent species that have clearly been misplaced in genera they do not belong to (i.e., *Eriophora* or *Araneus*) and these represent undescribed genera (in that study listed as "NGEN01" for *Eriophora transmarina* (Keyserling, 1865), "NGEN02" for *Araneus recherchensis* Main, 1954 and "NGEN05" for *Araneus senicaudatus* Simon, 1908). Until these species have been revised and placed in new or existing genera, *Novakiella* cannot be diagnosed against them.

*Novakiella* distinctly differs from the revised backobourkiine genera by overall somatic morphology. The abdomen is subtriangular with strong humeral humps (Figs 1A, 3A, 4A, 6A), while it is rounded with small humerals in *Backobourkia* (i.e., Framenau et al. 2010, fig. 5), slightly elongated in *Plebs* (e.g. Joseph and Framenau 2012, figs 6, 7, 10), and strongly elongated in *Lariniophora* (Framenau 2011, figs 2, 3). Males can be differentiated by the presence of a tibial apico-prolateral spur carrying a thick spine (or macroseta) on leg II (Figs 1E, 4C). *Verrucosa* McCook, 1888 and *Carepalxis* also have a spur on leg II, but in both genera it carries two spines (Levi 2002: p. 546; Lise et al. 2015: p. 5; VWF pers. obs.). In addition, *Verrucosa* is limited to the Neotropics and not part of the backobourkiines (Scharff et al. 2020). There are distinct differences in the male pedipalp morphology between *Novakiella* and other backobourkiines. *Novakiella* males have a stout median apophysis that is drawn out into a basally pointing acute projection (Figs 1C, 2B, 4D, E, 5); in contrast, the median apophysis has a basal flange in *Backobourkia* (Framenau et al. 2010) (absent in *Novakiella*), is elongate transverse with two apical tips in *Plebs* (Joseph & Framenau, 2012, e.g. figs 4B, 8A), and has a two-humped lobe in *Lariniophora* (Framenau, 2011, fig. 4). All backobourkiines appear to have a basal extension of the conductor (discussed in Framenau et al. 2010 and there termed paramedian apophysis), but in *Novakiella* it is very different to all other described backobourkiines and much more conspicuous; we here propose a new term, conductor lobe (CL), which extends apically well past the radix (Figs 1C, 2A, B, 4D–F, 5).

Females of *Novakiella* have an elongated triangular scape without terminal pockets, as is typical for all backobourkiines above; however, these genera lack the subtriangular base plate with its transverse and lateral wrinkles (Figs 3C, 6C; Framenau et al. 2010, e.g. figs 6D, F; Framenau 2011, fig. 6; Joseph and Framenau 2012, e.g. figs 4D, 8E).

**Description**. Medium-sized (TL males ca. 5–9, females 8–12) orb-weaving spiders with males on average slightly smaller than females. Carapace longer than wide, pear-shaped; cephalic area similar in shape in both sexes (Figs 1A, 3A, 4A, 6A); fovea longer than wide in males and wider than long in females, and with a dark spot in both sexes (Figs 1A, 3A, 4A, 6A); colouration (of ethanol preserved specimens) varying from reddish-brown to yellowish-brown, with black patches along carapace borders (Figs 1A, 3A, 4A, 6A). Eyes ringed in black, anterior median eyes largest, posterior eye row slightly recurved, lateral eyes almost touching, posterior lateral eyes separated from posterior median eyes by more than their diameter and located on small tubercles at the clypeus border (Figs 1A, 3A, 4A, 6A). Chelicera paturon with dark hue, fangs reddish-brown. Labium wider than long, subtriangular, with front end bulging and beige (Figs 1B, 3B, 4B, 6B). Endites rounded, inner portion beige (Figs 1B, 3B, 4B, 6B). Sternum almost as long as wide with dark contour (Figs 1B, 3B, 4B, 6B). Legs (Figs 1A, B, E, 3A, B, 4A–C, 6A, B): Leg formula IV > I > II > III, all longer than body's length with dark spots on joints; tibia II of males with apico-prolateral spur bearing a thick macroseta or spine (less pronounced in *N. boletus* sp. nov.). Abdomen subtriangular, longer than wide, with two distinct humeral humps and posterior tip reaching beyond spinnerets (Figs 1A, 3A, 4A, 6A); folium pattern distinct; sides varying in colour from yellowish-brown to black (Figs 1A, 3A, 4A, 6A), venter light coloured, generally mottled dark (Figs 1B, 3B, 4B, 6B). Male genitalia (Figs 1C, D, 2A, B, 4D–F, 5): male pedipalp patella with a single strong macroseta; paracymbium well-developed and hook-like; cymbium longer than wide; radix thick and elongated, reaching from the base of median apophysis to near the cymbium tip; conductor lobe conspicuous and projected apically, being composed of two distinct lobes (*N. trituberculosa*) or mushroom-shaped (*N. boletus* sp. nov.); terminal apophysis wider than long, rounded and tapering terminally; conductor well-developed, subquadrate; embolus uncapped, elongated, pointed and almost straight; median apophysis stout, with an acute basally pointing tip. Female genitalia (Figs 3C–E, 6C): epigyne plate wider than long, subtriangular; scape much longer than wide and extending posteriorly beyond plate (but length not known in *N. boletus* sp. nov.), generally broken off. Spermathecae spherical and occupying most of genital area.

**Composition.** *Novakiella trituberculosa* (Roewer, 1942) and *N. boletus* sp. nov.

**Remarks.** The nomenclatural history of *Novakiella* is convoluted. *Novakiella trituberculosa* was first described as *Epeira tri-tuberculata* by Urquhart (1887), before Roewer (1942) replaced the species-group name as it is a junior primary homonym of *Epeira trituberculata* Lucas, 1846, currently listed as a junior synonym of *Cyclosa insulana* (Costa, 1834). Court and Forster (1988) described the genus *Novakia* to accommodate this species; however, this new genus-group name was also preoccupied, by *Novakia* Strobl, 1893 (Diptera) and *Novakia* Tolmachoff, 1926 (Mollusca). Court and Forster, in Platnick (1993), proposed *Novakiella* Court & Forster, 1993 as a replacement name.

**Distribution.** Australia and New Zealand (Figs 7, 8).

### *Novakiella trituberculosa* (Roewer, 1942)

Figs 1–3, 7, 8

*Epeira tri-tuberculata* Urquhart 1887: 78–79, pl. 7, fig. 2; pl. 8, fig. 1 (preoccupied by *Epeira trituberculata* Lucas, 1846; currently listed as junior synonym of *Cyclosa insulana* (Costa, 1834).

*Epeira tri-tuberculata* Urquhart 1888: 120–121, pl. 11, figs 7, 8.

*Aranea trituberculosa* Roewer 1942: 834 (replacement name).

*Novakia trituberculata* Court and Forster 1988: 119–124, figs 359, 553–562.

*Novakiella trituberculosa* Court and Forster, in Platnick 1993: 457.

**Type material.** Syntypes of *Epeira tri-tuberculata* Urquhart, 1887, 2 females, Karaka, New Zealand (37°06'S, 174°53'E), A.T. Urquhart (CMNZ 2005.135.112). Examined.

**Other material examined. AUSTRALIA: Australian Capital Territory**: 1 female, 1 juvenile, Canberra, 35°18'S, 149°08'E (SAM); 1 female, Kaleen, Canberra, 35°17'S, 149°13'E, 25.iv.1990 (SAM 31192); 1 male, 1 female, Kaleen, Canberra, 35°17'S, 149°13'E, 21.v.1988 (SAM); 1 male, Red Hill, 14 Pera Place, 35°20'S, 149°08'E, 24.ii.1982, M. S. Harvey leg. (WAM T73527); 1 male, same locality, 01.iv.1983, M. S. Harvey leg. (WAM T73571); **New South Wales:** 1 male, Beecroft, 33°45'S, 151°04'E, 25.i.1999, J. Noble leg. (AM KS58630); 1 female, Botany, 33°57'S, 151°12'E, 04. vii.1961, R. Mascord leg. (AM KS32651); 1 female, same locality, 26.vii.1966, R. Mascord leg. (AM KS32652); 1 female, Coonabarabran, 'Smokey Hole', 31°16'S, 149°17'E, 04.x.1978, E. Edmondson leg. (AM KS7545); 1 male, Dulwich Hill, 33°54'S, 151°08'E, 17. ii.1977, H. Ehmann leg. (AM KS0741); 1 male, Epping Strip, 33°46'S, 151°05'E, 10.i.1996, J. Noble leg. (AM KS49920); 1 male, Hillsdale, Sydney, 33°57'S, 151°13'E, 03.ii.1972, R. Mascord leg. (AM KS34148); 1 male, Khancobin, 26.iv.1990 (SAM); 1 female, Lord Howe Island, Erskine Valley Transect, 31°34'58"S, 159°04'45"E, pitfall traps, 01.xi.1978, T. Kingston leg. (AM KS88175); 1 male, Lord Howe Island, Goat House Cave, 31°33'54"S, 159°05'18"E, 10.ii.1971, M. Gray leg. (AM KS20999); 1 female, Mount Colah, 33°40'S, 151°07'E, M. Gray leg. (AM KS48820); 1 male, Park Beach, Coffs Harbour, 30°18'S, 153°07'E, 23–24.v.1986 (SAM), 1 male, Punchbowl, 33°56'S, 151°03'E, 02.xii.1940, Ms Levitt leg. (AM KS33550); 1 male, The Rock Nature Reserve, 30km SW Wagga Wagga, 35°16'S, 147°05'E, sweeping/beating, 13.xii.2000, C. A. Car leg. (AM KS93847); **Queensland:** 1 male, Bardon, Brisbane, 27°27'S, 152°58'E (QM); 1 female, Burleigh Heads, 28°06'S, 153°26'E (QM); 1 male, 1 juvenile, Endfield Station, 27°55'S, 149°43'E (QM); 1 female, Eurimbula, 24°11'S, 151°50'E, C. Horseman leg. (AM KS12771); 1 female, Gatton, Queensland Agricultural College, 27°34'S, 152°20'E, S. Pearce leg. (QM S66755); 1 male Hurdle Gully, 13km WSW Monto, 24°54'00"S, 150°59'55"E, 23.ix–20.ix.1997, G. Monteith leg. (QM); 1 male, Jerons St Park, 27.xi.2009, R. Whyte leg. (WAM S84676); 1 male, Jevons, 26.xii.2009, R. Whyte leg. (QM S84670); 1 female, same locality, 01.i.2010, R. Whyte leg. (QM S84672); 1 male, Masthead Island, Great Barrier Reef, 23°32'S, 151°44'E, C. Hedley leg. (AM KS32650); 1 male, Mt Gavial, 1km S, 23°36'S, 150°29'E, 17.xii.1998, D. J. Cook leg. (QM S69354); 1 male, Oakey, 27°27'S, 151°42'E (QM); 1 male, Teewah Creek, Cooloola, 26°02'S, 153°03'E (QM); 1 female, Walton Bridge Reserve, 20.xi.2009, R. Whyte leg. (QM S84666); **South Australia:** 1 male, Baird Bay, 33°09'S, 134°22'E, 12.i.1995, J. M. Waldock & P. Payne leg. (WAM T73553); 1 male, 1 female, Beautiful Valley Caravan Park, adjacent, near Wilmington, 32°39'S, 138°06'E, 14.iv.1993 (SAM); 1 male, Belalie Creek, Jamestown, 33°12'S, 138°36'E, 10.iv.1993, D. Hirst leg. (SAM); 2 males, Cape Gantheaume, 1km N Point Tinline, Kangaroo Island, 35°59'S, 137°37'E, 10.xi.1987, D. Hirst leg. (SAM); 1 male, Caracoorte Cave Reserve, 37°05'S, 140°47'E, 25.iv.1979, D. Lee leg. (SAM); 1 female, Caroline Forest, 'Snowgum Reserve', 37°56'S, 140°56'E, 20.iv.1979, G. Grass leg. (SAM); 1 male, Coromandel Valley, Mt Lofty Ranges, 32°05'S, 138°38'E, 02.iii.1996, L. N. Nicolson leg. (SAM); 1 female, Dog Lake Road, SE Langhorne Creek, 35°17'S, 139°02'E, J. Eckert leg. (SAM); 1 female, Eucla, 77km E, 31°28'S, 129°37'E, 23.ii.1978, B. Y. Main leg. (WAM T87424); 1 female, Forestville, 34°57'S, 138°35'E (SAM); 1 male, Gluepot Reserve, 11.3km W Gluepost Homestead, 33°45'16"S, 139°59'58"E, 26.xi.–06.xii.2000 (SAM NN19454); 1 female, Kangaroo Island, 35°45'S, 137°37'E (SAM); 1 male, Kangaroo Island, Western River Wilderness Protection Area, Waterfall Creek near waterfall, 35°41'44"S, 136°54'37"E, beating, 09–10.v.2010, M. G. Rix & D. Harms leg. (WAM T102789); 1 male, Lake Gilles Conservation Park, 33°05'S, 136°39'E, 21.xi.1995 (SAM); 1 female, Langhorne Creek, 35°18'S, 139°02'E, C. Wilson leg. (SAM); 1 female, 1 juvenile, Marino Rocks to Halletts Cove, near railway line, 35°04'S, 138°30'E, 05. ix.1967, H. M. Cooper leg. (SAM); 2 females, Melrose, camping area, 32°49'S, 138°11'E, 14.iv.1987, D. Hirst leg. (SAM); 1 male, Mitcham, 34°59'S, 138°37'E, 21.i.1979, R. V. Southcott leg. (SAM); 2 females, Muston, Kangaroo Island, 35°49'S, 137°44'E, 03.vii.1967, H. M. Cooper leg. (SAM); 1 female, Nappyalla, 35°20'S, 139°07'E, J. Eckert leg. (SAM); 1 female, Port Wakefield, 34°11'S, 138°09'E, 14.iii.2004, B. S. Pavey leg. (SAM); 1 female, Port Wakefield, T-junction W, 34°11'S, 138°09'E, B. Pavey leg. (SAM); 1 male, Pyap, 34°27'S, 140°29'E, 02–09.vi.1990, L. N. Nicolson leg. (SAM); 1 female, Sellicks-Aldinga Scrub, 35°17'S, 138°27'E, 22.ix.1987, D. Hirst leg. (SAM); 1 male, Serpentine Lakes, 28°30'S, 129°00'E, 16.iv.1994, D. Hirst leg. (SAM); 1 female, St Peters, Adelaide, 33°55'S, 151°11'E, 27.i.1975, P. Walker leg. (AM KS32104); 1 male, Walkerville, Adelaide, 34°53'S, 138°37'E, 20.vi.1984, J. Thurmer & D. Hirst leg. (SAM); 1 male, Windsor Gardens, Adelaide, 34°52'S, 138°39'E, 20.vi.1988, D. Hirst leg. (SAM); 2 males, East Risdon, 42°50'S, 147°21'E, 27.iv.1961, V. V. Hickman leg. (AM KS28582); **Tasmania:** 3 males 1 juvenile, Launceston, 41°27'S, 147°10'E, 06.iv.1928, V. V. Hick-

man leg. (AM KS28538), 1 female, same locality, 03. ix.1929, V. V. Hickman leg. (AM KS28547); 1 female, Liberty Creek Track, S side Macquarie Heads, 42°12'S, 145°12'E (QM); 3 males, 2 females, 2 juveniles, New Town, 42°53'S, 147°19'E, 10.ii.1934, V. V. Hickman leg. (AM KS28699); 1 female, St. Columba Falls, 41°19'17"S, 147°55'34"E, 07.iii.2006, G. Hormiga leg. (NHMD); **Victoria:** 1 female, Balwyn, 24 Yandilla Street, 37°48'S, 145°04'E, 05.iv.1981, M. S. Harvey leg. (WAM T73551); 1 male, same locality, 31.iii.1981, M. S. Harvey leg. (WAM T73572); 1 male, same locality, 12.i.1981, M. S. Harvey leg. (WAM T73573); 1 male, same locality, 05. iv.1981, M. S. Harvey leg. (WAM T73574); 1 male, same locality, 28.xii.1982, M. S. Harvey leg. (WAM T73577); 1 male, Beaconsfield, 38°03'S, 145°15'E, 07.xi.1893 (MV K9424); 1 female, Cann River, 20km N, 37°17'S, 149°12'E, 17.vi.1987, R. J. Raven leg. (QM S13154); 1 female, Canterbury, 7 Quantock St, 37°49'S, 145°04'E, 13.vi.1981, M. S. Harvey leg. (WAM T73556); 3 females Croydon, 37°47'S, 145°16'E, 28.ii.1999, S. W. Fulton leg. (MV K10088); 1 male, 1 female, 1 juvenile, Echuca, 36°08'S, 144°45'E, 01.xi.1955 (MV K9427); 1 female, Emerald, 37°56'S, 145°27'E, 13.v.1948, C. Oke leg. (AM KS32504); 1 female, Frankston, 38°08'S, 145°08'E, 30. iv.1994 (SAM); 1 male, Nyah to Kooloonong (no exact location), B. Harvey leg. (MV K9451); **Western Australia:** 1 female, Attadale, 32°01'S, 115°48'E, 20.vii.1962, A. R. Main leg. (WAM T87192); 1 female, Bannister, 32°39'S, 116°33'E, 15.vi.1985, B. Y. Main leg. (WAM T73564); 1 female, Bannister, 32°39'S, 116°33'E, 15. vi.1985, B. Y. Main leg. (WAM T73565); 1 female (WAM T73566); 1 male, Boolathana Station, 24°24'49"S, 113°44'41"E, pitfall trap, 15.i.–31.v.1995, J. M. Waldock et al. leg. (WAM T73548); 1 female, Boya, Helena Valley, 31°54'S, 116°03'E, 06.x.1982, B. Y. Main leg. (WAM T73561); 1 female (WAM T73562); 1 female, same locality, 08.x.1982, P. Hussey leg. (WAM T73563); 1 male, Commonwealth Road, West, 32°44'13"S, 118°16'16"E, wet pitfall trap, 30.x.1997–15.v.1998, N. A. Guthrie leg. (WAM T74852); 1 female, Durokoppin Nature Reserve, 31°24'S, 117°46'E, 03.vi.1989, B. Y. Main leg. (WAM T73567); 1 female, same locality, 05.v.1987. B. Y. Main leg. (WAM T73568); 1 male, Grass Patch, Fitzg. Loc. 41, 32°13'56"S, 121°46'00"E, 29.xi.1978, A. F. Longbottom leg. (WAM T73578); 1 male, Hurlstone Nature Reserve, 32°32'32"S, 119°22'42"E, wet pitfall trap, 30.x.1997–20.v.1998, P. van Heurck et al. leg. (WAM T74854); 1 male, Jarrahdale (Alcoa) Mine area, 31°16'S, 116°06'E, K. E. C. Brennan leg. (WAM T48214); 1 female, vacuum collector, M. L. Moir leg. (WAM T48215); 1 female, N of Lake King-Norseman Road, 33°04'54"S, 119°59'53"E, wet pitfall trap, 15.x.1999–25.x.2000, N. A. Guthrie leg. (WAM T74863); 1 male, Lake Morgan, Helms Arboretum, 33°43'09"S, 121°48'29"E, wet pitfall trap, 15.x.1999–01.xi.2000, P. van Heurck et al. leg. (WAM T74853); 1 male, Mount Gibson iron-ore mine, 29°36'02"S, 117°12'25"E, pitfall trap, 15–30.iv.2005, S. Thompson leg. WAM T67918); 1 male, Roe Plain, between Mundrabilla and Madura, 32°04'S, 126°31'E, 26. ii.1990, B. Y. Main leg. (WAM T87431); 1 female, Stirling Range National Park, Moingup Spring, 34°24'S, 118°06'E, 10.vi.1993, J. M. Waldock & A. Sampey leg. (WAM T74423); 1 female, Torbay Hill, Lot 40, 35°04'S, 117°37'E, 07.x.1983, B. Y. Main leg. (WAM T73569); 1 female, same locality, 06.x.1993, B. Y. Main leg. (WAM T73570); 1 female, Two Peoples Bay Nature Reserve, track near coast, 34°59'25"S, 118°09'35"E, 01.v.2008, M. Rix & M. S. Harvey leg. (WAM T81707). **NEW ZEALAND: North Island:** 1 male, Paranui Scenic Reserve, 35°05′S 173°27′E, at night, 16.ii.2000, G. Hall leg. (NZAC 03038710); 1 male, Karaka, 37°06'S, 174°53'E, A.T. Urquhart leg. (CMNZ 2005.135.112); 1 male, Hamilton, 37°48′S 175°18′E, 2014, B.N. McQuillan leg. (LUNZ 00012963); 1 female, Hamilton, 37°48′S 175°18′E, 2014, B.N. McQuillan leg. (LUNZ 00012964); **South Island:** 1 female, Kaituna Valley, 43°44′S 172°42′E, 24.v.1975, R.R. Forster leg. (NZAC 03038712).

**Diagnosis.** Male *N. trituberculosa* can easily be distinguished from *N. boletus* sp. nov. by the much stronger apico-prolateral spur on tibia of leg II and the morphology of key pedipalp sclerites (Figs 1C–E, 2A, B, 4D–F, 5): median apophysis with a longer basal portion and a smaller pointed curved and acute tip in *N. trituberculosa* (projection to the tip flattened, much longer, reaching beyond the radix base and tip rounded in *N. boletus* sp. nov.), conductor lobe two-lobed in *N. trituberculosa* (mushroom-shaped in *N. boletus* sp. nov.), and embolus almost straight in *N. trituberculosa* (with tip strongly bent, thin and very acute in *N. boletus* sp. nov.). Females of *N. trituberculosa* differ from those of *N. boletus* sp. nov. by details in the epigyne plate (Figs 3C, 6C), specifically its subtriangular shape in *N. trituberculosa* (trapezoidal in *N. boletus* sp. nov.); the transverse and short wrinkles, mainly laterally visible in *N. trituberculosa* (more pronounced and crossing the plate in *N. boletus* sp. nov.); copulatory openings less conspicuous in *N. trituberculosa* (clearly visible in *N. boletus* sp. nov.); and bridge thin and longer in *N. trituberculosa* (subtriangular, much wider at posterior margin in *N. boletus* sp. nov.).

**Redescription.** *Male* (WAM T73571 [images]; WAM T73573 [measurements]): Total length: 5.3. Carapace (Fig. 1A) 2.7 long, 2.1 wide, light brown with large black bands on lateral margins and yellowish setae throughout except from fovea to pedicel; cephalic area subquadrate; fovea longer than wide and bearing a long black spot. Eyes ringed in black, lateral ones located on small tubercles (Fig. 1A). AME 0.22, ALE 0.11, PME 0.14, PLE 0.11; row of eyes: AME 0.58, PME 0.40, PLE 0.86. Chelicerae with paturon light brown and fangs reddish-brown; three promarginal teeth, central one largest, two or three retromarginal teeth, basal one largest. Legs (Fig. 1A, B, E) yellow with dark brown spots on joints; tibiae II with strong apico-prolateral spur that carries a stout spine; metatarsi and tarsi of leg II slightly curved; leg formula IV > I > II > III; length of segments (femur + patella + tibia + metatarsus + tarsus = total length): I – 3.97 + 1.30

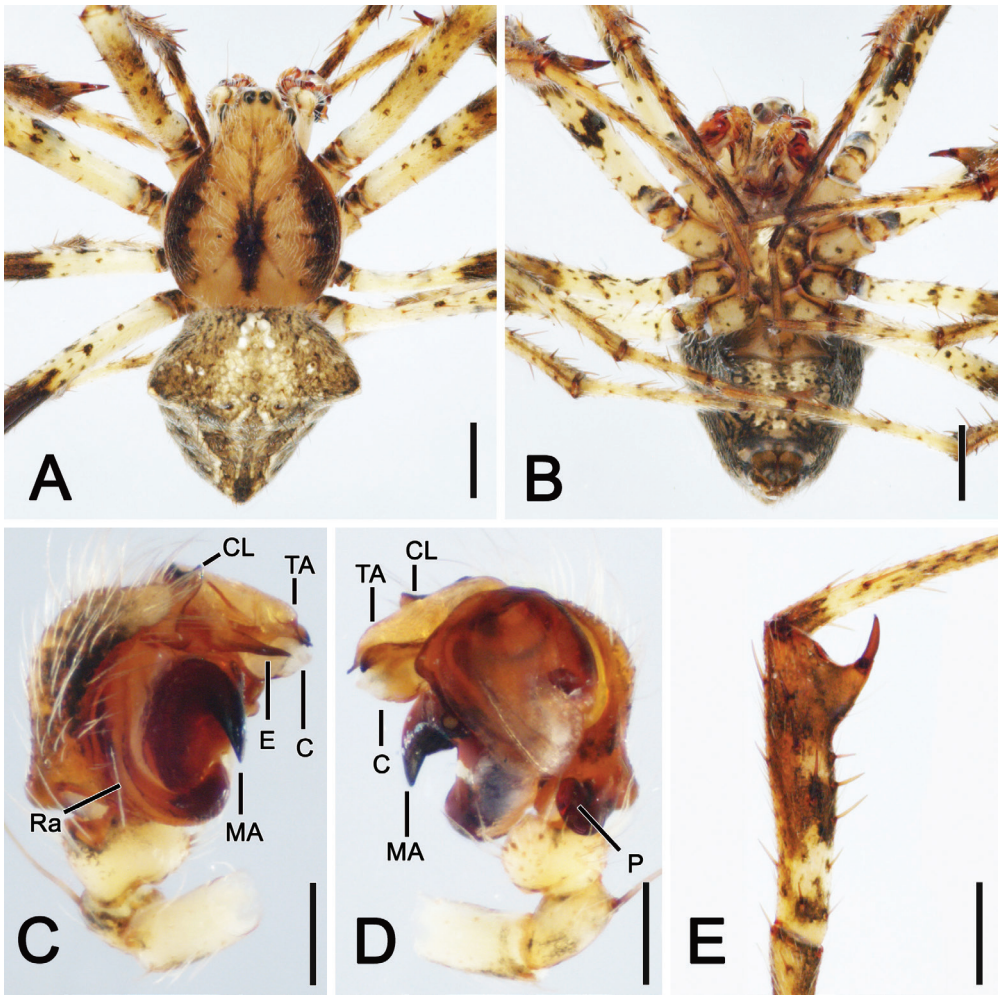


**Figure 1.** *Novakiella trituberculosa*, male (WAM T73571). **A.** Dorsal habitus; **B.** ventral habitus; **C.** Left pedipalp, mesal view; **D.** Left pedipalp, ventral view. Abbreviations: C, conductor; CL, conductor lobe; E, embolus; MA, median apophysis; P, paracymbium; Ra, radix; TA, terminal apophysis. Scale bars: **A, B,** 1 mm; **C, D,** 0.5 mm; **E,** 1.2 mm.

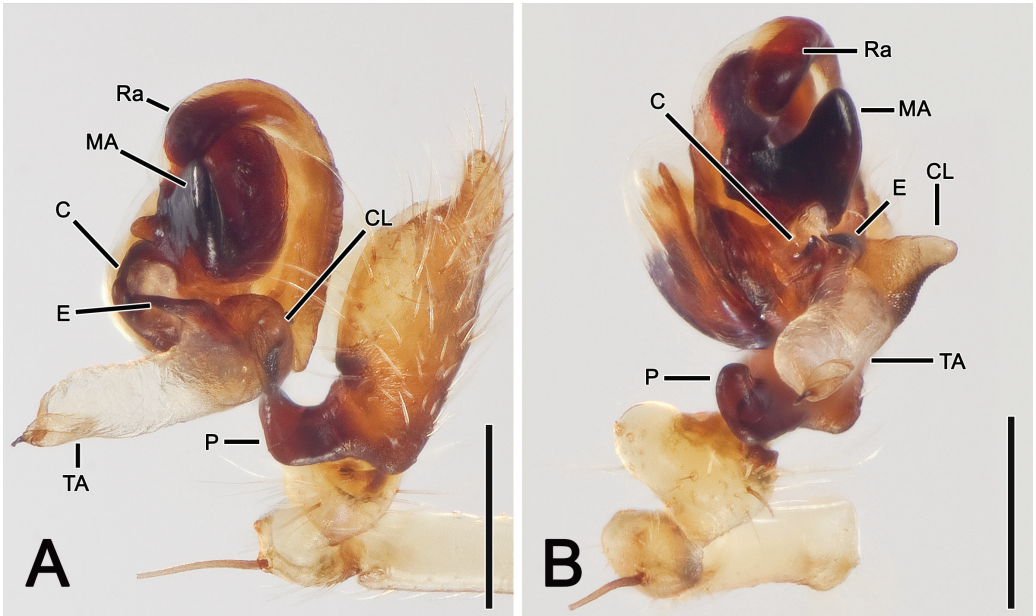


**Figure 2.** *Novakiella trituberculosa*, male (WAM T73571), expanded left pedipalp. **A.** Ventral view; **B.** Retrolateral view. Abbreviations: C, conductor; CL, conductor lobe; E, embolus; MA, median apophysis; P, paracymbium; Ra, radix; TA, terminal apophysis. Scale bars: 0.5 mm

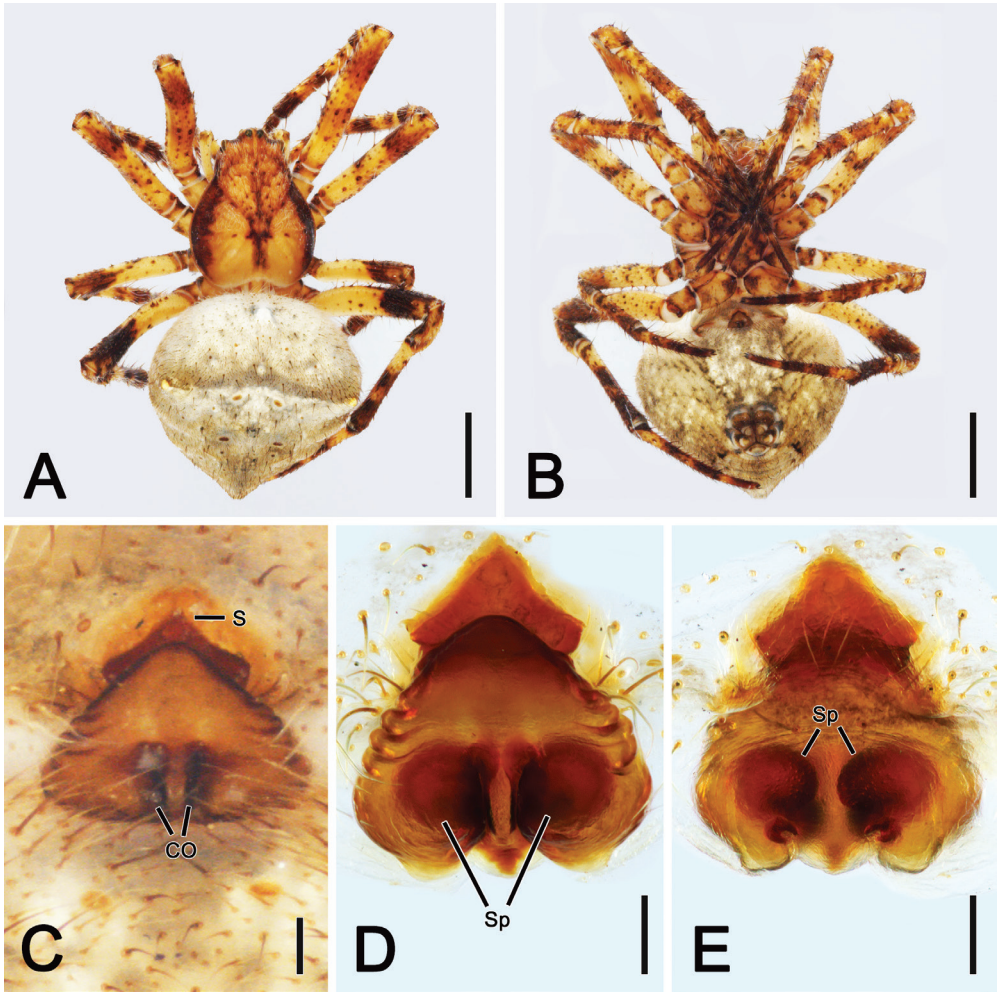


**Figure 3.** *Novakiella trituberculosa*, female (WAM T73556). **A.** Dorsal habitus; **B.** Ventral habitus; **C.** Epigyne, ventral view; **D.** Cleared epigyne, ventral view; **E.** Cleared epigyne, dorsal view. Abbreviations: CO, copulatory openings; S, scape; Sp, spermatheca. Scale bars: **A, B,** 2 mm; **C–E,** 0.1 mm.

+ 3.25 + 2.86 + 0.97 = 12.35, II – 2.73 + 1.17 + 2.21 + 0.90 + 0.97 = 7.98, III – 2.34 + 0.71 + 1.23 + 1.30 + 0.72 = 6.30, IV – 3.51 + 0.97 + 2.14 + 2.14 + 0.84 = 9.62. Labium wider than long, subtriangular, brown (Fig. 1B); endites rounded, light brown (Fig. 1B). Sternum slightly longer than wide, light brown, with thick black edges and large anterior guanine white spot (Fig. 1B). Abdomen (Fig. 1A, B) 3.06 long, 2.47 wide; subtriangular, longer than wide, abdominal humps strong and posterior end reaching over spinnerets; dorsum beige with posterior darker folium pattern; sparsely covered with long brown setae; sides dark olive-grey; venter beige and irregularly mottled with brown spots. Pedipalp (Figs 1C, D, 2A, B) length of segments (femur + patella + tibia + cymbium = total length): 0.58 + 0.26 + 0.26 + 0.78 = 1.88; conductor lobe bilobed, basal lobe rounded and apical lobe pointed and connected to a wide and concave lateral expansion, whose ectal border has a dense black field of scale-like structures; terminal apophysis with an inflated and membranous body, ending in curved and pointed well-sclerotised tip; embolus slightly sinuous in mesal view, tapering apically towards conductor when expanded (Fig. 2A, B); conductor very conspicuous and subquadrate with a projected and sclerotised tip; median apophysis stout, heavily sclerotised, forming an arch over the radix, medially curved and extended into a basally pointing tip.

*Female* (WAM T73556): Total length: 9.0. Carapace 3.5 long, 2.8 wide, eyes, chelicerae, legs, labium, endites and sternum essentially as in male (Fig. 3A, B). Eye measurements: AME 0.18, ALE 0.09, PME 0.14, PLE 0.14; row of eyes: AME 0.63, PME 0.47, PLE 1.46. Pedipalp length of segments (femur + patella + tibia + cymbium = total length): 1.12 + 0.48 + 0.48 + 1.20 = 3.28. Leg formula IV > I > II > III; length of segments (femur + patella + tibia + metatarsus + tarsus = total length): I – 3.92 + 1.60 + 3.20 + 2.96 + 0.96 = 12.64, II – 3.68 + 1.52 + 2.88 + 0.90 + 0.88 = 9.86, III – 2.40 + 0.88 + 1.44 + 1.44 + 0.80 = 6.96, IV – 4.08 + 1.36 + 2.56 + 2.48 + 1.04 = 11.52. Abdomen (Fig. 3A, B) 5.00 long, 4.80 wide, similar to male in shape and colour, but slightly more rounded, with less projected humeral humps. Epigyne plate subtriangular, with smooth anterior portion near the basis of scape and lateral wrinkles; scape broken off (Fig. 3C–E) (as in all specimens examined by us). If present, scape

triangular with indistinct transverse wrinkles (Court and Forster 1988, figs 554, 555). Spermathecae spherical and very large, occupying most of the genital area, medially connected to copulatory openings (Fig. 3D, E).

**Remarks.** The study was conducted over many years and at different institutions and therefore imaging and descriptive work based on variable specimens (plural) availability at the time. This explains why the male *N. trituberculosa* is here redescribed based on two specimens; one imaged many years ago, but not measured, and the measurements added for a second specimen more recently.

**Habitat preferences and life history.** In Australia, mature males of *N. trituberculosa* were found between November and June, with peaks in January and April. Mature females were found all year round with the lowest numbers of records in November and December. Here, the species is mainly found in "pastoral habits" and constructs a horizontal orb-web amongst low grasses or weeds, with the centre pulled up by stabilizing threads. The webs are up to 0.1 m above ground. Additionally, habitat descriptions on specimen labels include "woodland", "open forest", "shrubs near ground", "in long grass", but the species also seems to occur in more disturbed habitats such as "among garden rubbish", "ex toilet", "walking on wall at night", "inside house on wall", "stationary on door knob". In New Zealand it is mostly found in pastoral habitats (Court and Forster 1988), which suggests that it is introduced.

**Distribution.** *Novakiella trituberculosa* has been recorded from all Australian states, except Northern Territory, south of ca. 22°S Latitude (Fig. 7). In New Zealand the species is more frequently found in the North Island but it has also been found in some South Island localities (Court and Forster 1988) (Fig. 8).

### *Novakiella boletus* sp. nov.

http://zoobank.org/2181EC8F-7BE5-4ECD-92EC-9FB21DD4B177

Figs 4–7

**Type material.** Holotype male from Maits Rest, 10 km W of Apollo Bay, Otway Ranges, Victoria, AUSTRALIA, 38°45'S, 143°34'E, 16.iii.1992, G. Milledge leg. (MV K9867).

**Other material examined. AUSTRALIA: New South Wales:** 1 male, Coolah Tops National Park, off Gemini Road Loop, 31°48'59"S, 150°10'31"E, beating, 12–13.iv.2010, M. G. Rix & D. Harms leg. (WAM T102788); **South Australia:** 1 female, Kelly Hill Caves camping area, Kangaroo Island, 35°59'S, 136°54'E, 09.xi.1987, D. Hirst leg. (SAM); 1 male, Loftia Recreation Park, 35°02'S, 138°42'E, pitfall traps, 20–27.iii.1990, D. Hirst leg. (SAM). **Tasmania:** 1 male, Junction Creek, Arthur Plains West, 43°5'S, 146°16'E, 08.ii.1966, A. Neboiss leg. (MV K9862); 3 females, 3.8 km SE of Beechford, 41°02'50.6"S, 146°59'20.94"E, May 2021, vehicle vibration (QVM:2021:13:0514–5, 2021:13:0517) (examination by image). **Victoria:** 1 male, Sherbrook Forest, 37°53'S, 145°21'E (MV K9864).

**Etymology.** The specific epithet is a Latin noun in apposition – *boletus* – meaning mushroom and it refers to the distinctly mushroom-shaped conductor lobe that is reminiscent of a chanterelle (*Cantharellus* spp.).

**Diagnosis.** Male *N. boletus* sp. nov. can be distinguished from *N. trituberculosa* by the weaker apico-prolateral spur on the tibia of leg II (Fig. 1E vs Fig. 5C) and the morphology of key pedipalp sclerites, specifically the mushroom-shaped conductor lobe (two-lobed in *N. trituberculosa*) (Fig. 1C *vs* Fig. 5F). Females of *N. boletus* sp. nov. differ from those of *N. trituberculosa* by details in the epigyne plate, specifically its transverse wrinkles that are more pronounced and mainly limited to the lateral margins in *N. trituberculosa* (Fig. 3C vs Fig. 6C).

**Description.** *Male* (based on holotype, MV K9867): Total length: 6.44. Carapace (Fig. 4A) 2.41 long, 2.08 wide, reddish-brown with black lateral margins, and yellowish setae mainly on the subquadrate cephalic area, fovea longer than wide, covered by a long black spot. Eyes ringed in black, lateral ones located on small tubercles (Fig. 4A). AME 0.22, ALE 0.10, PME 0.16, PLE 0.13; row of eyes: AME 0.58, PME 0.36, PLE 0.85. Chelicerae with paturon dark brown and fangs reddish brown; four promarginal teeth with the apical and third largest, three retromarginal teeth of equal size (Fig. 4B). Legs (Fig. 4A, B) yellowish-brown, mottled with chestnut brown spots; tibia of leg II with spur represented by a small apico-prolateral bulge that carries a strong macroseta; femur IV darker than other legs; leg formula IV > I > II > III; length of segments (femur + patella + tibia + metatarsus + tarsus = total length): I – 4.16 + 1.56 + 3.70 + 3.25 + 1.17 = 13.84, II – 3.38 + 1.36 + 2.92 + 0.90 + 1.11 = 9.67, III – 2.34 + 0.78 + 1.36 + 1.30 + 0.78 = 6.56, IV – 3.51 +0.97 + 2.40 + 2.21 + 0.91 = 9.94. Labium wider than long, subtriangular and brown, with beige apical portion (Fig. 4B); endites rounded, light brown with beige edges (Fig. 4B). Sternum a little longer than wide, reddish brown, with thick darker and wavy contour and yellowish centrally placed guanine patch (Fig. 4B). Abdomen (Fig. 4A, B) 3.58 long, 2.34 wide; subtriangular, longer than wide, humeral humps conspicuous and posterior end reaching over spinnerets; dorsum yellowish-brown, with diamond-shaped patch with dark contour and a black longitudinal median line from pedicel towards posterior end, meagerly covered with long brown setae; sides beige with sparse black lines and yellow setae; venter beige irregularly covered with black spots. Pedipalps (Figs 4D–F, 5) length of segments (femur + patella + tibia + cymbium = total length): 0.78 + 0.32 + 0.26 + 1.04 = 2.40; radix thick; conductor lobe mushroom-shaped, with a projected base ending in a rounded tip and a large apical lamellar portion, which is concave at its middle portion, expanded into a wide rounded mesal projection and with its ectal border bearing a dense and dark field of scale-like structures; terminal apophysis apically projected, and longer than wide, slightly twisted and tapering to its tip; conductor rectangular and projected from behind embolus into a flat tip; embolus very thick and long, ending in a very sclerotised

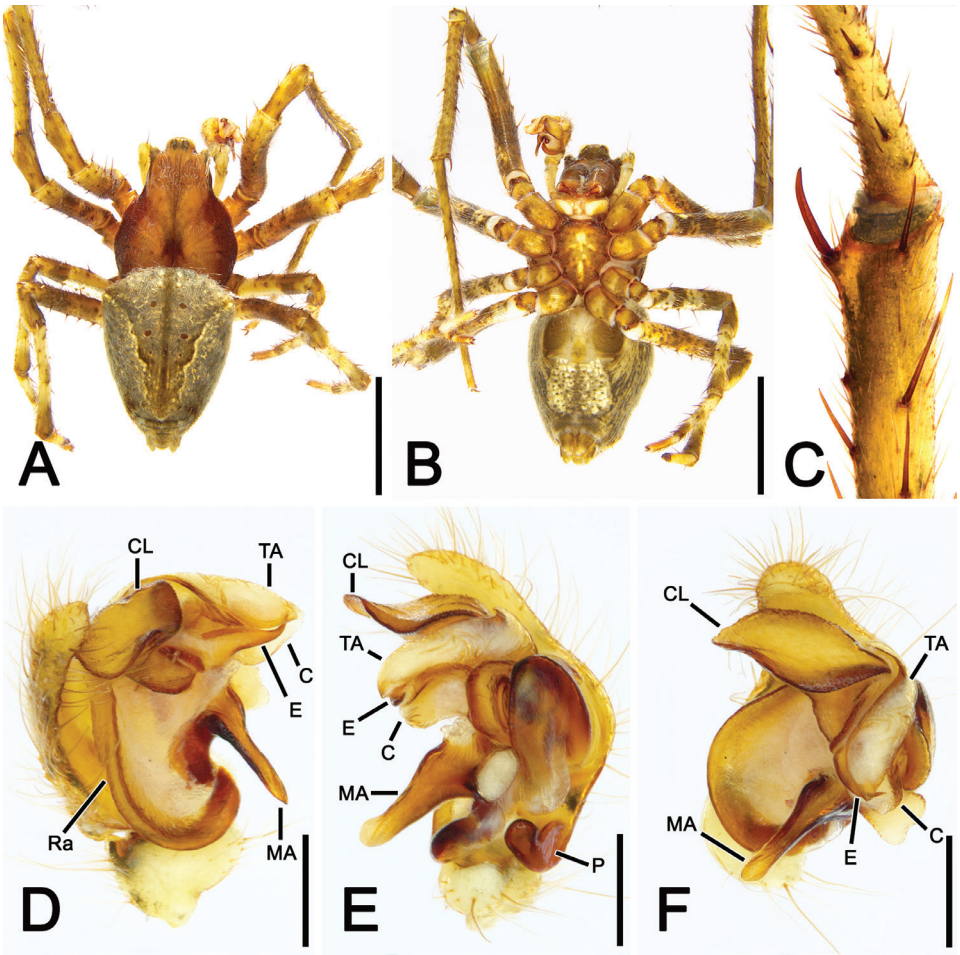


**Figure 4.** *Novakiella boletus* sp. nov., male holotype (MV K9867). **A.** Dorsal habitus; **B.** Ventral habitus; **C.** Left tibia, ventral view; **D.** Left pedipalp, mesal view; **E.** Left pedipalp, ventral view, **F.** left pedipalp, apical view. Abbreviations: C, conductor; CL, conductor lobe; E, embolus; MA, median apophysis; P, paracymbium; Ra, radix; TA, terminal apophysis. Scale bars: **A, B,** 2 mm; **C,** 1 mm; **D–F,** 0.5 mm.

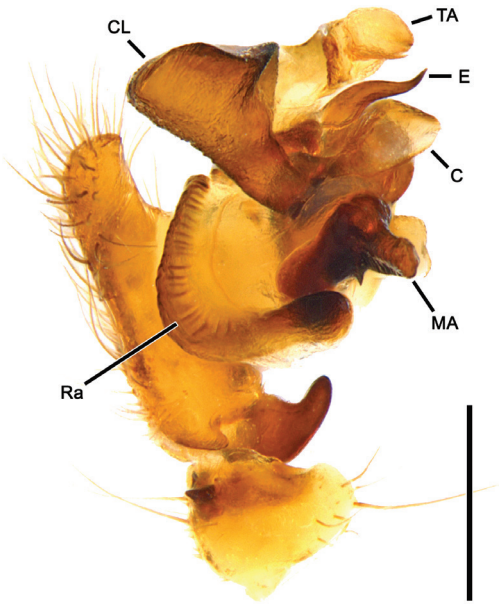


**Figure 5.** *Novakiella boletus* sp. nov., male holotype (MV K9867), expanded left pedipalp. mesal view. Abbreviations: C, conductor; CL, conductor lobe; E, embolus; MA, median apophysis; Ra, radix; TA, terminal apophysis. Scale bar: 0.5 mm.

distally curved tip; median apophysis stout, with a smaller basal portion and a strong median curvature, ending in a long and flattened basally pointing acute projection.

*Female* (SAM; from Kelly Hill Caves camping area, Kangaroo Island): Total length 8.5. Carapace (Fig. 6A) 3.7 long, 2.9 wide, as in male but with larger anterior portion. Eyes, chelicerae, legs, labium, endites and sternum generally as in male (Fig. 6A, B). Eye measurements: AME 0.2, ALE 0.11, PME 0.14, PLE 0.15; row of eyes: AME 0.68, PME 49, PLE 1.46. Pedipalp length of segments (femur + patella + tibia + cymbium = total length): 1.14 + 0.50 + 0.49 + 1.18 = 3.31. Leg formula IV > I > II > III; and length of segments (femur + patella + tibia + metatarsus + tarsus = total length): I – 4.00 + 1.62 + 3.20 + 3.00 + 1.02 = 12.84, II – 3.75 + 1.60 + 2.89 + 0.96 + 0.99 = 10.19, III – 2.40 + 0.91 + 1.44 + 1.45 + 0.86 = 7.06, IV – 4.12 + 1.40 + 2.58 + 2.53 + 1.05 = 11.68. Abdomen (Fig. 6A, B) 4.5 long, 4.5 wide, with a more pronounced subtriangular shape than the male, dorsum with colour similar to male, except for the lighter folium and absent median line; venter as in male. Epigyne (Fig. 6C) plate trapezoidal with a rectangular anterior portion, crossed by long transverse wrinkles; scape broken off, but with a wide rectangular

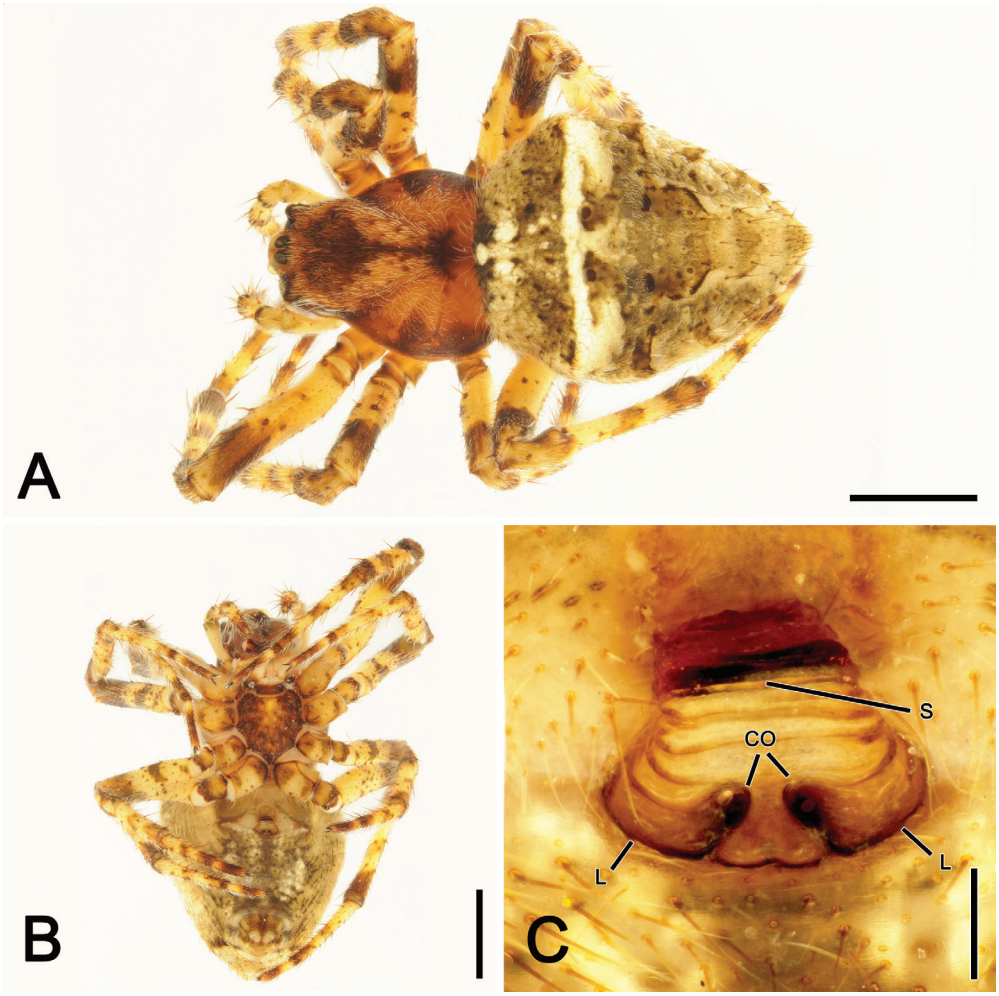


**Figure 6.** *Novakiella boletus* sp. nov., female (SAM). **A.** Dorsal habitus; **B.** Ventral habitus; **C.** Epigyne, ventral view. Abbreviations: CO, copulatory openings; L, lips; S, scape. Scale bars: **A, B,** 2 mm, **C,** 0.2 mm.

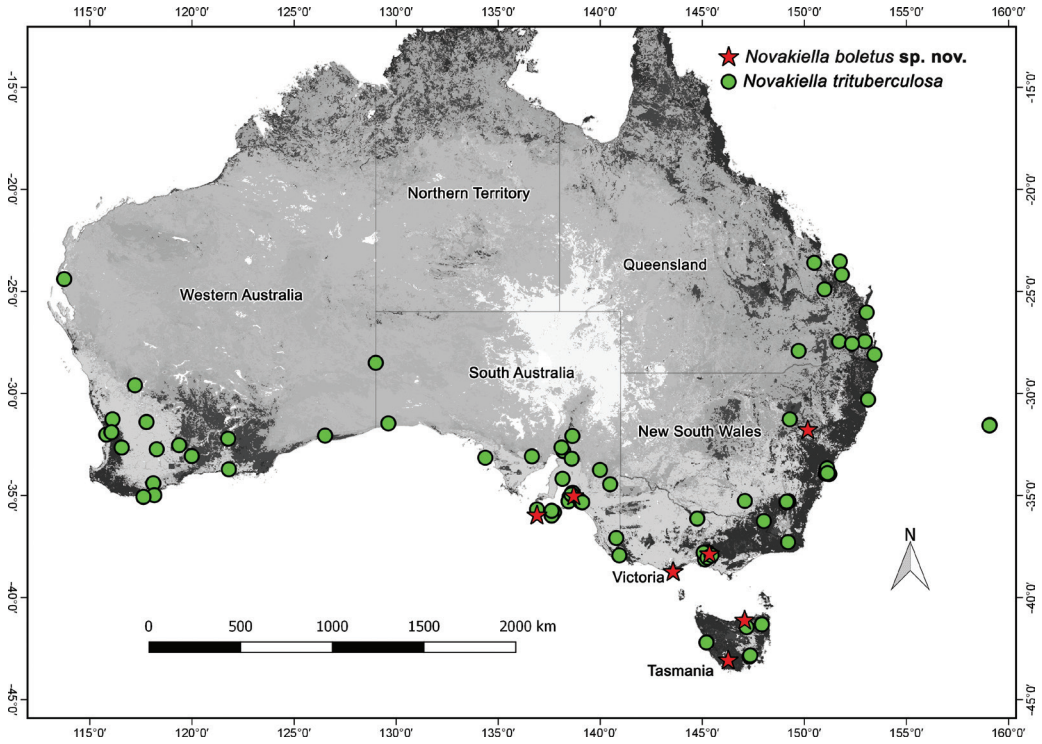


**Figure 7.** Distribution records of *Novakiella trituberculosa* (green circles) and *Novakiella boletus* sp. nov. (red stars) in Australia.

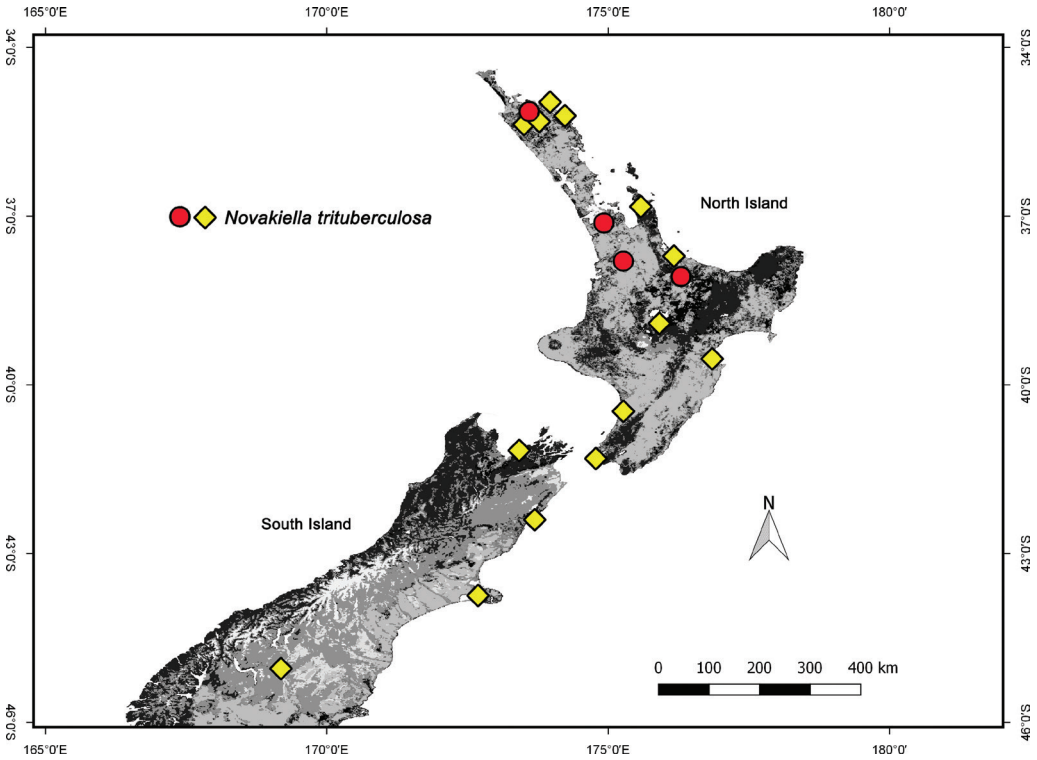


**Figure 8.** Distribution records of *Novakiella trituberculosa* in New Zealand; red circle points to material examined for this study and yellow diamonds point to records literature records (Court and Forster 1988).

torn basis (Fig. 6C). Spermatheca was not dissected to preserve the only available female specimen.

**Habitat preferences and life history.** Mature males of *N. boletus* sp. nov. were collected between February and April, females were found in May and November. Habitat descriptions include *Nothofagus cunninghamii* (Myrtle Beech) forest, *Eucalyptus amygdalina* coastal forest, and "eucalypt forest with tree fern gully", suggesting this species occurs predominantly in temperate forests and rainforests.

**Remarks.** Males and female *N. boletus* sp. nov. have not been found together, but somatic features such as size range, carapace (Fig. 5A vs Fig. 6A), sternum (Fig. 5B vs Fig. 6B) and leg (Fig. 5A vs Fig. 6A) colouration match well and they are currently assumed to be the same species.

**Distribution.** This new species is only known from Australia, specifically New South Wales, South Australia, Victoria and Tasmania (Fig. 7).

## Discussion

Araneidae are a highly diverse family with generally complex male pedipalp morphology which is traditionally used to infer phylogenetic relationships within this family as well as in other spiders (Scharff and Coddington 1997 and references therein). Developing homology hypotheses on the various pedipalp sclerites is a prerequisite to infer phylogenetic relationships using morphological data, subsequently tested by the phylogeny, but homology hypothesis based on the classical homology criteria of congruence, conjunction and similarity (Patterson 1988) is not straightforward. For example, the name paramedian apophysis has been used for structures in the male pedipalp that are very different in shape and with different position on the male pedipalp. For instance, the lobe of the conductor seen in *Micrathena* Sundevall, 1833 (Levi 1985, figs 6–9) was considered homologous with the separate sclerite seen in *Gasteracantha* Sundevall, 1833 (Levi, 1978, figs 83, 84) and named paramedian apophysis by Levi (1978, 1985). The term paramedian apophysis was first used by Comstock (1910, pp. 179, figs 18, 19) for an extra sclerite in *Eriophora ravilla* (C.L. Koch, 1844). He writes "in this species there is an apophysis which like the median apophysis is joined by a flexible articulation to the tegulum within the cuplike cavity formed by the distal margin of the tegulum; this may be termed the paramedian apophysis". Scharff and Coddington (1997, fig. 95) tested the homology of the paramedian apophysis (in the broad definition of Levi) on a phylogeny based on morphological characters and found that the paramedian apophysis had developed several times independently within Araneidae. The same results are obtained if the character is mapped on the new molecular phylogeny of Scharff et al. (2020). Each presence of a paramedian apophysis therefore has to be considered individually and probably represent different non-homologous structures. Interestingly, a paramedian apophysis in the form of a separate sclerite inserting on the tegulum next to the median apophysis, is a possible synapomorphy for the clade called gasteracanthines in Scharff and Coddington (1997) and Scharff et al. (2020). In other backobourkiines (i.e. *Backobourkia*; Framenau et al., 2010, figs 6A, 10A; *Plebs*, Joseph & Framenau, figs 8A, 11A), the paramedian apophysis is clearly connected basally to the conductor and thus not homologous to the one

in *Micrathena*, and could thus be better termed conductor lobe. Levi (1985) considered the paramedian apophysis as a synapomorphy that could group different genera like *Eriophora*, *Parawixia*, *Alpaida* O. Pickard-Cambridge, 1889 and *Wagneriana* F. O. Pickard-Cambridge, 1904.

In *Novakiella*, a basal conductor lobe is also present, but it is shaped very differently to that in other backobourkiines and the homology of a variety of structures in araneids in such a position remains unclear (Scharff and Coddington 1997). In *Novakiella* the conductor lobe is a very prominent structure that originates between the basis of the distal hematodocha and the stipes and fills the lateral space between the terminal apophysis and the embolus basis reaching far apically of the radix and connecting to the conductor from under the embolus. We initially thought it was a structure similar to the subterminal apophysis, which is a bubble-shaped structure that was first cited as a synapomorphy for *Eustala* Simon, 1895 and *Metazygia* F. O. Pickard-Cambridge, 1904 (Levi 1977) and then also cited for *Larinia* Simon, 1874 (Harrod et al. 1991), a member of the "Nuctenines" (sensu Scharff et al. 2020). However, it looks more related to the paramedian apophysis of *Eriophora* and *Backobourkia* cited above due to its origin at the base of the conductor and its shape. A large well-sclerotized transverse structure similar to the conductor lobe of *Novakiella* appears to be present in other "backobourkiine" genera, such as *Acroaspis* (see Framenau 2019: fig. 1B for *A. lancearia* (Keyserling, 1887). Testing homologies of the various pedipalp sclerites within the backobourkiines and to develop a generalized ground plan for this group will be a prerequisite to develop homology hypotheses to other major clades of the Araneidae as identified in Scharff et al. (2020). This can only be conducted once the apparently highly diverse backobourkiines have been taxonomically revised.

*Novakiella trituberculosa* was originally described from New Zealand, but Court and Forster (1988) considered the species to also occur in Australia, so the biogeographic origin of the genus remained ambiguous. The finding of a second species of *Novakiella* in Australia suggests that the genus evolved there and that *N. trituberculosa* is a natural or human-induced introduction to New Zealand. This is also consistent with *Novakiella* being part of the backobourkiines, a clade with likely Australian origin (Scharff et al. 2020). Likewise, *Eriophora pustulosa* (Walckenaer, 1841) is the only New Zealand species of a group of backobourkiines with a number of otherwise Australian representatives (VWF, PSC, CJV unpublished data).

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PENSOFT

für Natur MUSEUM FÜR NATURKUNDE BERLIN

# A type catalogue of the reed frogs (Amphibia, Anura, Hyperoliidae) in the collection of the Museum für Naturkunde Berlin (ZMB) with comments on historical collectors and expeditions

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## Abstract

We present a commented catalogue of the type specimens of the Afro-Malagasy frog family Hyperoliidae at the herpetological collection of the Museum für Naturkunde Berlin (ZMB). In current publications and databases, many names based on ZMB primary types are listed as synonyms of other species, the types often declared as lost. Consequently, the respective names are often no longer considered in current taxonomic work. We traced 146 nominal taxa of the family Hyperoliidae in the ZMB collection of which currently 130 are presented by primary types (88 holotypes, 10 lectotypes and 32 taxa based on syntype series); 50 of these taxa are currently considered as valid. Primary types of nine taxa could not be located during our inventory of the collection holdings. Seven taxa are exclusively represented by secondary types (paratypes). Many of these types comprise taxa where types have been thought to be lost. As a further service to the community, we provide important details about collectors and their travel routes, as well as respective documents stored in the collection of the Department of Historical Research at ZMB. This should make it easier to potentially compare the ZMB types in future taxonomic revisions.

## Key Words

Africa, colonies, historical collections, Hyperoliidae, type specimens, Zoologisches Museum Berlin

## Introduction

The amphibian collection of the Museum für Naturkunde Berlin (ZMB) is one of the richest in the world, comprising about 60,000 specimens of ca. 2,000 species. One of the largest and most diverse families in the collection is the African frog family Hyperoliidae with approximately 3,500 wet preserved specimens, including about 860 type specimens. These specimens have been used by various authors as a basis for 145 first descriptions, reflecting several periods of research on reed frogs by staff herpetologists and external researchers associated with the ZMB collection.

The oldest specimens from this family present in the collection are from South Africa. These vouchers, sent by G. L. E. Krebs (1792–1844), are two *Hyperolius horstockii* (ZMB 3061) from the "Cap". These specimens either were part of a shipment by Krebs containing 90 "Amphibien", in addition to other natural history specimens, and arrived at the museum on 21 June 1830, or they were acquired by the Zoologisches Museum Berlin from Krebs' collections, auctioned after that date until April 1840 (Lichtenstein "Eingangsjournal ZMB" [acquisition catalogue], see also Bauer 2000). Other old representatives of reed frogs are the species described by W. C. H. Peters (1815–1883) from his Mozambican collections, i.e. *Hyperolius variegatus* collected first in June 1843 on the Cabaceira Peninsula (see below and Peters 1882b) and five syntypes of *H. picturatus* Peters, 1875 (ZMB 3063, 76991–76994) collected at "Boutre" [Butre (Bootry), Ahanta West District, Western Region,

Ghana] by the zoologist and colonial administrator of the Dutch Gold Coast, H. S. Pel (1818–1876). Pel, who stayed in the Gold Coast (modern Ghana), from March to April 1841, March 1842 to December 1843, in January 1844, and from September 1847 to November 1850, collected natural history objects for the Rijksmuseum van Natuurlijke Historie in Leiden (Holthuis 1968). In March 1849, the Leiden Museum probably exchanged Pel's specimens together with other herpetological and ichthyologcial objects with the Zoologisches Museum Berlin (Lichtenstein "Eingangsjournal ZMB"). The first mention of *Hyperolius* species from the ZMB was in the catalogue published by Lichtenstein and Martens (1856: 36) "Nomenclator Reptilium et Amphibium Musei Zoologici Berolinensis".

During the second half of the 19th century, the collection was enriched by material collected, for example, by R. W. Buchholz (1837–1876) and G. A. E. Reichenow (1847–1941) in Cameroon, by C. C. von der Decken (1833–1865) in East Africa and by H. W. L. Dohrn (1838–1913) in Liberia. Several objects from the collection of J. A. de Oliveira Anchieta (1832–1897) from Angola were received in exchange with J. V. Barbosa du Bocage (1823–1907) from Lisbon. Wilhelm Peters undertook the first scientific work on the Berlin *Hyperolius* material based on his own collections from Mozambique and specimens purchased from the aforementioned collectors (Peters 1854, 1867, 1875, 1876, 1877a, 1878, 1882a, b).

However, most hyperoliid frogs now present in ZMB were collected during the period from 1873 to 1918 in Germany's African colonial territories, and during further expeditions to other regions of the African continent, such as the "Loango–Expedition" (1873–76), J. M. Hildebrandt's Expeditions to East Africa (1873–77), the "Emin Pasha Expedition" (1890–92), the "Togo–Hinterland–Expedition" (1894–95), the "Nyassa–See- und Kinga–Gebirgs–Expedition" (1898–99), the "Kunene–Sambesi–Expedition" (1899–1900), the "German–French Border Expedition" (1901–02), the "Lübecker Pangwe–Expedition" (1907–09), and the "Lagone–Pama–Expedition" (1912–13). Important collectors which need to be mentioned in this context are K. M. E. G. W. Adametz (1877–year of death unknown), E. R. R. Baumann (1871–1895), R. Böhm (1854–1884), L. F. W. E. Conradt (life dates unknown), C. A. Denhard (1852–1929), C. V. H. von Erlanger (1872–1904), F. G. H. H. Fülleborn (1866–1933), R. Grauer (1870–1927), H. Grote (1882–1951), J. M. Hildebrandt (1847–1881), P. Hösemann (1868–1922), R. F. P. Hübner (1869–1961), R. Kandt (1867–1918), G. Martienssen (life dates unknown), F. W. A. von Mechow (1831–1904), O. R. Neumann (1867–1946), W. A. von Raven (1875–1928), G. R. O. Scheffler (life dates unknown), J. G. H. Schubotz (1881–1955), P. W. H. Spatz (1865–1942), F. L. Stuhlmann (1863–1928; Fig. 1), G. T. Tessmann (1884–1969), W. Tiesler (life dates unknown), and G. A. Zenker (1855–1922).

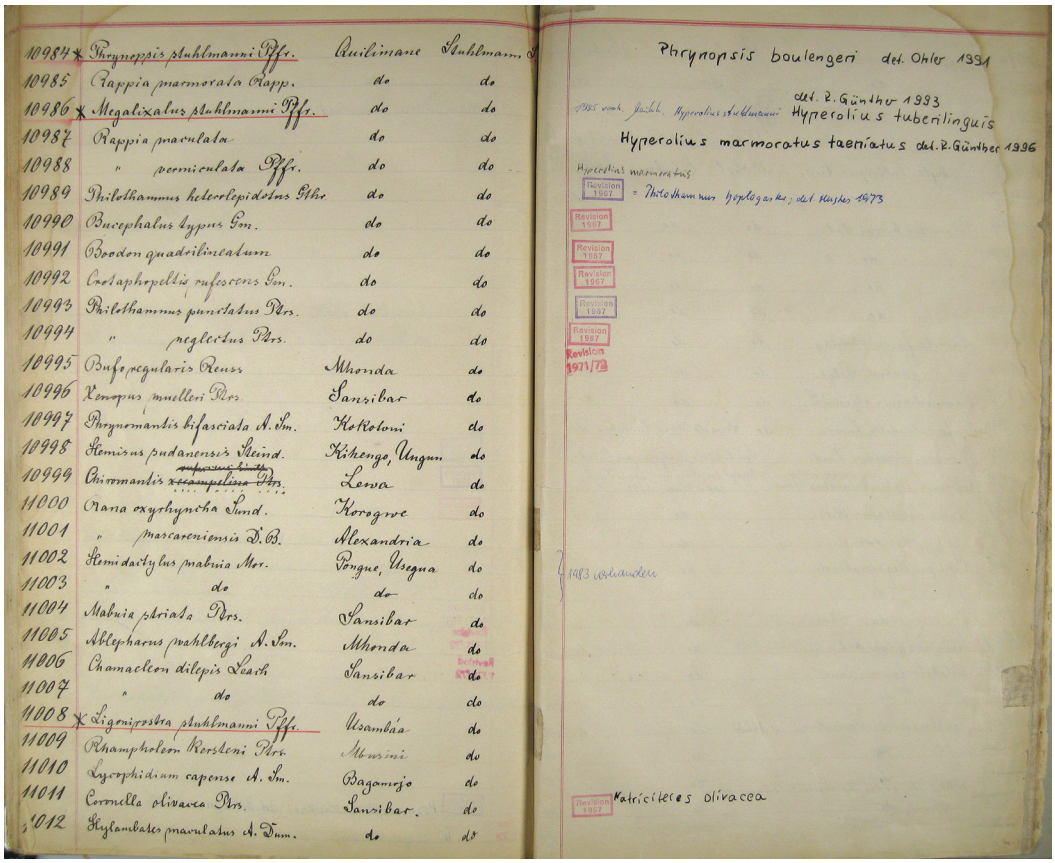


**Figure 1.** Examplary page from the first numerical inventory catalogue of the herpetological collection at ZMB, with entries of material collected by F. L. Stuhlmann in East Africa.

At the end of the 19th century, Gustav Tornier (1859–1938), at the time curator of the amphibian and reptile collection at the ZMB, took over the task of a systematic analysis of the herpetological material collected from colonial German East Africa. In addition to the Berlin collection, he also had access to F. Stuhlmann's collections at the Museum Hamburg. Johann Georg Pfeffer (1854–1931) from Hamburg, who had originally been assigned this task, resigned for health reasons (Tornier 1896, see also remarks on *H. flavoguttatus*). The extensive colonial material in Berlin and Hamburg, which was particularly rich in reed frogs, enabled Tornier to compile an overview of the "Farbkleid der Rappienhaut" [color pattern of the reed frog skin] (Tornier 1896, plate 4, reproduced here in Fig. 2). In contrast to Ahl, who studied the Berlin reed frog collection three decades later (see below), with one exception, Tornier did not introduce new species names, but rather reported cautiously with regard to the number of (new) species present in the material examined (1896: 156): *Sobald die von mir aus Afrika erwarteten Rappiensendungen eingetroffen sind und ich das Rappienmaterial gesehen habe, welches die Hauptmuseen Europas beherbergen, komme ich in einer besonderen Arbeit auf diese Frage zurück*. [As soon as the expected *Rappia* shipments from Africa have arrived, and I have seen the *Rappia* material housed by the major European museums, I will return to this question in a special study]; however, this study was never compiled and published.

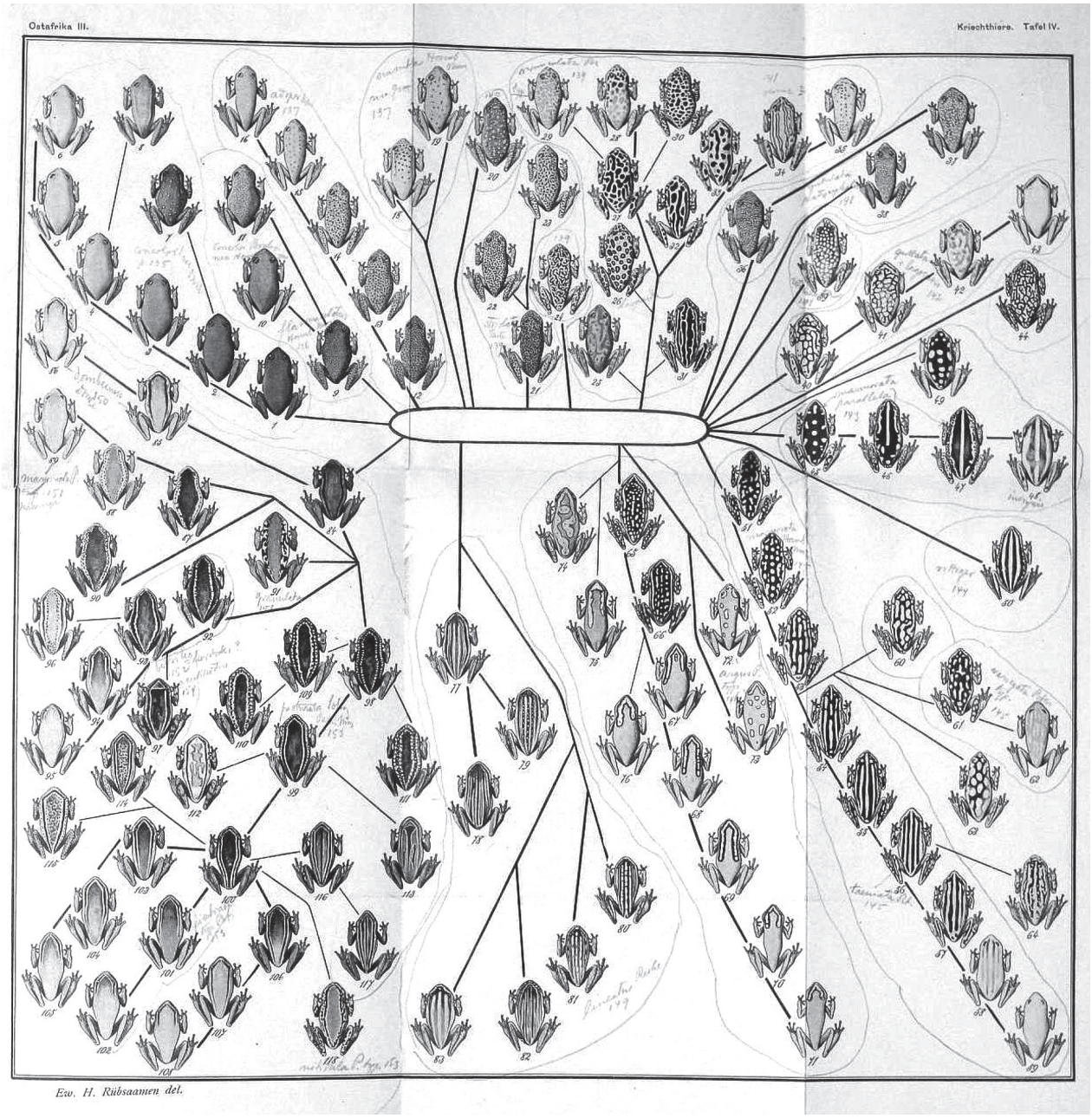


**Figure 2.** "Farbkleidmuster der Gattung *Rappia*" [color pattern of the genus *Rappia* (*Hyperolius* species)], reproduced from Tornier (1896, pl. 4).

A decade later, at the beginning of 20th century, large scale research expeditions such as the first and second "Deutsche–Zentral–Afrika–Expedition" [German-Central-African-Expedition] from 1907–08 and 1909–10, provided rich new material. Together with specimens obtained from the German colony Cameroon, further remarkable collections of amphibians and reptiles, including considerable numbers of reed frogs, were sent to ZMB, and were partly described by Friedrich Erich Gottlieb (called Fritz) Nieden (1883–1942) (Nieden 1910a, 1913; see also Günther and Bischoff 2018).

In December 1921, Christoph Gustav Ernst Ahl (1898–1945) started to work as a voluntary scientific assistant in the ichthyological and herpetological department of ZMB. After Tornier's retirement, Ahl became an official assistant from February 1923 to September 1927. From October 1927 until his dismissal in March 1941, he was employed as senior scientific assistant in the herpetological department of ZMB. During his career, Ahl published a total of 302 new descriptions of amphibians and reptiles (Paepke 2013). Over a period of seven years, he described 106 nominal taxa from the family Hyperoliidae (Ahl 1924, 1930a-d, 1931a), and worked on this family monographically, published as part 55 of the "Tierreich" [animal kingdom] series (Ahl 1931b). However, Ahl's work is overshadowed by a large number of scientifically flawed descriptions. A selection of Ahl's *Hyperolius* types were sent in exchange to the Museum of Comparative Zoology at Harvard University (MCZ) in 1932, and this material allowed Barbour and Loveridge (1947) and Loveridge (1957) to synonymize 50 of Ahl's species. Arthur Loveridge (1957: 157, 324) called Ahl a "reckless describer" and complained about his insufficient knowledge of intra-specific variability within the Hyperoliidae as well as his inadequate comparisons with already described species. Ahl's working methods and the quality of his descriptions have subsequently been commented on, e.g. by Glaw and Vences (1992), Paepke (1995, 2013), Rieck (2001), and Adler (2007). In addition to Ahl's questionable scientific approach, many of the (type) specimens mentioned by him (Ahl 1931a, b) were not inventoried and placed in the systematic collection during his tenure; instead, these were often placed on the shelves of undetermined material, sometimes with handwritten notes. In the 1950s Heinz Wermuth (1918–2002), then curator of herpetology at ZMB, at the request of Raymond Ferdinand Louis-Philippe Laurent (1917–2005), endeavored to locate Ahl's type material. In September 1958 and January 1959, a total of 244 *Hyperolius* specimens, including the locatable types, were sent on loan to Laurent at the "Université Officielle du Congo-Belge, Elisabethville" [Lubumbashi, Democratic Republic of the Congo]. Based on this material Laurent (1961) published a paper on the *Hyperolius* and *Afrixalus* in the ZMB. He reviewed some of Ahl's taxa and mentioned for the first time respective inventory numbers. In the introduction of this paper he suggested that it would be desirable to declare all descriptions based on the activity of this individual zoologist [Ahl] as *nomina confusa* (see also comments in Liedtke et al. 2014: 254).

Several 'Ahl species' were synonymized by Loveridge (1942, 1957); Laurent (1943, 1958, 1961) and Barbour and Loveridge (1947). Other herpetologists studied Berlin hyperoliid specimens in the second half of the 20th century. Among them were Arne Schiøtz (1932–2019) from the Zoological Museum Copenhagen, who got material on loan in 1960 and 1961, and visited the ZMB in 1968 in order to study the East African frog collection, and Jean-Luc Perret from the Natural History Museum Geneva, who borrowed a number of Ahl types in 1962, and visited the ZMB collection in 1974. As a result of their studies, further 'Ahl species' were synonymized (see e.g. Schiøtz 1967, 1975). Only 24% of the reed frogs described by Ahl are still considered valid (see below; Paepke 2013; Frost 2021).

After 2000, ZMB received new hyperoliid vouchers from West and Central Africa, as well as from Mozambique, mainly through the collections of the working group of Mark-Oliver Rödel, curator of herpetology at ZMB since 2007. J. M. Dehling (University Koblenz-Landau) provided new vouchers from Rwanda, V. Mercurio (Berlin) collected in Malawi, and A. Channing (University of the Western Cape) sent vouchers from South and East Africa. The study of these new collections and the re-evaluation of historic specimens resulted in a number of revisions and new descriptions (see e.g. Lötters et al. 2004; Rödel et al. 2003, 2009, 2010; Dehling 2012; Channing et al. 2013; Frétey et al. 2014; Liedtke et al. 2014). These and other studies, often based on 'new' molecular technologies, also revealed that the diversity within the Hyperoliidae might be much higher than previously assumed and comprise a large number of cryptic taxa (i.e. Channing et al. 2013; Portik et al. 2019). Thus it is likely that some of the ZMB 'synonyms' actually refer to valid species (see Rödel et al. 2010). Current researchers describing new African reed frogs tend to ignore the availability of these names because these are listed as either synonyms or lost by Frost (2021). However, the collection of hyperoliid frogs in ZMB was never fully reviewed and the status of several taxa and the presence of many type specimens remained unevaluated. For instance, no fewer than 17 nominal taxa have been reported lost or not traced by Frost (1985) or listed as originally present in ZMB but without inventory number (Frost 2021).

We present a list of existing and so far unlocated type specimens of 146 nominal taxa of the family Hyperoliidae from the ZMB collection of which 130 are primary types (88 holotypes, 10 lectotypes and 32 taxa based on series of syntypes), 50 of which are currently considered valid. Primary types of nine taxa could not be located. Seven taxa are represented by secondary types (paratypes) only. This summary is not intended to resolve pressing taxonomic issues that in many cases will require dedicated research using molecular genetic approaches (see Scherz et al. 2020 for a promising example). In some cases, however, we added observations of taxonomic value (e.g.

measures and character descriptions), clarified priority of names, and identified and corrected type localities when possible. Our aim is to present what is available for study to aid future research that describes and names new taxa in the Hyperoliidae. This contribution is the third catalogue on the amphibian types held by ZMB. Previous catalogues include the caecilians and salamanders (Bauer et al. 1993), as well as the hemisotid, microhylid, myobatrachid, pelobatid and pipid frogs (Bauer et al. 1996).

## Methods

From 2017 to 2020, all specimens from the family Hyperoliidae in the collection of the Museum für Naturkunde Berlin (ZMB; in some publications the Museum für Naturkunde is also abbreviated with MfN or other acronyms (see Sabaj 2020); to avoid confusion, we apply the traditional use of ZMB for the herpetological collection) were systematically digitally registered, including all specimens not previously inventoried. Lots—jars with specimens all carrying the same accession number—were individualized and each specimen was assigned an individual number. Details on the original field labels were compared to catalogue entries, information from the accession catalogues and the data were completed where necessary. ZMB numbers always refer to the final inventory catalogue numbers. In addition, we sometimes mention 'accession numbers'. These are separate catalogues used in the herpetological department from ca. 1856 to April 1940, to record the accession of new material, often registered in lots with "C-Catalogue" numbers (Fig. 3). Only after having been assigned a 'ZMB number' are vouchers finally inventoried. The terms 'Register Catalogue' or 'ZMB Register' refer either to the accession catalogues of the Zoological Library or to Lichtenstein's 'Eingangsjournal' for the entire Zoological Museum, the latter archived in the Department of Historical Research at the Museum für Naturkunde (see Unpublished Sources). Our digital accessioning of the specimens, in connection with information on collectors, localities and collection periods, made it possible to search for previously unlocated type specimens of the species described by Ahl (1931a). For primary type specimens, the type localities and the collectors were identified whenever possible. Secondary types from the Berlin collection are likewise listed with locality and collector. If not stated otherwise, we follow Frost (2021) regarding the currently valid names. Concerning type localities, we always provide first the original spelling (in quotation marks) followed by the currently applied name of the locality in brackets, as well as further geographic data, such as province and country.

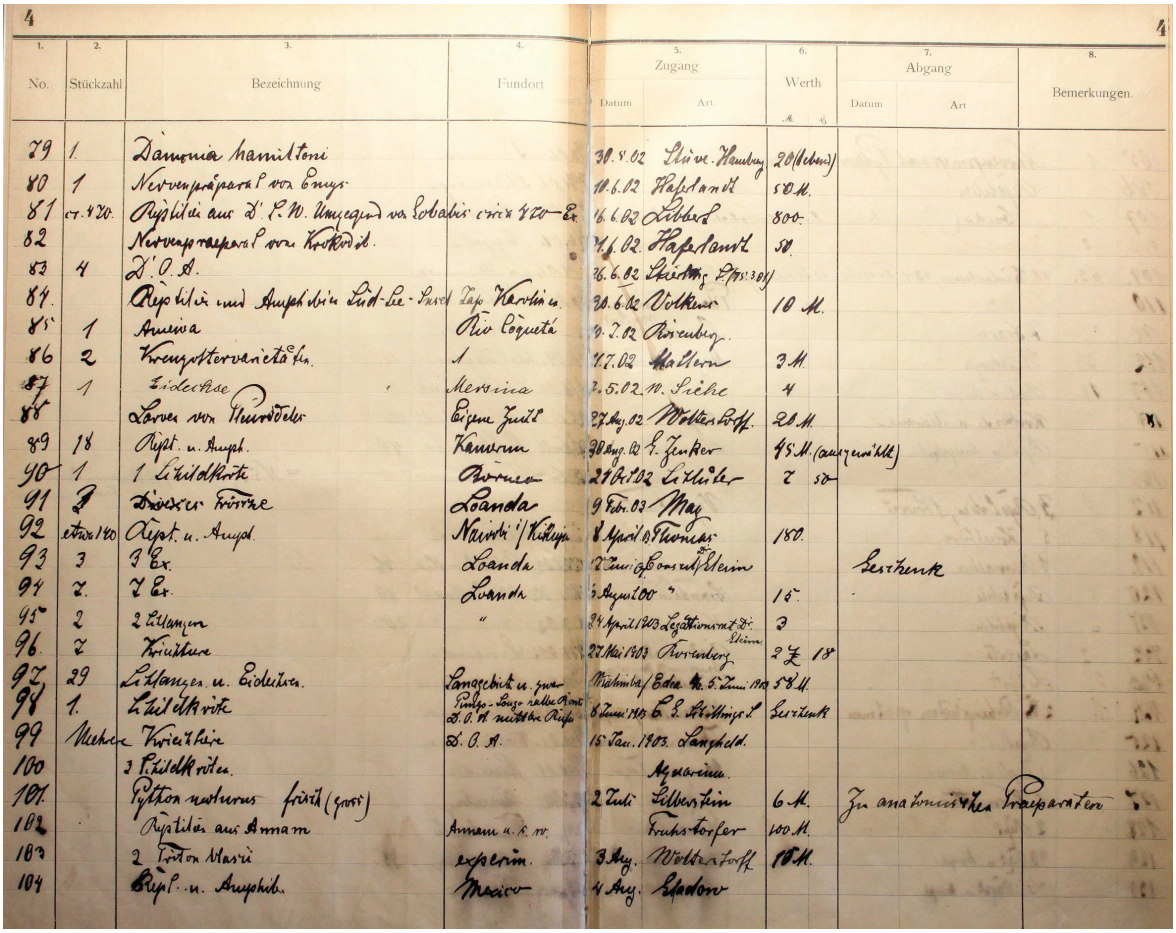


**Figure 3.** Examplary page from the second numerical accession catalogue (= "C" catalogue) of the herpetological collection at ZMB, with entries of African material collected by e.g. O. Gleim, W. Langheld, C. May, F. Thomas and G. A. Zenker.

We list all types in alphabetic order, using the original name in the description. The present taxonomic status and generic association is given under 'present name' in each species account. Remarks in the individual species accounts contain information on illustrations of the type material, the activities of the collectors, and the collection periods as well as information taken from secondary literature on additional type material that is not housed in the ZMB collection. We omit providing information on the history of the synonymy of the respective taxa. This is provided by Frost (2021) and can be consulted at: https://amphibiansoftheworld.amnh.org/index.php. It is important to note that we herein do not take any taxonomic decisions; we present the available type specimens and their current taxonomic name. For valid names we refer, with few exceptions, to Frost (2021). Until the complex taxonomy of the *H. marmoratus* species group is solved and allows a more accurate assignment, we tentatively follow Marques et al. (2018) and list Angolan taxa previously considered as synonyms of *H. marmoratus* Rapp, 1842 or *H. parallelus* Günther, 1858 as belonging to *Hyperolius angolensis* Steindachner, 1867. Ceríaco et al. (2020: 395) confirm assignment of Angolan frogs of the *H. marmoratus* group to *H. angolensis.* In contrast Frost (2021) and Baptista et al. (2019) regard *H. angolensis* as a junior synonym of *H. parallelus* Günther, 1858. An even more conservative approach has recently been applied by Channing and Rödel (2019), treating almost all populations of the *Hyperolius viridiflavus/marmoratus*-complex as *H. viridiflavus*.

Recently, Dubois et al. (2021) suggested 'new concepts and methods for phylogenetic taxonomy and nomenclature', using Lissamphibia as a template. To avoid causing further confusion in an already complex and taxonomically confusing group of tropical tree frogs, we – without assessing the new system – refrain herein from following Dubois et al. (2021). The future will show if researchers will accept and apply this new concept.

In the chapter "Specimens erroneously marked as types in ZMB inventory catalogues", we mention names which are labeled as types in the inventory catalogues of the Herpetological Department of ZMB. To our knowledge, these names were never published by the authors to which these names are attributed, nor by anyone else. These names are thus placed in quotation marks and not italicized to indicate that they are not used as valid. Since these names were sporadically used (e.g. Schiøtz 1975) we list them here and clarify the identity of the specimens if possible.

It is important here to provide commentary on the publication history of Ahl's (1931a, b) works on hyperoliid frogs and how that relates to priority of the species names published therein. In 1931, Ahl published two monographs: the paper "Zur Systematik der afrikanischen Baumfroschgattung *Hyperolius* [Towards the systematics of the African tree frog genus *Hyperolius*] (Ahl 1931a)" as well as Lieferung [issue] 55 „Anura III" from the 'animal kingdom' series ["Das Tierreich"] (Ahl 1931b). The later work covered all known frog species of what today is accepted to be the family Hyperoliidae. However, it has never been fully clarified which of these monographs was published first, and thus which of these two publications serves as the original publication for making available the *Hyperolius* species names described by Ahl in 1931. We thus researched respective entries in the zoological main library of ZMB and in the reprint collection of the herpetological department. On the front covers of both publications we found the following notes on publications dates: volume 17, issue 1 of the "Mitteilungen aus dem Zoologischen Museum in Berlin" (Ahl 1931a) was published on 1 April 1931; Lieferung (volume) 55 of Anura III of the series "Das Tierreich" (Ahl 1931b) was published in March (März) 1931 (Fig. 4). Later, these publication dates were often overlooked or ignored. Hence numerous authors gave priority to the names published in Ahl's Lieferung 55 of the „Das Tierreich" (e.g. Laurent 1941, 1958; Loveridge 1942, 1953, 1957; Barbour and Loveridge 1946; Manaças 1949, Perret and Mertens 1957; Schiøtz 1975; Frost 1985; Channing and Howell 2006; Pickersgill 2007a; Seniagbeto et al. 2007; Mercurio 2011; Dehling 2012; Paepke 2013; Frétey et al. 2014; Liedtke et al. 2014; Marques et al. 2018), others however, regarded the names published in the "Mitteilungen vol. 17(1)" as having priority (e.g. Loveridge 1936a, b; Laurent 1943; Schiøtz 1967; Perret 1976b; Pakenham 1983; Rödel 1996; Lötters et al. 2004; Rödel et al. 2010; Amiet 2012). Frost (2021) states "The description in Ahl, 1931[a], Mitt. Zool. Mus. Berlin, 17 […], appeared a few weeks later according to unpublished notes by A. Loveridge (fide R. Laurent)." These personal notes and comments by Arthur Loveridge and Raymond Laurent correspond with the printed publication dates on the front covers of the two publications, as has been already commented on by Barbour and Loveridge (1946, p. 126).

However, it needs to be emphasized that, without doubt, Ahl intended to publish the paper "Zur Systematik […]" (Ahl 1931a) ahead of the "Anura III" (Ahl 1931b). For instance: i) in the preface of his paper" Zur Systematik […]" he refers to the ‚soon to be published monograph within the ‚animal kingdom series' [„demnächst erscheinende Monographie im "Das Tierreich""]; ii) in the paper he added „spec. nov." to the new names, in the species accounts of "Das Tierreich" this is not added, and iii) lastly comments concerning the distribution of species are generalized in "Das Tierreich" and data concerning type material and collectors are lacking completely (but are provided in "Zur Systematik […]").

To finally clarify the history of both publications (Ahl 1931a, b), we checked the original prints and Eingangsregister (accession catalogues) in the department of herpetology and the zoological library at ZMB. Based on the receipt stamps on the original prints (journal issue and reprints) as well as the notes in the Eingangsregister [entry register] of the zoological library, it is obvious that volume 17, issue 1 of the "Mitteilungen aus dem Zoologischen Museum in Berlin" was received on 11 April 1931. In contrast Lieferung 55, Anura III from "Das Tierreich" was only received a month later on 11 Mai 1931. Article 21.4 of the `Code' (ICZN 1999) clarifies that "If

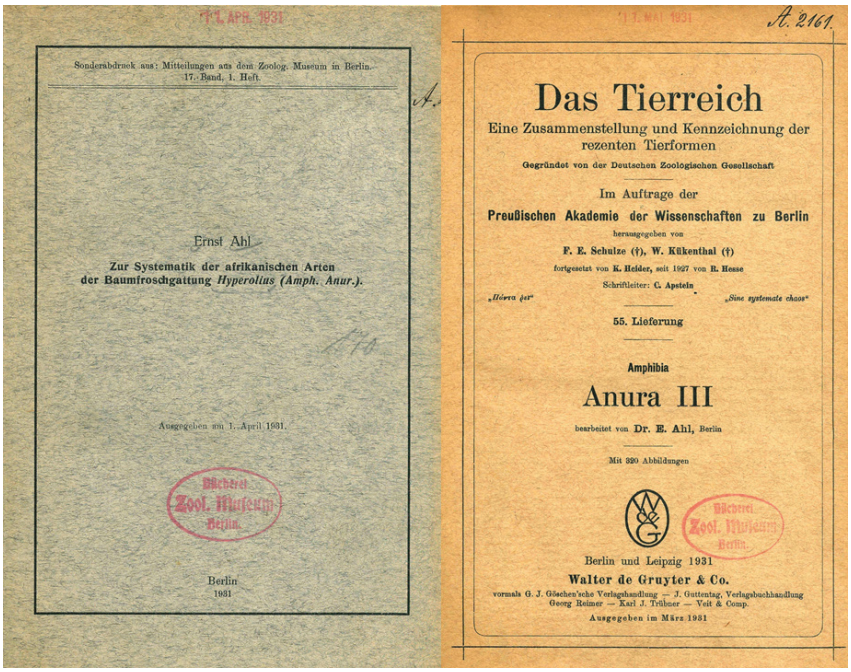


**Figure 4.** Titlepages of Ahl (1931a) "Mitteilungen…" and Ahl (1931b) "Das Tierreich" with printed dates of publication at the bottom and receipt stamps of the zoological library at ZMB with date of availability at the upper edge; compare text.

the date of publication specified in a work is found to be incorrect, the earliest day on which the work is demonstrated to be in existence as a published work is to be adopted". Furthermore, Recommendation 21D states that "A librarian should not remove, or allow to be removed by a binder, the cover or pages bearing information relevant to the date of publication, the contents of the work or its parts, or the day or dates of receipt by the library." This makes the receipt dates from the ZMB library relevant in this context. We thus follow Recommendation 21F of the `Code' and correct the publication dates which gives the names published by Ahl (1931a) in the "Mitteilungen […]" priority.

## Abbreviations

Institutional codes following Sabaj (2020):

BMNH [NHMUK] Natural History Museum, London (formerly: British Museum (Natural History));
MCZ Museum of Comparative Zoology, Harvard University, Cambridge;
MBL Museu Bocage [Museu Nacional de História Natural], Lisboa;
MHNG Muséum d'Histoire naturelle, Genève; MHNN – Naturhistorisches Museum Mainz;
MNHN Muséum national d'histoire naturelle, Paris;
MSNG Museo Civico di Storia Naturale "Giacomo Doria" [Civic Museum of Natural History], Genova [Genoa];
NMW Naturhistorisches Museum Wien, Vienna;
PEM Port Elizabeth Museum, Bayworld, Port Elizabeth;
RMNH Naturalis Biodiversity Center (formerly Rijksmuseum van Natuurlijke Historie], Leiden;
SAIAB South African Institute of Aquatic Biodiversity, Grahamstown;
SMF Senckenberg Forschungsinstitut und Naturmuseum, Frankfurt am Main;
SMNS Staatliches Museum für Naturkunde, Stuttgart;
ZFMK Zoologisches Forschungsmuseum Alexander Koenig, Bonn;
ZMB Museum für Naturkunde Berlin, Leibniz Institute for Evolution and Biodiversity Science (formerly: Zoologisches Museum Berlin);
ZMG Zoologisches Institut und Museum der Universität Greifswald;
ZMH CeNak (Center of Natural History), Zoologisches Museum, Universität Hamburg (formerly: Zoologisches Museum Hamburg; presumably from 1 July 2021 on, ZMH and ZFMK will fuse to LIB: Leibniz-Institut zur Analyse des Biodiversitätswandels);
ZMUC Universitets København, Zoologisk Museum, København;
ZSM Zoologische Staatssammlung München, Munich.

# Results

## Extant types

*Afrixalus fornasini*
see *Hyperolius bivittatus*.

*Afrixalus brevipalmatus*
see *Hyperolius brevipalmatus.*

*Afrixalus dorsalis*
see *Hyperolius dorsalis.*

*Afrixalus dorsimaculatus*
see *Megalixalus dorsimaculatus.*

*Afrixalus laevis*
*see Megalixalus laevis* (unlocated type specimens).

*Afrixalus stuhlmanni*
see *Hyperolius pygmaeus*, *Hyperolius unicolor*, *Megalixalus stuhlmanni.*

*Afrixalus uluguruensis*
see *Megalixalus uluguruensis.*

*Afrixalus vittiger*
see *Hyperolius vittiger.*

***Acanthixalus sonjae* Rödel, Kosuch, Veith & Ernst, 2003: 44.**

**Paratypes.** ZMB 74985 and ZMB 79340, "SRET [Station de Recherche en Écologie Tropicale] station transect X, large water-filled tree stump, secondary forest, Taï National Park, Ivory Coast, 5°50'N, 7°20'W", coll. Raffael Ernst and Mark-Oliver Rödel, 16.IX.2000.

**Present name.** *Acanthixalus sonjae* Rödel, Kosuch, Veith & Ernst, 2003.

**Remarks.** Holotype: SMNS 09573, "SRET station transect X, large water-filled tree stump, secondary forest, Taï National Park, Ivory Coast, 5°50'N, 7°20'W", coll. Raffael Ernst and Mark-Oliver Rödel, 16.IX.2000. Additional paratypes: SMNS 09574.1–2, "Noe-Grid, Taï National Park, Ivory Coast, 5°50'N, 7°20'W", coll. Raffael Ernst and Mark-Oliver Rödel, 16.IX.2000 and SMNS 09575.1–28, same collecting data as for the holotype; ZSM 9080/2001, same collecting data as for the holotype (Glaw and Franzen 2006); PEM A7414, "Forêt Classé de Haute Dodo, 4°54'03"N, 7°19'3"W, coll. William Roy Branch and Mark-Oliver Rödel, 15.III/2002 (Conradie et al. 2015); and "three males, two females and four juveniles alive, same data as holotype; numerous tadpoles alive". The two Berlin paratypes (ZMB 79340 and 74985) were formerly part of the aforementioned mentioned series of uncatalogued paratypes.

*Acanthixalus spinosus*
see *Hyperolius spinosus.*

***Cystignathus argyreivittis* Peters, 1854: 626.**

**Lectotype.** ZMB 4426, "Boror" [Companhia do Boror, Zambezia Province, Mozambique], coll. Wilhelm Carl Hartwig Peters.

**Paralectotypes.** ZMB 10106 and ZMB 85708 (formerly part of ZMB 10106), "Cabaceira" [Peninsula Cabaceira, Mossuril District, Nampula Province, Mozambique], coll. Wilhelm Carl Hartwig Peters.

**Present name.** *Kassina senegalensis* (Duméril & Bibron, 1841).

**Remarks.** Peters (1854) does not mention the number of specimens available to him for the description, but later he specified that he found "[…] drei weibliche Exemplare, eins auf der Halbinsel Cabaceira, zwei in Boror, während des Märzmonats, in feuchtem Grase." [… three female specimens, one on the Cabaceira Peninsula, two in Boror, during the month of March, in wet grass] (Peters 1882b: 158). One of the former syntypes is depicted in Peters (1882b, pl. 22, fig. 2, and on pl. 26, fig. 3, sternum). Bauer et al. (1995: 43) consider the specimens inventoried under ZMB 4426 and ZMB 10106 to be syntypes. Frost (2021) mentioned only ZMB 4426 as syntype and refers to other unnumbered syntype(s) in the ZSM collection. However, Glaw and Franzen (2006) do not mention syntypes of *Cystignathus argyreivittis* present at ZSM. Ahl (1930c: 283) denotes [ZMB] 4426 as "Typus" and [ZMB] 10106 (two specimens) as "Cotypen" of *Cystignathus argyreivittis*. This constitutes a lectotype designation of ZMB 4426 from "Boror" (ICZN 1999: Art. 74.5).

W. C. H. Peters, a zoologist, anatomist and later director of ZMB (from 1857 to 1883) undertook a journey to Mozambique from September 1842. Via Portugal and Luanda (Angola) he reached Mozambique Island on 17 June 1843. During his stay in Mozambique, he undertook various short trips, e.g. to Zanzibar, Anjuan (Comores), Saint Augustin (Madagascar) and South Africa. On August 7, 1847 he left Mozambique and sailed via Goa and Mumbai (India), Candy (Sri Lanka) and Egypt to return to Berlin in early 1848 (itinerary and map in Bauer et al. 1995). Already during his journey, Peters regularly sent his collections back to Berlin, some of which were donated to the Anatomical Museum, the majority was given to the ZMB and a number of doublets ("Doubletten") were sold (Brauer 1910; Bauer et al. 1995).

*Heterixalus betsileo*
see *Hyperolius friedrichsi.*

*Heterixalus variabilis*
see *Megalixalus variabilis.*

***Hyperolius acuticephalus* Ahl, 1931a: 131.**

**Holotype.** ZMB 30999; "Ngoto, Lobajegebiet" [Kembé, Basse-Kotto Prefecture, Central African Republic]; coll. Günther Theodor Tessmann, 30.X.1913.

**Present name.** *Hyperolius acuticephalus* Ahl, 1931.

**Remarks.** Depicted in Ahl (1931b: 419, fig. 291). The German botanist, ethnologist and explorer Tessmann travelled to Cameroon in 1904, where he worked until 1905 for the West African plantation company Bibundi as a supervisor on a cocoa plantation. Afterwards he travelled to the Cameroon Hinterland [‚Hinterland' is a term in the colonial literature, used in various languages;

it does not specify a specific geographic region but refers generally to regions being away from the coast or provincial towns] and to Yaoundé and founded his own plantation in the border area between German-Cameroon and Spanish-Guinea. From 1907 to 1909 he was the head of the "Lübecker Pangwe–Expedition" to South Cameroon and Equatorial Guinea and in 1913 he led an expedition to "Neu Kamerun". During the First World War he fled to Spanish Guinea and was interned by the Spanish on Fernando Pó [Bioko]. Later, he turned to South America, travelled through Peru, and emigrated to Brazil in 1936, where he settled in the state of Paraná and got a position at the Museu Paranaense. During his stay in Africa he collected large numbers of zoological, botanical and ethnological objects, most of which were sent to the museums in Berlin and Lübeck (Dinslage and Templin 2012; Dinslage 2015; Templin 2015).

*Hyperolius acuticephalus* could be conspecific with either *H. igbettensis* Schiøtz, 1963 or *H. adsperus* Peters, 1877. Type locality and shape of head better fit *H. igebettensis* (fide Channing et al. 2013); concerning webbing of feet *H. acuticephalus* is intermediate between *H. igbettensis* and *H. adspersus* (fide Channing et al. 2013); the ratio of head width/snout-vent length speaks in favor of *H. adspersus* (fide Amiet 2012); and the ratio of head length/head width points again to *H. igbettensis* (fide Amiet 2012); finally the value for the length of the snout/head width surpasses both *H. igebettensis* and *H. adspersus*.

***Hyperolius acuticeps* Ahl, 1931a: 29.**

**Syntypes.** ZMB 36039 and ZMB 65176 (formerly part of ZMB 36039), "Konde-Nika" [Region at the northern tip of Lake Malawi, Mbeya and Njombe Region, Tanzania], coll. Friedrich Georg Hans Heinrich Fülleborn, 02.VI.1900.

**Present name.** *Hyperolius microps* Günther, 1864.

**Remarks.** Drawing in Ahl (1931b: 282, fig. 153). For the location of "Konde-Land" we refer to Fülleborn (1906: 268 ff.), who describes it as a small area at the northern tip of Lake Malawi as follows: limited in the east by Lake Malawi, in the northeast by the slopes of the Livingstone Mountains [Kipengere Range], in the southeast by the Untali and Malila Mountains, in the north by Rungwe Volcano and in the south by the lower reaches of the Ssongwe (Songwe River).

In 1896, the German physician, doctor of tropical medicine, and explorer Fülleborn joined the colonial "Schutztruppe" in German East Africa, where he was active as a government physician until 1901. From April 1897 to the beginning of 1898, he participated in the military campaigns against the Wangoni and Wahehe of the "Ungoni", "Uhehe" and "Ubena" regions, in present day southern Tanzania. From 1898 to 1899 Fülleborn was stationed in Langenburg [Lumbira, Mbeya Region, Tanzania] in the north of Lake Malawi and undertook numerous excursions in the surrounding area, which took him to the southern end of Lake Malawi, through the "Schire-Hochländer" [Shire Highlands, southern Malawi], and on the Shire and Zambezi River to Quelimane and afterwards to the Island of Mozambique. In 1899 he was commissioned to research the "German-Nyassa" region from a zoological and ethnological-anthropological point of view. Together with W. Goetze he participated in the "Nyassa–See- und Kinga–Gebirgs–Expedition" (Engler 1902; Fülleborn 1906; see also comments on *Hyperolius goetzei*). During this time Fülleborn also surveyed Lake Malawi and the lakes in northern Nyasaland (Rukwa, Chungruru, Itende) and collected a considerable number of mammals, about 800 birds, more than 1000 fishes, amphibians and reptiles, thousands of insects and other invertebrates, and particularly plankton (Fülleborn 1900a, b; Paepke and Seegers 1995). The majority of these collections were donated to ZMB. Fülleborn's extensive herpetological collections were partly studied in the first third of the 20th century by former curators of herpetology at ZMB (e.g. Ahl 1929, 1931a, c; Tornier 1900, 1902, 1905). However, many specimens remained unexamined on the shelves within the 'undetermined material'. On the basis of Fülleborn's diary Hans Paepke (curator emeritus Department of Ichthyology at ZMB) compiled a list of the places where Fülleborn stayed between April 1897 and October 1899. The diary and this list are archived in the Department of Historical Research at ZMB (Zool. Mus. Sign. S III, "Fülleborn, F.").

***Hyperolius acutirostris* Buchholz & Peters in Peters, 1875: 207, pl. 2, fig. 4.**

**Syntypes.** ZMB 8470 and ZMB 65177 (formerly part of ZMB 8470), "Cameruns" [Douala, Region Littoral, Cameroon], coll. Reinhold Wilhelm Buchholz.

**Present name.** *Hyperolius acutirostris* Buchholz & Peters, 1875.

**Remarks.** Perret (1966: 408) considered the type material of *H. acutirostris* lost and designated MHNG 965.12 as neotype. Bauer et al. (1995: 43) could only locate one of the two syntypes. The type locality was corrected to "Douala" by Frétey et al. (2014); for further information see also remarks on *Hyperolius guttatus*.

The German explorer, zoologist and anatomist Buchholz went to Equatorial Africa from 1872 to 1875. He was accompanied by the Berlin ornithologist Georg Anton Eugen Reichenow and Reichenow's friend, fellow student and zoologist Wilhelm Lühder. On 1 June 1872 they set off from Bremerhaven to "Akkrá on the Gold Coast" [Accra, Ghana], which they reached on 29 July 1872. The first collecting tours took place in the surroundings of Accra and Aburi (29 July to 16 October 1872). On 16 October they left Accra for "Camaroons" [today part of present day Douala city] where they stayed until 2 November. Then they travelled to Bimbia, Victoria and Bonjonjo (2 November 1872 to 9 December 1873). On 12 March 1873 Lühder died of malaria in 'Camaroons'. Reichenow, also suffering from malaria, returned via Gabon to Germany in April 1873.

Buchholz was on his own from then on. He travelled between Victoria and `Camaroons' with intermediate stops on Fernando Pó to get his collections to Camaroons in early December.

Thereafter he went to Abo (9 December 1873 to 24 March 1874) and from Mungo via Balong he returned again to 'Camaroons' (5 April to 11 August 1874). He left `Camaroons' for a stay in Gabon where he also explored the Rembo River (12 August to 9 November 1874). After his return to the Gabon coast he again explored the area around Mungo and Jenssoki (9 November 1874 to 11 January 1875), and again visited Fernando Pó, the Gabon coast and the Ogowe (or Ogooué) River (11 January to 31 August 1875). On 3 September he started from Gabon on his way back to Greifswald where he arrived during the beginning of November 1875 (Reichenow 1874; Heinersdorff 1880; Weidmann 1894; Stresemann 1943). Buchholz' collections went to the zoological museums in Greifswald and Berlin and his herpetological material has been described by Wilhelm C. H. Peters (Peters 1875, 1876).

***Hyperolius ademetzi* Ahl, 1931a: 37.**

**Lectotype.** ZMB 20794, "Bamenda" [Mezam Department, Northwest Region, Cameroon], coll. First Lieutenant Karl Moritz Ernst Gustav Wilhelm Adametz, VI/1909.

**Paralectotypes.** ZMB 77729–77733 and ZMB 77749, same collecting data as for the lectotype.

**Present name.** *Hyperolius ademetzi* Ahl, 1931a.

**Remarks.** Drawing in Ahl (1931b: 296, fig. 171). Originally eight specimens according to the original publication. Lectotype designation by Perret (1962: 244, fig. 2) who provided a photograph of the lectotype. Another paralectotype MCZ A-17626 was sent in exchange from ZMB in 1932 (Barbour and Loveridge 1946: 126). Adametz was a first lieutenant in the German `Schutztruppe' for Cameroon and head of the colonial station in Bamenda. He was involved in surveying the Hinterland of the Kamerun-Nordbahn in the Bamenda region. In summer 1912, he also took part in an operation against the Baminge (Bamije–Expedition) at the eastern frontier of the present day Manyu Division, Southwest Region, Cameroon (Nkwi 1989; Hoffmann 2007; Hafeneder 2008).

***Hyperolius adolphi-friederici* Ahl, 1931a: 116.**

**Holotype.** ZMB 36114, "Rugegewald, 2000 m Höhe" [Nyungwe Forest, Cyangugu Prefecture, West Province, Rwanda], collected during the first "Deutsche Zentral–Afrika–Expedition", VIII/1907.

**Present name.** *Hyperolius castaneus* Ahl, 1931a.

**Remarks.** Drawing in Ahl (1931b: 399, fig. 274). Under the leadership of Adolf Friedrich, Duke of Mecklenburg, the first "Deutsche Zentral–Afrika–Expedition" had the goal of scientifically investigating the areas of the western branch of the East African Rift Valley. Among the expedition members who collected herpetological material were Schubotz and Grauer (meeting at Lake Kivu, see also remarks on *Hyperolius callichromus*) as well as von Raven. On 29 May 1907, the expedition started in Mombasa [Kenya], led via "Port Florence" [Kisumu at the northeastern coast of Lake Victoria, Kenya] to "Bukoba" [June 1907, Bukoba Urban District, Kagera Region, Tanzania] on the western shore of Lake Victoria. From here, almost 600 expedition members headed west to "Kifumbiro" [June 1907, a German military post at the ferry over the Kagera River], and to "Rufua" [July 1907, a military post in the northern Mpororo Region, Ntungamo District, Western Region, Uganda]. From here the expedition moved south to the "Mohasi See" [July 1907; Lake Mohasi, Rwanda] and "Niansa" [August 1907] and from there in western direction to the military station "Ischangi" [August 1907; Shangi, Gafunzo, Ruhango District, Southern Province, Rwanda] at the southern tip of Lake Kivu.

Then, the caravan turned north, crossed Lake Kivu with a stop at "Kwidschwi" Island [September 1907; Idjwi (Ijwi) Island, Lake Kivu, Democratic Republic of the Congo] and reached "Kissenji" [September 1907; Gisenyi on the northeast shore of Lake Kivu, close to the border of Democratic Republic of the Congo, Rwanda]. From there they went to Rutschurru [December 1907, Rutshuru, North Kivu Province, Democratic Republic of the Congo] and further north to Vitshumbi [December 1907] at the southern tip of Lake Edward. The expedition continued further along the west coast of Lake Albert to reach the Rwenzori Mountains via Kasindi [January 1908]. From Fort Beni [January to February 1908] on the western slopes of the Rwenzori Mountains the expedition went to Kassenje [March 1908] on the southwestern shore of Lake Albert. From here, the expedition turned west. Via Mawambi [April 1908] on the Ituri River and Avakubi [April 1908], it went along the left bank of the Aruwimi River to Basoko [May 1908; Tshopo Province, Democratic Republic of Congo] to the confluence with the Congo River, where the expedition ended in June 1908 (Schubotz 1909, 1912; Bamps 1975). The extensive zoological-botanical collections made during this expedition, including nearly 3000 vertebrates, were deposited at the ZMB and the Botanical Museum in Berlin. Most of the herpetological results of the expedition were published by Nieden (1913, Amphibia) and Sternfeld (1913, Reptilia).

From 1909 to 1910 a second "Deutsche Zentral–Afrika–Expedition", also under the leadership of Adolf Friedrich, Duke of Mecklenburg, extended along a main route from Cameroon via Spanish Guinea [Equatorial Guinea], Gabon, the Congo and the Ubangi River up to Fort de Possel [Possel, Central African Republic], and from there further north to Lake Chad and back via North Cameroon to the Niger Delta. Schubotz, who accompanied this expedition, deviated along the Ubangi River eastwards, in order to follow the White Nile in southern Sudan and returned via Khartoum and Egypt to Germany (Mecklenburg 1921).

***Hyperolius adspersus* Peters, 1877a: 619, pl., fig. 6.**

**Holotype.** ZMB 9176, "Chinchoxo (Westafrika)" [Cabinda Province, Angola], don. Africanische Gesellschaft.

**Present name.** *Hyperolius adspersus* Peters, 1877a.

**Remarks.** The "Africanische Gesellschaft", or formally "Deutsche Gesellschaft zur Erforschung Aequatorial-Africas", sponsored the "Loango–Expedition" from 1873–1876 under the leadership of the German geographer and explorer Richard Paul Wilhelm Güssfeldt. The expedition had the task to establish a station at the Loango coast (at Chinchoxo), which was to serve as a depot for the material collected during the expedition. Geographic-topographical explorations into the interior of the African continent were also intended to be carried out. With an interdisciplinary research team, comprising the medical officer and zoologist Julius Falkenstein, the geographer Eduard Pechuël-Loesche, the geodesist von Görschen, Reserve Lieutenant Hans von Hattorf, the mechanic Otto Lindner, the botanist Herman Soyaux, and the topographer Major Alexander von Mechow, Güssfeld travelled for two years, starting in July 1873. They mainly followed the coastal area of Cabinda, on the Kouilo river, the Chiluango river, and on the lower course of the Nyanga river. From March 1874, they turned to Luanda, on the Cuango to Dondo and to the rapids of Cambambe, as well as to Quicombo and Novo Redondo (Güssfeldt et al. 1879, Weidmann 1894, Heintze 2007, Marques et al. 2018). The amphibians and reptiles collected during these trips were sent to ZMB and described by Peters (1877a, b).

***Hyperolius albifrons* Ahl, 1931a: 81.**

**Holotype.** ZMB 36095, "Afrika (ohne genaueren Fundort [without precise locality])", collector and/or donor unknown.

**Present name.** *Hyperolius marmoratus* Rapp, 1842.

**Remarks.** Drawing in Ahl (1931b: 355, fig. 230).

***Hyperolius albofrenatus* Ahl, 1931a: 53.**

**Holotype.** ZMB 86012, "Deutsch-Ost-Afrika (genauerer Fundort unbekannt [without precise locality])" [probably Tanzania], coll. Ule, 22.XI.1912.

**Present name.** *Hyperolius albofrenatus* Ahl, 1931a.

**Remarks.** Drawing in Ahl (1931b: 315, fig. 189). There remains confusion about the collector and, related to this, the likely place of collection. A man by the name of Ernst Heinrich Georg Ule collected in Brazil and donated two frogs to the herpetological collection, one with the accession catalogue number C-581 (from November/December 1912) without further data, and a second one (C-145) on 21 May 1904, collected on the Upper Amazon. The frog thus might actually be a South American tree frog and not a hyperoliid. However, another person named Dr. Ferdinand Uhl was a member of the "Deutsche Schutztruppe" in East Africa who collected the holotype of *Hyperolius guttolineatus* Ahl, 1931 (see below, unlocated type specimens). Lastly, a person with the surname Uhle collected in Sumatra, Bolivia and Argentina. Thus, neither the identity of the frog, nor its geographic origin and collector can be determined with certainty.

***Hyperolius albolabris* Ahl, 1931a: 33.**

**Holotype.** ZMB 58748, "Kwa Buosch oder Bnorch (Deutsch-Ost-Afrika)", located in "Kwa Buosch in Süd Kavirondo" [near Lake Victoria, Migori district, southwestern Kenya] according to Neumann (1898: 242), coll. Oscar Rudolph Neumann, 26.II.1894.

**Present name.** *Hyperolius glandicolor* Peters, 1878.

**Remarks.** Depicted in Ahl (1931b: 288, fig. 161).

***Hyperolius alticola* Ahl, 1931a: 106.**

**Lectotype.** ZMB 39008, "Ruwenzori, 1800 m hoch" [Rwenzori Mountains, Democratic Republic of the Congo], collected during the first "Deutsche Zentral–Afrika–Expedition", II/1908.

**Paralectotype.** ZMB 74944, same collecting data as for the lectotype.

**Present name.** *Hyperolius discodactylus* Ahl, 1931a.

**Remarks.** Depicted in Ahl (1931b: 380, fig. 255). Lectotype designation by Liedtke et al. (2014) who rediscovered the type specimens in the ZMB collection.

*Hyperolius angolensis*

see *Hyperolius decorates*, *Hyperolius insignis*, *Hyperolius nossibeensis*, *Hyperolius vermiculatus.*

***Hyperolius argentophthalmus* Ahl, 1931a: 83.**

**Holotype.** ZMB 36092, "ohne genauen Fundort" [without specified locality], collector or donor unknown.

**Present name.** *Hyperolius concolor* (Hallowell, 1844).

**Remarks.** Depicted in Ahl (1931b: 357, fig. 233).

***Hyperolius argentovittis* Ahl, 1931a: 72.**

**Holotype.** ZMB 85718, "Ujiji (Udjidji, Tanganyika-See, Deutsch-Ost-Afrika)" [Ujiji, Kigoma Province, Tanzania], coll. Paul Hösemann (Fig. 5).

**Present name.** *Hyperolius marginatus* Peters, 1854.

**Remarks.** Drawing in Ahl (1931b: 346, fig. 220).

Dr. Hösemann served in the colonial German Schutztruppe as medical officer [Stabsarzt], undertook anthropological studies, and between 1897 and 1907, collected zoological objects on the northeastern shore of Lake Tanganyika (Udjidji), in the Kissaka Region (southeast of Lake Mugesera, Ngoma and Kirehe Districts, Eastern Province, Rwanda] and between Mwanza and Moshi [northern Tanzania] (Hösemann 1897, Hafeneder 2010). Among others, he participated in the "German–French

**Figure 5.** Holotype of *Hyperolius argentovittis* Ahl, 1931a, ZMB 85718 from "Ujiji (Udjidji, Tanganyika-See, Deutsch-Ost-Afrika)", coll. Hösemann.

Border Expedition" (October 1901 to December 1902) to define the southern border of Cameroon, during which he mapped the area from the camp Nyengwe, south of Kampo, to the Ngoko station in the Sanga Ngoko area (Danckelmann 1901; Fitzner 1901).

***Hyperolius argus* Peters, 1854: 628.**

**Syntypes.** ZMB 4807 (two specimens according to Peters 1882b and ZMB inventory catalogue), "Boror" [Companhia do Boror, Zambezia Province, Mozambique], coll. Wilhelm Carl Hartwig Peters.

**Present name.** *Hyperolius argus* Peters, 1854.

**Remarks.** Peters (1882b: 165) refers to two syntypes which he found in March 1846 in a bush at the edge of the forest near Boror. Depicted in Peters (1882b, pl. 22, fig. 6) and mentioned and depicted by Tornier (1896: 146, pl. 4, fig. 72 [=ZMB 4807]). Only one syntype could be located.

*Hyperolius argus*
see *Hyperolius flavoviridis*, *Hyperolius tettensis.*

***Hyperolius asper* Ahl, 1931a: 49.**

**Holotype.** ZMB 36106, "Nairobi" [Kenya], coll. Felice Thomas.

**Present name.** *Hyperolius marmoratus* Rapp, 1842.

**Remarks.** Between 1896 and 1903 the engineer and transport officer of the "Mombasa–Uganda Railway" in British East Africa, Felice (sometimes Felix) Thomas sent several shipments, containing amphibians and reptiles, from the Kenyan coast province (Mombasa and Takanugu) and from Nairobi to ZMB.

***Hyperolius baumanni* Ahl, 1931a: 34.**

**Syntypes.** ZMB 84956, coll. 26.VII.1894, ZMB 90925–90926, coll. 07.V.1894, all from "Misahöhe, Togo" [Missahomé, Agou Prefecture, Plateau Region, Togo], all coll. Ernst Richard Reinhold Baumann.

**Present name.** *Hyperolius baumanni* Ahl, 1931a.

**Remarks.** Drawing in Ahl (1931b: 291, fig. 167). According to Ahl (1831a: 35) the original series consisted of four specimens, i.e. one collected on 26 July 1894 and three on 07 May 1893. However, the latter year given by Ahl is most probably a typographical error. According to Baumann's preserved original field label, the date of collection was the "7. Mai 1894". Another paratype MCZ A-17627 from "Misahöhe, Togo", coll. Baumann on 07 May 1894, was sent in exchange from ZMB in 1932 (Barbour and Loveridge 1946: 127). The latter specimen was erroneously regarded as a holotype by Seniagbeto et al. (2007: 77).

The natural scientist and cartographer Baumann joined the German Colonial Service in 1893. He worked at Klein Popo [Aného, Lacs Prefecture, Maritime Region, Togo] and was later stationed at Misahöhe [Agou Prefecture, Plateau Region, Togo] where he was deputy station chief from 1894–95. In November 1894, he accompanied the "Togo–Hinterland–Expedition" headed by the colonial officer Hans Gruner along the Volta River to Kete Kratschi [Kete Krachi, Oti Region, Ghana] and returned to Misahöhe. In the hinterland of the station (Agome Region) he collected zoological, botanical and ethnological objects, which were given to the museums in Berlin. In 1895 he returned to Germany where he died on 4 September as a result of malaria that he contracted on his return journey (Danckelmann 1895; Reichenow 1897; Heß 1902; Hafeneder 2008).

*Hyperolius bicolor*
see unlocated type specimens'.

***Hyperolius bitaeniatus* Ahl, 1931a: 58.**

**Holotype.** ZMB 39004, "Konde-Nika, Deutsch-Ost-Afrika" [Region at the northern tip of Lake Malawi, Mbeya and Njombe Region, Tanzania], coll. Friedrich Georg Hans Heinrich Fülleborn.

**Paratypes.** ZMB 11919, "Deutsch-Ost-Afrika", coll. Oscar Rudolph Neumann and ZMB 85835–85840, "Konde-Nika", coll. Friedrich Georg Hans Heinrich Fülleborn.

**Present name.** *Hyperolius mariae* Barbour & Loveridge, 1928.

**Remarks.** Drawing in Ahl (1931b: 322, fig. 196). Another paratype MCZ A-17628 from "Konde-Nika", coll. Fülleborn was sent in exchange from ZMB to MCZ in 1932 (Barbour and Loveridge 1946: 127).

***Hyperolius bivittatus* Peters, 1854: 627.**

**Syntypes.** ZMB 4529 and ZMB 52503–52509 (formerly part of ZMB 4529), "Boror" [Companhia do Boror, Zambezia Province, Mozambique], coll. Wilhelm Carl Hartwig Peters.

**Present name.** *Afrixalus fornasini* (Bianconi, 1849).

**Remarks.** Depicted in Peters (1882b, pl. 24, fig. 2 and pl. 26, fig. 6, sternum). Peters (1882b: 161) specified that he found this species in March 1846, often in grass and on bushes in the Prazo [estate] Boror northwest of Quellimane. Poynton and Broadley (1987: 192) incorrectly state that the description of *H. bivittatus* is based on a holotype.

***Hyperolius brachiofasciatus* Ahl, 1931a: 87.**

**Holotype.** ZMB 77723, "Ngoto, Lobaje-Gebiet, Westafrika" [Lobaye Prefecture, Central African Republic]", coll. Günther Theodor Tessmann.

**Present name.** *Hyperolius brachiofasciatus* Ahl, 1931a.

**Remarks.** Depicted in Ahl (1931b: 361, fig. 237).

***Hyperolius breviceps* Ahl, 1931a: 54.**

**Holotype.** ZMB 86026, "Tschimbo, Port. Ost-Afrika" [Chemba, Sofala Province, Upper Zambezi, Mozambique], coll. Wilhelm Tiesler, 11.XI.1905.

**Paratypes.** ZMB 39012 and ZMB 77753–77754 (formerly part of ZMB 39012), all from "Eldama River Station, südöstlich vom Baringo-See, Britisch-Ost-Afrika" [Eldama Ravine, Baringo County, Kenya], all coll. Hermann Grote.

**Present name.** *Hyperolius marmoratus* Rapp, 1842.

**Remarks.** Depicted in Ahl (1931b: 316, fig. 190). Another paratype, MCZ A-17629 from "Eldama River Station", coll. Grote was sent in exchange from ZMB in 1932 (Barbour and Loveridge 1946: 127).

In October 1905 and November 1907, Tiesler sent two shipments, including nearly 300 amphibians and reptiles, to ZMB. This material was collected between November 1904 and January 1906 in Portuguese East Africa [Mozambique] and described by Nieden (1915). The vouchers of this collection originated from the following localities: Cabayra, Chifumbazi, Chinta, Costa, Lukunga, Marazi, Missala, Tschimbo, Tschinoupe and from the Zambezi River without any exact locality data.

***Hyperolius brevipalmatus* Ahl, 1931a: 25.**

**Holotype.** ZMB 24499, "Sangmelina, Süd Kamerun" [Sangmélina, Lobo Division, South Province, Cameroon], purch. Franz Hermann Rolle.

**Present name.** *Afrixalus brevipalmatus* Ahl, 1931a.

**Remarks.** Drawing in Ahl (1931b: 279, fig. 150). Perret (1976b: 21) listed two specimens, i.e. ZMB 24499 and ZMB 20132, as syntypes of *H. brevipalmatus* Ahl. However, Ahl's description is clearly based on a single specimen "1 Stück [piece]" from "Sangmelina" purchased from "Rolle". Furthermore, the collection data of ZMB 20132 from "Bipindi" [Bipindi village, Océan Department, South Province, Cameroon], coll. Georg August Zenker, do not match the information provided in the original description.

Rolle was a well-known dealer of zoological and enthnological objects. He maintained a worldwide network of collectors and suppliers and acquired several important collections. From 1889 onwards, he supplied private collectors as well as important European museums with zoological objects from Berlin. In later years, he traded objects under the name of the natural history institute "Kosmos".

*Hyperolius buchholzi*

see "unlocated type specimens".

***Hyperolius callichromus* Ahl, 1931a: 99.**

**Holotype.** ZMB 78576, "Westliches Russisi-Ufer und Nordwest-Ufer des Tanganyika" [West Bank of Ruzizi River, Democratic Republic of the Congo], coll. Rudolf Grauer (Fig. 6).

**Paratypes.** ZMB 78577–78583, same data as for the holotype, ZMB 85841–85844 "Usumbura" [Bujumbura, Bujumbura Mairie Province, Burundi], coll. Rudolf Grauer; ZMB 85854 "Kililana" [opposite of Manda Island, Lamu District, Coast Province, Kenya], coll. Clemens Andreas Denhard; ZMB 86000, "Kawende" [region in south Kigoma and northwest Katawi Division, eastern Tanzania], coll. Robert Reichert; ZMB 85869–85872, "Dar-es-Salaam" [Dar es Salaam, Tanzania], collector unknown.

**Present name.** *Hyperolius marginatus* Peters, 1854.

**Remarks.** Drawings illustrating the variation of this taxon are given by Ahl (1931b: 373, fig. 248). Ahl (1931a: 101) mentioned 27 specimens, of which we could not locate the material collected by Schubotz and Paulus in "Bagamojo" and "Zentral Afrika". Two paratypes, MCZ A-17630–17631 from "Westliches Russisi-Ufer und Nordwest-Ufer des Tanganyika", coll. Grauer, were sent in exchange from ZMB in 1932 (Barbour and Loveridge 1946: 126).

The Austrian hunter and Africa explorer Grauer undertook several expeditions to Eastern Africa, e.g. to British East Africa [Uganda] (February to May 1904 and September to November 1905) and to Tanganyika in 1907, where he met the first "Deutsche Zentral–Afrika–Expedition" at Lake Kivu in August. Upon this meeting he handed the zoological material he had collected in the "Zwischenseengebiet" [Region between Lake Victoria, Lake Kivu and Lake Malawi, Tanzania] for ZMB and the Walter Rothschild Zoological Museum (now the Natural History Museum at Tring), to the German expedition. Grauer then turned south, travelled along the west bank of Lake Tanganyika and returned to Europe in early 1909 (Schubotz 1909, 1912; ÖAW 1959; Riedl-Dorn 2001). In November 1909, he returned to Africa, on behalf of the Natural History Museum Vienna (NMW) and travelled to Lake Victoria and Lake Malawi. From there he turned further north along the African Rift Valley to Beni [North Kivu Province, Democratic Republic of the Congo], from where he returned to Austria in May 1911. About 250 herpetological objects (mainly reptiles) collected during

**Figure 6.** Holotype of *Hyperolius callichromus* Ahl, 1931a, ZMB 78576 from "Westliches Russisi-Ufer", coll. Grauer.

this expedition are in the collection of NMW, collected mainly in South Kivu, North Kivu and Orientale Province of D. R. Congo (Silke Schweiger in litt. 5 August 2020). The herpetological collections of Grauer's last expedition were partly described by Steindachner (1911) and Werner (1924). We refer also to Gemel et al. (2019) for information about the type material collected by Grauer and deposited in the NMW collection.

***Hyperolius castaneus* Ahl, 1931a: 31.**

**Holotype.** ZMB 60230, "Vulkangebiet nord-östlich des Kivu-See's" [volcano region northeast of Lake Kivu, Virunga Mountains, along the border between Rwanda and the Democratic Republic of the Congo], coll. Werner Alborus von Raven, X/1907.

**Present name.** *Hyperolius castaneus* Ahl, 1931.

**Remarks.** Drawing in Ahl (1931b: 286, fig. 159). The German medical doctor von Raven, who specialized in bacteriology and tropical medicine, accompanied the first "Deutsche Zentral–Afrika–Expedition" under the leadership of Adolf Friedrich, Duke of Mecklenburg from 1907 to 1908 (Schubotz 1909). For expedition information see account on *Hyperolius adolphi-friederici*.

*Hyperolius castaneus*
see *Hyperolius adolphi-friederici*, *Hyperolius latifrons*, *Hyperolius rugegensis*, *Hyperolius ventrimaculatus.*

***Hyperolius chabanaudi* Ahl, 1931a: 124.**

**Holotype.** ZMB 18228, "Beniló, Französischer Kongo" [Benito River, Equatorial Guinea], don. William Frederic Henry Rosenberg.

**Present name.** *Hyperolius phantasticus* (Boulenger, 1899).

**Remarks.** The English ornithologist and entomologist Rosenberg collected mainly for the British Museum of Natural History (Günther 1906). In the accession catalogues of the herpetological department at ZMB, it is documented that Rosenberg on multiple occasions sent amphibians and reptiles to ZMB between 1900 and 1925. These vouchers were collected in Columbia, Peru, Ecuador, Venezuela, Cameroon, and Equatorial Guinea.

***Hyperolius coeruleopunctatus* Ahl, 1931a: 76.**

**Holotype.** ZMB 36115, "Nairobi" [Kenya], coll. Felice Thomas.

**Paratypes.** ZMB 77540–77542, "Nairobi", coll. F. Thomas and ZMB 85884 "Kibwezi" [Makueni County, Kenya], coll. Georg R. O. Scheffler.

**Present name.** *Hyperolius glandicolor* Peters, 1878.

**Remarks.** Depicted in Ahl (1931b: 351, fig. 225).

*Hyperolius concolor*
see *Hyperolius argentophthalmus*, *Hyperolius depressus*, *Hyperolius guineensis*, *Hyperolius moseri* (unlocated type specimens), *Hyperolius narinus*, *Hyperolius petersi*, *Hyperolius togoensis.*

*Hyperolius concolor guttatus*
see *Hyperolius guttatus*, *Hyperolius hildebrandti*, *Hyperolius maximus*, *Hyperolius pulcher.*

***Hyperolius decipiens* Ahl, 1931a: 120.**

**Syntypes.** ZMB 39003 and ZMB 77763–77765 (formerly part of ZMB 39003), "Westliches Russisi-Ufer und Nordwest-Ufer des Tanganyika" [West Bank of Ruzizi River and northwest bank of Lake Tanganyika, Democratic Republic of the Congo], coll. Rudolf Grauer 1908–1911.

**Present name.** *Hyperolius marginatus* Peters, 1854.

**Remarks.** Depicted in Ahl (1931b: 405, fig. 280). Another paratype, MCZ A-17633 from "Westliches Russisi-Ufer und Nordwest-Ufer des Tanganyika", coll. Grauer, was sent to MCZ in exchange in 1932 (Barbour and Loveridge 1946: 126).

***Hyperolius decoratus* Ahl, 1931a: 78.**

**Lectotype.** ZMB 36112, "Longa" [Longa River, Angola], coll. Ludwig J. Brühl or Otto Gleim.

**Paralectotypes.** ZMB 31905–31906, "Angola", coll. Brühl; ZMB 38255 and ZMB 77797 (formerly part of ZMB 38255), "Longa, Angola", coll. Brühl or Gleim; ZMB 77752, "Angola" coll. Gleim.

**Present name.** *Hyperolius angolensis* Steindachner, 1867 (fide Marques et al. 2018).

**Remarks.** Lectotype designation by Perret (1962). Another paralectotype MCZ A-17632 from "Longa", coll. Brühl and Gleim, was sent in exchange from ZMB

in 1932 (Barbour and Loveridge 1946: 127). Drawing in Ahl (1931b: 352, fig. 227). Ahl (1931a: 80) stated that seven specimens were collected by "Brühl and Gleim". However, these two people were not active in Angola at the same time (see below). It is possible that specimens of both collectors were stored together. Thus, it is no longer possible to assign the specimens to one collector.

Gleim was Deputy Governor of the German Colony of Togo from 1896 to 1898. From 1899, he was sent to São Paolo de Loanda by the "Kolonialabteilung des Auswärtigen Amtes", where he served as the first professional consul for Angola and French Congo. From 1910 to 1911 he was Governor of Cameroon (Schnee 1920a). On his return from Angola to Germany in 1901, he donated various collections of vertebrates and invertebrates to ZMB. In 1928 Prof. Dr. Brühl, at that time custodian at the Institut für Meereskunde Berlin, donated the insects and vertebrates he collected in Mossamedes (Angola) from 1922 to 1923 to ZMB.

***Hyperolius depressus* Ahl, 1931a: 61.**

**Holotype.** ZMB 43554, "Misahöhe, Togo" [Missahomé, Agou Prefecture, Plateau Region, Togo], coll. Ernst Richard Reinhold Baumann.

**Present name.** *Hyperolius concolor* (Hallowell, 1844).

**Remarks.** Depicted in Ahl (1931b: 326, fig. 200).

***Hyperolius dermatus* Ahl, 1931a: 108.**

**Holotype.** ZMB 85999, "Cabayra (Port. Ost-Afrika)" [? Cabaíra, Cahora Bassa District, Tete Province, Mozambique], coll. Wilhelm Tiesler, 20.VII.1905.

**Present name.** *Hyperolius marmoratus* Rapp, 1842.

**Remarks.** Ahl (1931a: 109) incorrectly names "Teisler" as collector. The exact position of the type locality remain dubious. There is also a Cabaia in the Zambézia province, district of Namacurra, area of Macuze, but it is unclear if this locality was intended.

***Hyperolius dintelmanni* Lötters & Schmitz, 2004: 150.**

**Paratype.** ZMB 79543,"Edib Hills (ca. 1,200 m above sea level) Bakossi Mountain, Southwest Cameroon (4°57'N, 9°39'E)" [type locality], coll. Oliver Euskirchen and Andreas Schmitz, 03.XII.1997.

**Present status.** *Hyperolius dintelmanni* Lötters & Schmitz, 2004.

**Remarks.** Holotype ZFMK 67871 and ten paratypes ZFMK 67441, 67443–447, ZFMK 67453, ZFMK 67872–67873 and ZFMK 67890 all from the type locality. ZMB 79543 (formerly ZFMK 67442), was given in exchange to ZMB on 18.X.2013 (see also Böhme 2014).

***Hyperolius discodactylus* Ahl, 1931a: 89.**

**Holotype.** ZMB 36089, "Rugegewald" [Nyungwe Forest, Cyangugu Prefecture, West Province, Rwanda], coll. Rudolf Grauer.

**Present name.** *Hyperolius discodactylus* Ahl, 1931.

**Remarks.** Drawing in Ahl (1931b: 364, fig. 239). According to Ahl (1931a: 90) the original series consists of seven specimens from "Rugegewald", including the "Type" and from "westlich des Albert-Edward-Sees's" [west of Lake Edward, Democratic Republic of the Congo], all coll. Grauer. A paratype MCZ A-17634 from Lake Edward, coll. Grauer was sent in exchange to MCZ in 1932 (Barbour and Loveridge 1946: 128). The remaining five paratypes could not be located. Liedtke et al. (2014) regarded ZMB 36089 as lectotype and restricted the type locality to "Nyungwe Forest (most likely Rwasenkoko [Uwasenkoko])".

*Hyperolius discodactylus*
see *Hyperolius alticola.*

***Hyperolius dorsalis* Peters, 1875: 206, pl. 1, fig. 2.**

**Syntypes.** ZMB 4488, "Boutry" [Butre (Bootry), Ahanta West District, Western Region, Ghana], don. Hermann Schlegel (Museum Leyden), and ZMB 8850 "Victoria" [Limbe, Fako Division, Southwest Region, Cameroon], coll. Ernst Richard Reinhold Baumann.

**Present name.** *Afrixalus dorsalis* (Peters, 1875).

**Remarks.** Peters' (1875) description was based on an unknown number of syntypes. He mentioned several specimens found in a pond in Victoria of which we could locate only one specimen. Mertens (1938) restricted the type locality to "Boutry".

***Hyperolius fimbriolatus* Buchholz & Peters in Peters, 1876: 121.**

**Syntypes.** ZMB 8830 and ZMB 65178 (formerly part of ZMB 8830), "Limbareni am Ogowe" [Lambaréné on the river Ogooué (or Ogowe), Moyen-Ogooué Province, Gabon], coll. Reinhold Wilhelm Buchholz.

**Present name.** *Hyperolius olivaceus* Peters, 1876.

**Remarks.** Depicted in Tornier (1896, pl. 4, fig. 100 and 101) and partly redrawn in Ahl (1931b: 332, fig. 205).

The name *Rappia fimbriata* Tornier (1896: 153, pl. 4, figs 100, 101) is categorized as *nomen inquirendum*, "Name(s) unassigned to a living or extinct population" by Frost (2021). Tornier (1896) attributed the authorship of this name to "B e P" which refer to Buchholz and Peters instead of "Duméril and Bibron" as claimed by Frost (2021). Tornier (l.c.) mentioned the type material as collected at "Gowe Limbareni", a writing error for "Limbareni am Ogowe [river]" (Peters 1876). However, Buchholz and Peters never together described a reed frog with

the specific epithet "*fimbriata*". Tornier's name *fimbriata* does not meet the requirements of Art. 33.2 of the 'Code' (ICZN 1999) for an "emendation". We therefore consider *fimbriata* Tornier, 1896 as an incorrect subsequent spelling of the specific epithet *fimbriolata* Buchholz & Peters in Peters, 1876.

### *Hyperolius flavoguttatus* Ahl, 1931a: 96.

**Holotype.** ZMB 39011, "Bukoba" [Bukoba Urban District, Kagera Region, Tanzania], coll. Franz Ludwig Stuhlmann.

**Paratypes.** ZMB 75607 (formerly part of ZMB 39011), from "Bukoba", coll. Stuhlmann and ZMB 85757, "Kenia", coll. Johann Georg Kolb, 1894.

**Present name.** *Hyperolius viridiflavus* (Duméril & Bibron, 1841).

**Remarks.** Depicted in Tornier (1896: 136, fig. 1) and redrawn in Ahl (1931b: 370, fig. 245). According to Ahl (1931a: 97) the original series consists of five specimens from "Bukoba", including the "Type" and from "Kenia", collected by Stuhlmann and Kolb. Another paratype MCZ A-17635 from "Bukoba", coll. Stuhlmann, was sent to MCZ in exchange in 1932 (Barbour and Loveridge 1946: 128). The fourth paratype could not be located.

The German zoologist, cartographer, explorer and colonial official Stuhlmann spent a total of 14 years in East Africa. With the financial support of the Akademie der Wissenschaften [Academy of Sciences] zu Berlin, he investigated the coastal regions of Zanzibar and the adjacent mainland including "Usegúa" and "Ungúu" in present-day Tanzania in the summer of 1888; then, until mid-1889, the area of the Zambezi estuary around Quelimane in Mozambique. From April 1890 to 1892, Stuhlmann participated as a scientist, together with Lieutenant Wilhelm Langheld, on the expedition of Mehmed Emin Pasha [also known as Eduard Karl Oskar Theodor Schnitzer] to the German East African colonial area. The expedition led them from Bagamoyo (26 April 1890) via Tabora (29 July) to Bukoba on Lake Victoria (November 1890). From here, Stuhlmann undertook a trip on Lake Victoria to Murchison Bay in Uganda (December 1890) and reached Mengo Mountain (26 to 29 December) via Manjongo [Rubaga Division, Kampala District, Central Region, Uganda]. After his return to Bukoba, he set off (12 February 1891) towards the west in the Karagwe Region, and after crossing the Kagera River (06 April 1891), the expedition reached the southwestern tip of Lake Edward in early May 1891. The expedition turned west of Lake Edward another 250 km to the north, but was terminated in mid-September 1891 due to insurmountable difficulties. With a group of 27 askaris (local soldiers serving in European colonial armies) and 100 porters Stuhlmann went back to Bukoba, where he arrived on 17 March 1891. Emin Pasha, in contrast decided to stay behind with sick expedition members, turned southwest towards the Congo River and was murdered by Arab slave traders 80 km from this destination at Kinene on Mwiko River on 20 October 1892. Stuhlmann's herpetological collections from these expeditions were sent to Johann Georg Pfeffer at the Zoologische Museum Hamburg, who published the first results (Pfeffer 1889, 1893). Parts of these collections, including "Doubletten" [doublets], were later donated to ZMB (Stuhlmann 1893; Tornier 1896). In July 1892 Stuhlmann returned to Bagamoyo on the East African coast, where he engaged in cartography and other scientific activities in Dar-es-Salaam and its surroundings until 1901. Between December 1900 and June 1901 Stuhlmann visited India, Sri Lanka, Singapore, and Indonesia. After returning to Africa, he was offered the post of the director of the "Biologisch-Landwirtschaftliche Institut Amani" [Agro-biological Institute Amani] in Usambara in July 1901, a post he took up in June 1903 and held until the end of 1905. During his last stay in Africa from December 1906 to January 1908, he worked in Amani primarily on the completion of his "Kulturgeschichte von Ostafrika" [Cultural history of East Africa] published in 1909. After various tropical diseases, he left the African continent at the age of 43 years with his health "exhausted" on 27 January 1908, and returned to Germany (Stuhlmann 1891, 1893, 1894, 1909; Danckelmann 1891, 1892; Weidmann 1894; Schnee 1920b; Bindseil 2008; Schabel 1990; Wenzel Geißler et al. 2020).

### *Hyperolius flavoviridis* Peters, 1854: 628.

**Holotype.** ZMB 6631, "Boror" [Companhia do Boror, Zambezia Province, Mozambique], coll. Wilhelm Carl Hartwig Peters.

**Present name.** *Hyperolius argus* Peters, 1854.

**Remarks.** Depicted in Peters (1882b, pl. 22, fig. 4). Bauer et al. (1995: 44) regarded two specimens, i.e. ZMB 6631 and ZMB 6632 as syntypes of *H. flavoviridis*. However, Peters (1854: 628) mentioned only material from "Boror" in his original description and he specified later (1882b: 164) that he got only one male from that locality, which corresponds to ZMB 6631. Although ZMB 6632 from "Halbinsel Cabaceira" [Peninsula Cabaceira, Mossuril District, Nampula Province, Mozambique], collected in June 1843, is marked by Peters' hand as type of *H. flavoviridis* in the ZMB inventory catalogues, the stated locality does not correspond with the type locality. Another two specimens from "Tette" donated from ZMB to the collection in Leiden (RMNH RENA-1780 and 1785) have been regarded as possible syntypes of *H. flavoviridis* (Bauer et al. 1995: 44, Gassó Miracle et al. 2007: 36). Both specimens can be excluded as types of *H. flavoviridis* because of the locality information, being different from the type locality. Likewise they cannot be the types of *H. tettensis* because of the single female type specimen mentioned by Peters (1882b: 164) is ZMB 4812 (see below).

***Hyperolius friedemanni* Mercurio and Rödel in Channing, Hillers, Lötters, Rödel, Schick, Conradie, Rödder, Mercurio, Wagner, Dehling, Du Preez, Kielgast & Burger, 2013: 20, fig. 4D, fig. 6, second row left.**

**Paratype.** ZMB 76095, "Karionga, Malawi, 9°55'59.6"S, 33°56'44.6"N, 472 m a.s.l." [Karonga District, Northern Region, Malawi], coll. Vincenzo Mercurio, 07.II.2007.

**Present name.** *Hyperolius friedemanni* Mercurio and Rödel 2013.

**Remarks.** Holotype: SMF 85694 from "Karionga, Malawi, 9°55'59.6"S, 33°56'44.6"N, 472 m a.s.l.", coll. Mercurio, 07.II.2007 and additional paratypes: SAIAB 186000 (two juveniles) from "Monkey Bay, Malawi", collector not mentioned.

***Hyperolius friedrichsi* Ahl, 1930d: 67.**

**Holotype.** ZMB 30637, "Antananarivo, Madagaskar" [Analamanga Region, Madagascar], coll. Karl Friedrichs [sic] aus Rostock.

**Present name.** *Heterixalus betsileo* (Grandidier, 1872).

**Remarks.** Depicted in Ahl (1931b: 422, fig. 295). From October 1914 until the end of 1915, the German zoologist and colonial officer Prof. Dr. Friederichs, who was a prisoner of war during the First World War, collected in the courtyard of the French Fort Duchesne (on a hill opposite of Antananarivo, ca. 1400 m a.s.l.). Later, he continued collecting until 1916 on Kap Diego [Cap Diego, Antsiranana I District, Diana Region, Diego Suarez Province] in northern Madagascar (Schultheß 1918; Friederichs 1919).

***Hyperolius fuelleborni* Ahl, 1931a: 75.**

**Syntypes.** ZMB 71184–71186 and 85925–85927, "Neu Helgoland" [Pugulo (or Papaya Island), a small rock island in Lake Malawi, Mbinga District, Ruvuma Region, Tanzania]; ZMB 77465–77468, 85919–85921, 85928, 85964–85971, 86138, 90972, "Langenburg" [Lumbira, Mbeya Region, Tanzania]; ZMB 85922–85924, "Langenburg – Nordende des Nyassa" [Lumbira at the northern shore of Lake Malawi]; ZMB 85972–85973, 90928, "Miramba bei Langenburg" [Miramba near Lumbira]; ZMB 85929–85963, 85974–85988, 86017, 86132–86137, 90929–90948, "Rugwe" [Rungwe village, Mbeya Region, Tanzania]; ZMB 86128–86131, "S'ongwe" [Songwe, at the border to Malawi on the northwestern tip of Lake Malawi, Kyela District, South Mbeya Region, Tanzania]; ZMB 86126–86127 "D.O.A." [German East Africa], all coll. Friedrich Georg Hans Heinrich Fülleborn, 1897–1899 (Fülleborn 1900a, b) (Fig. 7).

**Present name.** *Hyperolius marmoratus* Rapp, 1842.

**Figure 7.** Syntype of *Hyperolius fuelleborni* Ahl, 1931a, ZMB 71186 from "Neu Helgoland" coll. Fülleborn.

**Remarks.** Two drawings showing the variation of this taxon are presented by Ahl (1931b: 349, fig. 224). According to Ahl (1931a: 76) 199 specimens were originally present. Two paratypes, MCZ A-17636–17637 from "Miramba bei Langenburg", coll. Fülleborn, were sent to MCZ in 1932 (Barbour and Loveridge 1946: 128).

***Hyperolius fusciventris* Peters, 1876: 122.**

**Syntypes.** ZMB 6635, "Liberia", don. Stephen Allen Benson, and ZMB 8668, "Liberia", coll. Heinrich Wolfgang Ludwig Dohrn.

**Present name.** *Hyperolius fusciventris* Peters, 1876.

**Remarks.** Peters (1876: 122) explicitly mentions the inventory numbers for the two syntypes in ZMB. The Prussian zoologist H. Dohrn travelled between 1864 and 1866 in West Africa where he collected mainly vertebrates. He exchanged his duplicates with MSNG, NMW, RMNH and ZMB (Pfaffl 2017).

*Hyperolius fusciventris*
see *Hyperolius oeseri*, *Hyperolius rosaceus*, *Hyperolius trifasciatus.*

***Hyperolius glandicolor* Peters, 1878: 209, pl. 2, fig. 9.**

**Syntypes.** ZMB 9299 and ZMB 77768 (formerly part of ZMB 9299), "Taita" [Taita Hills, Taita-Taveta County, Kenya], coll. Johann Maria Hildebrandt.

**Present name.** *Hyperolius glandicolor* Peters, 1878.

**Remarks.** Depicted in Ahl (1831b: 345, fig. 219), reprinted from Peters (1878).

In March 1872, Hildebrandt travelled from Berlin via Egypt to the southwest coast of the Arabian Peninsula and to Aden, where he stayed until the end of 1872. In spring 1873, he went from Zanzibar to Karachi and travelled the Indus upwards. After returning to Zanzibar in July 1873, he travelled the Wami and Kingani (Rufu)

Rivers in present-day Tanzania together with the animal trader and director of the Hamburg Zoo, Carl Gottfried Wilhelm Heinrich Hagenbeck, and then visited the southern Somali coast alone. He returned to Europe in August 1874.

In February 1875, Hildebrandt arrived again in Aden and visited the "Serrut Mountains" [Somaliland]. Then he went to Zanzibar and the Comoros (Johanna Island [Anjouan], June to September 1875). Back in Zanzibar he prepared his expedition into the Inner Africa, via Pangani [Tanzania], Lamu, through the South Gala countries up the Tana River. He had to return to Mombasa due to illness in December 1875. In November 1876 he started again from Zanzibar via Mombasa (10 January 1877) in the direction of Mount Kenya. He travelled the Taita, Ukamba and Kitui areas, but had to return to Mombasa without reaching his actual destination Mount Kenya, from which he was only a three days' march away. He arrived again in Mombasa in August 1877 (Kurtz 1877). The type material of *H. glandicolor* was collected from June to July 1877 during Hildebrandt's stay in the Taita region (Hildebrandt 1877).

*Hyperolius glandicolor*
see *Hyperolius albolabris*, *Hyperolius coeruleopunctatus*, *Hyperolius goetzei*, *Hyperolius pulchromarmoratus*, *Hyperolius scheffleri*, *Hyperolius striolatus*, *Hyperolius bergeri* (unlocated type specimen).

***Hyperolius goetzei* Ahl, 1931a: 128.**

**Holotype.** ZMB 53181, "Uhehe" [Uhehe Highlands, Iringa Region, Tanzania], coll. Walter Goetze, 1899.

Paratype: ZMB 53182, "Massai-Nyika" [Massai Steppe, Tanzania], coll. Oscar Rudolph Neumann, 1893.

**Present name.** *Hyperolius glandicolor* Peters, 1878.

**Remarks.** Drawing in Ahl (1931b: 413, fig. 286). From 1898 to November 1899 the gardener and botanist Goetze travelled from Uhehe [Iringa Region] to Langenburg [Lumbira at the northern shore of Lake Malawi] and collected in the mountainous region between Lake Rukwa and Lake Malawi, particularly in the Kinga Mountains [Kipengere Range SW Tanzania] (Engler 1902; Urban 1917).

***Hyperolius granulosus* Peters, 1867: 891, footnote.**

**Syntypes.** ZMB 4811 and ZMB 75652 (formerly part of ZMB 4811), "Mossambique", coll. Wilhelm Carl Hartwig Peters.

**Present name.** *Hyperolius marmoratus* Rapp, 1842.

**Remarks.** Peters (1882b: 162) specified the locality for the two syntypes as "Capanga am Flüsschen Mutizi östlich von Tette" [Capanga, Maravia District, Tete Province, Mozambique] where he collected on August 8, 1845. One of the specimens is depicted in Peters (1882b, pl. 22, fig. 3).

The name *Rappia granulata* Tornier (1896: 151) is categorized as *nomen inquirendum*, "Name(s) unassigned to a living or extinct population" by Frost (2021) who placed the type locality "Tette" mistakenly in Tanzania. Tornier (1896) attributed the authorship of this name to Peters and mentioned the type specimens by number (ZMB 4811). However, Peters never described a reed frog with the specific epithet "*granulata*". Tornier's name *granulata* does not meet the requirements of Art. 33.2 of the 'Code' (ICZN 1999) for an "emendation". We therefore consider *granulata* Tornier, 1896 as an incorrect subsequent spelling of the specific epithet *granulosus* Peters, 1867.

***Hyperolius graueri* Ahl, 1931a: 131.**

**Holotype.** ZMB 85758, "Westliches Russisi-Ufer und Nordwestufer des Tanganyika-See's" [West Bank of Ruzizi River, Democratic Republic of the Congo and northwestern shore of Lake Tanganyika], coll. Rudolf Grauer 1908–1911.

**Present name.** *Hyperolius marginatus* Peters, 1854.

**Remarks.** Drawing in Ahl (1931b: 420, fig. 292).

***Hyperolius guineensis* Ahl, 1931a: 30.**

**Holotype.** ZMB 77464, "Guinea", don. Hermann Schlegel (Museum Leyden).

**Present name.** *Hyperolius concolor* (Hallowell, 1844).

**Remarks.** Drawing in Ahl (1931b: 285, fig. 158).

***Hyperolius gularis* Ahl, 1931a: 125.**

**Holotype.** ZMB 83544, "Loanda" [Luanda, Angola], coll. Carl May.

**Present name.** *Hyperolius gularis* Ahl, 1931.

**Remarks.** Drawing in Ahl (1931b: 408, fig. 281).

First Lieutenant May collected between 1901 and 1903 in Luanda and surroundings, e.g. in Mubella near Funda on the Bengo River [Municipality of Cacuaco, Luanda Province, Angola] (Matschie 1906). He donated the collected zoological objects as gifts to ZMB from 1902 to 1903 (Anonymous 1903, 1904). Recently, the type was erroneously mentioned as probably lost by Marques et al. (2018).

***Hyperolius guttatus* Peters, 1875: 207, pl. 2, fig. 3.**

**Lectotype.** ZMB 8378, "Cameruns" [Douala, Region Littoral, Cameroon], coll. Georg Anton Eugen Reichenow, don. Reinhold Wilhelm Buchholz.

**Paralectotype.** ZMB 4489. "Boutry" [Butre (Bootry), Ahanta West District, Western Region, Ghana], coll. Hendrik Severinus Pel, don. Hermann Schlegel (Museum Leiden).

**Present name.** *Hyperolius concolor guttatus* Peters, 1875, according to Frétey et al. (2014).

**Remarks.** Lectotype by subsequent designation of Laurent (1961: 73). Frétey et al. (2014) corrected the type locality to "Douala" based on an account and a map of Buchholz' Central African travels provided by Heinersdorff (1880). According to the latter, Buchholz visited "Cameroons" between October 1872 and August 1874. According to Frétey et al. (2014) the collection in RMNH holds four additional paralectotypes (RMNH RENA 1788 A–D) from "Boutry", coll. Pel (not listed by Gasso Miracle et al. 2007). Drawing in Ahl (1931b: 354, fig. 229) figuring paralectotype ZMB 4489. For the origin, history and status of *Hyperolius guttatus* and drawings, photographs and redescriptions of the ZMB type specimens we refer to the revision by Frétey et al. (2014).

The Berlin ornithologist Reichenow travelled together with Lühder and Buchholz from spring 1872 on a one year collecting trip to "Akkrá" on the Gold Coast [Accra, Ghana] and the region around "Camaroons" [Douala Region, Cameroon] (Reichenow 1874; Heinersdorf 1880; Weidmann 1894; Stresemann 1943; see also remarks on *Hyperolius acutirostris*). Reichenow was assistant in the fish and reptile department in ZMB until Wilhelm Peters' death. From 1883 he worked as an administrator and assistant in the mammal and reptile department. In 1888 he became curator for the reptile, bird and mammal exhibition in the new ZMB building on the Invalidenstraße. After the retirement of his father-in-law Jean Louis Bennoit Cabanis in 1892, and after almost twenty years conducting various activities at ZMB, Reichenow took over as the curator of the ornithological collection (Stresemann 1943).

### *Hyperolius hieroglyphicus* Ahl, 1931a: 126.

**Lectotype.** ZMB 20793, "Bamenda, Kamerun" [Mezam Department, Northwest Region, Cameroon], coll. First Lieutenant Karl Moritz Ernst Gustav Wilhelm Adametz.

**Paralectotypes.** ZMB 20795, 77728 (formerly part of ZMB 20793), 77798–77801 (formerly part of ZMB 20795), coll. Adametz, 1909; ZMB 27270, 77756–77757 (formerly part of ZMB 22270), coll. Hans Glauning, X–XI/1907; ZMB 22321, coll. Lieutenant Naumann, 1911; all specimens from "Bamenda".

**Present name.** *Hyperolius riggenbachi* (Nieden, 1910).

**Remarks.** Drawing in Ahl (1931b: 409, fig. 282) modified from Nieden (1910: 243, fig. 3). Lecotype designation by Laurent (1961: 76). Photograph of the lectotype in Perret (1962: 243, fig. 1). Another paratype MCZ A-17638 from "Bamenda", coll. Adametz, was sent to MCZ in exchange from ZMB in 1932 (Barbour and Loveridge 1946: 128).

### *Hyperolius hildebrandti* Ahl, 1931a: 64.

**Holotype.** ZMB 8378, "Kamerun" [Douala, Region Littoral, Cameroon], coll. Georg Anton Eugen Reichenow, don. Reinhold Wilhelm Buchholz.

**Present name.** *Hyperolius concolor guttatus* Peters, 1875, according to Frétey et al. (2014).

**Remarks.** Depicted in Ahl (1931b: 334, fig. 207), copied from Peters (1875, pl. 2, fig. 3). The same specimen that is the holotype of *H. hildebrandti* is also the lectotype of *H. guttatus* Peters, 1875. For the origin, history and status as well as type localities, drawings, photographs and redescriptions of the ZMB types of *H. guttatus* and *H. hildebrandti*, we refer to the revision by Frétey et al. (2014).

### *Hyperolius houyi* Ahl, 1931: 101.

**Holotype.** ZMB 39099, "SW-Ussagara (Neu-Kamerun)" [partly in error, see remarks below], coll. Reinhardt Houy, 29.XI.1911.

**Present name.** *Hyperolius houyi* Ahl, 1931a.

**Remarks.** Depicted in Ahl (1931b: 374, fig. 249). The type locality indicated by Ahl (1931a: 102) is misleading and composed of two different regions which are far apart. Houy was a member of the "Lagone–Pama–Expedition 1912–13", which he accompanied as government doctor and zoologist to "Neu-Kamerun", and several natural history objects from this expedition were sent by him to ZMB. Together with the topographer and First Lieutenant Otto Tiller, he also accompanied the "Expedition ins Zwischenseengebiet in Ostafrika" [region between Lake Kivu and Lake Victoria] in 1911, the expedition directed by the colonial geographer Hans Heinrich Josef Meyer. According to the original label, the holotype of *H. houyi* was collected on 29 November 1911, at the end of Meyer's expedition to East Afrika (see also Urban 1917). On the basis of the map showing the expedition route (Meyer 1913), the corrected type locality for *H. houyi* has to be "SW-Ussagara" [southern Kilosa District, Morogoro Region, Tanzania].

### *Hyperolius insignis* Bocage, 1868: 844, fig. 2.

**Syntype.** ZMB 6462, "Benguella" [Benguela, Angola], coll. José Alberto de Oliveira Anchieta, don. José Vicente Barbosa du Bocage.

**Present name.** *Hyperolius angolensis* Steindachner, 1867 (fide Marques et al. 2018).

**Remarks.** Drawing in Ahl (1931b: 284, fig. 157), copied from Bocage (1868: 844, fig. 2). The Berlin syntype was sent in 1869 in exchange from Lisbon by Bocage and was mentioned and depicted by Tornier (1896: 143, pl. 4, fig. 48). The syntypes MBL T. 21-164, 27-167 from "Benguella", coll. Anchieta and "St. Salvador du Congo" coll. António José de Sousa Barroso were destroyed by a fire in the Museu Bocage on 18 March 1978 (Marques et al. 2018: 90). Perret (1976a: 28) corrected the type locality to "São Salvador do Congo, Angola, and Novo Redondo, Angola". The Berlin syntype is not mentioned by Marques et al. (2018), but probably is the only remaining syntype.

***Hyperolius inyangae* Channing in Channing, Hillers, Lötters, Rödel, Schick, Conradie, Rödder, Mercurio, Wagner, Dehling, Du Preez, Kielgast & Burger, 2013: 322, fig. 6 second row right, fig. 12 C and D.**

**Holotype.** ZMB 77276, "Rhodes Dam in the Nyanga National Park, Zimbabwe, 18°17'20.3"S, 32°43'24.4"E", coll. Alan Channing, 14.XI.2009.

**Paratypes.** ZMB 77277–77279, same collecting data as for the holotype.

**Present name.** *Hyperolius inyangae* Channing in Channing, Hillers, Lötters, Rödel, Schick, Conradie, Rödder, Mercurio, Wagner, Dehling, Du Preez, Kielgast & Burger, 2013.

***Hyperolius ipianae* Ahl, 1931a: 43.**

**Holotype.** ZMB 36091, "Ipiana" [Ipyana (Ipanya) on Kiwira River, at the northwestern tip of Lake Malawi, Kyela District, South Mbeya Region, Tanzania], coll. Adolf Ferdinand Stolz.

**Present name.** *Hyperolius kivuensis* Ahl, 1931a.

**Remarks.** Drawing in Ahl (1931b: 301, fig. 175). Stolz was a mission trader and planter, working as head of the missionary station of the Moravian Church (Herrnhuter Brüdergemeinde) at Ipyana from 1898 to1903. Afterwards, and until 1914, he collected botanical and zoological objects in Kiymbila and Rungwe (Urban 1917; Jones et al. 2000). Amphibians and reptiles from his collection arrived at ZMB on 8 June 1901.

***Hyperolius irregularis* Ahl, 1931a: 114.**

**Syntypes.** ZMB 36105 and 75606 (formerly part of ZMB 36105), "Mohasi-See, Ruanda" [Lake Muhazi, Eastern Province, Rwanda], coll. Johann Gustav Hermann Schubotz, VII/1907.

**Present name.** *Hyperolius viridiflavus* (Duméril & Bibron, 1841).

**Remarks.** Type specimens depicted in Ahl (1931b: 396, fig. 272). The syntypes were collected during the first "Deutsche Zentral–Afrika–Expedition", 1907–1908; see also remarks on *Hyperolius adolphi-friederici*.

***Hyperolius jackie* Dehling, 2012: 54, figs 1, 2.**

**Holotype.** ZMB 77476, "a natural pond at Karamba (2°28'44.28"S, 29°06'44.50"E, 1940 m a.s.l.), Nyungwe National Park, Rwanda", coll. Jonas Maximilian Dehling, 20.III.2011.

**Paratypes.** ZMB 77477–77480, coll. 19.–20. III.2011; ZMB 77481, coll. 3.IV.2011; ZMB 77782, coll. 18.III.2012; ZMB 77783, coll. 24.III.2012; otherwise same collecting details as holotype.

**Present name.** *Hyperolius jackie* Dehling, 2012.

***Hyperolius jacobseni* Channing in Channing, Hillers, Lötters, Rödel, Schick, Conradie, Rödder, Mercurio, Wagner, Dehling, Du Preez, Kielgast & Burger, 2013: 327, fig. 6, third row left, fig. 12 B.**

**Holotype.** ZMB 77280, "near Gatiko, Central African Republic, 5°4'43"N, 20°40'2"E", coll. Niels Jacobsen, 29.VIII.2006.

**Paratypes.** ZMB 77281–77298 same collecting data as for the holotype.

**Present name.** *Hyperolius jacobseni* Channing in Channing, Hillers, Lötters, Rödel, Schick, Conradie, Rödder, Mercurio, Wagner, Dehling, Du Preez, Kielgast & Burger, 2013.

***Hyperolius kandti* Ahl, 1931a: 62.**

**Holotype.** ZMB 46526, "Kivu-See" [Lake Kivu, Rwanda and Democratic Republic of the Congo], coll. Richard Kandt.

**Present name.** *Hyperolius viridiflavus* (Duméril & Bibron, 1841).

**Remarks.** The Prussian medical officer and discoverer of one of the sources of the Nile, Richard Kandt (who used Kantorowicz until 1894) explored the northwestern part of German East Africa from October 1897 to January 1898, and the region around Lake Kivu between 1898 and 1902 (Kandt 1899, 1900, 1921; Bindseil 1988).

***Hyperolius karissimbiensis* Ahl, 1931a: 74.**

**Holotype.** ZMB 46525, "Bambusurwald und Waldwiesen ca. 2400 m hoch, beim Dorf des Mhcabu Gahama am Karissimbi" [Mount Karisimbi, Muzanze District, Northern Province, Rwanda; bamboo jungle and forest meadows at 2400 m a.s.l.], coll. Johann Gustav Hermann Schubotz.

**Present name.** *Hyperolius viridiflavus* (Duméril & Bibron, 1841).

**Remarks.** Depicted in Ahl (1931b: 348, fig. 223). The holotype was collected during the first "Deutsche Zentral–Afrika–Expedition", 1907–1908.

***Hyperolius kivuensis* Ahl, 1931a: 26.**

**Holotype.** ZMB 36098, "Kivu-See" [Lake Kivu, Rwanda and Democratic Republic of the Congo], coll. Richard Kandt.

**Present name.** *Hyperolius kivuensis* Ahl, 1931a.

**Remarks.** Depicted in Ahl (1931b: 280, fig. 151); see also remarks under *H. kandti*.

*Hyperolius kivuensis*

see *Hyperolius bituberculatus* (unlocated type specimens), *Hyperolius ipianae*, *Hyperolius multifasciatus*, *Hyperolius raveni*, *Hyperolius simus.*

***Hyperolius koehli* Ahl, 1931a: 121.**

**Holotype.** ZMB 26089, "Kissenji, Deutsch-Ost-Afrika" [on the northeast shore of Lake Kivu close to the border of Democratic Republic of the Congo, Rwanda], coll. Franz Koehl (Köhl).

**Present name.** *Hyperolius viridiflavus* (Duméril & Bibron, 1841).

**Remarks.** First Lieutenant, later Captain, Köhl served from 1912 on in the colonial "Schutztruppe" of Deutsch Ostafrika at Kissenji, and from 1916 on in various missions under General Paul Emil von Lettow-Vorbeck, e.g. at Taveta [Kenya], Port Amelia [Pemba, Cabo Delgado Province, Mozambique] and Medo [Metoro, Mozambique] (Haup 1988; Fecitt 2011).

***Hyperolius kwidjwiensis* Ahl, 1931a: 38.**

**Holotype.** ZMB 52449. "Insel Kwidjwi im Kivu-See" [Idjwi (Ijwi) Island, Lake Kivu, Democratic Republic of the Congo], coll. Johann Gustav Herrmann Schubotz, VI/1909.

**Present name.** *Hyperolius viridiflavus* (Duméril & Bibron, 1841).

**Remarks.** Drawing in Ahl (1931b: 296, fig. 172).

***Hyperolius laticeps* Ahl, 1931a: 69.**

**Holotype.** ZMB 46529, "Togo", coll. Leopold Fritz Wilhelm Edmund Conradt, 17.XII.1892.

**Present name.** *Hyperolius laticeps* Ahl, 1931a.

**Remarks.** Depicted in Ahl (1931b: 342, fig. 216). Conradt was a German planter and colonial officer, who was working in "Derema" [Derema, Usambara Mountains, Korogwe District, Tanga Region, Tanzania] at the end of 1891. His collections of vertebrates made during this time were described by Matschie (1892). Later he went to Togo, being stationed in Bismarckburg [Sotouboua Prefecture, Centrale Region, Togo] from VII/1892–XII/1893 (Weidmann 1894; Conradt 1896). See also remarks on *Megalixalus laevis* concerning his activities in Tanzania and Cameroon.

The specimen is a juvenile *Hyperolius* and cannot be assigned confidently to a particular West African species.

***Hyperolius latifrons* Ahl, 1931a: 65.**

**Holotype.** ZMB 50278, "Bambusurwald und Waldwiesen ca. 2400 m hoch, beim Dorf des Mhcabu Gahama am Karissimbi" [Mount Karisimbi, Muzanze District, Northern Province, Rwanda; bamboo jungle and forest meadows at 2400 m a.s.l.], coll. Johann Gustav Hermann Schubotz.

**Present name.** *Hyperolius castaneus* Ahl, 1931a.

**Remarks.** Depicted in Ahl (1931b: 335, fig. 208). The holotype was collected during the first "Deutsche Zentral–Afrika–Expedition", 1907–1908.

***Hyperolius leptosomus* Peters, 1877a: 619, pl., fig. 5.**

**Holotype.** ZMB 9175, "Chinchoxo (Westafrika)" [Cabinda Province, Angola], don. Africanische Gesellschaft.

**Present name.** *Afrixalus* "*quadrivittatus*" Pickersgill, 2007b.

**Remarks.** See also remarks on *Hyperolius adspersus.*

***Hyperolius lupiroensis* Channing in Channing, Hillers, Lötters, Rödel, Schick, Conradie, Rödder, Mercurio, Wagner, Dehling, Du Preez, Kielgast & Burger, 2013: 330, fig. 6, third row second right, fig. 12 G.**

**Holotype.** ZMB 77299, "near Lupiro, 8°25'29.3"S, 36°41'33.1"E, Ifakara district, Tanzania", coll. A. Danby, 9.VII.2007.

**Paratype.** ZMB 77300, same collecting data as for the holotype.

**Present name.** *Hyperolius lupiroensis* Channing in Channing, Hillers, Lötters, Rödel, Schick, Conradie, Rödder, Mercurio, Wagner, Dehling, Du Preez, Kielgast & Burger, 2013.

***Hyperolius macrodactylus* Ahl, 1931a: 95.**

**Holotype.** ZMB 39100, "Kivu-See" [Lake Kivu, Rwanda and Democratic Republic of the Congo], coll. Richard Kandt.

**Present name.** *Hyperolius viridiflavus* (Duméril & Bibron, 1841).

**Remarks.** Depicted in Ahl (1931b: 369, fig. 244); see further comments under *H. kandti*.

***Hyperolius marginatus* Peters, 1854: 627.**

**Holotype.** ZMB 4806, "Macanga" [Makanga Region, Tete Province, Mozambique], coll. Wilhelm Carl Hartwig Peters.

**Present name.** *Hyperolius marginatus* Peters, 1854.

**Remarks.** Depicted in Peters (1882b, pl. 22, fig. 8) and Tornier (1896, pl. 4, fig. 89). Bauer et al. (1995: 44) erroneously listed ZMB 1806 as holotype. Peters visited the Macanga region north and northwest of Tete because of its goldmines. Here he also collected the holotype of *H. marginatus* on the Pomfe River (one of the northern tributaries of the Zambezi) on 12 June 1845 (Hand 1848; Peters 1882b: 166; map in Futterer 1895).

*Hyperolius marginatus*
see *Hyperolius argentovittis*, *Hyperolius callichromus*, *Hyperolius decipiens*, *Hyperolius graueri.*

***Hyperolius mariae* Barbour & Loveridge, 1928: 217.**

**Paratype.** ZMB 38029 [ex MCZ, former inventory number unknown], "Derema bei Amani, Usambara Mts., Tan-

ganyika Territorium" [Derema, Korogwe District, Tanga Region, Tanzania], coll. Mary V. Loveridge, 30.XI.1926.

**Present name.** *Hyperolius mariae* Barbour & Loveridge, 1928.

**Remarks.** Holotype: MCZ A-13267; Paratypes MCZ A-13262–13266 and MCZ A-13268–13276, all from "Derema nr. Amani, Usambara Mtns., Tanganyika Territory", coll. Mary V. Loveridge, 30.XI.1926. ZMB 38029 was donated by A. Loveridge (MCZ) in the 1930s and inventoried in 1958.

*Hyperolius mariae*

see *Hyperolius bitaeniatus*, *Hyperolius melanophthalmus*, *Hyperolius noblei*, *Hyperolius renschi* (unlocated type specimens), *Hyperolius rubriceps*, *Hyperolius udjidjiensis.*

*Hyperolius marmoratus*

see *Hyperolius albifrons*, *Hyperolius asper*, *Hyperolius breviceps*, *Hyperolius dermatus*, *Hyperolius fuelleborni*, *Hyperolius granulosus*, *Hyperolius guttolineatus* (unlocated type specimens), *Hyperolius marungaensis*, *Hyperolius microstictus*, *Hyperolius nyassae*, *Hyperolius taeniatus*, *Hyperolius variegatus*, *Hyperolius vermicularis.*

***Hyperolius marungaensis* Ahl, 1931a: 77.**

**Holotype.** ZMB 10736, "Marunga, Angola" [in error, see remarks], coll. Richard Böhm.

**Present name.** *Hyperolius marmoratus* Rapp, 1842.

**Remarks.** Depicted in Ahl (19831b: 351, fig. 226). Ahl (1931a, b) placed the locality "Marunga" erroneously within Angola because a village of this name exists in the province of Cuando Cubango (see also Marques et al. 2018: 93).

The zoologist and anatomist Böhm, together with the explorer Paul Reichard, travelled on behalf of the "Africanische Gesellschaft" from Zanzibar via Bagamojo [27 July 1880] to Tabora, which they reached two-and-a half months later. From here they turned to Kakoma [southeast of Tabora, Tabora Division, Tanzania], where they stayed for over a year. Then they continued to Jagonda [just northeast of Kakoma]. From Jagonda, Böhm and the topographer Emil Kaiser went on a journey to Lake Tanganyika, lasting several months. They reached Karema on the western shore of the lake [Mpanda District, Katavi Region, Tanzania] and returned to Jagonda on December 23, 1881. In March 1882 they travelled along the Wala River. Dr. Kaiser died during an expedition to Lake Rukwa near Upia on 27 October 1882. Towards the end of December 1882, Böhm and Reichard left Jadonda for Karema, crossed Lake Tanganyika to Mpala (at the mouth of the Lufuku River, Tanganyika Province, Democratic Republic of the Congo) and reached the "Marunga Land" in July 1883. From here they turned southwest and discovered Lake Upemba in the Urua region [Upemba, Bukama Region, Haut-Lomami Provinz, Democratic Republic of the Congo]. On 27 March 1884 Böhm died in southern Urua, south of the Lake Upemba (Schalow 1888, Weidmann 1894). Based on Böhm's itinerary, it is clear that he found the holotype of *H. marungaensis* in the Marunga Highlands, where he collected extensively in summer 1883 (Schalow 1886, 1888). Therefore, we correct the type locality to "northern Marunga or Marungu Region southwest of Lake Tanganyika, Kalemie Territory, Tanganyika Province, Democratic Republic of the Congo".

***Hyperolius maximus* Ahl, 1931a: 91.**

**Holotype.** ZMB 36113, "Ossidinge" [Ossidinge station (Mamfe), on the left bank of the Cross River, Southwest Region, Cameroon], coll. Alfred Mansfeld.

**Paratypes.** ZMB 43548–43552, "Busa" [sic], Buea [Fako District, Southwest Region, Cameroon], coll. Paul Preuss (Preuß).

**Present name.** *Hyperolius concolor guttatus* Peters, 1875, according to Frétey et al. (2014).

**Remarks.** Depicted in Ahl (1931b: 366, fig. 241). Ahl (1931a: 92) mentioned eight specimens from "Ossidinge, Busa [sic, Buea], Guinea". One paratype MCZ A-17639 from "Guinea", coll. Pel, don. Schlegel (Leiden) was sent to MCZ in exchange in 1932 (Barbour and Loveridge 1946: 128); another paratype could not be located. The colonial officer and ethnologist Mansfeld, who collected the holotype, arrived in Ossidinge on 30 August 1904 and was stationed there until 1907 (Mansfeld 1908).

***Hyperolius melanophthalmus* Ahl, 1931a: 68.**

**Syntypes.** ZMB 85670–85672, "Zanzibar" [Unguja Island, Tanzania], coll. Moriz Tup.

**Present name.** *Hyperolius mariae* Barbour & Loveridge, 1928.

**Remarks.** Depicted in Ahl (1931b: 342, fig. 215). Another paratype, MCZ A-17640 from "Zanzibar", coll. Tup, was sent in exchange in 1932 (Barbour and Loveridge 1946: 128).

*Hyperolius micops*

see *Hyperolius acuticeps.*

***Hyperolius microstictus* Ahl, 1931a: 80.**

**Syntypes.** ZMB 36100 and ZMB 77762 (formerly part of ZMB 36100), "Longa, oberhalb Minnescra [sic]" [above Minnesera, today Cuito Cuanavale, on left bank of Longa River (a right tributary of Cuito river) and confluence with Quiriri (Kuarliri) River, Cuando Cubango Province, Angola, ca. 1250 m a.s.l.], coll. [Hugo Baum, see below], 14.I.1900.

**Present name.** *Hyperolius marmoratus* Rapp, 1842.

**Remarks.** Depicted in Ahl (1931b: 353, fig. 228). Ahl (1931a: 81) mentioned no collector or donor for the type specimens. However, the syntypes were mentioned earlier by Sokolowski (1903: 541 f.) who described two *Rappia* specimens collected by the botanist Baum on 14 January 1900 "am Longa oberhalb Minnesera" during the "Kunene–Sambesi–Expedition 1899–1900" led by Pieter van der Kellen. Based on Baum's notes, Sokolowsy (l. c.) almost literally described the same observations, as was later repeated by Ahl (1931a: 81), i.e. "[…] kleine auf Blättern von Sträuchern nach Art unserer Laubfrösche sitzende Fröschchen […]" […small frogs sitting on leaves of bushes like our tree frogs …]. Baum's expedition route in Angola was illustrated by Heintze (2007, map 2).

***Hyperolius mohasicus* Ahl, 1931a: 85.**

**Holotype.** ZMB 36094, "Mohasi-See, Ruanda" [Lake Muhazi, Eastern Province, Rwanda], coll. Johann Gustav Hermann Schubotz, 29.VII.1907.

**Present name.** *Hyperolius viridiflavus* (Duméril & Bibron, 1841).

**Remarks.** Depicted in Ahl (1931b: 360, fig. 236). The holotype was collected during the first "Deutsche Zentral–Afrika–Expedition", 1907–1908.

***Hyperolius monticola* Ahl, 1931a: 102.**

**Holotype.** ZMB 39010, "Niansa, Ruanda, 1500 m hoch" [Nyanza (Nyabisindu), Nyanza District, Southern Province, Rwanda, 1500 m a.s.l.], coll. Johann Gustav Hermann Schubotz, 10.VIII.1907.

**Present name.** *Hyperolius viridiflavus* (Duméril & Bibron, 1841).

**Remarks.** Depicted in Ahl (1931b: 377, fig. 251). The holotype was collected during the first "Deutsche Zentral–Afrika–Expedition", 1907–1908.

***Hyperolius multicolor* Ahl, 1931: 94.**

**Syntypes.** ZMB 39002, 39005, 74953–74956, "Bambusurwald und Waldwiesen ca. 2400 m hoch, beim Dorf des Mhcabu Gahama am Karissimbi" [Mount Karisimbi, Muzanze District, Northern Province, Rwanda; bamboo jungle and forest meadows at 2400 m a.s.l.], coll. Johann Gustav Hermann Schubotz.

**Present name.** *Hyperolius viridiflavus* (Duméril & Bibron, 1841).

**Remarks.** Drawing in Ahl (1931b: 368, fig. 243). The syntypes were collected during the first "Deutsche Zentral–Afrika–Expedition", 1907–1908. Another syntype MCZ A-17641 was sent in exchange to MCZ in 1932 (Barbour and Loveridge 1946: 128).

***Hyperolius multifasciatus* Ahl, 1931a: 24.**

**Holotype.** ZMB 36109, "Missionsstation Rungwe" [station of the Moravian Church (Herrnhuter Brüdergemeinde), Rungwe village, Mbeya Region, Tanzania], coll. Friedrich Georg Hans Heinrich Fülleborn.

**Present name.** *Hyperolius kivuensis* Ahl, 1931a.

**Remarks.** Drawing in Ahl (1931b: 278, fig. 149).

***Hyperolius narinus* Ahl, 1931a: 109.**

**Holotype.** ZMB 36090, "Togo (Misahöhe)" [Missahomé, Agou Prefecture, Plateau Region, Togo], coll. Julius Smend, 9.II.1903.

**Paratypes.** ZMB 36121 (two larvae), same collection data as for holotype.

**Present name.** *Hyperolius concolor* (Hallowell, 1844).

**Remarks.** Drawing in Ahl (1931b: 383, fig. 258). First Lieutenant Smend served from 1901 as district manager at the German colonial station Misahöhe.

*Hyperolius nasutus*
see *Rappia dombeensis.*

***Hyperolius ngoriensis* Ahl, 1931a: 60.**

**Syntypes.** ZMB 85760–85763, "Krater des Ngori-See's [sic] (Deutsch-Ost-Afrika)" [Ngozi Crater Lake, Poroto Mountains range, Rungwe District, Mbeya Region, Tanzania], coll. Friedrich Georg Hans Heinrich Fülleborn.

**Present name.** *Hyperolius pictus* Ahl, 1931a.

**Remarks.** Depicted in Ahl (1931b: 324, fig. 198).

***Hyperolius nigropalmatus* Ahl, 1931a 104.**

**Holotype.** ZMB 85764, "Lolodorf, Kamerun" [Lolodorf, Océan Division, South Province, Cameroon], coll. Oswald Rudolf Johannes Gerhard Jacob, 1907.

**Present name.** *Hyperolius phantasticus* (Boulenger, 1899).

**Remarks.** Drawing in Ahl (1931b: 378, fig. 253). In 1907 First Lieutenant Jacob (also spelled Jakob) served in the "Schutztruppe für Kamerun" as manager of the German Colonial Station Lolodorf (Hoffmann 2007).

***Hyperolius nitidulus* Peters, 1875: 209, pl. 3, fig. 4.**

**Holotype.** ZMB 7729, "Yoruba (Lagos)" [Nigeria], don. Christian Ferdinand Friedrich von Krauss.

**Present name.** *Hyperolius nitidulus* Peters, 1875.

**Remarks.** Depicted in Tornier (1896, pl. 4, fig. 118). The traveler, botanist and malacologist Krauss became

director of the Königliche Naturalienkabinett in Stuttgart in 1890. He studied and collected southern African flora, fauna and geological samples between 1838 and 1840.

***Hyperolius noblei* Ahl, 1931a: 118.**

**Holotype.** ZMB 85765, "Kilwa (Deutsch-Ost-Afrika)" [Kilwa (Kivinje), Kilwa District, Lindi Region, Tanzania], coll. Julius Vosseler, VI/1907.

**Present name.** *Hyperolius mariae* Barbour & Loveridge, 1928.

**Remarks.** Drawing in Ahl (1931b: 400, fig. 275). On behalf of the Prussian government, the German zoologist and later director of the Hamburg Zoological Garden, Vosseler went to Deutsch-Ostafrika where he worked at the "Biologisch-Landwirtschaftliche Institut Amani" from 1903 to 1908, together with Stuhlmann (see above) (Schnee 1920b; Grimpe 1931; Wenzel Geißler et al. 2020).

***Hyperolius nossibeensis* Ahl, 1930d: 66.**

**Syntypes.** ZMB 50098–50100, "Nossi-Bé" [Nosy Be (island), Diana Region, Madagascar], don. Senckenberg Museum [in error]; corrected here to "Lunda" [Lunda Sul Province, Angola], coll. Max Buchner, XII/1979–VI/1880 (see below).

**Present name.** *Hyperolius angolensis* Steindachner, 1867 (fide Marques et al. 2018).

**Remarks.** Depicted in Ahl (1931b: 421, fig. 294, probably ZMB 50089). The three type specimens of *H. nossibeensis* were originally inventoried in 1882 as "3 [specimen] *Hyperolius vermiculatus* Pts." under inventory number ZMB 10100. According to the ZMB inventory catalogue the specimens were collected by "Dr. M. Buchner" at "Lunda".

Because of a reading error, assuming ZMB 10100 instead of ZMB 10101, a new label was written for this collection jar in the 1920s, for which erroneously the information of ZMB 10100 was adopted, viz. "Nossi-Bé" and "Museum Senckenberg". This transmission error and the specimens became the basis for Ahl's (1930) new description of *H. nossibeensis*. In 1992, Frank Glaw (ZSM) located the syntypes of *H. nossibeensis* in the ZMB collection. The jar with the label from the 1920s mentioned *Mantidactylus granulatus* from Nosy Be, ZMB 10100. Glaw and Vences (1993: 216) discussed the status and identity of *H. nossibeensis*, synonymized it with *Hyperolius marmoratus* and corrected the terra typica to "das Äthiopische Afrika" [Ethiopian Africa]. Subsequently the three syntypes were re-inventoried as ZMB 50098–50100. This was necessary as the inventory number ZMB 10100 had already been assigned to a specimen of "*Mantidactylus granulatus*" (= paralectotype of *Limnodytes granulatus* Boettger, 1881) from "Nosy Bé, don. Museum Senckenberg" (see Glaw and Vences 1993).

The physician Dr. Buchner arrived in Luanda on 5 December 1878 and travelled via Dondo (20 December 1878) and Malanje (30 January to 22 July 1879) to Mussumba in the Lunda Empire (11 December 1879 to June 1880). He returned to Malanje (28 February 1881) and via Golungo and Cazengo travelled back to Luanda, where he arrived at the end of August 1881. He finally returned to Berlin in January 1882 (Heintze 2007).

***Hyperolius nyassae* Ahl, 1931a: 66.**

**Holotype.** ZMB 39006, "Langenburg" [Lumbira, Mbeya Region, Tanzania], coll. Friedrich Georg Hans Heinrich Fülleborn.

**Paratypes.** ZMB 77766–77767 (formerly part of ZMB 39006), "Langenburg"; ZMB 85885–85889, "Rugwe"; ZMB 90953–90989, "Rugwe am Nyassa (D.O.A.)"; ZMB 90980–90992, "Rugwe, D.O.A."; ZMB 90993–90995, "Konde-Nika (D.O.A.)"; ZMB 90996–90999, "Neu-Helgoland", all coll. Fülleborn.

**Present name.** *Hyperolius marmoratus* Rapp, 1842.

**Remarks.** Drawing in Ahl (1931b: 340, fig. 213). Ahl (1931a: 67) mentioned 133 specimens from Langenburg (including the type), Rugwe, Mirambo [sic; Miramba], Konde-Nika, Ipiana, Transvaal, Neu Helgoland and Lindi, collected by Fülleborn and Wilms. A paratype (MCZ A-17642) from "Rugwe" coll. Fülleborn, was sent to MCZ in 1932 (Barbour and Loveridge 1946: 128). Seventy-seven other paratypes, including specimens from Miramba, Ipiana, Transvaal and Lindi, as well as material collected by Wilms could not be located.

***Hyperolius obstetricans* Ahl, 1931a: 90.**

**Holotype.** ZMB 77755, "Bipindihof" [Bipindi village, Océan Department, South Province, Cameroon], coll. Georg August Zenker.

**Present name.** *Hyperolius obstetricans* (Ahl, 1931a).

**Remarks.** Photo in Ahl (1931b: 365, fig. 240, horizontally mirrored) showing the specimen on a leaf with 39 eggs. The type previously was regarded as lost, however, we rediscovered the specimen, still sitting on the leaf with the eggs (see Fig. 8). In accordance with Article 75.8 of the International Code of Zoological Nomenclature (ICZN 1999), the rediscovery of the holotype in the ZMB collection in 2012 renders the neotype designation by Perret invalid (1966: 410; MHNG 995.48 from "Foulassi, rivière Lobô"). Since 1988 the species was a member of the genus *Alexteroon* Perret, 1988. However, recently Ernst et al. (2021) revised the systematic position of *Alexteroon* and assigned the three species to the genus *Hyperolius*.

The German naturalist, botanist and gardener Zenker joined the German colonial service as taxidermist in 1889. He was manager of the colonial station Jaunde (Yaoundé, Mfoundi Department, Centre Region, Cameroon) from 1890–1895 (Zenker 1890). In 1896 he settled in Bipindi on the Lokundje River where he collected natural history and ethnological objects extensively and managed different plantations until his death on 6 Febru-

**Figure 8.** Holotype of *Hyperolius obstetricans*, ZMB 77755 from "Bipindihof", coll. Zenker.

ary 1922. The main part of his zoological collection is at ZMB (Mildbraed 1923; Frahm and Eggers 2001).

***Hyperolius oculatus* Ahl, 1931a: 103.**

**Holotype.** ZMB 58570, "Balaibo am Duki-Ufer" [Balaibo on Duki River, southwest of Lake Albert, Ituri Province, northeastern Democratic Republic of the Congo], coll. Franz Ludwig Stuhlmann, 9.XI.1891.

**Paratype.** ZMB 85766, "Golei-See [sic]" [Lake Solei or Solai, Nakuru county, Rift Valley Province, Kenya], coll. Arthur Berger, 2.II.1908.

**Present name.** *Hyperolius viridiflavus* (Duméril & Bibron, 1841).

**Remarks.** Depicted in Tornier (1896, pl. 4, fig. 20), reproduced in Ahl (1931b: 377, fig. 252). From April 1890 to July 1892, Stuhlmann accompanied Mehmed Emin Pasha's [actually Eduard Karl Oskar Theodor Schnitzer] expedition to the East African lake region as a zoologist. This took him as far as the northeastern part of today's Democratic Republic of the Congo (Stuhlmann 1894). Today, his extensive zoological collections are mainly housed at ZMB and in the Zoologisches Museum der Universität Hamburg. The German physician, explorer and hunter Dr. Berger travelled to areas of British East Africa, Uganda, from July 1908 to 1909. He visited the border area with Democratic Republic of the Congo and Sudan, and returned to Germany via Egypt (Berger 1924, 1942). A large part of the zoological objects he collected is at ZMB.

***Hyperolius oeseri* Ahl, 1931a: 51.**

**Holotype.** ZMB 31867, "Grand Bassa, Liberia" [Grand Bass County, Liberia], coll. Richard Oeser.

**Present name.** *Hyperolius fusciventris* Peters, 1876.

**Remarks.** The German physician Dr. Oeser undertook various journeys, e.g. to East Asia and Indonesia (1923 as a ship's doctor), to the USA (1925), to Central America and northern South America (1931–32) as well as to Cameroon (1936). In spring 1928 he undertook a journey along the West African coast, collecting in Benin, Nigeria, Fernando Pó, Sao Tomé and Principe, Angola, Namibia and Liberia. He collected the type material of *H. oeseri* and *Hyperolius trifasciatus* Ahl (see below). A large part of his collection was sold through the zoological wholesaler "Scholze & Peotzschke" in Berlin (Mertens 1975).

***Hyperolius olivaceus* Buchholz & Peters in Peters, 1876: 120.**

**Syntypes.** ZMB 8829 and ZMB 53264–53265 (formerly part of ZMB 8829), "Limbareni am Ogowe" [Lambaréné on the river Ogooué (or Ogowe), Moyen-Ogooué Province, Gabon], coll. Reinhold Wilhelm Buchholz.

**Present name.** *Hyperolius olivaceus* Buchholz & Peters in Peters, 1876.

*Hyperolius olivaceus*
see *Hyperolius fimbriolatus*.

***Hyperolius petersi* Ahl, 1931: 23.**

**Holotype.** ZMB 5573, "Mombas" [Mombasa, Kenya], coll. Carl Claus von der Decken.

**Present name.** *Hyperolius concolor* (Hallowell, 1844).

**Remarks.** Depicted in Ahl (1931b: 274, fig. 144). The German explorer, geographer, zoologist and botanist von der Decken arrived in East Africa (Zanzibar) in September 1860. Until 1865 he undertook several expeditions to Kilwa, the Malawi Lake region, the Usambara Mountains and Mount Kilimanjaro in Tanzania and to Bardera [Baardhere, southwestern Somalia] (Decken 1869; Verdcourd 2002).

*Hyperolius phantasticus*
see *Hyperolius chabanaudi*, *Hyperolius nigropalmatus.*

***Hyperolius phrynoderma* Ahl, 1931a: 71.**

**Syntypes.** ZMB 39000 and ZMB 77734–77736 (formerly part of ZMB 39000), "Zentrales Deutsch-Ost-Afrika"

[Central German East Africa, Central Tanzania, see comment below] collected during the first "Deutsche Zentral–Afrika–Expedition", 1907–1908.

**Present name.** *Hyperolius* cf. *viridiflavus* (Duméril & Bibron, 1841).

**Remarks.** Depicted in Ahl (1931b: 344, fig. 218). Another syntype, MCZ A-17643 with identical collecting data was sent in exchange to MCZ in 1932 (Barbour and Loveridge 1946: 130). Laurent (1961: 83) erroneously presumed that the five subadult specimens inventoried under ZMB 13701 could be the types of *Hyperolius phrynoderma*, but these specimens were collected at "Bukoba" by Stuhlmann and do not correspond with the locality given by Ahl for the types. Ahl's (1931a: 72) vague locality information for the *H. phrynoderma* types is probably wrong and mainly based on the transfer of the expedition name to a region, i.e. "Central German East Africa" which would be geographically equivalent to today's central Tanzania. However, the zoological collections of this expedition mainly originate from northeast Tanzania, Rwanda and the adjacent Democratic Republic of the Congo, more precisely from the region between Bukoba on the western shore of Lake Victoria, Ischangi in the south of Lake Kiwu and Irumu in the Ituru Province of northeastern Democratic Republic of the Congo (see comments in Barbour and Loveridge 1946: 130; map in Schubotz 1909).

Currently, the status of this taxon is uncertain. In comparison to species in the *H. marmoratus / viridiflavus* group, the body is comparatively slender and the snout more pointed. Dorsal warts are distinct, and arranged very regularly, even in the single adult frog (ZMB 39000). Juveniles of the *H. marmoratus / viridiflavus* group have warty skin, adults usually have smooth skin). Drewes (1997) described a superficially similar-looking frog from the Serengeti, *Hyperolius orkarkarri*, which is currently regarded as a synonym of *H. glandicolor* (see Channing and Howell 2006).

Under the leadership of Adolf Friedrich, Duke of Mecklenburg, the first "Deutsche Zentral–Afrika–Expedition" was carried out from 1907 to 1908, to scientifically investigate the area of the African Rift Valley (see also remarks on *Hyperolius adolphi-friederici*).

***Hyperolius picturatus* Peters, 1875: 206, pl. 2, fig. 2.**

**Syntypes.** ZMB 3063 and ZMB 76991–76994 (formerly part of ZMB 3063), "Boutry" [Butre (Bootry), Ahanta West District, Western Region, Ghana], coll. Hendrik Severinus Pel, don. Hermann Schlegel (Museum Leyden).

**Present name.** *Hyperolius picturatus* Peters, 1875.

**Remarks.** Depicted in Ahl (1931b: 333, fig. 206) copied from Peters (1875, pl.2, fig. 2).

***Hyperolius pictus* Ahl, 1931a: 44.**

**Holotype.** ZMB 86001, "Krater des Ngori-See's [sic]" [Ngozi Crater Lake, Poroto Mountains range, Rungwe District, Mbeya Region, Tanzania], coll. Friedrich Georg Hans Heinrich Fülleborn.

**Paratypes.** ZMB 85767–85781, ZMB 86002–86005, „Krater des Ngori-See's", coll. Fülleborn; ZMB 46533, ZMB 85782–85818, ZMB 85876–85878, "Nairobi", coll. F. Thomas; ZMB 85819–85823, "Uhehe", coll. Goetze; ZMB 85824, "Rungwe", coll. Goetze; ZMB 85825, "Rugwe" and ZMB 77720, "Nyassa See", coll. Fülleborn; ZMB 85826–85827, "Rugegewald", coll. Grauer; ZMB 90454–90456, "Bukoba", coll. Schubotz; ZMB 90457–90479, "Bukoba", coll. Deutsche Zentralafrica Expedition, Schubotz, 15.VI.1907; ZMB 90480–90483, "Bukoba", coll. ? Stuhlmann, III/1892.

**Present name.** *Hyperolius pictus* Ahl, 1931a.

**Remarks.** Five drawings showing the variation of this taxon are provided by Ahl (1931b: 302, fig. 176). Two paratypes, MCZ A-17644–17645 from "Uhehe", coll. Goetze were sent in exchange to MCZ in 1932 (Barbour and Loveridge 1946: 128). Ahl (1931a: 45) mentioned 114 specimens of which we could not locate the material collected at "Iringa", "Kivu-See", "Kissenji" and "Kinga-Gebirge".

*Hyperolius pictus*
see *Hyperolius ngoriensis.*

***Hyperolius pulcher* Ahl, 1931a: 48.**

**Holotype.** ZMB 36088, "Japoma, Kamerun" [suburb east of Douala, Region Littoral, Cameroon], coll. Hans Schäfer, 1.X.1910.

**Present name.** *Hyperolius concolor guttatus* Peters, 1875, according to Frétey et al. (2014).

**Remarks.** Drawing in Ahl (1931b: 308, fig. 183), reproduced by Frétey et al. (2014, fig. 6), who also provided a photograph and redescribed the holotype. In 1910 the naval physician Schäfer collected various botanical and zoological objects in Cameroon, e.g. at Mount Manengouba, Mount Cameroon (Fako) and Japoma, that are accessioned at ZMB and the Botanische Museum Berlin-Dahlem [Botanical Museum and Garden Berlin-Dahlem] (Urban 1917).

***Hyperolius pulchromarmoratus* Ahl, 1931a: 92.**

**Holotype.** ZMB 77751, "Britisch Ostafrika" [Kenya], coll. Richard Fritz Paul Hübner [later Huebner].

**Present name.** *Hyperolius glandicolor* Peters, 1878.

**Remarks.** Drawing in Ahl (1931b: 367, fig. 242). Huebner worked from 1894 to 1913 as a merchant, banker, farmer and administrator and from 1901 to 1903 as Municipal Commissioner of Nairobi in (British) East Africa. He was active in Zanzibar (1894–1896), Mombasa (1896–1899), Nairobi (1899–1905), Kibwezi (1905–1908, together with G. R. O. Scheffler), and Voi (1908–1913), and undertook a journey from Mombasa to Kampala from June to November 1899. In 1913 he

travelled to Germany for a convalescent stay because of health problems. However, his already planned return to Kenya was thwarted by the beginning of the First World War. In his spare time he was engaged in nature observations and collected interesting zoological objects, which he sent to ZMB (Sieberg 1998).

***Hyperolius punctatissimus* Ahl, 1931a: 41.**

**Syntypes.** ZMB 39013, 79403–79439 and 80407 (formerly part of ZMB 39013), coll. Johann Gustav Hermann Schubotz; ZMB 43553, 43584–43590 and 79402, coll. Franz Ludwig Stuhlmann; all from "Bukoba" [Bukoba Urban District, Kagera Region, Tanzania],

**Present name.** *Hyperolius viridiflavus* (Duméril & Bibron, 1841).

**Remarks.** Two drawings showing the variation of this taxon are illustrated by Ahl (1931b: 299, fig. 174). Ahl (1931a: 42) mentioned 89 specimens and explicitly states that the types ["die Typen"] are among the material from "Bukoba" without specifying a number or the collector. Therefore we do not regard specimens listed by Ahl (18931a: 42) from "Rugege-Wald", "Vulkangebiet nord-östlich des Kivu-See's", "Sisse" [sic], "W-Niansa" [Sesse or Ssese Archipelago, Lake Victoria, Uganda], and "Njamagelo" as part of the type series, and restrict the type series to those frogs from Bukoba.

***Hyperolius pygmaeus* Ahl, 1931a: 22.**

**Holotype.** ZMB 36102, "Tanga" [Tanga Region, Tanzania], coll. Georg Martienssen.

**Present name.** *Afrixalus stuhlmanni* (Pfeffer, 1893).

**Remarks.** Drawing in Ahl (1931b: 273, fig. 143). *Hyperolius pygmaeus* Ahl, 1931a, is a junior homonym of *Hyperolius pygmaeus* Meyer, 1875 (=*Litoria pygmaea*).

Between December 1896 and June 1899, the German planter Martienssen sent amphibians and reptiles from the German colony in East Africa to ZMB. The locality "Tanga" as given by Ahl (1931a) most likely refers to today's Tanga region. It is clear from Martienssen's correspondence with ZMB that the amphibians he sent to Berlin were collected, with few exceptions (e.g. Ukami), exclusively in "Magrotto" [plantation in southern part of Mlinga Mountains, East Usambara, Tanga Region] and "Plantation Schöller" [Bondei County near Ngomeni, east of the Mlinga Mountains, ca. 25 km SW of Tanga town] (see Gvoždík et al. 2014). Martienssen also supported the African expedition to Kilimanjaro undertaken by Yngve Sjöstedt from 1905–1906 by providing porters (Sjöstedt 1910: 3). The correspondence between Martienssen and the ZMB curators (especially Gustav Tornier), kept in the archives of the Historical Department at ZMB, reveals that "Laubfrösche" [Tree frogs, *Hyperolius* spp. (s. l.)] sent by Martienssen to ZMB were all collected on the "Magrotto" plantation between 17 April and 18 May 1897.

***Hyperolius quadratomaculatus* Ahl, 1931a: 127.**

**Holotype.** 36108, "Mohorro, Deutsch-Ost-Afrika" [Mohoro (Muhoro), Pwanai Region, Tanzania], coll. Karl Grass, 22.II.1901.

**Present name.** *Hyperolius quadratomaculatus* Ahl, 1931.

**Remarks.** Depicted in Ahl (1931b: 413, fig. 285). The Imperial District Administrator Graß (sometimes Grass) served his colonial service as forestry assessor: in 1899 at the forest bureau "Usimbe" [Rufiji District, Pwani Region, Tanzania], and from 1900 onwards at the joint forest and district administrative office "Mohorro" (Graß 1904; Schabel 1990). Until 1901, he sent zoological specimens to ZMB (he was stationed in Africa longer).

***Hyperolius raveni* Ahl, 1931a: 36.**

**Holotype.** ZMB 77750, "Vulkangebiet nord-östlich des Kivu-See's" [volcano region northeast of Lake Kivu, Virunga Mountains, along the border between Rwanda and the Democratic Republic of the Congo], coll. Werner Alborus von Raven, X/1907.

**Present name.** *Hyperolius kivuensis* Ahl, 1931a.

**Remarks.** Drawing in Ahl (1931b: 292, fig. 168). The holotype was collected during the first "Deutsche Zentral–Afrika–Expedition", 1907–1908.

*Hyperolius riggenbachi*
see *Hyperolius hieroglyphicus*, *Rappia riggenbachi.*

***Hyperolius rosaceus* Ahl, 1931a: 105.**

**Holotype.** 36104, "Klein-Popo, Togo" [Anhéo (Anecho or Popovi), Lacs Prefecture, Maritime Region, Togo], coll. Julius Graf von Zech auf Neuhofen.

**Present name.** *Hyperolius fusciventris* Peters, 1876.

**Remarks.** Depicted in Ahl (1931b: 379, fig. 254). In 1895, the German colonial officer Count von Zech went to Togo. Here he was assigned the management of the administrative station in Kete Krachi, a position which he held until 1900. He undertook several expeditions into the Togo Hinterland. In 1900 he was appointed District Administrator of the district Anecho in Klein Popo on the Togo coast. From 1905 to 1910 he was the governor of the German colony of Togo (Schnee 1920b).

***Hyperolius rubripes* Ahl, 1931a: 88.**

**Lectotype.** ZMB 36110, "Kililana" [opposite Manda Island, Lamu District, Coast Province, Kenya], coll. Clemens Andreas Denhard, 1896.

**Paralectotype.** ZMB 57530 (formerly part of ZMB 36110), same collecting data as for the lectotype.

**Present name.** *Hyperolius mariae* Barbour & Loveridge, 1928.

**Remarks.** Lectotype designation by implication by Laurent (1961: 87) who considered ZMB 36110 (adult male) as "Holotype". In 1878, together with his brother Gustav and Dr. med. Gustav Adolf Fischer, the German engineer and colonial economist C. A. Denhard undertook a research expedition to explore the Tana River (Kenya). One year later he explored the coastal area from Mombasa (Kenya) to Pangani (Tanzania). In 1885 an expedition led him to Lamu Island (Kenya). In 1885 he acquired land from the Sultan of Witu on the mainland coast southwest of Lamu, on which he established plantations and later (1886) ceded parts of it to the German Witu Society. In accordance with the Helgoland-Zanzibar Treaty, the "Wituland", which was under German protectorate from 1885 onwards, was declared a British protectorate on 18 June 1890 (Schnee 1920a).

***Hyperolius rugegensis* Ahl, 1931a: 82.**

**Syntypes.** ZMB 77721–77722, "Rugege-Wald, 2000 m hoch" [Nyungwe Forest, Cyangugu Prefecture, West Province, Rwanda], collected during the first "Deutsche Zentral–Afrika–Expedition", VIII/1907.

**Present name.** *Hyperolius castaneus* Ahl, 1931a.

**Remarks.** Depicted in Ahl (1931b: 355, fig. 231).

***Hyperolius rwandae* Dehling, Sinsch, Rödel and Channing in Channing, Hillers, Lötters, Rödel, Schick, Conradie, Rödder, Mercurio, Wagner, Dehling, Du Preez, Kielgast & Burger, 2013: 337, fig. 6, lower row, second right, fig. 9 E.**

**Holotype.** ZMB 77221 (field no: JMD 562, now missing), "from a pond in farmland on the eastern outskirts of Butare, Huye District, South Province, Rwanda (2°37'10.79"S, 29°45'08.45"E)", coll. Jonas Maximilian Dehling, 13. IX. 2010.

**Paratypes.** ZMB 77222, same collecting data as for the holotype; ZMB 77223, "from the Mugesera wetland south of Lac Mugesera, Bugesera Province, southeastern Rwanda (2°12'18.92"S, 30°16'18.18"E)", coll. J. M. Dehling, 27.III.2011; ZMB 77224, "from the Mugesera wetland, Bugesera Province, southeastern Rwanda (2°12'15.95"S, 30°15'49.25"E)", coll. Bonny Dumbo and J. M. Dehling, 27.III.2011; ZMB 77225, "from a wetland of the Akagera River, Kihere Province, southeastern Rwanda (2°13'27.63"S, 30°49'39.06"E)", coll. J. M. Dehling, 31.III.2011; ZMB 77423–77429, "from farmland on the eastern outskirts of Butare, Huye Province, southern Rwanda", coll. Katrin Lümkemann, Katharina Rosar and Christiane Schwarz, X/2009; ZMB 77686–77689, "from farmland on the eastern outskirt of Butare /2°35'44.1"S, 29°45'25.6"E)", coll. J.M. Dehling, 27.II.2012; ZMB 77683–77685, "from the Mugesera wetland, Bugesera Province, southeastern Rwanda", coll. J. M. Dehling, 26.II.2012; ZMB 77746–77748, "from a swamp in farmland on the eastern outskirt of Ruhengeri, Musanze District, North Province, Rwanda (1°30'25.73"S, 29°39'12.11"E)", coll. J. M. Dehling, 30.II.2012.

**Present name.** *Hyperolius rwandae* Dehling, Sinsch, Rödel and Channing in Channing, Hillers, Lötters, Rödel, Schick, Conradie, Rödder, Mercurio, Wagner, Dehling, Du Preez, Kielgast & Burger, 2013.

**Remarks.** The type material of *H. rwandae*, inventoried prior to the publication of the paper, was sent to ZMB in August 2017, however, without containing the holotype (ZMB 77221). On written request, we were informed that the holotype could not be found (J. M. Dehling in litt. 28 August 2017) and must therefore be regarded as lost.

***Hyperolius scheffleri* Ahl, 1931a: 111.**

**Holotype.** ZMB 85759, "Kibwezi, Britisch-Ost-Afrika" [Kibwezi Division, Makueni County, Kenya], coll. Georg Richard Otto Scheffler, 28.-29.XII.1905.

**Present name.** *Hyperolius glandicolor* Peters, 1878.

**Remarks.** Scheffler worked from 1899 to 1900 as a horticulturist on plantations of the German East African Society in Usambara (Nguelo and Derema), in the Useguha region [Tanzania] and from 1905 until his death on 10.VI.1911 as a farm manager under managing director Paul Huebner in Kibwezi, British East Africa (Urban 1917; Sieberg 1998).

***Hyperolius scriptus* Ahl, 1931a: 32.**

**Holotype.** ZMB 36087, "Tanga" [Tanga Region, Tanzania], coll. Georg Martienssen.

**Present name.** *Hyperolius substriatus* Ahl, 1931a.

**Remarks.** Drawing in Ahl (1931b: 287, fig. 160). For Martienssen's activities in East Africa and locality information and collecting dates see remarks on *H. pygmaeus* (above) and Gvoždík et al. (2014).

***Hyperolius simus* Ahl, 1931a: 46.**

**Lectotype.** ZMB 36111, "Usumbura, Tanganyika-See" [Bujumbura, Bujumbura Mairie Province, Burundi], coll. Rudolf Grauer.

**Paralectotypes.** ZMB 65179–65180 (formerly part of ZMB 36111), same collection data as for the holotype.

**Present name.** *Hyperolius kivuensis* Ahl, 1931a.

**Remarks.** Drawing in Ahl (1931b: 306, fig. 181). Lectotype designation by Laurent (1961: 82, ZMB 36111a = ZMB 36111).

***Hyperolius spatzi* Ahl, 1931a: 123.**

**Lectotype.** ZMB 32602, "Bakel-Kidira (Oberes Senegalgebiet)" [Kidira town near Malian border, Bakel Depart-

ment, Tambacounda Region, East Senegal], coll. Paul Wilhelm Heinrich Spatz.

**Paralectotypes.** ZMB 74853–74876 (formerly part of ZMB 32602), same locality data as for the lectotype.

**Present name.** *Hyperolius spatzi* Ahl, 1931.

**Remarks.** Lectotype designation by Rödel et al. (2010: 185). Another paratype (MCZ A-17646) from "Bakel-Kidira", coll. Spatz, was sent in exchange to MCZ in 1932 (Barbour and Loveridge 1946: 131).

The German trader and zoologist Spatz carried out various hunting and collecting expeditions in the northern Sahara during which he also collected ethnological and prehistoric objects. In 1884 he travelled to Tunisia and Algeria for the first time. Further journeys to Tunisia were as follows: in spring 1891, together with Alexander Koenig and Koenig's wife; in 1893 and from November 1896 to July 1898 together with Carlo von Erlanger; and from 1904 to 1906 together with Otto Eduard Graf von Zedlitz und Trützschler and Alfred Blanchet. In the early 1920s he travelled to Mauritania, to the lower Senegal River and the Spanish colony of Rio de Oro. The latter he visited again with the Berlin taxidermist Fritz Bock in spring 1926. On behalf of ZMB he made a collecting trip from Dakar to the lower Senegal River from February to July 1928, then accompanied by his son Richard (Spatz 1926 1930; Schulz-Parthu 1997). It was probably on this journey that the holotype of *Hyperolius spatzi* was collected.

### *Hyperolius spinosus* Buchholz & Peters in Peters, 1875: 208, pl. 1, fig. 3.

**Syntypes.** ZMB 8359 and ZMB 59353–59355 (formerly part of ZMB 8359), "Cameruns" [Douala, Region Littoral, Cameroon], coll. Georg Anton Eugen Reichenow, don. Reinhold Wilhelm Buchholz (Fig. 9).

**Present name.** *Acanthixalus spinosus* (Buchholz & Peters, 1875).

**Figure 9.** Syntype of *Hyperolius spinosus* Buchholz & Peters in Peters, 1875, ZMB 59354 from "Cameruns", coll. Buchholz.

**Remarks.** Depicted in in Nieden (1910b: 58, fig. 124), copied by Ahl (1931b: 446, fig. 310).

Type locality corrected to "Douala" by Frétey et al. (2014); for further information see also remarks on *Hyperolis guttatus*. Herrmann (1989: 13) reported two additional syntypes without inventory numbers in the collection of the Zoologisches Museum Greifswald (ZMG) from "Bonjongo" [Southwest Region, Cameroon], coll. Buchholz. However, although the collector is identical, the locality of the two ZMG specimens does not correspond to the type locality "Cameruns" [= part of the present Douala, see above] given by Peters (1875: 209), and thus the ZMG specimens should not be regarded as syntypes.

### *Hyperolius stenodactylus* Ahl, 1931a: 21.

**Holotype.** ZMB 85834, "Bipindi, Kamerun" [Bipindi village, Océan Department, South Province, Cameroon], coll. Georg August Zenker.

**Present name.** *Hyperolius stenodactylus* Ahl, 1931.

**Remarks.** Drawing in Ahl (1931b: 271, fig. 140). For Zenker's activities in Cameroon, see the remarks on *Hyperolius obstetricans*.

### *Hyperolius striolatus* Peters, 1882a: 9.

**Holotype.** ZMB 9300, "Taita" [Taita Hills,Taita-Taveta County, Kenya], coll. Johann Maria Hildebrandt.

**Present name.** *Hyperolius glandicolor* Peters, 1878.

**Remarks.** Depicted in Tornier (1896: pl. 4, fig. 21) and redrawn in Ahl (1931b: 313, fig. 187, right specimen).

### *Hyperolius stuhlmanni* Ahl, 1931a: 113.

**Holotype.** ZMB 13008, "Vitschumbi, Südende des Albert-Eduard-See's" [Witschumbi on the southern tip of Lake Edward, North Kivu Province, Democratic Republic of the Congo], coll. Franz Ludwig Stuhlmann.

**Present name.** *Hyperolius viridiflavus* (Duméril & Bibron, 1841).

**Remarks.** Drawing in Tornier (1896, pl. 4, fig. 24) reprinted by Ahl (1931b: 396, fig. 271).

### *Hyperolius substriatus* Ahl, 1931a: 84.

**Holotype.** ZMB 36099, "Magrotto bei Tanga" [plantation in southern part of Mlinga Mountains, East Usambara, Tanga Region, Tanzania], coll. Georg Martienssen (Fig. 10).

**Paratypes.** ZMB 23087, "Songea" [Songea district, Ruvuma Region, Tanzania], coll. P. Preuss; ZMB 85719, "Udjidji" [Ujiji, Kigoma Province, Tanzania], coll. Stabsarzt Hösemann; ZMB 85859, "Magrotto bei Tanga", ZMB 85996–85998, "Tanga" [Region], coll. G. Martienssen; ZMB 85858 and ZMB 85863–85865, "Usam-

bara" [Usambara Mountains], coll. Otto Küttner; ZMB 85860, "Marakiras (1500 m)" and ZMB 85861–85862, "Uhehe", coll. W. Goetze; ZMB 85866 and ZMB 85875, "Amani" [East Usambara Mountains], coll. J. Vosseler; ZMB 85867–85868, "Mwa Mkoro [sic]" [Kwa Mkoro (Kwamkoro, Prinz Albrecht Plantations), Tanga Region], coll. H. Glauning; ZMB 85873, "Dar-es Salaam", coll. F. L. Stuhlmann; ZMB 86018, "Nguelo" [Ngwelo, East Usambara Mountains, Lushoto District, Tanga Region], coll. Auguste Kummer, 1898–99; ZMB 86006–86011 and ZMB 86019–86025, no collecting data.

**Present name.** *Hyperolius substriatus* Ahl, 1931.

**Remarks.** Drawings illustrating the variation of this taxon were published by Ahl (1931b: 358, fig. 234), reprinted from Tornier (1896, pl. 4, figs 65, 67, 69, 71). Ahl (1931a: 85) mentions all together 65 specimens of which we could not locate the material collected from "Konde Nika" and "Derema". For Martienssen's activities in East Africa and locality information and collecting dates, see remarks on *H. pygmaeus*, as well as Gvoždík et al. (2014).

**Figure 10.** Holotype of *Hyperolius substriatus* Ahl, 1931a, ZMB 36099 from "Magrotto bei Tanga", coll. Martienssen.

*Hyperolius substriatus*
see *Hyperolius scriptus.*

***Hyperolius taeniatus* Peters, 1854: 627.**

**Holotype.** ZMB 4531, "Boror" [Companhia do Boror, Zambezia Province, Mozambique], coll. Wilhelm Carl Hartwig Peters.

**Present name.** *Hyperolius marmoratus* Rapp, 1842.

**Remarks.** Peters (1882b: 167) mentions that he received only a single specimen from "Boror" in March 1846 and depicted it in plate 22, fig. 7. An additional drawing of the holotype is shown by Tornier (1896, pl. 4, fig. 56).

***Hyperolius tettensis* Peters, 1854: 628.**

**Holotype.** ZMB 4812, "Tette" [Tete Province, Mozambique], coll. Wilhelm Carl Hartwig Peters.

**Present name.** *Hyperolius argus* Peters, 1854.

**Remarks.** Peters (1882b: 164) mentioned the sole female type specimen collected by him at "Tette" and synonymized it with a species he described, *H. flavoviridis.* The latter was later considered by Laurent (1961: 74) as a synonym of another of Peters' species, namely *Hyperolius argus*. The type is depicted by Peters (1882b, pl. 22, fig. 5). See also remarks on *Hyperolius flavoviridis*.

***Hyperolius thoracotuberculatus* Ahl, 1931a: 98.**

**Holotype.** ZMB 36097, "Afrika (ohne genauen Fundort)" [Africa, without locality information], collector or donor unknown.

**Present name.** *Hyperolius thoracotuberculatus* Ahl, 1931a.

**Remarks.** Laurent (1961: 68) erroneously gives "360097" as the inventory number for the male holotype.

***Hyperolius togoensis* Ahl, 1931a: 112.**

**Holotype.** ZMB 39009, "Togo (Genauerer Fundort unbekannt)" [Togo, without precise locality information], collector or donor unknown.

**Present name.** *Hyperolius concolor* (Hallowell, 1844).

**Remarks.** Depicted in Ahl (1931b: 390, fig. 264).

***Hyperolius tornieri* Ahl, 1931a: 45.**

**Holotype.** ZMB 85833, "Ukami (Deutsch-Ost-Afrika)" [Udzungwa Mountains,Tanzania], coll. Georg Martienssen.

**Present name.** *Hyperolius tornieri* Ahl, 1931.

**Remarks.** Drawing in Ahl (1931b: 305, fig. 179). The holotype of *H. tornieri* was sent by Martienssen in April 1898 to ZMB. For Martienssen's activities in East Africa, locality information and collecting dates, see remarks on *H. pygmaeus*, as well as Gvoždík et al. (2014).

***Hyperolius trifasciatus* Ahl, 1931a: 119.**

**Syntypes.** ZMB 31868–31869 and ZMB 77976 (formerly part of ZMB 31869), "Grand Bassa, Liberia" [Grand Bass County, Liberia], coll. Richard Oeser.

**Present name.** *Hyperolius fusciventris* Peters, 1876.

**Remarks.** Depicted in Ahl (1931b: 303, fig. 278). Oeser collected the type specimens together with the holotype of *H. oeseri* in Liberia, during his journey along the West African coast in 1928, see remarks above.

***Hyperolius udjidjiensis* Ahl, 1931a: 97.**

**Holotype.** ZMB 36101, "Udjidji", [Ujiji, Kigoma Province, Tanzania], coll. Paul Hösemann.

**Paratype.** ZMB 85832, "Kibwezi, Britisch-Ost-Afrika" [Kibwezi Division, Makueni County, Kenya], coll. Richard Fritz Paul Hübner [later Huebner], 5.III. 1906.

**Present name.** *Hyperolius mariae* Barbour & Loveridge, 1928.

**Remarks.** Depicted in Ahl (1931b: 370, fig. 246). For Huebner's activities in (British) East Africa see remarks on *Hyperolius pulchromarmoratus*; for Hösemann, see remarks on *H. argentovittis*.

***Hyperolius unicolor* Ahl, 1931a: 122.**

**Holotype.** ZMB 86013, "Ipiana" [Ipyana (Ipanya) on Kiwira River, at the northwestern tip of Lake Malawi, Kyela District, South Mbeya Region, Tanzania], coll. Adolf Ferdinand Stolz.

**Present name.** *Afrixalus stuhlmanni* (Pfeffer, 1893).

**Remarks.** For activities of Stolz in East Africa, see remarks on *H. ipianae*.

***Hyperolius variabilis* Ahl, 1931a: 39.**

**Holotype.** ZMB 36122, "Bukoba" [Bukoba Urban District, Kagera Region, Tanzania], coll. Johann Gustav Hermann Schubotz, 15.VI.1907.

**Paratypes.** ZMB 77802–77813 (formerly part of ZMB 36122), "Bukoba", coll. Schubotz, 17.VI.1907; ZMB 36116, "Mohasi See", coll. Schubotz; ZMB 46518–46519, "NW-Buddu-Wald" [Minziro Forest, NW of Bukoba, Missenyi District, Kagera Region, Tanzania], coll. Schubotz, VI/1907; ZMB 46521, "Insel Kwidjwi (Kivu See)" [Idjwi (Ijwi) Island, Lake Kivu, Democratic Republic of the Congo], coll. Schubotz; ZMB 47210, "Sisse [sic], W-Niansa" [Sesse or Ssese Archipelago, Lake Victoria, Uganda], coll. Stuhlmann; ZMB 78564, "Udjidji" [Ujiji, Kigoma Province, Tanzania], coll. Dr. Hösemann; ZMB 85831, "Kagera-Ufer" [Tanzania], coll. Stuhlmann; ZMB 85879–85881, "Bukoba", coll. Dr. Eggel; ZMB 85882, "Mpororo" [Region in southern Nyagatare District, Eastern Province, Rwanda], coll. Schubotz; ZMB 85890–85892 and ZMb 91000–91002, "Bukoba", coll. Stuhlmann.

**Present name.** *Hyperolius viridiflavus* (Duméril & Bibron, 1841).

**Remarks.** Ahl (1931a: 40) mentioned a series of 47 specimens from "Bukoba" including the "Type". We failed to locate 23 of these specimens. He also lists 11 additional specimens that we could not locate, as well as a second specimen from "Mohasi See", coll. Schubotz and two specimens without locality or collector information. Drawings of seven specimens showing the variation of this taxon are published by Ahl (1931b: 298, fig. 173). These have been copied from Tornier (1896, pl. 4, specimen no. 26–28 and 30–33). Another two paratypes, MCZ A-17648 and 17626, from "Bukoba" collected during the first "Deutsche Zentral–Afrika–Expedition", 1907–1908 were sent in exchange to MCZ in 1932 (Barbour and Loveridge 1946: 129).

***Hyperolius variegatus* Peters, 1882a: 8.**

**Syntypes.** ZMB 10249 and 75602–75604 (formerly part of ZMB 10249), "Cabaceira" [Peninsula Cabaceira, Mossuril District, Nampula Province, Mozambique], coll. Wilhelm Carl Hartwig Peters.

**Present name.** *Hyperolius marmoratus* Rapp, 1842 [part] and *Afrixalus* sp. [part]

**Remarks.** *Hyperolius variegatus* was described by Peters (1882a) from an unknown number of specimens originating from "Mocambique (Cabaçeira, Quellimane, Inhambane)". Peters (1882b: 168) mentions that he first saw small specimens of this species on bushes on the "Cabaceira" peninsula in June 1843, an adult specimen on a mulberry tree in "Quellimane" in January 1846. He also lists an observation from "Prazo [estate] Boror" in March 1846, but did not mention the locality "Inhambane" in this second publication. Tornier (1896: 145, pl. 4, figs 61, 62) mentioned and depicted the two syntypes from "Quellimane, Mozambique", both inventoried together under ZMB 4530. These could not be traced by Bauer et al. (1995: 46), nor by us. The other syntype(s) from "Inhambane" with unknown inventory number could also not be located. Laurent (1961: 67) suggested that one of the specimens under 10249 is actually a specimen of *Afrixalus fornasini* (Bianconi, 1849). Our examinations revealed that ZMB 10249 und ZMB 75602 are *H. marmoratus*, whereas ZMB 75603 and ZMB 75604 are juvenile specimens of *Afrixalus*. Identification on the species level was not possible for the latter two frogs.

***Hyperolius veithi* Schick, Kielgast, Rödder, Muchai, Burger & Lötters, 2010: 27, fig. 4 A.**

**Paratype.** ZMB 79542, "a flooded area in the middle of primary forest away from rivers and streams in Salonga National Park (02.88°S, 20.41°E, ca. 415 m above sea level), Province of Bandundu, Equateur Kasaï Oriental and Occidental, Democratic Republic of the Congo", coll. Jos Kielgast, 24.–26.I.2008.

**Present name.** *Hyperolius veithi* Schick, Kielgast, Rödder, Muchai, Burger & Lötters, 2010.

**Remarks.** Holotype: ZFMK 89607, "a flooded area in the middle of primary forest away from rivers and streams in Salonga National Park (02.88 S, 20.41 E, ca. 415 m above sea level), Province of Bandundu, Equateur Kasaï Oriental and Occidental, Democratic Republic of the Congo", coll. J. Kielgast, 24.I.2008. Paratypes ZFMK 89608–89645 and ZMUC R771393–771412, same locality data as for the holotype, coll. J. Kielgast, 24.–26.I.2008. ZMB 79542 (formerly ZFMK 89631), was given in exchange to ZMB in October 2013 (see also Böhme 2014).

***Hyperolius ventrimaculatus* Ahl, 1931a: 107.**

**Holotype.** ZMB 78563, "Vulkangebiet nord-östlich des Kivu-See's" [volcano region northeast of Lake Kivu,

Virunga Mountains, along the border between Rwanda and the Democratic Republic of the Congo], coll. Werner Alborus von Raven, X/1907.

**Present name.** *Hyperolius castaneus* Ahl, 1931a.

**Remarks.** Drawing in Ahl (1931b: 381, fig. 256). The holotype was collected during the first "Deutsche Zentral–Afrika–Expedition", 1907–1908.

***Hyperolius vermicularis* Ahl, 1931a: 24.**

**Syntype.** ZMB 10988, "Zanzibar" [Unguja Island, Tanzania], coll. Franz Ludwig Stuhlmann.

**Present name.** *Hyperolius marmoratus* Rapp, 1842.

**Remarks.** *Hyperolius vermicularis* Ahl is a *nomen novum* pro *Rappia vermiculata* Pfeffer, 1893 which is preoccupied by *Hyperolius vermiculatus* Peters, 1882a. A copy of Pfeffer's (1893, pl. 1, fig. 12) drawing is depicted by Ahl (1931b: 275, fig. 145). Pfeffer (1893) mentions two specimens (catalogue no. "352") from "Sansibar, Insel", collected by Fülleborn on 6 August 1888. The type specimens of *Rappia vermiculata* are not mentioned in the herpetological type catalogues of ZMH (Hallermann 1998, 2006). A new search in ZMH collection also failed to locate the type material (Jakob Hallermann in litt. 29 July 2020). ZMB 10988 was inventoried as "*Rappia vermiculata* Pffr." collected by Stuhlmann, allegedly at "Quillimane". However, Tornier (1896: 141) stated that the locality is "Zanzibar" and depicted this juvenile specimen on plate 4, figure 34.

***Hyperolius vermiculatus* Peters, 1882a: 8.**

**Lectotype.** ZMB 10050, "Malange (Angola)" [Malanje, Malanje Province, Angola], coll. Friedrich Wilhelm Alexander von Mechow.

**Paralectotype.** ZMB 9408, "Malange", coll. Benedictus Ludwig Heinrich Otto Schütt.

**Present name.** *Hyperolius angolensis* Steindachner, 1867 (fide Marques et al. 2018).

**Remarks.** Tornier (1896, pl. 4, fig. 29) depicted the paralectotype; figure copied by Ahl (1931b: 338, fig. 211). Lectotype by subsequent designation through Laurent (1961: 88).

The Prussian explorer and topographer Major Mechow participated in the first "Loango–Expedition" from 1873–1875 under Paul Güssfeldt (Güssfeld et al. 1879). On a second "Kuango–Expedition" (1878–81), led by himself, he and two companions, the botanist Julius Eduard Teusz and the shipwright of the Imperial Navy Jess Bugslag (or Buslag), travelled from Luanda via Malanje (June 1888) to the confluence of the Luhemba and the Cuango River (November 1880). He returned via Malanje (February 1881) to Luanda and arrived in Berlin in August 1881 (Mechow 1882; Weidmann 1894; Heintze 2007, 2018; Teusz 2018).

From 1878 to 1879, the expedition of engineer Schütt was carried out together with the architect Paul Gierow on behalf of the "Afrikanische Gesellschaft in Deutschland". The expedition aimed at compiling topographic reconnaissance and producing maps. They started on 4 January 1878 in Luanda and reached the lower Luachimo River (3 to 9 February 1879) via Malanje (22 February to 4 July 1878), and Quimbundo (12 November to 1 December 1878). They almost reached Mai Munene. From Quimbundo they turned back through the Lunda area and reached Luanda again via Malanje (12 to 24 May 1879) on 21 June 1879 (Heintze 2007).

*Hyperolius viridiflavus*

see *Hyperolius flavoguttatus*, *Hyperolius irregularis*, *Hyperolius kandti*, *Hyperolius karissimbiensis*, *Hyperolius koehli*, *Hyperolius kwidjwiensis*, *Hyperolius macrodactylus*, *Hyperolius mohasicus*, *Hyperolius monticola*, *Hyperolius multicolor*, *Hyperolius oculatus*, *Hyperolius phrynoderma*, *Hyperolius punctatissimus*, *Hyperolius schubotzi* (unlocated type specimens), *Hyperolius stuhlmanni*, *Hyperolius variabilis*, *Hyperolius wettsteini.*

***Hyperolius vittiger* Peters, 1876: 122.**

**Holotype.** ZMB 8669, "Liberia", coll. Heinrich Wolfgang Ludwig Dohrn.

**Present name.** *Afrixalus vittiger* (Peters, 1876).

**Remarks.** Peters (1876: 123) explicitly mentions the inventory number of the holotype; it was also mentioned and depicted by Tornier (1896: 144, p. 147, fig. K 50, and pl. 4, fig. 50).

***Hyperolius wettsteini* Ahl, 1931: 70.**

**Holotype.** ZMB 36103, "Bukoba" [Bukoba Urban District, Kagera Region, Tanzania], coll. Johann Gustav Hermann Schubotz, 15.VI.1907.

**Present name.** *Hyperolius viridiflavus* (Duméril & Bibron, 1841).

**Remarks.** Depicted in Ahl (1931b: 343, fig. 217). The holotype was collected during the first "Deutsche Zentral–Afrika–Expedition", 1907–1908.

***Kassina deserticola* Ahl, 1930c: 280.**

**Syntype.** ZMB 23397 (formerly two specimens, one present in ZMB collection), "Windhuk" [Windhoek, Namibia], coll. Leonhard Scheben.

**Present name.** *Kassina senegalensis* (Duméril & Bibron, 1841).

**Remarks.** Ahl (1930c: 281) exclusively designated two male specimens from "Windhuk" (ZMB 23397) as types ("Typen"). Another syntype MCZ A-17650 (formerly the second specimen under ZMB 23397) was sent in exchange to MCZ in 1932 (Barbour and Loveridge 1946: 132). Between 1909 and 1913, the German gov-

ernment veterinarian and farmer Scheben sent several amphibian and reptile collections from the former colony "Deutsch-Südwestafrika" to ZMB. Scheben collected in "Windhuk", "Klein Nauas" and "Rehobot".

*Kassina maculifer*
see *Megalixalus maculifer.*

***Kassina modesta* Ahl, 1930c: 281.**

**Holotype.** ZMB 27374, "Mariannhill Natal" [Trappist Mission Station Mariannhill, ca. 16 km east of Durban, today eThekwini Metropolitan Municipality, KwaZulu-Natal Province, South Africa], donated by the Mariannhill Mission, leg. 11.XII.1912.

**Present name.** *Kassina senegalensis* (Duméril & Bibron, 1841).

**Remarks.** The holotype was probably collected by Rev. Father Pascalis Boneberg of the Trappist Mariannhill Mission who sent several specimens to ZMB in 1913.

*Kassina senegalensis*
see *Cystignathus argyreivittis*, *Kassina deserticola*, *Kassina modesta.*

***Megalixalus dorsimaculatus* Ahl, 1930b: 92.**

**Holotype.** ZMB 13696, "Magrotto bei Tanga" [plantation in southern part of Mlinga Mountains, East Usambara, Tanga Region], coll. Georg Martienssen.

**Present name.** *Afrixalus dorsimaculatus* (Ahl, 1930b).

**Remarks.** For Martienssen's activities in East Africa and locality information and collecting dates, see remarks on *H. pygmaeus*, as well as Gvoždík et al. (2014).

***Megalixalus maculifer* Ahl, 1924: 7.**

**Holotype.** ZMB 26911, "Ganda Ali, Annia Galla" [south of Bia Woraba in the Ennia Galla county, East Harerge Zone, Oromia Region, Ethiopia], coll. Carl Viktor Heinrich Freiherr von Erlanger and Oskar Rudolph Neumann, 28.–29.V.1900.

**Present name.** *Kassina maculifer* (Ahl, 1924).

**Remarks.** From 1900 onwards, the ornithologists, mammalogists and explorers von Erlanger and Neumann undertook a two-year journey through Somaliland to the south of Ethiopia. They were accompanied by the physician and collector of botanical objects Dr. Hans Ellenbeck, the cartographer Johann Holtermüller and the taxidermist Carl Hilgert. From Zeila at the Gulf of Aden [Zeylac District, Awdal Region, Somaliland] they started their journey on 12 January 1900 and travelled via Djeldessa [Jaldessa, Sitti zone, Somali Region, Ethiopia] (3 March 1900), Harar (1 April 1900 ), Biar-Woraba [Bia-Woraba, East Harerge Zone, Oromia Region, Ethiopia] (23 May 1900), the Wabbi River [Webi Shebeli] (passage on 10 June 1900), to Addis Ababa (16 August 1900). During that journey they climbed Abu-el-Kassim [Abul Kasim, Arsi Zone, Oromia Region, Ethiopia] on 16 July 1900. From Addis Ababa Neumann went alone to Shoah [Shewa Kingdom, a region present day in Central Ethiopia] and southern Sudan, then returned to Cairo via Khartoum. Erlanger went on to Lake Turkana (Neumann 1902a, b; Erlanger 1904; Kleinschmidt 1905; Kobelt 1905). During this expedition (map with the route in Neumann 1902b) he collected thousands of zoological objects (manly insects and vertebrates), which are stored in ZMB, SMF, NHMM, and the Walter Rothschild Zoological Museum (now the Natural History Museum at Tring; Stresemann 1947; Hildebrandt 2004).

***Megalixalus Stuhlmanni* Pfeffer, 1893: 99.**

**Syntypes.** ZMB 10986 and ZMB 11015, ? "Quillimane" [Quelimane, Angoche District, Zambezia Province, Mozambique], coll. Franz Ludwig Stuhlmann.

**Present name.** *Afrixalus stuhlmanni* (Pfeffer, 1893).

**Remarks.** Pfeffer (1893: 100) mentions 26 specimens from "Sansibar, Insel" [Zanzibar Island], collected by Stuhlmann on 6.VIII.1888. Ahl (1930b: 96) listed under "*Megalixalus stuhlmanni*" two specimens from "Quillimane, Stuhlmann leg." and denotes one of them as type: "No. 1" [= ZMB 10986] "Type der Art"; the specimen "No. 2" is inventoried under ZMB 11015. These two specimens are part of a collection of amphibians and reptiles (ZMB 10983–11015), which was donated to ZMB in 1893 by ZMH in agreement with Stuhlmann. This donation contains "Doubletten" from the Stuhlmann collection, which was described by Pfeffer (1893) (see also Kirchhof et al. 2016: 181).

However, the location "Quillimane" as mentioned in the ZMB inventory catalogue does not correspond to the type locality given by Pfeffer (1893). It is unclear whether this is a transmission error of the locality information for the ZMB specimens, and "Quillimane" instead of "Sansibar" is correct. At least for the Berlin syntype of *Rappia vermiculata*, the locality information has been mixed up, as shown by Tornier (1896: 141, see below).

*Megalixalus stuhlmanni* and its type material is not mentioned in the herpetological type catalogues of ZMH (Hallermann 1998, 2006). During a renewed search, no further type material could be located (Jakob Hallermann in litt. 29 July 2020).

***Megalixalus uluguruensis* Barbour & Loveridge, 1928: 231.**

**Paratype.** ZMB 38031 (ex MCZ, previous inventory no. unknown), "Vituri, Uluguru Mtns., Tanganyika Territory" [Uluguru Mountains, Tanzania], coll. Arthur Loveridge, 30.X.1926.

**Present name.** *Afrixalus uluguruensis* (Barbour & Loveridge, 1928).

**Remarks.** Holotype: MCZ A-13311; Paratypes MCZ A-13312–13320, all from "Vituri, Uluguru Mtns.," coll. A. Loveridge, 27.31.X.1926; MCZ A-13321, from "Bumbuli, Usambara Mtns.", coll. A. Loveridge, 14.XII.1926 and MCZ A-13368, from "Derema, Usambara Mtns.", coll. A. Loveridge, XII/1926. ZMB 38031 was donated to ZMB by A. Loveridge (MCZ) in the 1930s and was inventoried in 1958.

***Megalixalus variabilis* Ahl, 1930a: 526.**

**Syntypes.** ZMB 7856 and ZMB 50108–50117 (formerly part of ZMB 7856), "Nossi Faly, bei Madagascar" [Nosy Faly, SW Ambaro Bay, Ambanja District, Diana Region, Madagascar], coll. François Paul Louis Pollen.

**Present name.** *Heterixalus variabilis* (Ahl, 1930a).

**Remarks.** One of the 12 syntypes mentioned by Ahl (1930a: 527) could not be located. Pollen, a Dutch merchant and naturalist, undertook expeditions to Madagascar and its offshore islands in the Mozambique Channel, as well as trips to the Comoros, Mascarenes and Réunion. He collected various botanical and zoological objects between 1863 and 1866 (Pollen 1867, 1868; Rosenberg 1886). His collections are held today by the BMNH, RMNH and ZMB.

***Morerella cyanophthalma* Rödel, Assemian, Kouamé, Tohé and Perret in Rödel, Kosuch, Grafe, Boistel, Assemian, Kouamé, Tohé, Gourène, Perret, Henle, Tafforeau, Pollet & Veith, 2009: 29.**

**Paratypes.** ZMB 71566 (cleared and stained), "Banco National Park, near forest school, 05°23'.104"N, 04°03.072"W, Ivory Coast" coll. N. Emmanuel Assemian, N'Goran G. Kouamé, Blayda Tohé and Mark-Oliver Rödel, 4.IX.2003; ZMB 71588–71590 and ZMB 73271, "Banco National Park, swampy forest with shallow puddles near river and open area near fish culture ponds, 05°25'N, 04°03'W, Ivory Coast", coll. same as above, 23.IX.2004.

**Present name.** *Morerella cyanophthalma* Rödel, Assemian, Kouamé, Tohé & Perret, 2009.

**Remarks.** Holotype MHNG 2131.44, "Banco National Park, 05°25'N, 04°03'W, Ivory Coast", coll. Jean-Luc Perret, 1980. Additional paratypes as follows MHNG 2131.36–43 and MHNG 2131.45–55 same collecting data as for the holotype; SMNS 11939–11940, "Banco National Park, near forest school, 05°23'.104"N, 04°03.072"W, Ivory Coast" coll. Assemian, Kouamé, Tohé and Rödel, 4.IX.2003; ZFMK 82796 same collecting data as for SMNS 11939 (Böhme 2014).

*Paracassina kounhiensis*
see *Tornierella pulchra.*

***Rappia dombeensis* Tornier, 1896: 150, pl. 4, fig. 86.**

**Holotype.** ZMB 6465, "Dombe" [Dombe Grande, Benguela Province, Angola], coll. José Alberto de Oliveira Anchieta, don. José Vicente Barbosa du Bocage.

**Paratypes.** ZMB 9173 and ZMB 74945 and 75448 (both formerly part of ZMB 9173), "Chinchoxo" [Cabinda Province, Angola], don. Africanische Gesellschaft.

**Present name.** Not assigned to a valid name according to Frost (2021); see below.

**Remarks.** As far as we are aware this nomen was not used again as valid after its introduction by Tornier (1896). It is not mentioned in recent compilations of the Angolan herpetofauna (e.g. Marques et al. 2018).

In a letter sent to Wilhelm Peters, dated 12 June 1869, Bocage announced a shipment containing 30 species of "Reptiles et Batraciens". He listed under no. 21 a "*Hyperolius dombeensis* n. sp. [from] Dombe". The letter is archived in the Historical Research department at the Museum für Naturkunde Berlin (ZMB). The specimens were inventoried by Peters as ZMB 6456 under the name "*Hyperolius dombeensis* Bocage" and marked as type specimen. Tornier (1896: 150) attributed the authorship to Bocage as well. However, to the best of our knowledge, Bocage never described a reed frog with the specific epithet "*dombeensis*". Furthermore, the name of this "new species" used by Bocage in his letter to Peters, is not accompanied by a description or drawing. Because of this, the criteria of Article 50.1.1 of the Code (ICZN 1999) are not fully met and the authorship should be attributed to Tornier (1896) who first published the name together with a short description and figure.

Beside the specimen from "Dombe" which Tornier regarded as "Type", Tornier also mentioned "identical" specimens from "Chinchoxo", ZMB 9173 [originally three specimens] and a specimen from "Port Natal" [Durban, KwaZulu-Natal Province, South Africa] in the "Hamburger Museum". The Hamburg specimen, inventoried under ZMH R16591, which we consider as paratype too, is currently determined as *Hyperolius m. marmoratus* (Jakob Hallermann in litt. 6 July 2020), and was collected by W. Joost on 23 April 1893. Jost also collected invertebrates at "Lourenço Marques" [Maputo, Mozambique] and "Delagoa-Bai" [Maputo Bay, Mozambique] (Wassmann 1922; Harms and Dupéré 2018; Jakob Hallermann in litt. 31 July 2020).

Our examination of the holotype revealed that it is a member of the 'sharp-nosed reed frogs', *Hyperolius nasutus* complex. Without genetic data, identification to species is not possible (compare Channing et al. 2013).

***Rappia riggenbachi* Nieden, 1910a: 244, fig. 4.**

**Holotype.** ZMB 20435, "Banjobezirk" [Mayo-Banyo Department, Adamawa Region, Cameroon], coll. Fritz Wilhelm Riggenbach.

**Present name.** *Hyperolius riggenbachi* (Nieden, 1910a).

**Remarks.** Depicted in Nieden (1910b: 61, fig. 128) and Ahl (1931b: 391, fig. 266), copied from Nieden (1910a: 245, fig. 4). The holotype of *R. riggenbachi* was collected in January 1909 by the zoologist Riggenbach who accompanied the "Zoologisch–Botanische Kamerun–Expedition, 1908–1909" into the hinterland of Cameroon. The expedition started in Jabassi on Wuri River (15 to 16 November 1908) and went via Bamenda (17 December), the Bansso Mountains (29 December), Banjo (12 January 1909), the Genderu Mountains (20 February to 3 March), Gorua (13 to 23 April), the Lagdo Mountains (26 June) to Garua (11 to 20 August 1909). A map and itinerary of the expedition can be found in Reichenow (1911).

***Tornierella pulchra* Ahl, 1924: 10.**

**Syntypes.** ZMB 26917, "Garamulata, ca. 2800 m hoch, im Wald" [Gara Muleta Mountain, East Harerge Zone, Oromia Region, Ethiopia, ca. 2800 m a.s.l., in forest], coll. Carl Viktor Heinrich Freiherr von Erlanger and Oskar Rudolph Neumann, 31.III.1900, and ZMB 26918, "Somaliland" [Eastern Oromia Region, Ethiopia], same collectors as above.

**Present name.** *Paracassina kounhiensis* (Mocquard, 1905).

**Remarks.** For information on the expedition period and route of Erlanger and Neumann, see remarks on *Megalixalus maculifer*.

Unlocated type specimens

***Hyperolius bergeri* Ahl, 1931a: 73.**

**Holotype.** ZMB unknown; "Guaso Narok (Englisch-Ostafrika)" [Uaso Narok, Nyandarua North District Laikipia County, Kenya]; coll. Arthur Berger.

**Present name.** *Hyperolius glandicolor* Peters, 1878.

**Remarks.** Drawing in Ahl (1931b: 347, fig. 221).

***Hyperolius bicolor* Ahl, 1931: 129.**

**Holotype.** ZMB unknown; "Farenda [sic] Bango, Loanda", [Fazenda Bango, Cuanza Norte Province, Angola]; coll. Lieutenant Karl May, 1903.

**Present name.** *Hyperolius bicolor* Ahl, 1931.

**Remarks.** Drawing in Ahl, 1931b: 414, fig. 287.

***Hyperolius buchholzi* Ahl, 1931a: 56.**

**Holotype.** ZMB unknown, "Accra" [Ghana], coll. Reinhold Wilhelm Buchholz.

**Present name.** *Hyperolius guttulatus* Günther, 1858.

**Remarks.** Depicted in Ahl (1931b: 320, fig. 194).

***Hyperolius guttolineatus* Ahl, 1931a: 57.**

**Holotype.** ZMB unknown, "Deutsch-Ost-Afrika (näherer Fundort unbekannt)" [German East Africa, exact locality unknown], coll. Ferdinand Uhl.

**Present name.** *Hyperolius marmoratus* Rapp, 1842.

**Remarks.** Drawing in Ahl (1931b: 321, fig. 195). Chief medical officer Dr. Uhl carried out his colonial service at the "Schutztruppe" from 1896 to 1901. In January 1900, he was commanded to Langenburg [Lumbira, Mbeya Region, Tanzania] to replace Stuhlmann (Stuhlmann 1906).

***Hyperolius bituberculatus* Ahl, 1931a: 27.**

**Holotype.** ZMB unknown, "Mohasi-See, Ruanda" [Lake Mohasi, Rwanda], coll. Johann Gustav Hermann Schubotz, VII/1907.

**Present name.** *Hyperolius kivuensis* Ahl, 1931a.

**Remarks.** Drawing in Ahl (1931b: 281, fig. 152). The holotype was collected during the first "Deutsche Zentral–Afrika–Expedition", 1907–1908.

***Hyperolius moseri* Ahl, 1931a: 50.**

**Holotype.** ZMB unknown, "Misahöhe, Togo", [Missahomé, Agou Prefecture, Plateau Region, Togo], coll. Ernst Richard Reinhold Baumann.

**Present name.** *Hyperolius concolor* (Hallowell, 1844).

**Remarks.** Depicted in Ahl (1931b: 311, fig. 186).

***Hyperolius renschi* Ahl, 1931a: 115.**

**Holotype.** ZMB unknown, "Zanzibar" [Unguja Island, Tanzania], coll. Oscar Rudolph Neumann.

**Present name.** *Hyperolius mariae* Barbour & Loveridge, 1928.

***Hyperolius schubotzi* Ahl, 1931a: 63.**

**Holotype.** ZMB unknown, "Kissenji" [on the northeast shore of Lake Kivu close to the border of Democratic Republic of the Congo, Rwanda], coll. Johann Gustav Hermann Schubotz, X/1907.

**Present name.** *Hyperolius viridiflavus* (Duméril & Bibron, 1841).

**Remarks.** Depicted in Ahl (1931b: 329, fig. 202). The holotype was collected during the first "Deutsche Zentral–Afrika–Expedition", 1907–1908.

***Megalixalus laevis* Ahl, 1930b: 93.**

**Holotype.** ZMB unknown, "Kamerun" [Cameroon], coll. Leopold Fritz Wilhelm Edmund Conradt, 8.V.1896.

**Present name.** *Afrixalus laevis* (Ahl, 1930b).

**Remarks.** The German colonial officer and planter Conradt was stationed at "Lolodorf" [Océan Division, South Province, Cameroon] and worked as station manager at "Johann Albrechtshöhe" [southeast of Lake Barombi Mbo, near Kumba, Southwest Region, Cameroon] from 1895 to 1899 (Schnee 1920a). In February 1897, a shipment containing amphibians and reptiles collected by him at "Albrechtshöhe, Kamerun" arrived at ZMB. See also remarks on *Hyperolius laticeps* for Conradt's activities in Togo.

## Specimens erroneously marked as types in ZMB inventory catalogues

***Hyla horstockii* Schlegel, 1837: 24, footnote 1.**

ZMB 3064 (originally 3 specimens), "Cap" [Cape Province, South Africa], coll. Georg Ludwig Engelhard Krebs.

**Present name.** *Hyperolius horstocki* (Schlegel, 1837).

**Remarks.** The original catalogue entry of ZMB 3064 made by Martin Hinrich Carl Lichtenstein around 1858 is marked as type of "*Hyla Horstockii* Schlegel*" from "Cap", collected by "Krebs". The original entry was later crossed out and changed to "[*Hyperolius*] *modestus*, Boutry, Goldküste, [coll.] Pel, [don.] Schlegel" by the same. With this "correction" Lichtenstein probably was referring to *Eucnemis modestus* Lichtenstein & Martens, 1856 (p. 36, *nomen nudum*). Likely following the new generic allocation proposed by Tschudi (1838: 35), Lichtenstein and Martens (1856: 36) listed a specimen under the name "*Eucnemis Horstockii* Schleg." (= *Hyperolius horstockii*) with the locality given as "Cap" but without mentioning collector or donor. We reviewed the documents archived in the Historical Research department of the ZMB concerning the correspondence and exchange files of Lichtenstein with Heinrich Boie, Hermann Schlegel and Coenraad Jacob Temminck from the Rijksmuseum van Natuurlijke Historie in Leiden. We could not find any evidence that specimens of the original type series of *Hyla horstockii* were given to the ZMB. Most probably the specimens under ZMB 3064 (as originally indicated) were collected by the German apothecary and collector of natural history objects Krebs who regularly sent specimens to the ZMB collection during the first half of the 19th century (Bauer 2000, 2004) and then were erroneously marked as types.

**"Hyperolius callodermatus" attributed to "Ahl" according to ZMB inventory catalogue.**

ZMB 36096, "Ukami" [Udzungwa Mountains, Tanzania], coll. Georg Martienssen.

**Status.** Unpublished name.

**Present determination.** A *Hyperolius* which, due to the usual preservation state of these frogs – showing few diagnostic characters – cannot be assigned with certainty to any species.

**"Hyperolius cinctopunctatus" attributed to "Ahl" according to ZMB inventory catalogue.**

ZMB 31663, "Kibwezi" [Makueni County, Kenya], coll. Georg Richard Otto Scheffler.

**Status.** Unpublished name.

**Present determination.** *Hyperolius viridiflavus ferniquei* fide Schiøtz (1975).

**"Hyperolius janenschi" attributed to "Ahl" according to ZMB inventory catalogue.**

ZMB 36118 and 36119 from Rugwe [Rungwe village, Mbeya Region, Tanzania], and ZMB 36120 and 77033 from "S'ongwe" [Songwe, at the border to Malawi on the northwestern tip of Lake Malawi, Kyela District, South Mbeya Region, Tanzania], all coll. Friedrich Georg Hans Heinrich Fülleborn.

**Status.** Unpublished name.

**Present determination.** Most likely *Hyperolius substriatus* Ahl, 1931a.

**"Hyperolius nairobiensis" attributed to "Ahl" according to ZMB inventory catalogue.**

ZMB 36107 from "Nairobi" [Kenya], coll. Felice Thomas.

**Status.** Unpublished name.

**Present determination.** *Hyperolius viridiflavus ferniquei* fide Schiøtz (1975).

**"Rappia femoralis"** attributed to "Matschie" according to ZMB inventory catalogue.

ZMB 11088, "Borombi" [Colonial station, from 1895 under the name "Johann-Albrechtshöhe", southeast of Lake Barombi Mbo, near Kumba, Southwest Region, Cameroon], coll. Captain Karl Ludwig Zeuner.

**Status.** Unpublished name.

**Present determination.** unknown, specimen not located.

**"Rappia ocularis"** attributed to "Matschie" according to ZMB inventory catalogue.

ZMB 11131, "Kribi" [Océan Department, South Province, Cameroon], coll. Major Curt Ernst Morgen.

**Status.** Unpublished name.

**Present determination.** unknown, specimen not located.

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PENSOFT

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# Lost, forgotten, and overlooked: systematic reassessment of two lesser-known toad species (Anura, Bufonidae) from Peninsular India and another wide-ranging northern species

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## Abstract

We rediscovered two species of toads, *Bufo stomaticus peninsularis* and *Bufo brevirostris*, which were described from Peninsular India 84 and 101 years ago, respectively, but have not been reported since. Because the name-bearing types of both species are either damaged or lost, we provide detailed redescriptions, morphological comparisons, and insights into phylogenetic relationships with closely related members of the genus *Duttaphrynus* sensu lato, based on new material from the type locality of each species. We clarify and validate the identity of *D. brevirostris*, which was rediscovered from multiple localities in the Malenadu and adjoining coastal regions of Karnataka. We also demonstrate that *Bufo stomaticus peninsularis*, which was considered a synonym of *Duttaphrynus scaber*, is a distinct species. *Bufo stomaticus peninsularis* differs from *Duttaphrynus scaber* morphologically and genetically, and is more closely related to members of the *Duttaphrynus stomaticus* group. We also clarify the identity of the namesake species of the *Duttaphrynus stomaticus* group, which is reported widely in India and neighbouring countries, but lacks sufficient taxonomic information due to its brief original description and reportedly untraceable type material. We located and studied the complete syntype series of *D. stomaticus*, probably for the first time in over a century, and we report on the status of available specimens, provide detailed description of a potential type, compare it to related species, and clarify the species' geographical range. Our molecular analyses suggest that *D. stomaticus* is minimally divergent from, and possibly conspecific with, *D. olivaceus*. Our analyses also clarify its relationship to the closely-related *D. peninsularis* **comb. nov.**, with which it was previously confused. Finally, our study provides other insights into the phylogenetic relationships and genetic differentiation among various species of *Duttaphrynus* toads.

## Key Words

Amphibia, *Bufo stomaticus peninsularis*, distribution, *Duttaphrynus brevirostris*, *Duttaphrynus stomaticus* group, *Firouzophrynus*, molecular phylogeny, redescription, rediscovery, taxonomy

## Introduction

The genus *Duttaphrynus* sensu lato, comprising 26 recognised Asian species, is a widely-distributed and commonly-occurring group of toads, found at elevations from sea level up to 2500 m asl (Frost et al. 2006; Van Bocxlaer et al. 2009; Portik and Papenfuss 2015). The genus is represented by 19 species in India, 16 of which were described with type localities designated in the country. Among the Indian *Duttaphrynus* species, nine occur in Peninsular India and of these, six are endemic to the region. Although the wide-ranging species (*D. melanostictus*, *D. stomaticus*, *D. hololius*, and *D. scaber*) are frequently studied and reported from Peninsular India (Sarkar et al. 1993; Dutta 1997; Biju 2001; Chanda 2002; Van Bocxlaer et al. 2009; Dinesh et al. 2009; Srinivasulu et al. 2013; Ganesh et al. 2020), the taxonomic status of the endemic species has not been thoroughly investigated

subsequent to their original descriptions (Dubois and Ohler 1999; Biju 2001). These include five recognised species—*D. beddomii* (Günther, 1876), *D. brevirostris* (Rao, 1937), *D. microtympanum* (Boulenger, 1882), *D. parietalis* (Boulenger, 1882), and *D. silentvalleyensis* (Pillai, 1981). Their identities remain somewhat doubtful, due to reasons such as either brief or cursory original descriptions, unavailability of type specimens, or absence of new topotypic collections (Dubois and Ohler 1999; Biju 2001). In addition, identification of *Duttaphrynus* species is challenging, due to their overall phenotypic similarities and substantial intraspecific morphological variability (Inger 1972; Dubois and Ohler 1999; Biju 2001; Van Bocxlaer et al. 2010; Wogan et al. 2016; Jayawardena et al. 2017). Another four available names from Peninsular Indian regions exist as junior subjective synonyms (Dubois and Ohler 1999). Given such complex nomenclatural histories, misidentifications of *Duttaphrynus* species in museum specimens (S.D.B., personal observation) and regional biodiversity reports (Ray and Deuti 2008; Gururaja 2012; Hegde 2012; Seshadri et al. 2012; Ganesh et al. 2020) are frequent.

Two *Duttaphrynus* toads were described by C. R. Narayan Rao (15 August 1882–2 January 1960), who was among the most notable amphibian taxonomist in southern India during the colonial and post-colonial periods of the twentieth century. He described a total of 27 new species of frogs, including subspecies and varieties, largely from the states of Karnataka and Tamil Nadu (Rao 1920, 1922, 1937). However, a large number of his types (19 species; deposited in the Central College, Bangalore) are lost (Dubois 1984; Biju 2001). Seventeen of Rao's species currently are recognised as valid; nine of these have had their name-bearing type status stabilised through designation of neotype specimens (e.g., Bossuyt and Dubois 2001; Biju et al. 2011, 2014a, 2014b; Garg et al. 2018). Similarly, the fate of Rao's bufonid species has remained precarious: (1) *Bufo brevirostris* Rao, 1937 was described based on a single specimen from "Kempholey, Hassan District, Mysore State," which subsequently was reported to be lost (Dubois 1984; Biju 2001). Hence, this species is known only from its original description. Dubois and Ohler (1999) discussed the problematic taxonomic status of this taxon, and, later Van Bocxlaer et al. (2009) transferred it to *Duttaphrynus* based on DNA sequences from a single specimen, without further information or discussion. The species continues to be recognised in the literature, albeit in the absence of new reliable records, photographs, or voucher specimens (Dutta 1997, Chanda 2002; Dinesh et al. 2009; Subramanian et al. 2013; Jayawardena et al. 2017). Additionally, (2) *Bufo stomaticus peninsularis* Rao, 1920 was described as a new variety of "*Bufo stomaticus*" from "Mavkote and Watekolle, Coorg," based on a specimen (ZSIC 19176) designated as the holotype by Chanda et al. (2001 "2000"). This taxon was considered a synonym of *Duttaphrynus stomaticus* (Daniel 1963; Daniels 2005), until Srinivasulu et al.'s (2013) correction of some photograph-based misidentifications of "*D. scaber*" (not *Duttaphrynus stomaticus peninsularis*) as "*D. stomaticus*," which was implicitly considered as the transfer of *Bufo stomaticus peninsularis* into the synonymy of *Duttaphrynus scaber* (Schneider, 1799) by Frost (2021). However, most recently Ganesh et al. (2020) made a cursory statement referring to the identity of this taxon as "status: incertae sedis" without any clarification.

The confusing taxonomic status of Rao's variety *Bufo stomaticus peninsularis* is also undeniably linked to its originally assigned species—*Duttaphrynus stomaticus* (Lütken, 1864). Although Srinivasulu et al. (2013) reported on misidentifications of *D. stomaticus* from Peninsular India, no studies to date have provided direct and conclusive evidence for either resolving the identity of *Bufo stomaticus peninsularis* or clarifying the occurrence of *Duttaphrynus stomaticus* in Peninsular India. The latter is considered as a widely distributed species in south and southwest Asia, with its range encompassing nearly the whole of India and the neighbouring Bangladesh, Nepal, Pakistan, Afghanistan, and Iran (Stöck et al. 2006; Rastegar et al. 2008; Van Bocxlaer et al. 2009; Shaikh et al. 2014; Portik and Papenfuss 2015; Nepali and Singh 2018; Frost 2021) (Suppl. materal 1: Table S1). However, *Duttaphrynus stomaticus* was originally described from "ostindiske" (= East Indies or East India) (Lütken 1864), where its type locality was subsequently restricted to "Assam" (Boulenger 1891). Since type specimens were reported as untraceable (Dutta 1997), the identification of this species in recent literature is apparently based only on its brief original description, rather than examination of name-bearing types, or detailed redescription of topotypic material.

The present study was undertaken to conclusively resolve the taxonomic identity and stabilise the nomenclatural status of the two lesser-known *Duttaphrynus* toads from Peninsular India (*Bufo brevirostris* Rao, 1937 and *Bufo stomaticus peninsularis* Rao, 1920) and another wide-ranging northern species (*Bufo stomaticus* Lütken, 1864). We do so based on morphological comparison with original descriptions and available type specimens (except for *D. brevirostris*), as well as molecular and morphological insights gathered from new topotypic material, arguably rediscovered for the first time since both species' original descriptions. We also aimed to infer phylogenetic relationships of the focal species, as well as gather insights on patterns of genetic differentiation among all known members of the genus *Duttaphrynus* that are characterised by known localities, and represented by accompanying vouchered molecular data.

## Materials and methods

### Field study

Surveys were carried out for sampling the target species from regions encompassing their type localities in the Indian states of Karnataka, Andhra Pradesh, Tamil Nadu, and Assam. Additionally, some populations of

'*Duttaphrynus stomaticus*' were randomly sampled from regions across India to understand intra and interspecific relationships. A total of 15 newly sampled populations are included in the study (Suppl. materal 1: Tables S2 and S3). Surveys and sampling were conducted both during day and night hours, mostly during the pre-monsoon and monsoon months (April–August), but occasionally also at other times of the year (March and October). The sampled individuals were photographed to document colouration and characters in life, followed by euthanisation using Tricaine methanesulphonate (MS-222). Tissue samples were taken from the thigh muscle or liver, preserved in absolute ethanol, and stored at -20 °C for molecular studies. Locality information was recorded using a GPS with the WGS84 datum system. Distribution maps were prepared in QGIS version 2.6.1 (http://www.qgis.org).

## Morphological study

Sex and maturity were determined by examining the gonads through a small lateral or ventral incision, or by the presence of secondary sexual characters (such as nuptial pads and vocal sacs in males). The following measurements were taken to the nearest 0.1 mm with digital slide-calipers: SVL (snout-vent length), HW (head width, at the angle of the jaws), HL (head length, from rear of mandible to tip of snout), SL (snout length, from tip of snout to anterior orbital border), EL (eye length, horizontal distance between bony orbital borders), IFE (internal front of the eye, shortest distance between the anterior orbital borders), IBE (internal back of the eyes, shortest distance between the posterior orbital borders), IUE (inter upper eyelid width, the shortest distance between the upper eyelids), UEW (maximum upper eyelid width), IN (internarial distance), NS (distance from the nostril to the tip of the snout), EN (distance from the front of the eye to the nostril), PD (minimum distance between parotoids), PL (maximum parotoid length), PW (maximum parotoid width), TYD (greatest tympanum diameter), TYE (distance from the tympanum to the back of the eye), FAL (forearm length, from flexed elbow to base of outer palmar tubercle), HAL (hand length, from base of outer palmar tubercle to tip of third finger), TL (thigh length, from the vent to the knee), SHL (shank length, from knee to heel), FOL (foot length, from base of inner metatarsal tubercle to tip of fourth toe), TFOL (total foot length, from heel to tip of fourth toe), ITL (inner toe length), OMTL (length of outer metatarsal tubercle), and IMTL (length of inner metatarsal tubercle). Digit number is represented by roman numerals I–V in subscript. All measurements provided in the taxonomy section are in millimetres (mm). Measurements and associated terminology follow Dubois and Ohler (1999) and Biju and Bossuyt (2009). The webbing formulae follow Savage and Heyer (1967) as modified by Myers and Duellman (1982). The amount of webbing relative to subarticular tubercles is described by numbering the tubercles 1–3, starting from the base. For the convenience of discussion, webbing is additionally defined as basal, small, medium, or large, following Garg and Biju (2017).

To ascertain the degree of morphometric differentiation among the three Indian members of the *Duttaphrynus stomaticus* group, a multivariate analysis was performed using 21 morphometric characters from male specimens. The data for each character was expressed as the ratio of the respective SVL so as to reduce the impact of allometry, and subjected to Principal Component Analysis (PCA), a dimensionality reduction technique. Furthermore, Box and Whiskers plots were created for a univariate analysis of SVL and five morphometric characters that yielded the most significant contribution to the PCA, in order to visualise differences among the species. The analyses were performed in R (R Development Core Team 2008) using the package MASS and the plots were made using the ggplot2 and ggfortify packages.

## Molecular study

Genomic DNA was extracted from the new samples using Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocols. A short fragment of the mitochondrial 16S rRNA (~540 bp) was PCR-amplified using previously published primer sets 16Sar and 16Sbr (Simon et al. 1994). Purified PCR products were sequenced with the same primers using BigDye Terminator v3.1 Cycle Sequencing Kit on ABI 3730 automated DNA sequencer (Applied Biosystems). Raw sequences were checked and assembled in ChromasPro v1.34 (Technelysium Pty Ltd.) and deposited in the NCBI GenBank under accession numbers MZ816170–MZ816184.

We reconstructed phylogenetic relationships among major distinct evolutionary lineages representing known or putative *Duttaphrynus* species (Van Bocxlaer et al. 2009; Portik and Papenfuss 2015). DNA sequences for nine mitochondrial gene regions (12S ribosomal RNA, $tRNA^{Val}$, 16S ribosomal RNA, $tRNA^{Leu}$, NADH dehydrogenase subunit 1, $tRNA^{Ile}$, $tRNA^{Gln}$, $tRNA^{Met}$, and NADH dehydrogenase subunit 2) and two nuclear genes (NCX1 and CXCR4) from previously published studies (Biju and Bossuyt 2003; Van Bocxlaer et al. 2009; Portik and Papenfuss 2015; Liedtke et al. 2016) were retrieved from the GenBank and assembled along with selected new sequence data (Suppl. materal 1: Table S2). Sequences were aligned using ClustalW in MEGA 6.0 (Tamura et al. 2013). Alignments for coding DNA were checked by comparison with amino acid sequences, whereas the alignment for non-coding sequences was visually optimised and the ambiguously aligned regions were subsequently excluded from phylogenetic analyses. A character set of total 5,737 bp assembled for 18 taxa was used for the Maximum Likelihood (ML) and Bayesian Inference (BI). Appropriate models of sequence evolution were determined for each gene by implementing

Akaike Information Criteria in ModelTest 3.4 (Posada and Crandall 1998). Maximum Likelihood (ML) searches were performed on a partitioned dataset using the GTRGAMMA model with 2,000 independent runs executed alongside 10,000 rapid bootstrap replicates in RAxML 7.3.0 (Stamatakis et al. 2008) as implemented in raxmlGUI 1.1 (Silvestro and Michalak 2012). Bayesian analyses were performed using the best-fit General Time Reversible (GTR) model with a proportion of invariant sites (+I) and gamma-distributed rate variation among sites (+G) independently for each gene partition, with all parameters estimated. Bayesian searches were executed in MrBayes (Ronquist and Huelsenbeck 2003) with two parallel runs of four Metropolis-Coupled Markov Chain Monte Carlo (MCMCMC) chains executed for 10 million generations using uniform priors and sampling frequency of trees after every 1,000 generations. Convergence of the parallel runs was determined by split frequency standard deviations of less than 0.01 and ~1.0 potential scale reduction factors for all model parameters. Bayesian posterior probabilities (BPP) for the clades were summarised after discarding the first 2,500 trees (25 percent) as burn-in from each run (Huelsenbeck et al. 2001).

We further assessed relationships using available homologous mitochondrial 16S rRNA sequences from GenBank and our new samples (Suppl. materal 1: Table S3). Sequences were aligned using ClustalW in MEGA 6.0 (Tamura et al. 2013) and the alignment was manually checked for the presence of any ambiguous or doubtful sites. Certain short GenBank sequences and sequences or positions that showed low confidence for homology were excluded from phylogenetic analyses. A character set of 524 bp from 137 taxa, including an outgroup, was subjected to ML and BI analyses. The ML search was executed in RAxML based on 500 independent runs using the GTRGAMMA model and clade support was assessed through 1,000 rapid bootstrap replicates. The Bayesian analysis was performed with two parallel runs of four MCMCMC chains executed for 10 million generations using the GTR+I+G model, with a sampling frequency of 1,000 and 25 percent burn-in. The resultant ~15,000 trees were summarised to determine clade support (BPP). The details of the analyses were as described above for the multi-gene dataset. Additionally, the ML phylogram was used as input for performing species delimitation analyses by Bayesian implementation of the Poisson Tree Processor (PTP) method (Zhang et al. 2013) on the bPTP webserver (https://species.h-its.org). Intra- and interspecific uncorrected pairwise genetic distances for the 16S rRNA were computed in PAUP* (Swofford 2002). A Median-Joining (MJ) network was further constructed using the software Network 4.6.1.0 (www.fluxus-engineering.com) to evaluate relationships and possible mutation steps among 42 haplotypes recovered from 133 sequences of the 16S rRNA after performing the PHASE algorithm (Stephens et al. 2001) in DnaSP version 5 (Librado and Rozas 2009).

## Abbreviations

Museum acronyms and other abbreviations used herein are as follows: **BNHS** (Bombay Natural History Society, Mumbai); **CCB** (Central College, Bangalore); **CSPT** (Chennai Snake Park Trust, Chennai); **ICZN** (The International Code of Zoological Nomenclature); **SDBDU** (Systematics Lab, University of Delhi, India); **ZMUC** (Universitets København, Zoologisk Museum, Denmark); **ZSIC** (Zoological Survey of India, Kolkata, India).

# Results and discussion

## Taxonomic accounts

### *Duttaphrynus brevirostris* (Rao, 1937)

Figs 1–4; Table 1; Suppl. materal 1: Tables S1–S4

Kempholey Toad

**Original name and description.** *Bufo brevirostris* Rao, 1937. Rao, C. R. N. 1937. On some new forms of Batrachia from S. India. Proceedings of the Indian Academy of Sciences. Section B 6: 387–427. **Type locality.** "Kempholey, Hassan District, Mysore State," Karnataka, India. **Current status of specific name.** Valid name, as *Duttaphrynus brevirostris* (Rao, 1937).

**Material studied.** ***Topotype*.** An adult male, BNHS 6126 (SVL 45 mm), from Kempholey Ghat region in Sakleshpur taluk, Hassan district, Karnataka State, India, collected by S. D. Biju and Sonali Garg in June 2013. ***Other referred specimens*.** An adult male, SDBDU 2008.410 (SVL 48.6 mm), from Bhagamandala, Kodagu district, Karnataka State; an adult male, SDBDU 2015.3075 (SVL 46 mm), from Manipal, Udupi district, Karnataka State; and a subadult, SDBDU 4714 (SVL 25 mm), from Someshwara, Udupi district, Karnataka State.

**Rediscovery and validation of taxonomic status.** This species was described based on a single specimen ("snout to vent, 27.00 mm") deposited in the Central College, Bangalore (CCB). This original name-bearing type specimen is considered lost (Dubois 1984; Biju 2001) and the species currently is known only from its original description. Rao (1937) enumerated several morphological character states to describe this taxon, but did not provide comparisons with other species. Our collection from a region of Kempholey Ghat in Sakleshpur taluk, that is part of the type locality (Rao 1937), is comparable with the original description with respect to several mentioned characters such as "canthus rostralis angular," "nostril nearer to the end of the snout than to the eye," "first finger equal to the second," "parotoids elongate, moderately prominent," and "upper surface of the skin covered with small uniformly distributed tubercles; with a small row of larger warts on the median line of the back." The primary inconsistencies between Rao's described specimen and our new collection involve snout-vent length, SVL 45 mm (vs. "27.00 mm") and weakly developed or


**Figure 1.** Morphological characters for topotype of *Duttaphrynus brevirostris* (Rao, 1937), topotype of *D. peninsularis* (Rao, 1920), and syntype of *D. stomaticus* (Lütken, 1864) in preservation. **A–G.** *Duttaphrynus brevirostris*, BNHS 6126: **A.** Dorsal view; **B.** Ventral view; **C.** Lateral view of head; **D.** Dorsal view of hand showing brown nuptial pad on fingers I, II, and III; **E.** Ventral view of hand; **F.** Ventral view of foot; **G.** Schematic illustration of webbing on foot. **H–N.** *Duttaphrynus peninsularis*: **H.** Holotype, ZSIC 19176; **I–N.** Topotype, SDBDU 6370: **I.** Dorsal view; **J.** Ventral view; **K.** Lateral view of head; **L.** Ventral view of hand; **M.** Ventral view of foot; **N.** Schematic illustration of webbing on foot. **O–T.** *Duttaphrynus stomaticus*, ZMUC 131137 (ex 196): **O.** Dorsal view; **P.** Ventral view; **Q.** Lateral view of head; **R.** Ventral view of hand; **S.** Ventral view of foot; **T.** Schematic illustration of webbing on foot.

inconspicuous cephalic ridges (vs. "crown without bony ridge"). The cephalic ridges in our new collection are relatively smooth, depressed, or less conspicuous (Figs 1A, C, 2A) when compared to other species of the *Duttaphrynus melanostictus* group from Peninsular India. Hence, presence or absence of this character may be considered a matter of interpretation depending on degree of its prominence. Furthermore, the body size disparity between our collection and that of Rao (1937) also suggests that the type specimen he described could have been a subadult. We examined another subadult specimen from Someshwar (SDBDU 4714; SVL 25 mm), previously

**Table 1.** Morphometric measurements for specimens included in the study. Measurement abbreviations and museum acronyms are provided in the Material and methods section. ST = Syntype; TT = Topotype; RS = Referred specimen. All measurements are in millimeters (mm).

| *Duttaphrynus brevirostris* (all males, from Karnataka) | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Voucher No | Status | SVL | HW | HL | TYD | SL | EL | TYE | MN | EN | NS | IUE | UEW | IN | FAL | HAL | TL | SHL | FOL | TFOL | IMTL | OMTL |
| BNHS 6126 | TT | 45.0 | 16.9 | 14.0 | 2.6 | 6.1 | 5.9 | 0.7 | 12.0 | 3.2 | 1.7 | 5.1 | 4.1 | 3.0 | 10.8 | 11.3 | 17.8 | 18.8 | 18.5 | 28.1 | 1.6 | 3.1 |
| SDBDU 2008.410 | RS | 48.4 | 16.9 | 15.4 | 2.9 | 6.6 | 5.3 | 0.9 | 13.3 | 3.3 | 2.1 | 5.1 | 4.4 | 3.4 | 11.8 | 13.8 | 19.1 | 18.8 | 20 | 30.5 | 1.3 | 2.9 |
| SDBDU 2015.3075 | RS | 45.7 | 16.2 | 13.8 | 2.9 | 5.9 | 4.7 | 0.6 | 11.7 | 2.9 | 2.0 | 4.9 | 4.1 | 3.1 | 10 | 11.1 | 17.8 | 18.9 | 19.2 | 28.2 | 1.5 | 2.9 |
| | Mean | 46.4 | 16.7 | 14.4 | 2.8 | 6.2 | 5.3 | 0.7 | 12.3 | 3.1 | 1.9 | 5.0 | 4.2 | 3.2 | 10.9 | 12.1 | 18.2 | 18.8 | 19.2 | 28.9 | 1.5 | 3.0 |
| | SD | 1.8 | 0.4 | 0.9 | 0.2 | 0.4 | 0.6 | 0.2 | 0.9 | 0.2 | 0.2 | 0.1 | 0.2 | 0.2 | 0.9 | 1.5 | 0.8 | 0.1 | 0.8 | 1.4 | 0.2 | 0.1 |
| *Duttaphrynus peninsularis* (all males, from Karnataka and Tamil Nadu) | | | | | | | | | | | | | | | | | | | | | | |
| Voucher No | Status | SVL | HW | HL | TYD | SL | EL | TYE | MN | EN | NS | IUE | UEW | IN | FAL | HAL | TL | SHL | FOL | TFOL | IMTL | OMTL |
| SDBDU 2006.6370 | TT | 50.8 | 18.0 | 14.0 | 3.1 | 5.8 | 4.9 | 1.0 | 12 | 3.4 | 1.7 | 6.2 | 4.5 | 3.7 | 11.5 | 10.9 | 19.9 | 17.8 | 18.4 | 28.3 | 1.6 | 1.8 |
| SDBDU 2006.4018 | TT | 52.0 | 17.6 | 14.0 | 2.8 | 5.9 | 4.7 | 0.9 | 12.4 | 3.5 | 2.2 | 5.4 | 4.3 | 3.6 | 10.9 | 9.9 | 20.5 | 19.4 | 18.3 | 27.4 | 1.5 | 7.5 |
| SDBDU 2006.4019 | TT | 45.2 | 15.5 | 10.4 | 2.3 | 5.2 | 3.9 | 1.0 | 9.8 | 2.9 | 1.8 | 5.0 | 3.6 | 3.0 | 10.6 | 10.0 | 18.1 | 16.8 | 17.2 | 26.1 | 1.4 | 1.8 |
| SDBDU 2006.4020 | TT | 47.7 | 16.4 | 11.8 | 2.5 | 5.6 | 4.1 | 1.0 | 10.5 | 3.3 | 1.6 | 5.3 | 3.8 | 3.2 | 10.2 | 9.2 | 18.3 | 17.1 | 17.1 | 26.1 | 1.6 | 1.3 |
| SDBDU 2006.4021 | TT | 47.4 | 15.6 | 13.2 | 2.6 | 5.5 | 4.1 | 1.0 | 10.8 | 3.1 | 1.5 | 5.9 | 3.7 | 3.5 | 10.6 | 9.6 | 18.2 | 15.7 | 14.1 | 21.8 | 1.6 | 1.4 |
| | Mean | 48.6 | 16.6 | 12.7 | 2.7 | 5.6 | 4.3 | 1.0 | 11.1 | 3.2 | 1.8 | 5.6 | 4.0 | 3.4 | 10.8 | 9.9 | 19.0 | 17.4 | 17.0 | 25.9 | 1.5 | 2.8 |
| | SD | 2.7 | 1.1 | 1.6 | 0.3 | 0.3 | 0.4 | 0.0 | 1.1 | 0.2 | 0.3 | 0.5 | 0.4 | 0.3 | 0.5 | 0.6 | 1.1 | 1.4 | 1.7 | 2.5 | 0.1 | 2.7 |
| *Duttaphrynus stomaticus* (all males, from Assam) | | | | | | | | | | | | | | | | | | | | | | |
| Voucher No | Status | SVL | HW | HL | TYD | SL | EL | TYE | MN | EN | NS | IUE | UEW | IN | FAL | HAL | TL | SHL | FOL | TFOL | IMTL | OMTL |
| ZMUC 131136 | ST | 55.0 | 18.9 | 15.3 | 3.1 | 6.3 | 6.3 | 1.7 | 13.7 | 3.5 | 1.8 | 5.7 | 3.6 | 3.5 | 12.2 | 12.2 | 20.5 | 20.3 | 19.4 | 30.5 | 3.7 | 2.3 |
| ZMUC (untagged) | ST | 59.2 | 20.4 | 16.2 | 3.6 | 6.4 | 6.3 | 1.6 | 13.8 | 4.7 | 2.1 | 6.7 | 4.6 | 4.5 | 12.3 | 12.5 | 20 | 20.3 | 22.5 | 31.6 | 3.3 | 2.1 |
| SDBDU 2018.4109 | RS | 57.6 | 19.7 | 16.9 | 4.2 | 6.5 | 5.4 | 1.5 | 14.4 | 3.4 | 1.8 | 6.2 | 4.1 | 3.9 | 12.2 | 12.4 | 20.8 | 22.5 | 21.4 | 32.9 | 2.1 | 2.9 |
| SDBDU 2018.4110 | RS | 69.2 | 23.7 | 18.5 | 4.5 | 6.2 | 6.2 | 1.6 | 14.9 | 5.0 | 2.1 | 6.6 | 5.6 | 4.5 | 13.7 | 13.9 | 27.8 | 27.6 | 24.5 | 36.0 | 3.4 | 2.3 |
| SDBDU 2018.4111 | RS | 55.1 | 18.5 | 151 | 3.5 | 6.7 | 6.6 | 1.3 | 13.2 | 3.8 | 1.7 | 5.5 | 2.9 | 2.9 | 11.6 | 11.8 | 20.2 | 20.2 | 20.6 | 31.1 | 3.0 | 2.4 |
| | Mean | 59.2 | 20.2 | 43.6 | 3.8 | 6.4 | 6.2 | 1.5 | 14.0 | 4.1 | 1.9 | 6.1 | 4.2 | 3.9 | 12.4 | 12.6 | 21.9 | 22.2 | 21.7 | 32.4 | 3.1 | 2.4 |
| | SD | 5.2 | 1.8 | 53.7 | 0.5 | 0.2 | 0.4 | 0.1 | 0.6 | 0.6 | 0.2 | 0.5 | 0.9 | 0.6 | 0.7 | 0.7 | 3.0 | 2.8 | 1.7 | 2.0 | 0.5 | 0.3 |
| *Duttaphrynus hololius* (all males, from Tamil Nadu) | | | | | | | | | | | | | | | | | | | | | | |
| Voucher No | Status | SVL | HW | HL | TYD | SL | EL | TYE | MN | EN | NS | IUE | UEW | IN | FAL | HAL | TL | SHL | FOL | TFOL | IMTL | OMTL |
| SDBDU 2006.4242 | RS | 43.0 | 15.6 | 13.9 | 3.5 | 5.5 | 3.5 | 0.7 | 10.2 | 3.5 | 1.8 | 5.6 | 3.2 | 3.7 | 9.8 | 10.1 | 17.6 | 17.4 | 16.3 | 24.3 | 1.5 | 1.5 |
| SDBDU 2006.4243 | RS | 45.2 | 17.1 | 14.5 | 3.8 | 5.4 | 3.7 | 0.8 | 10.1 | 3.6 | 1.7 | 5.4 | 3.1 | 3.7 | 9.9 | 10.2 | 17.5 | 18.8 | 16.8 | 26.9 | 1.5 | 1.4 |
| SDBDU 2015.2892 | RS | 46.2 | 17.2 | 13.3 | 3.5 | 5.9 | 3.4 | 0.9 | 10.3 | 3.5 | 1.9 | 5.7 | 3.4 | 3.8 | 9.3 | 9.8 | 17.7 | 17.2 | 16.1 | 22.3 | 1.7 | 1.5 |
| SDBDU 2015.2894 | RS | 42.7 | 16.4 | 13.1 | 3.5 | 5.4 | 3.6 | 0.8 | 10.9 | 3.3 | 1.6 | 5.3 | 3.2 | 3.2 | 9.2 | 9.9 | 18.7 | 17.5 | 16.1 | 23.5 | 1.4 | 1.4 |
| SDBDU 2015.2895 | RS | 47.0 | 17.5 | 13.2 | 3.4 | 5.9 | 3.5 | 1.9 | 10.2 | 3.2 | 1.8 | 5.5 | 3.5 | 3.8 | 9.9 | 11.2 | 18.9 | 17.8 | 18.6 | 26.5 | 1.7 | 1.4 |
| | Mean | 44.8 | 16.8 | 13.6 | 3.5 | 5.6 | 3.5 | 1.0 | 10.3 | 3.4 | 1.8 | 5.5 | 3.3 | 3.6 | 9.6 | 10.2 | 18.1 | 17.7 | 16.8 | 24.7 | 1.6 | 1.4 |
| | SD | 1.9 | 0.8 | 0.6 | 0.2 | 0.3 | 0.1 | 0.5 | 0.3 | 0.2 | 0.1 | 0.2 | 0.2 | 0.3 | 0.3 | 0.6 | 0.7 | 0.6 | 1.1 | 2.0 | 0.1 | 0.1 |


**Figure 2.** Topotype of *Duttaphrynus brevirostris* (Rao, 1937), topotype of *D. peninsularis* (Rao, 1920), and referred specimens of *D. stomaticus* (Lütken, 1864) in life. **A.** *Duttaphrynus brevirostris* (BNHS 6126) from Kempholey Ghat region in Sakleshpur taluk. **B.** *Duttaphrynus peninsularis* (SDBDU 6370) from Wattakolli. **C–F.** *Duttaphrynus stomaticus*: **C.** SDBDU 2015.2909 from Assam; **D.** SDBDU 2012.2170 from Rajasthan; **E.** SDBDU 2012.2172 from Delhi; and **F.** SDBDU 2012.2268 from Bihar.

reported along with DNA sequence data (Van Bocxlaer et al. 2009), and found some comparable characters such as "a small row of larger warts on the median line of the back," "a network of dark lines," and "a dark temporal line extending to the sides," which can usually also be observed in subadults of *Duttaphrynus melanostictus* group species (S.D.B., personal observations). The Someshwar specimen is genetically identical to our Sakleshpur collection. Together, these two populations are also morphologically and genetically similar to our additional collections from other localities within the Malenadu (Malnad) and adjoining coastal regions of Karnataka (see 'Material studied'). Altogether, we consider the available morphological and molecular evidence reliable for assigning all the mentioned populations to *D. brevirostris* (Rao, 1937).

Since the absence of a name-bearing type has contributed towards poor knowledge and uncertainty regarding the taxonomic identity of this taxon, as evident from the absence of new records, below we provide a detailed description of a newly-collected voucher specimen from the original type locality (Kempholey Ghat region in Sakleshpur taluk, Hassan district, Karnataka State, India: BNHS 6126), which is largely consistent with what is known of the former name-bearing type (Rao 1937). The topotype description provided below, augmented by a range of variation observed in vouchered specimens and genetic data from additional localities (Table 1; Suppl. materal 1: Tables S3, S4), validate the identity of *D. brevirostris* and also serve as a redescription of this poorly known species for the benefit of future taxonomic work.

**Description of topotype, BNHS 6126** (measurements in mm). A medium-sized, robust adult male (SVL 45.0); head of moderate size, wider (HW 16.9) than long (HL 14.0); snout subovoid in dorsal and ventral view, not pro-

jecting, its length (SL 6.1) longer than horizontal diameter of eye (EL 5.9); loreal region obtuse with sharp canthus rostralis; distance between posterior borders of the eyes (IBE 13.9) 2.2 times the distance between the anterior borders (IFE 6.3); interorbital space 1.2 times wider (IUE 5.1) than upper eyelid width (UEW 4.1); nostril oval without lateral flap of skin, closer to tip of snout (NS 1.7) than to eye (EN 3.2); tympanum distinct (TYD 2.6), vertically oval, 44.1% of eye diameter (EL 5.9), tympanum to eye distance (TYE 0.7); pineal ocellus absent; vomerine ridge and teeth absent; tongue small, oval, entire, median lingual projection absent; parotoid glands present, oval, flat, without spines and warts, longer (PL 6.2) than wide (PW 3.4), shorter than distance between them (PD 8.7); supraorbital and postorbital ridges weakly developed.

Forelimbs short; forearm length (FAL 10.8) shorter than hand length (HAL 11.3); fingers rather thin, $FL_I$ nearly equal to $FL_{II}$, $FL_{III}$ longest (6.3); relative length of fingers: I=II<IV<III; tips of fingers rounded; subarticular tubercles prominent, single on fingers I, II, IV, double in finger III, oval, all present; prepollex oval, distinct; single rounded prominent palmar tubercle; numerous supernumerary tubercles irregularly set on palm.

Hind limbs relatively long and thin, thigh length (TL 17.8) shorter than shank length (SHL 18.8) and foot length (FOL 18.5); relative length of toes: I<II<V<II-II<IV; tips of all toes rounded, without discs; webbing between toes present, small: I1$^{+}$–2II1$^{+}$–3III2–3⅔IV3⅔–2V; well-developed dermal fringes present on all toes; subarticular tubercles rather distinct, oval, all present; inner metatarsal tubercle present, prominent, its length (IMT 1.6) nearly half the length of outer metatarsal tubercle (OMT 3.1); numerous supernumerary tubercles irregularly set on foot.

***Skin.*** Dorsal and lateral surfaces of head and snout, and skin between eyes relatively smooth; anterior and posterior parts of back with flat and smooth glandular projections; flanks glandular without horny spinules or warts; dorsal surfaces of thigh, shank, and tarsus with smooth glandular warts. Ventral surfaces of throat, chest, belly, and thighs glandular.

***Secondary sexual character.*** Male: light brown granular projections on lateral surfaces of fingers I, II, and III.

***Colour in preservation.*** Dorsum and limbs slate grey to buff coloured; lateral surfaces of head, flank, and groin slightly lighter than dorsum; ventral surfaces (including limbs) off-white; throat with a faint light bluish-grey calling patch (Fig. 1). ***Colour in life*****:** dorsum uniformly golden yellow with a brown tinge; limbs darker than dorsum; ventral surfaces white with a prominent bluish-yellow calling patch on throat.

**Variation.** Adult size range: SVL 45–49 mm. Morphometric data from three adult males, including the described topotype, is given in Table 1. Dorsal colour varies from dark brown to golden yellow with a brown or reddish tinge; prominence of cephalic ridge varies from being inconspicuous to rather prominent; parotoid glands more prominent in life and relatively flattened in preservation; dorsal skin texture varies from having smooth glandular projections to glandular warts.

**Comparisons.** *Duttaphrynus brevirostris* differs from other congeners that have relatively prominent cephalic ridges (*D. chandai*, *D. himalayanus*, *D. kiphirensis*, *D. mamitensis*, *D. manipurensis*, *D. melanostictus*, *D. microtympanum*, *D. mizoramensis*, *D. nagalandensis*, *D. parietalis*, *D. scaber*, *D. silentvalleyensis*, *D. stuarti*, *D. wokhaensis*, *D. crocus*, *D. kotagamai*, *D. noellerti*, and *D. totol*) by its relatively smooth and inconspicuous cephalic ridges (vs. prominent and often with carotenoid margins or spinules), and smooth glandular dorsal skin (vs. presence of prominent glandular warts with horny spinules). Specifically, it also differs from the Indian species by the following characters: from *D. chandai*, by its shorter male snout-vent length, SVL 45–49 mm (vs. longer, SVL 67–89 mm), absence of canthal, parietal, and cranial ridges (vs. present), and distinct tympanum (vs. inconspicuous externally); from *D. himalayanus*, *D. kiphirensis*, *D. mamitensis*, *D. manipurensis*, *D. melanostictus*, *D. microtympanum*, *D. mizoramensis*, *D. nagalandensis*, *D. parietalis*, *D. scaber*, *D. silentvalleyensis*, and *D. wokhaensis*, by absence of canthal, preorbital, and supratympanic ridges (vs. present), relatively flat parotoid glands (vs. prominently raised), and ventral surfaces of hand, fingers, foot, and toes with smooth tubercles (vs. raised and spinular tubercles); and from *D. beddomii*, *D. hololius*, *D. peninsularis*, and *D. stomaticus* by the presence of supraorbital and postorbital ridge (vs. absent). *Duttaphrynus brevirostris* specifically also differs from *D. beddomii* by its finger and toe tips lacking expanded discs (vs. with weakly-expanded discs), relatively reduced foot webbing, I1$^{+}$–2II1$^{+}$–3III2–3⅔IV3⅔–2V (vs. extensive, I1–1II1–1III1–2IV2–1V), and absence of prominently glandular warts or horny spinules on dorsum (vs. present); from *D. hololius*, by its robust body (vs. dorso-ventrally flattened body), absence of mid-dorsal line (vs. present), sharp canthus rostralis (vs. rounded), snout rounded in lateral view (vs. acute), and more extensive foot webbing, I1$^{+}$–2II1$^{+}$–3III2–3⅔IV3⅔–2V (vs. rudimentary); from *D. stomaticus*, by its shorter male snout-vent length, SVL 45–49 mm (vs. longer, SVL 54–69 mm), snout subovoid in dorsal view (vs. rounded), canthus rostralis sharp (vs. rounded), and relatively reduced foot webbing, I1$^{+}$–2II1$^{+}$–3III2–3⅔IV3⅔–2V (vs. more extensive: I1–1II1–2$^{-}$III1–3IV3–1V); and from *D. peninsularis*, by its canthus rostralis sharp (vs. rounded), snout length longer than eye diameter, SL/EL ratio 1.2–1.3 mm (vs. nearly equal), and relatively reduced foot webbing, I1$^{+}$–2II1$^{+}$–3III2–3⅔IV3⅔–2V (vs. more extensive: I1$^{+}$–2II1$^{+}$–3$^{-}$III1½–3IV3–1½V).

**Phylogenetic relationships and genetic distances.** *Duttaphrynus brevirostris* is a member of the *Duttaphrynus melanostictus* group (Fig. 3), within which it is more closely related to *D. melanostictus*, *D.* cf. *microtympanum* (*D.* "sp", Van Bocxlaer et al. 2009), and *D. parietalis* (Fig. 3). All populations of *D. brevirostris* exhibit intraspecific distances of 0–0.2% in 16S. The sequence

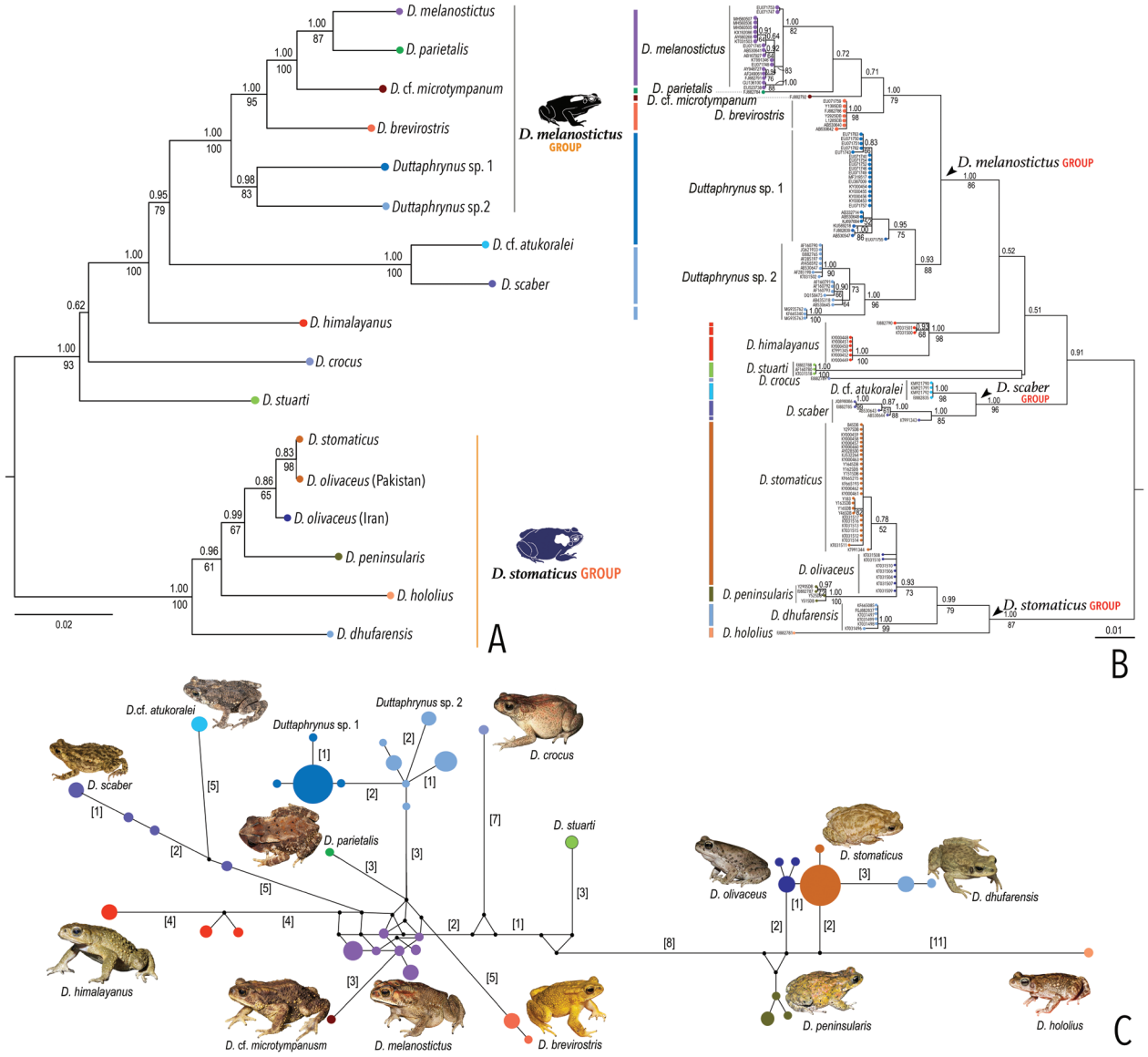


**Figure 3.** Phylogenetic relationships and genetic differentiation in the genus *Duttaphrynus*. **A.** Maximum Likelihood phylogenetic tree based on 5,737 bp DNA comprising nine mitochondrial gene regions and two nuclear genes, showing phylogenetic relationships between the major species-level lineages. Values above and below the branches indicate Bayesian Posterior Probabilities (BPP) and RAxML Bootstrap Support (BS), respectively; **B.** Maximum Likelihood barcoding tree based on 524 bp of the mitochondrial 16S rRNA sequences. BPP and BS support values are indicated above and below the branches, respectively. Coloured vertical bars outside the terminal node labels indicate putative species delimited in the bPTP analysis; **C.** Median-Joining haplotype network based on 42 haplotypes recovered from 133 sequences of the 16S gene (420 bp). Size of the coloured circles is proportional to the number of haplotypes; black circles indicate median vectors; each branch represents a single mutation step; additional mutational steps are indicated by values in parentheses; photo credits: *D. crocus* (Guinevere O. U. Wogan), *D. olivaceus* (Parham Beyhaghi), and *D. dhufarensis* (Todd W. Pierson).

divergence for *D. brevirostris* from other members of the *Duttaphrynus melanostictus* group was as follows: 2.1–3.3% from *D. melanostictus*, 2.2–2.6% from *D.* cf. *microtympanum*, 2.8–3.2% from *D. parietalis*, 3.0–4.3% from *Duttaphrynus* sp. 1, and 2.4–5.6% from *Duttaphrynus* sp. 2 (Suppl. materal 1: Table S4).

**Distribution and natural history.** *Duttaphrynus brevirostris* is endemic to the Western Ghats, where it currently is known only from the State of Karnataka. Here, we report this species from Hassan district (Sakleshpur taluk, encompassing the type locality Kempholey Ghat), Kodagu district (Bhagamandala), and Udupi district (Someshwara and Manipal). Furthermore, we confirm the following available DNA sequences for this species: Someshwara (FJ882786, Van Bocxlaer et al. 2009), specimen examined herein; Bajipe (AB530640) and Shirva (AB530642), specimen vouchers unavailable and reportedly released (Hasan et al. 2014); and another sample

EU071759 from an unknown locality in India (Shouche and Ghate, unpublished GenBank data). Based on available evidence, *D. brevirostris* is confirmed to occur in Malnad or Malenadu regions as well as coastal regions (districts of Mangalore and Udupi) of Karnataka State and, therefore, has a wider distribution than previously surmised (Fig. 4).

Most individuals were located during night searches (between 17:00–21:00 hours) in secondary forests or open urban areas. Calling males, usually with yellow dorsal colouration, were observed in June, away from the bodies of water. Specimens found closer to water were generally greyish-brown. A cursory tadpole description was provided along with the original description (Rao 1937).

### *Duttaphrynus peninsularis* (Rao, 1920), comb. nov.

Figs 1–5; Table 1; Suppl. materal 1: Tables S1–S5

Peninsular Toad

**Original name and description.** *Bufo stomaticus peninsularis* Rao, 1920. Rao, C. R. N. 1920. Some South Indian batrachians. "Journal of the Bombay Natural History Society" 27: 119–127. **Holotype.** ZSIC 19176, SVL 45.1 mm (designated by Chanda et al. 2001 "2000"), from "Mavkote and Watekolle, Coorg," Karnataka State, India.
**Current status of specific name.** Valid name, as *Duttaphrynus peninsularis* (Rao, 1920), comb. nov.

**Material studied. *Topotype*.** An adult male, SDBDU 6370 (SVL 50.8 mm), collected by S. D. Biju, from Wattakolli, Karnataka State. ***Other referred specimens*.** Four adult males, SDBDU 4018 (SVL 51.8 mm), SDBDU 4019 (SVL 45.5 mm), SDBDU 4020 (SVL 49.5 mm), and SDBDU 4021 (SVL 46.5 mm), from Coimbatore, Tamil Nadu State.

**Reassessment and validation of taxonomic status.** Rao (1920) described a new variety of *Bufo stomaticus* from "Mavkote and Watekolle, Coorg" as "*Bufo stomaticus peninsularis* var. nov." The original description mentioned two specimens ("Type and syntype in the Indian Museum") and subsequently Chanda et al. (2001 "2000") proposed ZSIC 19176 to be the holotype. Currently a single specimen is available in the ZSIC (Kolkata) collection (S.D.B., personal observation). It is noteworthy that, prior to describing this taxon, Rao (1920) took an opinion from Boulenger (then Curator, British Museum Natural History, London), who was not in favour of separating this collection from *D. stomaticus*. However, Rao being unconvinced mentioned "no doubt about their being racially distinct" in the original description and went on to formally describe *Bufo stomaticus peninsularis* as a new variety of *D. stomaticus*. This nomen was considered to be a synonym of *Bufo stomaticus* (= *Duttaphrynus stomaticus*) by Daniel (1963), without any justification or comparison, other than considering the characters mentioned by Rao (1920) as variation, based on examination of *D. stomaticus* specimens from Bombay. This action was followed by Dubois (1974) and Dutta (1997). In later years, regional anuran lists reported *Duttaphrynus stomaticus* from Peninsular India based on earlier reports and photographs, without citing any voucher specimens (Hegde 2012; Ramachandra et al. 2012; Seshadri et al. 2012). Srinivasulu et al. (2013) identified the "captioned-photographs" of Seshadri et al. (2013) and Hegde (2012) as belonging to *D. scaber*, a species that is widely distributed in Peninsular India (Dutta 1997; Chanda 2002; Daniels 2005; Dinesh et al. 2009; Padhye et al. 2013). Srinivasulu et al.'s (2013) notes concerning the misidentifications of *D. scaber* as *D. stomaticus* (and not *D. peninsularis*) was by implication considered as a synonymisation action of *Bufo stomaticus peninsularis* with *D. scaber* by Frost (2021).

In order to verify the above, we compared the type specimen and the original description of *Bufo stomaticus peninsularis* Rao, 1920. Although the holotype (ZSIC 19176) was found to be in a severely damaged and dehydrated condition (Fig. 1), the head portion was relatively better preserved. Diagnostic morphological characters, such as absence of prominent cephalic ridges, weakly developed parotoid glands, distinct tympanum (about 63% of the eye), and the relatively smooth skin texture of the head and dorsum, match with the original description of *Bufo stomaticus peninsularis* Rao, 1920. Additionally, Rao (1920) clearly stated six differences between his new variety and the typical form of *Bufo stomaticus* from "Indian Museum nos., 16067, 16068, 17254 and 17274" (see the detailed comparison section), which we further re-examined to confirm distinctness of the two taxa.

We examined specimens from two populations of *Duttaphrynus* "*stomaticus*," sampled from different localities (including Wattakolli) in Peninsular India, which were found to be comparable to the original description and type specimen of *Bufo stomaticus peninsularis* Rao, 1920 with respect to snout-vent length, absence of cephalic ridges, weakly developed parotoid glands, and relatively smooth skin. Based on re-examination of the holotype and assessment of newly-collected material, and molecular data, we conclude that *Bufo stomaticus peninsularis* Rao, 1920 and *Bufo stomaticus* Lütken, 1864 represent two distinct species, both individually diagnosable from other Indian congeners and each other. Hence, we formally resurrect *Bufo stomaticus peninsularis* Rao, 1920, as a distinct species: *Duttaphrynus peninsularis* (Rao, 1920), comb. nov. Furthermore, since the holotype is poorly preserved, we also provide a detailed redescription of this species, based on new topotypic material from Wattakolli, which matches the original description and the type.

**Description of topotype, SDBDU 6370** (measurements in mm). A medium-sized, robust adult male (SVL 50.9); head of moderate size, wider (HW 18.0) than long (HL 14.0); snout truncate in dorsal and ventral view, rounded in lateral view, projecting beyond the mouth, its length (SL 5.8) nearly equal to horizontal diameter of eye (EL 5.7); loreal region acute with rounded canthus rostralis; distance between posterior borders of the eyes (IBE 13.9) 1.6 times the distance between the anterior

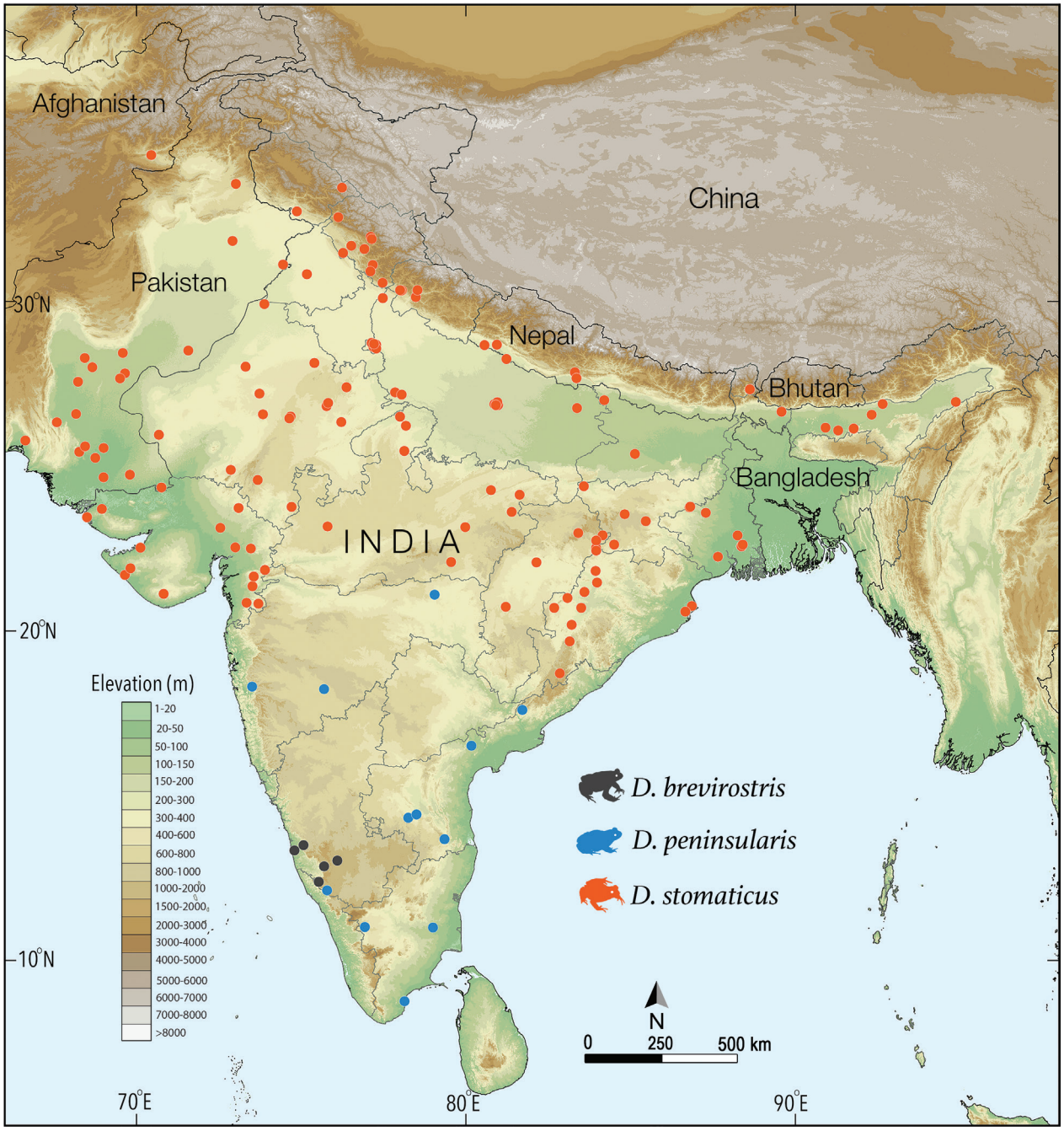


**Figure 4.** Geographical distribution of *Duttaphrynus brevirostris* (dark grey), *D. peninsularis* (blue), and *D. stomaticus* (orange).

borders (IFE 8.2); interorbital space about 1.4 times wider (IUE 6.2) than upper eyelid width (UEW 4.5); nostril oval without lateral flap of skin, closer to tip of snout (NS 1.7) than eye (EN 3.2); tympanum distinct (TYD 3.1), vertically oval, about 56.4% of eye diameter (EL 5.5), tympanum to eye distance (TYE 1.0); pineal ocellus absent; vomerine ridge and teeth absent; tongue small, oval, entire, median lingual projection absent; parotoid glands present, oval, flat, without spines and warts, slightly longer (PL 10.4) than wide (PW 5.5), distance between them (PD 6.2) more than the width.

Forelimbs short; forearm length (FAL 11.5) longer than hand length (HAL 10.9); fingers rather thin, $FL_I$ longer than $FL_{II}$, $FL_{III}$ longest (5.6); relative length of fingers: II<IV<I<III; tips of fingers rounded; subarticular tubercles prominent, single, all present; prepollex oval, distinct; single rounded prominent palmar tubercle; numerous supernumerary tubercles irregularly set on palm.

Hind limbs relatively long and thin, thigh length (TL 19.7) longer than shank (SHL 17.8) and foot (FOL 18.4) length; relative length of toes: I<II<V<III<IV; tips of all toes rounded, without discs; webbing between toes present, small: $I1^{+}$–$2II1^{+}$–$3^{-}III1½$–3IV3–1½V; dermal fringes present on all toes; subarticular tubercles rather weakly developed, oval; inner metatarsal tubercle present, prominent, its length (IMT 1.6) shorter than outer metatarsal

tubercle (OMT 1.8); numerous weakly developed supernumerary tubercles set on foot.

***Skin*.** Dorsal and lateral surfaces of head and snout, and skin between eyes relatively smooth to sparsely granular; anterior and posterior parts of back with flat and smooth glandular projections; flanks glandular without horny spinules or warts; dorsal surfaces of thigh, shank, and tarsus with smooth glandular warts. Ventral surfaces of throat, chest, belly, and thighs glandular.

***Male secondary sexual character*.** Light brown granular projections on the lateral surfaces of fingers I, II, and III.

***Colour in preservation*.** Dorsum and limbs greyish-brown without any prominent markings; lateral surfaces of head, flank, and groin slightly lighter than dorsum; ventral surfaces (including limbs) greyish-white, throat with a faint light blue calling patch (Fig. 1). ***Colour in life*:** dorsum yellowish-brown with reddish patches; limbs yellowish brown; ventral surfaces white with a prominent bluish-yellow calling patch on throat (Fig. 2).

**Variation.** Adult size range: male SVL 45–52 mm. Morphometric data from five adult males, including the described topotype, is given in Table 1. The dorsal colour is highly variable in life: SDBDU 4018: light brown with light grey patches, SDBDU 4019: light brown with reddish blotches, and SDBDU 4020: uniformly olive green.

**Comparisons.** *Duttaphrynus peninsularis* differs from the Indian congeners: *D. chandai*, *D. himalayanus*, *D. kiphirensis*, *D. mamitensis*, *D. manipurensis*, *D. melanostictus*, *D. microtympanum*, *D. mizoramensis*, *D. nagalandensis*, *D. parietalis*, *D. silentvalleyensis*, *D. scaber*, *D. stuarti*, and *D. wokhaensis*, and species from other regions: *D. crocus* (Myanmar), *D. kotagamai* and *D. noellerti* (Sri Lanka), and *D. totol* (Indonesia), by the absence of conspicuous cephalic ridges (vs. present), absence of prominent or raised parotoid glands (vs. present), and dorsal skin without distinct glandular warts or horny spinules (vs. present in all species). Due to the lack of conspicuous cephalic ridges *D. peninsularis* could be confused with four Indian species *D. beddomii*, *D. brevirostris*, *D. hololius*, and *D. stomaticus*. However, it differs from *D. beddomii* in having a relatively larger tympanum (vs. smaller), finger and toe tips without discs (vs. with weakly developed discs), relatively reduced foot webbing, I1$^{+}$–2II1$^{+}$–3$^{-}$III1½–3IV3–1½V (vs. extensive, I1–1II1–1III1–2IV2–1V), and absence of prominent glandular warts or horny spinules on dorsum (vs. present). *Duttaphrynus peninsularis* differs from *D. hololius* by its robust body (vs. dorso-ventrally flattened), absence of mid-dorsal line (vs. present), snout rounded in lateral view (vs. acute), tympanum smaller than eye diameter (vs. nearly equal), and more extensive webbing between toes, I1$^{+}$–2II1$^{+}$–3$^{-}$III1½–3IV3–1½V (vs. rudimentary). *Duttaphrynus peninsularis* differs from *D. stomaticus* by its relatively shorter snout-vent length, male SVL 45–52 mm (vs. longer, male SVL 54–69 mm), its snout truncate in dorsal and ventral view (vs. rounded), snout longer than eye diameter (vs. nearly equal), dorsal skin granulation relatively smooth (vs. with prominent glandular warts), and relatively reduced foot webbing, I1$^{+}$–2II1$^{+}$–3$^{-}$III1½–3IV3–1½V (vs. more, I1–1II1–2$^{-}$III1–3IV3–1V). For comparisons to *D. brevirostris*, see the respective comparison section.

We quantitatively assessed the degree of morphometric differentiation of *Duttaphrynus peninsularis* from the other two Indian members of the *Duttaphrynus stomaticus* group (*D. hololius* and *D. stomaticus*). An ordination of the first two principal components resulted in formation of three distinct clusters, what we consider to be three species (Fig. 5). The first two principal components (PC) accounted for 50.73% of the total variance, of which PC1 was able to explain 32.08%, and PC2 explained 18.65% of the variation in the dataset. Variables with the highest factor loadings for PC1 were HW, TYD, EL, IUE, and IN, while PC2 was highly loaded for UEW. The third and fourth principal components (PC3 and PC4) accounted for 9.37% and 9.07% of the total variance, respectively, taking the cumulative variance for the first four components to 69.17% (Suppl. materal 1: Table S5). The Box and whiskers plots of the five most significant characters recovered from PCA showed diagnostic differences between the three species (Fig. 5). Of the three species, *D. hololius* was more distinct for all the studied characters, whereas *D. peninsularis* and *D. stomaticus* could be clearly delineated based on SVL, EL/SVL, TYD/SVL, and IN/SVL.

**Phylogenetic relationships and genetic distances.** *Duttaphrynus peninsularis* is a member of the *Duttaphrynus stomaticus* group (Fig. 3), within which it is more closely related to *D. stomaticus* and *D.* '*olivaceus*' than to *D. dhufarensis* and *D. hololius*. The studied populations of *D. peninsularis* exhibit intraspecific distances of 0–0.4% in 16S. The sequence divergence of *D. peninsularis* from other members of the *Duttaphrynus stomaticus* group was as follows: 2.3–3.8% from *D. dhufarensis*, 5.2–5.4% from *D. hololius*, 1.3–2.6% from *D. stomaticus*, and 1.0–1.5% from *D.* '*olivaceus*' (Suppl. materal 1: Table S4).

**Distribution and natural history.** *Duttaphrynus peninsularis* is currently known only from the Peninsular Indian States of Karnataka, Tamil Nadu, and Maharashtra. Genetically confirmed records are from Karnataka: Kodagu district (Wattakolli); Tamil Nadu: Coimbatore district (Coimbatore); and Maharashtra: Solapur district (Barshi and Solapur). We have also observed this species at Namakkal district (Kolli Malai) of Tamil Nadu. DNA sequences of this species were previously reported as *D. stomaticus* (FJ882787, Van Bocxlaer et al. 2009). Another genetically identical sample from an unknown locality in India is currently available (EU071742, Shouche and Ghate, unpublished GenBank data). Given that this species currently has a disjunct distribution based on available genetically confirmed records, it is likely to be more widely distributed in the intervening regions of Peninsular India (Kerala, Tamil Nadu, and Karnataka, up to southern Maharashtra). Furthermore, its most closely related congener *D. stomaticus* is frequently and widely reported in Peninsular India, which could be misidentifications of *D. peninsularis*; hence the identity of all '*D.*

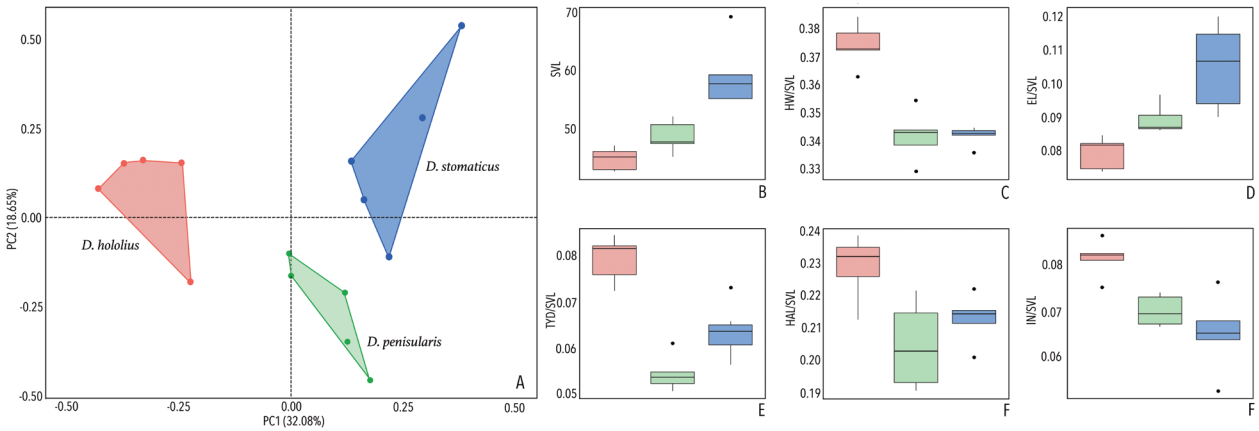


**Figure 5.** Morphometric analyses for Indian members of the *Duttaphrynus stomaticus* group. **A.** Principal component analysis showing distinct clusters for three species in a scatter plot of the first two principal components; **B–G**. Box and whiskers plots depicting the most significant diagnostic characters for the three species.

*stomaticus*' records from this region require further verification. Based on the present study, the geographical boundary between *D. peninsularis* (southern species) and *D. stomaticus* (northern species) could lie in the northern Western Ghats regions of Maharashtra state, where we have observed and genetically confirmed the presence of both these species (see Distribution and Natural History section of *D. stomaticus*). Further extensive sampling will be necessary to understand the patterns of population structure and delineate the ranges of these two species, using integrative approaches focusing on quantified ranges of phenotypic variation, traditional morphology, bioacoustics, ecological information, and phylogeny.

Most individuals reported here were located during night searches (between 17:00–21:00 hours) largely in vegetated urban areas. The species were also found in secondary forest patches adjacent to human settlements. Ganesh et al. (2020) reported this species as *D. stomaticus* from Tuticorin, Tamil Nadu.

### *Duttaphrynus stomaticus* (Lütken, 1864)

Figs 1–5; Table 1; Suppl. materal 1: Tables S1–S5

Marbled Toad

**Original name and description.** *Bufo stomaticus* Lütken, 1864. Lütken, C. F. 1864 "1863." Nogle ny Krybyr og Padder. Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening i Kjøbenhavn, Serie 2, 4: 292–311. ***Syntypes.*** Three adult females, ZMUC 131137 [ex 196], ZMUC 131365 [ex 198], and one unnumbered, from "Assam;" two adult males, ZMUC 131136 [ex 195] and one unnumbered, from "Assam;" and three subadults, ZMUC 131366 [ex 199] from "Hoogly," ZMUC 131363 [ex 193] from "Calcutta," and ZMUC 131364 [ex 194] from "Calcutta." **Type locality.** "Assam," India, based on two specimens used in the original description (Lütken, 1864). **Current status of specific name.** Valid name, as *Duttaphrynus stomaticus* (Lütken, 1864).

**Material studied. *Syntypes*:** Three adult females, ZMUC 131137 [ex 196] (SVL 60.9 mm), ZMUC 131365 [ex 198] (SVL 55.2 mm), and one unnumbered (SVL 61.4 mm), from "Assam;" two adult males, ZMUC 131136 [ex 195] (SVL 55 mm) and one unnumbered (SVL 59.2 mm), from "Assam;" and three subadults, ZMUC 131366 [ex 199] (SVL 26.4 mm) from "Hoogly," ZMUC 131363 [ex 193] (SVL 33.4 mm) from "Calcutta" (Kolkata), and ZMUC 131364 [ex 194] (SVL 30.0 mm), from "Culcutta" (Kolkata). ***Other referred specimens*:** three adult males, SDBDU 2018.4109 (SVL 57.6 mm), SDBDU 2018.4110 (SVL 69.2 mm), and SDBDU 2018.4111 (SVL 55.1 mm), from Sonitpur district, Assam State; two adult males, SDBDU 2018.3717 (SVL 56.2 mm) and SDBDU 2018.3750 (SVL 54.2 mm), from Dehradun, Uttarakhand State; an adult female, SDBDU 2012.2172 (SVL 67.5 mm), from Delhi; an adult female, SDBDU 2012.2269 (SVL 68.7 mm), from Kaitha in Banka district, Bihar State; an adult male, SDBDU 2012.2170 (SVL 51.0 mm), from Jaipur, Rajasthan State.

**Taxonomic history of *Bufo stomaticus* Lütken, 1864.** In the original description, Lütken (1864) mentioned that the Zoological museum, Copenhagen received six specimens of a toad from "Hr. Grosserer Westerman" (= Mr. Wholesales man Westermann) from "ostindiske" (= East India). Subsequent researchers stated the type locality of this species to be 'East India' where it was later restricted to Assam (Boulenger 1891). Dutta (1997) stated that the type specimens are untraceable. We (SDB and SG) studied the types that are available at ZMUC, Copenhagen, and found a total of eight specimens (see 'Other material studied'). According to the museum catalogue and bottle labels, all the adult animals are from "Assam," one juvenile from "Hoogly," and two juveniles from "Culcutta" (Kolkata). All the specimens belong to the same species and the morphological characters were in agreement with the brief original description. Boulenger (1891) had mentioned after examining the syntypes that the exact locality from where these were procured

is unknown and believed they originated from Assam or "they are perhaps from Bengal." However, while describing *Bufo stomaticus* Lütken (1864) provided four measurements from two specimens, without mentioning the voucher numbers—"en Han" (one male) and "en Hun" (one female) "Fra Snudespidsen til Gattet" (= from snout to cloaca) 54 mm and 61 mm, respectively. Among the eight located syntypes, two similar-sized specimens were found bearing small tags on the hind limbs stating 'type'.

Based on the available information, it is apparent that only two specimens, ZMUC 131137 [ex 196] and ZMUC 131136 [ex 195], were used for Lütken's (1864) description of *Bufo stomaticus*; hence only these can be considered as potential syntypes. However, since the type series contains both adult and subadult specimens originating from different localities, it has led to confusion regarding the type locality and type status (Boulenger 1891). In order to clarify the taxonomic status of *B. stomaticus*, we provide a detailed redescription for one potential syntype, ZMUC 131137 [ex 196], an adult female, SVL 60.9 mm, from "Assam." The below redescription, along with live photographs, interspecific comparisons, and enumeration of diagnostic characters, may be useful for differentiating this taxon from other known *Duttaphrynus* species. We also provide additional information on new topotypic material, including live photographs, genetic data, inferred phylogenetic relationships, and extended geographical records, based on morphologically-characterised and genetically-confirmed records—all of which shows that *D. stomaticus* (as understood here) is consistent with what is known of the name-bearing types.

**Description of syntype, ZMUC 131137 [ex 196]** (measurements in mm). A medium-sized, robust adult female (SVL 60.9). Head of moderate size, wider (HW 22.7) than long (HL 17.8); snout rounded in lateral, dorsal, and ventral view, projecting beyond the mouth, its length (SL 6.8) longer to horizontal diameter of eye (EL 6.0); loreal region acute with rounded canthus rostralis; distance between posterior borders of the eyes (IBE 16.2) 1.8 times the distance between the anterior borders (IFE 9.2); interorbital space concave, 1.3 times wider (IUE 6.6) than upper eyelid width (UEW 5.0); nostril oval without lateral flap of skin, closer to tip of snout (NS 1.8) than to eye (EN 3.5); tympanum distinct (TYD 3.6), rounded, 58.1% of eye diameter (EL 6.2), tympanum to eye distance (TYE 1.6); pineal ocellus absent; vomerine ridge and teeth absent; tongue small, oval, entire, median lingual projection absent; parotoid glands present, oval, elongate, without spines and warts, longer (PL 13.9) than wide (PW 6.5) and distance between them (PD 10.0) wider than their width; cephalic ridges absent.

Forelimbs short; forearm length (FAL 11.5) shorter than hand length (HAL 13.7); fingers rather thin, $FL_I$ longer to $FL_{II}$, $FL_{III}$ longest (7.1 mm); relative length of fingers: I<II<IV<III; tips of fingers rounded; subarticular tubercles prominent, single, all present; prepollex oval, distinct; single rounded prominent palmar tubercle; numerous supernumerary tubercles irregularly set on palm.

Hind limbs relatively long and thin, thigh length (TL 21.3) shorter than shank (SHL 21.8) and foot (FOL 22.6) length; relative length of toes: I<II<V<III<IV; tips of all toes rounded without discs; webbing between toes present, small: I1–1II1–2–III1–3IV3–1V; dermal fringes present on all toes; subarticular tubercles rather well-developed, oval; inner metatarsal tubercle present, prominent, its length (IMT 3.1) shorter than outer metatarsal tubercle (OMT 3.7); numerous weakly developed supernumerary tubercles set on foot.

***Skin*.** Dorsal surfaces of head sparsely granular; lateral surfaces of head shagreened with scattered tubercles; upper eyelids with glandular warts possessing horny spinules; anterior and posterior parts of back with glandular warts possessing horny spinules, larger warts towards posterior back; flanks glandular without warts or horny spinules; dorsal surfaces of thigh, shank, and tarsus glandular. Ventral surfaces of throat, chest, belly, and thighs with fine glandular projections without horny spinules or warts.

***Secondary sexual characters*.** Female (ZMUC 131137): ova white, pigmented on pole (diameter 0.8–1.0 mm, N = 20); Male (SDBDU 2018.4111): light brown granular projections on the lateral surfaces of fingers I, II, and III. ***Colour in preservation*:** dorsal surfaces of head and body uniformly fawn, some spines brown; dorsal surface of fore-and hind limbs light fawn; ventral surfaces of head, body, and limbs light grey (Fig. 1). ***Colour in life*** (based on other material studied): dorsum yellowish-brown, straw, light brown, or olive green, with or without grey or brown patches; and a pair of faint discontinuous dorsolateral lines; ventral surfaces greyish-white (Fig. 2).

**Variation.** Adult size range: male SVL 54–69 mm, female SVL 60–72 mm. Morphometric data from five adult males, including the described syntype, is given in Table 1. Dorsal colouration varies from light grey or brown to olive green; the amount and degree of prominence of granulation on dorsal skin variable.

**Comparisons.** *Duttaphrynus stomaticus* differs from the Indian species: *D. chandai*, *D. himalayanus*, *D. kiphirensis*, *D. mamitensis*, *D. manipurensis*, *D. melanostictus*, *D. microtympanum*, *D. mizoramensis*, *D. nagalandensis*, *D. parietalis*, *D. silentvalleyensis*, *D. scaber*, *D. stuarti*, and *D. wokhaensis*, and other species found outside: *D. crocus* (Myanmar), *D. kotagamai* and *D. noellerti* (Sri Lanka), and *D. totol* (Indonesia), by the absence of cephalic ridges, absence of prominent or raised parotoid glands, and absence of distinct glandular warts or horny spinules (vs. present in all species). Due to the absence of cephalic ridges *D. stomaticus* could be confused with three Indian species *D. beddomii*, *D. hololius*, and *D. peninsularis*. However, *D. stomaticus* differs from *D. beddomii* in having a tympanum larger than eye diameter (vs. smaller), finger and toe tips lacking expanded discs (vs. with weakly-expanded discs), relatively reduced foot webbing, I1–1II1–2–III1–3IV3–1V (vs. more extensive, I1–1II1–1III1–2IV2–1V), and less prominent glandular

warts or horny spinules on dorsum (vs. more prominent); from *D. hololius*, in having a stout body (vs. flattened or dorso-ventrally compressed), absence of a prominent or broad mid-dorsal line (vs. present), snout rounded in lateral view (vs. acute), dorsum with relatively more prominent smooth or spinular warts (vs. less prominent and scattered smooth tubercles), and moderate foot webbing, I1–1II1–2–III1–3IV3–1V (vs. rudimentary). For comparisons to *D. brevirostris* and *D. peninsularis*, see the respective comparison sections of those species.

**Phylogenetic relationships and genetic distances.** *Duttaphrynus stomaticus* is a member of the *Duttaphrynus stomaticus* group (Fig. 3), within which it is more closely related to *D.* '*olivaceus*' and *D. peninsularis* than to *D. dhufarensis* and *D. hololius*. The studied populations of *D. stomaticus* exhibit intraspecific distances of 0–0.4% in 16S. The sequence divergence of *D. stomaticus* from other members of the *D. stomaticus* group is as follows: 0.2–0.6% from *D.* '*olivaceus*', 1.3–2.6% from *D. peninsularis*, 1.5–3.0% from *D. dhufarensis*, and 3.4–5.6% from *D. hololius* (Suppl. materal 1: Table S4).

**Relationships within *Duttaphrynus stomaticus* group.** The close phylogenetic relationship of *Duttaphrynus stomaticus* with *D. dhufarensis*, *D. hololius*, *D. olivaceus*, and *D. peninsularis* is well-supported (Van Bocxlaer et al. 2009; Portik and Papenfuss 2015; present study). Martin (1972) also discussed the absence of conspicuous cephalic ridges as a potential morphological synapomorphy for these species. Within this group, subsequently referred to as the *Duttaphrynus stomaticus* group (Inger 1972; Dubois and Ohler 1999; Silva and Mendelson 1999; Van Bocxlaer et al. 2009), the taxonomic identity of *D. olivaceus* has been questionable due to the lack of sufficient morphological distinctness (Dubois 1984; Balletto et al. 1985; Minton 1966) as well as shallow genetic divergence (Portik and Papenfuss 2015; present study). Eiselt and Schmidtler (1973) regarded *D. olivaceus* as the subspecies of *D. stomaticus*. However, subsequent workers treated *D. olivaceus* as a distinct species closely related to *D. stomaticus* with relatively weak and variable morphological diagnostic characters, such as differences in the size of parotoid glands, number of subarticular tubercles on finger III, and weakly or well-developed tibial gland and tarsal folds (Schmidtler and Schmidtler 1969; Khan 1987; Auffenberg and Rehman 1997). The available genetic data for *D. stomaticus* and *D. olivaceus*, along with new samples reported in this study for various *D. stomaticus* populations from India (including topotypic sequences) show a shallow divergence of 0.2–0.6% between the two species (Fig. 3).

Recently, Safaei-Mahroo and Ghaffari (2020) discussed the taxonomic status of *D. olivaceus* (Frost 2021). This study also proposed a new genus name *Firouzophrynus* Safaei-Mahroo & Ghaffari, 2020 to accommodate a single species *Duttaphrynus olivaceus* (Blanford 1874), which rendered the genus *Duttaphrynus* paraphyletic (Frost 2021). Subsequently, based on phylogenetic evidence from selected taxa, Dubois et al. (2021) redelimited *Firouzophrynus* as a genus, while also stating the possibility of considering it as a subgenus, to include members of the *Duttaphrynus stomaticus* group as defined by Inger (1972) and Dubois and Ohler (1999). However, as noted by Frost (2021), there continues to be lack of clarity regarding the morphological and phylogenetic affinities of some other members of the group, which may have implications on the monophyly of *Firouzophrynus*. The composition of *Duttaphrynus stomaticus* species group and its phylogenetic position have been discussed by numerous studies (Inger 1972; Martin 1972; Maxson 1981; Van Bocxlaer et al. 2009; Portik and Papenfuss 2015). However, only five species (*D. stomaticus*, *D. dhufarensis*, *D. hololius*, *D. olivaceus*, and *D. peninsularis*) currently are included in this group based on morphological (Inger 1972; Martin 1972; Dubois and Ohler 1999; present study) and phylogenetic analyses (Frost et al. 2006; Van Bocxlaer et al. 2009; Portik and Papenfuss 2015; this study). At least two other species from Indonesia, *D. valhallae* and *D. sumatranus*, that are known to lack cephalic ridges, a characteristic of the group (Inger 1972; Dubois and Ohler 1999), require further studies to establish their systematic relationships. Although we do not doubt that *Firouzophrynus* could be recognised as a genus or subgenus, we currently consider the taxonomic status of this taxon uncertain, pending additional studies which may provide clarity, because of its cursory description and lack of a clear definition. Because it is beyond the scope of the present work to address this question, we have provisionally referred our focal taxa to the genus *Duttaphrynus*, sensu lato, and make use of previously defined species-groups, which could easily be adopted to an alternate classification, as more evidence concerning the recognition of *Firouzophrynus* becomes available.

**Distribution and natural history.** *Duttaphrynus stomaticus* is one of the most widely-distributed species of the genus, occurring between elevations of sea-level to 2500 m asl in India (through Indo-Gangetic Plains, upper and lower Indus Valleys) and the neighbouring Bangladesh, Nepal, Pakistan (Balochistan), Afghanistan, and Iran (Suppl. materal 1: Table S1). This species is known to occur in varying climatic conditions and habitats, ranging from dry scrub forests, arid and semi-arid regions, hot and humid mixed forests, plains, and grasslands to drier and colder regions, montane woodlands and forests (Choudhury et al. 2001; Mehta 2005; Deuti et al. 2014; Safaei-Mahroo et al. 2015). Genetically confirmed records of this species exist from India, Afghanistan, and Pakistan (Suppl. materal 1: Table S3). In the present study, we specifically confirm the presence of *D. stomaticus* in the Indian States of Assam, Bihar, Delhi, Punjab, Rajasthan, and Uttarakhand (Suppl. materal 1: Table S3) and also clarify the identity of some previously published DNA sequences from Peninsular India (Van Bocxlaer et al. 2009; Shouche and Ghate 2007, unpublished GenBank data) as belonging to *D. peninsularis*. Hence, records of *D. stomaticus* from Peninsular India (south

of Maharashtra and possibly Odisha) are currently presumed to be doubtful and will require verification of all known populations (see *D. peninsularis* for discussion). The reports of *D. stomaticus* from Karnataka and Tamil Nadu States (Hegde 2012; Ramachandra et al. 2012; Seshadri et al. 2012; Ganesh et al. 2020) likely refer to *D. peninsularis*. A report of *D. olivaceus* from Gurgaon, India (Ray and Deuti 2008) is also questionable (Heydari and Rastegar-Pouyani 2010) and considered to represent *D. stomaticus* based on our fresh collections from Delhi and surrounding North Indian regions.

*Duttaphrynus stomaticus* is predominantly a nocturnal species. In this study, we found individuals of this species in urban, rural, and secondary forested areas during the breeding season (usually between May–August). Calling and breeding activities were observed in agricultural fields and temporary puddles in urban and rural landscapes, whereas inside secondary forests breeding was observed in shallow parts of flowing streams.

## Phylogenetic relationships and genetic differentiation in the genus *Duttaphrynus*

Our reanalysis of the multilocus data derived from previous studies (primarily Van Bocxlaer et al. [2009] and Portik and Papenfuss [2015]), with 16S data for our newly-sampled populations, support the monophyly of the *Duttaphrynus melanostictus* group and the *Duttaphrynus stomaticus* group (Fig. 3A), as shown in these previous studies. Among the focal taxa of our study, *D. brevirostris* was nested in the *Duttaphrynus melanostictus* group, with high support for the recovered phylogenetic position, whereas *D. peninsularis* and *D. stomaticus* were recovered in the *Duttaphrynus stomaticus* group with variably-supported relationships (weak or high) in the ML and BI analyses. The genetic differentiation at the species level, based on an expanded mitochondrial 16S rRNA dataset, however, is relatively shallow as compared to other wide-ranging anuran groups in South Asia, such as dicroglossids, microhylids, ranids, and rhacophorids (Biju et al. 2014b, 2020; Vijaykumar et al. 2014; Dinesh et al. 2015; Garg and Biju 2017; Garg et al. 2018, 2019). The maximum intraspecific divergence within the recognised or putative species reaches up to 2.1% in the *Duttaphrynus melanostictus* group (Fig. 3B; Suppl. materal 1: Table S4). At the same time, low interspecific distances of 1.0–6.0% are observed in both species groups. The interspecific divergence between *D. stomaticus* and *D. olivaceus* species is rather shallow (0.2–0.6%) but, together, these two taxa are more extensively differentiated from their sister species *D. peninsularis* (1.0–2.6%). In general, interspecific divergences among some members of the *Duttaphrynus stomaticus* group (*D. stomaticus* + *D. olivaceus*, *D. dhufarensis*, and *D. peninsularis*) trend towards the lower extent of the spectrum (1.0–1.5%) of genetic divergences observed in other *Duttaphrynus* species groups (Fig. 3B; Suppl. materal 1: Table S4).

Our species delimitation analyses for the *Duttaphrynus stomaticus* group recovered only four species: *D. dhufarensis*, *D. hololius*, and *D. peninsularis*, and *D. stomaticus* + *D. olivaceus* (as a single species) (Fig. 3). Hence, our results indicate the need for a future comprehensive phenotypic assessment for all members of the group from its entire range, in order to clarify the taxonomic status of unsupported populations of '*D. olivaceus*,' for which specimens were not available in our study for imparting a conclusive morphological evaluation. Furthermore, the results of species delimitation also suggest the presence of additional putative species among other known members of the genus *Duttaphrynus* (Fig. 3B): within the *Duttaphrynus melanostictus* group, one additional putative species was recovered, apart from two previously known and unidentified taxa (*Duttaphrynus* sp. 1 and *Duttaphrynus* sp. 2); within the *Duttaphrynus scaber* group, three putative species were recovered; finally, the *D. himalayanus* lineage comprised of three potential candidate species. These results indicate the possible presence of potentially undescribed cryptic species diversity within the genus, which requires further investigation.

The mitochondrial 16S gene median-joining network, however, did not show sharing of any haplotypes among the studied populations of various recognised or putative species of the genus *Duttaphrynus* (Fig. 3C). The *Duttaphrynus stomaticus* and *D. melanostictus* groups formed distinct species clusters separated by nine mutation steps. At the species-level, members of *D. stomaticus* group were separated by a minimum of one to five mutation steps between *D. olivaceus–D. stomaticus* and *D. peninsularis–D. olivaceus*, respectively, and a minimum of 15 steps between *D. hololius* and the remaining species of the group. Within the *Duttaphrynus melanostictus* group, the putative *Duttaphrynus* spp. 1 and 2 were separated by three mutation steps, followed by four steps between *D. melanostictus–D. parietalis* and *D. melanostictus–D.* cf. *microtympanum*, and up to a minimum of 10 steps between *D. melanostictus–D.* sp. 1. All other known members of the genus—*D. scaber* group species (*D.* cf. *atukoralei* and *D. scaber*), *D. himalayanus*, *D. stuarti*, and *D. crocus*—were separated from species of the *D. melanostictus* group and *D. stomaticus* group by at least eight mutation steps (Fig. 3C).

Altogether, our various analyses were congruent with respect to the distinctness and phylogenetic position of *D. brevirostris* and *D. peninsularis*. We suggest a further detailed population-level investigation of the *D. stomaticus* + *D. olivaceus* clade, for which the name *D. stomaticus* (Lütken 1864) holds priority, if *D. olivaceus* (Blanford 1874) is confirmed to be conspecific by evaluation of phenotypic data. Our results also shed light on the degrees of mitochondrial differentiation among members of the *D. stomaticus* group, as well as the other known species of the genus; these and other data will facilitate future taxonomic and phylogenetic studies on toads of the genus *Duttaphrynus*.

## Conclusions

The results of this study resolve long-standing uncertainty regarding the identities and taxonomic status of two toad species described from Peninsular India. *Bufo brevirostris* Rao, 1937 was considered a problematic taxon, because its original name-bearing types are lost. *Bufo stomaticus peninsularis* Rao, 1920 was long forgotten as an available name for Peninsular Indian populations closely related to *Duttaphrynus stomaticus*. We substantiate *D. peninsularis* to be a distinct species, which is both morphologically diagnosable and phylogenetically distinct. Taxonomic redefinition of both of these species was achieved not just by examining the original literature and available types, but also through an effort to rediscover new material from each species' respective type locality. The redescription of *Bufo brevirostris* Rao, 1937 based on new topotypic material, along with detailed comparisons to related taxa, objectively clarifies its identification for future reference. Similarly, topotypic material for *Bufo stomaticus peninsularis* Rao, 1920 enabled a detailed re-evaluation of its taxonomic status in the absence of a well-preserved type. Altogether, our results emphasise that new collections from type localities of historically available names should be attempted when taxonomic resolution is not feasible on the basis of original descriptions or type specimens (Bailey 1933; Garg and Biju 2016).

The present work clarified the taxonomic identity of another species, *Duttaphrynus stomaticus*, which was overlooked due to its presumed wide distribution. This taxon was known only from its brief original description, and the available, original name-bearing types remained unexamined due to literature-based misconceptions concerning their untraceability (Dutta 1997; Ganesh et al. 2020). We located the well-preserved eight original type specimens, and clarified the status of name-bearing types and the identity of this species, which we redescribed to facilitate future taxonomic studies. This action also aided our objective of resolving the taxonomic status of *D. peninsularis*, which was originally defined as a variety of *D. stomaticus*. Our results have important implications concerning the taxonomy and geographical ranges of the two species. Hereafter, *D. stomaticus* should be considered as a species found in the northern regions of South Asia, whereas its sister taxon *D. peninsularis* should be recognised as a Peninsular Indian form (Fig. 4; Suppl. materal 1: Table S3). Detailed redescriptions provided in this study will enable proper identification and range delineation, and serve as the basis for future conservation action. Knowledge of phenotypic variation and phylogenetic affinities of both species will also facilitate a better understanding of patterns of genetic differentiation within the genus, particularly among the species of the *Duttaphrynus stomaticus* group.

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# Supplementary material 1

## Supplementary tables S1–S5

Authors: Karan Bisht, Sonali Garg, A. N. D. Akalabya Sarmah, Saibal Sengupta, S. D. Biju

Data type: species data



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PENSOFT

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# Contribution to the trout of Euphrates River, with description of a new species, and range extension of *Salmo munzuricus* (Salmoniformes, Salmonidae)

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## Abstract

In an effort to reveal the Euphrates trout taxonomy, the Karasu River, which is one of the eastern drainages of the river, was investigated and three independent populations were identified. Result revealed that two populations belonged to *Salmo munzuricus*, which was known only in Munzur River, while the other population belonged to an unnamed species. *Salmo baliki*, a new species, is described from the Murat River, a drainage of Euphrates River. It differs from *Salmo* species in adjacent water by the combination of the following characters: a grayish body; commonly one, rarely two pale black spots behind eye and on cheek; two to seven black spots on opercle; a few black spots on back and upper part of flank, missing on predorsal area; few to numerous large irregular-shaped red spots in median, upper and lower part of flank, surrounded by a large irregular-shaped white ring; the number of black and red spots not increasing in parallel with size; maxilla short and narrow; adipose-fin medium size, no or rarely one or two red spot its posterior edge; 107–118 lateral line scales; 24–28 scales rows between dorsal-in origin and lateral line; 18–22 scale rows between lateral line and anal–fin origin; maxilla length 7.7–9.1% SL in males, 8.2–9.6 in females. Finally, the genetic study of the Cyt *b* mitochondrial gene confirmed the morphological data, suggesting the separation of *S. baliki* from other *Salmo* species.

## Key Words

Anatolia, cytochrome b, freshwater fish, *Salmo*, taxonomy

## Introduction

Anatolia has a high level of species richness and endemism, thus it has been classified as a European biodiversity "*hot-spot*" (Kosswig 1955; Şekercioğlu et al. 2011), which has also positively reflected in salmonid biodiversity (Bardakçı et al. 2006). *Salmo trutta* L. 1758 is the most widely distributed freshwater fish native to the Palearctic region. Its natural habitat extends from Northeast Russia and Norway, southward to the Atlas Mountains, also, from the spring waters of the Aral Sea to Iceland (Bernatchez 2001; Lobón-Cerviá 2018 and references there in). Initially, all Anatolian trout had been grouped within the *S. trutta* or its subspecies (e.g. Kuru 1975; Geldiay and Balık 2007). Further studies based on morphology (Turan et al. 2012, 2014a, 2014b, 2017; Turan and Bayçelebi 2020) and genetic-aided morphology (Turan et al. 2010, 2011, 2020) of Anatolian trout have revealed a much more complex species structure. Overall, fourteen species have been identified in Anatolia within the last decade. Based on our current knowledge, the upper Euphrates River is one of the most species-rich areas for the genus *Salmo* genus including four well-described species: *Salmo euphrataeus* Turan, Kottelat & Engin, 2014 from the streams Şenyurt, Kuzgun, Rizekent, Ağırcık and Sırlı, northern Euphrates; *S. okumusi* Turan, Kottelat & Engin, 2014 from the streams Göksu, Gökpınar and Sürgü, western Euphrates; *S. munzuricus* Turan, Kottelat & Kaya,

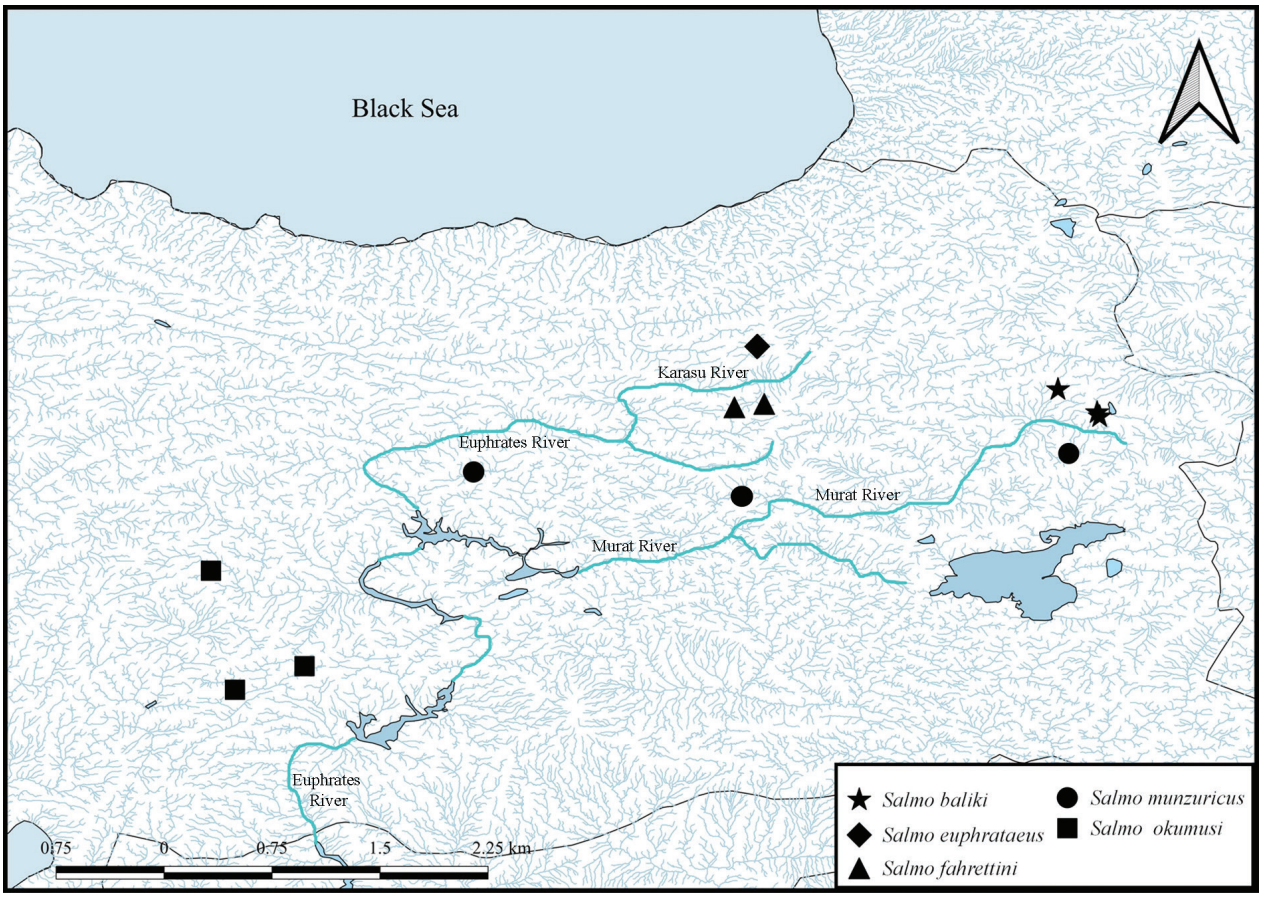


**Figure 1.** Distribution of *Salmo* species in the Euphrates River basin.

2017 from the stream Munzur, northwestern Euphrates; *S. fahrettini* Turan, Kalaycı, Bektaş, Kaya & Bayçelebi, 2020 from the streams Ömertepe suyu and Tekke, northern Euphrates.

Traditionally, five major evolutionary lineages of brown trout were described based on their origin, and phylogenetic; including the AD (Adriatic origin), AT (Atlantic), DA (Danubian), MA (Marmaratus) and ME (Mediterranean) (Bernatchez 2001). Further investigations identified new lineages as Duero from Spain (DU; Suárez et al. 2001), TI from Turkey (Tigris; Sušnik et al. 2005; Bardakçı et al. 2006), Dades from Morocco (Snoj et al. 2011) and from Northern Africa (Tougard et al. 2018). Additional molecular studies have placed the trout species from the Euphrates River drainages in the Danubian (*S. euphrataeus* and *S. fahrettini*), and the Adriatic (*S. okumusi* and *S. munzuricus*) lineages providing the significant species diversity in the Euphrates.

In the scope of this study, three additional trout populations in the Murat River were determined. To reveal the taxonomic status of these novel populations, morphologic and molecular studies were carried out to compare them with the previously identified species in the adjacent waters. Our studies demonstrated that two of these populations belonged to the *S. munzuricus*, which was previously known from a single locality, while the other population belongs to an unnamed species within the Adriatic lineage.

## Material and methods

The field work was carried out by following the guidelines of the Local Ethics Committee of RTE University for the use of animals in scientific experiments with a permit reference number of 2014/72. Samples were collected from the stream Sinek, drainage of the Murat River, Ağrı, and eastern Turkey (Figure 1). This water is known to be one of the uppermost tributaries of the Euphrates River. Samples were caught using an electrofishing device (Samus, 1000). First, live photographs were taken in an aquarium, filled with the water of the sampling reservoir so as to capture the natural coloration and patterns of the specimens. Then, anesthesia was performed using tricaine methane sulphonate solution (MS222). Subsequently, fin clips were collected from one of the pelvic fins, placed into 96% ethanol, for molecular work. Following a surgical procedure, samples were fixed in 4% formaldehyde in the vertical position. These specimens were taken to FFR, Zoology Museum of the Faculty of Fisheries, Recep Tayyip Erdogan University, Rize for detailed morphologic analysis.

## Morphological analyses

Turan et al. (2010) was used as a guideline for morphometric analysis. All measurements were carried out in the form of point to point approach (projections were refused) using a dial caliper calibrated to 1 mm. Specific to the present study, the last two branched rays articulating on a single pterygiophore in the anal and dorsal fins were counted as "1½". Comparative materials used in this study were listed in Turan et al. (2010, 2011, 2012, 2014a, 2017, 2020).

## DNA extraction, PCR and sequencing

Total genomic DNA was extracted from the ethanol-fixed fin clips using DNeasy Blood & Tissue Kit (Qiagen, USA) following the manufacturer's protocol carried out in the Qiacube Automated DNA purification system. The DNA concentration and purity of each sample were assessed by spectrophotometry (Nanodrop, 2000/c, Thermo Scientific, USA), while the integrity was assessed by 0.8% agarose gel electrophoresis. Mitochondrial cytochrome b (Cyt *b*) gene was amplified using SsaL14437 (Warheit and Bowman 2008) and StrCBR (Turan et al. 2010) primer pairs following the PCR conditions specified in Turan et al. (2020). The amplicons were visualized on UV Quantum–Capt ST4 system (Vilber Lourmat, France) and sequenced in both directions by Macrogen Inc. (Amsterdam, Netherlands).

## Phylogenetic analysis

A total of 65 Cyt *b* sequences were assessed from the *Salmo* species (Table 2) inhabited in the Tigris, Euphrates and Kura River drainages as well as the Black Sea and eastern Mediterranean Sea basins. Generated sequences were aligned using BioEdit 7.2.5 (Hall 1999) with Clustal W (Thompson et al. 1994). Trimming was essential thus, applied to both ends of the fragments to set the equal lengths of 993 bp for each and every fragment. The phylogenetic relationships among *Salmo* species were assessed by maximum likelihood (ML) approach in MEGA X software (Kumar et al. 2018) and by Bayesian analysis (BI) in MrBayes v3.2.1 (Ronquist et al. 2012).

The most appropriate evolution model of nucleotide substitution was selected by the Akaike Information Criteria (AIC) and Bayesian Information Criteria (BIC) approaches in jmodelTest 0.1.1 (Posada 2008). ML tree was generated by selecting TrN+I+G (Tamura and Nei 1993) model according to AIC and 1000 bootstrap replicates applied. The BI was generated according to the TrN+I+G (Tamura and Nei 1993) model that the evolution model was selected by the lowest BIC score. For BI, analyses were run for $1\times10^6$ generations with Metropolis coupled Monte Carlo Markov Chains (MCMC) sampled every 1000 generations.

*Salmo salar* Linnaeus, 1758 (JX960834) was used as an outgroup so as to root the phylogenetic tree. The new sequences generated in the present study were deposited

**Figure 2.** *Salmo baliki*, FFR 3242, holotype, 212 mm SL, male; Turkey: stream Sinek, a tributary of Murat River.

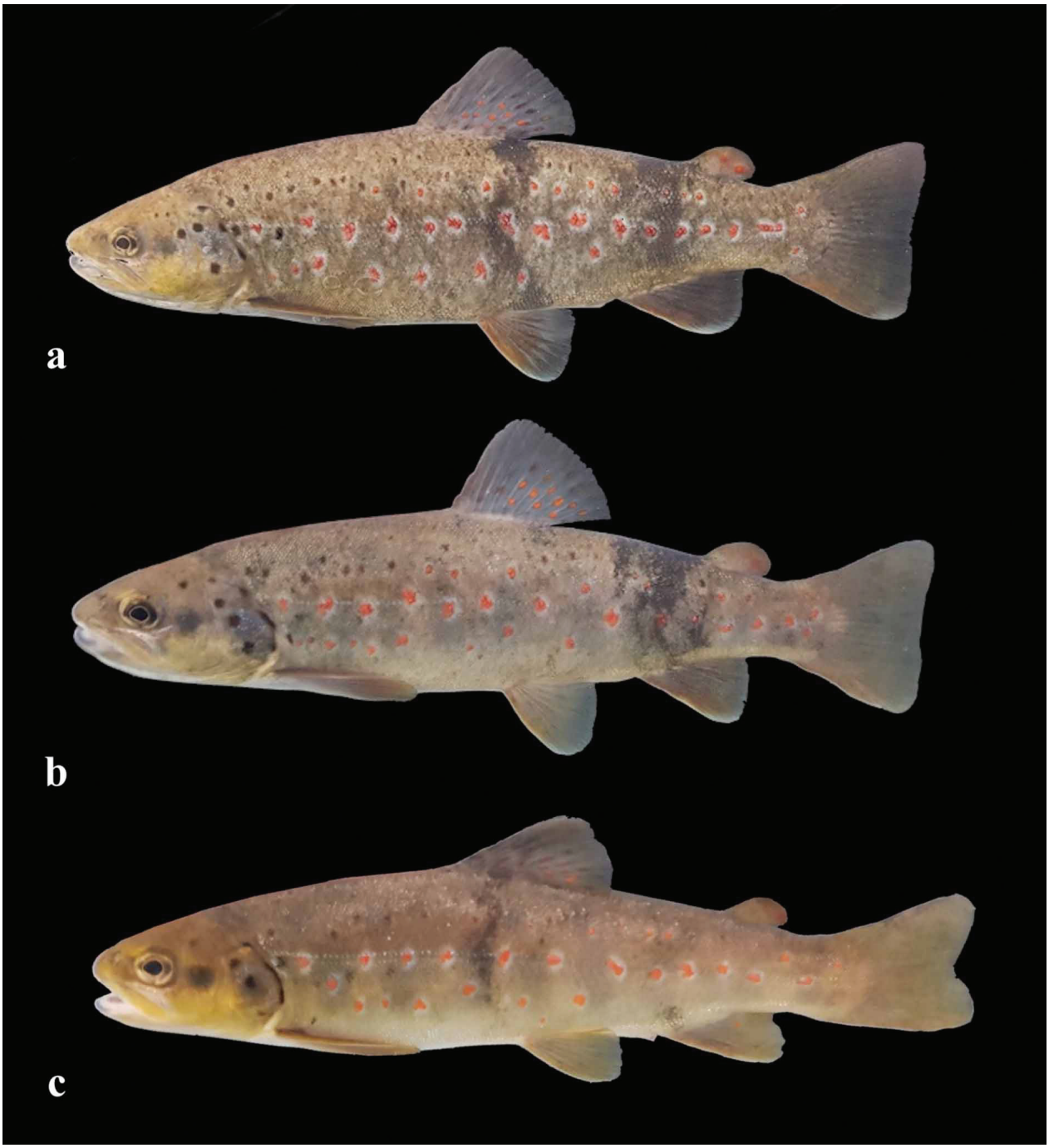


**Figure 3.** *Salmo baliki*, FFR 3234, paratypes, **a.** 216 mm SL, male; **b.** 170 mm SL, male; **c.** 164 mm SL, female; Turkey: stream Sinek, a tributary of Murat River.

to GenBank under the accession numbers: MW366844–MW366860 and MW382946–MW382950 (Table 2).

## Results

***Salmo baliki* sp. nov.**

http://zoobank.org/69483E41-85FA-42AF-AACF-2E69BBE83B0A

Figures 2–4

**Holotype.** FFR 3242, 212 mm SL; Turkey: Ağrı Province: stream Sinek a tributary of Murat River at Taşlıçay, 39.758749°N, 43.464480°E.

**Paratypes.** FFR 3234, 6, 132–276 mm SL; same data as holotype. —FFR 3205, 3, 175–267 mm SL; Turkey: Ağrı Province: a tributary of Murat River 39.730705°N, 43.481869°E.

**Additional record.** Turkey: Ağrı Province: stream Cuma at Cumaçay, 39.919118°N, 43.192272°E.

**Diagnosis.** *Salmo baliki* differs from the other species of trout recorded from the Euphrates and Tigris River drainages (*S. euphrataeus*, *S. okumusi*, *S. munzuricus*, *S. fahrettini* and *S. tigridis*) by having large and irregular-shaped red spots on its body (red spots larger than pupil, vs. smaller than pupil). *Salmo baliki* further differs from *S. euphrataeus* by the general body color silvery in

live (vs. brownish), a shorter head in the male (length 24–27% SL, vs. 27–31), a shorter maxilla in the male (length 8–9% SL, vs. 10–11), and a shorter mouth gape (12–14% SL in male, 11–12 in female, vs.14–17 in male, 12–15 in female). *Salmo baliki* further differs from *S. munzuricus* by having fewer black spots in postorbital and suborbital areas (commonly 1, rarely 2, vs. 3–24); fewer black spots on the body (less than 30, vs. more than 80 in adult specimens), whose number does not increase with size (vs. number increasing with size); the black spots circular (vs. irregularly shaped); commonly plain or rarely two roundish red spots on posterior edge of the adipose-fin in male and female (vs. plain or the adipose-fin with a very narrow white margin, then a red submarginal band, then a white band or spots, then a red band again in males, Figures 3, 5), a smaller adipose-fin (8–9% SL in male, 7–8 in female, vs. 9–12 in male, 8–10 in female) a deeper anal-fin in females (16–18% SL, vs. 13–16), a greater anal-fin base (10–12% SL, vs. 8–10), a longer median caudal-fin rays (14–16% SL, vs. 11–14), a greater mouth gape in females (12–13% SL, vs. 10–12), a deeper maxilla in females (maximum maxilla depth 3–4% SL, vs. 2–3), a smaller distance between the adipose and caudal fins in males (15–16% SL, vs. 16–18), and fewer lateral line scales (107–118, vs. 116–123). *Salmo baliki* further differs from *S. okumusi* by having one or two pale black spot in postorbital and suborbital area (vs. 1–22), fewer black spots on opercle (3–7, vs. 8–17), fewer black spots on the body in specimens smaller than 210 mm SL (less than 30, vs. more than 90), presence black spot on body in all size (vs. the body with black dots in specimens larger than about 230 mm SL), the shape of the black spots ocellated (vs. irregularly shaped), the black spots scatter on back and upper part of flank (vs. whole flank covered black spots or dots), no black spots on top head (vs. 5–18), one or two dark bands on posterior part of the flank (vs. four dark bands in specimens larger than about 230 mm SL), the parr marks vertically oblong (vs. vertically elongate), the shape of the black spots ocellated (vs. irregularly shaped), a more slender dorsal-fin in males (16–17% SL, vs. 17–19), a shorter pectoral-fin in males (17–19% SL, vs. 19–21), a smaller eye diameter (4–5% SL in males, 4–6 in females; vs. 5–7 in males, 6–8 in females), a shorter maxilla in males (8–9% SL, vs. 9–11) (see Figures 3, 6). *Salmo baliki* further differs from *S. tigridis* by having fewer scale rows between the dorsal-fin origin and the lateral line (24–28, vs. 32–35); fewer scale rows between the end of the adipose-fin base and the lateral line (13–15, vs. 19–20), a slenderer caudal peduncle depth (11–12% SL, vs. 12–13). *Salmo baliki* further differs from *S. fahrettini* by having fewer black spots on its body (less than 30, vs. more than 80); the black spots scatter on back and upper part of flank (vs. scattered on back, middle and upper part of flank, and anterior part of lower half of flank), their number not increasing with size (vs. increasing with size), fewer red spots on body (fewer than 42 in adult specimens, vs. more than 70 in adult specimens), their number not increasing with size (vs. increasing with size). See Discussion for comparison with other trout in adjacent waters of Anatolia.

**Table 1.** Morphometry of *Salmo baliki* (holotype, FFR 3242; paratypes FFR 3205, n = 3 and FFR 3234, n = 6). The calculations include the holotype.

| | Holotype | Paratypes | | | |
|---|---|---|---|---|---|
| **Sex** | **male** | **male** | | **Female** | |
| Number of specimens | | n = 4 | | n = 5 | |
| Standard length (mm) | 212 | 164–250 | | 132–267 | |
| In percentage of standard length | | Range (mean) | SD | Range (mean) | SD |
| Head length | 26.9 | 24.1–26.9 (25.7) | 1.1 | 24.2–26.0 (25.2) | 0.7 |
| Predorsal length | 47.4 | 45.0–49.7 (47.7) | 1.8 | 46.2–48.2 (47.4) | 0.8 |
| Prepelvic length | 52.7 | 52.7–55.6 (54.3) | 1.1 | 52.9–55.3 (54.1) | 1.0 |
| Preanal length | 72.7 | 72.7–76.0 (74.8) | 1.3 | 72.6–77.7 (75.2) | 2.0 |
| Body depth at dorsal-fin origin | 23.9 | 23.6–26.4 (24.7) | 1.2 | 22.2–25.8 (24.3) | 1.4 |
| Body depth at anal-fin origin | 19.1 | 18.5–20.8 (19.5) | 1.2 | 17.4–20.1 (18.9) | 1.2 |
| Depth of caudal peduncle | 10.6 | 10.6–11.7 (11.0) | 0.5 | 10.6–11.9 (11.2) | 0.5 |
| Length of caudal peduncle | 17.3 | 16.1–17.9 (17.2) | 0.7 | 15.8–17.9 (17.0) | 0.9 |
| Distance between adipose- and caudal-fins | 16.0 | 15.4–16.4 (15.8) | 0.4 | 16.1–17.9 (16.6) | 0.7 |
| Body width at anal-fin origin | 11.9 | 9.6–11.9 (10.6) | 0.9 | 8.9–11.7 (10.5) | 1.1 |
| Length of dorsal-fin base | 12.2 | 12.2–14.8 (13.5) | 1.0 | 12.9–14.0 (13.5) | 0.4 |
| Depth of dorsal-fin | 16.7 | 16.0–17.2 (16.6) | 0.5 | 12.8–18.2 (15.9) | 2.0 |
| Length of pectoral-fin | 17.8 | 17.0–19.0 (17.9) | 0.8 | 17.2–20.3 (19.1) | 1.2 |
| Length of adipose-fin base | 4.2 | 4.0–5.3 (4.7) | 0.6 | 3.9–5.0 (4.4) | 0.4 |
| Depth of adipose-fin | 8.4 | 7.5–8.7 (8.2) | 0.5 | 7.1–8.3 (7.7) | 0.6 |
| Length of pelvic-fin | 14.5 | 13.8–15.8 (14.8) | 0.8 | 14.0–15.2 (14.7) | 0.5 |
| Depth of anal-fin | 16.9 | 15.8–18.0 (16.9) | 0.8 | 16.3–18.3 (17.4) | 0.8 |
| Length of anal-fin base | 10.3 | 10.3–11.3 (10.9) | 0.4 | 10.2–11.8 (10.9) | 0.6 |
| Length of upper caudal-fin lobe | 17.0 | 15.3–17.2 (16.3) | 0.8 | 15.2–18.3 (16.7) | 1.1 |
| Length of median caudal-fin rays | 14.3 | 13.5–14.7 (14.3) | 0.4 | 13.5–15.7 (14.2) | 0.9 |
| Length of lower caudal-fin lobe | 17.5 | 15.6–18.2 (17.0) | 1.2 | 14.4–19.2 (16.9) | 1.9 |
| Snout length | 8.1 | 6.3–8.3 (7.2) | 0.9 | 6.6–7.6 (7.1) | 0.5 |
| Distance between nasal openings | 4.5 | 3.7–5.0 (4.4) | 0.5 | 4.0–4.7 (4.3) | 0.2 |
| Eye diameter | 4.0 | 3.6–4.9 (4.4) | 0.5 | 3.6–5.8 (5.1) | 0.9 |
| Interorbital width | 7.9 | 7.1–9.3 (8.3) | 0.8 | 6.9–8.1 (7.5) | 0.5 |
| Head depth through eye | 12.3 | 11.2–13.4 (12.5) | 0.9 | 11.4–13.5 (12.5) | 0.9 |
| Head depth at nape | 16.6 | 15.0–17.7 (16.3) | 1.0 | 16.0–18.6 (16.8) | 1.0 |
| Length of maxilla | 7.7 | 7.7–9.1 (8.4) | 0.5 | 8.2–9.6 (8.7) | 0.6 |
| Maximum height of maxilla | 3.1 | 2.5–3.1 (2.9) | 0.3 | 2.6–3.9 (3.2) | 0.5 |
| Width of mouth gape | 9.4 | 8.6–10.5 (9.6) | 0.7 | 8.7–10.1 (9.1) | 0.6 |
| Length of mouth gape | 13.9 | 11.7–13.9 (12.8) | 0.9 | 11.6–12.6 (11.8) | 0.3 |

**Description.** The general appearance is shown in Figures 2–4, morphometric data are in Table 1. Body deep, compressed laterally, its depth approximately equal to head length. Dorsal profile markedly arched and ventral profile less arched than the dorsal profile. Head short, upper profile

**Table 2.** Materials used in genetic analysis.

| Species | Sample | Locality | Accession number | Reference |
|---|---|---|---|---|
| *S. baliki* | 5 | Turkey: Agri, Sinek stream, Murat River, Euphrates River | MW366856–MW366860 | This study |
| *S. munzuricus* | 3 | Turkey: Tunceli, Munzur Stream, Euphrates | MN815914 | Turan et al. 2020 |
| | 5 | Turkey: Agri, Murat River, Euphrates River | MW382946–MW382950 | This study |
| *S. okumusi* | 3 | Turkey: Sivas, Gökpinar Stream, Euphrates | MN815915 | Turan et al. 2020 |
| *S. opimus* | 3 | Turkey: K.Maras, Göksun, Ceyhan River drainage | MW366853–MW366855 | This study |
| *S. chilo* | 3 | Turkey: Sivas, Akdere stream, Ceyhan River drainage | MW366850–MW366852 | This study |
| *S. labecula* | 3 | Turkey: Nigde, Ecemis stream, Seyhan River drainage | MW366847–MW366849 | This study |
| *S. platycephalus* | 3 | Turkey: Kayseri, Pinarbasi stream, Seyhan River drainage | MW366844–MW366846 | This study |
| *S. fahrettini* | 3 | Turkey: Erzurum, Omertepesuyu Stream Euphrates | MN815913 | Turan et al. 2020 |
| *S. coruhensis* | 3 | Turkey: Rize, Cayeli Kanlidere Stream | MN815912 | Turan et al. 2020 |
| *S. rizeensis* | 3 | Turkey: Rize, Kangel stream | MN815910 | Turan et al. 2020 |
| | 3 | Turkey: Rize, Alakoz stream | MN815910 | Turan et al. 2020 |
| *S. euphrataeus* | 3 | Turkey: Erzurum, Sirli Stream, Euphrates | MN815911 | Turan et al. 2020 |
| *S. caspius* | 2 | Turkey: Ardahan, Toros Stream, Kura River drainage | MN815909 | Turan et al. 2020 |
| | 2 | Turkey: Ardahan, Derindere stream, Kura River drainage | MN815909 | Turan et al. 2020 |
| | 2 | Turkey: Ardahan, Karaman stream, Kura River drainage | MN815909 | Turan et al. 2020 |
| *S. tigridis* | 3 | Turkey: Van, Catak Stream | MN815916 | Turan et al. 2020 |
| *S. trutta* | 1 | Italy: Flumendosa | LT617538 | Tougard et al. 2018 |
| | 1 | France: Vidourle | LT617535 | Tougard et al. 2018 |
| | 1 | Slovenia: Volaja | LT617539 | Tougard et al. 2018 |
| | 1 | United Kingdom: Camel | LT617540 | Tougard et al. 2018 |
| | 1 | Austria: Kleiner Kamp | KF985687 | Schenekar et al. 2014 |
| | 1 | Norway: Leksa | JX960836 | Crête–Lafrenière et al. 2012 |
| | 2 | Turkey: Van, Arpet Stream, Tigris | MT981164–MT981165 | Kaya 2020 |
| | 2 | Turkey: Bitlis, Sapur Stream, Lake Van | MT981168–MT981169 | Kaya 2020 |
| *S. obtusirostris* | 1 | Bosnia and Herzegovina: Neretva | JX960841 | Crête–Lafrenière et al. 2012 |
| *S. ohridanus* | 1 | Macedonia: Lake Ohrid | AF053590 | Sušnik et al. 2006 |
| *S. salar* | 1 | Norway: Ims | JX960834 | Crête–Lafrenière et al. 2012 |


**Figure 4.** *Salmo baliki*, FFR 3205, paratypes, **a.** 250 mm SL, male; **b.** 267 mm SL, female; Turkey: stream Sinek, a tributary of Murat River.

straight both on the snout and above the eye in male, straight above the eye and convex on snout in female. Mouth small, terminal or slightly subterminal in male, subterminal in female. Tip of lower jaw slightly curved upwards, pointed, with a slightly-developed process at symphysis in male larger than 200 mm SL. Maxilla short, reaching slightly beyond posterior margin of the eye in males and female larger than about 200 mm SL. Snout somewhat long, with

**Figure 5.** *Salmo munzuricus*: **a.** FFR 3226, 211 mm SL, male; Turkey: Tunceli Prov., stream Kalan; **b.** FFR 3241, 205, male; Turkey: Muş Prov., stream Mengel; **c.** FFR 3226, 240, male; Turkey: Ağrı Prov., stream Alakoçlu.

pointed tip in male, rounded in female. Adipose fin somewhat large, its height 7.5–8.7% SL in males and 7.1–8.3 in females. Largest observed specimen 250 mm SL.

Dorsal fin with 3–4 unbranched and 8–10 branched rays, its distal margin slightly convex. Pectoral fin with 1 unbranched and 10–11 branched rays, its external margin slightly convex. Pelvic fin with 1 unbranched and 7–8 branched rays, its external margin slightly convex. Anal fin with 3 unbranched and 7–9 branched rays, its distal margin convex anteriorly and straight or concave posteriorly. Caudal fin deeply emarginated in specimens less than 160 mm SL, slightly emarginated or truncate in specimens larger than about 200 mm SL, lobes slightly pointed. Lateral line with 107–118 scales; 24–28 scale rows between dorsal-fin origin and lateral line; 18–22 scale rows between anal-fin origin and lateral line; 13–15 scale rows between origin of the adipose fin and lateral line. Gill rakers 16–18 on first gill arch.

**Coloration.** In formalin: General coloration of freshly preserved specimens silvery on back and flank, yellowish on the belly. One pale black spot in postorbital and suborbital areas, greater than pupil; three to seven black spots on opercle, approximately smaller than pupil. Black spots on body few (fewer than 30), smaller than the pupil, ocellated, scattered on the upper part of flank (missing in back). No black spot on top of the head. Red spots few (fewer than 30), large (greater than pupil), irregularly-shaped, surrounded by an irregularly shaped narrow ring, organized in two to four irregular longitudinal rows on median part of the body, and half

**Figure 6.** *Salmo okumusi*, FFR 3157, 260 mm SL, male; Turkey: stream Gökpınar, a tributary of Tohma River.


**Figure 7. a.** Stream Sinek, Murat River, Turkey; type locality of *Salmo baliki*: **b.** Stream Alakoçlu; **c.** Stream Mengel, Murat River Turkey, two new localities of *S. munzuricus*.

of lower part of the flank. The number of black and red spots on flanks does not increase with increasing size. Dorsal fin grey, with three or four rows of black spots (smaller than pupil) and one or two rows of red spots (smaller than pupil). Caudal fin dark gray; pectoral, anal pelvic fins greyish. Adipose-fin plain grayish, rarely one or two red spots on its posterior edge. Eight to nine oblong parr marks on the body, distinct in specimens up to about 190 mm SL. 1–2 vertical bands on posterior half of flank in most specimens.

**Distribution and habitat.** *Salmo baliki* inhabits clear and moderately swift-flowing water, with a substrate of stones and pebbles. The observed material for this species has only been collected from stream Sinek, drainage of Murat River (Figures 1, 7a). The species has not been found in adjacent waters except stream Cumaçay (39.919118°N, 43.192272°E) that is located approximately 32 km northwest of Sinek, another drainage of Murat River. However, there was no opportunity to carry out survey in the stream Cumaçay location. Following solid evidences of shape and size of the spots from the video records shown by local people, this location will further be investigated in the near future.

**Conservation status.** There is serious pressure on the populations of *Salmo baliki* due to overfishing. The species is taken for curative purposes, hence demand is very

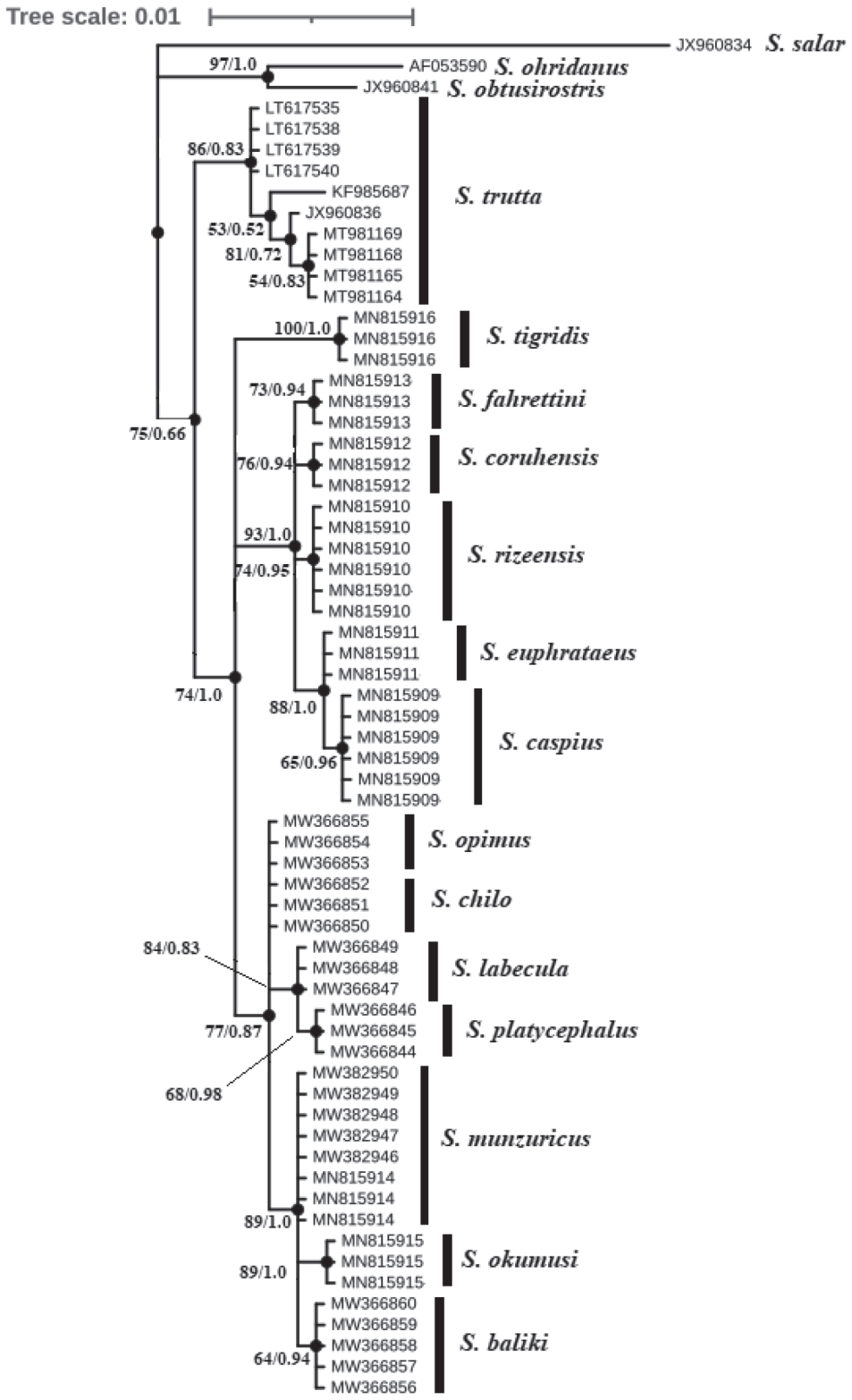


**Figure 8.** Bayesian inference (BI) phylogenetic tree based on Cyt *b* sequences of *Salmo* species. ML and BI methods generated the similar topologies and therefore only the BI tree is shown. The bootstrap values of ML and posterior probability values of BI are indicated on nodes (ML/BI).

high. Within the first fieldwork in the area which was carried out in 2006, in total 3 specimens were found in the middle of the stream (39.730705°N, 43.481869°E), however no specimens were detected in the same location during the recent survey, only a small population observed about 4 km upstream (a restricted area, far from the villages, and the only transportation is provided through a rough and muddy road). Taking all these factors into account, endemic *S. baliki* is stuck in a very limited area, thought to be under a serious threat. There-

fore, there is a need for the species to be conserved under international legislation.

**Sexual dimorphism.** The snout of the male is more pointed than that of the female. The depth of the adipose-fin and the length of the mouth gape in male are greater than those of the female.

**Etymology.** The species is named after Dr. Süleyman Balık (Turkey), taxonomist, in appreciation of his contributions to the freshwater fish fauna of Turkey.

**Phylogenetic placement of *Salmo baliki*.** We analyzed a total of 65 sequences (22 new sequences in this study and 43 sequences from GenBank; Table 2) to assess if the phylogenetic relationship among *Salmo* sp. *Salmo baliki* new species is genetically different from the other *Salmo* species. The nodes separating the species in the phylogenetic tree topology of the Cyt *b* gene were supported by high posterior probability and bootstrap values. (Figure 8). The bootstrap values in ML analysis are relatively low compared to the posterior probability values in BI analysis. However, the two tree topologies do not contradict each other. According to the result of phylogenetic analysis, *Salmo baliki* is a sister taxon to *S. munzuricus* (Figure 8). For *Salmo* species, phylogenetic tree topology corresponds exactly with the fiction formed as a result of morphological data.

**Range extension of *Salmo munzuricus*.** Within the aim of the present study a geographic range extension for *Salmo munzuricus* was also recorded. This species was previously only described from Munzur River, north western Euphrates (Turan et al. 2017). Here, two new localities have been identified for *S. munzuricus* (Figure 7b, c). These new localities in the Murat River, located 140 and 340 km east of the previously known distribution range of the species, reveal the scarce bio-geographic knowledge of the species in the Euphrates basin.

## Discussion

The population of *Salmo baliki* has been experiencing a serious pressure caused by local people and fisherman. The trout inhabiting stream Sinek is thought to be a 'healer fish', as appeared in local and national press, thus sold for higher prices throughout the country. This is the main reason behind the relatively low number of specimens investigated in the present study as opposed to standard morphometric studies (10 fish versus 25 fish). However, evidence, discussions with local people and video recordings suggest an additional locality for *S. baliki*, soon to be confirmed.

Trout inhabit cold, well-oxygenated waters where the flow is relatively high and species get restricted to such locations. This leads to ecological isolation from the other populations inhabiting same water bodies. Hence, this, in turn, has a significant effect on speciation. Although there are significant morphological differences among the trout species, relatively lower genetic distances in mtDNA sequences indicate an early stage of speciation taking place in *Salmo* genus within the course of evolution. In total, fifteen native trout species have been identified in Turkey, six of which are known from the Euphrates and Tigris drainages, namely; *Salmo tigridis*, *S. okumusi*, *S. euphrataeus*, *S. munzuricus*, *S. fahrettini* and *S. baliki* (in the present study). Those of *Salmo baliki*, *S. munzuricus* and *S. okumusi* belong to Adriatic linage. Molecular distance among these species is not very distinct, however, the remarkable morphological differences easily separate these species which are presented above in the diagnosis section (see also Figure 2–4, 5 and 6).

*Salmo baliki* is easily distinguished from *S. platycephalus*, *S. chilo*, *S. labecula* and *S. opimus*, all from streams draining to the Mediterranean, by zero to two dark bands on the posterior part of the flank (vs. four dark bands on flank), a smaller eye in males (eye diameter 4–5% SL, vs. 6–7), in having more scale rows between the anal-fin origin and the lateral line (18–22, vs. 15–18).

*Salmo baliki* further differs from *S. platycephalus* and *S. labecula* by having fewer gill rakers on the first gill arch (16–18, vs. 21–25), a shorter head in males (head length 24–27% SL, vs. 27–29) and the presence of red spots in specimens larger than about 70 mm SL (vs. absence). In *S. baliki*, the top of the head is not flattened, while the top of the head is flattened in *S. platycephalus*,

*Salmo baliki* further differs from *S. chilo* by the dorsal profile of the head being straight in the interorbital area and at the level of the nostrils (vs. strongly convex), the snout slightly pointed in the male (vs. blunt), the maxilla and lower lip are not fleshy (vs. flesh), fewer black spots behind the eye (always one, vs.up to 12) and fewer black spots on the opercle (3–7, vs. 7–13). *Salmo baliki* also differs from *S. chilo* by the number and position of the black spots on the body in males. In *S. baliki*, black spots are fewer (less than 30) and located on the upper part of the flank. In *S. chilo*, there are numerous (more than 40) black spots which are scattered on the middle part of the body, mostly on the anterior part; however, these are missing on the back in specimens larger than 140 mm SL.

*Salmo baliki* further differs from *S. opimus* by having a slenderer body in male (23–26% SL, vs. 26–29), a shorter maxilla in male (maxilla length 8–9% SL, vs. 9–10) with the black circular (vs. irregularly shaped) spots. Additionally, the top of the head is straight in male (vs. convex) and the mouth is located terminally or slightly subterminal in male (vs. conspicuously subterminal).

*Salmo baliki* is most notably distinguished from *S. caspius* (from Kura River drainage) by having fewer gill rakers on the outer side of the first gill arch (16–18, vs. 19–21) and no black spots on the top of the head (vs. small black spot on top of head). It further differs from *S. caspius* by having a greater distance between adipose and caudal fins in male (15–18% SL, vs. 14–15), a shorter head in male (24–27% SL, vs. 27–31), a shorter and narrower maxilla in male (maxilla length 8–9% SL, vs. 9–11; maxilla width 2–3% SL, vs. 3–4). In the male specimen of *Salmo baliki*, the anal- and adipose-fins do not reach the caudal-fin base (vs. reaching in specimens larger than 200 mm SL) and the general body color is silvery in live (vs. brownish).

*Salmo baliki* differs from *S. rizeensis* by the general body color being silvery in live (vs. brownish) and the absence of black spots on the back (vs. presence). *Salmo baliki* also differs from *S. rizeensis* by having more scale rows between anal-fin origin and lateral line (24–28, vs. 18–22), less branched dorsal-fin rays (7–9, mode 9, vs. 9–12, mode 10), a shorter head in male (24–27% SL, vs. 29–31), a deeper caudal peduncle (11–12% SL, vs. 10–11), a greater adipose fin (length of base of adipose-fin 4–5% SL, vs. 3–4), a smaller maxilla in male (length of maxilla 8–9% SL, vs. 10–12), and a smaller mouth gape in male (length of mouth 12–14% SL, mean 12.8, vs. 13–18, mean 15.5).

*Salmo baliki* is immediately distinguished from *S. coruhensis* by the number and distribution of the black and red spots on the body and the way they vary with increasing size. In *S. baliki*, the black spots are few, small, ocellated and restricted to the upper part of the flank and missing on the back. The red spots are few, large, irregularly shaped and scatter on the half of the lower and the upper, and median part of the flank. The number of black spots does not increase with increasing size. In *S. coruhensis*, the black spots are numerous, from medium to large, ocellated, and present on the whole upper half of the flank and on the anterior part of the lower half. The red spots are irregularly shaped and ocellated, and do not increase with increasing size. The number of both kinds of spots increase with increasing size and age. *Salmo baliki* usually has a single pale black spot behind the eye (on cheek and preopercle) at all sizes in both sex; 3 to 7 spots on the opercle. *Salmo coruhensis* has two or three spots on the cheek and the preopercle in most specimens, rarely a single one spot found and this number increases to 4–17 in large adult male; 5–14 spots on the opercle.

*Salmo baliki* is also distinguished from *S. trutta* by having fewer lateral line scales (107–118, vs. 117–128), fewer scale rows between lateral line and dorsal–fin origin (27–30, vs. 30–34), a shorter head in males (24–27% SL, vs. 28–31), a smaller maxilla (length of maxilla 8–10% SL, vs. 10–12), a smaller mouth gape in males (length of mouth gape 11–14% SL, vs. 14–16), a deeper caudal peduncle (11–12, vs. 10–11) and greater adipose-fin in males (8–9% SL, vs. 7–8). *Salmo baliki* further differs by body color and pattern. In *S. baliki*, red spots are large (greater than eye diameter, vs. smaller than eye diameter) and irregular-shaped (vs. roundish); black spots are few (less than 30, vs. more than 70) and scatter on upper part of flank (vs. numerous and scatter on back, upper part and middle part of flank, sometimes lower part of flank).

Results of genetic work was in correspondence with the morphological observations. Although a single mtDNA region (Cyt *b*) was used to assess phylogenetic relationship among *Salmo* sp. in comparison with *S. baliki*, support of high bootstrap and posterior probability values indicated separation among *Salmo* species inhabiting Anatolia. *Salmo* sp. are known to be recently diverged (Lobón-Cerviá 2018 and references therein) within the course of evolution, thus diversification of species is still an ongoing process, and mostly supported by lower genetic distances among species. However, phylogenetic analysis of the present study indicated a separate branch for *Salmo baliki*, when compared with the *S. euphrataeus*, *S. fahrettini*, *S. munzuricus* inhabits the same basin. Additionally, each of these species formed unique haplotypes, supporting differences among closely related species of *S. munzuricus* and *S. okumusi*.

## Comparative material

See Turan et al. (2010, 2011, 2012, 2014a, 2017, 2020) for additional comparative materials examined.

***Salmo munzuricus*:** FFR 3235, 13, 170–253 mm SL; Turkey: Ağrı province: stream Alakoçlu, a tributary of Euphrates River at Taşlıçay, 39.475000°N, 43.267000°E. —FFR 3241, 4, 108–205 mm SL; Turkey: Muş province: stream Mengel at Alabalık village, a tributary of Murat River, 39.313686°N, 41.162689°E. —FFR 3226, 11, 123–211 mm SL; Turkey: Tunceli province: stream Kalan at Sarıtaş village, a tributary of Munzur River, 39.249975°N, 39.489062°E.

***Salmo trutta*:** Germany, 7, 111–156 mm SL; Rhine River, Plesibach Stream at Niederpleiss.

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PENSOFT

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# A fantastic new species of secretive forest frog discovered from forest fragments near Andasibe, Madagascar

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## Abstract

We describe a fantastic new species of forest frog (Mantellidae: *Gephyromantis*: subgenus: *Laurentomantis*) from moderately high elevations in the vicinity of Andasibe, Madagascar. This region has been surveyed extensively and has a remarkably high anuran diversity with many undocumented species still being discovered. Surprisingly, by exploring areas around Andasibe that lacked biodiversity surveys, we discovered a spectacular and clearly morphologically distinct species, previously unknown to science, *Gephyromantis marokoroko* **sp. nov.**, documented for the first time in 2015. The new species is well characterised by a very rugose and granular dorsum, dark brown skin with bright red mottling, sparse light orange to white spots on the ventre, vibrant red eyes and femoral glands present only in males that consist of eight medium-sized granules. Bioacoustically, the new species has a quiet advertisement call that differs from related species by having a moderate call duration, 2–4 strongly pulsed notes and a slow note repetition rate. Furthermore, it has substantial differentiation in mitochondrial DNA, with pairwise distances of 7–9% to all other related species in sequences of the mitochondrial 16S rRNA marker. Additional evidence is given through a combined four mitochondrial markers and four nuclear exons concatenated species tree, strongly supporting *G. striatus* as the sister species of the new species in both analyses. The discovery of this new species highlights the need for continued inventory work in high elevation rainforests of Madagascar, even in relatively well-studied regions.

## Key Words

Amphibia, Anura, Andasibe, *Gephyromantis marokoroko*, Mantadia, new species, taxonomy

## Introduction

Madagascar hosts an impressively diverse and almost entirely endemic amphibian fauna, diversified into a multitude of different habitats and micro-habitats (Glaw and Vences 2007). Amongst the small, brown leaf litter frogs, members of the genus *Gephyromantis* Methuen, 1920 are well represented in Madagascar. Most small, brown, leaf litter frogs in Madagascar are members of Microhylidae, but Mantellidae has some representatives in the genus *Gephyromantis* Methuen, 1920. *Gephyromantis* is a genus within the Malagasy-Comoran endemic family Mantellidae (Glaw and Vences 2007). Within *Gephyromantis*, there are 47 recognised species belonging to six subgenera (Glaw and Vences 2006; Vences et al. 2017; AmphibiaWeb 2021), which are supported by molecular and morphological criteria (Glaw and Vences 2006; Wollenberg et al. 2011; Kaffenberger et al. 2012).

Recently, Madagascar's unique biota has been the focus of intensive field surveys (e.g. Goodman and Benstead 2003; D'Cruze et al. 2009; Hutter et al. 2015; Scherz et al. 2017a), with many previously unknown species discovered, typically through extensive molecular identification of candidate species lineages (Vieites et al. 2009; Perl et al. 2014). This is true for *Gephyromantis*, which has several candidate lineages identified (Scherz et al. 2017a, 2017b; Vences et al. 2017), some of which have recently been described (Scherz et al. 2017b, 2018). Despite these barcoding efforts, entirely new species, not previously identified from molecular barcoding, are still being discovered and described (Scherz et al. 2017a; Scherz et al. 2018), suggesting that many new species to science remain elusive.

Herein, we describe another *Gephyromantis* species new to science from the subgenus *Laurentomantis* that has not been previously identified through molecular barcoding studies. This new species is not morphologically cryptic and was immediately recognisable as a new species upon discovery in recent expeditions to remote high-elevation forests surrounding the village of Andasibe that lack biodiversity surveys. Despite the Andasibe region being one of the most well-studied areas in Madagascar (Vieites et al. 2009), this study shows that clearly recognisable new species that have not been barcoded are still being discovered by recent surveys. As a result of these discoveries, we emphasise that continued exploration and surveys in Madagascar are needed, even in relatively well-studied regions. Conservation of small forest fragments is especially important, as many undiscovered species may remain undetected.

# Materials and methods

## Terminology

We follow the unified concept of species (i.e. general lineage concept), which defines a species as a separately evolving lineage (Simpson 1961; Wiley 1978; de Queiroz 1998, 2005, 2007). We use multiple lines of evidence (i.e. secondary criteria) in assessing species boundaries, combining data from morphology, phylogenetics, bioacoustics and biogeography (de Queiroz 2007; Padial et al. 2010; Vences et al. 2013). This evidence is then considered equally and used as support for the hypothesis that a given population is an independently evolving lineage and, thus, a distinct species. Family and generic names follow the taxonomy proposed by Glaw and Vences (2006). Geographic regions for biogeographic analyses are defined according to Boumans et al. (2007). According to this zonation, the Andasibe area is within a region named the "Northern Central East" of Madagascar.

## Specimen collection and morphological measurement

Specimens were collected at night through targeted searches of the new species' microhabitat. Specimens were euthanised using Tricaine, fixed in ~ 10% formalin (buffered with sodium phosphate to ~ 7.0 pH) for 24 hours and then stored in 70% ethanol for long-term preservation. We deposited and examined alcohol-preserved specimens from the amphibian collections at the Biodiversity Institute of the University of Kansas (KU) and Département de Biologie Animale, Antananarivo (UADBA) (Appendix I). Additional collection acronyms used herein are FAZC, ZCMV, FGZC and LR (field number series of F. Andreone, M. Vences, F. Glaw and L. Raharivololoniaina, respectively), FGMV (field number series shared between M. Vences and F. Glaw) and ZSM (Zoologische Staatssammlung München, Germany). All photographs were taken by CRH, unless otherwise noted.

Morphological measurements were taken by ZFA with a Mituyo digital caliper (precision 0.01 mm) rounded to 0.1 mm. Terminology and measurements largely follow Glaw et al. (2001) and we used the following: (1) snout-vent length (SVL); (2) head width at the greatest point (HW); (3) head length (= rostrum) from snout tip to posterior edge of tympanum (HL); (4) horizontal eye diameter (ED); (5) interorbital distance (IOD); (6) eye-snout tip distance (ESD); (7) eye-nostril distance (END); (8) distance from nostril-snout tip (NSD); (9) distance between nostrils (NND); (10) horizontal tympanum diameter (TD); (11) upper arm length (humerus), from the articulation of the arm with the trunk to the elbow (UAL); (12) lower arm length (= radioulna), from the humerus-radioulna articulation point (elbow) to carpal-metacarpal articulation (LAL); (13) hand length from carpal-metacarpal articulation to tip of longest finger (HAL); (14) forelimb length, sum of UAL, LAL and HAL (FORL); (15) forearm length, summed from and UAL and LAL (FARL); (16) Finger I length from outer margin of palmar tubercle to tip of Finger I (FIL); (17) Finger II length from outer margin of palmar tubercle to tip of Finger II (FIIL); (18) femur length from femur-tibia articulation (knee) to cloaca (FEML); (19) tibia length from femur-tibia articulation to heel, measured along the shank (TIBL); (20) tarsus length from heel to base of foot (TARL); (21) foot length from tarsal-metatarsal articulation to tip of longest toe (FOL); (22) length of femoral gland, horizontal across the thigh (FGL); (23) width of femoral gland (FGW); and (24) the number of femoral gland clusters on each thigh (FGC).

## DNA sequencing and phylogenetics

Following euthanasia, we extracted whole livers and left hind limb muscles and stored the tissues in 95% ethanol. We obtained new genetic data for four specimens of the new species and one specimen from five other species in *Laurentomantis* from the 3' fragment of the 16S rRNA mitochondrial marker widely used for molecular comparisons and species barcoding in Mantellidae (e.g. Vieites et al. 2009). The methods for DNA extraction, primers used, PCR amplification and sequencing are described

in Hutter et al. (2018). Finally, additional mitochondrial and nuclear markers from one specimen of the new species (KU 343230) were acquired by extracting the target markers from samples sequenced using the FrogCap Ranoidea-V1 probe-set (Hutter et al. 2021; available at: https://github.com/chutter/FrogCap-Sequence-Capture). Probe design, sequencing and analytical methods are described in Hutter et al. (2021) in detail. After sequencing, DNA data were manually edited for quality in Geneious R9 (Biomatters 2016). Sequences were deposited in GenBank and their associated voucher specimens and accession numbers are provided in Appendix I.

We aligned the new sequences with 16S sequences from Kaffenberger et al. (2012) to confirm the subgeneric relationship of the new species (tree not shown). We next chose sequences for 16S from all *Laurentomantis* and several representatives from other species in *Gephyromantis* and the distantly-related *Mantella madagascariensis* as outgroups. In total, we supplemented these new data with 182 published sequences of *Gephyromantis* specimens from GenBank. The distantly-related *Mantella madagascariensis* was used as an outgroup to root the phylogeny. GenBank accession numbers and their associated specimen data are included in Appendix I.

The 16S rRNA sequence data were first aligned with MAFFT v.7.3 using the RNA alignment algorithm Q-ins-I (Katoh and Stanley 2013). We used Maximum Likelihood (ML) in IQ-Tree v.1.5.5 (Nguyen et al. 2015) to conduct phylogenetic tree reconstruction with default options selected. We used ModelFinder (Kalyaanamoorthy et al. 2017) to find a best-fit partitioning scheme and selected models of molecular evolution for each partition considering all models. We assessed support using 1000 ultrafast bootstrap replicates (Minh et al. 2013). Strongly supported nodes are those with 95 or higher bootstrap (BS).

For Bayesian Inference (BI), we used MrBayes 3.2 (Ronquist et al. 2012) and the best partitions and models selected above. We used reversible jump Markov Chain Monte Carlo to accommodate uncertainty in model selection (parameter set: *nst=mixed*). The analysis was run for two independent runs of 50 million generations sampling every 1000 generations. Chain mixing and stationarity were assessed by examining the standard deviation of split frequencies and by plotting the -lnL per generation using Tracer 1.5 software (Rambaut and Drummond 2007), where we discarded 25% of the generations as burn-in. Finally, results were combined using logCombiner 1.10 software (Rambaut and Drummond 2007) to obtain a 50% majority rule consensus tree and node posterior probabilities. Strongly supported nodes are those with a posterior probability (PP) of 0.95 or higher.

## Bioacoustics

Advertisement calls were recorded in the field with a Marantz PMD 661 MKII Field Recorder and a Sennheiser MKH 8060 shotgun microphone. The calls were recorded in WAV format with a sampling rate of 44.1 kHz/s with 16 bits/sample. Advertisement calls analysed here have been deposited on FigShare (10.6084/m9.figshare.16728994). Calling males were recorded while inside plastic collecting bags at ~ 100 cm because we could not approach them close enough to record them in the field (we did not perceive a difference between the captive and *in situ* advertisement calls). We measured call parameters using RavenPro 1.5 (K. Lisa Yang Center for Conservation Bioacoustics 2014). Frequency information was obtained through Fast Fourier Transformation (FFT; width 1012 points). A Hanning window (512 bands) was used to create the spectrogram. Measures are reported as the range followed by the mean ± two standard deviations from the mean. Terminology generally follows Köhler et al. (2017), with a call defined as the entire assemblage of acoustic signals emitted in sequence and notes are subunits separated by temporally distinct segments of background noise between each note.

We chose the following relevant call variables, generally following the call-centred definitions of Köhler et al. (2017) and Hutter et al. (2013; Table 1): (1) number of notes per call; (2) call duration (ms); (3) call interval duration (ms); (4) note duration (ms); (5) inter-note interval duration (ms); (6) note repetition rate within call (notes/s); (7) pulse rate (/s); (8) dominant frequency, measured at peak amplitude (Hz); and (9) frequency bandwidth (Hz), measured as 90% of the sound energy.

Finally, we evaluated the amount of bioacoustic differences between species following Vieites et al. (2009). We considered differences in general call structure (e.g. pulsed/tonal notes, consistent note arrangements, amplitude envelope shape; Ryan and Rand 1990) and such temporal variables that are putatively less influenced by temperature, body size and behaviour (e.g. note duration, pulse rate; Gerhardt et al. 2000) to be important traits for distinguishing species.

# Results

We discovered a morphologically distinct new species belonging to the subgenus *Laurentomantis* from *Gephyromantis* in the Andasibe area of Northern Central East Madagascar (Fig. 1), present at high elevations in several small forest fragments. The new species can be readily identified morphologically through its rugose and granular dorsal texture with prominent ridge elements, red dorsal colouration on a dark brown background, bright red eyes, the relatively large number of eight granules within each femoral gland and absence (or indistinction) of vertebral stripe (Fig. 2). Finally, comparisons of the uncorrected raw genetic distances give a minimum distance of 6–9% with *G. ventrimaculatus* in the mitochondrial marker 16S rRNA and greater distances with other species in *Laurentomantis* (Fig. 3). Phylogenetically, the new species position is poorly supported in 16S rRNA, but strongly supported sister to *G. striatus* in the combined nine marker mitochondrial and nuclear phylogeny (BS = 98; PP = 1.00;

Fig. 4). Furthermore, the genetic distances between *G. striatus* and the new species are 7–9%. The new species also has an advertisement call similar to that of other *Laurentomantis*, but can be distinguished through the combination of a moderate call duration, differing note structure with 2–4 clearly defined pulses and slower note repetition rate when compared to related species. Given the strong evidence, we describe the new species as follows:

### *Gephyromantis marokoroko* sp. nov.

http://zoobank.org/3A22A655-D3B9-4C69-BF6F-C8F1E9595D84

Common English name: The Rugose Forest Frog.

Common Malagasy name: Ny sahon'ala marokoroko.

**Holotype.** KU 343230 (field number CRH 1110), an adult male collected by Carl R. Hutter, Shea M. Lambert and Zo F. Andriampenomanana collected on 5 January 2016, at Vohidrazana Forest (18.976°S, 48.499°E; ca. 1150 m a.s.l.) in mid-altitude rainforest near Andasibe in Northern Central East Madagascar (Fig. 1).

**Paratypes.** Adult male KU 343229 (CRH 1108), adult female UADBA-CRH 472 and adult male UADBA-CRH 1061 collected on 5 January 2016, with same collection data as holotype. Adult female KU 343218 (CRH 1397) collected on 18 January 2016, at Tavolobe (19.005°S, 48.461°E; ca. 1100 m a.s.l.) by Carl R. Hutter, Shea M. Lambert, Ginah Tsiorisoa Andrianasolo and Kerry A. Cobb. Adult male UADBA-CRH 1626, Adult female UADBA-CRH 1819 collected on 6 January 2017 at Vohidrazana Forest, adult female KU 347328 (CRH 1923) collected on 14 January 2017 at Andasibe-Mantadia National Park (Belakato: 18.821°S, 48.439°E; ca. 1050 m a.s.l.) and adult female KU 347329 (CRH 2019) collected on 21 January 2017 at Vohimana (18.926°S, 48.489°E; ca. 1050 m a.s.l.), collected by Carl R. Hutter, Jary Harinarivo and Robin K. Abraham.

**Available names.** There are no junior synonyms available that could be assigned to the new species from the subgenus *Laurentomantis*.

**Etymology.** The specific epithet *marokoroko* is a Malagasy word meaning "rugose" or "rugged". The name was chosen to describe the rugose skin texture of this species. The name is to be treated as an invariable noun in apposition.

**Diagnosis.** *Gephyromantis marokoroko* (Fig. 2) is a member of the family Mantellidae, subfamily Mantellinae, as diagnosed by Glaw and Vences (2006). The new species can be diagnosed to the genus *Gephyromantis* morphologically through its granular dorsum, moderately enlarged fingertips, absence of foot webbing, bifid tongue and small femoral glands present only in males as a small number of large granules (type 2; Glaw et al. 2000). Within *Gephyromantis*, the new species can be diagnosed to

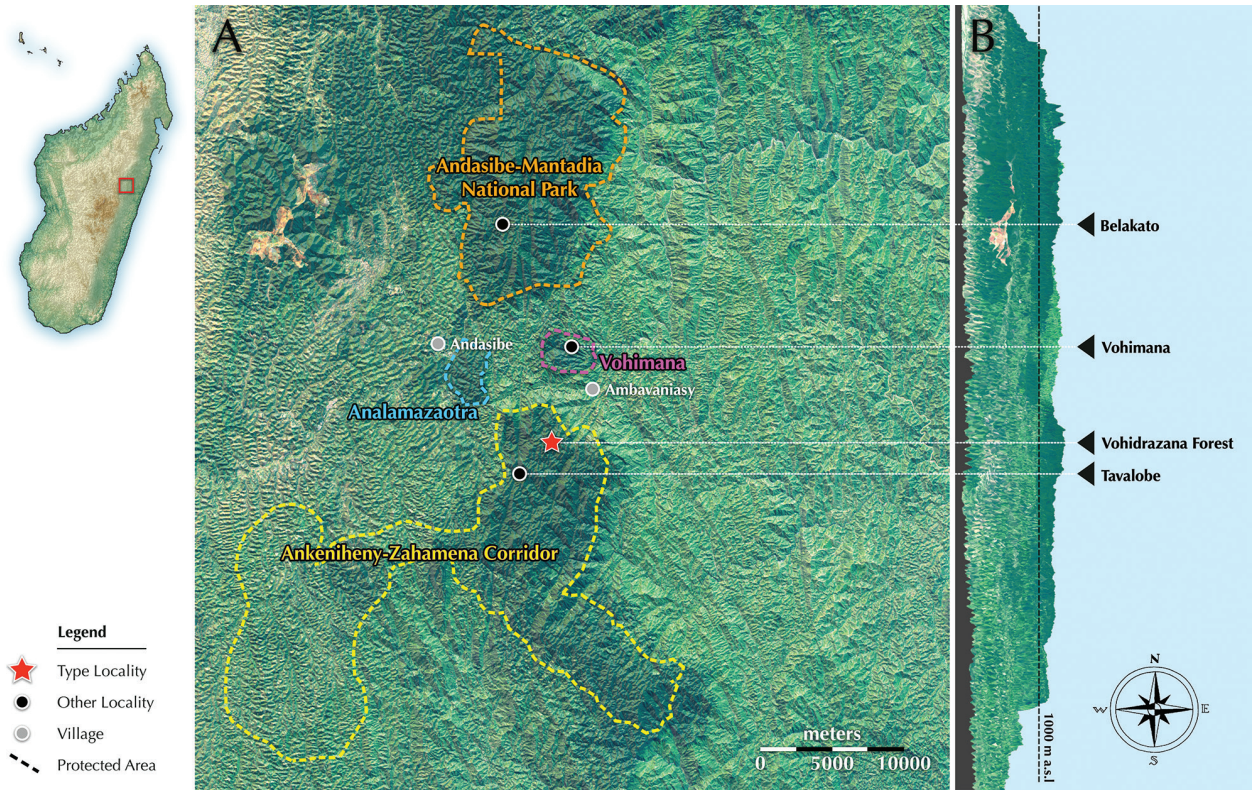


**Figure 1.** The distribution of *Gephyromantis marokoroko* sp. nov. in east-central Madagascar, view from above (**A.**) and from a profile view (**B.**). The black star marker indicates the type locality at Vohidrazana Forest where the black circle "locality" markers indicate other confirmed localities for the new species. *Gephyromantis marokoroko* sp. nov. is also found at high elevations and, thus, is likely distributed at other high elevation sites not surveyed. Elevational and satellite imagery data acquired from the USGS Earth Explorer (http://earthexplorer.usgs.gov).

the subgenus *Laurentomantis* through its irregular and rough granular dorsum, single subgular vocal sac in males, completely connected lateral metatarsalia, inner and outer metatarsal tubercle present and tympanum is the same size in male and female.

*Gephyromantis marokoroko* is characterised by bright red eyes, prominent ridge elements on dorsum, life colouration with a dark brown ground colour with mottled red and grey, hind-limbs dark brown containing red cross-bands, absence of red colouration on the sides of thighs and ventre, white spots on grey-coloured ventre and males with bulbous type 2 femoral glands with eight granules in two rows of four on each thigh. Furthermore, the new species is characterised by an advertisement call with a moderately long call duration (1095–1431 ms), 22–28 notes/call, 2–4 strong amplitude-modulated pulses per note and a dominant frequency of 2250–2812 Hz. Finally, *Gephyromantis marokoroko* has a large genetic distance of 6% or greater amongst related species in the 16S rRNA marker and has strongly supported reciprocal monophyly to all other species in *Laurentomantis* (Fig. 3).

*Gephyromantis marokoroko* can be distinguished from other members of *Laurentomantis* morphologically (Table 1; Fig. 5). The rugose and granular dorsal texture with prominent ridge elements and red mottled colouration and the larger number of eight prominent femoral gland granules per femur readily characterise this species from other *Laurentomantis* (Figs 3 and 5). The new species is easily distinguished from *G. horridus* (Boettger 1880), *G. malagasius* (Methuen and Hewitt 1913) and *G. ranjomavo* (Glaw and Vences 2011) by lacking tibial glands, its larger number of femoral gland granules and its rugose and granular dorsal texture with prominent ridge elements. Furthermore, the new species is easily distinguished from *G. ventrimaculatus*, where *G. marokoroko* has eight distinct femoral gland granules on each thigh (eight irregularly-shaped femoral gland granules in *G. ventrimaculatus*), by the dark grey and red dorsal colouration (light brown in *G. ventrimaculatus*) and by lacking blue marbling on the ventral surfaces (Fig. 5). The most similar species morphologically is *G. striatus* (Vences et al. 2002), but the new species differs from *G. striatus* through its larger number of femoral gland granules (8 vs. 3–6), the vertebral stripe is absent or indistinct and short (always distinct in *G. striatus*), bright red eye (orange-brown in *G. striatus*) and its prominent and strong ridge elements, as well as the dark grey and red colouration on the dorsum (weak ridge elements and brown and orange colouration on the dorsum in *G. striatus*).

Bioacoustically, the advertisement call of *Gephyromantis marokoroko* is similar to other species in *Laurentomantis* and can be distinguished from all other species in this subgenus through the following combination of continuous call characters: (1) moderately long call duration (1095–1431 ms); (2) 2–4 strongly amplitude-modulated pulses per note; and (3) a note repetition rate of 14–20 notes/s. *Gephyromantis striatus*, *G. malagasius* and *G. horridus* have overlapping call durations with the new


**Figure 2.** Ex-situ dorsal-lateral, dorsal and ventral photographs of **A.** Male *Gephyromantis marokoroko* sp. nov. (holotype, KU 343230) and **B.** Female (paratype, KU 343218) in life.

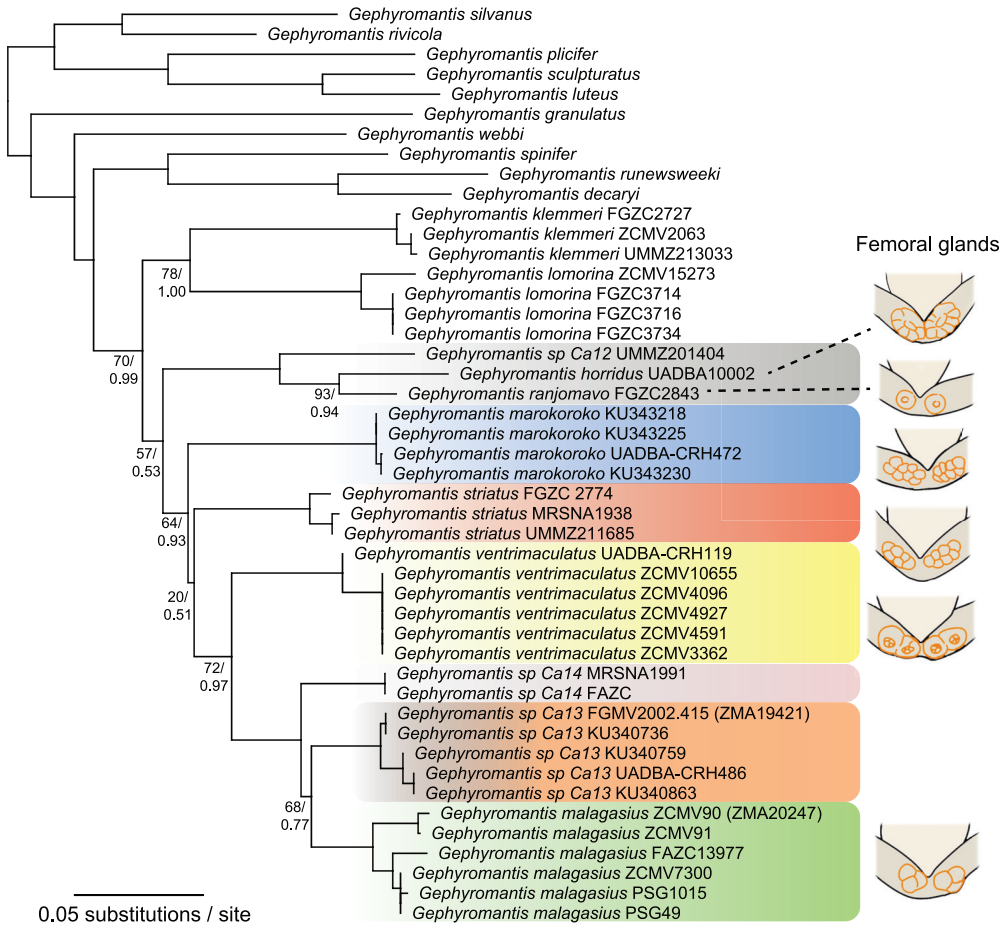


**Figure 3.** Results of phylogenetic analysis of the mitochondrial 16S rRNA barcode 3' marker for Maximum Likelihood (ML) and Bayesian Inference (BI). Topology is a consensus tree from IQ-Tree. The support values are shown as Bootstrap on top and Posterior Probability on the bottom only for nodes that were not perfectly supported. Note that *Gephyromantis marokoroko* sp. nov. placement in the clade is weakly supported in both analyses.

**Table 1.** Comparison of distinguishing characters used to differentiate species within *Laurentomantis*. Table adapted from Vences et al. (2002) combined with new data. Genetic distances are uncorrected and taken from the 16S rRNA mitochondrial marker.

| | Species | | | | | |
|---|---|---|---|---|---|---|
| **Character** | ***G. marokoroko* sp. nov.** | ***G. striatus*** | ***G. ventrimaculatus*** | ***G. horridus*** | ***G. malagasius*** | ***G. ranjomavo*** |
| **Male SVL (mm)** | 24.0–27.0 | 22.2–23.8 | 23.0–25.0 | 26.0–28.1 | 20.2–24.0 | 23.5–25.8 |
| **Female SVL (mm)** | 23.9–24.6 | 23.9–26.9 | 29.1 | 35.4 | 23.2–25.7 | n/a |
| **Tibial gland** | Absent | Absent | Absent | Present | Present | Present |
| **Granules in femoral glands (per femur)** | 8 | 3–6 | 9 | 5–6 | 1–4 | 1 |
| **Dorsal skin texture** | Strongly granular; strong ridge elements | Moderately granular; weak ridge elements | Strongly granular; strong ridge elements | Strongly granular; weak ridge elements | Strongly granular; weak ridge elements | Moderately granular, weak ridge elements |
| **Ventral skin texture** | Slightly granular | Smooth | Smooth | Granular | Slightly granular | Smooth |
| **Red colour on hind-limbs** | Absent | Absent | Absent | Absent | Present | Absent |
| **Dorsal colour** | Dark grey with bright red marbling | Dark grey with brown and orange marbling | Dark grey with brown marbling | Dark grey with brown marbling | Brown with lighter brown marbling | Brown with yellow mottling, orange limbs |
| **Ventral colour** | Dark-grey brown with light spotting | Dark grey-brown with few light spots | Brown with blue marbling | Dark with grey marbling | Brown with light marbling | Light brown with yellow, light spots |
| **Vertebral stripe** | Absent or indistinct | Present | Absent | Absent or indistinct | Absent | Absent |
| **Advertisement call duration (ms)** | 1095–1431 | 440–1266 | 407–455 | 1271–2521 | 768–1468 | n/a |
| **Advertisement call note repetition rate /s** | 14–20 | 29–40 | 21–24 | 13 | 18–36 | n/a |
| **Genetic distance (from *G. marokoroko*)** | 0.25–1.5% | 7–9% | 6–9% | 10–11% | 8–11% | 12% |

species and overlapping note repetition rates, except for *G. striatus*, which has the fastest note repetition rate (Table 1). Despite these similarities, the clearly pulsed notes alone distinguish the new species from all other *Laurentomantis*, except *G. ventrimaculatus* (Angel 1935), which has ca. 5–6 pulses/note; however, *G. ventrimaculatus* differs by having the shortest call duration non-overlapping with other *Laurentomantis* species at 407–455 ms and a slightly faster note repetition rate of 21–24 notes/s. Temperature is not likely to be an important factor in the characteristic differences described here, as structural characters, such as clearly defined pulses, would not be affected by temperature (Schneider 1974).

Motivation might affect number of notes emitted and, thus, call duration; however, the recording of *G. ventrimaculatus* is of a highly motivated male (i.e. many calls emitted in a short time) while the call of the new species was recorded from males which did not appear to be very motivated, emitting only 1–2 calls within an hour. Finally, comparisons could not be made to *G. ranjomavo* as calls were not available; however, the new species is clearly morphologically distinct (see above).

**Description of the holotype.** Fixed in 10% buffered formalin solution, preserved in 70% ethanol, in good state of preservation, except for skin loss near the anterior dorsum, with left thigh muscle removed for tissue sample. Adult male, SVL 26.0 mm. Body very slender; head longer than wide HL 33.4% of SVL; slightly wider than body, HW 33.7% of SVL; snout of moderate length, ESD 16.2% of SVL; snout rounded in dorsal and lateral view; nostrils directed laterally, slightly protuberant, nearer to snout tip than eye; ED larger than END; canthus rostralis indistinct, concave; loreal region slightly concave; single subgular vocal sac; gular glands absent. Tympanic annulus distinct and round, small, TD 64.5% of ED; supratympanic fold indistinct and irregular, tympanic membrane lighter than ground colouration. Vomerine teeth not visible on the buccal roof, present under mucosal skin; choanae small, rounded. Tongue longer than wide; ovoid in shape, posteriorly bifid. Dermal fold along lower jaw absent. Arms slender, subarticular tubercles single; outer and inner metacarpal tubercles present, indistinct. Fingers without webbing; nuptial pads absent; relative finger length 2 < 1 < 4 < 3; second finger distinctly shorter than fourth finger, only slightly shorter than finger one; finger discs distinctly enlarged, larger on third and fourth finger. Hind limbs slender; lateral metatarsalia connected; inner metatarsal tubercle distinct, outer metatarsal tubercle small, but recognisable; TIBL 55.2% of SVL; FOL 45.2% of SVL. Tibial glands absent. Toes without webbing; relative toe length 1 < 2 < 5 < 3 < 4; toe three distinctly longer and broader than toe five; toe discs distinctly enlarged. Femoral glands large, well delimited, having eight distinct clusters on each femur of almost the same size, in two rows of four. Skin coarsely granular and heavily rugose on dorsal surfaces; large and sharply elevated tubercles and ridges irregularly distributed across dorsal surfaces, with less distinct ridges on the lower back; some ridges are symmetrical, larger tubercles and short ridges present on

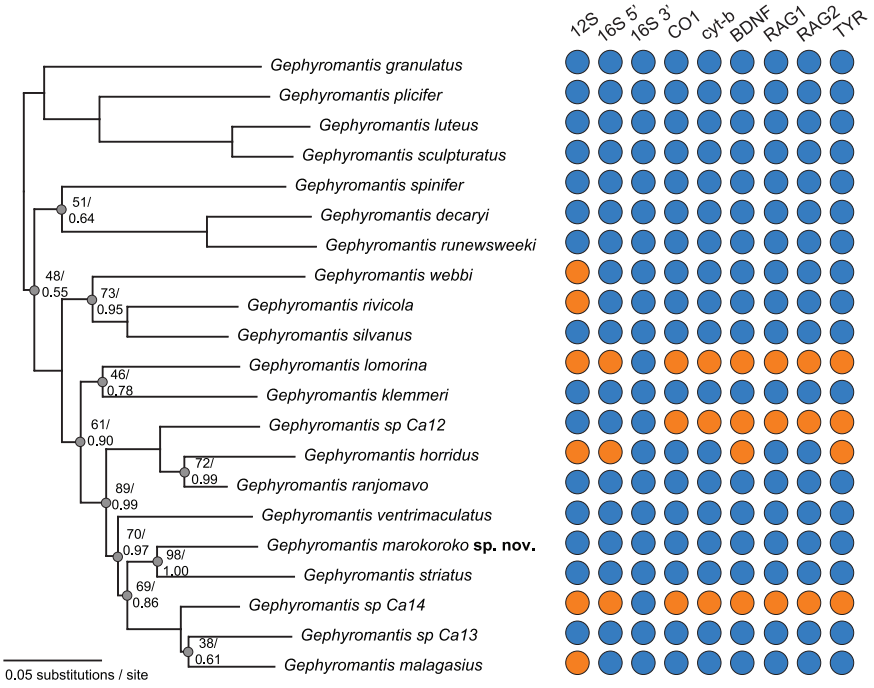


**Figure 4.** Results of phylogenetic analyses of the concatenated alignment of five mitochondrial and four nuclear markers for Maximum Likelihood (ML) and Bayesian Inference (BI). Topology is a consensus tree from IQ-Tree. On the right, the dots represent markers that were present in blue and absent in orange for each sample. The notes marked with a circle are those that did not receive perfect support (Bootstrap = 100; Posterior Probability = 1.00) from ML and BI, with the support values as BS on top and PP on the bottom. Note that *Gephyromantis marokoroko* sp. nov. has strong support in both analyses for a sister relationship to *G. striatus*.


**Figure 5.** Ex-situ dorsal-lateral, dorsal and ventral photographs of **A.** Male *Gephyromantis marokoroko* sp. nov. (holotype, KU 343230); **B.** *Gephyromantis striatus* (Marojejy, ZCMV 15140; photographs by Mark D. Scherz); and **C.** *Gephyromantis ventrimaculatus* (Ranomanfana, KU 340917).

head and anterior dorsal region. Ventral skin granular on stomach, throat and limbs.

After four years in preservative, dorsal ground colouration is a uniform dull brown including forelimbs and hind-limbs. The red colouration has faded to become light brown. Lighter coloured spots on ventral surfaces are still present.

In life (Fig. 2), dorsal colouration is a dark grey ground colour with thick, bright red mottling distributed on the dorsum. Many of the raised ridges are dark grey with bright red edges. Lighter red stripe present short distance up the dorsum. Lateral head the same as dorsum, tympanum a lighter brown. Flanks are also dark grey, but have less bright red colouration, typically only found on ridges. Forelimbs have same colouration as dorsum, except bright red colouration is more spotted, with a few lighter red spots. Hind-limbs have same colouration as forelimbs, except with red crossbands present on the dorsal surface. A whitish annulus is present before the terminal disc on fingers and toes, fingers and toes light brown. Ventral surfaces brown, with no red present. White and light-yellow spots are present and scattered moderately along the ventre. Ventral sides of arms and hind-limbs brownish-grey, with light red spotting. Femoral glands lighter brown than surrounding limb surfaces. Single subgular vocal sac is light grey, with some light-yellow spotting down the centre. Jaw has scattered light-red spots along the lip. The pupil is black with a bright red iris, with black reticulations around the outer margin of the iris.

**Variation.** All paratypes resemble the holotype in morphology and colouration. In life, dorsal colouration varies slightly in the amount and intensity of red present.

Spotting on the ventral surfaces varies in the colouration of the spots being white, light-yellow, light-orange or light-red. The vertebral stripe varies from being absent in some individuals to indistinct in others. Females lack femoral glands and have a granular texture on the femur.

**Morphometry of type series.** Measurements of the holotype and paratypes are shown in Table 2.

**Bioacoustics.** We recorded three calls from two males at Vohidrazana Forest after collection at ca. 02:00 hr on 6 January 2016. Males call infrequently with extremely quiet calls from the upper surfaces of leaves up to 50 cm above the ground. The recorded male was captured and placed in a separate plastic collecting bag. Males would not call when we were within recording distance, so we placed the microphone 100 cm away from the bag near where it was captured and moved several metres away. Calls were recorded during light rain at a temperature of 20.4 °C.

The advertisement call of this species sounds like a heavily pulsed trill or 'groan' to the human observer, emitted irregularly. We define each groan as a call (Fig. 6A–C) with a duration of 1095.1–1431.9 (1221.5 ± 183.5; *n* = 3) ms. Each call consisted of a series of 22–28 (24 ± 3.46; *n* = 72) short notes with a duration of 12–29 (19.9 ± 4.3; *n* = 72) ms and an inter-note duration of 15.5–43.7 (32.3 ± 5.6; *n* = 71) ms. Note rate within each call was 14.4–20.1 (17.9 ± 3.1; *n* = 3) note/s. Each note was strongly pulsed, with 2–4 (3.1 ± 0.783; *n* = 72) pulses per note and a pulse rate of 111.1–235.3 (156.3 ± 25.8) pulses/s (Fig. 6D–F). The call was strongly amplitude-modulated, beginning at a lower amplitude and increasing to the middle of the call, where the amplitude then decreased until the end of the call. The dominant frequency measured at peak amplitude of the call was 2390–2672 (2483 ± 162; *n* = 3) Hz, while the dominant frequency at the peak amplitude of the note was 2250–2813 (2458 ± 149; *n* = 72) Hz. For notes, the 90% bandwidth was from 1453–2297 (1942 ± 177; *n* = 72) Hz to 3000–4125 (3749 ± 290; *n* = 72) Hz. No harmonic frequencies were visible on the spectrogram (Fig. 6).

**Phylogenetics.** The phylogenetic results support the morphological diagnosis by placing *Gephyromantis marokoroko* within the *Laurentomantis* subgenus with strong support. At the species level, *G. marokoroko* is monophyletic with strong support in ML and BI analyses (BS = 100, PP = 1.00; Fig. 3). Uncorrected p-distances, using the 16S fragment, indicate that *G. ventrimaculatus* has the lowest distance to the new species, at ~ 6–9%. The combined nine marker multi-locus dataset places the new species sister to *G. striatus* with strong support (BS = 98; PP = 1.00) in both BI and ML analyses (Fig. 4). Overall, these results provide strong evidence that the species is a separately evolving lineage and strong evidence for the new species phylogenetic placement.

**Distribution.** *Gephyromantis marokoroko* is known from several sites in the forests in the vicinity of Andasibe, but has only been found at high elevation sites (~

**Table 2.** Morphometric measurements (in mm) of the holotype and paratypes of *Gephyromantis marokoroko* sp. nov. Femoral Gland Clusters (FGC) shown as "left, right" count.

| | Specimen | | | | | |
|---|---|---|---|---|---|---|
| **Type status** | **Holotype** | **Paratype** | **Paratype** | **Paratype** | **Paratype** | **Paratype** |
| Field Number | CRH 1110 | CRH 1108 | CRH 1397 | CRH 1061 | CRH 1923 | CRH 2019 |
| Museum Number | KU 343230 | KU 343229 | KU 343218 | UADBA CRH1061 | KU 347328 | KU 347329 |
| Sex | M | M | M | M | F | F |
| SVL | 26.0 | 25.3 | 27.0 | 24.0 | 24.6 | 23.9 |
| HW | 8.8 | 9.4 | 8.6 | 8.6 | 8.4 | 8.6 |
| HL | 8.7 | 10.0 | 8.8 | 8.2 | 8.7 | 8.6 |
| ED | 3.8 | 3.6 | 3.5 | 3.8 | 3.6 | 3.9 |
| IOD | 2.5 | 2.7 | 2.7 | 2.5 | 2.8 | 2.5 |
| ESD | 4.2 | 4.0 | 4.4 | 4.4 | 4.1 | 4.3 |
| END | 3.0 | 3.5 | 3.3 | 3.1 | 2.3 | 2.9 |
| NSD | 1.2 | 1.5 | 1.7 | 1.5 | 1.9 | 1.4 |
| NND | 2.4 | 2.6 | 2.5 | 2.3 | 2.4 | 2.2 |
| TD | 2.5 | 1.6 | 2.4 | 2.5 | 2.0 | 2.2 |
| FIL | 5.1 | 14.9 | 6.3 | 6.2 | 4.9 | 5.0 |
| FIIL | 6.8 | 5.7 | 8.1 | 8.2 | 6.4 | 6.6 |
| FEML | 13.9 | 14.1 | 14.3 | 13.4 | 14.1 | 14.1 |
| TIBL | 14.4 | 14.3 | 14.4 | 13.3 | 14.6 | 13.8 |
| FOL | 11.8 | 11.3 | 12.2 | 11.2 | 12.1 | 11.3 |
| TARL | 8.1 | 8.2 | 8.4 | 7.6 | 8.4 | 8.0 |
| HAL | 8.4 | 8.3 | 7.9 | 8.2 | 8.3 | 7.6 |
| LAL | 7.7 | 7.4 | 8.3 | 7.4 | 8.1 | 8.2 |
| UAL | 6.1 | 6.2 | 6.2 | 6.0 | 6.2 | 6.0 |
| FORL | 22.2 | 21.8 | 22.3 | 21.6 | 22.6 | 21.7 |
| FGL | 6.4 | 5.8 | 10.1 | 5.7 | - | - |
| FGW | 2.8 | 2.4 | 3.3 | 3.2 | - | - |
| FGC | 8; 8 | 8; 8 | 8; 8 | 8; 8 | - | - |

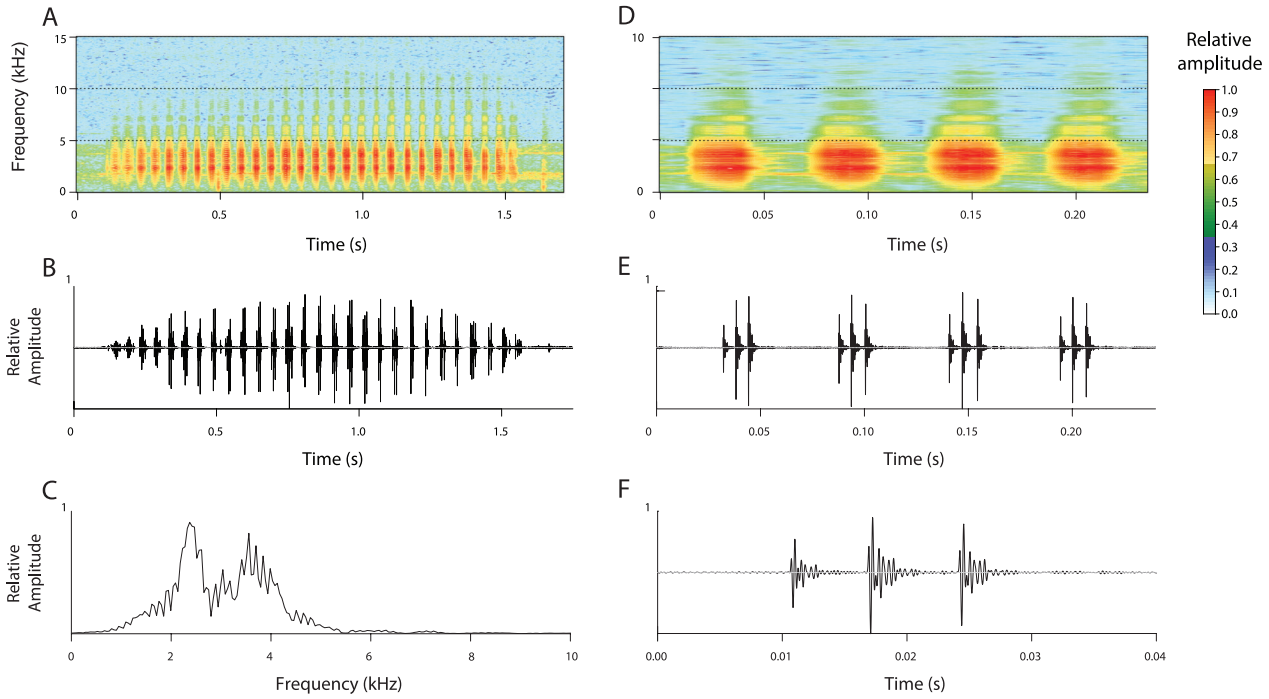


**Figure 6.** Oscillograms and spectrograms of the call of *Gephyromantis marokoroko* sp. nov. (Holotype: KU 343230). **A.** The entire call spectrogram and **B.** Entire call oscillogram; **C.** Power spectra/frequency spectrogram of a single note; **D.** A close-up spectrogram of four notes and **E.** Corresponding oscillogram; and **F.** an individual note taken from the middle of the call.

1000–1200 m a.s.l.; Fig. 1). The new species is known from the following localities: Vohidrazana Forest (18.976°S, 48.499°E), Tavalobe (19.005°S, 48.461°E), Vohimana (18.926°S, 48.489°E) and Andasibe-Mantadia National Park (Belakato: 18.821°S, 48.439°E).

**Natural history.** *Gephyromantis marokoroko* is apparently locally rare and, thus far, only found within undisturbed, primary forests at highland elevations (ca. 1000–1200 m). Individuals of the species were perched on the surfaces of vegetation less than 50 cm in height (Fig. 7). The species was infrequently encountered, always after moderate to heavy rain, with multiple individuals occasionally grouped in small clusters (~ 20 $m^2$). The species' call is very quiet and irregular and is barely audible to a human observer, even within three metres of a calling individual. Individuals of the new species were often found syntopically with another *Laurentomantis*, *G.* sp. Ca13, which is a candidate species identified in Vieites et al. (2009). Other syntopic *Gephyromantis* include *G. eiselti*, *G. salegy*, *G.* sp. aff. *plicifer* (not yet assessed for a candidate species number) and *G. cornutus*.

**Conservation status.** The new species is known from Andasibe-Mantadia National Park and several other managed areas (e.g. Vohimana, the community managed Vohidrazana Forest and Tavalobe). However, as currently understood, the distribution of this species is severely fragmented and restricted to only four known high-elevation localities (~ 1000–1200 m), which are very small patches with no connectivity (Fig. 1). Many other high elevation sites in the region have been surveyed by the authors over three field seasons. Furthermore, Vohidrazana Forest and Tavalobe face ongoing threats that result in the reduction of quality and extent of habitat. For example, slash-and-burn agriculture and forest products are frequently extracted directly from this species' habitats that are outside protected areas. Given this information, we categorise this species as "Endangered" [B1ab(iii-iv)] following IUCN Criteria (IUCN 2001).

## Discussion

*Gephyromantis* (*Laurentomantis*) *marokoroko* sp. nov. is a clearly distinct species, as evidenced through morphology, bioacoustics and molecular phylogenetics. The new species can be readily distinguished from other members in *Laurentomantis* by its heavily rugose granular skin, vibrant red eyes, bright red body colouration and distinctive femoral glands. The call of *G. marokoroko* also differs from all other *Laurentomantis* through its moderately long call duration, clearly pulsed notes and slower note repetition rate. Phylogenetic analyses strongly support the new species as monophyletic in the 16S rRNA mitochondrial marker multi-sample dataset. Additionally, the single-sample per species dataset of nine-markers (five mitochondrial and four nuclear) and both phylogenetic analyses strongly supported *G. striatus* and *G. marokoroko* as sister species (Fig. 4). In addition, morphological similarity in the number and shape of femoral glands and the occasional presence of vertebral stripe support this relationship.

*Gephyromantis marokoroko* is a remarkable discovery that was immediately obvious as a new species in the field

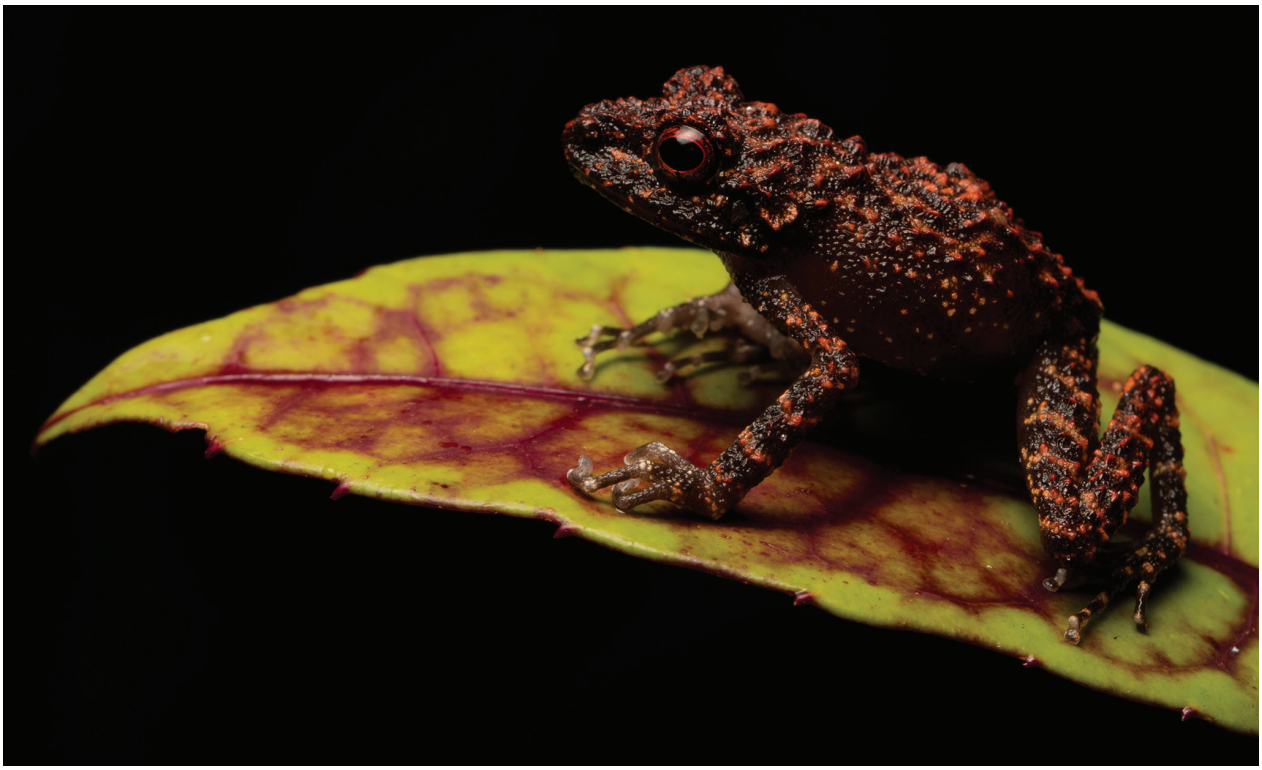

**Figure 7.** In-situ photograph of *Gephyromantis marokoroko* sp. nov. (UADBA-CRH1629).

as its general appearance is very distinct and spectacular, with several clear morphological differences from related species. Distinctive new species are typically discovered in poorly unexplored areas and *G. marokoroko* was discovered in the well-explored vicinity of Andasibe. In addition, the species had never been barcoded before, eluding past herpetological surveys. This new species highlights the importance of continued fieldwork in Madagascar, as the discovery of previously undocumented new species is occurring frequently (Lambert et al. 2017; Scherz et al. 2017a; Scherz et al. 2018), despite the extensive past barcoding efforts for Malagasy frogs (e.g. Vieites et al. 2009; Perl et al. 2014). Typically, such species have low population densities, small geographic or elevational ranges and/or are in areas that have not been extensively surveyed. Many recent species descriptions of Malagasy frogs are from previously-known candidate species and/or are cryptic lineages that required molecular evidence to diagnose (e.g. Hutter et al. 2015; Scherz et al. 2017c; Vences et al. 2017) and it is uncommon to find new species that have not already been documented through barcoding efforts (e.g. Vieites et al. 2009; Perl et al. 2014). These discoveries are rare and there are only a few recent examples of new discoveries that include *G. lomorina* (Scherz et al. 2018) and *Boophis masoala* (Glaw et al. 2018). The continuation of basic field inventories is, therefore, clearly necessary to fully understand the patterns of species richness and complete evolutionary histories of frogs in Madagascar and other tropical regions.

The discovery and conservation of these new and unique species is critically important as habitat loss continues, especially in the study area. The distribution of *G. marokoroko* is severely fragmented and restricted to only four locations and occurs in small habitat patches (Fig. 1). While the species is protected within Andasibe-Mantadia National Park and Vohimana Special Reserve, it has only been found in low abundance in single, very small habitat patches. The localities Vohidrazana Forest and Tavalobe face ongoing threats from slash-and-burn agriculture and forest products are frequently extracted directly from this species' habitat that are outside protected areas. Furthermore, climate change could exacerbate these risks reducing further the suitable habitat for this already micro-endemic species.

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## Author contributions

CRH and SML conceived the study. CRH wrote the first draft of the manuscript and the other authors provided input. ZFA collected phenotypic data. All the co-authors were involved with fieldwork, data collection and logistics.

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