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A new species of genus *Urophonius* Pocock, 1893 (Scorpiones, Bothriuridae), from Andean Mauline Chilean forests, with a phylogenetic re-analysis of the genus

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Abstract

Urophonius trewanke **sp. nov.** is described from the Mauline Andean woods of northern Chilean Patagonia. This species belongs to the *granulatus* species group, which includes the most basal species within the genus. This species is only active in summer as in all species of its group. We performed a phylogenetic analysis of the genus *Urophonius* based on morphological characters to establish the position and relationships of the new species in the genus.

Key Words

Bothriuridae, Chile, Mauline woods, new species, phylogeny

Introduction

The scorpion genus *Urophonius* Pocock, 1893 comprises small burrowing species from southern South America. In the last two decades, there has been a remarkable increase in the knowledge of this genus; in this period, the number of described species has almost doubled (Acosta 2003; Ojanguren-Affilastro and Cheli 2009; Ojanguren-Affilastro et al. 2010, 2011, 2020), the first phylogenetic analysis of the genus has been performed (Ojanguren-Affilastro et al. 2020) and, more recently, the historical time frame of its evolution and diversification has been unveiled using transcriptomes and UCEs (Ojanguren-Affilastro et al. 2023). *Urophonius* is remarkable amongst all known scorpion genera from temperate and cold areas because of its adaptations to low temperatures (Maury 1969, 1973; Ojanguren-Affilastro et al. 2020; Garcia et al. 2021). This genus reaches the southernmost and colder part of South America (Maury 1979; Ojanguren-Affilastro et al. 2020) and most *Urophonius* species exhibit surface activity in winter, contrary to other species from the region (and temperate areas of the world), which are active during the warmer period of the year (Pizarro-Araya et al. 2011; Ojanguren-Affilastro and Kovarik 2013).

The first morphological phylogeny of the genus (Ojanguren-Affilastro et al. 2020) suggested a single and relatively

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early origin of winter activity in *Urophonius*. A posterior dated phylogeny, based on diverse phylogenomic datasets (Ojanguren-Affilastro et al. 2023), supported this early origin of the winter activity period and dated it between 68 and 42 million years ago (MYA), before the most important uplift of the Andes (Ghosh et al. 2006; Garzione et al. 2008).

On the other hand, some species of the genus still retain the summer activity period, which is common to most species of the family and the order (Ojanguren-Affilastro and Kovarik 2013). These summer *Urophonius* species are grouped in the *granulatus* species group and occur exclusively in cold areas of southern South America, both in steppes and temperate woods.

In a recent comprehensive study of arthropods conducted at the "Fundo La Escuadra" (Figs 1a, 2), a small well-preserved area located within the Cipreses River Basin, a tributary of the Maule River Basin in the Maule Region of Chile, our research team made notable discoveries. The upper Maule River Basin is partially isolated by the Andes and has proven to harbour several endemic species of arthropods and even vertebrates (Corbalán et al. 2010; Correa et al. 2018, 2020; Ojanguren-Affilastro et al. 2020). This study allowed us to identify and collect numerous species of arachnids and insects not previously documented. In particular, amongst these newly-discovered taxa, we found a hitherto unknown species of scorpion that belongs to the genus Urophonius Pocock, 1893. This research, rooted in the rich and highly endemic biodiversity of the area, contributes significantly to our understanding of the local arthropod fauna. The identification and documentation of a new scorpion species underlines the ecological importance of the ecosystem preserved at "Fundo La Escuadra". The findings of this study not only expand our knowledge of regional arthropod diversity, but also emphasise the need for further research and conservation efforts in this ecologically important area.

In this contribution, we describe *Urophonius trewanke* sp. nov. (Fig. 1b) from the Maule Valley in the upper Maule River Basin (Fig. 2b). *Urophonius trewanke* sp. nov. is the second known endemic *Urophonius* from this area, the other species being *Urophonius pehuenche* Ojanguren-Affilastro & Pizarro-Araya, 2020, which, contrary to the new species, is only active in winter (Ojanguren-Affilastro et al. 2020). We also perform a phylogenetic analysis, based in morphological characters to clearly stablish the phylogenetic position of the new species in the genus.

Methods

Cladistic analysis

Taxa. The matrix in the cladistics analysis comprises a total of 21 species, 17 species of *Urophonius* and four outgroups. We used the same species as Ojanguren-Affilastro et al. (2020), including all known species of

Urophonius, together with the new species herein described: U. trewanke sp. nov. Outgroups belonging to four other genera in the family Bothriuridae include: one species of the Australian bothriurid Cercophonius, which, according to all previous phylogenetic analyses, is the sister genus of Urophonius (Prendini 2000, 2003; Ceccarelli et al. 2016; Ojanguren-Affilastro et al. 2020, 2023; Santibañez-López et al. 2023); one species of Phoniocercus Pocock, 1893, which has also been suggested as closely related to Urophonius (Acosta 1988); one species of Thestylus Simon, 1880, which, according to previous phylogenetic analysis, is the most basal split in the American bothriurids (Prendini 2000, 2003; Ceccarelli et al. 2016); and one species of Centromachetes Lonnberg, 1897, which also shares several morphological characters with Uropho-

nius (Ojanguren-Affilastro and Kovarik 2013). The tree was rooted on *Thestylus aurantiurus* Yamaguti & Pinto-da-Rocha, 2003, based on previous evidence for the relationships amongst bothriurid genera (Prendini 2000, 2003).

Materials

Specimens examined are deposited in the following collections: Museo Argentino de Ciencias Naturales "Bernardino Rivadavia," Buenos Aires, Argentina (MACN-Ar, Martín J. Ramírez); Museo Nacional de Historia Natural, Santiago, Chile (MNHN, Mario Elgueta Donoso); Museo de Zoología de la Universidad de Concepción (MZUC, Laura Tavera Martínez); Laboratorio de Entomología Ecológica, Universidad de La Serena, Chile (LEULS, Jaime Pizarro-Araya). A table with exemplars and locality data used for the analyses are presented as Suppl. material 1.

Characters

We used a matrix, based in 114 morphological characters. The complete list of characters and the matrix are available as supplementary material online, as Suppl. materials 2, 3, respectively. We used the same matrix as in Ojanguren-Affilastro et al. (2020) with the sole addition of the new species herein described.

Analyses

Analyses were made with TNT 1.5 (Goloboff et al. 2008), using parsimony under equal weights and implied weights, exploring the sensitivity of the results to a range of values of the concavity constant k from 1 to 99. Branch support was estimated with 1000 pseudoreplicates of symmetric resampling. Tree searches were made with 100 random addition sequences (RAS) followed by tree bisection-reconnection (TBR) branch swapping; since all

the RAS+TBR replicates reached the same result, it is likely that optimal trees were found.

Systematics

All new material reported here was collected by the authors; most specimens were collected at night by UV detection. Some specimens were also collected in daytime under logs or stones. Measurements, taken using an ocular micrometer, are recorded in mm. Descriptive terminology follows Mattoni and Acosta (2005) for hemispermatophores; Vachon (1973) for trichobothria; Francke (1977) for metasomal carinae abbreviated as follows: DL: dorsolateral; LIM: lateral inframedian; LSM: lateral supramedian; LM: lateral median; VSM: ventral submedian; VL: ventrolateral; VM: ventromedian; and Prendini (2000) for pedipalp carinae, abbreviated as follows: DI: dorsal internal; DE: dorsal external; VI: ventral internal; VE: ventral external; D: digital; E: external; IM: internomedian; EM: externomedian; V: ventral; VM: ventral median; DM: dorsal marginal; DS: dorsal secondary. Illustrations were produced with a Leica M165C stereomicroscope and a camera lucida. Digital images of pigmentation pattern and habitus were taken under visible light, images of external morphology under UV light, using a digital camera (Leica DFC290 or Nikon DS-Fi1) attached to a stereomicroscope (Leica M165C or Nikon SMZ1500) and the focal planes combined with Helicon Focus 3.10.3 (http://helicon.com.usa/heliconfocus/). Point locality records were georeferenced in the field with portable Global Positioning System devices (Garmin® Etrex Vista and Etrex Vista C). The distribution map was generated using https://www.simplemappr.net/.

Results

Cladistic analyses

The analysis under equal and implied weights resulted in highly concordant trees. All the analyses under implied weights with concavity constant below 20 produced the resolution of Fig. 3. The support values were calculated under implied weights with k = 15.

In our phylogenetic tree (Fig. 3), the genus *Urophonius* appears as monophyletic and *Cercophonius* appears as the sister genus of *Urophonius*, as in previous phylogenetic analyses (Prendini 2000; Ojanguren-Affilastro et al. 2020).

We recovered two major clades confirming the results of Acosta (1988) and Ojanguren-Affilastro et al. (2020). One of these corresponds to the concept of *granulatus* species group (Fig. 3) and includes all species with summer activity period and the new species herein described. In our analyses, *Urophonius trewanke* sp. nov. groups with *Urophonius tregualemuensis* Cekalovic, 1981, as expected by their external similarities and environmental proximity, both inhabiting southern Chilean woods. Species from the Patagonian steppe of the granulatus group, Urophonius granulatus Pocock, 1898, Urophonius somuncura Acosta, 2003 and Urophonius araucano Ojanguren-Affilastro & Pizarro-Araya, 2020 form a separate clade. Urophonius pizarroi Ojanguren-Affilastro, Ochoa, Mattoni & Prendini, 2010, fits in the granulatus group, but it is not part of either clade.

On the other hand, we recovered all species with winter activity as another monophyletic group (Fig. 3), with two internal clades corresponding to *exochus* and *brachycentrus* groups (Acosta 1988; Ojanguren-Affilastro et al. 2020). Urophonius mondacai Ojanguren-Affilastro, Pizarro-Araya & Prendini, 2011 appears as basal to the species with winter activity; however, its actual activity period is in debate. The original records of this species are from spring, but it has recently been collected also in winter by the authors, raising more doubts about the position of this enigmatic species.

Systematics

Urophonius trewanke sp. nov.

https://zoobank.org/50D20A04-1E7A-47E1-9EF3-3C60F4BA8922 Figs 1–8; Table 1

Type material. *Chile*, *Maule Region (VII)*, Maule Valley, Fundo La Escuadra: Holotype \mathcal{S} (MNHN 8411), Bocatoma-Ojos de Agua (35°46'06.1"S, 70°47'44.4"W), 1009 m a.s.l.; 14–17/X/2022, Pizarro-Araya, Alfaro & Calderón coll. Paratipes: 2 \mathcal{S} , same data as holotype (MACN); Laguna Invernada (35°43'16.1"S, 70°47'04.9"W), 1260 m a.s.l.; 14–17/X/2022, Pizarro-Araya, Alfaro & Calderón coll. 2 \mathcal{Q} , 10 \mathcal{S} (LEULS); 2 \mathcal{Q} , 2 \mathcal{S} (MACN). Woods of *Quillaja saponaria* and *Cryptocarya alba* (35°46'04.1"S, 70°47'45.6"W), 1020 m a.s.l.; 10/XII/2023, Pizarro-Araya, Alfaro & Calderón coll. 3 \mathcal{Q} (LEULS); 3 \mathcal{Q} , 6 \mathcal{S} (MACN). Bocatoma-Ojos de Agua (35°46'06.1"S, 70°47'44.4"W), 1009 m a.s.l.; 9/XII/2023, Pizarro-Araya, Alfaro & Calderón coll. 4 \mathcal{Q} , 2 \mathcal{S} , 1 juvenile (LEULS); 2 \mathcal{Q} , 3 \mathcal{S} (MACN).

Etymology. The specific epithet "*trewanke*" is a noun in apposition meaning scorpion in *Mapungudun*, the language of the Mapuche people, the original inhabitants from most parts of southern and central Chile.

Diagnosis. Urophonius trewanke sp. nov. is most closely related to U. tregualemuensis from south-central Chile (Fig. 2a). Both species can be easily separated by their pigment pattern; in U. trewanke sp. nov. the dorso-submedian spots of tergites are poorly developed, being reduced to small triangles in the posterior half of the segment (Figs 4a, 5a), whereas in U. tregualemuensis, these spots are much more developed, occupying almost the whole median part of the segment (Fig. 5b).

Both species can also be separated by the shape of pedipalp chela, which is stouter in *U. trewanke* sp. nov. (Fig. 6b, f). Pedipalp chela length/height ratio varies from



Figure 1. a. Habitat of *Urophonius trewanke* sp. nov., woods at Fundo La Escuadra, Maule Valley, Maule Region, Chile; b. Couple of *Urophonius trewanke* sp. nov. during courtship in its natural environment; c. *Urophonius trewanke* sp. nov. male, living specimen.

3.15–3.38 (n = 11, mean = 3.29) in *U. trewanke* sp. nov. males and between 3.96 and 4.24 (n = 12, mean = 4.09) in *U. tregualemuensis* males; it varies between 3.31 and 3.61 (n = 3, mean = 3.45) in *U. trewanke* sp. nov. females and between 4.06 and 4.33 (n = 10, mean = 4.19) in *U. tregualemuensis* females; length/width ratio varies between 3.35 and 3.70 (n = 11, mean = 3.54) in *U. trewanke* sp. nov. males and between 4.13 and 4.24 (n = 12, mean = 4.09) in *U. tregualemuensis* males; and varies between 3.70 and 3.93 (n = 3, mean = 3.80) in *U. trewanke* sp. nov. females and between 4.48 and 4.82 (n = 10, mean = 4.63) in *U. tregualemuensis* females.

They can also be separated by some details of the hemispermatophore; in *U. tregualemuensis*, the bifid lobe of the internal lobe is connected to the superior concavity

of the basal portion by a thick carina (Fig. 8b), that is very subtle in *U. trewanke* sp. nov. (Fig. 8a). Additionally, the distal margin of capsular concavity of the basal lobe is arranged more distally in *U. trewanke* sp. nov. (Fig. 8c, d) than in *U. tregualemuensis* (Fig. 8e, f).

There are also some differences in the development of the ventral carinae of metasomal segments I and II, which are clearly more developed in *U. tregualemuensis* (Fig. 7d, e) than in *U. trewanke* sp. nov. (Fig. 7a, b), particularly in males, in which these carinae are barely visible in *U. trewanke* sp. nov. (Fig. 7a) and well developed in *U. tregualemuensis* (Fig. 7d).

Description. Based on the holotype \circ (MNHN) and the paratypes \circ (LEULS, MACN-Ar).

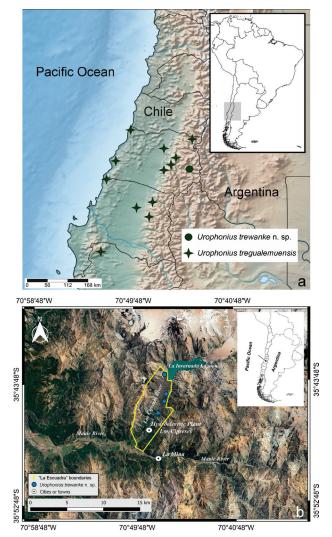


Figure 2. a. Map of central Chile with the distribution of *Urophonius trewanke* sp. nov. and its closest species, *Urophonius tregualemuensis*; **b.** Map of the study area, marking the collection sites, the Cipreses River and the Maule River.

Total length: 30–41 mm in \bigcirc (n = 12; mean = 34.7); 34.5–41 mm in \bigcirc (n = 4; mean = 38.88).

Colour: Base colour yellowish, with dark brown spots (Figs 1b, c, 4). Chelicerae with reticulate pigmentation on dorsal and retrolateral surface of manus, densely pigmented on the retrolateral margins of the fingers. Carapace, densely pigmented (Fig. 5a); anterior margin pigmented, with two broad, dark lateral stripes, extending from lateral sides of the anterior margin to the ocular tubercle and the anterior part of posterior longitudinal sulcus, with two other lateral dark stripes placed more posteriorly and reaching the lateral margins; median ocular tubercle and area around lateral ocelli dark brown; with two posterolateral triangular dark spots covering most of latero-posterior margin, leaving a median unpigmented area in the postocular furrow. Tergites I-VI each with four dark spots (Fig. 5a), two external-lateral forming a stripe along the segment and two submedian-subtriangular in the posterior half of the segment; lateral and median spots can be connected by pigment in the posterior margin, me-

dian area of the segment always unpigmented; tergite VII with two postero-lateral spots on each side, posterior to dorso-median and dorso-lateral carina, respectively. Sternum, genital opercula, pectines and pleura unpigmented. Sternites: sternites III, IV and V unpigmented medially (Fig. 5c), pigmented in the lateral margins; sternites VI and VII, lateral margins pigmented, posterior margin with faint posterior VL spots and a VM posterior spot that can continue in a thin median stripe, particularly in segment VII. Metasomal segments I-III: dorsal surface with two triangular posterior spots and two anterior small spots, with a thin stripe over the DL carina, with faint paired median spots that can be absent in some specimens; lateral surfaces densely pigmented between LM and LIM carinae; ventral surface: with VL stripes well marked, extending the entire length of the segment, slightly thicker posteriorly, without VSM stripes, VM stripe thin and extending the entire length of the segment, not connecting with VL stripes; segment IV similar to III, but the dorsal spots are elongated; segment V clearly darker than the rest of the segments, with a dark reddish base colour, with faint dorsal submedian stripes; with a lateral stripe along the LM carina, connecting with the VL stripe in the second half of the segment, postero-lateral margins densely pigmented, ventrally similar to remaining segments, but with VL stripes connected to lateral stripes. Telson, general colour dark reddish-brown, as metasomal segment V, dorsal gland of males barely paler than the rest of the vesicle; aculeus dark brown. Pedipalps, trochanter dorsally pigmented; femur with DI, DE and VE wide stripes across the whole segment, fusing in the articulation with patella. Patella, with a DI reticular stripe extending the entire length of the segment, but with an unpigmented median area, with a DE thin stripe in the proximal third of the segment and a retrolateral median stripe extending the entire length of the segment. Chela with seven dark stripes which seem to correspond to DI, DM, DS, D, E, V and VM carinae; area near articulation of fingers and fingers, pigmented. Legs: coxae unpigmented; trochanter with a median retrolateral spot; femur, retrolateral margin with anterior and posterior elongated spots; patella with retrolateral and dorsal stripes; tibia with dorsal and ventral spots; basitarsi with ventral and dorsal spots near articulation with tibia; telotarsi with a dorsal faint spot near articulation with basitarsi.

Carapace: lateral surfaces granular ($\mathscr{S} \ Q$), medially smooth (\mathbb{Q}) or slightly granular (\mathscr{S}). Anterior margin straight. Anterior longitudinal sulcus shallow; interocular sulcus weakly developed; posterior longitudinal and lateral sulci well developed. Median ocular tubercle not very pronounced, median ocelli large, ca. one diameter apart; with one macroseta behind each eye and one microseta in front of each eye. Three pairs of small lateral ocelli on each side of carapace, posterior ocellus slightly smaller than the rest of the ocelli; anterior and median ocelli almost in the same horizontal axis, posterior ocellus situated clearly dorsal to others; lateral ocelli pattern type 3A (Loria and Prendini 2014).

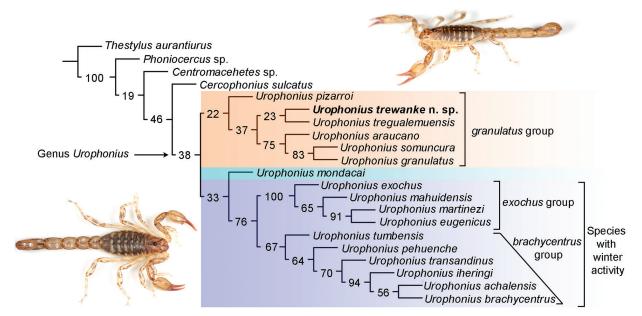


Figure 3. Phylogeny of the genus *Urophonius* Pocock, 1893 estimated with morphological characters under implied weights (k = 15), with Jackknifing frequencies indicated inside branches.

Pedipalps: Femur with DI, DE and VI carinae granular, extending the entire length of segment (Fig. 6h); with some sparse coarse granules in the anterior margin of the segment; with one macroseta related to trichobothria d and *i*; trichobothrium *e* situated distal to dorsal macroseta M1 (Fig. 6h). Patella with smooth tegument and without distinct carinae (Fig. 6i). Chela manus robust, more so in \mathcal{J} , length/width ratio varying from 3.15–3.38 in \mathcal{J} (n = 11, mean = 3.29) and from 3.31–3.61 in Q (n = 3, 1)mean = 3.45); length/height ratio varies from 3.35-3.70 in \bigcirc (n = 11, mean = 3.54) and from 3.70–3.93 in \bigcirc (n = 3, mean = 3.80; acarinate (Fig. 6a–e, g), prolateral surface with a pronounced, subtriangular projection and a shallow depression near articulation of movable finger (Fig. 6b), with a group of granules near the base of the movable finger (\mathcal{O}); all of them absent in \mathcal{Q} (Fig. 6c, g); fingers elongated, median denticle row medially uneven in the basal quarter of its length, with five pairs of accessory granules.

Pectines: Tooth count: 15–17 in \bigcirc (n = 12, median = 16) and 14–15 in \bigcirc (n = 4, mean = 15).

Legs: Surfaces smooth in \bigcirc , granular in \bigcirc . Basitarsi each with two well developed, equal length, pedal spurs. Telotarsi elongated, shallow, each with well-developed ventromedian row of hyaline setae and paired rows of ventrosubmedian spiniform setae with the following counts on each telotarsus: I: 1/1, II: 2/2, III: 5–6, IV: 6–6/6–7. The only pair of spines of telotarsus I and the first pair of spines of telotarsus II are less sclerotised than the remaining spines, the rest are well sclerotised. Ungues slightly curved, equal in length.

Tergites: Surfaces, I–VI: anterior area smooth, posterior and lateral margins finely granular; more so in \mathcal{S} ; VII with sparse, coarse granules in posterolateral margins, with paired dorso-submedian carinae in posterior third and paired dorso-lateral carinae in posterior two-thirds of the segment.

Sternites Surfaces, III–VI smooth, with small elliptical spiracles; VII, surface sparsely granular, more so in \Im ; in \Im with two VM and two VL barely visible carinae in posterior third of the segment, not conspicuous in \Im (Fig. 7a, b).

 Table 1. measurements in mm of the holotype male (MNHN)

 and a female paratype (MACN) of Urophonius trewanke sp. nov.

	Urophonius trewanke sp. nov.	
	Holotype 🖒	Paratype ♀
Total length	32.53	38.72
Carapace, length	3.71	5.01
Carapace, anterior width	2.58	3.39
Carapace, posterior width	4.12	5.17
Mesosoma, total length	8.88	9.69
Metasoma, total length	19.94	21.02
Metasomal segment I, length	1.94	2.58
Metasomal segment I, width	2.42	3.23
Metasomal segment I, height	2.02	2.58
Metasomal segment II, length	2.34	2.82
Metasomal segment II, width	2.18	2.91
Metasomal segment II, height	1.85	2.50
Metasomal segment III, length	2.58	3.47
Metasomal segment III, width	2.15	2.74
Metasomal segment III, height	1.85	2.42
Metasomal segment IV, length	3.07	4.04
Metasomal segment IV, width	2.02	2.58
Metasomal segment IV, height	1.85	2.42
Metasomal segment V, length	4.68	5.41
Metasomal segment V, width	2.10	2.83
Metasomal segment V, height	1.77	2.42
Telson, length	5.33	5.70
Vesicle, width	1.77	2.26
Vesicle, height	1.53	1.82
Chela, length	6.06	7.62
Chela, width	1.85	1.94
Chela, height	1.64	2.10

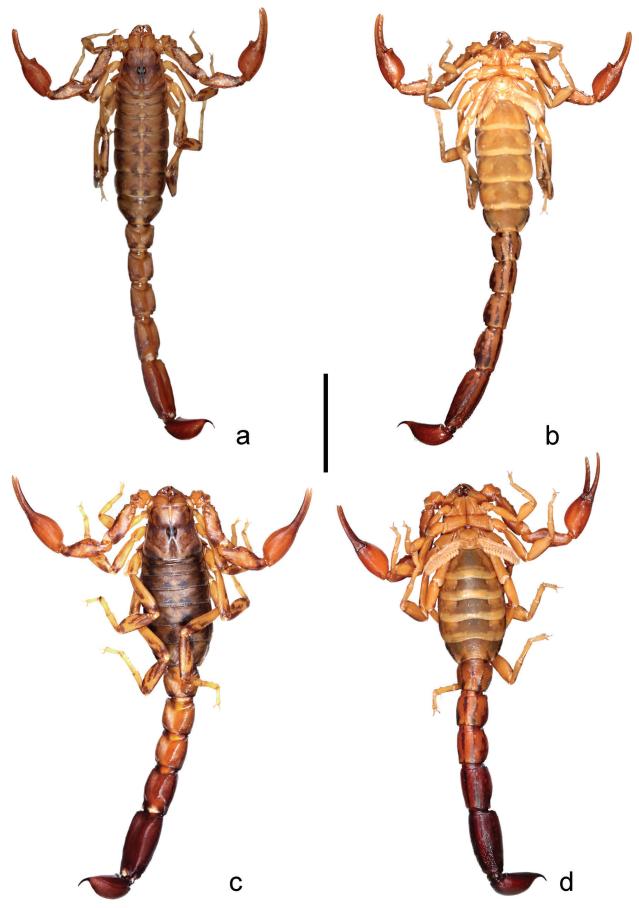


Figure 4. Urophonius trewanke sp. nov., a, b. Holotype \Im ; c, d. Paratype \Im ; a, c. Dorsal aspect; b, d. Ventral aspect. Scale bar: 10 mm.

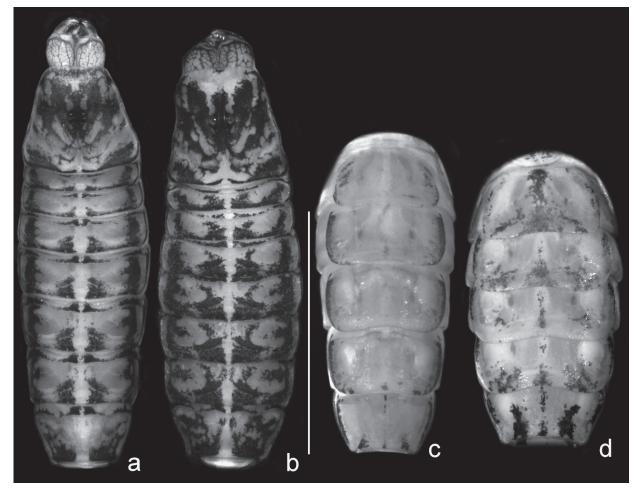


Figure 5. Pigment pattern. a, c. Urophonius trewanke sp. nov. a. Carapace and tergites; c. Sternites; b, d. Urophonius tregualemuensis Cekalovic, 1981; b. Carapace and tergites; d. Sternites.

Metasoma: Metasomal segment I, dorsal surface finely granular; DL carinae granular, extending the entire length of segment with anterior and posterior granules more developed than the rest; dorso-lateral margins granular, LSM carinae represented by some tiny granules in the posterior part of the segment, LM carina with an anterior blunt small keel and a granular part extending the posterior two-thirds of the segment; LIM carinae granular, restricted to the posterior half of the segment, with one macroseta; ventral surface smooth, VL carinae extending the entire length of the segment, granular in \mathcal{Q} , as an elevation of the tegument in *d*, VSM carinae well developed and granular in \mathcal{Q} , barely visible in \mathcal{J} , with two pairs of VSM macrosetae and three pairs of VL macrosetae (Fig. 7a, b). Metasomal segment II, similar to I, but with less developed carinae, being the ventral carinae barely visible in \mathcal{F} ; with a DL, a LM and a LIM macroseata and with three pairs of VSM and VL macrosetae. Metasomal segment III similar to segment II, but with less developed carinae, without ventral carinae, LIM carina restricted to posterior third of the segment; metasomal segment IV elongated, LIM carina absent, the rest similar to segment III. Segment V elongated (Fig. 7d, e); dorsal and lateral margins smooth; DL carinae restricted to some well-developed granules in the anterior third of the segment, with two dorsal macrosetae and four lateral macrosetae; VL carinae granular, extending the posterior two-thirds of the segment, with five pairs of VL macrosetae, being the posterior pair in the posterior margin; ventral surface densely granular in the posterior two-thirds of the segment, so that VSM and VM carinae are not conspicuous between the granulation (Fig. 7c, f), with four pairs of VSM macrosetae, being the posterior pair in the posterior margin.

Telson: Vesicle, shallow, more lobular in \mathcal{D} ; ventral surface with medium sized granules in \mathcal{D} , less granular in \mathcal{D} ; dorsal surface smooth, with (\mathcal{D}) or without (\mathcal{D}) an elliptical median well-developed depression corresponding to the telson gland. Aculeus short, shallowly curved (Fig. 7g, h).

Hemispermatophore: Basal portion well developed. Distal lamina well developed, ca. 30% shorter than basal portion; distal crest almost straight, orientated almost in same direction to the posterior margin of the DL; frontal crest (distal posterior flexure) present; internal lobe with two well-developed denticles, not connected with the distal lamina (Fig. 8a), external denticle ca. twice larger than internal denticle. Lobe region poorly developed (Fig. 8c, d); basal lobe well developed, barely protruding, with a flat internal laminar extension, with an internal concavity; genital plug poorly developed, barely exceeding the capsular concavity.

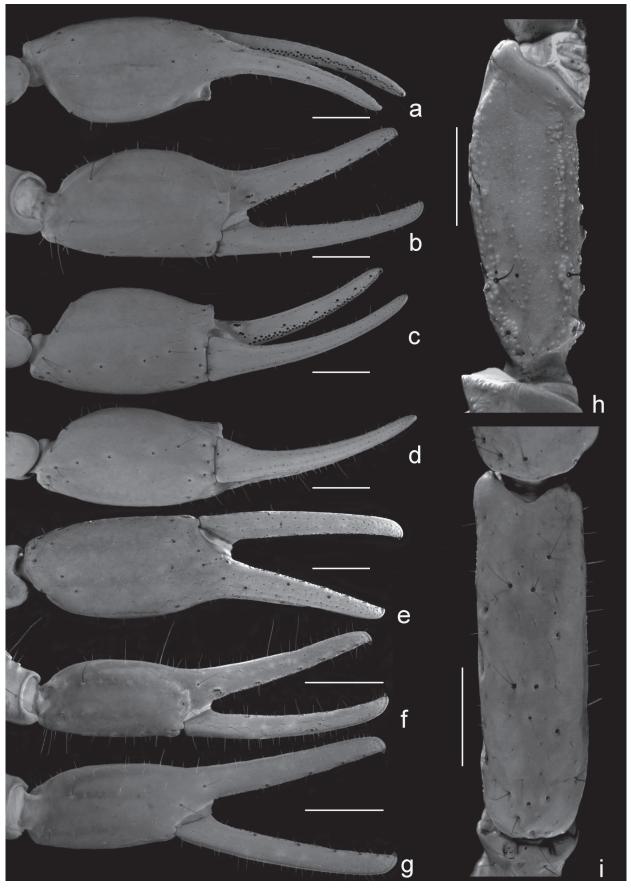


Figure 6. Left pedipalp. **a–e, g–i.** *Urophonius trewanke* sp. nov. **a–e.** Chela, ♂; **a.** Dorsal aspect; **b.** Prolateral aspect; **c.** Ventro-prolateral aspect; **d.** Ventral aspect; **e.** Retrolateral aspect; **g.** Chela ♀, prolateral aspect; **h.** Femur, ♂, dorsal aspect; **i.** Patella, ♂, retrolateral aspect; **f.** *Urophonius tregualemuensis* Cekalovic, 1981. Left pedipalp chela, ♂, prolateral aspect. Scale bars: 1 mm.



Figure 7. a–c, f–h. Urophonius trewanke sp. nov., **a, b.** Sternite V and metasomal segments I and II, ventral aspect **a**. \Diamond and **b**. \Diamond ; **c**. metasomal segment V, \Diamond , ventral aspect; **f**. Metasomal segment V, \Diamond , ventral aspect; **g**. Telson, \Diamond , lateral aspect; **h**. Telson, \Diamond , lateral aspect; **d**. *e*. Urophonius tregualemuensis Cekalovic, 1981, sternite V and metasomal segments I and II, ventral aspect; **d**. \Diamond ; **e**. \Diamond . Scale bars: 1 mm.



Figure 8. a, c, d, *Urophonius trewanke* sp. nov., **a.** Left hemispermatophore, external aspect; **c.** Left hemispermatophore, lobe region, internal aspect; **d.** Right hemispermatophore, lobe region, internal aspect; **b, e, f**, *Urophonius tregualemuensis* Cekalovic, 1981; **b.** Left hemispermatophore, external aspect; **e.** left hemispermatophore, lobe region, internal aspect; **f.** Right hemispermatophore, lobe region, internal aspect. Scale bars: 1 mm.

Distribution. This species has only been collected in its type locality, in the small preserved area of "Fundo La Escuadra", 35°46'06.1"S, 70°47'44.4"W (Fig. 1). This

area is placed in the Maule Valley, in the Maule Region, Chile, close to the Pehuenche international pass which connects Chile with Argentina (Fig. 2). **Ecology.** The area where *Urophonius trewanke* sp. nov. has been collected is located within the landscape of the "Estepa de los Andes Maulinos" (Mauline Andean Steppe) Botanical Formation. This distinctive formation represents the southernmost extension of the high Andean steppes. From this point, towards the south, a change in ecological conditions is perceived, characterised by an increase in precipitation and snowfall. This transition marks a natural limit for the distribution of numerous southern and boreal species (Gajardo 1993).

The ecological matrix of this region is made up of various types of vegetation, which has defined its heterogeneity. Dry forests intersect with shrub steppes, creating a plant mosaic. Notable vegetation components include species such as *Chuquiraga oppositifolia* D.Don, *Gochnatia foliorosa* D.Don and *Proustia cuneifolia* D.Don (Asteraceae), each of which plays a role in shaping the unique habitat of *Urophonius trewanke* sp. nov. (Fig. 1a).

In addition, the landscape presents different herbaceous steppes, where species such as Acaena alpina Poepp ex. Walp. (Rosaceae) and Festuca acanthophylla Desv. (Poaceae) contribute to the overall floral composition. In the midst of this botanical diversity, the presence of the Cordillera cypress (Austrocedrus chilensis (D.Don) Pic-Serm. & Bizzarri.) stands out, which adds to the ecological tapestry with its characteristic shape and contributes to the general microhabitat where Urophonius trewanke sp. nov. occurs. This environment, with its varied types of vegetation and the inclusion of notable species, such as the Cypress of the mountain range, highlights the ecological importance of the "Estepa de los Andes Maulinos" Botanical Formation as a unique and valuable habitat for the diversity of arachnids, providing crucial information on the ecology and habitat preferences of Urophonius trewanke sp. nov.

The Maule Valley and its associated area along the Maule River in the Pehuenche Andean Pass, seems to constitute an area of endemism for the epigean fauna, separated from surrounding valleys by transverse mountain chains. This area is already known to harbour an endemic and highly restricted species of Anuran, *Alsodes pehuenche* Cei, 1976 (Corbalán et al. 2010; Correa et al. 2018, 2020).

In nearby localities outside the Maule Valley, but with similar habitats, *U. trewanke* sp. nov. is replaced by *U. tregualemuensis*, another species of the *granulatus* group, which occupies similar environments and niches as *U. trewanke* sp. nov., but has a wider distribution in south central Chile (Fig. 2a) (Ojanguren-Affilastro et al. 2011). *Urophonius trewanke* sp. nov. has a spring/summer activity period, as in all species within the *granulatus* species group, therefore retaining what we consider the ancestral condition of the genus. In winter, in nearby areas of the Pehuenche Valley, the only active species of *Urophonius* (and of the whole order) is *U. pehuenche*, also endemic from the Pehuenche Valley (Ojanguren-Affilastro et al. 2020).

Urophonius trewanke sp. nov. has been collected in sympatry with an undescribed species of *Brachistosternus*,

which also seems to be endemic of the Pehuenche Pass (Ojanguren-Affilastro et al. in prep.).

Discussion

The description of U. trewanke sp. nov., a rare species belonging to the granulatus group from southern Chile, supports our previous hypothesis that the few species of Urophonius with a summer activity period, are restricted to central and southern Chile and to the colder areas of southern South America, whereas the species with a winter activity period are more widely distributed (Ojanguren-Affilastro et al. 2020, 2023). Our analyses included all known species of the genus and most of the characters used in all recent morphological phylogenies in the family; the lack of major changes in the general topology respect to our previous phylogeny in the genus (Ojanguren-Affilastro et al. 2020) despite the inclusion of a new species to our analysis, provides more support to our previous hypothesis about the relationships inside the genus.

Urophonius trewanke sp. nov. has been collected in "Fundo La Escuadra", meaning La Escuadra farm or ranch, a small preserved area currently under management by ENEL ("Empresa Nacional de Energía Eléctrica" or National Electric Energy Company). This area has been part of the "Cipreses" operational system of the Chilean Electric National System since 1955 and has, therefore, had highly restricted access for the last seventy years. This inaccessibility has resulted in an unintended, but yet remarkable, degree of preservation. This area is placed in the partially isolated Maule Valley which has proved to harbour an exceptional number of endemics, but lacks any kind of formal protection. We hope that the description of the actual diversity of this area could shed light on the need to preserve this important biological resource and lead to future preservation of the endemic species of the Maule Valley.

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Supplementary material 1

Studied material *Urophonius* phylogeny

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Data type: docx

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Supplementary material 2

Characters Urophonius phylogeny

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Supplementary material 3

Matrix Urophonius phylogeny

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Data type: txt

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