Zoosyst. Evol. 91 (1) 2016, 79–102 | DOI 10.3897/zse.91.7616

PENSOFT

museum für naturkunde

Four new species of the genus *Orobdella* from Shikoku and Awajishima island, Japan (Hirudinida, Arhynchobdellida, Orobdellidae)

Takafumi Nakano[1,2]

1 *Department of Science Education, Graduate School of Education, Hiroshima University, 1-1-1 Kagamiyama, Higashihiroshima 739-8524, Japan*

2 *Department of Zoology, Graduate School of Science, Kyoto University, Kitashirakawa-oiwakecho, Sakyo-ku, Kyoto 606-8502, Japan*

http://zoobank.org/04BCA088-96F0-4202-A36F-56FFF1DF2298

Corresponding author: *Takafumi Nakano* (tnakano@hiroshima-u.ac.jp)

Received 24 December 2015
Accepted 22 February 2016
Published 17 March 2016

Academic editor:
Michael Ohl

Key Words

Hirudinea
Orobdella
new species
gastroporous
molecular phylogeny
Japan

Abstract

Four new species of the genus *Orobdella* Oka, 1895 from the mountainous regions of Shikoku and Awajishima island, Japan are described. These new species consist of one quadrannulate, two sexannulate and one octannulate species. The quadrannulate *Orobdella brachyepididymis* **sp. n.** is a small species with a body length reaching only ca. 5 cm. The sexannulate *Orobdella okanoi* **sp. n.** was collected from Shikoku, and the other sexannulate species, *Orobdella yamaneae* **sp. n.**, inhabits Awajishima island. The octannulate *Orobdella nakahamai* **sp. n.** is a large species with a body length greater than 20 cm and is only the second large octannulate species known within this genus. Phylogenetic analyses using nuclear 18S rRNA and histone H3, as well as mitochondrial cytochrome *c* oxidase subunit I, $tRNA^{Cys}$, $tRNA^{Met}$, 12S rRNA, $tRNA^{Val}$, 16S rRNA, $tRNA^{Leu}$ and NADH dehydrogenase subunit 1 markers, indicated that *O. brachyepididymis* is a sister species of the quadrannulate *O. naraharaetmagarum* Nakano, 2016, while the other three new species formed a clade closely related to *O. masaakikuroiwai* Nakano, 2014 and *O. whitmani* Oka, 1895. The ranges of the distant phylogenetic lineage groups of *Orobdella* overlap in Shikoku and adjacent islets.

Introduction

The genus *Orobdella* Oka, 1895 is a terrestrial macrophagous leech taxon that inhabits the Japanese Archipelago, Korean Peninsula and Taiwan (Nakano and Lai 2012, Nakano and Seo 2014, Sawyer 1986). The latest taxonomic study on this genus showed that *Orobdella* contains 13 species (Nakano 2016) split into three groups based on their mid-body somite annulation: eight species in the quadrannulate (four annuli per one somite) group and four species in the sexannulate (six annuli) group. The other species *O. octonaria* Oka, 1895 is an octannulate (eight annuli) taxon.

Additionally, these 13 species can be also split into another three types based on the body lengths of mature leeches (Nakano 2016): three quadrannulate species belong to the small-type (of which the body lengths of mature leeches are shorter than 5 cm); the other five quadrannulate and all sexannulate species are designated the middle-type (reaching ca. 10 cm); and the octannulate species is assigned to the large-type. The body length of the octannulate *O. octonaria* has been described as being greater than 20 cm (Nakano 2012c, Richardson 1971). Molecular phylogenetic analyses of the *Orobdella* species clearly showed that these morphological groups or forms do not reflect phylogenetic relationships (e.g. Nakano 2016): the sexannulate mid-body somite annulation and the small-type body length have evolved in parallel in the genus.

Additional *Orobdella* leeches were collected from Shikoku and Awajishima island, Japan. The specimens collected from Shikoku comprised three morphological units: a quadrannulate small-type, a sexannulate middle-type and an octannulate large-type. Meanwhile, *Orobdella* leeches on Awajishima island belonged to the sexannulate middle-type. Each of these four units is described here as a new species. In addition, the phylogenetic positions of these new species were estimated using

nuclear 18S rRNA and histone H3, as well as mitochondrial cytochrome *c* oxidase subunit I, $tRNA^{Cys}$, $tRNA^{Met}$, 12S rRNA, $tRNA^{Val}$, 16S rRNA, $tRNA^{Leu}$, and NADH dehydrogenase subunit 1 sequence data.

Materials and methods

Sampling and morphological examination

Leeches were collected from five localities in Shikoku, and one locality on Awajishima island, Japan (Fig. 1). When possible, elevation and geographical coordinates for localities were obtained using a Garmin eTrex® GPS unit.

Almost all of the specimens were relaxed by the gradual addition of absolute ethanol to freshwater. For DNA extraction, botryoidal tissue was removed from the posterior part of the body around the caudal sucker of every specimen, and then preserved in absolute ethanol. The remainder of the body was fixed in 10% formalin and preserved in 70% ethanol. Four measurements were taken: body length (BL) from the anterior margin of the oral sucker to the posterior margin of the caudal sucker, maximum body width (BW), caudal sucker length (CL) from the anterior to the posterior margin of the sucker and caudal sucker width (CW) from the right to the left margin of the sucker. Examination, dissection, and drawing of the specimens were conducted using a stereoscopic microscope with a drawing tube (Leica M125). Specimens used in this study have been deposited in the Zoological Collection of Kyoto University (KUZ).

The numbering convention is based on Moore (1927): body somites are denoted by Roman numerals, and the annuli in each somite are given alphanumeric designations.

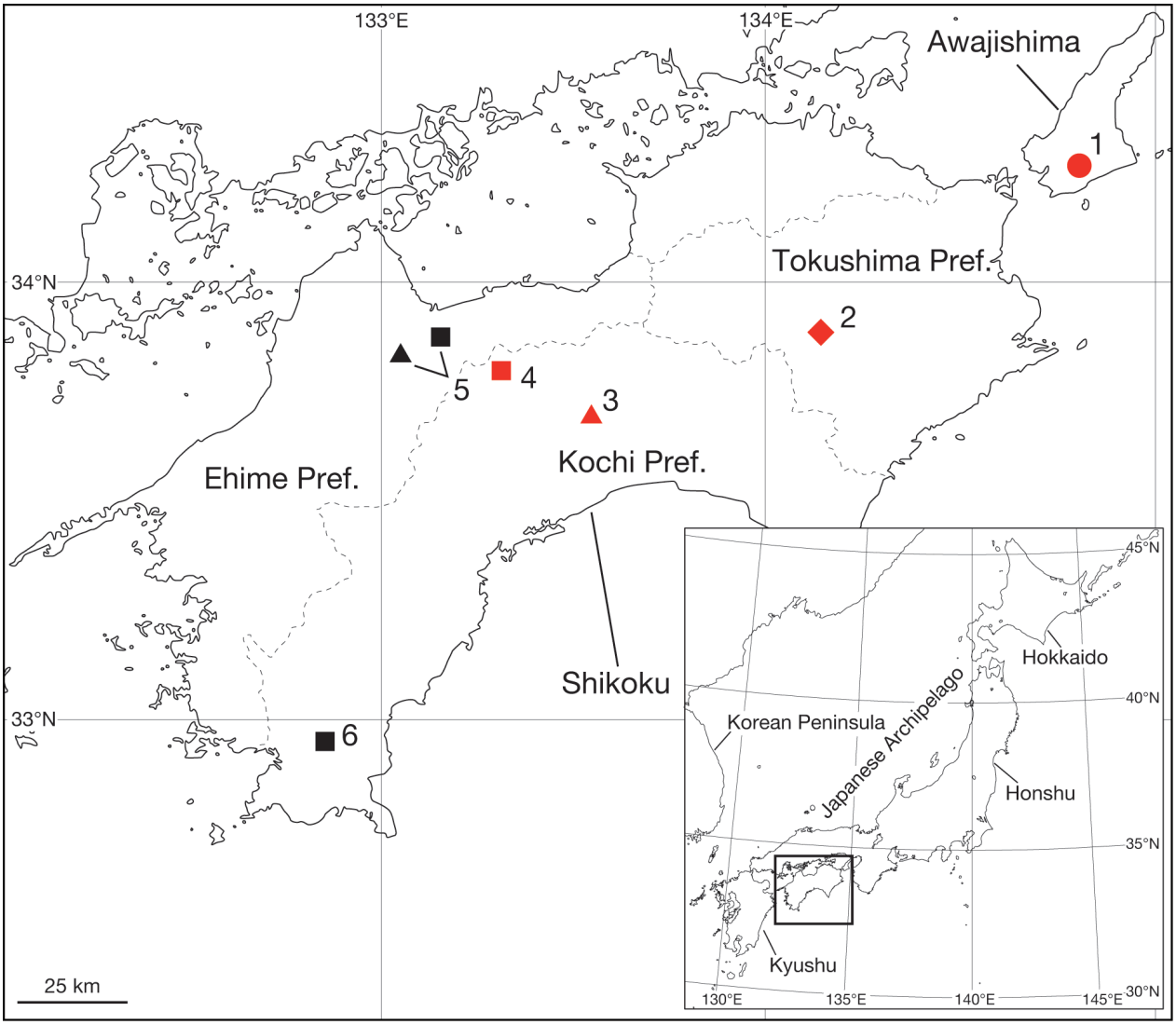

Figure 1. Map showing the collection localities of the specimens examined in this study. The closed diamond (2) denotes the locality of *Orobdella brachyepididymis* sp. n., closed squares (4–6) show the localities of *Orobdella nakahamai* sp. n., closed triangles (3, 5) designate the localities of *Orobdella okanoi* sp. n., and the closed circle (1) specifies the locality of *Orobdella yamaneae* sp. n. Symbols in red indicate the type locality of each of the new species.

The following morphological abbreviations are used in the figures: **ac**—atrial cornu; **af**—annular furrow; **an**—anus; **at**—atrium; **cl**—clitellum; **cod**—common oviduct; **cp**—crop; **ed**—ejaculatory duct; **ep**—epididymis; **fg**—female gonopore; **gd**—gastroporal duct; **gp**—gastropore; **mg**—male gonopore; **np**—nephridiopore; **od**—oviduct; **ov**—ovisac; **ph**—pharynx; **ts**—testisac.

RCP and DNA sequencing

The extraction of genomic DNA from botryoidal tissues preserved in absolute ethanol followed Nakano (2012b). Primer sets for the PCR and cycle sequencing (CS) reactions used in this study were as follows: for 18S rRNA, A and L (PCR and CS), C and Y (PCR and CS), as well as O and B (PCR and CS) (Apakupakul et al. 1999); for histone H3 (H3), H3aF and H3bR (PCR and CS) (Colgan et al. 1998); for cytochrome *c* oxidase subunit I (COI), LCO1490 (PCR and CS) and HCO2198 (CS) (Folmer et al. 1994), and LCO-in (CS) and HCO-out (PCR and CS) (Nakano 2012b) or HCO-outout (PCR and CS) (Nakano 2012a); for $tRNA^{Cys}$, $tRNA^{Met}$, 12S rRNA, $tRNA^{Val}$ and 16S rRNA ($tRNA^{Cys}$–16S), 12SA-out and 12SB-in (PCR and CS), and 12SA-in and 12SB-out (PCR and CS) (Nakano 2012b); for $tRNA^{Leu}$ and NADH dehydrogenase subunit 1 (ND1) ($tRNA^{Leu}$–ND1), LND3000 and HND1932 (PCR and CS) (Light and Siddall 1999). The PCR reactions and DNA sequencing were performed using the modified method mentioned in Nakano (2012a). The PCR reactions were performed using a GeneAmp PCR System 2700 and 9700 (Applied Biosystems) as well as a T100 Thermal Cycler (Bio-Rad). The PCR mixtures were heated to 94°C for 5 min, followed by 35 cycles at 94°C (10 s each), 52°C for 18S and H3, 50°C for the anterior and posterior parts of $tRNA^{Cys}$–16S, 48°C for COI or 42°C for $tRNA^{Leu}$–ND1 (20 s), and 72°C (1 min 12 s for COI, 24 s for H3 or 42 s for the other markers), and a final extension at 72°C for 6 min. The sequencing mixtures were heated 96°C for 2 min, followed by 40 cycles at 96°C (10 s each), 50°C (5 s each) and 60°C (42 s each). The obtained sequences were edited using DNA BASER (Heracle Biosoft S.R.L.). The DNA sequences listed in Table 1 were newly obtained in this study and were deposited with the International Nucleotide Sequence Database Collaboration (INSDC) through the DNA Data Bank of Japan (DDBJ). The GenSeq nomenclature proposed by Chakrabarty et al. (2013) was followed in Table 1 to show the reliability of the obtained DNA sequences.

Molecular phylogenetic and genetic distance analyses

Eighty-five published sequences were obtained from the INSDC for use in molecular phylogenetic analyses (Table 1). In addition to 13 known *Orobdella* species, the following four erpobdelliform species were used as outgroup taxa: *Erpobdella japonica* Pawłowski, 1962 (Erpobdellidae), *Gastrostomobdella monticola* Moore, 1929 (Gastrostomobdellidae), *Mimobdella japonica* Blanchard, 1897, and *Odontobdella blanchardi* (Oka, 1910a) (both Salifidae).

The phylogenetic relationships of the newly identified *Orobdella* species within the genus was estimated based on 18S, H3, COI, $tRNA^{Cys}$–16S and $tRNA^{Leu}$–ND1 sequences. The alignments of H3 and COI were trivial, as no indels were observed. 18S, $tRNA^{Cys}$–16S, and $tRNA^{Leu}$–ND1 were aligned using MAFFT v. 7.266 L-INS-I (Katoh and Standley 2013). The lengths of the 18S, H3, COI, $tRNA^{Cys}$–16S, and $tRNA^{Leu}$–ND1 sequences were 1,844, 328, 1,267, 1,135, and 635 bp, respectively. The concatenated sequences thus yielded 5,209 bp of aligned positions.

Phylogenetic trees were constructed using maximum likelihood (ML) and Bayesian inference (BI). ML phylogenies were constructed using RAxML v. 8.1.5 (Stamatakis 2014) with the substitution model set as GTRCAT, immediately after nonparametric bootstrapping (Felsenstein 1985) conducted with 1,000 replicates. The best-fit partitioning scheme for the ML analyses was identified with the Akaike information criterion (Akaike 1974) using PartitionFinder v. 1.1.1 (Lanfear et al. 2012) with the "greedy" algorithm: 18S/the 1st and 2nd positions of H3/the 3rd position of H3/the 1st position of COI/the 2nd position of COI/the 3rd positions of COI and ND1/the 1st position of ND1/the 2nd position of ND1/12S/16S/$tRNA^{Cys}$, $tRNA^{Met}$, $tRNA^{Val}$ and $tRNA^{Leu}$.

BI and Bayesian posterior probabilities (BPPs) were estimated using MrBayes v. 3.2.5 (Ronquist et al. 2012). The best-fit partition scheme and models for each partition were selected based on the Bayesian information criterion (Schwarz 1978) using PartitionFinder with the "greedy" algorithm: for 18S and the 1st position of H3, K80+I; for the 2nd position of H3, JC69; for the 3rd position of H3, HKY85+G; for the 1st position of COI, GTR+G; for the 2nd positions of COI and ND1, HKY85+I+G; for the 3rd positions of COI and ND1 plus 16S, HKY85+I+G; for the 1st position of ND1, 12S, $tRNA^{Cys}$, $tRNA^{Met}$, $tRNA^{Val}$ and $tRNA^{Leu}$, GTR+I+G. Two independent runs of four Markov chains were conducted 12 million generations, and the tree was sampled every 100 generations. The parameter estimates and convergence were checked using Tracer v. 1.6.0 (Rambaut and Drummond 2009) and the first 40,001 trees were discarded based on the results.

Nodes with bootstrap support (BS) values higher than 70% were considered sufficiently resolved (Hillis and Bull 1993). Nodes with BPPs higher than 0.95 were considered statistically significant (Leaché and Reeder 2002).

Pairwise comparisons of uncorrected *p*-distances for nine COI sequences (1,267 bp) obtained from specimens of the new species in this study and sequences from *Orobdella masaakikuroiwai* Nakano, 2014 and *Orobdella naraharaetmagarum* Nakano, 2016 were calculated using MEGA6.06 (Tamura et al. 2013) in accordance with the results of the molecular phylogenetic analyses.

Table 1. Samples used for the phylogenetic analyses. The information on the vouchers is accompanied by the collection locality numbers for the new species of *Orobdella* described in this study (see Fig. 1) and the INSDC accession numbers. Sequences marked with am asterisk were obtained for the first time in the present study. Acronyms: KUZ, the Zoological Collection of Kyoto University; UNIMAS, the Universiti Malaysia Sarawak.

Species	Voucher (locality number)	INSDC #					GenSeq Nomenclature
		18S	Histone H3	COI	$tRNA^{Cys}$–16S	$tRNA^{Leu}$–ND1	
Orobdella brachyepididymis sp. n.	KUZ Z1673 Holotype (2)	LC106319*	LC106321*	LC106320*	LC106318*	LC106322*	genseq-1 18S, H3, COI, $tRNA^{Cys}$, $tRNA^{Met}$, 12S, $tRNA^{Val}$, 16S, $tRNA^{Leu}$, ND1
Orobdella brachyepididymis sp. n.	KUZ Z1674 Paratype (2)			LC106324*	LC106323*	LC106325*	genseq-2 COI, $tRNA^{Cys}$, $tRNA^{Met}$, 12S, $tRNA^{Val}$, 16S, $tRNA^{Leu}$, ND1
Orobdella nakahamai sp. n.	KUZ Z1352 Paratype (5)			LC106327*	LC106326*	LC106328*	genseq-2 COI, $tRNA^{Cys}$, $tRNA^{Met}$, 12S, $tRNA^{Val}$, 16S, $tRNA^{Leu}$, ND1
Orobdella nakahamai sp. n.	KUZ Z1672 Holotype (4)	LC106330*	LC106332*	LC106331*	LC106329*	LC106333*	genseq-1 18S, H3, COI, $tRNA^{Cys}$, $tRNA^{Met}$, 12S, $tRNA^{Val}$, 16S, $tRNA^{Leu}$, ND1
Orobdella nakahamai sp. n.	KUZ Z1680 (6)			LC106335*	LC106334*	LC106336*	genseq-3 COI, $tRNA^{Cys}$, $tRNA^{Met}$, 12S, $tRNA^{Val}$, 16S, $tRNA^{Leu}$, ND1
Orobdella okanoi sp. n.	KUZ Z1491 (5)			LC106338*	LC106337*	LC106339*	genseq-3 COI, $tRNA^{Cys}$, $tRNA^{Met}$, 12S, $tRNA^{Val}$, 16S, $tRNA^{Leu}$, ND1
Orobdella okanoi sp. n.	KUZ Z1671 Holotype (3)	LC106341*	LC106343*	LC106342*	LC106340*	LC106344*	genseq-1 18S, H3, COI, $tRNA^{Cys}$, $tRNA^{Met}$, 12S, $tRNA^{Val}$, 16S, $tRNA^{Leu}$, ND1
Orobdella yamaneae sp. n.	KUZ Z1358 Paratype (1)			LC106346*	LC106345*	LC106347*	genseq-2 COI, $tRNA^{Cys}$, $tRNA^{Met}$, 12S, $tRNA^{Val}$, 16S, $tRNA^{Leu}$, ND1
Orobdella yamaneae sp. n.	KUZ Z1678 Holotype (1)	LC106349*	LC106351*	LC106350*	LC106348*	LC106352*	genseq-1 18S, H3, COI, $tRNA^{Cys}$, $tRNA^{Met}$, 12S, $tRNA^{Val}$, 16S, $tRNA^{Leu}$, ND1
Orobdella dolichopharynx Nakano, 2011b	KUZ Z120 Holotype	AB663665	AB698876	AB679680	AB679681	AB828558	
Orobdella esulacata Nakano, 2010	KUZ Z29 Holotype	AB663655	AB698873	AB679664	AB679665	AB828555	
Orobdella ijimai Oka, 1895	KUZ Z110 Topotype	AB663659	AB698877	AB679672	AB679673	AB828559	
Orobdella kawakatsuorum Richardson, 1975	KUZ Z167 Topotype	AB663661	AB698878	AB679704	AB679705	AB828561	
Orobdella ketagalan Nakano & Lai, 2012	KUZ Z208 Holotype	AB704785	AB704786	AB704787	AB828582	AB828563	
Orobdella koikei Nakano, 2012b	KUZ Z156 Holotype	AB698883	AB698882	AB679688	AB679689	AB828560	
Orobdella masaakikuroiwai Nakano, 2014	KUZ Z694 Holotype	AB938003	AB938013	AB938006	AB937997	AB938016	
Orobdella mononoke Nakano, 2012a	KUZ Z224 Holotype	AB698868	AB698869	AB698866	AB698867	AB828564	
Orobdella naraharaetmagarum Nakano, 2016	KUZ Z1652 Holotype	LC087143	LC087145	LC087144	LC087142	LC087146	
Orobdella octonaria Oka, 1895	KUZ Z181 Topotype	AB698870	AB698871	AB679708	AB679709	AB828562	
Orobdella shimadae Nakano, 2011b	KUZ Z128 Holotype	AB663663	AB698875	AB679676	AB679677	AB828557	
Orobdella tsushimensis Nakano, 2011a	KUZ Z134 Holotype	AB663653	AB698872	AB679662	AB679663	AB828554	
Orobdella whitmani Oka, 1895	KUZ Z45 Topotype	AB663657	AB698874	AB679668	AB679669	AB828556	
Erpobdella japonica Pawłowski, 1962	KUZ Z178	AB663648	AB698879	AB679654	AB679655	AB828542	
Gastrostomobdella monticola Moore, 1929	UNIMAS/ A3/ BH01/10	AB663649	AB698880	AB679656	AB679657	AB828543	
Mimobdella japonica Blanchard, 1897	KUZ Z179	AB663650	AB698881	AB679658	AB679659	AB828544	
Odontobdella blanchardi (Oka, 1910a)	KUZ Z180	AB663651	AB938012	AB938004	AB937995	AB938014	

Results

Taxonomy

Family Orobdellidae Nakano et al., 2012

Genus *Orobdella* Oka, 1895

Orobdella Oka, 1895: 278–280; Oka 1910b: 177; Soós 1966: 377, 381, 382; Richardson 1975: 42; Lukin 1976: 463, 464; Sawyer 1986: 680; Nakano 2010: 881; Nakano 2011b: 3.

Kumabdella Richardson 1971: 590, 591 (type species, *Orobdella octonaria* Oka, 1895 by original designation).

Type species. *Orobdella whitmani* Oka, 1895 by subsequent designation of Soós (1966).

Diagnosis. Body firm and muscular, elongate, with constant width in caudal direction, dorsoventrally compressed. Somite I completely merged with prostomium. Somite II uniannulate, not separated from I. Mid-body somite annulation variable, complete quadr-, sex- or octannulate. Post-anal annulus absent. Male gonopore in posterior part of XI. Female gonopore in anterior part of XIII. Pappilae numerous, minute, hardly visible, one row on every annulus. Pharynx agnathous, euthylaematous. Crop tubular, acaecate. Gastropore, when present, in anterior part of XIII. Gastroporal duct generally lying on female organ. Intestine tubular, acaecate. Rectum tubular, thin-walled, straight. Testisacs multiple. Ejaculatory bulbs absent. Male median reproductive system in posterior part of XI, without penis or penis sheath. Ovisacs globular. Oviducts thin-walled. Common oviduct thin-walled, short. Female median reproductive system essentially lacking.

Orobdella brachyepididymis sp. n.

http://zoobank.org/2A527AE0-4B23-4D87-B4EB-83C5A2F00520
Figs 2–5

Type materials. Holotype: KUZ Z1673, dissected, collected from under a rock along a road (33.87151°N, 134.12016°E; Elev. ca. 990 m; locality #2, see Fig. 1) at Mt. Ichinomori, Mima, Tokushima Prefecture, Japan, by TN on 7 July 2015. Paratype: KUZ Z1674, dissected, collected from under a rock along a road (33.87119°N, 134.12233°E; Elev. ca. 1014 m) at the type locality, by TN on 7 July 2015.

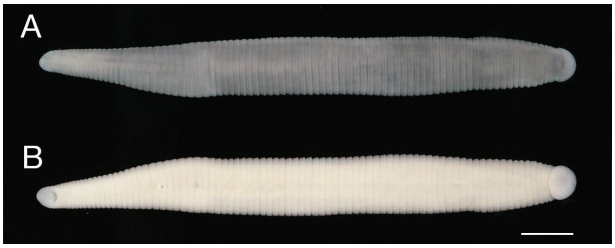

Figure 2. *Orobdella brachyepididymis* sp. n., holotype, KUZ Z1673. **A** dorsal, **B** ventral views. Scale bar: 5 mm.

Type locality. Japan, Tokushima Prefecture: Mima, Mt. Ichinomori (Shikoku).

Diagnosis. Body length of mature individual reaching to ca. 5 cm. Somite IV uniannulate, somites VIII–XXV quadrannulate. Clitellum in XI b5 to XIII a2. Male gonopore in middle of XI b6, female gonopore in anterior margin of or slightly anterior to middle of XIII a1, behind gastropore, gonopores separated by 1/2 + 4 [+ (< 1/2)] annuli. Pharynx reaching to XIV a1–a1/a2. Gastropore conspicuous, in anterior margin of or slightly anterior to middle of XIII a1. Gastroporal duct tubular, slightly bulbous at junction with gastropore. Paired epididymides in XX to XXI, occupying four annuli (one somite). Atrial cornua small ovate.

Description of holotype. BL 51.6 mm, BW 5.2 mm (Fig. 2). Caudal sucker ventral, elliptic, CL 3.0 mm, CW 3.4 mm (Figs 2B, 3D).

Somites III, IV uniannulate; IV with slight dorsal furrow (Fig. 3A). Somite V biannulate, (a1 + a2) = a3; a3 forming posterior margin of oral sucker (Fig. 3A, B). Somites VI and VII triannulate; VI, a1 < a2 > a3, a2 with slight dorsal furrow; VII, a1 = a2 = a3 (Fig. 3A, B). Somites VIII–XXV quadrannulate, a1 = a2 = b5 = b6 (Fig. 3A–E). Somite XXVI triannulate; dorsally a1 > a2 < a3, a3 with slight furrow; ventrally a1 > a2 = a3; a3 being ventrally last complete annulus (Fig. 3C, D). Somite XXVII uniannulate with slight dorso-lateral furrows (Fig. 3C). Anus behind somite XXVII (Fig. 3C).

X b5 and XIII a2, respectively, being first and last annuli of clitellum (Fig. 3E).

Male gonopore in middle of XI b6 (Fig. 3E). Female gonopore in anterior margin of XIII a1, inconspicuous, located posterior to gastropore (Fig. 3E, F). Gonopores separated by 1/2 + 4 annuli (Fig. 3E).

Anterior ganglionic mass in VI a2 and a3. Ganglion VII in a2. Ganglion VIII in a2 and b5. Ganglia IX and X, of each somite, in a2. Ganglion XI in a2 and b5 (Fig. 4A). Ganglia XII–XVI, of each somite, in a2 (Fig. 4A). Ganglia XVII–XX, of each somite, in a1 and a2 (Fig. 4A). Ganglia XXI (Fig. 4A) and XXII, of each somite, in a2. Ganglion XXIII in a2 and b5. Ganglia XXIV and XXV, of each somite, in a1 and a2. Ganglion XXVI in XXV b6 and XXVI a1. Posterior ganglionic mass in XXVI a2 and a3.

Eyes in three pairs, first pair dorsally on posterior margin of II, second and third pairs dorsolaterally on posterior margin of V (a1 + a2) (Fig. 3A).

Nephridiopores in 17 pairs, one each situated ventrally at posterior margin of a1 of each somite in VIII–XXIV (Fig. 3B, D, E).

Pharynx reaching to XIV a1 (Fig. 3G). Crop reaching to XIX b5/b6. Gastropore conspicuous, ventral, in anterior margin of XIII a1 (Fig. 3E, F). Gastroporal duct tubular, but slightly bulbous and winding at junction with gastropore, joining with crop in XIV a1 (Fig. 3G). Intestine reaching to XXIV/XXV.

Testisacs (Fig. 4A); on right side, in XXI b6 to XXV b5, in total approx. 27 testisacs, 2 in XXI, 7 in XXII, 6 in

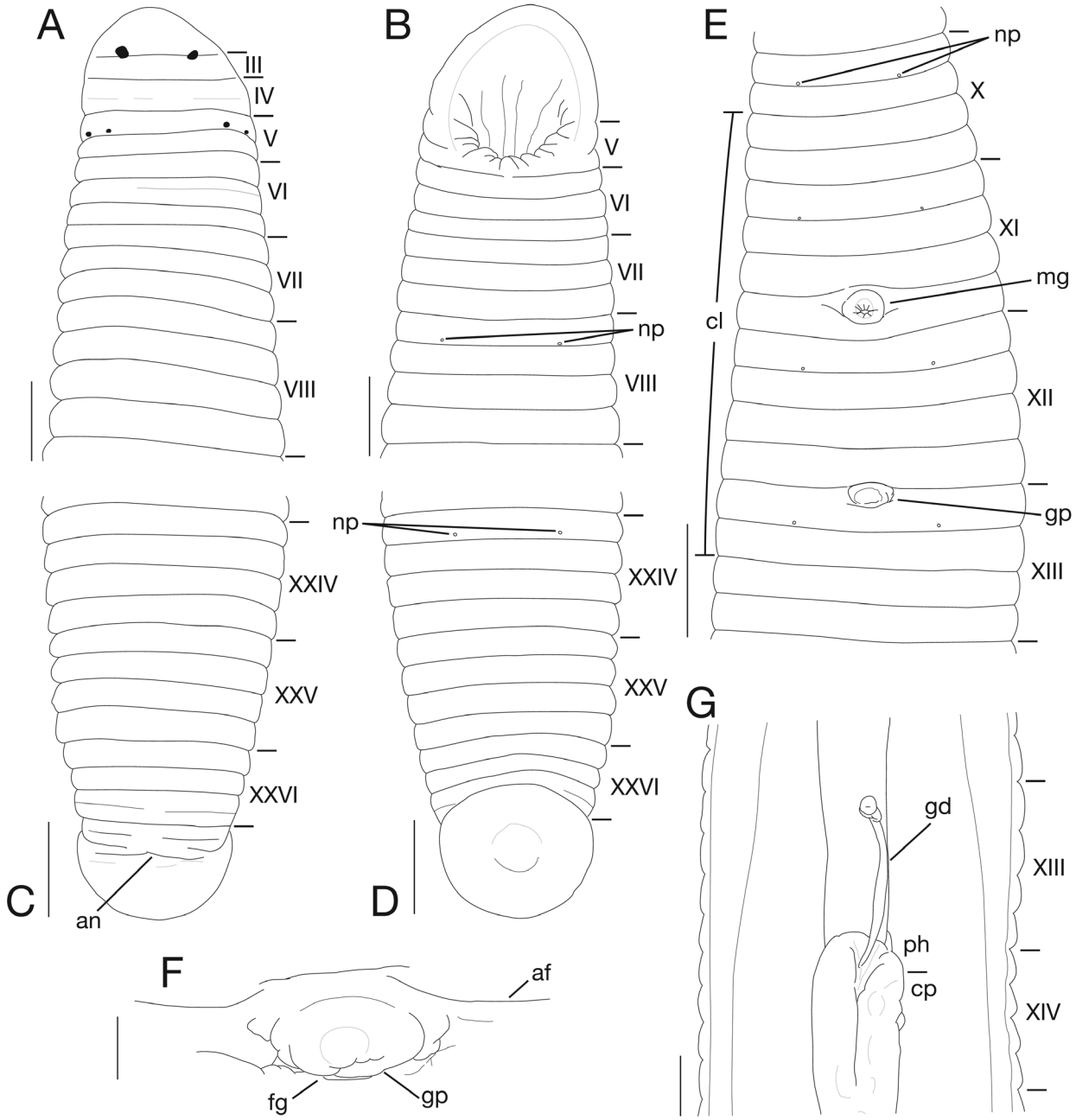

Figure 3. *Orobdella brachyepididymis* sp. n., holotype, KUZ Z1673. **A** dorsal, **B** ventral views of somites I–VIII; **C** dorsal, **D** ventral views of somites XXIV–XXVII and caudal sucker; **E** ventral view of somites X–XIII; **F** ventral view of gastropore and female gonopore; **G** ventral view of gastroporal duct. Scale bars: **A, B, G** = 1 mm; **C–E** = 2 mm; **F** = 0.25 mm.

XXIII, 7 in XXIV, 5 in XXV; on left side, in XXI b6 to XXV b6, in total approx. 25 testisacs, 2 in XXI, 5 in XXII, 7 in XXIII, 5 in XXIV, 6 in XXV. Paired epididymides; right epididymis in XX b5/b6 to XXI b5/b6, occupying 4 annuli; left epididymis in XX b6 to XXI b5/b6, occupying 4 annuli (Fig. 4A). Paired ejaculatory ducts, thick; right duct in XI b5 to XX b5/b6; left duct in XI b5 to XX b6; loosely coiled in position posterior to ovisacs; each widening from respective junction with epididymis, narrowing at junction with atrial cornua, then turning gradually inward toward atrial cornua without pre-atrial loop (Fig. 4A–D). Pair of muscular atrial cornua small ovate, in XI b5 and b6 (Fig. 4A–D). Atrium short, muscular, globular in XI b5 and b6 (Fig. 4A–D).

Paired ovisacs in XIII a2 and b5 (Fig. 4A, E). Oviducts; right oviduct crossing ventrally beneath nerve cord; both oviducts converging into common oviduct in XIII a1 (Fig. 4A, E). Common oviduct directly descending to female gonopore (Fig. 4E).

Variation. BL 36.3 mm, BW 3.5 mm, CL 1.8 mm, CW 2.2 mm. Somite IV uniannulate Somite VI triannulate, a1 = a2 = a3. Male gonopore in middle of XI b6, female gonopore in slightly anterior to middle of XIII a1, go-

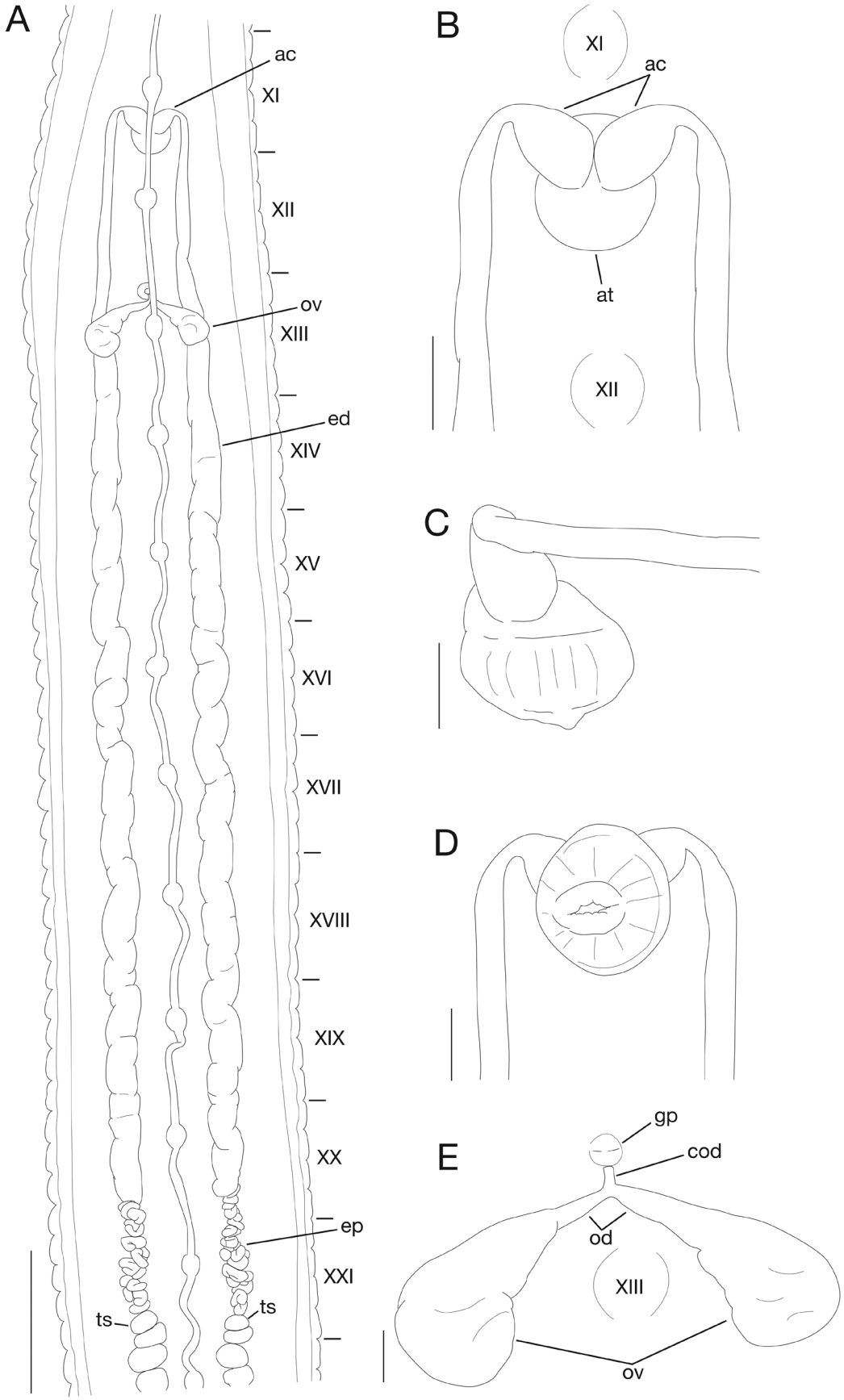

Figure 4. *Orobdella brachyepididymis* sp. n., holotype, KUZ Z1673. **A** dorsal view of reproductive system including ventral nervous system; **B** dorsal (including positions of ganglia XI and XII), **C** lateral, **D** ventral views of male atrium; **E** dorsal view of female reproductive system including position of ganglion XIII. Scale bars: **A** = 3 mm; **B–D** = 0.5 mm; **E** = 0.25 mm.

Table 2. Comparisons of morphological characters between *Orobdella brachyepididymis* sp. n. and eight quadrannulate congeneric species.

Character	Body length	Somite IV	Somite XXV	Gastroporal duct	Annuli between gonopores	Epididymides	Atrial cornua
Orobdella brachyepididymis sp. n.	less than or reaching to ca. 5 cm	uniannulate	quadrannulate	tubular	1/2 + 4 [+ (< 1/2)]	XX to XXI	small, ovate
Orobdella esulcata	reaching to ca. 10 cm	uniannulate	quadrannulate	tubular, but bulbous at junction with gastropore	2/3 + 4 + 1/3	XVI to XX	developed, ovate
Orobdella kawakatsuorum	reaching to ca. 10 cm	biannulate	quadrannulate	simple tubular	6	XVI to XVII	undeveloped
Orobdella ketagalan	reaching to ca. 10 cm	uniannulate	quadrannulate	simple tubular	1/2 + 4 + 1/2	absent	undeveloped
Orobdella koikei	less than 4 cm	uniannulate	triannulate	bulbous	1/2 + 4 + 1/2	XV to XX	developed, ovate
Orobdella masaakikuroiwai	less than 4 cm	uniannulate	quadrannulate	bulbous	1/2 + 4 + 1/2	XVI to XVIII	developed, ovate
Orobdella naraharaetmagarum	less than 5 cm	uniannulate	quadrannulate	bulbous	1/2 + 4 + 1/2	XV to XX	developed, ellipsoid or ovate
Orobdella tsushimensis	reaching to ca. 10 cm	uniannulate	quadrannulate	bulbous	1/2 + 5	XVII to XIX	developed, ovate
Orobdella whitmani	reaching to ca. 10 cm	uni- or biannulate	quadrannulate	bulbous	1/2 + 4 + 1/2	XVI to XVIII	developed, ovate

Figure 5. *Orobdella brachyepididymis* sp. n., holotype, KUZ Z1673. Dorsal view of live animal. Scale bar: 5 mm.

nopores thus separated by 1/2 + 4 + (< 1/2) annuli. Pharynx reaching to XIV a1/a2. Crop reaching to XX a1/a2. Gastropore in slightly anterior to middle of XIII a1. Gastroporal duct tubular, but slightly bulbous at junction with gastropore, joining with crop in XIV a2/b5. Intestine reaching to XXIV a1/b5. Testisacs undetectable. Paired epididymides; right epididymis in XX b5 to XXI a2/b5; left epididymis in XX a2/b5 to XXI a2; each occupying four annuli. Atrium in XI b6. Left oviduct crossing ventrally beneath nerve cord.

Colouration. In life, dorsal surface grayish (Fig. 5); ventral surface whitish red; clitellum, when obvious, whitish grayish pale ochre (Fig. 5). Color faded in preservative.

Etymology. The specific name is a compound noun in apposition derived from the Greek words transliterated into Latin, brachys (short) and epididymis (epididymis), referring to the fact that the epididymides of this species occupy only four annuli.

Distribution. This species was found only from its type locality.

Natural history. This species was found curled up under rocks in moist mountainous habitats. As oligochaete worms were observed in the digestive tract of a dissected specimen, KUZ Z1674, this species is an earthworm-eater, as are the other known *Orobdella* species.

A mature leech, KUZ Z1673, was collected on 7 July. Therefore, *Orobdella brachyepididymis* is considered to enter its reproductive season before early July.

Remarks. The specimens were small (up to 52 mm), but the holotype was determined to be mature because it possessed an obvious clitellum and developed testisacs.

According to taxonomic studies (Nakano 2010, 2012b, 2014, 2016, Nakano and Gongalsky 2014, Nakano and Lai 2012, Nakano and Seo 2014), the new species is distinguished from the eight quadrannulate species (i.e. *O. esulcata* Nakano, 2010, *O. kawakatsuorum* Richardson, 1975, *O. ketagalan* Nakano & Lai, 2012, *O. koikei* Nakano, 2012b, *O. masaakikuroiwai*, *O. naraharaetmagarum*, *O. tsushimensis* Nakano, 2011a and *O. whitmani*) by the following combination of characteristics (Table 2): body length less than or reaching ca. 5 cm, IV uniannulate, XXV quadrannulate, gonopores separated by 1/2 + 4 [+ (< 1/2)] annuli, gastroporal duct tubular, epididymides in XX to XXI and atrial cornua small ovate.

Orobdella nakahamai sp. n.

http://zoobank.org/47B528A0-16E3-40D6-BAA8-E7035C167A56
Figs 6–9

Type materials. Holotype: KUZ Z1672, dissected, collected from under rocks of a small mountain stream at Mt. Takanosuyama (33.788°N, 133.271°E; Elev. ca. 1500 m; locality #4), Ino, Kochi Prefecture, Japan, by Ryosuke Okano on 4 July 2015. Paratype: KUZ Z1352, dissected, collected from under rocks of a small mountain stream at Mt. Iwagurosan

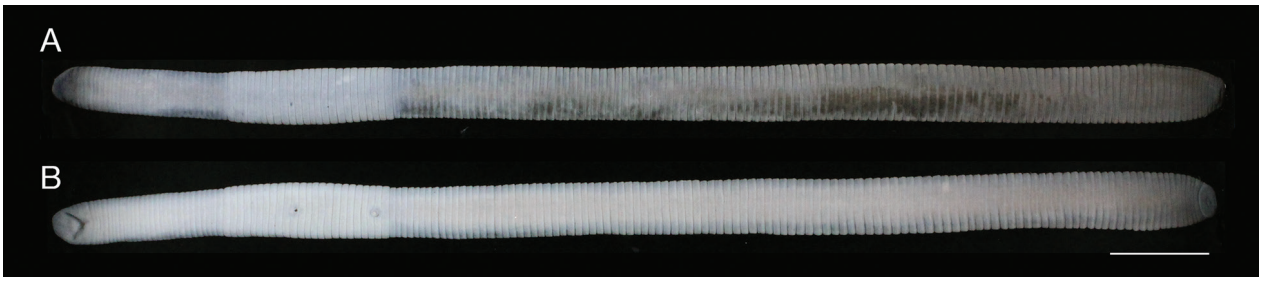

Figure 6. *Orobdella nakahamai* sp. n., holotype, KUZ Z1672. **A** dorsal, **B** ventral views. Scale bar: 2 cm.

(33.7516°N, 133.1533°E; Elev. ca. 1600 m; locality #5), Kumakogen, Ehime Prefecture, Japan, by Naoyuki Nakahama on 10 August 2010. For locality numbers, see Fig. 1.

Additional material. KUZ Z1680, collected from Kaminodoi (32.942714°N, 132.841350°E; Elev. ca 140 m; locality #6, see Fig. 1), Shimanto, Kochi Prefecture, Japan, by Ryosuke Okano on 2 November 2015.

Type locality. Japan, Kochi Prefecture: Ino, Ishizuchi Mountains, Mt. Takanosuyama (Shikoku).

Diagnosis. Body length of mature individual greater than 15 cm. Somites IX–XXV octannulate. Clitellum in X c9 to XIII b4. Male gonopore in slightly posterior to middle of XI c11 or XI c10/c11, female gonopore in XIII b2/b3, behind gastropore, gonopores separated by 1/2 + 11 or 12 annuli. Pharynx reaching to XIV b1–b3/b4. Gastropore conspicuous, in XIII b2/b3. Gastroporal duct bulbous, slightly winding at junction with gastropore. Paired epididymides in XV to XVII, occupying 12 or 13 annuli (one and half somites). Atrial cornua ovate or ellipsoid.

Description of holotype. BL 237.7 mm, BW 11.3 mm (Fig. 6). Caudal sucker ventral, elliptic, CL 3.9 mm, CW 6.5 mm (Figs 6B, 7D).

Somites III–V biannulate; III and V, (a1 + a2) = a3; IV, (a1 + a2) > a3; V a3 forming posterior margin of oral sucker (Fig. 7A, B). Somite VI dorsally quinquannulate, b1 = b2 < a2 > b5 < b6; ventrally quadrannulate, a1 = a2 > b5 < b6 (Fig. 7A, B). Somite VII quiquannulate, a1 > b3 = b4 = b5 = b6, a1 with slight lateral furrow on each side (Fig. 7A, B). Somite VIII dorsally septannulate, a1 (with obvious secondary furrow, b1 = b2) > b3 = b4 = c9 = c10 = c11 = c12; ventrally octannulate, b1 = b2 = b3 = b4 = c9 = c10 = c11 = c12 (Fig. 7A, B). Somite IX octannulate, b1 = b2 = b3 < b4 = c9 = c10 = c11 = c12. Somites X–XXV octannulate, b1 = b2 = b3 = b4 = c9 = c10 = c11 = c12 (Fig. 7C–E). Somite XXVI octannulate, b1 = b2 = b3 = b4 = c9 = c10 = c11 > c12. Somite XXVII comprises three annuli, second and third annuli, respectively, with slight dorsal furrow; first annulus being ventrally last complete annulus (Fig. 7C, D). Anus behind somite XXVII (Fig. 7C).

X c9 and XIII b4, respectively, being first and last annuli of clitellum (Fig. 7E).

Male gonopore in slightly posterior to middle of XI c11 (Fig. 7E). Female gonopore in XIII b2/b3, inconspicuous, located posterior to gastropore (Fig. 7E, F). Gonopores separated by 1/2 + 11 annuli (Fig. 7E).

Anterior ganglionic mass in VI b5 and b6. Ganglion VII in a1. Ganglia VIII–X, of each somite, in b3 and b4. Ganglion XI in b4 (Fig. 8B). Ganglion XII in b3 and b4 (Fig. 8B). Ganglion XIII in b4 (Fig. 8B). Ganglia XIV–XVI, of each somite, in b3 and b4 (Fig. 8B). Ganglion XVII in b3 (Fig. 8B). Ganglion XVIII in b3 and b4. Ganglion XIX in b3. Ganglia XX and XXI, of each somite, in b3 and b4. Ganglia XXII–XXV, of each somite, in b3. Ganglion XXVI in b2. Posterior ganglionic mass in XXVI c10–c12.

Eyes undetectable.

Nephridiopores in 17 pairs, one each situated ventrally at posterior margin of b2 of each somite in VIII–XXIV (Fig. 7B, D, E).

Pharynx reaching to XIV b1 (Fig. 8A). Crop reaching to XXII c9. Gastropore conspicuous, ventral, in XIII b2/b3 (Fig. 7E, F). Gastroporal duct bulbous, slightly winding at junction with gastropore, joining with crop in XIII c12 (Fig. 8A). Intestine reaching to XXV/XXVI.

Testisacs (Fig. 8B); on right side, in XVII c9 to XXV c11, in total approx. 164 testisacs, 7 in XVII, 18 in XVIII, 24 in XIX, 21 in XX, 21 in XXI, 20 in XXII, 23 in XXIII, 16 in XXIV, 14 in XXV; on left side, in XVII b4 to XXV c12, in total approx. 160 testisacs, 6 in XVII, 15 in XVIII, 24 in XIX, 20 in XX, 22 in XXI, 18 in XXII, 18 in XXIII, 21 in XXIV, 16 in XXV. Paired epididymides in XV c9 to XVI/XVII, occupying 12 annuli (Fig. 8B). Paired ejaculatory ducts in XI c9 to XV c9; coiled in position posterior to ovisacs; each duct crossing ventrally beneath each ovisac, then running straight in position anterior to ovisacs; each widening from respective junction with epididymis, narrowing at junction with atrial cornua, then turning sharply inward toward atrial cornua without pre-atrial loop (Fig. 8B–E). Pair of muscular atrial cornua ovate, in XI c9–c11 (Fig. 8B–E). Atrium short, muscular, globular in XI c10 and c11 (Fig. 8B–E).

Paired ovisacs in XIII b3 and b4 (Fig. 8B, F). Oviducts; right oviduct crossing ventrally beneath nerve cord; both oviducts converging into common oviduct in XIII b3/b4 (Fig. 8B, F). Common oviduct directly descending to female gonopore (Fig. 8B, F).

Variations. BL 162.3–180.4 mm, BW 7.6–98 mm, CL 3.3–4.3 mm, CW 5.6 mm. Somite VI quadrannulate, a1

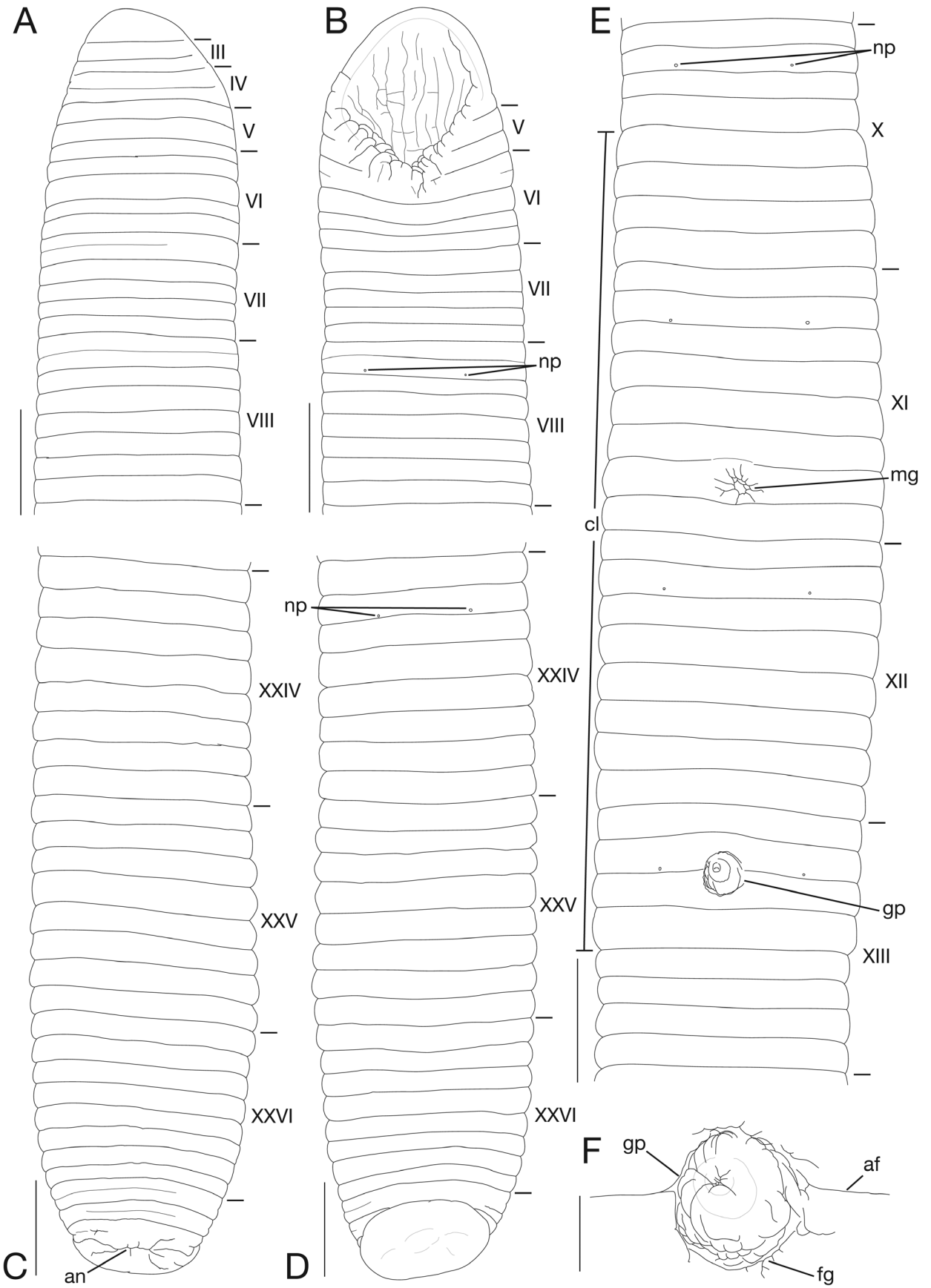

Figure 7. *Orobdella nakahamai* sp. n., holotype, KUZ Z1672. **A** dorsal, **B** ventral views of somites I–VIII; **C** dorsal, **D** ventral views of somites XXIV–XXVII and caudal sucker; **E** ventral view of somites X–XIII; **F** ventral view of gastropore and female gonopore. Scale bars: **A–E** = 5 mm; **F** = 1 mm.

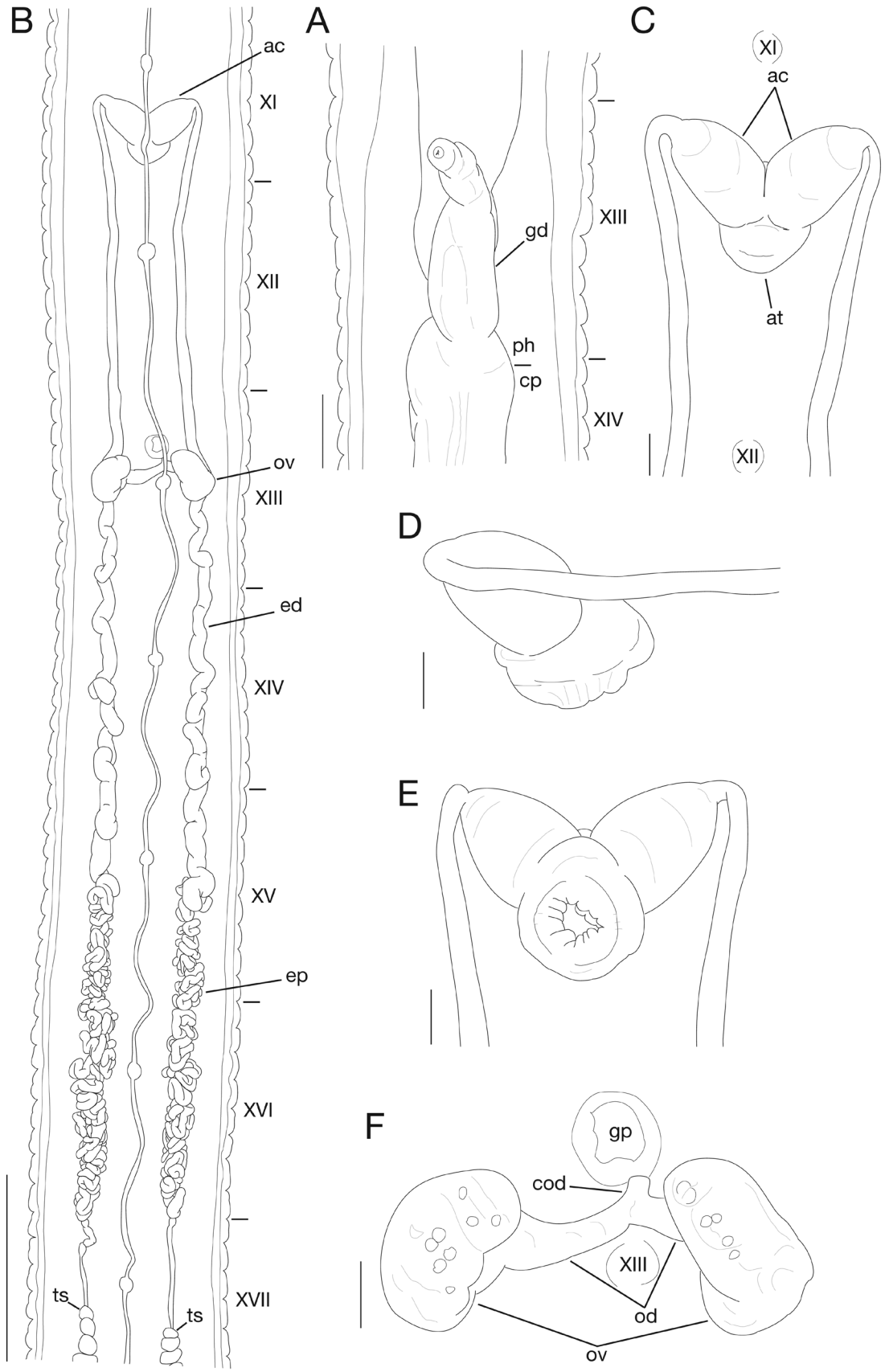

Figure 8. *Orobdella nakahamai* sp. n., holotype, KUZ Z1672. **A** ventral view of gastroporal duct; **B** dorsal view of reproductive system including ventral nervous system; **C** dorsal (including positions of ganglia XI and XII), **D** lateral, **E** ventral views of male atrium; **F** dorsal view of female reproductive system including position of ganglion XIII. Scale bars: **A** = 3 mm; **B** = 1 cm; **C–F** = 1 mm.

Figure 9. *Orobdella nakahamai* sp. n., holotype, KUZ Z1672. Dorsal view of live animal. Scale bar: 1 cm.

(dorsally b1 = b2) = a2 (dorsally b3 = b4) > b5 < b6, or dorsally quinquannulate, b1 = b2 < a2 > b5 < b6. Somite VII quinquannulate, a1 (dorsally b1 = b2 in KUZ Z1680) > b3 = b4 = b5 = b6. Somite VIII octannulate, b1 = b2 < b3 < b4 = c9 = c10 = c11 = c12, or ventrally septannulate, a1 (b1 = b2) > b2 = b3 = b4 = c9 = c10 = c11 = c12. Somite IX octannulate, b1 = b2 = b3 = b4 = c9 = c10 = c11 = c12. Somite XXVI dorsally octannulate, b1 = b2 = b3 = b4 > c9 = c10 = c11 = c12, ventrally septannulate, b1 = b2 = b3 = b4 > c9 = c10 < b6, or sexannulate, b1 = b2 = b3 = b4 = b5 < b6 (c11 = c12). Somite XXVII comprises two annuli, first annulus with slight three dorsal furrows, or comprises four annuli. Eyes in one pair, dorsally on posterior margin of II (KUZ Z1352). Pharynx reaching to XIV b3/b4. Crop reaching to XXII b1/b2. Gastroporal duct joining with crop in XIV b3. Intestine reaching to XXV b4. Male gonopore in XI c10/c11 (KUZ Z1680), thus gonopores separated by 12 annuli. Testisacs in XVII c10 to XXV c11; on right side, in total approx. 122 testisacs, 5 in XVII, 18 in XVIII, 17 in XIX, 18 in XX, 16 in XXI, 15 in XXII, 14 in XXIII, 10 in XXIV, 9 in XXV; on left side, in total approx. 126 testisacs, 4 in XVII, 16 in XVIII, 19 in XIX, 21 in XX, 17 in XXI, 13 in XXII, 13 in XXIII 11 in XXIV, 12 in XXV. Paired epididymides in XV c10/c11 to XVII b3/b4, occupying 13 annuli. Pair of muscular atrial cornua ellipsoid. Left oviduct crossing ventrally beneath nerve cord; both oviducts converging into common oviduct in XIII b3.

Colouration. In life, dorsal surface bluish gray (Fig. 9); ventral surface bluish white; clitellum, when obvious, paler than other body parts (Fig. 9). Color faded in preservative.

Etymology. The specific name is a noun in the genitive case formed directly from the name of Mr Naoyuki Nakahama, who collected a specimen of this new species.

Distribution. This species was collected from the Ishizuchi Mountains and south-western part of Kochi Prefecture. The lowest elevation among the localities was ca. 140 m, and the highest was ca. 1600 m. The locality data for this species suggested that it is distributed in mountainous regions in the western part of Shikoku, Japan. Its distribution may not be restricted by habitat elevation.

Natural history. Mature leeches with an obvious clitellum, KUZ Z1352 and Z1672, were collected on 4 July and 10 August, respectively, in the Ishizuchi Mountains. Therefore, the reproductive season of *O. nakahamai* in the mountains begins before or during July, and then continues at least to early August.

Remarks. *Orobdella nakahamai* is only the second octannulate large-type species known within the genus. According to Nakano (2012c), this species can be distinguished from another octannulate species *O. octonaria* by the following combination of characteristics (Table 3): female gonopore in XIII b2/b3, gonopores separated by 1/2 + 11 or 12 annuli and epididymides in XV to XVII. *Orobdella nakahamai* is clearly distinguishable from the quadrannulate and sexannulate species of this genus because it possesses octannulate mid-body somites.

Orobdella okanoi sp. n.

http://zoobank.org/28E2B152-07C6-4E03-8A92-5F91E7353881
Figs 10–13

Type material. Holotype: KUZ Z1671, dissected, collected from under a rock along a mountain trail at Mt. Kuishiyama (33.67636°N, 133.51556°E; Elev. ca. 910 m; locality #3, see Fig. 1), Kochi, Kochi Prefecture, Japan, by Yoshiko Yamane on 5 July 2015.

Table 3. Comparisons of morphological characters between octannulate *Orobdella nakahamai* sp. n. and *Orobdella octonaria*.

Character	Female gonopore	Annuli between gonopores	Epididymides
Orobdella nakahamai sp. n.	XIII b2/b3	1/2 + 11 or 12	XV to XVII
Orobdella octonaria	middle of XIII b2	1/2 + 10 + 1/2	XVII to XIX

Additional material. KUZ Z1491, dissected, collected from in soil along a road at Mt. Iwagurosan (33.755278°N, 133.148333°E; Elev. ca 1510 m; locality #5, see Fig. 1), Saijyo, Ehime Prefecture, Japan, by Yoshiko Yamane on 22 August 2013.

Type locality. Japan, Kochi Prefecture: Kochi, Tosayamatakakawa, Mt. Kuishiyama (Shikoku).

Diagnosis. Dorsal surface reddish. Somite VII quinquannulate. Somite VIII–XXVI sexannulate. Male gonopore in slightly posterior to middle of XI c11/c12, female gonopore in middle of XIII b2, behind gastropore, gonopores separated by 8 + 1/2 annuli. Pharynx reaching to XIV b1–b2/a2. Gastropore conspicuous, in middle of XIII b2. Gastroporal duct bulbous, slightly winding at junction with gastropore. Paired epididymides in XV to XVII, occupying 8–11 annuli (one and half to almost two somites). Pre-atrial loop absent. Atrial cornua ellipsoid.

Description of holotype. BL 95.2 mm, BW 5.8 mm (Fig. 10). Caudal sucker ventral, elliptic, CL 2.3 mm, CW 3.7 mm (Figs 10B, 11D).

Somite III uniannulate (Fig. 11A). Somites IV and V biannulate; IV, (a1 + a2) > a3 (Fig. 11A); V, (a1 + a2) = a3; V a3 forming posterior margin of oral sucker (Fig. 11A, B). Somite VI triannulate, a1 (dorsally b1 = b2) > a2 = a3 (Fig. 11A, B). Somite VII quinquannulate, b1 = b2 = a2 = b5 = b6 (Fig. 11A, B). Somites VIII–XXVI sexannulate, b1 = b2 = a2 = b5 = c11 = c12 (Fig. 11A–E). Somite XXVII comprises two annuli; first annulus being ventrally last complete annulus (Fig. 11C, D). Anus behind somite XXVII (Fig. 11C).

Male gonopore in XI c11/c12 (Fig. 11E). Female gonopore in slight posterior to middle of XIII b2, inconspicuous, located posterior to gastropore (Fig. 11E, F). Gonopores separated by 8 + 1/2 annuli (Fig. 11E).

Anterior ganglionic mass in VI a1 and VII b1. Ganglion VII in b2 and a2. Ganglion VIII in a2. Ganglion IX in a2 and b5. Ganglion X in a2. Ganglion XI in a2 and b5 (Fig. 12A). Ganglion XII in a2 (Fig. 12A). Ganglion XIII in a2 and b5 (Fig. 12A). Ganglia XIV and XV, of each somite, in a2 (Fig. 12A). Ganglion XVI in b2 and a2 (Fig. 12A). Ganglia XVII (Fig. 12A) and XVIII, of each somite, in a2. Ganglion XIX in b2 and a2. Ganglia XX–XXII, of each somite, in a2. Ganglia XXIII–XXV, of each somite, in b2 and a2. Ganglion XXVI in b1 and b2. Posterior ganglionic mass in XXVI b5–c12.

Eyes in three pairs, first pair dorsally on posterior margin of II, second and third pairs dorsolaterally on posterior margin of V (a1 + a2) (Fig. 11A).

Nephridiopore in 17 pairs, one each situated ventrally at posterior margin of b2 of each somite in VIII–XXIV (Fig. 11B, D, E).

Pharynx reaching to XIV b1 (Fig. 11G). Crop reaching to XXII b1. Gastropore conspicuous, ventral, in slightly posterior to middle of XIII b2 (Fig. 11E, F). Gastroporal duct bulbous, slightly winding at junction with gastropore, joining with crop in XIV b1 (Fig. 11G). Intestine reaching to XXV b5/c11.

Testisacs (Fig. 12A); on right side, in XVII b5 to XXV c12, in total approx. 94 testisacs, 5 in XVII, 13 in XVIII, 12 in XIX, 13 in XX, 11 in XXI, 12 in XXII, 10 in XXIII, 10 in XXIV, 8 in XXV; on left side, in XVII c11 to XXIV c12, in total approx. 86 testisacs, 3 in XVII, 15 in XVIII, 11 in XIX, 13 in XX, 13 in XXI, 10 in XXII, 11 in XXIII, 10 in XXIV. Paired epididymides; right epididymis in XV c11/c12 to XVII b2, occupying 9 annuli; left epididymis in XV c11/c12 to XVII b1/b2, occupying 8 annuli (Fig. 12A). Paired ejaculatory ducts in XI b5 to XV c11/c12; slightly coiled in position posterior to ovisacs; each duct crossing ventrally beneath each ovisac, then loosely curved in position anterior to ovisacs; each widening from respective junction with epididymis, narrowing at junction with atrial cornua, then turning sharply inward toward atrial cornua without pre-atrial loop (Fig. 12A–D). Pair of muscular atrial cornua ellipsoid, in XI b5–c12 (Fig. 12A–D). Atrium short, muscular, globular in XI c11 and c12 (Fig. 12A–D).

Paired ovisacs; right ovisac in XIII a2 and b5; left ovisac in XIII a2–c11 (Fig. 12A, E). Oviducts; left oviduct

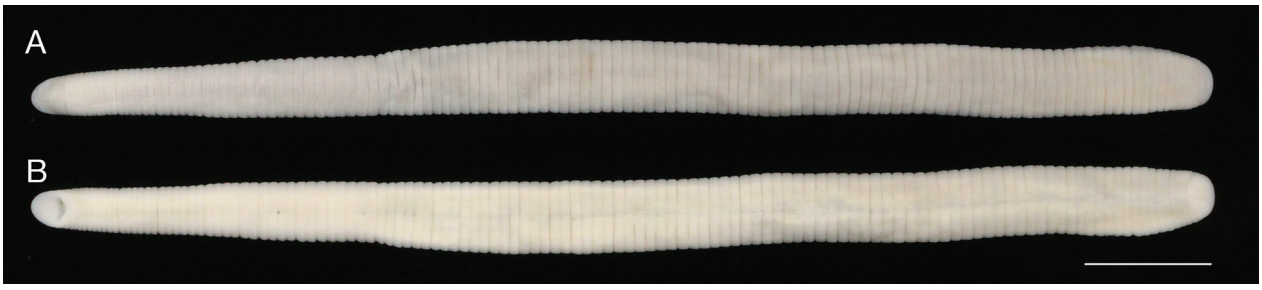

Figure 10. *Orobdella okanoi* sp. n., holotype, KUZ Z1671. **A** dorsal, **B** ventral views. Scale bar: 1 cm.

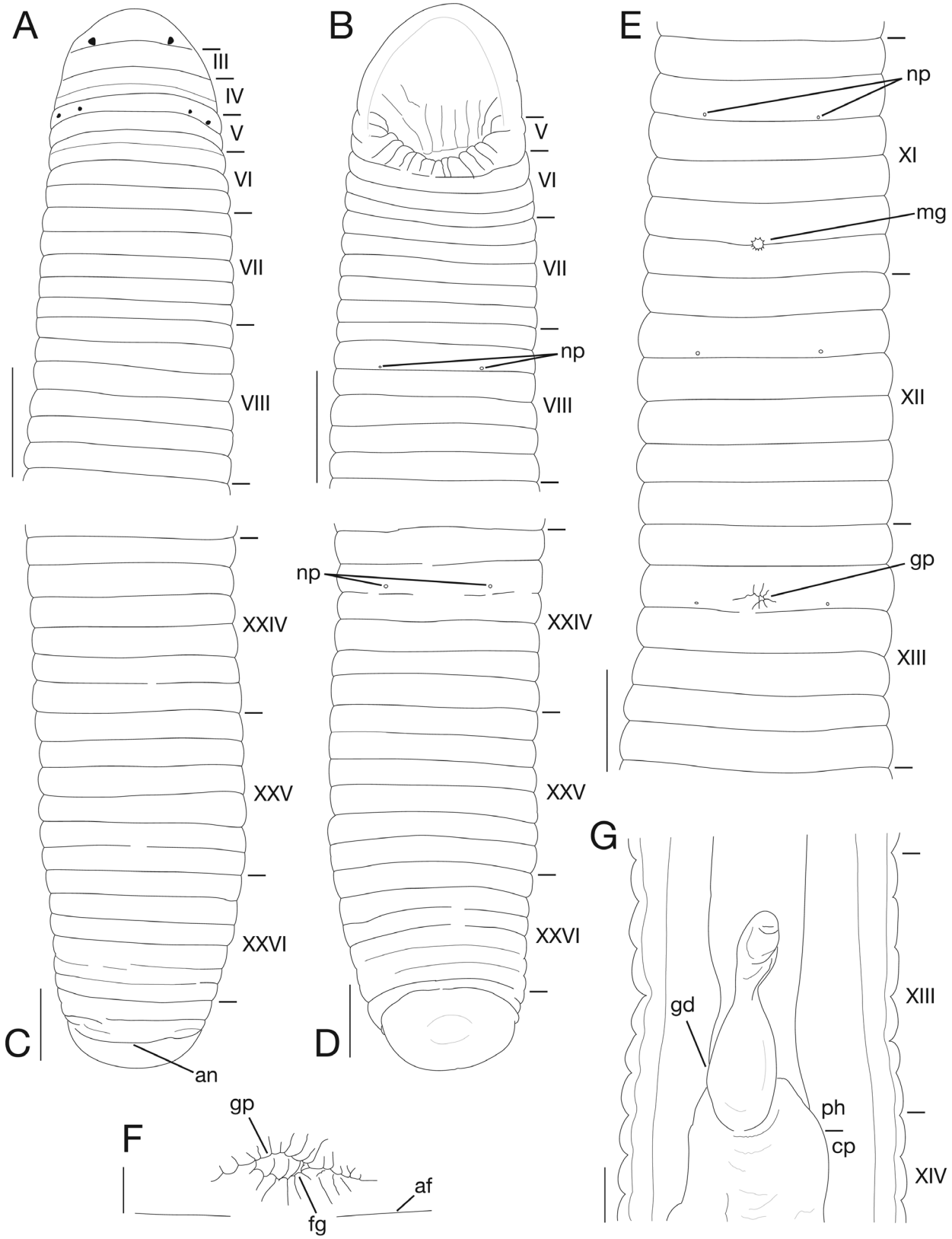

Figure 11. *Orobdella okanoi* sp. n., holotype, KUZ Z1671. **A** dorsal, **B** ventral views of somites I–VIII; **C** dorsal, **D** ventral views of somites XXIV–XXVII and caudal sucker; **E** ventral view of somites XI–XIII; **F** ventral view of gastropore and female gonopore; **G** ventral view of gastroporal duct. Scale bars: **A–E** = 2 mm; **F** = 0.25 mm; **G** = 1 mm.

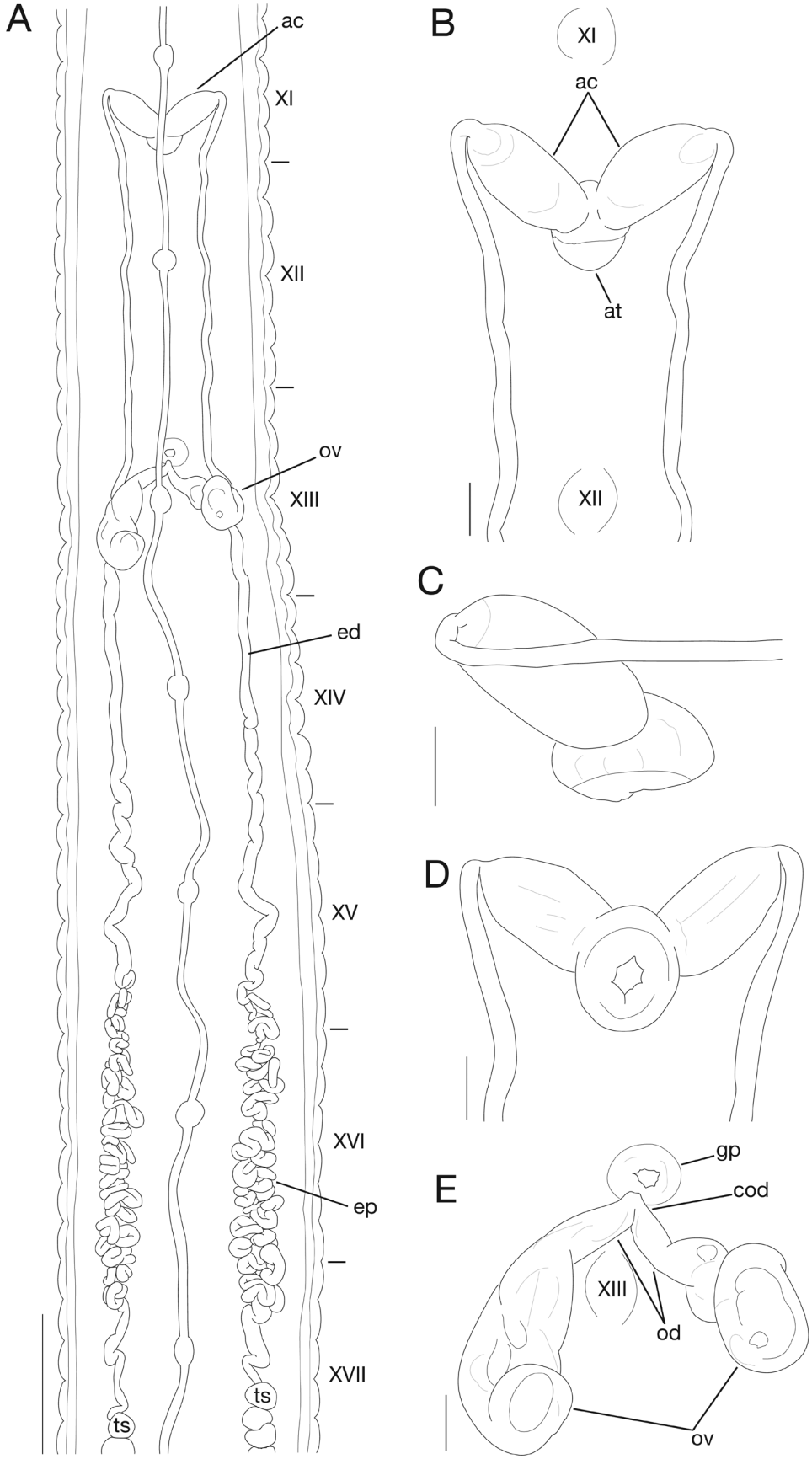

Figure 12. *Orobdella okanoi* sp. n., holotype, KUZ Z1671. **A** dorsal view of reproductive system including ventral nervous system; **B** dorsal (including positions of ganglia XI and XII), **C** lateral, **D** ventral views of male atrium; **E** dorsal view of female reproductive system including position of ganglion XIII. Scale bars: **A** = 3 mm; **B–E** = 0.5 mm.

Figure 13. *Orobdella okanoi* sp. n., holotype, KUZ Z1671. Dorsal view of live animal. Scale bar: 5 mm.

crossing ventrally beneath nerve cord; both oviducts converging into common oviduct in XIII a2 (Fig. 12A, E). Common oviduct directly descending to female gonopore (Fig. 12E).

Variation. BL 143.2 mm, BW 7.6 mm, CL 3.1 mm, CW 4.0 mm. Somite III uniannulate with slight dorsal furrow. Somite XXVII comprises two annuli, each annulus with slight dorsal furrow. Male gonopore in posterior margin of XI c11. Female gonopore in middle of XIII b2. Eyes in three pairs, firs pair dorsally on anterior margin of III. Pharynx reaching to XIV b2/a2. Crop reaching to XXII a2. Gastropore in middle of XIII b2. Gastroporal duct joining with crop in XIV b2. Intestine reaching to XXIV c12. Testisacs; on right side, in XVII b2 to XXI b5, in total approx. 64 testisacs, 8 in XVII 13 in XVIII, 18 in XIX, 17 in XX, 8 in XXI; on left side, in XVII b2 to XXIV c11, in total approx. 99 testisacs, 7 in XVII, 10 in XVIII, 17 in XIX 17 in XX, 15 in XXI, 13 in XXII, 11 in XXIII, 9 in XXIV. Paired epididymides; right epididymis in XV a2 to XVI/XVII, occupying 10 annuli; left epididymis in XV a2 to XVII b1, occupying 11 annuli. Paired ejaculatory ducts curved in position anterior to ovisacs. Paired atrial cornua; right cornu in XI c12 and XII b1; left cornu in XI b5 and c11. Paired ovisacs in XIII a2 and b5.

Colouration. In life, dorsal surface red-purple (Fig. 13) or pinkish gray; ventral surface grayish red-purple or whitish red. Color faded in preservative; dark line present from VI a1 (b2) to XIV b5 in KUZ Z1491.

Etymology. The specific name is a noun in the genitive case formed directly from the name of Mr Ryosuke Okano, who collected valuable specimens of *Orobdella* leeches.

Distribution. The type locality of this species is located in the central region of Shikoku, Japan. In addition, this species was collected from the Ishizuchi Mountains. According to the collection localities, this species is considered to inhabit the central mountainous region of Shikoku.

Natural history. This species was found curled up under a rock or in soil in moist mountainous habitats. Oligochaete worms were found in the digestive tract of the holotype, and thus this species is an earthworm-eater. The reproductive season of *O. okanoi* remains unclear because no individuals of this species with a clitellum have been collected.

Remarks. According to taxonomic studies on sexannulate *Orobdella* species (Nakano 2011b, 2012a), *Orobdella okanoi* differs from the four known sexannulate species, *O. dolichopharynx* Nakano, 2011b, *O. ijimai* Oka, 1895, *O. mononoke* Nakano, 2012a and *O. shimadae* Nakano, 2011b, as well as the new sexannulate species described below in having the following characteristics (Table 4): dorsal surface reddish, somite VII quinquannulate, somite VIII sexannulate, 8 + 1/2 annuli between gonopores, pharynx reaching to XIV, gastroporal duct bulbous, epididymides in XV to XVII, pre-atrial loop absent and atrial cornua ellipsoid. *Orobdella okanoi* is clearly distinguished from quadrannulate and octannulate species in having sexannulate mid-body somites.

The right atrial cornu of one specimen, KUZ Z1491, is caudad. Because its right side testisacs only reach somite XXI, the right side of its male genital organ may be a result of abnormal development.

Orobdella yamaneae sp. n.

http://zoobank.org/CCAC9A26-3497-4325-9B2C-5E2EB2A3BDD3
Figs 14–18

Type materials. Holotype: KUZ Z1678, dissected, collected from under fallen leaves along a forest road, "Yuzuruha Forest Road" (34.24741°N, 134.80791°E; Elev. ca. 195 m; locality #1, see Fig. 1), at Mt. Yuzuruhasan, Awajishima island, Japan, by Yoshiko Yamane on 8 July 2015. Paratypes: six specimens collected from the type locality; KUZ Z1358 (34.245889°N, 134.811861°E; Elev. ca. 210 m), and Z1359 (34.246000°N, 134.812000°E; Elev. ca. 210 m), from under a rock along the forest road, by TN on 17 June 2011; KUZ Z1675–Z1677 (34.24743°N, 134.80777°E; Elev. ca. 195 m), and Z1679 (34.24711°N, 134.80884°E; Elev. ca. 195 m), from under fallen leaves along a forest road, by Yoshiko Yamane on 8 July 2015; three specimens, KUZ Z1358, Z1676 and Z1679, dissected.

Additional material. KUZ Z1488, collected from under fallen leaves along the forest road (34.247222°N, 134.808611°E; Elev. ca. 215 m) at the type locality, by Yoshiko Yamane on 20 August 2013.

Type locality. Japan, Hyogo Prefecture: Minamiawaji, Mt. Yuzuruhasan (Awajishima island).

Diagnosis. Dorsal surface purplish. Somite VII quinquannulate. Somite VIII–XXVI sexannulate, b1 = b2 = a2 = c9 = c10 = b6. Male gonopore in middle of XI b6, female gonopore in slightly posterior to middle of XIII

Table 4. Comparisons of morphological characters between *Orobdella okanoi* sp. n., *Orobdella yamaneae* sp. n. and four sexannulate congeneric species.

Character	Dorsal colour	Somite VII	Somite VIII	Annuli between gonopores	Pharynx	Gastroporal duct	Epididymides	Pre-atrial loop	Atrial cornua
Orobdella okanoi sp. n.	reddish	quinquannulate	sexannulate	8 + 1/2	reaching to XIV	bulbous	XV to XVII	absent	ellipsoid
Orobdella yamaneae sp. n.	purplish	quinquannulate	sexannulate	1/2 + 7 + 1/2	reaching to XIV	bulbous	XVI to XVIII	extending to anterior of XI c9	ovate
Orobdella dolichopharynx	yellowish green	quadrannulate	quinquannulate	8	reaching to XVI	tubular, reaching to XVI	absent	extending to ganglion XI	absent
Orobdella ijimai	yellowish green	quadrannulate	sexannulate	1/2 + 7 + 1/2	reaching to XIV	bulbous	XVI to XIX	absent	ellipsoid
Orobdella mononoke	anterior and posterior parts grayish purple, mid-body amber	quadrannulate	sexannulate	8 + 1/2	reaching to XIV	tubular, but bulbous at junction with crop	XV to XIX	absent	ovate
Orobdella shimadae	yellowish green	triannulate	quinquannulate	9	reaching to XVI	tubular, reaching to XV	absent	extending to ganglion XI	absent

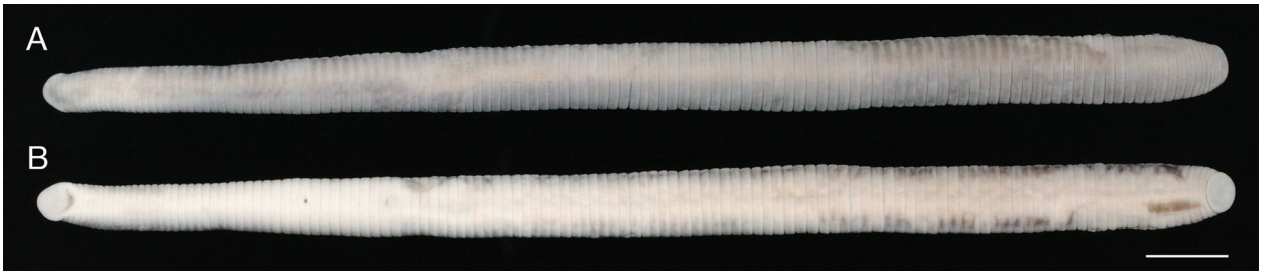

Figure 14. *Orobdella yamaneae* sp. n., holotype, KUZ Z1678. **A** dorsal, **B** ventral views. Scale bar: 1 cm.

b2, behind gastropore, gonopores separated by 1/2 + 7 + 1/2 annuli. Pharynx reaching to XIV a2/c9–c9. Gastropore conspicuous, in slightly posterior to middle of XIII b2. Gastroporal duct bulbous, slightly winding at junction with gastropore. Paired epididymides in XVI to XVIII, occupying 8–11 annuli (one and half to almost two somites). Pre-atrial loop present. Atrial cornua ovate.

Description of holotype. BL 142.2 mm, BW 8.1 mm (Fig. 14). Caudal sucker ventral, elliptic, CL 3.0 mm, CW 5.1 mm (Figs 14B, 15D).

Somites III–V biannulate; III and IV, (a1 + a2) > a3; V, (a1 + a2) = a3; V a3 forming posterior margin of oral sucker (Fig. 15A, B). Somite VI triannulate, a1 (dorsally b1 = b2) > a2 > a3 (Fig. 15A, B). Somite VII quinquannulate, a1 = a2 = c9 = c10 = b6 (Fig. 15A, B). Somites VIII–XXV sexannulate, b1 = b2 = a2 = c9 = c10 = b6 (Fig. 15A–E). Somite XXVI sexannulate dorsally, b1 = b2 = a2 > c9 = c10 = b6, quinquannulate ventrally, b1 = b2 = a2 < b5 (c9 = c10) > b6 (Fig. 15C, D). Somite XXVII comprises two annuli, first annulus with slight dorsal furrow; first annulus being ventrally last complete annulus (Fig. 15C, D). Anus behind somite XXVII (Fig. 15C).

X c9 and XIII a2, respectively, being first and last annuli of clitellum (Fig. 15E).

Male gonopore in middle of XI b6 (Fig. 15E). Female gonopore in slightly posterior to middle of XIII b2, inconspicuous, located posterior to gastropore (Fig. 15E, F). Gonopores separated by 1/2 + 7 + 1/2 annuli (Fig. 15E).

Anterior ganglionic mass in VI a2 and a3. Ganglion VII in a2. Ganglia VIII and IX, of each somite, in b2 and a2. Ganglia X–XII, of each somite, in a2 (Fig. 16A). Ganglion XIII in a2 and c9 (Fig. 16A). Ganglia XIV–XVIII, of each somite, in a2 (Fig. 16A). Ganglion XIX in b2 and a2. Ganglia XX–XXIV, of each somite, in a2. Ganglion XXV in b2 and a2. Ganglion XXVI in b1 and b2. Posterior ganglionic mass in XXVI c9–b6.

Eyes in three pairs, first pair dorsally on II/III, second and third pairs dorsolaterally on posterior margin of V (a1 + a2) (Fig. 15A).

Nephridiopores in 17 pairs, one each situated ventrally at posterior margin of b2 of each somite in VIII–XXIV (Fig. 15B, D, E).

Pharynx reaching to XIV a2/c9 (Fig. 15G). Crop reaching to XXI b6. Gastropore conspicuous, ventral, in slightly posterior to middle of XIII b2 (Fig. 15E, F). Gastroporal duct bulbous, slightly winding at junction with gastropore, joining with crop in XIV a2/c9 (Fig. 15G). Intestine reaching to XXIV c9/c10.

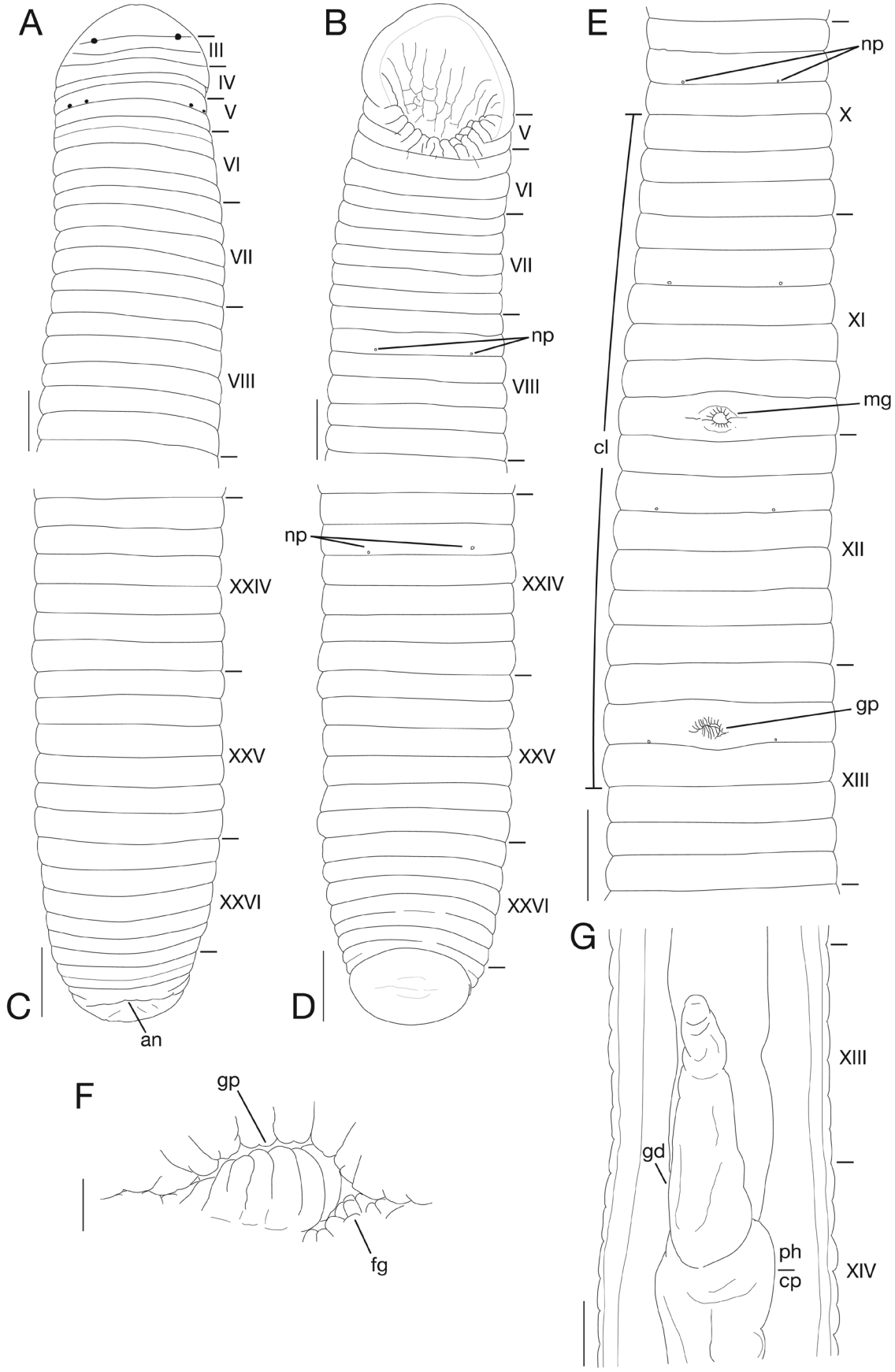

Figure 15. *Orobdella yamaneae* sp. n., holotype, KUZ Z1678. **A** dorsal, **B** ventral views of somites I–VIII; **C** dorsal, **D** ventral views of somites XXIV–XXVII and caudal sucker; **E** ventral view of somites X–XIII; **F** ventral view of gastropore and female gonopore; **G** ventral view of gastroporal duct. Scale bars: **A, B, G** = 2 mm; **C–E** = 3 mm; **F** = 0.25 mm.

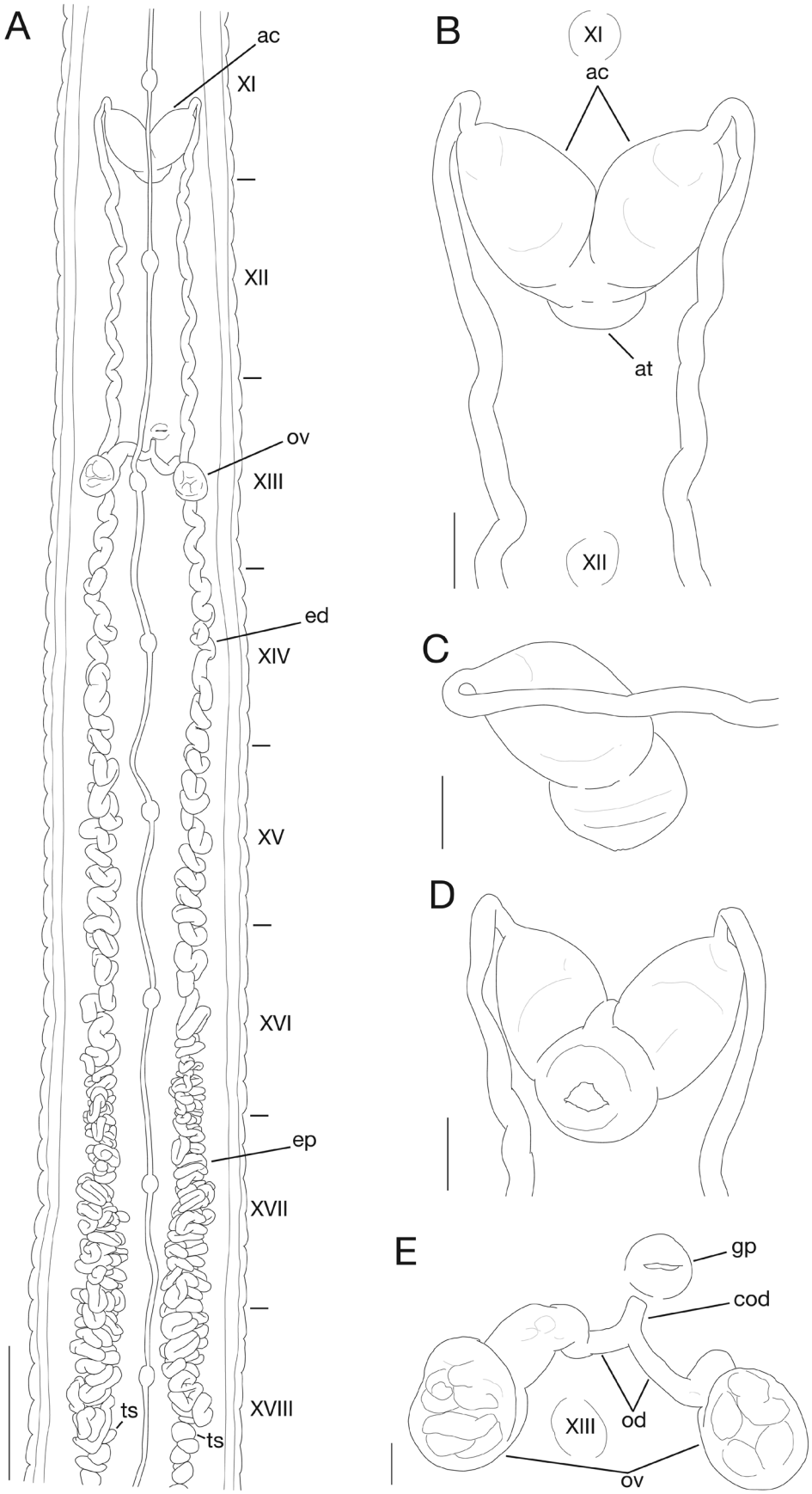

Figure 16. *Orobdella yamaneae* sp. n., holotype, KUZ Z1678. **A** dorsal view of reproductive system including ventral nervous system; **B** dorsal (including positions of ganglia XI and XII), **C** lateral, **D** ventral views of male atrium; **E** dorsal view of female reproductive system including position of ganglion XIII. Scale bars: **A** = 5 mm; **B–D** = 1 mm; **E** = 0.5 mm.

Testisacs in XVIII c9 to XXVI b1 (Fig. 16A): on right side, in total approx. 107 testisacs, 7 in XVIII, 13 in XIX, 16 in XX, 17 in XXI, 14 in XXII, 14 in XXIII, 14 in XXIV, 11 in XXV, 1 in XXVI; on left side, in total approx. 108 testisacs, 5 in XVIII, 12 in XIX, 19 in XX, 15 in XXI, 15 in XXII, 13 in XXIII, 14 in XXIV, 14 in XXV, 1 in XXVI. Paired epididymides; right epididymis in XVI c9 to XVIII c9, occupying 13 annuli; left epididymis in XVI c9/c10 to XVIII c9, occupying 12 annuli (Fig. 16A). Paired ejaculatory ducts; right duct in XI c9 to XVI c9; left duct in XI c9 to XVI c9/c10; coiled in position posterior to ovisacs; each duct crossing ventrally beneath each ovisac, then curved in position anterior to ovisacs; each widening from respective junction with epididymis, narrowing at junction with atrial cornua, then turning sharply inward toward atrial cornua with atrial loop extending to anterior of XI c9 (Fig. 16A–D). Pair of muscular atrial cornua ovate, in XI c9–b6 (Fig. 16A–D). Atrium short, muscular, globular in XI c10 and b6 (Fig. 16A–D).

Paired ovisacs in XIII a2 and c9 (Fig. 16A, E). Oviducts; left oviduct crossing ventrally beneath nerve cord; both oviducts converging into common oviduct in XIII a2 (Fig. 16A, E). Common oviduct directly descending to female gonopore (Fig. 16A, E).

Variations. BL 62.4–97.6 mm, BW 4.0–6.5 mm, CL 1.6–2.8 mm, CW 2.5–3.6 mm. Somite III uniannulate with slight dorsal furrow, [(a1 + a2) > a3]. Somite IV uniannulate with slight dorsal furrow, [(a1 + a2) > a3] in KUZ Z1359 (Fig. 17A). Somite VII quadrannulate in KUZ Z1359, a1 = a2 < b5 (ventrally c9 = c10) > b6 (Fig. 17A, B). Somite VIII quinquannulate in KUZ Z1359, b1 = b2 = a2 < b5 (c9 = c10) > b6 (Fig. 17A, B). Somite XXVI generally sexannulate, b1 = b2 = a2 = c9 = c10 = b6, or b1 = b2 = a2 = c9 > c10 = b6; KUZ Z1359, quinquannulate, b1 = b2 = a2 < b5 > b6. Somite XXVII comprises 2–4 annuli. XXVI b6, or first annulus of XXVII being ventrally last complete annulus. Female gonopore rarely in posterior margin of XIII b2, gonopores thus rarely separated by 1/2 + 8 annuli. First pair of eyes generally dorsally on posterior margin of II. Pharynx reaching to XIV a2/c9–c9. Crop reaching to XXI c9–XXII c9/c10. Gastroporal duct often tubular, slightly bulbous at junction with gastropore, joining with crop in XIV b1/b2–b2/a2. Intestine reaching to XXIV/XXV–XXV a2/c9. Testisacs hardly detected. Paired epididymides: right epididymis in XVI b2–b2/a2 to XVII c9–XVIII b1, occupying 8–11 annuli; left epididymis in XIV b1/b2–XVI b2/a2 to XVII c9–XVIII b1, occupying 9–11 annuli. Paired ejaculatory ducts running straight, or nearly straight in position anterior to ovisacs; pre-atrial loop reaching to XI a2/c9–c9. Pair of muscular atrial cornua fusiform or ellipsoid, often in XI c10 and b6. Paired ovisacs generally in XIII a2 and c9; KUZ Z1358, in XIII c9. Right or left oviduct crossing ventrally beneath nerve cord.

Colouration. In life, dorsal surface grayish purple or red-purple (Fig. 18); ventral surface whitish blue or grayish purple; clitellum, when obvious, paler than other body parts (Fig. 18). Color faded in preservative.

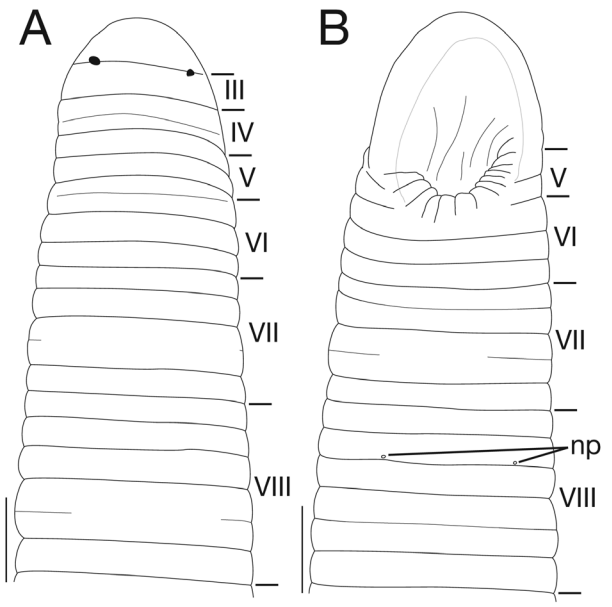

Figure 17. *Orobdella yamaneae* sp. n., paratype, KUZ Z1359. **A** dorsal, **B** ventral views of somites I–VIII. Scale bars: **A, B** = 1 mm.

Etymology. The specific name is a noun in the genitive case formed directly from the name of Ms Yoshiko Yamane, who collected specimens of this new species.

Distribution. This species was collected only from its type locality.

Natural history. This species was found curled up under fallen leaves in moist mountainous habitats. A mature leech, KUZ Z1678, was collected on 8 July. Therefore, *Orobdella yamaneae* is considered to enter its reproductive season before early July.

Remarks. The mid-body somite annulation of the known sexannulate *Orobdella* species was described as being composed of b1, b2, a2, b5, c11 and c12 (Nakano 2011b, 2012a). However, the annulation of somites VII [a1 = a2 < b5 (ventrally c9 = c10) > b6] and VIII [b1 = b2 = a2 < b5 (c9 = c10) > b6] of one specimen, KUZ Z1359, clearly suggests that b5 is divided into c9 and c10, and b6 remains undivided in *O. yamaneae*. The sexannulation of this species is thus b1 = b2 = a2 = c9 = c10 = b6.

In addition to its unique sexannulation, *O. yamaneae* is distinguishable from the four known sexannulate species and *O. okanoi* by the following characteristics (Table 4): dorsal surface purplish, somite VII quinquannulate, somite VIII sexannulate, 1/2 + 7 + 1/2 annuli between gonopores, pharynx reaching to XIV, gastroporal duct bulbous, epididymides in XVI to XVIII, pre-atrial loop present and atrial cornua ovate. *Orobdella yamaneae* obviously differs from quadrannulate and octannulate species of this genus in its mid-body somite annulation.

Except for the holotype, all dissected individuals possess the following characteristics of the male genital or-

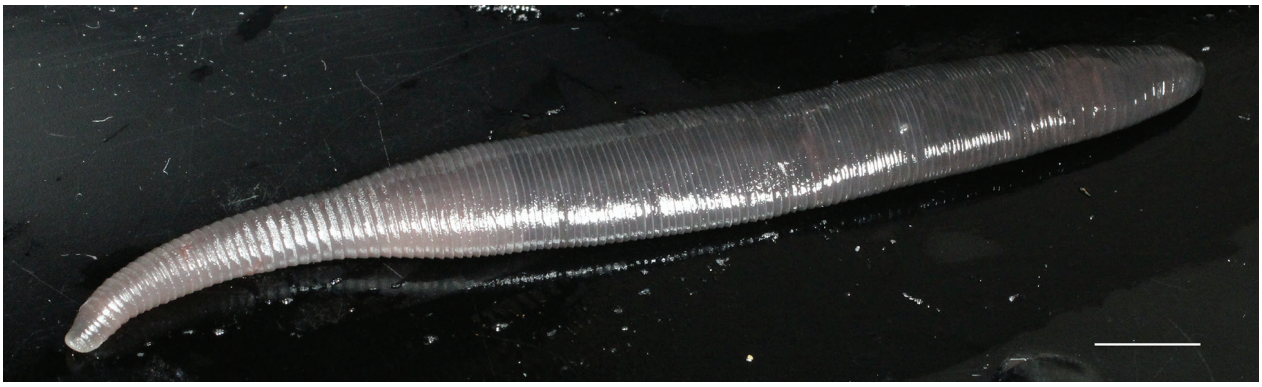

Figure 18. *Orobdella yamaneae* sp. n., holotype, KUZ Z1678. Dorsal view of live animal. Scale bar: 1 cm.

gan: ejaculatory ducts in position anterior to ovisacs running straight and male atrial cornua ellipsoid or fusiform. However, they seem to be immature leeches because all of them have undeveloped and undetectable testisacs. Therefore, straight ejaculatory ducts and ellipsoid or fusiform testisacs are considered to be immature characteristics of *O. yamaneae*.

Molecular phylogenies and genetic distances

The obtained BI tree (Fig. 19) had an almost identical topology to that of the ML tree (ln L = −27703.79; not shown). In the ML phylogeny, *Orobdella tsushimensis* formed a monophyletic lineage with the clade containing *O. dolichopharynx*, *O. esulcata*, *O. ketagalan*, *O. mononoke*, *O. naraharaetmagarum*, *O. shimadae* and *O. brachyepididymis* (BS = 41%).

Orobdella brachyepididymis formed a monophyletic clade with *O. naraharaetmagarum* (BS = 100%, BPP = 1.0). This clade is a sister lineage to *O. esulcata* (BS = 100%, BPP = 1.0). The monophyly of the two specimens identified as *O. brachyepididymis* was fully supported (BS = 100%, BPP = 1.0). The other three new species, *O. nakahamai*, *O. okanoi* and *O. yamaneae* formed a monophyletic clade (BS = 100%, BPP = 1.0). The monophyly of this clade and *O. masaakikuroiwai* was revealed, but this relationship was not strongly supported in the BI tree (BS = 87%, BPP = 0.90). Within the three new species, *O. okanoi* and *O. yamaneae* formed a monophyletic lineage (BS = 78%, BPP = 0.94). The monophyly of the specimens of the three new species was fully recovered (*O. nakahamai*, BS = 98%, BPP = 1.0; *O. okanoi*, and *O. yamaneae*, respectively, BS = 100%, BPP = 1.0).

The pairwise COI uncorrected *p*-distances within each of the new species were as follows: in *O. brachyepididymis*, 0.5%; *O. nakahamai*, 2.1–4.6% (mean = 3.7%); *O. okanoi*, 2.4%; and *O. yamaneae*, 3.9% (Table 5). The genetic divergence between *O. brachyepididymis* and *O. naraharaetmagarum* was 4.7%. The genetic distances among the three new species were as follows: *O. nakahamai* and *O. okanoi*, 5.6–6.2% (mean = 5.8%); *O. nakahamai* and *O. yamaneae*, 5.6–5.8% (mean = 5.7%); and *O. okanoi* and *O. yamaneae*, 5.8–6.0% (mean = 5.9%) (Table 5). Those between *O. masaakikuroiwai* and each of the three new species were as follows: *O. nakahamai*, 5.9–7.2% (mean = 6.8%); *O. okanoi*, 7.1–7.3% (mean = 7.2%); and *O. yamaneae*, 6.6–6.8% (mean = 6.7%).

Discussion

According to the morphological characteristics of the four new species, each of them can be well defined and distinguished from each other and from the previously known species of *Orobdella*. However, the genetic divergences of the COI sequences showed small interspecific divergences. The genetic distance between *Orobdella brachyepididymis* and its sister species *O. naraharaetmagarum* (4.7%) was equivalent to the largest intraspecific COI divergence of the latter species indicated by Nakano (2016). Although no clear genetic distance gap exists between *O. brachyepididymis* and *O. naraharaetmagarum*, the new species is clearly distinguished from *O. naraharaetmagarum* by the following combination of morphological characters (Table 2): morphology of the gastroporal duct, number of annuli between gonopores, lengths of epididymides and morphology of the male atrial cornua. Clearly, *O. brachyepididymis* is a distinctive new species within the genus *Orobdella*.

Calculated interspecific COI distances among the three other new species, *O. nakahamai*, *O. okanoi* and *O. yamaneae*, each of which is well defined by morphological characteristics, were also small. These values and the obtained phylogenetic trees indicated that these three species are closely related to each other. *Orobdella nakahamai* is sympatric with *O. okanoi* on Mt. Iwagurosan in the Ishizuchi Mountains (locality #5, see Fig. 1). Moreover, specimens of each new species collected from Mt. Iwagurosan belonged to its respective clade. The present genetic analyses clearly support that *O. nakahamai* and *O. okanoi* are well-defined species. The COI divergences between *O. yamaneae* and *O. okanoi* (mean = 5.9%), and those between *O. yamaneae* and *O. nakahamai* (mean = 5.7%), were almost equal to those between *O. nakahamai* and *O. okanoi* (mean = 5.8%). Therefore, these distance values confirmed the distinctiveness of *O. yamaneae*.

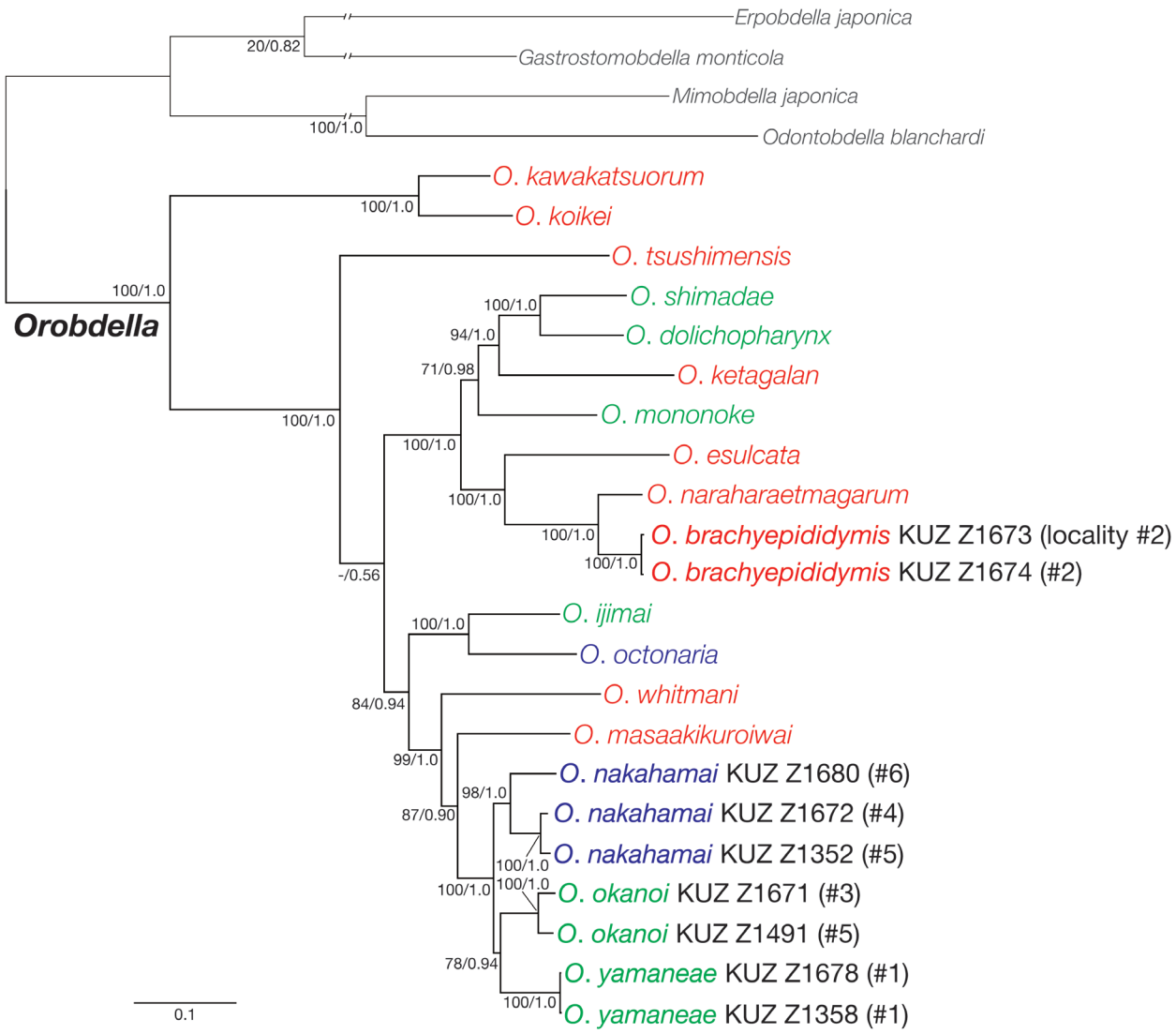

Figure 19. Bayesian inference tree for 5,209 bp of nuclear 18S rRNA and histone H3 and mitochondrial COI, $tRNA^{Cys}$, $tRNA^{Met}$, 12S rRNA, $tRNA^{Val}$, 16S rRNA, $tRNA^{Leu}$ and ND1 markers. Numbers on nodes represent bootstrap values for maximum likelihood and Bayesian posterior probabilities. A species name of *Orobdella* in red indicates a quadrannulate species; green, a sexannulate; and blue, an octannulate species. Locality numbers are shown in Fig. 1.

According to the obtained molecular phylogenies, mid-body somite annulation and body size of mature leeches within *Orobdella* clearly evolved in parallel. Nakano (2012b) stated that sexannulate mid-body somite annulation had evolved in parallel within this genus. The present finding of the second octannulate species, *O. nakahamai*, and its phylogenetic position indicate that octannulate mid-body somite annulation also evolved in parallel within *Orobdella*. Note that both of the octannulate species formed a clade with the sexannulate species. These phylogenetic relationships shed light on a possible evolutionary correlation between sexannulation and octannulation in *Orobdella* leeches. Richardson (1971) erected the genus *Kumabdella* Richardson, 1971, which has been considered subjective junior synonym of *Orobdella*, only for *O. octonaria*, based principally on its octannulate mid-body somite annulation. In the redescription of *O. octonaria*, Nakano (2012c) briefly reviewed the taxonomic history of *Kumabdella* and concluded that *Kumabdella* should retain its status as a junior synonym of *Orobdella*. The presence of the second octannulate species within this genus, and the fact that these two octannulate species are phylogenetically distant, fully support the present taxonomic treatment for the genus *Kumabdella*.

The sexannulation of *O. yamaneae* and the fact that this species is genetically quite close to *O. okanoi* imply the possibility that the sexannulation of *O. okanoi* also follows b1 = b2 = a2 = c9 = c10 = b6, and not the b1 = b2 = a2 = b5 = c11 = c12 observed in the other known sexannulate species of *Orobdella*. The annulation pattern of *O. yamaneae* suggests that annular formulae can vary among *Orobdella* species possessing the same annulation. The quadrannulate somite of *Orobdella* obviously consists of a1, a2, b5 and b6, according to the positions of paired

Table 5. Uncorrected *p*-distances for the 1267 bp for the COI sequences of specimens of *Orobdella nakahamai* sp. n., *Orobdella okanoi* sp. n. and *Orobdella yamaneae* sp. n., with associated collection locality numbers (see Fig. 1).

Species	Specimen (locality #)	1	2	3	4	5	6
Orobdella nakahamai sp. n.	1: KUZ Z1352 (5)						
	2: KUZ Z1672 (4)	0.021					
	3: KUZ Z1680 (6)	0.043	0.046				
Orobdella okanoi sp. n.	4: KUZ Z1491 (5)	0.058	0.056	0.056			
	5: KUZ Z1671 (3)	0.062	0.058	0.058	0.024		
Orobdella yamaneae sp. n.	6: KUZ Z1358 (1)	0.056	0.057	0.058	0.059	0.059	
	7: KUZ Z1678 (1)	0.057	0.056	0.058	0.058	0.060	0.004

nephridiopores and the ventral ganglion in each somite. However, the annulation pattern of sexannulate and octannulate *Orobdella* species should be determined based on several specimens, including immature individuals.

Nakano (2014, 2016) stated that the small-type body length also evolved in parallel within *Orobdella*. In addition to small size, the present phylogenies indicated that the large-type body length evolved in parallel within this genus. The close relation of the small- and large-types to mid-body somite annulation in *Orobdella* leeches is highly possible. According to current knowledge on *Orobdella* species, the small-type only occurs in the quadrannulate species, i.e. *O. brachyepididymis*, *O. koikei*, *O. masaakikuroiwai* and *O. naraharaetmagarum*, while the large-type species consist of octannulate species, *O. nakahamai* and *O. octonaria*. Future phylogenetic studies, including an ancestral state reconstruction, will elucidate the character state evolution of *Orobdella* leeches.

The present phylogenetic tree showed that 17 *Orobdella* species consist of four main lineages: a Hokkaido lineage containing two species, *O. kawakatsuorum* and *O. koikei*; an *Orobdella tsushimensis* lineage; a western lineage comprising four species inhabiting the Ryukyu Islands and Taiwan, along with *O. esulcata* distributed in Kyushu, *O. naraharaetmagarum* in the Chugoku district, western Honshu, as well as the new species *O. brachyepididymis* from Shikoku; and an eastern lineage comprising four species known from the eastern to central parts of Honshu, along with the other three new species, *O. nakahamai* and *O. okanoi* collected from Shikoku and *O. yamaneae* from Awajishima island. Therefore, the range of the western lineage group overlaps that of the eastern lineage group in Shikoku and adjacent islets. Because species belonging to both lineage groups are distributed in Shikoku, the species diversity of *Orobdella* in this region may be quite high compared to other regions. The new *Orobdella* species inhabiting Shikoku and Awajishima island would offer a suitable opportunity to reveal speciation events, as well as species coexistence mechanisms in the genus *Orobdella*.

Acknowledgements

The author is grateful to Naoyuki Nakahama (Kyoto University; KU), Ryosuke Okano (Ehime University), and Yoshiko Yamane (KU) for providing specimens of the new species, to Professor Hidetoshi Nagamasu (The Kyoto University Museum) for his helpful advice on a specific name of the new species. The author expresses his sincere thanks to Dr Yi-Te Lai (National Taiwan University) and Dr Michael Ohl (Museum für Naturkunde) for their constructive comments on this manuscript. A part of this study was financially supported by Grants for Biodiversity and Evolutionary Research of Global COE (A06) and for Excellent Graduate Schools, both from MEXT, Japan, to KU, and JSPS Grants-in-Aid for JSPS Fellows (#15J00720) and Young Scientists (B) (#26840127) to the author. The open access publication of this manuscript was supported by the Museum für Naturkunde.

References

Akaike H (1974) A new look at the statistical model identification. IEEE Transactions on Automatic Control 19(6): 716–723. doi: 10.1109/TAC.1974.1100705

Apakupakul K, Siddall ME, Burreson EM (1999) Higher level relationships of leeches (Annelida: Clitellata: Euhirudinea) based on morphology and gene sequences. Molecular Phylogenetics and Evolution 12(3): 350–359. doi: 10.1006/mpev.1999.0639

Blanchard R (1897) Hirudinées du Musée de Leyde. Notes from the Leyden Museum 19(1–2): 73–113.

Chakrabarty P, Warren M, Page L, Baldwin C (2013) GenSeq: An updated nomenclature and ranking for genetic sequences from type and non-type sources. ZooKeys 346: 29–41. doi: 10.3897/zookeys.346.5753

Colgan DJ, McLauchlan A, Wilson GDF, Livingston SP, Edgecombe GD, Macaranas J, Cassis G, Gray MR (1998) Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. Australian Journal of Zoology 46(5): 419–437. doi: 10.1071/ZO98048

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39(4): 783–791. doi: 10.2307/2408678

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3(5): 294–299. doi:

Hillis DM, Bull JJ (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Systematic Biology 42(2): 182–192. doi: 10.1093/sysbio/42.2.182

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability.

Molecular Biology and Evolution 30(4): 772–780. doi: 10.1093/molbev/mst010

Lanfear R, Calcott B, Ho SYW, Guindon S (2012) PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. Molecular Biology and Evolution 29(6): 1695–1701. doi: 10.1093/molbev/mss020

Leaché AD, Reeder TW (2002) Molecular systematics of the eastern fence lizard (*Sceloporus undulatus*): a comparison of parsimony, likelihood, and Bayesian approaches. Systematic Biology 51(1): 44–68. doi: 10.1080/106351502753475871

Light JE, Siddall ME (1999) Phylogeny of the leech family Glossiphoniidae based on mitochondrial gene sequences and morphological data. The Journal of Parasitology 85(5): 815–823. doi: 10.2307/3285816

Lukin EI (1976) Fauna USSR. Leeches. Nauka, Leningrad, 484 pp.

Moore JP (1927) The segmentation (metamerism and annulation) of the Hirudinea. In: Harding WA, Moore JP. The Fauna of British India, including Ceylon and Burma. Hirudinea. Taylor & Francis, London, 1–12.

Moore JP (1929) Leeches from Borneo with descriptions of new species. Proceedings of the Academy of Natural Sciences of Philadelphia 81: 267–295.

Nakano T (2010) A new species of the genus *Orobdella* (Hirudinida: Arhynchobdellida: Gastrostomobdellidae) from Kumamoto, Japan, and a redescription of *O. whitmani* with the designation of the lectotype. Zoological Science 27(11): 880–887. doi: 10.2108/zsj.27.880

Nakano T (2011a) A new species of *Orobdella* (Hirudinida: Arhynchobdellida: Gastrostomobdellidae) from Tsushima Island, Japan. Species Diversity 16(1–2): 39–47.

Nakano T (2011b) Redescription of *Orobdella ijimai* (Hirudinida: Arhynchobdellida: Gastrostomobdellidae), and two new species of *Orobdella* from the Ryukyu Archipelago, Japan. Zootaxa 2998: 1–15.

Nakano T (2012a) A new sexannulate species of *Orobdella* (Hirudinida, Arhynchobdellida, Orobdellidae) from Yakushima Island, Japan. ZooKeys 181: 79–93. doi: 10.3897/zookeys.181.2932

Nakano T (2012b) A new species of *Orobdella* (Hirudinida, Arhynchobdellida, Gastrostomobdellidae) and redescription of *O. kawakatsuorum* from Hokkaido, Japan with the phylogenetic position of the new species. ZooKeys 169: 9–30. doi: 10.3897/zookeys.169.2425

Nakano T (2012c) Redescription of *Orobdella octonaria* (Hirudinida: Arhynchobdellida: Orobdellidae) with designation of a lectotype. Species Diversity 17(2): 227–233. doi: 10.12782/sd.17.2.227

Nakano T (2014) A new quadrannulate species of *Orobdella* (Hirudinida, Arhynchobdellida, Orobdellidae) from central Honshu, Japan. ZooKeys 445: 57–76. doi: 10.3897/zookeys.445.7999

Nakano T (2016) A new quadrannulate species of *Orobdella* (Hirudinida, Arhynchobdellida, Orobdellidae) from western Honshu, Japan. ZooKeys 553: 33–51. doi: 10.3897/zookeys.553.6723

Nakano T, Gongalsky KB (2014) First record of *Orobdella kawakatsuorum* (Hirudinida: Arhynchobdellida: Erpobdelliformes) from Kunashir Island, Kuril Islands. Biodiversity Data Journal 2: e1058. doi: 10.3897/BDJ.2.e1058

Nakano T, Lai Y-T (2012) A new species of *Orobdella* (Hirudinida, Arhynchobdellida, Orobdellidae) from Taipei, Taiwan. ZooKeys 207: 49–63. doi: 10.3897/zookeys.207.3334

Nakano T, Ramlah Z, Hikida T (2012) Phylogenetic position of gastrostomobdellid leeches (Hirudinida, Arhynchobdellida, Erpobdelliformes) and a new family for the genus *Orobdella*. Zoologica Scripta 41(2): 177–185. doi: 10.1111/j.1463-6409.2011.00506.x

Nakano T, Seo H-Y (2014) First record of *Orobdella tsushimensis* (Hirudinida: Arhynchobdellida: Gastrostomobdellidae) from the Korean Peninsula and molecular phylogenetic relationships of the specimens. Animal Systematics, Evolution and Diversity 30(2): 87–94. doi: 10.5635/ASED.2014.30.2.087

Oka A (1895) On some new Japanese land leeches. (*Orobdella* nov. gen.). The Journal of the College of Science, Imperial University, Japan 8(2): 275–306.

Oka A (1910a) Key to Japanese leeches. Dobutsugaku Zasshi 22(256): 56–64.

Oka A (1910b) Synopsis der japanischen Hirudineen, mit Diagnosen der neuen Species. Annotationes Zoologicae Japonenses 7(3): 165–183.

Pawłowski LK (1962) O występowaniu pijawki *Erpobdella octoculata* (L.) w Japonii. Zeszyty Naukowe Uniwersytetu Łódzkiego Seria II Nauki Matematyczno-przyrodnicze 12: 127–136.

Rambaut A, Drummond AJ (2013) Tracer v. 1.6. http://tree.bio.ed.ac.uk/software/tracer/

Richardson LR (1971) Gastrostomobdellidae f. nov. and a new genus for the gastroporous *Orobdella octonaria* Oka, 1895, of Japan (Hirudinoidea: Arhynchobdellae). Bulletin of the National Science Museum (Tokyo) 14(4): 585–602.

Richardson LR (1975) A new species of terricolous leeches in Japan (Gastrostomobdellidae, *Orobdella*). Bulletin of the National Science Museum Series A (Zoology) 1(1): 39–56.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539–542. doi: 10.1093/sysbio/sys029

Sawyer RT (1986) Leech Biology and Behaviour. Clarendon Press, Oxford, 1065 pp.

Schwarz G (1978) Estimating the dimension of a model. The Annals of Statistics 6(2): 461–464. doi: 10.1214/aos/1176344136

Soós Á (1966) Identification key to the leech (Hirudinoidea) genera of the world, with a catalogue of the species. III. Family: Erpobdellidae. Acta Zoologica Academiae Scientiarum Hungaricae 12(3–4): 371–407.

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30(9): 1312–1313. doi: 10.1093/bioinformatics/btu033

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 30(12): 2725–2729. doi: 10.1093/molbev/mst197