

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

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

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Abstract

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

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 I 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.

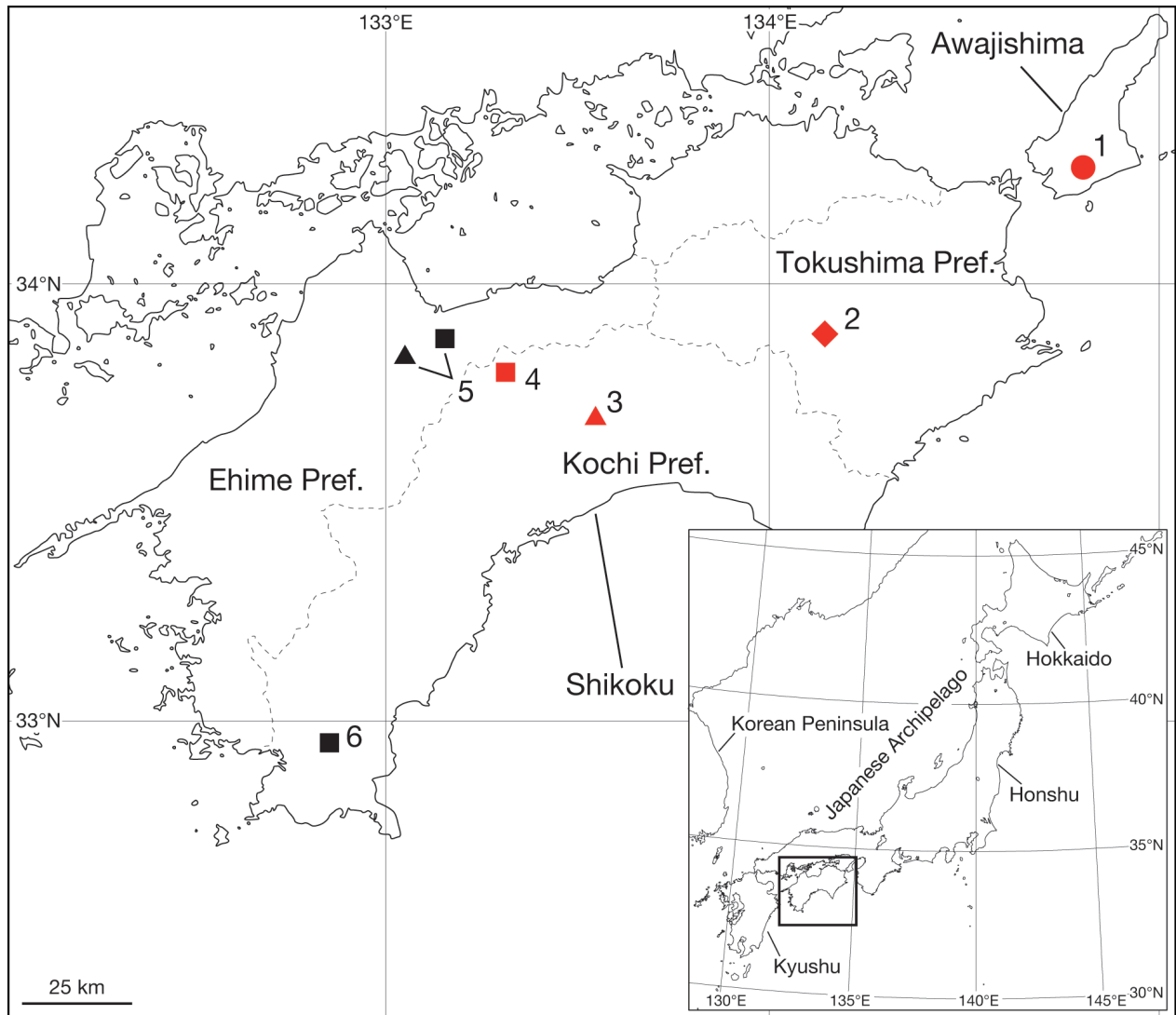


Figure 1. Map showing the collection localities of the specimens examined in this study. The closed diamond (2) denotes the locality of *Orobdella brachyepidymis* sp. n., closed squares (4–6) show the localities of *Orobdella nakahamai* sp. n., closed triangles (3, 5) designate the localities of *Orobdella 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 (Kato 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 *Orobodella* described in this study (see Fig. 1) and the INSDC accession numbers. Sequences marked with an 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 | |
| <i>Orobodella brachyepididymis</i> 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 |
| <i>Orobodella brachyepididymis</i> sp. n. | KUZ Z1674 Paratype (2) | | | LC106324* | LC106323* | LC106325* | genseq-2 COI, tRNA ^{Cys} , tRNA ^{Met} , 12S, tRNA ^{Val} , 16S, tRNA ^{Leu} , ND1 |
| <i>Orobodella nakahamai</i> sp. n. | KUZ Z1352 Paratype (5) | | | LC106327* | LC106326* | LC106328* | genseq-2 COI, tRNA ^{Cys} , tRNA ^{Met} , 12S, tRNA ^{Val} , 16S, tRNA ^{Leu} , ND1 |
| <i>Orobodella nakahamai</i> 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 |
| <i>Orobodella nakahamai</i> sp. n. | KUZ Z1680 (6) | | | LC106335* | LC106334* | LC106336* | genseq-3 COI, tRNA ^{Cys} , tRNA ^{Met} , 12S, tRNA ^{Val} , 16S, tRNA ^{Leu} , ND1 |
| <i>Orobodella okanoi</i> sp. n. | KUZ Z1491 (5) | | | LC106338* | LC106337* | LC106339* | genseq-3 COI, tRNA ^{Cys} , tRNA ^{Met} , 12S, tRNA ^{Val} , 16S, tRNA ^{Leu} , ND1 |
| <i>Orobodella okanoi</i> 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 |
| <i>Orobodella yamaneae</i> sp. n. | KUZ Z1358 Paratype (1) | | | LC106346* | LC106345* | LC106347* | genseq-2 COI, tRNA ^{Cys} , tRNA ^{Met} , 12S, tRNA ^{Val} , 16S, tRNA ^{Leu} , ND1 |
| <i>Orobodella yamaneae</i> 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 |
| <i>Orobodella dolichopharynx</i> Nakano, 2011b | KUZ Z120 Holotype | AB663665 | AB698876 | AB679680 | AB679681 | AB828558 | |
| <i>Orobodella esulacata</i> Nakano, 2010 | KUZ Z29 Holotype | AB663655 | AB698873 | AB679664 | AB679665 | AB828555 | |
| <i>Orobodella ijimai</i> Oka, 1895 | KUZ Z110 Topotype | AB663659 | AB698877 | AB679672 | AB679673 | AB828559 | |
| <i>Orobodella kawakatsuorum</i> Richardson, 1975 | KUZ Z167 Topotype | AB663661 | AB698878 | AB679704 | AB679705 | AB828561 | |
| <i>Orobodella ketagalan</i> Nakano & Lai, 2012 | KUZ Z208 Holotype | AB704785 | AB704786 | AB704787 | AB828582 | AB828563 | |
| <i>Orobodella koikei</i> Nakano, 2012b | KUZ Z156 Holotype | AB698883 | AB698882 | AB679688 | AB679689 | AB828560 | |
| <i>Orobodella masaakikuroiwai</i> Nakano, 2014 | KUZ Z694 Holotype | AB938003 | AB938013 | AB938006 | AB937997 | AB938016 | |
| <i>Orobodella mononoke</i> Nakano, 2012a | KUZ Z224 Holotype | AB698868 | AB698869 | AB698866 | AB698867 | AB828564 | |
| <i>Orobodella naraharaetmagarum</i> Nakano, 2016 | KUZ Z1652 Holotype | LC087143 | LC087145 | LC087144 | LC087142 | LC087146 | |
| <i>Orobodella octonaria</i> Oka, 1895 | KUZ Z181 Topotype | AB698870 | AB698871 | AB679708 | AB679709 | AB828562 | |
| <i>Orobodella shimadae</i> Nakano, 2011b | KUZ Z128 Holotype | AB663663 | AB698875 | AB679676 | AB679677 | AB828557 | |
| <i>Orobodella tsushimensis</i> Nakano, 2011a | KUZ Z134 Holotype | AB663653 | AB698872 | AB679662 | AB679663 | AB828554 | |
| <i>Orobodella whitmani</i> Oka, 1895 | KUZ Z45 Topotype | AB663657 | AB698874 | AB679668 | AB679669 | AB828556 | |
| <i>Erpobdella japonica</i> Pawłowski, 1962 | KUZ Z178 | AB663648 | AB698879 | AB679654 | AB679655 | AB828542 | |
| <i>Gastrostomobdella monticola</i> Moore, 1929 | UNIMAS/A3/BH01/10 | AB663649 | AB698880 | AB679656 | AB679657 | AB828543 | |
| <i>Mimobdella japonica</i> Blanchard, 1897 | KUZ Z179 | AB663650 | AB698881 | AB679658 | AB679659 | AB828544 | |
| <i>Odontobdella blanchardi</i> (Oka, 1910a) | KUZ Z180 | AB663651 | AB938012 | AB938004 | AB937995 | AB938014 | |

Results

Taxonomy

Family Orobdelellidae Nakano et al., 2012

Genus *Orobdelella* Oka, 1895

Orobdelella 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.

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

Type species. *Orobdelella 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.

Orobdelella 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.

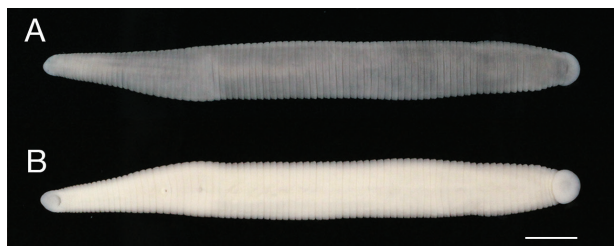


Figure 2. *Orobdelella brachyepididymis* sp. n., holotype, KUZ Z1673. **A** dorsal, **B** ventral views. Scale bar: 5 mm.

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

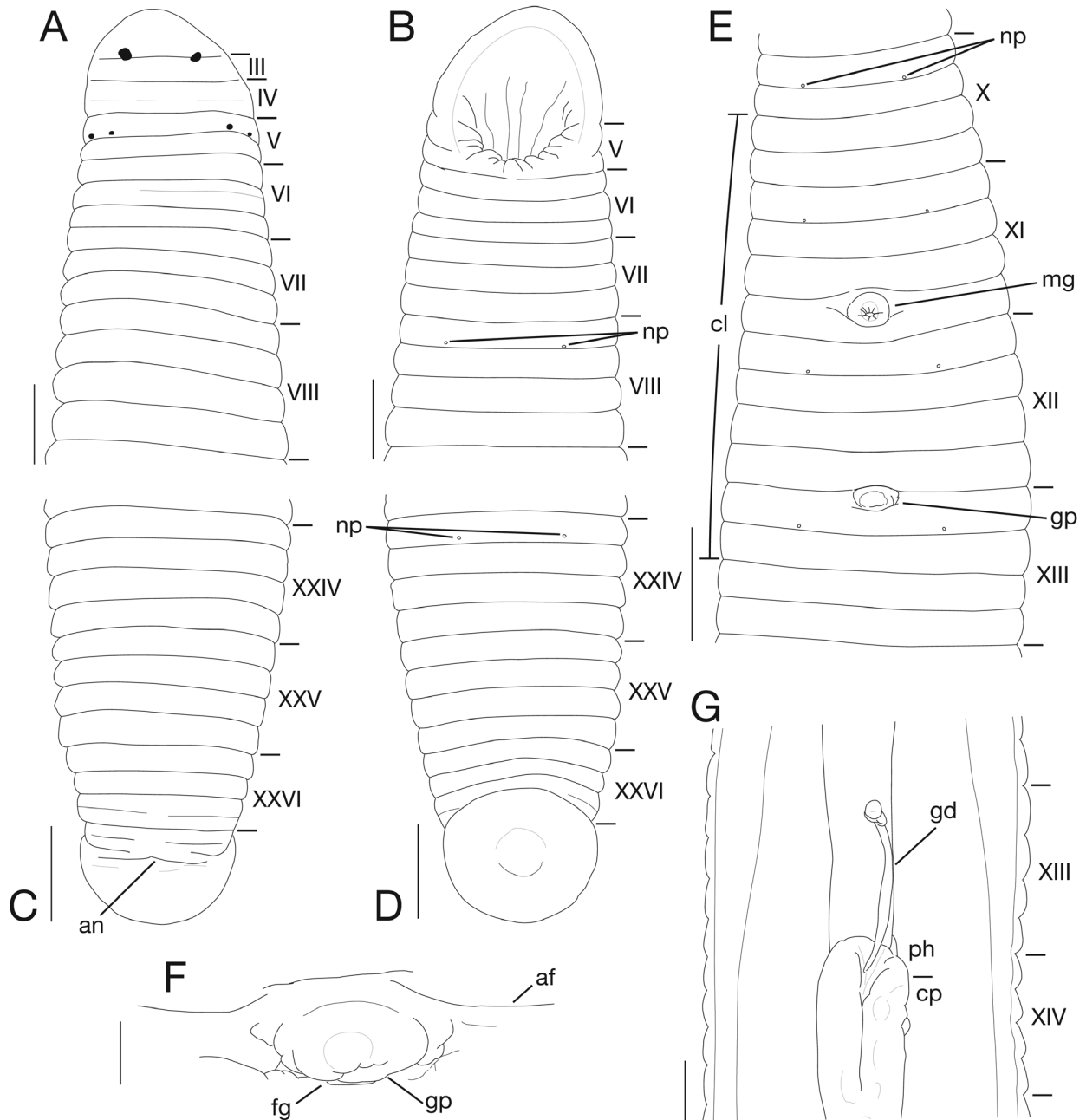


Figure 3. *Orobdella brachyepididymis* sp. n., holotype, KUZ Z1673. **A** dorsal, **B** ventral views of somites I–VIII; **C** dorsal, **D** ventral views of somites XXIV–XXVII and caudal sucker; **E** ventral view of somites X–XIII; **F** ventral view of gastropore and female gonopore; **G** ventral 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-

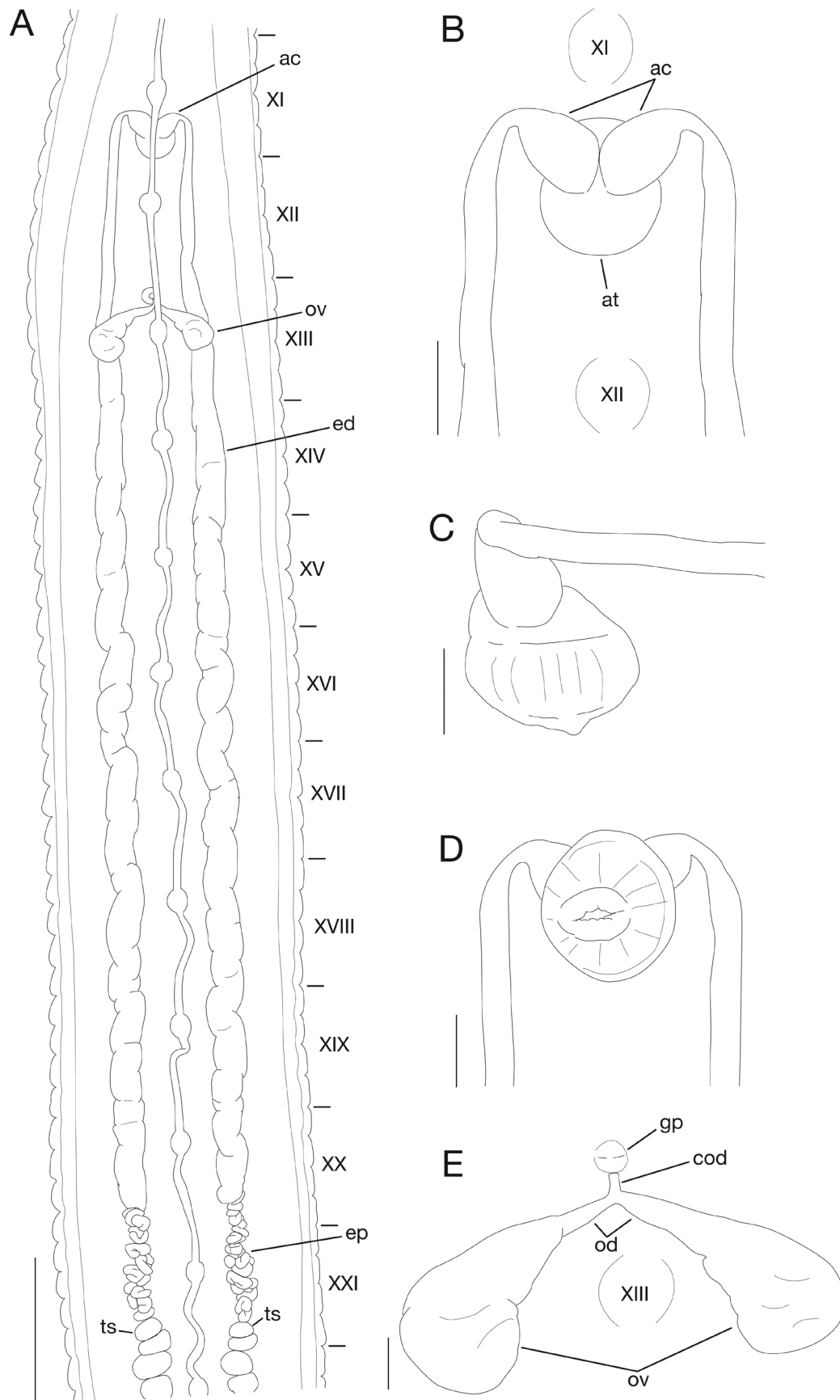


Figure 4. *Orobdella brachyepidymis* sp. n., holotype, KUZ Z1673. **A** dorsal view of reproductive system including ventral nervous system; **B** dorsal (including positions of ganglia XI and XII), **C** lateral, **D** ventral views of male atrium; **E** dorsal view of female reproductive system including 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 |
|------------------------------------------|-----------------------------------|--------------------|---------------|--------------------------------------------------|--------------------------|--------------|-------------------------------|
| <i>Orobdella brachyepididymis</i> sp. n. | less than or reaching to ca. 5 cm | uniannulate | quadrannulate | tubular | 1/2 + 4 [+ (< 1/2)] | XX to XXI | small, ovate |
| <i>Orobdella esulcata</i> | reaching to ca. 10 cm | uniannulate | quadrannulate | tubular, but bulbous at junction with gastropore | 2/3 + 4 + 1/3 | XVI to XX | developed, ovate |
| <i>Orobdella kawakatsuorum</i> | reaching to ca. 10 cm | biannulate | quadrannulate | simple tubular | 6 | XVI to XVII | undeveloped |
| <i>Orobdella ketagalan</i> | reaching to ca. 10 cm | uniannulate | quadrannulate | simple tubular | 1/2 + 4 + 1/2 | absent | undeveloped |
| <i>Orobdella koikei</i> | less than 4 cm | uniannulate | triannulate | bulbous | 1/2 + 4 + 1/2 | XV to XX | developed, ovate |
| <i>Orobdella masaakikuroiwai</i> | less than 4 cm | uniannulate | quadrannulate | bulbous | 1/2 + 4 + 1/2 | XVI to XVIII | developed, ovate |
| <i>Orobdella naraharaetmagarum</i> | less than 5 cm | uniannulate | quadrannulate | bulbous | 1/2 + 4 + 1/2 | XV to XX | developed, ellipsoid or ovate |
| <i>Orobdella tsushimensis</i> | reaching to ca. 10 cm | uniannulate | quadrannulate | bulbous | 1/2 + 5 | XVII to XIX | developed, ovate |
| <i>Orobdella whitmani</i> | 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.

gonopores 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. Gastropore 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. Takano-suyama (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

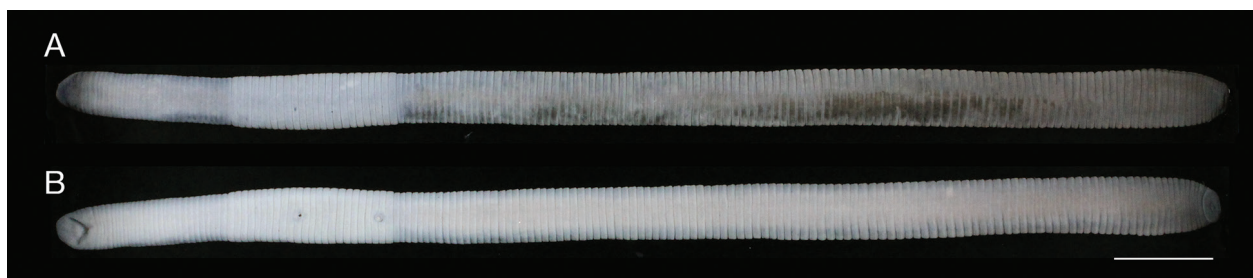


Figure 6. *Orobdella nakahamai* sp. n., holotype, KUZ Z1672. **A** dorsal, **B** ventral views. Scale bar: 2 cm.

(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 Kamimodoi (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 quinquannulate, $a1 > b3 = b4 = b5 = b6$, $a1$ 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

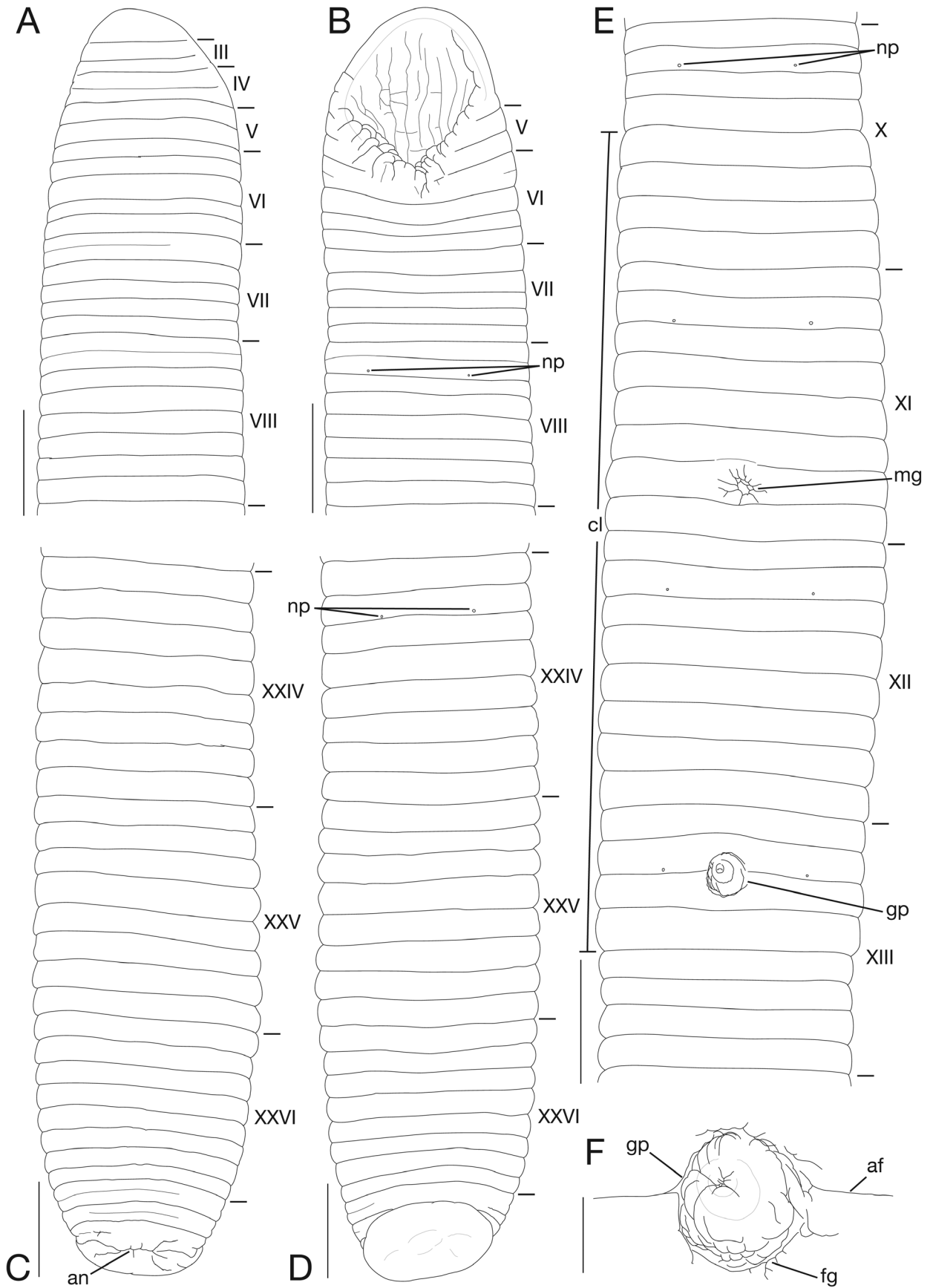


Figure 7. *Orobdella nakahamai* sp. n., holotype, KUZ Z1672. **A** dorsal, **B** ventral views of somites I–VIII; **C** dorsal, **D** ventral views of somites XXIV–XXVII and caudal sucker; **E** ventral view of somites X–XIII; **F** ventral view of gastropore and female gonopore. Scale bars: **A–E**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

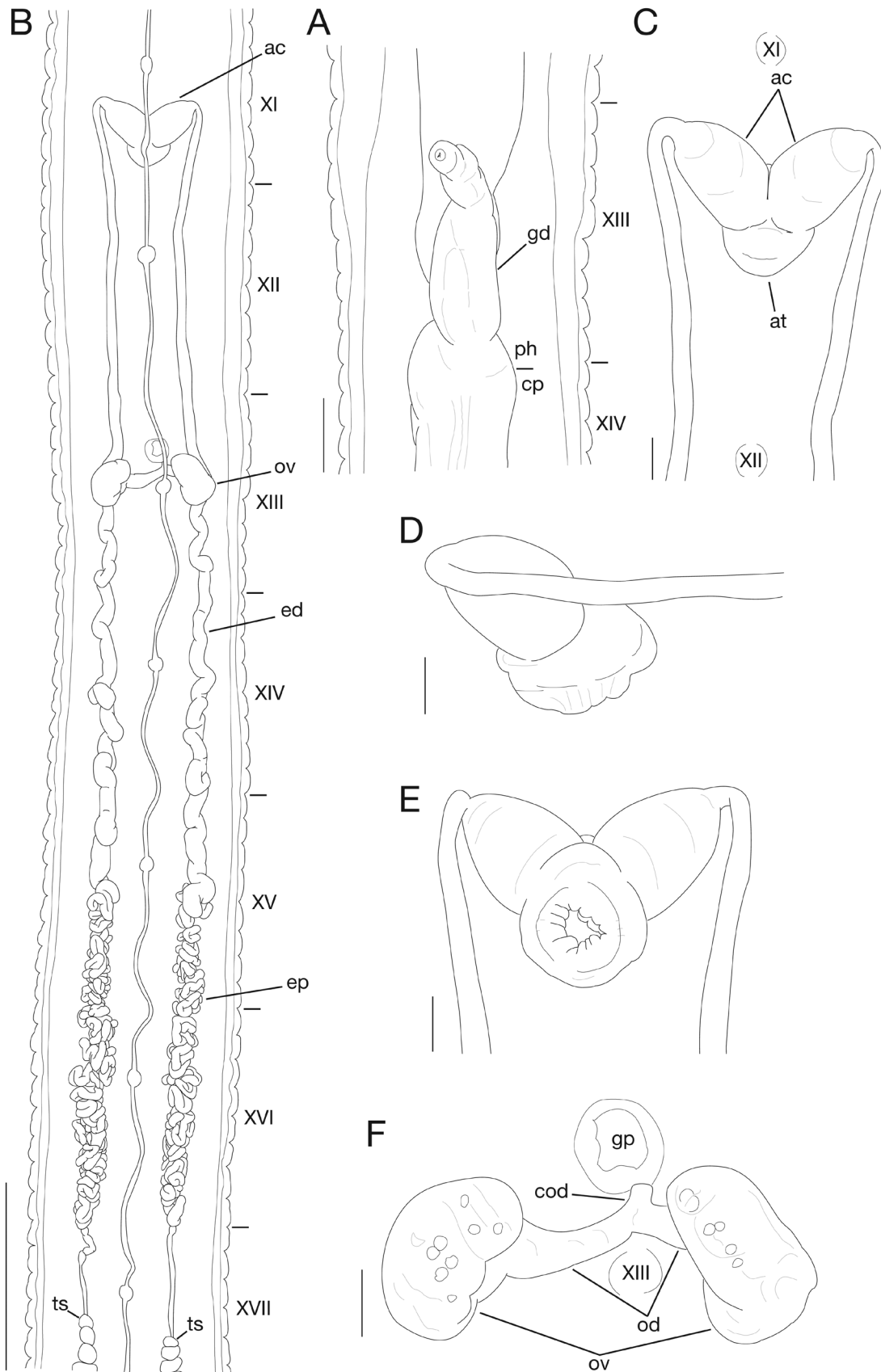


Figure 8. *Orobdella nakahamai* sp. n., holotype, KUZ Z1672. **A** ventral view of gastroporal duct; **B** dorsal view of reproductive system including ventral nervous system; **C** dorsal (including positions of ganglia XI and XII), **D** lateral, **E** ventral views of male atrium; **F** dorsal view 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 $b_1 = b_2$) = a_2 (dorsally $b_3 = b_4$) > $b_5 < b_6$, or dorsally quinquannulate, $b_1 = b_2 < a_2 > b_5 < b_6$. Somite VII quinquannulate, a_1 (dorsally $b_1 = b_2$ in KUZ Z1680) > $b_3 = b_4 = b_5 = b_6$. Somite VIII octannulate, $b_1 = b_2 < b_3 < b_4 = c_9 = c_{10} = c_{11} = c_{12}$, or ventrally septannulate, a_1 ($b_1 = b_2$) > $b_2 = b_3 = b_4 = c_9 = c_{10} = c_{11} = c_{12}$. Somite IX octannulate, $b_1 = b_2 = b_3 = b_4 = c_9 = c_{10} = c_{11} = c_{12}$. Somite XXVI dorsally octannulate, $b_1 = b_2 = b_3 = b_4 > c_9 = c_{10} = c_{11} = c_{12}$, ventrally septannulate, $b_1 = b_2 = b_3 = b_4 > c_9 = c_{10} < b_6$, or sexannulate, $b_1 = b_2 = b_3 = b_4 = b_5 < b_6$ ($c_{11} = c_{12}$). 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 b_3/b_4 . Crop reaching to XXII b_1/b_2 . Gastroporal duct joining with crop in XIV b_3 . Intestine reaching to XXV b_4 . Male gonopore in XI c_{10}/c_{11} (KUZ Z1680), thus gonopores separated by 12 annuli. Testisacs in XVII c_{10} to XXV c_{11} ; 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 c_{10}/c_{11} to XVII b_3/b_4 , occupying 13 annuli. Pair of muscular atrial cornua ellipsoid. Left oviduct crossing ventrally beneath nerve cord; both oviducts converging into common oviduct in XIII b_3 .

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 b_2/b_3 , 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 |
|-----------------------------------|-------------------|--------------------------|--------------|
| <i>Orobdella nakahamai</i> sp. n. | XIII b2/b3 | 1/2 + 11 or 12 | XV to XVII |
| <i>Orobdella octonaria</i> | 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, incon-

spicuous, 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

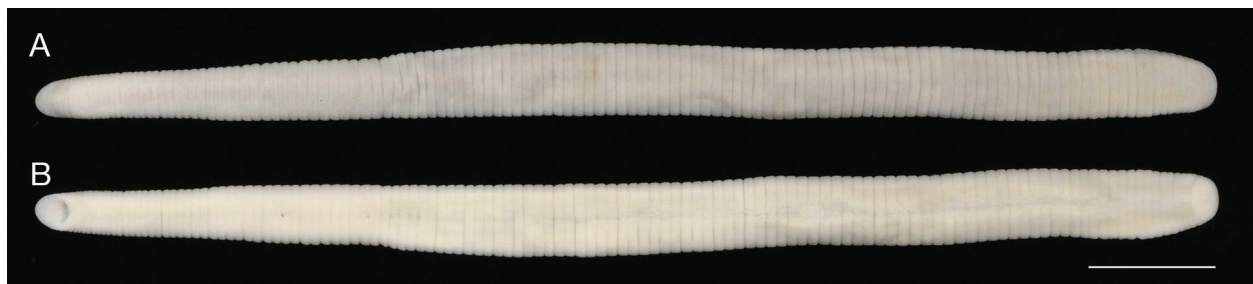


Figure 10. *Orobdella okanoi* sp. n., holotype, KUZ Z1671. **A** dorsal, **B** ventral views. Scale bar: 1 cm.

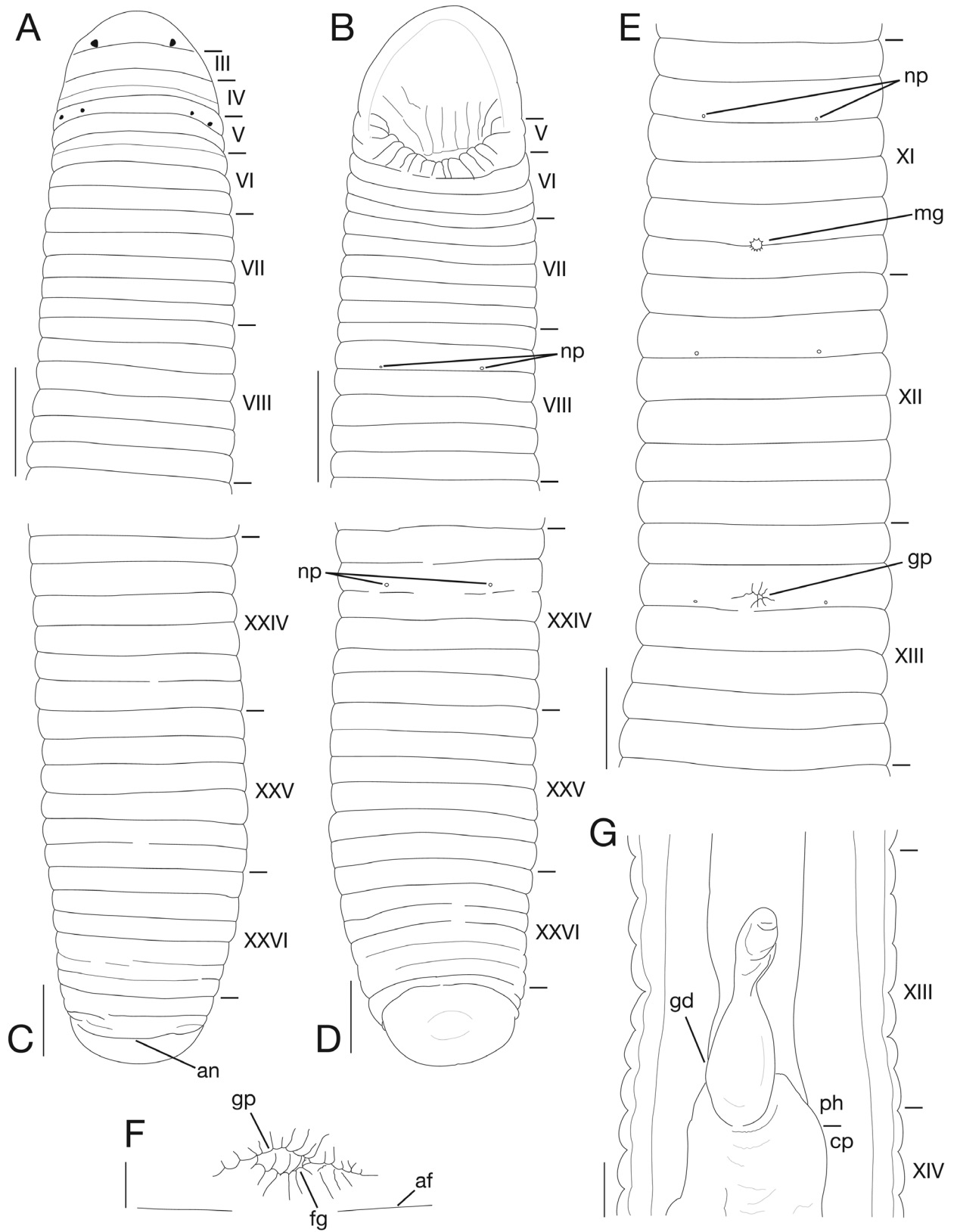


Figure 11. *Orobdella okanoi* sp. n., holotype, KUZ Z1671. **A** dorsal, **B** ventral views of somites I–VIII; **C** dorsal, **D** ventral views of somites XXIV–XXVII and caudal sucker; **E** ventral view of somites XI–XIII; **F** ventral view of gastropore and female gonopore; **G** ventral view of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

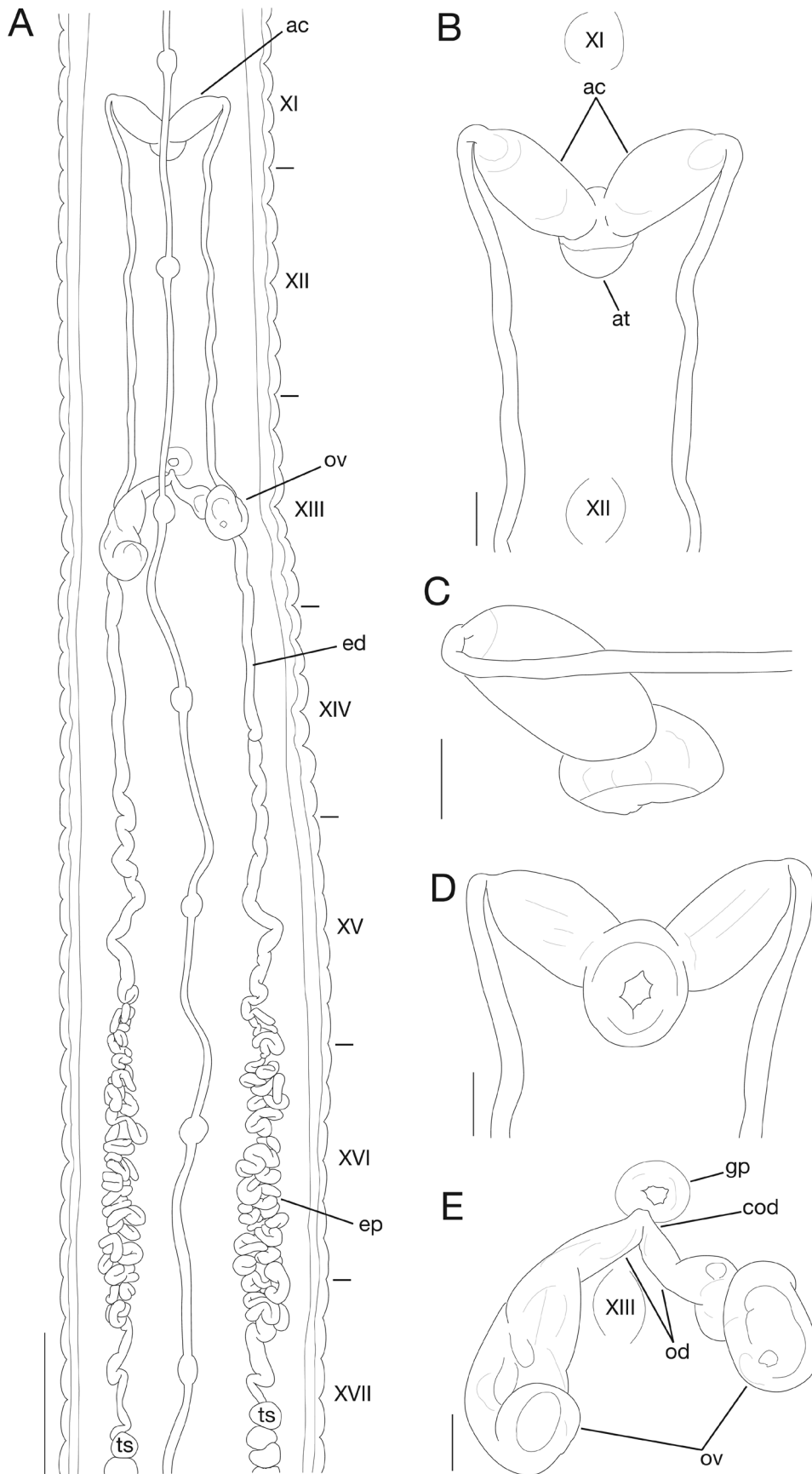


Figure 12. *Orobdella okanoi* sp. n., holotype, KUZ Z1671. **A** dorsal view of reproductive system including ventral nervous system; **B** dorsal (including positions of ganglia XI and XII), **C** lateral, **D** ventral views of male atrium; **E** dorsal view of female reproductive system including 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, first 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. Oli-

gochaete 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.

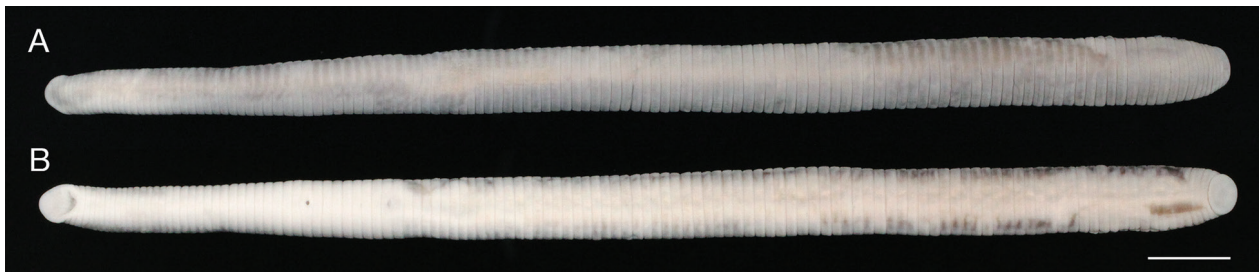
Additional material. KUZ Z1488, collected from under fallen leaves along the forest road (34.247222°N, 134.808611°E; Elev. ca. 215 m) at the type locality, by Yoshiko Yamane on 20 August 2013.

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 |
|----------------------------------|-------------------------------------------------------------|----------------|----------------|--------------------------|-----------------|--------------------------------------------|--------------|--------------------------------|---------------|
| <i>Orobdella okanoi</i> sp. n. | reddish | quinquannulate | sexannulate | 8 + 1/2 | reaching to XIV | bulbous | XV to XVII | absent | ellipsoid |
| <i>Orobdella yamaneae</i> sp. n. | purplish | quinquannulate | sexannulate | 1/2 + 7 + 1/2 | reaching to XIV | bulbous | XVI to XVIII | extending to anterior of XI c9 | ovate |
| <i>Orobdella dolichopharynx</i> | yellowish green | quadrannulate | quinquannulate | 8 | reaching to XVI | tubular, reaching to XVI | absent | extending to ganglion XI | absent |
| <i>Orobdella ijimai</i> | yellowish green | quadrannulate | sexannulate | 1/2 + 7 + 1/2 | reaching to XIV | bulbous | XVI to XIX | absent | ellipsoid |
| <i>Orobdella mononoke</i> | 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 |
| <i>Orobdella shimadae</i> | 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.

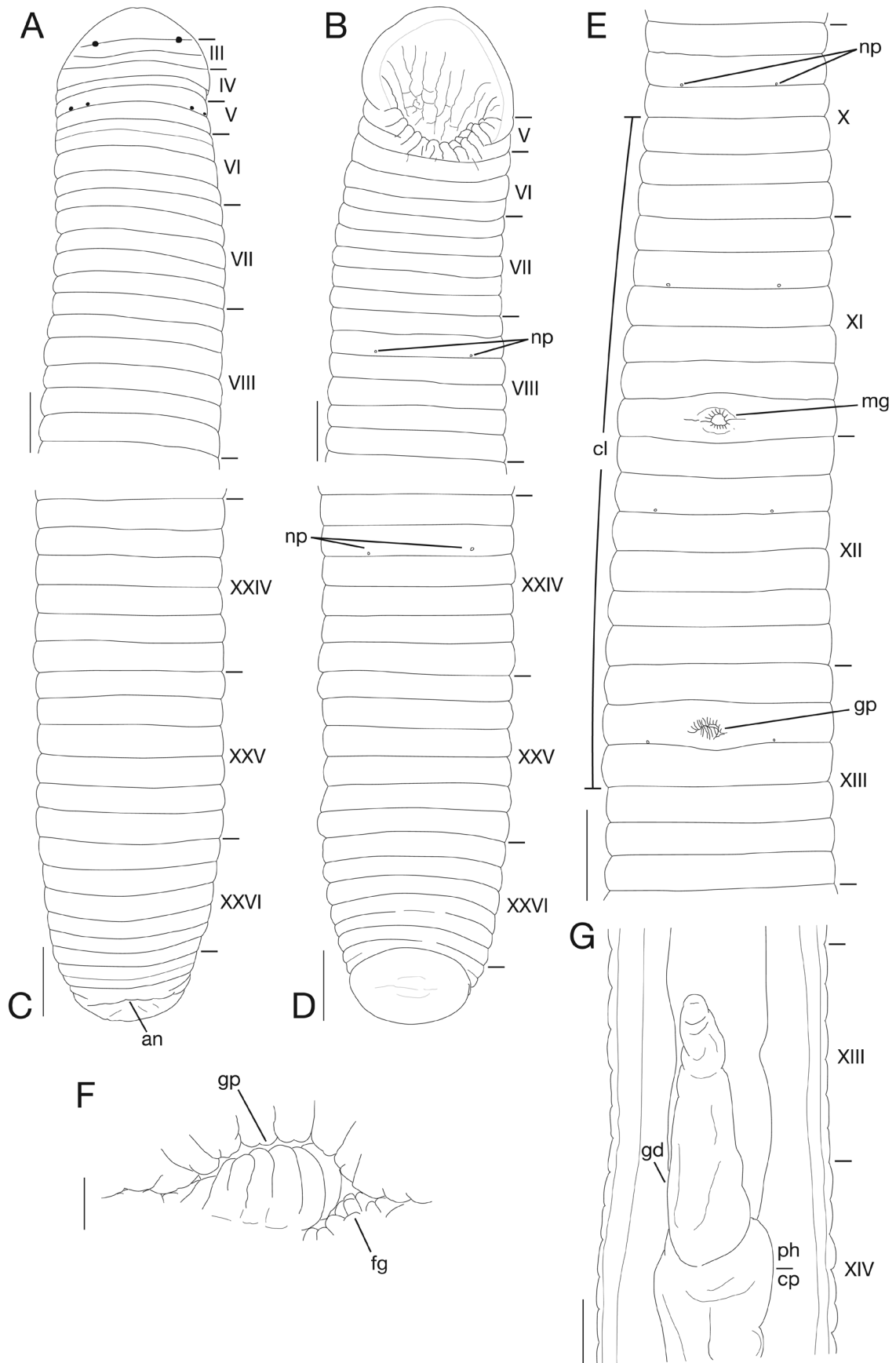


Figure 15. *Orobdella yamaneae* sp. n., holotype, KUZ Z1678. **A** dorsal, **B** ventral views of somites I–VIII; **C** dorsal, **D** ventral views of somites XXIV–XXVII and caudal sucker; **E** ventral view of somites X–XIII; **F** ventral view of gastropore and female gonopore; **G** ventral view of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

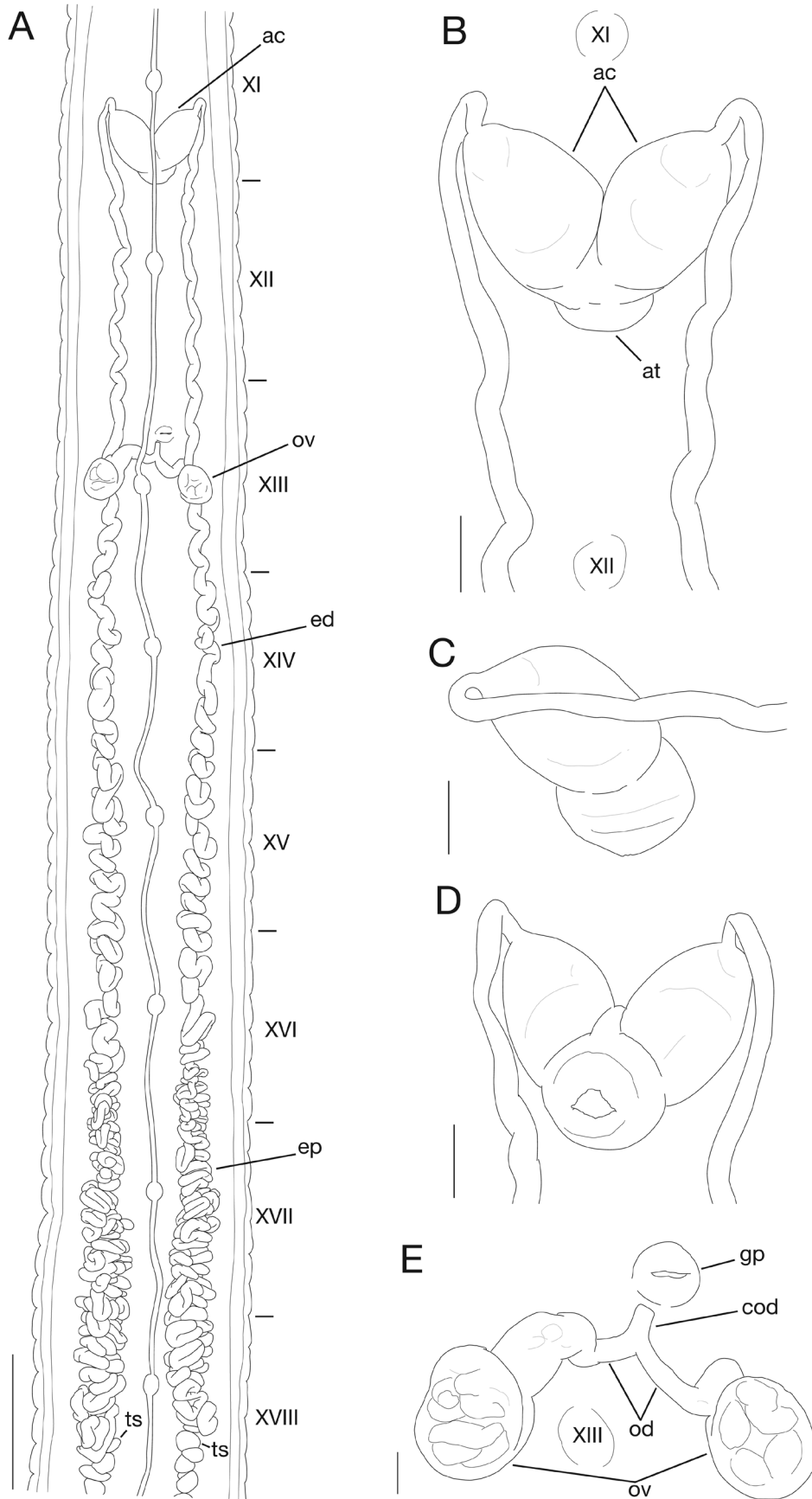


Figure 16. *Orobodella yamaneae* sp. n., holotype, KUZ Z1678. **A** dorsal view of reproductive system including ventral nervous system; **B** dorsal (including positions of ganglia XI and XII), **C** lateral, **D** ventral views of male atrium; **E** dorsal view of female reproductive system including 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.

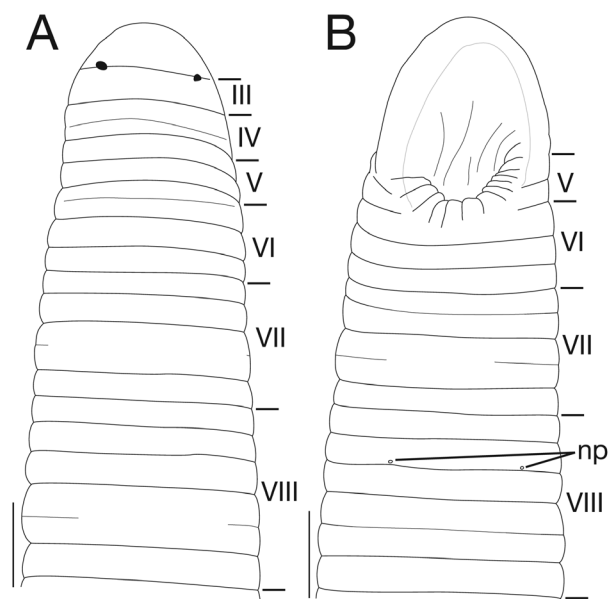


Figure 17. *Orobdella yamaneae* sp. n., paratype, KUZ Z1359. **A** dorsal, **B** ventral views of somites I–VIII. Scale bars: **A**, **B** = 1 mm.

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-

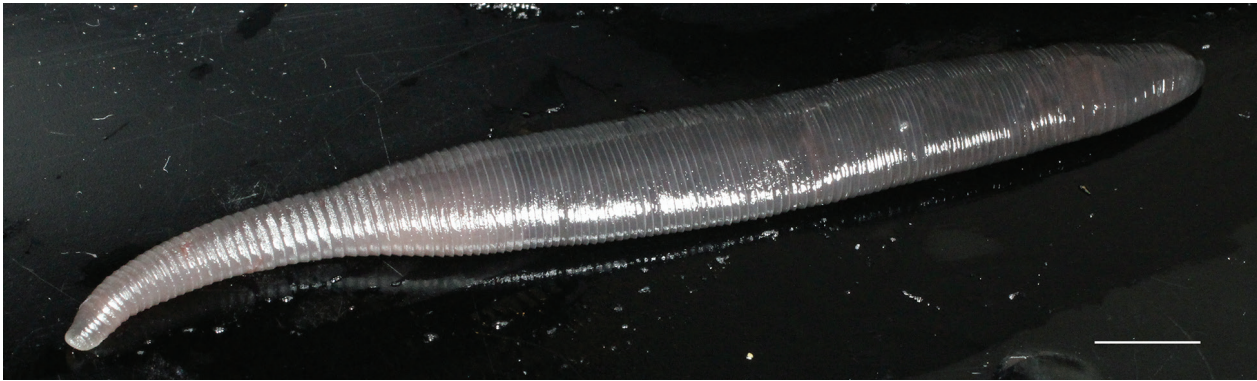


Figure 18. *Orobdella yamaneae* sp. n., holotype, KUZ Z1678. Dorsal view of live animal. Scale bar: 1 cm.

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*.

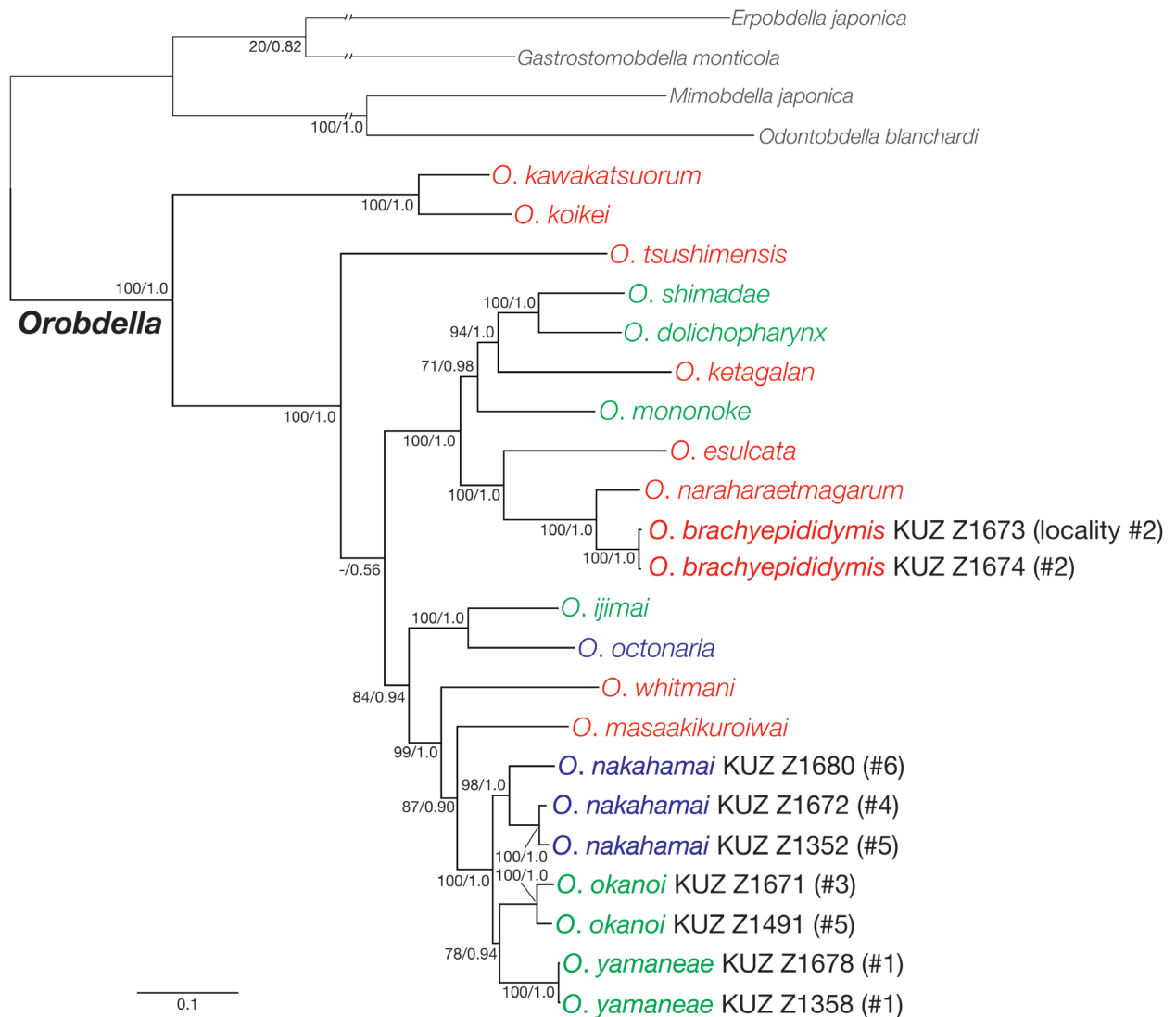


Figure 19. Bayesian inference tree for 5,209 bp of nuclear 18S rRNA and histone H3 and mitochondrial COI, tRNA^{Cys}, tRNA^{Met}, 12S rRNA, tRNA^{Val}, 16S rRNA, tRNA^{Leu} and ND1 markers. Numbers on nodes represent bootstrap values for maximum likelihood and Bayesian posterior probabilities. A species 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 |
|-----------------------------------|-----------------------|-------|-------|-------|-------|-------|-------|
| <i>Orobdella nakahamai</i> sp. n. | 1: KUZ Z1352 (5) | | | | | | |
| | 2: KUZ Z1672 (4) | 0.021 | | | | | |
| | 3: KUZ Z1680 (6) | 0.043 | 0.046 | | | | |
| <i>Orobdella okanoi</i> sp. n. | 4: KUZ Z1491 (5) | 0.058 | 0.056 | 0.056 | | | |
| | 5: KUZ Z1671 (3) | 0.062 | 0.058 | 0.058 | 0.024 | | |
| <i>Orobdella yamaneae</i> 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. masaakikuroi* 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.

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Jahr/Year: 2016

Band/Volume: [92](#)

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Artikel/Article: [Four new species of the genus Orobdella from Shikoku and Awajishima island, Japan \(Hirudinida, Arhynchobdellida, Orobdellidae\) 79-102](#)