

Two new sexannulate species of *Orobdella* (Hirudinea: Erpobdelliformes: Orobdellidae) from Kyushu, Japan

Takafumi Nakano

Department of Zoology, Graduate School of Science, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan

Corresponding author: Takafumi Nakano (nakano@zoo.zool.kyoto-u.ac.jp)

Received 8 July 2024; Accepted 27 July 2024

<https://zoobank.org/References/6A326197-A570-45EE-9A4E-61611BBF7F3E>

Abstract Two sexannulate leech species, *Orobdella imperfecta* sp. nov. and *O. kirishimayamensis* sp. nov., from Kyushu, Japan, are described. This study provides the first occurrence record of sexannulate species of *Orobdella* Oka, 1895, in Kyushu. Phylogenetic analysis using nuclear 18S rRNA, 28S rRNA, histone H3, mitochondrial cytochrome *c* oxidase subunit I, tRNA^{Cys}, tRNA^{Met}, 12S rRNA, tRNA^{Val}, 16S rRNA, tRNA^{Leu}, and NADH dehydrogenase subunit 1 markers showed that the two new species formed a monophyletic lineage with five quadrannulate congeners that are distributed across the western part of Honshu, Shikoku, and Kyushu.

Key words: Arhynchobdellida, macrophagous, mid-body somite annulation, molecular phylogeny, taxonomy

Introduction

The terrestrial leech genus *Orobdella* Oka, 1895 consists of three non-monophyletic groups based on the mid-body somite annulation patterns, namely, quadr- (four-), sex- (six-), and octannulate (eight-annulate) mid-body somite annulation (Nakano, 2017). Seven sexannulate and two octannulate species are endemic to the main islands of Japan and the Ryukyu Islands, while the remaining 15 quadrannulate species have been recorded from the whole range of this genus that comprises the Southern Russian Far East, Korean Peninsula, Japanese Archipelago, and Taiwan (Nakano, 2017; Nakano and Prozorova, 2024).

The sexannulate *O. ijimai* Oka, 1895 is distributed in eastern Honshu, Japan, and three closely related sexannulate species, *O. ibukifukuyamai* Nakano, 2022, *O. yamaneae* Nakano, 2016, and *O. okanoi* Nakano, 2016, are known from a small islet south of the Kii Peninsula, Awajishima Island, and Shikoku, respectively (Nakano, 2017, 2022). The other three sexannulate species, *O. mononoke* Nakano, 2012, *O. dolichopharynx* Nakano, 2011, and *O. shimadae* Nakano, 2011, are endemic to the Ryukyu Islands: *O. mononoke* is indigenous to Yakushima Island; *O. dolichopharynx* to Amamioshima Island; and *O. shimadae* to Okinawajima Island (Nakano, 2017).

Unidentified sexannulate specimens of *Orobdella* were

collected from Kyushu in the Japanese Archipelago. Their taxonomic accounts are herein clarified along with estimating their phylogenetic positions within this genus using nuclear and mitochondrial genetic markers.

Materials and Methods

Samples and morphological examination

Seven individuals were collected from six sites in Kyushu, Japan (Fig. 1). When possible, elevation and geographical coordinates for the locations were obtained using a Garmin eTrex® GPS unit. Leeches were relaxed by the gradual addition of absolute ethanol (EtOH) to freshwater, and then fixed in absolute EtOH. For DNA extraction, botryoidal tissue was removed from the posterior part around the caudal sucker of each specimen, and then preserved in absolute EtOH. The remainder of the body was fixed in 10% formalin, and preserved in 70% EtOH.

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

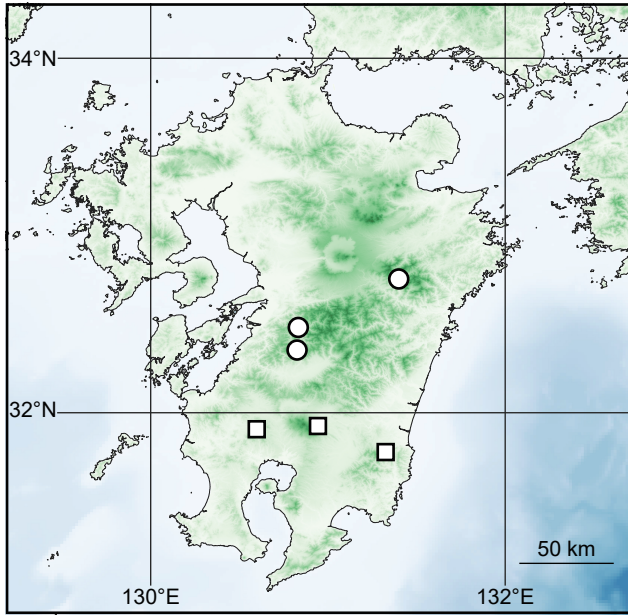


Fig. 1. Map showing the collection localities of the specimens examined in this study. Open circles represent the collection sites of *Orobdella imperfecta* sp. nov.; open squares represent the sites of *O. kirishimayamensis* sp. nov. Shoreline data were based on Wessel and Smith (1996).

microscope with a drawing tube (Leica M125C). All specimens examined in this study were deposited in the Zoological Collection of Kyoto University (KUZ), Kyoto, Japan.

The somite numbering convention is based on Moore (1927): body somites are denoted by Roman numerals, and the annuli in each somite are given alphanumeric designations. The following abbreviations are used for morphological characters in the figures: ac, atrial cornu; af, annular furrow; an, anus; at, atrium; cod, common oviduct; cp, crop; ed, ejaculatory duct; ep, epididymis; gd, gastroporal duct; gp, gastropore; mg, male gonopore; np, nephridiopore; od, oviduct; ov, ovisac; ph, pharynx; ts, testisacs.

Molecular analyses

The phylogenetic positions of the sexannulate *Orobdella* specimens from Kyushu were investigated based on the concatenated analyses of three nuclear markers: 18S rRNA, 28S rRNA, and histone H3; and on three mitochondrial markers: cytochrome *c* oxidase subunit I (COI), tRNA^{Cys}, tRNA^{Met}, 12S rRNA, tRNA^{Val} and 16S rRNA (tRNA^{Cys}-16S), and tRNA^{Leu} and NADH dehydrogenase subunit 1 (tRNA^{Leu}-ND1). Genomic DNA extraction, polymerase chain reaction, and cycle sequencing reaction methods were as previously described by Nakano and Lai (2016, 2017); a part of cycle sequencing products was sequenced on an

Applied Biosystems® 3500xL Genetic Analyzer (Thermo Fisher Scientific). In total, 24 sequences were newly obtained, and deposited with the International Nucleotide Sequence Databases (INSD) through the DNA Data Bank of Japan (Table 1).

In accordance with the previous study (Nakano and Prozorova, 2024), 192 sequences of 24 *Orobdella* species, and eight other erpobdelliform species, *i.e.*, one erpobdellid species, *Erpobdella japonica* Pawłowski, 1962, four gastrostomobdellid taxa, *Gastrostomobdella ampunganensis* Nakano, 2018, *G. extenta* Nakano & Jeratthitikul, 2018, *G. monticola* Moore, 1929 and *G. cf. monticola*, and three salifid species *Mimobdella japonica* Blanchard, 1897, *Odontobdella blanchardi* (Oka, 1910) and *Salifa motokawai* Nakano & Nguyen, 2015, which were selected as the outgroup, were retrieved from INSD, and included in the dataset for the analyses (Table 1). The H3 and COI alignments were trivial, as no indels were observed. The other markers sequences were aligned using MAFFT v. 7.520 L-INS-i (Katoh and Standley, 2013). The lengths of the 18S, 28S, H3, COI, tRNA^{Cys}-16S, and tRNA^{Leu}-ND1 aligned datasets were 1849, 2834, 328, 1271, 1177, and 640 bp, respectively. Our concatenated final dataset has 8099 bp of aligned positions.

Phylogenetic trees were reconstructed using maximum likelihood (ML) and Bayesian inference (BI). The best-fit partition scheme and optimal models were identified based on Bayesian information criterion using ModelFinder (Chernomor *et al.*, 2016; Kalyaanamoorthy *et al.*, 2017) implemented in IQ-Tree v. 2.2.2.6 (Minh *et al.*, 2020). The selected partition scheme and models are as follows: for all of 18S and the H3 1st positions, K80 + I; for all of 28S and the H3 2nd positions, GTR + I + G; for the H3 3rd positions, HKY + G; for the COI and ND1 1st positions, GTR + I + G; for the COI and ND1 2nd positions, GTR + I + G; for the COI and ND1 3rd positions, and all of 16S, GTR + I + G; and for all of tRNA^{Cys}, tRNA^{Met}, 12S, tRNA^{Val} and tRNA^{Leu}, GTR + I + G. The ML tree was inferred using IQ-Tree v. 2.2.2.6 with non-parametric bootstrapping (BS) conducted with 1000 replicates. BI tree and Bayesian posterior probabilities (PP) were estimated using MrBayes v. 3.2.7a (Ronquist *et al.*, 2012). Two independent runs with four Markov chains were conducted for 40 million generations, and the tree was sampled every 100 generations. Parameter estimates and convergence were checked using Tracer v. 1.7.1 (Rambaut *et al.*, 2018), and then the first 100001 trees were discarded (burn-in) based on the results.

Pairwise comparisons of uncorrected *p*-distances for COI sequences of the newly described *Orobdella* species (1267 bp,

Table 1. Samples used for phylogenetic analyses. Voucher information on the vouchers is accompanied by the International Nucleotide Sequence Databases (INSD) accession numbers of the markers. Sequences marked with an asterisk (*) were obtained for the first time in the present study. Acronyms: KUHE, Kyoto University, Human and Environmental Studies; KUZ, Zoological Collection of Kyoto University; MUMNH, Mahidol University Museum of Natural History; UNIMAS, Universiti Malaysia Sabah; VNMN, Vietnam National Museum of Nature; ZRC, Zoological Reference Collection, Lee Kong Chian Natural History Museum.

Species	Voucher	INSD#					
		18S	28S	Histone H3	COI	tRNACys–16S	tRNALeu–ND1
<i>Orobrella</i>							
<i>O. imperfecta</i> sp. nov.	KUZ Z1356 (holotype)	LC820567*	LC820568*	LC820566*	LC820565*	LC820563*	LC820564*
<i>O. imperfecta</i> sp. nov.	KUZ Z1542 (paratype)				LC820571*	LC820569*	LC820570*
<i>O. imperfecta</i> sp. nov.	KUZ Z5215				LC820577*	LC820575*	LC820576*
<i>O. kirishimayamensis</i> sp. nov.	KUZ Z1354 (holotype)	LC820561*	LC820562*	LC820560*	LC820559*	LC820557*	LC820558*
<i>O. kirishimayamensis</i> sp. nov.	KUZ Z1543				LC820574*	LC820572*	LC820573*
<i>O. kirishimayamensis</i> sp. nov.	KUZ Z5216				LC820580*	LC820578*	LC820579*
<i>O. angustata</i> Nakano, 2018	KUZ Z1439 (holotype)	LC323140	LC431606	LC323138	LC323139	LC323141	LC323137
<i>O. brachyepididymis</i> Nakano, 2016	KUZ Z1673 (holotype)	LC106319	LC274535	LC106321	LC106320	LC106318	LC106322
<i>O. dolichopharynx</i> Nakano, 2011	KUZ Z120 (holotype)	AB663665	LC274541	AB698876	AB679680	AB679681	AB828558
<i>O. esulcata</i> Nakano, 2010	KUZ Z29 (holotype)	AB663655	LC274538	AB698873	AB679664	AB679665	AB828555
<i>O. ganini</i> Nakano & Prozorova, 2024	KUZ Z5038 (holotype)	LC778511	LC778512	LC778514	LC778513	LC778510	LC778515
<i>O. ghilarovi</i> Nakano & Prozorova, 2019	KUZ Z2068 (holotype)	LC431608	LC431607	LC431612	LC431609	LC431616	LC431613
<i>O. ibukifukuyamai</i> Nakano, 2022	KUZ Z4031 (holotype)	LC687625	LC687626	LC687624	LC687620	LC687618	LC687622
<i>O. ijimai</i> Oka, 1895	KUZ Z110 (topotype)	AB663659	LC274542	AB698877	AB679672	AB679673	AB828559
<i>O. kanaekoikeae</i> Nakano, 2017	KUZ Z1747 (holotype)	LC184551	LC274533	LC184553	LC184552	LC184550	LC184554
<i>O. kawakatsuorum</i> Richardson, 1975	KUZ Z167 (topotype)	AB663661	LC274544	AB698878	AB679704	AB679705	AB828561
<i>O. ketagalan</i> Nakano & Lai, 2012	KUZ Z208 (holotype)	AB704785	LC274546	AB704786	AB704787	AB828582	AB828563
<i>O. koikei</i> Nakano, 2012	KUZ Z156 (holotype)	AB698883	LC274543	AB698882	AB679688	AB679689	AB828560
<i>O. masaakikuroiwai</i> Nakano, 2014	KUZ Z694 (holotype)	AB938003	LC274530	AB938013	AB938006	AB937997	AB938016
<i>O. meisai</i> Nakano & Lai, 2017	KUZ Z1917 (holotype)	LC314423	LC431605	LC314425	LC314424	LC314422	LC314426
<i>O. mononoke</i> Nakano, 2012	KUZ Z224 (holotype)	AB698868	LC274547	AB698869	AB698866	AB698867	AB828564
<i>O. montipumila</i> Nakano, 2021	KUZ Z3913 (holotype)	LC616674	LC616673	LC616672	LC616663	LC616667	LC616671
<i>O. nakahamai</i> Nakano, 2016	KUZ Z1672 (holotype)	LC106330	LC274534	LC106332	LC106331	LC106329	LC106333
<i>O. naraharaetmagarum</i> Nakano, 2016	KUZ Z1652 (holotype)	LC087143	LC274531	LC087145	LC087144	LC087142	LC087146
<i>O. octonaria</i> Oka, 1895	KUZ Z181 (topotype)	AB698870	LC274545	AB698871	AB679708	AB679709	AB828562
<i>O. okanoi</i> Nakano, 2016	KUZ Z1671 (holotype)	LC106341	LC274532	LC106343	LC106342	LC106340	LC106344
<i>O. shimadae</i> Nakano, 2011	KUZ Z128 (holotype)	AB663663	LC274540	AB698875	AB679676	AB679677	AB828557
<i>O. tsushimensis</i> Nakano, 2011	KUZ Z134 (holotype)	AB663653	LC274537	AB698872	AB679662	AB679663	AB828554
<i>O. whitmani</i> Oka, 1895	KUZ Z45 (topotype)	AB663657	LC274539	AB698874	AB679668	AB679669	AB828556

(continued on the next page)

Table 1. (Continued)

Species	Voucher	INSD#					
		18S	28S	Histone H3	COI	tRNACys-16S	tRNALeu-ND1
<i>O. yamaneae</i> Nakano, 2016	KUZ Z1678 (holotype)	LC106349	LC274536	LC106351	LC106350	LC106348	LC106352
Outgroup							
<i>Erpobdella japonica</i> Pawłowski, 1962	KUZ Z178	AB663648	LC274512	AB698879	AB679654	AB679655	AB828542
<i>Gastrostomobdella ampunganensis</i> Nakano, 2018	ZRC.ANN.0083 (holotype)	LC274517	LC274516	LC274552	LC274551	LC274564	LC274571
<i>G. extenta</i> Nakano & Jeratthitikul, 2018	MUMNH-Hir0001 (holotype)	LC274519	LC274518	LC274554	LC274553	LC274565	LC274572
<i>G. monticola</i> Moore, 1929	UNIMAS/A3/BH01/10	AB663649	LC274515	AB698880	AB679656	AB679657	AB828543
<i>G. cf. monticola</i>	KUHE 56264	LC274514	LC274513	LC274550	LC274549	LC274563	LC274570
<i>Mimobdella japonica</i> Blanchard, 1897	KUZ Z179	AB663650	LC274528	AB698881	AB679658	AB679659	AB828544
<i>Odontobdella blanchardi</i> (Oka, 1910)	KUZ Z180	AB663651	LC274529	AB938012	AB938004	AB937995	AB938014
<i>Salifa motokawai</i> Nakano & Nguyen, 2015	VNMN 2015.65 (holotype)	LC029434	LC274548	LC029435	LC029431	LC029432	LC029433



Fig. 2. *Orobdella imperfecta* sp. nov., holotype (A, B: KUZ Z1356), and from Mt. Dodake, Miyazaki (C: KUZ Z5215). A, dorsal view; B, ventral view; C, dorsal view of live animal. Scale bars: 10 mm (same bar for A, B).

but 1224 bp for the COI sequence of KUZ Z1542) were performed using MEGA11 (Tamura *et al.*, 2021) with pairwise deletion of missing data.

Taxonomic Accounts

Genus *Orobdella* Oka, 1895

Orobdella imperfecta sp. nov.

(Figs. 2–4)

Material examined

Holotype: near Nishinohara Bridge, Youra, Sagara, Kuma-gun, Kumamoto Prefecture, Japan (32.34167°N, 130.83944°E), elev. 242 m, 3 December 2010, Takafumi Nakano leg., KUZ Z1356.

Paratype: Hirasawatsudani Valley, Itsuki, Kuma-gun, Kumamoto Prefecture, Japan (32.47278°N, 130.84694°E), elev. 628 m, 15 September 2013, Yoshiko Yamane leg., KUZ Z1542.

Additional material: Mt. Dodake, Mitate, Hinokage, Nishi-Usuki-gun, Miyazaki Prefecture, Japan (approximately 32.76°N, 131.44°E), 30 May 2021, Ibuki Fukuyama leg., KUZ Z5215. All three specimens were dissected; genital organs in KUZ Z1542 undeveloped; testisacs in KUZ Z5215 undetectable.

Diagnosis

Body length of mature individuals reaching ~7 cm. Somite VII triannulate, somite VIII quadrannulate, somite IX quinquannulate, somites X–XXV sexannulate, $b1 = b2 < a2 = b5 > c11 = c12$. Male gonopore in slightly posterior to middle of, or in middle of somite XI c11, female gonopore in somite XIII b1/b2, posterior to gastropore, gonopores separated by $1/2 + 8$ annuli. Pharynx reaching to somite XIV c11/c12–XV b1/b2. Gastropore conspicuous, in somite XIII b1/b2. Gastro-

poral duct tubular. Paired epididymides in somites XVI–XX, occupying 13–18 annuli. Pre-atrial loop present, extending to posterior of somite XI a2. Atrial cornua developed, ovate. Dorsal surface grayish blue in life.

Description of the holotype

Body firm and muscular, elongated, with constant width in posterior direction, dorsoventrally compressed; BL 69.0 mm, BW 4.5 mm (Fig. 2A, B). Caudal sucker ventral, elliptic, CL 2.1 mm, CW 2.6 mm (Figs. 2B, 3C, D).

Somite I completely merged with prostomium (Fig. 3A). Somites II (= peristomium) and III uniannulate (Fig. 3A). Somite IV uniannulate, with obvious secondary furrow, $(a1 + a2) > a3$ (Fig. 3A). Somite V biannulate, $(a1 + a2) = a3$ (Fig. 3A); $a3$ forming posterior margin of oral sucker (Fig. 3B). Somites VI and VII triannulate (Fig. 3A, B); VI, $a1 = a2 = a3$; VII, $a1 = a2 < a3$, $a3$ with slight dorsolateral secondary furrows. Somite VIII quadrannulate, $a1 > a2 = b5 < b6$ ($c11 = c12$ dorsally) (Fig. 3A, B). Somite IX quinquannulate, $a1 > a2 = b5 > c11 = c12$, $a1$ with slight dorsal furrow (Fig. 3A). Somites X–XXV sexannulate, $b1 = b2 < a2 = b5 > c11 = c12$ (Fig. 3C–E). Somite XXVI dorsally quinquannulate, $b1 = b2 < a2 > b5 < b6$, $b6$ with slight dorsal furrow, ventrally quadrannulate, $b1 = b2 < a2 < a3$ ($b5 < b6$); ($b6$) being ventrally last complete annulus (Fig. 3C, D). Somite XXVII biannulate, with slight dorsal furrow (Fig. 3C). Anus on dorsal surface, posterior to somite XXVII; post-anal annulus absent (Fig. 3C).

Male gonopore slightly posterior to middle of XI c11 (Fig. 3E). Female gonopore in somite XIII b1/b2, inconspicuous, located posterior to gastropore. Gonopores separated by $1/2 + 8$ annuli (Fig. 3E).

Anterior ganglionic mass in somite VI a2 and a3. Ganglia VII–IX, XII, XIII, XV–XVII, and XIX–XXIV, of each somite, in a2 (Fig. 4A). Ganglia X, XI, and XIV, of each somite, in a2 and b5 (Fig. 4A). Ganglion XVIII in b2 and a2 (Fig. 4A). Ganglion XXVI in b1. Posterior ganglionic mass in somite

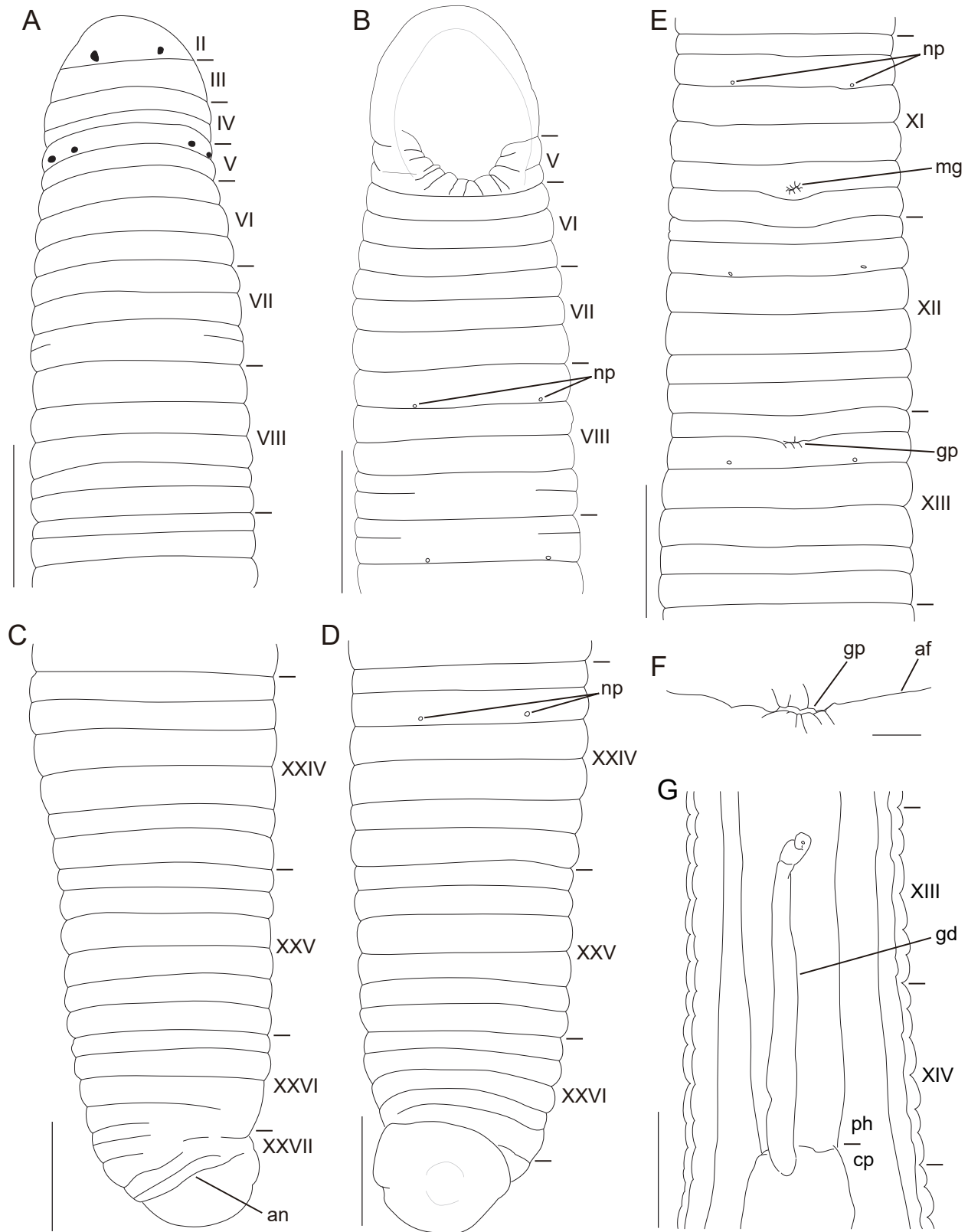


Fig. 3. *Orobiddella imperfecta* sp. nov., holotype (KUZ Z1356). A, dorsal view of somites I–VIII; B, ventral view of somites I–VIII; C, dorsal view of somites XXIV–XXVII and caudal sucker; D, ventral view of somites XXIV–XXVI and caudal sucker; E, ventral view of somites XI–XIII; F, ventral view of gastropore; G, ventral view of gastroporal duct. Scale bars: 2 mm (A–E, G), 0.2 mm (F).

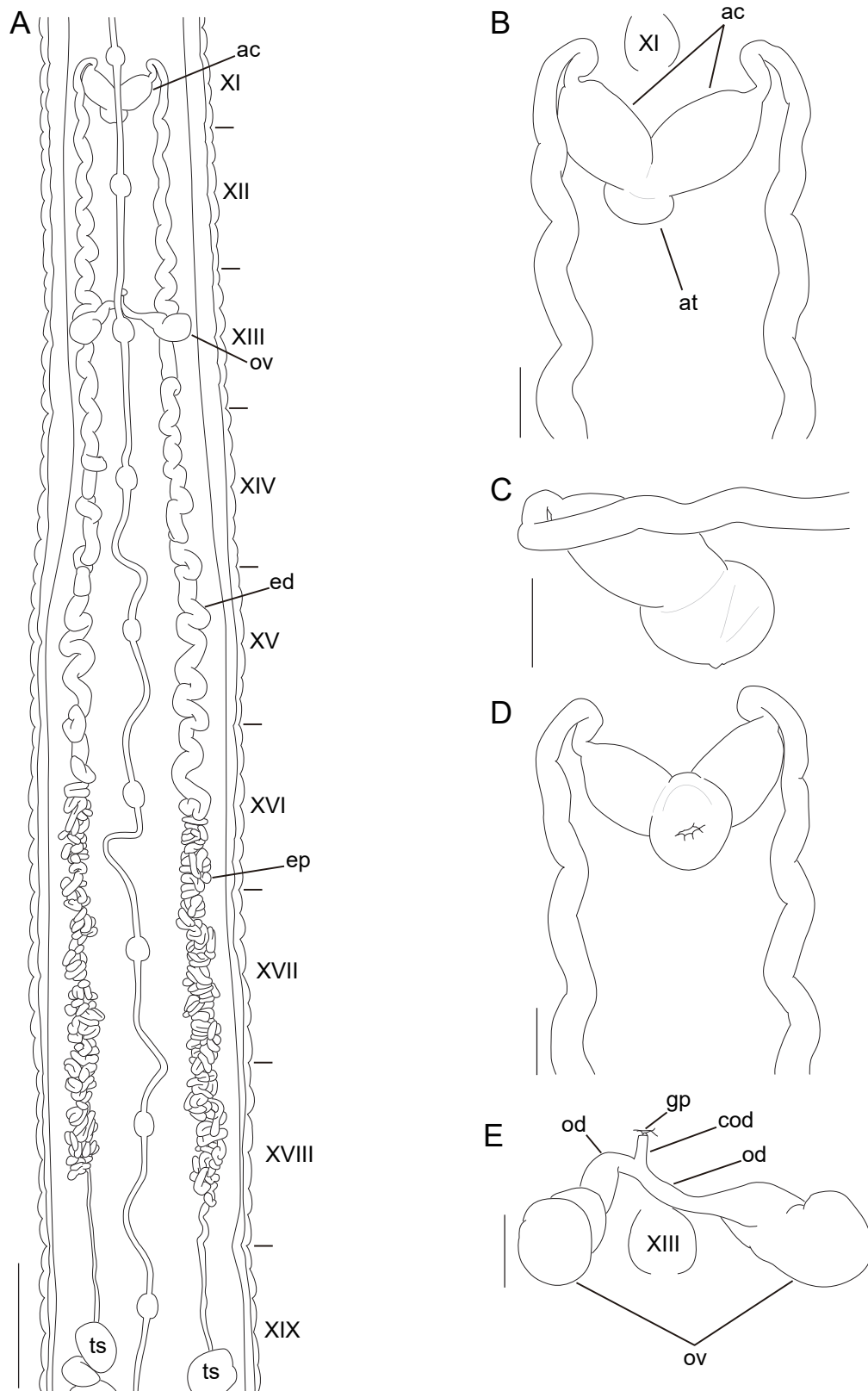


Fig. 4. *Orobdella imperfecta* sp. nov., holotype (KUZ Z1356). A, dorsal view of reproductive system, including ventral nervous system; B, dorsal view of male atrium including position of ganglion XI; C, left lateral view of male atrium; D, ventral view of male atrium; E, dorsal view of female reproductive system, including position of ganglion XIII. Scale bars: 2.5 mm (A), 0.5 mm (B–E).

XXVI a2 and (b5).

Eyespots in 3 pairs, 1st pair dorsally on posterior margin of somite III, 2nd and 3rd pairs dorsolaterally on posterior margin of somite V ($a1 + a2$) (Fig. 3A). Papillae numerous, minute, hardly visible, 1 row on dorsal surface of every annulus.

Nephridiopores in 17 pairs, each situated ventrally at posterior margin of a1 of each somite from VIII to IX, and of b2 of each somite from X to XXIV (Fig. 3B, D, E).

Pharynx agnathous, euthylaematous, reaching to somite XIV c11/c12 (Fig. 3G). Crop tubular, acecate, reaching to somite XXI b2/a2. Intestine tubular, acecate, reaching to somite XXIV b5. Rectum tubular, thin-walled, straight. Gastropore conspicuous, ventral, in somite XIII b1/b2 (Fig. 3E, F). Gastroporal duct tubular, slightly bulbous at junction with gastropore, reaching to somite XIV c12 and connecting with anteriormost part of crop (Fig. 3G).

Testisacs multiple, in somite XIX b5 to XXV c12 (Fig. 4A); on right side, in total ~54 testisacs, 2 in XIX, 7 in XX, 10 in XXI, 10 in XXII, 9 in XXIII, 8 in XXIV, 8 in XXV; on left side, in total ~55 testisacs, 4 in XIX, 9 in XX, 9 in XXI, 8 in XXII, 8 in XXIII, 9 in XXIV, 8 in XXV. Paired epididymides (Fig. 4A); right epididymis in somite XVI b5 to somite XVIII c11, occupying 13 annuli; left epididymis in somite XVI a2 to somite XVIII b5, occupying 14 annuli. Paired ejaculatory ducts, right duct in somite XI a2 to somite XVI a2, left duct in somite XI a2 to XVI b2 (Fig. 4A); coiled in position posterior to ovisacs; each duct crossing ventrally beneath each ovisac, then loosely coiled in position anterior to ovisacs; each winding from respective junction with epididymis, narrowing at junction with atrial cornua, then loosely turning proximally toward atrial cornua, with pre-atrial loop extending to posterior of somite XI a2. Pair of muscular atrial cornua developed, ovate, in somite XI b5 and c11 (Fig. 4A–D). Atrium short, muscular, globular in somite XI c11 and c12 (Fig. 4B–D).

Paired ovisacs globular, in somite XIII a2 and b5, left oviduct crossing ventrally beneath nerve cord; both oviducts converging into common oviduct in somite XIII b2/a2 (Fig. 4A, E). Common oviduct thin-walled, short, directly descending to female gonopore (Fig. 4A, E).

Variation

Measurements (mean $\pm$ 1SD, followed by ranges in parentheses; $n = 3$, including holotype): BL 58.2 ± 15.1 mm (41.0–69.0 mm), BW 3.9 ± 0.7 mm (3.1–4.5 mm), CL 1.8 ± 0.3 mm (1.4–2.1 mm), CW 2.2 ± 0.5 mm (1.6–2.6 mm). Somite IX quadrannulate, $a1 > a2 = b5 < b6$ ($c11 = c12$). Somites

X and XI quinquannulate, $a1 > a2 = b5 > c11 = c12$ (KUZ Z1542), or quadrannulate, $a1 > a2 = b5 < b6$ ($c11 = c12$) (KUZ Z5215). Somites XII–XIV dorsally sexannulate, $b1 = b2 < a2 = b5 > c11 = c12$, ventrally quinquannulate, $a1$ ($b1 = b2$) $< a2 = b5 > c11 = c12$ (KUZ Z1542), or quinquannulate, $a1$ ($b1 = b2$) $< a2 = b5 > c11 = c12$ (KUZ Z5215). Somite XXV quinquannulate, $a1$ ($b1 = b2$) $> a2 = b5 > c11 = c12$ (KUZ Z5215). Somite XXVI dorsally quadrannulate, $b1 = b2 < a2 < a3$, ventrally triannulate, $a1$ ($b1 = b2$) $> a2 < a3$ (KUZ Z1542), or quadrannulate, $a1$ ($b1 = b2$ dorsally) $> a2 = b5 < b6$. Somite XXVII uni- or biannulate. Male gonopore in posterior margin of somite XI c11. Gonopores separated by 1–1.5 annuli + somite XII + 1 annulus. Eyespots in 3 pairs, 1st pair dorsally on anterior margin of somite III (KUZ Z1542), or on somite II/III (KUZ Z5215). Pharynx reaching to somite XIV c11/c12–XV b1/b2. Crop reaching to somites XX/XXI. Intestine reaching to somite XXIV a2/b5–b5/c11. Gastroporal duct tubular, reaching to somite XIV c12–XV b1/b2. Epididymides in somite XVII a2–b5 to somite XX b2/a2–a2, occupying 18 annuli (KUZ Z5215). Paired ejaculatory ducts in somite XI b5 to somite XVII a2–b5; straight in position anterior to ovisacs; pre-atrial loop absent (KUZ Z5215). Paired ovisacs in somite XIII a2. Right or left oviduct crossing ventrally beneath nerve cord.

Coloration

In life, dorsal surface grayish blue (Fig. 2C); ventral surface whitish blue. Color faded in preservative.

Etymology

The specific name is a Latin adjective referring to the incomplete sexannulation in anterior mid-body somites (somites VIII–XIV) of this species.

Distribution and natural history

This species was collected from montane habitats in the central part of Kyushu, the known localities are located at Kyushu Mountains. Because one specimen, KUZ Z1542, with body length reaching up to 41.0 mm, did not bear developed male genital organs, this individual is likely immature. Body lengths of mature individuals seem to be longer than 50 mm, and may reach at least approximately 70 mm.

Molecular phylogenetic position and genetic distances

The ML (not shown) and BI (Fig. 5) trees had almost identical topologies, and were concordant with those of previous analyses (*e.g.*, Nakano and Prozorova, 2024). Two

Table 2. Uncorrected *p*-distances for the COI sequences of the sexannulate *Orobrella imperfecta* sp. nov. and *O. kirishimayamensis* sp. nov. from Kyushu, Japan. COI lengths: KUZ Z1542, 1224 bp; all other specimens, 1267 bp. Acronym: KUZ, Zoological Collection of Kyoto University.

Species	Voucher	(1)	(2)	(3)	(4)	(5)
(1) <i>O. imperfecta</i> sp. nov.	KUZ Z1356 (holotype)					
(2) <i>O. imperfecta</i> sp. nov.	KUZ Z1542 (paratype)	3.9%				
(3) <i>O. imperfecta</i> sp. nov.	KUZ Z5215	6.4%	6.5%			
(4) <i>O. kirishimayamensis</i> sp. nov.	KUZ Z1354 (holotype)	8.9%	8.9%	8.4%		
(5) <i>O. kirishimayamensis</i> sp. nov.	KUZ Z1543	8.4%	8.7%	8.6%	3.7%	
(6) <i>O. kirishimayamensis</i> sp. nov.	KUZ Z5216	7.7%	8.2%	7.7%	4.8%	4.1%

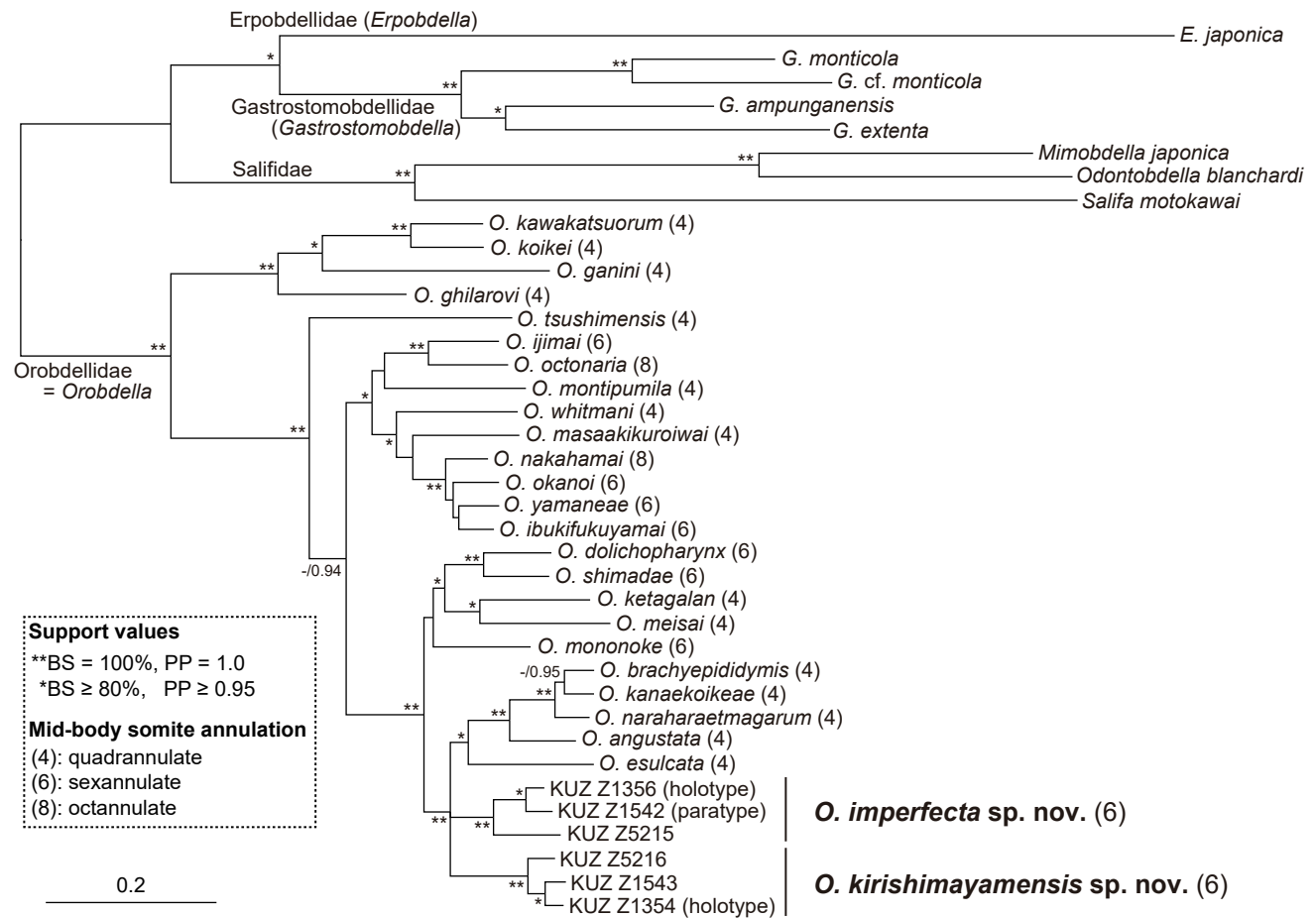


Fig. 5. Bayesian inference tree for 8099 bp of nuclear 18S rRNA, 28S rRNA, and H3, and mitochondrial COI, tRNA^{Cys}, tRNA^{Met}, 12S rRNA, tRNA^{Val} and 16S rRNA, tRNA^{Leu} and ND1 markers. Numbers on nodes indicate bootstrap (BS) values for maximum likelihood ≥ 70% and Bayesian posterior probabilities (PP) ≥ 0.90. Double asterisks denote nodes with BS = 100%, PP = 1.0; single asterisks denote nodes with BS ≥ 80%, PP ≥ 0.95. Numbers in parentheses represent the mid-body somite annulation of each species.

new sexannulate species from Kyushu, *O. imperfecta* and *O. kirishimayamensis*, formed a monophyletic lineage (BS = 100%, PP = 1.0) with five quadrannulate congeners: *O. esulcata* Nakano, 2010, known from northern to central Kyushu; *O. angustata* Nakano, 2018, endemic to the Kii Peninsula; *O. naraharaetmagarum* Nakano, 2016, known from the Chugoku Mountains in western Honshu; and *O. kanaekoikeae* Nakano,

2017 and *O. brachyepididymis* Nakano, 2016, endemic to Shikoku. Monophyly of the five quadrannulate species was well supported (BS = 84%, PP = 0.99), but nonetheless, the present analyses failed to reveal the relationships among the quadrannulate clade, *O. imperfecta* sp. nov., and *O. kirishimayamensis* sp. nov. Monophyly of the specimens of *O. imperfecta* sp. nov. was well supported (BS = 100%, PP = 1.0), and two

specimens from Kumamoto Prefecture (holotype and paratype) formed a monophyletic lineage (BS = 99%, PP = 1.0).

The pairwise COI uncorrected *p*-distances (Table 2) among the specimens of *O. imperfecta* sp. nov. were 3.9%–6.5%; the COI genetic divergences between the two specimens from Kumamoto (holotype, KUZ Z1356; paratype, KUZ Z1542) and the specimen from Miyazaki (KUZ Z5215) were 6.4% or 6.5%.

Remarks

Orobdella imperfecta sp. nov. differs from *O. kirishimayamensis* sp. nov. and the other seven sexannulate congeners in the following combination of characteristics (see also Nakano, 2022; Table 3): dorsal surface grayish blue in life, triannulate somite VII, quadrannulate somite VIII, quinquannulate somite IX, 1/2 + 8 annuli between gonopores, pharynx reaching to somite XIV, tubular gastroporal duct, epididymides in somites XVI–XX that occupy 13–18 annuli, pre-atrial loop present, and developed ovate atrial cornua. In those features, incomplete sexannulation of anterior somites in the new species, i.e., quinquannulate somite IX (sometimes somites IX–XIV), is a unique feature among the sexannulate *Orobdella* species. *Orobdella imperfecta* sp. nov. can be clearly distinguished from the quadrannulate and octannulate species of *Orobdella* by its sexannulate mid-body somites.

The calculated COI distance between the type specimens from Kumamoto (= western populations) and the specimen from Miyazaki (= an eastern population) was approximately 6.5%. This value is higher than the known interspecific variations calculated among the three closely related sexannulate species *O. ibukifukuyamai*, *O. yamaneae*, and *O. okanoi* (4.4%–6.0%; Nakano, 2022). Those three species can be differentiated from each other on the basis of morphological features (see Table 3). However, the specimen from Miyazaki was not clearly distinguishable from the type specimens of *O. imperfecta* sp. nov. using morphological characteristics. Thus, I have concluded that the Miyazaki specimen tentatively belongs to a new species. It is noteworthy that the Miyazaki specimen bears epididymides that occupy 18 annuli (vs. around 14 annuli in the holotype of *O. imperfecta* sp. nov.). Future taxonomic studies based on additional specimens of sexannulate *Orobdella* from the Kyushu Mountains should elucidate the taxonomic account of the eastern population(s) of *O. imperfecta* sp. nov.

Orobdella kirishimayamensis sp. nov.

(Figs. 6–8)

Material examined

Holotype: near Kirishimahigashi Jinja Shrine, Kamumata, Takaharu, Nishimurokata-gun, Miyazaki Prefecture, Japan (31.89083°N, 130.96388°E), elev. 400 m, 3 December 2010, Takafumi Nakano leg., KUZ Z1354.

Paratype: same data as for holotype (31.89111°N, 130.96417°E), elev. 390 m, KUZ Z1355.

Additional material (two specimens): Inohaekikoku Valley, Kitagocho-gonohara, Nichinan, Miyazaki Prefecture, Japan (31.72833°N, 131.36806°E), elev. 108 m, 17 September 2013, Yoshiko Yamane leg., KUZ Z1543; Mt. Eboshidake, Aira, Kagoshima Prefecture, Japan (31.86652°N, 130.59882°E), elev. 335 m, 2 August 2022, Taro Jonishi leg., KUZ Z5216. Three specimens, KUZ Z1354, Z1543, Z5216, were dissected; testisacs in KUZ Z1543 undeveloped.

Diagnosis

Body length of mature individuals reaching ~5 cm. Somite VII triannulate, somite VIII quinquannulate, somites IX–XXV sexannulate, $b1 = b2 = a2 = b5 = c11 = c12$ (especially mid-body somites). Male gonopore in middle of somite XI $c12$, female gonopore in somite XIII $b1/b2$, posterior to gastropore, gonopores separated by 1/2 + 7 annuli. Pharynx reaching to somite XIV $c11$ – $c12$. Gastropore conspicuous, in somite XIII $b1/b2$. Gastroporal duct tubular. Paired epididymides in somites XV–XIX, occupying 12–15 annuli. Atrial cornua developed, ellipsoid, without pre-atrial loop. Dorsal surface brownish in life.

Description of the holotype

Body firm and muscular, elongated, with constant width in posterior direction, dorsoventrally compressed; BL 46.4 mm, BW 3.2 mm (Fig. 6A, B). Caudal sucker ventral, elliptic, CL 1.6 mm, CW 1.9 mm (Figs. 6B, 7C, D).

Somite I completely merged with prostomium (Fig. 7A). Somites II (= peristomium) and III uniannulate, with slight dorsal furrow (Fig. 7A). Somite IV biannulate, $(a1 + a2) > a3$ (Fig. 7A). Somite V biannulate, $(a1 + a2) = a3$ (Fig. 7A); $a3$ forming posterior margin of oral sucker (Fig. 7B). Somites VI and VII triannulate (Fig. 7A, B); VI, $a1 = a2 = a3$; VII, $a1 = a2 < a3$, $a3$ with slight dorsal furrow. Somite VIII quinquannulate, $a1$ ($b1 = b2$ dorsally) $> a2 = b5 > c11 = c12$ (Fig. 7A, B). Somite IX–XX sexannulate (Fig. 7C–E); IX–XIV and

Table 3. Comparison of morphological characters between *Orobodella imperfecta* sp. nov., *O. kirishimayamensis* sp. nov., and seven sexannulate congeners.

Species	Dorsal color	Somite VII	Somite VIII	Somite IX	Annuli between gonopores	Pharynx length	Gastroporal duct	Epididymides	Pre-atrial loop	Atrial cornua
<i>O. imperfecta</i> sp. nov.	grayish blue	3	4	5	1/2 + 8	to XIV	tubular	XVI to XX (13–18 annuli)	to posterior of XI a2	ovate
<i>O. kirishimayamensis</i> sp. nov.	brownish yellow	3	5 (6)	6	1/2 + 7	to XIV	thick tubular	XV to XIX (12–15 annuli)	absent	ellipsoid
<i>O. dolichopharynx</i>	brownish	4	5	6	8	to XVI	rudimentary tubular, reaching to XVI	absent	to ganglion XI	absent
<i>O. ibukifukuyamai</i>	reddish brown	5	6	6	1/2 + 8	to XIV	thick tubular	XVI to XVIII (11–12 annuli)	absent	ovate
<i>O. iijimai</i>	yellowish green	4	6	6	1/2 + 7 + 1/2	to XIV	bulbous	XVI to XIX (12–17 annuli)	absent	ellipsoid
<i>O. okanoi</i>	reddish	5	6	6	8 + 1/2	to XIV	bulbous	XV to XVII (8–11 annuli)	absent	ellipsoid
<i>O. mononoke</i>	anterior and posterior parts grayish purple, mid-body amber	4	6	6	8 + 1/2	to XIV	tubular, but bulbous at junction with crop	XV to XIX (20–22 annuli)	absent	ovate
<i>O. shimadae</i>	yellowish green	3	5	6	9	to XVI	rudimentary tubular, reaching to XV	absent	to ganglion XI	absent
<i>O. yamaneae</i>	purplish	5	6	6	1/2 + 7 + 1/2	to XIV	bulbous	XVI to XVIII (8–11 annuli)	to anterior of XI c9	ovate



Fig. 6. *Orobdella kirishimayamensis* sp. nov., holotype (A, B: KUZ Z1354), and from Mt. Eboshidake, Kagoshima (C: KUZ Z5216). A, dorsal view; B, ventral view; C, dorsal view of live animal. Scale bars: 5 mm (same bar for A, B).

XXI–XXV, $b1 = b2 < a2 = b5 > c11 = c12$; XV–XX, $b1 = b2 = a2 = b5 = c11 = c12$. Somite XXVI quinquannulate, $b1 = b2 = a2 = b5 > b6$; $b6$ being ventrally last complete annulus (Fig. 7C, D). Somite XXVII biannulate, with slight dorsal furrow (Fig. 7C). Anus on dorsal surface, posterior to somite XXVII; post-anal annulus absent (Fig. 7C).

Male gonopore in middle of XI c12 (Fig. 7E). Female gonopore in somite XIII b1/b2, inconspicuous, located posterior to gastropore. Gonopores separated by $1/2 + 7$ annuli (Fig. 7E).

Anterior ganglionic mass in somite VI a2 and a3. Ganglia VII–IX, XI, XVI and XVII, of each somite, in a2 (Fig. 8A). Ganglia X and XII–XV, of each somite, in a2 and b5 (Fig. 8A). Ganglia XVIII–XXIV, of each somite, in b2 and a2 (Fig. 8A). Ganglion XXV in b2. Ganglion XXVI in somite XXV c11 and c12. Posterior ganglionic mass in somite XXVI b2–b5.

Eyespots in 3 pairs (Fig. 7A), 1st pair dorsally on somite II/III, 2nd and 3rd pairs dorsolaterally on posterior margin of somite V (a1 + a2). Papillae numerous, minute, hardly visible, 1 row on dorsal surface of every annulus.

Nephridiopores in 17 pairs, each situated ventrally at posterior margin of a1 of somite VIII, and of b2 of each somite from IX to XXIV (Fig. 7B, D, E).

Pharynx agnathous, euthylaematous, reaching to somite XIV c11/c12 (Fig. 7G). Crop tubular, acecate, reaching to somite XXI a2. Intestine tubular, acecate, reaching to somite XXIV a2/b5. Rectum tubular, thin-walled, straight. Gastropore conspicuous, ventral, in somite XIII b1/b2 (Fig. 7E, F). Gastroporal duct thick tubular, slightly bulbous at junction with gastropore, reaching to somite XV b1 and connecting with anteriormost part of crop (Fig. 7G).

Testisacs multiple, in somite XVIII c11 to XXV b2 (Fig. 8A); on right side, in total ~46 testisacs, 2 in XVIII, 6 in XIX, 7 in XX, 7 in XXI, 6 in XXII, 8 in XXIII, 8 in XXIV, 2 in XXV; on left side, in total ~47 testisacs, 2 in XVIII, 6 in

XIX, 7 in XX, 8 in XXI, 7 in XXII, 8 in XXIII, 7 in XXIV, 2 in XXV. Paired epididymides in somite XVI b5/c11 to somite XVIII b5 (Fig. 8A), occupying 12 annuli. Paired ejaculatory ducts, right duct in somite XI b5 to somite XVI b5/c11 (Fig. 8A); coiled in position posterior to ovisacs; each duct crossing ventrally beneath each ovisac, then also coiled in position anterior to ovisacs; each winding from respective junction with epididymis, narrowing at junction with atrial cornua, then sharply turning proximally toward atrial cornua without pre-atrial loop. Pair of muscular atrial cornua developed, ellipsoid, in somite XI b5–c12 (Fig. 8A–D). Atrium short, muscular, globular in somite XI c11 and c12 (Fig. 8B–D).

Paired ovisacs globular, in somite XIII a2 and b5, left oviduct crossing ventrally beneath nerve cord; both oviducts converging into common oviduct in somite XIII b2/a2 (Fig. 8A, E). Common oviduct thin-walled, short, directly descending to female gonopore (Fig. 8A, E).

Variation

Measurements (mean $\pm$ 1SD, followed by ranges in parentheses; $n = 4$, including holotype): BL 58.4 ± 15.1 mm (46.4–80.4 mm), BW 3.6 ± 0.4 mm (3.2–4.0 mm), CL 1.9 ± 0.3 mm (1.6–2.3 mm), CW 2.3 ± 0.4 mm (1.9–2.9 mm). Somite IV uniannulate, sometimes with slight dorsal furrow. Somite VIII quadrannulate, a1 ($b2 = b2$ dorsally) $> a2 = b5 < b6$ ($c11 = c12$) (KUZ Z5216). Somite IX quinquannulate, a1 ($b1 = b2$) $> a2 = b5 > c11 = c12$ (KUZ Z5216). Somites XXI–XXV sexannulate, $b1 = b2 = a2 = b5 = c11 = c12$. Somite XXVII uni- or biannulate. Female gonopore in anterior margin of somite XIII b2 (KUZ Z1355). Pharynx reaching to somite XIV c11–c12. Crop reaching to somite XXI a2/b5–c11. Intestine reaching to somite XXIV c12. Gastroporal duct, reaching to somite XIV c11. Epididymides in somite XV c11/c12–somite XVI c12 to somite XVII/XX–somite XIX b1/b2, occupying 13–15 annuli. Paired ejaculatory ducts in somite XI b5–c11 to somite XV c11/c12–somite XVI c12; loosely coiled

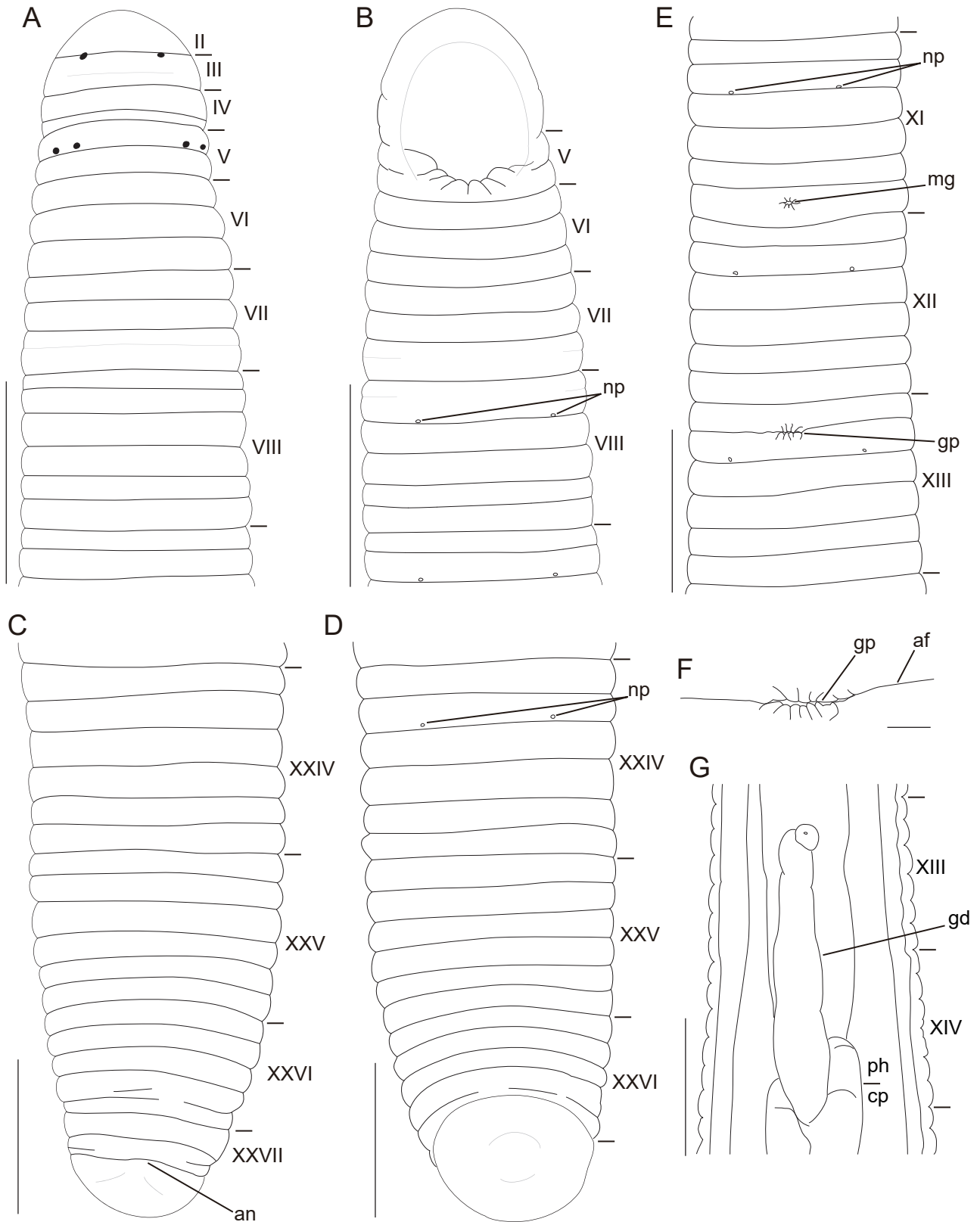


Fig. 7. *Orobdella kirishimayamensis* sp. nov., holotype (KUZ Z1354). A, dorsal view of somites I–VIII; B, ventral view of somites I–VIII; C, dorsal view of somites XXIV–XXVII and caudal sucker; D, ventral view of somites XXIV–XXVI and caudal sucker; E, ventral view of somites XI–XIII; F, ventral view of gastropore; G, ventral view of gastroporal duct. Scale bars: 2 mm (A–E, G), 0.2 mm (F).

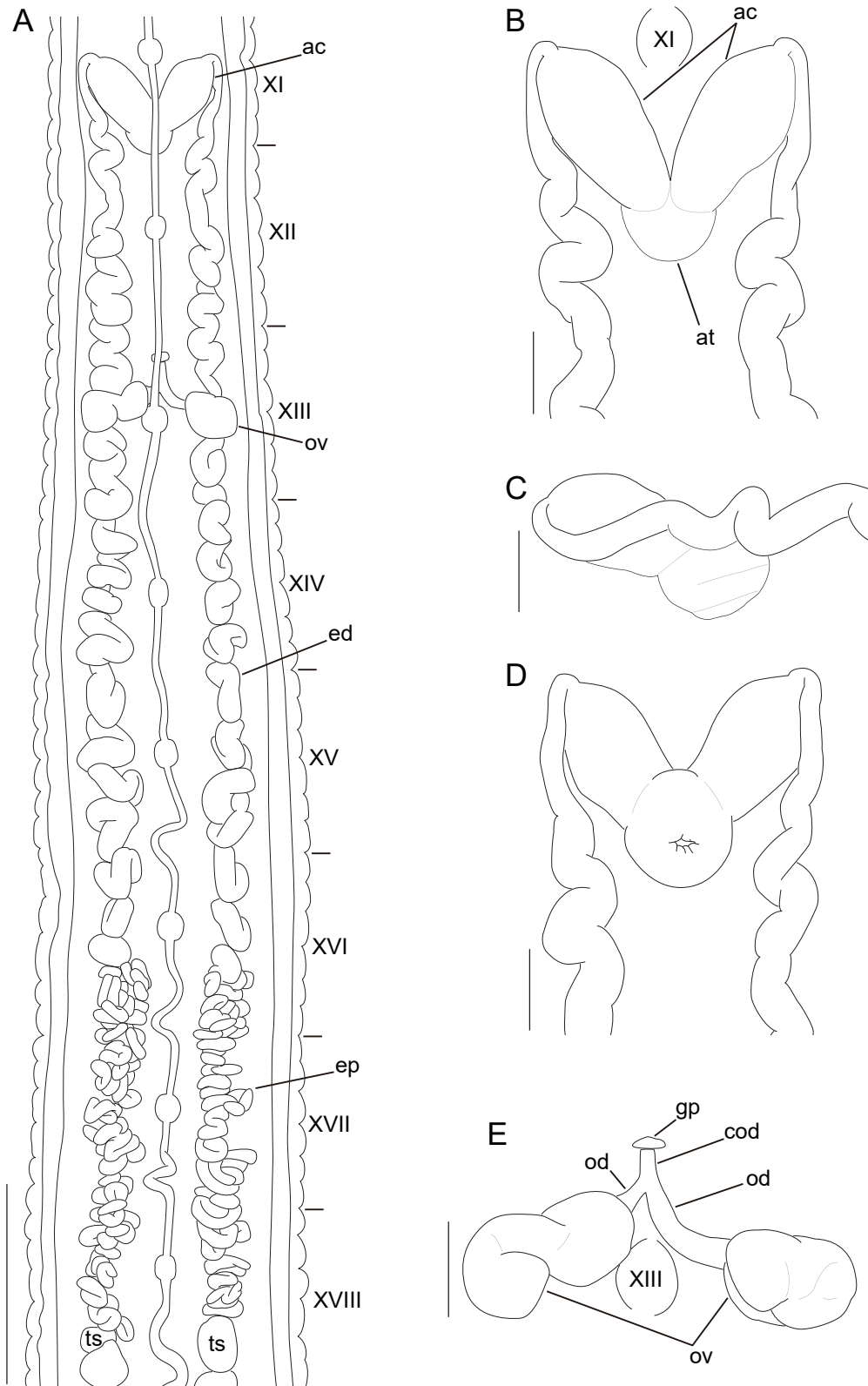


Fig. 8. *Orobdella kirishimayamensis* sp. nov., holotype (KUZ Z1354). A, dorsal view of reproductive system, including ventral nervous system; B, dorsal view of male atrium including position of ganglion XI; C, left lateral view of male atrium; D, ventral view of male atrium; E, dorsal view of female reproductive system, including position of ganglion XIII. Scale bars: 2.5 mm (A), 0.5 mm (B–E).

in position anterior to ovisacs. Paired ovisacs in somite XIII b2–c11. Right oviduct crossing ventrally beneath nerve cord.

Coloration

In life, dorsal surface brownish yellow (Fig. 6C); ventral surface whitish yellow. Color faded in preservative.

Etymology

The specific name is from the Kirishima Mountains (= Kirishima-yama), where the type locality of this species is located.

Distribution and natural history

This species was collected from montane habitats in the southern part of Kyushu. Although the holotype's body length does not reach 5 cm, it possesses developed male and female genital organs. Therefore, the holotype individual seems to be almost mature.

Molecular phylogenetic position and genetic distances

Three specimens of *O. kirishimayamensis* sp. nov. formed a well-supported clade (BS = 100%, PP = 1.0; Fig. 5). Monophyly of the holotype and the specimen (KUZ Z1543)—both specimens were collected from Miyazaki—was also supported (BS = 98%, PP = 1.0). The pairwise COI uncorrected *p*-distances (Table 2) among the specimens of *O. kirishimayamensis* sp. nov. were 3.7%–4.8%.

Remarks

Orobdella kirishimayamensis sp. nov. is clearly distinguishable from the other eight sexannulate congeners, including *O. imperfecta* sp. nov., by the following combination of the characters (see Remarks of *O. imperfecta* sp. nov. and Table 3): dorsal surface brownish yellow in life, triannulate somite VII, quinquannulate somite VIII, sexannulate somite IX, 1/2 + 7 annuli between gonopores, pharynx reaching to somite XIV, thick tubular gastroporal duct, epididymides in somites XV–XIX that occupy 12–15 annuli, and developed ellipsoid atrial cornua without pre-atrial loop. *Orobdella kirishimayamensis* sp. nov. is unquestionably distinguishable from the quadrannulate and octannulate congeners by its sexannulate mid-body somites.

The highest value of the calculated intraspecific COI distances (4.8%, in the range of 3.7%–4.8%) was almost concordant with the interspecific values between the sexannulate *O. ibukifukuyamai* and each of *O. yamaneae* and *O. okanoi*

(4.6%–4.9%, mean = 4.7%, between *O. ibukifukuyamai* and *O. yamaneae*; 4.4%–5.0%, mean = 4.7%, between *O. ibukifukuyamai* and *O. okanoi*; see Nakano, 2022). However, the present specimens identified as *O. kirishimayamensis* sp. nov. exhibited similar morphological features. Therefore, the detected COI divergences are herein deemed to be intraspecific variations within the new species.

Acknowledgments

The author is grateful to Dr. Ibuki Fukuyama (Hokkaido University), Taro Jonishi (Kyoto University; KU) and Yoshiko Yamane (KU) for providing valuable specimens of the new species. I also thank two anonymous reviewers and Dr. Taizo Nakamori (Yokohama National University) for their valuable suggestions on this manuscript, and Edanz (<https://jp.edanz.com/>) for editing a draft of this manuscript. A part of traditional sequencing was performed at the Medical Research Support Center, Graduate School of Medicine, KU. This study was supported by JSPS KAKENHI Grant Numbers JP13J00450, JP26840127, JP15J00720, JP18K14780, and JP22K06371, and by MEXT (Japan) grants for Biodiversity and Evolutionary Research of Global COE (A06) and for Excellent Graduate Schools to Kyoto University.

References

- Chernomor, O., von Haeseler, A. and Minh, B. Q., 2016. Terrace aware data structure for phylogenomic inference from supermatrices. *Systematic Biology*, 65: 997–1008.
- Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K. F., von Haeseler, A. and Jermini, L. S., 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods*, 14: 587–589.
- Katoh, K. and Standley, D. M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*, 30: 772–780.
- Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., von Haeseler, A. and Lanfear, R., 2020. IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution*, 37: 1530–1534.
- Moore, J. P., 1927. The segmentation (metamerism and annulation) of the Hirudinea. pp. 1–12, *In*: The Fauna of British

- India, Including Ceylon and Burma. Hirudinea (Harding, W. A. and Moore, J. P.), Taylor & Francis, London.
- Nakano, T., 2017. Diversity of leeches from Japan: recent progress in macrophagous and blood-feeding taxa. pp. 319–340, *In*: Species Diversity of Animals in Japan (eds. Motokawa, M. and Kajihara, H.), Springer Japan, Tokyo.
- Nakano, T., 2022. A new sexannulate species of *Orobdella* (Hirudinea, Arhynchobdellida, Orobdellidae) from Kii-Oshima Island, Japan. *Evolutionary Systematics*, 6: 135–142.
- Nakano, T. and Lai, Y.-T., 2016. First record of *Poecilobdella nanjingensis* (Hirudinida: Arhynchobdellida: Hirudinidae) from Taiwan and its molecular phylogenetic position within the family. *Species Diversity*, 21: 127–134.
- Nakano, T. and Lai, Y.-T., 2017. A new quadrannulate species of *Orobdella* (Hirudinida: Arhynchobdellida: Orobdellidae) from Pingtung, Taiwan. *Species Diversity*, 22: 143–150.
- Nakano, T. and Prozorova, L. A., 2024. Terrestrial predatory leeches of the genus *Orobdella* (Hirudinea: Erpobdelliformes: Orobdellidae) endemic to the Southern Russian Far East: a new species of the genus from Primorsky Krai, Russia. *Zootaxa*, 5424: 44–60.
- Oka, A., 1895. On some new Japanese land leeches. (*Orobdella* nov. gen.). *Journal of the College of Science, Imperial University, Japan*, 8: 275–306.
- Rambaut, A., Drummond, A. J., Xie, D., Baele, G. and Suchard, M. A., 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, 67: 901–904.
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A. and Huelsenbeck, J. P., 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61: 539–542.
- Tamura, K., Stecher, G. and Kumar, S., 2021. MEGA11: Molecular Evolutionary Genetics Analysis version 11. *Molecular Biology and Evolution*, 38: 3022–3027.
- Wessel, P. and Smith, W. H. F., 1996. A global, self-consistent, hierarchical, high-resolution shoreline database. *Journal of Geophysical Research: Solid Earth*, 101: 8741–8743.