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A New Small-Sized Species of *Orobdella* (Hirudinea: Erpobdelliformes: Orobdellidae) from Mount Hehuan, Central Taiwan

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A small-type quadrannulate leech species of *Orobdella* Oka, 1895, *O. minima* sp. nov., from Mt. Hehuan in central Taiwan is described. Phylogenetic analyses 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 revealed that *O. minima* forms a clade with the middle-type quadrannulate *O. ketagalan* Nakano and Lai, 2012, which is endemic to northern Taiwan. This clade was sister to the other Taiwanese middle-type quadrannulate *O. meisai* Nakano and Lai, 2017. Based on the phylogenetic relationships among the Taiwanese *Orobdella*, their morphological characteristics are briefly discussed in a phylogenetic context. A key to the three species of *Orobdella* endemic to Taiwan is also provided.

Key Words: Arhynchobdellida, leech, molecular phylogeny, soil fauna.

Introduction

The genus *Orobdella* Oka, 1895 consists of 26 species that are terrestrial predatory leeches distributed in East Asia (Sawyer 1986; Nakano 2017a, 2025). These species can be categorized into three morphological groups according to their mid-body somite annulation, i.e., quadr- (four-; 15 species), sex- (six-; nine species), and octannulate (eight-annulate; two species). In addition, they are grouped into small- (~5 cm; six quadrannulate species), middle- (~10 cm; nine quadrannulate and nine sexannulate species), and large-types (~20 cm; two octannulate species) based on the body lengths of mature leeches (Nakano 2017a, 2025). All sexannulate and octannulate species, as well as 10 quadrannulate species have been recorded in the Japanese Archipelago (Nakano 2025). Among the remaining five quadrannulate species, two occur in the Russian Far East (Nakano and Prozorova 2024), one inhabits the Korean Peninsula and adjacent islands including Tsushima Island (Nakano and Seo 2014), and two are known from Taiwan (Nakano and Lai 2017). Furthermore, all six small-type quadrannulate species appear to be restricted to the Japanese Archipelago (Nakano 2021).

Our better understanding of the species richness of Taiwanese *Orobdella* has been still hampered by the presence of several unidentified species in the island (see Nakano and Lai 2012). During recent surveys on Mt. Hehuan in the Central Mountain Range of Taiwan, small leeches of *Orobdella* were collected from the mountain. Based on morphological examination, they are described as a new small-type quadrannulate species of *Orobdella* herein. Its phylogenetic position within the genus was estimated using nuclear and mitochondrial genetic markers.

Materials and Methods

Sampling and morphological examination. Two leeches were collected from Mt. Hehuan in central Taiwan (Fig. 1). Elevation and geographical coordinates of the locations were obtained using a Garmin eTrex® GPS unit (Garmin Ltd., Olathe, KS, USA). Specimen and tissue sample preparation follows Nakano (2011); four body measurements, i.e., body length (BL), maximum body width (BW), caudal sucker length (CL), and caudal sucker width (CW), were taken following Nakano and Seo (2014). Examination, dissection, and drawing of the specimens were performed using a Leica M125 stereoscopic microscope with a drawing tube (Leica Microsystems, Wetzlar, Germany). The specimens examined in this study were deposited in the Zoological Collection of Kyoto University (KUZ), Kyoto, Japan, and in the Zoological Collection of the Biodiversity Research Museum, Academia Sinica (ASIZ), Taiwan.

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.

Molecular phylogenetic analyses. The phylogenetic position of the new species was investigated based on the concatenated dataset of three nuclear markers, i.e., 18S rRNA, 28S rRNA, and histone H3, and three mitochondrial markers, i.e., 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 were performed as previously described by Nakano and Lai (2016, 2017); some of the cycle sequencing products were sequenced on an Applied Biosystems® 3500xL Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA). In total, seven 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 a previous study (Nakano 2025), 204 sequences of 26 *Orobdella* species, and eight other erpobdelliform species, i.e., one erpobdellid, four gastrostomobdellid and three salifid species, 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 because no indels were observed. The other marker 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, 2835, 328, 1271, 1177, and 640 bp, respectively. Our concatenated final dataset had 8100 bp aligned positions. Because the COI sequences of the holotype (KUZ Z1471) and paratype (ASIZW0001115) were completely identical, the sequence of the latter specimen was removed from the dataset.

Phylogenetic trees were reconstructed using maximum likelihood (ML) and Bayesian inference (BI). Based on the 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 best-fit partition scheme and optimal models were identified as listed in Table 2. The ML tree was inferred using IQ-Tree v. 2.2.2.6, with non-parametric bootstrapping (BS) conducted with 1000 replicates. The 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 20 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 the first 100001 trees were discarded (burn-in) based on the results.

Orobdella minima sp. nov.

(Figs 1–4)

Diagnosis. Body length of mature individuals reaching ~3 cm. Somite IV uniannulate, somites VIII–XXV quadrannulate. Male gonopore in middle of somite XI b6, female in middle of somite XIII a1, behind gastropore, gonopores separated by 1/2 + 4 + 1/2 annuli. Pharynx reaching to somite XIII/XIV. Gastropore conspicuous, in middle of somite XIII a1. Gastroporal duct bulbous. Paired sperm duct bulbs, minute, in somite XVIII b6–XIX a1. Paired epididymides in somites XVI–XVIII, occupying 10–11 annuli. Atrial cornua developed, ovate.

Material examined. Holotype: KUZ Z1471, dissected, near High Altitude Experimental Station of Endemic Species Research Institute, Hehuanshan National Forest Recreation Area, Renai Township, Nantou County, Taiwan (24.16203°N, 121.28622°E; elev. 2960 m), by Yi-Te Lai, 9 February 2013. Paratype: ASIZW0001115, dissected, same locality and collector as for holotype (24.16439°N, 121.28571°E; elev. 2900 m), 21 March 2019.

Description. Body firm and muscular, elongate, with constant width in caudal direction, dorsoventrally compressed, BL 31.7 mm, BW 2.8 mm (Fig. 1A, B). Caudal sucker ventral, elliptic, CL 1.2 mm, CW 1.4 mm (Figs 1B, 2D).

Somite I completely merged with prostomium. Somite II (= peristomium), III, IV uniannulate (Fig. 2A). Somite V biannulate, (a1 + a2) = a3; a3 forming posterior margin of oral sucker (Fig. 2A, B). Somites VI and VII triannulate, a1 = a2 = a3 (Fig. 2A, B). Somites VIII–XXV quadrannulate, a1 = a2 = b5 = b6 (Fig. 2A–E). Somite XXVI triannulate, a1 = a2 < a3; a3 being ventrally last complete annulus (Fig. 2C, D). Somite XXVII uniannulate (Fig. 2C). Anus behind somite XXVII; post-anal annulus absent (Fig. 2C).

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

Anterior ganglionic mass in somite VI a2 and a3 and somite VII a1. Ganglion VII in a2 and a3. Ganglia VIII–XV and XIX, of each somite, in a2 and b5 (Fig. 3B). Ganglia XVI–XVIII and XX–XXIII, of each somite, in a2 (Fig. 3B). Ganglion XXIV in a1 and a2. Ganglion XXV in somite XXIV b6 and somite XXV a1. Ganglion XXVI in somite XXV b5. Posterior ganglionic mass in somite XXV b6 and XXVI a1 and a2.

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

Nephridiopores in 17 pairs, each situated ventrally at posterior margin of a1 of each somite in somites VIII–XXIV (Fig. 2B, D, E).

Pharynx agnathous, euthylaematous, reaching to somite XIII/XIV (Fig. 3A). Crop tubular, acecate, reaching to somite XX a1/a2. Intestine tubular, reaching to XXIV a2/b5. Rectum tubular, thin-walled, straight. Gastropore conspicuous, ventral, in middle of somite XIII a2 (Fig. 2E, F). Gastroporal duct thick, bulbous, winding at junction with gastropore, reaching to somite XIV a1 (Fig. 3A).

Testisacs multiple (Fig. 3B); on right side, in somite XIX a2 to somite XXV a1, in total ~22 testisacs, 3 in XIX, 2 in XX, 4 in each somite of XXI–XXIV, 1 in XXV; on left side, in somite XIX b6 to somite XXIV b6, in total ～20 testisacs, 1 in XIX, 4 in each somite of XX–XXIII, 3 in XXIV. Paired sperm duct bulbs minute; on right side, in somite XVIII b6; on left side, in somite XIX a1 (Fig. 3B). Paired epididymides; right epididymis in somite XVI a1 to somite XVIII b5/b6,

Table 1. Samples used for phylogenetic analyses. Voucher information on the specimens 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: ASIZ, Zoological Collection of the Biodiversity Research Museum, Academia Sinica, Taiwan; 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	tRNA$^{\mathrm{Cys}}$–16S	tRNA$^{\mathrm{Leu}}$–ND1
Orobdella							
O. minima sp. nov.	KUZ Z1471 (holotype)	LC868475*	LC868476*	LC868474*	LC868473*	LC868471*	LC868472*
O. minima sp. nov.	ASIZW0001115 (paratype)				LC868477*		
O. angustata Nakano, 2018	KUZ Z1439 (holotype)	LC323140	LC431606	LC323138	LC323139	LC323141	LC323137
O. brachyepididymis Nakano, 2016	KUZ Z1673 (holotype)	LC106319	LC274535	LC106321	LC106320	LC106318	LC106322
O. dolichopharynx Nakano, 2011	KUZ Z120 (holotype)	AB663665	LC274541	AB698876	AB679680	AB679681	AB828558
O. esulcata Nakano, 2010	KUZ Z29 (holotype)	AB663655	LC274538	AB698873	AB679664	AB679665	AB828555
O. ganini Nakano and Prozorova, 2024	KUZ Z5038 (holotype)	LC778511	LC778512	LC778514	LC778513	LC778510	LC778515
O. ghilarovi Nakano and Prozorova, 2019	KUZ Z2068 (holotype)	LC431608	LC431607	LC431612	LC431609	LC431616	LC431613
O. ibukifukuyamai Nakano, 2022	KUZ Z4031 (holotype)	LC687625	LC687626	LC687624	LC687620	LC687618	LC687622
O. ijimai Oka, 1895	KUZ Z110 (topotype)	AB663659	LC274542	AB698877	AB679672	AB679673	AB828559
O. imperfecta Nakano, 2025	KUZ Z1356 (holotype)	LC820567	LC820568	LC820566	LC820565	LC820563	LC820564
O. kanaekoikeae Nakano, 2017	KUZ Z1747 (holotype)	LC184551	LC274533	LC184553	LC184552	LC184550	LC184554
O. kawakatsuorum Richardson, 1975	KUZ Z167 (topotype)	AB663661	LC274544	AB698878	AB679704	AB679705	AB828561
O. ketagalan Nakano and Lai, 2012	KUZ Z208 (holotype)	AB704785	LC274546	AB704786	AB704787	AB828582	AB828563
O. kirishimayamensis Nakano, 2025	KUZ Z1354 (holotype)	LC820561	LC820562	LC820560	LC820559	LC820557	LC820558
O. koikei Nakano, 2012	KUZ Z156 (holotype)	AB698883	LC274543	AB698882	AB679688	AB679689	AB828560
O. masaakikuroiwai Nakano, 2014	KUZ Z694 (holotype)	AB938003	LC274530	AB938013	AB938006	AB937997	AB938016
O. meisai Nakano and Lai, 2017	KUZ Z1917 (holotype)	LC314423	LC431605	LC314425	LC314424	LC314422	LC314426
O. mononoke Nakano, 2012	KUZ Z224 (holotype)	AB698868	LC274547	AB698869	AB698866	AB698867	AB828564
O. montipumila Nakano, 2021	KUZ Z3913 (holotype)	LC616674	LC616673	LC616672	LC616663	LC616667	LC616671
O. nakahamai Nakano, 2016	KUZ Z1672 (holotype)	LC106330	LC274534	LC106332	LC106331	LC106329	LC106333
O. naraharaetmagarum Nakano, 2016	KUZ Z1652 (holotype)	LC087143	LC274531	LC087145	LC087144	LC087142	LC087146
O. octonaria Oka, 1895	KUZ Z181 (topotype)	AB698870	LC274545	AB698871	AB679708	AB679709	AB828562
O. okanoi Nakano, 2016	KUZ Z1671 (holotype)	LC106341	LC274532	LC106343	LC106342	LC106340	LC106344
O. shimadae Nakano, 2011	KUZ Z128 (holotype)	AB663663	LC274540	AB698875	AB679676	AB679677	AB828557
O. tsushimensis Nakano, 2011	KUZ Z134 (holotype)	AB663653	LC274537	AB698872	AB679662	AB679663	AB828554
O. whitmani Oka, 1895	KUZ Z45 (topotype)	AB663657	LC274539	AB698874	AB679668	AB679669	AB828556
O. yamaneae Nakano, 2016	KUZ Z1678 (holotype)	LC106349	LC274536	LC106351	LC106350	LC106348	LC106352
Outgroup							
Erpobdella japonica Pawłowski, 1962	KUZ Z178	AB663648	LC274512	AB698879	AB679654	AB679655	AB828542
Gastrostomobdella ampunganensis Nakano, 2018	ZRC.ANN.0083 (holotype)	LC274517	LC274516	LC274552	LC274551	LC274564	LC274571
G. extenta Nakano and Jeratthitikul, 2018	MUMNH-Hir0001 (holotype)	LC274519	LC274518	LC274554	LC274553	LC274565	LC274572
G. monticola Moore, 1929	UNIMAS/A3/BH01/10	AB663649	LC274515	AB698880	AB679656	AB679657	AB828543
G. cf. *monticola*	KUHE 56264	LC274514	LC274513	LC274550	LC274549	LC274563	LC274570
Mimobdella japonica Blanchard, 1897	KUZ Z179	AB663650	LC274528	AB698881	AB679658	AB679659	AB828544
Odontobdella blanchardi (Oka, 1910)	KUZ Z180	AB663651	LC274529	AB938012	AB938004	AB937995	AB938014
Salifa motokawai Nakano and Nguyen, 2015	VNMN 2015.65 (holotype)	LC029434	LC274548	LC029435	LC029431	LC029432	LC029433

Table 2. The selected partition scheme and optimal models for the phylogenetic analyses in this study.

Partition	Model
18S and H3 1st position	K80 + I
28S and H3 2nd position	GTR + I + G
H3 3rd position	HKY + G
COI and ND1 1st positions	GTR + I + G
COI and ND1 2nd positions	GTR + I + G
COI and ND1 3rd positions and 16S	GTR + I + G
tRNA$^{\mathrm{Cys}}$–tRNA$^{\mathrm{Val}}$* and tRNA$^{\mathrm{Leu}}$	GTR + I + G

* tRNA$^{\mathrm{Cys}}$, tRNA$^{\mathrm{Met}}$, 12S, and tRNA$^{\mathrm{Val}}$.

occupying 11 annuli; left epididymis in somite XVI a1/a2 to somite XVIII/XIX, occupying 11 annuli (Fig. 3B). Paired ejaculatory ducts in somite XI b5 to somite XVI a1; coiled in position posterior to ovisacs; each duct crossing ventrally beneath each ovisac, then coiled in position anterior to ovisacs; each widening from respective junction with epididymis, narrowing at junction with atrial cornua, then sharply turning proximally toward atrial cornua without pre-atrial loop (Fig. 3B). Pair of muscular atrial cornua developed, ovate, in somite XI b5 and b6 (Fig. 3B–E). Atrium short, muscular, globular in somite XI b5 and b6 (Fig. 3C–E).

Paired ovisacs globular, in somite XIII a2 and b5 (Fig. 3B, F). Oviducts thin-walled, left oviduct crossing ventrally be-

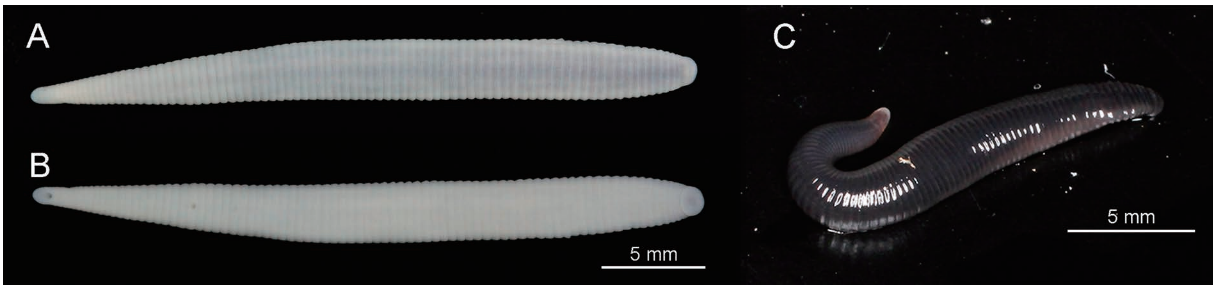

Fig. 1. *Orobdella minima* sp. nov., holotype, KUZ Z1471 (A, B), and paratype, ASIZW0001115 (C). A, Dorsal view of the preserved specimen; B, ventral view of the preserved specimen; C, dorsal view of live animal.

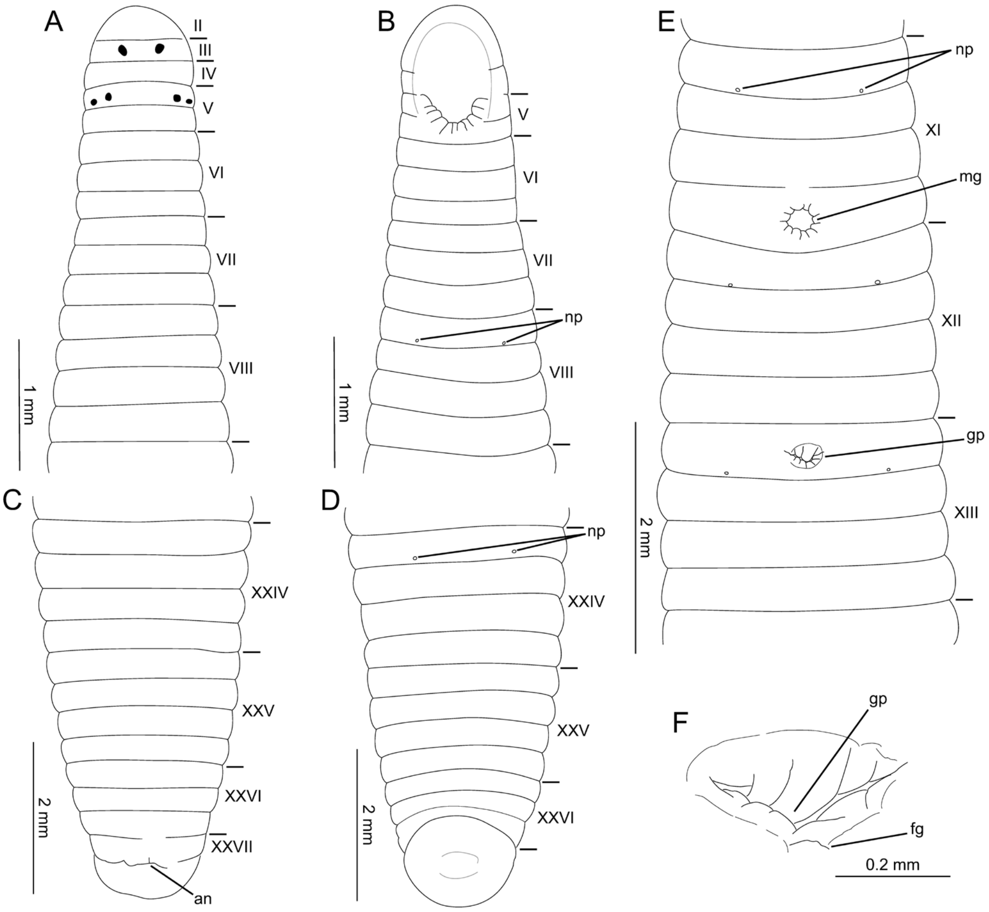

Fig. 2. *Orobdella minima* sp. nov., holotype, KUZ Z1471. A, Dorsal view of somites I–VIII; B, ventral view of somites I–VIII; C, dorsal view of somites XXIV–XXVII and caudal sucker; D, ventral view of somites XXIV–XXVI and caudal sucker; E, ventral view of somites XI–XIII; F, ventral view of gastropore. Abbreviations: an, anus; fg, female gonopore; gp, gastropore; mg, male gonopore; np, nephridiopore.

neath nerve cord (Fig. 3B, F); both oviducts converging into common oviduct in somite XIII a2. Common oviduct thin-walled, short, directly descending to female gonopore (Fig. 3F).

Variation. Measurements (n = 1, paratype only): BL 26.8 mm, BW 2.5 mm, CL 1.0 mm, CW 1.2 mm. Crop reaching to somite XIX/XX. Intestine reaching to somite XXIII a2/b5. Testisacs; on right side, ~14 sacs in somite XIX a2 to somite XXIV b5; on left side, ~12 sacs in somite XIX a2 to somite XXIV b6. Paired sperm duct bulbs (Fig. 4); right bulb in somite XIX a1; left bulb in somite XVIII b6. Paired epididymides; right epididymis in somite XVI b5 to somite XVIII/XIX, occupying 10 annuli; left epididymis in somite XVI a1/a2 to somite XVIII b6, occupying 11 annuli. Paired ejaculatory ducts; right duct in somite XI b5 to somite XVI b5; left duct in somite XI b5 to somite XVI a1. Paired ovisacs in somite XIII a1–b6. Right oviduct crossing ventrally beneath nerve cord.

Coloration. In life, dorsal surface bluish gray (Fig. 1C); ventral surface yellowish or grayish white. Color faded in preservative.

Distribution and natural history. This species was collected only from the high-elevation area of Mt. Hehuan in the Central Mountain Range of Taiwan. Because both speci-

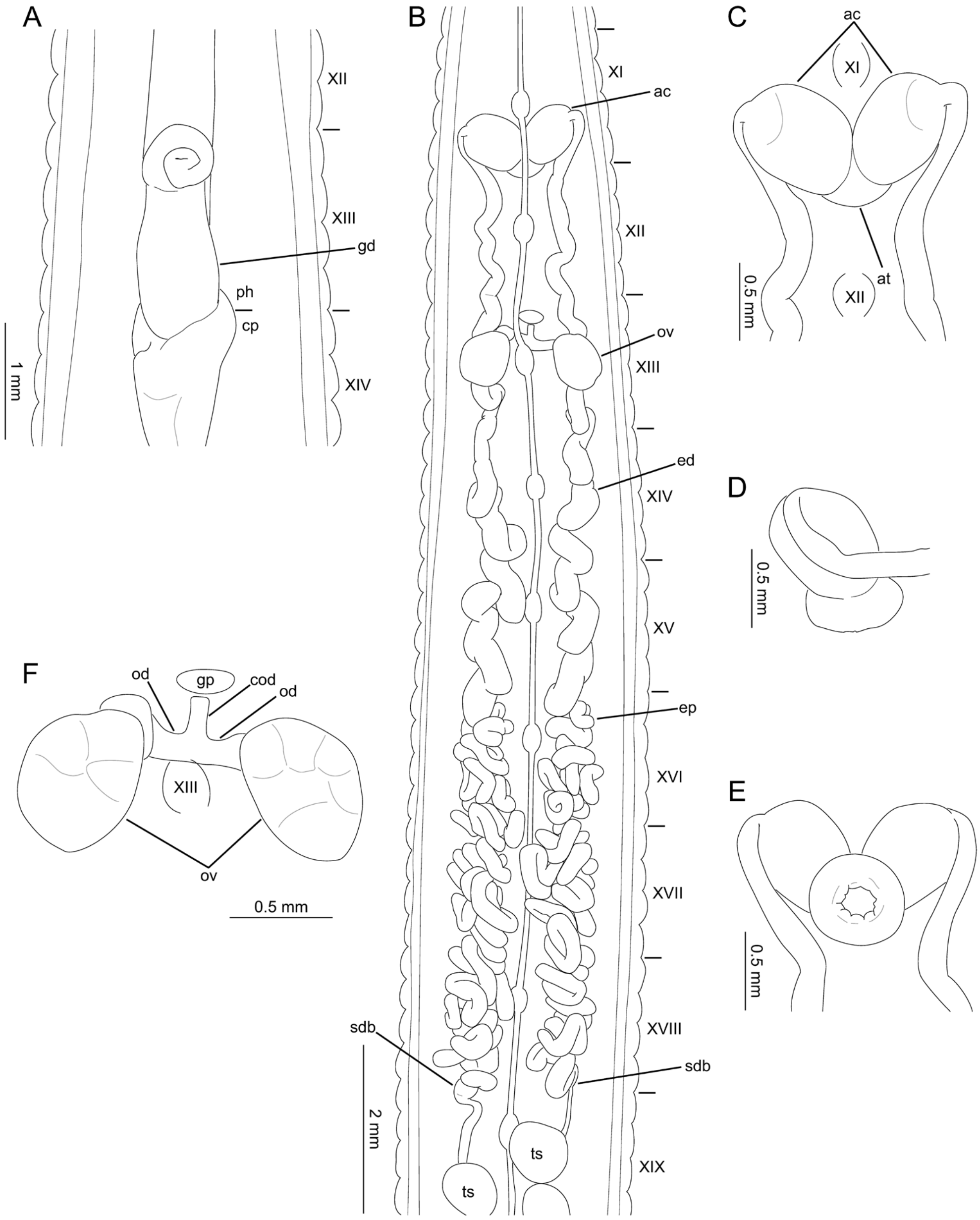

Fig. 3. *Orobdella minima* sp. nov., holotype, KUZ Z1471. A, Ventral view of gastroporal duct; B, dorsal view of reproductive system, including ventral nervous system; C, dorsal view of male atrium including positions of ganglia XI and XII; D, left lateral view of male atrium; E, ventral view of male atrium; F, dorsal view of female reproductive system, including position of ganglion XIII. Abbreviations: ac, atrial cornu; at, atrium; cod, common oviduct; cp, crop; ed, ejaculatory duct; ep, epididymis; gd, gastroporal duct; gp, gastropore; od, oviduct; ov, ovisac; ph, pharynx; sdb, sperm duct bulb; ts, testisacs.

mens possessing fully developed genital organs were collected in February and March, we estimate that the reproductive season of this species begins after March at the type locality.

Molecular phylogenetic position. The ML (not shown) and BI (Fig. 5) trees had almost identical topologies, and were concordant with those of previous analyses (e.g., Nakano 2025). *Orobdella minima* formed a well-supported clade with the Taiwanese *O. ketagalan* Nakano and Lai, 2012 and *O. meisai* Nakano and Lai, 2017 (BS = 99%, PP = 1.0). The monophyly of *O. minima* and *O. ketagalan* was fully supported (BS = 100%, PP = 1.0).

Etymology. The specific name is given based on its small body size, which is likely to be the smallest *Orobdella* species in Taiwan.

Remarks. *Orobdella minima* clearly belongs to the genus *Orobdella* because this new species possesses the generic diagnostic characteristics defined by Nakano (2016). The obtained trees also corroborate that this new species is explicitly classified as a member of this genus. The clitellum was not observed in any of the present specimens of *O. minima*, but nonetheless, they possessed fully developed male and female genital organs. Additionally, the body length of both individuals was up to ~3 cm. Therefore, we concluded that *O. minima* can be determined as a small-type species within *Orobdella*.

Orobdella minima differs from the nine sexannulate and two octannulate species by its mid-body somites, which are quadrannulate. Except for *O. ketagalan*, *O. minima* is clearly distinguishable from the 14 quadrannulate congeners by the possession of paired sperm duct bulbs (vs. without sperm duct bulbs in 14 congeners; see Nakano and Prozorova 2024). *Orobdella minima* is readily distinguished from *O. ketagalan* by the presence of epididymides, which are absent in the latter. It also differs in having a developed bulbous gastroporal duct and ovate, well-developed atrial cornua (vs. a simple tubular duct and undeveloped coniform cornua in *O. ketagalan*) (cf. Nakano and Lai 2012).

The present phylogenies recovered the monophyly of three Taiwanese quadrannulate species, *O. minima*, *O. ketagalan*, and *O. meisai*. Additionally, *O. minima* formed a clade with the middle-type *O. ketagalan*, and this clade was sister to the middle-type *O. meisai*. A previous study stated that the small-sized body length has evolved in parallel within the Japanese *Orobdella* (Nakano 2016, 2021). The obtained trees confirmed that the small body length has also evolved independently in the Taiwanese *Orobdella*. The present results also suggested that *O. minima* would retain the epididymides as a plesiomorphic feature among the Taiwanese *Orobdella*, because both *O. ketagalan* and *O. meisai* do not possess epididymides in their male genital organs (Nakano and Lai 2012, 2017).

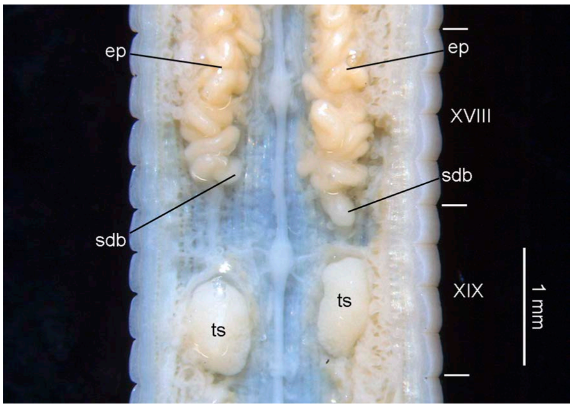

Fig. 4. *Orobdella minima* sp. nov., paratype, ASIZW0001115, dorsal view of male sperm ducts in somites XVIII and XIX including ventral nervous system. Abbreviations: ep, epididymis; sdb, sperm duct bulb; ts, testisac.

The absence of epididymides is not limited to *O. ketagalan* and *O. meisai*, but also observed in *O. shimadae* Nakano, 2011 and *O. dolichopharynx* Nakano, 2011 from the central Ryukyu Islands, which formed a monophyletic group with the Taiwanese species (Fig. 5). These two Ryukyu species also possess rudimentary gastroporal ducts (Nakano 2011). Given that the gastroporal duct serves as the spermatophore-receiving organ (Nakano 2017b), this combination may suggest a functional relationship between a reduced or undeveloped gastroporal duct and the absence of epididymides. Supporting this idea, *O. kawakatsuorum* Richardson, 1975 and *O. brachyepididymis* Nakano, 2016, from Hokkaido and Shikoku, Japan, respectively, possess simple, tubular gastroporal ducts and also retain only short epididymides (Nakano 2012, 2016). Thus, although exceptions may exist, the presence and degree of development of epididymides may be functionally linked to the morphol-

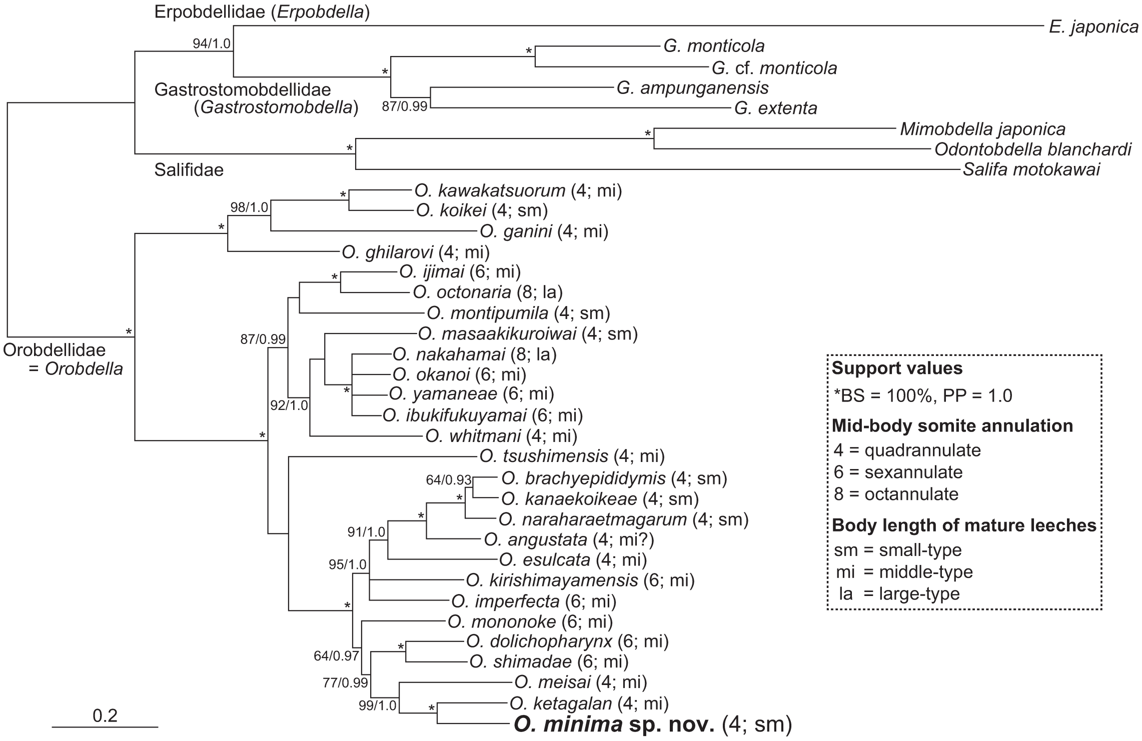

Fig. 5. Bayesian inference tree for 8100 bp of nuclear 18S, 28S, and H3, and mitochondrial COI, tRNA^{Cys}, tRNA^{Met}, 12S, tRNA^{Val}, 16S, tRNA^{Leu} and ND1 markers. Numbers on nodes indicate bootstrap (BS) values for maximum likelihood ≥ 60% and Bayesian posterior probabilities (PP) ≥ 0.90. Asterisks denote nodes with BS = 100%, PP = 1.0. Numbers and abbreviations in parentheses indicate, respectively, the mid-body somite annulation and the body length of mature individuals for each species.

ogy of the gastroporal duct, potentially reflecting variations in spermatophore formation or copulatory behavior across species. Furthermore, the phylogenetic position of the new species suggested that the possession of sperm duct bulbs may be a synapomorphic character between *O. minima* and *O. ketagalan* that formed a fully supported clade within the genus.

Key to Taiwanese Species of *Orobdella*

1. Body length more than 5 cm; gastroporal duct tubular; epididymides lacking; atrial cornua undeveloped or lacking...2

— Body length less than 5 cm; gastroporal duct developed, bulbous; epididymides present in somites XVI–XVIII; atrial cornua developed, ovate............. *O. minima* sp. nov.

2. Gonopores separated by 1/2 + 4 + 1/2 annuli; pharynx reaching to posterior of somite XIV; atrial cornua undeveloped coniform.. .... *O. ketagalan* Nakano and Lai, 2012

— Gonopores separated by 5 + 1/2 annuli; pharynx reaching to posterior of somite XV; atrial cornua lacking; dorsal-to-lateral surface with irregular brownish markings......... .... *O. meisai* Nakano and Lai, 2017

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Authors Contributions

Takafumi Nakano: Writing – original draft; Writing – review & editing; Visualization; Investigation; Formal analysis; Funding acquisition. Yi-Te Lai: Writing – review & editing; Resources; Validation.

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Declarations

Competing interests. The authors declare no conflicts of interest.

References

Chernomor, O., Haeseler, A. v., and Minh, B. Q. 2016. Terrace aware data structure for phylogenomic inference from supermatrices. Systematic Biology 65: 997–1008. doi:10.1093/sysbio/syw037

Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K. F., Haeseler, A. v., and Jermiin, L. S. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods 14: 587–589. doi:10.1038/nmeth.4285

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. doi:10.1093/molbev/mst010

Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., Haeseler, A. v., 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. doi:10.1093/molbev/msaa015

Moore, J. P. 1927. The segmentation (metamerism and annulation) of the Hirudinea. Pp. 1–12. *In*: Harding, W. A. and Moore, J. P. *The Fauna of British India, Including Ceylon and Burma. Hirudinea*. Taylor & Francis, London.

Nakano, T. 2011. Redescription of *Orobdella ijimai* (Hirudinida: Arhynchobdellida: Gastrostomobdellidae), and two new species of *Orobdella* from the Ryukyu Archipelago, Japan. Zootaxa 2998: 1–15. doi:10.11646/zootaxa.2998.1.1

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

Nakano, T. 2016. Four new species of the genus *Orobdella* from Shikoku and Awajishima island, Japan (Hirudinida, Arhynchobdellida, Orobdellidae). Zoosystematics and Evolution 92: 79–102. doi:10.3897/zse.91.7616

Nakano, T. 2017a. Diversity of leeches from Japan: recent progress in macrophagous and blood-feeding taxa. Pp. 319–340. *In*: Motokawa, M. and Kajihara, H. (Eds) *Species Diversity of Animals in Japan*. Springer Japan, Tokyo. doi:10.1007/978-4-431-56432-4_12

Nakano, T. 2017b. A new species of *Orobdella* (Hirudinida: Arhynchobdellida: Orobdellidae) from Japan reveals the function of the *Orobdella* gastroporal duct. Zoological Science 34: 161–172. doi:10.2108/zs160167

Nakano, T. 2021. A new species of *Orobdella* (Hirudinida, Arhynchobdellida, Orobdellidae) from the Tsukuba Mountains in Japan. Soil Organisms 93: 105–113. doi:10.25674/so93iss2id154

Nakano, T. 2025. Two new sexannulate species of *Orobdella* (Hirudinea: Erpobdelliformes: Orobdellidae) from Kyushu, Japan. Edaphologia 116: 1–16.

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

Nakano, T. 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. doi:10.12782/sd.21.2.127

Nakano, T. and Lai, Y.-T. 2017. A new quadrannulate species of *Orob-*

della (Hirudinida: Arhynchobdellida: Orobdellidae) from Pingtung, Taiwan. Species Diversity 22: 143–150. doi:10.12782/specdiv.22.143

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. doi:10.11646/zootaxa.5424.1.2

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

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. doi:10.1093/sysbio/syy032

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. doi:10.1093/sysbio/sys029

Sawyer, R. T. 1986. *Leech Biology and Behaviour*. Clarendon Press, Oxford, 1065 pp.