A New Quadrannulate Species of *Orobdella* (Hirudinida: Arhynchobdellida: Orobdellidae) from Pingtung, Taiwan

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A quadrannulate leech species, *Orobdella meisai* sp. nov., from southern Taiwan is described. This new species is characterized by its camouflage coloration, which is unique among the known *Orobdella* species. *Orobdella meisai* is the second species of the genus to be described from Taiwan. Phylogenetic analyses using nuclear 18S 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 *O. meisai* and another Taiwanese species, *O. ketagalan* Nakano and Lai, 2012, formed a supported clade. According to the results of the morphological examination and the phylogenetic position of *O. meisai*, morphological-phylogenetic relationships of this species and close congeners are briefly discussed.

Key Words: Erpobdelliformes, terrestrial, macrophagous, molecular phylogeny.

Introduction

The genus *Orobdella* Oka, 1895 comprises 18 known species inhabiting terrestrial habitats in the Japanese Archipelago, Korean Peninsula, Taiwan, and adjacent islets (Nakano 2017a, b); an *Orobdella* leech, whose taxonomic status remains uncertain, has been recorded from the Russian Far East (Gilyarov *et al.* 1969; Nakano 2012a). *Orobdella* species can be identified by the following three characteristics (Nakano 2017a): 1) the possession of a gastroporal duct, which is an accessory copulatory organ that receives a spermatophore during copulation (Nakano 2017b); 2) mid-body somite annulation that can be subdivided into quadrannulate (four annuli per one somite; ten species), sexannulate (six annuli; six species), and octannulate (eight annuli; two species); and 3) the body length of mature individuals, grouped into small (the body length is ~5 cm; five species), middle (reaching ~10 cm; 11 species), and large types (reaching ~20 cm; two species). Whereas only quadrannulate species are assigned to the small type, the two octannulate species are designated large types (Nakano 2017a, b).

Orobdella leeches are particularly diversified in the Japanese Archipelago. All sexannulate, octannulate and small-type quadrannulate species are indigenous to the archipelago (Nakano 2017a). By contrast, only one middle-type quadrannulate species *O. ketagalan* Nakano and Lai, 2012 is known from Taiwan (Nakano and Lai 2012); and only *O. tsushimensis* Nakano, 2011 (middle-type quadrannulate), is

indigenous to the Korean Peninsula (Nakano and Seo 2012, 2014). However, several undescribed species of *Orobdella* have been recognized in Taiwan (Nakano and Lai 2012).

Additional *Orobdella* leeches, which were designated as middle-type quadrannulate, were collected from Pingtung County, Taiwan, and they are described herein as a new species. Additionally, the phylogenetic position of the new species within *Orobdella* was estimated using nuclear and mitochondrial genetic markers.

Materials and Methods

Sampling and morphological examination. Leeches were collected from a locality in Pingtung County, Taiwan (Fig. 1). When possible, elevation and geographical coordinates for the collection site were obtained using a Garmin eTrex® GPS unit.

Specimens were relaxed by the gradual addition to absolute ethanol (EtOH) to freshwater, or directly fixed in absolute EtOH. For DNA extraction, botryoidal tissue was removed from the posterior part around the caudal sucker of every 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 caudal sucker, and caudal sucker

Fig. 1. Map showing the collection locality (=closed diamond) of the specimens examined in this study.

width (CW) from the right to the left margin of the caudal 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.

PCR and DNA sequencing. Genomic DNA was extracted from botryoidal tissues preserved in absolute EtOH following the method described by Nakano (2012b). Primer sets for the PCR and cycle sequencing reactions for the nuclear 18S rRNA (18S), histone H3 (H3), and the three mitochondrial regions, *i.e.*, 1) cytochrome *c* oxidase subunit I (COI), 2) tRNA^{Cys}, tRNA^{Met}, 12S rRNA, tRNA^{Val}, and 16S rRNA (tRNA^{Cys}–16S), and 3) tRNA^{Leu} and NADH dehydrogenase subunit 1 (tRNA^{Leu}–ND1), used in this study are shown in Nakano (2016a). The PCR reactions and DNA sequencing were performed using a modified version of a method mentioned in Nakano (2012a), and the detailed conditions were identical to those in Nakano (2016a). In total, ten sequences from the unidentified *Orobdella* specimens were newly obtained in this study, and deposited with the International Nucleotide Sequence Database Collaboration (INSDC) through DNA Data Bank of Japan (Table 1).

Molecular phylogenetic analyses. The phylogenetic position of the newly identified *Orobdella* species within the genus was estimated based on the 18S, H3, COI, tRNA^{Cys}–16S, and tRNA^{Leu}–ND1 sequences. The four erpobdelliform outgroup taxa were identical to those used in the phylogenetic analyses by Nakano (2017b) (Table 1). The alignments of H3 and COI were trivial, as no indels were observed. The sequences of 18S, tRNA^{Cys}–16S, and tRNA^{Leu}–ND1 were aligned using MAFFT v. 7.310 L-INS-i (Katoh and Standley 2013). The lengths of the 18S, H3, COI, tRNA^{Cys}–16S, and tRNA^{Leu}–ND1 sequences were 1844, 328, 1267, 1133, and 635 bp, respectively. The concatenated sequences yielded 5207 bp of aligned positions.

Phylogenetic trees were constructed using maximum likelihood (ML) and Bayesian inference (BI). The ML phy-

logeny was reconstructed using RAxML v. 8.2.8 (Stamatakis 2014) with the substitution model set as GTR+I+G, immediately after nonparametric bootstrapping (BS) was conducted with 1000 replicates. The best-fit partition scheme and the substitution model (=GTR+I+G) were identified with the Akaike information criterion using PartitionFinder v. 2.1.1 (Lanfear *et al.* 2017) with the “greedy” algorithm: 18S and H3 2nd position/H3 1st position/H3 3rd position/COI 1st position/COI 2nd position/COI 3rd position/tRNA^{Cys}, tRNA^{Val} and tRNA^{Leu}/tRNA^{Met}/12S/16S/ND1 1st position/ND1 2nd position/ND1 3rd position. BI and Bayesian posterior probabilities (PPs) were estimated using MrBayes v. 3.2.6 (Ronquist *et al.* 2012). The best-fit partition scheme and models for each partition were selected with the Bayesian information criterion using PartitionFinder with the “greedy” algorithm: for 18S and H3 1st position, K80+I; for H3 2nd position, JC+I; for H3 3rd position, HKY+G; for COI 1st position, GTR+G; for 2nd position of COI and ND1, HKY+I+G; for 3rd positions of COI and ND1, and 16S, HKY+G; and for tRNA^{Cys}, tRNA^{Met}, 12S, tRNA^{Val}, tRNA^{Leu}, and ND1 1st position, GTR+I+G. Two independent runs of four Markov chains were conducted for 20 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 2013), and the first 50001 trees were discarded based on the results.

Orobdella meisai sp. nov.

(Figs 2–4)

Diagnosis. Body length of mature individual exceeding 6 cm. Somite IV uniannulate, somites VIII–XXV quadrannulate. Clitellum in somite XI b5 to somite XIII a2. Male gonopore in somite XI b5/b6, female gonopore slightly posterior to anterior margin of somite XIII a1, behind gastropore, gonopores separated by 5 + 1/4 annuli. Pharynx reaching to somite XV b5–b6. Gastropore conspicuous, slightly posterior to anterior margin of somite XIII a1. Gastroporal duct rudimentary tubular. Paired vasa deferentia thick. Epididymis lacking. Pre-atrial loop present. Atrial cornu lacking.

Material examined. Holotype: KUZ Z1917, dissected, collected from under a stone along the Jinshui Ying Old Trail, Fanliao, Pingtung County, Taiwan (22.40730°N, 120.758568°E; elev. 1470 m) by Takafumi Nakano (TN) on 9 March 2017. Paratypes (12 specimens collected from around the type locality): KUZ Z1908 (22.407°N, 120.756°E; elev. 1490 m) by Wei-Ren Liang on 26 August 2014; KUZ Z1909–Z1910 (22.407°N, 120.756°E; elev. 1490 m) by Fu-Sheng Huang on 23 February 2014; KUZ Z1911–Z1912 (22.40824°N, 120.75264°E; elev. 1650 m), KUZ Z1914–Z1915 (22.40790°N, 120.75304°E; elev. 1620 m) by Yi-Te Lai (YL) on 9 March 2017; KUZ Z1913 (22.40809°N, 120.75288°E; elev. 1620 m), KUZ Z1916 (22.40772°N, 120.75662°E; elev. 1450 m) by TN on 9 March 2017; KUZ Z1918–Z1919 (22.407933°N, 120.756530°E) by YL on 22 January 2017; and KUZ Z1920 (22.409192°N, 120.754820°E) by Kao-Shr Shiang on 9 December 2016.

Table 1. Samples used for the phylogenetic analyses. The information on the vouchers is accompanied by 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; and UNIMAS, the Universiti Malaysia Sarawak.

Species	Voucher	INSDC accession #				
		18S	H3	COI	tRNA ^{Cys} -16S	tRNA ^{Leu} -ND1
<i>Orobdella</i>						
<i>O. meisai</i> sp. nov.	KUZ Z1915 Paratype	LC314418*	LC314420*	LC314419*	LC314417*	LC314421*
<i>O. meisai</i> sp. nov.	KUZ Z1917 Holotype	LC314423*	LC314425*	LC314424*	LC314422*	LC314426*
<i>O. brachyepididymis</i> Nakano, 2016	KUZ Z1673 Holotype	LC106319	LC106321	LC106320	LC106318	LC106322
<i>O. dolichopharynx</i> Nakano, 2011	KUZ Z120 Holotype	AB663665	AB698876	AB679680	AB679681	AB828558
<i>O. esulcata</i> Nakano, 2010	KUZ Z29 Holotype	AB663655	AB698873	AB679664	AB679665	AB828555
<i>O. ijimai</i> Oka, 1895	KUZ Z110 Topotype	AB663659	AB698877	AB679672	AB679673	AB828559
<i>O. kanaekoikeae</i> Nakano, 2017	KUZ Z1747 Holotype	LC184551	LC184553	LC184552	LC184550	LC184554
<i>O. kawakatsuorum</i> Richardson, 1975	KUZ Z167 Topotype	AB663661	AB698878	AB679704	AB679705	AB828561
<i>O. ketagalan</i> Nakano and Lai, 2012	KUZ Z208 Holotype	AB704785	AB704786	AB704787	AB828582	AB828563
<i>O. koikei</i> Nakano, 2012	KUZ Z156 Holotype	AB698883	AB698882	AB679688	AB679689	AB828560
<i>O. masaakikuroi</i> Nakano, 2014	KUZ Z694 Holotype	AB938003	AB938013	AB938006	AB937997	AB938016
<i>O. mononoke</i> Nakano, 2012	KUZ Z224 Holotype	AB698868	AB698869	AB698866	AB698867	AB828564
<i>O. nakahamai</i> Nakano, 2016	KUZ Z1672 Holotype	LC106330	LC106332	LC106331	LC106329	LC106333
<i>O. naraharaetmagarum</i> Nakano, 2016	KUZ Z1652 Holotype	LC087143	LC087145	LC087144	LC087142	LC087146
<i>O. octonaria</i> Oka, 1895	KUZ Z181 Topotype	AB698870	AB698871	AB679708	AB679709	AB828562
<i>O. okanoi</i> Nakano, 2016	KUZ Z1671 Holotype	LC106341	LC106343	LC106342	LC106340	LC106344
<i>O. shimadae</i> Nakano, 2011	KUZ Z128 Holotype	AB663663	AB698875	AB679676	AB679677	AB828557
<i>O. tsushimensis</i> Nakano, 2011	KUZ Z134 Holotype	AB663653	AB698872	AB679662	AB679663	AB828554
<i>O. whitmani</i> Oka, 1895	KUZ Z45 Topotype	AB663657	AB698874	AB679668	AB679669	AB828556
<i>O. yamaneae</i> Nakano, 2016	KUZ Z1678 Holotype	LC106349	LC106351	LC106350	LC106348	LC106352
Outgroup						
<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, 1910)	KUZ Z180	AB663651	AB938012	AB938004	AB937995	AB938014

Fig. 2. *Orobodella meisai* sp. nov., holotype, KUZ Z1917. A, dorsal view; B, ventral view; C, dorsal view of live animal. Scale bars: 5 mm.

(KUZ Z1910, Z1914, and Z1915 were dissected).

Description. Body firm and muscular, elongate, with constant width in caudal direction, dorsoventrally compressed, BL 62.5 mm, BW 4.7 mm (Fig. 2A, B). Caudal sucker ventral, slightly elliptic, CL 2.7 mm, CW 2.9 mm (Figs 2B, 3D).

Somite I completely merged with prostomium (Fig. 3A). Somites II (=peristomium), III and IV uniannulate (Fig. 3A); somite II not separated from somite I. Somite V biannulate, (a1+a2)=a3; a3 forming posterior margin of oral sucker (Fig. 3A, B). Somites VI and VII triannulate, a1=a2=a3 (Fig. 3A, B). Somites VIII–XXV quadrannulate, a1=a2=b5=b6 (Fig. 3A–E). Somite XXVI triannulate, a1>a2<a3; a3 being ventrally last complete annulus (Fig. 3C, D). Somite XXVII uniannulate with slight dorsolateral furrow on respective sides (Fig. 3C). Anus behind somite

XXVII; post-anal annulus absent (Fig. 3C).

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

Male gonopore in somite XI b5/b6 (Fig. 3E). Female gonopore slightly posterior to anterior margin of somite XIII a1, inconspicuous, located posterior to gastropore (Fig. 3E, F). Gonopores separated by 5+1/4 annuli (Fig. 3E).

Anterior ganglionic mass in somite VI a2 and a3. Ganglion VII in a1 and a2. Ganglia VIII–XIX, of each somite, in a2 (Fig. 4B). Ganglia XX–XXII, of each somite, in a1 and a2. Ganglion XXIII in a2. Ganglia XXIV–XXV, of each somite, in a1 and a2. Ganglion XXVI in somite XXV b6. Posterior ganglionic mass in somite XXVI a2 and a3.

Eyes in 3 pairs, 1st pair dorsally on anterior margin of somite III, 2nd and 3rd pairs dorsolaterally on posterior mar-

Fig. 3. *Orobiddella meisai* sp. nov., holotype, KUZ Z1917. 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 X–XIII; F, ventral view of gastropore and female gonopore. Scale bars: 1 mm (A, B); 2 mm (C–E); 0.25 mm (F). Abbreviations: af, annular furrow; an, anus; cl, clitellum; fg, female gonopore; gp, gastropore; mg, male gonopore; np, nephridiopore.

gin of somite V (a1 + a2) (Fig. 3A). Papillae numerous, minute, hardly visible, on row on every annulus.

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

Pharynx agnathous, euthylaematous, reaching to somite XV b5 (Fig. 4A). Crop tubular, acecate, reaching to somite

XXI a2. Gastropore conspicuous, ventral, slightly posterior to anterior margin of somite XIII a1 (Fig. 3E, F). Gastroporal duct rudimentary tubular, reaching to somite XV a1/a2 (Fig. 4A); its pore opening to crop hardly detectable. Intestine tubular, acecate, reaching to somite XXIV a1/a2. Rectum tubular, thin-walled, straight.

Testisacs multiple (Fig. 4B); on right side, in somite XVIII

Fig. 4. *Orobdella meisai* sp. nov., holotype, KUZ Z1917. A, ventral view of gastroporal duct; B, dorsal view of reproductive system including ventral nervous system; C, dorsal view of male atrium including position of ganglion XI; D, dorsal view of female reproductive system including position of ganglion XIII. Scale bars: 1 mm (A); 2 mm (B); 0.25 mm (C); 0.5 mm (D). Abbreviations: at, atrium; cod, common oviduct; cp, crop; ed, ejaculatory duct; gd, gastroporal duct; od, oviduct; ov, ovisac; ph, pharynx; ts, testisac; vd, vas deferens.

a2 to somite XXV a2, in total ~40 testisacs, 4 in XVIII, 5 in XIX, 6 in XX, 6 in XXI, 5 in XXII, 5 in XXIII, 6 in XXIV, 3 in XXV; on left side, in somite XVIII a2 to somite XXIV

b6, in total ~35 testisacs, 3 in XVIII, 4 in XIX, 7 in XX, 5 in XXI, 5 in XXII, 5 in XXIII, 6 in XXIV. Paired vasa deferentia thick (Fig. 4B). Epididymis absent. Paired ejaculatory

ducts in somite XI a2 to somite XVIII a1 (Fig. 4B); coiled in position posterior to ovisacs; each duct crossing ventrally beneath each ovisac, then coiled in position anterior to ovisacs; each winding from respective junction with vas deferens, narrowing toward atrium with pre-atrial loop extending to middle of somite XI a2. Atrial cornu absent. Atrium small, globular, in somite XI b5 and b6 (Fig. 4B, C). Penis sheath and penis absent.

Paired ovisacs globular, in somite XIII a2 and b5 (Fig. 4B, D). Oviducts thin-walled (Fig. 4D); both oviducts converging in to common oviduct in somite XIII a1; dorsal surface of junction of both oviducts covered with ventral nerve cord. Common oviduct thin-walled, short, directly descending to female gonopore (Fig. 4D).

Variation. Measurements (mean $\pm$ 1SD, followed by ranges in parentheses; $n=13$, including holotype): BL 48.9 ± 16.3 mm (27.4–73.1 mm), BW 4.2 ± 1.5 mm (2.1–7.2 mm), CL 2.1 ± 0.68 mm (1.2–3.0 mm), CW 2.5 ± 0.79 mm (1.4–4.2 mm). Somite XXVI a3 sometimes with slight dorsal furrow, or dorsolateral furrow on respective sides. Somite XXVII generally uniannulate without secondary furrow, or rarely biannulate. First pair of eyes rarely dorsally on posterior margin of somite II, or on somites II/III; small unpaired eye dorsally on left posterior margin of somite III (KUZ Z1912). Pharynx reaching to somite XV b5/b6–b6. Crop reaching to somite XXI a1–a2. Gastroporal duct reaching to somite XV a2–b5/b6. Intestine reaching to somite XXIII b5/b6–somite XXIV a2/b5. Testisacs; right side, ~34–42 sacs in somite XVII b5–b6 to somite XXV a1–a2; left side, ~36–42 sacs in somite XVII b5–b6 to somite XXIV b6–somite XXV a2. Paired ejaculatory ducts in somite XI a2 to somite XVII a2/b5–b5. Paired ovisacs in somite XIII a2, or in somite XIII a2 and b5.

Coloration. In life, dorsal-to-lateral surface yellowish brown with irregular brownish markings without distinct pattern (Fig. 2C); ventral surface brownish white with irregular dark markings, ventral median region rarely with markings. Color faded in preservative, but irregular markings on surface remain (Fig. 2A, B).

Distribution. This species was collected only from its type locality. The present locality is the southernmost habitat within the known range of *Orobdella* species.

Natural history. This species was found curled up under rocks in moist mountainous habitats, and is thus terrestrial. A mature individual bearing a slight clitellum (=holotype) was collected on 9 March 2017. This finding suggests that the reproductive season of this species begins in early-to-middle March.

Molecular analyses results. The ML tree (ln $L=-28606.84$; Fig. 5) for estimating the phylogenetic position of the new species had an identical topology to that of the BI tree (mean ln $L=-28193.30$; not shown). *Orobdella meishai* belonged to a well-supported clade (BS=100%, PP=1.0) containing the other eight species inhabiting the western part of Honshu to Taiwan. The clade was divided into two subclades: a well-supported lineage (BS=99%, PP=1.0) consisted of four species, i.e., *O. esulcata* Nakano, 2010, *O. naraharaetmagarum* Nakano, 2016, *O. kanaekoikeae* Na-

Fig. 5. Maximum likelihood tree for 5207 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.

kano, 2017 and *O. brachyepididymis* Nakano, 2016, inhabiting the western part of Honshu, Shikoku and Kyushu islands of Japan; another lineage was not fully supported in the ML tree (BS=49%, PP=0.92), comprising three species, i.e., *O. mononoke* Nakano, 2012, *O. shimadae* Nakano, 2011 and *O. dolichopharynx* Nakano, 2011, from the Ryukyu Islands, and the two Taiwanese *O. ketagalan* and *O. meishai*. The monophyly of *O. shimadae*, *O. dolichopharynx*, *O. ketagalan* and *O. meishai* was supported (BS=83%, PP=1.0). The two Ryukyu species, *O. shimadae* inhabiting Okinawajima island, and *O. dolichopharynx* known from Amamioshima island, formed a fully supported clade (BS=100%, PP=1.0). Monophyly of the Taiwanese *O. ketagalan* and *O. meishai* was also well-supported (BS=92%, PP=1.0).

The ND1 sequences (LC314421 and LC314426) obtained from the holotype and paratype of *O. meishai* exhibit unique genetic variations compared with those of the other known *Orobdella* leeches. The ND1 sequences of *O. meishai* lack 97th–111th and 249th–260th bp positions in those of the other congeners. Since those missing positions are counted from the first position of the ND1 sequences, the former deletion indicates that the 33rd–37th codons are missing from the *O. meishai* ND1 sequences, and the latter deletion shows that the 83rd–87th codons are compacted into a single DNA triplet.

Etymology. The specific name is from a Japanese word *meishai* (=camouflage) referring to the dorsal coloration of the new species. The specific name is thus treated as indeclinable.

Remarks. *Orobdella meishai* clearly belongs to *Orobdella*, because it possesses the generic diagnostic characteristics defined by Nakano (2016a): post-anal annulus absent; male gonopore in posterior part of somite XI; female gonopore in anterior part of somite XIII; gonopores separated by more than one full somite; pharynx agnathous euthylaematous; gastropore in anterior part of somite XIII; gastroporal duct

Table 2. Comparisons of morphological characters between *Orobdella meisai* sp. nov. and ten quadrannulate congeneric species.

Species	Body length	Somite IV	Somite XXV	Annuli between gonopores	Pharynx length	Gastroporal duct	Epididymides	Atrial cornua
<i>O. meisai</i>	middle	1	4	5+1/4	to posterior XV	rudimentary tubular	absent	absent
<i>O. brachyepididymis</i>	small	1	4	1/2+4[+($<1/2$)]	to anterior XIV	tubular	XX to XXI	small, ovate
<i>O. esulcata</i>	middle	1	4	2/3+4+1/3	to anterior to posterior XIV	tubular, but bulbous at junction with gastropore	XVI to XX	developed, ovate
<i>O. kanaekoikeae</i>	small	1	4	1/2+4+1/2	to posterior XIII to anterior XIV	bulbous	XIV to XVIII	developed, ovate
<i>O. kawakatsuorum</i>	middle	2	4	6	to middle to posterior XIV	simple tubular	XVI to XVII	undeveloped
<i>O. ketagalan</i>	middle	1	4	1/2+4+1/2	to posterior XIV	simple tubular	absent	undeveloped
<i>O. koikei</i>	small	1	3	1/2+4+1/2	to posterior XIII to anterior XIV	bulbous	XV to XX	developed, ovate
<i>O. masaakikuroiwai</i>	small	1	4	1/2+4+1/2	to anterior to middle XIV	bulbous	XVI to XVIII	developed, ovate
<i>O. naraharaetmagarum</i>	small	1	4	1/2+4+1/2	to posterior XIII	bulbous	XV to XX	developed, ovate
<i>O. tsushimensis</i>	middle	1	4	1/2+5	to posterior XIII to posterior XIV	bulbous	XVII to XIX	developed, ovate
<i>O. whitmani</i>	middle	1 or 2	4	1/2+4+1/2	to anterior to posterior of XIV	bulbous	XVI to XVIII	developed, ovate

lying on female organ; testisacs multiple; male atrium in posterior part of somite XI, without penis sheath or penis; ovisacs globular in somite XIII; female median reproductive system essentially lacking.

According to taxonomic studies (Nakano 2010, 2011b, 2012a; Nakano and Lai 2012; Nakano 2014; Nakano and Seo 2014; Nakano 2016a, b, 2017b), the new species is distinguishable from the ten quadrannulate species (*O. brachyepididymis*, *O. esulcata*, *O. kanaekoikeae*, *O. kawakatsuorum* Richardson, 1975, *O. ketagalan*, *O. koikei* Nakano, 2012, *O. masaakikuroiwai* Nakano, 2014, *O. naraharaetmagarum*, *O. tsushimensis*, and *O. whitmani* Oka, 1895) by the following combination of characters (Table 2): body length, annulation of somites IV and XXV, number of annuli between gonopores, pharynx length, gastroporal duct, epididymidal lengths and atrial cornua. *Orobdella meisai* can clearly differ from these known quadrannulate species in having 5+1/4 annuli between the gonopores, the pharynx reaching to the posterior part of somite XV, the rudimentary tubular gastroporal duct, and in lacking atrial cornua in the male median reproductive system.

The new species is obviously distinguished from the six sexannulate species, i.e., *O. dolichopharynx*, *O. ijimai* Oka, 1895, *O. mononoke*, *O. okanoi* Nakano, 2016, *O. shimadae* and *O. yamaneae* Nakano, 2016, and two octannulate species, i.e., *O. nakahamai* Nakano, 2016 and *O. octonaria* Oka, 1895, by its mid-body somites that are quadrannulate.

Discussion

Orobdella meisai possesses a rudimentary gastroporal duct and an undeveloped male median reproductive system without atrial cornua. These characteristics are congruent with the recent finding that the *Orobdella* gastroporal duct is a secondary copulatory organ that receives a spermatophore (Nakano 2017b); a leech spermatophore is secreted from the male atrium (Brumpt 1900). It remains uncertain whether the gastroporal duct of *O. meisai* plays a role as a spermatophore receptor. However, it is highly possible that the rudimentary gastroporal duct of *O. meisai* has lost its function, and that this species transfers spermatozoa directly to the

female organ.

Undeveloped gastroporal duct and male atrium are shared characteristics within a clade comprising four *Orobdella* species distributed in the Ryukyu Islands and Taiwan. *Orobdella shimadae* and *O. dolichopharynx*, which are respectively indigenous to Okinawajima and Amamioshima islands, possess a rudimentary gastroporal duct, and they completely lack an atrial cornu (Nakano 2011a). Although the Taiwanese *O. ketagalan* bears atrial cornua and a functional gastroporal duct, these organs are generally undeveloped (Nakano and Lai 2012). Since *O. mononoke* and the other phylogenetically related species, e.g., *O. esulcata*, *O. kanaekoikeae* and *O. naraharaetmagarum*, possess a developed gastroporal duct and atrial cornua (Nakano 2010, 2012b, 2016b, 2017b), the undeveloped gastroporal duct and atrium may be synapomorphic characters within the clade containing *O. shimadae*, *O. dolichopharynx*, *O. ketagalan* and *O. meisai*. Moreover, the pharynx of *O. shimadae*, *O. dolichopharynx*, and *O. meisai*, of which the gastroporal duct is vestigial, is relatively longer than those of the close congeners with a developed gastroporal duct (Nakano 2011a, 2012b; Nakano and Lai 2012). The pharyngeal elongation and degeneration of the gastroporal duct within *Orobdella* species may correlate with each other.

The obtained phylogenies showed that *O. meisai* is genetically highly diverged from *O. ketagalan* compared with the phylogenetic relationships of *O. shimadae* and *O. dolichopharynx*. These relationships suggest that the diversification of the Taiwanese *Orobdella* species had occurred prior to the speciation between *O. shimadae* and *O. dolichopharynx*. A biogeographical study with divergence time estimates of *Orobdella* species will help to improve our understanding of the evolutionary history of this East-Asian specific terrestrial leech group.

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