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A new species of Orobdella (Hirudinida, Arhynchobdellida, Orobdellidae) from Taipei, Taiwan

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Abstract
A new quadrannulate species of Orobdella, Orobdella ketagalan sp. n., from Taipei, Taiwan, is described. This is the first record of Orobdella and the family Orobdellidae from Taiwan. This new species possesses small, paired sperm duct bulbs in the male reproductive system. In addition to these bulbs, the following combination of characters distinguishes this new species from other quadrannulate species: somite IV uniannulate, male gonopore at XI b6, female gonopore at XIII a1, 1/2 + 4 + 1/2 between gonopores, simple tubular gastroporal duct, lacking epididymides, and undeveloped atrial cornua. Phylogenetic analyses using nuclear 18S rDNA and histone H3 as well as mitochondrial COI, 12S rDNA, tRNAVal, and 16S rDNA markers showed that O. ketagalan is related to the two Ryukyu Archipelago species Orobdella dolichopharynx Nakano, 2011 and Orobdella shimadae Nakano, 2011.

Keywords
Hirudinida, Orobdellidae, Orobdella, new species, first record, gastroporous, Taiwan
Introduction

Species of the genus *Orobdella* Oka, 1895 are large annelids that feed on earthworms. They are usually 10–20 cm in length (except for *O. koikei* Nakano, 2012, approx. 5 cm) and they inhabit the banks of mountain streams in East Asia (Nakano 2012a, Oka 1895). The systematic position of the genus *Orobdella* has been contentious. *Orobdella* was initially included in the family Gastrostomobdellidae along with the Southeast Asian terrestrial macrophagous leech genus *Gastrostomobdella* Moore, 1929 (Richardson 1971, Sawyer 1986). Although Sawyer (1986) placed Gastrostomobdellidae under Hirudiniformes, recent molecular phylogenetic studies reclassified the family under Erpobdelliformes (Nakano et al. 2012, Oceguera-Figueroa et al. 2011). Nakano et al. (2012) split Gastrostomobdellidae into two families based on phylogenetic analyses as well as morphological discontinuity, and the monotypic family Orobdellidae was erected for *Orobdella*.

Taxonomic and inventory studies on *Orobdella* have progressed recently, and this genus now includes ten species (Nakano 2010, 2011a, b, 2012a, b, in press, Oka 1895, Richardson 1975). *Orobdella* leeches exhibit various types of mid-body somite annulation; three types have been documented thus far: quadrannulate, sexannulate, and octannulate (Sawyer 1986). The quadrannulate mid-body somite is a plesiomorphy of this genus, and the sexannulate form is considered to have evolved in parallel (Nakano 2012a, b, Nakano et al. 2012).

All of the known *Orobdella* species have been described based on specimens collected from Japan, and eight of the ten species have been reported only from Japanese islands (Sawyer 1986). Outside Japan, *O. whitmani* Oka, 1895, which is the type species of the genus, has been recorded from Primorsky Krai, Russia (Gilyarov et al. 1969). However, Nakano (2012a) noted that this specimen from Russia was misidentified as *O. whitmani*, and that it should be considered a new, undescribed species. In addition, *O. tsushimensis* Nakano, 2011 was recently collected from Gageodo Island, Korea (Nakano and Seo in press). In Taiwan, no studies have investigated the species diversity of terrestrial macrophagous leeches. Taiwanese leech species were catalogued by Lai and Chen (2010), but *Orobdella* leeches were not included. Recently, quadrannulate *Orobdella* specimens were collected from Taipei, Taiwan. These materials clearly differ from the other known quadrannulate *Orobdella* species. Therefore, in the present study, *Orobdella* leeches from Taipei are described as a new species. This is the first record of orobdellid leeches from Taiwan. In addition, their phylogenetic position is estimated using nuclear 18S and histone H3 (H3) and mitochondrial COI, 12S, tRNAVal, and 16S rDNA (12S–16S) sequence data.

Materials and methods

Leeches were collected from Taipei, Taiwan (Fig. 1). Botryoidal tissue was taken from specimens, which were fixed in ethanol, for DNA extraction. All of the specimens were preserved in 70% ethanol. Two measurements were taken: body length (BL) from the
A new species of Orobdella (Hirudinida, Arhynchobdellida, Orobdellidae)...

anterior margin of the oral sucker to the posterior margin of the caudal sucker, and maximum body width (BW). Examination, dissection, and drawings of the specimens were accomplished under a stereoscopic microscope with a drawing tube (Leica M125). The specimens have been deposited in the Zoological Collection of Kyoto University (KUZ).

We used the numbering convention of Moore (1927): body somites are denoted by Roman numerals, and annuli in each somite are given alphanumeric designations.

The extraction of genomic DNA followed (Nakano 2012a). The primer sets used in this study are listed in Table 1: for 18S, A and L, C and Y, and O and B (Apakupakul et al. 1999) were used; for H3, H3aF and H3bR (Colgan et al. 1998); for COI, LCO 1490 and HCO 2198 (Folmer et al. 1994), and LCO-in and HCO-out (Nakano 2012a); for 12S–16S, 12SA-in and 12SB-out (Nakano 2012a). The DNA sequencing methods for the above four markers followed Nakano (2012a). The following DNA sequences were newly obtained and deposited in GenBank (Table 2): 18S and H3 sequences from the holotype (KUZ Z208) of the new species, and COI and 12S–16S sequences from the holotype (KUZ Z208) and three paratypes (KUZ Z209–Z211) of the new species. The DNA sequences of the holotype (KUZ Z208) were analyzed in the following phylogenetic analyses. The other sequences were taken from GenBank (Table 2). For the outgroup, three Erpobdelliformes leeches were included in the phylogenetic analyses: Erpobdella japonica Pawlowski, 1962 (Erpobdellidae), Gastrostomobdella monticola Moore, 1929 (Gastrostomobdellidae), and Mimobdella japonica Blanchard, 1897 (Salifidae).

H3 and COI sequences were aligned by eye because there were no indels. Nuclear 18S and mitochondrial 12S–16S sequences were aligned using MAFFT X-INS-I (Hofacker et al. 2002, Katoh and Toh 2008, McCaskill 1990, Tabei et al. 2008) taking into account RNA secondary structure information, and then refined with GBLOCKS (Castresana 2000). Aligned sequences of 18S was 1787 bp in length; those of H3, COI, and 12S–16S were 327, 1266, and 410 bp, respectively. The concatenated sequences thus yielded a total of 3790 bp positions.
Phylogenetic trees were constructed using maximum likelihood (ML) and Bayesian inference (BI). ML phylogenies were calculated using TREEFINDER v October 2008 (Jobb et al. 2004) with the tool package Phylogears v 2.0 (Tanabe 2008), and then non-parametric bootstrapping (Felsenstein 1985) was conducted with 500 replicates. The best-fit models for each partition were selected using the Akaike information criterion (Akaike 1974) by using Kakusan 4 (Tanabe 2011): for 18S, the Jobb 2008 model (J2) with gamma distribution (+G) and proportion of invariant sites (+I) was selected; for H3 1st position, the Tamura-Nei model (TN93); for H3 2nd position, the Jukes-Cantor model (JC69); for H3 3rd position, J2+G; for COI 1st position, TN93+G+I; for COI 2nd position, the transversion model (TVM)+I; for COI 3rd position, HKY85+G; for 12S, the general time reversal model (GTR)+G; for tRNAVal, the Hasegawa-Kishino-Yano model (HKY85)+G; and for 16S, the transition model (TIM)+G. BI and Bayesian posterior probabilities (BPPs) were estimated using the MPI version of MrBayes v 3.1.2 (Altekar et al. 2004, Huelsenbeck et al. 2001, Ronquist and Huelsenbeck 2003). The best-fit models for each partition were identified using the Bayesian information criterion (Schwarz 1978) also by using Kakusan 4: for 18S, the Jobb 2008 model (J2) with gamma distribution (+G) and proportion of invariant sites (+I) was selected; for H3 1st position, the Tamura-Nei model (TN93); for H3 2nd position, the Jukes-Cantor model (JC69); for H3 3rd position, J2+G; for COI 1st position, TN93+G+I; for COI 2nd position, the Felsenstein 1981 (F81) model+I; for COI 3rd position, HKY85+G; for 12S, GTR+G; and for tRNAVal and 16S, HKY85+G. Two independent runs of four Markov chains were conducted for 20 million generations and the tree was sampled every 100 generations. The parameter

### Table 1. PCR and cycle sequencing (CS) primers used in this study. Sources: aApakupakul et al. (1999), bColgan et al. (1998), cFolmer et al. (1994), dNakano (2012a).

| Gene   | Primer name | Reaction | Primer sequence (5’ → 3’) |
|--------|-------------|----------|---------------------------|
| 18S    |             |          |                           |
| 1 Aa   | PCR & CS    | AACCTGGTTGATCCTGCCAGT |
| 1 La   | PCR & CS    | CCAACTACAGGCTTTTTAACTG |
| 2 Cb   | PCR & CS    | CGGTAATTCAGCTCAATAG |
| 2 Yb   | PCR & CS    | CAGACAAATCGCTCACAACAC |
| 3 Ob   | PCR & CS    | AAGGGGACCACCAAGGATGGAG |
| 3 Bb   | PCR & CS    | TGATCCTTCCGCAGGTTACCT |
| Histone H3 |          |          |                           |
| H3aFb  | PCR & CS    | ATGGGCTCTGTAACACAGACVG |
| H3bRb  | PCR & CS    | ATATCCTTRGGCATRATRGAGC |
| COI    |             |          |                           |
| 1 LCO1490c | PCR & CS    | GGTCACAATACTCATAAGATATTG |
| 1 HCO2198d | CS          | TAAACTTCAGGTTGACCAAAAAATCA |
| 2 LCO-ind | CS          | TCCAGAAGTAGATCATATT |
| 2 HCO-outd | PCR & CS    | TCTGGGTAGTCAGAAT |
| 12S–16S |             |          |                           |
| 12SA-ind | PCR & CS    | AATTTAACAAGGATTAGATAAC |
| 12SB-outd | PCR & CS    | AACCATAATGCAAAAGGTAC |
A new species of Orobdella (Hirudinida, Arhyynchobdellida, Orobdellidae)...

estimates and convergence were checked using Tracer v 1.5 (Rambaut and Drummond 2009), and based on the results the first 50,001 trees were discarded.

Nodes with bootstrap (BS) values higher than 70% were considered sufficiently resolved (Hillis and Bull 1993). Nodes with BPPs higher than 95% were considered statistically significant (Leaché and Reeder 2002).

Taxonomy

Family Orobdellidae Nakano, Ramlah & Hikida, 2012
urn:lsid:zoobank.org:act:5F5BABE8-BD26-4FC7-9593-F73E62E26122

Genus Orobdella Oka, 1895
urn:lsid:zoobank.org:act:FA8333ED-8C17-41FD-AFC1-62A4F98D4AC1

Orobdella ketagalan sp. n.
urn:lsid:zoobank.org:act:AFF291DF-E13F-46A3-A965-14B92E23F520
http://species-id.net/wiki/Orobdella_ketagalan
Figs 2–4

Diagnosis. Somite IV uniannulate, somites VIII–XXV quadrannulate. Pharynx reaching to posterior of XIV to anterior of XV. Gastropore conspicuous at XIII a1. Gas-
troporal duct simple, tubular. Male gonopore at XI b6, female gonopore at XIII a1, gonopores separated by 1/2 + 4 + 1/2 annuli. Small paired sperm duct bulbs in XV. Epididymis absent. Atrial cornua, coniform, undeveloped.

**Materials examined.** Holotype. KUZ Z208, mature specimen of 70.9 mm length, dissected, collected from Yangmingshan National Park (alt. 779 m, 25°11'07"N, 121°31'10"E), Taipei City, Taiwan, by Win-Je Chi on March 24, 2011. Paratypes (a total of five specimens collected from Taiwan in 2005–2011): KUZ Z197, from Jinsan Township, Taipei County (alt. 739 m, 25°11'01"N, 121°30'54"E), on March 18, 2005; KUZ Z207, from the type locality (alt. 776 m, 25°09'49"N, 121°33'10"E) by Chi-Lun Lee and Win-Je Chi on July 30, 2010; KUZ Z209 (alt. 779 m, 25°11'07"N, 121°31'10"E), dissected, KUZ Z210 (alt. 600 m, 25°11'11"N, 121°31'10"E), dissected, from the type locality by Win-Je Chi on March 24, 2011; and KUZ Z211 from the type locality (alt. 737 m, 25°10'55"N, 121°30'50"E) by Win-Je Chi on April 24, 2011.

**Etymology.** The specific name is taken from the native Taiwanese tribe Ketagalan. The type locality of this new species is in an area settled by this aboriginal tribe. The specific name is a native word, not a Latin or Latinized word.

**Description of holotype.** Body firm, muscular, elongated, gaining regularly in width in caudal direction, dorso-ventral depressed, sides nearly parallel from mid-length to point just anterior to caudal sucker, BL 70.9 mm, BW 6.4 mm (Fig. 2). Caudal sucker ventral, oval, diameter smaller than BW (Figs 2B, 3D). Color faded in preservative (Fig. 2).

Somite I completely merged with prostomium (Fig. 3A). Somite II uniannulate, not separated from I (Fig. 3A). Somites III and IV uniannulate (Fig. 3A). 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 quadran-nulate, \(a1 = a2 = b5 = b6\) (Fig. 3A–E); b5 of X being first annulus on clitellum, a2 of XIII being last annulus of clitellum (Fig. 3E). Somite XXVI triannulate, \(a1 > a2 > a3\), a3 being last complete annulus on venter (Fig. 3C, D). Somite XXVII incomplete uniannulate with slight furrow (Fig. 3C); anus behind it with no post-anal annulus (Fig. 3C).

Anterior ganglionic mass in VI a2 and a3. Ganglia VIII–XXI in a2 of each somite (Fig. 4A). Ganglion XIII in a2 and b5 (Fig. 4A). Ganglia XIV–XXIII in a2 of each
somite (Fig. 4A). Ganglia XXIV and XXV in a1 and a2 of each somite. Ganglion XXVI in b6 of somite XXV. Posterior ganglionic mass in XXVI a1–a3.

Eyes, three pairs, first pair dorsally in furrow of II/III, second and third pairs dorsolaterally on posterior margin of V (a1 + a2) (Fig. 3A). Nephridiopores, 17 pairs, ventrally at posterior margin of a1 of each somite of VIII–XXIV (Fig. 3B, E). Papillae numerous, minute, hardly visible, one row on every annulus.

Pharynx agnathous, euthylaematous, reaching to XV a1 (Fig. 3F). Crop tubular, acerate, in XV a1 to XXI a2. Gastropore conspicuous, ventral, located middle of XIII a1 (Fig.
3E, G). Gastroporal duct narrow, simple tubular, joining with crop in XIV/XV (Fig. 3F).

Intestine tubular, acecate, in XXI a2 to XXIV b5/b6. Rectum tubular, thin-walled.

Male gonopore located at middle of XI b6 (Fig. 3E). Female gonopore at middle of XIII a1, inconspicuous, located behind gastropore (Fig. 3E, G). Gonopores separated by $1/2 + 4 + 1/2$ annuli (Fig. 3E). Testisacs multiple, one or two testisacs on each side in each

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**Figure 4. Orobdella ketagalan** sp. n., holotype, KUZ Z208. A Dorsal view of reproductive system including ventral nervous system B lateral view of bulb of right sperm duct C dorsal D lateral, and E ventral views of male atrium: C including position of ganglion XI; and F dorsal view of female reproductive system including position of ganglion III. Scale bars, 1 mm (A), 0.5 mm (C–F), and 0.25 mm (B). Abbreviations: at, atrium; cod, common oviduct; gp, gastropore; o, ovisac; od, oviduct; sd, sperm duct; sdb, sperm duct bulb; and ts, testisacs.
A new species of Orobdella (Hirudinida, Arhybobdellida, Orobdellidae)...

Variation. Maximum BL 111.7 mm, maximum BW 10.3 mm (KUZ Z210). In life, dorsal surface grayish, slightly darker in first third of dorsum, ventral surface whitish. Somite XXVI dorsally quadranannulate, ventrally triannulate (KUZ Z197, Z207, Z211) or quadranannulate (KUZ Z210). Somite XXVII incomplete biannulate. Pharynx reaching to XIV a1/b5–b6. Crop reaching to XXI a2/b5–XXI. Gastroporal duct joining with crop in XIV b5–XIV b5/b6. Intestine reaching to XXIV a2/b5–XXV a2. Testisacs in XV a2–XVI b6 to XXIII a1–XXV a2. Paired sperm duct bulbs in XV a1 and a2 (KUZ Z209), in XV b5 (KUZ Z210). Right or left oviducts crossing ventrally beneath nerve cord.

Distribution. Known from Yangmingshan National Park and adjacent areas in northern Taipei City, Taiwan (Fig. 1).

Remarks. Orobdella ketagalan differs from the five other quadranannulate Orobdella species (i.e., O. esulcata Nakano, 2010, O. kawakatsuorum Richardson, 1975, O. koikei, O. tsushimensis, and O. whitmani) in the following combination of characteristics (Table 3): IV uniannulate, gonopores separated by 1/2 + 4 + 1/2 annuli, XXV quadranannulate, gastroporal duct simple and tubular, paired sperm duct bulbs in XV, epididymides absent, and atrial cornua undeveloped. Because O. ketagalan possesses

Table 3. Comparison of morphological characters between Orobdella ketagalan sp. n. and five quadranannulate congeneric species.

| Character                          | O. ketagalan sp. n. | O. esulcata | O. kawakatsuorum | O. koikei | O. tsushimensis | O. whitmani |
|-----------------------------------|---------------------|-------------|------------------|-----------|----------------|-------------|
| Annulation of IV                  | uniannulate         | uniannulate | biannulate       | uniannulate | uniannulate | uni- or biannulate |
| Number of annuli between gonopores| 1/2 + 4 + 1/2       | 2/3 + 4 + 1/3 | 6                | 1/2 + 4 + 1/2 | 1/2 + 5 | 1/2 + 4 +1/2 |
| Annulation of XXV                  | quadrannulate       | quadrannulate | quadrannulate   | triannulate  | quadrannulate | quadrannulate |
| Gastroporal duct                   | simple tubular      | tubular, but bulbous at junction with gastropore | simple tubular | tubular, but bulbous at junctions with gastropore and crop | bottle-shaped | bulbiform |
| Paired sperm duct bulbs            | in XV               | absent      | absent           | absent     | absent   | absent |
| Epididymides                       | absent              | XVI to XX   | XVI to XXVII    | XVII to XIX| XVI to XIX | XVI to XVIII |
| Atrial cornua                      | undeveloped         | ovate       | undeveloped     | ovate     | coniform | ovate |
quadrannulate mid-body somites, this new species is easily distinguishable from the four sexannulate species (i.e., *O. dolichopharynx* Nakano, 2011, *O. ijimai* Oka, 1895, *O. mononoke* Nakano, 2012, and *O. shimadae* Nakano, 2011) and one octannulate species, *O. octonaria* Oka, 1895.

**Phylogenetic analyses**

The BI tree (Fig. 5) was nearly identical to the ML tree with ln \( L = -12357.61 \) (not shown). Monophyly of the genus *Orobdella* was well supported (BS = 97%, BPP = 100%). *Orobdella* then divided into two clades: clade A (BS = 100%, BPP = 100%) consisted of two species from Hokkaido, Japan, *O. kawakatsurorum* and *O. koikei*; and clade B (BS = 94%, BPP = 100%) included the other nine *Orobdella* species. Clade B was split into three subclades: subclade B1 included only *O. tsushimensis* (from Tsushima Island, Japan); subclade B2 (BS = 83%, BPP = 100%) included *O. esulcata* (from Kyushu, Japan), *O. mononoke* (from Yakushima Island, Japan), *O. dolichopharynx* (from Amamioshima Island, Japan), *O. shimadae* (from Okinawajima Island, Japan), and *O. ketagalan* (from Taipei, Taiwan); and subclade B3 (BS = 69%, BPP = 99%) consisted of three species (from Honshu, Japan), *O. whitmani*, *O. ijimai*, and *O. octonaria*. Subclades B2 and B3 formed a monophyletic clade in both analyses, but with low support (BS = 67%, BPP = 89%).

In subclade B2, three species from the Ryukyu Archipelago, *O. mononoke*, *O. dolichopharynx*, and *O. shimadae*, and the Taiwanese *O. ketagalan* formed a monophyletic clade, but this clade was also not sufficiently supported (BS = 55%, BPP = 82%).

**Figure 5.** The BI tree of 3790 bp of nuclear 18S rDNA and histone H3, and mitochondrial COI, 12S rDNA, tRNA\(^{Val}\), 16S rDNA. A species name in green indicates a quadrannulate species; in red, sexannulate; and in blue, octannulate. The numbers associated with the nodes represent the bootstrap values for ML (BS) and Bayesian posterior probabilities (BPPs). BSs higher than 70 % and/or BPPs higher than 95 % are indicated. Abbreviations: JPN, Japan; and TWN, Taiwan.
Monophyly of *O. ketagalan*, *O. dolichopharynx*, and *O. shimadae* was supported in the BI analyses (BPP = 100%), but was not recovered in the ML analyses (BS = 46%). Monophyly of *O. dolichopharynx* and *O. shimadae* was confirmed (BS = 87%, BPP = 100%).

**Discussion**

The phylogenies obtained in this study are nearly identical to those obtained in other phylogenetic analyses of the genus *Orobdella* (Nakano 2012a, b, Nakano et al. 2012). The most ancestral clade of *Orobdella* (clade A in Fig. 5) is distributed in Hokkaido, Japan. The other species inhabit islands south of Hokkaido (clade B in Fig. 5) and are divided into three subclades (B1–3 in Fig. 5). In our analyses, however, the phylogenetic relationships of these subclades were not sufficiently resolved. Our phylogenetic trees clearly indicated that the quadrannulate mid-body somite annulation is a plesiomorphic character of *Orobdella*, and that sexannulate mid-body somites had evolved in parallel. This result was also mentioned in previous studies (Nakano 2012a, b, Nakano et al. 2012). Even in subclade B2, the sexannulate character was considered to have evolved in parallel. In this subclade, three sexannulate species from the Ryukyu Archipelago were included: *O. mononoke* is from Yakushima Island, which is located in the northern part of the Ryukyu Archipelago; *O. dolichopharynx* is from Amamioshima Island, which is located in the middle region of the Archipelago; and *O. shimadae* is from Okinawajima Island, which is also located in the middle region of the Archipelago, but south of Amamioshima Island. Our analyses showed that these three sexannulate species did not form a monophyletic clade. In contrast, two sexannulate species, *O. dolichopharynx* and *O. shimadae*, and the Taiwanese quadrannulate *O. ketagalan* formed a monophyletic clade. The other sexannulate species, *O. mononoke*, was not closely related to *O. dolichopharynx* and *O. shimadae*. This is in agreement with findings by (Nakano 2012b), who mentioned that *O. mononoke* was probably not very close to those two species. Our phylogenetic analyses supported his phylogenetic conclusion. According to the topologies of the ML and BI trees, *O. mononoke* is a sister taxon of a clade including *O. ketagalan*, *O. dolichopharynx*, and *O. shimadae*, but this phylogenetic position was not well resolved in either tree. To better understand the biogeographical history of *Orobdella* leeches, more robust trees for this genus based on either more DNA markers or specimens should be obtained.

*Orobdella ketagalan* possesses small, paired sperm duct bulbs in XV (Fig. 4A, B). Such small bulbs have never before been reported in *Orobdella*. Hence, small sperm duct bulbs could be considered an apomorphy of the Taiwanese *O. ketagalan*. *Orobdella* species generally possess epididymides in their male reproductive systems (Nakano 2010, 2011a, b, 2012a, b, in press). However, only *O. ketagalan*, *O. dolichopharynx*, and *O. shimadae* do not bear epididymides (Nakano 2011b). These three species formed a monophyletic clade in our phylogenetic analyses (Fig. 5). Therefore, lacking epididymides could be considered a synapomorphy within *O. ketagalan*, *O. dolichopharynx*, and *O. shimadae*. *Orobdella ketagalan* also possesses a simple, tubular gastroporal duct, which
is similar to that of *O. kawakatsuorum* (Nakano 2012a, Richardson 1975). This morphological similarity is clearly due to convergence, according to our phylogenetic analyses.

This is the first record of the genus *Orobdella* from Taiwan. Moreover, we collected several other specimens that appear to be undescribed species of *Orobdella* (Nakano and Lai, unpublished observation). Further faunal and systematic studies will reveal the species diversity of Taiwanese *Orobdella* and further elucidate the biogeographical and evolutionary history of these macrophagous leeches.

**Key to the known species of the genus *Orobdella***

1. Mid-body somites more than quadranulate .............................................. 2
   – Mid-body somites quadranulate ............................................................... 6

2. Mid-body somites sexannulate .................................................................. 3
   – Mid-body somites octannulate ....................................................... *Orobdella octonaria* Oka, 1895

3. Pharynx reaching to XIV ........................................................................ 4
   – Pharynx reaching to XVI .................................................................... 5

4. Gonopores separated by 1/2 + 7 + 1/2 annuli .... *Orobdella ijimai* Oka, 1895
   – Gonopores separated by 8 + 1/2 annuli .... *Orobdella mononoke* Nakano, 2012

5. Gonopores separated by 8 annuli .............................................. *Orobdella dolichopharynx* Nakano, 2011
   – Gonopores separated by 9 annuli ............ *Orobdella shimadae* Nakano, 2011

6. Color yellowish ........................................................................................ 7
   – Color grayish blue or brown .................................................................. 9

7. Gonopores separated by 1/2 + 4 + 1/2 annuli ........................................ 8
   – Gonopores separated by 1/2 + 5 annuli, gastroporal duct bottle-shaped ................................................................................. *Orobdella tsushimensis* Nakano, 2011

8. Gastroporal duct bulbiform, epididymides in XVI to XVIII .....................
   ........................................................................................................ *Orobdella whitmani* Oka, 1895
   – Gastroporal duct simple tubular, epididymides absent, small paired sperm duct bulbs in XV ................................................. *Orobdella ketagalan* sp. n.

9. Color grayish blue ................................................................................ 10
   – Color brown, gonopores separated by 1/2 + 4 + 1/2 annuli .................
   ........................................................................................................ *Orobdella koikei* Nakano, 2012

10. Gonopores separated by 2/3 + 4 + 1/3, gastroporal duct simple tubular but bulbous at junction with gastropore ...... *Orobdella esulcata* Nakano, 2010
    – Gonopores separated by 6 annuli, gastroporal duct simple tubular ........
    ........................................................................................................ *Orobdella kawakatsuorum* Richardson, 1975

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References

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

Altekar G, Dwarkadas S, Huelsenbeck JP, Ronquist F (2004) Parallel Metropolis coupled Markov chain Monte Carlo for Bayesian phylogenetic inference. Bioinformatics 20: 407–415. doi: 10.1093/bioinformatics/btg427

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

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

Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Molecular Biology and Evolution 17: 540–552. doi: 10.1093/oxfordjournals.molbev.a026334

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

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

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

Gilyarov MS, Lukin EI, Perel TS (1969) The first terrestrial leech—Orobdella whitmani Oka (Hirudinel, Herpobdellidae)—in the fauna of the USSR: A Tertiary relict of forests of the southern Maritime Territory. Doklady Akademii Nauk SSSR 188: 235–237.

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

Hofacker IL, Fekete M, Stadler PF (2002) Secondary structure prediction for aligned RNA sequences. Journal of Molecular Biology 319: 1059–1066. doi: 10.1016/S0022-2836(02)00308-X

Huelsenbeck JP, Ronquist F, Nielsen R, Bollback JP (2001) Bayesian inference of phylogeny and its impact on evolutionary biology. Science 294: 2310–2314. doi: 10.1126/science.1065889
Jobb G, von Haeseler A, Strimmer K (2004) TREEFINDER: a powerful graphical analysis environment for molecular phylogenetics. BMC Evolutionary Biology 4: 18. doi: 10.1186/1471-2148-4-18

Katoh K, Toh H (2008) Improved accuracy of multiple ncRNA alignment by incorporating structural information into a MAFFT-based framework. BMC Bioinformatics 9: 212. doi: 10.1186/1471-2105-9-212

Lai Y-T, Chen J-H (2010) Leech Fauna of Taiwan. National Taiwan University Press, Taipei, 118 pp.

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

McCaskill JS (1990) The equilibrium partition function and base pair binding probabilities for RNA secondary structure. Biopolymers 29: 1105–1119. doi: 10.1002/bip.360290621

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

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

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

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

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

Nakano T (2012a) 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/zook.eys.169.2425

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

Nakano T (in press) Redescription of Orobdella octonaria (Hirudinida: Arhynchobdellida: Orobdellidae) with designation of a lectotype. Species Diversity.

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

Nakano T, Seo H-Y (in press) First record of Orobdella tsushimensis (Hirudinida: Arhynchobdellida: Orobdellidae) from Korea (Gageodo Island) and its molecular phylogenetic position within the genus. Species Diversity.

Oceguera-Figueroa A, Phillips AJ, Pacheco-Chaves B, Reeves WK, Siddall ME (2011) Phylogeny of macrophagous leeches (Hirudinea, Clitellata) based on molecular data and
evaluation of the barcoding locus. Zoologica Scripta 40: 194–203. doi: 10.1111/j.1463-6409.2010.00465.x

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

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

Rambaut A, Drummond AJ (2009) Tracer 1.5: http://tree.bio.ed.ac.uk/software/tracer/

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

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

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. doi: 10.1093/bioinformatics/btg180

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

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

Tabei Y, Kiryu H, Kin T, Asai K (2008) A fast structural multiple alignment method for long RNA sequences. BMC Bioinformatics 9: 33. doi: 10.1186/1471-2105-9-33

Tanabe AS (2008) Phylogears. 2.0: http://www.fifthdimension.jp/

Tanabe AS (2011) Kakusan4 and Aminosan: two programs for comparing nonpartitioned, proportional and separate models for combined molecular phylogenetic analyses of multilocus sequence data. Molecular Ecology Resources 11: 914–921. doi: 10.1111/j.1755-0998.2011.03021.x