



Redescription of *Orobdella ijimai* (Hirudinida: Arhynchobdellida: Gastrostomobdellidae), and two new species of *Orobdella* from the Ryukyu Archipelago, Japan

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Abstract

Sexannulate *Orobdella* leech, *Orobdella ijimai*, is redescribed based on newly collected specimens from the type locality, Nikko, Tochigi Pref., Japan, since this species was originally described based on limited number of characters. In addition, two new sexannulate species, *Orobdella dolichopharynx* **sp. nov.** from Amamioshima Island in the Ryukyu Archipelago, Japan, and *Orobdella shimadae* **sp. nov.** from Okinawajima Island also in the Ryukyu Archipelago, are described. They resemble *O. ijimai* in the annulation of mid-body somites, but they differ from the latter in the annulation of somite VIII, position of gonopores, the lack of gastropore, the length of pharynx, the shape of gastroporal duct, and the morphology of male genital organ. Two new *Orobdella* species differ from each other in the annulation of somite VII, the position of gonopores, and the shape of gastroporal duct. In accordance with the two new species, the diagnosis of *Orobdella* is slightly emended.

Key words: Hirudinida, Gastrostomobdellidae, *Orobdella ijimai*, new species, gastroporous, Japan

Introduction

The terrestrial macrophagous leech genus *Orobdella* Oka, 1895 includes six species which are divided into three groups according to a mid-body somite annulation (Nakano 2011): 1) *Orobdella esulcata* Nakano, 2010 (quadrannulate); 2) *Orobdella ijimai* Oka, 1895 (sexannulate); 3) *Orobdella kawakatsuorum* Richardson, 1975 (quadrannulate); 4) *Orobdella octonaria* Oka, 1895 (octannulate); 5) *Orobdella tsushimensis* Nakano, 2011 (quadrannulate); and 6) *Orobdella whitmani* Oka, 1895 (quadrannulate). This genus was placed in the family Gastrostomobdellidae Richardson, 1971, together with the genus *Gastrostomobdella* Moore, 1929 based on the presence of the gastropore and gastroporal duct (Richardson 1971; 1975; Nakano 2010). Gastrostomobdellid species are mountainous terrestrial leeches distributed in Southeast and East Asia, and Hawaii (Oka 1895; Moore 1929; 1935; 1946; Gilyarov *et al.* 1969; Richardson 1971; 1975; Lukin 1976; Sawyer 1986). Sawyer (1986) placed this family in Hirudiniformes on account of the euthylaematous pharynx. Recent molecular phylogenetic work revealed that *Orobdella* is included into Erpobdelliformes (Oceguera-Figueroa *et al.* 2011). However, the phylogenetic position of *Gastrostomobdella*, the type genus of this family, still remains uncertain.

Among the six known *Orobdella* species, sexannulate species, *Orobdella ijimai*, was originally described based on ten specimens from Nikko, Tochigi Pref., Japan (Nikkō in his paper) along with *O. whitmani* and *O. octonaria* (Oka 1895). This species was later recorded from Amamioshima Island in the Ryukyu Archipelago, Japan (Oka 1910a; b). However, *Orobdella ijimai* was originally established based on limited number of features. In addition, the type series of *O. ijimai* are missing. Therefore, in order to re-evaluate the morphology and diagnostic characters for *Orobdella ijimai*, new specimens were obtained from the type locality, Nikko, in May, 2010, and from Amamioshima Island in 2009–2010. In addition, sexannulate *Orobdella* specimens were newly collected from Okinawajima Island in the Ryukyu Archipelago in 2010. Herein, *Orobdella ijimai* is made the emended description, and two new *Orobdella* leeches from the Ryukyu Archipelago are described.

Material and methods

Leeches were collected from the type locality of *Orobdella ijimai*, Nikko, Tochigi Pref., Japan, and from Amami-oshima Island and Okinawajima Island in the Ryukyu Archipelago, Japan (Fig. 1). Leeches were found under rocks or fallen leaves along roads or mountain trails, either crawling on the ground or preying on earthworms, especially at night. Altitude and coordinates for localities were obtained using a Garmin eTrex GPS unit.

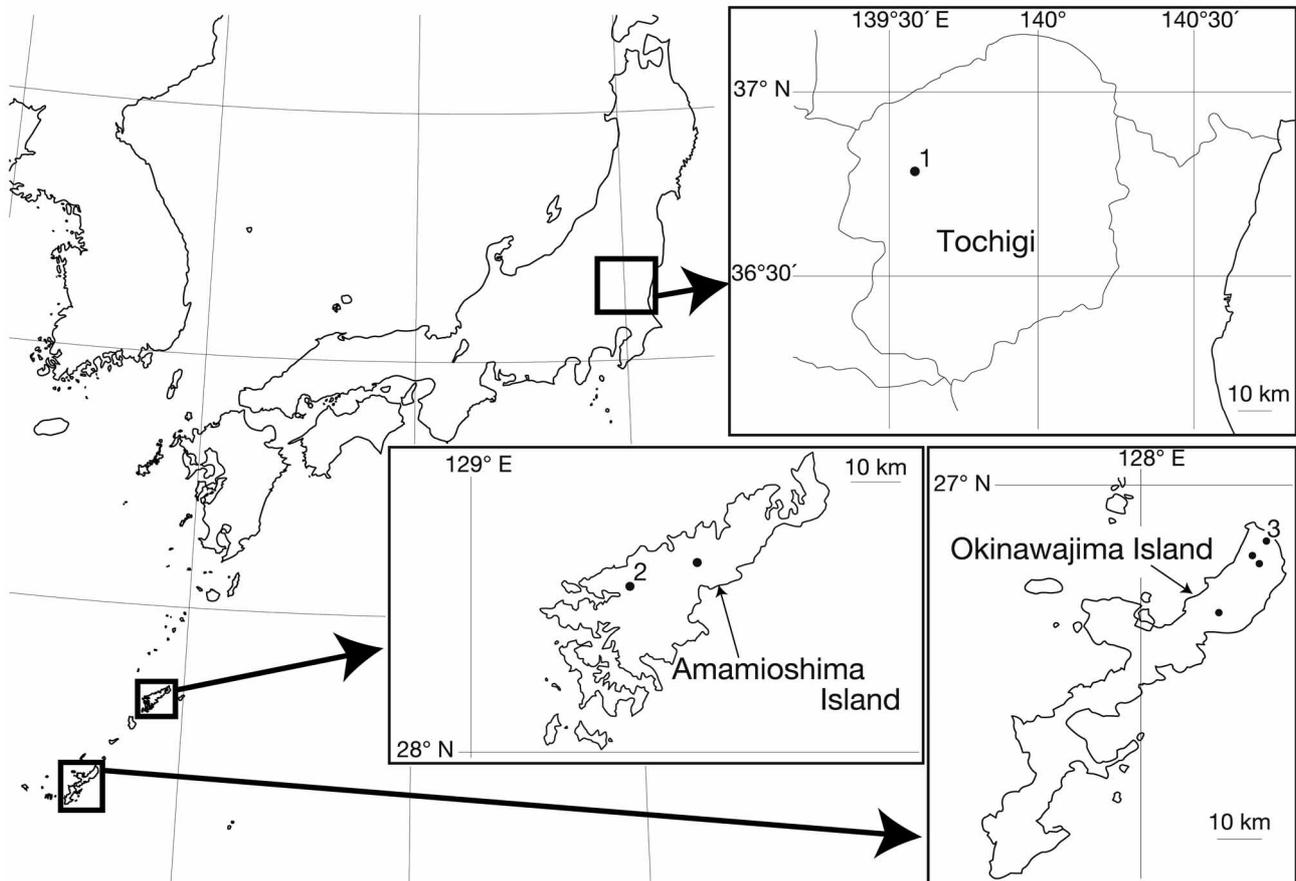


FIGURE 1. Location of collecting localities in this study. The locality numbers are referred to as follows: the type locality of, (1) *Orobdella ijimai* Oka, (2) *Orobdella dolichopharynx* sp. nov., and (3) *Orobdella shimadae* sp. nov.

Specimens were put into water and then relaxed with the gradual addition of 95% ethanol. Botryoidal tissue was taken from every specimen for future DNA extraction. Then they were fixed by 10% formalin for dissection and preserved in 70% ethanol. Two body measurements were taken after fixation: body length, from the anterior margin of the oral sucker to the posterior margin of the caudal sucker (BL), and maximum body width (BW). Examination, dissection and drawing of the specimens were accomplished under a stereoscopic microscope (LEICA S6E and WILD HEERBRUGG TYP 308700). Specimens used in this study have been deposited in the Zoological Collection of the Kyoto University Museum (KUZ).

The type series of *O. ijimai* were surveyed from Oka's collection housed at the National Museum of Nature and Science, Tokyo (NSMT) and in the zoological collection of The University Museum, The University of Tokyo (UMUTZ).

Numbering conventions are based on Moore (1927): 1) body somites are denoted by Roman numerals; and 2) annuli in each somite are given alphanumeric designation.

Abbreviations used in the figures are as follows: ac, atrial cornu; af, annular furrow; an, anus; at, atrium; cod, common oviduct; cp, crop; cs, caudal sucker; ed, ejaculatory duct; ep, epididymis; fp, female gonopore; g, ganglion; gd, gastroporal duct; gp, gastropore; in, intestine; mp, male gonopore; np, nephridiopore; o, ovisac; od, oviduct; ph, pharynx; t, testis; and vs, ventral sinus.

Results

Family Gastrostomobdellidae Richardson, 1971

Genus *Orobdella* Oka, 1895

Emended diagnosis. Annulation of mid-body somite variable (complete quadr-, sex-, or octannulate). Post-anal annulus absent. Pharynx terminating in XIV or XVI. Gastropore at the nephridioporale annulus of XIII or in the annular furrow between nephridioporale annulus and neural annulus of XIII., or absent. Gastroporal duct single, joining with crop in XIV, or not joining with crop. Nephridiopores variable, 17–20 pairs. Male gonopore in the last annular furrow of XI, at the last annulus of XI, or at the penultimate annulus of XI. Female gonopore at the nephridioporale annulus of XIII or in the annular furrow between nephridioporale annulus and neural annulus of XIII. Gonopores separated by one full somite. Testisacs multiple per somite. Male median reproductive system in XI, being weakly muscularized atrium with atrial cornua (eropbdelloid) or without atrial cornua, without penis sheath or penis. Ovisacs globular, in XIII. Oviducts short, converging into common oviduct. Female median reproductive system essentially lacking (micromorphic).

Remarks. The genus *Orobdella* differs from the type genus, *Gastrostomobdella*, in the following characteristics: post-anal annulus absent (present in *Gastrostomobdella*); Gastropore at XIII (XV); Gastroporal duct lies on female organ (behind female organ); ovisacs globular (elongated).

Type species. *Orobdella whitmani* Oka, 1895.

Orobdella ijimai Oka, 1895

(Figs. 2–4)

Orobdella ijimai Oka (1895): 284–285, pl. 28, figs. 2, 9–10, pl. 29, fig. B, pl. 30, figs. 2, 5–8; Oka (1910a): 19 (in part); Oka (1910b): 178 (in part); Oka (1935): 240; Oka & Nagao (1965): 574; Soós (1966): 376, 382, 397; Richardson (1971): 599–600; Lukin (1976): 466; Sawyer (1986): 680, 747.

Diagnosis. In life, dorsal surface yellowish green, ventral surface whitish yellow. Somite VII quadrannulate. Somites VIII–XXV complete sexannulate. Somite XXVI quadrannulate. Pharynx descending to XIII/XIV. Gastropore conspicuous at XIII b2. Gastroporal duct muscular, bulbiform, tortuous at junction with gastropore. Male gonopore at XI c12. Female gonopore inconspicuous at XIII b2, behind gastropore. Gonopores separated by 1/2 + 7 + 1/2 annuli.

Type specimens. The ten specimens, which were used in the original description of *O. ijimai* (Oka 1895), are automatically considered the syntypes in accordance with the Article 73.2 of the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature 1999). I surveyed leech collection at NSMT and UMUT (see Materials and Methods above), however, I could not find out any type series of *O. ijimai*.

According to the Art. 75.1 of the Code, a neotype should be designated when 1) no name-bearing type specimen is believed to be extant and 2) a name-bearing type is necessary to define the nominal taxon objectively (International Commission on Zoological Nomenclature 1999). The nomenclatural status of *Orobdella ijimai* does not apply the condition 2) above, because its type locality is only one place, Nikko, Japan and I did not collect any other sexannulate *Orobdella* species from the type locality. As shown at the Example of the Art. 75.2 of the Code, a neotype designation for such species like *O. ijimai* would be invalid (International Commission on Zoological Nomenclature 1999). Therefore, I have not designated a neotype for *O. ijimai* in this study.

Materials examined. A total of six specimens newly collected from around the Inarigawa Sand-tap Dams at the type locality, Nikko, Tochigi Pref., Japan by Takafumi Nakano. Four specimens collected on May 26, 2010: KUZ Z108 (alt. 960 m, 36°47'01"N, 139°34'54"E), dissected; KUZ Z109 (alt. 946 m, 36°46'59"N, 139°34'55"E); KUZ Z110, dissected, and KUZ Z111 (alt. 940 m, 36°46'59"N, 139°34'56"E). Two specimens collected on May 28, 2010: KUZ Z112 (alt. 990 m, 36°47'08"N, 139°34'54"E); and KUZ Z113, dissected (alt. 1014 m, 36°47'13"N, 139°34'51"E).

Emended description. Body firm, muscular, elongated, maximum BL 93.3 mm (KUZ Z111), maximum BW 6.3 mm (KUZ Z110) (Fig. 2). Caudal sucker ventral, oval, its diameter slightly smaller than maximum body width (Fig. 3D). In life, color variable, generally dorsal surface yellowish green, ventral surface whitish yellow, dorsal surface darker than ventral surface. Color faded in preservative, without any dorsal dark lines.

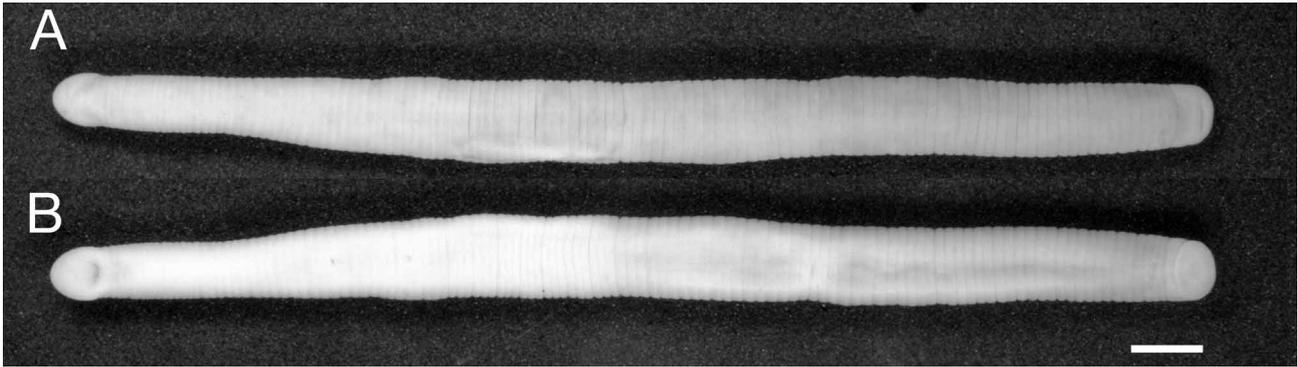


FIGURE 2. *Orobdella ijimai* Oka, KUZ Z108, from the type locality. (A) Dorsal, and (B) ventral surface. Scale bar, 5 mm.

Somite I completely merged with prostomium (Fig. 3A). Somite II uniannulate, slightly separated from I (Fig. 3A). Somite III biannulate, $(a1 + a2) > a3$ (Fig. 3A) or uniannulate (KUZ Z110). Somite IV biannulate, $(a1 + a2) > a3$ (Fig. 3A). Somite V biannulate, $a3$ forming posterior margin of oral sucker (Fig. 3A, B). Somites VI complete triannulate (Fig. 3A, B). Somite VII complete quadrannulate. Somites VIII–XXV complete sexannulate, $b1 = b2 = a2 = b5 = c11 = c12$ (Fig. 3A–E, H), X $b5$ the first annulus of the clitellum, XIII $a2$ the last annulus of the clitellum (KUZ Z110). Somite XXVI quadrannulate, $a1 = a2 = b5 > b6$, $b6$ the last complete annulus on the venter (Fig. 3C, D). Somite XXVII uniannulate or biannulate (Fig. 3C). Anus behind XXVII (Fig. 3C). Post-anal annulus absent (Fig. 3C).

Anterior ganglionic mass in VI $a1$ – $a3$. Ganglion VII in $a2$. Ganglia VIII–XXV in $a2$ of each somite, except ganglion XIV in $b5$ (Fig. 3H). Ganglion XXVI in $a1$. Posterior ganglionic mass in XXVI $b5$ and $b6$.

Eyes three pairs, first pair dorsally on posterior margin of II, second and third pairs dorsolaterally on posterior margin of V ($a1 + a2$) (Fig. 3A). Nephridiopores 17 pairs, ventrally at posterior margin of $b2$ of each somite, at VIII–XXIV (Fig. 3B, E). Papillae numerous, minute, hardly visible, one row on every annulus.

Pharynx agnathous, euthylaematous, reaching to XIII/XIV. Crop tubular, acecate, reaching to XX $c11/c12$ –XXI $a2$ (Fig. 4). Gastropore conspicuous, ventral, at middle of XIII $b2$ (Fig. 3E, G). Gastroporal duct muscular, tortuous at junction with gastropore, bulbiform, joining with crop in XIV $b1$ – $b2$ (Fig. 3F). Intestine tubular, acecate, reaching to XXIV $a2/b5$ – $c11$, one pair of undeveloped chambers at junction with crop or chamber absent (Fig. 4). Rectum tubular, thin-walled.

Male gonopore at the middle or posterior of XI $c12$ (Fig. 3E). Female gonopore at XIII $b2$, inconspicuous, diagonally behind gastropore (Fig. 3G). Gonopores separated by $1/2 + 7 + 1/2$ annuli (Fig. 3E). Testisacs multiple, two or three testisacs on each side in each annulus, in XVIII $c11$ –XIX $a2$ to XXV $b1$ – $b5$ (Fig. 3H). Epididymides in XVI $b5/c11$ – $c11/c12$ to XVIII $c11$ –XIX $a2$ (Fig. 3H). Ejaculatory bulbs absent. Ejaculatory ducts in XI $b5$ to XVI $b5/c11$ – $c11/c12$, loosely coiled, widen gradually from each junction with epididymis, narrow at each junction with atrial cornu, then turning sharply inward toward atrial cornu without pre-atrial loop (Fig. 3H–K). Atrial cornua in XI $b5$ to $c12$, muscular, nearly oviform (Fig. 3H–K). Atrium short, muscular, globular in XI $c12$ (Fig. 3I–K). Ovisacs one pair, thin-walled, globular, in XIII $a2$ to $c11$ (Fig. 3H, L). Oviduct thin-walled, left oviduct crossing ventrally beneath nerve cord, both oviducts converging into common oviduct in XIII $a2$ (Fig. 3H, L). Common oviduct thin-walled, short, directly descending to female gonopore (Fig. 3L).

Remarks. The original description of *O. ijimai* is accurate in the number of annuli, $1/2 + 7 + 1/2$ annuli, between gonopores (Oka 1895). This characteristic, possessing total 129 annuli and having complete sexannulate mid-body somites have been the key external features of *O. ijimai*. Characteristics of the specimens examined in this study were coincident with the above characteristics in the original description. Specimens newly collected from Nikko were thus identified as *O. ijimai*.

Oka (1895) and Soós (1966) treated *O. ijimai* without any reference to a gastroporal duct. But *Orobdella ijimai* has a conspicuous gastropore and bulbiform gastroporal duct (Fig. 3F, G). However, an inconspicuous female gonopore is distinguished beside a conspicuous gastropore only by a greater magnification. Therefore, a gastropore of *O. ijimai* is easily mistaken for a female gonopore.

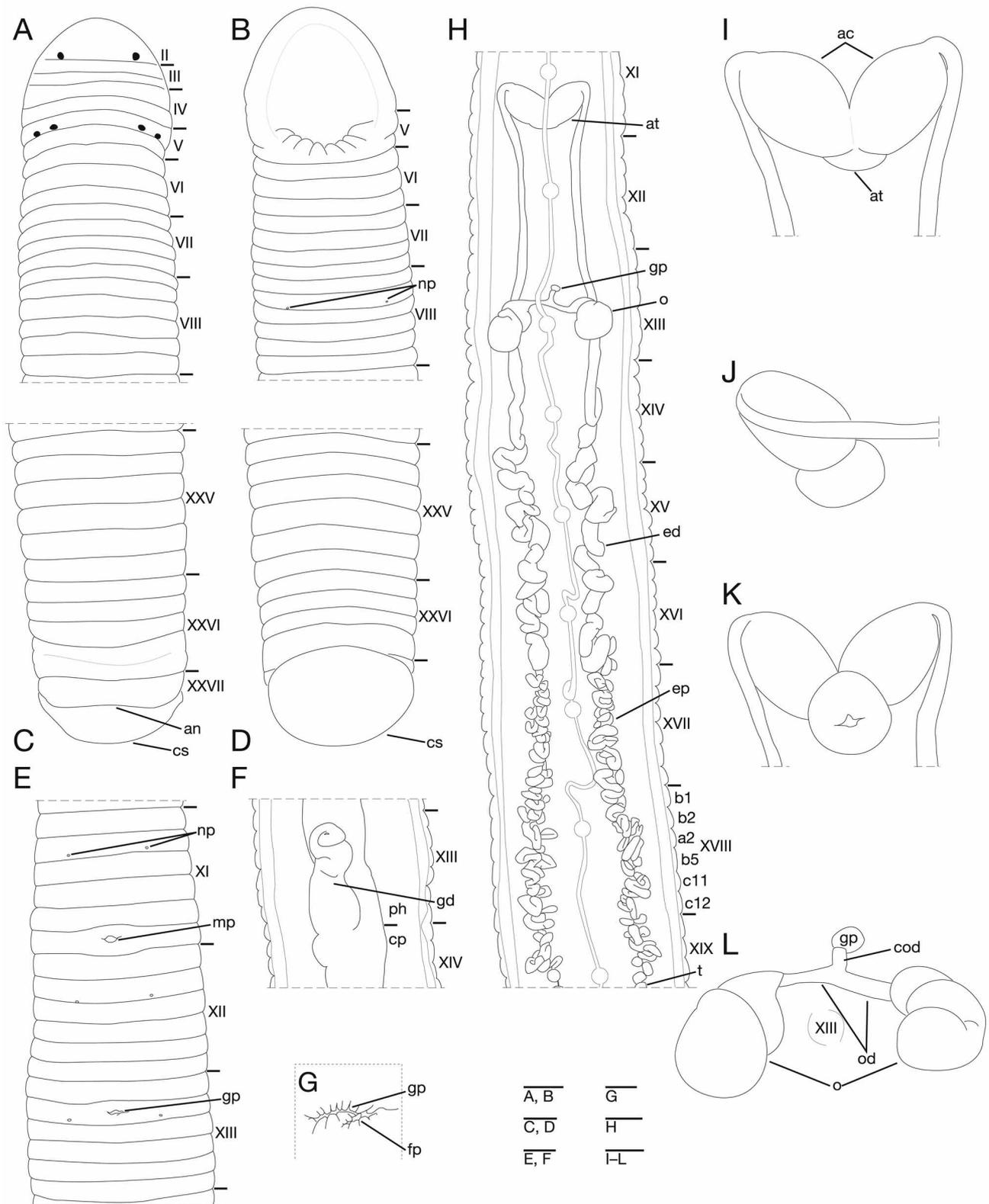


FIGURE 3. *Orobdella ijimai* Oka, KUZ Z108, from the type locality. (A) Dorsal, and (B) ventral views of somites I–VIII. (C) Dorsal, and (D) ventral views of somites XXV–XXVII and caudal sucker. (E) Ventral view of somites XI–XIII. (F) Ventral view of gastroporal duct. (G) Ventral view of gastropore and female gonopore. (H) Dorsal view of reproductive system including ventral nervous system. (I) Dorsal, (J) lateral, and (K) ventral views of male atrium. (L) Dorsal view of female reproductive system including position of ganglion XIII. Scale bars, 1 mm (A–F, H), 0.5 mm (I–L), 0.25 mm (G).

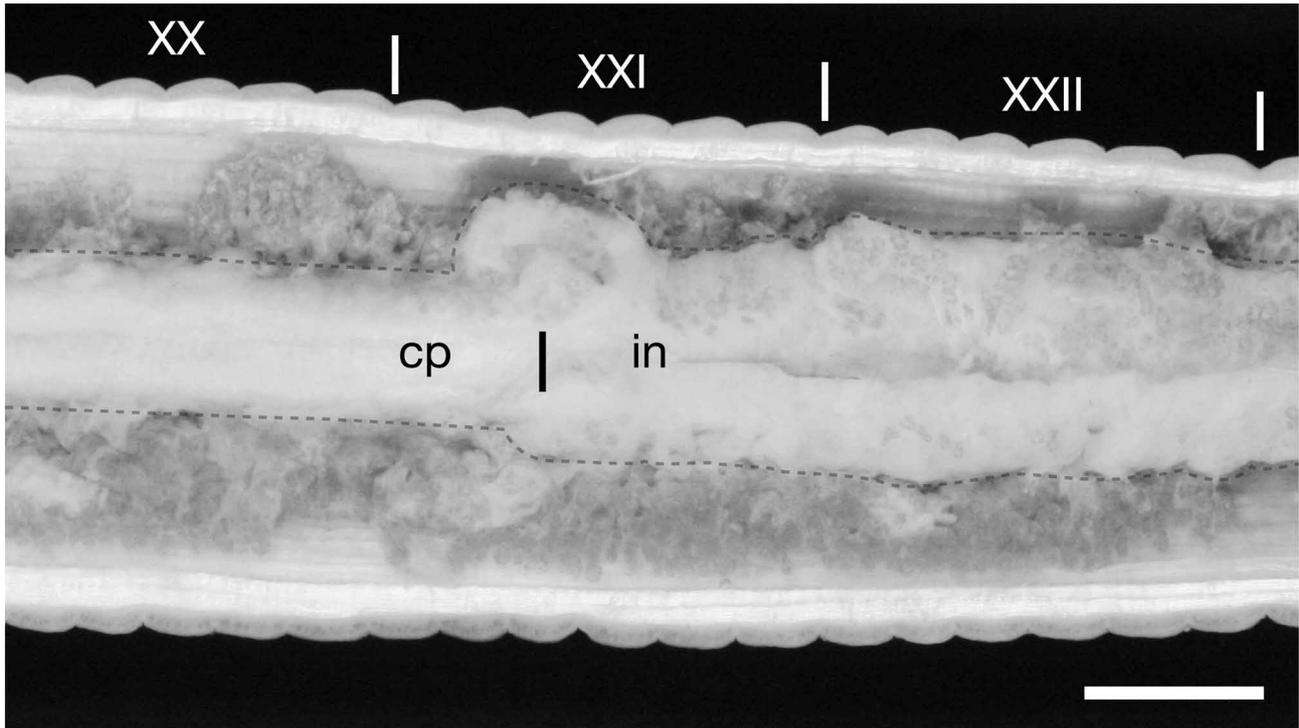


FIGURE 4. *Orobdella ijimai* Oka, KUZ Z108, from the type locality. Ventral view of crop and intestine. Scale bar, 2 mm.

Oka (1895) noted that this species had post-ceca. In the examined specimens of *Orobdella ijimai*, I could not establish any crop ceca or intestinal ceca. But the intestine has an undeveloped chamber at the junction with crop (Fig. 4). Therefore, the post-ceca in the original description by Oka (1895) might be those chambers as mentioned by Richardson (1975). The original description also noted that *O. ijimai* possesses the first pair of eyes on the anterior margin of III, the first pair of nephridiopores at IX b2 and the last pair of nephridiopores at XXV b2. However, the first pair of eyes is on the posterior margin of II (Fig. 3A), the first pair of nephridiopores is at VIII b2 (Fig. 3B) and the last pair of nephridiopores is at XXIV b2 in the examined specimens.

Distribution and ecology. Known from Honshu, Shikoku, Kyushu, Japan (Oka & Nagao 1965). Oka (1910a; b) recorded *O. ijimai* from Amamioshima Island in the Ryukyu Archipelago, Japan. However, the sexannulate *Orobdella* leech from that island is another species as described below. Leeches were found under rocks or fallen leaves.

***Orobdella dolichopharynx* sp. nov.**

(Figs. 5–7)

Orobdella ijimai: Oka (1910a): 19 (in part); Oka (1910b): 178 (in part).

Diagnosis. In life, dorsal surface yellowish green, ventral surface whitish yellow. Somite VII quadrannulate. Somite VIII quinquannulate. Somites IX–XXV complete sexannulate. Somite XXVI quinquannulate. Pharynx reaching to XVI. Gastropore absent. Gastroporal duct narrow, tubular, not joining with crop. Male gonopore in XI c11/c12 or at anterior of XI c12, close to c11/c12. Female gonopore in XIII b1/b2 or at anterior of XIII b2, close to b1/b2. Gonopores separated by eight annuli. Pre-atrial loop present, extending to ganglion XI. Atrial cornua absent.

Materials examined. *Holotype*. KUZ Z120, mature specimen of 106.7 mm length, dissected, collected from under fallen leaves along a road at Mt. Yuwandake (alt. 448 m, 28°17'11"N, 129°18'56"E), Uken, Amamioshima Island, Kagoshima Pref., Japan, by Takafumi Nakano on April 30, 2010. *Paratypes* (a total of nine specimens collected from Amamioshima Island in 2009–2010). Six specimens collected from the type locality by Takafumi Nakano: KUZ Z115 (alt. 520 m, 28°17'27"N, 129°18'58"E), dissected, on March 6, 2009; KUZ Z116 (alt. 536 m,

28°17'29"N, 129°19'00"E), dissected, on March 12, 2009; KUZ Z117 (alt. 440 m, 28°17'06"N, 129°18'55"E), on August 28, 2009; KUZ Z118 (alt. 606 m, 28°17'42"N, 129°19'06"E), dissected, on April 27, 2010; KUZ Z119 (alt. 434 m, 28°17'08"N, 129°18'55"E), dissected, and KUZ Z121 (alt. 448 m, 28°17'11"N, 129°18'56"E), dissected, on April 30, 2010. Three specimens collected from Kinsakubaru, by Masahiro Nishi on July 26, 2010: KUZ Z122, dissected, KUZ Z123 and KUZ Z124.

Etymology. The specific name is a compound noun in apposition derived from the Greek words transliterated into Latin, *dolicho* (long), and *pharynx* (pharynx), referring that the pharynx of this new species is reaching to somite XVI (those of the other known *Orobdella* species in Japanese islands north of the Ryukyu Archipelago are reaching to somite XIV).

Description of holotype. Body firm, muscular, BL 106.7 mm, BW 6.6 mm (Fig. 5). Caudal sucker ventral, oval, its diameter slightly smaller than maximum body width (Fig. 6D). In life, dorsal surface yellowish green, ventral surface whitish yellow, dorsal surface darker than ventral surface. Color faded in preservative, without any dark lines.

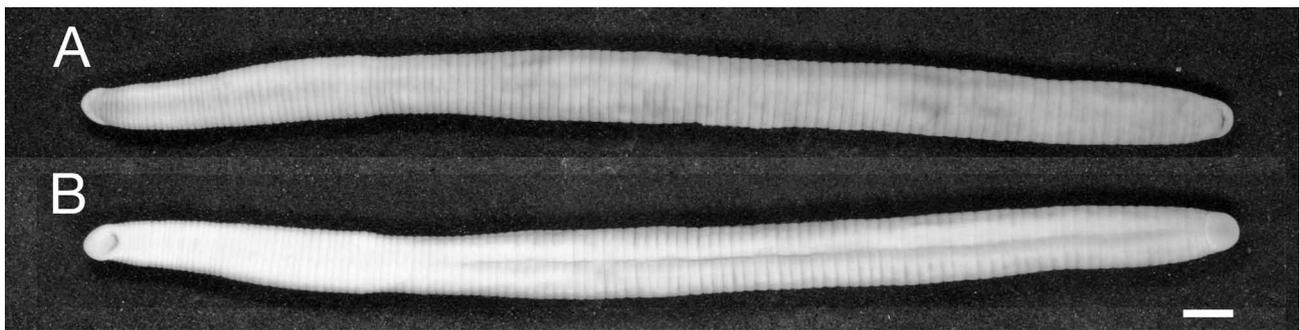


FIGURE 5. *Orobdella dolichopharynx* sp. nov., holotype, KUZ Z120. (A) Dorsal, and (B) ventral surface. Scale bar, 5 mm.

Somite I completely merged with prostomium (Fig. 6A). Somite II uniannulate, not separated from I (Fig. 5A). Somite III uniannulate (Fig. 6A). Somite IV biannulate (Fig. 6A). Somite V biannulate, a3 forming posterior margin of oral sucker (Fig. 6A, B). Somites VI triannulate (Fig. 6A, B). Somite VII complete quadrannulate, a1 = a2 > b5 = b6 (Fig. 6A, B). Somite VIII complete quinquannulate, a1 > a2 = b5 > c11 = c12 (Fig. 6A, B). Somite IX complete sexannulate, b1 < b2 = a2 = b5 > c11 = c12. Somites X–XXV complete sexannulate, b1 = b2 = a2 = b5 = c11 = c12 (Fig. 6C–E, H), X a2 the first annulus of the clitellum, XIII b5 the last annulus of the clitellum. Somite XXVI quinquannulate, b1 = b2 = a2 < b5 > b6, b6 the last complete annulus on the venter (Fig. 6C, D). Somite XXVII incomplete biannulate (Fig. 6C). Anus behind XXVII (Fig. 6C). Post-anal annulus absent (Fig. 6C).

Anterior ganglionic mass in VI a2 and a3. Ganglion VII in a2. Ganglia VIII–XXI in a2 of each somite (Fig. 6H). Ganglia XXII–XXV in b2 and a2 of each somite. Ganglion XXVI in b1 and b2. Posterior ganglionic mass in XXVI a2–b6.

Eyes three pairs, first pair dorsally in II, second and third pairs dorsolaterally on posterior margin of V (a1 + a2) (Fig. 6A). Nephridiopores 17 pairs, ventrally at posterior margin of a1 at VIII and b2 of each somite, at IX–XXIV (Fig. 6B, E). Papillae numerous, minute, hardly visible, one row on every annulus.

Pharynx agnathous, euthylaematous, reaching to XVI b2/a2. Crop tubular, acecate, in XVI b2/a2 to XXII a2. Gastropore absent (Fig. 6E, G). Gastroporal duct narrow, tubular but slightly bulbous at female gonopore, in XIII b1/b2 to XVI b2, not joining with crop (Fig. 6F). Intestine tubular, acecate, in XXII a2 to XXIV/XXV. Rectum tubular, thin-walled.

Male gonopore at anterior of XI c12, close to annular furrow of c11/c12 (Fig. 6E). Female gonopore at anterior of XIII b2, close to annular furrow of b1/b2 (Fig. 6E, G). Gonopores separated by 8 annuli (Fig. 6E). Testisacs multiple, two or three testisacs on each side in each annulus, in XVII b5 to XXV b5 (Fig. 6H). Epididymides absent. Ejaculatory bulbs absent (Fig. 6H). Ejaculatory ducts in XI a2 to XVII a2, coiled, widen from each junction with testisacs, narrow gradually toward each junction with atrium, with pre-atrial loop extending to ganglion XI (Fig. 6H, I). Atrial cornua absent. Atrium rudimentary in XI c11 and c12 (Fig. 6H, I). Ovisacs one pair, thin-walled, globular, in XIII a2 (Fig. 6H, J). Oviduct thin-walled, right oviduct crossing ventrally beneath nerve cord, both oviducts converging into common oviduct in XIII b2 (Fig. 6H, J). Common oviduct thin-walled, very short, directly descending to female gonopore (Fig. 6H, J).

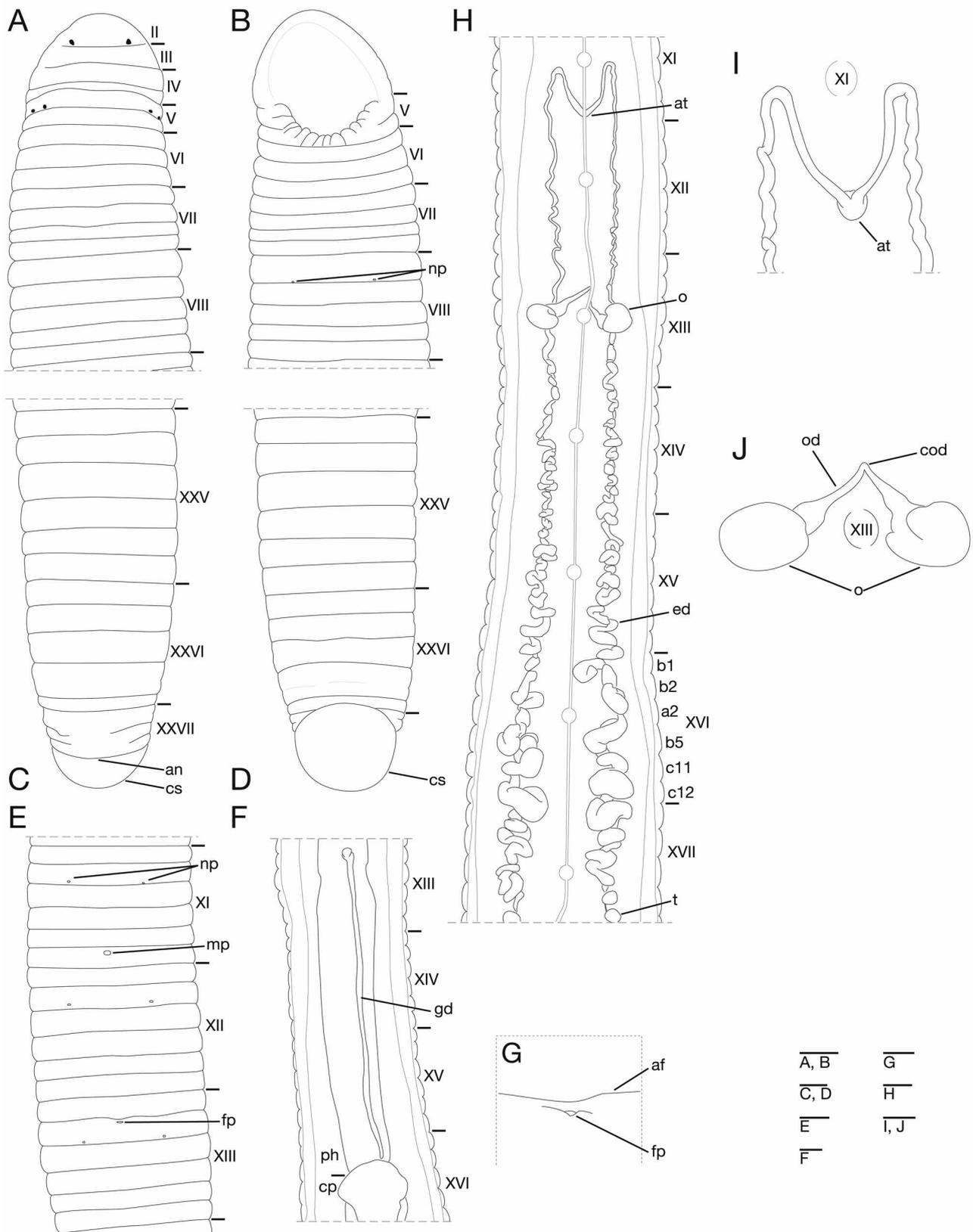


FIGURE 6. *Orobdella dolichopharynx* sp. nov., holotype, KUZ Z120. (A) Dorsal, and (B) ventral views of somites I–VIII. (C) Dorsal and (D) ventral views of somites XXV–XXVII and caudal sucker. (E) Ventral view of somites XI–XIII. (F) Ventral view of gastroporal duct. (G) Ventral view of female gonopore. (H) Dorsal view of reproductive system including ventral nervous system. (I) Dorsal view of male atrium. (J) Dorsal view of female reproductive system including position of ganglion XIII. Scale bars, 1 mm (A–F, H), 0.5 mm (I, J), 0.25 mm (G).

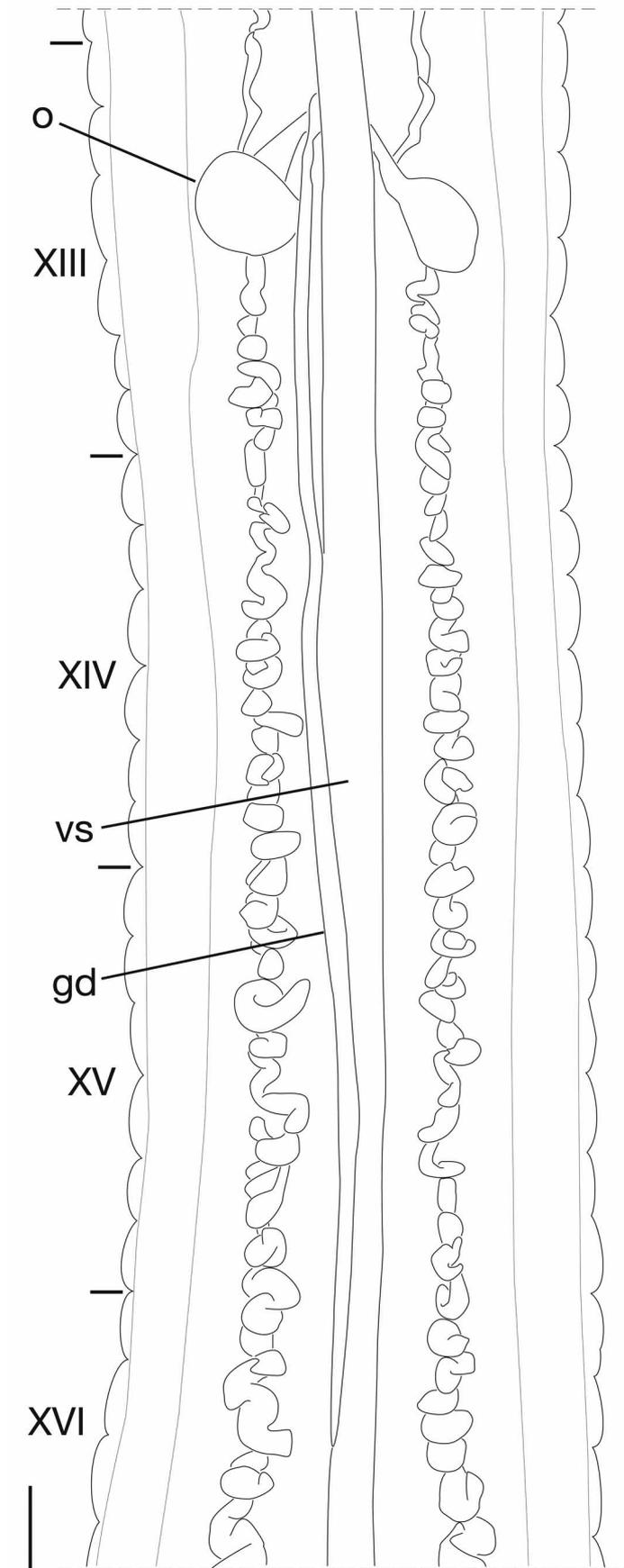


FIGURE 7. *Orobdella dolichopharynx* sp. nov., paratype, KUZ Z118, from the type locality. Dorsal view of gastroporal duct and reproductive system including ventral sinus. Scale bar, 1mm.

Variation. Somite VII triannulate (KUZ Z124) or ventrally triannulate (KUZ Z118, Z122). Somite XXVII incomplete biannulate or triannulate. Clitellum from X b5 to XIII a2–XIV b1. Pharynx reaching to XVI b1–c11. Crop reaching to XXII b1–XXIII b1. Gastroporal duct tends to attach ventral sinus (Fig. 7). Intestine reaching to XXIV c11–XXV b1/b2. Male gonopore in XI c11/c12 or at anterior XI c12. Female gonopore in XIII b1/b2 or at anterior of XIII b2. Testisacs in XVIII b1–XVIII b5 to XXV a2–c11.

Distribution and ecology. Known from the mountain region of Amamiyoshima Island in the Ryukyu Archipelago, Japan (Fig. 1). Leeches were found under rocks or fallen leaves.

Remarks. *Orobdella dolichopharynx* was considered to be *Orobdella ijimai* (Oka 1910a; b). However, *O. dolichopharynx* is easily distinguished from the latter by the number of annuli between the gonopores.

***Orobdella shimadae* sp. nov.**

(Figs. 8, 9)

Diagnosis. In life, dorsal surface greenish yellow, ventral surface whitish yellow. Somite VII triannulate. Somite VIII quinquannulate. Somites IX–XXV complete sexannulate. Somite XXVI quinquannulate. Pharynx reaching to XVI. Gastropore absent. Gastroporal duct narrow, tubular, descending to XV a2. Male gonopore in XI b5/c11 or at anterior of XI c11, close to b5/c11. Female gonopore in XIII b1/b2 or at anterior of XIII b2, close to b1/b2. Gonopores separated by nine annuli. Pre-atrial loop present, extending to ganglion XI. Atrial cornua absent.

Materials examined. *Holotype.* KUZ Z128, mature specimen of 100.9 mm in body length, dissected, collected from in the soil along the Okuyona Forest Road (alt. 100 m, 26°49.0' N, 128°16.5' E), Kunigami, Okinawajima Island, Okinawa Pref., Japan, by Taku Shimada on April 9, 2010. *Paratypes* (a total of nine specimens collected from Okinawajima Island, Japan). Six specimens collected by Taku Shimada: KUZ Z125 from Kunigami on February 15, 2010; KUZ Z126, dissected, and KUZ Z127, dissected, from Higashi on April 9, 2010; KUZ Z130, dissected, from near the type locality on June 17, 2010; KUZ Z137, and KUZ Z138, dissected, from the Okuni Forest Road (26°40.2' N, 128°11.2' E), Higashi, on September 6, 2010; and KUZ Z139 from the Okuyona Forest Road around the Benoki Dam (26°47.0' N, 128°16.3' E), Kunigami, on September 7, 2010. Two specimens collected from around the Benoki Dam, Kunigami on August 24, 2010: KUZ Z131 (alt. 176 m, 26°47'20" N, 128°15'22" E), dissected, by Takafumi Nakano; KUZ Z132 (alt. 191 m, 26°47'22" N, 128°15'21" E), by Keiko Tsubokawa.

Etymology. The specific name is a noun in the genitive case formed from the Latinized name of Mr. Taku Shimada who collected many valuable specimens of this species from Okinawajima Island.

Description of holotype. Body firm, muscular, BL 100.9 mm, BW 6.3 mm (Fig. 8). Caudal sucker ventral, oval, its diameter slightly smaller than maximum body width (Fig. 9D). In life, dorsal surface greenish yellow, ventral surface whitish yellow, dorsal surface darker than ventral surface. In preservative, color faded, without any dark lines.

Somite I completely merged with prostomium (Fig. 9A). Somite II uniannulate, not separated from I (Fig. 9A). Somite III uniannulate (Fig. 9A). Somite IV biannulate (Fig. 9A). Somite V biannulate, a3 forming posterior margin of oral sucker (Fig. 9A, B). Somites VI triannulate (Fig. 9A, B). Somite VII complete triannulate, a1 = a2 < a3 (Fig. 9A, B). Somite VIII complete quinquannulate, a1 > a2 = b5 > c11 = c12 (Fig. 9A, B). Somite IX complete sexannulate, b1 < b2 = a2 = b5 > c11 = c12. Somites X–XXV complete sexannulate, b1 < b2 = a2 = b5 > c11 = c12, or b1 = b2 < a2 = b5 > c11 = c12 (Fig. 9C–E), X b5 the first annulus of the clitellum, XIII a2 the last annulus of the clitellum. Somite XXVI sexannulate dorsally, b1 = b2 = a2 < b5 > c11 = c12, quinquannulate ventrally, b1 = b2 = a2 < b5 < b6, b6 with slight furrow and the last complete annulus on the venter (Fig. 9C, D). Somite XXVII incomplete triannulate (Fig. 9C). Anus behind XXVII (Fig. 9C). Post-anal annulus absent (Fig. 9C).

Anterior ganglionic mass in VI a2 and a3. Ganglion VII in VII a2. Ganglia VIII–XXIV in a2 of each somite (Fig. 9H). Ganglion XXV in XXV b2; ganglion XXVI in XXVI b1. Posterior ganglionic mass in XXVI a2–b6.

Eyes three pairs, first pair dorsally in II, second and third pairs dorsolaterally on posterior margin of V (a1 + a2) (Fig. 9A). Nephridiopores 17 pairs, ventrally at posterior margin of a1 at VIII and b2 of each somite, at IX–XXIV (Fig. 9B, E). Papillae numerous, minute, hardly visible, one row on every annulus.

Pharynx agnathous, euthylaematous, reaching to XVI a2/b5. Crop tubular, acecate, in XVI a2/b5 to XXII b2. Gastropore absent (Fig. 9E, G). Gastroporal duct narrow, tubular but slightly bulbous at female gonopore, in XIII b1/b2 to XV b2, not joining with crop (Fig. 9F). Intestine tubular, acecate, in XXII b2 to XXV b1. Rectum tubular, thin-walled.

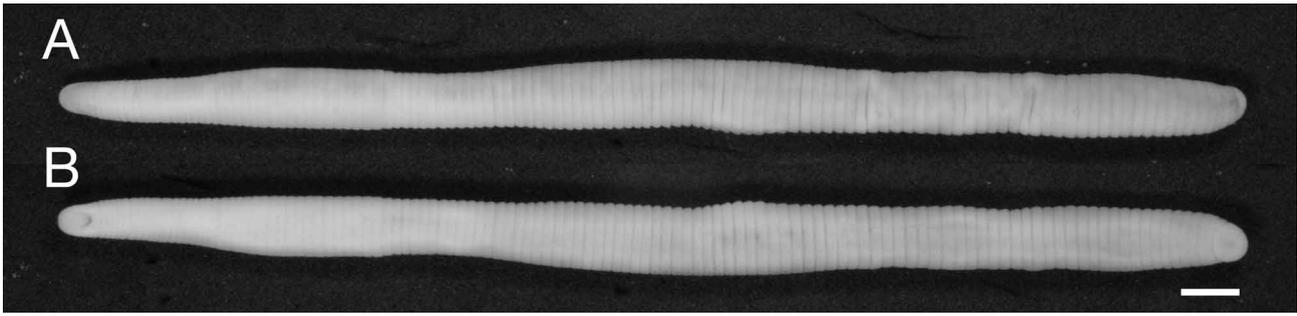


FIGURE 8. *Orobdella shimadae* sp. nov., holotype, KUZ Z128. (A) Dorsal, and (B) ventral surface. Scale bar, 5 mm.

Male gonopore at anterior of XI c11, close to annular furrow of b5/c11 (Fig. 9E). Female gonopore at anterior of XIII b2, close to annular furrow of b1/b2 (Fig. 9E, G). Gonopores separated by 9 annuli (Fig. 9E). Testisacs multiple, two or three testisacs on each side in each annulus, in XVIII b5 to XXV a2. Epididymides absent. Ejaculatory bulbs absent. Ejaculatory ducts in XI a2 to XVII c11, coiled, widen from each junction with testisacs, narrow gradually toward each junction with atrium, with pre-atrial loop extending to ganglion XI (Fig. 9H, I). Atrial cornua absent. Atrium rudimentary in XI b5 and c11 (Fig. 9H, I). Ovisacs one pair, thin-walled, globular, in XIII a2 and b5 (Fig. 9H, J). Oviduct thin-walled, right oviduct crossing ventrally beneath nerve cord, both oviducts converging into common oviduct in XIII b2 (Fig. 9H, J). Common oviduct thin-walled, very short, directly descending to female gonopore (Fig. 9H, J).

Variation. Median black line on dorsal surface present or absent in preservative. Somite XXVI quinquannulate or sexannulate (KUZ Z139). Somite XXVII incomplete biannulate or triannulate; clitellum from X b5 to XIII a2. Pharynx reaching to XVI a2/b5–c11/c12. crop reaching to XXII b2–XXII a2/b5. Intestine reaching to XXIV b5/c11–XXIV c12. Male gonopore in XI c11/c12 or at anterior XI c12, close to annular furrow of b5/c11. Female gonopore in XIII b1/b2 or at anterior of XIII b2, close to annular furrow of b1/b2. Testisacs in XVI c11–XVII a2/b5 to XXIV a2–XXV b2.

Distribution and ecology. Known from the mountain region of the northern part of Okinawajima Island in the Ryukyu Archipelago, Japan (Fig. 1). Leeches were found under rocks or fallen leaves.

Remarks. *Orobdella shimadae* is extremely similar to *Orobdella dolichopharynx* in Amamioshima Island. In *Orobdella shimadae*, however, its gastroporal duct is reaching only to XV b2 (to XVI b2 in *O. dolichopharynx*). In accordance with the strict morphological uniqueness, *Orobdella* leeches from Okinawajima Island and Amamioshima Island is treated as the two distinct species, *O. shimadae* and *O. dolichopharynx*, respectively in this study.

Discussion

Soós (1966) reported that the annulation of *Orobdella ijimai* was $b1 = b2 = b3 = b4 = b5 = b6$, because the mid-body somites were composed of six nearly equally long annuli. Certainly, all of the six annuli of the mid-body somite in *O. ijimai* are the same in length. If a2 of each mid-body somite were divided into b3 and b4, the ganglion would be in b3 and b4 of each somite in accordance with the annulation pattern by Moore (1927). However, ganglia of the mid-body somites of *O. ijimai*, *O. dolichopharynx* and *O. shimadae* are not in those two annuli. Furthermore, annulation of somites VII (quadrannulate, $a1 = a2 > b5 = b6$ or triannulate, $a1 = a2 < a3$) and VIII (quinquannulate, $a1 > a2 = b5 > c11 = c12$) in *O. dolichopharynx* (Fig. 6A, B) and *O. shimadae* (Fig. 9A, B) suggested that a3 was divided into b5 and b6, b6 was into c11 and c12, and then a1 was divided into b1 and b2 in those species (Fig. 10). The annulation of mid-body somites is evidently $b1 = b2 = a2 = b5 = c11 = c12$ in sexannulate *Orobdella* species.

Among the known *Orobdella* species, *Orobdella ijimai* was the only one where it was unclear whether it was a gastroporous species (Richardson 1971; Sawyer 1986; Nakano 2010). The examination of my specimens revealed that *Orobdella ijimai* is a gastroporous species. In the two new species, *O. dolichopharynx* and *O. shimadae*, the gastroporal duct is very narrow and opening neither to the body wall, nor to the crop (Figs. 6F–G, 9F–G). Their

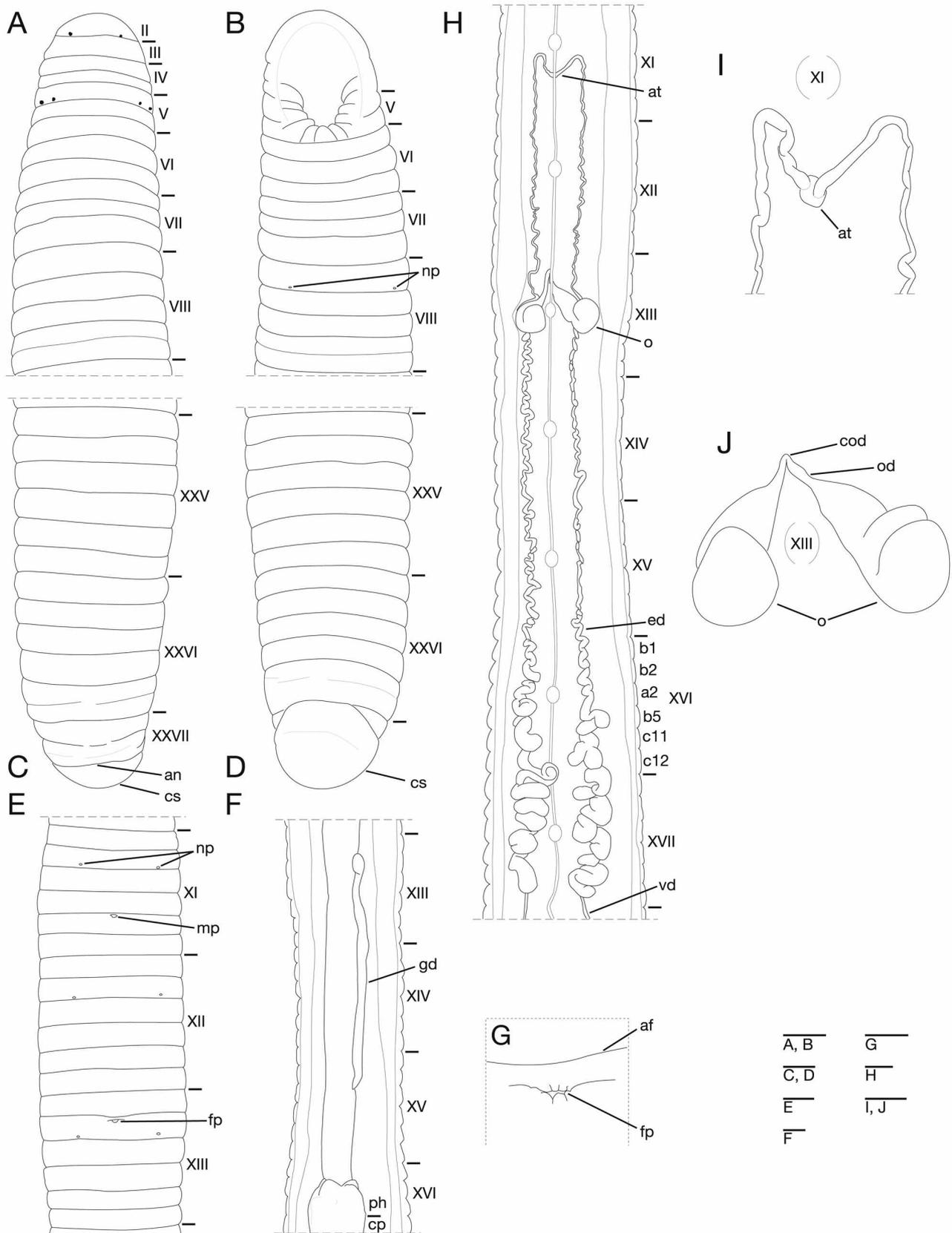


FIGURE 9. *Orobdella shimadae* sp. nov., holotype, KUZ Z128. (A) Dorsal, and (B) ventral views of somites I–VIII. (C) Dorsal and (D) ventral views of somites XXV–XXVII and caudal sucker. (E) Ventral view of somites XI–XIII. (F) Ventral view of gastroporal duct. (G) Ventral view of female gonopore. (H) Dorsal view of reproductive system including ventral nervous system. (I) Dorsal view of male atrium. (J) Dorsal view of female reproductive system including position of ganglion XIII. Scale bars, 1 mm (A–F, H), 0.5 mm (I, J), 0.25 mm (G).

rudimentary ducts apparently seem not to be a gastroporal duct. However, their ducts can be recognized as a gastroporal duct in accordance with their morphological and positional similarities with normally developed gastroporal ducts. The gastroporal duct is a shared character of the genus *Orobdella*.

Despite the discovery of mountainous gastroporous leeches by Moore (1929) about a century ago, the function of the gastropore and gastroporal ducts of terrestrial species is not understood (Nakano 2010). Richardson (1975) reported that a gastroporal duct of the paratype of *O. kawakatsuorum* did not descend to a gastropore. But, no rudimentary gastroporal duct has been reported in the genus *Orobdella* with the exception of *O. dolichopharynx* and *O. shimadae*. Besides, the pharynges of two *Orobdella* species from the Ryukyu Archipelago are longer than those of other known *Orobdella* species. There is a possibility of some correlations between those rudimentary gastroporal ducts and the long pharynges in *Orobdella* leeches in the Ryukyu Archipelago.

The male genital organs of the two new species are also unique. Unlike the other known *Orobdella* species, *O. dolichopharynx* and *O. shimadae* lack epididymides and atrial cornua, but possess pre-atrial loops. The loops reach anteriorly exactly the same length as the cornua of *O. ijimai*. Thus, there is a possibility that those loops are non-muscular atrial cornua. However, the loops of several specimens slightly coil just in front of the atrium (e.g. Fig. 9I). Therefore, the loops are recognized as the pre-atrial loops in this study. The rudimentary atrium without atrial cornua of the two new species would form a peculiar spermatophore. However, mating behavior or spermatophores of any *Orobdella* species have not been reported yet. Further ecological and ethological studies are needed to reveal mating behavior of *Orobdella* leeches.

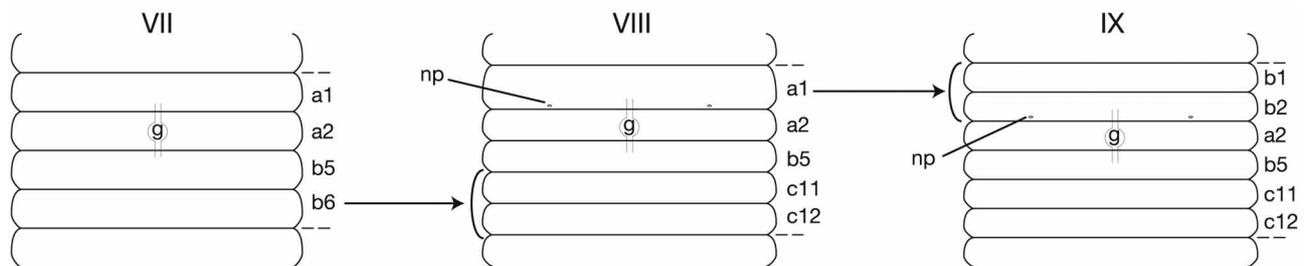


FIGURE 10. Schematic drawings of sexannulate annulation pattern in the genus *Orobdella* based on annulations of somites VII–IX of *O. dolichopharynx* **sp. nov.**

Comparisons

Two new sexannulate *Orobdella* species from the Ryukyu Archipelago, *Orobdella dolichopharynx* and *Orobdella shimadae*, differ from another sexannulate species, *Orobdella ijimai*, in the following characters: 1) annulation of VII; 2) male gonopore in XI c11/c12 or at anterior of XI c12, or in XI b5/c11 or at anterior of XI c11; 3) gastropore absent, female gonopore in XIII b1/b2 or at anterior of XIII b2; 4) pharynx descending to XVI; 5) gastroporal duct narrow tubular but slightly bulbous on female gonopore; 6) epididymis absent; and 7) atrium cornua absent, atrium rudimentary (Table 1). Two *Orobdella* species from the Archipelago can be distinguished from each other in three characters: 1) annulation of VII; 2) position of male gonopore; and 3) morphology of gastroporal duct (Table 1).

In the genus *Orobdella*, the annulation of mid-body somites is stable within a species (Nakano, unpublished data). Therefore, *Orobdella dolichopharynx*, *O. shimadae* and also *O. ijimai*, are easily distinguished from *O. esulcata*, *O. kawakatsuorum*, *O. octonaria*, and *O. whitmani*, in having mid-body somites that are sexannulate, while they are quadrannulate in *O. esulcata*, *O. kawakatsuorum* and *O. whitmani*, and octannulate in *O. octonaria*.

Acknowledgements

The author is grateful to Professor Tsutomu Hikida (Kyoto University) and Dr. Mark E. Siddall (American Museum of Natural History) for their helpful comments and suggestions, which improved this manuscript, and to Masahiro Nishi (Amami Mongoosebusters) and Taku Shimada (Ant Room) for sending me valuable *Orobdella* specimens from the Ryukyu Archipelago. I am also grateful to Dr. Rei Ueshima (The University of Tokyo) and

Dr. Hironori Komatsu (National Museum of Nature and Science, Tokyo) for allowing me to survey leech collections under their care, to Professor Boris Sket (University of Ljubljana) for his constructive comment on this manuscript, to Dr. Hidetoshi Nagamasu (The Kyoto University Museum) for his helpful comment on scientific names, and to Satoshi Ajitani, Koshiro Eto, Takuma Hashimoto (Japan Wildlife Research Center), Kazuaki Kimura, Tomoya Kishioka, Naoki Koike, Kazuki Kurita, Hirofumi Okumura, Kenji Suetsugu, Masashi Sugimoto, Nahoko Tokuyama, Nobuyuki Toyoda, Keiko Tsubokawa, Yoshiko Yamane and Yo Yamasaki for their help in collecting *Orobdella* specimens. This study was financially supported in part by a Grant-in-Aid for the Biodiversity and Evolutionary Research of the Global COE (A06), from the Ministry of Education, Culture, Sports, Science and Technology, Japan, to Kyoto University.

TABLE 1. Comparisons of morphological characters of *Orobdella ijimai* Oka, *Orobdella dolichopharynx* sp. nov. and *Orobdella shimadae* sp. nov.

Character	<i>O. ijimai</i>	<i>O. dolichopharynx</i> sp. nov.	<i>O. shimadae</i> sp. nov.
annulation of VII	quadrannulate	quadrannulate	triannulate
annulation of VIII	sexannulate	quinquannulate	quinquannulate
male gonopore	at XI c12 (middle)	in XI c11/c12 or at XI c12 (anterior)	in XI b5/c11 or at XI c11 (anterior)
gastropore	at XIII b2 (middle)	absent	absent
female gonopore	at XIII b2 (middle)	in XIII b1/b2 or at XIII b2 (anterior)	in XIII b1/b2 or at XIII b2 (anterior)
number of annuli between gonopores	1/2 + 7 + 1/2	8	9
pharynx	reaching to XIV	reaching to XVI	reaching to XVI
gastroporal duct	bulbiform, tortuous at junction with gastropore	narrow, tubular, descending to XVI (front of crop)	narrow, tubular, descending to XV b2
epididymides	in XIV–XIX	absent	absent
atrial cornua	developed	absent	absent
atrium	muscular	rudimentary	rudimentary

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