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Redescription of *Gyrinicola japonica*, a Tadpole-Endoparasitic Nematode from Japan, with Resurrection of the Family Gyrinicolidae (Nematoda: Oxyurina)

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The taxonomic account of the tadpole-parasitic nematode *Gyrinicola japonica* Yamaguti, 1938, which is the type species of the genus, was reassessed based on syntypes and newly-collected specimens from the type locality. Our redescription of *G. japonica* addresses the erroneous original description of a spicule in this nematode, and emends the diagnosis of the species. Additionally, molecular phylogenetic trees based on nuclear 18S and 28S rDNA sequences revealed that *G. japonica* forms a distinctive lineage within the suborder Oxyurina, and this tadpole-specialist is phylogenetically close to the lizard-parasitic nematodes that belong to the family Pharyngodonidae. The results of morphological examination with the aid of molecular phylogenetic trees highlight the systematic uniqueness of this tadpole-parasitic group within Oxyurina, and Gyrinicolidae is accordingly resurrected as a distinctive oxyurinan family, with redefinition of the family and the genus *Gyrinicola*.

Key words: Oxyuroidea, emended diagnosis, syntype, molecular phylogeny, *Rhacophorus arboreus*

INTRODUCTION

The genus *Gyrinicola* Yamaguti, 1938 is a group of anuran tadpole-endoparasitic nematodes classified in the Oxyurina, and currently contains five valid species (Planade et al., 2008). This genus is characterized by a didelphic genital tract with asymmetric usage of its two horns; i.e., one horn produces thick-shelled eggs, while the other produces thin-shelled eggs, embryos, or males (Yamaguti, 1938; Adamson, 1981a; Souza-Júnior and Martins, 1996; Planade et al., 2008). A developmental study of the Nearctic *G. batrachiensis* (Walton, 1929) revealed that thick-shelled eggs are used as transmission agents, whereas thin-shelled eggs are created for autoinfection (Adamson, 1981b). Additionally, previous studies of *G. batrachiensis* highlighted the unique reproductive and ecological features of this oxyurinan genus (Adamson, 1981b, c, d; Pryor and Bjorndal, 2005; Rhoden and Bolek, 2011; Childress et al., 2017; Pierce et al., 2018). Namely, *G. batrachiensis* had two reproductive strains that were parthenogenetic or haplodiploid and, moreover, this species appeared to accelerate its host's development. These unique characteristics may allow elucidation of the evolution and ecology of host–parasite interactions.

Gyrinicola japonica Yamaguti, 1938 was described based on specimens collected from a tadpole of a ranid frog, *Glandirana rugosa* (Temminck and Schlegel, 1838), obtained from Kyoto, Japan (Yamaguti, 1938). Although this species is fixed as the type species of *Gyrinicola* by original designation (Yamaguti, 1938), several morphological characters in the original description, e.g., paired unequal spicules in the male (oxyurinan males ordinarily possess a single spicule), are doubtful or insufficiently described. Moreover, the shape of the genital cone–tail junction, which is one of the present diagnostic characters of *Gyrinicola* species, was not provided in the original description of *G. japonica* (Yamaguti, 1938). Therefore, systematic clarification of the genus *Gyrinicola* has been hampered by the insufficient original description of its type species.

The family Gyrinicolidae is monogeneric, containing only its type genus, and was established by Yamaguti (1938), based on the aforementioned unique characteristic of the female genital tract. However, the taxonomic account of this family within Oxyurina has been controversial. Gyrinicolidae was once classified as a subfamily within Cosmocercidae by Chabaud (1978) without any reasonable morphological basis. Later, *Gyrinicola* was transferred to Pharyngodonidae with emendation of the diagnosis of *Gyrinicola* by Adamson (1981a) based on previously published keys (Petter and Quentin, 1976), and this systematic account of *Gyrinicola* has been adopted in recent taxonomic studies (Araujo and Artigas, 1983; Souza-Júnior et al., 1991; Souza-Júnior and Martins, 1996; Planade et al., 2008). Because all oxyurinan nematodes that parasitize "cold-blooded vertebrates" were classified within Pharyngodonidae by Petter and Quentin (1976), the systematic position of *Gyrinicola* established by Adamson (1981a) did not account for any unique morphological traits of this tadpole-endoparasitic nematode.

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To clarify the taxonomic status of the type species of *Gyrinicola*, newly collected *Gyrinicola* specimens from Japan and type specimens of *G. japonica* were examined, and the taxonomic status of *G. japonica* was fully clarified in the present study. Additionally, the phylogenetic position of *G. japonica* within Oxyurina was evaluated using nuclear 18S rDNA and 28S rDNA data. According to the morphological re-establishment of *G. japonica* and obtained molecular phylogenetic relationships, the systematic status of the genus *Gyrinicola* within Oxyurina is herein revisited.

MATERIALS AND METHODS

Sampling and morphological examination

Specimens of *Gyrinicola* were newly collected from tadpoles, which were obtained from Mt. Daimonjiyama, Kyoto, Japan. The host tadpole specimens were identified as rhacophorid *Rhacophorus arboreus* (Okada and Kawano, 1924) based on their dental characteristics according to Maeda and Matsui (1999). The tadpoles were euthanized by dipping in 10% ethyl alcohol (EtOH). Host body cavity was dissected with a longitudinal incision, and the digestive tract was removed. The excised organs were shredded with tweezers, and their contents were investigated. Nematodes obtained were fixed in a hot 5% solution of glycerin in 70% EtOH. To clear the nematode specimens, they were placed in 5% solution of glycerin in 30% EtOH and then incubated for two weeks in a desiccator at room temperature to slowly evaporate the EtOH. The cleared specimens were observed and drawn using a light microscope (OLYMPUS BX53) with a drawing tube. The micrograph of the mail caudal region was taken with a light microscope (OLYMPUS BX51) with a DP71 photo-system. A scanning electron micrograph of the female cephalic region was taken with a low-vacuum scanning electron microscope (HITACHI TM-1000).

Nematode specimens collected in the present study were deposited in the Zoological Collection of Kyoto University (KUZ) and tadpoles were deposited in the Graduate School of Human and Environmental Studies, Kyoto University (KUHE).

The original type material of *G. japonica*, which has been stored in Satyû Yamaguti's helminthological collection at Meguro Parasitological Museum (MPM), were re-examined using a light microscope (Nikon Eclipse 80i).

Measurements are provided herein as averages, followed by the associated ranges (integrating measurement of syntypes and topotypes). All measurements are in micrometers (µm), unless otherwise stated.

DNA sequencing

Genomic DNA of nematode specimens was extracted following the method described by Sata (2018). Partial sequences of the 18S rDNA (18S) and 28S rDNA (28S) containing variable domains 1–3 were amplified by polymerase chain reaction (PCR) using a TaKaRa *Ex Taq* kit (Takara Bio, Japan) and a GeneAmp PCR Systems 2700 instrument (Applied Biosystems, USA; ABI). The primer sets used for PCR and cycle sequencing reaction of 18S were Nem_18S_F and Nem_18S_R, as described in Floyd et al. (2005), and those for 28S were 28S rD1.2a and 28S B (Whiting,

Table 1. Nematode species and INSDC accession numbers of the 18S and 28S rDNA sequences used for the phylogenetic analyses. Sequences marked with an asterisk (*) were obtained for the first time in the present study.

Species	INSDC#		Species	INSDC#	
	18S	28S		18S	28S
Oxyurina			Pseudonymidae		
Gyrinicola japonica	LC456178*	LC489226*	*Pseudonymus islamabadi*	KJ632668	KF771648
Heteroxynematidae			*Pseudonymus spirotheca*	KJ632669	KF771649
Aspiculuris tetraptera	KY462828	MH215351	Thelastomatidae		
Hystrignathidae			*Blattophila peregrinata*	KX752427	KX752428
Coynema poeyi	MH577322	MH244509	*Cephalobellus brevicaudatus*	MF668724	MF668725
Hystrignathus rigidus	MH411156	MH411129	*Hammerschmidtiella keeneyi*	KX752429	KX752430
Oxyyuridae			*Leidynema appendiculata*	KY057031	KY057027
Enterobius vermicularis	JF934731	LC416069	*Thelastoma krausi*	EF180068	MG189597
Lemuricola pongoi	JF934731	LC416074	*Thelastoma* sp.	LC214830	LC214837
Oxyuris equi	KU180664	KU180675	Travassosinematidae		
Skrjabinema ovis	EF180060	KY990019	*Travassosinema claudiae*	KX844644	KX844645
Syphacia frederici	AB629697	AB500170	*Travassosinema* sp.	LC214829	LC214836
Trypanoxyuris pigrae	KU285458	KU285469	Outgroup		
Pharyngodonidae			Ascaridomorpha		
Ozolaimus linstowi	KJ632671	KJ632667	*Ascaridia galli*	EF180058	KY990014
Parapharyngodon bainae	MF102081	MF102080	*Cosmocercoides tonkinensis*	AB908160	AB908160
Parapharyngodon echinatus	JF829224	JF829241	*Cruzia americana*	U94371	U94757
Parapharyngodon micipsae	MH459194	MH459223	*Cucullanus grandistomis*	KX752094	KX752093
Skrjabinodon poicilandri	KX550024	KX550044	*Cucullanus opisthoporus*	KX752096	KX752095
Spauligodon auziensis	JF829225	JF829242	*Meteterakis amamiensis*	LC456174*	LC186007
Spauligodon extenuatus	MG573468	MG573502	*Strongyluris calotis*	LC133188	LC133188
Spauligodon saxicolae	KJ778084	KJ778093	*Truttaedacnitis truttae*	EF180063	KY857891
Thelandros tinerfensis	KJ778073	KJ778089	Rhigonematomorpha		
			Brumptaemilius justini	JX999733	JX999732

2002), and D2a and D3b (Nunn, 1992). PCR conditions were as follows: 94°C for 3 min, followed by 35 cycles of 30 s at 94°C, 30 s at 54°C, and 90 s at 72°C, and then a final extension at 72°C for 7 min for 18S; and 95°C for 5 min, followed by 35 cycles of 10 s at 94°C, 20 s at 44°C (for rD1.2a and B) or 54°C (for D2a and D3b), and 84 s at 72°C, and then a final extension at 72°C for 6 min for 28S. The PCR products were purified with ExoSAP-IT reagent (Affymetrix, USA). Sequencing reactions were performed using a BigDye Terminator Cycle Sequencing Kit v. 3.1 (ABI). The products were cleaned by ethanol precipitation and sequenced with an ABI 3130*xl* Genetic Analyzer. In total, three DNA sequences from *G. japonica*, and *Meteterakis amamiensis* Hasegawa, 1990 were newly obtained in the present study, and deposited with the International Nucleotide Sequence Database Collaboration (INSDC) through the DNA Data Bank of Japan (Table 1).

Phylogenetic analyses

The phylogenetic position of *Gyrinicola japonica* was estimated based on 18S and 28S sequences. In total 29 oxyurinan operational taxonomic units (OTUs) were included as ingroup taxa (Table 1). Additionally, eight Ascaridomorpha species and one Rhigonematomorpha species were selected as outgroup taxa. The sequences were edited with MEGA 5 (Tamura et al., 2011) and aligned with MAFFT v. 7.427 G-INS-i (Katoh and Standley, 2013). Regions difficult to align because of alignment gaps were removed manually; and thus the final sequences yielded 912 bp of aligned positions for 18S and 1143 bp for 28S.

Phylogenetic relationships were inferred with maximum likelihood (ML) method using RAxML v. 8.2.12 (Stamatakis, 2014), and Bayesian inference (BI) with posterior probabilities (PPs) using MrBayes v. 3.2.7a (Ronquist et al., 2012). The ML phylogeny was inferred with the substitution model set as GTRCAT, immediately after nonparametric bootstrapping (BS) was conducted with 1000 pseudoreplicates. The best-fit partition scheme was identified with AICc using PartitionFinder v. 2.1.1. (Lanfear et al., 2017) with the "all" algorithm: 18S/28S. The best-fit partition scheme and models for each partition for BI were selected with AICc using PartitionFinder with the "all" algorithm: GTR+I+G for 18S, and 28S, respectively. Two independent runs of four Markov chains were conducted for one million generations, and the tree was sampled every 100 generations. The parameter estimates and convergence were checked using Tracer v. 1.7.1 (Rambaut et al., 2018), and the first 2501 trees were discarded based on the results.

RESULTS

Taxonomy

Gyrinicola japonica Yamaguti, 1938
(Figs. 1–4)

Gyrinicola japonica Yamaguti, 1938, pp. 603–605, fig. 1, pl. 41, figs. 1–4; Baker, 1987, p. 32; Hasegawa and Asakawa, 2004, p. 33.

Emended diagnosis. Mouth opening of female surrounded by four small papillae and two prominent horn-like amphids; four large papillae (each papilla with two minute papillae) and two single large papillae present on outer circumference of cephalon. Projections with glandular cell adhering on anterior part of esophagus in male. Lateral alae present in male and absent in female. Didelphic, long horn containing thick-shelled eggs, and short horn forming pouch containing subadult males. Post-anal cuticular ridge developed in female. Transverse section of thick-shelled eggs triangular due to three thick lateral shell crests. Tail relatively short in both sexes. Genital cone supported by a sclerotized V-shaped gubernaculum. Shape of genital cone–tail junction of mail: trapezoid to triangular. One spicule with pointed tip.

Material examined. *Syntypes*: MPM Coll. No. 23960 (three prepared slides); since a single name-bearing type has not been designated for *G. japonica*, all of the original specimens examined in Yamaguti (1938) are thus automatically fixed as syntypes under Article 73.2 of the International Code of Zoological Nomenclature (hereinafter, Code; International Commission on Zoological Nomenclature, 1999). *Additional materials*: KUZ Z2278–Z2288, Z2558, 12 adult females, and KUZ Z2277, an adult male, obtained from digestive tracts of tadpoles of *Rhacophorus arboreus* (KUHE60764, 60766, 60768, 60770, 60772, 60773), collected from Mt. Daimonjiyama, Kyoto, Japan (35°01.685′N, 135°48.159′E) on 17 August 2018; KUZ Z2289, one prepared slide of the anterior end of KUZ Z2287; and KUZ Z2290, one prepared slide of the anterior end of KUZ Z2288.

Redescription. *Male* (n = 3; MPM Coll. No. 23960 and KUZ Z2277). Body short and slender, 896 (757–1070) long, maximum width 78 (n = 1). Body length/body width = 13.7 (n = 1). Cuticle with transverse annulations commencing short distance from anterior extremity. Narrow lateral alae present, commencing from region posterior to cephalic area and ending at region anterior to genital cone. Buccal cavity present, not forming chitinized capsule. Esophagus comprised of cylindrical portion and bulb, junction between cylindrical portion and bulb constricted. Pre-esophageal region consisting of transparent tissue. Bulb bearing valves. Pharynx absent. Total length of esophagus 132 (119–157) long with width of 14 (13–15). Body length/esophagus length = 6.8 (6.4–7.1). Several projections (may be four), each with a glandular cell, adhering on anterior part of esophagus. Several coelomocytes surrounding bulb. Esophageal bulb 35 long by 37 wide (n = 1). Nerve ring and excretory pore 73 (56–89) and 273 (n = 1), respectively, from cephalic end. Testis directed forward, flexed 144 (n = 1) behind esophageal bulb–intestine junction; no spermatozoa identified in seminal vesicle; vas deferens comprised of glandular region and ejaculatory duct, 54 (53–55) long (n = 2), surrounded by glandular cells. Rectum surrounded by glandular cells. Genital cone present with height of 23 and base 27 (n = 1), supported by one sclerotized V-shaped gubernaculum: upper side 19 and lower side 11 long (n = 1); shape of genital cone–tail junction trapezoid to triangular; two pairs of papillae present: one precloacal, one postcloacal. Single spicules present with pointed distal end, 37 long (n = 1), proximal end slightly bent ventrally. Tail bent ventrally, conical with pointed tip, 112 (99–135) long. Body length/tail length = 8 (7.4–8.7); one pair of caudal papillae present at middle of tail, 60 (50–70) (n = 2) from tip of tail. Caudal alae absent.

Female (n = 16; MPM Coll. No. 23960 and KUZ Z2278–Z2290, Z2558). Mouth with six small lips, two lateral, two subventral, and two subdorsal. Mouth opening roughly triangular, surrounded by four small papillae and two prominent horn-like amphids; four papillae (each papilla with two minute papillae) and two single papillae present on outer circumference of cephalon. Body short and relatively stout, length 2.59 mm (2.03–4.39 mm) (n = 15), maximum width 243 (194–329) (n = 10). Body length/body width = 11.2 (8.7–15.2) (n = 10). Shallow and relatively wide buccal cavity present, not forming chitinized capsule, with three sclerotized

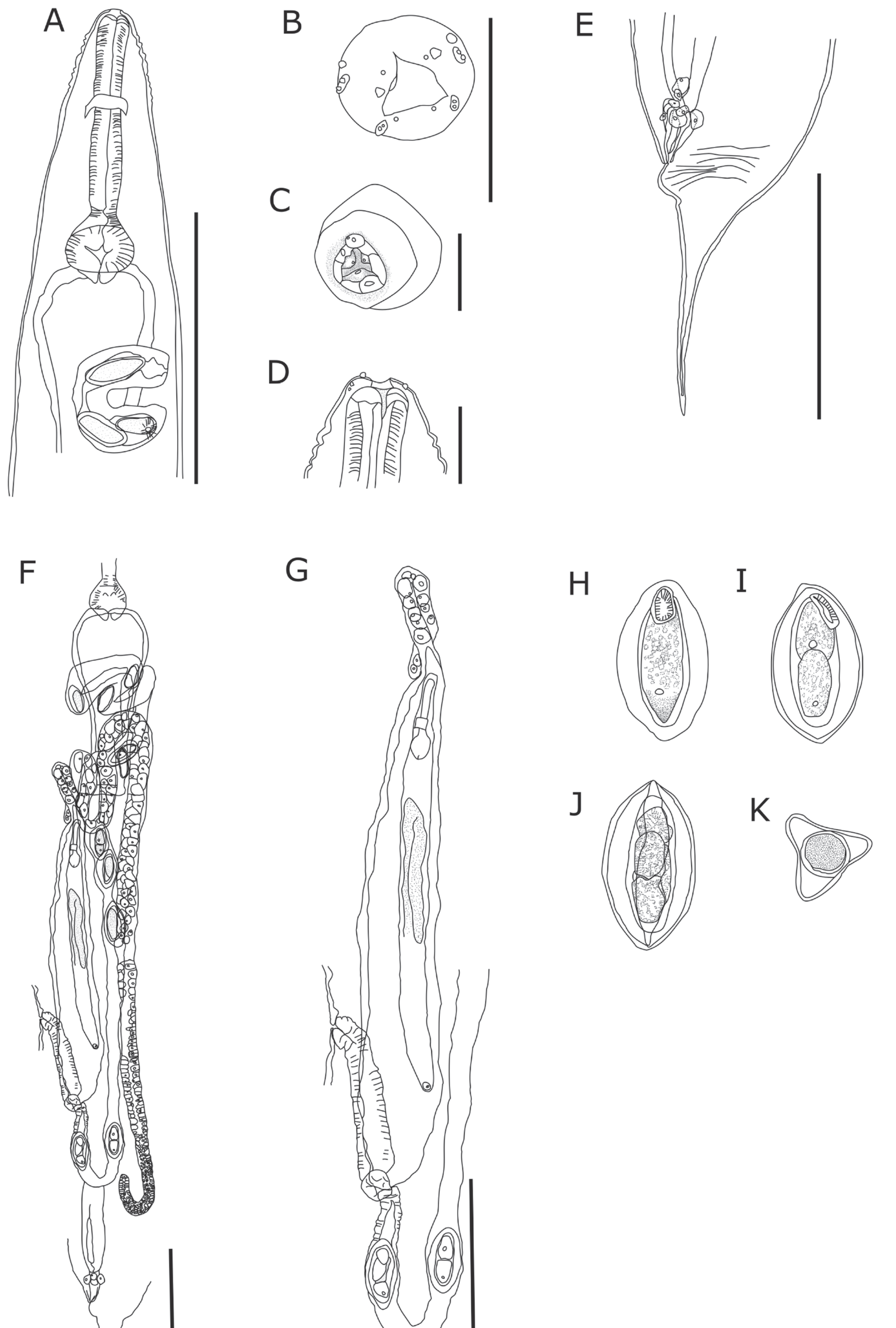

Fig. 1. *Gyrinicola japonica* Yamaguti, 1938, females, from the type locality (KUZ Z2281: **(A, D, H–K)** KUZ Z2289: **(B)** KUZ Z2283: **(E–G)**). **(A)** Anterior region, lateral view (left). **(B)** Cephalic papillae, apical view. **(C)** Mouth opening and lips, apical view. **(D)** Cephalic region, lateral view. **(E)** Caudal region, lateral view (left). **(F)** Whole genital tract, lateral view. **(G)** Muscular vagina and two uteri. **(H–K)** Thick-shelled egg, lateral view. **(K)** Transverse section of thick-shelled egg. Scale bars: 340 μm **(A)**, 50 μm **(B–D)**, 210 μm **(E)**, 240 μm **(F, G)**, and 90 μm **(H–K)**.

triangular pieces with single papilla. Esophagus comprised of cylindrical portion and bulb, junction between cylindrical portion and bulb constricted. Pre-esophageal region consisting of transparent tissue. Bulb bearing valves. Pharynx

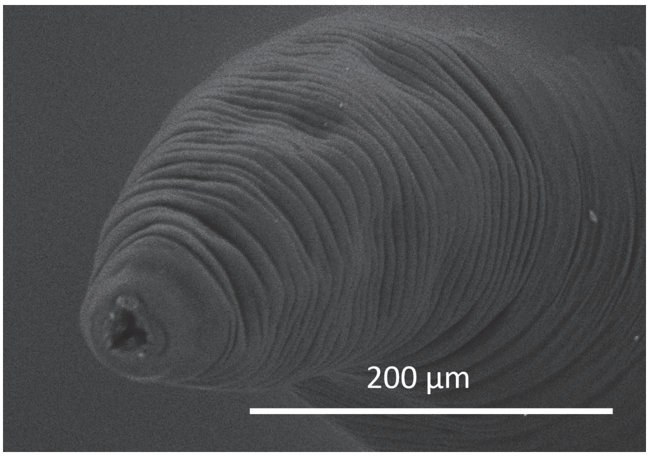

Fig. 2. Scanning electron micrograph of the mouth opening of female *Gyrinicola japonica* Yamaguti, 1938, from the type locality (KUZ Z2558).

Fig. 4. Light micrograph of a spicule and sclerotized V-shaped gubernaculum of male *Gyrinicola japonica* Yamaguti, 1938, from the type locality (KUZ Z2277).

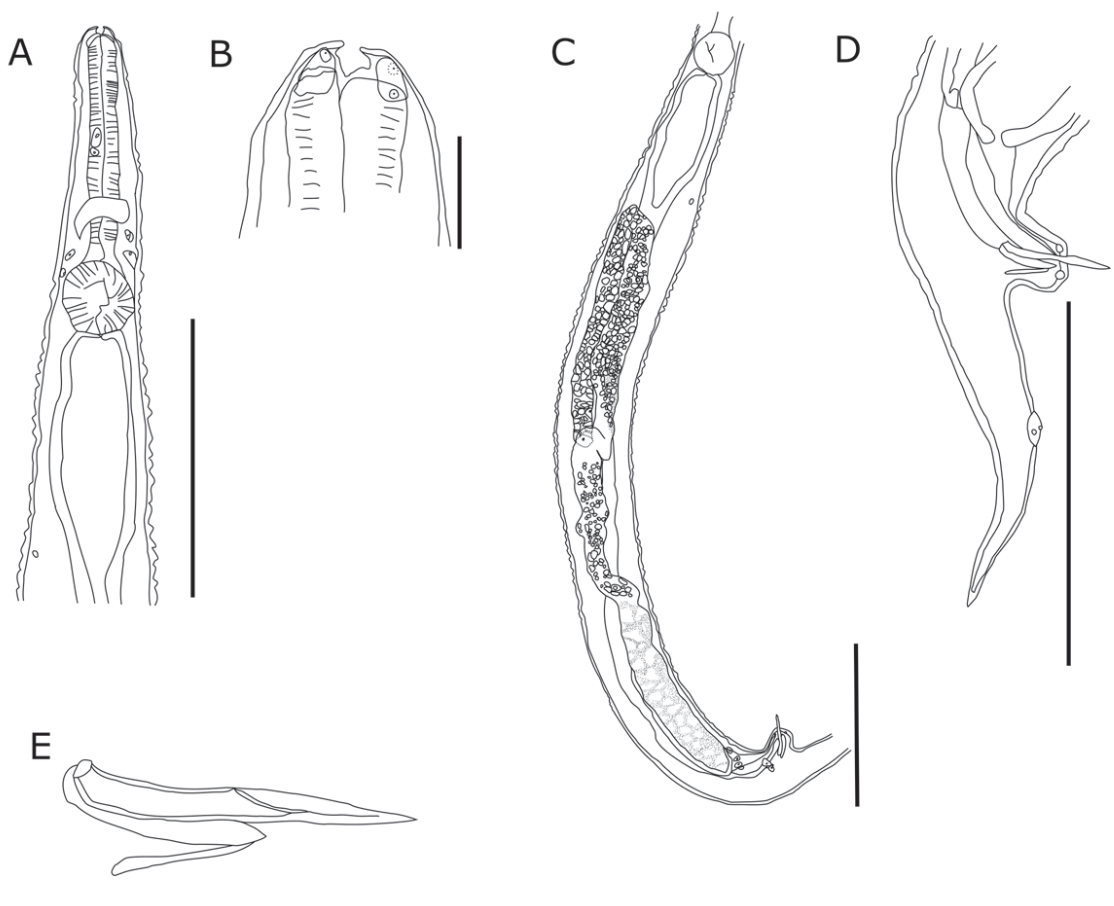

Fig. 3. *Gyrinicola japonica* Yamaguti, 1938, male, from the type locality (KUZ Z2277). **(A)** Anterior region, lateral view (left). **(B)** Cephalic region, lateral view. **(C)** Genital tract, lateral view (right). **(D)** Caudal region, lateral view (right). **(E)** Spicule and sclerotized V-shaped gubernaculum. Scale bars: 150 μm **(A, C)**, 15 μm **(B)**, 120 μm **(D)**, and 40 μm **(E)**.

absent. Total length of esophagus 332 (295–431) (n = 14) long with maximum width of 45 (35–53) (n = 15) at posterior region of cylindrical portion. Esophageal bulb 67 (55–78) (n = 10) long by 93 (80–107) (n = 10) wide. Body length/esophagus length = 7.6 (6.4–8.9) (n = 13). Cuticle with transverse annulations commencing short distance from anterior extremity and gradually becoming indistinct in posterior region. Lateral alae absent. Nerve ring and excretory pore 113 (89–130) and 617 (458–895) (n = 13), respectively, from cephalic end. Vulva 1.43 mm (1.14–1.84 mm) (n = 12) from cephalic end, and located at posterior half of body (57.2% [53.5%–67.3%] of body length) (n = 12). Didelphic genital tract comprising of a muscular vagina, 281 (219–343) (n = 11) long, and long and short horns connecting at bottom of vagina, each horn consisting of uterus and ovary; long-horn ovary originating from region anterior to anus with distal end turning forward, directing region posterior to excretory pore, then turning backward, forming S-shaped curve and linked to uterus; uterus coiled posterior to esophagus, then turning backward with a bend before reaching vagina, uterus containing large number of thick-shelled eggs; short horn directing anteriorly, uterus formed pouch containing subadult males; long-horn ovary substantially longer than short-horn ovary. Rectum surrounded by glandular cells. Post-anal cuticular ridge developed. Tail short, conical, attenuated, and 237 (210–273) long (n = 11). Body length/tail length = 10.6 (8.9–13.7) (n = 11). Caudal alae absent. Thick-shelled eggs, double shelled, outer shell elliptical, 88 (70–102) by 46 (36–52) (n = 42); inner shell elliptical, 74 (58–83) by 27 (21–33) (n = 42); containing two to four cells near vagina; surface dotted; transverse section triangular due to three thick lateral shell crests; operculum elliptical, subapical, 17 by 11 (n = 1).

Remarks. The newly collected materials exhibit near-identical measurements and the arrangement of lips and cephalic papillae to the original description of *G. japonica* (Yamaguti, 1938). Moreover, they also possess a genital tract with one of the two uteri forming a pouch containing subadult males, as described by Yamaguti (1938). Thus, the present samples were unquestionably identified as *G. japonica*.

Morphological examination of the *G. japonica* specimens revealed that this species possesses a single spicule, as well as a sclerotized V-shaped gubernaculum situated

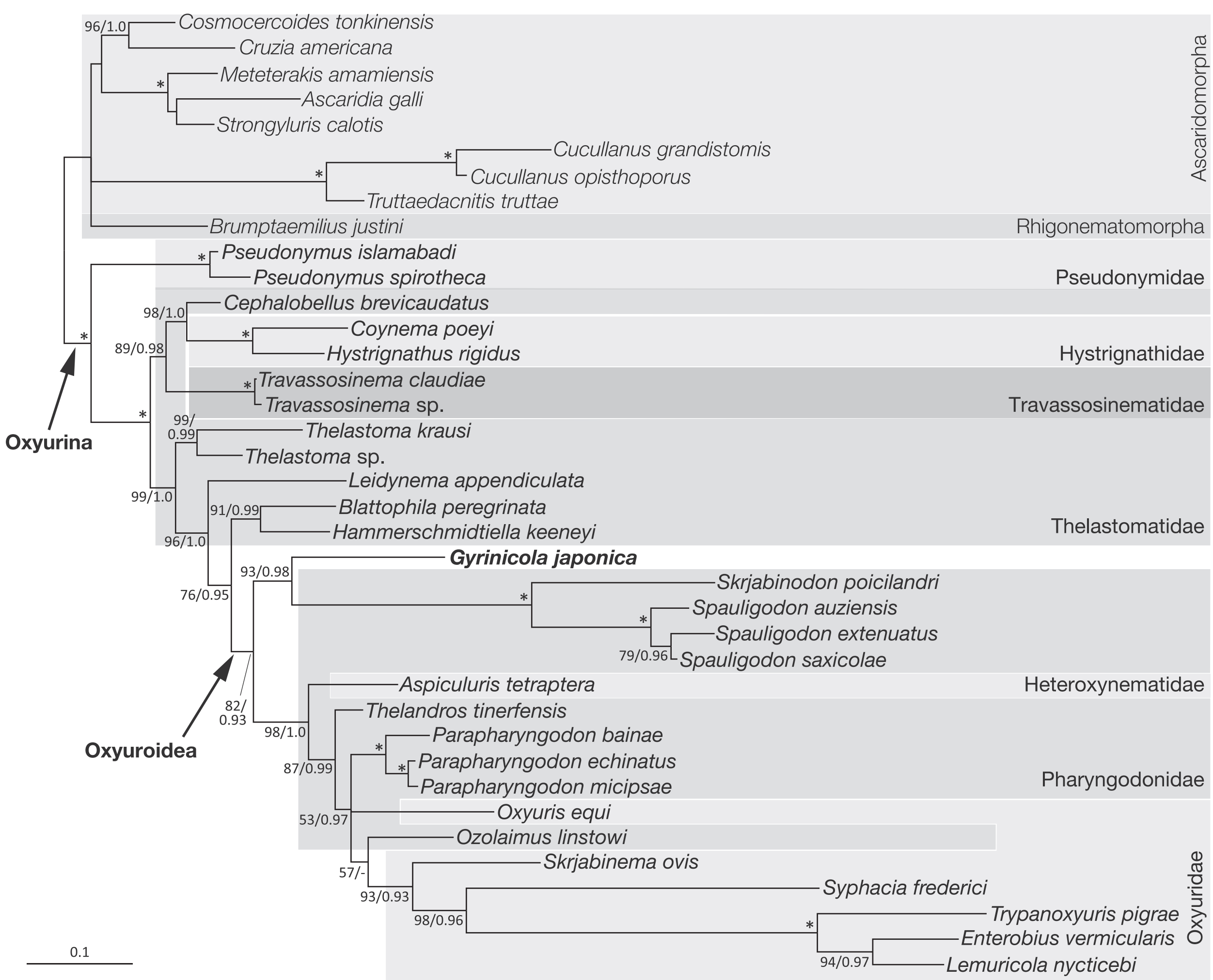

Fig. 5. Bayesian inference tree for 2055 bp of nuclear 18S and 28S rDNA markers. The numbers on nodes represent bootstrap values (BS; only values > 49%) for maximum likelihood and Bayesian posterior probabilities (PPs; only values > 0.89); asterisks (*) denote fully-supported nodes with BS = 100% and PP = 1.0.

under the spicule, and is longer than the gubernaculum. This V-shaped gubernaculum is highly likely to be concordant with “a pair of unequal spicules” noted for the male in the original description (Yamaguti, 1938), despite the fact that oxyurinan nematodes ordinarily possess a single spicule (Adamson, 1989). In reality, this species possesses a single spicule, as do other congeners; this is the first report of a sclerotized V-shaped gubernaculum in *Gyrinicola* species. The present study also revealed additional characteristics that were not stated in the original description (Yamaguti, 1938). The amended characteristics of *G. japonica* are as follows: the long-horn ovary is much longer than the short-horn ovary, the shape of the genital cone–tail junction of male is trapezoidal to triangular, and several projections each with glandular cells are adhered to the anterior part of the esophagus of male.

This species can be clearly discriminated from all other congeners by the presence of a sclerotized V-shaped gubernaculum in the male (Adamson, 1981a; Souza-Júnior et al., 1991; Souza-Júnior and Martins, 1996; Planade et al., 2008). This species is also distinguishable from all other congeners aside from *G. chabadamsoni* Planade and Bain in Planade et al., 2008 by having a uterine pouch that contains males (Adamson, 1981a, b; Souza-Júnior and Martins, 1996; Planade et al., 2008). Additionally, *G. japonica* differs from *G. chabadamsoni* by its possession of large papillae on the outer circumference of cephalon (Planade et al., 2008). Thus, the five known *Gyrinicola* species including *G. japonica* remain valid within this genus.

Molecular phylogeny

The BI tree (mean ln $L = -21358.451$) inferred from the combined data set of the partial 18S and 28S sequences is shown in Fig. 5; the topology of BI was identical to that of the obtained ML tree (ln $L = -21545.314$; not shown). The monophyly of Oxyurina was fully supported in both analyses (BS = 100%, PP = 1.0). The monophyly of Oxyuroidea, a vertebrate-parasitic oxyurinan superfamily, was also supported (BS = 82%, PP = 0.93). This clade nested within Thelastomatoidea, an invertebrate-parasitic oxyurinan superfamily, and formed a monophyletic lineage (BS = 76%, PP = 0.95) with a clade comprising of two thelastomatid species. Oxyuroidea was divided into two well-supported clades, i.e., one clade consisting of Heteroxynematidae, Oxyuridae and three pharyngodonid genera (BS = 98%, PP = 1.0), while the other clade composed of *G. japonica* and two pharyngodonid genera (BS = 98%, PP = 0.98). *Gyrinicola japonica* is highly diverged at the genetic level from other families within Oxyuroidea.

DISCUSSION

Host range of *Gyrinicola japonica*

Although oxyurinan nematodes generally exhibit narrow host ranges (Adamson, 1989), *Gyrinicola* species have been known to infect various anuran families, i.e., Bombinatoridae, Bufonidae, Hylidae, Leptodactylidae, Pelobatidae, and Ranidae (Baker, 1987; Souza-Júnior et al., 1991; Souza-Júnior and Martins, 1996; Planade et al., 2008). *Gyrinicola japonica* was exceptional in being reported only from the ranid *Gl. rugosa* (Yamaguti, 1938). However, the present study revealed that *G. japonica* could infect the tadpole of the rhacophorid *R. arboreus*, suggesting a wide host range concordant with its congeners. This is the first report of *Gyrinicola* nematodes infecting tadpoles of the family Rhacophoridae.

Tadpoles in the aforementioned seven families, including Rhacophoridae, share general morphological characteristics of the alimentary canal, e.g., a ventral and laterally broadened oral part with a simple digestive tract (Duellman and Trueb, 1986; Altig and McDiarmid, 1999; Viertel and Richter, 1999). Because ingestion of the sedimented eggs of *Gyrinicola* nematodes with food gives rise to infection of this nematodes in tadpole hosts (Adamson, 1981b), in both benthic and bottom-feeding tadpoles, which are typical life forms of anuran larvae of various species, could be potential hosts of *Gyrinicola* species. Thus, further helminthological study will shed light on the true host range of *G. japonica*; it is likely that this species infects tadpoles of a wide range of frog species inhabiting Japan.

Taxonomic account of *Gyrinicola*

The present phylogenies unquestionably revealed that *Gyrinicola* belongs to suborder Oxyurina, not to Cosmocercoidea as stated in Chabaud (1978) and Hasegawa and Asakawa (2004). The present phylogenetic trees supported Oxyurina, which includes *Gyrinicola*, forming a monophyletic group. A previous study indicated the monophyly of Oxyurina, but did not include any sequences from this tadpole-parasitic nematode (Nadler et al., 2007).

Within Oxyurina, the genus *Gyrinicola* has been classified as a pharyngodonid taxon (Adamson, 1981a). However, their precise phylogenetic position remained unresolved, since no DNA sequences of *Gyrinicola* nematodes had ever been assessed for molecular phylogenetic studies of Oxyurina nematodes. The present results suggested that *Gyrinicola* is highly possible to be a member of Oxyuroidea. The present phylogenies showed that Oxyuroidea comprised three families, i.e., Oxyuridae, Heteroxynematidae, and Pharyngodonidae, and the genus *Gyrinicola*, and that Pharyngodonidae was split into two major phylogroups, as a previous molecular phylogenetic study has shown (Pereira et al., 2018). Although *Gyrinicola* formed a monophyletic lineage with the pharyngodonid *Skrjabinodon* Inglis, 1968 and *Spauligodon* Skrjabin et al., 1960 species that parasitize reptiles, this genus formed a distinctive lineage within the superfamily Oxyuroidea.

The observed morphological characteristics of *Gyrinicola japonica* confirm that all *Gyrinicola* species possess a didelphic genital tract with asymmetric usage of its two horns. One of the horns produces thick-shelled eggs as transmission agents (Adamson, 1981b), and the other releases thin-shelled eggs, embryos, or males that are thought to contribute to autoinfection and/or oedipal mating (Adamson, 1981d; Adamson, 1989). This reproductive feature is unique among oxyurinan nematodes. Although several other pharyngodonid species are known to possess a didelphic genital tract bearing thick- and thin-shelled eggs, they always contain two types of females, each producing one type of eggs in both of the genital horns (Adamson, 1981b; Adamson and Petter, 1983; Adamson, 1988). In addition to the didelphic genital tract with asymmetric usage, *Gyrinicola* species possess two types of ovaries with a substantial difference in length. Moreover, their host specificity to tadpoles was also unique among Oxyurina.

The phylogenetic distinctiveness, morphological features of the reproductive organs, reproductive strategies, and host specificity of *Gyrinicola* nematodes led us to conclude that *Gyrinicola* holds distinctive family status within Oxyurina, and thus the family Gyrinicolidae erected by Yamaguti (1938) is resurrected as follows. Because of the possession of four cephalic papillae in the outer circle in females of *G. japonica* and other congeners and the phylogenetic position of the type species of *Gyrinicola*, this family should be classified within the superfamily Oxyuroidea sensu Adamson (1989).

Systematic conclusion

Superfamily **Oxyuroidea** Cobbold, 1864
Family **Gyrinicolidae** Yamaguti, 1938

Gyrinicolidae Yamaguti, 1938, p. 605; Skrjabin and Shikhobalova, 1951, p. 8.
Gyrinicolinae; Chabaud, 1978, pp. 5, 7 (within the family Cosmocercidae).
Pharyngodonidae; Adamson, 1981a, p. 1344; Araujo and Artigas, 1983, pp. 383–384; Baker, 1987, p. 25; Planade et al., 2008, p. 27.
Cosmocercidae; Hasegawa and Asakawa, 2004, pp. 32–33.

Emended diagnosis. Cephalic end simple. Cervical wing absent in both sexes. Esophagus relatively short in both sexes, typical oxyuroid esophagus. Didelphic, one horn of genital tract producing thick-shelled eggs, and another producing thin-shelled eggs or males. Two ovaries exhibiting extreme difference in length. Vulva located at middle to posterior half of body. Thick-shelled eggs elliptical to spindle-shaped, triangular in transverse section; operculum present and subapical. Single spicule and genital cone present in male. Gubernaculum absent or rarely sclerotized V-shaped gubernaculum present. Male tail, long and pointed, not bluntly truncate; caudal alae absent. Parasitize anuran tadpole.

Type and only included genus. *Gyrinicola* Yamaguti, 1938.

Remarks. This family clearly differs from Oxyuridae in the combination of the following characteristics: cervical wing absent in both sexes; vulva located in middle to posterior half of body; and male tail long and pointed, not bluntly truncate, and without caudal alae. Additionally, Gyrinicolidae can be discriminated from Pharyngodonidae by the following characteristics: vulva located in middle to posterior half of body; and male tail long and pointed, not bluntly truncate, and without caudal alae.

Although it is unquestionable that Gyrinicolidae belongs to Oxyuroidea based on its morphological features and phylogenetic position, this family is distinguishable from Cosmocercoidea by having a single spicule in the male.

Genus ***Gyrinicola*** Yamaguti, 1938

Gyrinicola Yamaguti, 1938, p. 605; Chabaud, 1978, p. 7.

Diagnosis. As for the family Gyrinicolidae.

Type species. *Gyrinicola japonica* Yamaguti, 1938, fixed by original designation.

Additional species. *Gyrinicola batrachiensis* (Walton, 1929); *G. chabadamsoni* Planade and Bain in Planade et al., 2008; *G. chabaudi* Araujo and Artigas, 1983; and *G. tba* (Dinnik, 1930).

Amendment to publication date. The date of publication of *G. chabaudi* has been mistakenly cited as 1982 (Souza-Júnior et al., 1991; Souza-Júnior and Martins, 1996; De Fabio et al., 2000; González and Hamann, 2005; Planade et al., 2008; Pierce et al., 2018). However, Volume 44/45 of the journal "Memórias do Instituto Butantan (Sao Paulo)", which includes the original description of *G. chabaudi*, was published on 23 June 1983; this publication date was specified as "EXPEDIDA EN 23-6-83" on the inside back cover of the issue. Therefore, the authorship of *G. chabaudi* should be attributed to "Araujo and Artigas, 1983" according to Article 21.2 of the Code.

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COMPETING INTERESTS

The authors have no competing interests to declare.

AUTHOR CONTRIBUTIONS

NS designed the study, and provided the taxonomic description as well as the figures of the nematodes. NS and TN obtained molecular sequence data, analyzed them, and wrote this manuscript.

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