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A new species of *Raphidascaris* (Nematoda: Raphidascarididae) infecting the fish *Gymnogeophagus balzanii* (Cichlidae) from the Pantanal wetlands, Brazil and a taxonomic update of the subgenera of *Raphidascaris* based on molecular phylogeny and morphology

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Research Paper

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Abstract

Raphidascaris (*Sprentascaris*) *andersoni* n. sp. (Nematoda: Raphidascarididae) collected in the intestine of the humphead cichlid *Gymnogeophagus balzanii* (Perugia) from the Pantanal wetlands, State of Mato Grosso do Sul (Brazil) is described and genetically characterized. The new species differs from its congeners mainly by having a conspicuous papilla-like formation slightly anterior to the cloacal aperture. Furthermore, males of *R.* (*S.*) *lanfrediae* and *R.* (*S.*) *mahnerti* have caudal alae, and *R.* (*S.*) *hypostomi* and *R.* (*S.*) *pimelodi* lack lateral alae, whereas in the new species caudal alae are absent and lateral alae present. The remaining congeners, namely, *R.* (*S.*) *marano* and *R.* (*S.*) *saltaensis* differ from *Raphidascaris* (*Sprentascaris*) *andersoni* n. sp. mainly because males have three pairs of postcloacal papillae (*vs* five pairs). In the phylogenetic reconstructions, using three nuclear genetic markers (18S, ITS1-5.8S-ITS2 and 28S rDNA) and one mitochondrial (*cox*1 mtDNA), the new species was separated from other representatives of Raphidascarididae, and the absence of monophyly in *Hysterothylacium* and *Raphidascaroides* was confirmed. Moreover, the subgenera *Sprentascaris* and *Ichthyascaris* appeared to be monophyletic. Therefore, even though *Raphidascaris* (*Raphidascaris*) was apparently not monophyletic, the subgenera of *Raphidascaris* should be re-erected as valid genera. The updated diagnoses of *Ichthyascaris*, *Raphidascaris* and *Sprentascaris* are given. The present study represents the first parasitological survey in *G. balzanii*.

Introduction

Phylogenetic studies based on genetic tools have generated important insights into the relationships among ascaridoid nematodes (Nadler and Hudspeth, 1998; Pereira and Luque, 2017; Li *et al.*, 2018). Discussion remains on the validity of the family Raphidascarididae Hartwich, 1954 and its independence from the closely related Anisakidae Railliet & Henry, 1912 (see Pereira and Luque, 2017). Similarly, the subdivision of the genus *Raphidascaris* Bloch, 1779 into three subgenera, namely *Raphidascaris* Bloch, 1779, *Ichthyascaris* Wu, 1949 and *Sprentascaris* Petter & Cassone, 1984, has also been debated (Petter and Cassone, 1984; Bruce, 1990; Moravec *et al.*, 1990; Petter, 1995; Moravec and Justine, 2012); however, there is no molecular approach focused on such a discussion.

Currently, *Raphidascaris* (*Sprentascaris*) includes six species parasitizing freshwater characiforms (Serrasalmidae), perciforms (Cichlidae) and siluriforms (Loricariidae and Pimelodidae) in the Neotropical region (Petter and Cassone, 1984; Ramallo, 2009; Luque *et al.*, 2011; Melo *et al.*, 2011; Zago *et al.*, 2013; Ailán-Choke *et al.*, 2017). None of these species have been characterized genetically.

During the first parasitological examination of *Gymnogeophagus balzanii* (Perugia, 1891) (Cichlidae), collected in the Pantanal wetlands, State of Mato Grosso do Sul, Brazil, several nematodes were found in the intestine of the fish. Detailed observations have revealed that these specimens represent a new species of *Raphidascaris* (*Sprentascaris*), described herein based on light and scanning electron microscopy (SEM), and characterized genetically based on four genetic markers. Additionally, phylogenetic reconstructions using representatives of Raphidascarididae were generated to evaluate the position of the new species within

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the family, its relationships with other taxa, especially with its congeners, and the validity of the subgenera of *Raphidascaris*.

Materials and methods

Collection and examination of nematodes

Twenty-one specimens of *G. balzanii* (total body length 50.2–109.5 mm) were collected using casting nets, from September 2015 to August 2017, in small lakes located at the margins of the State highway MS-184, which crosses the Pantanal wetlands, in the municipality of Corumbá, State of Mato Grosso do Sul, Brazil. Fish were kept alive in small water tanks with oxygen pumps, brought to the laboratory, euthanized and dissected. Host nomenclature and classification follow Froese and Pauly (2018). Nematodes found alive were washed in saline, fixed in hot 4% formaldehyde solution and preserved in 70% ethanol. For morphological examinations, nematodes were cleared in glycerine. The middle body parts of one male were excised and fixed in molecular-grade 96–99% ethanol for genetic studies; the anterior and posterior parts were fixed in 4% formalin for morphological identification, i.e. hologenophores (the voucher specimens from which the molecular sample is directly derived; see Astrin *et al.*, 2013, for more details).

Drawings were made using a drawing tube attached to an Olympus BX51 microscope. Measurements are given in micrometers, unless otherwise stated. Specimens used for SEM (two males, one of which the mid body was taken for genetic sequencing, and two females) were dehydrated through a graded ethanol series, dried by evaporation with hexamethyl disilazane, coated with gold and observed in a JEOL JSM 6460-LV at an accelerating voltage of 15 kV. The systematic classification of nematodes adopted follows the recent proposals of Pereira and Luque (2017) and Li *et al.* (2018), in which Anisakidae and Raphidascarididae are independent and valid. Parasite specimens were deposited in the Coleção Zoológica da Universidade Federal de Mato Grosso do Sul (ZUFMS).

Genetic procedures

Genomic DNA was isolated using a DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany), following the manufacturer's instructions. Four genetic regions were amplified: the 5′ end of the 18S rDNA, the nuclear ITS1-5.8S-ITS2, the D2 and D3 domains of the 28S rDNA and the *cox*1 of the mtDNA. All polymerase chain reactions (PCR), cycling conditions and primers are detailed in supplementary material S1. PCR products were purified through an enzymatic treatment with ExoProStar™ (GE Healthcare) and sent for sequencing at ACTGene (Ludwig Biotec, Rio Grande do Sul, Brazil) with the same primers used in the PCR reactions.

Contiguous sequences were assembled in Geneious v. 9.1.5 (created by Biomatters, available from http://www.geneious.com/) and deposited in GenBank (see taxonomic summary for accession numbers). A preliminary BLAST search of the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) was performed to confirm the genetic proximity between the present sequences and those from representatives of Raphidascarididae.

Sequences chosen for phylogenetic reconstructions were from all representatives of Raphidascarididae, of which genetic regions were congruent with those from the present study (see table 1). Sequences from the same isolate or clones with 100% genetic similarity were not included. The following sequences were excluded from the analyses because they interfered in the alignment, showing high incongruence with the other sequences: 18S and 28S rDNA sequences of *Hysterothylacium amoyense* (Hsü, 1933) (MF072695/MF094276) and of *Raphidascaris gigi* Fujita, 1928 (AB558482 and AB558483/AB558480 and AB558481), and *cox*1 mtDNA sequence of *Raphidascaris trichiuri* (Yin & Zhang, 1983) (FJ907318). The phylogenetic analyses were based on four different datasets, to compare the present sequences with those from almost all taxa of Raphidascarididae, with maximum accuracy: one including the 18S and 28S rDNA sequences concatenated, one consisting of the nuclear ITS1-5.8S-ITS2, one concatenating the whole 18S, ITS1-5.8S-ITS2 and 28S rDNA, and one with the *cox*1 mtDNA alone. The outgroups were chosen according to the recent phylogeny by Li *et al.* (2018), following the nature of each dataset (table 1). Sequences were aligned using M-Coffee (Notredame *et al.*, 2000), then evaluated by the transitive consistency score, to verify the reliability of aligned positions, and based on score values ambiguous aligned positions were trimmed (Chang *et al.*, 2014). Datasets were subjected to maximum likelihood (ML) and Bayesian inference analyses, using PHYML and MrBayes, respectively (Huelsenbeck and Ronquist, 2001; Guindon and Gascuel, 2003). The model of evolution and its fixed parameters for each dataset were chosen and estimated under the Akaike information criterion with jModelTest 2 (Guindon and Gascuel, 2003; Darriba *et al.*, 2012) and are detailed in supplementary table S1. Nodal supports for Bayesian posterior probability values were determined after running the Markov chain Monte Carlo (2 runs 4 chains) for 4×10^6 generations, with sampling frequency every 4×10^3 generations and discarding the initial ¼ of sampled trees (1×10^6) as burn-in. The same, but for ML, were based on 1000 bootstrap non-parametric replications.

Results

Ascaridoidea Baird, 1853; Raphidascarididae Hartwich, 1954; *Raphidascaris* (*Sprentascaris*) Petter & Cassone, 1984; *Raphidascaris* (*Sprentascaris*) *andersoni* n. sp.

Description

Small, thin, whitish nematodes. Cuticle thick with fine transverse striations throughout body (fig. 2F–H). Anterior end with three well-developed labia (one dorsal and two subventral), each bearing two long lateral, horn-shaped projections and three pairs of roughly triangular cuticular elevations (one dorsal and two subventral) at each labium base (figs 1C, D and 2D). Dorsal labium with two double papillae and pulp composed of two triangular anterior projections (figs 1C and 2A, B, D). Subventral bearing one double papilla and one amphidial papilla with transversal aperture, its pulp with cuticular projection of irregular edge (figs 1D and 2A–D). Well-developed lateral alae, *c.* 40 maximum width, originating at base of subventral labia (figs 1A–D and 2A, B, D), extending to near tail tip in females (figs 1G and 2H) and ending well anterior to first pair of caudal papillae in males (fig. 1J). Interlabia absent. Oesophagus muscular, slightly expanded at posterior end (fig. 1A, B). Nerve ring encircling oesophagus somewhat at end of its first 1/3 (fig. 1A, B). Excretory pore slightly posterior to level of nerve ring (fig. 1A). Ventriculus with irregular shape, transversely oval in some specimens; ventricular appendix short and posteriorly directed (fig. 1A, B).

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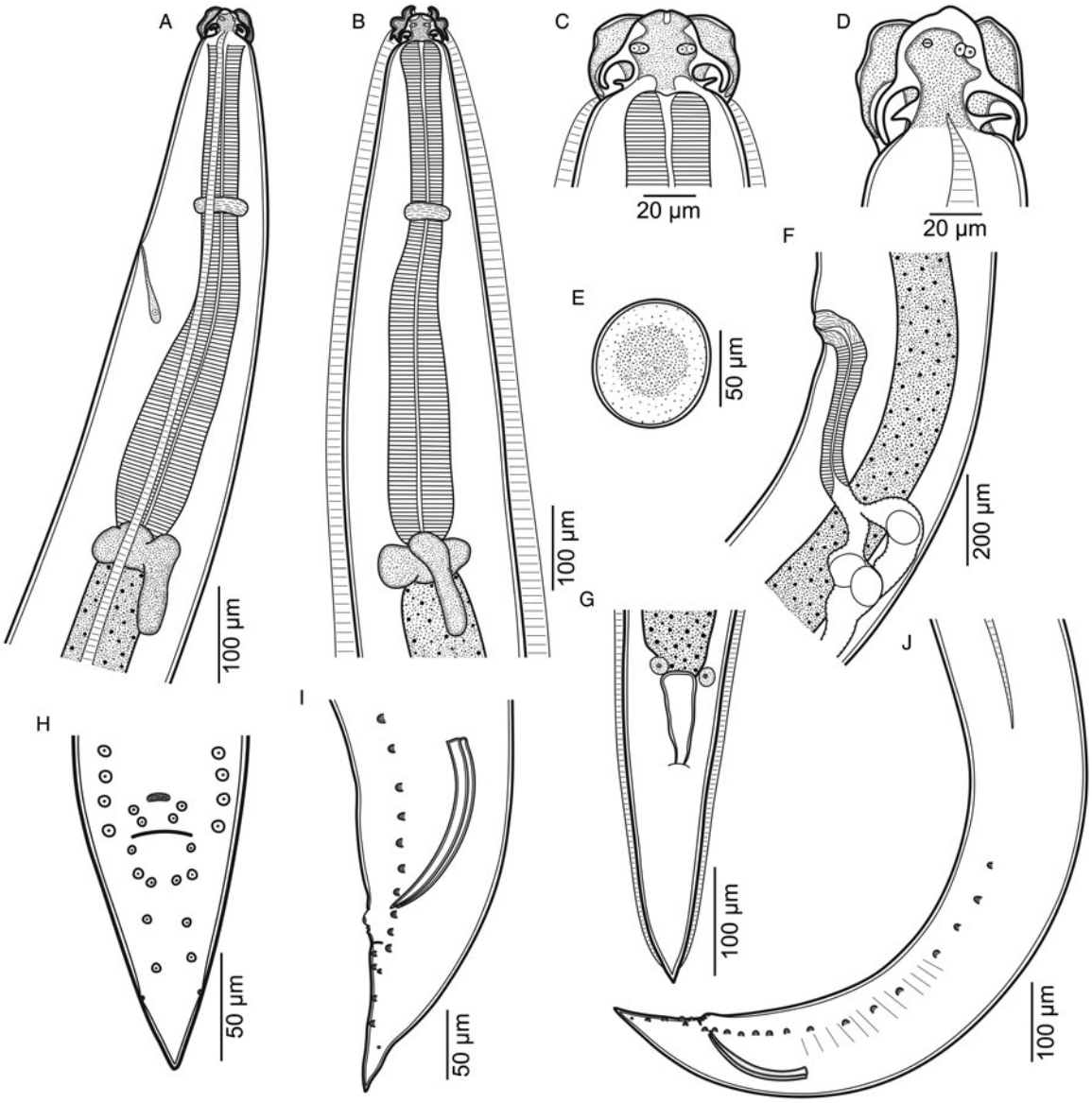

Fig. 1. *Raphidascaris* (*Sprentascaris*) *andersoni* n. sp. (A, B) anterior end, lateral and dorsoventral views, respectively; (C, D) cephalic end, male dorsal and female subventral views, respectively; (E) egg; (F) region of vulva, lateral view; (G) posterior end of female, ventral view; (H, I) tail of male, ventral and lateral views, respectively; (J) posterior end of male, lateral view.

Male (based on holotype and eight paratypes; measurements of holotype in parentheses). Body length 3.8–6.3 (5.1) mm, maximum width 171–232 (216). Labia 22–28 (28) long. Oesophagus 456–624 (624) long, with maximum width 63–91 (74). Entire length of oesophagus representing 9.6–12.6 (12.2) % of body length. Nerve ring and excretory pore 173–207 (204) and 203–242 (215), respectively, from anterior body end. Ventriculus 32–51 (35) long and 32–67 (45) wide; ventricular appendix 94–146 (94) long and 25–51 (34) wide. Spicules equal, well-sclerotized, with straight proximal end and sharp distal tip, 116–137 (117) long, representing 2.2–3.4 (2.3) % of total body length (fig. 1I, J). Gubernaculum and caudal alae absent. Precloacal papillae: 15 pairs similar in size, 13 of which are subventral and two ventral near cloacal aperture, somewhat asymmetric; one ventral unpaired papillae-like ellipsoid formation anterior to ventral pairs of precloacal papillae (figs 1H–J and 2E–G). Postcloacal papillae: five ventral pairs, asymmetrically arranged in two lines (figs 1H–J and 2E, F). Pair of lateral, small and inconspicuous phasmidial pores, posterior to last pair of postcloacal papillae (figs 1H–J and 2F). Tail conical, 103–132 (132) long (figs 1H–J and 2E).

Female (based on allotype and eight paratypes, all gravid; measurements of allotype in parentheses). Body length 4.4–8.7 (6.6) mm, maximum width 193–348 (264). Labia 21–39 (24) long. Oesophagus 549–797 (777) long, with maximum width 73–108 (86). Entire length of oesophagus representing 9.1–12.5 (11.7) % of body length. Nerve ring and excretory pore 172–285 (231) and 283–370 (296), respectively, from anterior body end. Ventriculus 34–58 (53) long and 45–70 (68) wide; ventricular appendix 94–160 (154) long and 21–44 (44) wide. Vulva 1.2–2.6 (2.2) mm from anterior end, at 28–35 (33) % of body length, vulval lips slightly elevated (fig. 1F). Vagina posteriorly directed, with striated musculature, followed by strong muscular ovijector *c.* 230 long and didelphic opisthodelphic uterus (fig. 1F). Eggs oval

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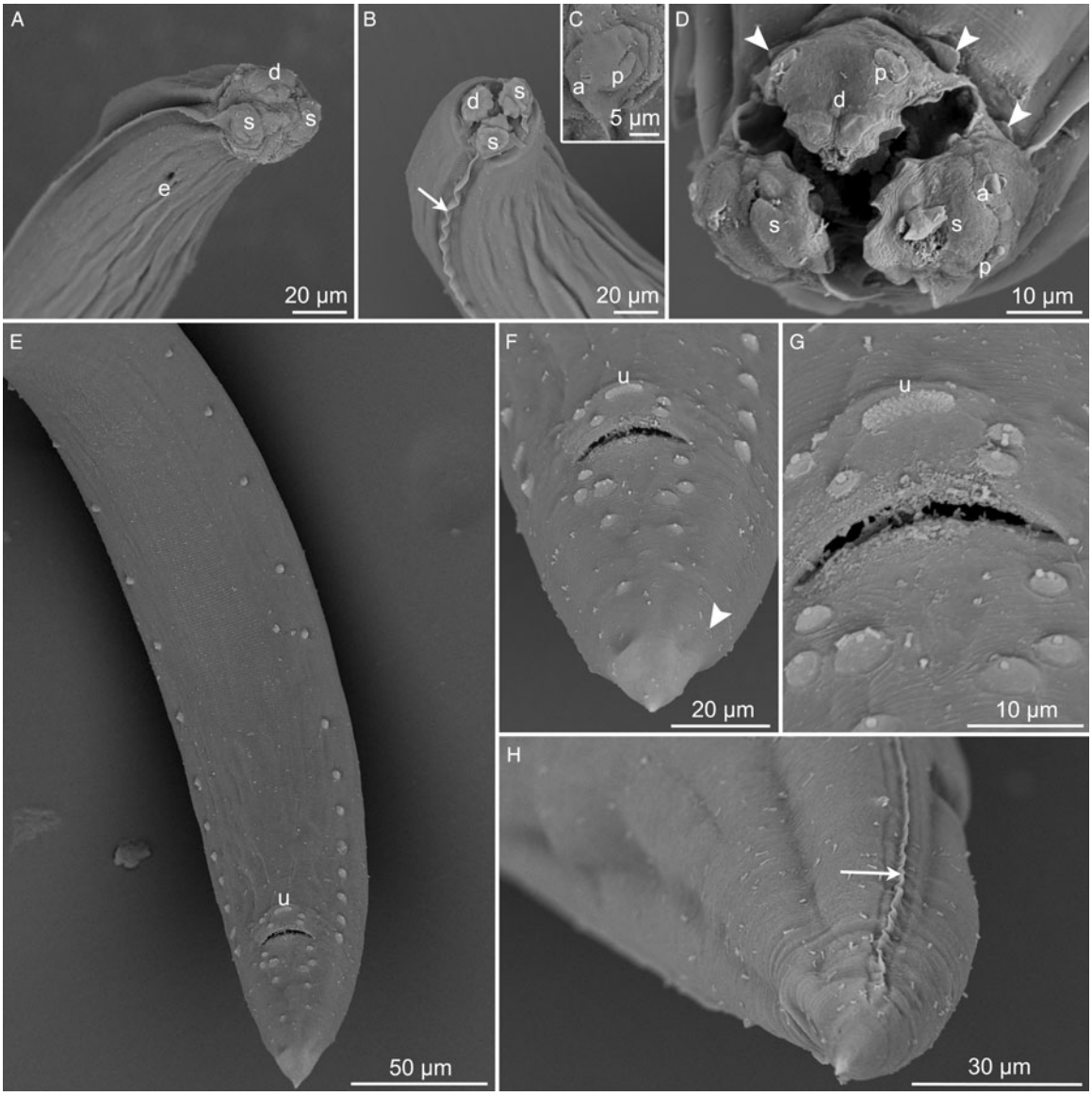

Fig. 2. SEM micrographs of *Raphidascaris* (*Sprentascaris*) *andersoni* n. sp. showing (A, B, D) cephalic end, apical views (arrow indicates lateral ala, arrowheads indicate postlabial cuticular elevations); (C) subventral labium, subapical view; (E) posterior end of male, subventral view; (F, G) tail and cloacal regions of male, ventral views, respectively (arrowhead indicates phasmidial pore); (H) tail of female, subapical view (arrow indicates lateral ala). Abbreviations: a, amphidial papilla; d, dorsal labium; e, excretory pore; p, double labial papilla; s, subventral labium; u, unpaired papilla-like formation.

with thin smooth shell, non embryonated, measuring 53–74 × 50–70 (from allotype) (fig. 1E). Tail conical with pointed end, 226–317 (317) long (figs 1G and 2H). Phasmids not observed.

Taxonomic summary

Type host. *Gymnogeophagus balzanii* (Perugia) (Actinopterygii: Cichlidae).

Site of infection. Intestine.

Type locality. Lakes on the margin of State highway MS-184, Pantanal wetlands, municipality of Corumbá, State of Mato Grosso do Sul, Brazil (19°28′S, 57°02′W).

Prevalence. Six infected fish of 21 examined (28.5%).

Mean intensity of infection (range). 5.16 ± 5.45 (1–17) nematodes per infected host.

Specimens deposited. Holotype male (ZUFMS-NEM00062), allotype female (ZUFMS-NEM00063), female paratypes (ZUFMS-00064), male paratypes (ZUFMS-00065).

Genetic data (GenBank accession numbers). 18S rDNA (MK141033), 28S (MK141031) rDNA, ITS1-5.8S-ITS2 (MK141032), *cox*1 mtDNA (MK14343).

Etymology. The specific name is a homage to Roy Clayton Anderson, who made important and valuable contributions to the knowledge of the nematode parasites of vertebrates.

Remarks

According to the conception of Moravec *et al.* (1990), the subgenus *Raphidascaris* (*Sprentascaris*) includes species with postlabial cuticular elevations and mature eggs in the uterus containing fully formed larvae. The present specimens have clear postlabial

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cuticular elevations and, even though no eggs were found containing larvae, they were allocated in *Raphidascaris* (*Sprentascaris*), as the subgenus is exclusive to freshwater fishes in the Neotropical region. Non-embryonated eggs were also reported in *R.* (*S.*) *lanfrediae* Melo, Santos, Giese, Santos & Santos, 2011 and in *R.* (*S.*) *pimelodi* (Petter & Cassone, 1984) (Petter and Cassone, 1984; Pereira and Luque, 2017) suggesting that this character may be unsuitable for subgeneric diagnosis, as it was amended by Moravec (1998).

Raphidascaris (*S.*) *andersoni* n. sp. is the only species in the subgenus that has a conspicuous unpaired papilla-like formation anterior to the cloacal aperture. A structure that may resemble it was described in *R.* (*S.*) *lanfrediae*, a parasite of cichlid fishes from northern Brazil (Pereira and Luque, 2017); however, this feature appears as a small button-like formation without striations in *R.* (*S.*) *lanfrediae*, whereas in the new species it is large and striated. Moreover, the new species differs from *R.* (*S.*) *lanfrediae* because the caudal alae are absent in males (*vs* present), the lateral alae end far anterior from the first pair of precloacal papillae (*vs* at level of ninth pair of precloacal papillae pairs), by having two pairs of subventral papillae slightly anterior to cloacal opening (*vs* only one pair) and vulva far posterior (at 28–35% *vs*14–22% of body length) (Melo *et al.*, 2011; Pereira and Luque, 2017), among other morphometric differences.

Of the remaining five species of *Raphidascaris* (*Sprentascaris*), *R.* (*S.*) *hypostomi* (Petter & Cassone, 1984) and *R.* (*S.*) *pimelodi* have no lateral alae, clearly differing from the new species (Petter and Cassone, 1984; Moravec *et al.*, 1990). Furthermore, *R.* (*S.*) *hypostomi* has digitiform conspicuous phasmids (*vs* pore-like inconspicuous in the new species) (Moravec *et al.*, 1990) and males of *R.* (*S.*) *pimelodi* have ventral cuticular surface on the posterior end of body ornamented (*vs* smooth in the new species) (Petter and Cassone, 1984). *Raphidascaris* (*S.*) *mahnerti* has well-developed lateral alae as in *R.* (*S.*) *andersoni* n. sp., but males of the first have long and conspicuous caudal alae, which are absent in the new species (Petter and Cassone, 1984; Moravec *et al.*, 1990). In contrast, *R.* (*S.*) *marano* Ramallo, 2009 and *R.* (*S.*) *saltaensis* Ailán-Choke, Ramallo & Davies, 2017, both parasites of loricariid fishes in Argentina, also have conspicuous lateral alae but males lack caudal alae as in the new species; however, these two species differ from *R.* (*S.*) *andersoni* n. sp. by having males with only three pairs of postcloacal papillae (*vs* five pairs), one pair of adcloacal papillae (*vs* absence of adcloacal papillae), larger spicules (220–300 µm at maximum *vs* 116–137 µm) and embryonated eggs with rugged shell (*vs* non-embryonated with smooth shell) (Ramallo, 2009; Ailán-Choke *et al.*, 2017).

Molecular characterization and phylogenetic analyses

The partial sequences of the 18S (779 bp) and 28S (728 bp) rDNA, nuclear region ITS1-5.8S-ITS2 (927 bp) and *cox*1 (384 bp) mtDNA, were obtained for the new species. As expected, *R.* (*S.*) *lanfrediae* was most genetically similar to *R.* (*S.*) *andersoni* n. sp. regarding the nuclear genes 18S and 28S rRNA (sequence identity 96.7%). Without *R.* (*S.*) *lanfrediae* in the alignment, *R.* (*S.*) *andersoni* n. sp. was most genetically similar to the two taxa labelled as *Raphidascaris* sp. in the dataset of the ITS1-5.8S-ITS2 (78.8% and 77.17%, respectively). When concatenating the whole nuclear region 18S, ITS1-5.8S-ITS2 and 28S rDNA, the new species was most genetically similar to *Raphidascaris* (*Raphidascaris*) *acus* (Bloch, 1779), followed by *Hysterothylacium tetrapteri* (Bruce & Cannon, 1989) and *Raphidascaris* (*Ichthyascaris*) *lophii* (Wu, 1949) (86.54%, 85.75% and 85.45%, respectively). In the alignment using sequences of the *cox*1 mtDNA, *R.* (*S.*) *andersoni* n. sp. was most similar genetically to *H. tetrapteri* (83.85%), *R.* (*I.*) *lophii* and *R.* (*I.*) *longispicula* Li, Liu, Liu & Zhang, 2012 (each 83.59%).

The phylogenetic reconstruction using the 18S and 28S rDNA sequences concatenated confirmed the separation of *R.* (*S.*) *andersoni* n. sp. from its closely related *R.* (*S.*) *lanfrediae*; both species formed a full-supported, monophyletic and external clade within Raphidascarididae (fig. 3A). In this phylogeny, species of the subgenus *Ichthyascaris* formed a monophyletic highly supported assemblage as sister group of *H. longilabrum* Li, Liu & Zhang, 2012. A similar pattern was noted in the phylogeny generated from sequences of the ITS1-5.8S-ITS2; after inclusion of *R.* (*I.*) *trichiuri* the clade formed by species of *Raphidascaris* (*Ichthyascaris*) continued with full support; however, two taxa identified only as *Raphidascaris* sp. were also in the same cluster (fig. 3B). In the same tree, the subgenus *Raphidascaris*, represented by two species, appeared as non-monophyletic (fig. 3B). The trees generated from the alignments of the 18S, ITS1-5.8S-ITS2, 28S rDNA concatenated and from the *cox*1 also showed the monophyly of *Raphidascaris* (*Ichthyascaris*) as sister group of *H. longilabrum* (fig. 4A), even though nodal support values were different according to each dataset (fig. 4A, B).

In all the phylogenetic reconstructions, *R.* (*S.*) *andersoni* n. sp. was separated from the other taxa, but its phylogenetic position within Raphidascarididae changed according to each dataset and the genus *Hysterothylacium* Ward & Magath, 1917 was not monophyletic (figs 3A, B and 4A, B). Similarly, *Raphidascaroides* Yamaguti, 1941 was not monophyletic in the tree generated from 18S and 28S rDNA sequences concatenated, appearing in two highly supported, separate clades (fig. 3A).

Discussion

Recent studies have shown the monophyly of Raphidascarididae and its clear separation from Anisakidae (Pereira and Luque, 2017; Li *et al.*, 2018). Such a discussion was not the focus here, but we agree with these previous studies and this was the reason why anisakids were used as outgroups in the present phylogenetic analyses.

The nuclear rDNA genes, which are more conserved than those from the mtDNA, seem to show more resolution pertaining to the phylogenetic relationships among genera (and subgenera) of Raphidascarididae according to the present phylogenies. In this sense, the absence of monophyly of *Hysterothylacium* and *Raphidascaroides* was confirmed, as shown in other studies (Nadler and Hudspeth, 2000; Pereira and Luque, 2017; Li *et al.*, 2018). Furthermore, it should be noted that the more taxa included in the analysis, the better the phylogenetic resolution.

When more than one taxon belonging to *Ichthyascaris* and *Sprentascaris* was included in the phylogenetic reconstructions, both subgenera formed well-supported monophyletic assemblages. It was interesting that *R.* (*S.*) *andersoni* n. sp. and *R.* (*S.*) *lanfrediae* clustered far from *Raphidascaroides brasiliensis* Moravec & Thatcher, 1997 and *Ro. moraveci* Pereira, Tavares, Scholz & Luque, 2015, even though all these species parasitize freshwater fishes from Brazil (fig. 3A) (Pereira *et al.*, 2015), indicating a clear separation between these genera. Conversely, the two *Raphidascaris* spp. isolated from a perciform fish off Brazil (Pantoja *et al.*, 2015), were closer to species of *Raphidascaris*

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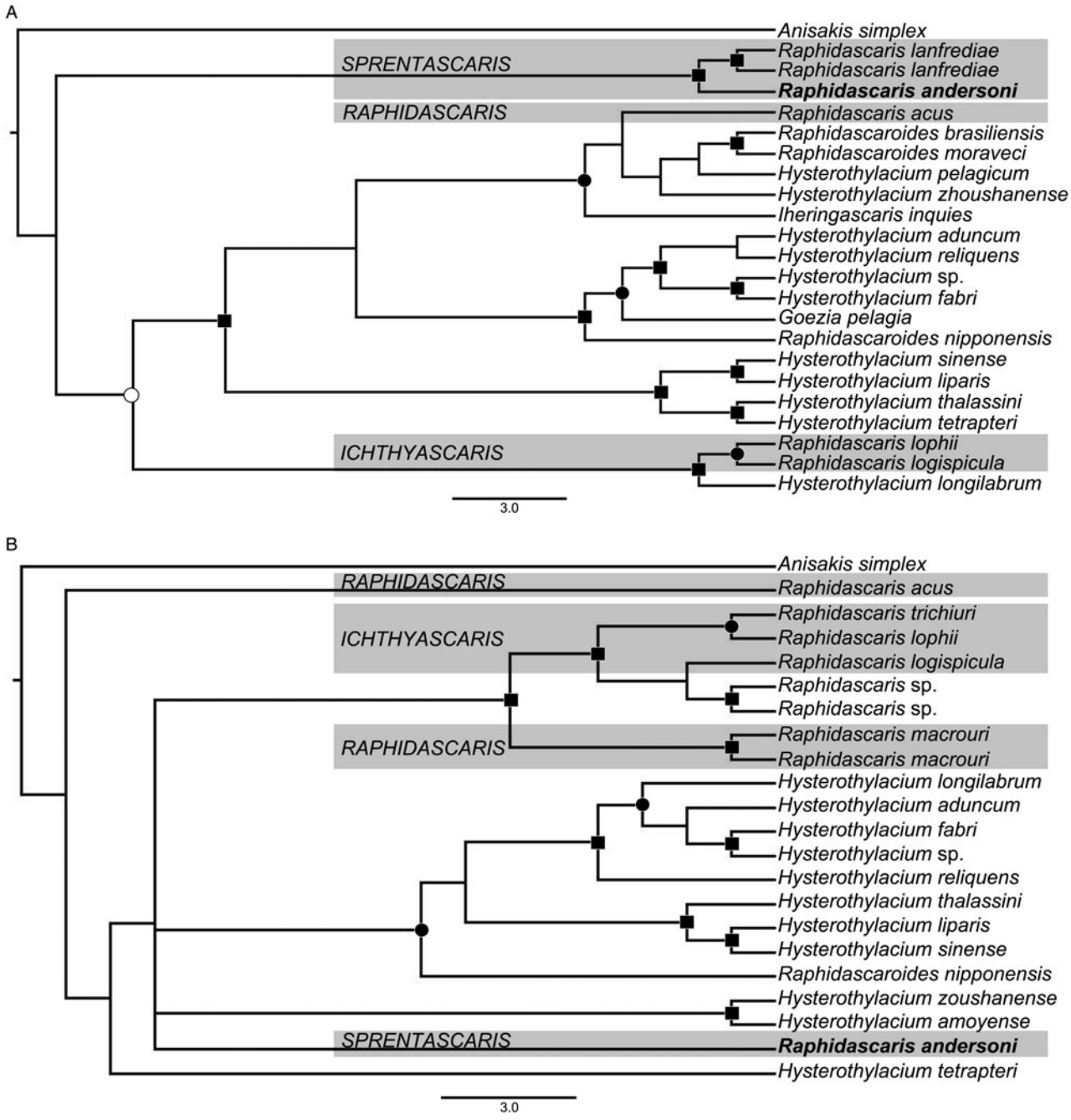

Fig. 3. Phylogenetic trees of Raphidascarididae generated from Bayesian inference (BI) analysis of sequences of the (A) 18S + 28S rDNA concatenated and (B) ITS1-5.8S-ITS2. Nodal supports of Bayesian posterior probabilities (BPP) were generated after running the Markov chain Monte Carlo (two runs four chains, 4×10^6 generations, sampling frequency = 4×10^3, burn-in = 1×10^6); those of maximum likelihood (ML) were generated after 1000 non-parametric bootstrap replications. Taxon in bold represents the new species; subgenera of *Raphidascaris* are highlighted. Squares represent BPP = 1 and ML = 100%; circles represent $0.96 <$ BPP < 1 and $90\% \leq$ ML $\leq 100\%$.

(*Ichthyascaris*) parasitic in marine fishes from different orders in Europe and Asia (Li *et al.*, 2012a; Xu *et al.*, 2012; Pérez-i-García *et al.*, 2015) (see fig. 3B and table 1). Both sequences of *Raphidascaris* sp. (KJ634267/KJ634266) were isolated from larval stages (L3) (Pantoja *et al.*, 2015), and they belong to *Ichthyascaris*. Therefore, at least the subgenera *Sprentascaris* and *Ichthyascaris* seem to have a consistent monophyly, different from *Raphidascaris*.

The lack of monophyly in the subgenus *Raphidascaris* observed in the phylogenetic reconstruction using the ITS1-5.8S-ITS2 dataset cannot be explained with certainty. However, it should be mentioned that *Raphidascaris* (*R.*) *acus* was isolated from a catadromous anguiliform fish in the eastern Mediterranean (off Turkey) (Simsek *et al.*, 2016) (KT633862), whereas the two isolates of *R.* (*R.*) *macrouri* Pérez-i-García, Constenla, Carrassón, Montero, Soler-Membrives & González-Solís, 2015 are from marine gadiform fishes in the western Mediterranean (off Spain) (Pérez-i-García *et al.*, 2015) (KR232377, KR232376). In contrast, there is consistent close relatedness of *R.* (*I.*) *longispicula* and *R* (*I.*) *lophii* with *H. longilabrum* (figs 3A and 4A), which may be explained by

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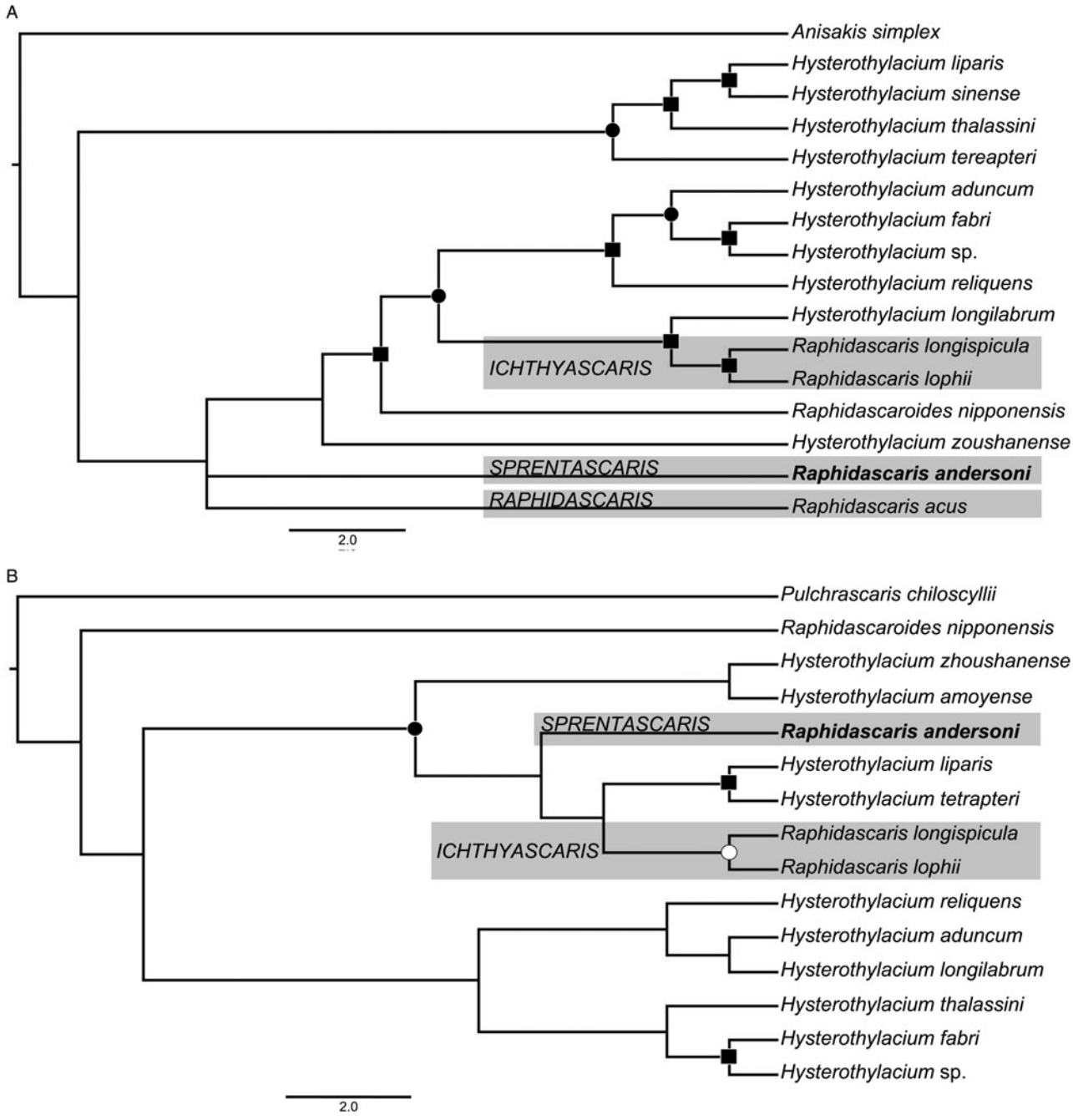

Fig. 4. Phylogenetic trees of Raphidascarididae generated from Bayesian inference (BI) analysis of sequences of the (A) 18S + ITS1-5.8S-ITS2 + 28S rDNA concatenated and (B) *cox*1 mtDNA. Nodal supports of Bayesian posterior probabilities (BPP) were generated after running the Markov chain Monte Carlo (two runs four chains, 4×10^6 generations, sampling frequency = 4×10^3, burn-in = 1×10^6); those of maximum likelihood (ML) were generated after 1000 non-parametric bootstrap replications. Taxon in bold represents the new species; subgenera of *Raphidascaris* are highlighted. Squares represent BPP = 1 and ML = 100%; filled circles represent $0.96 < \text{BPP} < 1$ and $90\% \leq \text{ML} \leq 100\%$; unfilled circle represents BPP = 0.94 and ML = 88%.

their same geographical origin (China) (Li *et al*., 2012a,b; Xu *et al*., 2012).

The validity of the subgenera of *Raphidascaris* has been discussed previously and some diagnostic features have been demonstrated to be inconsistent (e.g. egg development in ovijector, presence/absence of lateral alae). For example, Bruce (1990) and Bruce *et al*. (1994) re-established *Ichthyascaris* as an independent genus based on the structure of labia and the presence of lateral alae, but later Moravec & Nagasawa (2002) verified that several species were wrongly allocated in *Ichthyascaris* by the previous authors. Furthermore, one of the diagnostic features of *Raphidascaris* (*Sprentascaris*) is the presence of eggs containing larvae in gravid females (Moravec *et al*., 1990); however, in the new species, in *R.* (*S.*) *lanfrediae* and in *R.* (*S.*) *pimelodi*, the eggs are not embryonated (Petter and Cassone, 1984; Pereira and Luque, 2017; present study). Therefore, Moravec (1998) amended the subgeneric diagnosis of *Sprentascaris* as "eggs in uterus embryonated or non-embryonated."

Based on our results, mainly those of the phylogenetic reconstructions, *Raphidascaris*, *Ichthyascaris* and *Sprentascaris* should

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Table 1. Species whose sequences were obtained from GenBank and used in phylogenetic reconstructions, associated with their hosts (habitat), geographical origin, accession number and genetic regions. The 18S, ITS1-5.8S-ITS2, 28S refer to the rDNA and the *cox*1 to the mtDNA.

Parasite species	Host (Habitat)	Geographical origin	18S	28S	ITS1-5.8S-ITS2	*cox*1
*Anisakis simplex**	*Carcharhinus sorrah* (marine)	China	MF072711	MF094292	JX535521	–
Goezia pelagia	NM	NM	U94372	GPU94758	–	–
Hysterothylacium aduncum	*Lophius litulon* (marine)	China	MF072693	MF094277	KF736936	MF113231
Hysterothylacium amoyense	*Halieutaea stellata* (marine)	China	–	–	KP252131	MF113235
Hysterothylacium fabri	*Uranoscopus japonicus* (marine)	China	MF072709	MF094281	KF736944	MF113234
Hysterothylacium liparis	*Liparis tanakae* (marine)	China	MF072708	MF094280	KF601900	MF113233
Hysterothylacium longilabrum	*Siganus fuscescens* (marine)	China	MF072696	MF094285	JQ520159	MF113229
Hysterothylacium pelagicum	NM	NM	U94375	HPU94761	–	–
Hysterothylacium reliquens	NM	NM	U94376	MF094283	MF061682	MF113237
Hysterothylacium sinense	*Conger myriaster* (marine)	China	MF072694	MF094282	KX084795	–
Hysterothylacium tetrapteri	*Kajikia audax* (marine)	China	MF072705	MF094287	KF601901	MF113239
Hysterothylacium thalassini	*Priacanthus macracanthus* (marine)	China	MF072702	MF094278	JX982128	MF113232
Hysterothylacium zhoushanense	*Pseudorhombus oligodon* (marine)	China	MF072703	MF094279	JX028281	MF113230
Hysterothylacium sp.	*Uranoscopus tosae* (marine)	China	MF072698	MF094284	MF061683	MF113238
Iheringascaris inquies	NM	NM	U94377	U94763	–	–
*Pulchrascaris chiloscylii**	*Sphyrna lewini* (marine)	China	–	–	–	MF113245
Raphidascaris acus	*Esox lucius* (freshwater) *Anguilla anguilla* (freshwater/marine)	Finland Mediterranean Sea (off Turkey)	DQ503460	AY821772	KT633862	–
Raphidascaris lanfrediae	*Geophagus proximus* (freshwater)	Brazil	KX859077	KX859078	–	–
Raphidascaris lanfrediae	*Geophagus argyrostictus* (freshwater)	Brazil	KX859076	KX859075	–	–
Raphidascaris longispicula	*Uroconger lepturus* (marine)	China	MF072704	MF094288	JN102362	MF113241
Raphidascaris lophii	*Lophius litulon* (marine)	China	MF072692	MF094289	JF809816	MF113240
Raphidascaris macrouri	*Nezumia aequalis* (marine)	Mediterranean Sea (off Spain)	–	–	KR232377	–
Raphidascaris macrouri	*Trachyrincus scabrus* (marine)	Mediterranean Sea (off Spain)	–	–	KR232376	–
Raphidascaris trichiuri	*Muraenesox cinereus* (marine/freshwater)	China	–	–	FJ009682	–
Raphidascaris sp.	*Pinguipes brasilianus* (marine)	Brazil	–	–	KJ634267 / KJ634266	–
Raphidascaroides brasiliensis	*Platydoras costatus* (freshwater)	Brazil	KP726276	KP726277	–	–
Raphidascaroides moraveci	*Platydoras armatulus* (freshwater)	Brazil	KP726278	KP726279	–	–
Raphidascaroides nipponensis	*Halieutaea stellata* (marine)	China	MF072710	MF094286	KP271528	MF113242

*Used as outgroups in the phylogenetic analyses.
NM = not mentioned.

be considered as independent genera for a while, even though the subgenus *Raphidascaris* is apparently not monophyletic. In this sense, an updated generic diagnosis is give herein. It should be highlighted that this is the first parasitological survey in *Gymnogeophagus balzanii*.

Diagnosis of the genera *Raphidascaris*, *Ichthyascaris* and *Sprentascaris*, updated from Moravec *et al.* (1990) and Moravec and Nagasawa (2002):

Raphidascaris Bloch, 1779: Postlabial cuticular projections in interlabial space absent; only a feebly developed elevation may be present in ventral labial region. Lateral alae absent. Parasites of freshwater and marine fishes throughout the world.

Ichthyascaris Wu, 1949: Labia without lateral membranous flanges. Lateral alae present and united ventro-anteriorly at short distance from subventral labium base. Parasites of marine fishes throughout the world.

Sprentascaris Petter & Cassone, 1984: Labia with lateral membranous flanges. Three pairs of conspicuous postlabial cuticular projections, directed to interlabial space. Lateral alae present or absent. Parasites of freshwater fishes in the Neotropical region.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S0022149X18001153

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Conflict of interest. None.

Ethical standards. All procedures involving animal manipulation were permitted by Sistema de Informação e Autorização em Biodiversidade (license number 54895) and were in strict accordance with the recommendations of the Colégio Brasileiro de Experimentação Animal.

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