Parasitology

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Two names, one species: redescription and phylogenetic position of *Schrankiana formosula* Freitas, 1959 provides new insights into the evolutionary history of the Cosmocercidae

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Research Article

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Abstract

Schrankiana are gastrointestinal parasites of anurans, distributed throughout Central and South America. *Schrankiana formosula* and *Schrankiana inconspicata* are some of the most commonly reported species parasitising anurans from Brazil, and the morphological differences between them are unclear. In the present study, we redescribed *S. formosula* based on a re-examination of type series and newly collected material from the frog *Leptodactylus pentadactylus* in the state of Amapá, Brazil. Additionally, we re-examined the type series of *S. inconspicata*, and propose it as a junior synonym of *S. formosula*. We provide detailed morphological and morphometric data with intraspecific variation analyses and new molecular data for *S. formosula*. In the present phylogeny, *S. formosula* formed a well-supported clade with *Raillietnema* sp. and *Labeonema synodontisi*. Based on molecular phylogenetic analyses and some morphological similarities, our findings support the hypothesis that *Schrankiana* is a member of the family Cosmocercidae, not Atractidae. Additionally, we provide the first ultrastructural descriptions of *S. formosula*, and establish the species' phylogenetic position from the Cosmocercidae.

Introduction

Schrankiana Strand, 1942 are gastrointestinal parasites of anurans, distributed throughout Central and South America (Campião *et al.*, 2014; González and Hamann, 2014; González *et al.*, 2021*a*). To date, *Schrankiana* comprises 8 species, and most of them were reported parasitising anurans belonging to Leptodactylidae from Brazil (Baker and Vaucher, 1988; González and Hamann, 2014; Campião *et al.*, 2017; Carmo *et al.*, 2024).

The systematic status, phylogenetic position and evolutionary history of *Schrankiana* remain uncertain. The genus has been allocated in Atractidae (Chabaud, 2009; González and Hamann, 2014; Campião *et al.*, 2016; Da Graça *et al.*, 2017; González *et al.*, 2021*b*; Chero *et al.*, 2023). However, Adamson and Baccam (1988) and Gibbons (2010) transferred *Schrankiana* to Cosmocercidae.

Currently, the identification of *Schrankiana* spp. is based on meristic data with a few qualitative characters. These species exhibit morphological similarities, and their measurements often overlap. Freitas (1959) described *Schrankiana formosula* in *Leptodactylus fuscus* (Schneider, 1799) from the state of Rio de Janeiro and *S. inconspicata* in *Leptodactylus labyrinthicus* Spix (1824) from the state of Salvador, Brazil. Despite the comprehensive morphological description, the authors did not state clear differences between both species (see Freitas, 1959; Baker and Vaucher, 1988). Additionally, those 2 species are among the most commonly reported parasitising anurans from Brazil (Goldberg *et al.*, 2007, 2009; Campião *et al.*, 2014, 2017).

In the present study, we found nematodes parasitising *Leptodactylus pentadactylus* (Laurenti, 1768) that resembled *S. formosula*. However, due to morphological similarities among *Schrankiana* spp. we re-examined the type series *S. formosula* and *S. inconspicata* and observed that the species are morphologically identical. Thus, we provide a redescription of *S. formosula*, including an analyses of the intraspecific morphological variability, scanning electron microscope (SEM) characterization and a proposal of the systematic relationships of the genus using DNA.

Materials and methods

Host collection, morphological studies and map of species distribution

During a helminthological survey carried out in September 2021, 12 specimens of *L. pentadactylus* were collected by an active search in the 'Beija-flor Brilho de Fogo' Extractive Reserve, Pedra Branca do Amapari municipality, state of Amapá, Brazil (0°47′30.6″N, 51°58′42.1″W).

After collection, the anurans were anaesthetised, measured, weighed and necropsied for helminthological examination. Nematodes were removed from the digestive tract, washed in saline solution (NaCl 0.9%), killed in heated 70% ethanol and preserved in the same solution at −20°C. For morphological and morphometric analyses, the nematodes were washed in distilled water, cleared in glycerin, mounted on temporary slides and examined under an Olympus BX41 microscope (Olympus, Tokyo, Japan) coupled with a drawing tube (without zoom adjustment). The illustrations were prepared using the CorelDraw 2021 software and processed using Adobe Photoshop Version 21.0.2 software.

We re-examined the type series of *S. formosula* from state of Rio de Janeiro (holotype: CHIOC 22595a; allotype: CHIOC 22595b and paratypes: CHIOC 22579a, c, d, f, g, h, i, j; 22596e) and *S. inconspicata* from state of Bahia (holotype: CHIOC 22578a; allotype: CHIOC 22578b and paratypes: CHIOC 22579 e, g, h, i, j, n; CHIOC 29579m–n) deposited in the helminthological collection of the Oswaldo Cruz Institute (CHIOC) of Rio de Janeiro, Brazil. The prevalence and mean intensity values are reported according to Bush *et al.* (1997). The amphibian hosts were identified according to Frost (2024).

A total of 4 nematodes (males and females) were post-fixed in 1% osmium tetroxide (OsO_4), dehydrated in an increasing ethanol series and critical-point dried in carbon dioxide (CO_2). Subsequently, the helminths were mounted on metallic stubs, coated with gold-palladium, and examined under a Vega3 (TESCAN, Brno, Czech Republic) SEM in the Laboratory of Structural Biology, Biological Sciences Institute, Federal University of Pará (UFPA), Brazil.

We conducted a bibliographic reference search to compile the records of *Schrankiana* species, using 7 electronic databases (Google, Google Scholar, PubMed, Scielo, Science Direct, Scopus and Web of Science). The keywords were combined amongst themselves: Atractidae, Cosmocercidae, *Schrankiana*, Helminths and Leptodactylidae. Species without specific diagnosis ('gr.', 'af.' and 'sp.') were excluded from our checklist. Additionally, a map illustrating the distribution of *Schrankiana* spp. was generated using a spreadsheet and QGIS 3.28 software (Quantum, 2024)). This compilation included published records, data available and information from the present study.

Comparative Data analyses

We also tested 22 variables in males and 26 variables in females of *S. formosula* from *L. pentadactylus* (present study) and re-examined the type series of *S. formosula* from *L. fuscus* and *S. formosula* (= *S. inconspicata*) from *L. pentadactylus* using a principal component analysis (PCA) to assess the importance of each variable within the dataset and its applied variance. We followed the methodology proposed by González *et al.* (2019).

After this ordination analyses, we reduced the multivariate dataset into a smaller group of composite variables with a limited loss of information (McGarigal *et al.*, 2000). Thus, we applied multivariate analysis of variance (MANOVA), including the most relevant components according to the PCA to test the hypothesis that there are differences between metric variables of males and females of the 3 groups analysed.

In cases of Pillai was significant, a 2-factor ANOVA was performed for each variable. Before the analyses, variables were logarithmically transformed [ln(x)] to fit a normal distribution. Analyses were performed using the PAST 4.11 (Hammer *et al.*, 2001).

Molecular analyses and phylogenetic study

A male specimen was preserved in 100% ethanol and stored in a freezer at −20°C for molecular analyses. Genomic DNA was extracted using NucleoSpin Tissue (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. The SSU rDNA nuclear gene (18S) was amplified using the protocol and primers described in Gomes *et al.* (2015). The resulting amplicons were visualized on 1.5% agarose gel electrophoresis with GelRed Nucleic Acid Stain (Biotium, Hayward, California, USA) under Uv light transilluminator. PCR products were purified Illustra GFX PCR DNA and Gel Band kit (GE Healthcare, Chicago, IL, USA) according to the manufacturer's instructions and sequenced using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, USA). Amplicons were sequenced on Applied Biosystems™ 3730 DNA Analyser at the DNA Sequencing Platform of the Oswaldo Cruz Foundation (RPT01A/PDTIS/FIOCRUZ).

Contiguous sequences were assembled in Geneious 7.1.3 (Kearse *et al.*, 2012) and deposited in the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nml.nih.gov). The 18S rDNA datasets were aligned and trimmed using Muscle (Edgar, 2004) in Geneious 7.1.3 software (Kearse *et al.*, 2012). We obtained the saturation-substitutions index of each aligned matrix using the software DAMBE 5 (Xia, 2013). The genetic divergence was conducted in the MEGA11 software package (Kimura, 1980; Tamura *et al.*, 2011). The most appropriate evolutionary nucleotide substitution model was GTR + I + G, determined by the Akaike Information Criterion in the jModelTest program (Posada, 2008).

Phylogenetic reconstructions were performed using Maximum Likelihood (ML) in RAxML 8.2.12 and Bayesian Inference (BI) in MrBayes 3.2.7a software, respectively (Guindon and Gascuel, 2003; Ronquist and Huelsenbeck, 2003). Both analyses were conducted in CIPRES Science Gateway (Miller *et al.*, 2010). ML inference was performed using bootstrap support values of 1000 repetitions, and only nodes with a bootstrap percentage greater than 70% were considered well-supported.

Bayesian analyses employed the following settings for the dataset: Iset nst = 6, rates = invgamma, ngammacat = 4, nucmodel = 4by4, code = universal, prset statefreqpr = dirichlet (1,1,1,1), shapepr = fixed (0.5390) and pinvar = fixed (0.5290). Markov chain Monte Carlo (MCMC) search chains were run with 10 000 000 generations, saving 1 tree every 1500 generations. The first 25 000 generations were discarded on the burn-in, and the consensus tree (majority rule) was estimated using the remaining topologies, and we added commands sumt relburnin = yes, and sump relburnin = yes. Only nodes with Bayesian posterior probabilities greater than 90% were considered well-supported. The trees were visualized and edited in the software FigTree v1.3.3 (Rambaut, 2009). We used *Ichtyobronema hamulatum* (Moulton, 1931) (access number: KY476351) as an outgroup in all phylogenies.

Results

Systematics

Family: Cosmocercidae Travassos, 1925
Genus: *Schrankiana* Strand, 1942
Species: *Schrankiana formosula* Freitas, 1959

Taxonomic summary

Type host: Leptodactylus fuscus (Schneider, 1799) (Amphibia: Leptodactylidae)

Additional hosts: Leptodactylus pentadactylus (Laurenti, 1768); *Leptodactylus labyrinthicus* (Spix, 1824); *Leptodactylus elenae* Heyer, 1978.

Type locality: state of Rio de Janeiro, Brazil (22°54′10″N, 43°12′28″W)

Additional localities: 'Beija-Flor Brilho de Fogo' Extractive Reserve, Pedra Branca do Amapari municipality, state of Amapá, Brazil (0°47′30.6″N, 51°58′42.1″W) (present study);

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state of Bahia, Brazil (24°3′47.47″S, 54°18′50.14″W); Salto del Guiara municipality, department of Canindeyú, Paraguay (24° 1′12″N, 54°20′24″W); Arroyo Itabo Guazu municipality, department of Alto Paraná, Paraguay (25°4′60″S, 54°40′0″W); Coronel Oviedo municipality, department of Caaguazú, Paraguay (25° 26′60.00″S, 56°01′0.01″W)

Site of infection: large intestine

Parasitological descriptors: Prevalence 33% (4 of 12 analysed); mean intensity (230.5) and abundance (76.8). The infection parameters will be based only on the material collected in Amapá.

Voucher material: 15 males (MPEG 000289) and 15 females (MPEG 000290) were deposited in the Emílio Goeldi Paraense Museum.

GenBank Accession number: PP669822

Description (morphological description based on re-examination of type series and new material collected). Small slender nematodes (Fig. 1A and B). Cuticle with thin transverse striations (Fig. 2A and B). Sexual dimorphism evident, females larger than males (Fig. 1A and B). Lateral alae, weakly developed, extending from anterior third of oesophagus to just anterior to anus in both sexes (Fig. 2A). Somatic papillae, distributed over body surface. Oral opening triangular with 3 distinct lips; dorsal lip with a pair of papillae; subventral lips with 1 large papilla and 1 amphid each; all of them with cuticular flange overhanging mouth opening (Fig. 2B). Oesophagus divided into anterior short pharynx, cylindrical corpus, slightly narrower isthmus and well-developed posterior bulb with evident valvular apparatus (Fig. 1A and B). Nerve ring, located at middle portion of the oesophagus (Fig. 1A and B). Excretory pore large and slit-like anterior to bulb (Figs 1A, B, 2A and C). Viviparous. Monodelphic and prodelphic; vulva located slightly anterior to anus. Tail conical and sharply pointed in both sexes (Figs 1D, E and 2D). Males with short, equal and slightly sclerotised spicules curved ventrally, with proximal ends expanded, and sharply pointed distally (Fig. 3). Gubernaculum sclerotised, elongated, distal end pointed (Fig. 3). Caudal papillae arranged as follows: 3 pairs precloacal, 3 pairs slightly anterior to fringed cloacal lip plus unpaired papilla situated between them; 5–6 pairs postcloacal: anterior half of tail with 2 pairs of adjacent or *in tandem* subventral papillae and 1 pair lateral at same level of them; posterior half of tail with 1 lateral pair, 1 subventral pair present or absent and 1 pair subdorsal (Figs 1D, 2D, 4A and D). Posterior cloacal lip with cuticular comb-like fringe (Fig. 4A and D). Vagina well-developed, directed anteriorly, divided into *vagina vera*, and vagina uterina (Fig. 1B and E). Uteri with morulae embryonated (Fig. 1B). All the measurements obtained from the material collected in this study, re-examined in the type series and in previous studies, are given in Tables 1 and 2.

Metrical characters

The PCA analyses in males showed that the first (PCA1) and the second (PCA2) axis explained 26.58 and 17.16%, respectively. Combined, PCA1 and PCA2 explained 43.74% of the total variance. The first axis reflected the influence of the oesophagus, pharynx, corpus and tail lengths, while the second axis reflected the influence of oesophageal bulb width and the ratio of nerve ring to the body length (Table 3). In females, the first (PCA1) and the second (PCA2) axis explained 36.21 and 14.93%, respectively. Combined, PCA1 and PCA2 explained 51.14% of the total variance (Table 4). The first axis reflected the influence of excretory pore from anterior end, corpus and oesophagus lengths, while the second axis reflected the influence of nerve ring from anterior end, the ratio of tail to body length and morulae embryonated width.

The comparison between *S. formosula* (present study), re-examined type series of *S. formosula* and *S. formosula* (= *S. inconspicata*) showed statistical significance in both sexes (males: MANOVA Pillai = 1.434; $F = 6.328$; $P < 0.02$; females: MANOVA Pillai = 1.692; $F = 11.89$; $P < 0.00$). In the specific morphological comparison, males of *S. formosula* exhibited significant differences in 4 morphological characters (Table 5). Females of *S. formosula* exhibited significant differences in all morphological characters, except in the morulae embryonated width (Table 6).

Notes on Schrankiana *spp. distribution*

We found 7 *Schrankiana* spp. parasitizing 23 anuran hosts from 5 countries: Argentina, Brazil, Costa Rica, Paraguay and Peru. The Leptodactylidae has the highest number of *Schrankiana* species registered (7), followed by Hylidae (2), Bufonidae (2) and Brachycephalidae (1). All type hosts of *Schrankiana* spp. are anurans of the genus *Leptodactylus* Fitzinger, 1826.

Schrankiana formosula is the most common species, reported in 15 host species from 5 countries. Based on the present morphological analyses, we also considered *S. inconspicata* a synonym of *S. formosula* species in the distribution map (Table 7) (Fig. 5).

Molecular analyses and phylogenetic study

We sequenced the 18S rDNA gene from *S. formosula* and obtained a sequence with 786 pb. The alignment of nuclear gene 18S rDNA upon trimming to the shortest sequence length resulted in 748 pb. Xia's test provided no evidence for substitution saturation in the data matrix. Detailed information on nematode species included in the molecular analyses is provided in Table 8.

Pairwise genetic comparison of *S. formosula* showed low genetic divergence from *Raillietnema* sp. (0.81%) and *Labeonema synodontisi* (Vassiliadès, 1973) Koubková, Baruš, Hodová and Šimková, 2008 (2.61%) (see Supplementary 1). The topology of the phylogenetic trees performed on ML and BI revealed similar phylogenies among representatives of the Atractidae and Cosmocercidae (Fig. 6). The sequences of the atractids *Grassenema procaviae* Petter, 1959 and *Rondonia rondoni* Travassos, 1920 formed a well-supported monophyletic clade (100 bootstrap and 99 posterior probability).

We observed that sequences of species of Cosmocercidae (99 bootstrap and 100 posterior probability) formed 3 large clades. The first was composed of *Cosmocercoides* spp.+ *Cosmocerca longicauda* (Linstow, 1885) and *Nemhelix bakeri* Morand and Petter, 1986 (91 bootstrap and 99 posterior probability); the second grouped with 2 species of *Aplectana* Railliet and Henry, 1916 from China + a group consisting of 3 species of *Cosmocerca* spp. and *Aplectana chamaleonis* (Baylis, 1929) (62 bootstrap and 81 posterior probability); the latter clade is composed by *S. formosula*, *Raillietnema* sp. and *L. synodontisi* (100 bootstrap and 100 posterior probability) (Fig. 6).

Discussion

Schrankiana inconspicata *as a synonym of* S. formosula *and species differentiation*

The specimens studied herein were allocated in *Schrankiana* based on buccal and pharyngeal structures not elaborate, without specialized cuticularised formations; the morphology of the oesophagus divided into the pharynx, muscular corpus, isthmus well-marked and valved bulb; and spicules short, not much longer than gubernaculum, according to Chabaud (2009) those are the main characters used to identify this genus.

The main morphological characteristics used to distinguish species of *Schrankiana* include the morphology of the cephalic end; length of the oesophagus; lateral alae extension; vulva location; length of vagina, and male caudal characteristics (papillae,

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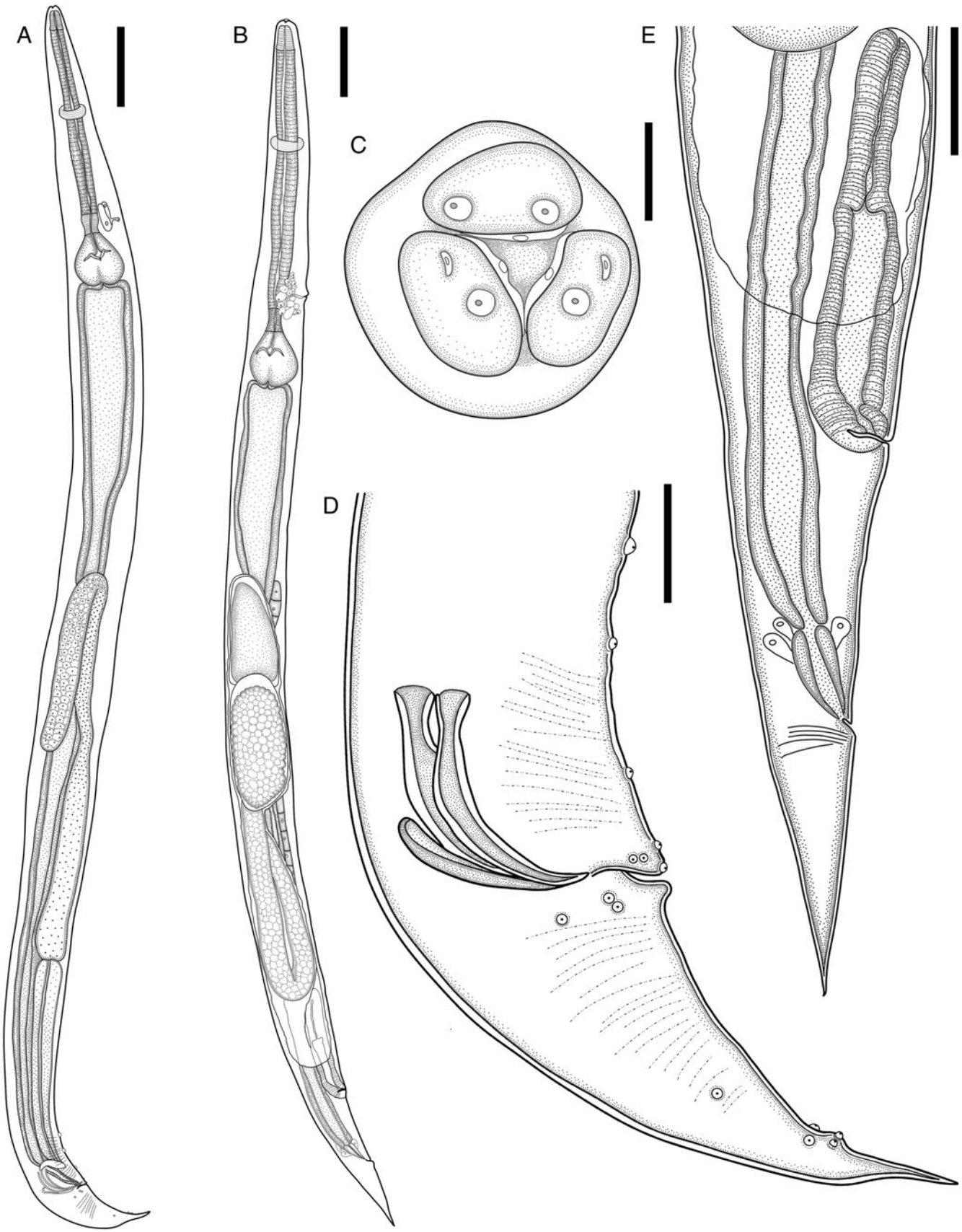

Figure 1. Line drawings of *Schrankiana formosula* from *Leptodactylus pentadactylus*. (A) Male specimen, lateral view; (B) female specimen, lateral view; (C) spicules and gubernaculum, lateral view; (D) caudal region of male, lateral view; (E) caudal region of female, lateral view. Scale bars: A–B = 200 μm; C = 50 μm; D = 100 μm; E = 50 μm.

gubernaculum and spicules) (Baker and Vaucher, 1988; González and Hamann, 2014; Draghi *et al*., 2020). Thus, *S. formosula* can be easily distinguished from *S. chacoensis*, *S. fuscus*, *S. freitasi* and *S. larvata* by the number of precloacal papillae (3 pairs in *S. formosula vs* 4–5 pairs in the other species), and resemble *S. inconspicata*, *S. schranki* and *S. brasili.*

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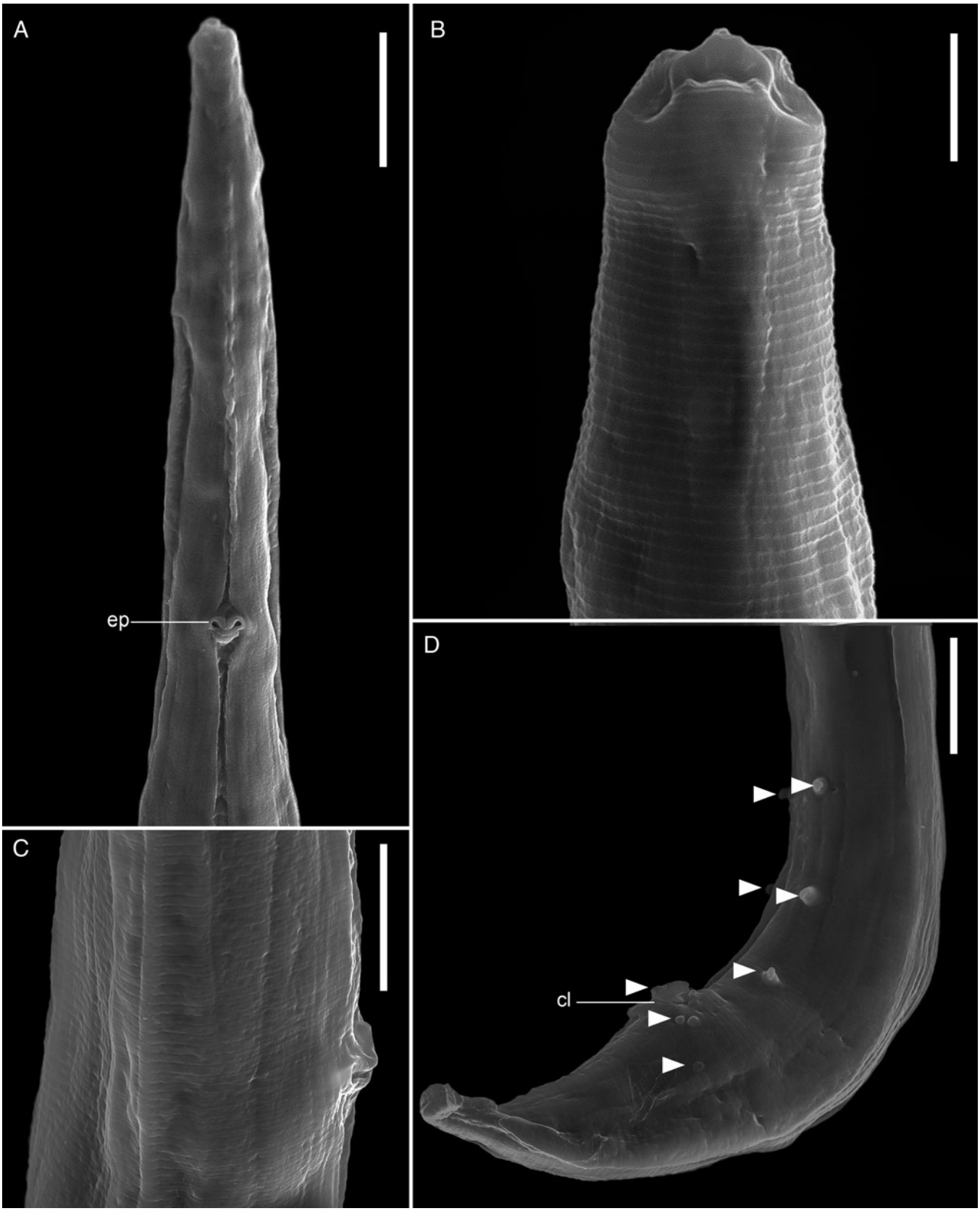

Figure 2. Scanning electron micrographs of *Schrankiana formosula* from *Leptodactylus pentadactylus*. (A) Anterior end, ventral view; (B) cephalic extremity, ventral view; (C) detail of excretory pore, ventral view; (D) posterior end of male, ventral view. Abbreviations: Cl, cloaca; ep, excretory pore; arrows, papillae. Scale bars: A = 100 μm; B–D = 20 μm.

Schrankiana formosula differs from *S. schranki* by having smaller spicules (42–86 in *S. formosula vs* 84–101 in *S. schranki*) and different number of postcloacal papillae (5–6 in *S. formosula vs* 3 pairs in *S. schranki*).

Schrankiana formosula differs from *S. brasili* by the smaller body size in males (1.44–2.30 in *S. formosula vs* 3.92–4.69 in *S. brasili*) and females (1.60–2.81 in *S. formosula vs* 4.62–6.53 in *S. brasili*); shorter oesophagus (325–560 in *S. formosula vs*

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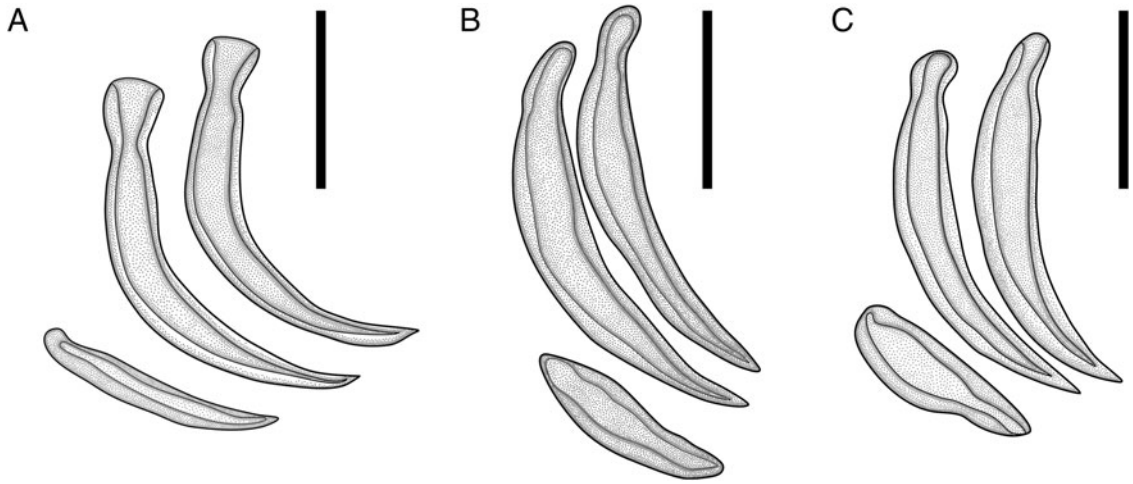

Figure 3. Spicules and gubernaculum of *S. formosula*. (A) Male from *L. pentadactylus*, lateral view (present study); (B) male from *L. fuscus* paratype *S. formosula*, ventrolateral view (CHIOC 14614c); male from *L. pentadactylus* paratype *S. formosula* (= *S. inconspicata*), ventrolateral view (CHIOC 1954c).

1000–1200 in *S. brasili*); absence of the corpus divided into procorpus and metacorpus (present in *S. brasili*); shorter distance from the vulva to the posterior end (230–390 in *S. formosula vs* 550–620 in *S. brasili*); shorter distance of the nerve ring (130–210 in *S. formosula vs* 340–370 in *S. brasili*) and excretory pore (280–340 in *S. formosula vs* 800–900 in *S. brasili*) from the anterior end.

The specimens resemble *S. inconspicata* in all measurements, except for the length of the oesophagus in females (325–560 in *S. formosula vs* 540–710 in *S. inconspicata*) (Table 1). The original description provided by Freitas (1959) does not detail the differences between *S. formosula* and *S. inconspicata*. However, Baker and Vaucher (1988) distinguished *S. inconspicata* by an unusual rod-shaped modification of the anterior end of the oesophageal corpus and the presence of an amuscular zone. Additionally, in females, *S. inconspicata* has lateral alae that end well anterior to the anus (while it is near to the anus in *S. formosula*) and an elongated uterine vagina (shorter in *S. formosula*).

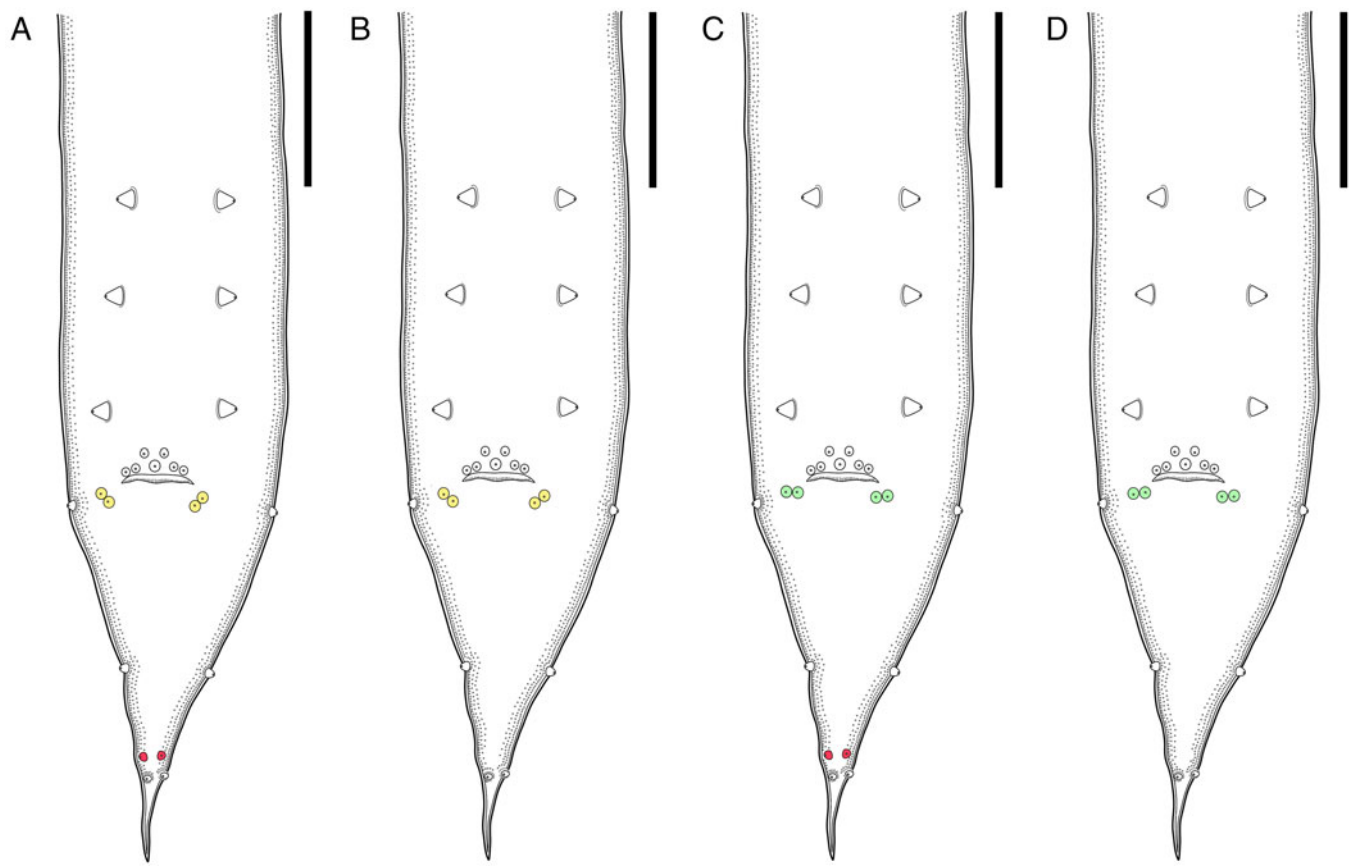

Figure 4. Variability of male caudal papillae of *Schrankiana formosula*, based on re-examined type series and newly collected material. The caudal papillae numbers are presented as pre cloacal: pairs of papillae slightly anterior to fringed cloacal lip plus unpaired papilla: post cloacal pairs. (A) Male caudal distribution pattern 3:3 + 1:6; (B) male caudal distribution pattern 3:3 + 1:5; (C) male caudal distribution pattern 3:3 + 1:6; (D) male caudal distribution pattern 3:3 + 1:5. Papillae in yellow represent the first post-cloacal pair that varies in position (green). Papillae presented in red are the fifth pair, which may be present or absent in some specimens. Scale bars: A–D = 100 μm.

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Table 1. Morphometric analyses of *S. formosula* from the present study, the type series of *Schrankiana formosula*, and *S. formosula* (= *S. inconspicata*)

	S. formosula (present study)		*S. formosula* (re-examined)		*S. formosula* (= *S. inconspicata* re-examined)	
Characters	Males ($n = 10$)	Females ($n = 10$)	Males ($n = 7$)	Females ($n = 5$)	Males ($n = 5$)	Females ($n = 5$)
Total length (mm)	1.60 (1.44–1.78)	1.78 (1.60–2.04)	1.58 (1.5–1.64)	1.8 (1.68–1.98)	1.91 (1.59–2.3)	2.32 (2.27–2.47)
Maximum width	87.7 (67–105)	127.8 (105–152)	90 (68–105)	143 (121–174)	98.8 (79–105)	133.6 (116–163)
Body width at oesophago-intestinal junction	78.6 (59–106.6)	111 (96–128)	83.8 (69–95)	97.6 (83–112)	92.8 (75–101)	120.4 (106–144)
Oesophagus length	432.7 (325–560)	494.1 (432–581)	380.2 (363–392)	393.8 (370–418)	525.6 (399–589)	621 (540–661)
Pharynx length	38.9 (35–42.6)	39.2 (32–45)	28.5 (24–40)	25.2 (19–30)	46 (35–53)	42.6 (40–45)
Pharynx width	20.9 (16–29.3)	22.3 (16–26)	18.1 (16–21)	15.8 (13–20)	18.4 (16–19)	22.6 (19–25)
Corpus length	324.3 (258.7–394.6)	353 (290–440)	261 (248–277)	271.8 (243–293)	310.8 (253–360)	454 (381–485)
Corpus width	42.4 (32–53.3)	33.3 (21–40)	29.2 (19–40)	36.8 (29–48)	40.6 (32–56)	37.8 (32–43)
Isthmus length	30.9 (24–48)	27.4 (21–32)	23.7 (19–27)	25.4 (19–31)	33 (29–42)	37.4 (32–48)
Isthmus width	23.4 (16–34.6)	26.4 (21–32)	19 (16–21)	18 (13–22)	22.6 (19–27)	28 (21–34)
Bulb length	73.4 (58.6–93)	77.1 (66–91)	70.4 (67–75)	71.4 (65–77)	68.2 (59–75)	86.8 (81–93)
Bulb width	70.9 (59–80)	83.1(72–96)	65.7 (53–80)	69.6 (66–75)	68.4 (59–80)	80.8 (67–92)
Nerve ring[a]	164.9 (130–202)	162.6 (139–200)	169.5 (160–179)	161.2 (140–173)	184.2 (168–202)	240.4 (229–253)
Excretory pore[a]	358 (312–400)	399.7 (339–467)	286.7 (280–297)	283.2 (247–307)	332.6 (310–373)	491 (400–522)
Tail length	104.8 (90–123)	125 (97–173)	94.8 (84–106)	108 (84–133)	118.8 (101–133)	112.6 (106–118)
Spicules	56.6 (45.5–67)	–	53.8 (42–64)	–	49 (50–53)	–
Gubernaculum	35.6 (27.2–45.4)	–	33.7 (28–38)	–	35.3 (29–47)	–
Vulva[a]	–	258 (220–320)		267 (198–304)	–	254 (240–290)
Distance vulva to anus		137.9 (99–181)		167.4 (133–208)		137.2 (129–146)
Vagina vera	–	91 (79–104)		138 (127–152)	–	146 (116–226)
Uterine vagina	–	248 (204–305)		–	–	161 (123–226)
Morulae length	–	217.3 (183–253)		126.4 (110–137)	–	151(103–213)
Morulae width	–	107.7 (100–120)		97.2 (86–109)	–	100.8 (64–147)
Oesophagus in % of body length	27.5 (22–34)	28 (22–32)	24 (23–25)	22 (21–24)	27.4 (25–31)	26.8 (24–28)
Excretory pore in % of body length	22 (20–26)	22 (17–26)	18 (17–19)	16 (14–17)	18 (16–20)	21 (18–23)
Nerve ring in % of body length	10 (9–14)	9 (7–11)	11 (10–11)	9 (9–9)	10 (8–11)	10 (9–11)
Tail length in % of body length	6.5 (5–8)	7 (6–10)	5.7 (5–6)	6 (5–7)	6.2 (6–7)	5 (5–5)
Spicules in % of body length	4 (3–4)		3 (3–4)		3 (2–3)	
Vulva in % of body length		14.6 (12–19)		14.8 (12–16)		11.2 (10–13)

All measurements are presented in micrometres, unless otherwise indicated.
[a]From the anterior end.

Table 2. Morphometric data of *Schrankiana formosula* from different studies

Characters	*S. formosula* (Freitas, 1959)		*S. formosula* (Baker, 1988)		*S. formosula* (= *S. inconspicata*) (Freitas, 1959)		*S. formosula* (= *S. inconspicata*) (Baker and Vaucher, 1988)	
	Males (*n* = 10)	Females (*n* = 10)	Males (*n* = 5)	Females (*n* = 5)	Males (*n* = 10)	Females (*n* = 10)	Males (*n* = 5)	Females (*n* = 5)
Total length (mm)	1.81–2.08	2.14–2.41	1.97–2.30	2.70–2.81	2.21–2.44	2.61–2.78	2.38–2.64	2.70–2.80
Maximum width	110–130	120–130	–	–	90–120	140–210	–	–
Oesophagus length	370–410	400–480	404–428	483–513	490–570	560–710	589–630	587–682
Pharynx length	25–29	25–31	–	–	38–45	45–56	–	–
Pharynx width	13–17	13–17	–	–	17–29	21–29	–	–
Corpus length	260–290	270–340	–	–	380–450	450–560	–	–
Corpus width	27–38	31–42	–	–	17–29	21–29	–	–
Isthmus length	38–63	42–80	–	–	34–59	38–63	–	–
Isthmus width	15–21	17–25	–	–	21	21–25	–	–
Bulb length	63–80	76–88	–	–	59–80	67–105	–	–
Bulb width	63–84	76–92	–	–	59–88	76–113	–	–
Nerve ring[a]	180–210	180–220	150–166	186–219	210–230	220–270	213–230	204–219
Excretory pore[a]	310–340	330–380	305–336	378–408	360–510	460–610	421–482	398–547
Tail length	90–140	110–160	128–135	126–134	110–140	130–150	157–181	168–190
Spicules	63–80	–	67–88	–	55–67	–	58–72	–
Gubernaculum	29–40	–	40–48	–	29–42	–	37–40	–
Vulva[a]	–	320–390	–	251–274	–	330–370	–	290–363
Vagina vera	–	140–220	–	120–200		110–160		120
Uterine vagina	–	–	–	–		–		300
Morulae length	–	165–235	–	–	–	200–287	–	–
Morulae width	–	104–174	–	–	–	130–200	–	–

All measurements are presented in micrometres, unless otherwise indicated.
[a]From the anterior end.

We re-examined the type series of *S. inconspicata* and did not observe the rod-shaped modification, and this structure was not highlighted by Freitas (1959). We noticed that the short and weakly developed amuscular zone of the oesophagus reported by Baker and Vaucher (1988) is an artefact that might appear during the processing of the specimens. In females, *vagina vera* length showed variation and overlapped values to those observed for *S. formosula* and *S. inconspicata* (see Table 1; Freitas, 1959; Baker and Vaucher, 1988). Males exhibit similar spicules (42–86 in *S. formosula vs* 49–72 in *S. inconspicata*) and gubernaculum lengths (27–52 in *S. formosula vs* 29–53 in *S. inconspicata*). The number and arrangement of caudal papillae of *S. inconspicata* are the same as described for *S. formosula* in the present study (Fig. 4) (Table 1).

Therefore, based on all morphological and morphometric similarities observed during re-examination of the type material, we consider *S. inconspicata* as a synonym of *S. formosula*, and the specimens that Baker and Vaucher (1988) identified as *S. inconspicata* might represent a different species.

In the Neotropical region, *Schrankiana* spp. are widely distributed in Brazil (Fig. 5) and also found in Argentina, Costa Rica, Paraguay and Peru (Table 7). The distribution and host record data suggest that these species have a low host specificity. Previous taxonomic studies suggested that specialist species appeared to be predominant in parasite communities (Combes, 2005; Agosta *et al.*, 2010; Engelstädter and Fortuna, 2019). However, parasite host specificity is not inflexible and can vary according to the composition of the host assemblage and the environment, with the parasite communities of anurans often composed of generalist species (Aho, 1990; Campião *et al.*, 2014; González *et al.*, 2019; Cardoso *et al.*, 2021; Euclydes *et al.*, 2021, 2022). Thus, we suggest that the paradox that parasites do not change in hosts may have led Freitas (1959) to describe *S. formosula* and *S. inconspicata* as different species due to their occurrence in distinct hosts.

Morphological and morphometric variation in Schrankiana formosula

We did not find intraspecific variation in most morphometric data based on the re-examination of the type series, the original description by Freitas (1959), and the study by Baker and Vaucher (1988), based on material from *L. fuscus* and *L. elenae* from Paraguay (see Tables 1 and 2). Of all the measured characters, 6 contributed to this variability in each sex (Tables 3 and 4). Of these, only 4 male characters and 5 female characters were statistically significant between the groups (Tables 5 and 6).

Among the variables that best-discriminated males were characteristics of the oesophagus (pharynx, corpus and oesophagus lengths), and tail length; while in females were characteristics of the oesophagus (corpus and oesophagus lengths), the ratio of the tail to body length, excretory pore and nerve ring from the

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Table 3. Results of principal component analysis of morphometric characters of males *Schrankiana formosula* ($n = 22$): coefficients for standardized measurements and percentage of explained variation

	PCA1	PCA2
Total length (mm)	0.29	−0.20
Maximum width	0.05	0.23
Body width at oesophago-intestinal junction	0.07	0.10
Oesophagus length	0.36	0.03
Pharynx length	0.31	−0.07
Pharynx width	0.07	0.22
Corpus length	0.30	0.18
Corpus width	0.24	0.13
Isthmus length	0.22	−0.01
Isthmus width	0.12	0.06
Bulb length	0.01	0.42
Bulb width	−0.02	0.42
Nerve ring[a]	0.20	0.09
Excretory pore[a]	−0.27	0.19
Tail length	0.33	0.01
Spicules	−0.08	0.26
Gubernaculum	−0.10	0.13
Oesophagus in % of body length	0.27	0.16
Excretory pore in % of body length	0.22	−0.04
Nerve ring in % of body length	−0.05	0.30
Tail length in % of body length	0.17	0.26
Spicules in % of body length	−0.18	0.29
Eigenvalue	5.84	37.7
Percentage of total variance explained	26.58	17.16
Cumulative percentage	26.58	43.75

[a]From the anterior end.

Table 4. Results of principal component analysis of morphometric characters of females *Schrankiana formosula* (20): coefficients for standardized measurements and percentage of explained variation

	PCA1	PCA2
Total length (mm)	0.21	0.24
Maximum width	−0.05	0.21
Body width at oesophago-intestinal junction	0.19	−0.12
Oesophagus length	0.31	0.05
Pharynx length	0.24	−0.08
Pharynx width	0.22	−0.08
Corpus length	0.30	0.05
Corpus width	0.05	0.04
Isthmus length	0.20	0.12
Isthmus width	0.27	−0.13
Bulb length	0.22	0.05
Bulb width	0.22	−0.19
Nerve ring[a]	0.18	0.31
Excretory pore[a]	0.31	−0.00
Tail length	0.04	−0.23
Vulva[a]	−0.09	0.06
Vagina vera	0.09	0.29
Uterine vagina	0.21	0.08
Morulae embryonated length	−0.00	0.29
Morulae embryonated width	−0.01	0.31
Oesophagus in % of body length	0.22	−0.16
Excretory pore in % of body length	0.24	−0.14
Nerve ring in % of body length	0.04	0.25
Vulva in % of body length	−0.22	−0.13
Tail length in % of body length	−0.08	−0.34
Distance vulva to anus	−0.31	0.24
Eigenvalue	9.14	3.88
Percentage of total variance explained	36.21	14.93
Cumulative percentage	36.21	45.35

[a]From the anterior end.

anterior end (Tables 7 and 8). In fact, the body length strongly influences these characteristics and variations in female growth.

Most of the characteristics proposed by Baker and Vaucher (1988) to differentiate *Schrankiana* species, such as cephalic end, extent of the lateral alae, location of the vulva, size of the vagina, spicules and gubernaculum presented uniformity in terms of morphology and did not significantly influence on the variability. The bulb width was not statistically significant, and the longest oesophagus was observed in the largest specimens. Thus, the metric difference is considered to reflect the variability of *S. formosula*.

Intraspecific variations have been reported in other species of the Cosmocercidae such as: *Aplectana hylambatis* Baylis 1927, *Aplectana hamatospicula* (Walton, 1940), *Cosmocercoides amapari* Rebêlo, Santos and Melo 2022, *Cosmocercoides variabilis* Harwood, 1930 and *Cosmocercoides pulcher* (Wilkie, 1930) (Vanderburgh and Anderson, 1987; Vhora and Bolek, 2013; González *et al.*, 2019; Rebêlo *et al.*, 2023). The authors report that the morphological and morphometric variations observed are usually related to generalist species and can be influenced by hosts or localities (González *et al.*, 2019). Thus, as *S. formosula* is a generalist species, the morphological and morphometric differences are considered intraspecific variations that can be associated with differences in host and environmental attributes. As proposed by González *et al.* (2019), our results reinforce the importance of examining the maximum number of specimens possible from different hosts and localities to identify intraspecific variations.

Table 5. Summary of the unidirectional analyses of the morphological characters of male *Schrankiana formosula*

Schrankiana formosula	*M*	*P*
Oesophagus length	8.80	**0.001**
Pharynx length	18.87	**0.003**
Corpus length	5.17	**0.016**
Bulb width	0.71	0.502
Tail length	5.81	**0.010**
Never ring % of body length	0.79	0.465

Bold values denote statistical significance.

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Table 6. Summary of the unidirectional analyses of the morphological characters of the female *Schrankiana formosula*

Schrankiana formosula	*F*	*P*
Oesophagus length	30.2	**0.000**
Corpus length	24.7	**0.000**
Nerve ring	33.6	**0.000**
Excretory pore	37.2	**0.000**
Morulae embryonated width	1.51	0.248
Tail length in % of body length	7.91	**0.000**

Bold values denote statistical significance.

The number and pattern of the caudal papillae in *S. formosula* are poorly established in the literature. In the original description, Freitas (1959) reports 3 pairs of sublateral precloacal papillae, 1 papillae on the anterior cloacal lip and 3 postcloacal papillae. Later, Baker and Vaucher (1988) describe 4–5 pairs of subventral precloacal papillae, 3 pairs of papillae on the anterior cloacal lip, with an unpaired papilla situated between them, and 5 pairs of postcloacal papillae.

The newly studied specimens exhibit 3 pairs of precloacal papillae, according to Freitas (1959). The other papillae were according to the description by Baker and Vaucher (1988): 3 pairs anterior to the cloacal lip, with an unpaired papilla between them, and 5–6 pairs of postcloacal papillae. However, Baker and Vaucher (1988)

Table 7. *Schrankiana* species list with host records, host family, and the geographic locality (country).

Species	Host species	Host family	Country	Reference
S. brasili (=*Schrankianella brasili*) (Travassos, 1927)	*Leptodactylus labyrinthicus* (Spix, 1824)	Leptodactylidae	Paraguay	Baker and Vaucher (1988)
	*Leptodactylus pentadactylus** (Laurenti, 1768)	Leptodactylidae	Brazil and Peru	Travassos (1927, 1931); Freitas (1959); Bursey et al. (2001)
	Rhinella diptycha (= *Bufo paracnemis*) (Cope, 1862)	Bufonidae	Paraguay	Baker and Vaucher (1988)
S. chacoensis González and Hamann, 2014	*Leptodactylus bufonius** Boulenger, 1894	Leptodactylidae	Argentina	González and Hamann (2014)
S. formosula (= *S. inconspicata*) Freitas, 1959	*Ischnocnema henselii* (Peters, 1870)	Brachycephalidae	Brazil	Euclydes et al. (2021)
	L. bufonius	Leptodactylidae	Argentina	González et al. (2021)
	Leptodactylus macrosternum (= *Leptodactylus chaquensis*) Miranda-Ribeiro, 1926	Leptodactylidae	Brazil	Campião et al. (2016)
	Leptodactylus elenae Heyer, 1978	Leptodactylidae	Paraguay	Baker and Vaucher (1988)
	Leptodactylus fragilis (= Brocchi, 1877)	Leptodactylidae	Costa Rica	Bursey and Brooks (2010); Goldberg et al. (2013)
	*Leptodactylus fuscus** (=*Leptodactylus typhonius*) (Schneider, 1799)	Leptodactylidae	Brazil and Paraguay	Freitas (1959); Baker and Vaucher (1988); Goldberg et al. (2007); Da Graça et al. (2017); Cardoso et al. (2021)
	L. labyrinthicus	Leptodactylidae	Paraguay	Baker and Vaucher (1988)
	Leptodactylus notoaktites Heyer, 1978	Leptodactylidae	Brazil	Euclydes et al. (2022)
	L. pentadactylus (= *Leptodactylus pentadactylus labyrinthicus*)	Leptodactylidae	Brazil and Costa Rica	Freitas (1959); Bursey and Brooks (2010)
	Leptodactylus poecilochilus (= Cope, 1862)	Leptodactylidae	Costa Rica	Bursey and Brooks (2010)
	Leptodactylus rhodonotus (Günther, 1869)	Leptodactylidae	Peru	Bursey et al. (2001)
	Leptodactylus vastus (= *L. pentadactylus labyrinthicus*) Lutz, 1930	Leptodactylidae	Brazil	Freitas (1959)
	Leptodactylus syphax Bokermann, 1969	Leptodactylidae	Brazil	Lins et al. (2017)
	Pithecopus azureus (= *Phyllomedusa azureus*) (Cope, 1862)	Hylidae	Brazil	Campião et al. (2016)
	Scinax fuscovarius (Lutz, 1925)	Hylidae	Brazil	Da Graça et al. (2017)

(*Continued*)

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Table 7. (*Continued.*)

Species	Host species	Host family	Country	Reference
S. freitasi Baker, 1982	*L. pentadactylus**	Leptodactylidae	Brazil	Baker (1982)
	L. mystaceus	Leptodactylidae	Brazil	Goldberg et al. (2007)
S. fuscus Baker and Vaucher, 1988	*L. fuscus**	Leptodactylidae	Brazil and Paraguay	Baker and Vaucher (1988); Goldberg et al. (2007); Campião et al. (2016); Campião et al. (2017)
S. larvata (Vaz, 1933) Fahel, 1952	*L. fuscus*	Leptodactylidae	Brazil	Freitas (1959); Goldberg et al. (2009)
	L. labyrinthicus	Leptodactylidae	Paraguay	Baker and Vaucher (1988)
	Leptodactylus latrans (= *Leptodactylus ocellatus*) (Steffen, 1815)	Leptodactylidae	Brazil	Goldberg et al. (2009)
	Leptodactylus mystaceus (Spix, 1824)	Leptodactylidae	Brazil and Peru	Bursey et al. (2001); Goldberg et al. (2009)
	*L. pentadactylus**	Leptodactylidae	Brazil and Peru	Vaz (1933); Freitas (1959); Bursey et al. (2001)
	L. vastus	Leptodactylidae	Brazil	Fahel (1952)
S. schranki (Travassos, 1925) Strand, 1942	*L. fuscus*	Leptodactylidae	Brazil	Oliveira et al. (2022)
	Leptodactylus latinasus Jiménez de la Espada, 1875	Leptodactylidae	Argentina	Hamann et al. (2006)
	L. mystaceus	Leptodactylidae	Peru and Ecuador	Dyer (1990); Bursey et al. (2001)
	*L. pentadactylus**	Leptodactylidae	Brazil and Ecuador	Travassos (1925); Freitas (1959); Dyer and Altig (1977)
	Leptodactylus rhodomystax Boulenger, 1884	Leptodactylidae	Brazil	Goldberg et al. (2007)
	Physalaemus cicada Bokermann, 1966	Leptodactylidae	Brazil	Oliveira et al. (2019)
	Physalaemus cuvieri Fitzinger, 1826	Leptodactylidae	Brazil	Oliveira et al. (2019)
	R. diptycha	Bufonidae	Brazil	Oliveira et al. (2022)
	Trachycephalus typhonius (Linnaeus, 1758)	Hylidae	Brazil	Oliveira et al. (2022)

*Indicate the type-host.

did not mention the intraspecific morphological variation in the number of postcloacal papillae, and the number of precloacal papillae resembles that of the original description. Additionally, we observed the same number, variation and arrangement of caudal papillae in the type series as described in the present study (Fig. 4).

We also observed that Baker and Vaucher (1988) represented, but did not comment in the manuscript, that the first 2 postcloacal papillae of *S. formosula* are slightly diagonal (*in tandem*) (see Fig. 1 in Baker and Vaucher, 1988), while in *S. inconspicata* those papillae are more in lateral position (see Figure 3 in Baker and Vaucher, 1988). However, we observed this variation in both type series (*S. inconspicata* and *S. formosula* deposited in CHIOC) and also in newly collected material. After analysing the material that we collected, we concluded that these differences might be artefacts of specimen positioning on the microscope slide.

Phylogeny and systematic position of Schrankiana

Our study presents the first molecular and phylogenetic analyses of the genus *Schrankiana*. The family Cosmocercidae included the genera *Aplectana*, *Cosmocerca* Diesing, 1861, *Cosmocercoides* Wilkie, 1930, *Nemhelix* Morand and Petter, 1986, *Schrankiana* and *Labeonema* Puylaert, 1970 (99 bootstrap and 100 posterior probability), while the family Atractidae was represented by *Grassenema* Petter, 1959 and *Rondonia* Travassos, 1920 (100 bootstrap and 99 posterior probability). These results are according to the classification proposed by Adamson and Baccam (1988) and Gibbons (2010), who transferred the genera *Labeonema* and *Schrankiana* from the Atractidae to the Cosmocercidae.

Schrankiana formosula formed a well-supported clade with *Raillietnema* sp. and *L. synodontisi* (100 bootstrap and 100 posterior probability), and previous molecular studies show a similar pattern (Fig. 6) (Pereira *et al.*, 2015; Cavalcante *et al.*, 2016; McElwain *et al.*, 2019; Saito *et al.*, 2021). *Labeonema synodontisi* and *Raillietnema* sp. share morphological similarities with *S. formosula*, such as cephalic, oesophageal and male caudal structures (size and shape of the spicules, and distribution of caudal papillae), reinforcing the close relationship between them (Fig. 6). The low divergence between *Raillietnema* sp. and *S. formosula* can be attributed to those species belonging to anuran hosts in the Neotropical region. In contrast, the higher divergence value observed in *L. synodontisi* may be associated with the

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Table 8. Nematode species, hosts, localities, GenBank accession numbers and references used in phylogenetic analyses

Families	Species	Host	Locality	Accession numbers	References
Atractidae	*Grassenema procaviae* Petter, 1959	*Procavia capensis* Storr, 1780	Japan	LC596375	Saito *et al.* (2021)
	Rondonia rondoni Travassos, 1920	*Pimelodus blochii* Valenciennes, 1840 and *Pterodoras granulosus* (Valenciennes, 1833)	Brazil and Peru	DQ442679	Wijová *et al.* (2006)
Cosmocercidae	*Aplectana dayaoshanensis* Chen, Ni, Gu, Sinsch and Li, 2021	*Hylarana spinulosa* (Smith, 1923)	China	OK045516	Chen *et al.* (2021*a*)
	Aplectana chamaeleonis (Baylis, 1929)	*Hyperolius kivuensis* Ahl, 1931	Germany	OK045518	Chen *et al.* (2021*a*)
	Aplectana xishuangbannaensis Chen, Ni, Gu and Li, 2021	*Polypedates megacephalus* Hallowell, 1861	China	MW329041	Chen *et al.* (2021*b*)
	Cosmocerca longicauda (Linstow, 1885)	Snail	–	OL468616	Unpublished
	Cosmocerca simile Chen, Zhang, Feng and Li (2020)	*Bufo gargarizans* Cantor, 1842	China	MN839758	Chen *et al.* (2020)
	Cosmocerca sp. 1	*Hoplobatrachus chinensis* (Osbeck, 1765)	China	MW329987	Chen *et al.* (2021*b*)
	Cosmocerca sp. 2	*Bufo melanostictus* (Schneider, 1799)	China	MW329990	Chen *et al.* (2021*b*)
	Cosmocercoides dukae (Holl, 1928)	*Deroceras panormitanum* Lessona, and Pollonera, 1882	USA	FJ516753	Ross *et al.* (2010)
	Cosmocercoides pulcher Wilkie, 1930	*Bufo formosus* Boulenger, 1883	Japan	LC018444	Tran *et al.* (2015)
	Cosmocercoides qingtianensis Chen, Zhang, Nakao, and Li (2018)	*B. gargarizans*	China	MH178321	Chen *et al.* (2018)
	Cosmocercoides tonkinensis Tran, Sato, and Luc (2015)	*Acanthosaura lepidogaster* Cuvier, 1829	Vietnam	AB908160	Tran *et al.* (2015)
	Labeonema synodontisi (Vassiliadès, 1973) Koubková, Baruš, Hodová and Šimková, 2008	*Synodontis ocellifer* Boulenger, 1900	Senegal	EF375487	Koubková *et al.* (2008)
	Nemhelix bakeri Morand and Petter, 1986	Snail	–	HM627010	Saito *et al.* (2021)
	Raillietnema sp.	–	–	DQ503461	Smythe *et al.* (2006)
	***Schrankiana formosula* Freitas, 1958**	***Leptodactylus pentadactylus* (Laurent, 1768)**	**Brazil**	**PP669822**	**Present study**
Quimperiidae (Outgroup)	*Ichtyobronema hamulatum* (Moulton, 1931)	*Lota lota* (Linnaeus, 1758)	Russia	KY476351	Sokolov and Malysheva (2017)

geographical distance between taxa and the different type of host, as this sequence is from a fish from Africa. However, the relationships between them remained unresolved.

Historically, *Schrankiana* was allocated in the family Atractidae by Chabaud (1957), who considered this classification only based on the monodelphic reproductive system of females and aspects of the life cycle such as viviparity and auto-infection. However, only these characteristics could not be decisive in allocating morphologically related genera, such as *Raillietnema* Travassos, 1927 and *Labeonema* (Koubková *et al.*, 2008). Later, Adamson and Baccam (1988) and Gibbons (2010) moved *Schrankiana* to Cosmocercidae based on morphological aspects of cephalic structure, arrangement of caudal papillae, oesophagus and excretory pore, hypothesizing that these characters are plesiomorphic, and autoinfection has arisen at least twice in the superfamily Cosmocercoidea. We observed that *S. formosula* grouped within representatives of Cosmocercidae, forming a sister group of *Labeonema* and *Railletnema*, giving an additional piece of evidence that these genera should be placed in Cosmocercidae.

In our phylogeny, the family Atractidae formed a monophyletic group, composed by the species *G. procaviae* and *R. rondoni* with high support. Previous studies also showed the monophyly of this group (Cavalcante *et al.*, 2016; Saito *et al.*, 2021; Chen *et al.*, 2021*a*, 2021*b*). However, Adamson and Baccam (1988) stated that the family Atractidae is characterized by a single synapomorphy: an oesophagus that is distinctly divided at the junction of corpus and isthmus, supported in phylogenetic analyses. The authors asserted that the group requires revision to identify more shared characteristics by the genera.

In our analyses, we did not recover the monophyly of the genus *Aplectana*, due to the clade *A. chamaleonis* and *Cosmocerca* spp., previous studies also received the same grouping (Sinsch *et al.*, 2020; Chen *et al.*, 2021*a*, 2021*b*; Harnoster *et al.*, 2022; Ni *et al.*, 2022; Svitin *et al.*, 2023). It is important to highlight the limited morphological data available in the literature to confirm *A. chamaeleonis* identification (see Sinsch *et al.*, 2020; Chen *et al.*, 2021*b*), which raises the hypothesis that the sequence could belong to the genus *Cosmocerca*.

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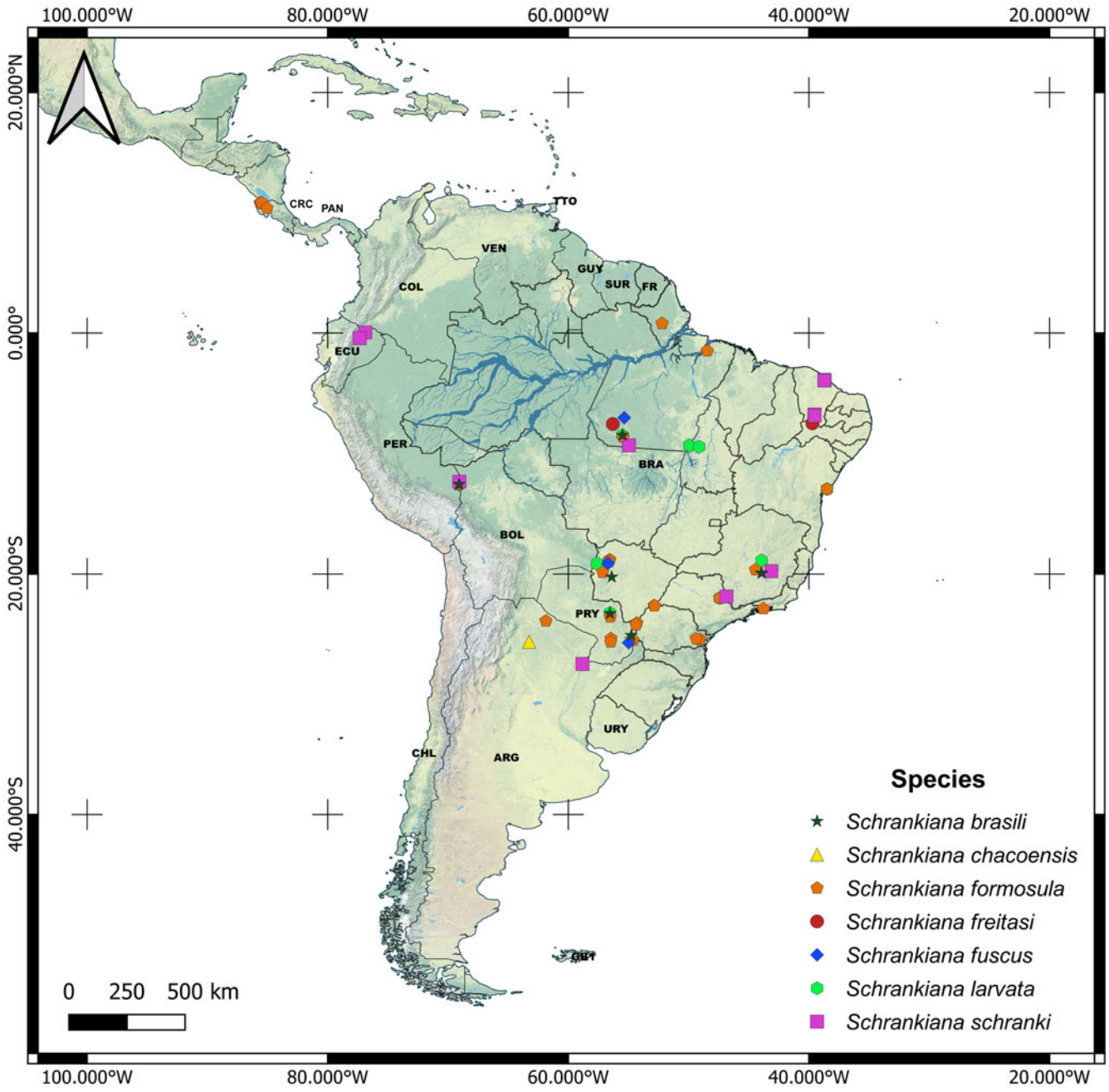

Figure 5. *Schrankiana* species distribution map and host records.

The sequence of *C. longicauda* grouped in a clade with *N. bakeri* as a sister group of *Cosmocercoides*. Recent molecular phylogenetic works showed that *Cosmocercoides* might be a monophyletic group (see Saito *et al.*, 2021; Harnoster *et al.*, 2022; Ni *et al.*, 2022; Svitin *et al.*, 2023; Tuschida *et al.*, 2023). However, these studies did not consider the sequence of *C. longicauda* and *N. bakeri.* Additionally, the recent work by Svitin *et al.* (2023) suggested that the authors misidentified *C. longicauda* (OL468616 and OL468682) and that the species should be placed in the genus *Cosmocercoides.*

We also checked the publication and information provided on GenBank about the sequence of *C. longicauda*. We could not find the published paper with possible morphological data to corroborate the species identification; based on the research project title, the host might be a snail or a slug. Thus, these data reinforce the potential misidentification of this species. Additionally, *Cosmocerca* spp. are rarely found in snails, unlike *Cosmocercoides* spp. (Svitin *et al.*, 2023).

Based on this, we reinforce the hypothesis of Svitin *et al.* (2023) that the *C. longicauda* sequences (OL468616, OL468682) belong to *Cosmocercoides*. To support this conclusion, we observed that *C. longicauda* shows lower genetic divergence in *Cosmocercoides* (0.81% divergence between the species) than in *Cosmocerca* (1.91–4.45% divergence between the species). Thus, the phylogenetic position of *Cosmocercoides* remains uncertain. Furthermore, future molecular studies from Cosmocercidae species, especially from different biogeographical regions, may clarify the phylogenetic position of the genus.

Final remarks

In this study, we redescribed the species *S. formosula* based on morphology, using light and scanning electron microscopy and molecular analyses. We also re-examined the type material, synonymizing *S. inconspicata* with *S. formosula*, one of the most prevalent species of parasites of *Leptodactylus* in Brazil.

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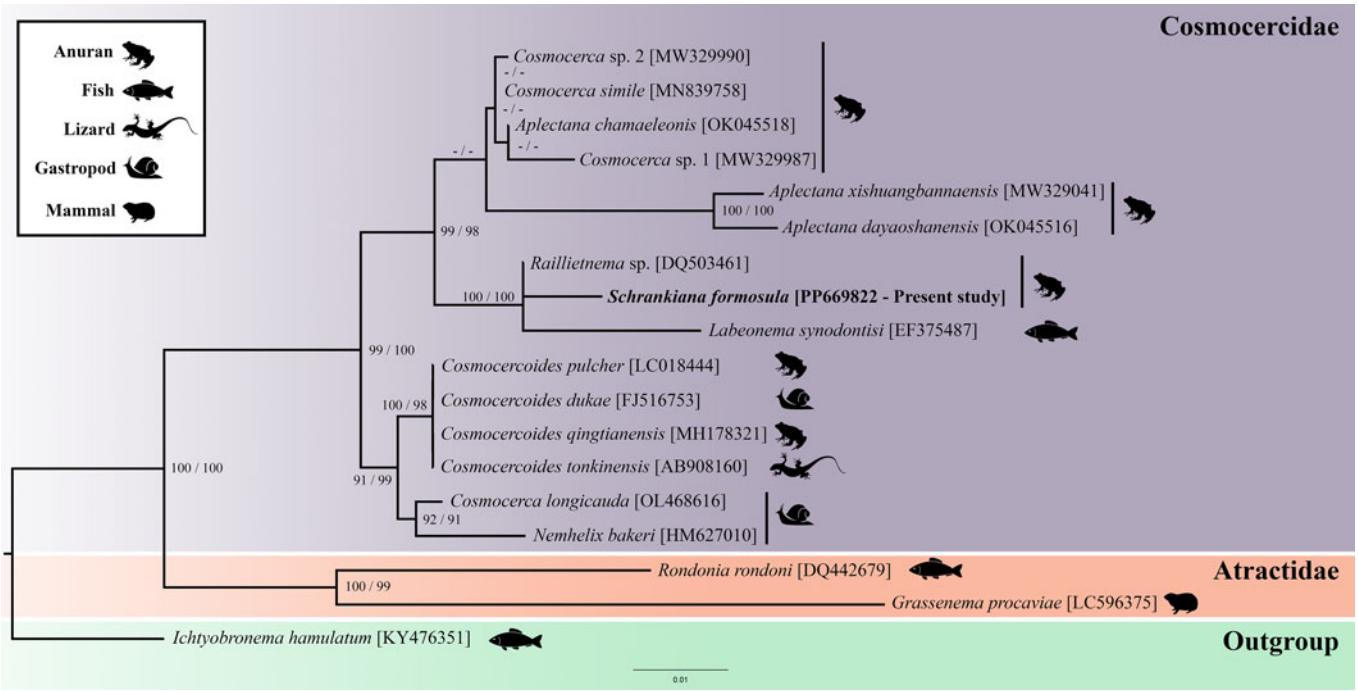

Figure 6. Maximum likelihood topology based on 18S rDNA using *Ichtyobronema hamulatum* as outgroup. GenBank accession numbers are indicated next to species names. Numbers beside the nodes represent support value by bootstrap for maximum likelihood analyses and posterior probabilities for Bayesian analyses, respectively (bootstrap scores >70 and posterior probabilities >90).

Therefore, the geographic distribution of *Schrankiana* is important for future investigations into the diversity and evolutionary history of the group.

Finally, we performed the first molecular analyses of *Schrankiana*, elucidating the phylogenetic position of the group, and demonstrated that *Schrankiana* is a member of the family Cosmocercidae and not Atractidae as previously classified.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S003118202400091X.

Data availability statement. All data from this study are available in the article and supplementary material. Additionaly, the specimens were deposited in the "Museu Paraense Emílio Goeldi" museum.

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Author contributions. A. J. S. Félix wrote the main draft and prepared images. A. N. Santos and C. E. Costa-Campos helped with specimen observations and SEM analyses, reviewing and writing the manuscript. A. Maldonado-Júnior, J. K. S. Neves and G. L. Rebêlo carried out PCR, sequencing and phylogeny. J. N. Santos, L. F. F. Trindade and F. T. V. Melo wrote the manuscript, revised and prepared the line drawings. All authors reviewed the manuscript.

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Ethical standards. All applicable institutional, national and international guidelines for the care and use of animals were followed. Host specimens were collected under permits Institute for the Environment and Renewable Resources – IBAMA/ICMBio (SISBIO: No 53527–4) and Ethics Committee on the Use of Animals of the Federal University of Pará (CEUA/UFPA: No 8341260821).

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