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Subulura eliseae sp. n. (Ascaridida: Subuluroidea), a parasite of *Marmosa* spp. from Amazon rainforest, Brazil

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Research Paper

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Abstract

The parasite biodiversity of mouse opossums in Brazil remains incompletely explored. We describe a new species of *Subulura* (Ascaridida: Subuluroidea) from the large intestine of the white-bellied woolly mouse opossum, *Marmosa constantiae*, based on the results of light and scanning electron microscopy (SEM). We also partially sequenced the mitochondrial cytochrome c oxidase I (MT-CO1) gene of the new species, using molecular phylogenetic analyses to determine its relationships within the Subuluroidea superfamily. As molecular data on subuluroid species are extremely limited, few inferences could be drawn from our phylogenies. Our SEM observations showed the detailed morphology of the cephalic extremity, precloacal pseudo-sucker, caudal papillae, phasmids and vulva. *Subulura eliseae* sp. n. differs from the other four *Subulura* parasites species of marsupials by the number of caudal papillae and the structure dimensions, and size of the spicule. Moreover, *S. eliseae* sp. n. has ten pairs of caudal papillae, which is unique compared to other species. We present morphometric and molecular data on this new species, contributing to future studies on subuluroids.

Introduction

The family Subuluridae Travassos, 1914 currently comprises six subfamilies: Allodapinae Inglis, 1958; Labiobulurinae Quentin, 1969; Parasubulurinae Berghe & Vuylsteke, 1938; Leipoanematinae Chabaud, 1957; Subulurinae Travassos, 1914; and Echidnonematinae Smales *et al.* 2020. This family is characterized by the structure of the buccal end in which the lobes of the oesophagus extend anteriorly to form a pharyngeal portion located between the true oral capsule and the oesophagus (Smales *et al.*, 2021). Recent work has adopted scanning electron microscopy (SEM) as a complementary tool for the identification and redescription of species belonging to the superfamily Subuluroidea, contributing to a refined description of the species (Smales, 2009; Baruš *et al.*, 2013; Du *et al.*, 2014; Guo *et al.*, 2019; Smales *et al.*, 2021).

Species of the genus *Subulura* Molin, 1860 (Ascaridida: Subuluroidea) occur in birds, lizards and mammals, with more than 60 valid *Subulura* species reported worldwide (Vicente *et al.*, 2000; Baruš *et al.*, 2013). To date, four *Subulura* species have been reported parasitizing marsupials from South America: *Subulura interrogans* Lent & Freitas, 1935, *S. Subulura* Foster, 1939, *Subulura trinitatis* Wolfgang, 1951 and *S. amazonica* Pereira & Machado Filho, 1968, with only one Australian species, *Subulura peramelis* Baylis, 1930, reported.

We recovered parasites of the white-bellied woolly mouse opossum, *Marmosa (Micoreus) constantiae* Thomas, 1904 in the Brazilian Amazon rainforest, municipality of Sinop, state of Mato Grosso (MT), Brazil. Examination of these parasites revealed a new species of subulurid. We describe the morphology of the species using light microscopy and SEM. In addition, we sequenced part of the mitochondrially encoded cytochrome c oxidase subunit I (MT-CO1) gene of this new species and performed a phylogenetic analysis.

Materials and methods

Marsupial collection

Fifty-three white-bellied woolly mouse opossums were captured in a fragmented landscape of the Amazon rainforest, as part of a larger study described elsewhere (De Mendonça *et al.*, 2020).

The phylogenetic study also included parasites of the long-furred woolly mouse opossum, *Marmosa* (*Micoreus*) *demerarae* (Thomas, 1905) from the Amazonia biome, municipality of Porto Acre, state of Acre (AC), Brazil and specimens of *Primasubulura jacchi* recovered from the white-tufted marmoset, *Callithrix jacchus* (Linnaeus, 1758), from the Atlantic Forest biome, municipality of Rio de Janeiro (RJ), state of Rio de Janeiro. All specimens were donated by Laboratório de Biologia e Parasitologia de Mamíferos Silvestres Reservatórios.

Recovery of helminths and morphological identification

All helminths were recovered and processed according to Hoffman, 1987, with 66 specimens in total (33 males and 33 females). In the laboratory, the helminth specimens were morphologically characterized. First, the specimens were clarified in phenol 90%. Drawings were then produced with the aid of a camera lucida attached to a Zeiss Scope Z1 light microscope (Zeiss, Göttingen, Germany). The structures observed were measured from digital images captured by a Zeiss Axio Cam HRC (Zeiss, Germany), using Carl Zeiss AxioVision Rel. 4.7 accessory software. All measurements were in millimetres (table 1). Identification followed the nematode keys of Vicente *et al.* (1997) and Anderson *et al.* (2009). The prevalence, abundance and mean intensity of parasitism for each helminth species recovered were calculated according to Bush *et al.* (1997). The type specimens of *S. interrogans* (CHIOC: 30,334 and 31,232 a–f) and *S. amazonica* (CHIOC: 30,337 and 30,336) used for comparisons were borrowed from the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC) of Rio de Janeiro, Brazil. Specimens of *P. jacchi* (Diesing, 1861) and *Primasubuura distans* (Rudolphi 1809) registered as. *Subulura jacchi* (CHIOC; 1108) and *Subulura distans* (CHIOC: 324), respectively, were analysed for comparison.

For SEM, six specimens (four males and two females) were processed according to a protocol modified by Souza *et al.* (2017). The helminths were dehydrated in a 70–100% ethanol gradient. First, the samples were dehydrated in 70% ethanol for 48 h and then 80%, 90% and absolute ethanol for 20 min, at each step. Finally, the samples were critical point dried in carbon dioxide, mounted on metal stubs and coated with gold (20 nm). The specimens were then examined using a microscope model JEOL JSM-6390 (JEOL, Tokyo, Japan) at the Rudolf Barth Electron Microscopy Platform Oswaldo Cruz Institute, Fiocruz PDTIS/FIOCRUZ.

Molecular and phylogenetic analyses

Genomic DNA of one specimen was extracted using a Qiagen QIAamp DNA Extraction Kit (QIAGEN, Venlo, The Netherlands) following the manufacturer's instructions.

Partial MT-CO1 gene fragments were amplified by polymerase chain reaction (PCR) using primer cocktails described by Prosser *et al.* (2013). PCR reactions were performed at a volume of 25 µl for each sample, with reagents in the following volumes: 12.5 µl of PCR Master Mix (Promega Corporation, Madison, WI, USA), 8.5 µl of ultrapure water, 0.5 µl of each forward and reverse primer cocktails (10 µM) and 3 µl of genomic DNA from the sample. Amplifications were performed on a Veriti Thermal Cycler (Life Technologies, CA, USA), following the parameters described by Prosser *et al.* (2013). Amplicons (PCR products) were visualized using GelRed (Biotium), on an ultraviolet transilluminator after 1.5% agarose gel electrophoresis.

The amplified products were purified using an Illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare, Little Chalfont, Buckinghamshire, UK) following the manufacturer's protocol. Individual primer sequences of the purified samples were obtained using a BigDye Terminator v3.1 Sequencing Kit (Applied Biosystems, USA). This resulted in six bidirectional sequencing reads for improved accuracy. Sequencing was performed using a 96-capillary 3730xl DNA Analyzer (Applied Biosystems) at the Genomic Platform, Fiocruz Technological Platforms Network Oswaldo Cruz Institute (RPT-Fiocruz) of Rio de Janeiro, Brazil.

Sequencing chromatograms were assembled into contigs and edited using the software package Geneious 9.1.8 (Kearse *et al.*, 2012), resulting in a consensus sequence. In addition to the consensus sequence of *Subulura eliseae* sp. n., we obtained MT-CO1 gene sequences from another specimen of *S. eliseae* sp. n. parasitizing *M. demerarae* and from a specimen of *P. jacchi*, parasite of *C. jacchus*. The DNA sequences obtained were compared using the BLASTn algorithm with sequences available in the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov).

Our partial MT-CO1 gene dataset included the sequence of a representative of the superfamily Subuluroidea, *Aulonocephalus pennula* Chandler, 1935. Sequences belonging to species of the superfamilies Ascaridoidea and Heterakoidea were included as outgroups (table 2).

Sequences of each dataset were aligned using the ClustalW multiple sequence alignment program (Thompson *et al.*, 1994). The resulting alignments of poorly aligned regions were manually trimmed using Mesquite, version 3.61 (Maddison & Maddison, 2018). The substitution saturation level of the matrix was evaluated according to the methods of Xia *et al.* (2003) and Xia & Lemey (2009) using DAMBE, version 6.4.79 (Xia & Xie, 2001). Maximum likelihood (ML) inference was performed using the online PhyML 3.0 (Guindon *et al.*, 2010). Nucleotide substitution model selection was executed using Smart Model Selection (SMS) (Lefort *et al.*, 2017) in PhyML under the Akaike information criterion. Node support in ML trees was determined using the approximate branch likelihood ratio test (aLRT) (Anisimova & Gascuel, 2006) and by nonparametric bootstrap percentages (ML-BP), with 1000 pseudoreplications, both implemented in PhyML 3.0.

For Bayesian inference (BI), MrBayes, version 3.2.6 (Ronquist *et al.*, 2012) on XSEDE was used, using the CIPRES Science Gateway platform (Miller *et al.*, 2010). Taking into account the different evolutionary processes at each codon position of the MT-CO1 gene, the substitution models were calculated separately for each position using the automated model selection in PAUP*, version 4.0a164 (Swofford, 2003), under the Bayesian information criterion, and implemented with unlinking base frequencies, models and parameters. Markov chain Monte Carlo samplings were performed over 10,000,000 generations, with four simultaneous chains in two runs. Node support in the BI trees was given by Bayesian posterior probability (BPP) calculated from trees sampled every 100 generations after the removal of a burn-in fraction of 25%. To assess the adequateness of our sampling, we used Tracer, version 1.7.1 (Rambaut *et al.*, 2014) to calculate the effective sample size of the parameters. Values above 200 effectively independent samples were considered robust.

Results

Zoobank registration: The Life Science identifier (LSID) for *S. eliseae* sp. n. urn: lsid: zoobank.org:act:726EBE11-2ABA-47F0-B8C2-E262390C747D

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Table 1. Morphometric data on species of the genus *Subulura* parasites of marsupials in the Americas.

Species	*Subulura interrogans*	*Subulura amazonica*	*Subulura lanigeri*	*Subulura trinitatis*	*Subulura eliseae*
Author	Lent & Freitas, 1935	Pereira & Machado Filho, 1968	Foster, 1939	Wolfgang, 1951	Present study
Host	*Caluromys philander*	*C. philander*	*Caluromys lanatus*[a]	*Caluromys p. trinitatis*	*Marmosa constantiae*
Type locality	Rio de Janeiro	Amazonas	Panama	Trinidad	Sinop
Male					
length (L)	6.1	14–15.5	10.2	6.1–6.9	9.0
width (W)	0.35	0.45–0.60	0.40	0.39	0.38
cervical alae (L)	1.073	not reported	absent	not reported	1.018
cervical alae (W)	0.057	not reported	absent	not reported	0.025
buccal cavity L × W	–	0.038 × 0.049	0.033 × 0.040	0.030 × 0.040	0.036 × 0.044
pharyngeal formations	6	6	3	3	3
cephalic papillae	6	6	4	4	4
oesophagus	1.073	1.400	1.200	1.260	1.055
bulb L × W	0.20	0.22	–	0.30	0.21
nerve-ring	–	0.31	–	0.24	0.30
excretory pore	–	0.57	–	0.36	0.45
spicules	1.144	1.620	1.850	1.280–1.580	1.431
gubernaculum	0.168	0.200	0.215	0.165	0.215
precloacal pseudo-sucker	0.672	0.750	0.520	–	0.622
cloaca	0.172	0.220	0.310	–	0.200
tail tip	0.08	0.06	0.10	0.15–0.26	0.07
pseudo-sucker	–	0.186	0.290	0.080–0.120	0.160
caudal alae	Present	not reported	not reported	present	absent
caudal papillae	12	9	11	11	10
Female					
length (L)	11.25–12.75	18.25–21	16.2	13.5–17.8	13.2
width (W)	0.40–0.50	0.60–0.75	0.60	0.68	0.49
cervical alae	–	–	–	–	1.250
cervical alae	–	–	–	–	0.025
buccal cavity L × W	0.040 × 0.028	0.055 × 0.037	–	–	0.045 × 0.043
oesophagus	1.52	1.56	–	1.50	1.16
bulb L × W	0.260	0.300	–	–	0.240 × 0.218
nerve-ring	0.310	0.328	–	–	0.330
excretory pore	0.55	0.60	–	–	0.54
vulva	5.50	9.10	–	–	5.20
tail tip	0.15	–	0.16	0.08–0.11	0.11
tail	–	–	–	0.855–0.975	1.031
eggs L × W	0.083 × 0.068	0.087 × 0.072	–	0.068 × 0.045	0.074 × 0.045

[a]this host may otherwise be *Caluromys derbianus*, based this species distribution.

***S. eliseae* sp. n.**

Type-host: *Marmosa (Micoreus) constantiae* Thomas, 1904

Type-locality: Municipality of Sinop, state of Mato Grosso (MT), Brazil.

Site-of-infection: large intestine.

Prevalence: 13.20% (seven of 53 hosts collected)

Mean intensity: 9.42 (66 helminths collected from seven infected hosts)

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Table 2. Species of Nematoda used in the present molecular analyses.

Superfamily	Family	Subfamily	Species	Accession number	Reference
Subuluroidea	Subuluridae	Subulurinae	*Subulura eliseae* sp. n.[a]	OM432014	present study
			S. eliseae sp. n.[b]	OM432016	present study
			Primasubulura jacchi	OM432015	present study
		Allodapinae	*Aulonocephalus pennula*	LC228775	Kalyanasundaram *et al.*, 2017
Ascaridoidea		Ascarididae	*Ascaris lumbricoides*	JN801161	Park *et al.*, 2011
			Baylisascaris procyonis	KJ698533	direct submission
Heterakoidea		Heterakidae	*Heterakis isoloncheae*	FJ009625	Zhao *et al.*, 2009
			Ascaridia sp.	JX624730	Liu *et al.*, 2013

[a]State of Mato Grosso, Brazil.
[b]State of Acre, Brazil.

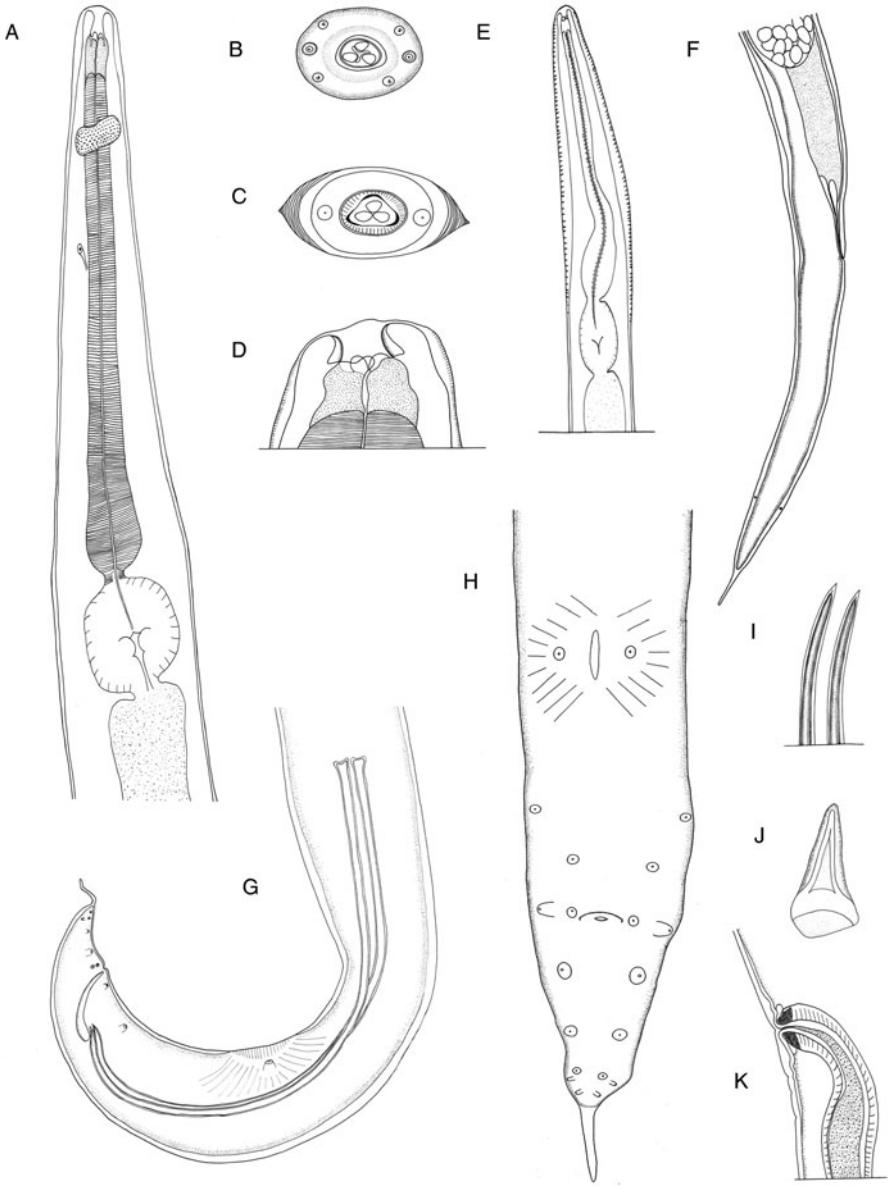

Fig. 1. *Subulura elisae* sp. n. from *Marmosa constantiae* in Brazil. (a) female anterior part, lateral view; (b, c) cephalic extremity apical view; (d) cephalic extremity lateral view; (e) male anterior part, ventral view; (f) female posterior end; (g) male posterior end, lateral view; (h) male posterior end, ventral view; (i) tip of spicule, lateral view; (j) gubernaculum, ventral view; (k) region of vulva, lateral view. Scale bars: (a, f, g, h) = 50 μm; (b, c, d, i, j, k) = 10 μm; (e) = 100 μm.

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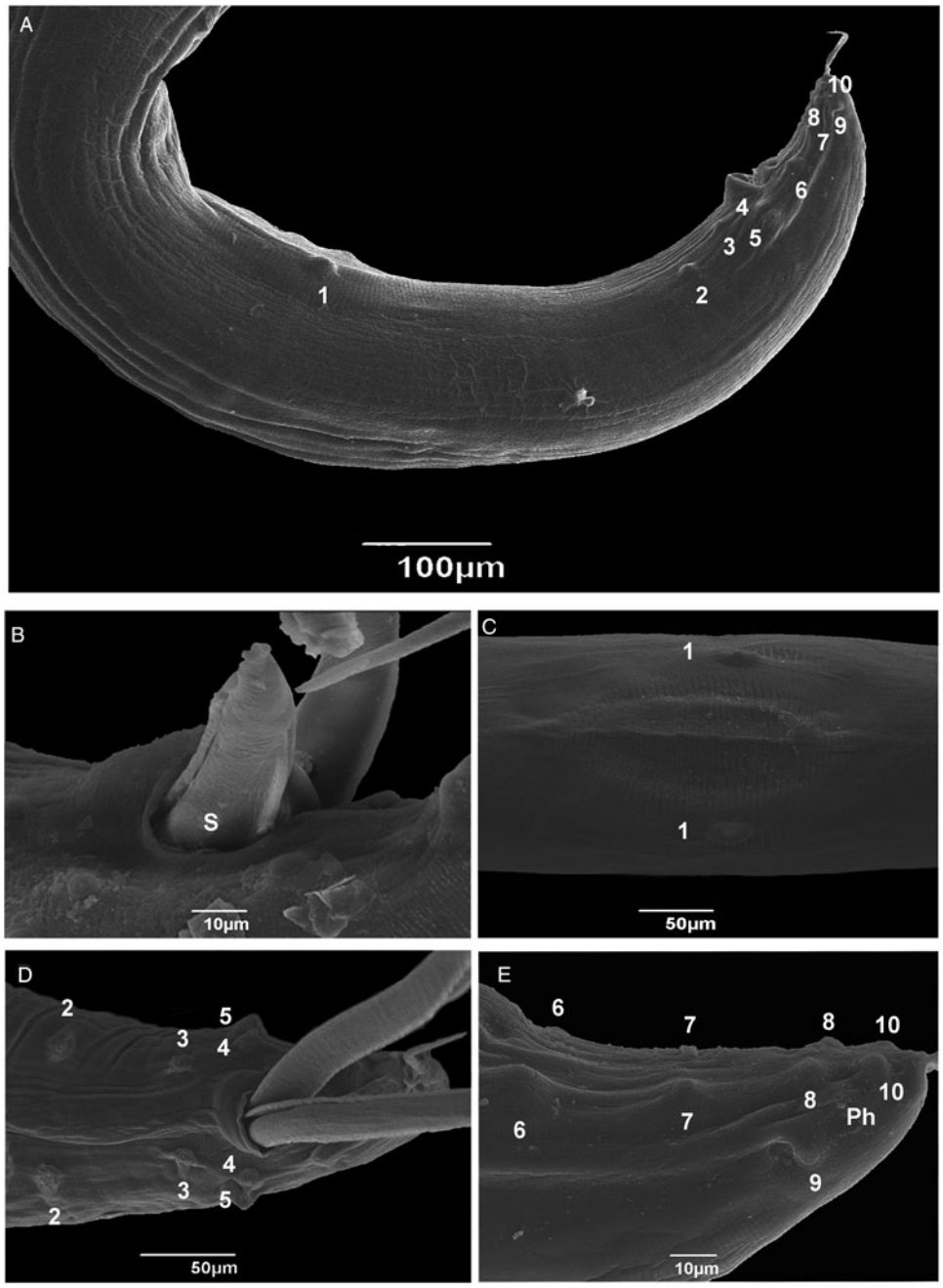

Fig. 2. Photographs of *Subulura elisae* sp. n. (a) vulva, lateral view; (b) gubernaculum, lateral view; (c) egg; (d) egg located in the ovijector. Scale bars: (a, b, d) = 50 µm; (c) = 10 µm.

Mean abundance: 1.24 (66 helminths collected from 53 hosts collected)

Specimen deposit: Helminthological Collection of the Oswaldo Cruz Institute (CHIOC) of Rio de Janeiro. The accession number of the holotype CHIOC number 39317a (male), accession number of the CHIOC number 39317b allotype (female) and accession numbers of paratype CHIOC number 39317c (one male and one female).

Other material studied: Specimens of *S. eliseae* sp. n. parasite of *M. demerarae,* from AC were deposited under the CHIOC number 39318 (one male and one female). Specimens of *P. jacchi* from the state of Rio de Janeiro were deposited under CHIOC number 39316 (one male and one female). All samples were deposited in a liquid medium.

Etymology: The new species is named in honour of Claudia Elise Xavier de Andrade, mother of the first author.

Description

Medium-sized, whitish nematodes. Cuticle finely striated. Maximum width at mid-body region. Cervical alae developed, starting at the base of cephalic plate and ending just after to posterior excretory pore (figs 1a, e and 2a). Deirids not observed. Cephalic end rounded, without developed lips. Oral aperture

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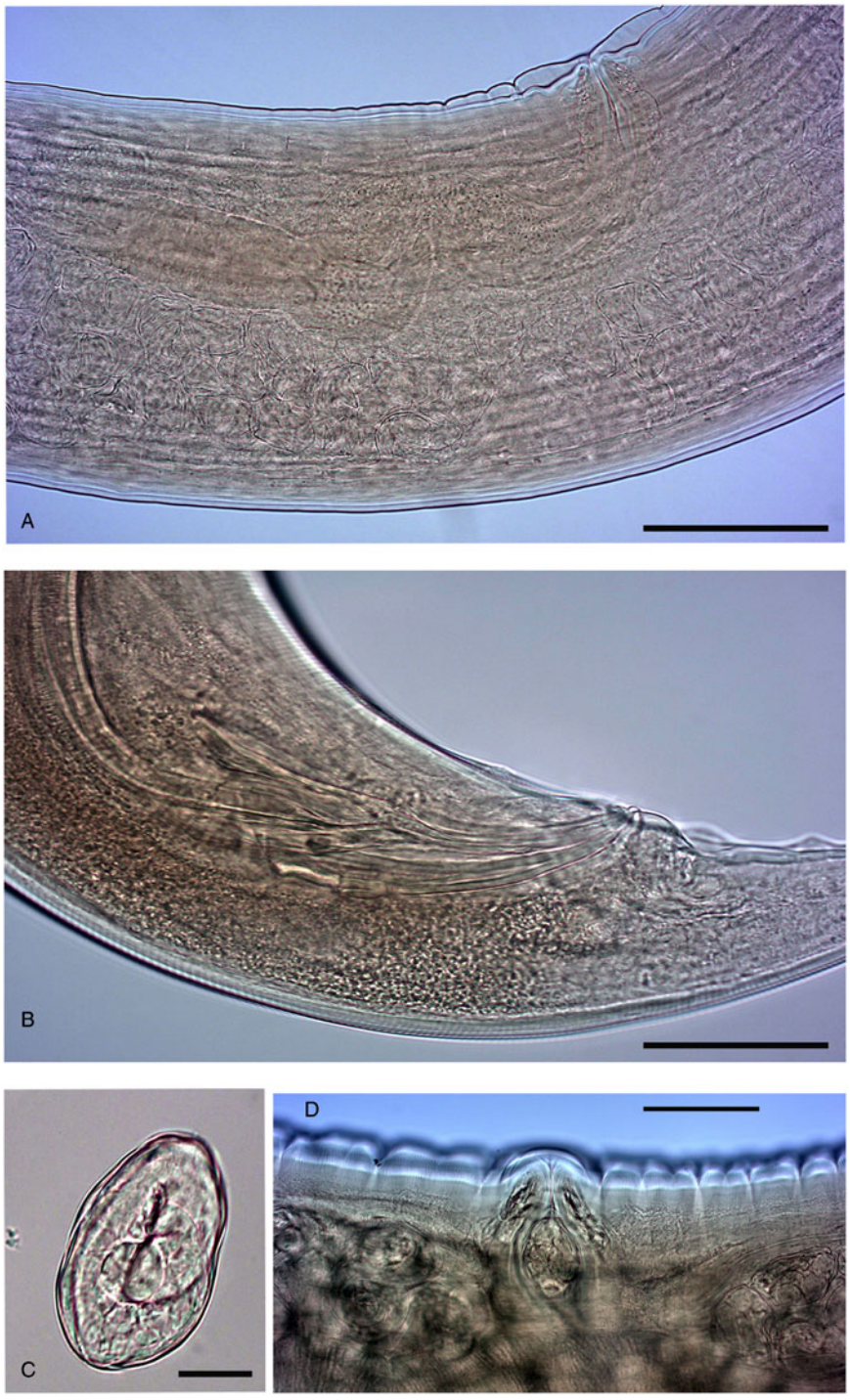

Fig. 3. Scanning electron photomicrographs of a specimen of *Subulura elisae* sp. n. (a) female anterior part, excretory pore (arrowed); (b) apical view, papillae (p) and amphids (am); (c) female posterior part, anus; (d) region of vulva, lateral view.

simple, circular, surrounded by four ovoid, submedial papillae, and two lateral amphids (figs 1b and 3b). Buccal cavity cylindrical, sclerotized walls and three small pharyngeal complex structures at the base (fig. 1c, d). Muscular oesophagus, terminating in conspicuous valved bulb (fig. 1a). Nerve ring at the first third of the body. Excretory pore slightly posterior to nerve ring (fig. 3a). Tail of both sexes conical, with digitiform tip (fig. 1f, g).

Male ($n = 4$): Posterior body ventrally curved, 8.56–9.39 (9.02) long, 354–398 (377) wide. Cervical alae 990–1.046 (1.018) mm long starting at the base of cephalic plate and ending just after to posterior excretory pore. Buccal capsule (fig. 1d), 42–47 (44.2) long, 34–39 (36.5) wide. Oesophagus 1.032–1.117 (1.055) mm long. Bulb rounded (fig. 1a), 193–219 (213.5) long, 182–205 (194) wide. Nerve ring and excretory pore 276–333 (308.5), and 399–510 (455.5), from anterior extremity, respectively. Curved caudal end (fig. 1h). Distance cloaca 133–241 (200.5) to the tail end. Precloacal sucker (figs. 1h and 4a, c), elliptical, 141–179 (159.7) long, 571–678 (622) from posterior extremity. Caudal alae absent. Sessile caudal papillae, ten pairs; three pairs pre-cloacal (fig. 4c, d), two pairs ad cloacal (fig. 4d), five pairs post-cloacal (fig. 4e, online Supplementary fig. S1). Last

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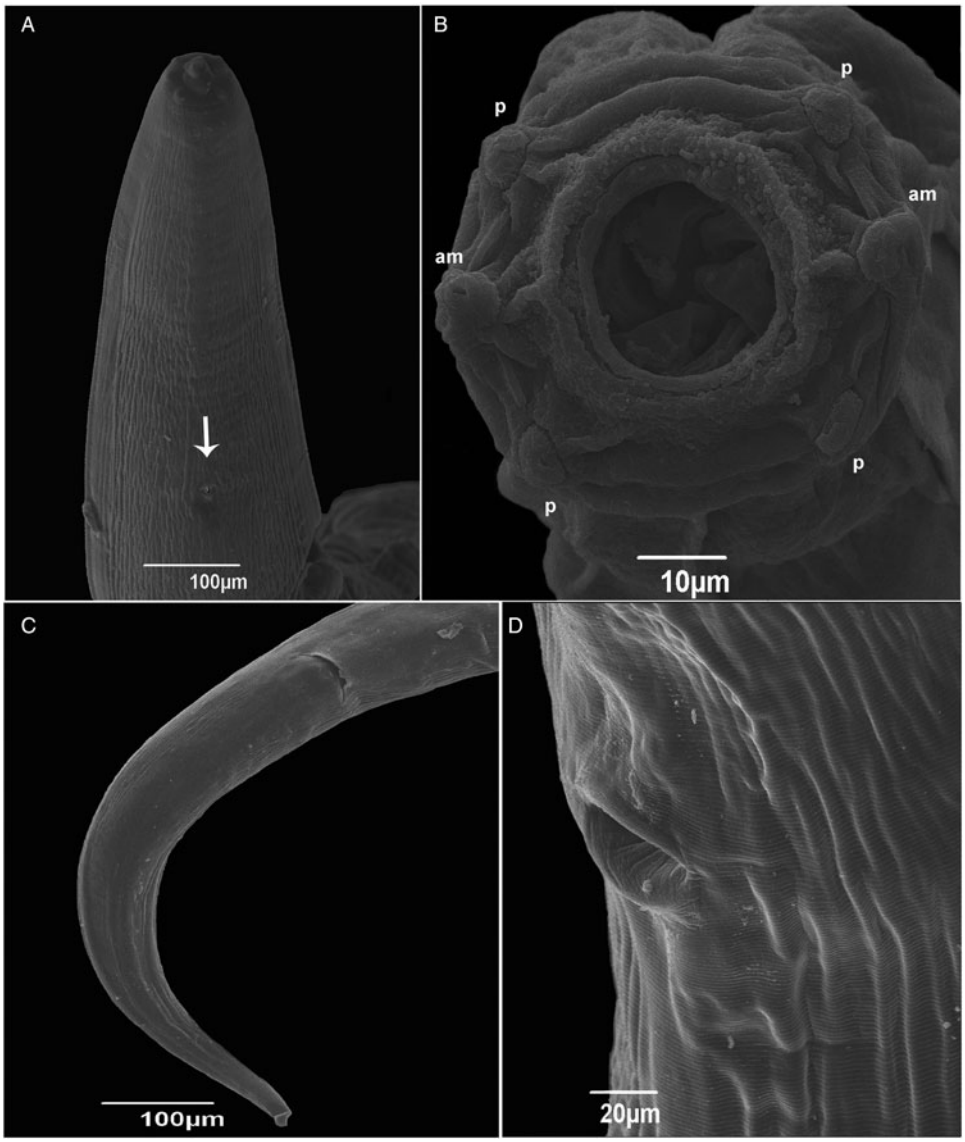

Fig. 4. Scanning electron photomicrographs of a specimen of *Subulura elisae* sp. n. (a) male posterior part, papillae (ten pairs); (b) spicule (s); (c) precloacal pseudo-sucker; ventral view, papillae (1st pair); (d) precloacal papillae (2nd and 3rd pairs); adcloacal papillae (4th and 5th pairs), ventral view; (e) postcloacal papillae (6th, 7th, 8th, 9th and 10th pairs) lateral view.

postcloacal papillae from the tail end 65–77 (72). Spicules similar, slender, slightly curvilinear, alate, distal tips pointed, 1.376–1.475 (1.431) mm long (figs. 1i and 4b). Gubernaculum triangular (figs. 1j and 2b), 190–231 (215.5).

Females (n = 5): Body curved ventrally, 12.345–13.942 (13.195) mm long, 435–548 (491.4) wide. Cervical alae 1.141–1.413 (1.250) mm long. Buccal capsule 43–49 (45.2) long, 40–45 (43) wide. Oesophagus, 1.007–1.245 (1.160) mm long. Bulb, 201–231 (218.8) long, 230–261 (240.8) wide. Nerve ring and excretory pore 275–365 (330.8), and 509–569 (542.7), from anterior extremity, respectively. Uterus filled eggs, with circumvolutions extended anteriorly from the vulva to the bulb, 2.323–3.973 (2.910) mm and posteriorly reaching the vulva and/or close to the anus, 5.652–6.063 (5.872) mm. Vulva small 4.331–5.496 (5.203) mm from the anterior extremity (figs. 1k, 2a and 3d). Tail and tip tail long with 910–1.141 (1.031) mm and 70–135 (110.8) (fig. 3c), respectively. Eggs, 71–77 (74) long, 41–50 (45) wide (fig. 2c, d).

Molecular analyses

The MT-CO1 gene sequence of *S. eliseae* sp. n. from Sinop, MT, Brazil is 601 base pairs (bp) in length. The sequence of the *S. eliseae* sp. n. from Porto Acre, AC, Brazil is 620 bp in length. To increase the representativeness of subuluroid taxa in our dataset, we amplified and sequenced the partial MT-CO1 gene from the subulurid *P. jacchi*, obtaining a sequence 612 bp in length. The MT-CO1 sequences of *S. eliseae* sp. n. (MT and AC) and *P. jacchi* were deposited in the GenBank database (http://www.ncbi.nlm.nih.gov) (accession numbers: OM432014, OM432016 and OM432015).

The MT-CO1 gene sequences from the present study, aligned with those retrieved from GenBank, resulted in a matrix of eight taxa and 579 characters. From these, 408 characters were constant, 76 were variable, and 95 were parsimony informative. In the ML analyses, the PhyML-SMS selected GTR + G as the best-fit nucleotide substitution model, with optimized ML frequencies,

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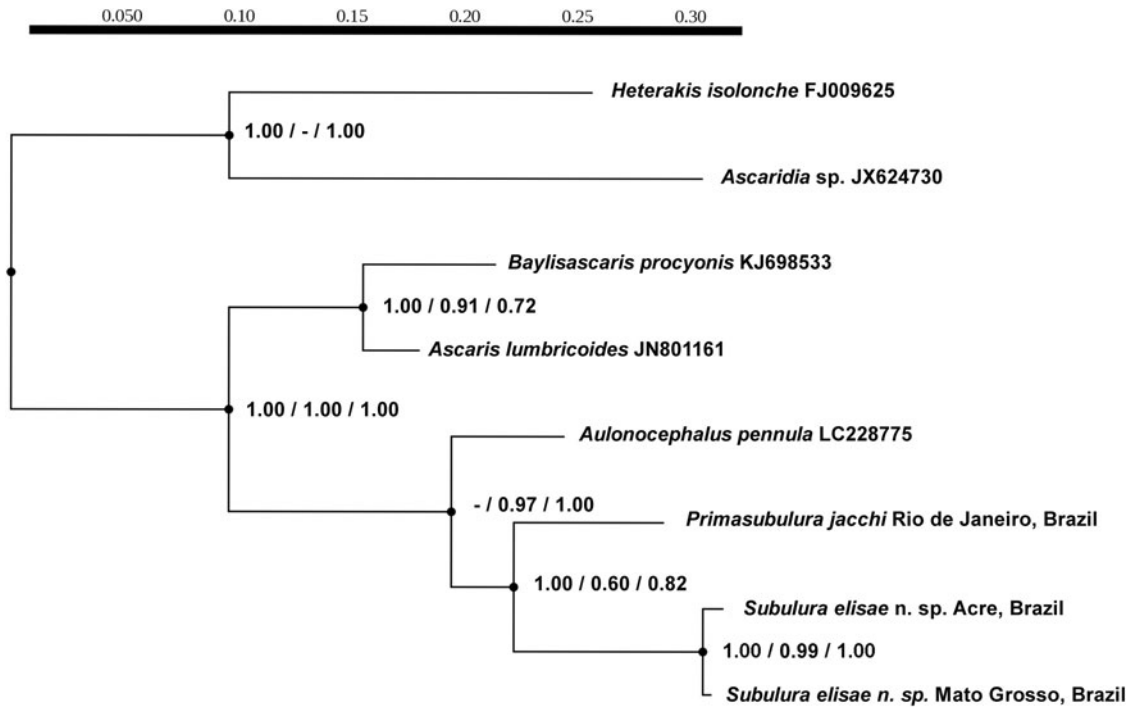

Fig. 5. Maximum likelihood tree (ML) based on an analysis of mitochondrial cytochrome c oxidase I sequences inferring a phylogenetic relationship between *Subulura elisae* sp. n. and other sequences from GenBank. Node values are aLRT, ML-BP, and BPP supports.

four rate categories and an estimated gamma-shape parameter of α=0.216. The best log-likelihood ML-tree score was -1893.108849. In the BI analyses, PAUP* selected the substitution models TrN + I for the first position, F81 for the second position, and HKY + G for the third position. The BI mean estimated marginal likelihood was -1704.5373 and the median was -1704.272. The effective samples sizes were well above 200 for all parameters.

The ML and BI phylogenies had similar topologies, with little variation in the nodes or support values in the matrix. In all the topologies, the sequences of Subuluroidea species formed a monophyletic group (aLRT= 1.00, ML-BP = 1.00, BPP = 1.00). All analyses confirmed that *S. eliseae* sp. n. from Acre state and *S. eliseae* sp. n. from Mato Grosso state formed a monophyletic group (aLRT = 1.00, ML-BP = 0.99, BPP = 1.00), with *P. jacchi* a sister to the monophyletic *S. eliseae* sp. n., with moderate to high support (aLRT = 1.00, ML-BP = 0.60, BPP = 0.82). *Aulonocephalus pennula* grouped with the clade formed by *P. jacchi* and *S. eliseae* sp. n., with moderate support (aLRT = -, ML-BP = 0.97, BPP = 1.00) indicating monophyly of the Subuluridae family (fig. 5).

Discussion

Currently, the identification of *Subulura* species is based mainly on morphological and morphometric characters, that is, body size, lengths of the oesophagus and spicules, number and arrangement of caudal papillae, morphology of the gubernaculum and position of the vulva (Guo *et al.*, 2019). We allocated the new species to the subfamily Subulurinae due to its complex pharyngeal structure with three pharyngeal portions, mouth opening simple and/or hexagonal and males with an elongated precloacal pseudosucker without a defined border.

The new species can be distinguished morphologically from the other four *Subulura* parasite species of marsupials by the number of caudal papillae, structure and dimensions (morphometric) and size of the spicule. *Subulura eliseae* sp. n. is smaller than *S. lanigeri*, but both species are longer than *S. interrogans* and *S. trinitatis*. It does not have caudal alae, a structure described in *S. interrogans* and *S. trinitatis*. The number of caudal papillae (ten pairs) found in *S. eliseae* sp. n. also differs from that found in other *Subulura* spp. described in South American marsupials.

In their description of *S. amazonica* and characterization the *S. interrogans* female allotype Pereira & Machado Filho (1968) mentioned three pairs of tooth-like pharyngeal projections. However, they seem not to have accurately documented an important *Subulura* characteristic. Quentin (1969) studied ontogenesis of the cephalic structure of the family Subuluroidea, describing three small pharyngeal portions of a complex structure at the base of the buccal capsule of the family Subuluridae species. Presuming that the pharyngeal projections described by Pereira & Machado Filho (1968) are the pharyngeal portions of the complex structure described by Quentin (1969), we consider that there are three pharyngeal formations in *Subulura* species, as suggested by Quentin (1969), instead of three pairs, as suggested by Pereira & Machado Filho (1968) (table 2).

In conclusion we describe a new *Subulura* species, *S. eliseae* sp. n., parasitizing mouse opossums *Marmosa* spp. As molecular data for the family Subuluridae species are insufficient, few inferences could be drawn from our phylogenies. As expected, our two sequences of *S. eliseae* sp. n. from Acre and Mato Grosso states formed a strongly supported clade, sister to the sequence of *P. jacchi*, thus supporting the subfamily Subulurinae, sister to *A. pennula*, which belongs to the subfamily Allodapinae, finally supporting the family Subuluridae.

Subulura eliseae sp. n. is the fifth species of the genus to be described in marsupials from the Americas, a genus that now comprises 61 species. This is only the second *Subulura* and the third Subuluridae species to be molecularly characterized.

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Adding to this, our sequence of *S. jacchi* is the fourth Subuluridae species thus far to be molecularly characterized. At present, morphological knowledge about many *Subulura* species is scattered or poorly reported. Thus, an integrative taxonomic approach comprising more species of the genus *Subulura* and family Subuluridae, including morphological, ecological and molecular characters, is essential to improve our understanding of their evolutionary and ecological associations with their hosts.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S0022149X22000244

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