Phylogenetic analyses based on molecular and morphological data reveal a new species of *Strigea* Abildgaard, 1790 (Digenea: Strigeidae) and taxonomic changes in strigeids infecting Neotropical birds of prey

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Abstract

Members of the genus *Strigea* Abildgaard, 1790 are endoparasites of birds distributed worldwide. Adults of an undescribed species of the genus *Strigea* were collected from the intestines of two hawk species (*Rupornis magnirostris* and *Accipiter coperii*). Other species identified as *Parastrigea macrobursa* that were described in Argentina were also recovered from two hawk species (*Buteogallus urubitinga* and *Buteogallus anthracinus*) in three localities along the coasts of Mexico. Specimens of the two species were sequenced for three molecular markers, the internal transcribed spacers locus (ITS1-5.8S rDNA- ITS2) and the domains D1–D3 from the large subunit from nuclear ribosomal DNA and the cytochrome *c* oxidase subunit 1 from mitochondrial DNA. The newly sequenced specimens were aligned with other strigeids sequences downloaded from GenBank. Maximum likelihood and Bayesian analyses inferred with each molecular marker revealed that our specimens of *Strigea* sp. formed an independent lineage, which is recognized herein as a new species, *Strigea magnirostris* n. sp., representing the first species in Mexico and the 16th in the Neotropical region. Morphologically, the new species is distinguished from other congeneric species from the Americas by having an oral sucker with several papillae around it, well-developed pseudosuckers (118–248 μm), a tegument covered with tiny spines, a larger cone genital (193–361 × 296–637) and a larger copulatory bursa (247–531 × 468–784). Our phylogenetic analyses revealed that *P. macrobursa* is not closely related to other members of the genus *Parastrigea* and is nested within *Strigea*, suggesting that *P. macrobursa* should be transferred to *Strigea* to form *Strigea macrobursa* n. comb., expanding its distribution range from Mexico to Argentina. Finally, the analyses also revealed that the taxonomy and systematics of *Strigea* should be re-evaluated, combining morphological and molecular characteristics.

Introduction

The cosmopolitan family Strigeidae Railliet, 1919 currently contains 13 genera with approximately 110 nominal species distributed worldwide (Niewiadomska, 2002). The type genus *Strigea* was established by Abildgaard, 1790 to accommodate species that have vitellarium uniformly distributed over both parts of the body and the presence of a pharynx (Dubois, 1968; Niewiadomska, 2002). Among strigeids, *Strigea* is considered the most diverse genus within the family, with approximately 47 nominal species associated mainly with strigiform, accipitriform, falconiform, ciconiiform, caprimulgiform, cariamiform, passeriform, gruiform, trogoniform and anseriform birds (Dubois, 1968; Drago *et al.*, 2014). Information on the life cycle of most species of *Strigea* is scarce, but it is thought to involve four hosts. Adult worms live and reproduce sexually in the digestive tracts of birds that serve as definitive hosts. Eggs are expelled into the environment with the faeces of the host. After the ingestion of the eggs by a planorbid snail, which serves as the first intermediate host, the parasites develop into cercariae. The cercariae emerge and swim to find and penetrate the second intermediate host (amphibians), where they develop into mesocercariae and in some cases it may cause severe morphological anomalies as the polydactyly (Sinsch *et al.*, 2019; Svinin *et al.*, 2020, 2023). The amphibian with the mesocercaria is ingested by the third intermediate host (an amphibian, reptile, bird, or small mammal) and then the parasite develops into an encysted, tetracotyle-type metacercaria. Finally, these amphibians, reptiles, birds and mammals

are some of the principal food resources of prey birds, in which the life cycle is completed (Pearson, 1959, 1972; Odening, 1967).

To date, *Strigea* contains 47 described species, nine of which are distributed in Asia, nine in Africa, five in Oceania and three in Europe (Dubois, 1968, 1988; Dubois & Beverley-Burton, 1971). In the Americas, 21 species have been described, of which six are in North America (*Strigea infundibuliformis* Dubois, 1934; *Strigea macroconophora* Dubois and Rausch, 1950; *Strigea elegans* Chandler and Rausch, 1947; *Strigea sphaerula macrosicya* Dubois and Rausch, 1950; *Strigea gruis* Dubois and Rausch, 1964 and *Strigea macropharynx* Dubois and Rausch, 1965); and 15 species in South America (*Strigea caryophylla* (Diesing, 1850) Mathias, 1925; *Strigea elliptica* (Brandes, 1888) Szidat, 1928; *Strigea bulbosa* (Brandes, 1888) Szidat, 1928; *Strigea nugax* Szidat, 1928; *Strigea vaginata* (Brandes, 1888) Szidat, 1928; *Strigea falconis brasiliana* Szidat, 1929; *Strigea caluri* Dubois, 1962; *Strigea sphaerocephala* (Westrumb, 1823) Dubois, 1937; *Strigea microbursa* Pearson and Dubois, 1985; *Strigea magniova* Dubois, 1988; *Strigea arcuata* Dubois, 1988; *Strigea meridionalis* Lunaschi and Drago, 2009; *Strigea inflecta* Lunaschi and Drago, 2012; *Strigea orbiculata* Lunaschi and Drago, 2013 and *Strigea proteolytica* Drago, Lunaschi and Draghi, 2014) (Dubois, 1968; Lunaschi & Drago, 2006, 2009, 2012, 2013; Drago *et al.*, 2014). The morphological identification of *Strigea* spp. is complex and problematic due to their small size and the difficulty in observing internal and external structures used for taxonomy and differentiation among species (Lunaschi & Drago, 2006, 2009, 2012, 2013; Drago *et al.*, 2014). Additionally, molecular data are scarce and only a few sequences of *Strigea* are currently available (Hernández-Mena *et al.*, 2017; Heneberg *et al.*, 2018; Svinin *et al.*, 2020). In Mexico, the metacercaria of *Strigea* was recorded for the first time by Vidal-Martínez (1995) in two cichlid fish species in south-eastern Mexico (Pérez-Ponce de León *et al.*, 2007). However, the specimens were not deposited, and, therefore, the records could not be verified. Hernández-Mena *et al.* (2017) recorded an adult of *Strigea* sp. in crested caracara (*Caracara cheriway* Jacquin) in Presa La Angostura, Chiapas, Mexico. Recently, strigeids in Mexico have started to receive attention and much effort has been made to incorporate morphological and molecular characteristics to describe and delineate the biodiversity of this group of parasites (Hernández-Mena *et al.*, 2014, 2017; López-Jiménez *et al.*, 2021, 2022).

In the current study, adult specimens of the genus *Strigea* were collected from the intestine of roadside hawk (*Rupornis magnirostris* Gmelin) and Cooper's hawk (*Accipiter cooperii* Bonaparte) in six localities from the Neotropical region of Mexico. After a careful morphological examination, the specimens were determined to correspond to an undescribed species of the genus *Strigea*. In addition, other strigeids collected from the intestine of the great black hawk (*Buteogallus urubitinga* Gmelin) and common black hawk (*Buteogallus anthracinus* Deepe) were identified as *Parastrigea macrobursa* Drago & Lunaschi, 2011, a species previously described in South America.

The objectives of the present research were: (a) to provide a morphological description of the new species; and (b) to test the systematic position of *P. macrobursa* by using sequences of the internal transcribed spacers (ITS1-5.8S rDNA- ITS2) and large subunit (LSU) of the nuclear DNA and of the cytochrome *c* oxidase subunit I (*cox 1*) gene of the mitochondrial DNA. We then used the resulting phylogenetic trees as a framework to discuss host–parasite associations and begin to understand the evolutionary history of this group of strigeids.

Materials and methods

Specimen collection

A total of 17 hawks were collected between December 2019 and December 2021 in nine localities from Mexico (fig. 1; table 1). Ten individuals of roadside hawk (*R. magnirostris*), one Cooper's hawk (*A. cooperii*), two individuals of great black hawk (*Buteogallus urubitinga*) and four common black hawks (*B. anthracinus*). Birds were identified following Howell & Webb (1995) and the American Ornithologist' Union (1998) guidelines. Strigeids were removed from the intestines of the birds and examined using a stereomicroscope. Digeneans collected were relaxed in hot distilled water and preserved in 100% ethanol for morphological and molecular analyses.

Morphological analyses

Digeneans preserved in 100% ethanol were stained with Mayer's paracarmine (Merck, Darmstadt, Germany), dehydrated in ethanol series, cleared in methyl salicylate and mounted in Canada balsam for morphological analysis. Specimens were examined using a compound microscope equipped with a bright field Leica DM 1000 light emitting diode microscope (Leica, Wetzlar, Germany). Measurements were taken using Leica Application Suite microscope software (Leica Microsystems GmbH, Wetzlar, Germany) and are given in micrometres and presented with the range followed by the mean in parentheses. Some specimens were dehydrated with an ethanol series, critical point dried, sputter coated with gold and examined with a Hitachi Stereoscan Model S-2469N scanning electron microscope operating at 15 kV. Voucher specimens from the present study were deposited in the Colección Nacional de Helmintos (CNHE) from Instituto de Biología, Universidad Nacional Autónoma de México (UNAM), Mexico City.

DNA isolation, amplification and sequencing

Strigeids preserved in 100% ethanol were placed individually in tubes and digested overnight at 56°C in a solution containing 20 mM sodium chloride, 10 mM Tris–hydrochloride (pH = 7.6), 100 mM ethylenedinitrilotetraacetic acid disodium salt dihydrate (pH = 8.0), 1% Sarkosyl and 0.1 mg/ml proteinase K. Following digestion, DNA was extracted from the supernatant using the DNAzol reagent (Molecular Research Center, Cincinnati, Ohio). The internal transcribed spacers (ITS1-5.8S rDNA- ITS2) of the nuclear ribosomal DNA were amplified using the forward primer BD1 5′-GTCGTAACAAGGTTTCCGTA- 3′ (Bowles & McManus, 1993) and the reverse primer BD2 5′-ATCTAG ACCGGACTAGGCTGTG-3′ (Bowles *et al.*, 1995). The partial fragments of domains D1–D3 of the large subunit of nuclear ribosomal RNA (LSU) were amplified with the forward primer 391 5′-AGCGGAGGAAAAGAAACTAA-3′ (Nadler *et al.*, 2000) and the reverse primer 536, 5′ -CAGCTATCCTGAGGGAAAC-3′ (García-Varela & Nadler, 2005). The complete gene of the cytochrome *c* oxidase subunit 1 (*cox 1*; 850 base pairs (bp)) was amplified using the forward primers AphaF, 5′-TAT GATTTTTTTYTTTTRATG-3′ and the reverse primer
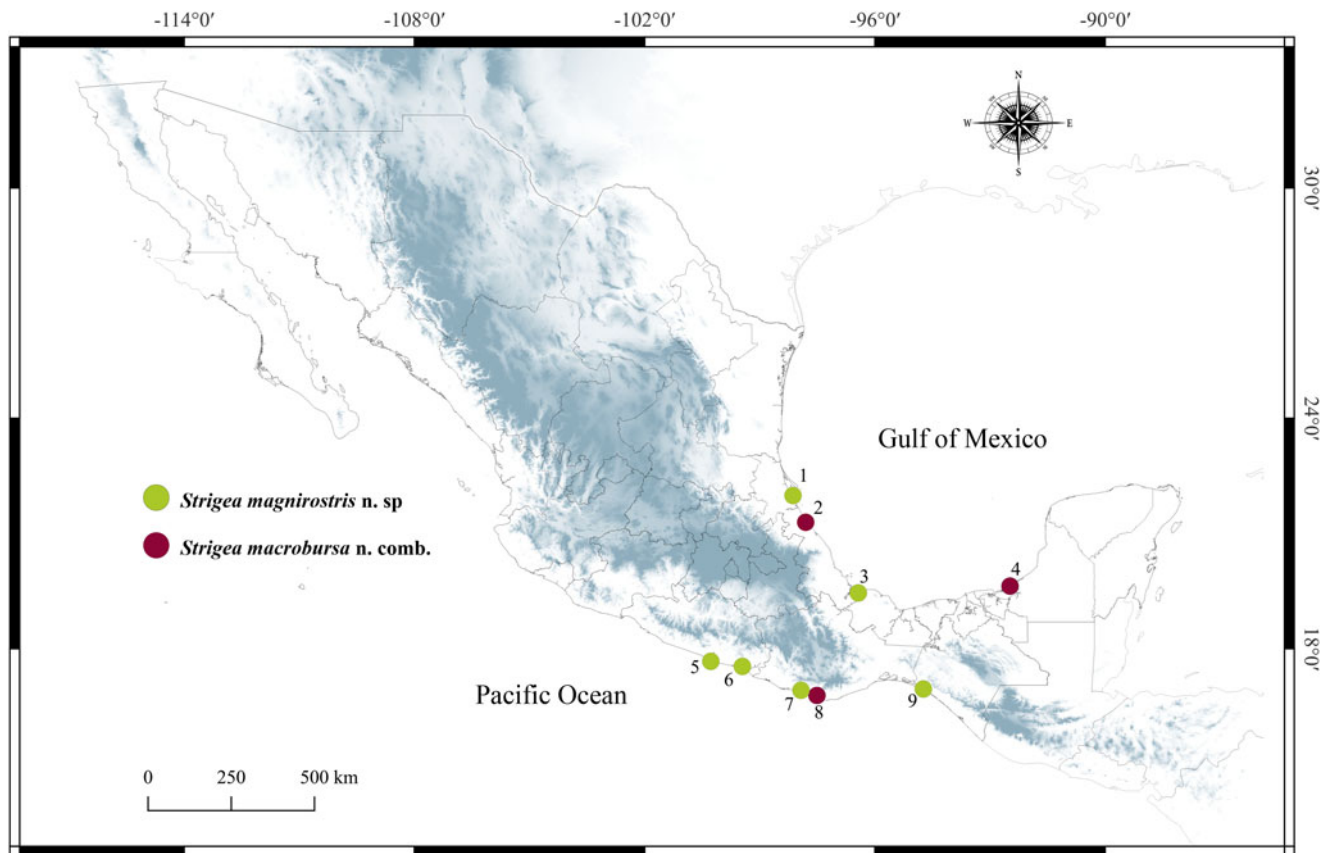
Fig. 1. Map of Mexico showing the sampled sites for the birds. Localities with a circle with colours green and red were positive for the infection with *Strigea magnirostris* n. sp. and *Strigea macrobursa* n. comb., respectively. Localities correspond to those in table 1.

JB4.5′-TAAAGAACATAATGAAATTG3′ (Bowles *et al.*, 1992). Polymerase chain reactions (PCRs) were carried out in 25 μl reactions, consisted of 1 μl of each primer, 2.5 μl of 10 × buffer, 1.5 μl MgCl2, 0.5 μl of dNTP mixture, 0.125 μl of Platinum Taq DNA polymerase (Invitrogen Corporation, São Paulo, Brazil) and 2 μl of genomic DNA. PCR cycling parameters for amplifications consisted of denaturation at 94°C for 1 min, 35 cycles of 94° C for 1 min, 50°C for 1 min and 72°C for 1 min, followed by a post-amplification incubation at 72°C for 10 min. Sequencing reactions were performed using ABI Big Dye (Applied Biosystems, Boston, Massachusetts) terminator sequencing chemistry and reaction products were separated and detected using an ABI 3730 capillary DNA sequencer. Contigs were assembled and base-calling differences resolved using Codoncode Aligner version 9.0.1 (Codoncode Corporation, Dedham, Massachusetts) and submitted to the GenBank dataset (table 1).

Alignments and phylogenetic analysis

Newly-generated sequences of ITS, LSU and *cox* 1 were aligned with other sequences of strigeids available in the GenBank dataset. Sequences of each molecular marker were aligned using the software CLUSTAL_X (Thompson *et al.*, 1997). The best nucleotide substitution model was selected for each molecular marker using jModelTest v2.1.7 (Posada, 2008) and applying the Akaike information criterion. The best nucleotide substitution model for the ITS and LSU dataset were TVM + I + G and for *cox* 1 the dataset was TIM3 + I + G. Phylogenetic trees were

reconstructed through maximum likelihood (ML) with the program RAxML v7.0.4 (Silvestro & Michalak, 2012), and Bayesian inference (BI) analyses were inferred with MrBayes 3.2.2 (Ronquist *et al.*, 2012) using the computational resource Cyberinfrastructure for Phylogenetic Research Science Gateway v3.3 (Miller *et al.*, 2010). ML analyses were inferred with the option GTRGAMMAI and 10,000 bootstrap replicates. BI analyses included Markov chain Monte Carlo searches of two simultaneous runs for 10 million generations, sampling every 1000 generations, a heating parameter value of 0.2 and a burn-in of 25%. Trees were drawn and edited using FigTree software v1.4.0 (Rambaut, 2012). Genetic divergences were estimated using *P* uncorrected distances with MEGA v.6 (Tamura *et al.*, 2013).

Results

Molecular characterization and phylogenetic analyses

Nuclear genes

The ITS dataset included 42 sequences with 1042 characters. The phylogenetic analyses performed with ML and BI showed that the genus *Strigea* is monophyletic and is subdivided into two major subclades (fig. 2). The first subclade was formed by 17 isolates of an undescribed species of *Strigea* sp. from the roadside hawk (*R. magnirostris*) and Cooper's hawk (*A. cooperii*) collected from six localities in Mexico. This clade is sister to another subclade formed by eight isolates identified morphologically as *P. macrobursa* recovered from the great black hawk (*Buteogallus urubitinga*) and common black hawk (*B. anthracinus*) from

Table 1. Specimens' information for *Strigea* spp., locality, state, geographical coordinates, host name, number of host examined/infected (prevalence of infection) and GenBank accession number for specimens studied in the current study.

Locality	State	Coordinates	Host	Host infected/ host revised	Species of *Strigea*	Internal transcribed spacers	Large subunit	Cytochrome *c* oxidase subunit 1
1. Tamiahua	Veracruz	21°18′02″ N 97°26′56.2″ W	*Rupornis magnirostris*	1/1	*S. magnirostris* n. sp.	OQ647944	OQ647911	OQ648146
						OQ647941	OQ647912	OQ648143
2. Tecolutla	Veracruz	20°33′49.8″ N 97°05′57.7″ W	*Buteogallus urubitinga*	1/1	*S. macrobursa* n. comb.	OQ647932	OQ647927	OQ648131
						OQ647933	OQ647928	OQ648130
						OQ647936	OQ647929	OQ648132
						OQ647935	OQ647930	OQ648133
3. Tlacotalpan	Veracruz	18°37′04.15″ N 95°38′56.10″ W	*R. magnirostris*	2/2	*S. magnirostris* n. sp.	OQ647942	OQ647913	OQ648142
						OQ647943	OQ647909	
						OQ647948	OQ647914	OQ648144
						OQ647940	OQ647910	OQ648145
4. Isla Aguada	Campeche	18°48′22.92″ N 91°28′03.68″ W	*B. urubitinga*	1/1	*S. macrobursa* n. comb.	OQ647937	OQ647923	
						OQ647938	OQ647924	
						OQ647939	OQ647925	
								OQ648128
							OQ647926	OQ648129
5. Tres Vidas	Guerrero	16°43′59.85″ N 99°42′48.99″ W	*R. magnirostris*	1/3	*S. magnirostris* n. sp.	OQ647949	OQ647917	OQ648135
			Accipiter cooperii	1/1		OQ647945	OQ647915	OQ648140
						OQ647946		OQ648141
						OQ647947	OQ647916	OQ648147
6. Marquelia	Guerrero	16°35′40.88″ N 98°50′37.90″ W	*R. magnirostris*	1/1	*S. magnirostris* n. sp.	OQ647950	OQ647918	OQ648136
						OQ647951	OQ647919	OQ648137
						OQ647952		OQ648138
7. Villa de Tututepec	Oaxaca	15°56′10.98″ N 97°13′38.87″ W	*R. magnirostris*	1/2	*S. magnirostris* n. sp.	OQ647953	OQ647920	OQ648139
			B. anthracinus	0/3				
8. Santa María, Cocotepec	Oaxaca	15°48′24.56″ N 97°00′49.79″ W	*B. anthracinus*	1/1	*S. macrobursa* n. comb.	OQ647934	OQ647931	OQ648134
9. El Zapotal	Chiapas	15°58′20.26″ N 93°51′23.04″ W	*R. magnirostris*	1/1	*S. magnirostris* n. sp.	OQ647954	OQ647921	
						OQ647955	OQ647922	
						OQ647956		OQ648148
								OQ648149
								OQ648150

The sample number for each locality corresponds with the same number in Figure 1.

three localities in Mexico. All these relationships were supported with well-supported bootstrap values and Bayesian posterior probabilities (fig. 2). The intraspecific genetic divergence among 17 isolates of *Strigea* sp. was low, ranging from 0 to 0.3%, whereas that for *P. macrobursa* ranged from 0 to 0.2% for ITS. The LSU dataset consisted of 21 terminals and 1208 characters. The tree topologies inferred using the LSU dataset from the nuclear DNA showed that the genus *Strigea* is paraphyletic because the genus was subdivided into two major clades. The first major clade was formed by two sequences identified as *Strigea robusta*
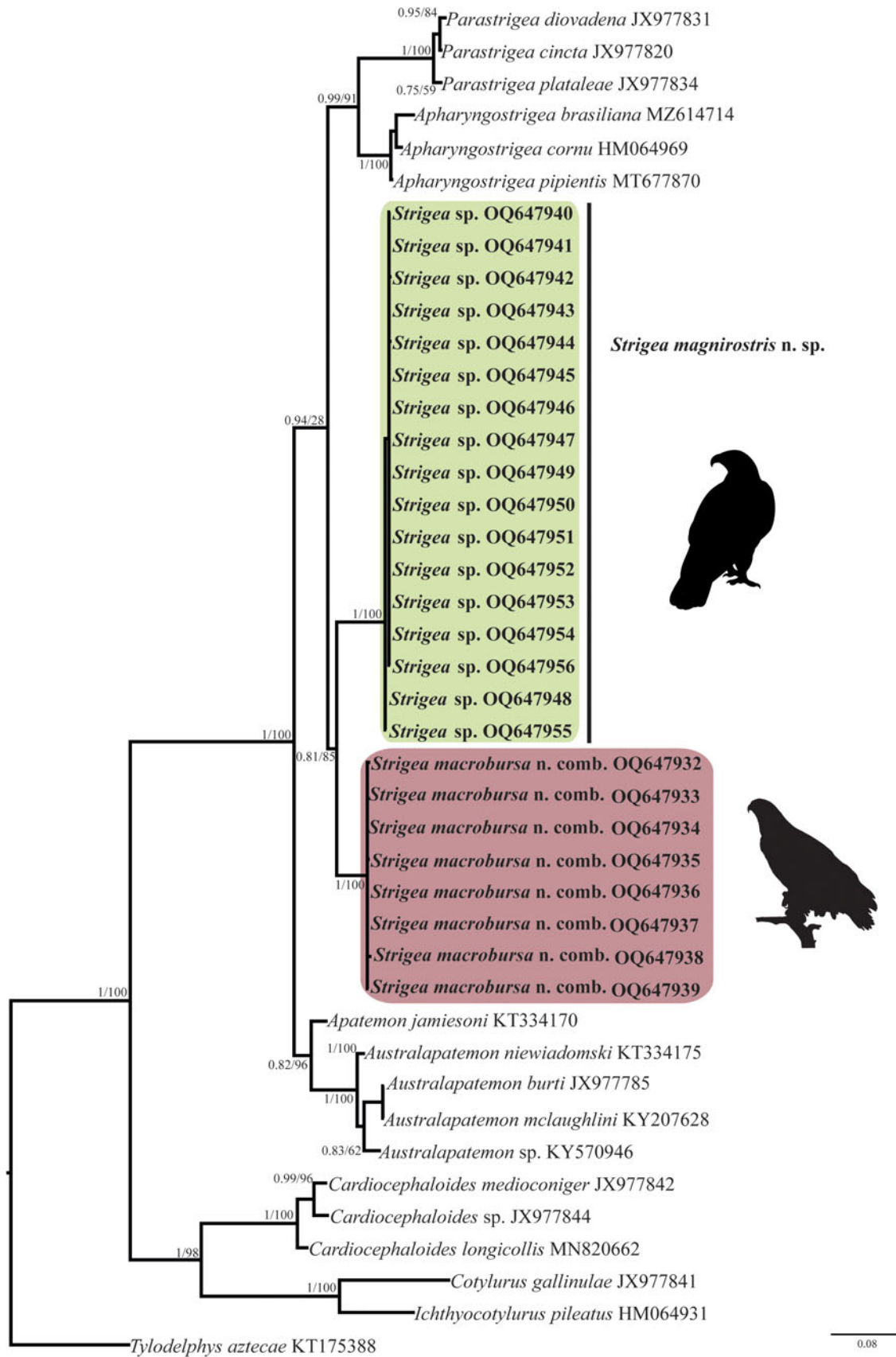
Fig. 2. Phylogenetic trees inferred with Maximum Likelihood (ML) and consensus Bayesian Inference (BI) with the internal transcribed spacers dataset. Numbers near internal nodes show maximum likelihood bootstrap percentage values and Bayesian posterior probabilities.
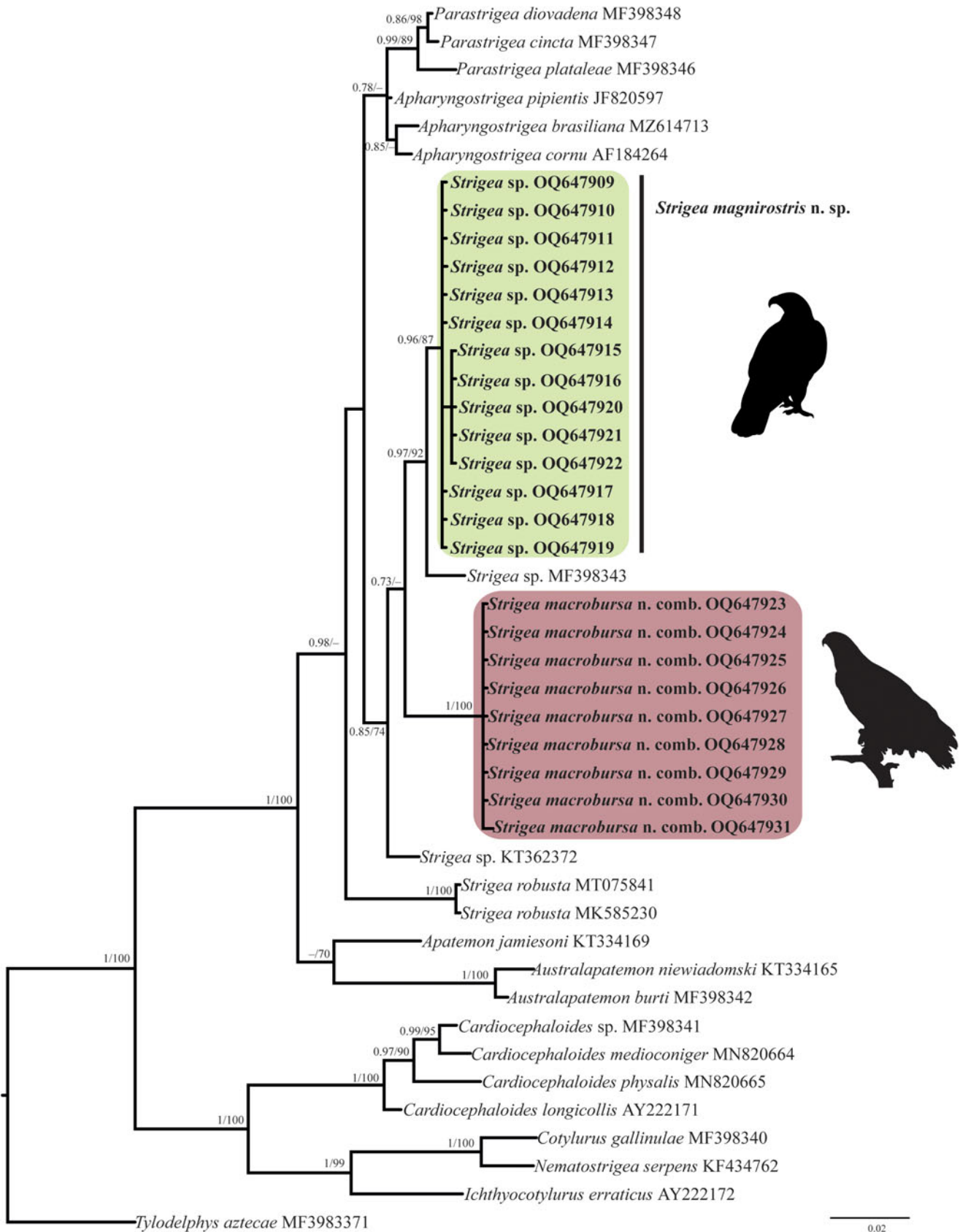
Fig. 3. Phylogenetics trees inferred with Maximum Likelihood (ML) and consensus Bayesian Inference (BI) with the large subunit dataset. Numbers near internal nodes show ML bootstrap percentage values and Bayesian posterior probabilities.

(MT075841 and MK585230) recovered from the marsh frog (*Pelophylax ridibundus*) and edible frog (*Pelophylax esculentus*), respectively, from Russia, and this clade was sister to a clade formed by species of the genera *Parastrigea* Szidat, 1928 and *Apharyngostrigea* Ciurea, 1927 (fig. 3). The second major clade was formed by four subclades. The first subclade contains an unidentified sequence of *Strigea* sp. (KT362372) from water frog (*Pelophylax* sp.) from France. The second subclade was formed by nine isolates of *P. macrobursa* from Mexico, which is a sister to the third subclade formed by a single sequence of an unidentified sample of *Strigea* sp. (MF398343) recovered from crested caracara (*Caracara cheriway*) from Presa La Angostura, Chiapas, Mexico. The fourth subclade was formed by 14 isolates of *Strigea* sp. recovered from the roadside hawk (*R. magnirostris*) and Cooper's hawk (*A. cooperii*) from six localities in Mexico (fig. 3). Finally, the intraspecific genetic divergence among 14 isolates of *Strigea* sp. was low, ranging from 0 to 0.10%, whereas that for nine isolates of *P. macrobursa* ranged from 0 to 0.08% for LSU.

Mitochondrial gene

The newly completed sequences from *cox 1* were aligned with other partial sequences downloaded from GenBank. The alignment included the first region of *cox 1* with 42 sequences and 374 characters. The phylogenetic analyses performed with ML and BI showed that the genus *Strigea* is monophyletic (fig. 4). The clade was subdivided into three subclades. The first subclade was formed by 16 isolates of an undescribed species of *Strigea* sp. from the Neotropical region of Mexico. The second subclade was formed by an isolate of an unidentified sequence of *Strigea* sp. (MF398319) from Presa La Angostura, Chiapas, Mexico. The third subclade was formed by seven isolates identified morphologically as *P. macrobursa*. All these relationships had high bootstrap values and Bayesian posterior probabilities (fig. 4). The intraspecific genetic divergence ranged from 0 to 1% among isolates of *Strigea* sp. and from 0 to 1.3% for *P. macrobursa* for *cox 1*.

In summary, the phylogenetic analyses performed with two nuclear markers and one mitochondrial molecular marker supported the monophyly of all new isolates of *Strigea* spp. from the Neotropical region (figs 2–4). The new ITS, LSU and *cox 1* sequences revealed that our specimens of *Strigea* sp. recovered from the roadside hawk (*R. magnirostris*) and Cooper's hawk (*A. cooperii*) from six localities in the Neotropical region of Mexico formed an independent lineage, which is recognized herein as a new species and is described next as *Strigea magnirostris* n. sp., representing the first species to Mexico and the 22nd to the Americas. In addition, the specimens identified as *P. macrobursa* collected from the intestine of the great black hawk (*B. urubitinga*) (type host) and common black hawk (*B. anthracinus*) formed a clade nested inside *Strigea*, and as a result, it should be transferred to *Strigea* to form *Strigea macrobursa* n. comb. (figs 2–4).

Morphological description

Family Strigeidae Railliet, 1919

Subfamily Strigeinae Railliet, 1919

Genus *Strigea* Abildgaard, 1790

Strigea magnirostris n. sp.

Type host: *R. magnirostris* (roadside hawk) (Accipitriformes: Accipitridae).

Other host: *A. cooperii* (Cooper's hawk) (Accipitriformes: Accipitridae).

Type locality: Tamiahua, Veracruz, Mexico (21°18′02″ N, 97°26′56.2″ W).

Other locality: Tres Vidas, Guerrero, Mexico (16°43′59.8″ N, 99°42′48.9″ W).

Site in host: Intestine.

Prevalence: eight of 11 (72%).

Type material: Holotype CNHE 11118; paratypes CNHE 11119; voucher CNHE 11120.

GenBank accession number: ITS, OQ647940–56; LSU, OQ647909–22; *cox 1*, OQ648135–50.

Etymology: The epithet of the species refers to the specific name of the type host.

Description (figs 5 and 6; table 2)

Description (based on 17 adult specimens) (figs 5 and 6): Body 3.03–4.43 mm (3.93 mm) in total length. Tegument spines on the surface of the forebody (fig. 6d). Forebody is longer than is wide, covered with tiny rounded spines, 562–872 (749) long by 400–690 (583) wide, representing 20% of body length (BL) (figs 5 and 6b–d). Hind-body long, strongly curved dorsally with tegument smooth, 2440–3591 (3176) long by 352–632 (492) wide, almost four times longer than the forebody, with a ratio of hind-body length to forebody length of 1: 3.3–5.3 (4.2). Oral sucker terminal, well developed, 77–109 (97) long by 80–115 (100) wide, with several papillae around it (fig. 6c). Ventral sucker well developed, larger than oral sucker, 150–240 (187) long by 124–188 (158) wide. Ratio of ventral sucker length to oral sucker length is 1: 1.45–2.42 (2.0). Pharynx 65–103 (80) long by 64–90 (72) wide. Ratio of pharynx length to oral sucker length is 0.84–1.10 (0.97). Pseudosuckers well developed with conspicuous folds in anterior section, 114–248 (185) long by 77–12 (101) wide (fig. 6b). Holdfast organ lobes can be projected beyond the anterior margin of the forebody, proteolytic gland at base of forebody, 182–225 (200) long by 83–119 (106) wide. Testes in tandem, bilobed, situated near posterior end of the body, anterior testis 272–476 (370) long by 261–496 (378) wide, posterior testis slightly larger than anterior testis at 346–497(420) long by 280–512 (400) wide. Seminal vesicle long, sinuous, postesticular, slightly overlapping with posterior testis. Ovary reniform, pretesticular 139–190 (165) long by 126–214 (170) wide. Mehlis' gland and vitelline reservoir in the intertesticular region. Vitelline follicles of different sizes in both body segments; in the forebody, small follicles extend into the holdfast organ and lateral body wall from the posterior margin of the sucker ventral, while in the hind-body, large follicles are mostly concentrated in the neck (pre-ovarian region) ventrally to the seminal vesicle or copulatory bursa (fig. 5). Copulatory bursa large triangle-shaped broadening in posterior end, 247–531 (390) long by 468–784 (630) wide (figs 5 and 6e). Muscular ring (*Ringnapf*) well developed. Genital cone large and well delimited from body parenchyma, 193–361 (280) long by 380–637 (512) wide, ejaculatory duct and uterus join at base of genital cone, forming hermaphroditic duct. Uterus with large and numerous eggs (20–50) (35), oval, 71–105 long by 40–65 (52) wide. Ratio of genital cone length to egg length is 1: 1.95–3.88 (2.9). Excretory pore terminal.

Remarks

Currently, 21 species of the genus *Strigea* have been described in the Americas that parasitize strigiform, ciconiiform, falconiform, caprimulgiform, passeriform, gruiform, trogoniform and anseriform birds. Of the 21 described species, only five species (*S. falconis brasiliana*, *S. elegans*, *S. microbursa*, *S. magniova* and *S. arcuata*) share morphological characteristics with *S. magnirostris*
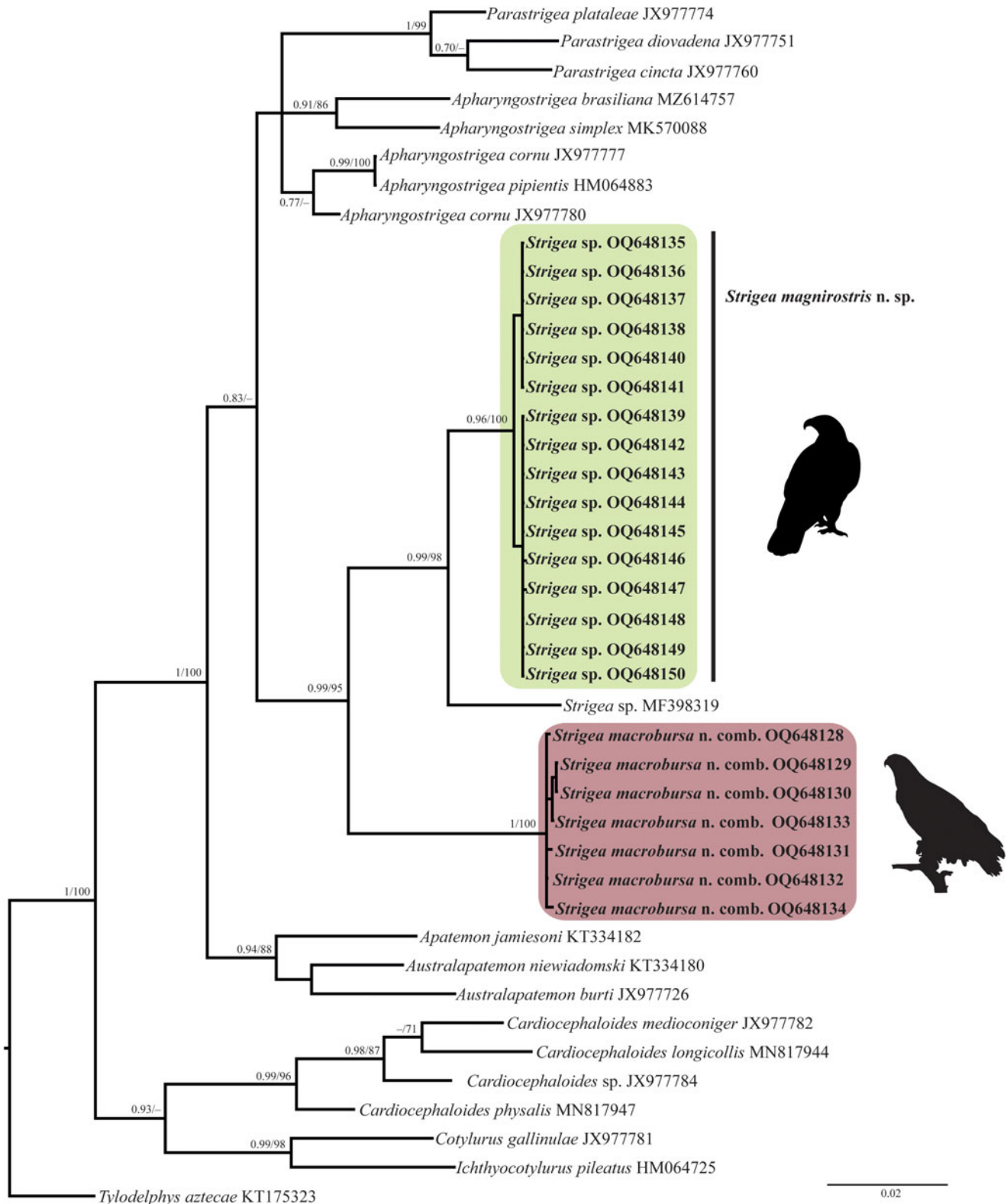
Fig. 4. Phylogenetics trees inferred with Maximum Likelihood (ML) and consensus Bayesian Inference (BI) with the *cox* 1 dataset. Numbers near internal nodes show ML bootstrap percentage values and Bayesian posterior probabilities.

n. sp., such as body shape, presence of a neck region in the hind-body and distribution of vitelline follicles in the forebody, which are scarce and extend into the lobes from the holdfast organ (Chandler & Rausch, 1947; Dubois, 1968, 1988; Pearson &

Dubois, 1985; Lunaschi & Drago, 2006, 2009). The new species most closely resembles *S. arcuata*, *S. microbursa* and *S. elegans* by having pseudosuckers that are well developed in the forebody. However, *S. arcuata* can be distinguished from *S. magnirostris*
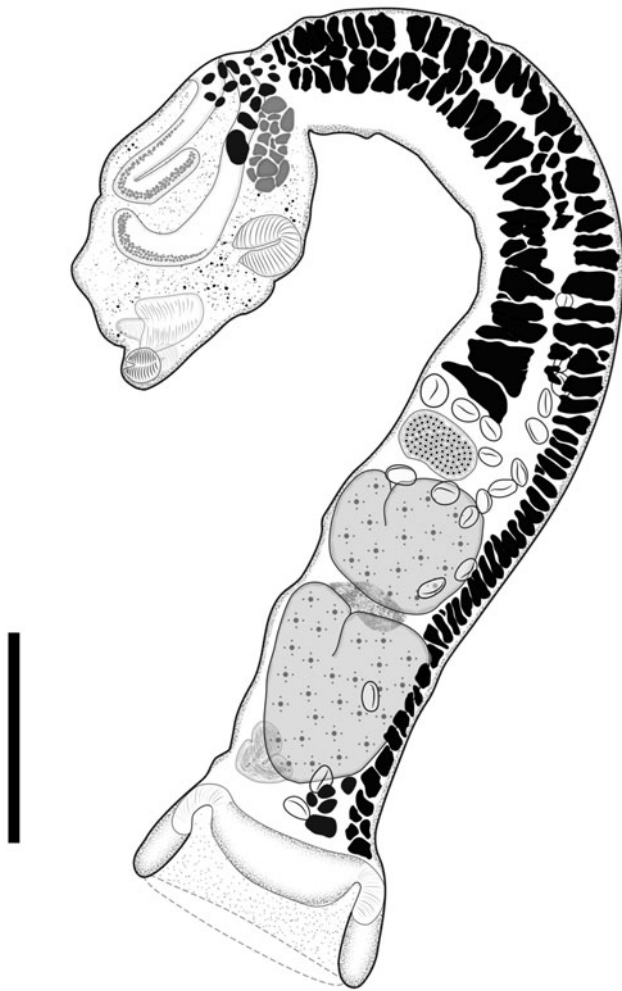
Fig. 5. Adult of *Strigea magnirostris* n. sp. from *Rupornis magnirostris*; whole worm, holotype, lateral view. Scale bars = 500 μm.

n. sp. by having a smaller genital cone included in a circular muscular formation (125 × 145 vs. 193–361 × 296–637 in *S. magnirostris*). In addition, *S. arcuata* possesses lower limits for the following characteristics: pseudosuckers (105 × 80 vs. 118–248 × 64–125 in *S. magnirostris*); hind-body width (180 vs. 352–632); anterior testes (255 × 185 vs. 272–476 × 261–496); and posterior testes (340 × 260 vs. 300–497 × 280–512). The species *S. microbursa* can be distinguished from *S. magnirostris* n. sp. by having a smaller genital cone (100–180 × 80–140 vs. 193–361 × 296–637). In addition, *S. microbursa* possesses lower limits for the following characteristics: pseudosuckers (85–95 × 90–95 vs. 118–248 × 64–125 in *S. magnirostris*); forebody width (230–300 vs. 400–690); ovary length (55–140 vs. 139–190); and ovary width (80–106 vs. 126–214). Finally, *S. elegans* can be distinguished from *S. magnirostris* n. sp. due to its smaller BL (1550–2450 vs. 3030–4437), smaller copulatory bursa (350 diam. vs. 247–531 × 468–784) and larger eggs (115–220 vs. 71–105) (see table 2).

Morphological redescription

Strigea macrobursa n. comb.

Syn. *Parastrigea macrobursa* Drago and Lunaschi, 2011

Host: *B. urubitinga* (great black hawk) (Accipitriformes: Accipitridae).

Other host: *B. anthracinus* (common black hawk) (Accipitriformes: Accipitridae)

Locality: Isla Aguada, Campeche, Mexico (18°48′22.92″ N, 91°28′03.68″ W).

Other localities: Tecolutla, Veracruz, Mexico (20°33′49.8″ N, 97°05′57.7″ W).

Site in host: Intestine.

Prevalence: 3 of 6 (50%).

Voucher specimens: CNHE 11121, 11122.

GenBank accession number: ITS OQ647932–39; LSU OQ647923–31; cox 1 OQ648128–34.

Description (figs 7 and 8; table 3)

Description (based on 26 adult specimens) (figs 7 and 8): Body 957–2.88 mm (1920 mm) in total length. Forebody tulip-shaped, 344–775 (560) long by 238–562 (400) wide (fig. 7b). Tegument spines on the surface of the forebody (fig. 8d). Hind-body slightly plump with tegument smooth, two to three times longer than the forebody at 609–2184 (1400) long by 256–759 (508) wide, with some specimens having a neck region (nine individuals) and some specimens lacking a neck region (17 individuals) (fig. 7a, c). Ratio of BL to forebody length is 1: 2.5–4.1 (1: 3.3). Ratio of hind-body length to forebody length is 1: 1.5–3.1 (1: 2.3). Oral sucker subterminal, well developed, 64–95 (80) long by 57–86 (71) wide (fig. 8c). Ventral sucker oval, 74–106 (90) long by 55–98 (76) wide. Prepharynx absent, pharynx 33–66 (52) long by 31–59 (47) wide. Holdfast organ lobes reaching anterior end (fig. 8b, c), proteolytic gland at base of forebody 75 long by 43 wide. Testes in tandem, large, not lobed, anterior testis oval 95–281 (190) long by 132–449 (290) wide, posterior testis slightly larger than anterior testis at 137–392 (260) long by 155–474 (314) wide. Seminal vesicle long, postesticular. Ovary oval, pre-testicular or slightly overlapping anterior testis, 52–188 (120) long by 72–193 (130) wide. Laure's canal, opening dorsally between ovary and anterior testis. Mehlis' gland and vitelline reservoir in the intertesticular region. Vitelline follicles similar in size in both body segments; in the forebody, they are in the dorsal lip of the holdfast organ forming two symmetrical masses situated between the ventral sucker and intersegmental constriction; in the hind-body, the vitelline follicles are concentrated in the pre-ovarian region, extending ventrally to the posterior testis or copulatory bursa (fig. 7a–c). Copulatory bursa large, delimited by pronounced constriction, occupying 30%–45% (40%) of hind-body length, 163–681 (422) long by 246–616 (430) wide (fig. 8e). Muscular ring (*Ringnapf*) absent. Genital cone well delimited from body parenchyma, 89–255 (170) long by 137–230 (180), ejaculatory duct and uterus join at base of genital cone forming hermaphroditic duct. Uterus with large and numerous eggs 3–50 (26) that are 72–117 (95) long by 45–67 (56) wide. Ratio of BL to egg length is 1: 10–28 (1: 19). Genital atrium very deep, genital pore terminal. Excretory pore dorso-subterminal at the level of the copulatory bursa (see table 3).

Remarks

This species was originally described as *P. macrobursa* by Drago & Lunaschi (2011) from the great black hawk (*B. urubitinga*) from Argentina. The specimens collected in the present study are similar to those of the original description by Drago & Lunaschi (2011). For instance, a forebody tulip-shaped and vitelline follicles distributed in two lateral expansions and a large well-delimited copulatory bursa, with a well-delimited genital cone and deep genital atrium. However, the newly collected specimens from the great black hawk (type host) and common black hawk in
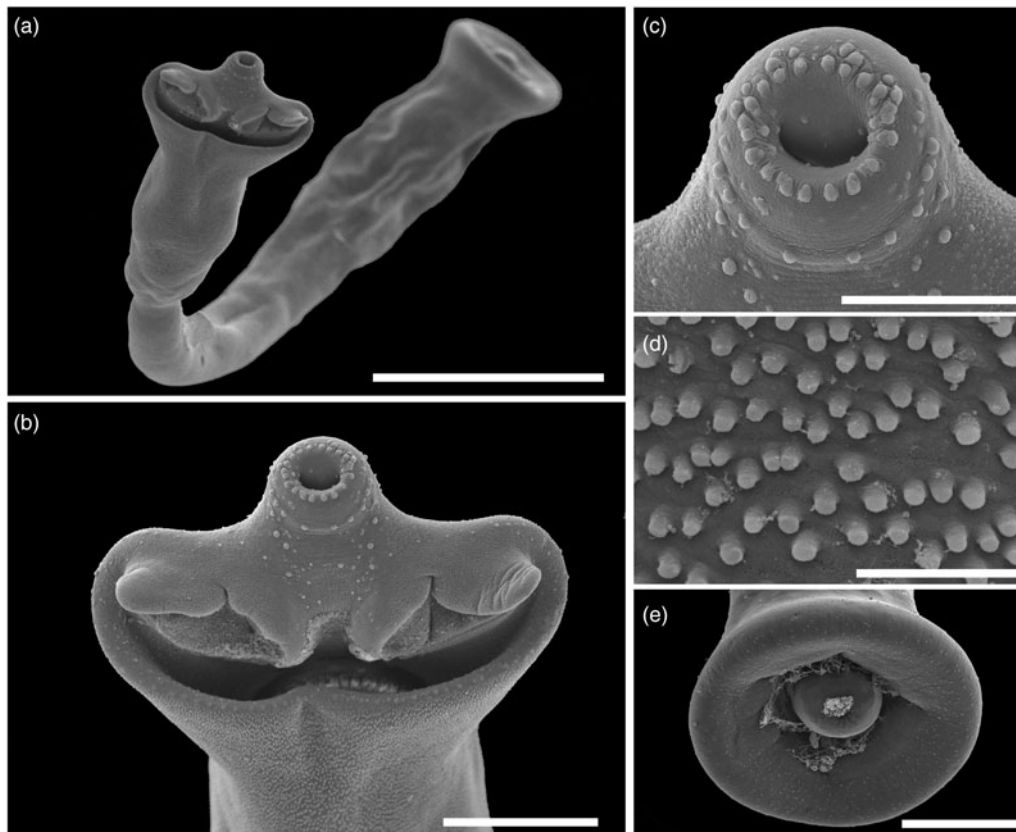
Fig. 6. Scanning electron micrographs of *Strigea magnirostris* n. sp. from *Rupornis magnirostris*. (a) Whole worm, ventral view; (b) forebody, ventral view showing pseudo-suckers; (c) oral sucker with papillae; (d) tegumental spines, ventral view of the forebody; (e) copulatory bursa showing cone genital. Scale bars: (a) 400 μm; (b, e) 100 μm; (c) 50 μm; (d) 10 μm.

three localities from the Neotropical region of Mexico showed some level of morphological intraspecific variation. For example, some of our specimens exhibit a neck in the hind-body, whereas other specimens do not. In addition, our specimens have tegumental spines that gradually diminish in size and number from the anterior to posterior region. However, apparently the presence or absence of spines could be related to the development of the worms. A similar pattern has been observed in specimens of two species, *S. falconis brasiliana* and *S. elliptica*, from the Neotropical region (Lunaschi & Drago, 2006, 2009). Finally, our specimens possess higher limits than original description for the following characteristics: hind-body length (609–2184 vs. 754–1451); ovary length (52–188 vs. 69–131); anterior testes width (132–449 vs. 188–262); and posterior testes width (155–474 vs. 193–304) (see table 3).

Discussion

The taxonomic history and species composition of the family Strigeidae have been complex and unsettled. Recent molecular evidence suggests that the family is paraphyletic. However, the genera *Apharyngostrigea*, *Parastrigea* and *Strigea* share a common ancestor (Blasco-Costa *et al.*, 2016; Blasco-Costa & Locke, 2017; Hernández-Mena *et al.*, 2017; Locke *et al.*, 2021; López-Jiménez *et al.*, 2022). The genetic library of some strigeid species of the genera *Apharyngostrigea*, *Parastrigea* and *Strigea* has recently increased and provides a large opportunity to clarify the taxonomy and species composition of these three genera

(Blasco-Costa *et al.*, 2016; Hernández-Mena *et al.*, 2017; Locke *et al.*, 2021; López-Jiménez *et al.*, 2022). In the current study, we combined morphological and molecular characteristics to describe a new species, *S. magnirostris* n. sp. that represents the first species in the Neotropical region of Mexico and the 22nd in the Americas. Morphologically, the new species is distinguished from other congeneric species from the Americas by having an oral sucker with several papillae around it, well-developed pseudosuckers, a tegument covered with tiny spines, a larger cone genital and a larger copulatory bursa. In addition, the phylogenetic trees established with three molecular markers supported that the isolates identified morphologically as *P. macrobursa* from *B. urubitinga* (type host) and *B. anthracinus* collected from three localities in Mexico are not closely related to other members of the genus *Parastrigea* because they were nested inside *Strigea*. Therefore, we transferred it to *Strigea* to form *S. macrobursa* n. comb., expanding its geographical distribution from Mexico to Argentina (Drago & Lunaschi, 2011), representing the first record in Mexico. Interestingly, our phylogenies established that *S. magnirostris* n. sp., *S. macrobursa* n. comb. and *Strigea* sp. were associated with accipitriform and falconiform birds from the Neotropical region on a clade, suggesting that at least two clades could be formed, one represented by the 22 described species from the Neotropical region and the second represented by the six species from the Nearctic region in the Americas. However, this hypothesis should be tested with more species from other biogeographical regions and primarily adult specimens because the sequences from the LSU available in

Table 2. Comparative measurements of *Strigea magnirostris* n. sp. and related species.

	Strigea magnirostris n. sp.	*Strigea arcuata*	*Strigea microbursa*		*Strigea elegans*	*Strigea magniova*	*Strigea falconis brasiliana*	
Source	Present study	Dubois (1988)	Pearson & Dubois (1985)	Lunaschi & Drago, (2009)	Chandler & Rausch (1947)	Dubois (1988)	Dubois (1968)	Lunaschi & Drago (2006)
Locality	Mexico	Paraguay	Indonesia	Argentina	United States	Paraguay	Brazil, Cuba	Argentina
Host	*Rupornis magnirostris Accipiter cooperii*	*Accipiter erythronemius Parabuteo unicinctus*	*Spilornis cheela*	*Buteogallus meridionalis*	*Bubo virginianus*	*R. magnirostris*	Accipitridae Falconidae	*R. magnirostris*
Body length	3030–4437	3700	1400–3600	1266–3021	1550–2450	1320	up to 2500	1305–1392
Forebody (Fo)	562–872 × 400–690	900 × 600	420–600 × 230–300	832–1083 × 328–551	560–1050 × 420–560	320–340 × 230–240	380–590 × 420–700	319–415 × 314–367
Hind-body (Hi)	2440–3591 × 352–632	2800 × 180	900–1200 × 170–220	1083–2102 × 232–435	980–1800 × 320–500	850–1000 × 160–260	1110–1830 × 340–580	890–1073 × 362–435
Pseudo-suckers	118–248 × 64–125	105 × 80	85–95 × 90–95	–		–	–	–
Oral sucker	72–109 × 80–115	110 × 105	68–117 × 70–127	69–107 × 74–117	110 × 130	48–68 × 48–57	100–125 × 85–115	76 × 55
Ventral sucker	142–240 × 119–188	200 × 135	65–122 × 73–138	107–143 × 116–143	198 × 220	55–63 × 70–78	160–235 × 140–200	152–162 × 71–105
Proteolytic gland	157–225 × 66–119	185 × 115	–	143–193 × 126–152	150 × 180	–	105–130 × 120–190	–
Pharynx	65–103 × 64–90	95 × 90	57–132 × 52–150	62–83 × 52–64	–	30 × 28	73–95 × 70–95	74 × 48
Ovary	139–190 × 126–214	140 × 170	55–140 × 80–106	105–138 × 88–217	150 × 165	52–65 × 60–80	110–200 × 175–300	59–68 × 101–107
Anterior testis	272–476 × 261–496	255 × 185	110–150 × 130–190	143–280 × 131–343	400 × 425	120–160 × 130–195	235–360 × 235–410	169–227 × 174–190
Posterior testis	300–497 × 280–512	340 × 260	110–190 × 150–190	179–241 × 157–314	400 × 430	130–150 × 140–220	275–370 × 235–420	197–217 × 179–241
Copulatory bursa	247–531 × 468–784	–	–	104–420 × 102–381	350 diameter	–	–	183–241 × 215–226
Genital cone	193–361 × 296–637	125 × 145	100–180 × 80–140	–	–	95–115 × 98–105	240–350 × 220–310	128–167 × 129–143
n eggs	8–50	32[a]	1–3	1–3	1–8	3–7	1–3	3–5
Eggs	71–105 × 40–65	84–96 × 55–63	100–105 × 50–57	83–129 × 52–98	115–220 × 65–73	105–115 × 52–60	67–91 × 42–55	82–88 × 48–52
Ratio Hi/Fo length	3.2–5.3	3.1[a]	2.0–2.1[a]	1.2–2.3	1.4–2.3	2.6–2.9[a]	1.8–3.6	2.1–3.4
Ratio Vs/Os	1.3–1.8	1.2[a]	1[a]	1.2–1.5[a]	1.6[a]	1.3–1.4[a]	1.6–1.7[b]	1.9
Ratio Ph/Os	0.7–0.9	0.8[a]	0.7–1.1[a]	0.7–1	–	0.4–0.5[a]	0.8[b]	0.9[b]
Ratio Hi/Gc	9.2–15.1	22.4[a]	6.6–9[a]	–	–	8.6–8.9[a]	4.6–5.2[b]	6.4–7[b]
Ratio Gc/E	1.9–3.8	1.4	1–1.7[a]	–	–	0.9–1[a]	3–5[b]	1.5–2[b]

Gc/eggs, genital cone length/egg length; Hi/Fo, hind-body length/forebody length; Hi/Gc, hind-body length/genital cone length; Ph/Os, pharynx width/oral sucker width; Vs/Os, suckers width ratio.
[a]Calculated from original descriptions.
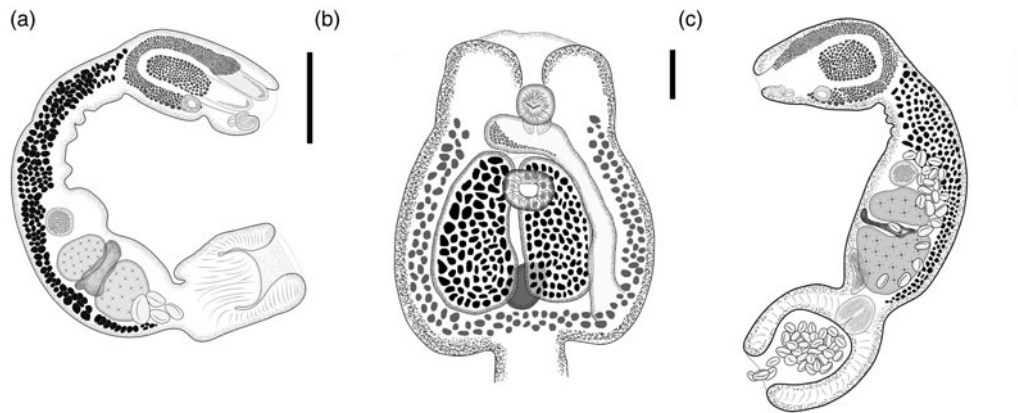[b]Calculated from original descriptions by Drago *et al.* (2014).

Fig. 7. Adult of *Strigea macrobursa* n. comb. from *Buteogallus urubitinga*. (a) Whole worm; (b) forebody, ventral view; (c) whole worm. Scale bars: (a) 300 μm; (b) 100 μm; (c) 250 μm.

GenBank are from larval forms of *Strigea* spp. (KT362372, MT075841 and MK585230) (Patrelle *et al.*, 2015; Svinin *et al.*, 2020).

Heneberg *et al.* (2018) performed one of the most comprehensive taxonomic reviews of strigeids that included samples of the genera *Strigea, Parastrigea, Apharyngostrigea, Cotylurus* Szidat, 1928 and *Apatemon* Szidat, 1928 from Central Europe. These authors sequenced the small subunit and the ITS2 from nuclear ribosomal DNA and the second region of the barcode from *cox 1* and nicotinamide adenine dinucleotide dehydrogenase subunit 1 from mitochondrial DNA. However, these authors could not compare their sequences with other sequences of strigeids previously analysed by Hernández-Mena *et al.* (2014, 2017) and Blasco-Costa *et al.* (2016) because these authors sequenced the ITS (ITS1-5.8S rDNA- ITS2), the domains D1–D3 of the LSU from nuclear DNA and the *cox 1* barcode, the first region from
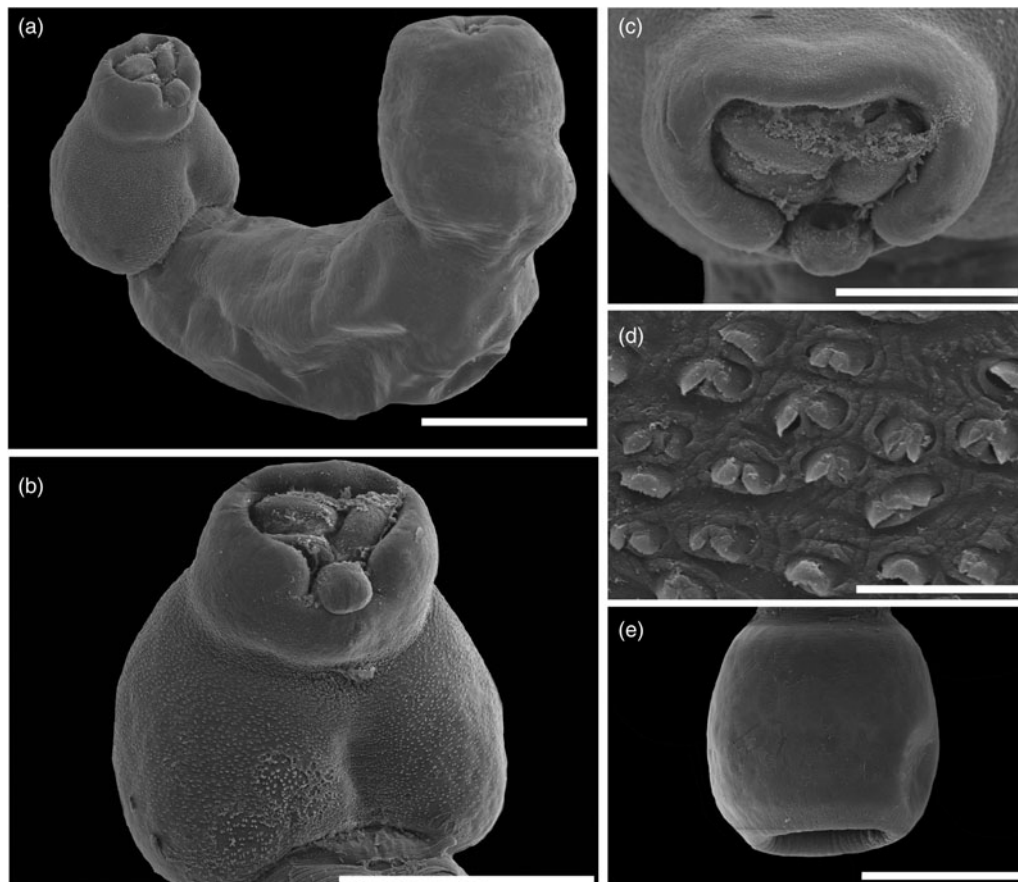

Fig. 8. Scanning electron micrographs of *Strigea macrobursa* n. comb. from *Buteogallus urubitinga*. (a) Whole worm, ventral view; (b) forebody, ventral view; (c) oral sucker; (d) tegumental spines; (e) copulatory bursa. Scale bars: (a) 500 μm; (b, e) 400 μm; (c) 200 μm; (d) 10 μm.

Table 3. Comparative measurements of adults *Strigea macrobursa* Drago & Lunaschi, 2011 recorded in the Americas.

	Strigea macrobursa n. comb.	*Strigea macrobursa* (syn. *Parastrigea macrobursa*)
Source	Present study	Drago & Lunaschi (2011)
Locality	Mexico	Argentina
Host	*Buteogallus urubitinga* *Buteogallus anthracinus*	*B. urubitinga*
Body length	957–2886	1189–2117
Forebody (Fo)	344–775 × 238–562	435–783 × 348–638
Hind-body (Hi)	609–2184 × 256–759	754–1451 × 391–658
Neck	130–576 × 94–291	–
Oral sucker	64–95 × 57–86	76–87 × 64–99
Ventral sucker	74–106 × 55–98	82–107 × 60–150
Proteolytic gland	75 × 53	64–83 × 60–76
Pharynx	34–66 × 31–59	44–60 × 39–60
Ovary	52–188 × 72–193	69–131 × 109–190
Anterior testis	95–281 × 132–449	97–155 × 188–262
Posterior testis	137–392 × 155–474	102–213 × 193–304
Copulatory bursa	163–681 × 246–616	290–648 × 280–532
Genital cone	89–255 × 137–230	117–179 × 107–176
n eggs	3–50	3–45
Eggs	72–117 × 45–67	92–143 × 57–77
Ratio Hi/Fo	1.5–3.1	1.7–3.1
Ratio BL/Fo	2.5–4.1	2.7–4.1
Ratio BL/E	10–28	10–20

BL/Fo, body length/forebody length; BL/E, body length/eggs length; Hi/Fo, hind-body length/forebody length.

the mitochondrial DNA. These three molecular markers have proven very useful for delineating species and inferring phylogenetic relationships at the genus level within Strigeidae. Herein, we compared the sequences of Heneberg *et al.* (2018) with other sequences available in GenBank and with the newly generated sequences. We generated two new alignments. The first includes 60 sequences of ITS2 with 320 bp, representing 30% (1042 bp) of our original dataset that contains ITS1-5.8S rDNA-ITS2. Our phylogenetic trees established with ITS2 were similar to the tree inferred by Heneberg *et al.* (2018), including the polyphyly of *Strigea*, with weak bootstrap support and posterior probabilities. In addition, the isolates of the new species *S. magnirostris* n. sp. plus *S. macrobursa* formed a clade together with *S. falconis* (MF628087) (see online supplementary fig. S1). The second alignment contained 46 sequences of *cox 1* (including the newly generated sequences in the current study) with 297 bp of the second region of the barcode. The phylogenetic trees placed all the species of *Strigea*, including the new species *S. magnirostris* n. sp. and *S. macrobursa* in a clade. However, the species *Parastrigea flexilis* Dubois, 1934 (MF628065) was nested inside *Strigea*, suggesting that *P. flexilis* should be transferred to *Strigea* (see online supplementary fig. S2). To clarify the taxonomy of the genera *Strigea*, *Parastrigea* and *Apharyngostrigea*, it is necessary to review the taxonomy of the species that share diagnostic characteristics among the three genera. For instance, *Parastrigea* is characterized by the distribution of vitellaria (two symmetrical masses on the forebody), which are present in *S. falconis*, *S. strigis* (Schrank,

1788) Abildgaard, 1790, *S. robusta* (Szidat, 1928) Heneberg and Sitko, 2018, *Apharyngostrigea brasiliana* Szidat, 1928 (Dubois, 1964) and *S. macrobursa* n. comb., (Dubois, 1968; Heneberg *et al.*, 2018; López-Jiménez *et al.*, 2022).

In summary our phylogenetic trees established with ITS and *cox 1* supported the monophyly of *Strigea*. However, the LSU tree showed that *Strigea* is paraphyly because two sequences of larval forms identified as *S. robusta* (MT075841 and MK585230) were nested inside other clades. In addition, the genetic divergence among the species of the first clade of *Strigea*, *S. magnirostris* n. sp., *S. macrobursa* and two isolates of *Strigea* sp. (MF398343 and KT362372), ranged from 0.6% to 1.6% and from 2.4% to 2.8% with respect to *S. robusta*. These high ranges of divergence are similar between *Strigea* and *Apharyngostrigea*, which ranged from 1.9% to 2.2% for the LSU marker. The phylogenetic analyses established with the LSU, in combination with the high genetic divergence, of the two sequences of larval forms identified as *S. robusta* suggests that they do not belong to *Strigea*. However, the ITS2 tree (see online supplementary file S1) placed five isolates of *S. robusta* of adult and larval forms (MF537205, MT075803, MK295777, MF537208 and MF628100) from Germany, Russia and Poland in a single clade that is a sister to the type species.

In the current study, we described a new species of *Strigea*, collected from the intestines of two hawk species (*R. magnirostris* and *A. coperii*) which is named *S. magnirostris* n. sp. In addition, the species *P. macrobursa* was transferred to *Strigea* to form

S. *macrobursa* n. comb. To clarify the taxonomy of the genus *Strigea*, it is necessary to sequence more species (including the type species, *S. strigis*) from diverse biogeographical regions with the ITS (ITS1-5.8S rDNA- ITS2), the D1–D3 domains from the LSU and the first region from the *cox 1* gene. Finally, the current integrative study represents a continuation of our effort in describing and understanding the biodiversity of strigeids in the Neotropical region.

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