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FISH PARASITOLOGY - ORIGINAL PAPER

Morphological and molecular data reveal a new species of *Lueheia* (Acanthocephala: Plagiorhynchidae) from *Turdus migratorius* (Turdidae) in central Mexico and its phylogenetic implications within the family

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Abstract

Members of the genus *Lueheia* Travassos, 1919, are endoparasites of birds, particularly passerines, throughout the Americas. Adults of *Lueheia* sp., (Plagiorhynchidae Golvan, 1960; Porrorchinae Golvan, 1956) were recovered from the intestine of the American robin (*Turdus migratorius phillipsi* Bangs) in Mexico City, and two other species of acanthocephalans identified as *Porrorchis nickoli,* (Plagiorhynchidae: Porrorchinae) Salgado-Maldonado and Cruz-Reyes, 2002 and *Centrorhynchus microcephalus* (Bravo-Hollis, 1947) Golvan, 1956 (Centrorhynchidae Van Cleave, 1916), were recovered from the Virginia opossum (*Didelphis virginiana* Allen) and groove-billed ani (*Crotophaga sulcirostris* Swainson), respectively in southeastern Mexico. Specimens of three species were sequenced at two molecular markers, the small subunit (SSU) and large subunit (LSU) of the nuclear rDNA and compared with other sequences available in GenBank. Maximum likelihood and Bayesian inference analyses of the combined (LSU + SSU) dataset and each individual dataset revealed that the specimens of *Lueheia* sp. formed an independent lineage, which is recognized herein as a new species, *Lueheia aztecae* n. sp., representing the fifth species of the genus in the Americas, and the second in the Nearctic region. The new species can be morphologically distinguished from the other five species in the genus by having a cylindrical proboscis, armed with 24–26 longitudinal rows with 9–10 hooks each. Phylogenetic inference performed with the combined dataset consisting of two genes (LSU + SSU) revealed that *Lueheia aztecae* n. sp. and *P. nickoli* belonging to subfamily Porrorchinae, formed two independent lineages, indicating that the subfamily is paraphyletic. *Porrorchis nickoli* and *C. microcephalus* formed a clade with other species of the genus *Centrorhynchus*, suggesting that *P. nickoli* should be transferred to genus *Centrorhynchus*, to form *C. nickoli* n. comb. In addition, we briefly discuss the ecological associations between the members of the families Plagiorhynchidae and Centrorhynchidae.

Keywords Acanthocephala · *Lueheia* · Central Mexico · Species description · Molecular markers · Phylogeny

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Introduction

Members of Plagiorhynchidae Golvan, 1960 are acanthocephalans that use birds, mammals, and rarely reptiles as definitive hosts and are distributed worldwide (Smales 2002; Amin 2013). Currently, the family includes three subfamilies: Porrorchinae Golvan, 1956, with six genera; Sphaerechinorhynchinae Golvan, 1956, represented by a single genus; and Plagiorhynchinae Meyer, 1931, with two genera (see Amin 2013). Golvan (1956) reviewed the taxonomy of Plagiorhynchidae and recognized the subfamily Porrorchinae with five genera. Currently, the subfamily includes approximately 37 species, classified into six genera:

Porrorchis Fukui, 1929; *Oligoterorhynchus* Monticelli, 1914; *Lueheia* Travassos, 1919; *Pseudolueheia* Schmidt and Kuntz 1967; *Owilfordia* Schmidt and Kuntz, 1967; and *Pseudogordiorhynchus* Golvan, 1957 (Amin 2013). The genus *Lueheia* is morphologically diagnosed by having a large cylindrical body with a small subglobular to semispherical proboscis armed with numerous strong hooks, a short and spineless neck, a double-walled cylindrical proboscis receptacle, a cerebral ganglion located in the middle of proboscis receptacle, and 4 to 10 long and slender lemnisci. The male possesses 2 spherical to oblique testes in tandem, placed in the anterior region of the body and long, tubular cement glands. The genital pore is terminal or subterminal, the eggs are oval or elongated, and the fertilization membrane exhibits polar prolongation (Smales 2013). The taxonomy of the genus *Lueheia* was evaluated recently by Smales (2013), recognizing five species: one distributed in North America (*Lueheia adlueheia*, (Werby 1938)), three in South America, (*Lueheia lueheia* Travassos, 1919; *Lueheia cajabambensis* Machado Filho and Nicanor Ibañez, 1967; *Lueheia inscripta* (Westrumb 1821)), and one in Asia (*Lueheia karachiensis* Khan, Bilqees and Muti-ur-Rahman, 2005).

The acanthocephalans in Mexico have received a great attention recently, and much effort has been made to incorporate morphological and molecular characters in order to describe and delineate the biodiversity of this group of parasites (see Monks 2001; García-Varela and Nadler 2005; Guillén-Hernández et al. 2008; García-Varela and Pérez-Ponce de León 2008; López-Caballero et al. 2015; Pinacho-Pinacho et al. 2018; García-Varela et al. 2019). In a checklist of acanthocephalans from Mexico, a total of 77 taxa were recognized (García-Prieto et al. 2010). In the current study, adult acanthocephalans were collected from the intestine of the groove-billed ani (*Crotophaga sulcirostris* Swainson), and were identified as *Centrorhynchus microcephalus* (Bravo-Hollis, 1947) Golvan, 1956. The acanthocephalans associated with the Virginia opossum (*Didelphis virginiana* Allen) were identified as *Porrorchis nickoli* Salgado-Maldonado and Cruz-Reyes, 2002, and finally, the acanthocephalans found in the American robin (*Turdus migratorius phillipsi* Bangs) corresponded to an undescribed species of the genus *Lueheia*.

The objectives of the present research were to (1) provide a morphological description of the new species (2) identify the systematic position of the genera *Lueheia* and *Porrorchis* belonging to subfamily Porrorchinae, and (3) reconstruct the phylogenetic relationships among members of the families, Centrorhynchidae and Plagiorhynchidae by using sequences of the near-complete small (SSU) and large (LSU) subunit of the nuclear rDNA. 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 acanthocephalans.

Material and methods

Specimens collecting, DNA isolation, and morphological analyses

During a helminthological survey in central and southeastern Mexico, acanthocephalans were collected from intestine of the groove-billed ani (*C. sulcirostris*), in Tlacatalpan, Veracruz (18° 36′ 0″ N; 95° 39′ 0″ W), the Virginia opossum (*D. virginiana*) in Los Tuxtlas, Veracruz (18° 34′ 21″ N; 95° 04′ 30″ W), and an American robin (*T. migratorius phillipsi*) found dead in Mexico City (see Table 1). The acanthocephalans recovered were immersed in distilled water for 10–12 h at 4 °C. Specimens were subsequently preserved in 100% ethanol and stored at 4 °C. For taxonomic identification, some specimens were stained with Mayer's paracarmine, dehydrated in graded ethanol series, cleared in methyl salicylate, and mounted as permanent slides using Canada balsam. All the specimens were examined using a bright-field Leica DM 1000 LED microscope (Leica, Wetzlar, Germany). Measurements were taken using the Leica Application Suite microscope software. The measurements are presented in micrometers (μm) unless otherwise stated; with the mean followed by the range in parenthesis. Drawings were made with the aid of a drawing tube. For scanning electron microscope (SEM) observations, some individuals were dehydrated through a graded series of ethyl alcohol, and then critical-point dried with carbon dioxide. These specimens were mounted on metal stubs with silver paste, coated with gold, and examined in a Hitachi Stereoscan model SU1510 (Hitachi High-Technologies, Tokyo, Japan) at 15 kV. The acanthocephalans recovered from groove-billed ani were identified as *Centrorhynchus microcephalus*, and the parasites from Virginia opossum were identified as *Porrorchis nickoli*. Finally, the specimens from the American robin were assigned to the genus *Lueheia*. All the specimens were deposited in the Colección Nacional de Helmintos (CNHE: *C.microcephalus* No. 7074; *P. nickoli* Nos. 9512, 9513), Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City. Definitive avian hosts were identified using the field guides of Howell and Webb (1995) and the American Ornithologists' Union (1998). The mammals were shot by local hunters or caught with tomahawk traps and then injected intraperitoneally with an overdose of sodium pentobarbital. The opossums were dissected within the following 4 h, and all organs were examined under a stereomicroscope (see Acosta-Virgen et al. 2015).

DNA extraction, PCR amplification, sequencing, and phylogenetic analyses

Specimens of each species were placed individually in tubes and digested overnight at 56 °C in a solution containing 10 mM Tris-HCl (pH 7.6), 20 mM NaCl, 100 mM Na2-EDTA (pH 8.0), 1% Sarkosyl, and 0.1 mg/ml proteinase K.

Table 1 Specimens analyzed in this study; host name and GenBank accession numbers of each molecular marker. Sequences in bold were generated in this study. Nd. not determined.

Species	Host	Genbank Access. SSU	Genbank Access. LSU	References
Plagiorhynchidae				
Plagiorhynchus (Plagiorhynchus) aznari	*Numenius americanus*	MN057693	MN057697	García-Varela et al. 2019
Plagiorhynchus (Plagiorhynchus) allisonae	*Transorchestia chiliensis*		KU922939	Lagrue et al. 2016
Plagiorhynchus (Prosthorhynchus) cylindraceus	*Porcilio saber*	AF001839	AY829102	García-Varela and Nadler 2005
Plagiorhynchus (Prosthorhynchus) transversus	*Sturnus vulgaris*	MN057694	MN057698	García-Varela et al. 2019
Lueheia aztecae n. sp.	*Turdus migratorious phillipsi*	**MT161620**	**MT161665**	**This study**
Porrorchis nickoli	*Didelphis virginiana*	**MT161621**	**MT161666**	**This study**
Centrorhynchidae				
Centrorhynchus aluconis	*Strix aluco*	MN057695	MN057699	García-Varela et al. 2019
Centrorhynchus globocaudatus	Nd	MN057696	MN057700	García-Varela et al. 2019
Centrorhynchus conspectus	Nd	U41399		Near et al. 1998
Centrorhynchus globirostris	*Centropus sinensis*		KM588207	Amin et al. 2015
Centrorhynchus microcephalus	*Crotophaga sulcirostris*	AF064813	**MT161664**	García-Varela et al. 2000 ; **This study**
Centrorhynchus nahuelhuapensis	*Strix rufipes*	MK411249	MK411250	Steinauer et al. 2019
Centrorhynchus sp.	*Falco peregrinus*	AY830155	AY829104	García-Varela and Nadler 2005
Polymorphidae				
Andracantha gravida	*Phalacrocorax auritus*	EU267802	EU267814	García-Varela et al. 2013
Arhythmorhynchus frassoni	*Eudocimus albus*	JX442165	JX442177	García-Varela et al. 2013
Bolbosoma turbinella	*Eschrichtius robustus*	JX442166	JX442178	García-Varela et al. 2013
Corynosoma enhydri	*Enhydra lutris*	AF001837	AY829107	Near et al. 1998; García-Varela and Nadler 2005
Ibirhynchus dimorpha	*Eudocimus albus*	GQ981436	GQ981437	García-Varela et al. 2011
Hexaglandula corynosoma	*Nyctanassa violacea*	EU267808	EU267817	García-Varela et al. 2009
Polymorphus trochus	*Fulica america*	JX442173	JX442185	García-Varela et al. 2013
Profilicollis altmani	*Enhydra lutris*	AF001838	AY829108	Near et al. 1998; García-Varela et al. 2013
Pseudocorynosoma constrictum	*Anas clypeata*	EU267800	EU267812	García-Varela et al. 2009
Southwellina hispida	*Tigrisoma mexicanum*	EU267807	EU267811	García-Varela et al. 2009
Outgroup				
Acanthocephaloides propinquus	*Gobius bucchichii*	AY830149	AY829100	García-Varela and Nadler 2005
Acanthocephalus lucii	*Perca fluviatilis*	AY830152	AY829101	García-Varela and Nadler 2005
Filisoma bucerium	*Kyphosus elegans*	AF064814	AY829110	Near et al. 1998; García-Varela and Nadler 2005
Pomphorhynchus bulbocolli	*Lepomis macrochirus*	AF001841	AY829096	Near et al. 1998; García-Varela and Nadler 2005
Echinorhynchus truttae	*Thymallus thymallus*	AY830156	AY829097	García-Varela and Nadler 2005
Koronacantha mexicana	*Pomadasys leuciscus*	AY830157	AY829095	García-Varela and Nadler 2005
Leptorhynchoides thecatus	*Lepomis cyanallus*	AF001840	AY829093	Near et al. 1998; García-Varela and Nadler 2005
Illiosentis sp.	Nd	AY830158	AY829092	García-Varela and Nadler 2005

Following digestion, DNA was extracted using DNAzol reagent (Molecular Research Center, Cincinnati, Ohio) according to the manufacturer's instructions. Two regions of nuclear ribosomal DNA (rDNA) were amplified using the polymerase chain reaction (PCR). Near-complete 18S rDNA (~ 1,800 bp) was amplified using 2 overlapping PCR fragments of 1,000 bp. The primers used for SSU amplicon 1 were forward 5′-AGAT TAAGCCATGCATGCGT-3′ and reverse 5′-AACT TTTCGTTCTTGATTAATG-3′; for amplicon 2, forward 5′-GCAGCGCGGTAATTCCAGCTC-3′ and reverse 5′-GCAG GTTCACCTACGGAAA-3′. Near-complete 28S rDNA (~ 2,900 bp) was amplified using 3 overlapping PCR fragments of 1200–1300 bp. Primers for LSU amplicon 1 were forward 5′-CAAGTACCGTGAGGGAAAGTTGC-3′ and reverse 5′-CAGCTATCCTGAGGGAAAC-3′; amplicon 2 were forward 5′-ACCCGAAAGATGGTGAACTATG-3′ and reverse 5′-CTTCTCCAACGTCAGTCTTCAA-3′; and for amplicon 3, forward 5′- CTAAGGAGTGTGTAACAACTCACC-3′ and reverse 5′-CTTCGCAATGATAGGAAGAGCC-3′ (García-Varela and Nadler 2005). The PCRs (25-μl final volume) consisted of 10 μM of each primer, 2.5 μl of 10× buffer, 2 mM $MgCl_2$, and 1 U of Taq DNA polymerase (Platinum Taq, Invitrogen Corporation, Carlsbad, California, USA). PCR cycling parameters for rDNA amplifications included denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C for 1 min, annealing at 50–58 °C (optimized for each rDNA amplification) for 1 min, and extension at 72 °C for 1 min, followed by a post-amplification incubation at 72 °C for 7 min. Sequencing reactions were performed with the primers mentioned above 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 5.1.5 (Codoncode Corporation, Dedham, Massachusetts). Sequences obtained in the current research for SSU and LSU of *C. microcephalus*, *P. nickoli*, and *Lueheia* sp. were aligned with other sequences downloaded from GenBank dataset (see Table 1). Sequences of each molecular marker were aligned separately using the software Clustal W (Thompson et al. 1997) after a combined alignment (LSU + SSU) was performed. A nucleotide substitution model was selected for each molecular marker and the combined dataset using jModelTest version 2.1.7 (Posada 2008) applying the Akaike criterion. The best nucleotide substitution models for each and the combined dataset were GTR + G + I. Phylogenetic trees were inferred through maximum likelihood (ML) with the program RAxML version 7.0.4 (Stamatakis 2006). A GTRGAMMAI substitution model was used, and 10,000 bootstrap replicates were run to assess nodal support. We also analyzed our data in a Bayesian framework using MrBayes 3.2.2 (Ronquist et al. 2012), with two Markov chain (MCMC) runs for 10 million generations, sampled every 1000 generations, a heating parameter value of 0.2 and burn-in of (25%). Trees were edited using FigTree version 1.4.0 (Rambaut 2012).

Results

Morphological description

Class Palaeacanthocephala Meyer, 1931.

Order Polymorphida Petrochenko, 1956.

Family Plagiorhynchidae Golvan,1960.

Subfamily Porrorchinae Golvan, 1956.

Lueheia aztecae n. sp. García-Varela and Andrade-Gómez; Figs. 1a–f

Description based on 14 specimens (seven males and seven females).

General: Porrorchinae with characters of the genus *Lueheia*. Living specimens of white color. Sexual dimorphism evident; females larger than males. Proboscis subglobular, armed with 24–26 longitudinal rows, with 8–10 hooks with simple roots per row; largest hooks located at the mid-proboscis (42–61 long). First hooks in row 29–34 long, last hooks in row 42–48 long, other hooks 38–52 long. Neck small and spineless, cone shaped. Proboscis receptacle double-walled, with an oval cephalic ganglion in the middle. Lemnisci 4–6, slender, of different lengths, inserted at the base of the neck. Genital pore subterminal in both sexes.

Male: (Based on seven mounted adult specimens and one analyzed by SEM.) Trunk 7.1 mm (4–10.5 mm) × 1.5 mm (1.0–2.3 mm); maximum width at hind-trunk level. Proboscis 430 (287–531) × 348 (291–520). Proboscis subglobular, armed with 24–26 longitudinal rows of hooks, with 8–10 hooks each row (Fig. 2a), largest hooks located at the mid-proboscis (42–61 long). First hooks in row 29–34 long, last two hooks in row 42–48 long, other hooks 38–52 long. Neck 228 (178–272) × 344 (244–433). Proboscis receptacle 1.13 mm (0.83–1.35 mm) × 287 (228–357). Lemnisci 1.9 mm (1.5–2.6 mm). Testes ovoid, in tandem, posterior to the proboscis receptacle. Anterior testis 1.18 mm (0.69–1.5 mm) × 501 (215–818). Posterior testis 1.09 mm (0.73–1.31 mm) × 469 (334–625). Cement glands, four tubular, 2.25 mm (1.6–3.1 mm) long. Säfftigen's pouch 729 (552–983) long. Copulatory bursa 519 (351–680) × 576 (364–700).

Female: (Based on seven mounted gravid specimens and one analyzed by SEM.) Trunk 12.4 mm (10.2–15.3 mm) × 2.0 (1.4–2.3 mm) (Fig. 1b). Proboscis 555 (505–628) × 365 (333–418). Proboscis subglobular, armed with 24–26 longitudinal rows of hooks, with 8–10 hooks each row. Largest hooks located at the mid-proboscis (43–48 long). First hooks in row 28–35 long, last two hooks in row 29–42 long, other hooks 32–53 long. Neck 260 (213–302) × 366 (343–400). Proboscis receptacle 1.3 mm (1.26–1.37 mm). Lemnisci of unequal lengths. Uterine bell with a thick body wall 539

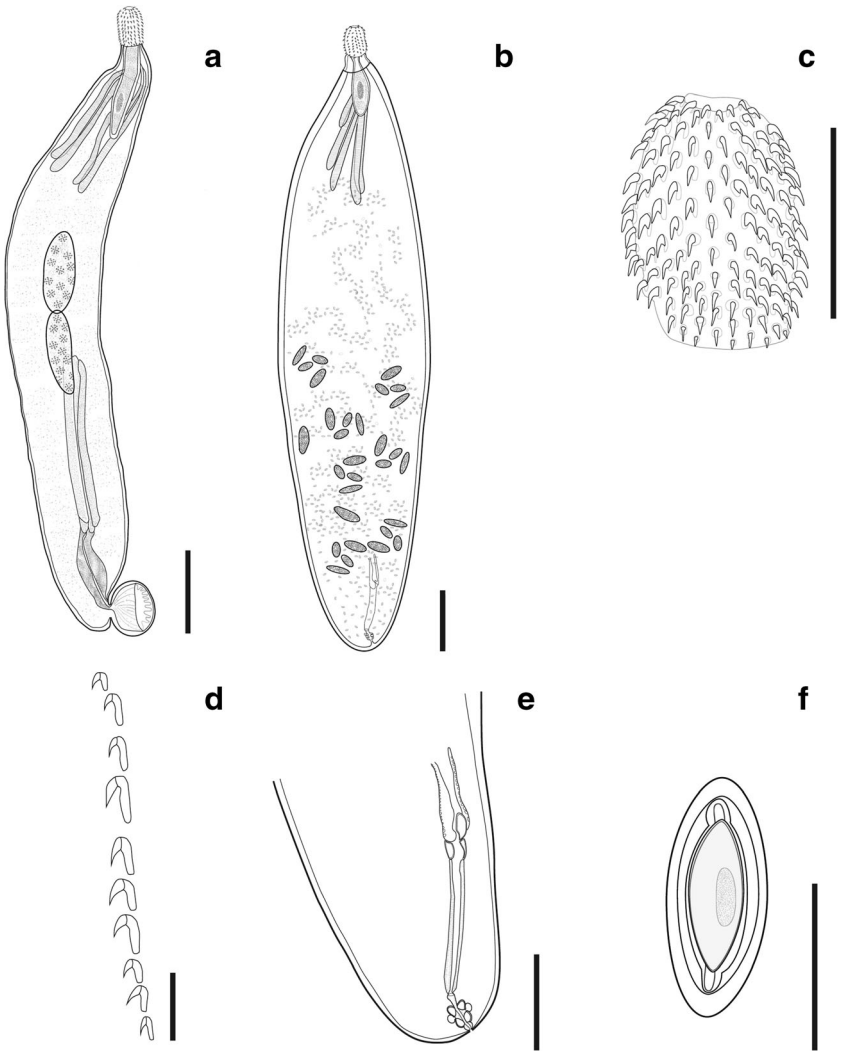

Fig. 1 *Lueheia aztecae* n. sp., from *Turdus migratorius phillipsi* **a** Adult male, whole worm (holotype), lateral view; **b** Adult female whole worm (allotype), lateral view; **c** Proboscis; **d** Hooks with roots; **e** Female reproductive system; **f** Egg
Scale bars = 1.0 mm (**a**, **b**); 400 μm (**c**, **d**); 500 μm (**e**); 20 μm (**f**)

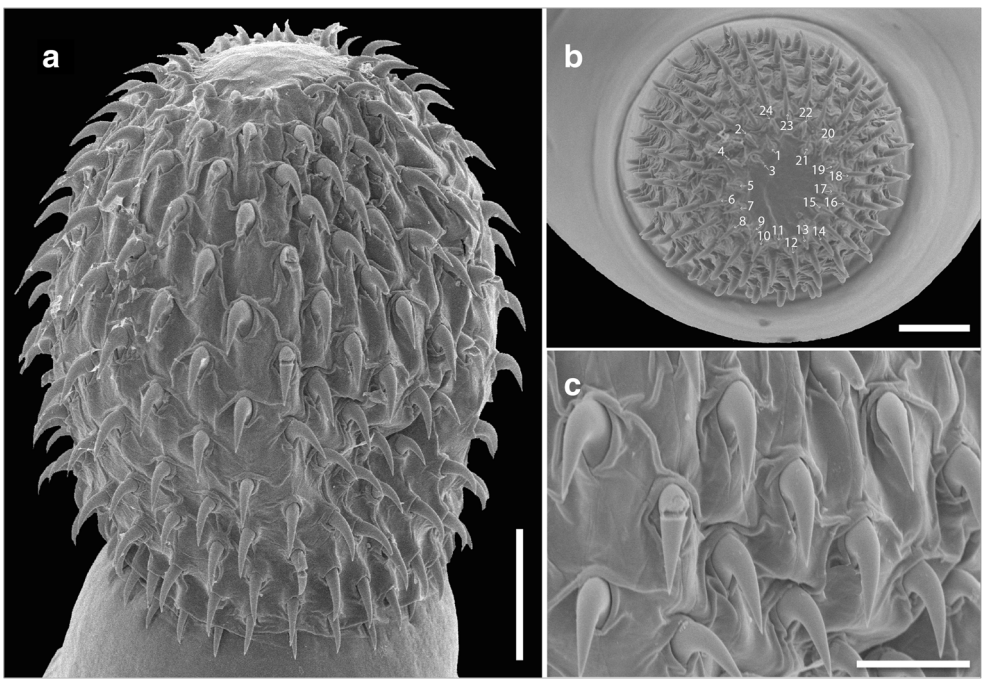

Fig. 2 Scanning electron micrographs of *Lueheia aztecae* n. sp., from *Turdus migratorius phillipsi*. **a** Proboscis adult male ventral view. **b** Proboscis adult male horizontal view. **c** Hooks. Scale bars = 300 μm (**a**, **b**); 50 μm (**c**). The hooks rows are numbered 1–24

(473–606) long. Uterus long 870 (723–964); vagina complex with two sphincter muscles 255 (220–276) long; gonopore subterminal (Fig. 3c). Mature eggs, containing a fully developed acanthor, fusiform, with polar prolongations in the middle fertilization membrane 67 (60–72) × 24 (22–27) (Fig. 3d).

Taxonomic summary

Host: *Turdus migratorius phillipsi* (Bangs) (American robin), Passeriformes.

Site of infection: Intestine.

Locality: Border between Tlalpan and Xochimilco municipalities, Mexico City, Mexico (19°16′ 34.63″ N 99° 08′ 30.46″ W).

Type-material: Holotype; (CNHE: 11247), allotype (CNHE: 11248); Paratype (CNHE: 11249).

Representative DNA sequences: MT161620 (SSU), MT161665 (LSU).

Etymology: The specific epithet refers to the Azteca, a Mesoamerican civilization who dominated Central Mexico during the early thirteenth century and founders of Tenochtitlán (where Mexico City is currently located).

ZooBank registration: The Life Science Identifier (LSID) of the article is urn:lsid: zoobank.org:pub: CE6ABC27-DF7D-4DE6-9815-BCF52538978B. The LSID for the new name *Lueheia aztecae* n. sp. is urn:lsid:zoobank.org:act: 1334EDE1-8E00-40BB-B25E-2CF833C66B03.

Taxonomic remarks

The new species belongs to the genus *Lueheia* in having a subglobular proboscis, small and spineless neck, double-walled proboscis receptacle with an oval cephalic ganglion in the middle, 4–6 slender lemnisci of different lengths inserted at the base of the neck and eggs with polar prolongations in the middle fertilization membrane. *Lueheia aztecae* n. sp. can be morphologically distinguished by its cylindrical proboscis armed with 24–26 longitudinal rows with 9–10 hooks each (vs 20–22 longitudinal rows with 8–9 hooks each in *L. lueheia* (Table 2), 28–30 longitudinal rows with 9–12 hooks each in *L. inscripta*, 28 longitudinal rows with 9–10 hooks each in *L. adlueheia*, and 20–40 longitudinal rows with 10–14 hooks each in *L. karachiensis*; Table 2). The new species also differs from *L. adlueheia* from North America by having larger eggs (60–72 × 22–27 vs 36–41 × 12–15; see Table 2).

Phylogenetic analyses

The combined dataset including two genes (LSU + SSU) consisted of 30 terminals with 4888 sites (including gaps), with GTR + G + I as the best model. The phylogenetic tree inferred with ML and Bayesian inference (BI) recovered Polymorphida as a monophyletic group with strong bootstrap support (100%) and Bayesian posterior probability (1.0). The phylogenetics trees showed three main clades (Fig. 3a). The first clade contained 10 genera of Polymorphidae, (*Andracantha* Schmidt, 1975; *Corynosoma* Lühe, 1904; *Bolbosoma* Porta, 1908; *Southwellina* Witenberg, 1932; *Hexaglandula* Petrochenko, 1950; *Ibirhynchus* García-Varela, Pérez-Ponce de León, Aznar and Nadler, 2011; *Arhythmorhynchus* Lühe, 1911; *Profilicollis* Meyer, 1931; *Pseudocorynosoma* Aznar, Pérez-Ponce de León and Raga 2006; and *Polymorphus* Lühe, 191, with strong bootstrap support (100%) and

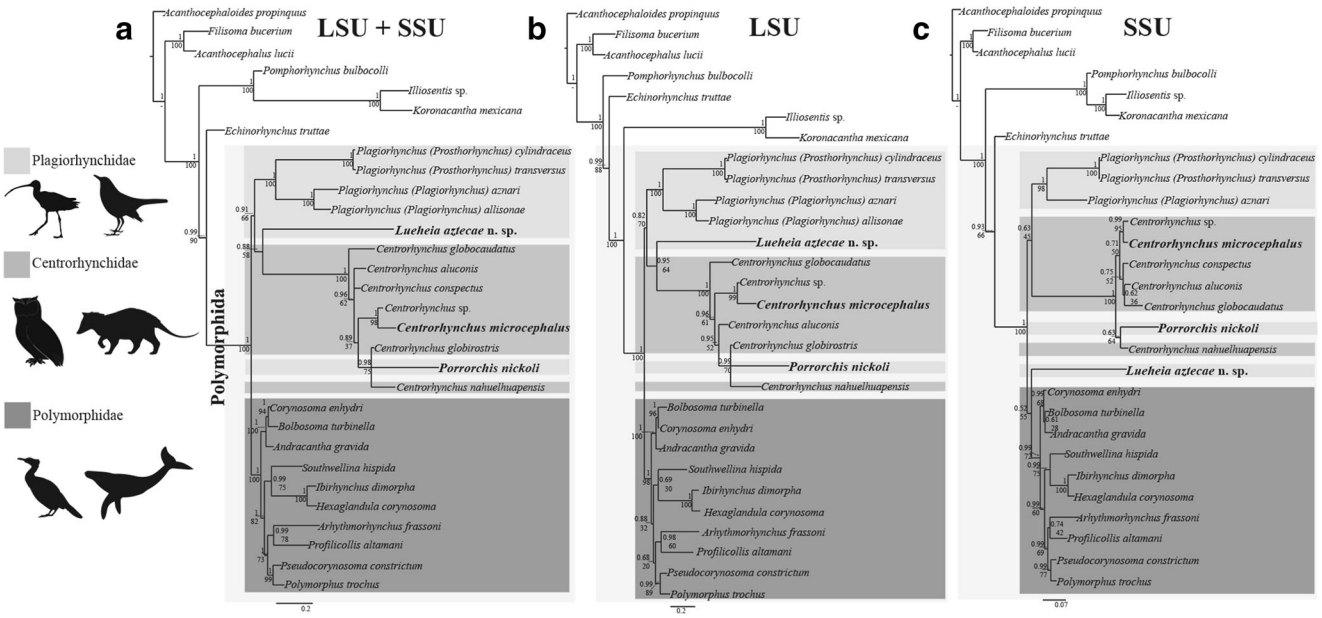

Fig. 3 Phylogenetic trees using maximum likelihood and consensus Bayesian Inference for the combined (LSU + SSU) data set (**a**), LSU data set (**b**), and SSU data set (**c**) Numbers near internal nodes show ML bootstrap percentage (BP) values and Bayesian posterior probabilities (BPP). Scale bars represent the branch length

Table 2 Comparative metrical data for male and female of adult species of *Lueheia*. Measurements in micrometers, unless otherwise indicated.

Species	*Lueheia lueheia*	*Lueheia inscripta*	*Lueheia inscripta*	*Lueheia adlueheia*	*Lueheia cajabambensis*	*Lueheia karachiensis*	*Lueheia aztecae* n. sp.
Reference	Travassos, 1919	Travassos, 1926	Smales, 2013	Werby,1938	Machado Filho and Nicanor Ibañez, 1967	Khan, Bilqees and Muti-Ur-Rahman, 2005	**This study**
Mele							
Trunk length (mm).	7–10 X 1.2–1.8	8 X1.2	8–11	3.5–9.2 X 0.8–1.7	14 X 2.9	13.6–18.4 X 0.7–1.04	4–10.5 X 1.0–2.3
Proboscis length	430–520 X 380–460	520–620 X 410–430	380–600 X 310–430	385–490 X 280–385	514 X 547		287–531 X 291–520
No. longitudinal rows	20–22	28–30	28–30	28		20–40	24–26
No. hooks per row	8–9	9–10	9–12	9–10		10–14	8–10
Neck			348 X 348	126–210			178–272 X 244–433
Proboscis receptacle	780–1000	1600 X 340	1375–1615 X 320–640	740–1190	1494 X 298	1000–1072 X 400–720	834–1355 X 288–357
Lemnicsi, number	6	4–6	6	3–5	4	4	4–6
Lemnicsi lenght	1900–2800		2250–4760	840–1820	4150	2400–3400	984–2655
Anterior testis	700	1000 X 400–500	1000–1205 X 400–630	231–1274 X 120–177	1743 X 1274	840–2480 X 400–1000	699–1571 X 215–818
Posterior testis			1000–1200 X 375–500	280–1267 X 154–776	1992 X 1162	840–2000 X 280–1040	736–1318 X 334–625
Cement glands		1900	1900–2550	700–3430	4834		1622–3178
Copulatory bursa everted			600–700			1330–1600 X 330–640	351–680 X364–700
Female							
Trunk length (mm)	10–12	9–15	7–15	11.3–15 X 1.8–2.5			10.2–15.3 X 1.4–2.3
Proboscis length			425–530 X 402–420	399–602 X 315–525			505–628 X 333–418
Reproductive system length		1900	1070–1140				1549–1673
Eggs size	78–80 X 28–31	63–78	59.5–78 X 23–28	36–41 X 12–15			60–72 X 22–27
Hosts	Thamnophilidae Furnariidae	Turdidae	Turdidae	*Turdus migratorius*	*Turdus fuscater*	*Accipiter badius cenchroides*	*Turdus migratorius phillipsi*
Locality	Brazil	Brazil	Paraguay	USA	Peru	Pakistan	México

Bayesian posterior probability (1.0). The second clade had strong bootstrap support (100%) and a high Bayesian posterior probability (1.0) and included *Plagiorhynchus* Luhë 1911 with two subgenera (*Plagiorhynchus* Luhë, 1911 and *Prosthorhynchus* Kostylew, 1915), which are members of Plagiorhynchidae. The third clade included six species of Centrorhynchidae (*Centrorhynchus globocaudatus* (Zeder, 1800); *Centrorhynchus aluconis* (Müller, 1780); *Centrorhynchus conspectus* Van Cleave and Pratt, 1940; *Centrorhynchus microcephalus; Centrorhynchus globirostris* Amin, Hechmann Wilson Keele and Khan, 2015*; Centrorhynchus nahuelhuapensis* Steinauer, Flores and Rauque 2020; and *Centrorhynchus* sp.) plus *Porrorchis nickoli* (Plagiorhynchidae), with a high bootstrap support (100%) and a high Bayesian posterior probability (1.0). The phylogenetic position of *Lueheia aztecae* n. sp., as sister to *Centrorhynchus* and *Porrorchis* was supported, with only 58% bootstrap in maximum likelihood analysis and an 0.88 posterior probability in the Bayesian analysis. The LSU dataset consisted of 29 terminals and 3093 sites (including gaps), with GTR + G + I as the best model. The tree topologies inferred with LSU dataset from rDNA (Fig. 3b) had the same branching order among the families from Polymorphida as the ML and Bayesians trees inferred with the combined (LSU + SSU) dataset, with minor differences regarding the position of *C. aluconis*. The SSU dataset consisted of 28 terminals with 1795 sites (including gaps), with GTR + G + I as the best model. The tree topologies inferred with SSU dataset were not the same because their taxon sampling differed. Nevertheless, they were similar to the topologies inferred with the combined (LSU + SSU) dataset, i.e., the SSU tree also recognized the three families from Polymorphida (Fig. 3c). One of the major differences between the trees inferred with SSU and combined (LSU + SSU) datasets was the systematic position of *Lueheia aztecae* n. sp. which was placed sister taxa to Polymorphidae with a weak bootstrap support (55%) and Bayesian posterior probability (0.52). The other taxa sampled in this study, namely, *C. microcephalus* and *P. nickoli* were consistently placed within the genus *Centrorhynchus* Lühe 1911, in all phylogenetic analyses (see Figs. 3a–c).

Discussion

The taxonomic history and species composition of *Lueheia* are complex and problematic, due in part to the incomplete morphological descriptions of the adult worms. Smales (2013) reviewed the taxonomy and recognized five species, *L. lueheia*, *L. adlueheia*, *L. cajabambensis*, *L. inscripta*, and *L. karachiensis*. However, the same author considered *L. karachiensis* to potentially belong to genus *Centrorhynchus* due to the morphological and ecological similarities between the two genera. Additionally, *L. karachiensis* was described from Asia, whereas the other congeneric species are distributed in the Americas. Given the current evidences, we agree with Smales (2013), that *L. karachiensis* may not belong to genus *Lueheia*; however, this hypothesis should be tested in a formal phylogenetic analysis. Therefore, the species described herein, *Lueheia aztecae* n. sp. represents the fifth species of the genus distributed in the Americas, and the second species of this genus recorded in the Nearctic region. Morphologically, *Lueheia aztecae* n. sp. is distinguished from *L. adlueheia*, which is also distributed in the Nearctic region by having a cylindrical proboscis, armed with 24–26 longitudinal rows with 9–10 hooks (vs 28 longitudinal rows with 9–10 hooks in *L. adlueheia*). The species *L. adlueheia* was described from the western robin (*Turdus migratorius propinquus* Ridgway), in Seattle, Washington, USA (Werby 1938). This bird occurs from British Columbia to southwestern Mexico (Kemper and Taylor 1981; American Ornithologists' Union 1998). *Lueheia aztecae* n. sp. was found in a subspecies of the American robin (*Turdus migratorius phillipsi*). This passerine bird has a distribution from central to southern Mexico (see American Ornithologists' Union 1998).

The phylogenetic analyses obtained with the combined dataset of two genes (LSU + SSU) revealed that *Lueheia aztecae* n. sp. and *P. nickoli*, both members of the subfamily Porrorchinae Golvan, 1956 were not grouped together. For instance, *Lueheia aztecae* n. sp. was placed in an independent lineage (Fig. 3a), whereas *P. nickoli* was nested within the genus *Centrorhynchus* (Fig. 3a). *Porrorchis nickoli* was originally described from the gray four-eyed opossum, (*Philander opossum* Linnaeus) in southeastern Mexico (Salgado-Maldonado and Cruz-Reyes 2002). This is the only recorded species of the genus in the Americas, whereas the other 21 species of *Porrorchis* Fukui, 1929 are distributed in Eurasia, Madagascar, and Australia, mostly in association with birds and rarely, with terrestrial mammals (Lisitsyna et al. 2012). Morphologically, our specimens of *P. nickoli* possessed a cylindrical trunk, a subglobular proboscis armed with 22–24 rows of 7–8 hooks each (Figs. 4a–c), a cylindrical and double-walled proboscis receptacle, a cerebral ganglion located at the mid-receptacle, lemnisci of equal size, oval testes arranged in tandem, four elongate cement glands, and elliptical eggs without polar prolongation. These characteristics of our newly collected material clearly correspond with those reported in the original description by Salgado-Maldonado and Cruz-Reyes (2002). In addition, Amin et al. (2015) reviewed and emended the genus *Centrorhynchus* to include species with a globular proboscis, a character also present in *P. nickoli*. Based on the current morphological evidence plus the phylogenetic position of *P. nickoli* in our analyses, *P. nickoli* should most likely be transferred to *Centrorhynchus* to form *Centrorhynchus nickoli* n. comb. To date, seven species of *Centrorhynchus* have been described from North America, primarily in association with birds of prey:

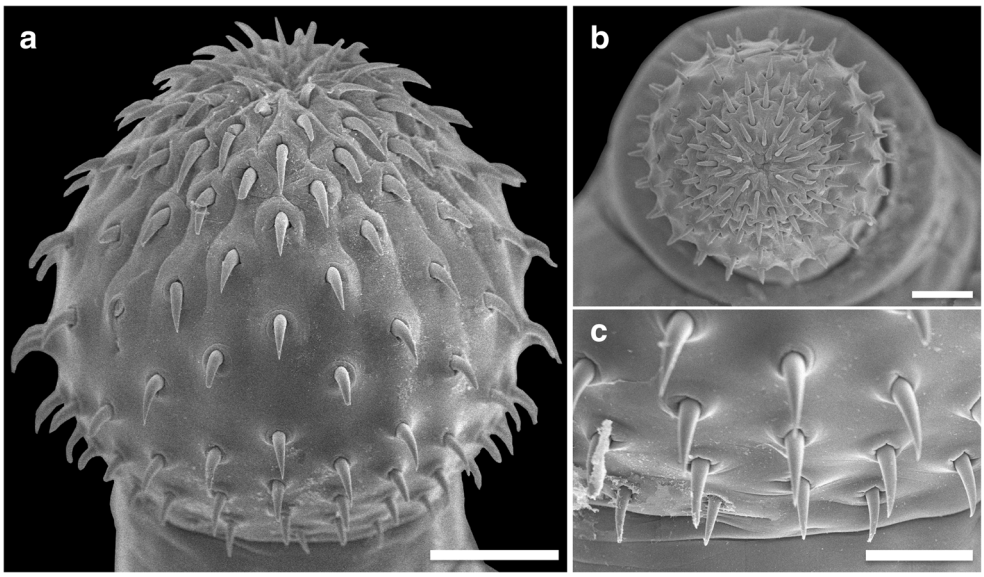

Fig. 4 Scanning electron micrographs of *Porrorchis nickoli* from *Didelphis virginiana*. **a** Proboscis adult male ventral view. **b** Proboscis adult male horizontal view. **c** Hooks. Scale bars = 300 μm (**a**, **b**); 50 μm (**c**)

Centrorhynchus spinosus (Kaiser, 1893) Van Cleave, 1924; *Centrorhynchus californicus* Millzner, 1924; *Centrorhynchus conspectus*, *C. microcephalus*; *Centrorhynchus wardae* Holloway, 1958 (junior synonym of *C. conspectus*); *Centrorhynchus kuntzi* Schmidt and Neiland, 1966, and *Centrorhynchus robustus* Richardson and Nickol, 1995 (Richardson and Nickol 1995; Amin 2013). In the current study, specimens of *C. microcephalus* were collected from groove-billed ani (*C. sulcirostris*). Morphologically, *C. microcephalus* is characterized by having a constricted, cylindrical proboscis armed with 30–33 longitudinal rows of 16–17 hooks each (Figs. 5a–c). The proboscis receptacle is double walled, the lemnisci are equal in size, and the cerebral ganglion is located at the mid-receptacle, two elliptoid testes, and four elongate cement glands. The eggs display polar prolongations (Bravo-Hollis, 1947; Richardson and Nickol 1995;

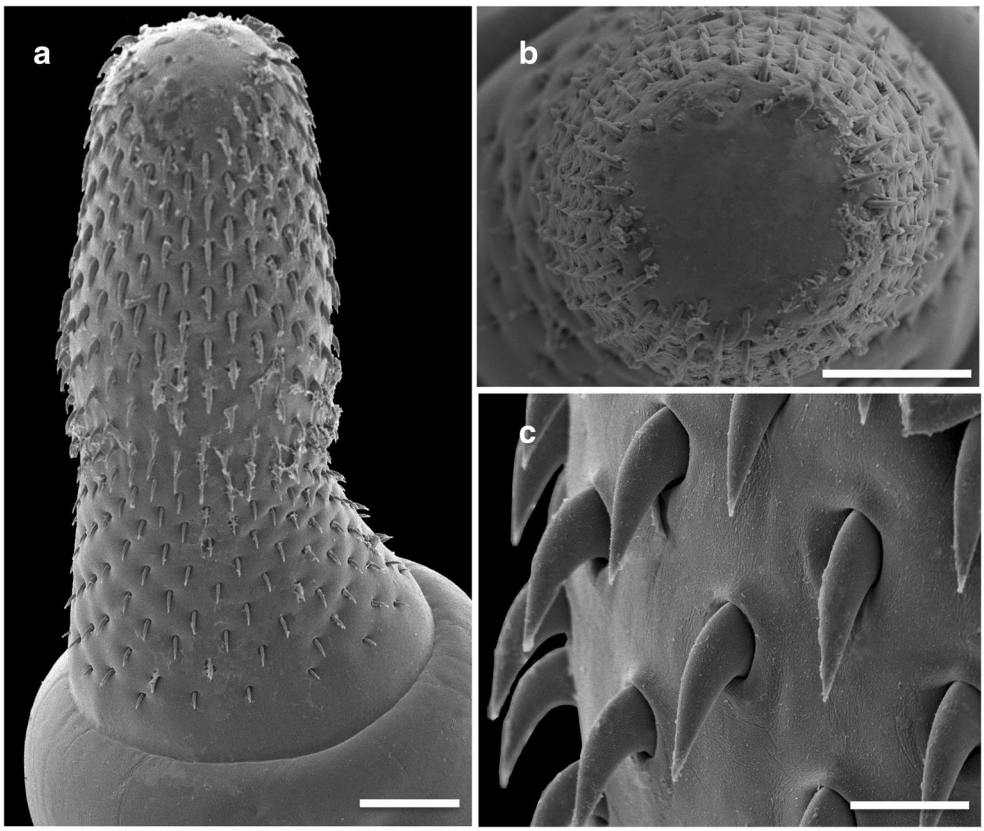

Fig. 5 Scanning electron micrographs of *Centrorhynchus microcephalus* from *Crotophaga sulcirostris*. **a** Proboscis adult male ventral view. **b** Proboscis adult male horizontal view. **c** Hooks. Scale bars = 100 μm (**a**, **b**); 20 μm **(c)**

Richardson et al. 2010). The phylogenetic analyses inferred with each molecular marker and the combined (LSU + SSU) dataset, consistently placed *C. microcephalus* within a clade with other species of *Centrorhynchus*, such as *C. nickoli* n. comb, *Centrorhynchus aluconis* (type species), *Centrorhynchus globocaudatus*, *Centrorhynchus conspectus*, *Centrorhynchus globirostris*, and *Centrorhynchus nahuelhuapensis*.

The phylogenetic relationships inferred with the combined dataset of two molecular markers, (LSU + SSU), represents the most compressive analysis to date and provides the first insight into the ecological associations between the parasites and their definitive hosts. We inferred that birds were the ancestral definitive hosts for Centrorhynchidae and Plagiorhynchidae with a secondary and independently event of colonization of mammals.

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