Journal of Helminthology

www.cambridge.org/jhl

A morphological and molecular study of adults and cystacanths of *Oncicola luehei* Travassos, 1917 (Acanthocephala: Archiacanthocephala), from the Neotropical region of Mexico

A.L. Sereno-Uribe[1], M.P. Ortega-Olivares[1], M.T. González-García[1], M.I. Grano-Maldonado[2] and M. García-Varela[1]

[1]Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, Ciudad Universitaria C. P. 04510, Ciudad de México, México and [2]Facultad de Ciencias del Mar, Universidad Autónoma de Sinaloa, Av. Claussen s-n, Mazatlán, Sinaloa, México

Research Paper

Cite this article: Sereno-Uribe AL, Ortega-Olivares MP, González-García MT, Grano-Maldonado MI and García-Varela M (2025). A morphological and molecular study of adults and cystacanths of *Oncicola luehei* Travassos, 1917 (Acanthocephala: Archiacanthocephala), from the Neotropical region of Mexico. *Journal of Helminthology*, **99**, e15, 1–11
https://doi.org/10.1017/S0022149X24000944

Received: 26 November 2024
Revised: 18 December 2024
Accepted: 19 December 2024

Keywords:
Archiacanthocephala; *Oncicola luehei*; *Nasua narica*; *Lithobates berlandieri*; *Lithobates vaillanti*; molecular markers

Corresponding author:
M. García-Varela;
Email: garciav@ib.unam.mx

Abstract

Members of the genus *Oncicola* Travassos, 1916 are generalist parasites able to infect a broad spectrum of carnivorous hosts, such as marsupials, procyonids, felids, and canids, and are distributed globally. Adult specimens were collected from the intestines of three white-nosed coatis (*N. narica*), whereas cystacanths (larval form) were found in the body cavities of two amphibian species (paratenic hosts) in localities from northern and southeastern Mexico. Morphologically, both stages were identified as *O. luehei* (Travassos, 1917) on the basis of the following features: trunk cylindrical, narrow anteriorly, enlarging midbody, tapering gradually to narrow posteriorly; proboscis globular with six circular rows of hooks with six hooks each, decreasing in size posteriorly; neck short with sensory papilla; tubular lemnisci long, extending to the posterior region; protonephridia dendritic type; and eight cement glands, compact with a single giant nuclei. Sequences from cytochrome c oxidase subunit 1 from mtDNA were aligned and compared with sequences available in GenBank. Phylogenetic analyses revealed that adults and cystacanths formed a clade with two other isolates identified as *Oncicola* sp. *and O. luehei* from Mexico. The intraspecific genetic divergence among the isolates was low, ranging from 0.0% to 3.0%, indicating that the two stages of the life cycle belong to the same species. The haplotype network was inferred with 11 sequences and revealed a lack of shared haplotypes between populations, suggesting a reduced recombination rate and a high pattern of genetic variation among individuals. Finally, these new records of *O. luehei* increase the distribution range of *O. luehei* on both coasts of Mexico.

Introduction

Members of the class Archiacanthocephala are parasites found in terrestrial mammals and birds and are distributed worldwide. The class is divided into four orders: Apororhynchida, Gigantorhynchida, Moniliformida, and Oligacanthorhynchida, each with a single family (Amin 2013; Bullock 1969). Archiacanthocephalans have an indirect life cycle using insects, myriapods, or other arthropods as intermediate hosts and terrestrial mammals or birds as definitive hosts. However, the participation of paratenic hosts, such as amphibians, turtles, snakes, and lizards, are key in the transmission of some parasites (Kenedy 2006; Nickol and Crompton 1985). Currently, the family Oligacanthorhynchidae Southwell and Macfine, 1925 is divided into 12 genera, and one of the most diverse groups within the family is *Oncicola* Travassos, 1916. This genus includes 24 species that are generalist parasites able to infect a broad spectrum of mammalian carnivorous hosts, such as marsupials, procyonids, felids, and canids, and are globally distributed (Amin 2013; Machado Filho 1950; Petrochenko 1958; Yamaguti 1963). Of the 24 described species of *Oncicola*, 16 of them are distributed in the Americas, representing 66.6% of the biodiversity of the genus. However, few species have been sequenced, limiting the understanding of the systematics of the family Oligacanthorhynchidae (García-Varela and Nadler 2006; Gazi *et al.* 2012; Near *et al.* 1998).

In the neotropical region of Mexico, adults of two species of *Oncicola* have been recorded on the coasts of the Pacific Ocean and Gulf of Mexico, including *O. luehei* (Travassos, 1917) obtained from the North American opossum (*Didelphis virginiana*, Kerr) in Veracruz state and *O. spirula* (Olfers, 1816) obtained from the white-nosed coati (*Nasua narica* L.) in Chiapas state, whereas cystacanths (larval form) have been identified as *Oncicola* sp. or *O. luehei* in at least four amphibian species (i.e., *Similisca cyanostica* Smith, *Lithobates forreri* Boulenger, *L. vaillanti* Brocchi, and *Rhinella marina* L.) (García-Prieto *et al.* 2010; Ortega-Olivares *et al.* 2013).

During a survey of parasitic helminths in northern and southeastern Mexico, adult specimens of an acanthocephalan were recovered from the digestive tract of a white-nosed coati (*N. narica*), whereas cystacanths were recovered from the body cavities of Vaillant's Frog (*L. vaillanti*) and the Rio Grande Leopard Frog (*Lithobates berlandieri* Baird). After a morphological examination of worms from both stages, the adults and cystacanths were identified as *O. luehei*. Therefore, the objectives of this study were to *i*) characterize morphologically adults and cystacanths recovered from the intestines of white-nosed coatis and from the body cavities of their paratenic hosts (amphibians) from northern and southeastern Mexico; *ii*) link both stages of adults and cystacanths by using sequences of cytochrome c oxidase subunit 1 (*cox1*) from mitochondrial DNA; and *iii*) test the systematic position of *O. luehei* within Archiacanthocephala by using small (SSU) and large (LSU) subunits from nuclear ribosomal DNA.

Materials and methods

Sample collection

During several field expeditions in northern and southeastern Mexico, three common white-nosed coati (*N. narica*) were found in three localities: Chamela, Jalisco (19° 27' 35.8" N, 104° 56' 11.4" W), Ciudad Guzmán, Jalisco (19° 44' 30. 934" N, 103° 28' 29.33" W), and Catemaco, Veracruz (18° 26' 14.43" N, 95° 04' 52.387" W), and seven adult male Rio Grande Leopard Frogs (*L. berlandieri*) and eight adult female Vaillant's Frogs (*L. vaillanti*) were collected in northern and southeastern Mexico (18° 35'–18 ° 36' N, 95 ° 05'–95° 06' W). The definitive and paratenic hosts were dissected, and the viscera were placed in separate Petri dishes with a 0.75% saline solution and examined under a dissecting microscope. The acanthocephalans were removed from the intestine (adult stage) and from the body cavity (encysted cystacanths) and washed in a 0.75% saline solution. Later, the unencysted cystacanths were placed in distilled water at 4°C overnight and subsequently were fixed and preserved in 70 or 100% ethanol.

Morphological analyses

A few acanthocephalans were gently punctured with a fine needle, stained with Mayer's paracarmine, destained in 70% acid ethanol, dehydrated in a graded ethanol series, cleared in methyl salicylate, and mounted on permanent slides with Canada balsam. Each slide with a cystacanth was deposited in the Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City, under numbers 12226–12228.

The acanthocephalans were analysed with a Leica DM 1000 LED microscope equipped with bright field (Leica, Wetzlar, Germany). Acanthocephalans were initially identified by conventional morphological criteria following the key of Yamaguti (1963) and the description of Machado Filho (1950). For scanning electron microscopy (SEM), two adults were individually dehydrated with an ethanol series, critical point dried with CO_2, sputter coated with gold, and examined with a Hitachi Stereoscan Model SU1510 scanning electron microscope operating at 15 kV at the Instituto de Biología, Universidad Nacional Autónoma de México (UNAM).

DNA sequence generation

A total of seven specimens identified as *O. luehei* were analyzed. Before DNA extraction, a tissue fragment was cut from two adults from northern and southeastern Mexico and two cystacanths (hologenophores, Pleijel *et al.* 2008), whereas the rest of the body was stained with Mayer's paracarmine and mounted on permanent slides with Canada balsam. The tissue of each specimen was placed individually in tubes and digested overnight at 56°C in a solution containing 20 mM NaCl, 100 mM Na_2 EDTA (pH 8.0), 10 mM Tris–HCl (pH 7.6), 1% sarkosyl, and 0.1 mg/ml proteinase K. Following digestion, genomic DNA was extracted from the supernatant using the DNAzol reagent (Molecular Research Center, Cincinnati, OH, USA) according to the manufacturer's instructions. A fragment of the cytochrome c oxidase subunit 1 (*cox 1*) from the mitochondrial DNA was amplified using the forward primer 5′-AGTTCTAATCATAA(R)GATAT(Y)GG-3′ and reverse primer 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′ (Folmer *et al.* 1994). PCR amplifications were performed in a total volume of 25 µl containing 2 µl of each primer, 10 pmol/ µl, 2.5 µl of 10X buffer, 1.5 µl of 2 mM $MgCl_2$, 2 µl of the genomic DNA, and 1U of Taq DNA polymerase (Platinum Taq, Invitrogen Corporation, California, United States). 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 40°C 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. Reaction products were separated and detected using an ABI 3730 capillary DNA sequencer. Contigs were assembled and base-calling differences were resolved using Codoncode Aligner version 11.0 (Codoncode Corporation, Dedham, Massachusetts).

Alignments, phylogenetic analyses, and haplotype network

Newly generated sequences *cox 1*, were aligned with published sequences for other members of Archiacanthocephala retrieved from the GenBank dataset (Table 1). Additionally, sequences from two nuclear genes from the SSU and LSU from Archiacanthocephalans were download from GenBank (Table 1) to test the systematic position of *Oncicola.* Alignments for each dataset (*cox 1*, SSU, and LSU) were constructed using the software Clustal W (Thompson *et al.* 1994). A nucleotide substitution model was selected for the dataset using jModelTest version 2.1.7 (Posada 2008). Phylogenetic analyses 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. In addition, a Bayesian analysis was carried out, using the program MrBayes 3.2.2 (Ronquist *et al.* 2012) with two Markov chain Monte Carlo (MCMC) runs for 10 million generations, sampling every 1,000 generations, a heating parameter value of 0.2, and a burn-in of 25%. The resulting phylogenetics trees were visualized and edited using FigTree version 1.4.2 (Rambaut and Drummond 2007). Finally, uncorrected *p* distances were estimated with the *cox 1* dataset by using the MEGA program (Kumar *et al.* 2016). To explore whether definitive and paratenic hosts from both coasts of Mexico share the same *cox* 1 haplotypes, an unrooted statistical network was constructed using PopART (Leigh and Bryant 2015) with the minimum spanning network option (Bandelt *et al.* 1999).

Results

Morphological identification

Adult acanthocephalans were recovered from intestines of three carcasses of white-nosed coati (*N. narica*) in Mexico (see Figure 1).

https://doi.org/10.1017/S0022149X24000944 Published online by Cambridge University Press

Table 1. Classification and GenBank accession numbers of the specimens used in the phylogenetic analysis and haplotype network. Sequences in bold were generated in this study

Class	Order	Species	SSU	LSU	cox 1	References
Rotifera						
Monogononta	Ploima	*Asplanchna sieboldi*	AF092434	–	–	García-Varela et al. (2000)
			–	AY829085	–	García-Varela and Nadler (2005)
			–	–	AF416994	García-Varela (unpublished data)
		Brachionus patulus	AF154568	–	–	García-Varela et al. (2000)
			–	AY829084	AF416995	García-Varela and Nadler (2005)
		Brachionus plicatilis	AY218118	–	–	Giribet et al. (2004)
		Lecane bulla	–	AY829083	–	García-Varela and Nadler (2005)
Pararotatoria	Seisonacea	*Seison nebaliae*	DQ089737	DQ089744	DQ089730	García-Varela and Nadler (2006)
Acanthocephala						
Archiacanthocephala	Gigantorhynchida	*Mediorhynchus africanus*	KC261353	–	–	Amin et al. (2013)
		Mediorhynchus gallinarum	KC261354	–	KC261352	Amin et al. (2013)
		Mediorhynchus sp.	AF064816	–	AF416996	García-Varela et al. (2000)
			–	AY829087	–	García-Varela and Nadler (2005)
		Mediorhynchus grandis	AF001843	–	–	Near et al. (1998)
	Moniliformida	*Gigantorhynchus echinodiscus*	–	MK635344	–	Gomes et al. (2019)
		Moniliformis kalahariensis	–	–	MH401040	Amin et al. (2019)
		Moniliformis moniliformis	HQ536017	–	–	Foronda (unpublished data)
			Z19562	–	–	Telford and Holland (1993)
			–	AY829086	–	García-Varela and Nadler (2005)
			–	–	AF416998	García-Varela (unpublished data)
		Moniliformis necromysi		MT808220	MT803593	Gomes et al. (2020)
		Moniliformis saudi	KU206782	–	KU206783	Amin et al. (2019)
		Moniliformis cryptosaudi	MH401043			Amin et al. (2019)
		Moniliformis ibunami	MW136271	MW136276	MW115575	Lynggaard et al. (2021)
			MW136272	MW136277	MW115576	
	Oligacanthorhynchida	*Macracanthorhynchus hirudinaceus*	LC350002	LC350002	LC350002	Kamimura et al. (2018)
					MZ683370–75	Mehmood and Varcasia (unpublished data)
		Macracanthorhynchus ingens	AF001844	–	–	Near et al. (1998)
			–	AY829088	–	(García-Varela and Nadler (2005)
			–	–	AF416997	García-Varela (unpublished data)
			–	–	ON197103	Najjari and Solgi (unpublished data)
			–	–	KT881246–49	Richardson et al. (2016)
			OR077291	OR077292	OR077684	Ortega-Olivares et al. (2023)
					OR077685	

(*Continued*)

https://doi.org/10.1017/S0022149X24000944 Published online by Cambridge University Press

Table 1. (*Continued*)

Class	Order	Species	SSU	LSU	cox 1	References
					OR077686	
					OR077687	
					OR077688	
					OR077689	
					OR077690	
					OR077691	
					OR077692	
		Oligacanthorhynchus microcephala	AF064817	–	–	García-Varela et al. (2000)
					KT881245	Richardson et al. (2016)
			–	AY829090	–	García-Varela & Nadler (2005)
					AF416998	
		Oncicola luehei	AF064818	–	–	García-Varela et al. (2000)
			–	AY829089	–	García-Varela and Nadler (2005)
			–	–	AF417000	García-Varela (unpublished data)
			–	–	OR077693	Ortega-Olivares et al. (2023)
					OR077694	
					NC102754	Gazi et al. (2012)
					PQ771482-	**This study**
					PQ771488	
		Oncicola venezuelensis		MK377341		Freeman (unpublished data)
				MK377342		
				KU521567		Santos et al. (2017)
		Prosthenorchis elegans	–	–	KT818504	Falla et al. (2015)
		Prosthenorchis sp.	–	–	KP997253	Sokolov et al. (unpublished data)
		Pachysentis canicola	MT864729			Chaudhary et al. (unpublished data)

The acanthocephalans showed similar morphological characteristics compared with those assigned to *O. luehei* by Travassos (1917) and Machado Filho (1950), including *i*) trunk cylindrical, narrow anteriorly enlarging midbody before tapering gradually to narrow posterior; *ii*) proboscis globular; *iii*) single-walled proboscis receptacle; *iv*) hooks in six alternative circles of six hooks each with roots robust, decreasing in size towards posterior; *v*) neck short with sensory papilla; *vi*) tubular lemnisci very long extending to the posterior region; *vi*) protonephridia dendritic type; *vii*) eight cement glands compact with single giant nuclei (Figures 2a–c). Compared to previous descriptions, our specimens exhibited variability in body size, proboscis, and hooks size (Table 2).

Taxonomy

Class: Archiacanthocephala Meyer 1931

Order: Oligacanthorhynchida Petrochenko 1956

Family: Oligacanthorhynchidae Southwell and Macfie 1925

Genus: *Oncicola* Travassos 1916

Species: *Oncicola luehei* (Travassos 1917) Schmidt 1972 (Figures 2, 3) (Adult)

Host: White-nosed coati *Nasua narica* Linnaeus

Site of infection: Intestine (prevalence 100%, (3/3)).

Paratenic host: Grande Leopard Frogs *Lithobates berlandieri* Baird; Vaillant's Frogs *Lithobates vaillanti* Brocchi

Site of infection: Body cavity

Locality: Chamela, Jalisco (19° 27' 35.8" N, 104° 56' 11.4" W); Ciudad Guzmán, Jalisco (19° 44' 30. 934" N, 103° 28' 29.33" W); Catemaco, Veracruz (18° 26' 14.43" N, 95° 04' 52.387" W).

Voucher: CNHE No. 12226–228

Representative DNA sequences: PQ771482–488.

Redescription

Adult (Figures 2, 3)

General:

Sexual dimorphism evident, females larger than males. Trunk cylindrical, narrow anteriorly enlarging midbody before tapering gradually to narrow posterior (Figures 2a, 3a). Proboscis globular covered with 36 hooks in 6 circles of 6 hooks each (Figures 2b, 3b, 3c). Neck short with a sensory papilla (Figures 2b, 3d). Proboscis receptacle single-walled. Lemnisci elongate, reaching posterior end trunk. Protonephridia dendritic type. Gonopore subterminal in both sexes.

https://doi.org/10.1017/S0022149X24000944 Published online by Cambridge University Press

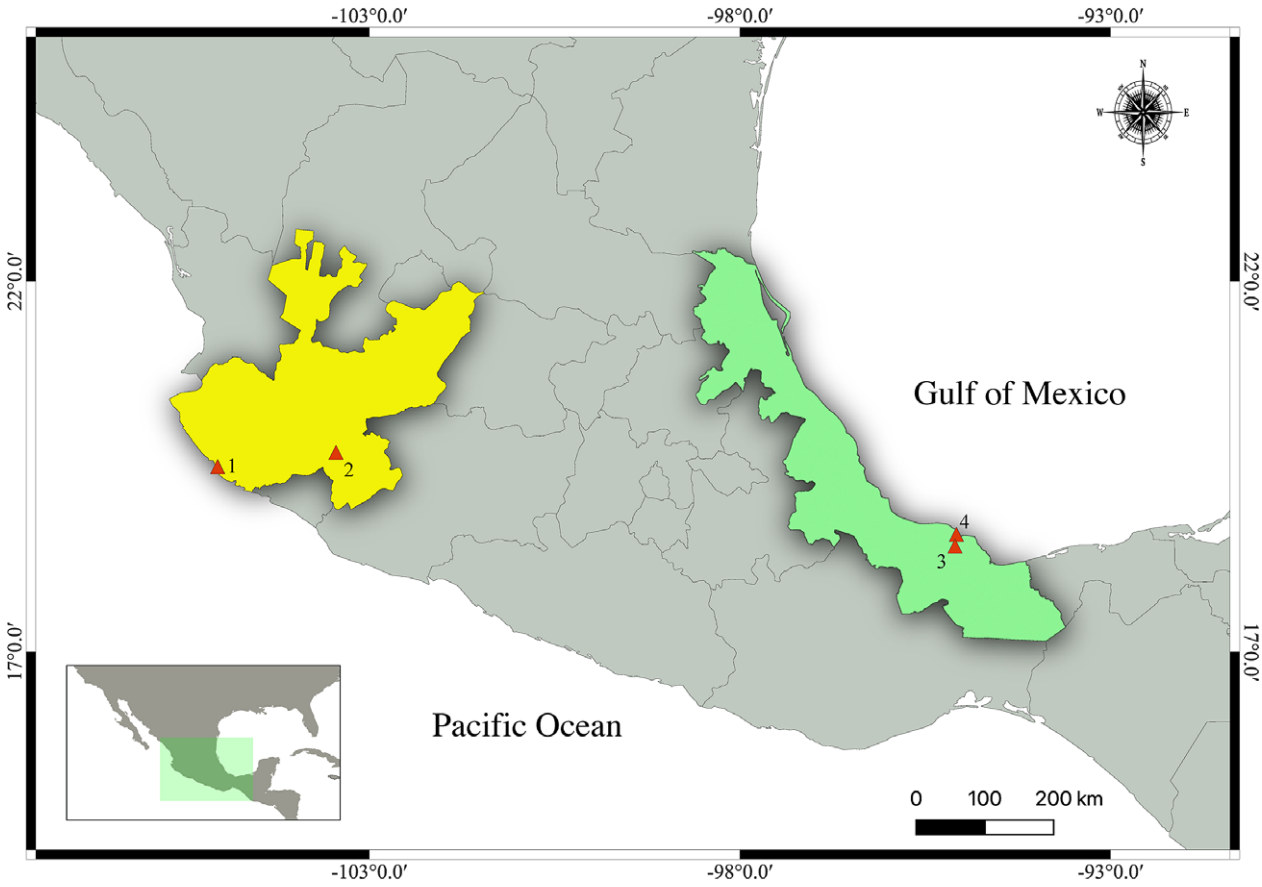

Figure 1. Sampling collection in Mexico. 1. Chamela, Jalisco (19° 27’ 35.8’’ N, 104° 56’ 11.4’’ W); 2. Ciudad Guzmán, Jalisco (19° 44’ 30. 934’’ N, 103° 28’ 29.33’’ W); 3. Catemaco, Veracruz (18° 26’ 14.43’’ N, 95° 04’ 52.387’’ W); 4. Los Tuxtlas, Veracruz (18° 35’–18 ° 36’ N, 95 ° 05’–95° 06’ W).

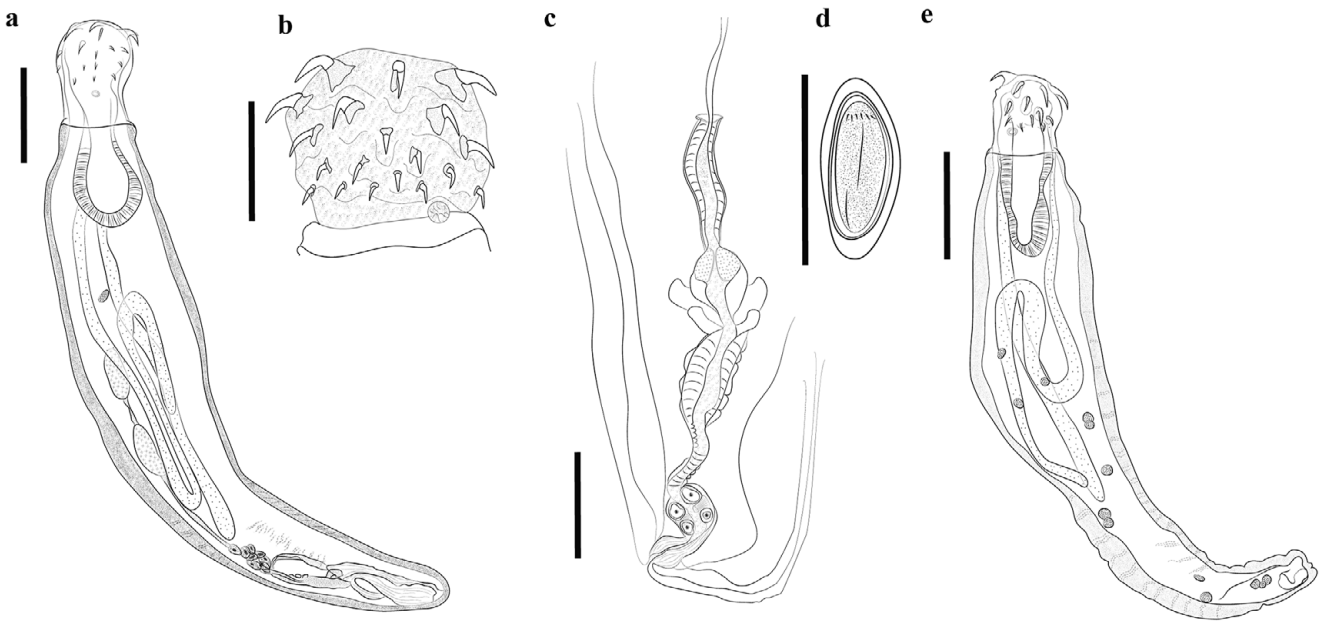

Figure 2. Drawing of *Oncicola luehei* from *Nasua narica.* Adult, total view (**a**); proboscis (**b**); Female reproductive system of *Oncicola luehei* (**c**); egg (**d**); cystacanth of *Oncicola luehei* from *Lithobates vaillanti* total view (**e**); Scale bars = 1.0 mm (**a**, **e**); 500 μm (**b**); 400 μm (**c**); 40 μm (**d**).

https://doi.org/10.1017/S0022149X24000944 Published online by Cambridge University Press

Table 2. Comparative metrical data for *Oncicola luehei.* Measurements in micrometres, unless otherwise indicated

Author	Travassos (1917)	Machado Filho (1950)	Oliveira *et al.* (2019)	This study
Country	Brazil	Brazil	Brazil	Mexico
Host	*Nasua narica*	*Nasua narica*	*Procyon cancrivorus*	*Nasua narica*
Trunk size mm				
Male length	20–30 × 2–3	10–20		6.0 × 1.2
Female length	40–50 × 4–5	25–35	15.8	8.2–27.3
Proboscis		760 × 70	710 × 810	
Proboscis male				713 × 605
Proboscis female				621–697 × 822–862
Proboscis receptacle		1360		1385–1512
No. longitudinal rows of hooks	5–6	6	6	6
No. hooks per row	6	6	6	6
Proboscis hooks size length				
				Male / Female
first	300	176		166–200 / 176 –206
second	280	210		151–155 / 151–185
third	150	147		110–112 / 121–149
fourth		105		92–105 / 96–123
fifth		94		71–89 / 70–94
sixth		76		62–65 / 50–58
Testes mm	2–3 × 0.5–1.0	2.1 × 0.54		
Anterior testis			1.4 × 0.53	0.55 × 0.18
Posterior testis			1.5 × 0.50	0.49 × 0.19
Cement glands	8	8	8	8
Eggs size	63–71 × 42	67 × 37		48–57 × 23–29

Male (based on 1 mounted specimen). Trunk, 6.1 mm long × 1.2 wide. Proboscis 713 × 605, with 36 hooks in 6 alternative circles of 6 hooks each with roots robust, decreasing in size towards posterior (Figures 2b, 3a). The first hook row 166–200 (187), second 151–155 (153), third 110–112 (111), fourth 92–105 (99), fifth 71–89 (77), sixth 62–65 (63). Neck 347 × 653. Proboscis receptacle 1164 long. Lemnisci tubular very long extending near posterior end of trunk (Figure 2a). Testes ovoid in tandem, anterior 558 × 186, posterior 497 × 191. Eight cement glands compact with single giant nuclei (Figure 2a).

Female (based on 5 mounted specimens). 8.2–27.3 mm long (14.8 mm) × 2.3–4.1wide (2.9). Proboscis 621–697 (648) × 822–862 (848), with 36 hooks in 6 alternative circles of 6 hooks each with roots robust, decreasing in size towards posterior. The first hook row 176–206 (194), second 151–185 (168), third 121–149 (135), fourth 96–123 (105), fifth 70–94 (79), sixth 50–58 (54). Proboscis receptacle 1385–1512(1448) long. Female reproductive system short (Figure 2c). Gonopore subterminal. Mature eggs subspherical 48–57 (52), × 23–29 (26) (Figure 2d).

Cystacanth (Figure 2e) (based on two immature mounted specimens, (hologenophores)).

Trunk narrow anteriorly enlarging to widest point near mid-body before tapering gradually to narrow posterior end; 6.1 mm (n=1) long by 1269 wide. Proboscis globular, 588–471 (529) long by 716–504 (610) wide; with 36 hooks in 6 alternative circles of 6 hooks each with roots robust, decreasing in size towards posterior. The first hook row 208–232 (224), second 178–207 (193), third 122–125 (123), fourth 66–92 (78), fifth 61–66 (63), sixth 56–60 (58). Neck 148–276 (212) long by 344–626 (485) wide; with pair of lateral sensory pits 47–54 (50) long by 44–67 (55) just posterior to root of last proboscis hook. Proboscis receptacle 1110 long by 522 wide (n=1) single walled with ventral cleft. Lemnisci tubular very long extending near posterior end of trunk, with small nuclei. Reproductive system primordial. Protonephridia dendritic type. Genital pore subterminal (see Figure 2e).

Phylogenetic analyses and haplotype network

The *cox 1* dataset included 664 sites and 49 sequences, and the best model was GTR + G + I. The tree inferred from the *cox 1* dataset showed that the phylogenetic relationships among the genera *Oligacanthorhynchus* Travassos, 1915, *Macracanthorhynchus* Travassos, 1917, *Oncicola* and *Prosthenorchis* Travassos, 1915 from Oligacanthorhynchidae are unresolved due a polytomy at the base of the tree (Figure 4a). Our phylogenetic trees showed that the seven newly isolates identified as *O. luehei* formed a subclade together

https://doi.org/10.1017/S0022149X24000944 Published online by Cambridge University Press

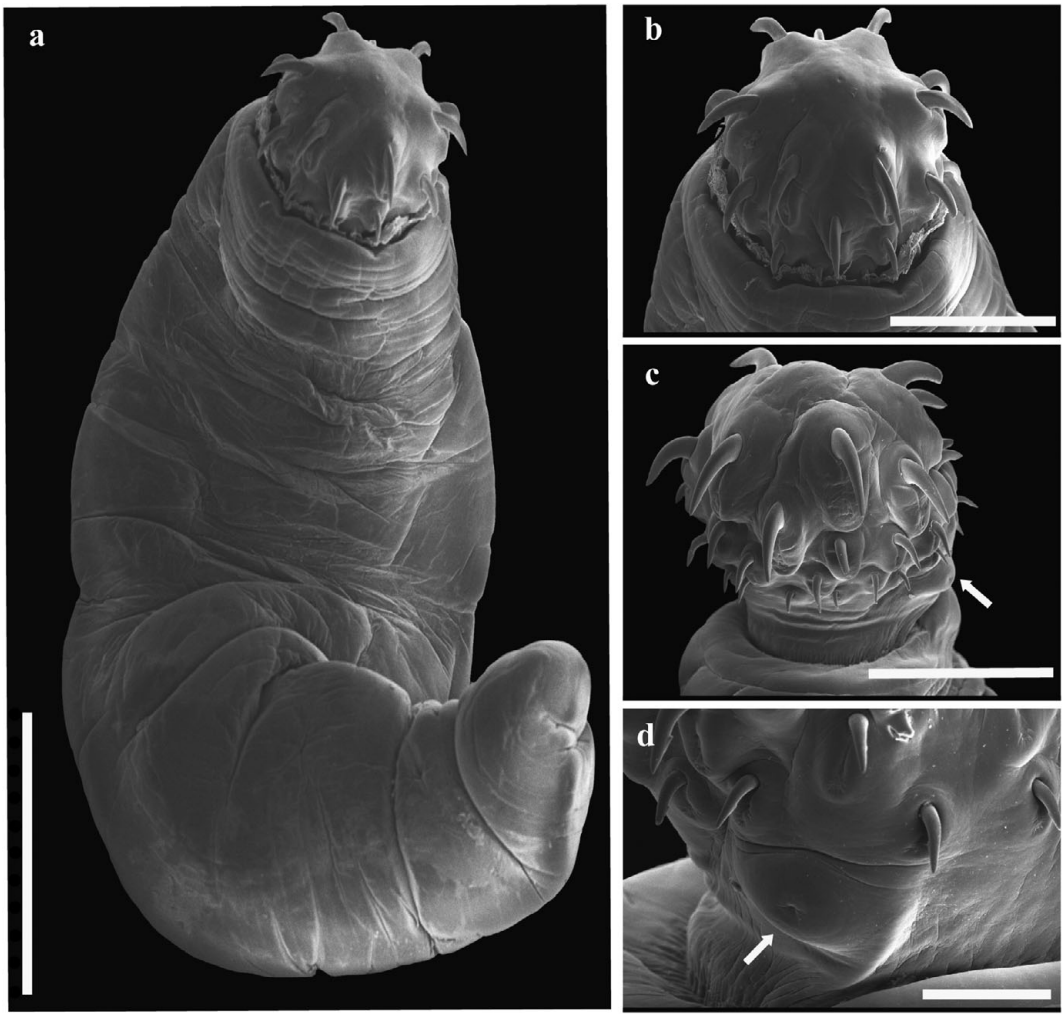

Figure 3. Scanning electron micrographs of adult specimen of *Oncicola luehei*, total view (**a**); proboscis (**b, c**); anterior region of proboscis (**d**). Arrows indicate a sensory papilla Scale bars = 1.0 mm (**a**); 500 μm (**b**); 400 μm (**c, d**).

with two isolates identified as *Oncicola* sp. and *O. luehei* downloaded from GenBank (AF41700, NC_102754), plus two other isolates identified as *Oncicola* sp. (ORO77693-694) recovered from the body cavities of two amphibian species (Vaillant's Frog (*L. vaillanti*) and Rio Grande Leopard Frog (*L. berlandieri*)) in southeastern Mexico (Figure 4a).

The uncorrected genetic divergence estimated with the *cox 1* dataset between the clade formed by the isolates of *Oncicola*, and *Prosthenorchis* its sister taxa in the *cox 1* phylogenetic tree, ranged 18%. The genetic divergence among our specimens of *O. luehei* recovered from three white-nosed coatis (*N. narica*) from northern and southeastern Mexico ranged from 0.03 to 1.5%; among the isolates recovered from the body cavities of their paratenic hosts, Vaillant's Frog, and Rio Grande Leopard Frog ranged from 0 to 3%. Finally, the genetic divergence between the two isolates identified as *Oncicola* sp. and *O. luehei* (GenBank: AF41700; NC_102754) ranged 2%. Monophyly and low genetic distances among sequences of *Oncicola* sp. and *O. luehei* from northern and southeastern Mexico suggest that all the isolates belong same species.

The haplotype network built in this study was inferred with 11 specimens and 661 characters. The network inferred herein recognized 11 haplotypes. The haplotypes were separated from each other by a maximum of 13 substitutions. The haplotype network did not show a phylogeographic structure; therefore, the haplotypes could not be grouped into their own geographic clusters (Figure 4b).

To test the systematic position of *O. luehei*, within Oligacanthorhynchidae, two nuclear genes from the SSU and LSU were analysed. A previous sequence identified as *Oncicola* sp. (AF064818) by García-Varela *et al.* (2000), now identified as *O. luehei*, was aligned together with 16 published SSU sequences from 12 species, plus four species from phylum Rotifera that were used as outgroup (see Table 1). The alignment contained 1,842 sites with 20 sequences. The best evolution model was TIM +I+G. This dataset included sequences representing four genera from Oligacanthorhynchidae:

Macracanthorhynchus, *Oligacanthorhynchus*, *Pachysentis* Meyer 1931, and *Oncicola*

The phylogenetic trees inferred with the SSU showed that Oligacanthorhynchidae is monophyletic and *Oligacanthorhynchus* is sister to *Oncicola* (Figure 5a). The LSU sequences of *O. luehei* (AY210467; AY829089) downloaded from GenBank were aligned together with 13 published sequences from eight species, plus four species from phylum Rotifera that were used as outgroup (see Table 1). The alignment contained 2,894 sites with 19 sequences. The best evolution model was GTR+G+I. This dataset included

https://doi.org/10.1017/S0022149X24000944 Published online by Cambridge University Press

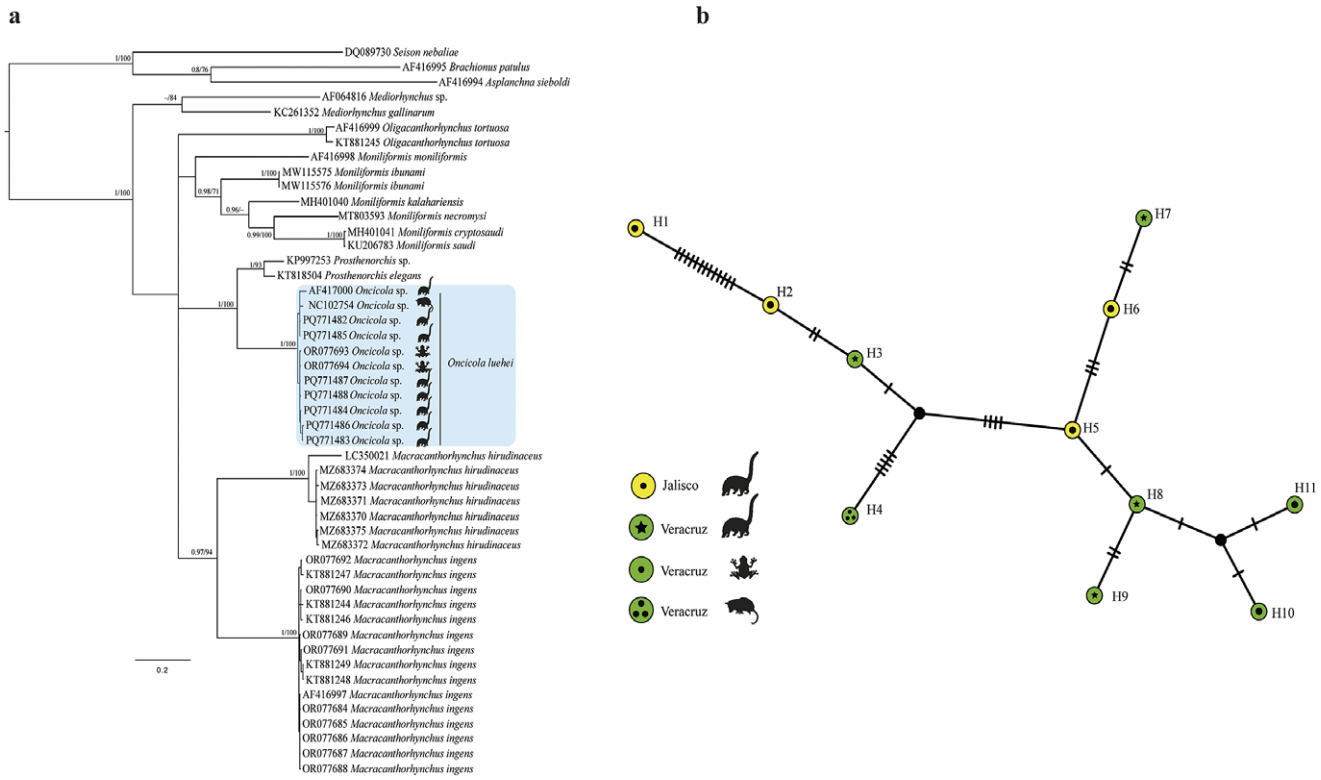

Figure 4. Phylogenetic trees using maximum likelihood (ML) and consensus Bayesian Inference for the *cox* 1 dataset (**a**). Numbers near internal nodes show ML bootstrap percentage values/ Bayesian posterior probabilities. Median-joining network of samples of *Oncicola luehei* built with the *cox* 1 gene (**b**). Each circle represents a haplotype, with size proportional to the haplotype's frequency in the populations.

sequences representing three genera from Oligacanthorhynchidae; *Macracanthorhynchus*, *Oligacanthorhynchus*, and *Oncicola*. The topologies inferred with the LSU dataset agree with the SSU tree because both trees placed all the genera from Oligacanthorhynchidae in a clade. However, the sequences representing two species from *Oncicola* – *O. luehei* (AY210467, AY829089) and *O. venezuelensis* Marteau, 1977 (MK377340-341; KU521567) – were nested in two independent subclades, suggesting that the genus is paraphyletic (Figure 5b).

Discussion

The acanthocephalan *O. luehei* obtained from the intestine of a white-nosed coati (*N. narica*) in Brazil was originally described as belonging to the genus *Prosthenorchis* (Travassoss, 1916). Later, it was validated in Yamaguti's key (Yamaguti 1963). In Schmidt (1972), its designation in the class Archiacanthocephala was revised, a taxonomic rearrangement at the genus level was proposed, and several species initially classified as *Prosthenorchis* were transferred to *Oncicola*, including *O. luehei*, which was later recognized in Amin's key (Amin 2013). This acanthocephalan is a parasite of American carnivorous and insectivorous mammals since it has been recorded in the ring-tailed coati (*Nasua nasua* L), the crab-eating raccoon (*Procyon cancrivorus* Cuvier), Tate's woolly mouse opossum (*Marmosa paraguaya* Tate), and the North American opossum (*D. virginiana*) in several countries from South America (Brazil, Argentina, and Paraguay) to North America (Mexico). In the present study, adult samples were collected from three white-nosed coatis (*N. narica*) in northern and southeastern Mexico, representing new records of this acanthocephalan in Mexico and extending its geographic range from northern Mexico to Argentina (Benatti *et al.* 2023; García-Prieto *et al.* 2010; Hernández-Orts *et al.* 2019; Oliveira *et al.* 2019; Vieira *et al.* 2008). In addition, Ortega-Olivares *et al.* (2023) reported that cystacanths were recovered from two frog species from southeastern Mexico. The cystacanths were initially identified as *Oncicola* sp.; morphologically, the specimens had a subglobular proboscis, covered with six hook rows with six hooks per row, and the lemnisci extended to the posterior region with small nuclei (Figure 2a–b). Nickol *et al.* (2006) reported that cystacanths from six genera of the family Oligacanthorhynchidae are characterized by having 36 proboscis hooks (*Macracanthorhynchus*, *Oligacanthorhynchus*, *Oncicola*, *Prosthenorchis*, and *Tchadorhynchus* Troncy, 1970). These genera can be distinguished at the species level primarily based on the features of the adult worms or molecular data (see Ortega-Olivares *et al.* 2023). In addition, the phylogenetic analyses of the *cox 1* dataset confirmed that the sequences of *Oncicola* sp. (ORO77693-694) from two cystacanths (hologenophores) from southeastern Mexico formed a clade together with two other sequences available in GenBank (AF41700 and NC_102754) identified as *Oncicola* sp. and *O. luehei*, respectively, plus the new sequences from adult specimens identified as *O. luehei* from northern and southeastern Mexico, confirming that the specimens are conspecific. The intraspecific genetic divergence among the isolates of *O. luehei* from northern and southeastern Mexico was very low, ranging from 0 to 3%. The level of intraspecific genetic variation found is similar to

https://doi.org/10.1017/S0022149X24000944 Published online by Cambridge University Press

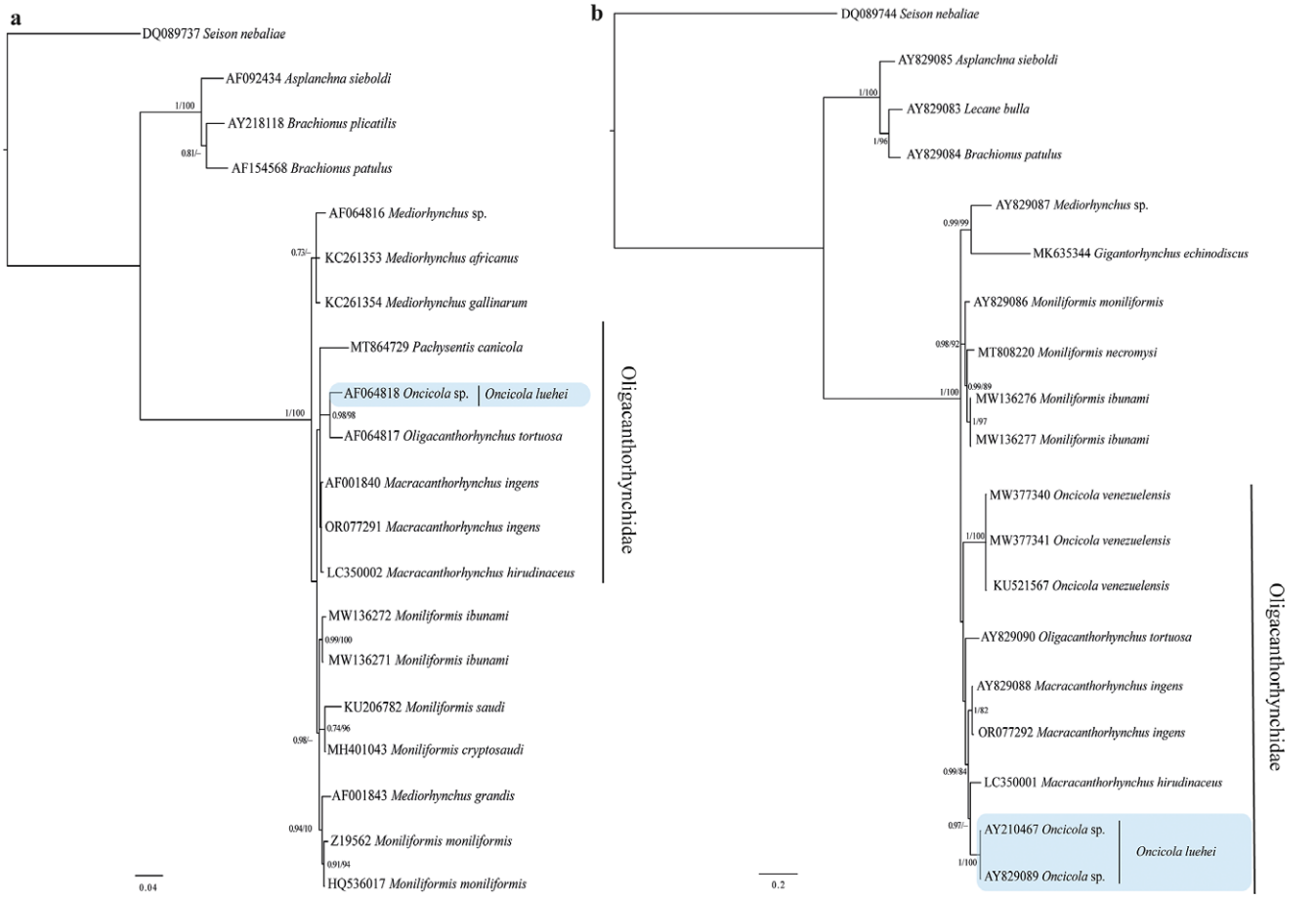

Figure 5. Phylogenetic trees using maximum likelihood (ML) and consensus Bayesian. SSU dataset (**a**), and LSU dataset (**b**). Numbers near internal nodes show ML bootstrap percentages/ Bayesian posterior probabilities.

that reported in other archicanthocephalans. For example, the genetic divergence among four isolates of *Mediorhynchus gallinarum* (Bhalerao 1937), a parasite of birds in Asia, was 0.2% (Rodríguez *et al.* 2022); among 37 isolates from *Prosthenorchis elegans* (Diesing 1815), a parasite of New World primates and carnivores in South America, the intraspecific genetic divergence ranged from 0 to 1.6% (Falla *et al.* 2015). Finally, among 15 isolates from *Macracanthorhynchus ingens* (von Linstow, 1879) Meyer 1932, a parasite of carnivores in North America, the intraspecific genetic divergence ranged from 0 to 2% (Ortega-Olivares *et al.* 2023).

Furthermore, the haplotype network analysis of *cox 1* revealed 11 distinct haplotypes obtained from 11 individual sequences. Therefore, the haplotypes could not be grouped into geographic clusters. The lack of shared haplotypes between populations in northern and southern Mexico suggested a reduced recombination rate and a high pattern of genetic variation among the specimens, possibly because the collection sites are separated by mountains that form geographical barriers, including Sierra Madre del Sur, Sierra Madre Occidental, Sierra Madre Oriental, and the central Trans-Mexicana Volcanic Belt (Morrone *et al.* 2017).

The cystacanths of *O. luehei* were found in two amphibian species (Vaillant's Frog and Rio Grande Leopard Frog), which serve as paratenic hosts. Although the complete life cycle of *O. luehei* is unknown, the current evidence suggests that adult worms of the genus *Oncicola* live and reproduce sexually in the digestive tracts of carnivorous hosts (Kennedy 2006). Female worms release eggs that are expelled into the environment with the faeces of the host. After the eggs are ingested by a termite that serves as an intermediate host, the parasite develops into the juvenile or cystacanth stage, at which point it is subsequently eaten by amphibians, lizards, and birds that serve as paratenic hosts until they are finally eaten by the appropriate definitive hosts (Nickol *et al.* 2006).

The taxonomic history of the species of *Oncicola* have been unstable in that some of them were initially described in the genus *Prosthenorchis* and were subsequently transferred to the genus *Oncicola* (Schmidt, 1972). The morphological traits that distinguish the two genera are the absence of a collar in *Oncicola* compared to the presence of a conspicuous festooned collar in the anterior region of the trunk in *Prosthenorchis.* The phylogenetic analyses based on the analysis of *cox 1* placed *Prosthenorchis* spp. as a sister taxon to *O. luehei* (see Figure 4a), supporting the close relationships between the two genera. However, the LSU tree placed the two species of *Oncicola* analysed herein (*O. luehei* and *O. venezuelensis*) in two independent clades (Figure 5b), suggesting that the genus is paraphyletic. Morphologically, *Oncicola* is divided into two groups: the first has roughly saccular or pyriform-shaped trunks that are less than 20 mm long, similar to *O. venezuelensis*, while the second has elongated trunks that may reach lengths of nearly 50 mm, similar to *O. luehei.* In addition, the protonephridial organ is key to identifying species of *Oncicola.* The feature has not

https://doi.org/10.1017/S0022149X24000944 Published online by Cambridge University Press

been described in *O. venezuelensis*, whereas *O. luehei* possesses a protonephridial organ, type dendritic, which has been observed in both adults and cystacanths (Nickol and Dunagan 1989; Schmidt 1972).

Acknowledgements. We are grateful to Laura Marquez and Nelly López for their help during the sequencing of the DNA fragments. We also thank Berenit Mendoza Garfias for her help in obtaining the scanning electron microphotographs.

Author contribution. ALSU and MGV conceived and designed the study. ALSU, MTGG, MPOO, MIGM, and MGV wrote and edited the article. ALSU, MPOO, and MGV collected the samples. ALSU, MPOO, MTGG, and MGV designed the methodology.

Financial support. This research was supported by the Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (PAPIIT-UNAM) IN201122.

Competing interest. No conflict of interest exists among the authors.

Consent to participate. All the listed authors have made significant contributions to the study and agreed to participate.

Consent for publication. All authors have read and agreed to the published version of the manuscript.

Ethical standards. The sampling in this work complies with the current laws and animal ethics regulations of México.

References

Amin OM (2013) Classification of the Acanthocephala. *Folia Parasitologica*, **60**, 273–305. doi:10.14411/fp.2013.031.

Amin OM, Evans P, Heckmann RA and El-Naggar AM (2013) The description of *Mediorhynchus africanus* n. sp. (Acanthocephala: Gigantorhynchidae) from galliform birds in Africa. *Parasitology Research* **112**, 2897–2906.

Amin OM, Heckmann RA, Sharifdini M and Albayati NY (2019) *Moniliformis cryptosaudi* n. sp. (Acanthocephala: Moniliformidae) from the long-eared hedgehog *Hemiechinus auritus* (Gmelin) (Erinaceidae) in Iraq; a case of incipient cryptic speciation related to *M. saudi* in Saudi Arabia. *Acta Parasitologica* **64**, 195–204.

Bandelt H, Forster P and Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* **16**, 37–48.

Benatti D, Moraes MFD, Pacheco CAA, Machado DMR, Oliveira WJ, Perin PP, Andrietti, LF, Cândido Júnior, JF, Vogliott, A, Tebaldi, JH, and Lux Hoppe, EG (2023) Endoparasites of marsupials in fragments of the Atlantic rainforest, western Paraná State, Brasil. *Brazilian Journal of Veterinary Parasitology* **32**, 1–11.

Bullock WL (1969) Morphological features as tools and pitfalls in acanthocephalan systematics. In Schmidt GD (ed), *Problems in Systematics of Parasites*. Baltimore, MD: University Park Press, 9–43.

Falla AC, Brieva C and Bloor P (2015) Mitochondrial DNA diversity in the acanthocephalan *Prosthenorchis elegans* in Colombia based on cytochrome c oxidase I (COI) gene sequence. *International Journal for Parasitology: Parasites and Wildlife* **4**, 401–407.

Folmer O, Black M, Hoeh W, Lutz R and Vrijenhoek R (1994) DNA primers for the amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**, 294–299.

García-Prieto L, García-Varela M, Mendoza-Garfias B and Pérez-Ponce de León G (2010) Checklist of the Acanthocephala in wildlife vertebrates of Mexico. *Zootaxa* **2419**, 1–50.

García-Varela M, Pérez-Ponce de León G, de la Torre P, Cummings MP, Sarma SS and Laclette JP (2000) Phylogenetic relationships of Acanthocephala based on analysis of 18S ribosomal RNA gene sequences. *Journal of Molecular Evolution* **50**, 532–540.

García-Varela M and Nadler SA (2005) Phylogenetic relationships of Palaeacanthocephala (Acanthocephala) inferred from SSU and LSU rDNA gene sequences. *Journal of Parasitology* **91**, 1401–1409.

García-Varela M and Nadler SA (2006) Phylogenetic relationships of Syndermata based on small subunit (SSU) and large subunit (LSU) of rRNA and cytochrome oxidase subunit I genes sequences. *Molecular Phylogenetics and Evolution* **40**, 61–72.

Gazi M, Sultana T, Min GS, Chul Park Y, García-Varela M, Nadler SA and Park JK (2012) The complete mitochondrial genome sequence of *Oncicola luehei* (Acanthocephala: Archiacanthocephala) and its phylogenetic position within Syndermata. *Parasitology International* **61**, 307–316.

Giribet G, Sorensen MV, Funch P, Kristensen RM and Sterrer W (2004) Investigations into the phylogenetic position of Micrognathozoa using four molecular loci. *Cladistics* **20**, 1–13.

Gomes APN, Cesário CS, Olifiers N, de Cassia BR, Maldonado A and Vilela RV (2019) New morphological and genetic data of *Gigantorhynchus echinodiscus* (Diesing, 1851) (Acanthocephala: Archiacanthocephala) in the giant anteater *Myrmecophaga tridactyla* Linnaeus, 1758 (Pilosa: Myrmecophagidae). *International Journal for Parasitology: Parasites and Wildlife* **10**, 281–288.

Gomes APN, Costa NA, Gentile R, Vilela RV and Maldonado A (2020) Morphological and genetic description of *Moniliformis necromysi* sp. n. (Archiacanthocephala) from the wild rodent *Necromys lasiurus* (Cricetidae: Sigmondontinae) in Brazil. *Journal of Helminthology* **94**, e138.

Hernández-Orts JS, Kutcha R, Semenas L, Cresco E, González RA and Aznar FJ (2019) An annotated list of the Acanthocephala from Argentina. *Zootaxa* **4663**, 001–064.

Kennedy CR (2006) *Ecology of the Acanthocephala*. New York: Cambridge University Press.

Kamimura K, Yonemitsu K, Maeda K, Sakaguchi S, Setsuda A, Varcasia A and Sato H (2018) An unexpected case of a Japanese wild boar (*Sus scrofa leucomystax*) infected with the giant thorny-headed worm (*Macracanthorhynchus hirudinaceus*) on the mainland of Japan (Honshu). *Parasitology Research* **117**, 2315–2322.

Kumar S, Stecher G and Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* **33**, 1870–1874.

Leigh JW and Bryant D (2015) POPART: Full-feature software for haplotype network construction. *Methods in Ecology and Evolution* **6**, 1110–1116.

Lynggaard C, García-Prieto L, Guzmán-Cornejo C and García-Varela M (2021) Description of a new species of *Moniliformis* (Acanthocephala: Moniliformidae) from *Peromyscus hylocetes* (Rodentia: Cricetidae) in Mexico. *Parasitology International* **83**, 102315.

Machado Filho DA (1950) Revisao do gênero *Prosthenorchis* Travassos, 1915 (Acanthocephala). *Memorias do Instituto Oswaldo Cruz* **48**, 495–544.

Morrone JJ, Escalante T and Rodríguez-Tapia G (2017) Mexican biogeographic provinces: Map and shapefiles. *Zootaxa* **4277**, 277–279.

Near TJ, Garey JR and Nadler SA (1998). Phylogenetic relationships of the Acanthocephala inferred from 18S ribosomal DNA sequences. *Molecular Phylogenetics and Evolution* **10**, 287–298.

Nickol BB and Crompton DWT (1985) *Biology of the Acanthocephala*. London: Cambridge University Press.

Nickol BB and Dunagan TT (1989) Reconsideration of the acanthocephalan genus *Echinopardalis*, with a description of adult *E. atrata* and a key to genera of the Oligacanthorhynchidae. *Proceedings of the Helminthological Society of Washington* **56**, 8–13.

Nickol BB, Fuller CA and Rock P (2006) Cystacanths of *Oncicola venezuelenzis* (Acanthocephala: Oligacanthorhynchidae) in Caribbean termite and various paratenic host in the U.S. Virgin Islands. *Journal of Parasitology* **92**, 539–542.

Oliveira WJ, Quagliatto Santos AL, Viotto de Souza W, Iannini Custódio AE, Guilherme Lux-Hoppe E and Rosalinski-Morales F (2019) *Oncicola luehei* in a wild crab-eating raccoon (*Procyon cancrivorus*) from the Brazilian cerrado savanna. *Revista Patologia Tropical* **48**, 243–248.

Ortega-Olivares MP, Velázquez-Urrieta Y, Sereno-Uribe AL, Harvey MB and García-Varela M (2023) A molecular and ecological study of *Macracanthorhynchus ingens* (von Linstow, 1879) (Acanthocephala: Archiacanthocephala), in its paratenic and definitive hosts in southeastern Mexico and the Eastern USA. *Systematic Parasitology* **100**, 543–556.

Petrochenko VI (1958) [*Acanthocephala of Domestic and Wild Animals*], vol. 2. Moscow: Izdatel'stvo Akad. Nauk SSSR. (In Russian.) (English translation by Israel Program for Scientific Translations, Ltd., Jerusalem, 1971, 478 pp.)

Pleijel F, **Jondelius U**, **Norlinder E**, **Nygren A**, **Oxelman B**, **Schander C**, **Sundberg P, and Thollesson M** (2008) Phylogenies without roots? A plea for the use of vouchers in molecular phylogenetic studies. *Molecular Phylogenetics and Evolution* **48**, 369–371.

Posada D (2008) jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution* **25**, 1253–1256.

Rambaut A and Drummond AJ (2007) Tracer v1.4. http://beast.bio.ed.ac.uk/Tracer (accessed 01/06/2024).

Richardson DJ, **Hammond CI and Richardson KE** (2016) The Florida ivory millipede *Chicobolus spinigerus* (Diplopoda:Spirobolidae): A natural intermediate host of *Macracanthorhynchus ingens* (Acanthocephala:Oligacanthorynchidae). *Southeastern Naturalist* **15**, 7–11.

Ronquist F, **Teslenko M**, **Van Der Mark P**, **Ayres DL**, **Darling A**, **Höhna S**, **Larget B**, **Liu L**, **Suchard MA and Huelsenbeck JP** (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**, 539–542.

Rodríguez SM, **Amin OM**, **Heckmann RA**, **Sharifdini M, and D'Elía G** (2022) Phylogeny and life cycles of the Archiacanthocephala with a note on the validity of *Mediorhynchus gallinarum*. *Acta Parasitologica* **67**, 369–379.

Santos EGN, **Chame M**, **Chagas-Moutinho VA and Santos CP** (2017) Morphology and molecular analysis of Oncicola venezuelensis (Acanthocephala: Oligacanthorhynchidae) from the ocelot *Leopardus pardalis* in Brazil. *Journal of Helminthology* **91**, 605–612.

Schmidt GD (1972) Revision of the class Archiacanthocephala Meyer, 1931 (Phylum Acanthocephala), with emphasis on Oligacanthorhynchidae Southwell et Macfie, 1925. *Journal of Parasitology* **58**, 290–297.

Stamatakis A (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**, 2688–2690.

Telford MJ and Holland PW (1993). The phylogenetic affinities of the chaetognaths: A molecular analysis. *Molecular Biology and Evolution* **10**, 660–676.

Thompson JD, **Higgins DG and Gibson TJ** (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**, 4673–4680.

Travassos L (1917) Contribuição para o conhecimento da fauna helmintológica brasileira, XVII. Revisão dos acantocéfalos brasileiros. Parte I. Fam. Gigantorhynchidae Hamann, 1982. *Memórias do Instituto Oswaldo Cruz* **9**, 5–62.

Vieira FM, **Luque JL and Muniz-Pereira LC** (2008). Checklist of helminth parasites in wild carnivore mammals from Brazil. *Zootaxa* **172**, 1–23.

Yamaguti S (1963) *Acanthocephala. Systema Helminthum*. New York, London: Wiley Interscience.

https://doi.org/10.1017/S0022149X24000944 Published online by Cambridge University Press