

***Mayarhynchus karlae* n. g., n. sp. (Acanthocephala: Neoechinorhynchidae), a parasite of cichlids (Perciformes: Cichlidae) in southeastern Mexico, with comments on the paraphyly of *Neoechinorhynchus* Stiles & Hassall, 1905**

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Abstract *Mayarhynchus* n. g. (Acanthocephala: Neoechinorhynchidae) is erected for *Mayarhynchus karlae* n. g., n. sp. described from the intestine of four species of cichlid fishes distributed from southeastern Mexico. The new genus placed in the family Neoechinorhynchidae (Ward, 1917) Van Cleave, 1928, is readily distinguished from the other 17 genera in the family by having a small proboscis armed with 45–46 relatively weak rooted hooks arranged in nine longitudinal rows of five hooks each. In addition,

Mayarhynchus n. g., n. sp. is diagnosed by the presence of a short trunk, body wall with five dorsal and one ventral giant hypodermal nuclei, proboscis receptacle nearly cylindrical with single layered wall, lemnisci broad and flat with large nuclei, testes in tandem, cement gland with eight large nuclei, and eggs elongate to oval. Partial sequences of the cytochrome *c* oxidase subunit 1 (*cox1*), internal transcribed spacers (ITS1 + 5.8S + ITS2), and the D2-D3 domains of the large subunit rRNA gene (28S) were obtained for five specimens of the new species and other species belonging to the Neoechinorhynchidae. Phylogenetic analyses confirmed that the new genus belongs to the Neoechinorhynchidae and indicated that the genus *Neoechinorhynchus* Stiles & Hassall, 1905 is not monophyletic. Comparison between three populations of the new species yielded nine variable sites for *cox1*, 11 for ITS and four for 28S.

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Introduction

Middle America, a geographical region that comprises eight countries (Mexico, Guatemala, Belize, El Salvador, Honduras, Nicaragua, Costa Rica and Panama) and sometimes the Antilles (see Choudhury et al., 2016) possesses a very diverse cichlid fauna, with over 120 species (Říčan et al., 2013, 2016). In this geographical region, these freshwater fishes serve as intermediate and definitive hosts of a large number of helminth parasites (e.g. Moravec, 1998; Mendoza-Franco et al., 2000; Vidal-Martínez et al., 2001; Razo-Mendivil et al., 2010; Sandlund et al., 2010; Pérez-Ponce de León et al., 2016). Five species of acanthocephalan have been recorded thus far in Middle-American cichlids: *Acanthocephalus amini* Salgado-Maldonado & Novelo-Turcotte, 2009 and *Dollfusentis chandleri* Golvan, 1969 in *Mayaheros urophthalmus* (Günther); *Octospiniferoides chandleri* Bullock, 1957 in *Petenia splendida* (Günther) and *Parachromis friedrichsthalii* (Heckel); *Neoechinorhynchus* (*Neoechinorhynchus*) *golvani* Salgado-Maldonado, 1978 from 36 species of cichlid fishes and *N. (N.) panucensis* Salgado Maldonado, 2013 in *Herichthys labridens* Pellegrin and *Herichthys cyanoguttatus* Baird & Girard (see Salgado-Maldonado et al., 1992; Scholz et al., 1996; Salgado-Maldonado et al., 1997; Martínez-Aquino et al., 2009; Salgado-Maldonado & Novelo-Turcotte 2009; Pinacho-Pinacho et al., 2015).

During a survey of the acanthocephalan parasites of fishes across Middle-America, we found several specimens in the intestine of three species of cichlids, i.e. *Thorichthys ellioti* Meek, *Paratheraps fenestratus* (Günther) and *M. urophthalmus* in southeastern Mexico. A combination of characters such the proboscis shape, proboscis receptacle and cement gland lead us to allocate the species into the family Neoechinorhynchidae, but the number and arrangement of the proboscis hooks set this species apart from all of the 17 genera currently recognised within the family. A new genus has to be erected to accommodate the new species. Both, the new genus and species are herein described based on morphological and molecular data.

Materials and methods

Sample collection

Fish were captured with seine nets and electrofishing, transported alive to the laboratory, and studied for

helminths a few hours after their capture; individual host were killed by spinal severance (pithing) following AVMA (2013), and all internal organs were examined for parasites under a dissecting microscope. Five specimens of *Mayaheros urophthalmus* from Silvituc Lagoon, Campeche (18°37'33.06"N, 90°17'10.15"W) were examined in 2013. In addition, 30 individuals of *Thorichthys ellioti* and five of *Paratheraps fenestratus* from Tlacotalpan, Veracruz (18°41'04.29"N, 95°38'47.47"W) were examined in 2014. Acanthocephalans were washed and relaxed in distilled water for 10–12 h at 4°C. Later, worms were fixed with hot (almost boiling) 4% formalin, washed and subsequently stored in 70% ethanol; some specimens were preserved in 100% ethanol, and stored at –80°C.

Morphological description

For morphological identification, acanthocephalans were punctured with a fine needle and stained with Mayer's paracarmine and eosin yellow wash following the procedure outlined by Hernández-Orts et al. (2012), dehydrated in a graded ethanol series, cleared with methyl salicylate, and mounted on permanent slides in Canada balsam. Illustrations of the specimens were made with the aid of a drawing tube. Specimens were examined using a bright field Leica DM 1000 LED microscope and the measurements were taken using the Leica Application Suite microscope software, and are presented in micrometres, with the range followed by the mean values in parentheses. Measurements of the eggs were made from fully developed eggs *in situ*. For scanning electron microscopical (SEM) observations, two specimens were dehydrated through an ethanol series, critical point dried, sputter-coated with gold and examined at 15 kV in a Hitachi Stereoscan Model SU1510 SEM (Hitachi Ltd., Tokyo, Japan) at the Instituto de Biología, Universidad Nacional Autónoma de México (UNAM). In addition, one male and one female were dehydrated through an ethanol series, critical point dried and coated with a gold-palladium alloy to a thickness of 250 nm and were examined with a Hitachi 4100 FE scanning electron microscope, operating at 20 kV, from the Central Service for the Support to Experimental Research (SCSIE) of the University of Valencia, Spain.

The holotype, allotype and paratypes were deposited in the Colección Nacional de Helminthos (CNHE),

Instituto de Biología, Universidad Nacional Autónoma de México. For morphological comparisons, type-specimens of the following species were examined: *Aactorhynchus duranguensis* Salgado-Maldonado, Aguilar-Aguilar & Cabañas-Carranza, 2005 ex *Cyprinodon meeki* Miller (CNHE 5214–5215, 6009, 6240); *Neoechinorhynchus* (*N.*) *golvani* (CNHE 601, 603, 604, 606, 631–632); *N.* (*N.*) *panucensis* (CNHE 8378, 8379, 8380–8381). Voucher specimens of *Floridosentis mugilis* (Machado, 1951) (CNHE 559–560); *F. pacifica* Bravo-Hollis, 1969 (CNHE 587) and *Octospinoferoides chandleri* (CNHE 662, 2688, 6761) were also examined.

Molecular data

Five adult acanthocephalans of the new species and three of *A. duranguensis* were 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. Following digestion, DNA was isolated from the supernatant using the DNAzol reagent (Molecular Research Center, Cincinnati, OH, USA) according to the manufacturer's instructions. Three separate gene fragments were PCR-amplified, including the cytochrome *c* oxidase subunit 1 (*cox1*) mitochondrial gene, the internal transcribed spacers (ITS1 + 5.8S + ITS2) and the D2-D3 domains of the large subunit ribosomal RNA gene (28S). PCR and sequencing protocols follow the procedures and use the primers described in Pinacho-Pinacho et al. (2012, 2014, 2015). PCR amplicons were sequenced in both directions; 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. Contiguous sequences were assembled and base-calling differences resolved using Codoncode Aligner version 5.0.2 (Codoncode Corporation, Dedham, Massachusetts) and submitted to GenBank.

Partial sequences obtained from the *cox1*, ITS and 28S genes (4, 5 and 4 sequences, respectively) were aligned in three independent datasets with sequences of other species belonging to the Neoechinorhynchidae available in the GenBank database using ClustalW with default parameters implemented in MEGA version 7.0 (Kumar et al., 2016).

The best-fitting nucleotide substitution models (*cox1*: GTR + G + I; ITS: GTR + G; 28S:

GTR + G + I) were estimated with the Akaike Information Criterion (AIC) implemented in MEGA version 7.0 (Kumar et al., 2016). The phylogenetic analyses were run on the three datasets individually and the combined datasets of 28S + *cox1* (because sequences are available for the same outgroups) under Bayesian inference (BI) criteria, employing the nucleotide substitution model identified for AIC. BI trees were generated using MrBayes v3.2 (Ronquist et al., 2012), running two independent MC³ runs of four chains for 5 million generations and sampling tree topologies every 1,000 generations. 'Burn-in' periods were set to 1 million of generations according to the standard deviation of splits frequencies values (< 0.01). Posterior probabilities of clades were obtained from 50% majority rule consensus of sample trees after excluding the initial 20% as 'burn-in'. The numbers of variable sites in the sequences of the new species were determined using the program MEGA version 7.0 (Kumar et al., 2016). Sequence divergences were estimated using the General Time Reversible (GTR) model with the program PAUP* 4.0b10 (Swofford, 2003).

Class Eoacanthocephala Van Cleave, 1936 **Order Neoechinorhynchida Southwell & Macfe, 1925**

Family Neoechinorhynchidae (Ward, 1917) Van Cleave, 1928

Subfamily Neoechinorhynchinae (Ward, 1917) Travassos, 1926

Mayarhynchus n. g.

Diagnosis

With characters of the family. Trunk short, aspinose, cylindrical. Body wall thick. Giant hypodermal nuclei irregular in shape, five dorsal and one ventral. Proboscis small, cylindrical, armed with 45–46 hooks. Proboscis hooks arranged in nine longitudinal rows of five hooks each. Hooks near tip of proboscis large, with weak roots, decreasing in size posteriorly. Neck short. Proboscis receptacle nearly cylindrical, with single layered wall. Ganglion near but not at base of proboscis receptacle. Lemnisci broad, flat, each with single large nucleus. Testes tandem, contiguous, cement gland syncytial, with eight large nuclei. Sperm ducts with two sperm vesicles. Spherical cement reservoir posterior to cement gland. Saeftigen's

pouch present. Eggs elongate to oval. Parasitic in the intestine of freshwater cichlids in southeastern Mexico. *Type-species*: *Mayarhynchus karlae* n. sp.

Etymology: The new genus epithet refers to the Mayan civilization, a Mesoamerican civilization that settled mainly in southeastern Mexico, and the Greek suffix “-rhynchus”, which means having a beak or snout and is commonly used to refer acanthocephalan genera.

Remarks

The new genus has all of the characters of the family Neoechinorhynchidae including an aspinose trunk, giant hypodermic nuclei, proboscis variable in shape, with hooks in longitudinal, spiral or transverse rows, cement gland with eight nuclei, and elongate eggs. Currently, there are 17 recognised genera within Neoechinorhynchidae according to Amin (2013): *Atactorhynchus* Chandler, 1935, characterised by having a proboscis armed with about eight diagonally transverse rows of hooks (Chandler, 1935); *Flori-dosentis* Ward, 1953 (proboscis with eight diagonally longitudinal rows of approximately seven hooks in each row; see Ward, 1953); *Tanaorhamphus* Ward, 1918 (proboscis with 20 circular row of 6–10 hooks in each row; see Van Cleave, 1913); *Eocollis* Van Cleave, 1947 (proboscis armed with three circles of six hooks each; see Van Cleave, 1947); *Gracilisentis* Van Cleave, 1919 (proboscis with three circles of hooks of approximately 12–14 hooks in each circle; see Jilek & Crites, 1979); *Pandosentis* Van Cleave, 1920 (proboscis with 12–14 longitudinal rows of three hooks each; see Smales, 2007); *Wolffhugelia* Mañé-Garzon & Dei-Cas, 1974 (proboscis with three circles of 15–19 hooks in each circle; see Lunaschi & Drago, 1995); *Dispiron* Bilqees, 1970 (proboscis arranged in two spiral rows of six hooks in each row; see Bilqees, 1970); *Gorytocephalus* Nickol & Thatcher, 1971 (proboscis armed with three circular rows of six hooks each; see Nickol & Thatcher, 1971); *Hexaspiron* Dollfus & Golvan, 1956 (proboscis armed with 6 hooks in each circle; see Golvan, 1988); *Microsentis* Martin & Multani, 1966 (proboscis armed with 16–20 longitudinal rows of hooks with 5–7 hooks per row; see Martin & Multani, 1966); *Neoechinorhynchus* (proboscis armed with three circles of hooks of six hooks in each circle; see Amin, 2002); *Octospinifer* Van Cleave, 1919

(proboscis with three circles of eight hooks each; see Van Cleave & Haderlie, 1950); *Octospiniferoides* Bullock, 1957 (proboscis armed with three circles of 8–10 hooks each; see Bullock, 1957); *Paraechinorhynchus* Bilqees & Khan, 1983 (proboscis with three spiral rows of four, six and eight hooks in each row; see Bilqees & Khan, 1983); *Paulisentis* Van Cleave & Bangham, 1949 (proboscis with six diagonal rows of five hooks each; see Van Cleave & Bangham, 1950 and *Zeylonechinorhynchus* Fernando & Furtado, 1963 (proboscis with seven oblique rows of four hooks in each row; see Fernando & Furtado, 1963). *Mayarhynchus* n. g. can be readily distinguished from all these genera by the number of rows and total number of hooks on the proboscis: 45–46 hooks arranged in nine longitudinal rows of five hooks each.

Mayarhynchus karlae n. sp.

Type-host: *Thorichthys ellioti* Meek (Perciformes: Cichlidae) (vernacular name ‘falso boca de fuego’).

Other hosts: *Paratheraps fenestratus* (Günther) (vernacular name ‘mojarra negra’); *Mayaheros urophthalmus* (Günther) (vernacular name ‘cíclido Maya’) and *Petenia splendida* (Günther), (vernacular name ‘tenguayaca’) (all Perciformes: Cichlidae).

Type-locality: Tlacotalpan (18°41′04.29″N, 95°38′47.47″W), River Papaloapan, Veracruz, Mexico.

Other localities: Silvituc Lagoon (18°37′33.06″N, 90°17′10.15″W), Campeche; River Champoton, (19°16′43″N, 90°37′26″W), Campeche, Mexico.

Site in host: Intestine.

Infection parameters: *T. ellioti*: prevalence: 36.66% (11 out of 30 infected), intensity: range 1–11, mean 2.5 worms per infected fish; *P. fenestratus*: prevalence: 20% (1 out of 5 infected), intensity: 1 worm in a fish from River Papaloapan; *M. urophthalmus*: prevalence 20% (1 out of 5 infected), intensity: 1 worm in a fish from Silvituc Lagoon, Campeche.

Type-material: The holotype (10289), the allotype (10290) and 16 paratypes (10291) have been deposited in the Colección Nacional de Helminthos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de México.

Representative DNA sequences: KY077083–KY077086 (*cox1*); KY077098–KY077102 (ITS1 + 5.8S + ITS2); and KY077066–KY077069 (28S).

ZooBank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the *International Code of Zoological Nomenclature* (ICZN, 2012), details of the new genus and new species have been submitted to ZooBank. The LSID for *Mayarhynchus* n. g. is urn:lsid:zoobank.org:act:D163BA61-CEC6-41F4-8A8C-49E086D04B2E and the LSID for *Mayarhynchus karlae* n. sp. is urn:lsid:zoobank.org:act:6B0137D2-B357-4DB8-A4EB-89E544643445.

Etymology: The species name refers to Karla Pinacho Pérez, daughter of the first author.

Description (Figs. 1–3)

General. [Based on 18 specimens ex *T. ellioti* in Tlacotalpan, including 4 specimens studied under SEM.] With characters of the genus. Small fusiform worms, wider at middle, with arched trunk usually

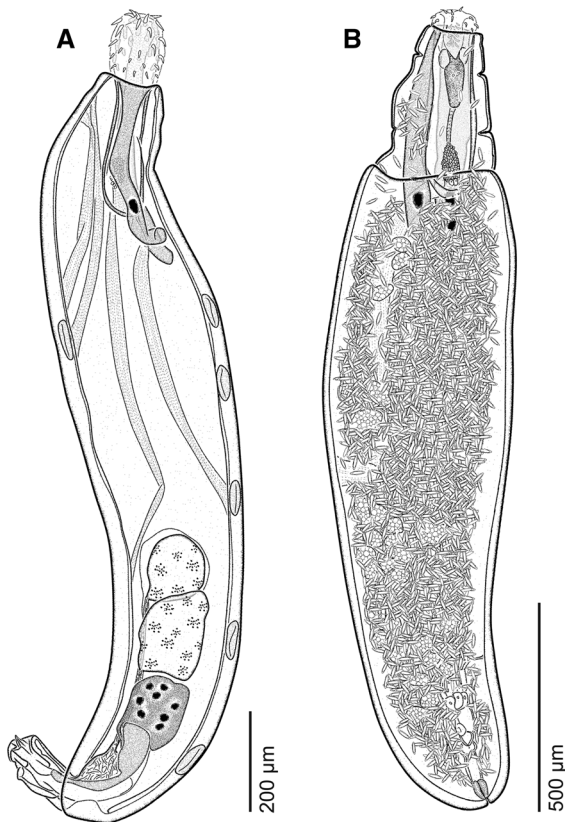


Fig. 1 *Mayarhynchus karlae* n. sp. ex *Thorichthys ellioti*. A, Male, whole mount, lateral view of the holotype; B, Female, whole mount, lateral view of the allotype

posteriorly, gradually tapering at both extremities. Body wall with 5 dorsal and 1 ventral giant hypodermal nuclei. Proboscis small, with an apical organ, cylindrical, slightly longer than wide, armed with 45–46 hooks arranged in 9 longitudinal rows of 5 hooks each. Hooks near tip of proboscis slightly larger than those in middle region and at base. All hooks rooted; roots simple, slender, blade-like, shorter than hooks, directed posteriorly. Neck prominent, markedly wider posteriorly. Proboscis receptacle single-walled, much larger than proboscis. Lemnisci of different length, longer than proboscis receptacle, narrow anteriorly, becoming strongly undulated and larger posteriorly, each with 1 prominent giant nucleus.

Male [Based on 6 mature males.] Total body length 1,772–2,366 (1,961), maximum width 317–437 (346). Trunk 1,360–2,167 (1,724) long, 317–437 (345) wide. Proboscis 98–142 (128) long, 83–107 (99) wide. Hooks near tip of proboscis 23–32 (28) long, 7–9 (8) wide; middle region hooks 19–31 (24) long, 5–8 (6) wide; hooks at base 11–18 (14) long, 4–5 (5) wide. Neck length 17–39 (29), maximum width 97–112 (105). Proboscis receptacle 113–307 × 21–108 (252 × 79). Longer lemniscus 212–638 (417) long, 37–81 (51) wide; shorter lemniscus 195–501 (365) long, 29–80 (47) wide. Reproductive system in posterior half of trunk; testes in tandem. Anterior testis 115–487 × 115–299 (248 × 168); posterior testis 161–284 × 123–290 (208 × 169). Cement gland 116–305 × 114–247 (211 × 164), with 8 giant nuclei; cement gland reservoir 86–225 × 53–159 (137 × 96). Seminal vesicle 91–172 × 28–71 (132 × 51). Bursa 141–188 (159) long.

Female. [Based on 2 gravid and 10 immature females.] Total body length of gravid females 1,741, maximum width 331–463 (397); body of juveniles 1,755–3,334 (2,310) long by 339–689 (492) wide. Trunk of gravid females 1,613–1,909 (1,761) long, 331–463 (397) wide; trunk of juveniles 1,530–3,196 (2,238) long, 339–689 (492) wide. Proboscis of gravid females 110 long, 80 wide; proboscis of juveniles 118–195 (145) long, 108–132 (118) wide. Hooks near tip of proboscis of gravid females 29 long, 9 wide; hooks near tip of proboscis of juveniles 25–33 (29) long, 4–9 (7) wide; middle region hooks of gravid females 23 long, 6 wide; middle region hooks of juveniles 20–26 (23) long, 5–7 (6) wide; hooks at base of gravid females 13

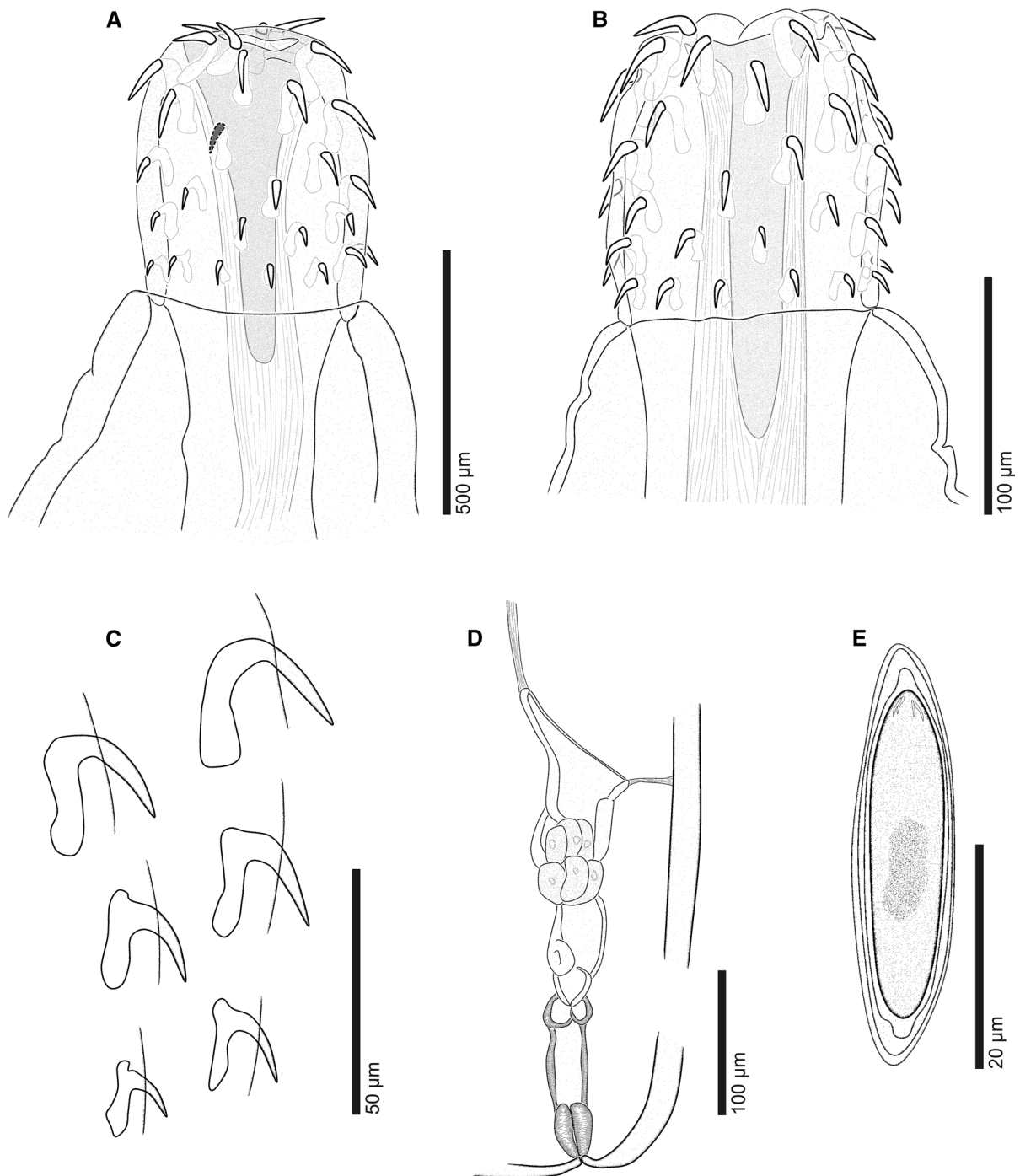


Fig. 2 *Mayarhynchus karlae* n. sp. ex *Thorichthys ellioti*. A, Male proboscis armature (holotype, lateral view); B, Female proboscis armature (paratype, ventral view); C, Hooks of male proboscis (holotype, lateral view); D, Female reproductive system (paratype), lateral view; E, Mature egg with acanthor

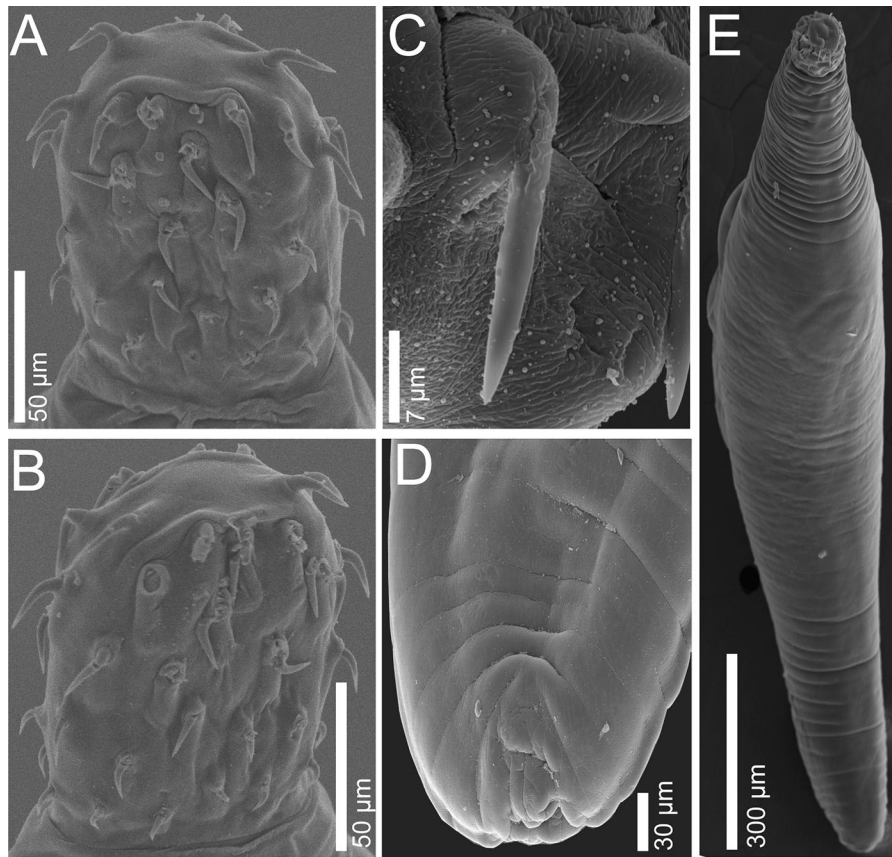


Fig. 3 Scanning electron microscopy of *Mayarhynchus karlae* n. sp. A, Male proboscis armature, ventral view; B, Male proboscis armature, dorsal view; C, Hook of male proboscis; D, End of the body of male; E, Female body, ventral view

long, 4 wide; hooks at base of juveniles 13–16 (14) long, 4–5 (4) wide. Neck length of gravid females 24, maximum width 95; neck length of juveniles 21–33 × 105–132 (27 × 116). Proboscis receptacle of gravid females 222–381 × 74–123 (302 × 99); proboscis receptacle of juveniles 251–453 × 88–142 (321 × 114). Longer lemniscus of gravid females 287–477 × 49–50 (382 × 49), longer lemniscus of juveniles 250–738 × 32–81 (477 × 55); shorter lemniscus of gravid females 264–443 × 50–59 (353 × 55); shorter lemniscus of juveniles 193–689 × 38–75 (466 × 56). Uterine bell of gravid females 95–119 × 39–60 (107 × 50); uterine bell of juveniles 76–222 × 46–77 (159 × 64). Uterus of gravid females 158–167 × 17–25 (163 × 21); uterus of juveniles 161–342 × 14–36 (241 × 25). Vagina of gravid females 16–23 × 20–26 (19 ×

23); vagina of juveniles 18–41 × 25–41 (30 × 31). Eggs 30–39 × 9–11 (36 × 10).

Remarks

Mayarhynchus karlae n. sp. is designated as the type-species of the new genus. The validity of this new species is supported by both morphological and molecular evidence. This species is characterised by the morphological characters of the new genus (see above) that sets it apart from any other member of Neoechynorhynchidae described to date. The type-host of *M. karlae* n. sp. is *T. ellioti*, a freshwater fish endemic to Papaloapan River basin, Mexico. In addition, this acanthocephalan was found in *P. fenestratus*, *M. urophthalmus* and *P. splendida*, three

species of freshwater fishes widely distributed in Middle-America.

Molecular data

Partial sequences of three molecular markers were obtained for five specimens of the new species from three host species in two localities of southeastern Mexico. The *cox1* sequences of three specimens from *T. ellioti* and one from *P. fenestratus* showed nine variable sites. The ITS and 28S sequences from Tlacotalpan (three in *T. ellioti* and one in *P. fenestratus*) and Silvituc Lagoon (one in *M. urophthalmus*) were submitted to searching in nucleotide Blast. These sequences showed similarities of 99 and 100% with a species identified as *Octospiniferoides* sp. from the cichlid *Petenia splendida* from Champoton River, Mexico (GenBank: FJ388978 for ITS and FJ388997 for 28S; data otherwise unpublished). Sequences of the ITS and 28S for *M. karlae* n. sp. were 685 bp (11 variable sites) and 754 bp (four variable sites) long, respectively.

The phylogenetic analyses the *cox1* dataset (546 bp long) included 26 sequences of *Neoechinorhynchus* spp., one of *Floridosentis mugilis* retrieved from GenBank, and two of *Atactorhynchus duranguensis* obtained in this study. One sequence of *Polyacanthorhynchus caballeroi* Diaz-Ungria & Rodrigo, 1960 and two of *Polyacanthorhynchus* sp. were used as the outgroup. The ITS dataset (c.971 bp) included 21 sequences of *Neoechinorhynchus* spp., two of *F. mugilis*, three of *A. duranguensis* and one sequence of *Acanthosentis cheni* Amin, 2005 used as the outgroup. Finally, the 28S dataset (c.834 bp long) included 29 sequences of *Neoechinorhynchus* spp., two of *F. mugilis*, two of *F. pacifica* and three of *A. duranguensis*. One sequence of *P. caballeroi* was used as the outgroup (Table 1).

Figure 4 shows the phylogenetic hypotheses yielded by BI analyses of the 28S, *cox1* and ITS separately, and the combined datasets (28S + *cox1*). The new species was found to be reciprocally monophyletic in all analyses, with strong nodal support of posterior probabilities in all trees. Regardless of the number the species included in each analysis, the phylogenetic relationships among species were largely incongruent in the four trees, showing that sampling of members of the family is still incomplete. However,

all analyses showed that *Mayarhynchus* n. g. and the genera *Floridosentis* and *Atactorhynchus* are nested within *Neoechinorhynchus*, suggesting that species in this genus distributed in freshwater fishes and turtles of Middle and North America might not be monophyletic; this is not unexpected considering that *Neoechinorhynchus* is the most species-rich genus within the Acanthocephala, with more than 113 species (Amin, 2013).

Genetic divergence values between *M. karlae* n. sp. and species of the other three genera of the Neoechinorhynchidae was very high, ranging between 23.4–44.3% for 28S, 36.3–69.5% for *cox1*, and 43.1–60.7% for ITS with respect to *Neoechinorhynchus* spp.; 44.5–45.7% for 28S, 42.4–43.6% for *cox1*, and 65.1–66.9% for ITS with respect to *Floridosentis* spp.; and 38.7–39.4% for 28S, 57.6–58.6% for *cox1*, and 58.4–60.2% for ITS with respect to *Atactorhynchus* spp. Furthermore, the divergence within *Neoechinorhynchus* ranged between 2.3–46.8% for 28S, 9.7–59.2% for *cox1*, and 4.7–61.9% for ITS.

Discussion

The new genus erected here is morphologically very distinct from the other 17 genera allocated into the family Neoechinorhynchidae. The most important feature of this genus, which had never been observed in other Middle-American acanthocephalans is the proboscis armed with 45–46 hooks in nine longitudinal rows of five hooks each. In Middle-America 16 species representing seven genera of the Neoechinorhynchidae have been reported in aquatic vertebrates, i.e. *Atactorhynchus duranguensis* in *Cyprinodon meeki* from northern Mexico (Salgado Maldonado et al., 2005; Martínez-Aquino & Aguilar-Aguilar 2008; Pérez-Ponce de León et al., 2009; Aguilar-Aguilar et al., 2014, 2015); *Floridosentis mugilis* and *F. pacifica* in mullets from Mexico (Rosas-Valdez et al., 2012); *Gracilisentis gracilisentis* (Van Cleave, 1913) in *Dorosoma cepedianum* (Lesueur) and *D. petenense* (Günther) from Nuevo León and Veracruz, Mexico; *Microsentis wardae* Martin & Multani, 1966 in *Gillichthys mirabilis* Cooper from Baja California Sur, Mexico; *Octospiniferoides chandleri* in *Petenia splendida*, *Parachromis friedrichsthalii*, *Poecilia petenensis* Günther and

Table 1 List of taxa included in the phylogenetic analyses

	Host	Locality	GenBank		References
			28S	ITS	
<i>Mayarhynchus</i> n. g.					
<i>Mayarhynchus karlae</i> n. g., n. sp.	<i>Thorichthys ellioti</i> Meek	Tlacotalpan, Veracruz, Mexico	KY077066– KY077068*	KY077083– KY077085*	KY077098– KY077100*
	<i>Paratheraps fenestratus</i> (Günther)	Tlacotalpan, Veracruz, Mexico	–	KY077086*	KY077101*
	<i>Mayaheros urophthalmus</i> (Günther)	Silvituc Lagoon, Campeche, Mexico	KY077069*	–	KY077102*
<i>Octospiniferoides</i> sp.**	<i>Petenia splendida</i> (Günther)	River Champoton, Campeche, Mexico	FJ388997	–	FJ388978
<i>Neoechinorhynchus</i> spp.					
<i>N. (N.) roseum</i> Salgado-Maldonado, 1978	<i>Citharichthys gilberti</i> Jenkins & Evermann	La Tovar, Nayarit, Mexico	FJ389000	JN830868	FJ388981
	<i>Achirus mazatlanus</i> (Steindachner)	Caimanero Lagoon, Sinaloa, Mexico	FJ388999	JN830867	FJ388980
<i>N. (N.) panucensis</i> Salgado-Maldonado, 2013	<i>Herichthys cyanoguttatus</i> Baird & Girard	River Purificacion, Tamaulipas, Mexico	KY077070– KY077072*	–	–
	<i>Herichthys</i> sp.	River Pantepec, Veracruz, Mexico	KR086335	KY077087*	KY077103*
<i>N. (N.) golvani</i> Salgado-Maldonado, 1978	<i>Paratheraps fenestratus</i> (Günther)	Lake Catemaco, Veracruz, Mexico	FJ388986; KR086272	KY077088*; JN830852	FJ388967; KC004224
<i>N. (N.) chimalapasensis</i> Salgado-Maldonado, Caspeta-Mandujano & Martínez-Ramírez, 2010	<i>Awacous banana</i> (Valenciennes)	River Negro, Santa Maria Chimalapa, Oaxaca, Mexico	KR086337; KR086336	KY077089*; KY077090*	KY077104*; KY077105*
<i>N. (N.) brentnickoli</i> Monks, Pulido-Flores & Violante-González, 2011	<i>Dormitator latifrons</i> (Richardson)	Tres Palos Lagoon, Guerrero, Mexico	FJ388991; KR086219	KY077091*; JN830808	FJ388972; KC004184
<i>N. (N.) mexicoensis</i> Pinacho-Pinacho, Sereno-Urbe & García-Varela, 2014	<i>Dormitator maculatus</i> (Bloch)	River Papaloapan, Tlacotalpan, Veracruz, Mexico	KR086299; KR086301	KY077092*; KY077093*	KY077106*; KY077107*
<i>N. (N.) mamesi</i> Pinacho-Pinacho, Pérez-Ponce de León & García-Varela, 2012	<i>Dormitator latifrons</i> (Richardson)	Joaquin Amaro Lagoon, Chiapas, México	JN830772; JN830774	JN830800 JN830799	KC004193; KC004191
<i>N. (N.) schmidti</i> Barger, Thatcher & Nickol, 2004	<i>Trachemys scripta</i> (Thunberg)	Centla, Tabasco, Mexico	HQ634786; HQ634785	–	KC004172; KC004173

Table 1 continued

	Host	Locality	GenBank		ITS		References
			28S	cox1			
<i>N. (N.) emyditoides</i> Fisher, 1960	<i>Trachemys scripta</i> (Thunberg)	Lake Catemaco, Veraacruz, Mexico	HQ634781; KR086315	KY077094*; KY077095*	KY077108*; KY077109*	This study	
<i>N. (N.) saginata</i> Van Cleave & Bangham, 1949	<i>Trachemys scripta</i> (Thunberg)	Purificacion River, Tamaulipas, Mexico	KY077082*;	-	-	This study	
<i>N. (N.) cyllindratas</i> (Van Cleave, 1914)	<i>Micropterus salmoides</i> (Lacépède)	River Purificacion, Tamaulipas, Mexico	AY829091	DQ089704	FJ388984	García-Yarela & Nadler 2005	
<i>N. (N.) salmonis</i> Ching, 1984	<i>Salvelinus malma</i> (Walbaum)	Lake Chistoe	KY077073- KY077076*	-	-	This study	
<i>N. (N.) tumidus</i> Van Cleave & Bangham, 1949	<i>Salvelinus alpinus</i> (Linnaeus)	Lake L'distoe; Lake Engteri, Russia	-	KF156887; KF156886	-	Malyarchuk et al. (2014)	
<i>N. (N.) beringianus</i> Mikhailova & Atrashkevich, 2008	<i>Pungitius pungitius</i> (Linnaeus)	Lake Chernoe; Grand Lake, Russia	-	KF156882	-	Malyarchuk et al. (2014)	
<i>N. (N.) simansularis</i> Roitman, 1961	<i>Salvelinus alpinus</i> (Linnaeus)	Lake Engteri, Russia	-	KF156890	-	Malyarchuk et al. (2014)	
<i>Neoechinorhynchus</i> sp.	<i>Coregonus nasus</i> (Pallas); <i>Prosopium cylindraceum</i> (Pennant); <i>Coregonus lavaretus</i> (Linnaeus)	River Kolymsa; Lake Rybnoe; River Yana, Russia	-	KF156884; KF156888; KF156885; KF156883	-	Malyarchuk et al. (2014)	
<i>Neoechinorhynchus</i> sp.	-	-	KY077077*; KY077078*	-	KY077110*; KY077111*	This study	
<i>Hebesoma violentum</i> Van Cleave, 1928	<i>Perccottus glenii</i> Dybowski	Primorye Region, Russia	HQ634789	-	KY077112*	García-Yarela et al. (2011)	
<i>Floridosentis</i> spp.	<i>Mugil cephalus</i> Linnaeus	Sontecomapan Lagoon, Veraacruz, Mexico	-	KF156893	-	Malyarchuk et al. (2014)	
<i>F. pacifica</i> Bravo-Hollis, 1969	<i>Mugil curema</i> Valenciennes	Tres Palos Lagoon, Guerrero, Mexico	JQ436497; JQ436495	DQ089723	KC004179; KC004178	Rosas-Valdez et al. (2012)	
<i>Atactorhynchus</i> spp.	<i>Cyprinodon meeki</i> Miller	Spring in the town 27 de Noviembre, Durango, Mexico	JQ436531; JQ436533	-	-	Rosas-Valdez et al. (2012)	
<i>Atactorhynchus duranguensis</i> Salgado-Maldonado, Aguilar-Aguilar & Cabañas-Carranza, 2005	-	-	KY077079- KY077081*	KY077096*; KY077097*	KY077113- KY077115*	This study	

Table 1 continued

	Host	Locality	GenBank		ITS		References
			28S	cox1	28S	ITS	
Outgroup							
<i>Polyacanthorhynchus caballeri</i> Diaz-Ungria & Rodrigo, 1960	<i>Caiman yacare</i> Daudin	-	DQ089738	DQ089724	-	-	García-Varela & Nadler (2005)
<i>Polyacanthorhynchus nigerianus</i> Echi, Suresh, Sanil, Iyaji, Nwani & Ejere, 2015	<i>Synodontis batensoda</i> Rüppell	Nigeria	-	KC904074	-	-	Echi et al. (2015)
<i>Polyacanthorhynchus echiyensis</i> Echi, Suresh, Sanil, Iyaji, Nwani & Ejere, 2015	<i>Synodontis batensoda</i> Rüppell	Nigeria	-	KC904075	-	-	Echi et al. (2015)
<i>Acanthosentis cheni</i> Amin, 2005	<i>Coilia nasus</i> Temminck & Schlegel	Yangtze River system, China	-	-	-	JX960752	Song et al. (2014)

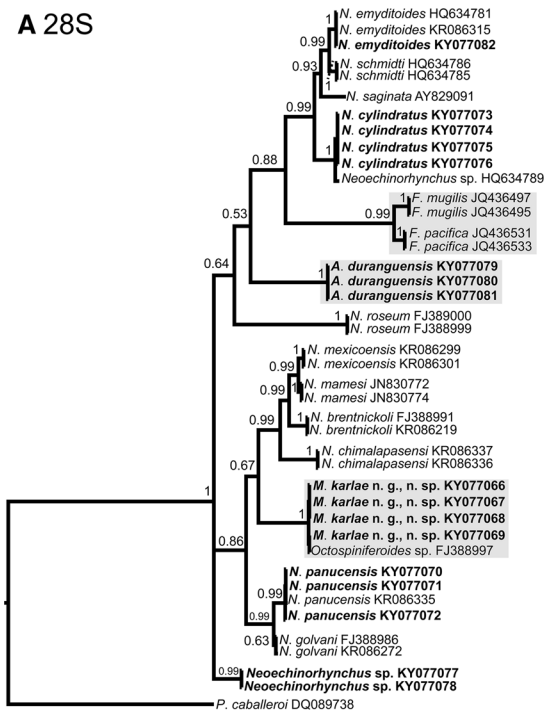
*Sequences generated in this study

**Source for sequences misidentified as *Octospiniferoides* sp.

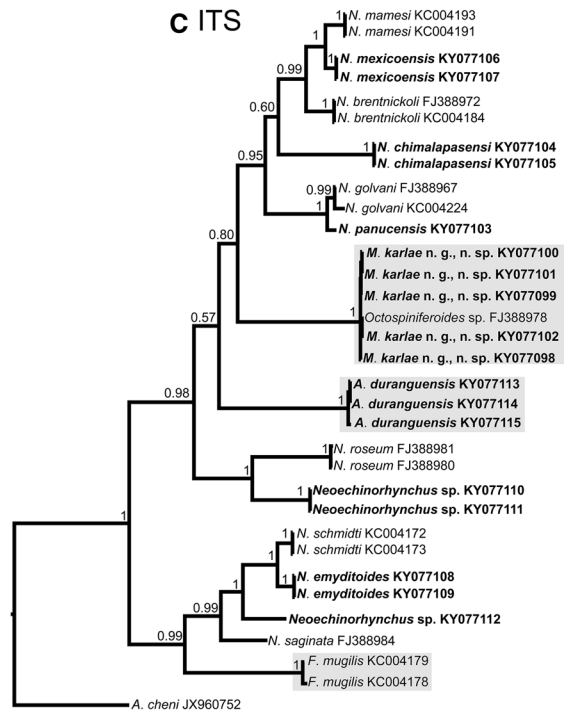
Pseudoxiphophorus bimaculatus Regan from south-eastern Mexico; *Paulisentis* sp. in *Gambusia marshi* Minckley & Craddock from Coahuila, Mexico (García-Prieto et al., 2010), and nine species of *Neoechinorhynchus* from freshwater fishes and turtles (see Pinacho-Pinacho et al., 2015). Unfortunately, only some species of the species-rich genus *Neoechinorhynchus*, two of *Floridosentis* and one of *Octospiniferoides* have been sequenced for at least one molecular marker. Sequences of *Octospiniferoides* sp. were identical with the sequences generated in this study, at least for the nuclear markers. These sequences are conspecific with the new species, and when taken with our observations on the morphology of the genus *Octospiniferoides* it is clear that there had been a misidentification of the specimens and that they should be referred to *M. karlae* despite there being no voucher specimens available for examination.

Bayesian phylogenetic analyses of all molecular markers analysed separately, and that of the combined *cox1* + 28S dataset show that the new genus represents an independent evolutionary unit, possessing a high nodal support and exhibiting high genetic divergence values in comparison with the other genera of the Neoechinorhynchidae for which sequence data are available. In all trees (Fig. 4), the new genus nests within a clade comprising six species of *Neoechinorhynchus* from Middle-American fishes, i.e. *N. (N.) mamesi* Pinacho-Pinacho, Pérez-Ponce de León & García-Varela, 2012, *N. (N.) mexicoensis* Pinacho-Pinacho, Sereno-Urbe & García-Varela, 2014, *N. (N.) golvani*, *N. (N.) chimalapasensis* Salgado-Maldonado, Caspeta-Mandujano & Martínez-Ramírez, 2010, *N. (N.) brentnickoli* Monks, Pulido-Flores & Violante-González, 2011 and *N. (N.) panucensis*. In most trees the topology is highly congruent, with the new genus as the sister group of all the other species, although in the 28S tree (Fig. 4) the sister group relationships are different. As an aside result of the present investigation, and being aware of the fact that the taxon sampling of this acanthocephalan group is still incomplete, we found that the species of *Neoechinorhynchus* for which sequences are available were not recovered as a monophyletic assemblage by any of the analyses using different molecular markers. This requires further investigation, although considering the high species diversity of the genus, it is likely that it does not represent a monophyletic group. Further sequencing work of more congeneric species, as well

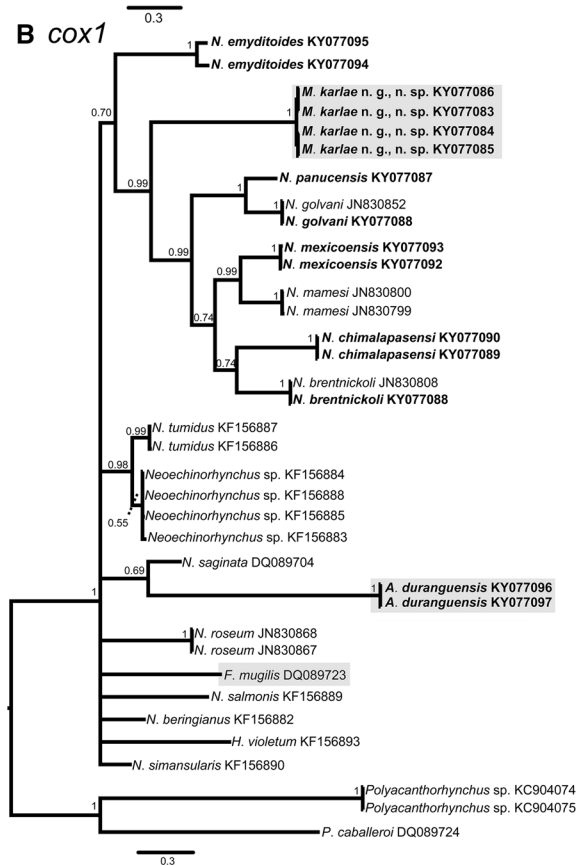
A 28S



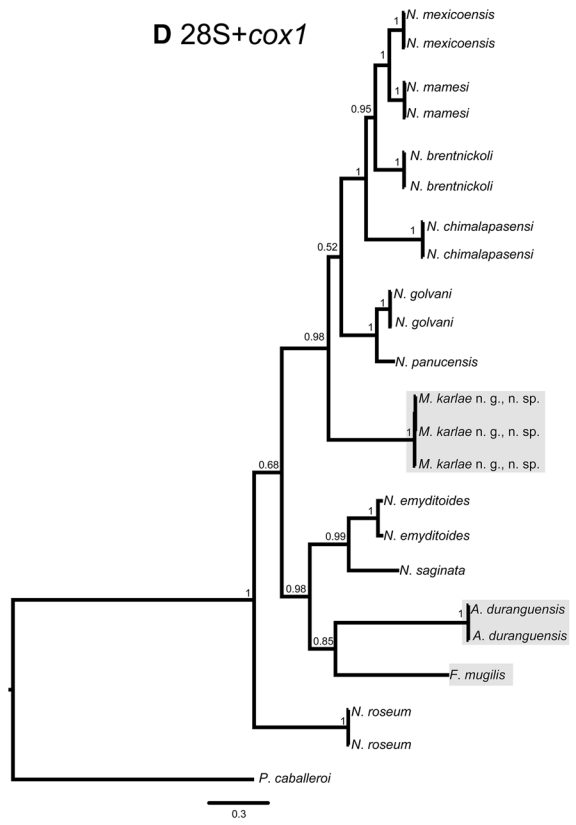
c ITS



B COX1



D 28S+cox1



◀ **Fig. 4** Phylogenetic hypothesis for the new genus. Phylogenetic trees inferred through Bayesian Inference (BI) of: A, the 28S dataset; B, the *cox1* dataset; C, the ITS dataset; and D, the combined 28S + *cox1* dataset. Numbers near internal nodes show the posterior probability clade frequencies. The scale-bars indicate the number of substitution per site. The newly generated sequences are indicated in bold

as sequencing of members of the other recognised genera within the family will be fundamental to corroborate this hypothesis, with the potential need to describe new genera to accommodate species and reach a stable classification scheme for this group of acanthocephalans.

The present erection of a new genus with a new species within the family Neoechinorhynchidae with unique morphological characteristics shows that the diversity of acanthocephalans in freshwater fishes across Middle America is still poorly known, and it is possible that some additional species will be described in the near future, even though that the inventory of this parasitic group is nearing completion, at least in Mexico (see Pérez-Ponce de León & Choudhury, 2010). Information on acanthocephalan diversity, especially in Middle America, is fragmentary, since just a few fish species have been studied for this group of parasites. Acanthocephalans are notably species poor in Middle America and the reason for that remains unclear (see Choudhury et al., 2016). The description of acanthocephalan diversity require the use of molecular data, since studies that follow an integrative taxonomy approach hold the best promise to obtain a more accurate estimate of parasite biodiversity and, as shown in this case, sequences from species of other genera of the Neoechinorhynchidae will be very helpful to understand the phylogenetic relationships among the genera and, based on that framework, reach a more stable classification system for the group.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable institutional, national and international guidelines for the care and use of animals were followed. Hosts were collected under the Cartilla Nacional de Colector Científico de Flora y Fauna Silvestre FAUT 0202 issued by the Secretaria del Medio Ambiente y Recursos Naturales (SEMARNAT), to MGv.

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