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A morphological and molecular study of *Pseudocorynosoma* Aznar, Pérez Ponce de León and Raga 2006 (Acanthocephala: Polymorphidae) from Mexico with the description of a new species and the presence of cox 1 pseudogenes

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ABSTRACT

Pseudocorynosoma tepehuanesi n. sp., is described from the intestine of the ruddy duck *Oxyura jamaicensis* Gmelin, 1789 from single locality from northern Mexico. The new species is mainly distinguished morphologically from the other five described species of *Pseudocorynosoma* from the Americas (*P. constrictum*, type species, *P. peposacae*, *P. anatarium*, *P. enrietti* and *P. iheringi*) associated with waterfowl species by possessing a proboscis with 15 longitudinal rows with 7–8 hooks each, a trunk expanded anteriorly and by having smaller lemniscus. Partial sequences of the mitochondrial gene cytochrome *c* oxidase subunit I (cox 1) and the large subunit (LSU) of ribosomal DNA including the domains D2 + D3 were used independently to corroborate the morphological distinction between the new species and other two congeneric species (*P. constrictum* and *P. anatarium*) from North America. The genetic divergence estimated among the new species and the other two species ranged from 15 to 18% for cox 1 and from 3.2 to 4% for LSU. The cox 1 alignment shows 24 sequences from *P. anatarium* with abnormalities, which were defined as pseudogenes due the presence of insertions, deletions and premature stop codons. Maximum likelihood and Bayesian inference analyses with each data set showed that the acanthocephalans from ruddy duck represent an independent clade with strong bootstrap support and posterior probabilities. The phylogenetic tree inferred with cox 1 gene placed all the pseudogenes from *P. anatarium* in single clade suggesting that those genes arose after speciation process within genus *Pseudocorynosoma*. The morphological evidence, plus the monophyly in both phylogenetic analyses indicate that the acanthocephalans collected from intestine of the ruddy duck from northern Mexico represent a new species.

1. Introduction

Member of the genus *Pseudocorynosoma* Aznar, Pérez Ponce de León and Raga 2006, are endoparasites that use waterfowl as definitive hosts and amphipods as intermediate hosts to complete their life cycle distributed in the America [1–4]. Morphologically, *Pseudocorynosoma* is distinct from other genera of Polymorphidae Meyer, 1931 by having spines covering the anterior part of the trunk, an ovoid or cylindrical proboscis with slightly swollen region, a truncated cone-shaped neck, 4 to 6 tubular cement glands and spines surrounding genital pore. The eggs of all species have a prominent polar protrusion in the middle fertilization membrane. Based on these morphological features the genus *Pseudocorynosoma* currently comprises five species: *P. constrictum* Van Cleave, 1918 (type species), *P. peposacae* Porta, 1914, *P. anatarium* Van Cleave 1945, *P. enrietti* Molfi and Fernandes, 1953 and *P. iheringi* Machado Filho, 1961 [4–6]. In Mexico two species of the genus *Pseudocorynosoma* have been recorded, i.e., *P. constrictum* associate to 5 species of dabbling ducks (*Anas crecca* Linnaeus, 1758, *Anas cyanoptera* Vieillot, 1816, *Anas diazi* Ridgway, 1886, *Anas strepera* Linnaeus, 1758 and *Anas clypeata* Linnaeus, 1758) and 2 species of diving ducks (*Aythya affinis* Eyton, 1838 and *Aythya americana* Eyton, 1838), and *P. anatarium* from a diving duck (*Bucephala albeola* Linnaeus, 1758) [5,7].

As part of an ongoing survey of helminth parasites of waterfowl species in both biogeographical regions of Mexico (Fig. 1), we collected three species of *Pseudocorynosoma*, two of which (*P. constrictum* and *P. anatarium*) have been previously recorded in this country [5,7]. Adult acanthocephalans determined as *Pseudocorynosoma* sp., were collected from intestine of the ruddy duck (*Oxyura jamaicensis* Gmelin, 1789) from Guatimape, Durango in northern Mexico (see locality 4 in Fig. 1).

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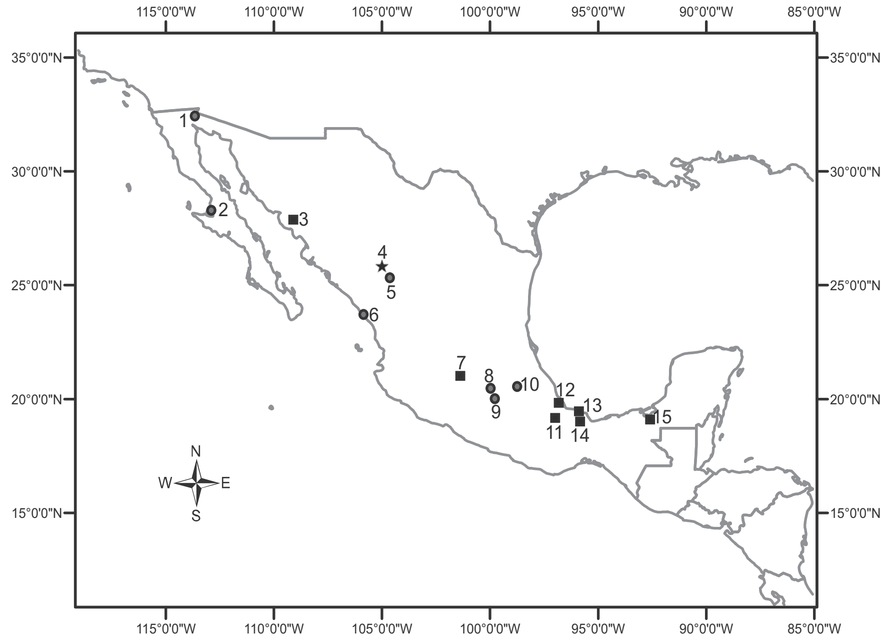

Fig. 1. Map of Mexico showing sampling sites for the waterfowl species. Localities with a circle were positive for the infection and with a square were negative for the infection with *Pseudocorynosma* spp. Type locality of *Pseudocorynosoma tepehuanesi* n. sp., is indicated by a start. 1. Cienaga de Santa Clara, Sonora; 2. Guerreo Negro, Baja California Sur; 3. La Esperanza, Sonora; 4. Guatimape Durango; 5. Nueva Ideal, Durango; 6. El Huizache, Sinalo; 7. Yuriria Guanajuato; 8. Ixtlahuca, Estado de México; 9. Almoloya del Rio, Estado de México; 10. Tecocomulco, Hidalgo; 11. Tuxtepec, Oaxaca; 12. El Bayo Veracruz; 13. Sontecomapan, Veracruz; 14. Catemaco, Veracruz; 15. Silvictuc, Campeche.

After of a morphological examination, these specimens represent an undescribed species, which is herein described and compared with the other 5 species of the genus. DNA sequences of two genes, the cytochrome oxidase subunit 1 (*cox 1*) of the mitochondrial DNA and the domains D2 and D3 of the Large Subunit (LSU) from nuclear ribosomal DNA were generated from *Pseudocorynosoma* sp., and compared with the other two species previously recorded in Mexico.

2. Materials and methods

2.1. Specimen collection and taxonomic identification

A total of 119 specimens from 15 waterfowl species from the genera: *Anas (A. clypeata*, *A. crecca*, *A. americana* Gmelin, 1789, *A. acuta* Linnaeus, 1758, *A. cyanoptera*, *A. strepera*, *A. diazi*, *A. platyrhynchos* Linnaeus, 1758 and *A. discors* Linnaeus, 1758) *Aythya* (*A. affinis*, *A. americana*, *A. collaris* Donovan, 1809, *A. marila* Linnaeus, 1761, and *A. valisineria* Wilson, 1814*) Anser* (*A. caerulescens* Linnaeus, 1758 and *A. albifrons* Scopoli, 1769) *Bucephala* (*B. albeola*), *Dendrocygna* (*D. bicolor* Vieillot, 1816 and *D. autumnalis* Linnaeus, 1758), and *Oxyura* (*O. jamaicensis*) were collected from 15 localities from both biogeographical regions of Mexico (Fig. 1). Birds were kept on ice and the digestive tract was excised and examined within 2 h after capture. Ducks and geese were identified using 2 field guides [8,9]. Of the 15 definitive hosts species examined only *Anas clypeata*, *A. crecca*, *A. americana*, *A. acuta*, *A. cyanoptera*, *A. diazi*, *A. discors*, *Aythya affinis*, *A. collaris*, *B. albeola* and *O. jamaicensis* were infected with *Pseudocorynosoma* spp. Acanthocephalans were relaxed in distilled water overnight and fixed in 70% ethanol, and stored at 4 °C.

For taxonomic identification, some specimens were stained with Mayer's paracarmine, dehydrated in a graded ethanol series, cleared with methyl salicylate, and mounted on permanent slides with Canada balsam. Illustrations of the specimens were made with the aid of a drawing tube. Measurements are given in micrometers (μm) unless otherwise stated and are presented as the mean, followed in parentheses by the ranges. Measurements of eggs were made from fully developed eggs ones measured in situ through the body wall of female worms.

Adult acanthocephalans of *P. constrictum* ($n = 2$), *P. anatarium* ($n = 2$) and *Pseudocorynosoma* sp. ($n = 2$) were placed individually in 4% formalin and dehydrated through a graded series of ethyl-alcohol and then critical point dried with carbon dioxide. The specimens were mounted on metal stubs with silver paste, coated with gold and examined in a Hitachi Stereoscan Model SU1510 at 10 kV to obtain micrographs of the proboscis, hooks and anterior trunk spines.

Specimens collected in the current study were deposited in the Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de Mexico, Mexico City, Mexico. For comparison, voucher material, deposited in the CNHE and in the Harold. W. Manter Laboratory of Parasitology, Nebraska (HWML) of the following specimens of *Pseudocorynosoma* were examined: *P. constrictum* (CNHE No. 5270, 5881, 6270–6271; HWML No. 34,108, 34,109, 34,714, 34,715, 34,716, 34,717, 34,718) and *P. anatarium* (CNHE No 5271, 10,197).

2.2. Isolation of genomic DNA

Eight specimens of *Pseudocorynosoma* sp., from ruddy duck, seven of *P. anatarium* from bufflehead duck and 15 specimens of *P. constrictum* from waterfowl species were placed individually in tubes and digested overnight at 56C in a solution containing 10 mM Tris–HCl (pH 7.6), 20 mM NaCl, 100 mM Na_2 EDTA (pH 8.0), 1% Sarkosyl, and 0.1 mg/ml proteinase K. Following digestion, DNA was extracted from the supernatant using the DNAzol reagent (Molecular Research Center, Cincinnati, Ohio) according to the manufacturer's instructions.

2.3. Amplification, cloning and sequencing of DNA

The two genes cox 1 and LSU including the domains D2 + D3 were amplified using the polymerase chain reaction (PCR). A partial fragment of 655 bp of the cytochrome *c* oxidase (cox 1) was amplified with the forward primer (507) 5′-AGTTCTAATCATAA(R)GATAT(Y)GG and reverse primer (588) 5′- TAAACTTCAGGGTGACCAAAAAATCA [10]. A partial fragment of approximately 820 bp that includes the domains D2 + D3 from LSU rDNA were amplified using the forward primer (502) 5′-CAAGTACCGTGAGGGAAAGTTGC 3′ and the reverse primer

(536) 5′-GTCGATAGGACTCCCTTTG 3′ [11]. PCR reactions (25 μl) consisted of 10 mM of each primer, 2.5 μl of buffer 10×, 50 mM $MgCl_2$, 0. 5 μl of dNTPS mixture (10 mM), 0.125 μl of Taq DNA polymerase (1 U/μl) (Platinum Taq DNA, Invitrogene Corporation, Brazil) and 2 μl of DNA. Thermocycling conditions included denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C for 1 min, annealing at 40/50 °C for 1 min by cox 1 and LSU respectively, and extension at 72 °C for 1 min, followed by a post-amplification incubation at 72 °C for 7 min. Each PCR product was cleaned up and filtered using Millipore columns (Amicon, Billerica, MA). PCR products were cloned by ligation into pGEM-T vector (Promega, Madison, Wisconsin) and used to transform competent *Escherichia coli* (JM109). Positive clones were identified by blue/white selection, and target insert size was confirmed by PCR of DNA extracts prepared from bacterial (clone) colonies. Liquid cultures for minipreps were grown in Luria broth (Lb) containing 50 μg/ml of ampicillin. Plasmids for DNA sequencing were prepared using commercial miniprep kits (Qiaprep, Qiagen, Valencia, California). Plasmids were sequenced for both DNA strands using two universal primers. Sequencing reactions were performed using ABI BigDye (PE Applied Biosystems, Boston, Massachusetts) terminator sequencing chemistry, and reaction products were separated and detected using an ABI 310 capillary DNA sequencer. Contigs were assembled and base-calling differences resolved using Codoncode version 5.1.5 (Codoncode Corporation, Dedham, MA). All sequences have been deposited in the Genbank dataset under numbers KX671827-KX671841 for LSU; KX688132-KX688148 for cox 1 and KX688108-KX688131 for the pseudogene.

2.4. Alignments

Sequences of *Pseudocorynosoma* spp., from cox 1 generated in this study were aligned using Clustal program [12] with other sequences of *Polymorphus trochus* Van Cleave, 1945 (JX442196), *Polymorphus minutus* Goeze, 1782 (EF467865), *Profilicollis altmani* Perry, 1942 (DQ089720) and *Profilicollis botulus* Van, Cleave 1916 (EF467862,

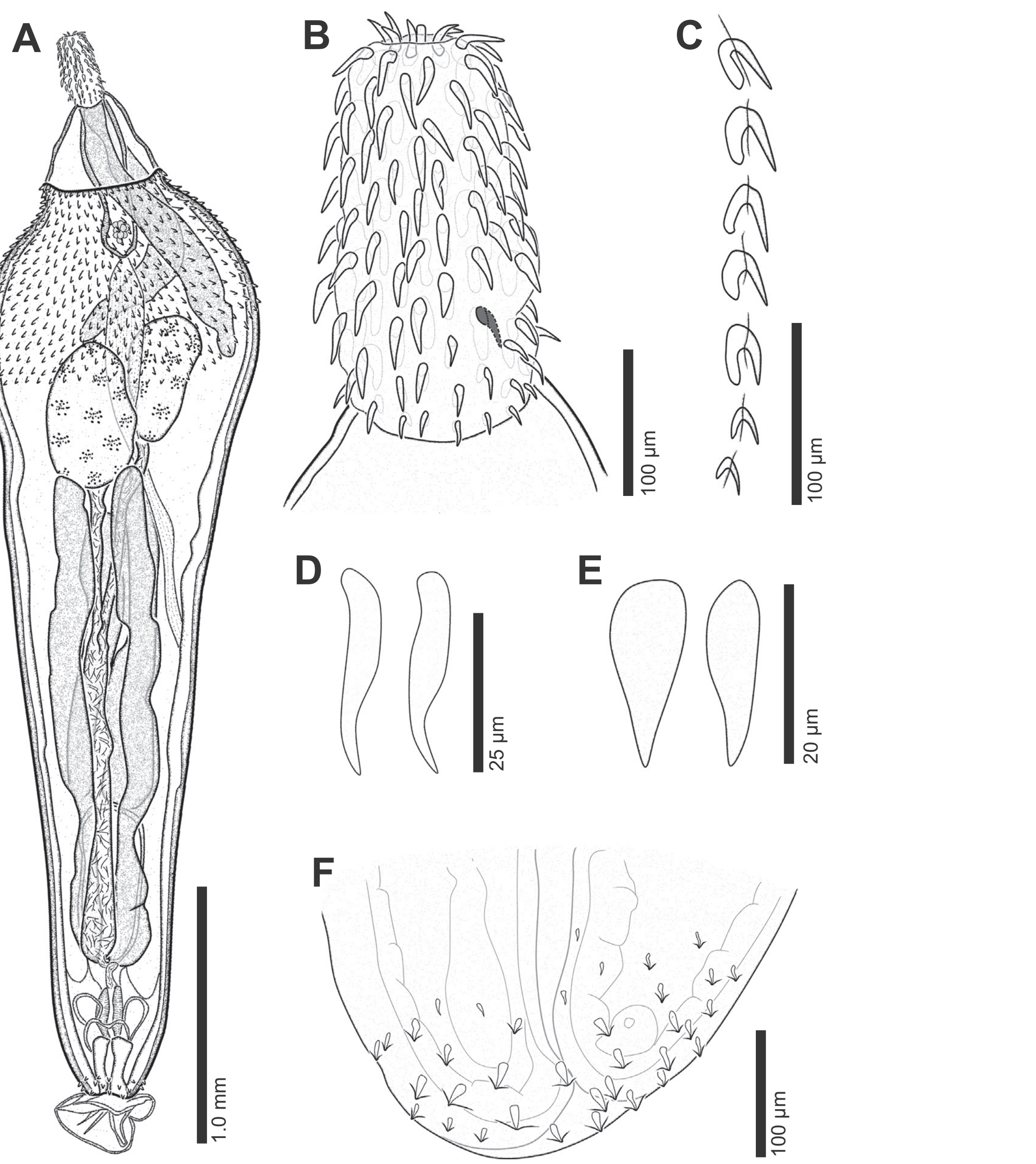

Fig. 2. *Pseudocorynosoma tepehuanesi* n. sp., from *Oxyura jamaicensis*. A. Adult male, whole worm (holotype), lateral view; B. Male proboscis armature (holotype), lateral view. Missing hooks have been reconstructed with a black shadowed area; C. Male row of hooks (holotype), lateral view; D. Male somatic spines (paratype), lateral view; E. Male genital spines (paratype), lateral view; F. Posterior end of a male showing complete spine armature (paratype), lateral view.

DQ089721) from Polymorphidae that were used as outgroup, due that these species are sister to *Pseudocorynosoma* in a previous phylogenetic analysis [5]. A second alignment was generated with the LSU sequences. This alignment contained 18 sequences of *Pseudocorynosoma* spp., plus other 4 sequences of *P. minutus* (EU267819), *P. altmani* (AY829108) and *P. botulus* (EU267818, AY829109) that were used as outgroup.

2.5. Phylogenetic analyses

The cox 1 and LSU alignments were analyzed independently. The akaike information criterion (AIC) was used to assess the fit of nucleotide substitution models for each alignment [13] through use of Modeltest version 3.0 [14]. The GTR model with invariable sites (+I), and rate heterogeneity (+G) [15], was determined to be the best fit for the alignments. Maximum likelihood analyses were conducted using parallel RAxML 7.2.7 [16], using GTR GAMMA for tree inference and the GTRCAT model approximation for bootstrapping, implemented via the CIPRES Science Gateway V. 3.1 [17]. Bayesian analyses were performed with the program MrBayes version 3.1.2 [18], with the GTR + I + G model. The settings were 2 simultaneous runs with 4 Markov chains and 10 million MCMC generations, sampling every 1000 generations, a heating parameter value of 0.2. The outputs of MrBayes were examined with Tracer Version 1.4 [19] to check for convergence of different parameters, and to determine the approximate number of generations at which log-likelihood values stabilized. A consensus tree was obtained after a conservative burn in of 25% was applied for each data set. Trees were drawn using FigTree version 1.3.1 [20]. The genetic divergence among taxa was estimated using uncorrected "p" distances with the program MEGA version 6 [21].

3. Results

3.1. Morphological description.

3.1.1. Pseudocorynosoma tepehuanesi n. sp. (Figs. 2–3).

General Polymorphidae, with characters of the genus *Pseudocorynosoma*. Living specimens of orange colour. Sexual

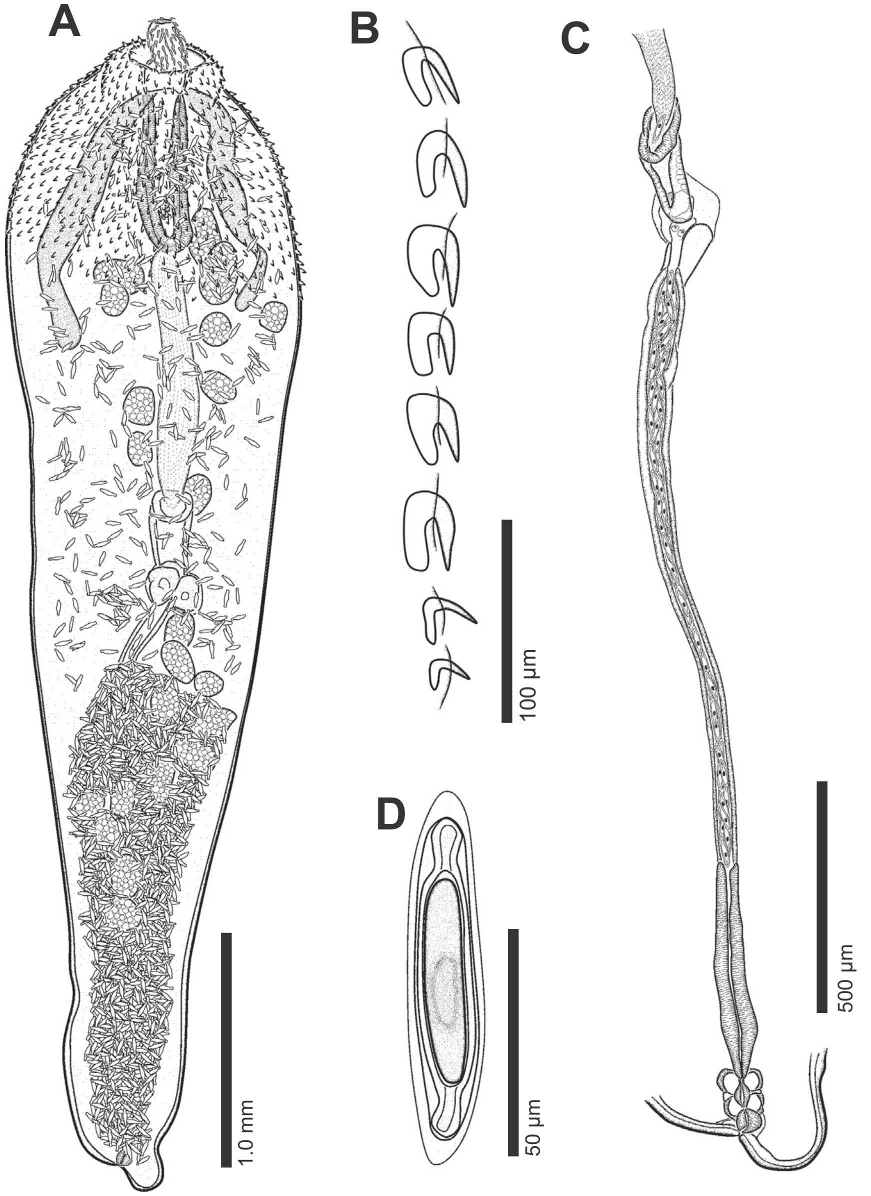

Fig. 3. *Pseudocorynosoma tepehuanesi* n. sp., from *Oxyura jamaicensis*. A. Adult female, whole worm (allotype), ventral view; B. Female row of hooks (paratype), lateral view; C. Female reproductive system (paratype), ventral view; D. Egg.

dimorphism evident females larger than males. Proboscis cylindrical armed with 15 longitudinal rows with 7–8 hooks per row; each row with 5–7 large rooted hooks, and 1–3 small basal hooks with small roots (Figs. 2-4) Measurements of hooks are presented in Table 1. Neck cone-shaped; Trunk expanded anteriorly (Figs. 2A, 5A); fore-trunk shorter, hind-trunk elongated posteriorly; spinose, single field, extending along ¾ of fore-trunk in males and females. Genital spines surround genital pore in males. Proboscis receptacle double-walled; cephalic ganglion sub-oval at its posterior end; lemnisci digitiform, longer than proboscis receptacle. Genital pore subterminal in both sexes.

Male (based on 8 mounted adult specimens, with sperm in the seminal vesicle and 1 for SEM). Trunk 3.8 mm (3.4–4.2 mm) × 801 (534–1.09); maximum width at hind-trunk level. Trunk spines 32 (31–34) × 9 (8–10). Proboscis 355 (315–445) × 140 (123–186). Neck 425 (359–480) × 481 (386–573). Proboscis receptacle 440 (324–590) × 178 (160–201). Lemnisci 856 (493–1.189 mm) × 107 (86–144) (Fig. 2). Testes ovoid, symmetrical, posterior to proboscis receptacle (Fig. 2A). Right testis 560 (489–632) × 301 (133–436). Left testis 556 (416–662) × 359 (247–416). Four tubular cement glands, 1285 (0.946–1.719 mm) × 160 (117–254). Säfftigen's pouch 663 (420–764) × 227 (189–262). Genital spines 20 (16–23) × 9 (8–10). Copulatory bursa 312 (239–433) × 412 (326–532) (Fig. 2A–F).

Female (based on 3 gravid mounted specimens and 1 for SEM). Trunk 4.6 mm (3.7–5.1 mm) × 896 (563–1.080); maximum width at hind-trunk level. Posterior end with distinctive rounded prolongation (Fig. 3 A, C). Trunk spines 34 (33–36) × 8 (8–9). Proboscis 337 (301–365) × 164 (143–178) (Fig. 3A). Neck 396 (302–490) × (336–618). Proboscis receptacle 696 (623–818) × 238 (219–256). Lemnisci 1.037 (805–1.195) × 99 (93–103). Uterine bell short with thick body wall; uterus long; vagina complex with four bulb connected to vagina; gonopore subterminal (Fig. 3C). Mature eggs, containing a fully developed acanthor, fusiform, with polar prolongations in middle fertilization membrane 82 (80–86) × 16 (17–32) (Fig. 3D).

3.1.2. Taxonomic summary

Type host: Oxyura jamaicensis Gmelin, 1789 (Aves: Anseriformes: Anatidae) ruddy duck.

Type locality: Guatimape, Durango, Mexico (24°49′45″N, 104°53′16″ N).

Site in the host: Intestine.

Type material: Holotype CNHE: No. 10,194; allotype CNHE: No. 10,195; paratypes CNHE: No. 10,196.

Prevalence: 100% (9 birds examined).

Etymology: the species is named after the Tepehuanes, an indigenous ethnic group inhabiting the northern Mexico, in the state of Durango, where the type locality is located.

3.1.3. Remarks

The new species belongs to the genus *Pseudocorynosoma* based on the distribution of somatic spines in the hind-trunk, and by having

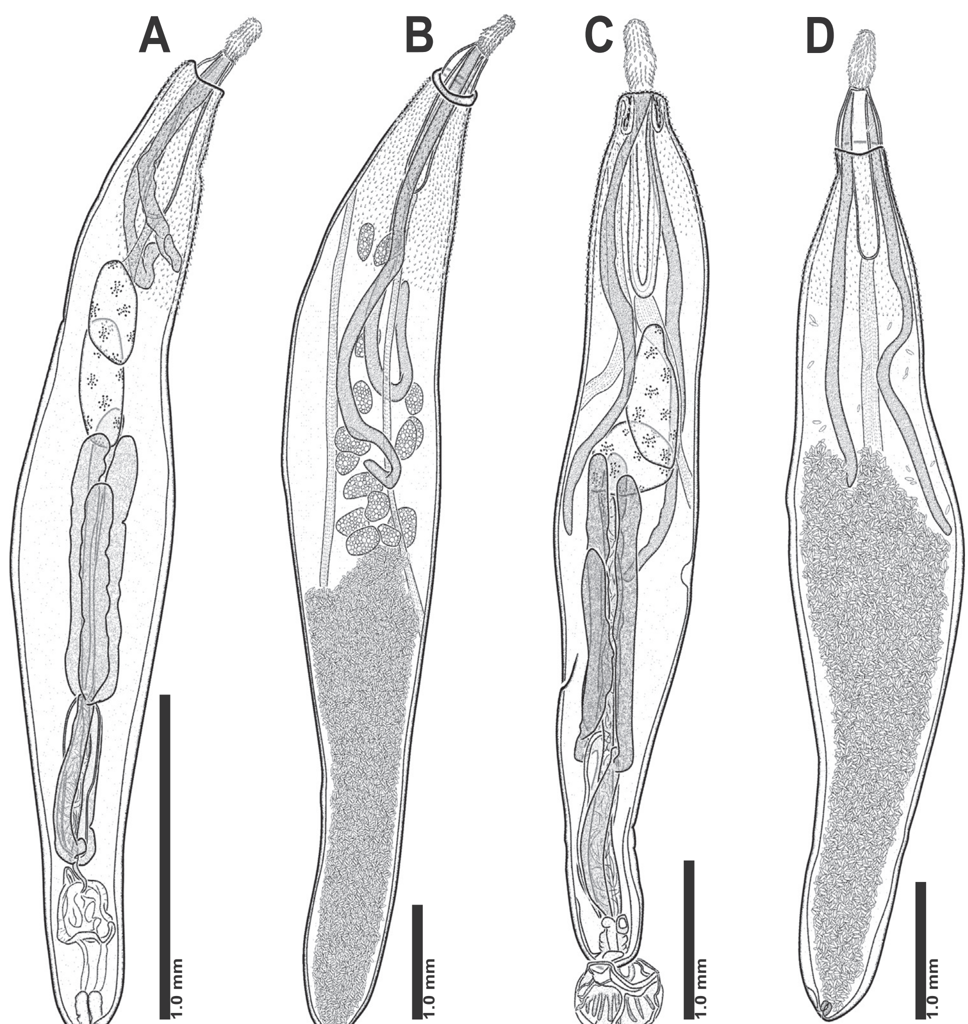

Fig. 4. *Pseudocorynosoma anatarium* from *Bucephala albeola*., A. Adult male, whole worm, voucher (CNHE 5721), lateral view; B. Adult female, whole worm, voucher (CNHE 5270), lateral view. *Pseudocorynosoma constrictum* from *Anas clypeata*., C. Adult male, whole worm, lateral view; D. Adult female, whole worm, voucher (CNHE 5721), lateral view.

Table 1
Comparative metrical data for males and females of species of *Pseudocorynosoma*. Measurements in micrometers, unless otherwise indicated.

Species Reference	*P. tepehuanesi* n. sp. This study	*P. constrictum* (Van Cleave, 1918) Van Cleave (1918)	*P. constrictum* Schmidt (1965)	*P. constrictum* This study	*P. anatarium* (Van Cleave, 1945) Van Cleave (1945)
General					
No. longitudinal rows of hooks	15	16	18–20	16	14
No. hooks per row	7–8	10–12	9–10	10	8–9
Male	$n = 8$			$n = 8$	
Trunk length (mm)	3.40–4.20 × 0.53–1.01 (3.8 × 0.801)	2.28–4.3 × 0.5–0.6	3.0–4.0 × 0.55–0.7	4.3–6.2 × 9.70–1.36 (5.6 × 1.13)	4.2–8.6 × 0.9–1.7
Proboscis	315–445 × 123–186 (355 × 140)		410–490 × 175–182	413–485 × 124–216 (439 × 179)	550 × 280–290
Apical hooks length	40–45 (42)	30–41	40–50	37–51 (44)	59–82
Middle hooks length	31–40 (33)	41–47	45–55	46–53(49)	–
Basal hooks length	19–27 (21)	35–41	40–43	30–33(32)	47–59
Proboscis receptacle	324–590 × 160–201 (440 × 178)		784–889 × 176–200	791–1.250 × 107–228 (1089 × 163)	
Leminiscus	493–1.189 × 86–144 (856 × 107)		1115	1.332–2.069 × 72–90 (1.796 × 77)	
Anterior testis	489–632 × 133–436 (560 × 301)		380 × 200	513–763 × 215–378 (639 × 294)	
Posterior testis	416–662 × 247–416 (556 × 359)			557–725 × 295–373 (673 × 332)	
Cement gland	946–1.719 × 117–254 (1.285 × 160)			1.120–1.635 × 95–169 (1.357 × 128)	
Säfftigen's pouch	420–764 × 189–262 (663 × 227)			744–1.033 × 99–211 (870 × 168)	
Females	$n = 3$			$n = 9$	
Trunk length (mm)	3.70–5.1 × 0.56–1.08 (4.60 × 0.89)	3.3 × 0.8	3.3 × 0.8	7.3–8.3 × 1.3–1.6 (7.6 × 1.5)	
Proboscis	301–365 × 143–178 (337 × 164)			422–483 × 183–190 (445 × 186)	
Apical hooks length	39–45 (42)			30–55 (46)	
Middle hooks length	36–38 (36)			38–45 (42)	
Basal hooks length	23–25 (23)			16–23 (19)	
Proboscis receptacle	623–818 × 219–256 (696 × 238)			1.193–1.563 × 150–223 (1.365 × 192)	
Leminiscus length mm	805–1.19 (1.03)			3.102–3.809 × 96–166 (3.460 × 124)	
Egg size	80–86 × 17–32 (82 × 16)			67–70 × 14–18 (68 × 15)	10–11 × 20–23

Species Reference	*P. anatarium* This study	*P. peposacae* (Porta, 1914) Porta (1914)	*P. ihering* (Machado Filho, 1961) Machado-Filho (1961)	*P. enrietti* (Molfie and Freitas-Fernandes, 1953) Molfie and Freitas-Fernandes (1953)
General				
No. longitudinal rows of hooks	14	14–18	20	20
No. hooks per row	8–9	12–14	8	8
Male	$n = 5$			
Trunk length (mm)	4.2–5.3 × 0.90–1.12 (4.8 × 1.05)	8.0–11.0	5.5–6.5	
Proboscis	371–540 × 165–200 (440 × 185)	340–430	330–340	
Apical hooks length	51–53 (52)	–	–	–
Middle hooks length	48–49 (48)	–	–	–
Basal hooks length	41–45 (42)	–	–	–
Proboscis receptacle	950–1.365 × 148–192 (1.131 × 172)			
Leminiscus	1.249–1.457 × 103–116 (1.368 × 108)		1662–1719 × 123	
Anterior testis	616–864 × 333–399 (772 × 367)		603 × 335	
Posterior testis	561–954 × 267–372 (738 × 311)		536 × 301	
Cement gland	1.174–1.364 × 127–150 (1.278 × 139)			
Säfftigen's pouch	471–724 × 104–213 (596 × 168)			
Females	$n = 7$			
Trunk length (mm)	4.8–6.9 × 1.03–1.82 (6.2 × 1.64)			
Proboscis	372–466 × 165–180 (433 × 172)			
Apical hooks length	55–62 (57)			
Middle hooks length	53–60 (56)			
Basal hooks length	40–44 (41)			
Proboscis receptacle	806–1.151 × 157–243 (968 × 204)			
Leminiscus	1.349–1.557 × 103–116 (1.368 × 108)			
Egg size	75–93 × 17–19 (83 × 18)	64–68 × 17–18		90–100 × 19–21

cylindrical proboscis, a cone-shaped neck, four tubular cement glands and eggs with a prominent polar prolongation in the middle fertilization membrane [1,4]. *Pseudocorynosoma tepehuanesi* n. sp. can be morphologically distinguished from the other five species from the Americas by having a trunk expanded anteriorly and a proboscis cylindrical, armed with 15 longitudinal rows with 7–8 hooks each vs 16–20 longitudinal rows with 10–12 hooks each in *P. constrictum* (Figs. 4, 5), 14–18 longitudinal rows with 12–14 hooks each in *P. peposacae*, 14 longitudinal rows with 8–9 hooks each in *P. anatarium* and 20 longitudinal rows with 8 hooks each in *P. enrietti* and *P. iheringi* [1,22,23] (see Table 1). The new species also differs from *P. constrictum* and *P. anatarium* by having smaller lemniscus (Figs. 3–4). The female of *P. tepehuanesi* n. sp. differs from the other five species, by having a small protuberance on the posterior extremity of the body (Figs. 2–4).

3.1.4. Alignments and phylogenetic analyses

A total of 62 sequences for cox 1 gene from the three species of *Pseudocorynosoma* (*P. constrictum*, *P. anatarium* and *tepehuanesi* n. sp.) were aligned with other sequences of *Polymorphus trochus*, *P. minutus*, *Profilicollis altmani* and *P. botulus* from Polymorphidae, conforming a data set of 673 sites. The alignment of these sequences revels 24 sequences belonging *P. anatarium* with abnormalities, which were defined as pseudogenes by the presence of insertions, deletions and premature stop codons. The length of these pseudogenes ranged from 643 bp to 656 bp, whereas the other 33 functional genes of cox 1 of *Pseudocorynosoma* spp., had a length of 655 bp. The genetic divergences among 24 types of pseudogenes range from 8 to 12%, whereas the genetic distances within *P. constrictum*, ranged from 0. 09 to 3%, *P. anatarium* from 0. 03 to 1.5% and *P. tepehuanesi* n. sp., ranged from 0 to 0.3%. Finally the genetic divergence among the new species and the other two congeneric species ranged from 15 to 18%. Phylogenetic analysis of this data set with ML and IB supported the monophyly of *Pseudocorynosoma*. This tree was composed of 4 main clades. The first clade contained 15 specimens of *P. constrictum* recovered from the intestine of ducks of the genera *Anas* and *Aythya* (localities 1, 4–6, 8–10 in Fig. 1). The second clade includes 8 specimens of *P. tepehuanesi* n. sp., recovered from single locality (locality 4 in Fig. 1). The third clade was composed of 10 specimens of *P. anatarium* recovered from bufflehead duck from three localities (localities 1, 2, 4 in Fig. 1). Finally the fourth clade was composed by pseudogenes of *P. anatarium*. The phylogenetic relationships among taxa received strong bootstrap support and Bayesian posterior probability values (Fig. 6).

The sequences of *P. tepehuanesi* n. sp., from LSU, were aligned with the same species of *Pseudocorynosoma* and with sequences of *P. minutus*, *Profilicollis altmani* and *P. botulus* available in the GenBank data set, conforming a data set of 811 sites. The genetic divergence among the three species of *Pseudocorynosoma* (*P. constrictum*, *P. anatarium* and *P. tepehuanesi* n. sp.), ranged from 3.2 to 4%. Phylogenetic analysis of LSU data set with ML and IB methods supported the monophyly of the three sub clades of *Pseudocorynosoma* representing the three species and the nodes among the branches received strong bootstrap support and Bayesian posterior probability values (Fig. 7).

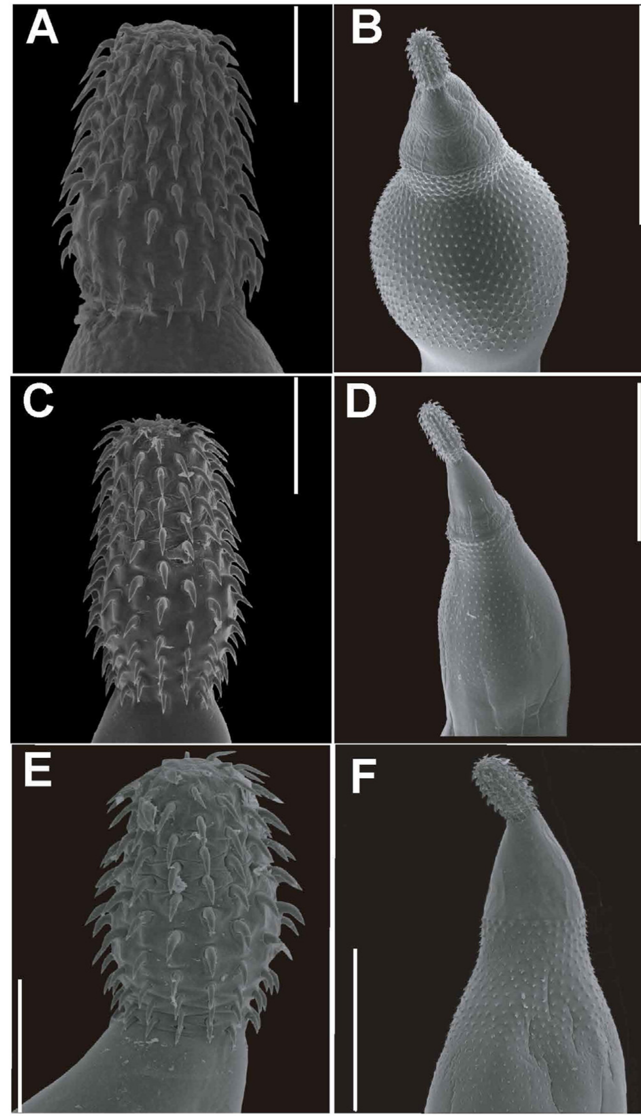

Fig. 5. Scanning electron micrographs of proboscis and trunk of adult male of *Pseudocorynosoma tepehuanesi* n. sp. (A–B), *Pseudocorynosoma constrictum* (C–D), *Pseudocorynosoma anatarium* (E–F). Scale bars: b, d, F, 1.0 mm; a, c, e, 200 μm.

4. Discussion

The phylogenetic trees obtained with LSU yielded three major clades, each one represents the three species of *Pseudocorynosoma*, which received high bootstrap and posterior probability values (Fig. 7). The phylogenetic tree inferred with cox 1 dataset yielded three clades, representing the three species of *Pseudocorynosoma*, and within of the clade of *P. anatarium* a sub-clade was conformed only with pseudogenes (Fig. 6). The genetic divergence among the pseudogenes ranged from 8 to 12%, these values are higher than those recorded between pseudogenes of *Acanthocephalus* Koelreuter, 1771, which had a divergence of 7% [24]. In contrast the genetic divergence found in cox 1 pseudogenes among individual of *Pomphorhynchus laevis* Müller 1776 from Sava river in Croatia ranged from 0 to 20.1% [25]. The presence of pseudogenes only in *P. anatarium* indicates that they arose after speciation process on the genus *Pseudocorynosoma*. Benesh et al. [24]. detected cox 1 pseudogenes in two species of the genus *Acanthocephalus* Koelreuter 1771 no related each other (*A. lucii* Müller, 1776 and *A. dirus* Van Cleave, 1931), indicating that the pseudogenes arose before these species diverged.

The nuclear mitochondrial pseudogenes exhibited a high degree of similarity with the mitochondrial DNA (numts). The presence of those genes is due two mechanisms 1) continual horizontal transfer from the mitochondria to the nucleus, 2) duplication within the nuclear genome after a transfer event [26,27]. The mitochondrial cox 1 pseudogenes were coamplified from total DNA by using conserved universal cox 1 primers [10] those pseudogenes have been found in the genomes of a diverse range of metazoan species. The cox 1 gene is a fragment that has been used successfully for the identification and delimitation of metazoan species, turning it into the core fragment for DNA barcoding [28–31]. However, the existence of cox 1 numts poses a serious challenge to DNA barcoding and may overestimate the diversity of the species on the ecosystems. To avoid the numts is necessary made a carefully examination of the sequences with the aim of detected insertions, deletions and premature stop codons [31].

Currently, *Pseudocorynosoma* contains five described species plus the new species from the Americas. During the migration of waterfowl species in North America four flyways have been proposed [32]. In the current study, waterfowl species from three flyways (Pacific, Central and Atlantic) were collected. Interestingly waterfowl from two flyways (Pacific and Central) were infected with acanthocephalans. The type species of the genus i.e., *P. constrictum* has been previously recorded in the four flyways in 20 waterfowl species and it is considered as one

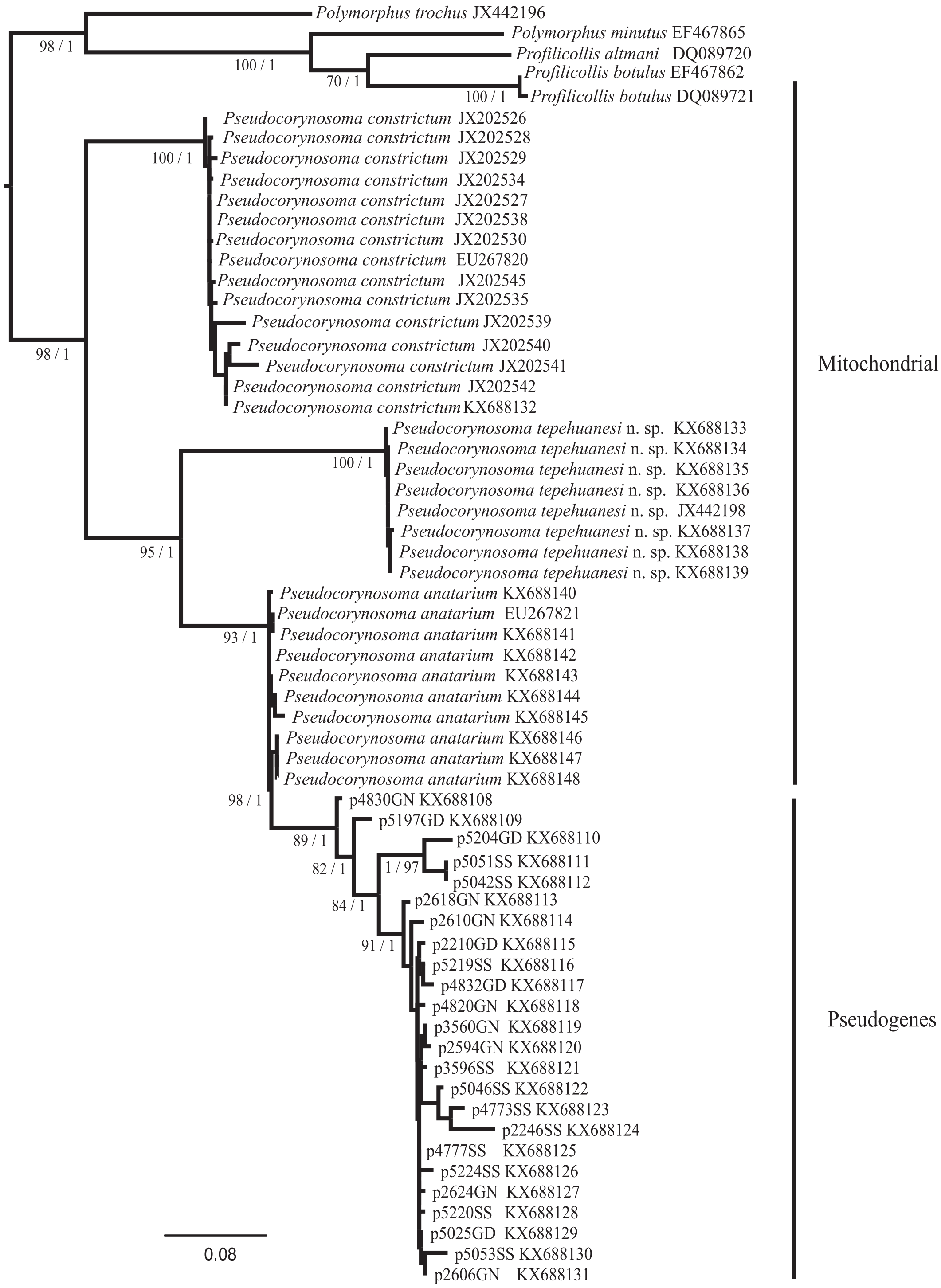

Fig. 6. Maximum likelihood tree and consensus Bayesian Inference trees inferred with cox 1 data set; numbers near internal nodes show ML bootstrap clade frequencies and posterior probabilities (BI).

generalist parasite, abundant and widely distributed in the Neartic region [1,7,33–36]. In contrast, *P. anatarium* was described from an undetermined duck in the state of Texas in the United States of North America [1]. Recently *P. anatarium* has been found on the Pacific and Central flyways from Mexico in the bufflehead duck (*Bucephala albeola*). The new species *P. tepehuanesi* n. sp., was recorder in the ruddy duck (*Oxyura jamaicensis*) a diving duck from single locality (see Fig. 1) of the Central flyway from Mexico. Species of *Pseudocorynosoma* apparently show some level of host-specificity, i.e., *P. constrictum*, has been found in waterfowl species from the genera *Anas* and *Aythya*, whereas *P. anatarium* and *P. tepehuanesi* n. sp., were only found in the genera *Bucephala* and *Oxyura* respectively.

The three species of *Pseudocorynosoma* from North America, *P. constrictum*, *P. anatarium* and *P. tepehuanesi* n. sp., were found in the same locality in Durango (locality 4, in Fig. 1). The other three species of *Psuedocorynosoma* from South America also occur in sympatry and show certain level of host-specificity, i.e., *P. peposacae* was recorded in the rosy-billed pochard (*Netta peposaca* Vieillot, 1816); *P. iheringi* in the Brazilian duck (*Amazonetta brasiliensis* Gmelin, 1789) and finally *P. enrietti* was recorded in the white-cheeked pintail (*Anas bahamensis*

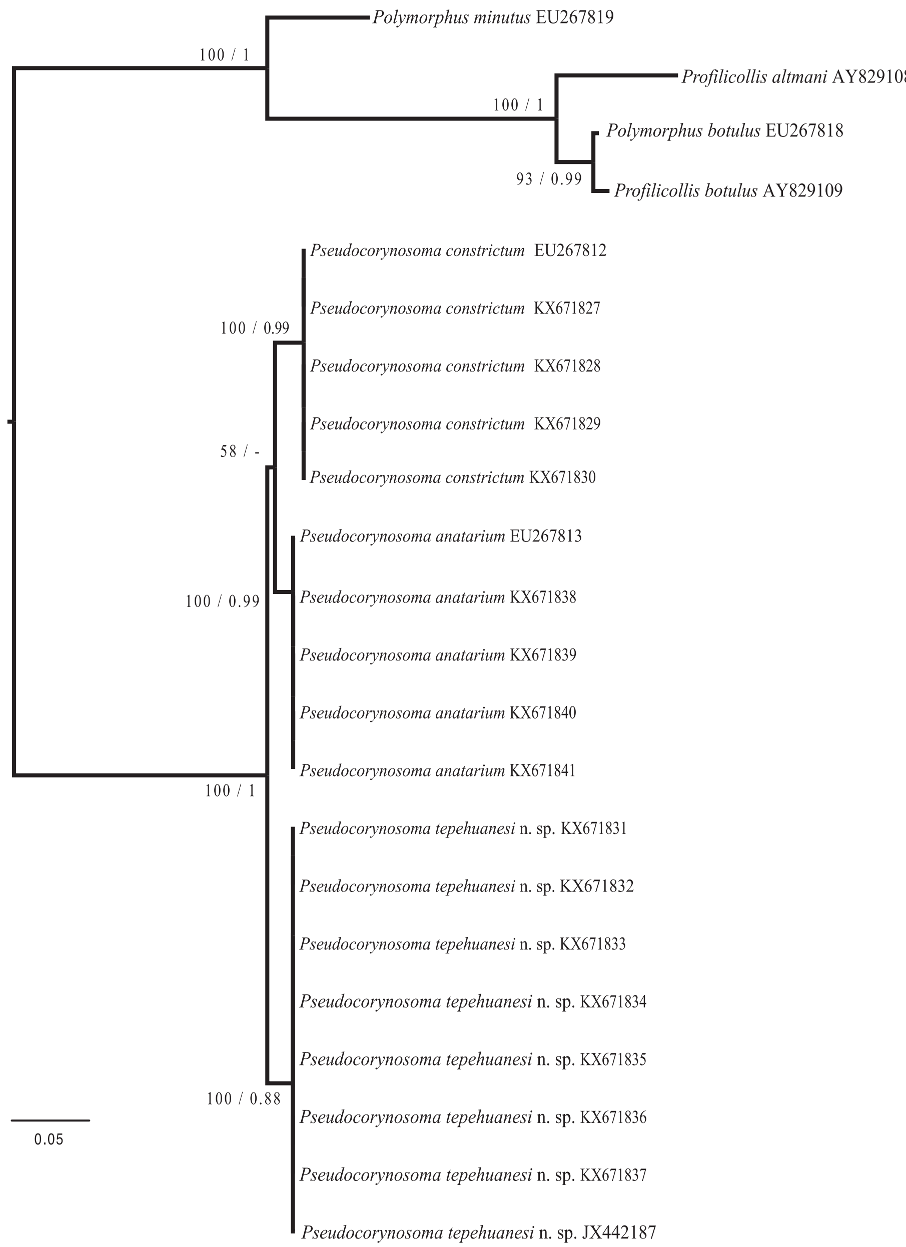

Fig. 7. Maximum likelihood tree and consensus Bayesian Inference trees inferred with LSU data set; numbers near internal nodes show ML bootstrap clade frequencies and posterior probabilities (BI).

Linnaeus, 1758) and Brazilian duck [23]. Therefore, the other three species of *Pseudocorynosoma* from South America are essential to understand the phylogenetic relationships among the six species of the genus *Pseudocorynosoma* and their relationships host-parasite.

5. Conclusions

Pseudocorynosoma tepehuanesi is the sixth species of the genus associated with waterfowl from northern Mexico. Morphologically, the new species is distinguished from the other five congeneric species described from Americas by possessing a proboscis with 15 longitudinal rows with 7–8 hooks each, a trunk expanded anteriorly and by having smaller lemniscus. These morphological distinctions were demonstrated with 2 phylogenetic analyses inferred with molecular data. We detected cox 1 pseudogenes only in *P. anatarium* suggesting that those genes arose after speciation process within genus *Pseudocorynosoma*.

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References

[1] H.J. Van Cleave, The acanthocephalan genus *Corynosoma* I. The species found in water birds of North America, J. Parasitol. 25 (1945) 332–340.
[2] J.S. Keithly, Life History of *Corynosoma constrictum* Van CleavePh.D. Thesis Iowa State University, Ames, Iowa, 1968 (203 p.).
[3] L.M. Duclos, B.J. Danner, B. Nickol, Virulence of *Corynosoma constrictum* (Acanthocephala: Polymorphidae) in *Hyalella azteca* (Amphipoda) throughout parasite ontogeny, J. Parasitol. 92 (2006) 749–755.

[4] F.J. Aznar, G.P.P. de Leon, J.A. Raga, Status of *Corynosoma* (Acanthocephala: Polymorphidae) based on anatomical, ecological and phylogenetic evidence, with the erection of *Pseudocorynosoma* n. Gen, J. Parasitol. 92 (2006) 548–564.
[5] M. García-Varela, G. Pérez-Ponce de León, F.J. Aznar, S.A. Nadler, Phylogenetic relationship among genera of Polymorphidae (Acanthocephala), inferred from nuclear and mitochondrial gene sequences, Mol. Phylogenet. Evol. 68 (2014) 176–184.
[6] O.M. Amin, Classification of the Acanthocephala, Folia Parasitol. 60 (2013) 273–305.
[7] L. García-Prieto, M. García-Varela, B. Mendoza-Garfias, G. Pérez-Ponce de León, Checklist of the Acanthocephala in wildlife vertebrates of Mexico, Zootaxa 2419 (2010) 1–50.
[8] S.N.G. Howell, S. Webb, A Guide to the Birds of México and Northern Central America, Oxford University Press, New York, 1995 (851 p.).
[9] American Ornithologists' Union (AOU), Check-list of North American, Birds, 7 th ed., 1998 (Washington, D.C.).
[10] O. Folmer, M. Black, W. Hoeh, R. Lutz, R. Vrijenhoek, DNA primers for the amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates, Mol. Mar. Biol. Biotechnol. 3 (1994) 294–299.
[11] M. García-Varela, S.A. Nadler, Phylogenetic relationships of palaeacanthocephala (Acanthocephala) inferred from SSU and LSU rDNA gene sequences, J. Parasitol. 91 (2005) 1401–1409.
[12] J.D. Thompson, H.G. Higgins, T.J. Gibson, CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice, Nucleic Acids Res. 22 (1994) 4673–4680.
[13] F. Rodríguez, J.F. Oliver, A. Marin, J.R. Medina, The general stochastic model of nucleotide substitution, J. Theor. Biol. 817-818 (1990).
[14] D. Posada, K.A. Crandall, Modeltest: testing the model of DNA substitution, Bioinformatics 9 (1998) 817–818.
[15] Z. Yang, Estimating the patterns of nucleotide substitution, J. Mol. Evol. 39 (1994) 105–111.
[16] W. Pfeiffer, A. Stamatakis, Hybrid mpi/pthreads parallelization of the raxml phylogenetics code, International Symposium on Parallel & Distributed Processing, Workshops and Phd Forum (IPDPSW) IEEE, IEEE, New York, USA 2010, pp. 1–8.
[17] M.A. Miller, W. Pfeiffer, T. Schwartz, Creating the CIPRES Science Gateway for inference of large phylogenetic trees, Proceedings of the Gateway Computing Environments Workshop (GCE) 2010, pp. 1–8 (New Orleans).
[18] J.P. Huelsenbeck, F. Ronquist, MrBayes: Bayesian inference of phylogeny, Bioinformatics 17 (2001) 754–755.
[19] A. Rambaut, A.J. Drummond, Tracer v1.4 2003–2007, MCMC Trace Analysis Package, 2007 (http://tree.bio.ed.ac.uk/software/tracer/).
[20] A. Rambaut, Tree Figure Drawing Tool Version 1.4.0, Institute of Evolutionary Biology, University of Edinburgh, 2006.
[21] K. Tamura, G. Stecher, D. Peterson, A. Filipski, S. Kumar, MEGA6: molecular evolutionary genetics analysis version 6.0, Mol. Biol. Evol. 30 (2013) 2725–2729.
[22] H.J. Van Cleave, The Acanthocephala of North America birds, Trans. Am. Microsc. Soc. 37 (1918) 19–48.
[23] E.M. McDonald, Key to Acanthocephala Report in Waterfowl, U. S. Fish and Wildlife Service, 1998 173.
[24] D. Benesh, P.T. Hasu, L.R. Suomalainen, E.T. Valtonen, M. Tiirola, Reliability of mitochondrial DNA in an acanthocephalan: the problem of pseudogenes, Int. J. Parasitol. 36 (2006) 247–254.
[25] I. Vardić Smrzlić, D. Valić, D. Kapetanović, V. Filipović Marijić, E. Gjurčević, E. Teskeredžić, *Pomphorhynchus laevis* (Acanthocephala) from the Sava River basin: new insights into strain formation, mtDNA-like sequences and dynamics of infection, Parasitol. Int. 64 (2015) 243–250.
[26] E. Hazkani-Covo, R. Sorek, D. Graur, Evolutionary dynamics of large Numts in the human genome: rarity of independent insertions and abundance of post-insertion duplications, J. Mol. Ecol. 56 (2003) 169–174.
[27] D. Bensasson, M.W. Feldman, D.A. Petrov, Rates of DNA duplication and mitochondrial DNA insertion in the human genome, J. Mol. Evol. 57 (2003) 343–354.
[28] A. Obwaller, R. Schneider, J. Walochnik, B. Gollackner, A. Deutz, K. Janitschke, H. Aspock, H. Auer, *Echinococcus granulosus* strain differentiation based on sequences heterogeneity in mitochondrial gene of cytochrome *c* oxidase-1 and NADH dehydrogenase-1, Parasitology 128 (2004) 569–575.
[29] S. Derycke, J. Vanaverbeke, A. Rigaux, T. Backeljau, T. Moend, Exploring the use of cytochrome oxidase c subunit I (COI) for DNA barcoding of free-living marine nematodes, PLoS One 5 (2010), e13716.
[30] J.V. López, N. Yuhki, R. Masuda, W. Modi, S. O'Brien, Numt, a recent transfer and tandem amplification of mitochondrial DNA to the nuclear genome of the domestic cat, J. Mol. Evol. 39 (1994) 174–190.
[31] H. Song, J.E. Buhay, M.F. Whiting, K.A. Crandall, Many species in one: DNA barcoding overestimates the number of species when nuclear mitochondrial pseudogenes are coamplified, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 13486–13491.
[32] P.A. Johnsgard, Waterfowl of North America, Revised ed. University of Nebraska Press Lincoln, 2010.
[33] V.I. Petrochenko, Acanthocephala of Domestic and Wild Animals, vol. II, Izdatel'stvo Akademii Nauk SSSR, Vsesoyuznoe Obshchestvo Gel'mintologov, Moscow, Russia, Moscow, 1958.
[34] H.N. Buscher, Dynamics of the intestine helminth fauna in three species of ducks, J. Wildl. Manag. 29 (1965) 772–781.
[35] J.D. Farias, A.G. Canaris, Gastrointestinal helminths of the Mexican duck, *Anas platyrhynchus diazi*, from north central México and Southwestern USA, J. Wildl. Dis. 22 (1986) 51–54.
[36] L.F. Mayberry, A.G. Canaris, J.R. Bristol, S.L. Gardner, Bibliography of Parasites and Vertebrate Hosts in Arizona, New Mexico and Texas (1893–1984), 2000.