GENETICS, EVOLUTION, AND PHYLOGENY - ORIGINAL PAPER

Redescription and molecular characterization of *Baruscapillaria spiculata* (Nematoda: Capillariidae) parasitizing the Neotropic cormorant *Phalacrocorax brasilianus* from two Argentinian lagoons

Lucas E. Garbin[1] · María C. Digiani[2] · María R. Robles[1] · Martín M. Montes[1] · Marcelo Knoff[3] · Daniela Fuchs[4] · Diego Montalti[5] · Julia I. Diaz[1]

Abstract

Two species of intestinal Capillariidae were hitherto known from the Neotropic cormorant *Phalacrocorax brasilianus*, *Baruscapillaria spiculata* (Freitas, 1933), and *Baruscapillaria appendiculata* (Freitas, 1933). The original descriptions are very short and brief, and further reports of both species are scarce and/or confusing. This paper provides a morphological redescription and molecular characterization, based on the partial 18S rDNA gene, of *B. spiculata* specimens parasitizing the Neotropic cormorant in two continental lagoons from Buenos Aires province, Argentina. Both morphological and morphometrical differences between *B. spiculata* and *B. appendiculata* are highlighted on the examination of available type material. Additionally, two previous reports of *B. appendiculata* from Mexico and Brazil are discussed. A phylogenetic analysis conducted on specimens of *B. spiculata* and 46 other capillariid isolates available from the GenBank demonstrated a sister-taxon relationship between our specimens and the type species of *Baruscapillaria*. But, at the same time, significant genetic distances between both taxa showed an interesting variability of the genus *Baruscapillaria*. The probable division of this genus into multiple genera could probably be confirmed through integrative studies including more species.

Keywords Capillariidae · *Phalacrocorax brasilianus* · *Baruscapillaria* · Phylogenetic analysis

Introduction

The family Capillariidae (Trichocephalida: Trichinelloidea) consists of nearly 400 nominal species parasitizing six vertebrate classes distributed worldwide (Moravec 2001; Hodda 2011). Moravec (1982), based mainly on morphological features of the male caudal end, provisionally proposed 16 genera

to which another 11 were later added, either by the erection of new genera, revalidation of older genera, or raising of subgenera to genera (Moravec and Cosgrove 1982; Mas-Coma and Esteban 1985; Moravec 1987; Baruš and Sergeeva 1990a, b; Yu and Wang 1994; Moravec and Spratt 1998; Moravec et al. 1999; Moravec 2001; Moravec and Beveridge 2017). However, some of these proposed genera

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Lucas E. Garbin, María C. Digiani and Julia I. Diaz contributed equally to this work.

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✉ Lucas E. Garbin
lgarbin@cepave.edu.ar

1 Centro de Estudios Parasitológicos y de Vectores (CEPAVE-UNLP-CONICET), La Plata, Buenos Aires, Argentina

2 División Zoología Invertebrados (Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata) and Consejo Nacional de Investigaciones Científicas y Técnicas-CONICET, La Plata, Buenos Aires, Argentina

3 Laboratório de Helmintos Parasitos de Vertebrados, Instituto Oswaldo Cruz, Fiocruz, Avenida Brasil, 4365, Manguinhos, Rio de Janeiro, Rio de Janeiro, Brazil

4 Centro de Investigaciones Científicas y Transferencia de Tecnología a la Producción (CICyTTP-UADER-CONICET), Diamante, Entre Ríos, Argentina

5 Sección Ornitología, División Zoología Vertebrados (Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata) and Consejo Nacional de Investigaciones Científicas y Técnicas-CONICET, La Plata, Buenos Aires, Argentina

fell into synonymy and the later classification (Moravec 2001) listed 22 genera and 17 subgenera. The species parasitizing avian hosts have been included in the following genera: *Capillaria* Zeder, 1800; *Eucoleus* Dujardin, 1845; *Aonchotheca* López-Neyra, 1947; *Echinocoleus* López-Neyra, 1947; *Pseudocapillaria* Freitas, 1959; *Pterothominx* Freitas, 1959; and *Baruscapillaria* Moravec, 1982.

The Neotropic cormorant *Phalacrocorax brasilianus* (Gmelin, 1789) (Pelecaniformes: Phalacrocoracidae) lives in both freshwater and marine environments (Harrison 1985), and is widely distributed from southern South America, i.e. Argentina and Chile, and north to Texas, USA (Morrison et al. 1979; Araya and Millie 1991; Telfair and Morrison 1995). Two intestinal Capillariidae species have been described from the Neotropic cormorant to date from Rio de Janeiro, Brazil by Freitas (1933a, b): *Baruscapillaria spiculata* (Freitas, 1933) parasitizing the cormorant cloaca, and *Baruscapillaria appendiculata* (Freitas, 1933) parasitic in the large intestine. Both species were originally described as belonging to the genus *Capillaria* and later transferred to *Baruscapillaria* by Moravec (1982) according to morphological features of the male caudal end. The second species appeared in some literature (Moravec et al. 2000; Monteiro 2006; Monteiro et al. 2011) as *Ornithocapillaria appendiculata* (Freitas, 1933), but this combination was later dismissed due to the proposed synonymy of *Ornithocapillaria* with *Baruscapillaria* (Okulewicz 1993; Moravec 2001).

Although a few morphological studies of *B. appendiculata* exist (Moravec et al. 2000; Monteiro 2006), there is no study including a detailed description of *B. spiculata* since the first brief study by Freitas 1933a. The original descriptions of both *B. spiculata* and *B. appendiculata* are short and lacking several morphological details which may have caused confusion in identifying the capillariid species parasitizing cormorants. For instance, studies by Monteiro (2006) have reported *B. appendiculata* from *Ph. brasilianus* in Brazil, but the morphological description seems to belong to the species *B. spiculata*.

Hence in this study, we aimed to describe morphological details of *B. spiculata* parasitizing *Ph. brasilianus* in Buenos Aires province, Argentina, in order to clarify the species status. Also, a molecular characterization of this species using the partial 18S rDNA gene was performed.

Material and methods

Sampling

Nine *Ph. brasilianus* individuals from the San Miguel del Monte Lagoon (SMML) (35° 27′ 35.46″ S; 58° 48′ 11.05″ W) and another 7 ones from the Chis-Chis Lagoon (CCL) (35° 45′ 43.76″ S, 57° 57′ 6.75″ W), both from the Buenos Aires

province, Argentina, were collected as dead animals during 2014–2017. All birds were necropsied and their complete digestive tracts were kept frozen at −20°C until they could be examined. Digestive tracts were analyzed for helminths and capillariid nematodes were isolated from cloacae. Nematodes for morphological study were fixed in 5% formalin or 70% ethanol. Specimens used to amplify specific DNA fragments from both SMML and CCL were fixed in absolute ethanol.

Morphological study

Thirty-six adult capillariid nematodes from *Ph. brasilianus* were examined. Nematodes were cleared in lactophenol and studied with both light microscopes Leica DM2500® (Wetzlar, Germany) with a drawing attachment, and Olympus BX51® (Tokyo, Japan) with a camera *QImaging® Go-3*. Types of *Capillaria spiculata* Freitas, 1933 (CHIOC 2833 and 3079) were studied with a light microscope Carl Zeiss Axiophot equipped with a Canon Power Shot S80 camera at the Coleção Helmintológico do Instituto Oswaldo Cruz, Rio de Janeiro, Brazil .

Some specimens were processed for scanning electron microscopy, dried by the critical point method, and observed using a JEOL/JSMT 6360 LV® Scanning Electron Microscope (JEOL Ltd, Tokyo, Japan). Several characters considered diagnostic for capillariid nematodes (Freitas et al. 1959; Moravec 1982) were analyzed. Mean measurements are expressed in millimeters, except otherwise indicated, usually as the range followed by the mean in parentheses. Prevalence of infection was calculated for worm specimens recovered from both sampling sites according to Bush et al. (1997). Voucher specimens were stored in 70% ethanol and deposited in the Helminthological Collection of Museo de La Plata, La Plata, Argentina.

DNA extraction, amplification, and sequencing

DNA of 2 male and 10 female capillariid specimens from both sampling sites, kept in 70 % alcohol, was extracted using a Promega Wizard® Genomic DNA Purification Kit according to the manufacturer's instructions. The PCR amplification of overlapping 18S ribosomal RNA gene (18S rDNA) segments was performed using different primer combinations of forward and reverse universal eukaryotic primers as previously described by Sato et al. (2010) and Tamaru et al. (2015): (1) NSF4/18 and 18S-1192R/20, (2) NSF4/18 and NSR1438/20, (3) NSF573/19 and NSR1787/18, and (4) NSF573/19 and SSU18R. The DNA polymerase used was GoTaq® Green Master Mix following the manufacturer's instructions and PCRs in 20–25-μl reaction solution were conducted in a thermal cycler Eppendorf[AG] (Mastercycler® Nexus) using the following cycling protocol: 2 min at 94 °C, then 40 cycles at 94 °C for 30 s, 64 or 62 °C for 30 s, and 72 °C for 90 s, followed by a final extension at 72 °C for 7 min. The

PCR products were purified using a Promega Wizard® SV Gel and PCR Clean-Up System; and sequenced by Macrogen Inc. (Seoul, Korea) directly with the primers for amplification. The nucleotide sequences reported in the present study are available from the DDBJ/EMBL/GenBank databases under the accession numbers MT068208, and MT068209.

Phylogenetic analysis

Contrasting 18S rDNA sequences from 46 capillariid isolates with more than 80% identity were analyzed using the Standard Nucleotide BLAST from GenBank. The 18S rRNA sequences of capillariid nematodes obtained in this study were optimized by eye using Geneious R11 under free-trial (http://www.geneious.com) (Drummond et al. 2012), and compared with other capillariid sequences from GenBank. Alignments were assembled using the online version of MAFFT v.7 (Katoh and Standley 2016). The online Gblocks v0.91 (Castresana 2000; Talavera and Castresana 2007) was used to detect and exclude from the analyses the hypervariable regions in the 18S rRNA.

The best partitioning scheme and substitution model for the 18S rRNA was chosen under the Bayesian Information Criterion (BIC) (Schwarz 1978) using the "greedy" search strategy in Partition Finder v.1.1.1 (Lanfear et al. 2012). The appropriate nucleotide substitution model implement for the 18S rRNA matrix resulting after Gblock TVMef+I+G. The phylogenetic analysis was performed by Bayesian Inference (BI) through MrBayes v. 3.2.1 (Ronquist et al. 2012) using the compared capillariid sequences and *Trichuris suis* (EU790668) as outgroup. The phylogenetic trees were reconstructed using two parallel analyses of Metropolis-Coupled Markov Chain Monte Carlo (MCMC) for 10 million generations each to estimate the posterior probability (PP) distribution. Topologies were sampled every 1000 generations, once the average standard deviation of split frequencies was less than 0.01. The robustness of the clades was assessed using Bayesian PP, where PP > 0.95 was considered strongly supported. A majority consensus tree with branch lengths was reconstructed after discarding the first 25% sampled trees. The final trees were visualized in FigTree software v 1.4.2 (Rambaut 2009; Rambaut et al. 2018). The proportion (*p*) of absolute nucleotide sites (*p*-distance) (Nei and Kumar 2000) was obtained to compare the genetic distance among and between linages using Mega X, with bootstrap method (1000 replicates) and with nucleotide substitution (transition + transversions) uniform rate (Kumar et al. 2018). In addition, the evolutionary history was inferred by using the Maximum Likelihood (ML) method based on the Jukes-Cantor model (Jukes and Cantor 1969). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are

collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The analysis involved 52 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 948 positions in the final dataset. Evolutionary analyses were conducted on MEGA X.

Results

Baruscapillaria spiculata (Freitas, 1933) Moravec 1982
=*Capillaria spiculata* Freitas, 1933

Morphological description

General (based on 18 males and 18 females from SMML and CCL): Body long and thin. Maximum body width at esophago-intestinal junction. Males smaller than females. Anterior end narrow and rounded. Oral aperture terminal, slit-like, oriented dorsoventrally. Nerve ring barely visible situated mostly within proximal fifth of muscular esophagus. Dividing line between muscular esophagus and stichosome not always observed (Fig. 1a). Stichosome composed of single row of stichocytes subdivided into 9–11 annuli, provided with large nuclei (Fig. 1b, c). Two medium-sized glandular cells or pseudo-coelomocytes observed at esophago-intestinal junction (Fig. 1c). Two distinct lateral bacillary bands present (Fig. 1d).

Male (measurements in Table 1): Spicule well sclerotized, with proximal end widely expanded and distal end rounded (Fig. 2a, d). Spicular sheath bearing ornamentations with 4 distinct sections with a regular pattern: proximal section with annulations fine and compact (Fig. 2a); second section with annulations wide and reticulate (Fig. 2b); third section with annulations wide and loose, sometimes oblique to the longitudinal axis giving the appearance of a spiral lining (Fig. 2c); fourth and distal section with annulations fine and compact becoming wider and looser when the spicular sheath is extruded (Fig. 2d–f). Cloacal opening subterminal (Fig. 2e, f). Caudal end with well-developed membranous bursa composed of one dorsal and two lateroventral lobes, these latter bearing one large papilla each. Dorsal lobe shorter than lateroventral ones (Fig. 2c, d). Caudal lateral alae absent.

Female (measurements in Table 2): Vulvar appendage present in all females, arising basally as a protrusion of the anterior vulvar lip and continuing as a heart-shaped cuticular fold (Fig 1c, d). Oval thick-walled eggs with polar plugs

Fig. 1 *Baruscapillaria spiculata*
(Freitas, 1933) parasitizing
Phalacrocorax brasilianus from
the San Miguel del Monte
Lagoon, Buenos Aires province,
Argentina, observed by light
(LM), and scanning electronic
(SEM) microscopies. **a** Anterior
end, nerve ring (black arrow),
muscular esophagus, first
stichocyte (white arrow) by LM.
b Stichosome with stichocytes by
LM. **c** Three terminal stichocytes,
vulvar appendage, glandular cells
or pseudo-coelomocytes (black
arrow) by LM. **d** Vulvar append-
age, bacillary band (double white
arrow) by SEM. Scale bars are
given in micrometers (μm)
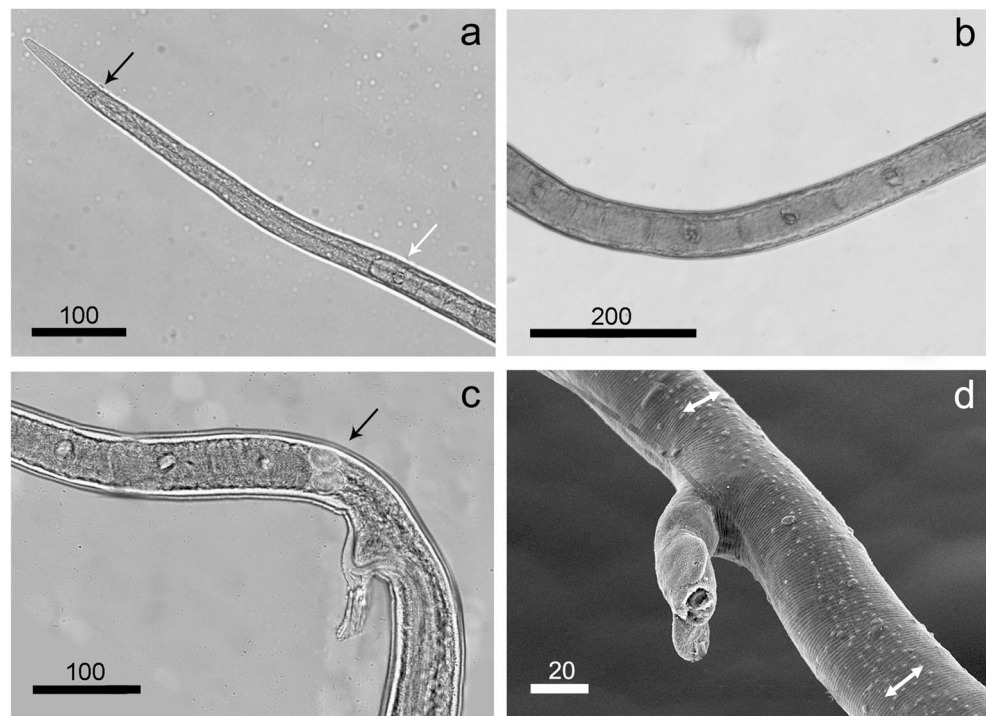
protruding in immature eggs, less protruding in fully mature
eggs. Content of mature eggs uncleaved. Posterior extremity
rounded. Anus subterminal.

Taxonomic summary

Host: *Phalacrocorax brasilianus* (Gmelin, 1789) (type host).
   New localities: San Miguel del Monte Lagoon (SMML)
(35° 27′ 35.46″ S, 58° 48′ 11.05″ W), San Miguel del
Monte; Chis-Chis Lagoon (CCL) (35° 45′ 43.76″ S, 57° 57′
6.75″ W), Chascomús, Buenos Aires province, Argentina.
   Site of infection: Cloaca.
   Prevalence: 88.8% in hosts from SMML, 42.8% in hosts
from CCL.
   Mean intensity: 25.8 in hosts from SMML; 34 in hosts from
CCL.
   Specimens deposited: Helminthological Collection of
Museo de La Plata (MLP-He 7718 and MLP-He 7719), La
Plata, Buenos Aires, Argentina.
   Remarks: the specimens studied in this work strongly re-
semble *B. spiculata* as described by Freitas (1933a). The mor-
phology of the membranous bursa, the spicule measurements,
the distance between the vulva and the esophago-intestinal
junction, and the site of infection allowed us to identify our
specimens as *B. spiculata* rather than to *B. appendiculata*
(Tables 1 and 2). Freitas (1933a) did not describe in details
the spicular sheath ornamentation of *B. spiculata* but he men-
tions a "spiral striation," in contrast to the spicular sheath of *B.
appendiculata,* which is described as smooth (Freitas 1933b).
The third section of the spicular sheath of our specimens

exhibited wide and loose annulations sometimes oblique to
the main axis of the spicule giving the appearance of a spiral
lining. Moreover, the specimens of *C. spiculata* examined
from the CHIOC (type male, Fig. 3a–d) showed the same
pattern of ornamentation on the spicular sheath—four distinct
sections with a regular pattern—present in our specimens.
Based on all these observations, we identified the capillariids
present in SMML and CCL as *B. spiculata*.

Molecular characterization and phylogenetic analysis

Only two 18S rDNA consensus sequences of 1623 and 1624
bp—isolates MT068208 and MT068209, respectively—were
obtained from two specimens of *Baruscapillaria spiculata* from
SMML. The amplicons from CCL specimens failed on sequenc-
ing. Both isolates showed 99.45 % identity among each other
(1615/1624), and only nine nucleotide substitutions were ob-
served at different positions. The pairwise genetic distance
among both isolates was 0.1 % (Table 3). Consequently, these
specimens can be considered belonging to the same species.
   Available molecular characterizations of *Baruscapillaria*
species only concerned the type species, *Baruscapillaria
obsignata* (Madsen, 1945). Therefore, we conducted a phylo-
genetic analysis including the newly obtained sequences of
B. spiculata in order to confirm the taxonomic status and
generic assignment of this latter species. With this aim, we
largely relied on the work of Tamaru et al. (2015), and that
of Sakaguchi et al. (2020) who compared several 18S rDNA
sequences of different capillariid species parasitizing mainly
birds and mammals. The BLAST searching revealed that our

Table 1 Comparative morphology and morphometry of males of the two species of intestinal capillarids described from *Phalacrocorax brasilianus*. In bold, main features differentiating *Baruscapillaria spiculata* and *B. appendiculata*

Source	Freitas 1933a male type	Freitas 1933b	Moravec et al. 2000	Monteiro 2006	This work	This work
Former identification	Capillaria spiculata (description)	Capillaria appendiculata (description)	Ornithocapillaria appendiculata	Ornithocapillaria appendiculata		
Present identification	Baruscapillaria spiculata	Baruscapillaria appendiculata	?	Baruscapillaria spiculata	Baruscapillaria spiculata	Baruscapillaria spiculata
Locality	Rio de Janeiro, Brazil	Rio de Janeiro, Brazil	Lake Pátzcuaro, Mexico	Lago Guaíba, Rio Grande do Sul, Brazil	Laguna Chis-Chis, Buenos Aires, Argentina	Laguna San Miguel del Monte, Buenos Aires, Argentina
Host	Ph. brasilianus	Ph. brasilianus	unknown(*)	Ph. brasilianus	Ph. brasilianus	Ph. brasilianus
Specimens measured	2 (one fragmented)	2	1	8	10 (except indication)	8
Body L	16.0	fragmented	9.25 (posterior fragment)	12.9–16.1 (14.7)	10.75–17.60 (14.36)	12.55–17.25 (14.42)
Maximum W	0.070	0.064	0.048	0.042–0.060 (0.050)	0.050–0.060 (0.055)	0.046–0.060 (0.054)
BB W (μm)	-	-	24	-	15–18 n=3	17–20
NR to apex (μm)	-	-	-	70–90 (79)	30–75 (49) n=7	35–65 (52)
Esophagus L	6.0–7.0	-	-	4.6–5.7 (5.3) (sic)	4.25–5.95 (5.29)	3.85–6.05 (5.49)
ME L	-	-	-	0.360–0.477 (0.412)	0.255–0.560 (0.390)	0.280–0.580 (0.412)
Stichosome L	-	-	-	4.6–5.7 (5.3) (sic)	3.85–5.64 (4.89)	4.27–5.61 (5.01)
Stichocyte number	-	-	-	45–49 (46)	32–46 n=9	34–44
First stichocyte (μm)	-	-	-	-	42–70 (57) x 19–28 (22) n=5	50–70 (57) x 20–35 (28)
Terminal stichocyte (μm)	-	-	-	-	65–120 (87) x 28–45 (33) n=9	80–120 (94) x 35–50 (42)
Anterior BL:posterior BL	1:2.66	-	-	-	1:1.03–2.07 (1:1.74)	1:1.30–2.55 (1:1.65)
E L/BL (%)	-	-	-	32.2–40	32.6–39.2 (35.7)	28.11–43.42 (38.55)
ED L	-	-	-	-	1.00–1.58 (1.28) n=6	0.97–1.38 (1.19)
Entire cloaca L	-	-	-	-	2.55–3.11 (2.87)	2.50–3.07 (2.65)
Pre-spicular cloaca L	-	-	-	-	0.180–0.385 (0.275)	0.300–0.550 (0.360)
Spicular cloaca L	-	-	-	-	2.37–2.81 (2.56)	2.0–3.5 (2.62)
Sp L	**2.33**	**1.77**	2.31	2.0–3.9 (2.4)	2.20–2.60 (2.32)	2.20–2.53 (2.30)
Sp L/BL (%)	14.5	-	-	-	13.6–21.0 (16.5)	14.20–17.52 (15.76)
SpS ornamentation	**Four ornamented sections**	**Smooth**	Smooth	Four ornamented sections	Four ornamented sections	Four ornamented sections
SpS - total L	-	1.024	-	2.7–7.3 (5.2)	1.30–2.81 (2.34)	2.40–2.75 (2.48)
SpS section I	-	-	-	-	0.550–0.750 (0.622)	0.620–0.800 (0.680)
SpS section II	-	-	-	-	0.165–0.370 (0.275)	0.240–0.420 (0.320)
SpS section III	-	-	-	-	0.110–0.420 (0.222)	0.250–0.400 (0.300)
SpS section IV	-	-	-	-	0.890–1.680 (1.283)	0.910–1.300 (1.090)
Site of infection	**Cloaca**	**Large intestine**	Intestine?	Cloaca/large intestine	Cloaca	Cloaca

Abbreviations: B body, *BB* bacillary band, *E* esophagus, *ED* ejaculatory duct, *L* length, *ME* muscular esophagus, *NR* nerve ring, *Sp* spicule, *SpS* spicular sheath, *W* width

(*)Accidental in *Chirostoma estor* Jordan (Pisces: Atherinopsidae) *sensu* Moravec et al. 2000; labeling confusion *sensu* Moravec et al. 2001

isolates differed from the other sequences available in the GenBank. The closest matches were species of the family Capillariidae (80–96% identity) with a sequence length coverage greater than 80%. The top matches corresponded to sequences of *Baruscapillaria obsignata* (GenBank Acc. Nos. LC052336 and LC052337). The phylogenetic analysis based on 46 sequences of 18S rDNA from 20 Capillariidae species was performed by BI and ML producing nearly identical well-resolved topologies (Fig. 4). Pairwise DNA analyses revealed that the interspecific variability among different capillariid sequences expressed as pairwise genetic distance (p-distance) ranged from 0.3 to 9.0% (Table 3).

Isolates MT068208 and MT068209 formed a well-supported clade (Clade B, bootstrap 100) with both isolates of *B. obsignata* parasitizing chickens in Kagoshima, Japan, and captive swans in Yamaguchi, Japan, respectively (Tamaru et al. 2015).

Discussion

As stated, *Baruscapillaria spiculata* and *B. appendiculata* are the only two species of intestinal Capillariidae described from the Neotropic cormorant. Reports of both species after their original descriptions are scarce. Fedynich et al. (1997) reported *B. spiculata* parasitizing both cormorants *Ph. brasilianus* and *Phalacrocorax auritus* (Lesson, 1831) from Texas (USA), although their report is not accompanied by either a description or illustration. Instead, there are a few studies available for *B. appendiculata* (Moravec et al. 2000; Monteiro 2006; Monteiro et al. 2011). However, the morphological and morphometric descriptions described in these reports do not agree with the original description of *B. appendiculata*, which causes confusion in the discrimination of these two species (Tables 1 and 2). For example, in the work of Moravec et al. (2000), morphological and metrical differences with respect to the

Fig. 2 *Baruscapillaria spiculata* (Freitas, 1933) parasitizing *Phalacrocorax brasilianus* from the San Miguel del Monte Lagoon, Buenos Aires province, Argentina, observed by light (LM) and scanning electronic (SEM) microscopies. **a** Spicular sheath, proximal section by LM. **b** Second section by LM. **c** Third section by LM. **d** Distal section with annulations by LM. **e-f** Male cloacal opening, membranous bursa with dorsal and lateroventral lobes, caudal papillae, everted cirrus by LM and SEM, respectively. Scale bars are given in micrometers (μm)
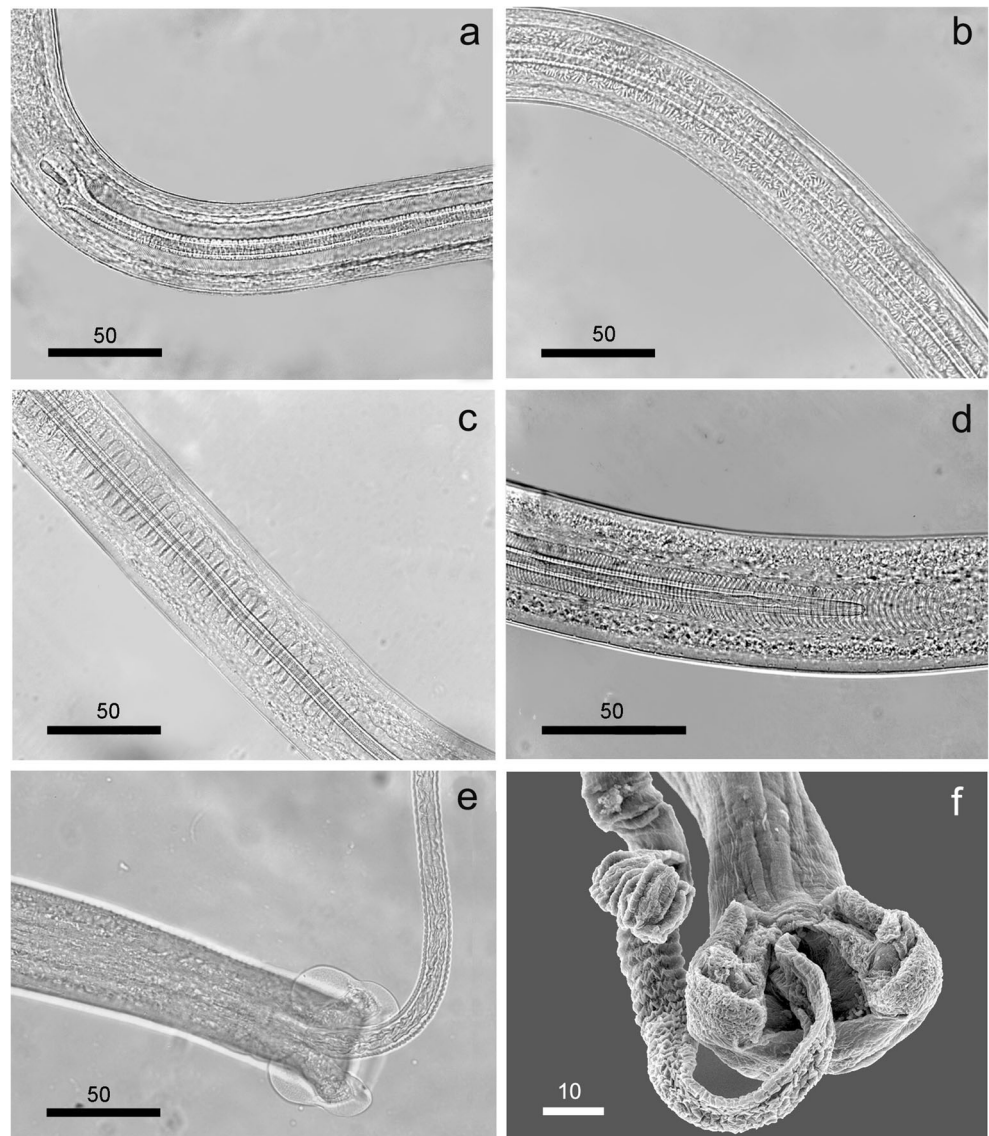
Table 2 Comparative morphology and morphometry of females of the two species of intestinal capillariids described from *Phalacrocorax brasilianus*. In bold, main features differentiating *Baruscapillaria spiculata* and *B. appendiculata*

Source	Freitas 1933a	Freitas 1933b	Moravec et al. 2000	Monteiro 2006	This work	This work
Former identification	*Capillaria spiculata* (description)	*Capillaria appendiculata* (description)	*Ornithocapillaria appendiculata*	*Ornithocapillaria appendiculata*		
Present identification	*Baruscapillaria spiculata*	*Baruscapillaria appendiculata*	?	Probably mixed infection of *B. appendiculata* and *B. spiculata*	*Baruscapillaria spiculata*	*Baruscapillaria spiculata*
Locality	Rio de Janeiro, Brazil	Rio de Janeiro, Brazil	Lake Pátzcuaro, Mexico	Lago Guaíba, Rio Grande do Sul, Brazil	Laguna Chis-Chis, Buenos Aires, Argentina	Laguna San Miguel del Monte, Buenos Aires, Argentina
Host	*Ph. brasilianus*	*Ph. brasilianus*	unknown (*)	*Ph. brasilianus*	*Ph. brasilianus*	*Ph. brasilianus*
Specimens measured	2	not specified	6	6	10 (except indication)	8 (except indication)
Body L	28.0	22.8	14.88–22.13	20.7–25.9 (24.1)	24.00–28.18 (25.85)	23.5–27.8 (25.10)
Maximum W	0.100	0.088–0.096	0.060–0.081	0.065–0.090 (0.072)	0.060–0.080 (0.073)	0.055–0.078 (0.069)
BB W (µm)	-	-	27–36	-	22–45 (34) n=9	20–43 n=6
NR to apex (µm)	-	-	84–153	75–95 (79)	55–105 (77) n=9	45–95 (72) n=5
Esophagus L	6.0–7.0	6.3	4.54–6.11	4.9–7.9 (6.6)	6.35–7.98 (6.99)	5.80–7.50 (6.35) n=7
ME L	-	-	0.315–0.435	0.410–0.530 (0.466)	0.435–0.670 (0.514) n=9	0.390–0.630 (0.495) n=7
Stichosome L	-	-	4.21–5.79	4.4–7.5 (6.2)	5.90–7.12 (6.36) n=9	6.10–7.32 (6.45)
Stichocyte number	-	-	40–45	43–45 (44)	37–48 n=7	35–45 n=6
First stichocyte (µm)	-	-	-	-	57–95 (78) x 20–28 (23) n=9	55–90 (68) x 30–45 (38) n=7
Terminal stichocyte (µm)	1:5.33	1:3.66	-	-	100–150 (120) x 45–60 (51)	90–160 (130) x 40–55 (48) n=6
Anterior BL:posterior BL	-	27.6	27–41	20.4–34.4	1:2.34–3.03 (1:2.71)	1:2.40–3.14 (1:2.81)
E L/BL (%)	-	-	-	-	24.8–29.9 (27.0)	25.3–30.3 (27.5)
Vulva to anterior end	-	-	-	6.1–7.6 (6.9)	6.45–8.13 (7.11)	6.25–7.90 (7.05)
Vulva to esophago-intestinal junction	**0.130**	**0.072**	0.093–0.171	0.057–0.137 (0.081)	0.090–0.150 (0.120)	0.080–0.135 (0.112)
Cuticular membranes on vulvar appendage	**Two (one triangular, other palm shaped)**	**Two well developed Not always present**	Two directed anteriorly	Enlarging towards the apex Heart shaped on figure	Heart shaped	Heart shaped
VA L	0.040	0.048	0.081–0.090	0.070–0.090 (0.080)	0.050–0.110 (0.085)	0.060–0.110 (0.088)
VA W	0.024	0.016–0.024	0.030–0.036	-	0.038–0.050 (0.042) n=4	0.040–0.055 (0.045)
Vagina vera L	-	-	-	-	0.320–0.530 (0.430) n=8	0.300–0.520 (0.410) n=7
Tail L (µm)	-	-	9–12	2–4 (3)	10–18 (15)	12–20 (16)
Egg L x W (µm)	48 x 36	56 x 32	57–60 x 27	45–52 x 17–27	48–55 x 20–27 n=9	50–56 x 20–30
Site of infection	**Cloaca**	**Large intestine**	Intestine?	Cloaca/large intestine	Cloaca	Cloaca

Abbreviations: *B* body, *BB* bacillary band, *E* esophagus, *L* length, *ME* muscular esophagus, *NR* nerve ring, *VA* vulvar appendage, *W* width

(*)Accidental in *Chirostoma estor* Jordan (Pisces: Atherinopsidae) *sensu* Moravec et al. 2000; labeling confusion *sensu* Moravec et al. 2001

original description of *B. appendiculata* are evident which renders, in our opinion, the report of *B. appendiculata* from Mexico rather dubious. The specimens described by Monteiro (2006) from Brazil seem to agree with *B. spiculata* rather than with *B. appendiculata* (Tables 1 and 2). Monteiro et al. (2011) again listed *B. appendiculata*, without a description in a survey of helminths parasitizing the Neotropic cormorant, although it is clear that the specimens concerned were those described in 2006.

Redescription of *B. appendiculata* is necessary to solve this problem but the types of the taxon originally described as *Capillaria appendiculata* Freitas, 1933, deposited in the CHIOC (Acc. Numb. 7469) are lost at present, and then unavailable for comparative purposes. In absence of the type material, any attempt to identify this species should be based on the original description by Freitas (1933b). At the same time, a significant sampling effort of the type host and type

locality, as well as a thorough taxonomic work should be done in order to increase the chances of finding the species originally described as *C. appendiculata* (Freitas 1933b)

As mentioned above, the combination *Ornithocapillaria appendiculata* was used previously by some authors (Moravec et al. 2000; Monteiro 2006; Monteiro et al. 2011) The genus *Ornithocapillaria* Baruš and Sergeeva, 1990 was erected to gather some species of avian capillariids until then included in *Baruscapillaria*. Baruš and Sergeeva (1990a) stated that the main feature differentiating *Ornithocapillaria* and *Baruscapillaria* was the shape of the processes on the posterior end of male body, distinctly rounded and more caudally orientated in *Baruscapillaria* species; conical and more laterally orientated in *Ornithocapillaria* species. In addition, the membranous pseudobursa in males is larger in *Ornithocapillaria* than in *Baruscapillaria*. Other main

Fig. 3 *Capillaria spiculata* Freitas, 1933, male specimen, CHIOC 2833, syntype. **a** Spicular sheath, proximal section. **b** Second section. **c** Third section. **d** Distal section with annulations. **e** Caudal end, dorsal view. **f** Caudal end, ventral view showing lateroventral lobes of membranous bursa. Scale bars are given in micrometers (μm)
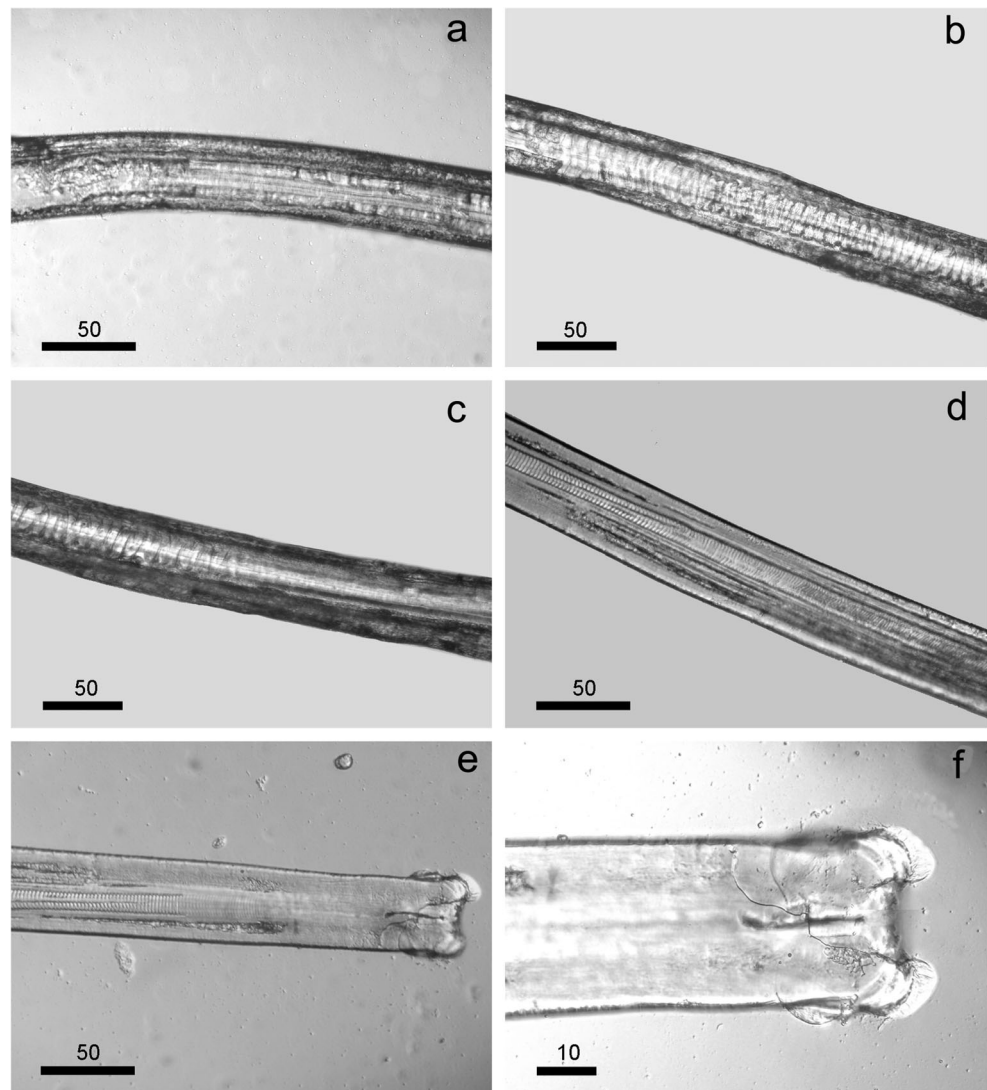
Table 3 Pairwise genetic distance (%) from 18S ribosomal RNA gene sequences (18S rDNA) among specimens of *Baruscapillaria spiculata* (Freitas, 1933) parasitizing *Phalacrocorax brasilianus* from San Miguel del Monte lagoon, Buenos Aires province, and other Capillariidae species

	outgr	B sp1	B sp2	B obs	Pe sp.	P pl1	A put	A eri	P pl2	C hep	A sp.	A mus	A anu	P xen	P tom	E sp.	E dis	E aer	C mad	C ana	C pud
outgr																					
B sp1	11.4																				
B sp2	11.2	0.1																			
B obs	10.7	1.4	1.2																		
Pe sp.	10.6	2.2	2	1.5																	
P pl1	10.5	2	1.8	1.2	0.5																
A put	10.4	2.6	2.4	1.8	0.9	0.6															
A eri	10.5	2	1.8	1.2	0.3	0	0.6														
P pl2	10.5	2	1.8	1.2	0.3	0	0.6	0.7													
C hep	10.3	2.5	2.3	1.8	0.8	0.5	1.1	0.5	0.5												
A sp.	10.6	2.3	2.1	1.6	0.6	0.3	0.7	0.3	0.3	0.7											
A mus	10.2	2.3	2.1	1.6	1	0.7	1	0.7	0.7	1.2	0.7										
A anu	10.2	2.3	2.1	1.6	0.6	0.4	0.6	0.4	0.4	0.9	0.4	0.7									
P xen	10.3	3.4	3.2	2.7	2.9	2.7	3.2	2.7	2.7	2.9	3	2.7	2.7								
P tom	10.9	2.9	2.7	2.1	2.6	2.3	2.9	2.3	2.3	2.9	2.6	2.3	2.7	2							
E sp.	11.3	6.7	6.5	5.9	6.3	6	6.2	6	6	6.4	5.8	5.8	6	7.5	7.1						
E dis	11.1	6.8	6.6	6.1	6.5	6.2	6.5	6.2	6.2	6.6	5.9	6.1	6.2	7.5	7.1	1.5					
E aer	10.9	7.1	7	6.1	6.5	6.2	6.5	6.2	6.2	6.6	5.9	6.1	6.2	7.5	7.1	1.2	0.4				
C mad	12.5	7.5	7.3	7.3	7	7.1	7.4	7.1	7.1	7.3	7	7.5	6.8	8.6	8.2	8	7.5	7.5			
C ana	12.1	7.8	7.7	7.5	7.7	7.5	8	7.5	7.5	8	7.8	7.8	7.5	8.7	8	8.5	8.2	8	5		
C pud	12.7	7.5	7.3	6.8	7.6	7.5	8	7.5	7.5	8	7.8	7.7	7.8	8.7	7.7	8.4	8.2	8	6.1	5.2	
C ten	12.5	7.7	7.5	7	7	6.8	7.3	6.8	6.8	7	7.1	7	7.1	8	7.7	6.3	5.9	5.7	5.7	5.3	5.3

Outgr, *Trichuris suis*; *B sp1*, *Baruscapillaria spiculata* specimen #1 (MT068208); *B sp2*, *Baruscapillaria spiculata* specimen #2 (MT068209); *B obs*, *Baruscapillaria obsignata*; *Pe sp.*, *Pearsonema* sp.; *P pl1*, *Pearsonema plica* (=*Capillaria plica*); *A put*, *Aonchotheca putorii*; *A eri*, *Aonchotheca erinacei*; *P pl2*, *Pearsonema plica*; *C hep*, *Calodium hepaticum*; *A sp.*, *Aonchotheca* sp.; *A mus*, *Aonchotheca musimon*; *A anu*, *Aonchotheca annulosa*; *P xen*, *Pseudocapillaroides xenopi* (=*Capillaria xenopi*); *P tom*, *Pseudocapillaria tomentosa*; *E sp.*, *Eucoleus* sp.; *E dis*, *Eucoleus dispar*; *E aer*, *Eucoleus aerophilus*; *C mad*, *Capillaria madseni*; *C ana*, *Capillaria anatis*; *C pud*, *Capillaria pudendotecta*; *C ten*, *Capillaria tenuissima*
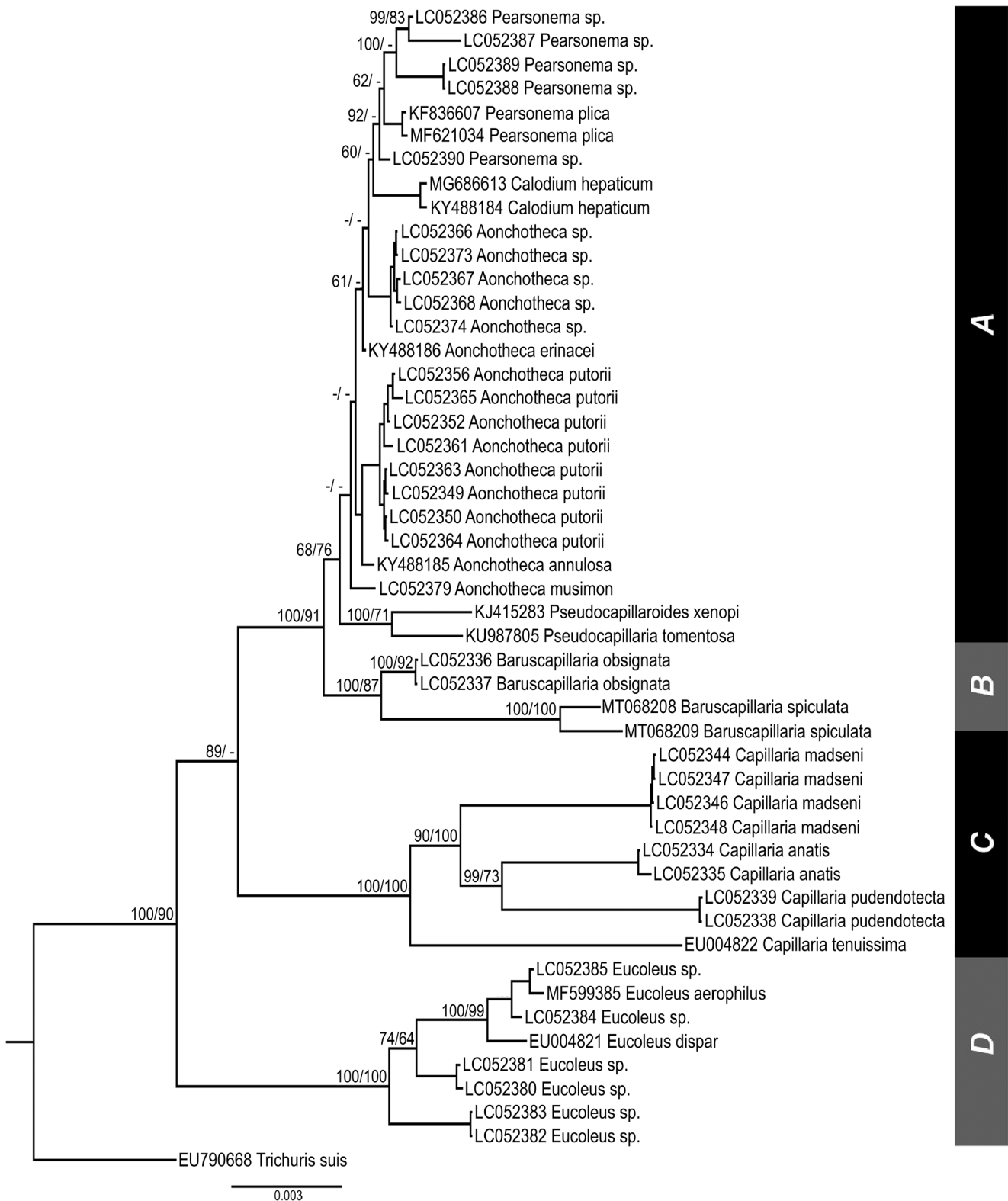
Fig. 4 Consensus phylogenetic tree of Capillariidae species mainly from mammals and birds based on 18S ribosomal RNA gene (18S rDNA). Inferred using both Bayesian Inference and Maximum Composite Likelihood. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown on the branches (Bayesian Inference/Maximum Composite Likelihood). Bootstrap values lower than 60% are not shown

features defining *Ornithocapillaria* species were the female vulva always possessing a long tubular vulvar appendage, and the presence of four bacillary bands.

However, Okulewicz (1993) considered unjustified the separation between both genera and synonymized *Ornithocapillaria* with *Baruscapillaria*. This author, studying several species of capillariids from Palearctic birds, observed that the main characters argued to separate *Baruscapillaria* and *Ornithocapillaria* could be found in species attributed to either genus. She also remarked that the original definition of *Ornithocapillaria* included only non-specific features and even characters that may be considered intraspecific such as the shape and inclination of the lateral processes supporting the pseudobursa. Moreover, Okulewicz (1993) stated that the number, position and size of bacillary bands lack a diagnostic value at a generic level assuming that most bird capillariids possess four bacillary bands: two lateral (wide), one ventral and one dorsal (narrower).

Moravec et al. (2000), based on the presence of a vulvar appendage and the morphology of the male caudal end, transferred three species parasitizing cormorants from *Baruscapillaria* to *Ornithocapillaria*, and proposed the combinations *Ornithocapillaria appendiculata*, *Ornithocapillaria carbonis* (Rudolphi, 1819), and *Ornithocapillaria phalacrocoraxi* (Borgarenko, 1975). However, shortly after Moravec et al. (2001) adopted the synonymy of *Ornithocapillaria* with *Baruscapillaria* proposed by Okulewicz (1993), and the combinations *B. appendiculata* and *Baruscapillaria carbonis* were readily reported, especially by some European authors (Moravec et al. 2001; Frantová 2001; Kanarek and Zaleśny 2014; Moravec and Scholz 2016).

All species shown in Fig. 4 clustered robustly according to the generic arrangement of Moravec (1982). The same was observed in the surveys of Tamaru et al. (2015), and Sakaguchi et al. (2020). In these latter works, the genus *Baruscapillaria* was only represented by isolates of *B. obsignata* since until then no sequences were available for other species of the genus. The present work brings, as expected, a sister-taxon relationship between the isolates of *B. obsignata* and *B. spiculata*.

The pairwise genetic distance between our isolates and *B. obsignata* ranged between 1.2 and 1.4%. These distances may be seen as relatively high considering that most distances between species belonging to the same genus were usually lower than 1% (Table 3, Fig. 4). There were as well some avian *Capillaria* spp. which hold greater genetic distances to each other compared with the mean distances observed between species belonging to the same genus (Table 3, Fig. 4). The same pattern was remarked by Tamaru et al. (2015) and Sakaguchi et al. (2020) for species of avian *Capillaria* which could not be easily discriminated on morphological grounds, although their genetic characterization of 18S rDNA clearly differentiated them from one another (Sakaguchi et al. 2020). These distances are then not completely unexpected, since Moravec (1982) remarked that further studies will apparently

result in breaking up the present genus *Capillaria* into additional independent genera. Similarly, Okulewicz (1993) considered the genus *Baruscapillaria* as the most heterogeneous and speciose among the bird capillariids and concluded that it seems to require revision. Therefore, we largely agree with Sakaguchi et al. (2020) that integrative approaches are highly recommended in capillariid worms.

Indeed, thorough morphological examination is necessary and should be accompanied with other approaches including different molecular genetic analysis and assessment of the geographical distribution and host ranges of multiple species. Variability within *Baruscapillaria* and its probable division into more genera should also be confirmed with the inclusion of more species.

Declaration

Ethics approval Neotropic cormorant individuals were captured according to permissions, legal procedure rules, and the animal protection law from the Dirección de Flora y Fauna, Ministerio de Asuntos Agrarios, Buenos Aires province. Expediente N° 22500-31095/15.

Conflict of interest The authors declare no competing interests.

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