Journal of Helminthology

cambridge.org/jhl

Research Paper

Cite this article: Sardella CJ, Mancini M, Salinas V, Simões RO, Luque JL (2020). A new species of *Contracaecum* (Nematoda: Anisakidae) found parasitizing *Nannopterum brasilianus* (Suliformes: Phalacrocoracidae) and *Hoplias argentinensis* (Characiformes: Erythrinidae) in South America: morphological and molecular characterization of larval and adult stages. *Journal of Helminthology* **94**, e184, 1–11. https://doi.org/10.1017/S0022149X20000644

Received: 3 May 2020
Revised: 16 July 2020
Accepted: 22 July 2020

Key words:
Fourth-stage larvae; mitochondrial cytochrome oxidase II; parasites; scanning electron microscopy; third-stage larvae

Author for correspondence:
J.L. Luque, E-mail: luqueufrrj@gmail.com

CAMBRIDGE UNIVERSITY PRESS

A new species of *Contracaecum* (Nematoda: Anisakidae) found parasitizing *Nannopterum brasilianus* (Suliformes: Phalacrocoracidae) and *Hoplias argentinensis* (Characiformes: Erythrinidae) in South America: morphological and molecular characterization of larval and adult stages

C.J. Sardella[1], M. Mancini[2], V. Salinas[2], R.O. Simões[3] and J.L. Luque[3]

[1]Programa de Pós-Graduação em Ciências Veterinárias, Universidade Federal Rural do Rio de Janeiro, Rodovia BR 465, km 7, 23890-000 Seropédica, RJ, Brazil; [2]Ecología y Acuicultura, Facultad de Agronomía y Veterinaria, Universidad Nacional de Río Cuarto, Argentina and [3]Departamento de Parasitologia Animal, Universidade Federal Rural do Rio de Janeiro, 23851-970 Seropédica, RJ, Brazil

Abstract

Nematode species of the genus *Contracaecum* Railliet & Henry, 1912 have been reported around the world in many species of fish-eating birds and seals. Here, *Contracaecum jorgei* n. sp. is morphologically described using light and scanning electron microscopy for adults and fourth-stage larvae (L4) found in the bird *Nannopterum brasilianus* and third-stage larvae (L3) found in the freshwater fish *Hoplias argentinensis*, both from the province of Córdoba, Argentina. Additionally, sequences of cytochrome *c* oxidase subunit II were obtained from these specimens and molecular phylogenetic analysis was used to determine its relationships within the genus. The present species is distinguished from other species by the number and disposition of cephalic papillae; shape and size of the interlabia; length of the spicules; and number and arrangement of papillae in the posterior end of the male. Furthermore, in the molecular analyses, sequences obtained from adult L4 and L3 specimens of *C. jorgei* n. sp. were similar and grouped, forming an independent lineage, thus confirming it as a distinct species. Thus, morphological characteristics associated with molecular data support the proposal of a new species.

Introduction

Several species of the genus *Contracaecum* Railliet & Henry, 1912 have been reported around the world in many species of fish-eating birds, including those belonging to the family Phalacrocoracidae (Abollo *et al.*, 2001). Their life cycle is heteroxenous, involving piscivorous birds or pinnipeds (definitive hosts), small crustaceans (first intermediate host) and fish (intermediate hosts). Birds are infected by eating fish containing third-stage larvae (L3), and the fish acquire them by ingesting other fish and/or crustaceans containing second-stage larvae or L3 (Anderson, 2000). Adults are found parasitizing the intestine and proventriculus of the definitive host, where they often cause pathological lesions (Garbin *et al.*, 2011; Violante-González *et al.*, 2011).

The Neotropical cormorant *Nannopterum brasilianus* (Gmelin, 1789) (syn. *Phalacrocorax brasilianus*) (Suliformes: Phalacrocoracidae) occurs from Patagonia to the coast of Texas (Fedynich *et al.*, 1997; Amato *et al.*, 2006; Conde-Tinco & Iannacone, 2013, Kennedy & Spencer, 2014). This bird inhabits freshwater and marine environments and is the only one within the family found throughout the Neotropical region (Kalmbach & Becker, 2005).

Five species of *Contracaecum* have been reported parasitizing *N. brasilianus*: *Contracaecum rudolphii* Hartwich, 1964 (syn. *C. spiculigerum*) in Brazil, Chile, Cuba and USA (Baruš, 1966; Fedynich *et al.*, 1997; Torres *et al.*, 2000; Amato *et al.*, 2006); *Contracaecum multipapillatum* Drasche, 1882 in Mexico (Vidal-Martínez *et al.*, 1994; Violante-González *et al.*, 2011); *Contracaecum australe* Garbin, Mattiucci, Paoletti, González-Acuña and Nascetti, 2011 in Chile and Argentina (Garbin *et al.*, 2011; Biolé *et al.*, 2012); *Contracaecum travassosi* Gutierrez, 1943 in Uruguay (Lent & Freitas, 1948); and *Contracaecum caballeroi* Bravo-Hollis, 1939 in Uruguay (Lent & Freitas, 1948).

In this study, we describe a new species of *Contracaecum* using light and scanning electron microscopy (SEM) for adults and fourth-stage larvae (L4) found in *N. brasilianus* and L3 found in the freshwater fish *Hoplias argentinensis* Rosso, Mabragaña, González-Castro, Bogan, Cardoso, Mabragaña, Delpiani & Díaz de Astarloa, 2018, both from the province of Córdoba, Argentina. Additionally, molecular phylogenetic data were used to determine its relationships within the genus.

Material and methods

Parasite collection

Adult nematodes and L4 were collected from the intestine and proventriculus of two specimens of *N. brasilianus*, and L3 specimens were collected from the intestine of one specimen of *H. argentinensis* from a shallow Pampean lake in central Argentina (33°25′S, 62°54′W), between September and December 2017. Birds were transported to a field laboratory where they were weighed and necropsied. During necropsy, their entire digestive tracts were removed and analysed. The contents were washed with water over a sieve with mesh of 0.25 mm and the sediment was placed in Petri dishes to collect nematodes. The fish specimen was kept in an ice chest to ensure good morphological condition of the parasites and to protect them during transport to the Pharmacology Laboratory of Universidad Nacional de Río Cuarto, where the specimen was weighed, measured and subsequently necropsied, through a ventral incision from the anal opening to the operculum line to expose the body cavity. All internal organs were individualized and placed in Petri dishes containing saline solution (0.65% sodium chloride), and subsequently examined with the aid of a stereoscopic microscope to collect the parasites. The isolated nematodes were fixed and stored in 70% ethanol.

Morphological study

Adult nematodes and L4 collected from *N. brasilianus* and L3 collected from *H. argentinensis* were morphologically examined. Later, the anterior and posterior parts of the specimens were clarified in glycerine. Measurements were taken in millimetres (means followed by the range in parentheses). Morphological analyses were conducted using an Olympus BX51 light microscope (Tokyo, Japan). Nematode identification was made according to keys of Anderson (2000) and Luque *et al.* (2011). Holotype, allotype and paratypes were deposited in the Helminthological Collection of the Instituto Oswaldo Cruz, Rio de Janeiro, Brazil (CHIOC).

For SEM analysis, one adult male and female, one L4 and one L3 were dehydrated through a graded ethanol series, dried in hexamethyldisilazane, coated with gold and examined in a JEOL JSM-740 1 F, at an accelerating voltage of 4 kV. The samples were mounted on aluminium stubs, coated with a 20 nm layer of gold and examined with a JEOL JSM 6390LV scanning electron microscope (operating at 15 kV) (JEOL, Akishima, Tokyo, Japan) at the Rudolf Barth Electron Microscopy Platform of Instituto Oswaldo Cruz, Rio de Janeiro.

Molecular and phylogenetic analyses

The middle part of two adult nematodes, one L4 and one L3 were separated from the rest of the body and used to characterize genetically the individual specimens by sequencing of the mitochondrial (mt) DNA cytochrome *c* oxidase subunit II (*cox*-2) gene. Genomic DNA was isolated using the phenol/chloroform protocol as in Billings *et al.* (1998). The *cox*-2 region of the mtDNA was amplified using the primers 211 (forward 5′ TTTTCTAGTTAT ATATAGATTGRTTTYAT 3′) and 210 (reverse 5′CACCAACT CTTAAAATTATC 3′) (Nadler & Hudspeth, 2000). Polymerase chain reaction (PCR) reactions were performed in a volume of 25 µl with 20 mM of Tris hydrochloride (Tris-HCl) at pH 8.4; 50 mM of potassium chloride (KCl); 250 lM of each phosphated deoxyribonucleotides (dNTPs); 1.5 mM of magnesium chloride ($MgCl_2$); 0.5 lM of each oligonucleotide primer; 1.25 U of Platinum Taq DNA polymerase (Invitrogen, Carlsbad, California, USA); and 2 µl of genomic DNA. PCR was performed using the cycling parameters specified in previous reports (Zhu *et al.*, 1998; Nadler & Hudspeth, 2000). PCR products were visualized with Sybergreen (Invitrogen, Eugene, Oregon, USA) staining before electrophoresis on 1.5% agarose gels. The amplified products were purified with Exo-SAP-IT Kit (GE Healthcare Life Sciences, Grens, Switzerland) following the manufacturer's instructions, sequenced using an ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems-Perkin Elmer, Waltham, MA, USA) in a MegaBACE sequencer (GE Healthcare Life Sciences). All molecular analyses were performed at the Laboratory of Molecular Biology from the Universidade Federal Rural of Rio de Janeiro.

To examine the phylogenetic relationships, the nucleotide sequences obtained in this study and those retrieved from GenBank were aligned with the CLUSTAL W algorithm of the Bioedit Package (Thompson *et al.*, 1994; Hall, 1999). Sequences of *Toxascaris leonina* (NC023504) and *Ascaris lumbicoides* (AF179907) were used as the outgroups. The best evolutionary model for *cox*-2 gene was inferred by the Bayesian inference (BI) criterion using MEGA 7.0 software for both analysis, maximum likelihood (ML) and BI as optimality criterion using the Hasegawa–Kishino–Yano model plus gamma distribution and invariable sites (HKY) + G + I (Tamura *et al.*, 2011). The same software was used for construction of genetic distances using Kimura two parameters (Kimura, 1980). The statistical support for the *cox*-2 tree was determined by a heuristic search with 1000 bootstrap replicates (Guindon & Gascuel, 2003) and posterior probability (PP) for BI run in MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2005) with the uncorrelated relaxed clock (Drummond *et al.*, 2006) and the conditioned reconstructed process (Yule process; Gernhard, 2008). The Markov Chain Monte Carlo simulations were run for 8^9 generations and sampled at every 8^5 generations, with a burn-in of 10%, in BEAST version 1.8.4 (Drummond & Rambaut, 2007). The nodal support of the topology of each gene was calculated by bootstrap replication and the PP with a *cut-off* of 50%.

The phylogenetic analyses of the ML and BI trees were done with the mtDNA *cox*-2 of larvae (L3 and L4) and adults of *Contracaecum* sp. Sequences obtained were deposited at GenBank under the accession numbers MT304462 (adult), MT304463 (L4) and MT304464 (L3).

Results

Contracaecum jorgei *n. sp.*

Description

General morphology. Body robust, filiform with fine transversal cuticular striations along the body, more evident on the anterior and posterior region. Interlabia subtriangular in shape, distal tip not bifurcate and the same length as the lips with one shallow apical notch (figs 1B and 2C). Lips with two conspicuous and lobed auricles, each with two prominent sensory pits at external end (fig. 2C). Dorsal lip larger than ventrolateral lips with two

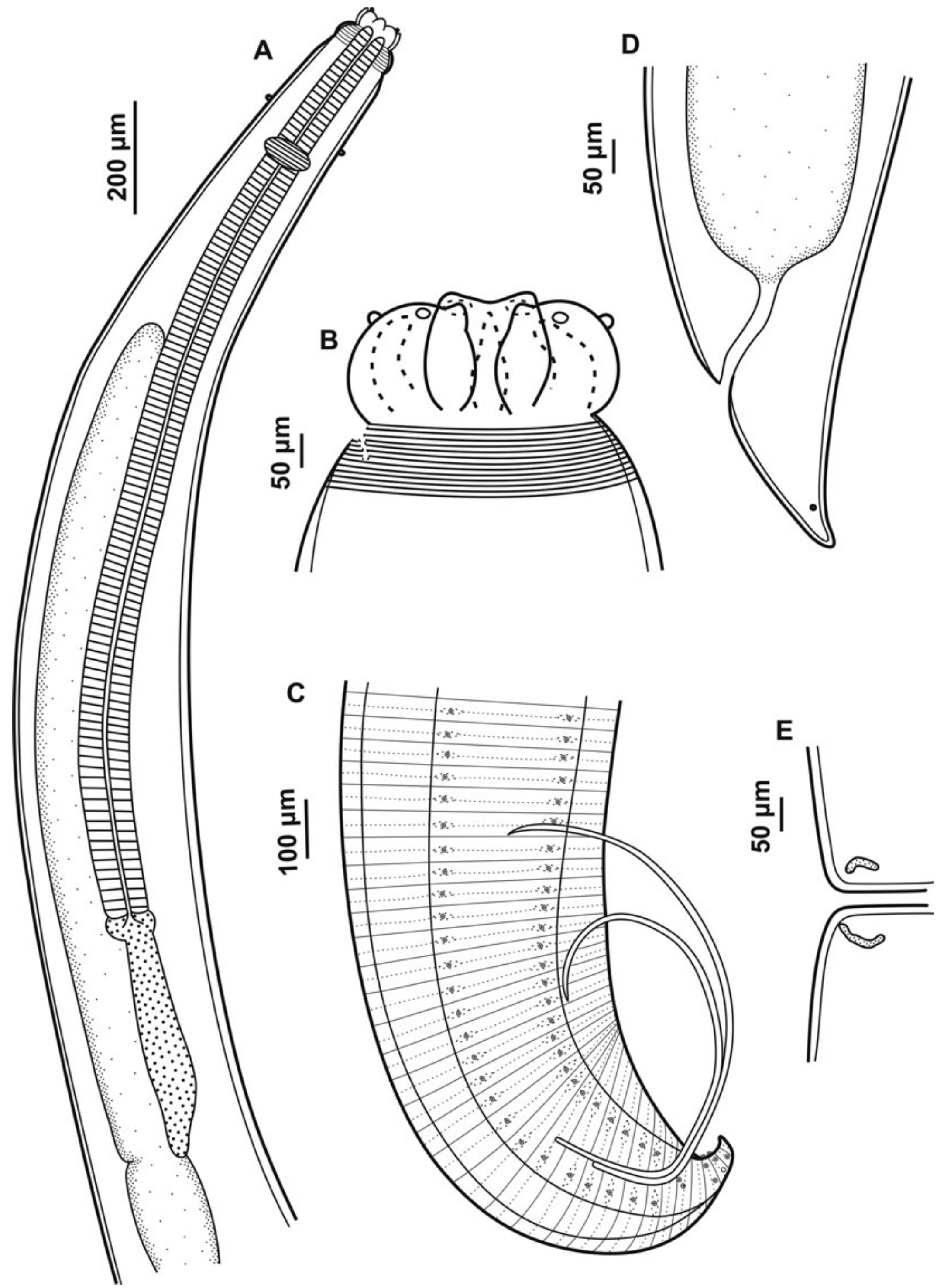

Fig. 1. Adults of *Contracaecum jorgei* n. sp. (A) Anterior end, dorsoventral view showing lips, cephalic collar, cephalic papilla, nerve ring, oesophagus, intestinal cecum, ventriculus and ventricular appendix. (B) Dorsal and ventrolateral lips, interlabia and cephalic collar. (C) Male, posterior end showing pre- and postcloacal papilla distribution, spicules and cloaca. (D) Female, posterior end showing cloaca and phasmid. (E) Female, median region of the body, vulva.

sublateral double papillae, each ventrolateral lip with one small externo-lateral papilla with irregular cuticular ornamentation and separate pore-like opening of amphid in addition to large double papilla (fig. 2C, D). Conspicuous cephalic collar with V-shaped lateral region without striations (fig. 2A, C). Oesophagus with reduced globular ventriculus (fig. 1A). Excretory pore at base of lips. Deirids slightly anterior to the nervous ring (figs 1A and 2A, B), rounded shape, not visible on all specimens. Ventriculus with posterior appendix (fig. 1A), intestinal caecum well developed, longer than ventricular appendix with final portion rounded (fig. 1A). Male and female presenting conical tail with pointed tip (figs 1C, D and 2E, F).

https://doi.org/10.1017/S0022149X20000644 Published online by Cambridge University Press

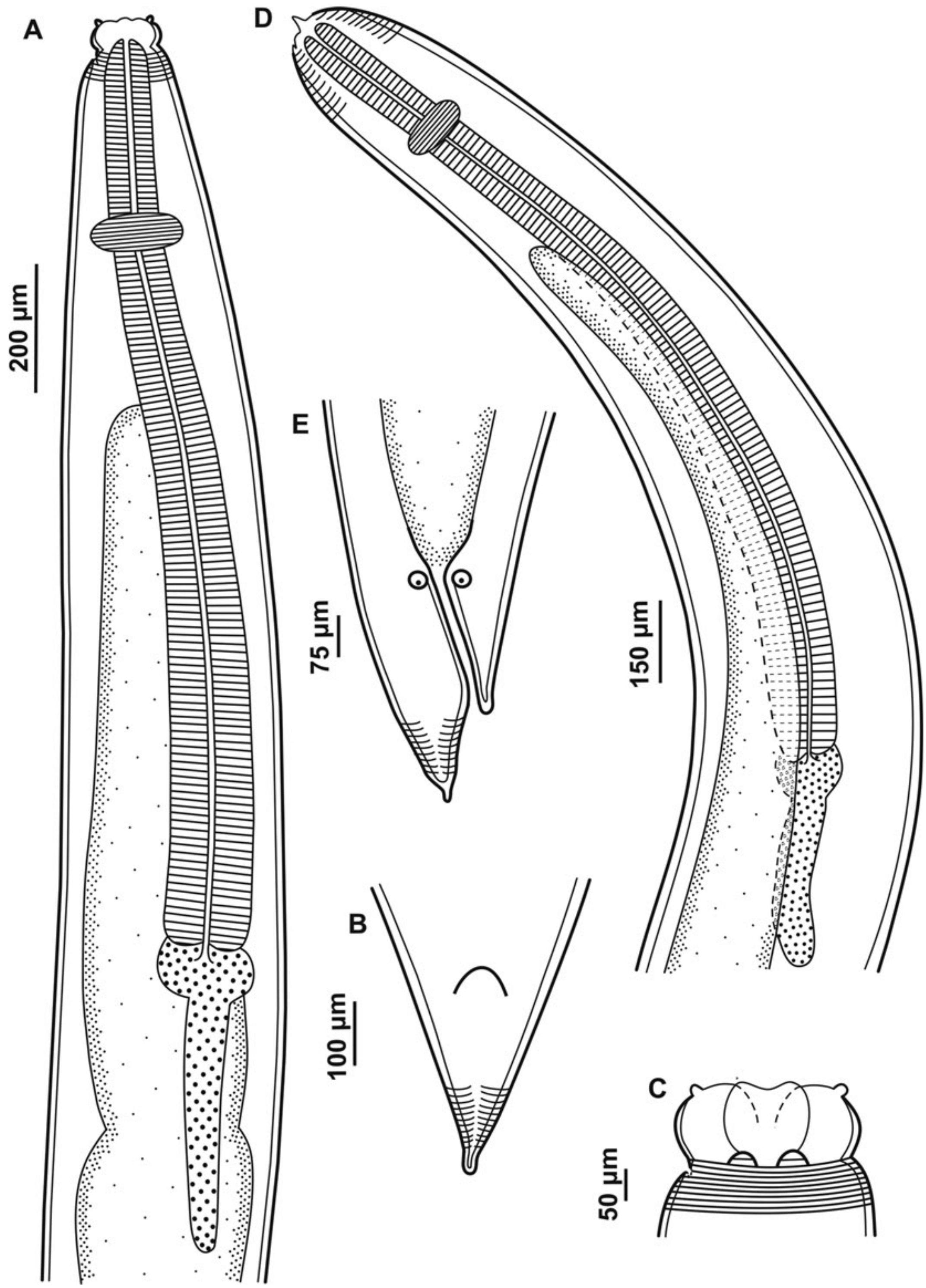

Fig. 2. Larvae of *Contracaecum jorgei* n. sp. (A) Fourth larval stage, anterior end, lateral view showing cephalic collar, cephalic papilla, nerve ring, oesophagus, intestinal cecum, ventriculus and ventricular appendix. (B) Fourth larval stage, posterior end showing a small projection with cuticle striated transversely. (C) Fourth larval stage, dorsal and ventrolateral lips, cephalic collar and cephalic papilla. (D) Third larval stage, anterior end, lateral view showing larval tooth, cuticle striated transversely, nerve ring, oesophagus, intestinal cecum, ventriculus and ventricular appendix. (E) Third larval stage, posterior end showing a small projection with cuticle striated transversely.

Male. Based on holotype and eight paratypes. Body length 14.31–20.98 (15.95), width at the intestinal caecum level 0.29–0.59 (0.38). Distance from anterior end to nerve ring 0.29–0.46 (0.37). Distance from anterior end to deirids 0.29 ($n = 1$). Oesophagus length 2.01–3.88 (2.62) and width 0.09–0.19 (0.12). Intestinal caecum length 1.19–3.32 (1.94) and width 0.07–0.15 (0.09). Length of intestinal caecum to body length ratio 8.00–15.83%. Ventriculus length 0.03–0.09 (0.05) and width 0.04–0.07 (0.06). Ventricular appendix length 0.29–0.57 (0.40) and width 0.04–0.09 (0.06). Ventricular appendix length representing

https://doi.org/10.1017/S0022149X20000644 Published online by Cambridge University Press

1.82–3.41% of body length. Tail length 0.15–0.21 (0.17). Caudal extremity presenting 26 precloacal pairs of papillae equidistant, forming two subventral lines extending anteriorly (figs 1C and 2F). Six postcloacal pairs of papillae: the first pair of subventral proximal papillae posterior to cloaca, the second pair of large paracloacal papillae (the pair of paracloacal papillae are asymmetrically displaced to the first pair proximal, first pair more anterior than the second pair) and four distal pairs of papillae (two subventral pairs and two sublateral pairs) (fig. 2I). One pair of phasmids situated between the sublateral pairs of papillae. Median plaque (median papilla) barely visible on anterior cloaca rim (fig. 2H). Spicules are subequal, distal tip pointed (figs 1C and 2G). Right and left spicule length 2.28–3.63 (2.81) ($n = 4$) and 2.03–3.18 (2.61) ($n = 4$), representing 13.47–19.72% and 13.17–17.28% of total body length, respectively.

Female. Based on allotype and five paratypes. Body length 15.25–18.77 (16.64) and width at intestinal caecum level 0.32–0.50 (0.40). Distance from anterior end to nerve ring 0.30–0.39 (0.36). Deirids were not visualized. Oesophagus length 2.50–3.81 (2.96) and width 0.08–0.12 (0.10). Intestinal caecum length 1.60–2.10 (1.90) and width 0.07–0.15 (0.10). Length of intestinal caecum to body length ratio 10.53–11.18%. Ventriculus length 0.02–0.07 (0.04) and width 0.02–0.07 (0.05). Ventricular appendix length 0.40–0.61 (0.49) and width 0.07–0.09 (0.07).Ventricular appendix length representing 2.63–3.26% of body length. Vulva in anterior half of body (fig. 1E). Distance from anterior end to vulva 6.57–8.48 (7.86). Ratio of vulva to body length 43.11–45.17%. Tail length 0.17–0.25 (0.22). One pair of phasmids on the tail end (figs 1D and 2E).

L4. Based on six paratypes. Large-sized nematodes white or yellow-white in colour. Cuticle striated transversely and more distinct at the extremities of the body (fig. 3A–C). Cephalic extremity rounded with three well-developed lips (one dorsal and two ventrolateral) (figs 3C and 4A). Lips with two conspicuous and lobed auricles, absence of interlabia (fig. 4A). Lips smooth, without cuticular and auricular striations, presenting cephalic papillae on each ventrolateral lip, with a single oval papilla and lateral amphid; well-developed cephalic collar. Body length 14.01–16.67 (15.17) and width at the caecum level 0.21–0.52 (0.30). Excretory pore located between the ventrolateral lips, difficult to visualize. Deirids not visible. Distance from anterior end to nerve ring 0.28–0.42 (0.32). Oesophagus length 1.99–2.91 (2.24) and width 0.08–0.12 (0.09) (fig. 3A). Intestinal caecum length 1.31–2.26 (1.56) and width 0.08–0.17 (0.11) (fig. 3A). Length of intestinal caecum to body length ratio 9.35–13.57%. Oval ventriculus length 0.05–0.10 (0.08) and width 0.05–0.13 (0.10) (fig. 3A). Ventricular appendix length 0.31–0.50 (0.42) and width 0.05–0.12 (0.07) (fig. 3A). Length of ventricular appendix representing 2.27–2.99% of body length. Conical tail with pointed tip with 0.10–0.18 (0.14) in length (figs 3B and 4B).

L3. Based on four paratypes. Medium-sized nematodes white in colour with cuticle striated transversely (fig. 4C). Body length 11.10–12.23 (11.56) and width at the caecum level 0.24–0.39 (0.29). Cephalic extremity rounded with three underdeveloped lips, one lip with a small larval tooth (figs 3D and 4C, D). Excretory pore situated below the ventral cephalic tooth 0.04–0.05 (0.05) in length (fig. 3D). Nerve ring located 0.18–0.28 (0.25) from the anterior end (fig. 3D). Oesophagus length 1.63–1.89 (1.76) and width 0.06–0.16 (0.10) (fig. 3D). Ventriculus small and oval, length 0.03–0.05 (0.04) and width 0.04–0.06 (0.05) (fig. 3D). Intestine distinct and dark. Ventricular appendix length 0.30–0.37 (0.34) and width 0.04–0.07 (0.06) (fig. 3D). Length of ventricular appendix representing 2.76–3.07% of body length. Intestinal caecum longer than ventricular appendix, length 0.98–1.30 (1.08) and width 0.10–0.12 (0.12). Length of intestinal caecum representing 8.86–11.57% of body length. Posterior extremity with two anal glands (fig. 3E). Conical tail with pointed tip with 0.11–0.13 (0.13) in length (figs 3E and 4E, F).

Taxonomic summary

Type host. *Nannopterum brasilianus* (Gmelin, 1789) (Suliformes: Phalacrocoracidae).

Site of infection. Intestine and proventriculus of *N. brasilianus* and intestine of *H. argentinensis*.

Type locality. Pampean shallow lakes, Unión Department, province of Córdoba, Argentina (33°25′S, 62°54′W).

Prevalence. 50% (one infected/two host specimens examined) in *N. brasilianus*; 100% (one infected/one host specimen examined) in *H. argentinensis*.

Intensity. 22 in *N. brasilianus* and four in *H. argentinensis*.

Specimens deposited. Holotype (adult male specimen from *N. brasilianus*, CHIOC number 38925a). Allotype (adult female specimen from *N. brasilianus*, CHIOC number 38925b). Paratypes:3 adult males and 2 adult females (CHIOC number 38925c); 3 L4 from *N. brasilianus* (CHIOC number 38925d); and 2 L3 from *H. argentinensis* (CHIOC number 38926).

This article was registered in the Official Register of Zoological Nomenclature (ZooBank) as: urn:lsid:zoobank.org:act:1D6178CA-533C-417C-8F27-53E4E72CC3E9.

Etymology. The new species is named in honour of Antônio Jorge de Andrade Sardella, father of the first author.

Molecular and phylogenetic analyses

Genetic sequences from *Contracaecum jorgei* n. sp. were generated (519 bp). Pairwise/Basic Local Alignment Search Tool (BLAST)/National Center for Biotechnology Information (NCBI) comparisons with the GenBank mt *cox*-2 gene data set confirmed the species as the genus *Contracaecum*, but not the species identification with any *Contracaecum* spp. available. Intraspecific genetic distance of *C. jorgei* n. sp. sequences ranged from 0.000 (between L3 and L4) to 0.004 (between adult and L3; between adult and L4). The interspecific distances between *C. jorgei* n. sp. and the species of the nearest clade were 0.105–0.107 (*Contracaecum overstreeti*) and 0.118 (*Contracaecum gibsoni*).

Tree topologies produced with different optimality criteria (ML and BI) were similar, with little variation in node support values (fig. 5). The sequences belonging to L3, L4 and adult of *C. jorgei* n. sp. were similar and grouped forming a well-supported clade (ML = 100%; Bayesian posterior probabilities (BPP) = 100%). *Contracaecum jorgei* n. sp. formed a sister group with *C. gibsoni* and *C. overstreeti* that was well supported (ML = 65%; BPP = 96%).

Discussion

The proposed new species belongs to the genus *Contracaecum* according to the following morphological characteristics: oesophagus provided with ventriculus, presence of posterior ventricular appendix, anterior caecum and excretory pore situated at the level of lip bases, and adults presenting interlabia (Moravec, 1998).

When comparing *C. jorgei* n. sp. with the other species described from *N. brasilianus*, the species *C. rudolphii*, *C. australe* and *C. travassosi* are the most similar regarding the morphometry of the oesophagus, intestinal caecum and number and distribution of caudal papillae; however, they can be distinguished by the size of

https://doi.org/10.1017/S0022149X20000644 Published online by Cambridge University Press

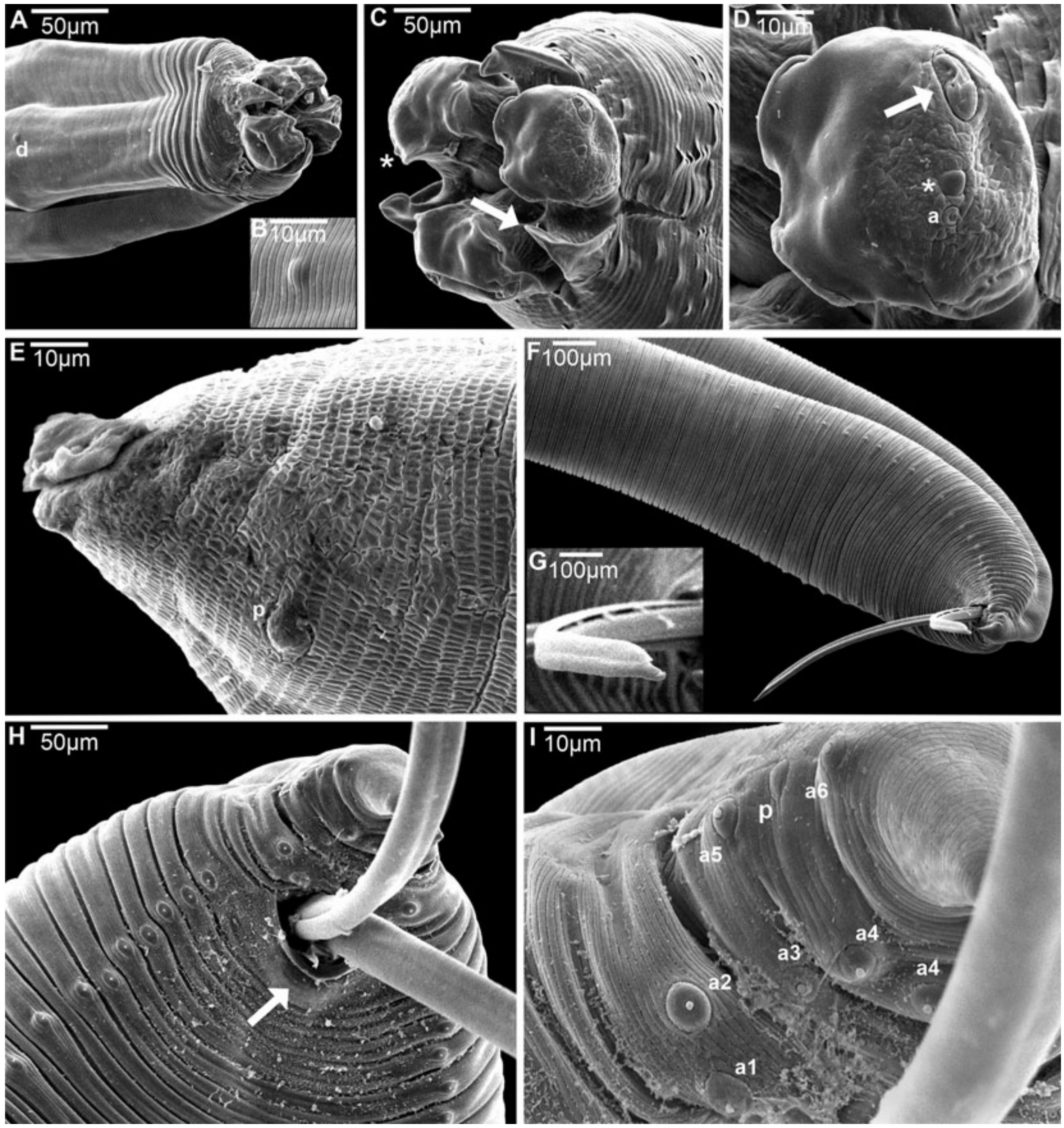

Fig. 3. Scanning electron microscopy of adults of *Contracaecum jorgei* n. sp. (A) Male, anterior end, lateral view showing cephalic collar and deirid. (B) Detail showing deirid. (C) Female, anterior end showing dorsal and ventrolateral lips, interlabia (arrow) and sensory pit (*). (D) Female, ventrolateral lip showing one large double papilla (arrow), one externo-lateral papilla (*) and amphid (a). (E) Female, posterior end showing conical tail with pointed tip. (F) Male, posterior end showing precloacal and postcloacal papillae, spicules and conical tail with pointed tip. (G) Distal spicule end, lateral view. (H) Male, ventral view posterior end showing precloacal papillae, postcloacal papillae and median papillae (arrow). (I) Male, ventral view showing postcloacal papillae. Abbreviations: d, deirid; p, phasmid; a1, proximal papilla pair; a2, paracloacal papilla pair; a3 and a4, distal subventral papillae; a5 and a6, distal sublateral papillae.

spicules. The new species has subequal and shorter spicules than *C. australe* and *C. travassosi*, which have equal and long spicules (9.60–15.88 mm and 7.70–11.10 mm, respectively). Moreover, *C. rudolphii* can be distinguished from the new species by having unequal and larger spicules (right 4.50–7.50 mm and left 5.90–8.20 mm), whereas *C. multipapillatum* presents equal and smaller spicules (0.89–1.11 mm and 0.90–1.09 mm) than the new species (right 3.63–2.28 mm and left 3.18–2.03 mm) and a greater number of caudal papillae (32–93 in *C. multipapillatum*; 61 in *C. caballeroi* and 32 in *C. jorgei* n. sp.). In addition, *C. rudolphii*, *C. australe* and *C. caballeroi* can be separated from the new species by the shape of the interlabia and arrangement of cephalic papillae. *Contracaecum rudolphii* and *C. australe* present bifurcation in the distal end of the interlabia, two double papillae on the dorsal lip, one double papilla and one amphid in each ventrolateral lip, while *C. caballeroi* possesses two large papillae in the dorsal lip and two papillae in each ventrolateral lip (one large ventral and one smaller lateral). In contrast, *C. jorgei* n. sp. has an interlabia without bifurcation with the same length as the lips and one additional small papilla in each ventrolateral lip. Furthermore, *C. multipapillatum* and *C. caballeroi* are differentiated from *C. jorgei* n. sp. by having interlabia smaller than the lips, longer length (20.63–32.34 mm in *C. multipapillatum*; 24.29–26.97 mm in *C. caballeroi*; 14.31–20.98 mm in the new species) and longer ventricular appendix (0.47–0.69 mm; 0.51–0.61 mm vs. 0.29–0.57 mm in the new species). Additionally, *C. multipapillatum* has a longer oesophagus (2.60–4.62 mm vs. 2.01–3.88 mm in the new species) and ventriculus (0.12–0.23 mm vs. 0.03–0.09 mm in the new species), while *C. caballeroi* has a conical tail without pointed tip (Lent & Freitas, 1948; Amato *et al.*, 2006; Garbin *et al.*, 2011; Violante-González *et al.*, 2011).

Other species of *Contracaecum* reported in the American continents have some similarities with the new species. *Contracaecum*

https://doi.org/10.1017/S0022149X20000644 Published online by Cambridge University Press

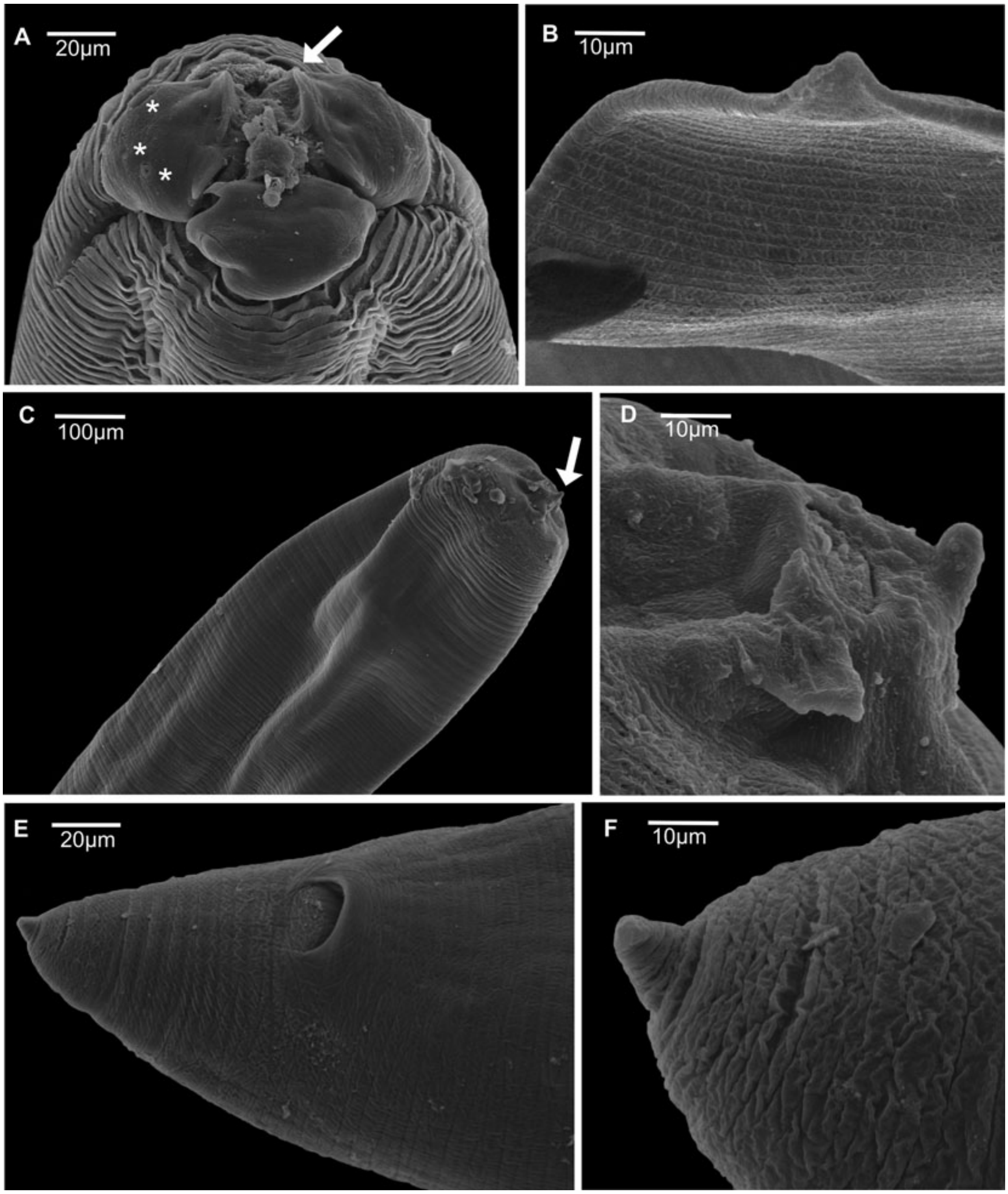

Fig. 4. Scanning electron microscopy of larval stages of *Contracaecum jorgei* n. sp. (A) Fourth-stage larvae, anterior end, lateral view showing cephalic extremity rounded with three well-developed lips (one dorsal and two ventrolateral), auricles (arrow) and cephalic papillae of ventrolateral lip. (B) Fourth-stage larvae, posterior end showing conical tail with pointed tip. (C) Third-stage larvae, anterior end, lateral view showing cephalic extremity rounded with one lip with a small larval tooth (arrow). (D) Third-stage larvae, detail showing larval tooth. (E) Third-stage larvae, posterior end showing cloacal opening. (F) Third-stage larvae, detail showing conical tail with pointed tip.

margolisi Mattiucci, Cianchi, Nascetti, Paggi, Sardella, Timi, Webb, Bastida, Rodríguez & Bullini, 2003 found in *Zalophus californianus* from Canada; *Contracaecum fagerholmi* D'Amelio, Cavallero, Dronen, Barros and Paggi, 2012 and *C. rudolphii* D'Amelio Cavallero, Dronen, Barros and Paggi, 2012 found in *Pelecanus occidentalis* from Texas; *Contracaecum bioccai* Mattiucci, Paoletti, Olivero-Verbel, Baldiris, Arroyo-Salgado and Garbin, 2008 found in *P. occidentalis* from Colombia; and *Contracaecum mirounga* Nikolskiy, 1974 found in *Spheniscus magellanicus* from Argentina have the same number and arrangement of cephalic papillae as *C. jorgei* n. sp.. They also have distal tip of interlabia not bifurcated, except *C. rudolphii* F and *C. bioccai*.

Contracaecum jorgei n. sp. most resembles *C. mirounga* due to body length (14.31–20.98 mm vs. 15.60–18.60 mm, respectively), distance from anterior end to nerve ring (0.29–0.46 mm vs. 0.35–0.43 mm) and size and shape of the tail (0.15–0.21 mm vs. 0.19–0.23 mm). However, *C. jorgei* n. sp. has much smaller spicules (right 2.28–3.63 mm; left 2.03–3.18 mm) than the other species: *C. fagerholmi* (spicules equal in length, 4.15–4.85 mm); *C. rudolphii* F (spicules equal in length 5.96–7.30 mm); *C. bioccai* (right 6.00–6.50 mm; left 5.80–6.20 mm); *C. mirounga* (right 8.12–10.71 mm; left 5.89–10.59 mm); and *C. margolisi* (right 6.10 mm; left 6.30 mm). The number of postcloacal papillae of *C. jorgei* n. sp. is greater than that of *C. bioccai* (six vs. four) and less than *C.*

https://doi.org/10.1017/S0022149X20000644 Published online by Cambridge University Press

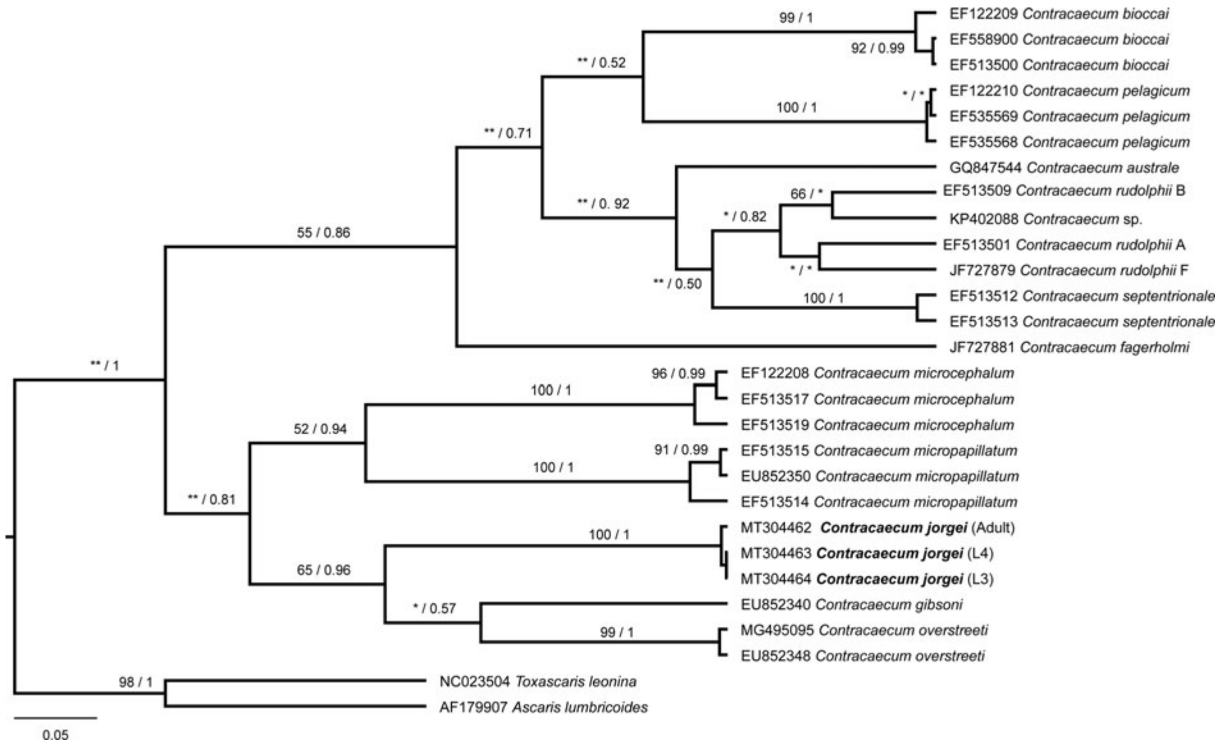

Fig. 5. Phylogenetic relationships within the *Contracaecum* genus based on the partial fragment of cytochrome oxidase II gene. The tree was inferred by using the maximum likelihood (ML) and Bayesian inference (BI) with the HKY + I + G model. The nodal support is described at the left by bootstrap replicates and at the right by posterior probability to each node represented. *Low nodal support in ML or BI; **incongruence between ML and BI. The scale bar represents the number of substitutions per site.

mirounga (six vs. 13 pairs), while *C. fagerholmi* and *C. margolisi* have the same number of postcloacal papillae and a single median papilla on the anterior cloaca rim (Mattiucci *et al.*, 2003, 2008; D'Amelio *et al.*, 2012; Garbin *et al.*, 2019).

The present species formed a well-supported clade with the sister group *C. gibsoni* Mattiucci, Paoletti, Solorzano and Nascetti, 2010 and *C. overstreeti* Mattiucci, Paoletti, Solorzano and Nascetti, 2010, but it is genetically and morphologically distinct. The sequences of *C. gibsoni* (EU852340) and *C. overstreeti* (EU852348) were obtained from adult worms recovered from *Pelecanus crispus* (L.) in Greece and *C. overstreeti* (MG495095) was obtained from larvae infecting *Mugil cephalus* in Turkey (Mattiucci *et al.*, 2010; Pekmezci & Yardimci, 2019). The adults of *C. gibsoni* and *C. overstreeti* are larger than *C. jorgei* n. sp. (30.00–47.00 mm; 44.00–50.00 mm vs. 14.31–20.98), but they resemble *C. jorgei* n. sp. by the disposition and number of cephalic papillae and absence of bifurcated interlabia. These species differ from the new species by presenting smaller spicules (*C. gibsoni*: right 2.15–2.30 mm and left 2.10–2.30 mm; *C. overstreeti*: right 2.78–2.86 mm and left 2.90–3.15 mm vs. right 3.63–2.28 mm and left 3.18–2.03 mm) and the number and arrangement of caudal papillae. *Contracaecum gibsoni* has 65–68 postcloacal papillae, while *C. overstreeti* presents 61–65 postcloacal papillae. In addition, both species differ from the present species by having four pairs of proximal papillae posterior to the cloaca, a single pair of double paracloacal papillae, four pairs of distal papillae and a single pair of very small papilla-like phasmids.

The larvae of *C. overstreeti* differ from larvae of *C. jorgei* n. sp. by larger size (20.10–22.60 mm vs. 11.10–12.23 mm); greater length of the oesophagus (2.23–2.91 mm vs. 1.63–1.89 mm); ventricular appendix (0.78–0.82 mm vs. 0.30–0.37 mm); intestinal caecum (2.18–2.22 mm vs. 0.98–1.30 mm); and longer tail (0.22–0.23 mm vs. 0.11–0.13 mm). In addition, the tail of *C. overstreeti* larvae have sharply pointed ends with spines, distinguishing them from *C. jorgei* n. sp.

There are several records of L3 of *Contracaecum* in freshwater fish from the Atlantic region of South America, some of them including a description of the morphotypes (Moravec *et al.*, 1993; Martins *et al.*, 2005; Luque *et al.*, 2011; Pinheiro *et al.*, 2019 in Brazil; and Mancini *et al.*, 2014 in Argentina). Moravec *et al.* (1993) described the larval type 1 of *Contracaecum* parasitizing *Hoplias malabaricus* in the Paraná River, Brazil, but this morphotype presents clear morphometric differences in relation to the new species (the total length, length of the oesophagus and length of the intestinal caecum are greater in the new species, while ventriculus length is shorter). Martins *et al.* (2005) described larvae of *Contracaecum* parasitizing *H. malabaricus* from northern Brazil with morphometric characteristics similar to the larval type 2 described by Moravec *et al.* (1993). The L3 of the new species can be separated from the type 2 and the larvae described by Martins *et al.* (2005) due to the shorter length of the body, oesophagus and intestinal caecum, while having a larger ventricle. The larvae of *Contracaecum* reported by Mancini *et al.* (2014) parasitizing nine freshwater fish species in Argentina, including *H. malabaricus*, were identified as type 2 (Moravec *et al.*, 1993), but the authors did not present morphometric information to allow comparison with *C. jorgei* n. sp. Unfortunately, there is no molecular information available about the larvae used in the morphological comparison, which could help to determine their specific identification.

The morphology of L4 of *Contracaecum* has not been described in detail until now. Torres *et al.* (2000) pointed to the

https://doi.org/10.1017/S0022149X20000644 Published online by Cambridge University Press

difficulty of distinguishing L4 larvae from immature adults, but the latter could be recognized by the presence of caudal papillae, spicules in males and well-developed vulvae in females. In addition, the main feature to distinguish L4 from the adults is the absence of interlabia, as confirmed by morphological descriptions of L4 in other *Contracaecum* species (Kreis, 1955; McClelland & Ronald, 1973; Semenova, 1974; Fagerholm *et al.*, 1996; Garbin *et al.*, 2007, 2008; Kanarek & Bohdanowicz, 2009), in agreement with the characteristics observed in the L4 of the present study. According to Kanarek & Bohdanowicz (2009), the lack of comprehensive analyses using molecular techniques to confirm the morphological criteria is a challenge to the identification of *Contracaecum* larvae. By integrating morphological and molecular studies of stages L3, L4 and adults isolated from a wide range of fish species (intermediate hosts) and birds and marine mammals (definitive hosts) will enable identifying specific larval stages of some species of *Contracaecum*.

There are only a few taxonomic descriptions of L3 and L4 of *Contracaecum* and studies using molecular techniques for larval identification, despite the high prevalence found in intermediate hosts in Argentina reported in recent years (Mancini *et al.*, 2014). Moreover, in the last 20 years, molecular genetic techniques have been important tools for species identification, mainly for larval stages of *Anisakis*, *Pseudoterranova* and *Contracaecum*, since morphological features are not enough to definitively distinguish species (Mattiucci & Nascetti, 2008; Mattiucci *et al.*, 2008; Garbin *et al.*, 2013).

In the life cycle of *Contracaecum*, eggs hatch and develop into second-stage larvae or L3 in water. These are ingested by aquatic arthropods and infect the body cavity of freshwater and marine fish when ingesting the infected aquatic arthropods (Dziekońska-Rynko & Rokicki, 2007). It is likely that microcrustaceans and fish can act as intermediate hosts (Moravec, 2009); thus, the high availability of zooplankton and fish density recorded in shallow Pampean lakes in Argentina (Mancini & Grosman, 2008) can increase the food supply for birds, enhancing the chance of infecting the definitive host. In the stomach of birds, L3 larvae develop to L4 and later become adults with sexual dimorphism (Køie & Fagerholm, 1995). Humans can accidentally be infected when consuming raw fish containing L3 of *Contracaecum*, as shown by Nagasawa (2012) with the species *Contracaecum osculatum*. The larvae can penetrate the stomach and intestine walls, causing a life-threatening condition that causes stomach pains, fever, diarrhoea and vomiting (Dorny *et al.*, 2009). Moreover, it is known that nematodes, both larvae and adults, can negatively affect host health and cause serious losses to fisheries (Dick *et al.*, 1987). For that reason, an accurate species identification of *Contracaecum* infective larvae found in fish used for human consumption is very important (Kanarek & Bohdanowicz, 2009).

The use of molecular techniques is fundamental not only to define the taxonomic status of these species, but also for their recognition. Due to its higher rate of base change and the genetic variability observed in the mt *cox*-2 gene, this gene is larger than ribosomal genes (Ceballos-Mendiola *et al.*, 2010; Mattiucci *et al.*, 2014) for species. The direct relationship between adults and larval stages of *C. jorgei* n. sp. and the interspecific variation found in this study show that mt *cox*-2 can be a valuable tool for species identification within the genus (Jex *et al.*, 2008; Borges *et al.*, 2014). Therefore, due to the biodiversity of Anisakidae nematodes, the analysis of molecular genetic markers has been shown to be an efficient tool for specific diagnosis and unequivocal identification of anisakid nematodes with zoonotic potential (Kanarek & Bohdanowicz, 2009). This is an essential requirement for epidemiological surveys. Thus, the phylogenetic analyses indicate that *C. jorgei* n. sp. belongs to a lineage independent of the other species of *Contracaecum*, confirming it as a separate species.

Acknowledgments. Our thanks to Douglas McIntosh, Laboratory of Molecular Biology, Department of Animal Parasitology, UFRRJ, for technical support in sequencing, and to Jonathan Gonçalves de Oliveira for his help in discussing the molecular analysis data. We also thank the Rudolf Barth Electron Microscopy Platform (PDTIS/FIOCRUZ) for the support in scanning photographs, and Santiago Gimenez, Martín Garro and Fernando Amaya for their collaboration during collections.

Financial support. This study was supported partially by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brasil (CAPES–Finance Code 001, grant number 88881.143550/2017-01) to CJS ('Programa Centros Associados da Pós-Graduação Brasil-Argentina'). JLL was partially supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico-Brasil (CNPq grant number 306952/2015-7).

Conflicts of interest. None.

Ethical standards. The authors declare that all applicable international, national and/or institutional guidelines for the care and use of animals were followed.

References

Abollo E, Gestal C and Pascual S (2001) Anisakid infection in the European shag *Phalacrocorax aristotelis*. *Journal of Helminthology* **75**, 209–214.

Amato JFR, Monteiro CM and Amato SB (2006) *Contracaecum rudolphii* Hartwich (Nematoda, Anisakidae) from the neotropical cormorant, *Phalacrocorax brasilianus* (Gmelin) (Aves, Phalacrocoracidae) in southern Brazil. *Revista Brasileira de Zoologia* **23**, 1284–1289.

Anderson RM (2000) *Nematode parasites of vertebrates: their development and transmission*. 2nd edn. 650 pp. Wallingford, Oxon, UK, CABI Publishing.

Baruš V (1966) Nemátodos parásitos de aves em Cuba. Parte I. *Poeyana Serie A* **22**, 1–37.

Billings AN, Yu X, Teel PD and Walker DH (1998) Detection of a spotted fever group rickettsia in *Amblyomma cajennense* (Acari: Ixodidae) in South Texas. *Journal of Medical Entomology* **35**, 474–478.

Biolé FG, Guagliardo SE, Mancini MA, Tanzola RD, Salinas V and Morra G (2012) Primer registro de *Contracaecum australe* (Nematoda: Anisakidae) em *Phalacrocorax brasilianus* (Aves: Phalacrocoracidae) de la región central de Argentina. *BioScriba* **5**, 1–11.

Borges JN, Santos HLC, Brandão ML, Santos EG, Miranda DF, Balthazar D, Luque JL and Santos CP (2014) Molecular and morphological characterization of *Contracaecum pelagicum* (Nematoda) parasitizing *Spheniscus magellanicus* (Chordata) from Brazilian waters. *Brazilian Journal of Veterinary Parasitology* **23**, 74–79.

Ceballos-Mendiola G, Valero A, Polo-Vico R, Tejada M, Abattouy N, Karl H, De Las Heras C and Martín-Sánchez J (2010) Genetic variability of *Anisakis simplex* parasitizing European hake (*Merluccius merluccius*) in the Little Sole Bank area in the Northeast Atlantic. *Parasitology Research* **107**, 1399–1404.

Conde-Tinco MA and Iannacone J (2013) Bioecology of *Phalacrocorax brasilianus* (Gmelin, 1789) (Pelecaniformes: Phalacrocoracidae) in South America. *The Biologist* **11**, 151–166.

D'Amelio S, Cavallero S, Dronen NO, Barros NB and Paggi L (2012) Two new species of *Contracaecum* Railliet & Henry, 1912 (Nematoda: Anisakidae), *C. fagerholmi* n. sp. and *C. rudolphii* F from the brown pelican *Pelecanus occidentalis* in the northern Gulf of Mexico. *Systematic Parasitology* **81**, 1–16.

Dick TA, Papst MH and Paul HC (1987) Rainbow trout (*Salmo gairdneri*) stocking and *Contracaecum* spp. *Journal of Wildlife Diseases* **23**, 242–247.

Dorny P, Praet N, Deckers N and Gabriel S (2009) Emerging food-borne parasites. *Veterinary Parasitology* **163**, 196–206.

Drummond AJ and Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC evolutionary biology* **7**, 214.

Drummond AJ, Ho SYW, Phillips MJ and Rambaut A (2006) Relaxed phylogenetics and dating with confidence. *PLoS Biology* **4**, 699–710.

Dziekońska-Rynko J and Rokicki J (2007) Life cycle of the nematode *Contracaecum rudolphii* Hartwig, 1964 (sensu lato) from northern Poland under laboratory conditions. *Helminthologia* **44**, 95–102.

Fagerholm HP, Overstreet RM and Humprey-Smith I (1996) *Contracaecum magnipapillatum* (Nematoda; Ascaridoidea): resurrection and pathogenic effect of a common parasite from the proventriculus from *Anous minutus* from the Great Barrier Reef, with a note of *C. variegatum*. *Helminthologia* **33**, 195–207.

Fedynich AM, Pence DB and Bergan JF (1997) Helminth community structure and pattern in sympatric populations of Double-crested and Neotropic cormorants. *Journal of the Helminthological Society of Washington* **64**, 176–182.

Garbin LE, Navone GT, Diaz JI and Cremonte F (2007) Further study of *Contracaecum pelagicum* (Nematoda: Anisakidae) in *Spheniscus magellanicus* (Aves: Spheniscidae) from Argentinean coasts. *Journal of Parasitology* **93**, 143–150.

Garbin LE, Diaz JI, Cremonte F and Navone GT (2008) A new anisakid species parasitizing the Imperial Cormorant *Phalacrocorax atriceps* from the north Patagonian coast, Argentina. *Journal of Parasitology* **94**, 852–859.

Garbin L, Mattiucci S, Paoletti M, González-Acuña D and Nascetti G (2011) Genetic and morphological evidences for the existence of a new species of *Contracaecum* (Nematoda: Anisakidae) parasite of *Phalacrocorax brasilianus* (Gmelin) from Chile and its genetic relationships with congeners from fish-eating birds. *Journal of Parasitology* **97**, 476–492.

Garbin LE, Mattiucci S, Paoletti M, Diaz JL, Nascetti G and Navone GT (2013) Molecular identification and larval morphological description of *Contracaecum pelagicum* (Nematoda: Anisakidae) from the anchovy *Engraulis anchoita* (Engraulidae) and fish-eating birds from the Argentine North Patagonian Sea. *Parasitology International* **62**, 309–319.

Garbin LE, Diaz JI and Navone GT (2019) Species of *Contracaecum* parasitizing the Magellanic penguin *Spheniscus Magellanicus* (Spheniscidae) from the Argentinean coast. *Journal of Parasitology* **105**, 222–231.

Gernhard T (2008) The conditioned reconstructed process. *Journal of Theoretical Biology* **253**, 769–778.

Guindon S and Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* **52**, 696–704.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium* **41**, 95–98.

Huelsenbeck J and Ronquist F (2005) Bayesian analysis of molecular evolution using MrBayes. pp. 183–226 *in* R Nielsen (*Ed.*) *Statistical methods in molecular evolution*. London, UK, Springer.

Jex AR, Waeschenbach A, Littlewood DTJ, Hu M and Gasser RB (2008) The mitochondrial Genome of *Toxocara canis*. *PLOS Neglected Tropical Diseases* **2**, e273.

Kalmbach E and Becker PH (2005) Growth and survival of neotropic cormorant (*Phalacrocorax brasilianus*) chicks in relation to hatching order and brood size. *Journal of Ornithology* **146**, 91–98.

Kanarek G and Bohdanowicz J (2009) Larval *Contracaecum* sp. (Nematoda: Anisakidae) in the Great Cormorant [*Phalacrocorax carbo* (L., 1758)] from north-eastern Poland: a morphological and morphometric analysis. *Veterinary Parasitology* **166**, 90–97.

Kennedy M and Spencer HG (2014) Classification of the cormorants of the world. *Molecular Phylogenetics and Evolution* **79**, 249–257.

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16**, 111–120.

Køie M and Fagerholm HP (1995) The life cycle of *Contracaecum osculatum* (Rudolphi, 1802) sensu stricto (Nematoda, Ascaridoidea, Anisakidae) in view of experimental infections. *Parasitology Research* **81**, 481–489.

Kreis HA (1955) *Contracaecum septentrionale*, ein neuer Parasit aus dem Kormoran; sein Lebenslauf, sowie Angaben über die Entwicklung der Anisakinae. *Zeitschrift fur Parasitenkunde* **17**, 106–121.

Lent H and Freitas JFT (1948) Uma coleção de nematódeos parasitos de vertebrados do Museu de Historia Natural de Montevideo. *Memórias do Instituto Oswaldo Cruz* **46**, 1–71.

Luque JL, Aguiar J, Vieira F, Gibson D and Santos C (2011) Checklist of Nematoda associated with the fishes of Brazil. *Zootaxa* **3082**, 1–88.

Mancini MA and Grosman F (2008) *El pejerrey de las lagunas pampeanas. Análisis de casos tendientes a una gestión integral de las pesquerías*. p. 449 Rio Cuarto, Ed Universidad Nacional de Río Cuarto – Universidad Nacional del Centro de la Provincia de Buenos Aires.

Mancini MA, Biolé FG, Salinas VH, Guagliardo SE, Tanzola RD and Morra G (2014) Prevalence, intensity and ecological aspects of *Contracaecum* sp. (Nematode: Anisakidae) in freshwater fish of Argentina. *Neotropical Helminthology* **8**, 111–122.

Martins ML, Onaka EM and Fenerick J Jr (2005) Larval *Contracaecum* sp. (Nematoda: Anisakidae) in *Hoplias malabaricus* and *Hoplerythrinus unitaeniatus* (Osteichthyes: Erythrinidae) of economic importance in occidental marshlands of Maranhão, Brazil. *Veterinary Parasitology* **127**, 51–59.

Mattiucci S and Nascetti G (2008) Advances and trends in the molecular systematics of Anisakid nematodes, with implications for their evolutionary ecology and host–parasite co-evolutionary processes. *Advances in Parasitology* **66**, 47–148.

Mattiucci S, Paoletti M, Olivero-Verbel J, Baldiris R, Arroyo-Salgado B, Garbin L, Navone G and Nascetti G (2008) *Contracaecum bioccai* n. sp. from the brown pelican *Pelecanus occidentalis* (L.) in Colombia (Nematoda: Anisakidae): morphology, molecular evidence and its genetic relationship with congeners from fish-eating birds. *Systematic Parasitology* **69**, 101–121.

Mattiucci S, Paoletti M, Solorzano AC and Nascetti G (2010) *Contracaecum gibsoni* n. sp. and *C. overstreeti* n. sp. (Nematoda: Anisakidae) from the Dalmatian pelican *Pelecanus crispus* (L.) in Greek waters: genetic and morphological evidence. *Systematic Parasitology* **75**, 207–224.

Mattiucci S, Cipriani P, Webb SC, Paoletti M, Marcer F, Bellisario B, Gibson DI and Nascetti G (2014) Genetic and morphological approaches distinguish the three sibling species of the *Anisakis simplex* species complex, with a species designation as *Anisakis berlandi* n. sp. for *A. simplex* sp. C (Nematoda: Anisakidae). *Journal of Parasitology* **100**, 199–214.

Mattiucci S, Cianchi R, Nascetti G, *et al.* (2003) Genetic evidence for two sibling species within *Contracaecum ogmorhini* Johnston & Mawson, 1941 (Nematoda: Anisakidae) from otariid seals of boreal and austral regions. *Systematic Parasitology* **54**, 13–23.

McClelland G and Ronald K (1973) In vitro development of the nematode *Contracaecum osculatum* Rudolphi, 1802 (Nematoda: Anisakinae). *Canadian Journal of Zoology* **52**, 847–855.

Moravec F (1998) *Nematodes of freshwater fishes of the neotropical region*. p. 464. Prague, Czech Republic, Academia Praha.

Moravec F (2009) Experimental studies on the development of *Contracaecum rudolphii* (Nematoda: Anisakidae) in copepod and fish paratenic hosts. *Folia Parasitologica* **56**, 185–193.

Moravec F, Kohn A and Fernandes BMM (1993) Nematode parasites of fishes of the Paraná River, Brazil. Part 2. Seuratoidea, Ascaridoidea Habronematoidea and Acuarioidea. *Folia Parasitologica* **40**, 115–134.

Nadler SA and Hudspeth DS (2000) Phylogeny of the Ascaridoidea (Nematoda: Ascaridida) based on tree genes and morphology: hypotheses of structural and sequence evolution. *Journal of Parasitology* **86**, 380–393.

Nagasawa K (2012) The biology of *Contracaecum osculatum* sensu lato and *C. osculatum* A (Nematoda: Anisakidae) in Japanese waters: a review. *Biosphere Science* **51**, 61–69.

Pekmezci GZ and Yardimci B (2019) On the occurrence and molecular identification of *Contracaecum* larvae (Nematoda: Anisakidae) in *Mugil cephalus* from Turkish waters. *Parasitology Research* **118**, 1393–1402.

Pinheiro RHS, Furtado AO, Santos JN and Giese EG (2019) *Contracaecum* larvae: morphological and morphometric retrospective analysis, biogeography and zoonotic risk in the Amazon. *Brazilian Journal of Veterinary Parasitology* **28**, 12–32.

Semenova MK (1974) The development of *Contracaecum microcephalum* (Anisakidae) in the definitive host. *Trudy Gel'mintologicheskoi Laboratorii* **24**, 153–60.

Tamura K, Peterson D, Peterson N, Stecher G and Nei M (2011) MEGA 5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* **28**, 2731–2739.

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

Torres PR, Valdivieso J, Schlatter R, Montefusco A, Revenga J, Marín F, Lamilla J and Ramallo G (2000) Infection by *Contracaecum rudolphii* (Nematoda: Anisakidae) in the Neotropic cormorant *Phalacrocorax brasilianus*, and fishes from the estuary of the Valdivia river. *Chile. Studies on Neotropic Fauna and Environment* **35**, 101–108.

Vidal-Martínez VM, Osorio-Sarabia D and Overstreet RM (1994) Experimental infection of *Contracaecum multipapillatum* (Nematoda: Anisakinae) from Mexico in the domestic cat. *Journal of Parasitology* **80**, 576–579.

Violante-González J, Monks S, Gil-Guerrero S, Rojas-Herrera A, Flores-Garza R and Larumbe-Morán E (2011) Parasite communities of the neotropical cormorant *Phalacrocorax brasilianus* (Gmelin) (Aves, Phalacrocoracidae) from two coastal lagoons in Guerrero state, Mexico. *Parasitology Research* **109**, 1303–1309.

Zhu XQ, Gasser RB, Podolska M and Chilton NB (1998) Characterization of anisakid nematodes with zoonotic potential by nuclear ribosomal DNA sequences. *International Journal for Parasitology* **28**, 1911–1921.

https://doi.org/10.1017/S0022149X20000644 Published online by Cambridge University Press