

1 RESEARCH NOTE

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3 *Ogmogaster antarctica* Johnston, 1931 (Digenea: Notocotylidae) infecting a dwarf minke whale
4 *Balaenoptera acutorostrata* (Lacépède, 1804) (Cetartiodactyla: Balaenopteridae) from Southwestern
5 Atlantic Ocean: supplementary morphological and molecular data

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27 Running title: Supplementary morphological and molecular data on *Ogmogaster antarctica* from South
28 Atlantic

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31 **Abstract**

32 Digeneans of the genus *Ogmogaster* Jägerskiöld, 1891 are intestinal parasites of whales and
33 pinnipeds. Due to the difficulty in recovering these parasites from opportunistic stranding events of their
34 hosts, very little morphological and molecular data are available on the species of this genus. During a
35 beach monitoring survey on the Southern Brazilian coast, a dwarf minke whale *Balaenoptera acutorostrata*
36 (Lacépède, 1804) was necropsied and thousands of digeneans were found in its intestine. Morphological
37 and molecular analyses based on the ribosomal DNA SSU and the mitochondrial DNA COI genes were
38 conducted. The morphological data and the phylogenetic reconstructions allowed the identification of
39 *Ogmogaster antarctica* Johnston, 1931. This is the first report of *O. antarctica* infecting a *B. acutorostrata*
40 on the South Atlantic Ocean. The supplementary morphological data, the molecular characterization and
41 the phylogenetic positioning of *O. antarctica* presented in this study contribute to the knowledge of the
42 helminth biodiversity of large whales.

43

44 **Keywords:** Notocotylidae, dwarf minke whale, molecular characterization, rDNA SSU gene, mtDNA COI
45 gene, Southwestern Atlantic ocean

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47 To better understand host-parasite relationships, detailed reports on helminth diversity are broadly
48 needed. Currently, the availability of parasites from large whales relies almost exclusively on their
49 strandings and subsequent necropsy to collect helminths [5]. Recovering these organisms from such
50 opportunistic stranding events of their large hosts is a challenging task and, as a result, they lack on detailed
51 morphological descriptions along with molecular information.

52 The minke whale *Balaenoptera acutorostrata* Lacépède, 1804 is a cosmopolitan worldwide
53 distributed species [28]. Their taxonomic status is yet to be resolved, but three different lineages have been
54 suggested based on morphological and genetic differences [26, 27, 32, 37]. The species lineage occurring
55 in the Southern hemisphere and in Antarctic waters is commonly referred to as dwarf minke whales [4, 15,
56 32].

57 The digenean *Ogmogaster antarctica* Johnston, 1931 is found in the intestine of baleen whales
58 and lobodont pinnipeds [30]. The species exhibits a worldwide distribution, been reported in several host
59 species from different geographical areas [7, 8, 10, 12, 14, 16, 20, 21, 23, 24, 29, 30, 34, 36, 38, 39]. In
60 South Atlantic waters, few records on *O. antarctica* are known. The digenean was first reported from
61 carcasses of a fin whale *Balaenoptera physalus* (Linnaeus, 1758) and a sei whale *Balaenoptera*

62 *borealis* Lesson, 1828 found washed ashore on the Brazilian coast in the 1960s [23]. A while later, this
63 trematode was once again found infecting a *B. borealis* off the Argentinian coast [17].

64 The helminth fauna of *B. acutorostrata* from South Atlantic ocean still remains poorly documented
65 [28, 41] and parasitological records are restricted to nematodes of the genus *Anisakis* Dujardin, 1845 and
66 *Pseudoterranova* Mozgovoi, 1951 [9, 33], and cestodes of the genus *Pylobothrium* Van Beneden, 1850 [9].

67 This study is the first reference of *O. antarctica* infecting a specimen of *B. acutorostrata* from
68 South Atlantic waters. The new record extends the knowledge on the helminth fauna of this unexplored
69 whale. Additionally, we provide supplementary information on the morphology, including a new
70 descriptive drawing, and the molecular characterization of the species based on the ribosomal DNA SSU
71 and mitochondrial DNA COI genes.

72 In October 2016 a 4.5 mt long adult male of *B. acutorostrata* was found washed ashore at the São
73 Francisco do Sul district, Santa Catarina State, South Brazil, Southwestern Atlantic (26°09'43.0"S,
74 48°34'01.6"W). During necropsy, the whale's small intestine was examined for helminths. The digeneans
75 were collected, cleaned in tap water to remove debris and then transferred directly to 70% ethanol.

76 For morphological analyses, unflattened specimens were stained with chloridric carmine,
77 dehydrated in a graded ethanol series, cleared with creosote, and mounted as temporary preparations. The
78 parasites were identified based on specific references [3, 14, 20, 30]. Morphometric analyses were made
79 using the Qwin Lite 3.1 (Leica) computerized system for image analysis. Drawing was made with a drawing
80 tube attached to a microscope and edited on CorelDRAW v. 18. Voucher specimens were deposited at the
81 Helminthological Collection of the Institute of Biosciences (CHIBB), UNESP, Botucatu, São Paulo state,
82 Brazil, under number xxx (will be deposited after manuscript acceptance).

83 The genomic DNA of three adult worms was extracted using the DNeasy® Blood and Tissue Kit
84 (Qiagen) according to the manufacturer's protocol, in a final volume of 30 µl. Partial fragments of the
85 ribosomal DNA SSU and the mitochondrial DNA COI genes were amplified. PCR amplifications were
86 performed using 3 µl of genomic DNA, 1.0 µl of each set of primers and Ready-to-Go PCR beads
87 (Pure Taq™Ready-to-Go™PCR beads, GE Healthcare) in a final volume of 25 µl. The primers used to
88 obtain partial fragments of nuclear and mitochondrial markers were: SSU, WormA (5'-
89 GCGAATGGCTCATTAATCAG-3') and WormB (5'-CTTGTTACGACTTTTACTTCC-3') [18] and
90 internal primers 300F (5'-AGGGTTCGATTCCGGGAG-3'), 930F (5'-
91 GCATGGAATAATGGAATAGG-3'), 1200F (5'-CAGGTCTGTGATGCCC-3') and 1270R (5'-

92 CCGTCAATTCCTTTAAGTTT-3') [18]; COI, JB3 (5'-TTTTTTGGGCATCCTGAGGTTTAT- 3') [22]
93 and JB4.5 (5'-TAAAGAAAGAACATAATGAAAATG-3') [31]. The thermocycling profile for the rDNA
94 SSU amplification included initial denaturation at 94 °C for 3 min, followed by 40 cycles of amplification
95 at 94 °C for 30 s, 56 °C for 30 s and 72 °C for 2 min, and a final extension step at 72 °C for 7 min [18]. For
96 the mtDNA COI amplification, the initial denaturation was at 94 °C for 5 min, followed by 40 cycles of
97 amplification at 92 °C for 30 s, 46 °C for 45 s and 72 °C for 90 s, and a final extension step at 72 °C for 10
98 min [28]. PCR amplicons (3 µl) were separated electrophoretically in 1% agarose gel with gel red (1 µl)
99 (Biotium Inc) and DNA bands were visualized under UV light. PCR products were purified using QIAquick
100 PCR Purification Kit (Qiagen) following the manufacturer's protocol and sequenced on an Applied
101 Biosystems ABI 3500 automated DNA sequencer using Big Dye v.3.1 Terminator (Applied Biosystems,
102 Foster City, CA, USA).

103 Forward and reverse sequences were assembled and edited using Sequencher v. 5.2.4. The new
104 rDNA SSU and mtDNA COI consensus sequences were aligned separately with selected sequences
105 belonging to members of close related families recovered from GenBank database (rDNA SSU: *Catatropis*
106 *indicus* Srivastava, 1935 (AY222114), *Pronocephaloidea* sp. Looss, 1899 (EU371601), *Notocotylus*
107 *pacifera* (Noble, 1933) Harwood, 1939 (AY245765), *Notocotylus* sp. Diesing, 1839 (AJ287547),
108 *Ogmogaster antarctica* (KM258669), *O. antarctica* (KY945915) [Notocotylidae], *Rhabdiopoeus taylori*
109 Johnston, 1913 (AY222113), *Taprobanella bicaudata* Cruz & Fernand, 1954 (AY222112)
110 [Rabdiopoeidae], *Labicola* cf. *elongata* Blair, 1979 (AY222115) [Labicolidae], *Lankatrema mannarensis*
111 Cruz & Fernand, 1954 (AY222116), *Opisthotrema dujonis* Fischer, 1883 (AY222117)
112 [Ophistotrematidae], *Macrovestibulum obtusicaudum* Mackin, 1930 (AY222111) [Pronocephalidae],
113 *Chiorchis fabaceus* (Diesing, 1838) Fiscoeder, 1901 (MF370224) [Cladorchiidae], *Diplodiscus japonicus*
114 Yamaguti, 1936 (KX506855), *Diplodiscus mehrai* Pande, 1937 (KX506857), *Diplodiscus subclavatus*
115 (Pallas, 1760) (AJ287502) [Diplodiscidae], *Heronimus mollis* (Leidy, 1856) (AY222118) [Heronimidae]
116 [1, 6, 11, 25]; COI: *Ogmocotyle sikae* (Yamaguti, 1933) (KR006934; NC027112), *Ogmocotyle* sp. Skrjabin
117 & Shulz, 1933 (KR006935), *Tristriata anatis* Belopolskaia, 1953 (KX833003; KX833006), *Hippocrepis*
118 *hippocrepis* (Diesing, 1850) (MN268535; MN268536), *O. antarctica* (KY945916) [Notocotylidae],
119 *Paramphistomum cervi* Schrank, 1790 (KF475773) [Paramphistomidae] [2, 13, 19, 40] using MUSCLE
120 algorithm implemented on Geneious v. 7.1 program, with default settings.

121 Phylogenetic relationships were inferred by Maximum Likelihood (ML) and Bayesian Inference
122 (BI), applying the model GTR + I + G selected as the best fitting model of nucleotide evolution by
123 jModelTest v.2.1 for both datasets. ML analyses were carried out using RAxML v.8 software on CIPRES
124 web portal, with bootstrap support calculated using 1000 replications. BI were made using MrBayes v.3.2
125 software implemented on CIPRES web portal, with the analyses performed by running two independent
126 MCMC runs of four chains for 1×10^7 generations and sampling tree topologies every 10^2 generations, the
127 first 25% of trees discarded as burn-in and the remaining trees used for calculating the Bayesian posterior
128 probabilities. The obtained ML and BI trees were visualized using FigTree v.1.3.1 and edited using
129 CorelDRAW v.18. Genetic distances were calculated with MEGA v.7 using Kimura-2-parameter model
130 with all ambiguous positions removed for each sequence pair.

131 Hundreds of adult digeneans were found in the lumen of the whale's intestine. Based on
132 morphological and molecular features, we have assigned the specimens as *Ogmogaster antarctica*. The
133 following observations and measurements were made based on 15 whole-mounted specimens.
134 Measurements are shown as the range followed by the mean in parentheses and are expressed in millimeters
135 unless otherwise stated.

136 **Morphological description**

137 Phylum Platyhelminthes Gegenbaur, 1859

138 Class Trematoda Rudolphi, 1808

139 Subclass Digenea Carus, 1863

140 Order Plagiorchiida La Rue, 1957.

141 Family Notocotylidae Lühe, 1909

142 Genus *Ogmogaster* Jägerskiöld, 1891

143 *Ogmogaster antarctica* Johnston, 1931 (Fig. 1)

144 Body oval, flattened, with the margins turned ventrally, slightly narrowed at the anterior extremity.
145 Lateral margin of the body with crenulations (number from 38 to 40). Spiny tegument, with parallel
146 longitudinal ridges on the ventral surface (number from 13 to 15). Body length 3.91 - 6.30 (5.33) and width
147 1.90 - 3.40 (2.73). Oral sucker terminal, muscular, 0.40 - 0.61 (0.51) long and 0.40 - 0.63 (0.54) wide.
148 Esophagus indistinguishable. Intestinal caeca extending posteriorly to the level of the ovary, curved along
149 its entire length, with small lateral branches. Testes symmetrically positioned, deeply lobed to dendritic,
150 extracaecal, situated in the posterior half of the body. Right testis 0.39 - 0.89 (0.69) long and 0.35 - 0.75

151 (0.59) wide. Left testis 0.44 - 0.93 (0.70) long and 0.35 - 0.77 (0.59) wide. Vas efferent joining anteriorly
152 to Mehlis' gland and forming a single medial vas deferens dorsal to the uterus, which forms an external
153 seminal vesicle before reaching the level of the posterior end of the cirrus sac. Cirrus sac wide, in the
154 midline of the body, 1.41 - 2.29 (1.85) long and 0.11 - 0.41 (0.21) wide, containing internal seminal vesicle,
155 prostate glands, and cirrus covered with tiny spines. Genital pore opening immediately posterior to oral
156 sucker. Ovary lobed, medially located between testes, 0.32 - 0.71 (0.56) long and 0.17 - 0.42 (0.32) wide.
157 Mehlis' gland preovarian, almost lobed. Vitellaria in groups of 15 to 18 follicles limited to each side of the
158 body, located between testes and the posterior end of the cirrus sac. Uterine field extending between anterior
159 margins of testes and region posterior to the genital pore, with numerous coils extracaecally. Metraterm
160 short, wide and muscular, with small spines. Eggs 15.20 - 22.70 μm (19.24) long, 8.30 - 13.20 μm (10.84)
161 wide, with 2 polar filaments 62.10 - 100.20 μm (73.48) long.

162 One partial rDNA SSU sequences (1.488 bp) and 3 partial mtDNA COI sequences (447 bp; 445
163 bp; 371bp) of *O. antarctica* were obtained. The aligned datasets of the newly generated sequences with
164 those previous selected on Genbank included 18 sequences comprising 1.279 nucleotides positions for
165 rDNA SSU and 11 sequences comprising 352 nucleotides positions for mtDNA COI.

166 For the rDNA SSU dataset, both BI and ML analyses resulted in consensus trees with identical
167 topologies (Fig. 2). Despite poorly supported in both analyses, the newly generated sequence formed a
168 clade with the other sequences of *O. antarctica* (KM258669 and KY945915), thus confirming their
169 conspecificity. The intraspecific genetic divergence in this clade was 0.1%. The *O. antarctica* clade formed
170 by the three sequences clustered as sister to the group formed by all other members of family Notocotylidae
171 used in the analyses.

172 The topologies resulting from ML and BI analyses of the mtDNA COI dataset were also identical
173 (Fig. 3). The three new generated sequences clustered together and along with another *O. antarctica*
174 sequence available in GenBank (KY945916) in a well-supported monophyletic group, which was also
175 placed within the Notocotylidae. The genetic divergence between *O. antarctica* sequences ranged from
176 0.0% (*O. antarctica* MN562653 x *O. antarctica* MN562654; both collected from *B. acutorostrata* from
177 Southwestern Atlantic) to 4.6% (*O. antarctica* KY945916 from *B. borealis* from Chilean Patagonia x
178 *O. antarctica* MN562654 from *B. acutorostrata* from Southwestern Atlantic).

179 The present study is the first reference of *O. antarctica* infecting the dwarf minke whale *B.*
180 *acutorostrata* in the South Atlantic Ocean. Whilst there is a bulk of scientific literature regarding the

181 helminth fauna of large whales, only a few records are related to *B. acutorostrata*, especially in South
182 Atlantic waters. To date, only one punctual parasitological record is known at this locality for this host
183 species [33]. Therefore, the new report on the occurrence of this digenean species extends our knowledge
184 on the distribution and biodiversity of helminths infecting whales.

185 Most references on *O. antarctica* are aged records and generally from specimens recovered in the
186 Northern hemisphere. The species have been revised by some authors [30, 35] but quality drawings and
187 detailed diagnostic descriptions were still lacking in a review. Reliable helminth identifications depend on
188 high quality and detailed morphological descriptions, thus the supplementary morphological data presented
189 here contributes greatly to the diagnosis of the species.

190 The phylogenetic reconstructions based on the rDNA SSU and mtDNA COI datasets consistently
191 showed that the new sequences studied herein are certainly ascribed to its correct assigned family
192 (Notocotylidae) and genus (*Ogmogaster*), thus corroborating with previous phylogenetic studies on
193 trematodes [25]. The low rDNA SSU genetic divergence (0.1%) found among the three sequences of *O.*
194 *antarctica* reinforces their wide geographical distribution and plasticity for its definitive host, as they were
195 recovered from different hosts from contrasting geographical locations (e. g., *B. borealis* from
196 Mediterranean x *B. borealis* from Chilean Patagonia x *B. acutorostrata* from South Atlantic).

197 Despite a sequence of the mtDNA COI gene of *O. antarctica* collected from a *B. borealis* in
198 Chilean Patagonia have already been submitted to Genbank, those authors have not published a
199 phylogenetic analysis nor any positioning in a phylogenetic tree. Therefore, here we provide new and first
200 mtDNA COI sequence of *O. antarctica* along with phylogenetic analyses. In addition, our new mtDNA
201 COI sequences and the analyses of intraspecific distances (0.0% to 0 4.6%) provide extra comparative data,
202 which can be applied in further investigations on the diversity, phylogenetic, taxonomic and evolutionary
203 relationships among species of *Ogmogaster* and, consequently, of their hosts.

204

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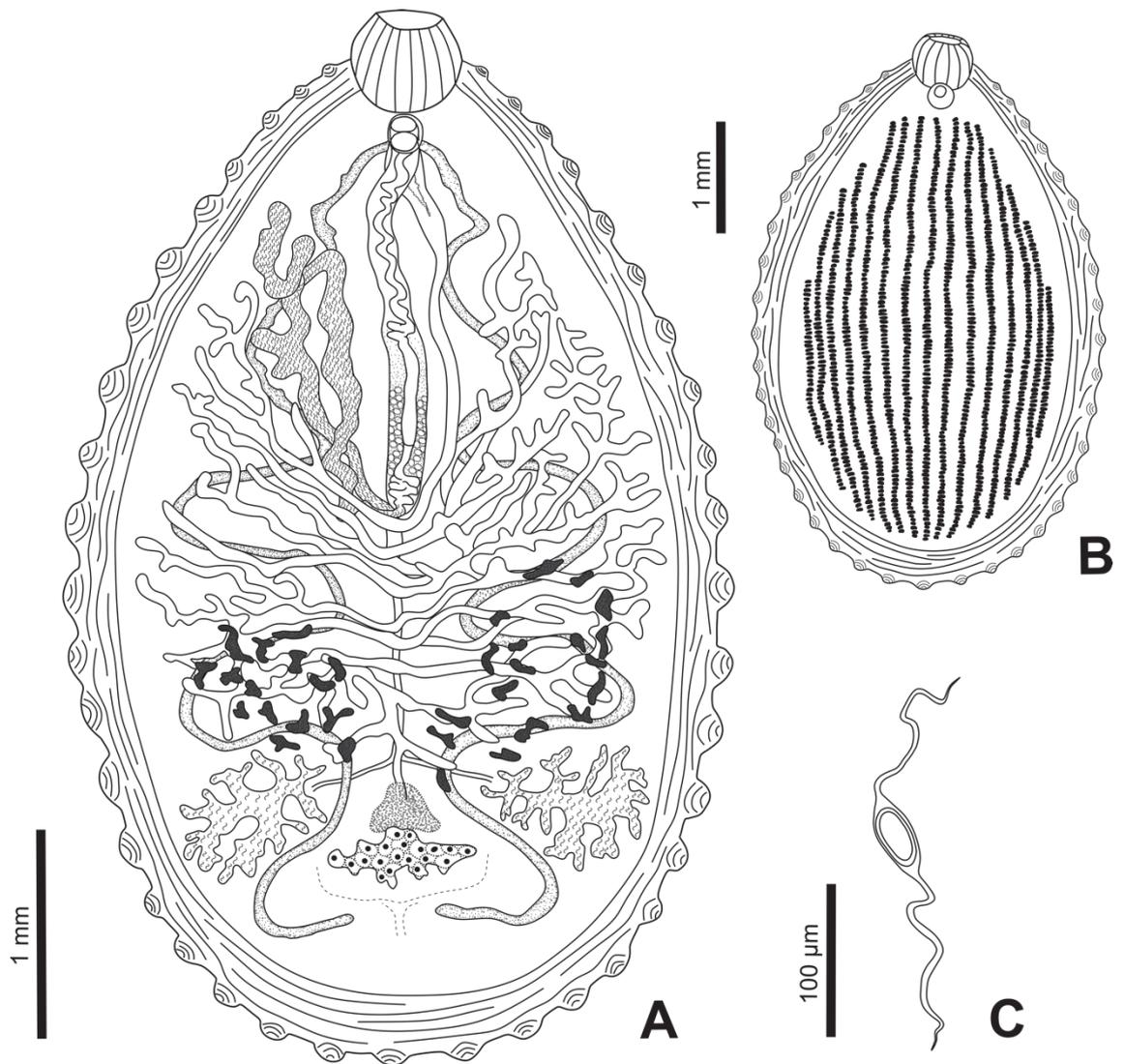
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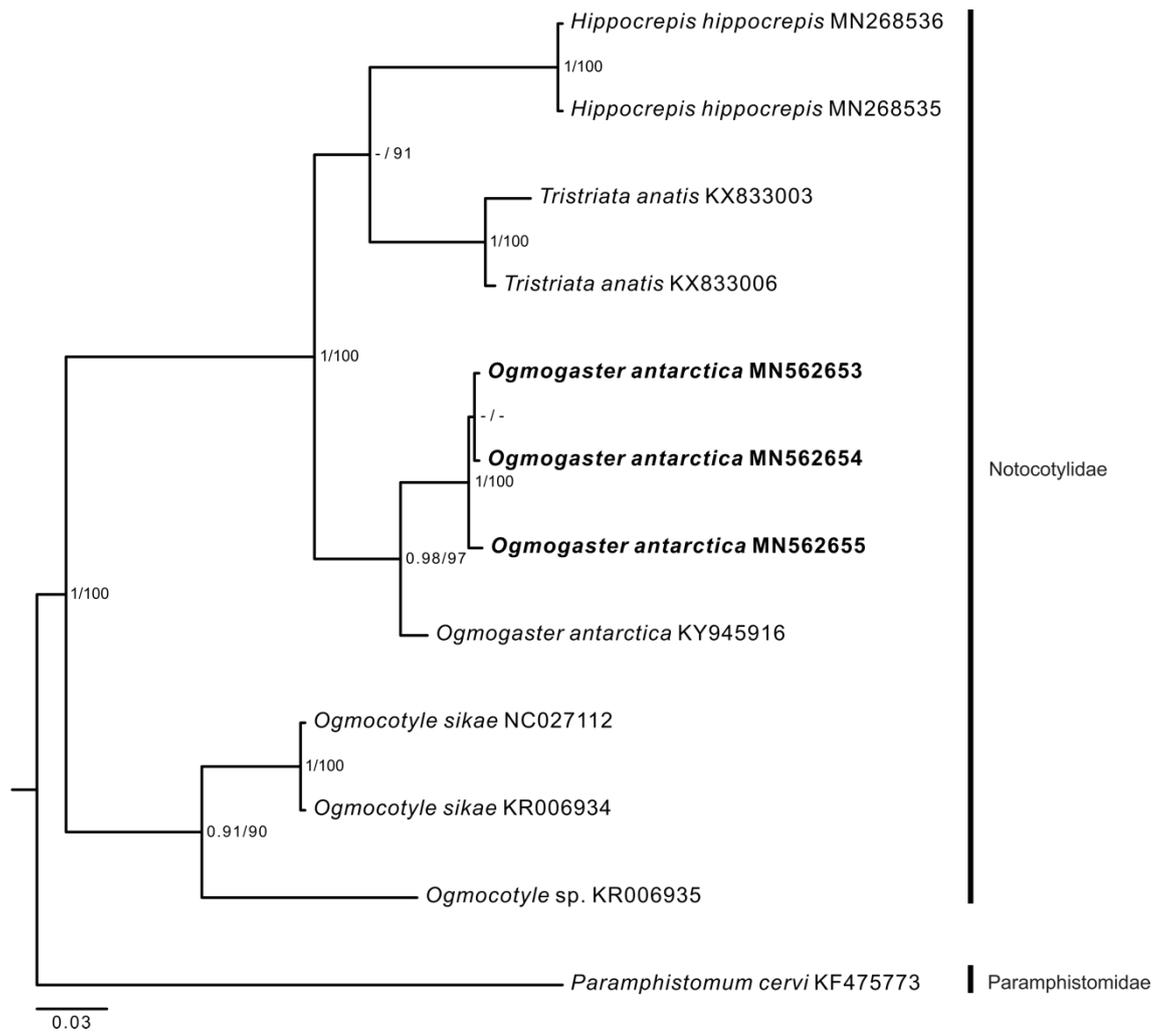
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Fig. 1 *Ogmogaster antarctica* from the intestine of a dwarf minke whale *B. acutorostrata* from Southwestern Atlantic Ocean. A: Whole worm, ventral view. B: Whole worm, disposition of ventral ridges. C: Egg, with polar filaments.



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Fig. 3 Bayesian phylogenetic tree based on partial mtDNA COI sequences of Notocotylidae. The support values at the branching points are shown as the Bayesian posterior probabilities followed by Maximum-likelihood bootstraps. The branch length scale bar indicates the mean number of substitutions per site.