

RESEARCH NOTE

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

Mariana Bertholdi Ebert^{1*}, Juliana Marigo², Guilherme Guerra Neto³, Marta Jussara Cremer³ and Reinaldo
José da Silva¹

¹ São Paulo State University (UNESP) Campus Botucatu, Institute of Biosciences, Department of
Parasitology, Rua Professor Doutor Antônio Celso Wagner Zanin, 250, Botucatu, São Paulo, Brazil -
8618689

² São Paulo University (USP), School of Veterinary Medicine and Animal Science, Department of
Pathology, Prof. Dr. Orlando Marques de Paiva, 87, São Paulo, São Paulo, Brazil – 05508270

³ Laboratory of Ecology and Conservation of Coastal and Marine Tetrapods, University of the Region of
Joinville (UNIVILLE), Duque de Caxias Road, Km 8, 6.365, São Francisco do Sul, Santa Catarina, Brazil
- 89240000

OrcID numbers:

Mariana Bertholdi Ebert: 0000-0001-8200-6411

Juliana Marigo: 0000-0002-3279-2909

Marta Jussara Cremer: 0000-0003-3521-1409

Reinaldo José da Silva: 0000-0002-3426-6873

*Corresponding author: mbe.bio@gmail.com; telephone number +55 14 38800530

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

Abstract

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

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

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

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

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

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

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

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

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

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

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

CCGTCAATTCCTTTAAGTTT-3') [18]; COI, JB3 (5'-TTTTTTGGGCATCCTGAGGTTTAT- 3') [22] and JB4.5 (5'-TAAAGAAAGAACATAATGAAAATG-3') [31]. The thermocycling profile for the rDNA SSU amplification included initial denaturation at 94 °C for 3 min, followed by 40 cycles of amplification 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 the mtDNA COI amplification, the initial denaturation was at 94 °C for 5 min, followed by 40 cycles of 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 min [28]. PCR amplicons (3 µl) were separated electrophoretically in 1% agarose gel with gel red (1 µl) (Biotium Inc) and DNA bands were visualized under UV light. PCR products were purified using QIAquick PCR Purification Kit (Qiagen) following the manufacturer's protocol and sequenced on an Applied Biosystems ABI 3500 automated DNA sequencer using Big Dye v.3.1 Terminator (Applied Biosystems, Foster City, CA, USA).

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

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

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

Morphological description

Phylum Platyhelminthes Gegenbaur, 1859

Class Trematoda Rudolphi, 1808

Subclass Digenea Carus, 1863

Order Plagiorchiida La Rue, 1957.

Family Notocotylidae Lühe, 1909

Genus *Ogmogaster* Jägerskiöld, 1891

Ogmogaster antarctica Johnston, 1931 (Fig. 1)

Body oval, flattened, with the margins turned ventrally, slightly narrowed at the anterior extremity. Lateral margin of the body with crenulations (number from 38 to 40). Spiny tegument, with parallel longitudinal ridges on the ventral surface (number from 13 to 15). Body length 3.91 - 6.30 (5.33) and width 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. Esophagus indistinguishable. Intestinal caeca extending posteriorly to the level of the ovary, curved along its entire length, with small lateral branches. Testes symmetrically positioned, deeply lobed to dendritic, extra-caecal, situated in the posterior half of the body. Right testis 0.39 - 0.89 (0.69) long and 0.35 - 0.75

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

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

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

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

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

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

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

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

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

Acknowledgments

We wish to thank the staff of PMP - UNIVILLE for the collection of the stranded whale and intestinal parasites. M. B. E. held a scholarship grant from CNPq (140873/2017-1). M. J. C. thanks CNPq for a research productivity scholarship (10477/2017-4). R. J. da S. was supported by CNPq (307808/2014-9), CNPq-PROTAX (440496/2015-2) and FAPESP 2016/50377-1. Financial support was also provided by Fundo de Apoio à Pesquisa FAP/UNIVILLE and PETROBRAS.

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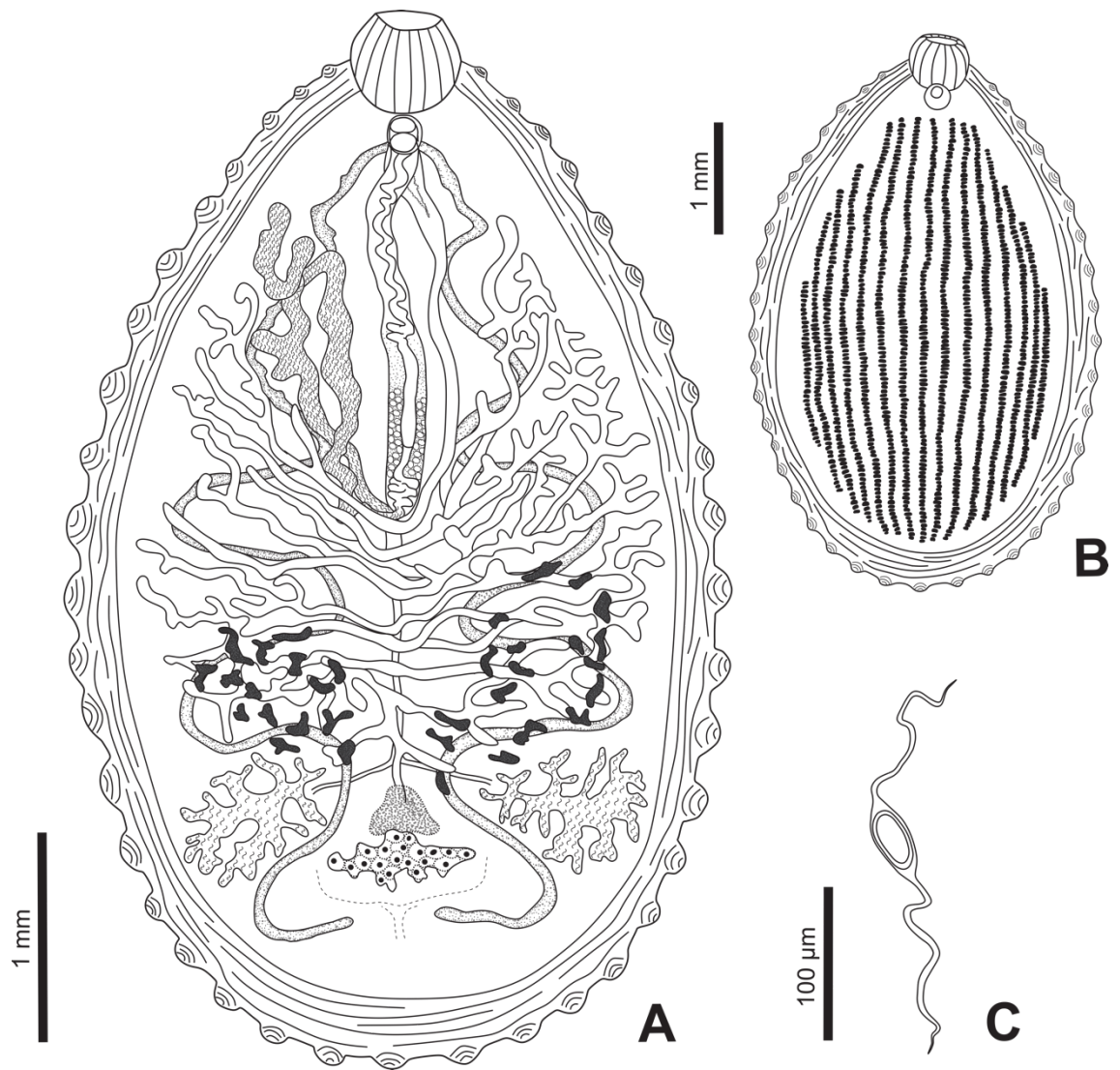
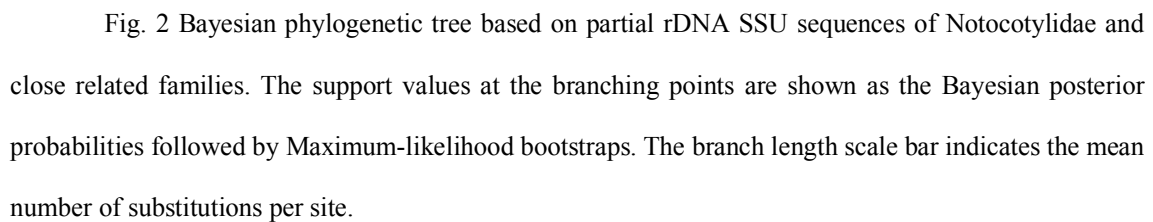


Fig. 1 *Ogmogaster antarctica* from the intestine of a dwarf minke whale *B. acutorostrata* from Southwestern Atlantic Ocean. A: Whole worm, ventral view. B: Whole worm, disposition of ventral ridges. C: Egg, with polar filaments.



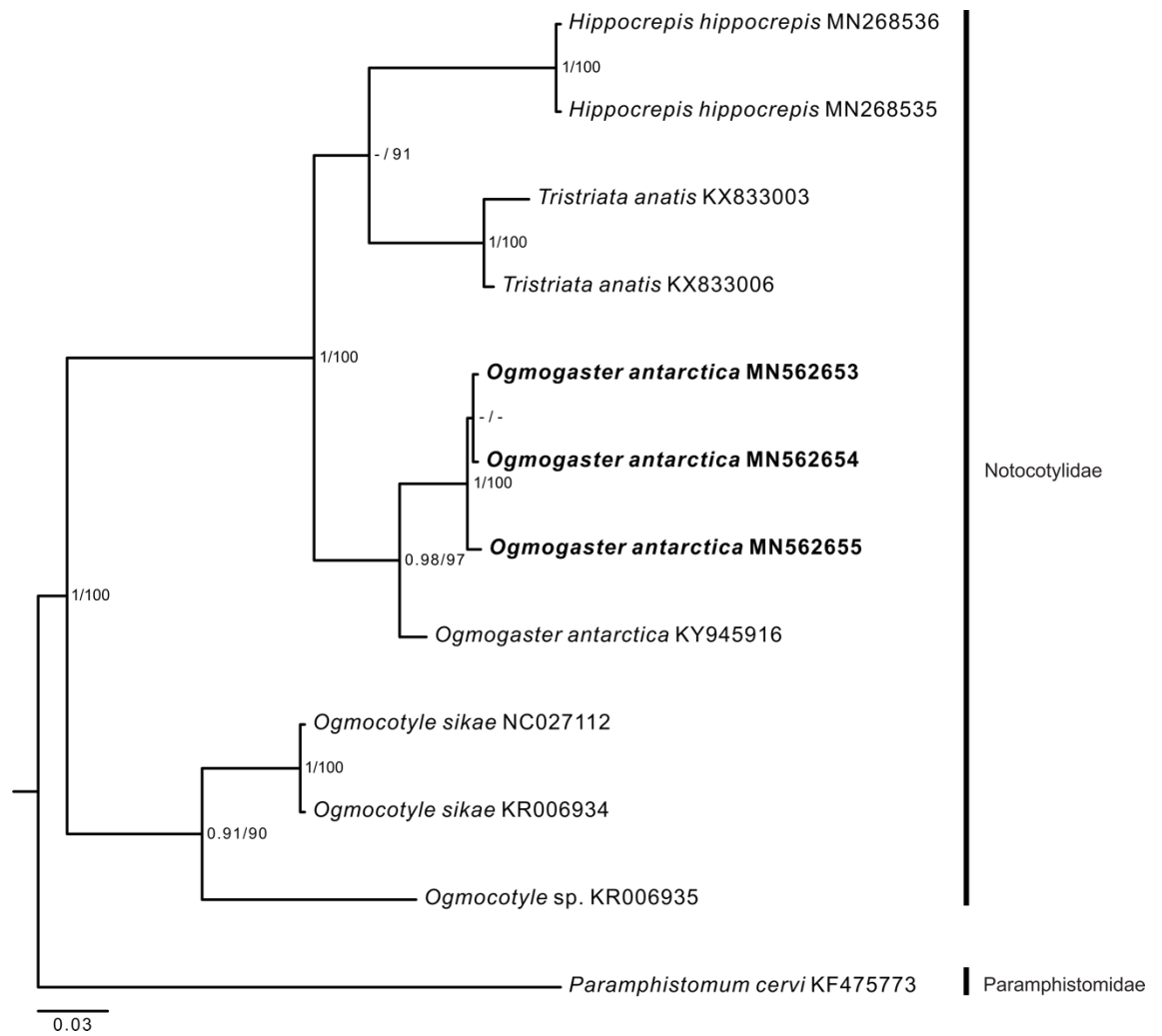


Fig. 3 Bayesian phylogenetic tree based on partial mtDNA COI sequences of Notocotylidae. The support values at the branching points are shown as the Bayesian posterior probabilities followed by Maximum-likelihood bootstraps. The branch length scale bar indicates the mean number of substitutions per site.