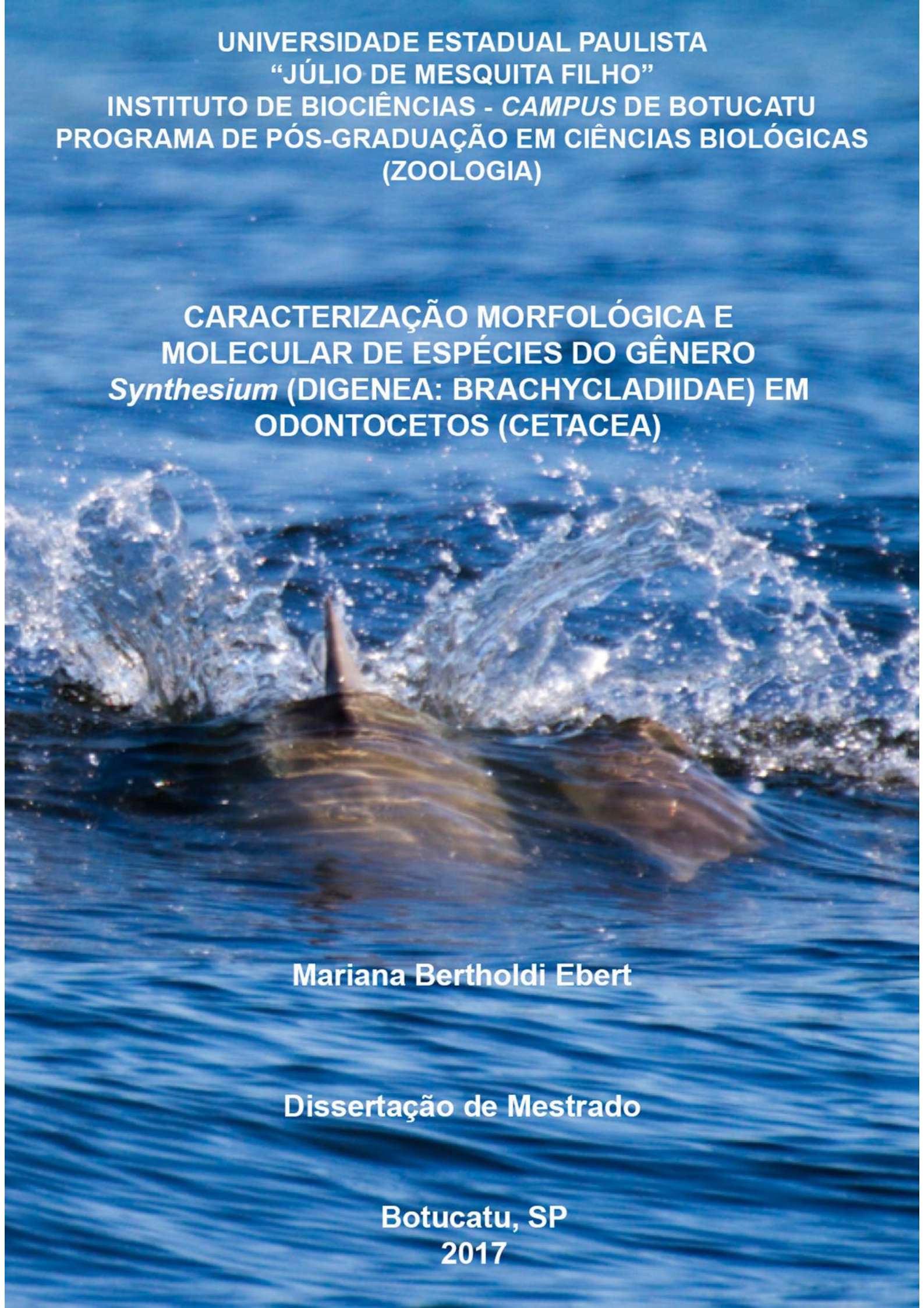


A photograph of a dolphin leaping from the water, creating a large splash. The dolphin's body is visible above the water, and its tail is partially submerged. The water is a deep blue color, and the splash is white and foamy.

UNIVERSIDADE ESTADUAL PAULISTA
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(ZOOLOGIA)

**CARACTERIZAÇÃO MORFOLÓGICA E
MOLECULAR DE ESPÉCIES DO GÊNERO
Synthesium (DIGENEA: BRACHYCLADIIDAE) EM
ODONTOCETOS (CETACEA)**

Mariana Bertholdi Ebert

Dissertação de Mestrado

**Botucatu, SP
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Orientador: Prof. Tit. Reinaldo José da Silva
Coorientadora: Dra. Juliana Marigo

Dissertação apresentada ao Programa de Pós-graduação em Ciências Biológicas (Zoologia) do Instituto de Biociências da Universidade Estadual Paulista, UNESP, *Campus* de Botucatu-SP, como parte dos requisitos para obtenção do título de Mestre em Ciências Biológicas (Zoologia).

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ATESTADO DE APROVAÇÃO - DEFESA

Atestamos que **MARIANA BERTHOLDI EBERT**, RA nº: 187132-1, RG nº 44.729.815-X, expedido pela SSP, defendeu, no dia 22/02/2017, a dissertação intitulada **"CARACTERIZAÇÃO MORFOLÓGICA E MOLECULAR DE ESPÉCIES DO GÊNERO *Synthesium* (DIGENEA: BRACHYCLADIIDAE) EM ODONTOCETOS (CETACEA)"**, junto ao Programa de Pós Graduação em CIÊNCIAS BIOLÓGICAS (ZOOLOGIA), Curso de Mestrado Acadêmico, tendo sido 'APROVADA'.

Atestamos ainda que a obtenção do título dependerá de homologação pelo Órgão Colegiado competente.

Botucatu, 22 de fevereiro de 2017


Hervaldo Mota Santos
Supervisor Técnico de Seção
Pós-Graduação-IBB-UNESP

***Dedico este trabalho a meus pais, Estela e Arlindo,
inspirações da minha vida.***

*“Deus ao mar o perigo e o abismo deu,
Mas nele é que espelhou o céu.”
(Fernando Pessoa)*

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RESUMO

Trematódeos do gênero *Synthesium* infectam o intestino de diversas famílias de odontocetos em todo o mundo. No entanto, a identificação e o status taxonômico de *Synthesium* ainda são confusos e, principalmente, baseados em análises morfológicas. As informações genéticas ainda são escassas. O presente estudo teve como objetivo investigar a diversidade de parasitos intestinais do gênero *Synthesium* colhidos de diferentes odontocetos, através de comparações morfológicas e moleculares. Foram estudados parasitos de golfinhos encontrados mortos em praias ou acidentalmente capturados em redes de pesca de diferentes regiões. Durante a necropsia, o intestino delgado foi removido e armazenado a -20°C. Posteriormente, os intestinos foram abertos, lavados em água corrente sobre peneira de 150 µm e o conteúdo examinado sob estereomicroscópio. Os trematódeos encontrados foram fixados e preservados em etanol 70% para análises morfológicas e moleculares. Os trematódeos foram preparados em lâminas temporárias e as análises morfométricas diagnósticas foram feitas em um sistema computadorizado para análise de imagens. O DNA genômico dos trematódeos foi extraído e amplificado utilizando-se primers com alvo no DNA ribossomal (menor subunidade do DNA ribossomal - gene SSU - e região intergênica - ITS1) e DNA mitocondrial (nicotinamida dinucleotídeo desidrogenase subunidade 3 - gene ND3 - e citocromo c oxidase subunidade I - gene COI). Os resultados morfológicos e moleculares apontam a existência de pelo menos quatro novas espécies de trematódeos do gênero *Synthesium*, sendo uma delas, *Synthesium neotropicalis*, descrita neste trabalho. As análises filogenéticas demonstram um complexo de espécies agregando *S. tursionis* e o posicionamento de *S. delamurei*, *S. subtile* e *S. seymouri* gera questionamentos sobre a correta inclusão destas espécies ao gênero, incentivando sua revisão através de abordagens morfológicas e moleculares. Os resultados obtidos representam uma importante contribuição para o conhecimento da helmintofauna de cetáceos.

Palavras-chave: Parasitos intestinais, Delphinidae, Identificação molecular, Morfologia, Taxonomia.

ABSTRACT

Trematodes of the genus *Synthesium* infect the intestines of a wide range of odontocete families worldwide. However, the identification and the taxonomic status of *Synthesium* are still confusing and mainly based on morphological analyses. Genetic information is still scarce. The present study aimed to investigate the diversity of intestinal parasites of the genus *Synthesium* collected from different odontocetes species, by means of morphological and molecular approaches. Parasites of dolphins found washed ashore or accidentally caught in fishing nets from different regions were studied. During necropsy, the small intestines were removed and stored at -20°C. The intestines were opened, washed in tap water over a 150 µm sieve and examined under a stereomicroscope. The recovered trematodes were fixed and preserved in 70% ethanol for morphological and molecular analyses. The parasites were prepared as temporary slides and diagnostic morphometric analyses were performed on a computerized imaging system. The genomic DNA of the trematodes was extracted and amplified using primers targeted at the ribosomal (small ribosomal subunit - SSU gene- and internal transcribed spacer 1 - ITS1) and mitochondrial DNA (NDH dehydrogenase subunit 3 - ND3 gene- and cytochrome c oxidase subunit 1 – COI gene). The morphological and molecular results indicate the existence of at least four new species of trematodes of the genus *Synthesium*. Phylogenetic analyses show a species complex aggregating *S. tursionis* and the phylogenetic position of *S. delamurei*, *S. subtile* and *S. seymouri* raises questionings about the correct inclusion of these species to the genus, suggesting a revision of the genus through morphological and specially molecular approaches. The obtained results represent an important contribution to the knowledge of the helminthofauna of cetaceans.

Keywords: Intestinal parasites, Delphinidae, Molecular identification, Morphology, Taxonomy.

INTRODUÇÃO GERAL

Estudos parasitológicos

Parasitos apresentam uma grande biodiversidade e potencialmente infectam a maioria dos metazoários de vida livre (POULIN & MORAND, 2000). Parasitos podem influenciar no funcionamento dos ecossistemas e fornecer informações sobre a história evolutiva e demográfica de seus hospedeiros, aspectos estes de grande importância para a conservação das espécies (AZNAR et al., 2010). Parasitos em cetáceos têm sido envolvidos em diversos estudos relacionados à delimitação de estoque populacional (BALBUENA et al., 1995; MARIGO et al., 2015), estrutura social (BALBUENA et al., 1995), história da população (KALISZEWSKA et al., 2005) e condição de saúde de seus hospedeiros (AZNAR et al., 2005). Assim, um modelo parasito-hospedeiro fornece uma ferramenta adequada para estudar a relação ecológica, sistemática, evolutiva e biogeográfica entre organismos (BROOKS & HOBERG, 2000).

Cetáceos

Os cetáceos (golfinhos, botos e baleias) são mamíferos marinhos completamente adaptados à vida aquática (DI BENEDITTO et al., 2010). A ordem Cetacea Brisson, 1762 é dividida em três subordens, Archaeoceti Flower, 1833 (apenas fósseis), Mysticeti Cope, 1864 e Odontoceti Flower, 1867. Os Mysticeti (cetáceos com barbatanas, conhecidos como baleias verdadeiras) estão divididos em quatro famílias e 14 espécies, sendo que oito delas ocorrem no Brasil. Os Odontoceti (cetáceos que possuem dentes, conhecidos como golfinhos e botos) dividem-se em nove famílias e 69 espécies, das quais 35 ocorrem no litoral brasileiro (ICMBio, 2010; DI BENEDITTO et al., 2010).

A distribuição dos cetáceos ao longo dos 8.000 km da costa brasileira é de extrema amplitude, ocorrendo de forma contínua. Isso se dá pela diversidade de ecossistemas presentes ao longo de nosso litoral (baías, manguezais, recifais etc), os quais representam habitats favoráveis para reprodução e alimentação destes animais (IBAMA, 2005).

A maioria das espécies listadas no Plano de Ação de Mamíferos Aquáticos do Brasil (IBAMA, 2001) está classificada na categoria DD (*Deficient Data*) da *IUCN Red List of Threatened Species* (<http://www.iucnredlist.org>), indicando que não existem informações adequadas para avaliação do *status* de conservação, ameaças sofridas e

outras características das espécies, inclusive sobre a diversidade parasitária abrigada ou a relação parasito-hospedeiro. Muito desta carência se deve a inexistência de estudos e destinação adequada de material biológico para fins acadêmicos e, consequentemente, dados substanciais a serem empregados em projetos que visam à conservação destes animais (IBAMA, 2005).

Parasitismo e diversidade de helmintos em cetáceos

Os primeiros registros de helmintos em cetáceos datam do início de 1930, época esta em que a caça comercial era permitida (BAYLIS, 1932; PRICE, 1932). Atualmente, a maioria dos registros utilizam carcaças de cetáceos provenientes de encalhes ocasionais ou capturas acidentais em redes de pesca (RAGA et al., 2008). Recentemente, alguns estudos têm utilizado técnicas não invasivas através da colheita de amostras de fezes para identificação de helmintos (KLEINERTZ et al., 2014; HERMOSILLA et al., 2016).

Estudos parasitológicos em cetáceos são limitados pela dificuldade de acesso tanto aos hospedeiros como seus parasitos em bom estado de conservação. Como consequência, há uma escassez de informações morfológicas e principalmente genéticas a respeito destes organismos (RAGA et al., 2008). Devido a esta insuficiência de informações disponíveis, principalmente em águas brasileiras, é importante o incentivo a estudos parasitológicos de caráter contínuo e padronizado. Estes trabalhos são inclusive citados junto aos projetos e ações prioritárias estabelecidas pelo Plano de Ação para os Mamíferos Aquáticos do Brasil (IBAMA, 2001).

A helmintofauna de cetáceos inclui atualmente cerca de 174 espécies divididas em quatro grandes grupos taxonômicos: os nematoides são o grupo mais diverso (62 espécies), seguido por trematódeos digenéticos (54 espécies), cestoides (38 espécies) e acantocéfalos (20 espécies) (FRAIJA-FERNÁNDEZ et al., in press).

As famílias Pseudaliidae Railliet & Henry, 1909, Anisakidae Skrjabin & Karokhin, 1945 e Tetrameridae Travassos, 1914 são as mais representativas entre os nematoides parasitos de cetáceos. Os cestoides estão representados principalmente pelas famílias Tetrabothriidae Linton, 1891, Diphylobothriidae Lühe, 1910 e Phyllobothriidae Braun, 1900. A família Polymorphidae Meyer, 1931 abriga todas as espécies de acantocéfalos infectantes de cetáceos. Já os trematódeos digenéticos estão distribuídos em quatro famílias: Brauninidae Wolf, 1903, Notocotylidae Lühe, 1909, Heterophyidae Leiper, 1909 e Brachycladiidae Odhner, 1905. A família Brauninidae é

monotípica, incluindo somente *Braunina cordiformis* Wolf, 1903. A família Notocotylidae inclui as espécies do gênero *Ogmogaster* Jägerskiöld, 1891. A família Heterophyidae é representada por uma única espécie, *Pholeter gastrophilus* (Kossack, 1910) Odhner, 1914. A família Brachycladiidae é a mais representativa, incluindo 10 gêneros e 42 espécies (GIBSON, 2005; FRAIJA-FERNÁNDEZ et al., in press).

Família Brachycladiidae

Digenéticos da família Brachycladiidae ocorrem nos ductos hepáticos e pancreáticos, intestinos, pulmões e seios nasais de mamíferos marinhos em todo o mundo (FERNÁNDEZ et al., 1998a,b; GIBSON, 2005). A maior parte da sua diversidade é encontrada em cetáceos (35 das 42 espécies descritas), mas algumas espécies também infectam pinípedes e lontras marinhas (AZNAR et al., 2001; DAILEY, 2007).

Pouco se sabe sobre a biologia da família Brachycladiidae. Nenhum hospedeiro intermediário ou paratênico foi identificado até o momento (FERNÁNDEZ, 1996). Análises filogenéticas e morfológicas indicam que a origem deste grupo e sua associação com mamíferos marinhos provavelmente resultou de eventos de mudanças entre hospedeiros a partir de um ancestral ocorrente em peixes, e que foi capaz de infectar odontocetos através da relação predador-presa (FERNÁNDEZ et al., 1998b; BRAY et al., 2005; FRAIJA-FERNÁNDEZ et al., 2015).

A organização taxonômica da família também é bastante controversa, incluindo muitas transferências de espécies e sinônimas. As identificações são principalmente baseadas em análises morfológicas de helmintos adultos e, recentemente, algumas espécies têm sido reavaliadas através de estudos moleculares (MARIGO et al., 2011).

Atualmente são reconhecidas duas subfamílias; Brachycladiinae Odhner 1905 incluindo *Brachycladium* Looss, 1899, *Hunterotrema* McIntosh 1960, *Oschmarinella* Skrjabin 1947, *Synthesium* Stunkard & Alvey 1930, *Campula* Cobbold 1858, *Zalophotrema* Stunkard & Avey 1929, *Orthosplanchnus* Odhner 1905 e *Odhneriella* Skrjabin 1915; e Nasitrematinae Ozaki, 1935, que agrega os gêneros *Nasitrema* Ozaki 1935 e *Cetitrema* Skrjabin 1970 (GIBSON, 2005).

Gênero *Synthesium*

Parasitos do gênero *Synthesium* infectam o intestino, sobretudo a porção anterior do duodeno, de praticamente todas as famílias de odontocetos (17 espécies registradas

dentro das famílias Pontoporiidae Gray, 1846, Delphinidae Gray, 1821, Monodontidae Gray, 1821 e Phocoenidae Gray, 1825), sugerindo um longo período de adaptações parasito-hospedeiros (FERNÁNDEZ, 1996).

O gênero *Synthesium* sofreu diferentes rearranjos taxonômicos nos últimos anos, com várias sinonímias entre gêneros morfológicamente relacionados como *Leucasiella* Krotov & Delyamure 1952, *Hadwenius* Price, 1932 e *Odhneriella* Skrjabin, 1915 (GIBSON, 2005, 2014). YAMAGUTI (1958) reconheceu *Leucasiella* como sinônimo de *Hadwenius*. Posteriormente, GIBSON (2005) considerou *Hadwenius* como sinônimo júnior de *Synthesium*.

Atualmente são reconhecidas oito espécies deste gênero; três aparentam especificidade de hospedeiros sendo *Synthesium seymouri* (Price, 1932) Marigo, Vicente, Valente, Measures & Santos, 2008, parasito de belugas *Delphinapterus leucas* (Pallas, 1776), *Synthesium delamurei* Raga & Balbuena, 1988, parasito de baleias piloto de peitorais longas *Globicephala melas* Traill, 1809, e *Synthesium pontoporiae* (Raga, Aznar, Balbuena & Dailey, 1994) Marigo, Vicente, Valente, Measures & Santos, 2008 encontrado em toninhas *Pontoporia blainvillei* Gervais & d'Orbigny, 1844. *Synthesium nipponicum* Yamaguti, 1951 é exclusivo de delfínídeos da família Phocoenidae, parasitando o golfinho de Dall *Phocoenoides dalli* True, 1885 e o golfinho do proto *Phocoena phocoena* Linnaeus, 1758. O restante das espécies, *Synthesium subtile* Skrjabin, 1959, *Synthesium mironovi* Krotov & Delyamure, 1952, *Synthesium elongatum* Ozaki, 1935 e *Synthesium tursionis* (Marchi, 1873) Stunkard & Alvey, 1930 são generalistas e apresentam uma grande variedade de hospedeiros registrados (FERNÁNDEZ, 1996; GIBSON, 2005, 2014).

Devido à dificuldade na obtenção de espécimes preservados e a fixação inadequada dos parasitos, muitos caracteres morfológicos são comprometidos (GIBSON, 2005), principalmente porque as espécies deste gênero são bastante similares e as diferenças morfológicas muitas vezes são sutis, o que dificulta a identificação e classificação através de abordagens morfológicas tradicionais. Além disso, alguns estudos apontam variação morfológica intraespecífica em diferentes populações de odontocetos (LAMOTHE-ARGUMEDO, 1988; FERNANDÉZ et al., 1994, 1995). O uso de métodos de identificação molecular para este gênero ainda é recente e os resultados estimulam novas investigações (MARIGO et al., 2011, 2015).

Um estudo integrando caracteres morfológicos e moleculares de espécies de *Synthesium*, coletados de diferentes odontocetos hospedeiros, gera informações

importantes para a identificação e caracterização correta das espécies deste gênero, contribuindo para um panorama mais preciso a respeito da biodiversidade e do *status* taxonômico de *Synthesium* e, em consequência, sua posição filogenética dentro da família Brachycladiidae.

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Objetivos

OBJETIVO

Objetivo Geral

Investigar a diversidade de espécies do gênero *Synthesium*, parasitos de odontocetos, ao longo da costa brasileira.

Objetivos específicos

- Avaliar os parasitos do gênero *Synthesium* provenientes de diferentes espécies de odontocetos hospedeiros com base em dados morfológicos e moleculares;
- Descrever espécies de digenéticos pertencentes ao gênero *Synthesium* através de comparações morfológicas e reconstruções filogenéticas e moleculares;
- Investigar a posição filogenética do gênero *Synthesium* dentro da família Brachycladiidae.

***A new Synthesium species (Digenea: Brachycladiidae)
from bottlenose dolphins Tursiops truncatus (Cetacea:
Delphinidae) in Southwestern Atlantic water***

**A new *Synthesium* species (Digenea: Brachycladiidae) from bottlenose dolphins
Tursiops truncatus (Cetacea: Delphinidae) in Southwestern Atlantic waters¹**

ABSTRACT

A new species of *Synthesium* from the bottlenose dolphin *Tursiops truncatus* in South Brazilian waters is described. Morphological and molecular identification were performed and phylogenetic analyses were carried out using the ribosomal SSU and ITS1 and the mitochondrial ND3 and COI genes. The main characteristics of the new species are the subterminal round-shaped oral sucker, the anterior distribution of vitellaria reaching the level of the ovary and the oval-shaped testes. The results obtained with the molecular markers supported the inclusion of the specimens into the genus *Synthesium*. The nucleotide divergence detected for the mitochondrial genes among the new species and others of the same genus supported the erection of a new species. This is the 9th species assigned to the genus and the third *Synthesium* species recorded in the South Atlantic Ocean.

KEYWORDS: *Tursiops truncatus*; Brachycladiidae; *Synthesium*; Morphology; Molecular identification; South Atlantic Ocean.

¹ The final publication is available at Springer via <http://dx.doi.org/10.1007/s00436-017-5421-2>

INTRODUCTION

The bottlenose dolphin *Tursiops truncatus* Montagu, 1821 is a cosmopolitan species found primarily in coastal and inshore regions in tropical and temperate oceans worldwide (Jefferson et al. 1993). This species is continuously distributed along the Brazilian coast, in the Southwestern Atlantic Ocean (SWA), inhabiting a variety of natural environments (Pinedo et al. 1992). In southern Brazil, bottlenose dolphins are observed in coastal waters, bays and offshore of the Santa Catarina and Rio Grande do Sul states (Cremer et al. 2009; Fruet et al. 2014).

The helminth fauna associated with *T. truncatus* has been surveyed in several geographical regions, mostly in the Northern Hemisphere (Dailey 1976; Raga et al. 1985; Fernández et al. 1994; Aznar et al. 2006, 2007; Kuwamura et al. 2007; Fauquier et al. 2009; Quiñones et al. 2013; Reboredo-Fernández et al. 2015), the Caribbean Sea (Mignucci-Giannoni et al. 1998; Colón-Llavina et al. 2009; Oliveira et al. 2011) and slightly in the South Atlantic (Tomo et al. 2010; Romero et al. 2014). Some studies regarding the diversity and composition of the helminth communities of different odontocete species, including bottlenose dolphins, are also recorded in Brazilian waters (Santos et al. 1996; Marigo et al. 2008, 2011; Carvalho et al. 2010).

Synthesium Stunkard and Alvey, 1930 is a genus of digenetic parasites within the family Brachycladiidae Odhner, 1905 (see Gibson 2005 for a taxonomical review), commonly found in the intestines of a wide range of odontocete families (Pontoporiidae, Delphinidae, Monodontidae and Phocoenidae) in most parts of the world (Fernández et al. 1998a). Their taxonomy is still confused due to the difficulty in obtaining preserved specimens from decomposed or frozen hosts, misleading morphological interpretations (Gibson 2005) and compromising molecular identifications.

In this study, we describe a new species of the genus *Synthesium* Stunkard & Alvey, 1930, family Brachycladiidae Odhner, 1905, collected from bottlenose dolphins *T. truncatus* off the Brazilian coast, SWA. The diagnosis is based on morphological and molecular analyses.

MATERIAL AND METHODS

Parasite sampling

The small intestines of three adult *T. truncatus*, two males and one female, stranded at the São Francisco do Sul district, Santa Catarina state, south Brazil (26°14'36"S, 48°38'17"O), between 2012 and 2014, were analyzed. During the necropsy, the gastrointestinal tracts were removed and stored at -20 °C for later examination. After thawing, the intestines were cut open, washed in tap water over a 150 µm sieve and the contents examined under a stereo microscope. Trematodes were cleaned in tap water, fixed and preserved in 70% ethanol for both morphological and molecular analyses.

Morphological analyses

The morphology of 35 specimens of the new species was studied by light microscopy. The worms were stained with chloridric carmine, dehydrated in a graded ethanol series, cleared with eugenol and mounted as temporary preparations. Three specimens of the new species were also histologically analyzed. The helminthes were placed in 2-hydroxyethyl-methacrylate (7022 18500 Leica historesin embedding kit). Transverse and longitudinal serial sections with a 4 µm thickness were made (Microtome Leica, model RM2165). These sections were stained with hematoxylin-eosin (HE). Morphometric analyses were done according to Fernández et al. (1995) in a computerized system for image analysis (Qwin Lite 3.1, Leica Microsystems, Wetzlar, Germany). Drawings were made with the aid of a camera lucida.

Molecular Characterization

Molecular analyses were conducted on five specimens collected from the three *T. truncatus*. Additionally, we obtained DNA from three specimens identified as *Synthesium tursionis* (Marchi, 1873) Stunkard & Alvey, 1930 collected from the small intestines of an adult Guiana dolphin *Sotalia guianensis* (Van Bénédén, 1864) stranded on the São Paulo coast of Brazil in 2012, and two specimens identified as *Synthesium delamurei* (Raga & Balbuena, 1988) collected from a long-finned pilot whale *Globicephala melas* (Traill, 1809) stranded on the Mediterranean coast of Spain in 2007.

Genomic DNA was extracted from each worm using the DNeasy® Blood & Tissue Kit (Qiagen) according to the manufacturer's protocol in a final volume of 30 µl. DNA fragments were amplified using primers for the ribosomal small subunit (SSU), internal transcribed spacer 1 (ITS1), NDH dehydrogenase subunit 3 (mtND3) and

cytochrome c oxidase subunit 1 (mtCOI). The primers and the cycling conditions are shown in Table 1.

PCR amplifications were carried out using 3 to 5 µl of genomic DNA, 1.0 µl (for SSU, mtND3 and mtCOI genes) or 1.25 µl (for ITS1) of each set of primers and Ready-to-Go PCR beads (Pure Taq™Ready-to-Go™PCR beads, GE Healthcare). The solution consisted of stabilizers, BSA, dATP, dCTP, dGTP, dTTP, ~2.5 units of puReTaq DNA polymerase and reaction buffer. With the reconstituted bead to a final volume of 25 µl, the concentration of each dNTP was 200 µM in 10 mM Tris-HCl, (pH 9.0 at room temperature), 50 mM KCl and 1.5 mM MgCl₂. Aliquots (3 µl) of individual PCR products were separated by electrophoresis using agarose gels (1%), stained with gel red (1 µl) (Biotium Inc) and visualized using ultraviolet transillumination. Gel images were captured electronically and analyzed using the program MULTI-ANALYST (v.1.1, Bio-Rad).

PCR amplicons were purified using a QIAquick PCR Purification Kit (Qiagen) following the manufacturer's instructions. Automated sequencing was directly performed on the purified PCR products using a BigDye v.3.1 Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA, USA) for cycle sequencing. The sequences were run on an Applied Biosystems ABI 3500 DNA genetic sequencer.

Contiguous sequences from each molecular marker were assembled and edited in Sequencher v. 5.2.4 (Gene Codes, Ann Arbor, MI). All sequences were subjected to BLAST analysis (<http://blast.ncbi.nlm.nih.gov>) to confirm their identity. All alignments were made using the MUSCLE software implemented in Geneious version 7.1.3 (Kearse et al. 2012).

Phylogenetic analyses

New SSU, ITS1, mtND3 and mtCOI sequences were used for pairwise estimates of divergence. Only the mtND3 sequences were used for phylogenetic inferences due to the considerable number of other Brachycladiidae sequences available in GenBank.

The genetic divergence between the sequences was calculated within the aligned portion using the Kimura-2-parameter distance model (Kimura 1980) in the MEGA6 program (Tamura et al. 2013).

The phylogeny of mtND3 was inferred by a Neighbor-Joining analyses (NJ) using the MEGA6 program, Bayesian Inference (BI) carried out in the BEAST program

(Drumond et al. 2012) and Maximum Likelihood (ML) performed with PhyML v3.0 (Guindon et al. 2010). The NJ analyses were performed using the Kimura-2-parameter model and 2000 bootstrap replicates. Prior to the ML analyses, the best fitting models of nucleotide substitution were determined based on the Akaike Information Criteria (AIC) using jModelTest 2.1.1 (Posada 2008) as TPM3uf+I+G. Supports for ML were determined by performing 100 bootstrap replicates. The BI analyses were run with a GTR nucleotide substitution model available in the BEAST program. BI Markov Chain Monte Carlo (MCMC) chains were run for 300 million generations, the log-likelihood scores were plotted, and only the final 70% of the trees were used to produce the consensus tree by setting the "burn-in" parameter at 3×10^7 generations. The phylogenetic trees were generated and edited in FigTree v1.3.1 (Rambaut 2009). The species, hosts and accession numbers used in this study are summarized in Table 2.

RESULTS

Description of *Synthesium neotropicalis* n. sp.

The observations and measurements were based on 35 whole-mounted and 3 serially sectioned specimens (see Fig. 1). Measurements (length x width) are shown as the range, with the mean in parentheses followed by the standard deviation, and are expressed in millimeters, unless otherwise stated.

Body elongated, slender, dorsoventrally flattened 11.43 - 29.61 (22.90 ± 5.42), maximum width behind posterior testes 0.36 - 0.73 (0.49 ± 0.08). Body spines not observed. Oral sucker subterminal, muscular, round-shaped, slightly oval 0.33 - 0.74 (0.56 ± 0.09) x 0.25 - 0.62 (0.44 ± 0.09). Ventral sucker muscular located in posterior region of the first third of body 0.31 - 0.59 (0.49 ± 0.07) x 0.33 - 0.61 (0.45 ± 0.09). Oral sucker / ventral sucker length ratio 1:1.14. Distance between suckers 2.32 - 7.83 (5.12 ± 1.60). Prepharynx variable in length, mostly long 0.38 - 2.18 (1.40 ± 0.53). Pharynx pyriform, strongly muscular 0.37 - 0.81 (0.50 ± 0.09) x 0.13 - 0.34 (0.20 ± 0.05). Esophagus very short or almost indistinguishable. Intestine H-shaped with anterior caeca reaching medial level of oral sucker and posterior caeca ending blindly close to posterior extremity of body. Uroproct absent. Genital pore preacetabular. Cirrus pouch long 1.84 - 3.31 (2.65 ± 0.42), extending well beyond ventral sucker, containing large seminal vesicle located at its extremity 0.33 - 1.07 (0.61 ± 0.20) x 0.10 - 0.30 (0.21 ± 0.04), pars prostatica and unarmed cirrus. Cirrus pouch opening into genital

pore. Ovary round to oval $0.14 - 0.45$ (0.30 ± 0.08) x $0.09 - 0.30$ (0.17 ± 0.05), postacetabular, pretesticular. Mehlis' gland preovarian. Vitelline reservoir ovoid, close to ovary. Laurer's canal not observed. Seminal receptacle absent. Distance between ovary and anterior testes $0.07 - 1.82$ (0.66 ± 0.37). Testes oval-shaped, tandem, with anterior testes situated in posterior region of middle third of body and posterior testes situated in anterior region of posterior third of body. Anterior testes $0.35 - 0.80$ (0.60 ± 0.11) x $0.13 - 0.37$ (0.30 ± 0.05) slightly smaller than posterior testes $0.37 - 1.06$ (0.71 ± 0.15) x $0.14 - 0.45$ (0.30 ± 0.07). Intertesticular distance $0.63 - 3.19$ (1.74 ± 0.61). Distance between posterior testes and extremity of body $2.77 - 9.12$ (6.00 ± 1.67). Vitellaria arranged in acinous bunches, profuse, commencing anteriorly to ovary and extending to posterior extremity of body. Distance between anterior margin of vitellaria and ovary $0.25 - 2.48$ (1.13 ± 3.54). Distance between anterior margin of vitellaria and ventral sucker $2.00 - 11.80$ (4.21 ± 2.42). Distance between anterior margin of vitellaria and extremity of body $5.64 - 16.10$ (11.29 ± 2.93). Uterus coils intercecally, widening into unarmed metraterm before opening into genital pore. Eggs oval slightly flattened at opercular pole, triangular in cross-section $47 - 66$ (55) x $28 - 41$ (34) μm .

Taxonomic summary:

Definitive host: *Tursiops truncatus* Montagu 1821, bottlenose dolphin.

Site: Small intestine.

Type locality: Babitonga Bay, São Francisco do Sul district, Santa Catarina state, Brazil, South Atlantic Ocean.

Specimens deposited: Holotype and paratypes specimens were deposited at Coleção Helminológica do Instituto Oswaldo Cruz - Fundação Oswaldo Cruz (CHIOC-Fiocruz) Rio de Janeiro, RJ, Brazil under number (will be deposited after the manuscript acceptance). Paratypes were deposited at Coleção Helminológica do Instituto de Biociências de Botucatu - UNESP (CHIB-UNESP), Botucatu, SP, Brazil under number (will be deposited after the manuscript acceptance).

Etymology: The specific epithet "*neotropicalis*" refers to the Neotropical region, location where the new species was first collected.

Remarks

A total of 1,174 *Synthesium neotropicalis* n. sp. were recovered from the intestine of the three *T. truncatus* hosts. The general morphology of the specimens

analyzed allowed its inclusion in the family Brachycladiidae and the subfamily Brachycladiinae Odhner, 1905 according to Gibson (2005). The following features placed the specimens into the genus *Synthesium*: body very elongated, well-developed pyriform pharynx, intestines with anterior and posterior caeca without diverticula, with posterior caeca ending blindly close to posterior extremity of body, ventral sucker in anterior third of the body, presence of cirrus sac, unarmed metratrem, testes in middle third or third quarter of body and vitellaria entirely in hindbody.

The principal morphological features that differentiate *Synthesium neotropicalis* n. sp. from the other *Synthesium* species are a combination of characteristics such as the subterminal round-shaped oral sucker, the anterior distribution of vitellaria reaching the level of ovary and the oval-shaped testes.

Synthesium neotropicalis n. sp. differs from *S. seymouri*, *S. nipponicum*, *S. mironovi*, and *S. subtile* in its shape (round *versus* cup-shaped) and position (subterminal *versus* terminal) of the oral sucker, the morphology of the pharynx (pyriform *versus* oval), the anterior distribution of vitellaria (at the level of the ovary in *Synthesium neotropicalis* n. sp.) and the egg size (smaller than in the other species). *Synthesium neotropicalis* n. sp. differs from *Synthesium tursionis* because the latter has a terminal cup-shaped oral sucker, lobed testes with shorter distance from the end of the body and anterior distribution of vitellaria reaching the seminal vesicle level. *Synthesium neotropicalis* n. sp. can also be distinguished from *Synthesium elongatum* because the latter presents lobe-shaped testes and vitellaria commencing at the seminal vesicle level. The new species resembles *S. pontoporiae* and *S. delamurei* in the shape and position of the oral sucker (round subterminal) and the shape of the testes (oval). However, *S. pontoporiae* shows a wider body size, vitellaria commencing at the seminal vesicle level and smaller oral and ventral suckers. Finally, *S. delamurei* presents gonads positioned close to the extremity of the body, anterior extent of vitellaria at the seminal vesicle level, and longer and wider testes and eggs compared to the new species. The morphological features differentiating all of the *Synthesium* species are summarized in Table 3.

Phylogenetic Analyses

Sequences of the SSU, ITS1, mtCOI and mtND3 genes were obtained from all specimens of the new species. We successfully sequenced the ITS1 and mtCOI genes

for the *S. tursionis* specimens. However, we could only obtain sequences of mtND3 for *S. delamurei* from the specimens utilized.

Partial SSU alignment (816 bp) comprised a newly generated sequence from *Synthesium neotropicalis* n. sp. (accession number will be deposited after manuscript acceptance) and the sequences from *S. pontoporiae* (FJ357162) and *S. tursionis* (FJ357163) retrieved from GenBank. The alignment of the mtCOI gene (406 bp) and ITS1 (534 bp) included newly generated sequences of *Synthesium neotropicalis* n. sp. (will be deposited after manuscript acceptance (COI) xxxx and (ITS1) xxx) and *S. tursionis* (will be deposited after manuscript acceptance (COI) xxxx and (ITS1) xxx), as well as *S. pontoporiae* (JX644156; JX644084) retrieved from GenBank. The mtND3 alignment (276 bp) consisted of 13 Brachycladiidae sequences retrieved from GenBank, newly generated sequences from *Synthesium neotropicalis* n. sp. (accession number will be deposited after manuscript acceptance) and *S. delamurei* (accession number will be deposited after manuscript acceptance), as well as *Tormopsolus orientalis* (KT180219) as an outgroup (Table 2).

The partial SSU pairwise distance analysis revealed that the *Synthesium neotropicalis* n. sp., *S. tursionis* and *S. pontoporiae* sequences included in the alignment are identical.

The ITS1 pairwise distance analysis showed only 1 nucleotide (0.2% of divergence) difference between *Synthesium neotropicalis* n. sp. and *S. pontoporiae*, suggesting a very close relationship. The nucleotide divergence between *Synthesium neotropicalis* n. sp. and *S. tursionis* was also very small (4 nucleotides, 0.9% of divergence). Additionally, *Synthesium pontoporiae* and *S. tursionis* diverged from each other by 3 nucleotides (0.7% of divergence).

The mtCOI pairwise distance analysis showed a nucleotide divergence between *Synthesium neotropicalis* n. sp. and *S. pontoporiae* of 9.1% (35 nucleotides) and between *Synthesium neotropicalis* n. sp. and *S. tursionis* of 14.6% (55 nucleotides). As for *S. pontoporiae* and *S. tursionis*, the divergence was 14.6% (55 nucleotides).

The mtND3 pairwise distance analysis revealed that the sequences of *Synthesium neotropicalis* n. sp. differed from *S. pontoporiae* by 14 nucleotides (5.3% of divergence), from *S. tursionis* by 35 nucleotides (14% of divergence) and from *S. delamurei* by 49 nucleotides (20.8% of divergence). The difference between *S. tursionis* and *S. delamurei* was 53 nucleotides (22.4%) and between *S. pontoporiae* and *S. delamurei* was 51 nucleotides (21.8% of divergence). Additionally, the sequences of *S.*

pontoporiae and *S. tursionis* are different for 43 nucleotides (17.8% of divergence). The genetic divergence estimated among Brachycladiidae genera had a mean of 19.6%, ranging from 11.9% (*Brachycladium atlanticum* (Abril, Balbuena & Raga, 1991) Gibson, 2005 versus *Campula oblonga* Cobbold, 1858) to 27.4% (*Synthesium pontoporiae* versus *Nasitrema globicephalae* Neiland, Rice & Holden, 1970).

The mtND3 phylogenetic analyses (Fig. 2) based on the BI, NJ and ML trees showed identical topologies with supported values in general. The ML analysis showed supported values on the terminal nodes except for the *Synthesium neotropicalis* n. sp. and the *S. pontoporiae* clade. In all topologies, *Synthesium neotropicalis* n. sp. and *Synthesium pontoporiae* are sister taxa and grouped with *S. tursionis* in a monophyletic clade. However, *S. delamurei* is placed in a different clade apart from all other *Synthesium* species.

DISCUSSION

In the past, the genus *Synthesium* has undergone various taxonomic rearrangements with several synonymies between morphologically closely related genera such as *Leucasiella* Krotov & Delyamure, 1952, *Hadwenius* Price, 1932 and *Odhneriella* Skrjabin, 1915 (see Gibson 2005, 2014). Yamaguti (1958) recognized *Leucasiella* as a synonym of *Hadwenius*. Later, Gibson (2005) considered *Hadwenius* as a junior synonym of *Synthesium*. The genus *Odhneriella* remains accepted with some species transferred to *Synthesium* (Adams and Rausch 1989; Gibson 2005). *Odhneriella* can be easily distinguished from *Synthesium* due to the presence of a uroproct, large and elongate testes and a metraterm armed with spines (Gibson 2005).

Synthesium neotropicalis n. sp. presented all the diagnostic characteristics to be included in the genus *Synthesium*, except for an armed cirrus. The presence of spines in the cirrus is a diagnostic characteristic of the genus *Synthesium* (Gibson, 2005). It is true that spines can be lost due to poor preservation, but we think that it is unlikely that we have missed the spines or, at least, their basal discs in the cirrus, given the large number of specimens we examined. Nevertheless, two other species assigned to the genus *Synthesium*, namely *S. delamurei* and *S. mironovi*, are described as possessing an unarmed cirrus. Originally these species were described as belonging to the genus *Leucasiella* due to the structure of the vitellaria and the lack of spines in the cirrus. Until now, no redescriptions of *S. delamurei* and *S. mironovi* have been carried out and a

morphological reassessment could reveal new observations that are different from their original descriptions. Because the genus *Synthesium* currently aggregates species with both an armed and unarmed cirrus, we think that spines in the cirrus must not be a relevant diagnostic characteristic and should be revised.

In the South Atlantic Ocean only two *Synthesium* species have been reported parasitizing odontocetes until now: *S. pontoporiae*, hosted by the endemic and endangered dolphin Franciscana *Pontoporia blainvillei* (Gervais & d'Orbigny, 1844) from Argentinian and Brazilian waters (Raga et al. 1994; Silva and Cousin 2004; Marigo et al. 2002, 2008), and *S. tursionis*, found in *S. guianensis* and *T. truncatus* from Brazil (Marigo et al. 2008, 2010) and Argentina (Romero et al. 2014). *Synthesium* spp. have also been reported in the intestine of *Cephalorhyncus commersoni* (Lacépède, 1804), *Lagenorhyncus obscurus* (Gray, 1828) and *Lagenorhyncus cruciger* (Quoy & Gaimard, 1824) off Argentina (Dans et al. 1999; Berón-Vera et al. 2001; Fernández et al. 2003). *Synthesium neotropicalis* n. sp. is the third *Synthesium* species recorded in the South Atlantic Ocean.

Regarding the molecular analyses, the sequences of the partial SSU did not exhibit any genetic variation between *Synthesium neotropicalis* n. sp., *S. pontoporiae* and *S. tursionis*. Thus, interspecific differences could not be inferred from this marker. The nucleotide variations in the ITS1 within our alignment were less than 1%. For the ITS region, previous studies have shown that the intraspecific divergence for species of digeneans is below 1% (Vilas et al. 2005; Nolan and Cribb 2005), with some exceptions (Galazzo et al. 2002; Jousson and Bartoli 2002). Nevertheless, the rate of change in a region of DNA may vary from one group to another. Kane and Rollinson (1994) found a 0.9% variation in the ITS region between species of *Schistosoma*. Also, Marigo et al. (2015) found identical ITS1 sequences among *S. pontoporiae* specimens, suggesting a single lineage of this species along the SWA coast. The nucleotide divergence observed in ITS1 of the species of *Synthesium* analyzed here was quite low and should be interpreted with caution rather it provides information at the species level for the genus. Although the ribosomal markers used in this study did not completely diagnose the *Synthesium* species employed here, the data generated will contribute to future phylogenetic analyses of the group.

The genetic divergence found among the species analyzed in this study as *Synthesium neotropicalis* n. sp, *S. pontoporiae*, *S. tursionis* and *S. delamurei* ranged from 9.1 to 14.6% for mtCOI and from 5.3 to 22.4% for mtND3. Previous studies have

suggested that trematode species show maximum intraspecific divergences to be considered one single species at approximately 2% for mtDNA (Vilas et al. 2005). This is below the observed values for the *Synthesium* species analyzed here. For mtCOI specifically, the mean pairwise divergence between the congeners is 19% (ranging from 3.9 to 25%) (Moszczyńska et al. 2009). Although this rate also might vary between the digenean groups our results corroborate these values. Moreover, the two recognized species coexisting along the Brazilian coast, namely *S. pontoporiae* and *S. tursionis*, presented interspecific genetic differences of 14.6% in mtCOI and 17.8% in mtND3. These results might be interpreted as a threshold to narrow the divergence between *Synthesium* species. Therefore, comparing the genetic divergence found between both species and *Synthesium neotropicalis* n. sp., we recognize the latter as a different taxon.

The phylogenetic position of *S. delamurei* caught our attention because we expected it to be placed within the *Synthesium* clade. The mtND3 genetic divergence between *S. delamurei* and its congeners (22.4%) was also higher than among the other *Synthesium* species. From our results, the mean mtND3 genetic divergence between the genera of the Brachycladiidae family is 19.6%. Fernández et al. (2000) also found genetic divergence in mtND3 between brachycladiids from separate genera such as *Nasitrema globicephalae* and *Synthesium tursionis* to be 26.5%. The specimens used here were morphologically identified as *S. delamurei* according to Raga and Balbuena (1988). However, given the phylogenetic inference and genetic distance results, a detailed study should be conducted to elucidate whether *S. delamurei* is correctly assigned to the genus *Synthesium*.

A combination of morphological traits and molecular tools using different genes is highly recommended for describing new species. The morphostructural and morphometrical features combined with the phylogenetic reconstruction and genetic distances presented here support the erection of a new parasite species infecting *T. truncatus*. The mtND3 phylogenetic inferences indicate the correct assignment of the new species in the genus *Synthesium*. We also added new SSU, ITS1, mtND3 and mtCOI gene sequences to the limited available Brachycladiidae sequence collection.

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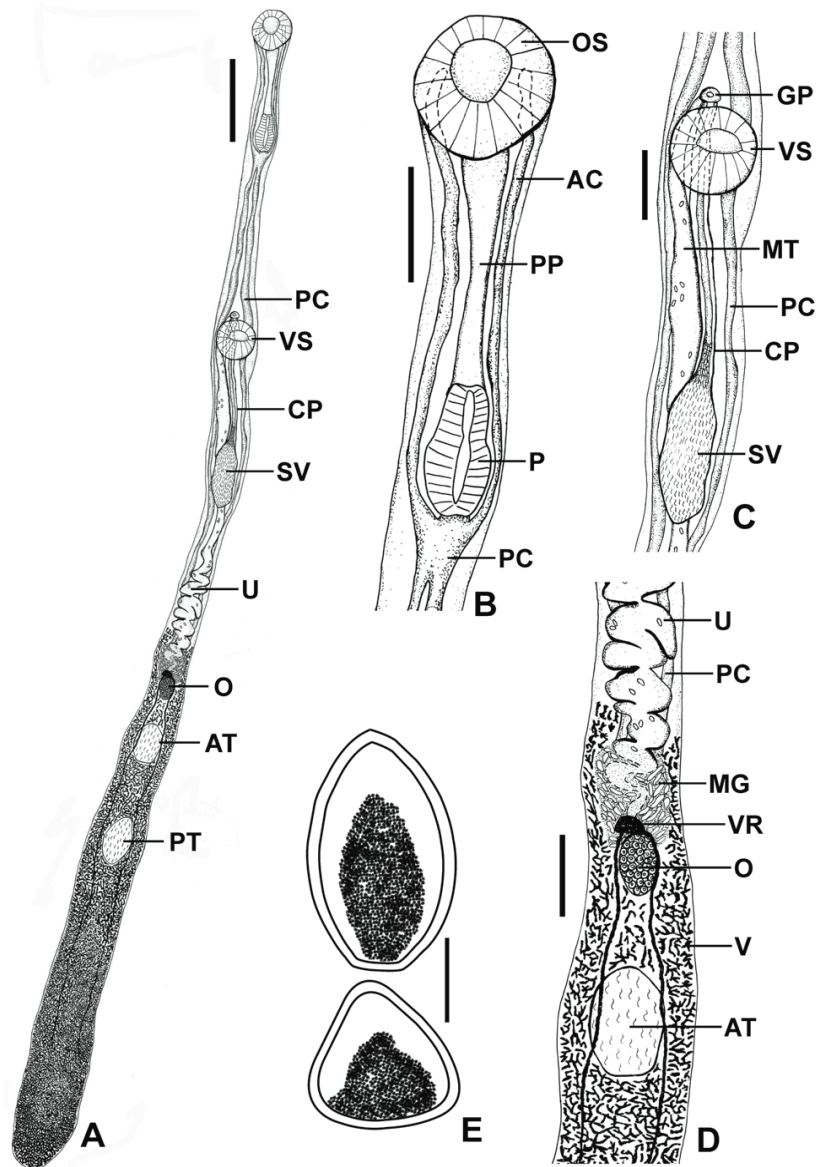


Fig 1 *Synthesium neotropicalis* n. sp. from the small intestine of *Tursiops truncatus* from Brazil. Ventral view. (A) Whole individual – Scale bar = 1 mm. (B) Detail of anterior extremity of body with anterior caeca reaching lateral of oral sucker – Scale bar = 200 µm. (C) Detail of cirrus punch, seminal vesicle and metraterm – Scale bar = 200 µm. (D) Detail of anterior extent of vitellaria, ovary and anterior testes – Scale bar = 200 µm. (E) Detail of eggs – Scale bar = 20 µm. Abbreviations: AC = Anterior Caeca, AT = Anterior Testis, CP = Cirrus Pouch, GP = Genital Pore, M = Metraterm, MG = Mehli's Gland, O = Ovary, OS = Oral Sucker, P = Pharynx, PC = Posterior Caeca, PP = Prepharynx, PT = Posterior Testes, SV = Seminal Vesicle, U = Uterus, V = Vitellaria, VR = Vitelline Reservoir, VS = Ventral Sucker.

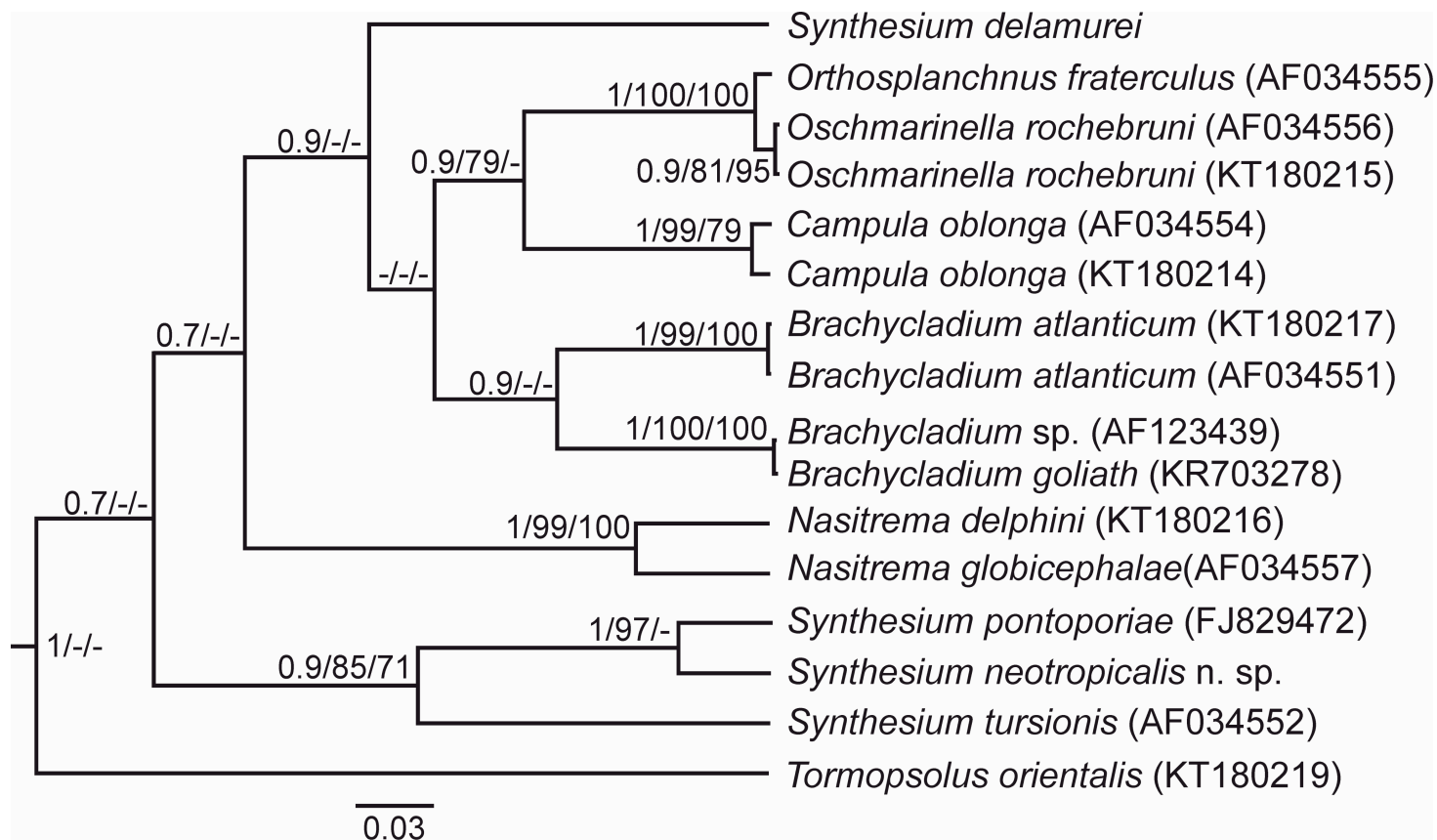


Fig 2 Bayesian tree based on the mtND3 sequences. The species names are followed by the GenBank accession numbers in parentheses. The support values at the branching points are shown as follows: Bayesian posterior probability/ neighbor-joining bootstrap/ maximum likelihood bootstrap. Dashes are shown for the branches not supported by the analyses (scored < 70%). The branch length scale bars indicate mean number of substitutions per site

Table 1 Primers, cycling conditions for PCR and respective bibliographic sources

Primer	Sequences 5'-3'	Cycling conditions	Source
SSU	18SF CGTATCTTTCAAATGTCTGCCC 18SR CCGATGACCTTGCTAAACC	Initial denaturation at 94°C for 5 min; 40 cycles of 94°C for 40 s, 55°C for 40 s and 72°C for 2 min; Final extension at 72°C for 10 min	This study (adapt. from Fernández et al. 1998b)
ITS1	ITS1F GACGACCAAACTTGATCATT ITS1R TGCGCTCTTCATCGACACACGA	Initial denaturation at 94°C for 3 min; 30 cycles of 94°C for 1 min, 48°C for 1 min and 72°C for 1 min and 30 s; Final extension at 72°C for 7 min	Marigo et al. 2015
COI	COIPRA TGGTTTTTTGTGCATCCTGAGGTTTA COIPRB AGAAGAACGTAATGAAAATGAGCAAC	Initial denaturation at 94°C for 5 min; 35 cycles of 94°C for 30 s, 47°C for 30 s and 72°C for 1 min; Final extension at 72°C for 7 min	Bessho et al. 1992
ND3	ND3F GCTTAATTKKTAAAGCYTTGRATTCTTACT ND3D CTACTAGTCCCACTCAACRTAACCYT	Initial denaturation at 95°C for 5 min; 35 cycles of 95°C for 30s, 47°C for 30s and 72°C for 50s; Final extension at 72°C for 7 min	Fernández et al. 1998a; Fernández et al. 2000

Table 2 Species, hosts, Genbank accession numbers and bibliographic sources of parasites sequences used for phylogenetic analyses

Parasite species	Host species (Common name)	GenBank accession no.	Source
SSU rDNA			
<i>Synthesium pontoporiae</i>	<i>Pontoporia blainvillei</i> (Franciscana)	FJ357162	Marigo et al. unpub. data
<i>Synthesium neotropicalis</i> n. sp.	<i>Tursiops truncatus</i> (Bottlenose dolphin)	*	This study
<i>Synthesium tursionis</i>	<i>Tursiops truncatus</i> (Bottlenose dolphin)	FJ357163	Marigo et al. unpub. data
ITS1 rDNA			
<i>Synthesium neotropicalis</i> n. sp.	<i>Tursiops truncatus</i> (Bottlenose dolphin)	*	This study
<i>Synthesium pontoporiae</i>	<i>Pontoporia blainvillei</i> (Franciscana)	JX644084	Marigo et al. 2015
<i>Synthesium tursionis</i>	<i>Sotalia guianensis</i> (Guiana dolphin)	*	This study
ND3 mtDNA			
<i>Brachycladium atlanticum</i>	<i>Stenella coeruleoalba</i> (Striped dolphin)	AF034551	Fernández et al. 1998a
<i>Brachycladium atlanticum</i>	<i>Stenella coeruleoalba</i> (Striped dolphin)	KT180217	Fraija-Fernández et al. 2016
<i>Brachycladium goliath</i>	<i>Balaenoptera acutorostrata</i> (Minke whale)	KR703278	Briscoe et al. 2016
<i>Brachycladium</i> sp.	<i>Balaenoptera acutorostrata</i> (Minke whale)	AF123439	Fernández et al. 2000
<i>Campula oblonga</i>	<i>Phocoena phocoena</i> (Harbour porpoise)	AF34554	Fernández et al. 1998a
<i>Campula oblonga</i>	<i>Phocoena phocoena</i> (Harbour porpoise)	KT180214	Fraija-Fernández et al. 2016
<i>Nasitrema globicephalae</i>	<i>Globicephala melas</i> (Long-finned pilot whale)	AF034557	Fernández et al. 1998a
<i>Nasitrema delphini</i>	<i>Delphinus delphis</i> (Common dolphin)	KT180216	Fraija-Fernández et al. 2016
<i>Oschmarinella rochebruni</i>	<i>Stenella coeruleoalba</i> (Striped dolphin)	KT180215	Fraija-Fernández et al. 2016
<i>Oschmarinella rochebruni</i>	<i>Stenella coeruleoalba</i> (Striped dolphin)	AF034556	Fernández et al. 1998a
<i>Orthosplanchnus fraterculus</i>	<i>Enhydra lutris</i> (Sea Otter)	AF034555	Fernández et al. 1998a
<i>Synthesium pontoporiae</i>	<i>Pontoporia blainvillei</i> (Franciscana)	FJ829472	Marigo et al. 2011
<i>Synthesium neotropicalis</i> n. sp.	<i>Tursiops truncatus</i> (Bottlenose dolphin)	*	This study
<i>Synthesium tursionis</i>	<i>Tursiops truncatus</i> (Bottlenose dolphin)	AF034552	Fernández et al. 1998a
<i>Synthesium delamurei</i>	<i>Globicephala melas</i> (Long-finned pilot whale)	*	This study
<i>Tormopsolus orientalis</i>	<i>Seriola dumerili</i> (Greater amberjack)	KT180219	Fraija-Fernández et al. 2016
COI mtDNA			
<i>Synthesium neotropicalis</i> n. sp.	<i>Tursiops truncatus</i> (Bottlenose dolphin)	*	This study
<i>Synthesium pontoporiae</i>	<i>Pontoporia blainvillei</i> (Franciscana)	JX644156	Marigo et al. 2015
<i>Synthesium tursionis</i>	<i>Sotalia guianensis</i> (Guiana dolphin)	*	This study

*Accession numbers will be included after manuscript acc

Table 3 Mean (range) of morphological measurements of *Synthesium neotropicalis* n. sp. compared to other species of the genus. Values are given as length × width in millimeters unless otherwise stated

	<i>S. neotropicalis</i> n. sp.	<i>S. delamurei</i>	<i>S. pontoporiae</i>	<i>S. elongatum</i>	<i>S. tursionis</i>	<i>S. seymouri</i>	<i>S. nipponicum</i>	<i>S. mironovi</i>	<i>S. subtile</i>
No. of Specimens	(n=35)	(n=4)	(n=20)	(n=2)	(n=15)	(n=10)	(n=10)	(n=2)	(n=20)
Host	<i>T. truncatus</i>	<i>G. melas</i>	<i>P. blainvillei</i>	<i>N. phocoenoides</i>	<i>T. truncatus</i>	<i>D. leucas</i>	<i>P. dalli</i> ; <i>P. phocoena</i>	<i>D. leucas</i>	<i>O. orca</i> ; <i>D. leucas</i> ; <i>G. melas</i>
Body Length	22.9 (11.4 - 29.6)	12.1 (9.7 - 16.8)	5.0 (3.6-7.1)	13.0 - 18.0	14.3 (8.85 - 21.31)	34.1 (27.2-38.1)	14.0 (13-15.9)	8.9 - 12.9	33.3 (14.0 - 38.3)
Max. Width	0.5 (0.4 - 0.7)	0.7 (0.6 - 0.8)	0.5 (0.3-0.7)	1 - 2.1	0.6 (0.5 - 0.8)	0.8 (0.6-1.0)	0.9 (0.8-1.0)	0.7 - 1.2	1.5 (1.2 - 2.0)
Oral Sucker Position	Subterminal	Subterminal	Subterminal	Subterminal	Terminal	Terminal	Terminal	Terminal	Terminal
Oral Sucker	0.6 x 0.4 (0.3-0.7 x 0.3-0.6)	0.5 x 0.4 (0.4-0.5 x 0.3-0.5)	0.2 x 0.1 (0.1-0.2 x 0.1-0.2)	0.4 x 0.5	0.6 x 0.5 (0.4-0.7 x 0.4-0.7)	2.0 x 1.6 (1.8-2.6 x 1.3-2.1)	0.8 x 0.6 (0.8-0.9 x 0.6-0.7)	0.3-1.0 x 0.5-1.1	1.5 x 1.8 (1.4-2.2 x 1.2-1.8)
Prepharynx Length	1.40 (0.4 - 2.2)	0.5 (0.3-0.7)	0.2 (0.7-0.3)	0.5 - 0.5	0.5 (0.2-0.8)	0.8 (0.4-1.3)	0.4 (0.3-0.6)	0.3	0.5 (0.05-1.8)
Pharynx Shape	Pyriform	Pyriform	Pyriform	Pyriform	Pyriform	Oval	Oval	-	Oval
Pharynx	0.5 x 0.2 (0.4-0.8 x 0.1-0.3)	0.2 (0.1-0.2)	0.6 x 0.1 (0.1-0.3 x 0.03-0.2)	0.4-0.6 x 0.3-0.3	0.5 x 0.2 (0.5-0.7 x 0.2-0.3)	1.0 x 0.8 (0.9-1.2 x 0.7-1.3)	0.3 x 0.3 (0.3-0.4 x 0.3-0.3)	0.3-0.4 x 0.3-0.4	0.7 x 0.7 (0.5-0.8 x 0.5-0.9)
Ventral Sucker Position	Anterior 1/3	Anterior 1/3	Anterior 1/3	Anterior 1/3	Anterior 1/3	Anterior 1/3	Anterior 1/3	Anterior 1/3	Anterior 1/3
Ventral Sucker	0.5 x 0.4 (0.3-0.6 x 0.3-0.6)	0.6 x 0.5 (0.5-0.8 x 0.4-0.6)	0.3 x 0.3 (0.2-0.4 x 0.2-0.4)	0.7 x 0.5	0.6 x 0.5 (0.4-0.8 x 0.4-0.7)	1.3 x 1.00 (1.1-1.5 x 0.8-1.4)	0.7 x 0.7	0.6-1.0 x 0.6- 0.8	0.8 x 0.9 (0.7-1.3 x 0.7-1.1)
Cirrus Pouch Length	2.6 (1.8 x 3.3)	2.6 (2.0 - 3.4)	0.9 (0.7 - 1.1)	0.9 - 1.5	2.2 (1.4-2.9)	3.2 (1.3-4.1)	1.2 (1.0-1.4)	-	4.4 (2.4 - 6.1)
Testes Shape	Oval	Oval	Oval	Lobed	Lobed	Oval	Oval	Oval	Oval
Ovary	0.3 x 0.2 (0.1-0.4 x 0.1-0.3)	0.3 x 0.2 (0.3-0.4 x 0.1-0.2)	0.2 x 0.1 (0.1-0.3 x 0.06-0.2)	0.3-0.4 x 0.2-0.3	0.2 x 0.2 (0.1-0.3 x 0.1-0.2)	0.3 x 0.2 (0.2-0.4 x 0.1-0.3)	0.22 x 0.22 (0.2-0.3 x 0.2-0.3)	0.1 - 0.3	0.4 x 0.3 (0.2-0.5 x 0.2-0.4)
Gonads Position	Medial 1/3	Posterior 1/3	Medial 1/3	Medial 1/3	Medial 1/3	Medial 1/3	Medial 1/3	Anterior 1/3	Medial 1/3
Anterior Testes	0.6 x 0.3 (0.3-0.8 x 0.1-0.4)	1.0 x 0.3 (0.8-1.2 x 0.2-0.4)	0.3 x 0.2 (0.2-0.5 x 0.1-0.2)	-	0.7 x 0.3 (0.5-1.1 x 0.3-0.6)	1.5 x 0.7 (1.3-1.8 x 0.5-1.0)	0.7 x 0.3 (0.6-0.8 x 0.2-0.3)	0.6-0.9 x 0.2-0.3	1.2 x 0.6 (0.7-1.8 x 0.4-0.8)
Posterior Testes	0.7 x 0.3 (0.4-1.0 x 0.1-0.4)	1.0 x 0.3 (0.9-1.1 x 0.3-0.4)	0.3 x 0.2 (0.2-0.5 x 0.1-0.3)	-	0.7 x 0.4 (0.5-1.1 x 0.3-0.6)	1.6 x 0.7 (1.3-1.9 x 0.5-0.8)	0.7 x 0.3 (0.6-0.8 x 0.3-0.4)	0.5-1.0 x 0.2-0.5	1.3 x 0.6 (0.8-1.9 x 0.4-0.9)
Anterior Extent of Vitellaria	Ovary	Seminal Vesicle	Seminal Vesicle	Uterine field	Seminal Vesicle	Anterior testes	Anterior Testes	Anterior Testes	Seminal Vesicle
Egg Size (µm)	55 x 34 (47-68 x 28-41)	67 x 40 (57-72 x 32-50)	53 x 26 (46-60 x 23-28)	47-55 x 25-31	53 x 29 (51-55 x 28-32)	91 x 55 (83-97 x 51-55)	70-82 x 35-45	72-90 x 33-37	89 x 49 (75-98 x 44-55)

***Phylogenetic relationship among species of Synthesium
(Digenea: Brachycladiidae), parasites of dolphins, based
on morphological and molecular data***

Phylogenetic relationships among species of *Synthesium* (Digenea: Brachycladiidae), parasites of dolphins, based on morphological and molecular data

ABSTRACT

The identification of *Synthesium* species is difficult due to their morphological similarity. Morphological and molecular analyses based on ribosomal and mitochondrial markers were conducted for species delimitation of *Synthesium* collected from delphinid hosts. The comparative analysis combining molecular and morphological data suggests the erection of three distinct putative species of *Synthesium* parasitizing dolphins from the Brazilian and Spanish coasts. Furthermore, new molecular data on *Synthesium tursionis* evidence the presence of cryptic species. The phylogenetic position of *Synthesium delamurei*, *Synthesium subtile*, and *Synthesium seymouri* reveals the need of taxonomic revision of the genus and potentially of the Brachycladiidae family.

Keywords: Trematode; Molecular characterization; Taxonomy; Biodiversity; Odontocete

INTRODUCTION

Species of *Synthesium* Stunkard & Alvey, 1930 are commonly found in the intestines of a wide range of odontocete (Cetacea) families (Pontoporiidae Gray, 1846, Delphinidae Gray, 1821, Monodontidae Gray, 1821 and Phocoenidae Gray, 1825) in most parts of the world (Fernández et al., 1998a).

In the past years the genus *Synthesium* has undergone different taxonomic rearrangements with several synonymies between morphologically close related genera such as *Leucasiela* Krotov & Delyamure 1952, *Hadwenius* Price, 1932, and *Odhneriella* Skrjabin, 1915 (See Gibson, 2005, 2014 for complete review). To date, nine valid species are recognized: the type-species *Synthesium tursionis* (Marchi, 1873) Stunkard & Alvey, 1930, *Synthesium pontoporiae* (Raga, Aznar, Balbuena & Dailey, 1994) Marigo, Vicente, Valente, Measures & Santos, 2008, *Synthesium seymouri* (Price, 1932) Marigo, Vicente, Valente, Measures & Santos, 2008, *Synthesium elongatum* Osaki, 1935, *S. nipponicum* Yamaguti, 1951, *Synthesium mironovi* Krotov & Delyamure, 1952, *Synthesium subtile* Skrjabin, 1959, *Synthesium delamurei* (Raga & Balbuena, 1988) (Gibson, 2014) and *Synthesium neotropicalis* Ebert, Müller, Marigo, Valente, Cremer & Silva, 2017.

Preserved specimens from decomposed or frozen hosts are problematic and usually leads to misidentifications (Gibson, 2005). Nevertheless, some *Synthesium* species present few morphological differences in adult stage, which compromises their correct identification. Such problems could be readily overcome by the use of molecular methods. Ribosomal and mitochondrial DNA regions can provide suitable markers for systematic studies of trematodes, helping to explore population variants and differentiation among species (Vilas et al., 2005; Nolan and Cribb, 2005). SSU rDNA and ND3 mtDNA sequences of Brachycladiidae have been used in previous phylogenetic studies (Fernández et al., 1998a,b, 2003; Marigo et al., 2011, 2015; Fraija-Fernández et al., 2016), whereas other markers such as ITS1 rDNA and COI mtDNA are still scarce (Marigo et al., 2011, 2015; Ebert et al., 2017) and only one study was performed up to date (Marigo et al., 2015). Thus, a molecular approach including different specimens of *Synthesium* may reveal a bulk of new species for this genus.

In the present study, we used DNA sequences of ribosomal and mitochondrial genes along with morphological data to discuss species boundaries and consequent identification of *Synthesium* and their phylogenetic relationships within the family Brachycladiidae.

MATERIAL AND METHODS

Parasite collection

We obtained *Synthesium* spp. samples from 14 dolphins found washed ashore along the South and Southeastern Brazilian coast, Southwestern Atlantic Ocean (one *Delphinus delphis* Linnaeus, 1758, one *Steno bredanensis* Lesson, 1828, three *Tursiops truncatus* Montagu, 1821, eight *Sotalia guianensis* Van Bénédén, 1864 and one *Stenella frontalis* Cuvier, 1829). During necropsy, the dolphins' gastrointestinal tracts were removed and stored at -20 °C until further examination. After thawing, the intestines were cut open, washed in tap water over a 150 µm sieve and the content examined under a stereo microscope. Trematodes were cleaned in tap water, fixed and preserved in 70% ethanol for both morphological and molecular analyses.

Additional samples of *S. delamurei* collected from a *Globicephala melas* (Traill, 1809) and *S. tursionis* from a *T. truncatus*, both found stranded on the Mediterranean Coast of Spain, *S. seymouri* collected from three *Delphinapterus leucas* (Pallas, 1776) from Saint Lawrence bay, Canada, and *S. subtile* collected from a *G. melas* from the Faroe Islands were also obtained and used for DNA sequencing. To implement the phylogenetic analyses, other Brachycladiidae species were also obtained and sequenced. Table 1 provides a taxonomic listing of specimens analyzed, identification voucher, their hosts, and collection localities.

Morphological examination and morphometric analyses

We studied the morphological aspects of the specimens from all Brazilian host by means of light microscopy. The worms were stained with chloridric carmine, dehydrated through a graded ethanol series, cleared with eugenol and mounted as temporary preparations. Diagnostic morphometric measurements were made according to Fernández et al. (1995) in a computerized system for image analysis (Qwin Lite 3.1, Leica Microsystems, Wetzlar, Germany).

Extraction of gDNA, PCR amplification and Sequencing

Total genomic DNA was extracted from whole worms using a Qiagen® DNeasy™ Blood & Tissue Kit following the manufacturer's recommended protocols, with a final elution volume of 30 µl.

Fragments were amplified using primers for the ribosomal internal transcribed spacer 1 (ITS1), and mitochondrial genes NDH dehydrogenase subunit 3 (mtND3) and cytochrome c oxidase subunit 1 (mtCOI).

PCR amplifications (25 µl) were performed using Ready-to-Go™ (GE Healthcare) PCR beads (each containing ~2.5 units of pureTaq DNA polymerase, 10 mM Tris-HCl at pH 9.0, 50 mM KCl and 1.5 mM MgCl₂ and 200 µM of each dNTP), 3 to 5 µl of genomic DNA and 1.0 µl of each PCR primer. Primers and cycling conditions are showed in Table 2.

PCR amplicons were purified using QIAquick PCR Purification Kit (Qiagen) following the manufacturer's instructions. Automated sequencing was performed directly on purified PCR products using BigDye v.3.1 Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems) for cycle sequencing. Sequences were run onto an Applied Biosystems ABI 3500 DNA genetic sequencer.

Alignments and phylogenetic analyses

Contiguous sequences from each molecular marker were assembled and edited using Sequencher v. 5.2.4 (Gene Codes, Ann Arbor, MI). Each sequence was subjected to BLAST analysis (<http://blast.ncbi.nlm.nih.gov>) to confirm identity.

Newly generated ITS1, mtND3 and mtCOI sequences were combined with other Brachyladiidae sequences retrieved from GenBank and aligned using MUSCLE software implemented on Geneious 7.1.3 (Kearse et al., 2012) with default settings. *Tormopsolus orientalis* Yamaguti, 1934 (Acanthocolpidae Lühe, 1906) was included as outgroup for ITS1 and ND3 alignments.

For species delimitation, we used the pairwise distance model and the GMYC model. The pairwise distance was calculated within each marker aligned portion using Kimura-2-parameter (Kimura, 1980) in MEGA6 program (Tamura et al., 2013). For GMYC analyses applied for the mtCOI dataset, a lognormal relaxed molecular clock tree was estimated using BEAST v.1.8.2 program (Drumond et al., 2012). The nucleotide evolutionary model used to estimate the ultrametric tree was the GTR model. The Markov Chain Monte Carlo (MCMC) were run for 1×10^7 generations, and a tree was sampled

every 100th generation. The GMYC analysis was performed within the online platform GMYC web server (<http://species.h-its.org/gmyc/>) with a single threshold method.

For phylogenetic inference, the obtained ITS1 and mtND3 datasets were analyzed individually by Maximum Likelihood (ML) and Bayesian Inference (BI), applying the model of nucleotide evolution GTR + I + G selected using PAUP v4a147 (Swofford, 2002) for both datasets. ML analyses were carried out using the program RAxML v. 8 (Stamatakis, 2014). The model parameters and bootstrap support values (1,000 repetitions) were estimated using RAxML. BI trees were generated using MrBayes v. 3.2 (Ronquist et al., 2012) running two independent MCMC runs of four chains for 1×10^7 generations and sampling tree topologies every 10^3 generations. Burn-in periods were set to the first 25,000 generations. MrBayes and RAxML analyses were carried out on CIPRES web portal (Miller et al., 2010). Phylogenetic trees were generated and edited in FigTree v1.3.1 (Rambaut, 2009).

RESULTS

Morphological analyses

Using traditional morphology, we could identify two different species of *Synthesium* in our samples: *S. tursionis* (in *S. guianensis* samples) and *S. neotropicalis* (in all *T. truncatus* samples). In four samples (*S. frontalis*, *S. guianensis* 353 and 537, *D. delphis*, and *S. bredanensis*), we could only identify the specimens until the genus *Synthesium*. In these samples and comparing to other *Synthesium* species, some morphological differences could be noticed in total length of body, shape and position of oral sucker, distance between sucker, distribution of vitellaria, shape of testes and distance between posterior testes and posterior extremity of body. Considering these differences, we defined 3 morphotypes: *Synthesium* morphotype 1 found in *S. guianensis* and *S. frontalis*; *Synthesium* morphotype 2, found in *D. delphis*; and *Synthesium* morphotype 3, found in *S. bredanensis* (Fig. 1). We also found co-infections with *S. tursionis* and *Synthesium* morphotype 1 in *S. guianensis*.

Synthesium morphotype 1: body elongated, slender, dorsoventrally flattened with maximum width behind posterior testes. The oral sucker is subterminal, muscular and slightly oval. The ventral sucker is muscular and located in the posterior region of the first third of body. The intestine is H-shaped with the anterior caeca reaching the medial level of oral sucker and posterior caeca ending blindly close to posterior extremity of

body. The cirrus pouch is long and extends well beyond the ventral sucker. A large seminal vesicle is located at cirrus pouch's extremity. Spines in cirrus are not observed. The ovary is round to oval. The testes are lobed, with the anterior testes presenting five lobes (in most specimens) and the posterior testes with six lobes (in most specimens). The initial position of vitellaria is at the pars prostatica region.

Synthesium morphotype 2: body length relatively short (when comparing to most *Synthesium* described species), elongated, slender, dorsoventrally flattened with maximum width behind posterior testes. The oral sucker is subterminal, muscular and slightly oval. The cirrus pouch is long and extends well beyond the ventral sucker. A large seminal vesicle is located at cirrus pouch's extremity. The cirrus is armed with spines. The ovary is round to oval. Distance between ventral sucker and ovary is very short. The testes are oval with irregular margin. The initial position of vitellaria is at the medial region of seminal vesicle.

Synthesium morphotype 3: body length relatively long (when comparing to most *Synthesium* described species), slender, dorsoventrally flattened with maximum width behind posterior testes. The oral sucker is subterminal, muscular and slightly oval. The ventral sucker is muscular and located in the posterior region of the first third of body. The cirrus pouch is long and extends well beyond the ventral sucker with a large seminal vesicle located at its extremity. Spine in cirrus not observed. The ovary is round to oval. The testes are lobed, with the anterior testes presenting five lobes (in most specimens) and the posterior testes with six lobes (in most specimens). The initial position of vitellaria is at the posterior end of seminal vesicle.

None of the morphotype were considered to be any of the nine *Synthesium* species already described. Table 3 provides the morphometric differences found in each morphotype considered and Table 4 provides the main morphological aspects that distinguish all *Synthesium* species and the resulted morphotypes.

Molecular and Phylogenetic Analyses

We obtained 64 sequences (18 ITS1 sequences; 24 mtND3 sequences; 22 mtCOI sequences) from 33 different Brachycladiidae specimens, of which 28 were *Synthesium* spp. specimens.

The ITS1 alignment (551 bp) included 20 sequences (18 newly generated and 2 retrieved from GenBank). The mtND3 alignment (252 bp) consisted of 46 Brachycladiidae sequences (24 newly generated and 22 retrieved from GenBank). The

mtCOI alignment (413 bp) comprised 32 sequences from Brachycladiidae (22 newly generated and 10 retrieved from GenBank). Table 5 provides a listing of all sequences used to construct the datasets.

The genetic distance analyses revealed that the *Synthesium* species and morphotypes present intraspecific divergence of less than 2% for mtCOI and mtND3, except between the Brazilian and Spanish *S. tursionis* specimens (9.0%), whereas the interspecific divergence showed higher genetic variation (minimum of 6.4% and maximum of 20.4% for mtCOI; minimum of 3.3% and maximum of 27.7% for mtND3), especially for the comparisons using *S. delamurei*, *S. seymouri*, and *S. subtile* (See Supplementary Table 1 and 2 for values). For ITS1 alignment, no intraspecific genetic distance between *Synthesium* specimens was observed, except between one *S. tursionis* specimen from Spain (StE3) compared to the all other *S. tursionis* (0.2%), and the interspecific distance ranged from 0.2% to 10.5% (See Supplementary Table 3 for values).

The molecular phylogenies (BI and ML) based on ITS1 and mtND3 datasets (Fig. 2 and 3) yielded similar topologies with most bootstrap values strongly supported. All *Synthesium* species, except for *S. delamurei* and *S. seymouri* were placed in a monophyletic group divided in two well defined clusters; one comprising all *S. pontoporiae*, *S. neotropicalis*, and *Synthesium* morphotype 2 (Clade A); and another with all *S. tursionis* from the Brazilian coast, *S. tursionis* from the Mediterranean Sea, *Synthesium* morphotype 1, and *Synthesium* morphotype 3 (Clade B). *Synthesium seymouri* and *S. delamurei* clustered apart from each other within the other Brachycladiidae species utilized in this study. As for ITS1, the topology obtained grouped *Oschmarinella rochebruni* with the *Synthesium* species, *Brachycladium atlanticum* and *Brachycladium goliath* did not form a monophyletic clade, with *Campula* as sister taxon of *B. atlanticum* and *S. delamurei* is unresolved.

The obtained results in the GMYC analysis for mtCOI (Fig. 4) suggested the recognition of 10 putative species, 2 of which contained a single individual (*B. goliath* and *N. attenuatum*), and the confidence limits for the estimated number of species ranged from 10 to 11. The GMYC analysis identified *S. pontoporiae*, *S. neotropicalis*, *S. tursionis* from the Brazilian coast, *S. tursionis* from the Spanish coast, *S. delamurei*, and *S. seymouri* + *S. subtile* as single entities. The analysis also identified two putative species that corresponded to *Synthesium* morphotypes 1 and 3.

DISCUSSION

Most diagnostic characters of the three morphotype support the hypothesis that they belong to the genus *Synthesium* (e.g. shape of body, well developed pyriform pharynx, intestines with anterior and posterior caeca without diverticula, with posterior caeca ending blindly close to posterior extremity of body, ventral sucker in anterior third of the body, presence of cirrus sac, unarmed metraterm, testes in middle third or third quarter of body and vitellaria entirely in hindbody). Indeed, these results are also confirmed by the molecular analyses, since they were grouped into the *Synthesium* clade.

Although an armed cirrus is also a diagnostic character of the genus *Synthesium* (see Gibson, 2005), we could only identify spines in the cirrus of *Synthesium* morphotype 2. Nevertheless, three other species assigned to the genus *Synthesium*, as follow: *S. neotropicalis*, *S. delamurei*, and *S. mironovi*, do not possess such structures. Considering that, we encourage a morphological reassessment of these species and *Synthesium* morphotypes 1 and 3 to confirm the lack of spines in cirrus. Also, a revision of this structure as a diagnostic character of the genus should be considered.

Synthesium morphotype 1 and 3 are very similar in several morphological aspects. The difference between them is that *Synthesium* morphotype 3 is much longer, present greater distance between ventral sucker and ovary and between posterior testes and the end of body, and the anterior region of vitellaria is located at the posterior margin of the seminal vesicle, while in *Synthesium* morphotype 1 it reaches the level of prostate glands. However, the genetic distances between these two morphotypes are significantly high (8.2% in mtCOI; 14.3% in mtND3).

The specimens assigned to *Synthesium* morphotype 1 are very similar morphologically to *S. elongatum* regarding the relative shape and body size, position and shape of oral sucker and shape of testes. They differ in the distribution of vitellaria, but the description of *S. elongatum* is quite unclear as it indicates that “the vitellaria extend from slightly caudal to acetabulum to extreme posterior end of body” (Ozaki, 1935). Since genetic material from *S. elongatum* is unavailable, a molecular comparison would not be possible. *Synthesium elongatum* was registered in the intestines of Indo-Pacific Finless Porpoises *Neophocoena phocoenoides* (Cuvier, 1829) in Japanese waters, North Western Pacific Ocean. *Neophocoena phocoenoides* are coastal water dolphins and restricted to the Northern Indian and Western Pacific Oceans (Wang and Reves, 2012). *Synthesium* morphotype 1 specimens were recovered from *S. guianensis* and *S. frontalis*. *Sotalia guianensis* is a coastal and estuarine water dolphin, endemic to the south coast of

Brazil until Honduras (Secchi, 2012). *Stenella frontalis* is endemic to the tropical and warm-temperate waters of the Atlantic Ocean (Perrin, 2002) and present discontinuous distribution in Southwestern Atlantic, suggesting the existence of an isolated population found on the southeastern and southern Brazilian coast (Moreno et al., 2005). In light of that, we think that it is unlikely that the same parasite is infecting different endemic dolphins from very distant geographical regions, as is the case. It would have to involve many different host switching events, from intermediate to definitive host, until the establishment of the same parasite in such different and isolated environments.

Surprisingly, one *Synthesium* specimen recovered from the *T. truncatus* from the Mediterranean coast was morphologically recognized as *Synthesium* morphotype 1. Indeed, the obtained sequence from this exemplar clustered with the other *Synthesium* morphotype 1 from *S. guianensis* and *S. frontalis* from Brazil. Also, a sequence identified as *S. tursionis* recovered from GenBank (KT180218) used for the mtND3 phylogeny, clustered with all other *Synthesium* morphotype 1 and apart from *S. tursionis* from Spain (see Fig. 3). We have considered the hypothesis that *Synthesium* morphotype 1 could be a morphological variation of *S. tursionis*, given the fact that they were often found co-infecting their hosts. However, the phylogenetic position of *Synthesium* morphotype 1, the significant genetic distance with *S. tursionis* (10.2% in mtND3; 9.7% in mtCOI), and the morphological differences are enough evidences that support both as different species.

Synthesium morphotype 2 resembles very closely *S. pontoporiae*. Morphologically, *S. pontoporiae* is much shorter and the oral sucker is smaller than in morphotype 2. Despite they present the lower interspecific genetic distance of all analyzed species (5.9% in mtND3), we also considered the result to be significant.

Two recognized species coexisting along the Brazilian coast, *S. pontoporiae* and *S. tursionis*, presented interspecific genetic differences of 14.6% in mtCOI and 17.8% in mtND3. The recently described *S. neotropicalis* diverge from *S. tursionis* and *S. pontoporiae* in 15.2% and 9.5% in mtCOI, respectively. The results of genetic distance found between these later three *Synthesium* species and the three morphotype are very similar. Previous studies have suggested that trematode species show maximum intraspecific divergences to be considered one single species at approximately 2% for mtDNA (Vilas et al., 2005). This is lower than the observed values for the *Synthesium* morphotypes and *Synthesium* species obtained.

For the ITS region, the interspecific distance ranged from 0.2% to 10.5% among the *Synthesium* species. Previous studies have shown that the intraspecific divergence for

species of digeneans is below 1% (Vilas et al., 2005; Nolan and Cribb, 2005). Our results do not corroborate this statement. The Bayesian topology presented was not resolved. It is true that there are some missing *taxa* to complete the phylogeny and the inclusion of more sequences from other Brachycladiidae species might alter the positions of some *taxa*. Nonetheless, we think that ITS1 is too conserved for the Brachycladiidae family and does not provide too much information at species level. Therefore, species that are more closely related might group in the same clade, as *O. rochebruni* and the *Synthesium* species and *Campula* and *Brachycladium*.

The distance values found between the Brazilian and Spanish specimens of *S. tursionis* are above the limit of intraspecific variation (comparing to values between specimens from Brazilian *S. tursionis* and *S. pontoporiae*). One could consider the possibility of Brazilian and Spanish specimens belong to different species, although they are identified as the same species morphologically. The possibility of *S. tursionis* contains cryptic species should be investigated.

The GMYC analysis corroborates the separation of two of the morphotypes (*Synthesium* morphotype 1 and 3). The analysis also split the Brazilian and Spanish *S. tursionis* as different entities, as well as the BI and ML analyses. Also, the complex *S. seymouri* + *S. subtile* should be better investigated. The *S. seymouri* and *S. subtile* specimens used in this study were morphologically identified and distinguished in accordance with Marigo et al. (2008) and Balbuena et al. (1989). However, their positioning suggests that they might not be members of *Synthesium*.

The division of the *Synthesium* clade in two groups (Clade A and Clade B) caught our attention. In Clade A, all species and morphotype share a small and robust body size, when comparing to the other *Synthesium* species, and present round to irregular shaped testes. In clade B, the species and morphotype share the lobed testes and long and slender bodies as an indicative for their close relationship.

In this instance, the current observations on morphological differences together with the genetic variation data presented here, provide strong evidence for 3 distinct putative species of *Synthesium* parasitizing dolphins from the Brazilian coast other than *S. tursionis*, *S. pontoporiae*, and *S. neotropicalis*.

Interestingly, the phylogenetic position of *S. delamurei* and *S. seymouri* seemed odd, since we expected them to be placed within the *Synthesium* clade. The specimens used were morphologically identified as *S. delamurei* and *S. seymouri* according to Raga

and Balbuena (1988) and Price (1932). However, given the obtained results, we raised different hypothesis:

I. *Synthesium* is a monophyletic group and the specimens identified as *S. delamurei* and *S. seymouri* are incorrectly assigned to the genus, considering that both species have received many taxonomic rearrangements. *Synthesium seymouri* was first described as *Odhneriella* and a comparison with other *Odhneriella* species to confirm their phylogenetic affinity would be interesting. Regarding *S. delamurei*, if indeed the specimens are not *Synthesium*, a revision on this species should be made to assign it to the correct genus. *Synthesium delamurei* appears to be more closely related with *Nasitrema* while *S. seymouri* appears to be more closely related with *Brachycladium*. Certainly, more sequences of *Synthesium* species would be required in order to elucidate such question.

II. The genus *Synthesium* is polyphyletic, despite most of the species morphologically assigned to *Synthesium* cluster together in the phylogeny. In this case, *S. delamurei* and *S. seymouri* would be more distant from other *Synthesium* from Brazil considering their geographical distance. However, if that is true, the *S. tursionis* from Spain should cluster together with them or at least, not in the same clade as the Brazilian *Synthesium* specimens, since they are more geographically close related.

III. The genus *Synthesium* is a polyphyletic group and the phylogenetic position of *S. delamurei* and *S. seymouri* is correct, but the Brazilian *Synthesium* are monophyletic group.

In all cases, the phylogenetic position of *S. delamurei* and *S. seymouri* raised some concerns about the current taxonomy of *Synthesium*.

In conclusion, we present in this study three new putative *Synthesium* species identified as *Synthesium* morphotype 1, collected from *S. guianensis* and *S. frontalis* from Brazil, *Synthesium* morphotype 2, collected from *D. deplhis* from Brazil and *Synthesium* morphotype 3, collected from a *S. bredanensis* from Brazil. We also report the presence of *Synthesium* morphotype 1 in a *T. truncatus* collected from the Mediterranean coast of Spain. Considering the molecular analyses implied here, *S. tursionis* should contain cryptic species. *Synthesium delamurei* and *S. seymouri* should be considered as a different genus, with *S. delamurei* as a different genus from *S. seymouri*. The species complex *S. seymouri* + *S. subtile* should be better investigated, since the similarity in mtCOI indicated them as a single entity. In light of the obtained results, we strongly encourage the taxonomic revision of all *Synthesium* to confirm their generic assignment. Finally, since

the current taxonomy of *Synthesium* is problematic, we propose the revision of the genus, and potentially of the Brachycladiidae family.

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Table 1 Specimens analyzed, identification voucher, their hosts, and collection localities.

Specimens	id Voucher	Host (Common name)	Collection Locality
<i>Syntesium</i> sp.	353	<i>Stenella frontalis</i> (Atlantic Spotted dolphin)	São Francisco do Sul, Santa Catarina, Brazil
<i>Syntesium</i> sp.	375a	<i>Sotalia guianensis</i> (Guiana dolphin)	São Francisco do Sul, Santa Catarina, Brazil
<i>Syntesium</i> sp.	375b	<i>Sotalia guianensis</i> (Guiana dolphin)	São Francisco do Sul, Santa Catarina, Brazil
<i>Syntesium</i> sp.	261	<i>Delphinus delphis</i> (Common dolphin)	Pontal do Sul, Paraná, Brazil
<i>Syntesium</i> sp.	25a	<i>Steno bredanensis</i> (Rough Toothed dolphin)	Praia Grande, São Paulo, Brazil
<i>Syntesium</i> sp.	25b	<i>Steno bredanensis</i> (Rough Toothed dolphin)	Praia Grande, São Paulo, Brazil
<i>Syntesium neotropialis</i>	392a	<i>Tursiops truncatus</i> (Bottlenose dolphin)	Rio de Janeiro, Brazil
<i>Syntesium neotropialis</i>	392b	<i>Tursiops truncatus</i> (Bottlenose dolphin)	Rio de Janeiro, Brazil
<i>Syntesium neotropialis</i>	1064	<i>Tursiops truncatus</i> (Bottlenose dolphin)	São Francisco do Sul, Santa Catarina, Brazil
<i>Syntesium neotropialis</i>	559	<i>Tursiops truncatus</i> (Bottlenose dolphin)	São Francisco do Sul, Santa Catarina, Brazil
<i>Syntesium neotropialis</i>	361	<i>Tursiops truncatus</i> (Bottlenose dolphin)	São Francisco do Sul, Santa Catarina, Brazil
<i>Synthesium tursionis</i>	StE1	<i>Tursiops truncatus</i> (Bottlenose dolphin)	Mediterranean Coast, Spain
<i>Synthesium tursionis</i>	StE2	<i>Tursiops truncatus</i> (Bottlenose dolphin)	Mediterranean Coast, Spain
<i>Synthesium tursionis</i>	StE3	<i>Tursiops truncatus</i> (Bottlenose dolphin)	Mediterranean Coast, Spain
<i>Synthesium tursionis</i>	234	<i>Sotalia guianensis</i> (Guiana dolphin)	Pontal do Sul, Paraná, Brazil
<i>Synthesium tursionis</i>	190	<i>Sotalia guianensis</i> (Guiana dolphin)	Pontal do Sul, Paraná, Brazil
<i>Synthesium tursionis</i>	243	<i>Sotalia guianensis</i> (Guiana dolphin)	Ilha das Peças, Paraná, Brazil
<i>Synthesium tursionis</i>	73	<i>Sotalia guianensis</i> (Guiana dolphin)	Praia Grande, São Paulo, Brazil
<i>Synthesium tursionis</i>	317	<i>Sotalia guianensis</i> (Guiana dolphin)	Rio de Janeiro, Brazil
<i>Synthesium tursionis</i>	78	<i>Sotalia guianensis</i> (Guiana dolphin)	Ilha Comprida, São Paulo, Brazil
<i>Synthesium tursionis</i>	414	<i>Sotalia guianensis</i> (Guiana dolphin)	Porto, Itapoá, Santa Catarina, Brazil
<i>Synthesium delamurei</i>	Sd1	<i>Globicephala melas</i> (Long-finned pilot whale)	Mediterranean Coast, Spain
<i>Synthesium delamurei</i>	Sd2	<i>Globicephala melas</i> (Long-finned pilot whale)	Mediterranean Coast, Spain
<i>Synthesium delamurei</i>	Sd3	<i>Globicephala melas</i> (Long-finned pilot whale)	Mediterranean Coast, Spain
<i>Synthesium seymouri</i>	Ss1	<i>Globicephala melas</i> (Beluga whale)	St. Lawrence Estuary, Quebec, Canada
<i>Synthesium seymouri</i>	Ss2	<i>Delphinapterus leucas</i> (Beluga whale)	St. Lawrence Estuary, Quebec, Canada
<i>Synthesium seymouri</i>	Ss3	<i>Delphinapterus leucas</i> (Beluga whale)	St. Lawrence Estuary, Quebec, Canada
<i>Synthesium subtile</i>	Ssub1	<i>Delphinapterus leucas</i> (Long-finned pilot whale)	Faroe Islands, Denmark
<i>Synthesium subtile</i>	Ssub2	<i>Globicephala melas</i> (Long-finned pilot whale)	Faroe Islands, Denmark
<i>Campula oblonga</i>	Co1	<i>Phocoena phocoena</i> (Harbour porpoise)	North Sea, United Kingdom
<i>Oschmarinella rochebruni</i>	Or1	<i>Stenella coeruleoalba</i> (Striped dolphin)	Mediterranean Coast, Spain

Table 1 Continued

Specimens	id Voucher	Host (Common name)	Collection Locality
<i>Brachycladum atlanticum</i>	Ba1	<i>Stenella coeruleoalba</i> (Striped dolphin)	Mediterranean Coast, Spain
<i>Nasitrema attenuatum</i>	Na1	<i>Sotalia guianensis</i> (Guiana dolphin)	Ilha Comprida, São Paulo, Brazil
<i>Nasitrema attenuatum</i>	Na2	<i>Sotalia guianensis</i> (Guiana dolphin)	Ilha Comprida, São Paulo, Brazil
<i>Nasitrema delphini</i>	Nd1	<i>Delphinus delphis</i> (Common dolphin)	Canary Islands, Spain
<i>Nasitrema</i> sp.	Nsp1	<i>Feresa attenuata</i> (Pygmy killer whale)	Mediterranean Coast, Spain

Table 2 Primers, cycling conditions used during PCR and respective bibliography sources

Primer	Sequences 5'-3'	Cycling conditions	Source
ITS1	ITS1F GACGACCAAACTTGATCATT ITS1R TGCGCTCTTCATCGACACACGA	Initial denaturation at 94°C for 3 min; 30 cycles of 94°C for 1 min, 48°C for 1 min and 72°C for 1 min and 30 s; Final extension at 72°C for 7 min	Marigo et al., 2015
COI	COIPRA TGGTTTTTTGTGCATCCTGAGGTTTA COIPRB AGAAGAACGTAATGAAAATGAGCAAC	Initial denaturation at 94°C for 5 min; 35 cycles of 94°C for 30 s, 47°C for 30 s and 72°C for 1 min; Final extension at 72°C for 7 min	Bessho et al., 1992
ND3	ND3F GCTTAATTKKTAAAGCYTTGRATTCTTAC T ND3D CTACTAGTCCCACTCAACRTAACCYT	Initial denaturation at 95°C for 5 min; 35 cycles of 95°C for 30s, 47°C for 30s and 72°C for 50s; Final extension at 72°C for 7 min	Fernández et al., 1998a; Fernández et al., 2000

Table 3 Morphometrical data of *Synthesium* morphotypes 1, 2 and 3 (in mm unless otherwise stated)

Morphological Characters	<i>Synthesium</i> morphotype 1		<i>Synthesium</i> morphotype 2		<i>Synthesium</i> morphotype 3	
	Mean (n=46) $\pm$ SD	Range (Min - Max)	Mean (n=5) $\pm$ SD	Range (Min - Max)	Mean (n=3) $\pm$ SD	Range (Min - Max)
Body length	19.36 $\pm$ 4.17	26.46 - 11.46	12.49 $\pm$ 2.98	8.83 - 16.90	30.21 $\pm$ 1.25	31.11 - 28.78
Maximum body width	0.69 $\pm$ 0.13	1.06 - 0.38	0.85 $\pm$ 0.09	0.74 - 1.01	0.87 $\pm$ 0.01	0.89 - 0.87
Oral sucker length	0.43 $\pm$ 0.08	0.65 - 0.25	0.46 $\pm$ 0.06	0.40 - 0.54	0.75 $\pm$ 0.04	0.79 - 0.69
Oral sucker width	0.35 $\pm$ 0.07	0.51 - 0.16	0.40 $\pm$ 0.07	0.33 - 0.50	0.66 $\pm$ 0.02	0.68 - 0.64
Ventral sucker length	0.63 $\pm$ 0.07	0.82 - 0.51	0.41 $\pm$ 0.04	0.35 - 0.45	0.97 $\pm$ 0.10	1.04 - 0.90
Ventral sucker width	0.41 $\pm$ 0.09	0.58 - 0.21	0.37 $\pm$ 0.07	0.30 - 0.45	0.80 $\pm$ 0.06	0.85 - 0.76
Prepharynx length	0.85 $\pm$ 0.37	1.93 - 0.39	0.19 $\pm$ 0.07	0.15 - 0.23	0.29 $\pm$ 0.01	0.30 - 0.28
Pharynx length	0.55 $\pm$ 0.18	0.99 - 0.32	0.43 $\pm$ 0.07	0.34 - 0.50	0.64 $\pm$ 0.02	0.67 - 0.62
Pharynx width	0.16 $\pm$ 0.04	0.30 - 0.07	0.23 $\pm$ 0.05	0.17 - 0.31	0.38 $\pm$ 0.02	0.40 - 0.36
Cirrus pouch length	3.31 $\pm$ 0.63	4.49 - 1.57	2.00 $\pm$ 0.36	1.50 - 2.65	4.30 $\pm$ 0.47	4.63 - 3.95
Seminal vesicle length	0.88 $\pm$ 0.19	1.33 - 1.56	0.56 $\pm$ 0.17	0.40 - 0.78	1.31 $\pm$ 0.00	1.37 - 1.22
Seminal vesicle width	0.26 $\pm$ 0.07	0.46 - 0.11	0.24 $\pm$ 0.03	0.19 - 0.28	0.42 $\pm$ 0.04	0.35 - 0.37
Ovary length	0.31 $\pm$ 0.05	0.50 - 0.13	0.30 $\pm$ 0.97	0.17 - 0.43	0.38 $\pm$ 0.12	0.47 - 0.30
Ovary width	0.21 $\pm$ 0.22	0.35 - 0.08	0.25 $\pm$ 0.08	0.14 - 0.34	0.26 $\pm$ 0.02	0.28 - 0.24
Anterior testes length	1.02 $\pm$ 0.13	1.76 - 0.58	0.48 $\pm$ 0.09	0.39 - 0.61	1.37 $\pm$ 0.15	1.54 - 1.22
Anterior testes width	0.46 $\pm$ 0.25	0.75 - 0.20	0.33 $\pm$ 0.09	0.22 - 0.48	0.58 $\pm$ 0.10	0.68 - 0.47
Posterior testes length	1.05 $\pm$ 0.11	1.69 - 0.53	0.56 $\pm$ 0.11	0.40 - 0.66	1.56 $\pm$ 0.14	1.73 - 1.47
Posterior testes width	0.44 $\pm$ 0.11	0.71 - 0.25	0.30 $\pm$ 0.06	0.19 - 0.40	0.68 $\pm$ 0.04	0.74 - 0.64
Intertesticular distance	1.38 $\pm$ 1.39	2.90 - 0.53	0.69 $\pm$ 0.15	0.32 - 1.40	1.70 $\pm$ 0.21	1.86 - 1.55
Distance posterior testes and extremity of body	5.08 $\pm$ 1.32	8.50 - 2.90	3.51 $\pm$ 0.23	2.74 - 4.23	9.43 $\pm$ 2.62	11.29 - 7.57
Distance oral sucker and ventral sucker	5.09 $\pm$ 1.69	8.64 - 2.01	2.74 $\pm$ 0.19	2.38 - 3.48	5.18 $\pm$ 1.64	6.33 - 4.02
Distance ventral sucker and ovary	4.95 $\pm$ 0.82	8.62 - 2.42	2.61 $\pm$ 0.71	1.51 - 4.29	7.59 $\pm$ 0.75	7.94 - 7.23
Distance ventral sucker and anterior margin of vitellaria	2.10 $\pm$ 0.80	4.49 - 0.96	1.24 $\pm$ 0.13	0.70 - 1.70	2.07 $\pm$ 1.64	2.60 - 1.57
Distance anterior margin of vitellaria and extremity of body	11.76 $\pm$ 2.51	16.76 - 6.16	7.03 $\pm$ 0.84	4.82 - 10.82	19.78 $\pm$ 1.25	20.95 - 18.62
Eggs length (μ m)	55	61 - 47	52	61 - 46	58	66 - 49
Eggs width (μ m)	31	35 - 28	31	39 - 25	34	38 - 29

Table 4 Morphological characters of all *Synthesium* species described. Values are given as length × width in millimeters unless otherwise stated

Species	No. of Specimens	Hosts found	Geographical Distribution	References	Body Length	Oral Sucker Position	Oral Sucker Shape	Pharynx Shape	Cirrus Pouch Length	Testes Shape	Anterior Extent of Vitellaria	Egg Size (μm)
<i>Synthesium</i> morphotype 1	(n=46)	<i>S. guianensis</i> ; <i>S. frontalis</i>	SWA	1*	19.4 (26.5 - 11.5)	Subterminal	Round	Pyriiform	3,31 (4.5 - 1.6)	Lobed	Pars Prostatica	55 x 31 (61-47 x 35-28)
<i>Synthesium</i> morphotype 2	(n=5)	<i>D. delphis</i>	SWA	1*	12.5 (8.8 - 16.9)	Subterminal	Round	Pyriiform	2.00 (1.5 - 2.6)	Oval	Seminal Vesicle	52 x 31 (61-46 x 39-25)
<i>Synthesium</i> morphotype 3	(n=3)	<i>S. bredanensis</i>	SWA	1*	30.2 (31.1 - 28.8)	Subterminal	Round	Pyriiform	4.3 (4.6 - 3.9)	Lobed	Seminal Vesicle	58 x 34 (66-49 x 38-29)
<i>S. neotropialis</i>	(n=35)	<i>T. truncatus</i>	SWA	2*	22.9 (11.4 - 29.6)	Subterminal	Round	Pyriiform	2.6 (1.8 x 3.3)	Oval	Ovary	55 x 34 (47-68 x 28-41)
<i>S. delamurei</i>	(n=4)	<i>G. melas</i>	NEA	3*	12.1 (9.7 - 16.8)	Subterminal	Round	Pyriiform	2.6 (2.0 - 3.4)	Oval	Seminal Vesicle	67 x 40 (57-72 x 32-50)
<i>S. pontoporiae</i>	(n=20)	<i>P. blainvillei</i>	SWA	4*	5.0 (3.6 - 7.1)	Subterminal	Round	Pyriiform	0.9 (0.7 - 1.1)	Oval	Seminal Vesicle	53 x 26 (46-60 x 23-28)
<i>S. elongatum</i>	(n=2)	<i>N. phocoenoides</i>	NWP	5*	13.0 - 18.0	Subterminal	Round	Pyriiform	0.9 - 1.5	Lobed	Uterine Field	51 x 28 (47-55 x 25-31)
<i>S. seymouri</i>	(n=10)	<i>D. leucas</i>	NEP, NWA	6*	34.1 (27.2 - 38.1)	Terminal	Cup-shape	Oval	3.2 (1.3-4.1)	Oval	Anterior Testes	91 x 55 (83-97 x 51-55)
<i>S. nipponicum</i>	(n=10)	<i>P. dalli</i> ; <i>P. phocoena</i>	NEP, NWP	7*, 8	14.0 (13-15.9)	Terminal	Cup-shape	Oval	1.2 (1.0-1.4)	Oval	Anterior Testes	70-82 x 35-45
<i>S. mironovi</i>	(n=2)	<i>D. leucas</i>	NEP	9*	8.9 - 12.9	Terminal	Cup-shape	-	-	Oval	Anterior Testes	72-90 x 33-37
<i>S. subtile</i>	(n=20)	<i>O. orca</i> ; <i>D. leucas</i> ; <i>G. melas</i>	NEA	10*	33.3 (14.0 - 38.3)	Terminal	Cup-shape	Oval	4.4 (2.4 - 6.1)	Oval	Seminal Vesicle	89 x 49 (75-98 x 44-55)
<i>S. tursionis</i>	(n=15)	<i>T. truncatus</i> ; <i>G. griseus</i> ; <i>S. bredanensis</i> ; <i>S. guianensis</i> ; <i>D. delphis</i> ; <i>C. eutropia</i> ; <i>P. spinnipinis</i> ; <i>L. obscurus</i> ; <i>C. commersonni</i> ; <i>L. cruciger</i>	NWP, NEP, SWP, NEA, SWA	6*, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20	14.3 (8.8 - 21.3)	Terminal	Cup-shape	Pyriiform	2.2 (1.4-2.9)	Lobed	Seminal Vesicle	53 x 29 (51-55 x 28-32)

NWP = North Western Pacif; NEP: Norte Eastern Pacif; SEP = South Easterns Pacif; NWA = North Eastern Atlantic; SWA = South Western Atlantic; *Original description; 1 = This Study; 2 = Ebert et al., accepted for publication; 3 = Raga and Balbuena 1988; 4 = Raga et al. 1994; 5 = Ozaki 1935; 6 = Price 1932; 7 = Yamaguti 1951; 8 = Ching & Robinson 1959; 9 = Delyamure 1955; 10 = Balbuena et al. 1989; 11 = Raga et al., 1985; 12 = Zam et al., 1971; 13 = Forrester & Robertson 1973; 14 = Tantalean & Escalante, 1987; 15 = Torres et al, 1992; 16 = Fernandez et al. 1994; 17 = Dans et al., 1999; 18 = Berón-Vera et al., 2001; 19 = Fernández et al., 2003; 20 = Marigo et al., 2010

Table 5 Parasite species, hosts, Genbank accession numbers and bibliographic sources of parasites sequences used for phylogenetic analyses

Trematode taxa	Host species	Collection Location	GenBank Accession No.		
			ITS1	ND3	COI
<i>Blachycladium goliath</i>	<i>Balaenoptera acutorostrata</i> (Minke whale)	North Sea, United Kingdom	KR703279	KR703278	KR703278
<i>Brachycladium atlanticum</i>	<i>Stenella coeruleoalba</i> (Striped dolphin)	Mediterranean Sea, Spain	*	KT180217	-
<i>Brachycladium</i> sp.	<i>Stenella coeruleoalba</i> (Striped dolphin)	Mediterranean Sea, Spain	-	AF123439	-
<i>Campula oblonga</i>	<i>Phocoena phocoena</i> (Harbour porpoise)	North Sea, United Kingdom	*	AF34554; KT180214	-
<i>Nasitrema attenuatum</i>	<i>Sotalia guianensis</i> (Guiana dolphin)	Off Brazilian Coast, SWAO	*	*, *	*
<i>Nasitrema delphini</i>	<i>Delphinus delphis</i> (Common dolphin)	Off Canary Islands, Spain	*	KT180216	-
<i>Nasitrema globicephalae</i>	<i>Globicephala melas</i> (Long-finned pilot whale)	Off Chilean Coast, SPO	-	AF034557	-
<i>Nasitrema</i> sp.	<i>Feresa attenuata</i> (Pygmy killer whale)	Off Canary Islands, Spain	*	-	-
<i>Orthosplanchnus fraterculus</i>	<i>Enhydra lutris</i> (Sea Otter)	Mediterranean Sea, Spain	-	AF034555	-
<i>Oschmarinella rochebruni</i>	<i>Stenella coeruleoalba</i> (Striped dolphin)	Mediterranean Sea, Spain	*	AF034556; KT180215	-
<i>Synthesium delamurei</i>	<i>Globicephala melas</i> (Long-finned pilot whale)	Mediterranean Sea, Spain	*, *	*, *, *	*, *
<i>Synthesium</i> morphotype 1	<i>Sotalia guianensis</i> (Guiana dolphin)	Off Brazilian Coast, SWAO	*	*, *	*, *, *
<i>Synthesium</i> morphotype 2	<i>Steno bredanensis</i> (Rough tooth dolphin)	Off Brazilian Coast, SWAO	*	*	*
<i>Synthesium</i> morphotype 3	<i>Delphinus delphis</i> (Common dolphin)	Off Brazilian Coast, SWAO	*	*	-
<i>Synthesium neotropicalis</i>	<i>Tursiops truncatus</i> (Bottlenose dolphin)	Off Brazilian Coast, SWAO	*	*, *, *, *	*, *, *
<i>Synthesium pontoporiae</i>	<i>Pontoporia blainvillei</i> (Franciscana)	Off Brazilian Coast, SWAO	JX644084	JX644113; JX644103; JX644116; JX644109; JX644112; JX644115; JX644106; JX644120; JX644121; FJ829472	JX644146; JX644152; JX644142; JX644134; JX644150; JX64414; JX644124; JX644140; JX644148
<i>Synthesium seymouri</i>	<i>Delphinapterus leucas</i> (Beluga whale)	Quebec, Canada	*	*, *, *	*, *, *
<i>Synthesium subtile</i>	<i>Globicephala melas</i> (Long-finned pilot whale)	Faroe Islands, NAO	-	-	*
<i>Synthesium tursionis</i>	<i>Sotalia guianensis</i> (Guiana dolphin)	Off Brazilian Coast, SWAO	*	*, *, *, *, *, *, *	*, *, *, *, *
<i>Synthesium tursionis</i>	<i>Tursiops truncatus</i> (Bottlenose dolphin)	Mediterranean Sea, Spain	*,	AF034552; *, KT180218	*
<i>Tormopsolus orientalis</i>	<i>Seriola dumerili</i> (Greater amberjack)	Mediterranean Sea, Spain	*	KT180219	-

*Accession numbers will be included after manuscript acceptance

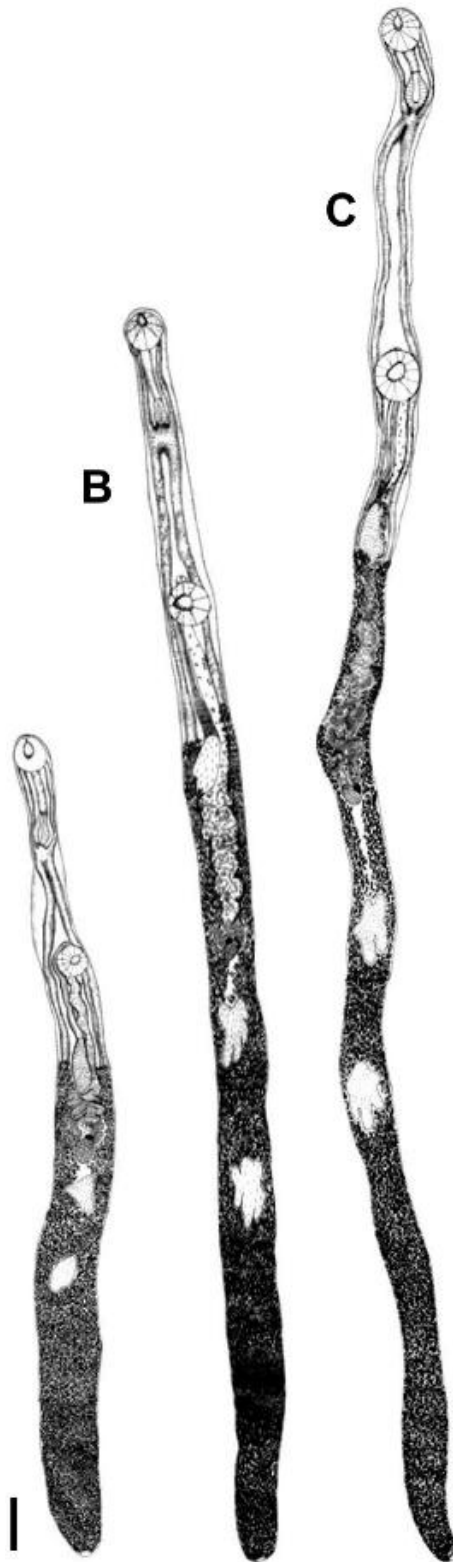


Fig 1 *Synthesium* morphotypes found in the intestines of dolphins from Brazil. (A) *Synthesium* morphotype 2 from the small intestine of *Delphinus delphis*. (B) *Synthesium* morphotype 1 from the small intestine of *Sotalia guianensis*. (C) *Synthesium* morphotype 3 from the small intestine of *Steno bredanensis*. Scale bar = 200 μ m.

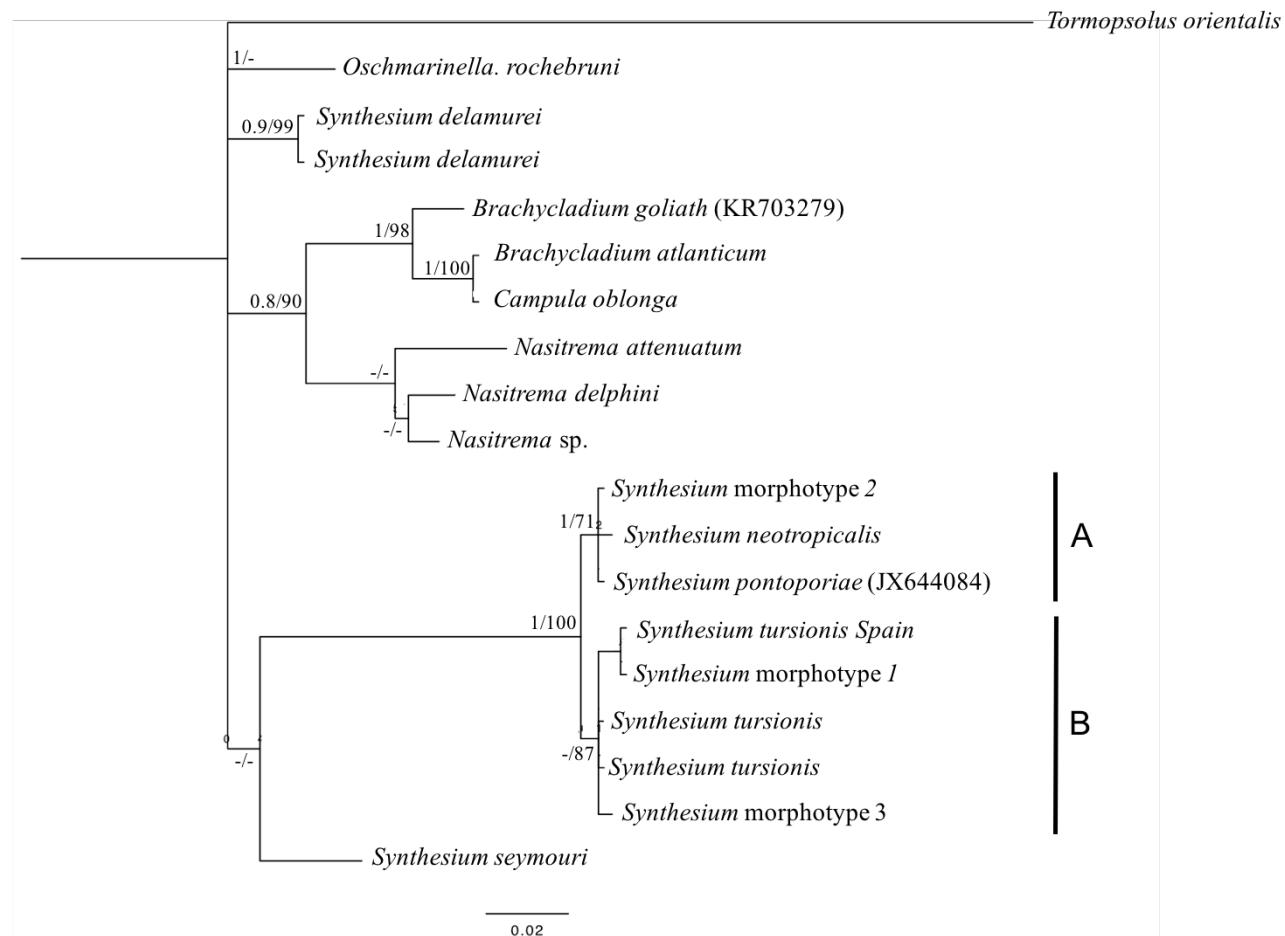


Fig 2 Bayesian Inference topology based on ITS1 rDNA sequences. Species names are followed by GenBank accession numbers in parentheses. Support values at branching points are shown as follows: Bayesian posterior probability/ Maximum likelihood bootstrap. Dashes are shown for the branches not supported by the analyses (scored < 70%). Branch length scale bars indicate mean number of substitutions per site

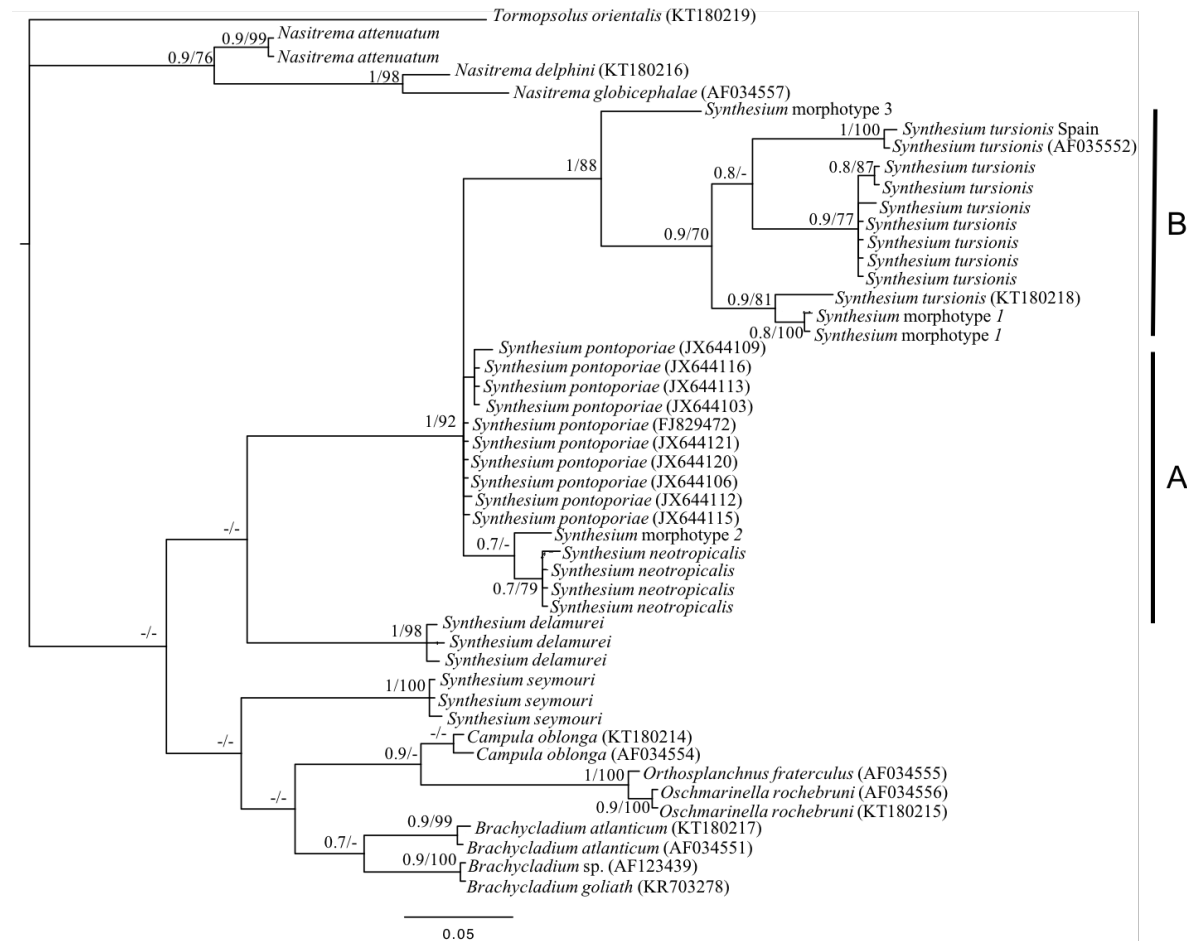


Fig 3 Bayesian Inference topology based on ND3 mtDNA dataset. Species names are followed by GenBank accession numbers in parentheses. Support values at branching points are shown as follows: Bayesian posterior probability/ Maximum likelihood bootstrap. Dashes are shown for the branches not supported by the analyses (scored < 70%). Branch length scale bars indicate mean number of substitutions per site

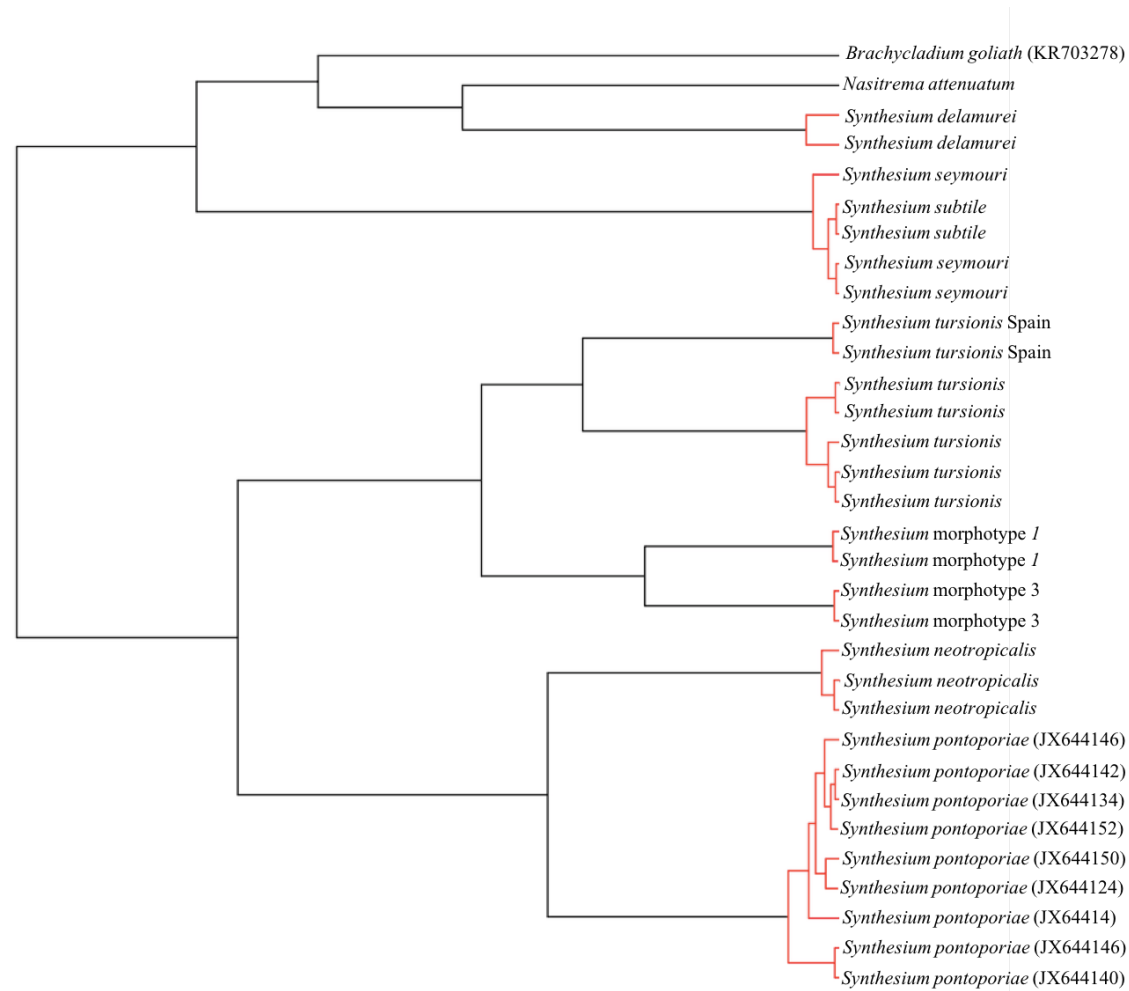


Fig 4 GMYC tree based on COI mtDNA dataset. Species names are followed by GenBank accession numbers in parentheses. Clusters in red indicate single entities identified by the analysis.

Supplementary Table 1 Pairwise genetic distances based on mtND3 alignments

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46				
1 <i>Tormopsolus orientalis</i> (KT180219)																																																		
2 <i>Synthesium</i> morphotype 3 (25a)	0.274																																																	
3 <i>Synthesium pontoporiae</i> (JX644109)	0.277	0.128																																																
4 <i>Synthesium pontoporiae</i> (FJ829472)	0.270	0.128	0.009																																															
5 <i>Synthesium pontoporiae</i> (JX644121)	0.270	0.128	0.009	0.000																																														
6 <i>Synthesium pontoporiae</i> (JX644120)	0.270	0.128	0.009	0.000	0.000																																													
7 <i>Synthesium pontoporiae</i> (JX644106)	0.270	0.128	0.009	0.000	0.000	0.000																																												
8 <i>Synthesium pontoporiae</i> (JX644116)	0.270	0.128	0.009	0.000	0.000	0.000	0.000																																											
9 <i>Synthesium pontoporiae</i> (JX644113)	0.270	0.128	0.009	0.000	0.000	0.000	0.000	0.000																																										
10 <i>Synthesium pontoporiae</i> (JX644103)	0.270	0.128	0.009	0.000	0.000	0.000	0.000	0.000	0.000																																									
11 <i>Synthesium pontoporiae</i> (JX644112)	0.277	0.128	0.017	0.009	0.009	0.009	0.009	0.009	0.009	0.009																																								
12 <i>Synthesium pontoporiae</i> (JX644115)	0.270	0.122	0.013	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004																																							
13 <i>Synthesium</i> morphotype 2 (261)	0.277	0.128	0.064	0.054	0.054	0.054	0.054	0.054	0.054	0.054	0.045	0.050																																						
14 <i>Synthesium neotropicalis</i> (392a)	0.270	0.139	0.054	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.036	0.040	0.036																																					
15 <i>Synthesium neotropicalis</i> (392b)	0.277	0.144	0.059	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.040	0.045	0.031	0.004																																				
16 <i>Synthesium neotropicalis</i> (559)	0.277	0.144	0.059	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.040	0.045	0.031	0.004	0.000																																			
17 <i>Synthesium neotropicalis</i> (1064)	0.277	0.144	0.059	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.040	0.045	0.031	0.004	0.000	0.000																																		
18 <i>Synthesium tursionis</i> (SEI)	0.293	0.178	0.187	0.176	0.176	0.176	0.176	0.176	0.176	0.176	0.164	0.170	0.159	0.137	0.142	0.142	0.142																																	
19 <i>Synthesium tursionis</i> (AF034552)	0.287	0.172	0.182	0.170	0.170	0.170	0.170	0.170	0.170	0.170	0.159	0.165	0.153	0.132	0.137	0.137	0.137	0.004																																
20 <i>Synthesium tursionis</i> (78)	0.280	0.145	0.178	0.178	0.178	0.178	0.178	0.178	0.178	0.178	0.166	0.172	0.166	0.155	0.160	0.160	0.160	0.118	0.113																															
21 <i>Synthesium tursionis</i> (234)	0.293	0.156	0.178	0.178	0.178	0.178	0.178	0.178	0.178	0.178	0.166	0.172	0.166	0.155	0.160	0.160	0.160	0.118	0.113	0.009																														
22 <i>Synthesium tursionis</i> (190)	0.280	0.145	0.178	0.178	0.178	0.178	0.178	0.178	0.178	0.178	0.166	0.172	0.166	0.155	0.160	0.160	0.160	0.118	0.113	0.000	0.009																													
23 <i>Synthesium tursionis</i> (473)	0.280	0.145	0.178	0.178	0.178	0.178	0.178	0.178	0.178	0.178	0.166	0.172	0.166	0.155	0.160	0.160	0.160	0.118	0.113	0.000	0.009	0.000																												
24 <i>Synthesium tursionis</i> (243)	0.280	0.145	0.178	0.178	0.178	0.178	0.178	0.178	0.178	0.178	0.166	0.172	0.166	0.155	0.160	0.160	0.160	0.118	0.113	0.000	0.009	0.000	0.000																											
25 <i>Synthesium tursionis</i> (414)	0.280	0.145	0.178	0.178	0.178	0.178	0.178	0.178	0.178	0.178	0.166	0.172	0.166	0.155	0.160	0.160	0.160	0.118	0.113	0.000	0.009	0.000	0.000	0.000																										
26 <i>Synthesium tursionis</i> (317)	0.280	0.145	0.178	0.178	0.178	0.178	0.178	0.178	0.178	0.178	0.166	0.172	0.166	0.155	0.160	0.160	0.160	0.118	0.113	0.000	0.009	0.000	0.000	0.000	0.000																									
27 <i>Synthesium</i> morphotype 1 (375a)	0.281	0.140	0.160	0.149	0.149	0.149	0.149	0.149	0.149	0.149	0.138	0.144	0.149	0.107	0.112	0.112	0.112	0.077	0.072	0.098	0.098	0.098	0.098	0.098	0.098	0.098																								
28 <i>Synthesium</i> morphotype 1 (375b)	0.281	0.140	0.160	0.149	0.149	0.149	0.149	0.149	0.149	0.149	0.138	0.144	0.149	0.107	0.112	0.112	0.112	0.077	0.072	0.098	0.098	0.098	0.098	0.098	0.098	0.098	0.000																							
29 <i>Nasirema attenuatum</i> (Na1)	0.260	0.293	0.226	0.214	0.214	0.214	0.214	0.214	0.214	0.214	0.220	0.214	0.191	0.191	0.185	0.185	0.185	0.218	0.212	0.261	0.261	0.261	0.261	0.261	0.261	0.261	0.230	0.230																						
30 <i>Nasirema attenuatum</i> (Na2)	0.260	0.293	0.226	0.214	0.214	0.214	0.214	0.214	0.214	0.214	0.220	0.214	0.191	0.191	0.185	0.185	0.185	0.218	0.212	0.261	0.261	0.261	0.261	0.261	0.261	0.261	0.230	0.230	0.000																					
31 <i>Nasirema delphini</i> (KT180216)	0.304	0.320	0.264	0.251	0.251	0.251	0.251	0.251	0.251	0.251	0.238	0.244	0.238	0.226	0.232	0.232	0.232	0.255	0.249	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.235	0.235	0.128	0.128																	
32 <i>Nasirema globicephalae</i> (AF034557)	0.351	0.343	0.314	0.299	0.299	0.299	0.299	0.299	0.299	0.299	0.285	0.292	0.271	0.258	0.265	0.265	0.265	0.288	0.282	0.293	0.293	0.293	0.293	0.293	0.293	0.293	0.287	0.287	0.156	0.156	0.089																			
33 <i>Synthesium seymouri</i> (Ss2)	0.303	0.263	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.218	0.224	0.211	0.181	0.187	0.187	0.187	0.218	0.213	0.220	0.220	0.220	0.220	0.220	0.220	0.220	0.208	0.208	0.199	0.199	0.222	0.217																		
34 <i>Synthesium seymouri</i> (Ss1)	0.303	0.263	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.218	0.224	0.211	0.181	0.187	0.187	0.187	0.218	0.213	0.220	0.220	0.220	0.220	0.220	0.220	0.220	0.208	0.208	0.199	0.199	0.222	0.217	0.000																	
35 <i>Synthesium seymouri</i> (Ss3)	0.311	0.270	0.230	0.230	0.230	0.230	0.230	0.230	0.230	0.230	0.224	0.230	0.218	0.187	0.193	0.193	0.193	0.224	0.219	0.226	0.214	0.226	0.226	0.226	0.226	0.226	0.226	0.226	0.226	0.226	0.214	0.214	0.205	0.205	0.229	0.223	0.004	0.004												
36 <i>Synthesium delamurei</i> (Sd1)	0.313	0.253	0.188	0.188	0.188	0.188	0.188	0.188	0.188	0.188	0.182	0.188	0.194	0.171	0.177	0.177	0.177	0.208	0.202	0.215	0.215	0.215	0.215	0.215	0.215	0.215	0.197	0.197	0.217	0.217	0.221	0.260	0.167	0.167	0.172															
37 <i>Synthesium delamurei</i> (Sd3)	0.305	0.246	0.182	0.182	0.182	0.182	0.182	0.182	0.182	0.182	0.177	0.182	0.188	0.165	0.171	0.171	0.171	0																																

Supplementary Table 2 Pairwise genetic distances based on mtCOI alignments

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1 <i>Synthesium</i> morphotype 3 (25a)																															
2 <i>Synthesium</i> morphotype 3 (25b)	0,000																														
3 <i>Synthesium</i> morphotype 1 (353)	0,067	0,067																													
4 <i>Synthesium</i> morphotype 1 (375a)	0,067	0,067	0,000																												
5 <i>Synthesium tursionis</i> Spain (StE1)	0,098	0,098	0,064	0,064																											
6 <i>Synthesium tursionis</i> Spain (StE2)	0,098	0,098	0,064	0,064	0,000																										
7 <i>Synthesium tursionis</i> (356)	0,124	0,124	0,098	0,098	0,090	0,090																									
8 <i>Synthesium tursionis</i> (386)	0,124	0,124	0,098	0,098	0,090	0,090	0,000																								
9 <i>Synthesium tursionis</i> (190)	0,115	0,115	0,089	0,089	0,090	0,090	0,008	0,008																							
10 <i>Synthesium tursionis</i> (317)	0,115	0,115	0,089	0,089	0,090	0,090	0,008	0,008	0,000																						
11 <i>Synthesium tursionis</i> (414)	0,115	0,115	0,089	0,089	0,090	0,090	0,008	0,008	0,000	0,000																					
12 <i>Synthesium pontoporiae</i> (JX644150)	0,147	0,147	0,142	0,142	0,133	0,133	0,161	0,161	0,156	0,156	0,156																				
13 <i>Synthesium pontoporiae</i> (JX64414)	0,142	0,142	0,142	0,142	0,133	0,133	0,161	0,161	0,156	0,156	0,156	0,008																			
14 <i>Synthesium pontoporiae</i> (JX644142)	0,147	0,147	0,142	0,142	0,133	0,133	0,161	0,161	0,156	0,156	0,156	0,004	0,004																		
15 <i>Synthesium pontoporiae</i> (JX644152)	0,147	0,147	0,142	0,142	0,133	0,133	0,161	0,161	0,156	0,156	0,156	0,004	0,004	0,000																	
16 <i>Synthesium pontoporiae</i> (JX644134)	0,147	0,147	0,142	0,142	0,133	0,133	0,161	0,161	0,156	0,156	0,156	0,004	0,004	0,000	0,000																
17 <i>Synthesium pontoporiae</i> (JX644146)	0,152	0,152	0,147	0,147	0,137	0,137	0,166	0,166	0,161	0,161	0,161	0,008	0,008	0,004	0,004	0,004															
18 <i>Synthesium pontoporiae</i> (JX644124)	0,142	0,142	0,137	0,137	0,137	0,137	0,156	0,156	0,151	0,151	0,151	0,008	0,008	0,004	0,004	0,004	0,008														
19 <i>Synthesium pontoporiae</i> (JX644140)	0,147	0,147	0,142	0,142	0,133	0,133	0,161	0,161	0,156	0,156	0,156	0,011	0,011	0,008	0,008	0,008	0,011	0,011													
20 <i>Synthesium pontoporiae</i> (JX644148)	0,147	0,147	0,142	0,142	0,133	0,133	0,161	0,161	0,156	0,156	0,156	0,011	0,011	0,008	0,008	0,008	0,011	0,011	0,000												
21 <i>Synthesium neotropicalis</i> (1064)	0,143	0,143	0,147	0,147	0,152	0,152	0,166	0,166	0,161	0,161	0,161	0,104	0,095	0,099	0,099	0,099	0,104	0,095	0,099	0,099											
22 <i>Synthesium neotropicalis</i> (361)	0,143	0,143	0,147	0,147	0,152	0,152	0,166	0,166	0,161	0,161	0,161	0,104	0,095	0,099	0,099	0,099	0,104	0,095	0,099	0,099	0,000										
23 <i>Synthesium neotropicalis</i> (559)	0,143	0,143	0,147	0,147	0,152	0,152	0,166	0,166	0,161	0,161	0,161	0,104	0,095	0,099	0,099	0,099	0,104	0,095	0,099	0,099	0,000	0,000									
24 <i>Nasitrema attenuatum</i> (Na1)	0,213	0,213	0,189	0,189	0,204	0,204	0,225	0,225	0,219	0,219	0,219	0,236	0,231	0,231	0,231	0,231	0,231	0,225	0,220	0,220	0,209	0,209	0,209								
25 <i>Brachycladium goliath</i> (KR703278)	0,170	0,170	0,146	0,146	0,175	0,175	0,165	0,165	0,160	0,160	0,160	0,180	0,175	0,180	0,180	0,180	0,186	0,175	0,180	0,180	0,181	0,181	0,181	0,180							
26 <i>Synthesium seymouri</i> (Ss1)	0,160	0,160	0,146	0,146	0,160	0,160	0,160	0,160	0,155	0,155	0,155	0,165	0,165	0,165	0,165	0,165	0,169	0,160	0,169	0,169	0,189	0,189	0,189	0,206	0,143						
27 <i>Synthesium subtile</i> (Ssub1)	0,165	0,165	0,151	0,151	0,165	0,165	0,160	0,160	0,155	0,155	0,155	0,169	0,169	0,169	0,169	0,169	0,174	0,165	0,174	0,174	0,194	0,194	0,194	0,200	0,148	0,004					
28 <i>Synthesium seymouri</i> (Ss3)	0,165	0,165	0,151	0,151	0,165	0,165	0,160	0,160	0,155	0,155	0,155	0,169	0,169	0,169	0,169	0,169	0,174	0,165	0,174	0,174	0,194	0,194	0,194	0,200	0,148	0,004	0,000				
29 <i>Synthesium seymouri</i> (Ss2)	0,165	0,165	0,151	0,151	0,165	0,165	0,160	0,160	0,155	0,155	0,155	0,169	0,169	0,169	0,169	0,169	0,174	0,165	0,174	0,174	0,194	0,194	0,194	0,200	0,148	0,004	0,000	0,000			
30 <i>Synthesium subtile</i> (Ssub2)	0,165	0,165	0,151	0,151	0,165	0,165	0,160	0,160	0,155	0,155	0,155	0,169	0,169	0,169	0,169	0,169	0,174	0,165	0,174	0,174	0,194	0,194	0,194	0,200	0,148	0,004	0,000	0,000	0,000		
31 <i>Synthesium delamurei</i> (Sd2)	0,155	0,155	0,141	0,141	0,146	0,146	0,199	0,199	0,194	0,194	0,194	0,165	0,175	0,170	0,170	0,170	0,175	0,165	0,160	0,160	0,180	0,180	0,180	0,165	0,129	0,142	0,147	0,147	0,147	0,147	
32 <i>Synthesium delamurei</i> (Sd1)	0,165	0,165	0,151	0,151	0,155	0,155	0,199	0,199	0,204	0,204	0,204	0,175	0,185	0,180	0,180	0,180	0,185	0,175	0,170	0,170	0,190	0,190	0,190	0,170	0,134	0,147	0,152	0,152	0,152	0,152	0,011

Supplementary Table 3 Pairwise genetic distances based on ITS1 alignments

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1 <i>Tormopsolus orientalis</i> (To1)																		
2 <i>Synthesium</i> morphotype 2 (261)	0,227																	
3 <i>Synthesium neotropica</i> (1064)	0,231	0,002																
4 <i>Synthesium pontoporiae</i> (JX644084)	0,227	0,000	0,002															
5 <i>Synthesium tursionis</i> Spain	0,231	0,007	0,009	0,007														
6 <i>Synthesium</i> morphotype 1 (537a)	0,231	0,007	0,009	0,007	0,000													
7 <i>Synthesium tursionis</i> (78)	0,227	0,005	0,007	0,005	0,002	0,002												
8 <i>Synthesium tursionis</i> (StE1)	0,227	0,005	0,007	0,005	0,002	0,002	0,000											
9 <i>Synthesium</i> morphotype 3 (25a)	0,230	0,007	0,009	0,007	0,005	0,005	0,002	0,002										
10 <i>Oschmarinella rochebruni</i> (Or1)	0,199	0,094	0,097	0,094	0,097	0,097	0,094	0,094	0,097									
11 <i>Synthesium seymouri</i> (Ss1)	0,190	0,100	0,103	0,100	0,103	0,103	0,100	0,100	0,103	0,050								
12 <i>Synthesium delamurei</i> (Sd1)	0,165	0,103	0,105	0,103	0,105	0,105	0,103	0,103	0,105	0,056	0,056							
13 <i>Synthesium delamurei</i> (Sd2)	0,165	0,103	0,105	0,103	0,105	0,105	0,103	0,103	0,105	0,056	0,056	0,000						
14 <i>Brachycladium goliath</i> (KR703279)	0,183	0,124	0,124	0,124	0,127	0,127	0,124	0,124	0,127	0,078	0,084	0,063	0,063					
15 <i>Brachycladium atlanticum</i> (Ba1)	0,198	0,133	0,133	0,133	0,136	0,136	0,133	0,133	0,135	0,078	0,065	0,076	0,076	0,033				
16 <i>Campula oblonga</i> (Co1)	0,198	0,133	0,133	0,133	0,136	0,136	0,133	0,133	0,135	0,078	0,065	0,076	0,076	0,033	0,000			
17 <i>Nasitrema attenuatum</i> (Na1)	0,181	0,130	0,133	0,130	0,133	0,133	0,130	0,130	0,133	0,103	0,101	0,098	0,098	0,063	0,071	0,071		
18 <i>Nasitrema delphini</i> (Nd1)	0,184	0,099	0,102	0,099	0,102	0,102	0,099	0,099	0,102	0,073	0,089	0,081	0,081	0,053	0,060	0,060	0,043	
19 <i>Nasitrema</i> sp. (Nsp1)	0,194	0,102	0,105	0,102	0,111	0,111	0,108	0,108	0,111	0,074	0,090	0,082	0,082	0,053	0,063	0,063	0,038	0,021

Considerações finais

CONSIDERAÇÕES FINAIS

A partir dos resultados obtidos neste estudo nota-se que a helmintofauna de odontocetos no Brasil é ainda subestimada. Estudos parasitológicos em cetáceos ainda são escassos, principalmente na costa brasileira, devido a falta de incentivo e conhecimento dos pesquisadores a respeito destes organismos. A identificação correta bem como estudos agregando dados ecológicos, biológicos e moleculares destes parasitos e seus hospedeiros contribuem para o conhecimento não somente da mortalidade destes animais, mas também de aspectos evolutivos e demográficos de ambos.

Os resultados morfológicos e moleculares obtidos revelam a existência de pelo menos quatro novas espécies de parasitos do gênero *Synthesium* ocorrentes em golfinhos da costa brasileira. Além disso, as análises filogenéticas obtidas indicam a existência de um complexo de espécies agregando *S. tursionis* provenientes da costa brasileira e espanhola, os quais devem ser melhor investigados. A posição filogenética das espécies *S. delamurei*, *S. subtile* e *S. seymouri* é incerta e levanta questionamentos sobre o correto posicionamento destas espécies no gênero *Synthesium*.

Finalmente, este estudo agrega novas informações moleculares para espécimes de braquicladídeos, principalmente para os marcadores rITS1 e mtCOI, os quais carecem de informações em banco de dados, informações estas essenciais para a elucidação do *status* taxonômico do gênero bem como da família Brachycladiidae.

Os resultados obtidos ressaltam a importância de trabalhos relacionados aos parasitos de cetáceos e incentivam a revisão dos espécimes pertencentes ao gênero *Synthesium* usando abordagens morfológicas e principalmente moleculares.