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***Nausithoe* Kölliker, 1853: propostas para  
delimitação morfológica das espécies e relações  
de parentesco do grupo (Nausithoidae,  
Coronatae, Scyphozoa)**

**Dissertação de Mestrado**

**São Paulo – SP**

**Julho 2019**

***Nausithoe Kölliker, 1853: propostas para diferenciação morfológica das espécies e relações de parentesco do grupo (Nausithoidae, Coronatae, Scyphozoa, Cnidaria)***

***Nausithoe Kölliker, 1853: proposals for morphological differentiation of species and relationships of the group (Nausithoidae, Coronatae, Scyphozoa, Cnidaria)***

Clarissa Garbi Molinari

Dissertação apresentada ao Instituto de Biociências da Universidade de São Paulo, para a obtenção de Título de Mestre em Ciências, na Área de Zoologia.

Orientador: Prof. Dr. André Carrara Morandini

São Paulo

2019

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71 páginas

Dissertação (Mestrado) - Instituto de Biociências da Universidade de São Paulo. Departamento de Zoologia.

1. Pólipo
2. Medusa
3. Tubo peridérmico
4. Taxonomia

Universidade de São Paulo. Instituto de Biociências. Departamento de Zoologia.

Comissão Julgadora:

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Prof. Dr. André Carrara Morandini

Orientador

## Epígrafe

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*“I have no dress except the one I wear every day. If you are going to be kind enough to give me one, please let it be practical and dark so that I can put it on afterwards to go to the laboratory.”*

Marie Curie

## Agradecimentos

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Ao meu orientador, Prof. Dr. André Carrara Morandini, pela confiança, parceria, incentivo e ensinamentos.

Aos meus colegas de laboratório, Anabelle, Arthur, Edgar, Gisele, Jonathan, Mayara e Max pela ajuda ao longo de todo o projeto e companheirismo.

Aos técnicos de laboratório do Departamento de Zoologia da USP, Beatriz, Manuel, Sabrina, Phillip e Ênio, pelo indispensável auxílio na obtenção dos dados.

Ao Dr. Allen G. Collins por me receber em seu laboratório no Museu Nacional de História Natural da Instituição Smithsonian (Washington, DC – EUA), além dos demais pesquisadores e funcionários do Smithsonian que me ajudaram durante minha estadia nos Estados Unidos.

Aos meus amigos pelo suporte nos momentos de dificuldade e por me ajudarem a acreditar no meu potencial.

À minha família pelo apoio durante todo meu percurso como pesquisadora; principalmente aos meus pais, Simone e Hélcio, por investirem na minha formação desde sempre, acreditando em mim e valorizando minhas escolhas profissionais.

Ao meu namorado, Henrique, pelo companheirismo, cuidado e carinho (além de me acompanhar ao laboratório nos finais de semana e ajudar na filtragem da água do mar).

À Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), pela concessão da bolsa (2017/04954-0) e da bolsa de estágio em pesquisa no exterior (BEPE - 2018/11763-9).

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) pela bolsa de auxílio à pesquisa concedida nos meses iniciais.

E a todos que em algum momento me ajudaram, seja por troca de conhecimento, apoio ou incentivo.

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## Disclaimer

None of the zoological names and nomenclatural acts in this thesis are published for purpose of zoological nomenclature. This is a disclaimer with reference to Article 8.2 of the International Code for Zoological Nomenclature (ICZN, 1999).

## Introdução geral

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Na presente dissertação tive três grandes objetivos: desbravar a diversidade morfológica do gênero *Nausithoe* Kölliker, 1853 baseado na comparação morfológica das 22 espécies do grupo (Capítulo 1); descrever uma nova espécie de *Nausithoe* para o litoral sudeste brasileiro (Capítulo 1); e propor uma relação evolutiva das famílias que compõem a ordem Coronatae Vanhöffen, 1892 (Scyphozoa, Cnidaria) a partir de dados moleculares (Capítulo 2).

### Estudando a morfologia das espécies de Coronatae

Os coronados são caracterizados pela presença de um tubo peridérmico na fase de pólipos, que envolve as partes moles (Russell 1970). A maioria dos coronados expressa ciclo de vida metagenético, com poucas exceções (Jarms *et al.* 1999). O estudo do ciclo de vida tem sido fundamental para a resolução da sistemática (Jarms 1990) e compreensão da evolução do grupo (Jarms 2010), permitindo unificar os sistemas de classificação de pólipos e medusas, garantindo uma identificação mais precisa das espécies e um sistema classificatório mais estável. Historicamente, exemplares preservados unicamente no estágio de pólipos eram identificados apenas como pólipos de Coronatae ou acabavam classificados dentro do grupo *Stephanoscyphistoma*, criado por Jarms (1990) para acomodar esses espécimes em um único grupo taxonômico.

Estudos recentes com a análise apenas da fase de pólipos foram capazes de promover avanços utilizando caracteres morfológicos do tubo peridérmico para a diferenciação de espécies (Morandini & Jarms 2005; 2010; 2012). Estes caracteres consistem em uma série de medições do tubo peridérmico (comprimento total, diâmetro da abertura e diâmetro a diferentes alturas do tubo), além da observação do formato dos espinhos e sua ornamentação secundária, número de espinhos por série e tipo de anelação externa do tubo. Entretanto, outros trabalhos (Molinari & Morandini, *submitted*) apontam a utilidade da comparação desses caracteres apenas para a identificação ao nível de gênero, sendo ainda necessário o estudo do ciclo de vida para diferenciação das espécies. Além da dificuldade de associação do pólipos à medusa, sem o acompanhamento do ciclo de vida, esbarramos em descrições extremamente antigas (muitas com mais de 100 anos), baseadas em caracteres possivelmente pouco informativos. A falta de material vivo para análise tem impossibilitado uma resolução mais clara da diversidade do grupo. Por fim, as recentes abordagens moleculares, tanto

na sistemática como na taxonomia do grupo, foram limitadas e não aportaram resultados conclusivos (como delimitação de espécies ou caracterização de novas linhagens).

Foquei aqui no gênero *Nausithoe*, pertencente à família Nausithoidae, devido: 1º - à disponibilidade de material em laboratório para comparação; 2º - ao grande número de espécies descritas para o grupo (22); 3º - à distribuição batimétrica ampla entre espécies, ocorrendo desde águas rasas até profundidades da ordem de 2.600m.

### Sistemática dos cifozoários coronados

A ordem Coronatae possui seis famílias: Atollidae Hickson, 1906 (1 gênero, 7 espécies); Atorellidae Vanhöffen, 1902 (1 gênero, 5 espécies); Periphyllidae Haeckel, 1880 (3 gêneros, 7 espécies); Paraphyllinidae Maas, 1903 (1 gênero, 3 espécies); Linuchidae Haeckel, 1880 (1 gênero, 2 espécies); e Nausithoidae Haeckel, 1880 (3 gêneros, 26 espécies). Propostas de relações de parentesco entre as famílias são escassas na literatura até o momento e desbravar a diversidade e sistemática da ordem Coronatae é fundamental para esclarecer questões mais amplas, como a ecologia de animais de mar profundo e a distribuição/riqueza de espécies crípticas (Bickford et. al. 2006).

Além da falta de estudos que elucidem a morfologia das espécies de coronados, poucos trabalhos fazem propostas mais concretas de relações interespecíficas. Bayha *et. al.* (2013) trazem sequências de genes ribossomais 18S e 28S para as seguintes espécies: *Atolla tenella* Hartlaub, 1909, *Atolla vanhoeffeni*, *Atolla wyvillei* Haeckel, 1880, *Atorella octogonos* Mills, Larson & Youngbluth, 1987, *Linuche aquila* (Haeckel, 1880), *Linuche unguiculata* (Swartz, 1788), *Nausithoe atlantica* Broch, 1914, *Nausithoe rubra* Vanhöffen, 1902, *Paraphyllina ransoni* Russell, 1956 e *Periphylla periphylla* (Péron & Lesueur, 1810). Embora o foco do trabalho não fosse a ordem Coronatae, os autores fazem especulações a partir das sequências obtidas, mas concluem que apenas com mais dados moleculares seria possível entender as relações de parentesco internas do grupo. Anteriormente, Hingston *et. al.* (2007) também haviam utilizado a análise de DNA para resolver a sistemática de cifozoários coronados, combinando análise morfológica com sequências ribossomais do gene 28S para entender a relação de parentesco de seis espécies previamente selecionadas.

Nesta dissertação, pretendi trazer a primeira proposta concreta de sistemática da ordem, levando em consideração todas suas famílias, a partir da análise ampliada das espécies e sequências de DNA.



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# Capítulo 1

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## **Revisão morfológica do gênero *Nausithoe* com a descrição de uma espécie nova do Atlântico Sul**

A ser submetido à *Zootaxa*, como “Research article”.

**Morphological review of the genus *Nausithoe* (Nausithoideae, Coronatae, Scyphozoa, Cnidaria) and description of *Nausithoe silveira* n. sp. from South Atlantic**

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**Abstract:** Here we explored and discussed the diversity of the scyphozoan jellyfish genus *Nausithoe* Kölliker, 1853 (Nausithoidea, Coronatae), questioning the feasibility of using some characters of the medusa stage to identify species of the group and filling gaps concerning the diversity and distribution of the genus. As most scyphozoans, the 22 valid *Nausithoe* species also have a metagenetic life cycle, but the similarity of the polyps within the genus highlights the need of studying the medusa stage. We studied morphological features on preserved and live *Nausithoe* specimens (polyp and medusae) comparing these data with the original descriptions. With our data we were able to validate twelve species, bringing new information for some of them and describing a new species: *Nausithoe silveira* n. sp..

**Keywords:** Deep-sea, medusae, polyp, periderm tube

**Resumo:** Neste artigo exploramos e discutimos a diversidade do gênero *Nausithoe* Kölliker, 1853 (Nausithoidea, Coronatae), questionando a viabilidade do uso de alguns caracteres do estágio medusa para identificar espécies do grupo e preenchendo lacunas de acordo com a diversidade e distribuição do gênero. Como a maioria dos cifozoários, as 22 espécies válidas de *Nausithoe* também possuem um ciclo de vida metagenético, mas a similaridade dos pólipos dentro do gênero destaca a necessidade de se estudar a medusa. Estudamos caracteres morfológicos de espécimes de *Nausithoe* (pólipo e medusa) preservados e vivos, comparando esses dados com as descrições originais. Com os dados obtidos, fomos capazes de validar doze espécies, adicionando informações para algumas delas e descrevendo uma espécie nova: *Nausithoe silveira* n. sp..

**Palavras-chave:** Mar profundo, medusas, pólipos, tubo de periderme

## Introduction

Among the scyphomedusae (Cnidaria, Scyphozoa) the order Coronatae Vanhöffen, 1892 comprises six families: Atollidae Hickson, 1906; Atorellidae Vanhöffen, 1902; Periphyllidae Haeckel, 1880; Paraphyllinidae Maas, 1903; Linuchidae Haeckel, 1880; and Nausithoidae Haeckel, 1880 totaling 59 species (Jarms & Morandini, *in press*). Most of the group diversity is represented by deep-sea animals (38 species) (Kramp, 1961). The genus *Nausithoe* Kölliker, 1853 (family Nausithoidae) consists of 22 species that have a wide range of distribution, occurring from shallow waters to 2,600m deep. Along the Brazilian coast, 8 species of coronates were recorded until now: *Atolla wyvillei* Haeckel, 1880; *Linuche unguiculata* (Swartz, 1788); *Nausithoe atlantica* Broch, 1914; *Nausithoe aurea* Silveira & Morandini, 1997; *Nausithoe punctata* Kölliker, 1853; *Nausithoe simplex* (Kirkpatrick, 1890); *Periphylla periphylla* (Péron & Lesueur, 1810); and *Stephanoscyphistoma corniformis* (Komai, 1936) (Goy, 1979; Silveira & Morandini, 1997; Morandini, 2003; Oliveira *et al.*, 2016). In addition, four other morphotypes occur in the country's coast, but only identified up to genus level as *Nausithoe* sp. or *Atorella* sp. (Jarms *et al.*, 2002a).

Several authors (*e.g.* Werner, 1973; Jarms, 1990; Silveira & Morandini, 1997) suggest studying life cycles as a means for unravelling the true taxonomic identity of coronate species. *Nausithoe* polyps have great morphological similarity, which makes it difficult to identify species by only examining this semaphoront of the life cycle. The identification of preserved polyps almost always remains at the family or generic level; although for certain species the polyp tubes can be identified to species (*e.g.* Morandini & Jarms, 2005, 2010, 2012). In general, only the study of medusae morphology can provide enough characters to compare and identify distinct species (Jarms, 1990).

Meanwhile, not all *Nausithoe* species exhibit the typical metagenetic life cycle. *Nausithoe planulophora* (Werner, 1971) and *Nausithoe racemosa* (Komai, 1936), for example, do not have the medusa stage in their biology (Werner, 1970; Werner & Hentschel, 1983). Other species, such as *Nausithoe wernerii* Jarms, 1990, *Nausithoe maculata* Jarms, 1990, and *Nausithoe aurea* exhibit typical metagenesis (Jarms, 1990; Silveira & Morandini, 1997). For those, it is imperative that the identification relies on the adult form: *i.e.* the medusa stage.

The goals of this project were to unravel the diversity and distinguish the species of the jellyfish genus *Nausithoe* (Scyphozoa, Coronatae, Nausithoidae) based on morphological characters and describe a new *Nausithoe* species, collected in the

southeast coast of Brazil. For this purpose, we observed approximately 380 medusae, 130 ephyrae, 112 solitary polyps and 5 colonies (with more than 50 polyps each) of the group, available in four different museums, and analyzed the morphology, molecular data and life cycle of the new Brazilian species.

## Methods

The study was conducted on two parallel approaches: I) morphological revision of the genus *Nausithoe* and II) description of a new species.

### I) Morphological revision of the genus *Nausithoe*

The analysis was composed of observation of specimens preserved in 4% formaldehyde or 70% alcohol solution from 4 different museums (Tab. 1). Observations were conducted at the Smithsonian's NMNH Museum Support Center facility and Invertebrate Zoology Department.

As Coronatae specimens are very similar and some identifications were made by non-experts in the group, we questioned all museums IDs and re-examined all specimens, following the main features recognized in Nausithoidae medusae as stated by Jarms *et al.* (2002) and represented in Fig. 1. Examinations considered: flexibility, color and thickness of the mesoglea; shape, length and number of marginal lappets; shape and size of mouthlips; rhopalium organization and form; thickness and shape of subumbrellar musculature; shape and subdivisions of gastrovascular cavity; shape, size and color of gonads; shape and size of tentacles; arrangement and number of gastric filaments; presence of nematocysts clusters; general shape of umbrella and central dome; besides additional features observed in the specimens.

With the observations on the specimens listed in Tab 1 we believe that we managed to cover most of the representative *Nausithoe* specimens available in Museum collections.

### II) New species' description

This part was performed in two facilities of the Department of Zoology - Biosciences Institute, University of São Paulo (IB – USP): *Laboratório de Cultivo e Estudos de Cnidaria* and *Laboratório de Evolução Molecular*. The 7 available polyps (here named just for differentiation as: AC01, AC02, AC08, AC10, AC17, AC18, and AC20) were kept in cultivation individually since

September 2002, in a 15°C chamber. These specimens were collected during the *Navio Oceanográfico Wladimir Besnard* Expedition to the coast of Cabo Frio (Rio de Janeiro – Brazil), at 200m depth. Along these years two polyps reproduced asexually by forming tissue balls from the ephyrae (according to Silveira *et al.* 2003) (AC01 and AC02) and one by budding (AC10).

We based our identification/description on four main aspects: i- life cycle; ii- external and internal morphology of polyps and medusae; iii- nematocyst observation by light microscopy; and iv- comparison of DNA sequences.

i- **Life cycle:** the polyps were fed *Artemia* sp. nauplii once a week. As ephyrae were released from strobila they were transferred to another dish (maximum of 20 individuals per dish) and fed at least once a day with a macerated of mussel gonad's (*Perna perna*). As soon as they grow enough to catch artemia we began to vary their diet adding 1-day hatched nauplii. Food was provided in abundance to medusae and ephyrae, and water was changed afterwards, not exceeding 1-2h.

The specimens were photographed during the whole life cycle and every growth stage was recorded for further comparisons.

ii- **Morphology:** all polyps were previously measured following the protocols established by Jarms (1990; 1991) and Jarms *et al.* (2002b). These measurements are: total tube length, tube diameter at 2mm above the base, tube diameter at 5mm above the base, diameter of the basal disc, diameter just above the basal disc and aperture diameter. The type of external ornamentation (number of transversal rings per 4mm length) was also noted for possible comparison, and we also considered whether these rings are more or less prominent with respect to an imaginary line tangential to the tube contour (according to Morandini & Jarms 2005). For the more translucent tubes we managed to observe the number of whorls of cusps and the number of cusps per whorl. We also performed Scanning Electron Microscopy (SEM) of the internal cusps of some polyps in order to observe their shape and ornamentation (if they had additional cusps on the margin and surface). The SEM observations were conducted on the *Laboratório de Biologia Celular e Microscopia* of the

IB - USP and the methodology to prepare the specimens followed the protocols of Morandini & Jarms (2005; 2010; 2012).

Ephyrae and medusae were measured and observed according to the characters represented on Fig. 1.

- iii- **Cnidome:** We measured the nematocysts of different life cycle stages considering the types and sizes (Mariscal, 1974; Östman, 2000). A total of 60 measurements were performed on each type per stage. Measurements were executed in a Nikon Eclipse 80i light microscope.
- i- **DNA:** as the strobilation of these specimens produced hundreds of ephyrae, most of the molecular tissue for DNA extractions came from them (and some from grown-up medusae). Apart from these specimens, we extracted DNA from three other *Nausithoe* species for comparison: *Nausithoe aurea*, *Nausithoe maculata* and *Nausithoe wernerii*. We chose this species due to material availability for culture and for being morphologically similar. DNA was extracted with ammonium acetate (protocol adapted from Fetzner, 1999). After some tests we established that the minimum number of *Nausithoe* ephyrae for a good extraction is around 30, considering the size of the ephyra. Three partial genes were amplified by Polymerase Chain Reaction (PCR): mitochondrial protein coding cytochrome oxidase subunit I (COI), and nuclear ribosomal markers 18S and 28S using published primers (Tab. 4). The products of these amplifications were purified with the Agencourt® AMPure® kit. The BigDye reaction was done using same primers and T<sub>m</sub> for each case; finally, the precipitated samples were sequenced at the “Laboratório de Sinalização de Redes Regulatórias de Plantas” in the Botanical Department, IB – USP, with a 3730xl DNA Analyzer. We used the BLAST algorithm to identify sequencing errors and/or contaminations. Sequence’s alignment and editing were performed with the Geneious® software (Kearse *et al.* 2012) using MAFFT (auto-mode; Katoh & Standley, 2013). Corrected distance values were obtained using MEGA X (Kumar *et al.*, 2018).



## Results

There were several issues concerning the animals examined from the collections. Some were not possible to identify because: of bad preservation; lack of diagnostic characters; and also for being too young (adult morphology not yet achieved). We also excluded from our list specimens wrongly identified as *Nausithoe* (e.g. *Paraphyllina* sp. - NHM 225).

### I) Morphological revision of the genus *Nausithoe*

More than 627 specimens were observed and the characters mentioned in the Methods section were annotated for each individual, except from: thickness and shape of subumbrellar musculature and shape and subdivisions of gastrovascular cavity. These characters were hardly visible in most specimens due to preservation techniques and time in formalin/alcohol. We also recorded pigmentation characters even though we know fixatives might impact them.

All species descriptions are presented below. For the ones with no material available or too damaged, we based our study only on their original description (i.e., *Nausithoe albatrossi*, *Nausithoe albida*, *Nausithoe clausi*, *Nausithoe hagenbecki*, *Nausithoe limpida* and *Nausithoe thieli*). We also relied on original descriptions for species with reduced or no medusa on the life cycle (i.e., *Nausithoe eumedusoides*, *Nausithoe planulophora*, and *Nausithoe racemosa*), and species based only on polyp descriptions (i.e., *Nausithoe simplex*, and *Nausithoe sorbei*).

#### ***Nausithoe albatrossi* (Maas, 1897)**

*Nauphanta albatrossi* Maas, 1897: 83, pl. XIV.

*Nausithoe albatrossi* – Vanhöffen, 1902: 29.

Material examined: None. Holotype not known, possibly not available.

Description: Based on Maas (1897), Bigelow (1928), Mayer (1910, and Kramp (1961). Adult medusa 35 to 40 mm in diameter and 35 mm high; narrow and elongate lappets, with rounded ends; smooth central disc; gonads oblong; gastric filaments organized in 16 clusters of 5 filaments (4 clusters per quadrant, a total of 80 filaments). No information about the rhopalia. No information about a polyp stage.

Type locality: Gulf of Panama.

Distribution: Pacific coast of South America.

Remarks: Probably a valid species due to the distinct organization of gastric filaments, not coincident to other *Nausithoe* species' description.

### ***Nausithoe albida* Gegenbaur, 1856**

*Nausithoe albida* Gegenbaur, 1856: 211-214.

Material examined: None. Holotype not known, possibly not available.

Description: Based on original description. Adult medusae: 3 to 4.5 mm in diameter; umbrella flat, transparent, with numerous dots; marginal lappets pointed, leaf-shaped; tentacles about 1.5 mm long; 20–28 gastric cirri; rhopalia with statocyst and pigmented bulb.

No information about polyp stage.

Type locality: Messina, Italy.

Distribution: Only known from type locality.

Remarks: Very superficial description of the medusa.

### ***Nausithoe atlantica* Broch, 1913**

(Fig. 2)

*Nausithoe atlantica* Broch, 1913: 9-10, pl. I figs 1-4.

Material examined: NHM 125, 128 (Portugal). Holotype not known, possibly not available.

Description: Based on Broch (1913), Russell (1956), and Kramp (1961). Adult medusa with up to 35 mm in diameter and 19 mm of central disc; umbrella smooth, dark yellowish-brown (almost black); somewhat arched central disc; slightly elongate marginal lappets with rounded margins; rhopalia with statocyst and deeply colored ventral sensory bulb; gonads oblong; more than 160 gastric filaments in total. No information about polyp stage.

Type locality: North Atlantic (36°53'N 29°47'W, 500 m depth).

Distribution: Only known from type locality.

Remarks: The only 2 specimens examined (NHM 125, 128) were partly damaged precluding the observation of important characters. Both animals had smooth umbrella

with remnants of red-brownish pigmentation (almost vanishing). Tentacles with a very thick base. Gonads disc-shaped (early development), elongated distally and near to the margin of coronal muscle. It was not possible to visualize the gastrovascular cavity and lappets, and the rhopalia were too damaged to be described. Tentacles were as long as the estimated total diameter of specimens, around 20 mm. All the morphological characteristics we observed were also coincident with *Nausithoe rubra*'s description, being both probably the same species, as was mentioned by previous authors (Russell, 1956, and Kramp, 1961).

***Nausithoe aurea* Silveira & Morandini, 1997**

*Nausithoe aurea* Silveira & Morandini, 1997: 236-239, figs 1-7, pls I-II.

Material examined: 10 specimens observed from cultive (polyps from São Sebastião – Brazil, collected in 2018, shallow waters). Holotype MNRJ 2899.

Description: Based on original description, Morandini & Silveira (2001a, b). Adult medusa with up to 5 mm in diameter and 3.69 mm of central disc; flattened transparent umbrella with a yellow pigment spot in the center of lappets; rounded lappets with pointed tips; rhopalia with statocyst and red ocellus; 12–24 gastric filaments in total; spherical gonads, from yellow to brown. Polyp solitary; living in shallow waters (3–9 m); 1.35–9.18 cm of total length; 16 cusps per whorl, from 2–7 whorls. Life cycle includes the production of a planuloid by the polyp, apart from the typical ephyra strobilation.

Type locality: Ilhabela, Brazil.

Distribution: Atlantic coast of South America (Brazil).

Remarks: Possibly *Nausithoe maculata* – the only difference between these species is the production of a planuloid by *N. aurea*, what was not observed in *N. maculata* until the present day.

***Nausithoe challenger* (Haeckel, 1880)**

(Fig. 3)

*Nauphanta challenger* Haeckel, 1880: 487. Haeckel, 1882: 103-111, pls XXVI-XXVII.

*Nausithoe challenger* – Vanhöffen, 1902: 28.

Material examined: NMNH 58264 (Mid North Atlantic Ocean, 1978, depth: 0-100m). Holotype NHM 1882.10.9.1.

Description: Based on original description, Bigelow, (1928), Mayer (1910), and Kramp (1961). Adult medusa 12 mm in diameter; deep annular furrow marking the transition from pedalia to central disc; somewhat less in diameter than bell-radius; thin and long tentacles (longer than bell-radius); large bean-shaped gonads; one cluster of gastric cirri per quadrant, each one with 24 filaments (96 total). No information about polyp stage.

Type locality: Tristan da Cunha island (32°24'S 13°5'W), 2,600m depth (central South Atlantic).

Distribution: Only known from type locality.

Remarks: The examined specimens (NMNH 58264) match the original description in most aspects. The animal had a very thick transparent umbrella, with high slightly quadratic dome. Gastrovascular cavity with 32 short gastric cirri in total and no manubrium (mouth opens directly to the cavity). Sixteen wide marginal lappets with pointed tips and eight rhopalia with statoliths and no ocellus. Eight yellowish-orange oval gonads. Total diameter up to 13 mm and tentacles up to 4 mm long (probably retracted). The absence of mouth lips is typical of *N. marginata*, however, the umbrella shape, the position of the gonads and the animal size resembles Haeckel's description of *Nausithoe challengerii*.

### ***Nausithoe clausi* Vanhöffen, 1892**

*Nausithoe clausi* Vanhöffen, 1892: 14, pl. IV figs 1-2.

Material examined: None. Holotype not known, possibly not available.

Description: Based on original description, Mayer (1910), and Kramp (1961). Adult medusa 9 mm in diameter and 5 mm of central disc; flat dome; exumbrella smooth; three times as wide as high; tentacles as long as bell-radius; marginal lappets short and wide; gonads spherical; numerous small gastric cirri arising independently. No information about polyp stage.

Type locality: North Pacific (13°30'N 156°E).

Distribution: Only known from type locality.

Remarks: All the morphological characteristics described for this species are coincident with *Nausithoe punctata*'s description, being both probably the same species, as was mentioned by previous authors (Kramp, 1961).

***Nausithoe eumedusoides* (Werner, 1974)**

*Stephanoscyphus eumedusoides* Werner, 1974: 439-461, figs 1-5.

*Nausithoe eumedusoides* – Jarms, 1990: 11.

Material examined: None. Holotype ZMH C9797.

Description: Based on Werner (1970) and original description. Medusoid 1–1.5 mm of umbrella width and 1–1.2 mm of height; kind of tetrameric form; lack of manubrium; rhopalia with sense organ; absence of coronal groove until the final moments of strobilation; no gastric filaments; reduced umbrella musculature with 4 thin longitudinal muscle strands; beating flagella on the epidermis; four gonads in total (eight merged in pairs), bean-shaped or oblong, with yellow to brown pigmentation; can be hermaphrodite (with even the production of both eggs and sperm cells at the same time in the same gonad) and/or single sexed. Polyp solitary; 22.2 mm in total length; 1–2 single cusps closer to the base (arranged vertically one above the other); produces 4–5 medusoids per strobilation.

Type locality: Submarine caves near Marseille, France.

Distribution: Marine caves probably of all Mediterranean.

Remarks: Only *N. eumedusoides* and *N. racemosa* have a medusoid described for the life cycle, but their polyps are extremely different from each other. *N. eumedusoides* is a solitary species while *N. racemosa* is colonial (and with significant differences on the soft body).

***Nausithoe globifera* Broch, 1914**

(Fig. 4)

*Nausithoe globifera* Broch, 1914: 10-11, pl. I figs 5-8.

Material examined: NHM 4 - 10 (France – Italy 1956), NHM 136 (Portugal 1959, depth: 1600m), NHM 138 (no info about locality, 1959).

Holotype: not known, possibly not available.

Description: Based on original description, Bigelow (1928), Kramp (1961) and Jarms (1997). Adult medusae 22 mm in diameter and 10 mm of central disc; umbrella smooth, transparent, with high-dome central disc; lappets wide and rounded; rhopalia with statocyst and slightly colored ventral bulb; 80 or more gastric filaments in total;

stomach deep-purple red, marginal tentacles, orange-red; gonads oblong, white to reddish brown. The polyp described for this specie was 6.86 mm long and 0.92 mm of aperture diameter. It had eight cusps per whorl and four whorls total. Soft body with a maximum of 40 tentacles. Strobilation produced 24 ephyrae.

Type locality: North Atlantic (45°26'N 25°45'W, 1.000m depth).

Distribution: Eastern part of North Atlantic.

Remarks: In the 4 examined specimens it was not possible to observe the structure of the rhopalia because they were too damaged. Eight oblong yellowish gonads located above coronal groove. Four triangular gastric septa with more than 15 gastric cirri each (apparently more than 60 in total). Transparent umbrella with deep-red central gastrovascular cavity and manubrium (some specimens lost colour completely). Tentacles were as long as the estimated total diameter of specimens, measuring around 20 mm long.

### ***Nausithoe hagenbecki* Jarms, 2001**

*Nausithoe hagenbecki* Jarms, 2001: 14-18, figs 1-5.

Material examined: None. Holotype ZMH C11659.

Description: Based on original description. Adult medusae with 5 mm in diameter, 3.7 mm between rhopalia and 1.6 mm of flat central disc; rhopalia with statolith and a dark red pigmented ocellus; stout and short tentacles (shorter than the lappets); spherical gonads arranged in pairs situated beneath the coronal groove. Polyp solitary with at least two whorls of 16 cusps; soft body with four lobes at the collar and mouth surrounded by four bigger (coming from the gastric septae) and 16 smaller (from the mouth margin) club-like lips; up to 8.2 mm long slender tentacles.

Type locality: found in the tropical aquarium of the Hagenbeck Zoo in Hamburg, Germany.

Distribution: Unknown, not yet found in the wild.

Remarks: This species appears to be different from others only for the polyp's soft body morphology (four lobes at the collar and mouth surrounded by four bigger and 16 smaller club-like lips)

### ***Nausithoe limpida* Hartlaub, 1909**

*Nausithoe limpida* Hartlaub, 1909: 474-476, pl. LXXVII figs 3, 5.

Material examined: None. Holotype not known, possibly not available.

Description: Based on original description, Bigelow (1928), and Kramp (1961). Adult medusae 16 mm in diameter and 6 mm of central disc; tentacles measuring 6 mm of length and with enlarged base; flattened (almost discoidal) umbrella with smooth central disc; manubrium violet; broad slightly elongated marginal lappets (3 mm long); rhopalia with statocyst and ocellus (not described, based on figure); 96 gastric filaments in total (4 groups of 24); gonads irregularly heart-shaped, females with many dark eggs.

Type locality: North Atlantic (75°47'N 12°59'W, 350m depth).

Distribution: Only known from type locality.

Remarks: This species might be synonymous to *N. rubra* because of the number of gastric cirri and gonads' shape.

### ***Nausithoe maculata* Jarms, 1990**

(Fig. 5)

*Nausithoe maculata* Jarms, 1990: 21-24, figs 15-17, pl. V.

Material examined: NMNH 57648, 57650, 57651, 57657, 57660, 57656, 57658 and 57659 (Puerto Rico 1975), NMNH 43306 (Dry Tortugas 1929), NMNH 57764, 57765, 57766, 57767, 57768, 57772 and 57773 (Belize 1978) and 14 medusae from culture (polyps from Cuba 2001, shallow waters). Holotype ZMH C11534.

Description: Based on original description. Adult medusa 4 mm in diameter and 2 mm of central disc; flattened transparent umbrella with a yellow pigment spot in the center of lappets; rounded lappets with pointed tips; rhopalia with statocyst and red ocellus; 7 gastric filaments per quadrant (28 in total); spherical gonads (female blue, male brownish). Polyp solitary; living in shallow waters (5–10 m); 13 mm of total length; 16 cusps per whorl; maximum of 8 whorls of cusps.

Type locality: Puerto Rico.

Distribution: Caribbean.

Remarks: Examined specimens had from three to seven gastric filaments per quadrant. The gonads were circular, varying in color from dark brown to yellowish-white. Specimens had a maximum total diameter of 6 mm and tentacles were about 3 mm long.

***Nausithoe marginata* Kölliker, 1853**

(Fig. 6)

*Nausithoe marginata* Kölliker, 1853: 323.

Material examined: ZMH C9774 and 9776 (France and Italy), NMNH 57649 (Puerto Rico 1975). Holotype not known, possibly not available.

Description: Based on original description, Jarms (1990). Adult medusa 4–5 mm in diameter and 2 mm of central disc; rhopalia with statoliths and no ocellus; marginal lappets triangular; mouth without lips; tentacles shorter than total body length, with yellowish spots; transparent umbrella; gonads oblong (egg-shaped). Polyp solitary; more than 5 mm in total length; aperture of 0.6–0.8 mm in diameter; 5 to 10 whorls of cusps with 8 cusps each.

Type locality: Messina, Italy.

Distribution: Mediterranean.

Remarks: Examined specimens were extremely damaged. Very delicate umbrella, transparent, with high dome. Gastrovascular cavity with 12 short gastric cirri in total (4 clusters in each quadrant) and no manubrium (mouth opens directly to the cavity). Eight white oblong gonads alternated in position. Total diameter up to 3 mm.

***Nausithoe picta* Agassiz & Mayer, 1902**

*Nausithoe picta* Agassiz & Mayer, 1902: 154-155, pl. 7 fig. 33.

Material examined: None. Holotype not known, possibly not available.

Description: Based on original description, Bigelow (1928), Mayer (1910), and Kramp (1961). Adult medusa 15 to 22 mm in diameter; wide, flat and smooth central disc; wide and pointed marginal lappets; rhopalia with statocyst; oval dark-brown gonads and ocelli; 4 clusters of 12 dark-blue gastric cirri (48 in total). No information about a polyp stage.

Type locality: Rangiroa Island, French Polynesia (15°22'S 147°57'W).

Distribution: French Polynesia and Malayan Archipelago.

Remarks: All the morphological characteristics described for this species are coincident with *Nausithoe punctata*'s description, being both probably the same species, as was mentioned by previous authors (Mayer, 1910; Kramp, 1961).



***Nausithoe planulophora* (Werner, 1971)**

*Stephanoscyphus planulophorus* Werner, 1971: 125-135, figs 2-8.

*Nausithoe planulophora* – Jarms, 1990: 11.

Material examined: None. Neotype ZMH C9742.

Description: Based on original description, and Werner & Hentschel (1983). No medusa. Polyp solitary; longest specimen measuring 20 mm in total length; 1 mm of aperture diameter; 0.2 mm of basal disc diameter; 16 cusps per whorl and 4–5 whorls; 30 tentacles; 10–20 m deep, in submarine caves. Planuloid less than 0.5 mm in diameter; body at first spherical, then ellipsoidal.

Type locality: Marseille, France.

Distribution: Mediterranean coast of France and Italy.

Remarks: As this species doesn't have a medusa or medusoid, its identification as a *Nausithoe* species might be incorrect.

***Nausithoe punctata* Kölliker, 1853**

(Fig. 7)

*Nausithoe punctata* Kölliker, 1853: 323.

*Stephanoscyphus mirabilis* Allman, 1874: 65, pl.XIV.

*Nausicaa phaecum* Haeckel, 1880: 485-486.

*Liniscus cyamopterus* Haeckel, 1880: 497-498.

*Nausithoe punctata* var. *pacifica* Agassiz & Mayer, 1899: 170.

Material examined: NHM 32, 195 (Naples, Italy 1902 and 1905), NHM 86-89 (Ceylon, Sri Lanka 1930), ZMH C7263, C7350 (Naples, Italy), NMNH 58427 (E. N. Atlantic Ocean 1978), NMNH 41723 (Bermuda 1914), NMNH 57649 (Puerto Rico 1975), NMNH 57652, 57654 (Saint Croix, USA Virgin Islands 1975), NMNH 57770, 57775, 57776, 57777, 57779 (Belize 1976). Holotype not known, possibly not available.

Description: Based on original description, Bigelow (1928), Mayer (1910), Vanhöffen (1913), Kramp (1961), and Werner (1970). Adult medusa 12 mm in diameter, 6 mm of central disc, 6 mm height; flattened (bell-shaped) transparent umbrella with pale pink disc and yellowish lappets; rounded marginal lappets; rhopalia with statocyst and ocellus; gonads can vary from white yellow (more immature) to dark brown or blue; females with 2 to 4 eggs per gonad. Polyp colonial; a primary polyp with a single small

basal disc holds the complete colony; individually, the polyps have a typical coronate polyp appearance; soft body with no zooxanthellae.

Type locality: Messina, Italy.

Distribution: Cosmopolitan species?

Remarks: In examined specimens, eggs are bigger in relation to the animal size when compared to those of *N. globifera* and *N. rubra*. Specimens had from 20 to 32 gastric cirri in total (5 to 8 per quadrant). The maximum total diameter was 9 mm and tentacles reached up to 4 mm long. The absence of pigmentation in the lappets was the character we used to differentiate these specimens from *N. maculata*, because all the other morphological features seem to be conflicting. Nevertheless, this pigmentation might vanish when the animal is kept in formalin for too long, so we also considered body size (*N. punctata* is slightly bigger) and excluded from analyses young specimens to avoid misidentifications.

### ***Nausithoe racemosa* (Komai, 1936)**

*Stephanoscyphus racemosus* Komai, 1936: 182.

*Nausithoe racemosa* – Jarms, 1990: 11.

Material examined: None. Holotype not known, possibly not available.

Description: Based on original description, and Werner (1970, 1971). A reduced medusa, medusoid with no gastric filaments, developed mouth or tentacles; males develop the gonads while in the strobilation chain, so that the upper distal medusoid releases the spermatozoa before the others (for having a full developed gonad already); females detach before shedding the eggs from the mouth, embedded in a nematocysts mucus; the medusoid has a well-developed muscular system, but are not able to swim. Polyp colonial; there is a central/primary basic polyp from which secondary polyps emerge, as a “stem with nodes”, not randomly, but in an oblique angle; soft body with enlarged crown, broad ring around the mouth and small delicate tentacles; the tentacle’s crown has kind of a square shape; dark yellow/brown color and thick tissue due to the large amount of zooxanthellae; occurs in shallow waters submerged cliffs.

Type locality: Seto, Japan.

Distribution: Japan, NW Pacific Ocean.

Remarks: This species polyp is extremely different from other *Nausithoe*, mainly the soft body.

***Nausithoe rubra* Vanhöffen, 1902**

(Fig. 8)

*Nausithoe rubra* Vanhöffen, 1902: 30-31, pl. I.

Material examined: NMNH 28128 and 28129 (Peru), NMNH 55931 (California, USA 1975, depth: 900m), AMNH 2113 (Tower Island 1925, depth: 372m). Holotype ZMB 14808.

Description: Based on original description, Bigelow (1928), Mayer (1910), and Kramp (1961). Adult medusa 15 mm in diameter; large pits over the exumbrella surface of central disc; pointed marginal lappets; tentacles and bell red-purple, stomach blue; septal nodes broadly triangular; narrow perradial gastric ostia. No information about a polyp stage.

Type locality: Not clearly stated, W of Seychelles, W of Sri Lanka, S of Congo River mouth.

Distribution: Cosmopolitan species?

Remarks: Examined specimens with brownish smooth umbrella, with narrow pointed lappets (in some specimens the marginal lappets were too damaged, making it difficult to verify their morphology). Eight rhopalia with statocyst and a darker brown bulb at the base. Triangular faint orange or dark purple gonads, pointing to the margin and containing hundreds of eggs. More than 50 gastric cirri in total, close to 12 per quadrant. Some specimens present in the central disc white dense “warts” at the surface (nematocysts?). The largest animal had 20 mm diameter and 15 mm long tentacles.

***Nausithoe simplex* (Kirkpatrick, 1890)**

*Stephanoscyphus simplex* Kirkpatrick, 1890: 14, pl. III.

*Stephanoscyphistoma simplex* – Jarms, 1990: 11.

*Nausithoe simplex* – Morandini & Jarms, 2012: 62-63, figs 1-4.

Material examined: Lectotype NHM 1878.3.26.11a.

Description: Based on original description, and Morandini & Jarms (2012). Only known from the polyp stage. Polyp with 4.56 mm of total length; 1 series of 4 internal cusps.

Type locality: S of Greenland (56°11'N 37°41'W), ~2650m depth.

Distribution: Only known from type locality.

Remarks: To be sure about the identification of this species it would be necessary to cut the tube and visualize the cusp shape and number (only remarkable characteristic of the polyp), what was not possible to do as there was only one polyp previously identified as *N. simplex* (NMNH 53831 – North Carolina/ USA 1966)

***Nausithoe sorbei* Jarms et al., 2003**

*Nausithoe sorbei* Jarms et al., 2003: 3-8, figs 3-5.

Material examined: None. Holotype ZMH C11682.

Description: Based on original description. Only known from polyp stage. Polyp solitary; 13.1 mm of total length; rings more prominent; 1–4 whorls of single cusp.

Type locality: Bay of Biscay.

Distribution: NE Atlantic Ocean (coast of Europe).

Remarks: The species occurs in the same area of *N. rubra* and *N. atlantica*; eventually it might represent the polyp stage of one of those.

***Nausithoe striata* (Vanhöffen, 1910)**

*Tubularia striata* Vanhöffen, 1910: 280, fig. 6.

*Scyphistoma striatum* – Vanhöffen, 1910: vii.

*Stephanoscyphus striatus* – Leloup, 1937: 64.

*Stephanoscyphistoma striatus* – Jarms, 1990: 11.

*Nausithoe striata* – Morandini & Jarms, 2005: 120-122, figs 7-9.

Material examined: None. Holotype ZMB CNI 14816.

Description: Based on original description, and Morandini & Jarms (2005). Only known from the polyp stage. Polyp solitary; 8.4 mm in total length; light brown color; 0.58 mm of basal disc; 0.14 mm of diameter just above the basal disc; 0.34 mm diameter at 2 mm height and 0.64 at 5 mm height; aperture of 0.96 mm in diameter; 5 whorls of 4 internal cusps.

Type locality: Antarctic Ocean (65°S 85°E), 2450m depth.

Distribution: Only known from type locality.

Remarks: This species was diagnosed as a new *Nausithoe* species only based on one polyp SEM, that showed to have 4 internal cusps per whorl. This might bring a misinterpretation of the animal's real identity.

***Nausithoe thieli* Jarms, 1990**

*Nausithoe thieli* Jarms, 1990: 17-21, figs 8-11, pl. IV.

Material examined: None. Holotype ZMH C11532.

Description: Based on original description. Polyp solitary; 19.58 mm of total length; 2, 4 and 8 cusps per whorl (number increases from base to aperture of tube) and maximum of 7 whorls; smooth cusps; 0.5 mm in diameter of basal disc; 0.08 mm in diameter above the basal disc; 30–40 tentacles; 20 ephyrae per strobilation. Newly released ephyra transparent; 2 mm diameter; 0.7 mm of central disc; rhopalia with statocyst and ocelli; 4 small buds of gastric cirri.

Type locality: Central Red Sea, 763m depth.

Distribution: Only known from type locality.

Remarks: Cultivation of ephyrae into mature medusae not yet successful.

***Nausithoe weneri* Jarms, 1990**

(Fig. 9 and 10)

*Nausithoe weneri* Jarms, 1990: 12-17, figs 1-7, pls I-III.

Material examined: ZMH C10693 (Portugal), ZMH C10602 (Morocco 1967), specimens kept in culture (polyps from Morocco 1980, 800-3.000m, and Mediterranean Sea 2008, 200m). Holotype ZMH C11530.

Description: Based on original description. Adult medusae 12 mm in diameter and 4.5 mm of central disc, with a high round dome umbrella (straw hat shape); marginal lobes partially overlapping, rounded; tentacles 4 mm long; rhopalia with statocyst and ocelli; rounded, translucent to opaque, gonads located above the coronal furrow (dioecious). Polyp solitary; 21.34 mm in total length; aperture diameter 0.51 mm; 0.1mm diameter just above the basal disc; 8 cusps per whorl and a maximum of 11 whorls; 40–50 tentacles; over 100 ephyrae per strobilation.

Type locality: Morocco coast, 415-420m depth.

Distribution: NE Atlantic Ocean.

Remarks: Examined specimens with a thimble-shaped transparent central disc, with 4 to 8 gastric cirri, made the *N. weneri* identification possible. Gonads are round and yellowish, located under or outside the coronal groove. Maximum diameter of specimens

was 7 mm and tentacles were up to 3 mm long. All specimens we managed to raise until maturation of the gonads turned out to be males.

II) Description of the new species

*Nausithoe silveira* n. sp.

(Figs. 11-15)

Holotype: MZUSP XXXX – one medusa (cultivated from polyp in 2018)

Paratypes: MZUSP YYYY – one polyp (Arraial do Cabo, Rio de Janeiro 2002); ZZZZ – one medusa (cultivated from polyp in 2018); WWWW – 50 ephyrae (released from polyp in 2019)

Examined material: Seven polyps kept in cultivation in the lab (AC01, AC02, AC08, AC10, AC17, AC18, and AC20). 850 ephyrae and 42 medusae.

Type locality: Off Arraial do Cabo (41°44.40'W, 23°45.80'S), 227m deep – Cabo Frio - Rio de Janeiro, Brazil. Collected by Navio Oceanográfico Wladimir Besnard on 15 September 2002 by Prof. Dr. Sergio A. Vanin.

Etymology:

This new species of the genus *Nausithoe* is dedicated in honor of the Brazilian cnidarian researcher Fábio Lang da Silveira (former professor at University of São Paulo), who devoted his career to study several groups of cnidarians and started the studies of coronates in Brazil back in the 1990's.

Diagnosis:

Nausithoidae species with solitary polyp about 15 mm in total length, dark to light brown pigmentation and 16 cusps per whorl close to the basal disc (with additional cusps) and 8 cusps per whorl upper in the tube (without additional cusps). Medusae completely translucent, with smooth flat umbrella, 9.5 mm in total length and 7.74 mm of central disc diameter, slightly elongated lappets with rounded margins, rhopalia with statocyst and red ocelli and gastrovascular cavity with 8 gastric cirri in total.

Species description:

Metagenetic species. Solitary polyp with typical periderm tube, dark to light brown in color, with conical shape and transverse rings on the surface with

longitudinal striations. Largest specimen 20.13 mm long. Basal disk 0.6 mm in diameter. Diameter of aperture 1.28 mm. Diameter just above the basal disc 0.14 mm. Diameter at 2 mm high 0.1 mm, and at 5 mm high 0.53 mm. From 3-10 whorls of internal cusps. Closer to the base the number of internal cusps per whorl is 16: 4 large (perradius) with additional cusps on the surface, 4 intermediate (interradius) and 8 small (adradius). Upper whorls presenting only 8 cusps: 4 large (perradius) with no additional cusps and 4 intermediate (interradius). Polyp with 26 to 37 filiform tentacles. Medusa entirely translucent with slightly flattened smooth umbrella, 16 slightly elongated lappets with rounded margins and 8 rhopalia with statocyst and red ocelli. Live specimens measuring: 9.5 mm total diameter; 7.74 mm central disk diameter; 2.83 mm coronal groove diameter; gastric cirri approximately 0.9 mm in length; and tentacle length up to half the total diameter of the medusa. Stomach with 4 pouches, each with 2 gastric filaments (8 in total). Polydisc strobilation, with more than 100 ephyrae at a time.

#### Life cycle:

During the period of study (feb/2017–jul/2019) all cultivated polyps strobilated at least one time, but only 5 had medusa that exhibited traces of gonad development. The growth rate of the species in laboratory was extremely low: some ephyrae can take more than two months to eat *Artemia* nauplii and some medusae were maintained for more than a year without developing the gonads. We assigned this low rate to the conditions the animal had to be submitted to, as small vessels and the lack of a more diversified diet.

Strobilation produced more than 100 ephyrae. Recently release ephyrae had 1.7 mm in total diameter, 1.2 mm of central disc and 0.75 mm of coronal groove. In general the time span when morphological characters start to appear (feeding once a day): first gastric cirri – 2 weeks; second gastric cirri – 13 weeks; tentacle's buds – 2 weeks; gonads – 24 weeks; ocelli – 3 weeks.

Ephyrae that were kept under unfavourable conditions or left unfed degenerated and produced tissue balls. A single ephyra can turn into a tissue ball that can regenerate a single polyp.

#### Polyp:

Solitary, with typical coronate periderm tube (Fig. 12): dark to light brown (usually darker close to the base), conical in shape and with transverse annulations (rings) on the surface and also showing some longitudinal striations. All polyps' measurements are detailed in Tab. 3, except the very tiny ones produced by tissue balls from AC01.

Polyp tube showing from 3 to 10 whorls of internal cusps. Closer to the base the number of internal cusps per whorl is 16 (Fig. 12A): 4 large (perradius) with additional cusps, 4 intermediate (interradius) and 8 small (adradius). In the upper whorls of the tube the number of cusps falls to 8 (Figs. 12C and 12D): 4 large (perradius) with no additional cusps and 4 intermediate (interradius). In one tube we found a malformation, presenting 4 cusps in a medium whorl (Fig. 12B).

Soft body with 26 to 37 tentacles and 4 mouth lips in the coronal disc (Fig 11), no further distinction in the mouth lips or collar.

#### Medusa:

All medusae that were cultivated and reached a later stage (Figs 13 and 14) had a complete translucent body, with delicate pigmentation on the lappets, corresponding to a concentration of nematocysts. Smooth umbrella, 16 slightly elongated lappets with rounded margins and 8 rhopalia with statocyst and red ocelli. While immature, the animal presents a short elevation on central disc (Fig. 14B), which gets flatter whereas it grows. Gastrovascular cavity with 4 pouches, each containing 2 gastric cirri. Some medusae never developed the second filament, probably because they were not large enough.

Measurements: total length = 5.7–9.5 mm; central disk diameter = 3.85–7.74 mm; coronal groove diameter = 1.8–2.83 mm; gastric cirri approximately 0.9 mm in total length and tentacles up to half the total diameter of the jellyfish. The manubrium is about 1 mm long and 2 mm wide when relaxed, nevertheless, lips can expand even more towards the margin (Fig. 13F-H).

#### Molecular data:

We were not able to amplify the three proposed markers for all polyps. Therefore, AC01 (no 28S info), AC17 (no COI info) and AC18 (no COI info) were not included in the molecular analyses. The polyps with all three genes sequenced were compared with each other and with *N. aurea* and *N. maculata* (Tab. 5, 6 and



7). AC02, AC08, AC10 and AC20 have less than 4% of genetic differences between them and more than 7% of genetic differences from *N. maculata* and *N. aurea*. Meanwhile, *N. maculata* showed less than 3% of differences from *N. aurea* (Tab. 5; multilocus approach). These main differences (*N. silveira* vs *N. aurea*, *N. silveira* vs *N. maculata*) are considered as proper “species gap” among scyphozoan species (Daglio & Dawson, 2019).

#### Cnidome:

The cnidome of the species is composed of only two nematocyst types: holotrichous isorhiza and heterotrichous microbasic eurytele (Fig. 15). Measurements were taken on the three life cycle stages (polyp, ephyra and medusa) and are summarized in Tab. 8.

#### Discussion

The order Coronatae is widely known as being composed of deep-sea medusae species, although part of the diversity also occurs on shallow waters. Some species of the genus *Nausithoe* have been described 100 years ago and were never found again (*i. e.*, *N. albatrossi*, *N. albida*, *N. clausi*, *N. limpida*, *N. picta*); therefore, their morphological features could not be reviewed mostly due to absence of available specimens for inspection. Comparing the data obtained by us with the original descriptions helped to elucidate the diversity of the group.

Although relatively poorly known, members of the genus *Nausithoe* are regularly found among samples of different studies either considering the medusa (Gershwin & Zeidler, 2003; León *et al.*, 2005; Pagès *et al.*, 2006; Neumann-Leitão *et al.*, 2008) or the polyp stage (Sterrer, 1986; Galil & Zibrowius, 1998; Cebrián & Ballesteros, 2004; Morandini & Jarms, 2010). Because they are not so common, it is hard to state (with the current knowledge) the importance of those species. But it should be noted that some pelagic stages can bloom in plankton communities (S. Toshino *pers. comm.* 2018), and some benthic species can colonize wide areas (ACM *pers. observ.* 2013); while others can inflict painful stings (Oiso *et al.*, 2004). In more recent years (since 2016), with the widespread use of smartphones and social media (mainly Facebook® and Instagram®), it has become common to find groups of amateur and professional divers and underwater photographers which from time to time post photos of *Nausithoe* polyps and medusae.

In general, studying the external morphology of gelatinous animals can be a difficult task. This is most evident because the preservation technique and substances can affect the main shape of the jellyfishes. Thus, relying on the shape and color of a preserved specimen can be somewhat tricky. Still, while studying the genus *Nausithoe* we noticed that the general shape of the bell, mainly the central disk conformation, could be important to differentiate species. In the case of *N. rubra* previous descriptions did not mention the shape of the central disk. However, when studying the preserved animals, we can infer that this species umbrella is flatter than in *N. globifera*, but not as flat as described for *N. punctata* or *N. maculata*. We faced ourselves with difficulties in describing the degree of elevation (height) of the central disk in relation to the rest of the umbrella. Thus, based on the lack of a proper nomenclature to help distinguishing bell outlines of Coronatae medusae and trying to start the discussion on this issue, we propose here a simple nomenclature (Fig. 16) to facilitate communication, taking into consideration four adult types: **hypodome** – dome smaller than the marginal disc radius; **isodome** – dome with about the same size of the marginal disc radius; **hyperdome** – dome higher than the marginal disc radius; and **catadiscus** – marginal disc facing down, perpendicularly to the coronal groove. The need of this terminology was also perceived when we found some inconsistencies while comparing descriptions with the specimen itself. Russel (1970) described *N. atlantica*'s umbrella as “somewhat flat” when compared to *N. globifera*, while we considered it as a “high dome” shaped central disc if compared to *N. maculata* or *N. punctata*. We are aware that the terminology proposed here is most applicable to recently collected or live animals, because the shape of specimens may be altered when preserved in formalin for long periods.

The importance of the bell shape to identify *Nausithoe* specimens was also apparent when comparing a museum specimen with Haeckel's (1882) description of *N. challengeri* (“cap-shaped; with horizontal apex and vertical side wall; sometimes as wide as high”). Haeckel's drawing also assembled the animal we observed (NMNH 58264), except from the presence of the manubrium. The specimen we analyzed lacked this structure, which is a feature so unique that it was diagnostic of *N. marginata*. However, all the other morphological characters are indeed similar to *N. challengeri*'s description. If our assumption is true, *N. challengeri* is a valid species, in conflict to what has been pointed out by some authors (Bigelow, 1928; Kramp, 1961), having a thimble-shaped umbrella (catadiscus) similar to members of the tropical genus *Linuche* Eschscholtz, 1829.

Another character we considered questionable or that needs further study based on our analysis was the number of gastric cirri. *N. punctata* and *N. maculata* may have the same number of gastric cirri, apparently depending on the size of the medusae. *N. rubra*, *N. atlantica* and *N. globifera*, which have more than a hundred gastric filaments (Russell, 1970), are recognized to be much larger than *N. maculata* and *N. punctata*. If the size of the animal is determinant for the amount of cirri, we should reconsider the utility of this feature as a diagnostic character for the group. It might be more informative to only take into consideration the arrangement of filaments within the gastrovascular cavity, or the general quantity of them (*e.g.* few, some, or many).

The gonads' shape was also a discrepancy point between our observations and the available literature. Depending on the degree of maturity, gonads might change in shape, in most cases varying from bean or oval-shaped to clearly round (more developed), as was observed in *N. maculata*, *N. punctata* and *N. weneri*. This type of incongruence was expected before our analysis of museum specimens due to the experience in cultivated animals, but also considering intraspecific variation observed for *N. aurea* (Morandini & Silveira, 2001).

It is well known that *Nausithoe* polyps are very similar and the morphological characters proposed by Jarms (1990) are not always enough to identify specimens (Molinari & Morandini, *submitted*), although for some species it is useful (Morandini & Jarms, 2005, 2012). The observation of the number and shape of internal cusps of a few individuals might bring an erroneous assumption of the true variation in morphology of the polyp. It would be necessary (and desired) to compare a significant amount of polyps, with an effective observation of all series of internal cusps (*e.g.* SEM) to avoid considering intraspecific variation of this character and diagnostic of erroneous separated species.

With the present study we validate the following *Nausithoe* species: *N. albatrossi*, *N. challengerii*, *N. eumedusoides*, *N. globifera*, *N. hagenbecki*, *N. maculata*, *N. marginata*, *N. planulophora*, *N. racemosa*, *N. rubra*, *N. sorbei* and *N. weneri*.

*Nausithoe albida*, *N. clausi*, *N. limpida* and *N. picta* are morphologically similar (although superficially described) and might all be specimens of *N. punctata*. *Nausithoe thieli*, *N. striata* and *N. simplex* were all describe based on few polyps morphological features, therefore we cannot affirm their validity here. We also acknowledge the possibility of *N. aurea* being the same species as *N. maculata*. Although *N. aurea* was described as having a reproductive approach not observed in *N. maculata* (the

production of a planuloid), their morphological features are the same, both polyp and medusa.

Our observations were not able to determine either if *N. rubra* and *N. atlantica* are synonymous, as suggested by Bigelow (1928) and Kramp (1961); although, based on the original descriptions and the specimens analyzed, we have to agree with the previous authors that those two are probably the same species (*Nausithoe rubra*). Vanhöffen (1902) described *Nausithoe rubra* as having a dark brown umbrella and a violet to velvet-black manubrium; narrow and pointed marginal lobes; and dotted central disc with a dense nettle of warts. Kramp's (1961) description of *Nausithoe rubra*, it is mentioned horseshoe-shaped gonads, facing inward, and the animal in the NMNH collection had a triangular gonad pointing to the coronal groove. Furthermore, the maximum diameter noted by Vanhöffen (1902) in the first description of this species was 15 mm, while one of the specimens we analyzed had more than 20 mm diameter and 15 mm long tentacles. These contrasts are probably due to the comparison of different ages specimens.

#### Description of *Nausithoe silveira* n. sp.

Among the Brazilian coast, eight species of coronate scyphozoans were described/registered: *Atolla wyvillei* Haeckel, 1880; *Linuche unguiculata*; *Nausithoe atlantica*; *Nausithoe aurea*; *Nausithoe punctata*; *Periphylla periphylla*; *Stephanoscyphistoma corniformis*; and *Stephanoscyphistoma simplex* (Goy 1979; Silveira & Morandini 1997; Morandini 2003; Oliveira *et al.* 2016).

Although not described for the region the bell shape, coloration and general measurements of *N. silveira* coincide with *N. weneri*'s morphology. Nevertheless, *N. weneri* has a more prominent central dome (hyperdome), while *N. silveira* n. sp. is thoroughly flat (hypodome). Also, the shape of internal cusps of *N. silveira* n. sp. is very similar to *N. weneri*, but in the first one we found 16 cusps in the lower whorls while in the later there are 8 cusps in all whorls. The occurrence of both species is also diverging: *N. weneri* was described for the North East and Central Atlantic and also Arctic Ocean and *N. silveira* n. sp. was found only in the South West Atlantic Ocean. We believe those morphological and geographic differences are sustainable enough to differentiate the species, recouping the absence of the COI gene sequence for *N. weneri*.

In relation to *N. maculata* and *N. aurea*, we have two major considerations to make. First, this species demonstrated an extremely low genetic difference (less than 3%), what indicates they are probably the same species. Second, we can affirm they are different species than *N. silveira* for three reasons: they have a higher number of gastric cirri (12 – 24, while *N. silveira* has 8); they have a yellow spot on the lappets; and their genetic difference to *N. silveira* is about 7% in the multilocus approach and ~18% considering the COI molecular marker (most effective one for taxonomy hypotheses) (Tab. 7).

The systematics of the genus *Nausithoe* is difficult, mostly because descriptions were historically based on a few specimens (not considering variation), and the characters used did not consider the array of species we know today. A somewhat similar condition would be defined for the previous molecular data, because coronates' sequences from GeneBank do not present vouchers (in most cases there is no description of the taxonomist related to the ID) and the majority of them were described as “sp.”; so, there is no clear way to check/validate these genetic source. To understand more deeply its diversity, it would be crucial to study recently collected specimens, as well as life cycle features as stated by several authors (*e.g.* Jarms, 1990; Silveira & Morandini, 1997). Observations on living specimens will also allow the inclusion of more coloration pattern and cnidome characters, apart from the possibility of sampling tissue for molecular analysis.

## **Conclusion**

Our works helped to understand and fill gaps concerning the diversity and distribution of the coronate jellyfish genus *Nausithoe*. By comparing the original descriptions with specimens from museum collections, we concluded that some of the morphological diagnostic characters traditionally used to identify coronate species should be questioned. We also described a new species, *Nausithoe silveira* n. sp., based on morphological, distribution, and molecular data (COI, 16S, 28S).

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## List of tables

**Table 1.** Number of lots analyzed from each museum.

Species	Museum	Locality	N° of lots
<i>N. rubra</i> , <i>N. punctata</i> , <i>N. maculata</i> , <i>N. marginata</i> , <i>N. simplex</i> , <i>N. racemosa</i> , <i>N. challengerii</i> (?)	Smithsonian's National Museum of Natural History (NMNH)	Washington, D.C., USA	79
<i>N. atlantica</i> , <i>N. globifera</i> , <i>N. punctata</i> .	Natural History Museum (NHM)	London, United Kingdom	12
<i>N. marginata</i> , <i>N. wernerii</i> , <i>N. punctata</i>	Zoological Museum Hamburg (ZMH)	Hamburg, Germany	17
<i>Nausithoe rubra</i>	American Museum of Natural History (AMNH)	New York, USA	1

**Table 2.** Updated list of the 23 species of the genus *Nausithoe* Kölliker, 1853 and brief information about life cycle (from Jarms & Morandini, in press), with the addition of the new species highlighted (bold characters), including new localities for some species as a result of this dissertation. \* not yet formally described.

Species name	polyp	medusa	Already known distribution	New localities
<i>Nausithoe albatrossi</i>	not known	present	E Pacific, deep sea	-
<i>Nausithoe albida</i>	not known	present	Mediterranean, shallow water	-
<i>Nausithoe atlantica</i>	not known	present	N Atlantic, deep sea	-
<i>Nausithoe aurea</i>	present	present	SW Atlantic, shallow water	-
<i>Nausithoe challengerii</i>	not known	present	S Atlantic, deep sea	Mid N Atlantic
<i>Nausithoe clausi</i>	not known	present	N Pacific, shallow water	-
<i>Nausithoe eumedusoides</i>	present	reduced	Mediterranean, submarine caves	-
<i>Nausithoe globifera</i>	present	present	NE Atlantic, deep sea	Mediterranean
<i>Nausithoe hagenbecki</i>	present	present	unknown, aquarium animal	-
<i>Nausithoe limpida</i>	not known	present	NW Atlantic, deep sea	-
<i>Nausithoe maculata</i>	present	present	Caribbean, shallow water	-
<i>Nausithoe marginata</i>	present	present	Mediterranean, shallow water	-
<i>Nausithoe picta</i>	not known	present	E Pacific, shallow water	-
<i>Nausithoe planulophora</i>	present	absent	Mediterranean, submarine caves	-
<i>Nausithoe punctata</i>	present	present	Worldwide?, shallow water	-
<i>Nausithoe racemosa</i>	present	reduced	Japan, shallow water	-
<i>Nausithoe rubra</i>	not known	present	Atlantic, Indian, E Pacific, deep sea	N Pacific; Antarctic
<i>Nausithoe simplex</i>	present	unknown	NW Atlantic, deep sea	-
<b><i>Nausithoe silveira n. sp.</i></b>	<b>present</b>	<b>present</b>	-	<b>SE Brazil, deep sea</b>
<i>Nausithoe sorbei</i>	present	present*	NE Atlantic, deep sea	-
<i>Nausithoe striata</i>	present	unknown	Antarctica, deep sea	-
<i>Nausithoe thieli</i>	present	present	Red Sea, deep sea	-
<i>Nausithoe wernerii</i>	present	present	NE Atlantic, deep sea	-

**Table 3.** *Nausithoe silveira* n. sp., measurements of the polyps according to Jarms (1990) and Jarms *et al.* (2002). Each specimen was measured twice, first in 2002 and second in 2018 (except from tissue ball and budding, only measured in 2018). Da = diameter at aperture, Dbd = diameter of the basal disc, Db = diameter just above the basal disc, D<sub>2mm</sub> = diameter at 2mm high, D<sub>5mm</sub> = diameter at 5mm high, L<sub>tot</sub> = total length, nwt = number of whorls of internal cusps, nw = number of cups per whorl, - = not measured. Mean and standard deviation (SD) correspond to the most recent annotations.

Polyp ID	L <sub>tot</sub> (mm)	Da (mm)	Ddb(mm)	Db (mm)	D <sub>2mm</sub>	D <sub>5mm</sub>	Da/L <sub>tot</sub>	Nwt	Nw
AC01 (in 2002)	6.6	0.7	-	-	0.35	0.45	0.1061	7	8 / 16
AC01 (in 2018)	13.46	1.29	-	-	0.13	0.68	0.0958		
AC02 (in 2002)	11.15	0.9	-	0.15	0.3	0.5	0.0807	8	8 / 16
AC02 (in 2018)	17.74	1.09	-	0.16	0.1	0.52	0.0614		
AC02 (tissue ball)	15.71	1.17	-	0.14	0.09	0.44	0.0746	6	8 / 16
AC08 (in 2002)	9.1		-	-	0.3	0.5	0.0769	9	8 / 16
AC08 (in 2018)	16.44	1.00	-	-	-	-	0.0608		
AC10 (in 2002)	14.25	1.15	-	0.15	0.2	0.4	0.0807	12	8 / 16
AC10 (in 2018)	15.33	1.15	0.63	0.15	0.1	0.53	0.0753		
AC10 (budding)	11.79	0.92	0.66	0.15	0.09	0.46	0.0782	6	8 / 16
AC17 (in 2002)	5.05	0.55	-	0.4	0.45	0.55	0.1089	9	8 / 16
AC17 (in 2018)	20.2	1.65	-	0.16	0.09	0.47	0.0818		
AC18 (in 2002)	10.5	0.95	-	0.15	0.35	0.5	0.0905	5	8 / 16
AC18 (in 2018)	12.27	1.02	-	0.12	0.08	0.39	0.0830		
AC20 (in 2002)	5.15	0.35	-	0.2	0.3	0.35	0.0680	10	8 / 16
AC20 (in 2018)	20.13	1.47	-	0.15	0.08	0.42	0.0733		
Mean±SD	15±4.2	1.28±0.36	0.6±0.03	0.14±0.02	0.1±0.02	0.53±0.14	0.076±0.02	8±3	8 / 16

**Table 4.** List of primers used to amplify each gene.

Gene	Primer	Primer sequencie 5'-3'	F/R	tm – base pair	Reference
COI	COXI-F2	TCGACTAATCATAAAGATATCGGCAC	F	52°C – 26bp	Ward <i>et al.</i> , 2005
	MEDCOXR	TGGTGNGCYCANACNATRAANCC	R	52°C – 23bp	Lawley <i>et al.</i> , 2016
	LCO1490JJ4	CIACIAAYCAYAARGAYATYGG	F	55°C+45°C – 22bp	Astrin <i>et al.</i> , 2016
	HCO2198JJ4	ANACTTCNGGRTGNCCAAARAATC	R	55°C+45°C – 25bp	Astrin <i>et al.</i> , 2016
	LCO1490JJ2	CHACWAAYCAYAARGAYATYGG	F	60°C+45°C – 22bp	Astrin <i>et al.</i> , 2016
	HCO2198JJ2	ANACTTCNGGRTGNCCAAARAATCA	R	60°C+45°C – 25bp	Astrin <i>et al.</i> , 2016
28S	F798	CCGTCTTGAAACACGGACC	F	55.5°C – 19bp	Medina <i>et al.</i> , 2001
	R798	GGTCCGTGTTTCAAGACGG	R	55.5°C – 19bp	Medina <i>et al.</i> , 2001
	F15	CTAACAAGGATTCCCCTAGTAACGGCGAG	F	55.5°C – 30bp	LEM lab *
	R1446	GTTGTTACACACTCCTTAGCGG	R	55.5°C – 22bp	Medina <i>et al.</i> , 2001
18S	18S – A	AACCTGGTTGATCCTGCCAGT	F	54°C – 21bp	Medlin <i>et al.</i> , 1988
	18S – L	CCAACACTACGAGCTTTTAACTG	R	54°C – 22bp	Apakupakul <i>et al.</i> , 1999
	18S – C	CGGTAATTCAGCTCCAATAG	F	54°C – 21bp	Apakupakul <i>et al.</i> , 1999
	18S – Y	CAGACAAATCGCTCCACCAAC	R	54°C – 21bp	Apakupakul <i>et al.</i> , 1999
	18S – O	AAGGGCACCACCAGGAGTGGAG	F	54°C – 22bp	Apakupakul <i>et al.</i> , 1999
	18S – B	TGATCCTTCCGCAGGTTACCT	R	54°C – 22bp	Medlin <i>et al.</i> , 1988

\* LEM = Laboratório de Evolução Molecular (IB -USP).

**Table 5.** Genetic differences (in number of bases and percentage) between each *Nausithoe silveira* n. sp. polyp (AC02, AC08, AC10, AC20), *Nausithoe aurea* and *Nausithoe maculata*. All markers combined.

	<i>N. aurea</i>	<i>N. maculata</i>	AC 02	AC 08	AC 10	AC 20
<i>N. aurea</i>		97.22%	94.01%	93.24%	93.93%	94.10%
<i>N. maculata</i>	41		93.57%	92.75%	93.45%	93.60%
AC 02	142	164		96.19%	97.52%	96.41%
AC 08	178	203	65		96.09%	95.73%
AC 10	184	212	50	111		96.31%
AC 20	139	164	54	87	100	

**Table 6.** Genetic differences (in percentage) between each *Nausithoe silveira* n. sp. polyp (AC02, AC08, AC10, AC20), *Nausithoe aurea* and *Nausithoe maculata*. 18S in white and 28S in grey.

	<i>N. aurea</i>	<i>N. maculata</i>	AC 02	AC 08	AC 10	AC 20
<i>N. aurea</i>		97.93%	97.36%	97.44%	97.36%	97.59%
<i>N. maculata</i>	96.43%		97.36%	97.44%	97.36%	97.59%
AC 02	95.48%	95.50%		97.49%	97.49%	97.44%
AC 08	95.63%	92.14%	93.06%		97.49%	97.52%
AC 10	95.16%	95.08%	96.01%	92.73%		97.44%
AC 20	92.19%	95.58%	96.51%	93.22%	96.16%	

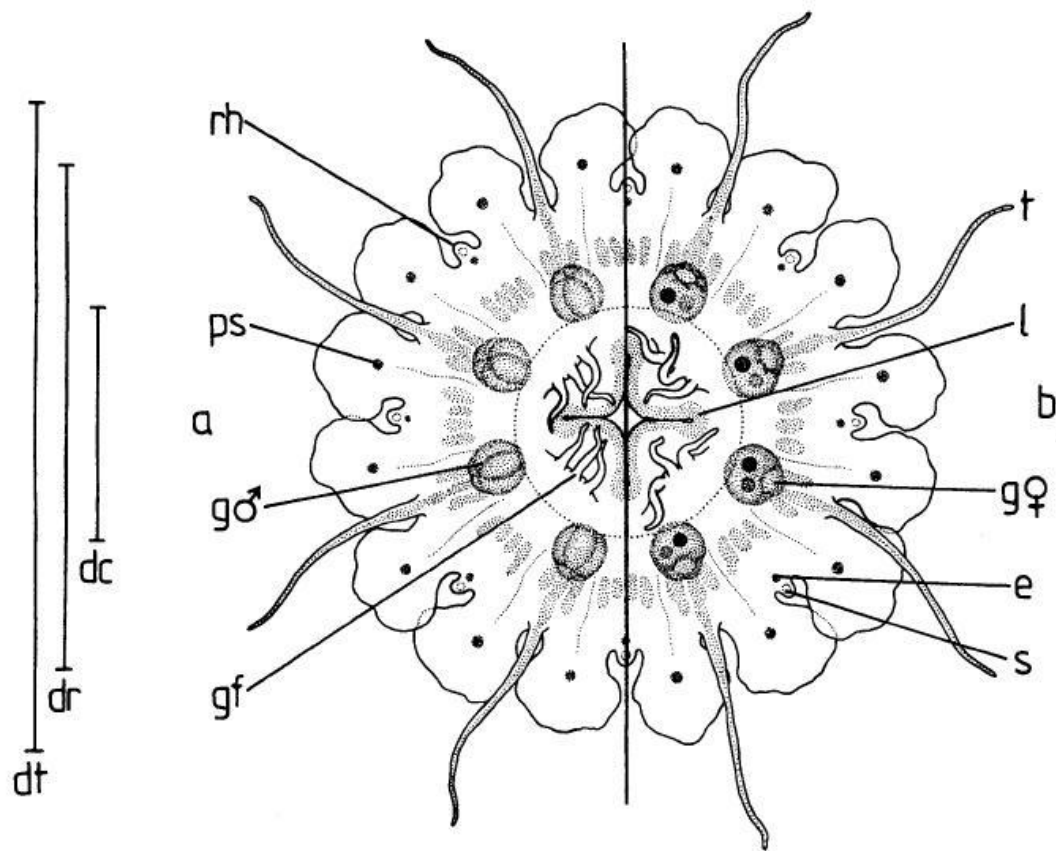
**Table 7.** Genetic differences (in number of bases and percentage) of COI between each *Nausithoe silveira* n. sp. polyp (AC02, AC08, AC10, AC20), *Nausithoe aurea* and *Nausithoe maculata*.

	<i>N. aurea</i>	<i>N. maculata</i>	AC 02	AC 08	AC 10	AC 20
<i>N. aurea</i>		96.34%	81.79%	82.44%	81.79%	81.34%
<i>N. maculata</i>	30		79.27%	79.76%	79.27%	78.66%
AC 02	112	135		97.24%	100.00%	93.21%
AC 08	108	132	17		97.24%	94.35%
AC 10	112	135	0	17		93.21%
AC 20	117	141	44	37	44	

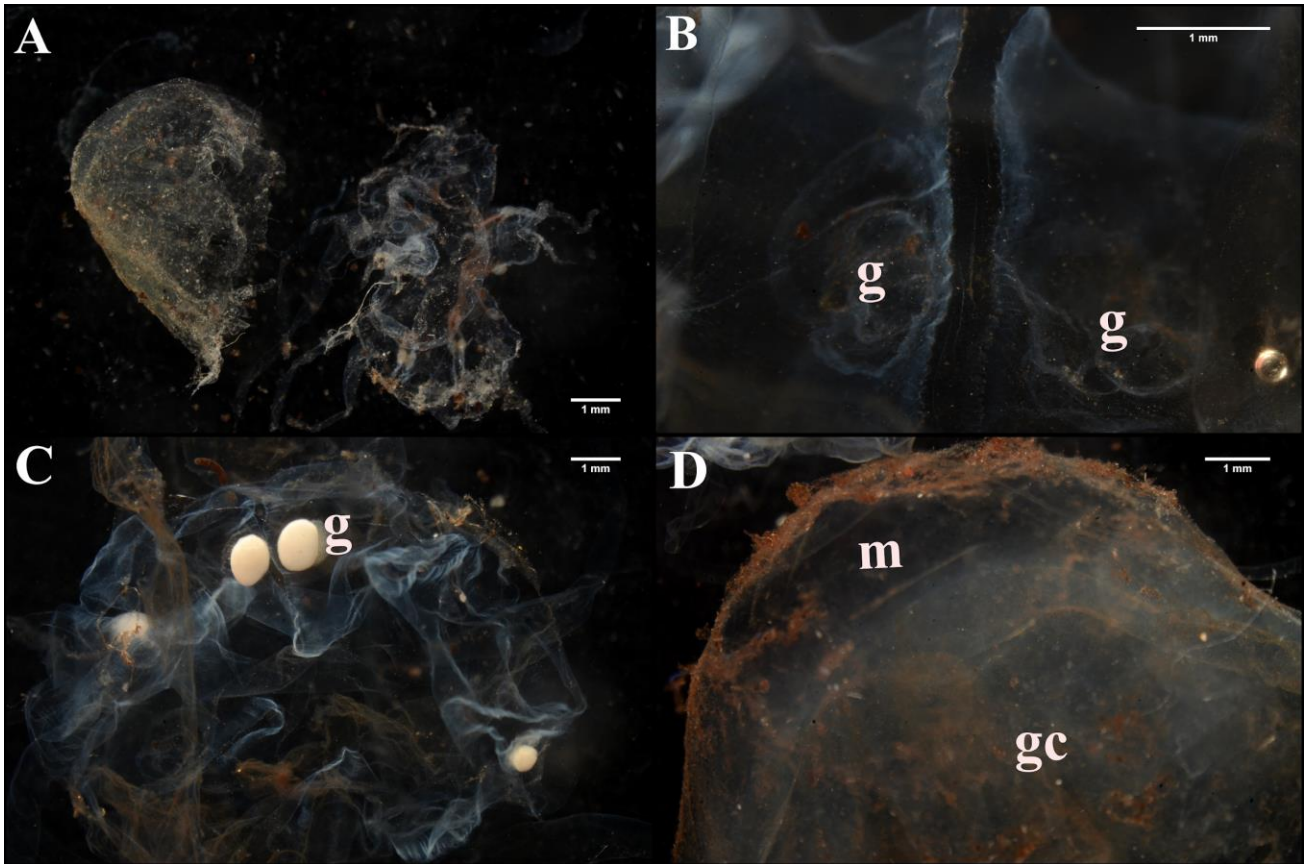
**Table 8.** Cnidome of *Nausithoe silveira* n. sp. The range was taken from 60 nematocysts of each type.

	<i>Holotrichous isorhiza</i>		<i>Heterotrichous microbasic eurytele</i>	
	Width (µm)	Length (µm)	Width (µm)	Length (µm)
<i>Medusa (tentacle)</i>	6.49 – 4.41	8.65 – 5.65	10.96 – 7.28	12.99 – 8.47
<i>Ephyra (whole)</i>	7.33 – 4.11	8.98 – 5.29	12.59 – 7.1	14.27 – 8.19
<i>Polyp (tentacle)</i>	7.58 – 5.86	9.67 – 8.24	11.54 – 9.58	13.53 – 11.28

## List of figures

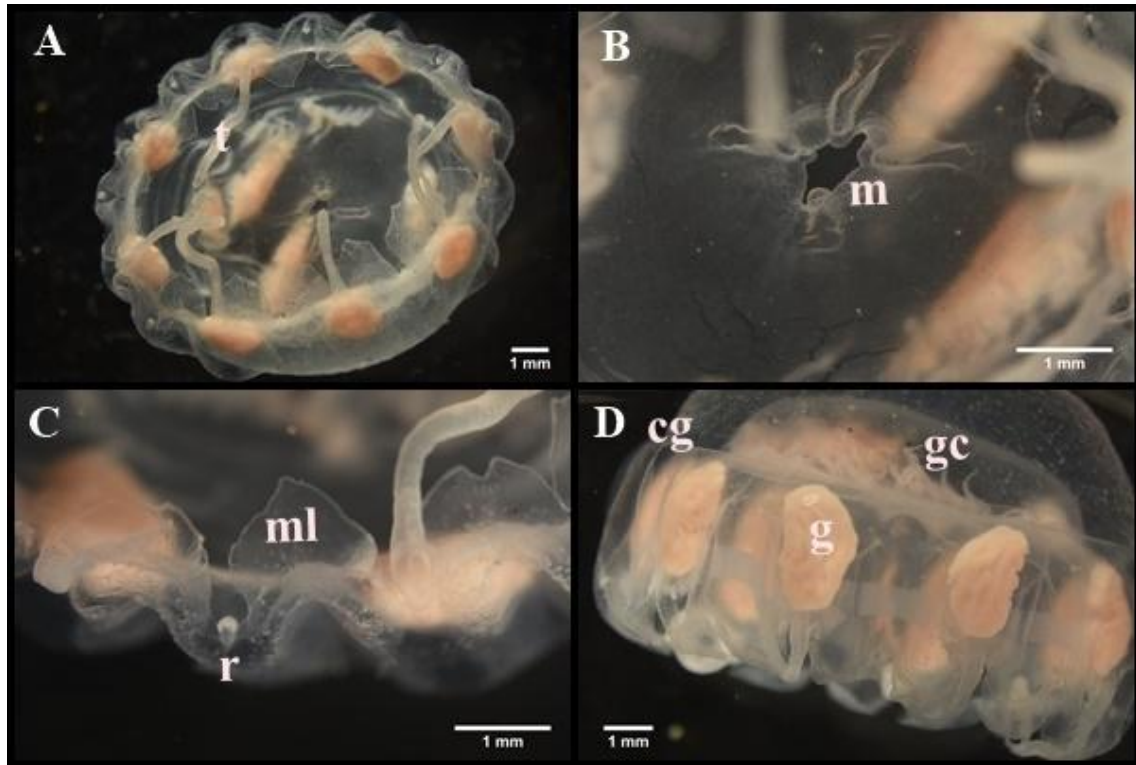


**Figure 1.** Schematic view of an adult medusa of the genus *Nausithoe* (based on the species *N. aurea*) illustrating the characters to be observed. Note that on the right side (**b**) it is represented a female medusa, and on the left side (**a**) a male individual. **dc** - diameter of the coronal furrow; **dr** - diameter between rhopalia; **dt** - total diameter; **gf** - bud of gastric filament; **l** - lips around mouth; **ml** - marginal lappets; **rh** - rhopalium; **t** - tentacle; **e** - eyespot; **g** - male and female gonads; **ps** - pigment spot; **s** - statolith; (adapted from Jarms *et al.*, 2002).

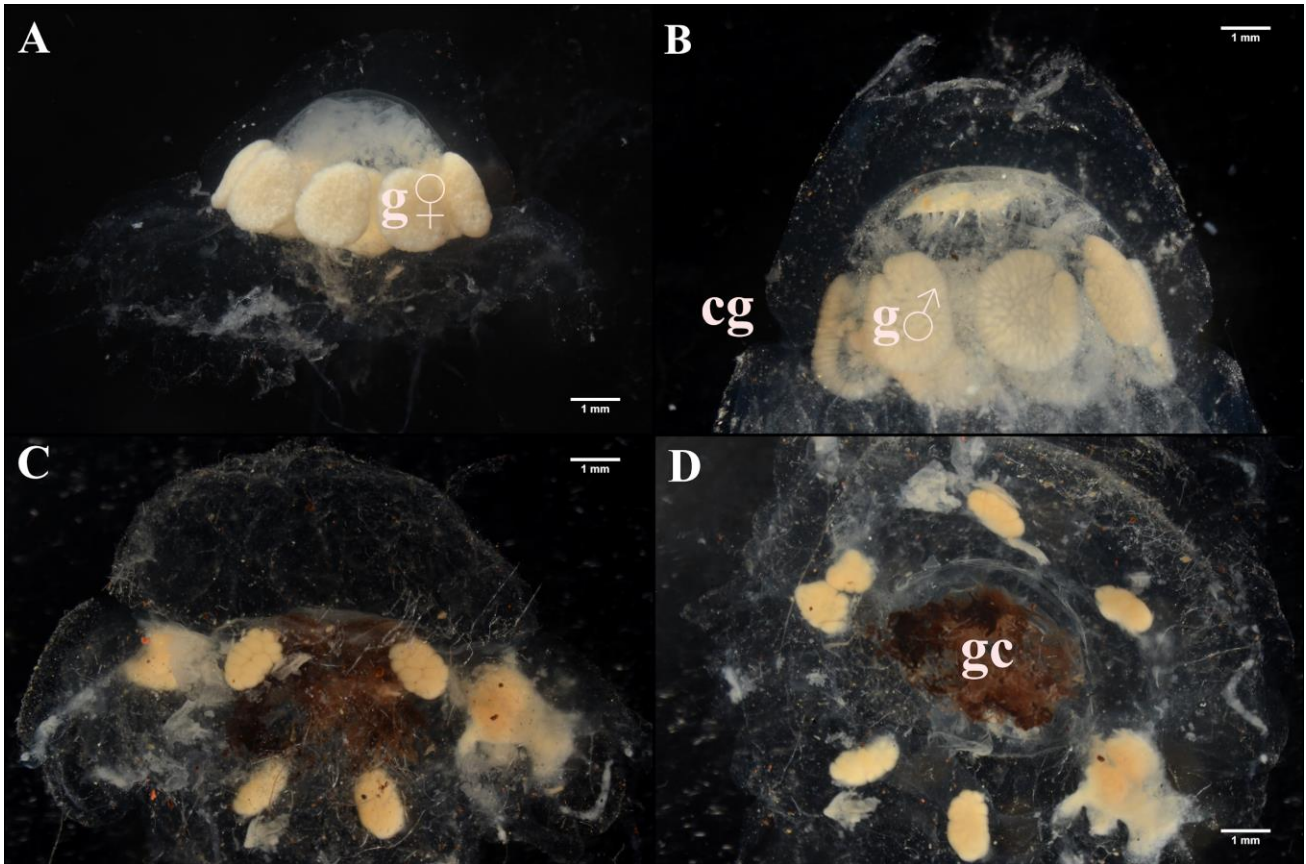


**Figure 2.** *Nausithoe atlantica* (A: NMH 128; B, C and D: NHM 125). **A** - Dome detached from the rest of the body; not possible to visualize gastric cirri and manubrium. **B** - Beginning of gonad (**g**) formation; note how pairs are separated by a narrow space. **C** - View of mature gonads. **D** - Exumbrellar red pigmentation vanishing; central disc with a high dome; possible to see the division between mesoglea (**m**) and gastrovascular cavity (**gc**).

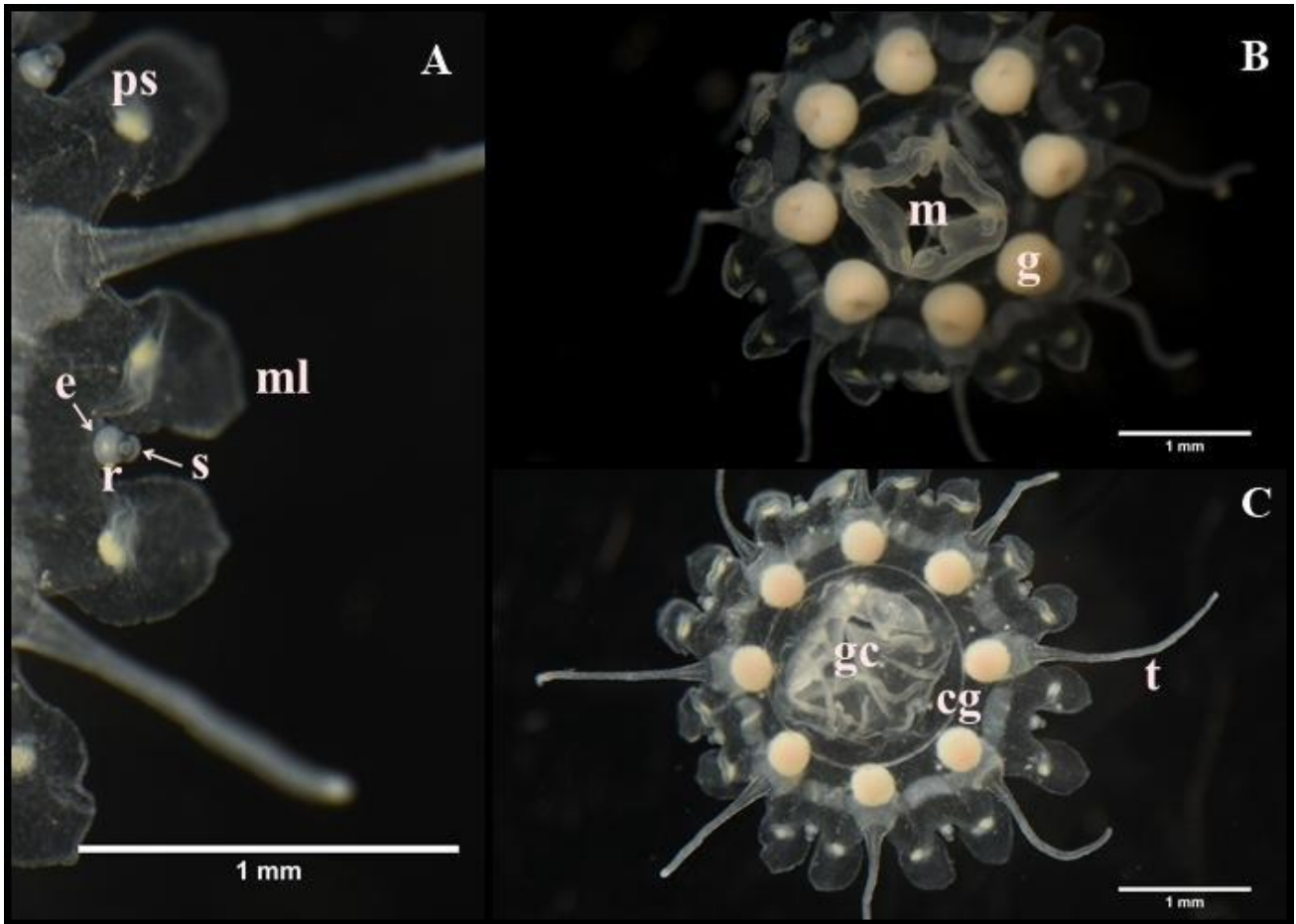




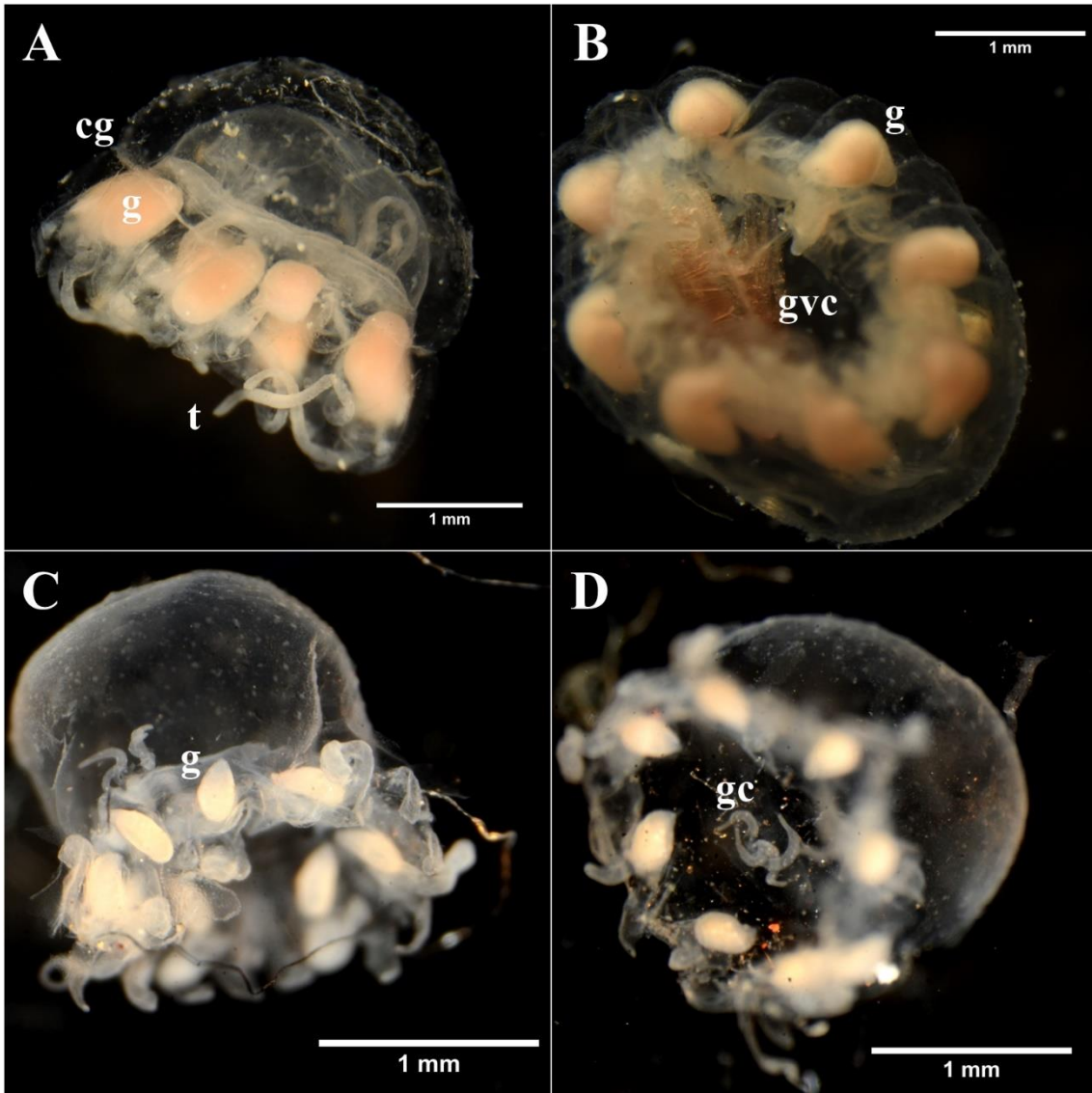
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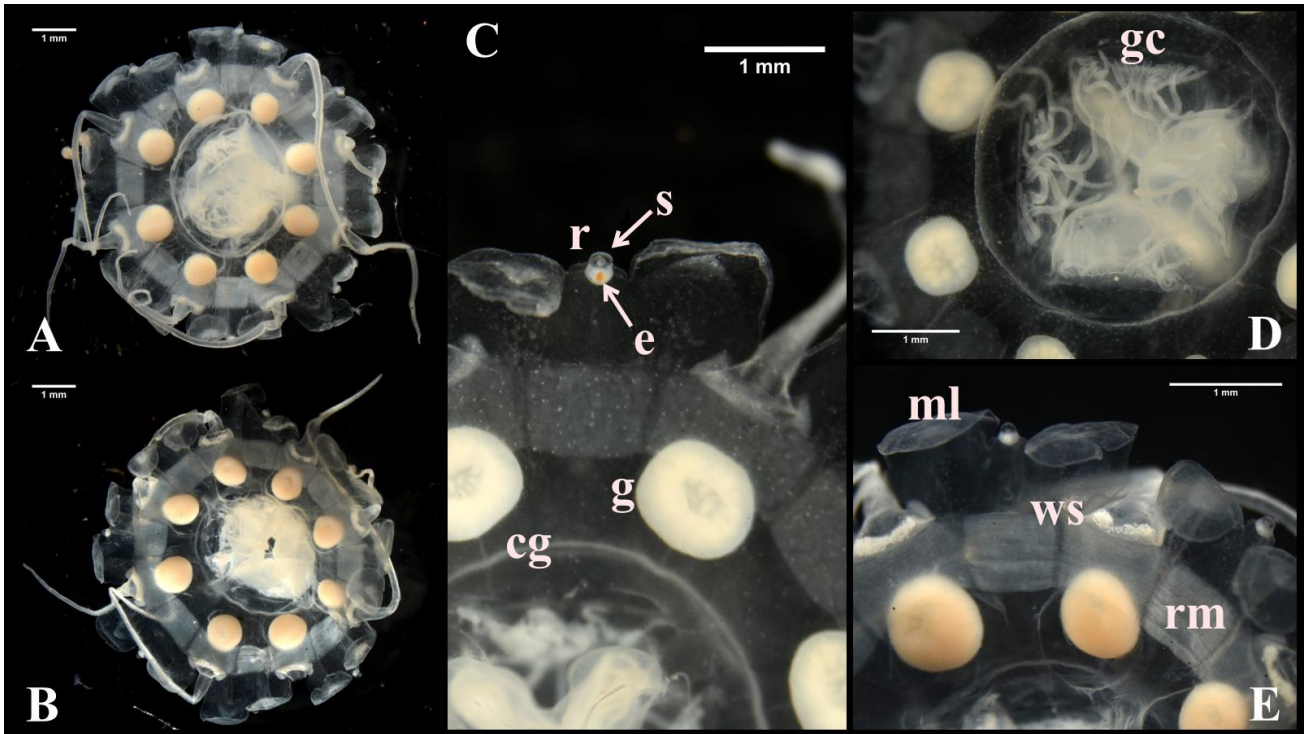
**Figure 4.** *Nausithoe globifera* (A and B: NHM; C and D: NHM 136). **A** - side view of a female; note the high dome shaped central disc and the oval gonads with hundreds of eggs (**g ♀**). **B** - side view of a male medusa, focusing on the deep coronal groove (**cg**) and gonads (**g ♂**); it is also possible to see a small crustacean prey inside the gastrovascular cavity. **C** - side view of a male with the red pigmentation of the gastrovascular cavity and the gastric cirri (**gc**) still preserved. **D** - aboral view of the same animal as C.



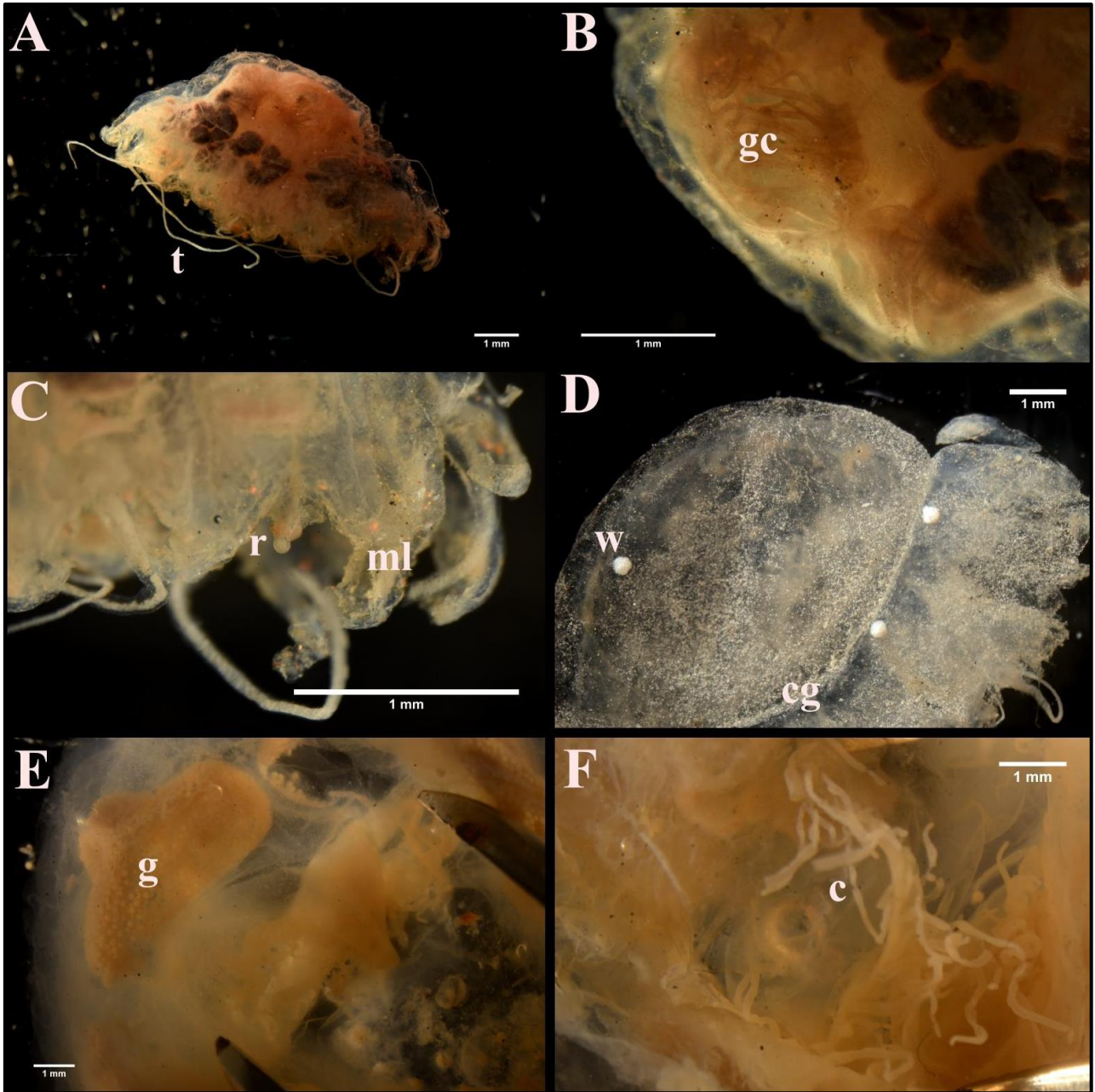
**Figure 5.** *Nausithoe maculata* (NMNH 57660). **A** - Detail of rhopalium (**r**), with statocyst (**s**) and ocellus (**e**), and marginal lappets (**ml**) with the yellow pigment spot (**ps**), essential for identifying this species. **B** - Oral view of the medusa, showing the mouth (**m**) and gonads (**g**). **C** - Aboral view of the medusa, showing the coronal groove (**cg**) and gastrovascular cavity (**gc**) with cirri.



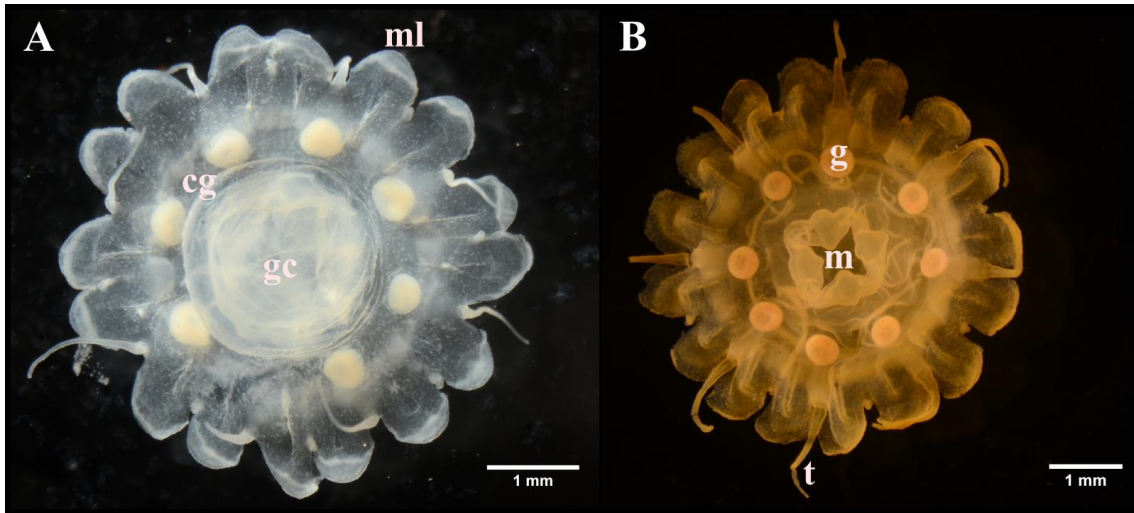
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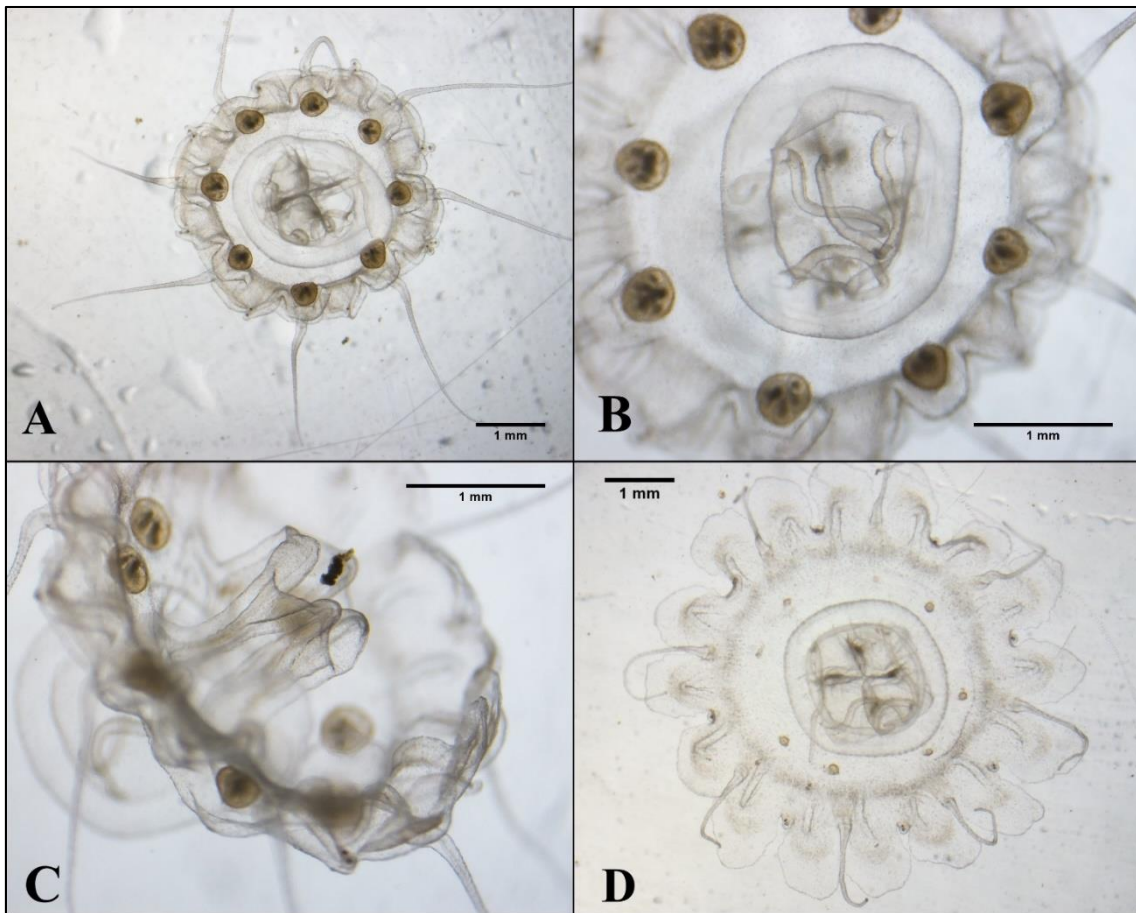
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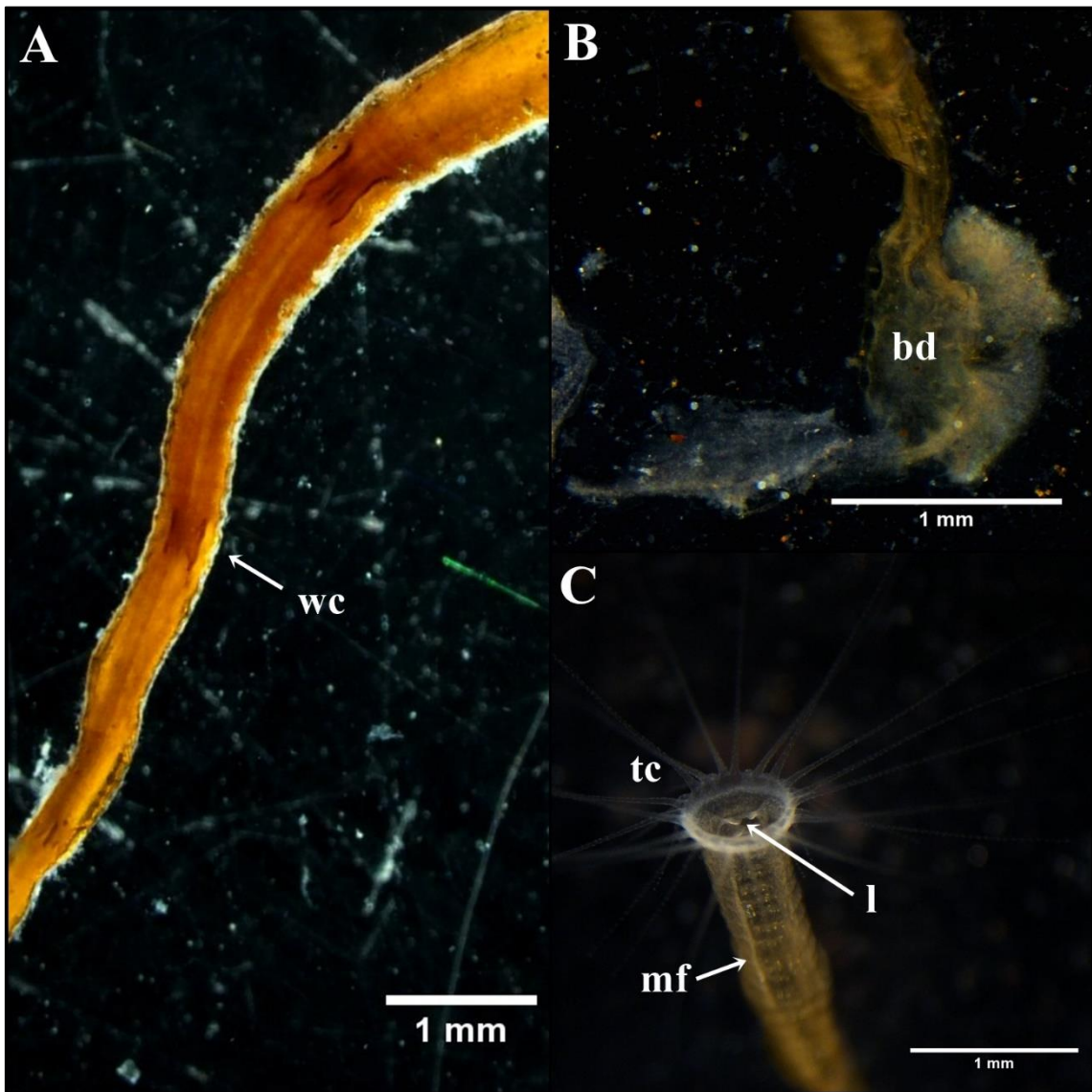
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**Figure 9.** Two specimens identified as *Nausithoe wernerii* (A: ZMH C10693; B: ZMH C10602). **A** - Aboral view showing the coronal groove (**cg**), gastrovascular cavity (**gc**) and marginal lappets (**ml**); **B** - Oral view, emphasizing the mouth (**m**), gonads (**g**) and tentacles (**t**).

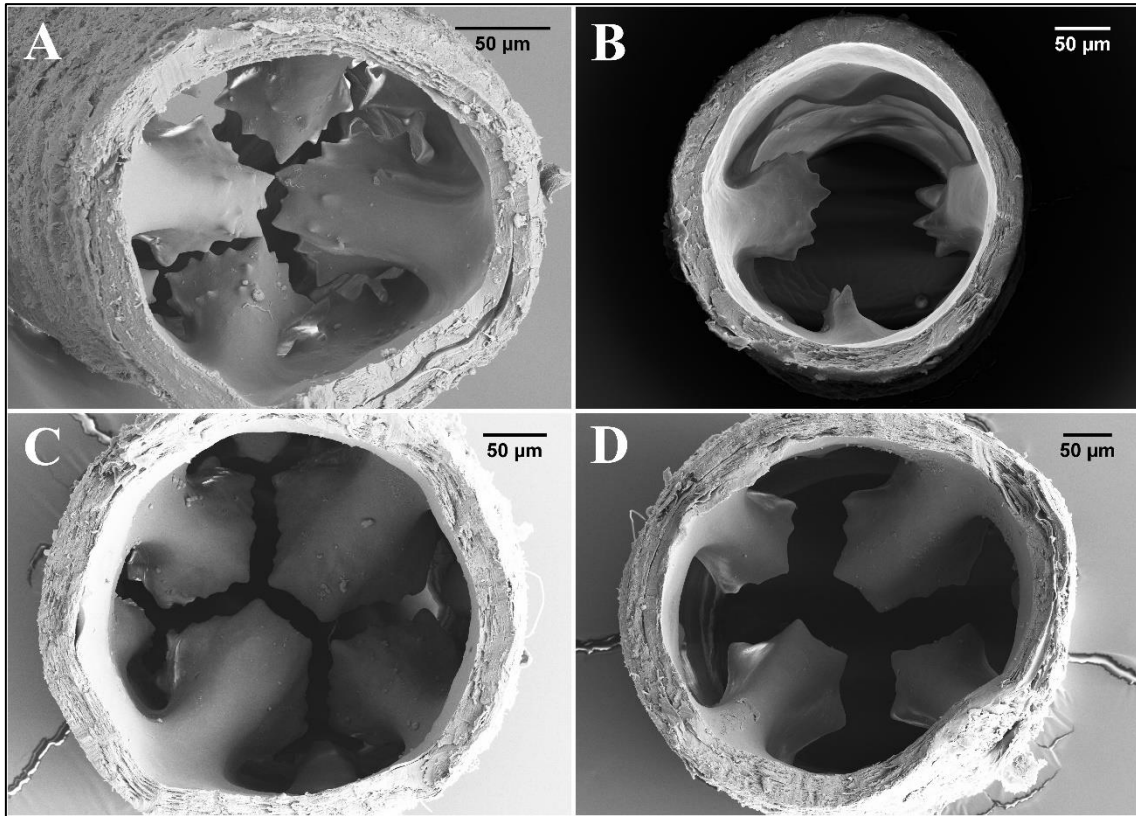


**Figure 10.** *Nausithoe wernerii* male medusae from culture (A, B and C – 5 months old; D – 3 months old). **A** – Aboral view of an adult medusa with mature gonads and contracted lappets. **B** - Detail of gastrovascular region focusing on the gastric cirri (only four developed at that point). **C** – Side view focusing on the lips and the extension of the manubrium. **D** – Beginning of gonads development (aboral view).

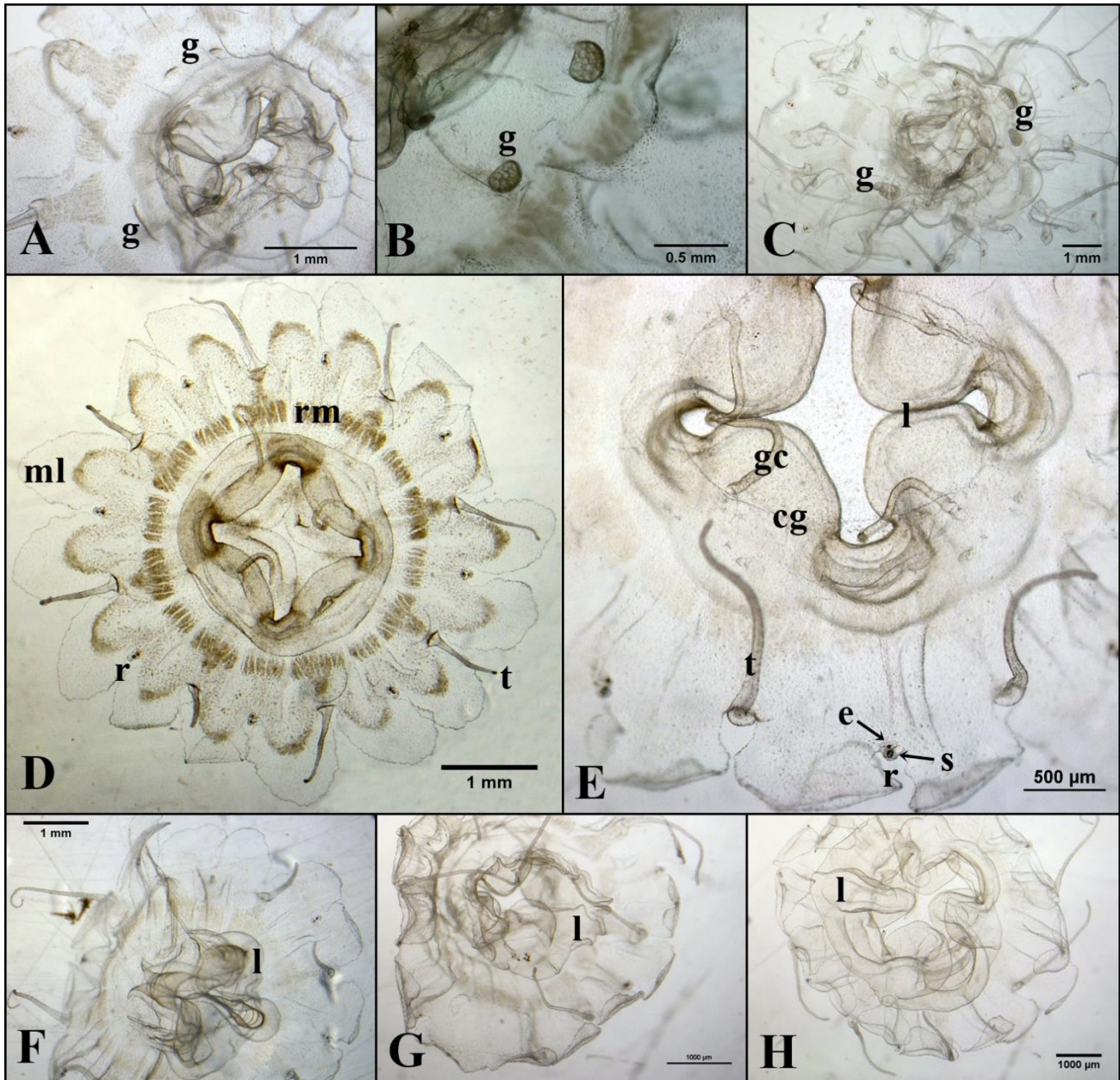


**Figure 11.** *Nausithoe silveira* n. sp. polyps. **A** – external view of polyp AC18 showing by transparency the internal whorls of cups (**wc**); **B** – tissue ball emerged polyp showing basal disc (**bd**), the ephyra that originated the tissue ball came from the polyp AC01; **C** – oral disc of the same polyp in **B**, highlighting the tentacles crown (**tc**) and the four lips (**l**) along with the muscle filaments in the gastric longitudinal septae (**mf**).

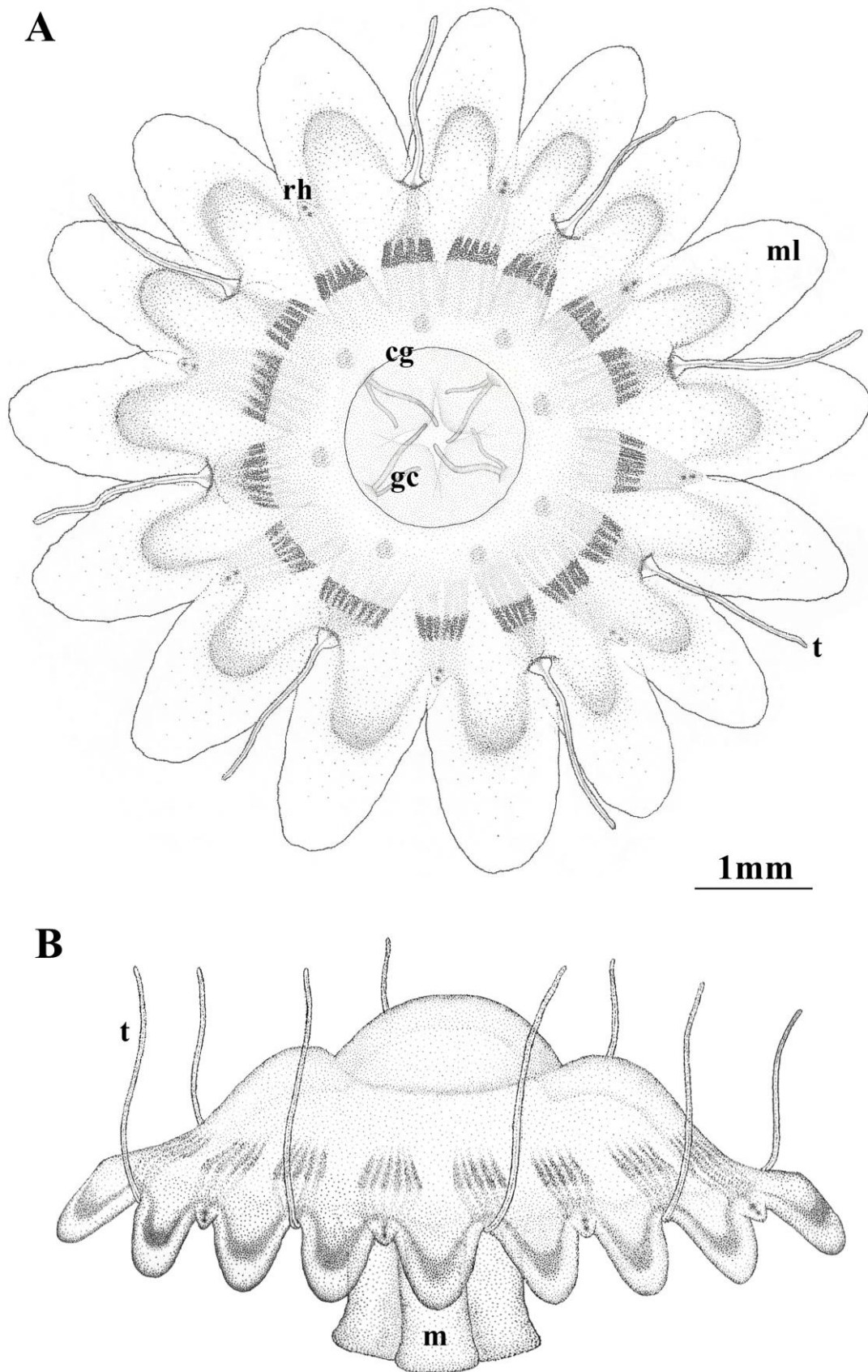




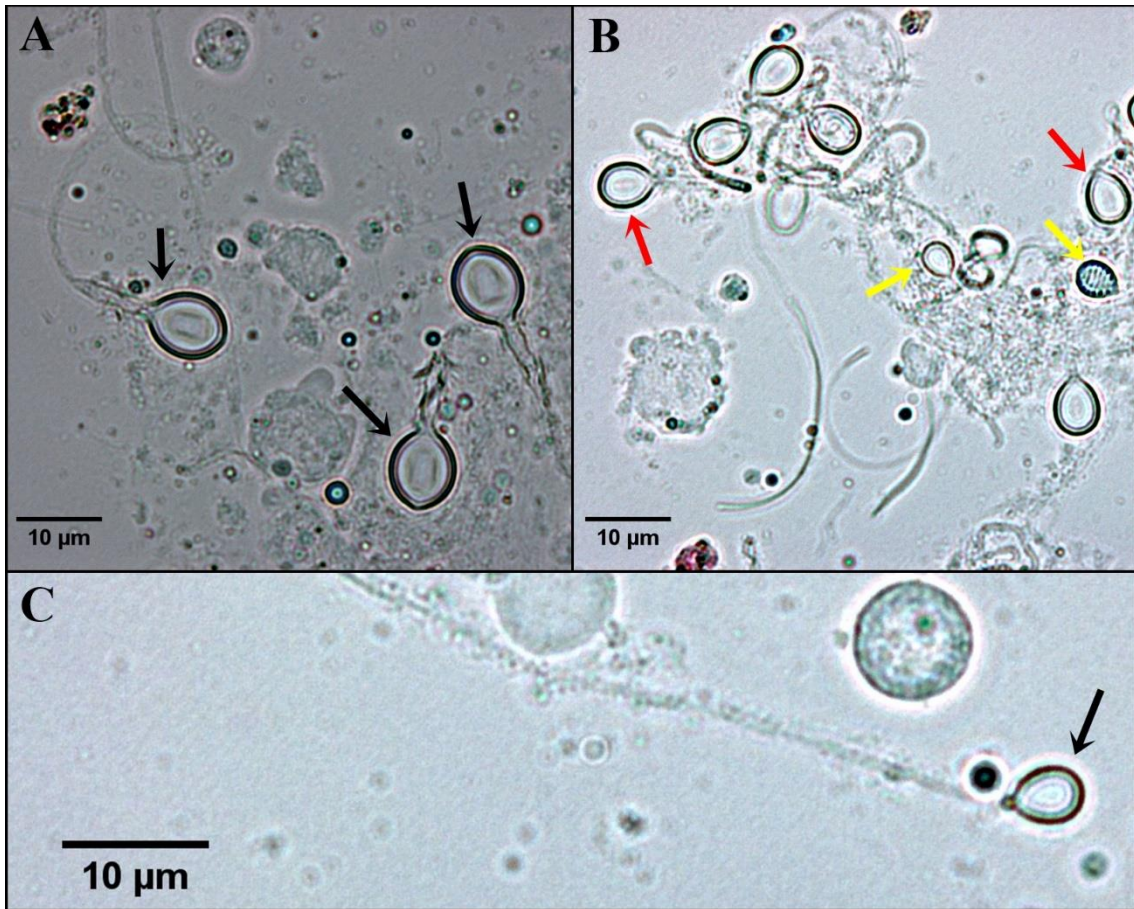
**Figura 12.** *Nausithoe silveira* n. sp. whorls of cusps seen by SEM (different heights of the polyp AC02). **A** – a more basal series, with 16 cusps and additional cusps over the 4 larger perradial ones; **B** – an intermediate series with a malformation, presenting only 4 irregular-shaped cusps; **C** and **D** – two more distal series, being the second the highest, both with 8 cusps..



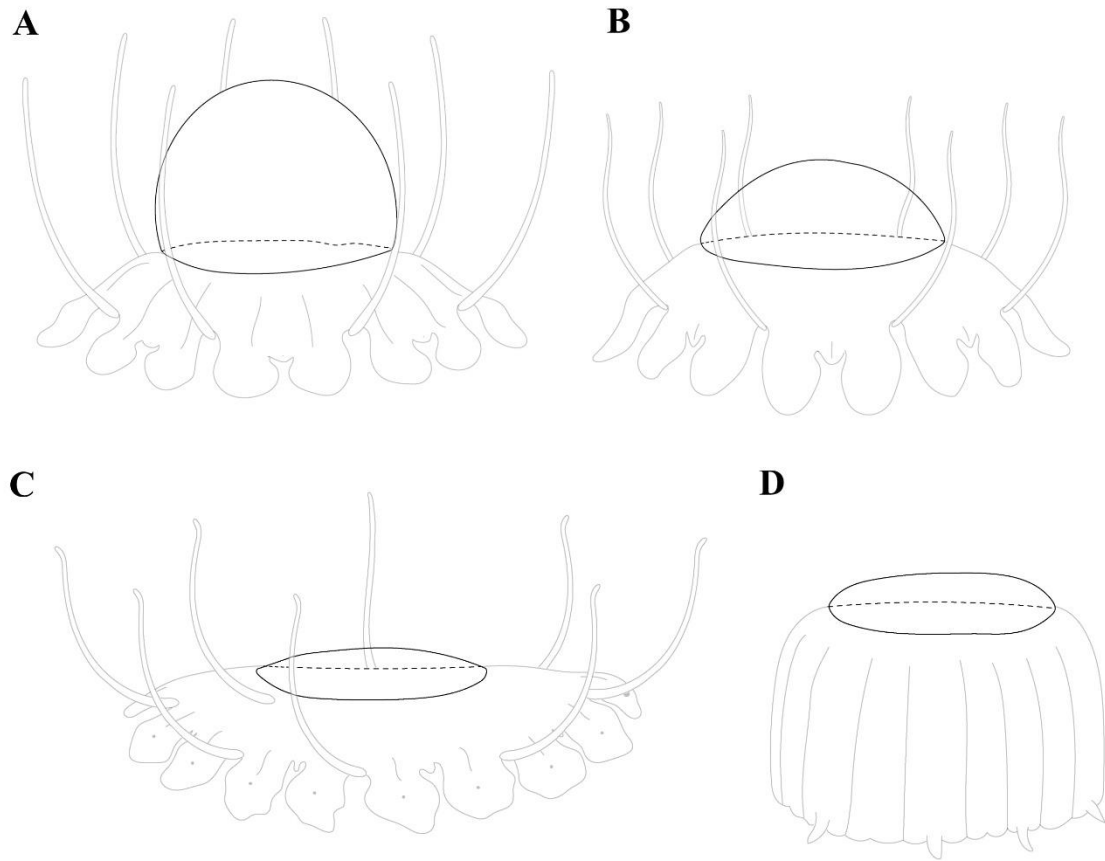
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**Figure 14.** Illustration of a *Nausithoe silveira* n. sp. grown medusa. **A** – aboral view. **B** – lateral view. **rh** – rhopalium; **ml** – marginal lappets; **m** – manubrium; **cg** – coronal groove; **gc** – gastric cirri; **t** – tentacle.



**Figure 15.** Photomicrographs of the two nematocyst types found in *Nausithoe silveira* n. sp. **A** – Three heterotranchous microbasic eurytele discharged. **B** – Holotranchous isorhiza capsules with two different sizes (bigger pointed in red and smaller pointed in yellow). **C** – One discharged holotranchous isorhiza.



**Figure 16.** Proposed nomenclature to distinguish umbrella outlines in Coronatae medusa according to the height of the central disc in comparison to the rest of the body. **A** – Hyperdome (dome higher than the marginal disc radius); **B** – Isodome (dome with about the same size of the marginal disc radius); **C** – Hypodome (dome smaller than the marginal disc radius) **D** – Catadiscus (marginal disc facing down, perpendicularly to the coronal groove).

## Capítulo 2

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**Relações filogenéticas na ordem Coronatae  
(Coronatae; Scyphozoa) com ênfase no gênero  
*Nausithoe* Kölliker, 1853**

A ser submetido à *Invertebrate Systematics*.

# Phylogenetic relationships within the order Coronatae (Coronatae; Scyphozoa), with emphasis on genus *Nausithoe* Kölliker, 1853

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**Abstract:** The use of genetic information to describe species, corroborate or refute parentage hypotheses and propose phylogenetic relationships among taxa has been increasingly accepted/required in the study of Scyphozoa's systematics. The morphological similarity of many species, along with 100 years old descriptions, has made it difficult to study the diversity and systematics of the group, being the application of molecular analysis essential to solve these gaps. In this article, we bring new DNA sequences from specimens of the order Coronatae and propose, for the first time, a phylogenetic relationship for the group.

**Keywords:** Systematics, cryptic species, COI, 28S, 18S.

**Resumo:** A utilização de informações gênicas para descrever espécies, corroborar ou refutar hipóteses de parentesco e propor relações filogenéticas entre táxons tem sido cada vez mais aceito/exigido no estudo da sistemática de Scyphozoa. A similaridade morfológica de muitas espécies, aliada a descrições centenárias, têm dificultado o estudo da diversidade e sistemática do grupo, sendo a aplicação da análise molecular essencial para resolver essas lacunas. Neste artigo, trazemos novas sequências de DNA de espécimes da ordem Coronatae e propomos, pela primeira vez, uma relação filogenética para o grupo.

**Palavras-chave:** Sistemática, espécies crípticas, COI, 28S, 18S

## Introduction

On the past three decades, the use of molecular data has been extremely helpful to solve the systematics of several marine invertebrates, especially the ones we have limited biological knowledge, few samples and/or more than one plausible classificatory proposal. In relation to the phylum Cnidaria, traditional data (18S, 28S, 16S or even COI) and recent phylogenomic analysis have allowed to corroborate, reject or propose new groups in the context of modern systematics (Collins *et al.* 2006, Kayal *et al.* 2018).

Based on this kind of data Bayha *et al.* (2013) brought evidences supporting the monophyly of the class Scyphozoa Goette, 1887, with two monophyletic groups within: Coronatae Vanhöffen, 1892 and Discomedusae Haeckel, 1880 (Semaestomeae L. Agassiz, 1862 and Rhizostomeae Cuvier, 1799). The authors also sampled Coronatae families previously supported by morphological characters but didn't manage to resolve interfamilial relationships. Low sampling did not allow to corroborate any relationship or even monophyly of minor groups.

In fact, only few studies on Coronatae families tried to elucidate species relationships within the group. Hingston *et al.* (2007) combined a morphological and 28S DNA analysis, considering only six species of the order Coronatae, and proposed three different groups: *Nausithoe punctata* Kölliker, 1853 + *N. weneri* Jarms, 1990 + *N. planulophora* (Werner, 1971); *Thecoscyphus zibrowii* Werner, 1984 + *N. eumedusoides* (Werner, 1974); and *Atolla vanhoeffeni* Russell, 1957 + *N. marginata* Kölliker, 1853. Unfortunately, the genetic sequences for this study were never available.

In this article, we obtained new data of DNA sequences from Coronatae species and propose a phylogenetic relationship of the families within the group.

## Methods

Tissues for molecular analyses were obtained from specimens in cultivation at the “Laboratório de Cultivo e Estudos de Cnidaria” and processed at the “Laboratório de Evolução Molecular” of the Zoology Department – IB, USP. Data from GenBank database was also included; however, some coronate vouchers available on GenBank were excluded from these analyses because we believe those species could be easily misidentified by non-experts in the group's taxonomy. Material for the extractions was preserved in alcohol 92% as vouchers.



For DNA extraction we used ammonium acetate; protocol adapted from Fetzner (1999). Three markers were amplified through Polymerase Chain Reaction (PCR): nuclear ribosomal 18S and 28S and mitochondrial protein coding COI. Primers successfully used for each gene were the same as mentioned in Chapter 1 of this Dissertation. Final concentration for PCR were: 0.4 nM of each primer, 2 mM MgCl<sub>2</sub>, 1x PCR buffer (Gotaq), 0,08 mM dNTPs (Fermentas), 0.25 units of TaqDNA polymerase (Gotaq G2 Flexi, Promega), in a final volume of 25 µL. Results were analyzed through agarose gel (1.5%) electrophoresis. Products of the amplification were purified with Agencourt® AMPure® kit. The BigDye reaction was done using same primers and T<sub>m</sub> for each case. Sequencing was performed in the “Laboratório de Sinalização de Redes Regulatórias de Plantas” in the Botanical Department, IB – USP, with a 3730xl DNA Analyzer. The sequence’s alignment and editing were performed with Geneious® software (Kearse *et al.* 2012). We used the BLAST algorithm to identify sequencing errors and/or contaminations. Taking into account our new sequences and those from GenBank (Coronatae and proper outgroups; Tab. 1), we created alignment of 18S and 28S markers, using the MAFFT (E-INS-i profile) plug-in in Geneious and for COI we used the MUSCLE (translation aware profile) plug-in. Final alignments achieved ~1800 bp for the 18S marker, ~1000 bp for the 28S marker and ~550 bp for COI marker.

Phylogenetic analyses were conducted on IQ-TREE 1.6.11 (Nguyen *et al.* 2015) by Maximum Likelihood approach (ML). Analyses considered both multilocus and single-gene datasets. For all cases, the clade stability from optimal trees were defined by four methods in IQ-TREE, two parametric (aBAYES, aLRT) and two non-parametric (standard bootstrap BS, SH-like, SH-aLRT). Using this approach, we try to circumvent obvious limitations from each support technique (Anisimova *et al.*, 2011). From our results we remark as high support for a certain clade if we have at least three of four support values with high values, where high values are aBAYES  $\geq 0.95$ , aLRT  $\geq 0.9$ , SH-aLRT  $\geq 0.85$ , BS  $\geq 75$ .

## Results

We analysed molecular data from 11 Coronatae species (“ingroup”: three Nausithoidae, two Linuchidae, one Atorellidae, three Atollidae, one Periphyllidae and one Paraphyllinidae), and other seven Discomedusae (“outgroup”: Table 1). Our main result of this study is summarized in Fig. 1 (multilocus analysis). The monophyly of the

order Coronatae is corroborated, where genus *Nausithoe* is sister group of a clade defined by the other 5 families (*Linuche* (*Atorella* (*Atolla* (*Periphylla*, *Paraphyllina*))). All main clades present high support.

As detailed in Chapter 1 of this dissertation, *N. aurea* and *N. maculata* have less than 3% of genetic differences from one to another, which indicates they are highly probable the same species. Those specimens were obtained from the wild (Cuba and Brazil), kept in our cultivation and their identification was precise. Taking into account *Linuche* specimens, they have almost 8% of genetic differences (see Appendix in the end of this dissertation), where only *L. unguiculata* has COI sequence. This might bring an erroneous interpretation of data because only more conservative markers were sampled (18S, 28S); in fact, when we observe the phylogram (Fig. 1) those specimens look extremely similar/closely related. Moreover, none of those vouchers have pictures of the specimens so their identification cannot be verified. Finally, the clade represented by *Atorella* (*Atolla* (*Periphylla*, *Paraphyllina*))) has low support but with no obvious alternative configuration; once again we attribute this result to the lack of COI sequences for three of six terminals.

Although single-gene analyses are limited because of data missing, they are useful to remark the importance of multilocus data matrix to infer better resolved clades for Coronatae.

## **Discussion**

The systematics of Coronatae species was historically based on morphological features of few specimens, described around 100 years ago. The difficulty of studying the external morphology of gelatinous animals (mostly because preservation techniques and substances can affect the main shape of jellyfishes) can also cause incorrect assumptions about species relationships. In such case, the use of molecular data could be an important toll. However, the lack of specialized taxonomists for the group makes the barcoding of coronate species questionable (Collins & Cruickshank 2013). This might be one of the reasons for the absence of a solid and robust phylogenetic proposal for the order until the present day.

We combined sequences from species cultivated in our laboratory with some of the few sequences available on GeneBank. Our results show the position of the genus *Nausithoe* as a basal group within the order Coronatae. Nevertheless, the high

morphological diversity of species described for this genus was not represented in our analyses, what we believe might be the weakest point of this study.

Because our results show that *N. maculata* and *N. aurea* have less than 3% of genetic differences (with all markers combined), we suggest that both species should be synonymized.

The monophyly of *Linuche* was also corroborated; these colonial scyphozoans are the sister group of *Atorella* + [*Atolla* + *Periphylla* + *Paraphyllina*]. The morphological data corroborates this result, taking into consideration the absence of a polyp stage in *Atolla*, *Periphylla* and *Paraphyllina* (Jarms *et. al.* 1999). For this reason, we propose to name this group as **Holoabyssi**.

As a macroevolutionary trend, we can remark the deep-water holoplanktonic clade as a derived group. These groups are highly difficult to study because of scarce sampling and for counting with just a few specialists worldwide able to properly identify specimens with certainty. It is fundamental to increase the barcoding of coronate species to help the comprehension of the group's phylogeny. A precise identification of specimens, with pictures and geographic occurrence of vouchers, is essential to achieve this goal. Apart from molecular information, it is crucial to expand our knowledge on species morphology, life cycle and geographic distribution. Elucidating molecular and morphological data of “non-standard” species (with remarkable different characteristics), as *Thecoscyphus zibrowii* Werner, 1984 and *Nausithoe eumedusoides* (Werner, 1974), both with a reduced medusa in the life cycle, would help to understand scyphozoans' evolutionary relationships.

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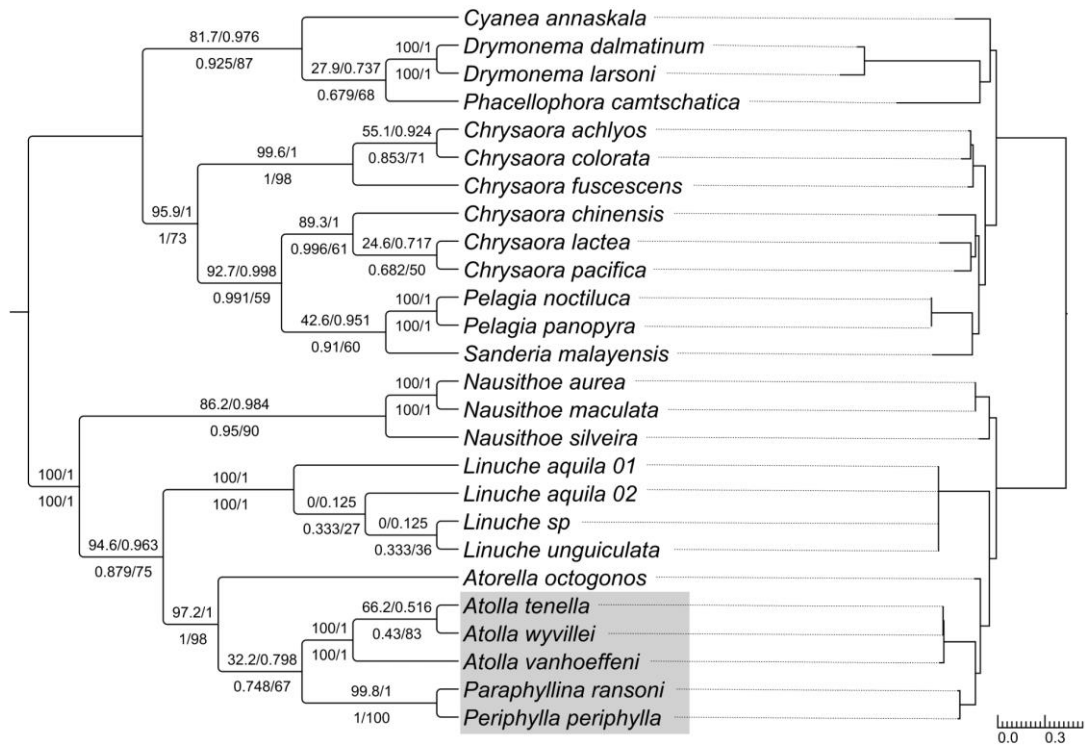
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**Table 1.** Species analyzed in this study. "Family (WoRMS)" indicates the family to which the genus or species belongs according to the WoRMS database. The column "nr" indicates total number of analyzed samples. "COI", "18S" and "28S" present GenBank accession numbers for each molecular marker; sequences generated in this study are in green. Molecular markers for a certain species obtained from different vouchers are highlighted (GenBank code, voucher and Country/Region). Code "no info" refers to those sequences where information is missing (i.e., voucher and/or country/region).

Family (WoRMS)	nr	Species	COI	18S info	28S info	Voucher	Country/Region
Nausithoidae	1	<i>Nausithoe aurea</i>	This study	This study	This study	CGM_NA1	Brazil/ São Paulo
	2	<i>Nausithoe maculata</i>	This study	This study	This study	CGM_NM1	Cuba
	3	<i>Nausithoe silveira</i>	This study	This study	This study	CGM_AC10	Brazil/ Rio de Janeiro
Linuchidae	4	<i>Linuche aquila</i>	No info	HM194772	HM194827	M0D00695S	Papua New Guinea/ Julian Reef
	5	<i>Linuche aquila</i>	No info	HM194777	HM194830	M0D00890F	Palau/ Melekeok
	6	<i>Linuche unguiculata</i>	JN700939	HM194806	HM194859	M0D13159C	Bermuda
	7	<i>Linuche sp.</i>	No info	This study	This study	CGM_L1	USA/ near Monterey Bay, CA
Atorellidae	8	<i>Atorella octogonos</i>	No info	HM194801	HM194854	M0D09903W	USA/ near Monterey Bay, CA
	9	<i>Atorella octogonos</i>	This study	This study	This study	CGM_AO1	Brazil/ Rio de Janeiro
Atollidae	10	<i>Atolla tenella</i>	No info	HM194799	HM194852	M0D06581C	Beaufort Sea
	11	<i>Atolla vanhoeffeni</i>	<b>GQ120084</b>	AF100942	AY026368	No info <b>Atol04</b>	No info <b>No info</b>
	12	<i>Atolla wyvillei</i>	<b>GQ120088</b>	HM194788	HM194841	M0D05955A <b>Atwy03</b>	USA/ Monterey Bay, CA <b>No info</b>
Periphyllidae	13	<i>Periphylla periphylla</i>	No info	HM194775	<b>HM194842</b>	M0D00742N <b>M0D05956B</b>	Norway/ Sognefjorden <b>Mexico/ Gulf of California</b>
Paraphyllinidae	14	<i>Paraphyllina ransoni</i>	No info	HM194787	HM194840	M0D05954Z	USA/ near Monterey Bay, CA
Cyaneidae	15	<i>Cyanea annaskala</i>	<b>AY902915</b>	HM194778	HM194831	M0D00908X <b>CHE4</b>	Australia/ Tasmania, Huon Estuary <b>Australia/ Tasmania, Huon Estuary</b>
Drymonematidae	16	<i>Drymonema dalmatinum</i>	HQ234621	HM194805	HM194858	M0D13158B	Turkey/ Foca
	17	<i>Drymonema larsoni</i>	HQ234650	HQ234652	HQ234661	M0D15759C	USA/ Dauphin Island, AL
Phacellophoridae	18	<i>Phacellophora camtschatica</i>	MF742360	<b>HM194822</b>	<b>HM194875</b>	USCAPIL <b>M0D02660H</b>	No info <b>USA: Morro Bay, CA</b>
Pelagiidae	19	<i>Chrysaora achlyos</i>	KY611221	KY610804	KY610948	M0D006019M_MXBSMAG	Mexico: Baja California Sur, Bahia Magdalena
	20	<i>Chrysaora fuscescens</i>	KY611230	<b>HM194815</b>	<b>HM194868</b>	M0D014619G_USCAMBQ	USA: California

					<b>M0D14619G</b>	<b>Aquarium of the Americas (New Orleans, LA)</b>
21	<i>Chrysaora chinensis</i>	KY611281	KY610842	KY610955	M0D022671Y_THKRPKOP	Thailand: Ko Panak
22	<i>Chrysaora colorata</i>	KY611228	KY610839	KY610946	M0D022666T_USCAMBQ	USA: California, Monterey Bay Aquarium
23	<i>Chrysaora lactea</i>	KY611264	HM194810	HM194863	M0D14610X	Brazil: Rio de Janeiro
24	<i>Chrysaora pacifica</i>	KY611276	KY610838	KY610952	M0D022681I_USCAMBQ	USA: California, Monterey Bay Aquarium
25	<i>Pelagia noctiluca</i>	KY611285	KY610844	KY610983	M0D021334N_CRPUDOM	Costa Rica: Punta Arenas, Dominical
26	<i>Pelagia panopyra</i>	KY611291	KY610848	KY610985	M0D001464H_IDPAGFC	Indonesia
27	<i>Sanderia malayensis</i>	KY611266	KY610808	KY610995	M0D022660N_USCTNOQ	USA: Norwalk Aquarium

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**Figure 1.** Phylogenetic tree of the order Coronate based on two ribosomal molecular markers (18S and 28S) and one mitochondrial molecular marker (COI). The number of markers per species is specified in Tab. 1. Supports values are aBAYES and aLRT (parametric, upper branch) together with SH-like and BS (non-parametric, lower branch) in clockwise fashion.

## Discussão Geral e Conclusões

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Tanto o gênero *Nausithoe* quanto os demais gêneros pertencentes à ordem Coronatae tiveram grande parte de suas espécies descritas há 100 anos ou mais (*e.g.* Kölliker, 1853; Haeckel, 1880; Maas, 1897). A utilização de caracteres muitas vezes não informativos e/ou não padronizados para cada descrição, juntamente com a grande similaridade morfológica do grupo, têm dificultado a resolução de sua sistemática, não havendo, até o presente momento, nenhuma proposta de parentesco entre as seis famílias de cifozoários coronados. Werner (1973) propõe uma relação evolutiva entre espécies de Nausithoidae baseado no padrão de formação das colônias de pólipos. Só considerando o gênero *Nausithoe*; Bigelow (1928) sugere três grupos morfológicamente distintos: I) *N. punctata*, *N. challengeri*, *N. picta* e *N. limpida* (grupo unido pela presença de ocelo e filamentos gástricos em séries contínuas, não em tufos); II) *N. rubra*, *N. atlantica* e *N. globifera* (filamentos gástricos em séries contínuas, não em tufos, mas sem ocelo); III) *N. albatrossi* (filamentos gástricos organizados em tufos).

O emprego de informações genéticas das espécies, alinhado aos dados morfológicos já existentes, tem ajudado a resolver a sistemática de diversos grupos de invertebrados marinhos. Infelizmente, poucos sequenciamentos moleculares de cifozoários coronados foram feitos até o momento, sendo grande parte deles com espécimes identificados apenas ao nível de gênero.

Nossos resultados, tanto morfológicos quanto moleculares trazem um direcionamento para a identificação correta das espécies do gênero *Nausithoe*. A partir de nossa análise morfológica, validamos doze espécies do gênero (*N. albatrossi*, *N. challengeri*, *N. eumedusoides*, *N. globifera*, *N. hagenbecki*, *N. maculata*, *N. marginata*, *N. planulophora*, *N. racemosa*, *N. rubra*, *N. sorbei* e *N. weneri*), sugerimos que algumas sejam sinonimizadas (*N. albida*, *N. clausi*, *N. limpida* e *N. picta* = *N. punctata*; *N. atlantica* = *N. rubra*; e *N. aurea* = *N. maculata*) e questionamos a validade de outras (*N. thieli*, *N. striata* e *N. simplex*). A comparação morfológica nos possibilitou, também, questionar alguns caracteres historicamente utilizados para descrever/identificar esses organismos, como o número de cirros gástricos e o formato do domo do disco central da umbrela.

A implementação dos dados de genética em nosso estudo se mostrou extremamente relevante para a descrição de uma nova espécie para o Atlântico Sul,



*Nausithoe silveira* n. sp., e comparação com outras do gênero. A partir desses dados, também foi possível unir nosso conhecimento na morfologia do grupo à base de dados já disponível no Genbank para propor uma relação filogenética entre as famílias da ordem Coronatae.

Pouco se sabe, ainda, sobre muitas das espécies de cifozoários coronados, sendo essencial que essas lacunas sejam preenchidas para que a compreensão da diversidade e sistemática do grupo seja clareada. Para isso, incentivamos o cultivo de espécimes, para que mais informações a respeito do ciclo de vida possam ser geradas (*e.g.* Jarms, 1990; Silveira & Morandini, 1997), já que muitas espécies são descritas apenas pelas medusas, sem informação da fase polipóide. É fundamental, também, que a informação genômica seja incorporada nos trabalhos de taxonomia, tanto dos exemplares cultivados em laboratório quanto provenientes de coletas, por auxiliarem na identificação de espécimes/descrição de espécies crípticas e compreensão da riqueza genética nas populações. Por último, embora ainda pouco utilizado como caractere taxonômico em Scyphozoa, a análise de nematocistos já se mostrou extremamente informativa para alguns grupos de cnidários (Mariscal, 1974; England, 1991; Östman, 2000).

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## Resumo Geral

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A ordem Coronatae Vanhoeffen, 1892 é comumente conhecida por suas medusas de profundidade, embora sua diversidade também abrange espécies de águas rasas. Em um âmbito geral, pouco se sabe sobre a maioria das espécies descritas para o grupo devido: à dificuldade de coleta dos espécimes, alguns por serem extremamente pequenos e outros por habitarem grandes profundidades; às descrições morfológicas antigas e pouco informativas; e ao baixo número de taxonomistas especialistas na ordem. Dentre os gêneros de cifozoários coronados, o gênero *Nausithoe* Kölliker, 1853 (Coronatae, Scyphozoa, Cnidaria) possui o maior número de espécies descritas. Na presente dissertação, trago uma revisão morfológica das espécies desse gênero, adicionando informações para algumas delas, a partir de exemplares provenientes de quatro diferentes museus. A partir desses dados, validamos doze espécies do gênero, sugerimos que algumas sejam sinonimizadas e questionamos a validade de outras. A comparação morfológica nos possibilitou, também, questionar alguns caracteres historicamente utilizados para descrever/identificar esses organismos, como o número de cirros gástricos e o formato do domo do disco central da umbrela.

Além disso, faço a descrição de uma nova espécie para o grupo a partir de pólipos coletados no estado do Rio de Janeiro (Brasil), a 227 m de profundidade. Foram utilizados como caracteres descritivos dados de ciclo de vida, morfologia do pólipo e da medusa, análise de nematocistos (pólipo, éfira e medusa) e sequências de DNA ribossomal e mitocondrial. Estendi a análise molecular para outras espécies da ordem, propondo, com a inclusão de dados disponíveis no GeneBank, uma relação de parentesco entre as famílias de coronados, algo inédito na literatura para o grupo.

## Abstract

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The order Coronatae Vanhöffen, 1892 is commonly known as a deep-sea jellyfish group, although its diversity also embrace species from shallow waters. In general, little is known about most species described for the group due to: the difficulty of sampling the specimens (some are extremely small and others inhabit great depths); old and non-informative morphological descriptions; and the low number of taxonomists working with the order. Among coronate scyphozoans, the genus *Nausithoe* Kölliker, 1853 (Coronatae, Scyphozoa, Cnidaria) has the largest number of species described. In this dissertation, I present a morphological review of the genus' species, adding information for some of them (obtained from specimens coming from four different museums). From these data, we validate twelve species of the genus, suggest some synonyms and questioned the validity of others. The morphological comparison also allowed us to inquiry some historically used characters to describe/identify these organisms, such as the number of gastric cirri and the central-disc dome shape.

In addition, I describe a new species for the group from polyps collected in the state of Rio de Janeiro (Brazil), 227 m deep. For that, I annotated information about life cycle, morphology of polyps and medusae, nematocysts types and measurements (polyp, ephyra and medusa) and ribosomal and mitochondrial DNA sequences. I extended the molecular DNA analysis to other species of the order, proposing, with the inclusion of data available on GeneBank, a phylogenetic relationship among Coronatae families, something never performed for the group.

## Anexo

Diferenças genéticas (em porcentagem) entre todas as espécies utilizadas para a construção das árvores (Capítulo 2), com combinação dos três marcadores (COI, 28S e 18S), quando disponíveis.

	<i>A. tenella</i>	<i>A. vanhoeffeni</i>	<i>A. wyvillei</i>	<i>Atorella octogonos</i>	<i>Chrysaora achlyos</i>	<i>Chrysaora chinensis</i>	<i>Chrysaora colorata</i>	<i>Chrysaora fuscescens</i>	<i>Chrysaora lactea</i>	<i>Chrysaora pacifica</i>	<i>Cyanea annaskala</i>	<i>Drymonema dalmatinum</i>	<i>Drymonema larsoni</i>	<i>Linuche aquila 1</i>	<i>Linuche aquila 2</i>	<i>Linuche sp</i>	<i>Linuche unguiculata</i>	<i>Nausithoe aurea</i>	<i>Nausithoe maculata</i>	<i>Nausithoe silveira</i>	<i>Paraphyllina ransoni</i>	<i>Pelagia noctiluca</i>	<i>Pelagia panopyra</i>	<i>Periphylla periphylla</i>	<i>Phacelophora camtschatica</i>	<i>Sanderia malayensis</i>
<i>Atolla tenella</i>		95.70	95.81	94.36	86.34	85.57	86.24	86.84	86.34	85.83	86.41	83.24	82.92	91.82	91.84	77.80	91.82	92.13	92.05	92.04	93.90	85.61	85.54	93.97	84.87	86.55
<i>Atolla vanhoeffeni</i>	95.70		95.52	96.85	84.70	84.96	84.86	86.18	85.88	85.52	85.11	81.80	82.13	94.20	94.22	78.74	89.54	90.94	90.47	91.10	96.38	84.41	84.26	92.78	84.53	85.48
<i>Atolla wyvillei</i>	95.81	95.52		96.88	84.02	84.13	84.03	85.43	85.00	84.84	84.42	80.92	81.37	94.31	94.32	78.84	88.59	89.67	89.14	90.10	96.42	83.69	83.52	91.99	83.46	84.42
<i>Atorella octogonos</i>	94.36	96.85	96.88		88.27	88.27	88.32	89.65	89.01	88.78	89.10	84.42	85.42	94.67	94.65	79.13	94.29	94.97	94.94	95.07	96.50	88.38	88.31	96.43	87.49	88.35
<i>Chrysaora achlyos</i>	86.34	84.70	84.02	88.27		90.15	94.85	95.08	90.80	91.28	89.79	84.68	84.51	87.48	87.50	73.76	85.27	84.69	84.19	85.12	87.87	90.56	90.50	85.63	88.05	90.61
<i>Chrysaora chinensis</i>	85.57	84.96	84.13	88.27	90.15		90.31	91.33	90.62	90.97	89.50	84.82	84.59	87.63	87.63	73.03	85.33	85.36	84.83	85.26	87.80	90.17	90.08	85.94	87.05	89.87
<i>Chrysaora colorata</i>	86.24	84.86	84.03	88.32	94.85	90.31		94.86	90.95	91.40	90.07	84.81	84.70	87.78	87.79	73.94	85.17	84.88	84.43	85.31	87.99	90.42	90.31	85.79	88.47	90.16
<i>Chrysaora fuscescens</i>	86.84	86.18	85.43	89.65	95.08	91.33	94.86		92.13	92.34	90.92	85.36	85.83	88.85	88.87	74.29	85.79	86.23	85.75	86.45	89.28	91.80	91.92	86.81	89.18	91.24
<i>Chrysaora lactea</i>	86.34	85.88	85.00	89.01	90.80	90.62	90.95	92.13		92.20	90.06	85.05	85.12	88.37	88.37	73.65	85.41	86.13	85.53	86.32	88.67	90.50	90.47	86.40	88.31	90.40
<i>Chrysaora pacifica</i>	85.83	85.52	84.84	88.78	91.28	90.97	91.40	92.34	92.20		89.80	84.65	85.29	87.93	87.93	74.12	84.99	85.34	84.81	86.12	88.38	90.32	90.32	85.81	88.46	90.99
<i>Cyanea annaskala</i>	86.41	85.11	84.42	89.10	89.79	89.50	90.07	90.92	90.06	89.80		84.98	85.16	88.43	88.45	73.88	85.26	85.45	85.17	85.94	88.48	89.75	89.69	86.29	88.56	89.01
<i>Drymonema dalmatinum</i>	83.24	81.80	80.92	84.42	84.68	84.82	84.81	85.36	85.05	84.65	84.98		91.76	84.47	84.47	71.46	81.68	81.08	81.05	81.24	84.63	84.26	84.24	82.34	83.87	84.47
<i>Drymonema larsoni</i>	82.92	82.13	81.37	85.42	84.51	84.59	84.70	85.83	85.12	85.29	85.16	91.76		85.40	85.40	71.46	82.10	81.78	81.76	82.03	85.45	84.48	84.51	82.99	84.40	84.10
<i>Linuche aquila 1</i>	91.82	94.20	94.31	94.67	87.48	87.63	87.78	88.85	88.37	87.93	88.43	84.47	85.40		98.28	82.51	97.92	93.21	93.18	93.38	94.07	88.15	88.08	94.03	87.30	87.61
<i>Linuche aquila 2</i>	91.84	94.22	94.32	94.65	87.50	87.63	87.79	88.87	88.37	87.93	88.45	84.47	85.40	98.28		82.53	97.93	93.25	93.22	93.41	94.05	88.17	88.10	94.05	87.32	87.61
<i>Linuche sp</i>	77.80	78.74	78.84	79.13	73.76	73.03	73.94	74.29	73.65	74.12	73.88	71.46	71.46	82.51	82.53		82.51	77.80	77.77	78.00	78.68	73.85	73.78	78.67	73.19	73.87
<i>Linuche unguiculata</i>	91.82	89.54	88.59	94.29	85.27	85.33	85.17	85.79	85.41	84.99	85.26	81.68	82.10	97.92	97.93	82.51		90.07	89.57	89.68	93.69	85.34	85.45	90.54	84.55	84.90
<i>Nausithoe aurea</i>	92.13	90.94	89.67	94.97	84.69	85.36	84.88	86.23	86.13	85.34	85.45	81.08	81.78	93.21	93.25	77.80	90.07		97.22	94.01	94.41	85.56	85.30	91.51	84.67	85.05
<i>Nausithoe maculata</i>	92.05	90.47	89.14	94.94	84.19	84.83	84.43	85.75	85.53	84.81	85.17	81.05	81.76	93.18	93.22	77.77	89.57	97.22		93.57	94.38	85.00	84.73	91.09	84.13	84.55
<i>Nausithoe silveira</i>	92.04	91.10	90.10	95.07	85.12	85.26	85.31	86.45	86.32	86.12	85.94	81.24	82.03	93.38	93.41	78.00	89.68	94.01	93.57		94.50	85.37	85.08	91.68	84.57	85.61

<i>Paraphyllina ransoni</i>	93.90	96.38	96.42	96.50	87.87	87.80	87.99	89.28	88.67	88.38	88.48	84.63	85.45	94.07	94.05	78.68	93.69	94.41	94.38	94.50		88.15	88.08	97.88	87.02	87.91
<i>Pelagia noctiluca</i>	85.61	84.41	83.69	88.38	90.56	90.17	90.42	91.80	90.50	90.32	89.75	84.26	84.48	88.15	88.17	73.85	85.34	85.56	85.00	85.37	88.15		97.94	85.28	87.86	89.73
<i>Pelagia panopyra</i>	85.54	84.26	83.52	88.31	90.50	90.08	90.31	91.92	90.47	90.32	89.69	84.24	84.51	88.08	88.10	73.78	85.45	85.30	84.73	85.08	88.08	97.94		85.28	87.77	89.90
<i>Periphylla periphylla</i>	93.97	92.78	91.99	96.43	85.63	85.94	85.79	86.81	86.40	85.81	86.29	82.34	82.99	94.03	94.05	78.67	90.54	91.51	91.09	91.68	97.88	85.28	85.28		84.67	85.29
<i>Phacellophora camtschatica</i>	84.87	84.53	83.46	87.49	88.05	87.05	88.47	89.18	88.31	88.46	88.56	83.87	84.40	87.30	87.32	73.19	84.55	84.67	84.13	84.57	87.02	87.86	87.77	84.67		88.10
<i>Sanderia malayensis</i>	86.55	85.48	84.42	88.35	90.61	89.87	90.16	91.24	90.40	90.99	89.01	84.47	84.10	87.61	87.61	73.87	84.90	85.05	84.55	85.61	87.91	89.73	89.90	85.29	88.10	