

UNIVERSIDADE ESTADUAL DE CAMPINAS INSTITUTO DE BIOLOGIA

BEATRIZ PEREIRA CUNHA

DIVERSITY OF FREE-LIVING MARINE NEMATODES: TAXONOMY AND SYSTEMATIC OF FAMILY CYATHOLAIMIDAE

DIVERSIDADE DE NEMATODAS MARINHOS DE VIDA LIVRE: TAXONOMIA E SISTEMÁTICA DA FAMÍLIA CYATHOLAIMIDAE

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DIVERSIDADE DE NEMATODAS MARINHOS DE VIDA LIVRE: TAXONOMIA E SISTEMÁTICA DA FAMÍLIA CYATHOLAIMIDAE

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Orientador: ANTÔNIA CECILIA ZACAGNINI AMARAL

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RESUMO

O Filo Nematoda é caracterizado pela enorme diversidade taxonômica, ecológica e geográfica, e pelo "astronômico" número de indivíduos. Apesar de sua importância ecológica, o conhecimento taxonômico do grupo é limitado devido principalmente à ausência de conhecimento sobre a homologia dos caracteres morfológicos e ao déficit de especialistas. Cyatholaimidae Filipjev, 1918 é uma família composta por táxons relativamente comuns e abundantes em ambientes marinhos. E assim como acontece com outros grupos de nematodas marinhos, a identificação desses organismos é desafiadora devido à sobreposição de alguns diagnósticos e à falta de conhecimento sobre a história evolutiva do grupo. O presente trabalho teve como objetivo realizar a revisão taxonômica da família e avaliar dados morfológicos e moleculares, buscando compreender as relações filogenéticas dentro do grupo, bem como, descrever as espécies novas identificadas e consequentemente ampliar o conhecimento da biodiversidade marinha do Estado de São Paulo. A revisão taxonômica de Cyatholaimidae aqui apresentada revelou, ao todo, 20 gêneros e 211 espécies consideradas válidas. No levantamento bibliográfico realizado foram encontrados 619 registros de espécies ao redor do mundo, principalmente nas regiões entremarés e sublitoral de praias e costões rochosos. Também existem espécies de Cyatholaimidae em ambientes de água doce e terrestre, todas classificadas no gênero Paracyatholaimus. Paralelamente foi realizada uma análise crítica dos padrões de distribuição geográfica e de habitat dessas espécies e elaboradas algumas perspectivas futuras sobre o estudo desses táxons. As análises filogenéticas baseadas nas regiões SSU e LSU rDNA indicaram a relação próxima entre Paracanthonchus, Paracyatholaimus e Praeacanthonchus, o que, apesar de não refletir a classificação atual, concorda com a proposta inicial de delimitação da subfamília Paracanthonchinae. O suplemento pré-cloacal em formato tubular e a abertura transversalmente orientada do poro complexo são prováveis sinapomorfias do clado. A parafilia de Cyatholaiminae foi confirmada e a validade de Pomponematinae não pode ser verificada já que há poucas sequências disponíveis de espécies classificadas nessa subfamília. O presente estudo apresenta a redescrição de duas espécies (*Paracanthonchus cochlearis* Gerlach, 1957 e Paracyatholaimus vitraeus Gerlach, 1957) e a descrição de três novas espécies (Biarmifer nesiotes Cunha, Fonseca & Amaral, 2023, Pomponema longispiculum Cunha, Fonseca & Amaral, 2023 e Longicyatholaimus sp. nov.), ampliando o número de registros no país para 14 gêneros e 23 espécies de Cyatholaimidae.

ABSTRACT

Phylum Nematoda is characterized by huge taxonomic, ecological, and geographic diversity, and by an astronomical number of individuals. Despite its ecological importance, the taxonomic knowledge about the group is limited mainly due to the lack of knowledge about morphological homology and specialists. Cyatholaimidae Filipjev, 1918 is a family composed of relatively common and abundant taxa in marine environments. As with other groups of marine nematodes, the identification of organisms is challenging due to the overlapping of some diagnoses and the lack of knowledge about the evolutionary history of the group. The present work aimed to carry out a taxonomic review of the family and evaluate the morphological and molecular data, seeking to understand the phylogenetic relationships within the group, as well as describe the new species identified, increasing the knowledge of marine biodiversity in the São Paulo State. The taxonomic review of Cyatholaimidae here presented revealed 20 genera and 211 species considered valid. In the bibliographic survey carried out, it was found 619 records of species around the world, mainly in the intertidal and sublittoral regions of beaches and rocky shores. Additionally, there are species of Cyatholaimidae found in freshwater and terrestrial environments, all classified in the genus Paracyatholaimus. A critical analysis of the geographic distribution and habitat patterns of these species was elaborated, together with some future perspectives on the study of these taxa. Phylogenetic analyzes based on the SSU and LSU rDNA regions indicated the close relationship between Paracanthonchus, Paracyatholaimus, and Praeacanthonchus, which, despite not reflecting the current classification, agrees with the initial proposal for delimiting the subfamily Paracanthonchinae. The tubular-shaped precloacal supplement and the transversely oriented opening of the pore complex are likely synapomorphies of this clade. The paraphyly of Cyatholaiminae was confirmed and the validity of Pomponematinae has not been verified due to the low number of sequences available. The present study presents the redescription of two species (Paracanthonchus cochlearis Gerlach, 1957 and Paracyatholaimus vitraeus Gerlach, 1957) and the description of three new species (Biarmifer nesiotes Cunha, Fonseca & Amaral, 2023, Pomponema longispiculum Cunha, Fonseca & Amaral, 2023 and Longicyatholaimus sp. nov.), expanding the number of records in the country to 14 genera and 23 species of Cyatholaimidae.

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a: rate of body length to maximum body diameter;

abd: anal body diameter;

Am: Amphideal fovea;

AZ: Abyssal Zone;

b: rate of body length to pharynx length;

BW: Brackish Water;

c: rate of body length to tail length;

c': rate of tail length to anal or cloacal body diameter;

cbd: corresponding body diameter;

CS: Continental Shelf;

CZ: Coastal Zone;

DT: Dorsal Tooth;

F: Freshwater;

Heterog.: Heterogeneous;

Homog.: Homogeneous;

HW: Hypersaline Water;

L: total body length;

L.d.: Lateral differentiation of cuticle;

LP: Lateral Pore-like structures;

n.a.: not available information;

P.b.: Pharynx bulb;

P.c.: number of longitudinal rows of pore complex in the cuticle.

PC: Pore Complexes;

S: Soil;

SEM: Scanning Electron Microscopy;

SL: Continental Slope;

Sp: precloacal supplements;

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INTRODUÇÃO

O filo Nematoda é um grupo formado por organismos altamente diversos e amplamente distribuídos, encontrados desde os ambientes terrestres até os marinhos, na forma de vida-livre ou parasitas de plantas e animais, inclusive do ser humano (Heip *et al.*, 1985). À primeira vista são animais simples, constituídos por "um tubo dentro de outro tubo", porém, em nível microscópico os nematodas são consideravelmente variáveis morfologicamente (Platt & Warwick, 1988). A grande diversidade estrutural da cavidade bucal das espécies de vida-livre, por exemplo, permite a exploração de recursos nos mais variados tipos de habitats e está relacionada a ocupação de diferentes posições na cadeia trófica (Heip *et al.*, 1982). A capacidade de ocupar ambientes distintos também parece estar ligada a alta taxa de sobrevivência ao estresse osmótico, a qual está associada a presença de cutícula mais permeável e sistema secretor-excretor simples, observados principalmente nas espécies marinhas (Holterman *et al.*, 2019).

No ambiente marinho bentônico, o grupo frequentemente corresponde ao principal componente, em termos de abundância e biomassa, em amostras de meiofauna (Higgins & Thiel, 1988; Kotwicki *et al.*, 2005; Giere, 2009). Apesar de sua importância, a diversidade dos nematodas marinhos ainda é pouco conhecida e estima-se que mais da metade das espécies existentes ainda não foram descritas (Appeltans *et al.*, 2012). Isso se deve, principalmente, à falta de conhecimento sobre a homologia dos caracteres morfológicos e ao déficit de taxonomistas do grupo (Hodda *et al.*, 2009; Fonseca *et al.*, 2017).

A delimitação de espécies de nematodas é, comumente, uma tarefa complexa devido à alta plasticidade fenotípica (Pereira *et al.*, 2010). Muitos dos caracteres morfométricos usados na delimitação das espécies, como por exemplo comprimento corpóreo, podem apresentar uma variação intraespecífica considerável de acordo com o local de origem ou características ambientais as quais a população está exposta, como a disponibilidade de alimento e a temperatura (Herman & Vranken 1988; Fonderie *et al.*, 2013; Hauquier *et al.*, 2017). A introdução de métodos moleculares nos estudos taxonômicos despontou como uma possível solução, porém, muitas vezes não é possível delimitar e identificar as espécies com base apenas em dados moleculares (De Ley *et al.*, 2005). Portanto, abordagens integrativas que unem evidências de múltiplos métodos podem ser a maneira mais eficiente de ampliar o conhecimento da biodiversidade dos nematodas (Fonseca *et al.* 2017).

Apesar de não resolver as questões relacionadas à taxonomia dos nematodas, a obtenção e análise de sequências de DNA foi fundamental para a reavaliação da sistemática do grupo nos níveis hierárquicos superiores (De Ley & Blaxter, 2002, 2004; Ahmed & Holovachov, 2021). Atualmente o filo é subdividido em três grandes grupos, com base em dados morfológicos e moleculares: Enoplia, Dorylaimia e Chromadoria. As relações filogenéticas dentro desses grupos ainda não são completamente compreendidas, especialmente dentro de Chromadoria, subclasse na qual a maioria das espécies marinhas são classificadas (Ahmed & Holovachov, 2021).

A ordem Chromadorida Chitwood, 1933 (incluída em Chromadoria) é composta por nematodas de vida livre principalmente marinhos, com raras ocorrências em ambientes de água doce ou terrestres (Tchesunov, 2014). O grupo é caracterizado por apresentar cutícula pontuada, doze hastes cuticularizadas que suportam o queilostoma (região anterior da cavidade bucal), fêmeas com ovários opostos e refletidos, e machos com dois testículos opostos ou apenas um anterior (Tchesunov, 2014). Atualmente, cinco famílias são classificadas dentro da ordem: Achromadoridae Gerlach & Riemann, 1973, Chromadoridae Filipjev, 1917, Cyatholaimidae Filipjev, 1918, Ethmolaimidae Filipjev & Schuurmans Stekhoven, 1941, e Selachinematidae Cobb, 1915. A familia Neothonchidae Wieser & Hopper, 1966, considerada na revisão de Tchesunov (2014), foi aceita como subfamília (Neothonchinae) dentro de Ethmolaimidae (Platt, 1982; Bezerra *et al.*, 2013). Embora a relação próxima entre Achromadoridae, Chromadoridae, Cyatholaimidae e Ethmolaimidae tenha sido recuperada por várias análises filogenéticas (*e.g.* Holterman *et al.*, 2008; Van Megen *et al.*, 2009), a posição da família Selachinematidae é incerta e a monofilia da ordem Chromadorida não foi recuperada pela maioria das análises filogenéticas do grupo (Leduc *et al.*, 2019; Ahmed & Holovachov, 2021).

As espécies de Cyatholaimidae estão, comumente, entre as mais abundantes nos levantamentos em diferentes ambientes e regiões geográficas (*e.g.* Vincx, 1990; Zeppilli & Danovaro, 2009; Pinto *et al.*, 2012; Zhao *et al.*, 2020). Contudo, a biodiversidade e a história evolutiva da família estão longe de ser compreendidas. Em 1918, Filipjev estabeleceu a subfamília Cyatholaimi dentro de Chromadoridae. Posteriormente, De Coninck e Schuurmans-Stekhoven (1933) consideraram Cyatholaimidae como uma família dentro da ordem Chromadoroidea (atualmente Chromadorida Chitwood, 1933). A posição dos dois círculos da sensila cefálica (também chamados de setas labiais externas e setas cefálicas) no mesmo nível em uma coroa única foi considerada a sinapomorfia de Cyatholaimidae por Lorenzen (1981, 1994). No entanto, este padrão de organização das setas também é observado em outras espécies

fora desta família, e o diagnóstico da família é feito com base na observação desse padrão em combinação com a presença de doze hastes distintamente cuticularizados no queilostoma, anfídeos multi-espirais (órgãos quimiorreceptores localizados na região anterior), cutícula coberta com pontuações (Fig. 1), e gônadas anteriores e posteriores sempre em lados opostos do intestino (Lorenzen, 1981, 1994). Comumente, as espécies de Cyatholaimidae apresentam um dente dorsal conspícuo na cavidade bucal, enquanto os dentes subventrais são pequenos ou ausentes (Lorenzen, 1981, 1994). Reconstruções filogenéticas baseadas em dados moleculares recuperaram a família como monofilética (Meldal *et al.*, 2007; Holterman *et al.*, 2008; Van Megen *et al.*, 2009); no entanto, as relações dentro do grupo são desconhecidas. As quatro subfamílias incluídas na família (Cyatholaiminae Filipjev, 1918, Paracanthonchinae De Coninck, 1965, Pomponematinae Gerlach & Riemann, 1973) não são definidas com base em sinapomorfias e possivelmente são parafiléticas (Lorenzen, 1981; Leduc & Zhao, 2016).



Figura 1. Desenho esquemático da região anterior de um indivíduo tipicamente classificado em Cyatholaimidae. **A**: Corte longitudinal; **B**: Vista superficial.

Para além da problemática relacionada as subfamílias, a falta de conhecimento sobre a relevância taxonômica dos caracteres dificulta a delimitação de espécies e a identificação a nível de gênero em Cyatholaimidae. Como é o caso dos gêneros Paracanthonchus Micoletzky, 1924 e Praeacanthonchus Micoletzky, 1924. A estrutura do gubernáculo (órgão do sistema reprodutor masculino) pareada em Paracanthonchus e não pareada em Praecanthonchus é a principal característica morfológica que distingue os dois gêneros, e os classifica nas subfamílias Paracanthonchinae e Cyatholaiminae, respectivamente (Tchesunov, 2014). Porém, de acordo com análises filogenéticas, Praecanthonchus está proximamente relacionado às espécies atualmente classificadas em Paracanthonchinae, inclusive do gênero Paracanthonchus, e esse caráter pode não ser significativo na separação de gêneros dessa família (Leduc & Zhao, 2016). Há mais de 50 espécies classificadas em *Paracanthonchus* e a variação morfológica entre essas espécies é considerável (Miljutina & Miljutin, 2015). O gênero não é delimitado a partir de caracteres morfológicos apomórficos e todas as características podem ser encontradas em outros gêneros de Cyatholaimidae; e a diagnose de *Paracanthonchus* é baseada na combinação única dos caracteres (Miljutina & Miljutin, 2015). Uma abordagem integrando dados morfológicos e moleculares bem como a reconstrução filogenética permitirá uma análise mais criteriosa do grupo.

Apesar da extensa linha costeira, pouco mais de 450 espécies de nematodas marinhos de vida livre foram registradas no Brasil (Venekey *et al.*, 2010; Venekey, 2017), número insignificante em relação às milhares de espécies normalmente encontradas em regiões litorâneas (Boucher & Lambshead, 1995). Considerando a família Cyatholaimidae, até o momento foram registrados 13 gêneros e 20 espécies no Brasil (Gerlach 1957a, 1957b; Venekey *et al.*, 2010; Oliveira *et al.*, 2017; Venekey, 2017; Cidreira *et al.*, 2019). Os estudos taxonômicos dos nematodas marinhos no país começaram em 1920 com Cobb e tiveram seu pico na década de 1950 com Gerlach (1954; 1956a; 1956b; 1957a, 1957b). A maioria desses estudos foi realizada na região costeira do Estado de São Paulo. O litoral paulista apresenta um grande potencial de revelar novas espécies por ser formada por um mosaico de ecossistemas não consolidados (Lamparelli, *et al.*, 1999) que proporcionam uma diversidade de habitats para os organismos bentônicos (Fonseca *et al.*, 2014).

O presente trabalho teve como objetivo principal revisar o conhecimento disponível sobre a diversidade e distribuição de Cyatholaimidae, bem como avaliar a relevância taxonômica de alguns caracteres e discutir as relações filogenéticas dentro da família. O trabalho foi dividido em três capítulos. O primeiro capítulo trata da revisão taxonômica da família, incluindo um levantamento dos registros das espécies nos diferentes habitats e regiões geográficas ao redor do mundo, além de uma lista das espécies válidas. No Capítulo 2 são descritas duas novas espécies encontradas na zona sublitoral da praia Pedras Miúdas, Ilhabela – SP. Além das descrições, o trabalho também inclui a discussão sobre a importância taxonômica de alguns caracteres morfológicos e apresenta tabelas de identificação para os gêneros *Biarmifer* Wieser, 1954 e *Pomponema* Cobb, 1917. Por fim, o Capítulo 3 tem como objetivo reavaliar as relações filogenéticas dentro de Cyatholaimidae a partir de sequências moleculares e inferir os possíveis caracteres relevantes para a delimitação dos clados observados. No capítulo também foram redescritas duas espécies conhecidas e descrita uma nova espécie para a ciência.

CAPÍTULO 1. DIVERSITY AND DISTRIBUTION OF CYATHOLAIMIDAE (CHROMADORIDA: NEMATODA): A TAXONOMIC AND SYSTEMATIC REVIEW OF THE WORLD RECORDS

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ABSTRACT

Nematoda is a very species-rich phylum that has successfully adapted to almost all types of ecosystems. Despite their abundance and ecological importance, the taxonomic knowledge of nematodes is still limited and the identification of species is not trivial. In Cyatholaimidae, a relatively common and abundant family of free-living nematodes, the identification of organisms is challenging due to the overlap of some generic diagnoses and the absence of updated systematic reviews. Here we systematically reviewed the knowledge about family diversity, providing a list of valid species, the diagnostic characters to genus level, and the geographical and habitat distribution of species. The review systematized a total of 619 records. The occurrences were classified into biogeographic ecoregions and habitats. Cyatholaimidae includes 211 valid species, classified into 20 genera. The genera can be differentiated based on six diagnostics characters, namely: pattern of cuticle ornamentation; number of longitudinal rows of pore-complex in cuticle; structures of the buccal cavity; presence/absence of pharyngeal bulb; precloacal supplements aspect; and the shape of gubernaculum. Cyatholaimidae includes mainly marine species, mostly occurring in the Coastal Zone. Four and three species were registered in freshwater and terrestrial habitats, respectively, all classified in the genus Paracyatholaimus. About 38% of the valid species occur in more than one type of habitat, under very different environmental conditions, suggesting a broad niche. The occurrence of congeneric species in different habitat types indicates that, throughout the evolutionary history of the family, multiple ecological shift events have occurred. The family occurs worldwide in 74 ecoregions, and the majority of the records and species are in the North Sea and Western Mediterranean. Most species are endemic to one ecoregion, and examples of broadly distributed ones may be a result of misidentifications or cases of long-distance dispersal, especially for those associated with biological substrates.

INTRODUCTION

Nematodes are the most diverse and abundant representative of meiofauna in many marine environments (Giere, 2009); yet, it is estimated that more than half of existent species are yet to be discovered (Appeltans et al., 2012). The taxonomic impediment is the result of a combination of different aspects, such as the small size of the individuals, the apparent few morphological change over the evolutionary time, and the limited number of specialists (Giere, 2009; Fonseca et al., 2017). Such a lack of taxonomic knowledge hampers the assessment of species inventories which are the basis of ecological, monitoring, and impact assessment studies (Hortal et al., 2015). In these surveys, the marine nematodes are commonly identified only at the genera or morphospecies level (e.g., Leduc et al., 2015; Corte et al., 2017; Netto & Fonseca, 2017; Spedicato et al., 2020), and even the identification to genus level may be challenging. This is particularly evident for the Cyatholaimidae Filipjev 1918, where descriptions of some genera overlap with each other and there is no information about the taxonomic importance of the diagnostic characters (Miljutina and Miljutin, 2015; Leduc and Zhao, 2016). Given that Cyatholaimidae is a relatively diverse group with more than 200 valid species, and is among the most abundant free-living marine nematodes (e.g., Coull et al., 1982, Zeppilli and Danovaro, 2009; Santos and Venekey, 2017; Zhao et al., 2020), the misidentification of specimens from this family may cause a substantial effect on the study's conclusions.

A recent and complete taxonomic review represents the first step in achieving a rigorous species delimitation (Dayrat, 2005). A review including character diagnosis and information on species geographical and habitat distribution can help taxa identification and support future taxonomic, systematic, and biogeography studies (e.g., Fonseca and Decraemer, 2008; Venekey *et al.*, 2014, 2019). Normally, in this type of survey, the data is presented following the political geographical units, a delimitation without biological meaning that may bias the conclusions (Whittaker *et al.*, 2005). The application of biogeographic classifications is thus fundamental to guiding conservation planning and providing a framework to analyze the patterns of biodiversity (Olson *et al.*, 2001; Spalding *et al.*, 2007).

Despite its broad distribution and diversity, there is no updated taxonomic review and a list of the valid species of Cyatholaimidae. The most recent list was published in the past century (Gerlach and Riemann, 1973). Since then, many new species have been described (e.g., Vincx *et al.*, 1982; Tchesunov, 2008; Huang and Xu, 2013) and many others were synonymized (e.g., Platt and Warwick, 1988; Cidreira *et al.*, 2019) or considered *inquerenda* (e.g., Miljutina and Miljutin, 2015). The information available about the family is sometimes incongruent and

incomplete, which represents an obstacle to species identification and descriptions and hinders the phylogenetic analysis of Cyatholaimidae.

In 1918, Filipjev established the subfamily Cyatholaimi within the Chromadoridae family. The systematic relationships of marine nematodes were reanalyzed by De Coninck and Schurmans Stekhoven (1933) and Cyatholaimidae was considered a family within Chromadoroidea order (currently Chromadorida Chitwood, 1933). The synapomorphy of Cyatholaimidae was defined as the position of the outer labial and cephalic setae in a single crown by Lorenzen (1981; 1994), however, this is not a unique characteristic within the order, and the diagnosis of the family is made with the combination of that character with the presence of twelve distinctly cuticularized rugae in the cheilostoma, multi-spiral amphids, cuticle covered with punctations, and anterior and posterior gonads always on opposite sides of the intestine (Lorenzen, 1981; 1994). Molecular phylogenies show that the group is monophyletic (Meldal *et al.*, 2007; Holterman *et al.* 2008; van Megen *et al.*, 2009); nevertheless, the four subfamilies that are included in the family (Cyatholaiminae, Paracanthonchinae, Pomponematinae, and Xenocyatholaiminae) are not supported by morphological synapomorphies and are probably paraphyletic (Lorenzen, 1981; Leduc and Zhao, 2016).

In the present study, we review and organize the knowledge about the family, with a list of valid species, comments about the species distribution, and for each genus, we provide the diagnostic characters as well as taxonomical issues. A critical analysis of the geographical and habitat distribution patterns of these species and some future perspectives on the study of these taxa are elaborated.

MATERIAL AND METHODS

The nematode checklist of Gerlach and Riemann (1973) was used as the starting point and all taxonomical studies published thereafter were considered in the present review. The diagnosis characters and comments about taxonomical issues are given for each genus. Since the relationship within the family is unknown and the subfamilies are probably nonmonophyletic, we listed the genera in alphabetical order. A valid species list is provided including the species synonymized in the present study.

To investigate the distribution of the species we included data from ecological and taxonomic publications with identification to species level. Studies recording species of the family were searched in Nemys database (Bezerra *et al.*, 2021) and Google Scholar. The search was made with each species name, full and abbreviated, within quotation marks (i.e.,

"Cyatholaimus gracilis" and "C. gracilis"). We also looked for records of synonymous species and did an active search for older publications. Given the lack of diagnostic characters of females and juveniles, we did not consider articles that recorded only them.

The habitat of occurrence of each species record was defined among eight categories: Soil (terrestrial environment), Hypersaline waters (hypersaline lakes), Freshwater (rivers and freshwater lakes), Brackish waters (estuaries, mangroves, brackish lagoons, and marshes), Coastal Zone (intertidal and subtidal regions of beaches, rocky shores and artificial coastal habitats, like pillars bridges), Continental Shelf (offshore until 200 m depth), Continental Slope (201 to 4000 m depth) and Abyssal Zone (more than 4000 m depth). The geographic distribution of each species recorded in Brackish waters, Coastal Zone, and Continental Shelf habitats was classified following the biogeographic regionalization for coastal and shelf areas proposed by Spalding *et al.* (2007). This system is composed of 12 Realms (largest spatial units), 62 Provinces (nested within the realms), and 232 Ecoregions (smallest-scale units with relatively homogeneous species composition, distinct from adjacent areas). The deep-sea environment (Continental Slope and Abyssal) was not considered in this classification, and we indicated the ocean and the hemisphere of occurrence for the species found there.

RESULTS

Since Gerlach and Riemann (1973), three new genera and more than 60 Cyatholaimidae species have been described (see the Taxonomic section and the list of valid species in the appendix). On average, almost two species per year have been described. The peak of taxonomic studies of the family happened between the decades of 1950 and 1970 when more than 80 species were described (Fig. 1). The main contributors to this "Golden-Age" for cyatholaimidae species considered valid today in materials from Italy (1953a), Chile (1953b), Madagascar (1953c), France (1954), El Salvador (1955), Germany (1956), Brazil (1957a, b), Egypt (1964a), Maldives (1964b) and Saudi Arabia (1967). Wieser also worked with material from different regions all over the world and described 28 species alone (1954a-Chile, 1954b-Italy, 1955-Japan, 1959-USA) and with Hopper (1967-USA).



Figure 1. Number of species descriptions of Cyatholaimidae along the years. The bars indicated the number of descriptions every five years and the line shows the accumulated data.

Taxonomic review: diagnosis and relationships of the family Cyatholaimidae

Class Chromadorea Inglis, 1983

Order Chromadorida Chitwood 1933

Family Cyatholaimidae Filipjev, 1918

Diagnosis (from Leduc and Zhao, 2016). Cuticle with transverse rows of punctations. Lateral punctations may be larger, irregular, or arranged in longitudinal rows. Longitudinal rows of circular or elliptical cuticular structures, called lateral pore-like structures, often present along mediolateral lines; up to 12 longitudinal rows of pore complexes may also be present. Inner labial sensilla often setiform; six outer labial setae and four cephalic setae in a single crown; outer labial setae longer than the cephalic setae. Multispiral amphideal fovea. Cheilostoma with twelve distinctly cuticularised rugae. Pharyngostoma with a large dorsal tooth, and usually with two smaller ventrosublateral teeth, which may be single or double. Pharynx usually without a posterior bulb. Female didelphic-amphidelphic with reflexed anterior and posterior gonads always on opposite sides of the intestine. Males usually with two testes, rarely with one. Precloacal supplements may be present or absent.

Phylogenetic relationships. Cyatholaimidae seems to be monophyletic according to different molecular phylogenies, however, all these studies used an average of five sequences from species of the family (Meldal *et al.*, 2007; Holterman *et al.* 2008; van Megen *et al.*, 2009; Avó *et al.* 2017). The non-monophyly of the subfamilies (Cyatholaiminae Filipjev, 1918; Paracanthonchinae De Coninck, 1965; Pomponematinae Gerlach & Riemann, 1973 and Xenocyatholaiminae Gerlach & Riemann, 1973) was suggested due to the absence of

synapomorphic characters (Gerlach & Riemann, 1973; Lorenzen, 1981, 1994), and it was corroborated by the molecular phylogenetic tree available for the family (Leduc and Zhao, 2016). This phylogeny was based on only a few rDNA sequences of the small subunit (SSU) and D2-D3 of the large subunit (LSU) from a few species (14 and 6 species, respectively).

Remarks. Drawings of heads and tails of typical species of Cyatholaimidae genera are available in the Handbook of Zoology (Tchesunov, 2014 – fig. 7.96; 7.97 – p.386 and 388). In this chapter, the genera Minolaimus Vitiello, 1970; Parapomponema Ott, 1972 and Propomponema, Ott, 1972 were listed within Cyatholaimidae; however, the first had previously been transferred to Comesomatidae family (Hope and Zhang, 1995), and the other two were later synonymized with Pomponema (Cidreira et al., 2019). Schemes of the principal structures that are necessary for the identification may be more informative than an illustration of the type species, given the great morphological variety of most genera. Six diagnostics characters are generally sufficient to differentiate the genera and are presented in the polytomous identification key (Table 1). The table is organized in alphabetical order. The different ornamentation patterns of the cuticle and the shape of precloacal supplements are schematized in figures 2 and 3, respectively. The shape of the gubernaculum (copulatory structure) is commonly considered in genus delimitation; however, this character hampers the identification of females and juveniles, and it can be very variable even within the genera (Fig. 4). The pore complex, a structure commonly present in the cuticle of Cyatholaimidae species, is frequently poorly described and currently, it is only considered to separate Longicyatholaimus and Marylynnia genus. Nevertheless, it was suggested that this character may be phylogenetic informative (Leduc and Zhao, 2016), thus we included it in the table.

Taxonomic review: genus diagnoses, distribution and relationships Genus *Acanthonchus* Cobb, 1920

Diagnosis. Cuticle with lateral differentiation absent or present of larger and more wide-spaced dots (Fig. 2A, B, C); precloacal supplements tubular (Fig. 3A), the anterior-most much larger than the others; and gubernaculum paired (Fig. 4A) usually dilated and dentated distally (similar to Fig. 4j4, j7) (Wieser, 1955). The species are differentiated mostly by the presence or absence of a dorsal tooth, the size of amphids in relation to the corresponding diameter of the body, the number of precloacal supplements, and the number of post-cloacal setae (Wieser, 1955).

Remarks. The *Acanthonchus* species were found in eight different realms, mostly in the Temperate Northern Pacific (six species). Except for *A. arcuatus*, *A. singaporensis* and *A.*

tridentatus which presents a distribution restricted to only one ecoregion, the other species have a broader distribution and occur in two to six ecoregions sometimes very far apart. *A. gracilis*, for example, was recorded in the North Sea (Ditlevsen, 1918; Gerlach, 1958), Baltic Sea (Allgén, 1929; 1953), Yellow Sea (Steiner, 1921), and Panama Bight (Allgén, 1947), and *A. viviparus* was registered in six ecoregions (Table 2). Most species were found on the Coastal Zone and/or in the Continental Shelf associated with algae. Wieser (1955) classified the subgenera *Acanthonchus* and *Seuratiella* which are distinguished only by the presence or absence of a dorsal tooth and ocelli. These previously separated genera need to be carefully studied and might prove to be synonymous (Wieser, 1955).



Figure 2. Cuticle pattern of Cyatholaimidae species. (A) Homogeneous, without lateral differentiation; (B) Homogeneous, with lateral differentiation of larger and more sparsely dots irregularly arranged; (C) Homogeneous, with lateral differentiation of larger and more sparsely dots regularly arranged; (D) Heterogeneous, with enlarged hexagonal punctations in the anterior end; (E) Homogeneous, with lateral differentiation of longitudinal rows of enlarged punctations with broad lateral fields between them; (F) Homogeneous, with lateral differentiation of longitudinal rows of punctations; (G) Heterogeneous, with alternating pattern of one or two rows of simple dots and one row of longitudinal bars consisting of two fused dots

in the anterior end; (H) Heterogeneous, with cuticle in the head thicker; (I) Heterogeneous, with cuticle in the head thicker, and with lateral differentiation of longitudinal rows of enlarged dots; (J) Homogeneous, with rings smooth, not dotted and adorned with ten marked longitudinal lines.

Genus Biarmifer Wieser, 1954

Diagnosis. Cuticle configuration heterogeneous, with enlarged hexagonal punctations in transverse rows from the anterior end to the nerve ring and transverse rows of simple punctations on the rest of the body (Fig. 2D), unique in the family; precloacal supplements cupshaped (Fig. 3B); and gubernaculum aspect variable, distally dilated or not, dentate or not (Fig. 4c1, j1) (Pastor de Ward, 2001). The species can be differentiated from each other mostly by the length of the head sensilla, the shape of copulatory organs, and the format of the tail.

Remarks. Until now, only three Biarmifer species were described, recorded in the Coastal Zone or Continental Shelf habitats on the Temperate South American realm. Here, we transfer three *Marylynnia* species to this genus (see section of the genus). *B. hopperi* (Sharma & Vincx, 1982) n. comb. were found on a coastal habitat of the Temperate Northern Pacific. *B. dayi* (Inglis, 1963) n. comb. and *B. punctata* (Jensen, 1985) n. comb. were described from the Continental Shelf of Temperate Southern Africa and Temperate Northern Atlantic, respectively. The species of the genus have been found in sublittoral sand, and *B. laminatus* was also found associated with algae.

Genus Craspodema Gerlach, 1956

Diagnosis. Presence of lateral differentiation in the cuticle very prominent as longitudinal rows of enlarged punctations with broad lateral fields between them (Fig. 2E); precloacal supplements complex, consisting of a stout cylindrical body having a flat cover with a central opening (Fig. 3C); and gubernaculum reduced (Fig. 4b2) or consisting of paired dorsal pieces joined (Fig. 4E) (Semprucci and Burattini, 2015). The species can be differentiated by amphideal fovea and buccal cavity structure (Semprucci and Burattini, 2015).

Remarks. There are two species described for the genus, both recently reanalyzed by Semprucci and Burattini (2015). *C. octogoniata*, first described for the Coastal Zone of the Western Mediterranean, was posteriorly found on the Continental Shelf (Boucher, 1976) and the Brackish water habitats of the ecoregion (Nasri *et al.*, 2015). *C. reflectans* was described on

Continental Shelf from the Maldives and was also found in Western Mediterranean, on the Continental Shelf (Beyrem and Aissa, 1998), and Coastal Zone (Boufahja *et al.*, 2015).



Figure 3. Precloacal supplements aspects of Cyatholaimidae species. (A) Tubular; (B) Cupshaped; (C) Complex, consisting of a stout cylindrical body having a flat cover with a central opening; (D) Papilla outer piece and inner tubular piece; (E) Plate-shaped outer piece and inner cup-shaped piece; (F) Pappiloid; (G) Setae-like; (H) Complex, with cuticle lamellated between supplements.

Genus Cyatholaimus Bastian, 1865

Diagnosis. Cuticle homogeneous without lateral differentiation (Fig. 2A); precloacal supplements absent; and gubernaculum unpaired (Fig. 4B), distally swollen, squarish, with large pointed teeth (similar to Fig. 4G) (Tchesunov, 2014). The differences between the species are mostly based on the body size, length of head sensilla, and reproductive male structures.

Remarks. The majority of species classified in the type genus of the family were described in the past century and many were transferred to other genera or considered taxon *inquerendum* (Gerlach and Riemann, 1973; Bezerra *et al.*, 2021). Here we add three more species as *inquerendae* for having been described based only on females and/or juveniles: *C. canariensis* Steiner, 1921; *C. crassus* Kreis, 1963; *C. unalaskensis* Allgén, 1957. Two-thirds of the valid species were first described in the Temperate Northern Atlantic realm. The majority seems to have a more restricted distribution and only *C. gracilis* was recorded in several locations, including Temperate South America and in the Southern Ocean (Allgén, 1959). Nevertheless, the records of this species outside the Temperate Northern Atlantic were considered unreliable based on the descriptions provided (Inglis, 1961). After that, *C. gracilis* was recorded from the Red Sea (Western Indo-Pacific realm - Riemann and Rachor, 1972). Most valid species were recorded in Coastal habitats, only one is also found on Continental Shelf (*C. microsetosus*) and a few were found in the Brackish water. The unique species of the family that is probably a parasite of an Isopoda is classified in this genus (*C. cirolanae*). The diagnostics characteristics of this genus are not always sufficient for classification, since it can also occur in other genera,

such as *Paracanthonchus*, and the arrangement of the gubernaculum is difficult to observe, visible only in ventral view (Wieser, 1954b). Therefore, the group urgently needs a systematic revaluation.



Figure 4. Examples of the different gubernaculum types of Cyatholaimidae species. (A) Ventral view showing a gubernaculum (a1) paired and an (a2) unpaired. (B)-(J), lateral view. (B)

Reduced, (b1) Paracyatholaimoides asymmetricus, (b2) Craspodema octogoniata, (b3) Xenocyatholaimus delamarei; (C) Distally not dilated nor dentate, (c1) Biarmifer laminatus, (c2)*Paracyatholaimoides mutispiralis*, (c3)Paracyatholaimus diva. (c4)Paralongicyatholaimus minutus; (D) With lateral structures, (d1) Nannolaimoides decoratus, (d2) Xyzzors fitzgeraldae, (d3) Pomponema reductum; (E) Paired dorsal pieces joined, Craspodema reflectans; (F) With ventral apophysis, Isacanthonchus obesus; (G) Distally massively swollen, dentate, *Praeacanthonchus punctatus*; (H) Distally dilated, not dentate, (h1) Paracanthonchus platypus, (h2) Paramarylynnia subventrosetata, (h3) Paracyatholaimus intermedius; (I) Distally not dilated, dentate, (i1) Paracanthonchus cochlearis, (i2) Marylynnia annae; (J) Distally dilated, dentate, (j1) Biarmifer madrynensis, (j2) Cyathoshiva amaleshi, (j3) Longicyatholaimus longicaudatus, (j4) Marylynnia preclara, (j5) Metacyatholaimus cylindribucca, (j6) Metacyatholaimus delicatus, (j7) Paracanthonchus macrodon, (j8) Paracanthonchus caecus, (j9) Paracyatholaimus pesavis, (j10) Phyllolaimus tridentatus.

Genus Cyathoshiva Datta, Miljutin, Chakraborty & Mohapatra, 2016

Diagnosis. Cuticle transversely punctated with lateral differentiation in form of slightly enlarged dots hardly visible along the body, except in the tail (Fig. 2C); buccal cavity with well-developed dorsal tooth and two subventral teeth; supplements having basal tubular part inserted mostly within the body and distal part in shape of indistinct papilla or short setae (Fig. 3D); and proximally unpaired and distally paired gubernaculum, dilated and dentate (Fig. 4j2) (Datta *et al.*, 2016).

Remarks. A monospecific genus recorded exclusively in the Coastal Zone of the Northern Bay of Bengal.

Genus Dispira Cobb, 1933

A monospecific genus found only once on a beach in the ecoregion Virginian. Here we considered *Dispira* as *incertae sedis* based on the organization of the head sensilla in three circles with six setae each, which goes against the synapomorphy of the family.

Genus Isacanthonchus Gagarin & Nguyen Vu Thanh, 2008

Diagnosis. Lateral differentiation of cuticle formed by two longitudinal rows of dots (Fig. 2F); precloacal supplement tubular (Fig. 3A) arranged in two rows; and gubernaculum paired (Fig. 4A) with ventral apophysis (Fig. 4F) (Gagarin and Nguyen Vu Thanh, 2008).

Remarks. The single species described in this genus was recorded on a mangrove in the Gulf of Tonkin, in Vietnam.

Genus Longicyatholaimus Micoletzky, 1924

Diagnosis. Presence of four complete longitudinal rows of pore complex in the cuticle; none or only one lateral pore-like structure on the tail; lateral differentiation of cuticle of larger and more wide-spaced dots (Fig. 2C) sometimes in form of longitudinal rows of punctations (Fig. 2F); precloacal supplement usually present, cup-shaped and non-sclerotized (Fig. 3B) (conical setae protruding from the body of the cup-shaped supplements in *L. maldivarum* Gerlach, 1964); and gubernaculum distally dilated with numerous denticles (Fig. 4j3) (Hopper, 1972; Semprucci *et al.*, 2017). The species can be differentiated mostly by the type of lateral differentiation of the cuticle, the length of the head sensilla, and the shape of the gubernaculum and spicules.

Remarks. The records of *Longicyatholaimus* species came from Brackish water, Coastal Zone, Continental Shelf, and Continental Slope habitats of six different realms from all oceans. The only species broader distributed is *L. longicaudatus* found in six ecoregions (see table 2). The classification in this genus of species with lateral fields arranged in form of longitudinal rows is debatable and their taxonomy position needs careful attention (see Hopper, 1972; Semprucci *et al.*, 2017).

Genus Marylynnia (Hopper, 1972) Hopper, 1977

Diagnosis. Presence of eight, ten, or twelve longitudinal rows of pore complex on the cuticle; lateral differentiation on the cuticle with larger and more widely spaced punctations (Fig. 2C); lateral pore-like structures present on the tail; precloacal supplements cup-shaped (Fig. 3B); and gubernaculum distally dentate, dilated or not (Fig. 4i2, j4). *Marylynnia* species can be differentiated from each other by the length of the head sensilla, the shape of the gubernaculum, and the format of the tail (Hopper, 1972).

Remarks. There are records of *Marylynnia* species in many regions all over the world, occurring on Brackish water, Continental Slope, and most on Coastal Zone and Continental Shelf habitats. Most species seem to have a restricted geographical distribution, and ten were recorded in more than one ecoregion. *M. annae* has a broader distribution and was found in five ecoregions nested in four different realms (Table 2). The genus was established by Hopper (1972) for *Longicyatholaimus* species having eight, ten, and twelve longitudinal rows of pore

complex on the cuticle. We here transfer the species *M. denticulata* Kim Tchesunov & Lee, 2015 for the genus *Paracanthonchus*, based on the presence of tubular precloacal supplements. *M. dayi* (Inglis, 1963) Hopper, 1977, *M. hopperi* Sharma & Vincx, 1982, and *M. punctata* Jensen, 1985 are transferred to *Biarmifer* genus by having the typical cuticle configuration of it, with enlarged hexagonal punctations in transverse rows in the anterior end. Also, *M. dayi* and *M. punctata* have only four longitudinal rows of pore complex.

Genus Metacyatholaimus Stekhoven, 1942

Diagnosis. Lateral differentiation of three to five longitudinal rows of punctations on the cuticle (Fig. 2F) (except in *M. effilatus*, which possesses 4–12 longitudinal rows of punctations); buccal cavity with small to medium-sized dorsal tooth and smaller subventral teeth usually present; pharynx with posterior bulb; precloacal supplements absent; and gubernaculum distally dilated or not, with or without denticles or horns (Fig. 4j5, j6) (Leduc and Zhao 2016). The species are distinguished among them mostly by the number of longitudinal rows of dots in the lateral differentiation of cuticle, position and the number of turns of amphideal fovea, and in the shape of spicules and gubernaculum (Vidakovic *et al.*, 2003; Leduc and Zhao, 2016).

Remarks. Despite the majority of species registers being from the Continental Shelf, there are representatives of *Metacyatholaimus* in all marine habitats categorized here, including *M. chabaudi* and *M. delicatus* from the deep sea. Only *M. cylindribucca* was recorded in the Brackish water (Jouili *et al.*, 2018), however, it had been first described from Continental Shelf habitat. Most species were found in the Mediterranean Sea, but there are also records in other regions of the world, like *M. delicatus* from Temperate Australasia realm and *M. spatiosus* from Temperate South America. The position of *M. brevicollis*, and *M. papillatus* in this genus is doubtful due to the presence of precloacal supplements, however, we opt to maintain them within the genus for now. Both possess complex supplements with several elements separated by a lamellated cuticle, a structure similar to supplements from individuals of the *Pomponema* genus.

Table 1. Diagnostics characters for Cyatholaimidae genus delimitation. Abbreviations: A: absent; Heterog.: Heterogeneous; Homog.: Homogeneous; L.d.: lateral differentiation of cuticle; n.a.: not available information; P: present; P.b.: pharynx bulb; P.c.: number of longitudinal rows of pore-complex in the cuticle.

	Cuticle	Р. с.	Buccal cavity	P. b.	Precloacal supplement	Gubernaculum
Acanthonchus	Homog.; with or without L.d. of larger dots irregularly arranged, more visible in post- anal region	4 or 8	Dorsal tooth; subventral teeth absent	A	Tubular, the anterior-most much larger than the others	Paired, distally dilated and dentate
Biarmifer	Heterog., with enlarged hexagonal punctations in transverse rows from the anterior end to the nerve ring and transverse rows of simple punctations on the rest of the body; with or without L.d. of widely spaced dots	4 (8 in <i>B.</i> hopperi)	Medium- sized dorsal tooth; two smaller subventral teeth	A	Cup-shaped, non- sclerotized	Distally dilated or not, dentate or not
Craspodema	Homog.; L.d. very prominent as longitudinal rows of enlarged punctations with broad lateral fields between them	n.a.	Large dorsal tooth; two smaller subventral teeth	A	Complex, consisting of a stout cylindrical body having a flat cover with a central opening	Reduced in <i>C.</i> octogoniata and consisting of paired dorsal pieces joined in <i>C. reflectans</i>
Cyatholaimus	Homog.; L.d. absent	4	Dorsal tooth present or absent; subventral teeth absent	A	A	Proximally unpaired; distally massively swollen and with teeth
Cyathoshiva	Homog.; L.d. of slightly larger dots, more visible in post-anal region	n.a.	Large dorsal tooth; two smaller subventral teeth	A	Basal part tubular inserted within the body and distal part in shape of indistinct papilla or short setae	Proximally unpaired; distally paired, dilated with serrated edges and two teeth

Table 1. (Continued)

	Cuticle	Р. с.	Buccal	P. b.	Precloacal	Guberna-
Isacanthonchus	Homog.; L.d. of larger dots irregularly arranged from anterior end to middle of pharynx, and organized in two longitudinal rows of dots from middle of pharynx to tail	n.a.	Large dorsal tooth; subventral teeth absent	A	Tubular, organized in two longitudinal rows	Paired, with ventral apophysis
Longicyatholaimus	Homog.; L.d. commonly of larger and more sparsely dots, and in a few species, dots are organized in longitudinal rows	4	Dorsal tooth; subventral teeth greatly reduced or absent	A	Cup-shaped, non- sclerotized	Distally dilated with numerous denticles
Marylynnia	Homog.; L.d. of larger and more sparsely dots	8, 10 or 12	Large dorsal tooth; two pairs of subventral teeth	A	Cup-shaped, non- sclerotized	Distally dilated or not, with teeth and/or numerous denticles
Metacyatholaimus	Homog., L.d. of three to five longitudinal rows of punctations	A* (4 in <i>M. delicatus</i>)	Small to medium- sized dorsal tooth; smaller subventral teeth usually present	Р	Absent in most species; complex, <i>Pomponema</i> -like in <i>M.</i> <i>brevicollis</i> and <i>M.</i> <i>papillatus</i>	Distally dilated or not, with or without denticles or horns
Nannolaimoides	Homog. or heterog. (alternating pattern of one or two rows of simple dots and one row of longitudinal bars consisting of two fused dots in anterior end); L.d. of dots irregularly arranged (present only in post-anal region in <i>N.</i> <i>effilatum</i>)	n.a.	Dorsal tooth weakly develop; subventral teeth absent	Р	Outer piece plate-shaped and an inner cup-shaped piece	Distally not dilated nor dentate, with lateral flanges

(Continued)

Table 1. (Continued)

	Cuticle	Р. с.	Buccal cavity	P. b.	Precloacal supplement	Guberna- culum
Paracanthonchus	Homog.; with or without L.d. of slightly larger and/or more sparsely, irregularly arranged dots.	8 (6 in <i>P</i> . <i>platti</i> ; 12 in <i>P. kamui</i> and <i>P.</i> <i>perspicuus</i>)	Dorsal tooth (absent in <i>P.</i> <i>angulatus</i>) ; smaller subventral teeth absent or present (from 1 to 4)	A	Tubular in most species (sucker-like in <i>P.longus</i> and papilloid in <i>P.digitatus</i>) or absent	Proximally paired (except in <i>P.</i> <i>elongatus</i>); often distally expanded and dentate
Paracyatholaimoides	Heterog., with cuticle in the head thicker; L.d. absent	4 (P. asymmetricu s); irregular (P. labiosetosus)	Small dorsal tooth; subventral teeth absent	A	Papilloid	Distally not dilated nor dentate
Paracyatholaimus	Homog.; with or without L.d. of slightly larger dots	4 or 8	Dorsal tooth; often with smaller subventral teeth; occasional ly other denticles present	A	Papilloid or setae-like	Distally dilated or not, commonly plate- shaped with denticles
Paralongicyatholaimus	Homog.; L.d. absent	A (4 incomplete in <i>P.</i> <i>mastigodes</i>)	Tooth absent	Р	А	Distally not dilated nor dentate
Paramarylynnia	Homog.; L.d. absent (heterog. with L.d. in <i>P.</i> <i>stenocervica</i>)	6	Dorsal tooth; paired subventral teeth	A	Present only in <i>P.</i> stenocervica with tubular aspect	Distally dilated, not dentate
Phyllolaimus	Homog.; L.d. absent	n.a.	Dorsal tooth; subventral teeth absent or present; lips with distinctive foliaceous develop- ment	A	Papilloid	Distally dilated, dentate, and heavily sclerotized

(Continued)
	Cuticle	Р. с.	Buccal cavity	P. b.	Precloacal supplement	Gubernaculum
Pomponema	Heterog., cuticle in the head may be thick with punctations appearing Y- shaped in lateral view; with or without L.d. of longitudinal rows of enlarged dots commencing some distance behind the head and occasionally with slit-like markings	A or 4	Large dorsal tooth; medium- sized subventral teeth; with or without additional minute denticles	A	Complex, consisting of several elements and cuticle lamellated between supplements	Distally with various structures, sometimes with L- shaped lateral plates or lateral flanges bearing blunt teeth
Praeacanthonchus	Homog.; with or without L.d. of slightly larger dots	4 (8 in <i>P.</i> <i>cygnis</i>)	Dorsal tooth (absent in <i>P.</i> <i>punctatus</i>); subventral teeth absent	А	Tubular	Proximally unpaired; distally massively swollen, and with teeth
Xenocyatholaimus	Homog., with rings smooth, not dotted and adorned with ten marked longitudinal lines; L.d. absent	n.a.	Dorsal tooth; subventral teeth absent	A	A	Reduced
Xyzzors	Homog.; L.d. of slightly larger and more sparsely, irregularly arranged dots	8	Dorsal tooth; smaller subventral teeth; two cuticular ridges encircling cup-shape region of the buccal cavity	A	Cup-shaped, non- sclerotized	Distally dilated with lateral process

*This feature may have been missed in other *Metacyatholaimus* species (Leduc and Zhao, 2016).

Genus Nannolaimoides Ott, 1972

Diagnosis. Cuticle homogeneous or heterogeneous with lateral differentiation irregular (Fig. 2B, G); buccal armature weakly developed with no denticles; precloacal supplements consisting of several elements (Fig. 3E); and lateral flanges in the distal end of gubernaculum (Fig. 4d1) (Ott, 1972). The species can be differentiated by the length of the head sensilla, tail length, and cuticle ornamentation homogeneous or heterogeneous throughout the body (Ott, 1972; Platt and Warwick, 1988).

Remarks. Despite there being only three species described of this genus, it presents a broad habitat distribution and was recorded on Brackish water, Coastal Zone, and Continental Shelf habitats. The type species is *N. armatus* described from a Continental Shelf habitat in Maldives ecoregions. *N. decoratus* and *N. effilatus* were first described in the Temperate Northern Atlantic realm and posteriorly registered in many other regions around the world. The first one, for example, was recorded in seven ecoregions included in four different realms (see Table 2).

Genus Nyctonema Bussau, 1993

This monospecific genus was considered unaccepted since it was described only in grey literature. Recently, the nomenclatural status of the nematode *nomina* included in this thesis was evaluated and considered valid (Holovachov, 2020). However, the six outer labial setae and four cephalic setae are organized in two separate crowns in *Nyctonema*, and here we considered this genus as *incertae sedis*.

Genus Paracanthonchus Micoletzky, 1924

Diagnosis. All characteristics used for identification can be found in other genera of the family, and it is diagnosed only by the unique combination of the characters. Cuticle homogeneous with or without lateral differentiation of slightly larger and/or more sparsely, irregularly arranged dots (Fig. 2A, B, C); buccal cavity in the vast majority of species with large pointed dorsal tooth and smaller ventrosublateral teeth; tubular supplements (Fig. 3A); and proximally paired gubernaculum (Fig. 4A), frequently distally dilated, very variable in shape (Fig. 4h1, i1, j7, j8) (Miljutina and Miljutin, 2015).

Remarks. Species of the *Paracanthonchus* genus were described from all oceans and latitudes, most of them recorded in the Temperate Northern Atlantic realm (20 species). More than 90% of species are described in the coastal habitats and a few were recorded from the Continental Shelf and Abyssal region. The species are found not only in soft sediments but also on algae as

well as on other hard substrates. This species-rich genus was recently revised by Miljutina and Miljutin (2015) where it listed 72 species, of which 20 were designated as species *inquirenda*. *P. medius* was omitted from this revision without explanation, and we here considered the species as valid. Since then, another four species have been described. Here we transfer two species to this genus (see sections of *Marylynnia* and *Paracyatholaimus*): *Marylynnia denticulata* Kim, Tchesunov & Lee, 2015 and *Paracyatholaimus duplicatus* Gerlach, 1964. Besides being commonly found in meiofauna surveys, the taxonomy of *Paracanthonchus* species is challenging mainly due to the absence of apomorphic morphological characters and the great morphological variation between species that in many cases lead to overlap between the descriptions and the diagnosis of other genera. The molecular phylogeny study of Cyatholaimidae species suggests that *Paracanthonchus* is not a monophyletic group (Leduc and Zhao, 2016).

Genus Paracyatholaimoides Gerlach, 1953

Diagnosis. Cuticle in the head region thickened (Fig. 2H); tight spiral turn of the amphid; conspicuous dimorphism sexual in the size of amphideal fovea; precloacal supplements papilloid (Fig. 3F); and gubernaculum distally not dilated nor dentate (Fig. 4b1, c2) (Gerlach, 1953a; Riemann, 1966). The species can be distinguished from each other by the form of buccal sensilla and the shape of the gubernaculum.

Remarks. There are three species of this genus described for the Temperate Northern Atlantic and only one, *P. serpens*, was first recorded in the Temperate South America realm on a Coastal habitat. The *Paracyatholaimoides* species seems to have restricted distribution, except by *P. multispiralis*, which was registered in the Coastal Zone and Continental Shelf habitats in five ecoregions nested in two different realms, Temperate Northern Atlantic (Gerlach, 1953a, 1953d, 1953e, 1953f; Somerfield *et al.*, 2007; Ellis *et al.* 2010) and Tropical Atlantic (Pérez-Garcia *et al.*, 2020). The systematic value of the characteristics on which the genus erection was based is controversial and a taxonomy review is needed (Riemann, 1966).

Table 2. Cyatholaimidae species most broadly distributed, recorded in more than five ecoregions nested in at least four different realms. The first ecoregion cited for each species is the local of the original description. ¹ example of works that registered the species in each one of the ecoregions cited. ² other records of *Paracanthonchus caecus* were considered invalid by Vincx *et al.* (1982). BW: Brackish water; CZ: Coastal Zone; CS: Continental Shelf; HW: Hypersaline water.

Species	Ecoregions	Habitat	References ¹
Acanthonchus viviparus	Southern California Bight, Manning-Hawkesbury, Southeastern Brazil, Gulf of Guinea South, Northern and Central Red Sea, Greater Antilles	CZ; CS	Cobb, 1920; Allgén, 1951; Gerlach, 1957a, c; Gerlach, 1964a; Riemann and Rachor, 1972; Armenteros <i>et al.</i> , 2009
Marylynnia annae	Floridian, South Kuroshio, Greater Antilles, Leeuwin, Maldives	CS	Wieser and Hopper, 1967; Kotta and Boucher, 2001; López-Canovas and Pastor de Ward, 2006; Hourston <i>et al.</i> , 2011; Semprucci and Balsamo, 2014
Nannolaimoides decoratus	Carolinian, Celtic Seas, Bermuda, Western Mediterranean, Leeuwin, Palawan/North Borneo, Adriatic Sea	BW; CZ; CS	Ott, 1972; Boucher, 1976; Gerlach, 1977; Palacín <i>et al.</i> , 1992; Long and Ross, 1999, 2008; Hourston <i>et al.</i> , 2009; Nasri <i>et al.</i> , 2015; Taheri <i>et al.</i> , 2015
Paracanthonchus caecus ²	North Sea, Baltic Sea, Western Mediterranean, Western Bassian, Northern Grand Banks-Southern Labrador, Gulf of St. Lawrence-Eastern Scotian Shelf, Black Sea, White Sea, Eastern India, North and East Barents Sea	BW; CZ; CS	De Man, 1889; Schneider, 1927(<i>P. caecus balgensis</i>); Palacín, 1985; Nicholas <i>et al.</i> , 1992; Reynolds and Finney-Crawley, 1999; Tita <i>et al.</i> , 2002; Vorobyova <i>et al.</i> , 2016; Fedyaeva and Tchesunov, 2017; Bhanu <i>et al.</i> , 2017; Krishnapriya <i>et al.</i> , 2019
Paracanthonchus hawaiiensis	Hawaii, Humboldtian, Central Chile, Araucanian, Chiloense, Central Kuroshio Current, Patagonian Shelf, Western India	BW; CZ; CS	Allgén, 1951; Wieser, 1954a, 1955; Pastor de Ward, 1985; Turpeenniemi <i>et al.</i> , 2001
Paracanthonchus longicaudatus	North Sea, Celtic Sea, Eastern India, Adriatic Sea, Sea of Japan, Greater Antilles	BW; CS	Warwick, 1971b; Boyd <i>et al.</i> , 2000; Ansari <i>et al.</i> , 2012; Semprucci <i>et al.</i> , 2014; Belogurova and Maslennikov, 2016; Pérez-Garcia <i>et al.</i> , 2020

Genus Paracyatholaimus Micoletzky, 1922

Diagnosis. Cuticle without lateral differentiation (Fig. 2A); precloacal supplements papilloid or as setae-like organs half inserted into the body (Fig. 3F, G); and gubernaculum hardly dilated at the distal end, without pronounced teeth (Fig. 4c3, h3), but sometimes plate-shaped with denticles (Fig. 4j9) (Platt and Warwick, 1988; Tchesunov, 2008). The species of *Paracyatholaimus* can be distinguished from each other mainly by the length of the head

sensilla, the number of turns in the amphideal fovea, supplementary organs, and copulatory apparatus shape (Tchesunov, 2008).

Remarks. Species classified in the Paracyatholaimus genus were recorded in all oceans and latitudes and were found in all types of habitats. P. intermedius has a remarkable distribution: it is a mainly brackish species (e.g., de Man, 1880; Warwick, 1971a), however, it was also recorded from marine (e.g., Kreis, 1963; Gerlach, 1965), freshwater (e.g., Riemann, 1966; Gusakov and Gagarin, 2012; Gagarin and Nguyen Vu Thanh, 2012) and terrestrial environment (Loof, 1961; Nzeako et al., 2019). Three species were found exclusively in the Freshwater habitats: P. lewisi Coomans, Vincx & Decraemer, 1985, P. paramonovi, and P. truncatus. Here we considered P. lewisi as species inquirenda because the original description is based on only females and juvenile specimens. There are also two species recorded in the abyssal zone, P. comatus from the Southeast Pacific Ocean and P. diva found in the Southeast Atlantic Ocean. Andrássy (1973) transferred the species Xyzzors inglisi Wieser & Hopper, 1967 to this genus based on the precloacal supplement and gubernaculum shape. This new combination was omitted by Tchesunov (2008) and Huang and Xu (2013) without explanations, however, we agree with Andrássy (1973). When describing P. duplicatus, Gerlach (1964b) raised the possibility that the species may be classified in Paracanthonchus genus due to the tubular aspect of precloacal supplements. Here we decide to make that transfer based on the shape of supplements and the strong expansion of the gubernaculum in the distal part, typical of Paracanthonchus.

Genus Paralongicyatholaimus Schuurmans Stekhoven, 1950

Diagnosis. Cuticle without lateral differentiation (Fig. 2A); buccal cavity without tooth; pharynx with posterior bulb; precloacal supplements absent; and the distal end of gubernaculum not dilated nor dentated (Fig. 4c4) (Warwick, 1971b). The species are differentiated mostly by the form of labial sensilla, the shape of gubernaculum, and the size of amphids in relation to the corresponding diameter of the body.

Remarks. Most *Paralongicyatholaimus* species were described for the Continental Shelf of Temperate Northern Atlantic realm, except for *P. complicatus* from the Coastal Zone in Temperate South America. *P. minutus* and *P. macramphis* are the broader distributed, recorded in five (Warwick, 1971b; Boyd *et al.*, 2000; Chinnadurai and Fernando, 2007; Sajan and Damodaran, 2007; Ansari *et al.*, 2012; Lampadariou and Eleftheriou, 2018) and three ecoregions (Lorenzen, 1972; Long and Ross, 1999; Pérez-Garcia *et al.*, 2020), respectively.

Genus Paramarylynnia Huang & Zhang, 2007

Diagnosis. Cuticle without lateral differentiation (Fig. 2A); buccal cavity with a dorsal tooth and paired subventral teeth; and distal region of gubernaculum dilated no dentate (Fig. 4h2) (Huang and Zhang, 2007). The precloacal supplements were observed only in *P. stenocervica* and the authors indicated the aspect tubular of the supplements, however, this is not clear on drawings and images of the description and the character needs to be interpreted with caution. The species are differentiated mostly by the tail shape and by cuticle ornamentation homogeneous or heterogeneous throughout the body (Huang and Sun, 2011).

Remarks. All three species of the genus were described and only recorded on the Continental Shelf of the Yellow Sea.

Genus Phyllolaimus Murphy, 1964

Diagnosis. Lips with distinctive foliaceous development, unique in the family; cuticle without lateral differentiation (Fig. 2A); precloacal supplements papilloid (Fig. 3F); and gubernaculum distally dilated, dentate, and heavily sclerotized (Fig. 4j10). They can be distinguished from each other by the number of amphid turns and the number of subventral teeth in the buccal cavity (Murphy, 1963).

Remarks. Both species of this genus were recorded on the intertidal region of beaches one in the Temperate Northern Atlantic and the other in the Temperate Northern Pacific realm. The two species were described based on a few specimens and the structure of the lips, on which the genus erection was based, may be artifacts of the fixation (Wieser, 1959), so the validity of the genus remains to be confirmed.

Genus Pomponema Cobb, 1917

Diagnosis. Cuticle heterogeneous, commonly thicker in head with the punctations appearing Y-shaped in lateral view and/or with more widely spaced dots, with or without lateral differentiation of longitudinal rows of enlarged dots occasionally with slit-like markings (Fig. 2C, H, I); buccal cavity armed with a pointed dorsal tooth, subventral teeth, and with or without additional minute denticles; precloacal supplements complex consisting of several elements and cuticle between supplements lamellated (Fig. 3H); and gubernaculum distally with various structures, sometimes with L-shaped lateral plates or lateral flanges bearing blunt teeth (Fig. 4d3) (Wieser and Hopper, 1967; Cidreira *et al.*, 2019). The valid species can be differentiated

mostly by the lateral differentiation of the cuticle, the presence or absence of denticles in the buccal cavity, the length of head sensilla, the number of amphideal turns, the gubernaculum shape, and the number of precloacal supplements.

Remarks. The *Pomponema* species were found in almost all oceans, but more than half of them were first described in the Temperate Northern Atlantic realm. They were recorded in all marine habitats here categorized, mostly in Coastal Zone (19 species) and Continental Shelf (18 species) habitats. *P. tautraense* seems to be the species more broadly geographically distributed of the genus and was recorded in eight ecoregions nested in two realms. Here we considered *P. multisupplementa* Huang & Zhang, 2014 as *incertae sedis* for having tubular supplements, amphideal fovea circular, and a pharyngeal bulb.

Genus Praeacanthonchus Micoletzky, 1924

Diagnosis. Cuticle without lateral differentiation (Fig. 2A); buccal cavity without subventral teeth; precloacal supplements tubular (Fig. 3A); and gubernaculum proximally unpaired with massive squarish distal ends with teeth (Fig. 4G) (Platt and Warwick, 1988). The main characteristics used to differentiate the species are the shape of spicules and gubernaculum.

Remarks. All *Praeacanthonchus* species are found in coastal habitats, however, a few species are also recorded on Brackish water and/or on Continental Shelf locations. They can be associated with algae substrate or live within the sediment. Most were first described from the province of Northern European Seas, and *P. cygnis* was described from the Southwest Australian Shelf. *P. kreisi* and *P. punctatus* were recorded in Temperate Northern Atlantic and Temperate South America, two realms that are very far apart. The first one was also recorded in the Southern Ocean (Allgén 1959).

Genus Xenocyatholaimus Gerlach, 1953

Diagnosis. Cuticle made up of rings smooth, not dotted, and adorned with ten marked longitudinal lines (Fig. 2J), configuration unique in the family; precloacal supplements absent; and gubernaculum reduced (Fig. 4b3).

Remarks. The single species of this genus was recorded in the Coastal Zone of Southeast Madagascar.

Genus Xyzzors Inglis, 1963

Diagnosis. Presence of irregular lateral differentiation in the cuticle (Fig. 2B); cuticular rings on the buccal cavity; spicules with double proximal ends; precloacal supplements cup-shaped (Fig. 3B); and gubernaculum with a lateral process (Fig. 4d2) (Inglis, 1963). The species are mostly differentiated by the shape of the copulatory apparatus and the length of the head sensilla.

Remarks. There are two valid *Xyzzors* species from the Atlantic Ocean. *X. fitzgeraldae* was recorded only in a Continental Shelf region in ecoregion Namaqua (Temperate Southern Africa realm). *X. iubatus* was found on coastal habitats in the ecoregion Carolinian (Temperate Northern Atlantic realm).

Habitat distribution

A total of 288 studies were considered in the present review, including the publications with the original taxonomic descriptions (see Supplementary Table 2¹). The data of geographical and habitat distribution retrieved from a total of 619 records are available in Supplementary material¹ Table 3. About 48% of valid species (101 out of 211) were recorded only from their original description. Cyatholaimidae species are mainly marine, but they are also found in freshwater and terrestrial environments, and one species, *Cyatholaimus cirolanae*, is possibly a parasite of Isopoda. The majority of species are recorded exclusively in one habitat (~62%), and 81 species are present in more than one (Fig. 5). The habitat distribution of species from most genera is broad, being that only *Metacyatholaimus*, *Pomponema*, and *Paracyatholaimus* occur in all marine habitats, and this last is the single one recorded in a terrestrial and freshwater environment (Fig. 6).

Of the three species recorded in Soil, two are found in sand dunes and are exclusive of terrestrial habitats (*Paracyatholaimus botosaneanui* and *Paracyatholaimus papillatus*). The third one is *P. intermedius*, which is considered a brackish species, but it was identified in clayey soil in Holland (Loof, 1961) and soil covered with grass from a hill in Uganda (Nzeako *et al.*, 2019). This species was also found in brackish and freshwater sediments of the Elbe River, Germany (Riemann, 1966) and sediments from rivers and freshwater lakes in Spain (Ocaña *et al.*, 1990), Russia (Gusakov and Gagarin, 2012), and Vietnam (Gagarin and Nguyen Vu Thanh, 2012). *P. ternus* is also a brackish species that was found in a Freshwater habitat (a river from Colombia – Riemann, 1970). Including the two species exclusive of this type of habitat (*P. paramonovi* and *P. truncatus*), there are a total of four species registered in

¹ Supplementary material 2 and 3 available at https://www.frontiersin.org/articles/10.3389/fmars.2022.836670/full

Freshwater. In the Hypersaline habitat, only two species were recorded: *P. pesavis*, and *C. gracilis*. The first was described based on specimens from the Coastal Zone of Floridian ecoregion and then it was found in the Continental Shelf (Tietjen, 1971), Continental Slope (Tietjen, 1976), and in the Salton Sea, a highly saline body of water in the U.S. state of California (Warwick *et al.*, 2002). The second one is mainly a coastal species (*e.g.*, De Man, 1889; Filipjev, 1918; Allgén, 1935), but it has been also recorded in the Continental Shelf (e.g., Allgén, 1959; Southern, 1914) and in the hypersaline lagoon of Bay Sivash (Shadrin *et al.*, 2019).



Figure 5. Habitat distribution of the Cyatholaimidae species. The panel above shows the number of species occurring in more than one habitat. Below are the numbers of species exclusive of each habitat. S: Soil; F: Freshwater; HW: Hypersaline Water; BW: Brackish Water; CZ: Coastal Zone; CS: Continental Shelf; SL: Continental Slope; AZ: Abyssal Zone.

The Coastal Zone is the habitat where most of the species occur, followed by the Continental Shelf (Fig. 5). The Continental Slope and Abyssal Zone are the marine habitats with fewer records, with only 10 species in each one. Of all 56 species recorded from the

Brackish waters, only 10 are exclusive to this habitat, the others also occur in the Coastal Zone and/or on the Continental Shelf. Among the exclusive species, only two were recorded more than once. The estuarine species *Longicyatholaimus falcatus* was also found in a brackish lagoon (Boufahja and Semprucci, 2015) and *Pomponema reductum*, as found in an estuary at the Celtic Seas and in an estuary from the North Sea.



Figure 6. The number of species registered in each habitat by genus. S: Soil; F: Freshwater; HW: Hypersaline Water; BW: Brackish Water; CZ: Coastal Zone; CS: Continental Shelf; SL: Continental Slope; AZ: Abyssal Zone.

Geographic distribution

There are records of Cyatholaimidae species in all the 12 Realms of coastal and shelf areas. The Temperate Northern Atlantic (TNA) has the largest number of records with 99 species from 16 genera (only the monospecific genera and *Paramarylynnia* were not recorded in TNA). At the Province level, there are records of the family in about 60% of the units (37 out of 62). The species were reported from 74 ecoregions, covering approximately 32% of the 232 marine ecoregions in the world (Fig. 7). The North Sea and Western Mediterranean, both nested on the realm TNA, are the richest ecoregions (43 and 39 species, respectively).

Most of the marine species are restricted/endemic to an ecoregion (~55%) and a few have a relatively wide distribution, for example, *Paracanthonchus caecus* occur in ten ecoregions

(Table 2). Other species are also widely distributed, despite occurring in two or three ecoregions. *Pomponema segregatum*, for example, described in the Puget Trough/Georgia Basin ecoregion (realm Temperate Northern Pacific), also occur in ecoregions Carolinian (realm Temperate Northern Atlantic; Tietjen, 1976) and Western India (realm Western Indo-Pacific; Sajan and Damodaran, 2007). Among all the non-monospecific genera, only *Paramarylynnia* have species exclusively in one realm, the Temperate Northern Pacific. All other genera have representatives in at least two realms (see Supplementary Table 2²). Considering the species from deep-sea (continental slope and abyssal zone), there are records in the Atlantic Ocean (13 species), Pacific Ocean (4 species), Arctic Ocean (2 species), and Southern Ocean (one species).

The species recorded exclusively in Soil habitats were found in Cuba (*Paracyatholaimus botosaneanui*) and Italy (*Paracyatholaimus papillatus*). There are two species restricted to the Freshwater: *Paracyatholaimus truncatus* from the USA (Cobb, 1914) and Colombia (Riemann, 1970); and *Paracyatholaimus paramonovi* from Russia (Gagarin, 2004).



Figure 7. World Map showing the marine ecoregions of Spalding *et al.* (2007) colored accordingly the number of Cyatholaimidae species recorded in each one.

DISCUSSION

Taxonomic issues

Like for other marine nematodes, the delimitation among Cyatholaimidae genera is not a trivial task and most are not defined by autoapomorphic characters (Lorenzen, 1994). The

² Available at https://www.frontiersin.org/articles/10.3389/fmars.2022.836670/full

weight given to each character is variable. The most important characters seem to be the cuticle pattern, which is sufficient to identify the genera *Biarmifer*, *Craspodema* and *Xenocyatholaimus*, and the male copulatory structures for the remaining genera. However, such importance is far from conclusive and other neglected traits may be more relevant. The descriptions of the pore patterns on the cuticle of cyatholaimids for example, are frequently inadequate and limited by light microscopy, and the use of advanced microscopies technics will certainly improve taxa diagnosis (Semprucci and Burattini, 2015; Leduc and Zhao, 2016).

The limitations of morphological data to taxon identification reveal the urgent need for high-quality reference sequence databases from free-living nematode species (Avó *et al.*, 2017). Although the initiatives for the acquisition of DNA sequences are increasing, the data is still reduced and limited to a few locations and habitats (Macheriotou *et al.*, 2019). Despite being abundant in many surveys, only a few Cyatholaimidae genera/species are found in high frequencies, and most of the taxa are considered rare (e.g., Maria *et al.* 2013, Santos *et al.* 2018), which makes it even more difficult to obtain the sequences. While the successful DNA barcoding of nematodes is not feasible, the use of robust and updated identification keys enables standardization and comparisons across studies.

Habitat transitions

The occurrence of congeneric species in different habitats, including soil, freshwater, and marine, indicates that multiple ecological shift events have occurred along with the evolutionary history of the family Cyatholaimidae. Interestingly, these changes seem to be linked with subtle morphological modifications. In *Paracyatholaimus*, for example, which contains species in soil, freshwater, brackish water, hypersaline and marine habitats, the differentiation from one another is based on slight morphological details (Tchesunov, 2008). *P. paucipapillatus* and *P. botosaneanui*, for example, are differentiated only by the length of the body and the number of precloacal supplements (Andrássy, 1973) and they occur in marine and terrestrial habitats, respectively. *Paracanthonchus bulbicola* and *Paracanthonchus longicaudatus* are also morphologically very similar, but the former occurs in the abyssal, while the latter is coastal. Habitats shifts between marine, freshwater, and terrestrial habitats at a low taxonomic level were also observed in genera from other families, such as *Theristus* Bastian, 1865 (Xyalidae), *Phillometra* Costa, 1845 (Philometridae), and *Procamallanus* Baylis, 1923 (Camallanidae) (Holterman *et al.*, 2019).

The phylum Nematoda arose from the marine environment and later colonized the land (Rota-Stabelli *et al.*, 2013). Nevertheless, the changes in habitat occurrence and ecological preferences happened a few times during the evolutionary history of nematodes and the frequency and directions of these habitat transitions vary within the group (Holterman *et al.*, 2019). The ecological transitions seem to be more common among free-living nematodes with highly water-permeable cuticles and a relatively simple secretory-excretory (S-E) system (Holterman *et al.*, 2019). Although the water permeability of the cuticle of Cyatholaimidae species is not yet studied, the S-E system consists of a single ventral gland cell, usually with a non-cuticularized terminal duct, or it can be absent in some species. It was suggested that the pore complex structure, commonly present in the cuticle of cyatholaimids, resembles a structure of trichuroid nematodes that may function in water/ion regulation (Wright, 1963), but their true role remains to be proven (Wright and Hope 1968).

The morphological variation can also be small within species that occupy very distinct habitats. The specimens of *Paracyatholaimus quadriseta* from an estuarine habitat, differ from individuals from the continental shelf only by a higher relation between body length and tail length (*c* rate), which may represent a local variation (Pastor de Ward, 1985). However, the acquisition of high-quality morphological data of organisms from populations of various habitats may reveal previously overlooked characters (Fonseca *et al.*, 2017). The use of advanced techniques, like confocal laser scanning microscopy, enabled the detailed observation of the morphology and the re-description of species, such as *Craspodema reflectans* (Semprucci and Burattini, 2015). For instance, the variations of pore complex and pore-like structures, may be meaningful for genera, species, or even population differentiation (Leduc and Zhao, 2016).

The case of *P. intermedius*, which was found in soil, freshwater, brackish water, and marine habitat, is worth noting. The majority of studies that recorded this species did not make a description and a comparison between the organisms found. Among those who did, a few morphological variations were observed. The most striking difference between some specimens from brackish water and terrestrial habitats, for example, was the number of precloacal supplements, varying between four to nine (De Man, 1880; Gerlach, 1953g; Paetzold, 1958; Loof, 1961). Tchesunov (2015) shows that the variability in the number and shape of this feature can be notable even within individuals of *Paracanthonchus olgae* from the same site. Therefore, he stated that this character may not be useful to species identification, and the same can be held for *Paracyatholaimus* species. Population genetics studies are the first step required to investigate the relationship between organisms occurring in different locations.

Understanding the evolutionary history of a taxon like *P. intermedius* may lead to a comprehension of mechanisms related to plasticity and adaptability to different environmental conditions.

Geographic distribution

The higher number of species registers in the northern hemisphere is common for inconspicuous marine species and it is related to the sampling intensity in this area, due to the long tradition of marine nematode taxonomic studies and a greater number of specialists in institutions from Europe and North America (Campos and Garraffoni, 2019; Garraffoni *et al.*, 2021). Thus, the knowledge about the biogeography of these organisms is biased by the absence of sampling in many regions all over the world (Venekey *et al.*, 2010). Despite this bias, the number of records from Cyatholaimidae in South America is similar to that from North America (9 vs 10 genera and 30 vs 47 species).

The high endemism observed for most cyatholaimid species suggests a low dispersal capability, however, there are a few examples of a relatively broad distribution (see Table 2). Examples of widely distributed meiofauna species may represent one of two distinct scenarios: false records or a high dispersal potential (Cerca *et al.*, 2018). The apparent morphological stasis in the meiofauna group and the tradition of using taxonomic keys and systematic reviews from European species, when the information for the study area is absent, lead to a greater number of species records that were proved to be incorrect (Cerca *et al.*, 2018). Nevertheless, species associated with biological substrates, for example, may present a high dispersal potential and be transported across the oceans by drifting (Thiel and Gutow, 2005). Until now, the only study dealing with morphological and molecular data of different populations of a Cyatholaimidae species suggested that *Paracanthonchus gynodiporata* have been able to disperse over large distances (Oliveira *et al.*, 2017). They found that the populations of *P. gynodiporata* are genetically similar, but with considerable differences between them in the morphometrics characters. *P. gynodiporata* is associated with seaweeds and was recorded more than 1000 km apart (Oliveira *et al.*, 2017).

CONCLUSIONS

Most Cyatholaimidae genera lack information about synapomorphic characters and are possibly non-monophyletic. Also, the definition of genus and species, or even of the subfamily, are based on the combination of multiple non-phylogenetic informative characters, which makes essential the systematic and taxonomic review. This issue difficult the identification of taxa and hamper the advance of studies in other disciplines that depend on taxonomic knowledge. All these caveats can be bypassed by embracing phylogenetic studies of the group, especially those integrating molecular and morphological data. Knowing the relationship within the family is fundamental to understanding the habitat transitions that occur along with the evolutionary history and may help the comprehension of the mechanisms that underlie the ecological flexibility of Nematoda (Holterman *et al.*, 2019).

The present work represents a foundation to uncover and understand the diversity and biogeographic patterns of the Cyatholaimidae family. For the first time, the compilation of distributional data of marine nematodes was based on the ecoregion system of classification. Cyatholaimidae is a family that includes organisms found worldwide, but most taxa are endemic to one ecoregion. The few examples of broadly distributed species might be misidentifications, however, long-distance dispersal may be possible in species associated with biological substrates, such as algae and turtles. The occurrence in more than one type of habitat, under very different environmental conditions, indicates that many cyatholaimid species have broad ecological niches.

APPENDIX

List of the valid genera and species of Cyatholaimidae. The type species of each genus are underlined.

Class Chromadorea Inglis, 1983 Order Chromadorida Chitwood 1933 Family Cyatholaimidae Filipjev, 1918 Genus Acanthonchus Cobb, 1920 Acanthonchus (Acanthonchus) arcuatus (Kreis, 1926) Wieser, 1955 Acanthonchus (Acanthonchus) cobbi Chitwood, 1951 Acanthonchus (Acanthonchus) duplicatus Wieser, 1959 Acanthonchus (Acanthonchus) setoi Wieser, 1955 Acanthonchus (Acanthonchus) viviparus Cobb, 1920 Acanthonchus (Seuratiella) gracilis (Ditlevsen, 1919) Wieser, 1955 Acanthonchus (Seuratiella) pugionatus Vitiello, 1970 Acanthonchus (Seuratiella) rostratus Wieser, 1959 Acanthonchus (Seuratiella) singaporensis Cheng-Ann, Nguyen Dinh Tu & Smol, 2015 Acanthonchus (Seuratiella) tridentatus Kito, 1976 Genus Biarmifer Wieser, 1954 Biarmifer cochleatus Wieser, 1954 Biarmifer dayi (Inglis, 1963) comb. n. syn. Marylynnia dayi (Inglis, 1963) Hopper, 1977 Biarmifer hopperi (Sharma & Vincx, 1982) comb. n. syn. Marylynnia hopperi Sharma & Vincx, 1982 Biarmifer laminatus Wieser, 1954 Biarmifer madrynensis Pastor de Ward, 2001 Biarmifer punctata (Jensen, 1985) comb. n. syn. Marylynnia punctata Jensen, 1985

Genus Craspodema Gerlach, 1956 Craspodema octogoniata (Gerlach, 1954) Gerlach, 1956 Craspodema reflectans Gerlach, 1964 Genus Cyatholaimus Bastian, 1865 Cyatholaimus cirolanae Adamson & Marcogliese, 1994 Cyatholaimus gracilis (Eberth, 1863) Bastian, 1865 Cvatholaimus microsetosus Wieser, 1954 Cvatholaimus minor Gagarin, 2012 Cvatholaimus ocellatus Bastian, 1865 Cyatholaimus papilliferus Allgén, 1929 Cyatholaimus prinzi (Marion, 1870) Inglis, 1961 Cyatholaimus scarratti Inglis, 1961 Cyatholaimus simulatus Kreis, 1924 Genus Cyathoshiva Datta, Miljutin, Chakraborty & Mohapatra, 2016 Cyathoshiva amaleshi Datta, Miljutin, Chakraborty & Mohapatra, 2016 Genus Isacanthonchus Gagarin & Nguyen Vu Thanh, 2008 Isacanthonchus obesus Gagarin & Nguyen Vu Thanh, 2008 Genus Longicyatholaimus Micoletzky, 1924 Longicyatholaimus cervoides Vitiello, 1970 Longicyatholaimus continus Filipjev, 1946 Longicyatholaimus egregius Hopper, 1972 Longicyatholaimus falcatus Vitiello, 1971 Longicyatholaimus longicaudatus (de Man, 1876) Micoletzky, 1924 Longicyatholaimus maldivarum Gerlach, 1964 Longicyatholaimus marilynae Hopper, 1972 Longicvatholaimus minor (Cobb, 1898) Micoletzky, 1924 Longicvatholaimus orientalis Gagarin & Nguven Vu Thanh. 2015 Longicyatholaimus subtenuis Gagarin & Nguyen Vu Thanh, 2007 Longicvatholaimus trichocauda Gerlach, 1955 Longicyatholaimus trichurus (Cobb, 1898) Micoletzky, 1924 Genus Marylynnia (Hopper, 1972) Hopper, 1977 Marylynnia annae (Wieser & Hopper, 1967) Hopper, 1977 Marylynnia bellula (Vitiello, 1970) Hopper, 1977 Marylynnia choanolaimoides (Schuurmans Stekhoven, 1942) Hopper, 1977 Marylynnia complexa (Warwick, 1971) Hopper, 1977 Marylynnia dubia (Filipjev, 1946) Hopper, 1977 Marylynnia effilata (Schuurmans Stekhoven, 1946) Hopper, 1977 Marylynnia eratos (Hopper, 1972) Hopper, 1977 Marylynnia gerlachi (Hopper, 1972) Hopper, 1977 Marylynnia gracila Huang & Xu, 2013 Marylynnia johanseni Jensen, 1985 Marylynnia macrodentata (Wieser, 1959) Hopper, 1977 Marylynnia mulsa (Hopper, 1972) Hopper, 1977 Marylynnia musharafii Nasira, Kamran & Shahina, 2007 Marylynnia oculissoma (Hopper, 1972) Hopper, 1977 Marvlvnnia preclara (Hopper, 1972) Hopper, 1977 Marylynnia puncticaudata Boufahja & Beyrem, 2014 Marvlvnnia stekhoveni (Wieser, 1954) Hopper, 1977 Marylynnia wieseri (Inglis, 1963) Hopper, 1977 Genus Metacyatholaimus Schuurmans Stekhoven, 1942 Metacyatholaimus adriaticus Vidakovic, Travizi & Boucher, 2003 Metacyatholaimus brevicollis (Cobb, 1898) Gerlach, 1964 Metacyatholaimus chabaudi Gourbault, 1980 Metacyatholaimus cylindribucca (Schuurmans Stekhoven, 1950) Wieser, 1954 Metacyatholaimus delicatus Leduc & Zhao, 2016 Metacyatholaimus effilatus de Bovee, 1973 Metacyatholaimus hirschi Schuurmans Stekhoven, 1942 Metacyatholaimus papillatus Vidakovic, Travizi & Boucher, 2003 Metacyatholaimus spatiosus Wieser, 1954 Genus Nannolaimoides Ott, 1972

Nannolaimoides armatus (Gerlach, 1964) Ott, 1972 Nannolaimoides decoratus Ott, 1972 Nannolaimoides effilatus (Boucher, 1976) Platt & Warwick, 1988 Genus Paracanthonchus Micoletzky, 1924 Paracanthonchus austrospectabilis Wieser, 1954 Paracanthonchus barka Inglis, 1962 Paracanthonchus batidus Gerlach, 1957 Paracanthonchus bipapillatus Kreis, 1928 Paracanthonchus bothnicus Schiemer, Jensen & Riemann, 1983 Paracanthonchus brevicaudatus Gagarin & Nguyen Vu Thanh, 2016 Paracanthonchus breviseta (Schuurmans Stekhoven, 1950) Hope & Murphy, 1972 Paracanthonchus bulbicola Bussau, 1993 Paracanthonchus caecus Micoletzky, 1924 Paracanthonchus canadensis Vincx, Sharma & Smol, 1982 Paracanthonchus cheynei Inglis, 1970 Paracanthonchus cochlearis Gerlach, 1957 Paracanthonchus cristatus Wieser, 1954 Paracanthonchus denticulata (Kim, Tchesunov & Lee, 2015) comb. n. syn. Marylynnia denticulata Kim, Tchesunov & Lee, 2015 Paracanthonchus duplicatus (Gerlach, 1964) comb. n. syn. Paracyatholaimus duplicatus Gerlach, 1964 Paracanthonchus elongatus (de Man, 1907) Micoletzky, 1924 Paracanthonchus filipjevi Micoletzky, 1924 Paracanthonchus gerlachi Vincx, Sharma & Smol, 1982 Paracanthonchus gynodiporata Oliveira et al., 2017 Paracanthonchus hartogi Inglis, 1970 Paracanthonchus hawaiiensis Allgén, 1951 Paracanthonchus heterocaudatus Huang & Xu, 2013 Paracanthonchus heterodontus (Schulz, 1932) Vincx, Sharma & Smol, 1982 Paracanthonchus kamui Kito, 1981 Paracanthonchus kartanum (Mawson, 1953) Wieser, 1959 Paracanthonchus latens Gourbault, 1980 Paracanthonchus lissus Gagarin & Klerman, 2008 Paracanthonchus longicaudatus Warwick, 1971 Paracanthonchus longispiculum Pastor de Ward, 1985 Paracanthonchus macrodon (Ditlevsen, 1918) Micoletzky, 1924 Paracanthonchus macrospiralis Allgén, 1959 Paracanthonchus major (Kreis, 1928) Wieser, 1954 Paracanthonchus mamubiae Miljutina & Miljutin, 2015 Paracanthonchus margaretae Inglis, 1970 Paracanthonchus medius Galtsova, 1976 Paracanthonchus micoletzkyi Schuurmans Stekhoven, 1943 Paracanthonchus micropapillatus Wieser, 1954 Paracanthonchus miltommatus Leduc & Zhao, 2016 Paracanthonchus multisupplementatus Gagarin, 2012 Paracanthonchus multitubifer Timm, 1961 Paracanthonchus mutatus Wieser, 1959 Paracanthonchus nannodontus (Schulz, 1932) Wieser, 1954 Paracanthonchus olgae Tchesunov, 2015 Paracanthonchus parahartogi Decraemer & Coomans, 1978 Paracanthonchus perspicuus Kito, 1981 Paracanthonchus platti Vadhyar, 1980 Paracanthonchus platypus Wieser & Hopper, 1967 Paracanthonchus quinquepapillatus Wieser, 1959 Paracanthonchus ruens Wieser, 1954 Paracanthonchus sabulicolus Bouwman, 1981 Paracanthonchus sandspitensis Nasira, Kamran & Shanina, 2007 Paracanthonchus sonadiae Timm, 1961 Paracanthonchus stateni Allgén, 1930 Paracanthonchus stekhoveni Wieser, 1954

Paracanthonchus steueri (Micoletzky, 1922) Micoletzky, 1924 Paracanthonchus sunesoni (Allgén, 1942) Wieser, 1954 Paracanthonchus thaumasius (Schulz, 1932) Vincx, Sharma & Smol, 1982 Paracanthonchus tyrrhenicus (Brunetti, 1949) Gerlach, 1953 Paracanthonchus uniformis (Schuurmans Stekhoven, 1950) Wieser, 1954 Genus Paracyatholaimoides Gerlach, 1953 Paracvatholaimoides asymmetricus Boucher, 1976 Paracyatholaimoides labiosetosus Riemann, 1966 Paracyatholaimoides multispiralis Gerlach, 1953 Paracyatholaimoides serpens Gerlach, 1957 Genus Paracyatholaimus Micoletzky, 1922 Paracyatholaimus arcticus Kreis, 1963 Paracyatholaimus botosaneanui Andrássy, 1973 Paracyatholaimus chilensis Gerlach, 1953 Paracyatholaimus comatus Bussau, 1993 Paracyatholaimus diva Tchesunov, 2008 Paracyatholaimus dubiosus (Bütschli, 1874) Micoletzky, 1922 Paracyatholaimus helicellus Wieser, 1954 Paracyatholaimus huanghaiensis Huang & Xu, 2013 Paracyatholaimus inglisi (Wieser & Hopper, 1967) Andrássy, 1973 syn. Xyzzors inglisi Wieser & Hopper, 1967 Paracyatholaimus intermedius (de Man, 1880) Micoletzky, 1922 Paracyatholaimus occultus Gerlach, 1956 Paracyatholaimus papillatus Vinciguerra & Orcelli, 1997 Paracyatholaimus paramonovi Gagarin, 2004 Paracvatholaimus paucipapillatus Gerlach, 1955 Paracvatholaimus pentodon Riemann, 1966 Paracyatholaimus pesavis Wieser & Hopper, 1967 Paracyatholaimus proximus (Bütschli, 1874) Micoletzky, 1924 Paracyatholaimus pugettensis Wieser & Hopper, 1967 Paracyatholaimus qingdaoensis Huang & Xu, 2013 Paracyatholaimus quadriseta (Wieser, 1954) Tchesunov, 2008 Paracyatholaimus rotundus Gerlach, 1964 Paracyatholaimus saradi Gerlach, 1967 Paracyatholaimus separatus Wieser, 1954 Paracyatholaimus spinulosus Jensen, 1985 Paracyatholaimus ternus Wieser, 1954 Paracyatholaimus truncatus (Cobb, 1914) Micoletzky, 1922 Paracyatholaimus vancouverensis Sharma & Vincx, 1982 Paracyatholaimus vitraeus Gerlach, 1957 Genus Paralongicyatholaimus Schuurmans Stekhoven, 1950 Paralongicyatholaimus complicatus (Gerlach, 1957) Platt, 1982 Paralongicyatholaimus macramphis Lorenzen, 1972 Paralongicyatholaimus mastigodes Schuurmans Stekhoven, 1950 Paralongicyatholaimus minutus Warwick, 1971 Paralongicvatholaimus zosterae (Allgén, 1933) Lorenzen, 1972 Genus Paramarylynnia Huang & Zhang, 2007 Paramarylynnia filicaudata Huang & Sun, 2011 Paramarylynnia stenocervica Huang & Sun, 2011 Paramarylynnia subventrosetata Huang & Zhang, 2007 Genus Phyllolaimus Murphy, 1963 Phyllolaimus dentatus (Wieser, 1959) Murphy, 1963 Phyllolaimus tridentatus Murphy, 1963 Genus Pomponema Cobb, 1917 Pomponema ammophilum Lorenzen, 1972 Pomponema astrodes Lorenzen, 1972 Pomponema clavicaudatum (Schuurmans Stekhoven, 1935) Riemann, 1972 Pomponema compactum Lorenzen, 1972 Pomponema concinnum (Wieser, 1954) Lorenzen, 1972 Pomponema coomansi Vincx, 1981

Pomponema corniculata Gourbault, 1980 Pomponema cotylophorum (Steiner, 1916) Lorenzen, 1972 Pomponema debile Lorenzen, 1972 Pomponema elegans Lorenzen, 1972 Pomponema foeticolum (Ott, 1972) Cidreira et al., 2019 Pomponema golikovi Platonova, 1988 Pomponema hastatum (Ott, 1972) Cidreira et al., 2019 Pomponema koesterae Jensen, 1992 Pomponema lineatum (Gerlach, 1953) Wieser, 1959 Pomponema litorium (Cobb, 1920) Lorenzen, 1972 Pomponema loticum Lorenzen, 1972 Pomponema macrospirale (Ott, 1972) Cidreira et al., 2019 Pomponema mirabile Cobb, 1917 Pomponema multipapillatum (Filipjev, 1922) Wieser, 1954 Pomponema polydontus Murphy, 1963 Pomponema proximamphidum Tchesunov, 2008 Pomponema reductum Warwick, 1970 Pomponema sedecima Platt, 1973 Pomponema segregatum Wieser, 1959 Pomponema stomachor Wieser, 1954 Pomponema syltense Blome, 1974 Pomponema tautraense (Allgén, 1933) Lorenzen, 1972 Pomponema tesselatum Wieser & Hopper, 1967 Pomponema veronicae Cidreira et al. 2019 Pomponema websteri (Sharma & Vincx, 1982) Cidreira et al. 2019 Genus Praeacanthonchus Micoletzky, 1924 Praeacanthonchus cvgnis Inglis, 1970 Praeacanthonchus inglisi (Coles, 1965) Platt & Warwick, 1988 Praeacanthonchus kreisi (Allgén, 1929) Gerlach & Riemann, 1973 Praeacanthonchus opheliae (Warwick, 1970) Gerlach & Riemann, 1973 Praeacanthonchus punctatus (Bastian, 1865) Micoletzky, 1924 Genus Xenocyatholaimus Gerlach, 1953 Xenocyatholaimus delamarei Gerlach, 1953 Genus Xyzzors Inglis, 1963 *Xyzzors fitzgeraldae* Inglis, 1963 Xyzzors iubatus Ott, 1972

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CAPÍTULO 2. TWO NEW SPECIES OF CYATHOLAIMIDAE (NEMATODA: CHROMADORIDA) FROM THE SOUTHEASTERN BRAZILIAN COAST WITH EMPHASIS ON THE PORE COMPLEX AND LATERAL PORE-LIKE STRUCTURES

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ABSTRACT

Cyatholaimidae is a common and diverse family of mainly marine nematodes, potentially, with a large number of species to be discovered. The taxonomy of the group is marked by a lack of information about the evolutionary history of the characters and of detailed descriptions of morphological structures that may be taxonomically relevant. Two new species of the family are described from a sublittoral region in Southeastern Brazil, emphasizing the importance of the distribution and morphology of pore complex and pore-like structures present on the cuticle. The taxonomic importance of the cuticle ornamentation and spicule shape for the Biarmifer species, as well as the precloacal supplements structures of Pomponema species, are discussed. Biarmifer nesiotes sp. nov. differs from other species of the genus by the presence of eight longitudinal rows of pore complex on the cuticle and by the shape of the copulatory structure. Pomponema longispiculum sp. nov. differs from the most similar species, P. stomachor Wieser, 1954, by the smaller number of turns of the amphidial fovea, the shorter tail and the beginning of the cuticle lateral differentiation (³/₄ of the pharynx length vs. end of the pharynx, respectively). We also obtained the SSU rDNA sequence from Pomponema longispiculum sp. nov., which is closely related to Pomponema sp. (MN250093) by about 91%. Updated tabular keys to species identification of each genus (Biarmifer and Pomponema) are included, containing morphometric data, characters related to cuticle ornamentation, and copulatory structures.

INTRODUCTION

Free-living marine nematodes are one of the most abundant and diverse groups of the meiofauna, however, the lack of phylogenetically informative morphological characters can

hamper the study of relationships between and within taxa (Pereira *et al.*, 2010). A careful observation of morphological characters through optical and electron microscopy combined with molecular data acquisition expands our knowledge about marine nematode taxonomy and allows the understanding of the evolution of the shape and form of species (Fonseca *et al.*, 2017).

The family Cyatholaimidae Filipjev, 1918 is a diverse group of mainly marine nematodes with 20 genera and 211 valid species distributed worldwide in different types of habitats (Cunha *et al.*, 2022). Based on morphology, it has been subdivided into four subfamilies (Cyatholaiminae Filipjev, 1918, Paracanthonchinae De Coninck, 1965, Pomponematinae Gerlach & Riemann, 1973 and Xenocyatholaiminae Gerlach & Riemann, 1973), however, none are supported by any synapomorphy (Lorenzen, 1981). The same is true for many genera of the family, which are delimited by characters that may not be phylogenetically relevant (Cunha *et al.*, 2022). Therefore, taxonomic studies of Cyatholaimidae are marked by changes in classifications, synonyms and transfers of species between genera (*e.g.*, Micoletzky, 1924; Wieser, 1954; Miljutin & Miljutina, 2015).

For instance, the genus Pomponema was first erected by Cobb (1917) and later reviewed by Lorenzen (1972), who provided a diagnosis of the genus, based on the complex precloacal supplements, the well-developed buccal cavity and cuticle differentiation not like in Craspodema Gerlach, 1956 (lateral differentiation in the pharynx region as longitudinal rows of enlarged punctations with broadly lateral fields between them; at the end of the pharynx, these punctations became smaller and eight additional longitudinal fields became evident throughout the body). The author considered the genera Anaxonchium Cobb, 1920, Nummocephalus Filipjev, 1946, Haustrifera Wieser, 1954, and part of the genera Cyatholaimus Bastian, 1865 and Longicyatholaimus Micoletzky, 1924 as synonyms of Pomponema. Recently, Parapomponema Ott, 1972 and Propomponema Ott, 1972 were synonymized with this genus (Cidreira et al., 2019). Unlike Pomponema, the genus Biarmifer Wieser, 1954 underwent fewer changes. The genus was erected by Wieser (1954) who described two species and, in 1959, the author erected the new species *Biarmifer gibber*, which was synonymized with Paracanthonchus longus Allgén, 1934 by Lorenzen (1972). The genus was only found again over 40 years later when Pastor de Ward (2001) described Biarmifer madrynensis. Based on the cuticle ornamentation, which is unique to this group, it was recently proposed the reclassification of three species belonging to Marylynnia (Hopper, 1972) Hopper, 1977 to *Biarmifer* (Cunha *et al.*, 2022).

Pore complexes and lateral pore-like structures (LP) are cuticle structures with unknown functions commonly observed in cyatholaimids (Leduc & Zhao, 2016). Despite being poorly known, the size, structure, and cuticularization of both structures seem to diverge among species and may be taxonomically relevant (Hopper, 1972; Leduc & Zhao, 2016). The pore complexes are ring-like structures of dense material in the middle cuticle layer and are commonly seen along the sublateral, subventral, and subdorsal lines. They are characterized as a channel with a slit-like aperture and an associated cell (Wright & Hope, 1968; Leduc & Zhao, 2016). These pores vary in number, size, and organization, being distributed irregularly along the body or in four to twelve longitudinal rows (Hopper, 1972). The lateral pore-like is rarely included in species description, mostly due to its inconspicuous nature, hardly seen in detail on light microscopy. They are usually observed along the mediolateral lines of the body and are composed of a circular cuticularized opening supported by punctations and with a central dome (Leduc & Zhao, 2016).

The number of marine nematode species recorded in Brazil is low considering its long and heterogeneous coastline of almost 7500 km. For the Cyatholaimidae family, only 20 species belonging to 13 genera have been registered (Venekey *et al.*, 2010; Venekey, 2017; Oliveira *et al.*, 2017; Cidreira *et al.*, 2019). However, some local surveys on different habitats of the country found a high diversity of the family. In the intertidal zone of a beach in the state of Rio de Janeiro, for example, six genera of the family were identified, being the most diverse taxa along with Chromadoridae (Maria *et al.*, 2013). More than ten morphospecies of Cyatholaimidae were found in Araçá Bay, a tidal flat located in the state of São Paulo (Vieira & Fonseca, 2019). These findings, together with the low number of studies, suggest that there are many species to be discovered.

In the present study, we describe two new Cyatholaimidae species from a subtidal region of a beach on the Southeast Brazilian coast using morphological and molecular (SSU rDNA) data. We also analyzed the distribution and morphology of the pore complex and pore-like structures using light and scanning electron microscopy (SEM) and discuss its variabilities on species from the family.

MATERIALS AND METHODS

Sampling and morphological data

Sampling was conducted in February 2020, in the subtidal zone of Pedras Miúdas Beach, which is less than 80 m long (Fig. 1). This beach is located at São Sebastião Island, the

main island of the Ilhabela Archipelago, with 337 km². It is separated from the mainland by the São Sebastião Channel, which in its narrowest part is approximately 1.8 km wide. The collection was approved by ICMBio (19887-1).

The specimens were extracted from qualitative samples from the top 5 cm of sediment (1 - 2 m water depth). The sediment was frozen at -20°C and, in the laboratory, it was thawed overnight before the fauna extraction using Ludox TM-50 with a density of 1.18 g.cm⁻³ (Heip et al., 1985). The nematode species were sorted using a stereomicroscope. Part of the specimens was fixed in DESS (Yoder et al., 2006), transferred to glycerin and mounted onto permanent slides for light microscopy under a ZEISS Imager.M2 microscope equipped with differential interference contrast (DIC). The measurements were performed using images taken by an AxioCam MRc5 camera and its software. All measurements are in micrometers (µm) and all curved structures are measured along the arc. In addition to standard body measurements, we provide information on the number of mediolateral pore-like structures and sublateral pore complexes in the pharyngeal, tail, and central body regions (between the end of the pharynx and anal region) following Hopper (1972). Those numbers were determined for the uppermost and the most visible row. Abbreviations of measurements are as follows: a, body length/maximum body diameter; abd, anal body diameter; b, body length/pharynx length; c, body length/tail length; c', tail length/anal or cloacal body diameter; cbd, corresponding body diameter; L, total body length; LP, lateral pore-like structures; PC, pore complexes; V, vulva distance from anterior end of body; %V, position of vulva as a percentage of body length from anterior end.



Figure 1. Map showing the location of Pedras Miúdas beach at São Sebastião Island, São Paulo, Brazil.

At least one adult individual of each species studied was fixed in 2.5 % glutaraldehyde solution with sodium cacodylate buffer for SEM observation. Specimens were then dehydrated in a graded ethanol series (25% during 30 min and 30, 50, 70, 80, 90, 95, first 100, and second 100% changing from one concentration to the following every 10 min) and submitted to critical point drying. Next, individuals were mounted onto stubs and coated with gold/palladium using a sputter coater. Observations were made using a JSM 5800LV tabletop scanning electron microscope at high vacuum mode at the Electron Microscope Laboratory of the Institute of Biology at the University of Campinas (LME/UNICAMP). Holotypes and paratypes were deposited on MDBio – Museum of Biological Diversity of the Institute of Biology at the University of Campinas, Brazil (ZUEC-NMA/Collection).

The information about measurements and character states presented in the tabular keys were retrieved from the original descriptions. If a character was not reported in the text of a description, it was measured/described based on the associated illustrations.

The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The LSID for this publication is: urn:lsid:zoobank.org:pub:2430E61E-EDC6-410B-B7CE-B24C1AA6CA0B. The online version of this work is archived and available from the following digital repositories: PeerJ, PubMed Central SCIE and CLOCKSS.

Molecular data

The small ribosomal subunit (18S) was selected since it has been traditionally used in taxonomy and metabarcoding studies of marine nematodes (e.g., Derycke *et al.*, 2010; Armenteros *et al.*, 2014). Previous to molecular analysis, one adult individual of each species was mounted in a drop of water on temporary slides to be photographed. The DNA extraction of each specimen was done with Worm lysis buffer following Derycke *et al.* (2005).

Primers for the rDNA SSU were forward primer 18S-CL-F, 5'-TCAAAGATTAAGCCATGCAT -3' (Carta & Li, 2018) and reverse primer 1912BR, 5'-TTTACGGTTAGAACTAGGG -3' (adapted from Holterman *et al.*, 2006). The PCR reaction was performed in total volumes of 20 µl containing 10 µl Go Taq® Green Master Mix (Promega Corporation, Madison, WI, USA), 1 µl (10 µM) each of forward and reverse primers, and 2 to 5 µl of DNA template, depending on the DNA concentration. Cycling conditions for the amplification were: denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 2 min, and a final extension at 72 °C for 10 min. The amplified products were sequenced using a Sanger ABI 3500xL sequencer at the Multi-User Genotyping and Sequencing Laboratory of the Institute of Biology at the State University of Campinas. The sequence was observed and edited in the Geneious Premium 2022.1 program (http://www.geneious.com). Unfortunately, the amplification of DNA for the Biarmifer species was not successful. The DNA sequence of the Pomponema species was deposited in the GenBank under the accession number OP548510. The light micrographics of this Pomponema individual used for molecular analyses was deposited on MDBio (Museum of Biological Diversity of the Institute of Biology at the University of **ZUEC-PIC** 700 Campinas) under the accession number (available at https://www2.ib.unicamp.br/fnjv/).

RESULTS

Taxonomy

Class Chromadorea Inglis, 1983

Order Chromadorida Chitwood, 1933

Family CYATHOLAIMIDAE Filipjev, 1918

Diagnosis: (from Leduc & Zhao, 2016) Cuticle with transverse rows of punctations. Lateral punctations may be larger, irregular or arranged in longitudinal rows. Longitudinal rows of circular or elliptical cuticular structures (termed 'lateral pore-like structures') often present along mediolateral lines; up to 12 longitudinal rows of pore complexes may also be present. Inner labial sensilla often setiform; six outer labial setae and four cephalic setae in a single crown (with some rare exceptions); outer labial setae longer than the cephalic setae. Multispiral amphidial fovea. Cheilostoma with twelve distinctly cuticularized rugae. Pharyngostoma with a large dorsal tooth, and usually with two smaller ventrosublateral teeth, which may be single or double. Pharynx usually without a posterior bulb. Female didelphic-amphidelphic with reflexed anterior and posterior gonads always on opposite sides of the intestine. Male usually with two testes, rarely with one. Precloacal supplements may be present or absent.

Subfamily CYATHOLAIMINAE Filipjev, 1918

Diagnosis: (emended from Decraemer & Smol, 2006) Body cuticle with homogeneous punctation (except in *Biarmifer*), with or without lateral differentiation. Precloacal supplements absent or cup-shaped (tubular in *Praeacanthonchus*); gubernaculum unpaired proximally, except in *Biarmifer* and *Marylynnia* (paired).

Genus Biarmifer Wieser, 1954

Diagnosis: (emended from Pastor de Ward, 2001) Cuticle heterogeneous, with enlarged hexagonal punctuations in transverse rows from the anterior end to the nerve ring and transverse rows of simple punctuations on the rest of the body. Lateral differentiation absent or of smaller and more widely spaced punctations; anterior punctuations without stellate processes. Pore complexes arranged in four or eight longitudinal rows, with slit-like aperture variable, at 0-90° angles to the longitudinal body axis. One median-sized dorsal tooth and two pairs of small subventral teeth present. Spicules with inner processes and simple distal end, gubernaculum distally expanded or not. Cup-shaped non-sclerotized precloacal supplementary organs. Tail conical with or without a filiform portion.

Remarks: the genus *Biarmifer* was considered as being part of the Paracanthonchinae subfamily by Gerlach & Riemann (1973). A revaluation of the literature led Pastor de Ward (2001) to transfer the genus to the subfamily Cyatholaiminae mostly due to the cup-shaped precloacal supplements (it is tubular in Paracanthonchinae). The cuticle ornamentation of enlarged hexagonal punctations in the anterior end is a unique characteristic of the genus. Based on that, the species *Marylynnia dayi* (Inglis, 1963) Hopper, 1972, *M. hopperi* Sharma & Vincx, 1982, and *M. punctata* Jensen, 1985 were transferred to *Biarmifer* by Cunha *et al.* (2022). Besides cuticle ornamentation, both genera may be differentiated by the number of longitudinal rows of pore complexes. *Marylynnia* species have up to 12 rows (with a few exceptions, like *M. oculissoma* (Hopper, 1972) Hopper, 1977), and *Biarmifer* presents four longitudinal rows. Of the three *Marylynnia* species transferred, only *M. hopperi* has eight rows of pore complexes, the other two species possess four rows.

Biarmifer nesiotes sp. nov. (Fig. 2-7, Table 1)

urn:lsid:zoobank.org:act:F5253EA9-84EA-4409-B033-34F53DDDCEE4

Type locality: Brazil, São Paulo State, São Sebastião Island, Pedras Miúdas beach (23°49'49"S, 45°23'27"W), subtidal zone, from sediment with gravel predominance

Type specimens: Holotype male (ZUEC-NMA 35, slide) and five males and seven females paratypes (ZUEC-NMA 36 – 43, slide), all from the type locality.

Etymology: The species name is derived from the Greek term *nesiotes* (= inhabitant of the island), as a reference to the type locality, São Sebastião Island.

Description: Holotype and paratype males. Body cylindrical, slightly narrower on the anterior extremity and tapering abruptly on the posterior end. Cuticle heterogeneous with bigger punctations surrounded by a hexagonal outline (Fig. 2B, 3B-C) from the anterior sensilla to the nerve ring, and transverse rows of smaller, simpler punctations on the rest of the body (Fig. 2D, 3E-F). Cuticle striations clearly visible from the anterior end to the middle region of the pharynx, with one striation between each row of punctations. Lateral differentiation consisting of larger and more spaced punctations, clearly visible from the posterior end of the pharynx to the tail tip. Lateral region with half the number of punctations rows when compared to the ventral and dorsal regions. Longitudinal row of lateral pore-like (LP) structures along each mediolateral line starting immediately after the excretory pore to the conical part of the tail; LPs consisting of a circular or elliptical opening supported by an unmodified punctation at the anterior and posterior extremities, with a central, non-cuticularized dome (Fig. 4B, 4D, 5); underlying gland cells absent. LPs more conspicuous and with a more cuticularized opening in the pharyngeal and tail region (~ $2.7 \times 2.2 \ \mu m$ in size) (Fig. 3D) when compared to the ones from the middle body region ($\sim 1.9 \times 1.7 \,\mu m$) (Fig. 3E). Eight longitudinal rows of circular pore complexes, situated subventrally (2 rows), subdorsally (2 rows), and sublaterally (4 rows), starting about three head diameters from anterior end to the conical part of the tail; the pores are $1.2 - 1.9 \times 1.3 - 1.9 \mu m$ in size and $\sim 4 - 16 \mu m$ a part, the sublateral ones more conspicuous; no differentiation in the distribution along the body. Pore complexes consisting of a cuticularized ring with a slit-like transversal oriented aperture, a few diagonally oriented (Fig. 5). Three somatic setae located on each sublateral axis on the pharyngeal region, 5 to 14 µm in length, at about 2.5 head diameter from the anterior end. Two longitudinal rows of setae on the tail.

Anterior sensilla arranged in two crowns; first crown consisting of six inner labial setae, 3 μ m long (2.3 – 3.4 μ m); second crown consisting of six slightly longer outer labial setae with a thinner tip (9 – 11.8 μ m long) and four cephalic setae (6.5 – 8.5 μ m long). Multispiral amphidial fovea with 5 turns and circular outline, corresponding to 50% of the cbd (46 – 50%), situated slightly posterior to the second crown of the anterior sensilla (Fig. 3B). Buccal cavity consisting of cup-shaped cheilostoma with 12 rugae and a narrow funnel-shaped
pharyngostoma with a large dorsal tooth and two pairs of ventrosublateral teeth (Fig. 2C, 3A). Pharynx cylindrical, muscular and glandular. Secretory excretory system present; excretory pore located about ¹/₄ of the pharynx length; renette cell observed in one male, 41 x 18 µm in size. Cardia not surrounded by intestinal tissue. Intestinal lumen with numerous small golden-brown granules.



Figure 2. *Biarmifer nesiotes* sp. nov. (A) Entire body, male. (B) Anterior body region, male, superficial view. (C) Cephalic region, male. (D) Cuticle in middle body region, male. (E) Posterior body region, male. (F) Middle body region of a female showing the reproductive system. (G) Gubernaculum, ventral view. Scale bars: $A = 200 \ \mu m$; $B = 20 \ \mu m$; C, D, $G = 10 \ \mu m$; $E = 50 \ \mu m$; $F = 100 \ \mu m$.

	Biarmij	^f er nesiotes s	o. nov.	Pomponema longispiculum sp. nov.			
-	Male	Males	Females	Male	Males	Females	
	Holotype	Paratypes	Paratypes	Holotype	Paratypes	Paratypes	
Ν	-	5	7	-	3	5	
L	1871 5	1252-1744	1488-1747	18544	1352-	1396-	
9	23.3	17 4-26 6	17 3-25 7	30	30 4-39 7	28-36.5	
a h	6.8	5 8-6 8	5 8-6 8	7	6 6-7	57-66	
C C	10.5	7 3-8 1	7-8.9	96	7 4-8	6-8.4	
c'	3.5	3.6.4.2	46.56).0 1 7	5 5 5 8	61.75	
c Pharvny length	276.6	215 3_285 7		265.6	206-242	244_285.2	
Pharyngeal diam. at	270.0	215.5 205.7	255.5 205.5	205.0	200 242	244 203.2	
base	32	24.4-28.3	22.3-32.3	25	17-20	18.3-26.7	
Pharynx cbd at base	74	52-81.6	49.3-80.7	43.3	41-44	39-47.3	
Max. body diam.	80.4	60-100	59-101	47.4	34-51	45-58	
Tail length	177.7	171.8-220.3	167-242	193.5	176-211.5	199.3-233.2	
Length of inner labial	2	2.2.4	2 4 2 5	5.4		1665	
setae Length of outer labial	3	2-3.4	2.4-3.5	5.4	4-0.4	4.0-0.3	
setae	10.5	9.1-11.8	9.3-12	14	12-15	11.5-14.6	
Length of cephalic setae	7.7	6.5-8.5	7-9.7	4.8	2.8-4.2	3.3-3.6	
Head diam. at cephalic							
setae	24.8	24-25	24.3-25.7	33	29-34	32.5-35	
Head diam. at amphids	30	27-30.5	27-30.8	33.6	30-32.7	34.3-37	
Amphid turns	5	5	4-5	5.5	6	4-5	
Amphid height	13	12.3-14	8.8-12.7	21,76	15.4-19	10.5-12	
Amphid width	15	12.8-14.7	11-13.5	19.2	17-19.5	10.8-11.3	
Amphid width/cbd (%)	50	47-48.2	40-46	57	54.8-59.6	29.2-32.9	
Amphid from anterior	97	7 5-10 8	6-9.2	11.2	8 1-10 7	6.6-10	
Excretory pore from).1	7.5 10.0	0 9.2	11.2	0.1 10.7	0.0 10	
anterior end	75.4	61-70	68.7-77.6	-	127*	-	
Nerve ring from	110.7	117 110	116 122 2	120.6	110 0 112	116 120 4	
Anterior end	60	54 57	54.57	120.0	25 41	110-130.4	
Nerve ring cou Spiculo longth	82.6	72 92 7	54-57	40.5	33-41 72 5 79	-	
Spicule length	62,0	60.70	-	10.6	12.5-10	-	
Gubernaculum length V	05	00-70	721 847	49.0	44-47.0	780 006 8	
v 0/ V	-	-	/31-84/	-	-	52 1 55 0	
70 V Vulval chd	-	_	68 5 99 2	-	-	13 3 54	
Abd	-	47_59	36-48.4	- 41 4	32_38.4	30_37 3	
Laterodorsal PC	51	т-37	50101	71.7	52-50.4	50-57.5	
(pharynx)	23	18-22	15-21	13	9	13-17	
Laterodorsal PC	1.4.5	114 100	100 155		75	(0, (1	
(central body)	145	114-129	108-155	//	/5	60-61	
Laterodorsal PC (tall) Lateroventral PC	2	6-8	5-9	/	6	5-7	
(pharynx)	25	15-27	13-20	18	8-15	10-17	
Lateroventral PC							
(central body)	138	118-141	116-139	71	60-61	55-69	

 Table 1. Morphometric data of the two new species.

(Continued)

Table 1. (Continued)

	Biarmi	fer nesiotes sp.	nov.	Pomponema longispiculum sp. nov.			
	Male	Males	Females	Male	Males	Females	
	Holotype	Paratypes	Paratypes	Holotype	Paratypes	Paratypes	
Lateroventral PC (tail)	7	6-8	6-8	7	4	5-7	
LP (pharynx)	11	11-13	11-16	5	5-6	5-7	
LP (central body)	45	38-52	30-40	29	29-33	26-41	
LP (tail)	3	3-5	5-8	2	2-3	2	

*Observed in only one specimen

Abbreviations: a, body length/maximum body diameter; abd, anal body diameter; b, body length/pharynx length; c, body length/tail length; c', tail length/anal or cloacal body diameter; cbd, corresponding body diameter; L, total body length; LP, lateral pore-like structures; N, number of specimens; PC, pore-complexes; V, vulva distance from anterior end of body; %V, position of vulva as a percentage of body length from anterior end.



Figure 3. *Biarmifer nesiotes* sp. nov., lightning micrographs, male. (A) Anterior body region. (B) Detail of the multispiral amphideal fovea. (C) Cuticle from the anterior region to nerve ring. (D) Cuticle from the nerve ring to end of pharynx. (E) Cuticle from the middle body region. (F) Posterior region and copulatory apparatus. Am: amphideal fovea; DT: dorsal tooth; LP: likepore structure; PC: pore complex; Sp: precloacal supplements; VT: ventrosublateral teeth. Scale bars $A-E = 10 \ \mu\text{m}$; $F = 50 \ \mu\text{m}$.

Reproductive system diorchic, gonads opposed. Spicules paired 83 μ m long, (72 – 83 μ m) without inner process, with prominences on the proximal end; fusiform in the distal half, dilated in the middle region, with a central groove, and pointed distal end (Fig. 6A). Gubernaculum complex (Fig. 6), proximally and distally paired (not fused), slightly shorter than the spicules, 65 μ m long (60 – 70 μ m), heavily cuticularized; forceps-shaped like in ventral or dorsal view (Fig. 2G); proximal end swollen and curved behind the spicules, mid-region with a lateral wing, distal end expanded with denticles. Five weakly developed cup-shaped precloacal supplements present, 17 – 27 μ m apart from each other, the anterior-most at about 120 μ m (88.2 – 122 μ m) from the cloacal opening. Tail conical-cylindrical with the conical portion representing approximately 45% (Fig. 2E).

Paratype females. Similar to males but with slightly smaller amphidial fovea, corresponding to 40 - 45% of cbd and with four to five turns (Fig. 7A). Renette cell observed in one female, at the level of the posterior end of the pharynx and anterior end of the intestine, with 40 x 20 µm in size. Reproductive system didelphic, amphidelphic. Vagina and vaginal glands heavily cuticularized (Fig. 2F, 7C). Vulva located slightly pre-median. Up to four eggs observed per individual, $54 - 79 \times 36 - 59$ µm in size.

Diagnosis. *Biarmifer nesiotes* sp. nov. is characterized by a heterogeneous cuticle with lateral differentiation. Longitudinal rows of LP along mediolateral lines and eight longitudinal rows of pore complexes situated subventrally, subdorsally, and sublaterally. Outer labial setae about 10 μ m long, and cephalic setae about 7 μ m. Amphids with five turns (50 % cbd) in males and 4 – 5 turns (40 – 45 %) in females. Buccal cavity with one dorsal tooth and two pairs of ventrosublateral teeth. Spicules 72 – 83 μ m long; gubernaculum 60 – 70 μ m long, proximally and distally paired (not fused), proximal end swollen and curved behind the spicules, midregion with a lateral wing, distal end expanded with denticles. Five weakly developed cupshaped precloacal supplements. Vagina and vaginal glands strongly cuticularized.



Figure 4. *Biarmifer nesiotes* sp. nov., scanning electron micrographs, male. (A) Anterior body region. (B) Anterior most lateral pore-like structures. (C) Detail of a pore complex in the pre-cloacal region. (D) Detail of a lateral pore-like structure in the pre-cloacal region. Scale bars: $A = 20 \ \mu m$; B-D = 5 \ \mu m.



Figure 5. Schematic drawing of pore complex (PC) and lateral pore-like structures (LP) from the pharyngeal region of *Biarmifer nesiotes* sp. nov. d: dome; p: punctation; sla: slit-like aperture; r: cuticularized ring.



Figure 6. Gubernaculum in different positions, from different specimens of *Biarmifer nesiotes* sp. nov., lightning micrographs. (A) Ventral view. (B) Dorsal view. (C) Dorsolateral view. (D) Lateral view. Scale bars: 10 μm.

Relationships: *Biarmifer nesiotes* sp. nov. differs from all species of the genus by the shape of copulatory organs (Fig. 8). It also can be differentiated from the other six valid *Biarmifer* species by the combination of the following characters include in the tabular key of the genus (Table 2): lateral differentiation of cuticle; number of longitudinal rows of pore complex; amphidial turns; amphid width (as % of the corresponding body diameter); spicule length; number of precloacal supplements; and the De Man morphometric ratios (a, b, c, c').

The new species is similar to *B. cochleatus* Wieser, 1954 in the number of supplements, conical-cylindrical tail and number of amphids turns, but differs by the smaller setae in the second crown of anterior sensilla $(10 + 7 \ \mu m \ vs. 23-24 + 16 \ \mu m)$ and by the presence of lateral differentiation in the cuticle. The strong cuticularized vagina of females and the presence of eight longitudinal rows of pore complex in the cuticle (there are four rows in the other species) were observed only in the new species and in *B. hopperi* (Sharma and Vincx, 1982) Cunha *et al.*, 2022 among all species of the genus. It differs from the new species by the absence of lateral differentiation in the cuticle and by the shape of the copulatory apparatus. Also, *B. hopperi* has a cluster of 25 - 36 lateral pore-like structures in the mid-pharyngeal region, which are not observed in the new species (only 11 - 13 LP in the pharynx, located on the mediolateral line).

The aperture of pore complexes is transversally or diagonally oriented throughout the body of *Biarmifer nesiotes* sp. nov., different from *B. madrynensis* Pastor de Ward, 2001, where the aperture variated from diagonal (oriented 30° from the longitudinal axis) on pharynx and tail and longitudinal on the middle of the body (oriented 0° the from the longitudinal axis).



Figure 7. *Biarmifer nesiotes* sp. nov, lightning micrographs, female. (A) Anterior body region. (B) Cuticle from the middle body region. (C) Detail of the vulva and vagina. Scale bars: $A = 20 \ \mu m$; B-C = 10 μm .



Figure 8. Copulatory apparatus of *Biarmifer* species. (A) *B. cochleatus*, adapted from Wieser, 1954. (B) *B. dayi*, adapted from Inglis, 1963. (C) *B. hopperi*, adapted from Sharma and Vincx, 1982. (D) *B. laminatus*, adapted from Wieser, 1954. (E) *B. madrynensis*, adapted from Pastor de Ward, 2001. (F) *B. punctata*, adapted from Jensen, 1985.

Table 2. Tabular key of *Biarmifer* species. When different from males, the information about females is provided between brackets. Abbreviations: a, body length/maximum body diameter; b, body length/pharynx length; c, body length/tail length; c', tail length/anal or cloacal body diameter; n.a, information not available; P.c., number of longitudinal rows of pore-complex; S.i.s., spicule inner septa. A, absent; P, present.

Species	Lateral differentiation of cuticle	P.c.	a	b	c	c'	Amphideal turns	Amphid cdb %	Spicule length (µm)	S.i.s.	N° Supplem.
B. cochleatus Wieser, 1954	А	4	20-28	5-7.5	6.1-9	4.5-5	4.5	37	60	Р	5
<i>B. dayi</i> (Inglis, 1963) Cunha <i>et al.</i> , 2022	А	4	31	4.8	12.4	3	3.75	20	65	n.a	0
<i>B. hopperi</i> (Sharma & Vincx, 1982) Cunha <i>et al.</i> , 2022	A	8	25.1- 36.5 (18- 25.5)	6.8- 7.8	9.9-10.2 (8.8-9.6)	3.3-5.2 (6.7)	5	45	88	n.a	5
B. laminatus Wieser, 1954	\mathbf{P}^1	4	40 (20.5 -25.9)	8 (5.2- 6.1)	12 (11.4- 15.6)	3.6 (2.75-3)	8.25 (5.5)	54 (50)	32	Р	n.a
<i>B. madrynensis</i> Pastor de Ward, 2001	Р	4	27.2- 45.3	4.7- 6.3	5.3-8	8-9	3.5	20-32.5	62-78	Р	3
B. nesiotes sp. nov.	Р	8	17.4- 26.6	5.8- 6.8	7.3-10.5	3.5-4.2	5 (4-5)	47-50 (40-46)	72-84	А	5
<i>B. punctata</i> (Jensen, 1985) Cunha <i>et al.</i> , 2022	Р	4	29-30	6.2 (4.9)	8.4 (6.6)	5 (6.5)	5	53	60	Р	6

¹Lateral differentiation was observed only on the tail.

Subfamily POMPONEMATINAE Gerlach & Riemann, 1973

Diagnosis: (from Decraemer & Smol, 2006) Body cuticle punctated, with lateral differentiation in ornamentation. Precloacal supplements knob-like or flattened, complex, consisting of several elements; gubernaculum paired proximally.

Genus Pomponema Cobb, 1917

Diagnosis: (modified from Cidreira *et al.*, 2019) Body cuticle heterogeneous with fine punctations, with or without lateral differentiation of longitudinal rows of enlarged punctations behind the cephalic region, and occasionally with slit-like markings. Cuticle in the cephalic region usually thick with punctations appearing in lateral view as elongated rods with Y-shaped ends. Inner labial sensilla setiform. Outer labial and cephalic sensilla setiform located in the same circle, usually jointed, with a tip section markedly narrower than the base, although this may be difficult to discern in some species. Outer labial setae larger than cephalic setae, but the four smaller setae are sometimes absent or at least so small and adherent to the outer labial setae that they cannot be detected. Amphidial fovea multispiral. Buccal cavity armed with a large pointed dorsal tooth, medium-sized ventrosublateral teeth, and with or without additional minute denticles. Pharyngeal bulb absent. Males with two opposed testes, rarely only one. Females didelphic-amphidelphic. Precloacal supplements complex, with several elements best seen in the ventral view. Gubernaculum with variously structured distal ends, sometimes with L-shaped lateral plates or lateral flanges bearing blunt teeth. Tail conical-cylindrical with a swollen tip.

Remarks: The species *Bolbolaimus punctata* Cobb, 1920 was transferred to the *Pomponema* genus by Luc & De Coninck (1959); however, it was re-established as *Bolbolaimus* species by Jensen (1978).

Pomponema longispiculum sp. nov. (Fig. 9-12, Table 1)

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Type locality: Brazil, São Paulo State, São Sebastião Island, Pedras Miúdas beach (23°49'49"S, 45°23'27"W), subtidal zone, from sediment with gravel predominance.

Type specimens: Holotype male (ZUEC-NMA 50, slide) and three male and five female paratypes (ZUEC-NMA 51 - 58, slide), all from type locality.

Etymology: The species name is derived from the Latin words *longis* (= big, long) and *spiculum* (=spicule), and refers to the long spicules of this species in comparison to the other species of the genus.

Description: Holotype and paratype males. Body cylindrical, slender (Fig. 9A). Cuticle heterogeneous; thicker in the cephalic region, with punctations appearing in the lateral view as Y-shaped. Transverse rows of larger punctations on the anterior region of the body, gradually decreasing posteriorly. Lateral differentiation consisting of two to four longitudinal rows of punctations, beginning approximately at ³/₄ of the pharynx length from the anterior end. Lateral pore-like structures (LP) consisting of a circular or elliptical strongly cuticularized and thick opening supported by unmodified punctations and with a central, non-cuticularized dome (Fig. 10C, 10F; 11C, 11E, 11F, 12); underlying gland cells absent. Four to five conspicuous lateral pore-like structures (LP), $2 - 3 \times 2.7 - 3.5 \mu m$ in size, located at mediolateral lines between amphidial fovea and the nerve ring, beginning approximately at two head diameters from the anterior end; opening supported by four to six punctations (Fig. 10C, 12). From the beginning of the lateral differentiation to the conical part of the tail, the LPs are slightly smaller, 1.5 - 2.8 \times 1.5 – 2.6 µm in size, and located between the mediolateral and laterodorsally lines (Fig. 9E; 10F), their opening is supported by two to four unmodified punctations. Four sublateral longitudinal rows of circular pore complexes, $\sim 1 - 1.8 \times 1.2 - 1.9 \,\mu\text{m}$ in size and $\sim 6 - 17 \,\mu\text{m}$ apart from each other, consisting of a cuticularized ring with a slit-like aperture, arranged 30° of the longitudinal axis (Fig. 12). Few somatic setae located sublaterally on the pharyngeal region $(7 - 15 \,\mu\text{m long})$ and four sublateral longitudinal rows of small setae along the body, sometimes difficult to differentiate from the pore complexes.

Anterior sensilla arranged in two crowns; first crown consisting of six inner labial setae, 5.4 μ m long (4 – 6.4 μ m); second crown consisting of six outer labial setae with a thinner tip, 14 μ m long (12 – 15 μ m), and four inconspicuous cephalic setae adherent to the outer labial setae (Fig. 11A), 4.8 μ m long (2.8 – 4.2 μ m). Multispiral amphidial fovea with 5.5 – 6 turns and circular in outline, representing 57% of the corresponding body diameter (54.8 – 59.6%), situated slightly posterior to the second crown of the anterior sensilla (Fig. 9B; 10B). Buccal cavity cup-shaped, cheilostoma with 12 rugae and a narrow funnel-shaped; pharyngostoma with a large dorsal tooth, two pairs of ventrosublateral teeth and three to four rows of denticles (Fig. 9C). Cylindrical pharynx, slightly enlarged anteriorly. Secretory excretory system observed in only one male; excretory pore located approximately at the middle pharynx, renette cell 69 x

20.4 µm long located after the end of the pharynx. Cardia not observed. Intestinal lumen with numerous small golden-brown granules.



Figure 9. *Pomponema longispiculum* sp. nov. (A) Entire body, male. (B) Anterior body region, male, superficial view. (C) Cephalic region, male. (D) Posterior body region, male. (E) Cuticle in the mid-body region, male. (F) Gubernaculum, ventral view. (G) Middle body region of a female showing the reproductive system. Scale bars: $A = 200 \mu m$; B, C, E, F = 10 μm ; D = 50 μm ; G = 100 μm .



Figure 10. *Pomponema longispiculum* sp. nov., lightning micrographs, male. (A) Anterior body region. (B) Surface view of the anterior body region. (C) Cuticle in the pharyngeal region. (D) Copulatory apparatus, ventral view. (E) Detail of precloacal supplements in ventral view. (F) Cuticle in the middle body region. Am: amphideal fovea; DT: dorsal tooth; G: gubernaculum; LP: lateral pore-like; PC: pore complex; S: spicule; VT: ventral teeth. Scale bars = $10 \mu m$.

Reproductive system diorchic, gonads opposed. Spicules arched with a central lamella (Fig. 10D), 75.7 μ m long (72.5 – 78 μ m), corresponding to 1.8 times (1.9 – 2.4) the cloacal body diameter. Gubernaculum fused, with lateral wings and distal end forked, bearing a pore in each tip (Fig. 9F, 10D, 11B). There is a short precloacal seta, ~3 μ m long. There are 22 (19 – 21) precloacal supplements extending 365 μ m (256 – 307 μ m) anteriorly from the cloaca. Supplements are composed of an outer plate, H-shaped in ventral view, with a pore running through the center (Fig. 10E). Above this plate, there is an elliptical cuticularized ring. Anteriorly and posteriorly of each supplement there is a cuticularized element of irregular shape. There are 4 to 7 bars (lamellated cuticle) between each supplement. Tail conical-cylindrical (Fig. 7D), corresponding to 4.7 times the anal body diameters.

Paratype Females. Similar to males but with a smaller amphidial fovea (5.5 - 6 turns) and 54.8 - 59.6% cbd in males *versus* 4 - 5 turns and 29.2 - 32.9% in females). Reproductive system didelphic, amphidelphic with the anterior reflexed ovary lying on the left of the intestine

and the posterior reflexed ovary lying on the right of the intestine (Fig. 9G). No mature eggs observed. Vulva located slightly post-median body region.



Figure 11. *Pomponema longispiculum* sp. nov., scanning electron micrographs, male. (A) Head in frontal view. (B) Cloacal region, showing the distal ends of the copulatory apparatus. (C) Like-pore structure and pore complex in the anterior region. (D) Pore complexes in the middle body region. (E) Like-pore structure in the middle body region. (F) Like-pore structure in the tail. Am: amphideal fovea; CS: cephalic seta; G: gubernaculum; LP: lateral pore-like; PC: pore complex; S: spicule. Scale bars: A, B = 5 μ m; C, D, F = 2 μ m; E = 1 μ m.

Diagnosis: *Pomponema longispiculum* sp. nov. is characterized by a heterogeneous cuticle with lateral differentiation consisting of two to four longitudinal rows of punctations. Four to five conspicuous lateral pore-like structures between the amphidial fovea and nerve ring and a longitudinal row of smaller LPs between the mediolateral and laterodorsally lines, from the beginning of lateral differentiation to the tail. Four longitudinal rows of pore complexes situated sublaterally. Six outer labial setae $12 - 15 \mu m$ long, and four inconspicuous cephalic setae, 2.8 – 4.8 μm long. Amphids with 5.5 – 6 turns (55 – 60 % cbd) in males and 4 – 5 turns (29 – 33 % cbd) in females. Buccal cavity with one dorsal tooth, two pairs of ventrosublateral teeth, and three to four rows of denticles. Spicules with lamella, with length corresponding to 1.8 - 2.4 times the anal cbd; 19 - 23 typical precloacal supplements; and gubernaculum 44 – 50 μm long, fused.

Relationships: *Pomponema longispiculum* sp. nov. can be differentiated from the other 31 valid *Pomponema* species (Cunha *et al.*, 2022) by the following characters included in the tabular key (Table 3): lateral differentiation of cuticle; presence or absence of ventral teeth; presence or absence of rows of denticles in the buccal cavity; amphid width (as % of the corresponding body diameter); amphidial turns; length of outer labial setae; spicules length; number of precloacal supplements; and De Man morphometric parameters (a, b, c, c').



Figure 12. Schematic drawing of pore complex (PC) and lateral pore-like structures (LP) from the pharyngeal region of *Pomponema longispiculum* sp. nov. d: dome; p: punctation; sla: slit-like aperture; r: cuticularized ring.

Pomponema longispiculum sp. nov. is similar to *P. stomachor* Wieser, 1954, which was described from the Chilean coast. The new species differs by the relative size of amphidial fovea (55 - 60 % cbd in males and 30% in females *vs.* 50% in both sexes in *P. stomachor*), the shorter tail in males (4.7 - 5.8 *vs.* 7 cloacal diameters in length), the shorter labial sensilla in males (inner labial setae 4 - 6 µm long *vs.* 9 µm; and outer labial setae 12 - 15 µm long *vs.* 18 µm), the presence of two pairs of subventral teeth *vs.* one pair in *P. stomachor*, and the beginning of the cuticle lateral differentiation ($\frac{3}{4}$ *vs.* end of the pharynx). Both species have spicules of similar length, however, it represents 1.8 - 2 anal diameters in *P. longispiculum* sp. nov. and 1.7 in the Chilean one. The shape of the gubernaculum is not described in detail and is not clearly visible on drawings of *P. stomachor*. The new species is also similar to *P. reductum* Warwick, 1970 regarding the gubernaculum shape, but they differ by the length of outer labial setae and the spicules (see Table S2), and by the cuticle ornamentation. In *P. reductum* the cuticle has smaller irregularly arranged punctations in the anterior region which

gradually increase in size towards the lateral fields. *Pomponema longispiculum* sp. nov. has bigger punctations in the anterior region and punctations in the lateral field of the same size throughout the body.

The general aspect of precloacal supplements from *P. longispiculum* sp. nov., *P. sedecima* and *P. tesselatum* Wieser & Hopper, 1967 are similar (Fig. 13). These structures differ among those species in the number of bars between each supplement, the absence of cuticularized elements of irregular shape in *P. sedecima*, and the absence of an outer plate, H-shaped in ventral view, in *P. tesselatum*

Pomponema tesselatum is the only species of the genus that presents detailed descriptions of the pore complexes and LPs structures. According to Hopper (1972), this species has the aperture of pore complexes longitudinally oriented and only 7 - 18 LPs throughout the body. In *P. longispiculum* sp. nov. the slit-like aperture is diagonally oriented and there are more LPs throughout the body (34 – 49).

Based on the SSU molecular sequence deposited in the Genbank, *Pomponema longispiculum* sp. nov. is closest to *Pomponema* sp. (MN250093) with 8.73% of difference (64 in 733 bp). The next two most similar sequences recovered are from *Praeacanthonchus punctatus* (MG669976 and MG669981), with 67 and 68 bases pairs different, respectively.



Figure 13. Schematic drawing of precloacal supplements from *Pomponema* species, in the ventral view. (A) *P. concinnum*, adapted from Wieser (1954). (B) *P. corniculata*, adapted from Gourbault (1980). (C) *P. hastatum*, adapted from Ott (1972). (D) *P. longispiculum* sp. nov. (E) *P. sedecima*, adapted from Platt (1973). (F) *P. tesselatum*, adapted from Platt & Warwick (1988). Note: The bars of lamellated cuticle are not represented by Ott in ventral view, but they are present in *P. hastatum*. However, the bars seem to be absent in *P. concinnum* and *P. corniculata*.

Table 3. Tabular key of *Pomponema* species. When different from males, the information about females is provided between brackets. Types of lateral differentiation of cuticle (L.d.): (1) Two to four longitudinal rows of dots not connected transversely, (2) Two to four longitudinal rows of dots connected transversely by lines, (3) Widely spaced dots, (4) Two longitudinal rows of bigger dots with wide space between them. Abbreviations: a, body length/maximum body diameter; b, body length/pharynx length; c, body length/tail length; c', tail length/anal or cloacal body diameter; cbd, corresponding body diameter; n.a, information not available; O.l.s: outer labial setae length; V.t.: ventral teeth; R.d.: rows of denticles on the buccal cavity. A: absent; P: present.

Species	L.d.	V.t.	R.d.	a	b	c	c'	Amphid. width (%)	Amphid. turns	Ο.l.s (μm)	N° cephalic setae	N° Supplem.	Spicule length (µm)
P. ammophilum Lorenzen, 1972	А	Р	Р	28-40	5.5-6.8	7.7-9	n.a	~55	5(4.5)	15-16	0	19-22	54-65
P. astrodes Lorenzen, 1972	1	Р	А	40-53	7	4.4-5.6	n.a	~51	6	6	4	11	33-35
P. clavicaudatum (Schuurmans Stekhoven, 1935) Lorenzen, 1972	1	А	A	48.1 (36)	7.3 (5.6)	8.7 (7.7)	4.5	50	5	14 (9)	4	22	48
P. compactum Lorenzen, 1972	3	Р	Р	36-41 (30-31)	4.7-5.3 (4.2- 4.5)	7.5-9.2 (5.9-6.7)	n.a	56-60 (46- 48)	5-5.25 (4-4.5)	11-16	0	13-16	30-35
P. concinnum (Wieser, 1954) Lorenzen, 1972	2	А	А	28	5.6	7	6	40	4	6	n.a	20	36
P. coomansi Vincx, 1981	2	Р	А	42.7- 47.5	6	4.7-6.2	11	38	5.3	16	4	2	n.a
<i>P. corniculata</i> Gourbault, 1980	4	Р	А	22.9- 23.7 (20.9- 22.4)	4.3-4.8	11.2-13.5	2.3-2.5 (3-3.4)	45	6	2.5-3	4	20	41-47
P. cotylophorum (Steiner, 1916) Lorenzen, 1972	4	А	А	30	5	12	n.a	n.a	n.a	A?	A?	15	n.a
P. debile Lorenzen, 1972	2	Р	Р	40-45	5.5-6.1	8.7-9.5	n.a	42-46	4-5	8.5-9.5	0	15-18	27-29
P. elegans Lorenzen, 1972	2	Р	Р	49-60 (41)	6.5-7.8	7.7-9.2 (5.4)	n.a	~70 (40)	6	8-9	0	12-15	30-34

(Continued)

 Table 3. (Continued)

Species	L.d.	V.t.	R.d.	a	b	c	c'	Amphid. width (%)	Amphid. turns	Ο.l.s (μm)	N° cephalic setae	N° Supplem.	Spicule length (µm)
<i>P. foeticolum</i> (Ott, 1972) Cidreira <i>et al.</i> , 2019	А	Р	А	42.9- 60.9	7-9.8	12.5- 18.3	3.1- 3.8 (4.9)	44-46 (38)	5 (3.5)	11-14	4	13-16	39-44
<i>P. golikovi</i> Platonova, 1988	А	Р	А	34.5	6.8	9.7	3.5	50	5	6.5	4	13	50
P. hastatum (Ott, 1972) Cidreira et al., 2019	4	Р	А	47.7- 55.5	4.7-5	17.9- 18.2 (14.5- 16.3)	3.1- 3.4 (4.7- 4.8)	53-54 (38)	3.25-3.75	11-13	4	19-20	25-28
P. koesterae Jensen, 1992	4	А	Р	24 (18- 21)	4.2- 6.1	7.3-7.6 (5.7- 7.9)	5.6-9	55	10	3-4	4	15-17	37-41
P. lineatum (Gerlach, 1953) Wieser, 1959	1	Р	А	38 (25)	5.9 (5.6)	8.8 (7)	5 (6- 7)	75	2.5	8-13	4	13	30
<i>P. litorium</i> (Cobb, 1920) Lorenzen, 1972	2	Р	Р	41.7	5.8	10	5.3	n.a	n.a	n.a	4	n.a	n.a
P. longispiculum sp. nov.	1	Р	Р	28- 39.7	5.7-7	6-9.6	4.7- 7.5	54.8-59.6 (29.2-32.9)	5.5-6 (4- 5)	11.5- 15	0 or 1	19-22	72.5-78
P. loticum Lorenzen, 1972	2	Р	Р	30.5- 31 (26)	5.2- 5.6 (4.9)	7.3-84	4.6-5 (5.4)	n.a	n.a	16	4	19	35-35.5
<i>P. macrospirale</i> (Ott, 1972) Cidreira <i>et al.</i> , 2019	4	Р	А	45.1- 49.7	4.2- 4.6	15.4- 16.1	3.4- 3.6	63-76	4.75-5.75	15-16	4	18	27-30
P. mirabile Cobb, 1917	n.a	Р	A?	52.6	4.8	13.7	4.9	~75	6	n.a	4	20	n.a
<i>P. multipapillatum</i> ¹ (Filipjev, 1922) Wieser, 1954	2	Р	Р	18- 30	5.5- 7.6	7.3-9.6	n.a	n.a	5	4-8	4	15-19	34-46
P. polydontus Murphy, 1963	1	Р	Р	43.1- 63.2	5.4- 7.4	9.9- 12.5	6 (5.2)	35	4.5	23	4	24	55
P. proximamphidum Tchesunov, 2008	4	Р	Р	27.6- 34	4.7- 6.2	4-6	7.4- 8.1	55-59	5	2-5.5	4	13-14	41-46

 Table 3. (Continued)

Species	L.d.	V.t.	R.d.	a	b	c	c'	Amphid. width (%)	Amphid. turns	O.l.s (µm)	N° cephalic setae	N° Supplem.	Spicule length (µm)
P. reductum Warwick, 1970	1	Р	Р	43.4- 46.2 (33.3- 42.6)	6.2-7	9.7- 10.9	4.5- 5.5 (5.6- 6)	37-42 (27- 31)	4-4.5 (3.5)	8-10	0	20-24	43-46
P. sedecima Platt, 1973	2	Р	Р	48.1- 49.5 (37.5)	7-7.4 (6.5)	12.2- 12.9 (10.4)	3.9- 4.9	32-35	3.5	9-10	0 (1 in one female)	15-17	~32
P. segregatum Wieser, 1959	4	Р	А	24	6	9.1	4.8	48	4	14	4	9	26
P. stomachor Wieser, 1954	1	Р	Р	35- 40.7 (31.6- 34.1)	6.2- 6.6 (6.5- 8.1)	7.1-8 (6.4- 7.3)	7	50	6-6.5 (4- 5)	18 (9)	4	23	75
P. syltense Blome, 1974	А	Р	Р	21-24 (17-19)	4.7- 5.2	4.8- 6.5	4.5- 6.5	37-43 (28- 29)	3.5	24	0	26-29	45-50
P. tautraense (Allgén, 1933) Lorenzen, 1972	А	А	А	46.6	7.3	8.3	n.a	~50	4.5	n.a	4	12	n.a
<i>P. tesselatum</i> Wieser & Hopper, 1967	1	Р	А	36.4	4.25	11.6	3.6	68	4.5	16	4	15	45
P. veronicae Cidreira et al., 2019	2	Р	Р	38.9- 45.9 (31.6- 35.8)	5.9- 6.2	6.9- 7.4 (5.7- 7.2)	6.8- 7.9 (8.9- 9.7)	44-50 (33- 34)	4.5 (3.5)	20-23 (18-19)	0	20	44-47
P. websteri (Sharma & Vincx, 1982) Cidreira et al. 2019	A	Р	A	80.5- 88.4 (64.4)	13.4- 14.9	7.1- 10.1 (5.7)	12 (18)	58 (41)	5.5	4	4	13-15	42

¹Data from Lorenzen (1972)

DISCUSSION

Prior to the present study, seven cyatholaimid species were described from Brazil, most of them in the 1950s (Gerlach, 1957a, 1957b; Oliveira *et al.*, 2017; Cidreira *et al.*, 2019). *Biarmifer nesiotes* sp. nov. is the first species of the genus recorded from the country. Five species of *Pomponema* were recorded in Brazilian coastal habitats so far (Venekey *et al.*, 2010; Venekey, 2017; Cidreira *et al.*, 2019): *P. corniculata* Gourbault, 1980; *P. cotylophorum* (Steiner, 1916) Lorenzen, 1972; *P. sedecima* Platt, 1973; *P. tautraense* (Allgén, 1933) Lorenzen, 1972 and *P. veronicae* Cidreira *et al.*, 2019, this last one described for Itapuã beach located on the Northeastern Brazilian coast. The records of *P. cotylophorum* and *P. sedecima* from Brazil (Silva, 2012) must be taken with caution since the specimens found have morphological differences from the original descriptions that could be considered interspecific variations. With the present work, the number of cyatholaimids registered in Brazil raised to 14 genera and 22 species.

Diagnostics characters within Cyatholaimidae

The principal issues in the Cyatholaimidae taxonomy are the relative aspect of the weight given to each character and the lack of detailed descriptions of morphological structures that may be taxonomically relevant (Cunha *et al.*, 2022). The pattern of ornamentation of the cuticle is very important for the delimitation of the *Biarmifer* genus, for example. However, for *Pomponema* identification, the buccal cavity and the precloacal supplements are the principal diagnostic characters, whereas the ornamentation of the cuticle is very variable between the species (see Table S2).

Wieser (1954) first erected the *Biarmifer* genus based on the presence of double spicules, with a median lacuna. Pastor de Ward (2001) described the spicules of *B. madrynensis* as having inner septa and updated the diagnosis of the genus as "spicules with inner processes". The researcher also noted the typical configuration of the cuticle in all species of the genus and emended the diagnosis. Here we considered the cuticle ornamentation as the main diagnostic character, since this is unique to the genus, and due to the lack of an accurate description of the spicules. In fact, all *Biarmifer* species described so far seem to have some kind of inner processes in the spicules, but they are variable. In *B. cochleatus* and *B. laminatus* Wieser, 1954 the spicules present a median lacuna, whereas the spicules of *B. dayi* (Inglis, 1963) Cunha *et al.*, 2022 and *B. punctata* (Jensen, 1985) Cunha *et al.*, 2022 have alae. The drawings of *B. hopperi* suggest that its spicules also have inner processes (Sharma and Vincx, 1982).

Accordingly, to the micrographs of *B. madrynensis* provided by Pastor de Ward (2001), instead of the inner septa described, the spicules seem to be similar to that from *Biarmifer nesiotes* sp. nov. with a central groove.

The supplements of *Pomponema*, as well as of *Craspodema*, are large and complex, formed by several elements and with the cuticle lamellated between them (type A *sensu* Wieser & Hopper, 1967). These complex structures may vary among species and ventral view observations reveal details that are not visible otherwise. However, until now, only the descriptions of five valid *Pomponema* species included illustrations of supplements ventral view: *P. concinnum* (Wieser, 1954) Lorenzen, 1972, *P. corniculata* Gourbault, 1980, *P. hastatum* (Ott, 1972) Cidreira *et al.*, 2019, *P. sedecima* Platt, 1973, *P. tesselatum* Wieser & Hopper, 1967 and *Pomponema longispiculum* sp. nov (see Fig. 13). Besides the variation in the number of bars between the supplements, the shape of the outer plate, and the position of the pore, is very diverse. An elliptical cuticularized ring above this plate is present in *P. corniculata*, *P. sedecima*, and *P. longispiculum* sp. nov., absent in *P. concinnum* and *P. hastatum*, and incomplete in *P. tesselatum*. Other elements, such as irregular-shaped structures, are present between the supplement and the cuticle bars of *P. tesselatum* and *P. longispiculum* sp. nov. Eventually these additional characteristics of the precloacal supplements may prove to be important to species delimitation.

Pore complex and lateral pore-like structures

The numbers of longitudinal rows of pore complexes present on the cuticle and the orientation of the slit-like aperture are features already used to differentiate between the genera *Longicyatholaimus* and *Marylynnia* (Hopper, 1972). However, these characteristics are not constant within other genera of the family. *Biarmifer* species, for example, have four or eight longitudinal rows of these structures, and the apertures are oriented transversely, longitudinally, or diagonally. The same is true for *Paracanthonchus* Micoletzky, 1924, where the common pattern is the presence of eight longitudinal rows, but with variations among species. For example, *P. platti* Vadhyar, 1980 has six rows, while *P. kamui* Kito, 1981 and *P. perspicuus* Kito, 1981 have twelve. The pore complex structure is rarely cited in *Pomponema* species descriptions, but for those that have been described, they appear as four sublateral longitudinal rows (Lorenzen, 1972; Ott, 1972; Blome, 1974; Gourbault, 1980), and the pore aperture may be longitudinally or diagonally oriented (Hopper, 1972; present study).

Lateral pore-like was analyzed in detail in only a few species of Cyatholaimidae, and their overall structure is similar among them: it is formed by a cuticularized open, supported by punctations fused or not, and with or without a central dome (present study; Leduc & Zhao, 2016). The variation is in the number and size of LPs throughout the body and in the number of punctations that supported it. The pattern that seems to be more common among the Cyatholaimidae species is the opening supported by unmodified punctations (not fused), as observed in *Biarmifer nesiotes* sp. nov., *L. cervoides* Vitiello, 1970, *L. complexus* Warwick, 1970, *Marylynnia bellula* (Vitiello, 1970) Hopper, 1977, *Metacyatholaimus adriaticus* Vidaković *et al.*, 2003 and *M. chabaudi* Gourbault, 1980. In these species, there is one punctation supporting each of the anterior and posterior extremities of the opening rings. However, in *M. bellula* and *L. cervoides* there are a few LPs throughout the body with three or four punctations supporting the ring. Different from all other species so far, in *Pomponema longispiculum* sp. nov. is related to the beginning of the lateral differentiation of longitudinal rows of punctations. Since those structures are rarely cited in species descriptions, it is not clear how usual this pattern of LPs distribution is within the family.

A second pattern was described for a few species, where the cuticularized ring is supported by modified (broad and curved) or possibly fused punctations. In *Metacyatholaimus delicatus* Leduc & Zhao, 2016 and *Paracanthonchus miltommatus* Leduc & Zhao, 2016, the opening of the LP is supported by two modified or fused punctations. The LP is not described in full detail in the description of *P. mamubiae* Miljutin & Miljutina, 2015, but based on their SEM photographs, the opening also seems to be supported by modified punctations.

CONCLUSIONS

The present study contributes to the growing knowledge of the diversity of free-living marine nematodes from the Brazilian coast. One of the new species described is classified in *Biarmifer*, a genus with few registers globally, and recorded for the first time in the country. The second is a *Pomponema* species, a genus very speciose and commonly found on surveys on the coast of Brazil. The taxonomy of the *Biarmifer* genus will be beneficiated from a detailed description and comparison of the structure of the spicules among species. For *Pomponema*, the characterization of the precloacal supplements in the ventral view may reveal previously overlooked features that may be taxonomically informative. The structures present in the cuticle can be useful to differentiate among some genera and species of the family. The organization of pore complex in longitudinal rows may be more relevant to genus identification and the

number of those structures by row seems to be useful in species delimitation. Nevertheless, there is still not enough information about these cuticle structures across the family to endorse their overall phylogenetic importance and detailed morphological data acquisition may help to clarify this issue.

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CAPÍTULO 3. DESCRIPTION OF ONE NEW AND REDESCRIPTION OF TWO KNOWN SPECIES, AND THE REASSESSMENT OF CYATHOLAIMIDAE PHYLOGENY (CHROMADORIDA: NEMATODA)

ABSTRACT

The systematic of Cyatholaimidae is unclear due to the lack of knowledge about phylogenetic informative characters and the shortage of molecular data. The specimens sampled along the Southeastern Brazilian coastline permitted a more detailed description of two known and one new species, as well as, the reassessment of the phylogenetic relationships within the family. Paracanthonchus cochlearis Gerlach, 1957 and Paracyatholaimus vitraeus Gerlach, 1957 were previously recorded only in their original description, and here was added new data about the pore complexes and like-pore structures observed in their cuticle. Longicyatholaimus sp. nov. differs from L. egregius Hopper, 1972 and L. trichocaudata Gerlach, 1955 mostly by the number of spines in the spicules, number of precloacal supplements, and number of pore complexes and like-pores structures. The phylogenetic inferences based on 39 SSU and 10 LSU rDNA sequences from Cyatholaimidae species indicated the monophyly of Paracanthonchinae as originally established, with high nodal support. The tubular shape of the precloacal supplements and the slit-like aperture of pore complexes transversally oriented are possible synapomorphies of the subfamily. Additionally, it was recovered a clade with high support including Pomponematinae species (*Pomponema*) and two unidentified species from Cyatholaiminae. The paraphyly of Cyatholaiminae was confirmed. For the monospecific Xenocyatholaiminae, no molecular data is available.

INTRODUCTION

Systematic studies of free-living nematodes are challenging due to the paucity of knowledge about the evolutionary history of morphological characters and the high incidence of convergent evolution (Lorenzen, 1981; Blaxter *et al.*, 1998). The continuous acquisition of molecular data throughout the last twenty years enlightened the understanding of the phylogenetic relationships within nematodes, but the placement of several taxa is still unclear (Ahmed & Holovachov, 2021). The situation at low taxonomic levels is even more critical, and few studies attempted to elucidate the relationships within families or genera (*e.g.* Tchesunov *et al.*, 2014; Abolafia & Penã-Santiago, 2018; Venekey *et al.*, 2019). The acquisition of new sequences from novel taxa, besides enhancing the accuracy of phylogenetic inferences (Pollock

et al., 2002), assists in species delimitation and systematic reviews (Macheriotou *et al.*, 2019; Pantó *et al.*, 2021).

Cyatholaimidae is one of the most diverse groups within Chromadorida and comprises more than 210 valid species recorded worldwide mainly in marine, but also in freshwater and terrestrial habitats (Cunha *et al.*, 2022). In addition to the broad habitat distribution, specimens of the family are commonly abundant in surveys of different environments (*e.g.* Pinto *et al.*, 2012; Santos *et al.*, 2018; Zhao *et al.*, 2020). The family was considered monophyletic according to different molecular analyses (Meldal *et al.*, 2007; Holterman *et al.*, 2008; Van Megen *et al.*, 2009), nevertheless, the phylogenetic relationships within it are still unclear, as well as the monophyly of the subfamilies (Cunha *et al.*, 2022). The first phylogenetic study of the family based on molecular data reveal two clades with low nodal support and also demonstrated the presence of several paraphyletic genera (Leduc & Zhao, 2016). The shortage of high-quality DNA sequences available is one of the main issues that hamper investigations on the subject (Leduc & Zhao, 2016; Cunha *et al.*, 2022).

According to the current systematic classification, Cyatholaimidae is subdivided into four subfamilies (Tchesunov, 2014): Cyatholaiminae Filipjev, 1918; Paracanthonchinae De Coninck, 1965; Pomponematinae Gerlach & Riemann, 1973; Xenocyatholaiminae Gerlach & Riemann, 1973. Cyatholaiminae lacks synapomorphies and it is composed of species that cannot be placed in the other subfamilies. Paracanthonchinae was erected to accommodate those genera with tubular precloacal supplements and buccal cavities usually with a large dorsal tooth. Finally, Pomponematinae includes the genera with complex precloacal supplements, and the monospecific Xenocyatholaiminae (*Xenocyatholaimus delamarei* Gerlach, 1953) differs from the other by the cuticle no punctated and adorned with ten marked longitudinal lines. Nevertheless, the current classification is not supported either by morphological synapomorphies or molecular data (Lorenzen, 1994; Leduc & Zhao, 2016).

The inclusion of new molecular and morphological data help to understand the phylogeny of the group and contribute to the systematic of the family. The objective of this study is to reappraise the phylogenetic relationships within Cyatholaimidae based on newly collected material from the Southeastern Brazilian coastline. In addition to the phylogenetic analysis, a new species of *Longicyatholaimus* Micoletzky, 1924 from a subtidal region is described and two species (*Paracanthonchus cochlearis* Gerlach, 1957 and *Paracyatholaimus vitraeus* Gerlach, 1957) previously recorded only in their original description are redescribed.

MATERIAL AND METHODS

Sampling and morphological data

The sampling was conducted in different habitats from the São Paulo State at the Southeastern Brazilian coastline (Fig. 1). Details of the sampling were summarized in Table 1. The qualitative samples of sediment were taken from the top five centimeters on the sandy beaches. At the mangroves, pieces of roots with algae associated were cut and stored in plastic bags. All samples were frozen at -20°C and thawed overnight at room temperature in the laboratory, previously the fauna extraction. The meiofauna was extracted from the sediment samples with Ludox TM-50 with a density of 1.18 g.cm⁻³ (Heip *et al.*, 1985), and by washing the mangrove roots under a continuous stream of filtered freshwater over a sieve with mesh sizes of 63 µm. The specimens of Cyatholaimidae were sorted using a stereomicroscope and fixed in DESS (Yoder et al., 2006). For morphological analysis, the specimens were transferred to glycerin and mounted onto permanent slides for light microscopy observations under a ZEISS Imager.M2 microscope equipped with differential interference contrast (DIC). The measurements were performed using images taken by an AxioCam MRc5 camera and its software. All measurements are in micrometers (µm) and all curved structures are measured along the arc. Information on the number of mediolateral pore-like structures (LP) and sublateral pore complexes (PC) was provided, following Hopper (1972).

At least two adult individuals of *Longicyatholaimus* sp. nov, *Paracanthonchus cochlearis* and *Paracyatholaimus vitraeus* were fixed in a 2.5 % glutaraldehyde solution with sodium cacodylate buffer for scanning electron microscopy observation. Specimens were dehydrated in a graded ethanol series and submitted to critical point drying. Next, individuals were mounted onto stubs and coated with gold/palladium using a sputter coater. Observations were made using a JSM 5800LV tabletop scanning electron microscope at high vacuum mode. Holotypes and paratypes were deposited in the MDBio - Museum of Biological Diversity from the State University of Campinas (ZUEC-NMA).

Molecular data

One adult individual of each species was selected for molecular analysis and it was previously mounted on a temporary slide to be photographed. The images were deposited on MDBio under the registration numbers ZUEC-PIC 786 – 790 (available at https://www2.ib.unicamp.br/fnjv/). The DNA was extracted as described in Derycke *et al.* (2005). Different primer sets were used for the amplification of the rDNA SSU. For

Longicyatholaimus forward primer 5'nov. the was 18S-CL-F, sp. TCAAAGATTAAGCCATGCAT -3' (Carta & Li, 2018), and the reverse primer 1912BR, 5'-TTTACGGTTAGAACTAGGG -3' (adapted from Holterman et al., 2006). For Paracanthonchus cochlearis and Paracyatholaimus vitraeus it was used the first fragment forward primer 1096 F, 5'-GGTAATTCTGGAGCTAATAC-3' and reverse primer 1912R, 5'-TTTACGGTCAGAACTAGGG-3', and the second fragment forward primer 1813 F, 5'-CTGCGTGAGAGGTGAAAT-3' and reverse 2646R, 5'-GCTACCTTGTTACGACTTTT-3' (Holterman et al., 2006). For Paracanthonchus sp. H, the forward primer G18S4, 5'-GCTTGTCTCAAAGATTAAGCC-3' 5'and SSU13R reverse GGGCATCACAGACCTGTTA-3' (Blaxter et al., 1998). Primers for LSU amplification were forward primer D2A, 5'- ACAAGTACCGTGAGGGAAAGT 3' and reverse primer D3B, 5'-TGCGAAGGAACCAGCTACTA 3' (Nunn, 1992). The PCR reactions were performed with Go Taq® Green Master Mix (Promega Corporation, Madison, WI, USA) in total volumes of 20 µl containing 1 µl (10 µM) each of forward and reverse primers and 2 to 5 µl of DNA template. Cycling conditions for the amplification of each primer set are detailed in Table 2. The amplified products were sequenced in both directions using a Sanger ABI 3500xL sequencer at the Multi-User Genotyping and Sequencing Laboratory of the Institute of Biology at the State University of Campinas. The complementary sequences were concatenated and edited in the Geneious Premium 2022.1 program (http://www.geneious.com). The sequences were deposited in the Genbank.

Phylogenetic analyses

Besides the sequences obtained in the present work, additional rDNA small subunit (SSU - 18S) and large subunit (LSU - 28S) sequences were retrieved from the Genbank database for phylogenetic analyses (Table 3). Sequences from species of each family within Chromadorida were selected as outgroups (Achromadoridae, Chromadoridae, Ethmolaimidae and Selachinematidae). Only sequences from GenBank of ingroup and outgroup taxa with more than 600 base pairs for SSU and 300 base pairs for LSU were used in the analyses. The sequence of *Xyzzors* sp. (Y16923) was not considered in the analyses for being too divergent from all other Cyatholaimidae sequences. The alignments of each region were made with L-ins-I algorithm implemented in the software MAFFT server v. 7 (Katoh *et al.*, 2019). After manual trim, the SSU alignment resulted in 1013 positions, and the LSU alignment in 724 positions. The ModelFinder (Kalyaanamoorthy *et al.*, 2017) was used to select the best substitution model

for each partition based on the Bayesian Information Criterion (BIC). The indels (insertiondeletion regions) generated in the SSU alignment were coded with the modified complex coding as implemented in the SeqState (Müller, 2006), which was included as an independent data partition.



Figure 1. Map of the sampling sites

The SSU and LSU regions were analyzed independently under model-based methods (Maximum likelihood and Bayesian inference). The Maximum Likelihood (ML) analysis was performed in the IQtree software v. 2.0.7 (Nguyen *et al.*, 2015) with 1000 replicates of the Ultrafast bootstrap (Hoang *et al.*, 2018). The DNA partition of SSU was analyzed under the model TIM2e+I+G4, whereas the MK+FQ+ASC+G4 model was used for the indel partition. For the LSU partition, it was selected the model TIM3+F+G4.

The Bayesian inference (BI) was conducted in MrBayes v. 3.2.7 (Ronquist *et al.*, 2012) using the Cipres Phylogenetic Portal (Miller *et al.*, 2010). For each region, it was performed two runs, each with four chains (three heated and one cold with a temperature of 0.1), throughout 50 million generations sampled by every 1000 replicates. The model considered were GTR+I+G for SSU, Mk1 model for the indel partition (Lewis, 2001), and GTR+G for LSU. The first 25% of each run was discarded as burn-in. Tracer v.1.7 (Rambaut *et al.*, 2018) was used to check the parameters of all analyses (Effective sample sizes > 200). The phylogenetic trees were observed in the FigTree software v. 1.4.2.

City	Location	Habitat	Substrate	Coordinates	Species
Bertioga	Guaratuba	Mangrove	Roots with algae	23°45'13"S 45°53'42"W	Paracyatholaimus vitraeus
Ilhabela	Pedras Miúdas	Beach Subtidal	ubtidal Sediment 23°49'49"S 45°23'27		Longicyatholaimus sp. nov.
					Nannolaimoides sp. C
Ilhabela	Ilhote	Beach Intertidal	Sediment	23°50'17"S 45°23'53"W	Paracanthonchus sp. H
Guarujá	Prainha Branca	Beach Intertidal	Sediment	23°52'14"S 46°08'11"W	Paracanthonchus cochlearis
São Sebastião	Boracéia	Beach Intertidal	Sediment	23°45'23"S 45°49'47"W	Paracanthonchus cochlearis
Ubatuba	Caçandoca	Beach Intertidal	Sediment	23°33'35"S 45°13'08"W	Paracanthonchus cochlearis
Ubatuba	Escuro	Mangrove	Roots with algae	23°29'24.7"S 45°09'52"W	Paracyatholaimus vitraeus

 Table 1. Sampling locations in São Paulo State and species found in each site.

Table 2. Cycle conditions for the amplification of each primer set.

rDNA	Primer set	PCR condition
SSU - 18S	18S-CL-F /	94°C for 5min + 35 cycles of (94°C for 30s + 50°C for 30s + 72°C for 2min) +
	1912BR	72°C for 10min
		95°C for 3min + 35 cycles of (94°C for 30s + 48°C for 30s + 72°C for 1min) +
	1096F / 1912R	72°C for 7min
		95°C for 3min + 35 cycles of (94°C for 15s + 53°C for 30s + 72°C for 45s) +
	1813F / 2646R	72°C for 7min
		94°C for 5min + 35 cycles of (94°C for 30s + 55°C for 30s + 72°C for 2min) +
	G18S4 / SSU13R	72°C for 10min
		95°C for 3min + 35 cycles of (94°C for 15s + 53°C for 30s + 72°C for 45s) +
LSU - 28S	D2A / D3B	72°C for 7min

Family	Species	SSU (18S)	LSU (28S)
Selachinematidae	Synonchium iranicum	MN158139	-
	Synonchiella rotundicauda	KU746883	KU746888
Chromadoridae	Chromadorina germanica	OK317200	OK317225
	Chromadorita sp.	OK31720	OK317226
Achromadoridae	Achromadora ruricola	AY593941	-
	Achromadora cf. terricola	AY593940	-
Ethmolaimidae	Gomphionema sp.	MK626810	_
	Ethmolaimus pratensis	AY593942	-
Cyatholaimidae	<i>Cyatholaimus</i> sp.	AM234618	-
2	<i>Cyatholaimus</i> sp.	AY854213	-
	<i>Cyatholaimus</i> sp.	JN968214	-
	Longicyatholaimus sp.	LK054720	-
	Longicyatholaimus sp.	MG669843	-
	Longicyatholaimus egregius	LK054728	-
	Longicyatholaimus sp. nov.	OR161039	-
	Longicyatholaimus subtenuis	MG669844	-
	Marylynnia sp.	MK626803	-
	Metacyatholaimus sp.	MG669863	-
	Metacyatholaimus delicatus	KX270433	KX270434
	Nannolaimoides sp. C	-	XXXXX
	Paracanthonchus caecus	AF047888	-
	Paracanthonchus caecus	MG669928	-
	Paracanthonchus cochlearis	OR161037	XXXXX
	Paracanthonchus gynodiporata	KX352221	KX352222
	Paracanthonchus sp. H	OR161040	XXXXX
	Paracanthonchus mamubiae	-	KJ638032
	Paracanthonchus miltommatus	KX270431	KX270432
	Paracyatholaimus sp.	KX944152	-
	Paracyatholaimus intermedius	AJ966495	-
	Paracyatholaimus intermedius	FJ969133	-
	Paracyatholaimus intermedius	JQ957906	-
	Paracyatholaimus intermedius	JQ957907	-
	Paracyatholaimus oistospiculoides	MG669944	-
	Paracyatholaimus oistospiculoides	MG669945	-
	Paracyatholaimus oistospiculoides	MG669946	-
	Paracyatholaimus vitraeus	OR161038	-
	Praeacanthonchus sp.	AF036612	-
	Praeacanthonchus sp.	AM234046	-
	Praeacanthonchus sp.	JN968256	-
	Praeacanthonchus sp.	KX944155	-
	Praeacanthonchus punctatus	-	AF210416

Table 3. DNA sequences used in the phylogenetic inferences with accession number inGenbank. The sequences obtained by the present work are in bold.

(Continued)

Table3. (Continued)

Family	Species	SSU (18S)	LSU (28S)
Cyatholaimidae	Praeacanthonchus punctatus	AY854214	-
	Praeacanthonchus punctatus	MG669976	-
	Praeacanthonchus punctatus	MG669977	-
	Praeacanthonchus punctatus	MG669978	-
	Praeacanthonchus punctatus	MG669979	-
	Praeacanthonchus punctatus	MG669980	-
	Praeacanthonchus punctatus	MG669981	-
	Pomponema sp.	-	DQ077763
	Pomponema sp.	MN250093	-
	Pomponema longispiculum	OP548510	OR148452

RESULTS

Taxonomy

Class Chromadorea Inglis, 1983

Order Chromadorida Chitwood, 1933

Family Cyatholaimidae Filipjev, 1918

Diagnosis – from Leduc & Zhao (2016). Cuticle with transverse rows of punctations. Lateral punctations may be larger, irregular or arranged in longitudinal rows. Longitudinal rows of circular or elliptical cuticular structures (termed 'lateral pore-like structures') often present along mediolateral lines; up to 12 longitudinal rows of pore complexes may also be present. Inner labial sensilla often setiform; six outer labial setae and four cephalic setae in a single crown (with some rare exceptions); outer labial setae longer than the cephalic setae. Multispiral amphideal fovea. Cheilostoma with twelve distinctly cuticularized rugae. Pharyngostoma with a large dorsal tooth, and usually with two smaller ventrosublateral teeth, which may be single or double. Pharynx usually without a posterior bulb. Female didelphic-amphidelphic with reflexed anterior and posterior gonads always on opposite sides of the intestine. Males usually with two testes, rarely with one. Precloacal supplements may be present or absent.

Subfamily Cyatholaiminae Filipjev, 1918

Diagnosis – from Cunha *et al.* (2023). Body cuticle with homogeneous punctation (except in *Biarmifer*), with or without lateral differentiation. Precloacal supplements absent or cup-shaped (tubular in *Praeacanthonchus*); gubernaculum unpaired proximally, except in *Biarmifer* and *Marylynnia* (paired).

Genus Longicyatholaimus Micoletzky, 1924

Diagnosis – emended from Hopper (1972). Cuticle lateral differentiation of larger and more wide-spaced punctations, sometimes in form of longitudinal rows of punctuations. Pore complex in four complete sublateral longitudinal rows; partial subventral and subdorsal rows at the anterior extremity in some species. Buccal cavity small, with a dorsal tooth and subventral teeth reduced or absent. Precloacal supplements present or absent; cup-shaped (conical setae protruding from the body of the cup-shaped supplements in *L. maldivarum*). Gubernaculum distally dentate, paired. Tail filiform.

Longicyatholaimus sp. nov. (Fig. 2–4, Table 4)

Locality. Brazil, São Paulo State, São Sebastião Island, Pedras Miúdas beach, subtidal zone, from sediment with gravel predominance

Type specimens. Holotype male (ZUEC-NMA 44, slide) and one male and four female paratypes (ZUEC-NMA 45 – 49, slide), all from the type locality.

Description. Holotype and paratype males. Body cylindrical and long, slightly narrower on the anterior extremity and tapering abruptly on the posterior end. Cuticle with transverse rows of punctations; with bigger and more spaced punctations on the cephalic region (Fig. 2B; 3A). Lateral differentiation consists of larger and more spaced punctations observed from the anterior end (1.4 head diameter) to the anal region; the lateral field present half the number of rows of punctations when compare to ventral and dorsal regions. Longitudinal row of conspicuous LP present along each mediolateral line from the nerve ring to the cloacal region; LPs consist of a circular or elliptical cuticularized opening supported by unmodified punctations (Fig. 2B), and with a central, non-cuticularized dome; underlying gland cells absent. Most LPs are supported by one punctation at each of the anterior and posterior extremities (Fig. 2B), but a few LPs in middle body region are supported by three or four punctations (Fig. 3F). LPs are $7-95 \mu m$ apart, $2-3.4 \times 2.1 - 3.4 \mu m$ in size throughout the body. Four longitudinal rows of elliptical pore complexes situated sublaterally, starting at a distance equivalent to about two head diameters from the anterior end and extending to the conical portion of the tail. Pore complexes (PC) consists of cuticularized ring in the middle cuticle layer with a slit-like aperture at an angle of approximately 45° in relation to the longitudinal body axis. Pore complexes are $3.1 - 4.5 \ge 2.6 - 4$ in size and $8 - 80 \ \mu m$ apart from each other; no differentiation in size and distribution along the body. Two circles with eight somatic setae (~17 µm length) each located
on the pharyngeal region, 1.5 and 2.5 head diameters from the anterior region, respectively. Few smaller somatic setae scattered throughout the body.

Anterior sensilla arranged in two crowns; first crown consisting of six inner labial setae, $\sim 4\mu m$ long; second crown consisting of six outer labial setae (19 – 20 µm) and four slightly shorter cephalic setae (13 – 14 µm). Multispiral amphideal fovea with five turns and circular in outline, corresponding to $\sim 30\%$ of cbd, situated slightly posterior to the second crown of the anterior sensilla. Buccal cavity consisting of a cup-shaped cheilostoma with 12 rugae, with a small dorsal tooth (Fig. 2A; 3D). Pharynx cylindrical with muscular and glandular cells. Secretory excretory system not observed. Cardia not surrounded by intestinal tissue. Intestinal lumen with numerous small golden-brown granules.



Figure 2. *Longicyatholaimus* sp. nov. **A**: Head, male; **B**: Anterior body region, male, superficial view; **C**: Posterior body region, male; **D**: Middle body region of a female showing the reproductive system. Scale bars: $\mathbf{A} = 10 \ \mu\text{m}$; \mathbf{B} , $\mathbf{C} = 50 \ \mu\text{m}$; $\mathbf{D} = 100 \ \mu\text{m}$.



Figure 3. *Longicyatholaimus* sp. nov. **A**: Anterior end, superficial view, male; **B**: Posterior body region and copulatory apparatus, male; **C**: Detail of cuticle in the middle body region, male; **D**: Anterior end, female; **E**: Middle body region, female; **F**: Detail of cuticle in the middle body region, female. Am: amphideal fovea; LP: like-pore structure; PC: pore complex; Sp: precloacal supplements; V: vulva. Scale bars: 20 μm.

Reproductive system diorchic, gonads opposed, outstretched. Spicules paired, arched, with the proximal end directed ventrally, and the distal end bearing 7 - 8 spine-like structures when looking with optical microscopy (Fig 5C), but they look serrated with SEM (Fig. 4C). Gubernaculum consists of a plate heavily cuticularized located laterally to each spicule. Each plate is covered by reduced spines all over its surface with the bigger ones in the distal border

(Fig. 4B). There are two narrow rod-shaped pieces, 48.9 μ m in length, lying posteriorly to the spicules. Three weakly developed cup-shaped precloacal supplements present, 35 – 50 μ m apart, the anterior-most at 115 – 123 μ m from the cloaca (Fig. 3B). Tail conoid anteriorly, and filiform posterior portion (Fig. 2C).

Paratype females. Similar to males, except with a higher number of pore-complex lateroventrally (81 - 82 vs. 67 - 78 in males). Reproductive system didelphic, amphidelphic, gonads reflexed. An egg 103 x 55 µm in size was observed in the uterus of one female (Fig. 2D, 3E). Vulva located slightly pre-median body region (%V=44%).



Figure 4. *Longicyatholaimus* sp. nov., scanning electron micrographs of a male. **A**: Anterior body region; **B**: Detail of the gubernaculum; **C**: Spines in distal end of spicules; **D**: Pore complex and lateral pore-like in the pre-cloacal region; **E**: Lateral pore-like structures of anterior region. LP: lateral pore-like; PC: pore complex. Scale bars: $\mathbf{A} = 10 \ \mu\text{m}$; $\mathbf{B}, \mathbf{D} = 5 \ \mu\text{m}$; **C**, $\mathbf{E} = 1 \ \mu\text{m}$.

	Male Holotype	Male Paratype	Females Paratypes
N	-	1	3
L	3170	3519	3214-3467
a	37.6	48.3	39-41.3
b	7	8.2	7-7.8
c	5	3.2	4-5.7
c'	10.8	19.7	9.7-15.8
Pharynx length	449	429.8	446.4-462.5
Pharyngeal diam. at base	34.6	31.6	30-33
Pharynx cbd at base	72.2	66.7	71.4-75
Max. body diam.	84.3	73	82.6-85.6
Tail lenght	648.7	1116	564-867.7
Length of inner labial setae	4.2	-	5-5.7
Length of outer labial setae	19	20	18.5-20
Length of cephalic setae	13	14	12.3-13
Head diam. at cephalic setae	40.5	40.6	38.4-41.5
Head diam. at amphids	47.8	45	41.8-47
Amphid turns	5	5	5
Amphid height	13	12.2	10.3-12.5
Amphid width	13,7	13.6	12.4-14.2
Amphid width/cbd (%)	28.7	30	27.7-32
Amphid from anterior end	10.8	9.7	10-11.3
Nerve ring from anterior end	164	-	156.5
Nerve ring cbd	64	-	66.5
Spicule length	108.6	110	-
V	-	-	1435-1534
% V	-	-	42.8-47.7
Vulval cbd	-	-	78.4-81.8
Abd	60	56.7	55-59.3
Laterodorsal PC (pharynx)	18	15	16-17
Laterodorsal PC (central body)	89	80	84-85
Laterodorsal PC (tail)	8	6	5-8
Lateroventral PC (pharynx)	15	14	14-16
Lateroventral PC (central body)	78	67	81-82
Lateroventral PC (tail)	4	5	5-7
LP (pharynx)	15	21	19-21
LP (central body)	44	30	31-45
LP (tail)	0	0	0

 Table 4. Morphometric data of Longicyatholaimus sp. nov.

Diagnosis. *Longicyatholaimus* sp. nov. is characterized by a cuticle with larger and more spaced punctations on the cephalic and lateral regions. Longitudinal rows of lateral pore-like structures along mediolateral lines and four longitudinal rows of pore complexes situated sublaterally. Outer labial setae $19 - 20 \mu m$ long, and cephalic setae $13 - 14 \mu m$ long. Amphids with five turns (corresponding to 30% of the body diameter). Buccal cavity with one dorsal tooth. Three weakly developed cup-shaped precloacal supplements; spicules arched, ~109 μm long, with 7 – 8 spines-like structures on the distal end; and gubernaculum with a plate-shaped covered by small spines.



Figure 5. Copulatory apparatus of *L. egregius* (A), *L. trichocaudata* (B), and *Longicyatholaimus* sp. nov. (C). A: adapted from Hopper, 1972; B: adapted from Gerlach, 1955.

Relationships. *Longicyatholaimus* specimens were already found on the Brazilian coastline, but they were unidentified at the species level (Venekey *et al.* 2010), then, here we made the first register of a species of the genus for the country. *Longicyatholaimus* sp. nov. can be differentiated from the other 12 valid species of *Longicyatholaimus* by a combination of characters (Table 5): lateral differentiation of cuticle; length of outer labial setae; number of spines in the spicules; number of precloacal supplements; and morphometric De Man parameters (a, b, c, c'). It is morphologically very closely related to *L. egregius* Hopper, 1972 and *L. trichocaudata* Gerlach, 1955. *Longicyatholaimus* sp. nov. differs from both species mostly by the shape of the gubernaculum (Fig. 5), the number of spines in the spicules (7 – 8 *vs.* 3 – 4 in *L. trichocaudata*, and more than 12 in *L. egregius*). The new species also has a higher number of like-pore structures when compared with *L. egregius* (59 *vs.* 30 – 36), and the numbers of pore complexes are similar between both species. The inverse is true for *L. trichocaudata*, which has similar numbers of like-pore structures, but a smaller number of pore

complexes when compared to *Longicyatholaimus* sp. nov. (74 - 97 vs. 97 - 115). Besides the four rows of pore complex, *L. trichocaudata* has two additional partial rows located subventrally, which are not present in *L. egregius* and in *Longicyatholaimus* sp. nov. The spicules are arched directed ventrally in the new species and in *L. trichocaudata*, different from *L. egregius*.

Based on the SSU sequence, *Longicyatholaimus* sp. nov. differed from an individual from *L. egregius* (LK054728) by 5.6% (39 in 700bp). When blasted in Genbank, the top three taxa recovered are an unidentified nematode (DQ504343) that differs from *Longicyatholaimus* sp. nov. by 4.98% (42 in 843 bp), and two sequences of *Paracyatholaimus oistospiculoides* (MG669945 and MG669944) by 5.8% (49 in 843 bp).

Species	L.d.	O.l.s	a	b	c	c'	N⁰ Suppl.	Spicule spines
L. cervoides	widely spaced	8-	69.7-	8.2-	3.4-	25.9-	8	А
	dots	8.5	82.6	10.1	3.6	27		
L. continus	slightly widely spaced dots	11	40	8.4	5-6	-	А	А
L. egregius	slightly larger and widely	20	40.6- 41.6	7.5	4-4.7	11	4-5	~12
I faloatus	spaced dots	117	55 0	0200	1 2	146	2	•
L. jaicalus	data	11./	50.2- 50.5	0.3-0.0	4.2-	14.0-	3	A
I low atoms dutient		-15	38.3	5676	4.0	1/./	2.2	
L. longicaudatus'	and widely	10- 12	22-31.7	5.6-7.6	3-5.3	10.7- 13	2-3	A
	spaced dots							
L. maldivarum ²	three longitudinal rows of dots	18	22-28	4.4-5.2	4.9-7	6-8	7-9	А
L. marilynae	А	18.5	38	6.9-7.6	4.75-7	6.2- 9.8	12-14	А
L. minor	four longitudinal rows of dots	10	28.6	6.25	7.7	4.6	6	А
L. orientalis	A	14- 18	39-56	-	3.5- 4 5	14.3- 20.2	9-10	А
L. subtenuis	А	8-9	24-40	6.3-8.2	5.8-8	5.7- 8.8	А	А
L. trichocauda	widely spaced	22	34-37	6-6.6	3.2- 3.4	14.3- 23.7	А	4
L. trichurus	longitudinal rows	15.5	31.25	8.2	7.7	5.4	А	А
Longicvatholaimus	widely spaced	18.5	37.6-	7-8.2	3.2-	9.7-	3	7-8
sp. nov.	dots	-20	48.3	, 0.2	5.7	19.7	5	, 0
L. maldivarum ² L. marilynae L. minor L. orientalis L. subtenuis L. trichocauda L. trichurus Longicyatholaimus sp. nov.	and widely spaced dots three longitudinal rows of dots A four longitudinal rows of dots A A widely spaced dots longitudinal rows of dots widely spaced dots	12 18 18.5 10 14- 18 8-9 22 15.5 18.5 -20	22-28 38 28.6 39-56 24-40 34-37 31.25 37.6- 48.3	4.4-5.2 6.9-7.6 6.25 - 6.3-8.2 6-6.6 8.2 7-8.2	4.9-7 4.75-7 7.7 3.5- 4.5 5.8-8 3.2- 3.4 7.7 3.2- 5.7	13 6-8 6.2- 9.8 4.6 14.3- 20.2 5.7- 8.8 14.3- 23.7 5.4 9.7- 19.7	7-9 12-14 6 9-10 A A A 3	A A A A 4 A 7-8

Table 5. Tabular key of *Longicyatholaimus* species. Data retrieved from original descriptions.L.d.: lateral differentiation of cuticle; O.l.s: outer labial setae length; A: absent.

¹ Data from De Man (1876) and Hopper (1972)

² Data from Gerlach (1964) and Semprucci et al. (2017)

Subfamily Paracanthonchinae De Coninck, 1965

Diagnosis – from Decraemer & Smol (2006). Homogeneously punctated body, with or without lateral differentiation in ornamentation. Pharynx without end bulb. Precloacal supplements variable, but mainly tubular; rarely absent. Gubernaculum paired proximally.

Genus Paracanthonchus Micoletzky, 1924

Diagnosis – from Miljutina & Miljutin (2015). Body cuticle with transverse rows of punctations, which laterally may be slightly enlarged and/or irregularly arranged. Buccal cavity with large pointed dorsal tooth (except in *P. angulatus* Schuurmans Stekhoven, 1950); smaller subventral teeth present (up to four) or absent. Gubernaculum proximally paired (proximal ends not connected by median piece), except in *P. elongatus* (de Man, 1907),

distally expanded and often dentate. Precloacal supplements usually tubular, except in *P. longus* Allgén, 1934 (sucker-like) and *P. digitatus* Gerlach, 1957 (papilloid) or seldom absent.

Paracanthonchus cochlearis Gerlach, 1957 (Fig. 6–7, Table 6)

Locality. Brazil, São Paulo State; Boracéia, Caçandoca and Prainha Branca beaches, intertidal zone.

Specimens. Six males from Boracéia beach; one male from Caçandoca beach; four males and two females from Prainha Branca beach.

Description. *Males.* Body cylindrical, slender, tapering slightly towards posterior extremities. Cuticle with transverse rows of punctations. Lateral differentiation consisting of larger punctations, clearly visible on the tail (Fig. 6A). Longitudinal row of lateral pore-like present along each mediolateral line beginning behind the amphideal fovea to halfway down the length of the tail. Lateral pore-like structures are inconspicuous, difficult to observe in light microscopy, but clearly visible in SEM (Fig. 7B). Those structures consist of a circular or elliptical cuticularized opening supported by a modified (or possibly fused) punctation at each of the anterior and posterior extremities; central dome not observed. Eight longitudinal rows of circular pore complexes, situated subventrally (2 rows), subdorsally (2 rows), and sublaterally (4 rows), extending from posterior to amphideal fovea to near tail tip. Pore complexes consist of a cuticularized ring in the middle cuticle layer with slit-like transverse pore. Sublateral somatic setae, $10 - 12 \mu m \log$, located at about 2.5 head diameters of the anterior end.

Anterior sensilla arranged in two crowns; first crown consisting of six inner labial setae, $3.5 - 6.8 \mu m \log$; second crown consisting of six outer labial setae with a thinner tip (9.5 – 14) μ m) and four slightly shorter cephalic setae (5.4 – 10.4 μ m). Multispiral amphideal fovea with 7 – 7.5 turns and circular outline, situated slightly posterior to the second crown of anterior sensilla (Fig. 6C; 7A). Buccal cavity consisting of a wide, cup-shaped cheilostoma with 12 rugae and a narrow funnel-shaped pharyngostoma with a large dorsal tooth and two pairs of ventrosublateral teeth (Fig. 6B). Cylindrical muscular pharynx, slightly enlarged in the posterior end, but not forming a bulb. Secretory excretory system present, excretory pore located about 5 head diameters to the anterior end, conspicuous renette cell, 32.1 – 42.4 x 11.7 – 12.3 μ m, located subventrally below the pharynx end.



Figure 6. *Paracanthonchus cochlearis* Gerlach, 1957, male. **A**: Posterior body region; **B**: Head; **C**: Anterior body region, superficial view. Scale bars: $\mathbf{A} = 20 \ \mu\text{m}$; \mathbf{B} , $\mathbf{C} = 10 \ \mu\text{m}$.

Reproductive system diorchic. Spicules paired, curved, with rounded capitulum on the proximal end; mid-region dilated, with a central groove; pointed distal end. Gubernaculum proximally and distally paired (not fused), slightly shorter than spicules, heavily cuticularized; distal end forked. Five to six weakly cuticularized precloacal supplements; the anterior-most at $72 - 93 \mu m$ from the cloaca. The distance between them gradually increases from the cloaca to

the anterior-most supplement (Fig. 6A). Tail conical (Fig. 7C), with two $9 - 17 \mu m$ long setae on the tip. Three caudal glands not restrict in the tail and spinneret present.



Figure 7. *Paracanthonchus cochlearis* Gerlach, 1957, scanning electron micrographs of a male. A: Anterior body region; **B**: Detail of the pore complex and lateral pore-like in the anterior body region; **C**: Tail. CL: cloaca; LP: lateral pore-like; PC: pore complex. Scale bars: $\mathbf{A} = 5 \ \mu m$; $\mathbf{B} = 1 \ \mu m$; $\mathbf{C} = 20 \ \mu m$.

Females. Similar to males but with smaller amphidial fovea, corresponding to 50% of cbd and with six to seven turns. Reproductive system didelphic, amphidelphic. Vulva located at the median body region. One egg was observed in one individual, 40.7 x 23 μ m in size. **Diagnosis.** *Paracanthonchus cochlearis* is characterized by a cuticle with larger punctations on the lateral region, clearly visible on the tail. Longitudinal rows of inconspicuous lateral pore-like structures along mediolateral lines and eight longitudinal rows of pore complexes. Outer labial setae $9.5 - 15 \mu$ m long, and cephalic setae $5 - 10 \mu$ m long. Amphids with 6 - 8 turns in males (corresponding to 67 - 94% of the body diameter), and with 6 - 7 turns in females (corresponding to 50% of the body diameter). Buccal cavity with one dorsal tooth and four ventrosublateral teeth. Five to six weakly developed precloacal supplements; spicules $35 - 44 \mu$ m long; and gubernaculum with a distal end forked, $29 - 34 \mu$ m long.

	Boracéia	Caçandoca	Prainha	Branca	Santos (Gerlach, 1957a)	
	Males	Male	Males	Females	Male	Female
Ν	6	1	4	2	1	1
L	970-1191.9	1105	1021.3	959-1013	1123	1162
a	28.4-38.9	29.1	23.7-37.2	28-30.5	31	28
b	5.1-6.4	5.5	5.4-6	5.3-5.6	5.9	5.3
c	8.1-10	9.5	8.1-9.5	7-7.4	10.9	7.6
c'	4.1-4.9	4.5	3.5-4.8	5-7	3.4	6.1
Dhammy langth	175.6-	201.5	147.3-	170.8-	100	219
Pharyngool diam at basa	12 / 15 5	201.5	107.5	109.5	190	210
Pharyny chd at base	26 1-29 4	15.5	20 5-33 0	24.2-26	31	- 33
Max body diam	26.1-29.4	38	20.5-55.9	24.2-20	36	55 41
Max. Douy diam.	109.7-	50	23.3-43.1	51.4-50.2	50	11
Tail lenght	123.3	116	94.5-111.6	137	103	152
Length of inner labial setae	4-6.8	5.4	3.5-5.1	3.9-4.9	6	6
Length of outer labial setae	11-14	13	9.5-12.4	11.2-13.4	15	15
Length of cephalic setae	6-10.4	6.5	5.4-8.6	6-7.8	10	10
Head diam. at cephalic setae	17.5-18.9	21.1	16.6-22.9	19.3	21	20
Head diam. at amphids	18-20.4	22.3	17.5-23.7	20	-	-
Amphid turns	7-8	7	7-7.5	6-7	6	-
Amphid height	14.8-19.4	16.6	15.2-17.7	10-10.8	-	-
Amphid width	15.8-18.8	15	14-16.2	10-10.4	17	-
Amphid width/cbd (%)	84.6-94	67.3	68.5-79	50-51.7	70	-
Amphid from anterior end Excretory pore from	5.7-7.4	7	6-10.7	7.3-8.6	-	-
anterior end	80.4-94	-	-	-	-	-
Spicule length	34.8-40.4	38.3	39.9-43.8	-	36	-
Gubernaculum length	29.3-31.4	30.4	31.9-34.4	-	30	-
V	-	-	-	497.2-510	-	613
V/total body lengh %	-	-	-	50.3-51.9	-	53
Vulval body diam.	-	-	-	30.7-32.4	-	41
Anal body diam.	24.2-26.7	25.9	22.5-30.8	19.6-27.7	30	25
Laterodorsal PC (pharynx) Laterodorsal PC (central	13-15	15	14	~11	-	-
body)	53-62	63	56	-	-	-
Laterodorsal PC (tail)	7-9	7	7-8	-	-	-
Lateroventral PC (pharynx) Lateroventral PC (central	11-13	13	12-13	11-14	-	-
body)	50-59	46	55-56	-	-	-
Lateroventral PC (tail)	3-4	5	5	-	-	-

Table 6. Morphometric data of *Paracanthonchus cochlearis* from each locality sampled in thepresent study, and from the type locality (data from Gerlach, 1957a).

Relationships. The specimens of *P. cochlearis* observed are very similar to those described by Gerlach (1957a) from Santos, about 60 to 120 km from the locations sampled in the present work. They differ from Santos individuals by a slightly smaller setae of the anterior sensilla and the individuals from Boracéia have a larger amphidial fovea (see Table 6). In the original description, it was cited the occurrence of five precloacal supplements in the male, but most individuals currently analyzed have six supplements.

Paracanthonchus cochlearis is similar to P. stateni Allgén, 1930 and P. heterocaudatus Huang & Xu, 2013 by the presence of the two curved teeth on the distal end of the gubernaculum, but it differs from both species mostly based on the bigger amphidial fovea (corresponding to 67 - 94 % of the body diameter in males vs. 33% in both sexes in P. stateni and about 60% in males of P. heterocaudatus). The precloacal supplements organization is also different: the two posterior supplements are smaller and close together in all three species, but the other supplements are equally distant from each other in P. stateni (five in total) and P. heterocaudatus (six in total), and in P. cochlearis the distance gradually increases to the anterior-most supplement. Contrary to P. cochlearis, there is no cuticular pore in P. heterocaudatus.

When blasted in the Genbank, the SSU rDNA sequence of *P. cochlearis* differs from *Cyatholaimus* sp. (AY854213) by 4.9%, from *Praecanthonchus punctatus* (AY854214) by 5.21% and from *Praecanthonchus* sp. (AM234046) by 5.4%. When the LSU sequence was blasted, the top two taxa recovered are *Paracanthonchus gynodiporata* Oliveira *et al.*, 2017 (KX352222) and *P. miltommatus* (KX270432), which differs from *P. cochlearis* by 9.29% and 9.58%, respectively. In the SSU rDNA phylogenetic trees, *P. cochlearis* is close related to *Paracyatholaimus oitospiculoides* (Allgén, 1935) Wieser, 1954 – MG669946 (pp. 0.92; ultrafast bootstrap 97); and in the reconstruction based on the LSU, the species is highly related to *Paracanthonchus mamubiae* Miljutina & Miljutin, 2015 (pp. 1; ultrafast bootstrap 100). Both species have few morphological similarities with *P. cochlearis*. P. *oitospiculoides*, previously included in the *Paracanthonchus* genus (Allgén, 1935), have a similar gubernaculum shape, with a distal end forked. The precloacal supplements of *P. mamubiae* are also small and barely visible.

Paracanthonchus sp. H

Locality. Brazil, São Paulo State, São Sebastião Island, Ilhote beach, intertidal zone. Specimen. One male. **Measurements.** Body length: 3732.4 μ m; a: 29; b: 6.4; c: 18.6; c': 2.1; Pharynx length: 580.2 μ m; Pharyngeal diameter at base: 41.3 μ m; Pharynx cbd at base: 103 μ m; Maximum body diameter: 128.6 μ m; Tail length: 201.2 μ m; Head diameter at cephalic setae: 56.2 μ m; Head diameter at amphids: 71.7 μ m; Amphid height: 41.9 μ m; Amphid width: 37.3 μ m; Amphid width/cbd: 52%; Amphid from anterior end: 19.9 μ m; Spicule length: 68.4 μ m; Gubernaculum length: 55.8 μ m.

Diagnosis. Body long, tapering abruptly in the tail. The cuticle is constituted of transversal rows of punctations, with bigger and more spaced punctations on the pharynx region and on the tail. Lateral differentiation of slightly more spaced punctations. Lateral pore-like structures located at the mediolateral lines, commencing behind the amphidial fovea to the conical part of the tail. LPs consist of a circular or elliptical cuticularized opening supported by one unmodified punctations at each of the anterior and posterior extremities. Eight longitudinal rows of pore complex, consisting of a cuticularized ring in the middle cuticle layer with a slit-like transversal pore. Anterior sensilla arranged in two crowns; first crown consisting of six inner labial setae, ~4 μ m long; second crown consisting of six outer labial setae (~18 μ m) and four slightly shorter cephalic setae (~11 μ m). Multispiral amphideal fovea with 4.5 turns and elliptical in outline, situated slightly posterior to the second crown of the anterior sensilla. Buccal cavity consisting of a cup-shaped cheilostoma with 12 rugae, with a dorsal tooth; ventrosublateral teeth not observed. Spicules paired, arched. Gubernaculum with trifurcated distal end. Nine tubular precloacal supplements present, 21 – 33 μ m apart, the anterior-most at 241.7 μ m from the cloaca. Tail conical-cylindrical.

Relationships. *Paracanthonchus* sp. H is probably a new species, however, as only one individual was found, the formal description will be made when more specimens are observed. The cuticle ornamentation of bigger punctations on the extremities is different from all other *Paracanthonchus* species but based on the tubular precloacal supplements and on the proximally paired gubernaculum, the specimen was classified in the genera until further information.

The top three SSU sequences recovered when blasted in Genbank are *Praecanthonchus* species (AF036612, AY854214, AM234046) which differ from *Paracanthonchus* sp. H by about 3%. Based on the LSU rDNA sequence, Paracanthonchus sp. H is closely related to two unidentified *Paracanthonchus* species (KY792385 by 95.2% and AF210414 by 94.91%) and to *Praecanthonchus punctatus* (AF210416 by 94.77%). In the SSU phylogenetic tree, *Paracanthonchus* sp. H is on Clade A and is the sister of a clade formed by *Praeacanthonchus*

sp., *Paracanthonchus cochlearis*, and *Paracyatholaimus oitospiculoides*, with low support (pp. 0.5; ultrafast bootstrap 47). However, based on the LSU phylogenetic tree, *Paracanthonchus* sp. H is closed related to *Praeacanthonchus punctatus*, with higher support (pp. 0.92; ultrafast bootstrap 82).

Genus Paracyatholaimus Micoletzky, 1922

Diagnosis – from Tchesunov (2008). Cuticle punctated, lateral differentiation hardly developed. Six outer labial sensilla and four cephalic sensilla setose, in a single circle. Amphidial fovea multispiral. Cheilostoma armoured with twelve rugae; distinct dorsal tooth, often supplemented with smaller subventral teeth and occasionally other denticles in the stegostoma. Precloacal ventromedian supplements as setae-like organs half inserted into the body. Gubernaculum hardly dilated at the distal end and devoid of denticles or serrations. Tail conical or with more or less slender cylindrical distal portion.



Figure 8. *Parayatholaimus vitraeus* Gerlach, 1957, male. A: Head; B: Anterior body region, superficial view; C: Posterior body region. Scale bars: $A = 20 \mu m$; B, C = 10 μm .

Paracyatholaimus vitraeus Gerlach, 1957 (Fig. 8–9, Table 7)

Locality. Brazil, São Paulo State, Guaratuba and Escuro, mangrove roots.

Specimens. One male and three females from Guaratuba; four males and two females from Escuro.

Description. *Males.* Body cylindrical, short. Cuticle finely punctated, without lateral differentiation. Transversal striation clearly visible in SEM (Fig. 9). Lateral pore-like structure irregularly distributed along the mediolateral line, starting behind the amphideal fovea until the tail end, $0.6 - 0.8 \ge 0.4 - 0.8 \ \mu m$ in size. LP is formed by a circular ring, strongly cuticularized (Fig. 9C, D), supported by one fused punctation in the anterior and posterior region; central dome not observed. Four longitudinal rows of pore complexes, which consist of a cuticularized ring in the middle cuticle layer with slit-like transverse pore. Sublateral longitudinal rows of somatic setae throughout the body.

Anterior sensilla arranged in two crowns; the first crown consisting of six short inner labial setae $\sim 1 \ \mu m$ long; the second crown consisting of six outer labial setae and four slightly shorter cephalic setae ($3.3 - 4 \ vs \ 2 - 3.2 \ \mu m$). Multispiral amphideal fovea with 3 - 4 turns and circular outline, situated slightly posterior to the second crown of anterior sensilla (Fig. 8B). Buccal cavity consisting of a cup-shaped cheilostoma with 12 rugae and funnel-shaped pharyngostoma with a large dorsal tooth and two pairs of reduced ventrosublateral teeth (Fig. 8A). Cylindrical muscular pharynx, slightly enlarged in the posterior end, but not forming a bulb. Secretory excretory system present, excretory pore located about midway between anterior end and pharynx end, rennete cell located behind the pharynx end.

Reproductive system diorchic. Spicules paired, curved, with a capitullum in the proximal end, and a pointed distal end (Fig. 8C). Gubernaculum proximally and distally paired (not fused), $14 - 17 \mu m$ in length, with a median process, distally with four teeth. Five to six unequally spaced setae-like precloacal supplements, weakly cuticularized. Tail conical, with 2.3 - 3.4 times the cloacal body diameters. Three caudal glands not restrict in the tail and spinneret present.

Females. Similar to males. Reproductive system didelphic, amphidelphic. An egg was observed in the uterus of two females, $35.3 - 46.2 \times 25.35 - 27 \mu m$ in size. Vulva is located in the median body region.

Diagnosis. *Paracyatholaimus vitraeus* is characterized by a cuticle finely punctated, without lateral differentiation. Longitudinal rows of lateral pore-like structures along mediolateral lines and four longitudinal rows of pore complexes located sublaterally. Outer labial setae $3 - 5 \mu m$

long, and cephalic setae $2 - 4 \mu m$ long. Amphids with 2.5 - 4 turns (corresponding to 21 - 33 % of the body diameter). Buccal cavity with one dorsal tooth and two pairs of reduced ventrosublateral teeth. Five to six setae-like precloacal supplements; spicules with a capitullum in the proximal end, $23 - 26 \mu m$ long; and gubernaculum with four teeth in the distal end and a median process, $14 - 17 \mu m$ long.



Figure 9. *Parayatholaimus vitraeus* Gerlach, 1957, scanning electron micrographs of a female. A: Entire body; **B**: Anterior body region; **C**: Pore complex and lateral pore-like in the pharynx region; **D**: Pore complex and lateral pore-like in the anal region. LP: lateral pore-like; PC: pore complex. Scale bars: $A = 50 \mu m$; $B, D = 5 \mu m$; $C = 2 \mu m$.

Relationships. The specimens analyzed in the present work were sampled in the same habitat type where *Paracyatholaimus vitraeus* was firstly described, about 250 to 350 km distant from the type locality. Compared with the specimen from the Cananéia mangrove described by Gerlach (1957b), the individuals from Guaratuba and Escuro are smaller (960 – 1010 *vs.* 505 -

743 μ m in body length). However, they are proportionally equal, with similar morphometric De Man parameters (a, b, c, c') as shown in Table 7.

Paracyatholaimus vitraeus differs from all other species of the genus mainly by the shape of the copulatory apparatus, which consists of spicules with a large capitulum on the proximal end and gubernaculum with a median process and four teeth on the distal end.

Table 7. Morphometric data of *Paracyatholaimus vitraeus* from each locality sampled in the present study, and from the type locality (data from Gerlach, 1957b).

	Escuro mangrove		Guaratuba mangrove		Cananéia mangrove (Gerlach, 1957b)		
	Males	Females	Male	Females	Male	Female	
N	4	2	1	3	1	1	
_	504.5-	610.4-	<		0.60		
L	568.7	742.5	650.2	609-622.6	960	1010	
a	19.3-23.9	19.9-21	21.2	18.8-20.8	20	27	
b	5.7-6.9	6.3-7.1	7.5	6.5-7.1	6.6	6.6	
c	8.7-11	10.1-10.3	9.1	8.5-9	10.7	11.5	
c'	2.3-3.4	3.1	3.1	3.4-3.7	2.9	3.4	
Pharynx length	80.6-91	96.9-104	86.4	87.3-93	145	154	
Pharyngeal diam. at base	10.7-13.1	13.1-14	14.5	14.7-16.2	-	-	
Pharynx cbd at base	20.8-25	25.5-27.2	26	25.3-27.2	37	31	
Max. body diam.	23.8-28.7	30.7-35.4	30.6	31.6-33	49	37	
Tail lenght	45.7-65.2	60.4-71.8	71.2	68.6-73.2	90	88	
Length of inner labial setae	-	-	1	1.1	-	-	
Length of outer labial setae	3.4-4	3-4.5	3.3	3.5-4.9	5	5	
Length of cephalic setae	2-3.2	2.5-4.1	2.8	2.8-3.8	3.5	3.5	
Head diam. at cephalic setae	11.7-15.4	13.7-15.4	14.4	13.8-14.4	18	18	
Head diam. at amphids	14.5-16.2	14.5-17.9	17	15.3-17.2	-	-	
Amphid turns	3	4	4	2.5-3	3.5	3.5	
Amphid height	3-4.2	4.7-4.9	4	2.5-3.6	-	-	
Amphid width	3.4-4.7	3.6-5.7	5.2	3.3-3.9	-	-	
Amphid width/cbd (%)	22.2-32.4	24.8-31.8	30.6	20.9-23.9		27-33*	
Amphid from anterior end	5.4-8.2	6.2-7.2	5	6.1-8.5	-	-	
Excretory pore from anterior end	38.3-44	47.9-50	-	43.4	68 23-	68	
Spicule length	22.4-25.4	-	24.3	-	26	-	
Gubernaculum length	13.6-17.2	-	14	-	15	-	
V	-	313.7- 361.9	-	296.2-313.7	-	522	
V/total body lengh %	-	48.7-51.4	-	48.6-50.4	-	52	
Vulval body diam.	-	27.5-30.6	-	27-30.7	-	37	
Anal body diam.	19.2-22.2	19.2-23.4	23.2	18.7-21.5	31	26	

*Gerlach (1957b) does not specify which amphid/width/cdb (%) value corresponds to male and female.

In Genbank, the SSU sequence of *P. vitraeus* appears similar to *Marylynnia* sp. (MK626803) by 95.35% and to *P. intermedius* (FJ969133, AJ966495, JQ957906, JQ957907) by about 94%. In the phylogenetic inference based on Maximum likelihood, the species was recovered as closely related to *Marylynnia* sp., but with low support (ultrafast bootstrap 38). In the Bayesian analyses, *P. vitraeus* is included in a clade formed by sequences of *P. intermedius* (de Man, 1880) Micoletzky, 1922 (pp. 0.89).

Subfamily Pomponematinae Gerlach & Riemann, 1973

Diagnosis – from Decraemer & Smol (2006). Body cuticle punctated, with lateral differentiation in ornamentation. Precloacal supplements knob-like or flattened, complicated, consisting of several elements; gubernaculum paired proximally.

Genus Nannolaimoides Ott, 1972

Diagnosis – emended from Ott (1972). Cuticle homogeneous or heterogeneous with lateral differentiation. Buccal cavity with weakly developed dorsal armature. Gubernaculum with lateral flanges without teeth or denticles, spicula strong. Precloacal supplements consist of several elements.

Nannolaimoides sp. C

Locality. Brazil, São Paulo State, São Sebastião Island, Pedras Miúdas beach, subtidal zone, from sediment with gravel predominance.

Specimen. One male.

Measurements. Body length: 2514.5 μ m; a: 69.8; b: 14.6; c: 4.2; c': 17; Pharynx length: 172 μ m; Pharyngeal diameter at base: 16.6 μ m; Pharynx cbd at base: 32.6 μ m; Maximum body diameter: 36 μ m; Tail length: 601 μ m; Head diameter at cephalic setae: 18.8 μ m; Head diameter at amphids: 23 μ m; Amphid height: 14 μ m; Amphid width: 14.75 μ m; Amphid width/cbd: 64 %; Amphid from anterior end: 9 μ m; Spicule length: 45 μ m; Gubernaculum length: 35.8 μ m.

Diagnosis. Body slender, tapering on the anterior and posterior end. Cuticle heterogeneous, thicker on the extremities of the body, with bigger and more spaced punctations. Cuticle with transverse rows of punctations arranged in an alternating pattern of one to three rows of simple dots and one row of longitudinal bars formed by two fused punctations. Lateral differentiation of two longitudinal rows of punctations, commencing behind the posterior end of the pharynx until the conical region of the tail. Longitudinal row of conspicuous LP present along each

mediolateral line, commencing 2.2 head diameters from the anterior end to the conical region of the tail; LPs consist of a circular or elliptical cuticularized opening supported by two unmodified punctations. Four longitudinal rows of elliptical pore complexes situated sublaterally. Four somatic setae (~13 μ m length) located sublaterally on the pharyngeal region, 2 head diameters from the anterior region. Anterior sensilla arranged in two crowns; first crown consists of six inner labial papillae; second crown consists of six outer labial setae (~9 μ m) and four slightly shorter and thinner cephalic setae (~8 μ m). Multispiral amphideal fovea with 6 turns and elliptical in outline, situated slightly posterior to the second crown of the anterior sensilla. Buccal cavity consists of a shallow cup-shaped cheilostoma with 12 rugae, and a small dorsal tooth; two to four sublateral projections of the cuticular lining in the conical part of the stoma. Spicules paired, arched. Gubernaculum with lateral flanges. Twelve precloacal supplements formed by a cup-shaped inner piece and a plate-shaped outer piece, the anteriormost at 253.8 μ m from the cloaca. Halfway between the cloaca and the first supplement, there is a ventral bristle, 3.7 μ m long. Tail conoid anteriorly, and filiform posterior portion.

Relationships. *Nannolaimoides* sp. C is probably a new species for science and it will be described in future work. It differs from all other species of the genus by the lateral differentiation of longitudinal rows of punctations (irregular punctations on other species) and by the tail filiform.

When blasted in Genbank, the LSU rDNA sequence of *Nannolaimoides* sp. C appears as closely related to seven unidentified nematode sequences (MK383033 - MK383039) by 92.18%, followed by *Praeacanthonchus punctatus* (AF210416) by 90.14%. In the phylogenetic inferences, the sequence is closely related to *Metacyatholaimus delicatus* (pp. 0.9; ultrafast bootstrap 82).

Phylogenetic relationships

The phylogenetic trees based on the SSU rDNA recovered by both methods were similar (Fig. 10 and Fig. 11). The Cyatholaimidae sequences clustered together in a monophyletic group well-supported (pp 1; ultrafast bootstrap 91). The relationships of the clade with the other families of Chromadorida were not resolvable. According to the Maximum Likelihood analyses (Fig. 10), Cyatholaimidae is a sister group of a low-supported clade composed of all other families (ultrafast bootstrap 43). In the phylogenetic tree generated by Bayesian analysis (Fig. 11), there is a polytomy formed by three clades: Achromadoridae, Cyatholaimidae, and a group including Chromadoridae + Ethomolaimidae.





Figure 11. Bayesian tree inferred by SSU sequences. The species are colored accordingly to the current classification: Cyatholaiminae (blue), Paracanthonchinae (green), and Pomponematinae (pink). Posterior probabilities values are given on each node.

The relationships within Cyatholaimidae clade were also unresolved and there is a polytomy of three groups. One with low nodal support included *Metacyatholaimus delicatus* Leduc & Zhao, 2016, *Longicyatholaimus* sp. (MG669843), and *Longicyatholaimus subtenuis* Gagarin & Nguyen Vu Thanh, 2007 (pp 0.6; ultrafast bootstrap 59). A second group with better support (pp 0.85; ultrafast bootstrap 90) composed only of *Longicyatholaimus* species (*Longicyatholaimus* sp. nov., *Longicyatholaimus* sp. - LK054720, and *L. egregius* Hopper, 1972). The third group with high nodal support (pp 0.97; ultrafast bootstrap 80) included two clades also with high support: Clade A (pp 1; ultrafast bootstrap 92) and Clade B (pp 1; ultrafast bootstrap 99). Clade A was formed by genera from Paracanthonchinae (*Paracanthonchus* Micoletzky, 1924 and *Paracyatholaimus* Micoletzky, 1922), including *Praeacanthonchus*

Micoletzky, 1924, two *Cyatholaimus* Bastian, 1865 sequences (AM234618 and AY854213), and a sequence of *Marylynnia* (Hopper, 1972) Hopper, 1977, all three taxa currently classified within Cyatholaiminae subfamily. Clade B comprises the *Pomponema* Cobb, 1917 species (Pomponematinae), a sequence of *Cyatholaimus* (JN968214), and *Metacyatholaimus* sp. (Cyatholaiminae). None of the genera represented by more than one sequence in the analyses were recovered as monophyletic.



Figure 12. Maximum likelihood tree inferred by LSU sequences. The species are colored accordingly to the current classification: Cyatholaiminae (blue), Paracanthonchinae (green), and Pomponematinae (pink). Ultrafast bootstrap (above) and Posterior probabilities (below) values are given on each node.

The Maximum likelihood and Bayesian inferences based on LSU rDNA data recovered identical trees (Fig. 12). The majority of Cyatholaimidae sequences formed a clade well-supported (pp 1; ultrafast bootstrap 94), however, the *Pomponema* sp. sequence (DQ077763) appears as closely related to Chromadoridae species (pp 0.6; ultrafast bootstrap 56). Within

Cyatholaimidae two groups were detected: one includes the *Paracanthonchus* species and *Praecanthonchus punctatus* (Bastian, 1865) Micoletzky, 1924 (pp 1; ultrafast bootstrap 97), and the second is formed by *Metacyatholaimus delicatus*, *Nannolaimoides* sp. C, and *Pomponema longispiculum* Cunha et al., 2023 (pp 0.86; ultrafast bootstrap 66).

DISCUSSION

Phylogenetic relationships within Cyatholaimidae: subfamilies

Compared to the phylogeny of the family presented by Leduc and Zhao (2016), 23 SSU and five LSU rDNA sequences from Cyatholaimidae were added to the analyses, including genera previously not represented, like *Marylynnia* (Hopper, 1972) Hopper, 1977, *Nannolaimoides* Ott, 1972, and *Pomponema* Cobb, 1917. In the SSU-based phylogenetic tree of the previous work, it was recovered two clades with low nodal support, roughly similar to the Cyatholaiminae and Paracanthonchinae subfamilies, respectively (Leduc & Zhao, 2016). With the inclusion of more sequences and new taxa in the present analyses, it was confirmed the close relationship of *Paracanthonchus*, *Paracyatholaimus*, and *Praeacanthonchus* (Clade A), with higher support in both genetic regions and both methods. However, conflicting with the previous work, Cyatholaiminae were paraphyletic and the species currently classified in the subfamily were placed in different clades on the phylogenetic trees.

Paracanthonchinae was first erected to include the species with tubular supplements and initially, the *Praeacanthonchus* genus was classified in the group (De Coninck, 1965). Nevertheless, currently, the genus is classified in the Cyatholaiminae subfamily based on the unpaired gubernaculum, which seems to be an irrelevant character in the systematic of genera and subfamilies of the family (Leduc & Zhao, 2016). Clade A, recovered by all analyses with high support, indicated the validity of the Paracanthonchinae as initially classified. The other three genera also classified in Paracanthonchinae (*Acanthonchus* Cobb, 1920, *Isacanthonchus* Gagarin & Nguyen Vu Thanh, 2008, and *Paracyatholaimoides* Gerlach, 1953) do not have DNA sequences available so far. The position of *Cyatholaimus* and *Marylynnia* sequences not identified at the species level within Clade A must be taken with caution, especially in the case of Cyatholaimidae, where the identification can be challenging even at the generic level (Cunha *et al.*, 2022). The misidentification of sequences in genetic data banks is common within the phylum, which impacts the resolution of molecular phylogenetics trees (Holovachov, 2016). Despite this issue, the data amount available would drastically reduce without the inclusion of the unidentified sequences in the analysis and may exclude correctly identified sequences, since

many were checked by experts (*e.g.*, Macheritou *et al.*, 2019). Ideally, all molecular data should be linked to specimen characterization and images to allow further evaluations.

Leaving aside the monospecific Xenocyatholaiminae, which has no molecular data, Pomponematinae is the subfamily with fewer DNA sequences available in databanks. There are only sequences of a few *Pomponema* species, and the present study provides the first LSU sequence from a species of *Nannolaimoides*. In the SSU-based analyses, both *Pomponema* species were recovered as part of Clade B, another group highly supported. However, they are grouped with unidentified sequences of *Metacyatholaimus* Schuurmans Stekhoven, 1942 and *Cyatholaimus* Bastian, 1865, both classified in Cyatholaiminae. In the LSU-based phylogeny, the relationship of the Pomponematinae species was also not resolved, and *Pomponema longispiculum* and *Nannolaimoides* sp. C forms a clade with relatively low nodal support including Cyatholaiminae species (*Metacyatholaimus delicatus*). Therefore, with the current data, it is not possible to indicate the monophyly or paraphyly of Pomponematinae.

The hypothesis of paraphyly of Cyatholaiminae subfamily was raised by several authors since there are no synapomorphies of the group (Gerlach & Riemann, 1973; Lorenzen, 1994). The species currently classified in the subfamily did not cluster together and were placed in all clades recovered in the present analyses, endorsing the paraphyly hypothesis. However, considering that the clades had low nodal support (except Clade A and B previously discussed) and no synapomorphy could be established for those, a new proposal of classification relies upon the obtaining of more robust data.

Morphological characters: hypotheses of synapomorphies

Commonly, adult male nematodes present auxiliary organs known as precloacal supplements located ventrally or subventrally, that are important in copulation and have a variety of forms (Lorenzen, 1994). The cup-shaped supplements are the pattern within the Chromadorida order, and the tubular form is only found within Cyatholaimidae (Tchesunov, 2014). The typical tubular shape is observed in species of *Acanthonchus*, *Isacanthonchus*, *Paracanthonchus*, and *Praeacanthonchus*. The setose or papillae precloacal structures of *Paracyatholaimus* and *Paracyatholaimoides* can be considered very fine and small tubules (Wieser, 1954; Wieser & Hopper, 1967). Then, the tubular shape of the precloacal supplement may be a synapomorphy of Clade A (Paracanthonchinae). In *Cyatholaimus* species, the supplements are absent and in *Marylynnia* they are cup-shaped. Considering that the

identification of the specimens from both genera was accurate, it means that it occurs a loss or a reversal of the character along the evolutionary history of Clade A.

Regarding Clade B, since it was formed only by four sequences, and three are not identified at the species level, it is impossible to make any assumption about a probable synapomorphy of the group. The precloacal supplements are complex with various elements in all *Pomponema* species (Cunha *et al.* 2022, 2023), and they are absent in *Cyatholaimus* and in the majority of the *Metacyatholaimus* species (Cunha *et al.*, 2022). Nevertheless, the supplements are complex in *M. brevicollis* (Cobb, 1898) Gerlach, 1964 and *M. papillatus* Vidakovic, Travizi & Boucher, 2003, similar to *Pomponema*. The inclusion of new sequences of species properly identified that have complex supplements in future analyses may assess the validity of this character as phylogenetic informative.

The pore complex structures frequently observed on the cuticle of Cyatholaimidae were considered as possible taxonomic relevant structures on the classification within the family (Hopper, 1972; Sharma et al., 1979; Leduc & Zhao, 2016). Most species possess four longitudinal rows of pore complexes throughout the body, but the detailed characterization of those structures is rarely given in the species descriptions (Cunha et al., 2022). The organization of the pore complex is important in genera delimitation (Hopper, 1972; Leduc & Zhao, 2016), but the number of rows of pore complexes is variable within the clades here recovered. Paracanthonchus species has, in general, eight rows, while Marylynnia may have up to twelve rows of pore complexes. The majority of Paracyatholaimus and Praeacanthonchus species present four rows, and a few have eight. Cyatholaimus also has four rows of those structures. In all other taxa included in the analyses, the pore complexes are absent or organized in four longitudinal rows. Considering the slit-like aperture of the pore complex, the transversal orientation seems to be the common pattern within Clade A. This orientation was observed in all Marylynnia species (Hopper, 1972), in all Paracanthonchus species included in the analyses, and in Paracyatholaimus vitraeus. There is no information about the aperture of the pore complex of the other species of Clade A. Outside this group, the aperture is longitudinally oriented (all Longicvatholaimus species and Pomponema longispiculum). The acquisition of more detailed data on these structures from different taxa is necessary to verify the possibility that the slit-like aperture with transverse orientation is a synapomorphy of Clade A.

Phylogenetic relationships within genera

Several genera of Cyatholaimidae lack morphological synapomorphies and were considered as probably paraphyletic, such as *Cyatholaimus* (Cunha *et al.*, 2022). The present phylogenetic inferences seem to confirm the paraphyly of all genera included, but, once again, given that some sequences may be misidentified, any conclusion should be considered with caution.

The non-monophyly of *Paracanthonchus* Micoletzky, 1924 had already been suggested based on morphological and molecular data (Miljutin & Miljutina, 2015; Leduc & Zhao, 2016), and was once more indicated in the present analysis. However, the paraphyly may not be entirely confirmed for other genera, such as *Paracyatholaimus*, here represented by sequences of three identified species. *Paracyatholaimus intermedius* and *P. vitraeus* form a clade with high nodal support in the Bayesian SSU-based tree (pp. 0.9), but the same relation was not recovered by the Maximum likelihood inference. *Paracyatholaimus oitospiculoides* (Allgén, 1935) Wieser, 1954 was considered *species inquerenda* by Tchesunov (2008) due to the poor description based on a single male, which hinders the genus determination. According to the phylogenetic inferences, it is not closely related to the other *Paracyatholaimus* species, indicating that the classification in the genus is inaccurate. Little can be said about *Praecanthonchus* since it was only represented by *P. punctatus* and unidentified sequences.

The genus *Longicyatholaimus* was first erected by Micoletzky (1924) to include *Cyatholaimus* species with lateral differentiation in the cuticle, pores conspicuous, small tooth, precloacal supplements non-tubular and tail filiform. Posteriorly, Hopper (1972) redefined the genus and established *Marylynnia* for those *Longicyatholaimus* species with eight or more longitudinal rows of pore complex. Until today there are still taxonomical issues with *Longicyatholaimus* diagnosis considering the inclusion of species with lateral differentiation of longitudinal rows of punctations in the cuticle (Semprucci *et al.*, 2017). The issue could not be resolved here, since none of *Longicyatholaimus* species analyzed have that kind of lateral differentiation. However, the analyses presented indicated that the genus is paraphyletic. The phylogeny recovered one clade with *L. egregius* and *Longicyatholaimus* sp. nov., which, together with *L. trichocaudata*, are the only species of the genus with spines on the distal end of spicules and a plate-shaped gubernaculum. *Longicyatholaimus subtenuis* appears as closely related to *Metacyatholaimus delicatus*. Both species are morphologically very different from each other, in terms of cuticle ornamentation and copulatory apparatus shape, but they have in common the absence of precloacal supplement (Gagarin & Nguyen Vu Thanh, 2007; Leduc &

Zhao, 2016). Both clades had relatively low nodal support, and the addition of new sequences may clarify the relationships of species currently classified in *Longicyatholaimus*.

CONCLUSIONS

The monophyly of Cyatholaimidae was once more confirmed by the phylogenetic analyses presented. Additionally, we have established the monophyly of Paracanthonchinae subfamily as originally erected. However, the position of unidentified sequences within this clade hampers a better resolution. The paraphyly of Cyatholaiminae was confirmed and the relationships within Pomponematinae remain unresolvable. The monophyly of the genera could not be verified and taxonomic reviews are urgently needed, especially for *Paracanthonchus* and *Longicyatholaimus*. The shape of the precloacal supplements and the orientation of the pore complex aperture seems to be taxonomic relevant features in the definition of subfamilies and genera within Cyatholaimidae. Those structures are rarely described in detail and must be carefully examined in future studies of species of the family.

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CONCLUSÕES FINAIS

Estado da arte de Cyatholaimidae

A partir da revisão taxonômica aqui apresentada foram considerados válidos 20 gêneros e 211 espécies. Com as descrições das três espécies novas apresentadas no presente trabalho (duas já publicadas – Capítulo 2), o número subiu para 214. No Brasil, o número de táxons registrados passou para 14 gêneros (primeiro registro do gênero *Biarmifer*) e 23 espécies (*Biarmifer nesiotes, Pomponema longispiculum e Longicyatholaimus* sp. nov.). A expectativa é de que o conhecimento da diversidade de nematodas marinhos de vida livre da costa brasileira cresça ainda mais nos próximos anos com a descrição das espécies novas encontradas (por exemplo, Nannolaimoides sp. C e *Paracanthonchus* sp. H; Capítulo 3) e com a ampliação dos estudos taxonômicos em áreas ainda não exploradas.

Cyatholaimidae é uma família que inclui organismos encontrados em todo o mundo, mas a maioria dos taxa é endêmica de uma ecorregião. Os exemplos de espécies amplamente distribuídas geograficamente podem ser erros de identificação, algo comum nos levantamentos de nematodas marinhos, já que as identificações são normalmente baseadas em chaves de organismos de regiões distantes das quais a amostragem foi realizada (Venekey *et al.*, 2010). Ainda assim podem ocorrer eventos de dispersão a longa distância por rafting, pela epibiose aos cascos de tartarugas marinhas ou por vetores antropogênicos, como água de lastro de navios (Ptatscheck & Traunspurger, 2020; Ingels *et al.*, 2020). Portanto, a confirmação ou não da ampla distribuição precisa ser estudada caso a caso e de preferência por meio de métodos moleculares de genética de populações (Oliveira *et al.*, 2017).

Além da ampla distribuição geográfica, os nematodas apresentam uma grande plasticidade e podem ser encontrados nos mais variados habitats. Apesar de serem em sua maioria marinhas, espécies de Cyatholaimidae também ocorrem em água doce, água salobra, água hipersalina e no ambiente terrestre. Isso faz com que a família seja um bom modelo de estudo para entender os mecanismos relacionados a flexibilidade ecológica do Filo Nematoda (Holterman *et al.*, 2019). No caso de espécies que foram registradas sob condições ambientais muito distintas, como *Paracyatholaimus intermedius*, estudos de genética de populações associados com a detalhada aquisição das variações morfológicas são essenciais para a verificação dessas ocorrências e compreensão da plasticidade fenotípica, frequentemente relatada para os nematodas.

Taxonomia, Sistemática e Filogenia de Cyatholaimidae

Muitos dos gêneros e subfamílias dentro de Cyatholaimidae não são definidos com base em caracteres apomórficos e alguns autores já haviam levantado a hipótese de que esses grupos não são monofiléticos (Gerlach & Riemman, 1973; Lorenzen, 1994; Miljutina & Miljutin, 2015; Leduc & Zhao, 2016). Com as reconstruções filogenéticas do presente trabalho foi possível fazer inferências a respeito das relações dentro da família. As análises indicaram a monofilia da subfamília Paracanthonchinae como originalmente estabelecida, ou seja, incluindo Praeacanthonchus. A subfamília foi definida com base na presença de suplemento pré-cloacal tubular. espécies de Acanthonchus, Paracanthonchus, caractere observado em Paracyatholaimus, Paracyatholaimoides e Praeacanthonchus. A parafilia de Cyatholaiminae foi confirmada e as relações dentro de Pomponematinae requerem uma melhor avaliação, pois há poucas sequências disponíveis de espécies classificadas nessa subfamília. Os gêneros Paracanthonchus e Longicyatholaimus foram recuperados como parafiléticos. Estudos anteriores já haviam levantado dúvidas sobre as relações entre as espécies desses dois gêneros (Miljutina & Miljutin, 2015; Semprucci et al., 2017), portanto, é importante revisar taxonomicamente esses grupos e realizar estudos filogenéticos.

Apesar de ainda haver questões a serem esclarecidas sobre as relações filogenéticas dentro de Cyatholaimidae e sobre a homologia dos caracteres, algumas estruturas morfológicas parecem ser informativas e merecem destaque nas futuras descrições de taxa da família. A forma dos suplementos pré-cloacais e a orientação da abertura dos poros complexos parecem ser características taxonômicas relevantes na definição de subfamílias e gêneros dentro de Cyatholaimidae. A organização dos poros complexos ao longo do corpo pode ser mais relevante para a identificação do gênero e o número dessas estruturas por fileira parece ser útil na delimitação das espécies. Contudo, essas estruturas raramente são descritas em detalhes, e ainda não há informações suficientes em toda a família para endossar sua importância filogenética geral. A taxonomia do gênero *Biarmifer* pode se beneficiar a partir de uma descrição detalhada e comparação da estrutura das espículas entre as espécies. Para *Pomponema*, a caracterização dos suplementos pré-cloacais em vista ventral pode revelar características anteriormente negligenciadas que podem ser taxonomicamente informativas.

O presente trabalho serve como base para compreensão da diversidade e dos padrões de distribuição da família Cyatholaimidae. Além disso, as relações filogenéticas dentro do grupo foram reavaliadas, levantando hipóteses sobre a história evolutiva da família. A tabela de identificação dos gêneros disponibilizada no Capítulo 1 é uma ferramenta útil para auxiliar

inclusive os não-taxonomistas, já que comumente os nematodas são identificados até o nível de gênero na maioria dos estudos ecológicos (*e.g.* Ingels *et al.*, 2020; Franzo *et al.*, 2022). Apesar de ainda haver questões em aberto sobre a taxonomia da família, a ferramenta reúne os dados disponíveis até o momento, facilitando o acesso à informação e permitindo a padronização entre os estudos.
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ANEXO 1 - Documento referente a Bioética e/ou Biossegurança



Ministério do Meio Ambiente CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Comprovante de Cadastro de Acesso

Cadastro nº A85A671

A atividade de acesso ao Patrimônio Genético, nos termos abaixo resumida, foi cadastrada no SisGen, em atendimento ao previsto na Lei nº 13.123/2015 e seus regulamentos.

Número do cadastro:	A85A671
Usuário:	UNICAMP
CPF/CNPJ:	46.068.425/0001-33
Objeto do Acesso:	Patrimônio Genético
Finalidade do Acesso:	Pesquisa
Espécie	
Impossibilidade de identificação	
Título da Atividade:	DIVERSIDADE DE NEMATÓIDES MARINHOS DE VIDA LIVRE: TAXONOMIA INTEGRATIVA DA FAMÍLIA CYATHOLAIMIDAE
Equipe	
Beatriz Pereira Cunha	UNICAMP
Antonia Cecilia Zacagnini Amara	UNICAMP

Data do Cadastro: Situação do Cadastro: 10/11/2019 21:23:13 Concluído

Conselho de Gestão do Patrimônio Genético Situação cadastral conforme consulta ao SisGen em 21:33 de 10/11/2019. SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO



SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO - **SISGEN** ANEXO 2 - Declaração Direito Autoral

Declaração

As cópias de artigos de minha autoria ou de minha co-autoria, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, que constam da minha Dissertação/Tese de Mestrado/Doutorado, intitulada DIVERSIDADE DE NEMATODAS MARINHOS DE VIDA LIVRE: TAXONOMIA E SISTEMÁTICA DA FAMÍLIA CYATHOLAIMIDAE, não infringem os dispositivos da Lei n.º 9.610/98, nem o direito autoral de qualquer editora.

Campinas, 28 de abril de 2023

Assinatura :

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