UNIVERSIDADE ESTADUAL DE CAMPINAS
INSTITUTO DE BIOLOGIA

CECÍLIA SOCHIARELLI DA COSTA

TAXONOMIA INTEGRATIVA DE ESPÉCIES DE CHAETONOTIDA (GASTROTRICHA) DULCÍCOLAS NO BRASIL

CAMPINAS – SP
2024

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Dissertação apresentada ao Instituto de Biologia da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Mestra em Biologia Animal, na área de Biodiversidade Animal.

Orientador: Prof. Dr. André Rinaldo Senna Garraffoni

ESTE ARQUIVO DIGITAL CORRESPONDE À VERSÃO FINAL DA DISSERTAÇÃO DEFENDIDA PELA ALUNA CECÍLIA SOCHIARELLI DA COSTA ORIENTADA PELO PROF. DR. ANDRÉ RINALDO SENNA GARRAFFONI

CAMPINAS

2024

Ficha catalográfica
Universidade Estadual de Campinas (UNICAMP)
Biblioteca do Instituto de Biologia
Mara Janaina de Oliveira - CRB 8/6972

So13t

Sochiarelli, Cecília Costa da, 1998-
Taxonomia integrativa de espécies de Chaetonotida (Gastrotricha) dulcícolas no Brasil / Cecília Sochiarelli da Costa. – Campinas, SP : [s.n.], 2024.

Orientador: André Rinaldo Senna Garraffoni.
Dissertação (mestrado) – Universidade Estadual de Campinas (UNICAMP), Instituto de Biologia.

1. Delimitação de espécies. 2. Perifíton. 3. Meiofauna. 4. Espécies crípticas. 5. Filogenia. 6. Gastrotricha - Classificação. 7. Microscopia eletrônica de varredura. I. Garraffoni, André Rinaldo Senna, 1977-. II. Universidade Estadual de Campinas (UNICAMP). Instituto de Biologia. III. Título.

Informações complementares

Título em outro idioma: Integrative taxonomy of freshwater Chaetonotida (Gastrotricha) species in Brazi
Palavras-chave em inglês:
Species delimitation
Periphyton
Meiofauna
Cryptic species
Phylogeny
Gastrotricha - Classification
Scanning electron microscopy
Área de concentração: Biodiversidade Animal
Titulação: Mestra em Biologia Animal
Banca examinadora:
André Rinaldo Senna Garraffoni [Orientador]
Thiago Quintão Araújo
Ariane Campos
Data de defesa: 02-12-2024
Programa de Pós-Graduação: Biologia Animal

Objetivos de Desenvolvimento Sustentável (ODS)
ODS: 14. Vida na água

Identificação e informações acadêmicas do(a) aluno(a)
- ORCID do autor: https://orcid.org/0009-0006-7308-8535
- Currículo Lattes do autor: http://lattes.cnpq.br/6205957168468492

Campinas, 02 de Dezembro de 2024

COMISSÃO EXAMINADORA

Prof. Dr. André Rinaldo Senna Garraffoni

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Prof. Dra. Ariane Campos

Os membros da Comissão Examinadora acima assinaram a Ata de Defesa, que se encontra no processo de vida acadêmica do aluno.

A Ata da defesa com as respectivas assinaturas dos membros encontra-se no SIGA/Sistema de Fluxo de Dissertação/Tese e na Secretaria do Programa (inserir o nome do programa de pós) da Unidade (inserir o nome da faculdade/instituto).

AGRADECIMENTOS

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoa de Nível Superior - Brasil (CAPES) - Código de Financiamento 001.

Agradecer é opcional em um trabalho acadêmico. No entanto, é uma das partes mais importantes de toda a dissertação, pois essa pesquisa é realizada por um ser humano, e seres humanos precisam de amigos para obterem conquistas.

Primeiramente, meus agradecimentos ao Professor André Garraffoni. Obrigada por acreditar em meu potencial, pela paciência, pelos ensinamentos, e por me contaminar com sua grande paixão pela ciência. Um agradecimento especial para o Axell, que me ensinou grande parte do que sei sobre gastrótricos, e pela amizade que formamos ao longo desses anos. Obrigada ao Nathan, grande amigo que levo para a vida, e que me ajudou desde o processo seletivo até o momento da submissão do trabalho. Luisa, Marlon, Emiliana, Danilo, Natália, muito obrigada pela amizade e pelo ombro amigo que foram durante todo esse processo.

Obrigada Ari, por ter me ensinado novas metodologias, mesmo que isso significasse ficar até as 22h na universidade. Obrigada Ana Lú, por todo o suporte como técnica de laboratório, e por tornar nosso dia-a-dia mais agradável com sua presença.

Agradeço a presença espiritual da minha mãe. Eu não estaria defendendo um mestrado agora sem seu exemplo, seus ensinamentos, e todo seu apoio em vida. De onde você estiver, mãe, muito obrigada. Tudo isso é por você.

Ao meu pai. Sem ele, nada disso seria possível. Obrigada por todo o apoio emocional, financeiro e espiritual. Obrigada por não reclamar de ouvir sobre gastrótricos nos cafés de manhã, e por fingir que estava entendendo o que eu estava dizendo. Obrigada por me levar nos lugares para as coletas, sem nem pensar duas vezes. Obrigada por tanto, pai.

Ao meu noivo, André Luis. Seu envolvimento foi tanto que poderia ser considerado um gastrotricólogo também. Obrigada por me acompanhar nas extensões, por ler meus textos, por ajudar com as mudanças do laboratório, pelos perrengues para coletar em rios, lagos e praias, pelas inúmeras vezes que ficamos nos sábados à noite triando material. Obrigada, meu querido.

À minha irmã amada, que mesmo longe sempre esteve torcendo por mim, sendo minha melhor amiga. Aos meus melhores amigos, Koga e Gabriel, por aguentarem áudios enormes que vocês não faziam ideia do que se tratava. Vocês são os melhores.

Obrigada a todos. Vocês foram indispensáveis nessa conquista.

O correr da vida embrulha tudo,
a vida é assim: esquenta e esfria,
aperta e daí afrouxa, sossega e depois desinquieta.
O que ela quer da gente é coragem.
João Guimarães Rosa

RESUMO

Os gastrótricos representam um filo de microinvertebrados aquáticos e limnoterrestres que fazem parte da comunidade meiofaunal. O estudo desses animais apresenta diversas lacunas relacionadas à biogeografia, taxonomia e filogenia. O filo tem sua distribuição considerada cosmopolita por terem sidos avistados em todas as regiões biogeográficas, mas é inegável a existências de um viés amostral, já que grande parte das coletas foram realizadas no Hemisfério Norte e diversas outras regiões não foram amostradas intensamente. Além disso, por serem pequenos e frágeis possuem coleta e identificação desafiadoras. Isso acaba resultando em delimitações de espécies imprecisas, informações morfológicas escassas, número reduzido de fotografias e ausência de material depositado em museus. Se focarmos nas lacunas taxonômicas, um dos principais problemas enfrentados atualmente é a realização da acurada delimitação e definição das espécies, uma vez que, espécies ditas antigamente como tendo amplas distribuições, podem ser, na verdade, mais de uma espécie em um fenômeno chamado "complexo de espécies". Também é visto que algumas espécies ditas como raras podem na verdade serem apenas pouco amostradas, como alguns integrantes semi-planctônicos, que vem recebendo maior atenção nos últimos tempos graças a sua morfologia diferenciada e o interesse evolutivo que a mesma acarreta. Considerando isso, o presente estudo descreve um novo gênero, contribuindo para a reorganização filogenética de *Chaetonotus*, um gênero reconhecidamente não monofilético e considerado o grupo taxonômico mais diversificado e problemático do filo. Além disso, descrevemos a terceira espécie do gênero *Ornamentula*, apresentando a segunda filogenia molecular do grupo. A inclusão de dados genéticos para um gênero que anteriormente possuía apenas uma espécie sequenciada permitiu testar a monofilia do grupo, objetivo alcançado com este trabalho.

Palavras-chave: Descrição de espécies, Semi-planctônico, Meiofauna, Complexo de espécies, MEV.

ABSTRACT

Gastrotrichs represent a phylum of aquatic microinvertebrates that are part of the meiofaunal community. The study of these animals presents several challenges related to biogeography, taxonomy, and phylogeny. They have a distribution considered cosmopolitan, as they have been observed in all biogeographic regions, but there is an undeniable sampling bias since most collections have been carried out in the Northern Hemisphere, with many other regions having been poorly sampled. Additionally, due to their small and fragile body, their collection and identification are quite complex. This often results in imprecise taxonomic delimitations, limited information, a reduced number of photographs, and a lack of material deposited in museums. Focusing on taxonomic shortfalls, one of the main issues currently faced is the accurate delimitation and definition of species, as species formerly considered to have broad distributions may actually represent more than one species in a phenomenon called a "species complex." It is also observed that some species previously considered rare may, in fact, be under-sampled, such as some semi-planktonic members, which have recently received more attention due to their unique morphology and the evolutionary interest it brings. Considering this, the present study describes a new genus, contributing to the phylogenetic reorganization of *Chaetonotus*, a genus widely recognized as non-monophyletic and regarded as the most diverse and problematic taxonomic group within the phylum. Additionally, we describe the third species of the genus *Ornamentula*, presenting the second molecular phylogeny of the group. The inclusion of genetic data for a genus that previously had only one sequenced species allowed us to test the monophyly of the group, an objective successfully achieved in this study.

Keywords: Species description, Semi-planktonic, Meiofauna, Species complex, SEM.

SUMÁRIO

1. INTRODUÇÃO

1.1.O filo Gastrotricha

Gastrotricha é um filo de diminutos metazoários invertebrados que podem medir de 60 µm a 3500 µm de comprimento e que se movimentam por cílios restritos à sua região ventral, fato este que dá nome ao grupo - do grego *gaster*, estômago; *trichos*, pêlo (Balsamo et al., 2008, 2014, 2020; Todaro et al., 2019). Possuem uma distribuição cosmopolita e desempenham um papel fundamental nas redes tróficas de ambientes dulcícolas, marinhos e salobros (Artois *et al.*, 2011; Balsamo *et al.*, 2020; Minowa et al., 2025). Os espécimes deste grupo alimentam-se de bactérias, algas microscópicas e protistas e são predados por protistas, cnidários, platelmintos, poliquetas e larvas de insetos (Strayer & Hummon, 1991). A reprodução dos espécimes deste filo pode acontecer por fertilização interna em organismos hermafroditas ou por partenogênese. Esses aspectos reprodutivos podem estar ligados ao ambiente em que vivem, considerando que a maioria das espécies marinhas são hermafroditas, enquanto a maioria das dulcícolas são partenogenéticas (Kieneke & Schimidt-Rhaesa, 2015).

Os gastrótricos fazem parte de um grupo monofilético, e suas principais características são: (1) cutícula com multicamada; (2) epicutícula cobrindo o corpo todo, incluindo os cílios locomotores e sensoriais; (3) saída dupla da glândula adesiva que é recoberta por cutícula e d) músculos em arranjo helicoidal circundando o canal alimentar (Hochberg & Litvaitis, 2001; Kieneke, Riemann & Ahlrichs, 2008).

O filo compreende atualmente ~895 espécies (Gammuto *et al.*, 2024) e são divididos entre duas ordens, Macrodasyida Remane, 1925 [Rao & Clausen, 1970] e Chaetonotida Remane, 1925 [Rao & Clausen 1970]. A ordem Macrodasyida possui cerca de 10 famílias, 36 gêneros e 377 espécies descritas. Seus representantes possuem forma vermiforme, presença de poros faríngeos e inúmeros tubos adesivos presentes em regiões anterior, dorsal, lateral, ventral e posterior do corpo (Todaro et al., 2019). A imensa maioria dos macrodasídeos são espécies marinhas; apenas quatro espécies foram coletadas em água doce (Ruttner-Kolisko., 1955; Garraffoni et al., 2010, 2019; Todaro et al., 2012; Araújo et al., 2013; Kånneby & Wicksten, 2014). Já os Chaetonotida apresentam duas subordens, oito famílias, 32 gêneros com quase 485 espécies (Todaro et al., 2019) e sua grande maioria vive em água doce na superfície de sedimentos e entre as vegetações de águas eutrofizadas, sendo 5 de 9 gêneros viventes de ambientes lóticos (Balsamo, 2014). Os quetonótidas são facilmente reconhecidos pelo seu formato de “pino de boliche”, corpo transparente, dividido em 3 regiões: cabeça distinta, tronco evidente e uma extremidade posterior furcada, presença de um par de tubos adesivo, ausência de poros faríngeos e uma faringe com lúmen em formato de “Y” (Garraffoni *et al.*, 2017). As

famílias que pertencem a essa ordem são: Chaetonotidae Gosse, 1864; Dasydytidae Daday 1905; Dichaeturidae Remane 1927; Muselliferidae Leasi & Todaro, 2008; Neogosseidae Remane, 1927; Proichthydidae Remane, 1927 e Xenotrichulidae Remane, 1927.

1.2.Taxonomia de Chaetonotidae

Atualmente a família Chaetonotidae possui 15 gêneros, 2 subfamílias e cerca de 400 espécies sendo a mais especiosa de todo o filo (Balsamo *et al*.2009; Gammuto *et al.*, 2024; Hummon & Todaro, 2010; Todaro & Hummon, 2008). Oito gêneros possuem espécies exclusivamente dulcícolas: *Arenotus* Kisielewski, 1987, *Bifidochaetus* (Kolicka *et al.*, 2016); *Cephalionotus* Garraffoni et al., 2017*; Fluxiderma* d'Hondt 1974*; Lepidochaetus* Kisielewski, 1991; *Polymerurus* Remane 1927a; *Rhomballichthys* Schwank, 1990 e *Undula* Kisielewski, 1991; dois são estritamente marinhos: *Caudichthydium* Schwank, 1990 e *Halichaetonotus* Remane, 1936; e cinco são dulcícolas e marinhos: *Aspidiophorus* (Voigt 1902), *Chaetonotus* Ehrenberg, 1830, *Heterolepidoderma* Remane, 1927a, *Ichthydium* Ehrenberg, 1830 e *Lepidodermella* Blake, 1933.

A morfologia dos integrantes de Chaetonotidae é caracterizada pela presença de uma cabeça distinta, podendo ser separadas em 3 ou 5 lobos; um par de tubos adesivos na região posterior e variações nos formatos das cutículas, que podem ser ausentes de escamas (epiderme lisa) ou apresentar uma diversidade grande de formatos da mesma (Kieneke & Schimidt-Rhaesa, 2015). A grande diversidade das estruturas cuticulares de Chaetonotidae é uma das principais características utilizadas para delimitar gêneros e espécies da família. No entanto, esses indicadores podem não ser muito confiáveis considerando que são características muito plásticas (Amato & Weiss, 1982).

Hochberg & Litivaitis (2000) realizaram o primeiro estudo filogenético morfológico e obtiveram que a família Dasydytidae está alocada dentro à família Chaetonotidae, acusando a não-monofilia desta última família. Posteriormente, Kånneby et al. (Kanneby, 2013) corroboraram essa informação com o o primeiro estudo filogenético multigênico (genes 18S, 28S, COI).

A presença de unidades não monofiléticas se estende à grande parte dos gêneros da família Chaetonotidae, e *Chaetonotus* é um dos grupos que tiveram sua validade questionada várias vezes (Kisielewski, 1997; Balsamo et al., 2009; Kånneby et al., 2013; Kieneke & Rhaesa, 2015; Minowa & Garraffoni, 2017; Kolicka *et al.*, 2020; Kolicka et al., 2020; Kolicka et al., 2020). *Chaetonotus* é o gênero mais especioso dentro do filo Gastrotricha, abrangendo mais de 200 espécies, que são, basicamente, definidas pela presença de escamas com espinhos (Todaro et

al., 2019).

1.3.Animais semi-planctônicos: família Dasydytidae

Ao contrário da maioria das espécies de Gastrotricha que possuem habitat bentônico, existem grupos taxonômicos que apresentam um estilo de vida diferenciado, caracterizados por habitarem regiões mais próximas à superfície da água associados ao perifíton de ambientes dulcícolas (Balsamo et al., 2014; Minowa & Garraffoni, 2022). Esses animais são conhecidos como semi-planctônicos, e somam cerca de quarenta espécies distribuídas em três famílias: Dasydytidae (33 espécies), Neogosseidae (10 espécies) e Chaetonotidae, 1864 (1 espécie) (Balsamo et al., 2014, 2020; Kånneby & Todaro, 2015; Minowa & Garraffoni, 2017, 2020).

Dasydytidae é composta por animais que possuem tais características: i) faixas cefálicas transversais que circundam a região da cabeça, ii) conjunto de tufos ciliares ventrais distribuídos ao longo do corpo, iii) longos espinhos inseridos ventro-lateralmente ou na extremidade posterior e iv) diminuição ou ausência completa dos tubos adesivos posteriores (Kisielewski, 1991; Kieneke & Ostmann, 2012; Balsamo et al., 2014; Kånneby & Todaro, 2015; Minowa & Garraffoni, 2020).

É bem aceito pela comunidade científica que o ancestral desses animais era um chaetonotidae com hábito bentônico, provavelmente pertencente ao grupo *Chaetonous,* hipótese que foi sustentada por dados tanto morfológicos quanto moleculares (Kånneby et al., 2013; Kånneby & Todaro, 2015; Kolicka et al., 2020; Minowa & Garraffoni, 2021). Em função disso, as características evolutivas desse grupo assumem especial importância, pois são base no entendimento na transição do hábito bentônico para o planctônico.

Dentro da família Dasydytidae temos o gênero *Ornamentula*, destacando-se por suas características morfológicas únicas entre outros animais pelágicos, como a presença marcante de escamas ornamentadas, largas e reforçadas. Foi descrito por Kisielewski (1991), que foi criado para acomodar uma única espécie, *Ornamentula paraensis* Kisielewski (1991). Posteriormente, Minowa & Garraffoni (2021) descreveram a segunda espécie encontrada no interior de São Paulo, *Ornamentula miyazakii*. Apesar da similaridade inconfundível dos representantes desse grupo, sua monofilia nunca havia sido testada

1.4. Complexo de Espécies

Complexo de espécies é um conceito onde ‘duas ou mais espécies distintas são erroneamente classificadas (e ocultas) sob um único nome de espécie’ (Klautau *et al.*, 1999; Bickford *et al.*, 2007; Magpali *et al.*, 2021). São espécies com unidades taxonômicas

semelhantes, mas, na verdade, são espécies distintas (Tulchinsky *et al*.2012). Isso se torna uma questão muito problemática ao considerarmos que a acurada delimitação de espécies é fundamental para a descrição da diversidade biológica e está intrinsecamente relacionada com diversas outras áreas do conhecimento, como ecologia, genética de populações, filogeografia, entre outros (Dayrat, 2005). Consequentemente, uma falha nessa área pode desencadear uma série de desinformações em cadeia.

Um exemplo disso é evidenciado na área de estudo da distribuição geográfica dos organismos meiofaunais. Por se tratarem de animais sem larva planctônica (o que facilitaria sua distribuição) e baixa capacidade locomotora, é esperado que tivessem uma distribuição mais restrita (Bohonak, 1999; Collin, 2001; Tulchinsky et al., 2012). No entanto, o que se verifica na literatura é uma distribuição cosmopolita de algumas espécies, estando presentes em um raio de distância tão amplo que podem abranger até mesmo continentes diferentes. Esse fenômeno é chamado de "paradoxo da meiofauna" e vem sendo questionado por diversos autores (Giere, 2008; Kånneby et al., 2012; Garraffoni & Balsamo, 2017;). Podemos, dessa forma, supor que o complexo de espécies contribui para a persistência desse paradoxo, e que o estudo das espécies de Gastrotricha com ampla distribuição geográfica pode representar uma valiosa abordagem para a resolução deste complexo.

Uma das espécies que se encaixa nesse contexto é *Chaetonotus acanthocephalus* (Valkanov, 1937). Originalmente reportada em várias localidades na Europa, foi encontrada no Brasil com algumas pequenas variações morfológicas em relação à descrição original (Kisielewski, 1991; Araújo et al., 2013). Com o auxílio de microscopia de luz (DIC) e microscopia de varredura (MEV), Vedovati (2019) apresentou hipóteses que justificam a distinção de dois morfotipos localizados em regiões distintas do Brasil, sendo elas encontradas nas cidades de São Carlos (SP) e Diamantina (MG); São João da Boa Vista (SP) e São Bernardos dos Campos (SP).

1.5. Déficit de estudos da biodiversidade

Existe uma notável predominância de estudos no Hemisfério Norte em contraste com a escassez de pesquisas no Hemisfério Sul, o que resulta em atrasos na compreensão das espécies e contribui para um déficit de estudos da biodiversidade do grupo Gastrotricha (Garraffoni & Balsamo, 2017; Balsamo et al., 2020; Garraffoni & Araújo, 2020; Garraffoni et al., 2021; Araújo et al., 2024).

No que diz respeito à América do Sul, vale ressaltar que 91 espécies de gastrótricos foram descritas na Argentina, Brasil, Guiana, Paraguai e Uruguai (Garraffoni & Araújo, 2020; Bosco

et al., 2020; Minowa and Garraffoni, 2020, 2021; Araújo & Garraffoni, 2021; Magpali *et al.*, 2021*).* No entanto, essas pesquisas possuem uma amostragem desigual em cada país, e o Brasil, apesar de ser o país mais bem amostrado da lista, ainda possui números insatisfatórios se comparados com países europeus, como Alemanha e Itália, e com os Estados Unidos (Garraffoni & Araújo, 2020; Araújo et al. 2024; Garraffoni et al., 2024).

A pesquisa nas regiões neotropicais enfrenta também a carência de detalhes e amostras coletadas, algumas das quais datam de mais de um século atrás. Essa carência está intrinsecamente ligada à falta de especialistas e à ausência de uma infraestrutura taxonômica adequada, considerando que a identificação e o manuseio dessas amostras são particularmente desafiadoras devido ao tamanho reduzido e à fragilidade das espécies (Ricci & Balsamo, 2000; Balsamo et al., 2020;~~,~~ Garraffoni & Araújo, 2020; Garraffoni et al., 2024). Isso é particularmente real quando se trata de gastróticos semi-planctônicos, já que além desses fatores gerais, eles também possuem maior sensibilidade ao ambiente.

2. OBJETIVOS

2.1. Objetivo geral

Ampliar o entendimento sobre a biodiversidade de gastrótricos da ordem Chaetonotida por meio de novas descrições baseadas em taxonomia integrativa.

2.2.Objetivos específicos

- Descrição espécies brasileiras utilizando técnicas para análises morfológicas (microscopia de luz com contraste de interferência diferencial e microscopia eletrônica de varredura) e moleculares (sequenciamento de genes nucleares e gene mitocondrial).
- Elucidar e delimitar os limites taxonômicos das populações brasileiras da espécie *Chaetonotus acanthocephalus.*
- Descrever a terceira espécie do gênero *Ornamentula.*
- Organizar um banco de dados fotográficos da diversidade de Chaetonotida no Brasil incluindo registro de microscópio de luz e microscópio eletrônico de varredura.
- Ampliar o número de sequências moleculares de espécies de gastrótricos brasileiros no GenBank.
- Depositar tanto material preservado quanto fotografias no Museu de Diversidade Biológica da UNICAMP (MDBio).

3. MATERIAIS E MÉTODOS

3.1.Coleta

Durante este estudo, várias coletas foram efetuadas com intuito de encontrar indivíduos da ordem Chaetonotida, sendo os locais de coleta listados abaixo. As amostras foram armazenadas em baldes e/ou pote no Laboratório de Evolução de Organismos Meiofaunais da Universidade Estadual de Campinas, e mantidas sob aeração através de um compressor de ar a uma temperatura em torno de 20ºC (Garraffoni & Araújo, 2010).

Locais com potencial para a presença de representantes de Chaetonotida:

- Represa Billings, Município de São Bernardo do Campo, SP (23°46’26”S 46°35’20”O) para água com sedimentos associados à raízes de plantas aquáticas. (Agosto/2022).
- Rio Jaguari, Cosmópolis, SP (22°41'12.6"S 47°07'42.2"O) para água com sedimentos associados à raízes de plantas aquáticas (Fevereiro/2023).
- Lago urbano, Município de Paulínia, SP (22°47'34.5"S 47°08'47.7"O) - para água com sedimentos associados à raízes de plantas aquáticas (Abril/2023).
- Represa do Broa, Município de São Carlos, SP (22°10’44”S 47°53’39”O); para água com sedimento arenoso/rochoso (Maio/2023).
- Represa das Graças, Cotia, SP (23°39'14.9"S 46°58'05.9"O) para água com sedimento arenoso/rochoso (Maio/2023).
- Rio do Peixe, Socorro, SP (22°32'55.5"S 46°34'42.4"O) para água com sedimentos associados à raízes de plantas aquáticas (Novembro/2023).
- Lago Limoeiro, Águas de São Pedro, SP (22°36'19.1"S 47°51'41.7"O) para água com sedimentos associados às raízes de plantas aquáticas e superfície do perifíton (Janeiro/2024).
- Represa das Palmeiras, Águas de São Pedro, SP (22°36'30.1"S 47°52'08.9"O) para água com sedimentos associados às raízes de plantas aquáticas e superfície do perifíton (Janeiro/2024).

Figura 1. Mapa das cidades onde foram realizadas as coletas durante o mestrado. **Fonte:** QGIS, v.3.34.

3.2. Análise Morfológica

3.2.1. Triagem

O processo de triagem do material foi realizado utilizando pequenas amostras de água, filtradas em uma malha de 45 µm para concentrar o material, sendo posteriormente colocadas em uma placa de Petri sob um estereomicroscópio Zeiss Stemi 2000. Os organismos encontrados foram isolados com micropipeta e anestesiados com solução de $MgCl^2$ a 2%. Os espécimes foram coletados com uma micropipeta e transferidos para uma lâmina com lamínula para observação ao microscópio.

3.2.2. Microscopia de luz (ML-DIC)

As lâminas foram observadas no microscópio de luz ZEISS Axio Imager M2 com Contraste de Interferência Diferencial (CID) e câmera AxioCam MRC5 acoplada. O programa ZenBlue foi empregado para capturar imagens e realizar medições em várias magnificações. Nas fotos foram adicionadas as escalas correspondentes a cada aumento e depois houve a elaboração de pranchas das imagens e a medição de determinadas estruturas, com auxílio do software MorphoJ.

O comprimento total e a posição dos órgãos serão descritos em (%) em relação a unidade

corporal, ou seja, o comprimento total a partir da região anterior (U00) para a posterior (U100) será de 100 unidades (Hummon *et al*. 1992; Hochberg & Atherton, 2010). A descrição será dividida de acordo com a região do corpo, da parte cefálica até as glândulas adesivas.

3.2.3. Fixação

Os organismos encontrados foram isolados utilizando uma micropipeta e anestesiados com uma solução de $MgCl^2$ a 2%. Após a identificação, uma parte dos organismos foi armazenada em microtubos contendo glutaraldeído diluído em tampão cacodilato a 4°C, visando futuras análises por microscopia eletrônica de varredura. Finalmente, alguns representantes foram separados para extração de DNA, sendo fixados em álcool absoluto a 100% e mantidos a -20°C.

3.2.4. Microscopia Eletrônica de Varredura (MEV)

Após a fixação em glutaraldeído 2,5%, os indivíduos foram lavados em tampão cacodilato 0,1M por 2 horas havendo ao menos sete trocas de tampão durante o período. Os espécimes passaram por uma desidratação alcoólica com porcentagens de 30%, 50%, 70%, 80%, 90%, 95% e 100% (com repetição da de 100%). Em seguida, os animais foram secados em ponto crítico com o Balzers CPD 030. Posteriormente os gastrótricos foram montados em stubs, metalizadas com ouro-paládio e observados no microscópio eletrônico de varredura Jeol - JSM 5800LV, de voltagem 10 kV, do Laboratório de Microscopia Eletrônica da Unicamp.

3.2.5. Pranchas e ilustrações

As imagens resultantes da microscopia de luz e microscopia eletrônica de varredura foram organizadas em pranchas através dos aplicativos Adobe Photoshop v1.24.4; Adobe Illustrator v24.1.2.408 e Adobe InDesign v17.1. No Illustrator, as imagens foram organizadas por indivíduos e diversos desenhos esquemáticos foram confeccionados a fim de manter a maior afinidade possível com as estruturas observadas. Por fim, os desenhos foram organizados em 2 únicas vistas por espécie: ventral e dorsal.

3.3. Análise molecular

3.3.1. Extração e amplificação de DNA (PCR)

Organismos inteiros foram coletados e submetidos à extração de DNA genômico utilizando o kit QIAmp DNA Micro (Qiagen), conforme as instruções do fabricante. O DNA

genômico foi então amplificado por PCR, em reações de 20 µL contendo 10 µL de 2x Taq PCR Master Mix (Qiagen), 5 µL de DNA genômico, 4,2 µL de água Miliq e 0,4 µL (4 pmol) de primers específicos. As sequências dos primers e as condições da PCR estão detalhadas na Tabela 1. Os produtos da amplificação foram analisados por eletroforese em gel de agarose a 1%, contendo SYBR Green (Life Technologies).

3.3.2. Sequenciamento

Os fragmentos de DNA amplificados foram sequenciados pelo método de Sanger utilizando o equipamento 3500xL Genetic Analyzer (Life Technologies), disponível no Centro de Biologia Molecular e Engenharia Genética (CBMEG). As sequências dos genes nucleares 18S rDNA, 28S rDNA e do gene mitocondrial COI mtDNA foram processadas com o software Geneious®, alinhadas por meio do programa Mafft v7.215 utilizando o parâmetro L-INS-I (Katoh & Standley, 2013), e posteriormente depositadas nos bancos de dados genéticos do GenBank.

3.4.Filogenia

Todas as espécies das famílias Chaetonotidae, Dasydytidae e Neogosseidae com sequências de 18S e 28S rDNA, e COI mtDNA listadas (Križanová & Vďačný, 2023) foram baixadas do GenBank (https://www.ncbi.nlm.nih.gov/genbank/), totalizando 43 terminais (números de acesso na Tabela Suplementar S2). Cada conjunto de dados de sequência foi alinhado separadamente no servidor MAFFT v.7 (https://mafft.cbrc.jp/alignment/server/) utilizando a abordagem L-INS-I (Katoh & Standley, 2013) e posteriormente aparado para o comprimento comum de todas as sequências no Geneious Prime v.2024.0.7 (Biomatters Inc., Auckland, New Zealand).

O conjunto concatenado foi feito através do programa Sequence Matrix v1.8 e analisado utilizando métodos de Máxima Verossimilhança (ML) e Inferência Bayesiana (IB). As análises de ML foram realizadas no IQ-TREE v.1.6.10 (Nguyen *et al.*, 2015) com o melhor modelo de substituição selecionado para cada marcador molecular separadamente (GTR+I+G.).

Nas análises de IB o aplicativo PAUP4 v. 4.3.99 foi utilizado para gerar árvores iniciais e testar modelos evolutivos. Posteriormente, esses resultados foram rodados no algoritmo MrBayes on XSEDE pela plataforma CIPRES (Ronquist *et al.*, 2012). As árvores de ML e IB foram enraizadas com o método do ponto médio no FigTree v.1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/). A árvore final foi ilustrada através do aplicativo Adobe Illustrator v24.1.2.408.

Tabela 1. Sequências de primer e regimes de PCR utilizados para amplificação e sequenciamento de 18S e 28S rDNA.

Regimes de Primer e PCR	Sequência primer (5' => 3')	Referência
18 S primers		
S30	GCTTGTCTCAAAGATTAAGCC	Norén and Jondelius (1999)
S30R	CTTCGGACCTCTGACTTTCG	Garraffoni et al. (2017)
PCR	94°C for 5 min, 40x (94°C for 30 s, 52.5°C for 30 s, 72°C for 60 s), 72°C for 7 min	-
1801	GATCTATTTTGTTGGTTTTCGG	Garraffoni et al., (2017)
1806	CCTTGTTACGACTTTTACTTCCTC	Norén and Jondelius (1999)
PCR	94°C for 5 min, 40x (94°C for 30 s, 52.5°C for 30 s, 72°C for 60 s), 72°C for 7 min	-
18SE F	CTGGTTGATCCTGCCAGT	Hillis & Dixon, (1991); Blanco-Bercial et al., (2011)
18SL R	CACCTACGGAAACCTTGTTACGACTT	
F-566 F	CAGCAGCCGCGGTAATTCC	
R-1200 R	CCCGTGTTGAGTCAAATTAAGC	
PCR	94 °C/4 min, 35x (95 °C/45 s, 51 °C/60 s, 72 °C/120s), 72 °C for 4 min	-
28 S primers		
28S.1R	CGATTAGTCTTTCGCCCCTA	Garraffoni et al., (2019)
PCR	94°C for 5 min, 40x (94°C for 30 s, 55°C for 30 s, 72°C for 60 s), 72°C for 7 min	-
28S.2F	GGACCCGAAAGATGGTGAAC	Garraffoni et al., (2019)
28S.2R	CAATTTGCCGACTTCCCTTG	Garraffoni et al., (2019)
PCR	94°C for 5 min, 40x (94°C for 30 s, 60°C for 30 s, 72°C for 60 s), 72°C for 7 min	-
COI primers		
LCOI490	GGTCAACAAATCATAAAGATATTGG	Folmer et al., (1994)
HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al., (1994)
PCR	94°C/5 min, 45x (94°C/30 s, 46°C/30s, 72°C/40 s), 72°C for 7 min	-

4. RESULTADOS

Os resultados da dissertação estão organizados em dois capítulos, que foram escritos em formato de artigos e serão submetidos a revistas especializadas após a defesa da dissertação.

4.1.Contribuição para um gênero novo e duas espécies novas de Chaetonotidae

Este capítulo foi iniciado em colaboração com a pesquisadora polonesa Małgorzata Kolicka, especialista em Gastrotricha, que assumiu a liderança como primeira autora, uma vez que a maioria das espécies a serem descritas ou realocadas são de origem europeia. Ficamos encarregados da descrição do novo gênero e de duas espécies brasileiras, que foram inicialmente delimitadas por Vedovatti et al., (2019) em seu mestrado. Kolicka seria responsável por descrever três espécies europeias e realocar *Chaetonotus acantocephalus* e *Chaetonotus armatus* no novo gênero. Entretanto, por motivos pessoais, ela não pôde concluir a tempo as seções sob sua responsabilidade, que incluíam o resumo, a introdução, o material e métodos, as descrições das espécies europeias e a discussão. Apesar disso, neste trabalho apresentamos nossa parte finalizada, que consiste nas descrições formais das espécies seguindo a estrutura proposta por Kolicka, além da produção de novas imagens e novos desenhos. O artigo será publicado assim que a pesquisadora retomar as atividades.

4.2.Espécie nova do gênero *Ornamentula*

O gênero *Ornamentula,* ~~com espécies endêmicas do Brasil~~contendo apenas espécies descritas para o Brasil, foi descrito orginalmente pelo prof. Jacek~~or~~ Kisielewski para realocar uma única espécie, *Ornamentula paraensis* Kisielewski (1991). Posteriormente, mais de 30 anos depois, Minowa & Garraffoni (2021) ~~–~~descreveram a segunda espécie *Ornamentula miyazakii*, encontrada em um lago urbano no estado de São Paulo, na cidade de Paulínia. Neste trabalho, é descrita a terceira espécie do gênero, *Ornamentula* sp. nov., também encontrada em um outro lago urbano no estado de São Paulo em Águas de São Pedro.

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6. Capítulo 1. New genus 'hidden' within *Chaetonotus* (Chaetonotidae, Gastrotricha) - taxonomic revision, reclassification and species description based on the integrative taxonomy approach.

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Abstract

We investigated the taxonomy of *Chaetonotus acanthocephalus* Valkanov, 1937, reported in Europe and Brazil. Using DIC and SEM, three morphotypes were identified in Brazil: two new species and one matching the original description. A new genus (*Gen. nov.*) is proposed, including *Gen. nov. acanthocephalus*, *Gen. nov. sp1*, and *Gen. nov. sp2*

Introduction

The phylum Gastrotricha are marine and freshwater meiofaunal organisms with a wide distribution in distinct habitats around the globe. However, gastrotrics have limited distribution due to their movement being restricted to ventral cilia, short life cycles and an absence of larval form, causing the so-called meiofauna paradox. One of these species that has a wide distribution is *Chaetonotus acanthocephalus* Valkanov 1937, originally described for Bulgaria and later reported to other european countries and southeastern Brazil. The taxonomic history of this species has been the subject of discussions among gastrotricologists due to the morphological variations observed between the different European and Brazilian populations. Thus, different authors have already opposed and favored the delimitation of a second species, but without ever having made a more detailed study of all observed morphotypes. The purpose of this study was to accurately delimit the species *Chaetonotus acanthocephalus* and to determine if such species has a wide distribution. For this, different morphological techniques were employed, such as interferential differential contrast light microscopy (DIC) and scanning electron microscopy (SEM). From the information obtained, we identified three morphotypes in Brazil. Two were confirmed as distinct species, while the third closely resembles the original description. Thus, as one of the first steps to reorganize the *Chaetonotus* genus, a reclassification was proposed

from the creation of a new genus that will contain Gen. nov. *acanthocephalus* (Valkanov, 1937), Gen. nov. sp1 and Gen. nov. sp2. Therefore, we hereby propose the establishment of gen. nov. and describe nov. sp1 and nov. sp1 from Brazil.

Material and methods

Study area

In Brazil, the specimens were found in:

- Urban lake, São João da Boa Vista/SP (21°57'S 46°44'W); sediments associated with aquatic plant roots.
- Billings Reservoir, São Bernardo do Campo/SP (23°46’26”S 46°35’20”W); sediments associated with aquatic plant roots.
- Urban lake, Paulínia/SP (22°42’27”S 47°14’51”W); sediments associated with aquatic plant roots.
- Mucugêzinho Stream, Mucugê/BA (13°00’44”S 41°22’09”W); interstitial.
- Soberbo River Stream, Diamantina/MG (17°33’40”S 43°49’25”W); interstitial.
- Broa Reservoir, São Carlos/SP (22°10’44”S 47°53’39”W); interstitial.

Sampling and documentation

Five-liter buckets were utilized to collect material from the upper 30cm of the water surface, among floating vegetation roots, and from the interstitial. The material was stored with continuing aeration, maintaining the temperature around 19 °C, and processed in fourteen days.

Morphological analyses

The sorting process involved 500 ml water samples filtered through a 30 μm mesh, then poured into Petri dishes and examined under a Zeiss Stemi 2000 stereomicroscope, focusing on the water column to identify semiplanktonic gastrotrichs. Each individual was isolated, anesthetized with 2% MgCl2, and mounted individually, with digital documentation performed using a Zeiss Axio Imager M2 light microscope equipped with DIC and an AxioCam MRC5 digital camera. Images were captured, and measurements were taken using the ZEN lite 2.5 2018 imaging software.

Specimens were preserved in 2% glutaraldehyde in sodium cacodylate buffer for storage. The samples were then rinsed in 0.1 M PBS and dehydrated through a graded ethanol series (30%, 40%, 50%, 60%, 70%, 90%, 95%, 100%, each step performed twice for 5 minutes). As described by Abolafia (2015), small containers were used to transfer the samples between alcohol solutions. Afterward, they were subjected to critical-point drying with CO2 using a Baltec CPD 030 dryer, mounted on aluminum stubs, and coated with gold-palladium using the

SCD-050 Sputter Coater. Observations were made using a JEOL JSM 5800LV scanning electron microscope at the State University of Campinas (UNICAMP).

The positions of morphological traits along the longitudinal axis are expressed as percentage units (U) of the total body length, measured from the anterior to the posterior end (Hummon et al. 1992).

Molecular data

DNA was extracted from 4 specimens using QIAmp DNA Micro Kit (Qiagen), following the manufacturer's instructions. The nuclear 18rRNA and 28S rRNA genes and mitochondrial cytochrome C oxidase subunit I (COI) were amplified using the polymerase chain reaction (PCR). The PCRs amplification was performed in 20 µl reaction mixture, containing 5 µl of extracted DNA template, 10 µl of GoTaq® Master Mix, and 0.4 µl (10 pmol/µl) of specific primers (Table S1), and 4.2 µl of H2O (Kieneke & Todaro, 2021). The amplification products were visualized by electrophoresis in 1% agarose gels containing SYBR® (Life Technologies). 5 µl of the post-PCR reaction product was mixed with 2ul of ExoSAPIT™ reagent. The DNA fragments were sequenced using BigDyeTerminator reactions in a 3500xL Genetic Analyzer (Life Technologies) at the Centro de Biologia Molecular and Engenharia Genética laboratory (CBMEG, Campinas, Brazil).

Results

Taxonomic account

Phylum GASTROTRICHA Mečnikow, 1865

Order CHAETONOTIDA Remane, 1925 [Rao & Clausen, 1970]

Suborder PAUCITUBULATINA d'Hondt, 1971

Family CHAETONOTIDAE Gosse, 1864 (*sensu* Leasi *et* Todaro, 2008)

Subfamily CHAETONOTINAE Gosse, 1864 (*sensu* Kisielewski, 1991)

Gen. nov.

Typ species. Comb. nov. described as *Chaetonotus acanthocephalus* Valkanov, 1937

***Locus typicus*.** Bulgaria.

Environment. Freshwater; benthic and epiphytic.

Diagnosis. Chaetonotidae with slender body measuring from 100 to 340 µm in length. Head complex with reduced central, unpaired cephalic plate (typical cephalion), two or three pairs of pleurae and three or five modified, peculiar cephalic scales ('spined cephalion'), which took over the role of typical cephalion. Peculiar cephalic scales large, thick, clearly visible in the

head outline. Hypostomium large, strongly reinforced with horn-like protuberances anterolaterally. Beneath hypostomium, a pair of large reinforced additional plates. Ocellar granules absent. Two pairs of cephalic ciliary tufts with anterior emerge nearly terminally. Mouth ring located subterminally, wide, strongly reinforced consisted of two cuticular rings with long internal lamellae and two cuticular teeth arising from the anterior pharynx region. Suboral bristles absent. The adhesive tubes simple, furcal branches set narrowly. Whole body, including furcal appendages covered by large and thick scales. Scales partially merged in cuticle, with anterior edges extraverted. Scales semi-triangle, semi-pentagonal, semi-rhomboidal to rhomboidal and heart-like, often asymmetrical, without or with shallow posterior notches. Scales generally keeled; keels strong, rhomboid-shaped located in scale centre. Scales located close to each other, juxtaposed or overlapping. Spines rigid and cone-like; lateral, spines of the ventrolateral and ventral head, neck and anterior trunk regions strongly curved; remaining spines mainly straight; without or with lateral denticles. Peculiar head scale spines and some body spines dual in structure – basal segment significantly thicker and cone-like; the second part uniformly thin and flexible. On the neck two pairs of spines and on the widen trunk region two pairs of spines or five spines longer and stronger than surrounding. Spines on lateral, ventrolateral and ventral surfaces of the head, neck and anterior trunk region and on ventral surfaces of the central and posterior trunk regions gradually tapering towards hair-like ends. Rigid parafurcal and intrafurcal spines. The entire ventral intraciliary field is covered with large, irregular scales; scales on the pharyngeal section keeled and spineless, on the intestinal section keeled and spined of the same type as the body scales. Two or three pairs of dorsal sensory bristles with a dual structure; basal segment thick, straighter and with pointed, cone-like ends, from which a very thin and flexible bristle part emerges. Pharynx cylindrical with slightly marked anterior dilatation, but without marked posterior dilatation or without anterior and posterior dilatations. Intestine straight with or without the anterior section differing in form and morphology.

Autopomorphies. Cephalion reduced to the small sized, very thin, terminally located plate. Epipleurae and hypopleurae shifted lateraly to ventraly, not visible in dorsal head outline. Anteriormost head region covered by strong, modified, peculiar scales with keels and spines – ‘spined cephalion’. Hypostomium wide, thick and reinforced with one pair of large and reinforced additional plates beneath. Mouth ring located terminally, wide with strong complex reinforcement formed by internal and external rings. Scales large, one-lobed, partially merged into cuticle with extraverted anterior edges. Peculiar head scales spins, part of remaining body spines and dorsal sensory bristles dual in structures – basal segment significantly thicker and

cone-like, the second part uniformly thin and flexible. Pharynx cylindrical without prominent anterior and posterior dilatation.

Remarks. Instead of adding newly founded species to the genus *Chaetonotus* Ehrenberg, 1830, we propose establishing a new genus because of the clear distinctiveness of this chaetonotidian lineages. Described and postponed species listed below, seemingly fully fit to be included as *Chaetonotus* representatives. They have a body covered by scales with spines which is considered as a characteristic for this genus. However, the genus *Chaetonotus* was established at the beginning of XIX centuries, when only a few gastrotrich species were known, on the base unspecific features very widely presented in various proportions in each genus of Chaetonotidae family. Currently, *Chaetonotus* grouped more than 230 species (Todaro 2020), which makes it the largest genus within the whole Gastrotricha. Contemporary analyses, both morphological and molecular (e.g., Kieneke et al. 2008; Kånneby et al. 2012, 2013; Kolicka et al. 2016, 2018, 2020) indicate the artificial and outdated status of this group. To avoid intensifying the well-known "wastebasket taxon", in which species with superficial resemblance are affiliated to the same higher ranking genus (Kieneke & Todaro 2020) we decided to extract this separate lineage as the first step to the re-systematization of genus *Chaetonotus* together with whole Chaetonotidae family.

spec. nov. 1

(Figs.1, 2, 4, 6, 8, 9, 10, 11)

Localities: Minas Gerais, Diamantina

Material.

Holotype. BRAZIL • adult (photographs, the specimen was destroyed); Ind. 06 - State of Minas Gerais, Diamantina.

Paratypes.

BRAZIL • Sixteen adult individuals collected from bottom sediments between the years 2009 and 2010 in the municipality of Diamantina, Minas Gerais state, Brazil.

BRAZIL • Six individuals collected in the São Carlos region, São Paulo state, Brazil.

Diagnosis. Slender body measuring 123-228 μm in length. Head complex with reduced cephalion and two pairs of pleurae and five modified, peculiar cephalic scales. The hypostomium is large, bean-shaped, with rounded lateral edges. A pair of additional symmetrical plates is located posterior to the main hypostomal plate. Ocellar granules absent.

Mouth ring wide, strongly reinforced with long internal lamellae and two cuticular teeth arising from the anterior pharynx region. Suboral bristles absent. Scales large and thick, partially merged in cuticle, with anterior edges extraverted. The scales are distributed in a total of 13 longitudinal rows in Dorsal (D), Lateral (L) and Ventral (V) sides (3D+2DL+2L+2VL+4V) with 16 scales in the central row, differing morphologically in the head, neck and trunk regions; scales semi-triangle, semi-rhomboidal to rhomboidal and heart-like, located close to each other, juxtaposed or overlapping. Spines rigid and cone-like; laterally, spines of the ventrolateral and ventral head, neck and anterior trunk regions strongly curved; remaining spines mainly straight; all without lateral denticles. Spines on lateral, ventrolateral and ventral surfaces of the head, neck and anterior trunk region and on ventral surfaces of the central and posterior trunk regions gradually tapering towards hair-like ends. Spine length gradually and slightly increases from head towards the widest trunk region, after which it shortens to the furcal base. Two pairs of neck lateral spines, one pair of trunk dorsal spines, one pair of trunk lateral spines and two pairs of parafurcal and one pair of intrafurcal spines significantly longer than remaining spines. The entire ventral interciliary field covered with small, irregular scales having a spine. Five pairs of ventral interciliary field terminal scales. Pharynx cylindrical without anterior and posterior dilatations. Intestine straight without anterior section differing in form and morphology.

Description.

Habitus

The new species has a slender body. The head is slightly wider than the neck, and the neck constriction is weakly marked. The neck has a diameter of 18-48 μm, and the trunk reaches a diameter of 26-55 μm. The trunk gradually dilates from *ca*. U30 to *ca*. U70, where it is at its maximum width, and then it gradually tapers towards the narrow furcal base (from U86 to U90) **(Figs. 6-A; 8-A)**. The furcal indentation (intrafurcal space) is parabolic in shape. The furcal branches are set narrowly apart and point slightly outwards. The adhesive tubes are straight and gradually taper towards their slightly rounded blunt ends **(Fig. 10).**

Head

The head is semitriangular in shape. It is complex with modified cephalic plates, in which the cephalion (U1) is reduced and present only on the top of the head **(Fig. 6-F).** Terminally on the head, a very small and flat epipleurae (U1) adheres to the cephalion. Covering the lateral, ventrolateral and ventral surfaces of the head, a large hypopleurae (U2-U7) is located

(Figs. 6-C, F; 8-B). The main head plates consist of five modified scales **(Figs. 6-B; 8-B).** These scales are large (U1-10) thick and located on the dorsal and dorsolateral head surfaces. Their anterior edges overlap the posterior edge of the cephalion, whereas the most dorsolateral pair laterally adheres to the posterior edges of the epipleurae and lateral edges of the hypopleurae. These peculiar scales are semi-triangular in shape and closely adhere to each other. The main hypostomium plate is large, bean-shape and its lateral edges are rounded **(Figs. 6-C; 8-C).** One pair of additional plates is located posterolaterally to the main hypostomium plate at U5 - U8. The anterior lateral edges of this pair are accentuated **(Figs. 6-C; 8-C).** Two pairs of cephalic ciliary tufts are present (U1). The ocellar granules are absent. The mouth ring is located terminally at U1. Suboral bristles are absent.

Scales

The entire body is closely covered with large and thick, one-lobed scales, partially merged in the cuticle, with anterior edges extraverted **(Figs. 2; 4; 6; 8).** The scales are distributed in a total of 13 longitudinal rows (3D+2DL+2L+2VL+4V) with 16 scales in the central row, differing morphologically in the head, neck and trunk areas. Longitudinal scale rows run straight, and are located very close to each other, in which most of these scales have overlapping edges.

Scales type 1: On the rostral head part, dorsally and dorsolaterally, five anteriormost scales are situated. These peculiar scales are modified and consist of a kind of 'cephalic plates' **(Figs. 6-B; 8-B,** see 'Head' subsection'). These scales are large and thick, elongated, triangular in shape without posterior notches; they adhere closely to other by lateral edges. The central scales are the largest and have bilateral symmetry, the remaining of these peculiar scales are slightly smaller and asymmetrical. These peculiar scales have strong, long keels, running from the anterior to posterior scale edges. Near the posterior scale edges, the keels turn into spines with a dual structure.

Scales type 2: Beneath these anteriormost peculiar scales, a pair of large and wide scales is located, adjacent to their posterior edges. These scales are shaped as a reverse semitriangle and have strong, wide, rhomboid-shaped keels in the centre. These keels are strong and the highest in their central axis, from the ends of which dual structured spines arise **(Figs. 6-B; 8-B).**

Scales type 3: Beneath these scales, a single semitrangle-shaped scale is located in the longitudinal dorsal central row of the scales. This scale has a pointed anterior edge, a rounded posterior edge and a shallow and narrow posterior notch. These scales have strong, short, wide, rhomboid-shaped keels in the center, the highest in their central axis, and they pass into a dual-structured spine **(Figs. 6-B; 8-B).**

Scales type 5: To support potential inferences and homologous comparisons within the genus, this specific trait is not assigned for this species, as no scales resembling those found in the other species could be identified.

Scales type 4: Laterally to these scales, on the dorsolateral and lateral head surfaces, one pair of rhomboid-shaped scales is present. These scales are adjacent to the posterior edges of the most lateral pair of peculiar scales and to the posterior edges of the hypopleurae. This scale has a shallow and narrow posterior notch. These scales have strong, wide, short rhomboid-shaped keels in the center, the highest in their central axis, and they pass into dual-structured spines **(Figs. 6-B, C; 8-B, C).**

Scales type 6: Lateral to this pair, on the ventrolateral head surface (around U10), a pair of small rhomboid-shaped scales is located. These scales are adjacent to the posterior edges of the hypopleura and the overlapping edges of the lateral scales. These scales have strong, short, wide and rhomboid-shaped keels situated anteriorly that pass into the cone-like spines gradually tapering seamlessly to the hair-like ends **(Figs. 6-C; 8-C)**.

Scales type 7: Two pairs of large, strongly rounded semitriangle-shaped scales are present dorsally, dorsolaterally, and laterally (around U10-U15) as subsequent scales of the head. These scales have shallow posterior notches and strong, short and wide rhomboidal shaped keels in the centre, the highest in their central axis, and they pass into dual-structured spines **(Figs. 6-F, 8-B, C)**.

Scales type 8: One pair of asymmetrical, oval-shaped scales is situated laterally to these scales, on the ventrolateral to ventral head surface. These scales do not have posterior notches and have strong wide rhomboid-shaped keels, the highest in their central axis, and they pass into the cone-like spines tapering seamlessly to the hair-like ends **(Figs. 6- C; 8-C)**.

Scales type 9: The remaining head and anterior neck dorsal, dorsolateral and lateral scales (from *ca.* U13 to *ca.* U23) are wide and oval-shaped (scales 9). These scales have shallow and narrow posterior notches and short and wide rhomboid-shaped keels, the highest in their central axis, and they pass into the strong cone-like spines **(Figs. 6-A; 8-D)**.

Scales type 10: To support potential inferences and homologous comparisons within the genus, this specific trait is not assigned for this species, as no scales resembling those found in the other species could be identified.

Scales type 11: On the ventrolateral to ventral surfaces, four pairs of asymmetrical, irregular scales are located. These scales do not have posterior notches and have strong wide rhomboid-shaped keels, the highest in their central axis, and they pass into the cone-like spines tapering seamlessly to the hair-like ends **(Fig. 6- C)**.

Scales type 12: Scales on the neck adjacent to the locomotory ciliary band on the ventral surface are much smaller and oval. These scales do not have posterior notches and have short and wide rhomboid-shaped keels, the highest in their central axis, and they pass into the cone-like spines tapering seamlessly to the hair-like ends **(Fig. 8- C)**

Scales type 13: Dorsally on the neck, at ca. U23-U25, one pair of asymmetrical, semitriangular-shaped scales is located. The lateral edge of these scales is oriented to the lateral body surface; they are shorter and more oval. These scales have slightly pointed anterior edges, unicentrally located posterior notches and short and wide rhomboid-like keels, the highest in their central axis, and they pass into the strong cone-like spines, located directly above the posterior notches **(Figs. 6- A; 8- A; 11-B).**

Scales type 14: On the neck, beneath this pair of scales, dorsally to dorsolaterally, another pair of asymmetrical scales is located (approximately at U28-U31). These scales are semitriangular in shape but wider and larger than the scales above. They have asymmetrical pointed anterior edges and very slightly pointed posterior edges, without posterior notches. Centrally located are strong, short, and wide rhomboid-shaped keels, highest along their central axis, which transition into cone-like spines **(Figs. 6-A, D; 8-A, D).**

Scales type 15: The remaining dorsal and dorsolateral scales on the neck, located approximately from U26 to U34, are large and wide, with a semi-rectangular shape. These scales do not feature posterior notches, and have centrally located strong, short, and wide rhomboid-shaped keels, highest along their central axis, which transition into cone-like spines **(Figs. 6-A; 8-A).**

Scales type 16: Dorsolaterally to laterally, at *ca.* U24 to *ca.* U34, two pairs of triangle-shaped scales, without any posterior notches, are present. These pairs of scales are similar in size to the neighbouring dorsolateral scales but longer and narrower. These scales have very strong, wide rhomboid-shaped keels, the highest in their central axis, which passes into the strong and long cone-like conspicuous spines with dual structure **(Figs. 6-A; 8-A).**

Scales type 17: Scales on the remaining neck dorsolateral, lateral, ventrolateral and ventral surfaces are wide, semioval-shaped, without posterior notches or only with very shallow notches. These scales have centrally located strong, short and wide rhomboid-shaped keels, the highest in their central axis, which passes into the cone-like spines or, on lateral, ventrolateral and ventral body surfaces gradually taper seamlessly to the hair-like ends **(Figs. 6-E; 8-E).**

Scales type 18: Scales in the trunk region, on the ventral surface, adjacent to the locomotory ciliary bands, are smaller and semitriangular with shallow posterior notches (scales 18). These scales have anteriorly located, short and wide, rhomboid-shaped keels, the highest in their

central axis, which passes into the cone-like spines gradually tapering seamlessly to the hair-like ends **(Figs. 6-E; 8-E).**

Scales type 19: Dorsally to dorsolaterally, on the anterior trunk region (at *ca*. U35-U37), one pair of slightly asymmetrical, triangle-shaped scales is situated. These scales have very shallow and narrow posterior notches and centrally located strong, short and wide rhomboid-shaped keels, the highest in their central axis, which passes into the cone-like spines **(Figs. 6-D; 8-D).**

Scales type 20: Dorsally (from *ca*. U40 to *ca*.U53) and dorsolaterally (from *ca.* U46 to *ca.* U50), triangle shaped scales are located. In the direction towards the widest trunk region, the length to width ratio of these scales changes – scales become longer and narrower. These scales have slightly pointed anterior edges, do not have posterior notches, and have centrally located, strong, short and wide keels, the highest in their central axis, and they pass into the cone-like spines **(Figs. 6-D; 8-D).**

Scales type 21: Beneath this scale type, in the central longitudinal row, from *ca*. U55 to *ca.* U65, two semiheart-shaped scales are located. These scales have pointed and rounded anterior edges and shallow posterior notches **(Fig. 8-F).**

Scales type 22: Laterally to them, on the dorsal, dorsolateral, lateral and ventrolateral trunk surfaces, asymmetrical, semitriangle-shaped scales are located (at *ca*. U52-64). Theses scales have pointed anterior and rounded posterior edges, without any notches. They have strong, centrally located, short and wide, rhomboidal-shaped keels, the highest in their central axis, which pass into the cone-like spines **(Figs. 6-E; 8-E, G).**

Scales type 23: Ventrolaterally to ventrally, on the same trunk region, strong asymmetrical, heart-shaped scales are located. These scales have deep posterior notches and centrally located strong, short and wide rhomboidal-shaped keels, the highest in their central axis, which pass into the cone-like spines **(Fig. 8-E, G).**

Scales type 24: Scales on the trunk adjacent to the locomotory ciliary band on the ventral surface are much smaller and oval. These scales do not have posterior notches and have short and wide rhomboid-shaped keels, the highest in their central axis, and they pass into the cone-like spines tapering seamlessly to the hair-like ends **(Figs. 6- E; 8- C, E, G).**

Scales type 25: Dorsally and dorsolaterally, on the wide trunk region, at *ca*. U63-U68, one pair of semiheart-shaped scales is present. These scales have asymmetrically pointed anterior and straight posterior edges. The keels of these scales are very strong and high, wide and semitriangle in shape. This pair of scales bears the longest conspicuous dorsal and dorsolateral spines **(Figs. 6-D; 8-D).**

Scales type 26: In the same transverse row of scales, on the widest body region (*ca.* U63-U68), laterally to ventrolaterally, one pair of semitriangle-shaped scales is present (scales 26). These scales are asymmetrical and have strongly rounded edges (Figs XX). Their keels are slightly rounded and cover large part of these scales, and they are very strong and high. These keels gradually pass into strongest and longest on the trunk, cone-like, conspicuous spines with dual structure. **(Figs. 6-D; 8-D).**

Scales type 27: Dorsally (from *ca*. U66 to *ca*. U83), dorsolaterally (from *ca*. U68 to *ca*. U77) and laterally (from *ca*. U65 to *ca*. U83), on the posterior trunk region, heart-shaped scales are present. These scales are symmetrical or slightly asymmetrical and they are longer and narrower compared to other trunk scales. In the central part of these scales, strong, short and wide, rhomboid-shaped keels, the highest in their central axis and passing into conspicuous spines are present **(Fig. 8-F).**

Scales type 28: Laterally to these scales, on the lateral and ventrolateral posterior trunk surfaces, semitriangle-shaped scales are located. These scales are distinctly asymmetrical and have rounded irregular posterior edges, with slightly pointed anterior edges. They have centrally located, strong, short and wide rhomboid-shaped keels, the highest in their central axis, and they pass into the cone-like spines **(Fig. 8-G).**

Scales type 29: On the ventrolateral to ventral surfaces, semitriangle-shaped and more asymmetrical and irregular scales are located. These scales have shallow and narrow posterior notches and anteriorly located strong, short and wide, rhomboid-shaped keels, the highest in their central axis, and they pass into the cone-like spines **(Fig. 8-G).**

Scales type 30: Dorsally to dorsolaterally, at *ca.* U77-U81, a pair of large, wide scales, bearing dorsal sensory bristles, are located. These scales are semirhomboid-shaped and constitute the widest body scales. Their anterior edges are asymmetrically, slightly pointed and posterior edges are shallowly notched. In the centre of these scales, two diagonal keels are located. These keels are asymmetrical – the more central ones are longer than those located dorsolaterally. Both of these keels pass into the cone-like spines, more central spines are longer **(Fig. 8-F).**

Scales type 31: Beneath the double-keeled and spined scales, dorsally and dorsolaterally, in the posterior trunk region and the furcal base (at *ca*. U83-U86), one pair of wide scales, semirhomboid-shaped, asymmetrical with irregular edges is located. These scales have strong,

short and wide rhomboid-shaped keels, the highest in their central axis and they pass into the cone-like spines **(Fig. 8-F).**

Scales type 32: Dorsally, at the furcal appendages, one pair of smaller, semitriangle-shaped scales is present (scales 32). The posterior edges of these scales are free and reach to the furcal indentation. These scales have strong, wide rhomboid-shaped keels passing into the cone-like spines reaching the intrafurcal indentation **(Fig. 8-F; 10).**

Scales type 33: Laterally to these centrally located scales, on the furcal base and furcal appendages (at U86-U90), from the dorsal to dorsolateral surfaces, one pair of rhomboid-shaped scales with rounded edges is present. These scales are regular, without any notches, and almost as long as wide. From the centre of these scales, almost along their entire length, strong, high keels run, extending to a triangular shape near the posterior edges and passing into very strong, rigid, slightly bent conspicuous spines reaching the intrafurcal indentation **(Fig. 8-F; 10).**

Scales Types 34: Laterally to these scales, two pairs of scales with parafurcal spines are present. The first of these pairs is located on the lateral surfaces of the furcal base (at U84-U87) and constitutes the second to the posteriormost pair of lateral scales. Scales of this pair are regular, rhomboid-shaped, without any notches, and have centrally located strong, high triangular keels **(Fig. 8-F; 10).**

Scales Types 35: The second of these pairs is located slightly posteriorly on the lateral and ventrolateral surfaces of the furcal base and furcal appendages (at U87-U89) and constitutes the posteriormost pair of the lateral scales. Scales of this pair are also regularly rhomboidal, but their edges are more rounded. From the centre of these scales arise very strong, rounded keels (scales 35). Both of these pairs bear very strong, rigid spines dual in structure **(Fig. 8-G; 10).**

Excluding the five peculiar head dorsal and dorsolateral scales, the size of the scales increases very slightly and gradually from the head towards the widest body region, and then decreases slightly and gradually towards the furcal base. Moreover, the scales of the dorsal, dorsolateral, lateral, ventrolateral and ventral surface of the head, neck and trunk decrease in size slightly and gradually towards the ventral ciliary bands. The scales in the ventral longitudinal rows located closest to the ciliary bands are distinctly smaller compared to other scales and their anterior edge is slightly oriented towards the bands.

Spines

All spines emerge from the keels located in the centre or less often in the anterior part of the scales and have no lateral denticles. In these species, six main types of spines can be distinguished (**Figs. 6-A; 8-A; 11)**.

The first type: dual structured spines on peculiar head scales **(Figs. 6-B; 8-B)**. This type of spines emerges from the rostrally located dorsal and dorsolateral, modified, peculiar head scales (scales 1). Spines emerge seamlessly from the straight, strong keels, in the posterior scale part. The basal segments are strong, short and cone-like, strongly tapering to the pointed ends. Second part of these spines arise from these pointed ends – long, uniformly narrow, hair-like along the entire length. This part of spines is very flexible and similar to the cephalic cilia in structure.

The second type: dual structured spines on dorsal and dorsolateral head scales. These types of spines emerge from the anterior head scales (scales 2, 3, 4, 7 and 9) located directly beneath the peculiar head scales, in total from 12 dorsal, dorsolateral and lateral scales. Scales with this type of spines are located by two in the central longitudinal row, by three in dorsal to dorsolateral longitudinal rows and by two scales located dorsolaterally to laterally in the longitudinal row **(Figs. 6-B; 8-B)**. These spines emerge smoothly from high, strong keels in the central scale part. The basal segments are strong, short and cone-like, strongly tapering to the pointed ends. The second part of these spines arise from these pointed ends – long, uniformly narrow, hair-like along the entire length.

The third type: cone-like spines tapering to the pointed ends. This is the main type of spines emerging from the head, neck and anterior trunk scales on the dorsal and dorsolateral surfaces and from the dorsal, dorsolateral, lateral and ventrolateral trunk surfaces. These spines arise from the strong, high keels in the centre of scales; they are straight, cone-like, and basally thick, then gradually tapering to the pointed ends **(Figs. 6-B; 8-B; 11-B)**. The length of these spines gradually increases from the head towards the widest trunk region, and then they become clearly shorter and decrease in length to the furcal base. Moreover, the length of the spines gradually decreases from the dorsal to dorsolateral surface on the head, neck and anterior trunk region, and from the dorsal surface to dorsolateral, lateral and ventrolateral surfaces on the trunk.

The fourth type: dorsolateral to ventrolateral long conspicuous spines of dual structure. This type of spines arise from two pairs of the neck dorsolateral to lateral scales (scales 16), trunk lateral to ventrolateral scales (scales 26), second to the posteriormost pair of the dorsolateral to lateral scales (scales 34), and the posteriormost lateral to the ventrolateral scales (scales 35). This spine type is strong, long and almost straight, formed of two segments **(Figs.**

6-D; 8-D). The first, much longer parts are cone-like, basally thick tapering to the pointed ends. The second part arising from their pointed ends is short, uniformly narrow and hair-like. These spines are significantly stronger and longer than the remaining body spines; the pair of the lateral to ventrolateral trunk scale spines is the longest conspicuous spine pair.

The fifth type: dorsal to dorsolateral long conspicuous spines with pointed ends. This type of spines arises from one pair of dorsal to dorsolateral trunk scales (scales 25) and one pair from the dorsal to dorsolateral scales of the furcal appendages (scales 33). These conspicuous spines are long and strong, significantly longer and stronger than the remaining one-segmented spines; they are rigid and slightly basally bent, thick and gradually tapering to their pointed ends.

The sixth type: cone-like spines gradually tapering seamlessly to the hair-like ends. These types of spines arise laterally, ventrolaterally and ventrally from the scales on the head, neck and anterior trunk region and ventrally from the scales on the central and posterior trunk regions. This type of spines arising from strong, high keels are basally straight and thick, cone-like, then strongly curved and gradually tapering seamlessly to the thin hair-like ends. These spines are longer than the cone-like shaped spines and their length gradually increases from the head towards the anterior trunk region on the lateral and ventrolateral surfaces, and to the widest trunk region on the ventral surface.

Dorsal sensory bristles

This species has two pairs of dorsal sensory bristles with a dual structure **(Fig. 6-D, F)**. The basal segment of each dorsal sensory bristle is significantly thicker, straighter and with pointed, cone-like ends, from which a very thin and flexible bristle part emerges. The first pair of sensory bristles is located on the dorsolateral surface of the neck at U24 and emerges from a distinct, rounded, spherical papilla. The second, posterior pair of sensory bristles arises from double-keeled scales (scales 30) located dorsally to dorsolaterally at U77–U81 in the posterior trunk region.

Ventral ciliary bands and ventral interciliary field

On the ventral surface, the longitudinal ciliary bands begin to adhere at U10 to the posterior edges of the additional hypostomium plates and run back to U90 **(Figs. 6-E;8-E, G).** The ciliary bands are narrow and slightly wider at the head than in other parts of the body. The scales of the ventral interciliary field are arranged in four parallel longitudinal rows that extend

the entire length, up to the region of the furca. All scales of the ventral interciliary field have outwardly curved edges and are partially fused into the cuticle. All scales are oval in shape, possessing short keels with small double-structured spines. They are unilobulate, with regular edges that exhibit minimal variation in size.

Internal morphology

The pharynx (from U2 to U33) is cylindrical and expands slightly towards its posterior end, without marked anterior and posterior dilatations **(Fig. 9-A).** The pharynx is connected through the pharyngeal-intestinal junction to the straight intestine. The pharyngeal-intestinal junction is clearly demarcated, short and wide (U34). The intestine does not have a separate anterior section differing in form and morphology. The X-organ of this species (observed in one specimen) is large and located at U83-U87 near the terminal part of the intestine. It is bilobed, built from two round-shaped extensions and connected by a thinner band located behind the intestine at the ventral side. The extensions have a granular appearance, while the cellular bridge connecting the extensions have a smooth and homogeneous structure. The sperm packets of this species are not observed.

Remarks. There is a variation between individuals of this species with regard to the size of the scales and the arrangement of the spines, where individuals from São Carlos have been found with additional conspicuous arrangements of spines, not seen in other specimens (Kisielewski, 1991). This species exhibits several distinguishing morphological features within the genus. The ventral head region is characterized by a broad and large hypostomium, accompanied by two additional plates positioned below it, with their upper tips directed outward. The interciliary ventral region is adorned with numerous small, spined scales arranged in four distinct columns extending to the furca region—a unique trait not observed in other species of the genus.

spec. nov. 2

(Fig.1, 3, 5, 7, 12, 13)

Localities: São Bernardo do Campo, SP; São João da Boa Vista, SP.

Material studied

Holotype

BRAZIL • adult (photographs, the specimen was destroyed); State of São Paulo.

Paratypes

• Five adults collected associated with roots of Ecchornia sp. on October 15th, 2017, in Billings Reservoir, São Bernardo do Campo municipality, São Paulo state, Brazil.

• Four specimens collected in substrate associated with roots of aquatic plants on February 13th, 2018, in an urban lake in São João da Boa Vista municipality, São Paulo state, Brazil.

Diagnosis. Slender body measuring 163-188 μm in lenght. Head complex with reduced cephalion and two pairs of pleurae and five modified, peculiar cephalic scales. Peculiar cephalic scales large, clearly visible in the head outline. Hypostomium large, strongly reinforced and kidney-shaped with horn-like protuberances anterolaterally. A pair of additional symmetrical plates is located posterior to the main hypostomal plate. Ocellar granules absent. Mouth ring wide, strongly reinforced with long internal lamellae and two cuticular teeth arising from the anterior pharynx region. Suboral bristles absent. Scales large and thick, partially merged in cuticle, with anterior edges extraverted. The scales are distributed in a total of 11 longitudinal rows (3D + 2DL + 2L + 2VL + 2V) with 16 scales in the central row differing morphologically in the head, neck and trunk regions., differing morphologically in the head, neck and trunk regions; scales semi-triangle, semi-rhomboidal to rhomboidal and heart-like, located close to each other, juxtaposed or overlapping. Spines rigid and cone-like; lateral, spines of the ventrolateral and ventral head, neck and anterior trunk regions strongly curved; remaining spines mainly straight; all without lateral denticles. Peculiar head scale spines, head anteriormost scale spines, two pairs of neck lateral scale spines, one pair of lateral trunk scale spines and two pairs of furcal base and furcal appendages scale spines dual in structure – basal segment significantly thicker and cone-like; the second part uniformly thin and flexible. The neck region has scales without spines, and this is a defining feature. Spines on lateral, ventrolateral and ventral surfaces of the head, neck and anterior trunk region and on ventral surfaces of the central and posterior trunk regions gradually tapering towards hair-like ends. Spine length gradually and slightly increases from head towards the widest trunk region, after which it shortens to the furcal base. Two pairs of neck lateral spines, one pair of trunk dorsal spines, one pair of trunk lateral spines and two pairs of parafurcal and one pair of intrafurcal spines significantly longer than those remaining spines. The entire ventral intraciliary field covered with small, irregular scales having a spine. Five pairs of ventral interciliary field terminal scales. Pharynx cylindrical without anterior and posterior dilatations. Intestine straight without anterior section differing in form and morphology.

Description

Habitus

Its head possesses a diameter larger than that of the neck, yielding a distinct appearance of constriction at the neck level **(Figs. 12-A, B; 13)**. The head has a diameter of 19µm, whereas the neck measures 12µm. From the neck (U25) to the widest area (U60), the body gradually increases its diameter, transitioning from 12µm to 18µm. Beyond this region, the trunk gradually begins to taper until reaching the furca region. The furca's base (U88) is short and continuous, gradually extending into relatively long furcal appendages (larger than those of species 1) **(Figs. 7-A, D; 11-C, D; 12- C, D)**. The indentation of the furca (U89) takes on a parabolic shape. The branches of the furca are slightly separated and project outward. The adhesive tubes are straight and gradually narrow toward their slightly rounded ends **(Figs. 7-A, D; 11-C, D; 13)**.

Head

The head is large and semi-rectangular, featuring modified cephalic plates. The cephalion (U1) is reduced and is present only at the top of the head, adhering to the head along its entire length **(Figs. 7-A; 12-A, E, F).** It is positioned anteriorly to the subterminal mouth, with its posterior margin free. Laterally on this large plate, a pair of pleurae are located. The hypopleurae (U5-U10) are large and convex **(Figs. 12-E, F)**. They cover the lateral, ventrolateral, and ventral faces in such a way that their major portion is situated on the ventral side, demarcated along the head's contour. The cephalic plates consist of five modified scales **(Figs. 12-A, E, F; 13-A).** These scales (U1-10) are large, thick, and positioned on the dorsal and dorsolateral surfaces of the head. Their anterior edges overlap with the posterior edge of the cephalion, while the dorsolateral pair adheres laterally to the lateral edges of the hypopleurae. These distinct scales are semi-triangular in shape and intimately adhere to each other, with the pair of scales adjacent to the central peculiar scale overlapping it. The presence of these scales forms the main shape and contour of the head. The hypostomium (U3) is elongated and kidney-shaped, bearing two anteriorly located horn-like protuberances **(Figs. 12-B; 13-C).** A pair of additional symmetrical plates is situated posteriorly to the main hypostomal plate. These plates (U5 - U8) are wide, with thick edges. The anterior lateral edges are more pronounced, thus serving as a distinguishing feature. A pair of sensory ciliary tufts (U1) is present **(Figs. 12-B; 13-C).** They emerge almost terminally, between the lateral cephalic edge and the anterior edge of the hypopleurae. Each tuft consists of five cilia. The cilia are slender and relatively short. Ocellar granules are absent. The oral ring is located terminally at U1; it is wide and exhibits long, robust,

and complex reinforcements **(Figs. 12-A, B, E, F; 13-B, C).** The reinforcements comprise an inner ring containing long segments and an outer ring formed by strong granular reinforcements overlapping the inner ring. Within the oral ring, there are long, wide, oval-shaped lamellae. Suboral bristles are absent.

Scales

The entire body is covered by large and thick scales, unilobulate and partially fused to the cuticle, with curved anterior edges **(Figs. 7; 12; 13)**. The scales are arranged in a total of 11 longitudinal rows (3D + 2DL + 2L + 2VL + 2V) with 16 scales in the central row, exhibiting morphological differences in the head, neck, and trunk regions. The longitudinal rows of scales, including the distinctive anterior head scales, run straight and are arranged almost in parallel from the anterior head region to the base of the furca. The scales are closely spaced. The edges of most scales meet and overlap, particularly those located dorsally, dorsolaterally, and laterally; only a few scales have overlapping edges on the ventrolateral and ventral surfaces **(Fig. 7)**.

Scales type 1: At the rostral part of the head, dorsally and dorsolaterally, five anterior scales are situated. These distinctive scales are modified and consist of a sort of cephalic plate **(Fig. 12-A, E; 13-A,** see 'Head' section). These scales are large and thick, elongated, and triangular without posterior notches. These unique scales have long keels, extending from the edges of the anterior to the posterior scales. Near the edges of the posterior scales, the keels transform into double-structured spines.

Scales type 2: Below these distinctive scales (around U9), a pair of large and wide scales is located adjacent to their posterior edges. These scales are shaped as reverse semitriangles and lack spines, only having protrusions located at the center **(Fig. 12-A, E; 13-A).**

Scales type 3: Beneath these scales (around U11), a single semitriangle-shaped scale is located on the longitudinal dorsal central line of scales **(Fig. 12-A, E; 13-A).** This scale has a slightly pointed anterior edges, without posterior notches. It lacks spine and has protrusions located at the center.

Scales type 4: Laterally to these scales, on the dorsolateral and lateral surfaces of the head (around U10), a pair of rhomboid-shaped scales is present. These scales are adjacent and overlap with the posterior edges of the lateral pair of distinctive scales and the overlapping

edges of the hypopleura **(Fig. 12-A, E; 13-A).** These scales have strong, wide, short keels in the center, tallest along their central axis, transitioning into double-structured spines.

Scales type 6: Lateral to this pair, on the ventrolateral head surface (around U10), a pair of small rhomboid-shaped scales is located. These scales are adjacent to the posterior edges of the hypopleura and the overlapping edges of the lateral scales **(Figs. 7-C; 12-B, F; 13-C).** These scales have strong, short, wide and rhomboid-shaped keels situated anteriorly that pass into the cone-like spines gradually tapering seamlessly to the hair-like ends.

Scales type 7: Two pairs of large, strongly rounded semitriangle-shaped scales are present dorsally, dorsolaterally, and laterally (around U10-U15) as subsequent scales of the head. These scales have shallow posterior notches and lack spines, only having protrusions located at the center **(Figs. 12-A, E; 13-A).**

Scales type 8: One pair of asymmetrical, pentagonal-shaped scales is situated laterally to these scales, on the ventrolateral to ventral head surface. These scales do not have posterior notches and have strong wide rhomboid-shaped keels, the highest in their central axis, and they pass into the cone-like spines tapering seamlessly to the hair-like ends **(Figs. 12-F; 13-C).**

Scales type 9: The remaining head and anterior neck dorsal, dorsolateral and lateral scales (from *ca.* U13 to *ca.* U23) are wide and oval-shaped. These scales have shallow and narrow posterior notches and lack spines, only having protrusions located at the center **(Fig. 12-E)**

Scales type 10: To support potential inferences and homologous comparisons within the genus, this specific trait is not assigned for this species, as no scales resembling those found in the other species could be identified.

Scales type 11: On the ventrolateral to ventral surfaces, four pairs of asymmetrical, irregular scales are located. These scales do not have posterior notches and have strong wide rhomboid-shaped keels, the highest in their central axis, and they pass into the cone-like spines tapering seamlessly to the hair-like ends **(Figs. 12-B, F; 13-C).**

Scales Types 12: The first four pairs of scales adjacent to the locomotory ciliary band on the ventral surface are much smaller and oval in shape. These scales do not have posterior notches and have short and wide rhomboid-shaped keels, the highest in their central axis, and they pass into the cone-like spines tapering seamlessly to the hair-like ends **(Fig. 12-F).**

Scales type 13: Dorsally on the neck, at ca. U23-U25, one pair of asymmetrical, semitriangular-shaped scales is located. The lateral edge of these scales is oriented to the lateral body surface; they are shorter and more oval. These scales have shallow, unicentrally located posterior notches and short and wide rhomboid-like keels, the highest in their central axis, and they pass into the strong cone-like spines, located directly above the posterior notches **(Figs. 12-A; 13-A).**

Scales type 14: On the neck, beneath this pair of scales, dorsally to dorsolaterally, another pair of asymmetrical scales is located (approximately at U28-U31). These scales are semitriangular in shape but wider and larger than the scales above. They have asymmetrical pointed anterior edges and very slightly pointed posterior edges, without posterior notches. Centrally located are strong, short, and wide rhomboid-shaped keels, highest along their central axis, which transition into cone-like spines **(Figs. 12-A; 13-A).**

Scales type 15: The remaining dorsal and dorsolateral scales on the neck, located approximately from U26 to U34, are large and wide, with a triangular shape. These scales feature very shallow posterior notches. Centrally located are strong, short, and wide rhomboid-shaped keels, highest along their central axis, which transition into cone-like spines **(Figs. 12-A; 13-A).**

Scales type 16: Dorsolaterally to laterally, at *ca.* U24 to *ca.* U34, two pairs of semitrangle-shaped scales, without any posterior notches, are present. These pairs of scales are similar in size to the neighbouring dorsolateral scales but longer and narrower. These scales have very strong, wide rhomboid-shaped keels, the highest in their central axis, which passes into the strong and long cone-like conspicuous spines with dual structure **(Figs. 7-A, B; 12-A, C).**

Scales type 17: Scales on the remaining neck dorsolateral, lateral, ventrolateral and ventral surfaces are wide, semioval-shaped, without posterior notches or only with very shallow notches. These scales have centrally located strong, short and wide rhomboid-shaped keels, the highest in their central axis, which passes into the cone-like spines or, on lateral, ventrolateral and ventral body surfaces gradually taper seamlessly to the hair-like ends **(Figs 7-A, C; 12-C).**

Scales type 18: Scales in the neck region, on the ventral surface, adjacent to the locomotory ciliary bands, are smaller and semitriangular with shallow posterior notches (scales 18). These scales have anteriorly located, short and wide, rhomboid-shaped keels, the highest in their central axis, which passes into the cone-like spines gradually tapering seamlessly to the hair-like ends **(Figs 7-A, C; 12-C).**

Scales type 19: Dorsally to dorsolaterally, on the anterior trunk region (at *ca.* U35-U37), one pair of slightly asymmetrical, triangle-shaped scales is situated. These scales have very shallow and narrow posterior notches and centrally located strong, short and wide rhomboid-shaped keels, the highest in their central axis, which passes into the cone-like spines **(Figs. 12-A. 13-A).**

Scales type 20: Dorsally (from *ca*. U40 to *ca.* U53) and dorsolaterally (from *ca.* U46 to *ca.* U50), triangle-shaped scales are located. In the direction towards the widest trunk region, the length to width ratio of these scales changes – scales become longer and narrower **(Figs. 12-A. 13-A).** These scales have pointed anterior edges, posterior notches and centrally located, strong, short and wide keels, the highest in their central axis, and they pass into the cone-like spines.

Scales type 21: Beneath this scale type, in the central longitudinal row, from *ca*. U55 to *ca.* U65, two semiheart-shaped scales are located (scales 21). These scales have pointed anterior edges and shallow posterior notches **(Figs. 12-A. 13-A).**

Scales type 22: Laterally to them, on the dorsal, dorsolateral, lateral and ventrolateral trunk surfaces, asymmetrical, semitriangle-shaped scales are located (at *ca*. U52-64). Theses scales have pointed anterior and rounded posterior edges with notches. They have strong, centrally located, short and wide, rhomboidal-shaped keels, the highest in their central axis, which pass into the cone-like spines **(Figs. 7-A, D; 12-B).**

Scales type 23: Ventrolaterally to ventrally, on the same trunk region, strong asymmetrical, triangular-shaped scales are located. These scales have deep posterior notches and centrally located strong, short and wide rhomboidal-shaped keels, the highest in their central axis, which pass into the cone-like spines **(Figs. 7-A, D; 12-B).**

Scales type 24: Ventral scales, adjacent to the locomotory ciliary bands on the neck region, are asymmetrical and lack spines, featuring only central protrusions. In the mid-trunk region, the scales are slightly asymmetrical, hexagonal in shape, and possess rhomboid-shaped keels situated anteriorly, which transition into cone-like spines **(Figs. 12-B).** Towards the furca, the scales become progressively smaller and more rounded, also having cone-like spines.

Scales type 25: Dorsally and dorsolaterally, on the wide trunk region, at *ca*. U63-U68, one pair of semiheart-shaped scales is present. These scales have asymmetrically pointed anterior and straight posterior edges. The keels of these scales are very strong and high, wide and

semitriangle in shape. This pair of scales bears the longest conspicuous dorsal and dorsolateral spines **(Figs. 7-D, E; 12-A; 13-A).**

Scales type 26: In the same transverse row of scales, on the widest body region (*ca.* U63-U68), laterally to ventrolaterally, one pair of semitriangle-shaped scales is present (scales 26). These scales are asymmetrical and have strongly rounded edges (Figs XX). Their keels are slightly rounded and cover large part of these scales, and they are very strong and high. These keels gradually pass into strongest and longest on the trunk, cone-like, conspicuous spines with dual structure **(Figs. 7-D, E; 12-A; 13-A).**

Scales type 27: Dorsally (from *ca*. U66 to *ca*. U83), dorsolaterally (from *ca*. U68 to *ca*. U77) and laterally (from *ca*. U65 to *ca*. U83), on the posterior trunk region, heart-shaped scales are present. These scales are symmetrical or slightly asymmetrical and they are longer and narrower compared to other trunk scales. In the central part of these scales, strong, short and wide, rhomboid-shaped keels, the highest in their central axis and passing into conspicuous spines are present **(Figs. 7-D, E; 12-A; 13-A).**

Scales type 28: Laterally to these scales, on the lateral and ventrolateral posterior trunk surfaces, semitriangle-shaped scales are located. These scales are distinctly asymmetrical and have rounded irregular posterior edges, with slightly pointed anterior edges. They have centrally located, strong, short and wide rhomboid-shaped keels, the highest in their central axis, and they pass into the cone-like spines **(Figs. 7-D, E; 12-B; 13-C).**

Scales type 29: On the ventrolateral to ventral surfaces, semitriangle-shaped and more asymmetrical and irregular scales are located. These scales have shallow and narrow posterior notches and anteriorly located strong, short and wide, rhomboid-shaped keels, the highest in their central axis, and they pass into the cone-like spines **(Figs. 7-D; 12- C).**

Scales type 30: Dorsally to dorsolaterally, at *ca*. U77-U81, a pair of large, wide scales, bearing dorsal sensory bristles, are located. These scales are semirhomboid-shaped and constitute the widest body scales. Their anterior edges are asymmetrically, slightly pointed and posterior edges are shallowly notched. In the centre of these scales, two diagonal keels are located. These keels are asymmetrical – the more central ones are longer than those located dorsolaterally. Both of these keels pass into the cone-like spines, more central spines are longer **(Fig. 12 – A).**

Scales type 31: Beneath the double-keeled and spined scales, dorsally and dorsolaterally, in the posterior trunk region and the furcal base (at *ca*. U83-U86), one pair of wide scales, semirhomboid-shaped, asymmetrical with irregular edges is located. These scales have strong, short and wide rhomboid-shaped keels, the highest in their central axis and they pass into the cone-like spines **(Fig. 12 – A).**

Scales type 32: Dorsally, at the furcal appendages, one pair of smaller, semitriangle-shaped scales is present. The posterior edges of these scales are free and reach to the furcal indentation. These scales have strong, wide rhomboid-shaped keels passing into the cone-like spines reaching the intrafurcal indentation **(Fig. 12 – A).**

Scales type 33: Laterally to these centrally located scales, on the furcal base and furcal appendages (at U86-U90), from the dorsal to dorsolateral surfaces, one pair of rhomboid-shaped scales with rounded edges is present. These scales are regular, without any notches, and almost as long as wide. From the centre of these scales, almost along their entire length, strong, high keels run, extending to a triangular shape near the posterior edges and passing into very strong, rigid, slightly bent conspicuous spines reaching the intrafurcal indentation **(Fig. 12 – A).**

Scales Types 34: Laterally to these scales, two pairs of scales with parafurcal spines are present. The first of these pairs is located on the lateral surfaces of the furcal base (at U84-U87) and constitutes the second to the posteriormost pair of lateral scales. Scales of this pair are regular, rhomboid-shaped, without any notches, and have centrally located strong, high triangular keels **(Fig. 12 – A, C, D).**

Scales Types 35: The second of these pairs is located slightly posteriorly on the lateral and ventrolateral surfaces of the furcal base and furcal appendages (at U87-U89) and constitutes the posteriormost pair of the lateral scales. Scales of this pair are also regularly rhomboidal, but their edges are more rounded. From the centre of these scales arise very strong, rounded keels (scales 35). Both of these pairs bear very strong, rigid spines dual in structure **(Fig. 12 – A, C, D).**

Excluding the five peculiar dorsolateral and dorsolateral head scales, the size of scales increases very slightly and gradually from the head towards the widest part of the body, and then decreases gently and gradually towards the base of the furca. The scales in the ventral longitudinal rows located closest to the ciliary bands are notably smaller compared to other scales.

Spines

All spines emerge from the keels located in the center or less often in the anterior part of the scales and have no lateral denticles. In these species, six main types of spines can be distinguished.

The first type: dual structured spines on peculiar head scales. This type of spines emerges from the rostrally located dorsal and dorsolateral, modified, peculiar head scales (scales 1). Spines emerge seamlessly from the straight, strong keels, in the posterior scale part. The basal segments are strong, short and cone-like, strongly tapering to the pointed ends. Second part of these spines arise from these pointed ends – long, uniformly narrow, hair-like along the entire length. This part of spines is very flexible and similar to the cephalic cilia in structure.

The second type: The second type of spines found in other species of the genus is absent in scales 2, 3, 4, and 9, which are bare and only exhibit a central prominence. Nevertheless, the dorsolaterally located scale 7 bears dual structured spines. These spines emerge smoothly from high, strong keels in the central scale part. The basal segments are strong, short and cone-like, strongly tapering to the pointed ends.

The third type: cone-like spines tapering to the pointed ends. This is the main type of spines emerging from anterior trunk scales on the dorsal and dorsolateral surfaces and from the dorsal, dorsolateral, lateral and ventrolateral trunk surfaces. These spines arise from the strong, high keels in the center of scales; they are straight, cone-like, and basally thick, then gradually tapering to the pointed ends. The length of these spines gradually increases from the head towards the widest trunk region, and then they become clearly shorter and decrease in length to the furcal base. Moreover, the length of the spines gradually decreases from the dorsal to dorsolateral surface on the head, neck and anterior trunk region, and from the dorsal surface to dorsolateral, lateral and ventrolateral surfaces on the trunk.

The fourth type: dorsolateral to ventrolateral long conspicuous spines of dual structure. This type of spines arise from two pairs of the neck dorsolateral to lateral scales (scales 16), trunk lateral to ventrolateral scales (scales 26), second to the posteriormost pair of the dorsolateral to lateral scales (scales 34), and the posteriormost lateral to the ventrolateral scales (scales 35). This spine type is strong, long and almost straight. The first, much longer parts are cone-like, basally thick tapering to the pointed ends. The second part arising from their pointed ends is short, uniformly narrow and hair-like. These spines are significantly stronger and longer than the remaining body spines; the pair of the lateral to ventrolateral trunk scale spines is the longest conspicuous spine pair.

The fifth type: dorsal to dorsolateral long conspicuous spines with pointed ends. This type of spines arises from one pair of dorsal to dorsolateral trunk scales (scales 25) and one pair from the dorsal to dorsolateral scales of the furcal appendages (scales 33). These conspicuous spines are long and strong, significantly longer and stronger than the remaining one-segmented spines; they are rigid and slightly basally bent, thick and gradually tapering to their pointed ends.

The sixth type: cone-like spines gradually tapering seamlessly to the hair-like ends. These types of spines arise laterally, ventrolaterally and ventrally from the scales on the head, neck and anterior trunk region and ventrally from the scales on the central and posterior trunk regions. This type of spines arising from strong, high keels are basally straight and thick, cone-like, then strongly curved and gradually tapering seamlessly to the thin hair-like ends. These spines are longer than the cone-like shaped spines and their length gradually increases from the head towards the anterior trunk region on the lateral and ventrolateral surfaces, and to the widest trunk region on the ventral surface.

Dorsal sensory bristles

This species has two pairs of dorsal sensory bristles with a dual structure. The basal segment of each dorsal sensory bristle is significantly thicker, straighter and with pointed, cone-like ends, from which a very thin and flexible bristle part emerges. The first pair of sensory bristles is located on the dorsolateral surface of the neck at U24 and emerges from a distinct, rounded, spherical papilla. The second, posterior pair of sensory bristles arises from double-keeled scales (scales 30) located dorsally to dorsolaterally at U77–U81 in the posterior trunk region.

Ventral ciliary bands and ventral interciliary field

On the ventral surface, the longitudinal ciliary bands begin to adhere at U10 to the posterior edges of the additional hypostomium plates and run back to U90**.** The ciliary bands are narrow and slightly wider at the head than in other parts of the body. The scales of the ventral interciliary field are arranged in four parallel longitudinal rows that extend the entire length, up to the region of the furca. All scales of the ventral interciliary field have outwardly curved edges and are partially fused into the cuticle. All scales are oval in shape, possessing short keels with small double-structured spines. They are unilobulate, with regular edges that exhibit minimal variation in size.

Internal morphology

The pharynx (from U2 to U33) is cylindrical and expands slightly towards its posterior end, without marked anterior and posterior dilatations**.** The pharynx is connected through the pharyngeal-intestinal junction to the straight intestine (running from U34 to U88). The pharyngeal-intestinal junction is clearly demarcated, short and wide (U34). The intestine does not have a separate anterior section differing in form and morphology. The X-organ of this species (observed in one specimen) is large and located at U83-U87 near the terminal part of the intestine. It is bilobed, built from two round-shaped extensions and connected by a thinner band located behind the intestine at the ventral side. The extensions have a granular appearance, while the cellular bridge connecting the extensions have a smooth and homogeneous structure. The sperm packets of this species are not observed.

Remarks. The configurations and dimensions of the scales located on the pharyngeal portion of the ventral interciliary field exhibit significant variability and irregularity, with each examined specimen displaying a unique pattern. This species is distinguished from others in the genus primarily by the absence of spines on both the dorsal and ventral regions of the neck, a characteristic that makes it unique within the group. Additionally, its conspicuous dorsal spines are slightly larger compared to those of other species.

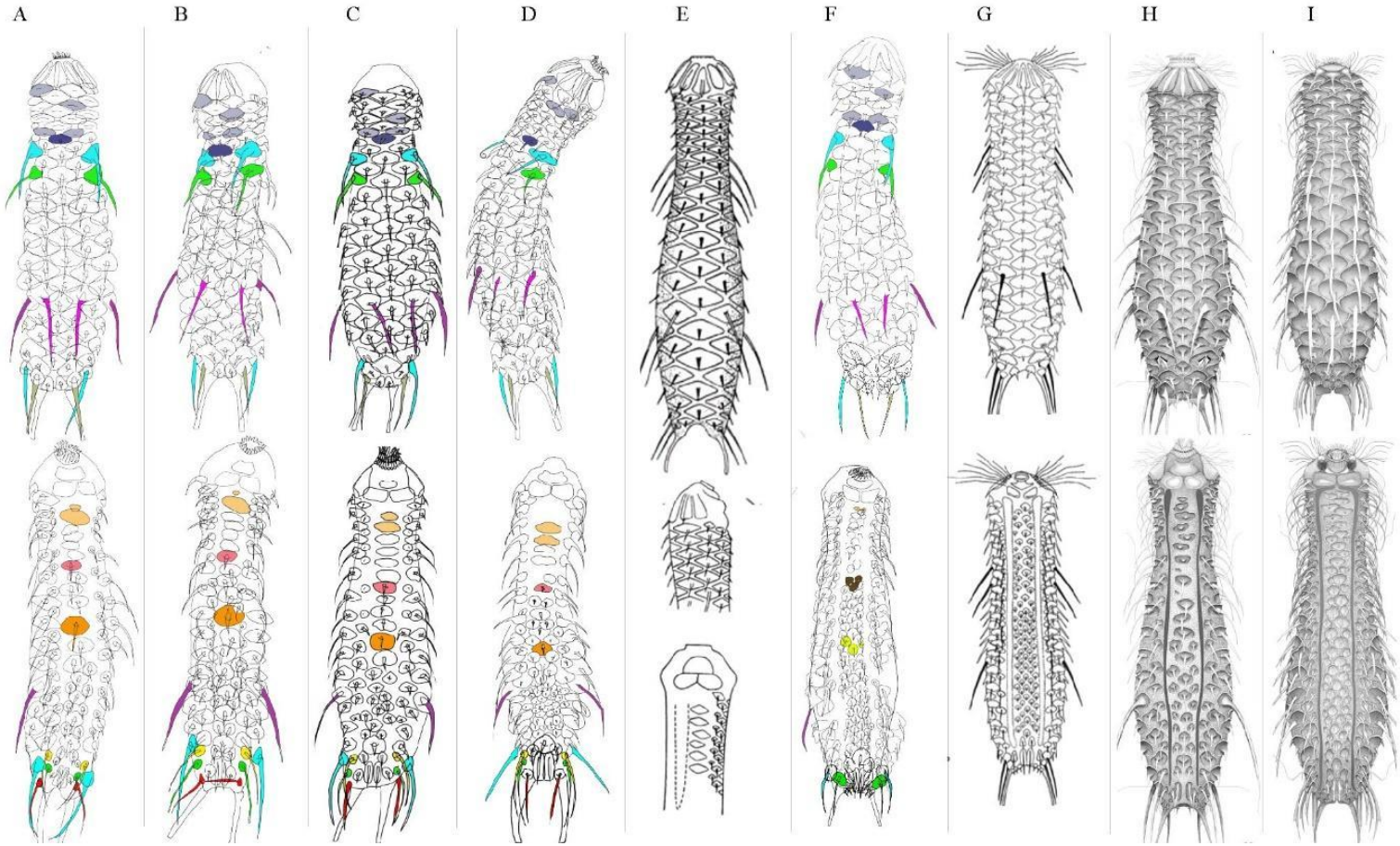

Figure 1. Drawing of specimens and their respective locations. Homologous structures in brazilian species highlighted ~~in~~with the color. **A** São João da Boa Vista/SP **B** São Bernardo dos Campos/SP **C** Paulínia/SP, **D** Mucugê/BA, **E** Bulgária (Screenshot of the original image from Valkanov's publication, 1937) **F** Diamantina/MG **G** São Carlos/SP (Screenshot of the original image from Kisielewski publication, 1991) **H-I** Islândia (Illustration by Polish researcher Malgorzata Kolicka).

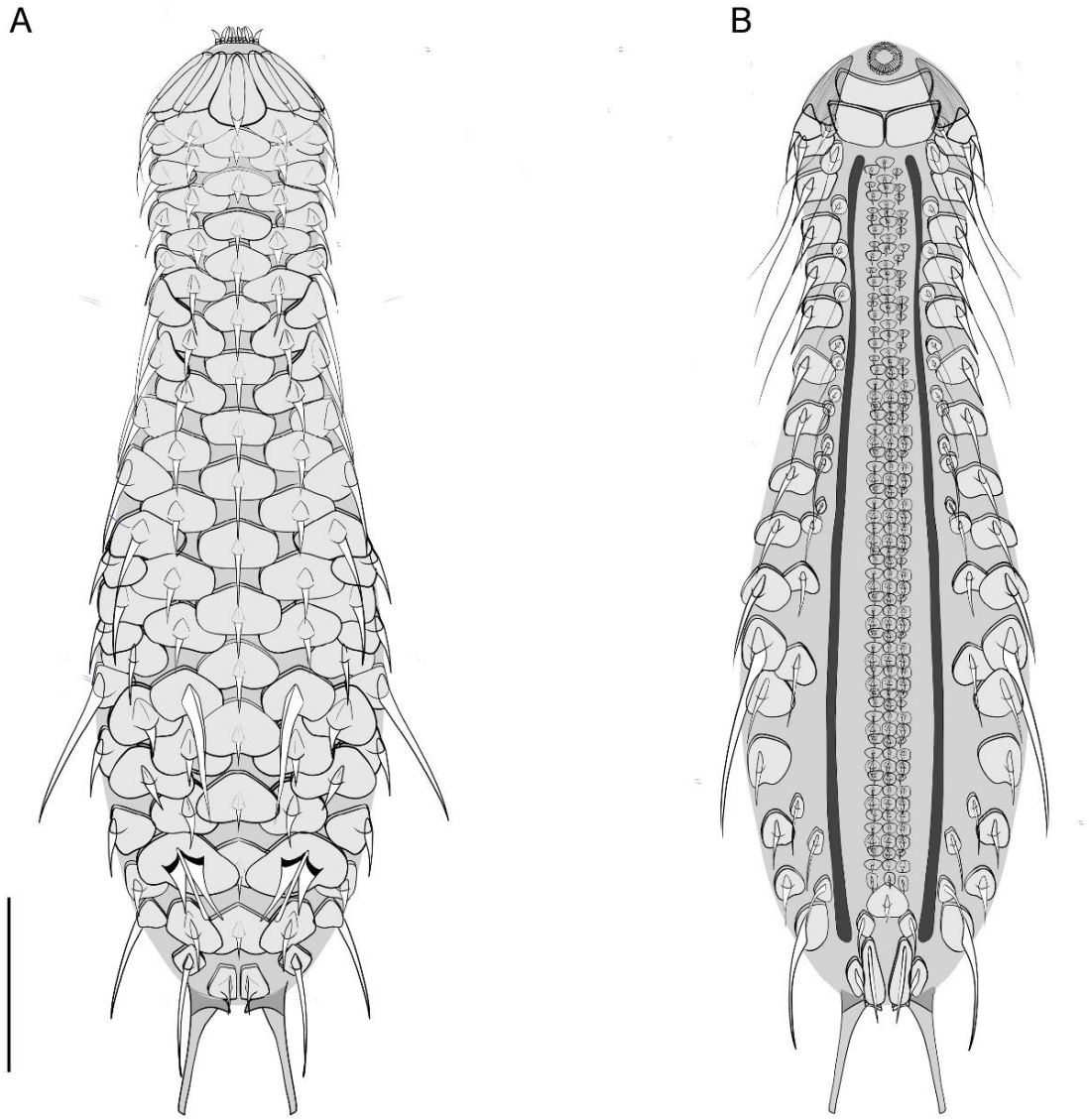

Figure 2. Schematic drawing of nov.sp.1 **A** Dorsal View ; **B** Ventral View. **Scale bar**: 50 µm

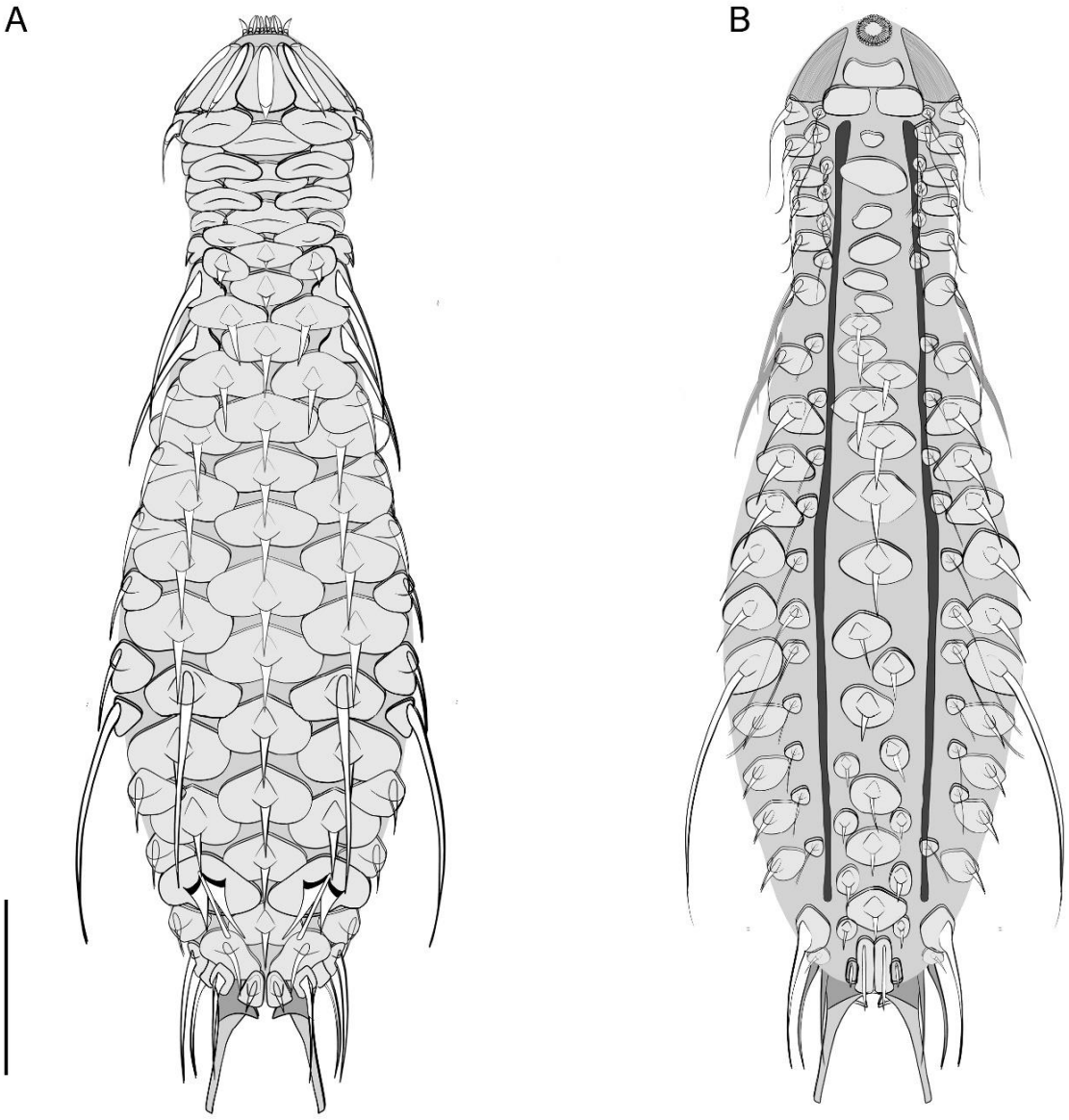

Figure 3. Schematic drawing of nov.sp.1 **A** Dorsal View; **B** Ventral View. **Scale bar**: 50 µm

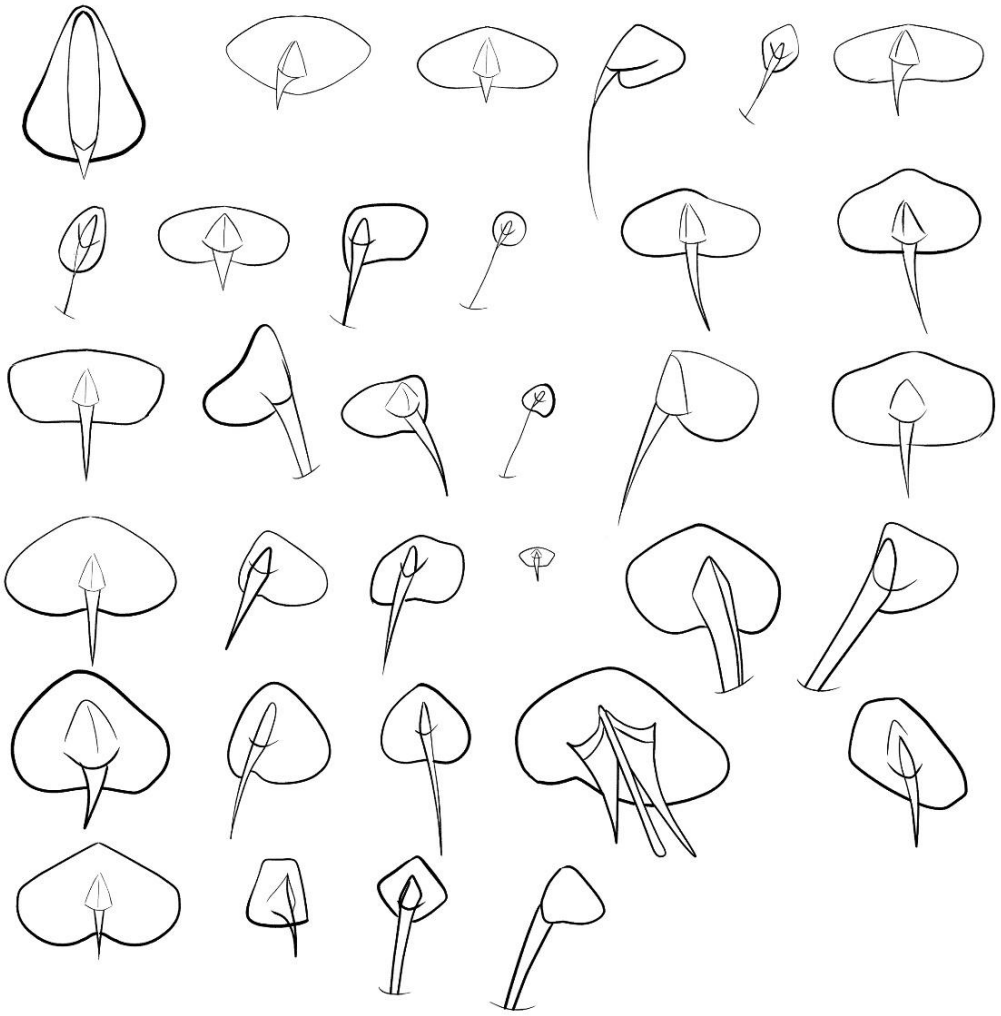

Figure 4. Schematic drawing of the scales of nov.sp. 1, arranged in ascending order from 1 to 33.

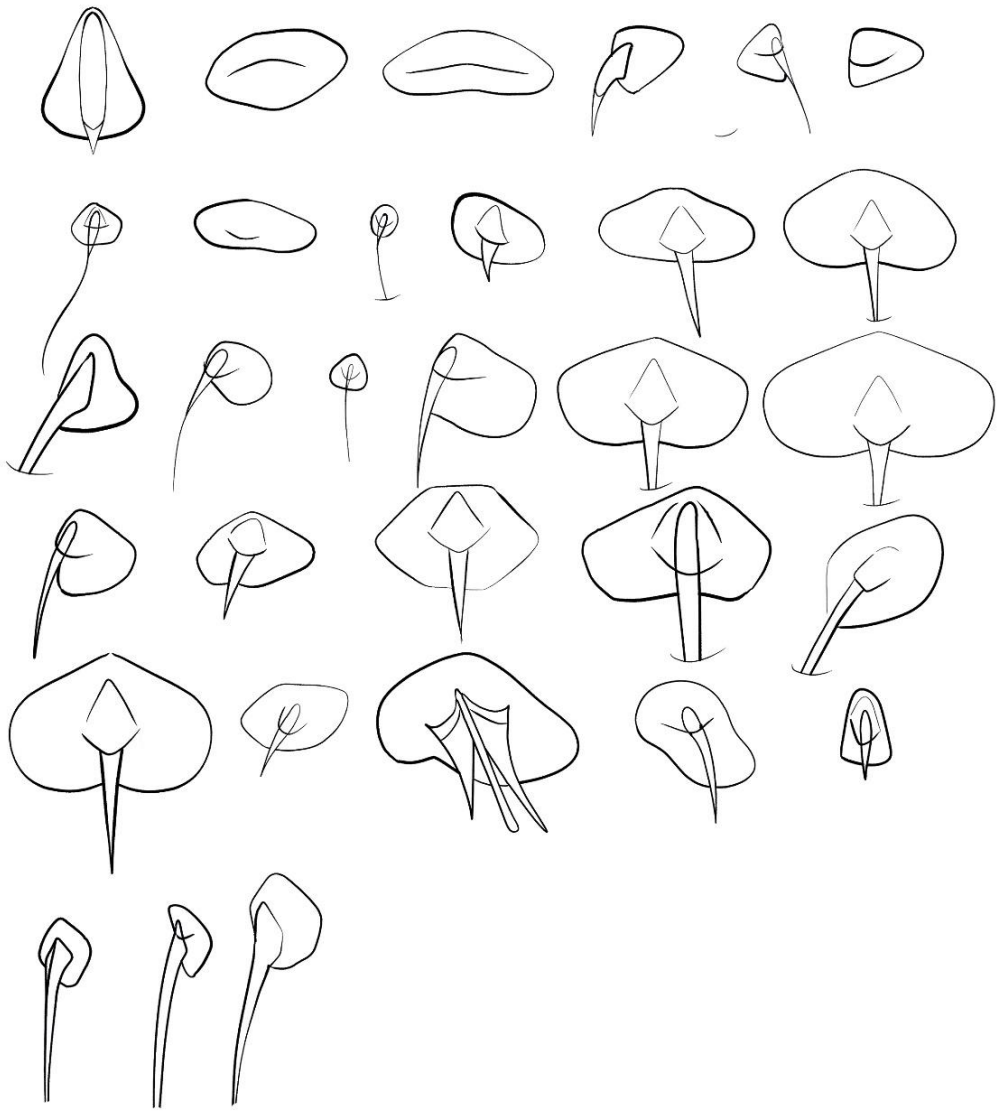

Figure 5. Schematic drawing of the scales of nov.sp. 2, arranged in ascending order from 1 to 33.

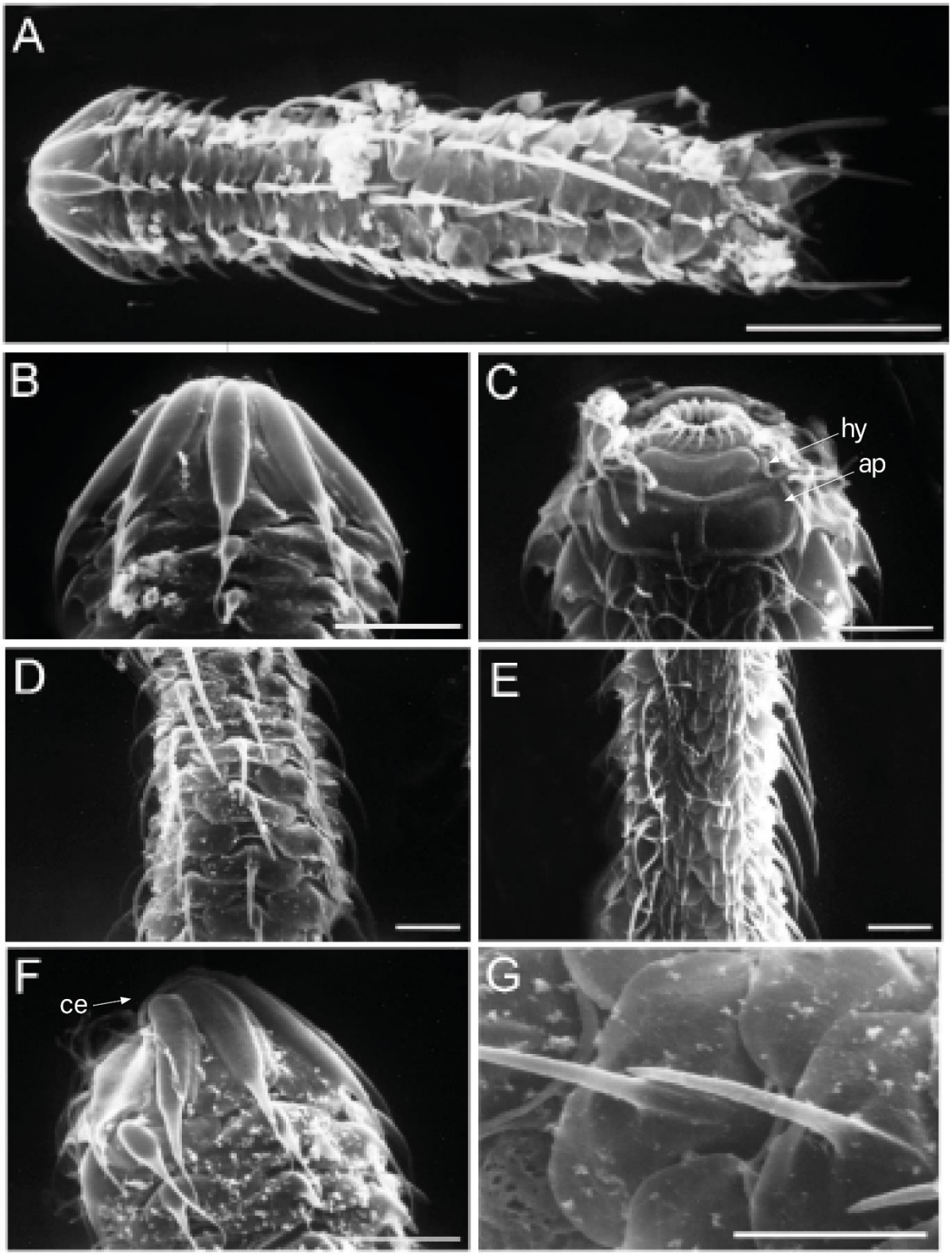

Figure 6. Scanning electron microscope (SEM) - sp. nov. 1 (Diamantina) **A** Habitus **B** Cephalic region dorsal view **C** Cephalic region ventral view **D** Median body region dorsal view **E** Medial region of the body ventral view. **F** Cephalic region dorso-lateral view. ce: cephalion**. G** Scale type 20. **Scales bars:** A 50 µm; B, C, D, E, 10 µm.

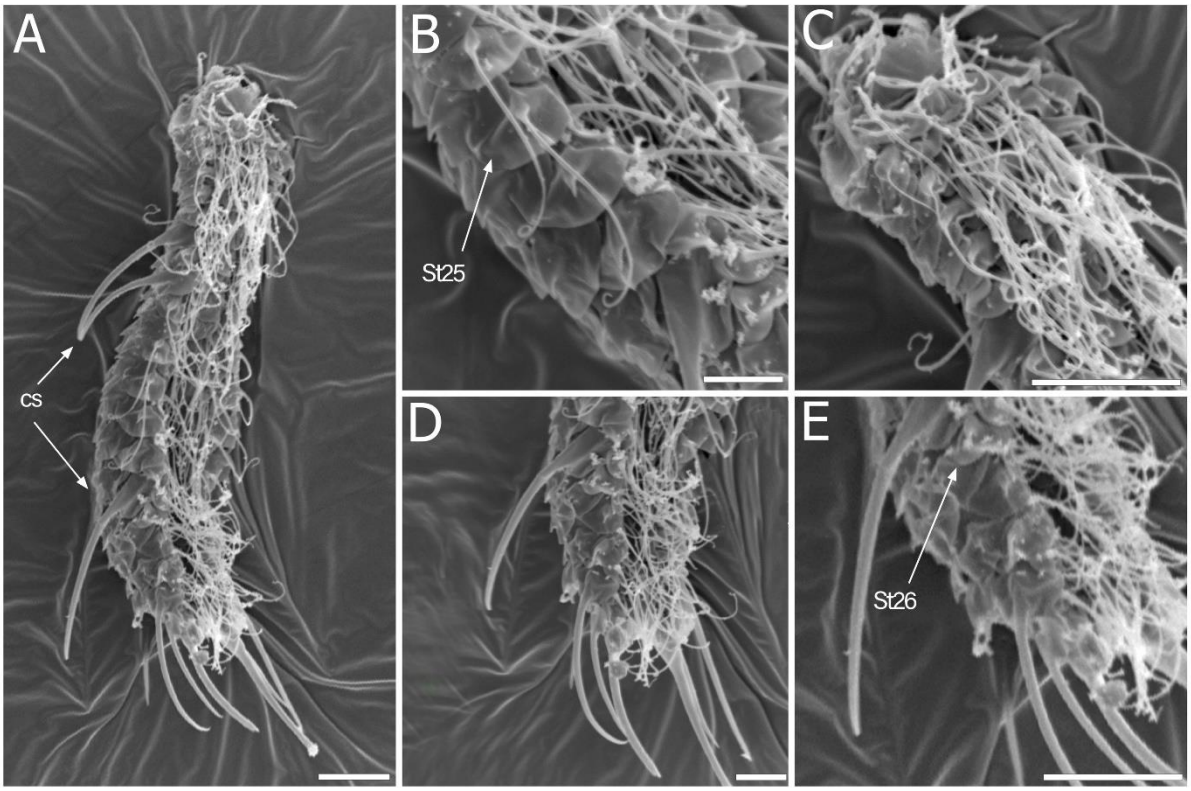

Figure 7. Scanning electron microscope (SEM) - sp. nov. 2 (Billings) **A** Habitus. cs: conspicuous spines **B** Scales, lateral view. St: scales type **C** Neck, ventral view **D** Furca, ventral view **E** Conspicuous spine, ventral view. **Scales bars:** A 10 µm; B 5 µm, C 10 µm, D 10 µm, E 10 µm.

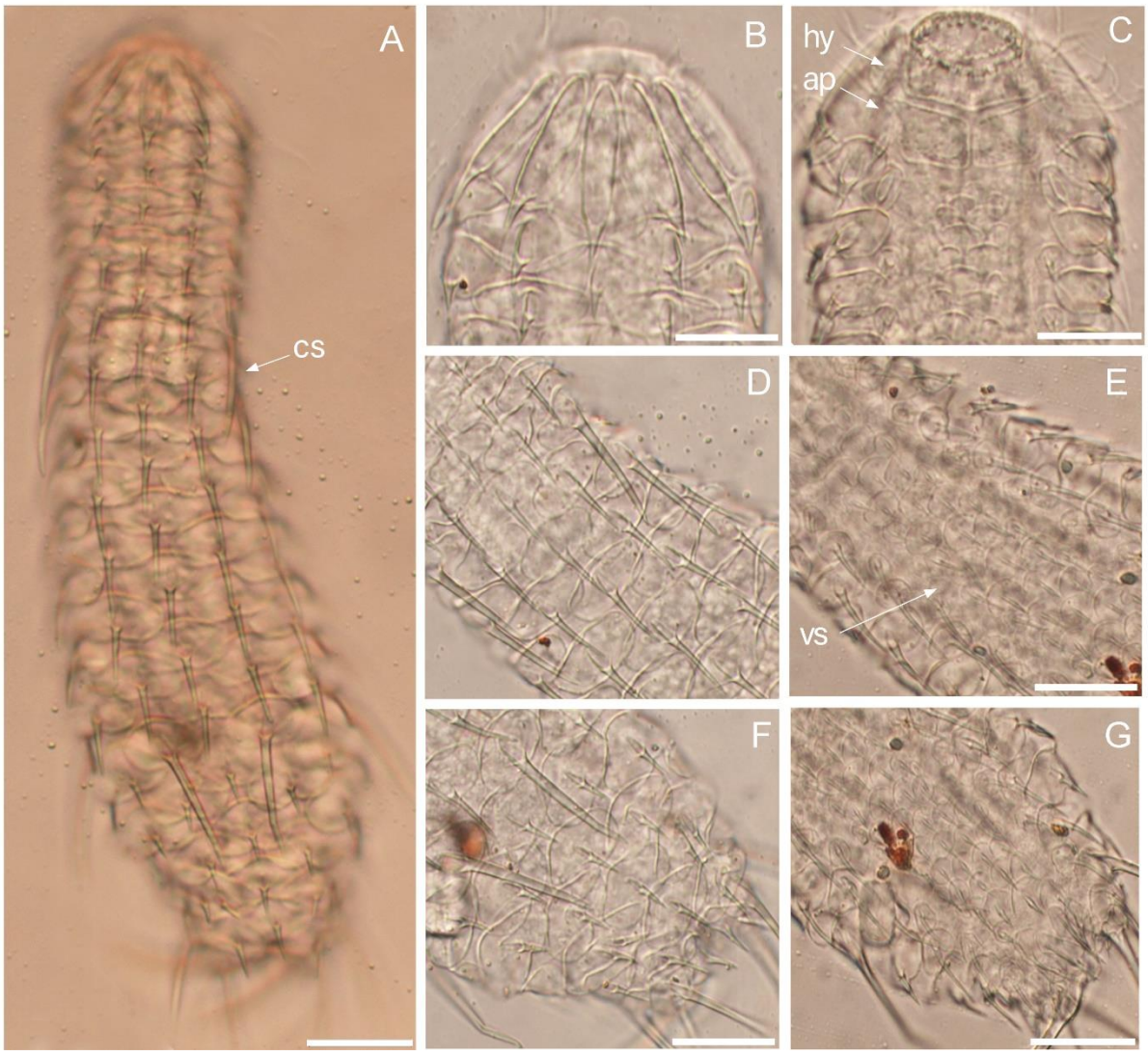

Figure 8. Differential interference contrast microscopy (DIC) - sp. nov. 1_(Diamantina) **A** Habitus, internal morphology; **B** Habitus, dorsal view; **C** Habitus, internal morphology. **Scales bars:** A - C 10 µm.

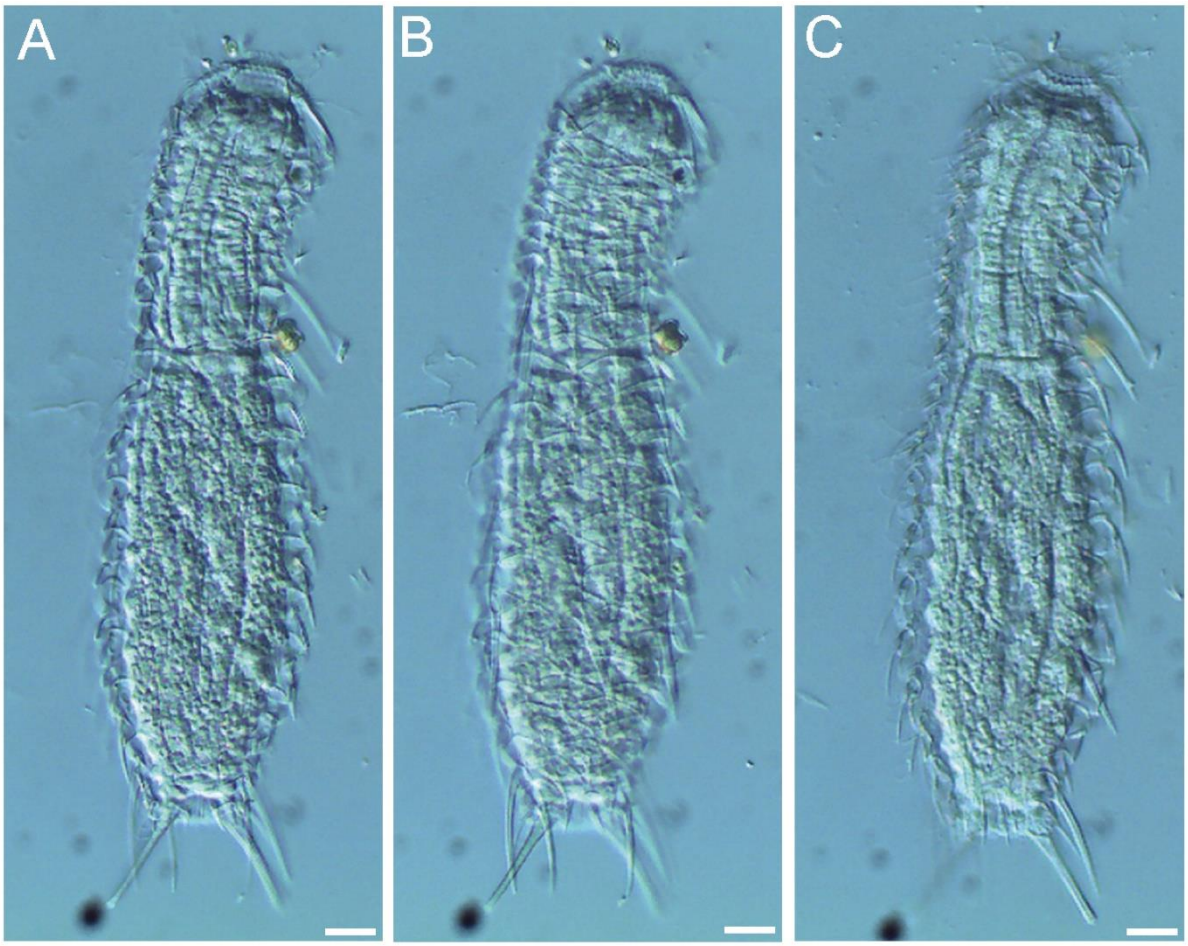

Figure 9. Differential interference contrast microscopy (DIC) - sp. nov. 1 (Diamantina) **A** Habitus, vista dorsal; **B** Cephalic region dorsal view; **C** Cephalic region ventral view; **D** Median region dorsal view **E** Median region ventral view **F** Posterior region dorsal view **G** Posterior region ventral view. **Scales bars:** A - G 10 µm.

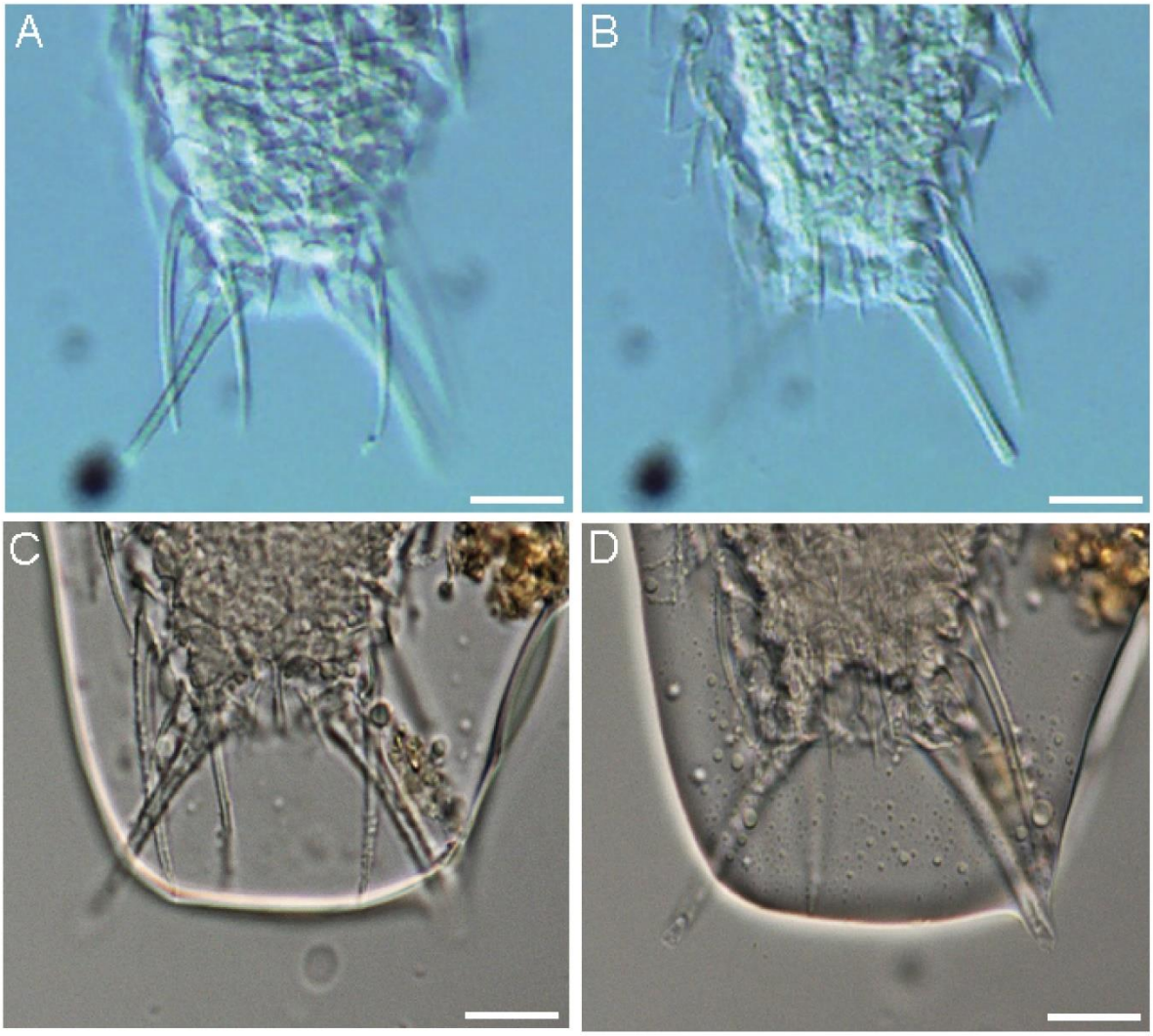

Figure 10. Differential interference contrast microscopy - DIC. sp. nov. 1 (Diamantina) **A** Furca, dorsal view **B** Furca, ventral view **C** Furca, dorsal view **D** Furca, ventral view **Scales bars:** A - C 10 µm.

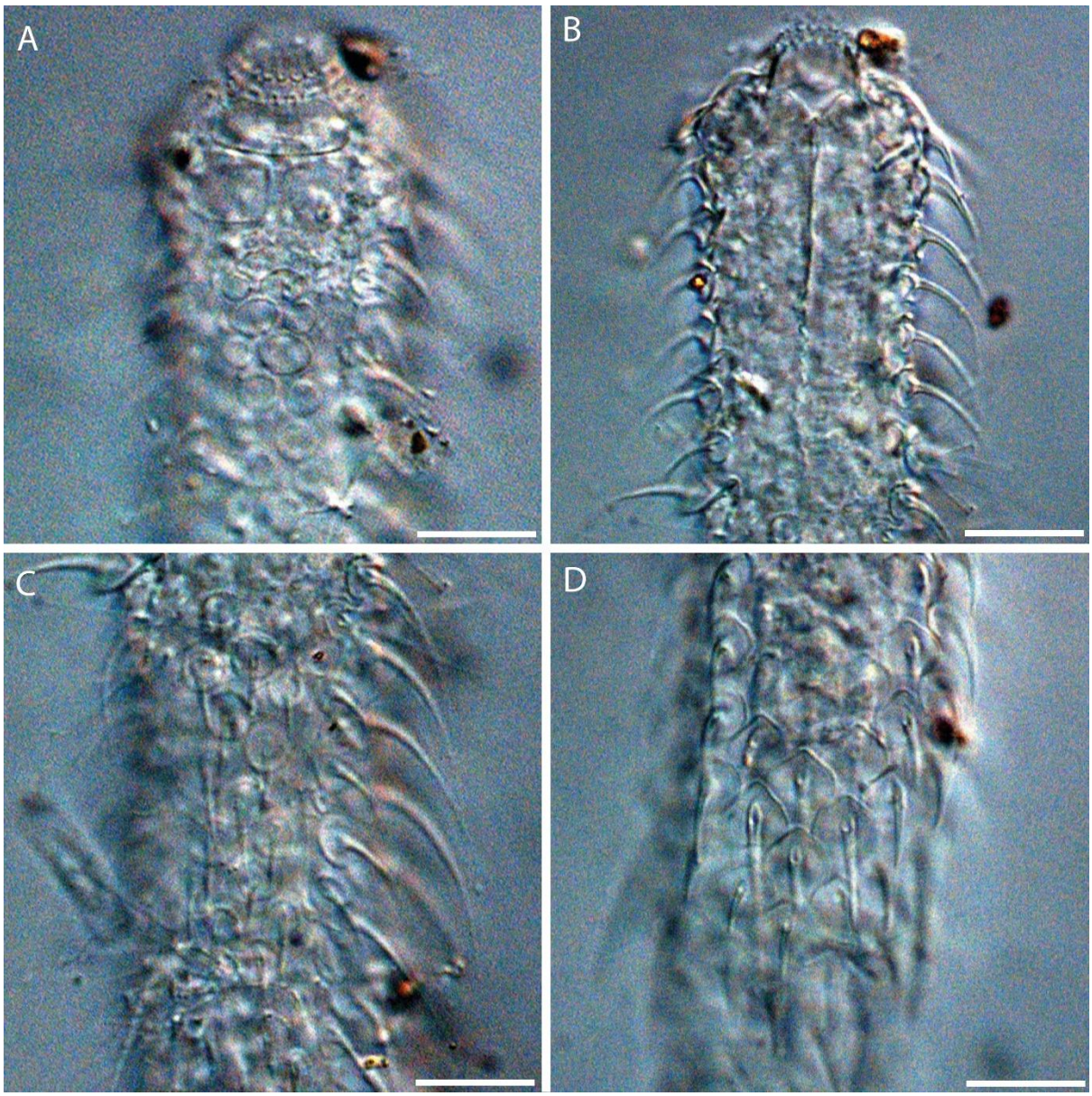

Figure 11. Differential interference contrast microscopy (DIC) - species. nov. 1(Diamantina) **A** Neck, ventral view **B** Neck, internal morphology **C** Trunk, ventral view **D** Trunk, dorsal view **Scales bars:** A - D 20 µm.

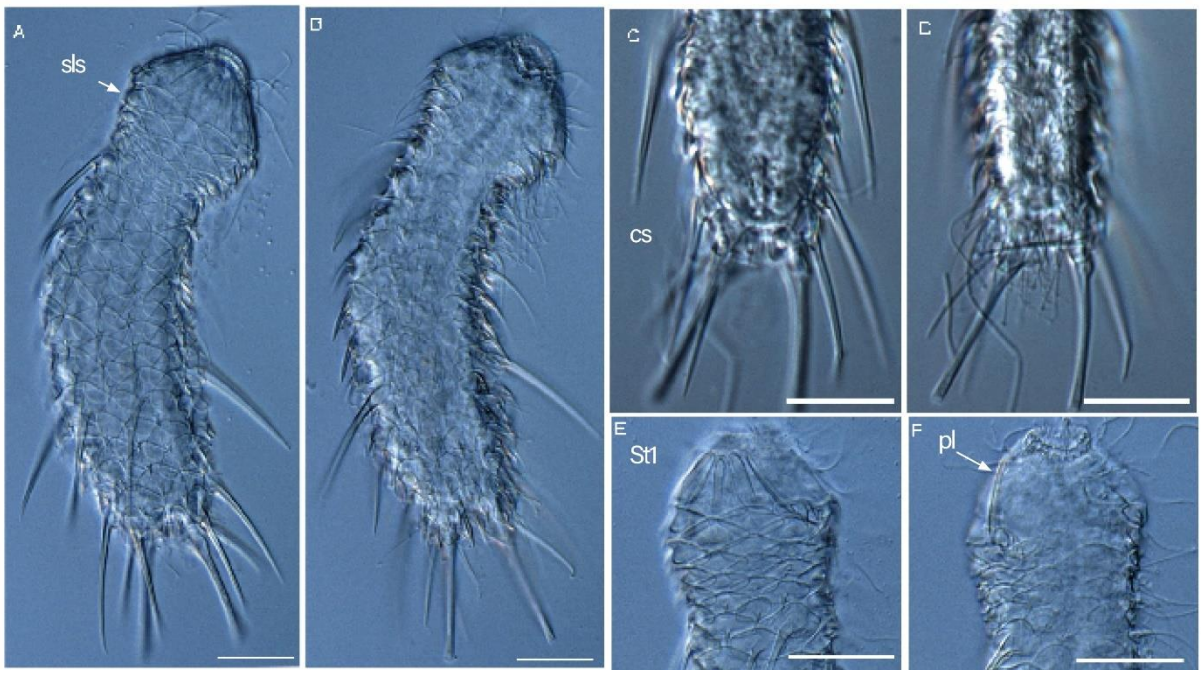

Figure 12. Differential interference contrast microscopy (DIC) - species. nov. 2 (Billings) **A** Habitus, dorsal view; **B** Habitus, ventral view; **C** Posterior region, dorsal view; **D** Posterior region, ventral view; **E** Anterior region, ventral view; **F** Posterior region dorsal view. **Scales bars:** A - F 20 µm.

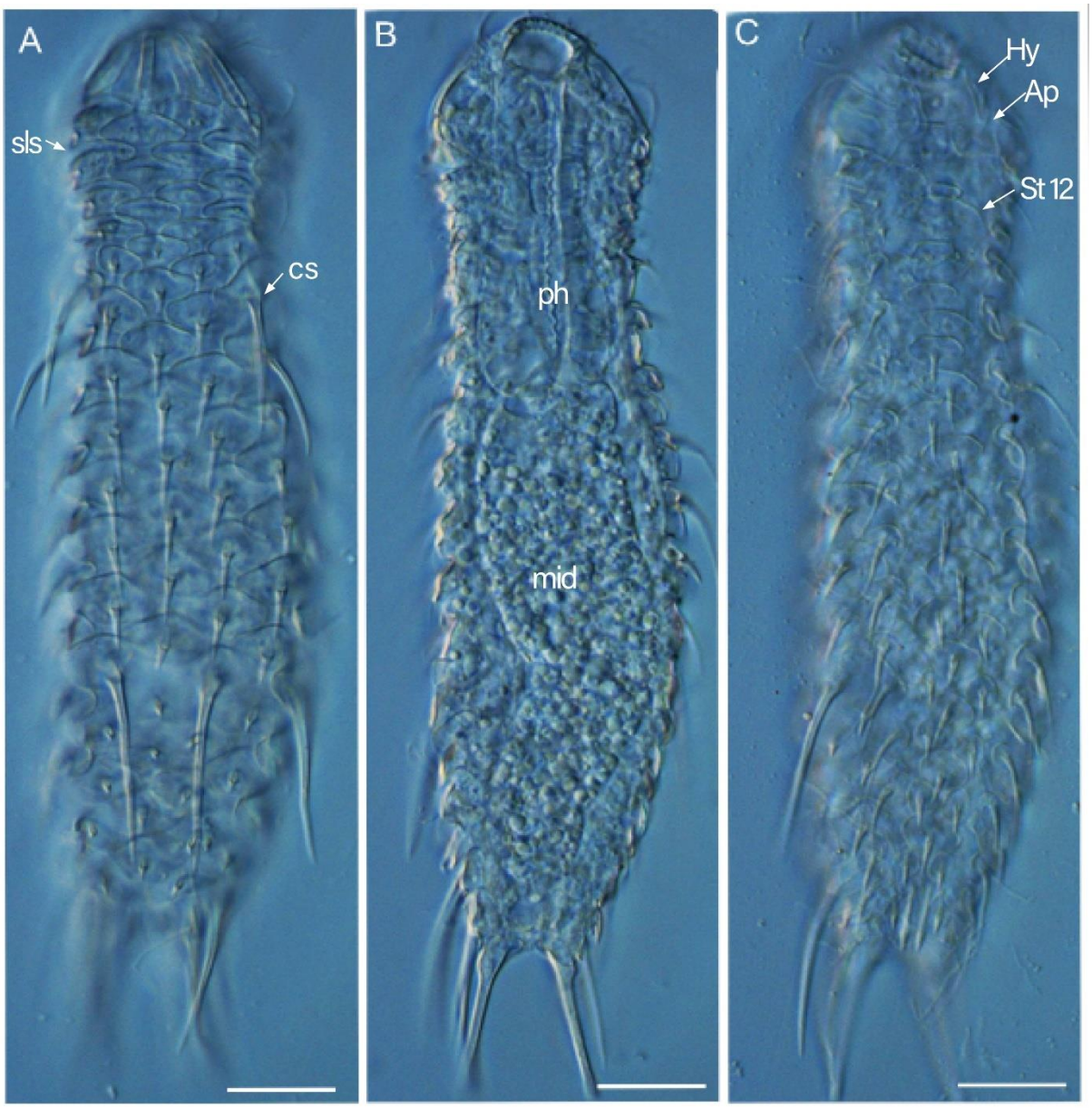

Figura 13. Differential interference contrast microscopy (DIC) - sp. nov. 2 (São João da Boa Vista) **A** Habitus, dorsal view. slc: scales lacking spines, cs: conscpicuous spines; **B** Habitus, view of internal morphology. ph: pharynx, mid: midgut; **C** Region Habitus, ventral view. hy: hypostomium, ap: additional plates, st: scales types. **Scales bars:** A - C 20 µm.

SUPPLEMENTARY TABLE 1. GenBank accession numbers of 18S rDNA, 28SrDNA and COI sequences.

Taxa	GenBank acess number			Referência
	18S	**28S**	**COI**	
sp.nov.1	OR738698.1	-	-	This Study
sp.nov.2	OR853698	OR853697	OR742314.1	This Study
Chaetonotus acanthocephalus aff.	OR853696	-	-	This Study

7. Capítulo 2. In search of hidden treasures: new species of the genus *Ornamentula* (Gastrotricha: Paucitubulatina)

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Abstract

Most gastrotrichs exhibit benthic or periphytic habits, but there is also some organisms which present an uncommon semi-planktonic lifestyle, such as members of the genus *Ornamentula* Kisielewski, 1991, belonging to the family Dasydytidae Daday, 1905. Herein, we describe the third species of the genus sampled in an urban lake from Brazilian southeast, using integrative taxonomy based on morphological and molecular analyses. *Ornamentula* sp. nov. can be distinguished from the congeneric species by the presence of asymmetrical dorsal scales, longest ciliature, the presence of an additional third transverse row of scales on the neck and additional scales on the ventral trunk. Furthermore, for the first time *Ornamentula* specimens were observed on scanning electron microscope and an updated identification key for the genus *Ornamentula* is also provided.

Key words. Semiplanktonic gastrotrich, taxonomy, freshwater, atlantic forest, urban lake.

Introduction

Gastrotricha are aquatic free-living microinvertebrates that represent an important role in trophic niches of marine, brackwish-water and freshwater (Balsamo *et al.*, 2014). The taxon consists of about 900 species grouped into two orders, Macrodasyida Remane, 1925 which is almost exclusively marine and Chaetonotida Remane, 1925, with both marine and freshwater representatives (Garraffoni & Balsamo, 2017; Todaro *et al.*, 2019a; Balsamo *et al.*, 2020; Saponi *et al.*, 2024)

Although most gastrotrichs have epibenthic or interstitial lifestyles, some of these animals can live in semiplanktonic environment (Balsamo et al. 2014). This last kind of lifestyle was only possible due to the acquisition of some synapomorphies, such as the absence of adhesive tubes and non-bilobed caudal end, lateral moveable spines and the arrangement of locomotory individual ciliation into transverse bands (Kieneke & Riemann, 2008; Kolicka *et al.*, 2020; Minowa & Garraffoni, 2020). These novelties are significant for understanding

evolution and adaptation, as they are associated with the shift from a benthic to a semipelagic lifestyle (Kisielewski, 1991; Kieneke & Ostmann, 2012; Kånneby & Todaro, 2015). Semiplanktonic species, however, are rare and particularly sensitive to environmental changes (Balsamo et al., 2014). The primary representatives of this group are the chaetonotida families Neogosseidae Remane, 1927 and Dasydytidae Daday, 1905 (Minowa & Garraffoni, 2020).

The genus *Ornamentula* Kisielewski, 1991 is a genus within Dasydytidae due to is large ornamented scales, feature never seen before within the phylum (Kisielewski, 1991). The first species described within this genus was *Ornamentula paraensis* Kisielewski,1991 sampled in a eutrophized urban lake in Pará state, Brazil. Later, Minowa & Garraffoni (2021) found *O. miyazaki* in an urban lake in São Paulo state, among floating vegetation roots. In this study, we describe the third species of the genus *Ornamentula* found in an urban lake in the state of São Paulo, Brazil. We employed different techniques for conducting studies on external and internal morphology, such as optical microscopy with differential interference contrast microscopy (DIC), scanning electron microscopy (SEM) for the first time in specimens of this genus, and sequenced nuclear ribosomal DNA (rDNA 18S and 28S) and mitochondrial (mtDNA COI) for molecular studies. Finally, an updated identification key for the genus *Ornamentula* is also provided.

Material and methods

Sampling Locations

Samples were collected in an urban lake called Limoeiro in Águas de São Pedro, São Paulo state, Brazil. This small lake has an area of 0.03km² (22°36'16"S 47°51'36"W) (Fig. 1) and it is surrounded by fragments of Atlantic rainforest. Five samples were made during the years 2023-2024, but the specimens of the new species occurred in high abundance only in material collected in February 2024 (rainy season in Neotropics). Five-liter buckets were utilized to collect material from the upper 30cm of the water surface, among floating vegetation roots. The material was stored with continuing aeration, maintaining the temperature around 19 °C, and processed in fourteen days. All maps were created and organized in a Geographical Information System (the open source Quantum Geographical Information System, QGIS version 3.12.1 www.qgis.org/en/site/)

Morphological data

The sorting process involved 500 ml water samples filtered through a 30 µm mesh, then poured into Petri dishes and examined under a Zeiss Stemi 2000 stereomicroscope, focusing

on the water column to identify semiplanktonic gastrotrichs. Each individual was isolated, anesthetized with 2% MgCl2, and mounted individually, with digital documentation performed using a Zeiss Axio Imager M2 light microscope equipped with DIC and an AxioCam MRC5 digital camera. Images were captured, and measurements were taken using the ZEN lite 2.5 2018 imaging software.

Twenty specimens were preserved in 2% glutaraldehyde in sodium cacodylate buffer for storage. The samples were then rinsed in 0.1 M PBS and dehydrated through a graded ethanol series (30%, 40%, 50%, 60%, 70%, 90%, 95%, 100%, each step performed twice for 5 minutes). As described by Abolafia (2015), small containers were used to transfer the samples between alcohol solutions. Afterward, they were subjected to critical-point drying with CO2 using a Baltec CPD 030 dryer, mounted on aluminum stubs, and coated with gold-palladium using the SCD-050 Sputter Coater. Observations were made using a JEOL JSM 5800LV scanning electron microscope at the State University of Campinas (UNICAMP).

The positions of morphological traits along the longitudinal axis are expressed as percentage units (U) of the total body length, measured from the anterior to the posterior end (Hummon et al. 1992).

Molecular data

DNA was extracted from 4 specimens using QIAmp DNA Micro Kit (Qiagen), following the manufacturer's instructions. The nuclear 18rRNA and 28S rRNA genes and mitochondrial cytochrome C oxidase subunit I (COI) were amplified using the polymerase chain reaction (PCR). The PCRs amplification was performed in 20 µl reaction mixture, containing 5 µl of extracted DNA template, 10 µl of GoTaq® Master Mix, and 0.4 µl (10 pmol/µl) of specific primers (Table S1), and 4.2 µl of H2O (Kieneke & Todaro, 2021). The amplification products were visualized by electrophoresis in 1% agarose gels containing SYBR® (Life Technologies). 5 µl of the post-PCR reaction product was mixed with 2ul of ExoSAPIT™ reagent. The DNA fragments were sequenced using BigDyeTerminator reactions in a 3500xL Genetic Analyzer (Life Technologies) at the Centro de Biologia Molecular and Engenharia Genética laboratory (CBMEG, Campinas, Brazil).

Phylogenetic analyses

For the phylogenetic analyses, we used available Chaetonotida data of 43 species representing the families Chaetonotidae (33), Dasydytidae (7) and Neogosseidae (3) based on data matrix of Kolicka et al. (2020) and Križanová & Vďačný (2023). Sequences obtained from GenBank

had been aligned using the L-ISC-I algorithm in MAFT v7.526 server (Katoh & Standley, 2013). The final alignment contains 4910 nucleotide positions (nps) for 47 terminals (table S2) (1741 nps for 18S rDNA, 2510 nps for 28S, and 657 nps for COI mtDNA) was concatenated using Sequence Matrix v1.8 (Vaidya et al., 2011). The best fit models of sequence evolution chosen by MrModelTest for rDNA 18S and 28S and mtDNA COI were GTR+I+G. Phylogenetic trees were constructed using IQTREE for Maximum likelihood analyses and MrBayes on XSEDE, implemented by the CIPRES platform, for Bayesian analyses. Tree editing was performed using the program FigTree v1.4.4 and Adobe Illustrator v24.1.2.408.

Results

Taxonomic account

Gastrotricha Metschnikoff, 1865

Chaetonotida Remane, 1925 [Rao & Clausen, 1970]

Paucitubulatina d'Hondt, 1971

Dasydytidae Daday, 1905

***Ornamentula* Kisielewski, 1991 (emended diagnosis)**

Dasydytidae of 97–152 μm in length. Body covered with very large and ornamented scales. Cephalion, hypostomion and pleurae occasionally present. Dorsal neck with one, two or three transversal rows of three spined scales. Dorsal trunk with two parallel columns of six large ornamented scales and a rearmost group made of three scales. Long cephalic and trunk dorsal spines present on three possible ways: in all scales, just on anterior three or in all scales except in fourth one. Each long spine provided with a single strong lateral denticle. Transverse band of cephalic cilia situated between large lateral plates. Four paired spines groups (ta-td) along the anterior trunk half, two pairs of rear spines (r1-r2) near the trunk end and in some cases a small pair of ventral rearmost spined scales. Posterior trunk half ventrally covered with fine ornamented and spined scales, oval or arrow-head in shape.

Three species: *Ornamentula paraensis* Kisielewski, 1991 (type species); *Ornamentula miyazakii* Minowa & Garraffoni, 2021 and *Ornamentula* sp. nov.

***Ornamentula* sp. nov**

(Fig.2-6; Table 1,2)

Type material. Holotype. Photographs of specimens collected from an urban lake in São Pedro, Brazil, in February 2024. The specimen was examined alive using a compound microscope with DIC. However, because of its fragile body, it was destroyed and is no longer available (Garraffoni et al. 2019). The holotype is in Figs 2-6 (accession number in

https://www2.ib.unicamp.br/museuvirtual/ ZUEC-PIC 1117 to 1147). Paratypes: 10 specimens collected from the same sampling site, with digital image data available under accession numbers x.

Diagnosis.

Ornamentula species 97-152 µm in body length (122-185 µm posterior spines included). Cone-shaped head, small cephalion. Two pairs of long sensory bristles. Three longitudinal scales transverse row at the dorsal neck region. Trunk dorsally covered by two columns each made of six large ornamented scales all spined, except for the fourth one. Ventral trunk side with small different shapes of spined scales between ciliary tufts. Trunk ventral ciliation with four paired tufts. Posterior end truncated; one pair of dorsal terminal scale with a thick spine.

Species-specific character. Neck with three transversal rows of spined ornamented scales. Rectangular shaped spined scales at the anterior part of the ventral trunk. Asymmetrical dorsal scales, which the fourth one is spineless. Three paired tufts of long cilia in the anterior part of the ventral trunk .

Description. Description based on characters and measurements of the holotype.

Head

The head is well distinct, conical in shape, featuring a small cephalion (15 µm wide) characterized by an irregular quadrilateral shape, with a rounded anterior edge and two invaginations at the posterior, similar to the symbol of a bat (Fig 5-A). The head also provides a wide hypostomion (15 µm wide) (Fig 4 -A, B, C; Fig 8-A). Presence of pleura (U05) (Fig 7-B; Fig 8-C, D). Narrow cylindrical pharynx (50 µm long) (Figs 3-B).

Two paired lateral tufts are arranged side by side, one more rostral, close to the mouth ring (U01), and the other closer to the conspicuous pair of cephalic spines (ca) (25 µm long) (U03) (Fig 4-A). A paired ventral tuft is located a little lower of the middle of the head (U14), containing each four transverse rows of cilia (Fig 5-B). The traverse ciliary band surrounds the head (U10), covering the dorsolateral, lateral, and ventrolateral parts, interrupted at the midpoint of both sides (Fig 4-A, F). This band is located between two lateral large granular scales (ga) that surround all the lateral portion of the head, just like the ciliation itself (Fig 4-B). A pair of long sensory bristles is present inserted between the second lateral cephalic ciliary tuft and lateral cephalic transversal ciliary bands, anteriorly to granular lateral scales (U05). Trunk ventral ciliation consists of four paired tufts (U25, U35, U50 and U85).

The anterior dorsal head is provided with twenty-two small cephalic spined scales (cs) arranged in five transverse lines, following a "V" arrangement (Fig 4-D, E). The first line of scales is composed of three triangular scales with pointed anterior edges and thin spines arising from it. The second line of scales is composed of three rounded scales with short spines arising from its middle. The third line of scales is composed of five scales, where the two most lateral scales have a diamond-shape with longer and thicker spines, and three of the middle follow the shape and size of the scales from the second line. The fourth line of head scales is composed of two pairs of rounded scales with thicker spines located at the most lateral part, and three scales in its middle with notched posterior edges and thicker spines. The fifth and last line of head scales brings four scales, where the two the most lateral ones are similar with the most lateral ones of the fourth line of scales, and the two others are similar as located at the middle of the fourth line.

At the neck, its arranged three transverse rows of ornamented scales with spines (sr) (U12 – U20) (Fig 3-A; 4-D). These scales have the same polygonal net as the other scales from the dorsum, but they are smaller and different in shape. The first row contains two scales with noted posterior edges, and the second and third row contain three and two scales each, with rectangular shaped ones.

Dorsal trunk

The dorsal trunk consists in two parallel columns of six large ornamented scales. These scales have a complicated polygonal net that looks like a reinforcement, being called as a rigid lorica by Kisielewski (1991). The first pair of dorsal large scales (s1) is asymmetrical with a lateral pointed anterior edge and bears a long, straight, thick and barbed spine at 4/5 of its length (d1, 27 μm long). (U20 – U28). The second pair (s2) is circular-shaped and also bears a long, straight, thick and barbed spine at 4/5 of its length (d2, 43 μm long) (U25 – U35). The third scale (s3) is wilder and longer than the other scales, asymmetrical, one-lobed, and bears a long, straight, thick and barbed spine at 4/5 of its length (d3, 40 μm long) (U33 – U53). The fourth scale (s4) is longer and wilder as s3, asymmetrical and has no spines (U45 – U62). The fifth scale (s5) is asymmetrical, longer, but less wild than s4 and s5, and brings posterior located barbed spine (32 μm long) (U60 – U76). This spine is mentioned as r1 by Kisielewski (1991) and it has a lateral scale as origin. The sixth scale (s6) is smaller, circular-shaped, and brings a short but thick spine pointed laterally (U75-U85). The posterior most paired scale is triangular-shaped, smaller than s6 and brings laterally located barbed spines (27 μm long) (U85 – U88).

This spine is mentioned as r2 by Kisielewski (1991) (Fig 3-A). On the furca, in its middle, there is a single scale with two small spines located at its posterior lateral edges (U88).

Ventro-lateral and ventral trunk

Ventrolateral trunk with thick and straight spines organized in four groups (ta, tb, tc, td) arranged in 5–3–2–1 spines each with a conspicuous denticle at 4/5 of its length, and inserted on triangular scales (Fig 5-A). The first set of spines (ta) may vary between species, with some possessing four spines and others five. In the middle of tb and tc, in its central portion, is inserted four asymmetrical transversal rows of three rectangular scales that increase in size from the anterior portion to the posterior most (Fig 5-C) (U35-U50). They bring thin and small spines, that follows the size of the scale. Right bellow then, there is three small rhomboid-scales with spines (U51-U60). Laterally to them, there is a pair of fish-scale followed by two pairs of large oval scales, all of them without spines. Following down the middle longitudinal line, there are two pairs of circular shaped scales with small spines, and in the middle of the posterior most pair there is a pentagonal spined scale (U60-U65). Right below, there is a pair of triangular scales with small spines (U70). Near to the trunk end medially, there is a pair of triangle-like scales which lie close one to the other and show rudimentary spines (U73-U80). Below them, there are twelve small rhomboid-circular scales, all with spines (Fig 6-F) (U82 – U85). Located slightly above the pair of posterior most scales (r2), there is a pair of scales with two diagonal keels in its center (U87).

Sexuality unknown, as we could not observe any specimen with eggs, nor the sexual organs.

Ecology and behavior.

Freshwater, periphytic and semiplanktonic among roots of floating vegetation mainly composed by *Eichhornia sp.,* and *Brachiaria subquadripara.*

Molecular diagnoses

DNA markers

The characteristics of the obtained markers of the *Ornamentula* sp. nov. are as follows.

- 18S rRNA: H1 H2 H3 H4; the uncorrected p-distance between the haplotypes: 0,6%
- 28S rRNA: H1 H2 H3 H4; the uncorrected p-distance between the haplotypes: 2,7%
- COI: H1 H2 H3; the uncorrected p-distance between the haplotypes: 0%

Genetic differential diagnosis

The ranges of uncorrected p-distances between the new species and *Ornamentula paraensis* (JQ798697.1) are as follows.

- 18S rRNA: 0% - 0.97% (0,05% on average) with the most similar being the haplotype number 2.
- 28S rRNA: 0,39% - 3,66% (0,02% on average) with the most similar being the haplotype number 3.
- COI: 32,67% - 33,23% (16,52% on average) with the most similar being the haplotype number 2.

Remarks.

Ornamentula sp. nov. shares specific features with the two other described *Ornamentula* species , such as large ornamented scales at the dorsal trunk; cephalic spines (ca); four paired spines groups (ta-td) along the anterior trunk half and two pairs of rear spines (r1-r2). The shape of the six dorsal ornamented scales is distinctive in the new species, being much more irregular and assymetrical when compared with the two previously described species. Also, an additional ciliary tuft located in the fourth paired spined group (td) is present in *Ornamentula* sp. nov., which is absent in the other two species. However, the new species also shows remarkable differences that can be easily distinguished from *O. paraensis* and *O. miyazakii.*

The most remarkable features between the new species with *O. paraensis* is located in the middle of the ventral-trunk to the furca end: while *O. paraensis* has 25 small spined scales oval and triangular-shaped arranged uniformly, *Ornamentula* sp. nov. has 42 small spined scales spread all over the trunk featuring rectangular, rhomboid, circular, pentagonal, and triangular shapes. Moreover, the new species has an additional row of spined scales at the neck. Furthermore, the shape of the six dorsal ornamented scales is distinctive in the new species, being much more irregular and asymmetrical when compared with the two previously described species. Also, an additional ciliary tuft located in the fourth paired spined group (td) is present in *Ornamentula* sp. nov., which is absent in the other two species.

The news species is distinguished from *O. miyazakii* also because of its ventral-trunk. The second one has symmetrical oval scales with spines, creating a significant contrast to the irregular scales of the new species. Besides, *O. miyazakii* exhibits only one row of spined scales at the neck, two less than the new species.

Discussion

The first species of *Ornamentula* was found in 1991, and for the next 26 years no additional representatives of the genus were found (Kisielewski, 1991; Minowa & Garraffoni, 2021). However, it was only to start intensifying sampling efforts focused on Gastrotricha that *Ornamentula miyazakii* was discoverded, follow by *Ornamentula* sp. nov. (Garraffoni *et al.*, 2021). This demonstrated that the concept of rare species in Gastrotricha may be influenced by sampling bias (Garraffoni *et al.*, 2021). Furthermore, if we consider the sampling effort in Brazil in relation to the number of recently described taxa, it becomes evident that a significant portion of this fauna is likely overestimated (Garraffoni et al., 2017; (Minowa & Garraffoni, 2020). This may suggest that increased data collections in certain environments can significantly enhance our understanding of *Ornamentula* diversity and Gastrotricha as a whole (Garraffoni, et al., 2017).

Ornamentula paraensis was first sequenced by Kanneby et al. (2013) and has since been included in most phylogenetic studies on the order Chaetonotida (Kanneby, 2013; Garraffoni *et al.*, 2017; Kolicka *et al.*, 2020; Minowa & Garraffoni, 2022; Križanová & Vďačný, 2023; Saponi *et al.*, 2024 . As *O. miyazakii* remained unsequenced, it had not been possible to confirm the monophyly of the genus, even though its morphology suggested monophyly due to the peculiar structure of its dorsal scales. This study confirmed the group's monophyly by sequencing the third species and aligning it with *O. parensis* and all other semi-planktonic species sequenced to date. Dasydytidae is setted non-monophyletic due to the inclusion of the taxon Neogosseidae, which separates the group into two clades: one comprising the species from *Dasydytes* and *Stylochaeta*, and the other containing the species from *Ornamentula* and *Haltydytes squamosus*. This result aligns with the findings of Garraffoni et al. (2017), Minowa & Garraffoni (2022), Križanová & Vďačný (2023) Gammuto et al., (2024) and Saponi et al., (2024), which positioned members of *Kijanebalola* and *Neogossea* between *O. paraensis* and *Haltydytes* species, separating them from other members of Dasydytidae. A similar result is also reported by Kisielwski (1991), who conducted a morphological analysis of the semi-planktonic species. In his analysis, *Setopus* is included with *Ornamentula* and *Haltydytes* (which was not included in recent phylogenetic analyses due to its absence in molecular databases). The major reason for this agroupment is the presence of large scales, groups of long spines with lateral denticles and rearmost spines in most individuals of the three genera.

Given this scenario, our results are particularly significant because it represents one of the few cases where molecular data and morphological traits are in agreement (Paps & Riutort, 2012). Such congruence is rare in Gastrotricha, a group known for its high morphological

plasticity and the challenges associated with interpreting morphological characters (Minowa & Garraffoni, 2022). The alignment between our molecular and morphological findings highlights the robustness of using both approaches to clarify taxonomic relationships within *Ornamentula* (Balsamo *et al.*, 2014; Atherton & Jondelius, 2020; Minowa & Garraffoni, 2020).

Taxonomic key

Minowa and Garraffoni (2021) provided a taxonomic key for *Ornamentula* species, which we updated here with the new species.

1a. Cephalic spined scaled simple; dorsal scales arranged in two parallel columns of six scales each, three anterior with long barbed spines, three posterior spineless, dorsal neck with two transversal rows of three spined scales .....*Ornamentula paraensis*

1b. Cephalic spined scaled barbed; dorsal scales arranged in two parallel columns of six scales each, dorsal neck with single or three transversal rows of spined scales..... 2

2a. All dorsal scales with long barbed spines, dorsal neck with single transversal row of three spined scales..... *Ornamentula miyazakii*

2b. Anterior three and two posterior dorsal scales with barbed spined, fourth spineless; dorsal neck with three transversal rows of spined scales ……..…. *Ornamentula* sp. nov.

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Table 1. Morphometric features of *Ornamentula* sp. nov. All measures in µm. x = average; ca: cephalic spine; ta, tb, tc, td: groups of trunk ventro-lateral long spine; r1-2: rear spines; d1-3: spines on dorsal scales; s1-7: dorsal scales. All measurements are expressed in micrometers (µm).

Characters		***Ornamentula* sp.nov.**										
		Ind1	**Ind2 (holotype)**	**Ind3**	**Ind4**	**Ind5**	**Ind6**	**Ind7**	**Ind8**	**Ind9**	**Juvenile**	**x**
Body length, spines excluded		111	152	97	131	122	119	118	132	128	101	121
Total body lenght		130	185	122	152	140	136	135	152	155	122	143
Maximum head width		23	43	22	32	35	31	31	32	26	32	31
Minimum neck width		17	29	15	22	25	23	20	21	13	17	20
Maximum trunk width		46	64	46	44	43	38	43	43	37	27	43
Pharynx length		44	50	-	42	37	-	-	43	35	40	42
Diameter of mouth ring		7	10	8	6	9	-	10	6	4	5	7
Cephalion length		-	-	10	-	-	-	-	-	-	-	10
Cephalion width		12	15	11	18	17	-	18	19	14	16	16
Spines length	ca1	15	25	24	17	17	22	21	20	20	14	20
	ta1	32	29	32	-	20	27	21	29	25	24	27
	ta2	36	33	33	-	23	30	27	35	32	-	31
	ta3	37	37	37	-	39	30	30	35	38	-	35
	ta4	-	41	41	38	43	30	26	39	41	-	37
	ta5	-	-	55	-	-	41	33	43	-	26	40
	tb1	40	36	41	-	-	33	40	36	46	34	38
	tb2	41	43	42	-	-	34	39	42	36	-	40
	tb3	40	39	43	40	40	33	41	40	26	-	38
	tc1	39	43	-	-		36	25	39	36	-	36
	tc2	38	36	-	-	40	37	33	39	31	32	36
	td	45	47	-	34	41	33	28	34	33	28	36
	r1	34	32	-	25	35	23	30	23	25	26	28
	r2	-	27	33	23	26	23	26	20	26	23	25
	d1	24	27	-	-	-	29	-	29	42	-	30
	d2	38	43	-	-	-	37	-	35	35	-	38
	d3	36	40	-	-	-	38	-	38	-	-	38
Scales length	s1	-	12	13	8	9	9	-	8	8	-	10
	s2	13	21	22	16	17	-	-	16	19	-	18
	s3	20	25	33	19	21	-	-	20	36	-	25
	s4	29	28	26	30	31	28	16	23	34	-	27
	s5	27	36	-	26	27	28	26	25	29	-	28
	s6	-	-	-	7	9	-	20	8	-	-	11
	s7	-	8	-	6	7	-	-	7	-	-	7
Scales width	s1	-	15	16	9	17	8	-	9	12	-	12
	s2	14	20	14	16	23	-	-	15	12	-	16
	s3	16	21	20	17	25	-	-	17	16	-	19
	s4	26	25	25	20	30	20	11	18	21	-	22
	s5	20	20	-	14	19	16	16	14	12	-	16
	s6	-	-	-	9	10	-	18	10	-	-	12
	s7	-	9	-	7	7	-	-	4	-	-	7

Table 2. Comparison of several morphological structures between *Ornamentula* sp. nov., and *Ornamentula miyazakii* and *Ornamentula paraensis*.

Species	Ornamentula santipeteri	Ornamentula miyazakii	Ornamentula paraensis
Cephalic ciliation	Two anterior tufts and two pairs of ventral medial tufts	Two anterior tufts	Two anterior tufts and two pairs of ventral medial tufts
Cephalic dorsal scales	22 spined scales between cephalic ciliary tufts	15 spined scales between cephalic plates	16 spined scales between cephalic ciliary tufts
Cephalic long spine (ca)	Extremely thick, barbed, inserted on scale	Extremely thick, barbed, inserted on small scale	Thin, barbed, inserted directly to the cuticle
Cephalic sensory bristle	A pair of dorsolateral long bristle	A pair of dorsolateral long bristle	No bristle
Neck's transverse scales row	Three scale transverse row	One scale transverse row	Two scale transverse row
Trunk ciliation	Four pairs of ventral tufts	Three pairs of ventral tufts	Three ventral tufts
Trunk dorsal scales parallel rows	Six pairs of dorsal scales, anterior three and two posterior spined, fourth spineless (s4)	Six pairs of dorsal scales, all spined	Six pairs of dorsal scales, anterior three spined, posterior spineless
Trunk lateral scales	Three lateral oval to fish-shaped scales posterior to each spine group	Three lateral oval to fish-shaped scales posterior to each spine group	Three lateral rounded scales posterior to each spine group
Trunk Ventral scales	42 small oval, triangular, rectancular and romboidal scales	12 small oval to round spined scales	25 small round to triangular spined scales
Rear spines (r)	One barbed spine iserted on dorsal scale, one barbed spine inserted on lateral scale	Two barbed spines inserted on lateral scales, one simple short spine inserted on ventral scale	Two barbed long spines inserted on lateral scales

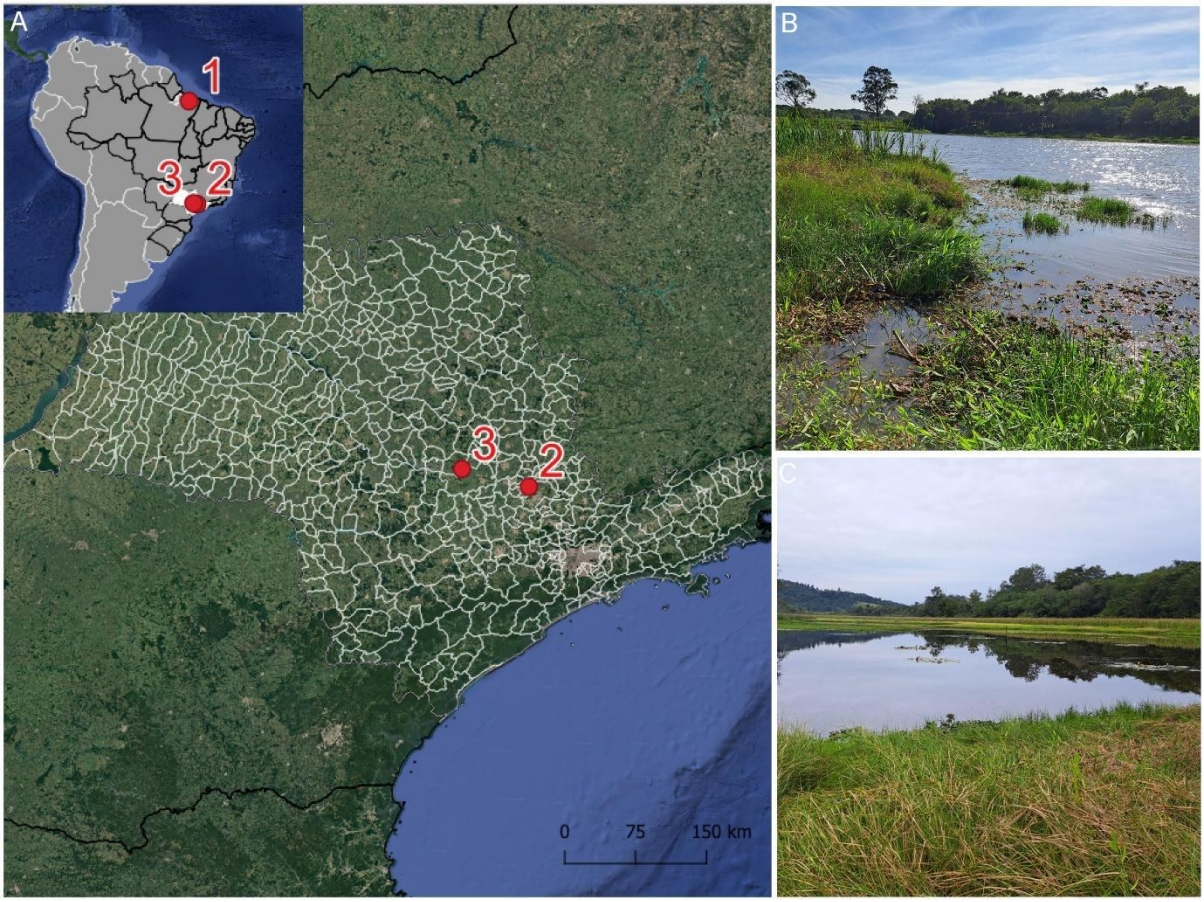

Figure 1. Sampling localities of *Ornamentula species*. **A 1**. Register of *Ornamentula paraensis* in Pará **2**. Register of *Ornamentula miyazakii* in Paulínia, São Paulo **3**. Register of *Ornamentula* sp. nov**.** in Águas de São Pedro, São Paulo. **B** Photos of type locality of sampling of *Ornamentula miyazakii*, an urban lagoon, Paulínia city. **C** Type locality of *Ornamentula* sp. nov., in a urban lagoon, Águas de São Pedro city

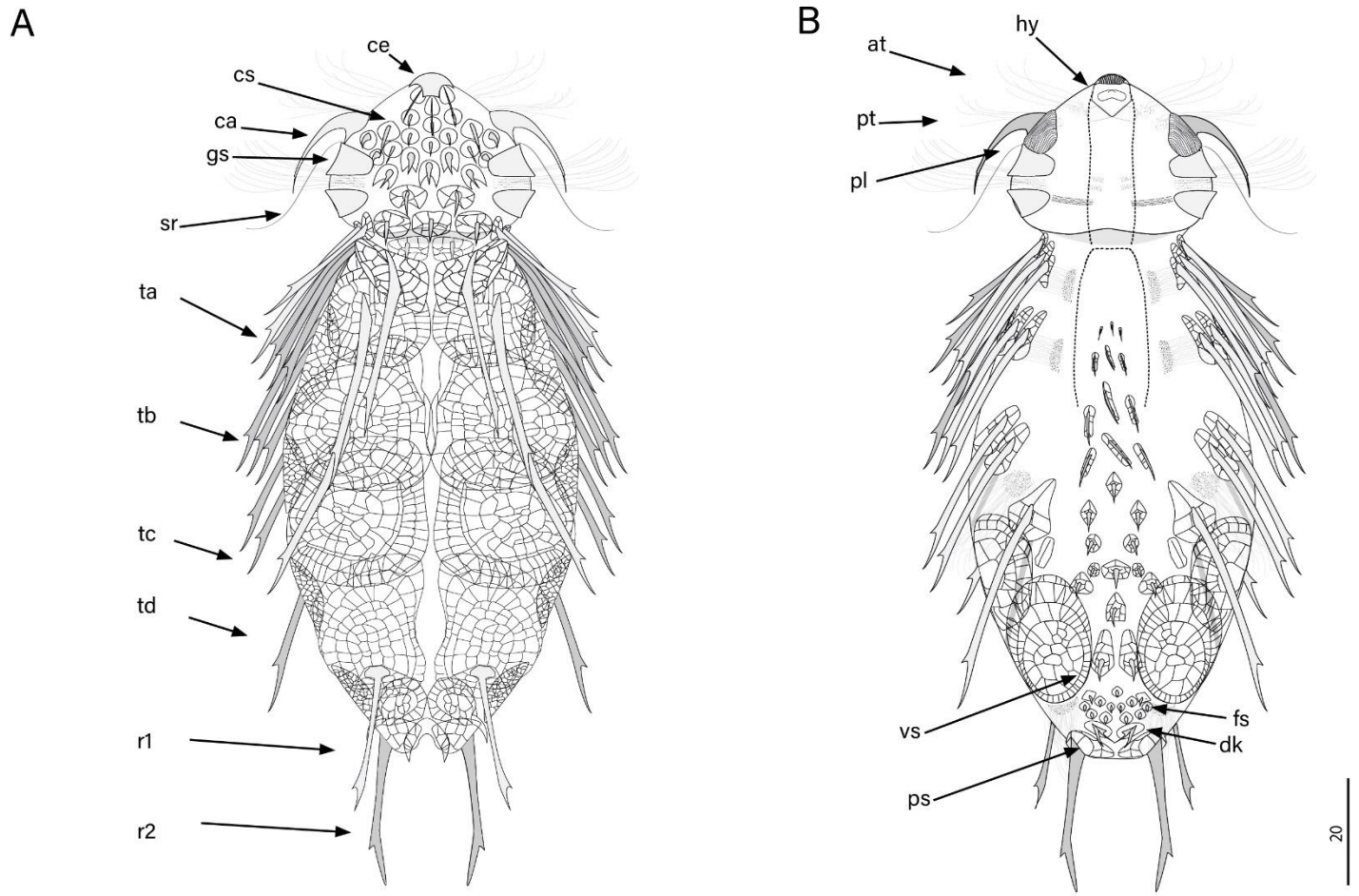

Figure 2. Schematic drawing of *Ornamentula* sp. nov. **A** Dorsal view. ce: cephalion; cs: cephalic scales; ca: cephalic long spine; gs: granular scales; sr: scales transverse row; ta-td: groups of trunk ventrolateral long spines; r1-r2: rearmost spines. **B** Ventral view. hy: hypostomium; at: anterior ciliary tufts; pt: posterior ciliary tufts; pl: pleurae; vs: ventral scales; ps: posterior scales; fs:furca scales; dk: dorsal keels.

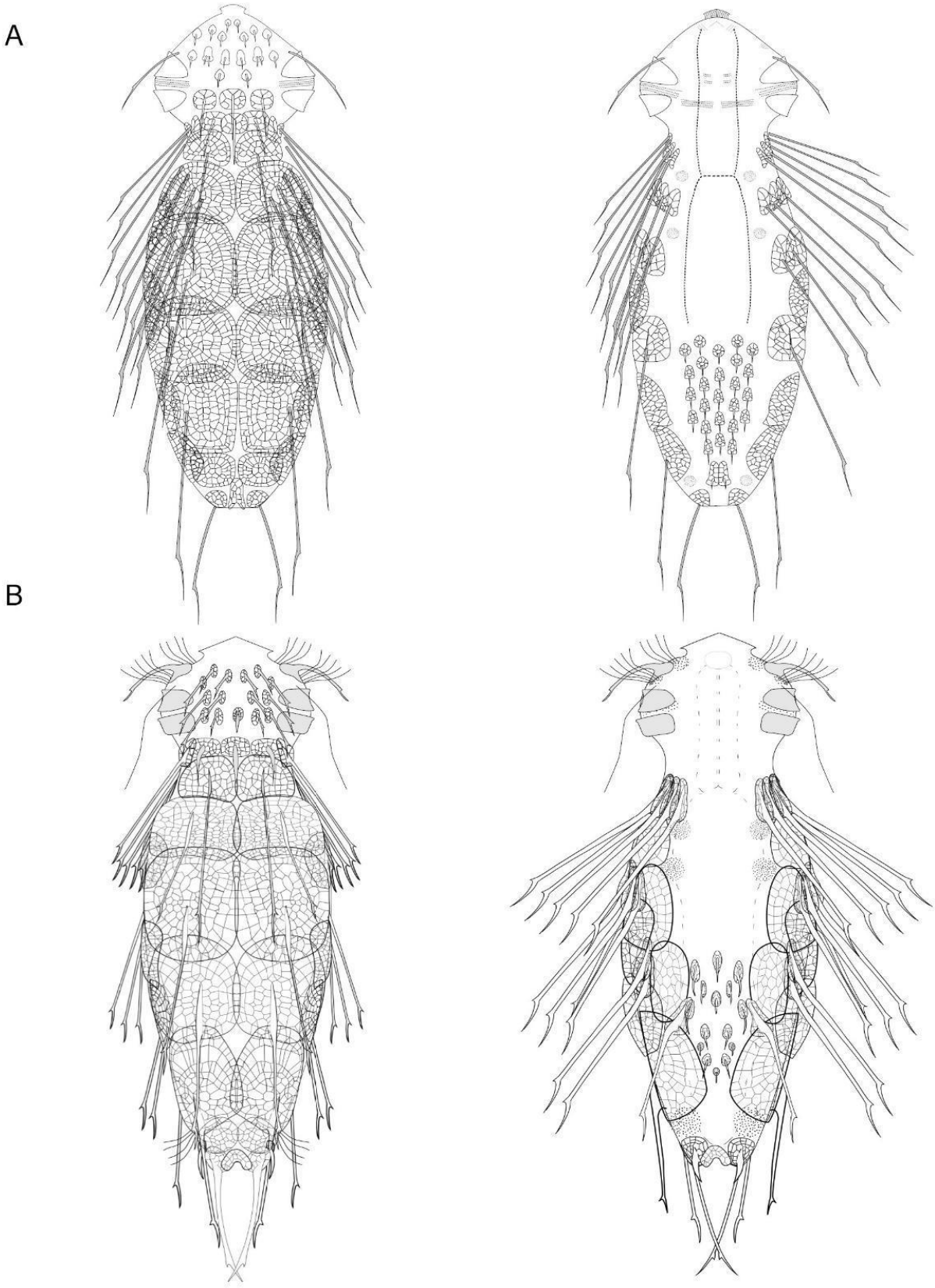

Figure 2. Schematic drawing of the two species described from *Ornamentula*. (left side) dorsal view; (right side) ventral view. **A** *Ornamentula paraensis.* **B** *Ornamentula miyazakii.*

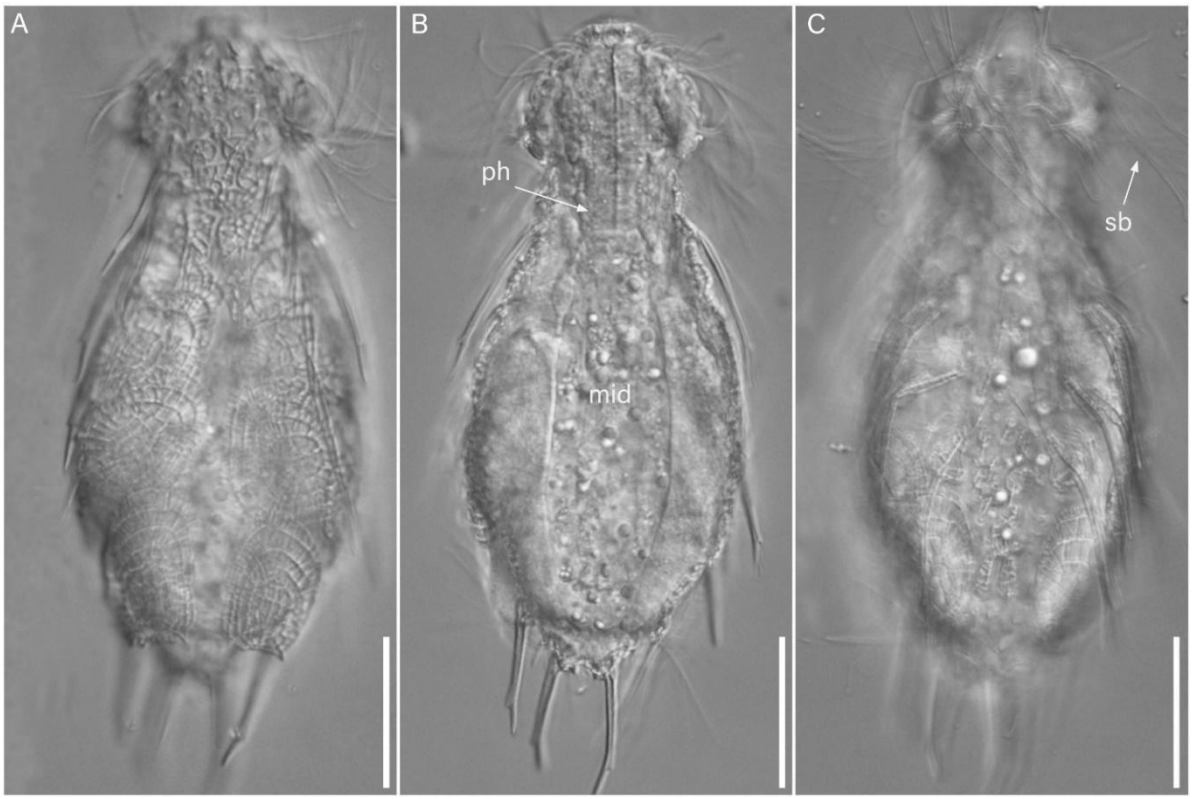

Figure 3. Habitus in differential interference contrast microscopy of *Ornamentula* sp. nov., holotype. **A** dorsal view. **B** internal view. ph: pharynx; mig: midgut. **C** ventral view. sb: sensory bristle. Scale bars: 20 µm.

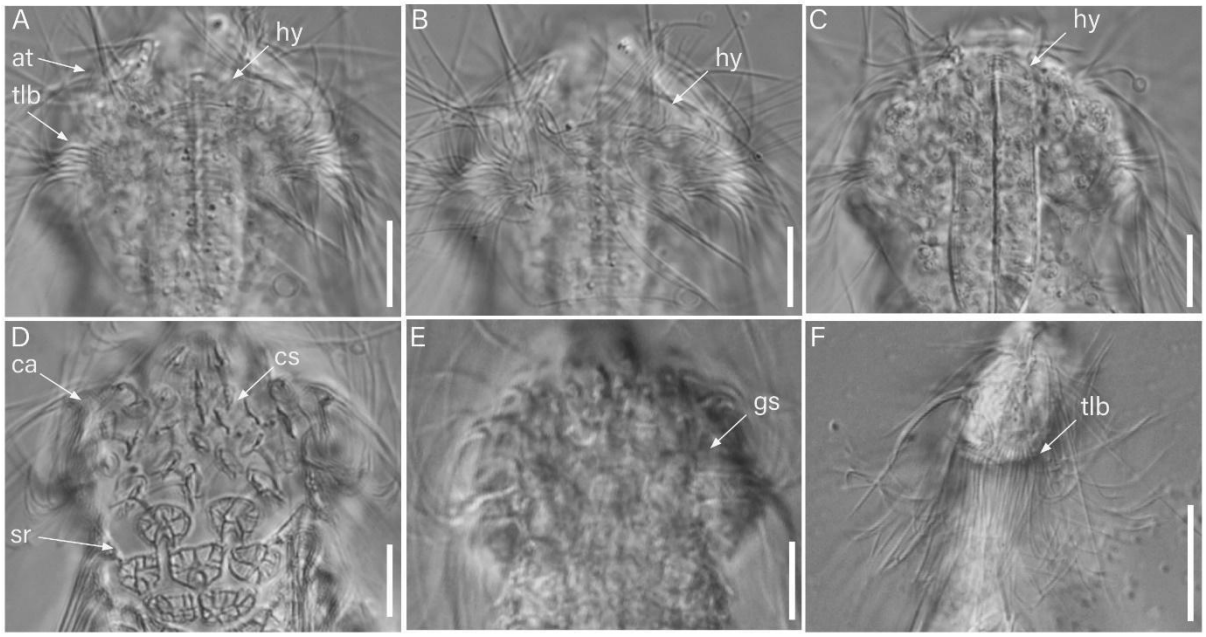

Figure 4. Head in differential interference contrast microscopy of *Ornamentula* sp. nov. **A** Holotype, ventral view. at: anterior ciliary tuft; tlb: transversal lateral band. **B** Holotype, ventral view. hy: hypostomion. **C** Holotype, ventral view. hy: hypostomion. **D** Holotype, dorsal view. ca: cephalic long spine; cs: cephalic scales. **E** Holoype, ventral view. gs: granular scales. **F** Paratype, lateral view. Scale bars: A, B,C, D, E: 10 µm; F: 20 µm.

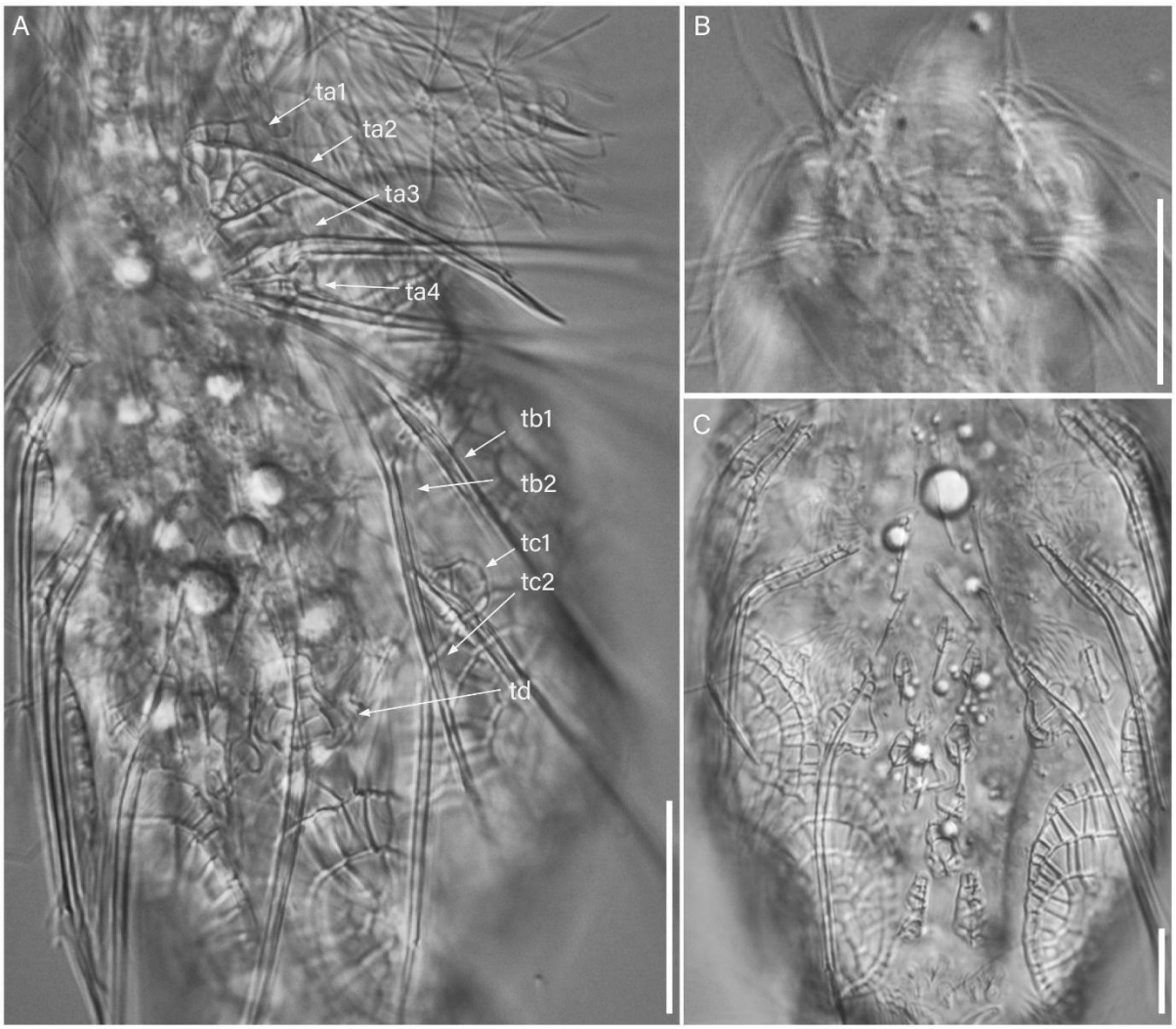

Figure 5. Ventral view in differential interference contrast microscopy of *Ornamentula* sp. nov. **A** Paratype, ventrolateral view. ta-td: groups of trunk ventrolateral long spines. **B** Holotype, head. **C** Holotype, trunk. **D** Holotype, habitus. Scale bars: A,C: 10 µm; B,D: 20 µm.

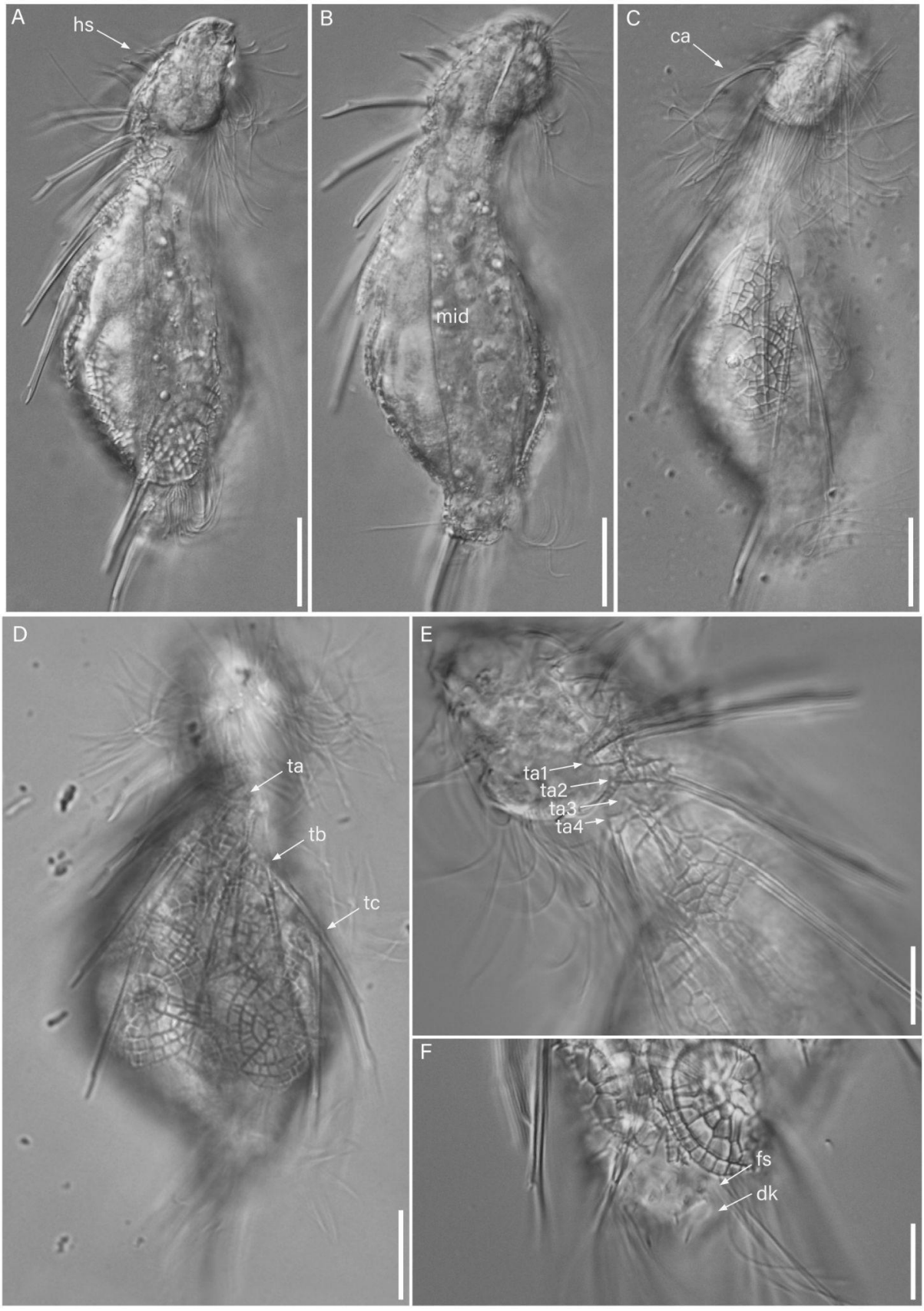

Figure 6. Photomicrographs in differential interference contrast microscopy of *Ornamentula* sp. nov. **A-C**: Paratype, lateral view. **D** Paratype, dorsolateral view. **E** Paratype, ventrolateral view. **F** Paratype, furca ventral view. fs:furca scales; dk: dorsal keels

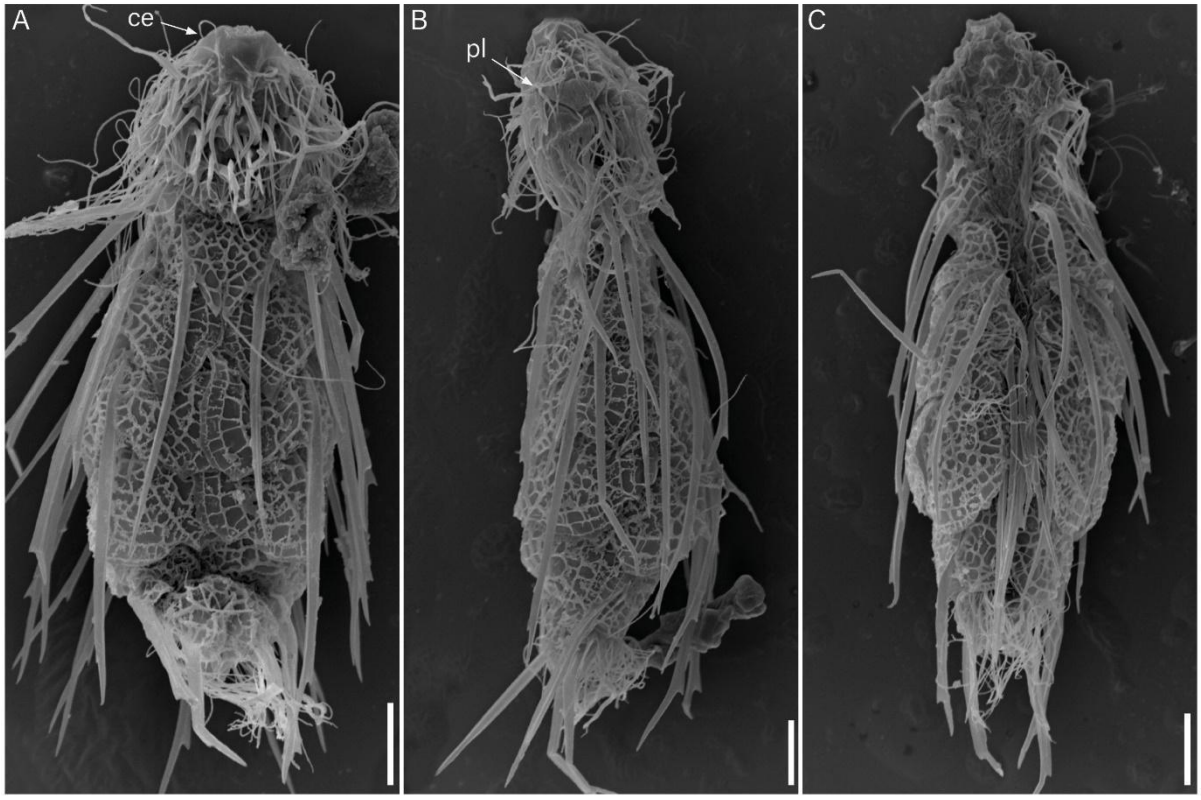

Figure 7. Habitus in scanning electron microscopy of *Ornamentula* sp. nov. **A** dorsal view. **B** lateral view. **C** ventral view. Scale bars: 10 µm.

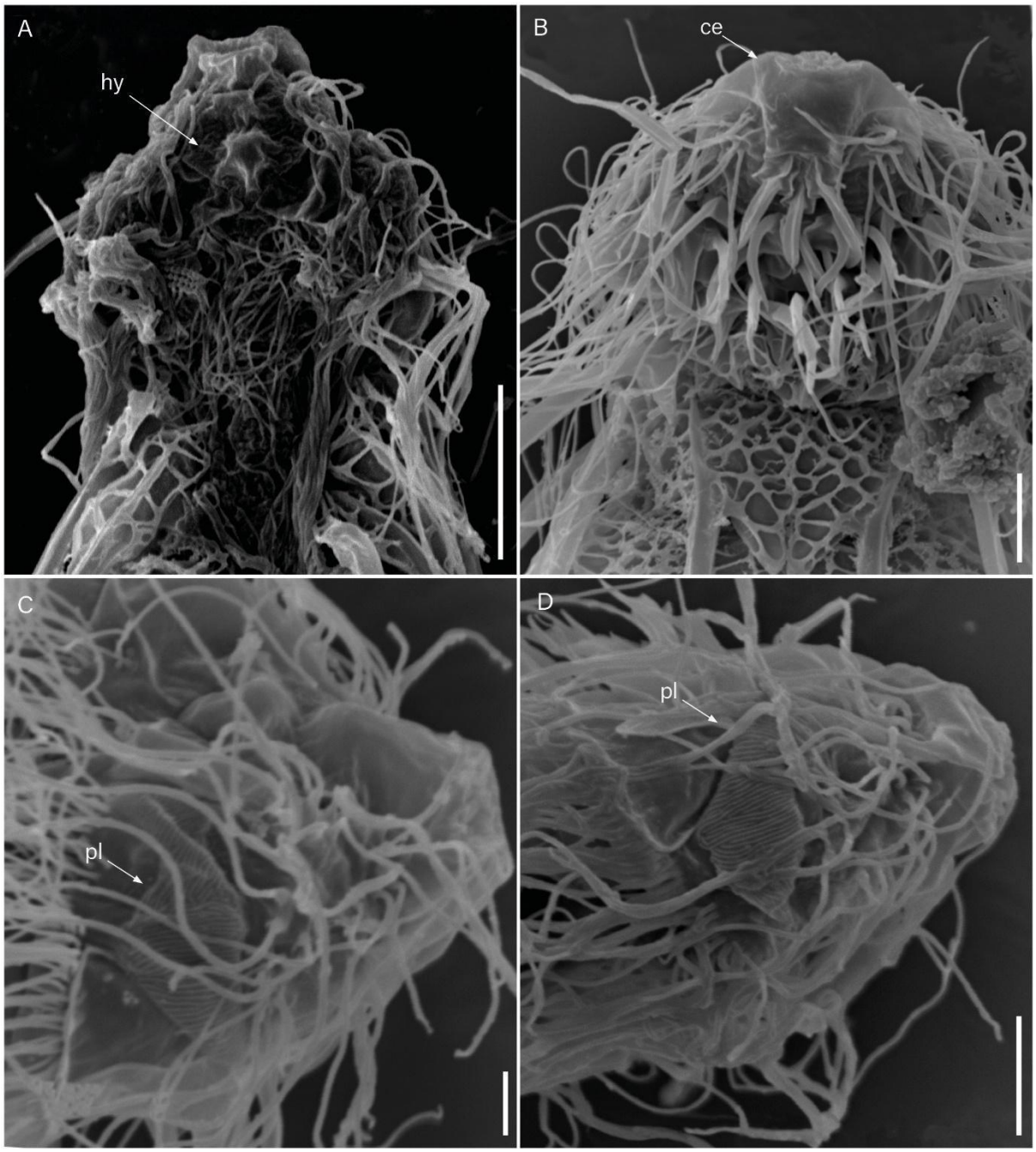

Figure 8. Habitus in scanning electron microscopy of *Ornamentula* sp. nov. **A** ventral view. hy: hypostomion . **B** dorsal view. ce: cephalion **C** lateral view. pl: pleura. **D** lateral view. pl: pleura. Scale bars: A - 10 µm; B – 5 µm; C - 2 µm; D - 5 µm.

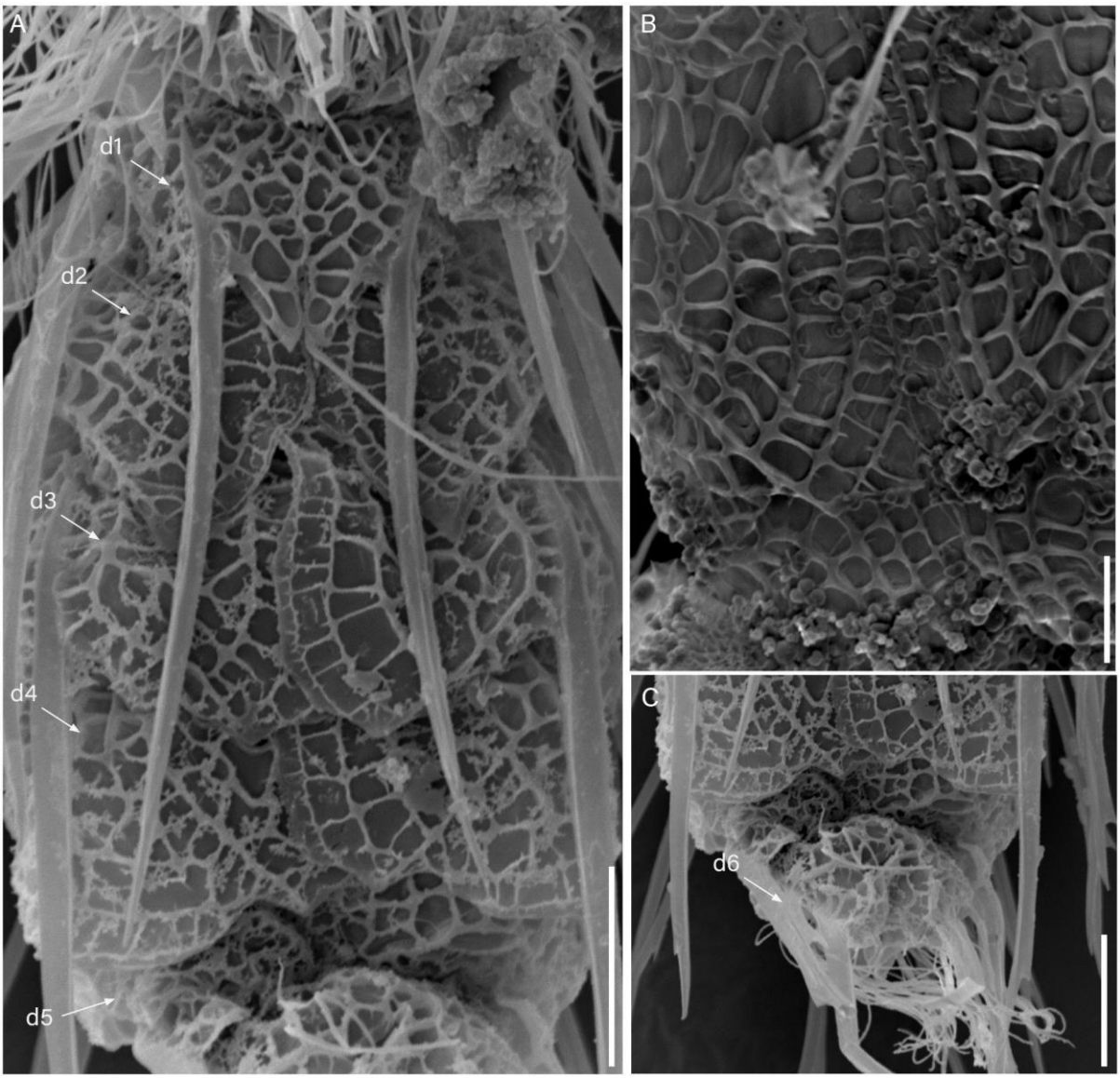

Figure 9. Dorsal scales in scanning electron microscopy of *Ornamentula* sp. nov. **A** dorsall view. d1-d5: dorsal scales. **B** dorsal view. **C** dorsal view. d6: dorsal scale. Scale bars: A - 10 µm..

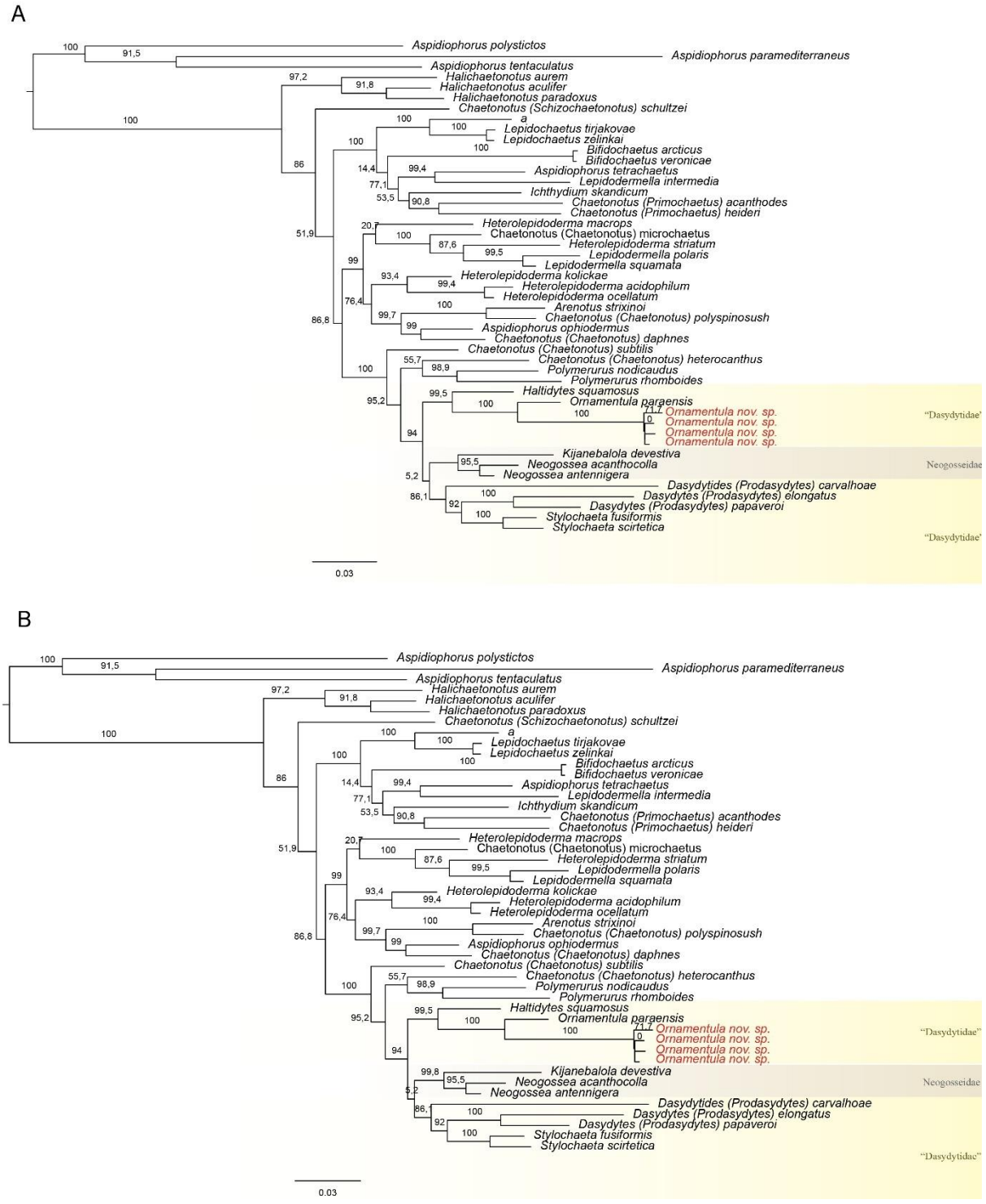

Fig 10. Phylogenetic relationships of Chaetonotida inferred from the maximum likelihood analysis **A** and Bayesian Analyses **B** of the concatenated data set. Yellow: "Dasydytidae", Brown: "Neogosseidae".

SUPPLEMENTARY TABLE 2. Primer sequences and PCR regimes used for 18S, 28S and COI rDNA amplification and sequencing.

Regimes de Primer e PCR	Sequência primer (5' => 3')	Referência
18 S primers		
18SE F	CTGGTTGATCCTGCCAGT	Hillis & Dixon, 1991; see Blanco-Bercial et al., 2011
18SL R	CACCTACGGAAACCTTGTTACGACTT	Hamby & Zimmer, 1988; see Blanco-Berical et al. 2011
F-566 F	CAGCAGCCGCGGTAATTCC	Hadziavdic et al., 2014
R-1200 R	CCCGTGTTGAGTCAAATTAAGC	Hadziavdic et al. 2014
PCR	94 °C/4 min, 35x (95 °C/45 s, 51 °C/60 s, 72 °C/120s), 72 °C for 4 min	-
28 S primers		
28S.2F	GGACCCGAAAGATGGTGAAC	Garraffoni et al., 2019
28S.2R	CAATTTGCCGACTTCCCTTG	Garraffoni et al. 2019
PCR	94°C for 5 min, 40x (94°C for 30 s, 60°C for 30 s, 72°C for 60 s), 72°C for 7 min	-
COI primers		
LCOI490	GGTCAACAAATCATAAAGATATTGG	Folmer et al., 1994
HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. 1994
PCR	94°C/5 min, 45x (94°C/30 s, 46°C/30s, 72°C/40 s), 72°C for 7 min	-

SUPPLEMENTARY TABLE 2. List of taxa used in this study. GenBank accession numbers of 18S rDNA, 28SrDNA and COI sequences. “NA” data not available.

Taxon	18S rDNA	28S rDNA	COI
Arenotus strixinoi	JQ798537	JQ798608	JQ798677
Aspidiophorus ophiodermus	JN185463	JN185510	JN185544
Aspidiophorus paramediterraneus	JQ798538	JQ798609	JQ798678
Aspidiophorus polystictos	JQ798597	JQ798664	JQ798726
Aspidiophorus tentaculatus	JQ798553	JQ798625	JQ798690
Aspidiophorus tetrachaetus	JN185505	JN185540	JN185576
Bifidochaetus arcticus	KP713403	KP713404	KP713406
Bifidochaetus veronicae	MN496207	MN496274	MN493714
Chaetonotus (Chaetonotus) daphnes	JQ798545	JQ798617	JQ798683

Chaetonotus (Chaetonotus) heterocanthus	JQ798543	JQ798615	JQ798681
Chaetonotus (Chaetonotus) microchaetus	JQ798546	JQ798618	JQ798684
Chaetonotus (Chaetonotus) polyspinosus	JQ798563	JQ798633	JQ798698
Chaetonotus (Chaetonotus) subtilis	MF325918	MF325895	MF374700
Chaetonotus (Primochaetus) acanthodes	JQ798544	JQ798616	JQ798682
Chaetonotus (Primochaetus) heideri	JQ798547	JQ798619	JQ798685
Chaetonotus (Schizochaetonotus) schultzei	JQ798596	JQ798663	JQ798725
Dasydytes (Prodasydytes) carvalhoae	JQ798570	JQ798639	JQ798702
Dasydytes (Prodasydytes) elongatus	JQ798568	JQ798638	JQ798700
Dasydytes (Prodasydytes) papaveroi	JQ798571	JQ798640	JQ798703
Halichaetoderma aureum	OQ358145	OQ358134	OQ354337
Halichaetonotus aculifer	JQ798550	JQ798622	JQ798688
Halichaetonotus paradoxus	JQ798599	JQ798666	JQ798728
Haltidytes squamosus	JQ798567	JQ798637	NA
Heterolepidoderma acidophilum	JN185462	JN185509	JN185543
Heterolepidoderma kolickae	OQ358136	OQ358125	OQ354328
Heterolepidoderma macrops	JN185469	JN185515	JN185548
Heterolepidoderma ocellatum	JN185475	JN185519	JN185554
Heterolepidoderma striatum	OQ358140	OQ358129	OQ354332
Ichthydium skandicum	JQ798573	JQ798645	JQ798705
Kijanebalola devestiva	KR822112	KR822117	KR822120
Lepidochaetus brasilense	JN185495	JQ798658	JN185568
Lepidochaetus tirjakovae	MW826075	MW826065	MW824657
Lepidochaetus zelinkai	JN185486	JN185527	JN185564
Lepidodermella intermedia	JN185468	JN185514	JN185547
Lepidodermella polaris	MF325919	MF325900	MF374702
Lepidodermella squamata	JN185472	JN185518	JN185551
Neogossea acanthocolla	KR822114	KR822119	KR822121
Neogossea antennigera	KR822110	KR822116	NA
Ornamentula paraensis	JQ798562	JQ798632	JQ798697
Ornamentula sp. nov	**PQ475800**	**PQ475804**	**PQ462534**
Ornamentula sp. nov	**PQ475801**	**PQ475805**	**PQ462535**
Ornamentula sp. nov	**PQ475802**	**PQ475806**	**PQ462536**
Ornamentula sp. nov	**PQ475803**	**PQ475807**	**NA**
Polymerurus nodicaudus	JN185460	JQ798614	JN185542
Polymerurus rhomboides	JN185467	JN185513	JN185546
Stylochaeta fusiformis	JN185471	JN185517	JN185550
Stylochaeta scirtetica	JN185491	JN185532	JN185566

8. CONSIDERAÇÕES FINAIS

Nossa pesquisa ressalta a importância de abordar os vieses de amostragem e integrar os métodos de delimitação de espécies. O estudo destaca que muitas espécies antes consideradas raras podem simplesmente estar subamostradas. Essa descoberta sugere que o aumento dos esforços de amostragem pode revelar uma imagem mais precisa da diversidade e distribuição das espécies, desafiando a noção de raridade nesses organismos.

Nosso estudo também enfatiza os desafios na identificação de espécies devido ao pequeno tamanho e fragilidade da Gastrotricha, o que complica a coleta e a análise morfológica. Isso geralmente resulta na delimitação imprecisa das espécies e na falta de dados morfológicos abrangentes. A pesquisa aborda esses desafios empregando uma abordagem integrativa, combinando análise morfológica usando contraste de interferência diferencial (DIC) e microscopia eletrônica de varredura (SEM) com dados moleculares dos genes 18S, 28S e COI. Essa abordagem contribui para uma estrutura filogenética mais robusta para o grupo.

Além disso, as descobertas do estudo destacam a necessidade de um esforço de amostragem global mais equilibrado, já que os dados atuais estão fortemente direcionados para o hemisfério norte. Esse viés limita nossa compreensão da verdadeira distribuição e diversidade de Gastrotricha.

Em conclusão, a pesquisa sobre Gastrotricha não apenas avança nossa compreensão da biodiversidade desse filo, mas também enfatiza a importância de abordagens taxonômicas integrativas e esforços globais de amostragem. Essas estratégias são cruciais para superar os desafios atuais na delimitação de espécies e para fornecer uma compreensão mais abrangente das relações evolutivas dentro de Gastrotricha. O estudo serve como um apelo à pesquisa e colaboração contínuas neste campo, garantindo que os tesouros escondidos da biodiversidade sejam totalmente explorados e apreciados.

9. ANEXOS

9.1. Declaração de bioética e Biossegurança

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DECLARAÇÃO

Em observância ao **§5º do Artigo 1º da Informação CCPG-UNICAMP/001/15**, referente a Bioética e Biossegurança, declaro que o conteúdo de minha Dissertação de Mestrado, intitulada "***TAXONOMIA INTEGRATIVA DE ESPÉCIES DE CHAETONOTIDA (GASTROTRICHA) DULCÍCOLAS NO BRASIL***", desenvolvida no Programa de Pós-Graduação em Biologia Animal do Instituto de Biologia da Unicamp, não versa sobre pesquisa envolvendo seres humanos, animais ou temas afetos a Biossegurança.

Assinatura: _____
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Assinatura: _____
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Data: 14/02/2025

9.2. Declaração de Direitos Autorais

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 **TAXONOMIA INTEGRATIVA DE ESPÉCIES DE CHAETONOTIDA (GASTROTRICHA) DULCÍCOLAS NO BRASIL**, não infringem os dispositivos da Lei n.° 9.610/98, nem o direito autoral de qualquer editora.

Campinas, 17 de Fevereiro de 2025

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