

UNIVERSIDADE FEDERAL DO PARANÁ

RICARDO IGNACIO CASTRO ÁLVAREZ

INTEGRATIVE TAXONOMY OF *NAINERIS* BLAINVILLE, 1828
(ANNELIDA, SEDENTARIA, ORBINIIDAE)

PONTAL DO PARANÁ

2023

RICARDO IGNACIO CASTRO ÁLVAREZ

INTEGRATIVE TAXONOMY OF *NAINERIS* BLAINVILLE, 1828
(ANNELIDA, SEDENTARIA, ORBINIIDAE)

Tese apresentada como pré-requisito para a
obtenção do título de Doutor em Sistemas
Costeiros e Oceânicos, Campus Pontal do
Paraná-Centro de Estudos do Mar,
Universidade Federal do Paraná.

Orientadores: Dr. Maikon Di Domenico,
Dr. Paulo Lana
Coorientadora: Dra. Nataliya Budaeva

PONTAL DO PARANÁ

2023

DADOS INTERNACIONAIS DE CATALOGAÇÃO NA PUBLICAÇÃO (CIP)
UNIVERSIDADE FEDERAL DO PARANÁ
SISTEMA DE BIBLIOTECAS – BIBLIOTECA DO CENTRO DE ESTUDOS DO MAR

Álvarez, Ricardo Ignacio Castro

Integrative taxonomy of *Naineris* Blainville, 1828 (Annelida, Sedentaria, Orbiniidae) / Ricardo Ignacio Castro Álvarez. – Pontal do Paraná, 2023.

1 recurso on-line : PDF.

Tese (Doutorado) – Universidade Federal do Paraná, Campus Pontal do Paraná, Centro de Estudos do Mar, Programa de Pós-Graduação em Sistemas Costeiros e Oceânicos.

Orientadores: Prof. Dr. Maikon Di Domenico.

Prof. Dr. Paulo da Cunha Lana.

Coorientadora: Profa. Dra. Nataliya Budaeva.

1. Taxonomia. 2. Cosmopolitismo. I. Di Domenico, Maikon. II. Lana, Paulo da Cunha. III. Budaeva, Nataliya. IV. Universidade Federal do Paraná. Programa de Pós-Graduação em Sistemas Costeiros e Oceânicos. V. Título.

Bibliotecária: Fernanda Pigozzi CRB-9/1151

ATA Nº010

MINUTES OF PUBLIC DEFENSE SESSION OF DISSERTATION TO OBTAIN THE DEGREE OF PHILOSOPHY DOCTOR IN SISTEMAS COSTEÍROS E OCEÂNICOS

On 10/11/2023 at 12:00, in Anfiteatro, Centro de Estudos do Mar, Universidade Federal do Paraná, Campus Pontal do Paraná, Unidade Pontal do Sul of the Federal University of Paraná, the activities pertaining to the rite of defense of work entitled: **Integrative taxonomy of *Naineris Blainville, 1828 (Annelida, Sedentaria, Orbiniidae)*** by RICARDO IGNACIO CASTRO ALVAREZ, were undertaken. The Examining Board, designated by the Faculty of the Graduate Program of the Federal University of Paraná in SISTEMAS COSTEÍROS E OCEÂNICOS was composed of the following Members: NATALIYA E BUDAEVA (UNIVERSITY OF BERGEN), JOANA ZANOL PINHEIRO DA SILVA (UNIVERSIDADE FEDERAL DO RIO DE JANEIRO), FABRICIUS MAIA CHAVES BICALHO DOMINGOS (UNIVERSIDADE FEDERAL DO PARANÁ), LEONARDO SANDRINI NETO (UNIVERSIDADE FEDERAL DO PARANÁ), ANNA ZHADAN (MOSCOW STATE UNIVERSITY), MAIKON DI DOMENICO (UNIVERSIDADE FEDERAL DO PARANÁ). The Board Chair initiated the rites as defined by the Faculty of the Program and, after the remarks of the members of the examining committee and the respective responses were addressed, the final evaluation of the examining board was read, which was decided by APPROVAL. This result should be made official by the Faculty of the Program, through the fulfillment of all recommendation and corrections requested by the examining board within the procedural deadlines defined by the program. The granting of the title of philosophy doctor is contingent upon the fulfillment of all the requirements and terms determined in the regulations of the Graduate Program. There being no further business to transact, the Chair adjourned the session, of which, I, NATALIYA E BUDAEVA, drew up these minutes, which are signed by me and the other members of the Examining Board.

Pontal do Paraná, November 10th, 2023.

Eletronic Signature

20/11/2023 09:19:16.0

NATALIYA E BUDAEVA

President of the Examining Board

Eletronic Signature

20/11/2023 11:16:39.0

JOANA ZANOL PINHEIRO DA SILVA

External Member (UNIVERSIDADE FEDERAL DO RIO DE JANEIRO)

Eletronic Signature

17/11/2023 10:06:13.0

FABRICIUS MAIA CHAVES BICALHO DOMINGOS

External Member (UNIVERSIDADE FEDERAL DO PARANÁ)

Eletronic Signature

17/11/2023 11:35:05.0

LEONARDO SANDRINI NETO

Internal Member (UNIVERSIDADE FEDERAL DO PARANÁ)

Eletronic Signature

17/11/2023 10:51:42.0

ANNA ZHADAN

External Member (MOSCOW STATE UNIVERSITY)

Eletronic Signature

20/11/2023 11:49:39.0

MAIKON DI DOMENICO

Internal Member (UNIVERSIDADE FEDERAL DO PARANÁ)

Av. Beira-Mar, s/n - Pontal do Paraná - Paraná - Brasil

CEP 83255-976 - Tel: (41) 3511-8642 - E-mail: pgsisco@ufpr.br

Documento assinado eletronicamente de acordo com o disposto na legislação federal Decreto 8539 de 08 de outubro de 2015.

Gerado e autenticado pelo SIGA-UFPR, com a seguinte identificação única: 328420

Para autenticar este documento/assinatura, acesse <https://siga.ufpr.br/siga/visitante/autenticacaoassinaturas.jsp>
e insira o código 328420



MINISTÉRIO DA EDUCAÇÃO
REITORIA
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO SISTEMAS COSTEIROS
E OCEÂNICOS - 40001016054P6

APPROVAL MINUTE

The Examining Board is designated by the Faculty of the Graduate Program of the Federal University of Paraná in SISTEMAS COSTEIROS E OCEÂNICOS where invited to argue the DISSERTATION of PHILOSOPHY DOCTOR by **RICARDO IGNACIO CASTRO ALVAREZ**, entitled: **Integrative taxonomy of *Naineris* Blainville, 1828 (Annelida, Sedentaria, Orbiniidae)**, which and after assessment of the candidate and the work, the Examining Board decided for the APPROVAL in the present rite.

The granting of the title of philosophy doctor is contingent upon the fulfillment of all the requirements indicated by the Examining Board and terms determined in the regulation of the Graduate Program.

Pontal do Paraná, November 10th, 2023.

Eletronic Signature
20/11/2023 09:19:16.0
NATALIYA E BUDAEVA
President of the Examining Board

Eletronic Signature
20/11/2023 11:16:39.0
JOANA ZANOL PINHEIRO DA SILVA
External Member (UNIVERSIDADE FEDERAL DO RIO DE JANEIRO)

Eletronic Signature
17/11/2023 10:06:13.0
FABRICIUS MAIA CHAVES BICALHO DOMINGOS
External Member (UNIVERSIDADE FEDERAL DO PARANÁ)

Eletronic Signature
17/11/2023 11:35:05.0
LEONARDO SANDRINI NETO
Internal Member (UNIVERSIDADE FEDERAL DO PARANÁ)

Eletronic Signature
17/11/2023 10:51:42.0
ANNA ZHADAN
External Member (MOSCOW STATE UNIVERSITY)

Eletronic Signature
20/11/2023 11:49:39.0
MAIKON DI DOMENICO
Internal Member (UNIVERSIDADE FEDERAL DO PARANÁ)

To Paulo, for believing in me.

ACKNOWLEDGEMENTS

To the Brazilian people, for allowing me to do an undergraduate degree without paying a cent out of my pocket, as well as a master's, doctorate, and PhD exchange with a scholarship. For maintaining the University Restaurants at most universities, including the wonderful RU at CEM. For all the public policies developed under the PT governments, which, my goodness, we have missed in recent years.

CAPES and its CAPES-Print program for the doctorate and sandwich doctorate scholarships abroad and for the facilities to conduct fieldwork in Paraná and other Brazilian states.

To everyone who accompanied me on this journey. To my first polychaete laboratory at UFRRJ for showing me this beautiful line of research and giving me the essential tools to become a taxonomist. To Ana, Vinícius, Andrielle, Iverson, Joao, Matheus, and everyone else who has helped me sort *Naineris* since my bachelor's.

To the friends I made at CEM's now-defunct Benthos Laboratory, Serena, Andrea, Estela, Aislyn, for all the collections, laughs, adventures, outbursts, quarrels, intrigues and fofocas shared, let's add beach to that.

To the USNM for granting me the Kenneth Jay Boss in Invertebrate Zoology award, which allowed me to visit the northeastern collections of the United States, including those of the YPM, MCZ, and AMNH.

To the LAC-AHF, for the Student Collections Study Award, which allowed me to study Olga Hartman's type material and spend some beautiful days with Leslie and David. Special thanks, of course, to Leslie and David, not only for hosting me but for all the extras I had during the stay, including a Bioblitz in Bodega Bay, visits to Californian vineyards, food of all kinds, collections in Morro Bay and partnerships with local researchers.

To all the curators of the museums I visited during this doctorate and to those of Brazilian and foreign museums for the loans of material. To Joana Zanol and Camila Messias (MNRJ), Monica Petti (ColBIO), Tatiana Steiner and Cecília Amaral (ZUEC-POL). In Mexico to Sergio Salazar-Vallejo (ECOSUR); in the US to Karen Osborn (USNM), Kirk Fitzhugh and Leslie Harris (LAC-AHF), Lily Berniker (AMNH), Eric Lazo-Wasem (YPM), Penny Benson (MCZ), and Gustav

Paulay (FM); in England, Emma Sherlock (NHML); in Australia, Stephen Keable (AM), Robin Wilson (MV), Shirley Sorokin (SAM); and in Germany, Andreas Schmidt-Rhaesa (ZMH) and Birger Neuhaus (ZMB).

To the researchers and partners who provided material for molecular studies, gave tips on finding animals, or helped sampling material. Thanks to Erica Keppel and Gregory Ruiz (SERC), Tony Phillips (SCAMIT), Miguel Meca (UiB), Isabel Carmona (UNMSM), Joao Gabriel (UFRRJ), José Souto (UFPE), Kalina Brauko (UFSC), Wagner Magalhães (UFBA), and Joachim Langeneck (CoNISMa). Sampling in Newport Dunes of specimens from the Invasion lab (SERC) were conducted through agreement No.: P1675035 between the California Department of Fish and Wildlife (CDFW) and SERC.

To Ivison for his partnership in collecting *Naineris* + *Spirobranchus* in the beautiful northeast of Brazil. For the animals, adventures, tapiocas, acarajés, bolo de rolo, cuscuz, cocadas, punhetas, moqueca, arrumadinhos, beers and laughs. For all the friendly people I met on the trips, like Julianna, Teresa Calado, and Wagner.

To the sailors of the CEM, for their willingness to take me to collect worms in the different bays of the CEP. Without their help I wouldn't have got anywhere near the *Naineris* de Pinheiros, how hidden they were!

To my family, especially my mother, for her generosity in letting me go for Brazil for what would only be an undergraduate degree. It has been ten years since then, and I am officially a resident.

To those caring for Margaret (dog), Wendy, and Tigresa (cats) during my trips. To Estela, Aislyn, Yan, Andi, and Kewinyn for taking such good care of them.

To Yan, for his friendship and for being my emotional support in 2022-2. Thank you for trying to make things easier.

To my lab friends in Norway, Martha, Polina, Miguel, and Monisha, for the coffees we shared and the exchange of experiences during the stay in Bergen. To the technicians at the DNA lab, especially Louise, for her training in the lab. I would also like to thank those who lived with me in that exotic house in Bergen, especially Ali and Ruben, for the weekend breakfasts and the multiple mountains climbs I did with Ruben. Mountain climbing has undoubtedly become one of my favorite pastimes, my feed says so.

To Maikon, for having taken on my guidance after the Lannister apocalypse. For his friendship, tips, and willingness to help whenever I needed it.

To Natasha (Nataliya) Budaeva, my co-supervisor. For taking me on a trip to Norway, allowing me to visit the Natural History Museum's collection, arranging the training at DNALab, and encouraging me in the final stretch after all that happened. Thanks for being an excellent person and advisor.

Finally, I would like to thank Paulo, the person responsible for this doctorate. I met Paulo at Vinícius Miranda's defense at UFF, where he was a panel member. Then, I met him a few weeks later at the V Silpoly in Costa Rica. After a presentation on Naineris, he invited me to do a doctorate with him from a different perspective, with an integrative approach. Shortly after starting my doctorate, we participated in the III Brazilian Symposium on Polychaeta with Serena, Andrea, and Estela. Going with Paulo to the congress was the coolest thing in the world. We learned, got to know the world, and laughed our asses off. Wow, how good was this congress! Thank you very much.

Regarding the work, I must admit that adapting to Paulo's hand was challenging. On the first revision of the manuscript, I had to go to the beach with Margaret and a caipirinha to swallow the corrections. That day, I swore that I would try my hardest not to cry for him again and to make him proud of me one day, not out of anger, but for myself. As time went by and the pandemic was declared over, we continued to work, albeit without much data, receiving fewer spankings and one or two compliments.

His support in fieldwork was outstanding, even if it was indirect. Everywhere I went, Paulo had friends who supported me somehow, whether in Pernambuco, Bahia, Rio de Janeiro, or Santa Catarina. Nevertheless, Paulo's influence went beyond Brazil, as he had acquaintances everywhere. Every time I copied him into an e-mail, people would magically reply. How good that power was! I miss him.

Paulo's support was essential whenever I wrote an award proposal, including the exchange fellowship. I remember his insistence that I try the CAPES-Print sandwich, even though I did not have a permanent visa in Brazil. I let a few calls go by because applying for something you did not meet the guidelines did not make sense. However, after his insistence, I decided to try,

totally unconfident. And it worked! Thank you for your insistence and the corrections we made the day before the deadline; they were fundamental to getting the scholarship.

On a Monday, Paulo decided to have a face-to-face meeting in Pontal, where we discussed some aspects of an experiment with the dorsal organs of *Naineris*. The meeting went very well, as we discussed a few missing things in the final stretch. He even held in his hands the holotype of a new species, which he had no name for at the time and which would be our object of study. Unfortunately, it was our last meeting; the next day, he left, and the animal he picked up became a species in his honor, probably the last polychaete he looked at.

I confess that the loss of Paulo was one of the hardest things I've been through, but I can only be grateful for the opportunity to work with him, I was very fortunate. His encouragement and pertinent comments have made me feel today that I have grown into a better person and researcher than the one who entered 2019. Thank you for everything!

*“... ... Cuando me preguntan a qué sector represento
Respondo que en verdad yo no entiendo
El sentimiento de estar ligado a un barrio
Al contrario que el salir de él pa' no ser marginado
... Yo soy ciudadano del planeta tierra
Ser humano que no cree en las fronteras
Tanto Squat, Cenzi, Anita vivieron fuera
Yo igual hermano, ah, ah
No es porque yo quiera, pero mi lugar
Es tanto aquí como donde sea, cuatro puntos cardinales
Cuatro cabezas, verás que la nacionalidad no es la gran cosa
Si no más bien girar con el viento como la rosa.”*

(La rosa de los vientos, Makiza)

RESUMO

Naineris é um gênero cosmopolita da família Orbiniidae Hartman, 1942 que inclui espécies de águas tropicais e temperadas. *Naineris* é reconhecida entre os demais gêneros da família por apresentar prostômio redondo ou truncado e uma clara distinção tórax-abdômen. As três espécies de *Naineris* mais bem estudadas são consideradas cosmopolitas na literatura corrente: *N. setosa* (Verrill, 1900), *N. dendritica* (Kinberg, 1867) e *N. laevigata* (Grube, 1855). Por outro lado, a literatura taxonômica mais recente tem questionado o presumido cosmopolitismo da maioria das espécies de poliquetas, ficando restrito apenas a algumas espécies invasoras e outras do mar profundo. A monofilia de *Naineris* também é controversa. Evidências morfológicas recuperaram *Naineris* como parafilético, mas análises moleculares recuperaram *Naineris* formando um clado junto com *Protoaricia*, ou ainda como parafilético. Frente a este histórico taxonômico, a revisão de *Naineris* com uma abordagem integrativa é necessária para uma melhor compreensão da evolução do gênero e para confirmar ou rejeitar o suposto cosmopolitismo dessas espécies, considerando atributos morfológicos e dados moleculares. No primeiro capítulo, nós realizamos a primeira revisão integrativa de *Naineris*, considerando dados morfológicos e moleculares. Nossa análise molecular incluiu 159 espécimes de *Naineris*, representando 25 espécies coletadas ao longo do globo. As filogenias (ambas Máxima Verossimilhança: MV e métodos de Inferência Bayesiana: IB) baseadas na matriz combinada de dois marcadores mitocondriais (COI e 16S) e um nuclear (28S) resultou em quatro grandes clados bem suportados, cada um suportado por sinapomorfias morfológicas. O clado *Naineris* s. str. inclui *Naineris quadricuspida* (espécie tipo) e compartilha a presença de prostômio arredondado a truncado, papila neuropodial torácica posicionada no meio do lobo, cílios dorsais entre as bases das brânquias, uncinos bífidos dispostos em fileiras múltiplas, ausência de subuncinos, órgãos sensoriais dorsais sempre pareados por segmento, início branquial fixo, com brânquias deslocadas medialmente. O clado *Protoaricia* combinou espécies com prostômio arredondado a truncado, papila neuropodial torácica em uma parte superior do lobo, presença de ciliacão entre as bases das brânquias, uncinos bífidos nunca presentes, uncinos dispostos em uma ou mais fileiras, subuncinos podem estar presentes, órgãos dorsais ovais pares ou múltiplos por segmento, início branquial desde segmentos torácicos ou abdominais, com início fixo ou variável, brânquias deslocadas lateralmente. O clado *Protoaricia* compreende três sub clados, nomeados de acordo a espécie mais bem conhecida de cada um deles, por conveniência. O sub clado “*Protoaricia bicornis*” combina espécies com prostômio redondo a truncado, ciliacão dorsal entre as bases das brânquias, ausência de estatocistos, papila neuropodial torácica em uma parte superior do lobo, presença de uma fileira ventral de uncinos neuropodiais digitiformes no tórax, fileira transversal de uncinos ausente, presença de subuncinos, órgãos sensoriais dorsais ovais e múltiplos por segmento, início branquial nos segmentos torácicos, brânquias torácicas lateralmente deslocadas. O sub clado “*Protoaricia setosa*” inclui três espécie com prostômio arredondado a truncado, ciliacão dorsal entre as bases das branquiais, papila neuropodial torácica em uma parte superior do lobo, ausência de uncinos e subuncinos, órgãos sensoriais dorsais pares e ovais, início branquial desde os segmentos torácicos, branquiais deslocadas

lateralmente, emergindo de um segmento fixo, e o sub clado "*Protoaricia laevigata*" compreende espécie com prostômio redondo a truncado, ciliacão dorsal entre as bases das brânquias, presença de estatocistos, papila neuropodial torácica em uma parte superior do lobo, fileira ventral de uncinos acuminados, fileira transversal de uncinos com pontas não divididas, presença de subuncinos, órgãos sensoriais dorsais ovais e pares por segmento, início branquial nos segmentos torácicos ou abdominais, brânquias torácicas deslocadas lateralmente. Nossos resultados sugerem a polifilia do gênero *Naineris*, com *Naineris* sensu stricto incluindo *N. quadricuspida* e *N. uncinata*, e o resto das espécies agrupadas em um clado separado junto com *Protoaricia*. Após revisão crítica de descrições, redescrições e outros registros, nós consideramos válidas 6 espécies de *Naineris*, 16 de *Protoaricia* e 3 de *Pettibonella*. Nós também transferimos *N. victoriae* para *Pettibonella* baseado em dados morfológicos, incrementando o número de espécies deste gênero a três. Para cada espécie foram fornecidas as listas de sinônimas, locação e número de tombo do material tipo, localidade tipo, distribuição geográfica, habitat, etimologia e disponibilidade de dados moleculares. No segundo capítulo, nós realizamos um estudo comparativo de materiais do complexo *Protoaricia setosa* comb. nov. dos oceanos Pacífico e Atlântico e reexaminamos o material tipo para testar o presumido cosmopolitismo de *P. setosa* comb. nov. A filogenia molecular a análise de delimitação de espécies baseados nos mesmos marcadores revelou a presença de três espécies. Um clado com ampla distribuição Amphi-Atlantica foi atribuído a *Protoaricia setosa* comb. nov. s. str. O Segundo clado do Atlantico restrito ao Sul e Sudeste do Brasil foi descrito como uma espécie nova, *Protoaricia lanai* sp. n. comb. nov. O terceiro clado, reportado no Pacífico Noroeste, foi identificado como uma espécie nova, mas não foi descrito formalmente devido a presença de apenas minhocas de tamanho juvenil no material estudado. Descrições morfológicas detalhadas de vários caracteres diagnósticos no complexo *Protoaricia setosa* comb. nov. foram fornecidas. Finalmente, no terceiro capítulo, nós realizamos uma abordagem integrativa a *Protoaricia laevigata* comb. nov. (Grube, 1855) para testar o seu presumido cosmopolitismo. Nós demonstramos que material sintipo de *Protoaricia laevigata* comb. nov. é uma mistura de duas espécies. Nós redescrevemos e designamos um lectótipo de *Protoaricia laevigata* comb. nov. e reestabelecemos *P. anserina* comb. nov. (Claparède, 1864). Baseado nas sequencias COI, 16S, e 28S de espécimes de diferentes localidades, nós reconstruímos a filogenia utilizando MV e métodos de IB. Os nossos resultados recuperaram quatro clados bem suportados, Florida, Mar Negro, e dois para o Mar Mediterrâneo, representando ambos morfotipos de Grube. A nossa análise reforça que outros registros em todo o mundo precisam ser reavaliados para rejeitar o cosmopolitismo de *P. laevigata* comb. nov.

Palavras-chave: distribuição; cosmopolitismo; taxonomia, marcadores moleculares.

ABSTRACT

Naineris is a cosmopolitan genus of the family Orbiniidae Hartman, 1942 that includes species from tropical to temperate waters. *Naineris* is distinguished for having a rounded or truncate prostomium and a clear distinction between thorax and abdomen. The three best-studied species of *Naineris* are considered cosmopolitan in literature: *N. setosa* (Verrill, 1900), *N. dendritica* (Kinberg, 1867), and *N. laevigata* (Grube, 1855); however, most of the "cosmopolitan species" are rare, being restricted to only a few invasive species and others from the deep sea. The monophyly of *Naineris* is also disputable. Morphological evidence recovered *Naineris* as paraphyletic, whereas molecular analysis recovered *Naineris* in a clade with *Protoaricia*, as polyphyletic. Facing this taxonomic history, the revision of *Naineris* with an integrative approach is necessary to better understand the evolution of the genus and to assess the distribution patterns of valid species, considering their morphology and molecular data. In the first chapter we performed the first integrative revision of *Naineris*, based on morphology and molecular data. Our molecular analysis includes 159 specimens of *Naineris* representing 25 species collected worldwide. Phylogeny (both Maximum Likelihood: ML and Bayesian Inference: BI methods) based on the combined matrix of two mitochondrial (COI and 16S) and one nuclear (28S) marker results in four large and well supported clades, each supported by morphological synapomorphies. Clade *Naineris* s. str. includes *Naineris quadricuspida* (type species) and shares the presence of rounded to truncate prostomium, thoracic neuropodial papilla in the middle of lobe, presence of dorsal cilia between branchial bases, bifid uncini arranged in several rows, absence of subuluncini, dorsal sensory organs always paired, branchial start fixed with branchiae medially displaced. Clade *Protoaricia* contained species with rounded to truncate prostomium, thoracic neuropodial papilla in upper part of lobe, presence of dorsal cilia between branchial bases, uncini never bifid, uncini arranged in one or two rows, subuluncini may be present, oval-shaped dorsal sensory organs paired or multiple per segment, branchial start from thoracic or abdominal segments, branchial start fixed or variable, with branchiae laterally displaced. *Protoaricia* clade comprises three subclades, named after the best-known species of each of them for convenience. "*Protoaricia bicornis*" subclade combined species with rounded to truncate prostomium, dorsal cilia between branchial bases, absence of statocysts, thoracic neuropodial papilla in upper part of lobe, ventral row of digitiform thoracic neuropodial uncini, transversal row of uncini absent, subuluncini present, dorsal sensory organs oval-shaped and multiple per segment, brachial start from thoracic segments, thoracic branchiae laterally displaced. "*Protoaricia setosa*" subclade included species with rounded to truncate prostomium, dorsal cilia between branchial bases in abdominal segments, thoracic neuropodial papilla in an upper position, thoracic neuropodial uncini and subuluncini absent, paired and oval-shaped dorsal sensory organs, branchial start from thoracic segments, branchiae laterally displaced, emerging from a fixed segment, and "*Protoaricia laevigata* subclade" was comprised of species with rounded to truncate prostomium, dorsal cilia between branchial bases, presence of statocysts, thoracic neuropodial papilla in upper part of lobe, ventral row of acuminate thoracic neuropodial uncini, transversal row of uncini with undivided tips, subuluncini present, dorsal sensory organs oval-shaped and

paired per segment, brachial start from thoracic or abdominal segments, thoracic branchiae laterally displaced. Our results suggest polyphyly of the genus *Naineris* with *Naineris sensu stricto* including *N. quadricuspida* and *N. uncinata*, and the rest of the species grouped in a separate clade with *Protoaricia*. After critical revision of descriptions, redescrptions, and other records, we considered 6 species of *Naineris*, and 16 species of *Protoaricia* as valid. We also transferred *N. victoriae* to *Pettibonella* based on morphological data increasing the number of species in the genus up to 3. For each species, we provide a synonym list, location, and catalog number of type material, type locality, geographic distribution, habitat, etymology, and availability of molecular data. In the second chapter, we performed a comparative study of the materials of the *Protoaricia setosa* comb. nov. complex from the Pacific and the Atlantic Oceans and re-examined the type material to test the presumed cosmopolitanism of *P. setosa* comb. nov. Molecular phylogenetic and species delimitation analyses based on the same three markers revealed the presence of three species. One clade with wide Amphi-Atlantic distribution was attributed as *Protoaricia setosa* comb. nov. s. str. The second Atlantic clade restricted to Southern and South-eastern Brazil was described as a new species, *Protoaricia lanai* sp. n. comb. nov. The third clade, reported from the Northwestern Pacific, was identified as a new species but was not formally described due to the presence of only juvenile-sized worms in the studied material. Detailed morphological descriptions of several diagnostic characters in the *Protoaricia setosa* comb. nov. complex is provided. Finally, in the third chapter, we performed an integrative approach to *Protoaricia laevigata* comb. nov. (Grube, 1855) to test its presumed cosmopolitanism. We demonstrate that the syntype material of *P. laevigata* comb. nov. comprises a mixture of two species. We redescribe and designate a lectotype for *P. laevigata* comb. nov and reinstate *Protoaricia anserina* comb. nov. (Claparède, 1864). Based on sequences COI, 16S, and 28S of specimens from different localities, we reconstructed a phylogeny utilizing ML and BI methods. Our result recovered four supported clades, Florida, the Black Sea, and two for the Mediterranean Sea, representing both Grube morphotypes. Our analysis reinforces that other records worldwide need to be reevaluated to reject the cosmopolitanism of *P. laevigata* comb. nov.

Keywords: distribution; cosmopolitanism; taxonomy; molecular markers.

LIST OF ABBREVIATIONS

AM	- Australian Museum, Australia
AMNH	- American Museum of Natural History, USA
CoIBIO	- Coleção Biológica Prof. Edmundo F. Nonato, Brazil
ECOSUR	- El Colegio de la Frontera Sur, Chetumal, Mexico
FM	- The Florida Museum of Natural History, USA
LACM-AHF Poly	- Natural History Museum of Los Angeles County, USA
MCZ	- Museum of Comparative Zoology, USA
MNRJP	- Museu Nacional do Rio de Janeiro, Brazil
MV	- Museums Victoria, Australia
NHML	- Natural History Museum London, England
SAM	- South Australian Museum, Australia
USNM	- Smithsonian National Museum of Natural History, USA
YPM	- Yale Peabody Museum of Natural History, USA
ZMH	- Zoologische Museum Hamburg, Germany
ZMB	- Museum für Naturkunde Berlin, Germany
ZMBN	- Invertebrate collection University Museum of Bergen, Norway
ZUEC-POL	- Museu de Diversidade Biológica, Zoologia, IB-UNICAMP, Brazil
FORV	-FORV Referral Centre, Centre for Marine Living Resources and Ecology, Cochin, Kerala, India

TABLE OF CONTENTS

1.	INTRODUCTION	17
2.	LITERATURE REVIEW OF CONCEPTS USED FOR INTEGRATIVE TAXONOMY OF <i>NAINERIS</i>.....	22
2.1.	PHYLOGENY OF <i>NAINERIS</i>	22
2.2.	REPRODUCTION IN <i>NAINERIS</i>	23
2.3.	INTEGRATIVE TAXONOMY	24
2.4.	SPECIES CONCEPT.....	26
2.5.	CRYPTIC SPECIES	26
2.6.	COSMOPOLITANISM	27
2.7.	REFERENCES	28
3.	CHAPTER I. PHYLOGENY OF <i>NAINERIS</i> BLAINVILLE, 1828 (ANNELIDA, SEDENTARIA, ORBINIIDAE) AND TRANSFER OF SPECIES TO <i>PROTOARICIA</i> AND <i>PETTIBONELLA</i>	41
3.1.	INTRODUCTION	44
3.2.	MATERIAL AND METHODS	46
3.2.1	Sample collection and morphology	46
3.2.2	Molecular dataset	47
3.2.3	Characters optimization	49
3.2.4	Morphological characters.....	49
3.2.5	Checklist	59
3.2.6	Terminology and abbreviations.....	61
3.3.	RESULTS	62
3.2.7	Molecular data analyses	62
3.2.8	Taxonomical review	72
3.4.	DISCUSSION	102
3.5.	CONCLUSION.....	108
3.6.	REFERENCES	108
3.7.	SUPPLEMENTARY MATERIALS	123
4.	CHAPTER II. HOW COMPLEX IS THE <i>NAINERIS</i> SETOSA SPECIES COMPLEX? FIRST INTEGRATIVE STUDY OF A PRESUMED COSMOPOLITAN AND INVASIVE ANNELID (SEDENTARIA: ORBINIIDAE)	
	161	
4.1.	INTRODUCTION	162

4.2.	MATERIAL AND METHODS	163
4.2.1	Sampling.....	163
4.2.2	Morphological study.....	164
4.2.3	Molecular analyses	166
4.3.	RESULTS	177
4.3.1	Phylogenetic reconstruction.....	177
4.3.2	Genetic distances	183
4.3.3	Taxonomic account.....	185
4.4.	DISCUSSION	199
4.5.	REFERENCES	204
5.	CHAPTER III. FIRST INTEGRATIVE APPROACH TO <i>PROTOARICIA</i>	
	<i>LAEVIGATA</i> (GRUBE, 1855). BUT WHICH OF THEM?	212
5.1.	INTRODUCTION	213
5.2.	MATERIAL AND METHODS	214
5.2.1	Sample collection and morphological analysis	214
5.2.2	DNA analysis	215
5.2.3	DNA extraction, amplification, and sequencing	215
5.2.4	Sequence alignment, phylogenetic analysis	219
5.3.	RESULTS	220
5.2.5	Phylogenetic reconstruction.....	220
5.2.6	Genetic distances	220
5.2.7	Taxonomy.....	223
5.4.	DISCUSSION	232
5.5.	REFERENCES	233
6.	CONCLUSION.....	236

1. INTRODUCTION

Orbiniids are a broad distributed family of marine annelids placed in Sedentaria (Bleidorn and Helm, 2019). They are found from shallow water to deep-sea habitats (Imajima, 2009; Blake, 2020). In shallow waters, they occur in sandy beaches, rocky shores, mangroves, and salt marshes, whereas in the deep sea, they can be found in gas hydrates and seep sediments (Bleidorn and Helm, 2019). In these habitats, the orbiniids are non-selective mobile deposit-feeder and act as bioturbators (Giangrande, 2002) by ingesting food particles, detritus, and associated organisms (Parkinson, 1978; Giangrande et al., 2001), as diatomaceous and foraminifera (Parkinson, 1978) adopting different strategies depending on the type of sediment (Francoeur and Dorgan, 2014).

The family comprises 20 valid genera (Bleidorn and Helm, 2019), distributed in four subfamilies: Methanoarticiinae, Orbiniinae, Microrbiniinae and Questinae (Read and Fauchald, 2022). Even though molecular studies question Blake's classification and the monophyly of most genera (Bleidorn, 2005; Bleidorn et al., 2009; Zhadan et al., 2015), four subfamilies are still accepted (Read and Fauchald, 2022).

Orbiniids size ranges from 4 to 300 mm, generally with an abrupt transition between a dorsoventrally compressed thorax and a cylindrical abdomen. The synapomorphy of the family is the shifted position of the parapodia in abdominal segments (Fauchald & Rouse, 1997). Moreover, the family is diagnosed by the presence of crenulated capillary chaetae, vascularized gills, hairy capillary chaetae in the distal region, lateral parapodia in the thorax and dorsal in the abdomen (Solís-Weiss et al., 2009). The prostomium is conic, as most of genera, round or square. Many taxa may possess eyes and paired chemosensory nuchal organs. The peristomium consists of one to two peristomial rings. The everted proboscis can be sack-like, dendritic or multilobed. The parapodia are biramous, sometimes with underdeveloped parapodial lobes, with capillary chaetae with simple or furcate tips, as well as acicular spines and uncini (Beesley et al., 2000).

Naineris Blainville, 1828 is among the genera recovered as paraphyletic (Blake 2000, Zhadan et al. 2015) *Naineris* is a cosmopolitan genus, with several species also showing wide geographical ranges. The species of *Naineris* have a

rounded or spatulate prostomium, with one or two rings in peristomium, thorax comprising 12–30 segments and branchiae between 2–23 chaetigers. Thoracic neurochaetae include crenulated capillaries, uncini, and subuluncini. The abdominal notochaetae can include furcate chaetae in addition to capillaries (Fauchald, 1977; Solis-Weiss et al., 2009; Blake, 2017).

To date, 20 species are considered valid (Read and Fauchald, 2022). The differentiation of species of *Naineris* is based mainly on the number of thoracic segments, the number of the segment in which the branchiae first appears, the shape of the parapodia, and morphology of the chaetae, as in the rest of the family (Solis-Weiss et al., 2009; Blake, 2017; Eisig, 1914).

Among the twenty species of *Naineris*, some have unsatisfactory descriptions for today's standards of taxonomic work, making their identification difficult, as seen in the first species descriptions of *Naineris* in Fabricius (1780) and Müller (1858). In some cases, the holotype is missing, as occurs in *Naineris brevicephala* Hartmann-Schröder, 1960 (Blake, 2017) and *Naineris aurantiaca* (Müller, 1858) (Pettibone, 1957; Álvarez et al., 2019). Other species have multiple records at different latitudes and oceans, despite the evident morphological differences in the descriptions themselves (Blake and Giangrande, 2011; Dean and Blake, 2015; Blake, 2017). Some orbiniids have been confirmed cosmopolitan based on molecular data, such as *Proscoloplos cygnochaetus* Day, 1954 (Meyer et al., 2008), although more recently some have been recognized as species complexes, such as *Scoloplos armiger* Müller, 1776 (Bleidorn et al., 2003; Kruse and Reise, 2003; Bleidorn et al., 2006). Due to this, the diversity of the orbiniids can be underestimated, being necessary to study the presumed cosmopolitan species, case by case (Bleidorn et al. 2009). For instance, in the case of Brazil, five species of *Naineris* have been recorded:

- (i) *N. dendritica* (Kinberg, 1866),

N. dendritica (type locality: Vancouver Island) (Disclaimer: as a result of Chapter 1 this species will be transferred to *Protoaricia*), a widely distributed species recorded in Brazil (Souza, 2006). The most recent taxonomical record of the species was in Southern California, 1.800 kilometers far from its type locality (Blake, 1996). It was described with the presence of different types of chaetae, missing the description of the chaetal arrangement in thoracic neuropodia (Blake, 1996).

(ii) *N. bicornis* Hartman, 1951,

Naineris bicornis (type locality: Gulf of Mexico) (Disclaimer: as a result of Chapter 1 this species will be transferred to *Protoaricia*), was distinguished from its congeners by the presence of two horns in anterior margins of prostomium. This prostomium shape, however, appears to be a fixation artifact, as can be seen when comparing alive and preserved animals from other species (Eisig, 1914; Álvarez et al., 2019). The original description does not furnish details on chaetal morphology.

(iii) *N. laevigata* (Grube, 1855),

N. laevigata (type locality: Naples) (Disclaimer: as a result of Chapter 1 this species will be transferred to *Protoaricia*), was recorded in Florida, Hawaii, and Peru. These descriptions show high variation in taxonomical characters, including the number of thoracic chaetigers and the chaetiger where branchiae first appeared (Hartman, 1957). Despite the detailed original description, morphology of uncini was neglected, currently observed under light microscopy, or scanning electron microscopy (SEM). In the Orbiniidae revision, Hartman (1957) stated that *N. laevigata* and *N. dendritica* are distinguished by the disjoint geographic distribution, the shape of thoracic neuropodial lobes and morphology of uncini.

(iv) *N. setosa* (Verrill, 1900),

Naineris setosa (type locality: Bermuda) (Disclaimer: as a result of Chapter 1 this species will be transferred to *Protoaricia*), is unique in having only crenulate capillaries in thoracic neuropodia. Despite the species being recorded in different oceans, its descriptions differ in several publications (Blake e Giangrande, 2011; Blake, 2017; Dean and Blake, 2015).

(v) *N. aurantiaca* (Müller, 1858),

N. aurantiaca (type locality: Desterro = Santa Catarina Island, Southern Brazil) (Disclaimer: as a result of Chapter 1 this species will be transferred to *Protoaricia*), was never recorded again since its description in 1858 (As a result of Chapter 1 this species will be transferred to *Protoaricia*). The original description is brief and poorly illustrated, and there is no type material deposited in museum collections. Recently the species was redescribed and a neotype was designated (Álvarez et al., 2019). Even though the status of *N. aurantiaca* was

reviewed, little is known about the biology of the species, which was not sequenced yet (Amaral et al. 2006, OBIS 2019, Pagliosa et al. 2014, Read & Fauchald 2022).

In addition to the species recorded in Brazil, there is *N. brevicephala* (type locality: Chorrillos, Peru), described without details of chaetae. Recently, the holotype was examined and found that there is a syllid inside the vial instead (Blake, 2017). Hence, Blake suggested the species should be treated as *incertae sedis* until located. Since then, the species is considered as *taxon inquirenda* in Worms Database (Read and Fauchald, 2023).

Detailed taxonomic descriptions of certain diagnostic characters would improve the knowledge on the records of species of *Naineris* worldwide, avoid misidentifications, and reevaluating the taxonomic status of the species. Some diagnostic characters show intraspecific and ontogenetic variation, but there are few studies focused on the development of *Naineris* species. Giangrande and Petraroli (1991) concluded that the chaetiger where branchiae first appear in *N. laevigata*, usually used as a diagnostic character, undergoes variations throughout the growth of the animals, which could explain the variation found in some species observed by Hartman (1957). The number of thoracic chaetigers also changes throughout development, as observed by Hoffmann and Hausen (2007) in *Scoloplos armiger*. Controversies also exist over the number of peristomial rings in the family; the presence of two peristomial rings has been observed in juveniles of *N. laevigata* (Giangrande and Petraroli, 1991) and *N. dendritica* (Blake, 1996) and in adults of *N. argentinensis* (Blake, 2017) and *N. quadricuspida* (Pettibone, 1963), with one peristomial ring traditionally being attributed to adults of *Naineris* (Hartman, 1957). Therefore, studies on the development of other species of the genus, in addition to *N. laevigata*, can assess the real suitability of the diagnostic characters currently used.

The monophyly of *Naineris* has been questioned in several studies. Blake (2000) performed a phylogenetic analysis of orbinids based on morphology including *Naineris quadricuspida* and *Naineris* sp, resulting in the paraphyly of *Naineris*. Later, molecular analyses of Bleidorn (2005) and Bleidorn et al. (2009) of gene fragments 16S, 18S, cox1, cox3, nad1 e nad4, recovered *Naineris* in a clade with *Protoaricia*, which comprises also *N. quadricuspida*, *N. laevigata* e *N. dendritica*. Zhadan et al. (2015) molecular analyses included COI fragments of

N. australis and recovered *Naineris* as polyphyletic. Although evidence supports the paraphyly of *Naineris*, only very few species have available sequences, so the genus is still considered valid (Bleidorn e Helm, 2019; Read e Fauchald 2023).

Facing this taxonomic history, this thesis revised the genus *Naineris* with integrative taxonomy, providing the delimitation of the species of the genus with a revision of the diagnostic characters, the inclusion of new taxonomical characters, associating sequences from their type localities to deposited material, and checking dubious records of the species of *Naineris*. In greater detail, the species of *Naineris* from the Brazilian coast were reviewed, using phenotypic (analysis of morphological characters) and genotypic (species delimitation based on phylogenetic inferences from molecular characters) information, as a way of analyzing the congruence between traditional taxonomic and the integrative approaches. The outcome of the thesis is:

- To carry out a taxonomic revision of *Naineris* species, based on their morphological characters (Chapter I);
- To investigate differential characters of specimens from the same taxonomic entity (belonging to different populations), using scanning electron microscopy, in order to detect candidates for possible cryptic species, which could be subject to further molecular analysis (Chapter I, II);
- To reconstruct the phylogenetic relationships of *Naineris* with integrative taxonomy, testing the monophyly of the *Naineris* + *Protoaricia* clade proposed by Bleidorn (2005, 2009) (Chapter I);
- To study the evolution of morphological characters optimized on the molecular phylogeny (Chapter I);
- To test the hypothesis of cosmopolitan distribution in selected species of *Naineris* (Chapter II, III);
- To describe species unknown to science based on combination of morphology and molecular data (Chapter II).

2. LITERATURE REVIEW OF CONCEPTS USED FOR INTEGRATIVE TAXONOMY OF *NAINERIS*

2.1. PHYLOGENY OF *NAINERIS*

The phylogenetic position of *Naineris* within Orbiniidae considering trees based on morphological and molecular characters was contrasting (Bleidorn et al., 2009). In one of the first revisions of the family, Hartman (1957) separated the orbiniids into the subfamilies Orbiniinae and Protoariciinae, using the number of peristomial rings as the main character, with *Naineris* placed within the Orbiniinae. Later, Blake (1996) questioned Hartman's (1957) division and proposed that some genera and species of Protoariciinae could be juveniles of Orbiniinae since the presence of two peristomial rings had been found in juveniles of *N. laevigata* and *N. dendritica*.

Blake (2000) then carried out a phylogenetic analysis of the family at genus level, using the type species of most genera as terminals. The species of *Naineris* used were the type species: *Naineris quadricuspida* Fabricius (1780), and *Naineris* sp. The result was two trees with different phylogenetic relationships within *Naineris*. Considering the number of peristomial rings as character, *Naineris quadricuspida* and *Naineris* sp. formed a monophyletic group. However, disregarding the number of peristomial rings, *Naineris* was paraphyletic. *Naineris quadricuspida* would be a sister group of *Protoariciella* Hartmann-Schröder, 1962, while *Naineris* sp. would be a sister group of a large clade containing several other genera. The genera *Leodamas* Kinberg, 1866 and *Methanoaricia* Blake, 2000 were erected, and the orbiniids were rearranged into the subfamilies Methanoariciinae, Orbiniinae and Microrbiniinae, considering the regionalization of the body and disregarding the number of peristomial rings. In this way, *Naineris* would also be included in the Orbiniinae. However, the support of these clades was questionable (Bleidorn, 2005).

Bleidorn (2005), using exclusively molecular data, reconstructed the internal relationships of the orbiniids. With the 18S nuclear and 16S mitochondrial rRNA sequences, *Protoaricia* and *Naineris* formed a monophyletic group with

high support, considering that *Protoaricia* (belonging to *Protoariciinae*) being a valid taxon, instead of representing juveniles of *Naineris*, as proposed by Blake (1996), which was corroborated after observing ovate specimens of *Protoaricia oerstedii* (Claparède, 1864).

By contrasting phylogenies based on morphological and molecular characters, Bleidorn et al. (2009) reconstructed the relationships within the family, once again recovering the clade (*Naineris* + *Protoaricia*) as the only monophyletic in the molecular analysis. Multiple polytomies and low support were obtained when only morphological characters were considered. The results of Bleidorn et al. (2009) corroborated the hypotheses supporting the *Naineris* + *Protoaricia* clade, although only a few species were studied (three species of *Naineris* and one of *Protoaricia* out of a total of 20 and 3, respectively).

Subsequently, Zhadan et al. (2015) sequenced the COI of *Naineris australis* Hartman, 1957 indicating that the species does not form a clade with the other species of *Naineris* and *Protoaricia*. However, considering the 16S and 18S genes, *N. australis* would belong to an unresolved clade that includes *Naineris*, *Phylo*, *Orbinia*, and some species from other genera, thus indicating that *Naineris* was paraphyletic.

An integrative approach, with a more significant number of species of *Naineris* (or all of them, if possible), will make it possible to clarify the internal relationships of the group as a way of determining whether *Naineris* is effectively a monophyletic group, according to the Bleidorn et al. (2009) or paraphyletic, according to Blake (2000) and Zhadan et al. (2015).

2.2. REPRODUCTION IN NAINERIS

Only two studies of *Naineris* reproduction are carried out with different populations of *Naineris laevigata*. Okuda (1946) studied a population in Japan, while Giangrande and Petraroli (1991) studied a population close to the type locality in the Mediterranean.

Okuda (1946) described that the eggs of *N. laevigata* form a ribbon-like cluster coated with a delicate film rather than a gelatinous mass as in other species of the family. This film containing the fertilized eggs is arranged on the

surface of the mud and adheres to the surrounding vegetation. In contrast to Okuda (1946), Giangrande and Petraroli (1991) observed that the Mediterranean population does form a gelatinous mass of eggs, like other orbiniid species. This single gelatinous mass per female, would have the function of protecting the eggs from predation and desiccation at low tides, given their large size (240µm).

Giangrande and Petraroli (1991) described some aspects of reproduction in greater detail than Okuda (1946). They observed that *N. laevigata* is a gonochoric species and that reproduction occurs synchronously within the population, unlike Okuda (1946), who described the reproduction of the Japanese population as asynchronous. Giangrande and Petraroli (1991) observed that the species does not show sexual dimorphism except when gametes accumulate in the coelomic cavity; they become evident through transparency at the base of the parapodia. In males, gametes are released into the coelom in the early stages of development; in females, oogenesis takes around seven months and is intraovarian. The larvae are of the lecithotrophic type, with a short life span. In addition, Giangrande and Petraroli (1991) recorded a reproductive effort of ~30% of the female's body volume, as well as a dormant stage in the face of unfavorable conditions.

These different reproductive patterns suggest that it is likely that these researchers worked with a different species. Thus, studying the embryonic development of species of *Naineris* in depth would not only help assess the suitability of diagnostic characters but would also allow us to detect biological and behavioral differences between different species.

2.3. INTEGRATIVE TAXONOMY

Taxonomy is the science that identifies, describes, classifies, and names biodiversity. It is estimated that since the implementation of Linnaean binomial nomenclature, two million species have been described (Stork, 1997), corresponding to only one-fifth of the planet's total biodiversity (Padial et al., 2010). Although the number of species described seems low, it is believed that the number of species currently considered valid may be even lower due to the various problems that morphological taxonomy has generated over time, such as

the high number of synonyms yet to be resolved (Stork, 1997). It is believed that the large number of these and other taxonomic errors is due in part to the lack of knowledge we have about intraspecific variations and the centralization of biological collections, which often prevents the comparison of specimens with standard material, leading to equivocal identifications (Stork, 1997).

In addition to the unfavorable ratio between described and undescribed species, there is an aggravating factor: the so-called taxonomic impediment or impasse. This states that under current conditions, it is challenging to know all the species on the planet (Salazar-Vallejo et al., 2018). Justifications for the taxonomic impediment include the low number of taxonomists, the uneven distribution of human and material resources around the world (Lana, 2003; Rodman and Cody, 2003), inefficient scientific practices, taxonomic production that is of little use to the public (Rodman and Cody, 2003) and the lack of reference collections (Lana, 2003). Among the recommendations that have been proposed to tackle this problem, we should increase the general population's knowledge of biodiversity and optimize the critical mass of researchers with the creation of research networks and taxonomic workshops that enable the training of new scientists and the cataloging of biodiversity at regional levels (Lana, 2003).

Despite the material difficulties and the scarcity of human resources, significant technological advances have promoted the revival of taxonomy as a basic science over the last few decades, making it more effective in dealing with taxonomic impediments. Among the new advances are the facilities for remote access to scientific collections, the improvement of DNA sequencing techniques, computer tomography, and geographic information systems, and the digitization of taxonomic literature due to globalization (Padial et al., 2010). This multidisciplinary approach is now called integrative taxonomy (Wake, 2003), or as defined by Dayrat (2005), "the science that delimits the unit of the diversity of life from multiple complementary perspectives, such as phylogeography, comparative morphology, population genetics, ecology, development, and behavior, among others".

2.4. SPECIES CONCEPT

Traditionally, species have been defined as the fundamental unit of biological diversity (Mayr, 1982; Barraclough, 2019) and are considered the practical unit of comparison between various fields of biology, such as anatomy, ethology, ontogeny, ecology, genetics, and systematics (de Queiroz, 2007). However, there is no consensus on the concept, with at least 34 different definitions (Zachos, 2018), which vary widely and are even incompatible with each other, including the biological, ecological, evolutionary, cohesion, phylogenetic, phenotypic, and genotypic cluster concepts (de Queiroz, 2007). Although there is a great deal of divergence in the definition of species, they all converge in that a species corresponds to lineages of metapopulations in independent evolution, thus giving rise to the concept of a unified species (de Queiroz, 2007).

In the present work, a species was approached not only as groups of organisms phenotypically and genetically different from other groups, evolving separately (Barraclough, 2019), but also as a hypothesis to be tested on the basis of as much evidence as possible, including morphological, genetic and ecological information whenever possible (Dayrat, 2005).

2.5. CRYPTIC SPECIES

Cryptic species are formally distinct, morphologically similar, or identical species (Struck et al., 2018), which occur in most phyla of living beings (Nygren, 2003, Jörger and Schrödl, 2013).

Cryptic species have been widely used but have different meanings and determination methods (Struck et al., 2018). In order to standardize and improve the delineation of cryptic species, Struck et al. (2018) proposed quantifying the extent of phenotypic disparity by comparing it with the degree of genetic divergence in order to differentiate true species complexes from taxonomic bias or malpractice (Struck and Cerca 2019).

Among the processes that could result in cryptic species are recent divergence, convergence, parallelism, and stasis (Swift et al., 2016; Struck et al., 2018). Recent divergence occurs when species need more time to accumulate phenotypic differences. In convergence and parallelism, cryptic species have developed identical phenotypes independently; the difference is that in convergence the most recent ancestors are dissimilar to each other and current species, whereas in parallelism, the ancestors are similar to each other but different from current species. Finally, in stasis, there is a retention of similar phenotypes for millions of years (Struck and Cerca, 2019).

This work will test for possible cryptic species, according to Struck and Cerca (2019), contrasting phenotypic diversity with genetic diversity. A species was considered cryptic when it exhibited a statistically lower degree of phenotypic divergence than non-cryptic species, given a particular divergence time from the most recent common ancestor.

2.6. COSMOPOLITANISM

Cosmopolitan species occur in all geographical areas of the planet (Hutchings and Kupriyanova, 2018). In the case of marine species, cosmopolitan would be species distributed throughout the oceans (Cox et al., 2016). However, for a species to be genuinely cosmopolitan, it must maintain gene flow throughout its distribution (Klautau et al. 1999), overcoming the barriers imposed by large ocean basins. In the marine environment, Spalding et al. (2007) identified 12 kingdoms, 62 provinces, and 232 biogeographical regions, which would largely explain the patterns observed in marine biodiversity, shaped by evolutionary history, dispersal, and isolation.

Until the 1980s, dozens of species of marine annelids were considered cosmopolitan since taxonomists from all over the world identified the global fauna based on identification keys from the northern hemisphere, mainly Europe. Over time, these taxonomic routines generated erroneous records for marine annelids from all regions of the world and, therefore introduced biases into what we know about the geographical distribution of species (Hutchings and Kupriyanova, 2018).

Many current studies show that cosmopolitan "polychaetes" exist, but mainly in the deep sea or shallow water fouling species (Hutchings and Kupriyanova, 2018), which is not the case with orbinids. These studies have shown that many species previously considered cosmopolitan correspond to complexes of morphologically close but perfectly distinct species (Hutchings and Kupriyanova, 2018). One of such cases has been confirmed within orbinids for a small, presumably invasive species, *Proscoloplos cygnochaetus* Day, 1954, which extremely wide distribution was explained by its capacity to anchor on vessels using mucous glands and chaetae possibly in combination with architomic reproduction (Meyer et al. 2008). Several species of *Naineris* currently have cosmopolitan status. Nevertheless, this should be tested based on a combination of molecular data and comparative morphological studies.

Five distribution patterns associated with "cosmopolitanism" are currently recognized: eucosmopolitan, which refers to species widely distributed due to natural processes; neocosmopolitan, which corresponds to cosmopolitans generated as a result of anthropogenic influence; provincial, native species found nowhere else or widely distributed in a biogeographic province; regionally introduced, species introduced outside their native range but within the same ocean; pseudocosmopolitan, which present multiple genetic lineages for a single taxon, keeping a single name (Darling and Carlton, 2018).

We would like to test if three *Naineris* species currently considered cosmopolitan in the literature, such as *N. setosa*, *N. dendritica* and *N. laevigata* (Hartman, 1948; 1957; Taylor, 1984; Blake and Giangrande 2011; Khedhri et al., 2014; Dean and Blake, 2015; Atzori et al., 2016, Choi et al., 2016; Zhadan, 2020), should be considered as such. According to Darling and Carlton (2018), they would all be species with unresolved cosmopolitanism, as even the most recent comparative studies have not explored their structure in detail, and molecular data has not yet been adequately generated and interpreted.

2.7. REFERENCES

ÁLVAREZ, R. C.; MIRANDA, V. R.; BRASIL A. C. D. B. Redescription of *Naineris aurantiaca* (Müller, 1858) and designation of a neotype from the Brazilian coast (Annelida: Orbinidae). **Zootaxa**, v. 4571, n. 1, p. 125–136, 25 mar. 2019.

Disponível em: <https://www.mapress.com/zt/article/view/zootaxa.4571.1.8>. Acesso em: 27 jul. 2023. DOI. [10.11646/zootaxa.4571.1.8](https://doi.org/10.11646/zootaxa.4571.1.8).

AMARAL, A.C.Z.; NALLIN, S.A.H.; STEINER, T.M.; FORRONI, T. O. & GOMES-FILHO, D.; ARAÚJO, G. R.; FREITAS, R.; COSTA, C.A.O.; RUTA, C.; GOMES, K.R.E. & BONALDO, R.O. **Catálogo das espécies de Annelida Polychaeta do Brasil**. 2006-2022. Disponível em: <https://intranet.ib.unicamp.br/intranet/polychaeta/apresentacao.php>. Acesso em: 27 jul. 2020.

ATZORI, G.; LÓPEZ, E.; ADDIS, P.; SABATINI, A.; CABIDDU, S. First record of the alien polychaete *Naineris setosa* (Scolecida; Orbiniidae) in Tyrrhenian Sea (Western Mediterranean). **Marine Biodiversity Records**, v. 9, n. 5, p. 1–6, 05 abr. 2016. Disponível em: <https://mbr.biomedcentral.com/articles/10.1186/s41200-016-0017-6>. Acesso em: 27 jul. 2023. DOI. [10.1186/s41200-016-0017-6](https://doi.org/10.1186/s41200-016-0017-6).

AUDOUIN, J. V.; MILNE-EDWARDS, H. Classification des Annélides et descriptions de celles qui habitent les côtes de la France. **Annales des Sciences Naturelles Paris**, v. 1, n. 29, p. 388–412, 1833. Disponível em: <https://www.biodiversitylibrary.org/part/8010>. Acesso em: 27 jul. 2023.

BARRACLOUGH, T.G. **The Evolutionary Biology of Species**. Oxford: Oxford University Press, 2019.

BLAINVILLE, H. de. **Dictionnaire des Sciences Naturelles**, 1828.

BLAKE, J.A. Chapter 1. Family Orbiniidae Hartman, 1942. In: BLAKE, J. A. HILBIG, B. & SCOTT, P.H. (Ed.). **Taxonomic Atlas of the Santa Maria Basin and Western Santa Barbara Channel**. Vol. 6. Annelida Part 3. Polychaeta: Orbiniidae to Cossuridae. Santa Barbara Museum of Natural History, Santa Barbara, California, 1998. p. 1–26.

BLAKE, J. A. A new genus and species of polychaete worm (Family Orbiniidae) from methane seeps in the Gulf of Mexico, with a review of the systematics and phylogenetic interrelationships of the genera of Orbiniidae. **Cahiers de Biologie Marine**, v. 41, p. 435–449, 2000.

BLAKE, J. A. Polychaeta Orbiniidae from Antarctica, the Southern Ocean, the Abyssal Pacific Ocean, and off South America. **Zootaxa**, v. 4218, n. 1, p. 1–145, 12 jan. 2017. Disponível em: <https://www.biotaxa.org/Zootaxa/article/view/zootaxa.4218.1.1>. Acesso em: 27 jul. 2023. DOI. [10.11646/zootaxa.4218.1.1](https://doi.org/10.11646/zootaxa.4218.1.1)

BLAKE, J. A. New species and records of deep-water Orbiniidae (Annelida, Polychaeta) from the Eastern Pacific continental slope, abyssal Pacific Ocean, and the South China Sea. **Zootaxa**, v. 4730, n. 1, p. 1–61, 3 fev. 2000. Disponível em: <https://www.mapress.com/zt/article/view/zootaxa.4730.1.1>. Acesso em: 27 jul. 2023. DOI. [10.11646/zootaxa.4730.1.1](https://doi.org/10.11646/zootaxa.4730.1.1).

BLAKE, J. A.; GIANGRANDE, A. *Naineris setosa* (Verrill) (Polychaeta, Orbiniidae), an American subtropical-tropical polychaete collected from an aquaculture facility in Brindisi (Adriatic Sea, Italy): A possible alien species. **Italian Journal of Zoology**, v. 78, p. 20–26, 25 jul. 2011. Disponível em: <https://www.tandfonline.com/doi/full/10.1080/11250003.2011.577982>. Acesso em: 27 jul. 2023. DOI. [10.1080/11250003.2011.577982](https://doi.org/10.1080/11250003.2011.577982).

BLEIDORN, C. Phylogenetic relationships and evolution of Orbiniidae (Annelida, Polychaeta) based on molecular data. **Zoological Journal of the Linnean Society**, v. 144, p. 59–73, 10 mai. 2005. Disponível em: <https://academic.oup.com/zoolinnean/article/144/1/59/2626917>. Acesso em: 27 jul. 2023. DOI. [10.1111/j.1096-3642.2005.00160.x](https://doi.org/10.1111/j.1096-3642.2005.00160.x).

BLEIDORN, C.; VOGT, L.; BARTOLOMAEUS, T. New insights into polychaete phylogeny (Annelida) inferred from 18S rDNA sequences. **Molecular Phylogenetics and Evolution**, v. 29, n. 2 p. 279–288, nov. 2003. Disponível em: <https://www.sciencedirect.com/science/article/abs/pii/S1055790303001076?via%3Dihub>. Acesso em: 27 jul. 2023. DOI. [10.1016/S1055-7903\(03\)00107-6](https://doi.org/10.1016/S1055-7903(03)00107-6).

BLEIDORN, C.; KRUSE, I.; ALBRECHT, S.; BARTOLOMAEUS, T. Mitochondrial sequence data expose the putative cosmopolitan polychaete *Scoloplos armiger* (Annelida, Orbiniidae) as a species complex. **BMC Evolutionary Biology**, v. 6, p. 1–13, 15 jun. 2006. Disponível em: <https://bmcecol.evol.biomedcentral.com/articles/10.1186/1471-2148-6-47>. Acesso em: 27 jul. 2023. DOI. [10.1186/1471-2148-6-47](https://doi.org/10.1186/1471-2148-6-47).

BLEIDORN, C.; HILL, N.; ERSÉUS, C.; TIEDEMANN, R. On the role of character loss in orbiniid phylogeny (Annelida): Molecules vs. morphology. **Molecular Phylogenetics and Evolution**, v. 52, p. 57–69, jul. 2009. Disponível em: <https://www.sciencedirect.com/science/article/abs/pii/S1055790309001018?via%3Dihub>. Acesso em: 27 jul. 2023. DOI. [10.1016/j.ympev.2009.03.022](https://doi.org/10.1016/j.ympev.2009.03.022).

BLEIDORN, C.; HELM, C. 7.1.4 Orbiniidae Hartman, 1942. In: PURSCHKE, G., BÖGGEMANN, M. e WESTHEIDE, W. (Ed.), **Handbook of Zoology**. Annelida. Vol. 1. Annelida Basal groups and Pleistoannelida, Sedentaria I. DeGruyter, Berlin, 2019. p. 251–269.

CARR, C. M.; HARDY, S. M.; BROWN, T. M.; MACDONALD, T. A.; HEBERT, P. D. N. A tri-oceanic perspective: DNA barcoding reveals geographic structure and cryptic diversity in Canadian polychaetes. **PLoS ONE**, v. 6, n. 7, 14 jul. 2011. Disponível em: <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0022232>. Acesso em: 27 jul. 2023. DOI. [10.1371/journal.pone.0022232](https://doi.org/10.1371/journal.pone.0022232).

CHOI, H. K.; KIM, J. G.; YOON, S. M. Two new Records of Scolecid Polychaetes (Annelida: Polychaeta: Scolecida) in Korean Fauna. **Animal Systematics, Evolution and Diversity**, v. 32, n. 4, p. 266–271, 31 out. 2016. Disponível em: <http://koreascience.or.kr/article/JAKO201634864496459.page>. Acesso em: 27 jul. 2023. DOI. [10.5635/ased.2016.32.4.039](https://doi.org/10.5635/ased.2016.32.4.039).

CLAPARÈDE, E. Glanures zootomiques parmi les annélides de Port-Vendres (Pyrénées Orientales). **Mémoires de la Société de physique d'histoire naturelle de Genève**, v. 17, p. 463–600, 1864. Disponível em: <https://www.biodiversitylibrary.org/bibliography/1972>. Acesso em: 27 jul. 2023.

CLEMENT, M.; POSADA, D.; CRANDALL, K. A. TCS: A computer program to estimate gene genealogies. **Molecular Ecology**, v. 9, p. 1657–1660, 25 dec. 2001. Disponível em: <https://onlinelibrary.wiley.com/doi/abs/10.1046/j.1365-294x.2000.01020.x>. Acesso em: 27 jul. 2023. DOI. [10.1046/j.1365-294x.2000.01020.x](https://doi.org/10.1046/j.1365-294x.2000.01020.x)

COX, C. B.; MOORE, P. D.; LADLE, R. J. **Biogeography: an ecological and evolutionary approach**. Chichester, UK; Hoboken, NJ: John Wiley & Sons, 2016.

DARLING, J. A.; CARLTON, J. T. A framework for understanding marine cosmopolitanism in the Anthropocene. **Frontiers in Marine Science**, v. 5, p. 293, 22 ago. 2018. Disponível em: <https://www.frontiersin.org/articles/10.3389/fmars.2018.00293/full>. Acesso em: 27 jul. 2023. DOI. [10.3389/fmars.2018.00293](https://doi.org/10.3389/fmars.2018.00293).

DAY, J. H. The Polychaeta of Tristan da Cunha. **Results of the Norwegian Scientific Expedition to Tristan da Cunha 1937-1938**, v. 29, p. 1-35, 1954. Disponível em: <https://www.marinespecies.org/polychaeta/aphia.php?p=sourcedetails&id=49610>. Acesso em: 27 jul. 2023.

DAYRAT, B. Towards integrative taxonomy. **Biological Journal of the Linnean Society**, v. 85, n. 3, p. 407–415, 24 jun. 2005. Disponível em: <https://www.marinespecies.org/polychaeta/aphia.php?p=sourcedetails&id=49610>. Acesso em: 27 jul. 2023. DOI. [10.1111/j.1095-8312.2005.00503.x](https://doi.org/10.1111/j.1095-8312.2005.00503.x)

DEAN, H. K.; BLAKE, J.A. The Orbiniidae (Annelida: Polychaeta) of Pacific Costa Rica. **Zootaxa**, v. 3956, n. 2, p. 183–198, 8 mai. 2015. Disponível em: <https://www.biotaxa.org/Zootaxa/article/view/zootaxa.3956.2.2>. Acesso em: 27 jul. 2023. DOI. [10.11646/zootaxa.3956.2.2](https://doi.org/10.11646/zootaxa.3956.2.2).

DE QUEIROZ, K. Species concepts and species delimitation. **Systematic Biology**, v. 56, n. 6, p. 879–886, 1 dez. 2007. Disponível em: <https://academic.oup.com/sysbio/article/56/6/879/1653163>. Acesso em: 27 jul. 2023. DOI. [10.1080/10635150701701083](https://doi.org/10.1080/10635150701701083).

EISIG, H. Zur Systematik, Anatomie und Morphologie der Arciiden nebst Beiträgen zur generellen Systematik. **Mitteilungen aus der Zoologischen Station Neapel**, v. 21, p. 153–600, 1914. Disponível em: <https://www.biodiversitylibrary.org/item/181156#page/7/mode/1up>. Acesso em: 27 jul. 2023.

FABRICIUS, O. **Fauna Groenlandica**. Hafniae et Lipsiae. 1780

FAUCHALD, K. **The Polychaete Worms. Definitions and Key to the orders, families and genera.** Natural History Museum of Los Angeles County, Science Series, 28, 1–190, 1977.

FELSENSTEIN, J. Confidence limits on phylogenies: an approach using the bootstrap. **Evolution**, v. 39, n. 4, p. 783–791, jul. 1985. Disponível em: <https://www.jstor.org/stable/2408678?origin=crossref>. Acesso em: 27 jul. 2023. DOI. [10.2307/2408678](https://doi.org/10.2307/2408678).

FITZHUGH, K. **A protocol for preparing polychaetes for SEM using HMDS.** p. 1–2, 2011.

FOLMER, O.; BLACK, M.; HOEH, W.; LUTZ, R.; VRIJENHOEK, R. DNA primers for amplification of mitochondrial cytochrome c oxidase I from diverse metazoan invertebrates. **Molecular Marine Biology and Biotechnology**, v. 3, n. 5, p. 294–299, out. 1994. Disponível em: https://www.mbari.org/wp-content/uploads/2016/01/Folmer_94MMBB.pdf. Acesso em: 27 jul. 2023.

FRANCOEUR, A. A.; DORGAN, K. M. Burrowing behavior in mud and sand of morphologically divergent polychaete species (Annelida: Orbiniidae). **The Biological Bulletin**, v. 226, n. 2, p. 131–145, 2014. Disponível em: <https://www.journals.uchicago.edu/doi/10.1086/BBLv226n2p131>. Acesso em: 27 jul. 2023. DOI. [10.1086/BBLv226n2p131](https://doi.org/10.1086/BBLv226n2p131).

GIANGRANDE, A.; PETRAROLI, A. Reproduction, larval development and post-larval growth of *Naineris laevigata* (Polychaeta, Orbiniidae) in the Mediterranean Sea. **Marine Biology**, v. 111, n. 1, p. 129–137, oct. 1991. Disponível em: https://link.springer.com/article/10.1007/BF01986354?awc=26429_1690483661_933ceadc9450c90f7b3a25b5bc5ef741&utm_medium=affiliate&utm_source=a_win&utm_campaign=CONR_BOOKS_ECOM_DE_PHSS_ALWYS_DEEPLINK&utm_content=textlink&utm_term=1035097. Acesso em: 27 jul. 2023. DOI. [10.1007/BF01986354](https://doi.org/10.1007/BF01986354).

GIANGRANDE, A.; LICCIANO, M.; FANELLI, G. Bioturbation behaviour in two Mediterranean polychaetes. **Journal of the Marine Biological Association of the United Kingdom**, v. 81, n. 2, p. 341–342, 14 jun. 2001. Disponível em: <https://www.cambridge.org/core/journals/journal-of-the-marine-biological-association-of-the-united-kingdom/article/abs/bioturbation-behaviour-in-two-mediterranean-polychaetes/F7E3C470FF78BCF3C04E8E574CF8132A>. Acesso em: 27 jul. 2023. DOI. [10.1017/S0025315401003836](https://doi.org/10.1017/S0025315401003836).

GIANGRANDE, A.; MONTRESOR, M.; CAVALLO, A.; LICCIANO, M. Influence of *Naineris laevigata* (Polychaeta: Orbiniidae) on vertical grain size distribution, and dinoflagellate resting stages in the sediment. **Journal of Sea Research**, v. 47, n. 2, p. 97–108, mar. 2002. Disponível em: <https://www.sciencedirect.com/science/article/abs/pii/S1385110101001034?via%3Dihub>. Acesso em: 27 jul. 2023. DOI. [10.1016/S1385-1101\(01\)00103-4](https://doi.org/10.1016/S1385-1101(01)00103-4).

GLASBY, C.J. Orbiniidae. In: Beesley, P. L.; Ross, G. J. B.; Glasby, C. J (Ed.). **Polychaetes and allies: the southern synthesis. Fauna of Australia.** Vol. 4A

Polychaeta, Myzostomida, Pogonophora, Echiura, Sipuncula. CSIRO publishing, 2000.

GRUBE, A.E. Beschreibungen neuer oder wenig bekannter Anneliden. **Archiv für Naturgeschichte**, Berlin, v. 21, p. 81–128, 1855.

HALL, T.A. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In: **Nucleic acids symposium series** (Vol. 41, No. 41, pp. 95-98). [London]: Information Retrieval Ltd., c1979-c2000, 1999.

HARTMAN, O. The marine annelids erected by Kinberg with some notes on some other types in the Swedish State Museum. **Arkiv för Zoologi**. v. 42A, n. 1, p. 1–137, 1948.

HARTMAN, O. The littoral marine annelids of the Gulf of Mexico. **Publications of the Institute of Marine Science, University of Texas**, v. 2, n. 1, p. 7–124, set. 1951. Disponível em: <https://repositories.lib.utexas.edu/handle/2152/22162>. Acesso em: 27 jul. 2023.

HARTMAN, O. Orbiniidae, Apistobranchidae, Paraonidae and Longosomidae. **Allan Hancock Pacific Expeditions**, v. 15, n. 3, p. 211–393, 1957. Disponível em: <https://www.biodiversitylibrary.org/part/7981>. Acesso em: 27 jul. 2023.

HARTMANN-SCHRÖDER, G. Zur Polychaetenfauna von Peru. Teil I. **Beiträge Zur Neotropischen Fauna**, v. 2, n. 1, p. 1–44, 1960. Disponível em: <https://www.biodiversitylibrary.org/part/7981>. Acesso em: 27 jul. 2023. DOI. [10.1080/01650526009380621](https://doi.org/10.1080/01650526009380621).

HARTMANN-SCHRÖDER, G. Zweiter Beitrag zur Polychaetenfauna von Peru. **Kieler Meersforschungen**, v. 18, n. 1, p. 109–147, 1962. Disponível em: <https://oceanrep.geomar.de/id/eprint/55635/>. Acesso em: 27 jul. 2023.

HOFFMANN, S.; HAUSEN, H. Chaetal arrangement in Orbiniidae (Annelida, Polychaeta) and its significance for systematics. **Zoomorphology**, v. 126, p. 215–227, 2007. Disponível em: https://link.springer.com/article/10.1007/s00435-007-0042-4?awc=26429_1690486416_1a367a2fae09f0269fe74184ff332b91&utm_medium=affiliate&utm_source=awin&utm_campaign=CONR_BOOKS_ECOM_DE_PH_SS_ALWYS_DEEPLINK&utm_content=textlink&utm_term=1018583. Acesso em: 27 jul. 2023. DOI. [10.1007/s00435-007-0042-4](https://doi.org/10.1007/s00435-007-0042-4).

HUTCHINGS, P.A.; KUPRIYANOVA, E. Cosmopolitan polychaetes—fact or fiction? Personal and historical perspectives. **Invertebrate Systematics**, v. 32, n. 1, p. 1–9, 8 jan. 2018. Disponível em: <https://www.publish.csiro.au/is/IS17035>. Acesso em: 27 jul. 2023. DOI. [10.1071/IS17035](https://doi.org/10.1071/IS17035).

JÖRGER, K.M. & SCHRÖDL, M. How to describe a cryptic species? Practical challenges of molecular taxonomy. **Frontiers in Zoology**, v. 10, p. 1–27, 27 set.

2013. Disponível em: <https://frontiersinzoology.biomedcentral.com/articles/10.1186/1742-9994-10-59>. Acesso em: 27 jul. 2023. DOI. [10.1186/1742-9994-10-59](https://doi.org/10.1186/1742-9994-10-59).

KATOH, K.; KUMA, K. I.; TOH, H.; MIYATA, T. MAFFT version 5: Improvement in accuracy of multiple sequence alignment. **Nucleic Acids Research**, v. 33, n. 2, p. 511–518, jan. 2005. Disponível em: <https://academic.oup.com/nar/article/33/2/511/2549118>. Acesso em 28 jul. 2023. DOI. [10.1093/nar/gki198](https://doi.org/10.1093/nar/gki198).

KHEDHRI, I.; LAVESQUE, N.; BONIFÁCIO, P.; DJABOU, H. AFLI, A. First record of *Naineris setosa* (Verrill, 1900) (Annelida: Polychaeta: Orbiniidae) in the Western Mediterranean Sea. **BioInvasions Records**, v. 3 n. 2, p. 83–88, jun. 2014. Disponível em: <https://www.reabic.net/journals/bir/2014/Issue2.aspx>. Acesso em: 27 jul. 2023. DOI. [10.3391/bir.2014.3.2.05](https://doi.org/10.3391/bir.2014.3.2.05).

KINBERG, J. Annulata nova. **Öfversight af Kungliga Vetenskaps–Adakemiens Förhandlingar, Stockholm**, v. 22, p. 239–258.

KLAUTAU, M.; RUSSO, C. A. M.; LAZOSKI, C.; BOURY-ESNAULT, N.; THORPE, J. P.; SOLÉ-CAVA, A. M. Does cosmopolitanism result from overconservative systematics? A case study using the marine sponge *Chondrilla nucula*. **Evolution**, v. 53, n. 5, p. 1414–1422, 1 out. 1999. Disponível em: <https://www.reabic.net/journals/bir/2014/Issue2.aspx>. Acesso em: 27 jul. 2023. DOI. [10.1111/j.1558-5646.1999.tb05406.x](https://doi.org/10.1111/j.1558-5646.1999.tb05406.x).

KRUSE, I.; REISE, K. Reproductive isolation between intertidal and subtidal *Scoloplos armiger* (Polychaeta, Orbiniidae) indicates sibling species in the North Sea. **Marine Biology**, v. 143, p. 511–517, 18 jun. 2003. Disponível em: <https://link.springer.com/article/10.1007/s00227-003-1112-x>. Acesso em: 27 jul. 2023. DOI. [10.1007/s00227-003-1112-x](https://doi.org/10.1007/s00227-003-1112-x).

KUEHN, E., STOCKINGER, A.W., GIRARD, J., RAIBLE, F., e ÖZPOLAT, B.D. (2019). A scalable culturing system for the marine annelid *Platynereis dumerilii*. bioRxiv, 714238. Disponível em: <https://www.biorxiv.org/content/10.1101/714238v1>. Acesso em: 27 jul. 2023. DOI. [10.1101/714238](https://doi.org/10.1101/714238).

KUMAR, S.; STECHER, G.; LI, M.; KNYAZ, C.; TAMURA, K. MEGA X: molecular evolutionary genetics analysis across computing platforms. **Molecular biology and evolution**, v. 35, n. 6, p. 1547–1549, jun. 2018. Disponível em: <https://academic.oup.com/mbe/article/35/6/1547/4990887>. Acesso em: 27 jul. 2023. DOI. [10.1093/molbev/msy096](https://doi.org/10.1093/molbev/msy096).

LANA, P.D.C. O valor da biodiversidade e o impasse taxonômico: a diversidade marinha como estudo de caso. **Desenvolvimento e Meio Ambiente**, n. 8, p. 96–104, jul./dez. 2003. Disponível em: <https://revistas.ufpr.br/made/article/view/22058>. Acesso em: 27 jul. 2023. DOI. [10.5380/dma.v8i0.22058](https://doi.org/10.5380/dma.v8i0.22058).

LE, H. L. V.; LECOINTRE, G.; PERASSO, R. A 28S rRNA-Based Phylogeny of the Gnathostomes: First Steps in the Analysis of Conflict and Congruence with Morphologically Based Cladograms. **Molecular Phylogenetics and Evolution**, v. 2, n. 1, p. 31–51, mar. 1993. Disponível em: <https://www.sciencedirect.com/science/article/abs/pii/S1055790383710055?via%3Dihub>. Acesso em: 27 jul. 2023. DOI. [10.1006/mpev.1993.1005](https://doi.org/10.1006/mpev.1993.1005).

LEIDY, J. Contributions towards a knowledge of the marine invertebrate fauna of the coasts of Rhode Island and New Jersey. **Journal of the Academy of Natural Sciences of Philadelphia**, v. 2, n. 3, 135–152, 1855. Disponível em: <https://www.biodiversitylibrary.org/part/245184>. Acesso em: 27 jul. 2023.

LEIGH, J. W.; BRYANT, D. POPART: full-feature software for haplotype network construction. **Methods in ecology and evolution**, v. 6, n. 9, p. 1110–1116, 2015. Disponível em: <https://besjournals.onlinelibrary.wiley.com/doi/10.1111/2041-210X.12410>. Acesso em: 27 jul. 2023. DOI. [10.1111/2041-210X.12410](https://doi.org/10.1111/2041-210X.12410).

LINNAEUS, C. **Systema Naturae**. Laurentii Salvii: Stockholm, 1758.

MADDISON, W.P.; MADDISON, D.R. MESQUITE: a modular system for evolutionary analysis. **Evolution**, v. 62, p. 1103-1118., 2006. Disponível em : <http://mesquiteproject.org>.

MAYR, E. **The growth of biological thought: Diversity, evolution, and inheritance**. Cambridge, MA: Harvard University Press, 1982.

MEYER, A.; BLEIDORN, C.; ROUSE, G. W.; HAUSEN, H. Morphological and molecular data suggest a cosmopolitan distribution of the polychaete *Proscoloplos cygnochaetus* Day, 1954 (Annelida, Orbiniidae). **Marine Biology**, v. 153, p. 879–889, fev. 2008. Disponível em: <https://link.springer.com/article/10.1007/s00227-007-0860-4>. Acesso em: 27 jul. 2023. DOI. [10.1007/s00227-007-0860-4](https://doi.org/10.1007/s00227-007-0860-4).

MINH, B. Q.; SCHMIDT, H. A.; CHERNOMOR, O.; SCHREMPF, D.; WOODHAMS, M. D.; VON HAESELER, A.; LANFEAR, R. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. **Molecular biology and evolution**, v. 37, n. 5. p. 1530–1534, mai. 2020. Disponível em: <https://academic.oup.com/mbe/article/37/5/1530/5721363>. Acesso em: 27 jul. 2023. DOI. [10.1093/molbev/msaa015](https://doi.org/10.1093/molbev/msaa015).

MÜLLER, O.F. **Zoologiae Danicae Prodromus, seu Animalium Daniae et Norvegiae indigenarum characteres, nomina, et synonyma imprimis popularium**. Havniae. 1776, xxxii + p. 282.

MÜLLER, F. Einiges über die Annelidenfauna der Insel Santa Catharina an der brasilianischen Küste. **Archiv für Naturgeschichte**, v. 24, p. 211–220, 1858. Disponível em: <https://www.biodiversitylibrary.org/page/7460059#page/233/mode/1up>. Acesso em: 27 jul. 2023.

NYGREN, A. Cryptic polychaete diversity: A review. **Zoologica Scripta**, v. 43, n. 2, p. 172–183, mar. 2014. Disponível em: <https://onlinelibrary.wiley.com/doi/abs/10.1111/zsc.12044>. Acesso em: 27 jul. 2023. DOI. [10.1111/zsc.12044](https://doi.org/10.1111/zsc.12044).

MÚRIAS DOS SANTOS, A.; CABEZAS, M.P.; TAVARES, A.I.; XAVIER, R.; BRANCO, M. TcsBU: A tool to extend TCS network layout and visualization. **Bioinformatics**, v. 32, n. 4, p. 627–628, fev. 2016. Disponível em: <https://academic.oup.com/bioinformatics/article/32/4/627/1744448>. Acesso em: 27 jul. 2023. DOI. [10.1093/bioinformatics/btv636](https://doi.org/10.1093/bioinformatics/btv636).

NGUYEN, L.T.; SCHMIDT, H.A.; VON HAESELER, A.; MINH, B.Q. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. **Molecular Biology and Evolution**, v. 32, n. 1, p. 268–274, jan. 2015. Disponível em: <https://academic.oup.com/mbe/article/32/1/268/2925592>. Acesso em: 27 jul. 2023. DOI. [10.1093/molbev/msu300](https://doi.org/10.1093/molbev/msu300).

NYGREN, A.; EKLÖF, J.; PLEIJEL, F. Arctic-boreal sibling species of *Paranaitis* (Polychaeta, Phyllodocidae). **Marine Biology Research**, v. 5, n. 4, p. 315–327, 16 jun. 2009. Disponível em: <https://www.tandfonline.com/doi/full/10.1080/17451000802441301>. Acesso em: 27 jul. 2023. DOI. [10.1080/17451000802441301](https://doi.org/10.1080/17451000802441301).

Ocean Biogeographic Information System (OBIS). Disponível em: <https://iobis.org/explore/#/taxon/474993>. Acesso em: 20 jul. 2019.

OKUDA, S. Studies on the development of Annelida Polychaeta I. **Journal of the Faculty of Science, Hokkaido Imperial University**, v. 9, n. 2, p. 115–219, 1946. Disponível em: https://eprints.lib.hokudai.ac.jp/dspace/bitstream/2115/27055/1/9%282%29_P1_15-219.pdf. Acesso em: 27 jul. 2023.

PADIAL, J.M.; MIRALLES, A.; DE LA RIVA, I.; VENCES, M. The integrative future of taxonomy. **Frontiers in zoology**, v. 7, n. 1, p. 1-14, 25 mai. 2010. Disponível em: <https://frontiersinzoology.biomedcentral.com/articles/10.1186/1742-9994-7-16>. Acesso em: 27 jul. 2023. DOI. [10.1186/1742-9994-7-16](https://doi.org/10.1186/1742-9994-7-16).

PAGLIOSA, P. R.; DORIA, J. G.; MISTURINI, D.; OTEGUI, M. B. P.; OORTMAN, M. S.; WEIS, W. A.; FARONI-PEREZ, L.; ALVES, A. P.; CAMARGO, M. G.; AMARAL, A. C. Z.; MARQUES, A. C.; LANA, P. DA C. NONATObase: A database for Polychaeta (Annelida) from the Southwestern Atlantic Ocean. **Database**, 2014.

PALUMBI, S. R. Nucleic acids II: the polymerase chain re- action. In: Hillis DM, Moritz C, Mable BK (Ed.) **Molecular systematics**, 2nd edn. Sunderland, MA: Sinauer, 1996, p. 205–247.

PARADIS, E. Pegas: An R package for population genetics with an integrated-modular approach. **Bioinformatics**, v. 26, n. 3, p. 419–420, fev. 2010. Disponível

em: <https://academic.oup.com/bioinformatics/article/26/3/419/215731>. Acesso em: 27 jul. 2023. DOI. [10.1093/bioinformatics/btp696](https://doi.org/10.1093/bioinformatics/btp696).

PARKINSON, G.T. Aspects of feeding, burrowing, and distribution of *Haploscoloplos elongatus* (Polychaeta: Orbiniidae) at Bodega Harbor, California. **Pacific Science**, v. 32, n. 2, p. 149–155, abr. 1978. Disponível em: <https://scholarspace.manoa.hawaii.edu/server/api/core/bitstreams/29bd7e7d-7d55-429c-b90a-baa63d997e38/content>. Acesso em: 27 jul. 2023.

PASSAMANECK, Y. J.; SCHANDER, C.; HALANYCH, K. M. Investigation of molluscan phylogeny using large-subunit and small-subunit nuclear rRNA sequences. **Molecular Phylogenetics and Evolution**, v. 32, n. 1, p. 25-38, jul. 2004. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S105579030400034X>. Acesso em: 27 jul. 2023. DOI. [10.1016/j.ympev.2003.12.016](https://doi.org/10.1016/j.ympev.2003.12.016).

PETTIBONE, M. H. North American genera of the family Orbiniidae (Annelida: Polychaeta), with descriptions of new species. **Journal of the Washington Academy of Sciences**, v. 47, n. 5, p. 159-167, mai. 1957. Disponível em: <https://www.biodiversitylibrary.org/part/70659>. Acesso em: 27 jul. 2023.

PETTIBONE, M. H. Marine polychaete worms of the New England region. **Bulletin of the United States National Museum**, v. 227, n. 1, p. 1–346, 1963. Disponível em: <https://repository.si.edu/handle/10088/3416>. Acesso em: 27 jul. 2023.

PULLANDRE, N.; LAMBERT, A.; BROUILLET, S.; ACHAZ, G. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. **Molecular Ecology**, v. 21, n. 8, p. 1864–1877, abr. 2012. Disponível em: <https://onlinelibrary.wiley.com/doi/10.1111/j.1365-294X.2011.05239.x#:~:text=Automatic%20Barcode%20Gap%20Discovery%20proposes,on%20an%20underlying%20genealogical%20tree>. Acesso em: 27 jul. 2023. DOI. [10.1111/j.1365-294X.2011.05239.x](https://doi.org/10.1111/j.1365-294X.2011.05239.x).

READ, G.B.; FAUCHALD, K. World Register of Marine Species. Disponível em: <http://www.marinespecies.org/polychaeta>. Acesso em 25 Jul. 2023.

RODMAN J. E.; CODY J. H. The taxonomic impediment overcome: NSF's Partnerships for Enhancing Expertise in Taxonomy (PEET) as a model. **Systematic Biology**, v. 52, n. 3, p. 428–435, jun. 2003. Disponível em: <https://www.jstor.org/stable/3651119>. Acesso em: 25 Jul 2023.

RONQUIST, F.; TESLENKO, M.; VAN DER MARK, P.; AYRES, D. L.; DARLING, A.; HÖHNA, S.; LARGET, B.; LIU, L.; SUCHARD, M. A.; HUELSENBECK, J.P. Mrbayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. **Systematic Biology**, v. 61, p. 539–542, mai. 2012. Disponível em: <https://academic.oup.com/sysbio/article/61/3/539/1674894>. Acesso em: 27 jul. 2023. DOI. [10.1093/sysbio/sys029](https://doi.org/10.1093/sysbio/sys029).

ROUSE, G. W.; FAUCHALD, K. Cladistics and polychaetes. **Zoologica Scripta**, v. 26, n. 2, p. 139-204, abr. 1997. Disponível em: <https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1463-6409.1997.tb00412.x>. Acesso em: 27 jul. 2023. DOI. [10.1111/j.1463-6409.1997.tb00412.x](https://doi.org/10.1111/j.1463-6409.1997.tb00412.x).

SALAZAR-VALLEJO, S. I.; GONZÁLEZ, N. E.; BARRIENTOS-VILLALOBOS, J.; CARBAJAL-MÁRQUEZ, R. A.; SCHMITTER-SOTO, J. J. El reto taxonómico de la biodiversidad en México. **Biología y Sociedad**, v. 1, n. 1, p. 65–74, jan./jun. 2018. Disponível em: <https://biologiaysociedad.uanl.mx/index.php/b/article/view/61>. Acesso em: 27 jul. 2023.

SANGER, F.; COULSON, A. R. The use of thin acrylamide gels for DNA sequencing. **FEBS letters**, v. 87, n. 1, p. 107-110, mar. 1978. Disponível em: [https://febs.onlinelibrary.wiley.com/doi/abs/10.1016/0014-5793\(78\)80145-8](https://febs.onlinelibrary.wiley.com/doi/abs/10.1016/0014-5793(78)80145-8). Acesso em: 27 jul. 2023. DOI. [10.1016/0014-5793\(78\)80145-8](https://doi.org/10.1016/0014-5793(78)80145-8).

SOLIS-WEISS, V.; HERMOSO-SALAZAR, M.; LÓPEZ-BARBOSA, A.; HERNÁNDEZ-ALCÁNTARA, P. Orbiniidae Hartman, 1942. In: DE LEÓN-GONZÁLEZ, J. A.; BASTIDA-ZAVALA, J. R.; CARRERA-PARRA, L. F.; GARCÍA-GARZA, M. E.; PEÑA-RIVERA, A.; SALAZAR-VALLEJO S. I.; SOLÍS-WEISS, V. (Ed.) **Poliquetos (Annelida: polychaeta) de México y América Tropical**. Universidad Autónoma de Nuevo León, Monterrey, México, vol. 33, 2009. p. 379 –390.

SOUSA, R.C. **Distribuição espacial dos poliquetas (Annelida, Polychaeta) dos recifes de arenito na praia da Pedra Rachada (Paracuru-Ceará)**. 70 f. Dissertação (Mestrado em Ciências Marinhas Tropicais) - Instituto de Ciências do Mar, Universidade Federal do Ceará, Fortaleza, 2005. Disponível em: <https://repositorio.ufc.br/handle/riufc/1576>. Acesso em: 27 jul. 2023.

SPALDING, M. D.; FOX, H. E.; ALLEN, G. R.; DAVIDSON, N.; FERDANA, Z. A.; FINLAYSON, N.; HALPERN, B. S.; JORGE, M. A.; LOMBANA, A.; LOURIE, S. A.; MARTIN, K. D.; MCMANUS, E.; MOLNAR, J.; RECCHIA, C. A.; ROBERTSON, J. Marine ecoregions of the world: a bioregionalization of coastal and shelf areas. **BioScience**, v. 57, n. 7 p. 573–583, jul. 2007. Disponível em: <https://academic.oup.com/bioscience/article/57/7/573/238419>. Acesso em: 27 jul. 2023. DOI. [10.1641/B570707](https://doi.org/10.1641/B570707).

STORK, N. E. Measuring global biodiversity and its decline. In: Rcaka-Kudla ML, Wilson DE, Wilson EO (Ed.) **Biodiversity II**. Washington , D.C.: Joseph Henry Press, 1997. p. 41–68.

STRUCK, T. H.; FEDER, J. L.; BENDIKSBY, M.; BIRKELAND, S.; CERCA, J.; GUSAROV, V. I.; KISTENICH, S.; LARSSON, K. H.; LIOW, L. H.; NOWAK, M. D.; STEDJE, B.; BACHMANN, L.; DIMITROV, D. Finding Evolutionary Processes Hidden in Cryptic Species. **Trends in Ecology and Evolution**, v. 33, n. 3, p. 153–163, mar. 2018. Disponível em: [https://www.cell.com/trends/ecology-evolution/fulltext/S0169-5347\(17\)30290-](https://www.cell.com/trends/ecology-evolution/fulltext/S0169-5347(17)30290-)

2? returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS0169534717302902%3Fshowall%3Dtrue. Acesso em: 27 jul. 2023. DOI. [10.1016/j.tree.2017.11.007](https://doi.org/10.1016/j.tree.2017.11.007).

STRUCK, T.H.; CERCA, J. Cryptic Species and Their Evolutionary Significance. **Encyclopedia of life sciences**, p. 1–9, jan. 2019. Disponível em: <https://onlinelibrary.wiley.com/doi/10.1002/9780470015902.a0028292>. Acesso em: 27 jul. 2023. DOI. [10.1002/9780470015902.a0028292](https://doi.org/10.1002/9780470015902.a0028292).

STRUCK, T.; PURSCHKE, G.; HALANYCH, K. Phylogeny of Eunicida (Annelida) and exploring data congruence using a partition addition bootstrap alteration (PABA) approach. **Systematic Biology**, v. 55, n. 1, p. 1–20, fev. 2006. Disponível em: <https://academic.oup.com/sysbio/article/55/1/1/2842874>. Acesso em: 27 jul. 2023. DOI. [10.1080/10635150500354910](https://doi.org/10.1080/10635150500354910).

SWIFT, HOLLY F.; DAGLIO, L. GÓMEZ; DAWSON, M. N. Three routes to crypsis: stasis, convergence, and parallelism in the *Mastigias* species complex (Scyphozoa, Rhizostomeae). **Molecular Phylogenetics and Evolution**, v. 99, p. 103–115, jun. 2016. Disponível em: https://www.sciencedirect.com/science/article/pii/S1055790316000579?casa_token=0jJfL14yyXYAAAAA:zOtkFtHEqbhHAPBa7wJYG-u_QZXEBUo5uU-wENeP9Bg-c3tax0gYWPe6DEHz8xS-qxcWjfTCruQ. Acesso em: 28 jul. 2023. DOI. [10.1016/j.ympev.2016.02.013](https://doi.org/10.1016/j.ympev.2016.02.013).

TAYLOR, J.L. Chapter1 Orbiniidae. In: UEBELACKER, J. M. e JOHNSON, P. G. (Eds.), **Taxonomic guide to the polychaete of the Northern Gulf of Mexico**. Vol. I. 1984. p. 1-1 to 1-38. [Barry A. Vittor & Associates.]. Disponível em: Acesso em: 27 jul. 2023.

THOMPSON, J. D.; HIGGINS, D. G.; GIBSON, T. J. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. **Nucleic Acids Research**, v. 22, n. 22, p. 4673–4680, 1 nov. 1994. Disponível em: <https://academic.oup.com/nar/article-abstract/22/22/4673/2400290>. Acesso em: 27 jul. 2023. DOI. [10.1093/nar/22.22.4673](https://doi.org/10.1093/nar/22.22.4673).

VERRILL, A. E. Additions to the Turbellaria, Nemertina and Annelida of the Bermudas, with revisions of some New England genera and species. **Transactions of the Connecticut Academy of Arts & Sciences**, v. 10, p. 595–671, 1900. Disponível em: <https://www.biodiversitylibrary.org/part/7035>. Acesso em: 27 jul. 2023.

WAKE, M. What is “Integrative Biology”? **Integrative and Comparative Biology**, v. 43, n. 2 p. 239–241, abr. 2003. Disponível em: <https://academic.oup.com/icb/article/43/2/239/609511>. Acesso em: 27 jul. 2023. DOI. [10.1093/icb/43.2.239](https://doi.org/10.1093/icb/43.2.239).

ZACHOS, F. E. (New) Species concepts, species delimitation and the inherent limitations of taxonomy. **Journal of Genetics**, v. 97, p. 811–815, sep. 2018.

Disponível em: <https://link.springer.com/article/10.1007/s12041-018-0965-1>. Acesso em: 27 jul. 2023. DOI. [10.1007/s12041-018-0965-1](https://doi.org/10.1007/s12041-018-0965-1).

ZHADAN, A.; STUPNIKOVA, A.; NERETINA, T. Orbiniidae (Annelida: Errantia) from Lizard Island, Great Barrier Reef, Australia with notes on orbiniid phylogeny. **Zootaxa**, v. 4019, n. 1, p. 773–801, 18 sep. 2015. Disponível em: <https://www.biotaxa.org/Zootaxa/article/view/zootaxa.4019.1.27>. Acesso em: 27 jul. 2023. DOI. [0.11646/zootaxa.4019.1.27](https://doi.org/10.11646/zootaxa.4019.1.27).

ZHADAN, A. Review of Orbiniidae (Annelida, Sedentaria) from Australia. **Zootaxa**, v. 4860, n. 4, p. 451–502, 14 out. 2020. Disponível em: <https://www.mapress.com/zt/article/view/zootaxa.4860.4.1>. Acesso em: 27 jul. 2023. DOI. [10.11646/zootaxa.4860.4.1](https://doi.org/10.11646/zootaxa.4860.4.1).

ZHANG, J.; KAPLI, P.; PAVLIDIS, P.; STAMATAKIS, A. A general species delimitation method with applications to phylogenetic placements. **Bioinformatics**, v. 29, n. 22, p. 2869–2876, 2013. Disponível em: <https://academic.oup.com/bioinformatics/article/29/22/2869/314968>. Acesso em: 27 jul. 2023. DOI. [10.1093/bioinformatics/btt499](https://doi.org/10.1093/bioinformatics/btt499).

3. CHAPTER I. PHYLOGENY OF *NAINERIS* BLAINVILLE, 1828 (ANNELIDA, SEDENTARIA, ORBINIIDAE) AND TRANSFER OF SPECIES TO *PROTOARICIA* AND *PETTIBONELLA*

Filogenia de *Naineris* Blainville, 1828 (Annelida, Sedentaria, Orbiniidae) e
transferência de espécies a *Protoaricia* e *Pettibonella*

Manuscript formatted according to Zoologica Scripta

ISSN: 1463-6409

Impact Factor: 2.5

Qualis: A1

Autores: RICARDO CASTRO-ÁLVAREZ^{1,5}, MAIKON DI DOMENICO¹, ERICA
KEPPEL⁴, XXXXXXXXXXXXXXXX, MIGUEL ANGEL MECA³, PAULO DA CUNHA
LANA^{1, 2, †} & NATALIYA BUDAEVA³

¹ Programa de Pós-Graduação em Sistemas Costeiros e Oceânicos (PGSISCO),
Universidade Federal do Paraná, Caixa Postal 61, 83255-976, Pontal do Paraná,
Paraná, Brazil.

² Centro de Estudos do Mar, Universidade Federal do Paraná, Caixa Postal 61,
83255-976, Pontal do Paraná, Paraná, Brazil.

³ Department of Natural History, University Museum of Bergen, University of
Bergen, Allégaten 41, 5007 Bergen, Norway

⁴ Smithsonian Environmental Research Center, Edgewater, MD, United States.
E-mail: KeppelE@si.edu

⁵Corresponding author. E-mail: ricastalvarez@gmail.com

Abstract

Naineris has a worldwide distribution, including species from tropical to temperate waters. *Naineris* is distinguished for having rounded or truncate prostomium, and a clear distinction thorax-abdomen. The three best-known species of *Naineris* are considered as cosmopolitan in literature: *N. setosa* (Verril, 1900), *N. dendritica* (Kinberg, 1867), and *N. laevigata* (Grube, 1855); however, to date cosmopolitan species are rare except for deep-sea and invasive species. We provide the first

comprehensive revision of *Naineris* using an integrative approach, considering morphology and molecular data. Our molecular analysis includes 159 specimens of *Naineris* representing 25 species collected worldwide. Phylogeny (both ML and BI) based on the combined matrix of two mitochondrial (COI and 16S) and one nuclear (28S) marker results in four large and well supported clades, each supported by morphological synapomorphies. Clade *Naineris* s. str. includes *Naineris quadricuspida* (type species) and shares the presence of rounded to truncate prostomium, thoracic neuropodial papilla in the middle of lobe, presence of dorsal cilia between branchial bases, bifid uncini arranged in several rows, absence of subuluncini, dorsal sensory organs always paired, branchial start fixed with branchiae medially displaced. Clade *Protoaricia* contained species with rounded to truncate prostomium, thoracic neuropodial papilla in upper part of lobe, presence of dorsal cilia between branchial bases, uncini never bifid, uncini arranged in one or two rows, subuluncini may be present, oval-shaped dorsal sensory organs paired or multiple per segment, branchial start from thoracic or abdominal segments, fixed or variable, with branchiae laterally displaced. *Protoaricia* clade comprises three subclades, named after the best-known species of each of them for convenience. “*Protoaricia bicornis*” subclade combined species with rounded to truncate prostomium, dorsal cilia between branchial bases, absence of statocysts, thoracic neuropodial papilla in upper part of lobe, ventral row of digitiform thoracic neuropodial uncini, transversal row of uncini absent, subuluncini present, dorsal sensory organs rounded and multiple per segment, brachial start from thoracic segments, thoracic branchiae laterally displaced. “*Protoaricia setosa*” subclade three included species with rounded to truncate prostomium, dorsal cilia between branchial bases in abdominal segments, thoracic neuropodial papilla in an upper position, thoracic neuropodial uncini and subuluncini absent, paired and rounded dorsal sensory organs, branchial start from thoracic segments, branchiae laterally displaced, emerging from a fixed segment, and “*Protoaricia laevigata* subclade” was comprised of species rounded to truncate prostomium, dorsal cilia between branchial bases, presence of statocysts, thoracic neuropodial papilla in upper part of lobe, ventral row of acuminate thoracic neuropodial uncini, transversal row of uncini with undivided tips, subuluncini present, dorsal sensory organs rounded and paired per segment, brachial start from thoracic or abdominal segments, thoracic branchiae laterally displaced. Our results suggest polyphyly of the genus *Naineris* with *Naineris sensu stricto* including *N. quadricuspida* and *N. uncinata*, and the rest of the species grouped in a separate clade with *Protoaricia*. After critical revision of descriptions, redescrptions, and other records, we reclassified some members of *Naineris* into two genera, *Protoaricia* and *Pettibonella*, and provided an amended description of all three genera. For each species, we provide a synonym list, location, and catalog number of type material, type locality, geographic distribution, habitat, etymology, and availability of molecular data.

Keywords: nuclear DNA, molecular phylogeny, new genus, species complex, mitochondrial DNA.

Resumo

Naineris, família Orbiniidae Hartman, 1942, é distribuída globalmente e inclui espécies tropicais e d'águas temperadas. *Naineris* se reconhece por apresentar prostômio redondo ou truncado e uma clara distinção tórax-abdômen. As três espécies mais conhecidas de *Naineris* são consideradas na literatura como cosmopolitas: *N. setosa* (Verrill, 1900), *N. dendritica* (Kinberg, 1867) e *N. laevigata* (Grube, 1855); entretanto, atualmente as espécies cosmopolitas são raras, exceto no caso das espécies invasoras e as de mar profundo. Apresentamos a primeira revisão abrangente de *Naineris*, como base em uma abordagem integrativa, considerando morfologia, moléculas, e aspectos ecológicos. A nossa análise molecular incluiu 159 espécimes de *Naineris*, representando 25 espécies amostradas ao longo do globo. A filogenia (MV e IB) baseada na matriz combinada de dois marcadores mitocondriais (COI e 16S) e um nuclear (28S) resultou em quatro grandes clados bem suportados, cada um suportado com sinapomorfias morfológicas. O clado *Naineris* s. str. inclui *Naineris quadricuspida* (espécie tipo) e compartilha a presença de prostômio arredondado a truncado, papila neuropodial torácica posicionada no meio do lobo, cílios dorsais entre as bases das brânquias, uncinos bífidos dispostos em fileiras múltiplas, ausência de subuncinos, órgãos sensoriais dorsais sempre pareados por segmento, início branquial fixo, com brânquias deslocadas medialmente. O clado *Protoaricia* combinou espécies com prostômio arredondado a truncado, papila neuropodial torácica em uma parte superior do lobo, presença de cílios entre as bases das brânquias, uncinos bífidos nunca presentes, uncinos dispostos em uma ou mais fileiras, subuncinos podem estar presentes, órgãos dorsais ovais pares ou múltiplos por segmento, início branquial desde segmentos torácicos ou abdominais, com início fixo ou variável, brânquias deslocadas lateralmente. O clado *Protoaricia* compreende três subclados, nomeados de acordo a espécie mais bem conhecida de cada um deles, por conveniência. O sub clado "*Protoaricia bicornis*" combina espécies com prostômio redondo a truncado, ciliacão dorsal entre as bases das brânquias, ausência de estatocistos, papila neuropodial torácica em uma parte superior do lobo, presença de uma fileira ventral de uncinos neuropodiais digitiformes no tórax, fileira transversal de uncinos ausente, presença de subuncinos, órgãos sensoriais dorsais ovais e múltiplos por segmento, início branquial nos segmentos torácicos, brânquias torácicas lateralmente deslocadas. O sub clado "*Protoaricia setosa*" inclui três espécie com prostômio arredondado a truncado, ciliacão dorsal entre as bases das brânquias, papila neuropodial torácica em uma parte superior do lobo, ausência de uncinos e subuncinos, órgãos sensoriais dorsais pares e ovais, início branquial desde os segmentos torácicos, brânquias deslocadas lateralmente, emergindo de um segmento fixo, e o sub clado "*Protoaricia laevigata*" compreende espécies com prostômio redondo a truncado, ciliacão dorsal entre as bases das brânquias, presença de estatocistos, papila neuropodial torácica em uma parte superior do lobo, fileira ventral de uncinos acuminados, fileira transversal de uncinos com pontas não divididas, presença de subuncinos, órgãos sensoriais dorsais ovais e pares por segmento, início branquial nos segmentos torácicos ou abdominais, brânquias torácicas deslocadas lateralmente. Nossos resultados sugerem a polifilia do gênero *Naineris*, com *Naineris sensu stricto* incluindo *N. quadricuspida* e *N. uncinata*, e o resto das espécies agrupadas em um clado separado junto com *Protoaricia*. Após revisão crítica de descrições, redescrições e outros registros, nós reclassificamos alguns membros de *Naineris* em dois gêneros, *Protoaricia* e

Pettibonella, e provemos uma descrição emendada dos três gêneros. Para cada espécie foram fornecidas as listas de sinônimas, locação e número de tombo do material tipo, localidade tipo, distribuição geográfica, habitat, etimologia e disponibilidade de dados moleculares.

Palavras-chave: DNA nuclear, filogenia molecular, gênero novo, complexo de espécies, DNA mitocondrial.

3.1. INTRODUCTION

Naineris is a genus of burrowing polychaete worms placed in Sedentaria. Most of their diversity is found in shallow waters and shelf habitats (Taylor 1984, Jumars 2015) with fewer records reported from deep sea (Imajima, 2009; Blake, 2020). In shallow waters, they can be found in estuaries or even in hard substrates such as rocky shores (Hartmann-Schröder 1960, Álvarez et al. 2019), whereas in deeper waters on gas hydrate and seep sediments (Blake 2020). Often *Naineris* individuals are found eelgrass and algal tufts (Kinberg 1867, Hartman 1957, Solís-Weiss & Fauchald 1989, Álvarez et al. 2019) since those environments reduce current velocity and increase sedimentation rates and food supplies (Hasegawa et al. 2008).

Blainville (1818) erected *Naineris* based on a single Greenlandic species, *Naineris quadricuspida* (Fabricius, 1780), originally described as belonging to *Nais* Muller, 1774, an oligochaete genus. Subsequently, descriptions of orbiniid species agreeing with *Naineris* added new species and records emphasizing the composition of thoracic neurochaetae, the number of thoracic segments, and the chaetiger where branchiae start (Hartman, 1957).

Eisig (1914) reviewed the orbiniids and explored their organs and systems, describing the family's internal and external morphology (Hartman, 1957). He synonymized *Theodisca* and *Anthostoma* with *Naineris* based on similarities in the everted proboscis, prostomium shape, and parapodia. He also placed several described species in synonymy with the "cosmopolitan" *N. laevigata* (Grube, 1855). Subsequently, most species described in the 18th century were synonymized with either *N. laevigata* or *N. dendritica*.

Hartman (1957), as part of the worldwide revision of the orbiniids, erected two subfamilies: Orbiniinae and Protoariciinae. The former has one peristomial

ring and includes *Naineris*, while the latter has two peristomial rings. In this revision, Hartman gave an updated key for the genus and described two species: *Naineris uncinata* (type locality: Oregon) and *Naineris australis* (type locality: Adelaide), keeping the wide distribution of several species. An output of Hartman's work established several similarities between *Naineris* and *Protoaricia*, distinguished mainly by the region of the body where branchiae first appeared. In *Naineris*, branchiae first appear on thoracic segments; in *Protoaricia*, in contrast, on abdominal segments. Blake (2017, 2020) and Zhadan (2020) recently performed extensive revisions of the orbinids for Southeast Pacific Ocean and Australia respectively. Both authors agreed on the need for a revision of cosmopolitan species, such as *Naineris laevigata*.

Twenty species of *Naineris* are considered valid (Read & Fauchald 2020). Some of them are widely distributed (Eisig, 1914; Hartman, 1957) and have been treated as “cosmopolitan” species until recently; as *Naineris setosa* (Verrill, 1900) (Blake & Giangrande, 2011; Khedhri et al., 2014; Dean & Blake, 2015; Atzori et al., 2016); *Naineris dendritica* (Kinberg, 1867) (Choi et al., 2016); and *Naineris laevigata* (Grube, 1855) (Hartman 1948, 1957; Taylor, 1984; Zhadan, 2020). Whereas some other species are known only from their original descriptions and type localities (Read & Fauchald, 2020).

Despite the controversial wide use of the term cosmopolitan in annelid's literature from 1980 until now, cosmopolitan species are rare except for deep-sea and invasive species (Hutchings & Kupriyanova 2018). According to the most recent terminology for cosmopolitanism in marine species, cosmopolitan species are those that occur in at least in both major basins. In *Naineris*, we have the examples of the species with unresolved cosmopolitanism, seeing that no species of *Naineris* was subjected to rigorous investigation (Darling & Carlton 2018).

Blake (2000) tested the validity of Hartman's classification, performing the first phylogeny for the whole family. He included two species of *Naineris*, recovering *Naineris* as paraphyletic. Conversely, the first molecular study of the orbinids based on 16S and 18S genes obtained *Naineris* in a clade with *Protoaricia* (Bleidorn, 2005). Later, Bleidorn et al. (2009) sequenced 18S, 16S, cox1, cox3, nad1, and nad4 fragments and corroborated the monophyly of *Naineris* + *Protoaricia*. Finally, Zhadan et al. (2015) sequenced 16S, 18S, and

COI fragments of *N. australis*, and obtained *Naineris* as polyphyletic. Hence, the phylogeny and taxonomy of this genus definitely is in need of a reappraisal (Álvarez et al. 2019, Bleidorn & Helm 2019, Blake 2020, Read & Fauchald 2023). This study presents the first comprehensive phylogeny of *Naineris* based on two mitochondrial (COI, 16S) and a nuclear marker (28S), and reviews the morphology of the obtained clades with light and scanning electron microscopy. We also compiled and reviewed the available records of the species in the world and performed up-to-date distribution maps. In addition, we provided a taxonomical checklist and reclassified some members of the genus into two genera.

3.2. MATERIAL AND METHODS

3.2.1 Sample collection and morphology

We conducted sampling of specimens of *Naineris* in 12 sites along the Brazilian coast from 2020 to 2022 (Supplementary Table I), including the intertidal zone from estuarine beaches, bays, embayments and rocky shores. First, the animals were anesthetized with magnesium chloride and fixed for morphological or molecular studies. Then, we fixed the specimens in 4% formalin for morphological studies, and later, transferred and preserved in 70% ethanol. Whereas for molecular studies, the animals were fixed in 99.5% ethanol.

The worms were examined under a Leica MZ stereomicroscope and a Leica DM 6000 B compound microscope. Photographs were taken using a Leica M205C stereomicroscope with a digital camera Leica DMC5400 attached and combined with the Z-stack function on Leica LAS software and edited in Adobe Photoshop C56. Methyl green and Shirlastain A were used to increase contrast. Temporary parapodia slides were mounted in glycerol to visualize the chaetal structure. Scanning Electron Microscopy (SEM) images were prepared in three institutions following different protocols. At the NMNH, the worms were run through an increasing ethanol series, and the critical point dried in a Bal-tec CPD030 with ethanol as the transition fluid. After that, they were mounted, coated

with gold particles, and examined using a Zeiss EVO MA15 scanning electron microscope housed in the SEM Laboratory (Smithsonian NMNH). Part of the Brazilian material was prepared similarly and photographed with a Jeol JSM 6360–LV Zeiss at the Center for Electron Microscopy (Federal University of Parana–UFPR). In Bergen, the specimens were run through an increasing ethanol /hexamethyldisilazane (HMDS) for drying, coated with gold particles, and examined under an FE-SEM model GEMINI, Supra 55VP, of Zeiss (University Museum of Bergen).

3.2.2 Molecular dataset

To generate the sequences, we extracted DNA from tissue samples using 100 µl of QuickExtract™, heating at 65 °C for 45 min, followed by 2 min at 90 °C in a thermocycler. Fragments of nuclear 28S and mitochondrial COI and 16S were amplified in a 25 µl reaction volume containing 2.5 µl 10xPCR buffer, 2 µl dNTP (2.5mM), 1 µl forward and reverse primers (1 µM), 0.15 µl TaKaRa Taq (5U/ µl) with 1 µl template DNA, and double-distilled water to bring the mix to a final volume of 25 µl. The primers and PCR conditions are depicted in Table S3. After that, we electrophoresed 4 µl of PCR products on 1% agarose gel for 30 min to visualize successful amplifications. The successful PCR products were sent to Macrogen Europe for sequencing. Contigs were automatically merged from chromatograms for forward and reverse sequences, BLASTed and checked by eye in Geneious (VERSION 2023.2) (<https://www.geneious.com>).

Whole genome shotgun sequencing was carried out to obtain COI, 16S and 28 fragments of *Leitoscoloplos* sp., *Leodamas* sp., *Phylo* sp., and *Protoaricia* spp., and problematic samples of *N. quadricuspida* (COI) and Mediterranean "*N. laevigata*" (28S). The genomic DNA was extracted using DNAeasy Tissue Kit (Qiagen) following the protocol supplied by the manufacturer and sequenced using an Illumina HiSeq X system (150 bp paired-end) at the Macrogen Inc. facilities (Amsterdam, The Netherlands). Genomes were assembled de novo using SPAdes v3.15.3 (Prjibelsiki et al., 2020). To identify mitochondrial genome and rRNA gene clusters contigs we performed BLAST+ v2.13.0 (Camacho et al., 2008) using, as a query files, the mitochondrial genome of *Platynereis dumerilii*

(Genbank accession AF178678; Boore and Brown, 2000), the 18S (Genbank accession AF412810; Struck et al., 2002) and 28S (Genbank accession AY366516; Jördens et al., 2004) of *Stygocapitella subterranea* and the 28S of *Scoloplos fragilis* (Genbank accession EU418863; Struck et al., 2008). The assembly and Blast pipelines used were run through the supercomputer Saga, placed at the Norwegian University of Science and Technology (NTNU) in Trondheim (Norway). Mitogenome annotations were conducted in MITOS2 web server (Donath et al., 2019) and, by means of the annotated fasta files generated by MITOS2, we compiled the genes of interest per sample.

The recovered sequences of individual markers were aligned in MAFFT with default parameters using L-INS-i strategy (<https://mafft.cbrc.jp/alignment/server/index.html>). The Phylogenetic analysis was undertaken using the combined dataset (COI+16S+28S) generated in MEGA 11 (Tamura et al. 2021), with Maximum likelihood (ML) and Bayesian inference (BI). The ML was conducted in IQ-Tree 2.2.6. (Minh et al. 2020) using the ultrafast bootstrap type (Hoang et al. 2018). The alignments were partitioned, the substitution models were automatically selected in ModelFinder (Kalyaanamoorthy et al. 2017) restricting them for MrBayes (COI: 1st codon, GTR+F+G4; 2nd codon, HKY+F+I; 3rd codon, GTR+F+G4; 16S: GTR+F+I+G4; 28S: SYM+I+G4) (Kalyaanamoorthy et al. 2017), and the statistical support of the branches was estimated with 10000 bootstrap replicates. The BI was conducted in MrBayes v. 3.27 (Ronquist et al. 2012) using the same substitutions models. We performed two independent and simultaneous runs with 5×10^6 generations and parameters sampled every 1000th generations. The first 25% were discarded as burn-in, the remaining trees were employed to build the final tree. The convergence analysis of the Markov chains Monte Carlo (MCMC) was carried out with the mean standard deviation of division frequencies <0.010 and using the logarithmic probability graph of the sampled trees plotted in the TRACER application, considering a mean standard deviation of split frequencies <0.010 .

3.2.3 Characters optimization

Our analysis was undertaken based on molecular data only. Selected morphological characters were optimized in MESQUITE v. 2.75 (Maddison & Maddison 2023) using a phylogenetic tree build based on 3 molecular markers. Representatives from 9 species of *Naineris* were used in the analysis, including *N. quadricuspida*, the type species (Supplementary Table1). We selected the taxa based on the availability of material for molecular studies. The species from the other orbiniid genera, namely *Protoaricia*, *Leitoscoloplos*, *Scoloplos*, *Phylo*, *Leodamas* were used as outgroups.

3.2.4 Morphological characters

The following morphological characters were scored to generate the matrix for character optimization (Table 1-2).

Table 1. List of Morphological Characters

-
- (1) Prostomium shape: 0, acutely pointed or sharply conical; 1, rounded to truncate anteriorly.
 - (2) Interramal cirri: 0, absent; 1, present.
 - (3) Dorsal cilia between branchial bases in abdominal segments: 0, absent; 1, present.
 - (4) Statocysts (pre and pos branchial papilla): 0, absent; 1, present.
 - (5) Thoracic neuropodial papilla: 0, absent; 1, present.
 - (6) Position thoracic neuropodial papilla: 0, middle of lobe; 1, upper part of lobe.
 - (7) Only one thoracic neuropodial papilla: 0, yes; 1, no.
 - (8) Thoracic neuropodial uncini: 0, absent; 1, present.
 - (9) Ventral rows of uncini in thoracic neuropodia: 0, absent; 1, present.
 - (10) Shape of uncini from ventral row: 0, digitiform uncini; 1, acuminate.
 - (11) Transversal rows of uncini in thoracic neuropodia: 0, absent; 1, present.
 - (12) Shape of uncini from transversal row: 0, bifid; 1, not bifid.
 - (13) Subuluncini on thoracic neuropodia: 0, absent; 1, present.
 - (14) Dorsal organs number per chaetiger: 0, paired; 1, multiple
 - (15) Dorsal organs shape: 0, oval-shaped; 1, semicircular or shield-shaped.
 - (16) Branchial start: 0, thoracic segments; 1, abdominal segments.
 - (17) Thoracic branchiae disposition: 0, medially displaced; 1, laterally displaced.
 - (18) Chaetiger where branchiae first appear: 0, fixed; 1, ranged.
-

Table 2. Morphology character matrix. “?” denotes missing data and “-” denotes inapplicable states.

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Outgroups:																		
<i>Leitoscoloplos</i> sp. (Lei 42; Cabo Frio, RJ, Brazil)	0	1	0	0	1	0	1	0	-	-	-	-	0	0	1	1	-	0
<i>Phylo kuwaitica</i> (Phy 29, Oman)	0	?	?	?	?	0	?	1	?	?	?	?	0	?	1	?	?	?
<i>Leodamas</i> sp. (Leo 38; Paranaguá Bay, PR, Brazil)	0	0	0	0	0	-	-	1	-	-	1	1	0	0	1	0	0	0
<i>Protoaricia oerstedii</i> (Pro 8)	1	0	?	?	?	?	?	?	?	?	?	?	?	?	?	1	?	?
<i>Protoaricia</i> sp. (Pro 9)	1	0	?	?	?	?	?	?	?	?	?	?	?	?	?	1	?	?
Ingroup:																		
<i>Naineris</i> sp. (Kuwait)	1	0	1	0	1	1	0	1	1	0	0	-	1	1	0	0	1	0
<i>Naineris</i> cf. <i>bicornis</i> (Cabo Frio, RJ, Brazil)	1	0	1	0	1	1	0	1	1	0	0	-	1	1	0	0	1	0
<i>Naineris</i> cf. <i>bicornis</i> (Todos os Santos Bay, BA, Brazil)	1	0	1	0	1	1	0	1	1	0	0	-	1	1	0	0	1	0
<i>Naineris</i> sp. 2 (Oman)	1	0	1	0	1	1	0	1	1	0	0	-	1	1	0	0	1	0
<i>Naineris</i> sp. 3 (5388BKON_0422; Hawaii, USA)	1	0	1	0	1	1	0	1	1	0	0	-	1	1	0	0	1	0
<i>N. laevigata</i> (5548BKON_2303; Hawaii, USA)	1	0	1	0	1	1	0	1	1	0	0	-	1	1	0	0	1	0
<i>Naineris australis</i> (W.44763.001, Australia)	1	0	1	0	1	1	0	1	1	0	0	-	1	1	0	0	1	0
<i>N. dendritica</i> (Sea of Japan)	1	0	1	1	1	1	0	1	1	1	1	1	1	0	0	0	1	1
<i>N. dendritica</i> (BC, Canada)	1	0	1	1	0	1	0	1	1	1	1	1	1	0	0	0	1	1
<i>N. dendritica</i> (California, USA)	1	0	1	1	0	1	0	1	1	1	1	1	1	0	0	0	1	1

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
<i>N. dendritica</i> (Bodega Bay, California, USA)	1	0	1	1	0	1	0	1	1	1	1	1	1	0	0	0	1	1
<i>Naineris</i> sp. 4 (002252BMOO_16164, French Polynesia)	1	0	1	1	1	1	0	1	1	1	?	?	?	0	0	0	1	0
<i>N. chilensis</i> (Peru)	1	0	1	1	1	1	0	1	1	1	1	1	1	0	0	0	1	1
<i>N. aurantiaca</i> (South and Southeastern Brazil)	1	0	1	1	1	1	0	1	1	1	1	1	1	0	0	0	1	1
<i>Naineris</i> sp. 5 (Florida, USA)	1	0	1	1	1	1	0	1	1	1	1	1	1	0	0	0	1	1
<i>Naineris</i> sp. 6 (Black Sea)	1	0	1	1	1	1	0	1	1	1	?	?	1	0	0	0	1	?
<i>N. anserina</i> (Ardenza, Italy)	1	0	1	1	1	1	0	1	1	1	0	-	1	0	0	0	1	1
<i>Naineris</i> sp. 7 (Malta, Mediterranean Sea)	1	0	1	1	1	1	0	1	1	1	0	-	1	0	0	0	1	?
<i>N. laevigata sensu stricto</i> (Western Mediterranean)	1	0	1	1	1	1	0	1	1	1	1	1	1	0	0	0	1	1
<i>Naineris</i> sp. 8 (California, USA)	1	0	1	1	1	1	0	0	-	-	-	-	0	0	0	0	1	0
<i>N. setosa</i> (Atlantic Ocean)	1	0	1	0	1	1	0	0	-	-	-	-	0	0	0	0	1	0
<i>N. lanai</i> sp. n. (South and Southeastern Brazil)	1	0	1	0	1	1	0	0	-	-	-	-	0	0	0	0	1	0
<i>N. quadricuspida</i> (Northern Atlantic and Arctic)	1	0	0	?	1	0	0	1	0	-	1	0	0	0	1	0	0	0
<i>N. uncinata</i> (MN816166-MN816169; Oregon, USA)	1	0	0	?	1	0	1	1	0	-	1	0	0	0	1	0	0	0

3.2.4.1 Description of characters in the present analysis

Prostomium shape (character 1)

Orbiniid prostomia may be of different shapes. Members of *Naineris* have rounded or spatulate prostomium [character state 1(1), Figure 1A]. Despite *Naineris bicornis* is distinguished by having two anterior horns, we observed that topotype specimens have spatulate prostomium (personal observations), hence this prostomium shape was disregarded. Solís-Weiss & Fauchald (1989) noticed that prostomium shape is size dependent in *Naineris setosa*, being rounded in juveniles and spatulate in big sized specimens. Here we considered both as a single state. The outgroups are considered to have either pointed or conical prostomium [character state 1(0), Figure 1B], except for *Protoaricia* which has rounded prostomium [character state 1(1)].

Interramal cirri (character 2)

Only one species of *Naineris* has been described to possess an interramal cirri, *Naineris victoriae*. However, this species was not included in our molecular analyses. Interramal cirri are absent [character state 2(0)] in the entire ingroup, but present in part of the outgroups [character state 2(1), Figure 1C], e.g., in *Leitoscoloplos* sp.

Dorsal cilia between branchial bases (character 3)

Dorsal cilia between branchial bases are present in orbiniids with branchiae placed widely apart from each other, such as in members of *Naineris* and *Protoaricia* [character state 3(1), Figure 1F]. They are associated with even raised or flat membranes, located between contralateral branchiae on abdominal segments (Blake, 2017). Dorsal cilia between branchial bases are absent [character state 3(0), Figure E] in the outgroups, except for *Protoaricia*.

Statocysts (character 4)

Statocysts, also described as pre and pos branchial papillae (Álvarez et al. 2019) or paired organs at branchial bases, are present in some species of *Naineris* [character state 4(1), Figure 1D]. Despite Blake & Giangrande (2011) considered these structures as dorsal organs, we agree with Hartan (1957) and consider both as distinct

structures. Statocysts are absent in the outgroups [character state 4(0)], except for *Protoaricia* [character state 4(1)].

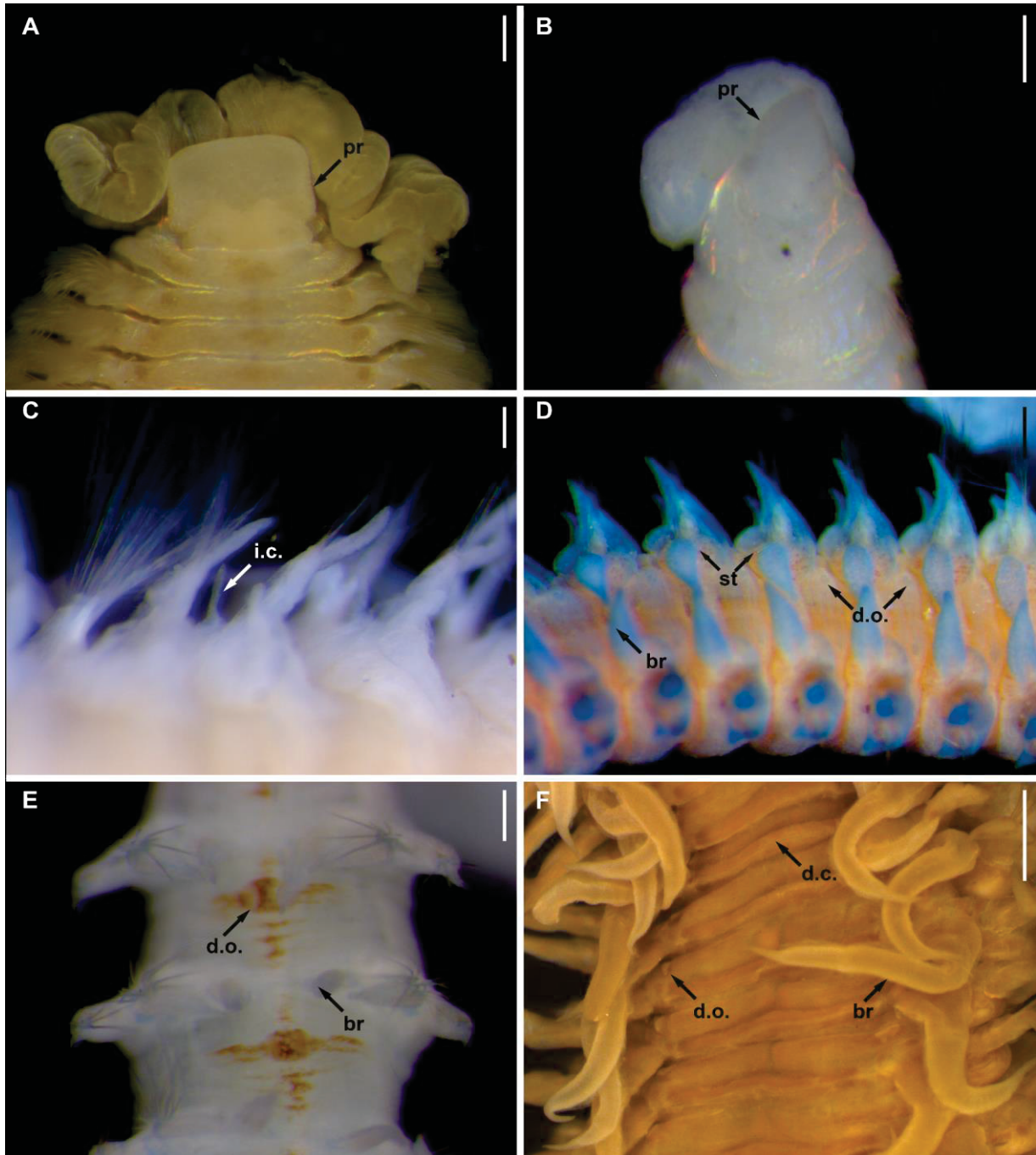


Figure 1. Orbiiniid morphological characters. (A) Anterior end of *Naineris* cf. *bicornis* (Cabo Frio, *****); (B) Anterior end of *Leodamas* sp. (Paranaguá Bay, *****); (C) Abdominal parapodia of *Leitoscoloplos* sp. (Cabo Frio, *****); (D) Abdominal parapodia of *Naineris aurantiaca* in dorsal view; (E) Abdominal segments of *Leodamas* sp. (Paranaguá Bay, *****); (F) Abdominal segments of *Naineris setosa* in dorsal view (Bahamas, UF 5895). br - branchiae, d.o. - dorsal organ, d.c.

dorsal cilia, i.c. - interramal cirri, pr - prostomium, st - statocysts. Scale bar; (A): 200 μm , (B-E): 500 μm , (F): 1 mm.

Thoracic neuropodial papilla (character 5–7)

The thoracic neuropodial papilla (character 5) is present [character state 5(1)] in species of *Naineris*, except for *N. dendritica* [character state 5(0)]. They are present in the outgroups *Protoaricia* and *Leitoscoloplos* sp. and absent in *Leodamas* sp. In most of the species of *Naineris* the papilla emerges (character 6) from the upper part of the lobe [character state 6(1), Figure 2B]. In other ingroup species and the outgroups, however, the papilla emerges in the middle of the lobe [character state 6(0), Figure 2C]. The number of thoracic neuropodial papillae may vary (subject 7) from a single unit per lobe [character state 7(0), Figure 2C] as in the entire ingroup, except for *N. uncinata*, which bears additional papillae per lobe [character state 7(1), Figure 2D]. *Leitoscoloplos* sp. have two neuropodial papilla lobe [character state 7(1)], in *Leodamas* sp. this character is unapplicable and unknown in the rest of the outgroups.

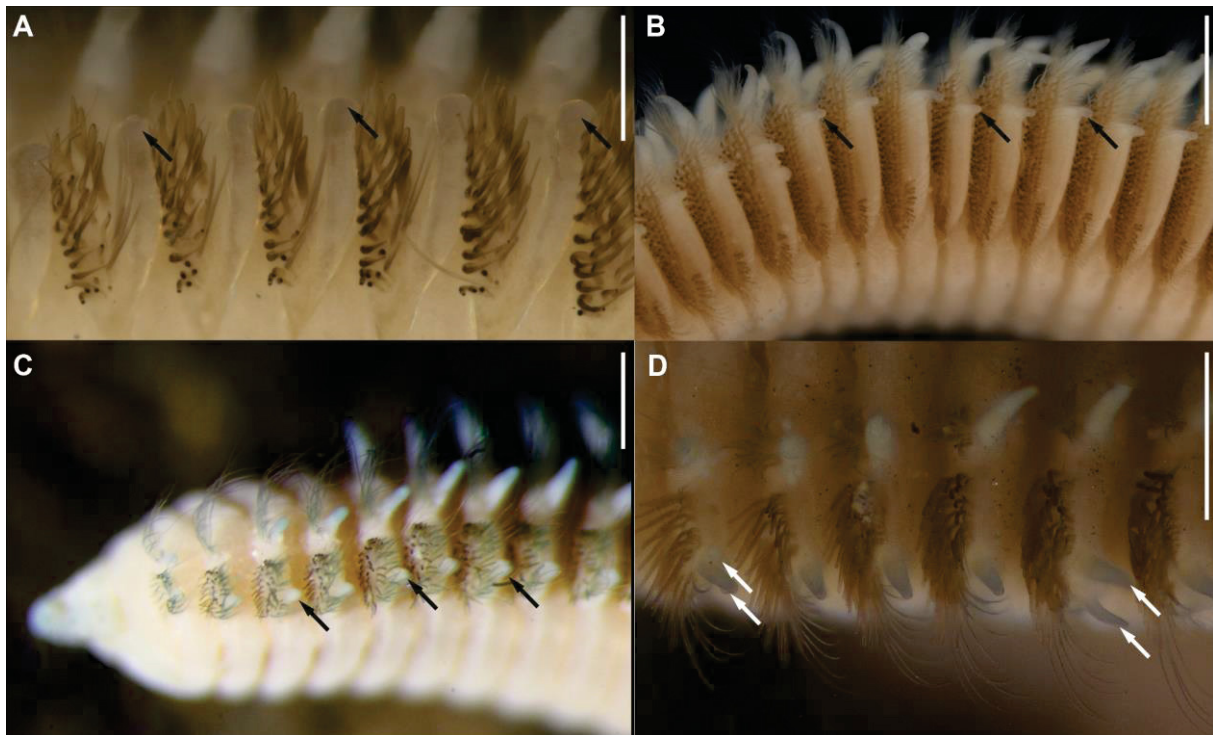


Figure 2. Thoracic neuropodial papilla in *Naineris* (arrows). (A) Thoracic neuropodia of *Naineris dendritica* (Japan, ZMBN *****); (B) Thoracic neuropodia of *Naineris australis* (Australia, ZMH *****); (C) Thoracic neuropodia of *Naineris quadricuspida* (Greenland,

USNM 342); (D) Thoracic neuropodia of *Naineris uncinata* (Paranaguá Bay, MCZ 153572). Scale bar; (A): 200 μ m, (B, D): 500 μ m, (C): 1 mm.

Characters of thoracic neurochaetae (character 8–13)

Uncini are present (character 8) in many species of *Naineris*. Their presence is considered a diagnostic character. Species of *Naineris* have uncini in thoracic neuropodia [character state 8(1), Figure 3B, C], except for *Naineris setosa* species complex [character state 8(0), Figure 3A]. Except for *Leitoscoloplos* sp. [character state 8(0)], they are present in the outgroup [character state 8(1)].

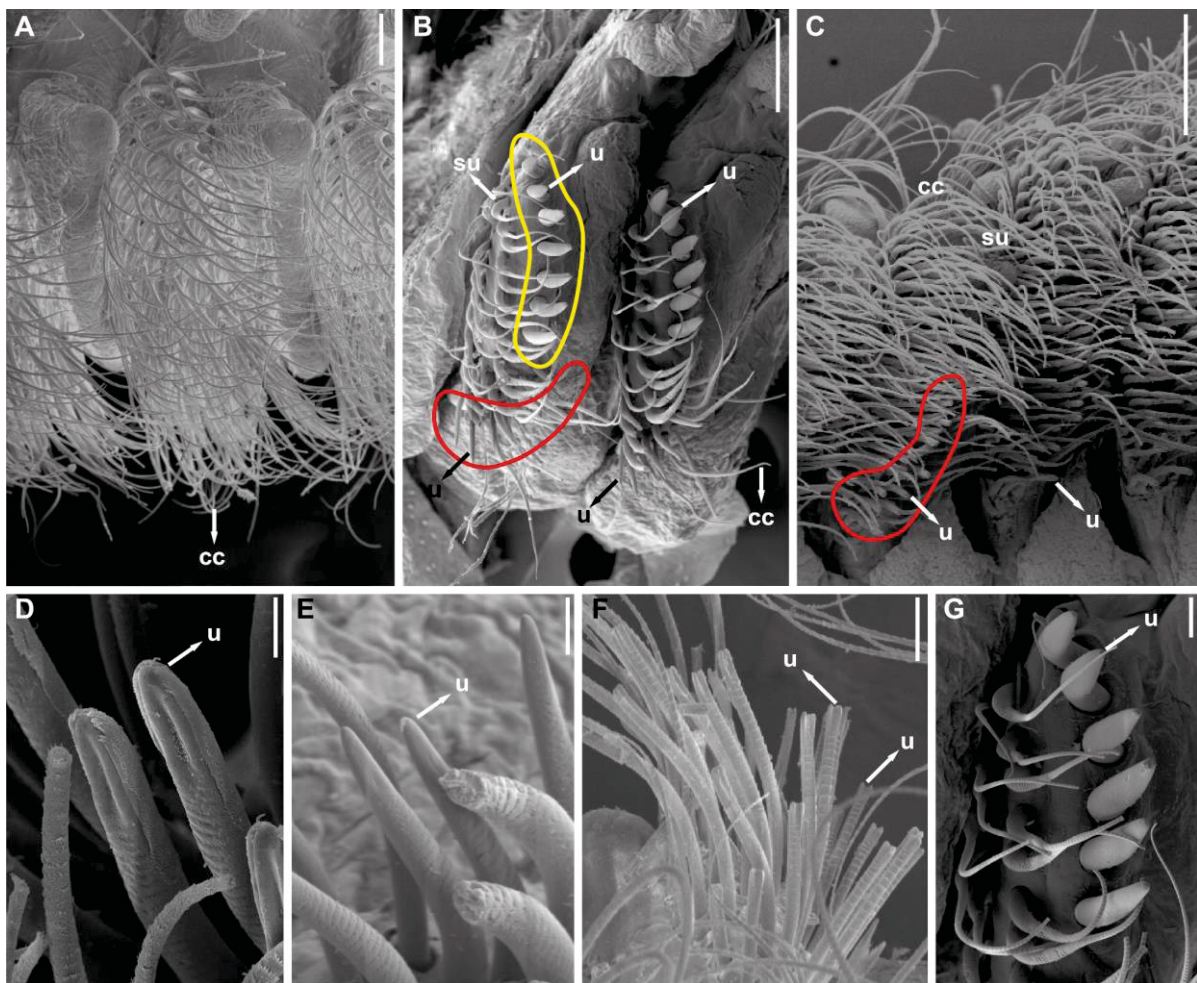


Figure 3. Thoracic neurochaetae in *Naineris* (transversal row of uncini is yellow colored, ventral in red). (A) Thoracic neurochaetae of *N. setosa* (Florida, USNM 3864); (B) Thoracic neurochaetae of *N. dendritica* (Vancouver Island, USNM 40198); (C) Thoracic neurochaetae of *N. bicornis* (Florida, USNM 1284137 165); (D) Digitiform thoracic neuropodial uncini of *N. bicornis* (Cabo Frio, MNRJP *****); (E) Acuminate thoracic neuropodial uncini of *N. dendritica* (Vancouver Island, USNM 40198); (F)

Bidentate thoracic neuropodial uncini of *N. quadricuspida* (Greenland, USNM 342); (G) Acuminate, recurved thoracic neuropodial uncini of *N. dendritica* (Vancouver Island, USNM 40198). Scale bars; (A-B): 100 μm , (C): 200 μm , (D-E): 10 μm , (F): 20 μm .

Uncini disposed in ventral rows (character 9) may be present [character state 9(1), Figure 3B, C] among ingroup members. Uncini are absent in *N. setosa* species complex and *Leitoscoloplos* sp., so this character is inapplicable. The outgroups do not possess the ventral row of uncini [character state 9(0)], except for *Protoaricia*. Whenever present, the ventral rows of uncini (character 10) may comprise digitiform [character 10(0), Figure 3CD] or acuminate [character state 10(1), Figure 3E] uncini. In members of the outgroups this character was not considered applicable, except for *Protoaricia* which has acuminate uncini in the ventral row.

As with ventral rows (character 9), transversal rows of uncini in thoracic neuropodia (character 11) can be present [character state 11(1), Figure B, G] or absent [character state 11(0)] in the ingroup and may be bifid [character state 12(0), Figure 3F] or acuminate, recurved [character state 12(1), Figure 3G]. Members of the outgroup possess transversal rows of uncini in thoracic neuropodia [character state 11(1)], acuminate recurved [character state 12(1)], except for *Leitoscoloplos*, in which characters 11 is absent and 12 inapplicable.

As with uncini, subuluncini (character 13) can be present [character state 13(1), Figure 3B, C] in the ingroup or absent [character state 13(0), Figure 3A] such as in the outgroups [character state 13(0)], except for *Protoaricia* [character state 13(1)].

Characters of dorsal sensory organs (character 14–15)

The dorsal sensory organs in *Naineris* may vary in number (character 14) being either paired [character state 14(0), Figure 4B, C] or multiple [character state 14(1), Figure 4A] per segment. They may also vary in shape (character 15), being oval-shaped [character state 15(0), Figure 4A, C] or semicircular / shield-shaped [character state 15(1), Figure 4B]. The outgroups possess paired [character state 14(0)] dorsal sensory organs, semicircular / shield-shaped [character state 15(1)].

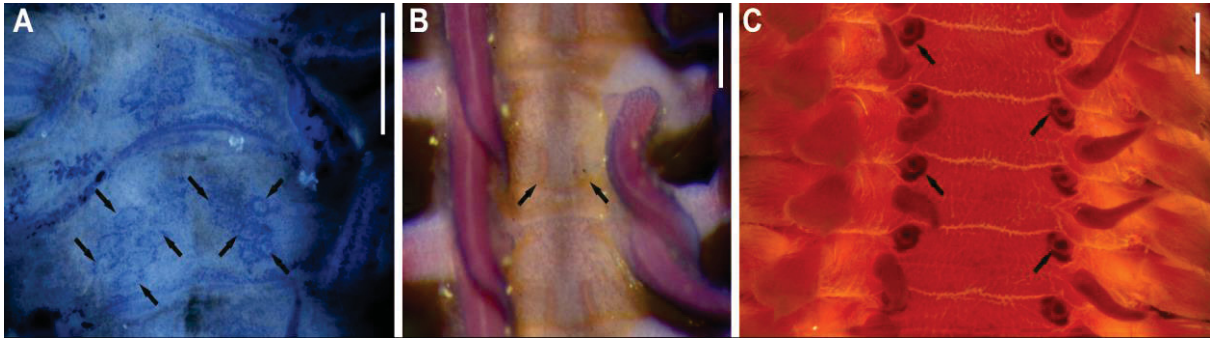


Figure 4. Dorsal sensory organs in *Naineris* (arrows). (A) Multiple dorsal sensory organs of *Naineris* sp. (Oman, ZMBN *****; (B) Semicircular, paired dorsal sensory organs of *Naineris uncinata* (Oregon, USNM 40182); (C) Oval shaped, paired dorsal sensory organs of *Naineris lanai* sp. n. (Paranaguá Bay, MNRJP 007623). Scale bar; (A): 250 μ m, (B): 500 μ m, (C): 1 mm.

Characters of branchiae (character 16–18)

The branchiae in the ingroup start (character 16) from thoracic [character 16(0), Figure 5A-C] segments. In members of the outgroups the branchiae can start either from thoracic segments [character state 16(0)], such as in *Leodamas* sp. or from abdominal segments [character state 16(1)], such as in *Protoaricia* and *Leitoscoloplos* sp. Thoracic branchiae also may be displaced (character 17) laterally [character state 17(0), Figure 5A, C] or medially [character state 17(1), Figure 5B]. The members of the outgroup possess branchiae medially displaced, in *Protoaricia* this character is inapplicable. The branchial start in the ingroup (character 18) may be fixed, starting always in the same chaetiger [character state 18(0)] or ranged [character state 18(1)]. The members of the outgroup possess fixed branchial start [character state 18(0)].

Eighteen characters were coded and optimized on the phylogenetic tree. Ingroup and outgroup taxa taken from GenBank were not included in the optimization, except when the DNA vouchers were studied. A summarized comparative morphology information of all species of *Naineris* is presented in Table S2.

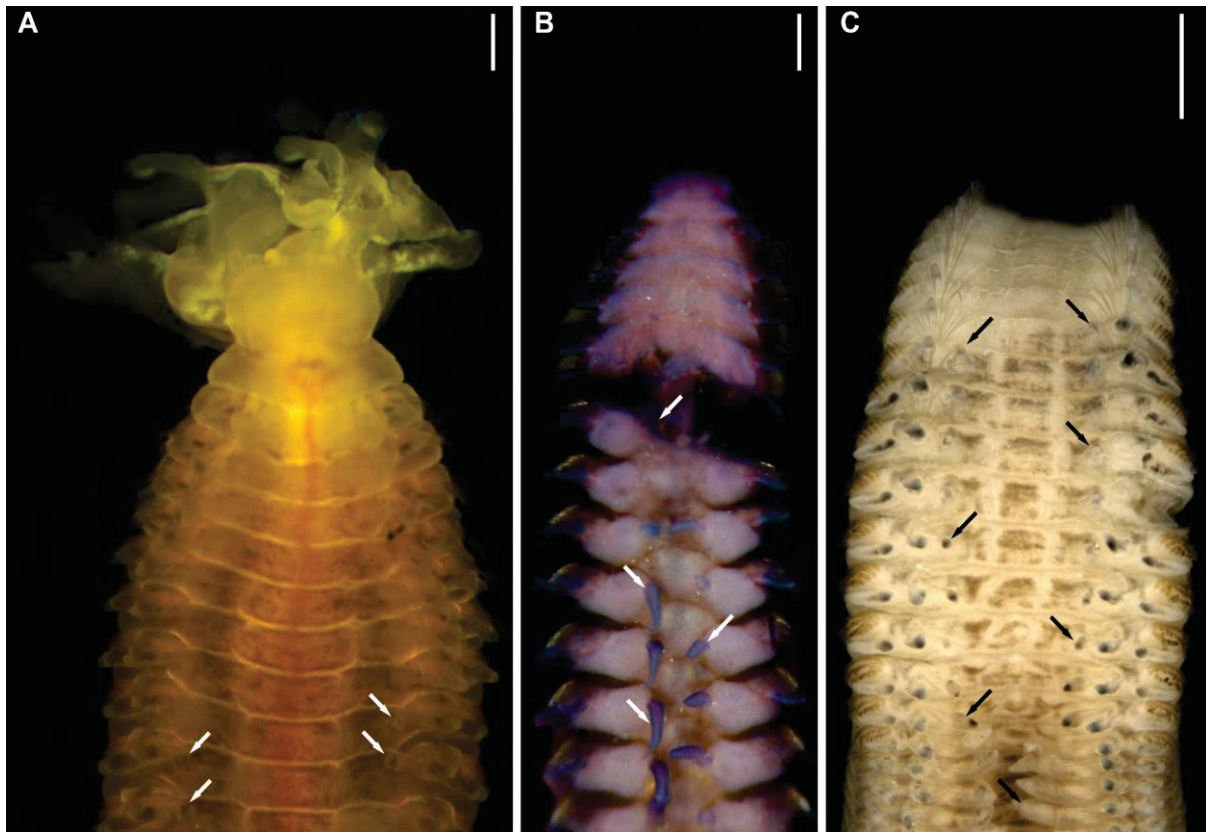


Figure 5. Branchial characters from thoracic segments in *Naineris* (arrows pointing to branchiae). (A) Anterior end of *Naineris aurantiaca* (Paraná, MNRJP *****); (B) Anterior end of *N. uncinata* (Oregon, USNM 40182); (C) Anterior end of *N. dendritica* (Sea of Japan, ZMBN *****). Scale bar; (A): 200 μ m, (B): 1 mm, (C): 500 μ m.

3.2.5 Checklist

We compiled the list of species based on accepted taxa from Worms Database (accessed on November 1, 2023), the species are listed in chronological order. Species descriptions, redescrptions, and taxonomical records were critically examined, doubtful records and synonymies were excluded and commented on respective notes section.

For each species, we provided full synonymies from Eisig (1914), Hartman (1957), Blake (1996, 2020), and Zhadan (2020); type material, including the location of type material, kind of primary type, catalog number and number of specimens; type locality; geographic distribution; habitat; etymology; molecular data; remarks (when appropriate); and map of distribution. The location of type material, kind of primary type, catalog number, and the number of specimens is based on original descriptions and data supplied by museum databases, or curators. Type locality and geographic coordinates (in decimal degrees) are given verbatim as appearing in original descriptions and enclosed in quotation marks, except in case of subsequent designation of neotypes and lectotypes; any information not part of the quoted information was enclosed in square brackets, as provincial and country names when not provided by authors; while additional explanatory information referring to the type locality were added in parentheses and information necessary to identify localities are added after the quoted material. Geographic distributions follow the provinces and ecoregions of Spalding *et al.* (2007). For habitat, we compiled information from original descriptions. For etymology, we based on original descriptions and dictionaries. For molecular data, we checked for its availability on the NCBI Taxonomy Database (<https://www.ncbi.nlm.nih.gov/taxonomy>) (Federhen 2012). Then we filtered COI and 16S and checked in our alignment. We included links to DNA sequence data posted on the platform, which correspond to the accession numbers of confirmed records. For maps of distribution, we took geographic coordinates from original descriptions; otherwise, we georeferenced based on notes that could help us identifying the locality using Google Earth Pro version 7.3 (Google Inc. 2020), or the gazetteer from Marineregions (<http://marineregions.org/>). We made maps of distribution in QGIS version 3.12.2 (QGIS Development Team 2016). All figures were done in Adobe Illustrator.

3.2.6 Terminology and abbreviations

The terms used follow those traditionally used in orbiniids systematics (Blake 1996, 2000, 2017, 2020, 2021; Zhadan *et al.* 2015, 2020).

In “location of type material,” we employed the following museum's collections acronyms:

ZMUC	Zoologisk Museum, Kobenhavn Universitet, Copenhagen, Denmark.
MPW	Muzeum Przyrodnicze Uniwersytetu Wrocławskiego, Wrocław, Poland.
ZMB	Museum für Naturkunde, Institut für Systematische Zoologie, Berlin, Germany.
ZMH	Zoologisches Museum - CeNak, Hamburg, Germany.
ZIN	Zoological Institute of Russian Academy of Sciences, Saint-Petersburg, Russia.
MNRJP	Museu Nacional, Rio de Janeiro, Brazil.
NR	Naturhistoriska riksmuseet, Stockholm, Sweden.
YPM	Yale Peabody Museum of Natural History, USA.
USNM	National Museum of Natural History, Washington D.C., USA.
AMNH	American Museum of Natural History, New York, USA.
LACM-AHF	Natural History Museum of Los Angeles County, USA.
(Poly)	
NHML	Natural History Museum, London, UK.
AM	Australian Museum, Australia.
NSTM	National Science and Technology Museum, Taiwan.
MZUC	Museo de Zoología de la Universidad de Concepción, Chile.
FORV	FORV Referral Centre, Centre for Marine Living Resources and Ecology, Cochin, Kerala, India

3.3. RESULTS

3.2.7 Molecular data analyses

We undertook a phylogenetic analysis of the combined partitioned dataset (COI: codon 1, 2, 3; 16S, and 28S). The combined alignment consisted of 2020 positions, represented by 654 (COI), 534 (16S), and 830 (28S) bases. Topologies and support values of both ML and BI trees were similar. Our ML tree of the combined dataset is depicted in Fig. 6, and the BI combined dataset in Fig. 7.

Polyphyly of *Naineris*. Both ML and BI analyses recovered *Naineris* as polyphyletic. Two major clades are proposed according to the topology of both trees, *Naineris sensu stricto* clade and *Protoaricia* clade. The *Naineris* s. str. clade was sister to a species of *Phylo* with moderate support. The *Protoaricia* clade contains *Protoaricia oerstedii*, the type species of the genus. Therefore, we suggest restricting the genus *Naineris* to the type species, *Naineris quadricuspida*, and its sister species *N. uncinata*. The rest of *Naineris* species in the present analysis are here considered as new combinations within *Protoaricia* clade.

***Naineris* s. str. clade.** Support values for the *Naineris* s. str. clade were 100% maximum likelihood bootstrap and 0.74 of posterior probability as shown in Figures 6-7. The diagnostic characters of this clade are the presence of rounded to truncate prostomium, thoracic neuropodial papilla in the middle of lobe, presence of dorsal cilia between branchial bases, bifid uncini arranged in several rows, absence of subuluncini, dorsal sensory organs always paired, branchial start fixed with branchiae medially displaced (Figures 8-12, S1-13).

***Protoaricia* clade.** Support values for the *Protoaricia* clade were 74% maximum likelihood bootstrap and 0.7 of posterior probability as shown in Figures 6-7. This clade comprises three well supported clades, “*Protoaricia laevigata*” clade, “*Protoaricia setosa*” clade and “*Protoaricia bicornis*” clade. The diagnostic characters of this clade are the presence of rounded to truncate prostomium, thoracic neuropodial papilla in upper part of lobe, presence of dorsal cilia between branchial bases, uncini never bifid,

uncini arranged in one or two rows, subuluncini may be present, oval-shaped dorsal sensory organs paired or multiple per segment, branchial start from thoracic or abdominal segments, fixed or variable, with branchiae laterally displaced (Figures 8-12, S1-13). Below, we are going to discuss the three subclades of the *Protoaricia* clade. Each of the subclades is named after the species that historically was the best known in the respective clade. We use quotation marks to identify the clade name and to distinguish it from a species name.

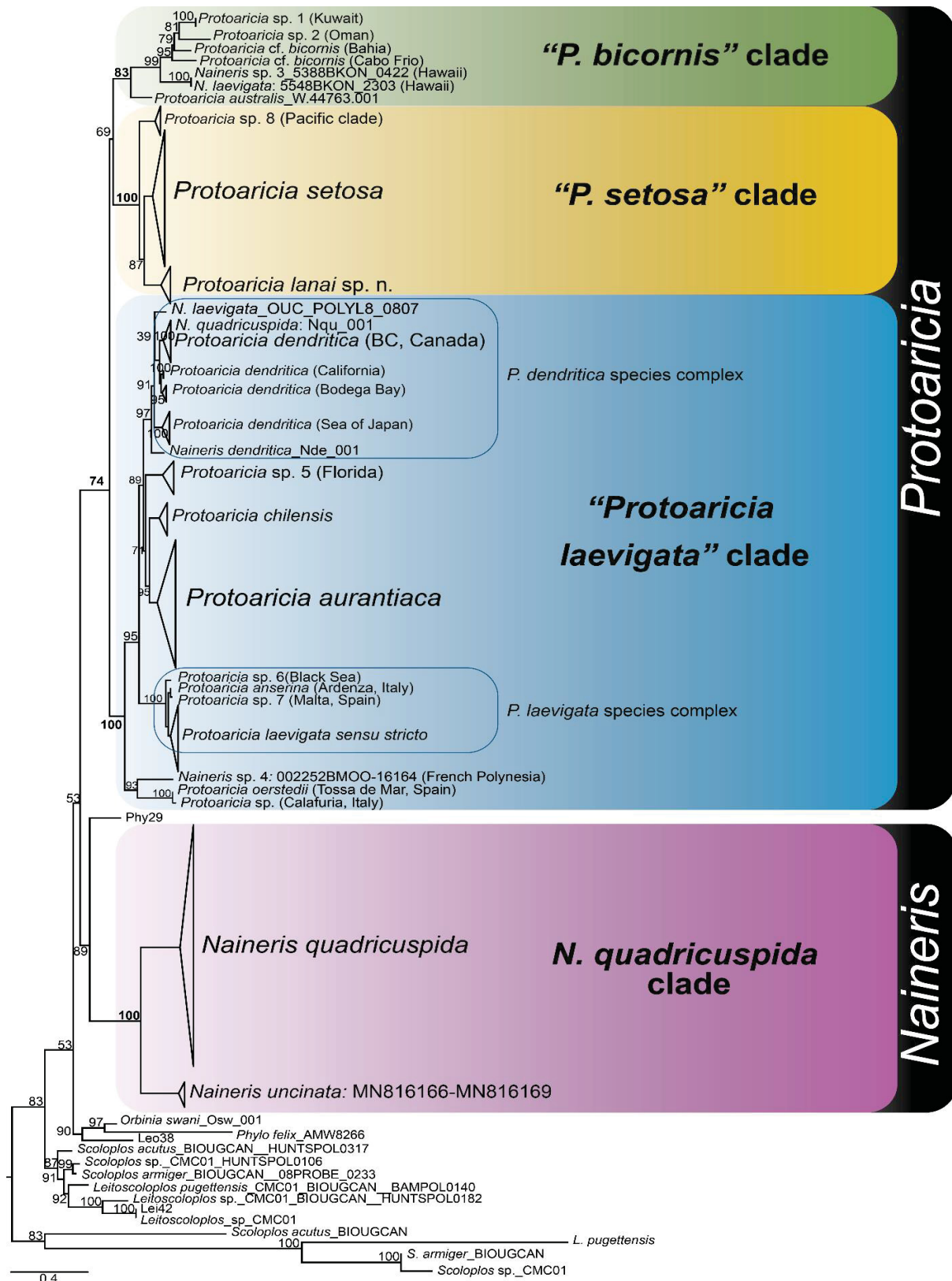


Figure 6. Maximum likelihood phylogenetic tree of combined dataset COI, 16S and 28S. Numbers at the nodes represent ultrafast bootstrap values from 10000 replicates.

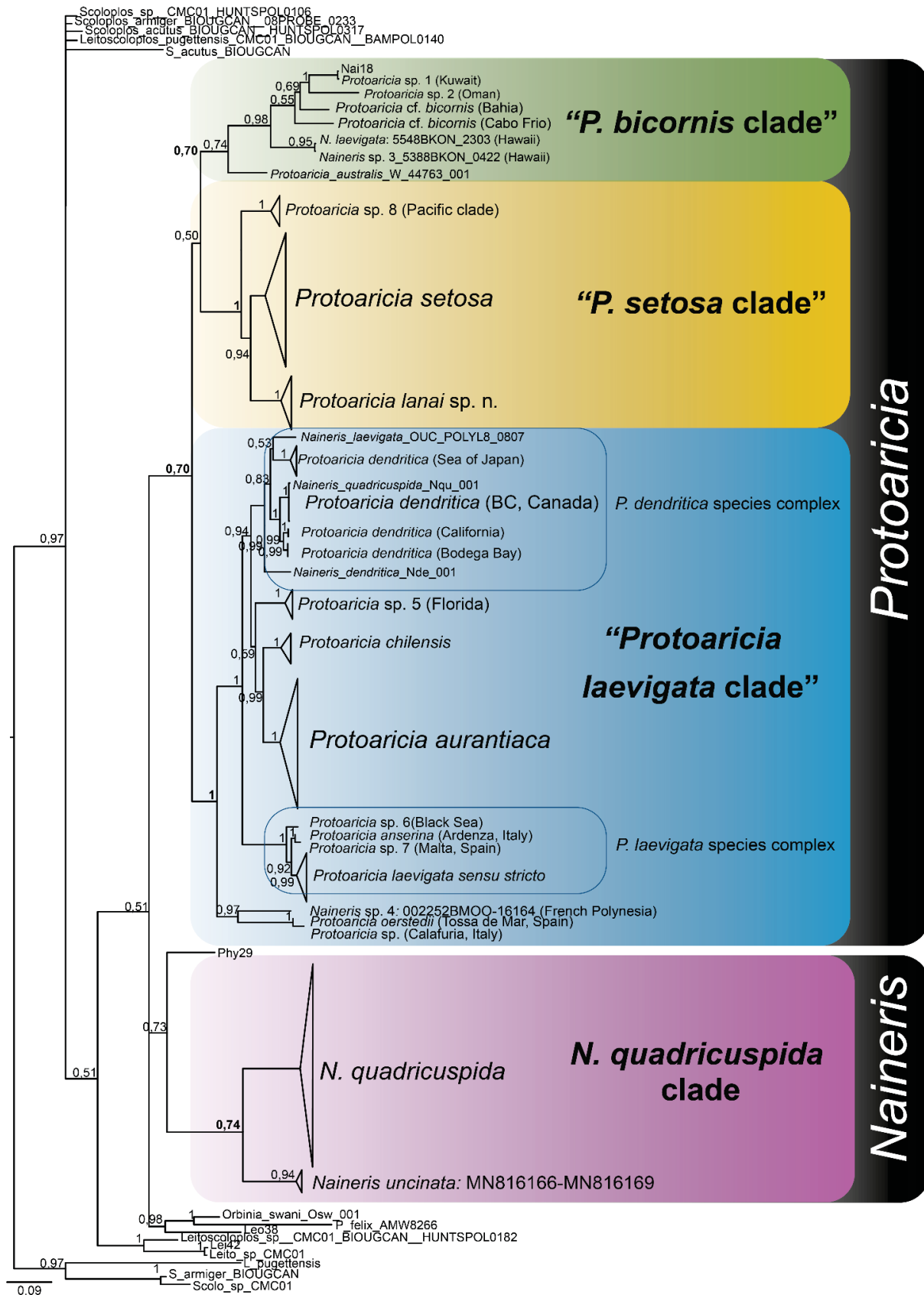
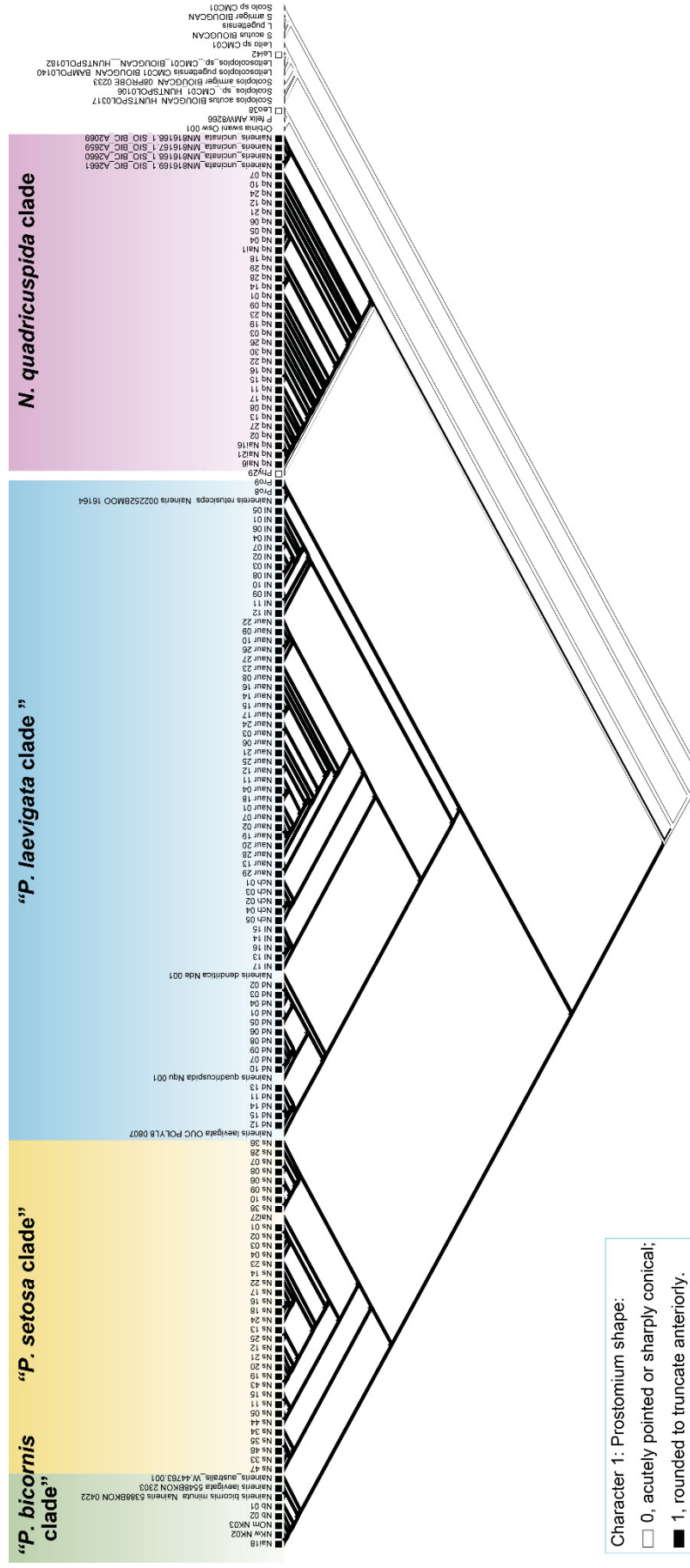


Figure 7. Bayesian Inference phylogenetic tree of combined dataset COI, 16S and 28S. Numbers at the nodes represent ultrafast bootstrap values from 10000 replicates.

“*Protoaricia laevigata*” subclade. This clade was recovered as sister group of “*P. bicornis*” and “*P. setosa*” clades and comprises *Protoaricia oerstedii* + *Protoaricia laevigata* comb. nov., *Protoaricia dendritica* comb. nov., *Protoaricia chilensis* comb. nov., *Protoaricia aurantiaca* comb. nov., *Protoaricia retusiceps* comb. nov. (ML = 100, BI = 1; Figures 6-7), and four new species to science, in blue. The diagnostic characters of this clade are the rounded to truncate prostomium, dorsal cilia between branchial bases, presence of statocysts, thoracic neuropodial papilla in upper part of lobe, ventral row of acuminate thoracic neuropodial uncini, transversal row of uncini with undivided tips, subuluncini present, dorsal sensory organs rounded and paired per segment, brachial start from thoracic or abdominal segments, thoracic branchiae laterally displaced (Figures 8-12, S1-13).

“*Protoaricia setosa*” subclade comprises *Protoaricia setosa* comb. nov., *Protoaricia lanai* comb. nov. sp. nov. and *Protoaricia* sp. (ML = 100, BI = 1; Figures 6-7), in dark yellow. The diagnostic characters of this clade are the presence of rounded to truncate prostomium, dorsal cilia between branchial bases in abdominal segments, thoracic neuropodial papilla in upper part of lobe, thoracic neuropodial uncini and subuluncini absent, paired and rounded dorsal sensory organs, branchial start from thoracic segments, branchiae laterally displaced, emerging from a fixed segment (Figures 8-13, S1-13).

“*Protoaricia bicornis*” subclade comprises species with multiple dorsal organs and similar chaetal structure, including *P. bicornis* comb. nov. and *P. australis* comb. nov. (ML = 83, BI = 0.7; Figures 6-7), in green. The diagnostic characters of this clade are the rounded to truncate prostomium, dorsal cilia between branchial bases, absence of statocysts, thoracic neuropodial papilla in upper part of lobe, ventral row of digitiform thoracic neuropodial uncini, transversal row of uncini absent, subuluncini present, dorsal sensory organs rounded and multiple per segment, brachial start from thoracic segments, thoracic branchiae laterally displaced (Figures 8-12, S1-13).



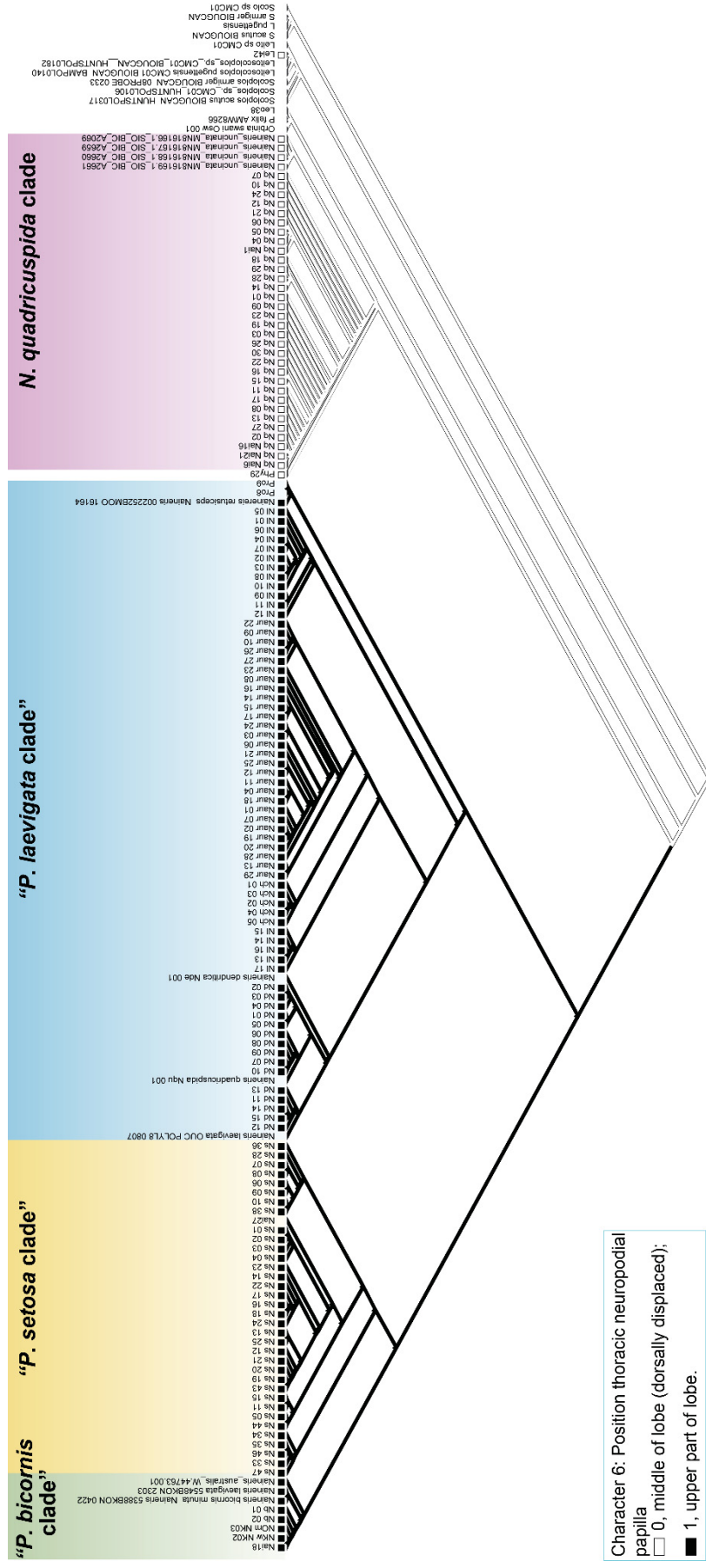


Figure 9. Ancestral state reconstructions of character 6 (position of thoracic neuropodial papilla) traced on the Maximum Likelihood reconstruction.

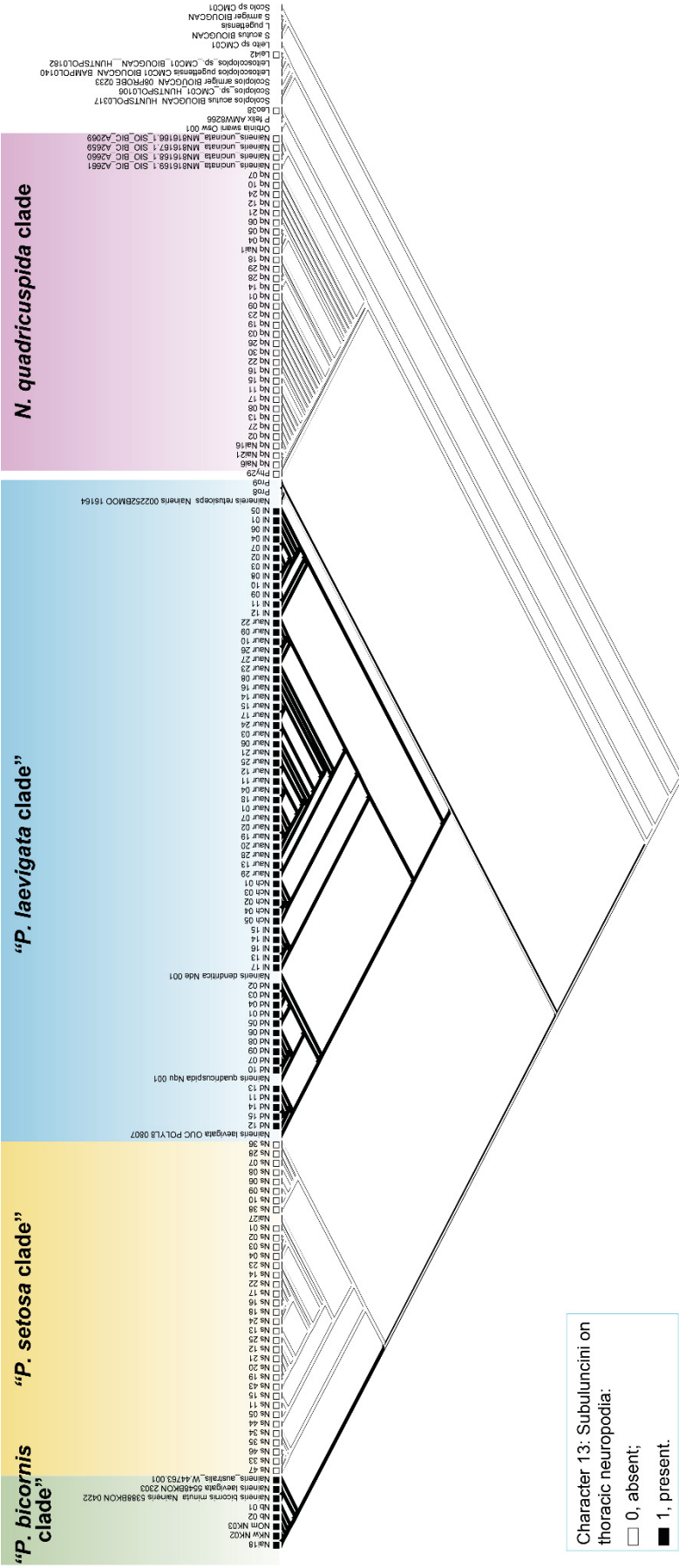


Figure 10. Ancestral state reconstructions of character 13 (subuluncini on thoracic neuropodia) traced on the Maximum Likelihood reconstruction.

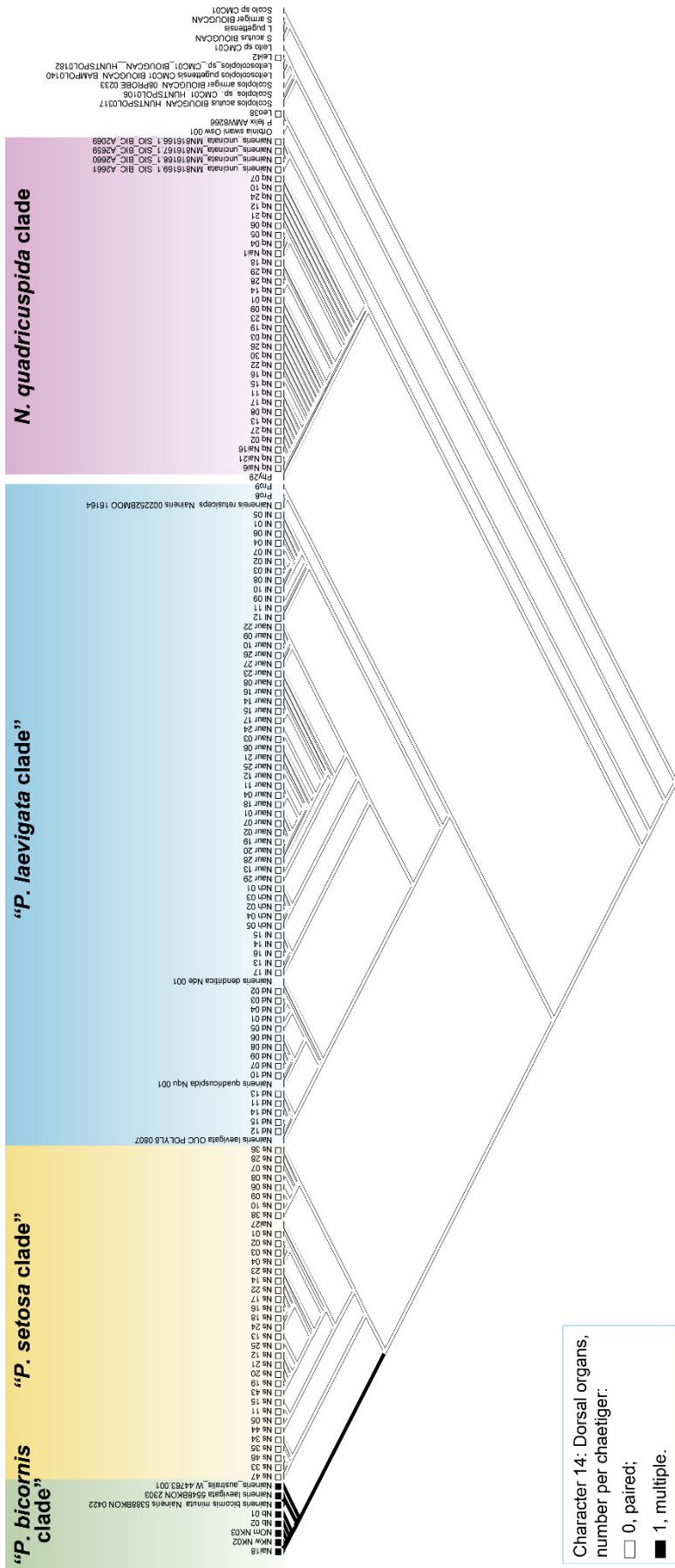


Figure 11. Ancestral state reconstructions of character 14 (dorsal organs, number per chaetiger) traced on the Maximum Likelihood reconstruction.

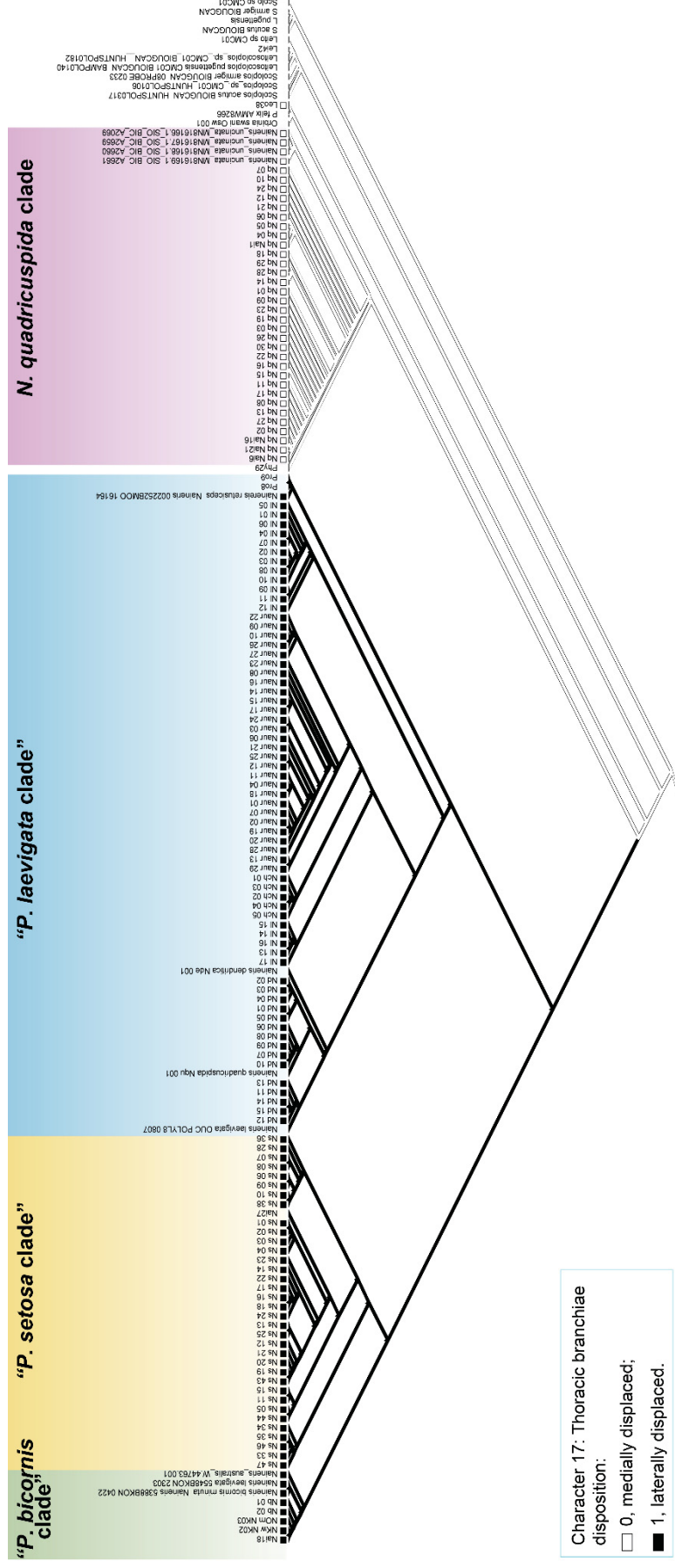


Figure 12. Ancestral state reconstructions of character 17 (thoracic branchiae disposition) traced on the Maximum Likelihood reconstruction.

3.2.8 Taxonomical review

Family ORBINIIDAE Hartman, 1942

Genus *Naineris* Blainville, 1828

Naineris Blainville, 1828. **Type species:** *Naineris quadricuspida* (Fabricius, 1780), by monotypy.

Variant spellings. *Naidonereis* Malmgren, 1867; *Nainereis* Mesnil & Caullery, 1898; *Naidoneris* Webster & Benedict, 1887.

Generic Diagnosis (amended after Blake 2020, Zhadan 2020)

Prostomium rounded or truncate on anterior margin. Peristomium with 1–2 achaetous rings. Thorax with 12–26 segments; branchiae first present from setiger 4–7, medially displaced. Thoracic neuropodia with 1–2 papilla in middle of postchaetal lobe, 1–2 additional papilla can be present: no subpodial lobes. Thoracic neurochaetae including several rows of uncini with numerous transverse ribs on the shaft and a bluntly rounded tip bearing a terminal notch and capillaries. Abdominal chaetae including capillaries and sometimes furcate chaetae in notopodia and capillaries and imbedded or protruding aciculae in neuropodia. Paired dorsal sensory organs present, oval-shaped or semicircular.

Remarks. Pettibone (1957) proposed the partition of *Naineris* into two subgenera: *Naineris* (*Naineris*) and *Naineris* (*Polynaineris*) based on the position of thoracic neuropodial papilla. In *Polynaineris* the papilla is placed in the upper part of the lobe (dorsally); whereas in *Naineris* the papilla is placed in middle of lobe, presenting up to two additional papillae. Except for Silva (1965), Pettibone's subdivision was not used and is currently unaccepted due to its lack of clarity (Worms Database, 2023).

Despite the problems in Pettibone's (1957) proposal, the position of thoracic neuropodial papilla is the chief difference between *Naineris* s. str. and *Protoaricia*. In species of *Naineris* s. str. the thoracic neuropodial papilla is placed in the middle of lobe, and may present one or two additional papillae, as in *N. uncinata*. In contrast, the thoracic neuropodial papilla in species of *Protoaricia*, if present, is placed in an upper position. Another difference between *Naineris* s. str. and *Protoaricia* is the presence, form, and arrangement of thoracic neuropodial uncini. In species of *Naineris* s. str. the neuropodial uncini are present in all the species; they are grouped in several transversal rows and adopt bidentate shape on tips. Whereas in *Protoaricia*, the thoracic neuropodial uncini, if present, are restricted to an inferior row intermixed with capillaries, adopting either digitiform or acuminate shape, undivided. Additionally, some species of *Protoaricia* present an additional, transversal, and posterior row of huge uncini. Dorsal organs between both genera also differ. In *Naineris* s. str. they are always paired per segment, being either oval shaped or as elongated semicircles. In contrast, dorsal organs of species of *Protoaricia* can be paired or multiple per segment, always oval shaped. The following six species of *Naineris* s. str. are recognized based on the diagnostic characters discussed above.

***Naineris quadricuspida* (Fabricius, 1780)**

Nais quadricuspida Fabricius, 1780: 315–316.

Naineris quadricuspida: Blainville, 1828: 490–491; Wesenberg-Lund 1950: 29–30; Hartman 1957: 296; 1959: 366; Pettibone 1963: 278–280, fig. 74 a–c; Reish 1965: 140; Hartmann-Schröder 1971: 274; Hobson & Banse 1981: 28, fig. 1f; Kirkegaard 1996: 58; Brunel *et al.* 1998: 147; Bellan 2001:222; Müller 2004:33; Viéitez *et al.* 2004: 116–120, fig. 40 A–F; Troot 2004:280.

Scoloplos minor Örsted, 1842: 125.

Scoloplos quadricuspida: Örsted, 1843: 200–201, figs. 106–110.

Aricia (Scoloplos) quadricuspida Leuckart, 1849: 198–200, pl. 3, fig. 11.

Naidonereis quadricuspida: Malmgren, 1867: 73; McIntosh 1878:504; Webster & Benedict 1887: 738–739, pl. VI, figs. 90–92; Whiteaves, 1901, p. 79.

Theodisca mamillata Cunningham & Ramage, 1888: 642–643, pl. XXXVIII.

Aricia quadricuspida: Birula 1897: 97.

Nainereis quadricuspida: McIntosh 1910: 517–519, pl. LXV, figs. 5 and 5a, pl. LXXXVI, figs. 2–2b, pl. LXXXVII, figs. 5–5b; Eisig 1914: 488; Fauvel, 1927: 22–24, fig. 8; Wesenberg-Lund 1951: 59; Howson & Picton 1997: 115.

Type material. No type material is known to be preserved for *N. quadricuspida*. Fabricius did not designate a type series, and the specimens no longer exist (Danny Eibye-Jacobsen *pers. com*). We refrain from designating a neotype because it is unnecessary to our knowledge.

Type locality. [Near Frederikshab (Paamiut), Sermerssoq,] “Groenl.” (Greenland) (Fig. 14).

Geographic distribution. Artic, Northern European Seas, and Cold Temperate Northwest Atlantic provinces (Fig. 14).

Habitat. Intertidal and infralittoral under rocks or between algae.

Etymology. Latin for “bearing four points” (*quadri* + *cuspidata*). Probably due for having four anal cirri, as mentioned in the species diagnosis (“quadrifid tail”).

Molecular data. COI: [MF121665.1](#), [MF121636.1](#), [MF121613.1](#), [MF121587.1](#), [MF121509.1](#), [MF121437.1](#), [MF121388.1](#), [MF121360.1](#), [MF121349.1](#), [MF121239.1](#), [MF121228.1](#), [MF121189.1](#), [MF121132.1](#), [MF121084.1](#), [MF121062.1](#), [MF121050.1](#), [MF121044.1](#), [MF121027.1](#), [MF121021.1](#), [MF121018.1](#), [MF120986.1](#), [MF120982.1](#), [MF120967.1](#), [GU672621.1](#).

Note. Fabricius (1780) gave the unspecific vernacular name *Pullateriak*. Despite the type locality was not well established, it is accepted that Fabricius research was conducted where he was based, Paamiut, SW Greenland (Blake, 2009).

Molecular data of 16S sequences [FJ612464.1](#) and [AY532346.1](#) are dubious, both matched with *P. dendritica* clades, and are probably misidentifications.

Material examined. **Greenland**, 10 spms, USNM 342; 3 spms. **USA**, MCZ 37092, 43.8576370°N–69.527920°W, 1955-08-14, rocky, algae, in sand among byssus threads of mussels (*Modiolus modiolus*); 1 spm., MCZ 70466, 44.9061570°N–66.984293°W; 3 spms, MCZ 70908, 42.5383200°N–70.887165°W, 1873-04-25; 34 spms, MCZ 70931, 44.9075890°N–66.993454°W, 1877, coll. Balfour H. Van Vleck, mud.

Description. A complete specimen with 16,5mm long, 1mm wide, for 84 chaetigers. Color in alcohol light tan. Thorax flattened and abdomen cylindrical (Fig. 13A), transition marked by the shifted position of parapodia in abdominal segments, also presenting a triannular pattern.

Prostomium rounded in anterior margin (Fig. 13A); eyespots not discernible; nuchal organs present. Peristomium with two annular rings (Fig. 13A); mouth opening ventrally, with striated lips; proboscis not everted in material examined.

Branchiae first present from the fifth chaetiger on both sides (Fig. 13A). The right one is absent, but a scar show they begin at the same chaetiger; cirriform, ciliated; well developed from first chaetigers, half times abdominal branchiae.

Dorsal sensory organs paired, rounded from chaetiger four onwards. Dorsal crest low reduced to a ciliary band (Figs 13B, C).

Thorax slightly flattened comprising 10 chaetigers. Parapodia biramous, lateral organs not observed. Notopodial lobes digitate to triangular (Fig. 13A); neuropodial lobes rounded with a rounded papilla in the middle, mamiliform-like. Abdominal notopodial lobes lanceolate; neuropodial lobes triangular (Fig. 13B).

Thoracic notochaetae with 20–25 crenulate capillaries and furcate chaetae in posterior segments. Abdominal notochaetae with crenulate capillaries and furcate chaetae (Fig. 13D). Thoracic neurochaetae with 5-7 rows of uncini and a ventral, oblique row of capillaries intermixed with a slightly smaller uncini: uncini with ribbed shaft and rounded tip bearing a terminal notch, bidentate (Fig. 13E). Abdominal neurochaetae with 3-5 acicular spines and crenulate capillaries (Fig. 13F). Pygidium bearing 4 anal cirri.

Remarks. The most similar species to *Naineris quadricuspida* is *Naineris grubei* by having rounded prostomium, one thoracic neuropodial lobe and two peristomial rings and branchial start from chaetigers 4-7. Both species differs,

however, in the number of thoracic chaetigers, which range from 12-17 in *N. quadricuspida* and from 17-20 in *N. grubei*.

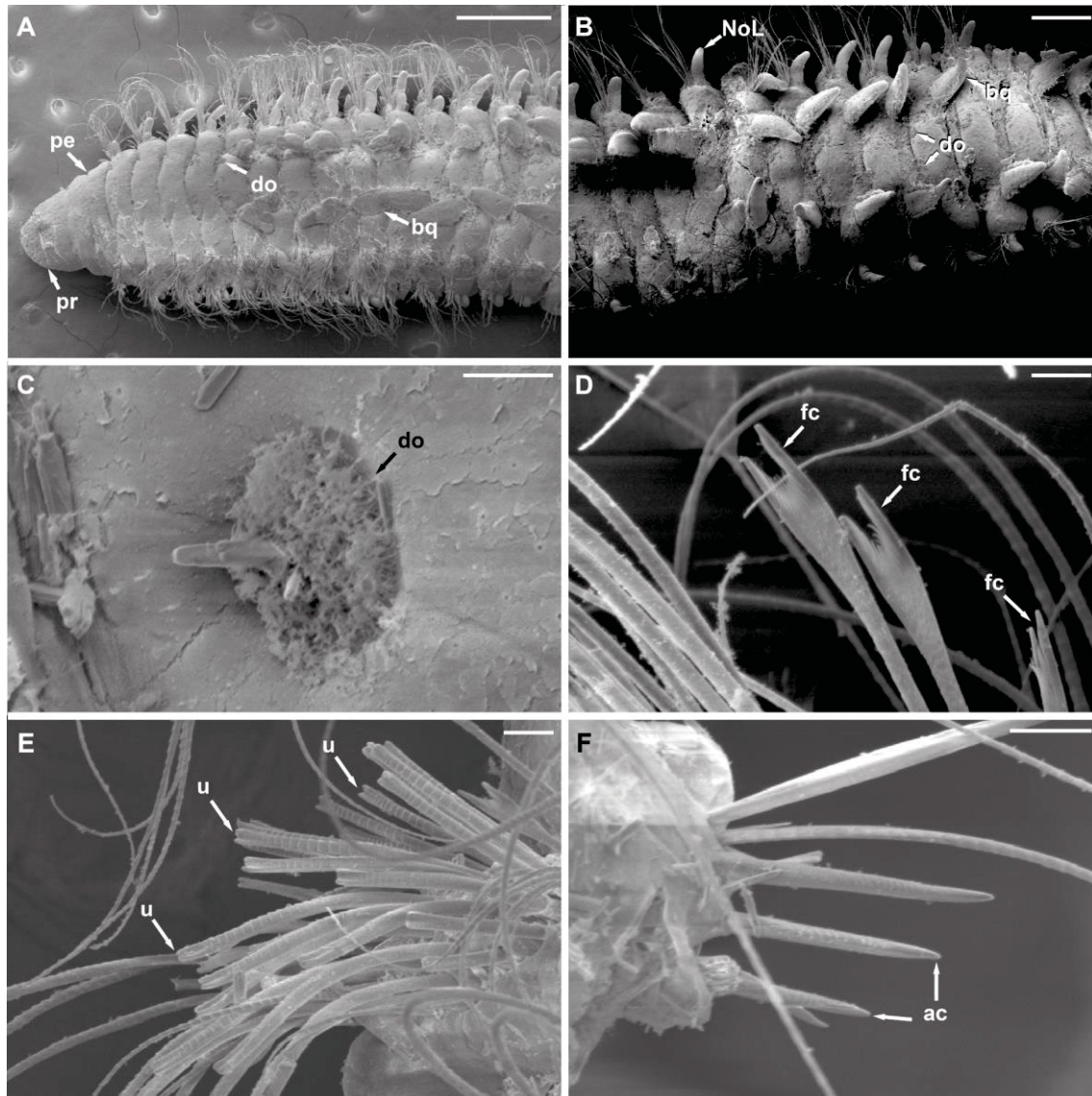


Figure 13. *Naineris quadricuspida* from Greenland (USNM 342). A, Anterior end, dorsal view; B, Abdominal segments, dorsal view; C, Dorsal sensory organ; D, Furcate chaetae; E, Bidentate thoracic neuropodial uncini; F, abdominal neuroacicula. Ac – abdominal neuropodial acicula, bq – branchia, do – dorsal organ, fc – furcate chaetae, NoL – notopodial lobe, pe- peristomium, pr – prostomium, u- uncini. Scale bars; (A, B): 200 μ m; (C, D, F): 10 μ m; (E): 20 μ m.

Naineris grubei (Gravier, 1908)

Scoloplos grubei Gravier, 1908: 42; 1909: 646–649, pl. XVIII, figs. 49–57.

Nainereis grubei: Eisig 1914: 499.

Naineris grubei: Hartman 1957: 303; 1959: 366; Tarazona 1974: 164; Blake 1991:86; Salazar-Vallejo & Londoño-Mesa 2004: 40; Blake 2017: 101–103, figs. 48A–I, 49A–D, 50A–E.

Type material: To our knowledge no type material remains for *N. grubei*.

Type locality: “Payta (Paita), [Piura,] Pérou” (Peru) (Fig. 14).

Geographic distribution: Warm Temperate Southeastern Pacific and Magellanic provinces (Fig. 14).

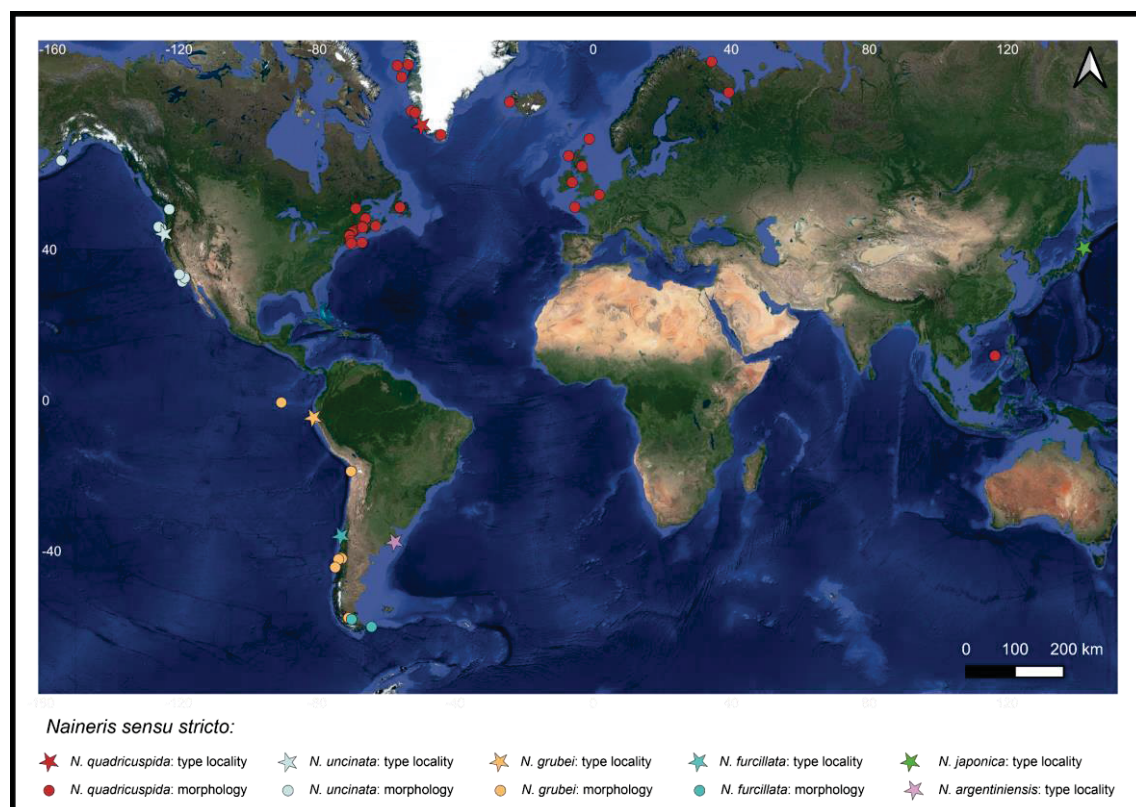


Figure 14. Distribution of species of *Naineris* s. str.

Habitat: Type habitat unknown. For specimens from Iquique (Chile) intertidal, red rocks in pools (Blake 2017).

Etymology: Gravier named the species in honor of Adolph-Eduard Grube, author of *Naineris laevigata* (Grube, 1855)

Molecular data: Available for specimens from indo-pacific (Ratnasingham & Hebert 2007).

Naineris uncinata Hartman, 1957

Naineris uncinata Hartman, 1957: 301–302, pl. 38, figs. 1–8; 1959: 366; 1969: 31–32, 6 figs; Hobson & Banse 1981: 28, figs. 1g–i.

Naineris (Naineris) berkeleyorum Pettibone, 1957: 165, fig. 4a–f.

Naineris cf. *grubei*: Blake 1996: 20–22, fig. 1.8A–F. Not Gravier 1908.

Naineris uncinata: Cadien & Lovell 2018:106; Blake 2020: 26–28, fig. 13A–F.

Type material: LACM. Holotype: LACM –AHF Poly 675. 1 specimen.

Type locality: 43°19'26"N–124°19'02"W “South Slough, Coos Bay, Oregon” (Fig. 14).

Geographic distribution: Cold Temperate Northeast Pacific province: Northern California, Oregon/Washington/Vancouver, and North American Pacific Fjordland ecoregions (Fig. 14).

Habitat Type habitat given only as hard-packed sand and eelgrass (Hartman 1957). Additional information about the type series habitat can be found in Fraser (1943), “intertidal, hard-packed sand and eelgrass; empire clam bed”. The most recently taxonomical record (Blake 2020) was collected from gas hydrate and seep sediments on the Cascadia Subduction Zone between 567–795 m (Blake 2020).

Etymology: Latin for hooked. Probably due to the sharply curved uncini in thoracic neuropodia.

Molecular data: COI. [MN816169.1](#), [MN816168.1](#), [MN816167.1](#), and [MN816166.1](#) (Blake 2020).

Note: The cytochrome oxidase I fragments above correspond to Blake's (2020) specimens. However, considering the discrepant bathymetry of Blake's and Hartman's (1957) records, the specimens may be a distinct species.

Naineris japonica Imajima, 2009

Naineris japonica Imajima, 2009: 109–111, figs. 34A–E, 35A–G.

Type material: NSTM. Holotype: NSMT Pol. H 510. 1 specimen. Paratype: NSMT Pol. P 511. 1 specimen. Paratype: NSMT Pol. P 512. 1 specimen.

Type locality: 40°16,88'N–142°13,51'E and 40°17,11'N–142°13,46'E “Off Sanriku”, [Honshu Island, Japan] (Fig. 14).

Geographic distribution: Cold Temperate Northwest Pacific: Northeastern Honshu province (Fig. 14).

Habitat: Depth Sea, range 354–424 m.

Etymology: Because the species was sampled in Japanese waters.

Molecular data: Not available.

***Naineris furcillata* Blake, 2017**

Naineris chilensis Carrasco, 1977: 70–72, figs. 5–12, homonym of *N. dendrítica chilensis* Hartmann-Schröder, 1965; Rozbaczyllo 1985: 130.

Naineris furcillata Blake, 2017: 97–98, fig. 45A–H.

Type material: MZUC. Holotype: 8316. 1 specimen.

Type locality: 36°38'07"S–73°03'58"W “Concepción Bay” (Quiriquina Island), [Talcahuano, Chile.] (Fig. 14).

Geographic distribution: Warm Temperate Southeastern Pacific and Magellan provinces (from Concepción Bay to Argentinian Patagonia) (Fig. 14).

Habitat: Type habitat given only as 10 m depth in fine gray sand.

Etymology: Latin for forked.

Molecular data: Not available.

***Naineris argentiniensis* Blake, 2017**

Naineris argentiniensis Blake, 2017: 103–105, fig. 51A–F.

Type material: NMNH. Holotype: USNM 1013686. 1 specimen. Paratypes: USNM 1013687. 3 specimens.

Type locality: “Rocas Pya Chica, Mar del Plata, Argentina” (Fig. 14).

Geographic distribution: Warm Temperate Southwestern Atlantic province: Uruguay–Buenos Aires Shelf ecoregion (Fig. 14).

Habitat: Type habitat given only as Intertidal.

Etymology: From Argentina, the type locality.

Molecular data: Not available.

Genus *Protoaricia* Czerniavsky, 1881

Type species: *Protoaricia oerstedii* (Claparède, 1864), by monotypy.

Generic Diagnosis (amended). Prostomium rounded or truncate on anterior margin. Peristomium with 1–2 achaetous rings. Thorax with 9–23 or more segments; branchiae first present either from thoracic or abdominal segments, laterally displaced. Thoracic neuropodia with 0–2 postchaetal lobes; no subpodial lobes. Thoracic neurochaetae only capillaries, or capillaries mixed with blunt-tipped uncini, sometimes hooded, and subuluncini. Abdominal chaetae including capillaries and sometimes furcate chaetae in notopodia and capillaries and imbedded or protruding aciculae in neuropodia. Rounded dorsal sensory organs present, paired per segment or multiple.

Remarks: *Protoaricia* was erected by Czerniavsky (1881). The genus was assigned to Protoariciinae by Hartman (1957) for having two peristomial rings, and *Naineris* to Orbiniinae. Despite erecting Protoariciinae with *Protoaricia* as the type genus, the similarities between *Naineris sensu lato* and *Protoaricia* are evident in Hartman's work. Blake (2000), in the phylogenetic analysis of the family, disregarded the number of peristomial rings from his character matrix, breaking up part of the protoariciin genera and transferring *Protoaricia* to Orbiniinae.

Interestingly, molecular phylogenies (Bleidorn 2005, Bleidorn *et al.* 2009, Zhadan *et al.* 2015) recovered *Protoaricia oerstedii* (the type species) forming a clade with only part of the species of *Naineris* with available sequences. Our results support the transference of most of the species of *Naineris sensu lato* to *Protoaricia*, based on COI, 16S, and 28S sequences and morphology. Disregarding *P. brevicephala* (see below), the following 16 species of *Protoaricia* are recognized.

***Protoaricia oerstedii* (Claparède, 1864)**

Protoaricia oerstedii, Claparède, 1864: 42–43., pl. IV, fig. 7.

Type material: No type material is known to be preserved for *P. oerstedii* (Solís-Weiss & Fauchald, 1989).

Type locality: Port-Vendres, France (Fig. 15).

Geographic distribution: Western Mediterranean (Fig. 15).

Habitat: Not stated. Specimens herein sequenced were sampled XXXX.

Etymology: Named for the renown Danish polychaetologist A. S. Örsted.

Molecular data: COI: [FJ612516.1](#). 16S: [AY532347.1](#)

***Protoaricia laevigata* (Grube, 1855) new combination**

Aricia laevigata Grube, 1855: 112–113 in part.

Naineris laevigata Grube 1855: 112–113 in part.

Type material. MPW. Syntype: 263 1 specimen. ZMB. Syntypes: 4689-Q. 5 specimens. ZIN. Syntype: 61/46662. 1 specimen.

Type locality. “Neapol” (Naples), [Campania, Italy] (Fig. 15).

Geographic distribution. Mediterranean Sea, from Italy to Tunisia (Fig. 15).

Habitat. No habitat information was given in description (Grube, 1855), however specimens near the type locality were found in Brackish waters, intertidal, and infralittoral (Giangrande & Fraschetti 1995).

Etymology. Latin for smooth, smoothed, not rough (*levis*).

Molecular data. Not available for *N. laevigata* s. str., except this study.

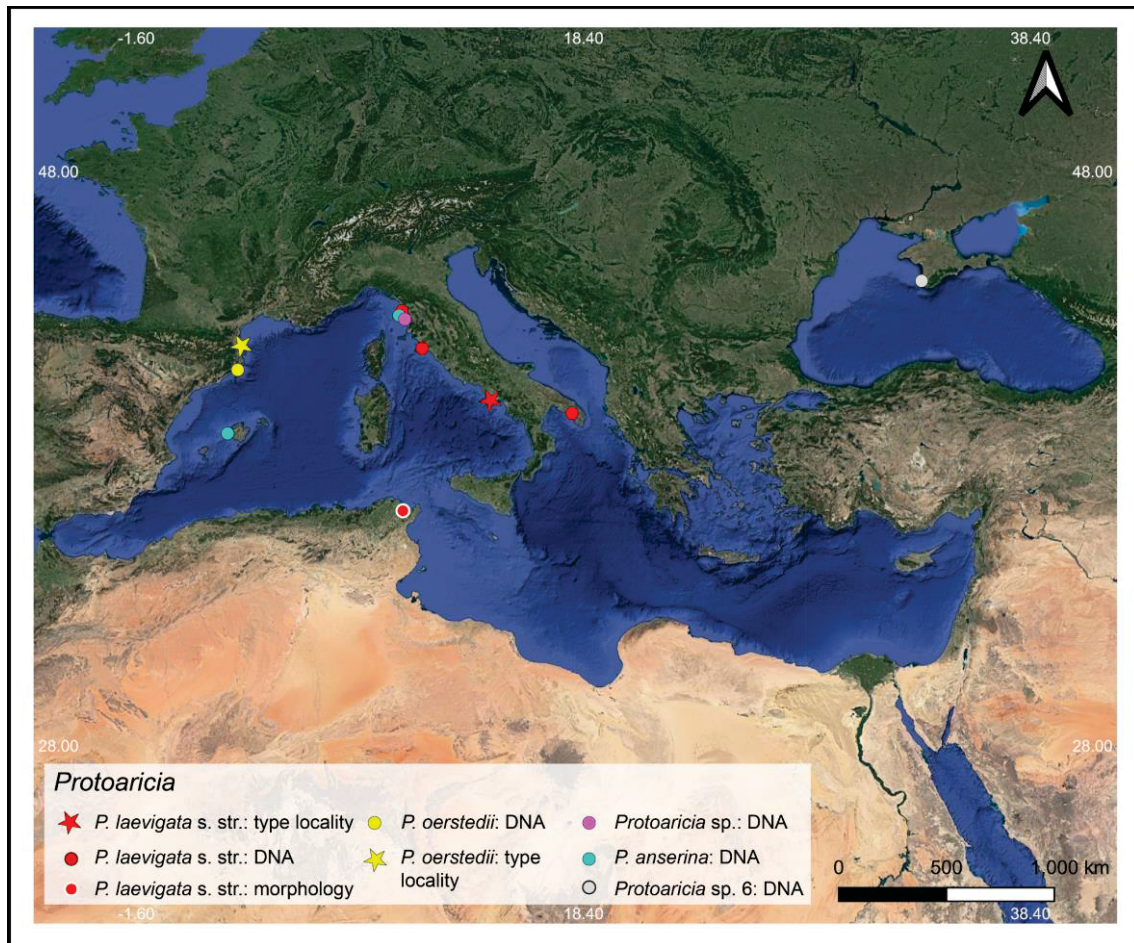


Figure 15. Distribution of Mediterranean species of *Protoaricia*.

Note. The type locality of *P. laevigata* needs to be better stated; it is usually assumed as contained in the Mediterranean Sea (Blake 2017, Zhadan 2020). Still some authors affirm that Nice is the type locality. This may be due to the fact that Grube (1855) mentioned that Nice specimens he studied in the past corresponded to *P. laevigata*. However, after examination of syntypes deposited at the ZMB, we noticed they were all sampled from Naples, which should be referred to as the type locality.

Moreover, we recognized two morphotypes among the syntypes, one with the posterior row of thoracic neurochaetae consisting of stout uncini, and the other with longer subuluncini. Our molecular dataset included both morphotypes and recovered them as distinct species.

Aricia armata Hansen, 1882 was described for Guanabara Bay, Rio de Janeiro, Brazil. Eisig (1914) synonymized *A. armata* with *P. laevigata*. The description of *A. armata* and drawings, however, agree with *P. setosa*. We requested the

material to Naturalis Biodiversity Center, but the material was in poor conditions, and the photos received do not permit us distinguishing any morphological character. We observed museum collections from the Guanabara Bay, and all the specimens from soft sediments in the bay bear only capillaries in thoracic neuropodia.

Molecular data of COI sequences [MN256577.1](#), [MN256576.1](#), [MN256575.1](#), and [GU362690.1](#) from GenBank are dubious, both matched with *P. dendritica* clades, not with Mediterranean representatives of *Protoaricia*. Similarly, 16S sequences [GU362680.1](#) and [FJ612463.1](#) did not match with *P. laevigata* s. str. The sequences here presented indeed correspond to *P. laevigata* s. str.

A particular problem are the potential distinct species synonymized with *P. laevigata* comb. nov. Among the species synonymized by Eisig (1914), we pointed out *P. hexaphyllum* comb. nov. (Schmarda, 1861) (type locality: South Africa), *P. ramosum* (Schmarda, 1861) comb. nov. (type locality: Jamaica), *Protoaricia fuscibranchis* (Grube, 1878) comb. nov. (type locality: Japan), *P. platycephala* (McIntosh, 1885) comb. nov. (type locality: Bermuda), *P. havaicus* (Kinberg, 1866) comb. nov. (type locality: Hawaii). Similarly, we consider that Australian specimens identified as *P. laevigata* comb. nov. (Day 1977, Zhadan 2020) need a revision. We examined collections from the AM and realized that the number of rows of subuluncini in thoracic neuropodia do not agree with the former species. Considering how problematic is the *P. laevigata* comb. nov. complex, joining efforts to sequence topotype materials will be essential to clarify their identity with integrative taxonomy.

***Protoaricia aurantiaca* (Müller, 1858) new combination**

Theodisca aurantiaca Müller, 1858: 216, pl. VI, figs. 13–15.

Theodisca aurantiaca: Quatrefages, 1866: 291.

Nainereis aurantiaca: Eisig, 1914: 498.

Naineris aurantiaca: Hartman 1957: 249; Hartman 1959: 366; Pagliosa *et al.* 2012: 32; Álvarez *et al.*, 2019: 125–134, figs. 2A–D, 3A–H, 4A–F.

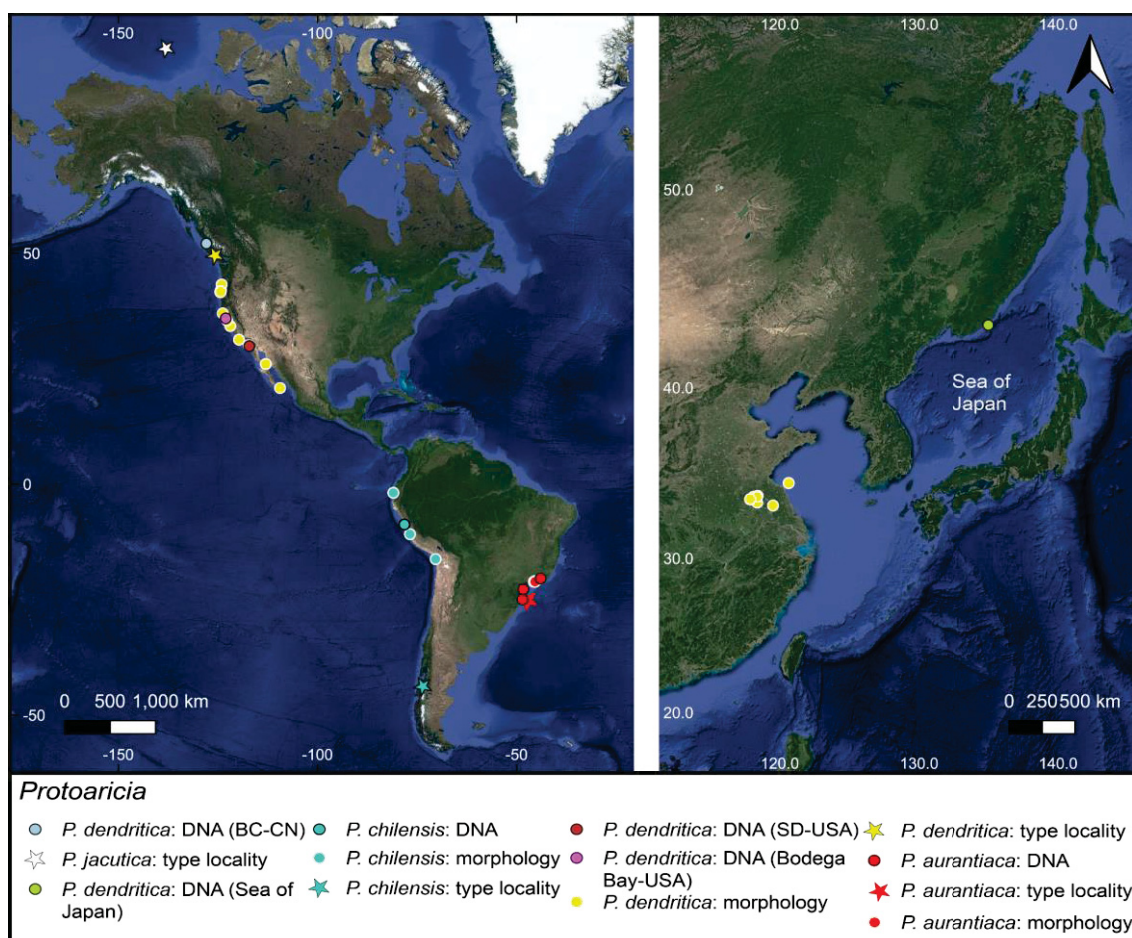


Figure 16. Distribution of the remaining species of the *P. dendritica*/*P. laevigata*-like species.

Type material: MNRJ. Neotype. MNRJP: 1956. 1 specimen.

Type locality: 27°47'2"S–48°30'25"W “Pântano do Sul Beach” (Florianopolis), Santa Catarina, Brazil” (Fig. 16).

Geographic distribution: Warm Temperate Southwestern Atlantic province: Southeastern Brazil ecoregion, from Santa Catarina (Müller 1858, Álvarez *et al.* 2019) to Rio de Janeiro states (Álvarez *et al.* 2019) (Fig. 16).

Habitat: Intertidal, type material found under rocks between oyster banks. Additional material, intertidal on sediment, and in deposited sediments among algae rhizoids and on sediments and fragments of shells in mussel banks (*Perna perna*).

Etymology: Latin for orange.

Molecular data: This study.

Note. *Protoaricia aurantiaca* comb. nov. was first described for Santa Catarina Island (Brazil), but it was never reported again until its redescription, probably

due to its vague and brief original description (Álvarez *et al.* 2019). The morphologically similar species, *P. laevigata* comb. nov. and *P. dendritica* comb. nov. were recorded several times instead (Álvarez *et al.* 2019). Among them, *Protoaricia aurantiaca* comb. nov. is more similar to *P. dendritica* comb. nov. by having fewer transversal rows of subuluncini. *Protoaricia aurantiaca* comb. nov. differs, however, in having a thoracic neuropodial papilla in thoracic neuropodia, which is absent in *P. dendritica* comb. nov.

As in *P. laevigata* comb. nov. and *P. dendritica* comb., in *Protoaricia aurantiaca* comb. nov., the number of thoracic chaetigers and branchial start is highly variable. Whereas the number of thoracic chaetigers ranged from 9–21 specimens from Southeastern Brazil (Álvarez *et al.* 2019), the branchial start ranged from 7–10 and is frequently unilateral (Álvarez 2019). Blake (2017) suggested that the wide variation in the branchial distribution of specimens from the Americas identified as *P. laevigata* comb. nov. may be due to the existence of multiple species. The present work provides sequences of *Protoaricia aurantiaca* comb. nov. for the first time, confirm its identity and distribution in South and Southeastern Brazil and indirectly support that the wide variation in morphological characters in *P. aurantiaca* comb. nov. complex is in fact intraspecific variation.

***Protoaricia dendritica* (Kinberg, 1867) new combination**

Anthostoma dendriticum Kinberg, 1867: 337.

Naineris robusta Moore, 1909: 262–264, pl. 8, figs. 34–37. *Fide* Hartman 1957.

Naineris longa Moore, 1909: 264–267, pl. VIII, figs. 38–42. *Fide* Hartman 1957.

Nainereis dendritica: Eisig 1914: 498.

Naineris nannobanchia Chamberlin, 1919: 260–261, pl. 2, fig. 10; pl. 3, fig. 1; 1969: 27–28, 2 figs

Naineris hespera Chamberlin, 1919: 14–15

Naineris laevigata: Berkeley & Berkeley 1941: 41; 1942: 196; Hartman 1944: 257.

Not Grube, 1855.

Naineris dendritica: Hartman 1948: 102–103; 1957: 299–300, pl. 36, figs. 1–3, pl. 37, figs. 1–7; 1959: 366; 1969: 25–26, 8 figs.; Blake 1975: 207, fig. 201; Hobson

& Banse 1981: 28, figs. 1c–e; Salazar-Vallejo 1991: 80; Bastida-Zavala 1995: 11; Blake 1996: 19, fig. 1.7A–I; Villalobos-Guerrero & Molina-Acevedo 2014: 104; Cadien & Lovell 2018:106.

Type material. NR. Holotype: SMNH type-6927. 1 specimen.

Type locality. “Insula Vancouveri” (Vancouver Island), [British Columbia, Canada] (Fig. 16).

Geographic distribution. Cold Temperate Northeast Pacific province (Fig. 16).

Habitat. Type material found in algae-covered with detritus, membranous tube.

Etymology. Greek for tree.

Molecular data. COI. CMC04 clade: [HM473485.1](#), [HM473484.1](#), [HM473488.1](#), [HM473489.1](#), [HM473487.1](#), [MF121580.1](#), [HM473486.1](#), [HM473483.1](#), [MF121380.1](#), [MF121380.1](#). **CMC03 clade:** [HM473482.1](#), [HM473481.1](#). **CMC02 clade:** [HQ932533.1](#), [HM473479.1](#), [HQ932535.1](#), [MH242859.1](#), [HM473480.1](#), **CMC01 clade:** [HM473478.1](#), [HM473474.1](#), [HM473475.1](#), [HM473473.1](#), [HM473476.1](#), [HM473477.1](#). **16S: CMC02 clade:** [AY532345.1](#). **Distinct clade:** [FJ612462.1](#).

Note. The *P. dendritica* comb. nov. species complex was first assessed by Carr et al. (2011). They found four different clades in Vancouver Island, the type locality (CMC01-CMC04). It is still necessary to determine which of them corresponds to Kinberg specimens. We examined the holotype of *P. dendritica* comb. nov., but unfortunately, our results were inconclusive. Our specimens matched with CMC01 and CMC02 clades and sampled two distinct clades: Bodega Bay and BC-Canada.

***Protoaricia capsulifera* (Bobretzky, 1870)**

Arícia capsulifera Bobretzky, 1870: 248–252, pl. XII, figs. 64–67.

Type material: Inexistent.

Type locality: Black Sea.

Geographic distribution: Known only from its type locality.

Etymology: Not stated, but it is probably due to the presence of paired dorsal sensory organs, referred as capsules by the author.

Molecular data: This study.

Note: The species is distinguished by having thoracic neuropodia with capillary chaetae only and branchial starting from the chaetiger sixth, disagreeing with the traditional genus diagnosis. Considering Bobretzky's description, we believe that the specimens identified as NI_12 in our phylogenetic reconstruction belong to *P. capsulifera*.

***Protoaricia setosa* (Verrill, 1900) new combination**

Aricia setosa Verrill, 1900: 651–653.

Anthostoma latacapitata Treadwell, 1902: 203-204, figs. 61–65.

Nainereis setosa: Treadwell 1936: 55.

Nainereis latacapitata: Treadwell 1939: 264, fig. 81.

Naineris setosa: Hartman 1942: 61, figs. 116–118; 1951: 67–70, pl. 17, figs. 1–6; 1957: 305, pl. 41, figs. 1–6; 1959: 366; Rioja 1960: 303; Perkins & Savage 1975: 43; Cubit & Williams 1983: 13; Vásquez-Montoya & Thomassin 1983:11; Amoreux 1985:102; Gillet 1986: 805; Solis-Weiss & Fauchald 1989: 774–778, figs. 2a–j; Salazar-Vallejo 1996: 26; Dueñas 1999: 12; Felder & Camp 2009: 764; Díaz-Díaz *et al.* 2012: 29–31, pl. 4, figs. E–I; Díaz-Díaz *et al.* 2014: 307; Díaz-Díaz *et al.* 2016: 29.

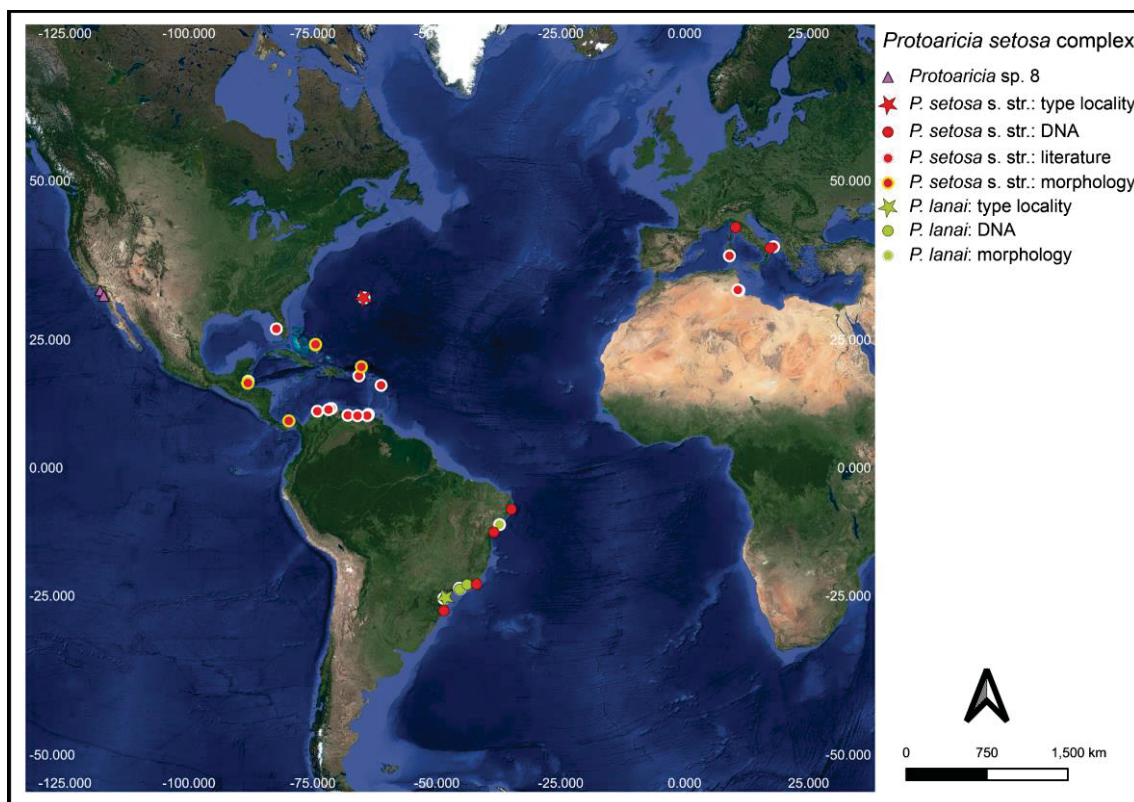


Figure 17. Distribution of species from the *P. setosa* complex.

Type material: YPM. Syntype: YPM IZ 001242.AN. 1 specimen.

Type locality: “Flatt’s Inlet beach”, [Smith’s Parish County,] Bermuda (Fig. 17).

Geographic distribution: Tropical Northwestern Atlantic, North Brazil Shelf, Tropical Southwestern Atlantic, Warm Temperate Southwestern Atlantic, Mediterranean Sea (Fig. 17).

Habitat: Type material found in shell-sand, at low tide.

Etymology: Latin for silky. It is probably due to the numerous chaetae of thoracic neuropodia.

Molecular data: Mitochondrion sequence: [OQ729897.1](#)

Note: *Protoaricia setosa* is known to be the only species of *Protoaricia* with only capillaries in thoracic neuropodia. However, as part of this review, we demonstrate that Pacific records examined are dubious, restrict *P. setosa* s. str. comb. nov. to Atlantic waters and the Mediterranean and describe a new species for the South and Southeastern Brazil (Chapter 2).

***Protoaricia retusiceps* Chamberlin, 1919 new combination**

Nainereis retusiceps Chamberlin, 1919: 355–357, pl. 65, figs 3–5.

Naineris returiceps: Hartman 1957: 296; Hartman 1959: 366.

Type material: NMNH. Holotype: USNM 19412. 1 specimen.

Type locality: 15°10'12.000"S–147°34'48.000"W “Mohican Reef., Rangiroa Island, Paumotu (Tuamotu) Islands”, French Polynesia (Fig. 18).

Geographic distribution: Southeast Polynesia province, Tuamotus ecoregion (Fig. 18).

Habitat: Type habitat given only as 0–50 m.

Etymology: Latin for blunt head (*retusus* + *ceps*). Probably because of the shape of prostomium, which is bluntly rounded.

Molecular data: Not available.

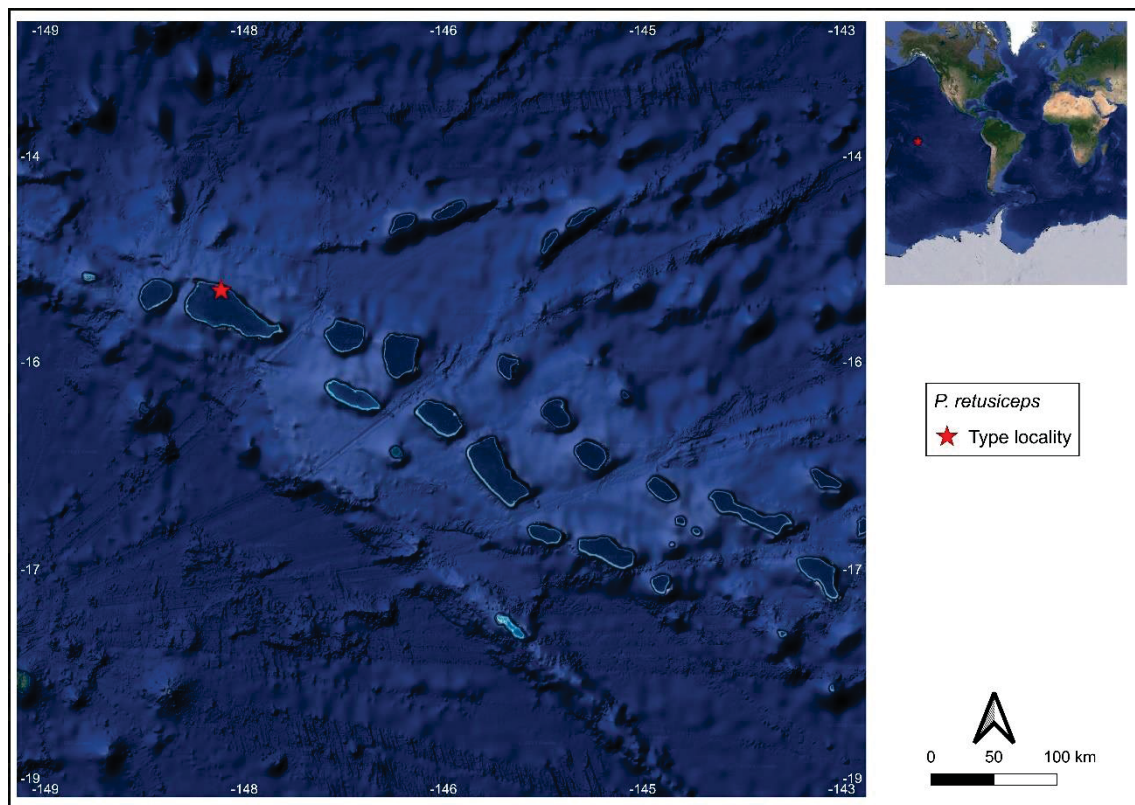


Figure 18. Distribution of *P. retusiceps* in French Polynesia.

***Protoaricia jacutica* Annenkova, 1931 new combination**

Nainereis jacutica Annenkova, 1931: 203–205, figs. 1–4; 1937: 140.

Naineris jacutica: Hartman 1957: 300; Hartman 1959: 366.

Type material: ZIN. Syntype: 1/25231-2/25232. 4 specimens.

Type locality: 73°06'N–138°4'W and 72°29'N–133°16'W “Laptev Sea” [Yacutia (Sakha), Far Eastern Federal District, Russia] (Fig. 16).

Geographic distribution: Artic, Laptev Sea ecoregion; Cold Temperate Northwest Pacific province (Fig. 16).

Habitat: Type habitat given only as Infralittoral (24 m) in the mud.

Etymology: Not stated. Perhaps Annenkova named the species because Yakutia is a state from Russia close to the Laptev Sea, the type locality. Also, it can refer to the expedition in which the specimens were sampled, “The Yacut Expedition of the U.S.S.R. Academy of Sciences”.

Molecular data: Not available.

***Protoaricia mutilata* Treadwell, 1931 new combination**

Nainereis mutilata Treadwell, 1931: 5–6, figs. 13–18.

Naineris mutilata: Hartman 1956: 290; 1957: 300–301; 1959: 366; Perkins & Savage 1975: 43; Vásquez Montoya & Thomassin 1983:11; Salazar-Vallejo 1996: 26.

Naineris mutilata: Cubit & Williams 1983: 13.

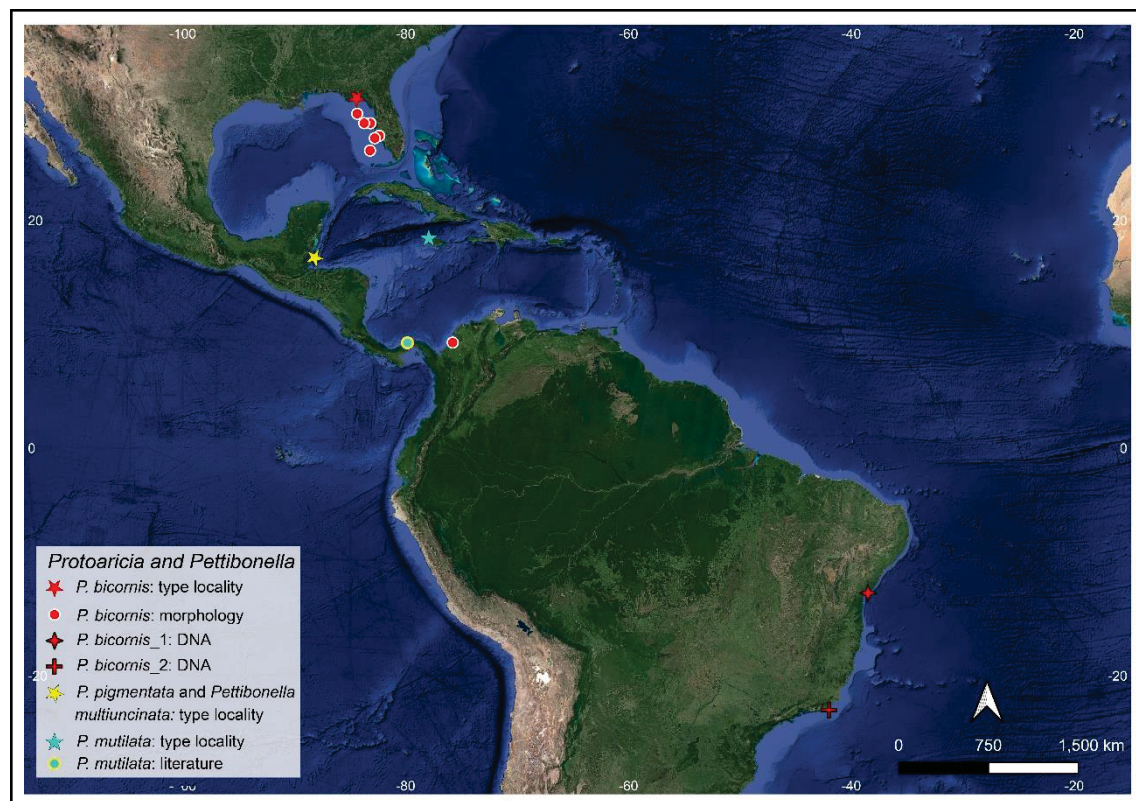


Figure 18. Distribution of *Protoaricia* in the Tropical Northwestern Atlantic.

Type material. AMNH. Holotype: 2070. 1 specimen.

Type locality. “Montego Bay, [St. James parish,] Jamaica” (Fig. 18).

Geographic distribution. Tropical Northwestern Atlantic province: Greater Antilles and Southwestern Caribbean ecoregions (Fig. 18).

Habitat. Type habitat unknown. Specimens from Panama were sampled in association with turtlegrass, *Thalassia testudinum* (Vásquez-Montoya & Thomassin 1983).

Etymology. Latin for mutilate. Perhaps because the type material was mutilated according to Treadwell, i.e., the holotype was an incomplete specimen,

Molecular data: Not available

Note. *Protoaricia mutilata* comb. nov. is very similar to *P. setosa* comb. nov. *Protoaricia mutilata* comb. nov. possess several rows of crenulate capillaries in thoracic neuropodia and paired oval-shaped dorsal sensory organs as *P. setosa* comb. nov. *Protoaricia mutilata* comb. nov. differ, however, in having an inferior row of acuminate uncini in thoracic neuropodia.

***Protoaricia bicornis* Hartman, 1951 new combination**

Naineris bicornis Hartman, 1951: 72–74, pl. 19, figs. 1–6; 1957: 304–305, pl. 40, figs. 1–6; 1959: 366; Perkins & Savage 1975: 43; Taylor 1984: 1–9, figs. 1–5, 1–6a–f; Salazar-Vallejo 1996: 26; Perkins 1998: 88; Dueñas 1999: 12; Báez & Ardilla 2003: 107; Felder & Camp 2009: 764.

Type material: LACM. Holotype: LACM—AHF Poly 674. 1 specimen.

Type locality: 29°54'36" N–84°23'43" W “Franklin Co., Alligator Harbor, Florida” (Fig. 18).

Geographic distribution: Warm Temperate Northwest Atlantic province: Northern Gulf of Mexico ecoregion and Tropical Northwestern Atlantic province: Floridian and Southern Gulf of Mexico ecoregions (Fig. 18).

Habitat: Type habitat given only as shallow water, in sand.

Etymology: Latin for with two horns (*bi* + *cornis*). Perhaps because of the shape of the prostomium, which is weakly bifid in the frontal margin.

Remarks: Nonato (1981) recorded *Naineris bicornis* in Ilha Grande, Brazil (unpublished). The species never was recorded again in that country, see Amaral *et al.* (2006). It is necessary to compare these specimens with material type and voucher specimens of *N. bicornis*.

Molecular data: Not available.

Note: *Protoaricia bicornis* comb. nov. was distinguished by having two horns in the anterior prostomium margin (Hartman 1951). Examining grab sampled collections of the USNM from the Gulf of Mexico, we noticed this condition in the holotype is an exception, probably due to fixation.

***Protoaricia australis* Hartman, 1957 new combination**

Naineris grubei australis Hartman, 1957: 303–304, pl. 39, figs. 1–4; 1959: 366; Day 1977: 238; Hutchings & Rainer 1979: 761; Hutchings & Murray 1984: 53; Zhadan *et al.* 2015: 793–797, figs. 10A–G, 11A–C, 12D.

Naineris grubei: Day 1977: 237–238.

Naineris australis: Blake 2017: 103; Zhadan 2020: 479–480, fig. 15A–E.

Type material: LACM. Holotype: LACM—AHF Poly 676. 1 specimen.

Type locality: 35°9'0" S–138°28'0" E “Port Noarlunga, [Adelaide,] South Australia” (Fig. 19).

Geographic distribution: Southwest Australian Shelf, Southeast Australian Shelf, East Central Australian Shelf, Northeast Australian Shelf provinces (Fig. 19).

Habitat: Hartman (1957) did not provide any information about type habitat. However, Leslie Harris, from the LACM, provided a species list with information of type material, they were found Intertidal to 1 m.

Etymology: Latin for southern.

Molecular data: COI: [KR920026.1](#). 16S: [KR920029.1](#)

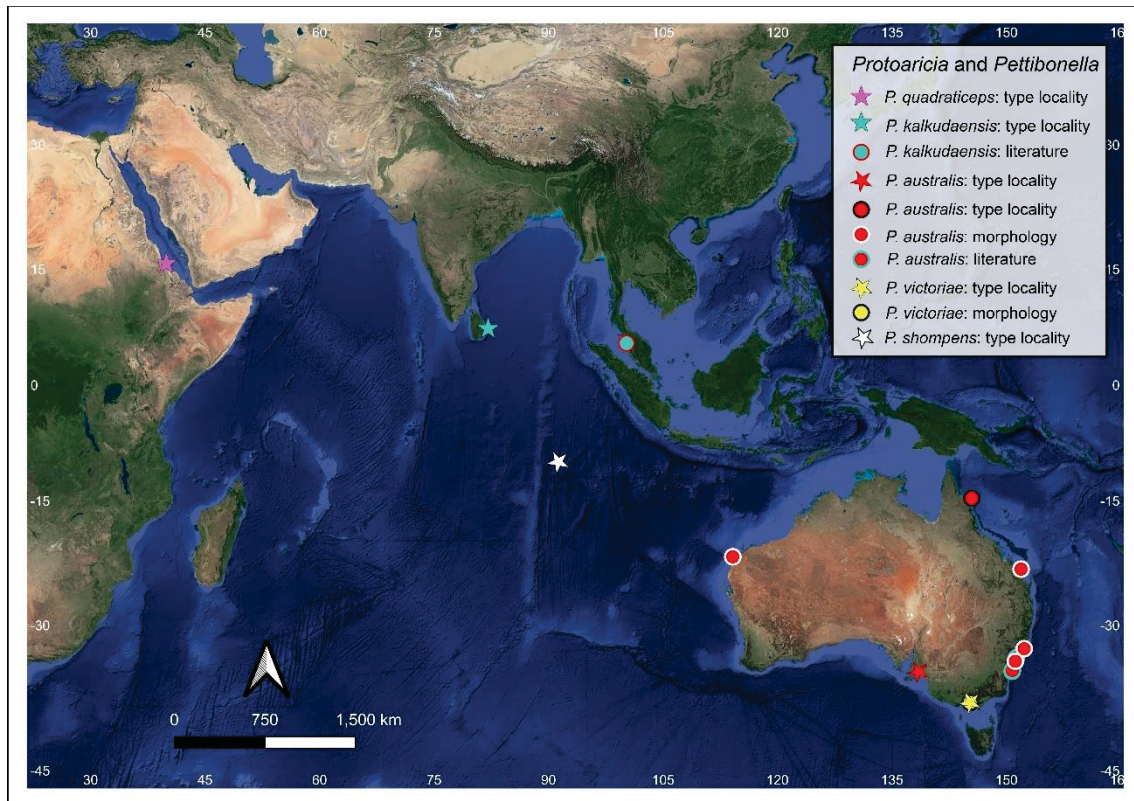


Figure 19. Distribution of *Protoaricia australis*, *Protoaricia quadraticeps*, *Protoaricia kalkudaensis*, *Pettibonella victoriae* and *Pettibonella shompens*.

Protoaricia brevicephala* Hartmann-Schröder, 1960? *incertae sedis

Naineris brevicephala Hartmann-Schröder, 1960: 32–33, figs. 72–75; Tarazona 1974: 164; Tasso *et al.* 2018: 8.

Type material: ZMH. Holotype: ZMH P-15275. 1 specimen. Paratype: ZMH P-15276. 1 specimen.

Type locality: “Zwischen La Herradura und La Chira bei Lima”, [Chorrillos, Peru].

Geographic distribution: Warm Temperate Southeastern Pacific province: Central Peru and Humboldtian ecoregions.

Habitat: Littoral, in rocky shore, associated with algae tufts and *Mytilus* banks.

Etymology: Latin for short head (*brevis* + *cephala*). Maybe due to the shape of prostomium, which is short and wide.

Remarks: Blake (2017) proposed to treat the species as *incertae sedis* because of the absence of specimens in the corresponding holotype vial. However, additional material type (paratypes) is still available according with Alexandra Kerbl, ZMH curator (*pers. com*).

Molecular data: This study.

***Protoaricia chilensis* Hartmann-Schröder, 1965 new combination**

Naineris dendritica chilensis Hartmann-Schröder, 1965: 195–197, figs. 179–180; Rozbaczylo 1985: 130.

Naineris chilensis Blake, 2017: 98–101, figs. 46A–G, 47A–D.

Type material. ZMH. Holotype: ZMH-P-15326. 1 specimen. Paratype: ZMH P-15327. Several specimens.

Type locality. ‘Puerto Aguirre, [Aysen region,] Chile’ (Fig. 16).

Geographic distribution: Warm Temperate Southeastern Pacific and Magellan provinces (Fig. 16).

Habitat. Type habitat given only as 10 m, mussel bank.

Etymology. From Chile, the type locality.

Molecular data. Not available.

Note. *Protoaricia chilensis* comb. nov. was described as *Naineris dendritica chilensis* by Hartmann-Schröder (1965). The species was recently redescribed (Blake 2017) and raised to full species status. *Protoaricia chilensis* comb. nov. have neuropodial postchaetal lobes foliaceous ridges with the upper part projected. In controversy, we observed that this character is inconsistent in the holotype.

***Protoaricia kalkudaensis* (De Silva, 1965) new combination**

Naineris (*Polynaineris*) *kalkudaensis* De Silva, 1965: 553, fig. 10A–F.

Naineris kalkudaensis: Ong 1995: 271–272, fig 8A–H.

Type material. NHML. Holotype: 1963.14.5. 1 specimen.

Type locality. “Kalkuda” (Kalkudah), [Ceylan, Sri Lanka] (Fig. 19).

Geographic distribution. West and South Indian Shelf province: South India and Sri Lanka ecoregion (Fig. 19).

Habitat. Not given by the author, but specimens near the type locality were sampled among tubes of *Diopatra neapolitana* (Idris & Arshad 2013). Ong (1995) recorded specimens in an intertidal sand flat from Malasia.

Etymology. From Kalkuda, the type locality.

Molecular data. Not available.

Note.

***Protoaricia quadraticeps* Day, 1965 new combination**

Naineris quadraticeps Day, 1965: 21–23, fig. 2a–h; Bellan 2001:222.

Type material: NHML. Holotype: 1963.13.2. 1 specimen.

Type locality: “Abiad Bay, Entedebir Island (Dahlak Archipelago), [Eritrea] (Fig. 19).

Geographic distribution: Red Sea and Gulf of Aden province: Southern Red Sea ecoregion (Fig. 19).

Habitat: Type habitat given only as intertidal, in coral sands.

Etymology: Latin for squared head (*quadrati+ceps*). The specific epithet refers to the square shape of the prostomium (Read & Fauchald 2020).

Molecular data: Not available

Note: Known only from the type locality.

***Protoaricia pigmentata* Solís-Weiss, 1989**

Type material: USNM. Holotype: 120950. Paratypes: USNM 120950 (2 spms), USNM 120951 (3 spms), NMH-London 1990.1-3 (3 spms).

Type locality: “Blue Ground Range, (Stan Creek), Belize” (Fig. 18).

Geographic distribution: Known only from its type locality (Fig. 18).

Habitat: Type habitat given as root-mat of *Rhizophora mangle*, covered with *Caulerpa verticillata*, 10-50 cm depth.

Etymology: Due to the pigmentation present on each notopodial lobe from thoracic and mid-abdominal segments.

Molecular data: Not available

Note: The species was distinguished based on the pigmentation pattern present in abdominal segments, the specimen's size, and the chaetiger where the branchiae start. *Protoaricia pigmentata* undoubtedly belong to the *P. oerstedii* clade.

***Protoaricia antarctica* Blake, 2017 new combination**

Haploscoloplos kerguelensis: Hartman 1978: 156 (in part, Sta. 69-1). Not McIntosh 1885.

Naineris antarctica Blake, 2017: 106–108, figs. 52A–I, 53A–F.

Type material: NMNH. Holotype and Paratypes: USNM 690405–6 (4 specimens). Paratype: USNM

1013906. 1 specimen. Paratype: USNM 1013907. 1 paratype.

Type locality: 72.05°S–172.63°E Ross Sea, East of Cape Adare, Antarctica

Geographic distribution: Continental High Antarctic province: the Weddell Sea and Ross Sea ecoregions.

Habitat: Type habitat given only as 344–923 m depth range.

Etymology: From Antarctica, the type locality (Blake 2017).

Molecular data: Not available.

Note.

Genus *Pettibonella* Solís-Weiss, 1989

Pettibonella Solís-Weiss, 1989. **Type species:** *Pettibonella multiuncinata* Solís-Weiss & Fauchald, 1989), by monotypy and original designation.

Generic Diagnosis (amended after Solís-Weiss, 1989)

Prostomium conical, distally rounded, usually with two eyespots. Peristomium with 2 achaetous rings. Thorax with 15-21 segments; branchiae from thoracic region, subdivided, deciduous. Thoracic neuropodia with 1 papilla in middle of postchaetal lobe. Thoracic neurochaetae including distinct huge capillaries and an inferior row of uncini. Notochaetae with capillaries only. Abdominal neurochaetae including capillaries and imbedded or protruding multidentate

aciculae in neuropodia. Paired, semicircular dorsal sensory organs may be present.

***Pettibonella multiuncinata* Solís-Weiss, 1989**

Pettibonella multiuncinata Solís-Weiss, 1989: 785–788, fig. 6a–l.

Pettibonella multiuncinata: Gopal et al., 2014: 1037, table I.

Type material: NMNH. Holotype: USNM 120971. Paratypes: USNM 120972 (1 specimen), USNM 120973 (3 specimens).

Type locality: “Twin Cays, West Bay, Belize” (Fig. 18).

Geographic distribution: Tropical Northwestern Atlantic province, Western Caribbean ecoregion (Fig. 18).

Habitat: Given only as 0.1 to 0.5m

Etymology: Refers to the distinctive hooks of the species.

Molecular data: COI: [FJ612510.1](#). 16S: [AY532339.1](#).

***Pettibonella victoriae* Day, 1977 new combination**

Naineris victoriae Day, 1977: 238–239, fig. 3k–p; Zhadan 2020: 481–483, fig. 17A–L.

Pettibonella sp. A Jacobsen 2002: 85–87, figs. 5A–D.

Type material: AM. Holotype: W.7358. 1 specimen. Paratype: W.7359 (1 specimen).

Type locality: 38°25'48"S–145°21'3"E “Westernport Bay, Victoria”, [Australia].

Geographic distribution: Southeast Australian Shelf province (Fig. 19).

Habitat: Type habitat given only as up to 12 m.

Etymology: From Victoria, the type locality.

Molecular data: Not available.

Material examined. Victoria: Westernport Bay, 1spm, MVF F 138362, -38.360804°, 145.254065° (Photo); 1 spm, MVF 138349, -38.360804°, 145.254065° (description); 2 spms, MVF 138345, -38.347447°, 145.225236° (last photos).

Description. A complete specimen with 25 mm long, 0.8 mm wide at chaetiger 50 for around 100 chaetigers. Color in alcohol light tan. Thorax and abdomen distinct, marked by dorsal displacement of chaetigers in abdomen. Dorsal surface from thoracic segments presents a longitudinal groove with evident intersegmental annular rings (Fig. 20B). Ventral groove from thoracic segments.

Prostomium wider than long, conical, rounded in anterior margin, but totally rounded in small specimens (Figs 20A, 21B); eyespots absent; nuchal organs present. Peristomium with two annular rings (Fig. 20A); proboscis not everted.

Branchiae are lost in most of the body, but scars first present from chaetiger eight (Fig. 20B); branchiae extremely long in abdominal segments, XX times segment length; proximal half ciliated; distal half without cilia, digitiform, tapering to rounded, flat papilla; distal portion longer than proximal, pigmented (Fig. 21B). Paired dorsal organs between brachial basis, medially displaced, represented by elongated semicircles (Fig. 21B). Dorsal crest between branchiae basis from abdominal segments, low (Fig. 21B).

Thorax comprises around 15 chaetigers, flattened (up to 1.5 times abdominal segments width). Parapodia biramous; lateral organ inconspicuous. Notopodial lobes elongate with wide basis (Fig. 21C). Neuropodial lobes mamilliform bearing a papilla in middle of lobe (Fig. 21C), last thoracic and first abdominal segments with additional papilla (Fig. 21D). Interramal cirri from last thoracic chaetigers. Abdominal notopodial lobes long, triangular (Fig. 21B); abdominal neuropodial lobes thin, triangular (Fig. 21B).

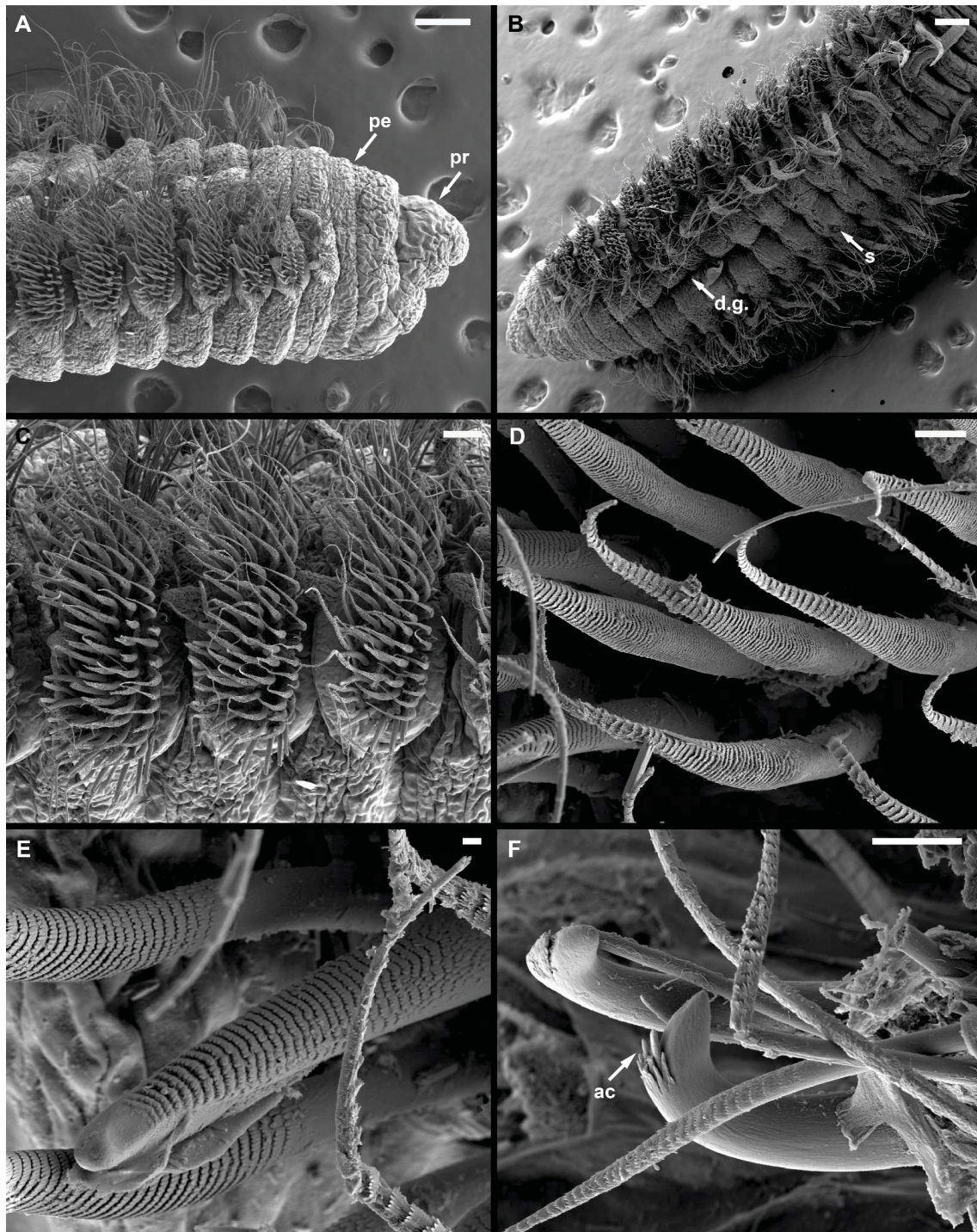


Figure 20. *Pettibonella victoriae* n. comb. (MVF F 138362) examined under SEM: (A) Anterior end, lateral view; (B) Thoracic segments, dorsal view; (C) Thoracic neuropodia, lateral view; (D) Thoracic neuropodial capillaries; (E) Thoracic neuropodial hooded uncini; (F) Abdominal neuropodial acicula. ac - multidentate aciculae, dg - dorsal groove, pe - peristomium, pr - prostomium, s - scars from branchiae. Scale bars; (A-B): 200 μ m; (C): 100 μ m; (D) 10 μ m; (E-F) 2 μ m.

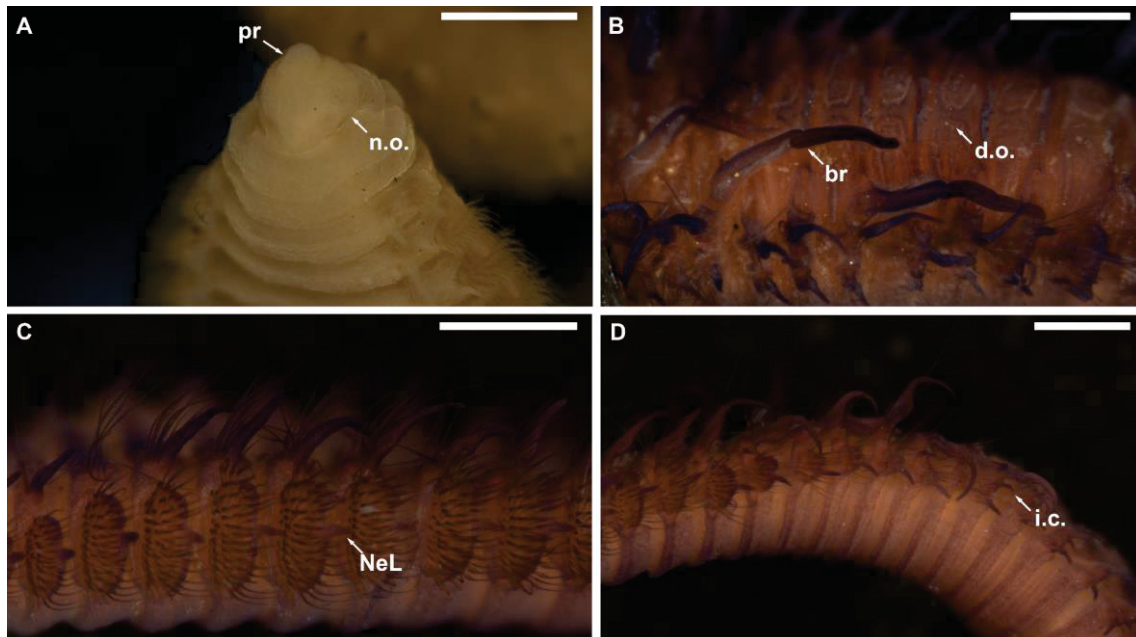


Figure 21. *Pettibonella victoriae* n. comb. (MVF F 138362) examined under SEM: (A) Anterior end, dorsal view; (B) Abdominal segments, dorsal view; (C) Thoracic parapodia, lateral view; (D) Transitional segments. br - branchiae, d.o. - dorsal organ, i.c. - interramal cirri, NeL - Thoracic neuropodial lobe, n.o. - nuchal organ, pr - prostomium. Scale bar; (A-D): 500 μ m.

Thoracic notochaetae crenulated capillaries (Fig. 21A). Abdominal notochaetae crenulated capillaries; furcate absent. Thoracic neurochaetae (Figs 20C, 21C) with 4-5 anterior, transversal rows of enlarged capillaries (Fig. 20D), and a posterior, ventral row of hooded uncini intermixed with capillaries; uncini (Fig. 20E) increasing in size from inferior to superior. Abdominal neurochaetae with capillaries, and thin serrated uncini (Figs 20F). Abdominal notopodia and neuropodia supported by aciculae; projected, swan shaped, and multidentate in neuropodia. Anus terminal with two, long anal cirri; last segments with long, digitate parapodial lobes, probably functioning as accessory branchiae.

Remarks: Examining topotype specimens of the recently redescribed *Naineris victoriae* (Zhadan 2020) revealed that the species does not show the typical characters of its congeners. *Pettibonella victoriae* is unique in having triangular prostomium; branchiae deciduous; enlarged capillaries in thoracic neuropodia (almost subuluncini); and multidentate, swan-shaped aciculae in abdominal neuropodia.

The aberrant triangular prostomium is uncommon in *Naineris sensu lato*. In the collections studied, we observed juveniles with rounded prostomium, which apparently becomes triangular in adults, supporting its incorrect inclusion in *Naineris sensu lato*.

Branchiae of *P. victoriae* were described as deciduous (Day, 1977). Effectively, most of the specimens studied lack branchiae, bearing scars instead. However, a few specimens studied kept branchiae after fixation, they are formed by a proximal, glandular part and a distal, pigmented, and projected part without cilia, separated by a constriction. This branchiae shape disagrees with other species of *Naineris* s. str. and *Protoaricia* but agrees with *Pettibonella* instead. Thoracic neurochaetae are also distinct. While species of *Protoaricia* have capillaries and intermediate forms of capillaries and uncini (subuluncini), *Pettibonella victoriae* has a different type of capillaries, oblanceolate, with several rows of small barbs.

Finally, abdominal neuroacicula were described as bidentate in earlier descriptions (Day, 1977; Zhadan et al., 2020). We noticed, however, that they are supported by a main fang and are clearly multidentate, as in species of *Proscoloplos* and *Pettibonella*. Based on these observations, we strongly recommend reallocating *Naineris victoriae* to *Pettibonella victoriae*.

Pettibonella victoriae is distinguished from its congeners *P. multiuncinata* Solis-Weiss & Fauchald, 1989 and *P. shompens* Gopal, Useph, Varghese & Narayana, 2014 in having interramal cirrus, proximal part of branchiae ciliated, and only 1-2 spines in abdominal neuropodia.

***Pettibonella shompens* Gopal, Useph, Varghese & Narayana, 2014**

Pettibonella shompens Gopal, Useph, Varghese & Narayana, 2014: 1033–2037, fig. 1–3.

Type material: FORV. Holotype: IO/SS/POL/00465. 1 specimen. Paratype: : IO/SS/POL/00466 (1 specimen).

Type locality: 9.299416°S–92.700239°E “Car Nicobar Island”, North Indian Ocean (Fig. 19).

Geographic distribution: Java Transitional province (Fig. 19).

Habitat: Type habitat given only as 50 m.

Etymology: The specific name refers to the Shompen tribe inhabiting the Nicobar Islands.

Molecular data: Not available.

3.4. DISCUSSION

Polyphyly of *Naineris*. Previous studies proposing *Naineris* being not a monophyletic taxon (Blake 2000, Zhadan et al. 2015) are corroborated by our results based on mitochondrial and nuclear DNA data. *Naineris* was split into two highly supported clades, *Naineris sensu stricto* clade (containing the type species, *N. quadricuspida*) and a supported clade containing most of the remaining species of “*Naineris*” + *Protoaricia*. Pettibone (1957) suggested rearranging *Naineris* into the subgenera *Naineris* and *Polynaineris*, after contrasting the position of thoracic neuropodial papilla of *N. quadricuspida* and *Anthostoma ramosum*, a junior synonym of *Naineris laevigata*. However, results here suggest that *N. laevigata*-like species cluster together with *Protoaricia*, forming a highly supported clade. Even though Pettibone's assumptions were not precise, they were the morphological baseline to split *Naineris* into *Naineris* s. str. and *Protoaricia* in the present work. Besides the position of the thoracic neuropodial papilla, both genera are distinguished by the shape and arrangement of thoracic neuropodial uncini and details of dorsal sensory organs. In this thesis, we distributed the valid species of *Naineris* between *Naineris* s. str. and *Protoaricia* and transferred *Naineris victoriae* to *Pettibonella* based on morphological characters, including the presence of multidentate hooks, prostomium shape, thoracic neurochaetae and the shape of branchiae. We amended generic definitions for all three genera.

***Naineris* s. str. clade.** The *Naineris* s. str. clade contains *Naineris quadricuspida* and other species sharing the following features: the presence of thoracic neuropodial papilla in the middle of the lobe, multiple rows of uncini, uncini with a ribbed shaft and rounded tip with a terminal notch, and dorsal sensory organs always paired. The most similar species to *N. quadricuspida*,

Naineris uncinata, was recovered with the type species in a highly supported clade. The redefined *Naineris* s. str. contains six species, including *N. quadricuspida*, *Naineris uncinata*, *Naineris grubei*, *Naineris japonica*, *Naineris furcillata*, and *Naineris argentiniensis*.

Protoaricia clade. *Protoaricia* clade contains species with thoracic neuropodial papilla at an upper position, reduced rows of uncini; uncini, whenever present, without ribbed shaft and rounded tip with a terminal notch; and paired or multiple dorsal sensory organs per segment. *Protoaricia* clade contains 23 species in our analysis, distributed in three major subclades, “*P. laevigata*” subclade, “*P. setosa*” clade, and “*P. bicornis*” subclade: with “*P. laevigata*” subclade being sister to (“*P. setosa*” subclade, + “*P. bicornis*” subclade). Among the 23 studied species, only 8 are considered valid and 15 are probably new to science. Our morphological reconstruction suggests different complexity patterns of thoracic neurochaetae, and dorsal sensory organs in *Protoaricia*. The species in the “*Protoaricia setosa*” clade do not possess neither uncini nor subuluncini in thoracic neuropodia. Whereas the species from the other two subclades, “*Protoaricia bicornis*” subclade and “*Protoaricia laevigata*” subclade have these types of chaetae. We also observed that the number of thoracic neurochaetae is considerably higher in the species from “*P. setosa*” and “*P. bicornis*” subclades, whereas in “*Protoaricia laevigata*” clade there is a reduction in number of rows and chaetae. This character should be addressed in future reconstructions.

“*Protoaricia laevigata*” subclade

The analysis of Bleidorn (2009) highlighted a clade containing *P. oerstedii*, *P. laevigata* comb. nov., and *P. dendritica* comb. nov. This clade, here referred as *Protoaricia* clade, was recovered in our analysis except for *N. quadricuspida* from Morro Bay (California) and Friday Harbor (Washington), which we consider a misidentification once it was not recovered within the *Naineris* s. str. clade. This misidentification certainly led to the misconception of the monophyly of *Naineris* in earlier studies (i.e., Bleidorn 2005, 2009), as shown by Zhadan et al.'s (2015) COI tree reconstruction.

“*Protoaricia laevigata*” clade contained several species for which we made taxonomical notes based on molecular results.

Protoaricia laevigata comb. nov. species complex (see the detailed results in the Chapter III) comprised 3 species with nearly identical morphology. Eisig

(1914) observed wide morphological variation in *Naineris laevigata* in terms of the number of thoracic chaetigers and branchial start, both considered as diagnostic characters. He also put under synonymy several species from very distant localities with *N. laevigata*. In this study, *P. laevigata* comb. nov. is restricted to the Mediterranean Sea. Other molecular clades represent the reinstated *Protoaricia anserina* comb. nov., also from the Mediterranean, and *Protoaricia* sp. from the Black Sea. A revision of other species previously synonymized with *Naineris laevigata* from more distant geographical localities is clearly necessary (Blake 2017, Zhadan 2020).

Protoaricia dendritica comb. nov. was described by Kinberg (1867) for Vancouver Island, Canada. Carr et al. (2011) sequenced topotype specimens and found four different clades near Vancouver Island indicating that *P. dendritica* comb. nov. represents a species complex. We sampled 2 of Carr's clades (CMC01 and CMC02, corresponding to Bodega Bay and British Columbia clades) and additionally found two new clades (California and the Sea of Japan). We examined the holotype of *P. dendritica*, but unfortunately, we could not determine which of the molecular clades corresponded to the type species as all the examined clades had very similar morphology. We suggest performing archival DNA extraction techniques for sequencing the holotype. This will allow comparison of the four topotype clades with the holotype DNA sequences to assign the *P. dendritica* species name to one of the obtained molecular clades.

Protoaricia chilensis comb. nov. was described by Hartmann-Schröder, 1965 for Puerto Aguirre, Chile, and was reported from its type locality to Ecuador (Blake 2017). In our study, we sequenced the specimens of *P. chilensis* comb. nov. sampled from off Peru, close to the type locality of *P. brevicephala* comb. nov. *Protoaricia brevicephala* comb. nov. originally described from Payta, Peru, has been considered a species with an uncertain status (Blake 2017). Blake (2017) revised the holotype of *P. brevicephala* comb. nov. and found a syllid inside the vial instead of an orbiniid, considering it as *incertae sedis*. Recently, we examined the only paratype of *P. brevicephala* comb. nov., but it consisted of a few very damaged fragments making it impossible to distinguish any morphological character. Consequently, we cannot consider *P. brevicephala* as a valid species. On the other hand, we examined the holotype of *P. chilensis* comb. nov., and found slight morphological differences with our specimens,

which can be size dependent. Our specimens of *P. chilensis* are considerably smaller than the holotype of *P. chilensis* comb. nov., have fewer abdominal neuroacacula and thoracic chaetigers. Waiting for more conclusive results, including molecular data of topotype specimens of *P. chilensis* comb. nov., we will keep our Peruvian records as *P. chilensis* comb. nov.

Protoaricia aurantiaca comb. nov. was described by Müller (1858) for Santa Catarina Island (Brazil), but it was never recorded again in Brazil until recently (Álvarez *et al.* 2019). Álvarez *et al.* (2019) revised different records from South-Southwestern Brazil, designated a neotype and redescribed the species, expanding its distribution beyond Santa Catarina, to São Paulo and Rio de Janeiro. In this study, we revisited most of the places sampled by them, and included samples from the Paraná state. Our results confirm *P. aurantiaca* comb. nov. being a distinct species with molecular data, occurring in South and Southeastern Brazil from Santa Catarina to Rio de Janeiro. *Protoaricia aurantiaca* comb. nov. inhabit soft sediments retained between algal tufts and mussel banks from hard substrates, as rocky shores (Álvarez *et al.* 2019). As we did not sample hard substrates of Northeast of Brazil, we cannot disregard that the species occurs also in lower latitudes.

Protoaricia retusiceps comb. nov. was described from French Polynesia. After examining the holotype (USNM 19412), we observed the voucher specimens *Naineris* 002252BMOO 16164 correspond to *Protoaricia retusiceps*. The species is very similar to the rest of the species of the complex, however, the inferior row of huge uncini arranged in a bow in thoracic neuropodia is a unique character of the species.

“*Protoaricia setosa*” subclade

Probably one of the most interesting results of the present study is the detection of *Protoaricia setosa* comb. nov. as a species complex. *Protoaricia setosa* comb. nov. has been subjected to morphological revisions in the past (i. e. Solís-Weiss & Fauchald 1989, Blake 2011, Dean & Blake 2015, Blake 2017), and was assumed to be a cosmopolitan species. Here, we revealed cryptic diversity in the species complex with three different lineages, two from the Atlantic and one from the Pacific Ocean (see detailed results and discussion in the Chapter 2).

Blake (2000) found that specimens of *Naineris* sensu lato without uncini in thoracic neuropodia, namely *P. setosa*-like, was distantly related to *Protoaricia*. Our results, however, disagree with Blake's (2000) conclusions, *P. setosa* species complex clade is placed within the *Protoaricia* clade, sister to "*P. bicornis*" clade.

"*Protoaricia bicornis*" subclade

Although poorly sampled, this clade was highly supported. All species in this clade are characterized by a unique taxonomical character, namely the presence of multiple dorsal sensory organs per segment. These organs were described for the first time in *P. australis* comb. nov. (Zhadan et al. 2015). However, our examination of the type materials of *P. bicornis* comb. nov., *P. kalkudaensis* comb. nov. and *P. quadraticeps* comb. nov. revealed that this feature was presumably overlooked in many original descriptions and present in all the species of the "*P. bicornis*" clade.

Taxonomical characters in *Naineris* s. str. and *Protoaricia*

Concerning *Naineris* s. str. and *Protoaricia*, previous phylogenetic reconstructions of the family based on morphological characters (i.e., Blake 2000, Bleidorn et al. 2009), considered the prostomium shape, thoracic neuropodia with elevated ridge, presence, and size of thoracic neuropodial uncini, and presence of statocysts as characters with high taxonomic value. From Blake's analyses it is possible to infer the monophyly of *Naineris* s. str. by the presence of elevated ridge in thoracic neuropodia, rounded prostomium and large conspicuous uncini in thoracic neuropodia (Blake 2000), if we consider only the terminal *Naineris* (= *N. quadricuspida*, the type species).

In the case of *Protoaricia*, Blake's results are difficult to interpret, since he obtained *Protoaricia* paraphyletic, if we consider *Naineris* sp. 1 (= *P. cf. setosa* comb. nov.) as part of *Protoaricia*). On the other hand, if we assume that the terminal *N. quadricuspida* in Bleidorn et al. (2009) correspond to a species of the *P. dendritica* complex instead, the presence of statocysts can be considered a synapomorphic character of *Protoaricia*.

As a result of tracing morphological characters in our phylogenetic tree, we consider that the position of thoracic neuropodial papilla, presence of subuluncini, number of dorsal sensory organs and thoracic branchiae disposition being potential characters of high taxonomic and diagnostic value in orbiniids.

Despite *Protoaricia* shows a high degree of variation in morphological characters, and only a few of them are shared among all the members of this genus, thoracic neuropodial papilla in an upper position is present in all members of *Protoaricia*, at least during a stage of their life cycle. Juveniles of *P. dendritica* comb. nov. may have a small papilla in an upper position (personal observation), which is inconspicuous in adults. Similarly, we observed in the holotype of *P. chilensis* comb. nov. that this structure was present in some of the thoracic chaetigers only. Unfortunately, developmental studies of *Protoaricia* species are scarce and did not assess the developmental changes in this structure (i.e., Giangrande & Petraroli 1991). All species from the *Naineris* s. str. have thoracic neuropodial papilla in middle position.

Presence of subuluncini was traced as a synapomorphy in *Protoaricia*, with their possible secondary loss is a unique apomorphy for the “*P. setosa*” subclade. Whereas in *Naineris* s. str. subuluncini were absent. Multiple dorsal sensory organs per segment, can be considered a unique apomorphy for “*P. bicornis*” subclade. It is worth noting that dorsal sensory organs are rarely described, therefore they were never included in orbiniids phylogenies (i.e., Blake 2000, Bleidorn *et al.* 2009), and only recently received more attention, after the discovery of multiple dorsal organs in *P. australis* comb. nov. (Zhadan *et al.* 2015). We suggest that efforts must be taken to fully illustrate the shape and number of dorsal sensory organs per chaetiger in orbiniids descriptions to test their significance in future phylogenetic hypotheses of the family.

Branchiae position in thoracic segments was first codified in this study. We observed that branchiae laterally displaced is a synapomorphic character in *Protoaricia*. Whereas in *Naineris* s. str. branchiae are medially displaced. Opposite to Bleidorn *et al.* (2009), statocysts can be considered a synapomorphic character only of the “*P. laevigata*” subclade, since they were absent in the “*P. bicornis*” and “*P. setosa*” subclades, as well as in *Naineris* s. str. clade. As cited above, these structures present in *N. quadricuspida* (sic) were considered a synapomorphy of *Protoaricia* by Bleidorn *et al.* (2009). However, we did not

observe statocysts in the specimens collected from Greenland and the North Sea, which confirm that the specimens they studied belong to *P. dendritica* comb. nov. species complex instead.

3.5. CONCLUSION

Our results support the polyphyly of *Naineris* and suggest restricting *Naineris* to the species with thoracic neuropodial papilla in the middle of the lobe, multiple rows of uncini, uncini with a ribbed shaft and rounded tip with a terminal notch, dorsal sensory organs always paired, and moving the remaining *Naineris* species into *Protoaricia*. Additionally, *Naineris victoriae* is suggested to be moved into *Pettibonella* based on a combination of unique morphological characters among the *Naineris sensu lato* species that agree with *Pettibonella* instead. This study increases the number of valid species of *Protoaricia* and *Pettibonella* to 16 and 4 respectively, whereas *Naineris* s. str. was reduced to only 6 species. Characters traditionally used in species diagnoses in *Naineris sensu lato* such as the number of thoracic chaetigers, and the chaetiger where branchiae first appear are shown to have low taxonomic value. Fixed character, such as the number and shape of dorsal sensory organs, the position of thoracic neuropodial papilla and the medial or lateral displacement of branchiae contain systematic information and may be extrapolated to other genera. Several clades within the re-defined *Protoaricia* were studied in detail, some of which will be scrutinized in separate papers.

3.6. REFERENCES

- Álvarez, R.C. (2019) Descrição de uma nova espécie de *Naineris* (Polychaeta, Orbiniidae) com comentários sobre a distribuição do gênero na costa brasileira. Monografia. Universidade Federal Rural do Rio de Janeiro, 32 pp.
- Alvarez, R.C., Miranda, V.R. & Brasil A.C. (2019) Redescription of *Naineris aurantiaca* (Müller, 1858) and designation of a neotype from the Brazilian coast (Annelida: Orbiniidae). *Zootaxa*, 4571(1), 125–136. <http://dx.doi.org/10.11646/zootaxa.4571.1.8>

Amaral, A.C.Z., Nallin, S.A.H., Steiner, T.M., Forroni, T. de O. & Filho, D.G. (2006) Catálogo das espécies de Annelida Polychaeta do Brasil. pp. 1–20. Available at: http://www.ib.unicamp.br/museu_zoologia/files/lab_museu_zoologia/Catalogo_Polychaeta_Amaral_et_al_2012.pdf. Accessed 10 Sep 2020.

Amoureux, L. (1985) Annélides benthiques récoltées à l'entrée de la lagune de la Manche-à-Eau, Guadeloupe (Antilles). *Bulletin du Muséum National d'Histoire Naturelle, Paris, 4e série, section A*, 1, 93–107.

Annenkova, N. P. (1931) Die Polychaeten in der sammlungen der Jakutischen Expedition der Akademie der Wissenschaften der USSR. *Zoologischer Anzeiger*, 95: 203–205.

Annenkova, N. P. (1937) Polychaete fauna of the northern part of the Japan Sea. *Issledovaniya fauny morei, Zoologicheskii Institut Akademii Nauk USSR*, 23, 139–216.

Atzori, G., López, E., Addis, P., Sabatini, A. & Cabiddu, S. (2016) First record of the alien polychaete *Naineris setosa* (Scolecida; Orbiniidae) in Tyrrhenian Sea (Western Mediterranean). *Marine Biodiversity Records*, 9 (5) 1–6. <https://doi.org/10.1186/s41200-016-0017-6>

Audouin, J.V. & Milne-Edwards, H. (1833) Classification des Annélides et descriptions de celles qui habitent les côtes de la France. *Annales des Sciences Naturelles Paris*, series, 1, 29, 388–412.

Báez, D.P. & Ardila, N.E. (2003) Poliquetos (Annelida, Polychaeta) del Mar Caribe Colombiano. *Biota Colombiana*, 4, 89–109.

Bastida-Zavala, J. R. (1995) Poliquetos (Annelida: Polychaeta) del arrecife coralino de Cabo Pulmo-Los Frailes, B.C.S., México. *Revista de Zoología, Universidad Nacional Autónoma de México*, 6, 9–29.

Bellan, G. (2001) Polychaeta. In: Costello, M.J., Emblow, C. & White, R. (Eds.), European Register of Marine Species: a check-list of the marine species in Europe and a bibliography of guides to their identification. *Patrimoines naturels*, 50, 214–231.

Berkeley, E., & Berkeley, C. (1941) On a collection of Polychaeta from Southern California. *Bulletin of the Southern California Academy of Sciences*, 40, 16–60.

Berkeley, E., & Berkeley, C. (1942) North Pacific Polychaeta, chiefly from the west coast of Vancouver Island, Alaska, and Bering Sea. *Canadian Journal of Research*, 20, 183–208.

Birula A. (1897) Researches on biology and zoogeography, chiefly in Russian Seas, collected by Dr A.L. Botkine in 1895 in the Gulfs of Yenesei and

Obi. *Annuaire du Musée zoologique de l'Académie des sciences de St. Pétersbourg*, 2: 78–116.

Blainville, H. de (1828) *Dictionnaire des Sciences naturelles*, 57, 368–501.

Blake, J. A. (1991) Chapter 3. The polychaete fauna of the Galapagos Islands. In: James, M.J. (Ed.). *Galápagos Marine Invertebrates: Taxonomy, Biogeography, and Evolution in Darwin's Islands*. Topics in Geobiology. Plenum Press. New York, 75–96. https://doi.org/10.1007/978-1-4899-0646-5_4

Blake, J.A. (1996) Chapter 1. Family Orbiniidae Hartman, 1942. In: Blake, J.A. Hilbig, B. & Scott, P.H. (Eds.). *Taxonomic Atlas of the Santa Maria Basin and Western Santa Barbara Channel*. Vol. 6. Annelida Part 3. Polychaeta: Orbiniidae to Cossuridae. pp. 1–26. Santa Barbara Museum of Natural History, Santa Barbara, California.

Blake, J.A. (2000) A new genus and species of polychaete worm (Family Orbiniidae) from methane seeps in the Gulf of Mexico, with a review of the systematics and phylogenetic interrelationships of the genera of Orbiniidae. *Cahiers de Biologie Marine*, 41, 435–449. <https://doi.org/10.21411/CBM.A.84F1D61E>

Blake, J.A. (2009) Redescription of *Capitella capitata* (Fabricius) from West Greenland and designation of a neotype (Polychaeta, Capitellidae). *Zoosymposia*, 2, 55–58.

Blake, J. (2017) Polychaeta Orbiniidae from Antarctica, the Southern Ocean, the Abyssal Pacific Ocean, and off South America. *Zootaxa*, 4218(1), 1–145. <https://doi.org/10.11646/zootaxa.4218.1.1>

Blake, J.A. (2020) New species and records of deep-water Orbiniidae (Annelida, Polychaeta) from the Eastern Pacific continental slope, abyssal Pacific Ocean, and the South China Sea. *Zootaxa*, 4730(1), 1–61. <https://doi.org/10.11646/zootaxa.4730.1.1>

Blake, J. & Giangrande, A. (2011) *Naineris setosa* (Verrill) (Polychaeta, Orbiniidae), an American subtropical–tropical polychaete collected from an aquaculture facility in Brindisi (Adriatic Sea, Italy): A possible alien species. *Italian Journal of Zoology*, 78 (S1), 20–26. <http://dx.doi.org/10.1080/11250003.2011.577982>

Bleidorn, C. (2005) Phylogenetic relationships and evolution of Orbiniidae (Annelida, Polychaeta) based on molecular data. *Zoological Journal of the Linnean Society*, 144, 59–73. <https://doi.org/10.1111/j.1096-3642.2005.00160.x>.

Bleidorn, C. Hill, N., Erséus, C. & Tiedemann, R. (2009) On the role of character loss in orbiniid phylogeny (Annelida): Molecules vs. Morphology. *Molecular Phylogenetics and Evolution*, 52, 57–69. <https://doi.org/10.1016/j.ympev.2009.03.022>

Bleidorn, C. & Helm, C. (2019) 7.1.4 Orbiniidae Hartman, 1942. In: Purschke, G., Böggemann, M. & Westheide, W. (Eds.), *Handbook of Zoology. Annelida. Vol. 1. Annelida Basal groups and Pleistoannelida, Sedentaria I*. DeGruyter, Berlin, pp. 251–269.

Boore, J. L., & Brown, W. M. (2000) Mitochondrial genomes of *Galathealinum*, *Helobdella*, and *Platynereis*: sequence and gene arrangement comparisons indicate that Pogonophora is not a phylum and Annelida and Arthropoda are not sister taxa. *Molecular Biology and Evolution*, 17(1), 87–106. <https://doi.org/10.1093/oxfordjournals.molbev.a026241>

Brunel, P., Lamarche, G., & Bossé, L. (1998) Catalogue of the marine invertebrates of the estuary and Gulf of St. Lawrence. *Canadian Special Publication of Fisheries and Aquatic Sciences*, 126, 1–405.

Cadien, D.B. & Lovell, L.L. (Eds.) (2018) *A Taxonomic Listing of Benthic Macro and Megainvertebrates from Infaunal and Epifaunal Monitoring and Research Programs in the Southern California Bight. 12th Edition*. Prepared by The Southern California Association of Marine Invertebrate Taxonomists, Los Angeles, California, 167 pp.

Camacho C., Coulouris G., Avagyan V., Ma N., Papadopoulos J., Bealer K., Madden T.L. (2009) “BLAST+: architecture and applications.” *BMC Bioinformatics*, 10, 1–9. <https://doi.org/10.1186/1471-2105-10-421>

Carr, C. M., Hardy, S. M., Brown, T. M., Macdonald, T. A., & Hebert, P. D. (2011). A tri-oceanic perspective: DNA barcoding reveals geographic structure and cryptic diversity in Canadian polychaetes. *PLoS one*, 6(7), e22232. <https://doi.org/10.1371/journal.pone.0022232>

Carrasco, F.D. (1977) Polychaeta (Annelida) de Bahía de Concepción, Chile. Familias. Orbiniidae, Cirratulidae, Cossuridae, Capitellidae y Ampharetidae, con la descripción de tres especies y una subespecie nuevas. *Boletín de la Sociedad de Biología de Concepción*, 51, 67–92.

Carus, J.V. (1863) Vermes. *Handbuch der Zoologie*, 2, 422–484.

Chamberlin, R.V. (1919) The Annelida Polychaeta (Albatross Expeditions). *Memoirs of the Museum of Comparative Zoology at Harvard College*, 48, 1–514. [<http://www.biodiversitylibrary.org/ia/memoirsofmuseumo4801harv>]

Choi, H. K., Kim, J. G., & Yoon, S. M. (2016) Two New Records of Scolecoid Polychaetes (Annelida: Polychaeta: Scolecida) in Korean Fauna. *Animal Systematics, Evolution and Diversity*, 32, 266–271. <http://doi.org/10.5635/ased.2016.32.4.039>

Çinar, M. E. (2005) Polychaetes from the coast of northern Cyprus (eastern Mediterranean Sea), with two new records for the Mediterranean Sea. *Cahiers de Biologie Marine.*, 46, 143–159.

Claparède, E. (1864) Glanures zootomiques parmi les annélides de Port-Vendres (Pyrénées Orientales). *Mémoires de la Société de physique d'histoire naturelle de Genève* 17, 463–600.

Claparède, É. (1869) Les Annélides Chétopodes du Golfe de Naples. Seconde partie. Ordre Ilme. Annélides Sédentaires (Aud. et Edw.). *Mémoires de la Société de Physique et d'Histoire Naturelle de Genève*. 20: 1–225.

Cubit, J. & Williams, S. (1983) The invertebrates of Galeta Reef (Caribbean Panama): a species list and bibliography. *Atoll Research Bulletin*, 269, 1–45.

Cunningham, Joseph Thomas; Ramage, G.A. (1888). The Polychaeta Sedentaria of the Firth of Forth. *Transactions of the Royal Society of Edinburgh*. 33, 635–684.

Darling, J. A. & Carlton, J. T. (2018) A framework for understanding marine cosmopolitanism in the Anthropocene. *Frontiers in Marine Science*, 5, 293.

Day, J. H. (1965) Israel South Red Sea Expedition, 1962, Reports no. 7. Some Polychaeta from the Israel South Red sea expedition, 1962. *Bulletin of the Sea Fisheries Research Station Haifa*, 38: 15–27.

Day, J.H. (1977) A review of the Australian and New Zealand Orbiniidae (Annelida: Polychaeta). In: Reish, D.J. & Fauchald, K. (Eds.), *Essays on Polychaetous Annelids in Memory of Dr. Olga Hartman*, pp. 217–246. [Allan Hancock Foundation, University of Southern California, Los Angeles.]

De Silva, P. H. D. H. (1965) New species and records of Polychaeta from Ceylon. *Proceedings of the Zoological Society of London*. 144: 537–563. <http://dx.doi.org/10.1111/j.1469-7998.1965.tb05196.x>

Dean, H. & Blake, J. (2015) The Orbiniidae (Annelida: Polychaeta) of Pacific Costa Rica. *Zootaxa*, 3956, 183–198. <http://dx.doi.org/10.11646/zootaxa.3956.2.2>

Díaz-Díaz, O., Vanegas-Espinosa, V., Cárdenas-Oliva, A., & Liñero-Arana, I. (2012) Orbiniidae Hartman, 1942 (Annelida: Polychaeta) de las costas de Venezuela. *Biota Colombiana*, 13 :3–20.

Díaz-Díaz, O. F., Bone, D., & López-Ordaz, A. (2014) Polychaetes associated to calcareous sediments Venezuela: Scolecida. *Pan-American Journal of Aquatic Sciences*, 9, 301–311.

Díaz, O. F. D., Bone, D., & López-Ordaz, A. (2016) Poliquetos de fondos blandos del Golfo de Venezuela: Clado Scolecida. *Acta Biológica*, 36, 119.

Donath, A., Jühling, F., Al-Arab, M., Bernhart, S. H., Reinhardt, F., Stadler, P. F., Middendorf, M. & Bernt, M. (2019) Improved annotation of protein-coding genes

boundaries in metazoan mitochondrial genomes. *Nucleic acids research*, 47(20), 10543-10552. <https://doi.org/10.1093/nar/gkz833>

Dueñas P. R. (1999) Listado de poliquetos colectados durante los años 1979 - 1999 en la bahía de Cartagena y Golfo de Morrosquillo. *Revista Milenio Facultad de Ciencias Básicas de la Universidad de Córdoba* 1(2): 9–18.

Eisig, H. (1914) Zur Systematik, Anatomie und Morphologie der Ariciiden nebst Beiträgen zur generellen Systematik. *Mittheilungen aus der Zoologischen Station zu Neapel*, 21, 154–600. [plates 10–27, 23 text figures.]

Fabricius, O. (1780) *Fauna Groenlandica*. Hafniae et Lipsiae. xvi, 452 pp., 12 figures.

Fauvel, P. (1927) Polychètes sédentaires. Addenda aux errantes, archiannélides, myzostomaires. *Faune de France* 16, 1–494.

Federhen, S. (2012) The NCBI Taxonomy Database. *Nucleic Acids Res.*, 40, D13–D25 (accessed 10 Sep 2020).

Felder DL, Camp DK. (2009) Gulf of Mexico Origins, Waters, and Biota: Biodiversity. Texas A & M Press, College Station, Texas, 1312 pp. <http://gulfbase.org/biogomx>

Giangrande, A., & Fraschetti, S. (1995) A population study of *Naineris laevigata* (Polychaeta, Orbiniidae) in a fluctuating environment (Mediterranean Sea). *Scientia Marina*, 59, 39–48.

Gillet, P. (1986) Contribution à l'étude des Annélides Polychètes des lagunes de la Manche-à-Eau et de Belle-Plaine (Guadeloupe). Description d'un nouveau Capitellidae: *Scyphoproctus guadalupensis* n. sp. *Bulletin du Muséum national d'Histoire naturelle, Paris*, 8, 803–817.

Google Inc. (2011) Software Google Earth. Available from: <http://www.google.com/earth/index.html> (accessed 1 May 2020)

Gopal, A., Koovapurath Useph, A. J., Aikkarakunnath Varghese, S., & Veloorkirakathil Narayana, S. (2014) A new species of polychaete, *Pettibonella shompens* sp. nov. (Orbiniidae), from the Nicobar Islands, North Indian Ocean. *Marine Biology Research*, 10(10), 1033-1037. <https://doi.org/10.1080/17451000.2013.879988>

Gravier, C. (1908) Sur les annélides polychètes rapportés par M. Le Dr Rivet, de Payta (Pérou). *Bulletin du Muséum d'Histoire Naturelle, Paris*, 14, 40–44.

Gravier, C. (1909) Annélides polychètes recueillis a Payta (Pérou) par M. le Dr. Rivet. *Archives de Zoologie Expérimentale et Générale*, séries 4, 10, 617–659, plates 16–17.

Grube, A. E. (1840) *Actinien, Echinodermen und Würmer des Adraitischen-und Mittelmeers nach eigenen Sammlungen beschrieben*, , Königsberg: J.H. Bon. 92 pp.

Grube, A.E. (1855) Beschreibungen neuer oder wenig bekannter Anneliden. Archiv für Naturgeschichte, Berlin, 21, 81–128. <https://doi.org/10.5962/bhl.part.13989>

Grube, A.E. (1878) Annulata Semperiana. Beiträge zur Kenntniss der Annelidenfauna der Philippinen, nach den von Herrn Prof. Semper mitgebrachten Sammlungen. *Mémoires de l'Académie Impériale des Sciences de St. Petersbourg*, (7) 25, 8, ix + 300 pp.

Jördens, J., Struck, T., & Purschke, G. (2004) Phylogenetic inference regarding Parergodrilidae and *Hrabeiella periglandulata* ('Polychaeta', Annelida) based on 18S rDNA, 28S rDNA and COI sequences. *Journal of Zoological Systematics and Evolutionary Research*, 42(4), 270-280. <https://doi.org/10.1111/j.1439-0469.2004.00265.x>

Hansen, G.A. (1882) Recherches sur les annélides receueillies par M. le professeur Édouard van Benedon pendant son voyage au Brésil et à la Plata. *Memoires Couronnes et Memoires des Savants Etrangers publies par L'Academie Royale des Sciences, des Lettres et des Beaux-Arts de Belgique*, 44(3), 1–29.

Hartman, O. (1942) A review of the types of polychaetous annelids at the Peabody Museum of Natural History, Yale University. *Bulletin of the Bingham Oceanographic Collections*, 8, 1–98.

Hartman, O. (1944) Polychaetous annelids from California, including the descriptions of two new genera and nine new species. *Allan Hancock Pacific Expeditions*, 10, 239–307.

Hartman, O. (1948) The marine annelids erected by Kinberg. With some notes on some other types in the Swedish State Museum. *Arkiv för Zoologi*. 42A (1), 1–137, 18 plates.

Hartman, O. (1951) The littoral marine annelids of the Gulf of Mexico. *Publications of the Institute of Marine Science, University of Texas*, 2, 7–124, 27 plates.

Hartman, O. (1956) Polychaetous annelids erected by Treadwell, 1891 to 1948, together with a brief chronology. *Bulletin of the American Museum of Natural History*, 109, 239–310.

Hartman, O. (1957) Orbiniidae, Apistobranchidae, Paraonidae, and Longosomidae. *Allan Hancock Pacific Expeditions*, 15 (3), 211–393, pls. 20–44, 1 chart.

Hartman, O. (1959) Catalogue of the polychaetous annelids of the world. Parts 1 and 2. *Occasional Papers of the Allan Hancock Foundation*, 23, 1–628.

Hartman, O. (1969) Atlas of the sedentariate polychaetous annelids from California. 1-812. *Allan Hancock Foundation, University of Southern California. Los Angeles*.

Hartman, O. (1978) Polychaeta from the Weddell Sea Quadrant, Antarctica. *Antarctic Research Series*, 26 (4), 125–223.

Hartmann-Schröder, G. (1960) Zur Polychaetenfauna von Peru. Teil I. *Beiträge Zur Neotropischen Fauna*, 2 (1), 1–44, 92 figures.

Hartmann-Schröder, G. (1965) Die Polychaeten des Sublitorals. In: Hartmann-Schröder, G. und Hartmann, G., (Eds), Zur Kenntnis des Sublitorals der chilenischen Küste unter besonderer Berücksichtigung der Polychaeten und Ostracoden. *Mitteilungen des Hamburgischen Zoologischen Museums und Instituts, Supplement*, 62, 59–305.

Hartmann-Schröder, G. (1971) Annelida, Borstenwürmer, Polychaeta. *Tierwelt Deutschlands*, 58, 1–594.

Hartmann-Schröder, G. (1980) Teil 4. Die Polychaeten der tropischen Nordwestküste Australiens (zwischen Port Samson im Norden und Exmouth im Süden). In: Hartmann-Schröder, G. & Hartmann, G. (Eds.), *Zur Kenntnis des Eulittorals der australischen Küsten unter besonderer Berücksichtigung der Polychaeten und Ostracoden. Mitteilungen aus dem Hamburgischen Zoologischen Museum und Institut*, 77, 41–110.

Hasegawa, N., Hori, M., & Mukai, H. (2008) Seasonal changes in eelgrass functions: current velocity reduction, prevention of sediment resuspension, and control of sediment–water column nutrient flux in relation to eelgrass dynamics. *Hydrobiologia*, 596 (1), 387–399.

Hobson, K.D. & Banse, K. (1981) Sedentariate and archiannelid polychaetes of British Columbia and Washington. *Canadian Journal of Fisheries and Aquatic Science*, 209, 1–144.

Hoffman, S. & Hausen, H. (2007) Chaetal arrangement in Orbiniidae (Annelida, Polychaeta) and its significance for systematics. *Zoomorphology*, 126, 215–227. <https://doi.org/10.1007/s00435-007-0042-4>

Howson, C. M., & Picton, B. E. (Eds.) (1997) *The species directory of the marine fauna and flora of the British Isles and surrounding seas* (No. 276). Ulster Museum.

Hutchings, P., & Rainer, S. (1979) The polychaete fauna of Careel Bay, Pittwater, New South Wales, Australia. *Journal of Natural History*, 13, 745–796.

Hutchings, P.A. & Murray, A. (1984) Taxonomy of polychaetes from the Hawkesbury River and the Southern Estuaries of New South Wales, Australia. *Records of the Australian Museum*, 36 (suppl. 3), 1–119.

Hutchings, P.A. & Kupriyanova, E. (2018) Cosmopolitan polychaetes—fact or fiction? Personal and historical perspectives. *Invertebrate Systematics*, 32, 1–9. <http://dx.doi.org/10.1071/IS17035>

Idris, I. & Arshad, A. (2013) Checklist of polychaetous annelids in Malaysia with redescription of two commercially exploited species. *Asian Journal of Animal and Veterinary Advances*, 8, 409–436. <http://dx.doi.org/10.3923/ajava.2013.409.436>

Imajima, M. (2009) Deep-sea benthic polychaetes off Pacific coast of the northern Honshu, Japan. *National Museum of Nature and Science Monographs* 39: 39–192.

Jumars, P.A., Dorgan, K.M. & Lindsay, A.M. (2015) Diet of worms emended: An update of polychaete feeding guilds. *Annual Reviews, Marine Science*, 7, 497–520. [Supplemental material] <https://doi.org/10.1146/annurev-marine-010814-020007>

Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K., Von Haeseler, A., & Jermini, L. S. (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature methods*, 14(6), 587–589. <https://doi.org/10.1038/nmeth.4285>

Khedhri, I., Lavesque, N., Bonifácio, P., Djabou, H. & Afli, A. (2014) First record of *Naineris setosa* (Verrill, 1900) (Annelida: Polychaeta: Orbiniidae) in the Western Mediterranean Sea. *BioInvasions Records*, 3 (2), 83–88. <http://dx.doi.org/10.3391/bir.2014.3.2.05>

Kinberg, J. (1867) *Annulata nova. Öfversigt af Kungliga Vetenskaps-Akademiens Förhandlingar, Stockholm*, 22, 239–258.

Kirkegaard, J. B. (1996) Bathyal and Abyssal Polychaetes (sedentary species I). *Galathea Report*. 17: 57–77.

Kurt Şahin, G. & Çinar, M.E. (2012) A check-list of polychaete species (Annelida: Polychaeta) from the Black Sea. *Journal of the Black Sea/Mediterranean Environment*, 18, 10–48. Available from: <http://dergipark.ulakbim.gov.tr/jbme/article/view/5000144348> (Accessed 2 Sept. 2016)

Leuckart, R. (1849) Zur Kenntnis der Fauna von Island. Erster Beitrag (Würmer). *Archiv für Naturgeschichte, Berlin*. 15(1), 149–208, plate 3.

Linnaeus, C. (1758) *Systema Naturae*, Ed. X. (*Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus*,

differentiis, synonymis, locis. Tomus I. Editio decima, reformata.) Holmiae. vol. 1, i–ii + 1–824.

W.P. Maddison & D.R. Maddison. (2007) Mesquite: a modular system for evolutionary analysis. Version 2.0. <http://mesquiteproject.org>.

Malmgren, A.J. (1867) Annulata Polychaeta Spetsbergiae, Groenlandiae, Islandiae et Scandinaviae hactenus cognita. *Öfversigt af Kungliga Vetenskaps—Adakemiens Förhandlingar, Stockholm*, 24, 127–235, pls. 2–15. <https://doi.org/10.5962/bhl.title.13358>

McIntosh, W. C. (1885) Report on the Annelida Polychaeta collected by. HMS Challenger during the years 1873–76. *Report of the Scientific Results of the Voyage of HMS Challenger 1873–76*, 12, 1–554.

McIntosh, W.C. (1878) On the annelida obtained during the cruise of H. M. S. "Valorous" to Davis Strait in 1875. *Transactions of the Linnean Society of London, Series 2, Zoology*, 1, 499–511. 10.1111/j.1096-3642.1878.tb00663b.x

McIntosh, W.C. (1910) *A monograph of the British Annelids. Polychaeta. Syllidae to Ariciidae*. Ray Society, London, 233–524.

Mesnil, F. & Caullery, M. (1898) Étude de morphologie externe chez les Annélides. La famille nouvelle des Levinseniens. Revision des Ariciens—affinités des deux familles. Les Aspistobranchiens. *Bulletin Scientifique de la France et de la Belgique*, 31, 126–150, pl. 6. [<https://biodiversitylibrary.org/page/10708498>]

Moore, J.P. (1909) Polychaetous annelids from Monterey Bay and San Diego, California. *Proceedings of the Academy of Natural Sciences, Philadelphia*, 61, 235–295, plates 7–9.

Müller, O.F. (1774) Vermium terrestrium et fluviatilium, seu animalium infusorium, helminthicorum et testaceorum, non marinorum, succincta historia, 1, 2, Havniae et Lipsiae, 72 pp.

Müller, F. (1858) Einiges über die Anneliden-Fauna der Insel Santa Catharina an der brasilianischen Küste. *Archiv für Naturgeschichte*, 24, 211–220.

Muller, Y. (2004) *Faune et flore du littoral du Nord, du Pas-de-Calais et de la Belgique: inventaire. [Coastal fauna and flora of the Nord, Pas-de-Calais and Belgium: inventory]*. France, Pas-de-Calais : Commission Régionale de Biologie Région Nord.

Nonato, E.F. (1981) Contribuição ao conhecimento dos anelídeos poliquetas bentônicos da Plataforma Continental Brasileira, entre Cabo Frio e o Arroio Tese de Livre Docência. Instituto Oceanográfico, Universidade de São Paulo, 248 pp.

Ong, B. (1995) Polychaetes of Telok Aling, Penang, Malaysia. *Raffles Bulletin of Zoology* 43: 257–283.

Örsted, A.S. (1842) Udtog af en Beskrivelse af Grönlands Annulata dorsibranchiata. *Naturhistorisk Tidsskrift, Köbenhavn*. 4, 109–127.

Örsted, A. S. (1843) Grönlands Annulata dorsibranchiata. *Det Kongelige Danske videnskabernes selskabs. Naturvidenskabelige og matematiske afhandlinger*. 10: 153–216.

Padua, A., Lanna, E. & Klautau, M. (2013) Macrofauna inhabiting the sponge *Paraleucilla magna* (Porifera: Calcarea) in Rio de Janeiro, Brazil. *Journal of the Marine Biological Association of the United Kingdom* 93, 889–898. <https://doi.org/10.1017/S0025315412001804>

Pagliosa, P. R., Doria, J. G., Alves, G. F., Almeida, T. D., Lorenzi, L., Netto, S. A., & Lana, P. D. C. (2012) Polychaetes from Santa Catarina State (southern Brazil): checklist and remarks on species distribution. *Zootaxa* 3486: 1–49.

Perkins, T.H. & Savage, T. (1975) A bibliography and checklist of polychaetous annelids of Florida, the Gulf of Mexico, and the Caribbean region. *Florida Marine Research Publications*, 14, 1–62.

Perkins, T.H. (1998) Checklist of shallow–water marine polychaetous Annelida of Florida. pp.79–122. In: D.K. Camp, D.K., Lyons, W.G. & Perkins, T.H. (1998) Checklists of selected shallow–water marine invertebrates of Florida. Florida Marine Research Institute, Technical Report TR–3, 238 pp.

Pettibone, M.H. (1957) North American genera of the family Orbiniidae. *Journal of the Washington Academy of Science*, 47, 159–167, 4 figs. [<https://biodiversitylibrary.org/page/39753528>]

Pettibone, M.H. (1963) Marine polychaete worms of the New England region. *Bulletin of the United States National Museum*, 227, 1–346. <https://doi.org/10.5479/si.03629236.227.1>

Prijbelski, A., Antipov, D., Meleshko, D., Lapidus, A., & Korobeynikov, A. (2020) Using SPAdes de novo assembler. *Current protocols in bioinformatics*, 70(1), e102. <https://doi.org/10.1002/cpbi.102>

QGIS Development Team (2016) QGIS Geographic Information System. Open Source Geospatial Foundation. Available from: <http://qgis.osgeo.org> (accessed 23 May 2020)

Quatrefages, A. De. (1866) Histoire naturelle des Annélides marines et d'eau douce. Annélides et Gephyriens. *Librairie Encyclopédique de Rôret*, Paris, 2, 1–336.

Ratnasingham, S. & Hebert, P. D. N. (2007) BOLD: The Barcode of Life Data System (www.barcodinglife.org). *Molecular Ecology Notes* 7, 355–364. DOI: [10.1111/j.1471-8286.2006.01678.x](https://doi.org/10.1111/j.1471-8286.2006.01678.x)

Read, G. & Fauchald, K. (2023) World Polychaeta database. *Naineris* Blainville, 1828. Accessed through: World Register of Marine Species. Available from: <http://www.marinespecies.org/aphia.php?p=taxdetails&id=129419> (accessed 16 May 2023).

Reish, D.J. (1965) Benthic polychaetous annelids from Bering, Chukchi, and Beaufort Seas. *Proceedings of the United States National Museum*, 117: 131–157.

Rioja, E. (1960) Estudios anelidológicos. XXIV. Adiciones a la fauna de Anelidos Poliquetos de las costas orientales de México. *Anales del Instituto de Biología, México*, 31, 289–316.

Rouse, G. (2001) 9 Orbiniidae Hartman, 1942. In: Rouse, G.W., & Pleijel, F. (Eds) *Polychaetes*. Oxford University Press, pp. 57–60.

Rouse, G. & Fauchald, K. (1997) Cladistics and polychaetes. *Zoologica Scripta*. 26, 139–204.

Rozbaczylo, N. (1985) Los Anélidos Poliquetos de Chile. Índice Sinonímico y distribución geográfica de especies. *Monografías Biológicas Facultad de Ciencias Biológicas Pontificia Universidad Católica de Chile, Santiago, Chile*, 3: 1–284.

Salazar-Vallejo, S. I. (1991) Poliquetos (Annelida: Polychaeta) de fondos blandos de Isla Rasa, Golfo de California. *Ciencias Marinas*, 16, 75–95.

Salazar-Vallejo, S. I. (1996) Lista de especies y bibliografía de poliquetos (Polychaeta) del Gran Caribe. *Anales del Instituto de Biología, Universidad Nacional Autónoma de México, Serie Zoología*, 67, 11–50.

Salazar-Vallejo, S.I. & Londoño-Mesa, M.H. (2004) Lista de especies y bibliografía de poliquetos (Polychaeta) del Pacífico oriental tropical. *Anales del Instituto de Biología, Universidad Nacional Autónoma de México, Serie Zoología*, 75, 9–97.

Sarda, R. (1991) Polychaete communities related to plant covering in the mediolittoral and infralittoral zones of the Balearic Islands (Western Mediterranean). *Marine Ecology*, 12, 341–360.

Savigny, J.C. (1820) Système des Annélides, principalement de celles des côtes de l'Égypte et de la Syrie, offrant les caractères tant distinctifs que naturels des

orders, families et genres, avec la description de espèces. Description de l'Égypte. *Historie naturelle*, Paris, 1 (3), 1–128.

Schmarda, L.K. (1861) *Neue wirbellose Thiere beobachtet und gesammelt auf einer Reise um die Erde 1853 bis 1857*. Vol. 1. Turbellarien, Rotatorien und Anneliden. Pt. 2, 1–164, 22 plates. Leipzig.

Solís-Weiss, V. & Fauchald, K. (1989) Orbiniidae (Annelida: Polychaeta) from mangrove root-mats in Belize, with a revision of the protoariciin genera. *Proceedings of the Biological Society of Washington*, 102 (3), 772–792.

Solís-Weiss, V., Barbosa-López, A., Hernández-Alcántara, P. & Hermoso-Salazar, M. (2021) 33. Orbiniidae Hartman, 1942. In: de León-González JA, Bastida-Zavala JR, Carrera-Parra LF, García-Garza ME, Salazar-Vallejo SI, Solís-Weiss V y Tovar-Hernández MA (Eds.). *Anélidos Marinos de México y América Tropical*. Universidad Autónoma de Nuevo León, Monterrey, México, 1054 pp.

Spalding, M.D., Fox, H.E., Allen, G.R., Davidson, N., Ferdaña, Z.A., Finlayson, N., Halpern, B.S., Jorge, M.A., Lombana, A., Lourie, S.A., Martin, K.D., McManus, E., Molnar, J., Recchia, C.A. & Robertson, J. (2007) Marine ecoregions of the world: a bioregionalization of coastal and shelf areas. *BioScience*, 57, 573–583. <https://doi.org/10.1641/B570707>

Struck, T., Hessling, R., & Purschke, G. (2002) The phylogenetic position of the Aeolosomatidae and Parergodrilidae, two enigmatic oligochaete-like taxa of the 'Polychaeta', based on molecular data from 18S rDNA sequences. *Journal of Zoological Systematics and Evolutionary Research*, 40(3), 155-163. <https://doi.org/10.1046/j.1439-0469.2002.00200.x>

Struck, T. H., Nesnidal, M. P., Purschke, G., & Halanych, K. M. (2008) Detecting possibly saturated positions in 18S and 28S sequences and their influence on phylogenetic reconstruction of Annelida (Lophotrochozoa). *Molecular phylogenetics and evolution*, 48(2), 628-645. <https://doi.org/10.1016/j.ympev.2008.05.015>

Struck, T. H., Golombek, A., Weigert, A., Franke, F. A., Westheide, W., Purschke, G., Bleidorn, C & Halanych, K. M. (2015). The evolution of annelids reveals two adaptive routes to the interstitial realm. *Current Biology*, 25(15), 1993-1999. <https://doi.org/10.1016/j.cub.2015.06.007>

Tarazona, J. (1974) Lista de poliquetos sedentarios hallados en el Perú. *Revista Peruana de Biología*, 1: 164–167.

Tasso, V., El Haddad, M., Assadi, C., Canales, R., Aguirre, L., & Vélez-Zuazo, X. (2018) Macrobenthic fauna from an upwelling coastal area of Peru (Warm Temperate South-eastern Pacific province-Humboldtian ecoregion). *Biodiversity data journal*, 6: 1–44. <https://doi.org/10.3897/BDJ.6.e28937>

Taylor, J.L. (1984) Chapter1 Orbiniidae. *In*: Uebelacker, J.M. & Johnson, P.G. (Eds.), Taxonomic guide to the polychaete of the Northern Gulf of Mexico. Vol. I. 1-1 to 1-38. [Barry A. Vittor & Associates.]

Treadwell, A.L. (1902) The polychaetous annelids of Porto Rico. *Bulletin of the United States Fisheries Commission, Washington*, 20, 181–210.

Treadwell, A.L. (1931) New species of polychaetous annelids from California, Mexico, Porto Rico, and Jamaica. *American Museum Novitates*, 482: 1–7.

Treadwell, A.L. (1936) Polychaetous annelids from the vicinity of Nonsuch Island, Bermuda. *Zoologica*, 21, 49–68, plates I–III.

Treadwell, A.L. (1939) Polychaetous annelids from Porto Rico and vicinity. *Scientific Survey of Porto Rico and the Virgin Islands* (New York Academy of Sciences), 16, 151–319.

Trott, T.J. (2004) Cobscook Bay inventory: a historical checklist of marine invertebrates spanning 162 years. *Northeastern Naturalist*, (Special Issue 2), 261–324.

Vásquez-Montoya, R., & Thomassin, B. (1983) Contribución al conocimiento de los anélidos poliquetos de las praderas de *Thalassia testudinum* y *Halodule* sp. del sector de Punta Galeta (Panamá, Provincia de Colón). *Anales del Instituto de Ciencias del Mar y Limnología UNAM*, 10: 1–10.

Verrill, A.E. (1900) Additions to the Turbellaria, Nemertina and Annelida of the Bermudas, with revisions of some New England genera and species. *Transactions of the Connecticut Academy of Arts & Sciences*, 10, 595–671, 1 plate.

Viéitez, J.M., Alós, C., Parapar, J., Besteiro, C., Moreira, J., Núñez, J., Laborda, A.J., San Martín, G. (2004) Annelida Polychaeta I. *In*: Ramos, M.A. *et al.* (Eds). Fauna Ibérica, Vol. 25. Museo Nacional de Ciencias Naturales, CSIC, Madrid, 530 pp.

Villalobos-Guerrero, T.F. & Molina-Acevedo, I.C. (2014) Lista de especies y estado de conocimiento de los poliquetos (Annelida: Polychaeta) de Sinaloa, golfo de California. *Boletín del Instituto Oceanográfico de Venezuela*, 53 (1), 79–109.

Webster, H.E. & Benedict, J.E. (1887) The Annelida Chaetopoda from Eastport, Maine. *The Annual Report of the Commissioner of Fish and Fisheries*, 1885, 707–755.

Wesenberg-Lund, Elise. (1950) Polychaeta. *Danish Ingolf-Expedition*. 4(14): 1–92, plates I–X.

Wesenberg-Lund, Elise. (1951) Polychaeta, in The zoology of Iceland, vol. 2, pt. 19, pp. 1–182, 12 figs., 62 charts.

Whiteaves, J.F. (1901) Catalogue of the marine Invertebrata of eastern Canada. Geological Survey of Canada Separate Report, 722, 1–271. <https://doi.org/10.4095/216086>

Wiktor, J. (1980) Type-specimens of Annelida Polychaeta in the Museum of Natural History of the Wroclaw University. *Annales Zoologici*, 35, 267–283.

Zaâbi, S., Gillet, P., Chambers, S., Afli, A., & Boumaiza, M. (2012) Inventory and new records of polychaete species from the Cap Bon Peninsula, north-east coast of Tunisia, Western Mediterranean Sea. *Mediterranean Marine Science*, 13, 36–48. <https://doi.org/10.12681/mms.22>

Zhadan, A., Stupnikova, A. & Neretina, T. (2015) Orbiniidae (Annelida: Errantia) from Lizard Island, Great Barrier Reef, Australia with notes on orbiniid phylogeny. *Zootaxa*, 4019(1), 773–801. <http://dx.doi.org/10.11646/zootaxa.4019.1.27>

Zhadan, A. (2020) Review of Orbiniidae (Annelida, Sedentaria) from Australia. *Zootaxa* 4860, 451–502. <https://doi.org/10.11646/zootaxa.4860.4.1>

3.7. SUPPLEMENTARY MATERIALS

Table S1. Specimens sequenced in the study, with museum collection registration numbers, localities, GenBank accession numbers for COI, 16S and 28S, Bold Sample and Process IDs.

Species	Voucher	Locality	Coordinates	Collector	COI	16SrRNA	28SrRNA	ID BOLD	Process BOLD ID
<i>N. setosa</i> s. str.	MNRJP 007629	Brazil, Santa Catarina	27.695°S; 48.565°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	Alvarez_NS01	NAI001-23
<i>N. setosa</i> s. str.	MNRJP 007630	Brazil, Santa Catarina	27.695°S; 48.565°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	Alvarez_NS02	NAI002-23
<i>N. setosa</i> s. str.	MNRJP 007631	Brazil, Santa Catarina	27.695°S; 48.565°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	Alvarez_NS03	NAI003-23
<i>N. setosa</i> s. str.	MNRJP 007632	Brazil, Santa Catarina	27.695°S; 48.565°W	R. Álvarez	NNNNNNNN	NNNNNNNN	–	Alvarez_NS04	NAI004-23
<i>N. setosa</i> s. str.	MNRJP 007633	Brazil, Santa Catarina	27.695°S; 48.565°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	Alvarez_NS05	NAI005-23
<i>N. setosa</i> s. str.	MNRJP 007639	Brazil, Rio de Janeiro	22.882°S; 42.002°W	R. Álvarez	NNNNNNNN	–	NNNNNNNN	Alvarez_NS11	NAI006-23
<i>N. setosa</i> s. str.	MNRJP 007640	Brazil, Rio de Janeiro	22.882°S; 42.002°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	Alvarez_NS12	NAI007-23
<i>N. setosa</i> s. str.	MNRJP 007641	Brazil, Rio de Janeiro	22.882°S; 42.002°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	Alvarez_NS13	NAI008-23
<i>N. setosa</i> s. str.	MNRJP 007642	Brazil, Rio de Janeiro	22.882°S; 42.002°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	Alvarez_NS14	NAI009-23
<i>N. setosa</i> s. str.	MNRJP 007643	Brazil, Rio de Janeiro	22.882°S; 42.002°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	Alvarez_NS15	NAI010-23
<i>N. setosa</i> s. str.	MNRJP 007644	Brazil, Bahia	12.910°S; 38.497°W	R. Álvarez	NNNNNNNN	–	NNNNNNNN	Alvarez_NS16	NAI011-23
<i>N. setosa</i> s. str.	MNRJP 007645	Brazil, Bahia	12.910°S; 38.497°W	R. Álvarez	NNNNNNNN	NNNNNNNN	–	Alvarez_NS17	NAI012-23
<i>N. setosa</i> s. str.	MNRJP 007646	Brazil, Bahia	12.910°S; 38.497°W	R. Álvarez	NNNNNNNN	–	NNNNNNNN	Alvarez_NS18	NAI013-23
<i>N. setosa</i> s. str.	MNRJP 007647	Brazil, Bahia	12.910°S; 38.497°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	Alvarez_NS19	NAI014-23

Species	Voucher	Locality	Coordinates	Collector	COI	16SrRNA	28SrRNA	ID BOLD	Process BOLD ID
<i>N. setosa</i> s. str.	MNRJP 007648	Brazil, Bahia	12.910°S; 38.497°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	Alvarez_NS20	NAI015-23
<i>N. setosa</i> s. str.	MNRJP 007649	Brazil, Pernambuco	8.355°S; 34.953°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	Alvarez_NS21	NAI016-23
<i>N. setosa</i> s. str.	MNRJP 007650	Brazil, Pernambuco	8.355°S; 34.953°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	Alvarez_NS22	NAI017-23
<i>N. setosa</i> s. str.	MNRJP 007651	Brazil, Pernambuco	8.355°S; 34.953°W	R. Álvarez	–	NNNNNNNN	NNNNNNNN	Alvarez_NS23	NAI018-23
<i>N. setosa</i> s. str.	MNRJP 007652	Brazil, Pernambuco	8.355°S; 34.953°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	Alvarez_NS24	NAI019-23
<i>N. setosa</i> s. str.	MNRJP 007653	Brazil, Pernambuco	8.355°S; 34.953°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	Alvarez_NS25	NAI020-23
<i>N. setosa</i> s. str.	ZMBN	Italy, Taranto	40.444°N; 17.241°E	J. Laganeck	NNNNNNNN	NNNNNNNN	NNNNNNNN	Alvarez_NS43	NAI021-23
<i>N. setosa</i> s. str.	ZMBN	Italy, Livorno	43.546°N; 10.302°E	J. Laganeck	NNNNNNNN	NNNNNNNN	NNNNNNNN	Alvarez_NS44	NAI022-23
<i>N. lanai</i> sp. n.	MNRJP 007634	Brazil, Paraná	25.408°S; 48.250°W	R. Álvarez	NNNNNNNN	–	NNNNNNNN	Alvarez_NS06	NAI023-23
<i>N. lanai</i> sp. n.	MNRJP 007635	Brazil, Paraná	25.408°S; 48.250°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	Alvarez_NS07	NAI024-23
<i>N. lanai</i> sp. n.	MNRJP 007636	Brazil, Paraná	25.408°S; 48.250°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	Alvarez_NS08	NAI025-23
<i>N. lanai</i> sp. n.	MNRJP 007637	Brazil, Paraná	25.408°S; 48.250°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	Alvarez_NS09	NAI026-23
<i>N. lanai</i> sp. n.	MNRJP 007638	Brazil, Paraná	25.408°S; 48.250°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	Alvarez_NS10	NAI027-23
<i>N. lanai</i> sp. n.	MNRJP 007654	Brazil, São Paulo	23.815°S; 45.406°W	R. Álvarez	NNNNNNNN	–	NNNNNNNN	Alvarez_NS28	NAI028-23
<i>N. lanai</i> sp. n.	MNRJP 007655	Brazil, Rio de Janeiro	22.939°S; 43.877°W	J. Gabriel	NNNNNNNN	–	NNNNNNNN	Alvarez_NS36	NAI029-23
<i>N. lanai</i> sp. n.	MNRJP 007656	Brazil, Rio de Janeiro	22.939°S; 43.877°W	J. Gabriel	NNNNNNNN	–	NNNNNNNN	Alvarez_NS38	NAI030-23
<i>Naineris</i> sp.	ZMBN	USA, California	33.620°N; 117.896°W	E. Keppel	NNNNNNNN	NNNNNNNN	NNNNNNNN	Alvarez_NS46	NAI031-23
<i>Naineris</i> sp.	ZMBN	USA, California	33.619°N; 117.895°W	E. Keppel	NNNNNNNN	–	NNNNNNNN	Alvarez_NS47	NAI032-23

Species	Voucher	Locality	Coordinates	Collector	COI	16SrRNA	28SrRNA	ID BOLD	Process BOLD ID
<i>Naineris</i> sp.	LACM-AHF Poly	USA, California	32.726°N; 117.192°W	K. Sorensen, C. Sosa	NNNNNNNN	–	NNNNNNNN	Alvarez_NS33	NAI033-23
<i>Naineris</i> sp.	LACM-AHF Poly	USA, California	32.726°N; 117.192°W	K. Sorensen, C. Sosa	NNNNNNNN	NNNNNNNN	–	Alvarez_NS34	NAI034-23
<i>Naineris</i> sp.	LACM-AHF Poly	USA, California	32.726°N; 117.192°W	K. Sorensen, C. Sosa	–	NNNNNNNN	NNNNNNNN	Alvarez_NS35	NAI035-23
<i>N. laevigata</i>	–	Tunisia		D. Martin	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	NL01
<i>N. laevigata</i>	–	Tunisia		D. Martin	–	NNNNNNNN	–	XXXXXXXXXX	NL02
<i>N. laevigata</i>	–	Tunisia		D. Martin	NNNNNNNN	NNNNNNNN	–	XXXXXXXXXX	NL03
<i>N. laevigata</i>	–	Tunisia		D. Martin	NNNNNNNN	NNNNNNNN	–	XXXXXXXXXX	NL04
<i>N. laevigata</i>	–	Tunisia		D. Martin	NNNNNNNN	NNNNNNNN	–	XXXXXXXXXX	NL05
<i>N. laevigata</i>	–	Italy, Orbetello	42.43299°N; 11.184909°E	J. Laganeck	–	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	NL06
<i>N. laevigata</i>	–	Italy, Orbetello	42.43299°N; 11.184909°E	J. Laganeck	NNNNNNNN	–	–	XXXXXXXXXX	NL07
<i>N. laevigata</i>	–	Italy, Pisa	43.655209°N; 10.281437°E	J. Laganeck	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	NL08
<i>N. laevigata</i>	–	Italy, Cesáreo	40.256343°N; 17.891929°E	J. Laganeck	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	NL10
<i>N. anserina</i>	–	Italy, Ardenza	43.510959°N; 10.317446°E	J. Laganeck	NNNNNNNN	NNNNNNNN	–	XXXXXXXXXX	NL09
<i>N. anserina</i>	ZMMSU 10680	Mediterranean Sea, Mallorca Island	–	A. Zhadan	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	NL11
<i>N. capsulifera</i> (Black Sea)	ZMMSU 11270	Black Sea, Crimea, Sebastopol Bay, Karantinnaya Bight	–	V. I. Radashevsky	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	NL12
<i>N. laevigata</i> (Florida)	SERC #255675	USA, Tampa Florida	27.8854°N; 82.53378°W	E. Keppel	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	NL13
<i>N. laevigata</i> (Florida)	SERC #255632	USA, Tampa Florida	27.88541°N; 82.53385°W	E. Keppel	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	NL14
<i>N. laevigata</i> (Florida)	SERC #255674	USA, Tampa Florida	27.88543°N; 82.53441°W	E. Keppel	NNNNNNNN	–	NNNNNNNN	XXXXXXXXXX	NL15

Species	Voucher	Locality	Coordinates	Collector	COI	16SrRNA	28SrRNA	ID BOLD	Process BOLD ID
<i>N. laevigata</i> (Florida)	SERC #255941	USA, St Petersburg Florida	–	E. Keppel	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	NL16
<i>N. laevigata</i> (Florida)	SERC #256071	USA, Picnic Island Florida	–	E. Keppel	NNNNNNNN	–	NNNNNNNN	XXXXXXXXXX	NL17
<i>Protoaricia oerstedii</i>		Spain, Catalonia, Tossa de Mar	–	J. Gil	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Pro8
<i>Protoaricia oerstedii</i>	–	Italy, Calafuria	43.474603°N; 10.330264°E	J. Laganeck	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Pro9
<i>Naineris dendritica</i>	–	USA, California, Bodega Bay	–	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nd02
<i>Naineris dendritica</i>	–	USA, California, Bodega Bay	–	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nd03
<i>Naineris dendritica</i>	–	USA, California, Bodega Bay	–	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nd04
<i>Naineris dendritica</i>	–	USA, California, Bodega Bay	–	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nd01
<i>Naineris dendritica</i>	LACM-AHF Poly	USA, California, Bodega Bay	–	B. Swope, C. Sosa	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nd05
<i>Naineris dendritica</i>	ZMMSU 10199	Canada, British Columbia, Calvert Island	–	V. I. Radashevsky	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nd06
<i>Naineris dendritica</i>	ZMMSU 10198	Canada, British Columbia, Calvert Island	–	V. I. Radashevsky	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nd07
<i>Naineris dendritica</i>	ZMMSU 10200	Canada, British Columbia, Calvert Island	–	V. I. Radashevsky	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nd08
<i>Naineris dendritica</i>	ZMMSU 10187	Canada, British Columbia, Calvert Island	–	V. I. Radashevsky	–	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nd09
<i>Naineris dendritica</i>	ZMMSU 10184	Canada, British Columbia, Calvert Island	–	V. I. Radashevsky	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nd10
<i>Naineris dendritica</i>	ZMBN	Japan, Sea of Japan	–	N. Budaeva	NNNNNNNN	NNNNNNNN	–	XXXXXXXXXX	Nd11

Species	Voucher	Locality	Coordinates	Collector	COI	16SrRNA	28SrRNA	ID BOLD	Process BOLD ID
<i>Naineris dendritica</i>	ZMBN	Japan, Sea of Japan	–	N. Budaeva	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXX	Nd12
<i>Naineris dendritica</i>	ZMBN	Japan, Sea of Japan	–	N. Budaeva	–	NNNNNNNN	NNNNNNNN	XXXXXXX	Nd13
<i>Naineris dendritica</i>	ZMBN	Japan, Sea of Japan	–	N. Budaeva	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXX	Nd14
<i>Naineris dendritica</i>	ZMBN	Japan, Sea of Japan	–	N. Budaeva	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXX	Nd15
<i>Naineris dendritica</i>	MNRJP 007617	Brazil, Santa Catarina, Pântano do Sul	27.783563°S; 48.506634°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXX	Naur01
<i>Naineris aurantiaca</i>	MNRJP	Brazil, Santa Catarina, Ingleses	27.783563°S; 48.506634°W	R. Álvarez	NNNNNNNN	–	NNNNNNNN	XXXXXXX	Naur02
<i>Naineris aurantiaca</i>	MNRJP	Brazil, Santa Catarina, Ingleses	27.783563°S; 48.506634°W	R. Álvarez	–	NNNNNNNN	–	XXXXXXX	Naur03
<i>Naineris aurantiaca</i>	MNRJP	Brazil, Santa Catarina, Ingleses	27.783563°S; 48.506634°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXX	Naur04
<i>Naineris aurantiaca</i>	MNRJP	Brazil, Paraná, Ilha do Mel, Bacia da Sereia	25.573386°S; 48.316551°W	R. Álvarez	NNNNNNNN	–	–	XXXXXXX	Naur06
<i>Naineris aurantiaca</i>	MNRJP	Brazil, Paraná, Ilha do Mel, Bacia da Sereia	25.573386°S; 48.316551°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXX	Naur07
<i>Naineris aurantiaca</i>	MNRJP	Brazil, Paraná, Ilha do Mel, Bacia da Sereia	25.573386°S; 48.316551°W	R. Álvarez	NNNNNNNN	–	–	XXXXXXX	Naur08
<i>Naineris aurantiaca</i>	MNRJP	Brazil, Paraná, Ilha do Mel, Bacia da Sereia	25.573386°S; 48.316551°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXX	Naur09
<i>Naineris aurantiaca</i>	MNRJP	Brazil, Paraná, Ilha do Mel, Bacia da Sereia	25.543970°S; 48.291922°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXX	Naur10

Species	Voucher	Locality	Coordinates	Collector	COI	16SrRNA	28SrRNA	ID BOLD	Process BOLD ID
<i>Naineris aurantiaca</i>	MNRJP	Brazil, Paraná, Ilha do Mel, Joaquim Manuel	25.543970°S; 48.291922°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Naur11
<i>Naineris aurantiaca</i>	MNRJP	Brazil, Paraná, Ilha do Mel, Joaquim Manuel	25.543970°S; 48.291922°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Naur12
<i>Naineris aurantiaca</i>	MNRJP	Brazil, Paraná, Ilha do Mel, Joaquim Manuel	25.543970°S; 48.291922°W	R. Álvarez	NNNNNNNN	NNNNNNNN	–	XXXXXXXXXX	Naur13
<i>Naineris aurantiaca</i>	MNRJP	Brazil, Paraná, Ilha do Mel, Joaquim Manuel	25.543970°S; 48.291922°W	R. Álvarez	NNNNNNNN	–	–	XXXXXXXXXX	Naur14
<i>Naineris aurantiaca</i>	MNRJP	Brazil, Paraná, Ilha das Palmas	25.517135°S; 48.285299°W	R. Álvarez	NNNNNNNN	–	–	XXXXXXXXXX	Naur15
<i>Naineris aurantiaca</i>	MNRJP	Brazil, Paraná, Ilha das Palmas	25.517135°S; 48.285299°W	R. Álvarez	NNNNNNNN	–	–	XXXXXXXXXX	Naur16
<i>Naineris aurantiaca</i>	MNRJP	Brazil, Paraná, Ilha das Palmas	25.517135°S; 48.285299°W	R. Álvarez	NNNNNNNN	–	–	XXXXXXXXXX	Naur17
<i>Naineris aurantiaca</i>	MNRJP	Brazil, Paraná, Ilha das Palmas	25.517135°S; 48.285299°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Naur18
<i>Naineris aurantiaca</i>	MNRJP	Brazil, Paraná, Ilha das Palmas	25.517135°S; 48.285299°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Naur19
<i>Naineris aurantiaca</i>	MNRJP	Brazil, Rio de Janeiro, Ilha da Marambaia, Praia Grande	23.057600°S; 43.991322°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Naur20
<i>Naineris aurantiaca</i>	MNRJP	Brazil, Rio de Janeiro, Ilha da Marambaia, Praia Grande	23.057600°S; 43.991322°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Naur21
<i>Naineris aurantiaca</i>	MNRJP	Brazil, Rio de Janeiro, Ilha da Marambaia, Praia Grande	23.057600°S; 43.991322°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Naur22
<i>Naineris aurantiaca</i>	MNRJP	Brazil, Rio de Janeiro, Ilha da Marambaia, Praia Grande	23.057600°S; 43.991322°W	R. Álvarez	–	NNNNNNNN	–	XXXXXXXXXX	Naur23

Species	Voucher	Locality	Coordinates	Collector	COI	16SrRNA	28SrRNA	ID BOLD	Process BOLD ID
<i>Naineris aurantiaca</i>	MNRJP	Marambaia, Praia Grande Brazil, Rio de Janeiro, Ilha da Marambaia,	23.057600°S; 43.991322°W	R. Álvarez	–	NNNNNNNN	–	XXXXXXXXXX	Naur24
<i>Naineris aurantiaca</i>	MNRJP	Praia Grande Brazil, Rio de Janeiro, Ilha da Marambaia,	23.046192°S; 43.974691°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Naur25
<i>Naineris aurantiaca</i>	MNRJP	Praia Suja Brazil, Rio de Janeiro, Ilha da Marambaia,	23.046192°S; 43.974691°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Naur26
<i>Naineris aurantiaca</i>	MNRJP	Praia Suja Brazil, Rio de Janeiro, Ilha da Marambaia,	23.046192°S; 43.974691°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Naur27
<i>Naineris aurantiaca</i>	MNRJP	Praia Suja Brazil, Rio de Janeiro, Ilha da Marambaia,	23.046192°S; 43.974691°W	R. Álvarez	NNNNNNNN	NNNNNNNN	–	XXXXXXXXXX	Naur28
<i>Naineris aurantiaca</i>	MNRJP	Praia Suja Brazil, Rio de Janeiro, Ilha da Marambaia,	23.046192°S; 43.974691°W	R. Álvarez	NNNNNNNN	NNNNNNNN	–	XXXXXXXXXX	Naur29
<i>Naineris chilensis</i>	MNRJP	Perú, Huarmey	–	I. Carmona	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nch01
<i>Naineris chilensis</i>	MNRJP	Perú, Huarmey	–	I. Carmona	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nch02
<i>Naineris chilensis</i>	MNRJP	Perú, Huarmey	–	I. Carmona	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nch03
<i>Naineris chilensis</i>	MNRJP	Perú, Huarmey	–	I. Carmona	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nch04
<i>Naineris chilensis</i>	MNRJP	Perú, Huarmey	–	I. Carmona	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nch05

Species	Voucher	Locality	Coordinates	Collector	COI	16SrRNA	28SrRNA	ID BOLD	Process BOLD ID
<i>Naineris bicornis</i>	–	Brazil, Rio de Janeiro, Cabo Frio	22.882752°S; 42.002578°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nb01
<i>Naineris bicornis</i>	–	Brazil, Bahia, Baía de Todos os Santos	12.910650°S; 38.497947°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nb02
<i>Naineris</i> sp. Kuwait	14685	Kuwait	–	A. Zhadan	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	NKw_NK02
<i>Naineris</i> sp. Oman		Oman	–	–	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nom_NK03
<i>Naineris quadricuspida</i>	ZMMSU 008255	Russia, Barents Sea, Dalnezelenetska ya Bay	–	–	–	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nq01
<i>Naineris quadricuspida</i>	ZMMSU 008255	Russia, Barents Sea, Dalnezelenetska ya Bay	–	–	–	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nq02
<i>Naineris quadricuspida</i>	ZMMSU 008255	Russia, Barents Sea, Dalnezelenetska ya Bay	–	–	–	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nq03
<i>Naineris quadricuspida</i>	ZMMSU 0013138	Russia, White Sea, Kandalakshab	–	A. Zhadan, M. Meca	–	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nq04
<i>Naineris quadricuspida</i>	ZMMSU 0013138	Russia, White Sea, Kandalakshab	–	A. Zhadan, M. Meca	–	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nq05
<i>Naineris quadricuspida</i>	ZMMSU 0013138	Russia, White Sea, Kandalakshab	–	A. Zhadan, M. Meca	–	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nq06
<i>Naineris quadricuspida</i>	ZMMSU 008243	Guba Bay	–	–	–	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nq07
<i>Naineris quadricuspida</i>	ZMMSU 008243	Guba Bay	–	–	–	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nq08

Species	Voucher	Locality	Coordinates	Collector	COI	16SrRNA	28SrRNA	ID BOLD	Process BOLD ID
<i>Naineris quadricuspida</i>	ZMMSU 008243	WS Guba Bay	–	–	–	NNNNNNNN	–	XXXXXXXXXX	Nq09
<i>Naineris quadricuspida</i>	ZMMSU 008243	WS Guba Bay	–	–	–	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nq10
<i>Naineris quadricuspida</i>	ZMMSU 008243	WS Guba Bay	–	–	–	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nq11
<i>Naineris quadricuspida</i>	ZMMSU 008243	WS Guba Bay	–	–	–	–	NNNNNNNN	XXXXXXXXXX	Nq12
<i>Naineris quadricuspida</i>	ZMBN_130403	Hardbunnsfauna	–	–	–	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nq13
<i>Naineris quadricuspida</i>	UiB	Kleivodden	–	–	–	–	NNNNNNNN	XXXXXXXXXX	Nq14
<i>Naineris quadricuspida</i>	UiB	Andoya	–	–	–	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nq15
<i>Naineris quadricuspida</i>	UiB	Kleivodden	–	–	–	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nq16
<i>Naineris quadricuspida</i>	UiB	Andoya	–	–	–	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nq17
<i>Naineris quadricuspida</i>	UiB	Andoya	–	–	–	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nq18
<i>Naineris quadricuspida</i>	UiB	Sommarøy	–	–	–	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nq19
<i>Naineris quadricuspida</i>	UiB	Sommarøy	–	–	–	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nq20
<i>Naineris quadricuspida</i>	UiB	Sommarøy	–	–	–	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nq21
<i>Naineris quadricuspida</i>	UiB	Sommarøy	–	–	–	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nq22
<i>Naineris quadricuspida</i>	UiB	St2	–	–	–	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nq23
<i>Naineris quadricuspida</i>	UiB	St2	–	–	–	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nq24
<i>Naineris quadricuspida</i>	UiB	St2	–	–	–	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nq25
<i>Naineris quadricuspida</i>	UiB	St2	–	–	–	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nq26
<i>Naineris quadricuspida</i>	UiB	Norway, Kvalsvik	–	–	–	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nq26

Species	Voucher	Locality	Coordinates	Collector	COI	16SrRNA	28SrRNA	ID BOLD	Process BOLD ID
<i>Naineris quadricuspida</i>	UiB	Norway, Kvalsvik	–	–	–	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nq27
<i>Naineris quadricuspida</i>	UiB	Norway, Kvalsvik	–	–	–	–	NNNNNNNN	XXXXXXXXXX	Nq28
<i>Naineris quadricuspida</i>	UiB	Norway, Kvalsvik	–	–	–	–	NNNNNNNN	XXXXXXXXXX	Nq29
<i>Naineris quadricuspida</i>	UiB	Norway, Kvalsvik	–	–	–	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nq30
<i>Naineris quadricuspida</i>	ZMMSU 0013138	WS Russia, White Sea, Kandalakshab	–	–	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nai1
<i>Naineris quadricuspida</i>	ZMMSU 08242	WS Russia, Barents Sea, Zelenetskaya Dalnyaya Guba Bay	–	–	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nai 6
<i>Naineris quadricuspida</i>	ZMMSU 08243	WS Russia, Barents Sea, Zelenetskaya Dalnyaya Guba Bay	–	–	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nai 16
<i>Naineris quadricuspida</i>		Norway, Kvalsvik			NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nai 21

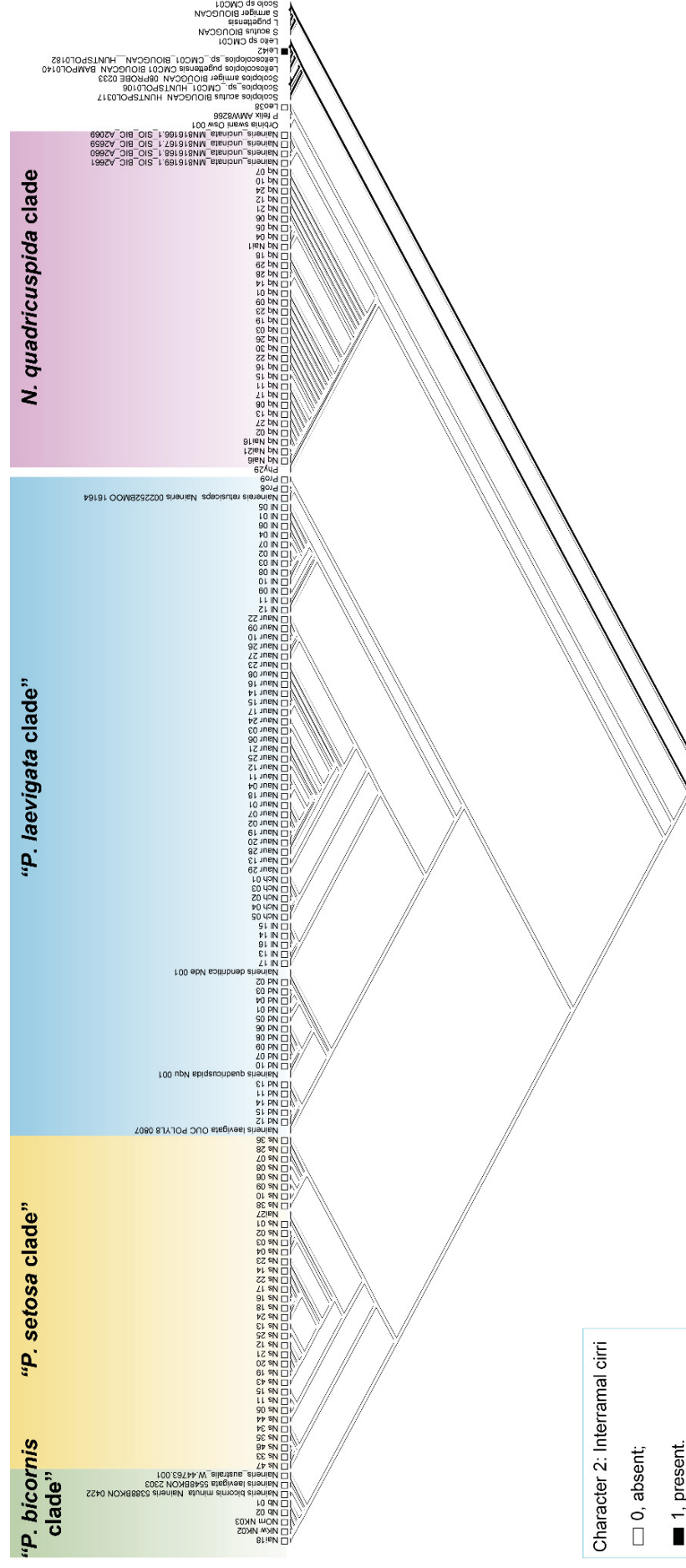


Figure S1. Ancestral state reconstructions of character 2 (interramal cirri) traced on the Maximum Likelihood reconstruction.

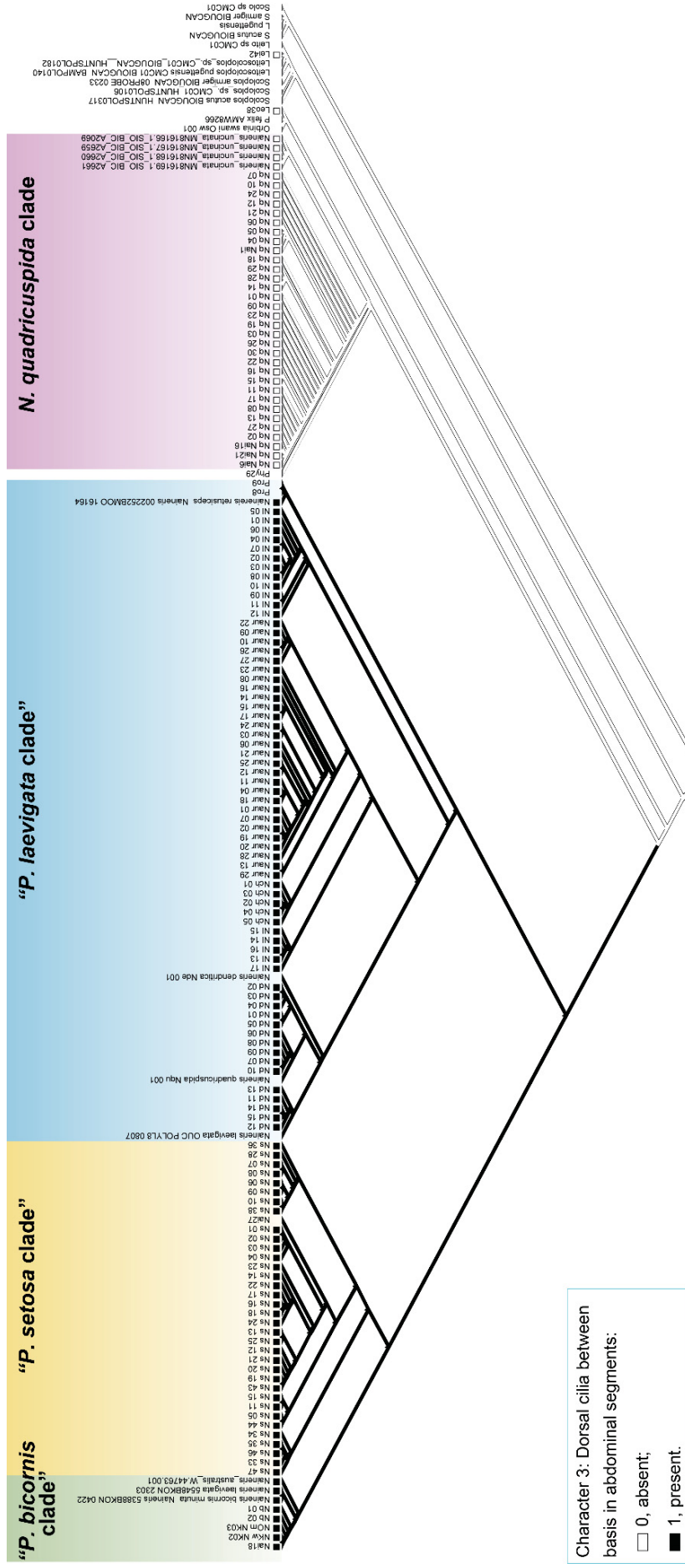
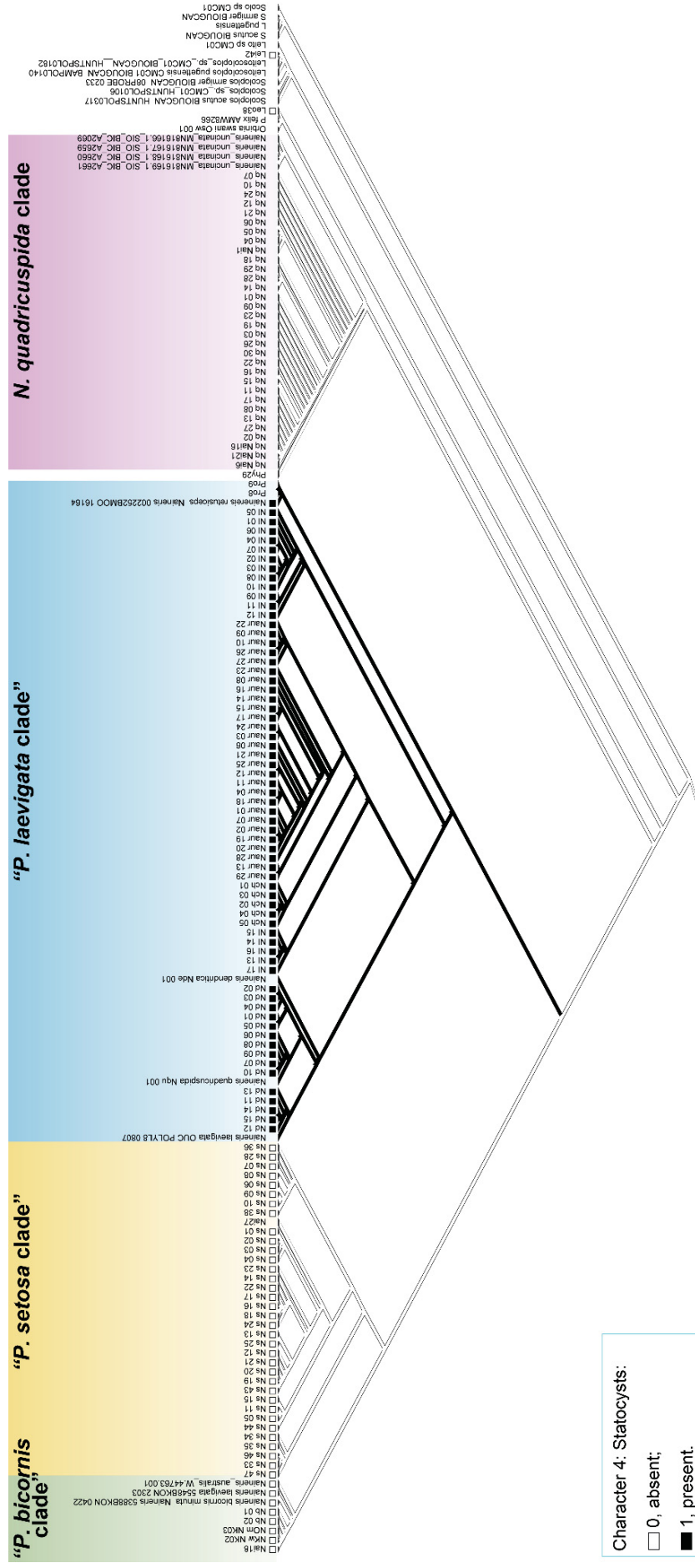


Figure S2. Ancestral state reconstructions of character 3 (dorsal cilia between branchial bases in abdominal segments) traced on the Maximum Likelihood reconstruction.



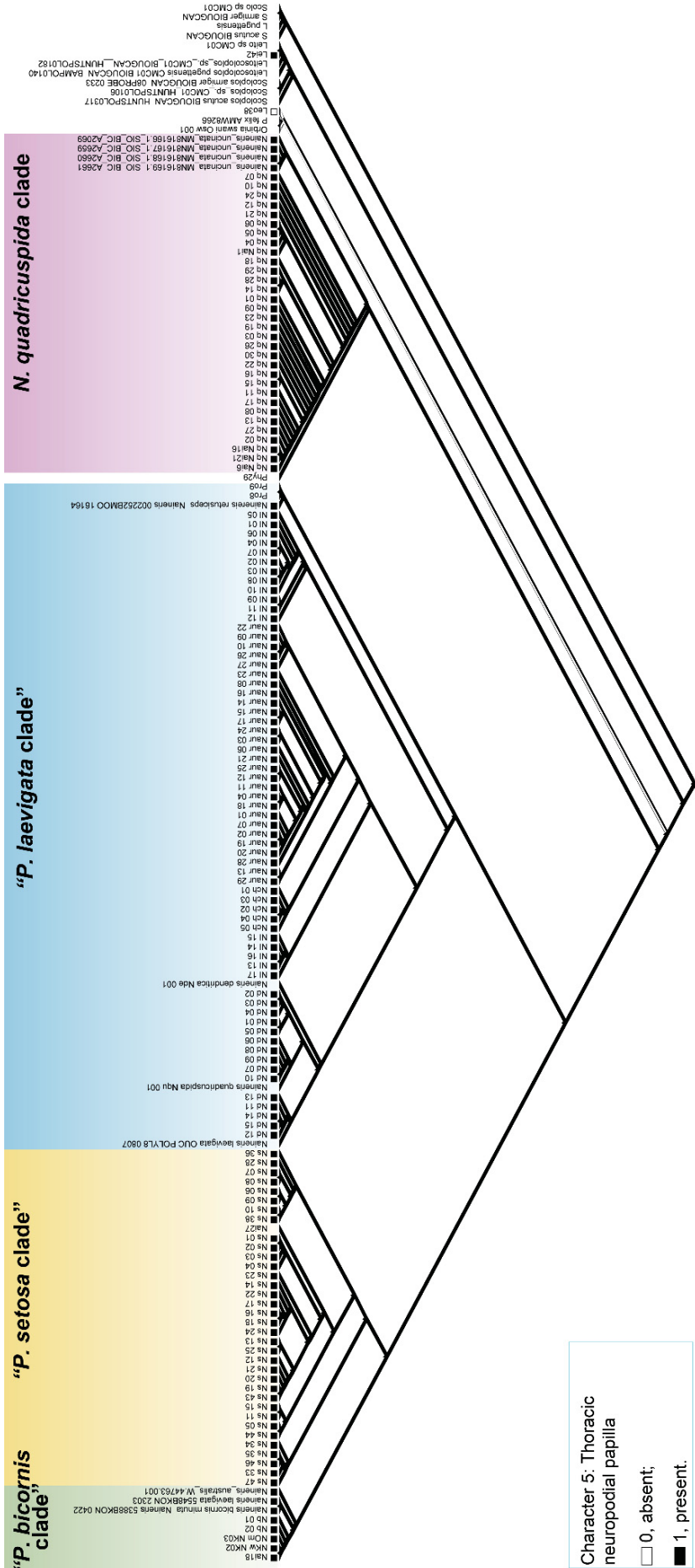


Figure S4. Ancestral state reconstructions of character 5 (thoracic neuropodial papilla) traced on the Maximum Likelihood reconstruction.

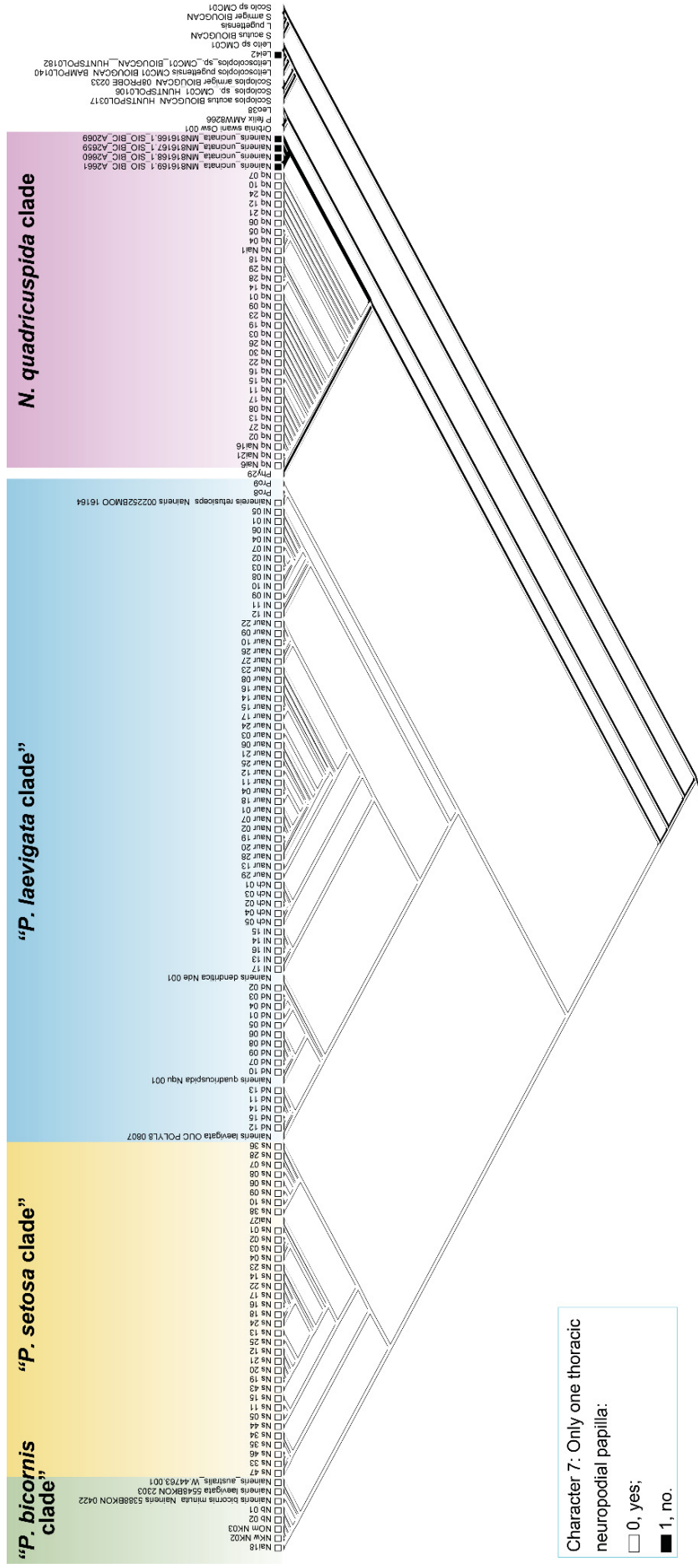


Figure S5. Ancestral state reconstructions of character 7 (only one thoracic neuropodial papilla) traced on the Maximum Likelihood reconstruction.

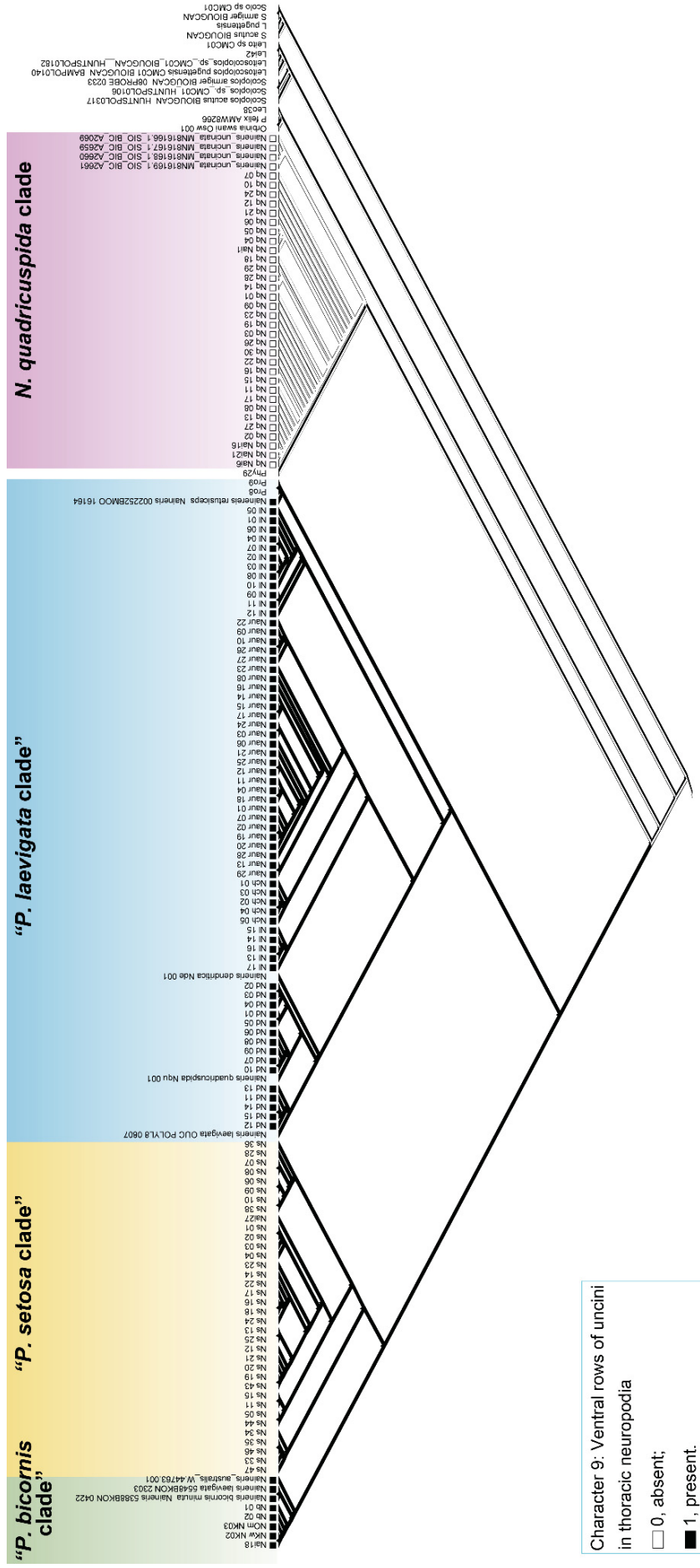


Figure S7. Ancestral state reconstructions of character 9 (ventral rows of uncini in thoracic neuropodia) traced on the Maximum Likelihood reconstruction.

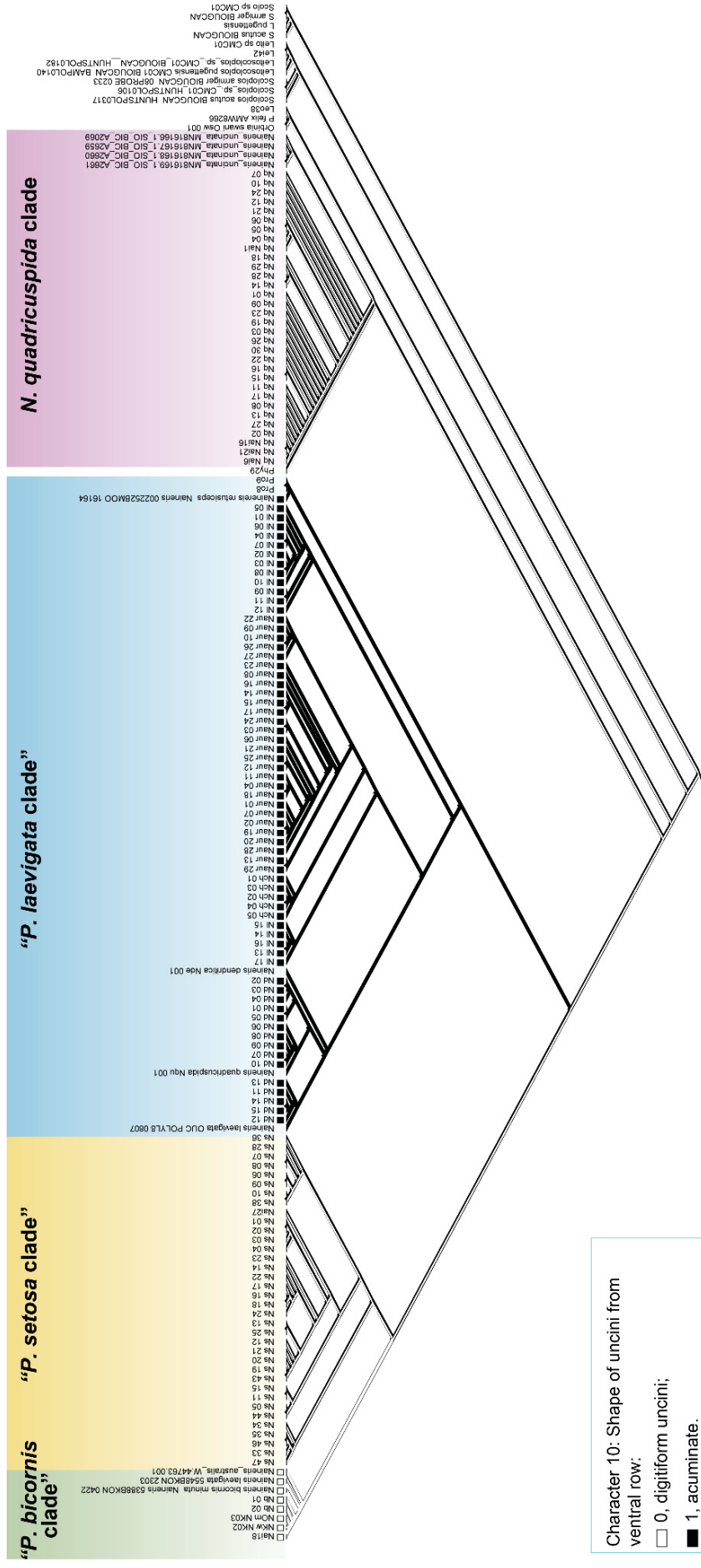


Figure S8. Ancestral state reconstructions of character 10 (shape of uncini from ventral row) traced on the Maximum Likelihood reconstruction.

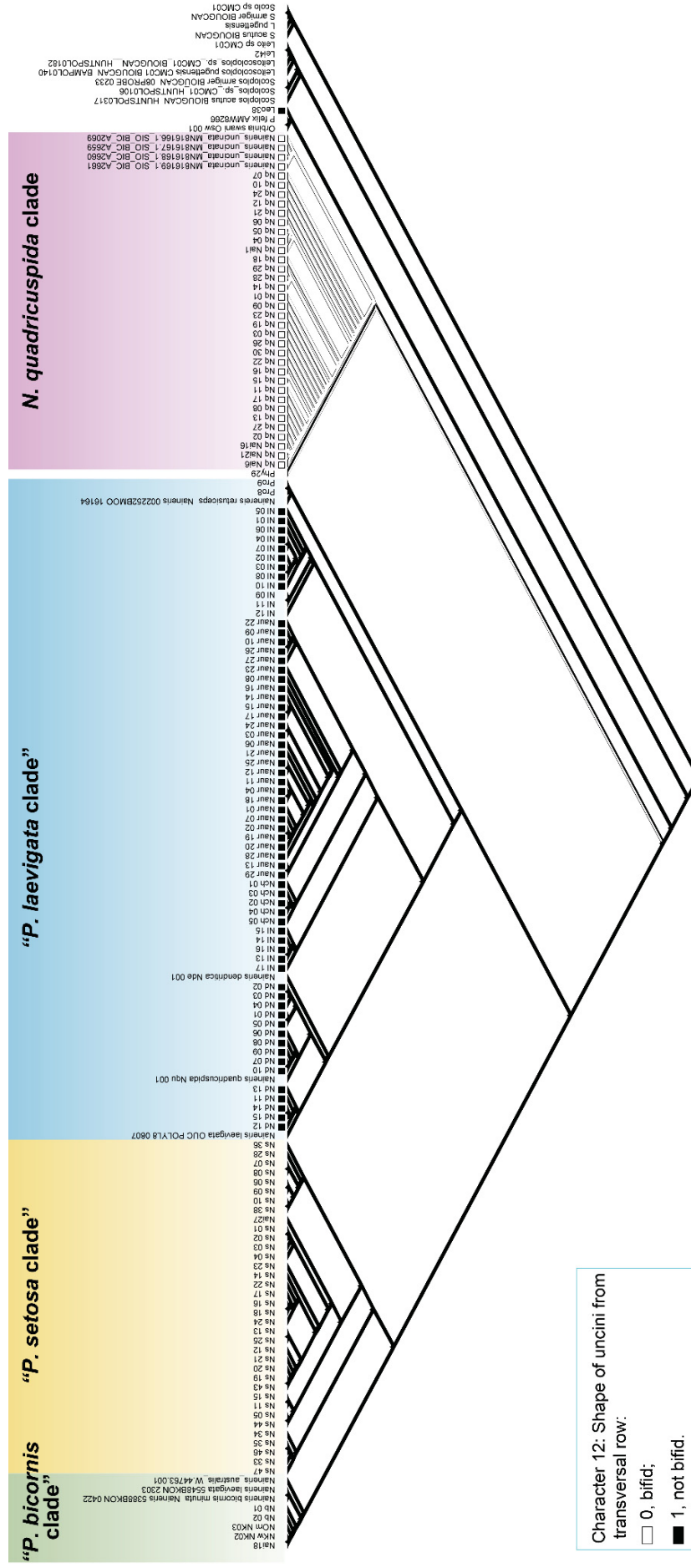


Figure S10. Ancestral state reconstructions of character 12 (shape of uncini from transversal row) traced on the Maximum Likelihood reconstruction.

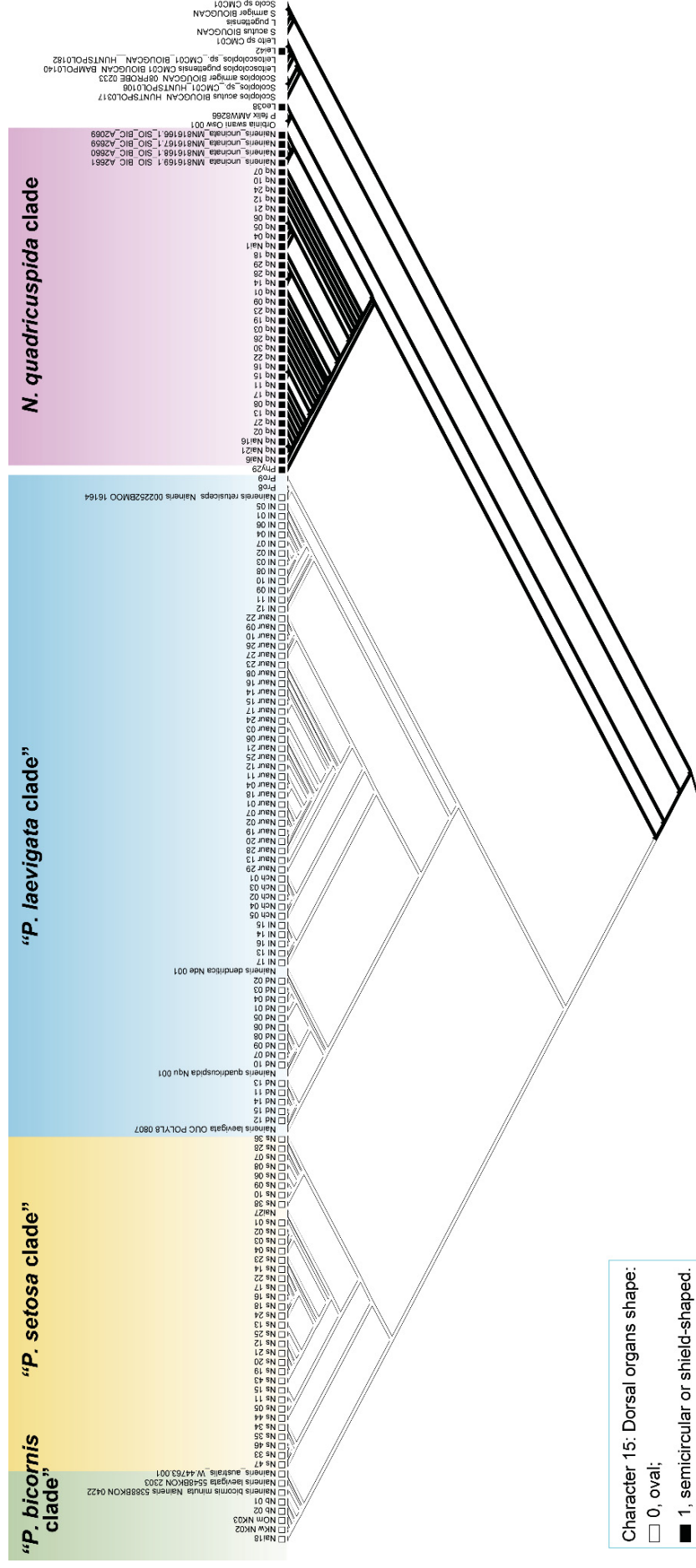


Figure S11. Ancestral state reconstructions of character 15 (dorsal organs shape) traced on the Maximum Likelihood reconstruction.

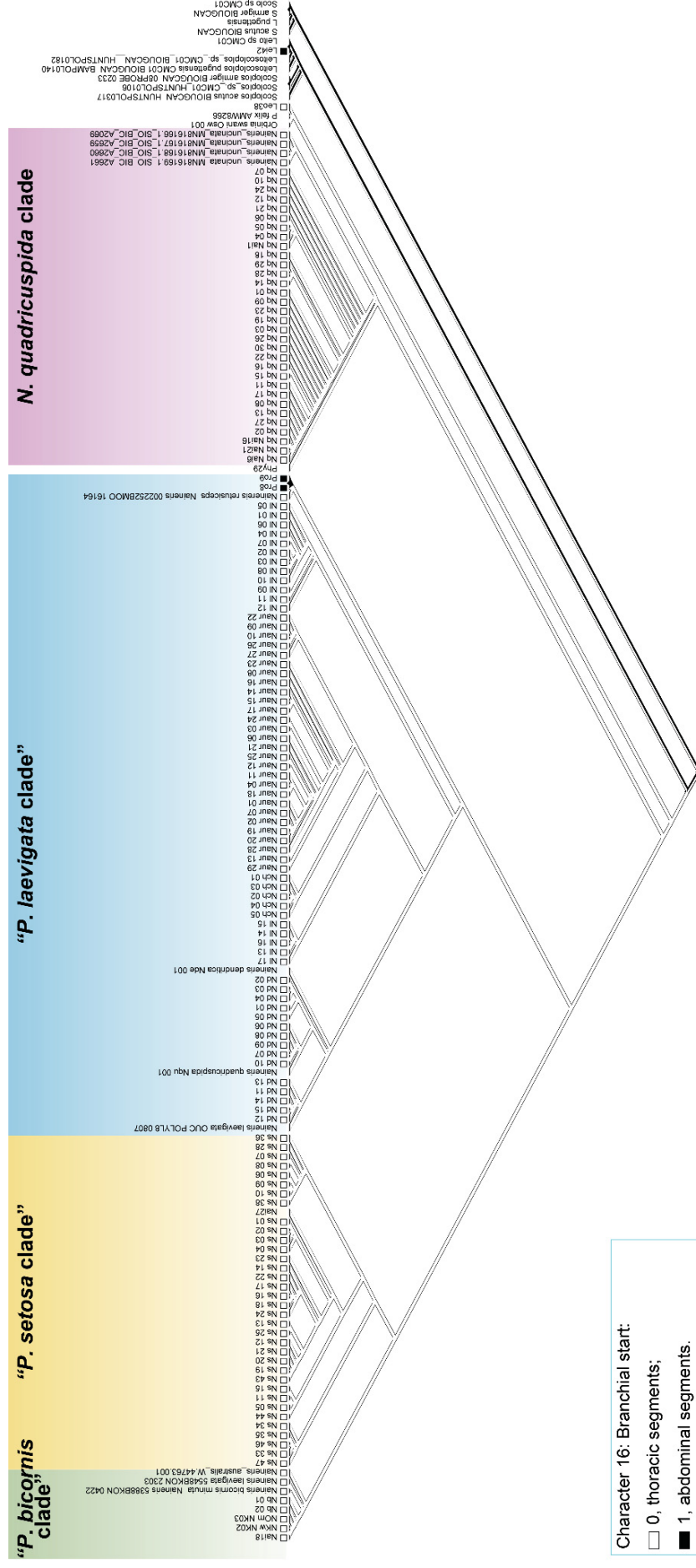


Figure S12. Ancestral state reconstructions of character 16 (branchial start) traced on the Maximum Likelihood reconstruction.

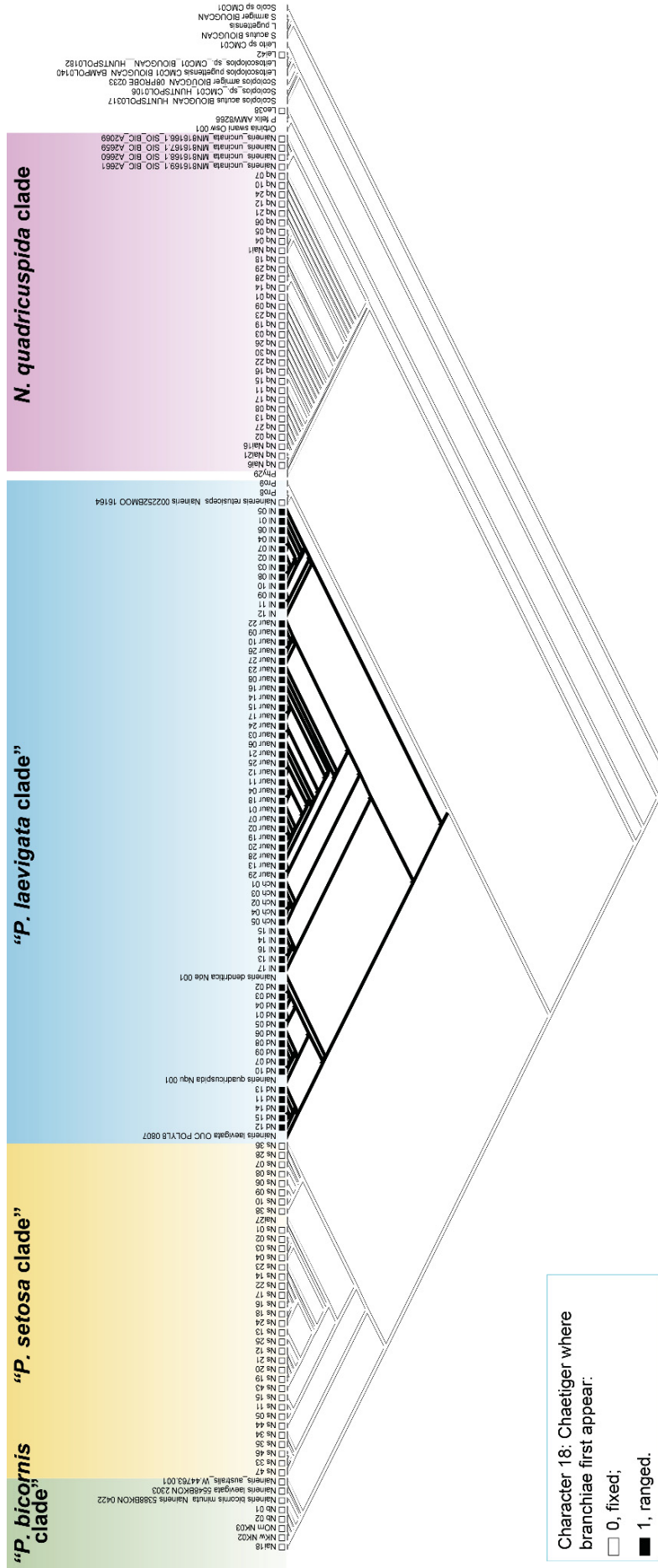


Figure S13. Ancestral state reconstructions of character 18 (chaetiger where branchiae first appear) traced on the Maximum Likelihood reconstruction.

Table S2. Comparison of species of *Naineris* based on literature (N/O - not observed, P – present, A – absent)

Species	Shape of prostomium	Nucal organs (P/A)	Eyesposts (ocelli)	Dorsal organs (P/A)	Dorsal crest (P/A)	Shape of proboscis	Branchial start (ch.)	Branchial Start abruptly	N° Thoracic ch.	Interramal papillae/Lateral organ
<i>Naineris quadricuspida</i> (Fabricius, 1780)	rounded	N/O	N/O	N/O	N/O	N/O	5	N/O	N/O	N/O
<i>Naineris quadricuspida</i> (Fauvel 1927)	rounded	N/O	P	N/O	N/O	N/O	4-7	abruptly	12-17	A
<i>Naineris quadricuspida</i> (López 2004)	rounded	P	P	P	N/O	lobed	4-5	abruptly	14-17	P
<i>Protoaricia laevigata</i> (Grube, 1855)	rounded	N/O	N/O	N/O	N/O	N/O	7	N/O	23-24	N/O
<i>Protoaricia laevigata</i> (Fauvel 1927)	rounded	N/O	P	P (1-23)	N/O	dendritic	4-11	not abruptly	N/O	N/O
<i>Protoaricia laevigata</i> (López 2004)	rounded	P	P	P	N/O	lobed	6-7	not abruptly	28	P
<i>Protoaricia aurantiaca</i> (Müller, 1858)	rounded	P	A	P (from 12 onwards)	A	dendritic, with finger-shaped lobes	9	not abruptly, they begin	13	P

Species	Shape of prostomiu m	Nucal organs (P /A)	Eyesposts (ocelli)	Dorsal organs (P/A)	Dorsal crest (P/A)	Shape of proboscis	Branchial start (ch.)	Branchial Start abrupt	N° Thoracic ch.	Interramal papillae/Lateral organ
(Álvarez et al. 2019)								as small spots		
<i>Protoaricia dendritica</i> (Kinberg, 1867)	rounded	N/O	N/O	N/O	N/O	N/O	N/O	N/O	18-19	N/O
<i>Protoaricia dendritica</i> (Blake 1996)	rounded	A	A	N/O	N/O	numerous branches	8 (-12)	N/O	19-28	N/O
<i>Protoaricia setosa</i> (Verrill, 1900)	rounded	N/O	P	P	P	*not mentioned . Sacklike in toptype material	6	abruptly, it appears normal in shape, a bit smaller than abdominal	20	N/O
<i>Protoaricia setosa</i> (Solis-Weiss 1989)	truncate/ T shaped	P	P	P	P	wide sacklike	6	abruptly	20	
<i>Naineris grubei</i> (Gravier, 1908)	rounded	N/O	A	N/O	N/O	well ramified	7	N/O	17	N/O
<i>Naineris grubei</i> (Blake 2017)	truncate, rounded	N/O	A	P	P	N/O	4	not abruptly, they are conical at first, then becoming	19-20	N/O

Species	Shape of prostomium	Nucal organs (P/A)	Eyesposts (ocelli)	Dorsal organs (P/A)	Dorsal crest (P/A)	Shape of proboscis	Branchial start (ch.)	Branchial Start abrupt	N° Thoracic ch.	Interramal papillae/Lateral organ
<i>Protoaricia retusiceps</i> Chamberlin, 1919	rounded	P	N/O	N/O	P	N/O	6	N/O	N/O	N/O
<i>Protoaricia jacutica</i> Annenkova, 1931	rounded	N/O	N/O	N/O	N/O	N/O	8-9	not abruptly	19-20	N/O
<i>Protoaricia mutilata</i> Treadwell, 1931	rounded	N/O	N/O	not mentioned, not evident in drawings	N/O	two rather thick, flattened lobes	2	not mentioned	N/O	N/O
<i>Protoaricia mutilata</i> (Hartman 1957)	N/O	N/O	N/O	N/O	N/O	N/O	3	not abruptly	29	N/O
<i>Protoaricia bicornis</i> Hartman, 1951	spatulate and bifid	N/O	P	P, paired within the branchial bases, from de fifth segment	N/O	broad, irregularly lobed	6	not abruptly	48	N/O

Species	Shape of prostomium	Nucal organs (P/A)	Eyesposts (ocelli)	Dorsal organs (P/A)	Dorsal crest (P/A)	Shape of proboscis	Branchial start (ch.)	Branchial Start abruptly	N° Thoracic ch.	Interramal papillae/Lateral organ
<i>Protoarcia australis</i> Hartman, 1957	truncate	N/O	A	N/O	P	N/O	6	abruptly	28	N/O
<i>Protoarcia australis</i> (Zhadan 2015)	rectangular	N/O	N/O	P, five per segment	P	N/O	6	abruptly	43 (18-43)	N/O
<i>Naineris uncinata</i> Hartman, 1957	broadly truncate to rounded	P	P, in juveniles	P, in front of branchiae bases	N/O	spacious and lobed, but not branched	5-6	not abruptly	26 (17-18)	N/O
<i>Naineris uncinata</i> (Blake 2020)	rounded to truncate	P	A	N/O	N/O	N/O	4-6	abruptly	14-15	N/O
<i>Protoarcia brevicephala</i> ? Hartmann-Schröder, 1960	rounded	N/O	N/O	N/O	N/O	lobed, fingerlike ramified	7	N/O	16-17	N/O
<i>Protoarcia chilensis</i> Hartmann-Schröder, 1965	rounded	N/O	N/O	P	N/O	ramified	7	not abruptly	27	N/O

Species	Shape of prostomium	Nucal organs (P/A)	Eyesposts (ocelli)	Dorsal organs (P/A)	Dorsal crest (P/A)	Shape of proboscis	Branchial start (ch.)	Branchial Start abruptly	N° Thoracic ch.	Interramal papillae/Lateral organ
<i>Protoaricia chilensis</i> (Blake 2017)	broadly rounded	A	A	N/O	N/O	large, multilobed	7-8	abruptly (see drawings)	15-30	N/O
<i>Protoaricia kalkudaensis</i> (De Silva, 1965)	rounded	N/O	A	N/O	N/O	faintly lobed	6	not abruptly	31	N/O
<i>Protoaricia quadraticeps</i> Day, 1965	square	N/O	A	N/O	N/O	large, frilly, not divided in lobes	6 (5-7)	abruptly	30-40	N/O
<i>Pettibonella victorinae</i> Day, 1977	rounded	N/O	N/O	N/O	N/O	N/O	8	N/O	22	present, well developed
<i>Pettibonella victorinae</i> (Zhadan 2020)	conical, rounded	N/O	N/O	N/O	N/O	N/O	8-9	N/O	16-20	present, well developed
<i>Naineris japonica</i> Imajima, 2009	spatulate and truncated	A	A	N/O	N/O	N/O	5	N/O	17	N/O
<i>Naineris argentinensis</i> Blake, 2017	rounded	N/O	A	N/O	N/O	N/O	5-6	N/O	12-13	N/O
<i>Protoaricia antarctica</i> Blake, 2017	pear-shaped, rounded	P	A	N/O	N/O	N/O	6-7	N/O	9-10	N/O

Species	Shape of prostomium	Nucal organs (P /A)	Eyesposts (ocelli)	Dorsal organs (P/A)	Dorsal crest (P/A)	Shape of proboscis	Branchial start (ch.)	Branchial Start abrupt	N° Thoracic ch.	Interramal papillae/Lateral organ
<i>Naineris furcillata</i> Blake, 2017	blunt, rounded	N/O	A	N/O	N/O	dendritic	4	N/O	17-19	absent

Table S2 (Continuation) . Comparison of species of *Naineris* based on literature (N/O - not observed, P – present, A – absent)

[illegible]

Species	Notopodial lobes in Thorax	Neuropodial lobes in Thorax	Notochaetae from Thorax	Neurochaetae from Thorax	Nopodial lobes in Abdomen	Neuropodial lobe in Abdomen	Notochaetae from Abdomen	Neurochaetae from Abdomen	Furcate chaetae (shape)
(Grube, 1855)									
<i>Protoaricia laevigata</i> (Clapere de 1864)	pyriform	lamella	crenulated capillaries	uncini, two types: curve and straight	N/O	N/O	crenulated capillaries	N/O	N/O
<i>Protoaricia laevigata</i> (Fauvel 1927)	pyriform	papillar	crenulated capillaries	capillaries, uncini, two types: curve and straight and subuluncini	pyriform	papillar	crenulated capillaries and 1-2 furcate chaetae	crenulated capillaries and 3-5 aciculae	unequal tynes
<i>Protoaricia laevigata</i> (Viéitez et al. 2004)	lanceolate	ridge	crenulated capillaries	subuluncini (anteriorly), uncini (heavily ridged and blunted - tipped) and crenulated capillaries	long, lanceolate	ridge	crenulated capillaries and furcate chaetae	crenulated capillaries and aciculae (5-7)	barbed shaft, unequal tines with distal huge needles
<i>Protoaricia aurantiaca</i> (Müller, 1858) (Álvarez et al. 2019)	pyriform	N/O	crenulated capillaries in semicircle (7-17)	subuluncini (2-3 transverse rows anteriorly), a transverse posterior row of heavily ridged uncini in an oblique row of blunted -tipped uncini	pyriform	pyriform	crenulated capillaries and a single furcate chaetae	crenulated capillaries mingled with aciculae	variably sized tines interspersed with thin needles, shaft with barbs

Species	Notopodial lobes in Thorax	Neuropodial lobes in Thorax	Notochaetae from Thorax	Neurochaetae from Thorax	Nopodial lobes in Abdomen	Neuropodial lobes in Abdomen	Notochaetae from Abdomen	Neurochaetae from Abdomen	Furcate chaetae (shape)
<i>Protoaricia dendritica</i> (Kinberg, 1867)	N/O	N/O	N/O	N/O	N/O	N/O	N/O	N/O	N/O
<i>Protoaricia dendritica</i> (Blake 1996)	long, triangular	resembling lamella	crenulated capillaries	and a row obliquely and ventrally of crenulated capillaries	slender, wide base	cirriform	crenulated capillaries and furcate chaetae	crenulated capillaries and aciculae	N/O
<i>Protoaricia setosa</i> (Verrill, 1900)	acuminate	foliaceous	crenulated capillaries	acute capillaries, with rather shafts	longer and narrower, falcate	papilliform	crenulated capillaries (10-12)	crenulated capillaries (6-8) and aciculae(3)	*N/O in description. In toptype specimens, it has unequal tynes with thin needles. Tynes with entire narrow tips with 4-6 needles on either side merging with tynes
<i>Protoaricia setosa</i> (Solís-Weiss 1989)	foliaceous	digitiform	crenulated capillaries	crenulated capillaries	digitiform	foliaceous	crenulated capillaries and furcate chaetae	crenulated capillaries and aciculae	spinous shaft and two distal tines

Species	Notopodial lobes in Thorax	Neuropodial lobes in Thorax	Notochaetae from Thorax	Neurochaetae from Thorax	Nopodial lobes in Abdomen	Neuropodial lobe in Abdomen	Notochaetae from Abdomen	Neurochaetae from Abdomen	Furcate chaetae (shape)
<i>Naineris grubei</i> (Gravier, 1908)	N/O	N/O	crenulated capillaries	crenulated capillaries (two types), and hooded uncini	N/O	N/O	crenulated capillaries	crenulated capillaries, aciculae	absent
<i>Naineris grubei</i> (Blake 2017)	fingerlike	triangular	30-35 crenulated capillaries	7-8 rows of uncini with ribbed shaft and bluntly rounded tip bearing a terminal notch and crenulated capillaries	fingerlike	bluntly rounded with ventral cirrus and ventral flange	15 or more capillaries and 5-6 furcate chaetae	1-2 protruding aciculae with curved blunted tips and 5-6 crenulated capillaries	unequal tynes with notched tips, connected by fine needles, shaft ribbed. Tynes openings on tips
<i>Protoaricia retusiceps</i> Chamberlin, 1919	subconical	shorter than notopodial, flattened	crenulated capillaries	crenulated capillaries and sigmoid spines on tip	slender, cirriform	N/O	N/O	N/O	N/O
<i>Protoaricia jacutica</i> Annenkova, 1931	foliaceous	not mentioned	crenulated capillaries	hooded uncini in an inferior row, huge uncini in a superior row and crenulated capillaries behind hooded uncini	not mentioned	not mentioned	not mentioned	not mentioned	not mentioned

Species	Notopodial lobes in Thorax	Neuropodial lobes in Thorax	Notochaetae from Thorax	Neurochaetae from Thorax	Nopodial lobes in Abdomen	Neuropodial lobe in Abdomen	Notochaetae from Abdomen	Neurochaetae from Abdomen	Furcate chaetae (shape)
<i>Protoaricia mutilata</i> Treadwell, 1931	lanceolate	with rounded apex	crenulated capillaries	hooded uncini and subuluncini	lanceolate with wide base	triangular	crenulated capillaries	heavy, straight aciculae and crenulated capillaries	not mentioned
<i>Protoaricia mutilata</i> (Hartman 1957)			crenulated capillaries	crenulated capillaries, acicular uncini 8-12			crenulated capillaries and furcate chaetae	crenulated capillaries and 4 aciculae	
<i>Protoaricia bicornis</i> Hartman, 1951	cirriform	short, digital	crenulated capillaries and three embedded aciculae	crenulated capillaries and acicular hooded spines (uncini) in a posterior row	bluntly rounded	bluntly rounded distally tapering preacicular and postacicular	crenulated capillaries and furcate chaetae	crenulated capillaries	unequal tyne and barbed shaft
<i>Protoaricia australis</i> Hartman, 1957	long, slender	ridge and longer when more developed	crenulated capillaries	crenulated capillaries and uncini, some are smooth and the others ridged. Can be hooded	long, slender	triangular, short	crenulated capillaries	crenulated capillaries and 3-5 aciculae decreasing in size ventrally	absent
<i>Protoaricia australis</i> (Zhadan 2015)	triangular, narrow tips	ridge with round or elongated papilla	crenulated capillaries	crenulated capillaries, subuluncini and straight hooded hooks	narrow, digitiform	long triangular, then reduced to inner lobe	crenulated capillaries	crenulated capillaries and 3-4 aciculae	

Species	Notopodial lobes in Thorax	Neuropodial lobes in Thorax	Notochaetae from Thorax	Neurochaetae from Thorax	Nopodial lobes in Abdomen	Neuropodial lobes in Abdomen	Notochaetae from Abdomen	Neurochaetae from Abdomen	Furcate chaetae (shape)
<i>Naineris uncinata</i> Hartman, 1957	long, slender	ridge with a papilla at the middle. Posterior half from thorax has a real lobe	crenulated capillaries	crenulated capillaries and ridged uncini (sharply curved)	long, slender	long and slender, but shorter than notopodial lobe	crenulated capillaries, furcate chaetae, and embedded acicula	crenulated capillaries and 1-2 aciculae (which may be projected)	unequal tines, long and with barbed shaft
<i>Naineris uncinata</i> (Blake 2020)	narrow, tapering postsetal lamella	thickened basally with an elongated postsetal lamella. A second lamella from chaetiger 10	crenulated capillaries	crenulated capillaries and hooded and ridged uncini with notched tip	narrow postsetal lobe	narrow, ending in bilobed tip	crenulated capillaries, 1-3 furcate chaetae	capillaries and 1-2 aciculae	unequal, blunt-tipped tynes with thin needles between tynes
<i>Protoaricia brevicephala</i> ? Hartmann-Schröder, 1960	pyriform	crenulated capillaries	crenulated capillaries	crenulated capillaries inferiorly, and uncini posteriorly	foliaceous (as a tongue)	swollen, low and rounded	crenulated capillaries	crenulated capillaries (drawings)	absent
<i>Protoaricia chilensis</i> Hartmann-Schröder, 1965	broadly triangular	foliaceous, uppermost prolonged	crenulated capillaries	crenulated capillaries, subuluncini and uncini	foliaceous, pyriform	less foliaceous	crenulated capillaries and furcate chaetae	crenulated capillaries and acicula	with thin needles
<i>Protoaricia chilensis</i> (Blake 2017)	broadly triangular	foliaceous, uppermost prolonged	crenulated capillaries	three rows of uncini (two types: smooth spines and weakly ribbed)	foliaceous, pyriform	less foliaceous	crenulated capillaries, furcate chaetae,	capillaries and 5-6 aciculae	unequal tynes, longest blunt on tip, pointed with 10 thin needles. Shaft with rows of barbs

Species	Notopodial lobes in Thorax	Neuropodial lobes in Thorax	Notochaetae from Thorax	Neurochaetae from Thorax	Nopodial lobes in Abdomen	Neuropodial lobe in Abdomen	Notochaetae from Abdomen	Neurochaetae from Abdomen	Furcate chaetae (shape)
				and subuluncini intermixed with capillaries (ventrally)			imbedded aciculae		
<i>Protoaricia kalkudaensis</i> (De Silva, 1965)	pyriform	semicircular, padlike with upper papilla	crenulated capillaries	crenulated capillaries and striated hooded uncini	pyriform	digitiform	crenulated capillaries and 1-3 furcate chaetae	crenulated capillaries	unequal tines flat, with thin needles. Tynes with entire narrow tips with 4-6 needles on either side merging with tynes
<i>Protoaricia quadratriceps</i> Day, 1965	pyriform (with a stout base and expanded in apex)	swollen	crenulated capillaries	anterior row serrated uncini, 1-2 rows of subuluncini, and a posterior row of crenulated capillaries	pyriform	triangular	crenulated capillaries and furcate chaetae	crenulated capillaries and 4 projecting aciculae	unequal tynes and serrated shafts
<i>Pettibonella victoriae</i> Day, 1977	cirriform	1-2 podal papillae	crenulated capillaries	broad bladed crenulated capillaries, long crenulated capillaries, and 6 serrated uncini	cirriform	slender	crenulated capillaries	broad-bladed crenulated capillaries supported by stout projecting and bidentate aciculae	N/O

Species	Notopodial lobes in Thorax	Neuropodial lobes in Thorax	Notochaetae from Thorax	Neurochaetae from Thorax	Nopodial lobes in Abdomen	Neuropodial lobe in Abdomen	Notochaetae from Abdomen	Neurochaetae from Abdomen	Furcate chaetae (shape)
<i>Pettibonella victoriae</i> (Zhadan 2020)	digitate, foliaceous	mammiform, a podal papillae in chaetiger 14	crenulated capillaries	crenulated capillaries and striated uncini	narrow	triangular	crenulated capillaries	crenulated capillaries and bidentate aciculae	N/O
<i>Naineris japonica</i> Imajima, 2009	digitate	swollen	crenulated capillaries	crenulated capillaries and ridged uncini with hood in 4-5 rows. 10-15 in well developed in a row. Uncini in anterior row shorter than posterior	distally tapering	bilobed, infracuticular lobe longest	crenulated capillaries and furcate chaetae	thin delicate capillaries	*not mentioned in description, but in drawings can see unequal tynes with thins needles. Tynes with entire narrow tips with 4-6 needles on either side merging with tynes
<i>Naineris argentinensis</i> Blake, 2017	elongate, fingerlike	swollen	crenulated capillaries	crenulated capillaries with spinelets along shaft and bidentate ribbed uncini 2-3 rows (two types: anterior row curved and the other straighter)	elongate, fingerlike	swollen, low and rounded	crenulated capillaries	crenulated capillaries and curved blunt-tipped unidentate uncini. Most posterior chaetigers with 2-3 unidentate aciculae	absent
<i>Protoaricia antarctica</i> Blake, 2017	elongated	swollen, forming rounded tori	crenulated capillaries, pointed chaetae and	crenulated capillaries and ribbed uncini in two rows	elongated	elongated with ventral cirrus	pointed chaetae, 1-2 smooth acicular	capillaries, and 1-2 heavy curved aciculae	unequal tynes with thins needles. Tynes with entire narrow tips with 4-5

Species	Notopodial lobes in Thorax	Neuropodial lobes in Thorax	Notochaetae from Thorax	Neurochaetae from Thorax	Nopodial lobes in Abdomen	Neuropodial lobe in Abdomen	Notochaetae from Abdomen	Neurochaetae from Abdomen	Furcate chaetae (shape)
			furcate chaetae				spines, and furcate chaetae		needles on either side merging with tynes. Barbed shafts
<i>Naineris furcillata</i> Blake, 2017	elongate, thin fingerlike. Forked in 17-18 chaetiger	short fingerlike	30-40 crenulated capillaries and 3-5 furcate chaetae	dense fascicles of uncini and crenulated capillaries	forked or undivided	N/O	few capillaries and furcate chaetae	few crenulated capillaries and 1-2 smooth, slightly curved aciculae	unequal tynes having blunted, bifid tips; tynes connected by membrane with fine needles, shafts with transverse ribs

Table S3. Primers and PCR conditions in this study

Marker	Cycle (PCR)			Primer name	Sequence (5'-3')	Direction	Reference
COI	1x	5x	1x				
	94°C - 40 s	94°C - 40 s	72°C - 120 s	72°C - 7 min	polyLCO	GAYTATWTTCAACAAATCATAAAGAT ATTGG	Carr et al. (2011)
	40x	48°C - 40 s	72°C - 120 s		polyHCO	TAMACTTCWGGGTGACCAAAARAATC A	Carr et al. (2011)
16S	1x	40x	1x				
	94°C - 3 min	94°C - 30 s (- 0.2C per cycle)	72°C - 70 s	72°C - 7 min	16SarL	CGCCTGTTTATCAAAAACAT	Palumbi et al. (1991)
					16SbrH (R)	CCGGTCTGAACTCAGATCACGT	Palumbi et al. (1991)
28S	1x	40x	1x				
	94°C - 40 s	94°C - 40 s	72°C - 120 s	72°C - 7 min	28v (28gg)	AAGGTAGCCAAATGCCCTCGTCATC	Hillis and Dixon (1991)
					28x (28ii)	GTGAATTCTGCTTCACAATGATAGGA AGAGCC	Hillis and Dixon (1991)
	94°C - 120 s	52°C - 40 s	72°C - 120 s	72°C - 7 min	Nai_28S_long	CGAATTCTGCTTCGCAATGATAGGAA GAGC	This study

4. CHAPTER II. HOW COMPLEX IS THE *NAINERIS SETOSA* SPECIES COMPLEX? FIRST INTEGRATIVE STUDY OF A PRESUMED COSMOPOLITAN AND INVASIVE ANNELID (SEDENTARIA: ORBINIIDAE)

Quão complexo é o complexo de espécies Naineris setosa? Primeiro estudo integrativo de um presumido anelídeo cosmopolita e invasor (Sedentaria: Orbiniidae)

* Disclaimer: This chapter was formatted as with *Naineris setosa* complex belonging to *Naineris sensu lato* because it was submitted before Chapter 1.

Accepted by the journal Zootaxa
ISSN: 1175-5326
Impact Factor: 1.091
Qualis: A4

Ricardo Álvarez 1,*, Nataliya Budaeva 2
R.A.: ORCID® 0000-0002-3509-0187
N.B.: ORCID® 0000-0001-9748-2285

1 Graduate program in Oceanic Coastal Systems (PGSISCO), Federal University of Paraná, Pontal do Paraná, Paraná, Brazil.

2 Department of Natural History, University Museum of Bergen, University of Bergen, Allégaten 41, 5007 Bergen, Norway

* Corresponding author: ricastralvarez@gmail.com

Abstract

We performed a comparative study of the specimens from the *Naineris setosa* complex from the Pacific and the Atlantic Oceans and re-described the syntype of *N. setosa*, including the selection of the lectotype. Molecular phylogenetic and species delimitation analyses based on two mitochondrial (COI and 16S) and one nuclear (28S) marker revealed the presence of three species. One clade with wide Amphiatlantic distribution was attributed as *Naineris setosa* s. str. The second Atlantic clade restricted to Southern and South-eastern Brazil was described as a new species, *Naineris lanai* sp. n. The third clade, reported from the Northwestern Pacific, was identified as a new species but was not formally described due to the presence of only juvenile-sized worms in the studied material. Detailed morphological descriptions of several diagnostic characters in the *Naineris setosa* complex are provided.

Keywords: Integrative taxonomy, 28S rRNA gene, mtDNA COI, 16S, cosmopolitanism, new species.

4.1. INTRODUCTION

Naineris setosa (Verril, 1900) is a “cosmopolitan” orbiniid species originally described from intertidal shell-sand sediment in Bermuda. Due to the apparent lack of consistent morphological differences among its records, *N. setosa* was for decades assumed to have an Amphi-American distribution with records both from the East Pacific (Dean & Blake 2015; Blake 2017) and in the Atlantic (Treadwell 1901; Hartman 1951; 1957; Rioja 1960; Solis-Weiss & Fauchald 1989; Amaral *et al.* 2006; Blake & Giangrande 2011). It is currently known that truly widespread annelids are rare (Hutchings & Kupriyanova 2018). One of such cases has been confirmed within orbiniids for a small, presumably invasive species, *Proscoloplos cygnochaetus* Day, 1954, and its extremely wide distribution was explained by its capacity to anchor on vessels using mucous glands and chaetae possibly in combination with architomic reproduction (Meyer *et al.* 2008). Several species of *Naineris* Blainville, 1828 currently have cosmopolitan status. Nevertheless, this should be tested based on a combination of molecular data and comparative morphological studies.

Naineris setosa is unique in lacking uncini and subuluncini in thoracic neuropodia, which makes it similar to *Leitoscoloplos* Day, 1977 and *Scoloplella* Day, 1963, but it has a rounded prostomium as in all other species of *Naineris*. Hartman (1957) equivocally considered *N. setosa* related to *Leitoscoloplos* based on the presence of only crenulated capillaries in thoracic neuropodia. Several attempts were made to reconstruct the phylogeny of Orbiniidae and to assess the position of *Naineris* among other genera. Blake (2000) proposed a phylogeny based on the morphological characters and recovered *Naineris* as a paraphyletic genus. Bleidorn *et al.* (2005, 2009) confirmed the monophyly of *Naineris* + *Protoaricia* based on molecular data suggesting a progenetic origin for *Protoaricia* either within or as a sister to *Naineris*. However, their most advanced analysis based on six markers included only three species of the genus, the type species *Naineris quadricuspida* (Fabricius, 1780), *Naineris dendritica* (Kinberg, 1867), and *Naineris laevigata* (Grube, 1855). Later, Zhadan *et al.* (2015) proposed several phylogenetic reconstructions of Orbiniidae based on three individual markers. They included four species of *Naineris* and

recovered the genus as paraphyletic or monophyletic, depending on the marker analyzed. No combined analysis was presented in the study, and the monophyletic status of the genus could not be assessed based on the combined dataset. Nevertheless, none of the phylogenetic studies included *N. setosa* in the analyses, and its phylogenetic placement within Orbiniidae remains unclear.

Even though most of the confirmed invasive polychaetes belong to Serpulidae and Spionidae, some orbiniids, such as *Scoloplos capensis* (Day, 1961), *Leitoscoloplos kerguelensis* (McIntosh, 1885), and *Naineris quadraticeps* Day, 1965 were also considered as invasive (Çinar 2013). *Naineris setosa* was repeatedly reported as an invasive species in the Mediterranean region (Blake & Giangrande 2011; Khedhri *et al.* 2014; Dean & Blake 2015; Atzori *et al.* 2016); however, because none of the studies was supported by molecular data, the species should be considered as cryptogenic.

In the present study, we re-examine the type material of *N. setosa* herein designated as lectotype according to Article 74 (ICZN 1999) and perform morphological and molecular analyses of the specimens collected from various localities in the Western Atlantic, the Mediterranean, and the Northeast Pacific. Based on the results, we explore this species' genetic and morphological diversity and describe one new species from Southern and South-eastern Brazil.

4.2. MATERIAL AND METHODS

4.2.1 Sampling

Specimens of the *Naineris setosa* species complex were collected intertidally along the Brazilian coast from 2020 to 2022 from Santa Catarina to Pernambuco states in different estuarine beaches, from bays and embayments (Fig. 1). The geographical localities of the collected samples are listed in Table 1. To avoid the common

fragmentation of the specimens, they were sorted, anesthetized with 8% magnesium chloride in seawater, and preserved in either 4% formalin or 99.5% ethanol directly in the field. Formalin-fixed samples were subsequently transferred into 70% ethanol.

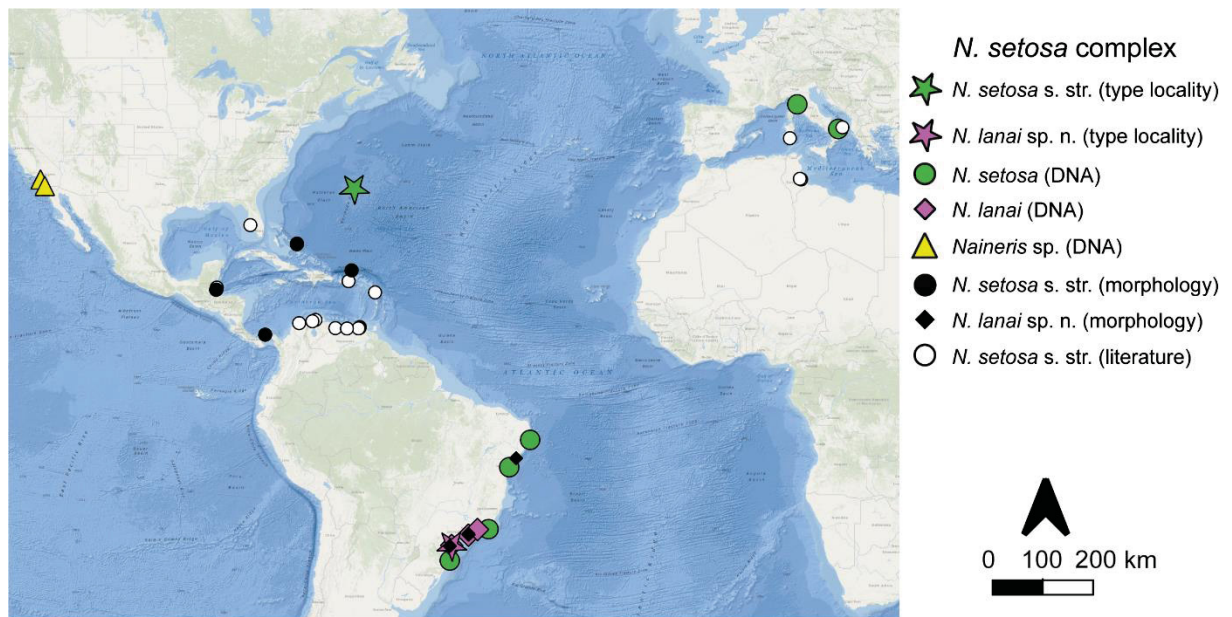


Figure 1. Distribution of the *Naineris* species described in this study.

4.2.2 Morphological study

The specimens were studied using a Leica MZ stereomicroscope and a Leica DM 6000 B compound microscope. Digital photos were made with a Leica M205C stereomicroscope with a digital camera Leica DMC5400 attached and combined with the Z-stack function using Leica LAS software. Methyl green and Shirlastain A dyes were used for increasing contrast and visualizing external morphological structures. Thoracic and abdominal parapodia were dissected and mounted on slides in glycerol to examine the chaetal morphology and composition. Scanning Electron Microscopy (SEM) images were prepared in three institutions following different protocols. At the SEM Laboratory of Smithsonian National Museum of Natural History, the museum specimens stored in 70% ethanol were run through an increasing ethanol series,

critical point dried in a Bal-tec CPD030 with ethanol as the transition fluid, mounted on an aluminum stub using double-sided tape, coated with a heavy metal, and examined using a Zeiss EVO MA15 scanning electron microscope. Brazilian material was prepared similarly and photographed with a Jeol JSM 6360-LV at the Center for Electron Microscopy, Federal University of Paraná. At the University Museum of Bergen, University of Bergen, hexamethyldisilazane (HMDS) was used for specimen dehydration, which was then coated with gold and examined under a Zeiss FE-SEM model GEMINI, Supra 55VP.

Terminology follows the main revisions of the family (Hartman 1957; Blake 2017, 2020, 2021). Additionally, the following morphological characters were described. Thoracic ventral groove, a continuous well-defined groove along the ventral side of the thoracic segments, is described for the first time for *Naineris*. Ventral groove is common in abdominal segments in orbinids, but its thoracic projection was not previously reported in the genus. Thoracic ventral longitudinal notches were discovered in specimens collected from muddy, possibly anoxic, sediments. They can almost reach consecutive annular rings or be less pronounced. Dorsal crest, following Blake (2017), is defined as ridges between abdominal branchiae. It may vary in shape from straight to folding and from inconspicuous to well-developed and can change in its shape along the body. Thoracic neuropodia can be of different shapes, varying from fleshy with rounded boundaries to thin and flattened with folding boundaries.

The segmental origin of branchiae and dorsal organs and the number of thoracic chaetigers were determined for 10 specimens from selected populations of the *N. setosa* complex from Brazil. Additionally, the following features were recorded at the chaetiger 50: total width disregarding parapodia (W), and total length of branchiae (Bq), dorsal crest (DC), abdominal notopodial (NoL), and neuropodial lobes (NeL).

The redescription of *N. setosa* is based on the syntype labeled as *Aricia setosa* Verrill, 1900, deposited in the annelids collection of the Yale Peabody Museum, USA (YPM). In the present study, this specimen is designated as the lectotype. We also studied comparative materials from the Natural History Museum of Los Angeles County, USA (LACM-AHF Poly); the Smithsonian National Museum of Natural History, USA (USNM); the American Museum of Natural History, USA (AMNH); the Florida

Museum, USA (FM); the reference collection of the Invasion laboratory at the Smithsonian Environmental Research Center, Maryland, USA (SERC); the Australian Museum, Australia (AM); Polychaeta Collection of the Museu Nacional, Universidade Federal do Rio de Janeiro, Brazil (MNRJP); Polychaeta Collection of the Museu de Diversidade Biológica, Instituto de Biologia, UNICAMP, Brazil (ZUEC-POL); Coleção Biológica Prof. Edmundo F. Nonato, Instituto Oceanográfico, Universidade de São Paulo, Brazil (ColBIO); the Invertebrate collection of the University Museum of Bergen, University of Bergen, Norway (ZMBN). The Brazilian specimens of *N. setosa* s. str. and type materials of *Naineris lanai* sp. n. are deposited in the MNRJ. Detailed information is provided in Table 1.

4.2.3 Molecular analyses

Thirty-five specimens from the *Naineris setosa* complex collected in the Eastern and Western Atlantic and the Northeastern Pacific were used for molecular analysis (Table 1). A single specimen of *Naineris aurantiaca* (Müller, 1858) from Pântano do Sul, Santa Catarina Island (Brazil), was used as an outgroup. Genomic DNA was extracted from ethanol-fixed tissue samples using 100 µL of QuickExtract™ solution, incubating in a thermocycler at 65 °C for 45 min, followed by 2 min at 90 °C. Fragments of a nuclear 28S rRNA and mitochondrial COI and 16S rRNA were amplified in a 25 µL reaction volume containing 2.5 µL 10x PCR buffer, 2 µL dNTP (2.5 mM), 1 µL forward and reverse primers (1 µM), 0.15 µL TaKaRa Taq (5 U/µL) with 1 µL template DNA, and double-distilled water. The primers and the PCR conditions are shown in Table 2. Obtained PCR products were run on 1% agarose gel for 30 min to visualize successful amplifications. The successful PCR products were sent to Macrogen Europe for purification and bidirectional Sanger sequencing (Applied Biosystems) using the same primers as in amplification. Contigs were automatically assembled from chromatograms for forward and reverse sequences and checked by eye in Geneious Prime (2023.1).

Table 1. List of specimens examined in the study with voucher museum numbers, localities, GenBank accession numbers for COI, 16S and 28S, Bold Sample and Process IDs.

<i>Naineris</i>	Voucher	Site	Coordinates	Collector	COI	16SrRNA	28SrRNA	BOLD Sample ID	BOLD Process ID
<i>N. setosa</i> s. str.	MNRJP 007629	Brazil, Santa Catarina	27.695°S; 48.565°W	R. Álvarez	OR732497	OR732526	OR732557	Alvarez_NS 01	NAI001-23
<i>N. setosa</i> s. str.	MNRJP 007630	Brazil, Santa Catarina	27.695°S; 48.565°W	R. Álvarez	OR732496	OR732525	OR732556	Alvarez_NS 02	NAI002-23
<i>N. setosa</i> s. str.	MNRJP 007631	Brazil, Santa Catarina	27.695°S; 48.565°W	R. Álvarez	OR732495	OR732524	OR732555	Alvarez_NS 03	NAI003-23
<i>N. setosa</i> s. str.	MNRJP 007632	Brazil, Santa Catarina	27.695°S; 48.565°W	R. Álvarez	OR732494	OR732523	–	Alvarez_NS 04	NAI004-23
<i>N. setosa</i> s. str.	MNRJP 007633	Brazil, Santa Catarina	27.695°S; 48.565°W	R. Álvarez	OR732493	OR732522	OR732554	Alvarez_NS 05	NAI005-23
<i>N. setosa</i> s. str.	MNRJP 007628	Brazil, Santa Catarina	27.695°S; 48.565°W	R. Álvarez	–	–	–	–	–
<i>N. setosa</i> s. str.	MNRJP 007639	Brazil, Rio de Janeiro	22.882°S; 42.002°W	R. Álvarez	OR732492	–	OR732553	Alvarez_NS 11	NAI006-23

<i>Naineris</i>	Voucher	Site	Coordinates	Collector	COI	16SrRNA	28SrRNA	BOLD Sample ID	BOLD Process ID
<i>N. setosa</i> s. str.	MNRJP 007640	Brazil, Rio de Janeiro	22.882°S; 42.002°W	R. Álvarez	OR732491	OR732521	OR732552	Alvarez_NS 12	NAI007-23
<i>N. setosa</i> s. str.	MNRJP 007641	Brazil, Rio de Janeiro	22.882°S; 42.002°W	R. Álvarez	OR732490	OR732520	OR732551	Alvarez_NS 13	NAI008-23
<i>N. setosa</i> s. str.	MNRJP 007642	Brazil, Rio de Janeiro	22.882°S; 42.002°W	R. Álvarez	OR732489	OR732519	OR732550	Alvarez_NS 14	NAI009-23
<i>N. setosa</i> s. str.	MNRJP 007643	Brazil, Rio de Janeiro	22.882°S; 42.002°W	R. Álvarez	OR732509	OR732537	OR732569	Alvarez_NS 15	NAI010-23
<i>N. setosa</i> s. str.	MNRJP 007626	Brazil, Rio de Janeiro	22.882°S; 42.002°W	R. Álvarez	–	–	–	–	–
<i>N. setosa</i> s. str.	MNRJP 007644	Brazil, Bahia	12.910°S; 38.497°W	R. Álvarez	OR732508	–	OR732568	Alvarez_NS 16	NAI011-23
<i>N. setosa</i> s. str.	MNRJP 007645	Brazil, Bahia	12.910°S; 38.497°W	R. Álvarez	OR732507	OR732536	–	Alvarez_NS 17	NAI012-23
<i>N. setosa</i> s. str.	MNRJP 007646	Brazil, Bahia	12.910°S; 38.497°W	R. Álvarez	OR732506	–	OR732567	Alvarez_NS 18	NAI013-23

<i>Naineris</i>	Voucher	Site	Coordinates	Collector	COI	16SrRNA	28SrRNA	BOLD Sample ID	BOLD Process ID
<i>N. setosa</i> s. str.	MNRJP 007647	Brazil, Bahia	12.910°S; 38.497°W	R. Álvarez	OR732505	OR732535	OR732566	Alvarez_NS 19	NAI014-23
<i>N. setosa</i> s. str.	MNRJP 007648	Brazil, Bahia	12.910°S; 38.497°W	R. Álvarez	OR732504	OR732534	OR732565	Alvarez_NS 20	NAI015-23
<i>N. setosa</i> s. str.	MNRJP 007627	Brazil, Bahia	12.910°S; 38.497°W	R. Álvarez	–	–	–	–	–
<i>N. setosa</i> s. str.	MNRJP 007649	Brazil, Pernambuco	8.355°S; 34.953°W	R. Álvarez	OR732503	OR732533	OR732564	Alvarez_NS 21	NAI016-23
<i>N. setosa</i> s. str.	MNRJP 007650	Brazil, Pernambuco	8.355°S; 34.953°W	R. Álvarez	OR732502	OR732532	OR732563	Alvarez_NS 22	NAI017-23
<i>N. setosa</i> s. str.	MNRJP 007651	Brazil, Pernambuco	8.355°S; 34.953°W	R. Álvarez	–	OR732531	OR732562	Alvarez_NS 23	NAI018-23
<i>N. setosa</i> s. str.	MNRJP 007652	Brazil, Pernambuco	8.355°S; 34.953°W	R. Álvarez	OR732501	OR732530	OR732561	Alvarez_NS 24	NAI019-23
<i>N. setosa</i> s. str.	MNRJP 007653	Brazil, Pernambuco	8.355°S; 34.953°W	R. Álvarez	OR732500	OR732529	OR732560	Alvarez_NS 25	NAI020-23

<i>Naineris</i>	Voucher	Site	Coordinates	Collector	COI	16SrRNA	28SrRNA	BOLD Sample ID	BOLD Process ID
<i>N. setosa</i> s. str.	MNRJP 007625	Brazil, Pernambuco	8.355°S; 34.953°W	R. Álvarez	—	—	—	—	—
<i>N. setosa</i> s. str.	ZMBN 157797	Italy, Taranto	40.444°N; 17.241°E	J. Langenck	OR732499	OR732528	OR732559	Alvarez_NS 43	NAI021-23
<i>N. setosa</i> s. str.	ZMBN 157798	Italy, Livorno	43.546°N; 10.302°E	J. Langenck	OR732498	OR732527	OR732558	Alvarez_NS 44	NAI022-23
<i>N. lanai</i> sp. n.	MNRJP 007634	Brazil, Paraná	25.408°S; 48.250°W	R. Álvarez	OR732481	—	OR732543	Alvarez_NS 06	NAI023-23
<i>N. lanai</i> sp. n.	MNRJP 007635	Brazil, Paraná	25.408°S; 48.250°W	R. Álvarez	OR732482	OR732515	OR732544	Alvarez_NS 07	NAI024-23
<i>N. lanai</i> sp. n.	MNRJP 007636	Brazil, Paraná	25.408°S; 48.250°W	R. Álvarez	OR732483	OR732516	OR732545	Alvarez_NS 08	NAI025-23
<i>N. lanai</i> sp. n.	MNRJP 007637	Brazil, Paraná	25.408°S; 48.250°W	R. Álvarez	OR732484	OR732517	OR732546	Alvarez_NS 09	NAI026-23
<i>N. lanai</i> sp. n.	MNRJP 007638	Brazil, Paraná	25.408°S; 48.250°W	R. Álvarez	OR732488	OR732518	OR732549	Alvarez_NS 10	NAI027-23

<i>Naineris</i>	Voucher	Site	Coordinates	Collector	COI	16SrRNA	28SrRNA	BOLD Sample ID	BOLD Process ID
<i>N. lanai</i> sp. n.	MNRJP 007618	Brazil, Paraná	25.408°S; 48.250°W	R. Álvarez	—	—	—	—	—
<i>N. lanai</i> sp. n.	MNRJP 007619	Brazil, Paraná	25.408°S; 48.250°W	R. Álvarez	—	—	—	—	—
<i>N. lanai</i> sp. n.	MNRJP 007620	Brazil, Paraná	25.408°S; 48.250°W	R. Álvarez	—	—	—	—	—
<i>N. lanai</i> sp. n.	MNRJP 007621	Brazil, Paraná	25.408°S; 48.250°W	R. Álvarez	—	—	—	—	—
<i>N. lanai</i> sp. n.	MNRJP 007622	Brazil, Paraná	25.408°S; 48.250°W	R. Álvarez	—	—	—	—	—
<i>N. lanai</i> sp. n.	MNRJP 007623	Brazil, Paraná	25.408°S; 48.250°W	R. Álvarez	—	—	—	—	—
<i>N. lanai</i> sp. n.	MNRJP 007624	Brazil, Paraná	25.408°S; 48.250°W	R. Álvarez	—	—	—	—	—
<i>N. lanai</i> sp. n.	MNRJP 007654	Brazil, São Paulo	23.815°S; 45.406°W	R. Álvarez	OR732487	—	OR732548	Alvarez_NS 28	NAI028-23

<i>Naineris</i>	Voucher	Site	Coordinates	Collector	COI	16SrRNA	28SrRNA	BOLD Sample ID	BOLD Process ID
<i>N. lanai</i> sp. n.	MNRJP 007655	Brazil, Rio de Janeiro	22.939°S; 43.877°W	J. Gabriel	OR732486	–	OR732547	Alvarez_NS 36	NAI029-23
<i>N. lanai</i> sp. n.	MNRJP 007656	Brazil, Rio de Janeiro	22.939°S; 43.877°W	J. Gabriel	OR732485	–		Alvarez_NS 38	NAI030-23
<i>Naineris</i> sp.	SERC 252007	USA, California	33.620°N; 117.896°W	E. Keppel	OR732477	OR732511	OR732539	Alvarez_NS 46	NAI031-23
<i>Naineris</i> sp.	SERC 252013	USA, California	33.619°N; 117.895°W	E. Keppel	OR732478	OR732512	OR732540	Alvarez_NS 47	NAI032-23
<i>Naineris</i> sp.	SERC 251805	USA, California	–	E. Keppel	–	–	–	–	–
<i>Naineris</i> sp.	SERC 251985	USA, California	–	E. Keppel	–	–	–	–	–
<i>Naineris</i> sp.	SERC 252000	USA, California	–	E. Keppel	–	–	–	–	–

<i>Naineris</i>	Voucher	Site	Coordinates	Collector	COI	16SrRNA	28SrRNA	BOLD Sample ID	BOLD Process ID
<i>Naineris</i> sp.	LACM-AHF Poly	USA, California	32.726°N; 117.192°W	K. Sorensen , C. Sosa	OR732479	OR732513	OR732541	Alvarez_NS 33	NAI033-23
<i>Naineris</i> sp.	LACM-AHF Poly	USA, California	32.726°N; 117.192°W	K. Sorensen , C. Sosa	OR732480	OR732514	OR732542	Alvarez_NS 34	NAI034-23
<i>Naineris</i> sp.	LACM-AHF Poly	USA, California	32.726°N; 117.192°W	K. Sorensen , C. Sosa	—	OR732510	OR732538	Alvarez_NS 35	NAI035-23
<i>N. aurantiaca</i>	MNRJP 007617	Brazil, Santa Catarina	27.783°S; 48.506°W	R. Álvarez	OR795632	OR795716	OR795717	Alvarez_NA 01	NAI036-23
<i>N. setosa</i> s. str.	YPM IZ 001242. AN	Bermuda, Flatt's Inlet beach	32.322°N; 64.739°W	—	—	—	—	—	—
<i>N. setosa</i> s. str.	YPM 1303	Bermuda	—	W. R. Coe	—	—	—	—	—
<i>N. setosa</i> s. str.	YPM 1384	Bermuda	—	A. E. Verril	—	—	—	—	—
<i>N. setosa</i> s. str.	AMNH 1972	Bermuda	—	—	—	—	—	—	—

<i>Naineris</i>	Voucher	Site	Coordinates	Collector	COI	16SrRNA	28SrRNA	BOLD Sample ID	BOLD Process ID
<i>N. setosa</i> s. str.	AMNH 2508A	Bermuda, St. David's Island	—	—	—	—	—	—	—
<i>N. setosa</i> s. str.	USNM 34092	Bermuda	—	—	—	—	—	—	—
<i>N. setosa</i> s. str.	USNM 181626	Panama, Colon	9.393°N; 79.838°W	STRI	—	—	—	—	—
<i>N. setosa</i> s. str.	USNM 181660	Panamá, Colon,	9.395°N; 79.849°W	STRI	—	—	—	—	—
<i>N. setosa</i> s. str.	USNM 174087	Bahamas, San Salvador Island	24.110°N; 74.461°W	R. Zottoli, C. Long	—	—	—	—	—
<i>N. setosa</i> s. str.	USNM 55607	P. Rico, Guanajibo	—	V. Vicente	—	—	—	—	—
<i>N. setosa</i> s. str.	FM 5895	Bahamas, San Salvador Island	24.109°N; 74.462°W	G. Paulay	—	—	—	—	—

<i>Naineris</i>	Voucher	Site	Coordinates	Collector	COI	16SrRNA	28SrRNA	BOLD Sample ID	BOLD Process ID
<i>N. setosa</i> s. str.	FM 5898	Bahamas, San Salvador Island	24.004°N; 74.476°W	G. Paulay	—	—	—	—	—
<i>N. setosa</i> s. str.	FM 5904	Bahamas, San Salvador Island	24.114°N; 74.462°W	C. Martin	—	—	—	—	—
<i>N. setosa</i> s. str.	FM 5902	Bahamas, San Salvador Island	24.110°N; 74.443°W	C. Martin	—	—	—	—	—
<i>N. setosa</i> s. str.	ColBIO IG 166	—	—	E. F. Nonato	—	—	—	—	—
<i>N. setosa</i> s. str.	ColBIO 168	—	—	E. F. Nonato	—	—	—	—	—
<i>N. setosa</i> s. str.	ColBIO NS20	—	—	E. F. Nonato	—	—	—	—	—
<i>N. cf. setosa</i>	AM W.22470	Australia, Sydney	33.873°S; 151.175°E	L. Smith	—	—	—	—	—

<i>Naineris</i>	Voucher	Site	Coordinates	Collector	COI	16SrRNA	28SrRNA	BOLD Sample ID	BOLD Process ID
<i>N. lanai</i> sp. n.	ZUEC-POL 3784	Brazil, São Paulo	23.623°S; 45.405°W	C. Amaral	—	—	—	—	—
<i>N. lanai</i> sp. n.	ZUEC-POL 3780	Brazil, São Paulo	23.623°S; 45.405°W	C. Amaral	—	—	—	—	—
<i>N. lanai</i> sp. n.	ZUEC-POL 3786	Brazil, São Paulo	23.623°S; 45.405°W	C. Amaral	—	—	—	—	—
<i>N. lanai</i> sp. n.	ZUEC-POL 16999	Brazil, São Paulo	23.747°S; 45.348°W	V. Radashevsky	—	—	—	—	—
<i>N. lanai</i> sp. n.	ZUEC-POL 3771	Brazil, São Paulo	23.627°S; 45.396°W	C. Amaral	—	—	—	—	—
<i>N. lanai</i> sp. n.	ZUEC-POL 2756	Brazil, São Paulo	23.748°S; 45.409°W	C. Amaral	—	—	—	—	—
<i>N. lanai</i> sp. n.	MNRJP 1909	Brazil, Sergipe	11.427°S; 37.335°W	J. Zanol	—	—	—	—	—

Each of the three markers was aligned individually using MAFFT with the automatically chosen L-INSI-i option (Kato *et al.* 2005). Three markers were concatenated in MegaX (Kumar *et al.* 2018). The phylogenetic analysis was done for individual markers and for the combined partitioned dataset (COI+16S+28S) using Maximum likelihood (ML) implemented in IQ-Tree 2.2.0. (Minh *et al.* 2020) with ultrafast bootstrap (Hoang *et al.* 2018). The substitution models were automatically selected (COI: 1st codon, TNe; 2nd codon, F81+F; 3rd codon, TPM2u+F; 16S: TIM2+F; 28S: TNe+I) (Kalyaanamoorthy *et al.* 2017), and the statistical support of the branches was estimated with 10000 bootstrap replicates. Mean uncorrected pairwise genetic distances (p-distances) for each of the main clades and between the main clades were calculated in MEGA11 (Tamura *et al.* 2021). Haplotype networks of each gene comprising specimens of the *Naineris setosa* complex were built using TCS (Clement *et al.* 2002) in PopART (Leigh & Bryant 2015). The single-marker trees generated in ML analyses were used as the input trees in the species delimitation analyses with Bayesian implementation of the Poisson Tree Processes (bPTP) model (Zhang *et al.* 2013). All analyses were run on the bPTP web server (<http://species.h-its.org/>) with default settings and pruned outgroups. Final species delimitation hypotheses were formulated based on the combined evidence from morphology and three independent bPTP analyses of individual molecular markers.

4.3. RESULTS

4.3.1 Phylogenetic reconstruction

We included 28 specimens of *Naineris* from the coast of Brazil, two specimens from the Mediterranean Sea, and five specimens from the Northeast Pacific. The independent COI, 16S, and 28S alignments were represented by 654, 512, and 626 bases respectively. The concatenated dataset consisted of 1792 bases. The results from the ML analysis are shown in Figure 2.

Table 2. Primers and PCR protocols used in this study.

Marker	Cycle (PCR)				Primer name	Sequence (5'–3')	Direction	Reference
	1x	5x	1x					
COI	94°C - 40 s	94°C - 40 s	45°C - 40 s	72°C - 7 min	polyLCO	GAYTATWTTCAACAAATCATAAAGATATTGG	Forward	Carr <i>et al.</i> (2011)
		40x		120 s				
	1x	40x	94°C - 40 s	48°C - 40 s	polyHCO	TAMACTTCWGGGTGACCCAAARAATCA	Reverse	
16S	94°C - 3 min	94°C - 30 s	51°C - 30 s (-0.2C per cycle)	70°C - 7 min	16SarL	CGCCTGTTTATCAAAAACAT	Forward	Palumbi <i>et al.</i> (1991)
	1x	40x			16SbrH (R)	CCGGTCTGAACTCAGATCACGT	Reverse	Palumbi <i>et al.</i> (1991)
				1x				
16S	94°C - 3 min	94°C - 30 s	52°C - 30 s	72°C - 7 min	AnnF	GCGGTATCCTGACCGTRCWAAGGTA	Forward	Sjölin <i>et al.</i> (2005)
				s	AnnR	TCCTAAGCCCAACATCGAGGTGCCAA	Reverse	Sjölin <i>et al.</i> (2005)
	1x	40x		1x				
28S	94°C - 40 s	94°C - 40 s	54°C - 40 s	72°C - 7 min	28v (28gg)	AAGGTAGCCAAATGCCTCGTCATC	Forward	Hillis & Dixon (1991)
				120 s	28x (28ii)	GTGAATTCTGCTTCACAATGATAGGAAGAGC	Reverse	Hillis % Dixon (1991)

The combined COI, 16S, and 28S analysis recovered three highly supported clades (BP = 98–100). Phylogenies inferred from the individual markers showed different topologies (Figure 3). A well-supported clade of the Northeastern Pacific specimens was recovered in all analyses and was highly supported (BP = 99, 94, 69; for COI, 16S, and 28S, respectively). In the 16S analysis, only two clades were recovered: all Atlantic specimens formed one clade (BP=100), while the Pacific specimens formed the second clade (BP=94). In the 28S phylogeny, part of the west Atlantic specimens and the specimens from the Mediterranean formed a well-supported clade (BP=88) sister to the Pacific clade (BP=69); however, several specimens from South and Southeastern Brazil were not recovered as a monophyletic group.

Species delimitation analysis resulted in a highly supported delimitation scheme of three putative species based on COI and two putative species based on 16S. The delimitation results of the 28S dataset were generally poorly supported, with the Pacific and one of the Atlantic clades delimited as two putative species and the second Atlantic clade split into six putative species (Fig. 2). Despite several conflicts in delimitation results from three molecular markers, the final species delimitation hypothesis also included information on the morphology and suggested the presence of three species in the dataset: *Naineris setosa* s. str. from the Western Atlantic and the Mediterranean, *Naineris lanai* sp. n. from the South and Southeastern coast of Brazil, and *Naineris* sp. from the Northeastern Pacific.

The TCS haplotype networks (Fig. 4) supported the presence of three species in the COI analysis, with *N. setosa* s. str. having the most frequent haplotype shared among different regions in the Atlantic, including the Mediterranean and the Brazilian coast. *Naineris lanai* sp. n. showed a high diversity of unique haplotypes found in one region – the South and Southeastern Brazilian coast. Two haplotypes of *Naineris* sp. were limited to the Northeastern Pacific locality. The analysis of 16S recovered only two haplotypes in the whole dataset; one shared among all the Atlantic specimens, including both *N. setosa* s. str. and *N. lanai* sp. n., and the second haplotype reported in the Pacific. In the 28S analysis, all *N. setosa* s. str. shared a single haplotype

reported in the Eastern and the Western Atlantic. The unidentified species from the Northeastern Pacific and *N. lanai* sp. n. showed the presence of two haplotypes in 28S.

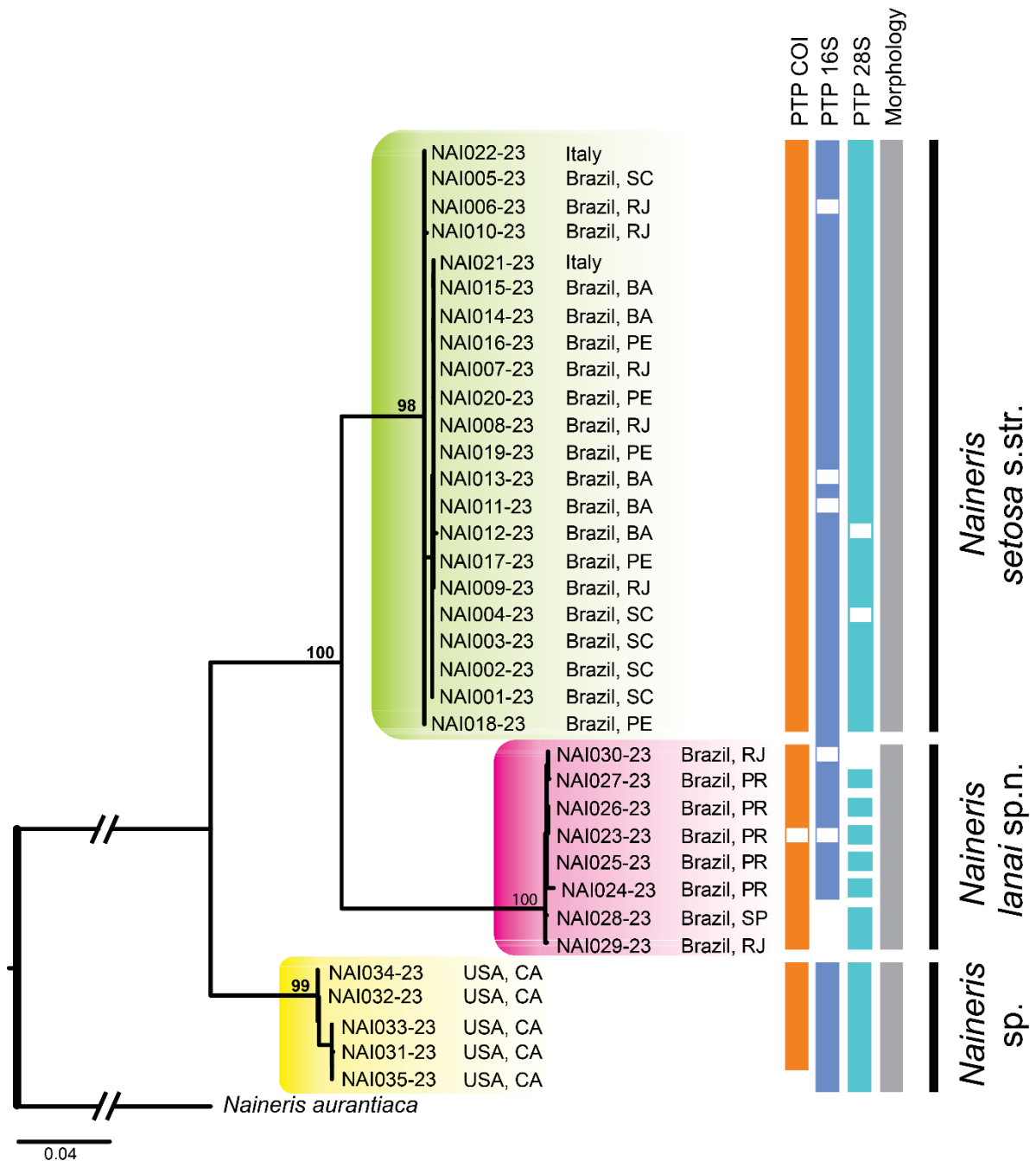
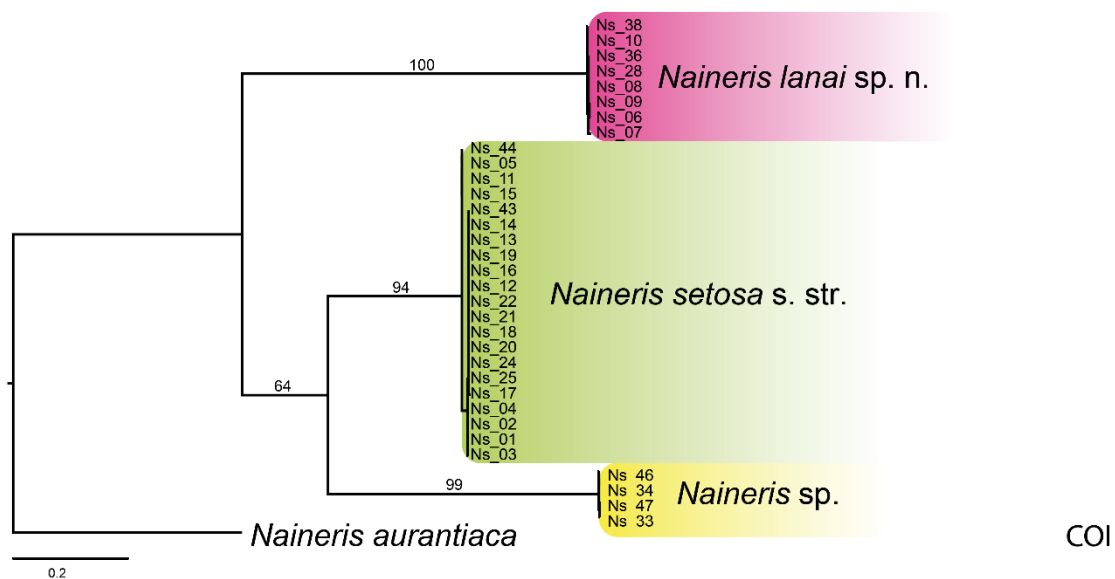
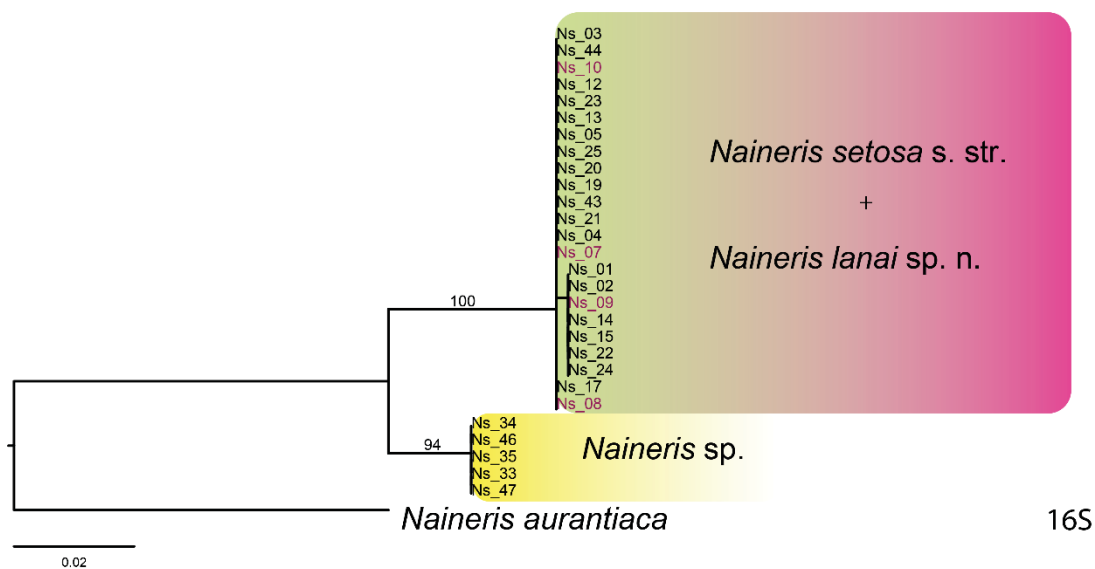


Figure 2. Maximum Likelihood tree of the combined COI, 16S, and 28S dataset; numbers on nodes indicate Bootstrap support values; species delimitation results inferred by morphology and DNA-based methods are indicated right to the consensus tree; bPTP was applied separately to the individual gene trees. White bars indicate missing data.

A



B



C

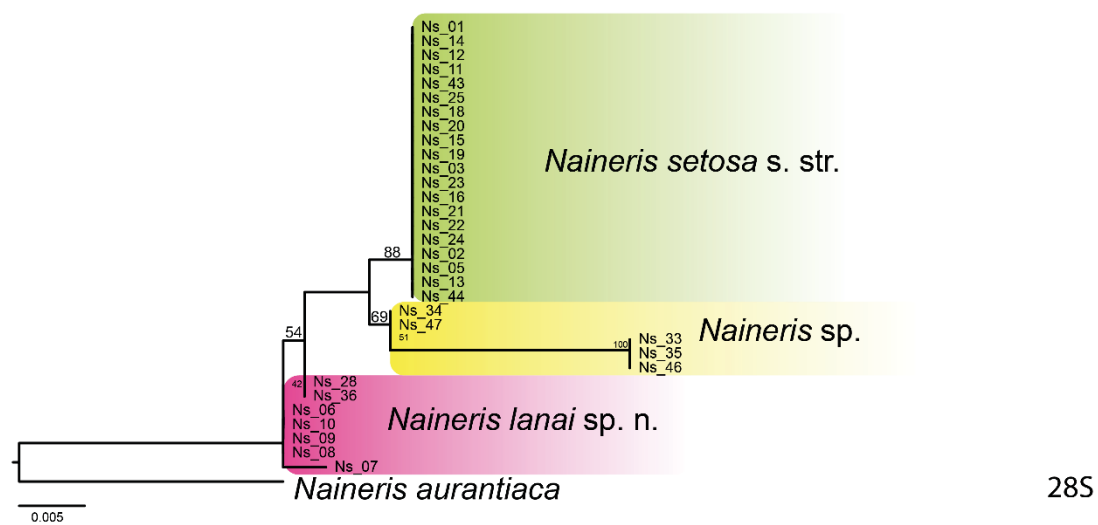


Figure 3. Maximum Likelihood trees of single COI, 16S, and 28S markers; numbers on nodes indicate Bootstrap support values.

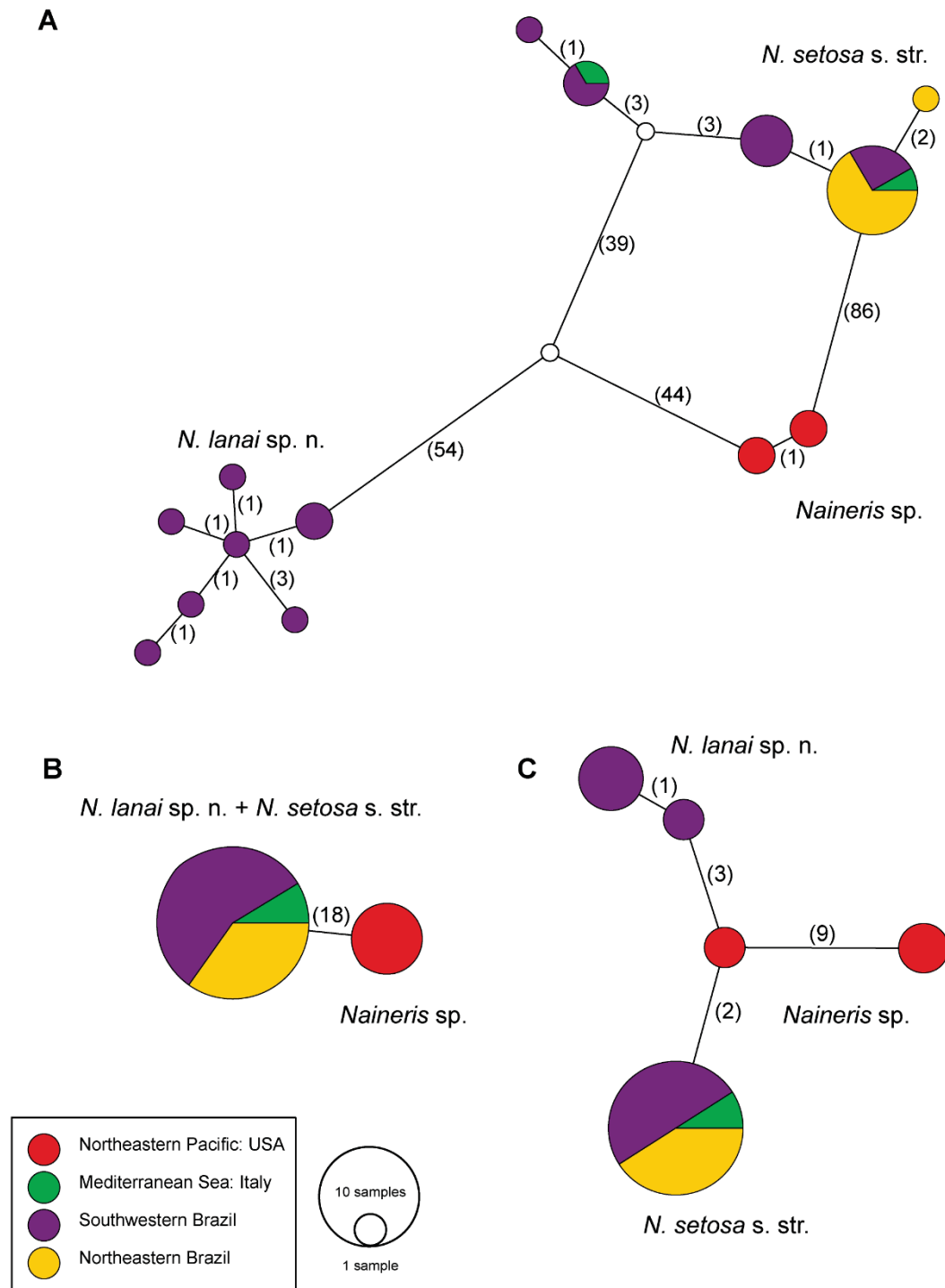


Figure 4. Haplotype networks of the *Naineris setosa* species complex based on COI (A), 16S (B), and 28S (C). Colors correspond to geographic areas. Circle sizes correspond to sample size. Numbers in parentheses show the number of substitutions between the haplotypes.

4.3.2 Genetic distances

The genetic distances are summarized in Tables 3–6. The estimated genetic divergences between the three clades in COI ranged from 13.7 to 16%, in 16S from 0 to 6.1%, and in 28S from 1.1 to 1.8%. The intraspecific genetic divergences in COI were 0.1–0.4% and in 28S 0–1.1%. There was no intraspecific divergence in 16S.

Table 3. Percentage of uncorrected p-distance matrix within clades of the *Naineris setosa* species complex based on COI, 16S, and 28S DNA sequences.

<i>Naineris</i>	COI	16S	28S
<i>Naineris</i> sp.	0.001	0,000	0,011
<i>N. setosa</i>	0.004	0,000	0,000
<i>N. lanai</i> sp. nov.	0.004	0,000	0.001

Table 4. Percentage of uncorrected p-distance matrix between clades of the *Naineris setosa* species complex based on COI sequences.

<i>Naineris</i>	<i>Naineris</i> sp.	<i>N. setosa</i> s. str.	<i>N. lanai</i> sp. nov.
<i>Naineris</i> sp.	-		
<i>N. setosa</i> s. str.	0.137	-	
<i>N. lanai</i> sp. nov.	0.160	0.155	-

Table 5. Percentage of uncorrected p-distance matrix between clades of the *Naineris setosa* species complex based on 16S sequences.

<i>Naineris</i>	<i>Naineris</i> sp.	<i>N. setosa</i> s. str.	<i>N. lanai</i> sp. nov.
<i>Naineris</i> sp.	-		
<i>N. setosa</i> s. str.	0.061	-	
<i>N. lanai</i> sp. nov.	0.061	0	-

Table 6. Percentage of uncorrected p-distance matrix between clades of the *Naineris setosa* species complex based on 28S sequences.

<i>Naineris</i>	<i>Naineris</i> sp.	<i>N. setosa</i> s. str.	<i>N. lanai</i> sp. nov.
<i>Naineris</i> sp.	-		
<i>N. setosa</i> s. str.	0.015	-	
<i>N. lanai</i> sp. nov.	0.018	0.011	-

4.3.3 Taxonomic account

Family ORBINIIDAE Hartman, 1942

Genus *Naineris* Blainville, 1828

Type species. *Naineris quadricuspida* (Fabricius, 1780)

Diagnosis (amended after Blake 2020; Zhadan 2020). Prostomium rounded, truncated, or weakly bifid on anterior margin. Peristomium with one or two achaetous rings. Thorax with 12–30 or more segments; branchiae first present from chaetigers 2–23. Thoracic neuropodia with 0–2 postchaetal lobes; no subpodial lobes. Thoracic neurochaetae include capillaries, or capillaries mixed with blunt-tipped uncini, sometimes hooded, or uncini and subuluncini. Abdominal chaetae include capillaries, sometimes furcate chaetae in notopodia, and capillaries and embedded or protruding aciculae in neuropodia. Dorsal sensory organs present in some species, being paired or multiple, rounded or as elongated semicircles. Dorsal cilia within branchiae bases present, either forming flat bands or crests.

***Naineris setosa* (Verril, 1900) s. str.**

(Figs 5–8)

Aricia setosa Verrill, 1900: 651–653.

Anthostoma latacapitata Treadwell, 1901: 203–204, figs 61–65.

Nainereis latacapitata Treadwell 1939: 264, fig. 81.

Naineris setosa: Treadwell 1936: 55; Hartman 1957: 305: pl. 41, figs 1–6 (in part); Nonato 1981: 149–150, figs 177–179; Solis-Weiss & Fauchald 1989: 774–778, figs 2a–j; Blake & Giangrande 2011: 20–26, figs 1–2; Khedhri *et al.* 2014: 83–88, fig. 2; Atzori *et al.* 2016: 2016: 1–6.

Material examined. Atlantic Ocean. Bermuda: YPM 001242.AN (lectotype), YPM 1303 (1 spm), YPM 1384 (1 spm), USNM 34092 (1 spm), AMNH 1972 (4 spms), AMNH 2508A (2 spms). **Panama, Caribbean Sea:** USNM 181626 (1 spm), USNM 181660 (1 spm). **Puerto Rico:** USNM 55607 (19 spms). **Bahamas. San Salvador Island, Oyster Pond:** FM 5895 (1 spm), USNM 174087 (10 spms). **Pigeon Creek Mangrove,** FM 5898 (1 spm). **Moon Rock Pond,** FM 5904 (1 spm). **Crescent Pond,** FM 5902 (2 spms). **Brazil. Pernambuco, Cabo de Santo Agostino:** MNRJP 007649 (DNA voucher Ns21), MNRJP 007650 (DNA voucher Ns22), MNRJP 007651 (DNA voucher Ns23), MNRJP 007652 (DNA voucher Ns24), MNRJP 007653 (DNA voucher Ns25), MNRJP 007625 (5 spms). **Rio de Janeiro. Cabo Frio:** MNRJP 007639 (DNA voucher Ns11), MNRJP 007640 (DNA voucher Ns12), MNRJP 007641 (DNA voucher Ns13), MNRJP 007642 (DNA voucher Ns14), MNRJP 007643 (DNA voucher Ns15), MNRJP 007626 (13 spms). **Ilha Grande:** ColBIO IG 166 (1 spm), ColBIO 168 (2 spms), ColBIO NS20 (7 spms). **Santa Catarina, Santa Catarina Island:** MNRJP 007629 (DNA voucher Ns1), MNRJP 007630 (DNA voucher Ns2), MNRJP 007631 (DNA voucher Ns3), MNRJP 007632 (DNA voucher Ns4), MNRJP 007633 (DNA voucher Ns5), MNRJP 007628 (4 spms). **Bahia, Salvador:** MNRJP 007644 (DNA voucher Ns16), MNRJP 007645 (DNA voucher Ns17), MNRJP 007646 (DNA voucher Ns18), MNRJP 007647 (DNA voucher Ns19), MNRJP 007648 (DNA voucher Ns20), MNRJP 007627 (8 spms). **Italy. Taranto:** ZMBN 157797 (DNA voucher Ns43). Livorno: ZMBN 157798 (DNA voucher Ns44). **Australia. Rozelle Bay, Sydney:** AM W.22470 (4 spms).

Type locality. Flatt's Inlet Beach, Bermuda. Shell-sand, low intertidal.

Diagnosis. Thoracic neurochaetae only crenulated capillaries, thoracic segments without ventral groove and notches, paired dorsal sensory organs present, low dorsal crest from abdominal segments, thoracic neuropodial lobe with upper papilla, as wide flanges with well-delimited boundaries.

Description (based on the lectotype). Lectotype (YPM IZ 001242.AN) incomplete, 149 chaetigers, 57 mm long, 0.7 mm wide at chaetiger 50. Color in alcohol light tan; (live Brazilian specimens pale yellow with reddish branchiae, bearing long fluorescent cilia along branchial axis and between them). Thorax and abdomen marked by dorsal

displacement of parapodia in abdomen (Figs 5A; 7A). Ventral surface smooth, ventral groove only present in abdominal segments (Fig. 8A, B), ventral notches absent.

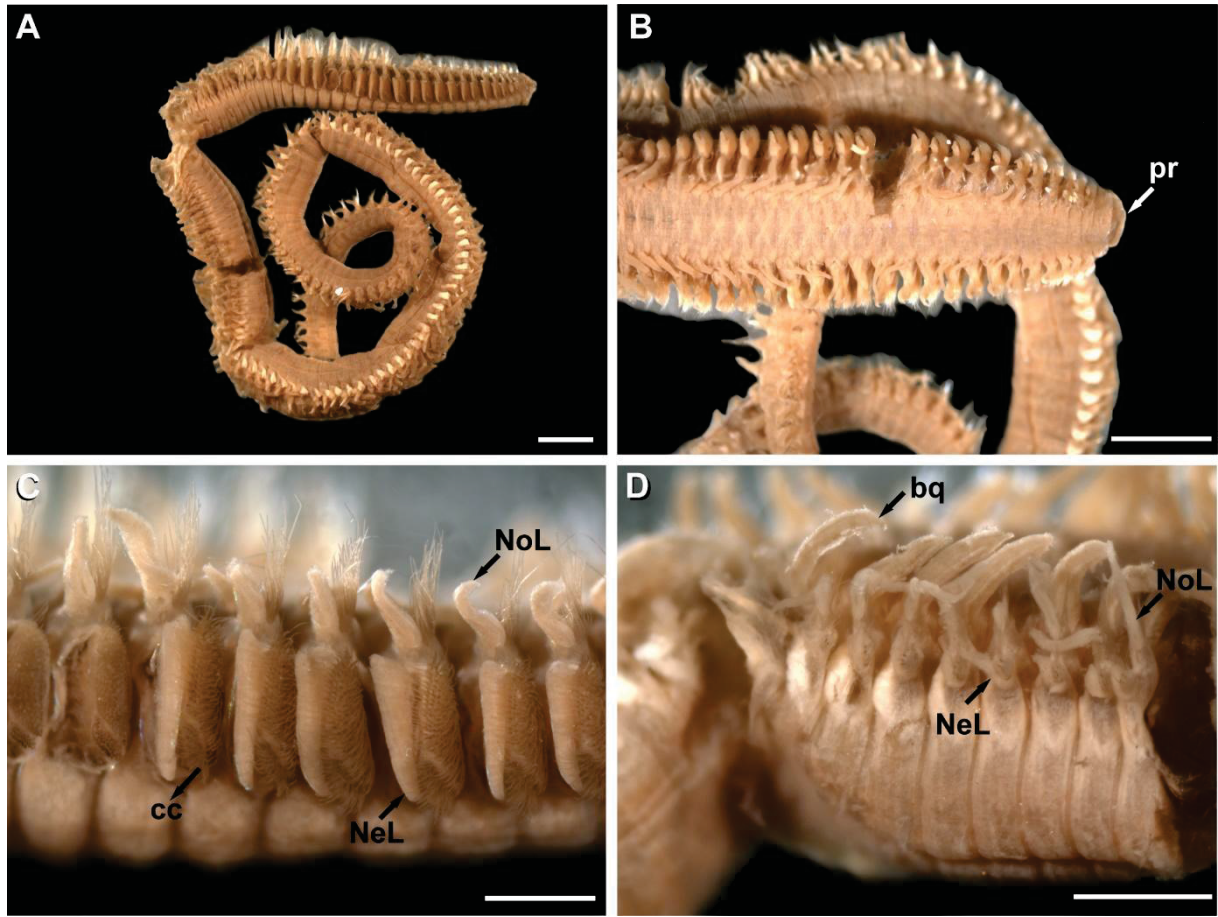


Figure 5. *Naineris setosa*, lectotype (YPM IZ 001242.AN): (A) Lateral view; (B) Anterior end, dorsal view; (C) Thoracic parapodia; (D) Abdominal parapodia. bq – branchia, cc – crenulate capillaries, NeL – neuropodial lobe, NoL – notopodial lobe, pr – prostomium. Scale bars; (A–B): 0.7 mm; (C–D): 0.35 mm.

Prostomium wider than long, more or less square and rounded in front and corners (Figs 5B; 7A); eyespots along lateral margins; nuchal organs discernible (Fig. 7A, B). Peristomium with two annular rings, only weakly marked; mouth opening situated ventrally and surrounded by striated lips; proboscis, not everted, presumably broad and lobed as in topotype specimens.

Branchiae first present from chaetiger 5–6, cirriform, tapering to sharp tip, with dense cilia, first branchiae long, reaching 1/3 of abdominal branchiae (Fig. 5B). Conspicuous dorsal sensory organs from chaetiger 7, oval-shaped and paired, with

dense cilia (Fig. 7C). Dorsal crest present from abdominal segments; straight, supported by thick base; low, approximately $\frac{1}{4}$ diameter of branchiae, ciliated.

Thorax comprises 20 chaetigers, moderately flattened (Fig. 5A). Parapodia biramous; interrampal papilla absent. Notopodial lobes lanceolate. Neuropodial lobes represented by wide flanges ending above digitate papillae, with well-delimited boundaries (Figs 5A, C; 6A). Abdominal notopodial and neuropodial lobes cirriform (Figs 5D; 6B); abdominal notopodial lobes longer or equal to thoracic notopodial lobes, shorter than branchiae (Figs 5D; 6B).

Thoracic notochaetae with two bundles of about 20–30 crenulated capillaries at different levels (Figs 5C; 6A). Abdominal notochaetae arranged in two groups of 10–15 crenulated capillaries and furcate chaetae (Figs 6B; 7F); furcate chaetae with unequal tines and tiny needles at different levels (Fig. 7F), placed in a lower position, difficult to observe; shaft bearing numerous crowded transversal rows of about 10–12 small barbs (Fig. 7F). Thoracic neurochaetae with around seven rows of numerous capillaries (Figs 5C; 6A, E) separated into two groups by oblique gap (Fig. 5C). Abdominal neurochaetae with 5–10 capillaries and three acicular spines (Figs 6B; 7D). Pygidium lacking.

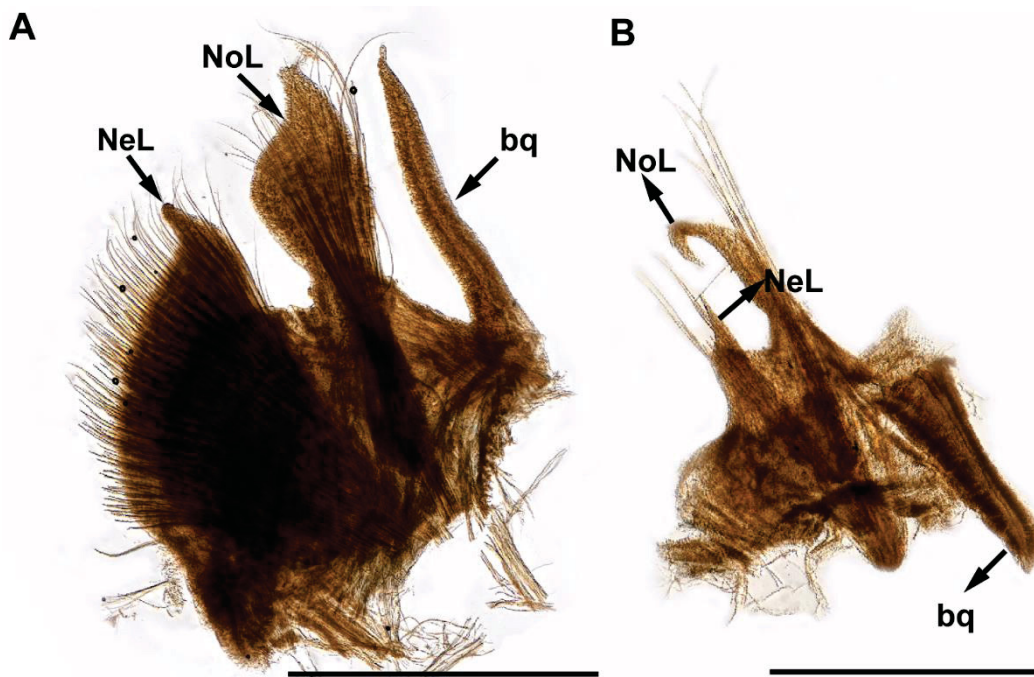


Figure 6. *Naineris setosa*, lectotype (YPM IZ 001242.AN): (A) Thoracic parapodium (ch. 11); (B) Abdominal parapodium (ch. 91). br – branchia (displaced), NeL – neuropodial lobe, NoL – notopodial lobe. Scale bars; (A–B): 1 mm.

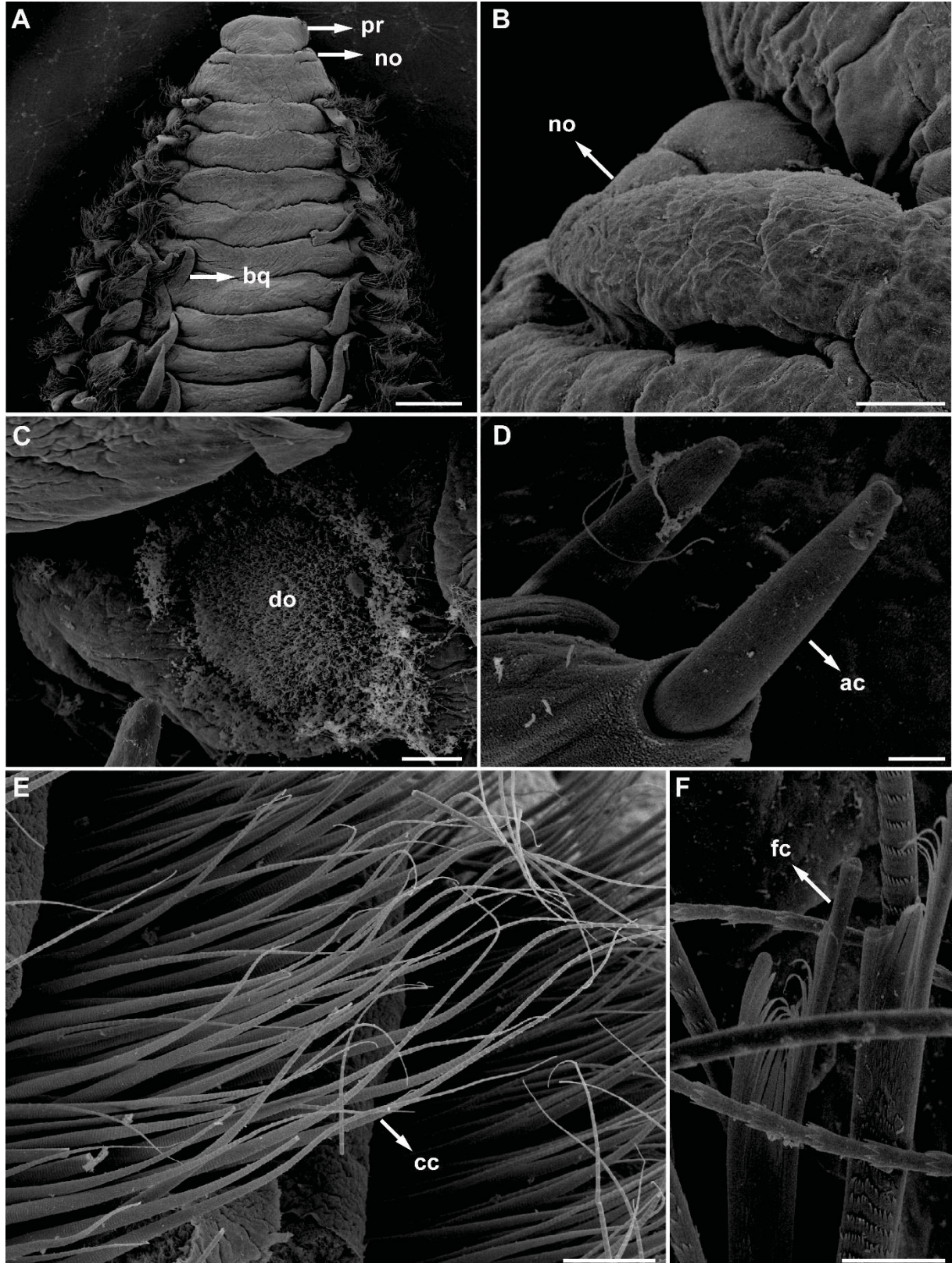


Figure 7. *Naineris setosa* from Pernambuco, Brazil (MNRJP *****) examined under the SEM: (A) Anterior end, dorsal view; (B) Nuchal organ; (C) Dorsal organ; (D) Acicular spines; (F) Furcate chaetae. *Naineris setosa* from Bahamas under SEM (FM *****): (E) Thoracic neurochaetae. ac – acicular spines, bq – branchia, cc – crenulate capillaries, do – dorsal organ, fc – furcate chaeta, no – nuchal organ, pr – prostomium. Scale bars; (A): 500 µm; (B): 50 µm; (C): 20 µm; (D): 5 µm; (E–F): 10 µm.

Remarks. Hartman (1951) examined specimens from the Gulf of Mexico and synonymized *Anthostoma lacapitata* Treadwell, 1901 with *Naineris setosa*. Solís-Weiss & Fauchald (1989) redescribed the syntype of *N. setosa* retaining its wide distribution in the tropical Northwest Atlantic, herein designated as the lectotype (ICZN 1999, Art. 74). Subsequently, Blake & Giangrande (2011) reported the species as invasive in the Mediterranean Sea. The species was also reported from the Adriatic and the Tyrrhenian Seas expanding its distribution to Tunisia and Taranto (Arduini *et al.* 2022, Rebai *et al.* 2022). The records from the Pacific, such as those from Galapagos and Australia, require further revision. We examined the specimen from Galapagos studied by Blake, but they were in poor condition preventing accurate identification.

Pygidium was not observed in the lectotype, but in the examined Brazilian specimens it agreed with Solís-Weiss & Fauchald's (1989) description, stating that *N. setosa* bears four anal cirri. Morphological variation of Brazilian populations of *N. setosa* s. str. is shown in Table 7.

Habitat. Shell-sand, intertidal.

Distribution. Intertidal and shallow subtidal (down to 1 m deep) zone of the Atlantic. Western Atlantic: Bermuda, Bahamas, Gulf of Mexico, Caribbean Sea, Panamá, Belize, Puerto Rico, and Brazilian coast. Presumably invasive in the Mediterranean Sea (Blake & Giangrande 2011; Khedhri *et al.* 2014; Dean & Blake 2015; Atzori *et al.* 2016).

Table 7. Measurements and morphological variation in different populations of *Naineris setosa* s. str. and *Naineris lanai* sp. n. from its type locality (Means+/-standard deviation (minimum-maximum)). Th. ch. – thoracic chaetigers, Width – with at chaetiger 50.

Species	Locality	Th. ch.	Width (mm)	Bq. start	Bq. length (mm)	DO start	DC (mm)	length (mm)	NoL (mm)	length (mm)	NeL length (mm)
<i>N. setosa</i> s. str.	Pernambuco	22.2+/-1.03 (21–24)	1.78+/-0.3 (1.4–2.2)	6+/-0 (6)	1.0+/-0.16 (0.7–1.2)	8.7+/-0.82 (8–10)	0.16+/-0.04 (0.1–0.2)	0.76+/-0.13 (0.6–1.0)	0.29+/-0.07 (0.2–0.4)		
<i>N. setosa</i> s. str.	Bahia	22.4+/-0.96 (21–24)	2.33+/-0.45 (2.0–3.4)	6+/-0 (6)	1.36+/-0.16 (1.1–1.5)	8.7+/-0.82 (8–10)	0.15+/-0.03 (0.1–0.2)	0.83+/-0.13 (0.7–1.1)	0.41+/-0.07 (0.3–0.5)		
<i>N. setosa</i> s. str.	Cabo Frio	23.5+/-1.35 (21–25)	2.25+/-0.29 (1.6–2.6)	6+/-0 (6)	1.28+/-0.3 (0.8–1.6)	8.9+/-1.1 (7–11)	0.15+/-0.07 (0.05–0.25)	0.78+/-0.15 (0.6–1.1)	0.38+/-0.08 (0.3–0.5)		
<i>N. setosa</i> s. str.	Santa Catarina	20.1+/-1.45 (18–23)	1.26+/-0.33 (0.7–1.8)	6+/-0 (6)	0.9+/-0.12 (0.7–1.1)	8.1+/-1.28 (7–10)	0.11+/-0.02 (0.1–0.15)	0.68+/-0.17 (0.5–1.1)	0.27+/-0.06 (0.2–0.35)		
<i>N. lanai</i> sp. n.	Paraná	29.4+/-1.07 (27–30)	3.79+/-0.69 (3.0–5.1)	6+/-0 (6)	2.35+/-0.24 (2.1–2.75)	9.2+/-0.91 (8.8–11)	0.45+/-0.05 (0.4–0.55)	1.42+/-0.28 (0.8–1.8)	0.55+/-0.13 (0.3–0.7)		

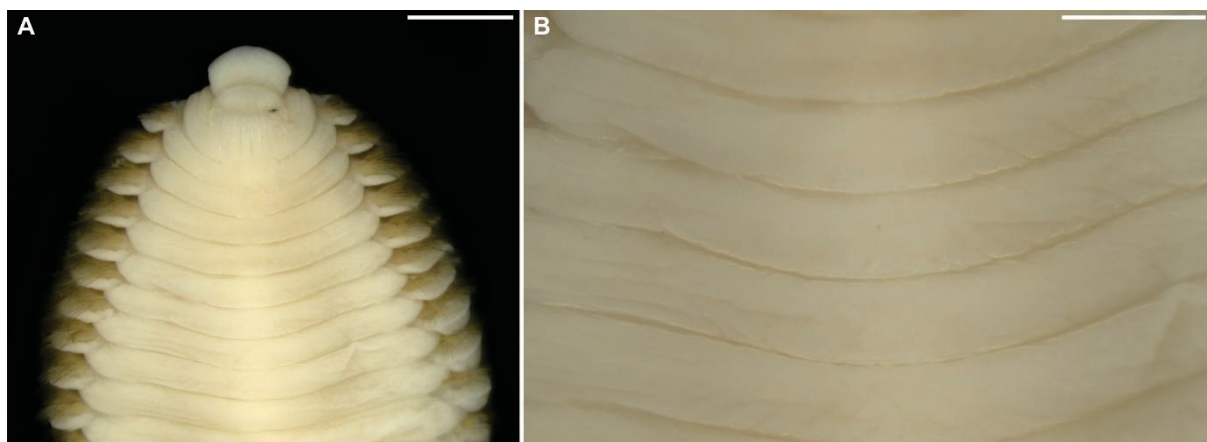


Figure 8. *Naineris setosa* from Taranto, Mediterranean Sea (ZMBN 157797): (A) Anterior end, ventral view; (B) Smooth, thoracic ventral surface. Scale bars; (A): 1 mm; (B): 500 μ m.

***Naineris lanai* sp. n.**

(Figs 9–11)

Material examined. **Brazil. Paraná, Pinheiros Bay:** MNRJP 007618 (holotype), MNRJP 007619 (2 paratypes), MNRJP 007624 (1 paratype), MNRJP 007622 (7 paratypes), MNRJP 007621 (10 paratypes), MNRJP 007634 (paratype, DNA voucher Ns06), MNRJP 007635 (paratype, DNA voucher Ns07), MNRJP 007636 (paratype, DNA voucher Ns08), MNRJP 007637 (paratype, DNA voucher Ns09), MNRJP 007638 (paratype, DNA voucher Ns10), MNRJP 007620 (6 paratypes). **Paraná, Paranaguá Bay:** MNRJP 007623 (2 paratypes). **Rio de Janeiro, Itacuruçá:** MNRJP 007655 (paratype Ns36), MNRJP 007656 (paratype Ns38). **São Paulo. Araçá:** MNRJP 007654 (paratype, DNA voucher Ns28). **Ilhabela,** ZUEC-POL 16999 (2 spms), ZUEC-POL 2756 (1 spm). **Caraguatatuba,** ZUEC-POL 3780 (1 spm), ZUEC-POL 3784 (1 spm), ZUEC-POL 3786 (1 spm), ZUEC-POL 3771 (1 spm). **Sergipe. Praia do Saco:** MNRJP 1909 (1 spm).

Type locality: Brazil: Paraná (Pinheiros Bay), 25.409° S, 48.251° W, intertidal, 20 cm depth, anoxic mud, close to *Spartina* sp.

Etymology. *Naineris lanai* is named in honor of R.A.'s late Ph.D. supervisor, the Brazilian scientist Paulo da Cunha Lana, in recognition of his substantial contribution to marine science and for believing in people.

Diagnosis. Thoracic neurochaetae including crenulated capillaries only; thoracic segments with ventral groove and notches; paired dorsal sensory organs present; ciliary dorsal crest in abdominal segments folded and hypertrophied, thoracic neuropodial lobes flattened and folded with irregular boundaries, with upper papilla; notopodial lobes undivided or forked.

Description. Large species (Figs 9A; 11E), holotype complete (MNRJP 007618), 263 mm long, 5 mm wide, consisting of 431 chaetigers. Color in alcohol dark brown, with dark segmental spots; live specimens dark brown to reddish, with dark pigmented branchiae and dorsal organs, and long fluorescent cilia along axis; eyespots and nuchal organs yellowish, dorsal crest bearing long fluorescent cilia. Body separated into two distinct regions of approximately same width (Fig. 9A); thorax and abdomen, with parapodia displaced dorsally in abdomen. Ventral surface of body rough, with prominent mid-ventral groove along most of body (Figs 9C; 11C), well-marked in thoracic segments, represented by longitudinal notch on each segment, almost reaching consecutive ring (Fig. 9C).

Prostomium wide, nearly square, spatulate in front (Fig. 9B); eyespots present on lateral margins of prostomium, organized in two groups; nuchal organs present, as two lateral notches between prostomium and peristomium (Fig. 9B), more conspicuous and globular in live specimens (Fig. 11A). Peristomium as single achaetous ring; mouth opening located ventrally; proboscis wide, thick, bearing triangular lobes (Figs 9B; 11A, B), densely ciliated (Fig. 10A).

Branchiae from chaetigers 5–6, continuing along entire body (Fig. 9A, B); elongate from first pair, widest basally tapering to narrow apex, with medial and lateral cilia. Thoracic branchiae 1/3 of longest abdominal branchiae. Paired dorsal sensory organs from mid-thoracic chaetigers, anterior and medial to branchial bases, oval-shaped, clearly raised (Figs 9B; 10B). Dorsal crest present in abdominal segments; straight at first, best developed and more extended in mid-abdominal segments

becoming folded, as long as or even exceeding basal width of branchiae (Figs 9A, D; 10D).

Thorax with 30 chaetigers (28–30 in paratypes), flattened. Parapodia biramous; interramal papilla absent. Thoracic notopodia with lanceolate lobes, more elongate in posterior chaetigers; forked or undivided. Neuropodial lobes wide, flat, with rough flanges with irregular borders, almost folded (Fig. 9C). Abdominal notopodial and neuropodial lobes similar in shape, triangular to lanceolate, with thin apex; notopodial lobes more prolonged than neuropodial lobes (Fig. 11D).

Thoracic notochaetae with two bundles of around 30–50 crenulated capillaries. Abdominal notochaetae with 15–20 crenulated capillaries in two bundles and furcate chaetae in lower position (Fig. 10C); each furcate chaeta with unequal tines, bearing tiny needles; shaft with small barbs. Thoracic neurochaetae with about 7–9 rows of capillaries separated into two groups by oblique gap. Abdominal neurochaetae with 15–20 capillaries and 2–3 acicular spines.

Pygidium with terminal anus, bearing four anal cirri with forked or undivided tips, and rounded base (Fig. 9E).

Remarks. *Naineris lanai* sp. n. is similar to *N. setosa* s. str. in having only crenulated capillaries in thoracic neuropodia, dorsal crest, and branchiae from chaetigers 5–6. It differs from *N. setosa* in the dorsal crest being long and folded, and having marked ventral groove in thoracic segments, flat and folded thoracic neuropodial lobes, and often divided abdominal notopodial lobes.

Considering the anoxic habitat of the species, the hypertrophied ciliary dorsal crest, and parapodial lobes, are probably an adaptation to this environment. Similar correlation was established for some species of Nereididae and Opheliidae living in anoxic conditions (Glasby et al. 2021). The shape of thoracic neuropodial lobes differs significantly in both species. In *Naineris setosa* s. str., neuropodial lobes are thick, elongated processes with rounded boundaries. In *N. lanai* sp. n., neuropodial lobes are enlarged, markedly flat, and folded with irregular boundaries. This may also be an adaptation to increase the surface area for oxygen uptake, such as in other

polychaetes (Hartman 1951; Nonato et al. 1986; Radashevsky & Lana 2009; Glasby et al. 2021).

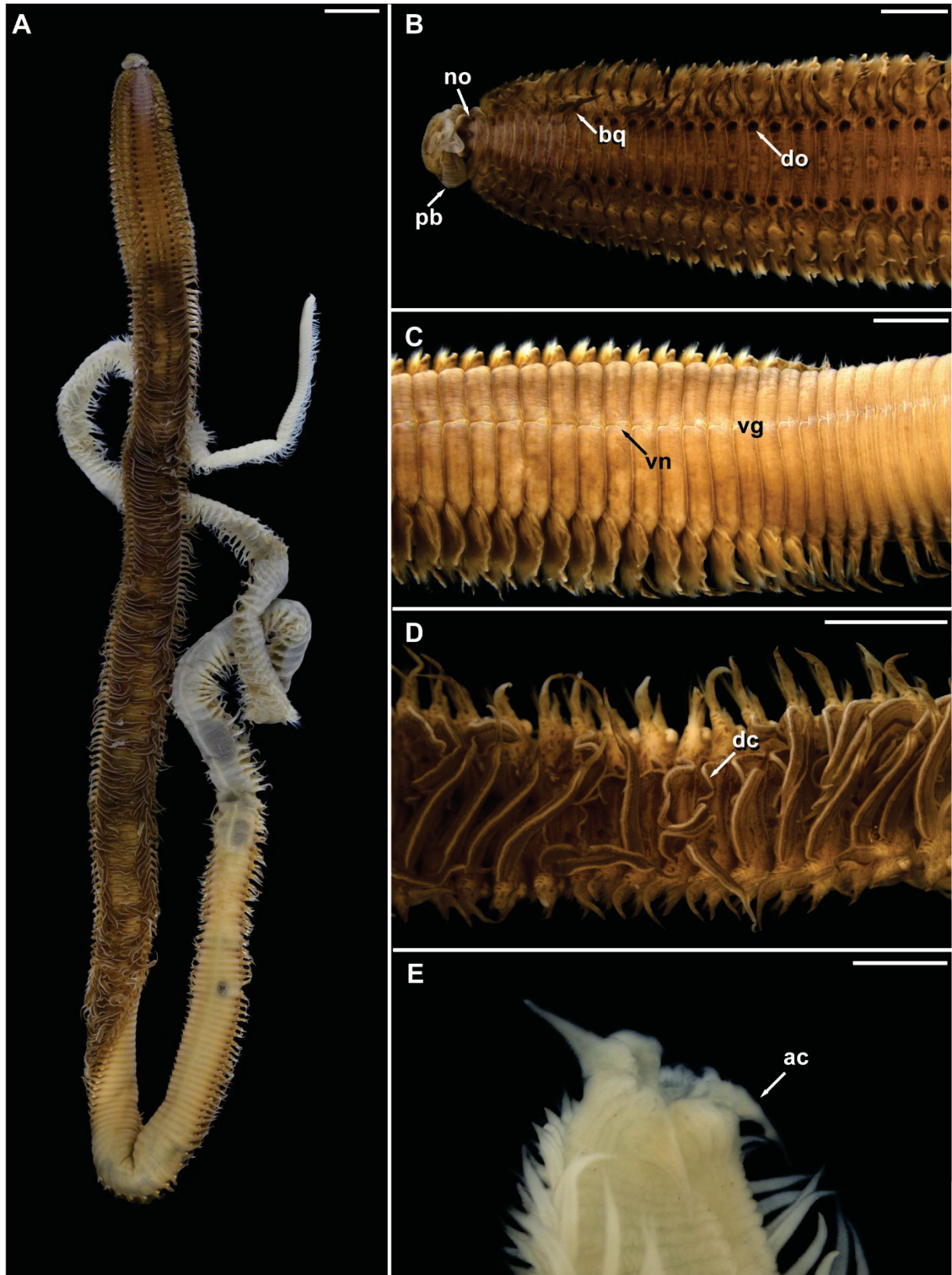


Figure 9. *Naineris lanai* sp. n., holotype (MNRJP 007618): (A) Complete specimen, dorsal view; (B) Anterior end, dorsal view; (C) Thoracic segments, ventrolateral view; (D) Abdominal segments, dorsal view; (E) Posterior end, dorsolateral view. ac – anal cirrus, br – branchiae, dc – dorsal crest, do – dorsal organs, no – nuchal organ, pb – proboscis, vg – ventral groove, vn – ventral notches. Scale bars; (A): 5 mm; (B–D): 2 mm; (E): 500 μ m.

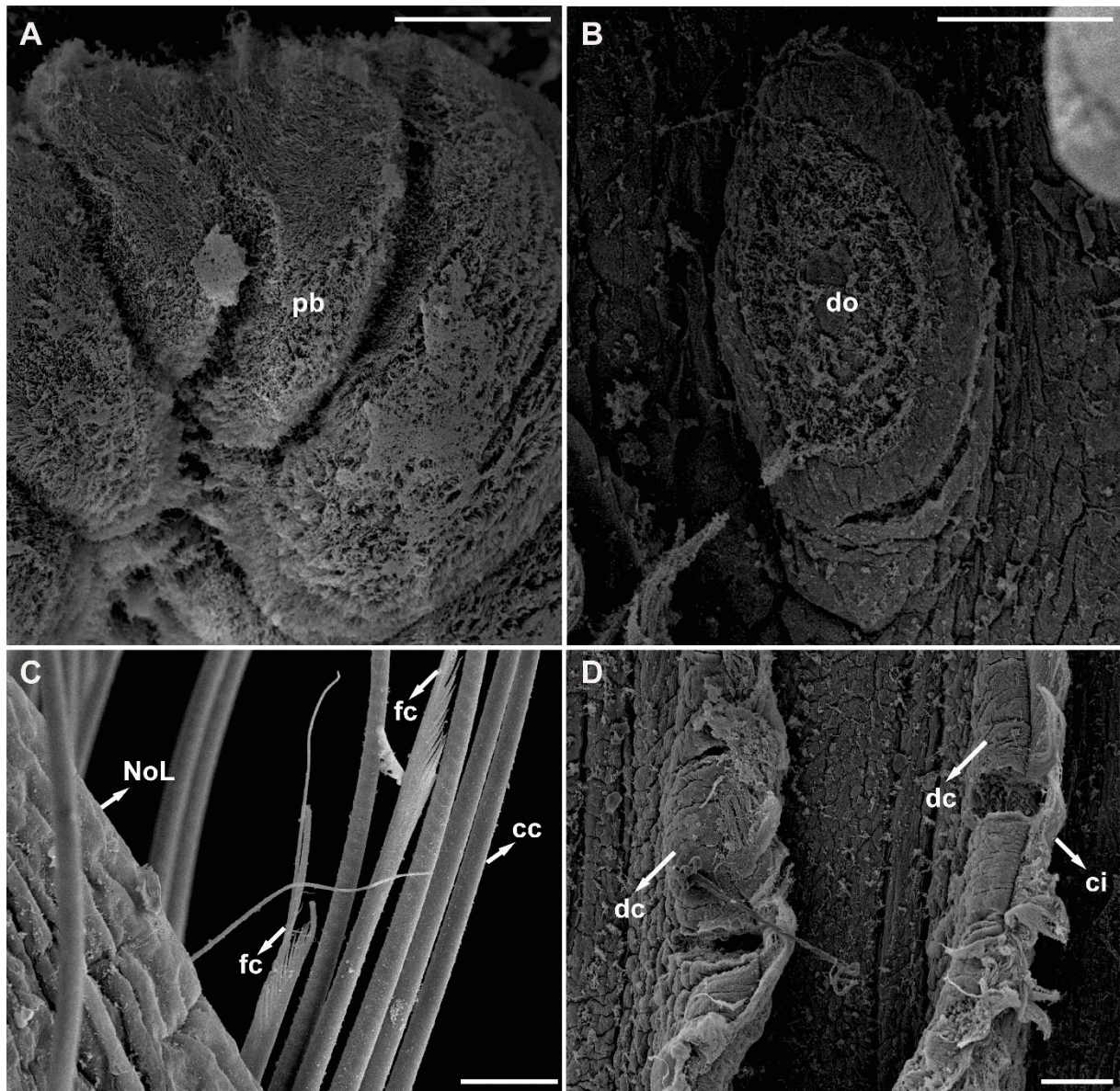


Figure 10. *Naineris lanai* sp. n. topotype specimen examined under the SEM: (A) Cilia in the everted proboscis, ventral view; (B) Dorsal organ in thoracic segment; (C) Furcate chaetae in an abdominal segment, lateral view; (D) Cilia in dorsal crest. cc – crenulate capillaries, ci – cilia, dc – dorsal crest, do – dorsal organ, fc – furcate chaetae pb – proboscis, NoL – notopodial lobe. Scale bars; (A): 50 mm; (B): 500 μ m; (C): 20 μ m, (D): 100 μ m.



Figure 11. Habitat (H) and live (A–D, I) and preserved (E–G; paratype MRNJP 007623) specimens of *Naineris lanai* sp. n.: (A) Anterior end with a partially everted proboscis, dorsal view; (B) Anterior end with a completely everted proboscis, dorsal view; (C) Thoracic segments, ventral view; (D) Abdominal segments, lateral view; (E–G) Furcation in abdominal notopodial lobes; (H) Tidal flat from Ilha das Peças, Pinheiros Bay, the type locality; (I) Specimens being sorted in the laboratory. bq – branchia, do – dorsal organ, e – eyespots, no – nuchal organ, pb – proboscis, vn – ventral notch (Arrows pointed furcate notopodial lobes). Scale bars; (A): 0.5 mm; (B): 2 mm (C): 0.2 mm; (D): 1 mm; (E–G): 0.1 mm.

Habitat. Large mature adults were sampled in black anoxic mud near *Spartina* sp. and mangroves from Pinheiros Bay, Paranaguá Estuarine Complex (Fig. 11E). Juveniles were sampled in mud from São Paulo and fouling communities from Rio de Janeiro.

Distribution. South and Southeastern Brazil, from Paraná to Rio de Janeiro, intertidal.

Naineris sp.

(Fig. 12)

Material examined. USA, California. SERC 252007 (DNA voucher Ns46, + 1 spm), SERC 252013 (DNA voucher Ns47, + 2 spms), SERC 251805 (1 spm), SERC 251985 (1 spm, 1 for SEM), SERC 252000 (2 spms), LACM-AHF: DNA vouchers Ns33, Ns34, Ns35.

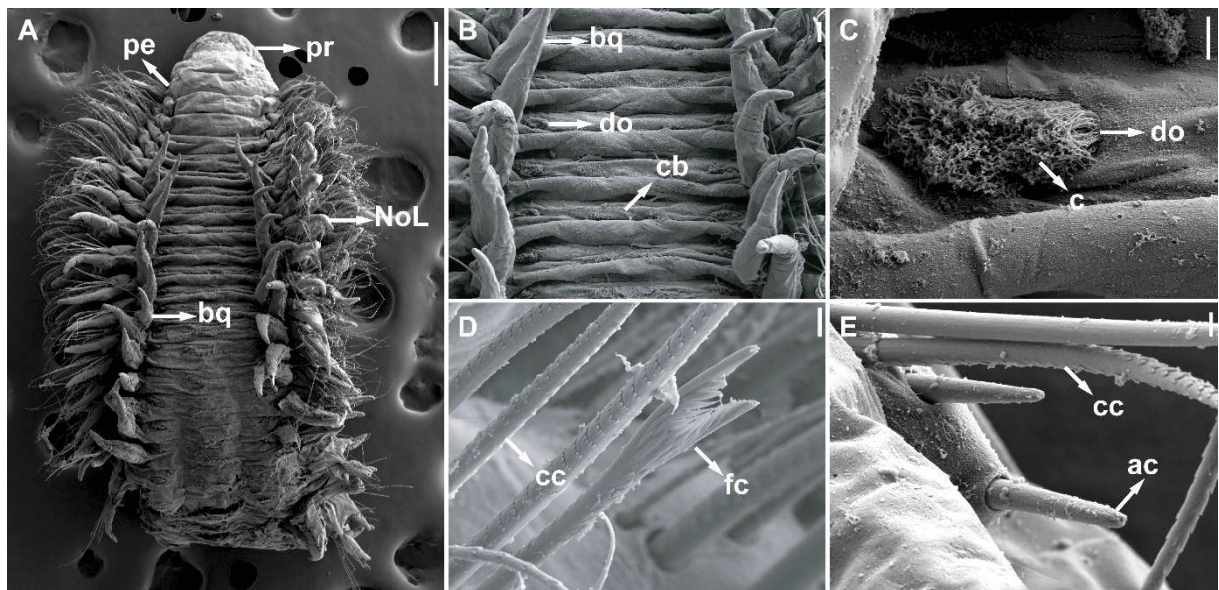


Figure 12. *Naineris* sp. examined under the SEM (SERC 252000): (A) Anterior end, dorsal view; (B) Thoracic segments, dorsal view; (C) Cilia in dorsal organs; (D) Furcate

chaetae in abdominal segment, lateral view; (E) Acicular spines in abdominal segments. bq – branchia, c – dorsal organ cilia, cb – ciliary band, cc – crenulate capillary chaeta, do – dorsal organ, fc – furcate chaeta, NoL – notopodial lobe, pe – peristomium, pr – prostomium. Scale bars; (A): 100 μm ; (B): 20 μm ; (C): 10 μm , (D–E): 2 μm .

Description. Small specimens, presumably juveniles (Fig. 12A). Color in alcohol light tan. Thorax and abdomen distinct. Prostomium anteriorly rounded (Fig. 12A); eyespots not discernible; nuchal organs present. Peristomium as 1–2 achaetous rings (Fig. 12A); mouth opening located ventrally; proboscis not everted.

Branchiae from chaetiger 6 (Fig. 12A), elongate from first pair. Paired dorsal sensory organs between branchial bases, oval-shaped (Fig. 12B, C). Dorsal crest as ciliary bands from first thoracic segment, straight (Fig. 12B).

Thorax dorsoventrally flattened. Parapodia biramous. Thoracic notopodial and neuropodial lobes lanceolate. Abdominal notopodial and neuropodial lobes triangular.

Thoracic notochaetae with crenulate capillaries only. Abdominal notochaetae including capillaries and furcate chaetae (Fig. 12D); furcate chaetae with unequal tynes and thin needles between; shaft consisting of several transversal rows of 5–7 barbs. Thoracic neurochaetae with capillaries only. Abdominal neurochaetae with capillaries and 1–2 acute acicular spines (Fig. 12E).

Pygidium with terminal anus, bearing anal cirri, presumably four cirri.

Remarks. Based on molecular data, the examined specimens represent an undescribed species. All specimens were of small size, presumably juveniles. The species is unique in having dorsal ciliary bands from the first thoracic segments. However, the species is not formally named here due to a lack of detailed information on the adult definitive morphological characters.

4.4. DISCUSSION

This study is the first step toward understanding the genetic and morphological diversity of the *Naineris setosa* complex. The cosmopolitan distribution of *N. setosa*

suggested by the earlier studies (Hartman 1951, 1957; Solis-Weiss & Fauchald 1989; Blake & Giangrande 2011; Dean & Blake 2015; Blake 2017) and its stagnated taxonomy over time can partly be explained by the fact that it was the only species of the genus bearing only capillary chaetae in thoracic neuropodia. Nevertheless, after careful examination, several morphological characters consistently differed in the worms from the studied Atlantic and Pacific populations, especially if examined in detail. For instance, the dorsal crest in *N. setosa sensu lato* is usually described in the literature as low. In the present study, we reported that the dorsal crest can vary in shape from straight to lobed, which can also change along the body. In addition, it can be hypertrophied, folded, and bear long cilia in *Naineris lanai* sp. n. collected from anoxic mud. At the same time, the species inhabiting regular marine aerated sediments show no such modifications (Blake & Giangrande 2011).

Similarly, we observed that parapodial lobes could be undivided or forked. Forked notopodial lobes were described as a unique character in *Naineris furcillata* Blake, 2017. Nonetheless, this character was also reported in *N. lanai* sp. n., with up to two furcations in some notopodial lobes (Figure 11D–G). Similar structures were reported by Hartman (1957), who described abdominal subpodial lobes in *N. setosa* specimens from the Gulf of Mexico. Solis-Weiss & Fauchald (1989) considered additional lobes a malformation. We suggest that subdivision of the parapodial lobes is characteristic for the specimens from muddy sediments with possibly reduced oxygen levels. According to Glasby *et al.* (2021), parapodial modifications may represent an adaptation to increasing surface area for oxygen uptake in anoxic environments.

Phylogenetic analysis utilizing several unlinked loci can aid in uncovering genetic diversity within complexes of species with similar morphology (Salicini *et al.* 2011; Liu *et al.* 2017, Qiu *et al.* 2020). In the present study, we attempted to delimit the species within the *Naineris setosa* complex by analyzing two mitochondrial and one nuclear marker. Phylogenetic analysis based on the COI marker and the combined dataset of three markers recovered three highly supported clades in the study material. Nevertheless, the individual 16S and 28S gene trees had different topologies. In the 16S analysis, only two clades were recovered, one combining the specimens from the Atlantic and the other from the Pacific. The analysis based on 28S recovered the North

Pacific clade sister to the clade representing *N. setosa* s. str. specimens. In contrast, the South and Southeastern Brazil specimens did not form a clade. Similar results were obtained by the haplotype network analysis. Furthermore, the haplotype network of 28S showed the presence of two significantly different haplotypes in the Pacific population, with 9 substitutions present in a generally very conservative nuclear marker.

The interspecific COI distances between the species in the *N. setosa* complex ranged from 13 to 16%, which was comparable with other studies on annelids using COI data for species discrimination (Taboada *et al.* 2015, 2017; Seixas *et al.* 2021). For 16S, the interspecific distances ranged from 0 to 6%, which was considerably lower than in other species complexes of polychaetes (Drennan *et al.* 2019, Radashevsky *et al.* 2020). Still, they were comparable with those in Clitellata (Martinsson *et al.* 2020). All Atlantic specimens shared the same haplotype in 16S, which could be explained by the low evolutionary rates in 16S in the *Naineris setosa* complex and possible recent speciation events between the two Atlantic species. For 28S, the distances ranged from 1.1% to 1.8%, which was similar to other polychaete species complexes (Grosse *et al.* 2020, Radashevsky *et al.* 2020, Kolbasova & Neretina 2021) and can be considered significant, given that 28S evolves at low rates.

Mitochondrial discordance is expected in the animal kingdom (Rokas *et al.* 2003; Toews & Brelsford 2012; Dávalos *et al.* 2012) and was reported for several groups of organisms (Degnan & Rosenberg 2009; Debiasse *et al.* 2014; Hirano *et al.* 2019; Ortiz *et al.* 2021; Formaggioni *et al.* 2022), including annelids (Liu *et al.* 2018; Elgetany *et al.* 2020). Phylogenies reconstruction based on a single marker may conflict as no single gene can capture all nuances of the phylogenetic relationships of a taxon (Rokas *et al.* 2003). In our study, one of the mitochondrial markers, 16S, provided less resolution than COI, indicating low evolutionary rates in the former in *Naineris*. The rates of evolution in different markers are poorly understood. Protein coding and non-coding genes show different evolution rates, which may also differ among codon positions within a coding gene (Liò & Goldman 1998). A phylogenomic approach, combining many genes, may help resolve conflicting topologies (Gee 2003; Rokas *et al.* 2003; Bleidorn 2017).

Even though our findings support that some of the North Pacific populations of former *Naineris setosa* represent a different species, the Galapagos population reported by Blake (2017) still needs to be studied with well-preserved specimens and molecular data to discard the Amphi-American distribution. Furthermore, a specimen of *Naineris* (identified as *N. australis* collected off Sydney) bearing only capillaries in thoracic neuropodia, paired dorsal organs, and similar parapodial structures was found in the Australian Museum collection (W.22470, unpubl.). After examining numerous specimens of the *Naineris setosa* species complex, we discovered that, in some cases, broken capillary chaetae might strongly resemble uncini, especially in the old specimens (R.A. personal observations). This could probably lead to misidentification of *Naineris* species in scientific collections. Presumably, other similar species were misidentified as *N. setosa* worldwide, requiring further verification and subsequent revision.

We confirmed the Amphi-Atlantic distribution of *Naineris setosa* s. str. with two records reported from the Tyrrhenian and the Ionian Seas. This corroborated the earlier records of the species in the Mediterranean and corroborated its alien status (Blake & Giangrande 2011; Khedhri *et al.* 2014; Atzori *et al.* 2016; Arduini *et al.* 2022; Rebai *et al.* 2022; Struck *et al.* 2023). The species was believed to have been introduced in the Mediterranean Sea due to aquaculture (Blake & Giangrande 2011) and shipping as in the case of possible regional introductions within the Mediterranean (Khedhri *et al.* 2014). *Naineris setosa* s. str. can be considered established in the Mediterranean since later records expanded its distribution to Egypt (Struck *et al.* 2023), Tunisia (Khedhri *et al.* 2014; Rebai *et al.* 2022), and the Adriatic (Blake & Giangrande 2011), Ionian (Arduini *et al.* 2022), and Tyrrhenian (Atzori *et al.* 2016) seas. *Naineris setosa* was first reported in Brazil by Nonato (1981); since then, the number of Brazilian records increased (Amaral *et al.* 2006–2022). Even though most of our sampling in Brazil was conducted in bays near the main ports, suggesting shipping as a possible reason for the range expansion, it is hard to determine whether Brazil is part of *N. setosa* s. str. native distribution range or represents a newly colonized area. Further studies, with additional North and Tropical Atlantic samples, including the type locality, may help better understanding of the species' bioinvasive

potentials, establish effective monitoring programs in affected areas, and avoid new invasions in susceptible regions.

Availability of data and material: All physical specimens used in this study are kept in the registered scientific collections. All sequences have been submitted to GenBank and BOLD (see Table 1 for accession numbers and BOLD process IDs). Alignments were uploaded to TREEBASE as nexus-formatted data (<http://purl.org/phylo/treebase/phyloids/study/TB2:S30855>).

Acknowledgments

Our gratitude to Joana Zanol, Camila Messias (MNRJP), Cecília Amaral (ZUEC-POL), Mônica Petti (ColBIO), Gustav Paulay (FM), Stephen Keable (AM), for the loan of specimens. We thank Karen Osborn (USNM), Lily Berniker (AMNH), Eric Lasso-Wasem and Lourdes Rojas (YPM). We would like to thank Phoebe Fu and Mr. Scott Whittaker (USNM, SEM Laboratory) for their assistance with SEM. RA is deeply indebted to Leslie Harris (LACM) and David for lodging him during his visit to the LACM and for providing specimens. A special acknowledgment to Tony Phillips for providing materials during the Bodega-Bay BIO BLITZ (SERC) and to Erica Keppel and Gregory Ruiz for loaning materials from the Invasion lab (SERC) collection. Thanks to Joachim Langeneck for providing ethanol-fixed specimens from the Mediterranean. We also thank Louise Lindblom for their help in molecular laboratory work. Christoph Bleidorn and an anonymous reviewer carefully read the manuscript and provided valuable comments that improved our manuscript. Finally, not surprisingly, RA would like to dedicate this paper to the memory of the most brilliant scientist Brazil ever produced, Paulo Lana, his late supervisor, whose passion for encouraging new generations was unsurpassed.

Funding

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - (Doctorate fellowship, process number: 88882.382993/2019-01) and CAPES-PRINT-UFPR (Ph.D. Exchange program, process number: 88887.696847/2022-00). RA was also supported by the USNM with the grant Kenneth Jay Boss Fellowship in Invertebrate Zoology and by the LACM with

the Students Collections Study Award. Sampling of Newport Dunes specimens was possible through agreement No.: P1675035 between the California Department of Fish and Wildlife (CDFW) and SERC.

4.5. REFERENCES

- Amaral, A.C.Z., Nallin, S.A.H., Steneir, T.M., Forroni, T. de O. & Gomes Filho, D. (2006) *Catálogo das Espécies de Annelida Polychaeta do Brasil*. Retrieved July 4, 2023, from http://www.ib.unicamp.br/museu_zoologia/files/lab_museu_zoologia/Catalogo_Polychaeta_Amaral_et_al_2012.pdf
- Arduini, D., Borghese, J., Gravina, M. F., Trani, R., Longo, C., Pierri, C. & Giangrande, A. (2022) Biofouling Role in Mariculture Environment Restoration: An Example in the Mar Grande of Taranto (Mediterranean Sea). *Frontiers in Marine Science*, 9, 842616. <https://doi.org/10.3389/fmars.2022.842616>
- Atzori, G., López, E., Addis, P., Sabatini, A. & Cabiddu, S. (2016) First record of the alien polychaete *Naineris setosa* (Scolecida; Orbiniidae) in Tyrrhenian Sea (Western Mediterranean). *Marine Biodiversity Records*, 9 (1), 1–6. <https://doi.org/10.1186/s41200-016-0017-6>
- Blainville, H. (1828) *Dictionnaire des Sciences naturelles, dans lequel on traite méthodiquement des différens êtres de la nature, considérés soit en eux-mêmes, d'après l'état actuel de nos connoissances, soit relativement a l'utilité qu'en peuvent retirer la médecine, l'agriculture, le commerce et les arts. Suivi d'une biographie des plus célèbres naturalistes*. FG Levrault, Strasbourg and Paris.
- Blake, J.A. (2000) A new genus and species of polychaete worm (Family Orbiniidae) from methane seeps in the Gulf of Mexico, interrelationships of the genera of Orbiniidae. *Cahiers de Biologie Marine*, 41 (4), 435–449. <https://doi.org/10.21411/CBM.A.84F1D61E>
- Blake, J.A. (2017) Polychaeta Orbiniidae from Antarctica, the Southern Ocean, the Abyssal Pacific Ocean, and off South America. *Zootaxa*, 4218 (1), 1–145. <https://doi.org/10.11646/zootaxa.4218.1.1>
- Blake, J.A. (2020) New species and records of deep-water Orbiniidae (Annelida, Polychaeta) from the Eastern Pacific continental slope, abyssal Pacific Ocean, and the South China Sea. *Zootaxa*, 4730 (1), 1–61. <https://doi.org/10.11646/zootaxa.4730.1.1>
- Blake, J.A. (2021) New species and records of Orbiniidae (Annelida, Polychaeta) from continental shelf and slope depths of the Western North Atlantic Ocean. *Zootaxa*, 4930 (1), 1–123. <https://doi.org/10.11646/zootaxa.4930.1.1>

Blake, J.A. & Giangrande, A. (2011) *Naineris setosa* (Verrill) (Polychaeta, Orbiniidae), an American subtropical-tropical polychaete collected from an aquaculture facility in Brindisi (Adriatic Sea, Italy): A possible alien species. *Italian Journal of Zoology*, 78 (sup1), 20–26. <https://doi.org/10.1080/11250003.2011.577982>

Bleidorn, C. (2005) Phylogenetic relationships and evolution of Orbiniidae (Annelida, Polychaeta) based on molecular data. *Zoological Journal of the Linnean Society*, 144 (1), 59–73. <https://doi.org/10.1111/j.1096-3642.2005.00160.x>

Bleidorn, C., Hill, N., Erséus, C. & Tiedemann, R. (2009) On the role of character loss in orbiniid phylogeny (Annelida): Molecules vs. morphology. *Molecular Phylogenetics and Evolution*, 52 (1), 57–69. <https://doi.org/10.1016/j.ympev.2009.03.022>

Bleidorn, C. (2017) *Phylogenomics: an introduction*. Springer, 1–217.

Carr, C.M., Hardy, S.M., Brown, T.M., Macdonald, T.A. & Hebert, P.D.N. (2011) A tri-oceanic perspective: DNA Barcoding reveals geographic structure and cryptic diversity in Canadian polychaetes. *PLoS ONE*, 6 (7), e22232. <https://doi.org/10.1371/journal.pone.0022232>

Çinar, M. E. (2013) Alien polychaete species worldwide: current status and their impacts. *Journal of the Marine Biological Association of the United Kingdom*, 93 (5), 1257–1278.

<https://doi.org/10.1017/S0025315412001646>

Clement, M., Snell, Q., Walker, P., Posada, D. & Crandall, K. (2002) TCS: estimating gene genealogies. In *Parallel and Distributed Processing Symposium, International*, 3, 0184–0184. IEEE Computer Society.

Dávalos, L.M., Cirranello, A.L., Geisler, J.H. & Simmons, N.B. (2012) Understanding phylogenetic incongruence: lessons from phyllostomid bats. *Biological Reviews*, 87 (4), 991–1024.

<https://doi.org/10.1111/j.1469-185X.2012.00240.x>

Day, J.H. (1961) The polychaete fauna of South Africa. Part 6. Sedentary species dredged off Cape coasts with a few new records from the shore. *Zoological Journal of the Linnean Society*, 44 (299), 463–560.

<https://doi.org/10.1111/j.1096-3642.1961.tb01623.x>

Day, J.H. (1963) The polychaete fauna of South Africa. Part 8: New species and records from grab samples and dredgings. *Bulletin of the British Museum (Natural History), Series Zoology*, 10 (7), 381–445. Retrieved from <https://www.biodiversitylibrary.org/>

Day, J.H. (1965) Israel South Red Sea Expedition, 1962, Reports no. 7. Some Polychaeta from the Israel South Red Sea Expedition, 1962. *Bulletin of the Sea Fisheries Research Station, Haifa*, 38, 15–27.

Day, J.H. (1977) A review of the Australian and New Zealand Orbiniidae (Annelida: Polychaeta). *Essays on Polychaetous Annelids in Memory of Dr. Olga Hartman*. Allan Hancock Found, Los Angeles, California, USA, 217–246.

Dean, H.K. & Blake, J.A. (2015) The Orbiniidae (Annelida: Polychaeta) of Pacific Costa Rica. *Zootaxa*, 3956 (2), 183–198.

<https://doi.org/10.11646/zootaxa.3956.2.2>

DeBiasse, M.B., Nelson, B.J. & Hellberg, M.E. (2014) Evaluating summary statistics used to test for incomplete lineage sorting: mito-nuclear discordance in the reef sponge *Callyspongia vaginalis*. *Molecular Ecology*, 23 (1), 225–238. <https://doi.org/10.1111/mec.12584>

Degnan, J.H. & Rosenberg, N.A. (2009) Gene tree discordance, phylogenetic inference and the multispecies coalescent. *Trends in ecology & evolution*, 24 (6), 332–340. <https://doi.org/10.1016/j.tree.2009.01.009>

Drennan, R., Wiklund, H., Rouse, G.W., Georgieva, M.N., Wu, X., Kobayashi, G., Yoshino, K. & Glover, A.G. (2019) Taxonomy and phylogeny of mud owls (Annelida: Sternaspidae), including a new synonymy and new records from the Southern Ocean, North East Atlantic Ocean and Pacific Ocean: challenges in morphological delimitation. *Marine Biodiversity*, 49, 2659–2697. <https://doi.org/10.1007/s12526-019-00998-0>

Elgetany, A.H., van Rensburg, H., Hektoen, M., Matthee, C., Budaeva, N., Simon, C.A. & Struck, T. H. (2020) Species delineation in the speciation grey zone—The case of *Diopatra* (Annelida, Onuphidae). *Zoologica Scripta*, 49 (4), 516–534. <https://doi.org/10.1111/zsc.12421>

Fabricius, O. (1780) *Fauna Groenlandica: systematice sistens animalia Groenlandiae occidentalis hactenus indagata, quoad nomen specificum, triviale, vernaculumque: synonyma auctorum plurium, descriptionem, locum, victum, generationem, mores, usum, capturamque singuli, prout detegendi occasio fuit: maximaque parte secundum proprias observationes*. impensis JG Rothe.

Formaggioni, A., Plazzi, F. & Passamonti, M. (2022) Mito-nuclear coevolution and phylogenetic artifacts: the case of bivalve mollusks. *Scientific Reports*, 12 (1), 11040.

<https://doi.org/10.1038/s41598-022-15076-y>

Gee, H. (2003) Ending incongruence. *Nature*, 425 (6960), 782–782. <https://doi.org/10.1038/425782a>

Glasby, C.J., Erséus, C. & Martin, P. (2021) Annelids in extreme aquatic environments: Diversity, adaptations and evolution. *Diversity*, 13 (2), 1–23. <https://doi.org/10.3390/d13020098>

Grosse, M., Bakken, T., Nygren, A., Kongsrud, J.A. & Capa, M. (2020) Species delimitation analyses of NE Atlantic *Chaetozona* (Annelida, Cirratulidae) reveals hidden diversity among a common and abundant marine annelid. *Molecular*

phylogenetics and evolution, 149, 106852.
<https://doi.org/10.1016/j.ympev.2020.106852>

Grube, A.E. (1855) Beschreibungen neuer oder wenig bekannter Anneliden. *Archiv fur Naturgeschichte, Berlin*, 21, 81–128. <https://doi.org/10.5962/bhl.part.13989>

Hartman, O. (1942) A review of the types of polychaetous annelids at the Peabody Museum of Natural History, Yale University. *Bulletin of the Bingham Oceanographic Collections*, 8, 1–98.

Hartman, O. (1951) The littoral marine annelids of the Gulf of Mexico. *Publications of the Institute of Marine Science, University of Texas*, 2, 7–124.

Hartman, O. (1957) Orbiniidae, Apistobranchidae, Paraonidae and Logosomidae. *Allan Hancock Pacific Expeditions*, 15 (3), 211–393, plates 20–44.

Hillis, D.M. & Dixon, M.T. (1991) Ribosomal DNA: molecular evolution and phylogenetic inference. *The Quarterly review of biology*, 66 (4), 411–453.

Hirano, T., Saito, T., Tsunamoto, Y., Koseki, J., Ye, B., Do, V.T, Miura, O., Suyama, Y. & Chiba, S. (2019) Enigmatic incongruence between mtDNA and nDNA revealed by multi-locus phylogenomic analyses in freshwater snails. *Scientific Reports*, 9 (1), 1–13.

<https://doi.org/10.1038/s41598-019-42682-0>

Hoang, D. T., Chernomor, O., Von Haeseler, A., Minh, B.Q. & Vinh, L.S. (2018) UFBoot2: improving the ultrafast bootstrap approximation. *Molecular biology and evolution*, 35 (2), 518–522.

<https://doi.org/10.1093/molbev/msx281>

Hutchings, P. & Kupriyanova, E. (2018) Cosmopolitan polychaetes - Fact or fiction? Personal and historical perspectives. *Invertebrate systematics*, 32 (1), 1–9.
<https://doi.org/10.1071/IS17035>

Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K., Von Haeseler, A. & Jermin, L.S. (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature methods*, 14 (6), 587–589.

<https://doi.org/10.1038/nmeth.4285>

Katoh, K., Kuma, K. I., Toh, H. & Miyata, T. (2005) MAFFT version 5: Improvement in accuracy of multiple sequence alignment. *Nucleic Acids Research*, 33, 511–518.
<https://doi.org/10.1093/nar/gki198>

Khedhri, I., Lavesque, N., Bonifácio, P., Djabou, H. & Afli, A. (2014) First record of *Naineris setosa* (Verrill, 1900) (Annelida: Polychaeta: Orbiniidae) in the Western Mediterranean Sea. *BioInvasions Records*, 3 (2), 83–88.
<http://dx.doi.org/10.3391/bir.2014.3.2.05>

Kinberg, J.G.H. (1867) *Annulata nova. Öfversight af Kungliga Vetenskaps-Akademiens Förhandlingar, Stockholm*, 22, 239–258.

Kolbasova, G. & Neretina, T. (2021) A new species of *Pelagobia* (Lopadorrhynchidae, Annelida), with some notes on literature records of *Pelagobia longicirrata* Greeff, 1879. *Zootaxa*, 5023 (1), 77–92. <https://doi.org/10.11646/zootaxa.5023.1.4>

Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular biology and evolution*, 35 (6), 1547. <https://doi.org/10.1093/molbev/msy096>

Kvist, S. (2016) Does a global DNA barcoding gap exist in Annelida?. *Mitochondrial DNA Part a*, 27 (3), 2241–2252. <https://doi.org/10.3109/19401736.2014.984166>

Leigh, J.W. & Bryant, D. (2015) POPART: full-feature software for haplotype network construction. *Methods in ecology and evolution*, 6 (9), 1110–1116. <https://doi.org/10.1111/2041-210X.12410>

Liò, P. & Goldman, N. (1998) Models of molecular evolution and phylogeny. *Genome research*, 8 (12), 1233–1244.

<https://doi.org/10.1101/gr.8.12.1233>

Liu, Y., Fend, S. V., Martinsson, S., Luo, X., Ohtaka, A., & Erséus, C. (2017) Multi-locus phylogenetic analysis of the genus *Limnodrilus* (Annelida: Clitellata: Naididae). *Molecular Phylogenetics and Evolution*, 112, 244–257.

<https://doi.org/10.1016/j.ympev.2017.04.019>

Liu, P., Xu, L., Xu, S. L., Martínez, A., Chen, H., Cheng, D., Dumont, H., Han, B-P. & Fontaneto, D. (2018) Species and hybrids in the genus *Diaphanosoma* Fischer, 1850 (Crustacea: Branchiopoda: Cladocera). *Molecular phylogenetics and evolution*, 118, 369–378.

<https://doi.org/10.1016/j.ympev.2017.10.016>

Martinsson, S., Klinth, M. & Erséus, C. (2020) Testing species hypotheses for *Fridericia magna*, an enchytraeid worm (Annelida: Clitellata) with great mitochondrial variation. *BMC Evolutionary Biology*, 20 (1), 1–12. <https://doi.org/10.1186/s12862-020-01678-5>

Meyer, A., Bleidorn, C., Rouse, G.W., & Hausen, H. (2008) Morphological and molecular data suggest a cosmopolitan distribution of the polychaete *Proscoloplos cygnochaetus* Day, 1954 (Annelida, Orbiniidae). *Marine Biology*, 153, 879–889. <https://doi.org/10.1007/s00227-007-0860-4>

Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., Von Haeseler, A. & Lanfear, R. (2020) IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Molecular biology and evolution*, 37 (5), 1530–1534.

<https://doi.org/10.1093/molbev/msaa015>

Müller, F. (1858) Einiges über die Annelidenfauna der Insel Santa Catharina an der brasilianischen Küste. *Archiv für Naturgeschichte*, 24 (1), 211–220.

Nonato, E.F. (1981) Contribuição ao conhecimento dos anelídeos poliquetas bentônicos da Plataforma Continental Brasileira, entre Cabo Frio e o Arroio Chuí. Livre Docência Thesis, Universidade de São Paulo, São Paulo, Brazil, 246 pp.

Nonato, E.F., Bolívar, G.A. & Lana, P. (1986) *Laonice branchiata*, a new species of Spionidae (Annelida; Polychaeta) from the southeastern Brazilian coast. *Revista Nerítica*, 1 (3), 22–25. <http://dx.doi.org/10.5380/rn.v2i1.84723>

Ortiz, D., Pekár, S., Bilat, J. & Alvarez, N. (2021) Poor performance of DNA barcoding and the impact of RAD loci filtering on the species delimitation of an Iberian ant-eating spider. *Molecular phylogenetics and evolution*, 154, 106997. <https://doi.org/10.1016/j.ympev.2020.106997>

Palumbi, S.R., Martin, A., Romano, S., McMillan, W.O., Stice, L. & Grabowski, G. (1991) The simple fool's guide to PCR, Special Publication. Department of Zoology and Kewalo Marine Laboratory, *University of Hawaii, Honolulu*.

Qiu, P. L., Liu, S. Y., Bradshaw, M., Rooney-Latham, S., Takamatsu, S., Bulgakov, T. S., Tang, S.R., Feng, J., Aroge, T., Li, Y., Wang, L.L. & Braun, U. (2020) Multi-locus phylogeny and taxonomy of an unresolved, heterogeneous species complex within the genus *Golovinomyces* (Ascomycota, Erysiphales), including *G. ambrosiae*, *G. circumfusus* and *G. spadiceus*. *BMC microbiology*, 20, 1–16.

<https://doi.org/10.1186/s12866-020-01731-9>

Radashevsky, V. I. & Lana, P. (2009) *Laonice* (Annelida: Spionidae) from South and Central America. *Zoosymposia*, 2, 265–295. <https://doi.org/10.11646/zoosymposia.2.1.19>

Radashevsky, V. I., Malyar, V.V., Pankova, V.V., Gambi, M.C., Giangrande, A., Keppel, E., Nygren, A., Al-Kandari, M. & Carlton, J.T. (2020) Disentangling invasions in the sea: molecular analysis of a global polychaete species complex (Annelida: Spionidae: *Pseudopolydora paucibranchiata*). *Biological Invasions*, 22 (12), 3621–3644.

<https://doi.org/10.1007/s10530-020-02346-x>

Rebai, N., Mosbahi, N., Dauvin, J. C. & Neifar, L. (2022) Ecological Risk Assessment of Heavy Metals and Environmental Quality of Tunisian Harbours. *Journal of Marine Science and Engineering*, 10 (11), 1625. <https://doi.org/10.3390/jmse10111625>

Rioja, E. (1960) Estudios anelidológicos. XXIV. Adiciones a la fauna de Anelidos Poliquetos de las costas orientales de México. *Anales Del Instituto de Biología, México*, 31, 289–316.

Rokas, A., Williams, B.L., King, N. & Carroll, S.B. (2003) Genome-scale approaches to resolving incongruence in molecular phylogenies. *Nature*, 425 (6960), 798–804. <https://doi.org/10.1038/nature02053>

Salicini, I., Ibáñez, C., & Juste, J. (2011) Multilocus phylogeny and species delimitation within the Natterer's bat species complex in the Western Palearctic. *Molecular Phylogenetics and Evolution*, 61 (3), 888–898.

<https://doi.org/10.1016/j.ympev.2011.08.010>

Seixas, V.C., Steiner, T.M., Solé-Cava, A.M., Amaral, A.C.Z. & Paiva, P.C. (2021) Hidden diversity within the *Diopatra cuprea* complex (Annelida: Onuphidae): Morphological and genetics analyses reveal four new species in the south-west Atlantic. *Zoological Journal of The Linnean Society*, 191 (3), 637–671. <https://doi.org/10.1093/zoolinnean/zlaa032>

Solis-Weiss, V. & Fauchald, K. (1989) Orbiniidae (Annelida: Polychaeta) from mangrove root-mats in Belize, with a revision of the protoariciin genera. *Proceedings of the Biological Society of Washington*, 102 (3), 772–792.

Struck, T. H., Golombek, A., Hoesel, C., Dimitrov, D., & Elgetany, A. H. (2023) Mitochondrial Genome Evolution in Annelida—A Systematic Study on Conservative and Variable Gene Orders and the Factors Influencing its Evolution. *Systematic Biology*, syad023.

<https://doi.org/10.1093/sysbio/syad023>

Taboada, S., Riesgo, A., Bas, M., Arnedo, M. A., Cristobo, J., Rouse, G.W. & Avila, C. (2015) Bone-eating worms spread: insights into shallow-water *Osedax* (Annelida, Siboglinidae) from Antarctic, Subantarctic, and Mediterranean waters. *PLoS One*, 10 (11), e0140341. <https://doi.org/10.1371/journal.pone.0140341>

Taboada, S., Leiva, C., Bas, M., Schult, N. & McHugh, D. (2017) Cryptic species and colonization processes in *Ophryotrocha* (Annelida, Dorvilleidae) inhabiting vertebrate remains in the shallow-water Mediterranean. *Zoologica Scripta*, 46 (5), 611–624.

<https://doi.org/10.1111/zsc.12239>

Tamura, K., Stecher, G. & Kumar, S. (2021) MEGA11: molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, 38 (7), 3022–3027. <https://doi.org/10.1093/molbev/msab120>

Toews, D. P. & Brelsford, A. (2012) The biogeography of mitochondrial and nuclear discordance in animals. *Molecular ecology*, 21 (16), 3907–3930. <https://doi.org/10.1111/j.1365-294X.2012.05664.x>

Treadwell, A.L. (1901) The polychaetous annelids of Porto Rico. *Bulletin of the United States National Fish Commission*, 20 (2), 183–210.

Treadwell, A.L. (1936) Polychaetous annelids from the vicinity of Nonsuch Island, Bermuda. *Zoologica*, 21 (1), 49–68.

Treadwell, A.L. (1939) *Polychaetous annelids from Porto Rico and vicinity*. *Scientific Survey of Porto Rico and the Virgin Islands* (New York Academy of Sciences), 16, 151–319.

Verrill, A.E. (1900) Additions to the Turbellaria, Nemertina and Annelida of the Bermudas, with revisions of some New England genera and species. *Transactions of the Connecticut Academy of Arts and Sciences*, 10, 595–671.

Zhadan, A., Stupnikova, A. & Neretina, T. (2015) Orbiniidae (Annelida: Errantia) from Lizard Island, Great Barrier Reef, Australia with notes on orbiniid phylogeny. *Zootaxa*, 4019(1), 773–801.

<https://doi.org/10.11646/zootaxa.4019.1.27>

Zhadan, A. (2020) Review of Orbiniidae (Annelida, Sedentaria) from Australia. *Zootaxa*, 4860 (4), 451–502.

<https://doi.org/10.11646/zootaxa.4860.4.1>

Zhang, J., Kapli, P., Pavlidis, P. & Stamatakis, A. (2013) A general species delimitation method with applications to phylogenetic placements. *Bioinformatics*, 29 (22), 2869–2876.

<https://doi.org/10.1093/bioinformatics/btt499>

5. CHAPTER III. FIRST INTEGRATIVE APPROACH TO *PROTOARICIA LAEVIGATA* (GRUBE, 1855). BUT WHICH OF THEM?

*Primeira abordagem integrativa a *Protoaricia laevigata* (Grube, 1855). Mas, qual deles?*

Manuscrito formatado segundo a estrutura da revista Zootaxa
ISSN: 1175-5326
Impact Factor: 1.091
Qualis: A4

Autores: RICARDO CASTRO ÁLVAREZ^{1,3} & NATALIYA BUDAEVA²

¹ Programa de Pós-Graduação em Sistemas Costeiros e Oceânicos (PGSISCO), Universidade Federal do Paraná, Caixa Postal 61, 83255-976, Pontal do Paraná, Paraná, Brazil.

² Department of Natural History, University Museum of Bergen, 5020 Bergen, Norway.

³ Corresponding author. E-mail: ricastralvarez@gmail.com

ABSTRACT

Protoaricia laevigata (Grube, 1855) is one of the most problematic species of *Protoaricia* Czerniavsky, 1881, probably from the orbiniids. The species was first described from off Naples, the Mediterranean Sea, based on twelve syntypes. Eisig (1914) placed several species from different localities under synonymy with *P. laevigata*, since then, the species has been treated as cosmopolitan in literature (Hartman 1948, 1957; Taylor 1984, Zhadan 2020). Using an integrative approach, we demonstrated that the syntype material of *Protoaricia laevigata* comprises a mixture of two species. We redescribed and designated a lectotype for *P. laevigata* and reinstated *P. anserina* (Claparède, 1864). Based on COI, 16S, and 28S sequences of specimens from different localities, we reconstructed a phylogeny utilizing Maximum Likelihood and Bayesian Inference methods. Our result recovered three clades, one from the Black Sea, and two from the Mediterranean Sea, representing both morphotypes found in the *P. laevigata* type series. Our delimitation results, however, show weak evidence to split *P. laevigata*. For stability purposes, we reinstate *P. anserina* based on morphology and, in part, for molecular data. Our analysis reinforces that other records worldwide need to be reevaluated to reject the cosmopolitanism of *P. laevigata*.

5.1. INTRODUCTION

Protoaricia laevigata was first described as *Aricia laevigata* by Grube (1855) based on syntypes from off Naples, the Mediterranean Sea, and then transferred to *Naineris*. However, new evidence supported the transfer of most of the species of *Naineris sensu lato* to *Protoaricia* Czerniavsky, 1881, including *P. laevigata* (Álvarez *et al.* in prep), which was adopted in this study. *Protoaricia laevigata* is undoubtedly one of the most problematic species of *Protoaricia*, and probably from the orbinids. The distinction between this species and part of its congeners is unclear, and four very similar species are considered valid. Most of them are differentiated by the number of thoracic chaetigers or the chaetiger where the branchiae first appear (Blake 2017). However, these characters are not fixed, and at least the number of thoracic chaetigers is related to the size of the specimens in the genus (Solís-Weiss & Fauchald 1989, Álvarez *et al.* 2019).

The best-known similar species, *Protoaricia dendritica* (Kinberg, 1867), was first described for Vancouver Island, Canada, and frequently is misidentified as *P. laevigata* in museum collections. Four different lineages represent the species in Vancouver (Carr *et al.* 2011). According to Hartman (1957), the distinction between *P. laevigata* and *P. dendritica* was mainly because of their distribution, details of uncini, and the different shapes of thoracic neuropodial lobes. Moreover, Álvarez *et al.* (2019) found that most of the specimens from South and Southeastern Brazil misidentified as *P. laevigata* or *P. dendritica* (Álvarez *et al.*, 2019), were *Protoaricia aurantiaca* (Müller, 1858), instead.

Protoaricia laevigata was recorded in different Oceans, including the Pacific and the Atlantic. In his extensive monograph, Eisig (1914) placed under synonymy with *P. laevigata* several very distant species from Brazil, South Africa, the Caribbean Sea, Japan, Bermuda, Hawaii, France, and Naples. Since then, *Protoaricia laevigata* was considered cosmopolitan (Hartman 1948, 1957; Taylor 1984, Zhadan 2020) despite the significant variation in diagnostic characters. Another Mediterranean Species, *P. anserina* (Claparède, 1864), has only subuluncini in the superior fascicle of thoracic

neuropodia, whereas *P. laevigata* also possesses huge uncini. Eisig (1914) considered those huge uncini as broken subuluncini synonymizing the former species with *P. laevigata*. Because of the species' wide distribution, different authors suggested the need for a revision of the species (López 2012, Blake 2017, Zhadan 2020). Moreover, considering there are several developmental (Giangrande & Petraroli 1881; Giangrande & Frascchetti 1995), behavioral (Giangrande & Licciano 2001), and ecological (Giangrande *et al.* 2002) studies on *P. laevigata*, clarification of its status will improve our understanding of its biology. A redescription, based on both molecular data and morphological data needed to assess *P. laevigata* geographical range and to compare the topotype specimens with several records outside the Mediterranean Sea.

In this study, we redescribe *P. laevigata*, restrict its geographical distribution and reinstate *P. anserina* based on morphology and molecular data, giving the first step toward clarifying the *P. laevigata* species complex worldwide.

5.2. MATERIAL AND METHODS

5.2.1 Sample collection and morphological analysis

Specimens identified as *Protoaricia laevigata* were sampled from different Mediterranean localities and in the Black Sea (Table 1). The specimens were relaxed in magnesium chloride and fixed in ethanol for molecular studies.

Specimens were studied with a Leica MZ stereomicroscope and a Leica DM 6000 B compound microscope. Digital photos were taken with a digital camera Leica DMC5400 mounted on a Leica M205C stereomicroscope and a Leica DM 6000 B compound microscope and combined with the Z-stack function on Leica LAS software. Images were edited in Photoshop® software. Methyl green saturated in 80% ethanol and Shirlastain A were used to elucidate taxonomical characters. A left parapodium from a mid-thoracic and midabdominal segment was removed and mounted in glycerol for chaetal counts and structure.

For nomenclature, we followed the main revisions of the group (Hartman 1957, Blake 2017). Syntypes of *P. laevigata* were borrowed from the Museum für Naturkunde, Berlin (ZMB). Additional material was studied in the collections of the Natural History Museum of Los Angeles County (LAC-ACF), the Smithsonian National Museum of Natural History (USNM), and Natural History Collections, University Museum of Bergen, University of Bergen, Norway (ZMBN).

5.2.2 DNA analysis

We included 11 specimens morphologically preliminary identified *P. laevigata*, and 20 specimens belonging to other *Protoaricia* species from the “*Protoaricia laevigata*” clade (Alvarez et al., in prep.) *Protoaricia setosa* (Verril, 1900) was chosen as the outgroup since the species was recently reviewed and is closely related to the *Protoaricia laevigata*-like species.

5.2.3 DNA extraction, amplification, and sequencing

We extracted total DNA with QuickExtractTM and sequenced three regions (nuclear 28S and mitochondrial COI and 16S). We conducted the polymerase chain reactions (PCRs) in 25 µl volume reactions containing 2.5 µl 10xPCR buffer, 2 µl dNTP (2.5mM), 1 µl each primer (1 µM), 0.15 µl TaKaRa Taq (5U/ µl) with 1 µl template DNA, and double-distilled water to bring the mix to a final volume of 25 µl. We used the primers polyLCO and polyHCO (Carr *et al.* 2011) for COI; 16SarL and 16SbrH (Palumbi 1996) for the 16S rRNA; and 28v (28gg) and 28x (28ii) (Hillis and Dixon 1991) for 28S.

Table 1. Specimens examined in the study, with museum collection registration numbers, localities, GenBank accession numbers for COI, 16S and 28S, Bold Sample and Process IDs.

<i>Protoarcia</i>	Voucher	Locality	Coordinates	Collector	COI	16SrRNA	28SrRNA	BOLD Sample ID	BOLD Process ID
<i>P. laevigata</i> s. str.	ZMBN *****	Tunisia	NNNNNNN	D. Martin	NNNNNN	NNNNNN	NNNNNN	NL01	NNNNNNNN
<i>P. laevigata</i> s. str.	ZMBN *****	Tunisia	NNNNNNN	D. Martin	NNNNNN	NNNNNN	NNNNNN	NL03	NNNNNNNN
<i>P. laevigata</i> s. str.	ZMBN *****	Tunisia	NNNNNNN	D. Martin	NNNNNN	NNNNNN	NNNNNN	NL04	NNNNNNNN
<i>P. laevigata</i> s. str.	ZMBN *****	Tunisia	NNNNNNN	D. Martin	NNNNNN	NNNNNN	NNNNNN	NL05	NNNNNNNN
<i>P. laevigata</i> s. str.	ZMBN *****	Italy, Orbetello	42.432°N; 11.184°E	J. Laganeck	NNNNNN	NNNNNN	NNNNNN	NL07	NNNNNNNN
<i>P. laevigata</i> s. str.	ZMBN *****	Italy, Pisa	43.655°N; 10.281°E	J. Laganeck	NNNNNN	NNNNNN	NNNNNN	NL08	NNNNNNNN
<i>P. laevigata</i> s. str.	ZMBN *****	Italy, Cesareo	40.256°N; 17.891°E	J. Laganeck	NNNNNN	NNNNNN	NNNNNN	NL10	NNNNNNNN
<i>P. anserina</i>	ZMBN *****	Italy, Ardenza,	43.510°N; 10.317°E	J. Laganeck	NNNNNN	NNNNNN	NNNNNN	NL09	NNNNNNNN
<i>P. anserina</i>	ZMBN *****	Spain, Malta	NNNNNNNN	J. Laganeck	NNNNNN	NNNNNN	NNNNNN	NL11	NNNNNNNN
<i>Protoarcia</i> sp. 3	WS11270	Black Sea	NNNNNNNN	V. I. Radashevs ky	NNNNNN	NNNNNN	NNNNNN	NL12	NNNNNNNN
<i>P. oerstedii</i>		Spain, Catalonia, Tossa de Mar		J. Gil	NNNNNN	NNNNNN	NNNNNN	XXXXXXXX	Pro8

<i>Protoaricia</i>	Voucher	Locality	Coordinates	Collector	COI	16SrRNA	28SrRNA	BOLD Sample ID	BOLD Process ID
<i>P. oerstedii</i>		Italy, Calafuria	43.474603°N; 10.330264°E	J. Laganeck	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Pro9
<i>P. laevigata</i>	SERC #255675	USA, Tampa Florida	27.8854°N; 82.53378°W	E. Keppel	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	NL13
<i>P. laevigata</i>	SERC #255632	USA, Tampa Florida	27.88541°N; 82.53385°W	E. Keppel	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	NL14
<i>P. laevigata</i>	SERC #255674	USA, Tampa Florida	27.88543°N; 82.53441°W	E. Keppel	NNNNNNNN	–	NNNNNNNN	XXXXXXXXXX	NL15
<i>P. laevigata</i>	SERC #255941	USA, St Petersburg Florida	NNNNNNNN	E. Keppel	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	NL16
<i>P. laevigata</i>	SERC #256071	USA, Picnic Island Florida	NNNNNNNN	E. Keppel	NNNNNNNN	–	NNNNNNNN	XXXXXXXXXX	NL17
<i>P. chilensis</i>	MNRJP	Perú, Huarmey	NNNNNNNN	I. Carmona	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nch01
<i>P. chilensis</i>	MNRJP	Perú, Huarmey	NNNNNNNN	I. Carmona	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nch02
<i>P. aurantiaca</i>	MNRJP 007617	Brazil, Santa Catarina, Pântano do Sul	27.783563°S; 48.506634°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Naur01
<i>P. aurantiaca</i>	NNNNNNNN	Brazil, Paraná, Ilha do Mel, Bacia da Sereia	25.573386°S; 48.316551°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Naur07
<i>P. aurantiaca</i>	NNNNNNNN	Brazil, Rio de Janeiro, Ilha da Marambaia, Praia Grande	23.057600°S; 43.991322°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Naur20
<i>P. dendritica</i>		USA, California, Bodega Bay	NNNNNNNN	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nd02
<i>P. dendritica</i>		USA, California, Bodega Bay	NNNNNNNN	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nd03

<i>Protoaricia</i>	Voucher	Locality	Coordinates	Collector	COI	16SrRNA	28SrRNA	BOLD Sample ID	BOLD Process ID
<i>P. dendritica</i>		USA, California, Bodega Bay	NNNNNNNN	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nd01
<i>P. dendritica</i>	LACM-AHF Poly	USA, California, San Diego	NNNNNNNN	B. Swope, C. Sosa	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nd05
<i>P. dendritica</i>	WS10199	Canada, British Columbia, Calvert Island	NNNNNNNN	V. Radashevsky	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nd06
<i>P. dendritica</i>	WS10198	Canada, British Columbia, Calvert Island	NNNNNNNN	V. Radashevsky	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nd07
<i>P. dendritica</i>	ZMBN	Japan, Sea of Japan	NNNNNNNN	N. Budaeva	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nd14
<i>P. dendritica</i>	ZMBN	Japan, Sea of Japan	NNNNNNNN	N. Budaeva	NNNNNNNN	NNNNNNNN	NNNNNNNN	XXXXXXXXXX	Nd15
<i>P. setosa</i>	MNRJP 007631	Brazil, Santa Catarina	27.695°S; 48.565°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	Alvarez_NS03	NAI003-23
<i>P. setosa</i>	MNRJP 007649	Brazil, Pernambuco	8.355°S; 34.953°W	R. Álvarez	NNNNNNNN	NNNNNNNN	NNNNNNNN	Alvarez_NS21	NAI016-23

PCR conditions for COI were, 94 °C for 40 s; five cycles of 94 °C for 40 s, 45 °C for 40 s, 72 °C for 2 min; 40 cycles of 94 °C for 40 s, 48 °C for 40 s, 72 °C for 2 min; and 72 °C for 7 min. For 16S, 94 °C for 3 min; 40 cycles of 94 °C for 30 s, 51 °C for 30 s (decreasing 0.2°C per cycle), 72 °C for 70 s; 72 °C for 7 min. For 28S, 94 °C for 40 s; 40 cycles of 94 °C for 40 sec, 54°C for 40 s, 72 °C for 2 min; 72 °C for 7 min.

The PCR products of successful amplifications were sent to Macrogen Europe. The new sequences were deposited in GenBank.

5.2.4 Sequence alignment, phylogenetic analysis

Sequences were assembled and edited in GENEIOUS R6 and aligned in MAFFT with the L-INS-i strategy (<https://mafft.cbrc.jp/alignment/server/index.html>). We checked gene sequences and concatenated the three markers in MEGA11 (Tamura et al. 2021). The ML was conducted in IQ-TREE VERSION IQ-Tree 2.2.0. (Minh et al. 2020) for individual markers and the concatenated partitioned matrix, with ultrafast bootstrap (Hoang *et al.*, 2018). The best substitution models were automatically selected in ModelFinder (COI: 1st codon, TIM2e+G4; 2nd codon, F81+F; 3rd codon, TIM2+F+G4; 16S: TIM2e+G4; 28S: TVMe+I) (Kalyaanamoorthy *et al.* 2017). The statistical support of the branches was estimated with 1000 bootstrap replicates. Pairwise distances (p-distances) for each target clade and between clades was estimated in Mega11. We delineated the species using Bayesian Poisson tree process (bPTP) (Zhang *et al.* 2013) from [http:// species.h-its.org/ptp/](http://species.h-its.org/ptp/) using the single markers ML trees.

5.3. RESULTS

5.2.5 Phylogenetic reconstruction

Our concatenated alignment of COI, 16S and 28S sequences comprised 1826 pb, with 654, 515 and 655 pb respectively. Mediterranean specimens of *P. laevigata* formed two different well supported clades in the combined COI, 16S, and 28S analysis (BP = 76-94), however single marker trees reconstructions were inconclusive. Despite both clades were also supported in the COI tree reconstruction, only one of them was recovered on 16S tree, while the second was not recovered. Moreover, in the reconstruction based on 28S all Mediterranean specimens were recovered in a single clade with maximum support (BP = 100). The specimen from the Black Sea (NI12) was sister to the Mediterranean clades in COI and 16S analyses and was missing in the 28S analysis.

Our species delimitation analysis resulted in a well-supported delimitation scheme of the three candidate species for COI only. The delimitation results on both 16S and 28S did not support our putative species, with all *P. laevigata* complex clustered in a single clade. Even though there were conflicts in our delimitation results considering all three markers, our final phylogenetic hypothesis suggested the presence of three species, which was highly supported with morphology. *Protoaricia laevigata* and *Protoaricia anserina* from the Mediterranean Sea, and *Protoaricia* sp. from the Black Sea.

5.2.6 Genetic distances

Genetic distances are depicted in Tables 2–5. Estimated genetic distances between the three clades in COI varied from 4.7 to 7.7%, in 16S from 1.7 to 3.7%, and in 28S there were no divergences. The intraspecific genetic divergences in COI were 0.7–1.1%, in 16S were 0.1–0.5%, and in 28S were 0%.

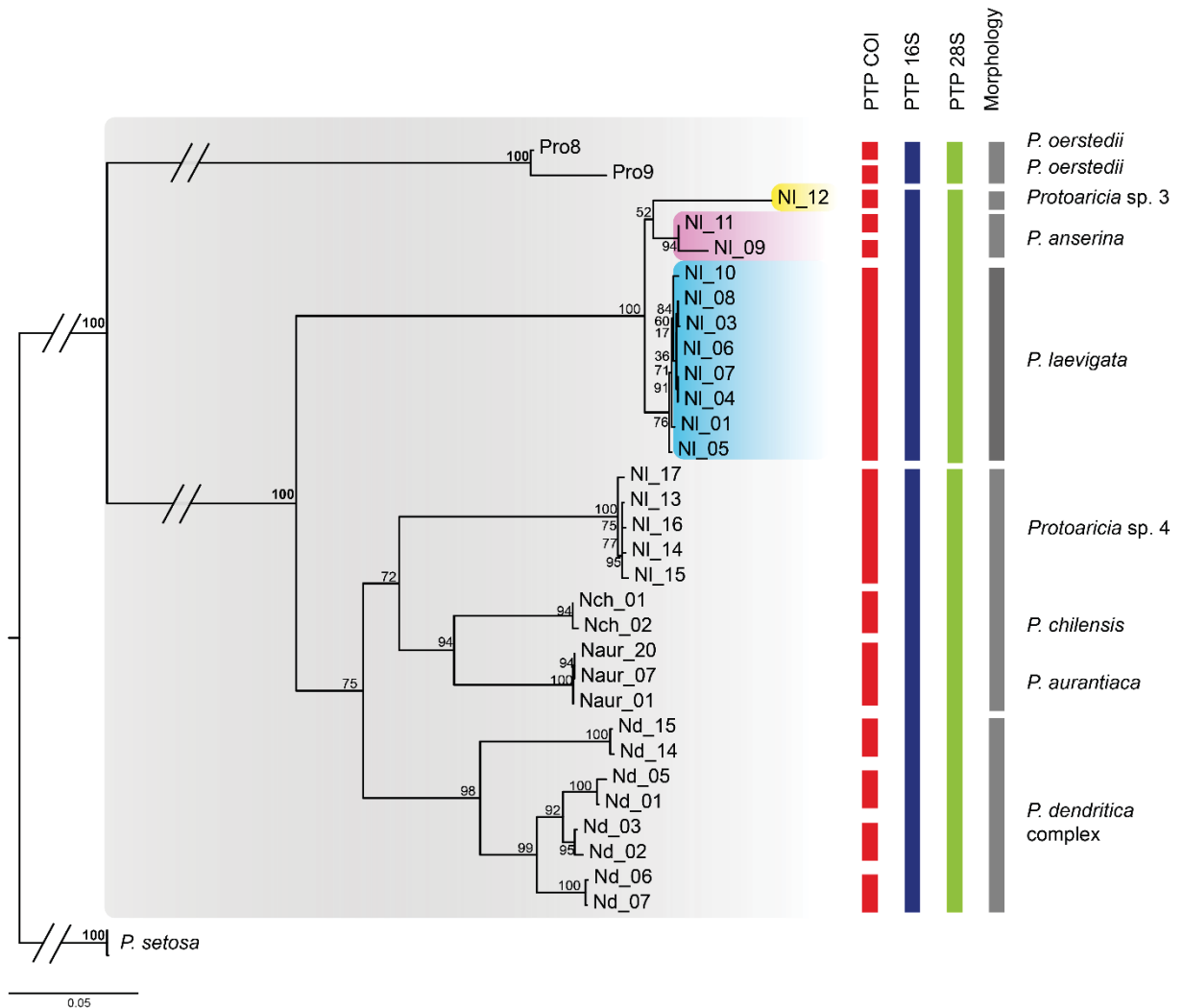


Figure 1. Maximum Likelihood tree of the combined COI, 16S, and 28S dataset; numbers on nodes indicate Bootstrap support values; species delimitation results inferred by morphology and DNA-based methods are indicated right to the consensus tree; bPTP was applied separately to the individual gene trees. White bars indicate missing data.

Table 2. Percentage of uncorrected p-distance matrix within clades of the *P. laevigata* species complex from the Mediterranean Sea based on COI, 16S, and 28S DNA sequences.

<i>Protoaricia</i>	COI	16S	28S
<i>Protoaricia</i> sp. 3	-	-	0.0000
<i>P. anserina</i>	0.0116	0.0058	0.0000
<i>P. laevigata</i>	0.0071	0.0014	0.0000

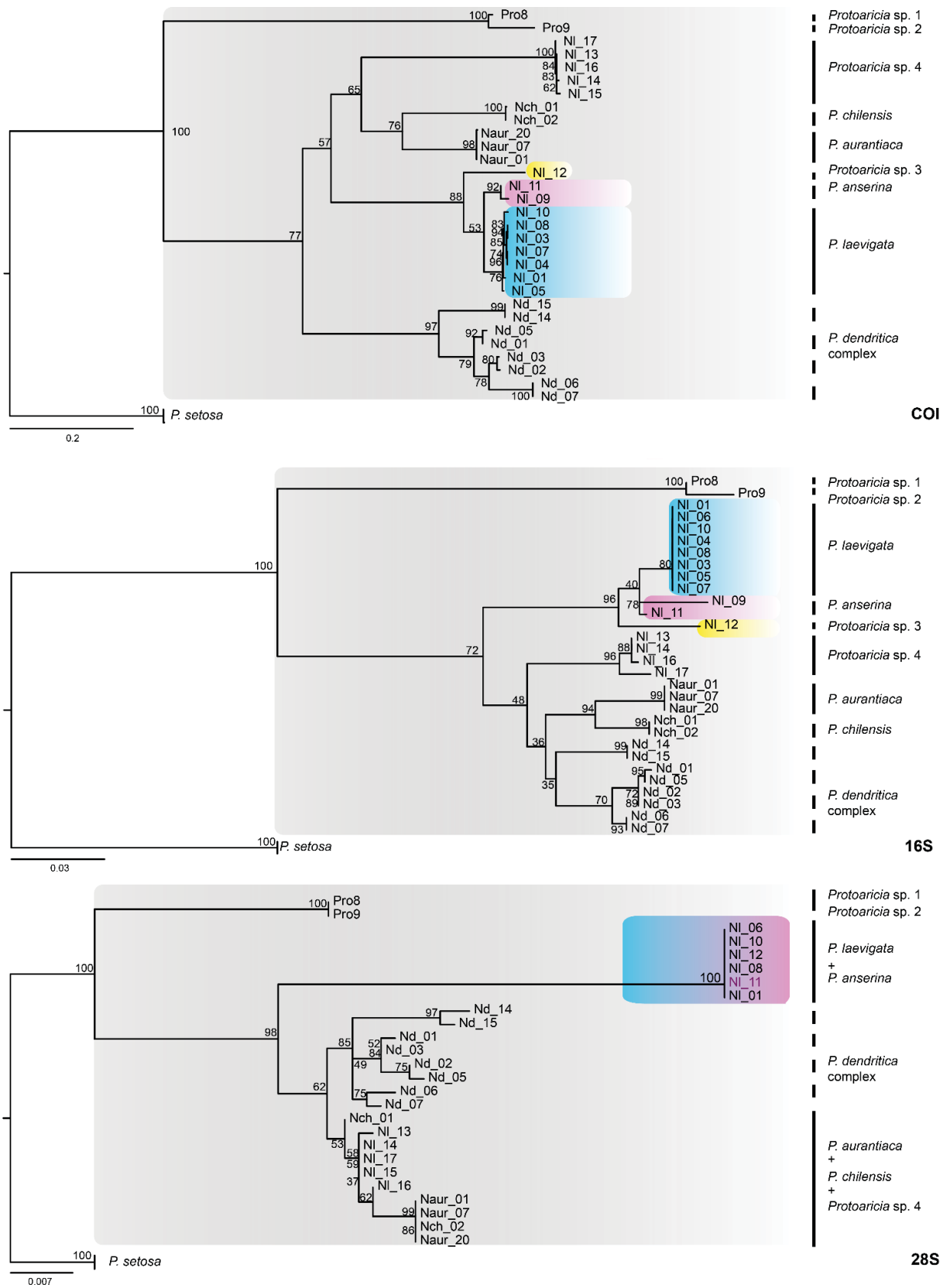


Figure 2. Maximum Likelihood trees of single COI, 16S, and 28S DNA markers; numbers on nodes indicate Bootstrap support values.

Table 3. Percentage of uncorrected p-distance matrix between clades of the *P. laevigata* species complex from the Mediterranean Sea based on COI sequences.

<i>Protoaricia</i>	<i>Protoaricia</i> sp. 3	<i>P. anserina</i>	<i>P. laevigata</i>
<i>Protoaricia</i> sp. 3	-		
<i>P. anserina</i>	0.0734	-	
<i>P. laevigata</i>	0.0771	0.0473	-

Table 4. Percentage of uncorrected p-distance matrix between clades of the *P. laevigata* species complex from the Mediterranean Sea based on 16S sequences.

<i>Protoaricia</i>	<i>Protoaricia</i> sp. 3	<i>P. anserina</i>	<i>P. laevigata</i>
<i>Protoaricia</i> sp. 3	-		
<i>P. anserina</i>	0.0307	-	
<i>P. laevigata</i>	0.0377	0.0170	-

Table 5. Percentage of uncorrected p-distance matrix between clades of the *P. laevigata* species complex from the Mediterranean Sea based on 28S sequences.

<i>Protoaricia</i>	<i>Protoaricia</i> sp. 3	<i>P. anserina</i>	<i>P. laevigata</i>
<i>Protoaricia</i> sp. 3	-		
<i>P. anserina</i>	0.0000	-	
<i>P. laevigata</i>	0.0000	0.0000	-

5.2.7 Taxonomy

Family ORBINIIDAE Hartman, 1942

GENUS *Protoaricia* Blainville, 1828

Type species. *Protoaricia oerstedii* (Claparède, 1864)

Diagnosis. Prostomium rounded or truncate anteriorly. Peristomium with 1–2 achaetous rings. Thorax with 9–23 or more segments; branchiae first present either from thoracic or abdominal segments, laterally displaced. Thoracic neuropodia with 0–2 postchaetal lobes; no subpodial lobes. Thoracic neurochaetae only capillaries, or capillaries mixed with blunt-tipped uncini, sometimes hooded, and subuluncini. Abdominal chaetae including capillaries and sometimes furcate chaetae in notopodia and capillaries and imbedded or protruding aciculae in neuropodia. Rounded dorsal sensory organs present, paired per segment or multiple.

***Protoaricia laevigata* (Grube, 1855)**

(Figures 3–4)

Aricia laevigata Grube, 1855: 112–113.*Naineris laevigata* Grube, 1855: 112–113 (in part).**Type material.** Lectotype: ZMB 4689-Q. Paralectotypes (3 specimens).**Additional material.** Tunisia: ZMBN____(DNA voucher NI01), ZMBN____(DNA voucher NI02), ZMBN____(DNA voucher NI03), ZMBN____(DNA voucher NI04), ZMBN____(DNA voucher NI05). Italy. Orbetello: ZMBN____(DNA voucher NI06), ZMBN____(DNA voucher NI07). Pisa: ZMBN____(DNA voucher NI08). Cesáreo ZMBN____(DNA voucher NI10).**Type locality:** Naples, Italy.**Diagnosis.** Thoracic neuropodia with a superior fascicle with around 6 anterior transversal rows of subuluncini, and one posterior transversal row of huge straight uncini, larger and thicker than accompanying subuluncini, with tips oriented anteriorly: inferior, oblique, semicircular fascicle of 1–2 rows of 10 small uncini together with around 10 long crenulated capillaries.**Description (based on lectotype).** Body long, cylindrical, lectotype complete (ZMB *****) with 52 mm long, 2.8 mm wide, for more than 200 chaetigers. Color in alcohol pale yellow. Body with two distinct areas marked by displacement of parapodia; thorax slightly flattened (Fig. 3A); abdomen cylindrical with parapodia directed dorsally (Fig. 3D). Space between branchial basis and notopodium equal to the distance between both notopodium and neuropodium. Ventral surface of thoracic segments more or less smooth (Fig. 3B); abdominal segments with longitudinal ventral groove (Fig. 3A).

Prostomium rounded (Fig. 3A-C), subtriangular; eyespots not observed, nuchal organ restricted to two lateral grooves. Peristomium with 1-2 achaetous rings, clearly 2 in ventral view (Fig. 3B); mouth with striated lips. Proboscis not observed in lectotype, but everted in paralectotypes, represented by four fusiform, unramified lobes, inferior pair longer (Fig. 3C).

Branchiae first present from chaetiger 6–7, tapering to acute tip, ciliated; first branchiae one third of notopodial lobes and one-fifth of mid-abdominal branchiae (Fig. 3D); branchiae triangular at first, fusiform well developed in mid-abdominal segments, reducing in size and triangular shape in posterior segments (Fig. 3D). Inconspicuous dorsal sensory organs present from mid-thoracic segments, rounded, paired (Fig. 3D). Dorsal crest present from abdominal segments, bearing low, straight ciliary bands (Fig. 3G); pre- and pos branchial papillae present. Lateral organ discreet (Fig. 3E).

Thorax comprising 17 chaetigers, partially flattened. Parapodia biramous. Thoracic notopodial lobes lanceolate; thinner, and elongated in abdominal segments, finger-like. Thoracic neuropodial lobes foliaceous, with upper subtriangular papilla (Fig. 3E-F); abdominal neuropodial lobes triangular, smaller (Fig. 3G).

Thoracic notochaetae with bundles of 15–20 crenulated capillaries. Abdominal notochaetae with 10–15 crenulated capillaries. Thoracic neurochaetae (Fig. 3E-F) with around 6 anterior transversal rows of subuluncini in different levels, increasing gradually in height from anterior to posterior, with tips oriented posteriorly; one transversal posterior row of huge, straight uncini (Fig. 4A), blunt, bigger than accompanying subuluncini, with tips oriented anteriorly; and inferior, oblique, semicircular fascicle of around 1–2 rows of 10 small uncini above 10 crenulated capillaries. Abdominal neurochaetae with 3–5 straight acicular spines and around 10 capillaries intermixed (Fig. 4B).

Pygidium with anus terminal, superiorly oriented; anal cirri absent (Fig. 3H).

Variation. The number of thoracic chaetigers in paralectotypes varied from 17 to 21 and the branchiae were first present from chaetigers 6-7.

Distribution. Type locality: Gulf of Naples, Mediterranean Sea.

Additional occurrence. Tunisia, Italy: Orbetello, Pisa, Porto Cesareo.

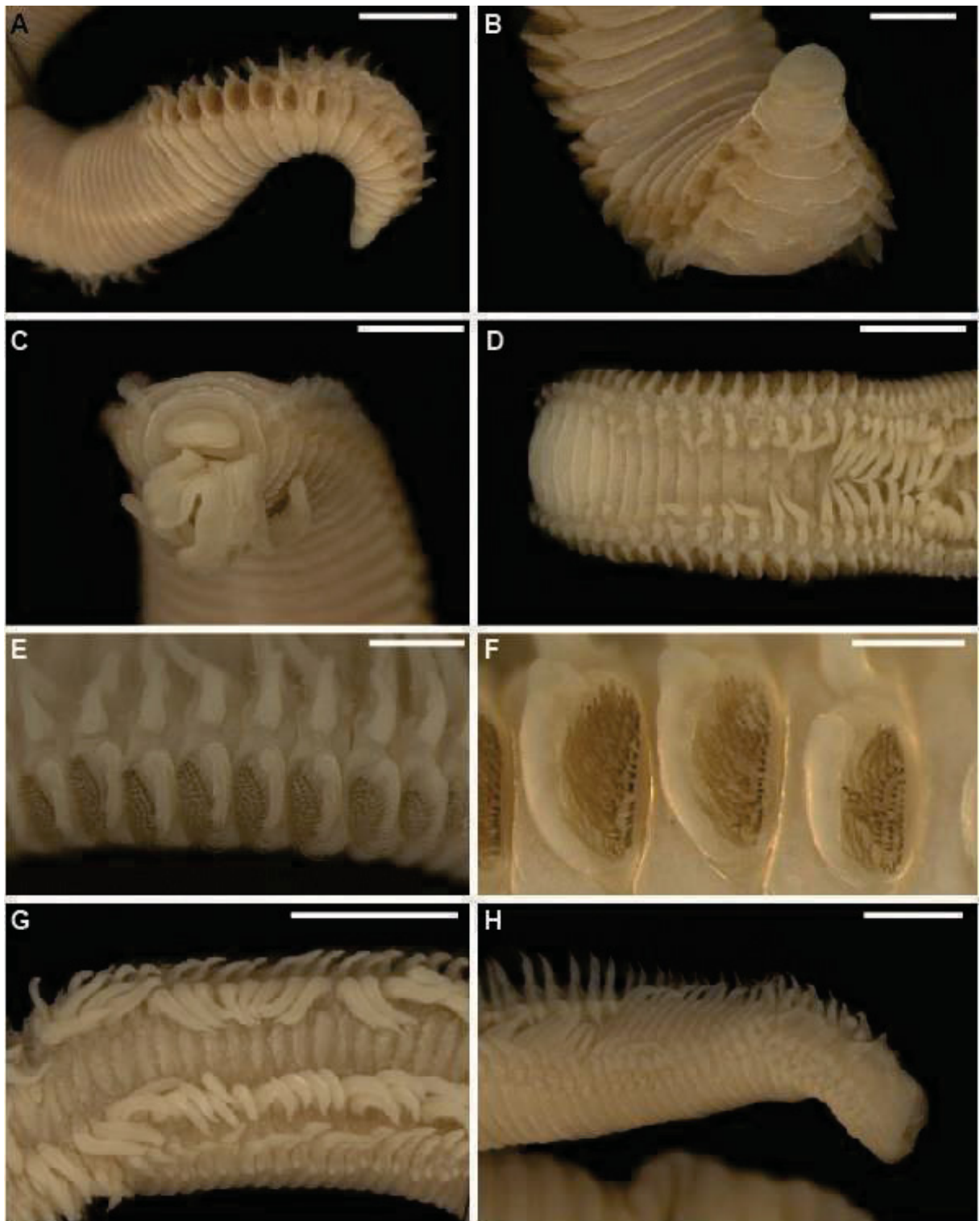


Figure 3. *Protoaricia laevigata*, lectotype (ZMB____): (A) Anterior end, lateral view; (B) Anterior end, dorsal view; (F) Thoracic neuropodia; (G) Abdominal segments, dorsal view; (H) Posterior end, dorsolateral view. Paralectotype (ZMB____): (C) Anterior end, ventral view; (D) Thoracic segments, dorsal view; (E) Thoracic parapodia, lateral view. Scale bars; (A, D, G): 2 mm; (E-F): 0.5 mm; (B-C, H): 1 mm.

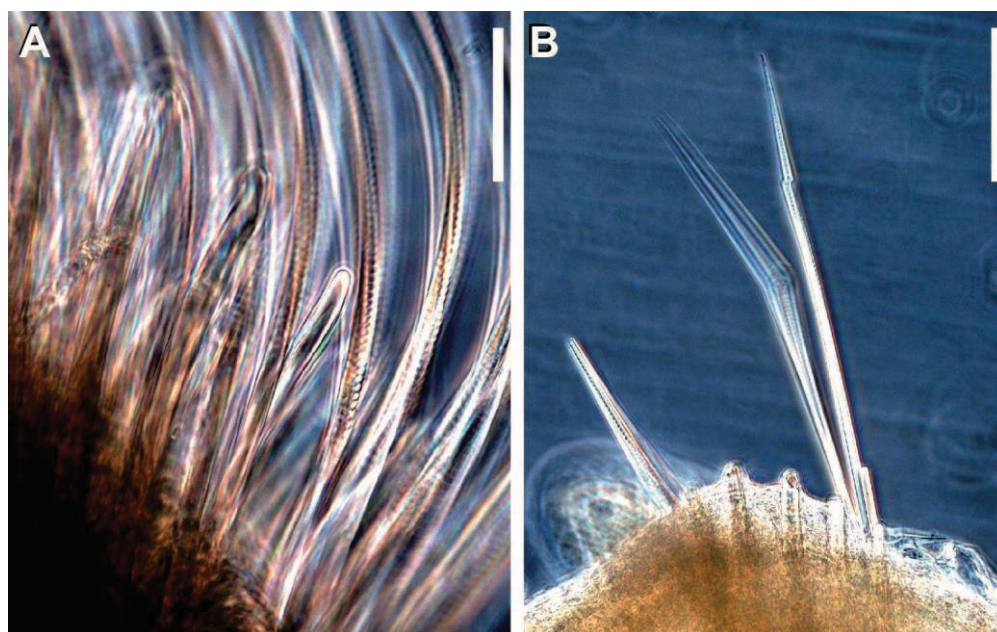


Figure 4. *Protoaricia laevigata*, paralectotype (ZMB*****): (A) Thoracic neurochaetae, huge uncini; (B) Abdominal neurochaetae. Scale bars; (A): 50 μm ; (B): 100 μm .

Remarks. We selected the lectotype for *P. laevigata* as the type series comprised two morphotypes. The second morphotype was identified as *P. anserina* (see the re-description of the species below).

It is believed that the type locality of *P. laevigata* is Nice (Villalobos-Guerrero & Molina-Acevedo 2014, Meca et al. 2021). However, all the syntypes were collected near Naples, and this locality should be used as the type locality of *P. laevigata*. Wiktor (1980) designated a lectotype, based on a loan received from the ZMB, consisting of a single syntype. In the absence of a formal description, it is impossible to distinguish if the selected specimen corresponded to *P. laevigata* or *P. anserina*, thus, we disregarded her designation.

Eisig (1914) considered *P. laevigata* a senior synonym of many similar species described, i.e., *Anthostoma hexaphyllum* Schmarda, 1861 (South Africa); *Anthostoma ramosum* Schmarda, 1861 (Jamaica); *Aricia (Scoloplos) fuscibranchis* Grube, 1878 (Japan); *Aricia armata* Hansen, 1882 (Brazil); *Aricia platycephala* McIntosh, 1885 (Bermuda); *Lacydes havaicus* Kinberg, 1866 (Hawaii); *Theodisca anserina* Claparède, 1864 (France, Mediterranean Sea); *Theodisca liriostoma* Claparède, 1869 (Naples) worldwide, expanding the distribution of the species to Jamaica, Brazil, Pacific and

Indian Oceans, besides Australia (Day 1977) equivocally. Our phylogenetic reconstruction included materials from the Atlantic and the Pacific oceans, however none of these specimens clustered together with the Mediterranean *P. laevigata* or *P. anserina*.

The most similar species to *P. laevigata* are *P. aurantiaca*, *P. chilensis*. *Protoaricia laevigata* differs, however, in having more numerous rows of subuluncini in thoracic neuropodia (5-7), whereas in *P. aurantiaca* and *P. chilensis* the number is reduced (3-4).

***Protoaricia anserina* Claparède, 1864, reinst., n. comb.**

(Figures 5–6)

Theodisca anserina Claparède, 1864: 504–505, Pl. IV, fig. 6.

Naineris anserina: Eisig 1914.

Type material. Neotype: (ZMB *****. 1 specimen). Paralectotypes: (ZMB *****. 5 specimens)

Additional material. Italy. Naples: ZMB ***** (7 specimens). Ardenza: ZMBN***** (DNA voucher NI09). Malta: ZMBN***** (DNA voucher NI11)

Diagnosis. Thoracic neuropodia with superior fascicle consisting of 5-7 transversal rows of subuluncini of the same width and inferior oblique semicircular fascicle of 10-15 small thin uncini, together with around 10 long crenulated capillaries. Thick uncini absent.

Description (based on neotype). Body long, neotype complete specimen (ZMB*****) 50 mm long, 2.8 mm wide, with 219 chaetigers. Color in alcohol light tan. Body with two distinct regions; thorax markedly flattened; abdomen cylindrical with parapodia dorsally displaced. Space between branchial basis and notopodium equal to the distance between notopodium and neuropodium. Ventral surface of thoracic segments smooth; abdominal segments with main, evident ventral groove (Fig. 5H).

Prostomium rounded, straight in front (Figs. 5A-C); eyespots absent; nuchal organ represented by two lateral grooves (Fig. 5A). Peristomium with 2 achaetous rings (Fig. 5A); mouth positioned ventrally, with striated lips. Proboscis not everted in neotype, but everted in topotype specimens, with long, wide, ligulate lobes.

Branchiae first present from chaetiger 6 (Fig. 5A); triangular in first, increasing in size in last thoracic chaetigers, best developed and adopting fusiform shape in abdominal segments, reducing in size in last segments; branchiae two times longer than accompanying notopodial lobes. Paired dorsal sensory organs from mid-thoracic segments, rounded (Fig. 5D). Dorsal crest absent, bearing straight ciliary bands instead (Fig. 5F); pre- and pos branchial papilla not observed. Lateral organ present.

Thorax comprising 23-24 chaetigers, flattened (Fig. 5B). Parapodia biramous. Thoracic notopodial lobes lanceolate (Fig. 5B); thinner in abdominal segments, subtriangular (Fig. 5G). Thoracic neuropodial lobes foliaceous, with rounded boundaries, bearing upper triangular papilla only in last 3-4 thoracic segments (Fig. 5E); abdominal neuropodial lobes triangular, smaller than thoracic, slightly longer than corresponding acicular spines (Fig. 5G).

Notochaetae including crenulated capillaries in two groups at different levels and furcate chaetae (Fig. 6A). Thoracic neuropodia with superior fascicle consisting of 5-7 superior transversal rows of subuluncini of the same width and inferior oblique semicircular fascicle of 10-15 small thin uncini, together with around 10 long crenulated capillaries (Fig. 5E). Abdominal neurochaetae with 3-5 acicular spines intermixed with around 10 crenulate capillaries (Figs. 5G, 6B).

Pygidium with terminal anus, posteriorly oriented; anal cirri absent (Fig. 5H).

Variation. The number of thoracic chaetigers in topotypes varied from 20 to 24 and the chaetiger where branchiae first appear ranged from 5 to 6.

Distribution. Type locality: Gulf of Naples, Mediterranean Sea.

Additional occurrence. Italy: Ardenza, Spain: Malta

Remarks. Eisig (1914) synonymized *P. anserina* with *P. laevigata* disregarding obvious differences in the shape of the posterior row of neuropodial thoracic uncini.

However, the posterior row of neurochaetae in the superior fascicle of thoracic neuropodia are different in both species. In *Protoaricia anserina* these chaetae are represented by long subuluncini, similar in width to the accompanying subuluncini. In *P. laevigata*, however, they consist of thick, straight, blunt, uncini, larger than accompanying subuluncini. Despite it is not clear if Claparède deposited the specimens and their condition, his description agrees with material herein described.

The Black Sea samples consisted of a single specimen in poor conditions. The only species described from the Black Sea is *P. capsulifera* (Bobretzky, 1870). Considering the similarities of *P. capsulifera* with our single specimen, it is possible that our specimens are the same species. However, as our single specimen is not in good condition and Bobretzky's material was not examined (if it still exists), we kept the genus level identification.

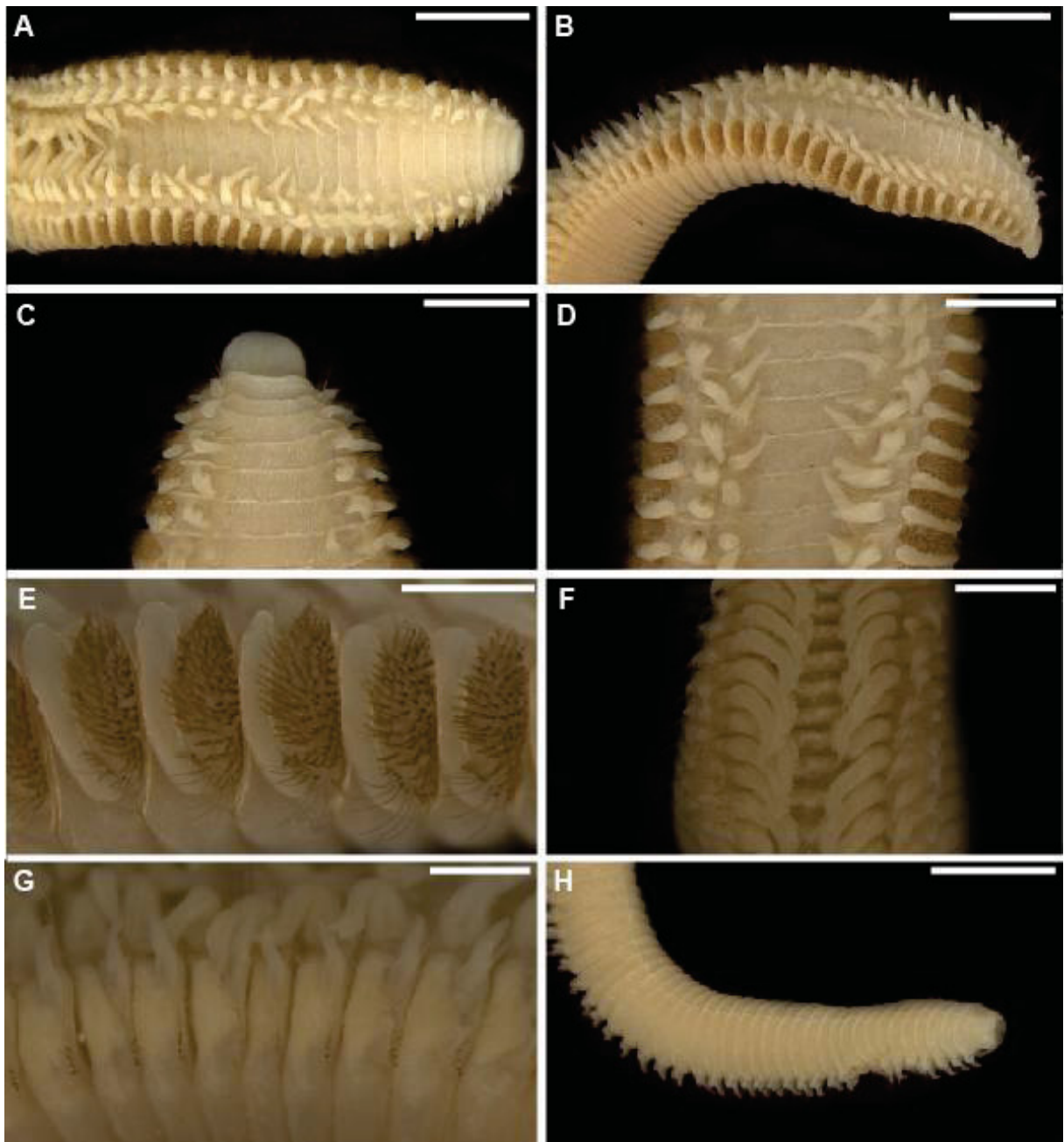


Figure 5. *Protoaricia anserina*, lectotype (ZMB****): (A) Thorax, dorsal view; (B) Thoracic segments, lateral view; (C) Anterior end, dorsal view; (D) Thoracic segments, dorsal view; (E) Thoracic neuropodia, lateral view; (F) Abdominal segments, dorsal view; (G) Abdominal parapodia, lateral view; (H) Posterior end ventrolateral view. Scale bars; (A, B): 2 mm; (C-D, F, H): 1 mm; (E, G): 0.5 mm.

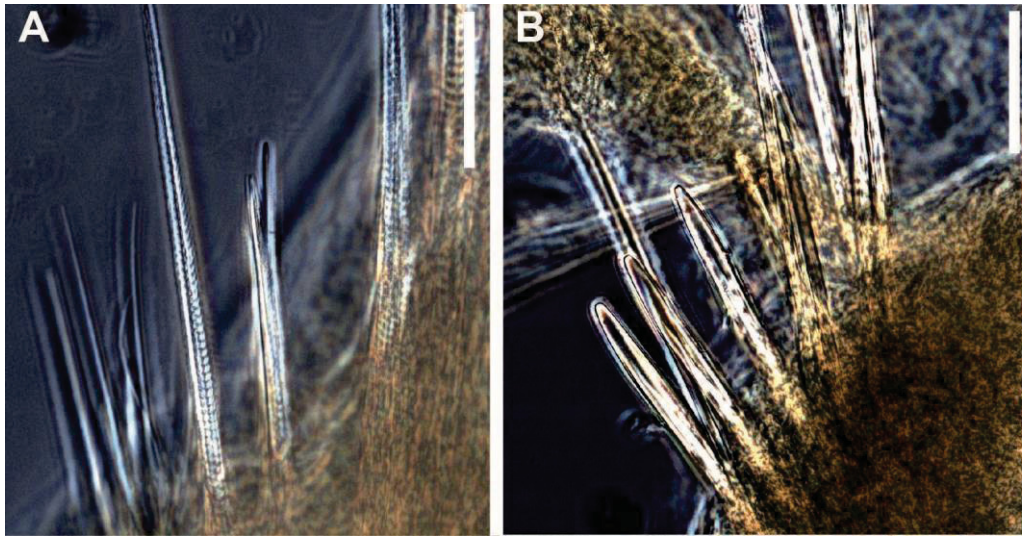


Figure 6. *Protoaricia anserina*, lectotype (ZMB*****): (A) Thoracic notochaetae, furcate chaetae; (B) Abdominal neurochaetae, acicula. Scale bars; (A-B): 50 μ m.

5.4. DISCUSSION

This work should not be considered the last word on *P. laevigata*; it is just one step towards the understanding its boundaries. The only time *P. laevigata* was subjected to revision was a hundred years ago, by Eisig (1914). By synonymizing a number of species from very distant localities and different morphology, some highly variable characters were disregarded, including the number of subuluncini rows, the shape of chaetae, the number of thoracic chaetigers and the chaetiger where branchiae are first present. We distinguished two different morphotypes in the type series of *P. laevigata*, corresponding to the original descriptions of *P. laevigata* and *P. anserina* respectively.

The morphological differences between *P. anserina* and *P. laevigata* were supported by the concatenated dataset tree, even though they were inconclusive when considering each marker separately. The genetic distances also supported the presence of two species from the Mediterranean Sea in our dataset. The divergence between both species was considerable for a species complex, with 4.73% for COI and 1.7% for 16S, but not for 28S (0%). Although 28S and 16S delimitation results did not delimit the species boundaries of the *P. laevigata* complex, we reinstated *P. anserina* based on solid morphological evidence supported by COI.

Considering that the Mediterranean and the Black Sea were part of a unique water mass in the past, we may assume that the species studied are in the gray zone of speciation (De-Queiroz 2007, Hausdorf 2011). The gray zone may span from 0.5% to 2%, independently of ecological features of the species (Roux et al. 2016, Ng & Tan 2021). Recent speciation events were reported in other annelids. Elgetany et al. (2019) obtained conflicting results among different loci in two species of *Diopatra* but decided to keep each species independent because of the taxonomic stability of use of the names in different geographical regions. Assuming *P. laevigata* is historically the most problematic of the “cosmopolitan” species of *Protoaricia*, that our candidate species are morphologically distinguished and supported for morphological data, the split of both species and subsequent designation of a lectotype for *P. laevigata* and a neotype for *P. anserina* are the best solution to keep taxonomical stability of *P. laevigata*. We reinforce that care must be taken when reporting *P. laevigata* in the Mediterranean, and especially outside this area.

Acknowledgements

The authors want to thank Birger Neuhaus (ZMB) for the loan of the syntypes. A special acknowledgement to Joachim Langeneck for providing ethanol-fixed materials from the Mediterranean for this study. We thank Louise Lindblom for the training to R.A. in the DNA-lab (UiB). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - (Doctorate fellowship, process number: 88882.382993/2019-01) and CAPES-PRINT-UFPR (Ph.D. Exchange program, process number: 88887.696847/2022-00).

5.5. REFERENCES

- Alvarez, R.C., Miranda, V.R. & Brasil A.C. (2019) Redescription of *Naineris aurantiaca* (Müller, 1858) and designation of a neotype from the Brazilian coast (Annelida: Orbiniidae). *Zootaxa*, 4571(1), 125–136. <http://dx.doi.org/10.11646/zootaxa.4571.1.8>
- Blainville, H. de (1828) *Dictionnaire des Sciences naturelles*, 57, 368–501.
- Blake, J. (2017) Polychaeta Orbiniidae from Antarctica, the Southern Ocean, the Abyssal Pacific Ocean, and off South America. *Zootaxa*, 4218(1), 1–145. <https://doi.org/10.11646/zootaxa.4218.1.1>

- Carr, C. M., Hardy, S. M., Brown, T. M., Macdonald, T. A., & Hebert, P. D. (2011) A tri-oceanic perspective: DNA barcoding reveals geographic structure and cryptic diversity in Canadian polychaetes. *PLoS one*, 6(7), e22232. <https://doi.org/10.1371/journal.pone.0022232>
- Claparède, E. (1864) Glanures zootomiques parmi les annélides de Port-Vendres (Pyrénées Orientales). *Mémoires de la Société de physique d'histoire naturelle de Genève*, 17, 463–600.
- De Queiroz, K. (2007) Species concepts and species delimitation. *Systematic Biology*, 56: 879–886. <https://doi.org/10.1080/10635150701701083>
- Eisig, H. (1914) Zur Systematik, Anatomie und Morphologie der Ariciiden nebst Beiträgen zur generellen Systematik. *Mittheilungen aus der Zoologischen Station zu Neapel*, 21, 154–600. [plates 10–27, 23 text figures.]
- Elgetany, A. H., van Rensburg, H., Hektoen, M., Matthee, C., Budaeva, N., Simon, C. A., & Struck, T. H. (2020) Species delineation in the speciation grey zone—The case of *Diopatra* (Annelida, Onuphidae). *Zoologica Scripta*, 49(4), 516–534. <https://doi.org/10.1111/zsc.12421>
- Grube, A. E. (1840) *Actinien, Echinodermen und Würmer des Adraitischen-und Mittelmeers nach eigenen Sammlungen beschrieben*, Königsberg: J.H. Bon. 92 pp.
- Hartman, O. (1948) The marine annelids erected by Kinberg. With some notes on some other types in the Swedish State Museum. *Arkiv för Zoologi*. 42A (1), 1–137, 18 plates.
- Hausdorf, B. (2011) Progress toward a general species concept. *Evolution*, 65 (4), 923–931. <https://doi.org/10.1111/j.1558-5646.2011.01231.x>
- Hoang, D. T., Chernomor, O., Von Haeseler, A., Minh, B. Q., & Vinh, L. S. (2018) UFBoot2: improving the ultrafast bootstrap approximation. *Molecular biology and evolution*, 35(2), 518–522. <https://doi.org/10.1093/molbev/msx281>
- Hartman, O. (1942) A review of the types of polychaetous annelids at the Peabody Museum of Natural History, Yale University. *Bulletin of the Bingham Oceanographic Collections*, 8, 1–98.
- Hartman, O. (1957) Orbiniidae, Apistobranchidae, Paraonidae, and Longosomidae. *Allan Hancock Pacific Expeditions*, 15 (3), 211–393, pls. 20–44, 1 chart.
- Hillis, D. M., & Dixon, M. T. (1991) Ribosomal DNA: molecular evolution and phylogenetic inference. *The Quarterly review of biology*, 66(4), 411–453.

Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K., Von Haeseler, A., & Jermini, L. S. (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature methods*, 14(6), 587–589. <https://doi.org/10.1038/nmeth.4285>

Kinberg, J. (1867) *Annulata nova. Öfversigt af Kungliga Vetenskaps-Akademiens Förhandlingar, Stockholm*, 22, 239–258.

López, E. (2012) Familia Orbiniidae Hartman, 1942. In: Parapar, J., Alós, C., Núñez, J., Moreira, J., López, E., Aguirrezabalaga, F., Besteiro, C., Martínez, A. (Eds.), *Annelida, Polychaeta* III. In: Ramos, M.A., Alba, J., Bellés, X., Gosálbez, J., Guerra, A., Macpherson, E., Martín, F., Serrano, J. & Templado, J. (Eds.) *Fauna Ibérica*, 36, 96–160. Museo Nacional de Ciencias Naturales. Editorial CSIC-CSIC Press: Madrid, Spain.

Meca, M. A., Zhadan, A., & Struck, T. H. (2021) The early branching group of Orbiniida sensu Struck et al., 2015: Parergodrilidae and Orbiniidae. *Diversity*, 13(1), 29. <https://doi.org/10.3390/d13010029>

Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., Von Haeseler, A., & Lanfear, R. (2020) IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Molecular biology and evolution*, 37(5), 1530–1534. <https://doi.org/10.1093/molbev/msaa015>

Müller, F. (1858) Einiges über die Anneliden-Fauna der Insel Santa Catharina an der brasilianischen Küste. *Archiv für Naturgeschichte*, 24, 211–220.

Ng, C. K. C., & Tan, J. (2021) Cryptic species and grey zone speciation of the *Barbodes binotatus* complex (Teleostei, Cyprinidae) in Sundaland. *Journal of fish biology*, 99(4), 1256–1273. <https://doi.org/10.1111/jfb.14829>

Palumbi, S. R., Martin, A. P., Romano, S., McMillan, W. O., Stice, L., & Grabowski, G. (1991) *The simple fool's guide to PCR, Special Publication. Depart 's Guide to PCR (Special Publication). Depart s Guide to PCR (Special Publication). Department of Zoology, University of Hawaii, Honolulu.*

Roux, C., Fraisse, C., Romiguier, J., Anciaux, Y., Galtier, N., & Bierne, N. (2016) Shedding light on the grey zone of speciation along a continuum of genomic divergence. *PLoS biology*, 14(12), e2000234. <https://doi.org/10.1371/journal.pbio.2000234>

Solís-Weiss, V. & Fauchald, K. (1989) Orbiniidae (Annelida: Polychaeta) from mangrove root-mats in Belize, with a revision of the protoariciin genera. *Proceedings of the Biological Society of Washington*, 102 (3), 772–792.

Taylor, J.L. (1984) Chapter1 Orbiniidae. In: Uebelacker, J.M. & Johnson, P.G. (Eds.), *Taxonomic guide to the polychaete of the Northern Gulf of Mexico*. Vol. I. 1-1 to 1-38. [Barry A. Vittor & Associates.]

Villalobos-Guerrero, T. F., & Molina-Acevedo, I. C. (2014) Lista de especies y estado de conocimiento de los poliquetos (Annelida: Polychaeta) de Sinaloa, Golfo de California. *Boletín del Instituto Oceanográfico de Venezuela*, 53(1), 79-109.

Zhadan, A. (2020) Review of Orbiniidae (Annelida, Sedentaria) from Australia. *Zootaxa*, 4860, 451–502. <https://doi.org/10.11646/zootaxa.4860.4.1>

Zhang, J., Kapli, P., Pavlidis, P., & Stamatakis, A. (2013) A general species delimitation method with applications to phylogenetic placements. *Bioinformatics*, 29(22), 2869–2876. <https://doi.org/10.1093/bioinformatics/btt499>

6. CONCLUSION

The thesis revision of the species of *Naineris* showed new taxonomical characters and listed systematic information for phylogenetic purpose.

The reconstruction of the phylogenetic relationships of *Naineris sensu lato* with integrative taxonomy, did not support the monophyly of the *Naineris* + *Protoaricia* as previously proposed (Bleidorn, 2005, 2009). *Naineris sensu lato* is polyphyletic. Thirteen species of *Naineris sensu lato* were found as *Protoaricia*, one species to *Pettibonella*, and *Naineris* s. str. was restricted to six species.

The morphological characters traced on the molecular phylogeny supported the clades:

Protoaricia comprise species with rounded to truncate prostomium, thoracic neuropodial papilla in upper part of lobe, presence of dorsal cilia between branchial bases, uncini never bifid, uncini arranged in one or two transversal rows, subuluncini may be present, oval-shaped dorsal sensory organs paired or multiple per segment, branchial start from thoracic or abdominal segments, fixed or variable, with branchiae laterally displaced. While *Naineris* s. str. includes species with thoracic neuropodial papilla in the middle of the lobe, multiple transversal rows of uncini, uncini with a ribbed shaft and rounded tip with a terminal notch, dorsal sensory organs always paired, and dorsal cilia between branchial bases.

The distribution of two selected species of *Protoaricia*, namely as *P. setosa* comb. nov. and *P. laevigata* comb. nov., showed the species restricted to the Atlantic Ocean and Mediterranean Sea respectively.

Protoaricia lanai sp. nov. comb. nov. were described, and *P. setosa* comb. nov. redescribed based on combination of morphology and molecular data. Additionally, we designated a lectotype for *P. laevigata* comb. nov. and reinstated *P. anserina* comb. nov. based on combination of morphology and molecular data.