Ocean and Coastal Research

ORIGINAL ARTICLE
Special Article Collection
Di Domenico, M.; Andriguetto-Filho, J.M. and Lopes, R.M. (Eds)
An ocean of science, worms, and humaneness:
a tribute to Paulo Lana
http://doi.org/10.1590/2675-2824072.23098
ISSN 2675-2824

Is *Namalycastis abiuma* (Grube, 1871) (Annelida: Nereididae) restricted to its type-locality? Evidence from morphological and molecular data

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ABSTRACT

Namalycastis abiuma has been recorded as a worldwide distributed species, found in most tropical and subtropical mangroves and estuarine environments. However, this status has been questioned in several publications, which indicate that several distinct species are being identified under the name *N. abiuma*. In this study, we perform a morphological analysis, along with a series of species delimitation tests and a phylogenetic analysis—using the molecular marker 16S—to evaluate whether analyzed populations previously identified as *Namalycastis abiuma* belong to the same species. We used sequences from the GenBank database in the analysis, as well as six newly sequenced specimens collected from the coast of Brazil, two of them from the *N. abiuma* type-locality. For species delimitation, we applied the Generalized Mixed Yule Coalescent (GMYC), the Assemble Species by Automatic Partitioning (ASAP), and the Multi-rate Poisson Tree Processes (mPTP) tests. Results from GMYC and ASAP suggest that *Namalycastis abiuma* may be endemic to the type-locality and that all other populations studied represent a second distinct species. However, mPTP indicates that all *Namalycastis* species included should be grouped into one single species. The mPTP results seem to be biased due to data limitation as it showed poor statistical support. Our morphological data, especially on the shape and dentition of the sub-neuroacicular falciger blades, support the GMYC and ASAP results, suggesting restricted endemism for *Namalycastis abiuma*. Based on these results, we conclude that *N. abiuma* is restricted to its type-locality and we provide a description of a new species, *Namalycastis lanai* sp. nov. occurring in Brazilian waters from 22°S to 27°S, including, at its southern range, an overlap with *N. abiuma* at Florianópolis. Finally, we provide a key to all *Namalycastis* species found in Brazil.

Keywords: Species delimitation; Molecular systematics; Namanereidinae

Submitted: 24-Jun-2023
Approved: 09-Jan-2024

Associate Editor: Maikon Di Domenico

INTRODUCTION

Namanereidinae (Annelida: Nereididae) comprises 50 valid species in two genera, from which *Namalycastis* is the most speciose with 26 recognized species (Read and Fauchald, 2023).

The type species of the genus, *Namalycastis abiuma* (Grube, 1871), is one of the most widely distributed taxa of the Namanereidinae subfamily, being recorded in tropical and subtropical estuarine regions worldwide (Hartman, 1959; Glasby, 1999). In his revision of the subfamily, Glasby (1999) recognized a large morphological variation within this name and proposed the *N. abiuma* species group concept, raising the possibility of this species being represented by a complex of cryptic species. The group was characterized by having a brown epidermal pigment on their dorsum; a trapezoidal prostomium with a cleft on anterior margin; presence of notochaetae; short and conical antennae; four pairs of tentacular cirri with distinct cirrophores; falcigerous and spinigerous chaetae in similar number (recorded for chaetiger 10); and coarsely serrated spinigerous chaetae in the posterior region of the body.

Since the revision of Glasby (1999), new species have been described that fit the concept of the species group and show a morphological resemblance with *N. abiuma*. Magesh et al. (2014a), for example, showed that *Namalycastis glasbyi* Fernando and Rajasekaram, 2007, is morphologically similar to *N. abiuma* and suggested that it may have been identified as the latter in previous studies. *Namalycastis jaya* Magesh, Kvist and Glasby, 2012, and *Namalycastis caetensis* Alves and Santos, 2016, were also described as having a morphological resemblance with *N. abiuma* species group (Magesh et al., 2012; Alves and Santos, 2016). The case that best illustrates the description of a distinct species within the morphological range of *N. abiuma* species group is the description of *Namalycastis rhodochorde* Glasby, Miura, Nishi and Junardi, 2007, in which the authors recognized the population as a novel species, being later formerly included in the *N. abiuma* species group in Glasby's revision (Glasby et al., 2007).

Some studies have used molecular markers to evaluate the boundaries of closely related and cryptic species in the Nereididae family. Many species complexes were investigated in the family, especially within the genus *Hediste* Malmgren, 1867 (e.g. Hateley et al., 1992; Abbiati and Maltagliati, 1996; Röhner et al., 1997; Sato and Nakashima, 2003; Audzijonyte et al., 2008; Cossu et al., 2012; Teixeira et al., 2022). Considering Namanereidinae species, Magesh et al. (2014b) found evidence for an incipient speciation process within *N. abiuma* species group using morphological and molecular data. Their results suggest that, within the specimens collected in southern India, at least four distinct lineages have been identified as *N. abiuma*.

Considering these findings, the species group hypothesis must be analyzed to understand the real distribution of the species. In this study, we used morphological and molecular data to compare populations of *Namalycastis abiuma* from Brazil, including specimens from the type-locality (Florianópolis, Santa Catarina) with other populations from Asia described as belonging to *Namalycastis abiuma*. This was performed to verify whether populations belong to the same species. We used phylogenetic analysis and species delimitation tests based on sequences of 16S rDNA gene to identify taxonomic units within the sample. As a result, we described one new species of *Namalycastis* from populations previously identified in *N. abiuma* species group *sensu* Glasby (1999).

METHODS

Collection sites

Samples were obtained from three coastline locations in Brazil, comprising one in the southeastern coastal region, in Guanabara Bay (Rio de Janeiro), at the Mauá mangrove region in the Magé municipality (22°41'18.70"S, 43°6'29.8"W); and two in the southern coastal region, in Florianópolis (Santa Catarina): one in Costeira beach (27°37'5.85"S, 48°31'50.28"W) and one in the Itacorubi mangrove region (27°34'50.37"S, 48°30'51.37"W) (Figure 1). The Itacorubi mangrove area is the type-locality of *Namalycastis abiuma* (Grube, 1871) and matches with the coordinates provided by Glasby (1999) for the holotype of the species. All specimens were obtained in mud sediment usually associated with decaying wood. Type specimens and other observed samples were deposited in the zoological collection of the Museu Nacional do Rio de Janeiro (MNRJP 007847 - 007851)

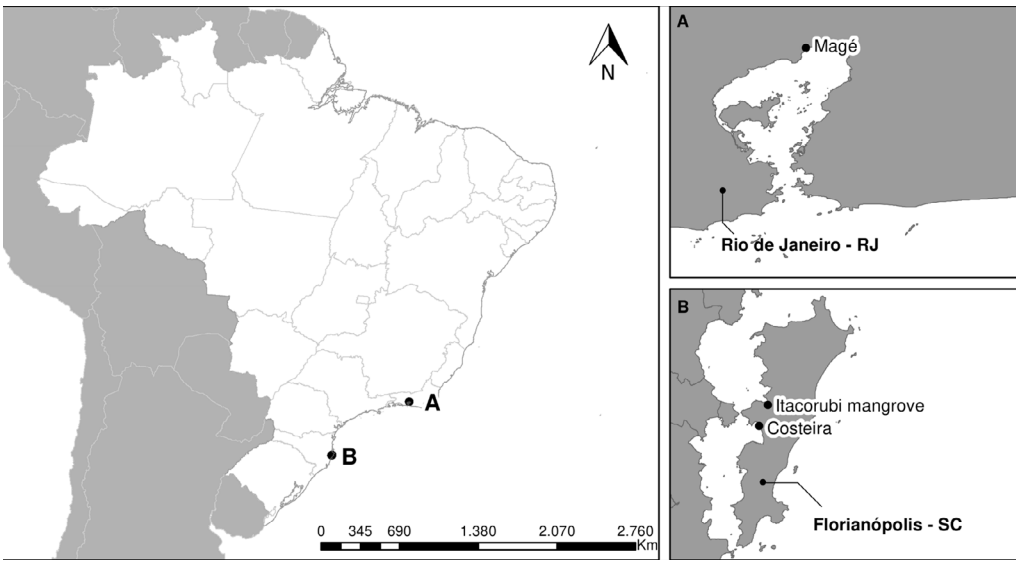

Figure 1. Location of collection sites.

Morphological analysis

A stereoscopic microscope was used to analyze specimens. They were examined alive for living coloration and after fixation for morphotype identification. At least three specimens from each collection site were fixed in formalin 4% for up to 48 hours and then stored in alcohol 70%. These specimens were used for microscopy slide preparation. For each specimen, three parapodia were examined, each of these were from chaetiger 3, chaetiger 10, and parapodia from chaetiger 90 up to chaetiger 250 (depending on the size of the specimen). Analysis also included slide images from parapodia of the holotype (ZMB Q3436; Museum für Naturkunde, Institut für Systematische Zoologie) of *Namalycastis abiuma*, prepared by CJG using an Olympus DP74 camera and cellSens v. 1.17 imaging software on an Olympus BX53 compound microscope. These images were used to verify whether collected specimens had the same parapodial and chaetal morphology as the holotype, especially for morphological features used to identify morphotypes in this study. Descriptions and terminology were based on Glasby (1999). Images of the recently collected material were taken with a Sony Cyber-shot DSC W-300 adapted for microscopy and plates were created using GIMP2 software. For each collection site, at least one specimen was prepared for scanning electron microscopy (SEM) using chemical dehydration with progressively stronger ethanol solutions (70%-100%), followed by a second step of chemical preparation using progressively stronger solutions of hexamethyldisilazane (HMDS) before being air-dried and sputter-coated with gold. Specimens were examined and photographed using a JEOL JSM-6390LV SEM system at Laboratório de Microscopia Eletrônica do Museu Nacional da Universidade Federal do Rio de Janeiro.

Molecular data

In total, eight specimens were selected for molecular analysis, four from Rio de Janeiro and four from Florianópolis—from which two specimens were collected in the Itacorubi mangrove and two from Costeira beach. Individuals were first stored in absolute alcohol for 48 hours, then transferred to a second vessel containing absolute alcohol to ensure most water was removed from tissues.

The mitochondrial large unit of rDNA gene (16S) was sequenced for these specimens. It is important to explain the decision of using only this marker in this study. Individuals were collected in 2014 as part of another study and kept stored in absolute alcohol since then. While the 16S marker was amplified during the original study, the cytochrome c oxidase subunit I (COI) was not sequenced at the time. Attempts to

amplify COI later using the same samples were unsuccessful, possibly due to problems with the DNA conservation of specimens. COI could be very informative to the question presented here; however, since returning to collection sites was not possible, only the 16S marker was used. The 16S has been used in parallel to COI as a specific mitochondrial marker for several annelid taxa, in addition to being shown to hold strong phylogenetic signal at the species level in Nereididae, for example, yielding 14.8% parsimony informative characters vs 16.9% for COI in *Perinereis* (Tosuji et al., 2019).

DNA was extracted following the protocol of Floyd et al. (2002) which consists in a first digestion in NaOH (0.25M) over 25 °C for 3 to 5 hours, followed by a heat step over 95 °C for 3 minutes. HCL (1.0M), Tris-HCL (0.5M), and Triton X-100 (2%) were added to each sample. The final step consists in another heat step over 95 °C for 3 minutes. Mitochondrial DNA was amplified by Polymerase Chain Reaction (PCR) using primers for large subunit ribosomal DNA (16S) described by Palumbi et al. (1991) and Zanol et al. (2010). PCR reactions were performed using the following volumes: H_2O (13.9 µL), Buffer 5X (2.5µL), $MgCl_2$ 25mM (3µL), BSA 10mg/ml (1µL), dNTP 2000µM (0.5µL), Primer Forward 10µM (1µL), Primer Reverse 10µM (1µL), Taq 5U/reaction (0.1µL), DNA (2µL).

The reactions were processed in a Veriti Thermal Cycler (Applied Biosystems) with the following cycles: 1 × 94 °C for 5 minutes/ 40 × 94 °C for 40 seconds + 48 °C for 1.5 minutes + 72 °C for 1.5 minutes / 1× 72 °C for 8 minutes, stabilizing at 15 °C. PCR products were verified by electrophoresis in agarose 1% gel using TBE 0.5× buffer. Sequencing was performed by Macrogen Inc. in an automatic sequencer ABI3500 (Applied Biosystems).

Besides the sequences collected in this study, we also included *Namalycastis* 16S sequences obtained from the NCBI GenBank, as well as sequences of *Platynereis dumerilii* (Audouin and Milne Edwards, 1833), *Neanthes glandicincta* (Southern, 1921), and *Tylorrhynchus heterochetus* (Quatrefages, 1866) as outgroups (Table 1), selected following Alves et al. (2020, 2023). The 16S sequences for the *Namanereis* species would be an appropriate option for outgroup composition due to being the sister taxon of *Namalycastis*; however, no 16S sequences for this taxon were found when analyses were performed. The sequences were aligned in Mafft v.7 (Katoh and Standley, 2013) using L-INS-i strategy and phylogenetic analyses were performed in IQtree 2 (Minh et al., 2020) using ultrafast bootstrap for support (Hoang et al., 2007) and ModelFinder (Kalyaanamoorthy et al., 2017) for model selection. Genetic distances were calculated in MEGA 11 software (Tamura et al., 2021) using p-distances and Kimura-2-Parameters as models.

In total, three approaches were used for molecular species delimitation: the Assemble Species by Automatic Partitioning (ASAP) (Puillandre et al., 2021), the Generalized Mixed Yule Coalescent (GMYC) (Fujisawa and Barraclough, 2013), and the Multi-rate Poisson Tree Processes (mPTP) (Zhang et al., 2013; Kapli et al., 2017). For all methods, the alignment provided was the same used for the phylogenetic analyses, including all outgroup species. For ASAP, the distance was calculated using the Kimura-two-parameters model (Kimura, 1980). For mPTP species delimitation, the same tree resulting from phylogenetic analyses was used. Support for mPTP was calculated using the Markov Chain Monte Carlo sampling method (mcmc option in mPTP software, Kapli et al., 2017) with 1 million generations and two runs. For GMYC, an ultrametric tree was provided, which was reconstructed using the BEAST program v.1.10.4 (Suchard et al., 2018) for 5 million generations. A consensus tree was calculated in TreeAnnotator on BEAST with 10% burn-in. Before running the species delimitation analyses, the possibility of the data violating the GMYC model was tested using the p2c2.gmyc package for R (Fonseca et al., 2021). Once confirmed that the model fit the data, the GMYC delimitation test was performed in R using the following packages: ape (Paradis et al., 2004), paran (Dinno, 2012), rncl (Michonneau et al., 2016), and splits (Ezard et al., 2017).

Table 1. GenBank accession number and locality of specimens included in phylogenetic analysis.

Species	Accession nº	Locality	Refs
Namalycastis abiuma	OQ652060	Itacorubi	This study
Namalycastis abiuma	OQ652061	Itacorubi	This study
Namalycastis abiuma	OQ652058	Costeira	This study
Namalycastis abiuma	OQ652059	Costeira	This study
Namalycastis abiuma	OQ652062	Magé	This study
Namalycastis abiuma	OQ652063	Magé	This study
Namalycastis abiuma	OQ652064	Magé	This study
Namalycastis abiuma	OQ652065	Magé	This study
Namalycastis abiuma group sp.	HM138705	India	Unpublished
Namalycastis abiuma	NC_030040	China	Lin et al. 2016
Namalycastis jaya	JX483870	India	Magesh et al. 2012
Namalycastis jaya	JX483868	India	Magesh et al. 2012
Namalycastis jaya	JX483869	India	Magesh et al. 2012
Namalycastis jaya	HM138706	India	Magesh et al. 2012
Namalycastis indica	GU230891	India	Unpublished
Namalycastis indica	MF959005	Myanmar	Bolotov et al. 2018
Namalycastis indica	MG759523	Myanmar	Bolotov et al. 2018
Namalycastis hawaiiensis	LC213728	Japan	Abe et al. 2017
Neanthes glandicincta	KY094478	China	Lin et al. 2017
Platynereis dumerilii	KP640622	-	Unpublished
Tylorrhynchus heterochetus	KM111507	China	Chen et al. 2016

RESULTS

Morphology

A total of 72 specimens were morphologically analyzed in all three sites (36 from Magé, 11 from Costeira, and 25 from Itacorubi). All obtained specimens were identified as belonging to *Namalycastis abiuma* species group and all variation observed was within the range of variation described by Glasby (1999). However, in this study, it was possible to observe a geographic pattern of variation, identified here as morphotypes of the species. The first morphotype was found in Itacorubi mangrove (Morphotype 1), and the second morphotype was found in both Costeira and Magé sites (Morphotype 2). Differences between the two morphotypes included a higher number of chaetigers in Morphotype 2; darker epidermal pigment in Morphotype 2; falcigers from subacicular fascicles with serrated blades in Morphotype 1 while blades were smooth or with few teeth proximally in Morphotype 2 (Figure 2). Parapodia from the holotype of *Namalycastis abiuma* showed the same features seen in Morphotype 1, that is, the blades of the subacicular falcigers showed clearly visible serrations (Figure 3). Table 2 highlights morphological features used to distinguish morphotypes and compares them with the same features from the *Namalycastis abiuma* holotype.

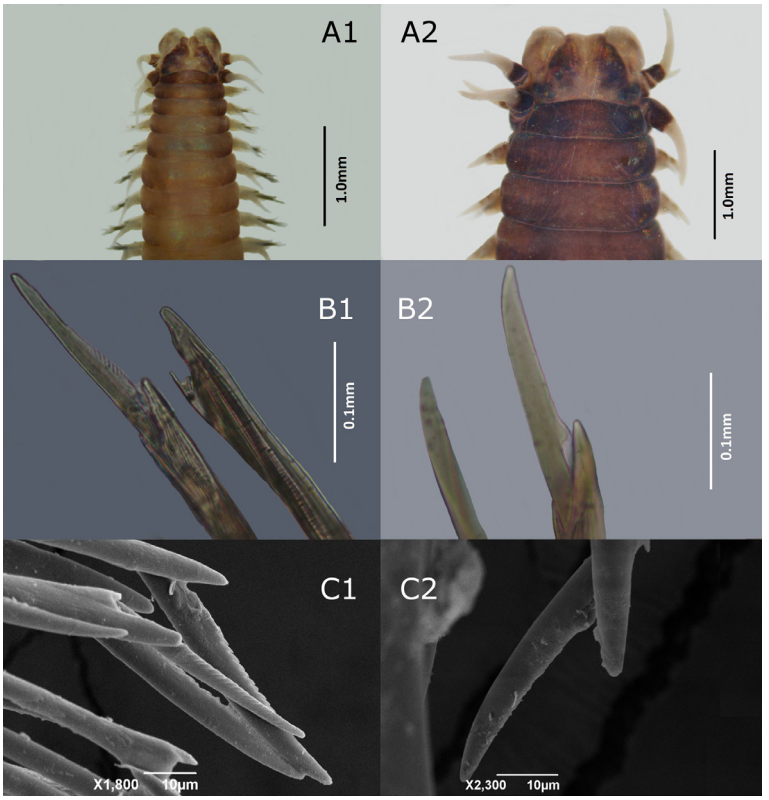

Figure 2. Morphological differences between morphotypes 1 and 2. A - Dorsal epidermal pigmentation; B - Subacicular falciger blades in chaetiger 10 (Light microscopy); C - Subacicular falciger blades in chaetigers 10 (Scanning Electron microscopy). 1 - Morphotype 1 (Itacorubi) 2 - Morphotype 2 (Costeira and Magé).

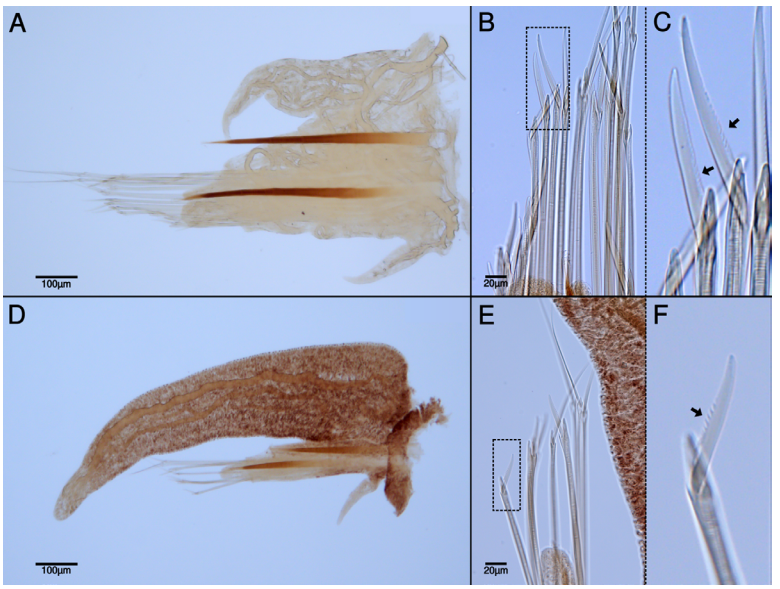

Figure 3. *Namalycastis abiuma* Holotype parapodia and chaetae A – C Chaetiger 10; D – F Chaetiger 120. Parapodia, anterior view, Neuroacicular chaetal bundle and details on subacicular falciger blades, respectively. Arrows point to blade serrations.

Table 2. Morphological differences and chaetae types count of *Namalycastis abiuma* holotype (retrieved from Glasby, 1999) and both morphotypes identified in the present study.

		Namalycastis abiuma (Holotype)			*Namalycastis abiuma* (Non-type/Morphotype 1)			*Namalycastis lanai* sp. nov (Holotype/Morphotype 2)		
	Number of chaetigers	141			123–145			259		
	Body length (mm)	45			27–29			167		
	Body width in chaetiger10 (mm)	2.2			1.0–1.9			2.8		
	Blades of subacicular falcigers	Serrated			Serrated			Smooth (few teeth)		
		Chaetiger 3	Chaetiger 10	Posterior region	Chaetiger 3	Chaetiger 10	Posterior region	Chaetiger 3	Chaetiger 10	Posterior region
Number of neurochaetae types	Supra-acicular postacicular sequigomph spinigers	2	4	3–4	2–3	5–7	3–4	2	3	2
	Supra-acicular preacicular heterogomph falcigers	3	3	1	1–3	1–3	1–2	2	2	1
	subacicular postacicular heterogomph spinigers	2	2	1	2–3	3–5	3–4	2	3	3
	subacicular preacicular heterogomph falcigers	8	6	3–8	6–8	6–8	3–4	7	7	4

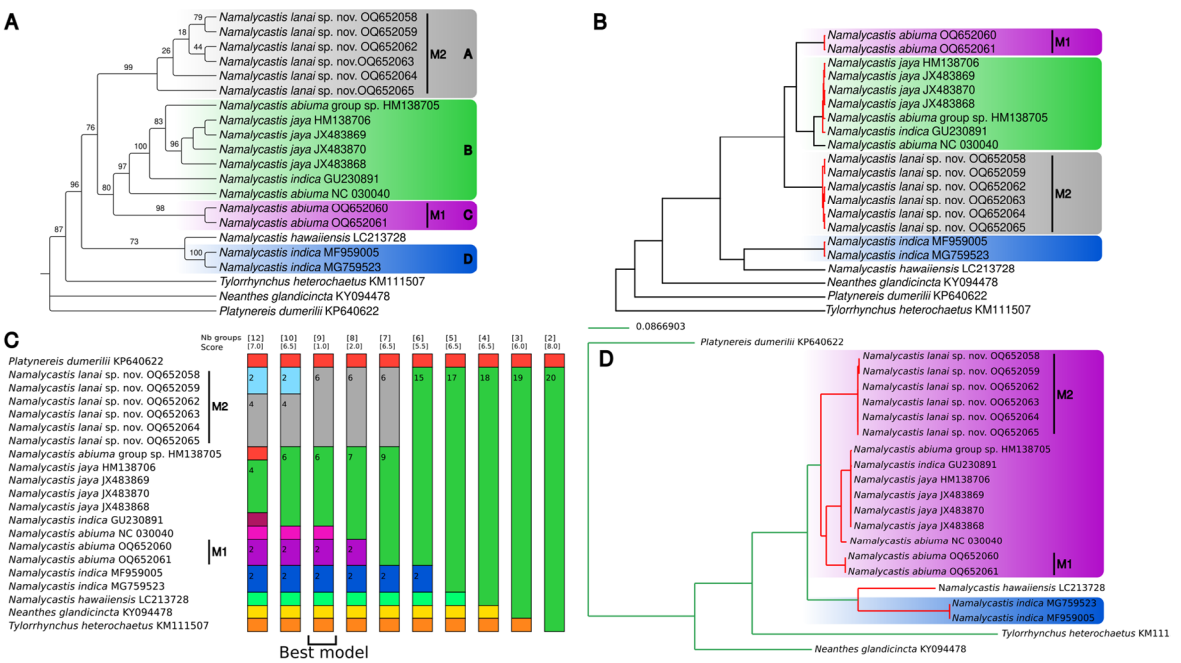

Figure 4. Phylogenetic and species delimitation results. A – Maximum likelihood tree, values in nodes are Bootstrap support values. B – GMYC, branches in red identifies delimited taxonomic units. C – ASAP groupings, number above columns identifies the number of groups and the score for each model. D – mPTP, branches in red identify delimited taxonomic units. M1 – Morphotype 1, M2 - Morphotype 2. Color boxes identify delimited groups.

Phylogenetic analysis

A total of 21 sequences were included in the analysis, 18 *Namalycastis* specimens and three outgroups. Final alignment added to 456 sites, with 135 being parsimony informative (29.6%). Genetic distances between all sequences included are presented in Table S1 (Supplementary Material). The resulting tree is presented in Figure 4A. The included *Namalycastis* sequences returned a monophyletic clade with high bootstrap support (96). Based on the maximum likelihood tree, four clades can be distinguished: Clade A containing six specimens collected in Brazil (Magé and Costeira) (support = 99); Clade B with seven Asian specimens, which includes three nominal species (97); Clade C with two specimens from Itacorubi, type-locality for *N. abiuma* (98); Clade D with two *N. indica* specimens from Myanmar (100). The *N. hawaiiensis* sample was not placed in Clade D due to low support (73).

Species delimitation tests

GMYC – Violation test performed with the p2c2.gmyc package (Fonseca et al., 2021) indicated that the present data did not violate the GMYC model (p-value = 0.65) and, thus, the model was applied. The test identified nine taxonomic units, from which three were the outgroup included and six included *Namalycastis* species (Figure 4B). Units delimited included Clades A, C, and D from phylogenetic analyses, while Clade B resulted in two distinct units in the GMYC test. The remaining unit represents the *N. hawaiiensis* sample.

ASAP – Models including up to 12 groups were tested (Figure 4C). The model with the best score (1.0) included nine groups. These groups are the same as the taxonomic units delimited in GMYC. The eight-group model held a similar score (2.0). In this model, Clade B resulted in a single group/unit.

mPTP – Only five taxonomic units were delimited in the mPTP test. These are the three outgroup species and only two *Namalycastis* units (Figure 4D). In this test, Clade A, B, and C are grouped in the same taxonomic unit, while Clade D and *N. hawaiiensis* compose another delimited unit. MCMC runs showed low support for this result (0.88/0.89).

Systematics

Based on morphological and molecular evidence, the *Namalycastis lanai* sp. nov. is proposed as a new species, described in the following section. A redescription of *Namalycastis abiuma* is also provided, based on non-type specimens collected at the Itacorubi mangrove.

Order Phyllodocida Dales, 1962
Family Nereididae Blainville, 1818
Subfamily Namanereidinae Hartman, 1959
Genus *Namalycastis* Hartman, 1959

Key to *Namalycastis* species found in Brazil

1. Heterogomph falcigers present in sub- and supra-preacicular fascicle in anterior parapodia 2
- Only heterogomph spinigers present in sub- and supra-preacicular fascicle in all parapodia .... *N. geayi*

2(1). Heterogomph falcigers present in sub- and supra-preacicular fascicle in all parapodia 3
- Heterogomph falcigers in anterior parapodia, replaced by heterogomph spinigers posteriorly in sub- and supra-preacicular fascicle 7

3(2). Heterogomph setae with boss not prolonged 4
- Heterogomph setae with boss extremely prolonged (see Glasby, 1999 for details on shaft head morphology in Namanereidinae) ..... *N. fauveli*

4(3). Heterogomph falcigers include serrated blade types ...5
- Heterogomph falcigers all with smooth blades; Supra-neuroacicular falcigers in parapodia of setiger 10 with smooth blades; supra-neuroacicular falcigers in parapodia of chaetiger 10 with blades less than 4× longer than width of shaft head ..... *N. brevicornis*

5(4). Body slightly shorter (usually up to 50 mm with 150 chaetigers), paler with brown epidermal pigment on head and anterior dorsum and/or posteriormost segments and pygidium; sub-neuroacicular falcigers in parapodia of chaetiger 10 with finely serrated blades 6
- Body longer (100-200 mm for 150-280 chaetigers), with darker brown epidermal pigment on head, dorsum, posteriormost segments and pygidium; Sub-neuroacicular falcigers in parapodia of chaetiger 10 smooth bladed or with few teeth basally in the blade ... . *N. lanai* sp. nov.

6(5). Brown epidermal pigment on head, anterior dorsum, posteriormost segments and pygidium; distinct tentacular cirrophores; dorsal cirri greatly increasing in posterior parapodia; heterogomph elongated falcigers (pseudospinigers) absent ..... *N. abiuma*
- Brown epidermal pigment only in posteriormost segments and pygidium; indistinct tentacular cirrophores; dorsal cirri same size throughout body or increasing slightly posteriorly; heterogomph elongated falcigers (pseudospinigers) present ..... *N. caetensis*

7(2). Acicular neuropodial ligule simple, subconical; supra-neuroacicular falcigers in parapodia of chaetiger 10 with blades smooth or only serrated basally, about 8× longer than width of shaft head ..... *N. siolii*
- Acicular neuropodial ligule bilobed; supra-neuroacicular falcigers in parapodia of chaetiger 10 with blades serrated over most of their length, 3.3 to 7.5× longer than width of shaft head 8

8(7). Dorsal cirri less than twice length of parapodium at setiger 3; dorsal-most sub-neuroacicular falcigers in parapodia of chaetiger 10 with blades having 16 to 30 teeth ..... *N. macroplatis*
- Dorsal cirri usually greater than twice (up to five times) length of parapodium at chaetiger 3; dorsal-most sub-neuroacicular falcigers in parapodia of chaetiger 10 with blades having 5 to 12 teeth ..... *N. senegalensis*

***Namalycastis abiuma* (Grube, 1871)**
Figures 2 (A1, B1, and C1) and 5, Table 2.
Lycastis abiuma Grube, 1871: 47-49.
Namalycastis abiuma – Glasby, 1999: 31, fig. 10.

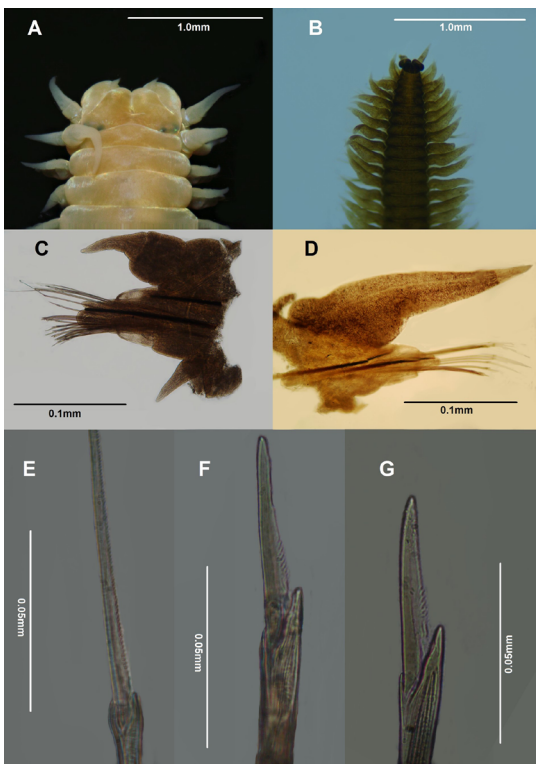

Figure 5. *Namalycastis abiuma*. (A) Anterior end, dorsal view. (B) Posterior end, dorsal view. (C) Parapodium from chaetiger 10, anterior view. (D) Parapodium from chaetiger 110, anterior view. (E) Supra-neuroacicular spiniger from chaetiger 10. (F) Supra-neuroacicular falciger from chaetiger 10. (G) Sub-neuroacicular falciger from chaetiger 10.

MATERIAL EXAMINED. Non-type specimens from Itacorubi mangrove, Florianópolis, Santa Catarina, Brazil (27°34'50.37"S, 48°30'51.37"W), 12 specimens (MNRJP 007851).

COMPARATIVE SPECIMEN. Microscope slide preparations of parapodia from holotype of *Namalycastis abiuma*, Desterro [=Florianópolis, Santa Catarina] (ZMB Q3436).

DESCRIPTION. A total of ten specimens observed were incomplete. Two complete specimens had 123-145 chaetigers, measuring 27-29 mm long and 1.0-1.9 mm wide at chaetiger 10. Long body with convex dorsum and flat ventral. Uniform anterior width, tapering posteriorly. Light brown color in 70% ethanol. Epidermal pigment present only in the posterior end and on pygidium.

Prostomium trapezoidal, with anterior cleft associated with a longitudinal groove that extends until mid-posterior prostomium. Short and subconical antennae, being in the species' lateral side and aligned over mid-palps insertion. It presents two pair of eyes, posterior pair smaller, aligned almost transversally to prostomium (Figure 5A).

Tentacular cirri with distinct cirrophores with smooth cirrostyles. Posterodorsal pair extending to chaetiger 3 (Figure 5A).

All parapodia with acicular neuropodial ligule bilobed. Notopodial cirrophores present and increasing in size posteriorly. Dorsal cirri 0.7-0.9× the length of parapodia in chaetiger 3, 1.0-1.3× in chaetiger 10, 1.4-1.7× in chaetiger 120. Ventral cirri with nearly the same size in all chaetigers, length of ventral cirri from posterior parapodia 1.0-1.1× length of ventral cirri from anterior parapodia (Figures 5C and 5D).

Notopodial chaetae absent. Supra-neuroacicular chaetae as sesquigomph spinigers in postacicular fascicles and heterogomph falcigers in preacicular fascicles. Sub-neuroacicular chaetae as heterogomph spinigers in postacicular fascicles and heterogomph falcigers in preacicular fascicles in all parapodia. Supra-neuroacicular falcigers in chaetiger 10 with finely serrated blades, 7-9 teeth, 5.6-6.1× longer than width of shaft head (Figure 5F). Sub-neuro acicular falcigers in chaetiger 10 with finely serrated blades, dorsal-most 5.0-5.2× longer than width of shaft head, ventral-most 4.7-5.1× longer than width of shaft head (Figure 5G). Subacicular spinigers in chaetiger 10 with moderately serrated blades (Fig. 5E). Sub-neuroacicular falcigers in posterior parapodia with

finely serrated blades. Sub-neuroacicular spiniger in posterior parapodia with blades moderately serrated. Chaetae pale, aciculae black.

Pygidium button-shaped with terminal anus. Two anal cirri subconical, smooth, ventrolateral, 0.6-0.8× width of pygidium (Figure 5B).

DISTRIBUTION. The species appears to be endemic to Santa Catarina.

REMARKS. See Discussion.

***Namalycastis lanai* sp. nov.**

urn:lsid:zoobank.org:act:0F7ECDF7-2BF7-404C-8B43-6614036AD85B

Figures 2 (A2, B2, and C2) and 6, Table 2.

Namalycastis abiuma – Lana, 1984: 275-276, figs 105-106. – Santos and Lana, 2001: 138-139 figs 1-6. – Liñero-Arana and Díaz, 2007: 157-158, fig. 3.

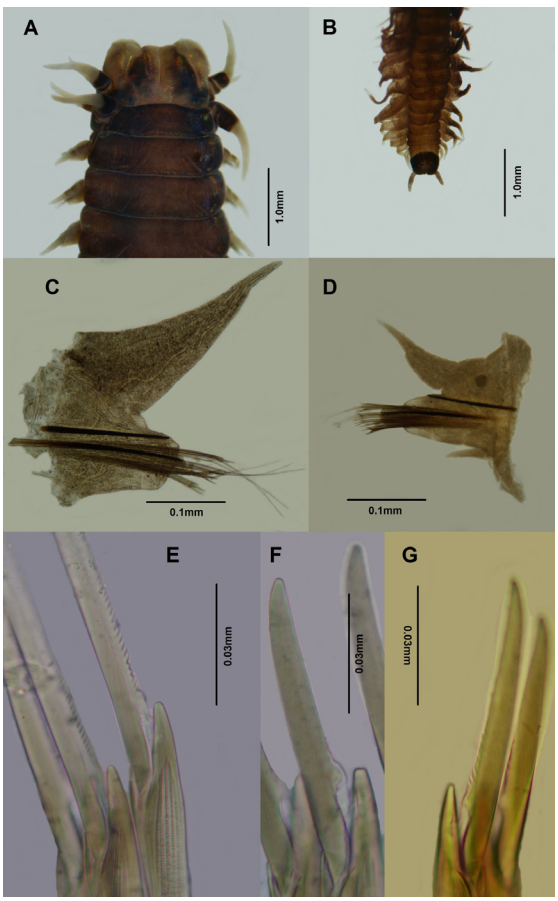

Figure 6. *Namalycastis lanai* sp. nov. (A) Anterior end, dorsal view. (B) Posterior end, dorsal view. (C) Parapodium from chaetiger 120, anterior view. (D) Parapodium from chaetiger 10, anterior view. (E) Sub-neuroacicular spinigers from chaetiger 10. (F) sub-neuroacicular falcigers from chaetiger 10. (G) Supra-neuroacicular falcigers from chaetiger 10.

TYPE MATERIAL. Holotype (MNRJP 007847), complete adult specimen, collected from mangroves associated with decomposing vegetal matter in Guanabara Bay, Magé, Rio de Janeiro, Brazil (22°41'18.70"S, 43°6'29.8"W). Paratypes (MNRJP 007848), two specimens from Guanabara Bay, Magé, Rio de Janeiro, Brazil.

OTHER MATERIAL OBSERVED. Non-type specimens from Gramacho, Duque de Caxias, Brazil (22°45'29.41"S, 43°15'58.18"W), two specimens, and Costeira, Florianópolis, Santa Catarina, Brazil (27°37'5.85"S, 48°31'50.28"W), seven specimens (MNRJP 007849/ MNRJP 007850).

DESCRIPTION. Holotype complete, 259 chaetigers, 167 mm long and 2.8 mm wide in chaetiger 10. Body long with dorsum convex and ventral flat. Uniform in width anteriorly, tapering posteriorly. Color Brown in 70% ethanol. Epidermal pigment present in dorsum, darker in posterior region and pygidium.

Prostomium trapezoidal and laterally indented, showing anterior cleft associated with a longitudinal groove that extends until mid-posterior prostomium. Antennae short and subconical, lateral and aligned over mid-palps insertion. two pair of eyes, posterior pair smaller, aligned obliquely to prostomium. Tentacular cirri with distinct cirrophores, cirrostyles smooth. Posterodorsal pair extending posteriorly to chaetiger 4. Brown pigmentation present, concentrated in prostomium margin and tentacular cirrophores (Figure 6A).

All parapodia with acicular neuropodial ligule bilobed. Notopodial cirrophores present and increasing in size posteriorly. Dorsal cirri 0.8× the length of parapodia in chaetiger 3, 1.1× in chaetiger 10, 1.9× in chaetiger 200. Ventral cirri with nearly the same size in all chaetigers, length of ventral cirri from posterior parapodia 1.1× length of ventral cirri from anterior parapodia (Figures 6C and 6D).

Notopodial chaetae absent. Supra-neuroacicular chaetae as sesquigomph spinigers in postacicular fascicles and heterogomph falcigers in preacicular fascicles. Sub-neuroacicular chaetae as heterogomph spinigers in postacicular fascicles and heterogomph falcigers in preacicular fascicles in all parapodia. Supra-neuroacicular falcigers in chaetiger 10 with blades finely serrated, showing few teeth only on proximal region of the blades,

6.3× longer than width of shaft head (Figure 6G). Sub-neuroacicular falcigers in chaetiger 10 with blades smooth, dorsal-most 5.3× longer than width of shaft head, ventral-most 5.2× longer than width of shaft head (Figure 6F). Subacicular spinigers in chaetiger 10 with blades moderately serrated (Figure 6E). Sub-neuroacicular falcigers in posterior parapodia with blades smooth. Sub-neuroacicular spiniger in posterior parapodia with blades moderately serrated. Chaetae pale, aciculae black.

Pygidium button-shaped with terminal anus. Two anal cirri subconical, smooth, ventrolateral, 0.9× width of pygidium (Figure 6B).

VARIATION. Some observed specimens had less pigmentation on dorsum, possibly faded due to the length of time in ethanol. It was observed that some specimens presented a few notopodial chaetae as sesquigomph spinigers in a few chaetigers, usually from the third. However, in most specimens, these notopodial chaetae were absent in many chaetigers along the body. Some specimens had sub-neuroacicular falcigers with blades showing few teeth in the basal region, usually no more than four small teeth on blades and sometimes occurring in the same fascicles with smooth-bladed falcigers.

ETYMOLOGY. The species was named after Dr. Paulo Lana, a renowned and prolific annelid specialist, with many contributions to the taxonomy and ecology of marine invertebrates and ecosystems.

HABITAT. Specimens were found in mangroves in brackish waters, usually associated with decomposing wood shallowly buried in sediment. This is the same habitat where *Namalycastis abiuma* can be found. However, we highlight that, in Magé, *N. lanai* sp. nov. could be found in high densities in a region in the early stages of succession after a restoration program. It seems that this species was able to quickly colonize these regions, dominating the area along with *Laeonereis* and *Alitta* species. At this stage, *N. lanai* sp. nov. could be found all over the mangrove, including sites a few meters away from any water source. After the same sites reached advanced stages of restoration, densities of *Namalycastis lanai* sp. nov. were strongly reduced and the species could only be found associated with decomposing wood and organic matter.

REMARKS. All populations included in the species *Namalycastis lanai* sp. Nov. were previously identified as *Namalycastis abiuma* species group and the only distinct character that was described by previous studies was the smooth blades in the falcigers. This character was not considered to be enough to establish a new species, especially since it was included in the species group denomination by Glasby (1999). However, with the recent recognition of a smooth-bladed form, *Namalycastis rhodochorde* from SE Asia, which was previously included in the *Namalycastis abiuma* species group (Glasby et al., 2007), it now appears as though individuals having smooth-bladed falcigers (i.e. no projecting fine teeth) that otherwise resemble the *N. abiuma* species group may represent undescribed species. Moreover, the molecular results discussed above showed that the small morphological differences characterized two lineages and are distinct enough to be considered different species (genetic distance = 0.095 in Jukes-Cantor model). Besides the shape and dentition (or not) of falciger blades, the two species can be distinguished by the number of chaetigers, the length of individuals (usually longer in *N. lanai* sp. nov., Table 2), and the body pigmentation (darker in *N. lanai* sp. nov.). We understand that body pigmentation may be strongly dependent on the fixation and preservation process, and the fact that it is fully observable only in complete individuals. Therefore, these are the reasons to focus on the form of the blades, a more informative character, to distinguish these species. We included previous records of *N. abiuma* on the South American coast in the new species based on the resemblance of the diagnostic character in these population's descriptions (see Discussion). Falcigers with smooth blades have also been described in *Namalycastis brevicornis* (Audouin & Edwards, 1833) and *Namalycastis kartaboensis* (Treadwell, 1926), with both species being already described in French Guiana, north coast of South America. However, *N. brevicornis* can be distinguished by the blades of falcigers, all having smooth blades while *N. lanai* sp. nov. presents supra-neuroacicular falcigers with serrated blades; and *N. kartaboensis* have

no epidermal pigment, prostomium without an anterior cleft, and faintly jointed cirrostyles of the tentacular cirri. The nomenclatural act referring to the new species described is registered in Zoobank with the accession urn:lsid:zoobank.org:act:0F7ECDF7-2BF7-404C-8B43-6614036AD85B.

DISCUSSION

Phylogenetic analyses and at least two of the species delimitation tests performed suggest that specimens identified as *Namalycastis abiuma* from Brazil do not represent a single species. The morphotypes identified in this study can be described as a distinct species with support from the tests performed. These results indicate that the specimens found in the type-locality (Itacorubi mangrove) are distinct from every other *Namalycastis* specimen sequenced and morphologically analyzed.

All specimens found in the present study match the range of variation identified by Glasby (1999). However, in order to facilitate future studies, Glasby (1999) provided two descriptions of *Namalycastis abiuma*. The first description follows the species designation and is based on the holotype of the species (Glasby, 1999, p. 31), characterized by prostomium shallowly cleft anteriorly, antennae extending to tip of palpophore, notochaetae present, though very few and not in every chaetiger; supra-neuroacicular falcigers in chaetiger 10 with blades moderately serrated, 11 teeth, teeth about uniform in length; sub-neuroacicular falcigers in chaetiger 10 showing 13 teeth and sub-neuroacicular spinigers in the posterior region with blades having coarse serrations proximally. The second description regards the species group, which encompasses a larger set of morphological variations (Glasby, 1999, p. 31-35), characterized by brown epidermal pigment, on the dorsum and on the pygidium; prostomium usually shallowly cleft anteriorly, antennae usually extending short of tip of palpophore; notochaetae present or absent; supra-neuroacicular falcigers in chaetiger 10 with blades finely to moderately serrated (very rarely lacking serrations), 4-15 teeth (very rarely 0-20), teeth about uniform in length; sub-neuroacicular falcigers in chaetiger 10 dorsally with blades showing up to 18 teeth and sub-neuroacicular spinigers in mid-posterior region with blades having coarse serrations proximally. The morphotypes identified in this study can both be included within the variation of the species group but only morphotype 1 closely resembles the holotype description provided by Glasby (1999) and Grube (1871). Moreover, chaetae from the holotype showed the same diagnostic feature identified for morphotype 1, which is the presence of serrations in the subacicular falciger blades. Since the holotype and the specimens identified as morphotype 1 were collected in the same mangrove and both share the same diagnostic feature, we understand both as belonging to the same species. Morphotype 2, however, shows significant differences to the *Namalycastis abiuma* holotype description and can only be identified as *N. abiuma* under the species group designation. Thus, it is described here as a new species, *Namalycastis lanai*.

Magesh et al. (2014b) also evaluated populations of *N. abiuma* species group from southern India and recognized six distinct morphotypes, which the authors divided in two subgroups. The same authors, in another contribution described a new species, *Namalycastis jaya*, from specimens they recognize that resembles *Namalycastis meraukensis* (Horst, 1918), which was previously included in the *N. abiuma* species group by Glasby's revision (Magesh et al., 2012). Subsequently, other two species were described from populations previously included in the species group designation: *Namalycastis rhodochorde* from South-east Asia and *Namalycastis caetensis* from Brazil (Glasby et al., 2007; Alves and Santos, 2016). Neither of the two morphotypes identified in this study matched any of these species.

Since many populations once included in the *Namalycastis abiuma* species group designation are now being described as new species, we suggest that the range of morphological variation of the species group should not be used to identify populations as *Namalycastis abiuma*. Rather, identifications should be matched against the description provided for the type specimen (i.e., Glasby 1999, p. 31) of the species. Populations

that fit the species group but do not match the type specimen of *N. abiuma* (or the other recently described species mentioned above) probably represent new species; they could be referred to as *Namalycastis* cf. *abiuma* pending formal description. Therefore, the use of the informal name '*Namalycastis abiuma* species group' should be used only when referring to the subset of species sharing the species group features mentioned above. Following this restricted species definition, it is clear that many circumtropical to subtropical populations from estuarine areas (particularly outside southern India and Brazil) that deviate from the type description of *N. abiuma* may represent new species in need of formal description.

The species *Namalycastis abiuma* has been recorded from many locations in the coastal regions of South America. Some descriptions were provided, and it is possible to recognize that most of the records resemble the morphotype 2. Lana (1984) reported the *N. abiuma* specimens with smooth falciger blades in the southern Brazilian coast, while Santos and Lana (2001) recorded species in the northeastern coast of Brazil. However, the specimens described by Santos and Lana (2001) had falciger blades with few teeth, although less than observed in morphotype 1. The position of the described chaetae was not provided in both studies and, regarding morphotype 2, only the subacicular chaetae were totally smooth. The specimens described by these studies resemble morphotype 2 in the remaining features. Liñero-Arana and Díaz-Díaz (2007) also recorded the occurrence of specimens with smooth blades on the coast of Venezuela; the authors recognized that this variation might represent a distinct population and referred to the specimen as *Namalycastis* cf. *abiuma*.

Based on morphology, it is possible to establish that most known populations, not only in the South American coast, show a subtle distinction from the holotype description of *N. abiuma*. Struck et al. (2017) argue that the best approach to study cases of crypticism is undergoing integrative evaluation of both morphological and molecular data. Our results show that the specimens from the type-locality can also be distinguished based on molecular data from other *Namalycastis* specimens included, with support from two of the species delimitation tests performed. As seen in Figure 4, the Itacorubi specimens form a distinct clade that is more related to other *Namalycastis* than with the nearest population (Costeira). On the other hand, the specimens from the other two collected sites resemble each other and group together in the analysis, being identified as the same species in all delimitation tests performed. Since all Itacorubi specimens were identified as morphotype 1 and Costeira and Magé specimens as morphotype 2, the molecular distinction reflects the observed morphological difference.

In this study, our focus was on *Namalycastis abiuma*, especially on its type-locality and relationships with other *Namalycastis* samples. However, a further note is needed on Clade B, as it included a *N. indica* specimen, leaving the species as non-monophyletic. The sequences for *N. indica*, as well as *N. abiuma* species group, that grouped in Clade B were all submitted to GenBank before the first description *Namalycastis jaya* (Magesh et al. 2012). Considering the distances found, it is possible that all these sequences belong to *N. jaya* specimens. Unfortunately, the authors did not include these sequences in their study, so this hypothesis still needs to be verified.

Results from GMYC and ASAP suggest that *Namalycastis abiuma* may be endemic to the type-locality and that all other populations studied represent distinct species, contradicting the current distribution of the species. While ASAP results are based on genetic distance between specimens (Puillandre et al., 2021), GMYC delimits species based on the likelihood of transitions between inter- and intra-specific processes (Fujisawa and Barraclough, 2013). GMYC and ASAP indicating that specimens from the type-locality are a distinct species suggest that these specimens not only show significant genetic distance to specimens from other locations, but also that these distances are most likely to be the result of these populations evolving independently. Genetic distances also support this interpretation (Table S1) since distances between the two morphotypes are above 9% for both models. Previous studies have described even lower distances (from about 2% using the

16S marker) between closely related species, including cryptic species of the nereidid *Perinereis anderssoni* Kinberg, 1866 (Paiva et al., 2019).

However, contrary to GMYC and ASAP, mPTP indicates that all *Namalycastis* species included should be grouped into two species. mPTP is similar to GMYC in considering inter- and intra- specific processes; however, mPTP allows distinct evolutionary rates to be traced in the phylogenetic tree (Kapli et al., 2017). Consequently, mPTP may be biased due to data limitation as more samples would improve rate estimation. Considering that our mPTP results showed low statistical support (both runs below 0.95), we consider that taxonomic units determined by the analysis may be biased due to the limited dataset.

Based on these results and considering that other species included in this study show significant morphological variation, being recognized as distinct species (i.e. *Namalycastis indica* Glasby 1999, *Namalycastis jaya* Magesh et al., 2012), we decided to follow the species delimitation provided by GMYC and ASAP, in which species delimitation reflects the morphological variation observed. These results imply that the population found in the Itacorubi mangrove, the type-locality for *Namalycastis abiuma*, belongs to a distinct species from all other populations studied. However, all other specimens sequenced for this study are part of a distinct new species, *Namalycastis lanai*.

CONCLUSION

Based on morphological and molecular data, we found that all populations studied are different from the species described by Grube (1871) under the name *Namalycastis abiuma*, except for individuals found at the type-locality. In this study, we described a new species of *Namalycastis*, understanding that the evidence found conclusively indicates that the population from Itacorubi is not the same species recorded for other locations. From the results obtained, the only known location where *N. abiuma* can be found is in its type-locality, in the Itacorubi mangroves. It would mean that the most widespread and studied species of the subfamily Namanereidinae must have its distribution changed from cosmopolitan to endemic for a single location.

ACKNOWLEDGMENTS

This work is a tribute to the memory of Dr. Paulo Lana who helped consolidate the science of marine annelids in Brazil, and who had the special ability to keep specimens of *Namalycastis* alive for a long period of time in his laboratory—a fact that he always proudly mentioned to PRA whenever they met at conferences and a poster with previous versions of this study was presented. Dr. Lana used to call these specimens *Namalycastis abiuma*. After this work, we know that the specimens he kept alive belong to another species: one justly named after him.

The authors are also thankful to Wilson Weis, Mariana Paz, and Paulo Pagliosa for the help with samples collection. We thank Torkild Bakken and another anonymous reviewer for the useful suggestions that improved this manuscript. P.R.A. and C.S.G.S. would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for financial support (process: PDSE 88881.188038/2018–01 and EST-SENIOR 99999.001299/2015–08), and C.J.G. thanks the Australian Biological Resources Study (Grant RG18-21) for funding. This study was financed by the Coordenação de Aperfeiçoamento de Pessoal de Nivel Superior—Brasil (CAPES)—Finance Code 001.

AUTHOR CONTRIBUTIONS

P.R.A.: Conceptualization; Investigation; Analysis; Writing – original draft; Writing – review & editing.

C.J.G.: Investigation; Writing – review & editing.

P.C.P.: Writing – review and editing.

C.S.G.S.: Conceptualization; Writing – original draft; Writing – review & editing.

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