PROCEEDINGS OF THE BIOLOGICAL SOCIETY OF WASHINGTON
127(3):455–465. 2014.

Redescription of *Namalycastis glasbyi* Fernando & Rajasekaran, 2007 (Annelida, Nereididae, Namanereidinae) from India

Mathan Magesh, Christopher J. Glasby, and Sebastian Kvist*

(MM) Department of Aquatic Biology and Fisheries, University of Kerala, Thiruvananthapuram, India, 695581. Present address: Department of Biochemistry, University of Calcutta, 35 Ballygunge Circular Road, Kolkata, India 700019, e-mail: maheshmathan2008@gmail.com;
(CJG) Museum and Art Gallery of the Northern Territory, GPO Box 4646, Darwin, Northern Territory 0801, Australia, e-mail: chris.glasby@nt.gov.au;
(SK) Museum of Comparative Zoology, Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, Massachusetts 02138, U.S.A., e-mail: skvist@fas.harvard.edu

Abstract.—*Namalycastis glasbyi* Fernando & Rajasekaran, 2007 was originally described from the creek-lined coast north of Mumbai in western India. However, the original description includes some oddities that make species recognition problematic and may confuse future taxonomic evaluations of the genus. Moreover, the type material of the species has been lost and, therefore, this species is herein redescribed, a neotype is assigned, and the distinguishing morphological features are clearly illustrated. The species shares some features with two other Indian species: *Namalycastis indica* Southern, 1921 and the *N. abiuma* Müller *in* Grube, 1871 species group. However, *N. glasbyi* differs from *N. indica* in having comparatively smaller antennae, shorter posterior dorsal cirri, a higher count of notochaetae (maximum of three) in the mid and anterior body, and finely serrated blades in sub- and supra-neuroacicular falcigers in the anterior and mid-body. Further, sub-neuroacicular spinigers have finely serrated blades in all parapodia in *N. glasbyi*, as opposed to the coarsely serrated spinigers from antero-mid body parapodia of *N. indica*. Moreover, *N. glasbyi* differs from the *N. abiuma* species group in lacking coarsely serrated sub-neuroacicular spinigers in the parapodia of mid and posterior parts of the body and in possessing a tripartite pygidium. In addition to these morphological differences, the mitochondrial COI sequence (DNA barcode) of the neotype of *Namalycastis glasbyi* clearly distinguishes it from other Indian species.

Keywords: DNA barcoding, India, morphology, *Namalycastis glasbyi*, Namanereidinae, neotype, Polychaeta, taxonomy

The polychaete family Nereididae (Annelida: Polychaeta) is one of the most abundant and diverse families of polychaetes and is found in a wide range of marine environments. Within Nereididae, the subfamily Namanereidinae is exceptional in that it represents one of the most highly adapted groups of polychaetes to freshwater and supralittoral coastal environments (Glasby 1999a, 1999b). Two major genera comprise Namanereidinae: *Namanereis* Chamberlin, 1919 and *Namalycastis* Hartman, 1959. The inclusion of a third genus, *Lycastoides* Johnson, 1903, remains questionable (Read & Fauchald 2013). With its 22 currently recognized species, *Namalycastis* is the most species-rich genus within the subfamily and is also

* Corresponding author.

one of the most successful polychaetes in organically enriched coastal waters (Glasby 1999a, Magesh et al. 2012, Conde-Vela 2013). Most of the Indian *Namalycastis* species have so far been collected from sulphide rich polluted areas associated with marine environments (Kalaiselvi & Ayyakkannu 1991, Magesh et al. 2012). Five species of *Namalycastis* are currently known from India: *N. fauveli* Nageswara Rao, 1981, *N. glasbyi* Fernando & Rajasekaran, 2007, *N. indica* Southern, 1921, *N. jaya* Magesh, Kvist & Glasby, 2012, and the *N. abiuma* Müller *in* Grube, 1871 species group.

The challenging morphology of *Namalycastis* species has led to the difficulty of establishing discriminating characters for several species. This is due to a reduced number of characters as compared to other nereidid species and the conservative nature of these characters across *Namalycastis*, particularly in the widespread species group *N. abiuma*. Glasby (1999a) suggested that the length of the antennae, tentacular and dorsal cirri, number and distribution of notochaetae, size of the serrations of the spinigers and falcigers, and the form of the pygidium are all major characteristics in identifying species of *Namalycastis*. In an attempt to circumvent the potentially deceptive morphology, Magesh et al. (2012) utilized a molecular identification approach, employing cytochrome *c* oxidase subunit I (COI), 16S rRNA, and 18S rRNA and concluded that these DNA markers represent yet another important tool in differentiating *Namalycastis* species. Currently, the combination of morphological and molecular tools offers the best approach to distinguishing species and highlights the need for molecular characterization of each specimen.

Namalycastis glasbyi was first reported from Gorai Creek, near Mumbai, India by Fernando & Rajasekaran (2007), and the species is thus far known only from the type locality. Fernando & Rajasekaran (2007) separated *N. glasbyi* from other *Namalycastis* species known at the time by its possession of coalescent and longitudinally arranged eyes, an anterior cleft in the prostomium, unjointed dorsal cirri that are extremely elongated in posterior segments, very few exposed teeth (2 or 3) in the jaws, by the presence of notopodial chaetae (1–3) in the anterior region of the body and, lastly, by "the unequal number of heterogomph spinigers and falcigers in the neuropodia" (i.e., more spinigers in the supra-neuroacicular fascicles and more falcigers in the sub-neuroacicular fascicles). Although *N. glasbyi* appears to have been well characterized and differentiated from other *Namalycastis* species, most of these characters are not diagnostic. Further, the type description, based on four specimens now lost (O. Fernando pers. comm.), does not include a nominated holotype (or syntypes) and therefore does not fully comply with recommendations in Articles 72 and 73 of the International Code of Zoological Nomenclature (ICZN).

In this paper, we designate a neotype for *N. glasbyi* from material collected very close to the type locality, clarify some points of ambiguity in the original description, and identify additional features (morphological and molecular) that distinguish the species.

Materials and Methods

Specimen collection.—Samples were collected from a marshy area of the Gorai Creek, north of Mumbai, near Manori village, off the western coast of India in March 2012 (Fig. 1). Sampling and identification followed the methods of Glasby (1999a) and Magesh et al. (2012). The samples were preserved in 80% ethanol. Subsequently, a small section of the posterior end (about 20 segments but avoiding the pygidium) was transferred to 95% ethanol to be used for DNA extraction. The redescription is based on the

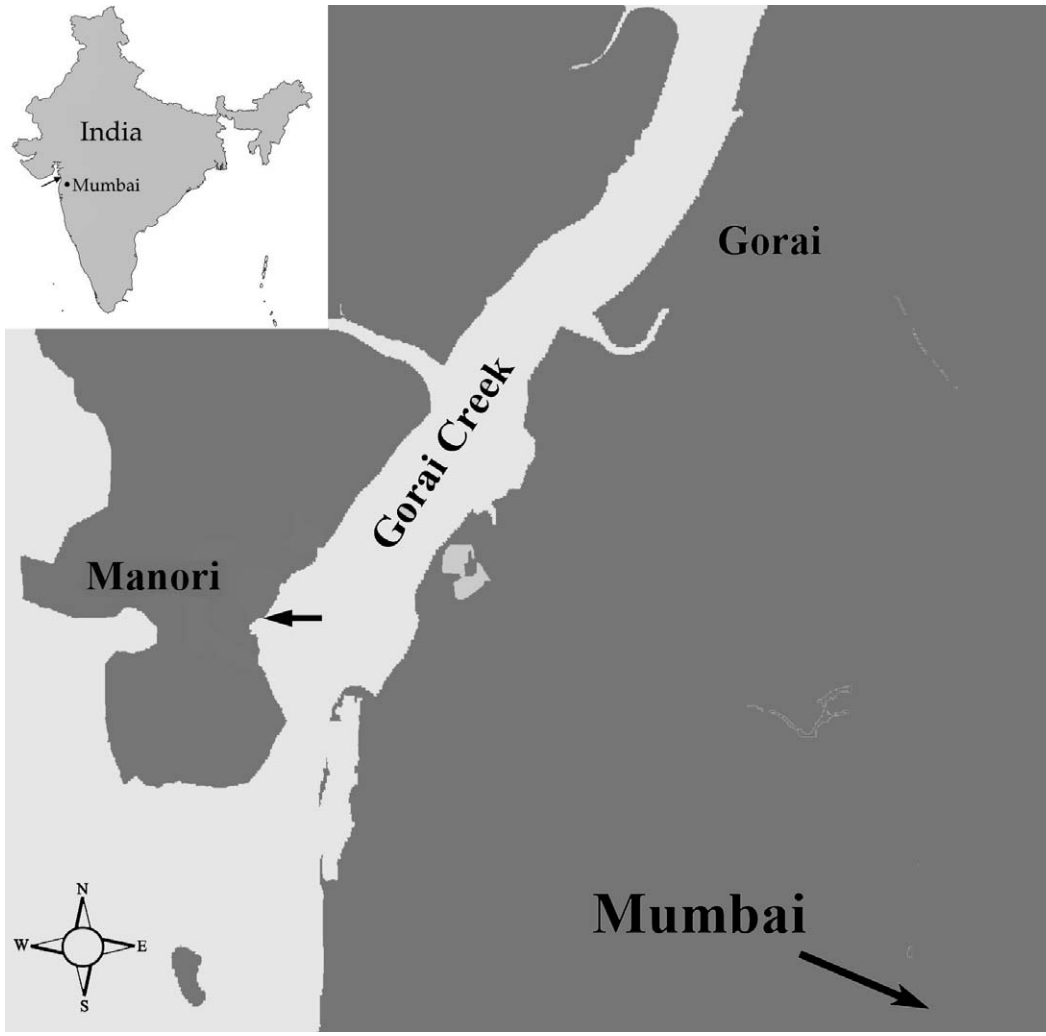

Fig. 1. Map showing collection locality of specimens of *Namalycastis glasbyi* studied herein. Arrow indicates neotype locality; Gorai Creek, about 2 km upstream from the sea, close to Manori (19°12′10.8″N, 72°47′31.2″E). Inset: outline drawing of India showing approximate position of collection locality (arrow).

neotype and eight other specimens collected in Gorai Creek. All specimens are deposited at the Zoological Reference Collection of the Zoological Survey of India (ZSI), in Kozhikode, Kerala.

DNA extraction, amplification, and alignment.—Total genomic DNA was isolated from tissue samples of three specimens of *Namalycastis glasbyi* (including the neotype, collection codes AQTT2–4) following the procedure of Miller et al. (1988). Partial sequences of mitochondrial COI were PCR-amplified, purified, and sequenced following the protocol used by Magesh et al. (2012).

Assembly of forward and reverse DNA strands was carried out using BioEdit ver. 7.0.5.2 (Hall 1999). Thereafter, a set of COI sequences representing both closely and more distantly related species was downloaded from GenBank (for accession numbers see Table 1) to establish the uniqueness of the newly generated COI sequences, as well as to estimate the

Table 1.—List of specimens included in the neighbor joining analyses, with taxonomic authorities, authors' collection codes, and GenBank accession numbers. Specimens in bold font were collected by the first author.

Species	Authority	Collection code	GenBank
Namalycastis			
glasbyi	Fernando & Rajasekaran, 2007	AQTT2	JX843802
glasbyi	Fernando & Rajasekaran, 2007	AQTT3	JX843801
glasbyi	Fernando & Rajasekaran, 2007	AQTT4	JX878494
abiuma	Müller *in* Grube, 1872	AQPA3	JQ081268
abiuma	Müller *in* Grube, 1872	AQPA4	JQ081269
abiuma	Müller *in* Grube, 1872	AQPA5	JQ081270
abiuma	Müller *in* Grube, 1872	AQMM5	JX843798
abiuma	Müller *in* Grube, 1872	AQMM6	JX867718
abiuma	Müller *in* Grube, 1872	AQMM7	JX867720
abiuma	Müller *in* Grube, 1872	AQMM8	JX843799
abiuma	Müller *in* Grube, 1872	AQMM9	JX843796
abiuma	Müller *in* Grube, 1872	AQMM52	JX843803
abiuma	Müller *in* Grube, 1872	AQMM62	JX843800
abiuma	Müller *in* Grube, 1872	AQMM63	JX867719
abiuma	Müller *in* Grube, 1872	AQMM92	JX843797
abiuma	Müller *in* Grube, 1872	K5	KF761615
abiuma	Müller *in* Grube, 1872	K24	KF761613
abiuma	Müller *in* Grube, 1872	K52	KF761616
abiuma	Müller *in* Grube, 1872	K53	KF761617
abiuma	Müller *in* Grube, 1872	K242	KF761614
jaya	Magesh, Kvist & Glasby, 2012	AQJ1	HQ456363
jaya	Magesh, Kvist & Glasby, 2012	AQJ2	JN790065
jaya	Magesh, Kvist & Glasby, 2012	AQJ3	JN790066
jaya	Magesh, Kvist & Glasby, 2012	AQJ4	JN790067
Platynereis			
bicanaliculata	Baird, 1863	N/A	GU362685
sp.	N/A	N/A	HM473638
Perinereis			
falklandica	(Ramsay, 1914)	N/A	HQ705185
longidonta	Rozbaczylo & Castilla, 1973	N/A	HQ705191
gualpensis	Jeldes, 1963	N/A	HQ705187
Hediste			
atoka	Sato & Nakashima, 2003	N/A	AB603864
japonica	(Izuka, 1908)	N/A	AB603758
Cheilonereis cyclurus	(Harrington, 1897)	N/A	HM473330
Nereis			
vexillosa	Grube, 1851	N/A	HM473510
heterocirrata	Treadwell, 1931	N/A	GU362684
Perinereis vallata	Grube, 1857	N/A	HQ705192
Marphysa sanguinea	(Montagu, 1815)	N/A	GQ497547

genetic distances between higher taxonomic ranks. Multiple sequence alignment of all sequences ($n = 36$) was accomplished using MAFFT ver. 7 (Katoh & Standley 2013), employing the G-INS-i strategy, which is recommended for datasets with $<$200 terminals and with global homology motifs; the remaining settings remained as default. In cases where the downloaded sequences extended beyond the 3′-end of the newly generated sequences, this end was cut in order to include only regions of shared homology for the comparisons. Sequence divergences (genetic distances) and standard deviations based on uncorrected *p*-distances, as suggested by Srivathsan & Meier (2012), were then estimated using PAUP* ver. 2.0b10 (Swof-

ford 2002) and Microsoft Excel, respectively. In addition, a distance-based neighbor joining (NJ) tree was constructed in PAUP*, employing uncorrected *p*-distances. The resulting tree was rooted at the only non-nereidid representative, the eunicid *Marphysa sanguinea* (Montagu, 1815).

Systematics

Family Nereididae
Subfamily Namanereidinae
Genus *Namalycastis* Hartman, 1959
Namalycastis glasbyi Fernando & Rajasekaran, 2007
Figs. 2, 3

Namalycastis glasbyi Fernando & Rajasekaran, 2007:65–66, Fig. 2a–h.

Type locality.—Gorai Creek (nearly 3 km upstream), Mumbai, India.

Type material.—Neotype AQTT2 (ZSI/WGRC/IR/INV 3191) collected in March 2012 by M. Magesh, from marshy area of the Gorai Creek, Mumbai (about 2 km upstream from sea, and less than 1 km from the type locality), off the western coast of India (19°12′10.8″N, 72°47′31.2″E). Neotype COI Barcode sequence GenBank accession number JX843802.

Additional material.—Five complete specimens, AQTT1, 3–6 (ZSI/WGRC/IR/INV 3193, 3258–3261), and three incomplete specimens AQTT7–9 (ZSI/WGRC/IR/INV 3255–3257). Specimens were collected in March 2012 from the neotype locality.

Description.—Neotype with body widest mid-anteriorly, gradually tapering towards posterior end. Total number of chaetigers, 137; width at chaetiger 10, 2.4 mm. Body color light brown throughout with lighter brown epidermal pigmentation anterodorsally. Prostomium with shallow anterior cleft (Fig. 2A) with narrow longitudinal groove extending from tip to mid-posterior prostomium. Prostomium almost triangular, slightly indented laterally. Antennae smooth, extending to just short of tip of palpophore, aligned over inner edge of palps. Two pairs of black eyes arranged obliquely with posterior pair smaller (Fig. 2A).

Posterodorsal tentacular cirri extending posteriorly up to chaetiger 4 or 5. Jaws with single robust terminal tooth, and 6 free subterminal teeth (5 teeth in a few specimens) (Fig. 2B). Acicular neuropodial ligule distally broad, bilobed (tenth parapodium; Fig. 2C). Dorsal cirri increasing in length posteriorly, 2 to 3 times as long as parapodial lobe. From 1 to 3 notochaetae (but occasionally absent) in mid and anterior body (Fig. 2D–F); notochaetae absent in posterior segments (~20 most posterior segments).

Sub-neuroacicular falcigers in chaetiger 10 with finely serrated blades (serrations equal in size), with 6 teeth (Fig. 3A). Supra-neuroacicular falcigers in chaetiger 10 with finely serrated blades (15–17 teeth of equal size) (Fig. 3C). Supra- and sub-neuroacicular spinigers in chaetiger 10 with finely serrated blades with teeth equal in size (Fig. 3B, E, respectively). Neuroacicular spinigers and falcigers in mid and posterior chaetigers with finely serrated blades present in all parapodia (Fig. 3B–H). Pygidium tripartite (Fig. 2G), anus terminal and anal cirri arising laterally.

Ecological note.—This species occurs in organically-enriched estuarine sediments having a sulfurous odor.

Remarks.—Designation of a neotype is deemed necessary in order to clarify the identity and taxonomic status of *N. glasbyi*. In particular, information provided in the type description and illustrations is contradictory; in general, the information in the description appears to be more accurate than that presented in the illustrations. We base our conclusion that the neotype designated here represents the same species described by Fernando & Rajasekaran (2007) on the close proximity of the type localities and on two features in particular: 1) the broad, bilobed parapodial acicular ligule, and 2) the compara-

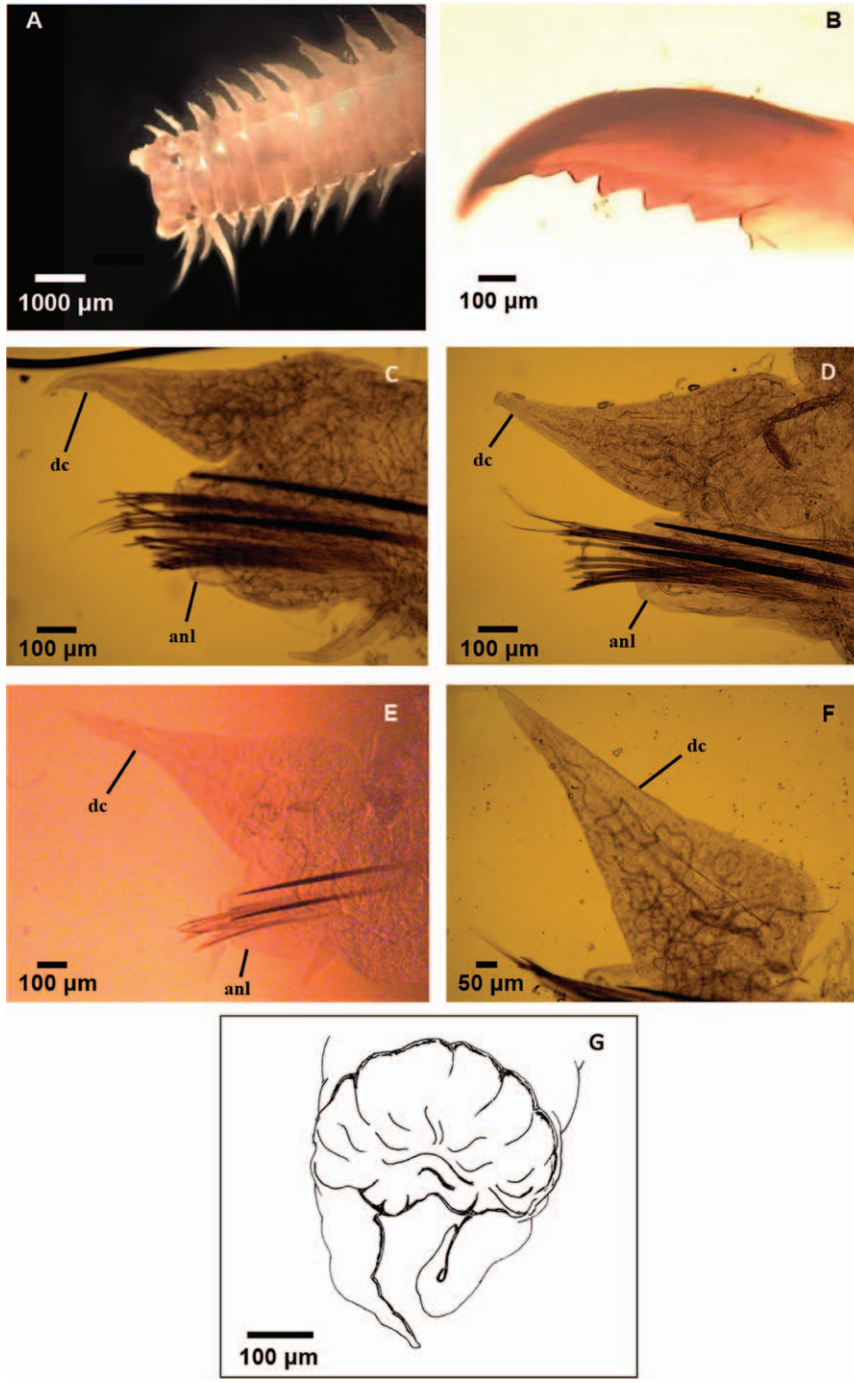

Fig. 2. *Namalycastis glasbyi*, neotype. A, anterior end, dorsal view; B, left jaw, ventromedial view; C, anterior parapodium, chaetiger 10; D, parapodium, chaetiger 51; E, parapodium, chaetiger 80; F, posterior parapodium, chaetiger 112; G, pygidium, dorsal view. Abbreviations: anl, acicular neuropodial ligule; dc, dorsal cirrus.

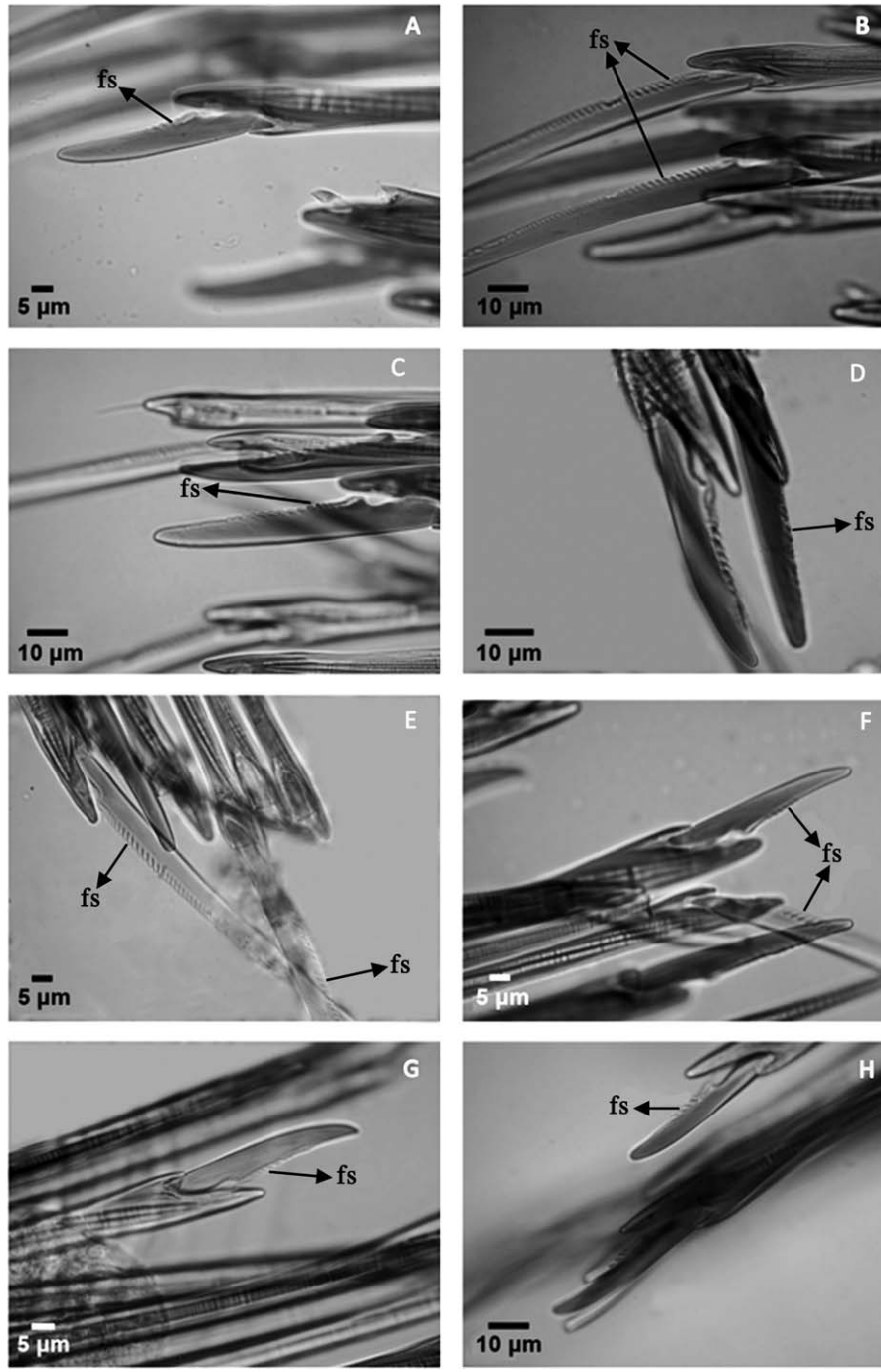

Fig. 3. *Namalycastis glasbyi*, neotype. A, sub-neuroacicular falciger, chaetiger 10; B, supra-neuroacicular spiniger, chaetiger 10; C, supra-neuroacicular falciger, chaetiger 10; D, supra-neuroacicular falciger, chaetiger 50; E, sub-neuroacicular spiniger, chaetiger 10; F, supra-neuroacicular falciger, chaetiger 80; G, sub-neuroacicular falciger, chaetiger 80; H, sub-neuroacicular falciger, chaetiger 110. Abbreviation: fs, fine serrations.

tively high number of notochaetae (1–3) in anterior parapodia.

Fernando & Rajasekaran (2007:Fig. 1a) illustrated the anterior pair of eyes as being smaller than the posterior pair and aligned almost longitudinally, which is highly unusual, whereas we found the posterior pair of eyes to be the smaller and the eyes to be aligned more laterally at the posterior margin of the prostomium (as stated in their type description). The neuropodial acicular ligule is described as being "bilobed with superior lobe papilliform and the inferior lobe globular" but illustrated as conical (Fernando & Rajasekaran 2007:Fig.1b–f); we confirm its bilobed nature. Further, important characters such as the form of the pygidium and serrations on the blades of the compound chaetae are not described by Fernando & Rajasekaran (2007). The form of the pygidium is found here to be tripartite, unlike the typical multi-incised type of most *Namalycastis* species, and the blades of the heterogomph falcigers and spinigers were finely serrated throughout.

Thus, additional characters have herein been identified, aiding in the separation of *N. glasbyi* from other congeneric forms in general, and those most similar from India (*N. indica* and the *N. abiuma* species group) in particular. *Namalycastis indica*, as opposed to *N. glasbyi*, possesses a slender, conical parapodial acicular ligule, coarsely serrated sub-neuroacicular spinigers from chaetiger 3 to 10 and subneuroacicular spinigers with coarsely serrated blades in the posterior region. *Namalycastis glasbyi* differs from the *N. abiuma* species group by the lack of coarsely serrated sub-neuroacicular spinigers in the parapodia of mid and posterior parts of the body and the presence of a tripartite pygidium.

There are other points of difference between the neotype and the specimens described by Fernando & Rajasekaran (2007), which are difficult to explain. For example, Fernando & Rajasekaran (2007) reported only a single subterminal tooth on the jaws and four teeth concealed (ensheathed) proximally, whereas we commonly found 6 subterminal teeth (1 or 2 ensheathed teeth in a few specimens). Additionally, the dorsal cirri of the neotype were only moderately longer than the parapodial lobes (about 2 to 3 times, longer posteriorly), whereas the original description reported the cirri to be 2 to 5 times as long as the parapodial lobe (longer posteriorly). These differences may reflect intraspecific variability, perhaps size related, or the latter difference may be related to fixation differences. Fernando & Rajasekaran (2007) do not state how their specimens were fixed or whether their observations were based on fixed or living animals. Their illustrations showing highly elongated posterior dorsal cirri and parapodial acicular ligules suggest the latter.

Genetic distances.—The final alignment occupied 658 sites and was devoid of internal insertions and deletions. All COI sequences derived from *N. glasbyi* specimens were identical, i.e., we found no intraspecific COI-variation for this species. However, the average COI-distance between *N. glasbyi* and the remaining sequences derived from *Namalycastis* species was 5.34% ± 0.30; the maximum value (5.84%) occurring between each of the comparisons between *N. glasbyi* and *N. jaya* (Supplementary Table 1). This corresponds well with the genetic variation reported between closely related yet distinct species in several other barcoding studies on various organisms (e.g., Hebert et al. 2003, Smith et al. 2005, Ratnasingham & Hebert 2007). Moreover, the average COI distance between the *N. glasbyi* sequences and those of the remaining nereidids was 23.13% ± 1.36, suggesting that genetic saturation has possibly occurred in COI for the sampled sequences. The separation of *N. glasbyi* is further supported by the NJ tree (Fig. 4), which places the *N. glasbyi* sequences in their

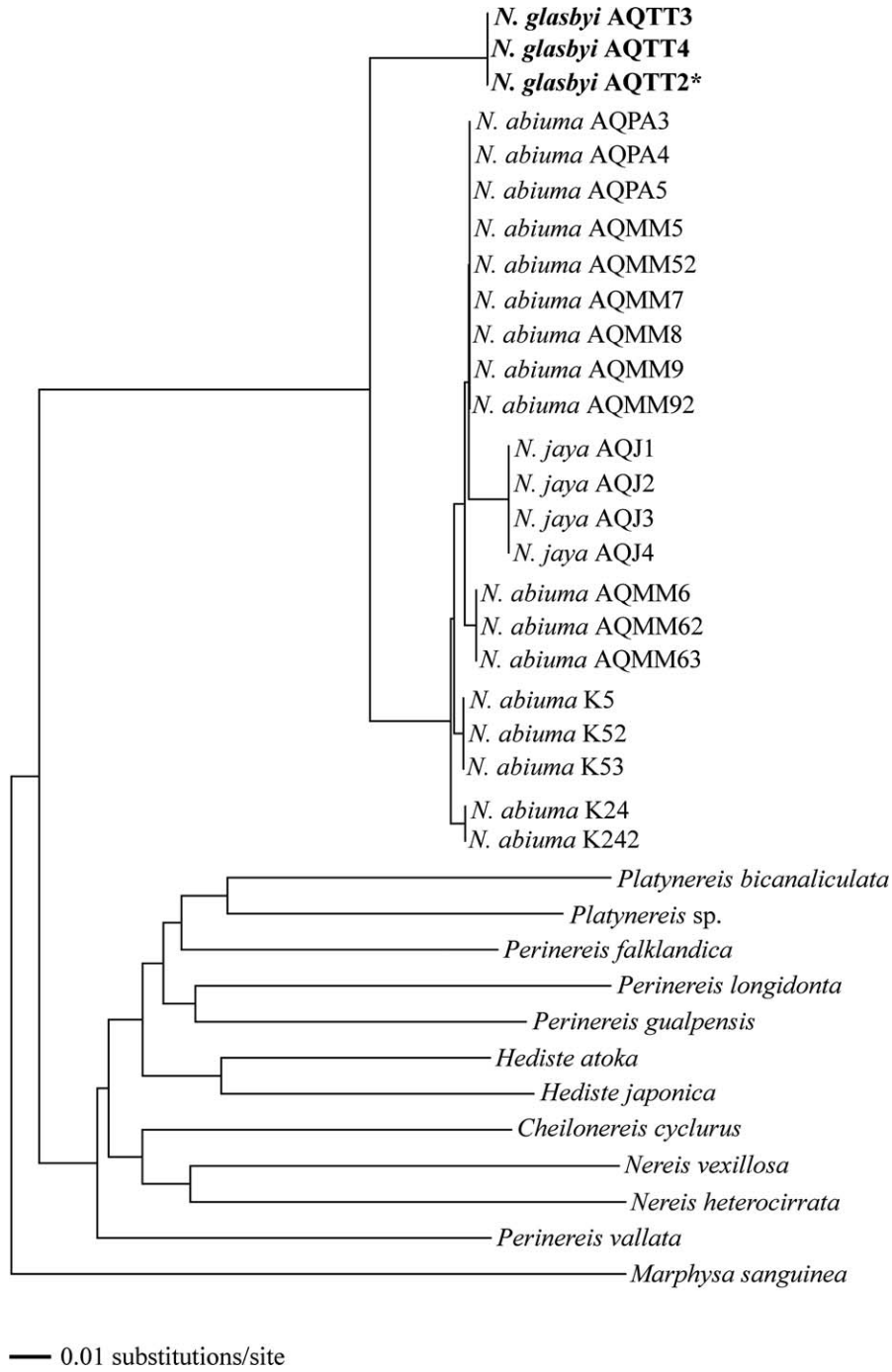

Fig. 4. Neighbor joining tree derived from the COI dataset comprising 36 sequences, including three newly generated sequences for *Namalycastis glasbyi* (bold). The neotype sequence is denoted with an asterisk. The tree indicates a distinct separation of the *N. glasbyi* clade from the remaining species of *Namalycastis* (see text for further details). Numbers following taxon names refer to the various collection codes; see Table 1 for GenBank accession numbers.

own clade, separate from the others; its sister cluster (based on genetic distance) is a larger clade containing specimens from both the *N. abiuma* species group and *N. jaya*. As such, molecular evidence clearly corroborates the morphological diagnosis in that *N. glasbyi* is distinct from the remaining nereidids.

Conclusions

Based on comparative features used for identifying the species of the genus *Namalycastis*, the species *N. glasbyi* can be distinguished by the following characters: 1) a broad, bilobed parapodial acicular ligule, 2) a comparatively high number of notochaetae (1–3) in anterior parapodia, 3) sub- and supra-neuroacicular falcigers of chaetiger 10 with finely serrated blades, 4) the presence of finely serrated teeth in subneuroacicular spinigers in all parapodia, 5) light brown colored pigmentation anterodorsally, and 6) pygidium tripartite. Complementary to the distinguishing morphological features, the genetic distance and composition of the COI sequences of *N. glasbyi* presented here clearly separates this species from Indian congeners.

Acknowledgments

We are grateful to Dr. Pat Hutchings, Australian Museum, Sydney, Australia, and Dr. Bijukumar, Aquatic Biology and Fisheries, Kerala University for their constant support and encouragement. This work was financially supported in part by the Wenner-Gren Foundation. Two anonymous reviewers provided helpful feedback that improved the manuscript.

Literature Cited

Conde-Vela, V. M. 2013. *Namalycastis occulta* n. sp. and a new record of *N. borealis* (Polychaeta: Nereididae: Namanereidinae) from the Northwestern Caribbean Sea. Zootaxa 3721(5):475–487.

Fernando, O. J., & R. Rajasekaran. 2007. A new species of Namanereidinae: *Namalycastis glasbyi* sp. nov. from Indian waters. Journal of the Bombay Natural History Society 104:64–67.

Glasby, C. J. 1999a. The Namanereidinae (Polychaeta: Nereididae). Part 1, taxonomy and phylogeny. Records of the Australian Museum, Supplement 25:1–129.

Glasby, C. J. 1999b. The Namanereidinae (Polychaeta: Nereididae). Part 2, Cladistic biogeography. Records of the Australian Museum, Supplement 25:131–144.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95–98.

Hebert, P. D. N., S. Ratnasingham, & J. R. deWaard. 2003. Barcoding animal life: cytochrome *c* oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society of London B: Biological Sciences (Suppl. 1) 270:S96–S99.

Kalaiselvi, R., & K. Ayyakkannu. 1991. Aspects of the ecology of *Lycastis* sp. (Polychaeta: Nereididae) from the southeast coast of India. P. 696 *in* M. E. Petersen & J. B. Kirkegaard, eds., Systematics, biology and morphology of world Polychaeta. Proceedings of the 2nd international polychaete conference, Copenhagen, 1986. Ophelia Supplement 5, 723 pp.

Katoh, K., & D. M. Standley. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30(4):772–780.

Magesh, M., S. Kvist, & C. J. Glasby. 2012. Description and phylogeny of *Namalycastis jaya* sp. n. (Polychaeta, Nereididae, Namanereidinae) from the southwest coast of India. ZooKeys 238:31–43.

Miller, S. A., D. D. Dykes, & H. F. Polesky. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research 16(3):1215.

Ratnasingham, S., & P. D. N. Hebert. 2007. BOLD: the barcode of life data system. Molecular Ecology Notes 7:355–364.

Read, G., & K. Fauchald. 2013. *Namanereidinae*. *In* G. Read & K. Fauchald, eds., World Polychaeta database. Accessed through: G. Read & K. Fauchald, eds., World Polychaeta database at: http://www.marinespecies.org/polychaeta/aphia.php?p=taxdetails&id=324345. (Last accessed on 19 July 2014.)

Smith, M. A., B. L. Fisher, & P. D. N. Hebert. 2005. DNA barcoding for effective biodiversity assessment of a hyperdiverse arthropod

group: the ants of Madagascar. Philosophical Transactions of the Royal Society B: Biological Sciences 360(1462):1825–1834.

Srivathsan, A., & R. Meier. 2012. On the inappropriate use of Kimura-2-parameter (K2P) divergences in the DNA-barcoding literature. Cladistics 28:190–194.

Swofford, D. 2002. PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods). Version 4.0b. Computer software and manual. Sinauer Associates, Sunderland, Massachusetts.

Associate Editor: Rick Hochberg.