VOL.67 NO.02 JULY-DECEMBER 2025 • PRINT ISSN 0025-3146 • ONLINE ISSN 2321-7898

JMBAI

JOURNAL OF THE MARINE BIOLOGICAL ASSOCIATION OF INDIA

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Marine Biological Association of India
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Available online at: https://www.mbai.org.in/jmbai doi:10.6024/jmbai.2025.67.2.2600-15

Distribution range extension of *Halimanemertes slacksmithae* (Nemertea) in the Indian ocean

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Received: 29 May 2025 Revised: 09 September 2025
Accepted: 11 September 2025 Published: 03 December 2025

Short Communication

Abstract

This study reports the range extension of the marine nemertean, *Halimanemertes slacksmithae*, Gibson, 1990, to Indian waters, from its type locality at Island, Albany, Australia, in the Indian Ocean to the rocky intertidal shores of Muttom, Kanyakumari, Tamil Nadu. Species identification was based on external body colouration and patterns, in conjunction with multilocus DNA analysis. In addition, the ultrastructure of the stylet apparatus is described and illustrated. Morphological and ecological characteristics of this hoplonemertean species are provided. This represents the second record of *H. slacksmithae*, with DNA barcoding carried out using mitochondrial cox1 and nuclear H3 loci. The genetic sequences obtained have been submitted to the GenBank database.

Keywords: *Nemertea, Halimanemertes, cytochrome oxidase subunit I, histone H3 gene*

Introduction

Members of the phylum Nemertea are elongated, unsegmented, and highly contractile invertebrates with soft bodies, ranging in length from a few millimetres to 30 meters (McIntosh, 1873; Turbeville, 2002; Sundberg and Strand, 2010). Although predominantly marine, 13 species have been recorded from terrestrial habitats and 22 species from freshwater environments (Ruppert and Barnes, 1994). Nemertea comprises approximately 1,300 valid species classified within 250 genera (Kajihara *et al.*, 2008; Gibson, 1995). Most nemertean species occur in European seas and in the northeastern and southern Asian seas (Herrera-Bachiller *et al.*, 2015; Chernyshev, 2016). The majority are predators, preying on small crustaceans, polychaetes, and molluscs. Forty species have been reported as symbionts or commensals on marine invertebrates; however, the nature of these associations remains unclear, being variously classified as commensalism, parasitism, or specialised egg predation (Berg and Gibson, 1996; Jensen, 2005).

A major diagnostic feature for nemertean species is the morphology of the mouth, proboscis, and stylets. Nemerteans possess an eversible proboscis, an elongate, tubular structure housed within a dorsal chamber called the rhynchocoel (Coe, 1901; Yamaoka, 1940; Gibson, 1970). The proboscis is divided into three distinct regions: (1) a relatively long anterior region; (2) a relatively short middle region; and (3) a posterior region of intermediate size. Within the middle region, the stylet bulb, which conceals the central and auxiliary stylets, is housed at its posterior and anterior parts, respectively. During prey capture, the proboscis is everted and the concealed central stylet is employed to inflict a wound, through which paralytic neurotoxins are injected (Kern, 1973). Reserve stylet sacs produce new stylets in case the central stylet is damaged or lost (Hyman, 1951).

Baseodiscus hemprichi (originally *Eupolia hemprichi* Ehrenberg, 1831) was the first nemertean described from India (Shrinivaasu *et al.*, 2011). More recently, a new species of the same genus, *Tetrastemma freyae*, was discovered from Kaneohe Bay, Oahu, Hawaii, and Kovalam Beach, Chennai, Tamil Nadu (Chernyshev *et al.*, 2020). To date, only eight species have been reported from the Indian seas (Kalita and Goswami, 2006; Shrinivaasu *et al.*, 2015; Chernyshev *et al.*, 2020).

In this study, we document the presence of *Halimanemertes slacksmithae* Gibson, 1990, a hoplonemertean of the family Emplectonematidae, in Indian waters for the first time. Previously, this species was known solely from its type locality in Western Australia. Species identification was confirmed based on distinct external morphological characteristics. A detailed image of the stylet ultrastructure is presented, and multilocus DNA barcoding using mitochondrial (cox1) and nuclear (H3) gene regions further supports the identification. The genetic sequences have been submitted to the NCBI GenBank database.

Material and methods

Two hundred monostiliferan worms were collected by extracting them from the marine algae, *Ulva lactuca* and *Dictyota* spp. The algae were initially placed in a clean tide pool of seawater for 15 minutes. Lack of hiding places forces the worms out due to temperature differences. The worms were then carefully picked up using tweezers. Collections were made from the intertidal region on or within the algaebeds along the Kanyakumari coast at Muttom rocky beach (8° 07′ 26.1″N, 77° 18′ 48.8″ E), Tamil Nadu, India (Fig. 1), between October 2018 and June 2020. Samples were separated, identified with standard keys (Gibson, 1990), measured for length, and maintained in petri dishes containing filtered (mesh size 0.22 μm) and sterilised seawater.

A subset of worms was anaesthetised and relaxed in 7.5% $MgCl_2$ for imaging, while other specimens were preserved in 95% ethanol for DNA extraction and voucher preparation. The proboscis was everted by gently applying pressure to the body, then removed and visualised using a Leica M165 FC stereo microscope equipped with a Leica DFC310 FX camera. The stylet was examined by placing the proboscis under a cover slip and imaging it with a Leica DM2000 LED upright microscope fitted with a Leica MC120HD camera. The stylet was carefully dissected from the proboscis, rinsed repeatedly in sterile seawater and distilled water, and air-dried for several hours. It was subsequently mounted on an alumina stub with double-sided carbon tape. Because the sample is nonconductive, it was coated with 10–15 nm of gold-palladium using a portable DC sputter coater, then imaged with a Carl Zeiss Supra 55 FESEM. The FESEM was operated at an extra high-tension voltage of 2.00 kV, with a working distance of 7.0 mm and SE2 detector signal, at a magnification of 1.20K×. The vacuum was maintained at 2.5×10^{-5} mbar during imaging.

Fig. 1. Sampling site showing the collection location (Muttom Coast, Kanyakumari, India)

Total genomic DNA was extracted using the OMEGA BIO-TEK E.Z.N.A. Blood and Tissue DNA Kit according to the manufacturer's instructions. Fragments of the cytochrome oxidase subunit I (cox1) and histone H3 genes were amplified using primers LCO1490 and HCO2198 for cox1 (Folmer *et al.*, 1994) and H3AF and H3AR for H3 (Colgan *et al.*, 1998). For the cox1 gene, PCR conditions were as follows: initial denaturation at 95 °C for 10 minutes; 35 cycles of 1 minute at 95 °C, 45 seconds at 54 °C, and 1 minute at 72 °C; with a final elongation at 72 °C for 10 minutes. For H3, PCR conditions were 5 minutes at 94 °C; 35 cycles of 40 seconds at 94 °C, 40 seconds at 56 °C, and 1 minute at 72 °C; followed by a final elongation at 72 °C for 10 minutes.

The PCR products were purified using a PCR purification kit and sequenced with an ABI Prism 3730 Genetic Analyser employing BigDye Terminator Chemistry. Sequence chromatograms were edited and assembled into contigs using BioEdit (Hall, 1999). The resulting sequences were compared with published sequences of various Emplectonematidae species available in GenBank. Sequences were aligned using MUSCLE as implemented in MEGA 7.0 (Kumar *et al.*, 2016), and pairwise genetic distances for cox1 were calculated using the Kimura 2-parameter model (Kimura, 1980). Both cox1 and H3 sequences of *H. slacksmithae* were submitted to GenBank (accession numbers: MT828541, MT840338). Voucher specimens (No. ZSI/MBRC/NE-357 and No. ZSI-MBRC/NE-361) were deposited in the National Zoological Collection of the Marine Biological Regional Centre, Zoological Survey of India, Chennai, India.

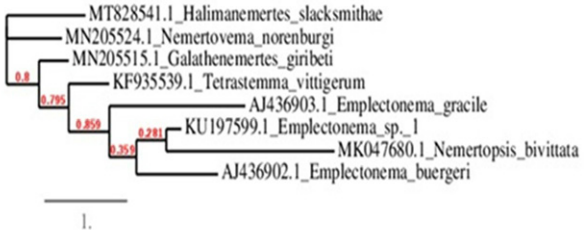

Fig. 2. Phylogenetic tree of selected nemertean species constructed using ClustalW multiple sequence alignment and maximum likelihood method. Bootstrap support values are shown at the nodes.

The nucleotide sequences of nemertean species were retrieved from GenBank (NCBI). All sequences were first checked for quality and trimmed wherever necessary. Multiple sequence alignment was performed using ClustalW with default parameters to align conserved regions across the selected taxa. The aligned dataset was then used to generate a phylogenetic tree using the maximum likelihood method in MEGA X. The resulting tree was visualised and annotated to display species names and bootstrap support values, as shown in Fig. 2.

Results and discussion

Systematics

Phylum: Nemertea Schultze, 1851
Class: Hoplonemertea Hubrecht, 1879
Family: Emplectonematidae Bürger, 1904
Species: *Halimanemertes slacksmithae* Gibson, 1990

Materials examined

Voucher materials: Voucher No. ZSI/MBRC/NE-357 and Voucher No. ZSI-MBRC/NE-361, collected from Muttom Beach, Kanyakumari coast, India (8° 07' 26.1" N, 77° 18' 48.8" E) on 4 October 2018. Specimens were obtained from rocky shores in the intertidal region on marine algae (*Ulva lactuca* and *Dictyota* spp.). Approximately 200 specimens were observed, collected, and examined between October 2018 and July 2020.

Description

External morphology: In live condition, worms measured between 30 and 80 mm in length and were less than 0.75 mm in width, with a consistent width along the body. The anterior end terminated in a blunt head and the posterior end in a blunt, rounded tail (Fig. 3A). The head was distinguished from the body by a transverse furrow. The body colour was yellow. A pair of large ocelli (eye spots) with 8–10 smaller ocelli was observed in the head region (Fig. 3B); in some specimens, several small ocelli were also present (Fig. 3C). The proboscis and cerebral glands were visible through the translucent body wall, with the cerebral glands immediately beneath the ocelli.

Internal morphology: The proboscis is a remarkably flexible structure that extends to about 0.4 times the total body length (Fig. 3C). Microscopic examination of the proboscis revealed the arrangement of stylets: one central stylet (total length: 149 μm; base length: 64 μm; needle length: 84.9 μm; needle tip width: 750 nm; base width: 30 μm) accompanied by two lateral stylet sacs as in Fig. 3D. The ultrastructure of stylet base is shown in Fig. 3E. The right stylet sac contained four stylets, while the left contained three. The needle base appears rigid and consists of an aggregation of several small, round, unidentified connected structures (Fig. 3F).

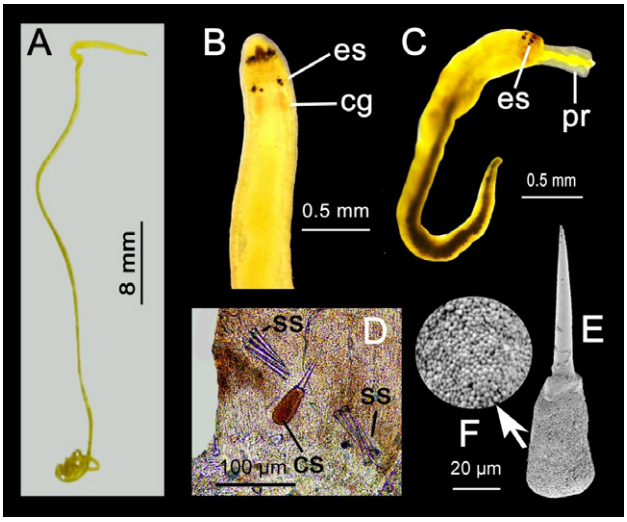

Fig. 3. *H. slacksmithae* Gibson, 1990. (A) Dorsal view of the entire worm with everted proboscis, (B) the dorsal view of the head, (C) lateral view of the whole worm with everted proboscis, (D) stylet along with accessory stylets, (E) ultrastructure of stylet base, (F) small aggregates forming the stylet base. pr-proboscis; es-eyespots; cg-cerebral ganglia; cs-central stylet; ss-secondary stylets

Remarks

This is the first record of *H. slacksmithae* Gibson, 1990 from India. This record extends the distribution range of the species from Australian (Indian Ocean) waters to the Indian marine waters. During field collection, the sting from the worm, less painful than an ant bite, caused mild, transient inflammation, which was noted on two occasions. Juveniles appeared to be particularly aggressive.

Genetic distance

A partial cox1 gene sequence (603 bp) obtained from this species contained 133 parsimony-informative sites. The phylogenetic tree of the selected species, constructed using ClustalW multiple sequence alignment and the maximum likelihood method, is shown in Fig. 2. The tree shows evolutionary relationships among *H. slacksmithae, Nemertovema norenburgi, Galathenemertes giribeti, Tetrastemma vittigerum, Emplectonema gracile, Emplectonema sp., Nemertopsis bivittata,* and *Emplectonema buergeri*. Bootstrap support values are shown at the nodes (Fig. 2). The calculated p-distance between *H. slacksmithae* and other closely related genera (*e.g.*, *Emplectonema* and *Nemertopsis* within Emplectonematidae) ranged from 18–20% (Table 1).

Table 1. Pairwise genetic distance calculated using COI sequences of *Halimanemertes slacksmithae* Gibson, 1990 and the closely related genera

Species name							
MT828541 *Halimanemertes slacksmithae* Gibson, 1990	0.00						
AJ436903 *Emplectonema gracile*	0.224						
KU197599 *Emplectonema sp.*	0.2	0.192					
AJ436902 *Emplectonema buergeri*	0.216	0.193	0.159				
MK047680 *Nemertopsis bivittata*	0.203	0.218	0.188	0.195			
MN205524 *Nemertovema norenburgi*	0.143	0.202	0.183	0.175	0.192		
MN205515 *Galathenemertes giribeti*	0.15	0.208	0.168	0.189	0.208	0.136	
KF935539 *Tetrastemma vittigerum*	0.162	0.208	0.168	0.187	0.215	0.178	0.142

Nemerteans remain a relatively understudied group in India, largely due to the taxonomic challenges and their cryptic lifestyles. To date, only eight species (7 marine and 1 freshwater) have been reported (Shrinivaasu *et al*., 2015; Kalita and Goswami, 2006; Chernyshev *et al*., 2020), suggesting that further intensive surveys along the extensive Indian coastline are likely to yield novel findings. Nemerteans not only contribute to our understanding of marine biodiversity but also provide potential sources for bioactive compounds; for example, pyridyl alkaloids from nemerteans have been shown to prevent barnacle settlement (Kem *et al*., 2003) and have antifouling properties (Kem *et al*., 2006). Additionally, various toxins produced by nemertean species are promising leads in novel drug discovery (Göransson *et al*., 2019).

Acknowledgements

The authors are grateful to Dr Hiroshi Kajihara (Hokkaido University, Japan) and Dr Jill Gibson (New Zealand) for confirming the identification of the specimen as *Monostilifera* sp. cf. *halimanemertes*. They also thank the anonymous reviewers for their constructive comments, which helped improve the quality of this manuscript.

Author contributions

Conceptualisation: RPJ, RRP; Methodology: RPJ, RRP; Data Collection: RPJ, RRP; Data Analysis: RPJ, RRP; Writing Original Draft: RPJ; Writing Review and Editing: RPJ, RRP; Supervision: RRP

Data availability

Both cox1 and H3 sequences of *H. slacksmithae* have been submitted to GenBank (accession numbers: MT828541, MT840338). Voucher specimens (No. ZSI/MBRC/NE-357 and No. ZSI-MBRC/NE-361) were deposited in the National Zoological Collection of the Marine Biological Regional Centre, Zoological Survey of India, Chennai, India.

Conflict of interest

The authors declare that they have no conflict of financial or non-financial interests that could have influenced the outcome or interpretation of the results.

Ethical statement

No ethical approval is required as the study does not include activities that require ethical approval or involve protected organisms, human subjects, the collection of samples, or protected environments.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Publisher's note

References

Berg, G. and R. Gibson. 1996. A redescription of Nemertoscolex parasiticus Greeff, 1879, an apparently endoparasitic heteronemertean from the coelomic fluid of the echiuroid Echiurus echiurus (Pallas). *J. Nat. Hist*., 30 (2): 163-173.

Chernyshev, A. V., N. E. Polyakova, M. S. Vignesh, R. P. Jain, P. Sanjeevi, J. L. Norenburg and R. P. Rajesh. 2020. A histology-free description of a new species of the genus Tetrastemma (Nemertea: Hoplonemertea: Monostilifera) from Hawaii and India. *Zootaxa*, 4808 (2): 379-383.

Chernyshev, A. V. 2016. Nemerteans of the coastal waters of Vietnam. Biodiversity of the Western Part of the South China Sea. (Eds AV Adrianov, and KA Lutaenko.) p. 279-314.

Coe, W. R. 1901. The nemerteans of Porto Rico. *Bull United States Fish Comm*., 20 (1): 223-229.

Colgan, D. J., A. McLauchlan, G. D. F. Wilson, S. P. Livingston, G. D. Edgecombe, J. Macaranas, G. Cassis and M. R. Gray. 1998. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Aust. J. Zool*., 46: 419-437.

Folmer, O., M. Black, W. Hoeh, R. Lutz and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Bio. Biotech*., 3: 294-299.

Gibson, R. 1970. The nutrition of Paranemertes peregrina (Rhynchocoela: Hoplonemertea). II. Observations on the structure of the gut and proboscis, site and sequence of digestion, and food reserves. *Biol. Bull*., 139 (1): 92-106.

Gibson, R. 1990. The macrobenthic nemertean fauna of the Albany region, Western Australia. In: The Marine Flora and Fauna of Albany, Western Australia. Proceedings of the Third International Marine Biological Workshop. Western Australian Museum, 1: 89-194.

Gibson, R. 1995. Nemertean genera and species of the world: an annotated checklist of original names and description citations, synonyms, current taxonomic status, habitats and recorded zoogeographic distribution. *J. Nat. Hist*., 29 (2): 271-561.

Göransson, U., E. Jacobsson, M. Strand and H. S. Andersson. 2019. The toxins of nemertean worms. *Toxins*, 11(2): 120.

Hall, T. A. 1999. January. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In: Nucleic acids symposium series. [London]: Information Retrieval Ltd., c1979-c2000. 41 (41): 95-98.

Herrera-Bachiller, A., F. A. Fernández-Álvarez and J. Junoy. 2015. A taxonomic catalogue of the nemerteans (phylum Nemertea) of Spain and Portugal. *Zool Sci*, 32(6): 507-522.

Hyman, L. H. 1951. Platyhelminthes and Rhynchocoela: The Acoelomate Bilateria. In: *The Invertebrates*, Vol. 2. McGraw-Hill Book Co., New York.

Jensen, K. S. P. 2005. Nemertea. In: Marine parasitology, Rohde K (ed). CSIRO publishing Victoria, Australia, p. 155-163.

Kajihara, H., A. V. Chernyshev, S. Sichun, P. Sundberg and F. B. Crandall. 2008. Checklist of nemertean genera and species (Nemertea) published between 1995 and 2007. Species Divers 13: 245-274.

Kalita, G. and M. M. Goswami. 2006. Record of occurrence of *Prostoma* Dujardin (Nemertea) in freshwater habitat of Assam, India. *Aquacult*., 7(2): 159-162.

Kalita, G. and M. M. Goswami. 2012. Record of occurrence of Prostoma Duges (Nemertea) in freshwater habitat of Assam, India. *Aquaculture*, 7 (2): 159-162.

Kem, W. R., F. Soti and D. Rittschof. 2003. Inhibition of barnacle larval settlement and crustacean toxicity of some hoplonemertine pyridyl alkaloids. *Biomol. Eng.*, 20 (4-6): 355-361.

Kem, W. R., F. Soti and D. Rittschof. 2006. Materials and methods for inhibiting fouling of surfaces exposed to aquatic environments. University of Florida and Duke University. U.S. Patent 7,087,106.

Kern. 1973 Biochemistry of nemertine toxins. In: Marine Pharmacognosy: Marine Biotoxins as Probes of Cellular Function. Martin, D. F. and Padilla, G. M. (eds.). Monogr Cell *Biol. Ser. Acad.* Press NY. p. 37–84.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* 16 (2): 111-120.

Kumar, S., G. Stecher and K. Tamura. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.*, 33 (7): 1870-1874.

McDermott, J. J. and P. Roe. 1985. Food, feeding behavior and feeding ecology of nemerteans. *Am. Zool.*, 25 (1): 113-125.

McIntosh, W. C. 1873. The Nemerteans. In: A monograph of the British annelids. Ray Society, London. https://doi.org/10.5962/bhl.title.54725

Ruppert, E. E. and R. D. Barnes. 1994. *Invertebrate Zoology: A Functional/Evolutionary Approach*, 6th ed. Saunders College Publishing, Philadelphia.

Shrinivaasu, S., K. Venkatraman and R. Mohanraju. 2011. Baseodiscus hemprichii (Ehrenberg, 1831) (phylum Nemertea) new distributional record from Andaman and Nicobar Islands, India. *Rec. Zool. Surv. India*, 111: 1-4.

Shrinivaasu, S., K. Venkataraman and C. Venkataraman. 2015. Checklist of Nemerteans with a new record in Indian coastal waters. In: Lesser known marine animals of India. Venkataraman K, Raghunathan C & Tamal Mondal RR (eds.) Zoological Survey of India, Kolkata. p. 173-175.

Sundberg, P. and M. Strand. 2010. Nemertean taxonomy-time to change lane?. *J. Zool. Syst. Evol. Res.*, 48 (3): 283-284.

Turbeville, J. M. 2002. Progress in nemertean biology: development and phylogeny. Integr *Comp. Biol.*, 42 (3): 692-703.

Yamaoka, T. 1940. Two nemerteans from the Riukiu Islands. *Annot. Zool. Japon*, 19: 13-18.