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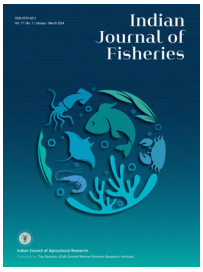
# DNA barcoding and phylogenetic analysis of deep-sea caridean shrimps (Decapoda: Caridea) from the southern coast of India

**Rekha Devi Chakraborty*, G. Kuberan, P. Purushothaman, G. Maheswarudu, A. P. Gayathri, S. Sreelakshmy and Aghana Muralidharan**
ICAR-Central Marine Fisheries Research Institute, Ernakulam North P.O., Kochi - 682 018, Kerala, India




## Abstract

Knowledge on the molecular and morphological traits of deep-sea caridean shrimps from Indian waters is still sparse. This study clarifies their phylogenetic relationships and builds a DNA barcode reference using two mitochondrial markers: COI and 16S rRNA. Specimens collected during 2014-2019 across major landing centres in Kerala and Tamil Nadu yielded 15 species spanning the genera *Plesionika, Heterocarpus, Acanthephyra, Oplophorus, Parapontocaris, Pontocaris, Pasiphaea,* and *Glyphocrangon*. The dataset includes 121 sequences (57 COI, 64 16S), all validated through NCBI BLAST searches. This forms the first comprehensive barcode library for deep-sea caridean shrimps from the region, supporting accurate species identification and future work on their evolution and biogeography and key for both ecological understanding and resource management.

## Introduction

Caridean shrimps form one of the most diverse decapod groups, with nearly 4,000 species across 36 families occupying habitats from shallow waters to abyssal plains (De Grave and Fransen 2011; Sun *et al*., 2024). Deep-sea carideans play key ecological roles in predation, scavenging, nutrient flux, and carbon cycling (Frolova *et al*., 2022). Several families, particularly Pandalidae, also support commercial fisheries, although deep-sea stocks are considered vulnerable owing to slow growth rates and low reproductive output (CMFRI, 2017; FAO, 2020). Traditional taxonomy based on rostral dentition and gill traits is often confounded by convergence and subtle variation, leading to misidentifications (Gan *et al*., 2024). Molecular markers, especially mitochondrial cytochrome oxidase subunit 1 (COI) and 16S ribosomal RNA (16S rRNA), have clarified species boundaries, revealed cryptic diversity, and reshaped caridean phylogeny (Hebert *et al*., 2004; Li *et al*., 2011; Liao *et al*., 2019), showing that many families are not monophyletic (Gan *et al*., 2024; Sun *et al*., 2024).

In India, most deep-sea shrimp studies remain morphology-based largely, with limited molecular evidence. Despite 156 species being recorded, no national DNA barcode reference library is available, which remains a critical gap (George and Rao, 1966; Mohamed and Suseelan, 1973; Radhika, 2004; Karuppasamy *et al*., 2006; Chakraborty *et al*., 2015, 2023; Kuberan *et al*., 2018). This study addresses this gap by integrating morphology with molecular analyses to generate deep-sea carideans' barcode dataset from the southern coast of India.

## Materials and methods

### Study site

Deep-sea caridean shrimps were collected between 2014 and 2019 from major commercial harbours along the southern coast of India: Kalamukku (9°59'02.91"N; 76°14'33.14"E) and Sakthikulangara (8°56'60.78"N; 76°32'34.27"E) in Kerala, and Tuticorin (8°47'40"N; 78°09'37"E) in Tamil Nadu (Fig. 1 ). Samples were collected

*Correspondence e-mail:
rekhadevi7674@gmail.com

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from deep-sea bottom trawlers operating at 200–800 m depth with a 20–26 mm cod-end mesh, trawled for 30–60 min at 3–4 knots. Specimens were immediately preserved in insulated ice boxes and transported to the laboratory for analyses.

Specimens were sorted and photo-documented. Morphological identification followed standard taxonomic references (Alcock 1901; Chace, 1985; Suseelan, 1985; Chan, 1996), ensuring accurate linkage between morphology and DNA data. Approximately 100 mg of pleopod tissue was excised from each species, preserved in 95% ethanol, and stored in pre-labelled Eppendorf tubes for molecular analysis. The integrated approach supports reliable species verification and downstream genetic studies.

## Mitochondrial DNA analysis

Total genomic DNA was extracted from pleopod tissue using the DNeasy® Blood and Tissue Kit (Qiagen) following the manufacturer's protocol. COI and 16S rRNA genes were amplified using universal primer sets: COI (Palumbi and Benzie,1991): 5'-CGCCTGTTTATCAAAAACAT-3'(F),5'-CCGGTCTGAACTCAGATCACGT-3'(R);and16S(Folmer *et al*.,1994):5'CGTCAACAAATCATAAAGATATTGG-3'(F), 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (R).

PCR amplification was carried out in 25 µl reactions containing 50 ng $\mu l^{-1}$ genomic DNA, 0.05 U $\mu l^{-1}$ Taq polymerase, 3 mM $MgCl_2$, 10 pmol of each primer, 200 µM dNTPs and 1X PCR buffer. The cycling profile included: 94°C for 5 min; 35 cycles of 94°C for 1 min, 52°C for 1 min, and 72°C for 1.5 min; followed by a final extension at 72°C for 5 min. Amplicons were checked on 1.8% TBE agarose gels, stained with ethidium bromide and visualised under UV. PCR products were purified with the XcelGen Gel/PCR Purification Kit and sequenced (BigDye Terminator v3.1) on an ABI Prism 3770 platform (Scigenome, India).

COI and 16S sequences were verified using ABISeq Editor v1.0 and edited in BioEdit v7.0.5.2 (Hall, 1999). Edited sequences were aligned with GenBank references using ClustalW. All sequence gaps were treated as missing data and the final sequences were submitted to GenBank. Phylogenetic analyses were performed in MEGA7 using Maximum Parsimony approach and phylogenetic trees were reconstructed applying the Subtree-Pruning-Regrafting (SPR) algorithm (Nei and Kumar, 2000), with 1,000 bootstrap replicates. Pairwise genetic distances were calculated in MEGA7. Maximum likelihood analyses were also performed for each gene, with the best-fit models selected by MEGA7: GTR+G+I for COI and TrN+G+I for 16S. ML trees were generated with 1,000 bootstrap replicates (Tamura *et al.,* 2013).

# Results

This study analysed 121 mitochondrial sequences [57 COI (665 bp); 64 16S (540 bp)] from 15 deep-sea caridean shrimp species representing 8 genera and 5 families. All sequences were validated through BLAST searches and deposited in GenBank (Accession numbers given in Table 1). The species studied include *Plesionika quasigrandis, P. narval, P. semilaevis, P. alcocki, P. reflexa, Heterocarpus chani, H. woodmasoni, Acanthephyra fimbriata, A. sanguinea, Oplophorus gracilirostris, Pasiphaea alcocki, Parapontocaris bengalensis, Pontocaris affinis affinis, P. propensalata,* and *Glyphocrangon investigatoris* (Fig. 2 ).

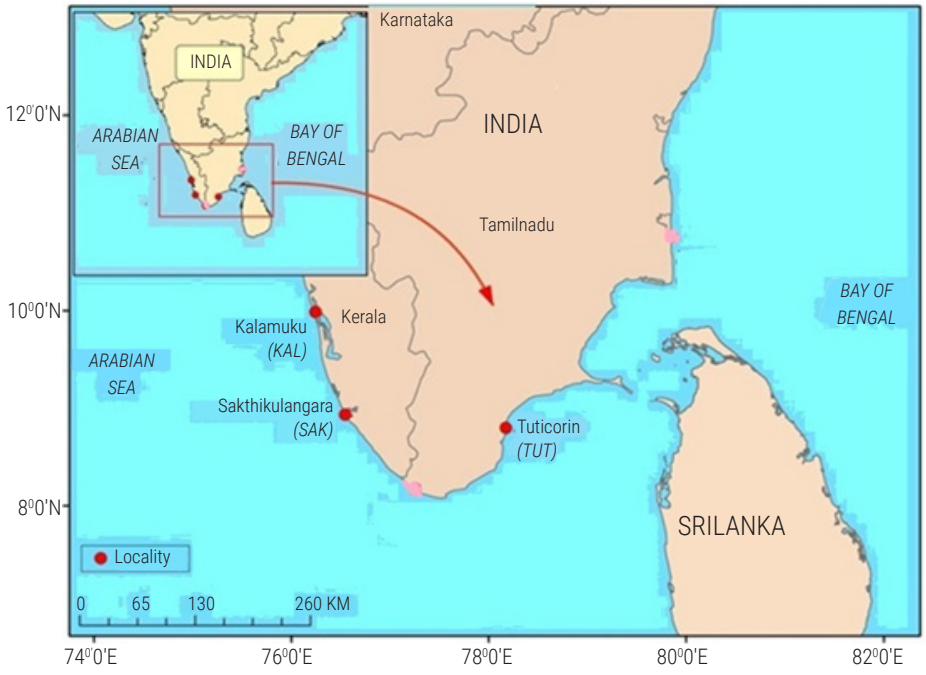


Fig. 1. Location of sampling areas along the southern coast of India

Table 1. Species, sampling locations, and GenBank accession numbers of deep-water caridean shrimps from the southern coast of India

| Sl. No. | Species | Sampling location | COI | 16S rRNA |
|---|---|---|---|---|
| 1 | *Plesionika quasigrandis* | Sakthikulangara | KM096444 | KM057395 |
| | | Sakthikulangara | KM096445 | KM057396 |
| | | Sakthikulangara | KM096446 | KM057397 |
| | | Sakthikulangara | KM096447 | KM057398 |
| | | Sakthikulangara | KM096448 | KM057399 |
| | | Sakthikulangara | KM096449 | KM057400 |
| | | Sakthikulangara | KM096450 | KM057401 |
| | | Sakthikulangara | KM096451 | KM057402 |
| | | Sakthikulangara | KM096452 | KM057403 |
| | | Sakthikulangara | KM096453 | KM057404 |
| | | Sakthikulangara | KM096454 | KM057405 |
| | | Sakthikulangara | KM096455 | KM057406 |
| | | Sakthikulangara | KM096456 | KM057407 |
| | | Sakthikulangara | KM096457 | KM057408 |
| | | Sakthikulangara | KM096458 | KM057409 |
| | | Sakthikulangara | KM096459 | KM057410 |
| | | Kalamuku | KM096460 | KM057411 |
| | | Kalamuku | KM096461 | KM057412 |
| | | Kalamuku | KM096462 | KM057413 |
| | | Kalamuku | KM096463 | KM057414 |
| | | Kalamuku | KJ401314 | KJ363166 |
| | | Kalamuku | KF938650 | KJ380892 |
| | | Tuticorin | KX838917 | KX838920 |
| | | Tuticorin | KX838918 | KX838921 |
| 2 | *Plesionika narval* | Kalamuku | KP398864 | KM057378 |
| | | Kalamuku | KP398863 | KP398866 |
| | | Kalamuku | | KM047390 |
| | | Kalamuku | | KM047389 |
| 3 | *Plesionika semilaevis* | Kalamuku | KX364192 | KX364190 |
| | | Kalamuku | KX364193 | KX364191 |
| 4 | *Plesionika alcocki* | Tuticorin | KX530799 | KX364188 |
| | | Tuticorin | KX530800 | |
| 5 | *Plesionika reflexa* | Sakthikulangara | MG958591 | MG958587 |
| | | Sakthikulangara | MG958592 | MG958588 |
| | | Sakthikulangara | | MG958589 |
| | | Sakthikulangara | | MG958590 |
| 6 | *Heterocarpus chani* | Sakthikulangara | KX364187 | KX364166 |
| | | Sakthikulangara | KX364180 | KX364167 |
| | | Sakthikulangara | KX364181 | KX364168 |
| | | Kalamuku | KX364182 | KX364169 |
| | | Kalamuku | KX364185 | KX364172 |
| | | Kalamuku | KX364186 | KX364173 |
| | | Tuticorin | KX364183 | KX364170 |
| | | Tuticorin | KX364184 | KX364171 |
| 7 | *Heterocarpus woodmasoni* | Sakthikulangara | KX364160 | KX364164 |
| | | Sakthikulangara | KX364161 | KX364165 |
| | | Sakthikulangara | MG879531 | |
| | | Sakthikulangara | MG958585 | |
| | | Sakthikulangara | MG958586 | |
| | | Sakthikulangara | MG879532 | |
| | | Kalamuku | MG879529 | |
| | | Kalamuku | MG879530 | |
| | | Tuticorin | KX838919 | KX838922 |
| | | Tuticorin | KX364162 | KX364174 |
| | | Tuticorin | KX364163 | KX364175 |
| 8 | *Acanthephyra fimbriata* | Sakthikulangara | KU055638 | KU055642 |
| | | Kalamuku | KU055639 | KU055643 |
| 9 | *Acanthephyra sanguinea* | Sakthikulangara | KU055644 | MF627729 |
| | | Sakthikulangara | KU055645 | MF627730 |
| | | Kalamuku | MF627727 | MF627731 |
| | | Kalamuku | MF627728 | MF627732 |
| 10 | *Oplophorus gracilirostris* | Sakthikulangara | KJ472213 | KJ819551 |
| | | Sakthikulangara | KJ472214 | |
| 11 | *Pasiphaea alcocki* | Sakthikulangara | MG748565 | MG748566 |
| 12 | *Parapontocaris bengalensis* | Sakthikulangara | MH045675 | MH045676 |
| 13 | *Pontacaris affinis affinis* | Sakthikulangara | MF996922 | MF996919 |
| | | Sakthikulangara | MF996923 | MF996920 |
| | | Sakthikulangara | | MF996921 |
| 14 | *Pontacaris propensalata* | Sakthikulangara | | MF996924 |
| | | Sakthikulangara | | MF996925 |
| 15 | *Glyphocrangon investigatoris* | Sakthikulangara | MH923239 | MH923238 |

Contd.....


Fig. 2. Deep-sea shrimps from the southern coast of India (a) *Plesionika quasigrandis*, (b) *Plesionika narval*, (c) *Plesionika semilaevis*, (d) *Plesionika alcocki*, (e) *Plesionika reflexa*, (f) *Heterocarpus chani*, (g) *Heterocarpus woodmasoni*, (h) *Acanthephyra fimbriata*, (i) *Acanthephyra sanguinea*, (j) *Oplophorus gracilirostris*, (k) *Pasiphaea alcocki*, (l) *Parapontocaris bengalensis*, (m) *Pontocaris affinis affinis*, (n) *Pontocaris propensalata* (o) *Glyphocrangon investigatoris*

## Systematics

Family Pandalidae Haworth, 1825
Genus *Plesionika* Spence Bate, 1888

*Plesionika quasigrandis* Chace, 1985
*Material examined:* 7 ovigerous females (CL 19–24 mm), 3 males (CL 18–20 mm), Sakthikulangara Fishing Harbour, Kollam (8°56'60.78" N; 76°32'34.27" E), 250-300 m depth, 2 February 2014; 1 ovigerous female (CL 20 mm), Kalamukku Fishing Harbour, Kochi (9°59'02.91" N; 76°14'33.14" E), 200–300 m depth, 12 February 2014.

*Distribution:* The species is widely distributed across the Indo–West Pacific, from the Gulf of Aden to Japan, Southeast Asia, Australia, and India.I In India it occurs along the southwest coast and the Gulf of Mannar. It inhabits the upper continental slope, typically at 200–500 m, with records ranging from 160 to 800 m depth.

*Plesionika narval* (Fabricius, 1787)
*Material examined:* 7 ovigerous females (CL 19–24 mm), 5 non–ovigerous females (CL 13–15 mm), 14 males (CL 10–15 mm), Kalamukku Fish Harbour, Kochi (9°59'02.91" N; 76°14'33.14" E), 200–300 m depth, 4 April 2014.

*Distribution:* The species is broadly distributed from the eastern Atlantic to the Mediterranean and occurs widely in the Indo–West Pacific, including the Red Sea, Arabian Sea, and Indian coast. It inhabits 35–400 m globally, with Indian records at 200–300 m on the southwest coast.

*Plesionika semilaevis* Spence Bate, 1888
*Material examined:* 2 ovigerous females (CL 20 mm), Sakthikulangara Fishing Harbour, Kollam (8°56'60.78" N; 76°32'34.27" E), 200–300 m depth, 8 February 2015; 2 males (CL 16–20 mm), Kalamukku Fishing Harbour, Kochi (9°59'02.91" N; 76°14'33.14" E), 200–300 m depth, 10 November 2014; 1 ovigerous female (CL 20 mm), 1 non–ovigerous female (CL 18 mm), 1 male (CL 17 mm), Tuticorin Fishing Harbour, Tamil Nadu (8°47'40" N; 78°09'37" E), 200–350 m depth, 20 January 2017.

*Distribution:* Philippines, Indonesia, South and East China Seas, Japan, and Australia. Also distributed in the south west and southeast coast of the Indian EEZ. Depth range: 200–350 m.

*Plesionika alcocki* (Anderson, 1896)
*Material examined:* 3 males (CL 22–25 mm), Tuticorin Fishing Harbour, Tamil Nadu (8°47'40" N; 78°09'37" E), 200–300 m depth, 8 January 2015.

*Distribution:* Indo–West Pacific from East Africa to Japan. Found at 287–1170 m globally; in India, recorded only from the south east coast (Bay of Bengal) at 200–300 m.

*Plesionika reflexa* Chace, 1985
*Material examined:* 1 ovigerous female (CL 15 mm) on 23 November 2015 and 1 male (CL 14.3 mm), Sakthikulangara Fishing Harbour, Kollam (8°56'60.78" N; 76°32'34.27" E), depth range 200–300 m.

*Distribution:* Indo-West Pacific, from the Red Sea and western Indian Ocean to the Philippines and Japan; in India, recorded from the south west coast (Arabian Sea). Occurs globally at 191–910 m, with Indian records at 200–300 m off Kollam.

Genus *Heterocarpus* A. Milne-Edwards, 1881

*Heterocarpus chani* Li, 2006
*Material examined:* Specimens were collected from 200–350 m depth at four sites: Sakthikulangara Fishing Harbour, Kollam (4 ovigerous females, CL 25–30 mm; 2 February 2014); Kalamukku, Kochi (3 males, CL 18–25 mm; 12 February 2014); Tuticorin Fishing Harbour (3 ovigerous females, 1 non-ovigerous female, 1 male; CL 17–25 mm; 8 January 2015); and Nagapattinam (1 ovigerous female, 1 non-ovigerous female, 1 male; CL 17–28 mm; 24 March 2017). One specimen was deposited as a voucher in the Marine Biodiversity Museum of ICAR-CMFRI, Kochi (E.D. 2.4.1.4), with the remainder retained in the working collection.

*Distribution:* Southern South China Sea, Bohol Sea and Sulu Sea (Philippines), at depths of 382–888 m; Bay of Bengal and Arabian Sea (India), at depths of 200–350 m.

*Heterocarpus woodmasoni* Alcock, 1901
*Material examined:* 3 ovigerous females (CL 22–28 mm), Sakthikulangara Fishing Harbour, Kollam (8°56'60.78" N; 76°32'34.27" E), 200–300 m depth, 18 March 2014; 3 Male (CL 20–25 mm), Kalamukku Fishing Harbour, Kochi (9°59'02.91" N; 76°14'33.14" E), 200–300 m depth, 28 March 2014; 2 ovigerous females (CL 20–25 mm), 1 non-ovigerous female (CL 18 mm), 3 males (CL 21–24 mm), Tuticorin Fishing Harbour (8°47'40" N; 78°09'37" E), 200–350 m depth, 8 January 2016.

*Distribution:* Northern Indian Ocean, India, and the Andaman Sea (318–485 m depth). From the southern coast of India (both east and west coasts) at a depth of 200–350 m.

Family Acanthephyridae Spence Bate, 1888
Genus *Acanthephyra* A. Mine-Edwards, 1881

*Acanthephyra fimbriata* Alcock and Anderson, 1894
*Material examined:* 3 males (CL 26–28 mm), Sakthikulangara Fishing Harbour, Kollam (8°56'60.78" N; 76°32'34.27" E), 200–300 m depth, 3 February 2015; 1 male (CL 30 mm), Kalamukku Fishing Harbour, Kochi (9°59'02.91" N; 76°14'33.14" E), 200–300 m depth, 18 November 2015.

*Distribution:* Gulf of Aden, Andaman Sea, Bay of Bengal, Laccadive Sea, and Arabian Sea: off Goa and the Philippines occurring between 412-1785 m. Off the Kerala coast between 200 and 350 m depths.

*Acanthephyra sanguinea* Wood-Mason and Alcock, 1892
*Material examined:* 2 ovigerous females (CL 23–25 mm), Sakthikulangara Fishing Harbour, Kollam (8°56'60.78" N; 76°32'34.27" E), 200–300 m depth, 18 March 2014; 3 males (CL 18–21 mm), Kalamukku Fishing Harbour, Kochi (9°59'02.91" N; 76°14'33.14" E), 200–300 m depth, 28 March 2014; 1 ovigerous female (CL 25 mm), 1 non-ovigerous female (CL 18 mm), 1 male (CL 17 mm), Tuticorin Fishing Harbour (8°47'40" N; 78°09'37" E), 200–350 m depth, 29 January 2017.

*Distribution:* Indo-West Pacific; Gulf of Aden and Eastern African coast, depth 3200 m. Southern coast of India at a depth of 200-350 m.

Family Oplophoridae Dana, 1852
Genus *Oplophorus* H. Milne Edwards, 1837

*Oplophorus gracilirostris* A. Milne Edwards, 1881
*Material examined:* 6 non-ovigerous females (CL 16–18 mm), Sakthikulangara Fishing Harbour, Kollam (8°56'60.78" N; 76°32'34.27" E), 200–300 m depth, 6 January 2014; 3 males (CL 20–25 mm); 13 non-ovigerous females (CL 17–20 mm), Tuticorin Fishing Harbour (8°47'40" N; 78°09'37" E), 400–800 m depth, 24 January 2014.

*Distribution:* Off south-eastern Africa, the Indian Ocean, Indonesia, the Philippines, southern Japan, Fiji Islands, Hawaii, the Gulf of Mexico, the Bahamas and Caribbean Sea. Specimen in the present study was from a depth of 400-800 m along the southern coast of India.

Family Pasiphaeidae Dana, 1852
Genus *Pasiphaea* Savigny, 1816

*Pasiphaea alcocki* Wood-Mason and Alcock, 1891
*Material examined:* 3 males (CL 20–23 mm), Sakthikulangara Fishing Harbour, Kollam (8°56'60.78" N; 76°32'34.27" E), 200–300 m depth, 16 November 2013.

*Distribution:* The species is widespread in the Indo-West Pacific and southwestern Atlantic; in India it occurs in the Arabian Sea and Bay of Bengal. Previously recorded from 335–1732 m, *P. alcocki* off Sakthikulangara at a shallower depth of 200–300 m.

Family Crangonidae Haworth, 1825
Genus *Parapontocaris* Alcocki, 1901

*Parapontocaris bengalensis* (Wood-Mason and Alcock 1891)
*Material examined:* 3 ovigerous females (CL 9–10 mm), 1 male (CL 9 mm), Sakthikulangara FishingHarbour, Kollam (8°56'60.78" N; 76°32'34.27" E), 200–300 m depth, 12 October 2013.

Genus *Pontocaris* Spence Bate, 1888

*Pontocaris affinis affinis* (Alcock, 1901)
*Material examined:* 20 non-ovigerous females (CL 9–11 mm), 18 males (CL 10–11 mm), Sakthikulangara Fishing Harbour, Kollam (8°56'60.78" N; 76°32'34.27" E), 200–300 m depth, 16 September 2014.

*Distribution:* Along the Indian coast, *P. affinis affinis* was previously reported from Bombay, the Bay of Bengal, and Madagascar at 33–175 m depth. Record from the Quilon Bank at 250–300 m indicates a much wider depth range in the Indian Ocean.

*Pontocaris propensalata* Spence Bate, 1888
Material examined: 2 non-ovigerous females (CL 10–11 mm), Sakthikulangara Fishing Harbour, Kollam (8°56'60.78" N; 76°32'34.27" E), 200–300 m depth, 10 December 2015.

*Distribution:* Distributed across the Andaman Sea, Philippines, Indonesia, and the south-west Pacific at depths of 100–525 m. The present specimens were collected at 250–300 m on the continental slope of the south-eastern Arabian Sea.

Family Glyphocrangonidae Smith, 1884
Genus *Glyphocrangon* A. Milne-Edwards, 1881

*Glyphocrangon investigatoris* Wood-Mason and Alcock, 1891
*Material examined:* 3 ovigerous females (CL 26–28 mm), Sakthikulangara Fishing Harbour, Kollam (8°56'60.78" N; 76°32'34.27" E), 200–300 m depth, 18 December 2017.

*Distribution:* Bay of Bengal, Arabian Sea, Myanmar, Sri Lanka; Depth 145–803 m.

A total of 57 COI sequences representing 14 deep-sea caridean shrimp species collected from 200–800 m depth along the southern coast of India were generated and deposited in GenBank. Sequence lengths ranged from 451–684 bp, yielding 17 haplotypes with high haplotype diversity (Hd = 0.82). The dataset showed an overall A+T bias (57.3%), highest at the third codon position (62.9%). Mean interspecific genetic distance across taxa was 26%, with maximum divergence (43%) between *P. quasigrandis* and *O. gracilirostris*, and minimum divergence (21%) between *Plesionika reflexa* and *H. chani*. Phylogenetic relationships were inferred using Maximum Likelihood analysis (GTR+G+I; 1000 bootstraps) (Fig. 3) .

Within *Plesionika*, five species were identified from 29 sequences, with a mean interspecific divergence of 13% and no intraspecific variation; notably, *P. quasigrandis* showed complete genetic homogeneity across the Arabian Sea and Bay of Bengal, confirming earlier misidentifications. Two *Heterocarpus* species exhibited low intrageneric divergence (1%) and moderate interspecific divergence (24%), while two *Acanthephyra* species showed 20% divergence. Several rare or data-deficient taxa (*O. gracilirostris*, *Pontocaris affinis affinis*, *Parapontocaris bengalensis*, *Pasiphaea alcocki*, and *Glyphocrangon investigatoris*) were also barcoded, all lacking intraspecific divergence, though high interspecific divergence (31%) was observed between *P. affinis affinis* and *P. bengalensis*.

In 16S rRNA analyses, sequence lengths varied from 314 bp in *O. gracilirostris* to 671 bp in *G. investigatoris*, followed by *P. reflexa* (650 bp) and *A. sanguinea* (628 bp). Multiple sequence alignment (BioEdit) produced a consensus length of 655 sites, including 61 variable sites (54 parsimony-informative, 7 singletons) and 34 invariable sites, with 465 gaps or missing data. Twenty haplotypes were identified. Mean nucleotide composition of the 16S rRNA dataset (n = 64) was A 32.2%, G 22.3%, C 11.0%, and T 34.5%. Mean pairwise K2P genetic distance was 31%, with maximum divergence between *P. narval* and *P. propensalata* (59%) and minimum between *P. propensalata* and *Pontocaris affinis affinis* (9%). Phylogenetic relationships inferred using Maximum Likelihood (GTR+G+I, 1000 bootstraps) are shown in Fig. 4 .

*Pandalidae:* Five *Plesionika* species (*P. quasigrandis, P. narval, P. alcocki, P. reflexa*, and *P. semilaevis*; n=33) were analysed, with sequence lengths ranging from 484 to 644 bp. Alignments yielded 525 conserved and 53 variable sites (48 parsimony-informative). Mean nucleotide composition was A 32.7%, G 23.3%, C 11.2%, and T 32.8% (A+T=65.5%). Mean genetic distance within *Plesionika* was 11%, with maximum divergence between *P. narval* and *P. semilaevis* (32%) and minimum between *P. reflexa* and *P. semilaevis* (15%). Intraspecific divergence was low (0–1%).

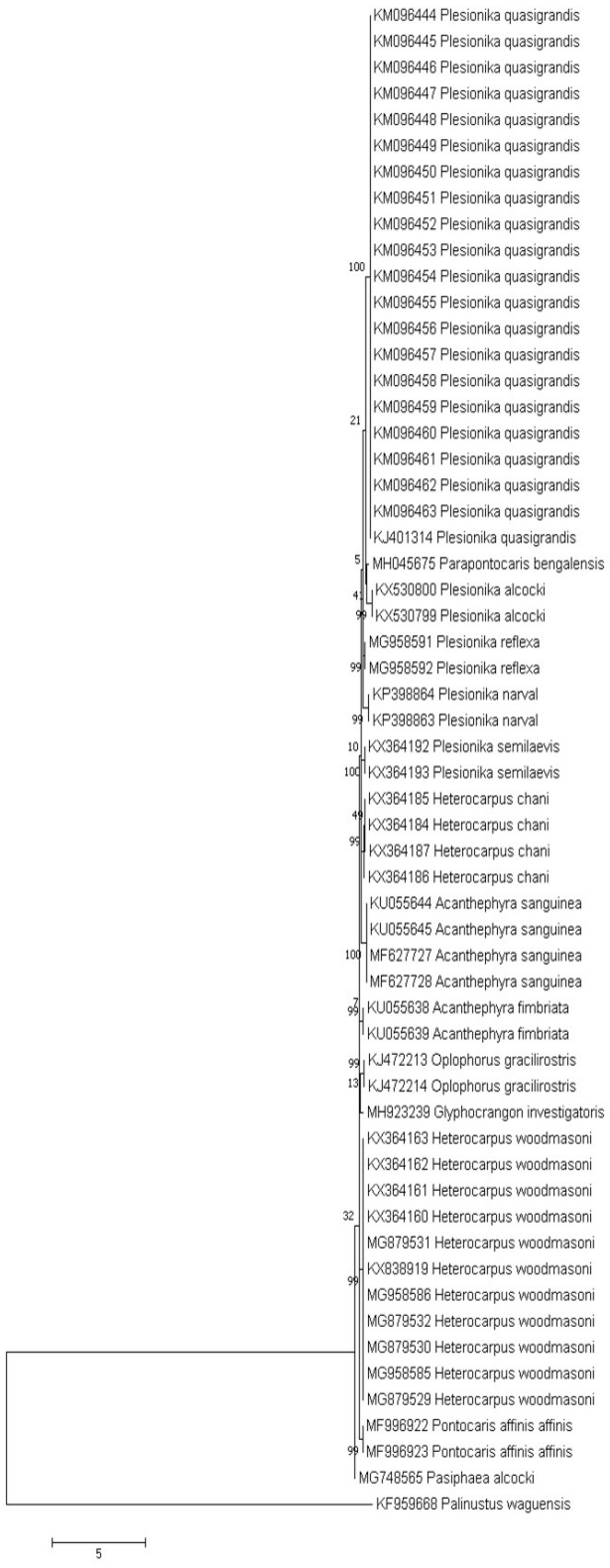


Fig. 3. Phylogenetic tree of the identified caridean shrimps using Maximum-Likelihood with 1000 bootstraps under the best fitting model GTR+G+I inferred from DNA Sequences of mitochondrial COI gene, Samples collected from the southern coast of India

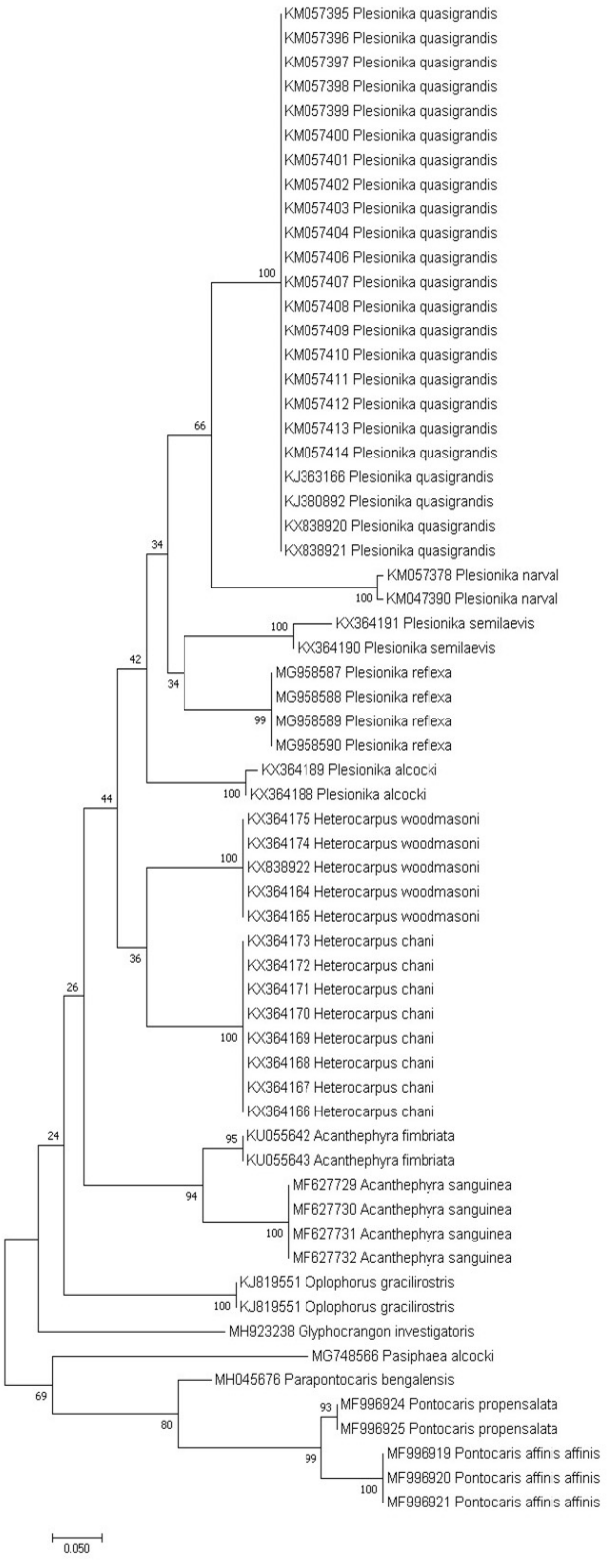


Fig. 4. Phylogenetic tree of the caridean shrimps from the southern coast of India using Maximum-Likelihood with 1000 bootstraps under the best fitting model GTR+G+I inferred from DNA sequences of mitochondrial 16S rRNA gene

Two *Heterocarpus* species (*H. chani* n=8; *H. woodmasoni* n=5) were analysed using 16S rRNA (376 bp). Of 367 sites, 35 were parsimony-informative. Mean nucleotide composition showed strong A+T bias (68.7%). Mean genetic distance was 5%, with 10% interspecific divergence and no intraspecific variation across regions.

*Acanthephyridae:* The family Acanthephyridae was represented by two *Acanthephyra* species, *A. fimbriata* (n=2) and *A. sanguinea* (n=4), analysed using 16S rRNA sequences (485 bp). Of the 344 aligned sites, 315 were invariable and 29 were parsimony-informative, with no singleton sites. Mean nucleotide composition was A 29.6%, G 22.5%, C 10.9%, and T 37.4% (A+T = 67%), increasing to 72.6% at the third codon position. The mean genetic distance within *Acanthephyra* was 5%, with an interspecific K2P distance of 9% and no intraspecific divergence detected in either species.

*Crangonidae:* Within Crangonidae, two genera were analysed: *Parapontocaris* (*P. bengalensis*, n=1) and *Pontocaris* (*P. affinis affinis* and *P. propensalata*). The 16S sequences ranged from 481 to 493 bp. Alignment yielded 469 sites, including 61 variable and 17 parsimony-informative sites. Mean nucleotide composition was A 30.5%, G 23.9%, C 11.8%, and T 33.8% (A+T = 64.3%), increasing to 66.6% at the third codon position. The mean genetic distance among species was 6%, with interspecific K2P distances ranging from 4% to 13%. The lowest divergence (4%) occurred between *P. propensalata* and *P. affinis affinis*, while no intraspecific divergence was detected in either species.

*Other carideans:* We also recorded three other caridean species, *O. gracilirostris* (n=2) from the family Oplophoridae, *G. investigatoris* (n=1) from the family Glyphocrangidae, and *P. alcocki* (n=1) from the family Pasiphaeidae. All of these species were collected from the south-west coast of India. For the COI gene, we observed amplified sequence lengths of 284 bp for *O. gracilirostris*, 540 bp for *G. investigatoris*, and 510 bp for *P. alcocki*. Based on the results of genetic analysis, we found that the intraspecific genetic distance for the specimens of *O. gracilirostris* was 0%, indicating a lack of genetic variation among these individuals.

## Discussion

Taxonomic resolution of deep-sea shrimps is frequently constrained by morphological complexity and convergence, necessitating the use of molecular tools for accurate species identification (Gomon *et al*., 2014). DNA barcoding, which relies on the principle that interspecific genetic divergence exceeds intraspecific variation, has proven to be a rapid and reliable approach for species delimitation across animal taxa, including crustaceans (Hebert *et al*., 2004; Goldstein and DeSalle, 2011). Deep-sea caridean shrimps remain among the least explored crustacean groups, with persistent taxonomic uncertainties across families, although previous studies employing mitochondrial, nuclear ribosomal, and protein-coding genes have improved understanding of higher-level relationships and species complexes within Carideans (Li *et al*., 2011). In this context, the present study provides a substantial contribution to deep-sea caridean shrimp diversity along the southern coast of India by integrating COI and 16S rRNA markers, generating COI barcodes for 14 species and 16S sequences for 15 species, and confirming 11 new regional records through a combination of morphological and molecular evidences.

This study provides robust molecular evidence for accurate species delimitation within the genus *Plesionika* from Indian waters. Phylogenetic analyses based on COI and 16S rRNA genes clearly resolved five well-supported clades in this genus: *P. quasigrandis*, *P. narval*, *P. semilaevis*, *P. alcocki*, and *P. reflexa*. Molecular comparison of Indian *P. quasigrandis* with the Philippine type material showed COI divergence of 5.8–8.4%, confirming its identity and correcting earlier misidentification as *P. spinipes* in Indian waters (Chakraborty *et al.,*2015). Specimens previously identified as *P. martia* from multiple deep-sea landing centres along the southern Indian coast were genetically and morphologically confirmed as *P. semilaevis*. Comparative analyses with GenBank sequences showed substantial divergence between *P. semilaevis* and *P. martia*, particularly in COI (up to 26.1%), exceeding the commonly accepted crustacean species threshold (>3%), conclusively establishing *P. semilaevis* as the species occurring in Indian waters.

Genetic comparisons of Indian *P. reflexa* with topotypic material of *P. ensis* and *P. reflexa* revealed high inter-regional COI divergence (9.3–14.5%), while divergence within Indian material remained low (0–0.3%). Despite limited genetic differentiation, consistent morphological differences, especially reduced or absent epipods on pereiopods III and IV, suggest that the Indian form may represent a distinct taxon (Chan *et al.,*2018). *Plesionika narval* from the south-west coast of India clustered distinctly in ML analyses and showed moderate genetic divergence from closely related species (*P. grandis, P. ensis, P. spinipes*, and *P. williamsi*). For *P. alcocki*, COI and 16S sequences showed no intraspecific divergence, and strong bootstrap support confirmed its stable presence along the Indian coast.

The genus *Heterocarpus* comprises commercially important deep-sea shrimps with a global diversity of about 30 species (Holthuis, 1980). This study reports the first COI and 16S rRNA sequences of *H. chani* and *H. woodmasoni* from Indian waters. *Heterocarpus chani*, previously misidentified as *H. gibbosus* in India, was earlier confirmed morphologically, and its COI and 16S sequences generated here were deposited in GenBank. Comparative analysis showed low intraspecific divergence in *H. chani* (0–2% for COI; 0% for 16S), while interspecific divergence from the closely related *H. gibbosus* was higher (12–13% for COI; 0–1% for 16S), providing molecular support for its occurrence in Indian waters. Similarly, *H. woodmasoni*, another commercially important species, was sequenced for COI and 16S and compared with available GenBank data. Low to moderate intraspecific divergence was observed (0-9% for COI; 0-4% for 16S), further validating its taxonomic identity in Indian waters.

This study analysed COI and 16S rRNA sequences of two *Acanthephyra* species (*A. fimbriata and A. sanguinea*) from Indian waters. *Acanthephyra fimbriata*, which shows morphological resemblance to *A. armata*, was compared with GenBank sequences. Intraspecific divergence between Indian and Philippine material (COI: KP076185; 16S: KP075895) was low (3% for COI; 0.3% for 16S), while Indian specimens showed complete genetic homogeneity (COI: KT222917, KT222918, MG029405; 16S: KP372713). Interspecific divergence from other *Acanthephyra* species ranged from 17.5–20.9% (COI) and 5.2–9.5% (16S), consistent with earlier findings.

*A. sanguinea*, commonly occurring along the southern coast of India, is reported here for the first time using molecular data, with sequences deposited in GenBank. Phylogenetic analyses based on COI and 16S showed no intraspecific divergence among Indian specimens. Comparisons with congeners revealed interspecific divergence of 7.4% from *A. fimbriata* (KU055642, KU055643) using 16S (KU055642, KU055643) and 22.9% using COI (KU055644, MF627727), supporting clear species-level separation.

In this study, we present the first report of the species *O. gracilirostris* from India, along with its molecular data (COI and 16S). To determine the genetic relationships and phylogeny, pairwise genetic distance and sequence identity analysis of COI and 16S genes were conducted, focusing on closely related species, namely *O. typus* and *O. spinosus*. Comparing COI sequences with *O. typus* and *O. spinosus* showed interspecies genetic distances of 0.020 (2%) and 0.131 (13%), respectively. Similarly, comparing 16S DNA sequences of *O. gracilirostris* available in NCBI GenBank with the present sequence of *O. gracilirostris* showed an intraspecies genetic distance of 0.03 (3%) and interspecies distance of 0.05 (5%) with *O. typus* (Chakraborty *et al.,*2014).

The genus *Parapontocaris* comprises six species with a global distribution. In this study, we focused on *P. bengalensis* for which mitochondrial gene sequencing of COI and 16S rDNA was performed and these sequences were subsequently deposited in GenBank. The sequence lengths for COI and 16S genes were determined to be 634 and 481 bp respectively. To assess the genetic relationships, we conducted interspecies divergence analysis, comparing the COI and 16S sequences from the present *P. bengalensis* specimen with other sequences retrieved from the NCBI database. The results revealed substantial genetic differences between *P. bengalensis* and *P. levigata* (KP759476, 9.1%) as well as *Parapontocaris* sp. (Accession No. KP759477, 8.8%) when analysing COI sequences. On the other hand, for 16S sequences, the genetic differences were observed to be in the range of 2.1–6.2% with *P. levigata*, *Parapontocaris* sp., and *P. aspera*.

Mitochondrial gene sequencing was conducted for *P. affinis affinis*, focusing on COI, with accession numbers MF996922 and MF996923, and for 16S rDNA with accession numbers MF996919, MF996920, and MF996921. Additionally, the 16S rDNA sequences of *P. propensalata* were analysed, and their accession numbers are MF996924 and MF996925. Upon comparing the 16S rDNA sequences, a genetic divergence of 4.1% was observed between *P. affinis affinis* and *P. propensalata* (Purushothaman *et al.,* 2019).

We retrieved COI and 16S sequences of twenty-two and nine species, respectively, from the NCBI database, including one intraspecies sequence of *P. alcocki*. For the COI gene, the following species were analysed: *P. sivado* (KP759488 and JQ306265), *P. telacantha* (KP759492), *P. multidentata* (JQ305978, FJ581855, and KF931036), *P. planidorsalis* (KP759483), *P. sirenkoi* (KP759484), *P. tarda* (DQ882139, JQ305981, and AF125439), *P. merriami* (MF197272), *P. pacifica* (DQ882135), and *P. hoplocerca* (JQ306169). For the 16S gene, the following species were considered: *P. sivado* (MF279526 and KP725631), *P. telacantha* (KP725635), *P. multidentata* (MF279519), *P. planidorsalis* (KP725624), *P. levicarinata* (MF279517 and GQ131899), *P. japonica* (MF279516), *P. sirenkoi* (MF279525 and KP725625), *P. merriami* (MF197216 and EU868700), *P. mclaughlinae* (MF279518), *P. americana* (MF279511), *P. acutifrons* (MF279508), *P. sinensis* (MF279524), *P. diaphana* (MF279512), *P. aequus* (MF279509), *P. falx* (MF279514), *P. romenskyi* (MF279522), *P. gelasinus* (MF279515), *P. scotiae* (MF279523), *P. orientalis* (MF279520), *P. pseudacantha* (MF279521), and *P. exilimanus* (MF279513). Interspecific divergence was higher for COI (18.5–27.9%) than for 16S (7.4–26.4%). Although genetic divergence between *P. sirenkoi* and the present *P. alcocki* isolate was relatively low (16S: 7.4%; COI: 18.5%), clear morphological differences were observed. The present specimen bears 10–11 spines on the merus of the second pereiopod, compared to a single spine in *P. sirenkoi*, supporting the substantial intraspecific divergence (21.2%) observed within *P. alcocki* (16S: MF279510)Mitochondrial COI and 16S rRNA sequences of *Glyphocrangon investigatoris* (618 bp and 464 bp, respectively) were generated and deposited in GenBank. Intraspecific comparison of COI sequences with available NCBI data showed low divergence (0.6–0.7%) from *G. investigatoris* (KJ143751, KJ143752). Comparable 16S data for this species were unavailable in GenBank. Interspecific comparisons with other *Glyphocrangon* species revealed higher divergence in COI (5.1–26.5%) than in 16S (1.1–8.1%). Among congeners, *G. regalis* showed the lowest divergence from the present material (COI: ~5%; 16S: 1.1%) (Kuberan *et al*., 2019).

The present study demonstrates the pivotal role of mitochondrial DNA (mtDNA) divergence in accurately identifying commercially important deep-sea shrimp species. The results highlight the effectiveness of molecular approaches in resolving species boundaries and clarifying phylogenetic relationships. Consistently lower intraspecific than interspecific genetic distances confirm the strong diagnostic value of mtDNA markers for distinguishing closely related shrimp species.

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