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Morphological and molecular identification of the marine copepod *Paramphiascella fulvofasciata* Rosenfield and Coull, 1974 (Crustacea: Harpacticoida) occurring in the Gulf of Mannar Biosphere Reserve, India, using 18S rRNA gene sequence data

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ABSTRACT

The harpacticoid copepod Paramphiascella fulvofasciata is a meiobenthic species of growing interest as a candidate live feed organism in marine aquaculture, yet no sequence-based identification of the species has been formally reported from Indian waters. Copepod specimens were collected from Mandapam coastal waters, Gulf of Mannar Biosphere Reserve, Tamil Nadu, India, and examined by light microscopy using standard zooplankton taxonomic manuals together with the original species description. Males measured 0.65 to 0.80 mm and females 0.70 to 0.85 mm in total length, with fifth leg length ranging from 0.08 to 0.12 mm in males and 0.10 to 0.15 mm in females; the fifth leg was leaf-like and enlarged in females, while males possessed geniculate antennules. A partial 18S ribosomal RNA gene sequence of 642 base pairs was generated and deposited in GenBank under accession number PP864741.1. The sequence grouped with the only other 18S rRNA sequence attributed to this species that we found in GenBank (EU380293.1), adjacent to Amphiascoides atopus, in a Maximum Likelihood tree (81% bootstrap support for the pair). Because dissected appendages and setal and spine counts were not examined, the identification should be regarded as provisional at species level. This study provides the first formal morphological and sequence-based account of P. fulvofasciata from Indian waters, extending a known distribution that previously included the USA, the Beaufort Sea, England, the German Bight, and South Korea.

Keywords: *Paramphiascella fulvofasciata; Harpacticoida; 18S rRNA; molecular identification; Gulf of Mannar Biosphere Reserve; India.*

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1. Introduction

Copepods are among the most abundant metazoans in marine ecosystems and occupy a central position in pelagic and benthic food webs [1,2]. Among them, harpacticoid copepods have attracted particular attention in aquaculture research because of their benthic lifestyle, tolerance of a wide range of environmental conditions, and suitability for high-density culture, which together make them promising live feed organisms for the larvae of fish and crustaceans with small mouth gapes [3,4,5,6,7,8].

The genus *Paramphiascella* Lang, 1944 (Miraciidae Dana, 1846) consists mostly of marine harpacticoids. It comprised 26 species at the time of Kim et al. [9] [10,11], and further species, including *P. aestuarii*, have been described since [12]. Three members live outside fully marine settings: two in brackish water in Thailand and one in fresh water in Venezuela [9]. Species of the genus have a semicylindrical, fusiform body with an indistinct boundary between prosome and urosome, and the genus is morphologically close to *Amphiascoides* Nicholls, 1941, from which it is separated mainly by the form of the male second swimming leg endopod (Kim et al. [9]). Sequence data point to a close relationship between the two genera, although their relationships remain poorly resolved with the sequences currently available [12].

Paramphiascella fulvofasciata was originally described by Rosenfield and Coull [13] from suspended sediment in tidal creeks and from wood bored by the isopod *Limnoria tripunctata* in Massachusetts Bay, USA, based on adult morphology and a complete account of naupliar and copepodite development. As compiled by Kim et al. [9], it has since been recorded from the Beaufort Sea, England, and the German Bight [14], and Kim et al. [9] reported it from sublittoral sandy sediment in Geoje, South Korea. Kim et al. [9] noted differences between their Korean material and the original description in the armature of the mouthparts, considered that some details of the original account might be inaccurate, and called for re-examination of type material to clarify the taxonomic status of the Korean specimens.

To our knowledge, none of these morphological accounts included molecular data. More recently, *P. fulvofasciata* collected from the Gulf of Mannar, India, was the subject of a culture optimisation study establishing species-specific rearing protocols for its use as a live feed organism [15]. That study deposited the 18S rRNA gene sequence reported here in GenBank (accession PP864741.1) but focused on culture performance and gave only a brief account of identification; the present study documents the morphological and molecular basis of the identification in more detail.

No molecular data for *P. fulvofasciata* from Indian waters have been formally documented in the taxonomic literature, and the species has not previously been reported in detail from the Gulf of Mannar Biosphere Reserve. Given the mouthpart variation already noted between the type of population and the Korean population, combined morphological and molecular evidence is particularly valuable for assessing species identity in a newly recorded population. The present study therefore provides a morphological account of *P. fulvofasciata* collected from the Gulf of Mannar Biosphere Reserve, India, supported by 18S rRNA gene sequencing and phylogenetic placement among related copepod taxa, and compares the Indian material with the previously described populations from the USA and South Korea.

2. Materials and Methods

2.1. Study area and sample collection

Copepod samples were collected from Mandapam coastal waters of Gulf of Mannar Biosphere Reserve, Tamil Nadu, India (9°15'13"N, 79°08'03"E), on 21st January 2022 by horizontal towing of a zooplankton net (158 µm mesh). Continuous aeration was provided using a battery-operated aerator during transport to the laboratory. On arrival, the sample was supplemented with a mixed microalgal diet and left undisturbed overnight. For isolation, the sample was diluted with filtered seawater and individual copepods were hand-picked using a fine brush.

2.2. Morphological identification

Isolated copepods were examined under an inverted phase-contrast microscope (Micros SUNDEW MCX1600; Micros, Austria) for initial morphological identification, with subsequent examination and photomicrography carried out using a Leica stereomicroscope fitted with a digital imaging system. Specimens were placed to genus using standard zooplankton taxonomic manuals [16,17,18,19], and species-level identity was assessed against the original description of Rosenfield and Coull [13], the redescription of Kim et al. [9], and the harpacticoid checklist and key of Wells [10]. Total length and fifth leg length were measured on three males and three females.

2.3. Preservation and molecular material

Following morphological identification and imaging, specimens designated for molecular analysis were preserved in 95% ethanol. Live individuals from the same collection were isolated separately and maintained in laboratory culture, forming the basis of a concurrent study on the culture requirements of the species [15]. No voucher specimens were deposited in a museum or institutional collection; the molecular record of the isolate is GenBank accession PP864741.1.

2.4. DNA extraction, amplification, and sequencing

Genomic DNA was extracted from a single ethanol-preserved specimen (isolate PSPS 02) using the DNeasy Blood and Tissue Kit (Qiagen) according to the manufacturer's protocol. A partial fragment of the 18S small subunit ribosomal RNA gene was amplified using the primer pair 18S rRNA F (5'-CAGCAGCCGCGGTAATTCC-3') and 18S rRNA R (5'-CCCGTGTTGAGTCAAATTAAGC-3'), as listed in GenBank record PP864741.1. PCR was carried out in a 25 µL reaction volume containing 1× buffer, 2.0 mM MgCl2, 0.2 mM of each dNTP, 0.4 µM of each primer, 1 U Taq DNA polymerase and approximately 50 ng of template DNA, with initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min, and a final extension at 72 °C for 7 min. The amplicon was sequenced bidirectionally by the Sanger dideoxy method at the Rajiv Gandhi Centre for Aquaculture (RGCA), Sirkali, Tamil Nadu, India. The forward and reverse reads were aligned and edited in MEGA 11 [20], with the reverse read converted to its reverse complement before alignment, to obtain a 642 base pair consensus sequence that was deposited in GenBank under accession number PP864741.1.

2.5. Sequence analysis and phylogenetic reconstruction

The developed sequence was compared against the NCBI nucleotide database using BLASTn [21] to assess similarity with existing records. Reference sequences of related harpacticoid and other copepod taxa were retrieved from GenBank in July 2024 for comparative phylogenetic analysis. The reference alignment comprised 81 GenBank sequences and 1872 aligned positions, including gaps, and PP864741.1 was added to give a dataset of 82 sequences. An inter-specific phylogenetic tree was constructed using the Maximum Likelihood method in MEGA 11 software [20] under the Tamura-Nei [22] substitution model with uniform rates among sites. All sites were used, with gaps and missing data retained, and the tree search used the nearest-neighbour-interchange heuristic from an automatically generated initial tree (Neighbor-Joining/BioNJ). Node support was assessed with 500 bootstrap replicates. Uncorrected pairwise p-distances over the 642 bp regions covered by PP864741.1 were calculated from the alignment, excluding positions with a gap or ambiguity code in either sequence.

3. Results

3.1. Systematics

Phylum: Arthropoda
Subphylum: Crustacea
Class: Hexanauplia
Subclass: Copepoda
Order: Harpacticoida
Family: Miraciidae
Subfamily: Diosaccinae
Genus: *Paramphiascella*
Species: *Paramphiascella fulvofasciata*

3.2. Description

The Gulf of Mannar Biosphere Reserve specimens were consistent with the generic diagnosis of *Paramphiascella* in body shape and segmentation, and with those characters of *P. fulvofasciata* detailed below that could be examined in whole-mount material (Fig. 1). The general body plan is shown in the line drawing in Fig. 2.

Males measured 0.65 to 0.80 mm in total length and females 0.70 to 0.85 mm (Table 1).

Females were larger than males in the type and Korean populations, and the ranges recorded here follow the same tendency, although with three specimens per sex and overlapping ranges this could not be tested. Measurement conventions differ among studies: Rosenfield and Coull [13] measured from the base of the rostrum to the tip of the caudal rami, and Kim et al. [9] from the anterior margin of the rostrum to the posterior margin of the caudal ramus, so the comparisons in Table 1 are approximate. The fifth leg (P5) was sexually dimorphic: in males it was smaller and more modified, measuring 0.08 to 0.12 mm in length, whereas in females it was larger and leaf-like, measuring 0.10 to 0.15 mm. Males possessed geniculate antennules, bent in a knee-like fashion and used for grasping females during mating, a feature typical of harpacticoid copepods [23] and consistent with the male antennule described by Kim et al. [9]. The P5 and the male antennule are not illustrated in the present figures.

Detailed setal armature of the antennule, antenna, mouthparts, and swimming legs P1 to P4 was not resolved to the level of individual seta and spine counts in the present material; this remains to be documented through dissection and scanning electron microscopy and is discussed further under Limitations in Section 4. The measured characters and gross body plan are consistent with the existing diagnosis of *P. fulvofasciata* [13,9]. However, the male second swimming leg endopod, which separates this species from the morphologically close *P. pacifica* [9], and other dissection-level characters were not examined, so the species-level identification is provisional and rests additionally on the sequence evidence presented below.

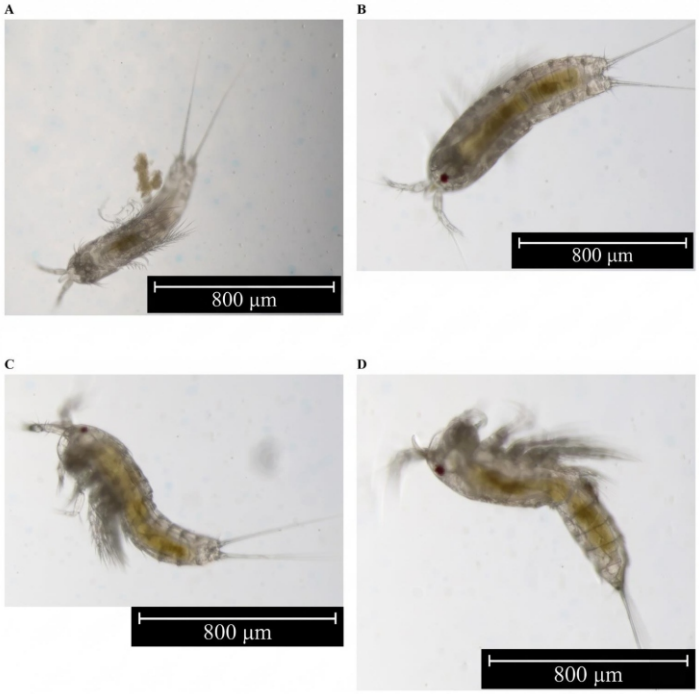


Fig. 1. Light microscope photomicrographs of adult Paramphiascella fulvofasciata, Gulf of Mannar material (unstained, whole-mount, live specimens). A. habitus of the whole specimen; B, anterior region showing the cephalosome, single medial eye spot, and antennules; C, mid-body region showing the prosome and swimming legs; D, posterior urosome and caudal rami bearing a long terminal seta. Scale bars are not shown because calibrated scale information was not available for the photographs; all four photographs were taken at the same zoom setting.

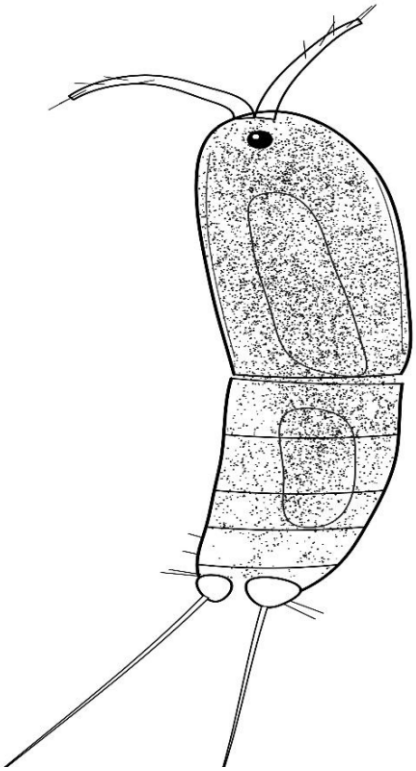


Fig. 2. Line drawing of an adult Paramphiascella fulvofasciata, Gulf of Mannar material, dorsal view, traced from a photomicrograph of a whole-mounted specimen. The outline, the cephalosome with the single medial eye spot, the junction between prosome and urosome, the four visible urosomal somite boundaries, the gut, the antennules, and the caudal rami with their long terminal setae were traced directly from the photograph; stippling approximates the tone of the specimen in the photograph. The sex of the specimen is not indicated. Segmentation of the antennules, setal and spine counts, pores, and surface ornamentation are not shown because they were not resolved in the photomicrographs (see Limitations, Section 4). No scale bar is shown (see Fig. 1).

Table 1. Comparison of adult total body length in known populations of Paramphiascella fulvofasciata

Population	Sex	n	Total length (mm)
Massachusetts, USA (type locality; Rosenfield and Coull [13])	Female	4	0.83 (one value reported for 4 females)
Massachusetts, USA (type locality; Rosenfield and Coull [13])	Male	1	0.78
Geoje, South Korea [9]	Female	3	0.67 to 0.83
Geoje, South Korea [9]	Male	5	0.60 to 0.71
Gulf of Mannar, India (present study)	Female	3	0.70 to 0.85
Gulf of Mannar, India (present study)	Male	3	0.65 to 0.80

3.3. Molecular identification

Genomic DNA amplification of the target 18S rRNA fragment yielded a clean 642 base pair sequence, deposited in GenBank under accession number PP864741.1 (isolate PSPS 02; submitted 1 June 2024). BLASTn comparison against the NCBI nucleotide database showed the highest sequence similarity, with 100% identity, 100% query coverage, and an E-value of 0.0, to a reference sequence identified as *Paramphiascella fulvofasciata*, which is consistent with the morphological identification. Because this is the only sequence attributed to the species and its provenance could not be verified, the match supports the identification but does not verify it independently. Over the 642 bp region, the sequence was identical to EU380293.1 and differed from the closest sequence of another genus, *Amphiascoides atopus* (KC815328.1), at only 3 of 642 positions (0.47%).

Differences from the other taxa in the broader group (*Robertgurneya*, *Rhyncholagena*, Normanellidae, *Sarsamphiascus*, and *Typhlamphiascus*) ranged from 10 to 13 positions (1.6 to 2.0%).

3.4. Phylogenetic analysis

In the Maximum Likelihood tree constructed from 18S rRNA sequences of related harpacticoid and other copepod taxa (Fig. 3), PP864741.1 grouped with EU380293.1, the only other GenBank 18S rRNA sequence identified as *P. fulvofasciata* that we found, although the provenance of EU380293.1 could not be confirmed from public metadata alone. This pair was adjacent to *Amphiascoides atopus* (KC815328.1), in agreement with the close relationship between *Paramphiascella* and *Amphiascoides* noted in the literature [9,12], and lay within a broader group containing *Robertgurneya* sp., *Rhyncholagena* sp., a member of Normanellidae, *Sarsamphiascus hawaiiensis*, *S. kawamurai*, and *Typhlamphiascus typhlops*. PP864741.1 and EU380293.1 clustered with 81% bootstrap support, and this pair grouped with *Amphiascoides atopus* with 97% support. Support for the nodes joining this group to other taxa was 69% or lower, and most deeper nodes had low support, so the tree is intended to indicate the placement of the new sequence, not to resolve relationships among genera or families. It does not include *P. aestuarii*, whose 18S sequence (PQ505074) was reported by Gómez et al. [12], and it contains few miraciid representatives, so it is compatible with, but does not test, the placement of *Paramphiascella* in Miraciidae. Gómez et al. [12] likewise found the relationships between *Amphiascoides* and *Paramphiascella* poorly resolved with 18S data. The low divergence among these taxa over the 642 bp region (Section 3.3) indicates that this fragment carries little phylogenetic signal at genus level.

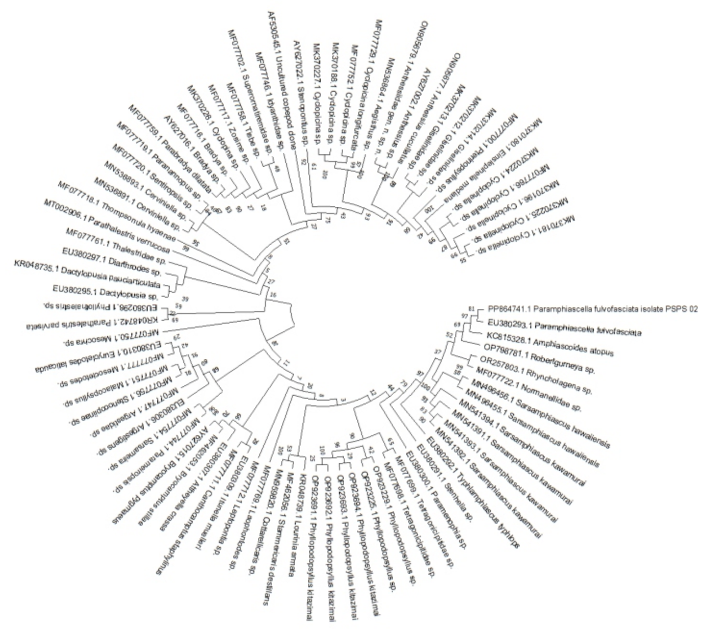


Fig. 3. Maximum Likelihood phylogenetic tree based on partial 18S rRNA gene sequences, showing the placement of Paramphiascella fulvofasciata isolate PSPS 02 (GenBank accession PP864741.1, this study) among related harpacticoid and other copepod taxa. Tips are labelled with GenBank accession number and taxon name. Numbers at nodes are bootstrap percentages from 500 replicates, and branch lengths are not proportional to genetic distance. The tree was inferred under the Tamura-Nei [22] substitution model in MEGA 11 [20].

4. Discussion and conclusion

The combined morphological and molecular evidence presented here supports the identification of the Gulf of Mannar specimens as *Paramphiascella fulvofasciata* and provides the first formal morphological and sequence-based account of the species from Indian waters. Its known distribution, which previously comprised the USA (Massachusetts), the Beaufort Sea, England, the German Bight, and South Korea [13,14,9], is thereby extended to the Indian coast of the Gulf of Mannar Biosphere Reserve.

The total length ranges recorded for the Gulf of Mannar material overlap those reported by Kim et al. [9] for Korean females and extend above them for males (upper limit 0.80 mm against 0.71 mm), and the upper limits for both sexes slightly exceed the single values given for the type population (0.83 mm for females and 0.78 mm for males; Rosenfield and Coull [13]). Those type dimensions were based on one male and four females taken from a collection of more than one hundred specimens, so they are not a robust baseline, and with three specimens per sex and differing measurement conventions among studies, the present data do not indicate a biologically meaningful size difference between populations.

Kim et al. [9] documented differences in mouthpart armature, specifically in the mandibular cutting-edge tooth count, the number of spines on the maxillule praecoxal arthrite, and the presence of an accessory seta on the maxilla, between the Massachusetts type population, material from the German Bight reported by Dahms [14], and their own Korean specimens. They considered it necessary to re-examine type material to clarify the taxonomic status of the Korean specimens, and the possibility that *P. fulvofasciata* as currently understood includes more than one species cannot be excluded without such work. Whether the Gulf of Mannar population shows comparable variation in these characters cannot be determined from the present data, since dissection-level examination of the mouthparts was not completed in this study. This is identified as a priority for future work, discussed further below.

To our knowledge, the only other 18S rRNA sequence attributed to *P. fulvofasciata* in public databases is EU380293.1. With only two sequences available, a meaningful intraspecific genetic distance could not be calculated, and none is presented here. The grouping of the two sequences is consistent with, but does not prove, conspecificity, because the 18S rRNA gene is highly conserved and has limited power to separate closely related taxa [24]: over the 642 bp region analysed here, *Amphiascoides atopus* differed from the sequence at only 3 of 642 positions (0.47%), in line with the poor resolution between *Amphiascoides* and *Paramphiascella* reported by Gómez et al. [12]. A faster-evolving marker such as cytochrome oxidase subunit I (COI) is needed for species-level delimitation and population-level comparison [25,27]. To our knowledge, no COI sequence is available for *P. fulvofasciata*, whereas Gómez et al. [12] generated COI data for its congener *P. aestuarii*; COI sequencing of the Gulf of Mannar material, using standard metazoan primers [26], and submission to the BOLD Systems barcode library [28], is therefore a clear priority.

Beyond its taxonomic significance, the presence of *P. fulvofasciata* in the Gulf of Mannar reinforces the value of this species as a candidate live feed organism for regional aquaculture, complementing the culture optimisation data already established for the same population [15].

This study has several limitations. The identification is provisional at species level because dissected appendages were not examined, and detailed appendage-level setal and spine counts for the antennule, antenna, mouthparts, and swimming legs were not resolved to the standard achieved in the original description and the Korean redescription. Scanning electron microscopy, which would allow direct character-by-character comparison with these prior accounts, was not undertaken, and the P5 and the male antennule are not illustrated. Molecular characterisation relied on a single nuclear marker from a single isolate, the provenance of the only comparison sequence (EU380293.1) could not be verified, and the phylogeny does not include the congener *P. aestuarii* and has low bootstrap support for most deeper nodes. Future work should prioritise detailed dissection and SEM-based documentation of the mouthparts and swimming legs of Gulf of Mannar material for direct comparison with the type and Korean populations, generation of a COI sequence, a phylogeny that includes congeners, and morphometric sampling of a larger series of individuals to establish more robust population-level size ranges.

Morphological examination combined with 18S rRNA gene sequencing supports the presence of *Paramphiascella fulvofasciata* in the Gulf of Mannar Biosphere Reserve, India, and provides the first formal morphological and sequence-based account of the species from Indian waters. The Gulf of Mannar specimens are consistent with the existing diagnosis in body form, sexual dimorphism of the fifth leg, and body length, and the generated sequence groups with the only other 18S rRNA sequence attributed to the species. Dissection-level morphological comparison, COI barcoding, and a phylogeny that includes congeners remain important next steps and would further strengthen the species-level identification.

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Ethics statement

No ethics committee approval was required for this study, as *Paramphiascella fulvofasciata* is a marine invertebrate not subject to institutional animal ethics regulations in India. All field collection was conducted in accordance with applicable national guidelines and did not involve any regulated or endangered species.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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Author contributions

Palani Sridhar: conceptualisation, methodology, formal analysis, data curation, visualisation, writing (original draft). Perumal Santhanam: supervision, validation, funding acquisition, writing (review and editing). Both authors read and approved the final manuscript.

Data availability statement

The 18S rRNA gene sequence generated in this study is available in GenBank under accession number PP864741.1. Other data are available from the corresponding author on reasonable request.

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