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Short Communication

First record of the cymothoid isopods, *Joryma malabaricus* Aneesh, Helna & Trilles, 2019 and *Nerocila arres* Bowman & Tareen, 1983, from West Bengal, along with the molecular phylogeny of *Joryma malabaricus* using the mtCOI gene

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Two species of parasitic isopods, *Joryma malabaricus* Aneesh, Helna & Trilles, 2019, and *Nerocila arres* Bowman & Tareen, 1983, were documented for the first time from the West Bengal coast. Molecular characterization of *J. malabaricus* was also conducted during the present study. *Joryma malabaricus* differs genetically from its congener sequences considered for the analysis with a K2P distance of 6.55 – 31.03 %. Additionally, a new host, *Protonibea diacanthus* (Lacepède, 1802), for *Nerocila arres* is reported in this manuscript.

[**Keywords**: Isopoda, Molecular analysis, New record, West Bengal]

Introduction

Parasitic isopods of the family Cymothoidae are parasites that attach to the buccal cavity, branchial cavity, and external surfaces of several fish species[1]. They are protandrous by nature and feed on the blood of host fish for their development[2-4]. Cymothoid isopods are found worldwide, with the exception of Arctic seas[5]. They can cause anaemia, skin lesions, and inflammation at the attachment site, leading to slower growth rates, impaired reproductive abilities, tongue degeneration, deformities in the opercular plate, damage to gill filaments, cardiovascular problems, and sometimes death in juvenile fishes[5-7]. The family Cymothoidae comprises 362 valid species worldwide[8]. According to Quddusi[9], there are 19 species of cymothoid isopods reported along the Pakistan coast. In contrast, no records of these species have been documented from Sri Lanka or Bangladesh. Meanwhile, India has reported a total of 70 species from this family to date[10]. From West Bengal coast, 13 species of cymothoid isopods have been documented[11].

The genus *Joryma* Bowman & Tareen, 1983, consists of 6 species worldwide[12]. This genus is characterised by a hunched body, an anterolateral angle of pereonite 1 that extends to the lateral side of the cephalon, a well-separated antennule base, a stout and enlarged mandibular palp, and coxae of pereonites 2 and 3 that are much larger and more inflated compared to the other coxae. The pleon and pereon are subequal in width, and the uropod rami do not extend to the posterior margin of the pleotelson[13,14]. The type taxon of the genus *Joryma* is *J. sawayah* Bowman and Tareen, 1983 and was described from the Gulf of Kuwait[13], while the type locality of *J. malabaricus* is from the Malabar coast, India[14].

The genus *Nerocila* Leach, 1818 comprises 43 species worldwide, with 14 species documented in India[15,16]. *Nerocila* can be identified by having dorso-ventrally flattened body, pleon narrower than pereon, its trilobed cephalon devoid of rostral process, and the presence of ventral processes on pleonites 1 and 2(refs. 1,17). The type taxon for the genus *Nerocila* is *N. blainvillei* Leach, 1818, with unknown type locality[18].

During this study, the molecular characterisation of species *J. malabaricus* Aneesh, Helna & Trilles, 2019, was carried out. Additionally, a new host, *Protonibea diacanthus* (Lacepède, 1802) is reported for *N. arres* Bowman & Tareen, 1983.

Materials and Methods

Specimens were collected from the Digha fish landing centre (21°38'14" N; 87°34'05" E) between November 2022 and March 2024 during routine field surveys along the West Bengal coastline. Daily fish catches were thoroughly examined for the presence of any cymothoid isopod species in the buccal cavity, branchial cavity, and on the external surfaces using a hand lens. Once an isopod was spotted, it was carefully removed using a pair of forceps. The collected isopod specimens were preserved in 99.9 % ethanol for further examination, while the host fish species were preserved in a 10 % formalin solution. The host fish were photographed using a Nikon D3500 DSLR camera, and the isopods were photographed using a Leica S9i stereo zoom microscope. Isopod identification was carried out using literature by Aneesh *et al.*[12] and Ravichandran *et al.*[1], while the host fish species were identified according to Talwar & Kacker[19] and Froese & Pauly[20]. The isopods were deposited in the National Repository of Estuarine Biology Regional Centre, Zoological Survey of India, Gopalpur-on-Sea.

DNA extraction and PCR amplification

DNA isolation from the muscle tissue present beneath the pereonites of the isopod species was performed using the HIMEDIA Blood and Tissue kit. The mitochondrial COI gene was amplified and sequenced with the universal primers LCO 1490: 5'-ggtcaacaaatcataaagatattgg-3' and HCO2198: 5'-taaacttcagggtgaccaaaaaatca-3'. The PCR reaction mixture for the COI gene consisted of Taq Master Mix (12.5 µL), isolated DNA (2 µL), forward primer (1 µL), reverse primer (1 µL), and a molecular-grade deionised water (8.5 µL), making a total volume of 25 µL. The PCR thermal cycling conditions included an initial denaturation at 94 °C for 3 min, followed by 35 cycles of 30 sec at 94 °C, 30 sec at 42 – 46 °C (gradient annealing temperatures), 45 sec at 72 °C for extension, with a final extension at 72 °C for 10 min, and an indefinite hold at 4 °C. Sanger sequencing of the amplified product was carried out by the Barcode Biosciences Pvt. Ltd., Bangalore, India. The sequence was assembled using BioEdit version 7.2 by Hall *et al.*[21]. Phylogenetic analysis was conducted on the obtained sequence to examine interspecific relationships. One mitochondrial COI gene sequence was submitted to NCBI and assigned the accession number PP066010.1 (621 bp). The GenBank accession numbers of mitochondrial COI gene sequences for other *Joryma* and cymothoid species considered in the analysis are: *J. sawayah* (OQ438522.1; 666 bp and OQ118337.1; 642 bp), *J. engraulidis* (ON181579.1; 246 bp), *J. hilsae* (MT876662.1; 675 bp and MT876659.1; 675 bp), *Cymothoa indica* (LC776736.1; 573 bp), *C. eremita* (MK430025.1; 658 bp), *C. frontalis* (ON661338.1; 612 bp), *Nerocila phaiopleura* (ON661341.1; 618 bp), *N. exocoeti* (ON661340.1; 621 bp), and *Alitropus typus* (KT445864.1; 662 bp). Using MEGA X by Kumar *et al.*[22], the comparable COI gene sequences obtained from the NCBI and BOLD databases underwent multiple alignment with K2P following Kimura[23] and ML tree analysis. The species *Alitropus typus* H. Milne Edwards, 1840 of the family Aegidae, with a K2P distance of 37.15 % was taken as an out-group.

Results

Order: Isopoda Latreille, 1816
Family: Cymothoidae Leach, 1814
Genus: *Joryma* Bowman & Tareen, 1983
Joryma malabaricus Aneesh, Helna & Trilles, 2018

Joryma malabaricus Aneesh, Helna & Trilles, 2018

Material examined: One female; Total Length (TL): 15.438 mm; width (at 6th pereonite): 7.1 mm; cephalon length: 0.8 mm; cephalon width: 1.1 mm; pleotelson length: 3.3 mm; pleotelson width: 2.8 mm; Host: *Escualosa thoracata* (Valenciennes, 1847); collection date: 23.11.2022; Regd. No: EBRC ZSI/Cr-16084.

Description: The body is hunched to one side. Cephalon is immersed in pereonite 1, and its anterior margin crosses the anterolateral margin of pereonite 1. Eyes large and visible. Antennule and antenna reach till the pereonite 1. The antennule is shorter than the antenna and composed of 8 articles. The article 8 with terminal setae. Antenna is a little longer than antennule and composed of 9 articles. Article 9 with little terminal setae. Maxillula has 4 recurved spines. Maxilla with 3 spines, and maxilliped has 4 spines. Brood pouch having 4 oostegites that arise from pereopods 2 – 4 and 6. The anterolateral angle of pereonite 1 is unilateral, without bilobed. Pereonite 1 is the longest, while pereonite 2 is the shortest. Coxa 2 is visible from the dorsal view and larger than the other coxae. Coxae 3, 6 and 7 are half overlapped by the previous pereonites. Pleonites subequal in width, and pleonite 5 is slightly longer than other pleonites. Pleonites 1 and 2 are laterally overlapped by pereonite 7. Pleotelson triangular with a caudo-medial lobe (Fig. 1a, b)

Distribution: Known to occur along the Malabar coast[14] and Odisha coast[24] of India.

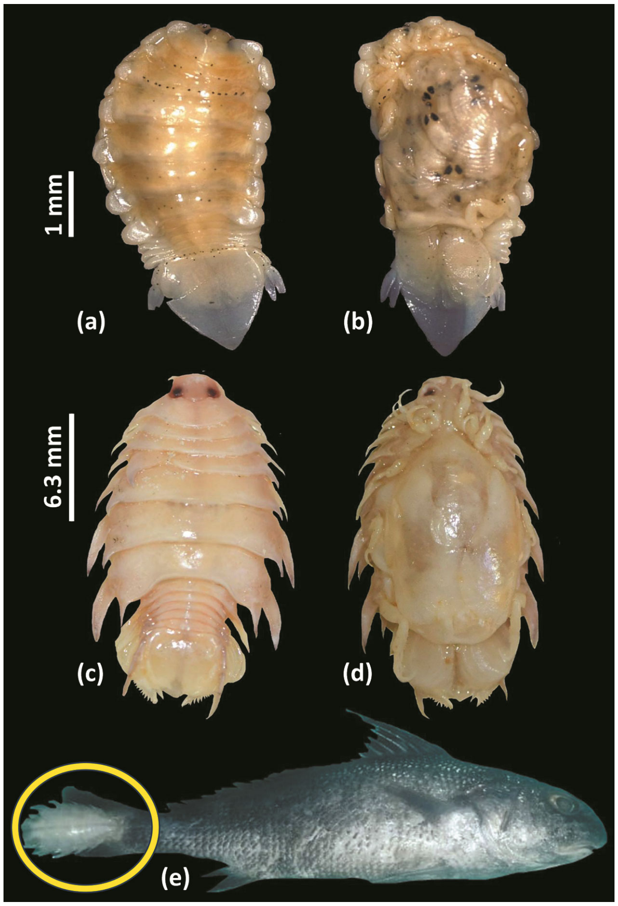

Fig. 1 — a,b) *Joryma malabaricus* Aneesh, Helna & Trilles, 2019: a) Dorsal view, and b) Ventral view; c,d) *Nerocila arres* Bowman & Tareen, 1983: c) Dorsal view, and d) Ventral view; e) New host record *Protonibea diacanthus* (Lacepède, 1802) for *N. arres*

Genus: *Nerocila* Leach, 1818

***Nerocila arres* Bowman & Tareen, 1983**

Material examined: One female; Total Length (TL): 25.40 mm; width (at 6th pereonite): 15.14 mm; cephalon length: 2.1 mm; cephalon width: 3.46 mm; pleotelson length: 4.53 mm; pleotelson width: 5.21 mm; exopod length: 3.55 mm; endopod length: 3.22 mm. Host: *Protonibea diacanthus* (Lacepède, 1802); collection date: 06.03.2024; Regd. No: EBRC ZSI/Cr-16727.

Description: Anterior margin of cephalon weakly convex, posterior margin tri-lobed. Antennule 9 segmented, antenna 8 segmented. The pereonite anterior margin has three depressions for the accommodation of the posterior lobes of the cephalon. Eyes moderately visible. All the coxae are visible in the dorsal view. Coxae 2, 6 and 7 cross the margin of respective pereonites. The width of the pereonites increases from pereonite 1 to 6. The width of pereonite 7 is lesser than that of pereonite 6. All the pleonites are visible. The ventral processes of pleonite 1 and 2 reach till the anterior part of the pleotelson. The merus, carpus and propodus of the 6th and 7th pereopod are having spines. The pleotelson lateral margins are convex in shape and the posterior margin has a caudo-medial point. The uropods cross the posterior margin of the pleotelson. The uropodal exopod taper at the distal point and the outer margin of the endopod is deep, serrated with a notch at the distal point (Fig. 1c, d).

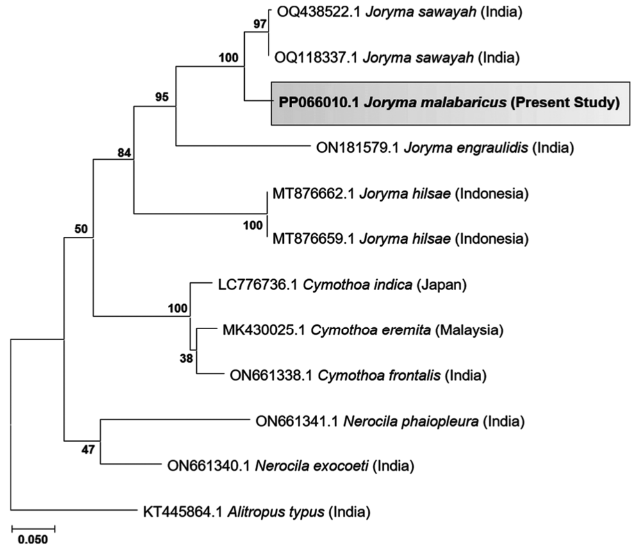

Fig. 2 — Phylogeny of *Joryma malabaricus* with *J. sawayah* showing both the species are phylogenetically related and have a common ancestor. The sequence of *Alitropus typus* is taken as an outgroup

Distribution: This species is known to occur in several countries *viz*. India, Iraq, Kuwait, Australia, and Malaysia[3,13,17,25-27].

Phylogeny of *Joryma malabaricus*

In the current study, *Joryma malabaricus* differs genetically from the sequences used for the analysis by a K2P distance of 6.55 – 31.03 % (Fig. 2). The species *J. malabaricus* is close to *J. sawayah* with a K2P distance of 6.55 – 6.88 %. In ML tree analysis, the sequence of *J. malabaricus* appears in a different cluster from its congeners, with strong bootstrap support of 100 %.

Discussion

The species *J. malabaricus* can be differentiated from *J. hilsae* and *J. engraulidis* by having overlapping pleonites and a triangular pleotelson (*vs*. *J. hilsae* and *J. engraulidis* have a rounded pleotelson and non-overlapping pleonites)[12]. It can be differentiated from *J. sawayah* and *J. brachysoma* by having the cephalon crossing the anterior margin of pereonite 1 (*vs.*

cephalon is small and does not cross the margin of pereonite 1). The species *J. malabaricus* have unilateral and non-bilobed anterolateral margin of pereonite 1 (*vs*. a bilobed anterolateral margin of pereonite 1 in the case of *J. sawayah*)[12,28]. The species, *J. malabaricus* differs from *J. tartoor* by having unilateral anterolateral angle of pereonite 1 (*vs*. bilateral and slightly bilobed antero-lateral angle of pereonite 1 in *J. tartoor*). Cephalon is not completely immersed to pereonite 1 in *J. malabaricus* (*vs*. cephalon is covered dorsally in *J. tartoor*). In *J. malabaricus*, the pleotelson is round triangular (*vs*. in *J. tartoor*, the pleotelson is acute triangular)[29].

The phylogenetic relationship of *J. malabaricus* with *J. sawayah* (Fig. 2) indicates that both species had a common ancestral origin. Earlier there was no sequence of *J. malabaricus* available in NCBI or other databases. This work provides a novel gene sequence for further analysis.

The species, *N. arres* looks similar to *N. serra* at first glance. *Nerocila arres* has deep serration in uropodal endopod (*vs*. shallow serration in *N. serra*). Morphologically it looks similar to *N. sigani*, with *N. arres* having a caudo-medial lobe (*vs*. no lobe in *N. sigani*). In *N. arres*, 15 – 16 teeth are present in the serration of uropodal endopod (*vs*. 10 teeth in *N. sigani*). In *N. arres*, 5 spines present on the maxilliped (*vs*. 6 spines in the maxilliped of *N. sigani*)[5]. *Nerocila arres* was earlier reported from *Nemipterus japonicus* (Bloch, 1791) and *Terapon puta* Cuvier, 1895(refs. 1,29). Presence of this species in *Protonibea diacanthus* (Lacepède, 1802) indicated this fish species as another new host for *N. arres*.

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Conflict of Interest

The authors declare no conflict of Interest.

Author Contributions

RM & DR: Sample collection, conceptualization and design; SKM & SR: Identification, molecular characterization and manuscript writing; DKT, JKS & AM: Data interpretation, and preparation of final manuscript draft.

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