



First record of a rare Axiid Shrimp *Eutrichocheles modestus* (Herbst, 1796) (Malacostraca: Decapoda: Axiidae) from Northwestern Bay of Bengal

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Abstract

A rare axiid shrimp *Eutrichocheles modestus* (Herbst, 1796) belong to the family Axiidae have been discovered from the Digha Coast, Northwestern Bay of Bengal, India. The presence of *E. modestus* in Northwestern Bay of Bengal constitutes extension of the known geographical range of the species in the Indo-Pacific. In addition, the molecular information of *E. modestus*, using the mitochondrial Cytochrome Oxidase subunit I (COI) gene was provided with the K2P genetic divergence matrix of the closely related genera in the family Axiidae. Thus, it is believed that further exploration of this rare axiid shrimp *E. modestus* in the Indian waters will fulfill the gaps on population as well as other biological information.

Keywords: Cytochrome Oxidase subunit I (COI), Digha, Genetic Divergence, Range Extension, Trawl Bycatch, West Bengal

Introduction

Burrowing lobsters of the family Axiidae Huxley, 1879 is diverse and currently comprises of more than 190 species belonging to 56 genera worldwide (Sakai, 2011; WoRMS, 2019). Among Axiidae, the genus *Eutrichocheles* Wood-Mason, 1876 contains seven species: *Eutrichocheles austrinus* (Sakai, 1994), *E. defenses* (Rathbun, 1901), *E. foveolatus* (Kensley, 1996), *E. granulimanus* (Kensley, 1996), *E. modestus* (Herbst, 1796), *E. spinipleurus* (Kensley, 1996) and *E. tuamotu* Ngoc-Ho, 1998 (Ngoc-Ho *et al.*, 2005; Sakai, 2011). *Eutrichocheles modestus* was described in 1796, and since then in the intervening 210 years only twelve specimens, all males, have been recorded (Ngoc-Ho, 1998; Ngoc-Ho *et al.*, 2005; Van Xuan & Ngoc-Ho, 2006). The habitat of *E. modestus* seems to be mud and sandy bottoms because of its burrowing ability. The average depth was noted to be 4-15 m and mainly prefers the estuarine areas of large rivers (van Xuan & Ngoc-Ho, 2006).

This species does not possess any importance in the commercial fishery because of its rare occurrence in the fishing nets or trawls. The present contribution is one such record on *E. modestus* from the Northwestern Bay of Bengal. Only five species of Axiid shrimps has been recorded till date from Indian waters, viz. *Eiconaxius andamensis* (Alcock, 1901), *E. laccadivensis* Alcock & Anderson, 1894, *Ambiaxius alcocki* (McArdle, 1900); *Calaxiopsis felix* Alcock & Anderson, 1899; *Eutrichocheles modestus* (Herbst, 1796) (Radhakrishnan *et al.*, 2012). However, knowledge on the diversity and taxonomic expertise of axiid shrimps are still lacking. Hence, to validate the species taxonomy, we attempted for an integrated approach by examining the morphology as well as DNA Barcoding technique based on the partial segment of mitochondrial Cytochrome Oxidase Subunit I (COI) gene. DNA barcoding has been considered as a valid tool for species identification in crustaceans (Madhavan *et al.*, 2020). It can effectively identify cryptic

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species by measuring the differences in their genetic characters (Bucklin *et al.*, 2007).

Material and Methods

During a routine survey, on 21st July 2019 at Digha Mohana fish landing centre, West Bengal, Northwestern Bay of Bengal, a single adult specimen of *E. modestus* was obtained. The specimen was collected from the trawl bycatch that was thrown as discarded at the landing centre. Therefore, the exact location of the sample was not recognized. The collected specimen was photographed to record the natural colour prior to preservation in 90% ethanol for further use. The preserved material was washed with 70% ethanol and key morphological characters were identified based on Chopra (1933), Ngoc-Ho *et al.* (2005), Van Xuan and Ngoc-Ho (2006). The material examined was deposited in the National Zoological Collection of the Zoological Survey of India, Marine Aquarium and Regional Centre, Digha as voucher specimen (MARC/ZSI/A6840). The size of the shrimps are measured and expressed as total length (TL in mm) from the tip of the rostrum to the posterior margin of the telson and carapace length (CL in mm) from the posterior orbital margin to the posterior margin of the carapace.

The representative specimen (1 no.) was also subjected to molecular identification. The tissue sample was collected from the propodal region of the third pereopod (Prakash & Amit Kumar, 2020) and the total genomic DNA was extracted using OMEGA BIO-TEK E.Z.N.A. Tissue DNA Kit (Omega Bio-tek, Norcross, GA) following the manufacturer's protocol. The PCR amplification was performed using LCO-1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO-2198 (5'-TAAACTTCAGGGTGACCAAAAATCA-3') primers (Folmer *et al.*, 1994). Each PCR contained 12.5 µL 2X PCR master mix (Ampliqon, Denmark), 2.5 µL each of the two primers (10 nM), and 2.5 µL of template DNA (10-20 ng) and water to make a final volume of 25 µL. PCR conditions were as follows: initial denaturation at 95°C for 10 min, 35 cycles of 95°C for 45 sec, 50°C for 45 sec, and 72°C for 45 min and final extension at 72°C for 10 min according to Prakash & Amit Kumar (2020). The PCR products were visualized on 1% agarose-gel electrophoresis and products with high intensity band were bi-directionally sequenced using ABI Prism 3730 Genetic Analyzer (Thermo Fisher Scientific, USA) based on BigDye Terminator Chemistry.

The chromatograms were visualized, edited, and contig was prepared using consensus sequences from both the forward and reverse strands in the BioEdit (Hall, 1999) software. The generated sequence (641 bp) obtained in the present study was deposited in NCBI GenBank. The generated sequence was compared with the published COI sequences of related taxa from NCBI GenBank using BLASTn tool (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Lastly, the percentage identity matrix was estimated for *E. modestus* along with other taxa in the family Axiidae through Clustal Omega 2.1 (Sievers *et al.*, 2011). The generated GenBank accession number of *Eutrichocheles modestus* (MN933767) was acquired from the GenBank global database.

Results

Order DECAPODA Latreille, 1802

Family AXIIDAE Huxley, 1879

Genus *Eutrichocheles* Wood-Mason, 1876

Eutrichocheles modestus (Herbst, 1796) (Figures 1, 2A–E).

1796. *Cancer modestus* Herbst, Versuch einer Naturgeschichte der Krabben und Krebse: nebst einer systematischen Beschreibung ihrer verschiedenen Arten: 173, pl. 43, Fig. 2.

2011. *Eutrichocheles modestus*: Sakai, *Crustacean Monographs*, 13: 112, Figs. 19, 20, 21A-G.

Material examined: Single individual, male (CL 35 mm, TL 76 mm), Digha Mohana fish landing centre (21°37'19.4916"N; 87°31'25.8276"E), Northwestern Bay of Bengal, India, coll. S. Balakrishnan & Sukanto Ghosh, 21st July 2019, MARC/ZSI/A6840.

Diagnosis: Rostrum acutely triangular and denticulate on lateral margins, with supraorbital spine at rostral base, extending posteriorly onto anterior half of gastric region as lateral carinae with tooth. Gastric region bearing smooth median carina with hepatic tubercle, and sub median carinae with 6 spines. Telson longer than wide, bearing proximal lobewith spine followed by 4 spines on lateral margin, median notch with or without spine on posterior margin, and paired divergent carinae with spines on dorsal surface. Uropodalendopod broad and armed with teeth on lateral margin and bearing median carina with 4 teeth dorsally (Figure 2A-E).

Colour pattern: Body generally orange brown, lateral surface of carapace yellowish-white, abdomen with three thick (1 median and 2 lateral) longitudinal white bands, median band slightly wider from the second abdominal



Figure 1. Burrowing shrimp *Eutrichocheles modestus* (Herbst, 1796), male from Digha Mohana Fish Landing Centre, Northwestern Bay of Bengal (MARC/ZSI/A6840). Scale bar = 2 mm.



Figure 2. Burrowing shrimp *Eutrichocheles modestus* (Herbst, 1796). **A**, carapace, lateral view; **B**, postero dorsal region of carapace showing rostrum; **C**, abdominal pleura of third to fifth segment, lateral view; **D**, dactylus and fixed finger of major cheliped showing numerous setae; **E**, telson and uropods, dorsal view. Scale bar: A-B = 2 mm; C-E = 1 mm.

segment and decreasing towards the last segment Eyes dark brown. Pereiopods 1 whitish and densely covered with dark brown setae, bearing a subdistal red spot on merus. Pereiopods 3 reddish brown with white rings at articulations Colouration of telson and uropodlike abdomen bearing numerous dark brown setae on the posterior margins (Figures 1, 2A-E).

Type locality: Indian Ocean

Distribution: India – Porto Novo (Balasubrahmanyam & Jacob, 1961; Sakai, 2011); Malacca; Malaysia – Pinang; Singapore; and Vietnam – Ha-tien (Ngoc-Ho, 1998; Sakai, 2011).

Molecular Identification

The nucleotide BLAST analysis revealed that the sequence of *E. modestus* exhibited 84.01% identity with existing COI sequence of Axiid shrimp *Leonardsaxius amurensis* in the NCBI GenBank database. The detailed genetic divergence of *E. modestus* along with other closely related genera within the family Axiidae were provided. This indicates that the percent identity matrix revealed that *E. modestus* has a lesser identity of 75.98% to *Axius stirychnus* and a maximum of 82.76% identity to *L. amurensis* (Table 2).

Lastly, our study was limited to only one individual from the Digha Mohana coast of Northwestern Bay of Bengal. Nevertheless, the information on the population assessment, species biology and ecology of *E. modestus* is

still lacking. Extensive sampling protocols along with the integrative approach of both morphology and molecular techniques will provide insight on the taxonomic ambiguity in the current classification of *E. modestus* within the family Axiidae.

Discussion

In India, it has been reported from Porto Novo (Balasubrahmanyam & Jacob, 1961) and Tranquebar (Herbst's type, according to Holthuis, 1986). Currently, the distribution range of *E. modestus* has been extended to the Digha Mohana coast of Northwestern Bay of Bengal. The present contribution on the extension of distribution range of *E. modestus* in the Digha coast of Northwestern Bay of Bengal has re-established its occurrence in the Indian peninsular waters after 1961 (Balasubrahmanyam & Jacob, 1961). The morphological description of *E. modestus* could be remarkably similar to its only congener *E. crosnieri*. Nevertheless, there are still certain morphological characters that have been overlooked while comparing *E. modestus* (Table 1). These characters provide an unambiguous separation of *E. modestus* from *E. crosnieri* to confirm the taxonomic ambiguity of the species (Table 1) Ngoc-Ho *et al.*, 2005; van Xuan & Ngoc-Ho, 2006). Lastly, the present species do not have any commercial importance. However, additional knowledge on biology and ecology of *E. modestus* will help to better manage the natural stock in the wild.

Table 1. Key diagnostic characters separating *E. modestus* from its congener *E. crosnieri*

Diagnostic characters	<i>Eutrichocheles modestus</i>	<i>E. crosnieri</i>
Anterolateral border of carapace	Bears a spine and a tubercle	Bears two spines
Post cervical region of carapace	Has a short median dorsal carina	Absent
Abdominal pleura	Third to fifth segment carry an anterior spine each	Absent
Antennal acicle	Bifid	Trifid
Fourth thoracic sternite	Armed with a lateral spine	Unarmed

Table 2. Percent identity matrix of *Eutrichocheles modestus* (Herbst, 1796) and its closely related genera in the family Axiidae as implemented in Clustal Omega 2.1 (Sievers *et al.*, 2011)

Gen Bank Accession No.	Species Name											
MG935030	<i>Calocaris macandreae</i>	72.17	100.00	83.96	71.52	74.49	77.17	77.81	77.20	77.83	73.65	72.99
DQ882037	<i>Calocaris investigatoris</i>	74.10	83.96	100.00	69.86	73.59	79.37	78.46	77.85	79.25	75.59	74.76
MG936412	<i>Axius serratus</i>	67.03	71.52	69.86	100.00	78.02	72.11	73.50	73.47	72.37	75.55	74.88
KT208525	<i>Axius stirynchus</i>	71.09	74.49	73.59	78.02	100.00	76.81	75.63	74.53	73.91	75.59	75.08
MG935012	<i>Calocarides coronatus</i>	73.86	77.17	79.37	72.11	76.81	100.00	78.25	78.27	78.74	76.63	76.11
KU707215	<i>Boasaxius princeps</i>	75.94	77.81	78.46	73.50	75.63	78.25	100.00	80.50	78.93	78.27	78.58
JN107918	Axiidae environmental sample	75.98	77.20	77.85	73.47	74.53	78.27	80.50	100.00	81.90	74.80	74.76
MN933767	<i>Eutrichocheles modestus</i>	76.44	77.83	79.25	72.37	73.91	78.74	78.93	81.90	100.00	79.06	78.21
KU707212	<i>Leonardsaxius amurensis</i>	73.23	73.65	75.59	75.55	75.59	76.63	78.27	74.80	79.06	100.00	92.16
DQ882035	<i>Calocarides spinulicauda</i>	73.51	72.99	74.76	74.88	75.08	76.11	78.58	74.76	78.21	92.16	100.00

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References

- Balasubrahmanyam, K. and Jacob, J. 1961. Occurrence of *Eutrichocheles modestus* (Herbst) in the near-shore waters of Porto Novo, South India. *Nature*, **191**: 830. <https://doi.org/10.1038/191830a0>.
- Bucklin, A., Wiebe, P.H., Smolenack, S.B., Copley, N.J., Beaudet, J.G., Bonner, K.G., Farber, L.J. and Pierson, J.J. 2007. DNA Barcodes for species identification of Euphausiids (Euphausiacea, Crustacea). *Jour. Plankton Res.*, **29**: 483-493. <https://doi.org/10.1093/plankt/fbm031>.
- Chopra, B. 1933. Further notes Crustacea Decapoda in the Indian Museum. V. On *Eutrichocheles modestus* (Herbst) (Family: Axiidae). *Rec. Indian Mus.*, **35**: 277-282, pl.6.
- Folmer, O., Black, M., Hoeh, W., Lutz, R. and Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome oxidase subunit I from diverse metazoan invertebrates. *Mol. Marine Biol. Biotech.*, **3**(5): 294-299.
- Hall, T.A. 1999. BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**: 95-98.

- Madhavan, A., Silvester, R., Prabhakaran, M.P., Naderloo, R., Radhakrishnan, C.K. and Menon, N.R. 2020. First barcode of *Ryphila cancellus* (Herbst, 1783), from the Southwest coast of India. *Reg. Stud. Marine Sci.*, **33**: 100910. <https://doi.org/10.1016/j.rsma.2019.100910>.
- Ngoc-Ho, N. 1998. Le genre *Eutrichocheles* Wood-Mason, 1876 (Crustacea, Decapoda, Thalassinidea) en Polynésie française et au Vietnam avec description de deux espèces nouvelles. *Zoosystema*, **20**: 363-378.
- Ngoc-Ho, N., Lin, F.J. and Chan, T.Y. 2005. New records for the axiid shrimp *Eutrichocheles modestus* (Herbst, 1796) with discussion on the genera *Eutrichocheles* Wood-Mason, 1876 and *Paraxiopsis* de Man, 1905 (Crustacea: Decapoda: Thalassinidea). *Proc. Biol. Soc. Washington*, **118**: 199-208. [https://doi.org/10.2988/0006-324X\(2005\)118\[199:NRFTAS\]2.0.CO;2](https://doi.org/10.2988/0006-324X(2005)118[199:NRFTAS]2.0.CO;2).
- Prakash, S. and Amit Kumar. 2020. Varying colour pattern, yet genetically similar: Pebble Crab *Seulocia vittata* (Stimpson, 1858) (Brachyura: Leucosiidae) from the Southeastern coast of India. *Jour. Threat. Taxa*, **12**(5): 15612-15618. <https://doi.org/10.11609/jott.5801.12.5.15612-15618>.
- Radhakrishnan, E.V., Deshmukh, V.D., Maheswarudu, G., Josileen, J., Dineshbabu, A.P., Philipose, K.K., Sarada, P.T., Lakshmi Pillai, S., Saleela, K.N., Chakraborty, R., Dash, G., Sajeev, C., Tirumilu, K., Sridhara, P., Muniyappa, B., Sawant, Y., Narayan, A.D., Vaidya, G., Dias Johnny, R., Verma, J.B., Baby, P.K., Unnikrishnan, C., Ramachandran, N.P., Vairamani, A., Palanichamy, A., Radhakrishnan, M. and Raju, B. 2012. Prawn fauna (Crustacea: Decapoda) of India - An annotated checklist of the penaeoid, sergestoid, stenopodid and caridean prawns. *Jour. Marine Biol. Assoc. India*, **54**: 50-72. <https://doi.org/10.6024/jmbai.2012.54.1.01697-08>.
- Sakai, K. 2011. Axioidea of the World and a Reconsideration of the Callianassoidea (Decapoda, Thalassinidea, Callianassida). *Crustaceana Monographs*, **13**: 1-616. <https://doi.org/10.1163/9789047424185>.
- Sievers, F., Wilm, A., Dineen, D., Gibson, T.J., Karplus, K., Weizhong Li., Lopez, R., McWilliam, H., Remmert, M., Söding, J., Thompson, J.D. and Higgins, D.G. 2011. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol. Syst. Biol.*, **7**: 539. <https://doi.org/10.1038/msb.2011.75>. PMID:21988835 PMCID:PMC3261699.
- van Xuân, N. and Ngoc-Ho, N. 2006. Some observations on the thalassinid shrimp, *Eutrichocheles modestus* (Herbst, 1796) from South Vietnam (Thalassinidea, Axiidae), with remarks on *Eutrichocheles crosnieri* Ngoc-Ho, 1998. *Crustaceana*, **79**(10): 1153-1166, Figs. 1-4. <https://doi.org/10.1163/156854006778859524>.
- WoRMS, 2019. Axiidae Huxley, 1879. Accessed at: <http://www.marinespecies.org/aphia.php?p=taxdetails&id=106798> on 2019-12-26.