Indian J. Fish., 61(4) : 124-127, 2014

Note

Report of *Gnathophausia ingens* (Dohrn, 1870) from bathypelagic region of Bay of Bengal, corroborated by DNA barcoding and 18S rRNA gene sequencing

B. GUNALAN AND C. PRASANNA KUMAR
Faculty of Marine Sciences, Centre of Advanced Study in Marine Biology, Annamalai University
Parangipettai - 608 502, Tamil Nadu, India

ABSTRACT

Gnathophausia ingens is a cosmopolitan bathypelagic large mysid belonging to the family Gnathophausiidae of the order Lophogastrida. Occurrence of this species in Bay of Bengal is reported in this paper. DNA barcodes as well as18S rRNA gene sequences were used to confirm the identity and to delineate the phylogeny of *G. ingens*. The mitochondrial CO1 sequence shared 97% similarity with *G. ingens* previously sequenced from deepsea waters of Canada. Phylogram precisely clustered *G. ingens* isolated from Bay of Bengal with the same species sequenced from Gulf of Mexico proving the similarity shared in DNA sequences despite the geographical distance. The sequences produced in this study will act as benchmark for precise identification of *G. ingens* from Indian waters.

Keywords: Bay of Bengal, Bathypelagic region, DNA barcode, *Gnathophausia ingens*, Phylogeny, 18S rRNA

The mysidacean genus *Gnathophausia* (Order: Lophogastrida; Family: Gnathophausiidae), is a strikingly crimson red crustacean abundant in mid-water trawl hauls from bathypelagic waters. All species of *Gnathophausia* are bathypelagic and are practically never encountered in shallow waters. Specimens of *Gnathophausia* have been collected and described as early as the Challenger Expedition in 1873-1876 (WillemoesSuhm, 1875; Sars, 1885) and also recorded from all parts of the world during other expeditions such as the Talisman, the Albatross, the Oceania and the Investigator. The Dana Expedition in 1928-30 and the Discovery Expeditions in the 1920s and 1930s have encountered specimens of this genus in greater numbers and reported from widespread locations throughout the world. The distribution and biology of *Gnathophausia* spp. were reported in detail by Fage (1941) in his study on Dana collections. Banner (1947) reported on one species of *Gnathophausia* from the north-eastern Pacific off Canada and Alaska. Banner (1954) discussed the distribution of two species of *Gnathophausia* from collections made off the California coast by the Allan Hancock Foundation. *Gnathophausia ingens* is a large ubiquitous bathypelagic species, which is negatively buoyant in seawater and known to be an active swimmer (Childress, 1975).

In the present investigation, *G. ingens* was collected during the 290th cruise of Sagar Sampada (FORV, CMLRE, Ministry of Earth science, Government of India) in 2011, from bathypelagic region, off east coast of India**.** The animal was encountered in the catch of a multiple plankton net (MPN) (Hydrobio, Germany), from a depth of 1023 m (Fig.1). The identity of the specimen was confirmed using morphological as well as molecular techniques.

The collected animal was carefully examined for morphogical characteristics and preserved in 95% ethanol. After 24 h, ethanol was replaced with fresh 95% ethanol. The specimen was stored at 4ºC until DNA extraction. The pleopods of the animal was excised and subjected to DNA isolation as per standard protocol (Prasannakumar *et al*., 2011). Pleopods were digested in lysis buffer with proteinase K and salted out using high molar sodium chloride solution. The DNA was then precipitated using cent percent ethanol and rinsed with 75% ethanol. DNA

Fig. 1. G. *ingens* collected from bathypelagic regions of Bay of Bengal

pellet was air dried at room temperature dissolved in Tris buffer and stored at -20ºC until it was used as template for PCR.

The primer pair LCO14905'-GGTCAACA AATCAT AAAGATATTGG-3'and HCO2198 5'-TAAACTTCAG GGTGACCAAAAAATC A-3' (Folmer *et al*., 1994) were used to amplify 650 bp fragment of the CO1 gene. PCR involved initial denaturation at 94ºC for 2 min, followed by 35 cycles of 94ºC for 1min, 54ºC for 30 sec and 72ºC for 1 min, with final extension for 7 min at 72ºC. The 18S rRNA gene (about 1800 bp) was amplified using the EMF (5'-TYC CTG GTT GAT YYT GCC AG-3') and EMR (5'-TGA TCC TTC CGC AGG TTC ACC T-3') primers (Weekers *et al*., 1994). Cycle conditions were 95ºC for 1 min, 55ºC for 1.5 min and 72ºC for 2 min for 35 cycles. Standard PCR reagent concentrations were used and standard agarose gel protocol was followed to detect the presence and concentration of PCR products (Prasannakumar *et al.,* 2011). The PCR amplicons were purified and sequenced in an ABI high throughput sequencer (Bioserve Biotechnologies Pvt. Ltd., India).

The sequences were read and manually double checked using ChromasLite ver.2.1 (www.technelysium com.au/chromas_lite.html*)*. CO1 and 18S rRNA gene sequences were referred with other DNA sequences available in DNA database of NCBI through Basic Local Alignment Searching Tool ver. 2.2.26 (Zhang *et al.*, 2000). Similar sequences were extracted for further analysis (Table 1). Putative amino acid composition of the translated DNA sequence was analysed using BioEdit ver. 7.9 (Hall, 1999). The sequences were aligned in Clustal X ver. 2.0.6 (Thompson *et al.*, 1997) and Molecular Evolutionary Ggenetic analysis (MEGA) ver. 4.1 was used for phylogenetic and pair-wise distance analysis (Tamura et al., 2007). The pair-wise distance was calculated as per Kimura-2 parametric distance model (Kimura, 1980).

The animal was caught at a depth of 1,023 m (14º34'84"N, 80º26'18"E) along the bathypelagic region of Bay of Bengal (temperature: 6.3ºC, salinity: 34.8 psu, water clarity: 93.8%, dissolved oxygen concentration: 0.8 ml l^{-1}, hydrostatic pressure: 105.5 MPa, conductivity: 35.3 m S cm^{-1}, water specific density: 27.3 kg m^{-3}) (Fig. 2). Morphologically the animal was found similar to all previous descriptions of this species.

The CO1 sequence (BoB1; GenBank Accession no. JQ638514) shared 97% similarity with the *G. ingens* samples (Fig. 3) previously sequenced from the deep sea waters of Canada (Costa *et al*., 2007). Three percent variation in CO1 sequence, between *G. ingens* from Bay of Bengal and Canada waters was observed due to single base pair synonymous substitution in 3rd codon position. No change in putative amino acid composition was observed by translating CO1 sequences of BoB1 and MaLap. Substitutions at 9th, 18th, 63rd, 75th, 156th, 159th, 190th, 297th, 423rd, 492nd, 498th, 519th, 540th, 546th, 606th and 615th positions of BoB1 were detected by placing CO1 sequence of MaLap as the reference sequence.

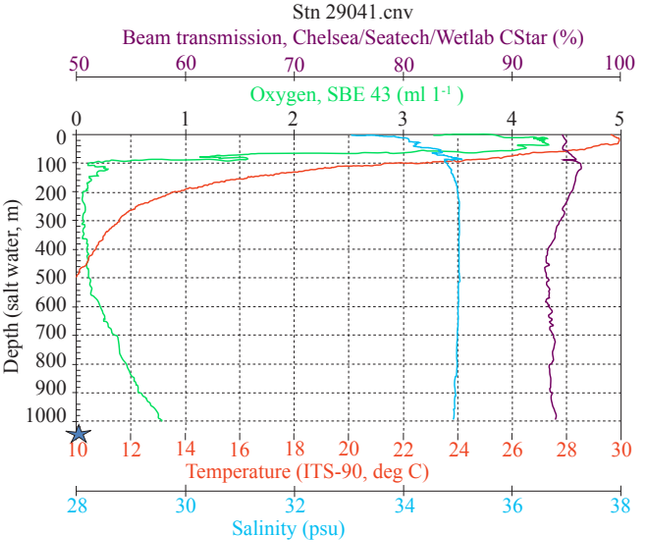

Fig. 2. Chart showing physico-chemical parameters of the collection site

Table 1. List of sequences used from GenBank for comparative analysis

Animal name	Strain name	Country	Accession no.	Gene name	Author
Gnathophausia ingens	MaLop000	Canada	DQ889115	CO1	Costa *et al*. (2007)
Eucopia grimaldii	UCONN:Cr21.1.1	USA	GU183795	CO1	Bucklin *et al*. (2010)
Gnathophausia ingens	Unknown	Gulf of Mexico	AY781416	18S rRNA	Spears *et al*. (2005)
Gnathophausia zoea	Unknown	Gulf of Mexico	AY781417	18S rRNA	Spears *et al*. (2005)
Gnathophausia zoea	ZMBM77816	Norway	AM422474	18SrRNA	Meland and Willassen (2007)
Eucopia sp.	TS2005	Gulf of Mexico	AY781418	18S rRNA	Spears *et al*. (2005)
Eucopia sculpticauda	ZMBM77814	Norway	AM422473	18S rRNA	Meland and Willassen (2007)
Lophogaster typicus	ZMBM77817	Norway	AM422476	18S rRNA	Meland and Willassen (2007)
Gnathophausia gigas	ZMBM77815	Norway	AM422475	18S rRNA	Meland and Willassen (2007)

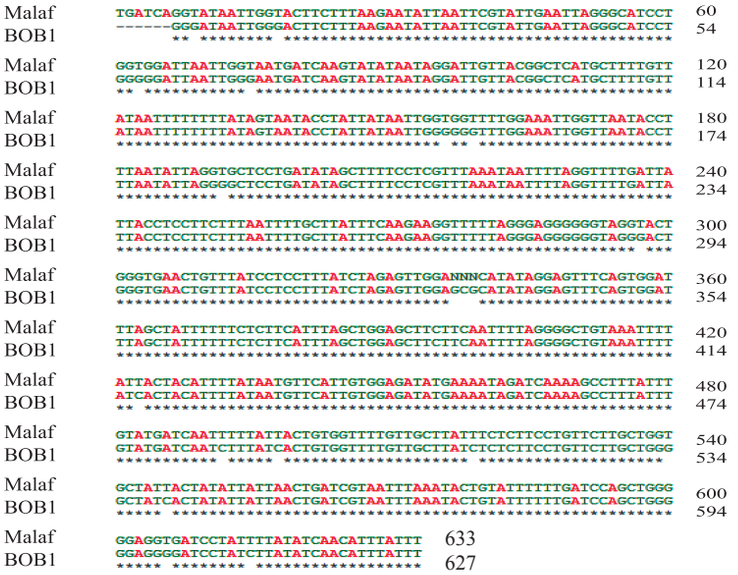

Fig. 3. Clustal W alignment of CO1 sequence of *Gnathophausia ingens* isolated from Bay of Bengal (BoB1, from the present study) and Canadian waters (MaLap, from Costa *et al.*, 2007).

The 18S rRNA gene sequence (BoB2, GenBank Accession no. JQ638515) was used to study the phylogeny of the animal. Similar 18S rRNA gene sequence from GenBank (Table 1) was aggregated for phylogram construction. The phylogram precisely clustered BoB2 from Bay of Bengal (Fig. 4) with *G. ingens* samples sequenced from Gulf of Mexico (Spears *et al*., 2005), proving the similarity shared in DNA sequences besides the geographical distance. *G. gigas*, and *G. zoea* were branched outside the cluster in the same node, proving the resolution of the DNA sequences at species level. *Eucopia* sp. formed the second clade from BoB2 clade and *Lophogaster typicus* formed the out-group.

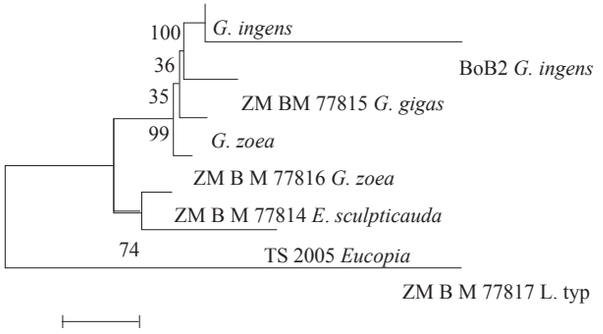

Fig. 4. 18S rRNA phylogeny of *Gnathophausia ingens* (BoB2) with similar species

The 18S rRNA pairwise distance was 0.017±0.004 between BoB2 and the strain from Gulf of Mexico and the distance was equal or more than 0.02 when compared to its sister taxa and other members of mysids (Table 2). The out-group was separated by maximum pairwise distance of 0.321±0.006 (mean value).

Occurrence of *G. ingens* in southern part of the Indian Ocean was reported by Tattersall (1955) and Mauchline and Murano (1977). However, recent reports on occurrence of *G. ingens* in bathypelagic depths of Bay of Bengal are rare. Studies on the environmental parameters and benthic

Table 2. 18S rRNA gene pair-wise distance of BoB2 strain compared with similar 18S rRNA sequences. Bottom (triangle) values represent the pair-wise distance and top values represent standard errors

Species	1	2	3	4	5	6	7	8
G. ingens (Gulf of Mexico)		0.004	0.001	0.001	0.002	0.002	0.003	0.006
G. ingens BoB2 (India)	0.017		0.004	0.004	0.004	0.004	0.005	0.007
G. zoea (Gulf of Mexico)	0.004	0.020		0.001	0.002	0.003	0.003	0.006
G. zoea (Norway)	0.004	0.020	0.003		0.002	0.002	0.003	0.006
G. gigas (Norway)	0.005	0.022	0.006	0.006		0.003	0.003	0.006
E. sculpticauda (Norway)	0.009	0.025	0.011	0.009	0.012		0.003	0.006
Eucopia sp. (Norway)	0.016	0.033	0.017	0.017	0.019	0.011		0.006
L. typicus (Norway)	0.042	0.060	0.043	0.041	0.046	0.042	0.046	

productivity along the mesopelagic to bathypelagic regions of Bay of Bengal have been ongoing from 2008 to 2012, through several cruises (cruise nos. 259, 269, 271, 275, 284, 290 and 293. In the course of these efforts, the species was spotted only during cruise no. 290 in September 2011 (present report) and hence DNA barcoding was used along with 18S rRNA gene sequencing to ascertain its identity and phylogeny. The sequences generated in this study can be used as bench mark sequences for precise identification of *G. ingens* from Indian waters.

Acknowledgements

The authors sincerely thank the reviewers for the critical suggestions. The authors are thankful to the Dean, Faculty of Marine Sciences, CAS in Marine Biology, Annamalai University, Parangipettai for the encouragement and for the facilities provided. Authors are also thankful to Dr. Sanjeevan (Former Director, CMLRE, Govt. of India) for providing the research vessel facility. The second author sincerely thank (DST)-INSPIRE program division (Ministry of Science and Technology, Govt. of India) for financial assistance.

References

Banner, A. H. 1947. *A taxonomic study of the Mysidacea and Euphausiacea (Crustacea) of the north-eastern Pacific, Part 1-3.* Royal Canadian Institute, 356 pp.

Banner, A. H. 1954. Some "schizopod" crustaceans from the deeper water off California. *Allan Hancock Found. Publ., Occas. Paper No. 13*: 68 pp.

Bucklin, A., Ortman, B. D., Jennings, R. M., Nigro, L. M., Sweetmen, J. C., Copley, N. J., Sutton, T. and Wiebe, P. H. 2010. A "Rosetta stone" for metazoan zooplankton: DNA barcode analysis of species diversity of the Sargasso Sea (North-west Atlantic Ocean). *Deep Sea Res., PTII*, 57(24-26): 2234-2247.

Childress, J. J. 1975. The respiratory rates of midwater crustaceans as a function of depth of occurrence and relation to the oxygen minimum layer off Southern California. *Comp. Biochem. Physiol*., 50A: 787-799.

Costa, F. O., DeWaard, J. R., Boutillier, J., Ratnasingham, S., Dooh, R. T., Hajibabaei, M. and Hebert, P. D. N. 2007. Biological identification through DNA barcodes: the case of the crustacea. *Can. J. Fish.Aquat. Sci*., 64: 272-295.

Fage, L. 1941. Mysidacea, Lophogastrida, 1. *Dana Report No.19,* p.1-52.

Folmer, O., Black, M., Hoeh, W., Lutz, R. and Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome C oxidase sub unit 1 from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol*., 3: 294-297.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.,* 41:95-98.

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

Mauchline, J. and Murano, M.1977. World list of the Mysidacea, Crustacea. *J. Tokyo Univ. Fish.,* 64: 39-88.

Meland, K. and Willassen, E. 2007. The disunity of "Mysidacea" (Crustacea). *Mol. Phylogenet. Evol*., 44(3): 1083-1104.

Prasannakumar, C., Akbar John, B., Ajmal Khan, S., Lyla, P. S., Murugan, S., Rozihan, M. and Jalal, K. C. A. 2011. Efficiency of universal barcode gene (cox1) on morphologically cryptic Mugilidae fishes delineation. *Tr. Appl. Sci. Res*., 6(9): 1028-1036

Sars, G. O. 1885. Challenger Reports, 13. Willemoes-Suhm, R. V. 1875. On some Atlantic crustacea from the Challenger Expedition. *Trans. Linn. Soc. Lond, Zool.,* 2(I): 23-58.

Spears, T., DeBry, R. W., Abele, L. G., Chodyla, K. and Boyko, C. B. 2005. Peracarid monophyly and interordinal phylogeny inferred from nuclear small-subunit ribosomal DNA sequences (Crustacea: Malacostraca: Peracarida). *Proc. Biol. Soc. Wash.,* 118 (1): 117-157

Tamura, K., Dudley, J., Nei, M. and Kumar, S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol.Biol. Evol.,*24:1596-1599.

Tattersall, O. S. 1955. Mysidacea. *Discovery Rep*., 28: 1-190.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. and Higgins, D. G. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.*, 25: 4876-4882.

Weekers, P. H. H., Gast, R. J., Fuerst, P. A. and Byers, T. J. 1994. Sequence variations in small-subunit ribosomal RNAs of *Hartmannella vermiformis* and their phylogenetic implications. *Mol. Biol. Evol*., 11: 684–690.

Zhang, Z., Schwartz, S., Wagner, L. and Miller, W. 2000. A greedy algorithm for aligning DNA sequences. *J. Comp. Biol.,* 7(1-2): 203-214.

Date of Receipt : 09.10.2012
Date of Acceptance : 02.05.2014