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ORIGINAL ARTICLE

Occurrence, morphology and molecular characterisation of Bopyrid parasite *Epipenaeon ingens* Nobili, 1906 (Isopoda: Bopyridae)

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Abstract *Epipenaeon ingens* occurs most commonly in Southeast Coast of India parasitized the penaeid shrimp. The present study provides the prevalence, morphological and molecular characterisation of *E. ingens* collected from the host *Metapenaeopsis stridulans.* In the present study highlights the significance of high prevalence and genetic diversity of *E. ingens* in *M. stridulans*. Intra specific analysis also revealed that *E. ingens* species detected in the present study can be easily distinguished from the other genospecies of bopyrids. Further application of this molecular tool to investigate the genetic variability among bopyrids detected in different hosts may facilitate our understanding of the significance of genetic diversity in relation to the infestation of bopyrid species in south coast of India.

Keywords Bopyridae · Penaeidae · Parasitism · Shrimp · India

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Introduction

Bopyrid isopods are holoparasites, having decapod crustaceans as their definitive hosts (Markham 1986). They are belonging to Orbioninae parasitize the branchial chambers of penaeid shrimps in the Indo-West Pacific from the Red Sea to Japan (Chopra 1923, 1930; Pillai 1954; Devi 1982; Markham 1986; Chu and Leong 1996; Trilles 1999; Ravichandran et al. 2000; Boyko 2003; Choi et al. 2004). Studies pertaining to bopyrids have been warranted in the past because high infestation levels may threaten host population recruitment. *Epipenaeon ingens* was one of the most common bopyrid, which was first described by Nobili (1906) on green tiger shrimp *Penaeus semisulcatus* (De Haan 1844) from the Red Sea. Bopyrids cause the parasitic castration of their hosts, which involves two associated but perhaps distinct phenomena: gonads of a female host do not mature and parasitized males are feminized (O'Brien and Van Wyk 1985). Host reproductive potential is always substantially reduced, and infection generally causes 'reproductive death' (Van Wyk 1982).

Epipenaeon ingens is mainly known from the Indian and western Pacific ocean. However a gradual expansion of this species in the Eastern Mediterranean happens since the Suez Canal was opened in 1869 (Markham 1986). It was also collected on *Penaeus japonicus*, *P. merguiensis*, *P. indicus*, *P. stylirostris* and *P. esculentus*. In the Indian coastal waters, Thomas (1977) reported *E. ingens* on *P. semisulcatus* from the Palk bay and the Gulf of Mannar and Ravichandran et al. (2000) on *P. monodon* from the Parangipettai Coast. Despite above morphological and prevalence of infestation studies there is still scarce and no attempt could be made to do molecular characterisation are compiled in the present paper. The main reason for presenting these preliminary data, however, is the fact that

DNA sequencing of the 18S rRNA gene of *E. ingens* from a *Metapenaeopsis stridulans* has been successful. The results of these first molecular studies on *E. ingens* a bopyrid from a *M. stridulans* are detailed in this paper.

Materials and methods

Collection and identification

Specimens were collected along the Parangipettai Coast (11°29′N, 79°96′E) in South India. Samplings were carried out at monthly intervals during the period from January 2014 to December 2014. The branchial cavities of shrimp were examined under a dissecting stereomicroscope for the presence of bopyrids. The parasites on each shrimp were numbered, sexed, weighed and measured to the nearest 0.1 mm; the presence of eggs in the gravid female brood pouch was also noted. The species was identified according to Kazmi and Tirmizi (1994) and Ravichandran et al. (2000). The prevalence was calculated according to Bush et al. (1997). Size, weight and maturity of the host were checked according to Kim et al. (2008).

DNA extraction from bopyrids specimens

Total genomic DNA was extracted from individual parasites used in this study. Briefly, parasites were cleaned by sonication for 3–5 min in 75 % ethanol and then washed twice in sterile distilled water. Afterwards, the specimen was dissected into pieces, placed in a microcentrifuge tube filled with 180-µl lysing buffer solution and then homogenized with a sterile tissue grinder. The homogenate was centrifuged at room temperature and the supernatant fluid was further processed using a Gen-Elute mammalian genomic DNA miniprep Kit (Sigma-Aldrich) as per manufacturer's instructions. After filtration, the filtrate was collected and the DNA concentration was determined spectrophotometrically using UV-1800 spectrophotometer (Schimadzu Corporation). The DNA was stored at −20 °C for further use.

DNA amplification by polymerase chain reaction (PCR)

DNA samples extracted from the tick specimens were used as a template for PCR amplification. A nested PCR was performed with primers designed to amplify the variable spacer region between two conserved duplicate structures. A specific primer set corresponding to 18S rRNA amplification were 18A1F (5′CCTACTTCTGGTTGATTCCTTGCCAGT3′) and 1800R (5′TAATGATCCTTCCGCAGGTT 3′) was designed and applied for the primary amplification, as described previously (Dreyer et al. 2001). In the nested PCR, the PCR mixture contained 2.5 µl of 10× buffer, 1 µl of each primer, 2.5 µl of 2.5 mM of each dNTP, 2.5 Units of Taq DNA polymerase and 1 µl Template DNA was used. The PCR amplification cycle consist of, a cycle of 5 min at 94 °C; 30 cycles of 45 s at 94 °C, 45 s at 57 °C, 1 min and 30 s at 72 °C; and 1 cycle of 5 min at 72 °C.

Sequence alignments and phylogenetic analysis

After purification with a PureLink™ Quick Gel Extraction Kit (K2100-12), sequencing reactions were performed with 25 cycles under the same conditions and it was further sequenced using ABI 3730XL sequencer and chromatogram was obtained. The primer used was 18A1F- 5′ CCTACTTCTGGTTGATTCCTTGCCAGT 3′ as the sequencing primer. The resulting sequences (959 bp) were initially edited by BioEdit software (V5.3) and aligned with the CLUSTAL W software. Afterwards, the aligned sequences (959 bp) were further analyzed by comparing with other bopyrid sequences that were available in GenBank. Phylogenetic analysis was performed by neighbour-joining (NJ) compared with maximum parsimony (MP) methods to estimate the phylogeny of the entire alignment using MEGA 5.0 software.

Results

Prevalence of infestation

Among the 4354 *M. stridulans* examined, 112 (84 females and 28 males) were parasitized by *E. ingens*. The highest prevalence's was observed in October 2012 (3.81 %) and minimum in the month of August 2012 (1.01 %) (Table 1). The prevalence was higher in females than in males. Seventy four *Epipenaeon* were attached to the right gill chamber (Fig. 1a) and 38 to the left side of *M. stridulans*. Seventy one gravid *Epipenaeon* females with eggs in their brood pouch and male parasites are always clinging with females (Fig. 1b). Infected shrimps were observed a characteristic bulge in the branchial chamber, no change in weight, growth retardation, and degeneration of the sex organs in the infested shrimps.

Morphological characters

Female

Body large, oval, larger than broad and slightly asymmetrical. Head distinct from thorax and pro1onged, into a lamina, anteriorly. Eyes rudimentary. Thoracic somites

Table 1 Prevalence of *Epipenaeon ingens* parasitizing *Metapenaeopsis stridulans* during the year 2014

Month of 2014	Number of shrimps examined	Number of shrimps infested	Prevalence (%)	Sex of the parasites		Number of gravid females	(%) Gravid females
				Male	Female		
January	389	9	2.31	2	7	7	100
February	412	14	3.39	3	11	9	81
March	483	18	3.72	5	13	11	84
April	217	6	2.76	1	5	5	100
May*	–	–	–	–	–	–	–
June	278	5	1.79	1	4	4	100
July	297	4	1.34	–	4	4	100
August	396	4	1.01	–	4	4	100
September	421	9	2.13	2	7	7	100
October	498	19	3.81	6	13	9	69
November	487	13	2.66	4	9	4	44
December	476	11	2.31	4	7	7	100

* Samples not taken from the periods

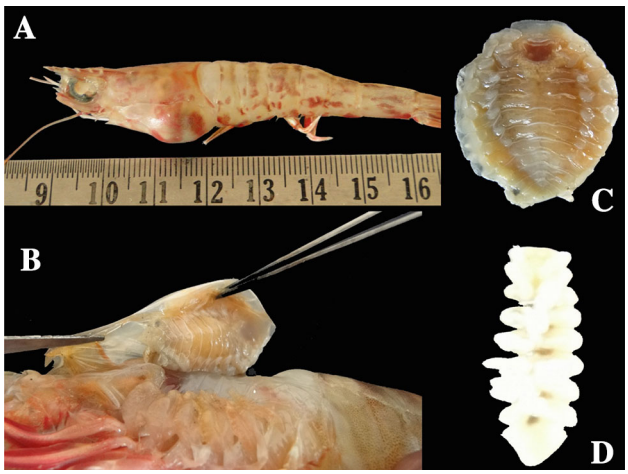

Fig. 1 *Epipenaeon ingens* parasitizing *Metapenaeopsis stridulans.* **a** Gill Bulge due to presence of bopyrid, **b** female *E. ingens* inside the gill region, **c** female *E. ingens*, **d** male *E. ingens*

well marked with clearly defined demarcating lines, epimera of these somities being highly developed with rounded outer margins. Abdomen one-third total length, with less developed pleural lamellae and without tubercles on dorsal surface. Only five abdominal somites visible dorsally, with paired biramous pleopods. Rami of abdominal appendages covered with warts and tubercles (Fig. 1c).

Male

Bodyelongated, twice as long as broad. Head small, distinct from thorax. Eyes extremely reduced. Antennules three-segmented. Thoracic somites distinct, with deeply notched lateral margins, first two pairs of lateral margins being directed anteriorly, while the outer one extends laterally. Abdominal somites completely fused to form, a sub-triangular, structure. No trace of pleopods or uropods. The mean size of female *E. ingens* ranged from 12.6 to 21.9 mm in length and 6.0 to 14.0 mm in width. The total length (TL) and width of males varied from 4.0 to 6.8 and from 2.0 to 4.0 mm, respectively (Fig. 1d).

Phylogenetic analysis of *E. ingens*

Phylogenetic relationships based on the 18S rRNA sequences were performed to analyze the genetic divergence among bopyrids investigated in this study. Bootstrap analysis was used to analyze the repeatability of the clustering of specimens represented in phylogenetic trees. Phylogenetic trees constructed by both NJ (Fig. 2) analyses showed congruent basal topologies with nine major branches of distinguished clades. Length of alignment of the 18S rRNA gene sequences for *E. ingens* was a 959 bp. MP tree of *E. ingens* comparison appears to indicate differences in phylogenetic history of bopyrids.

Discussion

One of the most common bopyrid occurs in the Indian and Western Pacific Ocean is *E. ingens* (Thomas 1977; Ravichandran et al. 2000; Gopalakrishnan et al. 2009). However, its presence has also been documented in the Mediterranean (Bourdon 1968; Overstreet 1986). *E. ingens* was originally found in *P. semisulcatus* in the Red Sea (Markham 1974). Thus, the opening of the Suez Canal in

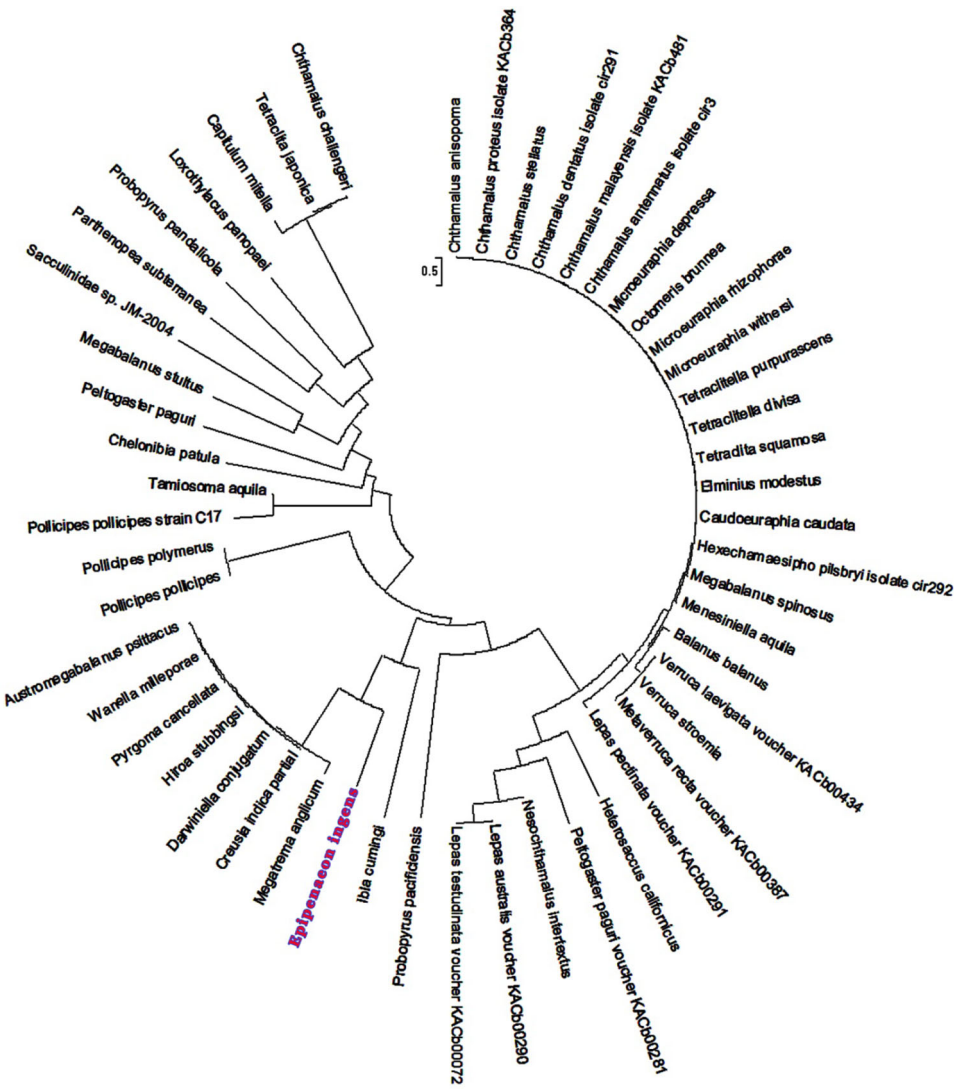

Fig. 2 Phylogenetic relationships based on the aligned sequences (959 bp) of 18s rRNA gene of *E. ingens*

1869 likely allowed the expansion of its range in the Eastern Mediterranean (Markham 1986). Penaeid shrimps may be divided into three major groups based on their level of infestation. The most heavily parasitized species is *P. semisulcatus* (Nobili 1906). *P. japonicus*, *P. merguiensis*, *P. indicus*, and *P. stylirostris* have low levels of infestation and *P. esculentus*, *P. monodon*, Western king prawn *P. latisulcatus* (Kishinouye 1896) Red spot king prawn *P. longistylus* (Kubo 1943) and *P. stylifera* are not or rarely infested (Monod 1933; Thomas 1977; Owens 1983; Overstreet 1986; Owens and Rothlisberg 1991; Courtney 1991; Ravichandran et al. 2000; Gopalakrishnan et al. 2009). Our results suggest that *M. stridulans* belongs in the second group. Clearly *E. ingens* exhibits stenoxenic parasitic specificity. The level of infestation varied among seasons. This relationship has also been noted in other hosts. For example, Owens and Glazebrook (1985) reported that the occurrence of *E. ingens* on *P. semisulcatus* peaked from April to June in Northern Australia.

Epipenaeon ingens was also more prevalent in female than in male *M. stridulans*. This is consistent with other reports, such as Owens and Glazebrook (1985). The branchial chamber of parasitized *M. stridulans* developed a characteristic bulge. This bulge increases the pressure on the gills and reduces the respiratory efficiency of the host (Chaplin-Ebanks and Curran 2005). Although such infestation does not immediately cause death, it is likely that the natural growth of the hosts is affected to some extent, leading to economic losses in commercial species. Furthermore, the primary and secondary sexual organs were degenerated in infested individuals. A number of authors have reported similar effects on the reproduction of penaeid shrimps infested by Orbionidae (Mathews et al. 1988, Courtney 1991, Chu and Leong 1996 Ayub and Ahmed 2004). Interestingly, the majority of female *E. ingens* had eggs in their brood pouches. This is consistent with the report of Ayub and Ahmed (2004). Infestation had no effect on host weight in our study. This contrasts with other

studies that have found marked decreases in host weight following infestation with Bopyrid parasites (Courtney 1991; Chu and Leong 1996). However, in the present study did note that infestation with *E. ingens* had a significant effect on reproduction in *M. stridulans*.

Phylogenetic relationships among bopyrids can be constructed and determined by analyzing their sequence homogeneity of a specific target gene. Indeed, the sequence analysis of 18 sr RNA *E. ingens* had been proved useful to evaluate the taxonomic relatedness of bopyrids derived from various biological and geographical sources. Although the variation of nucleotide sequence depends on the group diversity of bopyrids and may actually represent the genetic distance of phylogenetic divergence between or within the genospecies of bopyrids. In this study, phylogenetic analysis based on the sequences of 18s r RNA intergenic spacer amplicon gene of *E. ingens* from *M. stridulans* demonstrated a high sequence homogeneity among bopyrids from the gen bank. However, a high genetic heterogeneity within the genospecies of *Probopyrus pacificiensis* was also observed. Although a low intraspecific variation was observed among the same groups of bopyrids. The phylogenetic trees constructed by either NJ or MP analysis strongly support the discrimination recognizing the separation of different lineages of *E. ingens* detected.

This study provides the prevalence and first phylogenetic diversity of *E. ingens* collected from south coast of India. Further application of this molecular tool to investigate the genetic variability among bopyrids detected in different hosts may facilitate our understanding of the significance of genetic diversity in relation to the infestation of bopyrid species in south coast of India.

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References

Ayub Z, Ahmed M (2004) Study on the host-parasite relationship of *Parapenaeopsis stylifera* (H. Milne Edwards) (Decapoda: Penaeidae) and *Parapenaeon japonica* (Thielemann) (Isopoda: Bopyridae). Hydrobiologia 523:225–228

Bourdon R (1968) Les Bopyridae des Mers Européennes. *Mem Mus Natl Hist Nat Paris Nouvelle série Série A Zoologie L* (2 et dernier), 50:75–424

Boyko CB (2003) A new genus and species of bopyrid isopod (Crustacea, Isopoda, Bopyridae, Orbioninae) parasitic on Sicyonia (Crustacea, Decapoda, Penaeoidea) from New Caledonia. Zoosystema 25:593–600

Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis et al. revisited. J Parasitol 83:575–583

Chaplin-Ebanks SA, Curran MC (2005) The effect of the parasitic isopod, *Probopyrus pandalicola* (Packard, 1879) on tidal activity patterns of the grass shrimp, *Palaemonetes pugio* Holthuis, 1949. Crustaceana 78:1053–1061

Choi JH, Jamieson G, Han KH, Hong SY (2004) *Parapenaeon consolidatum* (Isopoda: Bopyridae) and the relative growth and reproduction of *Metapenaeopsis dalei* (Decapoda: Penaeidae) in South Korea. J Shell Res 23:237–242

Chopra B (1923) Bopyrid isopods parasitic on Indian Macrura. Rec Ind Mus 25(5):411–550

Chopra B (1930) Further notes on bopyrid isopods parasitic on Indian Decapoda Macrura. Rec Ind Mus 32(2):113–147

Chu KH, Leong FLS (1996) Occurrence and influence of *Orbione halipori* Nierstrasz and Brender a Brandis (Isopoda: Bopyridae) on *Metapenaeus joyneri* (Miers) (Decapoda: Penaeidae) from the Zhujiang Estuary, China. J Nat Hist 30:835–839

Courtney AJ (1991) Occurrence and influence of bopyrid parasites on female penaeid prawns from coastal waters of Central Queensland (Australia). Aus J Mar Fresh Res 42:615–623

Devi SL (1982) Bopyrid parasites of prawns at Kakinada. J Mar Biol Ass Ind 24:23–32

Dreyer B, Tranebjaerg L, Brox V, Rosenberg T, Möller C, Beneyto M, Weston MD, Kimberling WJ, Cremers CW, Liu XZ, Nilssen O (2001) A common ancestral origin of the frequent and widespread 2299delG USH2A mutation. Am J Hum Genet 69(1):228–234

Gopalakrishnan A, Rajkumar M, Ravichandran S, Trilles JP, Vasanthan TM (2009) Identification of *Parapenaeopsis stylifera* a new host for *Epipenaeon ingens*. J Environ Biol 30:1063–1064

Kazmi QB, Tirmizi NM (1994) A northern Indian Ocean record of a bopyrid, *Parapenaeon japonica* (Thielemann, 1910) parasitic on species of penaeid prawns (*Penaeus*). Pak J Mar Sci 3:171–175

Kim JC, Ma CW, Oh CW, Paik SG (2008) Reproduction and growth of the freshwater prawn, *Palaemon paucidens* (Decapoda: Palaemonidae) in a lake of Korea. J Environ Biol 29:163–168

Markham JC (1974) A systematic study of parasitic bopyrid Isopods in the West Indian faunal region. Ph.D. dissertation, University of Miami, Coral Gables

Markham JC (1986) Evolution and zoogeography of the Isopoda Bopyridae, parasites of Crustace Decapoda. Crustaceana 4:143–164

Mathews P, El-Musa M, Al-Hossaini M, Samuel M, Abdul-Gha AR (1988) Infestation of *Epipenaenon elegans* on *Penaeus semisulcatus* and their use as biological tags. J Crus Biol 8:53–62

Monod T (1933) Tanaidacea et Isopoda. Mission Robert-Ph Dollfus en Egypte. Mém. Inst. Egypt 21:161–264

Nobili B (1906) Nuovi Bopiridi. Atti Reale Accademia delle Scienze di Torino 41:1098–1113

O'Brien J, Van Wyk PM (1985) Effects of crustacean parasitic castrators (epicaridean isopods and rizocephalan barnacles) on growth of crustacean hosts. In: Wenner AM (ed) Crustacean issues 3: factors in adult growth. AA Balkema, Rotterdam, pp 191–218

Overstreet RM (1986) Solving parasite related problems in cultured crustacea. In: Howell MJ (ed) Parasitology, Quo Vadis?. Australian Academy of Science, Canberra, pp 309–318

Owens L (1983) Bopyrid parasite *Epipenaeon ingens* Nobili as a biological marker for the banana prawn, *Penaeus merguiensis* de Man. Aus J Mar Fresh Res 34:477–481

Owens L, Glazebrook JS (1985) The biology of bopyrid isopods parasitic on commercial penaeid prawns in northern Australia. In: Rothlisberg PC, Hill BJ, Staples DJ (eds) Second Australian National Prawn Seminar. Simpson Halligan, Cleveland, pp 105–113

Owens L, Rothlisberg PC (1991) Epidemiology of cryptonisci (Bopyridae: Isopoda) in the Gulf of Carpentaria, Australia. Mar Ecol Prog Ser 122:159–164

Pillai NK (1954) A preliminary note on the Tanaidacea and Isopoda of Travancore. Bull Cent Res Ins Univ Travancore 3:1–21

Ravichandran S, Soundarapandian P, Kannupandi T (2000) Infestation of isopod *Epipenaeon ingens* Nobili parasitized on *Penaeus monodon* from Parangipettai coastal environment. Adv Biosci Biotechnol 19:55–62

Thomas MM (1977) A new record of *Epipenaeon ingens* Nobili (Bopyridae: Isopoda) parasitic on *Penaeus semisulcatus* Dehann from Palk Bay and Gulf of Mannar. Ind J Fish 24:258

Trilles JP (1999) Ordre des isopodes, suborder of epicarids (Epicavidea, Latreille, 1825). In: Forest J (ed) Treatise of zoology, anatomy, systematic, biology (Pierre P. Grasse), vol. VII, Part-IIIA, Peracarid Crustaceans. Memorial Institute of Oceanography. Monaco, 19:279–352

Van Wyk PM (1982) Inhibition of the growth and reproduction of the porcellanid crab *Pachycheles rudis* by the bopyrid isopod, *Aporobopyrus muguensis*. Parasitology 85:459–473