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SHORT COMMUNICATION

First record on the occurrence of *Ryukyua circularis* (Pillai, 1954), a parasitic cymothoid (Crustacean: Isopoda) infesting the clupeid fish *Amblygaster sirm* (Walbaum) from Andaman Islands, India

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Abstract In the present study, occurrence of branchial parasitic cymothoid, *Ryukyua circularis* from the marine finfish, *Amblygaster sirm* is reported for the first time from Andaman Islands. Morphological characterization was carried out which confirmed the parasite as *R. circularis*. Molecular characterization using 28S rDNA revealed 443 bp sequence which has been submitted to NCBI GenBank under the accession no. KX090447. Ten female and one male parasite (*R. circularis*) were collected from the branchial cavity of the individual host fishes (*A. sirm*). The displacement of gill filament and cavity formation in the gill chamber of the host fish was observed. The report of this species from Andaman Islands gives further information on its geographic range extension, since they are currently known from West coast of India and Thailand.

Keywords Branchial cymothoid · Isopod · Andaman Islands · 28S rDNA sequence

Introduction

The parasitic cymothoids are known to be diverse and infest a wide array of tropical marine fishes (Bunkley-Williams and Williams 1998; Smit et al. 2014). Cymothoids infest different parts of the host body such as buccal cavity, gill chamber, body surface, fins and some burrow inside the host and feed on the blood (Trilles 1969, 1994; Bunkley-Williams et al. 2006; Ravichandran et al. 2010, 2011). Isopods cause deleterious effect in mariculture and cage culture of finfishes where the infested host exhibit retarded growth, emaciation, anorexia and mortality (Sievers et al. 1996; Horton and Okamura 2001; Rajkumar et al. 2005; Rameshkumar and Ravichandran 2014). The isopod, *Ryukyua circularis* was first described from Trivandrum coast of South India by Pillai (1954) as *Livoneca circularis* Pillai, 1954. Bruce (1990) revised the genus, *Livoneca*, Leach 1818 and noted that *L. circularis* did not belong in this genus. Williams and Bunkley-Williams (1994) included it in the newly erected genus *Ryukyua* as *R. circularis* (Pillai, 1954) (Syn. *Livoneca circularis*) based on the specimen collected from Thailand seas. There are no published reports on parasitic isopods from Andaman and Nicobar Islands, so the present study reports the first record on the occurrence of *R. circularis* in the clupeid fish *Amblygaster sirm* (Walbaum) from Andaman Islands based on morphological examination and molecular characterization.

Materials and methods

A total of 117 fishes including 72 numbers of *Amblygaster sirm* (Walbaum) and 45 numbers of *Amblygaster leiogaster* (Valenciennes) were collected from Dignabad landing center (11°40′31.1″N; 92°44′39.90″E) located at Port Blair, Andaman and Nicobar Islands during September, 2015 to February, 2016. Eleven isopods comprising of 1 male and 10 female were recovered from the branchial cavity of 10 individual specimens of *A.*

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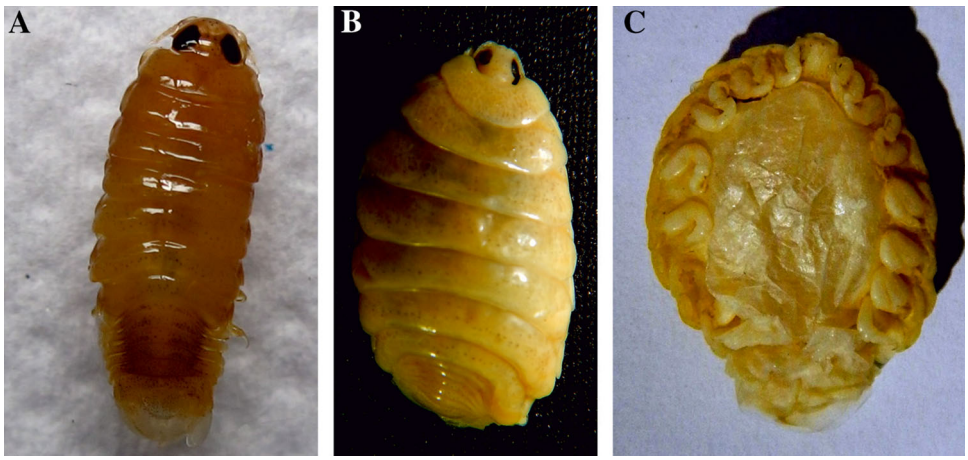

Fig. 1 *Ryukyua circularis*: **a** Male, **b** Female dorsal view, **c** Female ventral view

sirm. The male and female isopods were measured and preserved in 10 % formalin for morphological study and 90 % ethanol for molecular study. The isopods were identified based on their morphology described by (Pillai 1954, 1964; Williams and Williams 1986; Williams and Bunkley-Williams 1994) by using a Stereo-zoom microscope, Nikon SMZ 1500 and the photographs were taken by using Nikon E8400 digital camera attached with the microscope. The taxonomic classification of the fish host was carried out by following Froese and Pauly 2016 and WoRMS (http://www.marinespecies.org/).

Genomic DNA from the parasite was isolated by modified CTAB method of Bruce et al. (1993). PCR was performed to amplify a region of 28S rDNA using the universal primers (Roy et al. 2015), forward IsoF (5′-ACCCGCTGAATTTAAGCAT-3′) and reverse IsoR (5′CTCTTCAGAGTACTTTTCAAC-3′). PCR amplification reaction was performed in a final volume of 25 µl PCR mix consisting of 2.5 µl of 10× PCR buffer, 0.25 µl of 2 mM dNTP, 0.3 µl each of 50 pmol forward and reverse primers, 0.125 µl of 5 units μl^{-1} Taq polymerase, 1 µl template DNA with concentration of 1 µg and 20.525 µl of nuclease free water. Amplification reaction consisted of 95 °C for 5 min, 35 cycles of 95 °C for 30 s, 52 °C for 1 min, 72 °C for 1 min and finally 72 °C for 10 min in a thermal cycler (Bio-Rad, USA). The PCR products were analyzed on 1.5 % agarose gel containing ethidium bromide and visualized using a gel documentation system (Bio-Rad, USA). The amplified PCR products were sequenced using IsoF and IsoR primers in ABI 3500 DNA analyzer (Shrimpex Biotech Pvt. Ltd., Chennai). The homology of the generated sequence was analyzed using the Basic Local Alignment Search Tool (BLAST) program in the National Center for Biotechnology Information.

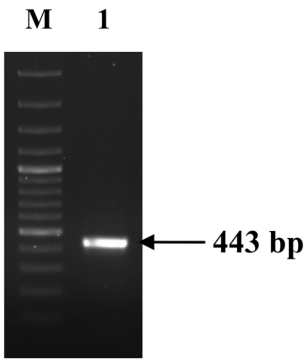

Fig. 2 PCR amplification using IsoF and IsoR primers. *Lane M* Molecular weight marker (100 bp); *Lane 1* PCR amplified product (443 bp)

Results

Morphological and molecular characterization of the parasite

One male (Fig. 1a) and 10 female (Fig. 1b, c) *R. circularis* were collected from 10 individual specimens of *A. sirm*. The male was small and elongated and the females were stout and wider in breadth compared to males. The female was located exactly on the posterior dorsal end of gill filaments in the gill chamber of the host and the male was located in the same position in the opposite gill chamber.

Molecular characterization was carried out using universal primer available from the conserved region of 28S rDNA to confirm the morphological identification. PCR amplification of 28S rDNA has revealed a product of 443 bp in length (Fig. 2). The amplified PCR products were sequenced, and while comparing with NCBI GenBank database, the obtained sequence was found to be a new one, which was then submitted to NCBI GenBank as

sequence of *R. circularis* based on its morphometric analysis under the accession no. KX090447.

Discussion

The isopod, *R. circularis* was first reported from the gill chamber of the clupeiod *Amblygaster leiogaster* (as *Clupea leiogaster*) by Pillai (1954) from Trivandrum coast of South India and from the Malabar coast by Panakkool-Thamban et al. (2015) and the same was reported in *A. sirm* from Thailand by Williams and Williams (1986). The Andaman Islands situated in the Bay of Bengal is 1200 km away from the East coast of India and it is also very near to the Thailand seas and hence the present report of this Genus from Andaman Islands gives further information on its geographic range extension, since they are currently known from Indian Ocean, South China Sea and Ryukyus in the Pacific (Williams and Williams 1986). Another species, *Ryukyua globosa* has been reported by Rameshkumar et al. (2015) from clupeid fishes of South East coast of India. *R. circularis* differs from *R. globosa* in having a triangular shaped pleotelson instead of rectangular one, an antenna reaching beyond the posterior region of the head whereas *R. globosa* with a short antenna. The head is not separated into a lobe in *R. circularis* whereas in *R. globosa* the head is produced into a lobe between the antennal bases.

In the present study, cavities in the gill chamber and displacement of the gill filaments were observed due to the pressure exerted by the lodged parasite. It is corroborated with the earlier studies that the lodging of this parasite on the gill filament caused considerable gill damage, necrosis of the gill filament (Williams and Williams 1986), cavities in the gill chamber (Provenzano 1983) and respiratory failure (Sarusic 1999). The molecular characterization of 28S rDNA sequence with the available universal primer proved to be an efficient tool, supplemented with the morphological study for the identification of *R. circularis.*

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