RESEARCH ARTICLE

MOLECULAR IDENTIFICATION OF THE PARASITE JORYMAHILSAE FROM RASTRELLIGER KANAGURTA (CUVIER, 1816)

* Sarlin, P. J., Jenifer Ann Thomas and Megha, M.

Department of Zoology, Fatima Mata National College (Autonomous), Kollam, India

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ABSTRACT

The parasitic infestation of isopod parasites in the commercially important fish Rastrelliger kanagurta was investigated. Genetic profiling of the Jorymahilsae from Rastrelliger kanagurta was the main aim of the study. The crustacean parasites along the south-west coast of India is high in R. kanagurta. Jorymahilsae occupying the entire branchial chamber of the Rastrelliger kanagurta may produce pressure on the gill surface and thus affect the efficiency of respiration. Although, the infestation does not cause immediate death, it affects the normal growth and appearance of the host fish. This may lead to economic losses among Rastrelliger kanagurta.

Key words: Infestation, Isopod, Parasite, Rastrelliger kanagurta, Genetic profiling – Blast Results, Jorymahilsae.

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INTRODUCTION

Cymothoids are obligatory parasites infesting many of the commercially important fishes. They are protandric hermaphrodites and bloodsuckers, living on the skin, gill filaments, or in the mouth of the fishes. These parasites retard growth and cause emaciation followed by death. Pathological conditions resulting from parasitic diseases in fishes reaches a high magnitude of epidemics under crowded and other unnatural conditions (Ravichandran et al., 2007). Isopod parasite of the family Cymothoidae has been reported in about 350 species of fishes. Over 80% of these are from tropical and subtropical seas, many are from the Indo-Malaysian archipelago (Lester and Roubal, 1995). Their life cycle involves only one host (holoxenec cycle) (Trilles, 1994) and usually these are large sized parasites, which can cause deleterious effects on the host fishes (Trilles, 1996). The information regarding cymothoid fauna of marine fishes from the Indian coasts is scanty (Pillai, 1954; Bal and Joshi, 1959; Veerapan and Ravichandran 2000). Most of the studies were from the east coast of India (Ravichandran et al., 1999, 2009; Ravichandran and Rameshkumar, 2004; Rajkumar et al., 2004, 2005; Ravichandran 2007; Rameshkumar and Ravichandran, 2010 a, b, Rameshkumar et al., 2011). Ravichandran et al. (2009) reported infestation of Rastrelliger kanagurta with cymothoid isopod, from Colachel, south-west coast of India.

Mance (juvenile parasitic stages of cymothoid) feed voraciously and kill fry and fingerlings of several species of fishes. Permanently attached adults parasites stun the growth of fish and retard reproduction process. Parasites in the gill chamber usually lead to stunted and anaemic gill conditions. Isopod infections can lead to severe economic loss in culture operations (Bragoni, 1984). According to Rameshkumar (2011) all the species of Joryma are reported from the North-western Indian Ocean. J. hilsae, J. engraulisid, J. tattorand J. brachysoma, from the South-western coasts of India and only one species J. sawayah from Kuwait. They are often abundant ectoparasites attached to the skin, gills or inside the buccal cavity. Single isopod can cause damage with their biting and sucking mouth parts. Heavy infestations of parasitic juveniles can kill small fish when they first attach (Noga, 2000). These cymothoids have a variety of pathogenic effects, causing direct damage not only to skin, gills and tongue at the site of attachment (Brusca, 1978; Adlard and Lester, 1994), but also indirectly affect host condition, physiological performance and reproductive output (Romestand, 1979; Ostlund-Nilsson et al., 2005). Studies pertaining to pathological effects of isopod parasites on the physiology of host are scanty, and few studies have been made along the Colachel coast environment. (Pillai, 1954; Bal and Joshi, 1959; Veerapan and Ravichandran 2000). Most of the studies were from the east coast of India (Ravichandran et al., 1999, 2009; Ravichandran and Rameshkumar, 2004; Rajkumar et al., 2004, 2005; Ravichandran 2007; Rameshkumar and Ravichandran, 2010 a, b, Rameshkumar et al., 2011). The present study reports the infection of an isopod parasites (J. hilsae) in the Indian Rastrelliger kanagurta which is a commercial important fish.
plays an important ecological role and maintains a balance ecosystem. Hence the present attempt was made to study the effect of isopod infestation on such fish. (Ravichandran et al., 2009). DNA barcoding aims to provide an efficient method for species-level identifications and, as such, will contribute powerfully to taxonomic and biodiversity research. As the number of DNA barcode sequences accumulates, however, these data will also provide a unique ‘horizontal’ genomics perspective with broad implications.

MATERIALS AND METHODS

The fishes for the study were collected from the three (Neendakara, Thangassery and Azheekal) main fish landing centers of Kollam District. Altogether 50 specimens of *R. kanagurta* ranging in size from 11 to 14cm (Fig.1) were collected soon after the mechanized and traditional fishing vessels moored at the harbor. The randomly selected fishes were carefully placed in Ice boxes and moved to the laboratory within an hour. The total length of each fish were measured in centimeters (cm) using measuring tape, while the weight of each fish was taken in grams (g) using a weighing balance. Then, the collected fish samples were dissected and the mesenteric cavity examined for parasites. The gastrointestinal tract was then dissected from the rectum to the oesophagus and parasites encountered were carefully detached from the stomach or intestinal mucosa. The internal organs of each fish were also examined for parasites or cysts. The parasites from each fish were then fixed in 70% alcohol. *Rastrelliger kanagurta* belongs to the classification *Rastrelliger kanagurta* (Cuvier, 1816) (Fig.1)

![Figure 1. Indian mackerel Rastrelliger kanagurta (Cuvier 1817)](image)

DNA Isolation

The parasite was ground with a pre-cooled (-20°C) mortar and pestle with liquid nitrogen until a fine powder obtained. Ground insect (2.0g) was resuspended in extraction buffer (12.5 ml) consisting of 200 mMTris – HCl (pH 8.5), 250 mMNaCl, 25 mM EDTA and 0.5 % SDS, after which phenol (pH 7.9) (8.75ml) preheated to 60°C was added followed by the addition of chloroform/isoamylalcohol [24:1 (v/v)] (3.75 ml)]. The suspension was carefully inverted a few times. After centrifugation (12000 rpm in a centrifuge, Sigma-Laboratory Centrifuge, Germany) for 60 min at 4°C the top liquid phase was removed containing the DNA. To remove excess RNA from the liquid phase 500 µl (5 mg/ml) RNase H was added and incubated for 15-20 min at 37°C. Equal volume of phenol was added to the mixture after incubation with the RNase and the mixture was again centrifuged (12000 rpm for 20 min) at 4°C. The liquid phase was removed and the DNA was precipitated with 1/10th volumes of isopropanol. The mixture was centrifuged (12000 rpm for 15 min) at 4°C and the resulting pellet was washed with 70 % (v/v) ethanol. The sample was centrifuged (12,000 rpm for 2 min) at 4°C after which the ethanol was aspirated and the pellet was dried under vacuum. The pellet containing the isolated DNA was then dissolved in 1 ml TE buffer 10 mMTris (pH 8.0) and 1 mM EDTA and stored at -20°C for further manipulation. After complete DNA isolation the presence of DNA was checked by Agarose Gel Electrophoresis.

- **PCR Amplification of CO1 gene Primers Used :**
  
  (Forward Primer) Sequence (5'-3'),
  TATTATAGACAAG AATCTGTTAAA,
  (Reverse Primer) Sequence (5'-3')
  AGGAAATGGTAGGAAGAAAGTAA

  The following sets of primers were used for the amplification of CO1 gene. The annealing temperature of the primers was also standardized by running at different temperatures.

  **PCR Amplification**

  PCR reactions for CO1 gene amplifications were carried out in Biorad Thermocycler, employing the CO1 gene primers

  (Forward Primer) 5'-
  TATTATAGACAAGAATCTGTTAAA -3’ and
  (Reverse Primer) 5'-
  AGGAAATGGTAGGAAGAAAGTAA -3’.

  PCR amplifications were performed in 25µl reactions containing 1X assay buffer with 1.5mM MgCl₂, 5p moles of each primer, 200µM dNTPs, 1.5UTaq DNA polymerase and 20ng of template DNA. To check DNA contamination, a negative control was set up omitting template DNA from the reaction mixture. Commercially available 100 base pair ladder was used as standard molecular weight DNA marker to determine the weight of amplified product.

  - **PCR-Product Electrophoresis:** Loaded 10µl of PCR product with 4µl loading dye in 1.5% Agarose gel. Ran the gel at constant voltage of 100V and current of 45A for a period of 1 hour 20 minutes till the bromophenol blue has travelled 6cm from the wells. Viewed the gels on UV transilluminator and Photograph of the gels was taken.
  - **Purification and DNA Sequencing of Samples:** The amplified products along with forward primer were sent for purification and DNA sequencing.
  - **Analysis of Data:** The sequenced data obtained were again confirmed with NCBI-BLAST program, the species identification

RESULTS

Infestation of Rastrelliger kanagurta

Out of 50 specimen of *R. kanagurta* collected from the Kollam coastal environment, and examined of which 20 fish were infested. Isopods, *Jorymahilsae* (Fig.2) inhabiting the buccal cavity and branchial chamber were identified. In *Rastrelliger kanagurta* the site of infection in the buccal cavity of the
parasite. The site of attachment of the parasite, indicative of mucus and blood feeding were found at the time of observation. The parasites were normally seen protruding through the mouth opening of the host. The studies have found that parasitic infection may reduced or interfere with the ability of the host. Gross lesions observed in the buccal cavity of infested fish showed small pin-holes in the tongue region, through which dactyls of pereopod’s penetrating claws dig into the host tissues.

The isolated parasite belonged to family cymothoidae. The parasite belongs to the following classification:

Kingdom : Animalia  
Phylum : Arthropoda  
Subphylum : Crustacea  
Class : Malacostraca  
Order : Isopoda  
Family : Cymothoidae  
Genus : Joryma  
Species : hilsae

Figure 2. Isopod parasite J.hilsae (a) branchial region and (b) buccal cavity of R. kanagurta

Isolation of parasite DNA

To confirm the species, the DNA of the parasite was isolated. After this, the DNA isolated are undergone Agarose Gel Electrophoresis with 0.8% Agarose. The result is shown in the figure (5). After the DNA isolation, the sample is undergone Polymerase Chain Reaction for the amplification of DNA sample isolated. The result of PCR is shown in the figure.

>SR873-CO  
CACCTTATATTTTATTTTATGGAGATCTGAGCTGGATTTC  
TAGGAGTAGCATTTTAGGTAATTATCCGGAGCTGAATT  
AGCTCAACCCGGTTCTATTCTATGGATAGGATCAAAAACCTATAATGCTATGTAACAGGCCACGCATTTATTATAA  
TTTTTCTTATTAGTTATACCTATTATAATTTGGAAGGGTTTT  
GGTTAGACTTGTCCCCTTTATAATAGACGACCCAG  
ATATAGCCCTCCACGAATAAACATATAAGAATTATTG  
ACTTTTACCCCCAGCTCTCCTACCTCTTCTGTGTAAGAG  
CTTTTGTAGAAGAAGGTGCAAGGCACAGGAGATGACCC  
TCTATCGCCCATATCCGGAGAATCAGCCCATAGAGG  
AGCTTCTGCGATTTTCAATTTCTCCTTTTCAATTTAG  
CAGGAAGTTTCTCCATCTTTAGGAGGAGCGTAAATTTAT  
TACACCAATTATCAATATAGACCTCTTTTACAATATT  
TTACACCAATATGCTATTAGTCATTGAGGAGGTGAT  
CCAATTTTATTTCAACATTTATT
NCBI Blast: Nucleotide Sequence (655 letters)

https://blast.ncbi.nlm.nih.gov/Blas.cgi#alnHdr_511106484

### Descriptions
Sequences producing significant alignments:

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<th>Description</th>
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<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Ident</th>
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<tr>
<td>Jocyna hisiae voucher CASMBALTRLC8 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial</td>
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<td>819</td>
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<td>0.0</td>
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<td>KCO96399.1</td>
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<td>405</td>
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<td>8e-124</td>
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<td>KUC64666.1</td>
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<td>455</td>
<td>455</td>
<td>99%</td>
<td>8e-124</td>
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<td>KCO38658.1</td>
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<td>3e-123</td>
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<td>KRO979184.1</td>
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<td>Endotylia sp. ANIC3 voucher 16ANIC-002033 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial</td>
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<td>KF409950.1</td>
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</table>
The above organism showed 99% similarity to *Jorymahilsae* a new species of parasite reported in 2011. From the blast results we find that the COI sequence shows 99% similarity to *Jorymahilsae*

**DISCUSSION**

The study based on examination of 50 specimens of *Rastrelliger kanagurta* collected from Neendakara, Kollam revealed the isopod parasite of the Indian mackerel to be fairly rich comprising *Cymothoaexigua*. The Indian Mackerel *Rastrelliger kanagurta* is an important food fish commonly consumed in South and Southeast Asian countries. Though the trans-boundary species is harvested by different nations, its population genetics is relatively unknown to the scientific world. It is of such importance that it contributed an average 8.8 per cent of the total marine fish production in the country. Its average annual catch was estimated to be 0.27 million tonnes, according to available data. Unambiguous identification of the parasites helps to assess the stock, evolve fisheries management methods and protective measures to sustain the regional fisheries; hence DNA bar coding of the parasites was done. Parasitic crustaceans are the largest fish parasites, which cause considerable damage to their hosts. Isopods inhabiting the buccal cavity and branchial chamber of the fish, inflict damage to gills through attachment and feeding and that the extent of damage is directly proportional to the size of the parasites and duration of settlement. *Jorymahilsae*, or the tongue-eating louse, is a parasitic isopod of the family Cymothoidae. This parasite enters fish through the gills, and then attaches itself to the fish's tongue. The female attaches to the tongue and the male attaches on the gill arches beneath and behind the female. Females are 8–29 millimetres (0.3–1.1 in) long and 4–14 mm (0.16–0.55 in) in maximum width. Males are approximately 7.5–15 mm (0.3–0.6 in) long and 3–7 mm (0.12–0.28 in) wide (*Richard C. Brusca*, 1981). The parasite severs the blood vessels in the fish's tongue, causing the tongue to fall off. It then attaches itself to the stub of what was once its tongue and becomes the fish's new tongue.

*Jorymahilsae* extracts blood through the claws on its front causing the tongue to atrophy from lack of blood. It appears that the parasite does not cause much other damage to the host fish (*R. C. Brusca*; *M. R. Gilligan*, 1983), but it has been reported by *Lanzing and O'Connor* (1975) that infested fish with two or more of the parasites are usually underweight (*Ruiz-Luna, Arturo, March 1992*). Once *Jorymahilsae* replaces the tongue, some feed on the host's blood and many others feed on fish mucus. This is the only known case of a parasite assumed to be functionally replacing a host organ.

**Acknowledgements**

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