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First report on the outbreak of *Argulus* sp. (Crustacea: Branchiura) at a carp seed farm from Andaman and Nicobar Islands, India

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Abstract

In the present study, a disease outbreak with 40% mortality in *Catla catla* and *Labeo rohita* was investigated at a carp seed farm located at South Andaman, India. Various symptoms like abnormal swimming, excessive mucus production, fin erosion and slight hemorrhage were observed in the affected fishes. Examination of moribund fishes revealed the causative agent as *Argulus* sp. The parasites were found attached to the skin and fins of all the examined fishes showing 100% prevalence. Heavy infestation of *Argulus* sp. was observed in *C. catla* and *L. rohita* with mean individuals of 90 to 130 parasites per fish. Molecular characterization of the obtained parasite using 28S rDNA (GenBank accession number MF926591) showed 94% sequence identity with *Argulus japonicus* and *A. foliaceus*, which further confirmed the causative agent. This is the first report on the outbreak of *Argulus* sp. from Andaman and Nicobar Islands, which also reveals the pandemic occurrence of *Argulus* sp. even in the freshwater aquatic system of these islands.

Keywords: *Argulus* sp., Carps, 28S rDNA, Andaman and Nicobar Islands

1. Introduction

Andaman and Nicobar Islands (ANI) is a union territory of India located in the Southeast Bay of Bengal. Freshwater fishes are in great demand in ANI with an average production of 194 tons per year from a total number of 2290 fish ponds ^[1]. Indian major carps, Chinese major carps and catfishes are mainly contributing to the total freshwater fish production of the islands. Though freshwater aquaculture is being practiced by the farmers of ANI since the Colonial British rule, factors such as improper pond management and feeding practices hinder the development of aquaculture in these islands.

Argulus sp. (Crustacea, Branchiura), also called as fish lice are an obligate ectoparasites of fishes causing economic loss in freshwater aquaculture. Globally, around 129 species of *Argulus* have been reported ^[2]. During early infestation of *Argulus*, the host fish exhibits behavioral abnormalities such as lethargy, irritation and loss of appetite and heavier infestation leads to excess mucus secretion, small hemorrhages, fin erosion and secondary infections by opportunistic pathogens ^[3]. These parasites inject digestive enzymes by inserting a preoral sting into the host body and suck out the liquefied body fluids from the skin or gill of the fish by using their proboscis-like mouth causing intense irritation and tissue damage ^[4]. *Argulus* may cause significant morbidity and mortality in cultured fish mainly during heavy infestation ^[5-7]. The total loss due to argulosis in Indian aquaculture has been estimated to be around US\$ 615 ha⁻¹ year⁻¹ ^[8].

Reports on the finfish and shellfish diseases of ANI are very scarce, with only very little documentation available on bacterial and viral diseases and a few incidences of parasites in marine finfishes ^[9, 10]. The reports on freshwater fish diseases are confined only to bacterial disease such as dropsy in *Catla* and *Mrigal* ^[11, 12]. Till date, there is no report available on parasitic outbreak in the freshwater fishes of ANI. With this background, an aquatic animal disease surveillance programme has been initiated to address the lack of knowledge on diseases in economically important fish and shellfishes of ANI. The first report, investigating an outbreak of *Argulus* sp. involving mortality and morbidity in *C. catla* and *L. rohita* is presented here.

2. Materials and Methods

A disease outbreak was observed in a carp seed farm (11°38'50.4"N 92°44'25.0"E) located at South Andaman, ANI during August, 2016 (Fig. 1). The outbreak occurred in a fish pond measuring 20x25x1.5 m stocked with *C. catla* (70 numbers) and *L. rohita* (130 numbers) in the size range of 60–80 g fishes. A total of 12 individuals of moribund *C. catla* and *L. rohita* were collected in aseptic condition for examination. The parasites found on the fish body were preserved in 70% ethanol for morphological identification and 90% ethanol for molecular study.

Morphological characterization of the parasites was carried out by following the appropriate literature [13]. The photographs of the isolated parasites were taken with the aid of stereo zoom microscope. Genomic DNA from the parasite was isolated by modified CTAB method [14]. PCR was performed to amplify a region of 28S rDNA using universal primers namely, forward 5'-ACCCGCTGAATTTAAGCAT-3' and reverse 5'-CTCTTCAGAGTACTTTTCAAC-3' by following the earlier method [15]. The amplified PCR products were sequenced commercially (ShrimpeX Biotech Pvt. Ltd., Chennai) using gene-specific primers. The generated sequence was analyzed using Basic Local Alignment Search Tool (BLAST) program in the National Center for Biotechnology Information (NCBI) for finding homology and multiple sequence alignment of the obtained sequence with the related sequences was constructed using clustal omega program.

3. Results and Discussion

Argulus are the ectoparasites, commonly known as carp lice, that causes a significant impact on aquaculture production mainly in carp hatcheries and seed production centers [16, 17]. In the present investigation, the infestation of *Argulus* sp. had lead to 40% mortality by killing 30 and 50 numbers of *C. catla* and *L. rohita*, respectively. During the examination, parasites were found attached to the skin and fins of all the examined fishes showing 100% prevalence (Fig. 2). It was reported that a carp louse may destroy a fingerling, while more numbers can emaciate and kill even large two-year-old fish [6]; which is evident in the present report. In aquaculture, mortalities are usually associated with hundreds of parasites per host fish [18, 19]. Similarly, in the present report, heavy infestation of *Argulus* sp. with mean individuals of 90 to 130 parasites per fish has lead to the mortality of young fishes.

The infested fishes exhibited symptoms like abnormal swimming, excessive mucus production, fin erosion and slight hemorrhage similar to the condition observed in the earlier report on *Argulus* infestation [3].

The mortality observed was higher in *L. rohita* than *C. catla*, owing to the fact that Rohu is more susceptible to argulosis [20]. In the present study, the outbreak occurred during the monsoon, which is the favorable period for *Argulus* infestation, as some studies have demonstrated that during monsoon, change in the physicochemical parameters of pond water and management practices trigger the parasitic attack [20, 21].

Molecular characterization of the parasite was also carried out using universal primers to ascertain its identity. The 28S rDNA was selected for molecular characterization since 18S and 28S rRNA genes have the densest coverage of known sequences in arthropods and are among the most popular markers for phylogenetic studies [22]. A product length of 443 bp was obtained in the PCR amplification of 28S rDNA of the parasite (Fig. 3). The sequence of the PCR product revealed 94% identity with 28S rDNA sequence of *Argulus japonicus* (GenBank accession number KF747850) and *A. foliaceus* (GenBank accession number EU370442). Multiple sequence alignment of the above said sequences were provided in Fig. 4. The 28S rDNA sequence of *Argulus* sp. obtained in this present study has been submitted to NCBI GenBank under the accession number MF926591.

The South Andaman district of ANI is a well-known place for carp culture. As per our knowledge, there is no report available on parasitic outbreaks causing mortality in fish ponds of ANI. The current outbreak of *Argulus* sp. is the first report from these islands which will serve as baseline information for all the future investigations on fish parasites. In the present study, fish mortalities had occurred as a consequence of heavy infestation, which alerts that, argulosis should be seriously considered as an economic problem in carp culture of the islands. The study draws attention towards the adoption of proper pond management during monsoon season, due to the high probability of parasitic infestations causing biomass loss in the fish pond. The present study calls for a detailed investigation on *Argulus* sp. occurring in the native waters and rain fed reservoirs of ANI, as appropriate morphological and molecular identification will aid in better understanding of its species diversity and biology which will help in devising better remedial and preventive measures.

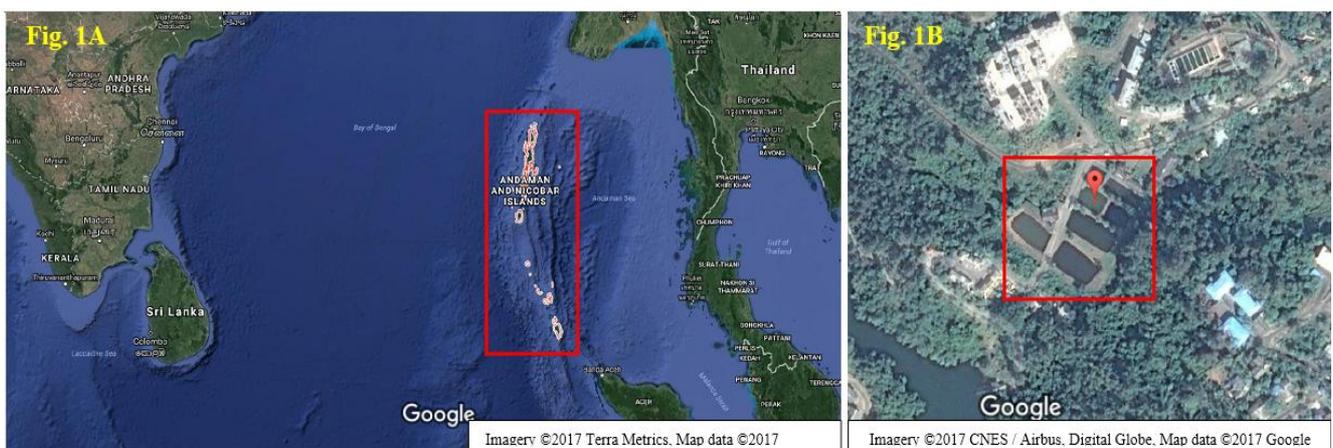


Fig 1: Map showing the location of ANI (Fig. 1A) and carp seed farm (11°38'50.4"N 92°44'25.0"E) located at South Andaman (Fig. 1B).



Fig 2: Argulus infestation on the body surface of rohu

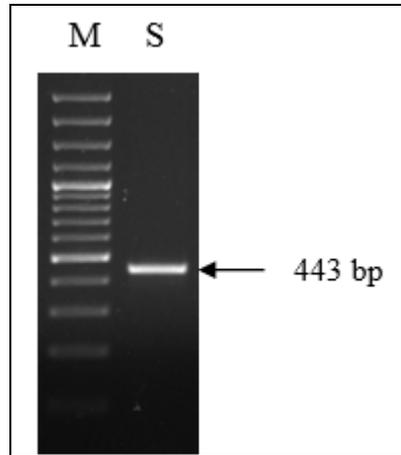


Fig 3: PCR for *Argulus* sp. using 28S rDNA primers. Lanes: M-100 bp marker; S-Sample.

MF926591_ <i>Argulus</i>	ACCCGCTGAATTTAAGCATATCACTAAGCGGAGGAAAAGAACTAACAAAGGATTCCCCTA	60
KF747850_ <i>A. japonicus</i>	ACCCGCTAAATTTAAGCATATCACTAAGCGGAGGAAAAGAACTAACAAAGGATTCCCCTA	60
EU370442_ <i>A. foliaceus</i>	ACCCGCTAAATTTAAGCATATCACTAAGCGGAGGAAAAGAACTAACAAAGGATTCCCCTA	60

MF926591_ <i>Argulus</i>	GTAACGGCGAGCGAACGGGGAGAGCCCGAGCACCACCCCTGCCGAAGTACAAGCGCCAG	120
KF747850_ <i>A. japonicus</i>	GTAACGGCGAGCGAACGGGGAGAGCCCGAGCACCACCCCTGCCGAAGTACAAGCGCCAG	119
EU370442_ <i>A. foliaceus</i>	GTAACGGCGAGCGAACGGGGAGAGCCCGAGCACCACCCCTGCCGAAGTACAAGCGCCAG	119

MF926591_ <i>Argulus</i>	GGAATGTGGTGTITGGGAGGGATCATTCTCGTGGTTCACCTTTCGGAGCGTTCGAAGTCCG	180
KF747850_ <i>A. japonicus</i>	GGAATGTGGTGTITGGGAGGGATCCTTCTCGCGGCT--TTCGGCTAACGTTCCAAGTCCG	177
EU370442_ <i>A. foliaceus</i>	GGAATGTGGTGTITGGGAGGGATCCTTCTCGCGGCT--TTCGGCTAACGTTCCAAGTCCG	177

MF926591_ <i>Argulus</i>	CTTGAAACGGCGCCATCGCCCGTAGAGGGTGACAGGCCCGTATGAACGTTCCGAGCCGCGG	240
KF747850_ <i>A. japonicus</i>	CTTGAAACGGCGCCATCGCCCGTAGAGGGTGACAGGCCCGTCTGAACGTTCCGAGTCCG	237
EU370442_ <i>A. foliaceus</i>	CTTGAAACGGCGCCATCGCCCGTAGAGGGTGACAGGCCCGTCTGAACGTTCCGAGTCCG	237

MF926591_ <i>Argulus</i>	GTTGATCTCTCCTTGGAGTCGGGTTGCTTGAGAGTGCAGCCCTAAGAGGGTGGTAAACTC	300
KF747850_ <i>A. japonicus</i>	GTTGATCCCTCCTTGGAGTCGGGTTGCTTGAGAGTGCAGCCCTAAGAGGGTGGTAAACTC	297
EU370442_ <i>A. foliaceus</i>	GTTGATCCCTCCTTGGAGTCGGGTTGCTTGAGAGTGCAGCCCTAAGAGGGTGGTAAACTC	297

MF926591_ <i>Argulus</i>	CATCTAAGGCTAAATACGACCACGAGCCGATAGCGAACAAAGTACCCTGAGGGAAAGTTG	360
KF747850_ <i>A. japonicus</i>	CATCTAAGGCTAAATACGACCACGAGCCGATAGCGAACAAAGTACCCTGAGGGAAAGTTG	357
EU370442_ <i>A. foliaceus</i>	CATCTAAGGCTAAATACGACCACGAGCCGATAGCGAACAAAGTACCCTGAGGGAAAGTTG	357

MF926591_ <i>Argulus</i>	AAARGTACTCTGAAGAGA	378
KF747850_ <i>A. japonicus</i>	AAARGAACTTTGAAGAGA	375
EU370442_ <i>A. foliaceus</i>	AAARGAACTTTGAAGAGA	375

Fig 4: Multiple sequence alignment of 28S rDNA sequence of *Argulus* sp. obtained in this present study (GenBank accession number MF926591) with that of other species such as *A. japonicus* (KF747850) and *A. foliaceus* (EU370442). Identical residues are indicated by asterisk (*) and dashes (-) indicate gaps in the aligned nucleotide sequences.

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