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RECORD OF FRESHWATER JELLYFISH BLOOMS OF INVASIVE *CRASPEDACUSTA SOWERBII* LANKESTER, 1880 (HYDROZOA, LIMNOMEDUSAE) FROM KERALA, INDIA

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Abstract: The freshwater jellyfish *Craspedacusta sowerbii*, native to China, has been introduced to various natural and artificial freshwater bodies globally. The blooms of this jellyfish was recorded from Chemeenchal pond, Vallakunnu, Thrissur district of Kerala, India. The jellyfish specimens were identified using integrative taxonomic approach. This forms the second report of *C. sowerbii* from Kerala and third report of this species from India. This paper provides an overview of freshwater jellyfish blooms and analyses the possible reasons for the blooms.

Keywords: Jellyfish bloom, *Craspedacusta*, medusa, Hydrozoa, phylogeny, Invasive species

INTRODUCTION

The freshwater jellyfish belonging to the phylum Cnidaria, class Hydrozoa and family Olindiidae (Bouillon and Boero, 2000a), are interesting group of less studied animals, often exhibiting unprecedented blooms (Dumont, 1994). About 20 extended species of freshwater jellyfishes, including 6 genera are recorded from the freshwater habitats around the globe (Jankowski, 2001). Reports on freshwater medusa exist in literature over the last 100 years only (Lankester, 1880) and are geologically young and polyphyletic group compared with other marine hydromedusae (Hadzi, 1928). The genus *Craspedacusta* Lankester, 1880 includes 11 species and *C. sowerbii* Lankester, 1880 is considered as a true freshwater jellyfish (Jankowski, 2001). *C. sowerbyi* was first described by Lankester (1880) based on specimens collected from water-lily tanks in Regents Park, London. Later studies revealed that this species is indigenous to the Yangtze River valley in China and it reached all over the world along with water hyacinth *Eichhornia crassipes* (Kramp, 1951; Slobodkin and Bossert, 1991). Currently *C. sowerbyi* represents the only cosmopolitan species occurring throughout the world with the exception of Antarctica and are very common in freshwater systems in subtropical to temperate regions (Dumont, 1994; Jankowski *et al.*, 2008). The other species belonging to the genus *Craspedacusta* are constrained to East Asia (Jankowski, 2001).

In India first record of freshwater jellyfish came from Kerala, where *Limnocnida indica* was from the Periyar River (Darling, 1935). This species has also been reported from other parts of India (Birsal, 1994). Indian records of fresh water jellyfish includes *Moerisia gangetica* from Calcutta (Kramp, 1958), *Craspedacusta sowerbii* from Poona (Joshi and Tonapi, 1965), and *Manasirella lacustris* from Jammu (Malhotra *et al.*, 1976). *Keralica idukkens* is a new species of freshwater jellyfish recorded from the Idukki reservoir (Khatri, 1984). Sarkar and Mude (2010) reported *Craspedacusta sowerbii* Lankester, 1880 from the rock quarry of Kunnampara near to Thiruvananthapuram in Kerala. According to Jankowski (2001) six species of freshwater medusa included in the three genera exist in the Indian subcontinent.

Modern systematics on freshwater jellyfish is based on embryological, developmental, morphological and nematocyst structures (Hartwich *et al.*, 1993; Bouillon and Boero, 2000a, b). This paper records the blooming of *Craspedacusta sowerbii* in Kerala and the species identification was done through integrative taxonomy.

MATERIALS AND METHODS

The occurrence of 'unusual' organisms in blooms from a pond in Thrissur district of Kerala was informed to us by a news editor from a Malayalam daily newspaper. We went to the site and found that these represent freshwater jellyfish. The specimens were then collected from the Chemeenchal pond (10°20'11.4" N; 76°15'50.4" E) near Vallakunnu in the Thrissur district of Kerala during November 2016. The pond is rectangular in shape having 15m length, 10m width with an average depth of 4 meter located at the centre of a rice field. About 20 specimens were collected with medium sized hand net directly from the pond and abundance of jellyfish was recorded by visual counts from water surface and through underwater videography.

The specimens were brought live to the wet lab of the department for further observations and few specimens were fixed in 95% ethanol for molecular analysis. Some medusae were maintained alive for about a week in aquarium for observation. Stereoscope microscopes were used for morphological studies. Nematocysts of fresh specimens were extracted from marginal tentacles. Tissues were squashed on slides under cover slip and measurements were taken at 1000x magnification using compound microscope**.** Nematocyst extraction and identification followed the methods of Östman (2000). The taxonomic identification was confirmed following the publications of Kramp (1951), Bouillon and Boero (2000a, b) and Jankowski (2001).

DNA extraction from the ethanol preserved tissue by using Dneasy Blood and Tissue Kit (QIAGEN, Hilden, Germany). A portion of the mitochondrial cytochrome oxidase I (COI) gene were amplified with the primers LCOjf (5'GGTCAACAA ATCA TAAAGATATTGGAAC-3'; Dawson 2005b) HCO2198 (5'-TAAACTTCAGGGTGACC AAAAA ATCA-3'; Folmer *et al.*, 1994). 25µl PCR reactions performed with Taq PCR master mix (QIAGEN, Hilden, Germany). Amplification of DNA made by using Eppendorf Thermocycler which needs six steps of 94°C for 8 min, 49°C for 2 min, 72°C for 2 min, 94°C for 4 min, 50°C for 2 min, 72°C for 2 min, followed by 33 cycles of 94°C for 45 s, 51°C for 45 s, and 72°C for 60s, then a final extension at 72°C for 10 min, before ending at 4°C (Dawson, 2005). Presence of DNA in the PCR product were visualized on 1% agarose gels and purified using Exo Sap IT (USB) (Affymetrix Inc., Santa Clara, USA). Sequencing was made bidirectionally using the corresponding PCR primers and Big Dye Terminator V.3.1 Cycle sequencing Kit (Applied Biosystems Inc., Foster City, USA) in an ABI 3730 capillary sequencer (Applied Biosystems Inc., Foster City, USA). The sequenced DNA were edited and aligned using BioEdit sequence alignment editor V.7.0.9.0. (lbisBiosciences, Carlsbad, USA., Hall, 1999). Sequence similarity of specimen was carried by using database GenBankhttp://www.ncbi.nlm.nih.gov/genbank/). Divergence in the sequence were analysed using the Kimura 2-Parameter distance model of MEGA (Version 7.0) (Kumar *et al.*, 2016). Maximum likelihood tree was selected for phylogeny analysis.

RESULTS AND DISCUSSION

Systematics

Phylum: Cnidaria
Class: Hydrozoa
Subclass: Trachylinae
Order: Limnomedusae
Family: Olindiidae
Genus: *Craspedacusta* Lankester, 1880
Craspedacusta sowerbii Lankester, 1880 (Fig. 1)

Medusae umbrella flatter than hemispherical with average bell diameter of 15 mm. Medusa with four simple radial canals. Four pouch-like gonad facing radial canal. Quadrangular manubrium in the centre of the sub umbrella joined together to form a lip. Five opaque-white canals, which form the gastrovascular cavity; four are radial and one is medially dorsoventral. Tentacles of varying lengths protrude from the upper margin of the velum, arranged with three to seven short tentacles between longer ones; total number of tentacles exceeds 200. Closed tubular statocysts situated in the velum. Number of the statocyst almost similar to the number of marginal tentacles. Medusa is translucent with a whitish tinge; gonads milky white.

Group of nematocyst from the marginal tentacle was identified as birhopaloids (Fig. 2). Largest nematocyst has a size of 15.28 x 7.19 µm. Coiled shaft visible under microscope with 1000X magnification.

Fig. 1. *Craspedacusta sowerbii* Lankester, 1880

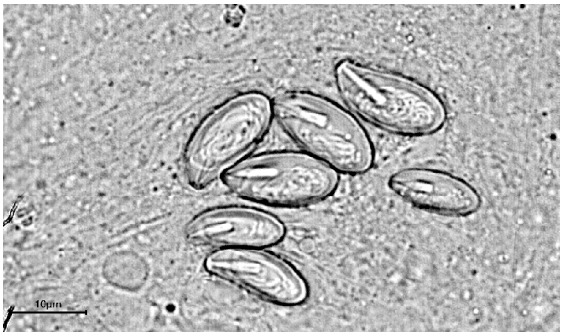

Fig. 2. Birhopaloid nematocyst of *Craspedacusta sowerbii*

The sequence data of the mitochondrial cytochrome oxidase I (COI) gene was used for confirming the identity of the species. The sequence length of *Craspedacusta sowerbii* of the present study was 571 bp. The A+T and G+C contents are 48.86% and 51.14% respectively. Pair-wise genetic distance of CO1 sequences was calculated using Maximum Composite Likelihood model. *Craspedacusta sowerbii* had genetically lesser distance (0.035) with *C. sowerbyi* (KP231217) which proves the confirmation of the species.

The phylogenic relationship of *C. sowerbii* was studied using Maximum Likelihood Method (Fig. 3). The ML tree was prepared and clade stability was estimated using 1000 non-parametric bootstrap replications. Phylogenetic tree revealed that *C. sowerbii* of the present study (shown in bold) has got clustered with the identical reference sequence of the *C. sowerbyi* (KP231217) from GenBank with highest boot strap value (100). Fresh water jellyfish was also analysed with marine jellyfish belong the same family were showing great variation with *C. sowerbii*. By interpreting the phylogenetic tree, it is clearly revealed that the jelly fish collected from Trissur is confirmed as *C. sowerbii*.

C. sowerbii entirely differ from other *Craspedacusta* species due to the arrangement of tentacle, structural pattern and nematocyst distribution. In India this may be an invasive species and its presence was reported earlier from Poona (Joshi and Tonapi, 1965) and Kerala, where the record was from a quarry pond (Sarkar and Mude, 2010). The native range of *C. sowerbii* is Yangtze River valley in China (Slobodkin and Bossert, 1991). Now this species has invaded many parts of the world, especially in temperate water bodies (Pennak, 1989). Though their presence has been reported from tropical freshwater systems, the extend of distribution remains to be fully studied (McKercher *et al.,* 2017). Throughout its invasive range *C. sowerbii* occurs in wide ranging habitats from natural water bodies such as rivers, ponds and lakes and man-made structures such as ornamental ponds, reservoirs, gravel pits, and quarries (Slobodkin and Bossert, 1991; Sarkar and Mude, 2010; McKercher *et al.*, 2017).

Jellyfish Blooms

The bloom of *C. sowerbii* was recorded from Chemeenchal pond near Vallakunnu in the Thrissur district of Kerala during November 2016 (Fig. 4). More than 2,000 specimens were counted in the bloom; the recorded density was 11 individuals per cubic meter on an average. The bloom was recorded during the post-monsoon season in Kerala. The sporadic blooms of jellyfish are part of the hydromedusa life cycle. *C. sowerbii* more often exist as microscopic podocysts (dormant "resting bodies"), frustules (larvae produced asexually by budding), planulae (larvae produced sexually by the hydromedusae), or as sessile polyps, which attach to stable surfaces and can form colonies consisting of two to four individuals measuring 5 to 8 mm

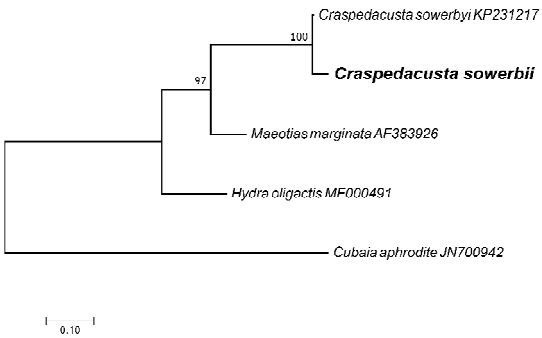

Fig. 3. Phylogenetic tree of *Craspedacusta sowerbii* generated using Maximum Likelihood analysis based on an alignment of partial mitochondrial C oxidase subunit 1 (CO1) gene.

(Angradi, 1998; Acker and Muscat, 1976; Pennak, 1989; Peard, 2002). In the present case the route of introduction of freshwater jellyfish in the isolated pond is unknown, though it may be assumed that the pond located inside the rice field may remain flooded during monsoon and the podocysts may get entry into the pond through the flooded water.

Rising temperature may be a reason for polyp formation of marine jellyfish (Mills, 2001; Purcell, 2005). Harder substratum in the freshwater environment may offer environment for the attachment of polyp. Another important route of jellyfish introduction to freshwaters is by migrating birds, which may carry the podocysts (Dumont, 1994). Kramp (1951) reported the transport of *C. sowerbii* along with water hyacinth *Eichhornia crassipes*. It may also be introduced through the transport of aquarium plants and of late, they are used in freshwater aquariums as well (Didžiulis and ¯urek, 2013).

The impact of *C. sowerbii* in the ecosystem has not been studied well. However, they feed on zooplankton (Dodson and Cooper, 1983; Dumont, 1994; Spadinger and Maier, 1999) and therefore the blooms would affect the zooplankton population in the ecosystem. Polyps are able to consume large variety of zooplankton and therefore their blooms may affect the food web and trophic interactions (Jankowski *et al.*, 2005) through increasing zooplankton mortality (Smith and Alexander, 2008).). Freshwater jellyfish is not considered dangerous to humans as its small

Fig. 4. Underwater photograph of *C. sowerbii* bloom

nematocysts are not likely to penetrate human skin, though its stings can paralyze macroinvertebrates and small fish (Peard, 2002). We recommend further studies on the distribution, ecology and ecosystem impacts of invasive *Craspedacusta sowerbii* in Indian water bodies.

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