ECTOPARASITIC CILIATES (PROTOZOA, CILIOPHORA) ON THE GIANT FRESHWATER PRAWN MACROBRACHIUM ROSENBERGII (CRUSTACEA: DECAPODA)

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Abstract
Protozoan ectoparasitic ciliates on the freshwater shrimp Macrobrachium rosenbergii were studied in different freshwater ponds and breeding grounds of Gajapathingaram, around Vizianagaram District of Andhra Pradesh, India. M. rosenbergii is the largest freshwater shrimp cultivated around the Vizianagaram district. Among them three ectoparasitic ciliates were identified Zoothamnium, Trichodina and Vorticella. These are located on the diverse areas of the surface and appendages of the shrimp. The colonial ciliates like the Zoothamnium and Vorticella, settle down on appendages and gill pouches and impair the feeding and the growth of the host. The solitary individuals such as the Trichodines having an adhesive disc can get attached to any vital organ and feed on the host. Present study contains information regarding morphological and taxonomical data of these ciliates along with their distribution.

INTRODUCTION
Protozoan’s are highly diverse group of organisms exhibiting different types of inter-specific relationships. The phylum Ciliophora is the most evaluated group having specialized organelles for their metabolism. Macro-organisms, as well other micro organisms that share same environment, will likely be impacted by members of this taxon. The external surfaces of crustaceans provide a wide array of microenvironment that attract the settlement and growth of algae, bacteria, fungi and numerous types of protists, including sessile and sedentary ciliates. Few of these are known to occur as ectoparasites on the shrimps. Ectoparasitism is a facultative, non symbiotic and interspecific association, where by one organism lives on the surface of another organism (Wahl, 1989, Wahl et al., 1997). This association can be temporary (Key et al., 1997) or last for the entire life span of the organism (Warner 1997, Buschbaum & Reise, 1999, Ozer&Erdem, 1998). Among the various protozoan ciliates, postomatids, peritichs, suctorians, heterotrichs and chonotrichs have been described as epibionts on crustaceans (Corliss, 1979; Batisse, 1986, Clamp, 1991; Batisse,1992). The role of ciliates as epibionts on crustaceans has been not studied as thoroughly as that of the organisms, such as hydrozoans, bryozoans and polychaetes (Sprague & Couch, 1971). There are 30 genera of peritiches described as ectoparasites of five classes of crustaceans:- viz Branchiopoda, Branchiura, Copepoda and Ostracoda (Fernandez – Leborans & Tato – Porto 2000a). One of these genera, Zoothamnium, infested almost the entire Zooplankton community (Valbonesi & Guglielmo 1988). The aim of the present study was to investigate ectoparasitic ciliates of the M. rosenbergii from different freshwater ponds of the Gajapathinagaram. This has become a major hindrance in hatcheries, resulting in mass mortalities. Their occurrence as solitary forms or colonial forms in vital parts are known to cause mass mortalities in post larval and early adults, it leads to reduced muscle gut ratio (MGR) in shrimps. This is an indicative phenomenon of the health status of the shrimp’s.

MATERIALS AND METHODS
The material for the present study was obtained from the ponds and natural breeding grounds from Gajapathinagaram, Vizianagaram District of Andhra Pradesh, India (A&B). The shrimps were maintained in aquaria tanks in the laboratory and were subjected to regular examination. In juveniles and post larval, clinical, signs of infection were evident with certain conformity of
diagnostic significance. The procedure followed to observe for the incidence of ectoparasitic ciliates was to remove the appendages, gills, slowly spread isolated appendage on to a clean glass slide using the edge of a cover slip or a fine needle and forceps. The contents were spread out and a wet smear was prepared. The wet mount was examined directly by a reduced bright field microscope with 10X and 45X objectives for Trophonts. Ectoparasites on the surface of the anatomical units of the shrimps were observed under stereoscopic and light microscopy. In order to identify the protozoan the procedure described by Fernandez – Leborans G & Castro de Zaldumbide (1986) was used. For histological studies of the ectoparasites the ciliates were fixed in Schaudin’s fluid under wet condition on an albuminised slide and stained with HIH (Heidenhain’s Iron Haematoxylin) or Delafields haematoxylin. Some of the fresh smears were air dried, treated with 2% silver nitrate and exposed to direct sunlight for one hour. This technique allowed impregnating silver oxide on the ciliary structure. Hence the motorium and all the ciliary structures were clearly visible by adopting this method. All measurements were taken with an ocular micrometer and drawings made to scale with the help of a camera Lucida.

RESULTS AND DISCUSSION
ZOOTHAMNIIUM (Fig.1. A & B):
Host : Macrobrachium rosenbergii
Site of infection : Gills
Locality : Fresh water pond in Gajapathinagaram
The presence of the group Zoothamnium species was reported from the fresh water pond of Gajapathinagaram and it is a frequent inhabitant of the surface of shrimp gill filaments of M.rosenbergii(Fig.1. A & B). When they become abundant on gills of shrimp ponds water shows the scarcity of oxygen content and suffocation can occur. The life of ecto-commensals involves continual duplication. The shrimp acquires an increasing burden of these protozoans until shedding of the exoskeleton provides relief. Zoothamnium colony was dichotomously branched with consistent cell size and shape, (generally 60-87μm & 35-30 μm) and slender vase-shaped. Body constricted slightly below the double-layered, thick peristomial lip; maximum width of cell mostly at oral border; large peristomial disc highly elevated. Pellicle very finely striated, which can be detected only using powerful objectives, yet completely smooth when observed at low magnification. Cytoplasm colorless to slightly grayish, usually containing several large greenish food vacuoles, and numerous small grayish granules which are 1-2μm in diameter. One large apically located contractile vacuole rather inactive and contracting at a rate of 3 times for 3 minutes.

TRICHODINA (Fig.2. A & B):
Host : Macrobrachium rosenbergii
Site of infection : Appendages
Locality : Fresh water pond in Gajapathinagaram
Trichodina species were observed on the appendages of M. rosenbergii, while examining for the occurrence of ciliate ectoparasites(Fig. 2. A & B). The barrel-shaped body was about 50 μm in diameter. Its height was measured 55 μm. The anterior part of the ciliate has three oral rings which show a fairly uniform ciliation while on the posterior part, internal to the aboral ring of cilia, a well developed adhesive disk with a denticulate skeletal complex could be seen for the body support on settlement. The texture of central area is finely granular and similar as rest of adhesive disc. The denticulate ring consists of 28-32 large denticles. There are 8-12 radial pins per denticle. The adoral cilia described a turn of about 380-385°. The blade of denticle is broad and curved, filling almost the entire spaces between y+1 axis, sometimes angular, which are also found in newly born individuals. The tangent point is blunt in most cases, slightly lower than distal margin.
VORTICELLA (Fig.3. A & B):

Host : Macrobrachium rosenbergii  
Site of infection : Gills  
Locality : Fresh water pond in Gajapathinagaram  

Vorticella sps was also reported from the gill region of M. rosenbergii and have polymorphic life cycle, the majority of which is spent in the feeding stage as a sessile stalked zooid called a trophont(Fig.3.A & B). This organism also assumes a motile form known as a telotroch. The trophont form will metamorphose into a telotroch when exposed to an unfavorable environment or following mitosis. Other minor forms exist as a byproduct of sexual reproduction. During the trophont stage Vorticella subsist by sweeping bacteria and other food particles into their mouths with swirling currents created by the rapid beating of peristomial cilia. The cell body (or zooid) of the trophont is a vase shaped mass, typically 40μm in length and 15μm wide. The zooid contains most of a Vorticella’s organelles, such as the food vacuoles, micro and macro nuclei, endoplasmic reticulum, and mitochondria. The anterior region of Vorticella consists of an oral region surrounded by cilia. These cilia are around the edge of the mouth. The posterior end of the cell narrows into a small region where a semi-permanent junction connects the long contractile stalk to the zooid. This junction is known as the scapular region. The contractile stalk is about 2.9 μm wide and can be anywhere from 20 μm -300μm long. The other end of the stalk attaches to a substrate, such as rocks, aquatic plants, and even aquatic animals via an adhesive pad.

DISCUSSIONS

The occurrence of ectoparasitic ciliates on the gills and appendages of shrimps is a common problem in natural as well as at culture practices. Colonial peritrichous ciliates are common and dominant in freshwater, especially in littoral eutrophic biotopes (Kahl, 1933; Kahl,1935; Sommer 1935, Stiller 1971). Other protozoan epibionts have not been described previously on the studied M. rosenbergii. The peritrich genus Zoothamnium has been described on amphiods, isopods and copepods (Morado & Small 1995; Fernandez – Leborans and Tato – Porto 2000b). Apart from some species, the other ectoparasites on the observed shrimp species belonged to ciliate protozoan genera well known as ectoparasites on crustaceans. However in the present study several of these ciliates showed particular morphological characteristics which might be related to the special conditions of high endemism of a shrimp as sites of attachment. Still lot of information has to be discovered about the diversity of ectocommensals on shrimp and their association with shrimp. Zoanthaminium species are frequent inhabitant of the surface of the shrimp gill filaments. The Trichodina species are more frequent on the appendages of the shrimp. The appendages of M. rosenbergii specimens were also found to be infected. Trichodinids are one of the most common parasitic ciliates that are well characterized by two main features: the morphology of the denticles in the adhesive and the development of the dorsal ciliary spiral (Lom, 1970; Lynn & Small 2000; Basson & Van 1989; Ozer 2003a; Ozer, 2003b). The finding of present study showed a rare appearance on the appendages, only one Trichodina on each appendages filament. Interestingly, their prevalence increased late, more than one protest was seen on each appendages filament. However, the peritrich genus Vorticella has been described on isopods, amphipods, cladocerns, copepods, branchiopods and isopods (Warren, 1986; Warren & Paynter 1991; Morado & Small1995; Fernandez–Leborans & Tato Porto 2000b) and on mysids (Precht, 1935; Fernandez Leborans, 2004). These are more frequent in the gills of M. rosenbergii sps. In natural environment ciliate epibiont rarely produce mortality in their crustacean basibionts. However these epibionts can cause gill and fouling disease in shrimps at high densities, typically under stress condition (Lightner 1996). In some cases they become opportunistic pathogens in culture ponds during period of low Oxygen concentration (Johnson 1978).

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