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An Investigation into Marine Ciliates with Establishment of a New Genus, *Phyllopharyngean americana* Nov. Gen., Nov. Spec.

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ABSTRACT

Marine fish contain a large diversity of parasitic ciliates. Among those, cyrtophorids constitute large populations and many species of these ciliates have not been yet identified. Therefore, morphology and infraciliature of a new marine cyrtophorid ciliate, *Phyllopharyngean americana* g. nov. sp. nov., which was isolated from the skin and gills of striped bass and the gills of white perch fish collected from the Chesapeake bay, Maryland, USA. Fish were collected and ciliates were isolated and examined using the protargol silver impregnation technique. The ciliate was characterized by burrowing motion of the live specimens, reniform shape and measured 30.2×22.9 (18-41×14-30) μm *in vivo*. The right side was ciliated and convex, while the left one was almost straight. There were 10 ridges on the dorsal surface and nine to eleven right ventral somatic kineties curved anteriorly. Two ovoid macronuclei and a single micronucleus were present. Two contractile vacuole pores detected between the second and the third right kineties and short transverse fibers deriving from the left kineties towards the middle of the body were present. Morphogenetic observations revealed cystic and precystic stages in the infected fish. It is concluded that, these data provide an evidence for the taxonomic placement of *Phyllopharyngean americana* as a new genus (nov. g.) and new species (nov. sp.) in the Cyrtophorida.

Key words: Ciliate, marine fish, morphology, *Phyllopharyngean americana*, protargol impregnation

INTRODUCTION

Protozoa can be established as motile unicellular eukaryotes capable of phagotrophy and constitute a diverse collection of organisms commonly grouped into flagellates, ciliates, amoebae and intracellular parasitic apicomplexans (Finlay and Esteban, 1998; Corliss, 2002). One essential problem for aquatic biologists is the inaccurate identification and enumeration of different taxa, although life observation is still essential for the determination of protists. The clear identification of several species also requires several cytological examinations, especially for ciliated protozoa, including light microscopy and scanning electron microscopy (Skibbe, 1994). It is difficult to reliably identify many protozoan species using ordinary stains, as these species may be fragile and inconspicuous. Consequently, it is challenging to determine whether or not the given morphological features are distinct (Fried *et al.*, 2002; Sims *et al.*, 2002; Caron *et al.*, 2004).

The majority of the methods used is time consuming and cannot be used for quantitative examination of the parasitic ciliates. Therefore, the Quantitative Protargol Staining (QPS) technique is required to reduce the gap between the quantitative and taxonomic methods (Montagnes and Lynn, 1987). It is based on protargol silver impregnation, one of the most valuable cytological stains used in ciliates taxonomy. The staining of ciliates is necessary in order to identify them at the species level, showing the arrangement of the ciliature and infraciliature in both somatic and oral structures. Different stains can be used to illustrate the ciliated structures. Silver salt is one of the most basic known stains that usually precipitate on the microtubular structures of the ciliate. Other recent methods that relied on the hybridization of molecular probes against microtubular structures are also available (Snoeyenbos-West *et al.,* 2004; Gong *et al.,* 2008).

The class Phyllopharyngea (phylum Ciliophora) is so named due to the presence of radially arranged microtubular structures, phyllae, surrounding the cytopharynx (Lynn and Small, 1997; 2002). In addition, these ciliates possess a reduced ciliature and a synapomorphic kinetid structure in some stages of the life cycle (Lynn and Corliss, 1991). Morphologically, species within the ciliates of class Phyllopharyngea are highly specialized. According to Adl *et al.* (2005) and Gong *et al.* (2008), the class is defined mainly by the ultrastructure of somatic kinetids and ontogenesis, including four subclasses; cyrtophoria, chonotrichia, suctoria and rhynchodia.

This study describes a new marine ciliate, *Phyllopharyngean americana* nov. g., nov. sp., belonging to the subclass Cyrtophoria, order Cyrtophorida, family Chilodonellidae. Morphological description of the isolated ciliate was based on staining using the protargol staining technique, as well as on camera lucida drawings.

MATERIALS AND METHODS

Sampling: Fish samples were obtained from the Chesapeake Bay (coordinates: 36°59'45"N75°57'34"W), Maryland, USA on the permission of Department of Natural Resources (Annapolis, Maryland).

Preparation and fixation: Wet smears were made from skin scrapings and from the gill mucous by scraping with a new razor blade and were examined microscopically to detect the living protozoa (Pritchard and Kruse, 1982). Following the exposure of the gill arch, the entire gill was put in vials containing modified fixative solution (glacial acetic acid and Bouin's fixative solution) and filtered water. The vials were gently shaken and stored for at least 24 h before staining (Coats and Heinbokel, 1982).

Protargol silver impregnation: Quantitative protargol silver impregnation staining technique was applied according to Lynn (1992) and Montagnes and Lynn (1993) and preparation of staining runs and filters was done according to Wilbert (1975) and Skibbe (1994). Measurements of the detected ciliate were done with a calibrated ocular micrometer. Drawing was done with a compound microscope (Zeiss, Inc. model RA), with the aid of a camera lucida device. Photomicrography was established with a microscope (Zeiss WL) equipped with a 35 mm camera (Wild-Leitz MPS 52).

RESULTS

Phyllopharyngean americana nov. g., nov. sp.

Type-host: Striped bass, *Morone saxatilis* (Walbaum 1792) (Perciformes: Moronidae) and white perch, *Morone americana* (Gmelin 1789) (Perciformes: Moronidae).

Type-locality: Chesapeake bay, Maryland, USA (36°59'45"N75°57'34"W).

Site of infection: The gills and skin of striped bass and the gills of white perch fish.

Prevalence and intensity of infection: The present study revealed that the intensity of infection with the detected ciliate was low (1-5 cells/filter) in both striped bass and white perch. Moreover, among the examined striped bass, the ciliate infection in the skin was 3.8% (4/104). In white perch, the infection rate was higher and gills were the target organ, with an infection rate of 10.5% (6/57).

Description: Cells were detected on the skin and gills of striped bass as well as on the gills of white perch. Live specimens characterized by a burrowing motion. The protozoan measured 30.2×22.9 (18-41×14-30) μm *in vivo*. Body shape flexible, usually reniform-shaped; right side convex and left side somewhat straight; the shape sometimes tapered at the forward end to the left side. Live specimens existed in a burrowing motion in the gill tissue. The body size averaged 30.2×22.9 (18-41×14-30 μm) (n = 20) with the dorsal surface traversed by 10 ridges. Nine to eleven Right Ventral Somatic monokinetids (RSK) were curved anteriorly. The right dorsal kineties extended backward a distance from the end of the body. Each kinety was supported by a ribbon of postciliary microtubules. A transverse Preoral Kinety (PK) extended anteriorly, together with two other circumoral (CK) short segments, formed a triangular area which encircled the cytostomial orifice. The mid-ventral area was free of kineties and contained 2 ovoid macronuclei, each about 7×6 μm in size. A globular micronucleus was located between the macronuclei and averaged 1.5-3 μm in diameter. Two pores of contractile vacuoles were seen between the second and the third kineties. Transverse fibers of about 4 μm originated from the left kineties toward the middle of the body. The cytoplasm contained many vesicles, tissue remnants and different-sized cells. Precystic as well as cystic stages were observed on the skin and gills. The cyst was usually semispherical to round and could be easily detected by the characteristic macronuclei and dorsal ridges. Some large-sized developmental stages with duplicated kineties were also observed (Figs. 1 and 2).

DISCUSSION

A group of morphological features including a distribution pattern of somatic kineties (two distinct left and right belts), oral ciliature, postciliary microtubules along the basal bodies of the somatic kineties, flattened body form, cyst formation and the absence of both adhesive organelles and post oral kineties put this genus in the family Chilodonellidae (Small and Lynn, 1985; Lom and Dykova, 1992). Following the key given by Small and Lynn (1985), two of six genera of this family share some morphological characteristics with the genus in the present study. The others show many taxonomic features that are entirely different. Of those two parallel genera, *Thigmogaster* (Deroux 1976) is discriminated from the studied genus by possessing reduced left ventral kineties, with right ones are curved to the left side, backward and forward. The left and right ciliary fields are slightly detached with a conspicuous cytostome (Foissner, 1988; Augustin and Foissner, 1989). Furthermore, genus *Chilodonella* (Strand 1928) is characterized by a posterior notch, single ovoid micronucleus, nearly equal numbers of right (7-15) and left (8-14) ventral kineties, central non-ciliated zone, spheroid to ellipsoidal macronuclei and 2 contractile vacuoles. However, some species (*C. calkins*) possess 4 contractile vacuoles and others (*C. bavariensis*) have five vacuoles (Golembiewska and Radzikowski, 1980; Lynn, 2003).

On the other hand, genus *Phascolodon* (Stein 1859) has numerous discrepancies comparing to the genus in the present investigation. It has more frequently shovel-like (vase-like) broad and cup-shaped oral region anteriorly with the posterior end tapered.

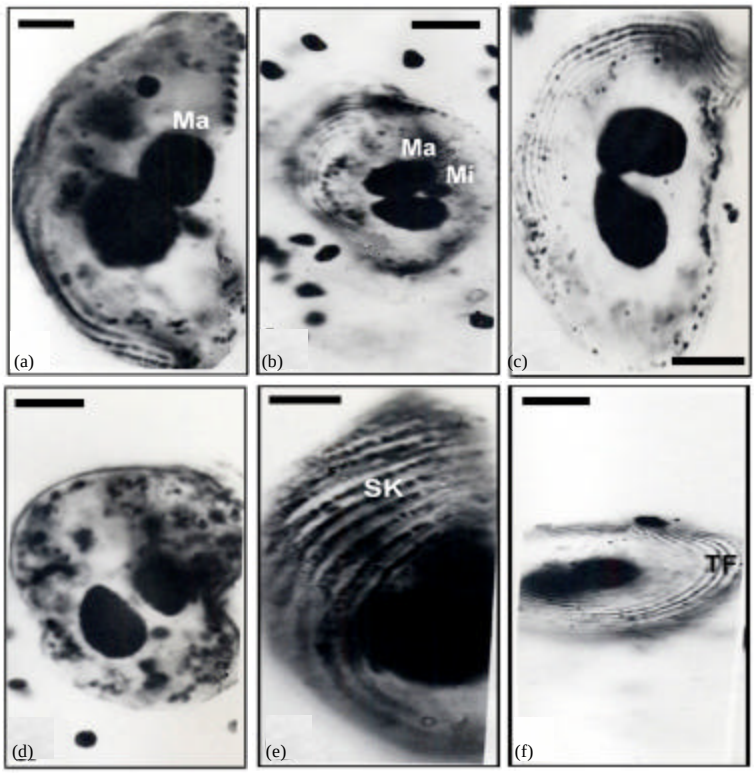

Fig. 1 (a-f): Different forms and developmental stages of *Phyllopharyngean americana*, (a) Elongated form (Scale bar = 5 μm), (b) Oval form (Scale bar = 20 μm) showing macronucleus (Ma) and micronucleus (Mi), (c) Early stage of morphogenesis (Scale bar = 5 μm), (d) Precystic stage (Scale bar = 5 μm), (e) *Phyllopharyngean americana* showing right somatic kineties (RSK) (Scale bar = 5 μm) and (f) Transverse fibers (TF) (Scale bar = 10 μm)

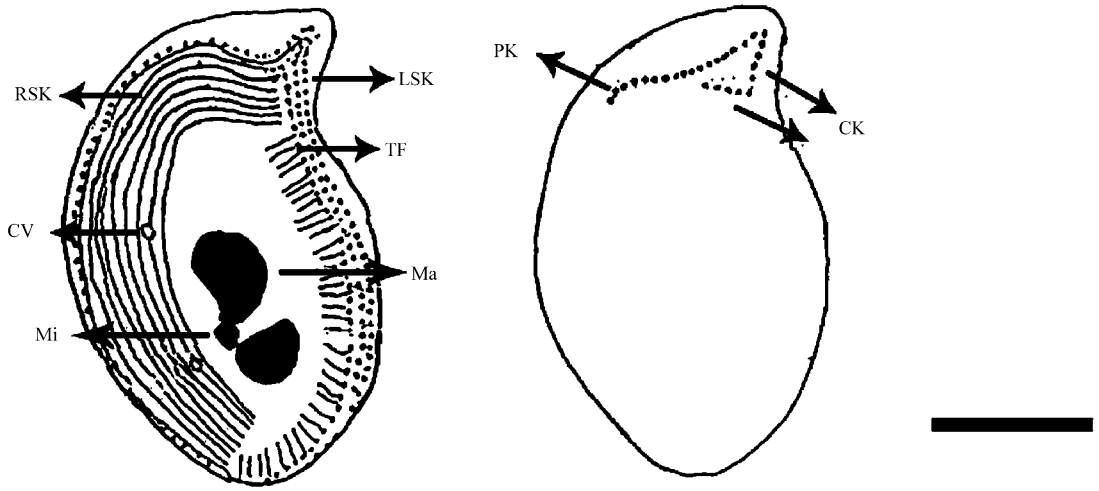

Fig. 2: Camera lucida-drawing of *Phyllopharyngean americana* showing RSK: Right Somatic Kinety, LSK: Left Somatic Kinety, TF: Transverse Fibers, CV: Contractile Vacuole, Ma: Macronucleus, Mi: Micronucleus, PK: Preoral Kinety and CK: Circumoral Kinety (Scale bar = 10 μm)

The ventral surface is concave and ciliated, while the dorsal one is much inflated over half its area from the posterior end and is non-ciliated. The cytopharynx is subcentral and tubular with conspicuous rods and two contractile vacuoles, one on each side of the cytopharynx (Lynn and Small, 2002). Also, genus *Trithigmostoma* (Jankowski 1967) exhibits some morphological variations. The ventral surface is flat with an arched dorsal surface, except for a flattened anterior region which carries a row of bristles. Cilia are often restricted to the ventral surface, consisting of many longitudinal kineties on the right side, most of which are curved to the anterior end of the body. An oval oral aperture which is supported by a protrusible basket of trichites. There is no central cilia-free zone, with several contractile vacuoles which are scattered throughout the entire body of the ciliate (Hofmann and Bardele, 1987; Bardele and Kurth, 2001). A third genus, *Chilodonatella* (Dragesco 1966), possesses different taxonomic features. The peculiar arrangement of somatic kineties is characteristic showing the right kinety field has 6-7 ciliary rows and the left field has only 5 rows. The four outer right kineties extend anteriorly to, more or less, communicating the outer 2-3 left kineties, thus, most of kineties are posteriorly located and giving a characteristic genus kinety arches. This pattern can be seen even in live specimens. A deep buccal cavity is also present (Colin, 1982; Becares and Foissner, 1994; Gong and Song, 2006). Moreover, genus *Pseudochilodonopsis* (Foissner, 1988) is differentiated from the present genus by being that the somatic cilia are restricted to the ventral surface. Left field of longitudinal kineties is separated from the right field by a large post-oral gap. There are 2 circumoral kineties close to the cytostome (Song, 1991).

Accordingly, a new genus with a single species is proposed to contain this ciliate with its distinctive morphology and the nomenclature *Phyllopharyngean americana* is suggested. The burrowing movement, the presence of intracellular cell debris and frequent cystic stages, appearing more often on the skin rather gills, indicate the parasitic nature of the ciliate on the gill tissues. In such situations, some degrees of damage may be observed (Lucky and Kral, 1982; Mitchell, 1984).

Regarding the type of fish examined, it has been found that striped bass was the most frequently infected with the ciliate. Moreover, adult fish were more frequently infected than young.

CONCLUSION

The present study reported a new genus and new species of the marine ciliate, *Phyllopharyngean americana*, in the skin and gills of striped bass and the gills of white perch fish collected from the Chesapeake bay, Maryland, USA. Detailed structure of the ciliate was described. Due to the expected parasitic residence of unknown ciliates in both marine and fresh water fishes, further studies, including morphological and molecular aspects, are needed.

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