Ultrastructural study of *Apiosoma piscicola* Blanchard, 1885 (Subclass: Peritrichia) on *Tachysurus fulvidraco* from China

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Abstract This paper gave detailed description of the fine structure of *Apiosoma piscicola* Blanchard 1885. These parasites were collected from the fry of *Tachysurus fulvidraco* during parasite surveys of fish at Jiangxia Fish Hatchery in Wuhan, Hubei province in May 2011. Its pellicle, peristomial apparatus, nuclei, trochal band and scopula were described, respectively. The relationship between these peritrich ciliates and their hosts was well discussed herein. The scopula of *A. piscicola* did not adhere directly to the surface of the host epithelial cells, but by means of a secreted adhesive pad of material according to the present study. Besides, no ultrastructural damage to host epithelial cells could be detected in our work. We concluded that these sessilids should be ectocommensals rather than ectoparasites, but not always harmless. In addition, their great variability of body shape and non-strict host specificity were also discussed.

Keywords *Apiosoma piscicola* · *Tachysurus fulvidraco* · Ultrastructure · Ectocommensal · Ectoparasite · Peritrich

Introduction

The *Apiosoma* spp. belong to the order Sessilida (Kahl 1933) in taxonomy that is characterized by possessing a scopula as the substrate-attaching organ. These peritrichs mainly live on the gills and body surface of aquatic organisms, especially the fry of freshwater fish. In fact, they are rather common members of the surface faunule of freshwater fish. In some case, they can badly endanger fish egg, fry and even adult fish and cause great losses to aquaculture (Li et al. 2008a, b). However, these special organisms were largely neglected over the past century according to great difficulties in studying-almost no reliable data can be available on the occurrence of their species (Lom 1973; Li et al. 2008a, b). And so far, they still remain groups of fish parasites that we should pay more attention to.

Apiosoma piscicola Blanchard, 1885 is a representative of the genus *Apiosoma*. It shows a quite wide limit of host variability and has a wide spread, such as Europe, Asia and South Africa (Li et al. 2007). And just for this reason, its great variability as a whole in shape and size and wide occurrence on various hosts in different localities result in many synonyms such as *Glossatella piscicola* (Blanchard 1885) Kahl (1935), *Glossatella cyprini* Šrámek-Hušek 1953, *Glossatella cylindriciformis* Chen (1955) and *Apiosoma magna* Banina (1968). Our former work (Li et al. 2008a, b) has given some suggestions on the taxonomic identification and put forward twelve morphometric parameters being used to describe these peritrichs in detail. Furthermore, we consider it an available mode of data measurements since these morphometric parameters are widely applicable to *Apiosoma* spp. However, the morphological data are still incomplete since no electron microscopy study is carried out so far. On the other hand, when it comes to the relationship between these peritrich

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ciliates and their hosts, two different opinions were expressed-harmless ectocommensals or harmful ectoparasites (Li et al. 2008a, b). The present study tries to examine the ultrastructural anatomy of *A. piscicola* aiming to contribute to the knowledge of this genus and to provide useful information for our understanding of the peritrich-host relationship at electronic microscopic level.

Materials and methods

During parasite surveys of fish at Jiangxia Fish Hatchery in Wuhan in May 2011, the fry of *Tachysurus fulvidraco* were found to be heavily invaded by *A. piscicola* in pond cultures. These fry were collected and transported alive to the laboratory, and kept temporarily in a 50-l water tank for further examination. Freshly killed fry were observed under a dissecting microscope Stemi SV6/AxioCam MRc5 (Zeiss, Germany). Their gills and fins occupied by numerous peritrichs were picked out for the preparation of electronic microscopic specimens.

For scanning electron microscopy (SEM), the specimens were washed twice with distilled water and then were fixed in 2.5 % Glutaraldehyde in 0.2 M phosphate buffered saline (PBS, pH 7.4). Then, the specimens were mounted on a clean glass slide (1 cm × 1 cm), previously treated with 0.1 % poly-L-lysine, and dried completely in air at room temperature (RT). After washed with PBS for three times, they were post-fixed in 1 % osmium tetroxide at 4 °C for 1 h, followed by serially dehydration in acetone and critical point drying using HCP-2 Critical Point Dryer. Then the glass slide was mounted on an aluminium-stub using a double-sided adhesive tape and sputter-coated with a thin layer gold in Eiko IB-3 Ion Coater before observing and photographing by using an Quanta 200 (FEI, Holand) SEM.

For transmission electron microscopy (TEM), the washed specimens were selected out and fixed directly in 2.5 % Glutaraldehyde in 0.2 M phosphate buffered saline (PBS, pH 7.4) for 2 h at 4 °C, then postfixed in 1 % (v/v) osmium tetroxide in PBS for 2 h at 4 °C, followed by serial dehydration in acetone and embedded in Araldite. Ultrathin sections were cut on a Leica Ultracut R ultramicrotome, stained with uranyl acetate and lead citrate before being observed in a JEM-1230 transmission electron microscope (JEOL, Japan).

Results

Populations of the fry of *T. fulvidraco* have suffered heavy mortality from Sessilinosis disease caused by *A. piscicola*, large numbers of which can be observed on the body surface, especially on the fins and mucus-covered gills with the aid of a dissecting microscope.

When expanded, the ciliate is somewhat cup-like in shape, with its peristome disc surrounded by thick adoral cilia. The peristomial lip is slightly vaulted, and is elevated some 5 µm above the infundibulum (Figs. 1a, 2a, b). Annular striations can be observed clearly in pellicle with numerous tiny particles dispersed over the body surface, both of which are characterised by argentophilic reaction and so called argentophilic annuli and particles (Fig. 1a). The peristomial lip is so contractile that it can cover the adoral cilia and peristomial disc completely and draw them inside the body (Figs. 1b, 3a).

As to the fine structures of the pellicle, two different types of arching mode are present herein. Type I exists in the oral part of the body-peristomial lip exactly. In this part, the cell membrane covers the peristomial lip with the outer and inner alveolar membranes located below and circumscribing the pellicular alveoli. Layer of microtubules with diameter of about 24 nm lies just beneath the inner alveolar membrane and parallels the epiplasm membranes (Fig. 3c). Thus, type I pellicle comprises these forementioned structures, with well-developed pellicular alveoli alternating with crest-like elevations of epiplasm. On the other hand, type II is present in the posteriormost of the body, where the inflated pellicular alveoli are actually absent (Fig. 3d).

Thick rows of adoral cilia are parallel to each other and surround the peristome disc, forming a distinct spiral and extending into the infundibulum (Fig. 2b–d). The macronucleus is triangular-shaped and situated at the back of the body (Fig. 2e). Its pointed end extends directly to the posterior end while the other end lies just above the level of the trochal band (Fig. 2f). And some ciliary organelles are embedded at this ring-like depression (Fig. 2f). The macronucleus is heteromorous with a prominent nucleolus near in the centre and heterochromatin occupying the karyoplasm (Fig. 2e).

The scopula is the holdfast organelle with its borders curved and exceeding the aboral end. Contrary to light microscope observations, the scopula does not adhere directly to the surface of the host epithelial cells. There is a pad of fine microfibrillar matrix beneath the scopular. Scopular cilia of about 1.2 µm in length are embedded in it (Fig. 3b).

In addition, the foreside of cytoplasm is abundant in bacteria and food particles, especially around the peristomial disc (Fig. 3e, f). Other cytoplasmic organelles such as mitochondria, endoplasmic reticulum, Golgi complex and etc. are typically ciliophoran that will not be described here.

Discussion

Two different opinions were expressed concerning the relationship between *Apiosoma* ciliates and their fish hosts-harmless ectocommensals or harmful ectoparasites. Schäperclaus (1954) once described a massive occurrence

Fig. 1 SEM images of *A. piscicola*. **a** Lateral view of the ciliate, showing its fully-expanded peristome disc surrounded by thick adoral cilia and annular striations (arrow) as well as small particles (arrowhead) over the pellicle. Scale bar 5 µm. **b** Frontal view of

the ciliate, showing the contraction form of *A. piscicola* with its peristomial lip completely covering the adoral cilia and peristomial disc. Scale bar 5 µm

of *Apiosoma* on carps and supposed these sessilids to be harmless ectocommensals since he could not find any evidence of necrosis or penetration of the host cells. Then, Fijan (1962) recorded losses on two-months old carp fry caused by massive epifaunation by *Apiosoma* (evidently *A. piscicola* according to his microphotographs) and defined these ciliates as genuine ectoparasites. Being unable to detect any pathological changes on the gills or skin, he ascribed the heavy mortality to suffocation. Banina (1968) also recorded an epizooty in young fish caused by *Apiosoma* ciliates and generalized in his brief review (1969) on mass development of *Apiosoma* as having disastrous effects on fish fry. Lom (1973) and Cone and Odense (1987) studied ultrastructural features of scopular attachment in TEM and SEM preparations of *A. piscicola*, indicating that no injury could be detected in host cells and so suggesting that these organism were ectocommensals.

According to our present study, the scopula of *A. piscicola* did not adhere directly to the surface of the host epithelial cells, but by means of a secreted adhesive pad of material. This fact was contrary to our former observations that the holdfast organelle with its curved borders firmly attached to the host surface at light microscopic level (Li et al. 2008a, b). Moreover, the results were concordant with those of aforementioned literatures since no ultrastructural damage to host epithelial cells due to *A. piscicola* could be detected even in intense infections in our work. On the other hand, the foreside of cytoplasm of *A. piscicola* specimens was abundant in bacteria and food particles, especially around the peristomial disc and cilia. In addition, their oral ends always turned towards the surrounding water, not hiding between the gill filaments or skin interspaces. Thus, these sessiline peritrichs should be host-independent in their food requirements, feeding on bacteria and particles dispersed in the water. They just use the host's surface

as an ecological niche but not a source of nutrition. Therefore, we prefer to conclude that *Apiosoma* ciliates are ectocommensals rather than ectoparasites. As to their harmful effects on host fish, we suspected that it depends on two factors, the water environment and the host itself. When the host is in good health, *Apiosoma* sessilids can be well kept under control and have little or no influence on the fish body. Otherwise, populations of these ciliates start flourishing on the surface of the fish, especially as the water environment and the host are both in poor conditions. In this case, the heavy invasion will cause great discomfort as well as excessively secreted mucus covering the gills and fish body. And this inevitably prevents the fish from normal breathing, feeding and moving. That is the reason that we always see the ill fish fry losing their balance, swimming slowly and gasping at the surface of the water and finally suffering heavy mortality due to lack of oxygen and food for a long time. So, these sessilids should be ectocommensals, but not always harmless. They can badly endanger the fish host indeed in particular case that we mentioned above.

As to the fine structures of the pellicle, the arching mode of the oral part was distinguished from that of the aboral end in our work since pellicular alveoli were actually absent in the latter. There is also a fact that only the oral part of the body is contractile, being capable of drawing the peristomial apparatus completely inside the body (Lom 1966; Li et al. 2008a, b). In our opinion, the reason for this phenomenon may be relative to the forementioned difference between the pellicular components of the two parts. It also gives us hint that the flexibility of the pellicle should be responsible for the great variability of *Apiosoma* ciliates as a whole in shape and size. On the other hand, kinetosomes of the cilia embedded at the transverse ciliary depression were observed in the present study. The

Fig. 2 TEM images of *A. piscicola*. **a** Longitudinal section of *A. piscicola* as a whole. Scale bar 10 μ m. **b** Cross section of the infundibulum, showing the numerous cilia existed in the inner membrane. Scale bar 2.5 μ m. **c** Longitudinal section of the cilia, showing the kinetosome (ks) embedded in the infundibulum. Scale bar 2 μ m. **d** Cross section of the cilia, showing the structure of kinetosome. Scale bar 0.5 μ m. **e** Longitudinal section of the macronucleus (ma), showing the nucleolus (nc) and heterochromatin (hc). Scale bar 5 μ m. **f** Cross section of the middle part, showing the macronucleus (ma) and trochal band (tcb). Scale bar 5 μ m

existence of these ciliary organelles in trophic *Apiosoma* can well account for the formation of a distinct wreath of locomotor cilia (generally named Pectinells) at the girdle of pectinells in its telotrochs (swarmers).

As a kind of widespread ectocommensals, *A. piscicola* shows a quite wide limit of host variability. However, to the best of our knowledge, the majority of the recorded host fish belong to the family Cyprinidae. It is reasonable

to suppose that *A. piscicola* is still host-specific to some extent and the surface properties of the cyprinids provide a more suitable condition than those of other hosts for *A. piscicola* to live on. In fact, these *Apiosoma* ciliates are quite unique and interesting organisms: they are host-independent in food requirements, but they are strongly influenced by physiological changes of the host; they do not cause pathological damage to host cells, but they can

Fig. 3 TEM images of *A. piscicola*. **a** Longitudinal section of the peristomial lip, showing its great flexibility to draw the adoral cilia and peristomial disc completely inside the body. Scale bar 2 μ m. **b** Longitudinal section of the scopula, showing the secreted pad of fine microfibrillar matrix (*mp*) with scopular cilia (arrow) embedded in it. Scale bar 2 μ m. **c, d** Images to show two different types of arching mode of the pellicle. Type I exists in the oral part of the body-peristomial lip exactly (**c**). The cell membrane covers the peristomial lip with the outer (*om*) and inner alveolar membranes (*im*) located below and circumscribing the pellicular alveoli (*pa*). Layer of microtubules (*ml*) lies just beneath the inner alveolar membrane and parallels the epiplasm membranes (*em*). Type II is present in the posteriormost of the body (**d**), where the inflated pellicular alveoli are actually absent. Scale bar in **c** and **d** 1 μ m. **e** Cross section of the oral part, showing the numerous bacteria (*ba*) that were filter-fed by the organism. Scale bar 2 μ m. **f** Image to show the bacteria (*ba*) and food particles (*fp*) within the body. Scale bar 0.5 μ m

lead to heavy mortality to young fish in some case; they attach to the surface of the host during most of their lifetime, but they transform into telotrochs for migration. These facts yield an interesting case from the viewpoint of the ciliate-host relations. And we hope that more and more protozoologists and parasitologists can pay their attention to these special organisms.

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References

- Banina NN (1968) *Apiosoma* from fresh-water fishes in the European part of the Soviet-Union. Acta Protozool VI:245–262
- Banina NN (1969) Ciliates of the *Apiosoma* genus. In: Progress in protozoology. abstracts of papers read at the 3rd international congress protozoology Nauka, Leningrad, pp 252–253

- Chen CL (1955) The protozoan parasites from four species of Chinese pond fishes: *Ctenopharyngodon idellus*, *Mylopharyngodon piceus*, *Aristichthys nobilis* and *Hypophthalmichthys molitrix*. I. The protozoan parasites of *Ctenopharyngodon idellus*. Acta Hydrobiol Sin 1:148–150
- Cone DK, Odense PH (1987) Occurrence of *Heteropolaria lwoffi* (Fauré-Fremiet, 1943) and *Apiosoma piscicola* Blanchard, 1885 (Ciliata) on *Salvelinus fontinalis* (Mitchill) in Nova Scotia, Canada. Can J Zool 65:2426–2429
- Fijan N (1962) Massive invasions of the young *Cyprinus Carpio* with protozoa of the genus *Glossatella*. Veterinarski Archiv 32:2–6
- Kahl A (1933) Ciliata libera et ectocommensalia. In: Grimpe G, Wagler E (eds) Die Tierwelt der Nord-und Ostsee. Lief. 23 (Teil II, C3), Leipzig, pp 29–146
- Kahl A (1935) Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria). 4: Peritricha und Chonotricha. Nachtrag. In: Dahl F (ed) Die Tierwelt Deutschlands. Gustav Fischer, Jena, pp 806–842
- Li M, Wang JG, Zhang JY, Zhu DL, Gu ZM, Gong XN (2007) Observation on the *Apiosoma piscicola* under optical and transmission electron microscope. Acta Hydrobiol Sin 31:208–213
- Li M, Wang JG, Gu ZM, Zhu DL, Zhang JY, Gong XY (2008a) Progress of genus *Apiosoma*-a group of epizootic protozoa. Acta Hydrobiol Sin 31:1–5
- Li M, Wang JG, Zhu DL, Gu ZM, Zhang JY, Gong XY (2008b) Study of *Apiosoma piscicola* (Blanchard 1885) occurring on fry of freshwater fishes in Hongze, China with consideration of the genus *Apiosoma*. Parasitol Res 102:931–937
- Lom J (1966) Sessiline peritrichs from the surface of some freshwater fishes. Folia Parasitol (Praha) 18:36–56
- Lom J (1973) The mode of attachment and relation to the host in *Apiosoma piscicola* Blanchard and *Epistylis lwoffi* Fauré-Fremiet, ectocommensals of freshwater fish. Folia Parasitol (Praha) 20:105–112
- Schäperclaus W (1954) Fischkrankheiten. Akademie Verlag Press, Berlin
- Šrámek-Hušek R (1953) Dva nové nálevníci z kůže kapra. Rozpravy ČSAV, řada MPV 63:37–41