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Research Article

A preliminary observation of a *Trichodina* sp. (Ciliophora: Peritricha) on the skin of *Elysia singaporensis* (Sacoglossa, Plakobranchidae)

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Abstract

The parasitic protozoa are a serious problem for aquatic animals both of natural and cultured condition. Therefore, the health checked investigation is necessitated. During the parasitological survey of sea slug species from Kranji mangrove forest of Singapore and tidal area of the mangrove forest in Bangtawa of Southern Thailand. Three species of sea slug (*Elysia singaporensis*, *E. bangtawaensis* and *E. leucolegnote*) were examined. A ciliate protozoan with flat disc shaped, 2 ciliary rows, denticulate ring presence. According to these characters, it was identifiled as *Trichodina* sp. This present study provides the first record of *Trichodina* sp. on the sea slug (*E. singaporensis*) host from Singapore.

Keywords: *Elysia singaporensis*, sea slug, SEM study, *Trichodina*

Introduction

Members of the genus *Trichodina* Ehrenberg, 1831, are ciliated protozoan ectoparasites classified in the phylum Protozoa, subclass Peritricha, order Peritrichida, suborder Mobilina, family Urceolariidae (after Kudo, 1977). They are regarded as an important biological indicator, whereby its density and prevalence can be used to show (water) pollution (Palm & Dobberstein, 1999).

Trichodinids are serious problem on fish because the sharp of their adhesive disc structure can lead to epithelial damage (Lom & Dykova, 1992). *Trichodina* can irritate the epithelial layer of cells in skin and gills of fish (Smith & Schwarz, 2009), and various species of *Trichodina* have been reported on gills of fish, such as *Trichodina tenuiformis* Stein, 1979 on *Cottus bairdi* (Girard, 1850) *Trichodina* sp. on *Oreochromis niloticus* (Linnaeus, 1758) and *O. aureus* (Steindachner, 1864), *T. cancilae* Asmat, 2001 on *Xenentodon cancila* (Hamilton, 1822), *T. gulshae* Asmat et al., 2003 on *Mystus cavasisus* (Hamilton, 1822), *T. domergue* Wallengren, 1897 on *Neogobius melanostomus* Pallas 1814, and *T. gobii* Raabe, 1959 on *Solea aegyptiaca* Chabanaud, 1927 (see Qi & Heckmann, 1995; Hassan, 1999; Asmat, 2001; Asmat et al., 2003; Özer, 2003; Yemmem et al., 2010).

Besides fish, *Trichodina* has also been reported from invertebrate hosts. *Trichodina* was found on the exoskeleton of the blue crab *Callinectes amnicola* Rochebrune, 1883 (see Ekanem et al., 2013), and on gills of gastropods (e.g., *Patella* spp.) and bivalves (e.g., *Mya*

arenaria, Linnaeus, 1758) (see Uzmann & Stickney, 1954; Aksit et al., 2008). For sea slugs, there have been numerous reports of parasites such as copepods (see Jensen, 1987, 1990; Schrödl, 1997, 2002; Abad et al., 2011; Uyeno & Nagasawa, 2012), Turbellaria flatworms (Sudo et al., 2011) and sea spiders (Arnaud, 1978), but none of *Trichodina* thus far.

Therefore, the researchers chose to observe the presence of *Trichodina* on sea slug as it has a short lifespan parasite and can be found in the general aquatic animals. In addition, it also benefit for predicting the collection area environment. For this study three species of sea slugs, *Elysia bangtawaensis*, *E. leucolegnote*, and *E. singaporensis* were examined for the presence of *Trichodina*.

Materials and methods

Ten specimens of each species were gathered as *Elysia bangtawaensis* and *E. leucolegnote* were collected with forceps from a waterway in the tidal area of a mangrove forest around the Pattani Bay, Thailand (06^0 53' 139" N, 101^0 16' 444" E). While, *E. singaporensis* was collected with forceps from Kranji mangrove forest, north region of Singapore (1^o 26' 47" N, 103^o 43' 57" E). (Figure 1) and kept in a bottle to be studied at the Zoology Lab of the Biology Division of the Department of Science, Faculty of Science and Technology, Prince of Songkla University, Pattani Campus. The specimens were narcotized in 7% $MgCl_2$ for 1 hour. They were preserved in seawater with 10% formalin before being transferred to a Petri dish with black wax layer. Fine needles and spines of cactus were used for fixing them under a stereo microscope. Next, *Trichodina* were observed from three *Elysia* under a stereo microscope and the *Trichodina* were only found on the skin of *E. singaporensis*. Ten trichodinids were transferred onto a slide for morphological study under an Olympus light microscope (BX 51), and photographs taken with attached Moticam 2000. As all measurements of *Trichodina* sp. were conducted according to the suggestion of Yemmen et al. (2011).

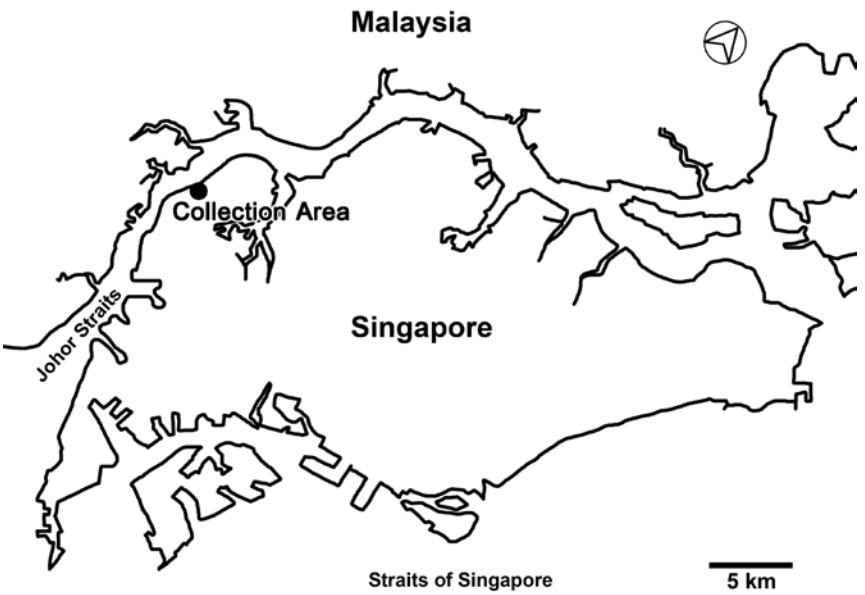

Figure 1. Study site in Singapore. Scale bars= 5 km (Line drawing by: Somsak Buatip)

For the electron microscope scanning, the skin of *E. singaporensis* was examined and soaked in 2.5% glutaraldehyde in 0.1 M Phosphate buffer saline (PBS), pH 7.8 at 4 ^{0}C for 4 hours before being fixed with 1% osmium tetroxide in 0.1M PBS, pH 7.4 at 4 ^{0}C for 2 hours. It was then washed off with 0.1M PBS pH 7.4 at 4 ^{0}C 3 times, each 5 mins., and dehydrated with ethyl alcohol of 70%, 80%, 90%, 95% (twice) and 100% (twice) for 30 mins., respectively. Next, it was dehydrated with the Critical-Point-Drying machine. A sample was put on a stub and coated with heavy metal before being put under an LEO scanning electron microscope (series 1450VP) and photographed.

Results

In this study, *Trichodina* was found on *Elysia singaporensis* only. In which, it confirmed from light microscopic and scanning electron microscopic analysis. The present study is the first to report on trichodinid existence on the skin of sea slug, especially in Genus *Elysia* as *E. singaporensis*. Detailed microscopic morphology of *Trichodina* is figured.

Electron microscope scanning clearly shows *Trichodina* attached to the epithelial layer of cells on the body of *E. singaporensis* on dorsal side, covering the head, pericardium and parapodium, as well as ventral side on the skin of its body (Figure 2). Interestingly, this protist was found on almost the whole body of the sea slug except for the sole. This fact that this body part is used for locomotion may explain the absence of the protist on this particular spot. Additionally, SEM analysis also shows the button-shape cell body of *Trichodina*. The picture reveals only the structure of cilia which is called locomotor ciliary wreath as a part of aboral ciliary complex (basal ciliary ring, locomotor ciliary wreath and marginal ciliary ring). The picture of *Trichodina* also shows the velum, which is a thick structure covering the base of the cilia of the aboral ciliary complex and separating the aboral ciliary complex from adoral ciliary spiral (Figure 3).

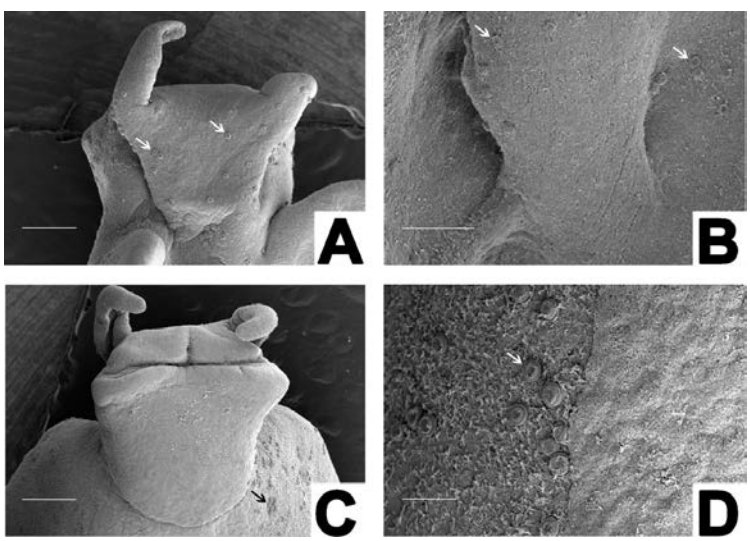

Figure 2. *Trichodina* on *Elysia singaporensis* (a few of them indicated with arrows). A, on dorsal side of head; B, on dorsal side of pericardium; C, on ventral side of its body; D, on dorsal side of parapodium. Scale bars= 400 µm [A-B], 100 µm [C], 200 µm [D] (Photographs by: Pattanasuda Sirinupong and Somsak Buatip [A-D])

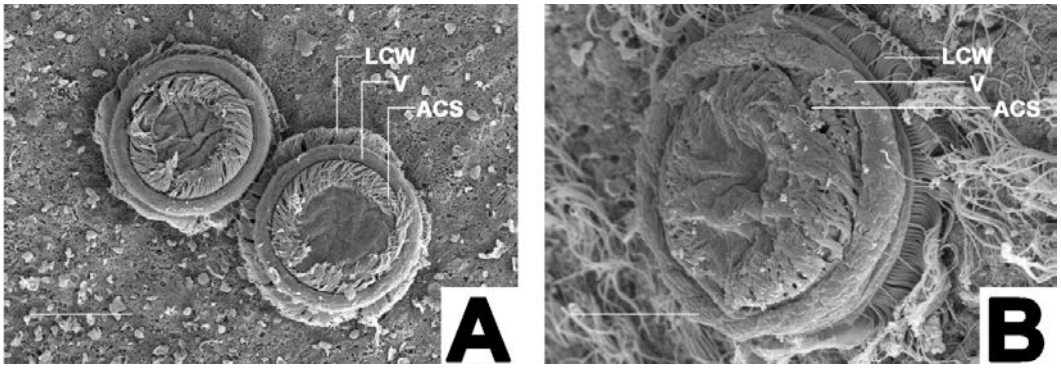

Figure 3. Scanning electron micrograph (SEM) imaging showing enlarged image of *Trichodina* on the skin of *Elysia singaporensis*. A, oral view; B, lateral view, Scale bars= 20 µm [A], 10 µm [B]. Abbreviations: ACS: adoral ciliary spiral; LCW: locomotor ciliary wreath; V: velum. (Photographs by: Pattanasuda Sirinupong and Somsak Buatip [A-B])

Light microscope shows that ten specimens of *Trichodina* between 47.73-58.76 µm (db), and the adhesive disc concave (da) was 38.24-47.73 µm in diameter. The denticulate ring was 33.38-33.62 µm in diameter (ddr) while the average denticle length were between 5.88-6.53 µm (dl). The average central circle diameter was between 17.54-18.40 µm (dcc). The central circle was dotted with small, irregularly rounded particles (Figure 4).

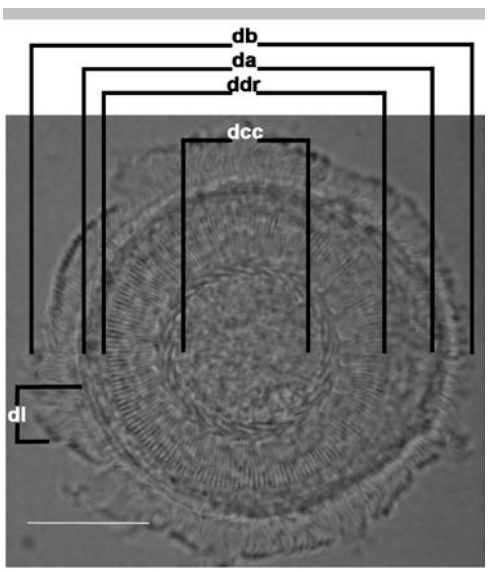

Figure 4. Photomicrograph of adhesive disc of *Trichodina* sp. from the skin of *Elysia singaporensis*. Scale bar= 15 µm, Abbreviations: da: diameter of the adhesive disc; db: diameter of body; dcc: central circle diameter; ddr: diameter of denticle ring; dl: denticle length (Photograph by: Pattanasuda Sirinupong and Somsak Buatip)

Discussion

The presence of *Trichodina* on *Elysia singaporensis* is presumed to be due to the fact that the area is suitable for their growth because the area is an abandoned shrimp pond (Swennen, 2011) or prawn aquaculture site before being declared a protected nature reserve (Cuong et al., 2005). Also, this mangrove forest is surrounded by industrial sites and residences, and it is likely that pollution from these communities has an impact on the water quality around this mangrove patch. This assumption is in accordance with the study of Cuong et al. (2005) who studied heavy metal contamination in Sungei Buloh Mangrove of Singapore. Their association of the discovery of such heavy metals as As, Cd, Cr, Cu, Ni, Pb, and Zn with the effect on the sea slug in the area agrees with the report of Mackenzie (2008) that this parasite could be an indicator of pollution in the marine environment. Khan et al. (1994) also stated that *Trichodina* might serve as an indicator of pollution, and reported that the increase of infection rate of *Trichodina* spp. In *Myoxocephalus* spp. is proportionate to crude oil, pulp, and paper mill effluent. Khan & Thulin (1991) revealed in their study of ectoparasite of aquatic animals, especially of fish, that numbers of ectoparasites such as trichodinid ciliates and monogeneans increased significantly on the gills following exposure to a polluted environments.

Trichodina is an ectoparasite which temporarily attaches to its host, and our examination under the Scanning Electron Microscope revealed that the parasite is quite abundant on the dorsal side of its host (head, pericardium, and parapodium) as well as the ventral side or the skin of its body, except for the foot area. Whereas, in other gastropods (e.g., *Patella* spp.), *Trichodina* was only observed in its gill area (see Aksit et al., 2008). The density of *Trichodina* on the host may irritate the skin and, consequently, injure the host. Lom (1973), who studied *Trichodinella epizootica* (Raabe, 1950) on fish reported high densities of the parasites' cilia into the gill surfaces and injured the fish. However, in this study, we did not found *Trichodina* on the skin of two *Elysia* that collected from the mangrove forest of Thailand. It is possible that the study area as found *E. bangtawaensis* and *E. leucolegnote* is situated far away from the industrial sites or pollution source. Therefore, it may be not suitable for the survival of *Trichodina*. Also, in accordance with the previous study of Lom & Dykova (1992) who revealed that the ciliated protozoa like to live in greatly polluted water.

Conclusion

The present study is possibly the first report of the discovery of *Trichodina* on a sea slug, especially, on *Elysia singaporensis* collected from Kranji mangrove forest, north region of Singapore in Singapore. Because *E. singaporensis* is a very small species (around 21-30 mm), it is difficult to notify if its skin has been damaged. Further studies are needed to provide more information on the possible detrimental effects of *Trichodina* on sea slugs.

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