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# Ultrastructure of Gametocyst of Parasitic Protozoan, *Nematopsis* sp. in Black Tiger Shrimp *Penaeus monodon* from the Gulf of Thailand

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**Abstract:** Ultrastructure of gametocyst of *Nematopsis* sp., a protozoa parasite of black tiger shrimp *Penaeus monodon* from the Gulf of Thailand is described. Ball-shaped gametocysts of about 110–160 μm diameter were found in close contact with the intestinal wall of shrimps. Surface of the gametocyst cyst wall or capsule is wrinkled with a circular bare area at one pole that contains a central pore 4–5 μm in diameter. The interior of the gametocyst is composed of numerous gymnospores and membranous sacs. Gymnospores varied in size with an average diameter of 6–8 μm. Ball-shaped gymnospores were composed of numerous, radially arranged, cone shaped sporozoites. Average width and length of sporozoites were 0.8–1.2 μm and 3–5 μm, respectively, with their rostral part pointing outward and caudal part, inward connecting to the residual cytoplasm in the centre of a gymospore. The rostral part of the sporozoite contains an oval nucleus, rough endoplasmic reticulum, mitochondria and a group of secretory granules. Membranous sacs were composed of two types of globular granules; large electron lucid granules and small dense granules.

**Key words:** *Nematopsis*, Protozoa, parasite, gametocyst, gymnospore, ultrastructure.

## INTRODUCTION

Eugregarine protozoans of the genus *Nematopsis* were identified first by Schneider (1892). More recently, marine bivalves and some gastropods have been recognized as intermediate hosts for these parasitic protozoans (Lauckner 1983) and decapods crustaceans as their definitive hosts (Prema and Janardanan 1990; Prasadan and Janardanan 1996, 2001; Shavanas *et al*. 1999; Lee *et al*. 2000; Jimenez *et al*. 2002). *Nematopsis* is widely distributed and occurs in wild and cultured penaeoid shrimp (Feigenbaum 1975, Jimenez 2002). The life cycle of this parasite has been the subject of several studies (see reviews by Prytherch 1940; Sprague 1970; Lauckner 1983; Azevedo and Cachola 1992; Bower *et al*. 1992; Belofastova 1996; Canestri-Trotti *et al*. 1998, 2000; Azevedo and Matos 1999; Carbellal *et al*. 2001; Padovan *et al*. 2003; Tuntiwaranuruk *et al*. 2004, 2008). Gametes (gametogony and sporogony) develop in bivalves (Prytherch 1940, Sprague and Orr 1955) and the vegetative gametocysts and gymnospores

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in shrimp and crabs (Prema and Janardanan 1990; Prasadan and Janardanan 1996, 2001; Shavanas *et al*. 1999; Jimenez *et al*. 2002).

The present study describes the morphology and ultrastructure of *Nematopsis* sp. gametocysts and gymnospores found in black tiger shrimp, *Penaeus monodon*, indigenous to the coast of the Gulf of Thailand. Ultrastucture is based on observations from scanning and transmission electron microscopy.

## MATERIALS AND METHODS

Live mature black tiger shrimps (n = 5) captured along the coast of the Gulf of Thailand (Chonburi province) were purchased from local fishermen and kept on ice for approximately a half hour when their digestive tracts were removed by dissection. Dark brown gametocysts (n = 15) of *Nematopsis* sp. were removed from the rectal wall with a small paint brush under a stereo microscope, fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer saline, pH 7.8, (PBS) at 4°C overnight, washed and stored in PBS at 4°C.

In preparation for scanning electron microscopy, fixed gametocysts (n = 5) were immersed in 1% osmium tetroxide in PBS for 2 hours at 4°C, dehydrated through a graded concentration series of ethanol, critical point dried and gold ion sputtering coated. Observations were made from a SEM (LEO, 1450vp), with a secondary electron detector. Remaining gametocytes (n = 10) were prepared for transmission electron microscopy (TEM). They were secondarily fixed in osmium tetroxide in PBS for 2 hours at 4°C, washed several times with 0.1 M PBS pH 7.4, dehydrated through a graded concentration series of ethanol. After that dehydrated samples were immersed twice with propylene oxide, each for 30 min and infiltrated with mixtures of propylene oxide and Araldite 502 resin at the ratios of 2:1 and 1:2, for 1 h and overnight, respectively. Thereafter, specimens were embedded in pure resin and polymerized in a hot air oven at 45 and 60°C, each for 2 days. Treated specimen blocks were sectioned at 500–700 nm (semithin) and 60–90 nm (ultrathin). Semithin sections were stained with 1% methylene blue or PAS, and observed under light microscopy. Ultrathin sections were stained with uranyl acetate saturated in 70% methanol and 0.1% aqueous lead citrate and examined by TEM at 80 kV (FEI, Tecnai 20).

## RESULT

*Nematopsis* gametocysts from the intestinal wall of black tiger shrimp were spherical in shape, 110–120 μm in diameter (n = 5) and covered by a thin cyst wall or capsule. Gametocyst surfaces were wrinkled with a circular bare area at one pole that contained a gametopore, 4–5 μm in diameter (n = 3; Figs 1–2). Each *Nematopsis* gametocyst contained numerous gymnospores and membranous sacs, each covered by a thin membrane (Figs 3–7). Gymnospores were spherical in shape, ranging from 6–8 μm in diameter (n = 10) and composed of numerous, radially arranged cone-shaped sporozoites, positioned with their rostral and caudal portions facing out- and inwardly, respectively (Figs 8–9). Widths and lengths of sporozites, measured under transmission electron microscopy, ranged from 0.8–1.2 and 3–5 μm, respectively (n = 10). An oval nucleus with moderate heterochromatin was present in the central part of the cytoplasm of each sporozoite. The rostral part of sporozites contained mitochondria, rough endoplasmic reticulum and a group of secretory vesicles, whereas the caudal part that connects with a central cytophore, contained only endoplasmic reticulum (Figs 10–11).

SEM images showed that membranous sacs in gametocysts had an average diameter of about 2 μm (n = 10), and contained numerous globular bodies with an average diameter of 0.7–0.8 μm (n = 10; Figs 6, 12). Internal structures of the membranous sacs examined by transmission electron microscopy indicated small amounts of cytoplasm and two types of granular structures, including large central-electron lucid granules, equivalent to the globular bodies found by SEM and similar to those of amylopectin granules and of the small electron dense granules (Fig. 12). Globular bodies, examined by light microscopy, stained positively with PAS.

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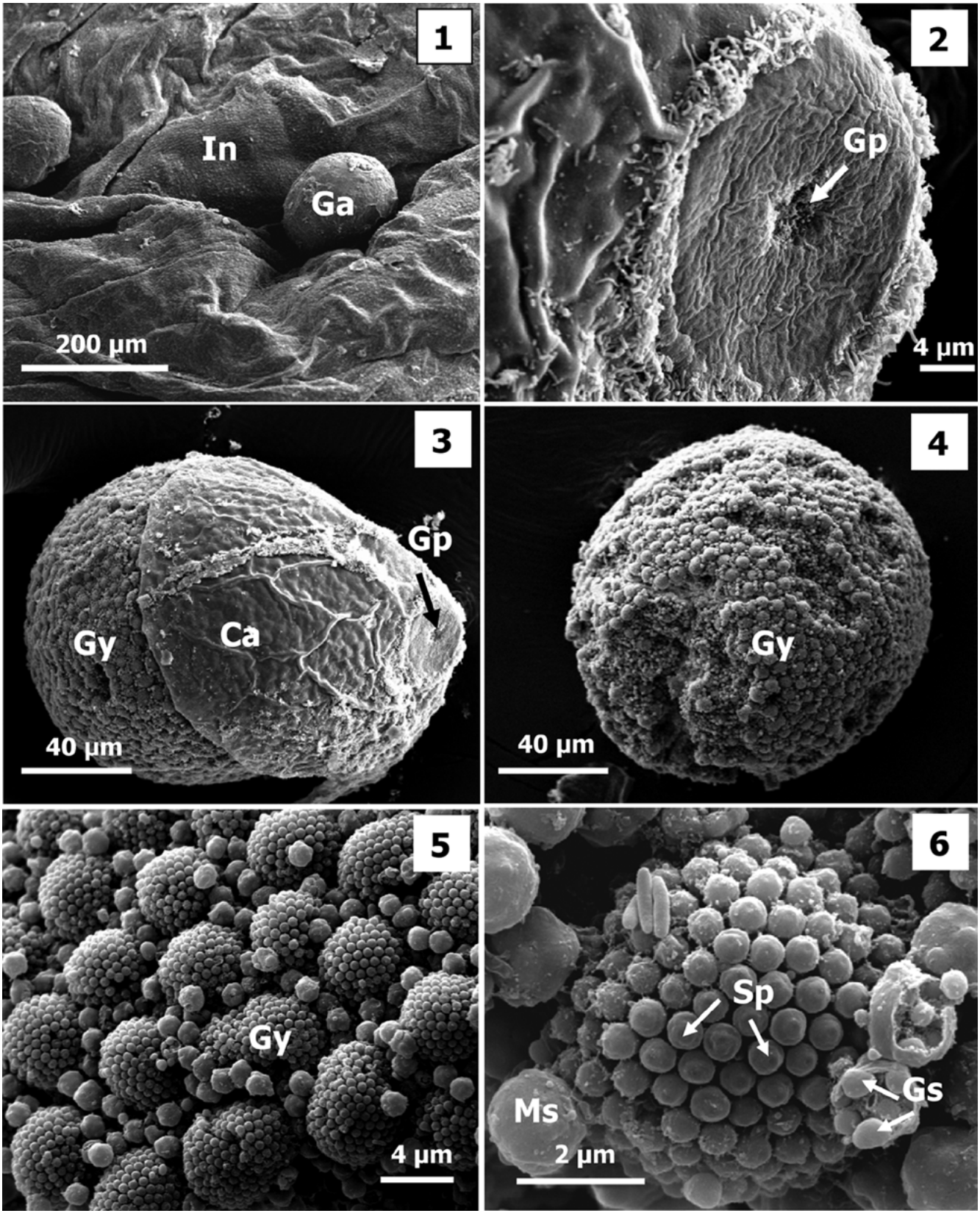
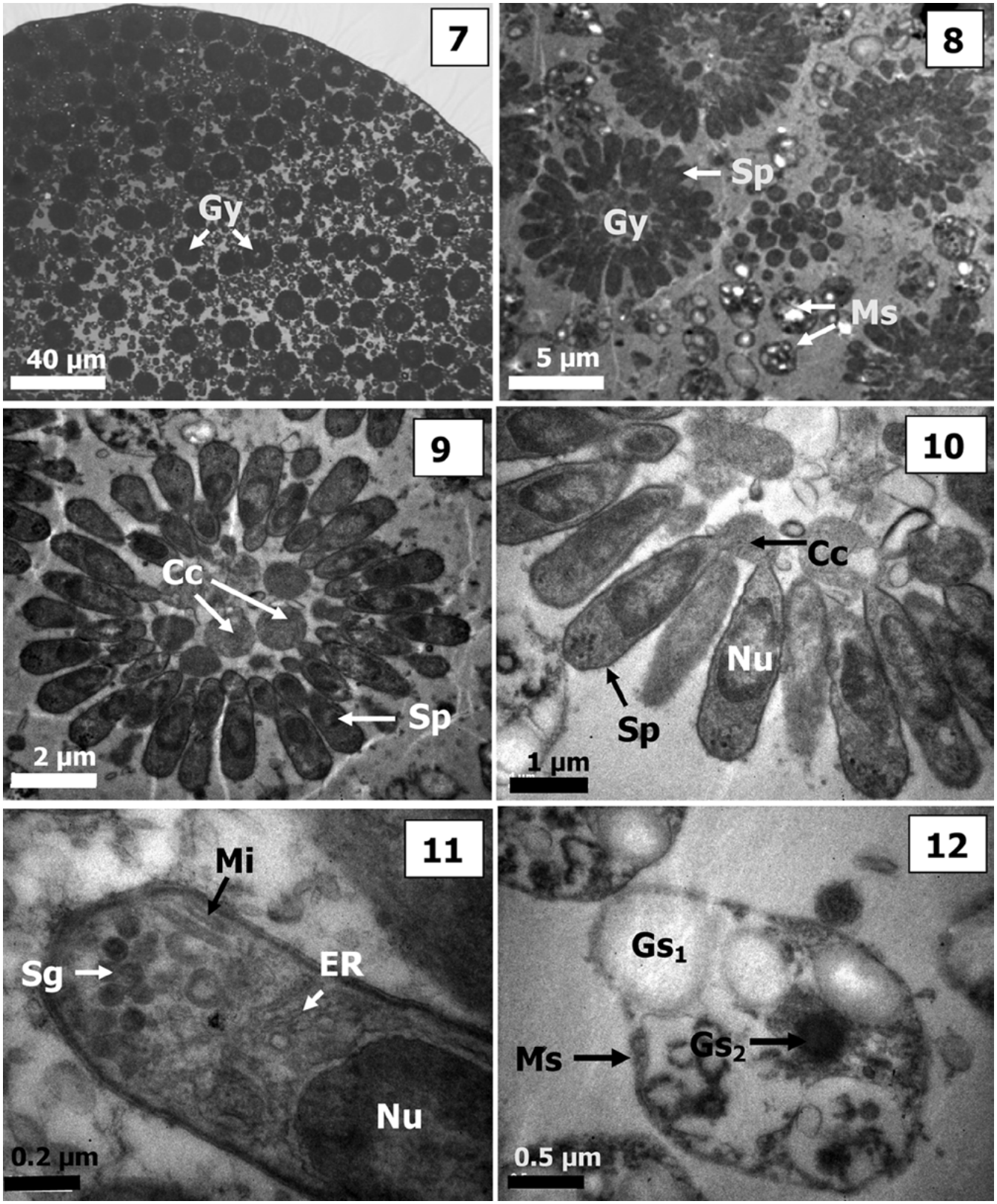
**Figs 1–6.** Scanning electron micrographs (SEM) of *Nematopsis* sp. gametocysts removed from the intestine of black tiger shrimp, *Penaeus monodon*. **1** – gametocyst (Ga) of *Nematopsis* sp. in close contact with the intestinal wall (In) of a shrimp; **2** – a low SEM micrograph of a gametocyst of *Nematopsis* sp. showing the external surface and gametopore (Gp) surrounded by a bare area; **3–4** – low magnification SEM micrographs of ruptured (figure 3) and capsule free (figure 4) gametocysts of *Nematopsis* sp. illustrating the external surfaces of the cyst wall or capsule (Ca), and numerous gymnospores (Gy) within the gametocysts interior; **5–6** – low and high magnification SEM micrographs of the interior of a gametocyst of *Nematopsis* sp. showing numerous gymnospores (Gy) composed of many sporoziotes (Sp) and membraneous sacs (Ms), the latter containing several globular structures (Gs).


1
In
Ga
200 µm
2
Gp
4 µm
3
Gp
Gy
Ca
40 µm
4
Gy
40 µm
5
Gy
4 µm
6
Sp
Gs
Ms
2 µm


7
Gy
40 µm
8
Sp
Gy
Ms
5 µm
9
Cc
Sp
2 µm
10
Cc
Nu
Sp
1 µm
11
Mi
Sg
ER
Nu
0.2 µm
12
Gs1
Ms
Gs2
0.5 µm

## DISCUSSION

Ultrastructure of gametocysts and gymnospores described in the present comunication corresponds to morphological characteristics of life cycle stages of organisms from the eugregarines genus, *Nematopsis*. Organisms in this genus were described as having spherical gametocysts with a thin cyst wall or capsule (Prasadan and Janardanan 2001, Jimenez 2002). Previous descriptions (Jimenez 2002) and schematic drawings (Prasadan and Janardanan 2001, Belofastova 1996) of gametocysts were based on observations under light microscopy. The present study provides the first description of the ultrastructure of gametocysts and gymnospores from taxa within this genus.

The most obvious differences with previous descriptions of gametocysts and gymnospores are the cyst wall, morphological characteristics of the gymnospores, and ultrastructural organization of the cytoplasm and internal structure of each sporozoite. The rostral portion of sporozoites may be the mechanism by which *Nematopsis* attach to bivalve tissue and release secretions from vesicles. Presumably these secretions contain enzymes that facilitate digestion and penetration into the host's hemolymph sinus where they develop to oocysts (Tuntiwaranuruk *et al*. 2008). The amylopectin-like granules found in the membranous sacs may increase hydrostatic pressure through water uptake and help to rupture the gametocysts after their release from the rectum of shrimp.

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**Figs 7–12** – a light micrograph of a gametocyst of *Nematopsis* sp. showing numerous gymnospores (Gy); **8** – a transmission electron micrographs (TEM) of a gametocyst of *Nematopsis* sp. showing gynnospore (Gy) and membranous sac (Ms); **9–10** – low (8) medium (9) and high (10) magnification TEM micrographs of a gymnospore (Gy) of *Nematopsis* sp. composed of many sporoziotes (Sp), with their rostral part facing outward and caudal part inward, and the central cytophore (Cc) connecting the caudal part of each sporoziote together; Nu – nucleus; **11** – a higher TEM micrograph of the rostal part of a sporoziote showing an oval nucleus (Nu), rough endoplasmic reticulum (ER), mitochondria (Mi), and secretory granules (Sg); **12** – a TEM micrograph of a membraneous sac (Ms) showing two types of globular structure ($Gs_1$, $Gs_2$) in its cytoplasm.

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