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[SHORT COMMUNICATION]

Discovery of the cement gland in the sea spider, *Nymphonella tapetis* (Pycnogonida, Ascorhynchidae)

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INTRODUCTION

Nymphonella tapetis Ohshima, 1927 is a curious form of pycnogonid endoparasitic on some bivalves during larval stages (Ohshima 1927, 1937; Miyazaki et al. 2010; Miyazaki and Yamada 2019). The sudden large-scaled outbreak of the species in the commercial bivalve (Asari) *Ruditapes philippinarum* and several other bivalves in Tokyo Bay occurred in April 2007. The simultaneous mass mortality of bivalves was the first example of serious fishery damage caused by pycnogonids (Miyazaki et al. 2010; Miyazaki and Yamada 2019).

In pycnogonids, adult males hold a mass of fertilized eggs usually until hatching with the deformed third head appendages (ovigers) gathered by a mucous material secreted by the cement glands in the male walking legs (King 1973; Arnaud and Bamber 1987; Bain and Govedich 2004; Brenneis et al. 2017). Similarly, adult male *N. tapetis* holds egg-mass(es) gathered by a transparent material (Fig. 1), but Ohshima (1935) wrote "Cement-glands could not be made out with certainty." No mention of the cement glands has been made yet in *N. tapetis*.

In the present study, the authors describe the cement gland and its associated pores in *N. tapetis* for the first time.

MATERIALS AND METHODS

About 150 free-living *Nymphonella tapetis* specimens were collected by hand at the Banzu tidal flat in Tokyo Bay, July 2018. Adult males were selected under a stereomicroscope.

Most specimens were fixed with 70% ethanol. Some ethanol-fixed specimens were stained with the chitin-binding fluorescent dye, Uvitex 2B (Disodium 4,4'-Bis(2-sulfostyryl) biphenyl) (Polysciences, Inc.) to enhance the cuticular outline (Yoshinaga et al. 2011). One percent Uvitex 2B in PBS was diluted five times with 70% ethanol. Specimens were immersed in the Uvitex 2B solution for 30 min, washed with 70% ethanol and then observed under a fluorescent microscope.

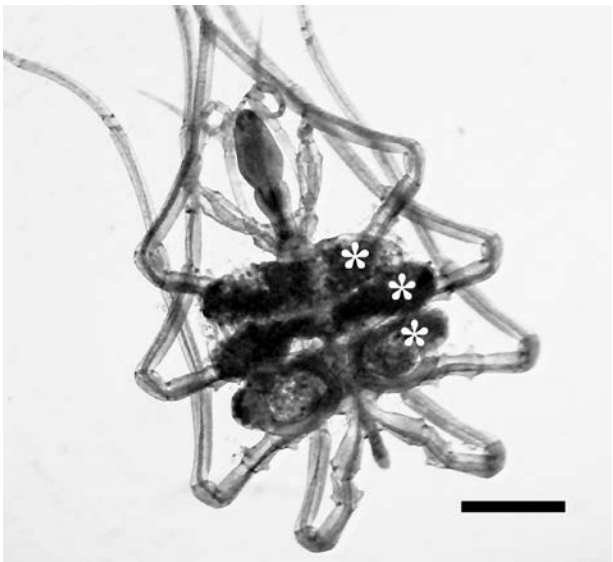

Fig. 1 Adult male *Nymphonella tapetis* holding three egg-masses (asterisks) on ovigers. Ventral view. Bar = 1 mm.

Some specimens were fixed with a seawater-Bouin's solution or a methanol-formalin based fixative, Ufix (Sakura Finetek Japan) for histology. In the fixative, the trunk and walking legs were separated using a razor blade. Each part was dehydrated and cleared with a graded ethanol-*n*-butanol series. After embedding in paraffin, serial sections of 5 μm thickness were prepared, and stained with Delafield's or Mayer's hematoxylin and eosin Y (H&E).

Some specimens kept in 70% ethanol were directly put into a 5–10% room temperature ionic liquid, HILEM IL-1000 (Ethyl (2-hydroxyethyl), dimethyl ammonium methane sulfonate) (Hitachi High-Technologies) diluted with distilled water for several minutes and were set on a stub for scanning electron microscope (SEM). After absorption of excess liquid, specimen was observed with a JEOL JSM-6330F at 15 kV.

RESULTS

Cement glands occurred in the entire length of the fourth and the fifth leg articles (femur and first tibia) as well as in the proximal half of the sixth one (second tibia) of all walking legs in adult male *Nymphonella tapetis*. The glands were situated dorsal to the pedal gut diverticulum (Fig. 2) and were directly connected with outside through pores (cement gland pores). The cement gland pores formed a row on the dorsal surface of the gland-bearing region (from femur to proximal half of second tibia) (Fig. 3). Each pore was a simple hole with a diameter between 5 and 20 μm (Fig. 4). The pores were counted between 15 and 35 in the femur and the first tibia and about 10 in the second tibia. The number of pores was variable even among the same article of different legs in the same specimen.

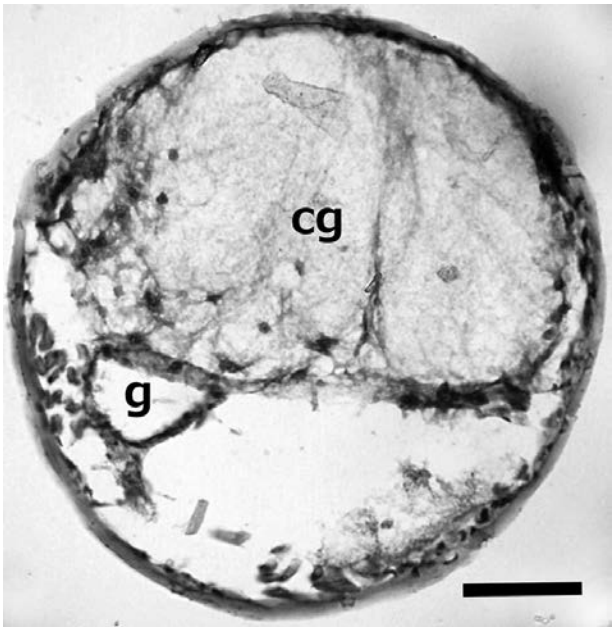

Fig. 2 Transverse section of femur in adult male *Nymphonella tapetis* (H&E). Dorsal to the top. cg: cement gland, g: pedal gut. Bar = 50 μm.

DISCUSSION

The cement gland is a male-specific tissue of pycnogonids, which provides a mucous material to attach the fertilized eggs to the ovigers. The glands are usually located in only the femur accompanied by pores, ducts, or slits as outlet openings (King 1973; Arnaud and Bamber 1987; Bain and Govedich 2004). Although some adult male *Nymphonella tapetis* carries the mass of eggs or embryos with the ovigers packed in a transparent material like other species of pycnogonids, the cement glands have not been described (Ohshima 1935). The present study shows the

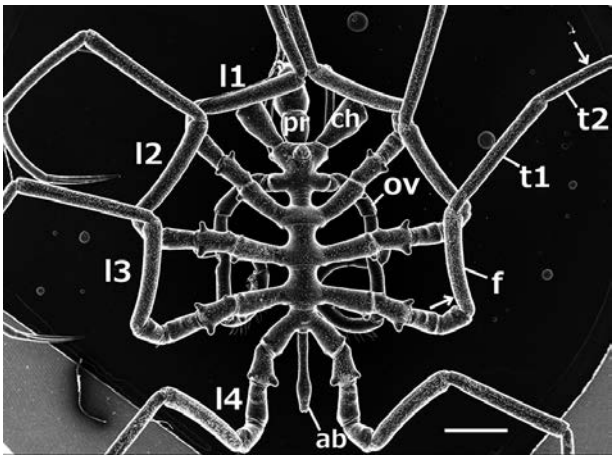

Fig. 3 SEM image of adult male *Nymphonella tapetis*. Dorsal view. Arrows indicate the position of the proximalmost and the distalmost cement gland pores. ab: abdomen, ch: chelifore, f: femur, l1–l4: first to fourth legs, ov: oviger, pr: proboscis, t1, 2: first and second tibiae. Bar = 1 mm.

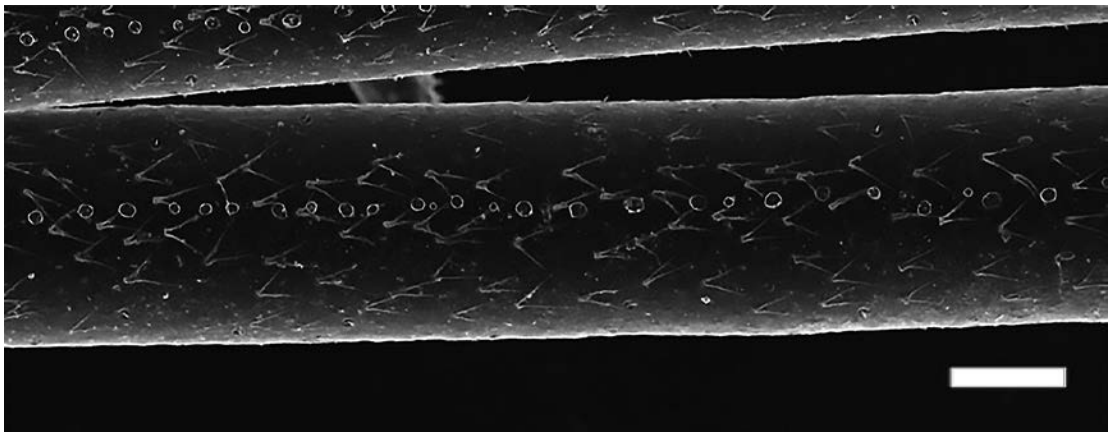

Fig. 4 SEM image of dorsal surface of femur in adult male *Nymphonella tapetis*, showing a row of cement gland pores. Bar = 100 μm.

existence of the cement gland and its outlet pores in *N. tapetis* for the first time.

In most pycnogonid species, the cement glands are limited within the femur (King 1973; Arnaud and Bamber 1987). Stock (1974), however, reported three exceptional cases on the position of cement gland pores. The pores were present in the femur and the first tibia of all legs in *Ascorhynchus armatus* and in those of the second to fourth legs in *Ascorhynchus pararmatus*. Another case was in *Nymphon caementarum,* where the pores were present not only in the femur but also in the first tibia and the second coxa (second leg article). *N. tapetis* shows a new distribution pattern of the cement glands extending from the femur to the second tibia of all legs. Such a wide distribution of cement glands in *N. tapetis* should be correlated with a higher activity of reproduction. Actually, *N. tapetis* shows the attribution of multiple matings as males often hold several egg-masses (up to seven; Miyazaki et al. 2010) stemming from different matings in pycnogonids (Brenneis et al. 2017).

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