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An electron microscope study on the peg-like predacious organ in *Zoophagus insidians* capturing rotifers

Masatoshi SAIKAWA* and Masao GOHO*

Department of Environmental Sciences

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Abstract

Electron micrographs of *Zoophagus insidians*, Zygomycota, revealed that numerous, membrane-bound, small dense-cored vesicles (ca. 0.1 μm in diameter) in the cytoplasm are released through one or more breaks of cell wall from the apex of a peg-like predacious organ of rotifers together with a homogeneous material derived from the large vesicles (ca. 1.0 μm in diameter). The small vesicles maintain their spherical in outline during release. A multi-vesiculate, foamy mass seen among the adhesive surrounding the apical portion of the peg would probably be originated from the membrane of the small vesicles.

Key words: adhesive, dense core, multi-vesiculate foamy mass, small vesicles

Department of Environmental Sciences, Tokyo Gakugei University, 4-1-1 Nukuikita-machi, Koganei-shi, Tokyo 184-8501, Japan

Zoophagus insidians Sommerstorff is classified in the Zygomycota (Kirk et al. 2008), although it has long been thought to be an oomycete (Sommerstorff 1911; Whisler and Travland 1974) mainly because the fungus is aquatic, unlike most species of Zygomycota. The fungus does not have septa in every living portion of underwater mycelium except the portions of its sexual and asexual reproduction (Mirande 1920; Gicklhorn 1922; Morikawa et al. 1993). The mycelium is composed of straight, weakly branched main hyphae that have numerous outgrowths of a trapping peg at a moderate interval to capture rotifers of a loricate-type by secretion of adhesive, or glue. The main hyphae do not grow at all until the fungus captures rotifers by the peg. Whisler and Travland (1974) showed the secretion of adhesive in ultrathin sections for the first time. In their observation, it is found that the secretion mechanism triggered by the rotifer is accompanied by the separation of the two layers of the cell wall, fusion of the large vesicles with the cell membrane and extrusion of the adhesive through pits in the apex of the inner wall of the peg. After capturing a rotifer, the peg grows as a haustorium into the body cavity of the animal. In the present study, the ultrastructural aspects of the fungus during release of adhesive are shown.

Materials and Methods

A mycelium of *Zoophagus insidians* was found in the water layer of a medium composed of water and water agar plate, in which the agar plate, 2 mm in depth, in a Petri dish, 90 mm in diameter, had been poured by 10 ml of water. The medium was

*Tokyo Gakugei University（4−1−1 Nukuikita-machi, Koganei-shi, Tokyo 184−8501, Japan）

incubated at room temperature (20−22 ˚C) for about 3 weeks with littoral detritus which had been collected from the surface of a fire reservoir, ca. 3×5 m, at Tokyo Gakugei University in December, 1996. For subculture of the fungus, the method by Karling (1952) using the onion (*Allium cepa* L.) skin was employed, i.e., the rotifers (*Lepadella oblonga* Ehrenb.), the hosts of the fungus, were multiplied in the same medium for the fungus, but added with a few pieces of onion skin (ca. 10×10 mm). When the culture was added by one or more pieces of onion skin bearing rotifers, the fungus in a starved condition grew again vigorously around the onion skin.

For electron microscopy, specimens were fixed in 2% (v/v) glutaraldehyde buffered with 0.1 M sodium phosphate (pH 7.2) for 1.5 h at room temperature, washed with the same buffer for 1.5 h, and post fixed in OsO_4 in the same buffer at 4 ˚C for 12 h. After dehydration through an acetone series, the fungal materials were embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate and observed with a JEOL 100CXII electron microscope operating at 80 kV. A Hitachi H-1250M high voltage electron microscope was also used operating at 1000 kV for 5-µm-thick sections.

Results

The main hyphae of *Zoophagus insidians* mycelium did not grow until the peg captured rotifers. Attempts to establish a non-axenic culture using antibiotics, for example, were unsuccessful and it was necessary to feed rotifers for cultivation of the fungus. The fungus grew during capturing rotifers and produced a number of pegs, 16.0−18.0×2.5 µm, at a moderate interval on main hyphae, 4.5−5.0 µm wide (Fig. 1). In a high-voltage electron micrograph both main hyphae and pegs are seen natural in outline, because most of them are not sliced but included in a 5-µm-thick section of an epoxy resin. They are surrounded by a numerous and variously-shaped bacteria (Fig. 1). In ultrathin sections, it was found that the apical portion of the peg is occupied by a number of electron-dense large vesicles (ca. 1.0 µm in diameter; Figs. 2, 4, 6−8), whereas in the other portions various organelles, such as mitochondria (Fig. 2), nuclei (Fig. 3) and vacuoles (Figs. 2, 3) are distributed in the cytoplasm as well as large electron-dense vesicles (Figs. 1−3, 10). In addition, electron-desne, small vesicles (ca. 0.1 µm in diameter) are also found in the cytoplasm. The small vesicles contain a dense core with transparent margins (Figs. 11, 12) and increase in number in the cytoplasm near the peg under release of adhesive (Fig. 10).

The large vesicles became to occupy wider range in the peg and some of the vesicles near the tip showed to be distorted by the dense accumulation of vesicles (Figs. 6, 7). The adhesive after release from the peg is composed of an electron-dense homogenous material and a multi-vesiculate, foamy mass (Figs. 9, 11) and both structures are considered to be derived only from secretion of the matrix of the large vesicles aggregated in the peg. Occasionally, the peg stops its activity on the way for release of adhesive (Fig. 8) due mainly to escape of a rotifer. In such a case, adhesive is seen still in the peg's cytoplasm as a homogeneous material (Fig. 8). The large volume of the homogeneous material in Fig. 8 is in contact with large vesicles, showing the vesicles being immediate before fusion with each other. The adhesive after release from the peg contained a large volumes of multi-vesiculate, foamy mass as well as an electron-dense, homogeneous material (Figs. 9−11).

The cell wall of hyphae including the wall of the peg was composed of an electron-dense outer layer and an electron-transparent inner layer (Figs. 7, 8, double arrow). The outer dense layer on the tip of the peg was organized into a number of fine ridges (Figs. 4−6, 11). Since the ridges run across the apex approximately parallel to the axis of the peg, the structures were occasionally obscure depending on the orientation of the peg (Figs. 7−9). At release of adhesive, the two layers of the cell wall at the apex of the peg separate from each other (Fig. 8) and the significant amount of homogeneous material of high-electron density remains between the two layers of the cell wall (Figs. 10−12).

In the present study, a peg surrounded by rotifer cilia was found in an ultrathin section under release of an adhesive material (Figs. 10−12). Although the peg had already begun to release adhesive (Fig. 10, double arrow), it is still releasing at the site exhibited by the arrow in Fig. 10, showing higher electron density than that already released. In addition, it was found that the releasing adhesive was composed of numerous electron-dense, spherical globules, approximately same in size as the dense-core of the membrane-bound, small vesicles seen in the cytoplasm of the peg (Figs. 11, 12).

Discussion

One of the remarkable ultrastructural aspects in thin sections of *Zoophagus insidians* mycelium is of the adhesive, or glue. The adhesive contains a multi-vesiculate, foamy

mass in addition to the electron-dense, homogeneous material derived from electron-dense large vesicles, ca. 1.0 μm, in diameter, accumulated in the cytoplasm of the peg (Whisler and Travland 1974; Morikawa et al. 1993). Such a multi-vesiculate structure seen outside of the traps of the fungus after release was reported in other species in the genus, *Zoophagus* (Saikawa and Morikawa 1985), but never reported from species in the Hyphomycetes that capture microscopic animals, such as nematodes (Dowsett and Reid 1977, 1979; Wimble and Young 1984; Saikawa 1985; Saikawa and Kaneko 1994; Kojima and Saikawa 2002). In the present study, a peg was found in an ultrathin section under release of an adhesive material surrounded by rotifer cilia. It is still releasing at the site exhibited by the arrow in Fig. 10. The adhesive under release shows higher electron density than that of the adhesive released already as exhibited by the double arrow in the same figure. In addition, it was found, for the first time, that the adhesive was composed of electron-dense spherical globules, approximately same in size (ca. 0.1 μm in diameter) as the dense-core of the small vesicles seen in the cytoplasm of the peg (Fig. 12). Thus, it is possible that the foamy vesiculate aggregation in an adhesive would be derived from the membrane of the small vesicles in the cytoplasm. The small vesicles increase in number in the cytoplasm (Fig. 10) after release of an adhesive material and are accumulated near the site of release of adhesive as shown by Whisler and Travland (1974).

Each of the ridges seen at the apex of pegs was different from that reported by Whisler and Travland (1974) and Morikawa et al. (1993) in the strain obtained from Washington, USA and Koganei, Tokyo, respectively, i.e., the ridge shown in the present study was sessile, whereas that reported by the latter authors had a stem which support a broader top portion. Although the fungus used in the present study and that used by Morikawa et al. were obtained from the same fire reservoir in the campus of Tokyo Gakugei University, the ultrastructure showed the morphology slightly different from each other.

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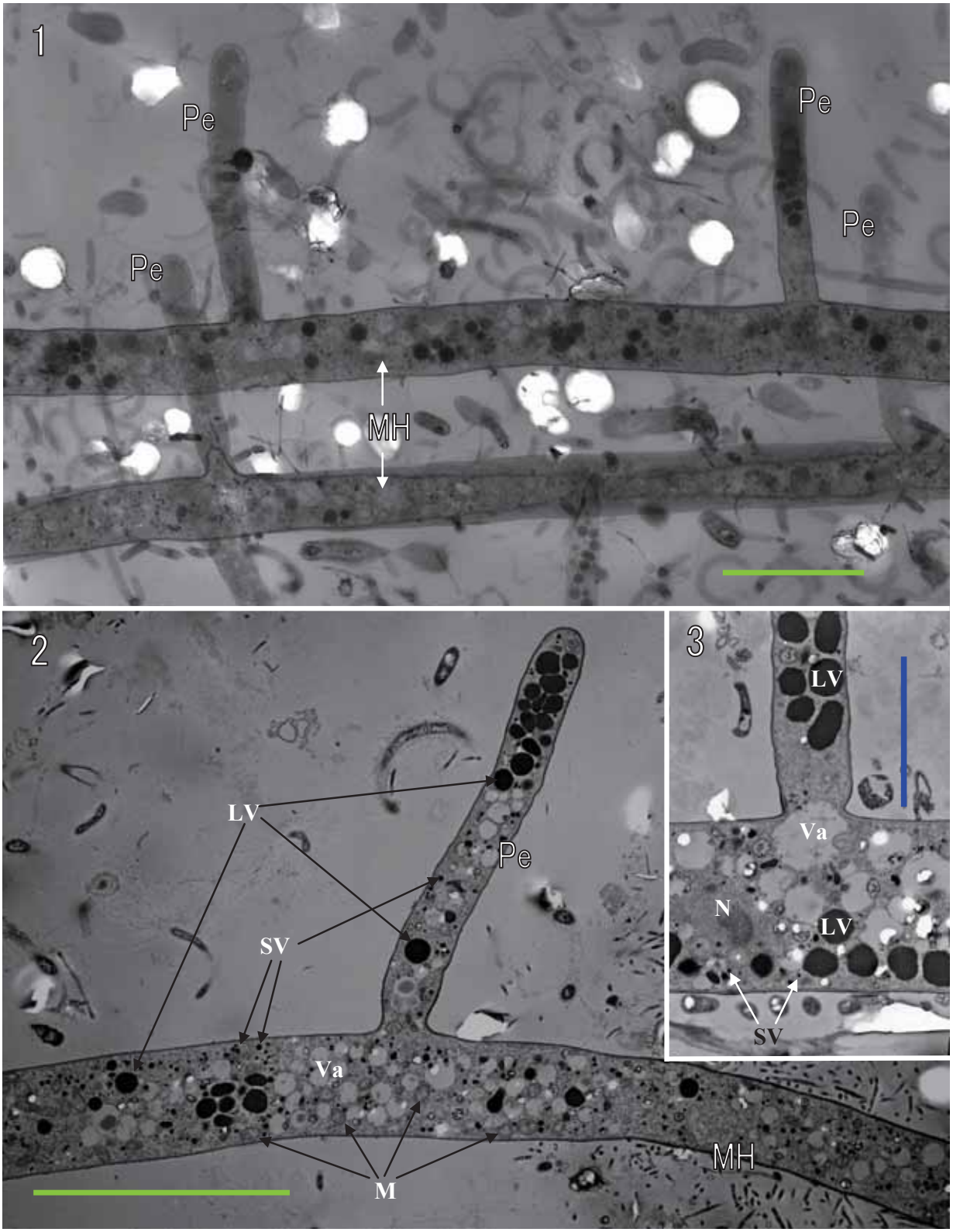
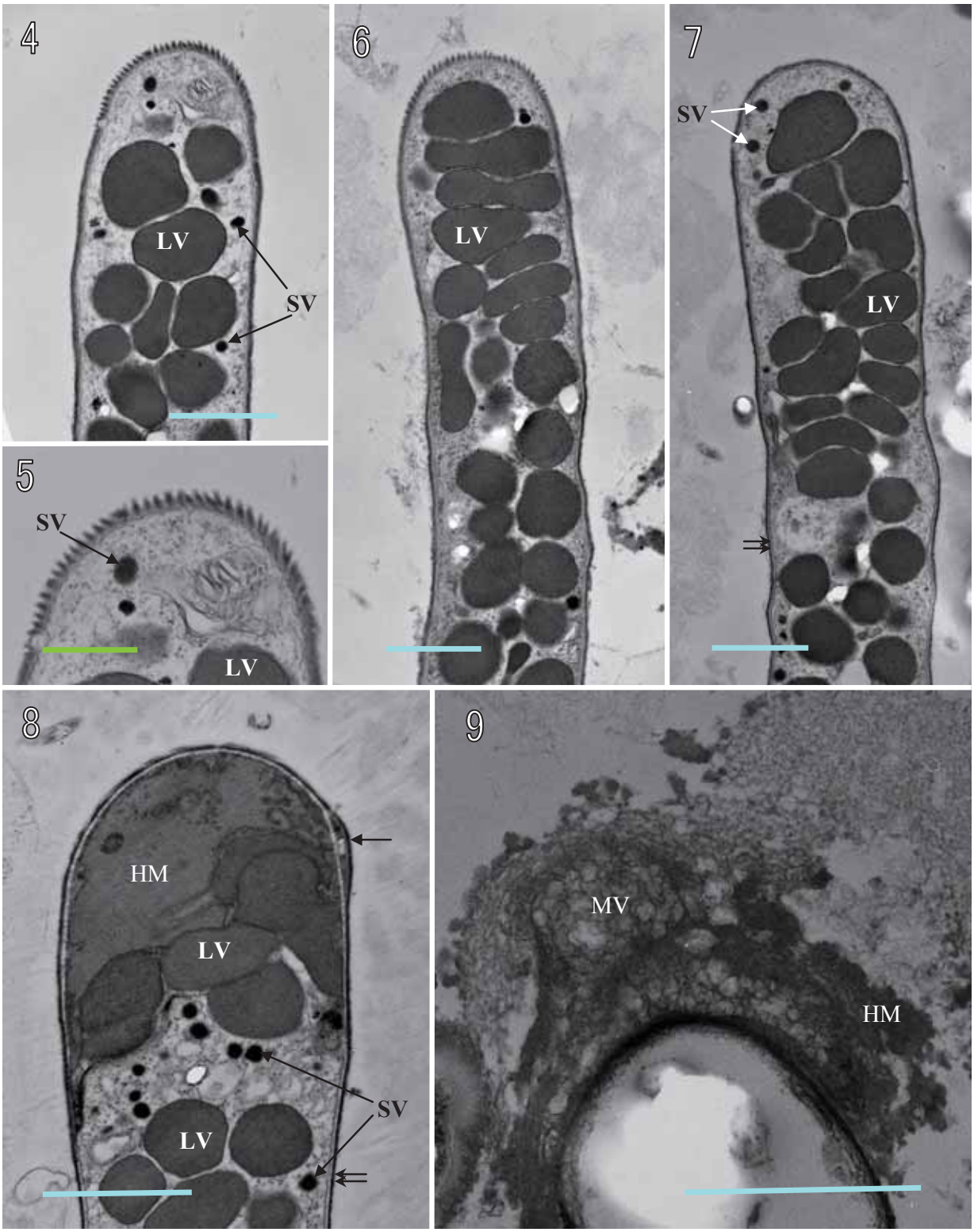
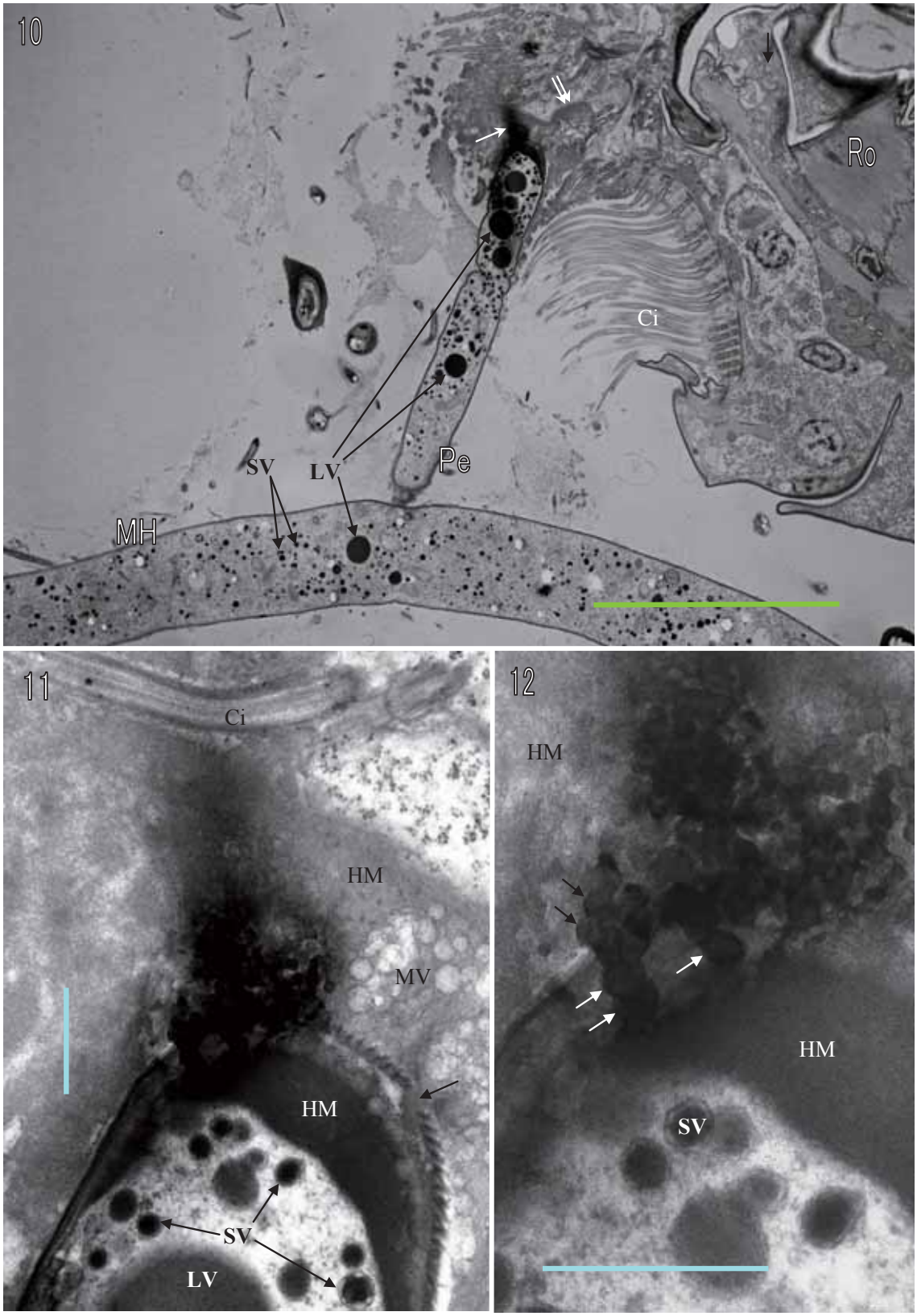


Figs. **1**—**3**. Mycelium of *Zoophagus insidians*. (1. A high-voltage electron micrograph of a 5-μm-thick section; 2, 3, Electron micrographs in ultrathin sections.) **1**. A portion of two main hyphae (MH), each bearing two adhesive pegs (Pe). The fungus is surrounded by a number of various species of bacteria. **2**. An adhesive peg (Pe) and a main hypha (MH). The apical portion of the peg is occupied by a number of electron-dense, large vesicles (LV), whereas in the other portion of the peg and also in main hypha, various organelles are distributed in the cytoplasm. M, mitochondria; SV, small vesicles; Va, vacuole. **3**. A basal portion of a peg. Cytoplasm is continuous between the peg and main hypha. The electron-dense large vesicles (LV) are seen in the cytoplasm of the peg and main hypha. N, nucleus; SV, small vesicles; Va, vacuole. Bars, 10 μm for Figs. 1, 2; 5 μm for Fig. 3.



Figs. **4**—**9**. Adhesive pegs of *Zoophagus insidians* in ultrathin sections. **4**. Apical portion of the peg seen in Fig. 2, showing electron-dense, large vesicles (LV) and small vesicles (SV). **5**. Photographic enlargement of the apical portion in Fig. 4. The outer layer of the cell wall shows ridge-like in appearance. LV, large vesicle; SV, small vesicle. **6**. An adhesive peg, occupied by large vesicles (LV). **7**. An adhesive peg, showing the ridge-like appearance of apical walls, being obscure due to the orientation of the peg. Double arrow shows the two-layered cell wall. LV, large vesicle; SV, small vesicle. **8**. Accumulation of homogeneous material (HM) in the apical portion of the peg. Arrow and double arrow show the beginning of separation of cell walls and the two-layered cell wall, respectively. LV, large vesicle; SV, small vesicle. **9**. Adhesive after release from the peg, showing electron-dense homogeneous material (HM) and multi-vesiculate, foamy mass (MV). Cytoplasm of the peg has completely been evacuated. Bars, 1 μm for Figs. 4, 6—9; 0.5 μm for Fig. 5.



Figs. **10**—**12**. An adhesive peg of *Zoophagus insidians* in an ultrathin section in releasing adhesive. **10**. Apical portion of a peg adhering to the mouth portion of a rotifer (Ro) with adhesive. The adhesive (double arrow) is seen to be composed of an electron-dense, homogeneous material and a multi-vesiculate foamy mass. Arrow shows the portion in releasing adhesive. Ci, cilia; LV, large vesicle; MH, main hypha; Pe, peg; SV, small vesicle. **11**. Apical portion of the peg in Fig. 10. Adhesive is seen as electron-dense homogeneous materials (HM) that contain several accumulations of a multi-vesiculate, foamy mass (MV). Arrow shows a break of the outer layer of cell wall. Ci, cilium; LV, large vesicle; SV, small membrane-bound vesicles. **12**. Photographic enlargement of Fig. 11. Electron-dense spherical globules (arrows) are rushing off the peg's cytoplasm through a break of the cell wall together with the electron-dense, homogeneous matrix (HM). The electron-dense material still in the small vesicles (SV) is bound by a membrane. Bars, 10 μm for Fig. 10; 1 μm for Figs. 11, 12.

ワムシ捕捉性*Zoophagus insidians*における ペグ形捕捉器官の電顕的研究

犀川　政稔・五寶　匡郎

環境科学分野

要　旨

Zoophagus insidians（接合菌綱）の電顕像の観察中，ペグの形をしたワムシ捕捉器官の先端から高い電子密度の芯をもった小ベシクル（直径約0.1 μm）が，同じく高電子密度で大ベシクル（直径約1.0 μm）由来の均質な物質とともに１～数か所の細胞壁の破れ目から粘着物として放出されているところを発見し，撮影した。放出中の小ベシクルは丸い形を保っていた。放出後の粘着物が含む泡の集合体は高電子密度の物質が抜けた後の小ベシクルの膜に由来するものと思われる。

キーワード: 粘着物，高電子密度の芯，泡の集合体，小ベシクル