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An electron microscope study on *Dactylella copepodii*, a hyphomycete capturing copepods

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Abstract

Ultrastructure of *Dactylella copepodii*, a hyphomycete capturing copepods, is reported for the first time. The outer layer of the cell wall of globose- and cylindrical adhesive knobs was known to be double layers of fibrous material. The fibrous material seems to be arisen from the innermost layer of the cell wall. Infection peg developed from an adhesive knob did not penetrate into the host immediately after the attachment with the cuticle by adhesive, but penetrate after the infection peg grew for some distance (～5 μm) on the cuticle. After consumption of the animal, hyphae became to have "intrahyphal hyphae" by which the fungus retrieves the nutrients.

Key words: cell wall layers of adhesive knob, infection peg, intrahyphal hyphae

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Introduction

Among various types of adhesive traps of hyphomycetes, adhesive knobs had already been studied ultrastructurally in *Dactylella drechslerii* (Tarjan) R. C. Cooke & Dickinson (Heintz and Pramer 1972), *Dactylaria candida* Drechsler (Dowsett and Reid 1977), *Dactylella lysipaga* Drechsler (Wimble and Young 1983, 1984), *Dactylella haptotyla* Drechsler (Saikawa and Kaneko 1994), *Dactylella ellipsospora* Grove (Kojima and Saikawa 2002) and *Arthrobotrys entomopaga* Drechsler (Saikawa et al. 2010). Except for *A. entomopaga*, all of these capture nematodes on agar plates. Another exception is *Dactylella copepodii* Barron. The fungus captures larval and adult stages of copepods under water by means of a globose- or a cylindrical adhesive knob. Ultrastructure of the fungus has not yet been reported and shown in the present study.

Materials and Methods

Dactylella copepodii capturing a species of copepods (*Phyllognathopus viguieri* Maupas) was recovered from a thin film (2 mm) of water in a Petri dish, 90 mm in diameter, incubated with about 10 g of a few pieces of wood (*Quercus serrata* Thunb.) for a week at room temperature (20－22 ˚C). The wood pieces were collected in the forest, Tama Forest Science Garden, Forestry and Forest Products Research Institute, Hachioji, Tokyo, Japan on 16, April, 1998 by one

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(Mak) of the authors. For multiplication of the copepods under water, those that have not yet been infected by the fungus were transferred to a fresh thin film of water, in which two pieces of onion skin (each ca. 1×1 cm) were added, the method fundamentally by Karling (1952) for multiplication of rotifers. Subculture was done at intervals of 15−20 days.

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.

Results

Inside body of adult and larval stages of copepod was seen to be occupied by assimilative hyphae at about 24 h after initiation of infection by *D. copepodii*. From the dead copepod one or a few hyphae (3.5−4.5 µm wide) developed and produced several thick branches, 40−50 µm long and 4.5−5.5 µm wide, tapering toward their basal portion where the branch was constricted slightly. The thick branch was terminated by a globose, adhesive knob, 7−8.5 µm in diameter (Fig. 1) or a swollen, cylindrical adhesive knob, 10−20×7−8.5 µm (Fig. 2). Proportion of the globose- and cylindrical adhesive knobs in a mycelium was about five to one. At the constricted basal portion of the thick branch, it was broken readily by a struggling copepod that moved away with the branch attached to its body. In ultrathin sections the cytoplasm of both globose- and cylindrical knobs contains a number of electron-dense, large vesicles, ca. 0.2−0.5 (−1.0) µm in diameter (Fig. 1). The cell wall of the adhesive structures was extremely thicker than that in other portions of hyphae. In the case in Fig. 2, the thickness of the wall at the apical portion (arrowhead) of the cylindrical cell is about several times thicker than that of the branch (double arrow), although it looks thicker than the real structure because it was cut obliquely to the main axis slightly. In magnified micrographs, the cell wall of adhesive structures was found to be composed of three layers of substances, i.e., the innermost cell wall were covered with double layers of fibrous materials that were arisen from the cell wall, in which the middle layer was slightly lower in electron density than the other two (Fig. 3). The septum of hypha was known to be the simple-pore type of Ascomycota, associating with a few Wononin bodies (Figs. 1, 2, 6, 8, 10).

Infection peg developed from an adhesive knob did not penetrate the host immediately after the attachment with the cuticle by adhesive. The penetration was done after the infection peg grew for some distance (～5 µm; Figs. 4, 5) on chitinous cuticle of the animal (Fig. 7). In addition, the portion of the infection peg outside of the animal became to have septa after penetration (Figs. 6, 7).

After consumption of copepod, the protoplasm of assimilative hyphae in copepod's body was moved toward outside of the animal through the portion of penetration by the development of new hyphae that grew inside of the old hyphae (Figs. 8−11). Such "intrahyphal hyphae" occupied most of the lumen of the enclosing hyphae and pushed aside septa (Fig. 10, arrows) to pass into the adjacent compartment of hypha. Spaces between new and old cell walls of both hyphae are seen clearly in the thin sections at the portions of assimilative hyphae (Fig. 9), adhesive knob (Fig. 10) and infection peg (Fig. 11), for examples.

Discussion

Saikawa et al. (2010) showed electron micrographs of *Arthrobotrys entomopaga* Drechsler, a hyphomycete that captures springtails by means of adhesive knobs. It was the only ultrastructural report on the adhesive knob in the Hyphomycetes that captures animals other than nematodes. Since springtails have a jumping ability, the knob of *A. entomopaga* exudes a considerable amount of adhesive that can be seen as a numerous mass of fine, fibrous materials in thin sections. *Dactylella copepodii* is known to be another example of a hyphomycete that captures animals other than nematodes by adhesive knobs. Although *Phyllognathopus viguieri*, a copepod host of the fungus, moves by jumping repeatedly under water, the amount of adhesive was not so much in thin sections as that in *A. entomopaga*. The homogeneous appearance of adhesive in thin sections was, however, similar to that in other species of nematode-capturing, adhesive knobs of hyphomycete species (Wimble and Young 1983, 1984; Saikawa and Kaneko 1994; Kojima and Saikawa 2002). The adhesive in ultrathin sections of *D. copepodii* did not contain the multi-vesiclulate foamy mass as seen in the species of *Zoophagus* in Zygomycota capturing rotifers (Whisler and Travland 1974; Saikawa and Morikawa 1985; Saikawa and Goho 2010), although *D. copepodii* has

aquatic in habit like the species of *Zoophagus*.

In hyphomycete species capturing nematodes by adhesive knobs, each knob develops an infection peg at the site of contact with the cuticle immediately after the exudation of adhesive to penetrate into the body of the nematode. Thus, the portion of the peg becomes constricted after penetration and there is no hyphal portion between assimilative hypha in the host and adhesive knob (Wimble and Young 1983, 1984; Saikawa and Kaneko 1994; Kojima and Saikawa 2002). On the other hand, the infection peg of *D. copepodii* is seen on the cuticle of the copepod as a short, narrow hypha, ～5 μm long. The peg will "find" the site suitable for penetration. The growth of infection peg from adhesive knob is somewhat similar in growth to the germ tube of urediospores in rust fungi (Littlefield and Heath 1979), in which the germ tube is "looking for" a stoma of the host plant to enter the stomatal cavity.

Intrahyphal hyphae have been thought to occur in certain adverse conditions of growth or in response to cellular damage, or by genetic mutations (Miller and Anderson 1961; Lowry and Sussman 1966; Kim et al. 2001; Bowman et al. 2006; Takeshita et al. 2006). In the case in *D. copepodii*, however, the intrahyphal hyphae seems to occur every time of infection of copepod when the fungus retrieves the nutrient from copepod in addition to a few hyphae that break the copepod's cuticle. Because of the thick cuticle, assimilative hyphae will not grow out of the animal easily, as in the case for the infection peg that does not penetrate until the peg comes at the site suitable for penetration.

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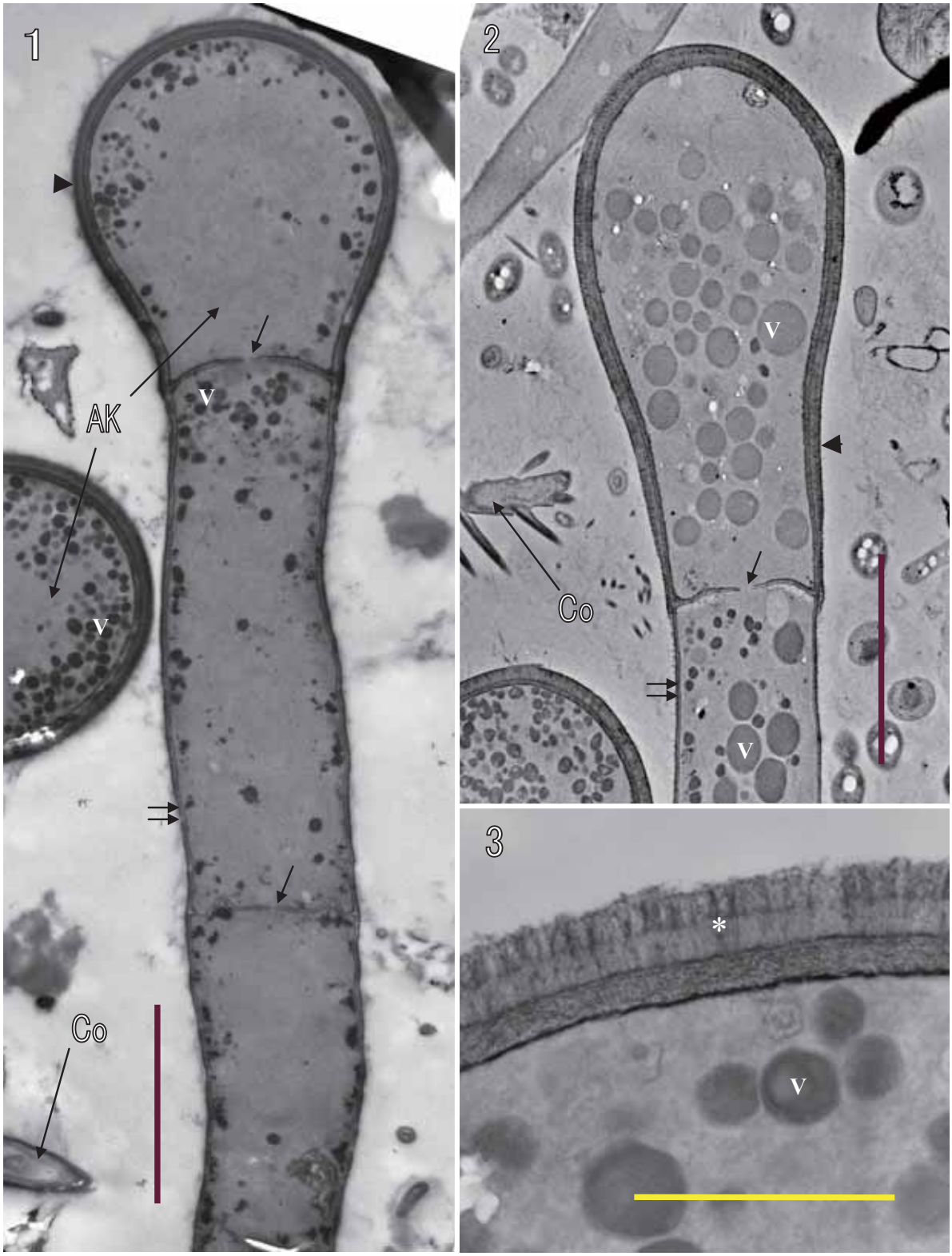
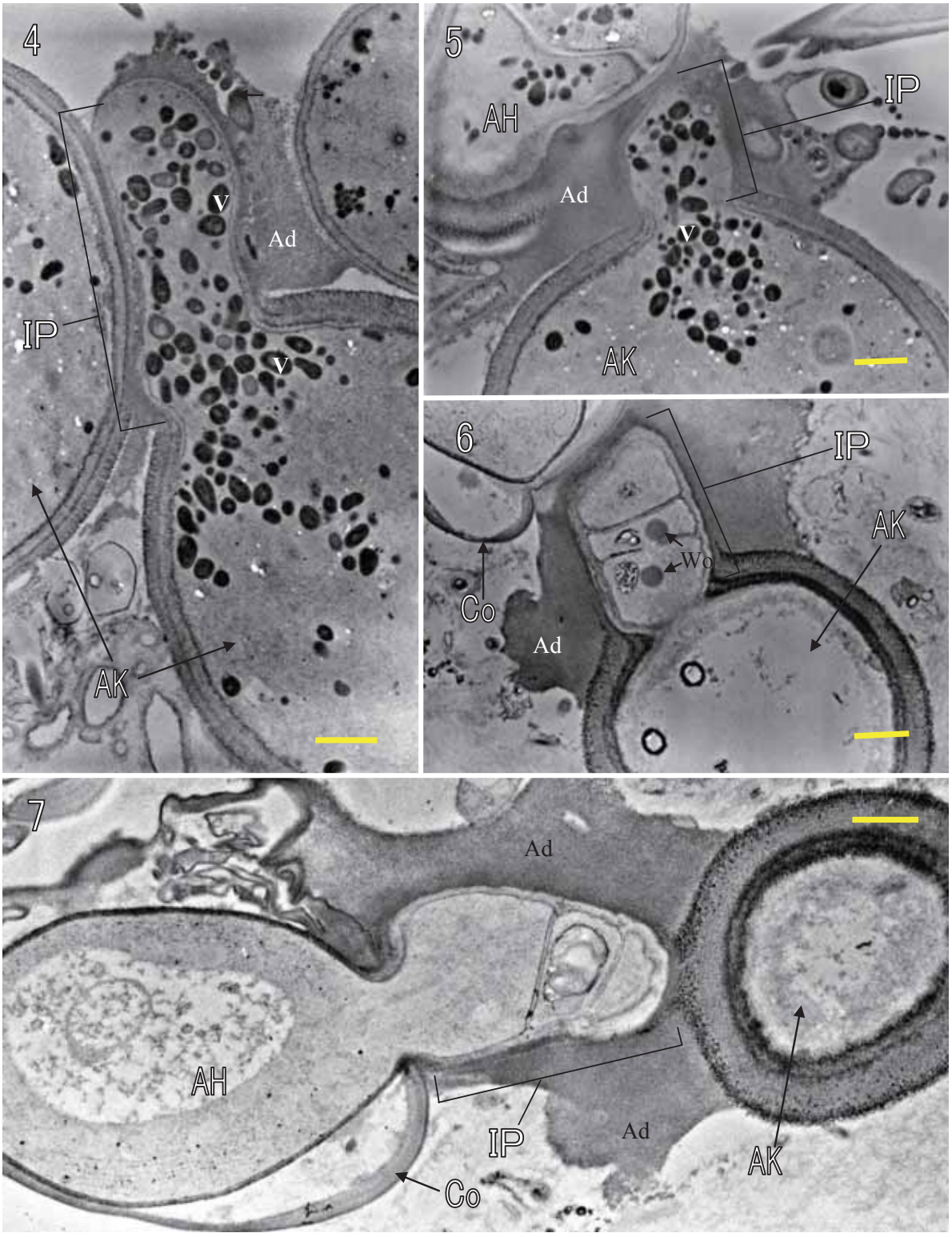
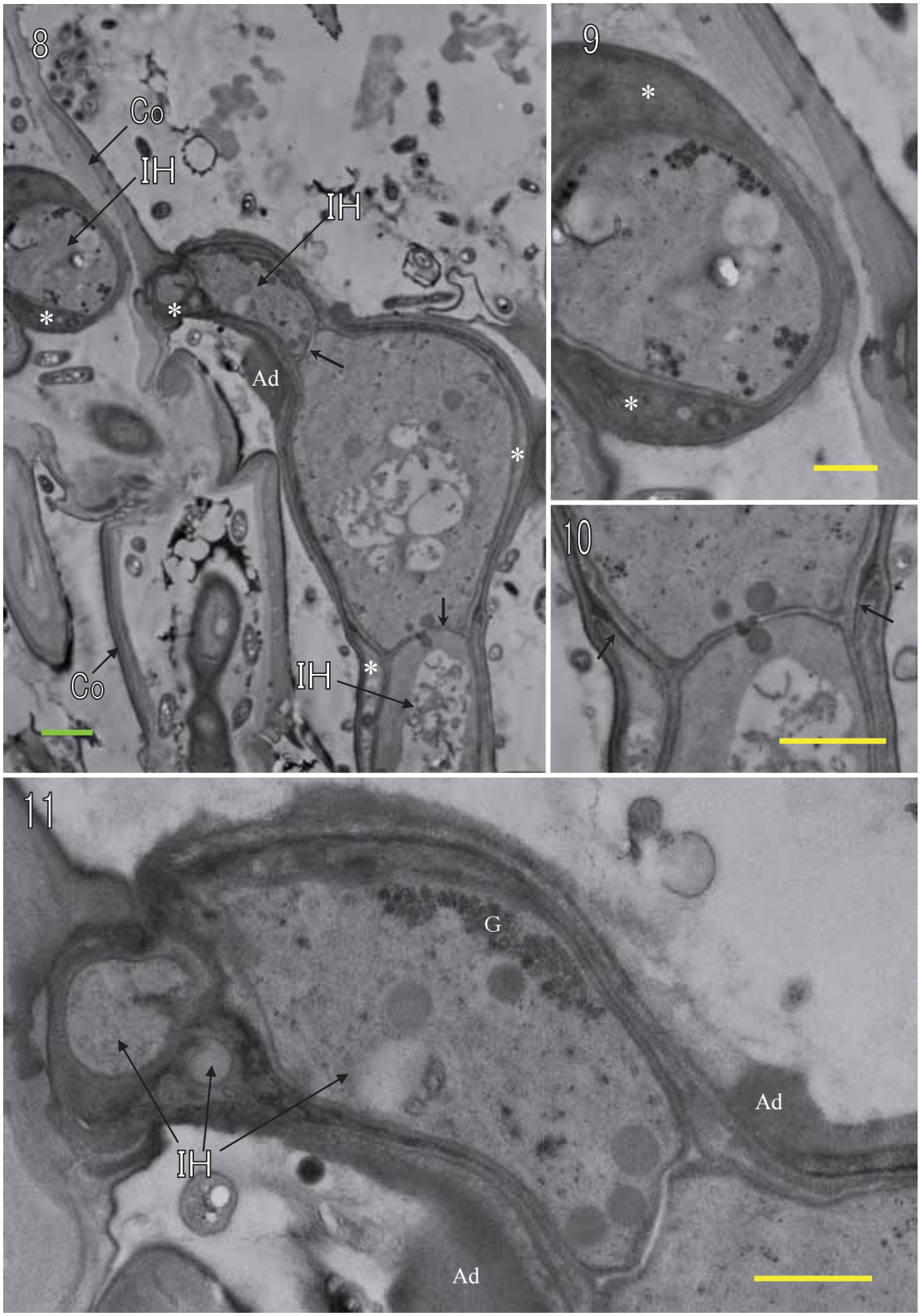


Figs. **1**—**3**. Globose- and cylindrical adhesive knobs of *Dactylella copepodii* in ultrathin sections. **1**. Distal portion of a thick branch of hypha terminated with a globose, adhesive knob (AK). The cell wall of the knob (arrowhead) is thicker than that of the branch (double arrow). A portion of another knob (AK) is also seen. Arrows show the simple pores of septa. Co, a portion of an appendage of a copepod; V, electron-dense vesicles. **2**. A cylindrical, adhesive knob on a thick branch of hypha. The cell wall of the knob (arrowhead) is thicker than that of the branch (double arrow). Arrow shows the simple pore of septum. Co, a portion of an appendage of copepod; V, electron-dense vesicles. **3**. Enlarged micrograph showing that the cell wall of adhesive knob was covered with double layers of fibrous material (asterisk) arisen from innermost layer of the cell wall. V, electron-dense vesicle. Bars, 5 μm for Figs. 1, 2; 1 μm for Fig. 3.



Figs. **4**—**7**. Penetration of copepod by infection pegs of *Dactylella copepodii* in ultrathin sections. **4**. Development of an infection peg (IP) from the adhesive knob (AK). Electron-dense vesicles (V) in the cytoplasm are moving toward the animal. A portion of another knob (AK) is also seen. Ad, adhesive. **5**. Development of an infection peg (IP) from the adhesive knob (AK). A portion of assimilative hyphae (AH) in the animal is seen. Ad, adhesive; V, electron-dense vesicles. **6**. Infection peg (IP) after consumption of copepod (Co). Two septa are seen in the peg. Ad, adhesive; Wo, Woronin bodies. **7**. Penetration portion by the infection peg (IP). The peg with two septa is continuous with the assimilative hypha (AH) in the copepod (Co). Ad, adhesive. Bars, 1 μm.



Figs. **8**—**11**. Intrahyphal hyphae in *Dactylella copedpodii*. **8**. Intrahyphal hyphae (IH) seen inside of the old hyphae of the various portions of mycelium. Asterisks exhibit the space between new and old cell walls of hyphae. Ad, adhesive; Co, a portion of an appendage of copepod. Arrows show septa. **9**. Photographic enlargement of Fig. 8. A portion of intrahyphal hyphae is seen in the old assimilative hypha in the animal. Asterisks show the space between new and old cell walls of hyphae. **10**. Photographic enlargement of Fig. 8. A portion of intrahyphal hyphae is seen in old hypha at the basal portion of an adhesive knob. Arrows show an old septum pushed aside by the backward growth of the intrahyphal hypha. **11**. Photographic enlargement of Fig. 8. A portion of intrahyphal hyphae (IH) is seen in old hypha of the infection peg, showing undulating in appearance. Ad, adhesive; G, glycogen granules. Bars, 2 μm for Fig. 8; 1 μm for Figs. 9—11.

ミジンコ捕食性不完全菌*Dactylella copepodii*の電顕的研究

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要　旨

ミジンコを捕捉する不完全菌*Dactylella copepodii*の微細構造をはじめて報告する。球形，および円筒形の粘着ノブの細胞壁の外層は２層の繊維性の物質でできていた。繊維性物質は最内層の細胞壁から生じているように見えた。粘着ノブから生じた感染ペグは宿主に直ちに侵入せず，動物のクチクラ上を最大5 μm伸長したあと侵入した。動物を消化したあとの菌糸は「菌糸内菌糸」をもっていた。この菌糸によって菌は栄養を引き上げる。

キーワード: 層状細胞壁，菌糸内菌糸，感染ペグ