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A light and electron microscope study on *Arthrobotrys entomopaga* capturing springtails

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Abstract

Arthrobotrys entomopaga was recovered from leaf mold collected in Japan. This is the first report of this fungus since its discovery by Drechsler (Drechsler 1944). The Japanese isolate showed the ability to capture springtails by means of one or more groups of many adhesive knobs that were aloft by a stalk-like basal cell. The adhesive knob, ovoid or ellipsoid in shape, was surrounded by a droplet of colorless, mucilaginous material like that in *Nematoctonus* spp. However, unlike in *Nematoctonus* the adhesive knobs were arisen primarily only from a web-shaped network system of procumbent hyphae, in which each mesh of the hyphal network mainly composed of straightly-grown, short hyphal branches. The fungus of *Arthrobotrys entomopaga* CBS 642.80 showed the morphology of *A. pauca* and did not capture springtails but nematodes by adhesive knobs, globose in shape. The knobs were not surrounded by a droplet of mucilaginous material. The species *Dactylellina entomopaga* (Drechsler) Scholler, Hagedorn & Rubner in the currently adopted classification for predatory orbiliaceous fungi is *A. pauca* and is completely different from *A. entomopaga*, mainly because *D. entomopaga* cannot capture springtails.

Key words: *Arthrobotrys pauca*, ATCC 28704, CBS 642.80, IMI 143686

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Introduction

Arthrobotrys entomopaga Drechsler is known as the only species in the genus *Arthrobotrys* that captures springtails in the order Collembola. The fungus was originally isolated by Drechsler (1944) from discolored rootlet of *Polygonum pennsylvanicum* L. collected moist ground near a brook in Arlington, Virginia. The fungus formed hyphal networks prostrate on the surface of the agar plate here and there. Adhesive knobs developed only from hyphae composing the network. Since Drechsler did not culture it axenically, Roxon and Jong (1975) redescribed the species, *A. entomopaga*, on the basis of a strain deposited in the Commonwealth Mycological Institute as *Arthrobotrys dactyloides* Drechsler

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IMI 143686. The fungus has been accessioned in the American Type Culture Collection and Centraal Bureau voor Shimmelcultures as ATCC 28704 and CBS 642.80, respectively, and the species was accommodated currently in the genus *Dactylellina* (Scholler et al. 1999) mainly because the fungus produces adhesive knobs and the species is named as *D. entomopaga* (Drechsler) Scholler, Hagedorn & Rubner. However, unlike those of *A. entomopaga*, the knobs of *D. entomopaga* are not surrounded by a droplet of mucilaginous materials like other known species of the genus *Dactylellina*. In this paper, light and electron micrographs of *A. entomopaga* capturing springtails will be shown.

Materials and Methods

Arthrobotrys entomopaga used in the present study appeared in October, 1999 on a water agar plate that was inoculated with a pinch (ca. 10 g) of leaf mold collected in Inashi, Nagano, Japan. The fungus was cultivated nonaxenically by adding various and many species of springtails weekly. A hand-made Berlese funnel trap was used for collection of springtails from leaf mold that was piled up near the farmer's house in the campus of Tokyo Gakugei University, Tokyo. *Arthrobotrys entomopaga* (CBS 642.80) obtained from Centraal Bureau voor Shimmelcultures was used for comparison.

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 osmium tetroxide in the same buffer at 4℃ for 12 h. After dehydration through a graduated acetone series, the fungal materials were embedded in Epon resin and polymerized at 65℃ for 48 h. Ultrathin sections were stained with uranyl acetate and lead citrate and observed with a JEOL 100CXII electron microscope operating at 80 kV.

Results

Arthrobotrys entomopaga captured various and many species of springtails, including *Pseudosinella* sp., *Folsomia* sp. and *Megalothorax* sp., by means of one or more groups of a number of adhesive knobs (Fig. 1). The adhesive knob, 8.0－12.8×6.0－7.6 μm, ovoid or ellipsoid in shape (Fig. 11), was aloft on a stalk-like basal cell, 8.0－18.0 μm long and 3.2－4.4 μm wide at the base, tapering upward (Fig. 2). The portion of the adhesive knob was surrounded by a droplet of colorless, mucilaginous material (Fig. 5), although the body of adhesive of the droplet became irregularly lobate envelope during observation under the microscope (Fig. 3). The group of adhesive knobs arose primarily only from a web-shaped network of hyphae, in which each mesh of the net was mainly composed of straightly-grown short hyphal branches (Fig. 4) and branches that changed their direction abruptly to anastomose with other branches at angles approaching a right angle (Fig. 4, arrows). In older cultures the network grew bigger (Fig. 5) and was composed of procumbent adhesive knobs and stalks, in addition to the branches of hyphae (Fig. 7). The fungus did not capture nematodes. The clusters of 2-celled conidia, 13－25×4.0－5.0 μm, were found on the apex of conidiophores (Fig. 6).

The droplet of adhesive surrounding the adhesive knob was fibrous in appearance in ultrathin sections (Figs. 10－14) and there was no such an image around the knob before maturation (Fig. 9). The central part of the knob was occupied by a number of vacuoles, 0.5－2.5 μm in diameter (Figs. 9－11) as well as plural nuclei (Fig. 10). Woronin bodies, characteristic to the fungi in the phylum Ascomycota were seen near the septum (Figs. 8, 11).

In magnified electron micrographs, numerous fibrils in the adhesive droplet were known to arise from the surface of the cell wall of adhesive knobs, i.e., fibrils still connecting with the cell wall grew at right angle to the wall (Fig. 12, arrow). However, the fibrils apart more than ca. 1 μm from the knob showed almost parallel to the knob's cell wall (Fig. 12, asterisk). When adhesive knobs became distorted, they looked as if it were composed of a few lobes. At the same time, the droplet surrounding the knobs reduced its thickness (Fig. 13), in which the peripheral part of the droplet was seen clearly by condensation of fibrils (Fig. 13). Outside of the condensed fibrils, however, fibrils from other knobs were seen in magnified electron micrographs (Fig. 14). At any rate, the fibrils keep parallel to each other (Fig. 12, 14) until dried up of the droplet.

Penetration of an ocellus of *Folsomia* sp. by *A. entomopaga* was seen in ultrathin sections (Fig. 15). The infection hypha in Fig. 15 pierced into an ocellus of eye patches through the smooth cornea and grew ca. 8 μm in the ocellus. A number of electron-dense vesicles, 0.5－1.0 μm in diameter, in the figure show the hypha under growing actively.

The fungus CBS 642.80 obtained from Centraal Bureau voor Schimmelcultures showed the morphology of *A. pauca* and did not capture any species of springtails but *Rhabditis*

nematodes in this study.

Discussion

Dactylella tylopaga Drechsler capturing amoebae is known to be an anamorph of the Basidiomycota, although the fungus does not have the clamp connection at the septum of hyphae. In electron micrographs of the fungus, the central perforation of the septum of hyphae was found to be of "dolipore" type of Basidiomycota associated with a pair of parenthesomes (Saikawa et al. 1994). Thus, it is probable that *A. entomopaga* is also a species in the Basidiomycota, because, except *A. entomopaga*, all known species of predaceous fungi that produce a glandular cell surrounded by a droplet of mucilaginous material are *Nematoctonus* spp. and their teleomorphs in the Basidiomycota (Barron and Dierkes 1977; Drechsler 1941, 1943, 1946, 1949, 1954; Giuma and Cooke 1972; Jones 1964; Saikawa and Arai 1986; Thorn and Barron 1986). The possibility was denied, however, in the present study that the septum in ultrathin sections of *A. entomopaga* was known to be of "simple-pore" type of Ascomycota, associating with a few Woronin bodies.

Van Oorshot (1985) observed the strain, CBS 642.80, named erroneously as *Arthrobotrys entomopaga* Drechsler, and made a discussion as follows. "In the absence of authentic material of *A. entomopaga*, Roxon & Jong (1975) redescribed the species on the basis of McCulloch's strain. This was apparently done without notifying Dr. McCulloch, who soon afterwards accommodated the same strain in a new species without any reference to Roxon & Jong's (1975) publication. As noted by Roxon & Jong (1975) the neotype deviates slightly from Drechsler's (1944) original description of *A. entomopaga* in having shorter conidiiferous denticles." The new species was *Arthrobotrys pauca* J.S. McCulloch and the fungus captures nematodes by means of spherical adhesive knobs (McCulloch 1977). Unfortunately, however, the fungus had been deposited in the Commonwealth Mycological Institute erroneously as *Arthrobotrys dactyloides* Drechsler IMI 143686 and had been accessioned in the American Type Culture Collection and Centraal Bureau voor Shimmelcultures as ATCC 28704 and CBS 642.80, respectively. Roxon and Jong found that the fungus was not *A. dactyloides*, because it did not make constricting ring traps but adhesive knobs, and considered the fungus to be *A. entomopaga*. They thought that the ovoid to ellipsoid in shape of adhesive knobs changed in pure culture to globose. In addition, they believed that the size and shape of conidia also became shorter and thicker by culture axenically. Thus, the species *Dactylellina entomopaga* (Drechsler) Scholler, Hagedorn & Rubner in the currently adopted classification for predatory orbiliaceous fungi (Scholler et al. 1999) is completely different from *Arthrobotrys entomopaga* Drechsler, mainly because *D. entomopaga* cannot capture springtails.

It is also important for identification of the species that the adhesive knobs of *A. entomopaga* arose only from one or more web-shaped networks of hyphae procumbent on the agar plate. Each mesh of the net was mainly composed of straightly-grown short hyphal branches and branches that changed their direction abruptly to anastomose with other branches at angles approaching a right angle. *Gamsylella gephyropaga* (Drechsler) Scholler, Hagedorn & Rubner also makes the network composed of straightly-grown short hyphal branches, but branches with abruptly changed their direction before anastomosis had never been seen in the network (Drechsler 1937; Abiko et al. 2005).

Saikawa and Arai (1986) reported that the droplet of adhesive knobs of conidium in *Nematoctonus pachysporus* Drechsler in ultrathin sections showed an irregular mass of randomly oriented fibrous material associated with the electron-dense, outer layer of the conidial cell wall. On the other hand, the droplet in *A. entomopaga* was known to be numerous fibrils arranged parallel to each other after liberation from the cell wall surface of adhesive knobs.

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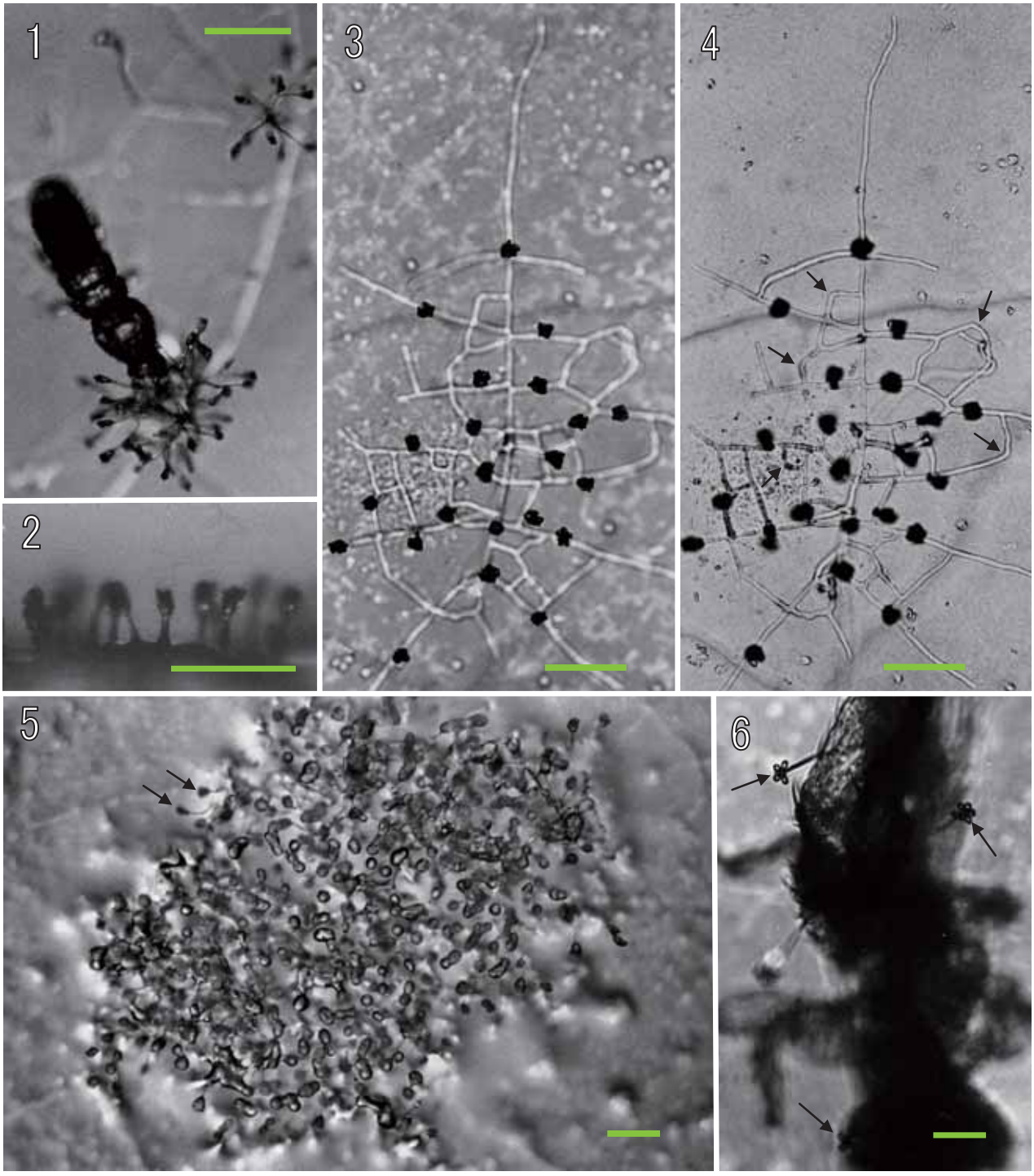
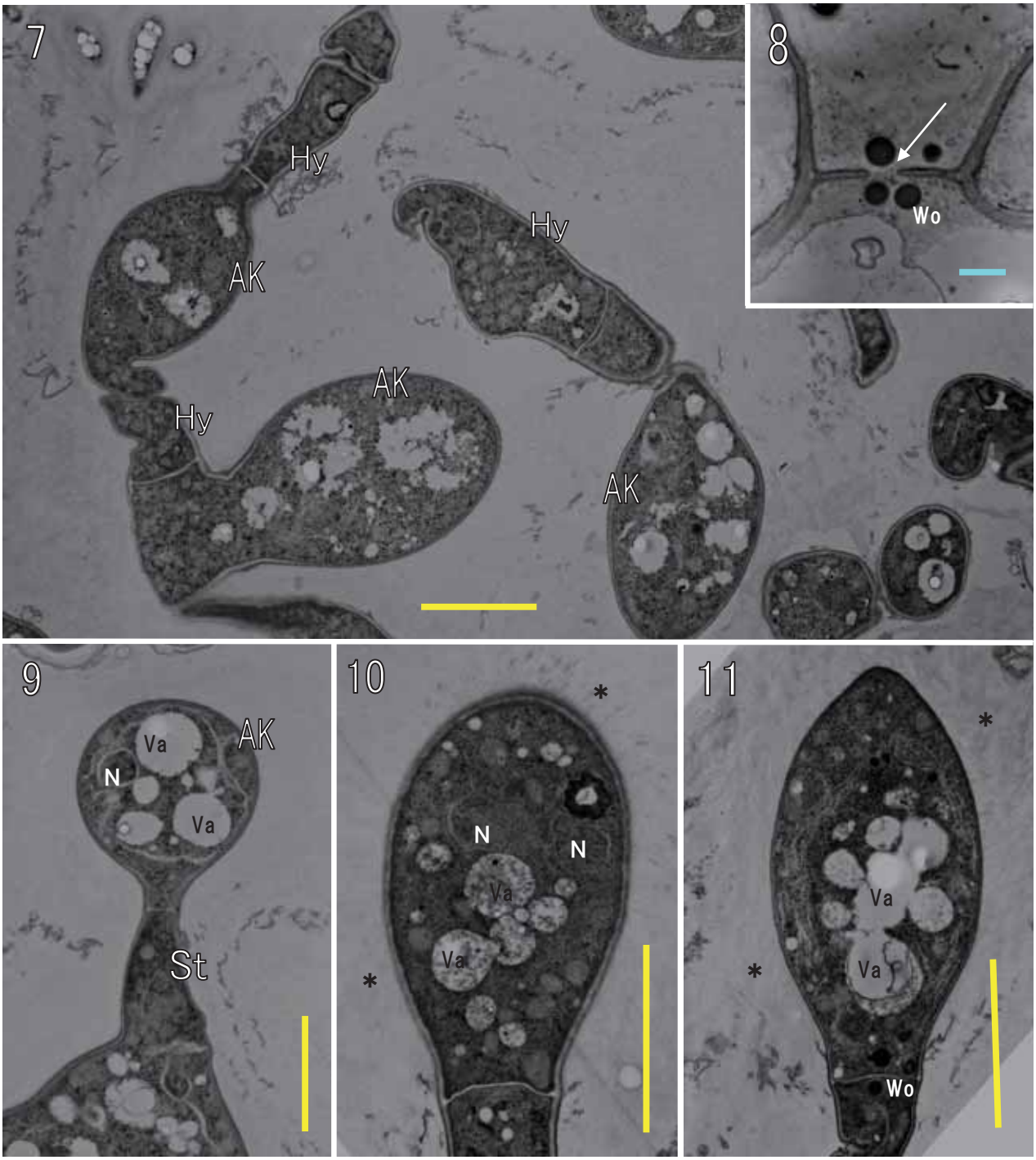
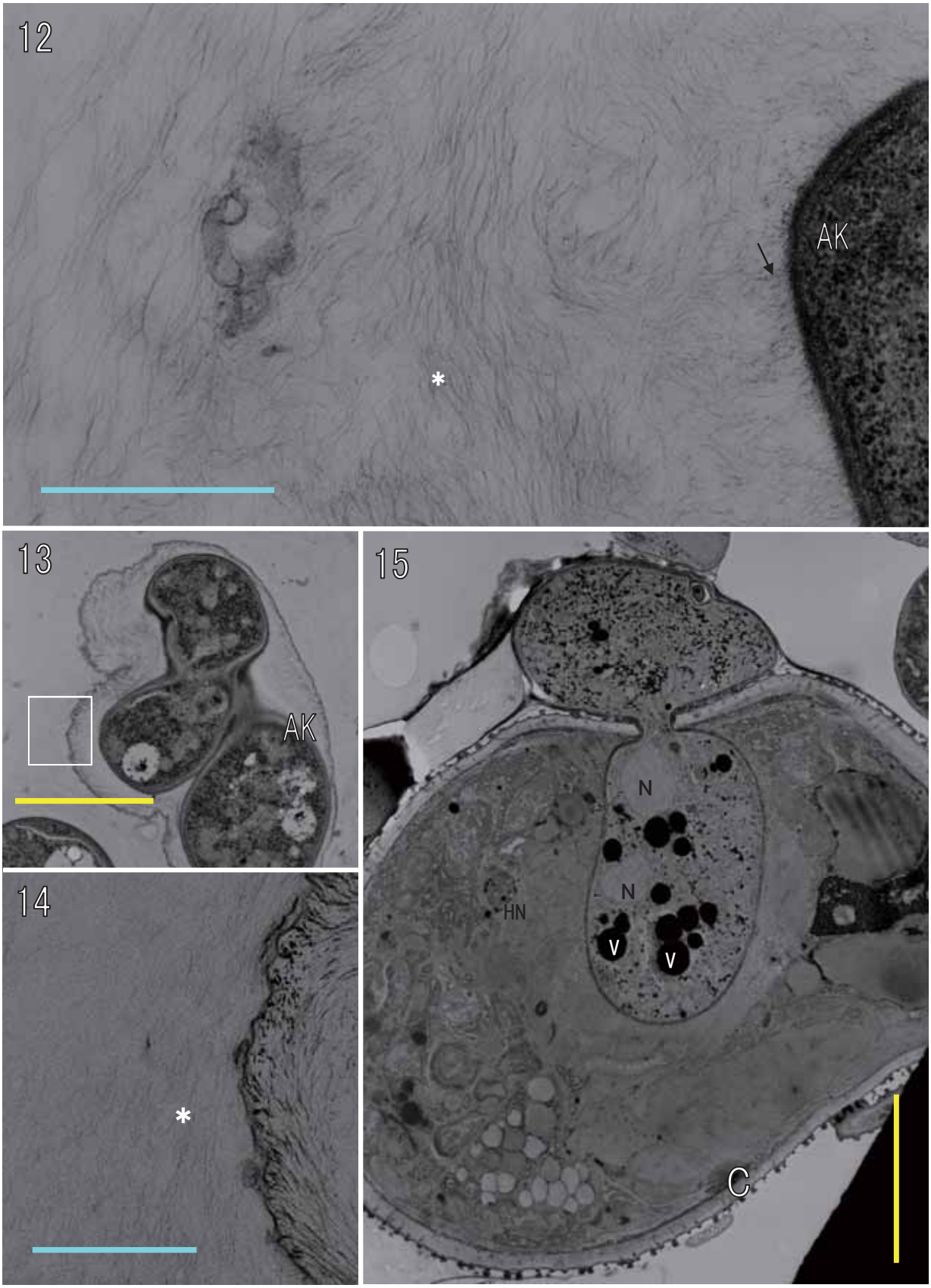


Figs. **1**—**6**. Light micrographs of *Arthrobotrys entomopaga*. **1**. A species of *Folsomia* sp., captured by a group of adhesive knobs. **2**. Side view of adhesive knobs and their stalks, the latter, tapering upward. **3**. Twenty-two adhesive knobs, each aloft by a stalk that was developed on a network of hyphae. The droplet of mucilaginous material surrounding the knob has already been disappeared by desiccation. **4**. The network system of procumbent hyphae in Fig. 3 in focus, showing several of hyphal branches changed abruptly their direction of growth (arrows) before their anastomoses with other branches. **5**. A group of adhesive knobs in older culture. The knobs are still surrounded by a mucilaginous droplet. Arrows show two prostrate adhesive knobs that loose droplets. **6**. Conidia and conidiophores (arrows) formed on a carcass of *Folsomia* sp. Bars, 50 μm.



Figs. **7**—**11**. Electron micrographs of *Arthrobotrys entomopaga*. **7**. A portion of a network in older culture, in which the net is composed of the procumbent adhesive knobs (AK) in addition to branches of hyphae (Hy). **8**. A septum between hyphae composing a network. Arrow shows the simple pore of ascomycetous type. Wo, electron-opaque vesicles of the Woronin body. **9**. Adhesive knob (AK) and the stalk cell (St) in an earlier stage of their development. N, nucleus; Va, vacuole. **10**. Matured adhesive knob sectioned perpendicular, but slightly eccentric to the axis. Fibrils of adhesive (asterisks) are seen. N, nucleus; Va, vacuole. **11**. Matured adhesive knob sectioned through its long axis. Fibrils of adhesive (asterisks) are seen. Va, vacuole; Wo, Woronin body. Bars, 5 μm for Figs. 7, 9—11; 1 μm for Fig. 8.



Figs. **12**—**15**. Electron micrographs of *Arthrobotrys entomopaga*. **12**. Adhesive (asterisk) developed from the cell wall of the adhesive knob (AK), showing fibrous in appearance. Although the fibrils arise almost at right angles from the cell wall (arrow) of the adhesive knob (AK), they become almost parallel to the knob's cell wall. **13**. An aged adhesive knob showing irregular in shape. Adhesive looks restricted to occur around the knob. **14**. Photographic enlargement of a part of Fig. 13, showing the existence of fibrils from other knobs (asterisk). **15**. Fungal penetration into an ocellus of *Folsomia* sp. C, smooth cornea; HN, host nucleus; N, nucleus of fungus; V, electron opaque vesicle. Bars, 5 μm for Figs. 13, 15; 1 μm for Figs. 12, 14.

トビムシ捕食菌*Arthrobotrys entomopaga*の光顕および電顕的研究

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要　旨

わが国で採取した腐葉土から*Arthrobotrys entomopaga* Drechslerが見つかった。これはDrechsler（1944）による発見以来はじめてのことである。この日本産菌株は柄の上に生じた粘着ノブの集まりでトビムシを捕捉した。粘着ノブは卵形または楕円形で，*Nematoctonus*の各種のように全体が無色で粘着性をもった液滴で包まれていた。しかし，*Nematoctonus*と異なり，粘着ノブは蜘蛛の巣状をした菌糸のネットからのみ生じ，ネットのメッシュは直線的に伸びた短い菌糸でできていた。*Arthrobotrys entomopaga* CBS 642.80は*A. entomopaga*ではなく*A. pauca*の形態を示し，球形をした粘着ノブでトビムシではなく，センチュウのみを捕えた。しかも，粘着ノブは液滴に包まれてはいなかった。現在*Dactylellina entomopaga* (Drechsler) Scholler, Hagedorn & Rubnerと呼ばれているトビムシを捕捉しない種は*A. entomopaga*とは別種である。

キーワード: *Arthrobotrys pauca*，ATCC 28704，CBS 642.80，IMI 143686