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FULL PAPER

Saori Abiko · Masatoshi Saikawa · Ratnawati

Capture of mites and rotifers by four strains of *Dactylella gephyropaga* known as a nematophagous hyphomycete

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Abstract Four strains of *Dactylella gephyropaga* obtained from Japan and Indonesia captured mites, as well as nematodes, by means of the adhesive hyphal network composed of columnar processes and rectangular meshes of hyphae, in which each of the meshes was made by additional growth of apex of a columnar process toward that of neighboring process and anastomosis with each other. This is the first report showing that a fungus captured and consumed mites. When immersed under water, the four strains captured rotifers also with the columnar processes by adhesion. The CBS178.37 used for comparison was not the strain of *D. gephyropaga*, and its adhesive network was produced only by repeating development and anastomosis of curved hyphae that captured neither mites and rotifers but only nematodes.

Key words *Dactylella gephyropaga* · Mite · Nematode · Rotifer

Introduction

Several examples have shown that one carnivorous fungus captured more than one species of microscopic animals, each classified in the different taxon of class or phylum. In the case for rotifer-capturing species in Zygomycetes, *Zoophagus insidians* Som. was shown to capture a species of the phylum Gastrotricha in addition to rotifers (phylum Rotifera) by means of short columnar adhesive processes of hyphae, each about 20 μm long and 3 μm wide (Sommerstorff 1911). *Zoophagus pectosporus* (Drechsler) M.W. Dick captured rotifers by a manner similar to that of *Z. insidians* when water including rotifers was poured over the fungus culture (Saikawa et al. 1988), although *Z. pectosporus* was originally known as a species capturing nematodes (phylum Nematoda; Drechsler 1962). Such examples were known also in Hyphomycetes; i.e., *Cephaliophora muscicola* Barron, Morikawa & Saikawa and *C. longispora* Barron, Morikawa & Saikawa, for example, captured tardigrades (phylum Tardigrada) as well as rotifers with predaceous columnar processes by adhesion (Barron et al. 1990). Until the study by Barron et al. (1990), *Cephaliophora* spp. had been thought to be *Z. insidians* (Cooke and Ludzack 1958; Cooke 1979) or a species in the genus *Zoophagus* (Pipes and Jenkins 1965), because their hyphal traps were quite similar in shape and size to those of *Z. insidians*.

In the case of nematophagous hyphomycetes, Drechsler (1937) found that *Dactylella bembicodes* Drechsler captured rotifers by means of the constricting-ring trap, in which three hyphal cells composed of a ring inflated suddenly when the animals entered the lumen of the ring. Drechsler (1944) also found *Arthrobotrys entomopaga* Drechsler captured a species of springtails (phylum Arthropoda), in addition to nematodes, with adhesive knobs.

In the course of our study on carnivorous fungi living in soil, we found that a strain of nematophagous *D. gephyropaga* Drechsler captured and consumed mites in the phylum Arthropoda with an adhesive network composed of rectangular predaceous meshes of hyphae. Then, we examined whether other strains of the species and a strain obtained from Centraalbureau voor Schimmelcultures, Utrecht, Netherlands (CBS strain) also captured mites. In the original report by Drechsler (1937), *D. gephyropaga* had already been known to capture individuals of a testate rhizopod, *Trinema enchelys* Ehrenb., as well as many species of nematodes belonging to genera *Acrobeles*, *Acrobeloides*, *Cephalobus*, *Diplogaster*, *Diploscapter*, *Plectus*, and *Rhabditis* (Drechsler 1937). However, there has been no report that the fungus captured mites by means of hyphal traps until the present time. Although several species in the genera *Neozygites* (Zygomycetes) and *Hirsutella* (Hyphomycetes) have already been known to

S. Abiko · M. Saikawa (✉)
Department of Biology, Tokyo Gakugei University,
Nukuikita-machi, Koganei, Tokyo 184-8501, Japan
Tel. +81-42-329-7514; Fax +81-42-329-7514
e-mail: saikawa@u-gakugei.ac.jp

Ratnawati
Department of Biology Education, University of Negri Yogyakarta,
Yogyakarta, Indonesia

destroy mites, they do not capture but rather parasitize the host by means of adhesion of conidium with their cuticle (Keller and Wuest 1983; Minter et al. 1983), and in fact *H. thompsonii* Fisher is used as an agent to control citrus rust mites in Florida (McCoy and Couch 1979).

Materials and methods

Four strains of *D. gephyropaga* used were obtained from leaf mold collected (1) in the campus of Tokyo Gakugei University, Koganei-shi, Tokyo, Japan in March 1998 (strain G), (2) in Inogashira Park, Mitaka-shi, Tokyo in October 1998 (strain I), (3) in the Purwodadi Botanical Garden, Lawang, Malang, Indonesia in January 1999 (strain M), and (4) at the roadside of Acacia-dori Avenue, Kodaira-shi, Tokyo in June 1998 (strain K). The strain CBS178.37, obtained from the Centraalbureau voor Schimmelcultures (CBS), Utrecht, Netherlands, was also used as a material for comparison. The strain was isolated from a nematode and deposited in CBS by Drechsler.

All fungal strains were cultured on water agar (WA) plates with nematodes (*Rhabditis* sp.), and a small portion of each agar plate was transplanted onto fresh agar plates at an interval of about 2 weeks. The nematodes were multiplied on SA agar (= "Saikawa & Aoki's agar": KNO_3, 0.2 g; $MgSO_4 \cdot 7H2O$, 0.02 g; KH_2PO_4, 0.1 g; K_2HPO_4, 0.3 g; $NaHCO_3$, 0.02 g; Na_2SiO_3, 0.02 g; agar, 20 g; distilled water, 1 liter; Saikawa and Kadowaki, 2002) together with bacteria. The interval of transplantation of nematodes was 4–6 days. Mites in the family Cunaxidae were multiplied by adding them on the agar plate of nematodes at about 3–4 days after inoculation of nematodes. Rotifers [*Lepadella oblonga* f. *oblonga* (Ehrenb.)] multiplied according to the method described by Karling (1952), in which a few pieces of onion (*Allium cepa* L.) skin were dipped in the water layer on WA to nourish rotifers. For optical microscopy, 5 × 5 mm of agar plate including fungus capturing microscopic animals was cut into an agar piece and put onto a glass slide. For observation of rotifers and rotifer-capturing fungus under water, the same preparation was adopted.

Results

The morphology of the four strains of fungi used in the present study essentially corresponded to that of the original description of *D. gephyropaga* by Drechsler (1937), with some differences; namely, the sizes of the top-shaped conidium in the four strains were somewhat larger than that (27–46 × 16–21 μm) in the original description, i.e., 48–61 × 16–22 μm in strain G, 47–62 × 15–20 μm in strain I, 47–64 × 21–25 μm in strain M, and 40–54 × 20–24 μm in strain K. Furthermore, the maximum number of septa in a conidium was five in the present four strains, although it was four in the fungus observed by Drechsler. In addition, the conidiophore in the four strains usually produced a single conidium, but up to ten or more conidia were produced on a single conidiophore in strain M (see Fig. 10). However, these differences are observed only on rare occasions and are recognized as exceptional cases within the range of variation of the species. Typically, the conidium had four septa (Fig. 1).

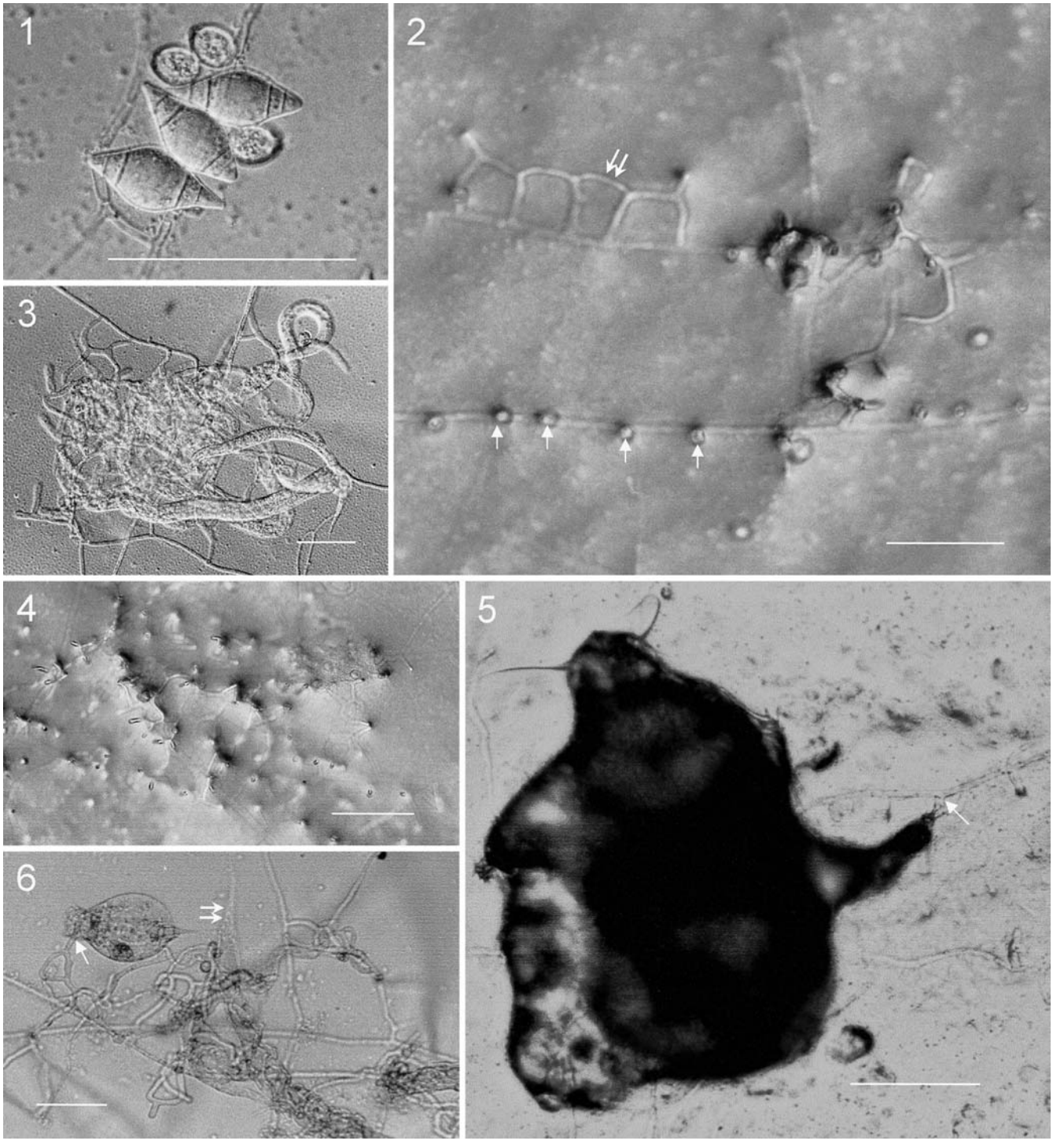
In earlier stages of culture, each strain of the species produced perpendicular columnar processes at close intervals on vegetative hyphae grown superficially on agar plates (Figs. 2, 7). These columnar processes had the ability to capture nematodes (Fig. 8). The apex of the columnar process grew toward that of a neighboring process to make a rectangular mesh (Figs. 2, 11, 14). Finally, the vegetative hyphae had a continuous series of rectangular meshes in a scalariform network, extending usually in one plane (Figs. 2, 11, 14). The scalariform arrangement may later be partly obliterated through the production of a new series of columnar processes (Figs. 4, 14). The network grew larger and larger by capturing nematodes (Fig. 3), followed by the superimposition of additional hyphal meshes, often in other planes than the primary meshes (Fig. 4). It was usually such a network of hyphae that captured mites. All the four strains of *D. gephyropaga* exhibited capture and consumption of mites in the family Cunaxidae (Figs. 5, 9, 12). When a mite came into the area of the larger network system, it was immobilized owing to the attachment of its body with erect adhesive processes in groups and rectangular meshes. Because the mite's body is not transparent, however, the attachment of hyphal traps to the mite's cuticle was not seen, except a portion of the appendages (Figs. 5, 9, 12), and penetration by the hyphae through cuticle and assimilative hyphae within the mite's body were not also seen in the present study. However, hyphae were seen breaking out through host cuticle 1–5 days after the mites were consumed by the fungus.

When the mycelia of the four strains were immersed into water, each strain also captured rotifers with columnar processes produced on the rectangular meshes of the hyphae (Fig. 6). In prolonged culture of the strains for 1–3 weeks, the columnar process newly developed on vegetative hyphae under water still captured rotifers (Fig. 13). Unfortunately, however, the four strains did not capture testate rhizopod, insofar as they were observed.

The strain CBS178.37 produced a network trap system for nematodes composed of circular-shaped meshes of adhesive hyphae (Fig. 15), in which each mesh is made by anastomosis with curved hyphae (Fig. 16). Such a trap formation is not found in any strains of *D. gephyropaga* and, in addition, the trap of the CBS strain did not capture mites nor rotifers but only nematodes in the present study.

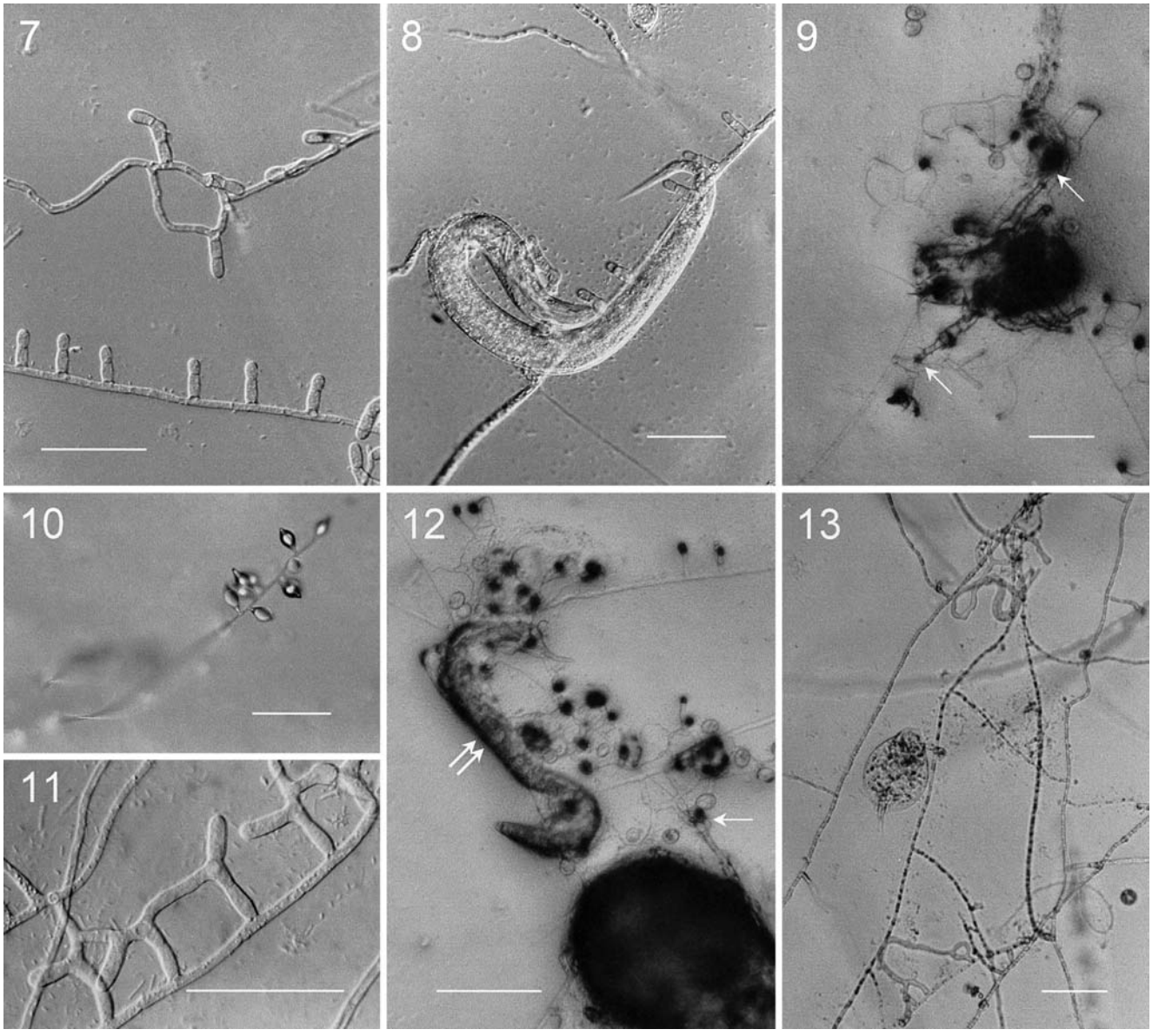
Discussion

Drechsler (1937) observed that strains of *D. gephyropaga* obtained from several localities in the United States captured many species of nematodes by means of a hyphal adhesive network that was composed of rectangular predaceous meshes and that one of the strains also captured a



Figs. 1–6. *Dactylella gephyropaga*. Strain G. **1** Three 4-septate, top-shaped conidia. **2** Columnar processes upright in position (*arrows*) and a network system composed of four rectangular meshes showing ladder-like configuration (*double arrow*). **3** A portion of mycelium capturing nematodes one after another by a hyphal network. **4** Numerous additional columnar processes developed on the hyphal network capturing nematodes. **5** A mite captured by the hyphal network under the mite's body, although most of the network is not seen in this figure. *Arrow* shows the distal portion of one mite appendage, with a columnar process adhering firmly. **6** A rotifer captured by a columnar process (*arrow*) under water. *Double arrow* shows the tail of a nematode that had already been consumed by the fungus. *Bars* 100 μm

testate rhizopod, *Trinema enchelys* Ehrenb., with a columnar process before forming the meshes. Each of the four strains of *D. gephyropaga* used in the present study was found to capture mites in the family Cunaxidae in addition to nematodes in the genus *Rhabditis*. Although the assimilative hyphae were not seen in the mite's body due to the lack of transparence, hyphae were seen breaking out through host cuticle after the mite was consumed by the fungus. This

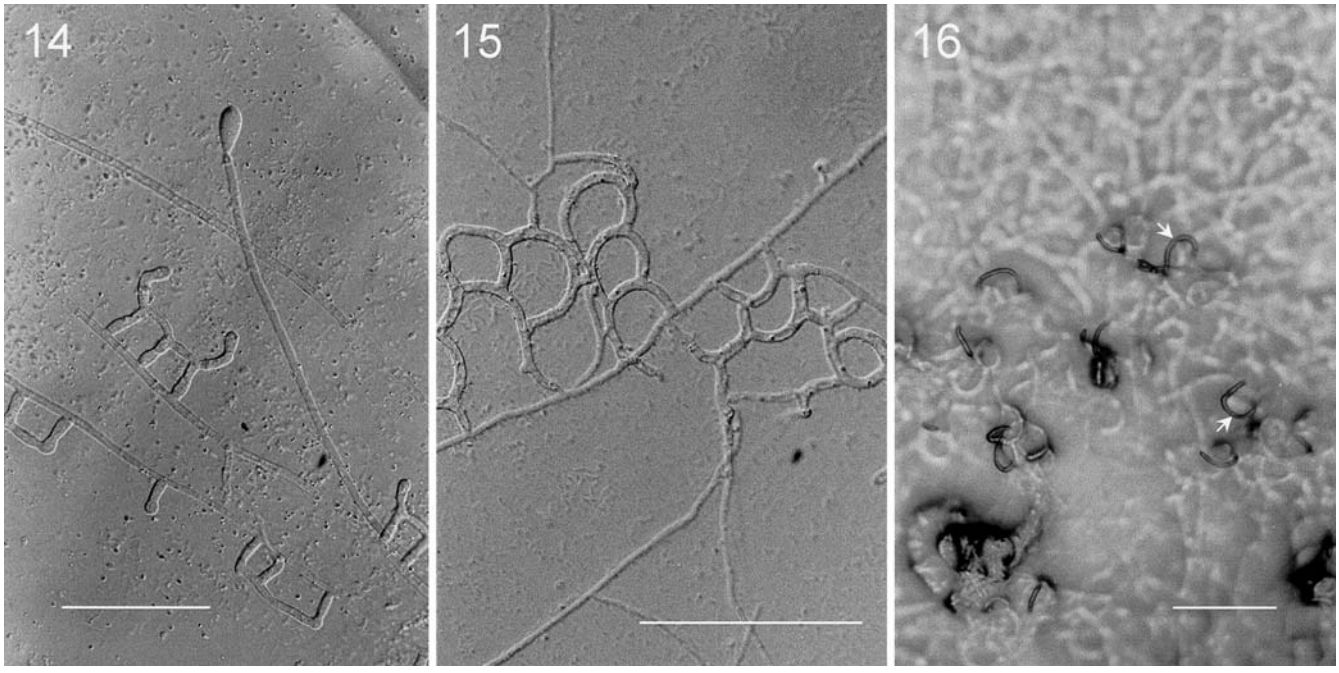


Figs. 7–13. *Dactylella gephyropaga*. Strain I (**7–9**) and strain M (**10–13**). **7** Two vegetative hyphae: each bears columnar processes at a moderate interval, although the hypha seen in the upper figure was distorted during preparation for microscopy. **8** A nematode captured by a few columnar processes. **9** A mite captured and almost consumed by a network system of hyphae. *Arrows* show two points of distal portion of a mite appendage to which the network of hyphae adhere firmly. **10** An aerial conidiophore bears ten conidia, although a conidiophore had usually only one conidium on the distal end. **11** A network system of hyphae composed fundamentally of rectangular meshes. **12** A nematode (*double arrow*) and a mite captured by the fungus. *Arrow* shows the point of connection between the distal end of mite appendage and a portion of the hyphal network. **13** A rotifer captured by a columnar process that was developed from vegetative hypha grown under water for 2 weeks. *Bars* 100 μm

is the first report showing that a fungus captures mites in the phylum Arthropoda by means of predaceous traps of hyphae, although several species of fungi in genera *Neozygites* (Zygomycetes) and *Hirsutella* (Hyphomycetes) have well been known to parasitize mites by means of a conidium that adheres to the cuticle of the worms before infection (Keller and Wuest 1983; Minter et al. 1983).

Up to the present time, *A. entomopaga* was the only known species that captured and consumed a worm in the phylum Arthropoda by means of adhesive knobs. The worm was a springtail of the genus *Sminthurides* in the class Insecta. When a group of adhesive knobs attached mainly to the ventral portion of the worm, they soon secreted a relatively large quantity of an adhesive liquid (Drechsler 1944). In the case for *D. gephyropaga* in the present study, however, the adhesive was scarcely recognized around the hyphal trap under the microscope, and it would be necessary for the fungus to make a larger network by capturing a number of nematodes before capture of mites.

Although *D. gephyropaga* was not found to capture testate rhizopods in the present study, it captured rotifers in the genus *Lepadella* when water containing rotifers was



Figs. 14–16. *Dactylella gephyropaga*. Strain K (**14**) and strain of CBS178.37 (**15, 16**). **14** A network system of rectangular meshes of hyphae and a conidiophore. The latter has an immature conidium on its distal end. **15** A network system composed of circular-shaped meshes of hyphae. **16** The circular-shaped meshes are made by anastomoses with curved hyphae (*arrows*). *Bars* 100 μm

poured over the mycelium on WA in which each of the rotifers was captured by its mouth on an adhesive columnar process just as a testate rhizopod in the genus *Trinema* observed by Drechsler (1937). Saikawa et al. (1988) ascertained that nematode-capturing *Z. pectosporus* also captured rotifers by means of adhesive columnar processes closely resembling those of *Z. insidians* (Sommerstorff 1911) and of *D. gephyropaga*.

Except for the size and shape of the conidium, the strain CBS178.37 did not correspond to that of *D. gephyropaga* in the original description by Drechsler (1937), although it was deposited by himself; i.e., the predaceous network system of hyphae was not composed of rectangular meshes of adhesive hyphae but of curved meshes made by repeated development and anastomosis of curved hyphae (Figs. 15, 16).

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