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FULL PAPER

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Shooting of sporidium by "gun" cells in *Haptoglossa heterospora* and *H. zoospora* and secondary zoospore formation in *H. zoospora*

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Abstract *Haptoglossa* spp. (Lagenidiales, Oomycetes) have been known to parasitize microscopic animals by means of a "gun" cell that shoots an infection cell, named the sporidium, into the body of the animal. A thallus grown from the sporidium changes into a zoosporangium at maturation to produce a number of zoospores that encyst after a swarming period, and the resulting cysts germinate to produce gun cells. In *Haptoglossa zoospora*, endoparasitic in nematodes, the cysts of primary zoospores that swam for about 5 min did not develop gun cells but produced secondary zoospores that swam for about 3 h. After encystment of the secondary zoospores, each secondary cyst germinated to produce a gun cell. In the present study, the secondary zoospores of the genus *Haptoglossa* could be recorded with a videocassette recorder for the first time. The videocassette recording also revealed the infection of a nematodes by *H. zoospora* and *H. heterospora* to be composed of two steps of injection of a sporidium by the gun cell, in which the gun cell came in contact with the cuticle of a nematode and produced a spherical adhesorium on the tip of the cell in 0.07–0.1 s in both species. The adhesorium was ~2 μm in *H. zoospora* and ~4 μm in *H. heterospora*. When the adhesorium inflated to full size, it shot the sporidium into the nematode's body in 0.5–0.65 s and in 0.2–0.5 (or rarely 1.0) s in *H. zoospora* and *H. heterospora*, respectively. After shooting, the empty gun cell with an empty cyst case was separated from the cuticle immediately in both species.

Key words Adhesorium · Gun cell · *Haptoglossa* · Sporidium · Videocassette recording

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Introduction

All known species in the genus *Haptoglossa* Drechsler attack microscopic animals such as nematodes, rotifers, and tardigrades (Drechsler 1940; Davidson and Barron 1973; Barron 1980, 1981, 1989, 1990; Glockling and Beaks 2000a,b; Saikawa et al. 1991). To initiate infection of the animal, the gun cell shoots the sporidium, an infection cellular unit, into the animal's body. The gun cell is produced by germination of the cyst after encystment of a zoospore. In the gun cell, the protoplasm is concentrated in the upper portion of the cell by a large vacuole that occupies the basal portion of the cell. When the tip of the cell comes in contact with the host cuticle, the gun cell shoots a sporidium by sudden evagination of a narrow tubelike structure that runs almost parallel to the axis of the cell (Robb and Lee 1986). Barron (1987) chanced to see the shooting of a sporidium by a gun cell in a rotifer-attacking species, *H. mirabilis* Barron, in which the gun cell mistakenly shot the sporidium outside the animal in about 0.1 s. The sporidium grows to be an endozoic thallus, cylindrical or ellipsoidal in shape with rounded ends. At maturation, the thallus becomes a zoosporangium that produces numerous zoospores and expels them through evacuation tubes, although some species omit the production of evacuation tubes or the zoospore state. The purpose of this study was to determine the mechanism of sporidium shooting by the gun cell in *H. heterospora* and *H. zoospora* in detail and to show the secondary zoospores in *H. zoospora* by videocassette recording.

Materials and methods

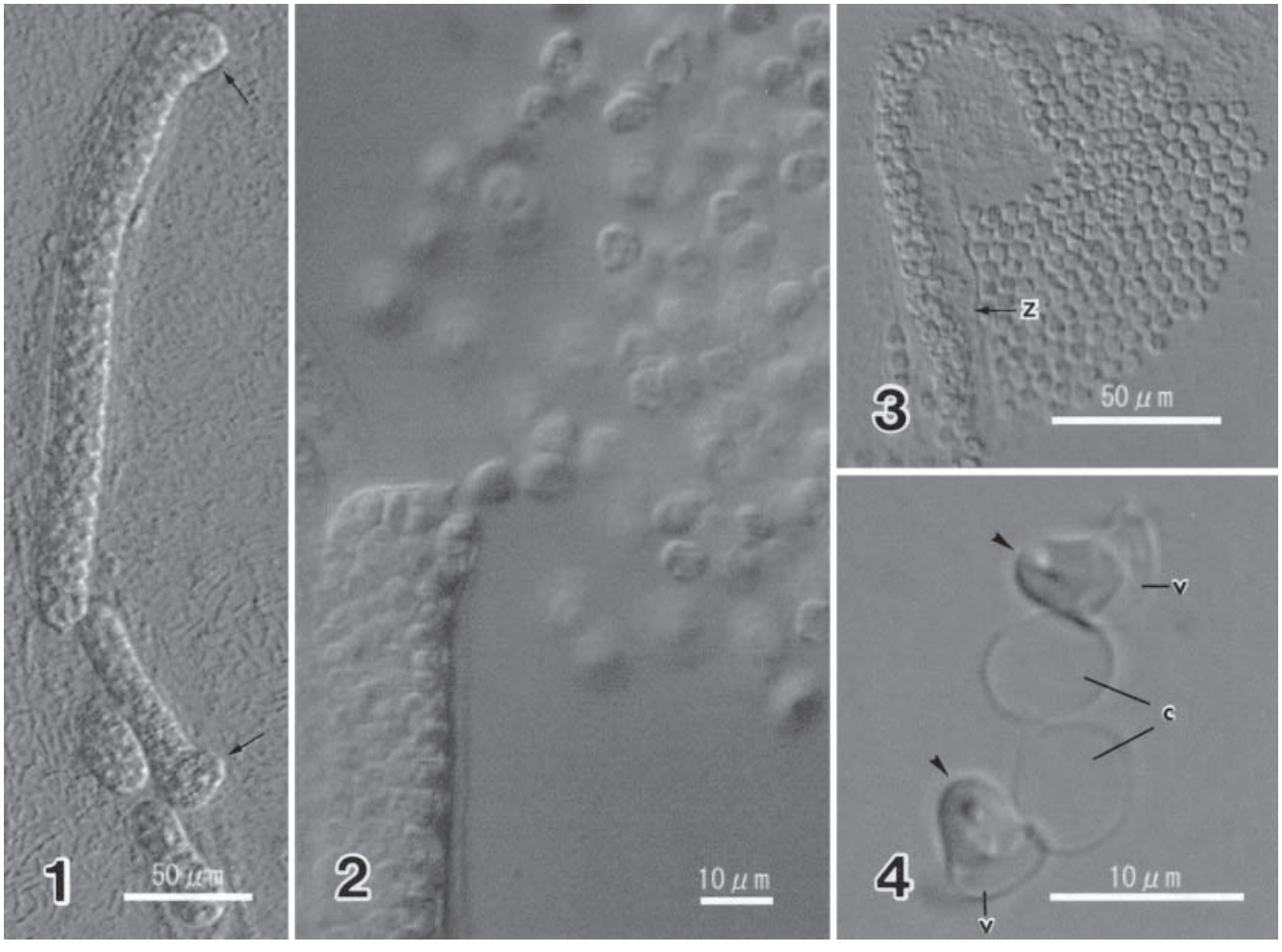
Haptoglossa heterospora Drechsler and *H. zoospora* Davidson & Barron were recovered from leaf mold collected in the Shinjuku Gyoen, an urban public park in Tokyo, Japan, in November 1999 and June 2000, respectively. Each of the cultures was maintained at room temperature on water agar with a small portion (~1 × 1 cm) of old

fungus culture and of nematode (*Rhabditis* sp.) culture added every 2 weeks. Nematodes were allowed to multiply on PDA diluted 1:10. After making microscopic preparations, photographs of fungi were taken with a digital video camera (Sony DXC-D30) that was connected to a videocassette recorder (Sony WV-D9000) and a personal computer (Sony PCV-L520). In the present study, weakened nematodes were chosen for observation because it was difficult to see infection by gun cells if the nematodes moved vigorously.

Results

Haptoglossa heterospora

The thallus [20–250 μm long and (7.5–) 25–30 μm wide] was cylindrical in shape with rounded ends. In a nematode's body, thalli that were infected earlier grew much longer than those that were infected later. At maturation, each thallus became a zoosporangium (Fig. 1) on which one (or, rarely, more) tiny papillae developed on the zoosporangial wall (Fig. 1, arrows). The numbers of papillae on a zoosporangium varied with size; i.e., a zoosporangium less than 50 μm long usually developed only one papilla. After breakdown of the cell wall of the papilla in this species, the zoosporangium expelled cysts or aplanospores 5.0–8.0 μm in diameter, in place of flagellated zoospores. Expulsion occurred continuously for smaller thalli, but often intermittently, at intervals of ~30–50 s, for thalli exceeding 100 μm in length (Fig. 2). However, many cysts remained in the zoosporangium after expulsion was finished (Fig. 3). Each cyst germinated to form a gun cell (5–8 × 4–7 μm; Fig. 4). During germination the protoplasm in the cyst moved into the gun cell (Fig. 4). After germination, the empty cyst remained as a cyst case attached to the gun cell. The gun cell of *H. heterospora* was somewhat triangular or resembled a three-cornered cushion in shape, in which one lobe, bent upward or downward, was slightly longer than the other two lobes. In the gun cell the protoplasm was pushed by a large basal vacuole that occupied the other two lobes and concentrated in the upper portion of the longer lobe. In the vacuole, there were usually one or rarely more particles (Fig. 4) that showed Brownian movement.



Figs. 1–4. *Haptoglossa heterospora*. **1** Four zoosporangia. *Arrows* show papillae. **2** Expulsion of cysts from an opening of papilla. **3** Many cysts still remaining in the zoosporangium (*z*) after finish of expulsion. **4** Two gun cells; each bears empty cyst case (*c*). The basal portion of the gun cell is occupied by a large vacuole (*v*) that pushes protoplasm toward the apical end (*arrowheads*) of the cell

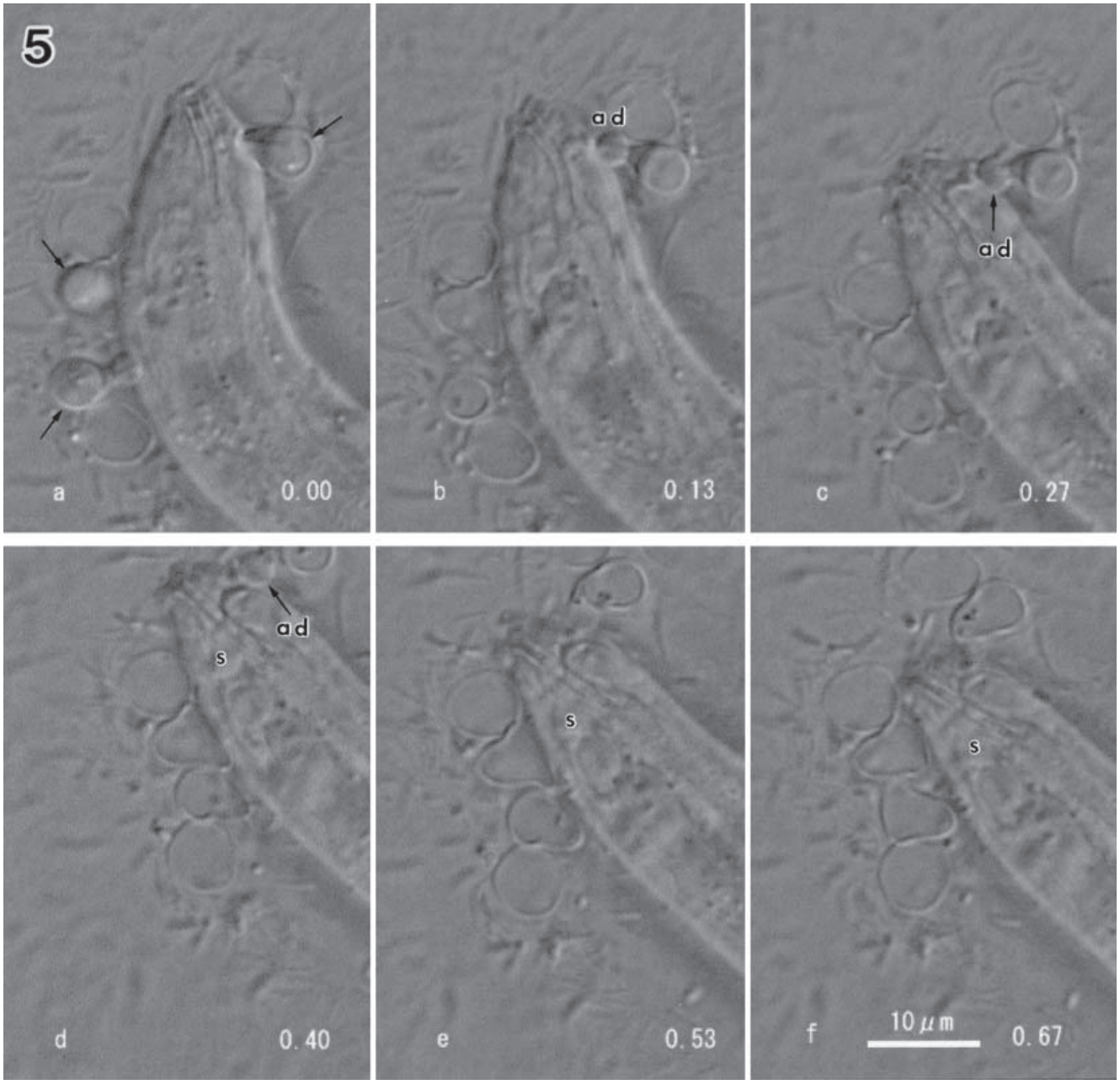


Fig. 5. *Haptoglossa heterospora*. Shooting of sporidia by three gun cells (*arrows*) in 0.67 s (**a–c**). Upper of the three gun cells in **a** produces an adhesorium (*ad* in **b–d**) that shoots sporidium (*s* in **d–f**)

Injection of nematodes by *H. heterospora* gun cells was observed when nematodes had been placed with a needle close to the place where many gun cells were scattered on the agar plate. However, it was difficult to see the injection clearly when a nematode moved vigorously, because it suddenly withdrew itself for a few seconds after being shot by a gun cell. Thus, weakened nematodes were mainly followed in the microscopic field during observation in this study. On the agar plate, the gun cell turns to and fro in the current caused by passing nematodes as these are added. When the distal end of a gun cell came into contact with a nematode's cuticle, a spherical adhesorium developed (~4 μm in diameter) at the tip of the gun cell in 0.07–0.1 s (Figs. 5–7), and the sporidium, ~4 × 2 μm, was shot not by the gun cell directly but by the adhesorium (Figs. 5–7). The gun cell became empty (Figs. 5–7) and was separated from the cuticle of the host immediately together with the adhesorium after shooting. Thus, there was no trace of fungal structure on the nematode cuticle after shooting of the gun cell.

In the present study, an adhesorium was observed to inject the sporidium unintentionally outside the nematode body (Fig. 7). The sporidium and empty gun cell, connected to each other with a tube, were seen immediately after separation of the gun cell from the nematode's cuticle (Fig.

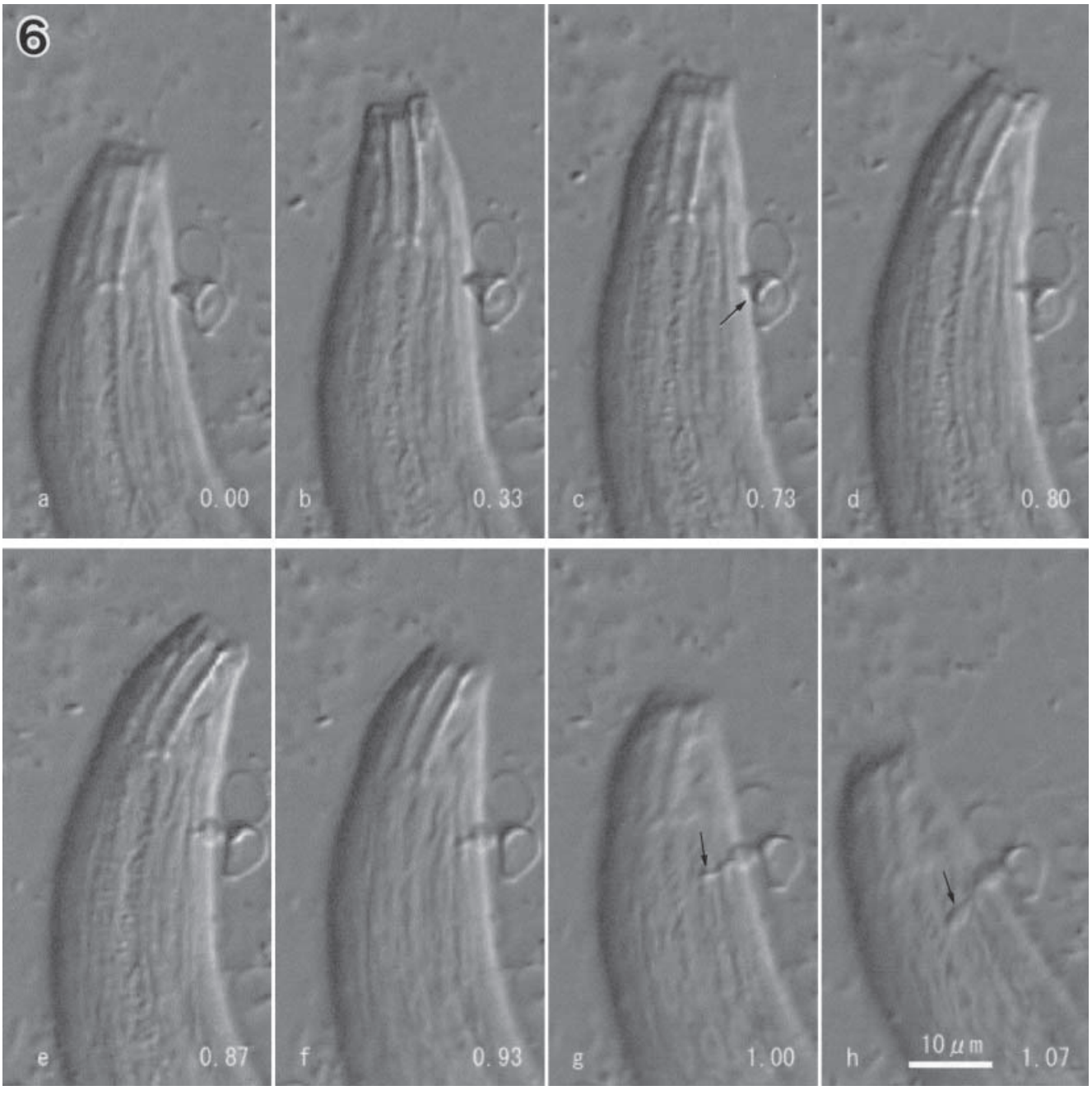


Fig. 6. *Haptoglossa heterospora*. Shooting of sporidium by a gun cell (*arrow* in **c**) in 1.07 s (**a–h**). The sporidium that is shot by the adhesorium is clearly seen (*arrows* in **g, h**)

7d). The direction of the sporidium in Fig. 7d became opposite to that in Fig. 7c because of struggling by the nematode ~20 s after separation.

Haptoglossa zoospora

The fungus attacked the same species of nematodes used for the aforementioned observation in *H. heterospora*. The endozoic thallus was cylindrical in shape, 20–210 μm long and 20–30 μm wide (Fig. 8). Matured zoosporangia (Fig. 9) had 1–6 evacuation tubes (20–50 μm long and 3–5 μm wide) in which the number of the tubes depended on the size of zoosporangia. Primary zoospores, 6.5–7.0 × 4.0–5.0 μm, had two heterokont flagella laterally (Fig. 10) and swam for ~5 min before their encystment. The resulting primary cysts, 5–7 μm in diameter, produced secondary zoospores, 6.0–7.5 × 4.5–6.0 μm (Fig. 11), after rest for 20–60 min. The secondary zoospore was quite similar to the primary one in morphology, but slightly larger in size. It swam for ~3 h before producing its secondary cyst, 4.7–6.0 μm in diameter. Gun cells, 7–8.5 × 3.5–5.0 μm (Fig. 13), developed

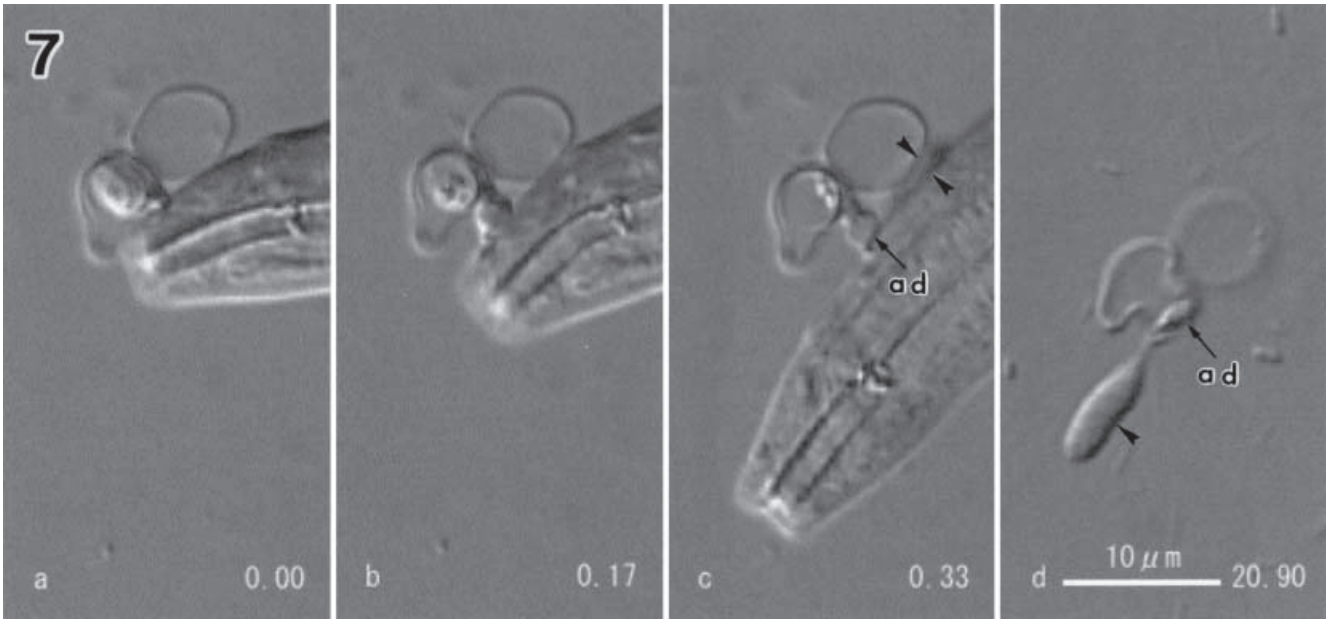
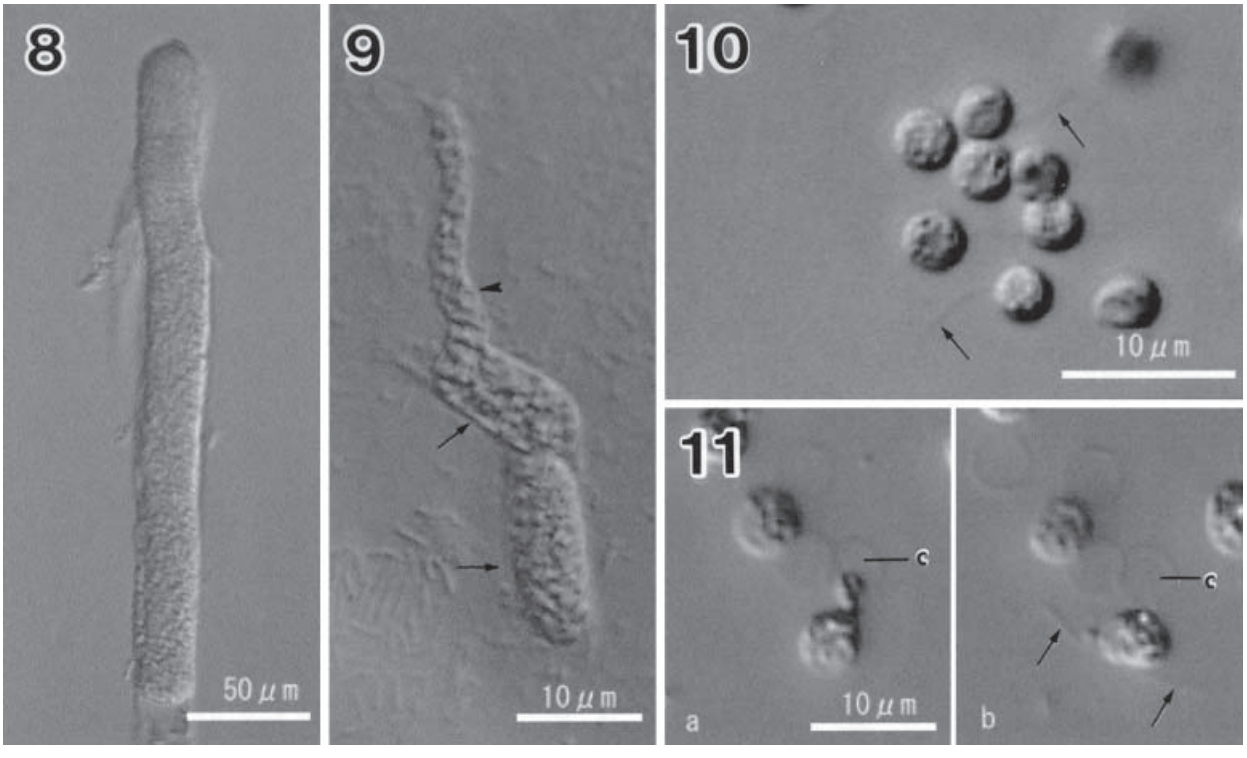


Fig. 7. *Haptoglossa heterospora*. Shooting of a sporidium by a gun cell in 0.33 s (**a–c**). In this observation the adhesorium (*arrows* in **c, d**) shot the sporidium (*arrowheads* in **c, d**) outside the nematode body. After shooting, the gun cell together with both a cyst case and the sporidium immediately separated from the nematode cuticle (**d**)



Figs. 8–11. *Haptoglossa zoospora*. **8** Thallus. Nematode body has already been appropriated by the fungus. **9** Two small zoosporangia (*arrows*), one of which extends an evacuation tube (*arrowhead*). **10** Primary zoospores in swimming for ca. 5 min before their encystment. Each arrow shows one of two flagella. **11** Secondary zoospore formation from cysts (*c*). A zoospore is going out from the cyst (**a**). *Arrows* in **b** show flagella

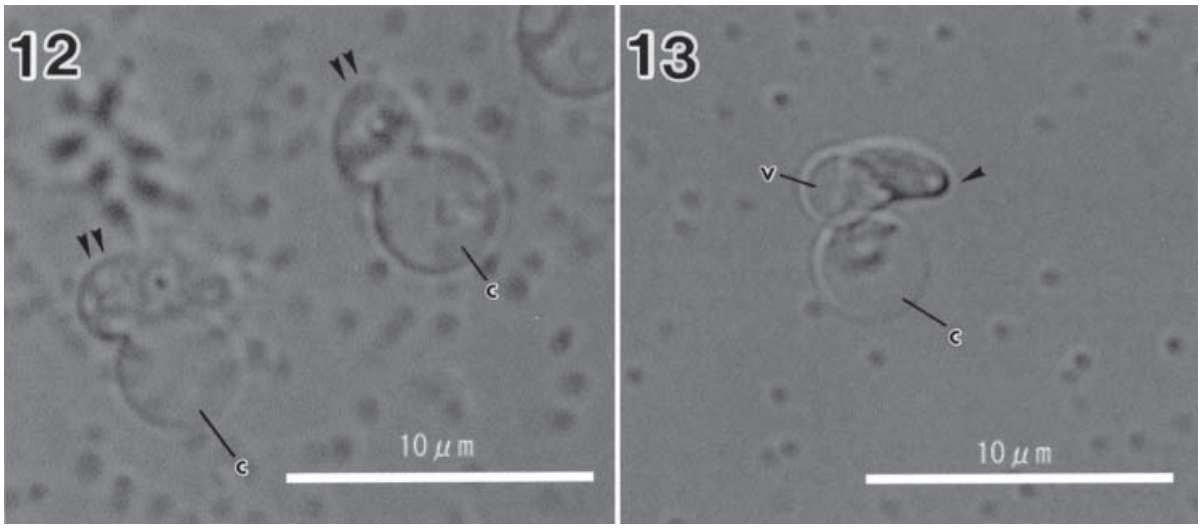


Fig. 12. *Haptoglossa zoospora*. Two cysts (*c*), each developing a gun cell (*double arrowheads*)

Fig. 13. *Haptoglossa zoospora*. Matured gun cell bearing an empty cyst case (*c*). The basal portion is occupied by a large vacuole (*v*) that pushes protoplasm toward the apical end (*arrowhead*) of the cell

immediately after germination of the secondary cysts in *H. zoospora* (Fig. 12).

Except for the difference in the size of adhesorium (~4 mm in diameter in *H. heterospora*; ~2 mm in *H. zoospora*), morphological changes in sporidium shooting were almost identical in the two species.

Discussion

Barron (1987) observed the injection of a sporidium by a gun cell of *H. mirabilis* under the microscope and estimated the injection time was ~0.1 s without using any instrumentation. Using videocassette recording in the present study, injection times for *H. heterospora* and for *H. zoospora* were estimated as 0.33–0.57 and 0.57–0.73 s, respectively, not so fast as *H. mirabilis*. In addition to injection time, the videocassette recording revealed that the infection was composed of two steps, i.e., adhesorium formation and injection of a sporidium from the adhesorium. The sequence of the series is quite similar to that for the plasmodiophoralean fungi *Polymyxa betae* Keskin (Keskin and Fuchs 1969) and *Plasmodiophora brassicae* Wor. (Aist and Williams 1971). The terminology of "adhesorium" in the present study was used by the latter authors. The morphological changes in adhesorium formation and penetration of a cabbage root hair by a single primary cyst are undoubtedly equivalent to the *Haptoglossa* gun cell, although the time series, ~1 min, was slower than those for penetration by *Haptoglossa* spp. As already discussed by Robb and Lee (1986), the relationships between the plasmodiophoralean fungi and *H. mirabilis* were recognized to be close ultrastructurally. Thus, all of these should be accommodated in the same group of organisms in future.

Barron (1987) considered that the mucous pad at the base of the *H. mirabilis* gun cell functions as an adhesive to anchor the cell to the substrate and to maintain the tip being elevated at an angle to the horizontal, and that the position of the cell would increase the chances of the cell meeting with *Adineta* rotifers underwater. In *H. heterospora*, however, the gun cell on the agar plate turns and moves back and forth quite easily in the water current made by passing nematodes. It would not be necessary for gun cells to keep their tips elevated because the sporidium was found in the present study to be shot not by the gun cell but by the adhesorium after its tentative attachment to the cuticle of the nematode.

Barron (1980) observed that *H. mirabilis* was diplanetic, in that secondary zoospores escaped occasionally from spherical cysts. According to his interpretation, both primary and secondary zoospores often encysted almost "immediately" after emergence from zoosporangia and cysts, respectively, and he could not confirm the morphology of the flagella in all cases precisely. On the other hand, in the present observation on *H. zoospora*, primary and secondary zoospores swam for ~5 min and ~3 h before encystment, respectively. In addition, we clearly showed that the primary and secondary zoospores were of the heterokont type.

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References

Aist JR, Williams PH (1971) The cytology and kinetics of cabbage root hair penetration by *Plasmodiophora brassicae*. Can J Bot 49:2023–2034

Barron GL (1980) A new *Haptoglossa* attacking rotifers by rapid injection of an infective sporidium. Mycologia 72:1186–1194

Barron GL (1981) Two new fungal parasites of bdelloid rotifers. Can J Bot 59:1449–1455

Barron GL (1987) The gun cell of *Haptoglossa mirabilis*. Mycologia 79:877–883
Barron GL (1989) Host range study for *Haptoglossa* and a new species, *Haptoglossa intermedia*. Can J Bot 67:1645–1648
Barron GL (1990) A new and unusual species of *Haptoglossa*. Can J Bot 68:435–438
Davidson JGN, Barron GL (1973) Nematophagous fungi: *Haptoglossa*. Can J Bot 51:1317–1323
Drechsler C (1940) Three fungi destructive to free living terricolous nematodes. J Wash Acad Sci 30:240–254
Glockling SL, Beaks GW (2000a) Two new species of *Haptoglossa*, *H. erumpens* and *H. dickii*, infecting nematodes in cow manure. Mycol Res 104:100–106
Glockling SL, Beaks GW (2000b) Video microscopy of spore development in *Haptoglossa heteromorpha*, a new species from cow dung. Mycologia 92:747–753
Keskin B, Fuchs WH (1969) Der Infektionsvorgang bei *Polymyxa betae*. Arch Mikrobiol 68:218–226
Robb J, Lee B (1986) Ultrastructure of mature and fired gun cells of *Haptoglossa mirabilis*. Can J Bot 64:1935–1947
Saikawa M, Oyama M, Yamaguchi K (1991) *Harposporium anguillulae* and *Haptoglossa intermedia* parasitizing tardigrades. Trans Mycol Soc Jpn 32:501–508