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Conidium Development of An Aero-aquatic Hyphomycete, *Peyronelina glomerulata*

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Summary

Conidium morphology and its developmental process in *Peyronelina glomerulata* are clarified by observing natural and cultured materials under a scanning electron microscope. Morphological characteristics of conidia and conidiophores show the adaptation of this fungus in the production and dispersal of conidia in aquatic habitats.

Keywords: aero-aquatic fungus, conidial development, *Peyronelina glomerulata.*

In the course of studies of aquatic fungi in the Bousou Peninsula, Chiba Pref., an aero-aquatic hyphomycete, *Peyronelina glomerulata* Arnoud ex Fisher, Webster & Kane, was found on a submerged decomposing culm of *Cyperus* sp. collected from the margin of a freshwater pond. *Peyronelina* is a monotypic genus and *P. glomerulata* has been reported only from France, U. K. and Canada (1,2,3). The fungus forms crown-shaped conidia comprised of curved arms surrounding a central pile of subglobose cells. Conidia at various stages of development were obtained by incubating the fungus on natural substrates in a moist chamber and by culturing the isolates on agar media. Morphology and the developmental process of the conidium was observed in detail under a scanning electron microscope (SEM).

Materials and Methods

Collection. Decomposing twigs and leaves submerged in water were collected from the Kamega-Jo pond, Misaki-cho, Isumi-gun, Chiba Pref., on 11 Dec. 1995. They were incubated in shallow water in a Petri dish at room temperature (20-25 °C). After several weeks, aquatic and aero-aquatic fungi, such as *Diplocladiella* sp., *Spirosphaera* sp. and *Canderabrum brocchiatum* Tubaki, appeared on aerated parts of twigs and culms. After three months of incubation, conidia of *P. glomerulata* were found on the surface of a wet culm of *Cyperus* sp. Continuing incubation by adding water enabled us to observe many

conidia at various developmental stages.

Isolation. Single conidia were isolated with a fine needle on Cornmeal agar (CMA, Nissui, Tokyo) plates containing 0.01% of penicillin and streptomycin.

Observation under SEM. Small pieces of the natural substrate and agar blocks with conidia were fixed with 1% osmium tetroxide at 4 °C for 12 h. Because the conidia were easily detached from the substrate and floated on the surface of the fixative fluid, they were fixed by putting the materials in a small chamber filled with the vapor of the fixative. The fixed material was dehydrated in ethanol and isoamyl acetate, then critical point dried before coating with platinum. Observation was carried out with a JSM-5400 (JEOL Ltd.) operated at 15 kv.

Results

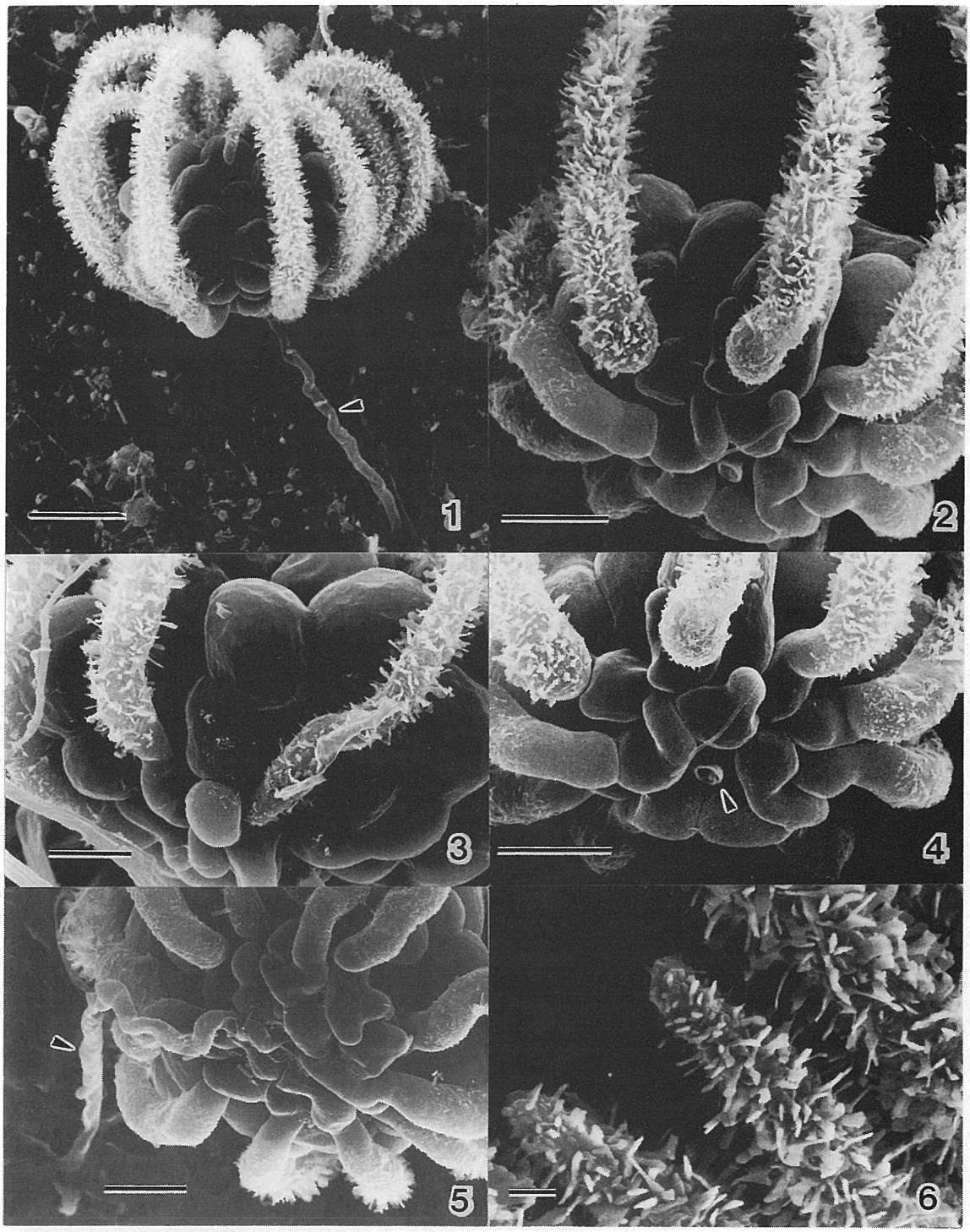
Morphology of conidia

Mature conidia are crown-shaped and composed of central subglobose cells and surrounding arms (Fig. 1). The subglobose cells, 4-7 μm in diam, originate from basal cells of the arms, and 20-30 cells are successively formed by budding (Fig. 3). From 7 to 17 arms arise from the base of the conidium. Each arm is composed of arm cells and a basal cell (Fig. 2). The latter cells are formed by repetitive branching of the primary cell of the conidium attached to the conidiophore (Figs. 4,5). The branched basal cells intricate together and form a disc at the base of conidium (Fig. 5). The arms, 30-60 X 2-3 μm, are attenuate and curved at the apex to gather at the center of the top of the conidium. The arm cells are covered with flat, flake-like spicules, 1-2 X 0.2-0.4 μm, though the basal part of the arm cells is poorly covered (Fig. 4) and the apical part is covered with short spicules (Fig. 6). Conidiophores, 30-45 μm long, arise from a creeping hypha in the substrate. The conidiophore twists or spirals and connects to the center of the basal disc of the conidium (Figs. 1,4,5).

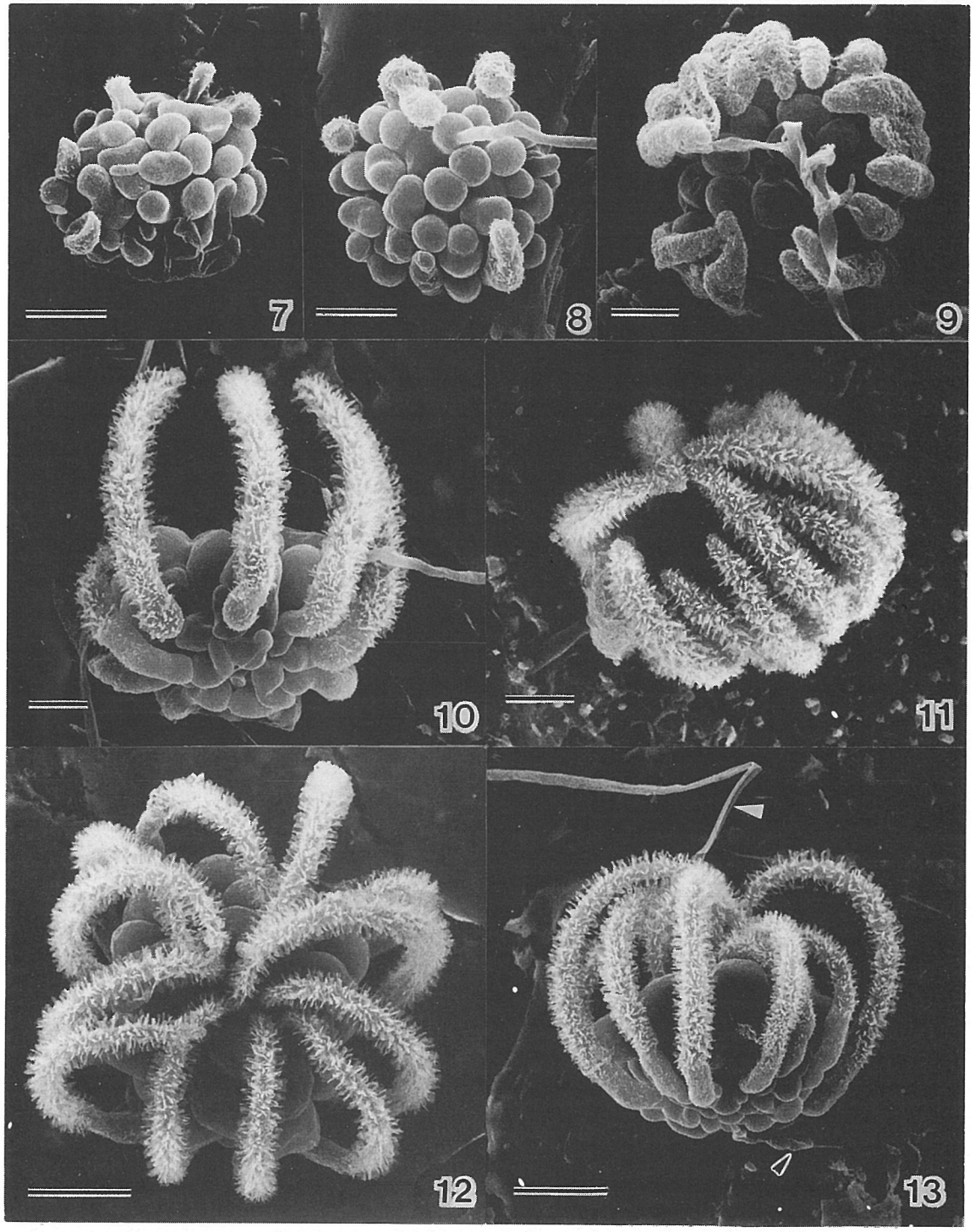
Developmental process of conidia

A conidium in the earliest developmental stage observed on the natural substrate is shown in Fig. 7. The conidium has subglobose cells on a basal disc which is composed of bulbous intricate cells. From the bulbous cells (= the arm basal cells), the subglobose cells emerge by budding, and several arm initials arise from the marginal part of the basal disc. The subglobose cells multiply by budding and the arms grow upward (Fig. 8). Many arms emerge from the basal disc (Fig. 9). At this stage, ornamentation on the arm cell has not yet developed. Then, the arms grow further upward and spicules begin to develop on the surface (Fig. 10). Elongated arms curve at the apices to enclose the pile of subglobose cells (Fig. 11). The tips of the arms gather at the center of the conidium (Fig. 12). The enclosed subglobose cells increased in number to 20-30 cells and in size to 4-7 μm in diam. Finally, the conidium becomes crown-shaped. The arms continue to grow to make a space inside the crown, which serves to entrap air (Fig. 13).

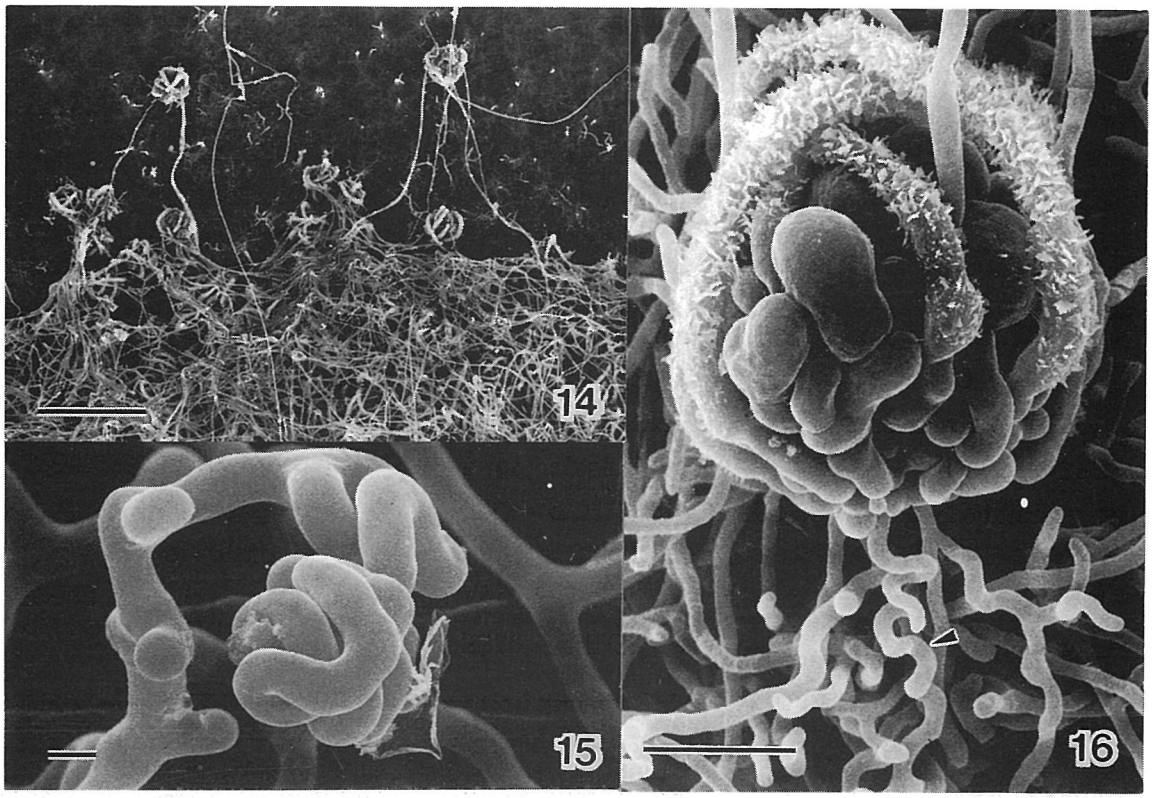
Conidia were observed to germinate from the central subglobose cells, never from the arm cells. Often, a hypha germinates from a subglobose cell, and elongates to more than



Figs. 1-6. *Peyronelina glomerulata*. **1.** A crown-shaped conidium produced on a conidiophore (arrow). **2.** Arms arising from arm basal cells. **3.** Subglobose cells inside the conidium multiplied by budding. **4 & 5.** A disc-shaped base of conidium, composed of intricately branching arm basal cells (arrow in Fig. 4, a detachment scar of conidiophore; arrow in Fig. 5, a twisting conidiophore attaching to the base of conidium). **6.** Apices of arms covered with flake-like spicules. (Bars: 1=10 μm; 2-5=5 μm; 6=1 μm)



Figs. 7-13. Conidium development of *Peyronelina glomerulata*. **7-13.** The develomental process is explained in the text (black arrow in Fig. 13, a conidiophore; white arrow in Fig. 13, hypha germinated from the subglobose cell of conidium). (Bars: 7-11=5 μm; 12,13=10 μm)



Figs. 14–17. *Peyronelina glomerulata* (IFO 32867) cultured on CMA plate. **14.** Colony edge forming conidia on the surface of the medium. **15.** An intricate hyphal ball formed on the surface of mycelium. **16.** Conidium produced in culture (arrow: twisting conidiophore). (Bars: 14= 100 μm; 15=1 μm; 16 = 10 μm)

100 μm and becomes erect (Fig. 13).

The single-conidium isolates IFO 32867 (AN-1505), AN-1506 and AN-1507 readily produced conidia on CMA plates (Fig. 14). In culture, an intricate hyphal ball (Fig. 15), which may be the initial structure of a conidium, was observed on the surface of mycelium. Conidia formed on the agar medium were similar to those on the natural substrate, but often the arms failed to enclose completely the central subglobose cells (Fig. 16). Conidiophores are twisted (Fig. 16), as seen in the specimen on the natural substrate.

Discussion

This is the first report describing the developmental process of the peculiarly shaped conidium of *P. glomerulata* and the first published report of this species from Japan, though two strains were previously deposited in Japan Collection of Microorganisms (JCM) as JCM 9266 and 9267 by Dr. Y. Tsurumi. The two strains were isolated from dead leaves submerged in ponds (pers. commun. from Dr. Tsurumi). *Peyronelina glomerulata* was originally isolated by Arnaud (1) from the surface of perithecia of *Lasiosphaeria* sp. on vegetative debris in France and was not suggested to be a member of aero-aquatic fungi. However, Fisher et al. (2) found this fungus from submerged plant

materials (wood blocks of Scots pine, *Pinus sylvestris;* pinnules of bracken, *Pteridium aquilinum;* leaf petiole of an unidentified tree) collected in U.K. and redescribed the species with Latin diagnosis to make the taxon name validly published. They considered it to be an aero-aquatic fungus. Our study supports their view, because this fungus produced conidia on the aerated parts of a dead culm of *Cyperus* sp. submerged in a shallow water, and the conidia float on the surface of water by entrapping an air bubble inside the arms of the conidium. Morphological characteristics of the fungus observed in this study mostly accord with those described by Fisher et al. (2). However, while they showed that branches (= arms) were straight under very moist conditions, our material did not show this property. They also mentioned that this fungus failed to sporulate on several agar media including corn meal agar, and had to be colonized on bracken leaf in aerated water and incubated on moist filter paper for sporulation. In contrast, our isolates readily produced conidia on the surface of CMA plates. In spite of these differences, the overall similarity in morphology and size of conidia warrants our identification.

Peyronelina glomerulata is an aero-aquatic fungus adapting well to aquatic habitats by forming a floatable propagule entrapping an air bubble inside its arms (3). The spiculate ornamentation on the arms was observed to be hydrophobic, as suggested by Fisher et al. (2), and may work effectively for keeping an air bubble inside the arms and floating on the surface of water. We observed a twisting conidiophore elongated up to 45 μm in accord with the conidium develompent. The flexible conidiophore may serve to keep the developing conidium on the surface of water if water level fluctuates, for example, due to rainfall. A single germinating hypha erecting into the air from a subglobose cell was often observed when the conidia were kept in a moist chamber. This phenomenon was also observed by Dr. Tsurumi on his materials (pers. commun. from Dr. Tsurumi). It is not clear whether this is just the germination of a conidium under moist conditions or the erect hypha works for dispersal or entrappment of a liberated conidium in the aquatic habitat.

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