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Dictyostelids in Japan. XIV. *Dictyostelium rosarium* Raper & Cavender and *Polysphondylium filamentosum* Traub, Hohl & Cavender

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Abstract Two species of dictyostelids, *Dictyostelium rosarium* and *Polysphondylium filamentosum*, were recovered in Japan. *D. rosarium*, characterized by sessile lateral sori and globose spores, is reported after an absence of 35 years and is redescribed here in detail. *P. filamentosum*, characterized by the rebranching of whorled branches and the filamentous elongation of sorophores, is described from indigenous Japanese material for the first time.
Key words: dictyostelids, cellular slime molds, *Dictyostelium rosarium*, *Polysphondylium filamentosum,* taxonomy, Japan.

Dictyostelium rosarium Raper & Cavender and *Polysphondylium filamentosum* Traub, Hohl & Cavender are among the most striking species of dictyostelid cellular slime molds. *D. rosarium*, first isolated from soil in Texas, USA and dung in Mexico and Washington, USA (Raper & Cavender, 1968), is distinguished by a combination of two characteristics, namely, sessile lateral sori and globose spores. Although widely distributed, the species tends to be rare. An exception is North America, where it is locally common in some substrates and regions, for examples in moderately dry or saline soils in southern California (Benson & Mahoney, 1977) and in soils from bat caves in West Virginia (Landolt & Stephenson, 1991). In Japan, it was previously known from only a single isolate taken from dung at Tama Zoo, Tokyo (Lee, 1970). *P. filamentosum*, originally described from isolates obtained from the soils of deciduous and conifer forests in Switzerland (Traub *et al.*, 1981), is characterized by the rebranching of whorled branches and the filamentous elongation of both sorophores and branches. This species is commonly recovered in Switzerland and Germany (Cavender *et al.*, 1995) and also in Hokkaido, Japan (Cavender & Kawabe, 1989), but rarely in North America (Swanson *et al.*, 1999).

In the course of this study on Japanese dictyostelids, *D. rosarium* and *P. filamentosum* were obtained from Honshu, Japan. Although both species have been previously reported from Japan (Lee, 1971; Cavender & Kawabe, 1989), these indigenous dictyostelids have neither been cultured nor observed by the methods followed in the present study. Descriptions of and observational notes on these species are presented here.

The procedures of isolation, cultivation, and observation were the same as those reported previously (Hagiwara, 1989). All the isolates examined were cultured at 20°C on non-nutrient agar with *Escherichia coli*. The technical terms used to describe *Polysphondylium* followed those in Harper (1929); that is, a point on the sorophore from which a whorl of branches grows out of is termed a "node"; a segment between nodes on the sorophore, an "internode"; and a portion of the sorophore above the uppermost node, the "terminal segment." The abbreviations relating to the morphology and dimensions of sorocarps are as follows:

B: Number of branches per whorl.

BB(max): Width of a branch base at the thickest point (μm).

BL: Branch length (μm).

MD: Mean size of spores per isolate (μm).

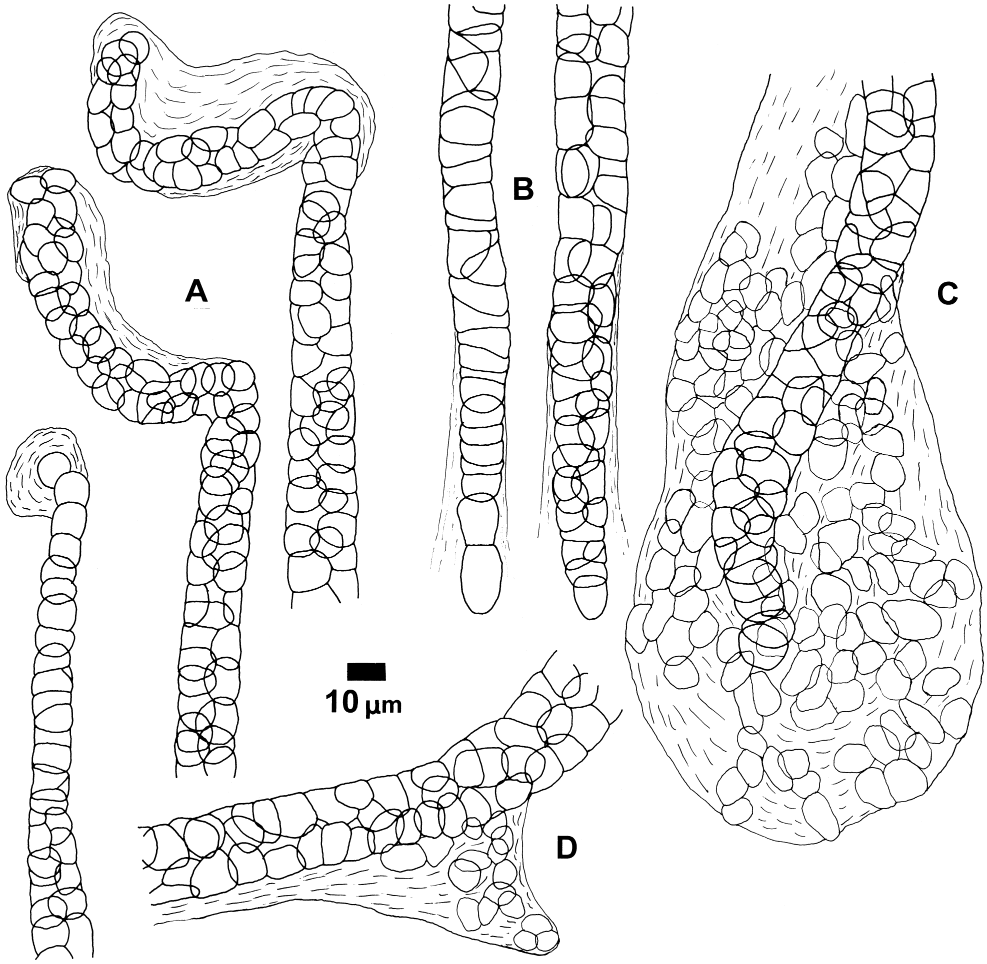

Fig. 1. *Dictyostelium rosarium*. A. Sorophore tips. B. Sorophore bases. C. Sorophore base surrounded by many vacuolated cells. D. Supporter.

N: Number of whorls per sorocarp.

SLL: Length of a sorophore with a lengthened terminal segment (μm).

STL: Width of a lengthened terminal segment at a level 50 μm below the apex (μm).

TLL: Length of a lengthened terminal segment (μm).

Dictyostelium rosarium Raper & Cavender, J. Elisha Mitchell Sci. Soc., **84**: 31 (1968).

(Figs. 1 & 2)

Sorocarps usually solitary but sometimes clustered, sometimes sparsely and irregularly branched, often prostrate. Sorophores colorless, 0.8–6.2μm in length, sometimes exceeding 8μm if prostrate, with 3–7 sessile lateral sori, sometimes with supporters if prostrate (Figs. 1D & 2H); bases acuminate or clavate (Figs. 1B & 2G), 16–24 μm in diam at thickest point; tips obtuse, simple to compound (Figs. 1A & 2F), 6–16 μm in diam at a level 50 μm below the apex. Sori white, globose to subglobose; terminal sori 40–290 μm in diam; lateral sori 30–260 μm in diam. Spores hyaline, globose, smooth, mostly 4.9–5.6 (MD: 5.3) μm in diam. Pseudoplasmodia radial (Fig. 2A), 0.5–23 mm in diam, centralized, not migrating without sorophore formation, sometimes producing plural sorogens.

Habitat: On dung and in field soil.

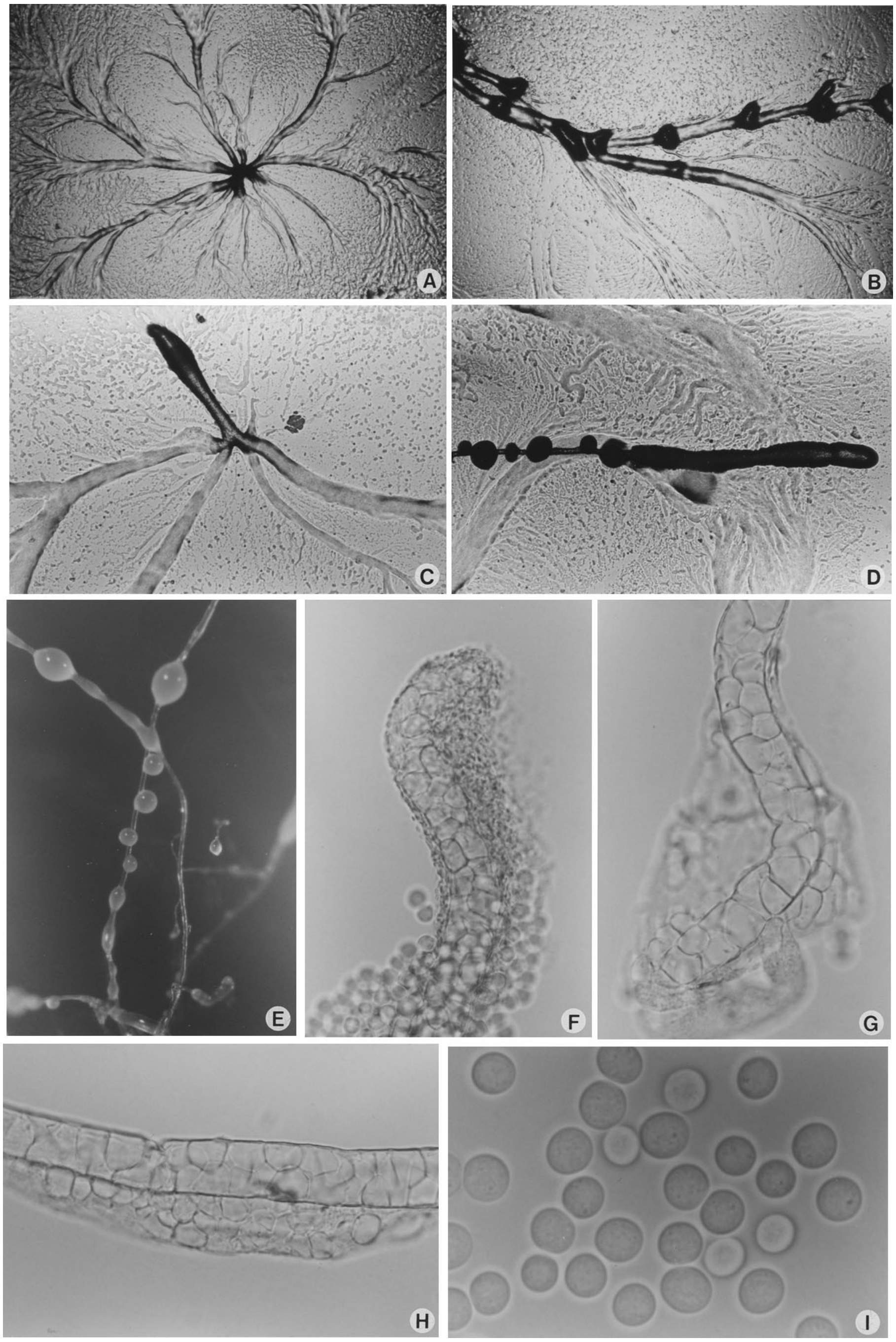

Fig. 2. *Dictyostelium rosarium*. A. Pseudoplasmodium. ×12. B. Nodules produced in the process of aggregation. ×12. C. Sorogen arising from a single aggregation. ×28. D. Part of an immature sorocarp. ×28. E. Lateral sessile sori along sorophores. ×20. F. Sorophore tip partially surrounded by slime materials. ×460. G. Sorophore base accompanying vacuolated cells. ×460. H. Supporter. ×460. I. Spores. ×1150. All figs., Strain S-12.

Isolate examined: S-12, from a field soil at ca. 60 m alt. in the suburbs of Fukaya-shi, Saitama Pref., 8 July 2005.

Distribution: Asia; Japan, Pakistan. N. America; Mexico, USA. Oceania; New Zealand.

Dictyostelium rosarium is easily distinguished from other dictyostelids by two characteristics, namely, its globose spores (Fig. 2I) and sessile sori borne at intervals along the upright sorophore (Fig. 2D & 2E).

As a third distinguishing characteristic of *D. rosarium*, Cavender *et al.* (2002) added the nodular aggregation pattern. Japanese isolate S-12 produced typical nodules during the inflow of myxamoebae into an aggregation center (Fig. 2B). In a moderate pseudoplasmodium, such nodules eventually flowed into the aggregation center and yielded a single sorocarp (Fig. 2C).

Dictyostelium rosarium is known to be widely distributed and common in North America (Raper, 1984). Cavender *et al.* (2002) found this species in New Zealand and confirmed that *D. rosarium* is scattered not only in the Northern Hemisphere but also in the Southern Hemisphere. In Japan, this species was first obtained from wallaby dung at Tama Zoo, Tokyo in 1970 (Lee, 1971). A second Japanese isolate was recovered in this study after an absence of 35 years. *D. rosarium* seems to be quite rare in Japan.

Polysphondylium filamentosum Traub, Hohl & Cavender, Amer. J. Bot., **68**: 169 (1981).

(Figs. 3–5)

Sorocarps usually solitary but sometimes clustered, with 1–4 nodes, with 2–14 branches per whorl, often with conspicuously lengthened terminal segments (Fig. 4G), sometimes prostrate. Sorophores colorless, stout, 1.1–2.3 (SLL 3.5–10.4) mm in length; bases robust, usually round (Figs. 3B & 5E), 22–68 μm in diam at the thickest point; tips acuminate (Figs. 3A & 5A), sometimes nearly obtuse if lengthened (Figs. 3C & 5B), simple, 3.6–9.6 (STL 3.2–8) μm in diam at a level 50 μm below the apex; terminal segments 231–462 (TLL 2000–5600) μm in length; internode segments 436–559 (–1103) μm in length. Branches colorless, stout, 255–627 μm in length, sometimes rebranching (Figs. 3F, 4E, 4F & 4I), sometimes producing filamentously lengthened tips; bases round, 11–40 μm in diam at the thickest point; tips acuminate (Figs. 3D & 5C), sometimes nearly obtuse if lengthened (Fig. 3E), simple. Sori white, globose; terminal sori 42–106 (–127) μm in diam, diminishing to almost zero if tips are lengthened; lateral sori 42–106 μm in diam. Spores hyaline, elliptical to slightly reniform, usually 2.0–2.4 times longer than broad, smooth, mostly 8.4–10.7×3.9–5.0 (MD: 9.2–9.9×4.1–4.7) μm, with conspicuous unconsolidated polar granules (Fig. 5G). Pseudoplasmodia radial (Fig. 4A), 0.15–6.5 (–8.5) mm in diam, centralized, not migrating without sorophore formation.

Habitat: In forest soil.

Isolates examined: CSB6, from soil of a deciduous forests at ca. 30 m alt., Boso Fudoki-no-oka, Chiba Pref., 2 July 1996; NYA55 & NYA65, from the soil of larch forests, 1300–1600 m alt., the Yatsugatake Mts., Nagano Pref., 8 May 2005.

Index values of sorocarp dimensions

N: 2.3±0.5 (n=52)

B: 5.3±2.3 (n=173)

BL: 434±100 (n=57)

BB(max): 26.3±6.5 (n=57)

Distribution: Asia; Japan. Europe; Germany, Switzerland. N. America; USA.

Polysphondylium filamentosum is characterized by very long filamentous terminal segments of the sorophores (Fig. 4G), the rebranching of whorled branches (Fig. 4F), and the fairly large elliptical spores with conspicuous, unconsolidated polar granules (Fig. 5G).

Polysphondylium filamentosum is easily distinguished from *P. pallidum* Olive, the most common species among white Polysphondylia, by its elongated terminal segments of the sorophores. Such an elongation has been reported in *P. candidum* Hagiwara (Hagiwara, 1973) and *P. psedocandidum* Hagiwara (Hagiwara, 1979). *P. candidum* closely resembles *P. filamentosum* not only in morphological features but also in habitat, and therefore, both the species are fairly difficult

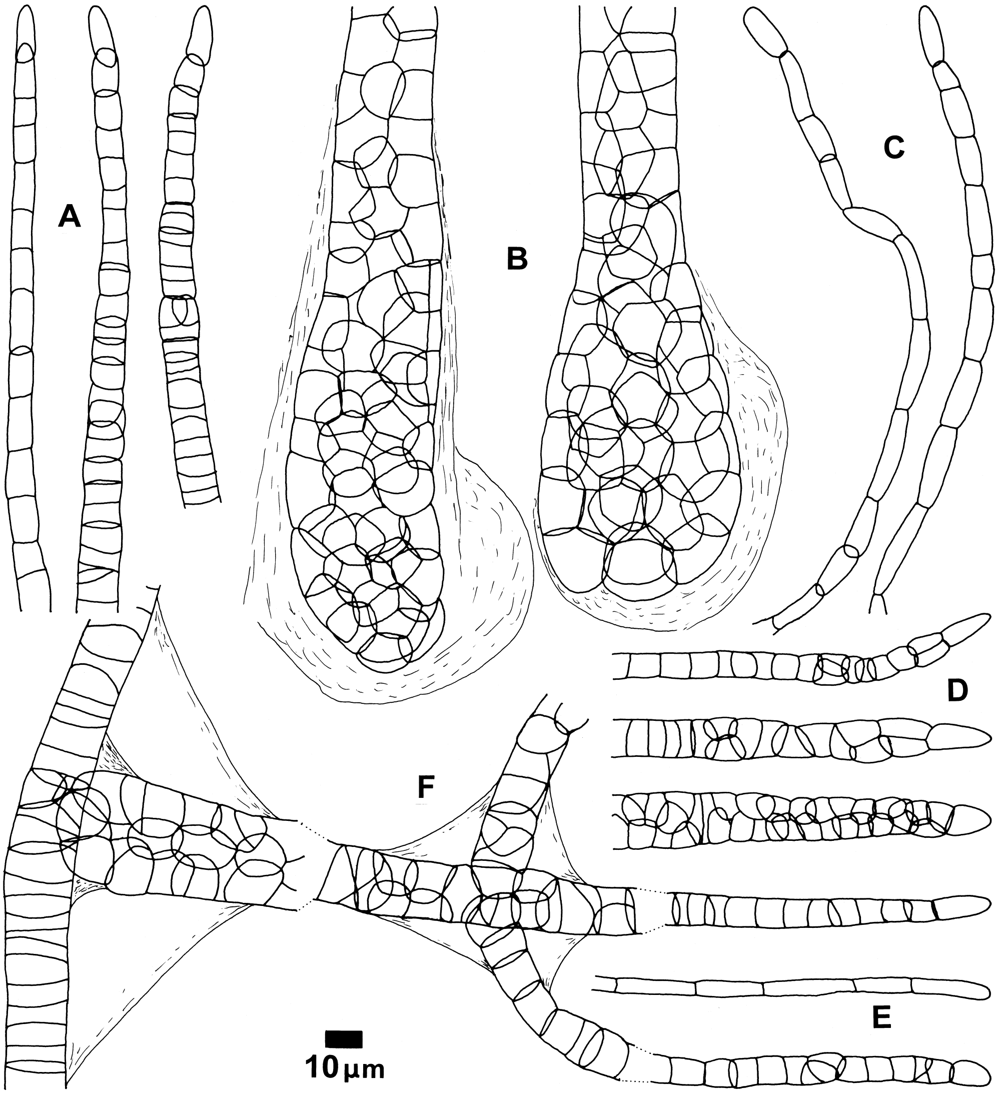

Fig. 3. *Polysphondylium filamentosum*. A. Sorophore tips. B. Sorophore bases. C. Lengthened sorophore tips. D. Branch tips. E. Lengthened branch tip. F. Branch with two secondary branches (branchlets).

to distinguish from each other. In *P. candidum*, however, the rebranching that characterizes *P. filamentosum* has not been reported. In addition, *P. candidum* exhibits a well-defined regular branching pattern with uniform branch lengths and uniform sorus size per whorl. In *P. pseudo-candidum*, rebranching has not been reported. This subtropical species is easily distinguished from *P. filamentosum* by its delicate sorocarps with small spores.

The pseudoplasmodia of the isolates examined typically had radial aggregation (Fig. 4A). Under certain conditions, however, many pseudoplasmodia were produced in a narrow area and their radial streams often anastomosed with each other (Fig. 4B). In other conditions, a large pseudoplasmodium yielded several satellite secondary aggregation centers and/or plural sorogens at the primary aggregation center (Fig. 4C). Such a large pseudoplasmodium often accompanied ring-like pseudoplasmodia with a vortex motion (Fig. 4D).

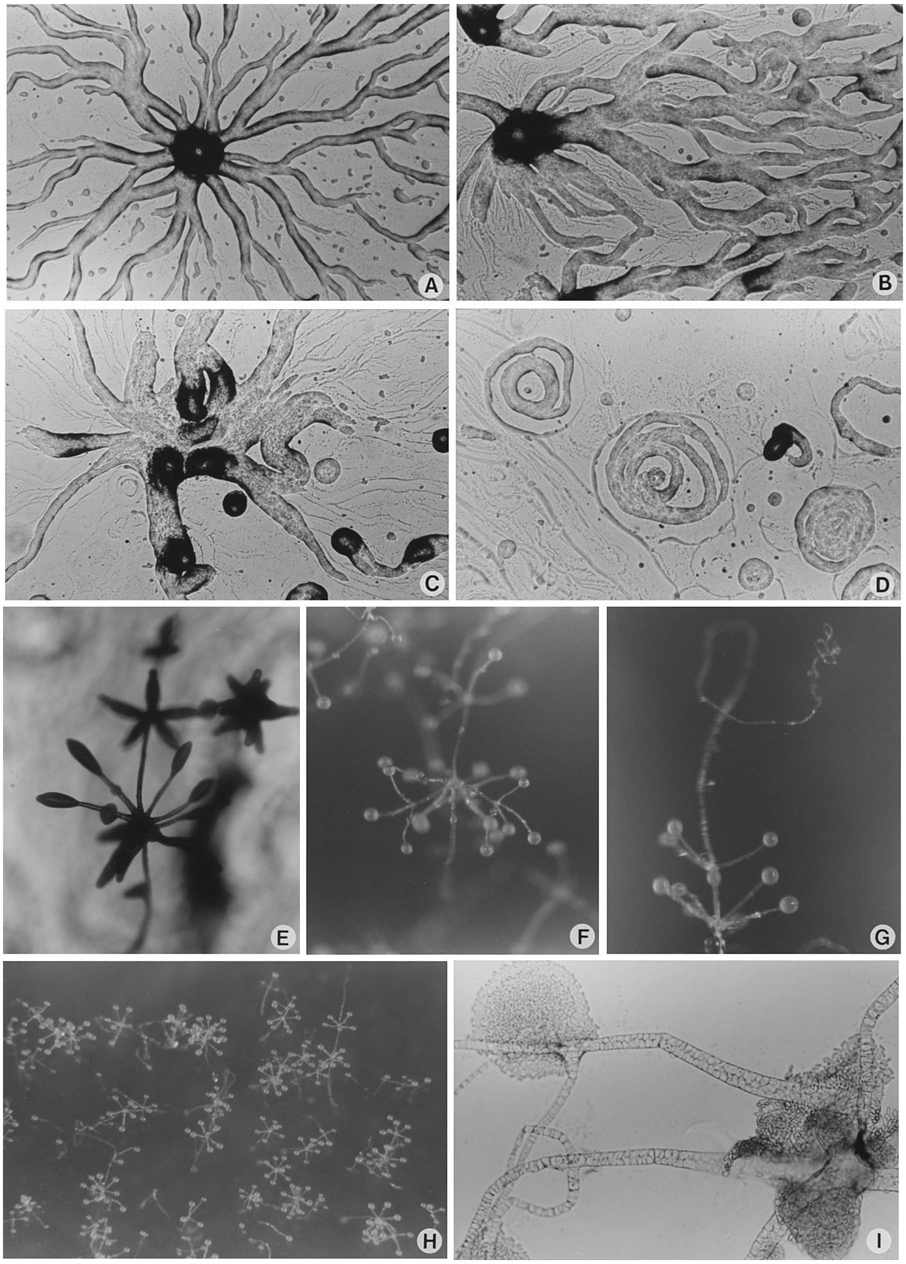

Fig. 4. *Polysphondylium filamentosum*. A. Pseudoplasmodium. ×28. B. Pseudoplasmodia developed in a narrow area. Their radial streams often anastomose each other. ×28. C. Sorogens irregularly produced at the center of a large aggregation. ×28. D. Ring-like pseudoplasmodia. Their streams flow like the vortex. ×28. E. Rebranching of whorled branches. ×28. F. Whorled branches with secondary branches (branchlets). ×20. G. Lengthened terminal segment of a sorophore. ×20. H. Growth habit. ×8. I. Two branches with whorls of branchlets. ×115. Figs. B, F, H & I, strain CSB6; Figs. C-E, strain NYA55; Figs. A & G, strain NYA65.

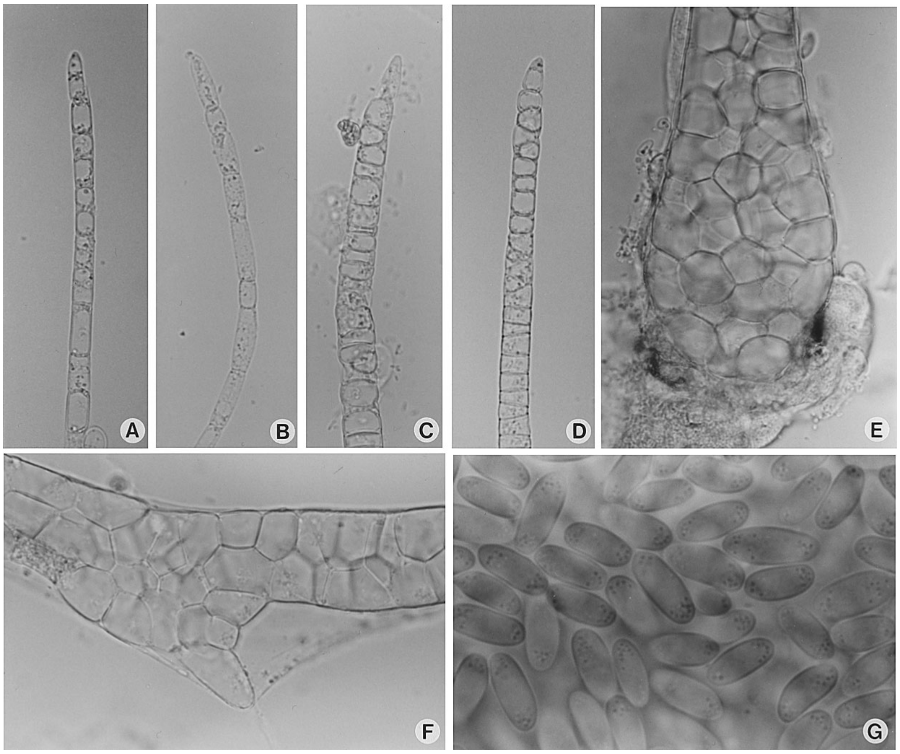

Fig. 5. *Polysphondylium filamentosum*. A. Tip of a sorophore. ×460. B. Tip of a lengthened sorophore. ×460. C. Tip of a branch. ×460. D. Tip of a branchlet. ×460. E. Sorophore base. ×460. F. Hook-like structure of a prostrate sorophore at the point of contact with agar. ×460. G. Spores. ×1150. Figs. A & D, strain CSB6; Fig. E, strain NYA55; Figs. B, C, F & G, strain NYA65.

Branches were usually stout or heavy at their bases and often longer than the unlengthened terminal segments of sorophores. The branch length and sorus size per whorl exhibited some degree of variation (Fig. 4H). Additionally, the branching angles from the sorophores were also variable. The observed variability could be attributed to the disproportionately large size of branch bases as compared with the thickness of the sorophore attached to the branches.

Rebranching is an important taxonomic criterion of *P. filamentosum*. Such a phenomenon is conspicuous in cultures grown on 0.1 LP agar (Traub *et al.*, 1981); however, it is not accentuated on non-nutrient agar even though terminal segments are often lengthened (Fig. 6).

Finally, it is noteworthy that prostrate sorocarps produced hook-like structures at the point of contact with agar (Fig. 5F). Such structures have been observed in both *D. rizopodium* Raper & Fennell and *D. vinaceo-fuscum* Raper & Fennell (Raper & Fennell, 1967), and also in *D. aureocephalum* Hagiwara (Hagiwara, 1991, 1998); however, they have not been seen in Polysphondylia.

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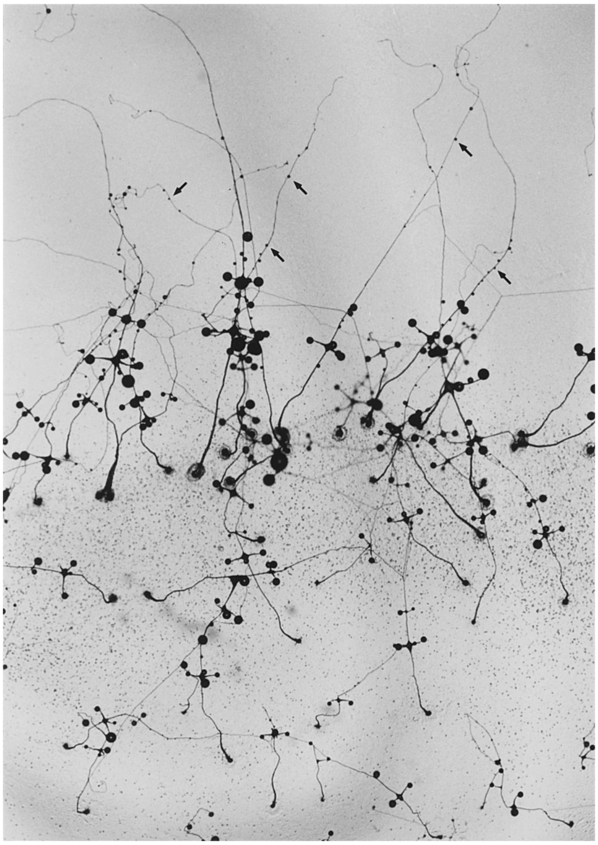

Fig. 6. *Polysphondylium filamentosum*. Growth habit of the type strain SH-1 (ATCC44370) cultured at 20°C on non-nutrient agar with *Escherichia coli*. The small black objects attached to the lengthened terminal segments of sorophores are droplets of water. Examples of these are indicated by arrows. ×8.

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