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A New Species of Cellular Slime Molds from Korea, *Dictyostelium floridum* sp. nov.

Hong, Jeong-Soo and Nam-Kee Chang
(*Department of Biology Education, Seoul National University, Seoul*)

韓國產 細胞性 粘菌의 1新種 *Dictyostelium floridum* sp. nov.

洪 廷 守 · 張 楠 基
(서울大學校 師範大學 生物教育科)

ABSTRACT

A new species of *Dictyostelium*, isolated from the subalpine coniferous forests of Halla mountain, was described. The most noteworthy morphological characteristics of this new species were broad petal-like aggregates, concentrated long branches near the bases, typically clustered and delicate sorocarps. *Dictyostelium floridum* sp. nov. was also characterized by 1) elliptical spores with distinctive polar granules, 2) strongly tapering and rough surface of sorophores, 3) frequent occurrence of expanded conical bases, 4) the congealed slime substrate where the several bases were anchored into, 5) compound and clavata tips. It was quite sensitive to environmental conditions, particularly temperature. Macrocysts and microcysts were not observed. This species can be cultured satisfactorily in assosiation with *Escherichia coli* upon agar media of weak nutrient content, 0.1% lactose-peptone, under diffuse light and darkness. Optimum temperature for growth and development was about 18-22℃, below that of most other species of the family.

INTRODUCTION

During the investigation of distribution of cellular slime molds in relation to the altitudes and forest types of Halla mountain of Korea (Hong *et al.*, 1992a, b), two *Dictyostelia* which differed subsequently from any of existing species were isolated from sample soils. These have been cultured repeatedly, and observed and compared in detail about for two years. One of these was previously reported as a new species, *Dictyostelium flavidum* Hong et Chang (1992a). The other species also have exibited several distinctive features which differed from the published species and here was identified and described as another new species. This was designated as *Dictyostelium floridum* Hong et Chang sp. nov., based on its petal-like aggregation and the flower-like appearance of gross morphology of the clustered fruiting body.

The taxonomic studies of dictyostelids were promoted coincidentally with ecological investigations. Up to the present, more than fifty species have been described (Raper, 1984; Hagiwara, 1989). Cellular slime molds were believed to be distributed differently in relation to the environmental conditions. Occurrence and distribution of these organisms have been investigated in many countries and various forest types (Traub *et al.*, 1981b; Cavender and Kawabe, 1989; Landolt and Stephenson, 1990; Stephenson *et al.*, 1991). From these studies, many new species were continually reported, and these species have been taxonomically reviewed (Bonner, 1967; Olive, 1975; Traub and Hohl, 1976; Cavender *et al.*, 1981; Raper, 1984; Hagiwara, 1989).

Studies on cellular slime molds of Korean forest soils

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were initiated more recently. Hong and Chang (1990, 1991; Hong *et al.*, 1992a, b) investigated the occurrence and distribution of these organisms in Korean forests. Cellular slime molds newly found in Korea were taxonomically described (Hong and Chang, 1992b, c). Until now, 20 Korean dictyostelid cellular slime molds have been identified and reported, including two new species.

The genus *Dictyostelium*, established by Brefeld in 1869, has characterized sorocarps that were typically erect or semierect and unbranched or sparsely and irregularly branched. The stalks consisted of strongly vacuolate parenchyma-like cells compacted within a continuous cellulose sheath, and they bore globose to citriform spore masses at their apices. Norberg (1971) studied the *Dictyostelium mucoroides* complex of species in which there has been considerable taxonomic confusion. These *Dictyostelia* have white sori, elliptical spores, and unbranched or irregularly branched sorophores. Six species and one variety were recognized on the basis of morphology: *D. mucoroides* Brefeld, *D. giganteum* Singh, *D. sphaerocephalum* (Oud.) Sacc. and March., *D. tenue* Cavender *et al.*, *D. minutum* Raper, *D. aureo-stipes* Cavender *et al.*, and *D. mucoroides* var. *stoloniferum* Cavender and Raper. Norberg includes a key which utilizes size and coloration but omits behavioral or cytological features.

Traub *et al.* (1981b) investigated distribution of the major types of Swiss forest soils, and they (1981a) reported three new species, *D. fasciculatum, D. polycarpum* and *P. filamentosum* which were originally isolated during the above ecological study. They presented a group key which utilizes behavioral or cytological features and Norberg's original morphological differentiation. *D. floridum* sp. nov. closely resembled *D. fasciculatum* and *D. polycarpum* found in Swiss forest soils in its gross morphology and primary habitat, respectively.

Raper (1984) have reviewed the historical background and systematics of dictyostelids in great detail. He contributed to systematize the taxonomical confusion of cellular slime molds. Hagiwara (1989) compiled the published information on all named taxa of Japanese dictyostelids. *D. floridum* sp. nov. exibited one or more distinctive morphological features and growth habit which did not fit into the above species. Therefore, it was described as a new species , based on the detail observation and comparison of developmental process.

MATERIALS AND METHODS

Cellular slime molds isolated were grown on weak nutrient media, 0.1% lactose-peptone agar (0.1 L-P), consisting of 1.0 g lactose, 1.0 g peptone, 1.5 g KH_2PO_4, 0.96 g $Na_2HPO_4 \cdot 12H_2O$ and 20 g agar/*l* distilled water, adjusted to pH 6. *Escherichia coli* were used as the most desirable food organism (Cavender, 1976) and were already cultured on the liquid medium for overnight with a rotary shaker at 37℃. These were centrifuged at 3000 rpm for 5 min, and the high suspension of fresh bacteria were prepared and stocked at 4℃. Spores were usually inoculated at the center of cross streaks made with a suspension of bacteria and were incubated at 18-22℃ in diffuse light and darkness. For the study of aggregation pattern, a suspension of spores and bacteria was spread as a broad band across the agar surface. Both growth and development were observed. Photographs were taken with a photomicroscope.

RESULTS

Description. *Dictyostelium floridum* Hong et Chang sp. nov.

Cultum ad 18-22℃ in diluto "lactose-peptone" agaro cum *Escherichia coli* in diffusa luce; sorocarpii typice gregarii aut solitarii, primo erecti vel semierecti, sinuosi, magnitudine saepissimae 1-4 mm, interdum ad 8 mm; sori globosi usque citriformes, lactei aut subflavidi, 40-200 μm, plerumque 100-200 μm; sorophora et rami ecolorata, latiore (11-40 μm in diametro) initio tenuem (4-6 μm) in ambitum exsurgentia, vel typice longa ramosi in basi, vel secundariorum; basi expansi; sporae ellipticae, cum typicis conspicuis polaribus granulis, 2-4×5-10 μm; index longitudinis per latitudinem 2.3-2.7; macrocystii et microcystii in nullo substrato obsevati.

Holotypus—Stirpes typicae HL-2 e solo Hallasan, Cheju-do, Korea.

韓國名: 꽃구슬팡이.

Cultured at 18-22℃ on low-nutrient agar media such as 0.1% lactose-peptone in association with *Escherichia coli* under diffuse light and darkness; sorocarps typically clustered or sometimes solitary, typically branched, at first erect or semierect, often sinuous and delicate, mostly 1-4 mm (ave. 2.5 mm), occasionally from 0.4 mm to 8 mm, quite sensitive to temperature; sori globose to citriform, milky-white to pale yellowish, 40-200 μm in diam; sorophores and branches colorless, strongly tapered from 11-40 μm at the base to 4-6 μm at the top, often bearing 2-5 irregularly concentrated long branches near the bases, surface very rough; bases often expanded conical to round, steadily anchored to a large slime deposit; aggregates typically showing petal-like appearances formed from small clumps of myxamoebaes; spores elliptical with distinctive polar granules, 2-4×5-10 μm (ave. 3.0×7 μm); length to breadth index 2.3-2.7; macrocysts and microcysts not observed.

Holotype—Type strain HL-2 isolated in 1990 from the subalpine coniferous forest soils of Mt. Halla 1600-1950 m in altitude, Cheju-do, Korea.

Obsevations. *Dictyostelium floridum* sp. nov. was charaterized by: 1) typically clustered sorocarps, 2) spores with distinctive polar granules, 3) a broad petal-like pattern of aggragation, 4) concentrated long branches near the bases, 5) often strongly tapered sorophores, 6) the frequent occurrence of expanded basal form, 7) compound and clavata tips.

Spores (Fig. 1) of this organism varied in size, namely 4.8-10.5×1.9-3.8, but mostly 5.7-7.7×2.1-3.0. L/B index varied from 2.0 to 3.0, but mostly 2.3-2.7. Spores germinated to produce myxamoebae. Aggregation began by inward migration of solitary myxamoebae after their vegetative stage (Fig. 2). Aggregation pattern of this species typically showed the three stages. Initially, a small mound appeared and became larger (Fig. 3), and began to divide inward into several segments, often showing wheel-like appearance (Fig. 4). As aggregation was continuing, this produced radiate patterns of typically broad 'petal-like' aggregates without conspicuous inflowing streams (Fig. 5). It was very interesting that this late aggregate showed several broad petal-like and radial segments. This petal-like form often remained even when the sorogens developed aerially because of unceasingly inflowing myxamoebae (Fig. 10). This unusual appearance of late aggregate was one of the most characteristic of this species. This interesting feature have not been showed in any other species. When spores and bacterial suspension were spread on a band over the agar surface, the agar surface often became a beautiful flower garden.

Occasionally, a mound-like slime mass which initially appeared in the post feeding myxamoebae produced a solitary or a few clustered sorocarps directly. However the majority of aggreagation presented typically large and broad petal-like form. Any aggregates did not show vigorous streaming, but sometimes radial short and thinner streams were faintly diffused in the outer region of peripheral 'petal'. A mound form of aggregation resembled that of *D. fasciculatum* (Traub *et al.*, 1981a). However *D. fasciculatum* did not show a broad petal-like aggregates.

While aggregation proceeded, a culmination papilla arised near the proximal end of each 'petal' (Fig. 6). Many pappillae of the proximal portions of broad aggregation segments synchronously gave rise to produce cylinder-like sorogens (Fig. 7). These rising vermiform sorogens were typically divided to produce multiple branches. Although some branches near the base of a sorophore could be produced by delayed streaming myxamoebae, the great majority of branches were formed synchronously from the cell masses that were divided from the top of rising sorogens (Fig. 8). A solitary sorogen also typically produced branches near the base, and often multiple branches from a common base (Fig. 9).

Sorogens continually erected (Fig. 10) to be elongated aerially (Fig. 11). Mature fruiting bodies were typically clustered (Figs. 12 and 14). Branches were mostly concentrated near the base. Therefore they particularly represented a complexity near the base, and showing a bushy form. Most branches of normal sorocarps were long and similar in size. Solitary sorocarps also typically bear 1-4 branches near the bases (Fig. 13). The sorophores were erect or semirect in normal temperature, but they were very delicate, flexous, and often droped off in higher temperature above 22℃. This sensitivity to temperature may be related to its habitat. The gross appearance of mature clustered sorocarps were flower-like and fascinating (Fig. 14).

Concentrated branches near the bases of sorophores were also most distinctive features of *Dictyostelium floridum* sp. nov. (Fig. 15). The multiple branches near the bases oriented away from each other. Therefore, they represented a radial (Fig. 15) or symmetrical (Fig. 16) form in a clustered sorophores. Sometimes sorophores bore secondary branches (Figs. 16 and 17). Sorophores are mostly moderately thick, but sometimes were quite heavy, up to 35-40 μm in diameter (Figs. 16 and 17). Sorophores were often noticeably tapered from the base to the top and were rough in surface (Figs. 16 and 17). These characteristics of sorophores, namely typically concentrated branches near the bases, often strongly tapering, and rough surface were the important criteria which could be compared with other species.

Bases of a clustered sorophore were anchored to a common substrate by a deposit of congealed slime. Therefore, when a cluster of sorocarps were removed from the agar surface and mounted in water droplet of the coverglass, usually the slime substance was tightly attached to the bases of sorocarp (Figs. 18 and 21). This slime substance often represented pale red color. Strong agitation made bases free from the slime mass, and the bases became distinctive (Fig. 19). Bases were often expanded conical (Figs. 19 and 20) but sometimes round (Figs. 21 and 22). Several bases often produced a coremiform (Fig. 21). Tips were usually clavate and compound (Fig. 23).

This isolate grew to fruit well on a weak nutrient agar media. Growth rate was fairly uniform at 15-22℃. However, growth and fruiting ability droped off sharply above 23℃. Temperature studies show that optimum temperature for the formation of fruiting body is around 20℃. These habitat and temperature condition somewhat resembled that of *Dictyostelium polycarpum* (Traub *et al.*, 1981b).

DISCUSSION

Traub and Hohl (1976) emphasized the importance of polar granules within spores. This spore character was highly correlated with less easily assessible features of life cycle such as the nature of the chemotactic response toward cAMP. They reported that many new strains were isolated which had the PG character and the cluster of associated features but produced sorocarps which were not regularly branched. According to them, this newly recognized group represented a clearly separate unit with common, defined properties. They discussed its taxonomic position in relation to the presently accepted genera of the Dictyosteliaceae. Therefore, they substantially presented a newly taxonomic concept of an intermediate group of species in the genus *Dictyostelium* which had Polysphondylium-like characteristics.

D. floridum sp. nov., isolated from Koean forest soils, have Polysphondylium-like characteristics and may be included into the above intermediate group. The most noteworthy morphological characteristics of this new species are broad petal-like aggregates and concentrated long branches near the bases. These unusual aggregation and branching pattern are major features which are clearly different from any other intermediate species typically branched and clustered. However, these are not sufficient warrant for species diagnosis. These can be identified by applying the combined criteria of spores with polar granules, typically clustered sorocarps, milky-white to pale yellow sori, colorless sorophores, expanded or round bases, and compound and clavata tips.

Dictyostelium floridum sp. nov. superficially resembled *Dictyostelium fasciculatum* (Traub *et al.*, 1981a) in gross appearance. The two species were closely similar in milky-white to pale yellowish sori, colorless sorophores, and clustered sorocarps. However, closer examination of *Dictyostelium floridum* sp. nov. revealed that this was a quite distinctive species. It was different from *D. fasciculatum* in aggregation pattern, branching formation, tapering and diameter of sorophores, and the basal form.

D. fasciculatum showed different patterns of aggregation depending on the culture condition. When spores and bacterial suspension were spread on a band over the agar surface, a small mound appeared and was surrounded by rather vigorous streams. When spores were inoculated on a bacterial streak, the primary center grew to produce the central mass often flattened with a slight depression in the middle. This aggregate was divided into many culmination pappillae. These aggregates did not show the broad petal-like appearances as in *D. floridum* sp. nov. This aggregation pattern appeared in both two culture conditions and did not show vigorous streams.

Sorophores of *D. fasciculatum* were unbranched or occasionally branched and mostly consist of a single tier of cells throughout their entire length (Raper, 1984). However, sorophores of *D. floridum* sp. nov. were typically concentratedly branched near the bases, much rather thick, and strongly tapered. In addition, the surface of sorophores was often rough in *D. floridum* sp. nov.

Based on the typically branched sorophores and the clustered sorocarps, *D. floridum* sp. nov. resembled *D. aureo-stipes* (Cavender *et al.*, 1979) and the varieties (Cavender *et al.*, 1979; Hagiwara, 1989; Hong and Chang, 1992b). However, *D. floridum* sp. nov. did not produce 'yellow-stalk'. And its branching pattern was different from *D. aureo-stipes* and the varieties. It did not show 'short and spaced, or crowded branches' along the developing sorophores but long branches concentrated near the base. In addition, *D. aureo-stipes* and the varieties did not produce the petal-like aggregation and expanded conical bases.

D. floridum sp. nov. was similar with *D. delicatum* (Hagiwara, 1989) in the typically branched sorophore, pigmentation, the tips, and delicate sorocarps. It was, however, clearly different from *D. delicatum* in the aggregative behavior, the branching pattern, the growth habit, and the basal form. An aggregation of *D. delicatum* had definite radial streams and then breaks up into many centers around the original center, subsequently forming sorocarps gregariously. It did not produce a cluster as in *D. floridum* sp. nov. The branching pattern of *D. delicatum* closely resembled, not that of *D. floridum* sp. nov., but that of *D. aureo-stipes* and the varieties.

Dictyostelium floridum sp. nov. was, in 1990, originally isolated from the subalpine conifer forest soils of Halla mountain 1600-1950 m in altitude. And it may be very sensitive to temperature. This was undoubtedly related to the nature of the soil habitat where it was principally found.

The occurrence of morphologically identical isolates of the same species from widely separated geographical areas indicates genetic stability (Cavender, 1976). It also lends support to the criteria which have been used to establish species in the cellular slime molds. However, as soils and habitats were investigated, apparently very rare organisms were found which do not fit into existing species but have characteristics of one or more of them. *D. floridum* sp. nov. was a good example of this, having some of *D. fasciculatum* and some of *D. aureo-stipes* and the varieties, but belonging to neither. In nature, there appears to be a continuum of cellular slime molds differing only slightly one from another (Cavender, 1976). Many species are probably very rare because of interspecific competition and can be found only in particular niches. Many studies until now have revealed that the soil habitat supported only a limited number of cellular slime molds. *D. floridum* sp. nov. is probably very rare and may be endemic to Korea.

적 요

漢拏山의 森林 土壤에서 앞서 보고된 新種 *Dictyostelium flavidum* Hong et Chang(노랑장대팡이)에 이어서 또하나의 新種이 記錄되었다. 이것은 특징적인 넓은 꽃잎 모양의 集合體와 기부부근에 집중적인 긴 가지를 갖는 꽃모양의 群體를 나타내어 *Dictyostelium floridum* sp. nov. Hong et Chang(韓國名 新稱 : 꽃구슬팡이)으로 명명되었다. *D. floridum* sp. nov.는 위의 특징 외에도, 1) 極囊이 뚜렷한 胞子, 2) 基部에서 頂端으로 올라갈수록 심하게 가늘어지는 자루, 3) 흔히 원뿔형이거나 구형인 基部, 4) 하나의 점액질에 단단히 묻혀 있는 여러 개의 기부, 5) 여러 층의 세포로 구성된 退型의 頂端에 의해 특징된다. 또한 이 種은 溫度에 매우 敏感한 반응을 보이며, macrocyst나 microyst는 형성하지 않는다. 이 종은 처음 토양으로부터 분리된 이후 계속적인 계대배양을 통하여 형태적 특징뿐만 아니라 발생과정 및 성장습성이 자세히 관찰되었으며, 이러한 특징들과 데이타는 다른 유사한 종과 비교되었다. 이 種은 1990년 해발 1600 m 이상의 한라산 침엽수림 토양에서 처음 발견되었으며, 약한 영양배지인 0.1% lactose-peptone 배지에서 *Escherichia coli*와 함께 18-22℃에서 이원배양되었다.

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Explanation of Figures

Fig. 1. Spores with distinctive polar granules, ×1000.
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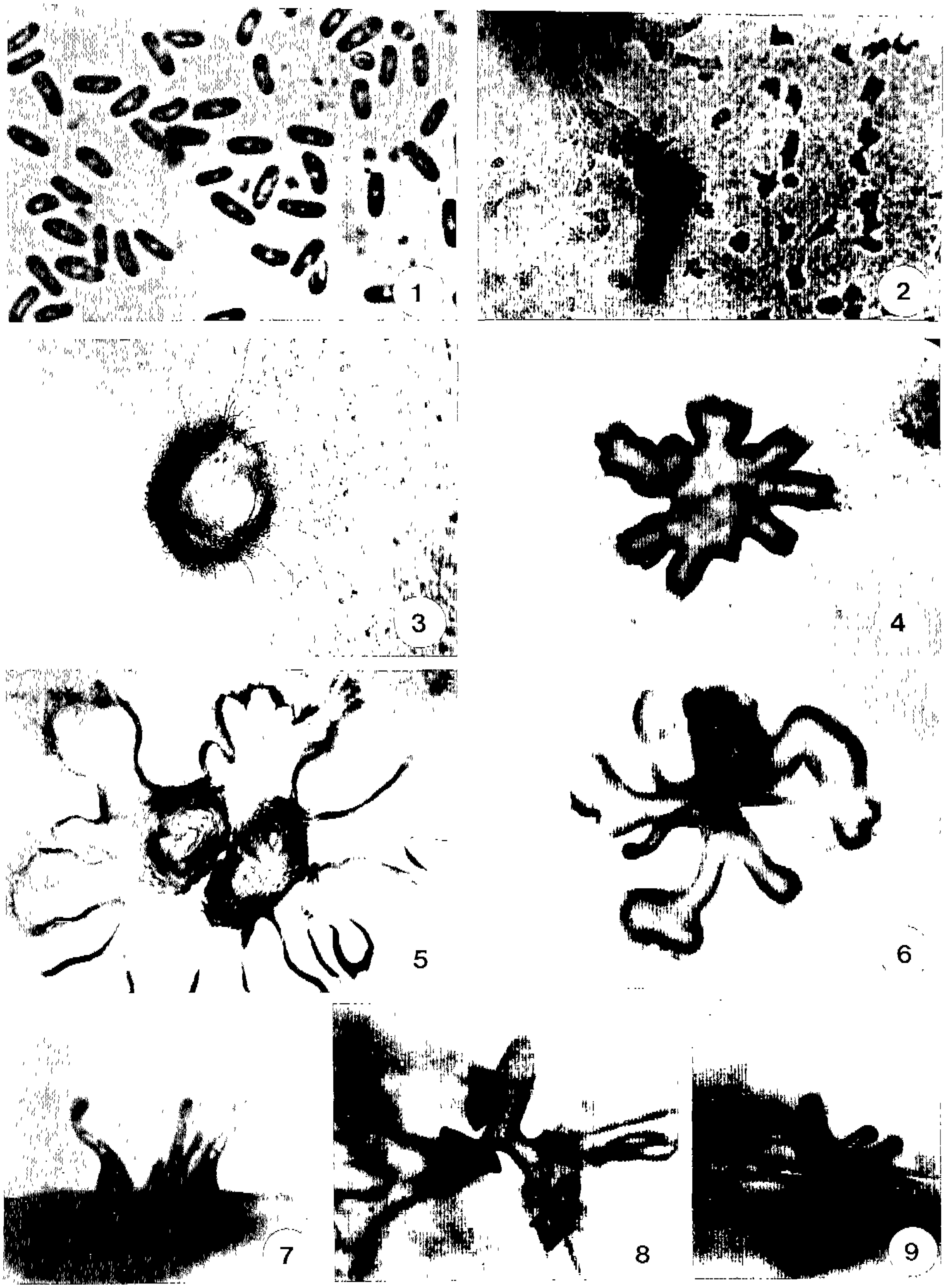
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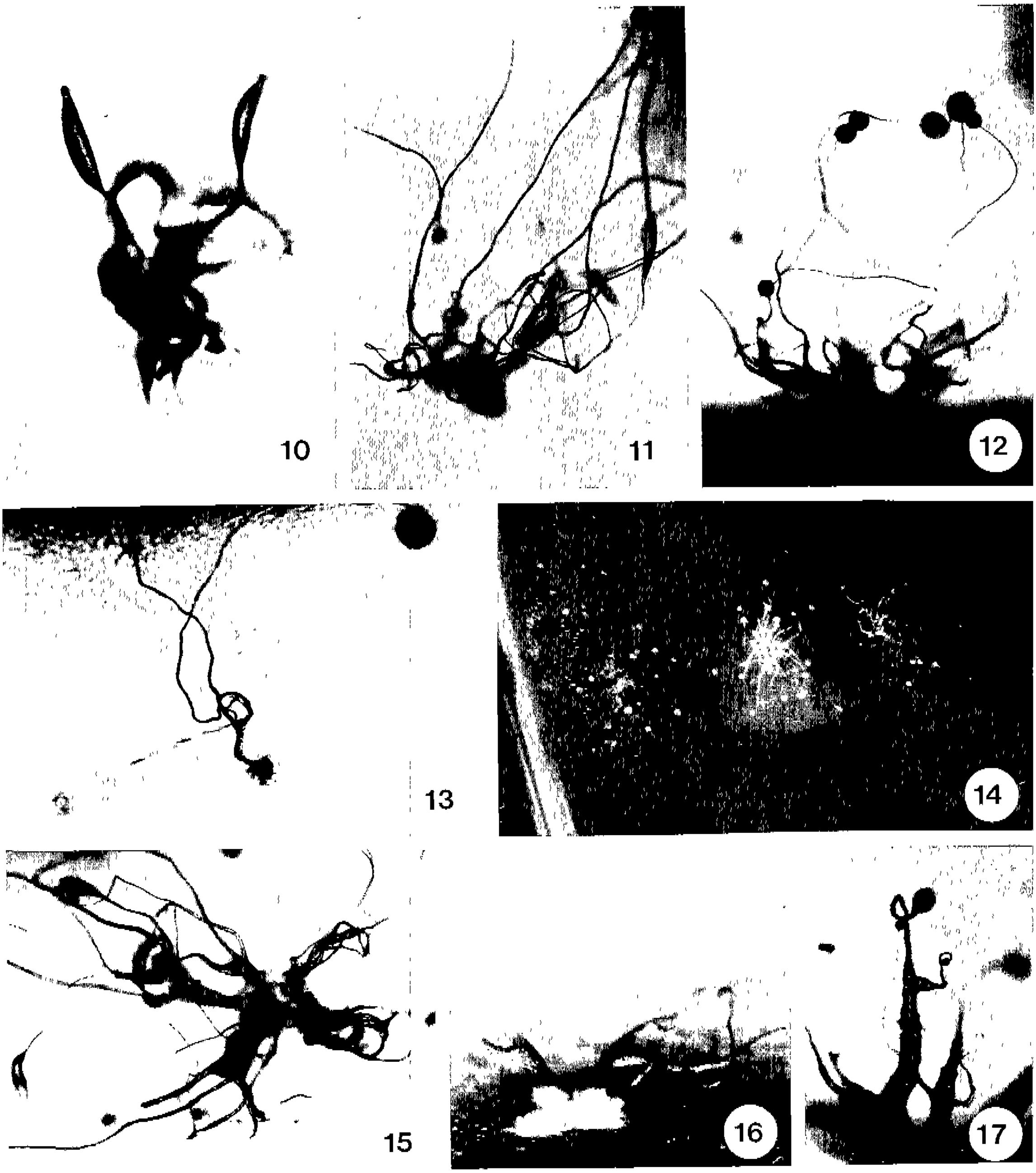
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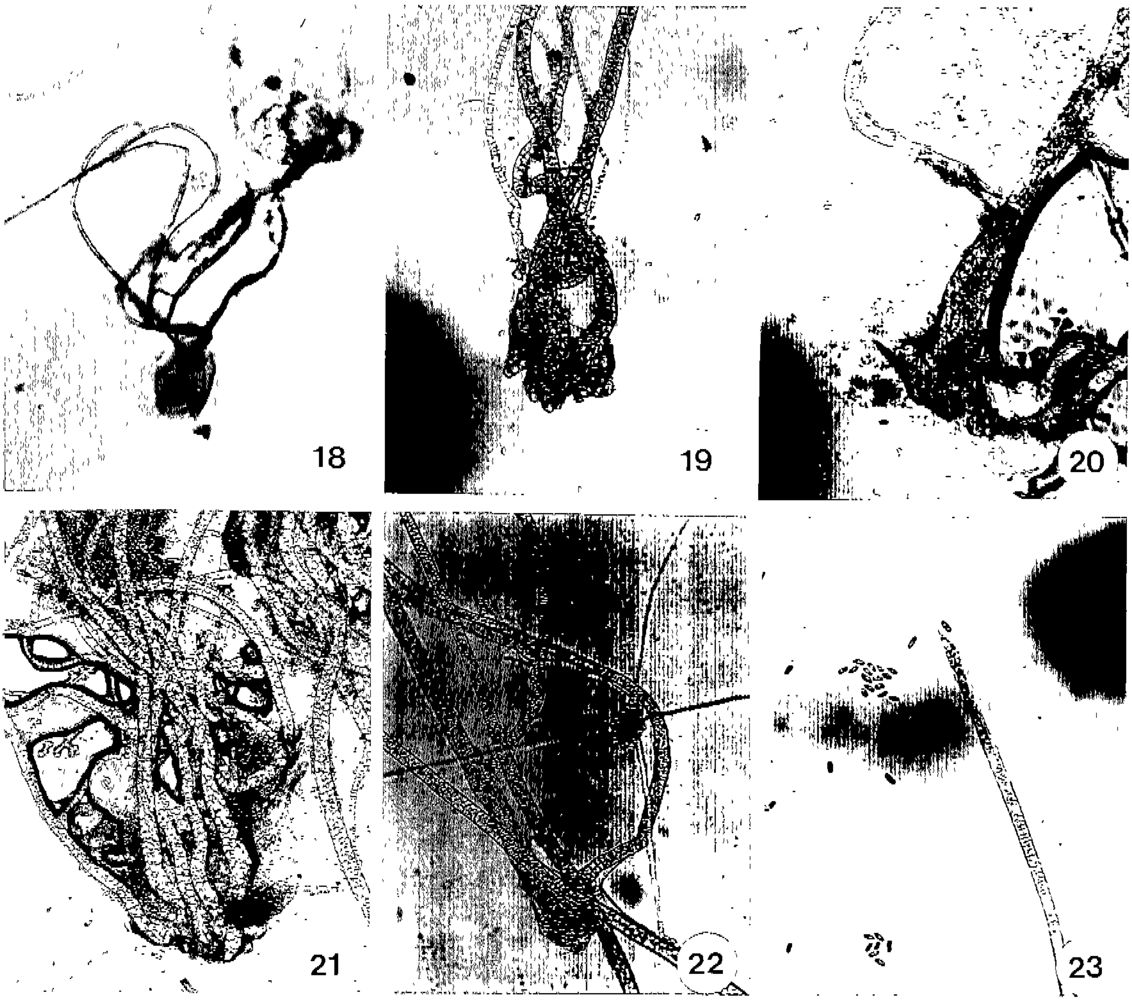
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