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Polysphondylium luridum, a new dictyostelid with unique spores

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Abstract

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A new species of *Polysphondylium* from Puerto Rican forest soil is described which has characteristics not found in any other member of the genus described to date. The myxamoebae of *Polysphondylium luridum* do not aggregate in the dark; development is halted and they transform themselves into microcysts. Charcoal added to the culture as well as exposure to light will release this inhibition. The myxamoebae will also pile up in undifferentiated pigmented masses in suboptimal conditions. The spores are unique among known dictyostelids in having numerous granules dispersed throughout the cytoplasm. Normally in *Polysphondylium* spore granules are polar while in *Dictyostelium* they may either be polar or dispersed but less dispersed or numerous than in *P. luridum.* The larger members of the Dictyostelia have dispersed granules and what appear to be ribosomes coating the mitochondria while *P. luridum* does not. This combination of characteristics puts *P. luridum* in an intermediate position taxonomically, and perhaps evolutionarily.

Key words: Dictyostelid, *Polysphondylium luridum,* spore.

Introduction

The genus *Polysphondylium,* established by Brefeld (1884), is characterized by sorocarps with a regularly whorled branching pattern, thus differentiating it from the non-branching or irregularly branching genus *Dictyostelium.* To this first isolate he gave the name *P. violaceum,* since it possesses vinaceous purple to pale violet sori. Another *Polysphondylium* was discovered by E. Olive (1901), the ubiquitous, unpigmented *P. pallidum.* Concurrent with this description, he described another unpigmented species, *P. album,* which differed from *P. pallidum* in having more numerous and slightly larger sori, smaller spores, and stalks that lie closer to the agar surface. Raper (1984) did not recognize *P. album* stating the characteristics used by Olive for separation of the two are too variable among the unpigmented Polysphondylia.

No new Polysphondylia were described until 1973 when Hagiwara described *P. candidum.* This is another unpigmented species but very striking in its regularity and uniformity of branching. Typically it has less than four whorls and possesses, under certain cultural conditions, a very long terminal sorophore segment with either a diminutive sorus or with no sorus. Hagiwara (1979) described a similar species *P. pseudo-candidum,* which possesses a more delicate growth habit with slightly smaller sori and spores. Although Raper (1984) recognized this species he questioned the separation of the two since many isolates now assigned to *P. pallidum* have as much variation in size as *P. candidum* and *P. pseudo-candidum.* Hagiwara (1979) also described a distinctive new species, *P. tenuissimum.* Although similar in form to *P. pallidum,* it produces many more whorls (up to 35) on long thin sorophores. The most recent species to be formally described is *P. filamentosum* (Traub et al. 1981). It produces whorled primary branches which have whorled secondary branches terminating in sori while the primary branches as well as the main axis are elongated with very reduced or absent terminal sori.

Traub und Hohl (1976) have suggested that most small members of the genus *Dictyostelium* share more characteristics with *Polysphondylium* than *Dictyostelium* but lack the one demarcating feature, the whorled branching pattern. These shared features consist of elliptical spores with polar granules, and smooth mitochondria* (Fig. 2), the ability to form microcysts, the absence of aggregative response to the acrasin cyclic AMP, and a clustering of sorogens arising from one aggregation. They designated this group PG+. Examples are *D. tenue* and *D. aureostipes* Cavender, Raper and Norberg, *D. polycephalum* Raper, *D. delicatum* Hagiwara and *D. fasciculatum* Traub, Hohl and Cavender. The features of the larger members of the genus *Dictyostelium* investigated by them have spherical or elliptical spores without polar granules and rough mitochondria (coated with ribosomes) (Fig. 1), no microcysts, response to cyclic AMP and limited clustering. They designated this group PG–. Examples are *D. discoideum* Raper, *D. mucoroides* Brefeld, *D. rosarium* Raper and Cavender and *D. aureum* Cavender, Worley and Raper.

Cavender (1970) in a survey of Puerto Rican tropical forest soils isolated a clone resembling *P. violaceum* in form but lacking vinaceous purple pigment. This was reported by Raper (1984) in his review of the genus *Polysphondylium* (p. 374). Recently it has been recultivated from lyophilized material (strain EY-1) and an investigation of the distinguishing characteristics of this new *Polysphondylium* has been undertaken and is described here.

Materials and methods

Culture requirements. Nutrient media employed were thin hay infusion agar; doublethin hay infusion; thin hay infusion with 0.05% peptone; 0.1% lactose + 0.1% peptone (.1 LP); 0.05% lactose + 0.05% peptone (.1 LP/2); 0.025% lactose + 0.025% peptone (.1 LP/4); 10% V8 juice (Campbell Soup Co.) + 0.1% $CaCO_3$ (V8); 5% V8 juice + 0.05% $CaCO_3$ (V8/2); 0.5% dextrose + 0.5% pep-

* The mitochondria do not have an electron dense ribosomal coating as in the PG– group of species. This feature (presence or absence of the mitochondrial coating in PG– or PG+ species, respectively) has been verified for the PG+ *P. violaceum* (U. P. Roos, unpublished), *P. pallidum,* and *P. minutum,* and for the PG– *D. aureum, D. discoideum, D. purpureum,* and *P. rosarium* (Hohl, unpublished).

tone + 0.1% Na_2 HPO_4 (SM/2); 0.1% lactose + 0.05% yeast extract; 0.05% lactose + 0.025% yeast extract; and non-nutrient agar (Raper 1951, Sussman 1951, Traub and Hohl 1976).

A stock culture of this *Polysphondylium* (EY-1) was maintained at 22–23 °C on double-thin hay medium (Cavender and Raper 1965). Mature sorocarps were kept at 4 °C for 2–2½ mo. between transfers. *Escherichia coli* B/r served as the nutrient source for all cultural studies. Optimum cultural conditions were determined using methods reported in similar studies, e.g. Cavender et al. (1981), Traub et al. (1981). Temperature requirements were investigated on a temperature gradient bar incubator ranging from 5 °C to 30 °C.

The slime mold cultures were routinely grown in diffuse fluorescent light. Total darkness and one-sided light were also employed in some experiments. Darkness was achieved by wrapping the petri dishes in aluminum foil. Uni-directional illumination was achieved by punching a small hole on one side of the aluminum foil surrounding the petri dish and orienting these perforations toward a light source.

With less than ideal cultural conditions improvement in fructification has been reported with the addition of charcoal to dictyostelid cultures at the onset of aggregation (Bonner and Hoffman 1963, Cavender et al. 1981). The cause of this benefit is unknown at this time although it has been shown that the charcoal absorbs some gas (e.g. NH_3) detrimental to sorocarp formation (Bonner and Dodd 1962, Bonner et al. 1986). The behavior of this cellular slime mold was examined for any beneficial effects from the addition of charcoal. Substitution of glazed clay covers for glass covers has been found to enhance fructification in some dictyostelids, e.g. *D. polycarpum* and *D. polycephalum.* This was also investigated.

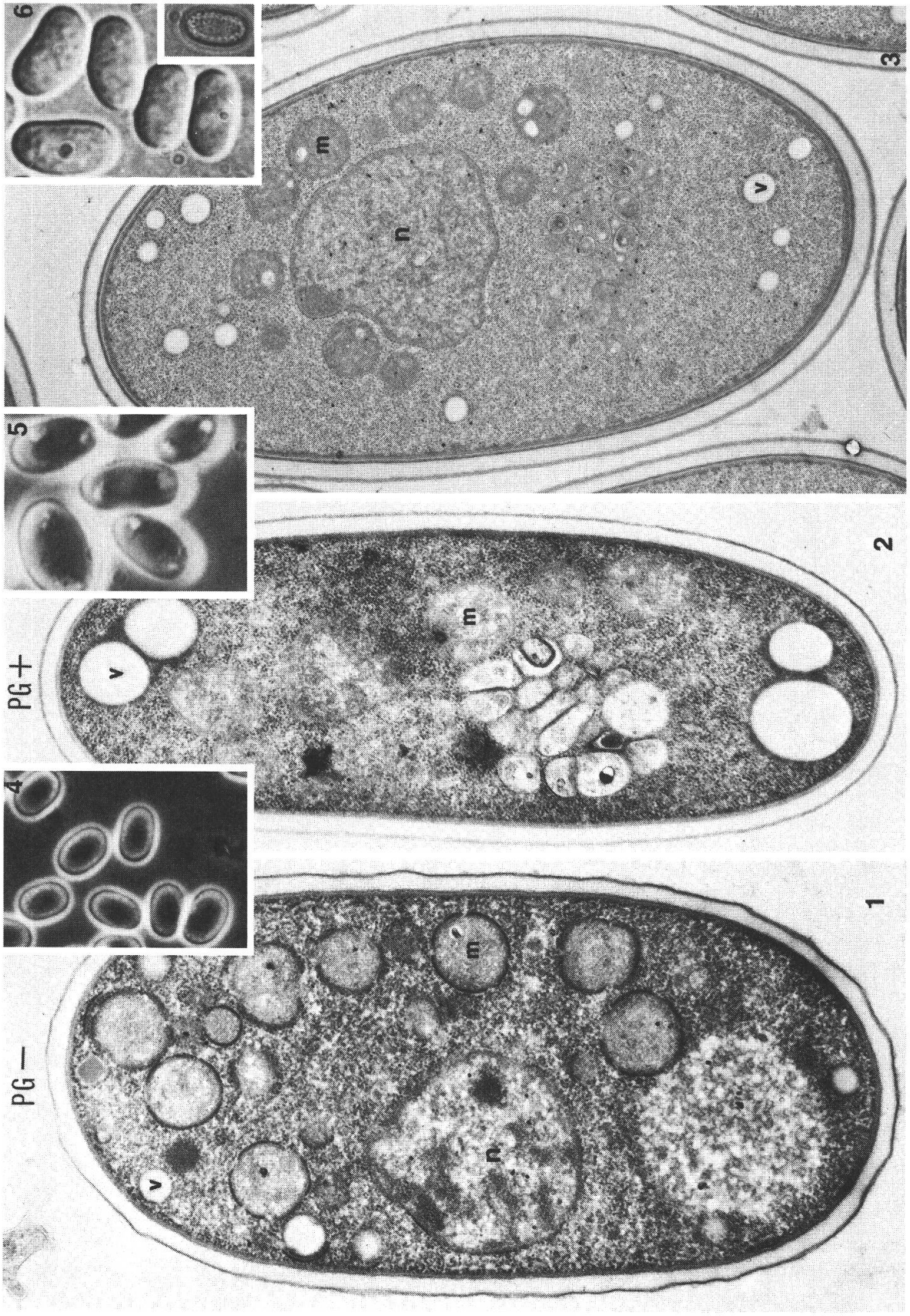
Response to Cyclic AMP. – Five microdroplets (0.05 ml) of a dense suspension of pregrown and washed aggregation-competent myxamobae were deposited separately upon 2% water agar in 100 ml petri dishes. Small 2% agar blocks containing the following concentrations of cAMP: 10^{-7} M, 10^{-6} M, 10^{-5} m, 0.05 mg/ml and 0.1 mg/ml were placed next to the myxamoebal drops, one next to each drop. Agar blocks containing no cAMP were used as controls. In addition *D. discoideum* myxamoebae which are known to be responsive to cAMP and *P. violaceum* myxamoebae which are not responsive to cAMP, were tested for comparison. Prominent streaming of myxameobae toward the agar block was determined to be a positive response to this acrasin.

Tests were also employed with mixed species of dictyostelids to determine if they coaggregate (Raper and Thom 1941). Coaggregation indicates that the two species probably possess the same acrasin. The new *Polysphondylium* was tested with *D. discoideum* which produces cAMP, *P. violaceum,* which produces glorin (Shimomura et al. 1982), *P. pallidum,* responsive to glorin, *P. filamentosum* and *D. aureo-stipes,* both of which produce unidentified acrasins. All tests were made on double-thin hay infusion agar as substrate with a thin suspension of *E. coli* spread over the entire surface. The two species tested were inoculated in separate corners and allowed to grow and meet along the whole midpoint of these cultures. Pseudoplasmodia forming along these common frontiers were examined approximately every hour to determine if the separate species developed mature sorocarps from a common aggregation.

Observations of spores were made and photographs taken with a Leitz phase contrast and a Zeiss differential interference contrast (DIC) light microscope, and a Hitachi HU-11E transmission electron microscope. For TEM, pelleted spores were fixed in phosphate buffer (0.07 M, pH 6.8) containing 1.25% glutaraldehyde and 1.5% acrolein, followed by 1% phosphate buffered osmium tetroxide for 30 min. The material was dehydrated in a graded series of ethanol and embedded in Spurr's medium.

Description

Polysphondylium luridum Kauffman, Cavender et Hohl sp. nov. Sorocarpia plerumque solitaria, erecta, ad 1.5–2 cm longa; sorophoria ecolorata, bases clavatae 18–30 μm diam., ad apicem leviter rotundatum vel abrupte angustatum ad 1.5–3.5 μm contracta. Nodi pro sorocarpio 3–6, omnes ramos 3–6 gerentes; pars terminalis axis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principalis deinde elongata, 2–8 mm longa, sed haud elongata quando culta sub luce. Rami laterales 180–300 μm longi; sori albi vel pallide viridicinerei, laterales 70–95 μm diam.; terminales more minores, 25–36 μm; sporae ellipsoideae vel late ellipsoideae, 8.5–11 μm × 4.5–6.5 μm, medio 9.7 × 5.2 μm, granulis multis per cytoplasma dispersis; microcystae praesentes in culturis illuminatis atque cultis in obscuritate, globosae, 5–12 μm, medio 7–8 μm, productum unicum differentiationes constituendae quando culta in obscuritate et carbone carente. Stirps typica EY-1.

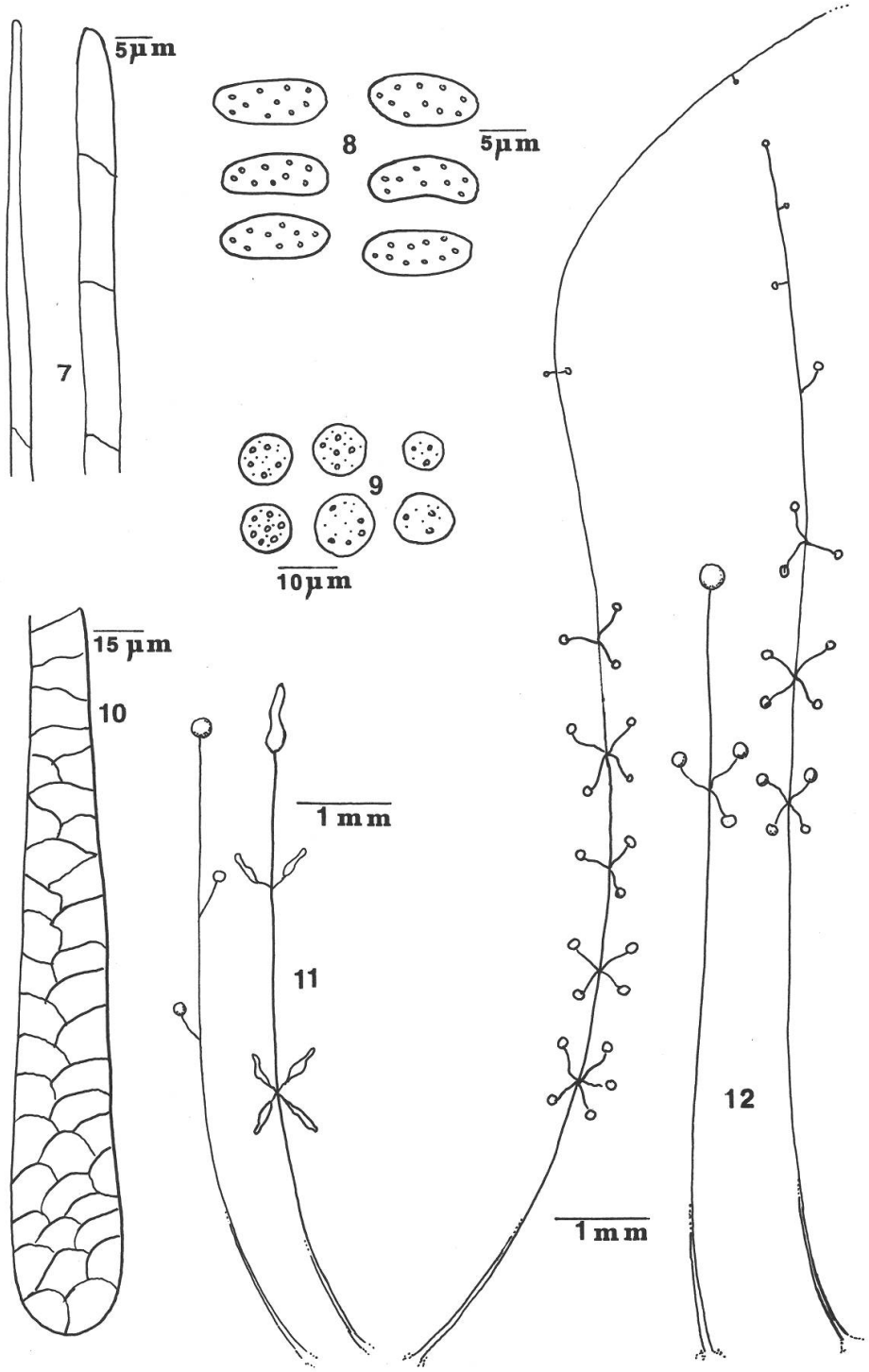
Cultivated in association with pregrown cells of *Escherichia coli* strain B/r on double-thin hay infusion agar at 22–24 °C. Sorocarps mostly solitary, erect or semi-erect, variable in length, up to 1 cm when incubated under overhead light although mostly 6.5–7 mm (Fig. 11); non-phototrophic but lengthening in unidirectional light or darkness to 2 cm (Fig. 12). Sorophores colorless, bases clavate to slightly expanded (Fig. 10), 18–30 μm in diameter near the base narrowing to 1.5–1.3 μm at the summit, slightly rounded to abruptly tapered at the apex (Fig. 7); the main axis bearing 1–3 whorls, mostly three branches per whorl, at times branching is opposite or alternate or not present; whorled branching more evident in darkness, 3–6 whorls present, each bearing 3–6 branches; terminal portions of main axis may be relatively short, 0.5–0.7 mm when grown in light or very long 2–8 mm when grown in darkness; branches 180–300 μm in length, the clavate bases 12–15 μm in width narrowing to 1.5–2.5 μm at the apex. Sori grayish-white changing to light greenish-gray or tannish-gray with age, globose; terminal sori mostly 150–200 μm in diameter when grown in light, 25–36 μm or occasionally absent when grown in darkness; lateral sori 70–95 μm in diameter. Spores variable in size and shape (Fig. 6), elliptical, 8.5–11 μm × 4.5–5 μm to broadly elliptical, 8.5–11 μm × 5–6.5 μm, occasionally slightly reniform, with numerous granules present and dispersed throughout the cytoplasm (Figs. 3, 6). Cell aggregations radiate in pattern, commonly 5.5–6.5 mm in diameter but up to 1–1.5 cm, the smallest forming single sorocarps and the largest fragmenting into parts which fruit separately. Myxamoebae variable in size mostly 15–20 × 14–15 μm, varying from 13–15 × 12–14 μm to, 22–24 × 20–22 μm, each containing 2–3 hyaline contractile vacuoles. Microcysts present in light and dark grown cultures, globose or nearly so (Fig. 9), variable in size from 5–12 μm. Microcysts are the sole morphogenetic structure produced when grown in darkness without activated charcoal. Macrocysts are not produced. Type strain: EY-1, deposited with The American Type Culture Collection, Rockville, Maryland.

Habitat: In leaf mold and surface soil, lower montane rain forest, Puerto Rico.

Results

The optimum temperature for development on the media tested was 22–23 °C. At temperatures higher than optimum, e.g. up to 30 °C, sorocarps had fewer whorls and became more entangled although myxamoebal consumption of bacteria was faster than

Figs. 1–6. Dictyostelid spore cytological characteristics. 1. Polar granule negative spore (PG–) of *Dictyostelium aureum.* TEM × 20,000. 2. Polar granule positive spore (PG+) of *D. aureo-stipes.* TEM × 20,000. 3. Spore of *Polysphondylium luridum.* TEM × 11,000. 4. Spores of *D. aureum* PC × 1100. 5. Spores of *D. tenue.* PC × 2000. 6. Spores of *P. luridum.* DIC × 1100. Inset PC × 900. v = vesicle; m = mitochondrion; n = nucleus



Figs. 7–12. *Polysphondylium luridum.* 7. Sorophore tips, light and dark grown. 8. Spores. 9. Microcysts. 10. Sorophore base. 11 Growth habit in light with charcoal. 12. Growth habit in darkness with charcoal.

at 22–23 °C. With lower temperatures than optimum, e.g. down to 15 °C, the sorocarp length became progressively shorter with decreased whorling. Myxamoebal consumption of bacteria was also slower.

Double-thin hay agar, one of the lowest nutrient media examined, provided the best substrate for the growth of this dictyostelid, the next best medium was 0.05% lactose + 0.025% yeast extract. Those cultures grown with more nutrients typically produced aberrant masses of undifferentiated myxamoebae and had fewer whorled branches. Powdered charcoal (American Norit Co.), if added to cultures at the beginning of aggregation, stimulated more frequent whorling plus larger mature sorocarps. The substitution of glazed clay covers showed no improvement over the usual glass petri dish lids.

Myxamoebae were not responsive to the varying concentrations of cyclic AMP. When myxamoebae were intermixed with myxamoebae of each of the other three Polysphondylia investigated no mixed aggregations were found. This was also true in mixed cultures with *D. discoideum* and *D. aureo-stipes.*

When grown on 0.1 LP or 0.1 LP/2 agar in the light, this *Polysphondylium* resembles a *P. violaceum* or a large *Dictyostelium* species in size with mainly alternate branching or an occasional whorl. However, a characteristic on 0.1 LP, also noted in certain strains of *P. pallidum,* was the production of numerous aberrant mounds of undifferentiated myxamoebae changing in color from a greenish-yellow to brown to deep golden brown, ranging in size from 0.4–0.8 × 0.3–0.6 mm. The individual myxamoebae are spherical to triangular in newly formed masses becoming isodiametric with strongly vacuolate protoplasm reminiscent of the partially differentiated myxamoebae occasionally found in *D. coeruleo-stipes* cultures (Raper and Fennell 1967). When grown in the light with charcoal on double-thin hay agar these aberrant masses are not produced and mature sorocarps have more frequent whorling although alternate and opposite branching patterns are also present (Fig. 11). Under the same culture conditions in total darkness more *Polysphondylium*-like characteristics are displayed, e.g. more frequent whorls and an elongation of the terminal portion of the main axis (Fig. 12). As light is diminished from full light to almost total darkness there is an elongation of the sorophore. However, in total darkness in the absence of charcoal, only microcysts are produced. No phototropism is shown by maturing sorocarps.

Discussion

The spores of this slime mold possess granules dispersed throughout the cytoplasm, in such a way as to be easily seen by phase contrast (PC) or differential interference contrast microscopy (DIC) (Fig. 6). The larger *Dictyostelium* species possess a more homogeneous spore cytoplasm (Figs. 1, 4) while almost all the smaller species plus all known *Polysphondylium* species have polar granules (Figs. 2, 5). These polar granules are formed by a group of tightly packed small vesicles (Hohl and Hamamato 1969). Although these vesicles are present in the larger dictyostelids, they are scattered throughout the spore (Fig. 1) and barely detectable with the light microscope (Traub and Hohl 1976). When viewing *D. discoideum,* one of the larger PG– dictyostelids, under DIC optics, granules can be seen in the spores. However, these granules are larger and not nearly as numerous as the granules found everywhere in the spores of this new *Polysphondylium.* The mitochondria are, in addition, smooth (Fig. 3), as are those of *P. pallidum, P. violaceum, D. minutum,* and other PG+ *Dictyostelium* species studied.

A combination of morphological, cytological and developmental characteristics, therefore, separate *Polysphondylium luridum* from other dictyostelids. These are: (1) the production of aberrant golden brown masses of undifferentiated myxameobae when grown on 0.1 LP or 0.1 LP/2 agar, (2) the absence of aggregation, with microcysts replacing spores when grown in the dark without charcoal, (3) the enhanced, robust *Polysphondylium*-like growth when cultured in the dark with charcoal, (4) the pale greenish or tannish-gray pigmentation of aged sori, and (5) the presence of numerous granules dispersed throughout the cytoplasm of the spores.

Only the last characteristic distinguishes this species from all known dictyostelids. While this characteristic does not support Traub and Hohl's taxonomic concept marking the small *Dictyostelium* species which are allied with *Polysphondylium,* it does not invalidate this interesting concept. This species may represent an intermediate between species we designate as either *Polysphondylium* or *Dictyostelium* on morphological grounds.

The unusual developmental characteristics noted are evidently the result of sensitivity to gas or gases produced which are removed by charcoal and counteracted by light.

The wan pigmentation provides a useful descriptive epithet as derived from the Latin *lurid* meaning pale.

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