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Physarum polycephalum: A Myxomycete from the Forest Soil of North Jordan*

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ABSTRACT. An acellular slime mold identified as *Physarum polycephalum* was isolated from surface soil collected at the Dibbin National Park forest in the north of Jordan. The myxomycete, the first to be isolated in Jordan, was isolated from dead organic matter incubated at room temperature in a moist chamber. It was characterized by small stalked gyrose sporangia, peridial lime deposists, branched capillitium and ornamented spores. It had a large yellowish phaneroplamodium which showed rhythmic shuttle streaming with a periodicity of approximately 1.3 min.

Acellular slime molds or myxomycetes are a strange group of microorganisms. They show plant and animal-like characteristics. The acellular migrating vegetative body, the plasmodium, is animal-like in structure and physiology. On the other hand, they are similar to plants when they form fruiting bodies with unicellular spores bounded by a cell wall. The acellular slime molds live in moist and cold areas in forests on dead leaves and on bark of dead and living trees. In these places a lot of organic matter and bacteria are available as food for the migrating stage of these organisms.

So far no data on myxomycetes occurring in Jordan have been published, and little is known about this group in the Middle East (Ramon 1968). In the course of investigations into the occurrence and distribution of acellular slime molds in forest soil in Jordan, a species of myxomycetes (order Physarales) was isolated and is here described.

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Materials and Methods

Description of Site

Samples were collected from surface soil rich in plant debris, fallen leaves and bark of trees in a forest near Dibbin National Park in the north of Jordan. The area has the following geographical characteristics (Al-Eisawi 1985):

Altitude: Over 700m over the sea level.

Rainfall: Annual mean of 500 mm.

Temperature: A mean annual maximum of 20°C but may drop below zero in the coldest months.

Bioclimate: Sub-humid mediterranean bioclimate, warm and cold varieties.

Soil: Terra Rossa and Rendzina.

Vegetation: Pine forests, dominants: *Pinus halepensis*, *Quercus calliprinus* and *Cistus* spp.

Collection of Samples

Samples for acellular slime mold isolation were collected in early September, 1986. Samples were collected from areas where the organic layer of the surface soil was thick and where the fallen dead bark and leaves seen to bear sclerotia of myxomycetes. Five different samples were placed in wide mouth glass vials, transported to the laboratory and kept in darkness until used.

Light Microscopy

Collected samples were examined in the laboratory under a binocular stereomicroscope (Zeiss) to look for the presence of sporangia or other stages in the life cycle of myxomycetes. The sclerotia and the stages of fructification of the isolate were observed and recorded under the binocular stereomicroscope, while the squeezed sporangia and the rest of the micrographs were taken under a phase contrast microscope (Zeiss).

Enrichment and Isolation

For isolation, two main procedures were applied:

1. The moist-chamber technique introduced by Gilbert and Martin (1933).
2. Sclerotia found on dead bark and leaves were allowed to grow on semidefined growth medium (Danial and Baldwin 1964).

Pure Culture

To obtain pure or axenic cultures, the technique of migration (Daniel and

Baldwin 1964) was used with modifications:

1. The plasmodia which developed through application of the procedures mentioned above were allowed to migrate over a sterile, 1.5% nonnutrient agar.
2. Pieces of a plasmodial front that has not intersected its own path during migration were excised and transferred to a second agar plate.
3. The process was repeated until the contaminants have been left behind the migrating plasmodium.
4. The advancing migrating fronts were removed and grown as follows:
   (a) On the surface of semidefined growth medium agar plates kept in a humid chamber at 25°C.
   (b) In a broth culture, plasmodia were grown on semidefined growth medium in Erlenmeyer flasks incubated in a controlled environment incubator shaker operating at 180 rpm at 25°C with a 1-inch radius of gyration.

Sterility Test

The purity of the culture was checked by transferring pits of the plasmodia to the following bacteriological culture media (Difco Manual 1984):

1. Bacto AC medium (incubated at 35°C for 18-48 hr).
2. Bacto AC broth (incubated at 35°C for 18-48 hr).
3. Bacto Czapek Dox solution agar (incubated at 30°C for 40-48 hr).
4. Bacto Czapek Dox broth (incubated at 30°C for 40-72 hr). No bacterial or fungal growth appeared and this was checked by Gram staining and by direct microscopic examination.

Spherulation (sclerotization)

The isolated organism was induced to spherulate as follows:

1. Microplasmodia from axenic culture were allowed to fuse and migrate on wet filter paper in Petri dishes.
2. Plates were left to dry gradually at room temperature.

Under these conditions spherula or sclerotia completed their development in 12-14 hr.

Fructification

After starvation for 5-7 days in darkness, plasmodia migrating on agar surface

were induced to sporulate by illuminating them once for 3-4 hr by means of four 25 W fluorescent lamps, emitting white light. The energy fluence rate measured at the level of the Petri dishes amounted to about 4 w/m^2. Competent plasmodia formed small papilla-like mounds (presporangial masses) which developed into complete sporangia in 10-12 hr.

Myxamoebae

Mature spores were induced to germinate and give rise to myxamoebae by submersion into one of the following solutions (Olive 1975):

1. Knop's solution.
2. Lactose-Yeast Extract solution.
3. Malt-Yeast Extract solution.

Observations and Discussion

Light Microscopy

Microscopic examination of collected samples did not reveal the presence of plasmodia or sporangia of myxomycetes. Only small and large sclerotia on dead bark (frequently) and on fallen leaves (occasionally) were observed. The leaves with sclerotia were located on the surface soil very close to the dead bark. The plasmodia which develop from sclerotia taken from both bark and leaves were identical. It seems that the isolate described represents one strain that could migrate and develop sclerotia frequently on the dead bark and occasionally on the fallen leaves.

Sclerotium

Varied in size from 0.1-5 cm^2 or even larger. It was orange to brown in color. In nature it was found on the surface of fallen leaves and bark of dead trees. In the laboratory it developed on filter paper (Figs. 1A, 2A), on agar surfaces and on other substrata.

Microplasmodium

Yellowish to orange in color in axenic culture, with various shapes and ranging from 20 to 200 μm in diameter (Figs. 1D, 2B).

Plasmodium

The organism was characterized by having a phagotrophic somatic phase, the phaneroplasmodium. It was a yellowish, creeping, multinucleate mass of protoplasm enveloped by a slimy sheath and was differentiated into two main regions: a massive front and a posterior network of connected veins (Figs. 1B, 2C). The

protoplasm in the veins exhibited reversible or shuttle streaming with a time period of approximately 1.3 min. (duration of a complete cycle *i.e.* from rest to "there" and back again to rest). The plasmodium grew well on agar plates with semi-defined growth medium.

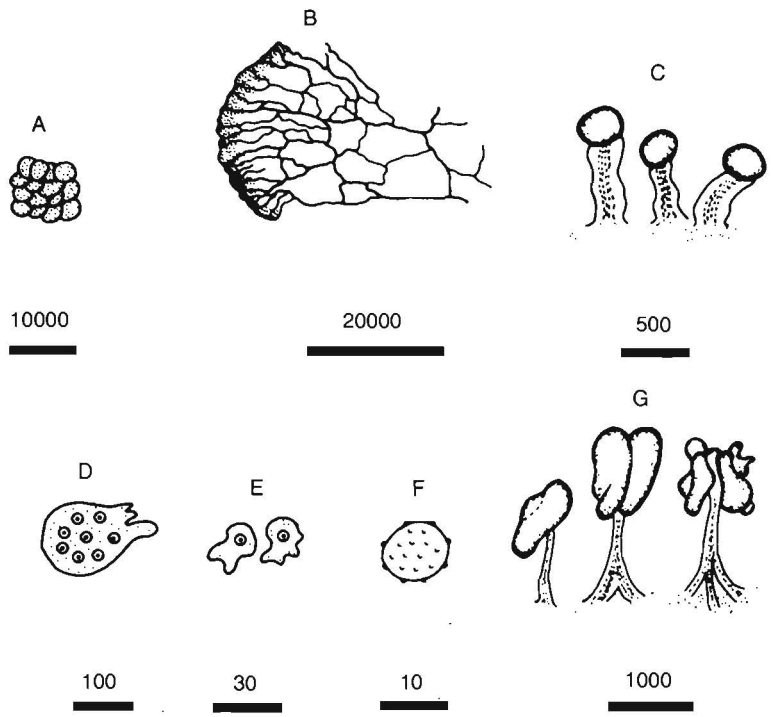

Fig. 1. A. Sclerotium. B. Phaneroplasmodium. C. Young sporangia. D. Microplasmodium. E. Myxamoebae. F. Mature spore. G. Mature sporangia. (Numerals in scales represent length in μm.)

Sporangium

The grayish stalked sporangium was typically gyrose and lobed, appearing to have a semi-compound nature (Figs. 1G, 3A, B). It was seated on a membranous hypothallus which persisted at the base. The peridium was continuous with the stalk and the hypothallus. Branched capillitial threads traversed the interior of the sporangium, separating and supporting the spores (Figs. 3D, E). Abundant granular lime was conspicuous on the peridium (Fig. 3C) and often found in the capillitial nodes (Figs. 3D, E). Spores in mass were black and brownish by transmitted light, minutely spinulose, rounded in shape, 9-11 μm in diameter (Figs. 1F, 3E).

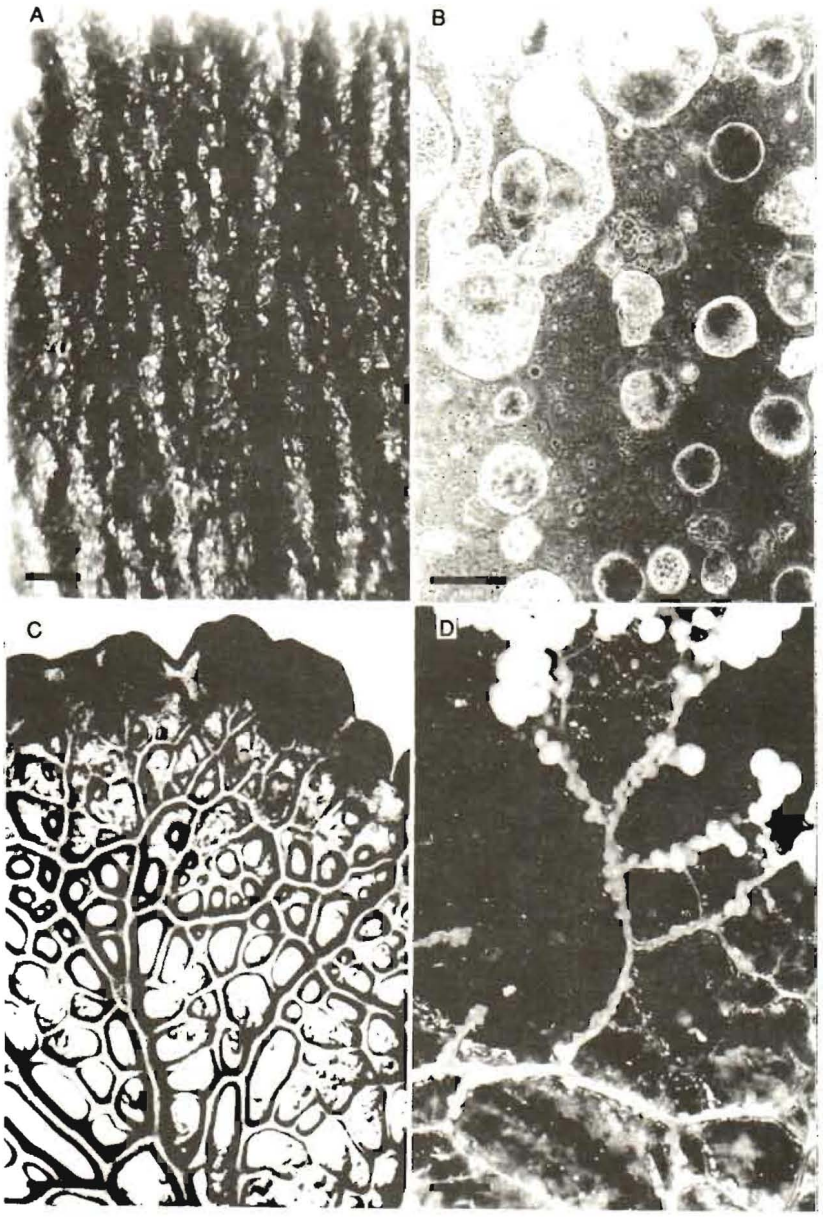

Fig. 2. A. Photomicrograph of a sclerotium on filter paper. (Bar = 1500 μm). B. Phase-contrast micrograph of microplasmodia grown in submerged axenic culture on the semidefined growth medium. (Bar = 100 μm). C. Phase-contrast micrograph of portion of a phaneroplasmodium supported on agar, showing front and veins. (Bar = 500 μm). D. Micrograph of a phaneroplasmodium giving rise to papilla-like presporangial masses. (Bar = 2000 μm.)

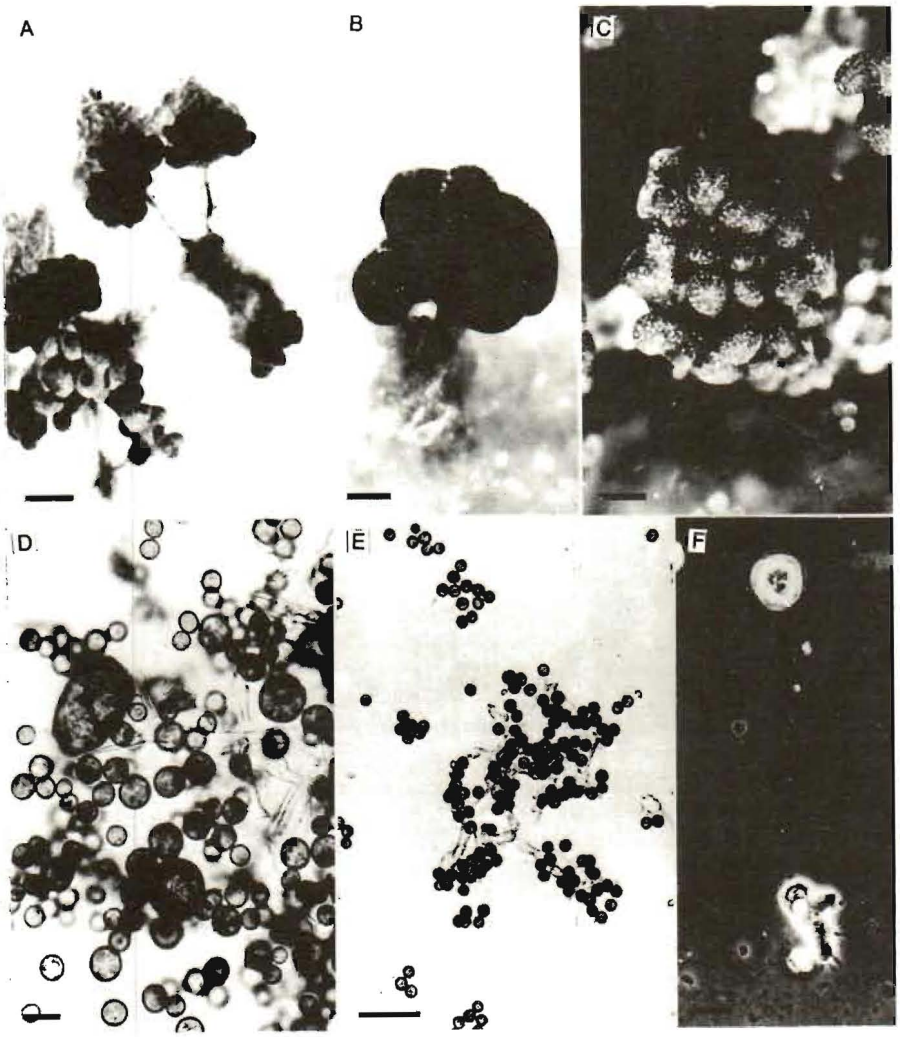

Fig. 3. A Grayish stalked sporangia, typically gyrose and lobed, appearing to have a semicompound nature.

B. Sporangia (side view) showing one stalk carrying many sporangial globules. (Bar = 200 μm).

C. Dry sporangium (top view) with many globules and lime deposit on the peridium. (Bar = 300 μm).

D. Phase-contrast micrograph of a sporangium 12 hr old (squeezed preparation) showing capillitial threads with calcareous nodes, mature (small) and immature (large) spores. (Bar = 20 μm).

E. Phase-contrast micrograph of mature sporangium (squeezed preparation) showing capillitial threads and spores with tiny spines. (Bar = 50 μm).

F. Phase-contrast micrograph of myxamoebae (bottom) and a spore (top) ready for germination. (Bar = 10 μm).

Identification of the Organism

The general characteristics of the plasmodium, sporangia and spores of the isolate resemble those of *Physarum polycephalum* (Order: Physarales; Family: Physaraceae) (Martin and Alexopoulos 1969).

A culture has been deposited in the Jordanian Culture Collection of Microorganisms (JCCM), Department of Biological Sciences, University of Jordan.

Acknowledgement

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الفطر الهلامي اللاخلوي «فيزاروم بوليسيفالم» من تربة الغابات في شمال الأردن

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تم عزل نوع هام من الفطور الهلامية اللاخلوية من غابات دبين في شمال الأردن. تقع هذه الغابات على ارتفاع يزيد على ٧٠٠ متر فوق سطح البحر وتسقط عليها الأمطار بمعدل سنوي يزيد على ٥٠٠ ملم ودرجة الحرارة القصوى حوالي ٢٠°م وتنخفض في الشتاء إلى ما دون الصفر المئوي. تتميز الغابات بوجود أشجار الصنوبر والبلوط وغيرها، وتربتها غنية بالمواد العضوية والمخلفات النباتية. جمعت العينات من مواقع مختلفة بعد أن تم التعرف على الطور الساكن للفطر الهلامي ملتصقاً بأوراق وقلف أشجار الصنوبر المتساقطة. تم عزل النوع في المختبر باستعمال طريقة الحاضنة الرطبة وبالنقل المتعاقب على أطباق الآجار ـ آجار المعقمة. وتمت تنمية العينات النقية المعزولة على بيئة غذائية خاصة.

تميز الفطر بوجود حاملات الأبواغ عديدة الرؤوس والمحمولة على ساق قصيرة واحدة، والغلاف المغطى بترسبات الكلس والأبواغ المستديرة المسننة. وتميز أيضاً بوجود طور خضري زاحف أصفر اللون من النوع «فانيروبلازموديوم» المميز بعروقه البروتوبلا زمية. تميز الطور الخضري بالحركة الانعكاسية الرتيبة للسيتوبلازم داخل عروقه بطور زمني تراوح حوالي ١,٣ دقيقة. أمكن الحصول على الطور الساكن للفطر من الطور الخضري في المختبر بطريقة التجفيف البطيئة، كما أمكن أيضاً الحصول على حاملات الأبواغ بعد تعريض الطور الخضري للضوء المناسب وتم أيضاً الحصول على الطور الأميبي للفطر بتنمية أبواغه الناضجة في بيئات غذائية مناسبة. وقد تم عزل هذا النوع من الفطور

لأول مـرة في الأردن، وبسبب تشابهـه مع الفـطر الهلامي الـلاخلوي «فيـزاروم بوليسيفالم» فقد أعطى هذا الاسم. وقد تم إيـداع عينة نقيـة من الفطر المعـزول لـدى المجموعـة الأردنية لـلأحيـاء الـدقيقـة ـ قسم العلوم الحيـاتيـة ـ الجـامعـة الأردنية.