Dictyostelid cellular slime moulds from Africa

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Dictyostelid cellular slime moulds (dictyostelids) are an understudied group in Africa, but several recent surveys carried out in the context of a Planetary Biodiversity Inventory project funded by the National Science Foundation of the United States have added a considerable number of new records from several areas of the continent. Since Edgar Olive isolated *Polysphondylium pallidum* from a sample of dung collected in Liberia at some point during the period between 1897 and 1900, at least 25 species of dictyostelids have been documented for continental Africa and about the same number for the island of Madagascar. These totals include an appreciable number of forms that appear to be new to science. Most of what is now known about the dictyostelids of continental Africa has been derived from study sites in East Africa and South Africa. Only limited data exist for West Africa and Central Africa, and we are aware of records of only two species of dictyostelids from all of North Africa.

Key words – *Dictyostelium* – distribution – Madagascar – *Polysphondylium*

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Introduction

Dictyostelid cellular slime moulds (dictyostelids) are single-celled, eukaryotic, phagotrophic bacterivores usually present and often abundant in terrestrial ecosystems. These organisms represent a normal component of the microflora in soils and apparently play a role in maintaining the natural balance that exists between bacteria and other microorganisms in the soil environment (Raper 1984). The primary habitat for dictyostelids is the leaf litter decomposition zone of forest soils (e.g., Swanson et al. 1999). However, they are known to occur in other types of soils. Among these are soils of cultivated regions (Agnihothrudu 1956), grasslands and prairies (Smith & Keeling 1968, Sutherland and Raper 1978), deserts (Benson & Mahoney 1977), and both alpine (Cavender 1983) and arctic (Cavender 1978, Stephenson et al. 1991) tundra. In addition, dictyostelids have been reported from the layer of soil-like material ("canopy soil") associated with the epiphytes that occur on the branches and trunks of tropical trees (Stephenson and Landolt 1998). Dictyostelids also occur on dung and were once thought to be primarily coprophilous (Raper 1984).

Africa (including the island of Madagascar), with a total area of 30.2 million km^2, is the second largest continent, occupying more than 20% of the earth's land surface. African vegetation types are diverse and range from moist tropical forests to grasslands, subtropical, Mediterranean, warm temperate shrubs and

woodlands, and deserts. Dictyostelids are an understudied group over most of Africa, but several recent surveys carried out in the context of a Planetary Biodiversity Inventory project funded by the National Science Foundation of the United States have added a considerable number of new records from several areas of the continent. The objective of this paper is to review all previous records of African dictyostelids and summarize the results obtained in these recent surveys.

Methods

Except for the early work by Olive (1901) and the more recent work of Kawakami and Hagiwara (2008a,b), the samples mentioned in this paper were collected from the soil/humus zone at the surface of the ground, stored in sterile plastic bags or other containers and transported to a laboratory for processing. In some instances, this involved mailing sample material back to the United States. In the laboratory, samples were processed following procedures similar to those described by Cavender and Raper (1965). In brief, this involves dispersing the sample material in sterile distilled water, then placing small measured aliquots of the resulting suspension in Petri dishes containing hay infusion agar (Raper 1984), and finally adding a suspension of *Escherichia coli* to each dish. Soil suspensions prepared in this manner contain viable dictyostelid propagules in the form of spores, active amoebae or encysted, resting amoebae called microcysts, and all of these have the potential of yielding clones that are capable of undergoing cell aggregation and fruiting on the agar surface (Raper 1984). Particular isolates derived from spores obtained from newly formed fruiting bodies then can be subcultured to facilitate identification and/or be conserved for future study.

The locations of sites from which samples for isolation of dictyostelids were collected are indicated in Fig. 1. The numbers provided in the figure refer to particular collecting sites listed and briefly described in the section that follows.

Results and Discussion

The first report of a dictyostelid from Africa was by Edgar Olive, who isolated a new species of *Polysphondylium* at some point during the period between 1897 and 1900 (Kawakami & Hagiwara 2008a,b). This new species was recovered from the dung of an ass collected in Liberia (1). At the time, Olive was working towards a Ph.D. degree at Harvard University under the direction of Roland Thaxter and William Farlow. In his Ph.D. dissertation, he referred to his isolate as *P. verticillatum*, but it was later (Olive 1901) formally described as *P. pallidum*.

While a faculty member at Wabash College (Indiana), senior author J.C. Cavender traveled to East Africa on a grant from the Research Corporation and collected soil samples for isolation of dictyostelids at 13 sites in Kenya, Uganda and Tanzania (Cavender 1969). These sites were Tsavo (2), a thorn-scrub woodland in Kenya; Mombasa (3), coastal deciduous forest in Kenya; Moshi (4) and Lake Manyara (5), both ground water forests in Tanzania; the Ituri forest (6) in the Congo Basin (near the Uganda-Congo border), the Budongo Forest Reserve (7) and Lake Victoria (8), all of which are evergreen or semi-evergreen rain forests in Uganda; Fort Portal (9) in Uganda, the Ngorogoro Crater (10) in Tanzania, the Aberdare Mountains (11) in Kenya, the Kilimanjaro Volcano (12) in Tanzania, and the Muhavura Volcano (13) in Uganda, all of which are montane tropical forests except at the very highest elevations, where temperate forests occur; and near the summit of Mt. Muhavura in Uganda, an alpine *Senecio-Lobelia* community located at an elevation of ca 4267 m [14000 ft]. No dictyostelids were recovered from the samples collected near the summit of Mt. Muhavura, but 10 species were recorded from the other collecting sites as well as lower on the mountain. These were *Dictyostelium mucoroides, D. giganteum, D. minutum, D. purpureum, D. polycephalum, D. lavandulum, D. tenue, Polysphondylium pallidum, P. violaceum* and *Acytostelium leptosomum*. Eight of these occurred in the rain forest collecting sites, although one species (*D. minutum*) was found only in montane temperate forests (Cavender 1969).

Hagiwara (1976), who collected samples from several sites in the Mount Margherita area (14) of East Africa during the summer of 1974,

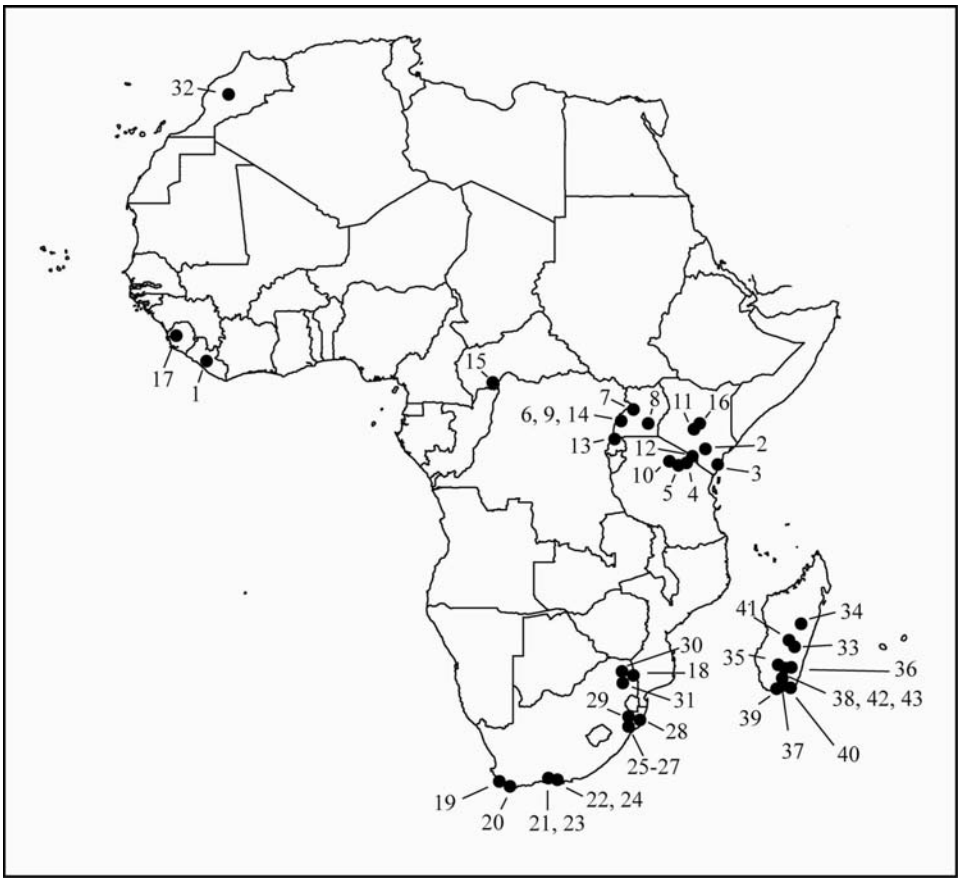

Fig. 1. – Locations of sites in continental Africa and Madagascar from which samples for isolation of dictyostelids have been collected. Numbers refer to particular collecting sites (1–43) referred to in the text.

recovered 11 species of dictyostelids. This total included five described species (*Dictyostelium minutum, D. monochasioides, D. mucoroides, D. purpureum* and *Polysphondylium violaceum*) along with six forms that could not be assigned to any described species.

After moving to Ohio University, Cavender and R. Bradshaw, a Ph.D. student who served as an interpreter, traveled to the Central African Republic on an International Programs grant from NSF and collaborated with Jacques-Paulin Regner and Thomas Damio (both at the University of Bangui). Their objective was to study the effects of slash and burn agriculture on the assemblages of dictyostelids in forest soils. A study area was selected in an evergreen rain forest near the village of Bagandu (15), which is adjacent to one of the Biosphere Reserves in the Central African Republic. Thirteen species were recorded in the study area. These were *Dictyostelium mucoroides, D. giganteum, D. polycephalum, D. purpureum, D. aureostipes, D. tenue, D. lavandulum, D. firmibasis, D. macrocephalum, P. violaceum, P. pallidum, P. tenuissimum* and *P. pseudocandidum*. After slash and burn, only four species were recovered. The number of dictyostelids (11 species) was greatest for a plot in which slash and burn had occurred 14 years earlier. At the time the samples were collected, the plot had a forest of small trees covering the soil. After 34 years, the number of species of dictyostelids had dropped to seven. At the forest edge there were nine species, and eight occurred within the mature forest (Cavender et al. 1993). These data suggested that disturbance may have actually stimulated dictyostelid diversity when the natural vegetation was allowed to recover. A total of 57 isolates, identified and unidentified, from this study and the previous study were preserved by lyophilization and are deposited in the Kenneth B. Raper Dictyostelid Collection housed at Ohio University.

S.L. Stephenson traveled to Kenya and Tanzania in the summer of 1990. While in Kenya, he collected a small series of samples from the alpine zone (>3962 m [13,000 ft]) on

Mt. Kenya (16). However, none of these samples yielded dictyostelids. As such, his results conformed to what Cavender had reported earlier for Mt. Muhavura in Uganda.

In 1995 Kawakami and Hagiwara (2008a, b) traveled to Sierra Leone near Freetown (17) in an effort to collect mating types of *Polysphondylium pallidum* that might possibly represent Olive's isolate. They were unable to reach Liberia because of the country's internal conflict but did obtain mating types of *P. pallidum* as well as those belonging to a new species that was later described as *P. multicystogenum* because of its numerous microcysts.

In 2005, Peter Njuguna, a graduate student at the University of Arkansas, collected a few samples at several sites in the Aberdare Mountains (11) of Kenya. These were processed by J.C. Landolt at Shepherd University. During the summer of the following year, G.G. Ndiritu collected soil samples from a number of additional sites located at various elevations throughout the same mountains. These soil samples were processed for dictyostelids by Cavender at Ohio University, by Landolt at Shepherd University and by Stephenson at the University of Arkansas. Ten species were identified from samples collected in the Aberdare Mountains. These were *Dictyostelium giganteum, D. leptosomum, D. mucoroides, D. minutum, D. sphaerocephalum, D. aureum, D. polycephalum, Polysphondylium violaceum, P. tenuissimum* and *Acytostelium* sp. In addition, there were a number of other isolates that could not be assigned to any known species. Isolates of these were deposited at the American Type Culture Collection (ATCC) and are currently being sequenced by Maria Romeralo at Uppsala University in Sweden. The most significant of these as yet unidentified isolates are Kenya 17B (a species that resembles *D. polycarpum*), Kenya 28 (a very small *Dictyostelium* with oval, consolidated PG+ spores), Kenya 17A (a small and delicate species of *Polysphondylium*), Kenya 19A (a small clustered *Dictyostelium* with PG- spores), Kenya MK-1A (a form that may be intermediate between *Dictyostelium* and *Polysphondylium*), and Kenya MK-10A (a species of *Polysphondylium* with oval spores).

In August of 2006, Jolanda Roux (University of Pretoria) collected samples from seven sites in Kruger National Park (18). These samples were processed by Cavender and Landolt. Six already described species of dictyostelids were isolated, along with an unknown species of *Acytostelium*, two unknown species of *Polysphondylium* and two unknown species of *Dictyostelium* (one of which, Krug 6-5A, resembles *D. aureostipes*). The described species were *D. giganteum, D. purpureum, D. sphaerocephalum, D. lavandulum, D. polycephalum and P. violaceum.* There also were numerous isolates of a light-spored species of *Polysphondylium* (Krug 6-5B, Krug 6C) that resembles *P. asymmetricum*, and this unknown species is currently being sequenced by Romeralo. This is also the case for two of the unknown species (Krug 15A and Krug 6-5A) of *Dictyostelium*.

In November of 2006, Cavender, working with Roux and Karin Jacobs (University of Stellenbosch) collected samples for dictyostelids throughout the forested areas of the Republic of South Africa. Collecting sites were six different regions on Table Mountain (19), Jonkershock and Stellenbosch (20), Knysna Big Tree (21), Tsitsikamma and Storm River (22), Monkeyland (23) and the Birds of Eden Sanctuary (24), Western Cape, in the more temperate region of the country; the Amatikulu Nature Reserve (25), Ongoye (26), Twin Streams (27), Raphia National Monument (28), and the Hluhluwe-Umfolozi Game Reserve (29) in subtropical vegetation in Zululand; and Makhado (30) and Barotta (31) in Limpopo, an area of montane forests. Very few clones of dictyostelids were recorded from the temperate region. Only six species (*Dictyostelium mucoroides, D. giganteum, D. leptosomum, D. implicatum, P. violaceum and P. pallidum*) were widely distributed, while *D. minutum* and two unknown species in the same genus (Eden 1 and Eden 2) were recovered from samples collected at the Birds of Eden Sanctuary. Subtropical soils were richer in dictyostelids and yielded a number of additional species. These were *D. polycephalum, D. purpureum, D. lavandulum, P.* cf. *asymmetricum* and *Acytostelium* sp. as well as a crampon-based species from Ongoye that resembles *D. vinaceo-fuscum* and an unknown species of *Polysphondylium* from Raphia National Monument. Isolates of these four

dictyostelids (Eden 1, Eden 2, Ong 2 and MZ 6) are currently being sequenced by Romeralo.

Earlier in 2006, through the efforts of Marianne Meyer and Khalid Yamni, a set of samples for possible isolation of dictyostelids was obtained in Morocco (32) during a collecting trip to that country. These samples, which were sent to Cavender for processing, yielded only *Dictyostelium mucoroides* and *D. sphaerocephalum.*

In May of 2009, samples for dictyostelids were collected by Carlos Lado (Real Jardin Botanico in Madrid, Spain) from 13 sites on the island of Madagascar as one component of a comprehensive survey of the eumycetozoans of the island. Collecting sites were in Ranomafana National Park (33) and Mandraka (34), both humid tropical forest; L'Isalo National Park (35), tropical forest and sclerophyllous forest; Andringitra National Park (36), secondary tropical forest and grassland; Berenty National Reserve (37), *Euphorbia* and *Alluaudia* dry forest; Ihosy (38), dry forest; Ambovombe (39), *Euphorbia* dry forest; Andohaela National Park (40), leguminose dry forest; Ambositra (41), sclerophyllous forest; Betroka-Beraketa (42), xerophyllous scrub and savannah; and Ihosy-Betroka (43), *Eucalyptus* forest and grassland. The samples were processed for dictyostelids by Cavender and Landolt. Madagascar proved to have unusually rich environments for dictyostelids. Thirteen described species (*Dictyostelium mucoroides, D. giganteum, D. sphaerocephalum, D. leptosomum, D. purpureum, D. lavandulum, D. vinaceo-fuscum, D. tenue, D. polycephalum, D. minutum, D. deminutivum, D. implicatum* and *Polysphondylium violaceum*) were identified. In addition, isolates of five different light-spored species of *Polysphondylium* (*P.* sp.1-MAD 29, *P.* sp. 2-MAD 44, *P.* sp. 3-MAD 14D, *P.* sp. 4-MAD 46, and *P.* sp. 5-MAD 25) were sent to Romeralo to be sequenced. Eight isolates of *Dictyostelium* also appear to be new to science. These are MAD 5A and MAD 14C (both with yellow pigmentation), MAD 52 (characterized by a very slimy sorus and spores), M26B (with very irregular development), M23A (a species similar to *D. implicatum*), M10A, M11A and M12A (all of which are small PG+ species).

Collectively, all of the efforts outlined above provide a reasonable body of data on the dictyostelid biota of only portions of the entire continent of Africa, with the Aberdare Mountains of Kenya probably representing the best known region. Most of what is now known about the dictyostelids of continental Africa has been derived from collecting sites in East Africa and South Africa. Only limited data exist for West Africa and Central Africa, and we are aware of records of only two species of dictyostelids from all of North Africa.

Clearly, considerable additional work remains to be done. This is certainly the case for the vast area of tropical rain forest that exists in the Congo Basin, which seems likely to support a particularly diverse assemblage of dictyostelids. Except for a few collecting sites on the north and east margins, this region of Africa remains virtually untouched.

Summary

Although Africa remains an understudied continent for dictyostelids, at least 25 species have been documented to occur in continental Africa and about the same total is now known for the island of Madagascar. These totals include an appreciable number of forms that are new to science but have yet to be formally described.

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References

Agnihothrudu V. 1956 – Occurrence of Dictyosteliaceae in the rhizosphere of plants in southern India. Experientia 12, 149–151.

Benson MR, Mahoney DP. 1977 – The distribution of dictyostelid cellular slime molds in southern California with taxonomic notes on selected species. American Journal of Botany 64, 496–503.

Cavender JC. 1969 – The occurrence and distribution of Acrasieae in forest soils. II.

East Africa. American Journal of Botany 56, 993–998.
Cavender JC. 1978 – Cellular slime molds in tundra and forest soils of Alaska including a new species, *Dictyostelium septentrionalis*. Canadian Journal of Botany 56, 1326–1332.
Cavender JC. 1983 – Cellular slime molds of the Rocky Mountains. Mycologia 75, 897–903.
Cavender JC, Bradshaw RA, Regner J-P, Damio T. 1993 – Response of soil dictyostelid slime molds to agricultural disturbance in a tropical environment. Biotropica 25, 245–248.
Cavender JC, Raper KB. 1965 – The Acrasieae in nature. I. Isolation. American Journal of Botany 52, 294–296.
Hagiwara H. 1976 – Cellular slime molds from Mount Margherita (Mts. Ruwenzor1), East Africa. Bulletin of the National Science Museum, Series B (Botany) 2, 53–62.
Kawakami S, Hagiwara H. 2008a – *Polysphondylium multicystogenum* sp. nov., a new dictyostelid species from Sierra Leone, West Africa. Mycologia 100, 347–351.
Kawakami S, Hagiwara H. 2008b – A taxonomic revision of two dictyostelid species, *Polysphondylium pallidum* and *P. album*. Mycologia 100, 111–121.
Olive EW. 1901 – A preliminary enumeration of the Sorophoreae. Proceedings of the American Academy of Arts and Sciences 37, 333–344.
Raper KB. 1984 – The Dictyostelids. Princeton University Press, Princeton, New Jersey.
Smith KL, Keeling RP. 1968 – Distribution of the Acrasiae in Kansas grasslands. Mycologia 60, 711–712.
Stephenson SL, Landolt JC. 1998 – Dictyostelid cellular slime molds in canopy soils of tropical forests. Biotropica 30, 657–661.
Stephenson SL, Landolt JC, Laursen GA. 1991 – Cellular slime molds in soils of Alaskan tundra. Arctic and Alpine Research 23, 104–107.
Sutherland JB, Raper KD. 1978 – Distribution of cellular slime molds in Wisconsin prairie soils. Mycologia 70, 1173–1180.
Swanson AR, Vadell EM, Cavender JC. 1999 – Global distribution of forest soil dictyostelids. Journal of Biogeography 26, 133–148.