First records of dictyostelids and protostelids from Ascension Island

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Landolt, J. C., Shadwick, J. D. & Stephenson, S. L. (2008) First records of dictyostelids and protostelids from Ascension Island. – Sydowia 60 (2): 235–245.

Ascension Island (7° 57' S, 14° 22' W) is a small (total area of only 97 km^2), geologically young and extremely isolated island in the South Atlantic, located approximately 1600 km from the coast of Africa. Although much of the biota is relatively well documented, some groups such as the eumycetozoans (slime moulds) remain understudied or even unknown. During early March of 2007, samples of soil/humus and dead plant material for laboratory isolation of two groups of eumycetozoans (dictyostelids and protostelids) were collected at a number of localities on Ascension Island. As a result of this effort, three species of dictyostelids and fourteen species of protostelids were recorded. There were no previous records for either group from the island.

Keywords: biodiversity, long-distance dispersal, slime moulds, South Atlantic

Ascension Island (7° 57' S, 14° 22' W) is a small (total area of only 97 km^2), geologically young and extremely isolated island in the South Atlantic, located approximately 1600 km from the coast of Africa. The island is a dependency of the British overseas territory of Saint Helena, which is situated 1287 km to the southeast. Ascension Island is the tip of a 3200 m high, 60 km wide shield volcano, and the oldest exposed rocks date to only about 1 million years ago. When the island was discovered in 1501, it was almost entirely barren, with a spare flora consisting of no more than about 20-30 species of vascular plants (Ashmole & Ashmole 2000). Since then, large-scale introductions of many different kinds of plants have occurred, and the island now represents a good example of "terraforming" as the term might be used in a terrestrial context (Wilkinson 2004).

Although many elements of the biota of Ascension Island are relatively well documented, others remain understudied or even unknown. The latter is the case for dictyostelids (cellular slime

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moulds) and protostelids, two groups of terrestrial amoeboid protists (eumycetozoans) that produce aerial spore-bearing structures (Raper 1984, Spiegel *et al.* 2004). Dictyostelids represent a normal component of the microflora of the surface humus and litter layers of most soils but are especially common in forest soils (Raper 1984, Cavender 1990). The canopy soil microhabitat (*sensu* Stephenson & Landolt 1998) of moist tropical forests also supports an assemblage of dictyostelids. The fruiting bodies of dictyostelids are essentially microscopic, and only rarely, and then under special conditions, are these structures observed directly in nature. Studies of dictyostelids invariably involve isolating these organisms in laboratory culture. There are approximately 130 described species of dictyostelids, and the majority of these have been described since 1970.

Protostelids, a group first recognized less than 50 years ago, are widely distributed in many different kinds of habitats (Olive 1975, Spiegel 1990, Spiegel *et al.* 2004). All of the approximately 40 species described to date produce microscopic fruiting bodies characterized by a delicate acellular stalk that supports one or a few spores. The fruiting bodies of protostelids are very small, with those of some species less than 10 µm tall. Because of their size, protostelids can be detected only by the microscopic examination of substrates under laboratory conditions (Spiegel *et al.* 2004).

The objective of the project described herein was to determine just which species of dictyostelids and protostelids were present on Ascension Island, where there appear to be no previous records for either group. A secondary objective was to obtain data relating to the distribution and ecology of the species present on the island and to consider the ways in which they may have been introduced.

Materials and Methods

During early March of 2007, samples of soil/humus and dead plant material for laboratory isolation of dictyostelids and protostelids were collected at a number of localities on Ascension Island. At each locality, samples were collected at more or less regular intervals along a transect that extended through a representative area of the particular habitat being examined. For protostelids, an effort was made to collect samples of as many different types of dead plant material as possible. Samples of dead plant material consisted of either ground litter or aerial litter (dead but still attached plant parts above the ground). All localities were referenced to geographic location through the use of the NAVSTAR Global Positioning System (GPS), with latitude and longitude determined by means of a portable GPS unit. Samples to be processed for dictyostelids were sent to the laboratory of the first author, whereas isolation procedures for protostelids were carried out at the University of Arkansas.

Isolation procedures for dictyostelids followed those described by Cavender and Raper (1965). Each sample of soil/humus or canopy soil was weighed and enough sterile distilled water added to obtain an initial suspension of 1:10. This mixture was shaken to disperse the material and to suspend the cells of dictyostelids present. A 5.0 mL volume of this initial suspension was added to 7.5 mL of sterile distilled water to create a 1:25 final dilution. Aliquots (each 0.5 mL) of this dilution were added to the surface of each of three 100 by 15 mm plastic disposable Petri dishes containing hay infusion agar (Raper 1984). Approximately 0.4 mL of a heavy suspension of *Escherichia coli* was added to each Petri plate, and the plates incubated under diffuse light at 22–25 °C. Each plate was examined carefully at least once a day following the appearance of initial aggregations, and the location of each aggregation marked. When necessary, particular isolates were subcultured to facilitate identification.

Protostelids were isolated using a modification (Spiegel *et al.* 2004) of the technique described by Olive (1975). Each sample of dead plant material was broken into small pieces, moistened with distilled water and plated onto a weak malt extract/yeast extract agar. Pieces were arranged in four rows on each plate in a cross shape. Observations were made with a compound light microscope using the 10X objective lens. Plates were examined once weekly, beginning 5 days after being plated out and continuing for a period of at least two weeks. Species of protostelids appearing in each plate were identified on the basis of the morphology of their fruiting bodies and amoebae (Olive 1975, Spiegel 1990) and their occurrence on a particular sample recorded.

Results

Three species of dictyostelids and fourteen species of protostelids were recorded from samples collected in the present study. There were no previous records for either group from the island. In addition, the acrasid cellular slime mould *Acrasis rosea* Olive & Stoianovitch was isolated from a sample of aerial litter collected from a clump of *Agave americana* L. in the lower portion of Grazing Valley along the road to the old NASA site.

Fourteen species of protostelids were recovered from aerial litter and 12 were recovered from ground litter. The aerial litter microhabitat was dominated by *Protostelium mycophagum*, which was recorded from 75% of all (both aerial and ground) samples, and *Soliformovum irregularis*, recorded from 42% of all samples. The ground litter microhabitat was dominated by the minute species *Schizoplasmodiopsis pseudoendospora* (39% of all samples), with both *Protostelium mycophagum* and *Schizoplasmodiopsis amoeboidea* recorded from 23% of all samples. Two species (*Nematostelium ovatum* and *Protostelium nocturnum*) occurred only on aerial litter, while five species (*Ceratiomyxella tahitiensis, Protostelium pyriformis, Schizoplasmodiopsis vulgare, Schizoplasmodium cavostelioides* and *Tychosporium acutostipes*) occurred only on ground litter. The total number of occurrences of all species on the 25 samples processed was 50, for an average of two species per sample.

Annotated list of species

In the list that follows, dictyostelids and protostelids recorded from Ascension Island are arranged alphabetically by genus and then species in each of the two groups. Information is provided on the source(s) of each record, including the microhabitat or substrate from which the species in question was cultured and the locality from which the sample material was collected. Additional comments are included for records of particular interest. Nomenclature follows Raper (1984) for dictyostelids and Olive (1975), Spiegel et al. (1994), and Spiegel et al. (1995) for protostelids.

Dictyostelids

Dictyostelium aureo-stipes Cavender, Raper & Norberg

Source of record. – soil/humus, mesic site along Elliot Trail on Green Mountain.

Comments. – Two clones of this species were recovered from a sample collected from one of the more mesic sites visited on the island.

Dictyostelium giganteum B.N. Singh

Sources of record. – soil/humus, mesic site along Elliott Trail on Green Mountain; soil/humus, a planted grove (The Pines) of *Araucaria excelsa* (Lamb.) R. Br. on the lower slope of Green Mountain.

Comments. – A single clone of *D. giganteum* was recovered from each of two sites located some distance apart and ecologically quite different. As such, it seems likely that this species occurs in other localities throughout the island.

Polysphondylium pallidum Olive

Source of record. – canopy "soil" collected from beneath mats of bryophytes on the branches of screwpine (*Pandanus utilis* Bory) trees along the trail to the summit on Green Mountain.

Comments: – Three different samples yielded a total of 30 clones of this species, which occurred at an estimated density (ca. 100 clones per gram) much higher than those recorded for either of the other dictyostelids recorded from Ascension Island. Interesting, values of pH recorded for samples of canopy "soil" (4.2 to 4.8) were appreciably lower than those (6.3 to 7.4) recorded for samples of soil/ humus on the ground.

Protostelids

Ceratiomyxella tahitiensis L.S. Olive & Stoian.

Sources of record. – ground litter, near Dew Pond at the summit of Green Mountain; ground litter, along Elliott Trail on Green Mountain.

Echinosteliopsis oligospora Reinhardt & L.S. Olive

Sources of record. – ground litter, near Dew Pond at summit of Green Mountain; aerial litter, clump of *Agave americana* in the lower portion of Grazing Valley along road to the old NASA site; ground litter, clump of *Agave americana* in the lower portion of Grazing Valley along road to the old NASA site.

Nematostelium ovatum (L.S. Olive & Stoian.) L.S. Olive & Stoian.

Source of record. – aerial litter, along Elliott Trail on Green Mountain.

Protostelium arachisporum L.S. Olive

Sources of record. – aerial litter, along road below Garden Cottage on Green Mountain; ground litter, along road below Garden Cottage on Green Mountain; ground litter, along Elliott Trail on Green Mountain.

Protostelium mycophagum L.S. Olive & Stoian.

Sources of record. – aerial litter, along road below Garden Cottage on Green Mountain; ground litter, along road below Garden Cottage on Green Mountain; aerial litter, along trail to summit of Green Mountain; ground litter, near Dew Pond at summit of Green Mountain; aerial litter, along trail to The Pines; along Elliott Trail on Green Mountain; aerial litter, clump of *Agave americana* in the lower portion of Grazing Valley along road to the old NASA site; ground litter, clump of *Agave americana* in the lower portion of Grazing Valley along road to the old NASA site.

Comments. – This species, generally considered as the most common and widespread of all protostelids, has been recorded from numerous different types of habitats worldwide. The most commonly encountered species on Ascension Island, it was isolated from 12 different samples. The species concept traditionally used for *Protostelium mycophagum* actually encompasses at least three different taxonomic entities (Shadwick, unpublished data), and all of these were represented among the isolates recovered from the samples collected on Ascension Island.

Protostelium nocturnum Spiegel

Sources of record. – aerial litter, along trail to the summit of Green Mountain; aerial litter, along road below Garden Cottage on Green Mountain.

Protostelium pyriformis L.S. Olive & Stoian.

Source of record. – ground litter, clump of *Agave americana* in the lower portion of Grazing Valley along road to the old NASA site.

Schizoplasmodiopsis amoeboidea L.S. Olive & K.D. Whitney

Sources of record. – aerial litter, along road below Garden Cottage on Green Mountain; ground litter, along road below Garden Cottage on Green Mountain; ground litter, along trail to the summit of Green Mountain; ground litter, Dew Pond on the summit of Green Mountain; aerial litter, along trail to The Pines.

Schizoplasmodiopsis pseudoendospora L.S. Olive, Martin & Stoian.

Sources of record. – ground litter, along road below Garden Cottage on Green Mountain; ground litter, Dew Pond on the summit of Green Mountain; aerial litter, along Elliott Trail on Green Mountain; ground litter, along Elliott Trail on Green Mountain; ground litter, clump of *Agave americana* in the lower portion of Grazing Valley along road to the old NASA site.

Schizoplasmodiopsis vulgare L.S. Olive & Stoian.

Sources of record. – ground litter, along road below Garden Cottage on Green Mountain; ground litter, near Dew Pond at the summit of Green Mountain.

Schizoplasmodium cavostelioides L.S. Olive & Stoian.

Source of record: – ground litter, along road below Garden Cottage on Green Mountain.

Soliformovum expulsum (L.S. Olive & Stoian.) Spiegel

Sources of record. – aerial litter, along trail to the summit of Green Mountain; aerial litter, along the trail to The Pines; ground litter, along Elliott Trail on Green Mountain.

Soliformovum irregularis (L.S. Olive & Stoian.) Spiegel

Sources of record. – ground litter, along road below Garden Cottage on Green Mountain; aerial litter, along the trail to the summit of Green Mountain; aerial litter, near Dew Pond on the summit of Green Mountain; ground litter, near Dew Pond on the summit of Green Mountain; aerial litter, along trail to The Pines.

Comments. – *Soliformovum irregularis*, which was isolated from seven different samples, was the second most common protostelid recorded on Ascension Island.

Tychosporium acutostipes Spiegel, Moore & Feldman

Sources of record: – ground litter, along road below Garden Cottage on Green Mountain; ground litter, near Dew Pond on the summit of Green Mountain.

Discussion

It has long been recognized that various small particles, including dust, spores, bacteria and other microbes, can be carried long distances by wind. For example, the British mycologist Berkeley (1857) concluded that "The trade winds, for instance, carry spores of fungi mixed with their dust, which may have traveled thousands of miles before they are deposited." In the mid-1930's, Meier (Meier & Lindbergh 1935) identified a variety of fungal spores, pollen, algae and diatoms from a series of samples collected over the North Atlantic by exposing sterile, oil-coated microscope slides directly to the air by way of a long metal arm extending from an airplane. A process that could transfer enormous numbers of microorganisms into the atmosphere was identified in the late 1990's, when satellite images revealed the astonishing magnitude by which desert soils are aerosolized into giant clouds of dust (Griffin *et al.* 2001, 2002; Kellogg & Griffin 2006). These clouds of dust frequently move across the Atlantic Ocean and reach the Caribbean, Central America, northern South America and the southeastern United States, where the particles they contain (including spores) are deposited. Similar long-range movements of dust have been demonstrated for other parts of the world, including from Asia across the Pacific Ocean to western North America and from Australia to New Zealand. One particularly large dust cloud originating in China actually moved eastward all the way across the Pacific, North America and the Atlantic to reach Europe, thus traveling most of the way around the world (Griffin *et al.* 2002). Clearly, airborne spores would have the potential of being dispersed by wind over considerable distances. If the spores of protostelids are largely wind-dispersed, as is likely to be the case (Olive 1975), then global wind patterns would give them considerable potential for long-distance dispersal over the expanses of ocean that separate Ascension Island from the continents of Africa and South America.

Although long-distance dispersal by wind undoubtedly accounts for the presence of many of the microorganisms found on an isolated land mass such as Ascension Island, this is not necessarily true for all groups. For example, the spores produced by dictyostelids are embedded in a mucilaginous matrix that dries and hardens. As such, the spores stand little chance of being dispersed by wind (Cavender 1973, Olive 1975). However, it has been clearly demonstrated that viable dictyostelid spores may be dispersed great distances by birds (Suthers 1985). Birds have an excellent chance of covering the long distances from neighboring land masses to Ascension Island, either on their own or with assistance of humans. In addition to a variety of seabirds and migratory shorebirds found on Ascension Island (McColloch 2004), Bourne and Simmons (1998) also listed such vagrant species as the common swift, barn swallow, common cuckoo, European nightjar, European roller and red-backed shrike. Birds that have been introduced to the island include the starling (now apparently extinct) and breeding populations of the common mynah, house sparrow, common waxbill and yellow canary. Other animals and plants introduced to the island by Europeans over the past two centuries also may have served as vectors for dictyostelids, allowing these organisms to reach Ascension Island from elsewhere on the globe. The three species of dictyostelids isolated in this study are common and globally widespread. This is especially true for *Dictyostelium giganteum* and *Polysphondylium pallidum* (Raper 1984). In addition, *P. pallidum* has been recovered from canopy soils collected in more than 15 other localities in tropical, subtropical and temperate forests throughout the world (Stephenson & Landolt 1998, unpublished data). The relative "indifference" of *P. pallidum* to climatic conditions was noted by Cavender (1973).

In addition to birds, the dispersal of dictyostelids from the ground soil habitat to canopy soil microsites on Ascension Island may have involved some other type of vector. There would seem to be a number of possibilities, since previous studies have demonstrated that many different kinds of animals are capable of transporting the spores of dictyostelids over distances of several meters or more. For example, Stephenson and Landolt (1992) isolated dictyostelids from the fecal material collected from bats, rodents and amphibians as well as birds and also (unpublished data) from the fecal material of arboreal land snails in Puerto Rico. Stephenson et al. (2007) demonstrated that cave crickets could carry dictyostelid spores both internally and externally. Presumably, various other insects, including forms known to be present on Ascension Island (Ashmole & Ashmole 1997) would be capable of doing the same thing. In general, dictyostelid species richness and density are somewhat lower in canopy soil compared to ground soils in the same forest (Stephenson & Landolt 1998).

Moore and Spiegel (2000a) placed sections of sterilized straw culms in the field to simulate naturally occurring aerial litter microhabitats for protostelids in temperate forests and grasslands. Straws left in place for periods ranging from two to 12 weeks, averaged 78% and 80% colonization in forests and grasslands, respectively (Moore & Spiegel 2000). The magnitude of the spore rain of the *Protostelium mycophagum*, a species of protostelid with deciduous spores, in a beech forest in Germany was determined to be at least one spore per 10 cm of leaf area over a six month period (Tesmer et al. 2005). These data suggest the occurrence of large numbers of protostelid spores in the air, even in grasslands with no tree canopy. Consequently, long-distance dispersal of spores by wind to relatively young land masses such as Ascension Island seems quite likely and probably occurs on a regular basis. However, the dominant species (*Schizoplasmodiopsis pseudoendospora*) recorded from ground litter is not characterized by deciduous spores, which makes it less likely to be dispersed in the air. This species could have been carried to Ascension Island by ground feeding birds or arrived in association with introduced animals or plants, as described above for dictyostelids.

Although the number of samples in this study is relatively small, the dominance of certain species in particular types of microhabitats conforms to what has been reported in other studies. For example, *Protostelium mycophagum* and *Soliformovum irregularis* have been found to dominate aerial litter, including both sterilized artificial substrates (Moore & Spiegel 2000a) and naturally occurring substrates (Aguilar *et al.* 2007). Moreover, *Schizoplasmodiopsis pseudoendospora* has been reported previously to dominate ground litter (Moore & Spiegel 2000a, Moore & Spiegel 2000b, Moore *et al.* 2000).

In summary, although Ascension Island is extremely isolated and geologically young, both dictyostelids and protostelids have managed to reach it. Whether this was the result of long-distance dispersal by wind, animal vectors or inadvertent introduction by human activities is unknown. Interestingly, protostelids, which are thought to be largely wind-dispersed, are better represented on the island than dictyostelids, whose spores probably depend largely upon animal vectors for their dispersal.

Acknowledgements

This study was supported in part by a grant from the National Science Foundation. Appreciation is extended to Susanna Musick and Stedson Stroud of Ascension Island Conservation for supplying information on collecting sites and various aspects of the biota of Ascension Island.

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(Manuscript accepted 9 June 2008; Corresponding Editor: R. Pöder)

Zeitschrift/Journal: Sydowia

Jahr/Year: 2008

Band/Volume: 60

Autor(en)/Author(s): Landolt John C., Shadwick John D., Stephenson Steven Lee

Artikel/Article: First records of dictyostelids and protostelids from Ascension Island. 235-245