Mycol Progress (2006) 5:231–241
DOI 10.1007/s11557-006-0515-8

ORIGINAL ARTICLE

Dictyostelids from Mediterranean forests of the south of Europe

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Received: 11 July 2006 / Accepted: 21 September 2006 / Published online: 18 October 2006

Abstract The first results of a study of dictyostelid diversity in soils of Mediterranean vegetation are presented. Surface soil samples were collected during 2003–2004 from different Mediterranean forests in Spain and Portugal and were plated out for cellular slime moulds. Isolated during this study were 14 species of dictyostelids. One species, *Dictyostelium firmibasis* is reported for the first time from Europe, and nine (*Dictyostelium aureo-stipes*, *Dictyostelium* sp. 1, *Dictyostelium fasciculatum*, *Dictyostelium giganteum*, *Dictyostelium implicatum*, *Dictyostelium leptosomum*, *Dictyostelium sphaerocephalum*, *Polysphondylium candidum*, *Polysphondylium pallidum*) are new records for the Mediterranean region. Members of the three genera *Dictyostelium* (ten species), *Polysphondylium* (three species) and *Acytostelium* (one species) are present in this region of the world. Comments on their diversity and distribution are included.

Introduction

The dictyostelids or cellular slime moulds are organisms, which live in soils of different types of vegetation such as forests, grasslands and cultivated fields where they selectively feed on bacterial cells (Cavender and Raper 1965b). They are microscopic, amoeboid organisms distributed all over the world (Bonner 1967; Raper 1984), but the species diversity, according to Cavender (1973) and Swanson et al. (1999), seems to increase with a decrease in latitude and altitude. The dictyostelids include three genera (*Acytostelium*, *Dictyostelium*, *Polysphondylium*) with about 100 species that are good bioindicators of microbial activity in nature (Raper 1984). Dictyostelids with protostelids and myxomycetes make up a monophyletic group, the Eumycetozoa (Olive 1975; Cavender 1990; Spiegel et al. 1995, 2004; Baldauf et al. 2000). Forest-humus is their favourite habitat because they need moist and aerobic places without the direct effect of sun and wind.

In the south of Europe, especially in the Mediterranean region, only six species of dictyostelids were reported (Cavender 1969; Swanson et al. 1999) (see Table 1). Two more species, *Dictyostelium rosarium* Raper and Cavender and *Polysphodylium filamentosum* Traub, Hohl and Cavender, also were recorded by Hagiwara (personal communication) from central Spain (Madrid), but the data remain unpublished.

The Mediterranean basin has the richest and most varied flora of Europe and one of the richest per square kilometre in the world (Quezel 1985). This region is considered as one of the 34 hotspots of biodiversity in the world, but very little information is known about the dictyostelids linked to this vegetation. The present study was undertaken to obtain new information on the species present in this region and the relation of cellular slime moulds to soils of Mediterranean vegetation.

Materials and methods

During the years 2003–2004, samples of soils were taken from forests, which are well-represented in the Iberian Peninsula. Especially, but not exclusively, the evergreen

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Table 1 Dictyostelids found in the south of Europe (Spain and Portugal) before 2003

Species	Locality	Habitat	Reference
Acytostelium leptosomum Raper	Bilbao	Mixed forest	Cavender (1969)
Dictyostelium lacteum Tiegh.	Bilbao	Mixed forest	Cavender (1969)
Dictyostelium minutum Raper	Cantabria	Oak forest	Cavender (1969)
	Bilbao	Mixed forest	Cavender (1969)
Dictyostelium mucoroides Bref.	Granada	Mixed forest (elm dominates)	Cavender (1969)
	Cantabria	Oak forest	Cavender (1969)
	San Sebastián	Mixed forest (oak dominates)	Cavender (1969)
	Bilbao	Mixed forest	Cavender (1969)
Dictyostelium purpureum Olive	Mallorca		Swanson et al. (1999)
Polysphondylium violaceum Bref.	San Sebastián	Mixed forest (oak dominates)	Cavender (1969)

forests (*Quercus* spp.) characteristic of the Mediterranean vegetation were studied. Samples were taken beneath the following substrates, *Arbutus unedo*, *Ceratonia siliqua*, *Cistus* spp., *Juniperus phoenicea*, *J. communis*, *J. oxycedrus*, *Olea europaea*, *Pinus halepensis*, *P. pinea*, *Pistacia lentiscus*, *Quercus canariensis*, *Q. coccifera*, *Q. faginea*, *Q. ilex*, *Q. pyrenaica* and *Q. suber.* In addition, substrates from some boreal forest of *Abies alba*, *Acer* spp., *Betula pendula*, *Castanea sativa*, *Corylus avellana*, *Fagus sylvatica*, *Fraxinus excelsior*, *Ilex aquifolium*, *Pinus pinaster*, *P. sylvestris*, *Populus* spp., *Quercus petraea*, *Thyllia* spp. were sampled for comparison. The range of altitude of the sampling sites was from 10 to 1700 m, and all sites were georeferenced with the aid of a GPS.

Soil samples of 20 g were collected in Whirl Pak® plastic bags. Samples were processed as soon as possible after collection. Procedures described by Cavender and Raper (1965a) were followed. A final soil dilution of 1/50 in distilled water was used for all samples. Soil pH was measured in the laboratory before the dilution. Culture plates were incubated under diffuse light at 20–25°C. Each plate was carefully examined at least once a day for 1 week after the appearance of initial aggregations and the location of each aggregate clone marked. This examination was done with a stereomicroscope Nikon SMZ 1000.

Cellular slime mould isolates were sub-cultured to facilitate identification. For this we prepared two-member cultures with the bacterium *Escherichia coli* or *Klebsiella aerogenes*. Isolates of each species were cultivated on non-nutrient agar (2%) and preserved by freezing with glycerol (20%) (http://www.dictybase.org).

For the morphological features of the spores, 20 spores per isolate were measured, and the mean ratio of length to width was calculated. Distilled water was used as a mounting medium. Photomicrographs were taken using an Eclipse 600 model Nikon microscope with DIC.

Taxonomic treatment used herein follows that of Raper (1984).

Results

Isolated from the soil samples in different places and substrates in the Spain and Portugal study were 14 different species of dictyostelids. A total of 150 isolates were recovered from 200 samples that were collected. Ten species isolated belong to the genus *Dictyostelium*, the most numerous and most widely distributed of the group, three to the genus *Polysphondylium* and one to *Acytostelium*, the genus of the dictyostelids with smallest species-size. One of the species (*Dictyostelium firmibasis* H. Hagiw.) is a new record for Europe and nine (*Dictyostelium aureo-stipes*, *Dictyostelium* sp. 1, *Dictyostelium fasciculatum*, *Dictyostelium giganteum*, *Dictyostelium implicatum*, *Dictyostelium leptosomum*, *Dictyostelium sphaerocephalum*, *Polysphondylium candidum*, *Polysphondylium pallidum*) are not previously reported from the south of Europe or the Mediterranean region. From the total, ten are new records for Spain and seven are new records for Portugal. Cavender 1969 reported three species for Portugal, *D. minutum*, *D. mucoroides* and *P. pallidum*. The total number of cellular slime moulds reported from these two countries is 18, to date.

List of species

Acytostelium leptosomum Raper

Portugal. Algarve: Monchique, Cortes, road EN-266, 37°17′N 8°33′W, 300 m, under *P. pinaster* in a mediterranean forest with *A. unedo*, *C. siliqua*, *Cistus salvifolius*, *Crataegus monogyna*, *Genista hirsuta*, *P. lentiscus*, *Quercus suber* and *Rhamnus alaternus*, 17-XI-2004, Romeralo 212.

This is the first record for Portugal. This species was found previously in the north of Spain by Cavender (1969). It is the only species of the genus that was found in Europe (Swanson et al. 1999).

A. leptosomum differs from other *Acytostelium* species by its gregarious to clustered habit, larger size (750–1500 μm in length) and globose spores (5–7 μm in diameter). The material studied matches the description made by Raper (1984) for this species. Their small size and resemblance to filamentous fungi (Cavender and Vadell 2000) make it more difficult to distinguish at first.

D. aureo-stipes Cavender, Raper and Norberg

Spain. Asturias: Pola de Somiedo, La Malva, 43°07′06″N 06°15′42″W, 737 m, under *Prunus laurocerasus*, 5-X-2005, Romeralo 282.

D. aureo-stipes has yellow pigmentation on its sorophores, typical cell aggregations (violaceum type), subdividing into many sorogens (Fig. 1a), polar granules are present on the spores, and the pattern of branching is irregular (Fig. 2a).

This isolate has clustered growth, intense yellow pigmentation, consolidated polar granules and the branching pattern is less developed. The spore size is 6–6.5×3–3.5 μm. It was cultured in the dark at 23°C.

This is the first record for the south of Europe.

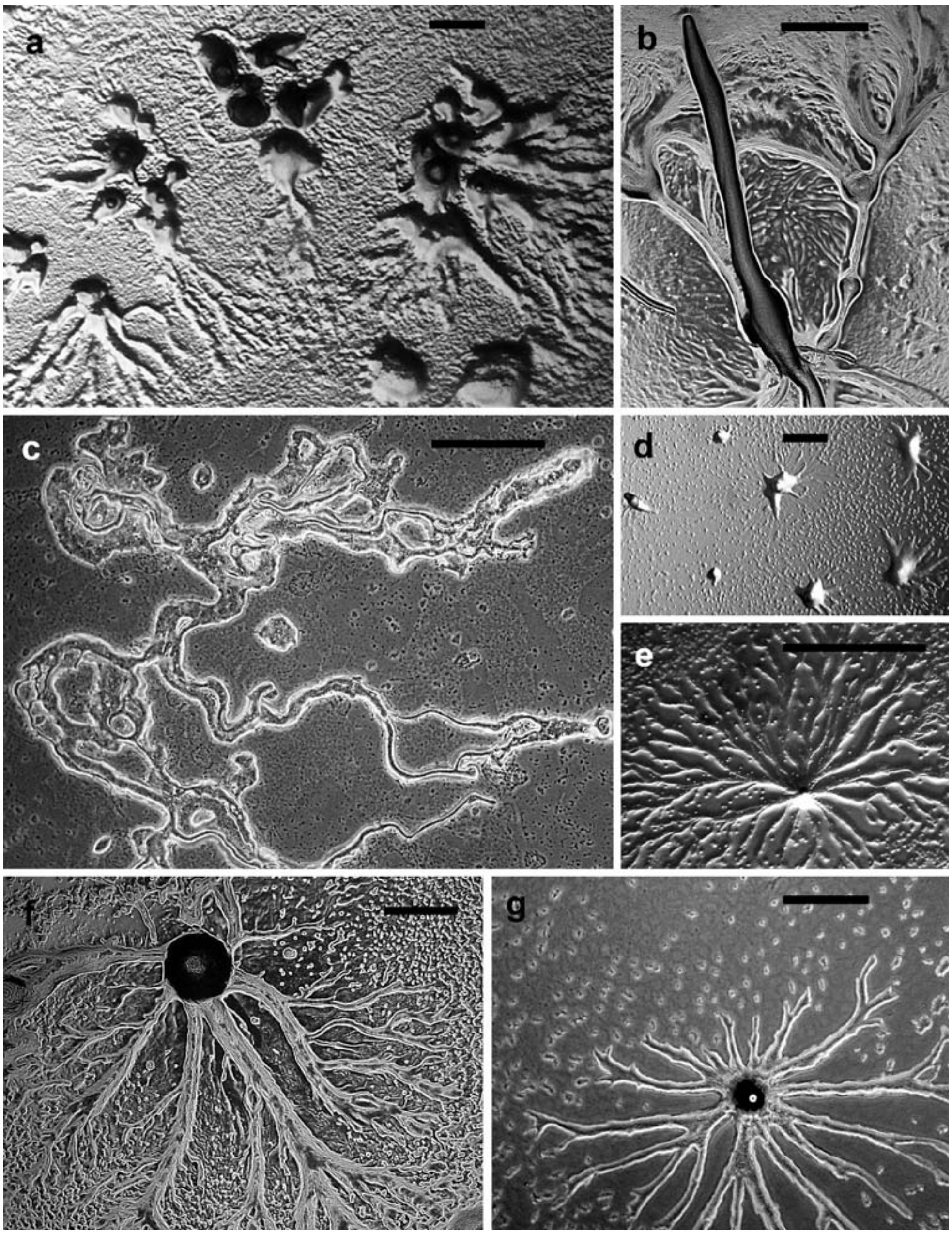

Fig. 1 **a** Aggregation of *D. aureo-stipes*, note the subdivision of the streams into many mounds, which result in many sorogens (isolate Romeralo 282) (scale: 100 μm). **b** Aggregation of *D. implicatum*, note the thick streams and the long pseudoplasmodium (isolate Romeralo 16A) (scale: 100 μm). **c** Aggregation of *D. minutum* (isolate Romeralo 11C) (scale: 100 μm). **d** Aggregation of *D. mucoroides*, small and radiate (isolate Romeralo 1B) (scale: 100 μm). **e** Aggregation of *D. sphaerocephalum*, large and radiate (isolate Romeralo 20B) (scale: 100 μm). **f** Aggregation of *P. candidum* with thick streams (isolate Romeralo 89A) (scale: 100 μm). **g** Aggregation of *P. pallidum*, note the thinner streams with respect to *P. candidum* (isolate Romeralo 23D) (scale: 10 μm)

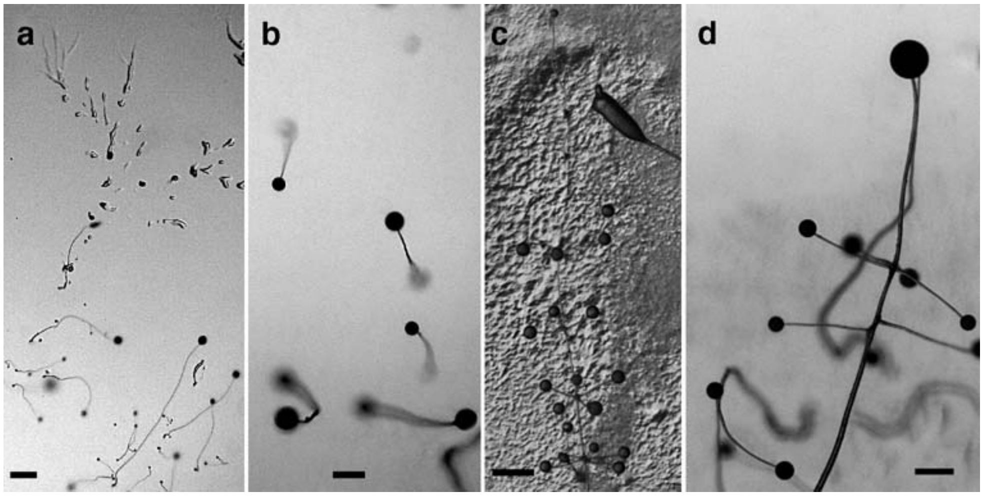

Fig. 2 **a** Pattern of growth of *D. aureo-stipes* with typical aggregation of the species (isolate Romeralo 282) (scale: 20 μm). **b** Sorocarps of *D. sphaerocephalum*, note the characteristic L-shape of the sorophore (isolate Romeralo 6E) (scale: 10 μm). **c** Sorocarp of *P. pallidum*, note the regular whorls along the sorophore (isolate Romeralo 23D) (scale: 10 μm). **d** Irregular whorls characteristic of *P. violaceum* (isolate 289A) (scale: 10 μm)

Dictyostelium sp. 1

Spain. Navarra: Yesa, Km. 1–2 of the road NA-2201, 42° 40′48″N 01°09′09″W, 870 m, under *Quercus ilex*, 4-VII-2004, Romeralo 160.

This isolate is similar to *D. delicatum* in some aspects, but different in others. In our isolate, sorocarps are usually gregarious, as in *D. delicatum*, sometimes solitary, but unbranched (branched in *D. delicatum*). Sorocarps are medium size and sometimes prostrate. Sorophores gradually tapering from bases to tips, colorless, tips capitate (not clavate as *D. delicatum*). Sori are white and globose. Spores are hyaline, elliptical, mostly 4–6×2.5–4 μm (average 5.15×2.8 μm) with consolidated polar granules (as *D. delicatum*). However the aggregations are different, of the violaceum type, typically sub-divided in aggregative behaviour, very similar to *D. aureo-stipes* aggregation. Pseudoplasmodia migrate while forming the sorophore like *D. delicatum*.

D. fasciculatum Traub, Hohl and Cavender

Spain. Cádiz: La Espera, Lagunas Zorrilla, 36°52′N 5°52′W, 100 m, under *P. lentiscus*, near *Chamaerops humilis*, 26-VI-2003, Romeralo 24. Badajoz: Calera de León, Km. 716 of the road N-630, 38°07′49″N 06°17′18″W, 670 m, under *Q. ilex*, 7-XI-2003, Romeralo 100. Huelva: El Collado, Puerto de Alajar, 37°53′10″N 6°39′42″W, 845 m, under *O. europaea* in an unproductive olive grown, 4-XI-2003, Romeralo 87. La Presa, Sendero la Urralera, 37°53′25″N 6°39′54″W, 825 m, sample taken in an evergreen forest under a mix of trees and bushes with *P. lentiscus*, *Q. ilex*, *A. unedo* and *Smilax aspera*, 4-XI-2003, Romeralo 90.

First record for the south of Europe, previously known from Switzerland (Traub et al. 1981a,b) and Germany (Cavender et al. 1995). This species was described from beech forests, but in Spain, it appears in typical Mediterranean vegetation with *Quercus faginea* and *O. europaea.* Raper (1984) considered that *D. fasciculatum* is probably cosmopolitan in distribution but Cavender et al. (1995) mention that it is not known anywhere outside Europe. Recently (Cavender et al. 2002), it was also found in New Zealand. In the south of Europe it seems related with limestone soils with slightly basic pH (6.49, 7, 7.22, 7.65, 7.81).

The distinctive features of this species are the clustered sorocarps, the strong phototropism, the spores, 5–6×2.2–3 μm with the presence of conspicuous polar granules and the absence of pigmentation in the sorocarps.

D. firmibasis H. Hagiw.

Portugal. Algarve: Loulé, Querença, road EN-396, 37°11′N 8°10′W, 238 m., under *O. europaea*, near *P. lentiscus* and *A. unedo*, 16-XI-2004, Romeralo 207.

Spain. Huelva: Santa Ana la Real, 37°52′02″N 6°42′40″W, 620 m, under *Quercus*, 4-XI-2003, Romeralo 86.

This species has solitary growth and unbranched sorocarps. Sorophores are colorless, often with a basal disk. Very characteristic is the conical base and the shape and size of spores (6.2–9.2×2.7–3.8 μm, narrowly elliptical) without polar granules and the length of sorophores (0.5–9.7 mm in length).

This species is similar to *D. mucoroides* and *D. giganteum* in the size and shape of the sorocarps but the size and shape of the spores are clearly different, in *D. mucoroides* the spores are more oblong with dimension averages of 6×3 μm. The ranges of these dimensions for *D. giganteum* are 5–6.5×3–3.5 μm (more elliptical) compared to the narrowly elliptical spores of *D. firmibasis*, which have dimension ranges of 6.2–9.2×2.7–3.8 μm (Hagiwara 1989). We have found *D. firmibasis* in soils of Mediterranean vegetation with acid to slightly basic pH (5.6–7.8).

This is the first record for Europe with isolates from the south–west of Spain and Portugal, in areas of Mediterranean vegetation but with Atlantic influence. According to Hagiwara (1989), this species is rare in warm temperate forests of the world.

D. giganteum B. N. Singh

Portugal. Algarve: Santa Barbara de Nexe, Goldra de Baixo, 37°06′N 7°58′W, 280 m, under *C. siliqua*, 16-XI-2004, Romeralo 197. Loulé, Querença, road EN-396, 37°11′N 8° 10′W, 238 m, under *O. europaea* with *P. lentiscus* and *A. unedo*, 16-XI-04, Romeralo 207. Aljezur, Bordeira, Francelhe, 37°18′N 8°48′W, 30 m, under *J. phoenicea*, Romeralo 227.

Spain. Cádiz: La Espera, Lagunas Zorrilla, 36°52′N 5°52′ W, 100 m, under *P. lentiscus* with *C. humilis*, 26-VI-2003, Romeralo 24. Ibidem, under *P. lentiscus* and *Quercus coccifera*, 26-VI-2003, Romeralo 25. Ciudad Real: Retuerta del Bullaque, Embalse de Torre de Abraham, Km. 43 of the road C-403, 39°22′56″N 4°13′44″W, 900 m, under *Q. ilex*, 23-VI-2003, Romeralo 3. Pueblo Nuevo del Bullaque, road to Cabañeros National Park, near Bullaque river bridge, 39°18′45″N 4°16′28″W, 655 m, under *Q. faginea*, 23-VI-2003, Romeralo 6. Ibidem, under *Q. faginea* mixed with *P. lentiscus*, *C. monogyna*, *Cistus laurifolius*, *Ruscus aculeatus*, *O. europaea*, 23-VI-2003, Romeralo 7. Huesca: Santa Cruz de la Serós, Monasterio San Juan de la Peña, 42°31′08″N 0° 41′18″W, 986 m, under *Q. ilex*, 5-VII-2004, Romeralo 176. Madrid: Lozoya, road M-604, 40°55′53″N 3°49′55″W, 1140 m, under *Fraxinus angustifolia*, 1-VI-2004, Romeralo 117. Sevilla: Cazalla, 4 km from Cazalla, 37°54′44″N 5°48′ 09″W, 512 m, under *Q. ilex*, 24-VI-2003, Romeralo 11. Soria: Montenegro de Cameros, Km. 23–24 of the road SO-831, 42°04′05″N 2°46′44″W, 1500 m, under *I. aquifolium* and *Juniperus oxycedrus*, 1-VII-2004, Romeralo 137.

All the isolates that we have found follow the same pattern: very long sorophores, absence of polar granules and size of spores quite uniform (6×3 μm). The material studied matches the description made by Raper (1984) for this species. It is easily recognized by the fact that it has no polar granules in the spores and its large and wavy sorocarps, mostly solitary and strongly phototropic.

This is the first record for the Mediterranean region. We have found *D. giganteum* under many different Mediterranean trees such as *Q. coccifera*, *Q. ilex*, *Q. faginea*, *P. lentiscus*, *C. humilis*, and usually jointly with *Dictyostelium mucoroides*. This species is probably worldwide in distribution and very common (Raper 1984).

D. implicatum H. Hagiw

Portugal. Algarve: Loulé, Salir, EN-503, Brasieira de Baixo, 37°08′N 8°20′W, 254 m, under *Erica arborea*, 16-XI-2004, Romeralo 208. Aljezur, Bordeira, Francelhe, 37°18′ N 8°48′ W, 172 m, under *Pinus pinea*, 18-XI-2004, Romeralo 225.

Spain. Cádiz: Puerto de Galiz, 36°33′28″N 5°35′33″W, 450 m, under *Q. suber*, 26-VI-2003, Romeralo 27. Ciudad Real: Retuerta del Bullaque, Embalse de Torre de Abraham, Km. 43 of the road C-403, 39°22′56″N 4°13′44″W, 900 m, under *Q. ilex*, 23-VI-2003, Romeralo 3. Huelva: La Presa, Sendero la Urralera, 37°53′25″N 6°39′54″W, 825 m, under *A. unedo* mixed with *P. lentiscus, Q. ilex* and *S. aspera*, 4-XI-2003, Romeralo 90. Aracena, road from Aracena to Carboneras, 37°55′13″N 6°39′54″W, 825 m, under *A. unedo*, 6-XI-2003, Romeralo 96. Madrid: Miraflores de la Sierra, Km. 4 of the road M-611, 40°47′24″N 3°46′22″W, 1037 m, under *Quercus pyrenaica*, 1-VI-2004, Romeralo 102. Canencia, 40°52′32″N 3°46′35″W, 1390 m, under *B. pendula*, 1-VI-2004, Romeralo 110. Rascafría, road M-604, 40°55′53″ N 3°49′55″W, 1140 m, under *Q. faginea*, 1-VI-2004, Romeralo 115. Sevilla: Cazalla, 37°58′00″N 5°52′07″W, 430 m, under *Q. coccifera*, 24-VI-2003, Romeralo 16.

The main characteristic of *D. implicatum* in this part of the world, is that spores germinate as soon as the sori fall on the agar. Secondary sorocarps are often produced from the collapsed sori of primary sorocarps without food bacteria. Also if a sorus comes in contacts with another in the air, some secondary sorocarps are produced from their collapsed sori (Hagiwara 1989). This species produces large and radiate aggregations with long and thick pseudoplasmodiums (Fig. 1b).

Another feature of *D. implicatum* is the tangled growth of the tops of the sorocarps, which are solitary, unbranched and phototropic. Sorophores taper from their bases to their tips. Also the large size of the spores (6.6–9.5×4–4.8 μm) without polar granules in them, and the conical bases of the sorocarps with acuminate tips, are characteristic of this species. *D. mucoroides* var. *stoloniferum* Cavender and Raper has the same stoloniferous habit, but this is a tropical species (Swanson et al. 1999).

This is the first record for the south of Europe where it appears in conifers, deciduous trees and Mediterranean woodlands.

D. leptosomum Cavender, Stephenson, Landolt and Vadell

Portugal. Algarve: Santa Barbara de Nexe, Goldra de Baixo, 37°06′N 7°58′W, under *C. siliqua*, 280 m, 16-XI-2004, Romeralo 198. Loulé, Querença, road EN-396, 37°11′N 8° 10′W, under *Q. suber*, 160 m, 16-XI-2004, Romeralo 201. Loulé, road EN-503, Brasieira de Baixo, 37°08′N 8°20′W, under *Q. suber*, 254 m, 16-XI-2004, Romeralo 210. Aljezur, Bordeira, Francelhe, 37°12′N 8°53′W, 30 m, under *Salix salvifolia*, 18-XI-2004, Romeralo 230.

Spain. Huesca: Ansó, road Ansó-Zuriza, 42°50′36″N 0° 49′28″W, under *Buxus sempervirens*, 800 m, mixed with *F. sylvatica*, *Q. ilex* and *Tilia platyphyllos*, 5-VII-2004, Romeralo 169. Ansó, Old path Ansó-Zuriza, 42°49′04″N

0°49′40″W, under *Acer campestre*, 985 m, 5-VII-2004, Romeralo 175. Navarra: Urbasa, Sierra de Urbasa, road NA-718, 42°50′16″N 2°10′47″W, under *F. sylvatica*, 920 m, 2-VII-2004, Romeralo143. Yesa, Monasterio de Leyre, fountain path, 42°38′17″N 1°10′22″W, 824 m, under *A. unedo*, 4-VII-2004, Romeralo 166. Soria: Montenegro de Cameros, Km. 23–24 of the road SO-831, 42°04′05″N 2°46′44″W, 1500 m, under *I. aquifolium* mixed with *J. oxycedrus*, 1-VII-2004, Romeralo 137.

D. leptosomum has three main characteristics: the slender sorophores (from 0.8 to 3 mm long), sorocarps solitary to clustered and very phototropic. The absence of polar granules on the elliptical spores and the type of aggregation of the myxamebas (mucoroides type), small and weakly radiate.

We have found it in soils beneath different boreal and Mediterranean forests such as *A. alba*, *A. campestre*, *A. unedo*, *B. pendula*, *C. sativa*, *C. siliqua*, *F. sylvatica*, *Ilex aquifolia*, *J. oxycedrus*, *Populus nigra*, *Q. coccifera*, *Q. ilex*, *Q. pyrenaica*, *Q. suber*, *Salix eleagnus*. The soils had different pH values (5.6, 6.18, 6.47, 6.53, 6.74, 7.09, 7.3, 7.68, 7.92).

This is the first record for the south of Europe. It was found in the south and the north of Spain. This species appears to be widely distributed, although it was described in 2002. It was probably listed as *mucoroides* in previous studies (Cavender et al. 2002).

Dictyostelium minutum Raper

Spain. Sevilla: El Pedroso, 4 km from Cazalla, 37°54′44″N 5°48′09″W, 512 m, under *Q. ilex*, 24-VI-2003, Romeralo 11C.

This is one of the smallest species in the group. *D. minutum* has small sorocarps (0.10–1.67 mm long), spores (5–6×3–3.50 µm) with polar granules, which are sometimes not very prominent in this species, and its pseudoplasmodium is mound-like. Its aggregation is also peculiar, small, rounded mounds without streams. Sometimes aggregations are radiate but in an uncommon way (Fig. 1c).

This isolate produced macrocysts in great number. It was found living together with members of the genus *Polysphondylium*, such as *P. pallidum*, from soil with pH of about 7 in an evergreen forest.

This is a species with worldwide distribution (Raper 1984). In Spain it was previously reported by Cavender (1969) from the boreal forests of the north, and now it appeared in the Mediterranean woodlands of the south.

D. mucoroides Bref

Portugal. Algarve: Santa Barbara de Nexe, Goldra de Baixo, 37°06′N 7°58′W, 280 m., 16-XI-2004, under *Q. ilex*, Romeralo 200B. Loulé, Querença, road EN-396, under *O. europaea*, 37°11′N 8°10′W 238 m., 16-XI-2004, Romeralo 206B.

Spain. Ciudad Real: Las Ventas con Peña Aguilera, Molinillo, road C-403, 39°33′13″N 4°14′27″W, 900 m, under *Q. ilex*, 23-VI-2003, Romeralo 1. Retuerta del Bullaque, Embalse Torre Abraham, road C-403, Km. 43, 39°33′13″N 4°14′27″W, 900 m, under *Q. ilex*, 23-VI-2003, Romeralo 3. Pueblo Nuevo del Bullaque, road to Cabañeros National Park, near Bullaque river bridge, 39°18′45″N 4°16′28″W, 655 m, under *Q. faginea*, 23-VI-2003, Romeralo 6. Ibidem, under *Q. faginea*, 23-VI-2003, Romeralo 7. Huelva: Jabugo, Km. 132, 37°54′39″N 6°43′39″W, 690 m, under *C. sativa*, 25-VI-2003, Romeralo 20. La Presa, Sendero la Urralera, 37°53′25″N 6°39′54″W, 825 m, under *A. unedo* in a mixed forest with *P. lentiscus, Q. ilex* and *S. aspera*, 4-XI-2003, Romeralo, 89. Huesca: El Collado, Puerto de Alajar, 37°53′10″N 06°39′42″W, 845 m, under *O. europaea*, 4-XI-2003, Romeralo 88. Ibidem, Km. 497 of the road N-260, near San Bartolomé ravine, forest track between Gavín and Yesero, 42°37′22″N 0°16′22″W, 924 m, under *B. sempervirens* with *C. avellana*, *C. monogyna* and *Pinus sylvestris*, 5-VII-2004, Romeralo 178. Madrid: Rascafría, 40°55′53″N 3°49′55″W, 1140 m, under *Q. faginea*, 1-VI-2004, Romeralo 115. Navarra: Urbasa, Sierra de Urbasa, road NA-718, 42°50′16″N 2°10′47″W, 920 m, under *F. sylvatica*, 2-VII-2004, Romeralo 143.

D. mucoroides is the most common species in this study. It has appeared in almost all the substrates under *Acer pseudoplatanus*, *C. sativa*, *F. sylvatica*, *F. excelsior*, *O. europaea*, *P. sylvestris*, *P. nigra*, *Nardus stricta*, *Q. coccifera*, *Q. ilex*, *Q. faginea*, *Q. pyrenaica*, *S. eleagnus*, and living together with many others species of cellular slime moulds and is recorded from Portugal for the first time.

In all the isolates we have found great variability in size and shape of sorocarps. According to Raper (1984), it is a highly variable species and unfortunately this type no longer exists.

The main characteristics of *D. mucoroides* are its solitary and erect sorocarps without branching, white sori, spores (4.5–6×2.5–3.5 µm) without polar granules but with vesicles and small, radiate aggregations (mucoroides type) (Fig. 1d). In this species, each aggregation forms one sorocarp. Hagiwara (1984, 1989), in his taxonomic study of Japanese dictyostelids, established another concept of *D. mucoroides*. But as mentioned before, the taxonomic criteria used herein follow that of Raper (1984).

D. sphaerocephalum (Oudem.) Sacc. and Marchal

Portugal. Algarve: Monchique, Ginjeira, 37°20′N 8°28′W, 580 m, under *C. sativa*, 17-XI-2004, Romeralo 215. Aljezur, Bordeira, Francelhe, 37°12′N 8°53′W, 30 m, under *S. salvifolia, 18-XI-2004, Romeralo 230 B.*

Spain. Ciudad Real: Las Ventas con Peña Aguilera, Molinillo, road C-403, 39°33′13″N 04°14′27″W, 900 m, under *Q. ilex*, 23-VI-2003, Romeralo 1. Pueblo Nuevo del Bullaque, road to Cabañeros National Park, near Bullaque river bridge, 39°18′45″N 4°16′28″W, 655 m, under *Q. faginea*, 23-VI-2003, Romeralo 6. Huelva: Jabugo, Km. 132, 37°54′39″N 6°43′39″W, 690 m, under *C. sativa*, 25-VI-2003, Romeralo 20. La Nava, Murtiga river, 37°58′20″ N 6°45′04″W, 370 m, under *Alnus glutinosa*, 6-XI-2003, Romeralo 97. La Presa, sendero la Urralera, 37°53′25″N 6° 39′54″W, 825 m, under *A. unedo* in a mixed forest with *P. lentiscus*, *Q. ilex* and *S. aspera*, 4-XI-2003, Romeralo 89.

D. sphaerocephalum is easily recognized because of the size of the sorocarps (2–4 mm long), commonly branched with solitary growth, the absence of polar granules at the spores (5.5–7×3–3.5 μm), the migration of the slugs, the presence of the collar near the apex of the sorophore, the radiate and large aggregation pattern (Fig. 1e) and the characteristic L-shape of the sorocarp (Fig. 2b). Another characteristic of the species is that sori are not completely removed by needle contact.

This is the first record for the south of Europe. This species was very common in soils of Mediterranean forests, especially from the south–west of the Iberian Peninsula, in areas with Atlantic influence. It also appeared in the centre and north of Spain where cold and boreal climatic conditions are present. *D. sphaerocephalum* was found in different substrates like *A. glutinosa*, *C. sativa*, *I. aquifolium*, *J. oxycedrus*, *O. europaea*, *P. lentiscus*, *Q. faginea* and *Salix* sp. The range of pH is quite stable around the neutral values (6.45–7.68). According to Raper (1984), *D. sphaerocephalum* is another very common species.

D. sphaerocephalum and *D. mucoroides* were found growing together many times in this study.

P. candidum H. Hagiw

Portugal. Algarve: Loulé, Querença, road EN-396, 37°11′N 8°10′W, 160 m, under *Q. suber*, 16-XI-2004, Romeralo 203.

Spain. Ciudad Real: Cabañeros, Embalse Torre Abraham, Km. 43 of the road C-403, 39°33′13″N 4°14′27″W, 900 m, under *Q. ilex*, 23-VI-2003, Romeralo 3. Pueblo Nuevo del Bullaque, road to Cabañeros National Park, near Bullaque river bridge, 39°18′45″N 4°16′28″W, 655 m, under *Q. faginea*, 23-VI-2003, Romeralo 7. Riofrío, 39°05′ 02″N 04°30′16″W, 640 m, under *B. pendula*, 23-VI-2003, Romeralo 9. Huelva: Jabugo, Km. 132, 37°54′39″N 6°43′ 39″W, 690 m, under *C. sativa*, 25-VI-2003, Romeralo 20. Doñana beach, 36°59′25″N 6°31′23″W, 20 m, in dunes, under *Juniperus thurifera* and mosses, 26-VI-2003, Romeralo 23. La Presa, sendero la Urralera, 37°53′25″N 6°39′54″W, 825 m, under *A. unedo* in a mixed forest with *P. lentiscus*, *Q. ilex* and *S. aspera*, 4-XI-2003, Romeralo 89. Sevilla: El Pedroso, a 4 km de Cazalla, 37°54′44″N 5°48′ 09″W, 512 m, under *Q. ilex*, 24-VI-2003, Romeralo 11. Cazalla, 37°58′00″N 05°52′07″W, 430 m, under *Q. coccifera*, 24-VI-2003, Romeralo 15.

This is the first record for the Mediterranean region. The species was found in soils under conifers, deciduous trees and was especially frequent under Mediterranean forests.

Features of the species are the solitary growth, the number of whorls (1–6) with branches (2–10), the sorocarps are not phototropic, colourless and the aggregations are large, radial with thick streams (Fig. 1f).

We have found this species in many different places and the morphological characters seem to be quite variable in all of them. One of the main characters, the presence or absence of sori or the elongation of the terminal segment varies with the isolates. We have found differences between isolates and also between sorocarps in the same petri dish. The polar granules are another variable feature between isolates. In some cases the granules are consolidated, in others unconsolidated and irregular in others. Most of the sorocarps are prostrate and not erect. The aggregations of the myxamoebas are relatively large and frequently they sub-divide to generate several sorogens. The spore dimensions vary between 8–10×3.5–5 μm. It is the most variable species found in this study.

P. pallidum Olive

Spain. Huelva: Doñana beach, 36°59′25″N 6°31′23″W, 20 m, in dunes under *J. thurifera* and mosses, 26-VI-2003, Romeralo 23.

The most relevant features of this species are the solitary sorocarps, the number of whorls (1–11) with branches between two and six (Fig. 2c), sorocarps without lengthened terminal segments, not phototropic, colorless, the spores with unconsolidated polar granules and the radial aggregation (Fig. 1g). Aggregation in this species are smaller and streams thinner than those of the species above.

Although Raper (1984) considers this species as having a worldwide distribution, this is the first record for the south of Europe.

P. pallidum appears to be a highly variable species. It shows variety not only in morphology but also in genetics (Eisenberg and Francis 1977). Hagiwara (1982) introduced the term “*P. pallidum* complex” for species similar to *P. pallidum*, i.e. Polysphondylia with white sori and elliptical spores without lengthened terminal segments.

Swanson et al. (1999) suggested that this species is more common than *P. candidum* but this is not the case in the south of Europe. We have found only one isolate of *P. pallidum* and that in the south of Spain from the dunes of Doñana National Park. This species is also uncommon in Germany (Cavender et al. 1995).

Polysphondylium violaceum Bref

Spain. Asturias: Vigidel, 43°08′49″N 6°08′28″W, 630 m, under *I. aquifolium*, 4-X-2005, Romeralo 260. Ibidem, Romeralo 261A. Pineda, 43°08′15″N 6°15′43″W, 600 m, under *C. sativa*, 6-X-2005, Romeralo 289. La Rebolleda, 43°08′49″N 6°19′56″W, 547 m, under *C. sativa*, 6-X-2005, Romeralo 299.

Our collections show sorocarps solitary, 3–8 whorls per sorocarp, sometimes ill-formed, 2–5 branches per whorl (Fig. 2d). Sori are of a very intensive colour, the spores are narrow and with prominent polar granules. Their typical aggregations are radiate and very big and sub-divide into several mounds. It is a very phototrophic species. Following Raper (1984), this is another very common species. Raper (1984) established the pH values of about 6–6.5 as the optimum. We have found *P. violaceum* in acid to almost neutral pH [4.85, 5.13, 5.22, 5.75, 6, 6.8, 6.9 (average: 5.8)].

This is one of the more variable species in the group. The best proportioned sorocarps are often seen in primary isolation plates. Some authors (Hagiwara 1989) think this could be another species complex.

This species was previously reported from soils of the north of Spain, under deciduous trees (Cavender 1969).

Discussion

A total of 14 species from three genera were isolated during this study, bringing the number of dictyostelids known from the south of Europe to 18. As these first and preliminary results show, the Mediterranean region seems to be a good place for dictyostelids to live. We have found specimens of the three genera, *Acytostelium* (one species), *Dictyostelium* (ten species) and *Polysphondylium* (three species). The combination of factors seem to result in a particular distribution of species around the Iberian Peninsula (a geographical region that comprise Spain and Portugal). For example, *D. mucoroides* as a cosmopolitan species appears in the north and in the south. On the other hand *D. minutum* and *P. pallidum* were found only in the south of Spain.

Of the 14 species, 10 are reported for the first time in Spain, and for the 8 species reported for Portugal, all are new records except *D. mucoroides*.

Both *D. giganteum* and *D. mucoroides* can withstand a wide range of differences in humidity and temperature (Cavender 1973). These differences are very common in the Mediterranean region where cold dry climatic conditions are mixed with warm humid ones. Our results support this because we have found these two species very frequently, both of them in the north and south of Spain, in soils of Mediterranean and boreal vegetation (see Table 2).

Traub et al. (1981b) established that *D. mucoroides* is the dominant species in all kinds of forests in Switzerland. Furthermore, Cavender (1969) established that this species is overwhelmingly abundant in Europe. We can confirm this for Spain as well. We have found this species in a number of different places, in soils under a variety of substrates (see Table 2) and in a wide range of pH values (4.62–8.1). It is one of the most abundant species together with *D. leptosomum*. *Dictyoselium leptosomum* was described from New Zealand (Cavender et al. 2002), but before this, it was known as *D. mucoroides* L-type (Traub 1972). Many records of *D. mucoroides* actually may be *D. leptosomum*.

Some species such as *D. sphaerocephalum* and *D. giganteum* have a wide distribution and others a more restricted one. *D. fasciculatum* was originally found only in Europe (Cavender et al. 1995) and has since been isolated in New Zealand in 2002 (Cavender et al. 2002). In this study, *P. candidum* was found more frequently than *P. pallidum*, which was only found by us in one place, the same happened in Germany (Cavender et al. 1995). Outside of Europe, the latter is more common in other parts of the world (Swanson et al. 1999). It is interesting to note that in the dunes of Doñana beach, in the south of Spain, we found both *P. candidum* and *P. pallidum* living together under the same substrate.

Some species of cellular slime moulds, are associated with different kinds of forests. For example, *D. minutum* with *Q. ilex* and *P. pallidum* with *J. phoenicea*, while others are less specific such as *D. leptosomum* and *D. mucoroides* (see Table 2). Almost all species were found in soils with different species of *Quercus* (*Q. coccifera*, *Q. faginea*, *Q. ilex*, *Q. pyrenaica*, *Q. suber*).

Pigmented species such as *D. aureo-stipes* (yellow sorophores) and *P. violaceum* (vinaceous sori) seem to appear in the clearings of the forest. This could be related to the greater amount of light in these areas, since they would have more protection against UV radiation. Substrate pH, as Leitner saw in 1987 (Cavender et al. 1995), is an important factor for the development of cellular slime moulds. Some species can live in a very wide pH range, e.g. *D. mucoroides* and *D. implicatum* (pH 3.86–7.81) and others like *D. fasciculatum* (pH 6.49–7.81) and *D. sphaerocephalum* (pH 6.45–7.68) are more restricted.

When the study of the order Dictyosteliales in the south of Europe was undertaken, it was presumed that species similar to those isolated from other areas of Europe would be found along with perhaps some species more typical of Mediterranean vegetation. The most recent study of dictyostelids in Europe was done in Germany by Cavender et al. (1995) who found 18 species. In our study, a total of 18 species were found in the Iberian Peninsula. Twelve of these species are common to the two countries, showing

Table 2 Dictyostelids found in the south of Europe (Spain and/or Portugal) compared with species from Germany and Switzerland (in bold, species in common)

Species	Soil under (Spain, Portugal)	Soil under (Germany)	Soil under (Switzerland)
Acytostelium leptosomum	***Pinus** pinaster*	*Fagus*	*Fagus sylvatica* *Picea abies* ***Pinus***
Dictyostelium aureo-stipes	*Prunus laurocerasus*	*Acer* *Betula*	
Dictyostelium sp. 1	*Quercus ilex*		
Dictyostelium fasciculatu	*Olea europaea* ***Quercus** faginea* ***Quercus** pyrenaica*	***Acer*** ***Betula***	***Acer*** ***Betula*** *Carpinus betulus* *Castanea sativa* *Fagus sylvatica* *Fraxinus excelsior* *Pinus* ***Quercus*** spp.
Dictyostelium firmibasis	*Arbutus unedo* *Olea europaea* *Quercus* sp.		
Dictyostelium giganteum	*Arbutus unedo* *Ceratonia siliqua* *Juniperus phoenicea* *Olea europaea* ***Quercus** coccifera* *Q. faginea* *Q. ilex*	*Betula* *Corylus* ***Fagus***	*Acer* *Carpinus betulus* *Castanea sativa* *Corylus avellana* ***Fagus** sylvatica* *Fraxinus excelsior* ***Quercus*** spp.
Dictyostelium implicatum	*Abies alba* *Arbutus unedo* *Betula pendula* ***Quercus** coccifera* *Q. faginea* *Q. ilex* *Q. pyrenaica* *Q. suber* ***Pinus** pinea* *P. sylvestris*	***Betula*** *Picea* ***Pinus*** ***Quercus***	
Dictyostelium leptosomum	*Abies alba* ***Acer** campestre* *A. pseudoplatanus* *Arbutus unedo* ***Betula** pendula* *Castanea sativa* *Ceratonia siliqua* *Fagus sylvatica* *Ilex aquifolium* *Juniperus oxycedrus* *Populus nigra* *Quercus coccifera* *Q. ilex* *Q. pyrenaica* *Q. suber* *Salix eleagnus*	***Acer*** ***Betula***	
Dictyostelium minutum	***Quercus** ilex*	***Quercus*** ***Betula*** ***Fagus*** ***Pinus***	*Acer* ***Betula*** *Carpinus betulus* *Castanea sativa* ***Fagus** sylvatica*

Table 2 (continued)

Species	Soil under (Spain, Portugal)	Soil under (Germany)	Soil under (Switzerland)
			Fraxinus excelsior ***Pinus*** ***Quercus*** spp. *Tilia* spp. *Ulmus glabra*
Dictyostelium mucoroides	*Acer pseudoplatanus* ***Castanea sativa*** ***Fagus*** *sylvatica* ***Fraxinus*** *excelsior* *Olea europaea* *Pinus sylvestris* ***Populus*** *nigra* Alpine grassland (*Nardus stricta*) ***Quercus*** *coccifera* *Q. Ilex* *Q. faginea* *Q. pyrenaica* *Salix eleagnus*	***Fagus*** ***Fraxinus*** ***Populus***	*Betula* ***Castanea sativa*** *Corylus avellana* ***Fagus sylvatica*** *Fraxinus excelsior* ***Pinus*** *Picea abies* ***Quercus*** spp.
Dictyostelium sphaerocephalum	*Alnus glutinosa* *Castanea sativa* *Ilex aquifolium* *Juniperus oxycedrus* *J. communis* *Olea europaea* *Quercus faginea* *Q. ilex* *Salix atrocinerea* *S. salvifolia*	***Quercus*** *Betula* ***Fagus*** ***Pinus***	*Abies alba* ***Fagus*** *sylvatica* ***Pinus***
Polysphondylium candidum	*Arbutus unedo* *Betula pendula* *Castanea sativa* *Quercus coccifera* *Q. ilex*	*Crataegus* *Fagus*	
Polysphondylium pallidum	*Juniperus phoenicea*	***Betula*** ***Fagus*** *Picea* ***Pinus*** ***Quercus***	*Acer* spp. ***Betula*** spp. *Carpinus betulus* ***Fagus*** *sylvatica* *Fraxinus excelsior* *Larix decidua* ***Pinus*** ***Quercus*** spp.
Polysphondylium violaceum	*Castanea sativa* *Ilex aquifolium* *Sorbus aria*	***Acer*** ***Betula*** ***Corylus avellana*** ***Fagus sylvatica*** ***Picea***	*Abies alba* ***Acer*** spp. ***Betula*** spp. *Carpinus betulus* ***Castanea sativa*** ***Corylus avellana*** ***Fagus sylvatica*** *Fraxinus excelsior* ***Picea*** *Pinus* *Quercus* spp. *Tilia* spp. *Ulmus glabra*

approximately a 67% similarity. Another country studied in Europe is Switzerland (Cavender 1969) from which 13 species were isolated, 8 in common with Spain and Portugal. This shows a 62% similarity. We have compared, in these countries, the substrates in which species of cellular slime moulds were found (see Table 2). We cannot find major trends in the substrates, but some things to note are that *D. firmibasis* was only found in Spain and under Mediterranean plant species such as *Arbutus*, *Olea* and *Quercus*. *D. minutum* was found in the three countries and in all of them under *Quercus* spp., although not exclusively. The increase of species diversity with a decrease in latitude (Cavender 1973; Swanson et al. 1999) seems confirmed, since we found 11 species in the south of the Iberian Peninsula (between 36° and 39°N) compared to 5 species in the north (between 40° and 43°N).

Sampling all over the region will be continued to test these working hypotheses with more data. Dictyostelids in Europe are still little known and information only exists about Austria, Germany, Sweden, Switzerland, Norway, Great Britain (Kawabe 1995), Spain and Portugal. It is premature to suggest patterns of European dictyostelid distribution. Much work on this group is still necessary in this part of the world, but some trends can be seen from this first approach, which also suggests at least a modestly rich diversity of dictyostelids.

Acknowledgements We would like to thank to Dr. J. C. Cavender (Ohio University, USA), Dr. J. Landolt (Shepherd University, USA), Dr. H. Hagiwara (National Science Museum, Tokyo, Japan) and Dr. E. Vadell (Museo de Ciencias Naturales, Buenos Aires, Argentina) for the examination of cultures, and Dr. J. Landolt for the helpful suggestions and critical reading of the manuscript. We also would like to thank Diana Wrigley de Basanta for the linguistic assistance. This research was supported by the Ministry of Education and Science of Spain (REN2002-00445/GLO and CGL2005-00320/BOS) and an I3P grant to M. Romeralo. The stay of the first author at the Universities of Ohio and Shepherdstown, USA, was supported by a grant from the National Science Foundation (GBE-0316284).

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