Microb Ecol
DOI 10.1007/s00248-010-9715-5

# Social Amoebae: Environmental Factors Influencing Their Distribution and Diversity Across South-Western Europe

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Received: 3 May 2010 / Accepted: 18 June 2010

**Abstract** The social amoebae (dictyostelids) are the only truly multicellular lineage within the superkingdom Amoebozoa, the sister group to Ophistokonts (Metazoa+ Fungi). Despite the exceptional phylogenetic and evolutionary value of this taxon, the environmental factors that determine their distribution and diversity are largely unknown. We have applied statistical modeling to a set of data obtained from an extensive and detailed survey in the south-western of Europe (The Iberian Peninsula including Spain and Portugal) in order to estimate some of the main environmental factors influencing the distribution and diversity of dictyostelid in temperate climates. It is the first time that this methodology is applied to the study of this unique group of soil microorganisms. Our results show that a combination of climatic (temperature, water availability), physical (pH) and vegetation (species richness) factors favor dictyostelid species richness. In the Iberian Peninsula, dictyostelid diversity is highest in colder and wet environments, indicating that this group has likely diversified in relatively cold places with high levels of water availability.

## Introduction

Dictyostelids, also called cellular slime molds or social amoebae, are a major group of soil microorganisms that hover at the borderline of true multicellularity. While they spend most of their life cycle as solitary amoebae, upon starvation they can aggregate in hundreds of thousands to form differentiated sporophores. Molecular studies in recent years have now firmly established Dictyostelia as a member of the eukaryotic superkingdom Amoebozoa, which is mainly comprised of solitary naked amoebae [1, 2, 15, 20]. Amoebozoa is the sister group to the Opisthokonta, the eukaryotic supergroup including Metazoa and Fungi [2, 3, 46]. Therefore, understanding the ecological factors that affect the distribution and diversity of this group may throw additional and independent light onto the understanding of distribution patterns of biodiversity on Earth. Understanding the factors that explain the diversity and distribution of species is one of the fundamental goals in ecological studies [17, 29, 37, 42, 50]. Microorganisms are influenced by a variety of environmental factors such as the habitat and its resources. However, the relative contribution of these factors is still poorly understood [52].

The dictyostelids are a common component of the soil microflora [9, 23], their fruiting bodies are microscopic (in length), so rarely seen except in laboratory cultures. Dictyostelid amoebas, as bacterial predators, could poten-

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tially play important roles in the ecology and health of soils by performing top-down control on the ecosystem processes in which bacterial populations are involved (e.g., decomposition). Their primary habitat is forest soils [9, 16, 35], as they seem to need wet places with organic material. More than 100 species of dictyostelids have been described so far [4, 22]. Some species are known to have a global distribution while others are much more restricted in their range [47]. In the Iberian Peninsula (Spain and Portugal), 19 species have been recorded so far (5, 38, 39, 41, 47, Hagiwara pers. comm.), comprising about 20% of the global dictyostelid diversity.

There are some accounts of dictyostelid ecology from the 70s and 80s. However, these papers were purely descriptive, and our current knowledge of the ecology of this extremely unique group is still very poor. Furthermore, almost no formal statistical analysis has been applied to relate ecological factors to the distribution of the diversity of this group. Some descriptive ecological information is available from North and Central America and East Africa [6, 7, 23]. However, very little is known about the ecology of dictyostelids from the European continent [5], with only some information available from Switzerland [48, 49] and Germany [12].

In this study, we apply statistical modeling to reveal the ecological factors (both biotic and abiotic) that may be responsible for the distribution and diversity of dictyostelid in temperate climates. We collected 300 samples from 90 localities across the Iberian Peninsula and compiled them along with a set of environmental data. Species descriptions from this dataset were previously published in both morphological and molecular terms [38, 40, 41], but the ecological factors affecting their distribution are analyzed for the first time in this study.

To date, this may be the most comprehensive study on the ecological factors that affect the presence of dictyostelids and their distribution, and as such it provides useful information (a) for where and when to find dictyostelids and the highest diversity of them, and (b) to contribute to revealing the ecological role that this group plays in natural ecosystems.

## Methods

### Study Area

The study area extends across two countries (Spain and Portugal) in the Iberian Peninsula (south of Europe), located between 36°–44°N latitude and 4°E and 10°W longitude. The Iberian Peninsula has two main vegetation regions, the Mediterranean region in the central and south, and the boreal or Eurosiberian region in the north. The Mediterranean region, in which the majority of representative tree and shrub species belong to the genera *Arbutus*, *Ceratonia*, *Cistus*, *Olea*, *Prunus*, *Pistacea*, or *Quercus*; and the Boreal region, where species within the genera *Abies*, *Acer*, *Alnus*, *Betula*, *Castanea*, *Fagus*, *Fraxinus*, or *Tilia* are well represented. Both regions were sampled. The samples were taken in a 0–2,000-m elevation range; i.e., from sea level to the timberline. A total of 90 localities (Appendix 1. Table 1.) were sampled, 300 soil samples were collected and 937 clones of dictyostelids were recovered.

### Sampling and Species Identifications

For this study we used material collected in several seasons during 2003–2005.

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 [9] 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. Cellular slime mold 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 nonnutrient agar (2%) and preserved by freezing with glycerol (20%). Taxonomic treatment used herein follows that of Raper [35].

For DNA extraction, colonies were grown on SM plates (standard medium: 20-g/L peptone, 2-g/L yeast extract, 20-g/L glucose, 2-g/L MgSO4, 3.8-g/L KH2PO4, 1.2-g/L K2HPO4, and 2% agar). Cells from the edge of plaques growing on these plates were collected with a sterile tip, mixed with DNA extraction solution from Epicentre and heated 30 min at 60°C followed by 8 min at 98°C. Cell lysates were used directly for PCR amplification.

For more detail about sampling and species identification, see Romeralo and Lado [38, 39] as well as Romeralo et al. [40].

### Species Accumulation Curve

To estimate the actual number of species and the extent to which the survey was exhaustive, we built a species accumulation curve [44, 45]. The sequence of samples was randomly permutated 100 times, and the means of the accumulated number of species were calculated using the program EstimateS. The plot of the mean accumulated number of species was regressed against the number of samples using the package Curve Expert 1.3, and taking as the formula for saturation the Michaelis–Menten equation:

**Table 1** List of localities sampled

| Locality | Coordinates | Elevation (m) | Substrate (under) |
|---|---|---|---|
| Spain | | | |
| Almería: Benizalón, road to Benizalón from Benitagla | 37°14′12″N 2°15′05″W | 1,032 | *Quercus ilex* |
| Almería: Mojácar, road to Turre | 37°08′34″N 1°55′59″W | 90 | *Chamaerops humilis*<br>*Stipa tenacissima* |
| Almería: Níjar, Rodalquilar, Cala del Toro, road to Rodalquilar | 36°49′20″N 2°02′36″W | 9 | *Chamaerops humilis*<br>*Cistus salviifolius*<br>*Pinus halepensis*<br>*Stipa tenacissima* |
| Almería: Níjar, San Jose, Monsul beach | 36°43′57″N 2°08′37″W | 12 | *Agave americana*<br>*Chamaerops humilis* |
| Almería: Tabernas, road N-340, Sierra Alhamilla | 37°02′35″N 2°25′03″W | 347 | *Tamarix africana* |
| Almería: Tahal, road A-349 to Tahal, Sierra de los Filabres | 37°12′58″N 2°17′51″W | 1,151 | *Pinus halepensis* |
| Asturias: Llanés, Purón river | 43°24′13″N 4°42′07″W | 10 | *Alnus glutinosa*<br>*Corylus avellana* |
| Asturias: Pola de Somiedo, road to Pineda | 43°08′15″N 6°15′43″W | 600 | *Acer pseudoplatanus*<br>*Castanea sativa*<br>*Tilia platyphyllos* |
| Asturias: Saliencia, Alto de la Farrapona | 43°03′38″N 6°06′00″W | 1,523 | *Sorbus aucuparia* |
| Asturias: Pola de Somiedo, Endriga | 43°05′26″N 6°09′17″W | 1,148 | *Corylus avellana*<br>*Fagus sylvatica* |
| Asturias: Pola de Somiedo, central eléctrica de La Malva | 43°07′06″N 6°15′42″W | 737 | *Prunus laurocerasus*<br>*Quercus ilex*<br>*Sorbus aria* |
| Asturias: Somiedo, La Rebolleda | 43°08′49″N 6°19′56″W | 547 | *Acer pseudoplatanus*<br>*Alnus glutinosa*<br>*Castanea sativa*<br>*Corylus avellana*<br>*Fraxinus angustifolia*<br>*Fraxinus excelsior*<br>*Quercus petraea* |
| Asturias: Teverga, San Lorenzo pass | 43°08′26″N 6°11′36″W | 1,310 | *Crataegus monogyna*<br>*Ilex aquifolium* |
| Asturias: Pola de Somiedo, Somiedo pass | 43°00′59″N 6°13′39″W | 1,437 | *Salix* sp. |
| Asturias: Pola de Somiedo, Saliencia | 43°04′43″N 6°07′50″W | 1,205 | *Sorbus aria*<br>*Sorbus aucuparia* |
| Asturias: Teverga, Vigidel | 43°08′49″N 6°08′28″W | 630 | *Castanea sativa*<br>*Ilex aquifolium* |
| Badajoz: Arroyo del Campo | 38°50′55″N 5°28′47″W | 392 | grasses |
| Badajoz: Calera de León, road N-630, km 716 | 38°07′49″N 6°17′18″W | 670 | *Quercus ilex* |
| Burgos: Cilleruelo de Bezana, road N-623, km 80–85 | 42°59′08″N 3°51′17″W | 865 | *Quercus petraea*<br>*Fagus silvatica* |
| Burgos: Riocavado de la Sierra, road BU 820, km 13, Sierra de la Demanda | 42°09′37″N 3°12′35″W | 1,220 | *Quercus pyrenaica*<br>*Fagus sylvatica* |
| Burgos: Santo Domingo de Silos | 41°54′31″N 3°28′00″W | 1,120 | *Juniperus communis*<br>*Juniperus oxycedrus*<br>*Juniperus sabina*<br>*Salvia officinalis* |

**Table 1** (continued)

| Locality | Coordinates | Elevation (m) | Substrate (under) |
|---|---|---|---|
| Cádiz: Grazalema, Llano del Laurel, road A-372, km 38, from Grazalema to El Bosque | 36°44′56″N 5°27′01″W | 705 | *Ceratonia siliqua* |
| Cádiz: La Espera, lagunas Zorrilla | 36°52′N 5°52′W | 100 | *Chamaerops humilis* |
| | | | *Pistacia lentiscus* |
| | | | *Quercus coccifera* |
| Cádiz: La Sauceda, Puerto de Galiz | 36°33′28″N 5°35′32″W | 450 | *Quercus faginea* |
| | | | *Quercus suber* |
| Cádiz: Ubrique, road to Ubrique | 36°34′06″N 5°32′33″W | 585 | *Arbutus unedo* |
| | | | *Cistus* sp. |
| | | | *Olea europaea* |
| Cantabria: Fontibre | 43°00′56″N 4°11′29″W | 915 | *Acer* sp. |
| | | | *Fraxinus* sp. |
| | | | *Ilex aquifolium* |
| Cantabria: Reinosa, road to Alto Campoó | 43°00′17″N 4°17′27″W | 1,150 | *Quercus pyrenaica* |
| Cantabria: Pechón, Tina Menor | 43°24′31″N 4°28′27″W | 50 | *Arbutus unedo* |
| | | | *Quercus ilex* |
| Ciudad Real: Las Ventas con Peña Aguilera, road C-403 to El Molinillo | 39°33′13″N 4°14′27″W | 900 | *Cistus ladanifer* |
| | | | *Quercus ilex* |
| Ciudad Real: Pueblo Nuevo del Bullaque, road to Cabañeros | 39°18′45″N 4°16′28″W | 655 | *Cistus* sp. |
| | | | *Crataegus* sp. |
| | | | *Olea europaea* |
| | | | *Pistacia lentiscus* |
| | | | *Quercus faginea* |
| | | | *Quercus ilex* |
| | | | *Ruscus aculeatus* |
| Ciudad Real: Retuerta del Bullaque, embalse Torre Abraham, road C-403, km 43 | 39°22′56″N 4°13′44″W | 715 | *Cistus* sp. |
| | | | *Crataegus* sp. |
| | | | *Olea europaea* |
| | | | *Pistacia lentiscus* |
| | | | *Quercus faginea* |
| | | | *Quercus pyrenaica* |
| | | | *Ruscus aculeatus* |
| Ciudad Real: Riofrío del Llano | 39°05′02″N 4°30′16″W | 640 | *Betula pendula* |
| Huelva: Alajar, Alajar pass | 37°53′10″N 6°39′42″W | 845 | *Olea europaea* |
| Huelva: Almonte, Matalascañas, Doñana,, | 36°59′25″N 6°31′23″W | 20 | *Juniperus* sp. |
| | | | *Pinus pinea* |
| | | | *Rosmarinus officinalis* |
| | | | *Cistus* sp. |
| Huelva: Aracena, road from Aracena to Carboneras | 37°55′13″N 6°33′37″W | 638 | *Arbutus unedo* |
| Huelva: Castaño del Robledo, sendero la Urralera | 37°53′32″N 6°42′59″W | 710 | *Quercus suber* |
| Huelva: El Talenque | 37°55′47″N 6°40′39″W | 663 | *Quercus pyrenaica* |
| Huelva: Jabugo, road N-35 to Huelva | 37°54′39″N 6°43′39″W | 690 | *Castanea sativa* |
| Huelva: La Nava, Múrtiga river | 37°58′20″N 6°45′04″W | 370 | *Alnus glutinosa* |
| | | | *Cistus ladanifer* |
| | | | *Quercus ilex* |
| Huelva: Río Tinto, Minas de Río Tinto | 37°42′N 6°36′ W | 400 | *Pinus pinea* |
| Huelva: Santa Ana la Real, Parque Natural Sierra de Aracena | 37°52′02″N 6°42′40″W | 620 | *Quercus suber* |
| | | | *Quercus* sp. |
| Huesca: Ansó, camino viejo Ansó-Zuriza | 42°49′04″N 0°49′40″W | 985 | *Abies alba* |

**Table 1** (continued)

| Locality | Coordinates | Elevation (m) | Substrate (under) |
|---|---|---|---|
| | | | *Acer campestre*<br>*Castanea sativa*<br>*Fagus sylvatica*<br>*Salix eleagnus* |
| Huesca: Ansó, road Ansó-Zuriza | 42°50′36″N 0°49′28″W | 800 | *Abies alba*<br>*Tilia platyphyllos* |
| Huesca: Biescas, road N-260 from Escuer to Arguisal | 42°35′14″N 0°19′31″W | 820 | *Fraxinus sp.*<br>*Populus nigra*<br>*Salix eleagnus* |
| Huesca: Biescas, road N-260, km 497, from Gavín to Yesero | 42°37′22″N 0°16′22″W | 924 | *Corylus avellana*<br>*Pinus sylvestris* |
| Huesca: Panticosa, Balneario de Panticosa | 42°45′27″N 0°14′51″W | 1,658 | *Acer pseudoplatanus*<br>*Fraxinus sp.*<br>*Pinus nigra*<br>*Populus tremula* |
| Huesca: Panticosa, Balneario de Panticosa | 42°45′39″N 0°14′17″W | 1,658 | *Fraxinus excelsior*<br>*Populus tremula* |
| Huesca: Panticosa, road A-2606, km 9–10 to Baños de Panticosa | 42°45′09″N 0°14′33″W | 1,200 | *Acer peudoplatanus*<br>*Salix atrocinerea* |
| Huesca: Santa Cruz de la Serós, road to monasterio de San Juan de la Peña | 42°31′08″N 0°41′18″W | 986 | *Quercus ilex* |
| Huesca: Sariñena, Los Monegros, road CHE 1410 from Sariñena to Cartuja de Monegros | 41°46′47″N 0°14′58″W | 309 | *Pinus halepensis*<br>*Tamarix africana* |
| Huesca: Sos del Rey Católico, road A-1602, km 15 | 42°40′36″N 0°47′00″W | 800 | *Quercus faginea* |
| León: Oseja de Sajambre | 43°07′15″N 5°01′06″W | 960 | *Corylus avellana*<br>*Fagus sylvatica*<br>*Quercus sp.* |
| Madrid: Canencia, road from Canencia pass to Lozoya | 40°52′32″N 3°46′35″W | 1,390 | *Betula pendula* |
| Madrid: Hoyo de Manzanares, road M-618, km 20 | 40°36′09″N 3°54′55″W | 943 | *Castanea sativa*<br>*Cistus ladanifer*<br>*Juniperus communis*<br>*Quercus ilex* |
| Madrid: Miraflores de la Sierra, road M-629, km 5, from Miraflores to Canencia pass | 40°50′56″N 3°45′57″W | 1,280 | *Castanea sativa*<br>*Cistus* sp.<br>*Quercus pyrenaica* |
| Madrid: Miraflores de la Sierra, road N- 611, km 10 | 40°49′26″N 3°47′37″W | 1,352 | *Quercus pyrenaica* |
| Madrid: Rascafría, road M-604, from Lozoya to Rascafría | 40°55′53″N 3°49′55″W | 1,140 | *Fraxinus* sp.<br>*Quercus faginea* |
| Madrid: Rascafría, road N-604, km 13 | 40°56′53″N 3°45′47″W | 1,108 | *Castanea sativa*<br>*Juniperus communis* |
| Madrid: Soto del Real, road M-611, km 4, to Miraflores de la Sierra | 40°47′24″N 3°46′22″W | 1,037 | *Quercus pyrenaica* |
| Murcia: Águilas, Cabo Cope | 37°27′06″N 1°28′57″W | 13 | *Aulaga* sp.<br>*Stipa tenacísima*<br>*Tamarix africana* |
| Murcia: Alhama de Murcia, Sierra Espuña | | 437 | *Pinus halepensis* |
| Murcia: Blanca, Sierra de la Muela | 38°11′07″N 1°20′37″W | 220 | *Pinus halepensis* |
| Murcia: Cieza, road to Jumilla | 38°28′03″N 1°347′20″W | 515 | *Pinus halepensis* |
| Murcia: Mazarrón, road N-332 to Ramonete | 37°31′07″N 1°28′00″W | 297 | *Olea europaea* |

**Table 1** (continued)

| Locality | Coordinates | Elevation (m) | Substrate (under) |
|---|---|---|---|
| | | | *Thymus* sp. |
| Navarra: Amescoa Baja, road NA-718, km 12 | 42°45′35″N 2°06′32″W | 510 | *Quercus faginea*<br>*Buxus sempervirens* |
| Navarra: Amescoa Baja, road NA-718, km 16–17, from Alto de Urbasa to Zudaire | 42°46′24″N 2°08′32″W | 709 | *Quercus faginea* |
| Navarra: Ochagavía, road from Ochagavía to Iratí and Muskilda | 42°58′52″N 1°06′33″W | 1,008 | *Abies alba*<br>*Fraxinus excelsior*<br>*Pinus nigra*<br>*Quercus petraea* |
| Navarra: Roncesvalles, road N-135, km 24, to Erro pass | 42°56′56″N 1°28′58″W | 700 | *Buxus sempervirens*<br>*Castanea sativa*<br>*Ilex aquifolium*<br>*Pinus sylvestris*<br>*Quercus* sp. |
| Navarra: Yerri, Venta de Urbasa, road NA-718, km 22 | 42°48′05″N 2°08′44″W | 923 | *Crataegus monogyna* |
| Navarra: Yerri, Venta de Urbasa, road NA-718, km 28 | 42°50′16″N 2°10′47″W | 920 | *Fagus sylvatica* |
| Navarra: Yesa, road NA- 2201, km 1–2 | 42°40′48″N 1°09′09″W | 870 | *Pinus sylvestris*<br>*Quercus ilex*<br>*Quercus petraea* |
| Navarra: Yesa, Monasterio de Leire, trail to La Fuente | 42°38′17″N 1°10′22″W | 824 | *Acer campestre*<br>*Arbutus unedo*<br>*Corylus avellana*<br>*Quercus faginea*<br>*Quercus ilex* |
| Segovia: Moral de Hornuez | 41°29′04″N 3°38′18″W | 1,160 | *Cistus ladanifer*<br>*Juniperus oxycedrus*<br>*Juniperus sabina*<br>*P. pinaster*<br>*Quecus faginea*<br>*Quercus ilex* |
| Sevilla: Cazalla | 37°58′00″N 5°52′07″W | 430 | *Quercus coccifera* |
| Sevilla: El Pedroso | 37°54′44″N 5°48′09″W | 512 | *Quercus ilex*<br>*Quercus suber* |
| Soria: Calatañazor, road SO-P-5026, km 3–4, to Muriel de la Fuente | 41°42′39″N 2°50′35″W | 1,040 | *Juniperus thurifera*<br>*Quercus ilex* |
| Soria: Montenegro de Cameros, road SO-831, km 23–24, Santa Inés pass | 42°04′05″N 2°46′44″W | 1,500 | *Ilex aquifolium*<br>*Juniperus oxycedrus* |
| Soria: Vinuesa, road SO-830, km 13 | 42°00′33″N 2°47′18″W | 1,447 | *Pinus sylvestris* |
| Soria: Vinuesa, road SO-840, km 6 | 41°50′07″N 2°47′11″W | 1,078 | *Pinus sylvestris* |
| Portugal | | | |
| Algarve: Aljezur, Bordeira, Francelhe | 37°12′N 8°53′W | 30 | *Salix salvifolia* |
| Algarve: Aljezur, Bordeira, Francelhe | 37°18′N 8°48′ W | 30 | *Juniperus phoenicea*<br>*Pistacia lentiscus*<br>*Salix salvifolia*<br>*Cistus ladanifer*<br>*Pinus pinea* |
| Loulé, Querença, road EN-396 | 37°10′N 8°0′W | 160 | *Chamaerops humilis*<br>*Myrtus communis*<br>*Quercus suber* |

**Table 1** (continued)

| Locality | Coordinates | Elevation (m) | Substrate (under) |
|---|---|---|---|
| Loulé, Querença, road EN-396 | 37°11′ N 8°10′W | 238 | *Olea europaea*<br>*Pistacia lentiscus*<br>*Arbutus unedo* |
| Loulé, Salir, road EN-503, Brasieira de Baixo | 37°08′N 8°20′W | 254 | *Erica arborea*<br>*Quercus suber* |
| Monchique, Alferce, road EN-267, km 32 | 37°20′N 8°28′ W | 441 | *Castanea sativa*<br>*Quercus canariensis* |
| Monchique, Cortes, road EN-266 | 37°17′N 8°33′ W | 300 | *P. pinaster* |
| Monchique, Ginjeira | 37°20′N 8°28′W | 580 | *Castanea sativa* |
| Monchique, Marmelete, road EN-267, Casais | 37°19′N 8°38′W | 450 | *Myrtus communis*<br>*Quercus suber* |
| Santa Barbara de Nexe, Goldra de Baixo | 29SNB 9008 | 280 | *Arbutus unedo*<br>*Ceratonia siliqua* |

$y = Ax/(B+x)$, where $x$ is the number of samples, $y$ is the number of species recorded, $A$ is the maximum expected number of species, and $B$ the number of samples needed to reach half the value of $A$.

## Climate Variables

For each sampling locality, we obtained the following potentially relevant climatic data from the “Atlas Climático Digital de la Península Ibérica” [33]: Mean Annual Rainfall in millimeter (RAIN), Minimum Annual Temperature in degree Celsius (MINT), Maximum Annual Temperature in degree Celsius (MAXT), Average Annual Temperature in degree Celsius (AVGT), Potential Annual Solar Radiation in 10 $\mathrm{kJ/m^2 \times day \times \mu m}$ (RADI), Elevation in meter (ELEV), Light in $100+100\times\cos$(angle of incidence) (LIGH) and shade in degrees (SHAD). We also constructed a new variable, Thermal Amplitude (TAMP) by taking the difference MAXT−MINT.

## Statistical Analysis

As the factors determining the diversity and the presence or absence of social amoebae are largely unknown, we did not have a good a priori predictive statistical model. Thus, we exploratorily searched for the best subset of explanatory variables predicting dictyostelid species richness across the studied area. Each sampling locality was considered as an independent data point in the analysis. To search for the best subset of explanatory variables, we used generalized linear models (GLMs) on dictyostelid species richness with the following potential explanatory variables: Month and Year of collection (which were entered as continuous variables with their quadratic terms), RAIN, MINT, MAXT, AVGT, RADI, ELEV, LIGH, SHAD, TAMP, plant species richness (PLANT_R), the number of samples taken in each location ($n$), which varied between 2 and 6, and pH, along with its quadratic term ($\mathrm{pH}^2$), as a hypothesis suggests top dictyostelid diversity at intermediate pH (26, Cavender pers. com.). In order to have control for spatial autocorrelation and to test whether there were potentially unmeasured environmental variables (i.e. Trend Surface Analysis, 25), we also allowed in the model the spatial coordinates (Longitude-$x$, Latitude-$y$ in UTMs) and a complex combination of them ($x^2$, $y^2$ and the interaction term $x \times y$). We used the package R for statistical analysis [36] and as recommended by Moya-Laraño and Wise [32] we used the Akaike’s Information Criterion (AIC) “step” algorithm within that package in order to select the model with the best subset of explanatory variables. Since this is largely an exploratory analysis to search for ecological predictors of the presence and diversity of amoebae, we did not include corrections for multiple tests, as this is advisable only for analyzing experimental responses [32].

We searched for the best-subset model explaining dictyostelid diversity, using a negative binomial distribution (with log-link function) in the GLM and then tested for significance of variables within the final model (within the MASS R package). The negative binomial accounts for dispersion (mean different than variance), and thus from departure from a Poisson distribution, by estimating an additional parameter. Also following recommendations by Moya-Laraño and Wise [32], we tested for the significance of each parameter estimate in the model using a type III hypothesis, which is more powerful than the conventional $t$ tests. To implement this test in R (function glm.nb in MASS), one has to take twice the difference between the log-likelihoods, one for the full model and another for the model without the variable of interest (a log-likelihood ratio test). For continuous variables the value is compared to the chi-square distribution with one degree of freedom. Since the final model did not converge and could not end parameter estimation, overestimating the YEAR effect by several orders of magnitude, we first centered YEAR by subtracting the mean prior to model estimation.

We also evaluated the ecological predictors for the presence/absence of social amoebae in the different locations using a GLM with binomial error and a logical link function. To implement this test in R, one has to take the difference between Residual Deviances—one for the full model and another for the model without the variable of interest. For continuous variables, the value is compared to the chi-square distribution with one degree of freedom. Depicting curvilinear patterns in multiple regression models can be achieved using partial residual plots [31]. However, in order to visualize if there was an optimal peak of dictyostelid diversity around neutral pH, we used a Combining Conditional Expectations and Residuals (CERES) plot [13] because it is an improved way to graphically depict (and even detect) curvilinear relationships by using LOWESS smoothing. A CERES plot on a binomial GLM shows an estimate of the partial (i.e., holding all other variables constant) of the probability of one or another event to occur (thus the binomial distribution) depending on the value of a target independent variable in the model. In our case, the dependent variable is the presence or absence of dictyostelids, and the independent variable is pH. Since the values on the *y*-axis are estimates based on residuals and serve to remove the effect of all the other independent variables in the model, the magnitude of the curvature of the line, and not the units, is of interest. To build the CERES plot, we used the package "art" in R.

## Results

A total of 300 soil samples from 44 different substrates were analyzed. From these samples, we isolated 15 different species of dictyostelids that together with the other four species previously reported described for the area (5, 47, Hagiwara pers. comm.) make a total of 19 species for the Iberian Peninsula. These species are distributed among the four different groups recognized by the first molecular phylogeny [43].

### Species Accumulation Curve

The sampling effort is shown in Fig. 1. We found that the maximum expected number of species (*A*) was 15.0 and that the number of samples necessary to reach half the value of *A* (i.e., *B*) was 10.2. As the number of species obtained in this study was 15, our sampling effort seems to be appropriate to include the entire diversity of dictyostelids present in the Iberian Peninsula.

### Statistical Analysis

Table 2 shows the results of the statistical analysis and the final models according to the AIC algorithm for best subsets in R. Both the final statistical models for species richness and that for presence/absence were highly significant ($\chi^2$=43.3, d.f.=14, $P$<0.0001, generalized-$R^2$=0.390; $\chi^2$=45.3, d.f.=12, $P$<0.0001, generalized-$R^2$=0.399). A significant outlier with strong leverage and long Cook distances was removed from the model of species richness. There were significant differences among collecting months on dictyostelid species richness (Table 2a), and a close inspection of the data (not shown) showed that in autumn (October–November) the

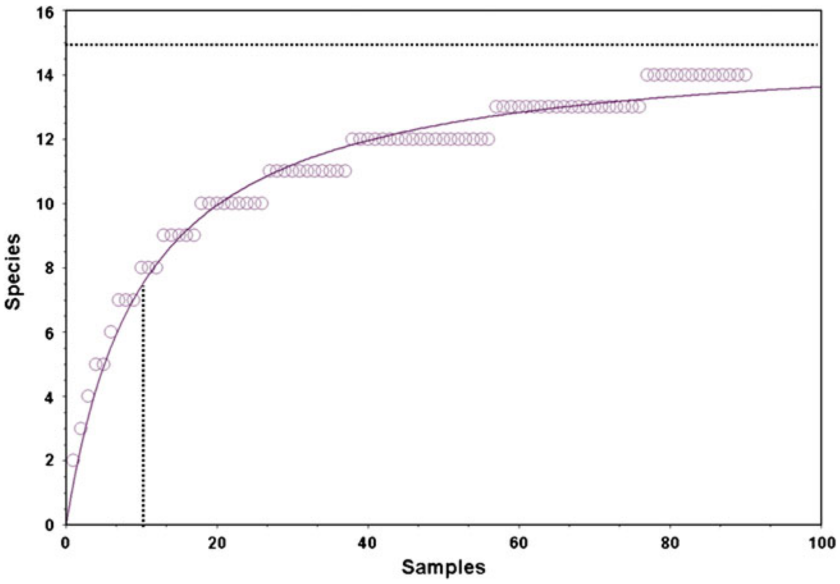

**Figure 1** Species accumulation curve (BS analysis) of the randomly permutated sequence of all samples studied fitted to the accumulated number of species (*open circles*). These values are the means of 100 runs. The *solid line* shows the non-linear regression fit using the saturation function $y=Ax/(B+x)$, where *A* is the expected maximum number of species (*horizontal dotted line*) and *B* is the number of samples needed to reach half of *A* (*vertical dotted line*)

**Table 2** GLMs predicting dictyostelid species richness and presence/absence across the Iberian Peninsula

| | Estimate | SE | Chi-square | d.f. | *P* value |
|---|---|---|---|---|---|
| Species richness (negative binomial) | | | | | |
| **Month** | **−1.5E−01** | **6.9E−02** | **4.8** | **1** | **0.028** |
| **Year** | **1.3E+00** | **4.6E−01** | **10.6** | **1** | **0.001** |
| Year$^2$ | 5.4E−01 | 3.6E−01 | 2.4 | 1 | 0.123 |
| **Longitude-*x*** | **−9.2E−05** | **4.4E−05** | **5.0** | **1** | **0.026** |
| **Latitude-*y*** | **8.3E−05** | **3.7E−05** | **5.6** | **1** | **0.018** |
| **Longitude-$x^2$** | **−1.3E−11** | **7.1E−12** | **3.7** | **1** | **0.053** |
| **Latitude-$y^2$** | **−1.1E−11** | **4.5E−12** | **6.3** | **1** | **0.012** |
| **MINT** | **−3.8E−01** | **1.6E−01** | **6.1** | **1** | **0.013** |
| **ELEV** | **−2.0E−03** | **8.8E−04** | **5.1** | **1** | **0.024** |
| **SHAD** | **3.5E−02** | **1.3E−02** | **6.3** | **1** | **0.012** |
| Plant richness | 5.8E−01 | 3.7E−01 | 2.4 | 1 | 0.118 |
| **pH** | **3.8E+00** | **1.7E+00** | **6.0** | **1** | **0.014** |
| **pH$^2$** | **−2.9E−01** | **1.3E−01** | **5.6** | **1** | **0.018** |
| **$x \times y$** | **2.3E−11** | **1.1E−11** | **4.8** | **1** | **0.029** |
| Presence/absence (binomial) | | | | | |
| **Year** | **2.9E+00** | **1.0E+00** | **10.6** | **1** | **0.001** |
| **Longitude-*x*** | **−1.6E−04** | **6.0E−05** | **8.9** | **1** | **0.003** |
| **Latitude-*y*** | **2.8E−04** | **1.5E−04** | **3.9** | **1** | **0.049** |
| **Longitude-$x^2$** | **−4.0E−11** | **1.8E−11** | **5.9** | **1** | **0.015** |
| **Latitude-$y^2$** | **−3.6E−11** | **1.7E−11** | **4.9** | **1** | **0.027** |
| **MINT** | **−1.8E+00** | **7.0E−01** | **8.3** | **1** | **0.004** |
| MAXT | −1.3E+00 | 9.7E−01 | 2.0 | 1 | 0.157 |
| **ELEV** | **−1.6E−02** | **6.6E−03** | **8.2** | **1** | **0.004** |
| **SHAD** | **1.3E−01** | **6.8E−02** | **5.9** | **1** | **0.015** |
| **pH** | **1.7E+01** | **5.3E+00** | **12.4** | **1** | **0.000** |
| **pH$^2$** | **−1.3E+00** | **4.1E−01** | **12.9** | **1** | **0.000** |
| **$x \times y$** | **4.4E−11** | **1.6E−11** | **8.8** | **1** | **0.003** |

Significant predictors are in bold

species richness was lower than in the summer (June–July). Year was also positive and significant, indicating that a higher diversity of dictyostelids was detected as the years passed. Longitude and its quadratic term were marginally significant and both negatively associated to dictyostelid diversity, indicating that the farther the distance from the Mediterranean Sea, the higher the diversity of dictyostelids. There was also a significant and positive correlation with latitude and a negative effect of the quadratic term, indicating that, once controlled for all other factors in the model, diversity is highest at intermediate latitudes. MINT showed a significant negative relationship with dictyostelid species richness, indicating that localities with severe winters tended to have higher dictyostelid diversity. ELEV was significant and negative, indicating that dictyostelid diversity decreases with altitude. SHAD had a positive effect on species diversity, likely indicating that a shady meso-climate with relatively high moisture enhances dictyostelid diversity. Relative plant richness in the sample entered the model positively but not reaching significance, suggesting just slightly that higher plant diversity may be linked to a higher diversity of social amoebas. Interestingly, dictyostelid richness was highest as intermediate values of pH, as the linear term was significant and positive and the quadratic term was significant and negative. Finally, the product longitude× latitude was significant, indicating that some source of unmeasured environmental variation that covariates with space can potentially affect dictyostelid species richness.

When analyzing the predictors for the presence/absence of dictyostelids (Table 2b), the pattern was very similar to that of diversity. We found a positive effect of year, indicating a trend towards increasing the probability of detecting dictyostelids as the years passed. As with species richness, the higher the distance from the Mediterranean, the higher is the probability of dictyostelid presence. Also, presence was highest at intermediate latitudes. MINT showed a significant negative relationship with dictyostelid presence, indicating that localities with severe winters tended to have a higher probability of finding social amoebas. Higher elevations, independently of winter severity, had a lower chance to hold dictyostelid populations. The amount of shade (and thus water availability) was positively related to the presence of dictyostelids. Also, the probability of detecting dictyostelids was highest at intermediate, close to neutral pH (Fig. 2). Again, the significant interaction "longitude × latitude" indicates covariance between important unmeasured predictors and spatial heterogeneity.

## Discussion

In south-west Europe (Iberian Peninsula), dictyostelid diversity and presence seem to be affected by a complex set of environmental variables. First, there seems to be temporal and spatial effects. Second, once controlled for the above effects, abiotic factors such as shade (local water availability), temperature, and pH seem important.

Our results suggest that the collecting season is important in the study of social amoebae (diversity being higher in summer as compare to autumn). These microorganisms have two cycles, the asexual which is the most studied and most common in laboratory cultures and which ends with the erection of a fruiting body, and the sexual, which is mostly unknown and which ends with the production of macrocysts. Dictyostelids can build two types of structures when entering diapause, macro- and microcysts, which they produce when environmental conditions are adverse [21]. These two forms of resistance are much more difficult to germinate in laboratory cultures than spores, and thus samples collected from places in which conditions are relatively harsh to dictyostelids will end up with a lower germination success when reared in the laboratory, therefore leading to an underestimation of dictyostelid diversity. As our results show, collecting only in a single year or in a single season would bias the estimates of diversity coming from growing spores in the laboratory.

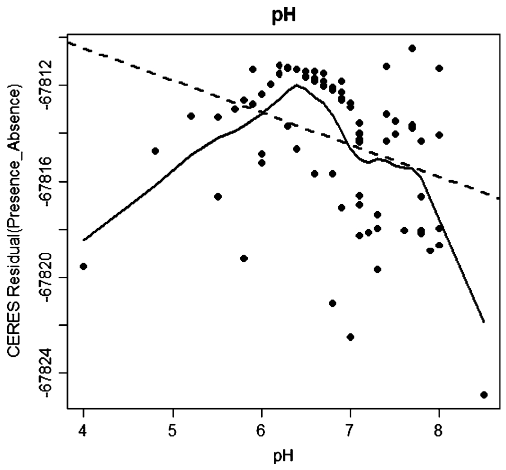

**Figure 2** CERES plot showing a peak of presence for social amoebae near-neutral pH. The units on the *y-axis* are CERES residuals and are not of interest. The *dashed line* shows the linear least squares fit for the residuals. The *solid line* shows the LOWESS smoothing fit

One possible explanation for the higher dictyostelid diversity and presence, related to the distance from the Mediterranean Sea could be the beneficial effect of humidity (in the form of mists and fogs) coming from the influence of the Atlantic Ocean. However, other effects may be important, as presence (and partially diversity) is highest at intermediate latitudes. Nevertheless, the amount of shade (which is directly related to ambient relative humidity) also positively explains dictyostelid diversity. Thus, it seems that this group of multicellular organisms was able to colonize the wettest terrestrial ecosystems, and as a consequence, they have proliferated in environments that are considerably humid.

Extremely low temperatures also seem to enhance diversity and presence of dictyostelids, suggesting that severe winters also allow for a higher diversity of coexisting dictyostelid species. Thus, other factors being equal (e.g., shade, latitude, and longitude), differences in minimum temperature are important in determining dictyostelid diversity.

Although the diversity of plants did not reach significance, it also seems plausible that this variable could expand the niche space for dictyostelids. The association between dictyostelid species and vegetation was first suggested by Cavender and Raper [10, 11] and Cavender and Kawabe [8]. Different plant species have different ecological requirements (such as pH values), and dictyostelid species seem to have adapted to and/or specialized in this relatively wide niche space facilitated by a higher diversity of plant species. Furthermore, some dictyostelids seem to be present only under certain plants and other species seem to be generalists, appearing in association with many different plants. Plants, on the other hand, may host several species of dictyostelids (e.g., *Dictyostelium implicatum* and *Dictyostelium leptosomum* were collected under 25 different plant species, *Dictyostelium mucoroides* under 19 species and *Dictyostelium giganteum* under 16 species) or just very few of them (e.g., *Pinus pinaster* with only one species) (data not shown).

However, both water availability and plant species richness could facilitate dictyostelid diversity indirectly, via their prey—bacteria. Different plant species have a different microflora of bacteria associated with them (e.g., [19], [27], [51]). Furthermore, the diversity of some soil bacteria has been found to be higher in relatively wet places (e.g. [24]), and in terrestrial ecosystems, diversity should be related to moisture, not only to temperature [30]. Thus, perhaps dictyostelids are merely responding directly to the higher diversity of bacteria (i.e., in a bottom-up control of diversity, see [18]). Surveys including bacteria, dictyostelids, and the factors discussed here could help disentangling whether the environmental effects that we found are direct or indirect, or if both, which are more important.

In other microbiological studies [14, 28], an abiotic soil factor such as pH was reported as one of the most important in determining community composition. Also in a recent paper [24], the authors looked at several soil characteristic (mean annual temperature, soil moisture deficit, soil texture, pH, etc.) and concluded that pH was the most important factor in determining bacterial community structure and diversity. Diversity was highest in soils with near-neutral pHs. However, although soil pH was the best predictor, a large amount of the variability in bacterial community structure remained unexplained. As they said, there are a number of soil characteristics that are directly or indirectly related to soil pH so the influence of other factors needs to be understood. Unfortunately, these authors did not apply a multivariate analysis to their data such as the one we have performed here, which would have allowed disentangling partial from total effects. In other words, the partial effect of a given factor (e.g., temperature) cannot be revealed if this factor is highly correlated with another (e.g., pH) and most of the explanatory power in the model can be assigned to the second (see Moya-Laraño and Wise [32] for further details). In our case, we found a complex pattern in the case of the social amoebae, and importantly, as the above authors found for bacteria, we found maximum diversity and presence of dictyostelids near-neutral pHs, strongly suggesting that the diversity and presence of dictyostelids follow that of their prey, bacteria.

Leitner [26] suggested that there is a relationship between soil pH and the diversity of dictyostelids. Indeed, it has been suggested that the general trend in dictyostelid species is that they prefer soils with pH values close to neutrality and that it is in these soils that one can find the highest diversity of species (Cavender pers. comm.). We did find evidence for such a trend. However, it is true that the pH range in which dictyostelids live is highly variable among species (4–8.5). Thus, perhaps dictyostelids are merely responding directly to the higher diversity of bacteria, which we did not measure here.

Our analysis was intended to be an exploratory one, determining for the first time which complex set of environmental variables determine the diversity and the presence and absence of dictyostelids. A potential drawback of our study is that it has not been validated with an independent sample. However, this sort of validation using GLMs has been rarely proven to be efficient [34]. In our case, the relatively low explanatory power of our models (both generalized-$R^2$ values$<0.4$) would hardly grant validation power. However, additional samples and further statistical modeling expanding the number of explanatory variables could help to understand the generality of our findings. For instance, we know that we did not include in our analysis some of the environmental determinants of dictyostelid diversity and dictyostelid presence or absence (e.g., diversity of bacteria), as suggested by the inclusion in the model of the significant product “longitude×latitude”, which points to unmeasured environmental heterogeneity [25]. Nevertheless, as a first approach, our findings do substantially contribute to achieving a global understanding of the ecological requirements of this group.

## Conclusions

Our results show, with statistical support for the first time, relevant information on the environmental factors that may contribute to social amoebae (dictyostelids) distribution and diversity. A combination of climatic (temperature, water availability), physical (pH), and vegetation (diversity) factors seems to favor species richness in the south-western of Europe (Iberian Peninsula). This diversity is substantially high, especially when we compare it with the diversity present in other central European countries such as Germany and Switzerland [12, 48, 49]. The high number of dictyostelid species present in the Iberian Peninsula could be explained by the presence of the two vegetation regions (Mediterranean and Eurosiberian), which are not found in the other countries studied and which may include a higher diversity of habitats and therefore more ecological conditions for different species to live in.

**Acknowledgements** We want to thank María Aguilar (Royal Botanical Garden, Madrid) for the help with the species accumulation curve and Allison Perrigo (EBC, Uppsala University, Sweden) for the linguistic assistance. This research was supported by a postdoctoral fellowship from the Ministry of Science and Innovation of Spain (0027-2007) to M. Romeralo, with funding also coming from the project CGL2005-00320/BOS and CGL2008-0720/BOS of this Ministry.

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