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Protostelids from deciduous forests: first data from southwestern Europe

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ABSTRACT

The first data of Protostelids from the southwest of Europe are presented in this paper. A total of 21 species were identified from samples collected in Somiedo Biosphere Reserve (Spain). This is the highest species richness recorded to date for Europe or for a latitude this high (>40°). Seven species (*Cavostelium apophysatum*, *Endostelium zonatum*, *Microglomus paxillus*, *Protosporangium fragile*, *Protostelium okumukumu*, *Soliformovum expulsum* and *Schizoplasmodiopsis micropunctata*) are new records for Europe. Approximately 65 % of the microscopic protostelid species described in the world have been found in this Reserve, a fact that increases the biological value of this protected area and suggests that Spain is an excellent location to study this group. A microhabitat study has been carried out finding differences in species composition and abundance between ground litter, aerial litter, and bark substrates. Comments on the distribution and ecology of the species, as well as illustrations of some species are included.

Introduction

Protostelids, a widespread group of simple mycetozoans (*Eumycetozoa*, *Amoebozoa*; Adl *et al.* 2005) producing microscopic fruiting bodies usually bearing a single spore at the tip of a delicate stalk, can be readily isolated from a great variety of substrates such as dead attached plant parts, soil, humus, dung, or bark (Olive 1975a). The first species of the group was found only 45 y ago by Olive & Stoianovitch (1960), who incidentally isolated *Protostelium mycophaga* on dead florets of *Phragmites australis* from Somerville (New Jersey), as they were attempting to culture *Acrasis rosea*. Since then, more than 30 species of protostelids have been described by studying material from several parts of the world (Spiegel *et al.* 2005; Hernández & Lado: *An on-line nomenclatural information system of Eumycetozoa*; http://www.nomen.eumycetozoa.com).

It is remarkable that Europe, one of the most studied territories of the world in terms of biodiversity, has hardly been surveyed for this group. The published works that contain European records are few: those carried out by Olive (1962, 1967, 1975b) more than 30 y ago, two records from Ukraine (Glustchenko *et al.* 2002), and a recent survey from beech forests of Germany (Tesmer *et al.* 2005). No studies have taken place in the southwest of Europe; this being the first study of protostelids made in this part of the world.

Information relating to ecology and distribution of the group has increased recently, but still relatively little is known. The data that are available would seem to indicate

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that compositional differences exist for the assemblages of species associated with particular types of microhabitats (Moore & Spiegel 1995, 2000a,b,c; Stephenson & Moore 1998).

The purpose of this paper is to establish a biodiversity survey of the protostelid species present in the southwest of Europe and to report their relationship to their environmental factors in order to guide further studies.

Materials and methods

Study area

This study is based on material collected in October 2005 in Somiedo Biosphere Reserve, which is located in the northwest of Spain, in the province of Asturias, between 43°00′- 43°11′N and 6°04′- 6°22′W. The entire 29 100 ha Reserve is located on the northern slope of the Cordillera Cantábrica, in a range of elevation between 395 m and 2194 m. The landscape is dominated by mountains, U-shaped valleys and glacial lakes, and the lithology is varied and peculiar, with alternating siliceous and calcareous formations. The climate is oceanic, with frequent fog, high humidity, rain and snow, having an annual medium precipitation of 1030–1350 mm; and the temperature (mean annual temperature 8 °C) is regulated by influence of the Cantabrian sea. In addition, the altitude causes a certain degree of continentality in the climate, so this area has transitional characteristics between the temperate climate of the northern areas of Spain, and the greater extremes of the central plain where the climate becomes Mediterranean. The predominant vegetation in the study area is boreal forests (mixed broadleaf forests with oaks, beeches, chestnut, and hazelnut), shrublands, and grasslands. This area has high human influence but most is based in sustainable uses as traditional agriculture and stockbreeding.

Sampling

A total of 12 localities (Table 1) was sampled. All samples were segregated according to microhabitat during the sampling by placing them in different paper bags and air drying. Then they were sent to the laboratory of the Real Jardín Botánico and designated as collection AS05 (AS for Asturias). Results in previous studies suggest the protostelid biota differs according to microhabitat in temperate regions (Moore & Spiegel 2000a,b,c; Best & Spiegel 1984). The samples were collected from three different microhabitats: bark from living trees, ground litter, and aerial litter. The ground litter microhabitat was defined as the layer of twigs, leaves, and other plant debris extending over the soil surface, whereas the aerial litter microhabitat was defined as the assemblage of dead but still attached parts of standing plants.

As a preliminary study, 68 samples were randomly selected from the 121 collected samples. These samples included, 30 from ground litter, 32 from aerial litter, and six from bark. Primary isolation plates were prepared between October 2005 and March 2006, using a modification of the technique described by Olive (1975a); (see also Moore & Spiegel 1995 and Spiegel *et al.* 2005). One plate per sample was prepared as follows: the material was cut into small (*ca* 1.5–2 cm) pieces with sterile forceps and then soaked in sterile water. Eight pieces from each sample were plated out forming a circle on a 9 cm Petri dish with a weak nutrient medium (wMY: 0.002 g malt extract, 0.002 g yeast extract, 0.75 g K_2HPO_4, 15 g agar l^{-1} of distilled water). The plates were incubated at ambient laboratory temperature (20–24 °C) and were surveyed for protostelids in the second week of culture. Species were identified on the basis of fruiting body morphology under the light microscope using the ×10 objective. When necessary and possible, fruiting bodies were also examined with ×20 objective to help confirm the identification. Isolations to culture were made, if necessary, to confirm the constancy of characters. Photomicrographs (Figs 1–2) were taken with a Nikon Eclipse E600 compound microscope using bright-field optics and a Nikon Digital Sight DS-5M digital camera.

Occurrences of species that were observed were recorded simply as present on a given sample of substrate (number of identifications). Although a species may have occurred in many patches in some samples and only once in others, we were interested in a simple survey of the protostelid biota, and did not design the survey to collect more detailed quantitative data.

Nomenclature used herein follows Olive (1975a) and Hernández & Lado www.nomen.mycetozoan.com. Identifications were made using both Spiegel *et al.* (2005) and original descriptions.

Data analysis

To estimate the extent to which the survey was exhaustive in terms of recorded species, a species accumulation curve was constructed (Schnittler 2001; Schnittler & Stephenson 2000). The sequence of samples was randomly permutated 100 times and the means of the cumulated number of species were calculated with a program developed in the laboratory of Real Jardín Botánico. The plot of the mean cumulated number of species *versus* the number of samples was subjected to a regression analysis, using the saturation formula

$$y = Ax/(B + x)$$

where x is the number of samples, y represents the number of species recorded, and the parameter A refers to the maximum number of species to be expected and B is the number of samples needed to reach half of the number of species to be expected.

Results

Ecology

A total of 164 occurrences, incorporating 21 species of protostelids, were recorded in this study. An estimate of 25 species ($A = 25$) to be expected was obtained from the BS analysis (Fig 3). Comparing the actual number of species with this estimation, the survey was complete to 84.2 %. Considering the different microhabitats (Fig 4) the survey was complete to 78.7 % ($A = 17.8$) for ground litter, and 73.2 % ($A = 21.9$) for aerial litter. Bark samples did not give a reasonable fit. It can be assumed that our sampling effort was sufficient for recovering all of

Table 1 – Sampled localities, their characteristics, and the code for samples deposited in the Departamento de Micología, Real Jardín Botánico

	Locality	Coordinates	Altitude	Sampling date	Vegetation	Simples
Loc. 1	Spain, Asturias, Teverga, Vigidel	43.14636° N 06.14100° W	630 m	4 Dec. 2005	Mixed forest with *Castanea sativa*, *Acer* sp., *Fagus sylvatica*	AS05-1 – AS05-12
Loc. 2	Spain, Asturias, Teverga, Puerto de San Lorenzo	43.14056° N 06.19333° W	1310 m	4 Dec. 2005	*Ilex aquifolia* forest and mountain grassland	AS05-13 – AS05-26
Loc. 3	Spain, Asturias, Somiedo, Las Viñas	43.15278° N 06.26472° W	740 m	4 Dec. 2005	Path with *Corylus avellana*, *Rubus* sp.	AS05-27 – AS05-40
Loc. 4	Spain, Asturias, Somiedo, Puerto de Somiedo	42.99541° N 06.20290° W	1427 m	4 Dec. 2005	Shrubland with *Erica* spp., *Juniperus* sp., *Calluna vulgaris*, *Vaccinium* sp.	AS05-41 – AS05-53
Loc. 5	Spain, Asturias, Somiedo, Saliencia, Endriga	43.10909° N 06.15511° W	1300 m	5 Dec. 2005	Mixed forest with *Corylus avellana*, *Fraxinus excelsior*, *Genista occidentalis*	AS05-54 – AS05-63
Loc. 6	Spain, Asturias, Somiedo, Saliencia, Endriga	43.09000° N 06.15475° W	1120 m	5 Dec. 2005	Mixed forest with *Fagus sylvatica*, *Corylus avellana*	AS05-64 – AS05-69
Loc. 7	Spain, Asturias, Somiedo, Braña Campa d'Abaxu	43.07860° N 06.13067° W	1202 m	5 Dec. 2005	Livestock farm	AS05-70 – AS05-71
Loc. 8	Spain, Asturias, Somiedo, Saliencia lakes	43.05541° N 06.09935° W	1610 m	5 Dec. 2005	Subalpine shrubland	AS05-72 – AS05-78
Loc. 9	Spain, Asturias, Somiedo, Alto de la Farragona	43.06147° N 06.09975° W	1549 m	5 Dec. 2005	Mixed forest with *Sorbus aria*, *S. aucuparia*, *Ilex aquifolia*	AS05-79 – AS05-84
Loc. 10	Spain, Asturias, Somiedo, La Malva electric power station	43.11275° N 06.24660° W	700 m	5 Dec. 2005	Planted trees	AS05-85 – AS05-95
Loc. 11	Spain, Asturias, Somiedo, La Venta Castru, road to Pineda	43.12916° N 06.26738° W	534 m	6 Dec. 2005	Path in mixed forest with *Castanea sativa*	AS05-96 – AS05-108
Loc. 12	Spain, Asturias, Somiedo, Río Pigüeña	43.14482° N 06.33294° W	569 m	6 Dec. 2005	Riverside forest	AS05-109 – AS05-121

the more common species in ground litter and aerial litter, whereas more sampling effort is needed for bark.

In 60 of the 68 samples of substrate that were plated (Table 2), one or more species of protostelids fruited, that makes an 88 % of positive cultures for protostelids (PCP = number of collections positive for protostelids × 100/number of collections plated). Of these 68 plates, 30 were prepared using ground litter samples, 32 came from aerial litter samples, and six were from bark. The mean number of species occurring per plate was 2.41 (range 0–9) and the ratio between number of species recorded and plates was 0.31.

In five of the studied localities (Table 3) PCP was 100 %, and in all localities it was more than 72 %, except for one locality (Loc. 7, a livestock farm) where only one collection was plated, yielding negative results. The PCPs vary between the three microhabitats studied (Table 2): 93 % for ground litter samples, 81 % for aerial litter samples, and 100 % for bark. The latter is only an approximation due to the small number of samples, and it cannot be reliably compared with the other microhabitats, but marks a tendency.

Aerial litter (Table 2) constitutes the microhabitat with the highest species richness (16) and number of identifications (species recorded as present on a given sample of substrate) (75), followed by ground litter with 14 species and 72 identifications. It is remarkable that bark has very high species richness (ten), if we take the number of collections plated (six) and the number of identifications (17) into consideration.

The most commonly encountered species (Table 2) are *Protostelium mycophaga* (Pm) with 33 identifications, representing a 20 % of the total number of occurrences, *Schizoplasmodiopsis amoeboidea* (Sa) with 22 identifications (13 %), *S. pseudoendospora* (Sps) with 22 identifications (13 %), and *Soliformovum irregulare* (Si) with 17 identifications (10 %). All these species together with *Cavostelium apophysatum* and *Schizoplasmodiopsis cavostelioides* have been found in the three microhabitats. *Protostelium mycophaga* and *S. irregulare* seem to have preference for aerial microhabitat. *Endostelium zonatum*, *Nematostelium gracile*, and *Protostelium okumukumu* were recovered only from aerial litter samples. *Microglomus paxillus*, *Protostelium arachisporum*, and *Protosporangium fragile* were found exclusively on bark. *Nematostelium ovatum* and *Schizoplasmodiopsis micropunctata* were recovered exclusively from ground litter samples.

Localities can not be reliably compared because the number of samples is different in each case, but some preliminary data can be obtained. Localities with the highest species richness (Table 3) are Loc. 11 (with 12 species), Loc. 6 (11 species), Loc. 12 (11 species) and Loc. 1 (ten species). All of them are mixed broadleaf forests, except Loc. 12 that is a riverside forest. The highest number of identifications was found in Loc. 11 (31 identifications), Loc. 12 (27 identifications), Loc. 1 (19 identifications) and Loc. 10 (18 identifications), followed by Loc. 6 with 17 identifications. The highest number of species recorded from one sample was nine, for the sample AS05-96 (Loc. 11, *Rubus* sp., aerial litter).

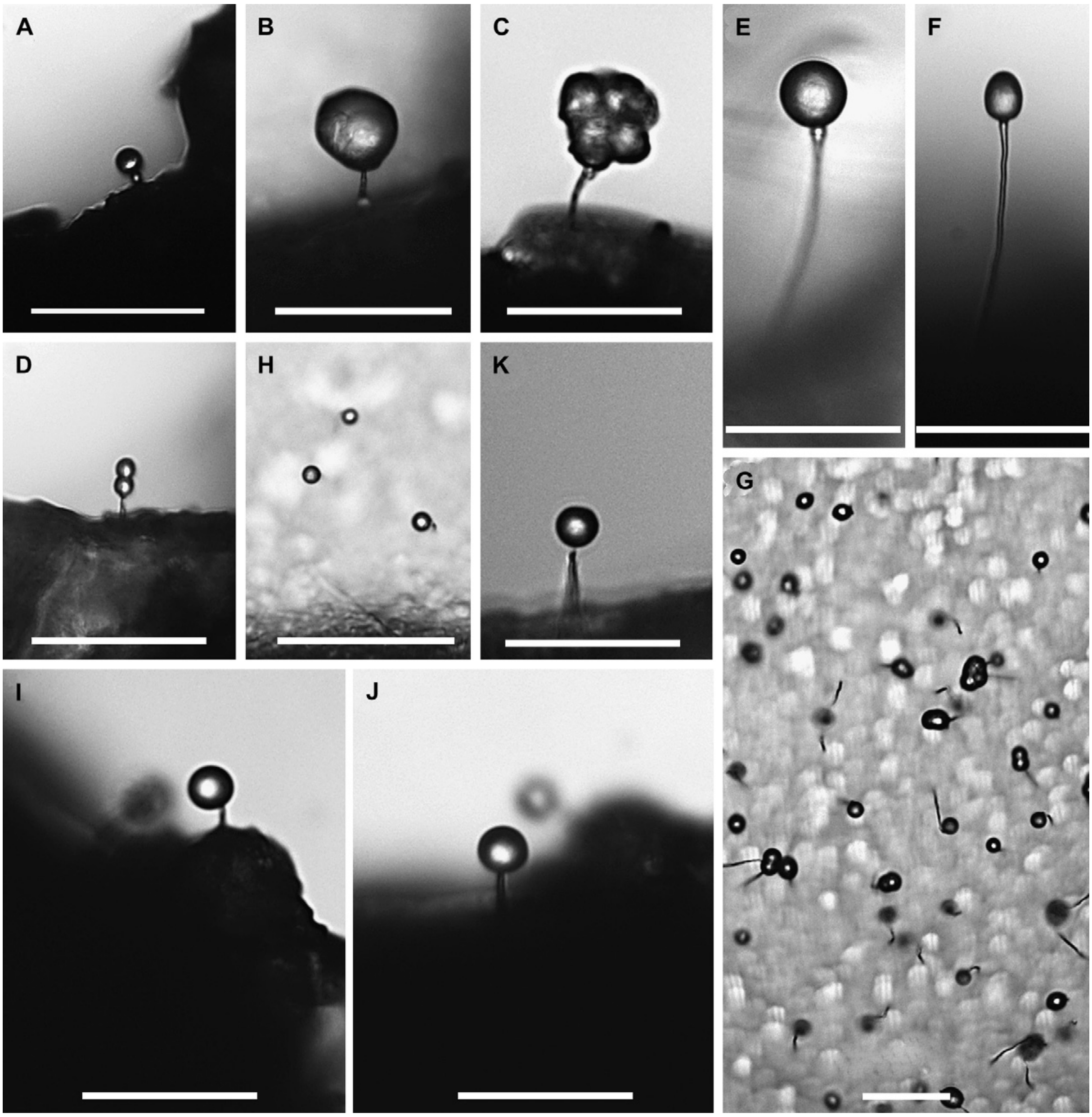

Fig 1 – Fruiting bodies of: (A) *Cavostelium apophysatum*; (B) *Echinosteliopsis oligospora* hydrated and (C) dried; (D) *Echinostelium bisporum*; (E) *Nematostelium gracile*; (F) *Nematostelium ovatum*; (G) *Protostelium mycophaga*; (H) *Protostelium nocturnum*; (I–J) *Schizoplasmodiopsis amoeboidea*; (K) *Schizoplasmodiopsis micropunctata*. Bars = 50 μm.

Taxonomy

A total of 21 species of protostelids were recorded. All of them are new records for southwestern Europe and seven are reported for the first time in Europe (noted with an asterisk).

Annotated species list

*__Cavostelium apophysatum__ L. S. Olive 1965

Loc. 1, ground litter of *Asteraceae*, AS05-12 (Fig 1A)

Loc. 3, aerial litter of *Lamiaceae*, AS05-39

Loc. 6, bark of *Fagus sylvatica*, AS05-66; aerial litter of *Erica* sp., AS05-68

Loc. 9, ground litter of *Cytisus* sp., AS05-84; ground litter of *Tilia* sp., AS05-105

The apophysis, although usually wider than the base of the stalk, is sometimes narrow such that the stalk appears to be equally thick for its entire length. The spore is rough and, when observed in apical view, it appears nearly opaque.

Echinosteliopsis oligospora D. J. Reinh & L. S. Olive 1967

Loc. 1, ground litter of *Asteraceae*, AS05-12 (Fig 1B–C)

Loc. 2, aerial litter of *Cytisus* sp., AS05-20

Loc. 3, aerial litter of *Cytisus* sp., AS05-31; aerial litter of *Quercus ilex*, AS05-37

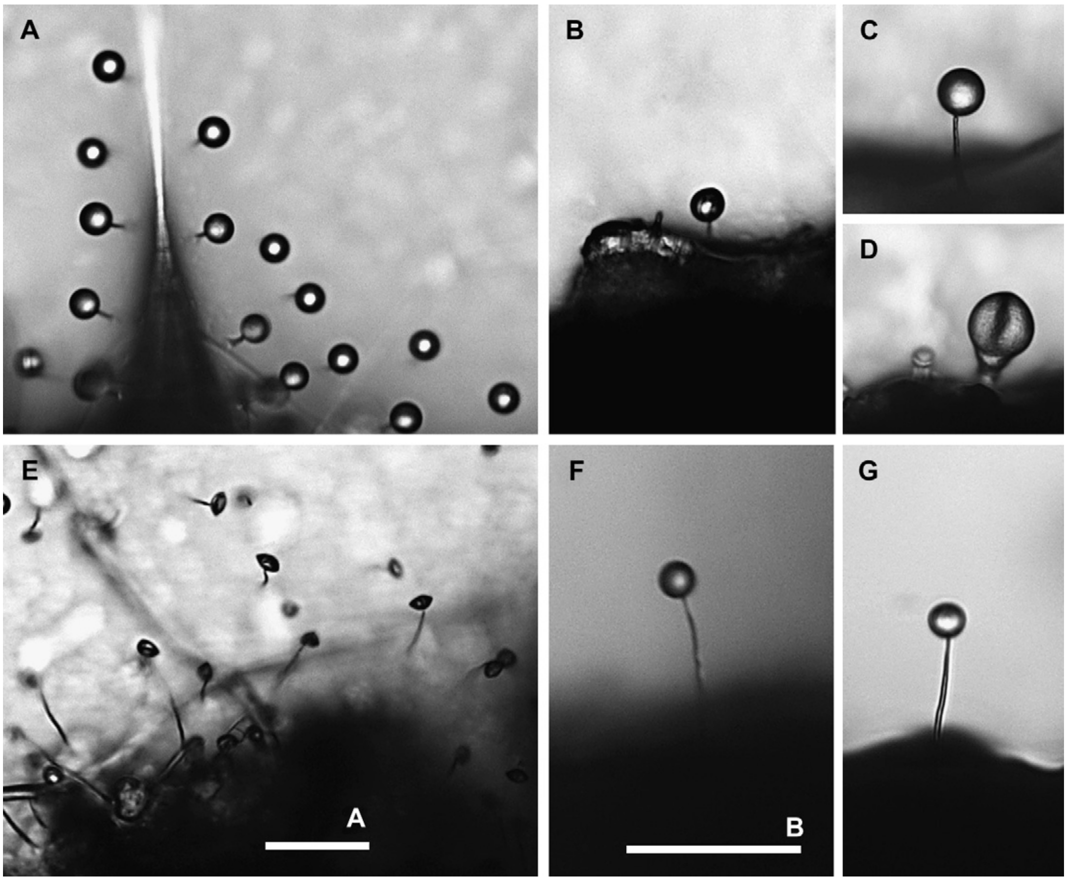

Fig 2 – Fruiting bodies of: (A–B) *Schizoplasmodiopsis pseudoendospora*; (C) *Schizoplasmodiopsis vulgare*; (D) *Schizoplasmodium cavostelioides*; (E) *Soliformovum irregulare* dried and (F) hydrated; (G) *Tychosporium acutostipes*. Bars = 50μm.

Loc. 11, ground litter of *Rubus* sp., AS05-97; ground litter of *Campanula* sp., AS05-101; ground litter of *Asteraceae*, AS05-103; aerial litter of *Tilia* sp., AS05-104

The number of spores is variable (4–8), and they are surrounded by a transparent, hygroscopic sheath. In conditions of high humidity the sheath appears as a spherical structure that contains the spores. In dryer conditions the sheath deflates and the sporangium becomes trefoil-shaped. In Europe this species has been reported previously from Germany (Tesmer *et al.* 2005).

Echinostelium bisporum (L. S. Olive & Stoian.) K. D. Whitney & L. S. Olive 1982 (Fig 1D)
Loc. 2, aerial litter of *Cytisus* sp., AS05-20
Loc. 10, aerial litter of *Poaceae*, AS05-87
Loc. 11, aerial litter of *Rubus* sp., AS05-96
Loc. 12, ground litter of *Rubus* sp., AS05-110

This mycetozoan was first described as a protostelid by Olive & Stoianovitch (1966) but it is now included in the myxomycetes (Spiegel & Feldman 1989; Whitney *et al.* 1982). It is usually studied under the same conditions as protostelids and usually grows intermixed with them. In Europe this species has been reported only from Germany (Tesmer *et al.* 2005).

***Endostelium zonatum** (L. S. Olive & Stoian.) W. E. Benn. & L. S. Olive 1984
Loc. 6, aerial litter of *Fagus sylvatica*, AS05-64

This species was found only once during our study but it presented the characteristic chain-like stalk and the pyriform spore that are typical of *E. zonatum* (Olive & Stoianovitch 1969).

***Microglomus paxillus** L. S. Olive & Stoian. 1977
Loc. 2, bark of *Crataegus monogyna*, AS05-26
Loc. 12, bark of *Alnus* sp., AS05-115

The 2–4 spores of this species can be observed through the sporangial sheath, that forms a round structure slightly flattened in the upper side.

Nematostelium cfr. **gracile** (L. S. Olive & Stoian.) L. S. Olive & Stoian. 1970 (Fig 1E)
Loc. 3, aerial litter of *Lamiaceae*, AS05-39

This species and *Ceratiomyxella tahitiensis* have identical fructifications and usually must be distinguished in culture. Unfortunately, all attempts to culture it failed, so its identity could not be confirmed. Spiegel *et al.* (2005) report that the vast majority of culture attempts are assigned to *N. gracile*, but all cultures from various parts of the world that have

Table 2 – Number of identifications per species in the three studied microhabitats

	Ca	Eo	Eb	Ez	Mp	Ng	No	Pfrag	Pa	Pm	Pn	Po	Ppyr	Sa	Sm	Sps	Sv	Sc	Se	Si	Ta	TI	R	NC	NP	PCP (%)
Ground litter	3	4	1	-	-	-	2	-	-	12	6	-	1	10	1	10	5	1	-	6	10	72	14	30	28	93
Aerial litter	2	4	3	1	-	1	-	-	-	20	5	1	3	8	-	8	1	3	1	10	4	75	16	32	26	81
Bark	1	-	-	-	2	-	-	1	1	1	-	-	-	4	-	4	-	1	1	1	-	17	10	6	6	100
TI	6	8	4	1	2	1	2	1	1	33	11	1	4	22	1	22	6	5	2	17	14	164	21	68	60	88

Ca, *Cavostelium apophysatum*; Eo, *Echinosteliopsis oligospora*; Eb, *Echinostelium bisporum*; Ez, *Endostelium zonatum*; Mp, *Microglomus paxillus*; Ng, *Nematostelium gracile*; No, *N. ovatum*; Pfrag, *Protosporangium fragile*; Pa, *Protostelium arachisporum*; Pm, *Protostelium mycophaga*; Pn, *P. nocturnum*; Po, *P. okumukumu*; Ppyr, *P. pyriforme*; Sa, *Schizoplasmodiopsis amoeboidea*; Sm, *Schizoplasmodiopsis micropunctata*; Sps, *S. pseudoendospora*; Sv, *S. vulgare*; Sc, *Schizoplasmodium cavostelioides*; Se, *Soliformovum expulsum*; Si, *S. irregulare*; Ta, *Tychosporium acutostipes*; TI, total number of identifications; R, species richness; NC, number of collections plated; NP, number of collections positive for protostelids; PCP, percentage of cultures positive for protostelids.

been established in the Spiegel laboratory in the last year have proven to have the amoeboflagellate state indicative of *C. tahitiensis* (Olive & Stoianovitch 1971). Further work is under way to clarify the taxonomy of protostelids with this sporocarp morphology.

In Europe, this species has been cited only from Germany (Tesmer *et al.* 2005).

Nematostelium ovatum (L. S. Olive & Stoian.) L. S. Olive & Stoian. 1970 (Fig 1F)

Loc. 6, ground litter of *Fagus sylvatica*, AS05-65

Loc. 10, ground litter of *Tilia* sp., AS05-94

This species has an ovoid or ellipsoid spore and a long, thick, robust stalk with a distinct apophysis. It has been recorded previously in Germany (Tesmer *et al.* 2005).

***Protosporangium fragile** L. S. Olive & Stoian. 1972

Loc. 2, bark of *Crataegus monogyna*, AS05-26

This species has a long, easily fragmented stalk that supports a four-spored sporocarp. It was found only once during our study.

Protostelium arachisporum L. S. Olive. 1962

Loc. 10, bark of *Pinus sylvestris*, AS05-95

The spores are very variable in shape, from almost spherical or ovate to elongate with one or more constrictions resembling the pod of a peanut. Tesmer *et al.* (2005) reported this species from Germany.

Protostelium mycophagum L. S. Olive & Stoian. 1960

Loc. 1, aerial litter of *Pteridium aquilinum*, AS05-5; (Fig 1G) ground litter of *Pteridium aquilinum*, AS05-6; ground litter of *Asteraceae*, AS05-12; aerial litter of *Asteraceae*, AS05-11

Loc. 2, aerial litter of *Cytisus* sp., AS05-20; ground litter of thistle, AS05-23; ground litter of *Crataegus monogyna*, AS05-25

Loc. 3, ground litter of *Cytisus* sp., AS05-32; aerial litter of *Hedera helix*, AS05-35; aerial litter of *Lamiaceae*, AS05-39

Loc. 4, aerial litter of *Erica* sp., AS05-48; aerial litter of *Mentha* sp., AS05-52; ground litter of *Mentha* sp., AS05-53

Loc. 5, aerial litter of *Corylus avellana*, AS05-62

Loc. 6, aerial litter of *Fagus sylvatica*, AS05-64

Loc. 9, aerial litter of *Lamiaceae*, AS05-81; aerial litter of *Lamiaceae*, AS05-82; aerial litter of *Cytisus* sp., AS05-83

Loc. 10, aerial litter of *Poaceae*, AS05-87; aerial litter of *Aesculus hippocastanum*, AS05-88; bark of *Pinus sylvestris*, AS05-95

Loc. 11, aerial litter of *Rubus* sp., AS05-96; ground litter of *Campanula* sp., AS05-101; aerial litter of *Asteraceae*, AS05-102; ground litter of *Asteraceae*, AS05-103; aerial litter of *Tilia* sp., AS05-104

Loc. 12, aerial litter of *Rubus* sp., AS05-109; ground litter of *Rubus* sp., AS05-110; aerial litter *Lamiaceae*, AS05-111; aerial litter of *Alnus* sp., AS05-113; ground litter of *Equisetum* sp., AS-121

Very variable in size and deciduousness of spores. Some individuals seemed to have stalks that move easily in air

Table 3 – Occurrence of protostelid species in the 12 studied localities

	Ca	Eo	Eb	Ez	Mp	Ng	No	Pfrag	Pa	Pm	Pn	Po	Ppyr	Sa	Sm	Sps	Sv	Sc	Se	Si	Ta	TI	R	NC	NP	PCP (%)
Loc.1	1	1	-	-	-	-	-	-	-	4	1	-	-	2	-	3	3	2	-	1	1	19	10	4	4	100
Loc.2	-	1	1	-	1	-	-	1	-	3	1	-	-	3	-	-	-	-	-	2	1	14	9	6	5	83
Loc.3	1	2	-	-	-	1	-	-	-	3	2	-	1	-	-	1	-	-	-	2	1	14	9	7	6	86
Loc.4	-	-	-	-	-	-	-	-	-	3	-	-	-	4	-	2	-	-	-	1	1	11	5	7	5	72
Loc.5	-	-	-	-	-	-	-	-	-	1	-	-	2	1	-	-	-	-	-	1	-	5	4	2	2	100
Loc.6	2	-	-	1	-	-	1	-	-	1	-	-	-	2	-	3	1	2	1	1	2	17	11	6	5	83
Loc.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	1	0	0
Loc.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	1	1	1	100
Loc.9	1	-	-	-	-	-	-	-	-	3	-	-	-	2	-	-	-	-	-	-	1	7	4	6	5	83
Loc.10	-	-	1	-	-	-	1	-	1	3	1	-	-	5	-	5	-	-	-	-	1	18	8	11	10	91
Loc.11	1	4	1	-	-	-	-	-	-	5	5	1	1	2	-	4	-	-	1	3	3	31	12	7	7	100
Loc.12	-	-	1	-	1	-	-	-	-	7	1	-	-	1	1	4	2	1	-	5	3	27	11	10	10	100
TI	6	8	4	1	2	1	2	1	1	33	11	1	4	22	1	22	6	5	2	17	14	164	21	68	60	88
NL	5	4	4	1	2	1	2	1	1	10	6	1	3	9	1	7	3	3	2	9	9					

Ca, *Cavostelium apophysatum*; Eo, *Echinosteliopsis oligospora*; Eb, *Echinostelium bisporum*; Ez, *Endostelium zonatum*; Mp, *Microglomus paxillus*; Ng, *Nematostelium gracile*; No, *N. ovatum*; Pfrag, *Protosporangium fragile*; Pa, *Protostelium arachisporum*; Pm, *Protostelium mycophaga*; Pn, *P. nocturnum*; Po, *P. okumukumu*; Ppyr, *P. pyriforme*; Sa, *Schizoplasmodiopsis amoeboidea*; Sm, *Schizoplasmodiopsis micropunctata*; Sps, *S. pseudoendospora*; Sv, *S. vulgare*; Sc, *Schizoplasmodium cavostelioides*; Se, *Soliformovum expulsum*; Si, *S. irregulare*; Ta, *Tychosporium acutostipes*; TI, total number of identifications; R, species richness; NL, number of localities in were the species was found; NC, number of collections plated; NP, number of collections positive for protostelids; PCP, percentage of cultures positive for protostelids.

currents, whereas others had stiffer stalks. Sometimes two-spored fruiting bodies were observed. Frequently, sporocarps were found where spores germinated *in situ* and refruited, forming a chain. Usually this species appears in big dense patches covering large areas of the plate. In Europe, this species has been reported from Holland (Olive 1962, 1967), Sweden (Olive 1962, 1967), Greece (Olive 1967) and Germany (Tesmer *et al.* 2005).

Protostelium nocturnum Spiegel. 1984 (Fig 1H)

Loc. 1, aerial litter of *Pteridium aquilinum*, AS05-5

Loc. 2, ground litter of thistle, AS05-23

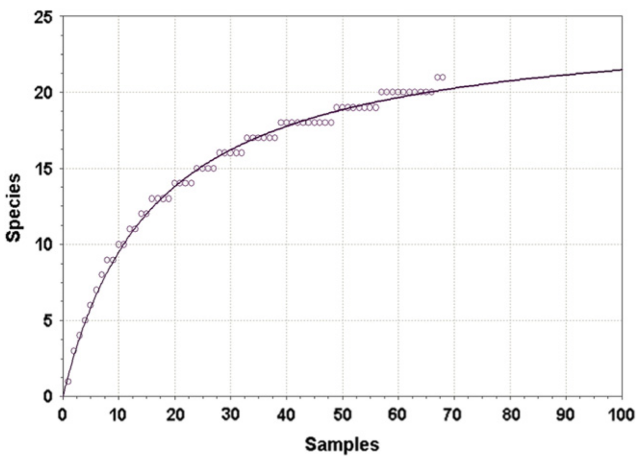

Fig 3 – BS analysis of the randomly permutated sequence of all samples studied versus cumulated species numbers (open circles). These values are the means of 100 runs. The solid line shows the results of regression analysis using a saturation function y = Ax/(B+x), where A is the maximum number of species to be expected and B is the number of samples needed to reach half of the number of species to be expected.

Loc. 3, ground litter of *Cytisus* sp., AS05-32

Loc. 10, ground litter of *Tilia* sp., AS05-94

Loc. 12, aerial litter of *Lamiaceae*, AS05-111

Loc. 11, aerial litter of *Rubus* sp., AS05-96; ground litter of *Campanula* sp., AS05-101; aerial litter of *Asteraceae*, AS05-102; ground litter of *Asteraceae*, AS05-103; aerial litter of *Tilia* sp., AS05-104

Most of the patches of this species fruited most heavily after sunset until early morning. Spores are soon actively released with the disappearance of the stalk. In Europe, this species has been only reported from Germany (Tesmer *et al.* 2005).

***Protostelium okumukumu** Spiegel, Shadwick & Hemmes. 2006

Loc. 11, aerial litter of *Tilia* sp., AS05-104

This species has a bipartite stalk that supports a spherical spore. The spore is actively shot from the stalk with the disappearance of the spherical apophysis such that only the rigid basal portion of the stalk remains. In a patch of sporocarps, there is typically a dense stand of these stalk bases (Spiegel *et al.* 2006). This is the first confirmed observation of this recently described species (Spiegel *et al.* 2006) outside of Polynesia.

Protostelium pyriforme L. S. Olive & Stoian. 1969

Loc. 3, aerial litter of *Quercus ilex*, AS05-37

Loc. 5, aerial litter of *Corylus avellana*, AS05-62; ground litter of *Corylus avellana*, AS05-63

Loc. 11, aerial litter of *Rubus* sp., AS05-96

Sporocarps are similar in size to those of *P. mycophaga*. The spore is obpyriform or campanulate, often waving in air currents. In Europe, it has been previously reported only from Germany (Tesmer *et al.* 2005).

Schizoplasmodiopsis amoeboidea L. S. Olive & K. D. Whitney. 1982 (Fig 1I–J)

Loc. 1, ground litter of *Pteridium aquilinum*, AS05-6; ground litter of *Asteraceae*, AS05-12

Loc. 2, ground litter of *Cytisus* sp., AS05-21; ground litter of thistle, AS05-23; bark of *Crataegus monogyna*, AS05-26

Loc. 4, ground litter of *Calluna vulgaris*, AS05-42; bark of *Cytisus* sp., AS05-45; aerial litter of *Erica* sp., AS05-48; ground litter of *Lamiaceae*, AS05-53

Loc. 5, aerial litter of *Corylus avellana*, AS05-62

Loc. 6, aerial litter of *Fagus sylvatica*, AS05-64; bark of *Fagus sylvatica*, AS05-66

Loc. 9, aerial litter of *Cytisus* sp., AS05-83; ground litter of *Cytisus* sp., AS05-84

Loc. 10, aerial litter of *Erica arborea*, AS05-90; aerial litter of *Poaceae*, AS05-91; ground litter of *Poaceae*, AS05-92; ground litter of *Tilia* sp., AS05-94; bark of *Pinus sylvestris*, AS05-95

Loc. 11, aerial litter of *Rubus* sp., AS05-96; aerial litter of *Tilia* sp., AS05-104

Loc. 12, ground litter of *Alnus* sp., AS05-114

Sporocarps of this species have the same proportions as those of *S. pseudoendospora*. Most of them are bigger in size and grow in sparse patches. The stalk gets suddenly thinner towards the apex, forming a sharp point. Tesmer *et al.* (2005) reported this species for the first time in Europe.

***Schizoplasmodiopsis** cf. **micropunctata** L. S. Olive & Stoian. 1976 (Fig 1K)

Loc. 12, ground litter of *Lamiaceae*, AS05-112

The stalk of this species gets thinner in the apex, forming a hair-like structure at the point of attachment with the spore. The stalk in this material is more robust than usual (Spiegel *et al.* 2005).

Schizoplasmodiopsis pseudoendospora L. S. Olive, M. Martin. & Stoian. 1967 (Fig 2A–B)

Loc. 1, aerial litter of *Pteridium aquilinum*, AS05-5; ground litter of *Pteridium aquilinum*, AS05-6; aerial litter of *Asteraceae*, AS05-11

Loc. 3, aerial litter of *Cytisus* sp., AS05-31

Loc. 4, ground litter of *Calluna vulgaris*, AS05-42; bark of *Cytisus* sp., AS05-45

Loc. 6, ground litter of *Fagus sylvatica*, AS05-65; bark of *Fagus sylvatica*, AS05-66; aerial litter of *Erica* sp., AS05-68

Loc. 10, ground litter of *Picea abies*, AS05-85; bark of *Picea abies*, AS05-86; ground litter of *Aesculus hippocastanum*, AS05-89; aerial litter of *Erica arborea*, AS05-90; ground litter of *Tilia* sp., AS05-94

Loc. 11, aerial litter of *Rubus* sp., AS05-96; ground litter of *Rubus* sp., AS05-97; aerial litter of *Tilia* sp., AS05-104; ground litter of *Tilia* sp., AS05-105

Loc. 12, aerial litter of *Rubus* sp., AS05-109; bark of *Alnus* sp., AS05-115

This species tends to fruit in big dense patches, and is usually smaller than *S. amoeboidea*. It has been cited for Germany (Tesmer *et al.* 2005) and Ukraine (Glustchenko *et al.* 2002).

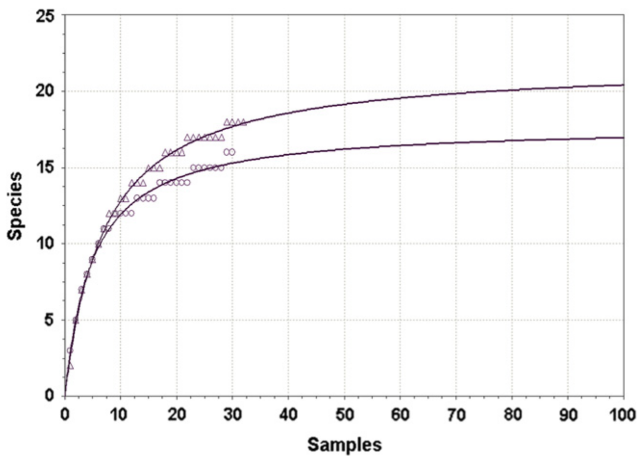

Fig 4 – BS analysis of the randomly permutated sequence of samples versus cumulated species numbers. Open circles represent the values for samples from the ground litter microhabitat and triangles represent the values for samples from the aerial litter microhabitat. These values are the means of 100 runs. The solid lines show the results of two regression analyses using a saturation function $y = Ax/(B+x)$, where A is the maximum number of species to be expected and B is the number of samples needed to reach half of the number of species to be expected.

Schizoplasmodiopsis vulgaris L. S. Olive & Stoian. 1976 (Fig 2C)

Loc. 1, aerial litter of *Pteridium aquilinum*, AS05-5; ground litter of *Pteridium aquilinum*, AS05-6; ground litter of *Asteraceae*, AS05-12

Loc. 6, ground litter of *Fagus sylvatica*, AS05-65

Loc. 12, ground litter of *Rubus* sp., AS05-110; ground litter of *Equisetum* sp., AS-121

The spores of this species are nearly spherical and the stalk length is variable. This species has been cited for England (Olive 1975b) and Germany (Tesmer *et al.* 2005).

Schizoplasmodium cavostelioides L. S. Olive & Stoian. 1966 (Fig 2D)

Loc. 1, ground litter of *Pteridium aquilinum*, AS05-6; aerial litter of *Asteraceae*, AS05-11

Loc. 6, bark of *Fagus sylvatica*, AS05-66; aerial litter of *Erica* sp., AS05-68

Loc. 12, aerial litter of *Rubus* sp., AS05-109

The spores attach to the stalk by a ring-shaped hilum that fits a distinct cup-shaped apophysis. In Europe, this species has been reported previously from Germany (Tesmer *et al.* 2005).

***Soliformovum expulsum** (L. S. Olive & Stoian.) Spiegel. 1994

Loc. 6, bark of *Fagus sylvatica*, AS05-66

Loc. 11, aerial litter of *Rubus* sp., AS05-96

The sporocarps are in the size range of *P. mycophaga*, but the stalk is bipartite with a broadly tapered basal section and a uniformly thin apical section. The stalk is usually reflexed at the junction of the two sections. The spores are forcibly discharged with the disappearance of the stalk. The presence of "fried egg"-shaped prespore cells helps to identify this species (Spiegel *et al.* 2005). It has been found only once during our study. Our material has an articulated stalk that bears a spherical spore that is typical of the species.

Soliformovum irregulare (L. S. Olive & Stoian.) Spiegel. 1994 (Fig 2E–F)

Loc. 1, aerial litter of *Asteraceae*, AS05-11

Loc. 2, aerial litter of *Cytisus* sp., AS05-20; bark of *Crataegus monogyna*, AS05-26

Loc. 3, aerial litter of *Cytisus* sp., AS05-31; aerial litter of *Lamiaceae*, AS05-39

Loc 4, aerial litter of *Mentha* sp., AS05-52

Loc. 5, ground litter of *Corylus avellana*, AS05-63

Loc. 6, aerial litter of *Erica* sp., AS05-68

Loc. 8, aerial litter of *Poaceae*, AS05-77

Loc. 11, aerial litter of *Rubus* sp., AS05-96; aerial litter of *Asteraceae*, AS05-102; aerial litter of *Tilia* sp., AS05-104

Loc. 12, ground litter of *Lamiaceae*, AS05-112; ground litter of *Alnus* sp., AS05-114; ground litter of *Equisetum* sp., AS-121

This is one of the tallest protostelids, and the stalks are usually very straight. Sometimes the hastate apophysis that is diagnostic of this species (Olive & Stoianovitch 1969; Spiegel *et al.* 1994) is not clearly obvious, and the stalk gets gradually thinner all the way to its apex. The deciduous spore can adhere to the side of the stalk after falling. When dried, it is "American football"-shaped. In Europe, this species have been cited only from Germany (Tesmer *et al.* 2005).

Tychosporium acutostipes Spiegel, D. L. Moore & J. Feldman. 1995 (Fig 2G)

Loc. 1, ground litter of *Pteridium aquilinum*, AS05-6,

Loc. 2, ground litter of *Cytisus* sp., AS05-21

Loc. 3, aerial litter of *Lamiaceae*, AS05-39

Loc. 4, ground litter of *Lamiaceae*, AS05-53

Loc. 6, aerial litter of *Erica* sp., AS05-68

Loc. 9, ground litter of *Gentiana lutea*, AS05-80

Loc. 10, ground litter of *Picea abies*, AS05-85

Loc. 11, aerial litter of *Rubus* sp., AS05-96; ground litter of *Campanula* sp., AS05-101; ground litter of *Asteraceae*, AS05-103

Loc. 12, aerial litter of *Lamiaceae*, AS05-111; ground litter of *Lamiaceae*, AS05-112

Our specimens have a stiff stalk that gets gradually thinner towards its apex and is characteristic of the species (Spiegel *et al.* 1995). The spore can be somewhat turbinate. *Tychosporium acutostipes* has been recently cited for the first time in Europe (Tesmer *et al.* 2005).

Discussion

This study area has shown the highest species richness (21 species) recorded to date for Europe (Spiegel, unpubl.) or for a latitude this high (>40°). This number of species represents approximately 65 % of the described microscopic protostelid species of the world. Comparable species richness has been reported for the island of Hawaii (32 spp.) and Puerto Rico (25 spp.) in the tropics (Spiegel *et al.* 2006; Stephenson *et al.* 2004) and unpublished work of Shadwick & Spiegel has recorded 22 species in the Great Smoky Mountains National Park, USA, a mix of temperate forest habitats. Of these, only the last was of an area of comparable latitude (36°N) and scale.

Previous studies that have been carried out at comparable scale and effort in other parts of the world show, for instance, these values: 16 species from Hueston Woods State Park, Ohio, USA (Best & Spiegel 1984), 17 species were recovered from samples from Costa Rica (Stephenson & Moore 1998), 16 species from Northwest Arkansas (Moore & Spiegel 2000a), 15 from Germany (Tesmer *et al.* 2005), 13 from Caribbean National Forest, Puerto Rico (Moore & Spiegel 2000b; Stephenson *et al.* 1999), and 12 from northern India (Shadwick & Stephenson 2004). These results are evidence consistent with a hypothesis that Biosphere Reserves, such as Somiedo, are as important for maintaining the biodiversity of microorganisms as they are for the diversity of macroscopic organisms (SIAPA, 2004: http://tematico.princast.es/mediambi/siapa/web/espacios/espacios/pnt/somiedo/). This high richness in Somiedo could be a result of its proximity to the Mediterranean Basin, one of the world biodiversity hotspots (http://www.biodiversityhotspots.org/xp/Hotspots).

The highest species richness and number of identifications were found in aerial litter microhabitat, as reported in many other study areas (Moore & Spiegel 2000b; Moore & Stephenson 2003; Olive 1975a). It has been suggested that this tendency may be because some species of protostelids are unable to cope with antagonistic microorganisms in substrates on the ground (Olive 1975a); however, ground litter microhabitats are richest at very high latitudes (Spiegel & Stephenson 2000), and certain species are more commonly found in ground litter than other microhabitats (Moore & Spiegel 2000a). Another possible cause of this phenomenon is that the much more fluctuating moisture gradient of aerial litter could favour protostelids due to their short life cycles (Tesmer *et al.* 2005).

Protostelium mycophaga, *Schizoplasmodiopsis pseudoendospora*, and *Soliformovum irregulare* are very frequently encountered species in the Somiedo Biosphere Reserve, as well as in other temperate areas (Best & Spiegel 1984; Moore & Spiegel 2000a; Tesmer *et al.* 2005), but *S. amoeboidea* appears more frequently here than has been reported in other parts of the world. Perhaps it was caused by the long drought period that had taken place before sampling. *Protostelium mycophaga* is possibly the most common protostelid worldwide, and appeared in all the substrate types examined and in ten of the 12 sampling localities as well.

These promising results, though still preliminary, can constitute a basis for further research and suggest that the north of Spain, a transitional area between boreal and Mediterranean vegetation, can be a very interesting place for further work. Spain, one of the European areas with highest biodiversity of other organisms, also appears to have high protostelid richness. It is an excellent location to study the biology of this group in more detail and its wide variety of habitats and climatic regions can help to increase the ecological information on these organisms.

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