

The Presence and Distribution of Protostelid Group Organisms in Bursa - Uludağ University Campus Area, Turkey

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ABSTRACT

This study is the first report of protostelids in Turkey. The listed 11 species, all of them new to Turkey, have been prepared in the laboratory cultures that contain samples of aerial plant parts and leaves, litter, woody twigs. The taxon of protostelids representing 3 families were recorded. These taxon are as follows; *Cavostelium apophysatum* Olive, *Ceratiomyxella tahitiensis* Olive and Stoian., *Echinosteliopsis oligospora* Reinhardt and Olive, *Echinostelium bisporum* (Olive and Stoian.) Whitney and Olive, *Nematostelium ovatum* (Olive and Stoian.) Olive and Stoian., *Protosporangium bisporum* Olive and Stoian., *Protostelium mycophagum* Olive and Stoian., *Schizoplasmodiopsis amoeboidea* Olive and Whitney, *Schizoplasmodiopsis pseudoendospora* Olive, Martin and Stoian., *Schizoplasmodiopsis vulgaris* Olive and Stoian., and *Soliformovum irregulare* (Olive and Stoian.) Spiegel.

Keywords: Anatolian, Biodiversity, Mycetozoa, Protosteliomycetes, Taxonomy, Turkey

INTRODUCTION

The protostelids, are characterized as having amoebae that form simple fruiting bodies, or sporocarps, comprised of a single acellular stalk with 1 or more spores (Olive 1975). Olive (1975) suggests that Protostelids are microbivorous predators feeding upon decomposers, including bacteria and fungi. On the other hand Olive (1975) have also reported that these protostelids are rarely found in soil habitats. Aquilar *et al.* (2011) have shown in his studies that, the protostelid's trophic phases are very diverse in that it consists of singular nucleus ameboid or multiple nucleus amoebflagellat phase or multiple nucleus retiform plasmodia which shows similarities with many Mycetozoan. Adl *et al.* (2012) states a general description of protostelids as "some sporophore producing protostelids have uninucleate trophic stages, they are greatly restricted in size and form since the spore requires the great majority of the resources present in the amoeba, plasmodial protostelids, on the other hand, may produce multinucleate single spores, multispored sporocarps, or a number of individual single spored units". Feest (1987) and Spiegel (1990), have stated in their studies that the Eumycetezoa and its' members Protosteliomycetes are eucaryotic and phagotrophic which possess an important role in the regulation of the bacteria population present in the soil and other microhabitation. Within the soil saprophyte community the decomposer community contains ameboids protosteliomycetes which feed on bacteria and mushrooms. 36 identified Protosteliomycet species are stated in Olive (1975, 1982) and Spiegel (1990)'s studies. Bacteria and mushrooms which exist in abundance on live and dead plant particles are crucial to the distribution and emergence of the Protosteliomycet group organisms as they make up their nutritional source. On the other hand, studies and evaluations have been conducted in regards to the Plasmodial and cellular slime molds and their connection with microarthropods used for disseminations (Blackwell 1984; Stephenson and Stempen 1994; Wheeler 1984, Moore and Spiegel 2000 a). In regards to the Myxomycetes group, Haskins (1990)'s remark "Due to their Plazmodial (uninucleate) structure and their fructification which carries only a few spores, affects their spore distribution mechanisms and allows it to function dormant which results in great importance for the distribution and emergence of the protostelid group organisms", can be referred to as the reason why the protostelid group has difficulties in creating a stable and strong niche opposite other competitive organisms in a micro environment. Taking into consideration the amount of numerous existing micro environments and their eco physiological conditions, the protostelids's life cycles, according to the biogeography distribution and other biological charecteristics, are more sensitive and more meticulous micro-environment needs as opposed to the other amoebozoan organisms. Moore and Spiegel (2000 a) states that on the performed work in Arkansas, "intended to microhabitat preferences are likely driven by the abiotic parameters influencing not only

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protostelids but their food source and dispersers as well". Also, Moore and Spiegel (2000 a) have stated in their studies that, it does not appear that the availability of food is what is limiting protostelid distributions.

Preliminary biogeographical studies of protostelids from Alaska, Puerto Rico, Hawaii, and Macquarie Island shows that the humidity and temperature may play a role in the microhabitat distribution of protostelids. Temperate aerial species are lacking from tropical mountain forests, where aerial and litter microhabitats are more similar, but temperate aerial species are frequently present in the litter microhabitat at high altitudes, where the litter microhabitat is more similar to the aerial microhabitat (Moore and Spiegel 2000 c, Spiegel and Stephenson 2000). This and similar studies shows us that, places on earth that are especially natural and with high entrance biomaterial are appropriate and fertile for the existence and distribution of the protosteliomycetes. However further detailed studies are needed.

A number of studies have been conducted in different habitats throughout the world in especially temperate areas (Aguilar *et al.* 2007, Aguilar *et al.* 2011, Best and Spiegel 1984, Brown and Spiegel 2008, Moore and Spiegel 1995, Moore and Spiegel 2000 a, Moore and Spiegel 2000 b, Shadwick and Stephenson 2004, Shadwick *et al.* 2009, Tesmer *et al.* 2005), tropical regions (Moore and Spiegel 2000 c, Moore and Stephenson 2003, Ndiritu *et al.* 2009, Powers and Stephenson 2006, Stephenson and Moore 1998, Stephenson *et al.* 1999), polar regions (Moore *et al.* 2000, Spiegel and Stephenson, 2000), and aquatic environments (Lindley *et al.* 2007, Tesmer and Schnittler 2009), but these studies of protosteloid amoebae communities on a large scale have not been updated.

The Campus area exists on 40°23'81" – 40°21'76" Northern latitude and 28°88'57" – 28°85'83" eastern longitude, 155 meters above sea level. The surveyed area is 691,65 hectares. The area consists of mixed woodlands with *Quercus robur* L. subsp. *robur*, *Quercus infectoria* Olivier subsp. *infectoria*, *Quercus pubescens* Willd., *Paliurus spina-cristi* Miller as the dominant vegetation. Also seen taxons are the, *Robus discolor* Weihe et Ness, *Rubus sanctus* Schreber which exist largely in shrubbery and *Salix alba* L., *Salix fragilis* L., *Typha latifolia* L. which exist in the watery and humid biotopes. Besides the natural vegetation, there exists a planted pine grove which also makes up an important part of the vegetation (Kaya 2002). Taking into consideration Thornthwaite and Trewartha's climatic classification (Universal temperature scale), the area is mesothermal, water shortage occurs in the summer with vaporization ratio at 53%, subhumid Mediterranean climate (Anonymous 2106).

MATERIALS AND METHODS

This qualitative study was carried out on 9 selected locations on random periods between May 2010 – May 2011 especially during the summer season on the Uludag University Campus. Collections were made in the field and samples of various substrates, such as dead parts of standing plants (aerial litter), ground litter, bark, and decaying or decayed plant debris for subsequent laboratory culture applications were collected. Specimens were put into breathable paper bags with appropriate descriptions enclosed for incubation and analysis in the laboratory. Laboratory applications have been completed using authorial publications (Shadwick *et al.* 2009, Tesmer *et al.* 2005). Potential substratum pieces have been cut into 2–2,5 cm long and 1cm wide strips using sterile scissors. After that, they have been soaked in distilled water for 15–20 minutes. Next they have been placed into Petri dishes which are 9 cm wide and contain wMY agar (1 litre distilled water, 0.002 g malt ekstrat, 0.002 g maya ekstrat, 0.75 g K₂HPO₄, 15 g agar/L) in a 8 radial plane position. With this 118 primer incubation petri (PIP) have been created. Petri dishes were maintained at room temperature (22–23 °C). Both petri media as well as the substrate material were observed microscopically every day for a period of three weeks. It concentrated on the edges of the petri materials, and also medium surfaces were observed carefully for the occurrence of protostelid fruiting bodies. The emergence of sporocarps have been monitored using 100 and 200 magnification on a light microscope. Identifications were made by using several keys, including Aguilar *et al.* 2011, Moore and Spiegel 2000 a, Moore and Stephenson 2003, Shadwick *et al.* 2009, Spiegel *et al.* 2007, Stephenson and Moore 1998, and Tesmer *et al.* 2005. Sample observations and image capturing were done using a Nikon Eclipse Ni light microscope coupled with a Nikon DS-Ri camera, supported by NIS-Elements AR. Taxonomic characterizations of samples were done by using basic data obtained from stereomicroscopical and

microscopical investigations of the specimens (Olive 1975, Spiegel 1990, Spiegel *et al.* 2007), web site of <http://comp.uark.edu/~fspiegel/protist.html> were used to identify and determine the morphologic and taxonomic details of the sporocarps that began emerging. The nomenclature treatment followed Lado (2005-2016, <http://www.nomen.eumycetozoa.com>) and Index Fungorum website: <http://www.indexfungorum.org/names/Names.asp>. To identify the fructification structures (Spiegel *et al.* 2007), certain characteristics were deemed important. Such as; The amount and shape of spores in the existing sporocarp, the relative size, the amount of effusive or non-effusive and passive or active spores, the existence and non-existence of hilum, and the features of the sheathes. Also, in regards to diagnosis and identification, following features were taken into account; The stalk being long (> 2 spor diameter long) or short (≤ 1.5 spor diameter long), thinning towards the tip, relatively thick or thin, straight or curvy, hard or flexible, the existence or non-existence of apophysis, observing the basic disc, and whether the stalk is permanent or temporary after the spores fall out (Spiegel *et al.* 2007).

A map of the study area, figure 1, and climate diagrapheme from nearest region for University campus area figure 2 was also indicated (<http://tr.climate-data.org/location/53103/>). Microscopic and stereoscopic measurements and evaluations for the diagnosis and identification were made and given in Figures 3-13.

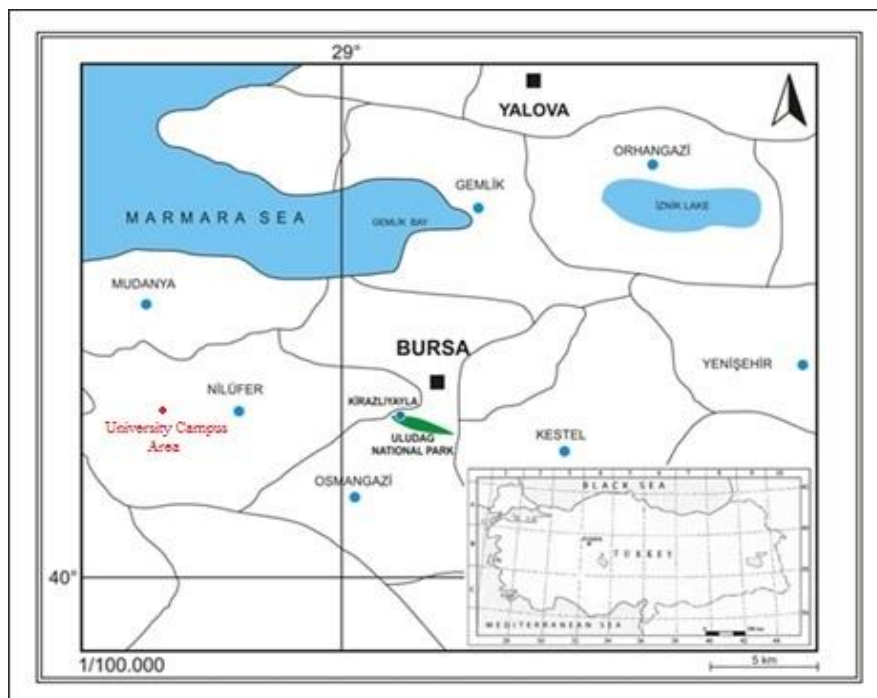


Figure 1. The study area on a map of Turkey with a grid system.

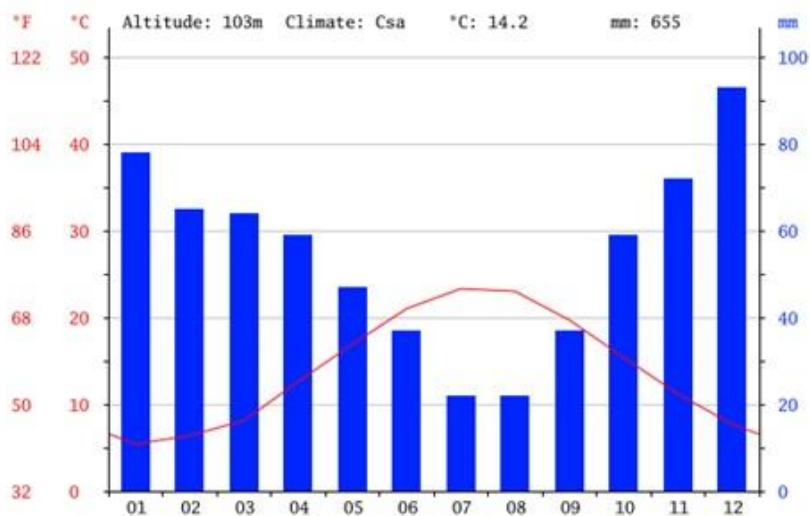


Figure 2. Climate diagram of the Nilüfer district for campus area.

RESULTS

This study was carried out periodically between May 2010 – May 2011, especially during the summer months (May, June, July) on the campus of Uludag University. Materials and samples were collected from 9 determined locations. From the natural substrates collected, 118 primer isolation petri (PIP) were created. The monitoring and the examination of these specimens resulted with determining 11 taxons in 8 genera (Figs 3-13). All of the specified taxon is a new record for Turkey. These taxon are: *Cavostelium apophysatum* Olive, *Ceratiomyxella tahitiensis* Olive and Stoian., *Echinosteliopsis oligospora* Reinhardt and Olive, *Echinostelium bisporum* (Olive and Stoian.) Whitney and Olive, *Nematostelium ovatum* (Olive and Stoian.) Olive and Stoian., *Protosporangium bisporum* Olive and Stoian., *Protostelium mycophagum* Olive and Stoian., *Schizoplasmodiopsis amoeboides* Olive and Whitney, *Schizoplasmodiopsis pseudoendospora* Olive, Martin and Stoian., *Schizoplasmodiopsis vulgaris* Olive and Stoian., and *Soliformovum irregulare* (Olive and Stoian.) Spiegel.

List of Species and Taxonomic Category

Domain: Eukarya
Kingdom: Protista
Phylum: Amoebozoa
Subphylum: Mycetozoa
Class: Protosteliomycetes
Subclass: Incertae sedis
Order: Protosteliales

Family 1: Cavosteliaceae

Species: *Cavostelium apophysatum* L.S. Olive, Mycologia 56(6):886 (1965)

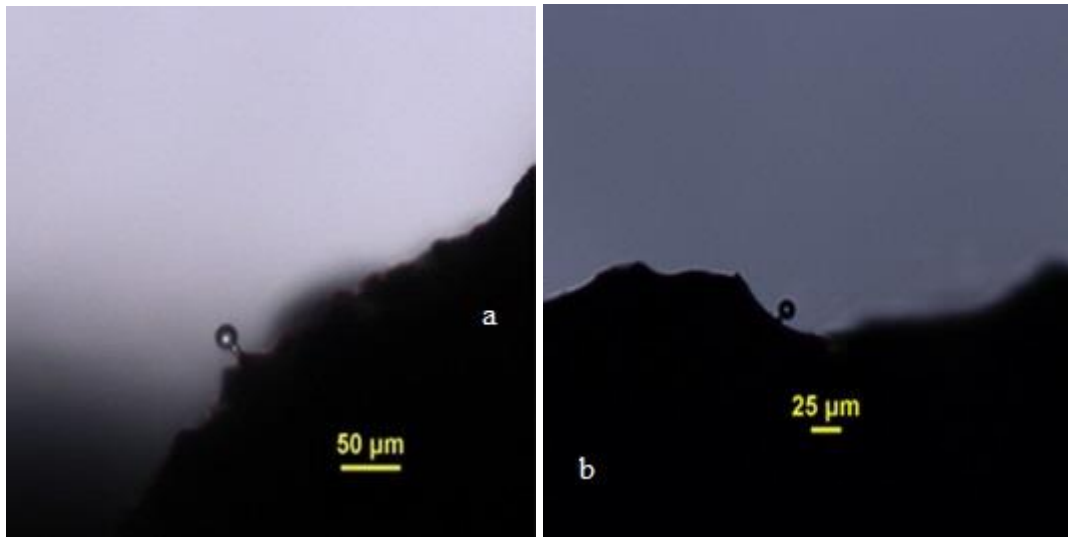


Figure 3 (a, b). Microscopic images from the *Cavostelium apophysatum*.

Comments: It is found widespread in tropical regions but also exist in mild climate regions. This specie was present in both aerial and litter microhabitats (Moore and Spiegel 2000 a). *C. apophysatum*, on a study in Costa Rica that was indicated on a third most abundant species of the total number all of the colonies, made up 15% (Moore and Stephenson 2003). It can be seen on dead plant particles or fallen materials. The stalk is very short, thick and has a cup shaped apophysis. Sporocarp is round and contains a non-deciduous single spore (Spiegel *et al.* 2007).

Species: *Ceratiomyxella tahitiensis* L.S. Olive & Stoian., Amer. J. Bot. 58(1):32 (1971)

Synonyms: *Ceratiomyxella tahitiensis* var. *neotropicalis* L.S. Olive & Stoian., Amer. J. Bot. 58(1):33 (1971)

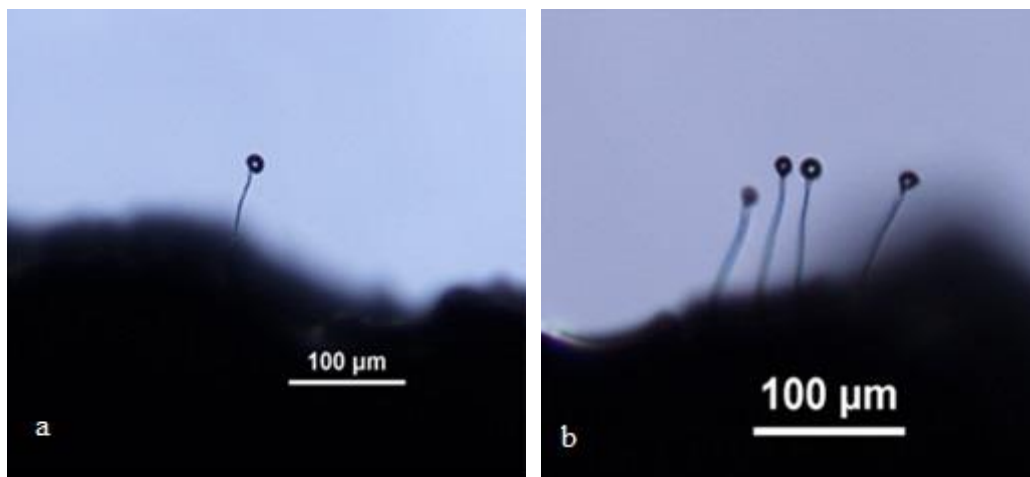


Figure 4 (a, b). Microscopic images from the *Ceratiomyxella tahitiensis*.

Comments: Exists on arial dead plant particles, live tree barks, fallen materials, feces and the earth soil. Glustchenko *et al.* (2002) have recorded the *C. tahitiensis* along with *Schizoplasmodiopsis pseudoendospora* on their study in Ukraine. The stalk is long, slightly curvy, (30-) 60-240 µm long, ends with an apex cup type

apophysis. Single spore carrying sporocarps which show round morphology features have spontaneous spores falling off (Spiegel *et al.* 2007).

Species: *Echinostelium bisporum* (L.S. Olive & Stoian.) K.D. Whitney & L.S. Olive, in Whitney, Bennett & Olive., Mycologia 74(4):680 (1982)

Synonyms: *Cavostelium bisporum* L.S. Olive & Stoian., Mycologia 58(3):440 (1966)

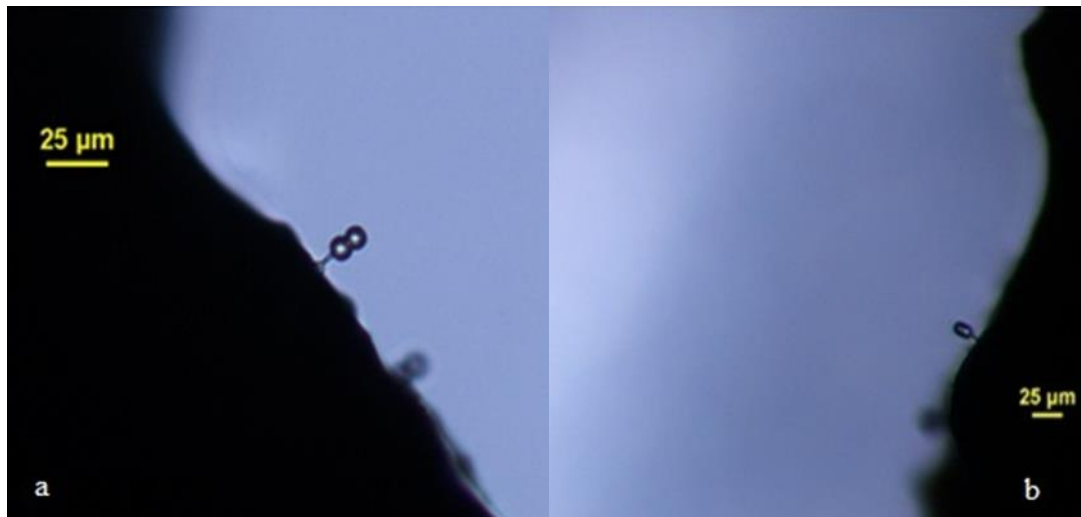


Figure 5 (a, b). Microscopic images from the *Echinostelium bisporum*.

Comments: Many of the species have been described as myxomycete for so many years and have been studied as such. Also they have long been treated as a protostelid - like myxomycete (Olive 1975). When the substrates in primer isolation petri dishes prepared for protostelids are observed, these species can often be seen in them. This type of species are often found in tropical regions and less in mild climate regions (Moore and Spiegel 2000 c). Moore and Stephenson (2003) suggest that the *E. bisporum*, a species rare in temperate litter microhabitats, was the single most abundant species in the aerial litter microhabitat and also it was the second most abundant species, representing 23% of the total number of all colonies. The stem is very short with a small protruding apophysis. It always has a globose or subglobose 2 spores. These spores exist back to back on a straight axis. They are deciduous and punctate. The spore that is closer to the tip is smaller (Spiegel *et al.* 2007).

Species: *Protosporangium bisporum* L.S. Olive & Stoian., J. Protozool. 19(4):565 (1972)

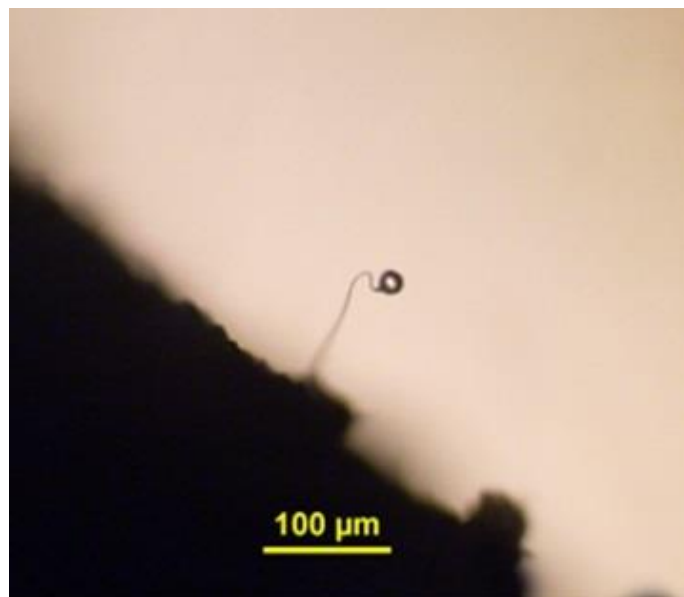


Figure 6. Microscopic images from the *Protosporangium bisporum*.

Comments: According to Olive (1975), this specie thrives on tree bark however the isolation and cultivation of it, is very difficult. The *Protosporangium bisporum* is one of the rare species. The stalk is very long, thin and flexible. The stalk has a curvy shape with sharp punctate angles that form at certain intervals. The taxon is the only type that is characterized by these properties. The spores are round and slightly elipsoidal (Spiegel *et al.* 2007).

Family 2: Echinosteliopsidaceae

Species: *Echinosteliopsis oligospora* Reinhardt & L.S. Olive, Mycologia 58(6):967 (1967)

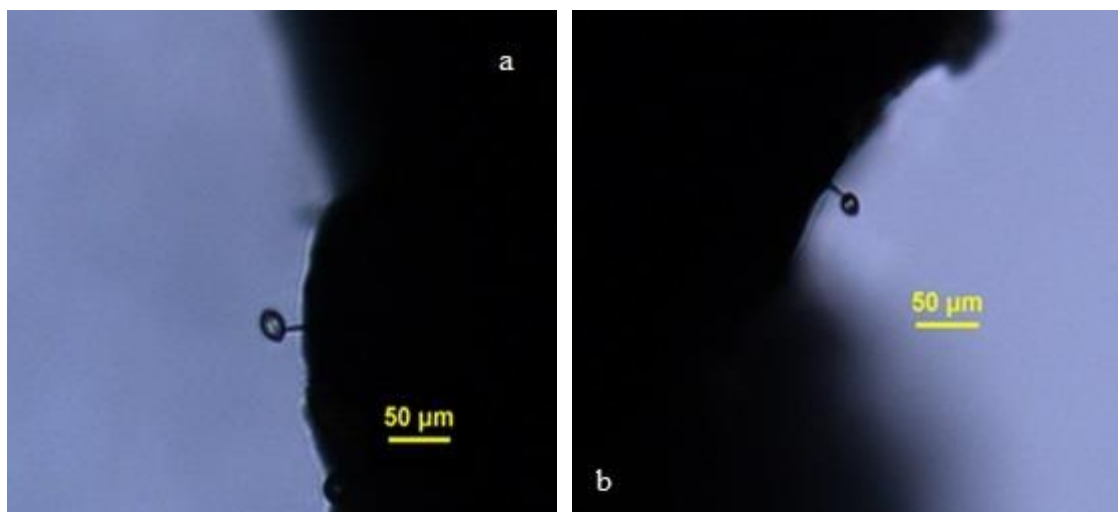


Figure 7 (a, b). Microscopic images from the *Echinosteliopsis oligospora*.

Comments: This species is very widespread on the surface of the Earth, it can be found on the primer tissue of live plants and on fallen materials. In statment of Moore and Spiegel (2000 a), “for the relative abundance of each species were calculated entire study, *E. oligospora* were uncommon and comprised 2–5% of

the total colonies relative abundance with, *Schizoplasmodiopsis vulgaris* and *Tychosporium acutostipes* in northwestern Arkansas”. Again, one noted that in the same study, *E. oligospora* were indicated more abundant in forests. It is characterized by its short stalks. At first glance, we can see the spore part enclosed with a hydrate cover. This cover allows the spore diameter seem larger than the stalk’s length. The spore range between 1 – 8 but mostly 4 in a single sporangium which is enclosed by this hydrate cover. The sporangium is usually globose in different dimensions or can be seen compressed from the sides (Spiegel *et al.* 2007).

Family 3: Protosteliaceae

Species: *Nematostelium ovatum* (L.S. Olive & Stoian.) L.S. Olive & Stoian., in Olive, Bot. Rev. (Lancaster) 36(1):68 (1970)

Synonyms: *Schizoplasmodium ovatum* L.S. Olive & Stoian., J. Protozool. 13:164 (1966)

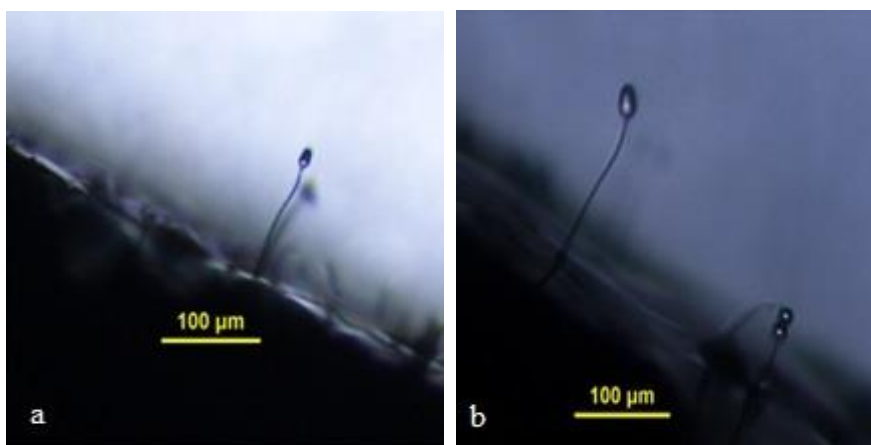


Figure 8 (a, b). Microscopic images from *Nematostelium ovatum*.

Comments: This species can be found widespread in mild temperature regions. Moore and Spiegel (2000 a) suggested on the study of protostelid communities from the leaf litter and aerial microhabitats of forests and grasslands in northwestern Arkansas that was characterized a more abundant species. Moore and Stephenson (2003) stated, *N. ovatum* as highly abundant in temperate habitats but rare and low abundance in the tropics. It is the most easily identified species among the Protosteliomycetes. Stalk is long, durable and distinct. It has a button shaped apophysis. The spore is single, deciduous, oval or elipsoid shaped (Spiegel *et al.* 2007).

Species: *Protostelium mycophagum* L.S. Olive & Stoian., Bull. Torrey Bot. Club 87(1):12 (1960)

Synonyms: *Protostelium mycophagum* var. *major* L.S. Olive, Amer. J. Bot. 49(3):299 (1962) *Protostelium mycophagum* var. *crassipes* L.S. Olive & Stoian., Amer. J. Bot. 56(9):98

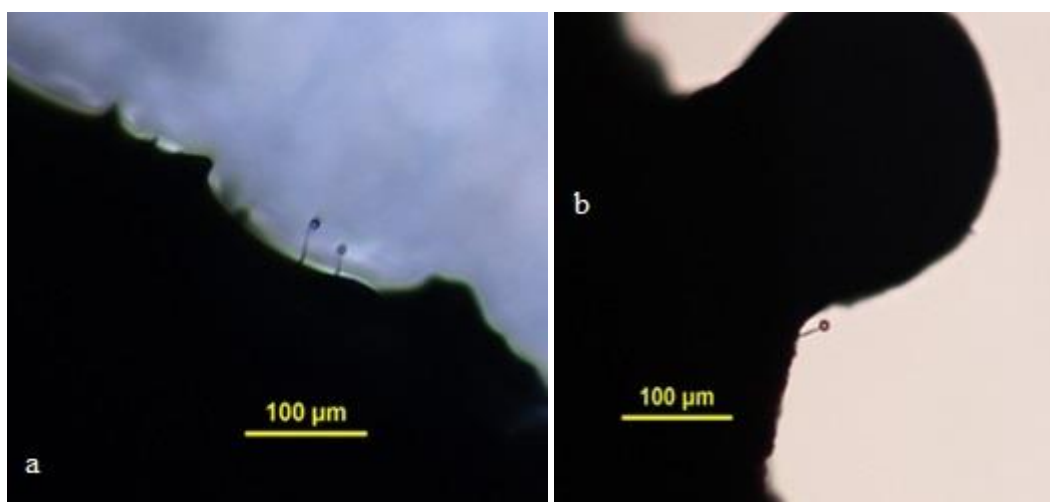


Figure 9 (a, b). Microscopic images from the *Protostelium mycophagum*.

Comments: Protosteliomycet is the most prevalent type in the World. Prefers the aerial microhabitat over the litter microhabitat but are more abundant in grassland aerial microhabitats (Moore and Spiegel 2000 a). Moore and Stephenson (2003) on stated *P. mycophagum*, are highly abundant in temperate habitats but rare and low abundance in the tropics. It was listed along with *Soliformovum irregulare* as the most recorded type in a study done by Shadwick and Stephenson (2004) on the Himalayas. In another study, done by Kosheleva (2009) in Siberia, it was listed as the most widespread along with *S. irregulare* on rotten plant particles, tree bark and fallen materials. Ndiritu *et al.* (2009) have listed the *Soliformovum irregulare* and *P. mycophagum* as the most widespread in Kenya – Aberdare. Powers and Stephenson (2006) have identified *P. mycophagum* as the most widespread type in dry coppice forest. The taxon of protosteliomycet can be found on every type of substrates however they are abundant on live plants primer ifarct. Sporocarps can be found separated or in groups in their isolation petri dishes. Their long and flexible stalks carry 1 small apophysis and 1 spore which is round and deciduous (Spiegel *et al.* 2007).

Species: *Schizoplasmodiopsis amoeboides* L.S. Olive & K.D. Whitney, Mycologia 74(4):655 (1982)



Figure 10 (a, b). Microscopic images from the *Schizoplasmodiopsis amoeboides*.

Comments: This species shows up single or in groups in the isolation petri dishes. It is a widespread species with wide substrate preference. According to Aquilar *et al.* 2011, on the study of deciduous forests from

southwestern Europe, *S. amoeboidea* that was indicated as a second most common species was represented with 22 identifications, 13% of the total number of occurrences. The stalk short and bigger than the 1,5 diameter spor. It is characterized by the way the stalk narrows abruptly towards the tip. The singular spores are round and non-deciduous. Commonly mistaken with *S. pseudoendospora*. However *S. amoeboidea*'s stalk has a narrowing on the apex part while the *S. pseudoendospora*'s sporocarp does not portray a narrowing (Spiegel *et al.* 2007).

Species: *Schizoplasmodiopsis pseudoendospora* L.S. Olive, M. Martin & Stoian., in Olive, Mycologia 59(1):19 (1967)

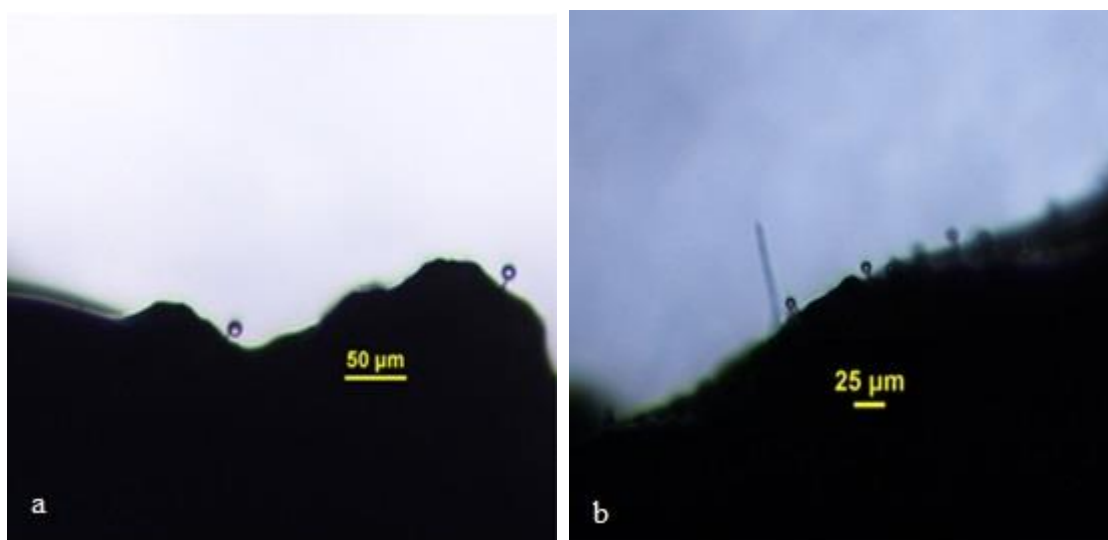


Figure 11 (a, b). Microscopic images from the *Schizoplasmodiopsis pseudoendospora*.

Comments: Even though this species is one of the smallest type of protostelids, it is one of the most widespread. *S. pseudoendospora* is a litter species that prefers the forest litter microhabitat over the grassland litter microhabitat and also typical of temperate litter was the overwhelming dominant of the forest floor litter (Glustchenko *et al.* 2002, Moore and Spiegel 1999, 2000 a, 2003). The sporocarps that develop from a single plasmodium have very short stems and are slightly larger than the spor diameter. In some cases the stalk disappears suddenly and do not narrow. The spores are round and non-deciduous (Spiegel *et al.* 2007).

Species: *Schizoplasmodiopsis vulgaris* L.S. Olive & Stoian., Mycologia 67(6):1092 (1975) ["vulgare"]

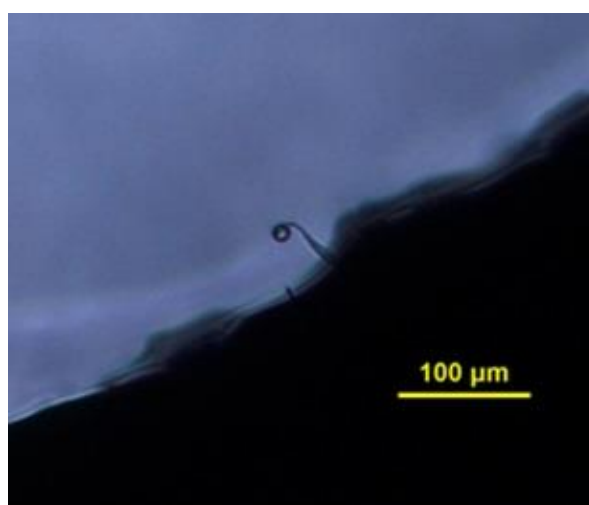


Figure 12. Microscopic images from the *Schizoplasmodiopsis vulgaris*.

Comments: This species can be found in almost every region on earth, lives on the rotting primer tissue of live plants and their fallen materials. Moore and Spiegel (2000 a) stated that the *S. vulgaris* was uncommon and comprised 2–5% of the total colonies with *Echinosteliopsis oligospora*, and *Tychosporium acutostipes*. It is the only kind that exists in cool and humid habitats. It has a long stalk which is thick all the way to its tip. The stalk does get thinner towards the end however it never ends with a sharp edge. Even though the stalk shows changes, it is still has the thickest stalk which makes this specie easy to identify. Single and non-deciduous spores have webby protruding spor walls. The spores are generally round and similar sized (Spiegel *et al.* 2007).

Species: *Soliformovum irregulare* (L.S. Olive & Stoian.) Spiegel, in Spiegel, Gecks & Feldman, J. Eukaryotic Microbiol. 41(5):518 (1994) ["irregularis"]

Synonyms: *Protostelium irregulare* L.S. Olive & Stoian., Amer. J. Bot. 56(9):983 (1969) ["irregularis"]

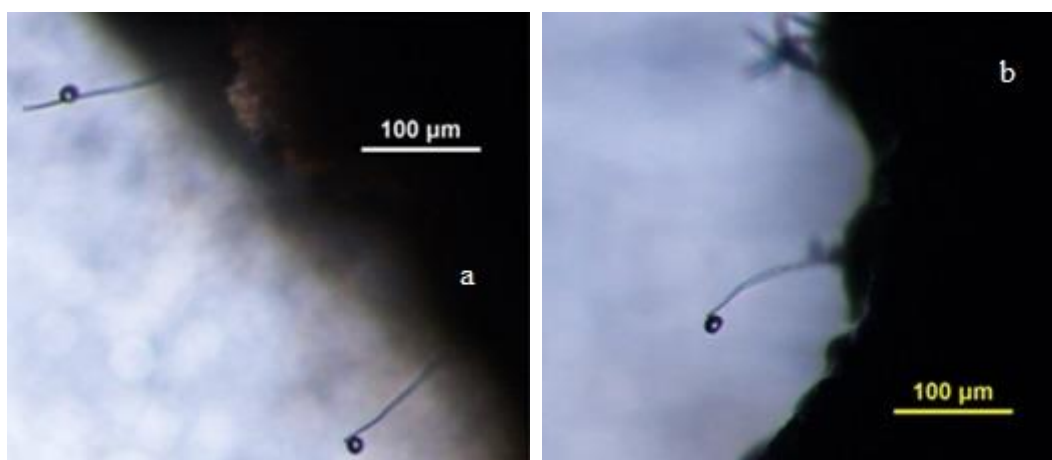


Figure 13 (a, b). Microscopic images from the *Soliformovum irregulare*.

Comments: These species are the most widespread in mild climate regions. They are especially found on the primer dead plant tissues. *S. irregulare* prefers the aerial microhabitat over the litter microhabitat but are more abundant in grassland aerial microhabitats (Moore and Spiegel 2000 a). It was listed along with *Protostelium mycophagum* as the most recorded type in a study done by Shadwick and Stephenson (2004) on the Himalayas. In another study, done by Kosheleva (2009) in Siberia, it was listed as the most widespread along with *S. irregulare*. Ndiritu *et al.* (2009) have listed the *S. irregulare* and *P. mycophagum* as the most widespread and conspicuous type in Kenya – Aberdare. They can easily be mistaken with other Protosteliomycet species, especially the *P. mycophagum*'s "fruiting bodies". They can easily be identified once the spear shaped apophysis and the spores on the tip of the stalk falls off. The stalk is straight or slightly curvy but it is not flexible. Supports only 1 spore which is round and deciduous. It was originally identified as the *Protostelium irregularis* (Spiegel *et al.* 2007)

DISCUSSION

This study has been carried out on protostelids in Turkey, on the Anatolian Peninsula that is dominated by Mediterranean transition climate condition. Aguilar *et al.* (2011) the state of Protosteloid amoebae communities have been investigated for the first time in the Mediterranean Basin. Also, the study includes the natural substrates collected from 13 localities, and a total of 1,504 colonies and 18 species were recorded from Espana. Partially, our study is related to Aguilar *et al.* (2011), *Cavostelium apophysatum*, *Echinosteliopsis oligospora*, *Echinostelium bisporum*, *Nematostelium ovatum*, *Protostelium mycophagum*, *Schizoplasmodiopsis amoeboidea*, *Schizoplasmodiopsis pseudoendospora*, *Schizoplasmodiopsis vulgaris* and *Soliformovum irregulare* are common

species in both studies. According to Spiegel (1990), studying more protostelid substrates in different regions will result in the identification of new types of this organism.

Turkey is a part of the three base phytogeographical regions (Mediterranean, Europe-Siberia, Iran-Turan), accomodating six ecoregions and 4 subregions that harbour a rich and diverse vegetation (Atalay 2008). Conducting protostelid researches on potential substrate and microhabitat diversities in Turkey which is home to distinctive biogeographical conditions in its' existing geography, will likely result in the increase of identified taxon. This study on the protostelids in Turkey will enable us to better elucidate the biogeographical distribution of protostelids both locally and globally.

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