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# INVESTIGATION OF MYXOMYCETES (MYXOMYCOTA) IN KIRIKHAN (HATAY PROVINCE)

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ABSTRACT. The present study was conducted with the samples collected in 10 different stations in Kırıkhan district and vicinity in 2012-2014. The samples were collected from tree barks, leaves, wood and other decayed plant material. The collected materials were used in Moist Chamber Culture to develop myxomycetes sporophores. Furthermore, myxomycetes were collected from their natural environment. In the field and laboratory studies, 45 taxa in 10 families and 22 genera were identified.

## 1. INTRODUCTION

Myxomycetes, known as plasmodial slime molds, true slime molds, or Mycetozoa are multinuclear fungus-like organisms without a cell wall that produce one or more spores and sporophores. Based on the current classification system, they are a member of the Mycetozoa group in Protista regnum and commonly found in terrestrial ecosystems. In the vegetative stage, they have a plasmodium, transparent adhesive sheath, and a pile of acellular protoplasm [1,2]. The plasmodium is a membrane-bound single cell that contains multiple nuclei. The generative stage includes four types of myxomycete fruiting bodies. The most common type is the sporangium. Fruiting bodies are usually composed of 6 parts: hypothallus, stalk, columella, peridium, capillitium and spores. In certain fruiting bodies, a pseudo-columella or a pseudo-capillitium may be present. Not all of these components are present in all fruiting body types. The columella is observed as an extension of the stalk into the spore mass, although it may not resemble the stalk. In a sessile fruiting body, the columella may be an area on the inside surface of the peridium where it contacts the substrate or may appear as a dome-shaped structure. A pseudo-columella is a columella that is not attached to the stalk. Capillitial elements may be attached to the columella or pseudo-columella. The capillitium includes thread like elements within the spore mass of a fruiting body. Several myxomycetes species have a capillitium, either as a single connected network,

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or as several free elements called elaters. Capillitial elements may be smooth, sculptured or spiny or they may appear to include several interwoven strands. Spores range in size between about 5 and 15 micrometers. Nearly all are round and most are ornamented to a certain degree. In fact, entirely smooth spores does not exist. Spore ornamentation could be reticulate, echinate, verrucose, or asperulate (with fine warts). Spore shape and size are very important in identification. Spores could be classified as either dark (found in the Stemonitales and Physarales) or light to brightly colored (all of the other orders) [3].

Known Myxomycetes count is 1017 globally [4], in Turkey the same figure is still 284 [5-16]. Turkey is located in a moderate climate and thus it has a very rich flora diversity and the number of available Myxomycetes is expected to be higher.

## 2. MATERIAL AND METHOD

### 2.1. Geographical location and vegetation

On the east, Kırıkhan is bordered by Syria and Kumlu, on the west, by Belen, on the north, by Hassa, and on the south by Antakya and Kumlu (Figure 1). Kırıkhan is located between 36-37 degrees north latitude and 36-37 degrees east longitude and the surface area of the district is 687.73 km². The Amik plateau region that extends in the north - south direction is an important passage between the Amanos and Kürd mountains. The most important passages include Yalangoz and Incirli to Syria in the east west direction, Gedik - Belen pass and Atik plateau in the west. The most important mountain in the district is Amanos mountains, which is an extension of the Taurus mountains. It extends to the southern Hatay province. The section in the Amanos where Ceyhan River crosses the mountain range is called Gâvur Mountains until the Belen Passage. The section that extends between the Belen Pass and Antakya is known as Kızıldağ. Gölbaşı Lake is the only lake in the district and exploited for aquaculture and irrigation. The Amik plain, formed by the dried Amik Lake, forms a part of the district. The Delibekir Stream, which passes through the town center, and Karasu that crosses the Amik plain, are the major streams in the district [17].

Forests are destroyed by irregular cutting, fire and grazing. Forests are usually found in mountainous areas. Vegetation on mountain slopes includes *Pinus* sp, *Quercus* sp, *Maquis* and wild olive trees in forests. The forests occupy a surface area of 9521 hectares. The largest green area in the district center is Vali Ürgen Landscaping with 168 hectares. Reeds and various marsh plants grow in the plain marshlands. Agricultural crops are cultivated on the entire plain. Nerium oleander is a poisonous plant that grows naturally. Kırıkhan residents mainly conduct cotton and wheat cultivation. Furthermore, olives, rice, pods, vines are grown. In addition, watermelon, melon, sesame, onion, pepper, tomato, eggplant, pumpkin, cucumber, spinach, lettuce, radish, okra, and beans are grown. The fruits grown in the region include figs, grapes, pears, apricots, pomegranates, plums, oranges, lemons, and mandarins. Fruit orchards are usually located in villages on the mountain slopes [17].

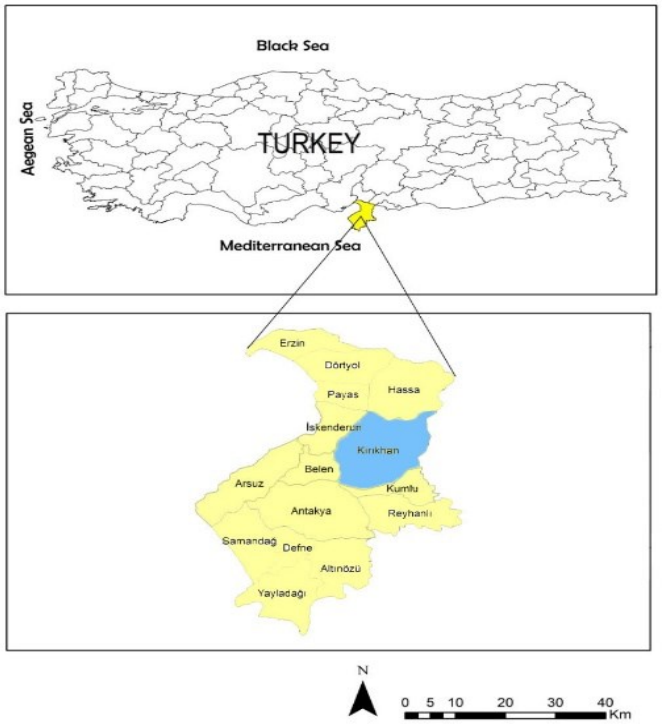


FIGURE 1. Research area

## 2.2. Climate of research area

The climate in the study area is Mediterranean. In the Mediterranean climate, daily and seasonal photoperiods are experienced, precipitation is usually during relatively cold seasons, and the dry season is summer. The climate is characterized by rains during the rainy season but the rainfall is sparse in other seasons. Most of the precipitation is in the form of a rain that runs on the soil and provides very little irrigation for the soil and plants. The average temperature is 7.31 degrees in winter and 32.3 degrees in summer [17].

## 2.3. Collection of samples

Samples were collected at 10 stations in Kırıkhan district and vicinity between 2012 and 2014, and the field trips were conducted in autumn, winter, spring and summer. Field study and sample collection locations and sample collection dates are presented in Table 1.

TABLE 1. Land trip dates and location information

| **Stations** | **Dates** | **Altitude(m)** | **Coordinates** |
|---|---|---|---|
| Alan way | 23.11.2013 | 1150 | 36° 36' 52" N; 36° 21' 27" E |
| Bektaşlı | 20.04.2013 | 450 | 36° 65' 48" N; 36° 40' 72" E |
| Ceylanlı | 02.02.2013/16.02.2014 | 280 | 36° 55' 92" N; 36° 38' 06" E |
| Delibekirli | 02.03.2013/16.03.2013 | 525 | 36° 53' 91" N; 36° 31' 45" E |
| Karataş | 15.12.2012/29.12.2012 | 300 | 36° 50' 15" N; 36° 33' 84" E |
| Kodallı | 30.03.2013/06.04.2014 | 95 | 36° 54' 36" N; 36° 40' 45" E |
| Kurtlusoğuksu | 18.11.2012/01.12.2012 | 85 | 36° 48' 66" N; 36° 29' 92" E |
| Saylak | 04.04.2013 | 250 | 36° 62' 37" N; 36° 41' 07" E |
| Taşoluk | 16.11.2013 | 450 | 36° 62' 66" N; 36° 38' 89" E |
| Telbizek | 05.01.2013/19.01.2013 | 260 | 36° 54' 29" N; 36° 36' 69" E |

Natural Myxomycete samples were collected from natural substrata, barks, woods, debris material. Samples were transferred to the laboratory in small carton boxes. Furthermore, of myxomycete fructifications were cultured in a moist chamber in the laboratory. All moist chamber cultures were prepared within a week after the field survey. Substrates were placed in petri dishes lined with filter paper. Distilled water was added to each petri dish and the samples were allowed to soak overnight. After 24 hours, excess water was removed. Cultures were maintained under diffuse light at room temperature (22-25°C) for approximately three months. All cultures were checked weekly for the presence of myxomycete plasmodia or fruiting bodies [7]. When myxomycete development was observed, the moist chamber was allowed to dry slowly and the myxomycetes were then dried further for one week. The samples were prepared as fungarium material and stored in the laboratory.

## 2.4. Identification of samples

Myxomycetes usually include 6 sections: hypothallus, stalk, columella, peridium, capillitium and spores. For the identification of the samples, a stereomicroscope and a high-resolution light microscope were used. Stereomicroscope examines the general structure, fructification

type, shape, color, macroscopic measurements, the presence or absence of lime or the color and shape of samples. With light microscopy, it is possible to observe whether the capillitium, pseudo-capillitium and columella or pseudo-columella were present, the shape, size and form of the capillitium, the branching form, whether the columella is free or attached to the stem, the characteristics of pseudo-capillitium, the shape, color, size and ornamentation of the spores in detail.

The Myxomycetes specimens were identified based on relevant references such as Martin and Alexopoulos [18], Farr [19], Thind [20], Farr [3], Martin et al. [21], Neubert et al. [22], Stephenson and Stempen [1], Alexopoulos et al. [23], Lado and Pando [24], Sesli et al. [12].

## 3. RESULTS AND DISCUSSION

Between 2012 and 2014, 377 samples were collected at 10 different stations in Kırıkhan and processed in the laboratory, revealing 208 myxomycetes. Identification of myxomycete samples collected in the natural environment and grown in moist chamber culture revealed a total of 45 species in 5 groups, 10 families, and 22 genera. Twenty-two samples were collected in the natural environment, 186 samples were obtained in the moist chamber culture, 11 samples were obtained in both the natural environment and the moist chamber culture. The water pH was measured before the sample water was discharged during the application of moist chamber technique and it was determined that the sample pH values were generally neutral. This finding was consistent with the findings reported by Härkönen and Uotila [25].

The relative abundance of fruit bodies of each species was determined by the categorization based on a modification of the method proposed by Stephenson et al. [26]. For this purpose, species represented in more than 3.0% of the collections were considered abundant (A), those that were represented between 1.5 % and 3.0 % were considered common (C), those that were represented between 1.5 % and 0.5 % were considered occasional (O), and those that were represented in less than 0.5 % were considered rare (R) [26]. The mean number of species per genus (S/G) was calculated based on the collected datasets in the study area. In the present study, it was determined that 9 species were abundant (A), 4 species were common (C), 7 species were occasional (O), and 25 species were rare (R) (Table 2). The mean number of species per genus (S/G) was calculated with the data collected in the study area and it was determined that the species/genus ratio (S/G) was 2.04. The Myxomycete biodiversity in Antakya was 3.64, and 2.3 in Kuseyr mountain [7]. This value was significant when compared to other study findings. For example, in a North American study, it was observed that S/G ratio in Mountain Lake was 3.65 and in Cheat Mountain, it was 2.24. Another study calculated S/G ratios in northwestern India and southern India as 3.04 and 4.13, respectively [26]. A low S/G reflects a higher overall diversity when compared to a high S/G rate.

TABLE 2. Myxomycetes name and occurrence

| No | Species | Occurrence | No | Species | Occurrence |
|---|---|---|---|---|---|
| 1 | *Echinostelium minutum* | O | 24 | *P. oblatum* | R |
| 2 | *Cribraria cancellata* | R | 25 | *P. robustum* | C |
| 3 | *C. intricata* | R | 26 | *Arcyria cinerea* | A |
| 4 | *Licea castanea* | R | 27 | *A. globosa* | O |
| 5 | *L. kleistobolus* | R | 28 | *A. incarnata* | O |
| 6 | *L. minima* | R | 29 | *A. minuta* | A |
| 7 | *L. pedicellata* | R | 30 | *A. pomiformis* | R |
| 8 | *Lycogala epidendrum* | R | 31 | *Perichaena vermicularis* | R |
| 9 | *Dictydiaethalium plumbeum* | R | 32 | *Trichia contorta* | R |
| 10 | *Diderma hemisphaericum* | R | 33 | *T. munda* | R |
| 11 | *Didymium bahiense* | A | 34 | *Collaria lurida* | R |
| 12 | *D. difforme* | C | 35 | *Comatricha ellae* | A |
| 13 | *D. megalosporum* | C | 36 | *C. laxa* | O |
| 14 | *D. melanospermum* | R | 37 | *C. nigra* | A |
| 15 | *D. squamulosum* | A | 38 | *C. pulchella* | R |
| 16 | *Badhamia macrocarpa* | R | 39 | *Enerthenema papillatum* | R |
| 17 | *B. panicea* | O | 40 | *Lamproderma arcyrioides* | R |
| 18 | *Craterium leucocephalum* | R | 41 | *Paradiacheopsis longipes* | R |
| 19 | *Leocarpus fragilis* | R | 42 | *Stemonitis fusca* | A |
| 20 | *Physarum album* | A | 43 | *Stemonitopsis amoena* | A |
| 21 | *P. contextum* | O | 44 | *S. typhina* | C |
| 22 | *P. leucopheum* | O | 45 | *Symphytocarpus* | R |
| 23 | *P. notabile* | R | | *sp.* | |

In the literature, myxomycete samples were mostly observed on decayed Gymnosperm woods, leaves and debris [1,3,11,23], and the number of field studies on myxomycetes have increased. However, myxomycete spores were collected and processed on different material that might have been infected. Liceales, Trichiales and Stemonitales are generally known to be present in coniferous forests [18, 27,28]. The majority of the specimens were identified on Gymnosperm rashes.

If myxomycete distribution is based on the substrate on which it was developed, corticolous myxomycetes develop on plant barks, lignicolous myxomycetes develop on rotten wood and barks, foliiaceous myxomycetes develop on leaves, fimicolous myxomycetes develop on animal manure, nivicolous myxomycetes are those with special needs for development [29]. In our study area, samples were collected only from bark and rotten wood.

Natural samples increased in winter in our study area, however myxomycetes species obtained with the moist chamber technique increased in the autumn. The best months for finding Plasmodial slime molds in our research area is winter and spring. Due to rain, relative humidity is apparently at an optimum level and temperatures are mild. Primary characteristics of these months in our research area include alternate rainy and sunny periods. These provide favorable conditions for adequate moisture levels and suitable temperatures that allow

Plasmodial slime molds complete their life cycle. The present study findings were consistent with previous studies conducted in Hatay, Turkey and other locations based on the seasonal distribution of myxomycetes.

The distribution of the species determined in our study area demonstrated that there were four families (*Stemonitidaceae*, *Physaraceae*, *Didymiaceae* and *Arcyriaceae*) that included 35 species. This figure constituted 76% of the species collected in our study area. This rate was determined as % 71 by Yağız [30], as 72.4% by Baba [31], as 70% by Baba et al., [11], and these findings were similar to our and several other studies conducted in Turkey.

The genera and species determined in our study area included 6 *Physarum*, 5 *Arcyria*, 5 *Didymium*, 4 *Comatricha*, 4 *Licea*, 2 *Badhamia*, 2 *Cribraria*, 2 *Stemonitopsis*, 2 *Trichia*, 1 *Collaria*, 1 *Craterium*, 1 *Dictydiaethalium*, 1 *Diderma*, 1 *Echinostelium*, 1 *Enerthenema*, 1 *Lamproderma*, 1 *Leocarpus*, 1 *Lycogala*, 1 *Paradiacheopsis*, 1 *Perichaena*, 1 *Stemonitis*, and 1 *Symphytocarpus* species.

Analysis of the distribution of myxomycete species demonstrated that *E. minutum, A. cinerea, A. denudata* and *S. fusca* were the most common species in the present study. Most myxomycetes species were cosmopolitan, the humidity and temperature were the main factors in diversity and abundance of these species. In most studies, it was observed that these species were commonly distributed across several substrates [1]. *A. pomiformis, A. cinerea, C. ellae* and *C. nigra* were identified in almost all stations. *Stemonitopsis amoena* is detected in 6 out of 10 stations. Most myxomycetes species grow globally and, in most studies, these species were observed to be prevalent in several substrates [30, 32-34].

Altitude is an important factor for various myxomycete families. According to Rojas and Stephenson [35], as the altitude increases, the type and number of myxomycetes significantly decrease. In high altitudes, substrate pH decerases. In the present study area, *Liceaceae* was most prevalent near sea level. Physaraceae prevalence exhibited a rapid increase above 750 m. Stemonitaceae were prevalent at almost every altitude. The highest Didymiaceae prevalence was observed at low altitudes. It was not possible to identify Arcyriaceae at low altitudes. It grows at altitudes between 20 and 750 m. Thus, it was found that Didymiaceae and Liceaceae were more prevalent at low altitudes and their prevalence decreased as altitude increased. Arcyriaceae was identified mostly in mid altitudes. Stemonitaceae could adapt to all altitudes except the coastline. However, although Physaraceae were observed at all altitudes, it could be suggested that the highest prevalence was observed at high altitudes. The sizes of *A. cinerea, P. corticalis, P. album* and *S. amoena* change at high altitudes. Furthermore, all studied species were highly prevalent at an altitude of 100-400 m. In contrast, certain Mycetozoa species prefer certain altitudes or are only present at these altitudes. *Didymium difforme* was prevalent between 50-100 m, *L. castanea* was prevalent between 20-300 m [36].

In the present study, most prevalent ornamented spore was verruculose or verrucose, followed by spinulose. The least common types of ornamentation were smooth, reticulate and echinulate. It was suggested that the net and thorn type ornaments should be more prevalent in wider surfaces, improving their attachment to the surface. Since flying ornamented spores attach to the surface better, this improves their susceptibility to germination. Comparison of the study samples based on sporophore types demonstrated that fructification types of the collected species were generally sporangium, 61% of the species were sporangium with stem. Pseudoaethalium, aethalium, and plasmodiocarpic fructification rates were almost identical.

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## 4. CONCLUSION

Middle Amanos mountains are located on one side of the Kırıkhan district and Amik plain on the other, and the district has cosmopolitan geographical, climatic and surface shapes, rich biodiversity and rich myxobiota. In the study area, climatic conditions and vegetation are suitable for myxomycetes, as demonstrated in the present study findings, and the present study added a total of 45 species to the regional and Turkish myxobiota.

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## NOMENCLATURE

Current myxomycetes names were checked from nomen eumycetozoa
http://eumycetozoa.com