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Notes on a rapid assessment of myxomycetes for Kabylie, Algeria

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Studies of myxomycetes in some regions of the world are limited. Northern Africa is one prominent example. For this reason, a rapid assessment of myxomycete diversity in four study areas within the Kabylie region of Algeria was carried out in July 2008. Fourteen different species were identified from specimens obtained in moist chamber cultures prepared with samples of dead plant material. Six of the species are taxa not previously reported for Algeria. Our preliminary data suggest that myxomycete assemblages in the region may show the influence of topographic and microclimatic factors.

Keywords: **distribution, Mediterranean, myxogastrids, northern Africa**

The myxomycetes (plasmodial slime molds or myxogastrids) are a group of amoeboid protists (Pawlowski & Burki 2009) found in practically all terrestrial ecosystems (Stephenson 2003). One of the primary characteristics of the group is the formation of a structure known as plasmodium, which feeds upon other microorganisms, at one point during their life cycle. The plasmodium gives rise fruiting bodies that contain spores, which have the potential for long distance dispersal (Martin & Alexopoulos 1969, Stephenson *et al.* 2007).

Results obtained in recent studies of the distribution and occurrence of myxomycetes have indicated that some species of myxomycetes appear to be globally distributed in a manner that apparently reflects the distribution of suitable habitats (Stephenson *et al.* 2007). This observation of biogeographical patterns for particular species of myxomycetes seems to support the so-called moderate endemicity model of distribution (see Foissner 2006). In this scenario, it would be possible for different regions of the world to be characterized by their own distinctive assemblages of species. However, the lack of information on myxomycete distribution for most ecosystems in the world makes the evaluation of such patterns problematic.

Global studies of this group of organisms clearly show the influence of historical patterns of human activity. As such, two of the best

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studied continents in the world are Europe and North America. Interestingly, the extent to which myxomycetes have been studied in Africa and South America, located directly south of Europe in one instance and North America in the other is rather limited (Lado & Wrigley de Basanta 2008, Ndiritu *et al.* 2009). This is especially true for Africa. For instance, Lado (1994) listed 81 publications relating to myxomycetes in the Mediterranean countries, whereas Ndiritu *et al.* (2009) included only 70 publications on myxomycetes for the entire African continent. Although these two recent reviews may not be totally comprehensive, they do reflect the level of study of myxomycetes in these two regions of the world.

In spite of the geographical proximity and common history of northern African territories and the European continent, myxomycete studies in former are scarce. For instance, the last published report on myxomycetes from Algeria apparently dates back to 1964 (Faurel *et al.* 1964), just after the county declared its independence from France. Although some specimens of myxomycetes collected by Jan Rammeloo in the early 1980's are deposited in the National Botanic Garden of Belgium (de Haan & Bogaerts 2009), no formal publication reporting the records represented by these specimens is available. This situation may reflect the limitations of being able to carry out studies in Algeria, which may be a partial product of the social-political constraints that have existed in the modern history of the country.

The Kabylie region of Algeria is located in the north central part of the country, which is within the Mediterranean basin (Peel *et al.* 2007). The presence of low- to mid-elevation mountains close to the coast provides this region with the environmental conditions necessary for the development of some areas of forests (Dahmani-Megrerouche 2002). In fact, the relatively high numbers of endemic and rare organisms found in this region seem to have been overlooked in the past (Vela & Benhouhou 2007). As would be expected from the limited amount of information available on the myxomycetes of Algeria, the assemblages of these organisms present in the Kabylie region have not been extensively studied.

In this context, the project described herein was carried out with the main objective of generating some baseline information on the myxomycete biota of the Kabylie region of Algeria. It is important to have such information if we are to develop a more complete understanding of the global biogeographical patterns of myxomycetes. Moreover, the data obtained in the project also represents a small contribution to our knowledge of African myxomycetes.

Materials and Methods

The present study was not intended to be an exhaustive survey of myxomycetes. Instead, it represents a rather limited contribution to

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the study of these organisms. However, due the exceedingly limited data available on the myxomycetes of the Kabylie region and the inherent difficult of carrying out research in this part of the world, we have decided to make the results we obtained available. For this study, the morphological concept of species was used, and species names follow the nomenclatural treatment provided by Lado (2005–2010).

Study areas

Four study areas in Algeria in northern Africa that represent an elevational, topographical gradient were selected for the present study which was carried out during 2008 (Fig. 1). All of these are located in the Wilaya (province) of Tizi Ouzou (also spelled Tizi Wezzu) in the heart of the Kabylie region, approximately 150 km east from the capital of Algiers. According to the Köppen classification of climate zones, these areas fall within the dry-summer subtropical category (Peel *et al.* 2007), often referred to as a "Mediterranean Climate".

The first study area selected (A) was in the general vicinity of the commune (town) of Tigzirt (also known as Tigzirt sur mer, coordinates: 36°53'39.48" N, 4° 7'31.44" E, elevation 10 m above sea level [a.s.l.], coastal habitat, date sampled: 15 July 2008). The second study area (B) was in and around the commune of El Kalâa (also spelled El Kalâa, coordinates: 36°50'51.72"N, 4° 7'35.26"E, elevation 445 m a.s.l., premontane habitat, date sampled: 15 July 2008). This study area is located approximately 5 km south of Tigzirt (see insert in Fig. 1). The remaining two study areas are located in the vicinity of the commune of Makouda (also spelled Makuda) and are located on the slopes of the Kabylie Mountains. These two areas represent the highest points examined in the present survey and correspond to (C) Makouda North (coordinates: 36°49'58.69"N, 4° 5'5.17"E, 685 m a.s.l., premontane habitat, date sampled: 16 July 2008) and (D) Makouda South (coordinates: 36°49'14.12"N, 4° 4'30.00"E, 676 m a.s.l., premontane habitat, date sampled: 16 July 2008).

Sampling methods and collecting specimens

The information generated in the present study is the product of one survey to the Kabylie region of Algeria carried out in the summer of 2008. In all four study areas, a series of four samples of ground litter (GL), aerial litter (AL) and woody twigs (TW) was collected, together representing a total of 48 substrate samples. Bark was not considered in this study due to the lack of woody plants in some of the sampled areas. The samples were taken back to the laboratory at the University of Arkansas and used to prepare a series of two moist chamber cultures per sample in the manner described by Stephenson & Stempen (1994). Following this method, the material for examination was placed in Petri dishes previously lined with filter paper and soaked in pH-neu-

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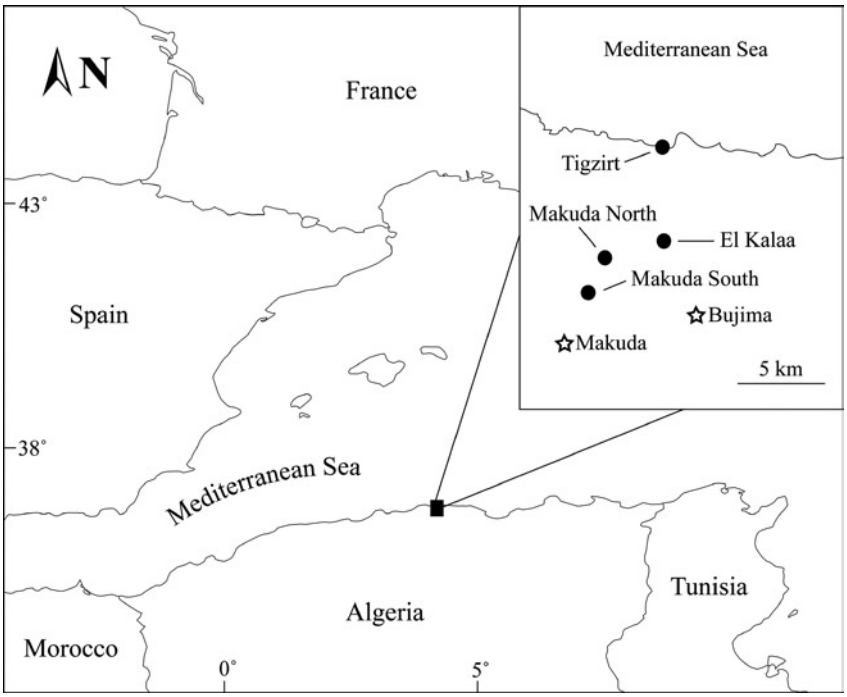

Fig. 1. – Geographical location of the general region of study and the actual study areas (as black circles in the insert) considered in the present study. Stars represent communes (towns).

tral water for a period of 24 h. After this time, the pH value of the substrate was recorded, excess water was poured off the plate, and a period of examination of 10 weeks began. During the examination period, extra water was added to the cultures when needed in order to maintain a moist environment.

When fruiting bodies were detected, these were carefully extracted from the moist chambers and glued to paper strips previously placed in small pasteboard boxes. These collection boxes were left at room temperature for drying for approximately two days, after which the process of identification was carried out. All specimens curated in this manner were deposited in the mycological herbarium of the University of Arkansas (UARK) for future reference.

Data Analysis

A number of basic parameters were calculated for each of the study areas. Among these were the number of species, the number of positive moist chambers and the range of pH values. In addition, specific analyses were also carried out to evaluate several aspects of possible ecological importance. For example, pH values determined for

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the various substrates placed in the moist chamber cultures and their possible relationship with the number of species per study area was evaluated by performing a simple correlation analysis, followed by an analysis of variance to test the randomness of the correlation. For these analyses the term F corresponds to the F value in an analysis of variance, P to the probability value and R^2 to the Pearson's product moment. When differences were found, a *post-hoc* Tukey test was performed to evaluate the directionality of the relationship. Also, the number of records found per substrate type was evaluated with a Pearson's Chi-square test. In every instance, null hypotheses were evaluated with a rejection probability of 0.05 by using the statistical package JMP, version 8.0.

The effectivity of the survey was assessed by calculating the maximum number of species that might be expected to be found according to the sampling parameters of the project. For this, both the Chao 1 and the Abundance-base Coverage Estimator (ACE) were calculated using the program SPADE (Chao & Shen 2003). Also, a species accumulation curve was generated for each study area using the Chao 2 estimator values as recommended by Unterseher *et al.* (2008). For this part, calculations were carried out using the program EstimateS, version 8.2.0 (Colwell 2006). The obtained curves were adjusted according to the formula $y = ax/(b + x)$ as suggested by Raaijmakers (1987).

In order to compare the distribution of species among study areas, values for the Morisita Similarity Index were calculated for all combinations of study areas and for all assemblages pooled together. These values were obtained by performing a beta-diversity analysis based on 200 runs in SPADE.

Results

A total of 60 records of specimens of myxomycetes representing 15 identified species were recovered from the samples collected in the project. All the specimens that could not be identified to species belong to the genus *Physarum*. Among the species recorded, *Badhamia affinis*, *Comatricha tenerrima*, *Didymium anellus*, *D. minus*, *Echinostelium minutum, Licea rugosa* var. *fujiokana* and *Physarum echinosporum* are taxa not previously reported for Algeria. All study areas except Tigzirt showed a high number of positive moist chambers, and the study area with the highest number of species was Makouda North. The two most abundant species were *Didymium difforme* and *E. minutum* (Tab. 1).

The analysis of pH values indicated that there were differences among the study areas (F [3.53] = 40.18, P = 0.0001, R^2 = 0.68) that seem to be related to the higher values associated with the two study areas located at the highest elevations (Tukey test, P = 0.0001 in both cases). However, differences were not observed when pH values and species richness were evaluated together (F (1,3) = 9.47, P = 0.09, R^2 = 0.66).

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Tab. 1. – Summary of the species recovered and the various parameters determined or evaluated in the present study. Data are arranged by study area. For details see Materials and methods.

Parameters	Study Area			
	Tigzirt	El Kalaa	Makouda South	Makouda North
Species				
Arcyria cinerea				1
Badhamia affinis	2	1		
Comatricha tenerrima		1		
Didymium anellus		1	5	
Didymium difforme	4	8	1	2
Didymium iridis	2			
Didymium minus				1
Didymium squamulosum		3	1	3
Diachea leucopodia				1
Echinostelium minutum		1	6	3
Licea rugosa var. fujiokana		1		
Perichaena depressa		2	1	1
Physarum cinereum				1
Physarum echinosporum			1	
Physarum sp.	3		1	
Stemonitis fusca				2
Survey characteristics				
Elevation (m)	10	445	676	685
Number of samples	12	12	12	12
Number of moist chambers	24	24	24	24
Positive moist chambers (%)	50	91	91	100
Range of pH values	6.6–7.9	6.2–7.7	4.8–6.7	5.6–7.0
Mean pH value	7.16	7.08	6.29	5.68
Evaluation of survey effectivity				
Chao1 estimator	4.0	13.0	17.0	12.3
ACE estimator	4.0	20.2	18.3	13.7
Observed species richness	4	8	7	9
Effectivity range (%)	100	39–61	38–41	65–73

Tab. 2. – Number of records of myxomycetes arranged by substrate (top) and values calculated in the analysis of study areas (bottom), including the Morisita Similarity Index (above right) and the number of species shared (below left) between study areas.

Parameter	Study area				Total
	Tigzirt	El Kalâa	Makouda South	Makouda North	
Substrates					
Aerial litter	0	5	10	5	20
Ground litter	2	8	2	4	16
Twigs	9	5	4	6	24
Study areas					
Tigzirt	1.00	0.84	0.40	0.78	
El Kalâa	2	1.00	0.20	0.35	
Makouda South	5	2	1.00	0.70	
Makouda North	4	1	4	1.00	

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Similarly, when substrates were examined, no differences were found in the number of records associated with each different type (Tab. 2, Chi-square (3) = 1.6, P =0.65, N = 60), even though twigs were the most productive.

The calculations for the effectivity of the survey indicate that moderate to high values were obtained for all study areas except for Makouda South. A similar pattern is apparent in the rarefaction-based species accumulation curves obtained using the Chao 2 estimator (Fig. 2). For this estimator, the only curve that does not appear to flatten out is the one for this study area.

The Morisita Similarity Index values calculated among study areas (Tab. 2) are moderate to high in all cases. Similarly, the value obtained for the comparison of species present among all the study areas is 0.53. The two areas with the most shared species were Makouda South and Tigzirt, whereas the pairwise combination of areas with the lowest values of species in common was Makouda North-El Kalâa. Basic information relating to all collections is provided in Tab. 3.

Tab. 3. – Species recovered and specimens studied with basic information relating to all collections. For abbreviations of substrates see Materials and Methods.

Species name	Specimens studied a	Study area	Substrate
Arcyria cinerea	2916	MN	TW
Badhamia affinis	3037, 3039, 3046, 3059	EK, TZ	AL, TW
Comatricha tenerrima	2899	EK	TW
Didymium anellus	3043, 3044, 3049, 3051, 3054, 3055	MS, EK	AL, GL, TW
Didymium difforme	2912, 2917, 2924, 2927, 2909, 2921, 2926, 2908, 2903, 2910, 2914, 2897, 2911, 2998, 2953, 2905, 2986, 2906	MN, MS, EK, TZ	AL, GL, TW
Didymium iridis	2922, 2925	TZ	TW
Didymium minus	3041	MN	AL
Didymium squamulosum	2913, 3045, 2919, 2923, 2893, 2900, 2915, 2918	MN, MS, EK, TZ	AL, GL, TW
Diachea leucopodia	2949	MN	AL
Echinostelium minutum	3038, *	MN, MS, EK, TZ	AL, GL, TW
Licea rugosa var. fujiokana	2901	EK	TW
Perichaena depressa	2907, 2902, 2920	MN, MS, EK, TZ	AL, GL, TW
Physarum cinereum	3040	MN	AL
Physarum echinosporum	2928	MS	AL
Physarum sp.	3056	MS, TZ	AL, TW
Stemonitis fusca	2895, 3042, 2894	MN	TW

Study area abbreviations: Makouda North = MN, Makouda South = MS, Tigzirt = TZ, El Kalâa = EK

[a] The numbers correspond to the first author's collecting number

[*] A large number of records from Echinostelium minutum were observed. However, most records were not collected.

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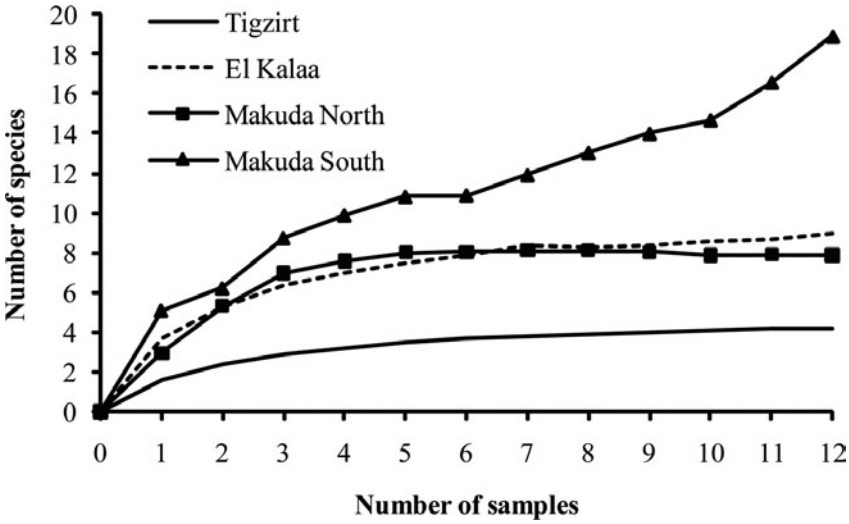

Fig. 2. – Species accumulation curves calculated for the data sets from the four study areas using the Chao 2 estimator values.

Discussion

It is obvious that a rapid biodiversity assessment of the type carried out in the present study does not reflect the actual myxomycete diversity present in the region investigated. The sampling effort was relatively limited, and it would require a much more intensive effort to generate a more robust dataset to evaluate such parameter. For that, consideration of higher numbers of samples, habitats and substrates would be necessary. However, due the limitations of carrying out research in this part of the world, the effort presented herein is not insignificant. The six new records of myxomycetes for the Algerian territory are evidence that this is the case.

These new records are not surprising, and they seem to be the product of previous undersampling in this region of the world. For instance, *Badhamia affinis*, *Comatricha tenerrima*, *Didymium anellus*, *D. minus* and *Echinostelium minutum* have been reported from a number of localities within the Mediterranean basin (see Lado 1994). The case of *Physarum echinosporum* is different, since this species has not been widely reported in the same region and seems to be more common in areas such as Central Africa (Ndiritu *et al.* 2009). This is probably not surprising, since the species is known to have a mostly tropical distribution (Martin & Alexopoulos 1969). However, it is interesting to note that this species also has been found in habitats where moisture is limited, occurring primarily on litter in North America (e. g. Bates & Barber 2008, Ndiritu *et al.* 2008). It is possible that in the region of study *P. echinosporum* occurs in moderately mesic habitats

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on particular substrates that represent a suitable environment, but testing this observation would require more data.

Interestingly, the high number of moist chamber cultures positive for myxomycetes reflects the widespread occurrence of the group in the region of study and certainly suggests the need for future studies. This is especially true when data from a particular study area is taken in consideration. Although pH values do not seem to have an effect on the species richness in the present study, it would be interesting to observe what patterns might exist as a result of putting an increased sampling effort in place. For example, in arid zones of Russia and Mexico, species of myxomycetes seem to display a strong response to pH (e.g. Novozhilov *et al.* 2006, Estrada-Torres *et al.* 2009), and the composition of particular assemblages appears to be determined largely by the pH values characteristic of plant species or particular substrates present in the area. In the present study, there were no readily apparent differences in the numbers of species associated with three substrates evaluated, but woody twigs yielded the highest number of specimens. In previous studies, it has been observed that this substrate is particularly productive for myxomycetes in non-tropical areas (Stephenson *et al.* 2008).

In any case, the present survey seems to have been adequate to obtain most of the species that might have been expected from the technique, effort and substrates used. Only the Makouda South study area seems to show that the effort was not sufficient. This is interesting, since this was the only area that had *Eucalyptus* sp., a non-native tree, as one of the dominant components of the forest. It is remarkable to observe that *P. echinosporum*, one of the new records for Algeria, was recovered in this area. However, it is interesting to note that this study area is also the only one on the slope of the mountain range facing south towards the continent, whereas the other three are located on the slope facing the Mediterranean Sea.

It is possible that topography and microclimates are playing a role in shaping the myxomycete assemblages as well. This has been observed in other parts of the world (see forest type discussion in Rojas *et al.* 2009). The highest values for the Morisita Index of Similarity were obtained for the within-slope combinations Tigzirt-El Kalâa and Tigzirt-Makouda North, whereas the lowest value for the across-slope combination of El Kalâa and Makuda South. Interestingly, the value obtained for the four study areas considered together indicates that their overall similarity is moderate. However, the limited dataset presented herein does not provide enough information to support such a conclusion.

The importance of this rather limited study relates to the fact that our knowledge of the distribution and ecology of microorganisms, and not just myxomycetes, is so very poor for the northern section of the African continent. In spite of the limitations of the survey described

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herein, the data obtained still represents a noteworthy contribution to the body of information available for a group of microorganisms on a continent that is woefully understudied.

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