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A study case of two myxomycete surveys in a fir forest of central Mexico

Berlia Beneric Salazar-Hernández[1], Randall Valverde[2] and Carlos Rojas[2,3]

[1] Facultad de Biología, Universidad Veracruzana, Xalapa, Veracruz, Mexico
[2] Engineering Research Institute, University of Costa Rica, San Pedro de Montes de Oca, 11501-Costa Rica
[3] Department of Biosystems Engineering, University of Costa Rica, San Pedro de Montes de Oca, 11501-Costa Rica

E-mail: bbshbio@hotmail.com

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Abstract: Field collections and laboratory isolation in moist chamber cultures are two complementary techniques widely used to record sporocarps of myxomycetes. The species recorded using either method tend to be different due to the distinct ecological pressures taking place in natural and artificial systems. The present study summarized the results of two myxomycete surveys, carried out in different decades and by different research teams, in the *Abies* forests of Cofre de Perote National Park. In both studies the two sampling techniques were used, and recorded data showed the complementarity of the methods and the importance of including both of them to minimize non-planned variability. In one survey, most of the results were obtained in moist chamber cultures whereas field collections represented most of the data in the other survey. When the general dataset was pooled together, 75 species of myxomycetes were recorded. As expected, the survey where most of the results were associated with moist chamber data showed a higher proportion of species of the genera *Didymium*, *Perichaena* and *Physarum*; whereas the survey where field collections represented most of the results showed a higher proportion of species within the genera *Arcyria*, *Cribraria* and *Trichia*. No structural differences were found in the data between the two surveys. This study demonstrates the complementary nature of the two recording techniques on myxomycete sporocarps and it shows very prominently the advantages of collaboration and communication among research teams to generate local lists of species. Having two different teams working in the same area at different times also minimized taxonomic skewness and increased the representativity of the obtained data.

Keywords: Cofre de Perote National Park, fir forests, high elevation, mountains, slime molds

Introduction

It is not common in myxomycete research, unless planned that way, that different authors visit the exact same location with two different sampling strategies. It is perhaps even more rare, that such events take place in different decades. During the period of 2006-2008, Rojas et al. (2011) carried out a study on myxomycetes in different parts of the northern Neotropics and established one sampling location in the fir forests of the Cofre de Perote National Park in central Mexico. One decade later, the first author of the present study visited the same fir forests and carried out a different survey on myxomycetes. Even though

the objectives of both studies were different and sampling methodologies were designed with those objectives in mind, these two surveys in the same area were largely complementary.

In both cases, field collections and the moist chamber technique were used to create the species lists, as it has been recommended for decades (Stephenson and Stempen 1994). However, results from moist chambers represented the primary results in the 2006-2008 survey. Given the susceptibility of sporocarps to processes mediated by environmental oscillations, using the moist chamber technique, creates more neutrally collected datasets, which are more suitable for assessments aimed at generating baseline data (Novozhilov et al. 2017). The moist chamber technique, as explained by Alexopoulos (1953), is a type of microcosm that has allowed myxomycete researchers to document species at a faster pace and it offers a neutralizing setting for several external – confounding – factors (see Rojas et al. 2021a). However, it is a type of chaotic system (Seifriz and Russell 1936) that has not been carefully examined in terms of constraints and its methodology requires a refinement based on empirical data to increase its general applicability.

During the second survey in the fir forests of Cofre de Perote, the number of visits to the field quadrupled in comparison with the first survey and most myxomycete records were field collections. As explained, sporocarps of myxomycetes are produced in response to environmental variables and a representative list of the myxobiota of a single location, based on field collections, is typically associated with a higher frequency of visits to sampling sites (Wrigley de Basanta and Estrada-Torres 2017). Field collections tend to be more robust and taxonomically representative (Alexopoulos 1953) and as expected, several species never produce sporocarps in moist chamber cultures making such technique unviable for the recording of several species. Even though field collecting takes considerably more time and perhaps economic resources, it is highly valuable in the process of documenting myxomycetes.

The short evaluation presented herein has been conceived to offer some insights on the use of the moist chamber and field collecting methods to generate data in one location in central Mexico based on two complementary individual surveys. Additionally, since this part of the world is well associated with a high myxomycete biodiversity (Lado and Wrigley de Basanta 2008), the evaluation of different methodological approaches in one single biological system allows for a pertinent analysis of the pros and cons associated with each one of them. Given the potential of myxomycetes, as biological entities, for the development of activities related with the Sustainable Development Goals of the United Nations (https://sdgs.un.org/goals), it is imperative to study these organisms under the framework of multidisciplinary agendas, for which clear recording methods are required.

Materials and methods

The present study was carried out in the Cofre de Perote National Park in central Mexico. In such location there are fir forests dominated by *Abies religiosa* (H.B.K.) Schl. & Cham. trees, locally known as “bosques de oyamel”. These forest stands are characterized by open canopies and understories with a simple vertical and horizontal structure (Fig. 1). In 2007-2008, two study sites were selected in these forests and during 2018-2019 three different sites were selected. All sites were located in a 4.5 km long semi-circular transect ranging in elevation between 3200-3900 m asl.

During the first survey, the two study sites were visited three times in July 2006, 2007 and 2008 at the beginning of the rainy season. In each visit, 12 samples each of ground litter, aerial litter, twigs and

bark were collected from each study site for a total of 96 substrate samples each year and 288 samples for the entire survey. Additionally, sporocarps of myxomycetes were examined and recorded in the field one day each year. During the second survey, the three study sites were visited 12 times between August 2018 and June 2019 and sporocarps of myxomycetes were surveyed in the field in each visit. Since the temporal range of the second survey encompassed both rainy and dry seasons, one time during each period, a series of 24 substrate samples of ground litter, twigs, bark and decayed wood at each study site were collected for a total of 288 substrate samples per season and 576 samples for the entire survey.

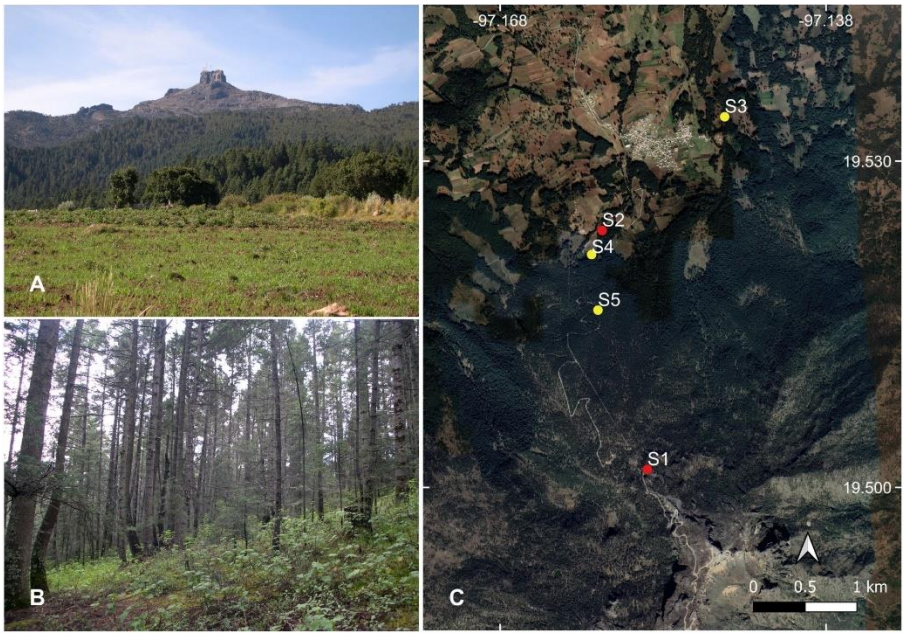

Figure 1. General aspect of the fir forests between lower grassy areas and upper open areas in Cofre de Perote National Park (A) and profile of the vertical structure of the forest in the Cofre de Perote National Park (B). The five study sites of the present investigation are shown on the right (C) and displayed based on the period (red shows 2007-2008 sampling sites, yellow shows 2018-2019 sites).

Field collections made in both surveys were directly placed in cardboard boxes, dried out in natural conditions, and stored for identification. The material for laboratory isolation were used to create a series of moist chambers following the basic methodology of Stephenson and Stempen (1994), after which, physical vouchers of myxomycetes were treated in the same manner as field collections. All material from the first survey was stored in the University of Arkansas herbarium (UARK) in USA, whereas all material from the second survey was stored in the herbarium of the Instituto de Ecología A.C. (XAL) in Veracruz, Mexico. All scientific names were based on Lado (2005-2021).

Records obtained using both collecting strategies were compiled during each individual survey and were pooled together in the present evaluation to create a representative picture of the myxobiota of the fir forests at the Cofre de Perote National Park. Results from the two surveys were compared using a) both the Simpson and Shannon indices, b) species richness and c) the taxonomic diversity index (number of species/number of genera). Two t-tests based on the diversity indices were performed with an alpha value of 0.05 to test the null hypothesis of no differences between datasets. In addition, a cluster analysis

based on Bray-Curtis distances was carried out with the results from field collections and moist chambers for both surveys in order to evaluate similarities. All analyses were carried out in PAST v4.06b (Hammer et al. 2001). Finally, a series of comparisons of the numbers of unique species associated with each survey and with each recording technique were carried out to evaluate the relative contribution of these two levels on the overall dataset.

Results

Overall, a total of 443 records of myxomycetes were compiled. From these, 198 records and 49 species were obtained during the first survey in 2006-2008 and 245 records and 39 species were observed in the 2018-2019 period. For the first survey, the average number of records and species per visit in the field were 8.3 and 4.6, respectively. The average number of records and species per visit in the field for the second survey were 15.1 and 2.7, respectively. Similarly, the average number of records and species per moist chamber were 0.6 and 0.12 for the first survey, and 0.1 and 0.02 for the second one. With the complete dataset, a total of 75 species of myxomycetes were recorded in the fir forests of Cofre de Perote National Park (Table 1).

The Shannon index of diversity was calculated as 3.27 for the first survey and 3.30 for the second with no significant differences between values (t=-0.71, $d.f.$=327, p=0.5). The Simpson index of diversity was calculated as 0.91 and 0.95 for the first and second surveys, respectively, with significant differences in the calculations (t=2.6, $d.f.$=230, p=0.009). The taxonomic diversity indices were 2.3 and 1.7 for the first and second surveys, respectively. A cluster analysis of the data showed that the highest affinity in subdatasets was observed between the moist chamber data from 2006-2008 and the field collections from 2018-2019 (Fig. 2). This analysis also showed that the most different set corresponded to the moist chamber results from 2018-2019.

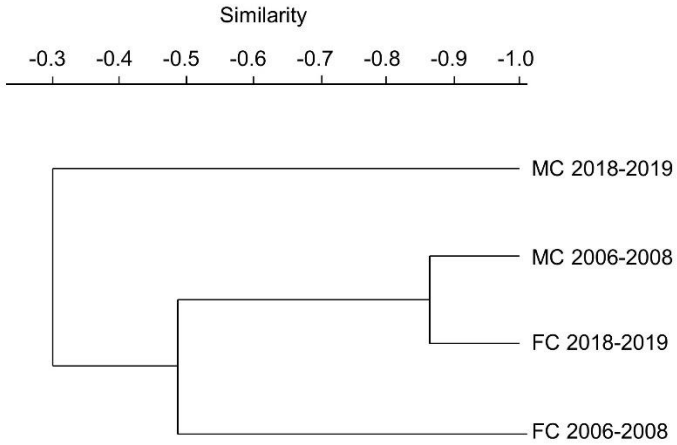

Figure 2. Cluster diagram showing the similarity among sub datasets associated with the two surveys carried out in Cofre de Perote National Park.

Table 1. Complete list of myxomycete species recorded in the two studied datasets from Cofre de Perote National Park, arranged by type of collection.

Species	**2006-2008 Survey**		**2018-2019 Survey**	
	Field Collection	**Moist Chambers**	**Field Collection**	**Moist Chambers**
Arcyria cinerea	1	4	2	17
Arcyria ferruginea			3	
Arcyria incarnata			1	
Arcyria pomiformis			2	
Badhamia crassipella			1	
Badhamia melanospora		8		
Badhamia utricularis	1		7	
Calomyxa metallica			2	
Ceratiomyxa fruticulosa	1		13	3
Collaria arcyrionema		1		
Comatricha elegans		1		
Comatricha laxa		1	1	
Comatricha nigra		23	21	
Comatricha pulchella		1	5	
Comatricha rigidiretta		1		
Craterium aureum		1		
Craterium minutum			5	
Cribraria lepida			3	
Cribraria microcarpa				3
Cribraria oregana			21	
Cribraria splendens	1			
Cribraria vulgaris	2		2	
Diachea leucopodia		1		
Dianema depressum			2	
Diderma asteroides			10	
Diderma effusum		1		
Diderma hemisphaericum		1		
Diderma umbilicatum			1	
Didymium bahiense		3		
Didymium clavus		6		
Didymium difforme		47		
Didymium dubium		1		
Didymium iridis		5		
Didymium nigripes			9	
Didymium serpula			1	
Didymium squamulosum		5		
Didymium vaccinum		1		
Echinostelium apitectum		1		
Echinostelium minutum		1		11
Fuligo septica	2			
Hemitrichia intorta			3	
Lamproderma scintillans		2		
Leocarpus fragilis			2	
Licea castanea				1
Licea pygmaea			3	4

Licea variabilis	1			
Lycogala epidendrum	4		11	2
Metatrichia floriformis			1	
Mucilago crustacea			18	1
Paradiacheopsis fimbriata		8		
Paradiacheopsis solitaria				5
Perichaena chrysosperma		6		
Perichaena corticalis		2		
Perichaena depressa		6		1
Perichaena liceoides		2		
Perichaena vermicularis		3		
Physarum album	1			
Physarum bivalve		5		
Physarum bogoriense			1	
Physarum cinereum		1		
Physarum compressum		8		
Physarum didermoides		4		
Physarum echinosporum		1		13
Physarum newtonii			2	
Physarum pusillum		1		
Physarum viride		1		
Prototrichia metallica			8	
Stemonitis axifera	1			
Stemonitis fusca		9		1
Stemonitis splendens	4			
Trichia decipiens	4		5	
Trichia lutescens			11	2
Trichia scabra	1			
Trichia subfusca			4	
Tubifera ferruginosa	1			

A total of 31 species (41.4%) were recorded exclusively in the field, including *Calomyxa metallica*, *Dianema depressum* and *Fuligo septica*. In addition, several species of the genera *Arcyria*, *Cribraria* and *Trichia* were recorded only in field conditions as well. In contrast, 35 species (46.6%) were recorded exclusively with the moist chamber technique including *Badhamia melanospora*, *Diachea leucopodia* and *Stemonitis fusca*. With this technique, several species of the genera *Didymium*, *Perichaena* and *Physarum* were recorded exclusively as well. Only nine species (12%) were recorded using both strategies, including *Arcyria cinerea*, *Ceratiomyxa fruticulosa* and *Comatricha nigra*. Finally, just 13 species (17.3%) were recorded in both surveys and the other 62 species (82.7%) were unique for one of the two studies.

Discussion

Every campaign intended to record myxomycetes is different. Researchers organize and plan their work with the main objective of maximizing the cost-benefit relationship, but various non-controlled variables affecting the life cycle and population dynamics of these organisms impose effects, both positive and negative, on the obtained results. In this manner, myxomycete surveys based on sporocarps, are always

influenced by external factors and in fact, much of the preparation is devoted to managing such non-planned external variability.

As noted by Wrigley de Basanta and Estrada-Torres (2017) field collecting can be a problem when visits do not coincide with the sporulation of a wide variety of myxomycetes, and moist chamber culturing has an inherent taxonomic bias. The best strategy to document a single location is to minimize both errors by increasing the effort associated with both techniques. However, this is not always possible. In a recent study in a tropical forest (Rojas et al. 2021) it took 30 consecutive months of sampling for researchers to reach a morphospecies accumulation "plateau" in a 28-hectare forest patch. Since most researchers do not have time, economic resources, or interest in doing so, a vast majority of myxomycete surveys are "snapshot studies" that only capture the variability of single temporal and spatial units. Despite the latter, such studies have been very important for the determination of biogeographical analyses (i.e., Schnittler et al. 2002).

In the present evaluation, the effort in the 2006-2008 survey was much lower than that of the 2018-2019 survey. Even though results showed 18% more records in the second one, they also showed 20% more recorded species in the first survey. Only the number of field records per visit was higher in the second survey, but these results do not mask the important feature in the second survey of having added 35% of the total number of species recorded herein. It was precisely from field collected myxomycetes that most of these additions were included, and such origin of the records (based on the collecting technique) was clearly responsible for the high level of unique species for either survey (more than 80% different). In other words, the first survey in 2006-2008 was highly efficient in recording myxomycete species, but its "snapshot" nature limited the variability of the data, a constraint that was only clear when the second survey was added. Stephenson (1988) had already shown such temporal variability to be a potential constraint for myxomycete data collected in the field and recent studies have also indicated that a larger temporal range of field detection could generate more conclusive (i.e., more complete) results (Treviño-Zevallos and Lado 2020).

The two surveys did not show differences in diversity using the Shannon Index but displayed significant differences when the Simpson Index was used. Since the calculation of the second one is more sensitive to dominance (see Somerfield et al. 2008) results simply suggest that the difference between the two surveys is related with a higher number of records associated with a lower number of species in the 2018-2019 survey. This observation is also supported by the lower taxonomic diversity index calculated with the same dataset, which indicated that less species per genus were recorded during that time. The basis of such result relies in the poor performance of the moist chambers during the second survey. As observed in the data, out of 576 cultures only 64 records were obtained (0.1 records per culture) in comparison with 173 records in 288 cultures (0.6 records per culture) for the first survey. Long term evaluations of moist chamber performance with data from both temperate and tropical conditions has also demonstrated higher productivity per moist chamber. For instance, Härkönnen and Ukkola (2000) evaluated 4793 moist chambers and obtained a value of 0.35 records per moist chamber. It is likely that the moist chambers during the second survey were affected by an external, unaccounted factor, but they still added three species (4%) to the general species list.

Such poor performance of the moist chambers during the second survey along with the limited field sampling during the first survey explained the pattern observed in the cluster analysis. In this analysis, the two "stronger" (and potentially the only representative) sub datasets were clustered. This is interesting because taxonomically, the sub datasets seemed different. As observed in the results, the represented

assemblages in each one of them differ in both composition and frequency of observations but as noted before, no differences were observed in the Shannon Index of Diversity. The same result was obtained when only these two "strong" datasets were compared (not shown before, t=-1.69, $d.f.$=309, p=0.09) suggesting that the clustering has structural validity.

Despite the latter, the results presented herein clearly showed the complementarity of recording techniques. The ratio of unique species recorded in moist chambers to those recorded in the field was 1.1, very close to a perfect 1.0 for a dataset where literally half the species are recorded with either technique. As mentioned before, several authors have pointed out to the importance of including both techniques when myxomycete surveys are carried out (Wrigley de Basanta and Estrada-Torres 2017) and the present evaluation is clear empirical basis for such advice. Remarkably, the collaborative effort organized in Cofre de Perote intended to increase the number of myxomycete species known for the fir forests also showed the positive effect of communication among researchers, a simple aspect that is not necessarily discussed in the myxomycete literature. The collaborative effort presented herein was possible because the three researchers coauthoring this note shared contextual information, data, and other important observations before the second survey was conducted, providing insight at the point of study design. This simple, but important fact, had a very positive effect on the documentation of the myxobiota in Cofre de Perote National Park.

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