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Myxomycetes of the Monte Alto Protected Zone in Costa Rica: a case study

Carlos Rojas[1,2], Pedro A. Rojas[1] and Miguel Méndez García[3]

[1] Instituto de Investigaciones en Ingeniería, Universidad de Costa Rica, San Pedro de Montes de Oca, 11501-Costa Rica
[2] Escuela de Ingeniería de Biosistemas, Universidad de Costa Rica, San Pedro de Montes de Oca, 11501-Costa Rica
[3] Zona Protectora Monte Alto, Área de Conservación Tempisque, Hojancha, Costa Rica

E-mail: carlos.rojasalvarado@ucr.ac.cr

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Abstract: A survey of myxomycetes was carried out during 2022 in the Monte Alto Protected Zone in Guanacaste, Costa Rica. Eighty samples of ground litter were collected from the premises around the headquaters and studied with the moist chamber technique. The resulting dataset was compared with an equivalent set from the Caribbean side of the country obtained in the same forest type in order to evaluate the previous observation that forests on the Pacific slope of Costa Rica seem to be associated with higher values of alpha diversity, when surveys are based on sporocaps and morphospecies. Results supported such observation to a degree, but also showed that the overlap among species assemblages within the same forest type could be affected by a different history of forest management. In the case of the Monte Alto Protected Zone, the observed diversity of myxomycetes suggested that the forests of this area have responded succesfully to the reforestation processes that took place more than 30 years ago.

Keywords: Área de Conservación Tempisque, tropical wet forest, Nicoya Peninsula, Guanacaste

Introduction

Costa Rica is famous for its reversion of deforestation in the late 1980s (Evans 1999) that derived in a scenario where more than 50% of its continental territory is currently forested (Canet 2015). During the first years of such reversion, many cattle farms were reforested with socio-economic incentives from the Payment for Environmental Services program of the Costa Rican government.

Within that context, the Monte Alto Protected Zone is an example of the efforts in the 1980s to reforest land in the Nicoya Peninsula of the Guanacaste Province (Madrigal et al. 2012) that resulted in the protection of 23% of the Hojancha Canton by the year 2000 (Yglesias 2011). Today, Monte Alto protects over 924 hectares of both tropical wet and premontane wet forests and is the main provider of environmental services for the community of Hojancha.

Based on the review of Lado and Rojas (2018), myxomycetes have been poorly documented in the Nicoya Peninsula, and most tropical wet forests studied so far in Costa Rica are located on the Caribbean side of the country. Interestingly, previous studies comparing the myxobiota of locations in both Costa Rican slopes (i.e., Rojas and Valverde 2015; Arenas-Taborda et al. 2021) have found that ecological indicators such as diversity, richness and evenness are all higher on the dryer areas of the Pacific side, but there are no studies comparing those variables using information from moister areas, such as those found in the Nicoya Peninsula.

In this manner, the present study was intended to compare ecological variables associated with species assemblages of myxomycetes in tropical wet forests of both Costa Rican slopes using a standard methodology and equivalent effort. As part of the task of supporting the documentation of Costa Rican Conservation Areas, the present study is also a contribution to the biological knowledge of both the Tempisque Conservation Area and the Nicoya Peninsula.

Materials and methods

This project was carried out in the Monte Alto Protected Zone (ZPMA hereafter) in Hojancha, Costa Rica (10.0118, -85.4025, 490 m). This area contains a section of wet forests located within the context of the more common moist and dry forests of the Guanacaste Province of Costa Rica. In this location, a series of 80 samples of ground litter were collected around the headquarters in the manner described by Wrigley de Basanta and Estrada Torres (2022).

Since the main idea of this investigation was to compare the results from the ZPMA with an equivalent dataset from the Caribbean side of Costa Rica, partial data from Experiment #1 in the study of Rojas et al. (2021) was retrieved. As such, the results from 72 ground litter samples collected in the Finca Experimental Interdisciplinaria de Modelos Agroecológicos (FEIMA hereafter) were used for comparison. Both sets of samples were collected during the dry season (January-March) in total areas of approximately 2000 m^2.

Both the ZPMA and FEIMA have tropical wet forests in a successional stage of about the same age (~32-35 years) but are on different slopes of Costa Rica (Fig. 1), have very different sizes and have slightly different conservation purposes. The ZPMA has 924 hectares and is administrated by the National System of Conservation Areas and the Fundación Pro Reserva Forestal Monte Alto with a focus on biodiversity conservation. FEIMA, on the other hand, is an experimental station that contains a 28-hectare forest patch reforested for watershed protection and is administrated by the University of Costa Rica Protected Area Network. The ZPMA receives more people since it is open to the public and FEIMA is basically a natural laboratory dedicated to research.

In both locations, after the collection of samples took place, the material was transported to a climate-controlled (~21°C and 50% humidity) laboratory at the Engineering Research Institute of the University of Costa Rica. In this place, all samples were placed on pieces of filter paper lining individual petri dishes, after which, they were saturated with distilled water for 24 hours. Following this period, excess water was discarded and the pH value of all the cultures was obtained. All moist chamber cultures were kept under investigation for 10 weeks by checking the presence of myxomycete activity on a regular basis. When sporocarps were detected, these were identified to morphospecies level and in some cases, they were extracted and glued to paper boxes for storage in the herbarium of the University of Costa Rica (USJ).

After such period, the two datasets were unified and analyzed in Past 4.06b (Hammer et al. 2001). All morphospecies were assigned an abundace category based on the ACOR classification (Stephenson et al. 1993), but using the quartile values of the distributions instead of the original values for partitioning. For comparison, the alpha diversity of each set was calculated using both the Shannon and the Simpson Diversity Indices. The Shannon´s evenness value was also calculated in each case. The beta diversity (β) was calculated using the formula from Whittaker (1960) $\beta = (S/\bar{a})-1$ and the Proportional Species Turnover (*PST*) was calculated using the formula from Williams (1996) $PST=1-(a_{max}/S)$; where S = total number of species, $\bar{a}$ = average number of species per location, and $a_{\max}$= maximum number of species per location. The first metric allowed the quantification of the overall species pool relative to individual species pools, which can be useful to understand the average weight of the individual locations for the general system under study. With the second metric it was possible to quantify the proportion of the overall species diversity that was not contained in the individual datasets. Finally, the levels of significance associated with four abundance distributions known as log-normal, geometric series, log-series and broken stick were obtained for each dataset using a Chi square test. With such levels, a high probability value did not indicate much but a low value implied a bad fit with the known model.

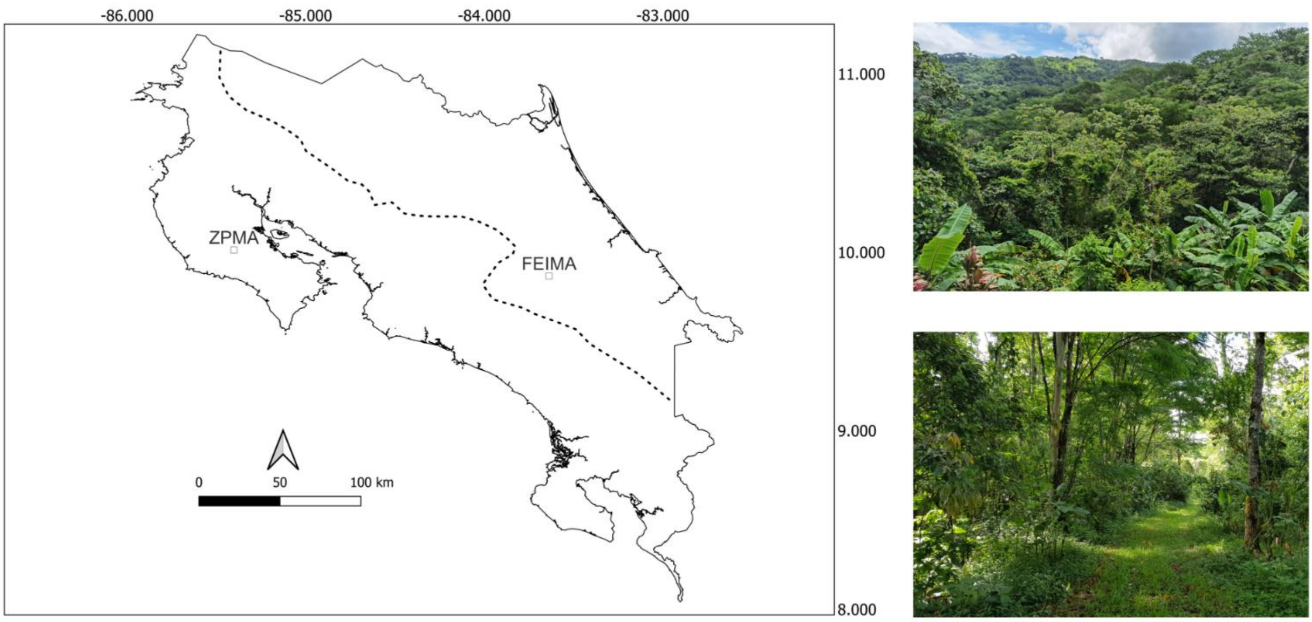

Figure 1. Map of Costa Rica displaying the location of both the ZPMA (top right picture) on the Pacific side of the country and FEIMA (bottom right picture) on the Caribbean. The dotted line indicates the approximate position of the Continental Line.

Results

A total of 54 sporocarps and 20 non-fruiting plasmodia were recorded from the 80 litter samples collected in the ZPMA (Table 1). Those results showed that 76% of the studied moist chambers, or 61 of them, displayed positive myxomycete activity. Overall, 23 morphospecies were identified from the same dataset. In contrast, results showed 72 myxomycetes within 20 morphospecies and one plasmodium in the

72 samples collected in FEIMA. In such case, the number of positive moist chambers accounting for any myxomycete activity was 80%.

Of the 31 morphospecies recorded in the studied tropical wet forests, eight of the them (26%) were not present in the ZPMA and 11 (35%) were not recorded in FEIMA. Notably, *Perichaena pedata*, *Hemitrichia pardina* and *Physarum oblatum* were not present on the pacific slope forests; whereas *Arcyria pomiformis*, *Collaria lurida*, *Hemitrichia calyculata* and *Physarum notabile* did not show up on the Caribbean slope.

Table 1. Myxomycete morphospecies recorded in the tropical wet forests of both the ZPMA and FEIMA arranged by abundance category. A=abundant, C=common, O=occasional, R=rare.

Morphospecies	ZPMA	FEIMA	Total	Abundance category
Arcyria cinerea	4	16	20	A
Arcyria afroalpina	1	11	12	A
Lamproderma scintillans	9	2	11	A
Didymium squamulosum	9		9	A
Comatricha tenerrima		8	8	A
Didymium minus	6	2	8	A
Arcyria insignis		6	6	C
Diderma hemisphaericum	3	3	6	C
Physarum compressum	3	3	6	C
Comatricha nigra	1	3	4	C
Cribraria violacea	2	2	4	C
Perichaena corticalis	1	3	4	C
Physarum sp.	1	3	4	C
Didymium difforme	2	1	3	R
Perchaena chrysosperma	1	2	3	R
Didymium dubium	2		2	R
Perichaena pedata		2	2	R
Arcyria pomiformis	1		1	R
Collaria lurida	1		1	R
Cribraria microcarpa	1		1	R
Didymium anellus		1	1	R
Didymium bahiense		1	1	R
Didymium nigripes	1		1	R
Hemitrichia calyculata	1		1	R
Hemitrichia pardina		1	1	R
Lamproderma sp.	1		1	R
Perichaena vermicularis		1	1	R
Physarum decipiens	1		1	R
Physarum notabile	1		1	R
Physarum oblatum		1	1	R
Stemonitis fusca	1		1	R
Unidentifed Plasmodia	20	1	21	A

The Shannon Diversity Index was slighlty higher in the ZPMA (2.96) than in FEIMA (2.72) with a 95% confidence range that showed a similar pattern (Fig. 2). Accordingly, the Simpson Diversity Index showed that the ZPMA had a higher (0.93) value than FEIMA (0.90). The evenness value was 0.84 for the ZPMA and 0.76 for FEIMA. The Whittaker Beta Diversity Index associated with both locations was 0.44 and the Williams Proportial Species Turnover Index was 0.26.

The significance values of the Chi square tests applied to the dataset distributions showed that, for the ZPMA, the significance decreased from log-normal (p=0.15), to geometric series (p=0.21), log series (p=0.96) and the broken stick model (p=0.97). In the FEIMA case, the significance decreased from log normal (p=0.05), to geometric series (p=0.45), broken stick (p=0.98) and log series model (p=0.99).

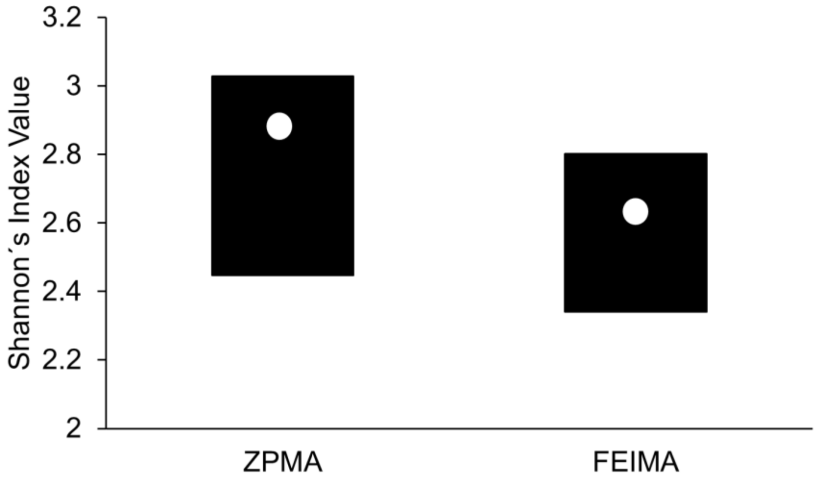

Figure 2. 95% Confidence interval (dark area) and mean value (white circle) of the Shannon Diversity Index calculated for the datasets of the ZPMA and FEIMA.

Discussion

The present study is a glimpse of the potential for myxomycete research in the Nicoya Peninsula of Costa Rica. Overall, for the Guanacaste area, researchers have already focused on studying the myxobiota of dryer parts such as the Santa Rosa National Park (Schnittler and Stephenson 2000) and the Palo Verde National Park (Rojas and Valverde 2015), but this is the first time an attempt is made in the wet forests of the peninsular region.

The results shown herein demonstrated that the complexity of myxomycete assemblages in the ZPMA is very similar to that of the better documented wet forests on Costa Rica´s Caribbean slope. For instance, a mid-term study in FEIMA during 2018-2019 (Rojas et al. 2021) yielded 32 morphospecies of myxomycetes from 864 substrate samples. As observed in the present study, a 91% reduction in such effort – only considering 72 samples – was associated with 20 morphospecies or 63% of the total number for the original location. If an equation of effort to results is constructed ($y=(ab+cx^d)/(b+x^d)$) with the FEIMA dataset, it is calculated that 80 samples would be associated with just 21 morphospecies or that in order to find 23 morphospecies an effort of 100 samples would be necessary.

As observed in the results, 23 morphospecies were found in the ZPMA with an effort of 80 samples – this is 20% less effort than expected by the predicting model – suggesting that such site would at least be equivalent to FEIMA in terms of generating myxomycete results with the collecting and recording parameters of the present study. In this manner, the general trend of higher ecological values of species richness, diversity and evenness previously observed in Costa Rica for the Pacific slope, was supported by the data presented herein. However, given the natural oscillations in sporocarp formation dynamics and data generation previously documented as well (Stephenson et al. 1993, Schnittler and Stephenson 2000), any pattern should be inconclusive until documented with more data from similar ecological situations. In other words, the present study supports the previously observed pattern but given the fact that it is only one point in the Nicoya Peninsula and the Pacific slope of Costa Rica, can only be used to evaluate such hypothesis in a future meta-analysis.

Both the Beta Diversity and the Proportial Species Turnover Indices showed that the contributions of each individual dataset (ZPMA and FEIMA) to the overall species pool for the ecosystem under study (tropical wet forest) were substantial and equivalent. In the first case, the value of 0.44 indicated that there were important ecological contributions from each dataset to the species pool associated with the ecosystem; for example, in the form of species detected in only one location but not in the other. In the second case, the value of 0.26 indicated that the proportion of the overall species diversity that was not contained in the individual datasets was calculated on the low third suggesting that both datasets were represented by a core of morphospecies complemented by other ones with particular distributions. Such results provided one more level of support to the idea that the ZPMA represents a quality ecosystem for the study of myxomycetes. Ironically, historical data (from Lado and Rojas 2018) showed that the general area encompassed by the Nicoya Peninsula (about 10% of the Costa Rican continental territory) has contributed to less than 3% the number of records of myxomycetes known for the country.

The results from the abundance distribution analyses indicated that both log-normal and geometric series models, assuming weak and strong competition, respectively (Alroy 2015) were the models with the worst fit to both datasets from the ZPMA and FEIMA. Constrastingly, log-series and broken stick, with weak and intermediate competition showed the highest values of probability not rejecting the null hypotheses. As such, results suggested that in both locations, but more so in the ZPMA (broken stick showed a higher probability than log-series), competition seems on the low end, indicating that tropical wet forests likely offer good conditions for myxomycetes to thrive. One more time, results suggested that the ZPMA is represented by an ecosystem with enough conditions to support a diversity microbiota, and is likely the product of good conservation practices.

It is very interesting though, that morphospecies present in the Caribbean wet forests such as *Perichaena pedata* and *Hemitrichia pardina* were not present in the ZPMA. These two morphospecies have been previously recorded in wet-moist transitional landscapes or deciduous forests (Cavalcanti et al. 2016). Similarly, it is very interesting that *Arcyria pomiformis* or *Hemitrichia calyculata* were recorded in the ZPMA in moist chamber culture. Both of the last morphospecies are much more commonly recorded in field surveys as historical data shows (Rojas et al. 2018). In this sense, it would be worthwhile to study myxomycetes using field techniques in the ZPMA to understand the impact of complementary methodologies and even to address phenological patterns in a wet forest on the northern Pacific coast of Costa Rica. With the promising preliminary data presented herein, it seems that this location is very adequate for the task.

What such taxonomic differences, and the values of the diversity indices might suggest, is that forest management could also play a role in determining the presence/absence of some myxomycetes – by means of recording morphospecies – via substrate availability. Previous assessments have determined that there are between 100-130 tons of biomass per hectare in FEIMA (Quesada-Chacón et al. 2020) and between 100-200 tons per hectare for the ZPMA (Gutierrez Pacheco et al. *unpublished*). Since substrate availability is a byproduct of biomass acumulation per area, it is possible that higher values in the ZPMA could be reflected in more complex myxomycete assemblages. However, in a similar manner as previously noted, field-based observations of myxomycetes in the ZPMA are required to estalish more accurate comparisons. In any case, the quantified effect of a more conservation-oriented policy in the ZPMA suggest a good response from the ecosystem to the original process of reforestation. The microbial data presented herein, also supports the idea that the forest is undergoing the successional process in an adequate manner.

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