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Rapid assessment of myxomycetes in Isla Calero and Isla Brava, Costa Rica

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Abstract: The geography of the northeasternmost section of Costa Rica corresponds to river islands sparsely populated and highly protected for biological conservation. Myxomycetes had not been studied in that part of such Central American country and the present rapid assessment project (RAP) represents the first attempt to do so. By studying moist chambers made with ground litter from both Isla Calero and Isla Brava, a total of 22 species of myxomycetes were identified in the lowland rainforests occupying this area. Of the total number of samples used to prepare moist chamber cultures, about 53% yielded positive results of myxomycete activity and the most commonly recorded species were *Lamproderma scintillans* and *Arcyria cinerea*. Surprisingly, *Physarum roseum* and *P. virescens*, two species more commonly found in the tropical dry forests of Costa Rica, were recorded as well. This study covered the section of the islands facing the inner part of the country and not the open Caribbean Sea, where the effect of winds and marine environments could be responsible for a different assemblage of fruiting myxomycetes.

Keywords: microorganisms, river island, slime molds, tropical rainforest, wetlands

Introduction

The myxomycetes of Costa Rica have been well studied during the last decades. However, the sampling effort has been timid in perhumid forests receiving more than 5000 mm of rain per year (Lado and Rojas 2018). One of such efforts, however, was a sampling trip that took place in the Northeastern section of Costa Rica during 2016, that albeit very localized, generated enough results to appear in country-wide analyses of myxomycete diversity (Rojas et al. 2018).

Northeastern Costa Rica is mainly represented by the district of Colorado – Pococí County – where lands are mostly dedicated to the protection of natural resources. From the 488 districts in Costa Rica, Colorado ranks 483rd in population density with only 4.1 citizens per square kilometer (INEC 2016) implying that forests in this area are mostly unaffected by human activities. The Costa Rican government considers this part of the country a Ramsar site with top wetland protection (SINAC-PNUD-GEF 2018) and the general area is occupied by four major islands known as Isla Calero, Isla Brava, Isla Machuca, and

Isla Portillos. The first one is the largest in Costa Rica with an area of 151.6 km^2 whereas the last one was part of an international conflict with environmental consequences between Costa Rica and Nicaragua solved in 2015 by the International Court of Justice (ICJ Reports 2018).

The present note aimed to present the list of myxomycetes recorded in both Isla Calero and Isla Brava that had not been explicitly included in such manner in any previous publication. Given the importance of these islands for the conservation of wetlands in Costa Rica, the documentation of the biodiversity in them is a top priority. Recent forecast models of coastal flooding in the Caribbean of Costa Rica also suggest that low elevation areas along the coast are highly vulnerable for destruction by 2100 based on climate change scenarios of sea level increase (Lizano-Araya and Lizano-Rodríguez 2022). This further necessitates the rapid assessment of biodiversity in these vulnerable islands.

Materials and methods

A surveying trip to Isla Calero and Isla Brava in Northeastern Costa Rica took place in January 2016. From the locality known as Delta Costa Rica (DCR, starting sampling location), in the mainland, six sampling locations were selected along the margins of the Colorado River and the Caño Bravo in areas within the Barra del Colorado Wildlife Refuge (see Fig. 1). Three of these locations were selected on Isla Calero (IC1, IC2, IC3, Table 1) and three on Isla Brava (IB1, IB2, IB3).

Table 1. Information about the sampling localities surveyed in the present study.

Locality	Coordinates	Elevation (m asl)
DCR – Delta Costa Rica	10.768889, -83.758333	10
IB1 – Isla Brava 1	10.746667, -83.729722	9
IB2 – Isla Brava 2	10.749722, -83.715556	9
IB3 – Isla Brava 3	10.753889, -83.740278	9
IC1 – Isla Calero 1	10.761389, -83.742778	13
IC2 – Isla Calero 2	10.756667, -83.7425	15
IC3 – Isla Calero 3	10.752222, -83.720278	19

In all locations (Fig. 2), including DCR, a series of 20 samples of ground litter were collected and transported in paper bags to a laboratory at the University of Costa Rica. Without quarantine period, all samples were used right away to set up one moist chamber per sample for a total of 140 moist chamber cultures in the manner described by Stephenson and Stempen (1994). The material corresponded to a mixture of decomposing leaves located on the ground of semi-disturbed forest patches with presence of *Prioria copaifera* and *Cecropia insignis* within the *Raphia taedigera*-dominated marshy areas on the edge of the islands, which had very similar conditions. The collected ground litter was already wet from the field and was not dried out in the laboratory before its use, simply not to disturb any biological processes (e.g., formation of plasmodia) already taking place on the substrates.

In this manner, all samples were placed on filter paper within standard size Petri dishes and soaked with water. After 24 hours, excess water was discarded, pH was measured, and the cultures were checked for myxomycete activity (either plasmodia or fruiting bodies) for a period of 10 weeks. When fruiting bodies were discovered, these were extracted with forceps, identified, and glued to matchboxes for storage in the biorepository of mycetozoans at the Engineering Research Institute (INII) of the University of Costa

Rica. Not all records were collected, and, on some occasions, due to minimal number of fruiting bodies, the only observed record was destroyed during the identification process. A total of 79 vouchers were generated for this collection.

A list of species was included to annotate the collecting numbers (all corresponding to the first author) associated with the deposited vouchers whenever possible. All scientific names follow the nomenclature provided in Lado (2005-2022). Simple calculations of completeness and relative distribution were obtained, and, in all cases, the total number of samples associated with the calculations was provided in the results.

Results

A total of 22 morphological species in 8 genera of myxomycetes were recorded in the studied locations (Table 2 and annotated list). The most commonly recorded species were *Lamproderma scintillans* and *Arcyria cinerea*, and single records were observed for *Comatricha nigra*, *Didymium dubium*, *D. iridis*, *D. nigripes*, *Diachea leucopodia*, *Hemitrichia serpula*, *Physarum auriscalpium*, *P. roseum,* and *P. virescens*. These singletons accounted for 41% of the overall 22 species.

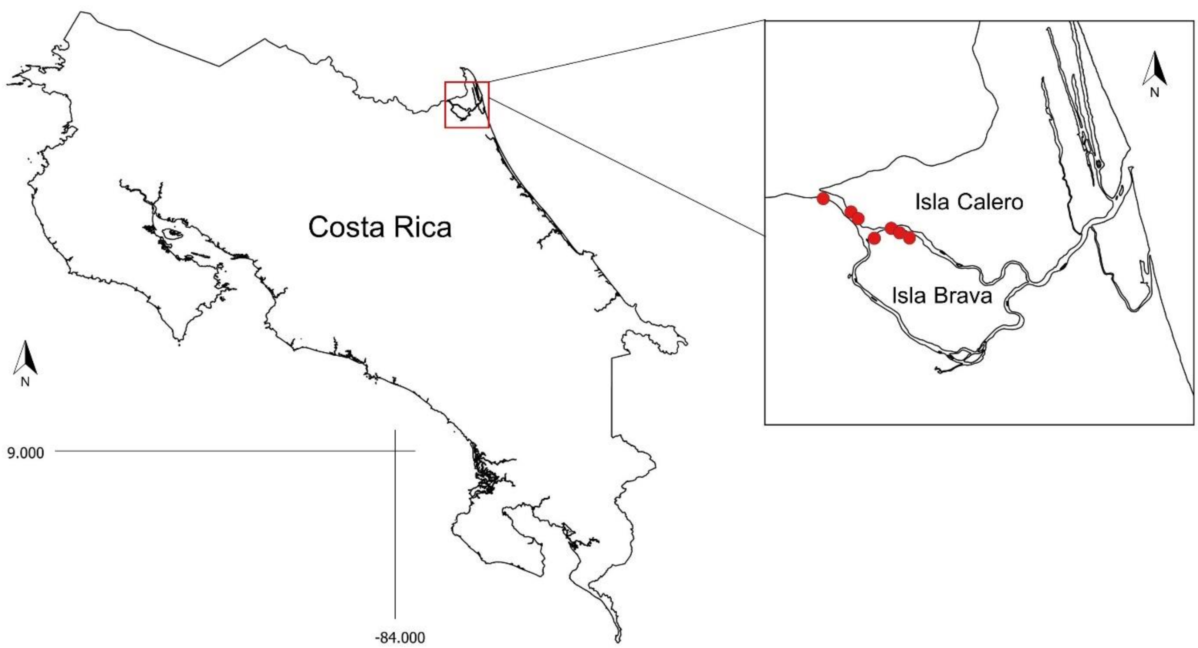

Figure 1. Map of Costa Rica (left) showing the Northeastern section where sampling for the present study took place (red square and detailed insert to the right). The insert shows the position of the seven sampling locations (red circles) in the western area of the Barra del Colorado wetland.

During the process of examination of cultures, 94 records of myxomycete activity were observed, including 10 non-fruiting plasmodia (10.6%) that remained in the moist chamber cultures after the period of study finalized. From the 140 individual substrate samples, 75 yielded positive results for a percentage of approximately 53% positive samples. All sampling locations were associated with a total number of records between 11 and 17 (this value does not include non-fruiting plasmodia), with an average of 8.4±1.1 species. Negative samples (those that did not produce either fruiting bodies or plasmodia) oscillated between seven (35%) and 11 (55%) out of the 20 samples collected per sampling location, with slightly more negative samples observed in the Isla Calero locations (10, 10 and 11 negative samples) than in the Isla Brava (seven, nine and nine negative samples).

Table 2. List of morphospecies of myxomycetes recorded in Delta Costa Rica (DCR), Isla Calero (IC1, IC2, IC3) and Isla Brava (IB1, IB2, IB3) along with total number of records per taxon for the complete survey. The number of non-fruiting plasmodia observed in the moist chamber cultures was added as well.

Morphospecies	**Sampling locations**							**Total**
	DCR	**IC1**	**IC2**	**IC3**	**IB1**	**IB2**	**IB3**	
Arcyria cinerea	4		1	5	1	1	2	14
Comatricha nigra							1	1
Comatricha tenerrima	1			2		1		4
Didymium bahiense		1		1	1			3
Didymium difforme	2	1	1			2		6
Didymium dubium						1		1
Didymium iridis			1					1
Didymium minus		3			3		2	8
Didymium nigripes			1					1
Didymium squamulosum	1	1		2	2	1		7
Diachea leucopodia						1		1
Diderma effusum			1		1			2
Diderma hemisphaericum	1		1	1				3
Hemitrichia serpula							1	1
Lamproderma scintillans				2	5	7	2	16
Physarum auriscalpium							1	1
Physarum compressum		1	1					2
Physarum melleum		1	3	1	1			6
Physarum pusillum				1	1			2
Physarum roseum			1					1
Physarum superbum						1	1	2
Physarum virescens	1							1
Non-fruiting plasmodium	2	3	1		1	1	2	10
Total	12	11	12	15	16	16	12	94

Of all the recorded species *Physarum roseum* and *P. virescens* had been mostly recorded in the tropical dry forests of the Pacific coast of Costa Rica. All other species recorded herein had been previously known, from several collections, to be associated with lowland rain forests of the Caribbean.

List of species

1. *Arcyria cinerea* (Bull.) Pers.

Recorded from both Isla Calero and Isla Brava (all locations except IC1). Deposited and studied vouchers correspond to numbers 8318, 8319, 8321, 8323, 8326, 8335, 8340, 8343, 8353, 8354, 8355 and 8356.

2. *Comatricha nigra* (Pers. ex J.F. Gmel.) J. Schröt.

Recorded only in Isla Brava (IB3). Deposited and studied voucher corresponds to number 8347.

3. *Comatricha tenerrima* (M.A. Curtis) G. Lister

Recorded in both Isla Calero and Isla Brava (DCR, IC3 and IB2). No vouchers were collected.

Figure 2. Images of the Río Colorado dividing Isla Calero and Isla Brava (A) in the northeastern wetlands of Costa Rica, and one sampled perhumid forest (B). Note that the survey was carried out during the "dry" season in January of 2016 even though the images may look like obtained during the wet season.

4. *Didymium bahiense* Gottsb.

Recorded in Isla Calero and Isla Brava (IC1, IC3 and IB1). No vouchers were collected.

5. *Didymium difforme* (Pers.) Gray

Recorded in Isla Calero and Isla Brava (DCR, IC1, IC2 and IB2). Deposited and studied vouchers correspond to number 8028, 8298 and 8359

6. *Didymium dubium* Rostaf.

Recorded only in Isla Brava (IB2). Deposited and studied voucher corresponds to number 8332.

7. *Didymium iridis* (Ditmar) Fr.

Recorded only in Isla Calero (IC2). Deposited and studied voucher corresponds to number 8301.

8. *Didymium minus* (Lister) Morgan

Recorded in both Isla Calero and Isla Brava (IC1, IB1 and IB3). Deposited and studied vouchers correspond to numbers 8291, 8292, 8295, 8305, 8312, 8313, 8314, 8315, 8341, 8342, 8344, 8345 and 8346.

9. *Didymium nigripes* (Link) Fr.

Recorded only in Isla Calero (IC2). No vouchers were collected.

10. *Didymium squamulosum* (Alb. & Schwein.) Fr. & Palmquist

Recorded in both Isla Calero and Isla Brava (DCR, IC1, IC3, IB1 and IB2). Deposited and studied vouchers correspond to numbers 8029, 8294, 8306, 8307, 8310, 8320, 8325 and 8358.

11. *Diachea leucopodia* (Bull.) Rostaf.

Recorded only in Isla Brava (IB2). Deposited and studied voucher corresponds to number 8030.

12. *Diderma effusum* (Schwein.) Morgan

Recorded in both Isla Calero and Isla Brava (IC2 and IB1). Deposited and studied vouchers correspond to numbers 8296 and 8297.

13. *Diderma hemisphaericum* (Bull.) Hornem.

Recorded in Isla Calero (DCR, IC2 and IC3). Deposited and studied voucher corresponds to number 8299.

14. *Hemitrichia serpula* (Scop.) Rostaf. ex Lister

Recorded only in Isla Brava (IB3). Deposited and studied voucher corresponds to number 8350.

15. *Lamproderma scintillans* (Berk. & Broome) Morgan

Recorded in both Isla Calero and Isla Brava (IC3, IB1, IB2 and IB3). Deposited and studied vouchers correspond to numbers 8025, 8027, 8031, 8032, 8308, 8309, 8311, 8316, 8317, 8322, 8327, 8328, 8330, 8331, 8333, 8334, 8336, 8337, 8338, 8339, 8348, 8349 and 8351.

16. *Physarum auriscalpium* Cooke

Recorded only in Isla Brava (IB3). No vouchers were collected.

17. *Physarum compressum* Alb. & Schwein.

Recorded only in Isla Calero (IC2 and IC3). Deposited and studied vouchers correspond to numbers 8293 and 8302.

18. *Physarum melleum* (Berk. & Broome) Massee

Recorded in both Isla Calero and Isla Brava (IC1, IC2, IC2 and IB1). No vouchers were collected.

19. *Physarum pusillum* (Berk. & M.A. Curtis) G. Lister

Recorded only in both Isla Calero and Isla Brava (IC3 and IB1). Deposited and studied vouchers correspond to numbers 8023, 8024, 8026 and 8324.

20. *Physarum roseum* Berk. & Broome

Recorded only in Isla Calero (IC2). Deposited and studied vouchers correspond to numbers 8022, 8303 and 8304.

21. *Physarum superbum* Hagelst.

Recorded only in Isla Brava (IB2 and IB3). Deposited and studied vouchers correspond to numbers 8329 and 8352.

22. *Physarum virescens* Ditmar

Recorded only in DCR and associated with both Isla Calero and Isla Brava. Deposited and studied voucher corresponds to number 8357.

Discussion

The present survey demonstrated the occurrence of myxomycetes in perhumid wetlands of northeastern Costa Rica. Of the total number of species, 11 were recorded in both Isla Calero and Isla Brava, five were only observed in the former island and six in the latter one. These results suggest that very specific conditions of each location (i.e. plant communities, microclimate) could have influenced either the presence/absence of myxomycete species on ground litter substrates or their development in moist chamber cultures, despite the homogeneity of landscape-level conditions. Similar results for other tropical forests have been observed previously in Costa Rica. For instance, Schnittler and Stephenson (2000) found 16 species in the wet forests of coastal Cahuita (Caribbean) with an effort of 63 samples and Arenas-Taborda et al. (2021) recorded 24 species in a mainland wet forest with an effort of 240 samples.

Localized surveys such as the one presented herein are important to characterize taxonomically the assemblages of myxomycetes in particular geographical locations (e.g. Schnittler and Stephenson 2000). Data analysis in the period between 2010 and 2014 showed that the northeastern section of Costa Rica had been undersampled at that time, but modeling of species distribution (Rojas et al. 2015) had shown this area to be good for myxomycetes. Such background justified the present survey in the wetlands of the northeastern section of Costa Rica. Interestingly, as part of the last study, researchers modelled the expected geographical distribution of five myxomycete species and all of them were likely to occur in the Isla Calero/Isla Brava area. After the survey carried out herein four of the expected morphospecies were confirmed in those islands (*A. cinerea*, *D. iridis*, *D. squamulosum* and *P. compressum*) and only *Hemitrichia calyculata* could not be recorded. However, the latter species is known to fruit mostly on woody substrates, which were not surveyed in the present assessment.

This study surveyed only the inner part of the islands facing the mainland. Other tropical studies have shown that myxomycete assemblages based on morphospecies may differ between windward and leeward zones (Rincón-Marín et al. 2021), presumably due to the effect of wind sustaining propagules in the air. As such, it is likely that the outer section of these islands facing the Caribbean Sea would show a partially different species assemblage, which would be likely based on the vegetation and microclimate differences between the zones directly subjected to coastal dynamics and those also influenced by mainland processes. In this manner, for future reference, it would be interesting to explore the coastal sections of these islands.

More complete surveys of myxomycete biodiversity require the combination of different sampling techniques (see Wrigley de Basanta and Estrada-Torres 2022) but simple straighforward surveys such as the present study are able to initiate the documentation of myxomycetes in understudied locations. In the case of Costa Rica, as discussed, the myxobiota of Isla Calero and Isla Brava had not been documented before and such limitation also implied that perhumid forests were less documented than other forest types. Currently, the same situation holds true but discrete attempts to document the biodiversity of myxomycetes such the present study alleviate, even just a little, the unbalance in sampling effort and results obtained from different ecosystems in tropical areas.

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