



## New additions to the myxobiota of Costa Rica

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### Abstract

The most recent checklist of myxomycetes from Costa Rica reported 208 species for the country. Informational gaps detected in that work and a sustained survey effort since the publication of the checklist increased the number of species to 218. In the study reported herein, we identified seven species not previously known from Costa Rica. In addition to increasing the number of species recorded to 225, this work also provided important information relating to potential distribution, ecological preferences and the role of isolation techniques for biodiversity surveys of myxomycetes. The fact that a region with only 0.05% of the terrestrial surface of the Earth accounts for approximately 25% of the global biodiversity of myxomycetes, even with the technical limitations of the isolation methods used up to date, shows one more time that this group of organisms is not only well established in terrestrial environments but widely distributed in the Neotropics.

**Key words** – biodiversity – biogeography – myxogastrids – Neotropics – slime molds

### Introduction

The myxomycetes (also called plasmodial slime molds or myxogastrids) have been studied more intensively in the Neotropics during the last 50 years than at any time in the past. This has partially been a product of the interest of researchers to document the myxobiota of this part of the world after realizing that most of the information already available had been derived from temperate regions in North America and Europe (see Stephenson et al. 2004). Even though the current effort to study such group of microorganisms in the Neotropics is not evenly distributed across the region, those areas where research has been carried out have provided important information for the evaluation of distributional patterns of species (i.e., Rollins & Stephenson 2011). As part of the overall effort to acquire information, Costa Rica has been a country characterized by a sustained effort to study the myxomycetes, and this has been maintained for the past two decades (see Schnittler & Stephenson 2000, Rojas et al. 2010, Walker et al. 2015).

In the process noted above, the number of species of myxomycetes known to occur in Costa Rica has gradually increased. However, in the interval of 2011-2015, the rate of recording new species for Costa Rica has slowed down in comparison with the decade of 2001-2010. For example, Rojas et al. (2010) reported 208 species, 62 of which were recorded during the period of 2001-2010, whereas in the interval of 2011-2015 only 10 new records were reported for Costa Rica (see

Rojas & Calvo 2014, Leontyev et al. 2014, Walker et al. 2015, Rojas et al. 2015), excluding the species presented herein. This phenomenon is comprehensible from a biodiversity-based perspective, since the species accumulation curve should effectively show slower rates of data accumulation over time as the collecting effort increased. However, in the case of Costa Rica, one noteworthy aspect of the species recorded in the interval of 2011–2015 is that they were rare species either collected in the field or obtained in moist chamber cultures using substrate material from previously studied areas (i.e., Cerro de la Muerte, Palo Verde National Park and the La Selva Biological Station). These results suggested that basic biodiversity surveys of myxomycetes in Costa Rica were not complete and still had the potential to yield interesting data for species distribution analysis, in particular when rare species were considered (see Rojas et al. 2015). In this manner and based on the information currently available, would researchers be able to find other non-previously recorded species for Costa Rica in poorly studied areas?

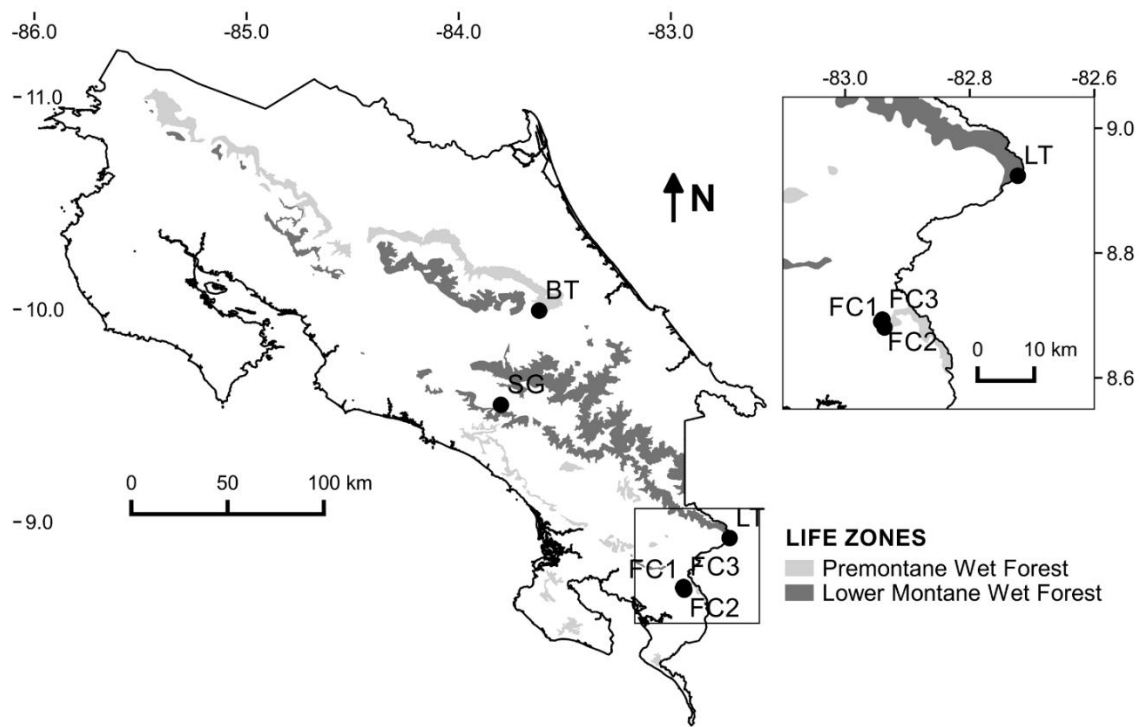
A potentially inviting quick hypothesis for such question is that poorly studied ecosystems would likely host different species assemblages than those previously studied due to the preference of particular species of myxomycetes for specific microhabitats and forest types, as noted from previous research (e.g., Schnittler & Stephenson 2000, Lado et al. 2013, Rollins & Stephenson 2013). However, there are limited data showing the potential of myxomycetes to migrate across forest stages, vegetation types and landscape-based variables. As such, the previous question is still current and remains to be appropriately tested. As a strategy to generate information on local distribution patterns, current efforts for studying myxomycete biodiversity in Costa Rica have been recently directed toward poorly studied ecosystems, geographical areas or microhabitats, and a higher number of isolation techniques have been used (from 2014 onwards). The idea of such an approach is to increase the body of data on myxomycetes for the different ecosystems, climatic and topographic zones in Costa Rica. Within that framework, seven new additions to the myxobiota of the country have been recorded in the last two years and they are presented herein.

## **Materials & Methods**

The present research effort was carried out in Costa Rica during 2014 and 2015. As previously mentioned, poorly studied ecosystems, forest types and microhabitats were targeted in an effort to continue generating myxomycete information for the country. In this manner and based on the data presented by Rojas et al. (2010), both premontane and lower montane wet forests have been studied, with particular focus on the mountainous southern Pacific and Caribbean sections. For the present study, selected research localities are shown in Figure 1 and included (1) the Las Tablas Protected Zone (abbreviated as LT and referred to as Las Tablas), (2) the Fila de Cal Mountains (FC, Fila de Cal) and (3) the Bonilla de Turrialba (BT, Bonilla). The first two localities are situated in the southern region of Costa Rica, within the County of Coto Brus and close to the towns of Ciudad Neily and San Vito. The third locality is situated on the central Caribbean region, in the Turrialba County and between the towns of Turrialba and Siquirres.

In all these localities, a series of samples of ground litter and twigs was obtained from different collecting sites and taken back to the Forest Resources Unit (ReForesta) of the University of Costa Rica for culturing and data recording. For this part of the study, the moist chamber technique as described by Stephenson & Stempen (1994) was used, and individual cultures were maintained for at least two months. After curation, all collected specimens were identified and deposited either in the Herbarium of the University of Costa Rica (USJ) or in the Myxogastrid Biorepository of the University of Costa Rica (BioRep) as required by Costa Rican legislation. The first repository has been used until 2014 and the second one, administrated by the Engineering Research Institute (INII) of the University of Costa Rica, has all myxomycete specimens obtained by the first author from 2015 onwards. The collecting sites within each locality shown in Figure 1 (labelled with numbers) correspond only to those specific sites that yielded the new records for Costa Rica presented herein.

In addition to the protocol recently explained, a series of unidentified collections deposited in the Herbarium of the University of Costa Rica were checked, identified and curated. Some of



**Fig. 1** – Map of Costa Rica showing the localities yielding the new records of myxomycetes from the country. Gray areas correspond to the extent of the forest types (life zones) studied and the insert map shows localities in the southern section of the country. Abbreviations are detailed in the Material and Methods section.

these myxomycetes were collected in (4) San Gerardo de Dota (abbreviated as SG, referred to as San Gerardo) during 2003 as part of an expedition of the first and third authors to that locality. From these collections, one specimen corresponding to a species not previously recorded in Costa Rica was identified and has been included in the present work. The last locality is within the County of Dota in the Central Pacific region.

In all cases, the morphological concept of species was used and the taxonomic treatment followed Lado (2005-2015).

## Results

As part of this survey, seven species representing new records for Costa Rica were identified. Five of those species were found on material collected in the southern section of the country, one in the Caribbean section and one in the Central Pacific region. These new records represent the species listed below.

### *Didymium melanospermum* (Pers.) T. Macbr.

From San Gerardo de Dota, 9.550443 N and 83.801267 W at 2430 m. Collected in the field on a decayed log in a successional section of a *Quercus* forest. In a tropical lower montane wet forest on the Pacific slope. Material collected on 1 February 2003. No pH was recorded. Collection Ro-333 (USJ 103556)

### *Didymium serpula* Fr.

From Fila de Cal, 8.680645 N and 82.936784 W at 602 m. Recorded in a moist chamber culture prepared with leaf litter from a disturbed area with a litter layer of 1 cm deep. In a tropical premontane wet forest on the Pacific slope. Material collected on 1 March 2014. pH = 7.4. Collection Ro-4159 (USJ 103059). New record for Central America.

***Licea castanea*** G. Lister

From Las Tablas Protected Zone, 8.923780 N and 82.722837 W at 1550 m. Recorded in a moist chamber culture prepared with leaf litter from the lower section of a ground litter layer 8 cm deep. In a tropical lower montane wet forest dominated by *Quercus* spp. on the Pacific slope of the country. Material collected on 20 April 2014. pH range = 4.31–5.44. Collections Ro-3968, 3969, 3974 and 3980 (USJ 103321, 103302, 103345 and 103226). New record for Central America

***Paradiacheopsis solitaria*** (Nann.-Bremek.) Nann.-Bremek.

Fig. 2

From Las Tablas Protected Zone, 8.923780 N and 82.722837 W at 1550 m. Recorded in a moist chamber culture prepared with leaf litter from the lower section of a ground litter layer 6 cm deep. In a tropical lower montane wet forest dominated by *Quercus* spp. on the Pacific slope. Material collected on 10 January 2014. pH = 4.31. Collection Ro-4920 (USJ 104006). New record for Central America.



**Fig. 2** – Images of two species not previously recorded in Costa Rica. Left. *Paradiacheopsis solitaria* (USJ 104006) from Las Tablas, scale is 100 µm. Right. *Physarum lateritium* (BioRep 6722) from Bonilla, scale is 1 mm. For details see the Materials and Methods section.

***Physarum aeneum*** (Lister) R.E. Fr.

From Fila de Cal, 8.689904 N and 82.94222 W at 714 m. Recorded in a moist chamber culture prepared with twigs found on the ground of a disturbed area. In a tropical premontane wet forest on the Pacific slope. Material collected on 1 March 2014. pH range = 6.91–6.93. Collections Ro-4295 and 4296 (USJ 103265, 103262). New record for Central America.

***Physarum galbeum*** Wingate

From Fila de Cal, 8.693301 N and 82.940177 W at 801 m. Recorded in a moist chamber culture prepared with twigs found on the ground of a disturbed area. In a tropical premontane wet forest on the Pacific slope. Material collected on 1 March 2014. pH = 4.73. Collection Ro-4208 (USJ 103004)

*Physarum lateritium* (Berk. & Ravenel) Morgan

Fig. 2

From Bonilla de Turrialba, 9.99433 N and 83.6210 W at 601 m. Recorded in a moist chamber culture prepared with leaf litter from a disturbed area. In a tropical premontane wet forest on the Caribbean slope. Material collected on 12 February 2015. pH = 6.32. Collection code BioRep 6277. New record for Central America.

## Discussion

Myxomycetes have been studied in Costa Rica for more than 100 years (see Rojas & Doss 2013). During this time, it has been only during the past 50 years or so that the increased effort has yielded the majority of species recorded in the country. For example, before the work of Alexopoulos & Sáenz (1975), there were only 15 species known from Costa Rica. Their work, carried out primarily during the late 1960's, brought the list up to 94 species. Thirty five years later Rojas et al. (2010) reported 208 species for the country. Even though many locations such as Cerro de la Muerte (including San Gerardo) have been revisited many times over this period of time, they still continue generating important information (see Leontyev et al. 2014) on the distribution of myxomycetes in Costa Rica and the Neotropics. However, a larger number of particular localities in Costa Rica have never been surveyed for myxomycetes, and the question as whether or not these places have the same assemblages as previously studied localities is still open and awaiting more distributional data. In the present study, Fila de Cal and Las Tablas represented two of those understudied localities, which after relatively little study seem to be very good areas for myxomycete research. The latter has already been explored to some extent in the recent past and has yielded good results (see Rojas & Calvo 2014), but this is apparently the first time Fila de Cal has been studied. Independent of the records from these two localities presented herein, about 90% of the moist chamber cultures from Fila de Cal were positive (either fruiting bodies or plasmodia were recorded) for myxomycetes (not shown before), also demonstrating that this is a good area for the study of myxomycetes in Costa Rica.

These results are particularly interesting due the geology and natural history of such a locality, which shows that these low-elevation mountains (the forearc of the Talamanca Mountain Range) appeared about 5 million years ago after heavy tectonic and seismic activity shaped southern Costa Rica (Zeilinga de Boer et al. 1995). Land colonization obviously took place more recently and was also determined by the migratory routes used by species from North and South America. If heavy land use in the area after humans arrived is also considered (see Clement & Horn 2001), it seems logical to think that both Fila de Cal and Las Tablas, but particularly the first locality, have represented areas with a high availability of niches for myxomycetes to occupy. After the present effort to study the myxomycete diversity in poorly studied locations in Costa Rica, the presence of new records for the country suggests that the southern section deserves additional study.

The last result may support the idea put forward by Schnittler & Stephenson (2000) that the conditions associated with different ecosystems support different assemblages of species, at least when the surveys are carried out at the morphological species level and do not consider molecular methods. Interestingly, according to Lado & Wrigley de Basanta (2008), five of the species presented herein also represent new records for the Central American region and have a limited distribution in the Neotropics. This is interesting, since it implies that a country like Costa Rica, with only 0.05% of the terrestrial surface of the earth, may account for approximately 25% of the global biodiversity of myxomycetes. Such results suggest that myxomycetes are indeed well adapted to the country, and perhaps by extension to the Neotropics. However, these results also demonstrate the need for continuing with basic myxomycete diversity surveys in tropical areas in order to better construct distributional models of the different species.

Interestingly, the premontane wet forest in which four of the seven species reported herein were found has already been noted as one of the more favorable life zones for myxomycete diversity in Costa Rica (Rojas et al. 2010). Once again, the implication of this result is that even

though there has been an increased effort to obtain biodiversity data on myxomycetes in Costa Rica during the last decades, there seems to be a lot of work that still needs to be carried out in order to reach a plateau in the species accumulation curve for the majority of life zones in the country. For the lower montane wet forest zone, the situation seems to be even more challenging, taking in consideration that less work has been carried out in this type of ecosystem. In spite of the latter, one important aspect of the present study is that it has also provided data on myxomycete distribution primarily in the southern section of the country, which has obviously helped fill in some informational gaps due to geographical undersampling. However, as already indicated, there is plenty of work on myxomycete diversity and distribution yet to be carried out in Costa Rica.

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### References

- Alexopoulos CJ, Sáenz JA. 1975 – The myxomycetes of Costa Rica. *Mycotaxon* 2, 223–271.
- Clement RM, Horn SP. 2001 – Pre-Columbian land-use history in Costa Rica: a 3000-year record of forest clearance, agriculture and fires from Laguna Zoncho. *The Holocene* 11, 419–426.
- Lado C 2005-2015 – An online nomenclatural information system of the Eumycetozoa. Real Jardín Botánico de Madrid. <http://www.nomen.eumycetozoa.com> (accessed on 14 July 2015).
- Lado C, Wrigley de Basanta D. 2008 – A Review of Neotropical Myxomycetes (1828-2008). *Anales del Jardín Botánico de Madrid* 65, 211–254.
- Lado C, Wrigley de Basanta D, Estrada-Torres A, Stephenson SL 2013 – The biodiversity of myxomycetes in central Chile. *Fungal Diversity* 59, 3–32.
- Leontyev D, Schnittler M, Moreno G, Stephenson SL, Mitchell D, Rojas C. 2014. The genus *Alwisia* (Myxomycetes) revalidated, with two species new to science. *Mycologia* 106, 936–948.
- Rojas C, Doss RG. 2013 – Brief research history and status of myxomycete conservation in the Neotropics. *Brenesia* 79, 37–43.
- Rojas C, Calvo E. 2014 – Additions to the myxobiota of Central America. *Mycosphere* 5(3), 488–495.
- Rojas C, Lado C, Valverde R. 2015 – First record of the myxomycete genus *Colloderma* in Central America. Checklist, DOI: 10.15560/11.4.1716.
- Rojas C, Schnittler M, Stephenson SL. 2010 – A review of the Costa Rican myxomycetes (Amebozoa). *Brenesia* 73–74, 39–57.
- Rollins AW, Stephenson SL. 2011 – Global distribution and ecology of myxomycetes. *Current Trends in Plant Biology* 12, 1–14.
- Rollins AW, Stephenson SL. 2013 – Myxomycetes associated with grasslands of the western central United States. *Fungal Diversity* 59, 147–158.
- Schnittler M, Stephenson SL. 2000 – Myxomycete biodiversity in four different forest types in Costa Rica. *Mycologia* 92, 626–637.
- Stephenson SL, Stempen H. 1994 – *Myxomycetes: a handbook of slime molds*. Timber Press, Oregon.
- Stephenson SL, Schnittler M, Lado C, Estrada-Torres A, Wrigley de Basanta D, Landolt JC, Novozhilov YK, Clark J, Moore DL, Spiegel FW. 2004 – Studies of Neotropical mycetozoa. *Systematics and Geography of Plants* 74, 87–108.

- Walker L, Rojas C, Stephenson SL. 2015 – The myxomycetes of the La Selva Biological Station. *Austrian Journal of Mycology* 24 (in press).
- Zeilinga de Boer J, Drummond MS, Bordelon MJ, Defant MJ, Bellon H, Maury RC. 1995 – Cenozoic magmatic phases of the Costa Rican island arc (Cordillera de Talamanca). *GSA Special Papers* 295, 35–56.