

First record of association of gall midges (Cecidomyiidae, Diptera) with a slime mold (*Fuligo candida*, Myxomycetes) in the tropics

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Abstract: Associations of Diptera with slime molds have hitherto been recorded only for the Palearctic region. Dipteran pupal exuviae were found on two fruiting bodies of the slime mold *Fuligo candida* on a living mango tree stem in the tropical lowlands of Panama. During incubation of the fruiting bodies, adult flies emerged which were identified as Cecidomyiidae, whereas a fruiting body of *F. rufa*, also from an epiphytic habitat nearby, did not yield Diptera. The possible specificity of this first record about gall midges associated with *Fuligo* slime molds is discussed. Since previous records of *Fuligo* in Panama identified only *F. septica*, the taxonomy of the slime mold specimens is presented in detail.

Key words: Fungivorous gnats, insect-fungus interaction, Myxomycota, neotropical.

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Few Diptera have been recorded to be associated with slime molds (myxomycetes) compared to other groups of fungi. Published reports include species of Anthomyiidae, Lonchaeidae, Mycetophilidae, Sciaridae and Sphaeroceridae from the plasmodia or fruiting bodies of *Fuligo septica* (L.) F. H. Wigg. (Krivosheina 2008; Ševčík 2010). Certain mycetophilids of the genera *Mycetophila* and *Platurocypta* seem to be specific to myxomycetes, whereas Anthomyiidae, Drosophilidae, Empididae, Lonchaeidae, Scatophagidae, Sciaridae, Sepsidae and Sphaeroceridae are considered nonspecific occasional colonizers of plasmodia or fruiting bodies of myxomycetes (Jakovlev 2011; Krivosheina 2008; Ševčík 2010). The single record of Cecidomyiidae from myxomycetes is from England (Buxton 1954). Information about the taxonomy and geographical distribution of fungivorous Cecidomyiidae is widely lacking for the tropics; even in Europe new taxa and records are continuously published (Sikora *et al.*

2017). The role of Cecidomyiidae as pollinators in the tropics is also poorly known (Damon & Salas-Roblero 2007).

In Panama, more than 130 species of Myxomycetes have been recorded (Lado & Wrigley de Basanta 2008; Martin 1938; Piepenbring 2006; 2013; Welden 1954). Among the species of *Fuligo*, hitherto only *F. septica* has been recorded (Piepenbring 2013). The taxonomy of the *F. septica* species complex is mainly based on color characteristics of fruiting bodies. Because of the high color variation from pure white to bright yellow and rusty red, several varieties or species have been segregated by some authors, whereas others consider these segregate taxa as a single species. In contrast to the advanced progress of DNA barcoding in true fungi, developing molecular tools for routine species identification in myxomycetes is in its pioneer phase, but first insights indicate cryptic taxa within morphospecies presently considered to have wide morphological

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variation and global distribution (Schnittler *et al.* 2017).

The study reported herein was carried out in the tropical lowland (altitude 36 m a.s.l.) of western Panama during the period July-August of the rainy season. Fruiting bodies (aethalia) of *Fuligo* species were photographed in situ and collected at the campus and adjacent forest of the Universidad Autónoma de Chiriquí, David, Chiriquí Province, Panama. The aethalia were kept in a moist chamber for one week. Insects emerging in the moist chamber were preserved in 95% ethanol and identified based on wing venation patterns under dissecting and light microscopes. Microscopic characteristics of the slime molds were investigated in 5% KOH. The fungal and insect specimens were deposited in the herbarium of the Universidad Autónoma de Chiriquí (UCH) and in the Museo de Peces de Agua Dulce e Invertebrados (MUPADI), respectively, both in David, Chiriquí Province, Panama.

Fuligo candida Pers. Figs. 1, 2. Fruiting bodies solitary, sessile, pulvinate, 20–32 mm long and 7–16 mm wide, and 5–7 mm high (n = 3). Cortex white, crustose, fragile. Hypothallus membranous to crustose, yellowish to rusty reddish, extending up to 5 mm from beneath the cortex. Capillitial threads filamentous, simple or branched, delicate, pure white under the dissecting microscope, colorless in the light microscope, lime nodes scattered, up to 4 µm wide, colorless. Spores black in mass, dark brown by transmitted light, minutely and densely verruculose, globose, 7–8 µm dia. (n = 30). Specimen examined: Panama, Chiriquí, David, Universidad Autónoma de Chiriquí, on bark of living stem of *Mangifera indica*, 29. July 2016, R. Kirschner 4303 (UCH).

Fuligo rufa Pers. Fig. 3. Fruiting body sessile, irregularly pulvinate with some compartments on a tree stem and the other ones extending to a living leaf touching the stem, all connected by thinner threads, compartments about 17–25 mm long, 8–17 mm wide, and 2–5 mm high (n = 2). Cortex reddish, crustose, scaly, fragile. Hypothallus membranous to crustose, yellowish to rusty reddish. Capillitial threads filamentous, simple or branched, delicate, dirty yellowish to pure white under the dissecting microscope, yellowish in the light microscope, lime nodes not found. Spores black in mass, dark brown by transmitted light, minutely and densely verruculose, globose, (6.5–) 7 (–7.5) µm dia. (n = 30). Specimen examined: Panama, David, Universidad Autónoma de Chiriquí, forest of Jardín Botánico, on

tree stem and adjacent living leaf, 5. August 2016, R. Kirschner *et al.* 4320 (UCH).

Three aethalia of *F. candida* were removed from the subtending bark and investigated. Two fruiting bodies appeared somewhat older by their comparatively smooth surface and compacted consistency, whereas the third had an intact rough surface and disintegrated during sampling. No insects were found in its remnants. The peridium of the two older ones each contained ca. 5–10 pupal exuviae with immersed base and protruding exit hole (Fig. 4). Four flies were reared from the two aethalia and identified as Cecidomyiidae based on the wing venation (Figs. 5–7), number and size of the tarsal segments and number of antennal flagellomeres (Gagné 1983). Based on our illustrations, the identification was confirmed by a taxonomic specialist (J. Ševčík, Czech Republic). No exuviae were found in the apparently younger aethalium of *F. candida* and in the aethalia of *F. rufa*.

For *Fuligo* species, only *F. septica* has been listed from Panama without differentiating between the typical yellow and deviating white and reddish forms (Martin 1938; Standley 1933; Welden 1954). Farr (1976) considered these forms as conspecific, whereas in other publications these forms are separated on the level of species or infraspecific taxa (Chen *et al.* 2005; Davison *et al.* 2017; Kryvomaz *et al.* 2017; Neubert *et al.* 1995; Poulain *et al.* 2011). While *F. candida* is distinguished by its white color, there are deviating concepts for *F. rufa* (Neubert *et al.* 1995). The taxonomical problem persists also in recent new Neotropical records of *F. septica* (Lima & Cavalcanti 2017). Piepenbring (2006) presented a photo labeled as *F. septica* from Panama showing a strongly diverticulated white fruiting body extending over living herbaceous plants, which is also reminiscent of *Mucilago crustacea* P. Micheli ex F. H. Wigg.

In this study, all *Fuligo* fruiting bodies were epiphytic on living plants, whereas in temperate regions, *Fuligo* species are predominantly found on the ground or dead plant remains and rarely on living trees up to two meters high (Everhart & Keller 2008). *Fuligo rufa* was spreading partly onto a tree stem and an accidentally attached living leaf of another plant and, therefore, exhibited an irregular shape. A similar growth and habitat was reported for a specimen of *F. septica* on leaves and trunk of a living tree in Ecuador (Lado *et al.* 2017). Whether or not *Fuligo* species in the tropics occur more frequently on living trees than in temperate



Fig. 1. Fruiting body of *Fuligo candida* on bark of living stem of mango tree.

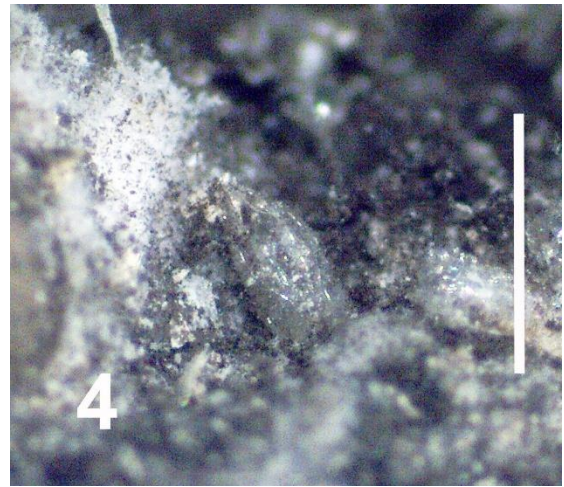


Fig. 4. Half-immersed pupal exuvia of undetermined Cecidomyiidae on the fruiting body of *F. candida* shown in Fig. 1. Scale bar 1 mm.

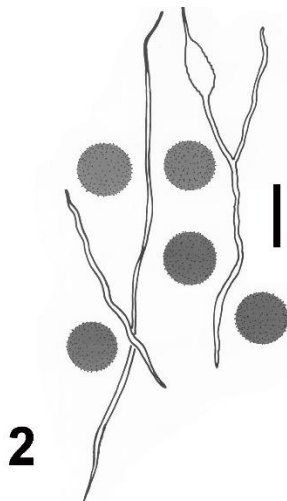


Fig. 2. Spores and capillitium of *F. candida*. Scale bar 10 µm.

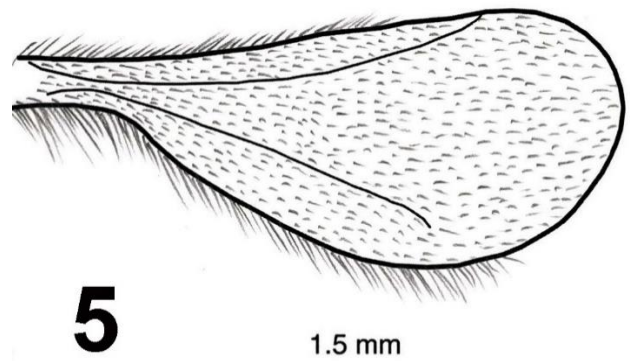


Fig. 5. Wing of undetermined Cecidomyiidae bred from *F. candida*. Drawing by Z. Serracin.



Fig. 3. Fruiting body of *Fuligo rufa* on bark of living stem of unidentified tree. Scale bar 15 mm.



Fig. 6. Undetermined Cecidomyiidae bred from *F. candida*, with focus on body.



Fig. 7. Undetermined Cecidomyiidae bred from *F. candida*, with focus on wings.

regions requires further study. Epiphytic slime molds are rarely investigated compared to epiphytic angiosperms and lichens (Joshi *et al.* 2016; Mondragón *et al.* 2015).

Size, shape and color of fruiting bodies are variable in *Fuligo* species, particularly in the *F. septica* species complex. Several collections labeled as *F. septica* in Germany appeared uniform with respect to the selected DNA region, but whether the species concept was broad or narrow, was not specified (Feng & Schnittler 2017). We expect that molecular tools will reveal significant characteristics for distinguishing taxa in the *F. septica* species complex and, therefore, presented the morphological annotations and anticipated distinct species names instead of merely lumping this information under the name "*F. septica*".

Since *F. septica* and two other slime molds are collected and consumed as food in Mexico, Stijve & Sobestiansky (2003) analyzed the nutritious values of these three species. They found similar compositions of lipids, proteins and other substances as present in common edible mushrooms, but only in *F. septica* a high content of minerals (35% of dried fruiting body) mainly containing calcium, but also zinc and manganese. Such particular nutritious values may be the basis for consumption of certain slime molds by specific insects.

Associations between Diptera and myxomycetes have hitherto only been studied in the Palearctic region (Krivosheina 2008). In spite of intensive research of Palearctic fungivorous Diptera, even in Central Europe new taxa and new records are continuously published (Sikora *et al.* 2017). Some Diptera appear to be highly specifically associated with slime molds (Jakovlev 2011), whereas several other species are only occasionally reared from myxomycetes (Krivosheina 2008). According to Krivosheina (2008), larvae of Cecidomyiidae rather live as epibionts on the surface of fungal mycelia than as endobionts within fungal fruiting bodies. Myxomycetes are not mentioned among the fungal substrates for the European Cecidomyiidae in Skuhrová & Skuhrový (2009). To our knowledge, Buxton (1954) presented the single record of Cecidomyiidae from myxomycetes. He reared two specimens of an unidentified gall midge and one specimen identified as *Brittenia fraxinicola* Edwards from two separate collections of *Arcyria incarnata* (Pers.) Pers. in Britain, and a single specimen of *Bremia* s. lat. (Diptera, not the homonymous genus of Oomycota!) from a single collection among 14 ones of *Lycogala epidendrum* (J. C. Buxb. ex L.) Fr. The midge *Brittenia fraxinicola* is usually found below the bark of trees (Mamaev & Krivosheina 1993). Because of the small number of gall midges per slime mold, incubation of the slime molds together with the subtending woody substrate, and missing observation of exuviae (Buxton 1954), it is not clear whether the insects had emerged from the fungus or the woody substrate. In our study, we found several exuviae on each of two fruiting bodies by direct observation, incubated the fruiting bodies without the supporting bark, and reared adult flies from them. In contrast to the dead woody substrate used by Buxton (1954), the thalli in our study were found on healthy bark of living stems so that accidental contamination with saprobic or mycelium-feeding larvae is less likely. Our observation, therefore, indicates a more specific association of Cecidomyiidae with myxomycetes than that from Buxton (1954).

While many slime molds have a worldwide distribution, the potentially associated insects are restricted to particular areas. While some Mycetophilidae are specific to slime molds in the Palearctic region, our study indicates that certain Cecidomyiidae may be specific for slime molds in

the tropics. More observations about this association and species identification of the gall midges are required.

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