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# Specific and promiscuous ophiostomatalean fungi associated with Platypodinae ambrosia beetles in the southeastern United States

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ABSTRACT

Ambrosia beetles in the subfamily Platypodinae (Coleoptera: Curculionidae) have been farming fungi for over 50 million y, yet they remain understudied and most of their fungal symbionts are unknown. We identified fungal communities associated with all four platypodine species native to the southeastern United States: *Euplatypus compositus*, *Euplatypus parallelus*, *Myoplatypus flavicornis*, and *Oxoplatypus quadridentatus*. Forty-eight samples were analyzed by quantitative culturing and DNA sequencing. Phylogenetic analyses of 28S rDNA sequences revealed that the four platypodines were routinely associated with several genera in the Ophiostomatales. *E. compositus* is associated primarily with *Raffaelea campbellii* 1 and *Raffaelea* sp. 6 and, to a lesser extent, *Raffaelea* sp. 2. *M. flavicornis* is associated with *Raffaelea* sp. 5. *E. parallelus* and *O. quadridentatus* are less specific; the latter mostly associated with *Raffaelea cyclorhipidia*. Three of the four beetle species were also associated with *Ceratocystiopsis* spp. This is the first report of *Raffaelea* associated with *E. parallelus*, which is invasive in Asia and Africa.


## 1. Introduction

The weevil subfamily Platypodinae comprises more than 1400 species, of which the vast majority are distributed in tropical regions and fewer than 10 species are found in wet temperate areas (Wood, 1993; Jordal, 2015). All but two species in the subfamily Platypodinae maintain nutritional symbioses with fungal cultivars (Jordal, 2014). Platypodinae and fungus-feeding Scolytinae have similar ecologies and together comprise the polyphyletic "ambrosia beetles". However, Platypodinae have a closer relationship with the weevil subfamily Dryophthorinae, which lacks fungal mutualists, than with the Scolytinae (McKenna et al., 2009; Gillett et al., 2014). The subfamily Platypodinae is estimated to have arisen in the mid-Cretaceous (119–88 Ma), much earlier than Scolytinae and the other fungus-farming insects (Jordal, 2015), and thus they represent the oldest known fungus-farming system.

As in scolytines, platypodine ambrosia beetles are typically attracted to dead or severely declining trees (Hubbard, 1896; Jordal, 2015). They cultivate communities of fungi in galleries in trees as the sole food for their larvae (Nobuchi, 1993; Jordal, 2014). Previous studies indicate that the symbiotic fungal communities from platypodines are dominated by ambrosia fungi in the genus *Raffaelea* (Ascomycota: Ophiostomatales) and other members of the Ophiostomatales, in addition to unrelated *Ambrosiozyma* yeasts (Beaver, 1989; Kubono, 2002; Belhoucine et al., 2011; Bellahirech et al., 2014; Dreaden et al., 2014; Musvuugwa et al., 2015; Yun et al., 2015; Hulcr and Stelinski, 2017). Symbiotic fungi function primarily as a nutritional resource but in several cases have been shown to be important plant pathogens (Six, 2003; Kobayashi and Ueda, 2005; Kinuura and Kobayashi, 2006). As vectors of pathogenic fungi, some platypodines are considered forest pests (Kile and Hall, 1988; Massoumi Alamouti et al., 2009; Inácio et al., 2012a). Their associated fungi cause staining of the wood around the galleries (Fig. 1), resulting in the downgrading of timber quality (Beaver, 2013). *Platypus quercivorus* and its fungal symbiont,

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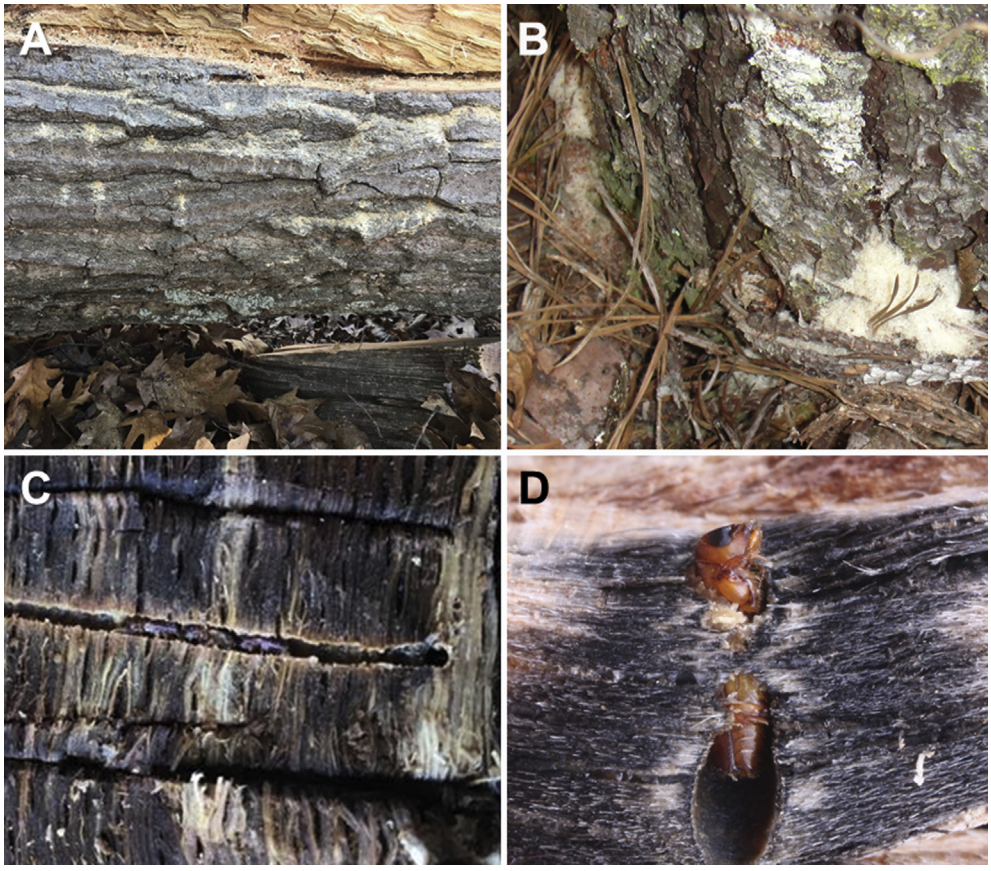


**Fig. 1.** Signs of platypodine infestation and associated ophiostomatalean fungal colonization. A. *Oxoplatypus quadridentatus* infesting a red oak (*Quercus rubra*); B. *Myoplatypus flavicornis* infesting a loblolly pine (*Pinus taeda*); C. associated fungal colonization and staining of surrounding *O. quadridentatus* galleries; and D. *Euplatypus parallelus* in gallery of *Acacia mangium* with associated staining of surrounding wood as a result of fungal colonization.

*Raffaelea quercivora,* cause significant mortality of oak trees in Japan (Kubono, 2002; Ito et al., 2003). Similarly, *Platypus cylindrus* is capable of killing European oaks, assisted by its nutritional symbiont *Raffaelea montetyi* (Belhoucine et al., 2011; Inácio et al., 2012a). *Megaplatypus mutatus*, which is associated with several *Raffaelea* species, attacks tree plantations in Argentina and Italy (Alfaro et al., 2007; Ceriani-Nakamurakare et al., 2016).

The study of ambrosia beetles and their symbiotic fungi is increasingly important as the international trade in lumber continues to grow and more exotic species are introduced (Brockerhoff et al., 2006). Although platypodines and their fungi have received some attention in Asia, Europe, and Oceania (Faulds, 1977; Kubono, 2002; Inácio et al., 2012a; Tarno et al., 2016), the fungal symbionts of the American fauna are poorly known (Batra, 1963; Farris and Funk, 1965; Ceriani-Nakamurakare et al., 2016). Compared with the high diversity of platypodine species in Asia, Africa, and the Neotropics, few species inhabit North America, with only seven species described to date (Wood, 1993). Four of these, *Euplatypus compositus*, *Euplatypus parallelus*, *Myoplatypus flavicornis*, and *Oxoplatypus quadridentatus*, are present in the southeastern United States (Atkinson, 2004).

The fungi associated with the four platypodines from the southeastern United States have not previously been studied. Two of these species, *M. flavicornis* and *O. quadridentatus,* are unusual in that their biology has never comprehensively been studied, let alone their relationships with symbiotic fungi. Based on previous collection information, *M. flavicornis* usually infests recently dead pine trees (*Pinus*) along with pine-feeding bark beetles (Curculionidae: Scolytinae), whereas *O. quadridentatus* prefers oak (Fagaceae) (Atkinson and Peck, 1994; Atkinson, 2004). *E. compositus* and *E. parallelus* are locally common and easily attracted to light traps. In the few reports of fungal isolations from southeastern platypodines, Batra (1963) and Verrall (1943) isolated the yeast fungus *Ambrosiozyma monospora* from *E. compositus* in Mississippi. *E. parallelus* is native throughout the tropical and subtropical regions in the Americas, but it has recently become an invasive species internationally and is now found throughout Africa, Asia, and parts of Oceania (Beaver, 2013; Gillett and Rubinoff, 2017). This beetle species has been reported to attack living rubber trees *Hevea brasiliensis* in Brazil (Pereira da Silva and Putz, 2013) and China (Li et al., 2018), as well as Indian rosewood, *Dalbergia sissoo,* in Bangladesh (Boa and Kirkendall, 2004). Several reports from Asia indicated it as a suspected vector of fungal pathogens of Burmese rosewood *Pterocarpus indicus* (Sanderson et al., 1996; Boa and Kirkendall, 2004; Bumrungsri et al., 2008; Tarno et al., 2016).

Studying the fungal symbionts of platypodine species is difficult for three reasons: (1) Platypodine species often colonize deep within the lower trunk of large trees (Fig. 1A) (Atkinson and Peck, 1994), making the collection of these beetles laborious. (2) The presence and location of mycangia (organs where the nutritional symbiont is concentrated and transported to new host trees) of most platypodine species are uncertain despite the presence of superficial pronotal pits on several of these species (Nakashima, 1975; Wood, 1993; Hulcr and Stelinski, 2017). (3) The phylogenetic placement of their most commonly reported fungal symbionts in the Ophiostomatales has not fully been resolved, leading to