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Association of *Geosmithia* fungi (Ascomycota: Hypocreales) with pine- and spruce-infesting bark beetles in Poland

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ABSTRACT

Bark beetles (Coleoptera, Scolytinae) are known to be associated with fungi, particularly species of the orders Ophiostomatales and Microascales. However, very little is known about other ectosymbionts of phloeophagous bark beetles on Pinaceae. In this study, we examined the *Geosmithia* species associated with eight bark beetle species infesting *Picea abies* and *Pinus sylvestris* branches in Poland. Fungi were isolated from 1 731 samples collected from 14 study sites. We identified a total of 653 isolates that were sorted into nine taxa based on their phenotypic similarity and phylogeny of their ITS-LSU regions of rDNA, β-tubulin, elongation factor 1α and the second-largest subunit of the RNA polymerase II gene. They represented nine species without formal names. There were large quantitative and qualitative differences in the composition of *Geosmithia* communities between *P. sylvestris* and *P. abies* trees. The proportion of samples infested with *Geosmithia* species suggests that this association is more widespread among bark beetles infesting branches of *P. sylvestris*. In addition, these beetles were vectors of different *Geosmithia* species compared with than the beetles that colonize *P. abies*. In mixed-conifer forests, the *Geosmithia* communities were more diverse and richer than in pure spruce or pine stands, where the insects *Pityogenes chalcographus* and *Pityophthorus pityographus* with low host-specificity play a distributing role for various *Geosmithia* species. Among eight bark beetle species examined, only *P. bidentatus*, *P. pityographus*, *P. chalcographus* and *Polygraphus poligraphus* acted as effective vectors for *Geosmithia* species. The following hypothesis emerges from these studies: changes in the composition of ectosymbionts of pine- and spruce-infesting bark beetles in Central Europe run along a gradient of thickness of the wood substrata preferred by insects.

Introduction

Many phloem-feeding bark beetles live in association with wood-inhabiting fungi, carrying them in their digestive tracts, directly on the body surface or in specialized ectodermal invaginations defined as mycetangia or mycangia (Francke-Grosmann, 1956). Bark beetles are commonly associated with the so-called ophiostomatoid fungi. These ascomycetes

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have morphologically similar sexual stages, occur in two phylogenetically unrelated orders, Microascales and Ophiostomatales, and are involved in various types of insect-fungus symbioses (from obligate mutualism to parasitism) (Kirisits, 2004; Six, 2012; De Beer and Wingfield, 2013). Fungi associated with bark beetles may provide a main or supplementary source of food for larvae and teneral adults, play an active role in recycling of limiting elements (C, N, P, S) from insect excrements and can convert beetle pheromones (Ayres et al. 2000; Harrington, 2005; Vega and Dowd, 2005). Ophiostomatoid fungi are introduced to trees during beetle attacks, which allows them to settle in almost sterile wood and thus avoid competition with other wood-colonizing fungi. However, only some ophiostomatoid species are aggressive tree-invaders capable of surviving in the very fresh tissues of infested trees (e.g., *Ophiostoma novo-ulmi* or *Leptographium wageneri*), and these species are considered as causal agents of dangerous tree diseases (Six and Wingfield, 2011).

In addition to ophiostomatoid species, the genus *Geosmithia* (Ascomycota: Hypocreales) includes many bark beetle associates. *Geosmithia* species develop stable symbiosies with different bark beetle species and even resemble ophiostomatoid fungi in their host affinity, life strategy evolution and pathogenicity (Kubátová et al. 2004; Kolařík, 2006; Kolařík et al. 2007, 2008; Kolařík and Kirkendall, 2010; Kolařík et al. 2011; Kolařík and Jankowiak, 2013). This association has been well studied for bark beetles infesting broad-leaved trees and conifers of the family Juniperaceae in temperate and Mediterranean Basin regions (Kolařík et al. 2007, 2008). In this area, *Geosmithia* communities contain 23 tentative species, of which six have been formally named so far.

Information about bark beetle-associated *Geosmithia* species on trees of the Pinaceae family is very limited. Only four published studies have described the occurrence of these fungi on Pinaceae in Europe (Kirschner, 2001; Kolařík et al. 2008; Jankowiak and Kolařík, 2010). Specific relationships are suggested for *Geosmithia* species and *Cryphalus piceae* infesting *Abies alba* (Jankowiak and Kolařík, 2010). Another *Geosmithia* species is commonly isolated from beetles and galleries of *Pityogenes bidentatus* in young managed Scots pine in Poland, suggesting a specific relationship (Jankowiak and Rossa, 2008). In a recent study, Kolařík and Jankowiak (2013) provided clear evidence of widespread and diverse associations among bark beetles living on plants of the Pinaceae family and *Geosmithia* species. They isolated 12 *Geosmithia* species from 85 conifer samples containing 23 subcortical insect species. Among them, ten *Geosmithia* species were found only in Pinaceae specimens. However, due to a high level of heterogeneity in the quality and size of the samples, the authors of this report were not able to determine the relationship among the *Geosmithia* species, their vectors and their tree hosts.

Previously, surveys of fungi obtained from bark beetles infesting coniferous trees have focused mainly on ophiostomatoid fungi because they cause significant economic losses to the forest product industry. *Geosmithia* species and other fungi that are also typically associated with these insects appear to have been systematically overlooked. Studies providing abundance data on all members of the cultivated mycobiome have shown that *Geosmithia* species are the predominant associates of *C. piceae* and *P. bidentatus* (Jankowiak and Rossa, 2008; Jankowiak and Kolařík, 2010). *Geosmithia* species, by contrast, have not been detected in a number of comprehensive surveys of the mycobiota associated with *Hylurgops palliatus* (Kirschner, 2001; Jankowiak, 2006a), *Tomicus piniperda* (Jankowiak, 2006b; Jankowiak and Bilański, 2007), *T. minor* (Jankowiak, 2008), *Dryocoetes autographus*, *Ips sexdentatus*, *P. chalcographus*, *Polygraphus poligraphus*, *Trypodendron lineatum* (Kirschner, 2001) and *I. typographus* (Kirschner, 2001; Jankowiak, 2005). These data indicate that species attacking conifers, or at least particular beetle populations, do not have stable associations with *Geosmithia* fungi. Jankowiak and Rossa (2008) proposed the hypothesis that bark beetles which breed in drier substrata (e.g., *P. bidentatus*) are unable to maintain mutualism with ophiostomatoid fungi and are involved in symbioses with *Geosmithia* species. This study indicated that these fungi are very well adapted for colonization of strongly heated and dried tree tissues (e.g., thin branches of trees). Moreover, it has been demonstrated that colonies of *Geosmithia* isolates originating from beetles and galleries of *P. bidentatus* grow well *in vitro* at 35 °C (Jankowiak, 2011). We therefore suspected that *Geosmithia* species are commonly associated with bark beetle species infesting branches of *Picea abies* and *Pinus sylvestris*. We also believe that the presence of some *Geosmithia* species is strongly correlated with specific host beetles, host trees or forest types.

The relationship between bark beetles and ophiostomatoid fungi on branches of *P. sylvestris* and *P. abies* investigated using the same research material has been described in previous reports (Jankowiak et al. 2009; Jankowiak and Kot, 2011). The results of these investigations showed that all bark beetle species on spruce branches except *Pityophthorus pityographus* are frequently associated with ophiostomatoid fungi. In contrast, except for *P. chalcographus*, pine-infesting bark beetles are rarely associated with these fungi.

The objective of the present study was to survey *Geosmithia* species associated with several bark beetle species that infest the branches of *P. sylvestris* and *P. abies* in Poland, and to define the relationships between them and their host trees, vectors and forest types. This is the first study to provide abundant data on *Geosmithia* species associated with bark beetle assemblages which attack the two most common conifers in Europe.

Materials and methods

Study areas and collection of bark beetles and galleries

Fourteen research sites, covering four forest types in southern Poland, were sampled to characterize the community of *Geosmithia* spp. associated with bark beetles inhabiting the branches of *P. abies* (Norway spruce) and *P. sylvestris* (Scots pine) (Fig 1). Four forest types, characterized by different proportions of pine, spruce and mixed (two types) tree species were studied (Table 1).

Overwintering adults had established brood galleries and gallery systems were collected from windblown trees at all of the sites. Samples were collected 2–4 weeks after the major