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Phylogenetic and phenotypic characterisation of *Sirococcus castaneae* comb. nov. (synonym *Diplodina castaneae*), a fungal endophyte of European chestnut

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ABSTRACT

In this paper we resolve the taxonomic status of the fungus *Diplodina castaneae* (Ascomycetes, Diaporthales, Gnomoniaceae) which occurs on the European chestnut (*Castanea sativa*) as endophyte and as the causal agent of Javart disease. Specimens from Switzerland, Spain, and Azerbaijan were sequenced at five nuclear loci (β-tubulin, EF-1α, ITS, LSU, and RPB2). Phylogenies were inferred to place *D. castaneae* in the Gnomoniaceae family. Moreover, growth rates and morphological characteristics on different agar media were assessed and compared to those of *Gnomoniopsis castaneae*, which can easily be confused with *D. castaneae*. Based on morphological and phylogenetic characteristics, we propose to reallocate *D. castaneae* to the genus *Sirococcus*, as *S. castaneae* comb. nov.

Introduction

The European chestnut (*Castanea sativa* Mill.) is a multipurpose tree species in Europe that has been cultivated for centuries for its edible nuts and valuable wood (e.g. high durability) (Conedera *et al.* 2004). Chestnut stands are thus considered as significant agro-forest ecosystems of great ecological, recreational, and cultural value. Unfortunately, several

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pathogens and pests affect *C. sativa* in Europe, among them the introduced chestnut blight fungus *Cryphonectria parasitica* (Murr.) Barr (Ascomycetes, Diaporthales, Cryphonectriaceae) and the chestnut gall wasp *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera, Cynipidae). *Cryphonectria parasitica* is a wound pathogen that infects the bark and cambium of susceptible chestnut (*Castanea*) species, causing perennial cankers on the stem and branches (Rigling & Prospero 2017). The pathogen was first introduced into Europe at the end of the 1930s and is now widespread in all of the major European regions where *C. sativa* occurs. *Dryocosmus kuriphilus*, a gall wasp native to China, is considered as the most significant pest for *Castanea* species worldwide (EPPO 2005). In Europe, this insect was detected for the first time in 2002 in Italy, and in the following years it rapidly spread to various countries (Brussino *et al.* 2002; Avtzis & Matošević 2013). The presence of galls is usually non-lethal for the affected tree, but leads to reduced growth and fruit production (e.g. Kato & Hijii 1997; Sartor *et al.* 2015).

When attempting to isolate *C. parasitica* from abandoned *D. kuriphilus* galls (i.e. necrotic, empty galls, after the emergence of the adults; Meyer *et al.* 2015) and chestnut blight cankers in Switzerland, Spain, and Azerbaijan, a fungus was recurrently recovered. On potato dextrose agar (PDA), this fungus showed a culture morphology similar to that of *Gnomoniopsis castaneae* Tamietti (syn. *G. smithogilvyi* L.A. Shuttleworth, E.C.Y. Liew & D.I. Guest), a chestnut endophyte which also causes brown rot of chestnut fruits (Sieber *et al.* 2007; Shuttleworth 2012; Visentin *et al.* 2012; Maresi *et al.* 2013; Shuttleworth *et al.* 2015; Dennert *et al.* 2015; Lione *et al.* 2016) and is frequently reported on necrotic chestnut galls (Magro *et al.* 2010; Vannini *et al.* 2017). Recently, Pasche *et al.* (2016) showed that *G. castaneae* can also induce bark cankers on European chestnut. Comparing the internal transcribed spacer (ITS) sequence of this unknown fungal species against reference sequences in GenBank (www.ncbi.nlm.nih.gov) revealed a complete match with the specimen *Gnomoniopsis* sp. ICMP14082 (KC145849). Hence, we initially defined this fungus as *Gnomoniopsis* sp. (Meyer *et al.* 2015). However, additional microscopic analyses that we performed using the key proposed by Bissegger & Sieber (1994) revealed that, based on morphology and size of the asexual spores (conidia), the unknown fungus was actually *Diplodina castaneae* Prill. et Del. (Ascomycetes, Diaporthales, Gnomoniaceae) rather than a *Gnomoniopsis* species.

Diplodina castaneae has been previously found as an endophyte or pathogen on *C. sativa* in several European countries, including Belgium, Bulgaria, England, France, Germany, Italy, Slovakia, and Switzerland (Saccardo 1895; Day 1930; Bissegger & Sieber 1994; Vanev *et al.* 1997; Adamčíková *et al.* 2013). In Japan, *D. castaneae* occurs on the Japanese chestnut *Castanea crenata* (Farr & Rossman 2013). In Europe, this species is the causal agent of the 'maladie de Javart', which has been documented in France since the end of the 19th century (Prillieux & Delacroix 1893). The disease is recognisable by deep, small, elongated cankers appearing as bright brown patches on the bark, frequently beginning at the stem base (Prillieux & Delacroix 1893; Day 1930). The infected bark seems to have been severely bruised and becomes depressed. Subsequently, it dries up and falls off in patches, exposing the wood. *Diplodina castaneae* is considered to be an endophyte of the European chestnut (Bissegger & Sieber 1994). However, in coppice stands it can eventually kill young shoots that have been weakened by other biotic or abiotic stresses (Prillieux & Delacroix 1893; Saccardo 1895; Day 1930; Berthon *et al.* 1953).

Grove (1935) and Ellis & Ellis (1997) considered *D. castaneae* to be the anamorphic state of *Cryptodiaporthe castaneae* (Tul. & C. Tul.) Whem., whereas Bissegger & Sieber (1994) found *D. castaneae* and *Discella castaneae* (Sacc.) Arx (the anamorphic state of *C. castaneae* according to Smith *et al.* (1988)) to be distinctive species based on culture morphology and conidial dimensions. *Diplodina* species do not form a taxonomically homogeneous group because they are synonyms of species in many other genera (e.g. *Ascochyta*, *Discella*, *Microdiplodia*, and *Phloeospora*). The type species of *Diplodina* Sutter is *Diplodina salicis* Westend. 1857 (Rosmann *et al.* 2015) which includes *Diplodina microsperma* B. Sutton 1977, known to be the anamorph form of *Plagiostoma apiculatum* (Wallr.) L. C. Mejía (Mejía *et al.* 2011). For this reason, Sogonov *et al.* (2008) used the name *Plagiostoma* instead of *Diplodina* in their analysis, even if the type species *Plagiostoma euphorbiae* (Fuckel) Fuckel was first described only in 1870 (Rossman *et al.* 2015).

The morphology of *D. castaneae* has been described by several authors (Prillieux & Delacroix 1893; Day 1930; Grove 1935; Bissegger & Sieber 1994; Ellis & Ellis 1997; Adamčíková *et al.* 2013). The taxonomic status of *D. castaneae*, however, is still unresolved. In this study, we aimed to shed light on the taxonomic position of *D. castaneae*, in particular regarding its relationship with the genera *Plagiostoma* and *Gnomoniopsis*. To achieve this goal, specimens from Switzerland, Spain, and Azerbaijan were sequenced at five nuclear loci (β-tubulin, EF-1α, ITS, LSU, and RPB2) and phylogenies were inferred using two previously published datasets (Sogonov *et al.* 2008; Walker *et al.* 2010). Moreover, for a subset of isolates, growth rates and morphological characteristics on different agar media were assessed and compared to *G. castaneae*, the other chestnut endophyte and potential pathogen, which can easily be confused with *D. castaneae*.

Materials and methods

Origin of the Diplodina castaneae *isolates*

Diplodina castaneae isolates were recovered from two different tissues of European chestnut trees, specifically abandoned galls of *Dryocosmus kuriphilus* and bark cankers caused by *Cryphonectria parasitica*. The galls all originated from eight chestnut stands in Switzerland and were sampled for a previous study in which we determined the incidence of *C. parasitica* on abandoned galls (Meyer *et al.* 2015). Of the stands used in this previous study, only those in the region Ticino were considered for canker samples (80 specimens) because the presence of *D. castaneae* was not recorded in the cankers from the other region (Chablais). Additionally, 14 cankers were sampled in 2014 in Gilly, Switzerland (Canton Vaud). In Azerbaijan, 257 bark cankers were sampled in 2015 in chestnut stands located in seven regions (Qabala, Sheki, Ismayilli, Oghuz, Qakh, Zagatala, Balakan). In Spain, 60 bark cankers were sampled in 2014 in four chestnut stands each located in a different county (Aller, Lena, Llanera, and Peñamellera