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FIRST REPORT OF *GEOSMITHIA PALLIDA* AND *G. LANGDONII* ASSOCIATED WITH *LIPARTHURUM COLCHICUM* IN CENTRAL ITALY

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Vitale S., Luongo L., Barzanti G.P., Binazzi F., Petrucci M., Galli M., Pennacchio F., Francardi V. – First report of *Geosmithia pallida* and *G. langdonii* associated with *Liparthrum colchicum* in central Italy.

Bark and wood-boring beetles feeding on coniferous and deciduous trees in different forest ecosystems are often associated with various species of fungi. In 2019, widespread attacks of *Liparthrum colchicum* Semenov (Coleoptera Curculionidae Scolytinae) were observed on *Laurus nobilis* L. in Tuscany (Italy). Samples of colonized terminal twigs were collected to investigate the presence of phytopathogenic fungi associated with the scolytid. Two different colonies of the *Geosmithia* genus were identified as *Geosmithia pallida* and *Geosmithia langdonii*. To our knowledge this is the first report of *G. pallida* and *G. langdonii* associated with *L. colchicum* on Bay tree, in Italy.

KEY WORDS: *Liparthrum colchicum*, *Laurus nobilis*, *Geosmithia* spp., Italy

INTRODUCTION

Bark and ambrosia beetles (Curculionidae: Scolytinae and Platypodinae) are pests threatening woody plants (e.g. timber plantations) all over the world. They are often associated with different communities of fungi including symbiont and phytopathogenic species. In the last two years (2019 – 2020), during extensive field surveys in different locations of Tuscany and Latium aimed at investigating the presence of a recently introduced scolytid, the black twig borer *Xylosandrus compactus* (Einchoff) on *Laurus nobilis* L., a second species, *Liparthrum colchicum* Semenov (Coleoptera Curculionidae Scolytinae), was identified in separate galleries on the same colonized twigs.

Liparthrum colchicum is a relatively unknown monophagous insect species primarily associated with thin dried twigs of about 0.5-1cm in diameter of *L. nobilis*. (JORDAL *et al.*, 2004) (Fig. I). This scolytid, which is currently considered a Mediterranean species, was reported for the first time in Italy as an intercepted organism alien to the country (RASSATI *et al.*, 2015). However, because of the frequency of records along the Tyrrhenian coasts and the surrounding areas, it might be considered as an established species or even as an insect native to Italy (FRANCARDI *et al.*, 2021). *L. colchicum* is normally attracted to moderate or even extremely dehydrated plant material and its populations have been overlooked so far due to their sporadic presence on the host plant. Nonetheless, *L. colchicum* infestations have recently increased becoming more evident due to the invasive presence on laurel trees of *X. compactus*, a much more harmful scolytid species that primarily attacks terminal twigs causing their progressive withering and thus increasing potential *L. colchicum* colonization. This species, which overwinters at larval or adult stages, is

monogynous with two generations per year. Females enter dried twigs through an entrance hole below 1 mm (0.6-0.7 mm) in diameter. In Tuscany the reproductive activity of *L. colchicum* wintering adults was observed between May and June (FRANCARDI *et al.*, 2021). *L. colchicum* attacks on twigs appear as typical lighter yellowish areas indicating the presence of maternal gallery under the epidermis where females have laid single eggs in niches. Larvae dig longitudinal feeding galleries that deeply excavate the surface of the sapwood. The possible role of *L. colchicum* as a vector of phytopathogenic fungi of the genus *Geosmithia* (Ascomycota, Hypocreales), in particular *Geosmithia putterillii* Thom. Pitt (Ascomycota, Hypocreales), has been reported by KOLAŘÍK *et al.* (2004, 2007) in France. More recently, fungi belonging to the genus *Geosmithia* associated with *L. colchicum* have been also recorded in Italy (BENVENUTI *et al.*, 2021).

This study aims to investigate in more detail the presence of phytopathogenic fungi associated with this scolytid.

MATERIALS AND METHODS

In Autumn 2019, terminal twigs of Bay trees with evident symptoms of *L. colchicum* attacks and fungal infections were collected in the park of Villa Salviati (Pisa), Tuscany. Adults of *L. colchicum* Semenov obtained from the wood galleries were kept in Petri dishes for about 1-3 h on Potato Dextrose Agar (PDA) medium; dishes were then incubated for 7 days at 24°C. After this period, fungal colonies cream to tan colored, and tan to yellow-tan on the reverse side of the plate were observed on half-strength PDA. Purified cultures were grouped according to culture morphology using a micro-

Fig. I - *Liparthrum colchicum*: adults within a typical maternal gallery

scope Zeiss Axio Imager A1 equipped with a Zeiss Axiocam 506 colour digital camera.

The DNA of two monosporic isolates (CREADC-F2469, CREADC-F2470) was extracted for molecular identification, following the protocol of the Wizard genomic DNA purification kit (Promega, Madison, WI, USA). The concentration of DNA was measured spectrophotometrically (Nanodrop ND-1000 Thermo Fisher scientific Inc., USA). The rDNA ITS region was amplified by conventional PCR using the primers ITS5 and ITS4 (WHITE *et al.*, 1990). Purified sequence reactions were analyzed on an ABI Prism 3730 Genetic Analyzer (Life Technologies). The sequences obtained after manual editing were blasted in NCBI database using the BLASTn program (<http://blast.ncbi.nlm.nih.gov>).

RESULTS

The *Geosmithia* genus was identified according to macro-and micro-morphological criteria. Growth was slow and restricted to room temperature, but it was enhanced at 30 °C, with colonies reaching 2-3 cm in diameter after 10 days at this temperature. Microscopic analysis revealed the presence of hyaline, rough-walled, septate, and often branched conidiophores. These conidiophores bore quaterverticillate- or terverticillate-

asymmetrical Penicillium-like and had phialides with a tapering tip that was parallel to the axis. Conidia were smooth-walled, hyaline, and cylindrical to ellipsoidal (Fig.II). Morphological identification of the two monosporic isolates (CREADC-F2469, CREADC-F2470) was confirmed by molecular sequence comparison with 100% identity with *Geosmithia pallida* (G. Sm.) M. KOLARIK, KUBATOVA & PAOUTOVA and 99% identity with *Geosmithia langdonii*, respectively as the highest identity percentages retrieved from BLAST searches. These sequence data have been submitted to the GenBank databases (ncbi.nlm.nih.gov/genbank) under the accession numbers MW627331 and MW627364.

DISCUSSION

Geosmithia species are cosmopolitan but understudied fungi. Most of them are associated with phloem-feeding bark beetles colonizing various woody hosts. *Geosmithia pallida* is an emerging pathogen of live oak in the United States (LYNCH *et al.*, 2014) showing affinities with a wide range of wood-boring insects, such as bark beetles and sawyer beetles (KOLARIK *et al.*, 2004). VANNINI *et al.* (2017) has recently found this fungal species on *Quercus ilex* L., *Pistacia lentiscus* L. and *Viburnum tinus* L., during a survey conducted in the Circeo National Park

Fig. II - Colonies of *Geosmithia langdonii* (1a and 1b) and *Geosmithia pallida* (2a and 2b) grown on Potato Dextrose Agar (PDA) medium. Conidia and branched conidiophores of *G. langdonii* (1c) and *G. pallida* (2c) respectively.

– Latium region (Italy). *Geosmithia langdonii*, on the other hand, was discovered in association with *Scolytus intricatus* on several oak species (KOLÁŘÍK *et al.*, 2005, 2007) and it was described as associated with *Platypus cylindrus* in wilted cork oaks (*Quercus suber* L.) in Algeria (BELHOUCINE *et al.*, 2011). More recently, it has been also isolated from a decaying elm (*Ulmus minor* Mill) in Switzerland (HÄNZI *et al.*, 2016).

To our knowledge this is the first report of *G. pallida* and *G. langdonii* associated with *L. colchicum*. Further research is, nonetheless, required to fully evaluate the potential role of this scolytid in the transmission of *Geosmithia* species.

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