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## The presence of *Prociphilus (Prociphilus) bumeliae* (Schrank, 1801) (Aphididae: Eriosomatinae: Pemphigini) in the Iberian Peninsula

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**Abstract.** *Prociphilus (Prociphilus) bumeliae* (Schrank, 1801) was recorded for the first time in the Iberian Peninsula, while developing colonies on the branches of *Fraxinus angustifolia* Vahl, attended by *Lasius niger* (Linnaeus, 1758) and a *Tapinoma* ant of *nigerrimum* group (Nylander, 1856). Relationships with these two ant species are new records. The identification was carried out using morphological, biological, and molecular characteristics.

**Key words.** Aphids, ash, Pemphigini, Barcoding, ant-aphid relationships, COI.

### INTRODUCTION

The genus *Prociphilus* Koch, 1857 groups over 50 species (Blackman & Eastop 2022; Favret 2022), which are characterized by being large aphids, usually without cornicles, covered in abundant filamentous wax produced by well-developed waxy glands also present in the fundatrices (Blackman & Eastop 2022).

In this genus, we can find species that have apparently recently moved their sexual phase from *Populus* species to other hosts such as Rosaceae, Caprifoliaceae and Oleaceae, as it happens in most Pemphigini, but they still maintain different conifers as secondary hosts, in which they can develop parthenogenetic populations (Zak 1965; Purkart et al. 2019).

Most species of genus *Prociphilus* are characterized by dioecious and holocyclic cycles, with a primary (or winter) host on which the fundatrix and winged fundatrigeniae develop, and a secondary (or summer) host in which winged virginogeniae develop and from which the sexuparae will leave to give birth on their primary hosts to the sexual morphs that, after mating, will produce overwintering eggs (Blackman & Eastop 2022). However, many species, like *Prociphilus tessellatus* (Fitch, 1851), have the capacity to develop anholocyclic populations despite living in areas where their primary hosts are present (Zak 1965; Purkart et al. 2019). The genus' complex

biological cycles and the polyphenism of its morphs have caused many species to be little known or their descriptions to be poor. Currently, their cycles (and all their morphs) are only fully known in three species (Blackman & Eastop 2022) and at a molecular level there are only COI sequences of ten species, making it a genus in need of a deep revision.

*Prociphilus* species are mainly distributed in the Northern Hemisphere (20 in North America, 7 in Europe, 3 in Central Asia and 15 in East Asia), basically restricted to the areas where their primary hosts develop, with the exception of the Asiatic *Prociphilus (P.) osmanthae* Essig & Kuwana, 1918 that has recently been introduced in Hungary (Ripka et al. 2020) and *Prociphilus (Meliarrhizophagus) fraxinifolii* (Riley, 1879), a species of Nearctic origin (Smith & Parron 1978; Maw et al. 2000), which has been expanding its distribution range due to the ornamental use of its hosts, and is now present practically throughout the Palearctic region, from Great Britain and Iberian Peninsula to China (Remaudière & Ripka 2003; Bieńkowski & Orlova-Bienkowskaja 2018), Mexico (Torres-Acosta & Sánchez-Peña 2015), South America (Chile) (Carrillo 1977) and South Africa (Müller & Schöll 1958).

Four species were known to date in the Iberian Peninsula: *Prociphilus (Prociphilus) fraxini* (Fabricius, 1777), *Prociphilus (Prociphilus) oleae* (Leach, 1826) and *Pro-*

*ciphilus* (*Stagona*) *pini* (Burmeister 1835) from the review carried out for the Iberian Fauna project by Pérez Hidalgo & Nieto Nafria (2003) and *Prociphilus* (*Meliarhizophagus*) *fraxinifolii* that was detected in 2011 (Pérez Hidalgo & Mier Durante 2012).

A colony of winged viviparous females collected on branches of *Fraxinus angustifolia* Vahl in Madrid and other colonies found on the same plant in the cities of Burgos and León allows us to mention, for the first time for the Peninsula, a fifth species, *Prociphilus bumeliae* (Schrank 1801).

## MATERIAL AND METHODS

Initially we studied several dozens of winged viviparous females and nymphs recorded on 10<sup>th</sup> June 2018 from the locality of San Agustín de Guadalix in the Province of Madrid, Spain (40.788270 N, 3.705692 W). The colony developed on branches of *F. angustifolia* (reference sample 2137) and was clearly attended by ants.

At first, the colony was identified using the identification keys of Blackman & Eastop (2022) and Heie (1980). The identification was confirmed by COI molecular analysis with the methodology exposed in Casiraghi et al. (2019).

During the spring of 2021, several *Prociphilus* colonies were also recorded on *Fraxinus angustifolia*, in several green areas of the cities of León (42.591725 N, 5.559641 W, 8<sup>th</sup> May 2021, reference sample 4017) and Burgos (42.358805 N, 3.681891 W, 15<sup>th</sup> May 2021, reference sample 4066), attended in both locations by *Lasius niger* (Linnaeus, 1758).

All associated ants were identified by Xavier Espadaler (University of Barcelona, Spain). Both aphid and ant specimens were deposited in the aphidological collection of the Institute for Integrative Systems Biology (University of Valencia-CSIC).

Furthermore, to investigate phylogenetic relationships of *Prociphilus* species, COI sequences identified as *Prociphilus* and *Pachypappa warshavensis* (outgroup) were downloaded from the NCBI nucleotide database (Nucleotide 2021) on November 14<sup>th</sup>, 2021 [queries: *prociphilus* AND “aphids”[porgn] AND (COI OR CO1 OR COX1) NOT COII; “*Pachypappa warshavensis*” AND “aphids”[porgn] AND (COI OR CO1 OR COX1) NOT COII].

After manually reviewing the fasta files, 106 sequences were obtained [1 problem sequence, 1 outgroup sequence, 103 template sequences]. Multiple sequence alignment was conducted with muscle ver. 3.8 (Edgar 2004).

Phylogeny was inferred using a Neighbour-Joining algorithm (Saitou & Nei 1987) with the F81 model (Felsenstein 1981) using the packages adegenet ver. 2.1.4 (Jombart 2008), ape ver. 5.5 (Paradis & Schliep 2018)

and phangorn ver. 2.7.1 (Schliep 2011) under R ver. 4.1.2 (R Core Team 2021). *Pachypappa warshavensis* (accession number MK936318.1) was used as outgroup, and node support was computed with 1000 bootstrap replicates. The tree was represented with ggtree package ver. 1.16.6 (Yu et al. 2018).

According to the obtained phylogeny, the problem sequence was identified as *Prociphilus bumeliae* (Schrank, 1801) (Fig. 2).

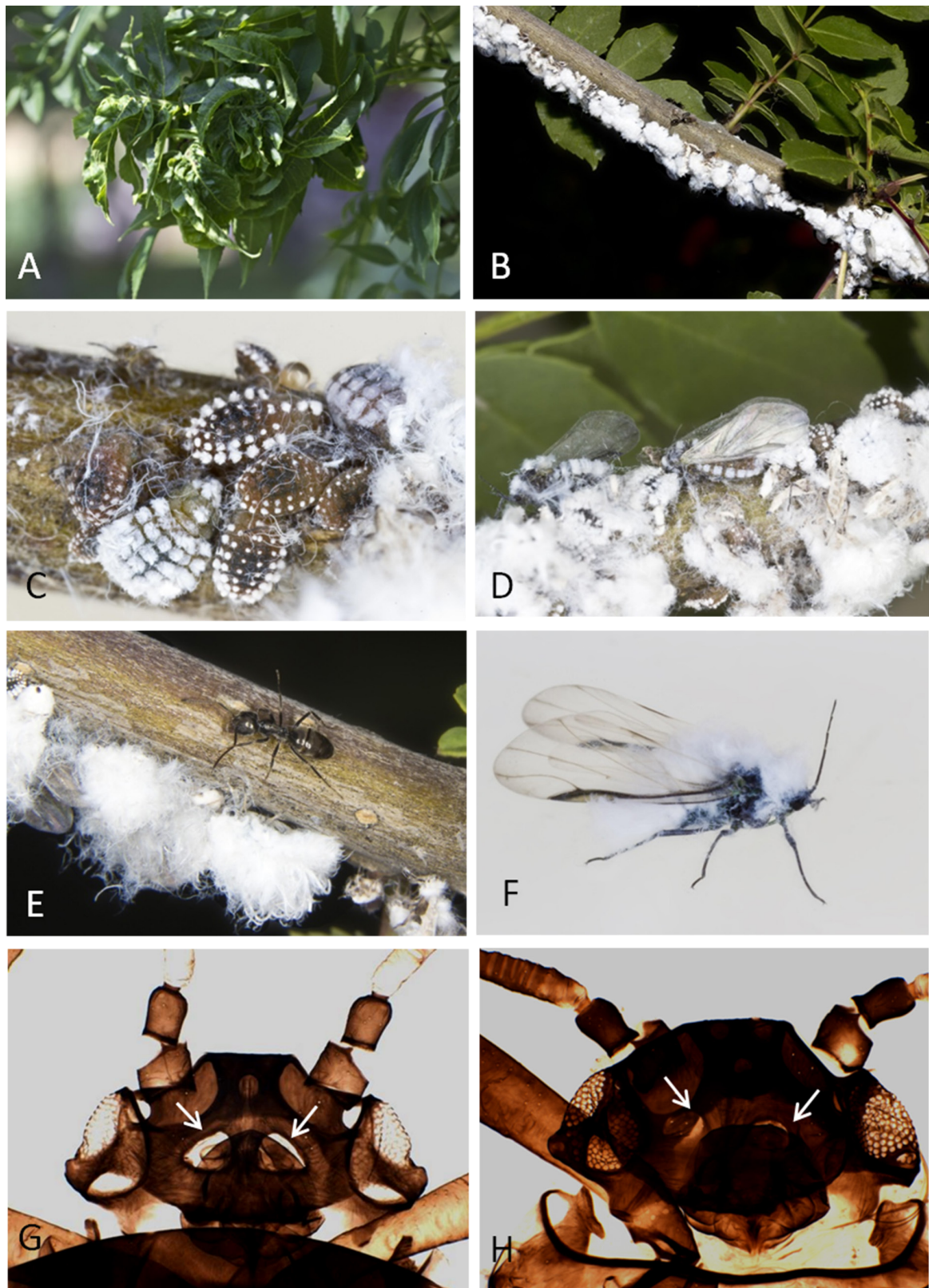
## RESULTS

Morphological characters and COI sequence (Fig. 2) confirm without doubt that the aphid colony on *F. angustifolia* was composed of *P. bumeliae*. Identification keys from Blackman & Eastop (2022) and Heie (1980) allow an easy distinction between *P. bumeliae* and *P. fraxini*, basically due to the presence of depigmented wax plates on the head of *P. bumeliae* (Fig. 1G). In *P. fraxini* these plates are badly defined (Fig. 1H).

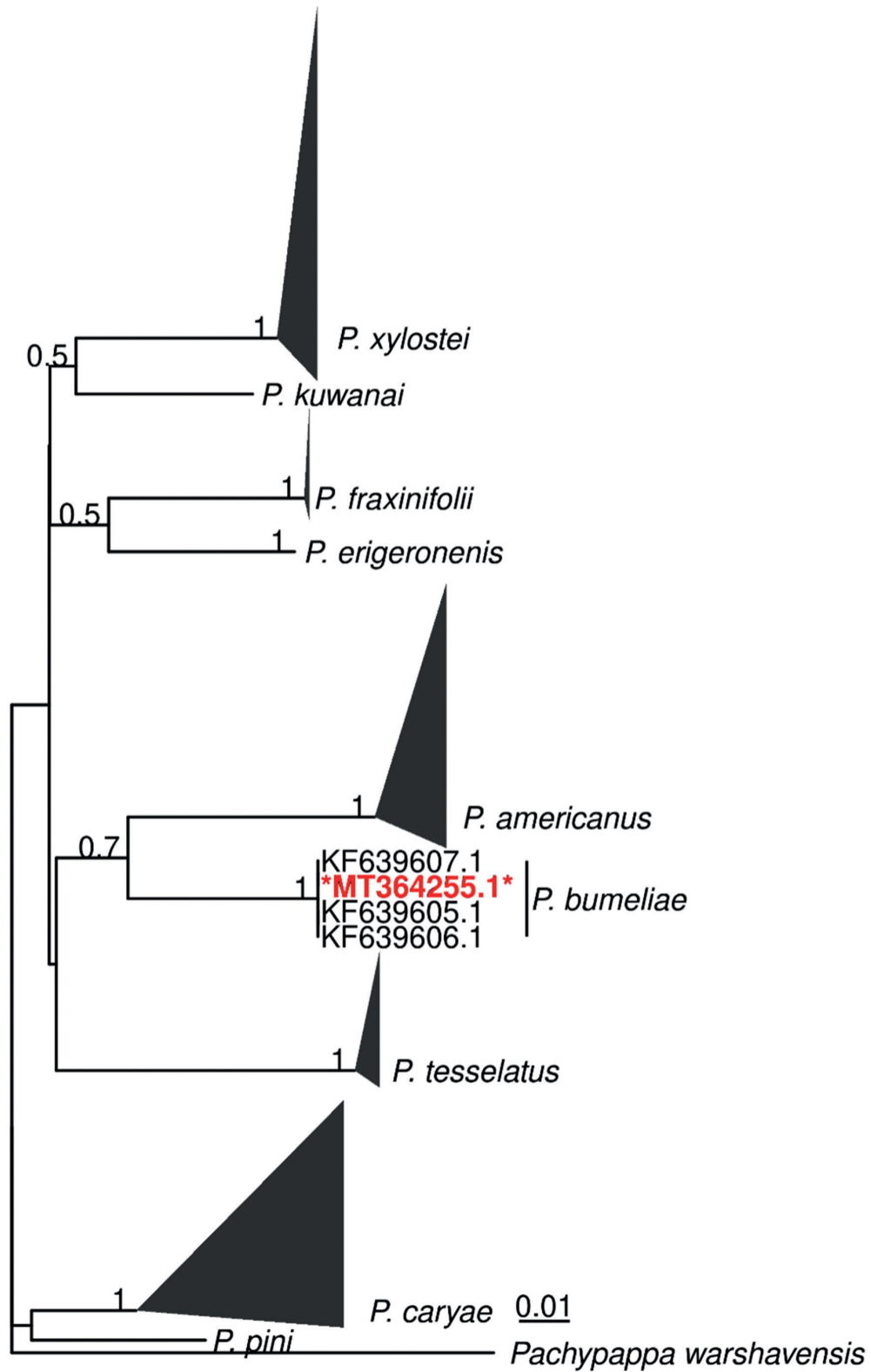
### Morphology

Heie (1980) gives a good description of the viviparous females of this species (fundatrix, winged fundatrigeniae, apterous virginogeniae and sexuparae), which we complement here with data on the only forms currently known in the Iberian Peninsula.

Winged fundatrigeniae (based on measurements of 10 specimens) measure 3.8–5.5 mm. In life, they have a reddish-brown coloration and are covered with abundant filamentous wax (Fig. 1B, 1F) that varies depending on the body distribution of their wax plates and their stage of development (Fig. 2C). In microscopic preparation, head and thorax are blackish brown, with lighter areas that correspond to the plates of the wax glands. The head presents a well-developed posterior pair of waxy gland plates (Fig. 1G) and, in very rare cases, two other small ones between the antennae in the anterior part of the head. Antennae measure 1.2–2.2 mm; they are formed by 6 segments and are 0.40–0.60 × the body. The antennal segment III measures 6.0–7.3 × the antennal segment II, and from 1.07–1.23 the sum of the antennal segments IV and V; the IV<sup>th</sup>–VI<sup>th</sup> antennal segments measure 0.31–0.38, 0.29–0.37 and 0.36–0.42 mm, respectively, and the terminal process 0.15–0.20 × the base of the VI<sup>th</sup> antennal segment. The antennal segments III–IV present 16–24 and 2–9, respectively, secondary sensoria, with a transverse oval shape, while the VI<sup>th</sup> lacks them. The rostrum measures 0.20–0.32 × the body and its apical segment carries about 6 complementary setae, and it is 3.25–4.25 × as long as its basal width and (0.6) 0.9–1.04 × the second segment of the hind tarsi. This morph lacks siphunculi and the cauda is rounded and carries 7–10 setae and in the first tarsal segment they usually have 4 setae (exceptionally 3).



**Fig. 1.** *Prociphilus (P.) bumeliae* (Schrank, 1801). **A.** leaves nest produced by the fundatrix and the winged fundatrigeniae in *Fraxinus angustifolia* Vahl. **B–D.** Colonies on branches with different degrees of detail. **E.** Same colony attended by an ant of the *Tapinoma nigerrimum* group. **F.** Aspect of the winged fundatrigeniae. **G.** Detail of its head (arrows indicate waxy plates). **H.** *Prociphilus (Prociphilus) fraxini* (Fabricius, 1777). Detail of the head of the winged fundatrigeniae (arrows indicate waxy plates).



**Fig. 2.** Phylogenetic tree with studied samples, based on mitochondrial marker COI, obtained with NJ method (K2P), bootstraps: 1000. Sample indicated in bold red corresponds to the record of the present study. Abbreviation: *P.* = genus of *Prociphilus* Koch, 1857.

### Biology

*Prociphilus (Prociphilus) bumeliae* presents a dioecious holocyclic cycle, with species of *Fraxinus* (usually *Fraxinus excelsior* L., and exceptionally *Fraxinus ornus* L., *Fraxinus angustifolia* subsp. *oxycarpa* (Willd.) and *Fraxinus pallisae* Wilmott), *Ligustrum vulgare* L. and *Syringa vulgaris* L. as primary hosts, and roots of *Abies* (usually *Abies alba* Mill. and, optionally, *Abies balsamea* (L.) Mill. and *Abies veitchii* Lindley) as secondary hosts (Holman 2009). Fundatrices make leaf curls resembling bird nests in early spring (Fig. 1A), but as colonies develop, they can move to trunk bark and thin branches (Fig. 1B). Winged females migrate to the roots of their secondary hosts where they form colonies covered with abundant waxy secretion and from which the winged sexuparae will emerge in late August–September to return to their primary host (Mordvilko 1935; Blackman & Eastop 2022).

Associations between *P. bumeliae* and the ants *T. cf. nigerrimum* and *L. niger* are new aphid-ant relationship for this species.

### Distribution

*Prociphilus bumeliae* is a widely recorded species throughout Europe (Holman 2009; Nieto Nafria et al. 2013), but there are some citations from East Asia that need confirmation because they could be other species (see Blackman & Eastop 2022). As for now, these records from the province of Madrid and Castilla y León are the first of *P. bumeliae* in the Iberian Peninsula, but it is likely to be distributed in other parts, where its host plants are present both natural and ornamentally.

## DISCUSSION

### Taxonomic-bionomic problems

*Prociphilus* is a genus of aphids that presents complex taxonomic and biological problems (Smith & Stroyan 1972), specifically due to the complexity of their life cycles and their ability to develop paracycles in their secondary hosts, as it happens in other groups of Pemphigini and Eriosomatini. However, thanks to the use of molecular tools combined with traditional taxonomy, some of the abovementioned problems can be solved (Pike et al. 2012).

As it happens with various Eriosomatinae, the identification of *P. bumeliae* presents some problems: *P. (Prociphilus) fraxini* is also widely distributed in Europe and very similar in aspect and size to *P. bumeliae* (Matošević 2004; Blackman & Eastop 2022). This species also alternates between *F. excelsior* and *A. alba* and its apterous viviparous females (exules) are practically indistinguishable from those of *P. bumeliae* (Blackman & Eastop 2022). The two species can only be well differentiated thanks to the winged stage corresponding to winged fun-

datrigeniae developing inside the leaf nest, which we used to determine the new record (Dransfield & Brightwell 2021).

### Faunistic studies

With our record, there are five *Prociphilus* species recorded in the Iberian Peninsula (*P. fraxini*, *P. bumeliae*, *P. oleae*, *P. (S.) pini* and *P. (M.) fraxinifolii* (Pérez Hidalgo & Nieto Nafria 2003; Pérez Hidalgo & Mier Durante 2012). With the exception of *P. fraxinifolii*, which is beginning to be widely distributed, due to the ornamental use of *Fraxinus pennsylvanica* Marshall, the rest of samples are due to few specimens captured on their hosts or with traps.

The distribution of *P. fraxini* in the Iberian Peninsula corresponds to the natural area of its secondary host *A. alba* (Pérez Hidalgo & Nieto Nafria 2003), but the presence of *P. bumeliae* on *F. angustifolia* requires further studies to confirm whether the ranges of both species are limited to that of their hosts or may be broader.

It is important to consider that *F. excelsior* is scarce in the Sierra de Guadarrama, however *F. angustifolia* is a very common species in this area, so it is to be supposed that when it develops also on this species it is more widespread, being able to carry out its root cycle in the firs scattered throughout the garden areas.

On the other hand, the discovery of *P. bumeliae* in the green spaces of the cities, where its secondary hosts (*Abies* and *Ligustrum*) are also present, may suggest that it may be much more widespread in the peninsula than one might initially think.

Normally, in contrast to the invasive *P. fraxinifolii* (Hałaj & Osiadacz 2017), European *Prociphilus* species are not considered harmful for their *Fraxinus* hosts, despite the impressive leaf malformations and the great amount of honeydew produced (Pérez Hidalgo & Mier Durante 2012). However, different authors sustain the hypothesis of an indirect damage from *P. bumeliae* and *P. fraxini* sucking activity, which could lead to facilitate ash pathogens infections, like those of the fungus *Hymenoscyphus fraxineus* (*Chalara fraxinea*) Queloz et al., 2011 (Jankovský & Holdenrieder 2009; Longauerová et al. 2013).

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