

Research article

First records of *Microstroma album* (Desmazières) Saccardo, 1878 (Basidiomycota: Microstromataceae) and *Phylloxera glabra* (von Heyden, 1837) (Hemiptera: Phylloxeridae) in oak stands in Bulgaria

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Abstract: The current study reports the first records of an aphid-like pest and leaf pathogen on oak species in Bulgaria. In 2020–2023, leaf samples presenting characteristic yellow spots and lesions were collected from mature trees of *Quercus petraea*, *Q. cerris*, *Q. frainetto* and *Q. robur* in different localities of the country. Damage caused by oak obligate leaf pathogen *Microstroma album*, the leaf phylloxera, *Phylloxera glabra* (Hemiptera: Phylloxeridae), and the oak lace bug, *Corythucha arcuata* (Hemiptera: Tingidae) were identified on the lower surface of oak leaves. *M. album* was identified on all studied *Quercus* species. Angular white patches, the fruiting stage of the fungus, appeared on the lower surfaces of the leaves. *P. glabra* caused yellowish mosaic discoloration on the leaf surface of *Quercus cerris*, *Q. frainetto* and *Q. petraea*. Sucking damage displayed a stippled pattern of yellow, brown, or whitish dots. In places where *M. album* develops, the invasive oak lace bug (*Corythucha arcuata*) was often found as the only sucking species on oak leaves, which is most likely also a vector of the disease. Severe damage caused by established both obligate pathogen (*M. album*) and sucking insect pests (*P. glabra*, *C. arcuata*) could result in a weakening of the oak trees. On sufficiently weakened trees, secondary fungal pathogens or boring insects, commonly infested oak trees, can cause their death.

Keywords: *Corythucha arcuata*, Eastern Balkans, oak leaf phylloxera, obligate tree pathogens, new records, plant parasite fungi, *Quercus*

Introduction

Oak forests in Bulgaria occupy about one third of the country's forest area (Marinov et al., 1995). For years, the health condition of oak forests has been deteriorated. They were negatively affected by various abiotic and biotic factors, such as insect pests (Kere-midčiev, 1965; Zlatanov, 1968, 1971; Tsankov et al.,

1997, etc.) and pathogens (Rossnev & Petkov, 2000; Rossnev et al., 2006, 2010). In the context of climate change, the most significant biotic threats include the attacks of alien species and the expansion of ranges of native pests and pathogens. The current study reports the first records of *Microstroma album* (Desmazières) Saccardo, 1878 and *Phylloxera glabra* (von Heyden, 1837) in oak stands in Bulgaria.

Table 1. Main characteristics of the studied areas.

No.	Region	Locality	Geographical coordinates	Altitude, m a.s.l.	Year of study	Tree species
1	Kyustendil	Kyustendil	N42.26153°, E22.68095°	829	2020	<i>Quercus frainetto</i>
2	Pazardzhik	Draginovo Vill.	N42.11594°, E24.03344°	963	2021	<i>Quercus petraea</i>
3	Pazardzhik	Panagyurski Kolonii Vill.	N42.55819°, E24.19633°	818	2022	<i>Quercus petraea</i>
4	Smolyan	Bosilkovo Vill.	N41.69718°, E24.95663°	1098	2022	<i>Quercus petraea</i>
5	Burgas	Primorsko	N42.24311°, E27.76049°	26	2022	<i>Quercus cerris</i> , <i>Quercus frainetto</i>
6	Burgas	Tsarevo	N42.14539°, E27.81781°	109	2022	<i>Quercus cerris</i> , <i>Quercus frainetto</i> , <i>Quercus petraea</i>
7	Sofia	Lesnovo Vill.	N42.64266°, E23.64012°	553	2022	<i>Quercus robur</i>
8	Sofia	Vladaya Vill.	N42.63393°, E23.18833°	859	2023	<i>Quercus cerris</i> , <i>Quercus frainetto</i> , <i>Quercus petraea</i>

Material and methods

The studies were conducted during the period 2020–2023 in oak stands or oak solitary trees in urban areas in different regions in Bulgaria (Osogovo Mt, Western Rhodopes, Sredna Gora Mts, Black Sea Coast, Sofia Lowland). The main characteristics of the studied areas are pointed out in Table 1.

On the field, the extent of damage caused by *Microstroma album*, *Phylloxera glabra* and other insect pests on studied oak leaves, was assessed in 10% steps according to the methodology of the International Cooperative Programme Forests (Eichhorn et al., 2020). In each locality, 3–5 trees were studied.

Samples of *M. album* (symptomatic leaves showed characteristic downy mycelium spots of the pathogen) were collected in sterile bags and transferred to the laboratories of phytopathology at the Forest Research Institute – Sofia and Forest Protection Station – Plovdiv. Symptoms were characterised by whitish, mycelium containing fungal structures and spores on the underside of the affected leaf surface, often concentrated along the veins with polygonal shape and a yellowish discolouration on the corresponding upper surface of the leaf. Symptomatic leaves were surface washed with tap water for 5 min, sterilised for 1 min in 96% ethanol, subsequently rinsing them once in sterile distilled

water. Sterilised parts were placed in Petri dishes on moistened filter paper and incubated at 22±2°C under artificial light. Isolations of fungal pathogen *Microstroma album* were made by placing small pieces of surface-sterilised leaf tissue with mycelium structures onto malt extract agar Difco (MEA: 20 g malt extract; 16 g agar-agar; 1000 ml tap water). The shape and size of 100 pycnidia and basidia were measured at magnification 40× using an ocular micrometer of Zeiss Stemi 305 and 100 conidia and basidiospores at 250× using Carl Zeiss NU2 light microscopes equipped with a digital camera DinoEye AM-423X.

Collected insects on oak leaves were transported to the laboratory of entomology in Forest Research Institute – Sofia. A part of *Phylloxera glabra* was preserved in 75% ethanol. Mature specimens were mounted on slides following the methods of Blackman & Eastop (2000). The slides were photographed by digital camera ProgResCT5 attached to the microscope Zeiss AX10.

The identification of *P. glabra* was made by the keys Blackman & Eastop (2021, online), and *Corythucha arcuata* – by the keys of Dobрева et al. (2023).

The biological material is deposited in the entomological collections of the Forest Research Institute and the Agricultural Faculty of University of Forestry in Sofia.

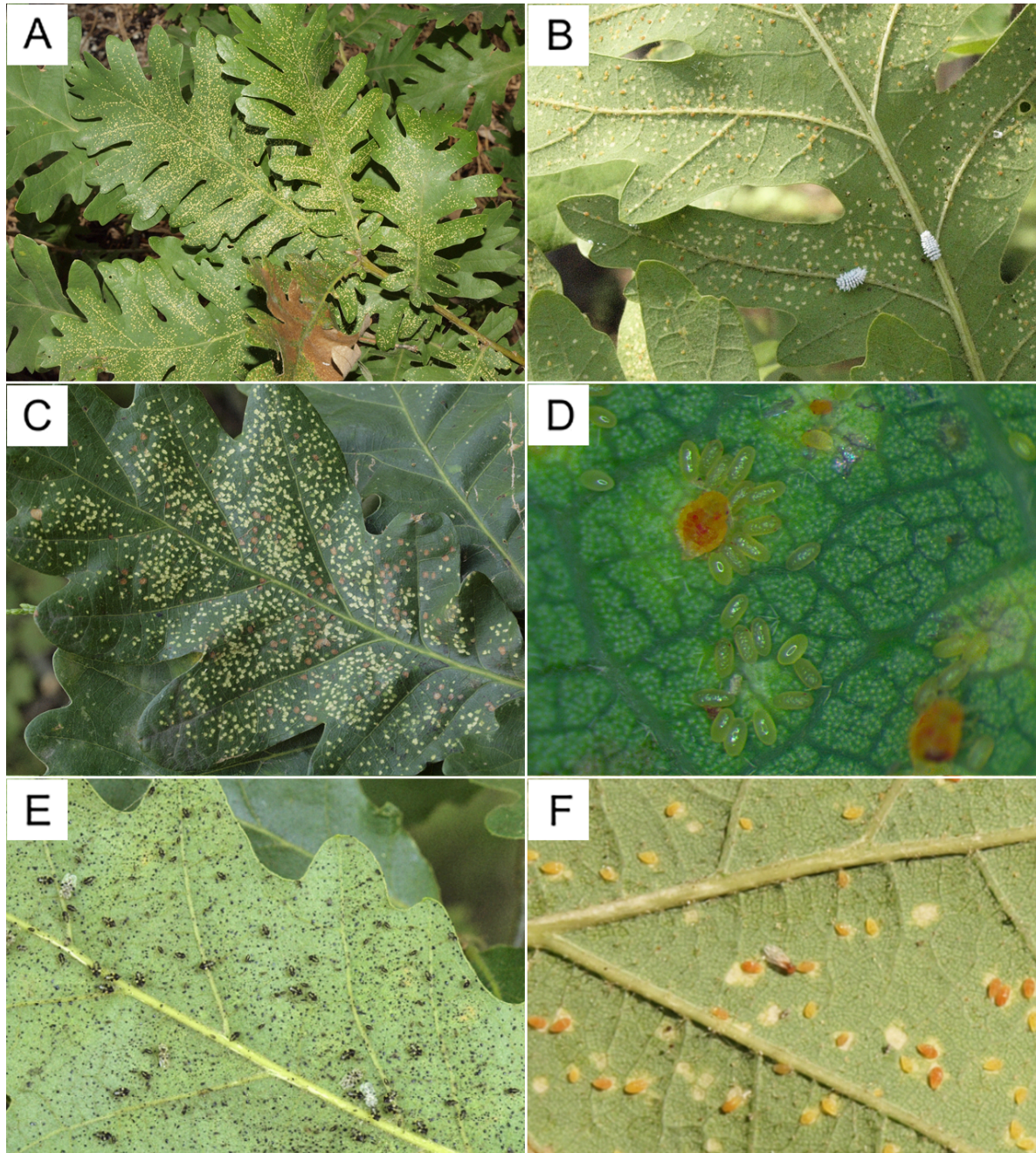


Fig. 1. *Microstoma album* and sucking insects on oak leaves: A – yellowish spots of *M. album* on upper surface of *Quercus frainetto* (Kyustendil, 2.09.2020); B – angular white patches of *M. album*, *Phylloxera glabra* first-instar larvae and *Scymnus* sp. larvae on the lower surfaces of *Quercus frainetto* (Kyustendil, 2.09.2020); C – yellowish spots of *M. album* on upper surface of *Quercus petraea* (Bosilkovo Vill., 23.07.2022); D – apterus females and eggs of *Phylloxera glabra* on the lower surfaces of *Quercus petraea* (Bosilkovo Vill., 23.07.2022); E – eggs, larvae and adults of *Corythucha arcuata* on upper surface of *Quercus cerris* (Tsarevo, 15.07.2022); F – an alate female and first-instar larvae of *Phylloxera glabra* on the lower surfaces of *Quercus petraea* (Vladaya Vill., 4.09.2023).

Results

Material examined: Kyustendil, 2.09.2020, *Microstoma album* on leaves on *Quercus frainetto* Ten.

(20–80%), alate adults and first-instar larvae of *Phylloxera glabra* (0–20%), single larvae of *Scymnus* sp. (Coleoptera: Coccinellidae); Draginovo Vill., 25.08.2021, *M. album* on leaves of *Quercus petraea*

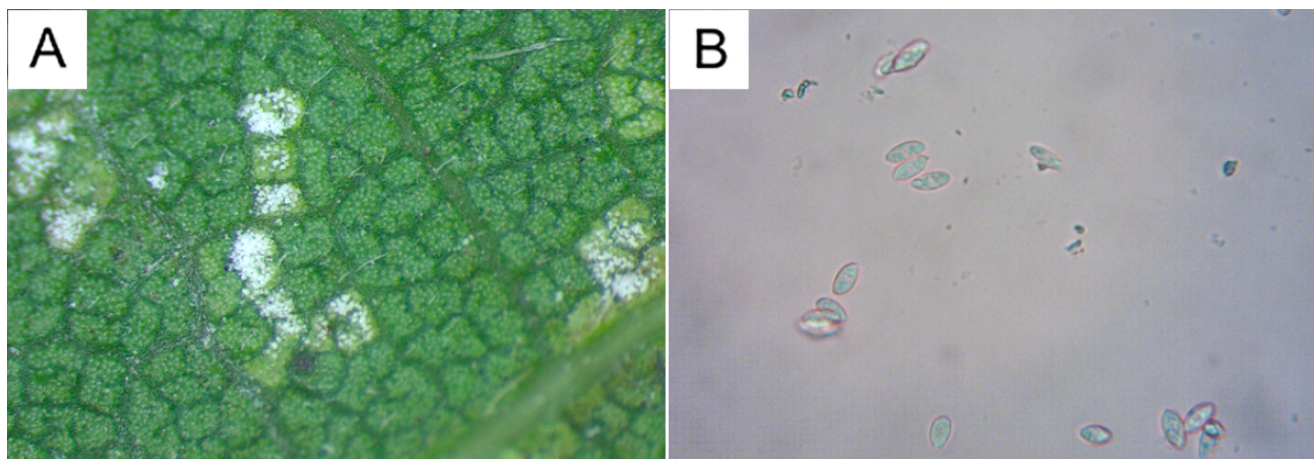


Fig. 2. *Microstroma album*: A – basidia on the leaf surface: B – basidiospores.

(Matt.) Liebl. (10–30%); eggs, larvae and adults of *Corythucha arcuata* (Say, 1832) (Hemiptera: Tingidae) (10–20%); Bosilkovo Vill., 23.07.2022, *M. album* on leaves of *Q. petraea* (30–80%); apterus females and eggs of *P. glabra* (30–50%); Primorsko, 13.07.2022, *M. album* on leaves of *Quercus cerris* L. and *Q. frainetto* (0–30%), eggs, larvae and adults of *C. arcuata* (0–60%); Tsarevo, 13.07.2022, *M. album* on leaves of *Q. cerris*, *Q. frainetto* and *Q. petraea* (0–20%), eggs, larvae and adults of *C. arcuata* (0–70%); Lesnovo Vill., 2.08.2022, *M. album* on leaves of *Quercus robur* L.; eggs, larvae and adults of *C. arcuata* (0–50%); Panagyurski Kolonii Vill., 22.06.2022, *M. album* on leaves of *Q. petraea*, apterus females and eggs of *P. glabra* (0–20%); Vladaya Vill., 4.09.2023, *M. album* on leaves of *Q. frainetto*; alate adults and first-instar larvae of *P. glabra* (10–30%), single adults of *C. arcuata* (0–10%).

Microstroma album (Desmazières) Saccardo, 1878 (Basidiomycota: Microstromataceae) was found on all studied oak species (*Quercus cerris*, *Q. frainetto*, *Q. petraea*, *Q. robur*). Characteristic lesions, yellow leaf spots on the upper surface and white mycelium on the lower surface corresponded to basidia and masses of basidiospores. The upper surface becomes yellowish and mottled (Fig. 1A, D). Angular white patches, the fruiting stage of the fungus, appeared on the lower surfaces of the leaves (Fig. 1B, C). On the lower surfaces of oak leaves, colonies of *Phylloxera glabra* (Fig. 1B, F) and *Corythucha arcuata* (Fig. 1D) were registered. In addition, early-instar unidentified aphid larvae on the

lower surfaces of oak leaves were observed in the region of Kyustendil and Vladaya Vill., as well as predatory larvae of *Scymnus* sp. (Coleoptera: Coccinellidae) on the lower surfaces of oak leaves in the region of Kyustendil (Fig. 1B).

Whitish colonies of *M. album* appeared on leaf spots, 200–600 µm in diameter (Fig. 2A), with columns of thick-walled cylindrical basidia 15–20 µm long and 3–4 µm in diameter. Basidiospores were $5.5\text{--}8 \times 2\text{--}3.5$ µm in size, ellipsoidal to cylindrical-ellipsoidal or ovoid, hyaline, aseptate, smooth, thin-walled, with two oil drops. (Fig. 2B).

Phylloxera glabra (von Heyden, 1837) (Hemiptera: Phylloxeridae) was found as alate females and early-instar larvae on *Quercus frainetto* and *Q. petraea*, and apterus females, eggs and larvae on *Quercus cerris*, *Q. frainetto* and *Q. petraea*. It caused yellowish mosaic discolouration on the leaf surface. Sucking damage displayed a stippled pattern of yellow, brown or whitish dots.

Oak leaf phylloxera adults and nymphs (immatures) are oblong or pear shaped and tapered toward the rear end. They are yellow to orange yellow coloured, 1 mm or less in length and a hand lens is required to clearly distinguish the individual insects.

Adult apterae of *P. glabra* is yellowish orange and feeds on undersides of leaves (Fig. 1F). According to Footitt & Richards (1993), the species have the following main differential characteristics: the body length of apterus female (Fig. 3A) varies from 0.7–0.85 mm; abdominal tergites from 2 to 5 have spiracles (Fig. 3B); antenna is 3 segmented with a

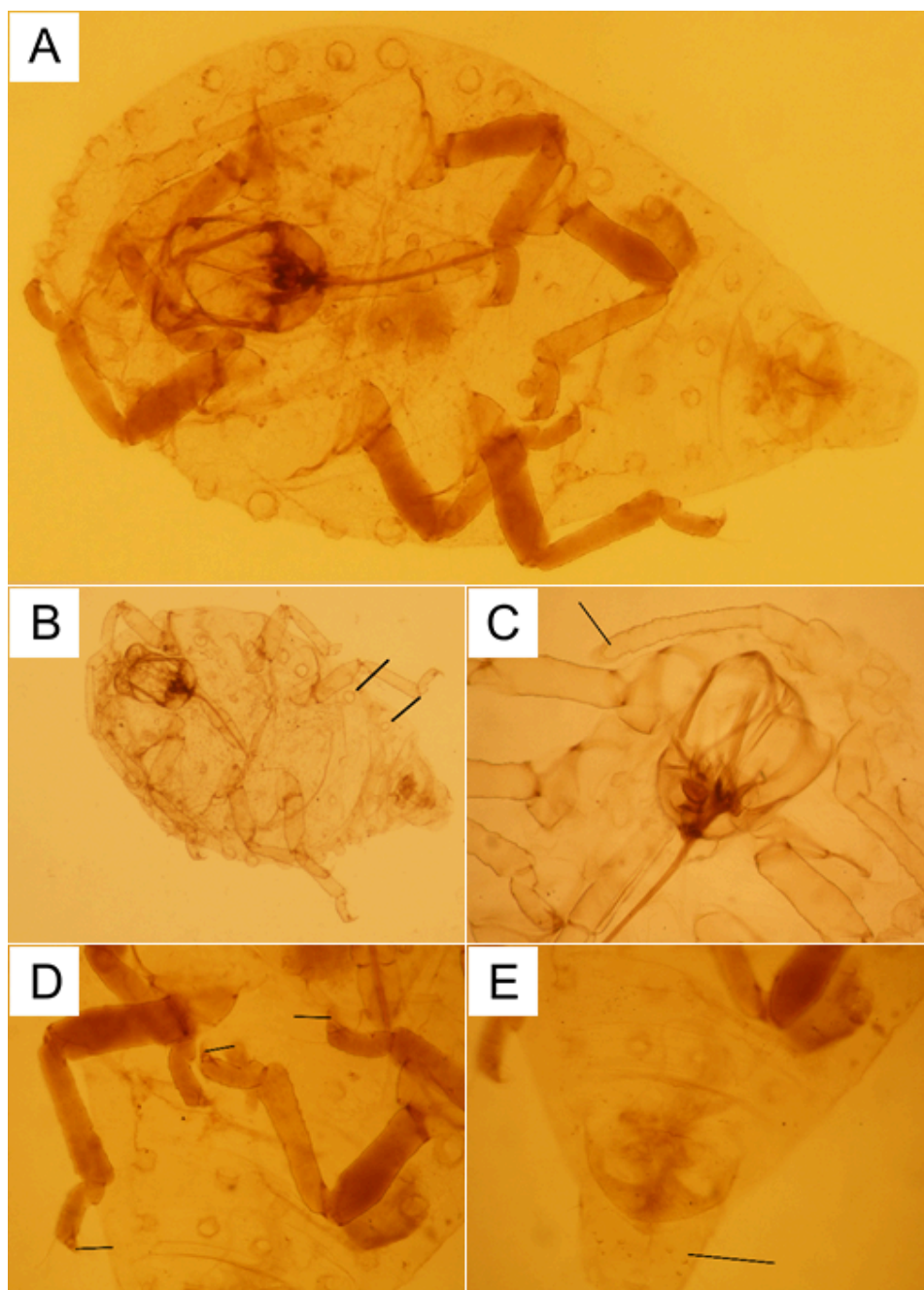


Fig. 3. Microscope view of *Phylloxera glabra*: A – apterus female; B – spiracles on abdominal tergites 2-5; C – primary rhinarium on the third antennal segment; D – simple claws; E – cauda.

developed primary rhinarium on the third antennal segment (Fig. 3C); claws are simple (Fig. 3D); cauda is bluntly rounded (Fig. 3E).

A probable vector of the disease is also *Corythucha arcuata* (Say, 1832) (Hemiptera: Tingidae), which has been registered at a high population density along the Black Sea coast of Bulgaria (Primorsko, Tsarevo) (Fig. 1D).

Discussion

In Romania, *M. album* was found in oak stands dominated by *Quercus cerris*, *Q. robur*, *Q. petraea*, *Q. pubescens* and *Q. frainetto* (Fodor & Hâruga, 2014). According to the authors, it was also found on *Quercus robur* and *Q. robur x Quercus canariensis* Willd. in New Zealand, *Q. robur*, *Quercus pubescens*

Willd., *Quercus macranthera* Fisch. & C.A.Mey. ex Hohen. in USA and Austria, *Quercus garryana* Douglas ex Hook. in Canada, *Quercus lusitanica* Lam. in Algeria, and *Quercus acutissima* Carruth. in Japan. Fodor & Hâruga (2014) pointed out numerous galling insects from *Andricus*, *Chilaspsis*, *Cynips*, *Dryomyia*, *Janetia* and *Neuroterus* genera, associated with *M. album*. However, no such associations have been observed in Bulgaria.

Phylloxera glabra is native to Europe (England, France, Germany, Italy, Hungary, Poland, Slovakia) (Ripka et al., 1998; Lubiarz, 2007; Wojciechowski et al., 2016; Blackman & Eastop, 2021, online), and introduced to New Zealand (Sunde, 1984) and Canada (Swiecki, Bernhardt, 2006). It is connected mainly with *Quercus robur* (Blackman & Eastop, 2021, online), but was also found on *Quercus boissieri* Reut. in Israel (Halperin et al., 1988), *Quercus dentata* Thunb. in New Zealand (Sunde, 1984) and UK (the Royal Botanic Gardens, Kew, London) (Wieczorek et al., 2019), and *Quercus garryana* Douglas ex Hook. in Canada (British Columbia) (Swiecki, Bernhardt, 2006). *Phylloxera glabra* overwinters as immature stages in bark crevices (Lubiarz, 2007). In spring, after the eggs have hatched, the larvae gather on the underside of young leaves. The aphids of the early spring generation cause 'ear galls' (Ellis, 2022). According to the author, later generations do not cause any galling, but their suction activities create numerous small yellow spots. The apterus females deposit large numbers of shining yellow eggs in a concentric circle (Fig. 1D). In autumn, the sexual adults appear and the oviparae lay their eggs following fertilisation (Fig. 1F). Another species, *Phylloxera quercina* (Ferrari, 1872) (syn. *Phylloxera spinulosa* Targiani-Tozzetti, 1875) has been previously found on *Quercus cerris* in field protective forest belts in Northeastern Bulgaria (Zlatanov, 1960, 1964).

The other sucking insect found in our studies, the oak lace bug (*Corythucha arcuata*), penetrated in Bulgaria in 2012 (Dobрева et al., 2013). In a short time, the species spread everywhere in the oak forests of Bulgaria (Georgiev et al., 2017; Simov et al., 2018).

In conclusion, the finding of *M. album* and *P. glabra* enriches the mycota and entomofauna of Bulgaria. However, severe damage caused by both established pathogen and its insect vectors (*P. glabra*, *C. arcuata*) could result in a weakening of the oak trees. On sufficiently weakened trees, secondary

fungal pathogens or boring insects, commonly infested oak trees, can cause their death.

Acknowledgments

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