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RESEARCH PAPER

First records of the North American leafhopper *Gyponana mali* (Hemiptera: Cicadellidae) invading urban gardens and agroecosystems in Europe

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Abstract. The Nearctic leafhopper species *Gyponana* (*Gyponana*) *mali* DeLong, 1942 is reported from Europe for the first time and represents the first record of the tribe Gyponini Stål, 1870 (Hemiptera: Cicadellidae: Iassinae: Gyponini) for the Palearctic Region. Specimens were collected in southern Switzerland (Ticino) and two regions of northern Italy (Lombardy and Veneto) in 2015–2019. The preferred host plant in these areas appears to be *Cornus sanguinea* L. Phylogenetic analysis of the COI barcode sequences grouped one of the European specimens with three individuals of *G.* (*G.*) *mali* from Ontario, Canada. Morphological study indicated that the male genitalia of the European population are intermediate between *G.* (*G.*) *mali* and *G.* (*G.*) *extenda* DeLong, 1942.

Key words. Hemiptera, Auchenorrhyncha, Cicadomorpha, Cicadellidae, exotic species, viticulture, ornamental plants, Palearctic Region

Zoobank: http://zoobank.org/urn:lsid:zoobank.org:pub:7D0297D2-7D73-4D7D-B82C-18DA5F7D9977

Introduction

The endemic New World leafhopper tribe Gyponini Stål, 1870 is the largest tribe in the subfamily Iassinae (Hemiptera: Cicadellidae), comprising 65 genera, 101 subgenera, and more than 1,300 species (Dmitriev 2003 onward). Although this group was long treated as a separate subfamily (Oman et al. 1990), phylogenetic analyses supported placement of Gyponini as a tribe of Iassinae (Dietrich 1999, Dietrich et al. 2005). A recent comprehensive phylogenetic analysis of Iassinae recovered Gyponini as monophyletic with the following apomorphies: ocelli on crown well separated from anterior margin and eyes, head striations extended well ventrad of antennal ledges, anteclypeus apex no wider than at midlength, clypeal suture obsolete at least medially, front femur intercalary row of setae uniseriate, male sternite VIII no longer than sternite VII, male pygofer without group of fine ventrolateral setae, subgenital plate flattened and depressed but not expanded, and aedeagus with paired distal processes (Krishnankutty et al. 2016).

The genus *Gyponana* Ball, 1920 is distributed and widespread in the Nearctic and Neotropical Regions, and comprises four subgenera: *Gyponana* s. str., *Pandara* DeLong & Freytag, 1972, *Spinanella* DeLong & Freytag, 1972, and *Sternana* DeLong & Freytag, 1964. The genus presently includes 93 species, with 88 species in the nominotypical subgenus (Dmitriev 2003 onward). The species-level taxonomy of *Gyponana* has traditionally been based primarily on the structure of the male genitalia, particularly the shape of the aedeagus and style, with many species originally described based on few specimens and only slight morphological differences. Hamilton (1982) reviewed the Nearctic species of the nominotypical subgenus and placed several previously described species as junior synonyms based on an interpretation of intraspecific variation from a study of larger series of specimens.

During three surveys conducted from 2015 to 2019 in Switzerland and in Italy, some specimens of *Gyponana*, a genus not previously recorded in the Palearctic Region, were collected. As most species included in this genus have similar external appearance and structure of the genital apparatus, with a high intraspecific variability (Hamilton 1982), we complemented a morphological study of these European specimens with molecular analyses with the aim to assign a species identity. The specimens were identified as *Gyponana* (*Gyponana*) *mali* DeLong, 1942 and are described below.

Material and methods

The specimens examined were collected in Switzerland and Italy (Fig. 1) using two different techniques: sweeping of vegetation and yellow sticky traps installed on the vegetation canopy.

The abdomens of voucher specimens (4 males and 2 females) collected from different localities in Italy were dissected to study the genitalia (DeLong 1942) under an Olympus SZX10 stereoscopic microscope. The species was identified using published taxonomic keys and related literature (DeLong & Freytag 1964, Hamilton 1982). The specimens were also compared with specimens of *G. mali* and other closely related species (e.g., *Gyponana extenda* DeLong, 1942) including paratypes of *G. mali* deposited in the collection of the Illinois Natural History Survey (INHS; University of Illinois, Champaign, IL, USA). Habitus photographs of voucher specimens were taken at INHS by C. H. Dietrich with a Canon SLR camera and 65 mm macro lens mounted on an automated lift, which allowed capture and stacking of images at different focal planes. Photographs of genitalia preparations were taken using a Q-Imaging Micropublisher camera mounted on an Olympus BX40 compound microscope. The specimens are deposited at the INHS collection.

Total DNA was extracted from the abdomen of a voucher specimen designated as I01/17 following the method of Gatineau et al. (2001) and deposited at the Research Centre for Viticulture and Enology (CREA–VE, Italy). The barcoding region of the mitochondrial cytochrome oxidase subunit I (mtCOI) gene was amplified using the primer pair LCO1490/HCO2198 (Folmer et al. 1994), using the protocol described by Trivellone et al. (2017). Amplicons were visualized on 1% agarose gel stained with GelRed (Biotium Inc.) under a GelDoc XR UV transilluminator (Biorad). Sequencing was carried out in both directions using automated equipment (BMR Service, Padua, Italy), with the same primers used for amplification. Nucleotide sequence obtained in this study was deposited in the DDJB/ENA/GenBank databases under accession number MH394187. Sequence alignment was conducted using the Muscle algorithm using the default settings and a maximum likelihood (ML) tree under Kimura 2-parameter model was constructed with 1,000 bootstrap replicates in MEGA v 7.0.26 (Kumar et al. 2016). The same software was used to calculate uncorrected p-distance among sequences, using the method of "complete deletion". The phylogeny obtained from MEGA was confirmed by running a maximum likelihood analysis under the GTR + Gamma model in RAxML (Stamatakis 2014) with 1,000 bootstrap replicates.

The distribution of *G. mali* in Europe from 2015 to 2019 (Fig. 1) was mapped using QGIS v. 3.10 software.

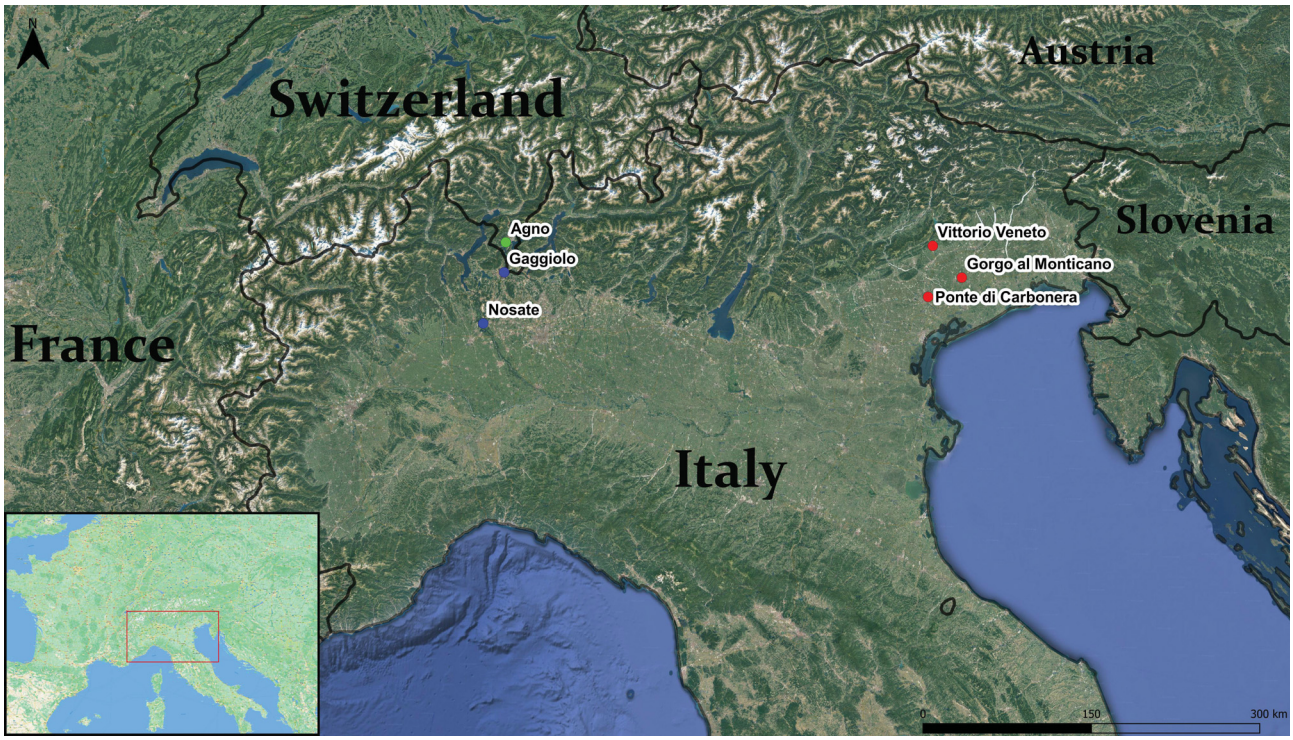

Fig. 1. Currently known distribution of *Gyponana mali* DeLong, 1942 in Europe. Colored dots represent the habitat type: green – urban green; blue – natural woody area; red – agroecosystem.

Results

Gyponana (*Gyponana*) *mali* DeLong, 1942

(Figs 2A–G, 3A–G, 4A–B)

Gyponana mali DeLong, 1942: 44.

Gyponana (*Gyponana*) *mali*: Oman (1949): 46; Metcalf (1962): 92; DeLong & Freytag (1964): 96; Hamilton (1982): 549, 555.

Material examined. SWITZERLAND: Ticino: Agno, 45°59′45.1″N 8°54′09.0″E, 277 m a.s.l., 4.vi.2015, on *Nerium oleander* (ornamental plant in urban green), 1 ♀. **ITALY: Lombardy:** Varese province: Gaggiolo, 45°49′44.6″N 8°53′22.7″E, 408 m a.s.l., 7.viii.2017, 1 ♀ 1 ♂ (I01/17) on the border of woody area intermixed with annual crop; Milano province: Nosate, 45°32′35.6″N 8°43′19.7″E, 149 m a.s.l., 12.vii.2015, on woody plants along the Ticino riverbank, 5 ♀♀ 1 ♂; same data but 45°32′38.3″N 8°43′22.0″E, 7.vii.2019, 10 ♀♀ (I03/19), 5 ♂♂ (I02/19). **Veneto:** Treviso province: Gorgo al Monticano, 45°47′58.0″N 12°33′08.5″E, 7 m a.s.l., 3.viii.2016, 1 ♀; 14.ix.2016, on *Vitis vinifera*, 1 ♀; 22.vii.2016, on hedgerows surrounding vineyards, 2 ♂♂, 1 nymph; same data but 45°48′02.5″N 12°33′08.0″E, 6.vii.2017, on *Cornus sanguinea* in a windbreak row surrounding vineyards, 2 ♀♀ 3 ♂♂; Vittorio Veneto, 45°58′35.0″N 12°19′11.7″E, 113 m a.s.l., 29.vi.–26.vii.2018, on *V. vinifera*, 1 ♀ 2 ♂♂; Carbonera, 45°41′30″N 12°16′57″E, 18 m a.s.l., 29.vi.–26.vii.2018, on *V. vinifera*, 1 ♂.

Diagnosis. Species belonging to the subgenus *Gyponana* s. str. vary from 7 to 13 mm in total length including forewings at rest, with external habitus depressed and pale green in overall coloration (fading to yellow in preserved specimens). Head with anterior margin declivous and subfoliaceous, narrower than pronotum. Pronotum transversely striate and widest posteriorly. Forewings opaque, venation moderately to strongly reticulate (Figs 2A–B, 3A–B).

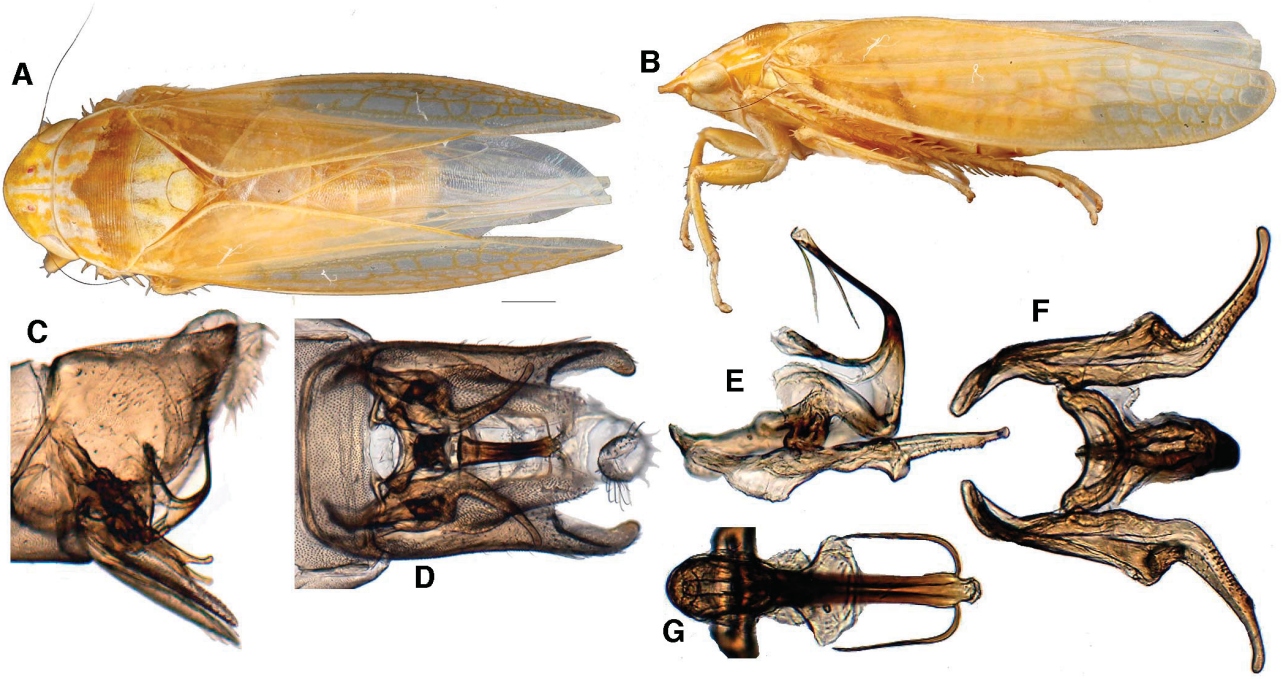

Fig. 2. *Gyponana mali* DeLong, 1942, male (voucher I02/19). A – dorsal view; B – lateral view; C – pygofer, valve, subgenital plate, and aedeagus, lateral view; D – pygofer, valve, subgenital plate, and aedeagus, dorsal view; E – styles, connective, and aedeagus, lateral view; F – styles, connective, and aedeagus, dorsal view; G – aedeagus, ventral view. Scale bar 1.0 mm.

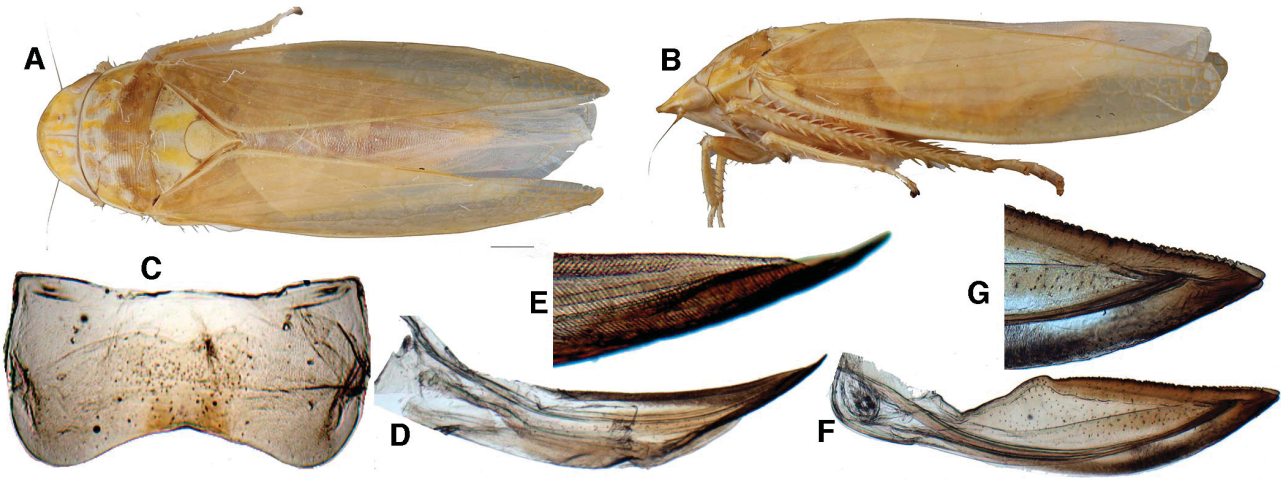

Fig. 3. *Gyponana mali* DeLong, 1942, female (voucher I03/19). A – dorsal view; B – lateral view; C – sternite VII, ventral view; D – first valvula, lateral view; E – first valvula, apical portion; F – second valvula, lateral view; G – second valvula, apical portion. Scale bar 1.0 mm.

Male (Fig. 2A, voucher I02/19). Body length 10.0 mm. Pygofer 1.5 times as long as its maximum height in lateral view, with anteroventral margin rounded and apex abruptly pointed; macrosetae dispersed on the posteroventral quadrant and fine setae present near ventral margin (Figs 2C–D). Aedeagus simple, without basal processes, constricted toward the middle, apex enlarged, produced and rounded; a pair of preapical lateral processes curved basally (Figs 2E, G). Style elongated, with the tip narrowed beyond a broad pointed tooth on the ventral margin; connective Y-shaped in dorsal view (Fig. 2F).

Female. Body length of specimens examined from Italy (Fig. 3, voucher I03/19) and Switzerland (voucher CH04/15) 11 and 13 mm, respectively. Sternite 7 slightly longer laterally than at midlength, with posterior margin concavely emarginate (Fig. 3C). Ovipositor surpassing pygofer apex. Valvula I (Figs 3D–E), in lateral view, about 4 times longer than high and slightly convex; apex pointed with fine denticles; dorsal sculptured area strigate and distributed submarginally. Valvula II (Figs 3F–G) abruptly broadened medially, with teeth small and rounded, present on distal half.

Comparative notes. Species of *Gyponana* bear no close resemblance to any other leafhopper taxa recorded in the western Palearctic region until now. In overall size and shape, *Gyponana* somewhat resembles the widespread Old-World grass-feeding genus *Glossocratus* Fieber, 1866 (Cicadellidae: Deltocephalinae) but is easily distinguishable by the green coloration and placement of the ocelli on the crown distant from the anterior margin and eyes.

Identification of species of *Gyponana* using morphology can be challenging. In an earlier attempt to review the related genera and subgenera of Gyponini using the genital apparatus, Ball & Reeves (1927) concluded that the aedeagal variation was not suitable to separate the species. Many species originally described based on slight differences in the structure of the male style and aedeagus were synonymized by Hamilton (1982) who reviewed the genus and provided a pictorial key to species. According to the morphological criteria of Hamilton (1982) the aedeagal shaft in posterior view is tapered distally in *G. mali* (Fig. 4A), but broadened preapically in *G. extenda* DeLong, 1942 (Fig. 4C). Moreover, the position of the gonopore is preapical on the ventral surface in *G. mali*, but situated at the apex of the distal extension in *G. extenda*. However, the male specimens examined from Italy appear to be morphologically intermediate between these two named North American species in the structure of the aedeagus (Fig. 2G). For example, in the voucher specimen I02/19, although the aedeagus is expanded well beyond the distal processes (Fig. 2G), as in *G. extenda* (*sensu* Hamilton 1982: Fig. 4C and *sensu* DeLong & Freytag 1964: Fig. 4D), the gonopore is distinctly preapical, as in *G. mali* (*sensu* Hamilton 1982: Fig. 4A and *sensu* DeLong & Freytag 1964: Fig. 4B).

Phylogenetic analysis. The barcoding sequence of mtCOI from the European specimen subjected to BLAST analysis (www.ncbi.nlm.nih.gov/BLAST) revealed 99.54% identity with a sequence of *G. mali* (accession number KF919958.1) collected from Canada (Ontario). The sequence collected during the present study was aligned with 28 mtCOI sequences downloaded from GenBank belonging to six species of *Gyponana* (*G. extenda*; *G. mali*; *G. salsa* DeLong, 1942; *G. parallela* DeLong 1942; *G. cacumina* DeLong, 1942; and *G. striata* (Burmeister, 1839)) (Supplementary material: Table S1) showing a percentage of identity higher than 98%. Alignments consisted of 692 nucleotide positions. Relationships among the six species of *Gyponana* recovered by the mtCOI ML tree (Fig. 5) were not fully congruent with morphological identifications. Our European sample groups with three out of five specimens of *G. mali* from Canada (Fig. 5, Gr. 4), but with low support. No species were recovered as monophyletic, and all grouped with low support (Fig. 5, Gr. 1–3), except four specimens of *G. extenda* from Canada (Fig. 5, Gr. 5) and two specimens of *G. cacumina* (Fig. 5, Gr. 6) with support of 87% and 93%, respectively. The comparisons with other sequences showed that the sequence obtained from Italy is very close to the other mtCOI sequences of *G. mali* (p-distances 0.00), *G. extenda* (0.01) and *G. striata* (0.00–0.01), all collected in Ontario (Canada), with

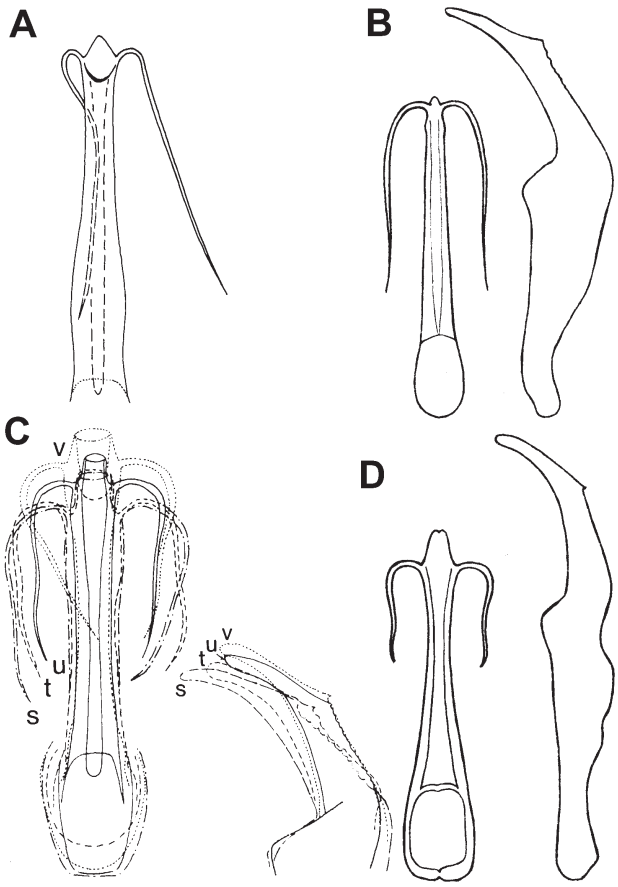

Fig. 4. *Gyponana mali* DeLong, 1942: A – aedeagus, caudal view (reprinted from Hamilton 1982); B – aedeagus, ventral view (left) and style, lateral view (right; reprinted from DeLong & Freytag 1964). *Gyponana extenda* DeLong, 1942: C – aedeagus, caudal view and style, lateral view, with structural variation from "s" to "v" form (reprinted from Hamilton 1982); D – aedeagus, ventral view (left) and style, lateral view (right; reprinted from DeLong & Freytag 1964).

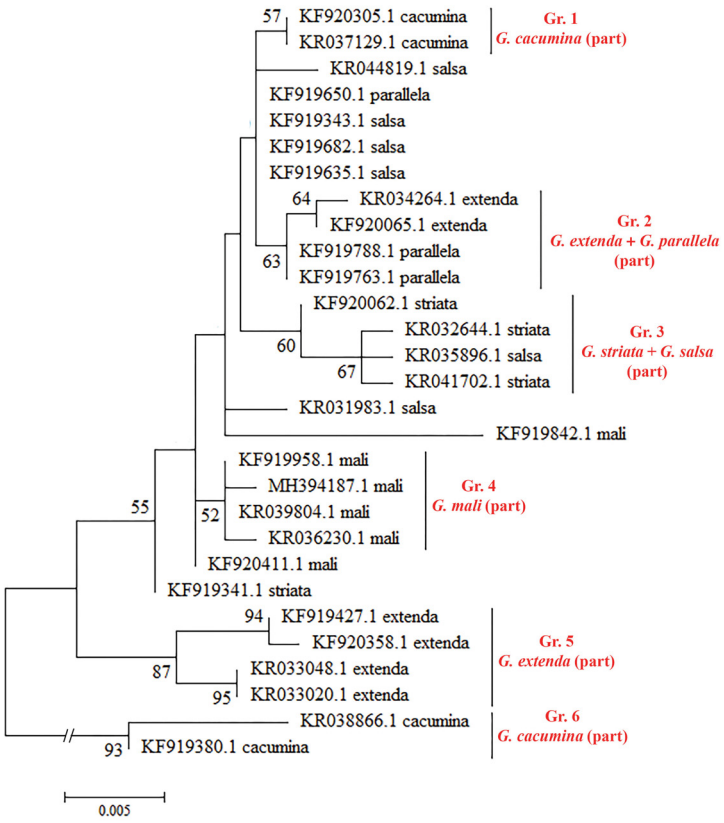

Fig. 5. Maximum likelihood tree of *Gyponana* spp. based on mtCOI gene. Bootstrap values are shown if >50. A total of 692 positions were included in the final dataset. The branch marked with "//" was shortened, with the real length of 0.016. Gr. – group. The sequence from the present study (GenBank accession number MH394187.1) is in Gr. 4.

the exception of *G. mali* (KF919842.1) which showed a higher p-distance (0.02) compared to the other *G. mali* specimens in Gr. 4. The divergence with specimens of the other three species was also very low, ranging from 0.01 to 0.02 (Supplementary Table S1). The mtCOI ML tree built in RAxML under the GTR + Gamma recovered the same topology and similar branch support (data not shown).

Discussion

Based on our morphological and molecular studies, all of our specimens from Europe can be assigned to the species *G. mali*. The European specimens studied here are morphologically similar to specimens (including paratypes) identified as this species from its native range in North America and also appear to be genetically similar based on the barcode region of the mitochondrial COI gene. Nevertheless, our analysis of the COI barcode data suggests that this molecular marker may not reliably distinguish some previously named species of *Gyponana*. This may indicate either that this gene region is not sufficiently variable in the genus, or that some species previously recognized based on slight differences in the male genitalia are not distinct and should be treated as synonyms. Further studies of the genus are needed to determine which molecular or morphological characters are the most useful for delimiting and diagnosing species of *Gyponana*.

This is the first record of the tribe Gyponini for the Palearctic Region. This group is widely distributed in North and South America, most probably with a Neotropical origin. Other records of Gyponini outside of the western hemisphere are very rare. Kirkaldy (1905) described the species *Gypona kangrensis* Kirkaldy, 1905 based on one specimen from northern India but the identity of this species and its placement in Gyponini have not been confirmed. An unidentified species of the genus *Curtara* DeLong & Freytag, 1972 was reported from the west coast of Africa, probably representing an anthropogenic introduction (Nielson & Knight 2000), but no further reports of this species have appeared.

In Europe, the first specimen was incidentally collected in Switzerland in 2015 by the first author: it was a female collected using a sweep net on an ornamental plant in a regularly managed urban green area (Fig. 6A). During an independent survey, more specimens were collected by an Italian amateur entomologist (Dr. Danilo Mario Piccolino) in 2015 and then regularly until 2019. All the specimens were collected from a riparian habitat located in the Natural Preserve "Parco del Ticino" which is located about 55 kilometers far away from Agno (Switzerland) and separated by the Lombardian Prealps (1097–1959 m a.s.l.) and the Ceresio Lake. The co-authors from CREA–VE (Conegliano, Italy) captured more specimens on yellow sticky traps placed in vineyards, routinely investigated to verify the occurrence of leafhopper vectors of quarantine phytoplasmas associated with grapevine yellows diseases (Flavescence dorée). This material remained unidentified until the present study due to the difficulty of species identification in this genus using only morphology.

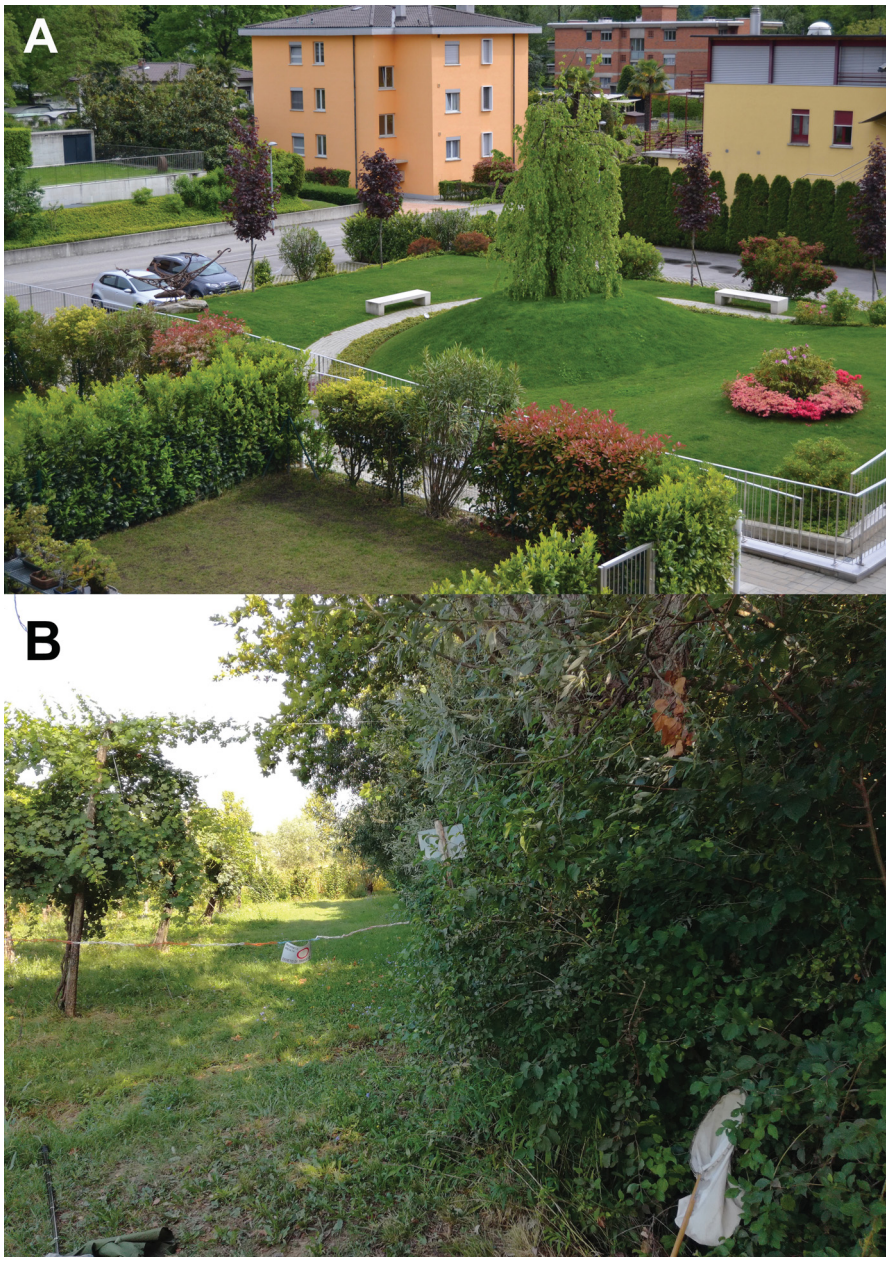

Fig. 6. Two collection sites of *Gyponana mali* DeLong, 1942 in Europe. A – ornamental plants in privately managed urban green in Switzerland (Agno, Ticino) where *G. mali* was accidentally collected in 2015; B – plants of *Cornus sanguinea* in a windbreak row (on the right) surrounding a vineyard plot (on the left) in Italy (Gorgo al Monticano, Treviso) where larvae and adults of *G. mali* were sampled in 2017.

Following these incidental collections, we planned to extend the survey to verify the host plants in the habitats of occurrences. Despite further surveys in 2017 and 2018, no additional specimens were collected from the site of the first record in Switzerland. However, in Italy, after the first record in 2015, we collected more specimens on grapevine (*Vitis vinifera*) from three additional locations in North Eastern Italy (Gorgo al Monticano, Vittorio Veneto and Carbonera). Using a sweep net, we also inspected every single plant of the windbreak surrounding the vineyard in Gorgo al Monticano (*Acer campestre*, *Alnus glutinosa*, *Betula* sp., *Cornus sanguinea, Corylus avellana*, *Crataegus monogyna, Carpinus sp., Platanus sp., Quercus sp.,* and *Salix* sp.). We collected specimens of *G. mali* only from *Cornus sanguinea* (Fig. 6B). Further sampling in Gaggiolo, a site located between Agno (Switzerland) and Nosate (Italy), yielded additional specimens of the exotic species in the border of a woody patch where *C. sanguinea* also occurred.

In the native range of North America, *G. mali* seems to have a narrower distribution than the other closely related species, being recorded in Georgia, Illinois and Ohio (USA) (Metcalf 1962). The species was commonly recorded from apple (*Malus* spp.) by DeLong (1942) but is also likely associated with other trees or shrubs. Little information about host plants of most *Gyponana* (*s. str.*) species is available in their native area of distribution. Various species have been recorded from woody host plants, including *Alnus*, *Carya*, *Malus*, *Pinus*, *Quercus*, *Salix*, *Tsuga*, and various herbaceous dicots (Hamilton 1982).

Altogether, our observations provide evidence for a recent introduction and spreading of this species in southern Switzerland and central northern Italy. We suggest that, after a possible initial introduction with ornamental plants, the species spread across different agroecosystems with a preference for semi-natural, less disturbed habitats and wild plants (e.g., *C. sanguinea* in the present study). Lately,

several records of exotic leafhoppers introduced from the Nearctic or Asian Regions were reported in the literature and all of them were presumably linked to the trade of ornamental plants (e.g., Seljak 2013; Trivellone et al. 2015, 2017; D'Urso et al. 2019).

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We thank Dr. Danilo Mario Piccolino who collected the material from Nosate Lombardy (Italy) and kindly provided us more specimens to examine and compare with our collections. This study was partially supported by the Swiss National Science Foundation (P2NEP3_168526) and US NSF grant DEB-1639601.

Digitally archived data

Supplementary Table 1. Estimates of evolutionary divergence (uncorrected p-distance) of mtCOI sequences of six species of *Gyponana*. Gr. – group; Acc. Number – GenBank accession number. The sequence from the present study was collected in Italy (Acc. Number MH394187.1), other specimens of *G. mali*, *G. extenda* and *G. striata* were collected in Canada (Ontario), *G. salsa* in Canada (Acc. Number starting for KR) and USA, Minnesota (KF), *G. parallela* in USA (Virginia), *G. cacumina* in Canada (KR) and USA, Pennsylvania (KF).

The .xls file is deposited on the web page of the journal (www.aemnp.eu) as well as in the Zenodo scientific archive (https://zenodo.org/) at https://doi.org/10.5281/zenodo.4733948.

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