Osbornellus auronitens (Hemiptera: Cicadellidae: Deltocephalinae), an introduced species new for the Palaearctic region

Valeria Trivellone,[1] Milana Mitrović, Christopher H. Dietrich, Ivo Toševski

Abstract—The introduction of invasive species may result in important ecological, environmental, and economic impacts. Extensive study of Auchenorrhyncha (Hemiptera) fauna in a wine-growing region in southern Switzerland revealed, for the first time, presence of the Nearctic leafhopper *Osbornellus auronitens* (Provancher) (Hemiptera: Cicadellidae) in the vicinity of Stabio (Canton of Ticino). The species identity of the collected specimens was confirmed using morphological and molecular characters. All specimens of *O. auronitens* were collected in a forest of *Castanea sativa* Miller (Fagaceae), *Corylus avellana* Linnaeus (Betulaceae), and *Alnus glutinosa* (Linnaeus) Gaertner (Betulaceae) intermixed with *Cornus sanguinea* Linnaeus (Cornaceae), *Salix* Linnaeus (Salicaceae), and *Rubus* Linnaeus (Rosaceae). In its native range this leafhopper is polyphagous and a relatively common visitor in vineyards. Based on analysis of the barcoding region of the mitochondrial cytochrome oxidase subunit I gene of the collected *O. auronitens*, 100% identity with specimens of the same species originating from Canada was determined. *Osbornellus auronitens* is morphologically similar to *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae), another introduced Nearctic leafhopper, which was involved in severe outbreaks of disease caused by the Grapevine flavescence dorée phytoplasma (Bacteria: Acholeplasmataceae) in European viticultural regions since the 1960s. In this paper, we report the morphological features to distinguish *O. auronitens* from *S. titanus*, and discuss the possible implications of its expected spread across the Old World.

Introduction

According to the results of the Delivering Alien Invasive Species Inventory for Europe project (Roques *et al.* 2009), alien insects and other terrestrial arthropods are the most numerous invaders in Europe, manifesting various effects, particularly in agricultural and forest ecosystems. Impacts range from the negative environmental effects commonly combined with economic losses, to negative social impacts when they affect a human well being (Binimelis *et al.* 2007). Once established into a new environment, alien terrestrial arthropods, especially insects, may undergo rapid dispersal followed by exponential population growth due to a lack of natural enemies that regulate their populations in their native areas, also referred to as enemy release hypothesis (Keane and Crawley 2002).

According to Roques *et al.* (2009), a total of 1296 alien terrestrial invertebrates originating from other continents are documented to have established in Europe; of which arthropods, mostly insects, represent 94% of the introduced species. The number of introduced species is probably even higher, taking into account the fact that identification and recognition of newly introduced alien species is often delayed until populations of introduced insects become well established and begin producing distinct

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V. Trivellone,[1] Swiss Federal Institute for Forest, Snow, and Landscape Research (WSL), Biodiversity and Conservation Biology, Zürchertrasse 111, Birmensdorf 8903, Switzerland; and Agroscope Swiss Federal Research Station, A Ramél 18, Cadenazzo 6594, Switzerland
M. Mitrović, Department of Plant Pests, Institute for Plant Protection and Environment, Banatska 33, Zemun 11080, Serbia
C.H. Dietrich, Illinois Natural History Survey, Prairie Research Institute, University of Illinois, Champaign, Illinois, 61820, United States of America
I. Toševski, Department of Plant Pests, Institute for Plant Protection and Environment, Banatska 33, Zemun 11080, Serbia; and CABI, 1 Rue des Grillons, Delémont 2800, Switzerland

[1]Corresponding author (e-mail: valeria.trivellone@gmail.com).
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environmental or economic impacts. Lack of taxonomic expertise in determination of the alien species may cause delays in recognition of the taxa, especially with regards to native and/or previously introduced congeneric species with high morphological similarity.

Over the past 50 years, several alien species in the suborder Auchenorrhyncha (Hemiptera) originating from the Nearctic Region caused various negative impacts in Europe by inducing substantial economic losses and environmental disturbance (Arzone *et al.* 1987; Foissac and Wilson 2009). The most striking example is a North American leafhopper *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae), which was first reported in 1958 in the vineyards of southwestern France (Bonfils and Schvester 1960). The species quickly spread to southeastern France (Schvester *et al.* 1963), northwestern Italy (Vidano 1964), and southern Switzerland (Baggiolini *et al.* 1968). Presently, *S. titanus* colonises vineyards in all Mediterranean countries including the temperate regions of Austria and southeastern Europe. Introduced from eastern parts of North America to Europe (Papura *et al.* 2012), this leafhopper became a causal vector in transmission of a quarantine disease, Grapevine flavescence dorée (Bacteria: Acholeplasmataceae) (Caudwell *et al.* 1970), caused by a phytoplasma belonging to the 16SrV taxonomic group (Angelini *et al.* 2004; Arnaud *et al.* 2007). It is worth noting that species of the genus *Scaphoideus* Uhler are also considered as potential vectors in transmitting diverse phytoplasma diseases in North America (Hill and Sinclair 2000; Beanland *et al.* 2006). In several large and small outbreaks from 1970 to 2006, Grapevine flavescence dorée severely affected vineyards in France, Spain, Portugal, Italy, and Serbia causing major economic losses due to deleterious effects on grapevine and the costs of control measures against the vector and disease spread.

Another well-known exotic species is the planthopper *Metcalfa pruinosa* (Say) (Hemiptera: Flatidae), also introduced from North America into the Mediterranean and temperate regions of Europe (Zangheri and Donadini 1980). Besides the great invasiveness of this planthopper in Europe, its presence is associated with serious damage to various field crops, gardens, and ornamental plants. The gregarious feeding behaviour of its nymphs and the massive secretion of epicuticular wax and honeydew favour the development of fungal diseases in the affected plants (Byrne and Bellows 1991).

In the present paper, we document for the first time a species of the genus *Osbornellus* Ball (Hemiptera: Cicadellidae) identified as *Osbornellus auronitens* (Provancher) introduced to the Palaearctic from the Nearctic region based on morphological and molecular evidence. We report morphological features to distinguish it from the most closely related species in Europe, *S. titanus*. Finally, we discuss the possible implications of its expected spread across the Old World and possible interactions with native plants in its new environment.

Materials and methods

Insect sampling

During extensive study of Auchenorhyncha fauna in a wine-growing region in Canton Ticino (Southern Switzerland), a total of three males of a leafhopper species resembling *Scaphoideus* were collected on 11 August 2016 in a woody patch scattered within a wine-growing area (Stabio, 45°51'17.2'N, 8°55'38.1'E). At the collection site, *Castanea sativa* Miller (Fagaceae), *Corylus avelana* Linnaeus (Betulaceae), and *Alnus glutinosa* (Linnaeus) Gaertner (Betulaceae) are the dominant trees intermixed with scarce *Cornus sanguinea* Linnaeus (Cornaceae), *Salix* Linnaeus (Salicaceae), and *Rubus* Linnaeus (Rosaceae). The site was revisited on 7 and 9 September, and three more specimens (two females and one male) belonging to the same species were collected. All specimens were collected using a sweep net and mouth aspirators.

Morphological study

The abdomen of a voucher specimen designated as Oa1 (male) was dissected to study the genitalia (Knight 1965), thereafter identified by using published taxonomic keys and related literature (Beamer 1937; DeLong and Knull 1941; DeLong 1941a; DeLong and Martinson 1976a, 1976b; Dlabola 1984, 1987a, 1987b). Photographs were taken with a Leica MC 170AD digital camera on the Leica M165C stereomicroscope (Wetzlar, Germany). Terminology follows Viraktamath and Mohan (2004). Voucher specimen Oa1 is deposited in the collection of the Natural History Museum in Lugano (Canton Ticino, Switzerland). The rest of the sampled material is deposited in the private collection of the first author.

Molecular analysis

For molecular analysis, DNA was extracted from a voucher specimen designated as Oa2. The barcoding region of mitochondrial cytochrome oxidase subunit I gene (mtCOI) was used to confirm the identity of the sampled leafhoppers. Genomic DNA was extracted using the DNeasy® Blood & Tissue Kit (Qiagen Inc., Valencia, California, United States of America) following the manufacturer's instructions. The barcoding region of the mtCOI gene was amplified using the primer pair LCO1490/HCO2198 (Folmer *et al.* 1994). Each polymerase chain reaction was carried out in a volume of 20 µL [1 µL of DNA, 11.8 µL of H_2O, 2 µL of high yield reaction buffer A with 1x Mg, 1.8 µL of $MgCl_2$ (2.25 mM), 1.2 µL of dNTP (0.6 mM), 1 µL of each primer of the pair LCO1490/HCO2198 (0.5 µM), and 0.2 µL of KAPATaq DNA polymerase (0.05 U/µL) (Kapa Biosystems, Wilmington, Massachusetts, United States of America)]. The polymerase chain reaction protocol consisted of an initial denaturation at 95 °C for five minutes, 35 cycles each consisting of three steps, *i.e.*, one minute at 94 °C, one minute at 54 °C, and 1.5 minute at 72 °C, with a final extension step at 72 °C for seven minutes at the end of amplification protocol. Amplified products were run on 1% agarose gel, stained with ethidium bromide and visualised under an ultraviolet transilluminator. Polymerase chain reaction amplicons were sequenced by Macrogen (Seoul, Korea). To obtain 658 base pairs of the barcoding region sequencing was performed with both, forward and reverse primers. Sequence data are deposited in GenBank (www.ncbi.nlm.nih.gov) under accession number KY006567.

Results

According to morphology and molecular analysis, the specimens collected in Stabio (Canton Ticino, Switzerland) were identified as *O. auronitens*. The barcoding sequence of mtCOI subjected to BLAST analysis (www.ncbi.nlm.nih.gov/BLAST) revealed 100% identity with a sequence of *O. auronitens* (accession number KR043272), originating from Churchill, Prince Edward Island, Canada (Gwiazdowski *et al.* 2015).

Osbornellus auronitens (Provancher)

Material examined. Four males (designated as voucher specimens Oa1–Oa4), Stabio, Ticino, Switzerland (45°51'17.2'N, 8°55'38.1'E), 11.viii.2016 and 9.ix.2016, V. Trivellone; two females (Oa5, Oa6) Stabio, Ticino, Switzerland, 7. ix.2016 and 9.ix.2016, V. Trivellone. Voucher specimens Oa1 is deposited in the Natural History Museum in Lugano, while voucher specimens Oa2–Oa6 are in the private collection of V.T.

Differential diagnosis. *Osbornellus auronitens* is morphologically similar to *S. titanus*, a well-known vector of phytoplasmas belonging to the elm yellows ribosomal group and introduced into the Palaearctic region in the 1960s. The overall size, shape, and colouration bear a striking resemblance between the two species, but they can be distinguished using distinctive external morphological characters.

In *O. auronitens*, the anterior margin of the head has two dark brown, transverse bands; an incomplete brown preapical band on the vertex; and an arcuate, red-orange fascia between the eyes more posterad (Fig. 1A–B). In *S. titanus*, the head has four transverse, brown bands – two on the upper part of the face below the ocelli and two on the vertex, and a transverse light-orange fascia between the eyes with a median angulate projection anteriorly (Fig. 1D–E). The forewings of the two species are similar in colouration and venation but *O. auronitens* has relatively few dark brown markings and vein R has only three branches (Fig. 1A–B) while *S. titanus* has the dark markings more extensive and vein R has five branches (Fig. 1D–E). As in other species of *Osbornellus*, the male subgenital plate of *O. auronitens* has an elongate, slender, filamentous distal portion (Fig. 1C). In contrast, the subgenital plate of *S. titanus* is broadly rounded distally and lacks an elongated distal portion (Fig. 1F). All of the above traits are visible without dissection.

In Fig. 2, dissected parts of the male genitalia of *O. auronitens* are shown. The aedeagus of *O. auronitens* in lateral view is slender and thin, slightly curved dorsally, with a pair of broad processes arising laterally near the base of the

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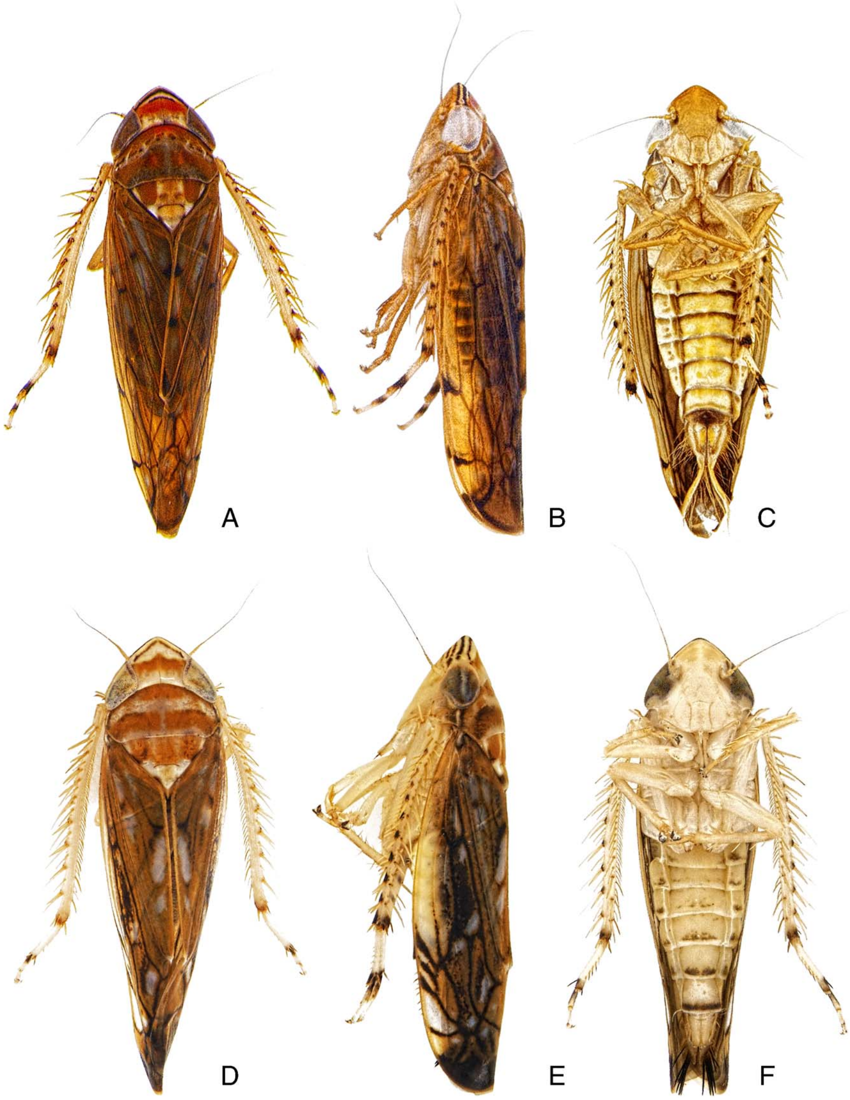
Fig. 1. Habitus of *Osbornellus auronitens* (Provancher), voucher specimen Oa1, **A–C** and *Scaphoideus titanus* Ball from Serbia (Bela Crkva, Banat region), **D–F** in dorsal, lateral view and ventral view respectively.



shaft, flaring slightly, extending just beyond the apex of the shaft to dorsally pointed apices (Fig. 2A–C). In *S. titanus*, the aedeagus in lateral aspect is generally triangular with the base generally pear shaped; the paired posterior processes arising from the connective stem are membranous, digitiform, and divergent in ventral view, usually not reaching the apex of the aedeagus (della Giustina 1989). The style in *O. auronitens* has the distal section stout, parallel sided, and relatively long, and the apex rounded and slightly concave on laterally near the apex (Fig. 2G–I). In *S. titanus*, the distal portion of the style is distinctly tapered and curved laterally with two setae near the middle and the apex is sharply pointed (della Giustina 1989). The shape of the male pygofers of *O. auronitens* and *S. titanus* are rather similar.

Discussion

Early detection of an alien organism is of crucial importance to allow observation of the vast array of

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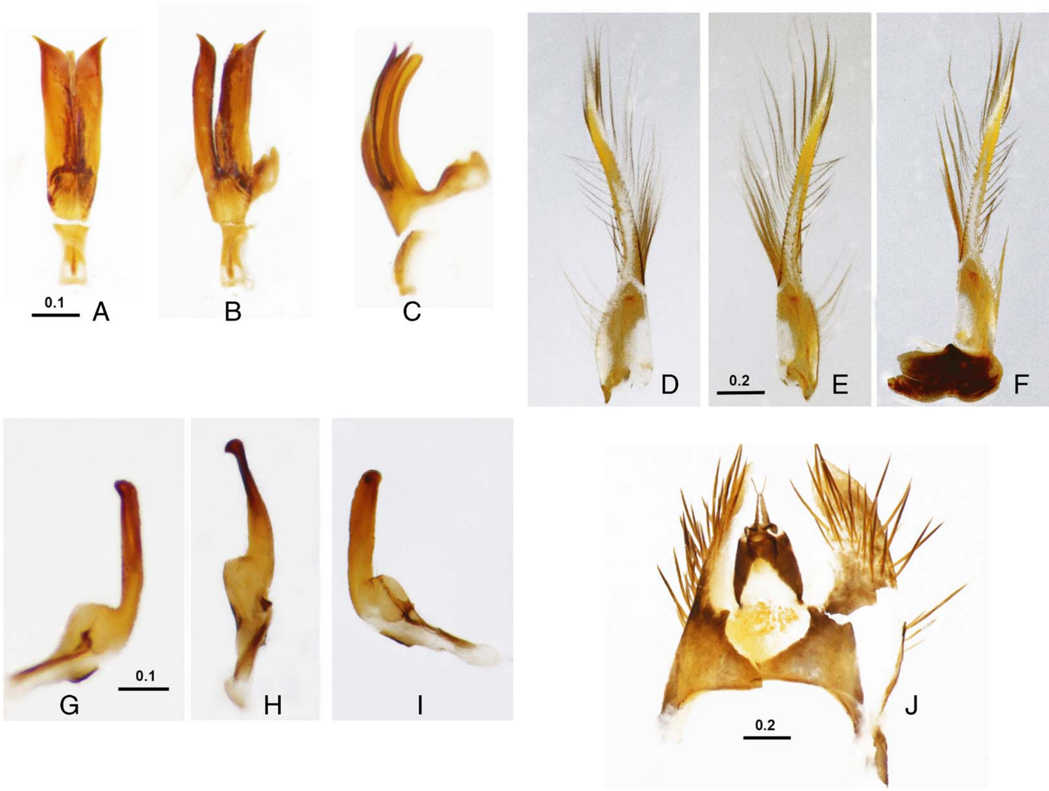
Fig. 2. Male genitalia of *Osbornellus auronitens* (Provancher) voucher specimen Oa1. **A**, Aedeagus, dorsal view; **B**, aedeagus, rotate 30°; **C**, aedeagus, lateral view; **D**, left subgential plate, dorsal view; **E**, left subgential plate, lateral view; **F**, valve and right subgential plate; **G**, left stylus, dorsal view; **H**, left stylus, ventral view; **I**, left stylus, lateral view; **J**, pygofer, dorsal view.



possible interactions between the introduced organism and its new environment. Developing a monitoring strategy is also important for minimising possible consequences of such an introduction. Most leafhopper species are small, inconspicuous animals that when introduced into new areas, may remain unnoticed for some time. Early detection may also be hindered in cases, such as the one presented here, when a newly introduced species is morphologically similar to another species already present. Unfortunately, due to a lack of systematic survey and monitoring programmes designed to detect exotic species, the presence of such species is most often noticed as a result of their negative environmental or economic impacts after they have become well established.

Although *O. auronitens* is morphologically similar to *S. titanus*, as noted above, in Europe it is possible to distinguish these species reliably using external morphological traits. So far, only one other species of *Osbornellus*, *O. horvathi* (Matsumura) (Hemiptera: Cicadellidae), and one other species of *Scaphoideus, S. dellagiustinai* Webb and Viraktamath (Hemiptera: Cicadellidae), have been reported from western Europe. *Osbornellus horvathi*, which is reported from Algeria, Sicily, and Spain, is similar to *S. titanus* in external colour pattern but easily distinguishable by the structure of the male genitalia (Hermoso de Mendoza *et al.* 2012). This species has recently been shown to be capable of transmitting '*Candidatus* Phytoplasma asteris' to broad bean (*Vicia faba* Linnaeus; Fabaceae) and periwinkle (Apocynaceae) in experimental trials (Rizza *et al.* 2016). *Scaphoideus dellagiustinai*, which is, so far, only recorded from the coast of southern France and Burkina Faso, is smaller and paler in colouration compared with both

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O. auronitens and *S. titanus*; has only two transverse, brown bands on the head, both on the face below the ocelli; the vertex is white with a single, bright red, transverse band; and the male genitalia are distinctive (see figures in Webb and Viraktamath 2007).

Within their native range of eastern North America, both *O. auronitens* and *S. titanus* species can be confused with congeneric species occurring in the same deciduous forest habitats that are similar in size, colour pattern, and wing venation. Thus, it is important to confirm the identities of these species by examining features of the male genitalia that are diagnostic at the species level. *Osbornellus* and *Scaphoideus* are large genera, each with over 100 documented species worldwide and it is quite possible that additional species of both genera could eventually become established in Europe.

The genus *Osbornellus* is presently included in the tribe Scaphoideini within the large cicadellid subfamily Deltocephalinae. The genus was originally established by Ball (1932) based on type species *Scaphoideus auronitens* Provancher. Ball's (1932) decision to recognise additional genera in the group was based on observations of morphological differences and distinct feeding behaviour and ecological relationships. The genera were originally distinguished based on the size of the ocelli and characteristics of the veins and cells of the forewing. Beamer (1937) revised *Osbornellus* in the United States of America, highlighting the difficulty of distinguishing species. Subsequent authors added nearly 100 species from the Nearctic and Neotropical regions (*e.g.*, DeLong and Berry 1937; DeLong 1941a, 1941b, 1942, 1976; DeLong and Knull 1941; Metcalf 1954; Linnavuori 1959; DeLong and Martinson 1976a, 1976b) mainly distinguished using features of the male genitalia. Martinson (1977) completed a taxonomic revision of the genus as a doctoral dissertation, but this work was never published. Dominguez and Godoy (2010) recently added 37 new *Osbornellus* species from Central America.

Nine species of *Osbornellus* have been recorded from the Palaearctic, four of which have been assigned to the endemic western Asian subgenus *Mavromoustaca* Dlabola and one to the nominotypical subgenus (Matsumura 1908; Vilbaste 1976; Dlabola 1984, 1987a, 1987b). However, the phylogenetic status of the genus, and relationships of subgenera and species presently included in the genus, has never been explored through phylogenetic analyses (Nielson and Knight 2000).

Within its native range, *O. auronitens* is widespread in the deciduous forests of eastern North America from Canada (southern Ontario and Québec) to the southeastern United States of America (Florida to Oklahoma) (Martinson 1977). Its introduction into the Palaearctic Region may have occurred through contamination of imported live plant material (Bertin *et al.* 2007), especially because *O. auronitens* hibernates as eggs inserted into the bark of woody plants. This is also the case with mosaic leafhopper *Orientus ishidae* (Matsumura) (Hemiptera: Cicadellidae), a polyphagous species native to the eastern Palaearctic Region but also widespread and well established in eastern North America (Valley and Wheeler 1985), and reported for the first time in Europe from Switzerland in 2002 (Günthart and Mühlethaler 2002). Over the subsequent 15 years, the mosaic leafhopper was recorded in Germany (Nickel and Remane 2003), Slovenia (Seljak 2004), Czech Republic (Malenovský and Lauterer 2010), Austria (Nickel 2010), France (Mifsud *et al.* 2010), Hungary (Koczor *et al.* 2013), and Serbia (V.T., unpublished data). Several authors also suggested that *O. ishidae* had probably been introduced into Europe by trading of plants (Malenovský and Lauterer 2010; Mifsud *et al.* 2010). The situation with *O. ishidae* is a typical case of rapid range expansion of the introduced alien leafhopper with possible economic impacts, especially after Grapevine flavescence dorée was identified recently in mosaic leafhopper adults from Italy, Slovenia, and Switzerland (Mehle *et al.* 2010; Gaffuri *et al.* 2011; Trivellone *et al.* 2016).

Data regarding capability of the species from the tribe Scaphoideini to serve as vectors in transmission of phytoplasmas are scarce in literature (Weintraub and Beanland 2006). Besides *S. titanus*, which is a documented vector transmitting the phytoplasma-associated disease Grapevine flavescence dorée on grapevine in Europe, *O. auronitens* was noted as a possible vector involved in transmission of grapevine yellows diseases in vineyards of Virginia, United States of America (Beanland *et al.* 2006).

Although it is not known whether *O. auronitens* will disperse into new areas in the Palaearctic, almost 60 years ago, *S. titanus*, a species with

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similar food preferences in its native range, was introduced in France and is now widespread from 35 to 48 degrees north latitude and from Portugal to Romania. *Scaphoideus titanus* adjusted well to its new European environment, and has colonised both wild and cultivated grapevine to become the major vector of phytoplasma causing the Flavescence dorée disease on grapevine. *Osbornellus auronitens* collected in Switzerland has colonised the ecotone strip between vineyards and forest. This suggests that it poses a potential risk, as already reported from Virginia, where *S. titanus*, *O. auronitens*, and *Jikradia olitorius* (Say) (Hemiptera: Cicadellidae) exhibit seasonal movement into vineyards from nearby forest vegetation, which could account for the high incidence of diseased vines observed near the vineyard edge (Beanland *et al.* 2006). Thus, presence of *O. auronitens* and its potential spread across viticultural regions in Europe is of objective concern because polyphagous adults can acquire different phytoplasmas from adjacent plant vegetation and launch new epidemiological cycles inside vineyards. To prevent such risk, setting controlled conditions for import of all plant material from foreign countries is of utmost importance. Scientific and economic concern that the spread of this exotic leafhopper species could lead to tremendous economic losses is justifiable because a similar scenario has already occurred in Europe with introduction and establishment of the related species, *S. titanus*.

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