

# New records and updates on alien Auchenorrhyncha species in Serbia

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## SUMMARY

During a 2019 survey of the Auchenorrhyncha fauna in Serbia, three alien species were recorded for the first time: a leafhopper species of Asian origin *Orientus ishidae* (Cicadellidae, Deltocephalinae) and two North American species, *Acanalonia conica* (Acanaloniidae) and *Erasmoneura vulnerata* (Cicadellidae, Typhlocybinae). Besides these first findings, the presence of the leafhopper *Phlogotettix cyclops* (Cicadellidae, Deltocephalinae) was confirmed in Serbia after its initial record more than a century ago. Other allochthonous species already known to be present in local entomofauna were also found: *Japananus hyalinus* (Cicadellidae, Deltocephalinae), *Scaphoideus titanus* (Cicadellidae, Typhlocybinae), *Stictocephala bisonia* (Membracidae) and *Metcalfa pruinosa* (Flatidae). The newly detected Auchenorrhyncha species in Serbia, *O. ishidae*, *A. conica* and *E. vulnerata*, as well as *Ph. cyclops*, are recognized as either tentative or true grapevine pests in Europe that cause physical damage or induce phytoplasma-borne diseases. Our findings reveal a potential phytosanitary risk that can emerge in Serbian vineyards in upcoming years and support refocused research of Auchenorrhyncha diversity in natural habitats, rather than in agroecosystems and their immediate surroundings.

**Keywords:** Auchenorrhyncha, diversity, alien species, Serbia

## INTRODUCTION

Whether considered as opportunists or victims of anthropogenic factors and climate change, invasive insects are a major threat to their new environment. They can disrupt the equilibrium in newly populated ecosystems and spread plant diseases, devastate crops, forests and food stocks, resulting in 20-30% of agricultural yield losses, which makes them the costliest

animal group to humanity (Bradshaw et al., 2016). Of more than 2000 European Auchenorrhyncha species, mostly those designated as pests have been thoroughly researched (Mifsud et al., 2010). So far, a total of 32 alien Auchenorrhyncha species have been detected in Europe, and the presence of four of them has been documented in Serbia: *Japananus hyalinus* (Osborn, 1900) (Cicadellidae, Deltocephalinae), *Scaphoideus titanus* Ball, 1932 (Cicadellidae, Typhlocybinae), *Stictocephala*

*bisonia* Kopp & Yonke, 1977 (Membracidae) and *Metcalfa pruinosa* (Say, 1830) (Flatidae) (Tanasijević, 1962; Janković, 1975; Magud & Toševski, 2004; Mihajlović, 2007; D'Urso et al., 2019). Although all four species are tentative pests of cultivated and ornamental plants, major agroecological damage has been induced in vineyards in Serbia and across Europe by a vector-phytoplasma duo consisting of accidentally introduced American grapevine leafhopper *S. titanus* and Europe-native *Flavescence dorée* (FD) phytoplasma (16SrV) (Arnaud et al., 2007; Krnjajić et al., 2007; Jeger et al., 2016).

Over the past few years, a new leafhopper linked to FD phytoplasma has been detected in Europe, the mosaic leafhopper *Orientalus ishidae* (Matsumura, 1902) (Cicadellidae, Deltocephalinae) (EPPO, 2015). After it was first detected in Italy, Switzerland and Germany in the early 2000s, this species of Asian origin quickly spread across the continent by moving north towards the Netherlands and spreading eastwards to Romania (Günthart & Mühlethaler, 2002; Nickel & Remane, 2003; Guglielmino, 2005; Den Bieman & Van Klink, 2015; Chireceanu et al., 2017). Interest in *O. ishidae* grew after FD phytoplasma was detected in European populations in vineyards and its vectoring potential was experimentally confirmed (Mehle et al., 2010; Lessio et al., 2016). Furthermore, this species was already a suspected vector of 'Candidatus Phytoplasma pruni' (16SrIII) in the USA (Rosenberger & Jones, 1978). The presence of Nearctic species *Acanalonia conica* (Say, 1830) (Acanaloniidae) in Europe was first reported in Italy, and soon after that detected in Switzerland, Slovenia and Romania (D'Urso & Uliana, 2006; Trivellone et al., 2015; Chireceanu et al., 2017; Seljak, 2018). Although widely considered as a polyphagous species and not related to crops, *A. conica* was observed ovipositing on grapevines in the USA (Wilson & McPherson, 1981). In Europe, it was recorded in urban areas, natural habitats and associated with several arboreal, shrubby and herbaceous plants bordering vineyards, but not on grapevines (Nicoli Aldini et al., 2008). The presence of another *Vitis*-related leafhopper, *Erasmoneura vulnerata* (Fitch, 1851) (Cicadellidae, Typhlocybinae) was also observed in Italy for the first time, followed by records from Slovenia (Duso et al., 2005; Seljak, 2011). This typhlocybin species from the temperate North America (Dietrich & Dmitriev, 2006) completes two or three

generations per annum in Europe and successfully colonizes several European grapevine cultivars, showing a pest potential (Duso et al., 2019).

This study focused especially on verifying the presence of the leafhopper *Phlogotettix cyclops* (Mulsant & Rey, 1855) (Cicadellidae, Deltocephalinae), which has been trending recently in phytoplasma epidemiological research as an inhabitant of European vineyards and a potential FD vector (Chuche et al., 2010; Chireceanu et al., 2017; Strauss & Reizenzein, 2018; Plavec et al., 2019). Despite rising concerns over this leafhopper as an invasive Asian pest that spreads across Europe, its presence in several European countries has been documented for more than a 100 years (Metcalf, 1967). Although occasionally perceived as an allochthonous species spreading in European fauna (Chuche et al., 2010), it is important to highlight that some authors identify *Ph. cyclops* as a palearctic/amphiboreal species and only representative of the genus *Phlogotettix* Ribault, 1942 in Europe (Gnezdilov, 2003; Meshram et al., 2015). The first traceable record of *Ph. cyclops* in Serbia was made by Horváth (1903) and it specified Niš as the collecting locality, but without plant or habitat data.

Comprehensive research of Auchenorrhyncha diversity in Serbia was conducted during the 1970s and 1980s (e.g. Janković, 1975; Janković, 1978; Janković & Papović, 1985), while more recent publications mainly focused on agroecosystems (Jović, 2009; Cvrković et al., 2010; Jakovljević et al., 2013). Thus far, at least 524 Auchenorrhyncha species and 35 varieties have been recorded in Serbia (Janković, 1975; Janković, 1978; Janković & Papović, 1985; Magud & Toševski, 2004; Mihajlović, 2007; Stojanović & Marković, 2014; Kosovac et al., 2020), but due to a gap of some 50 years in systematic faunistic studies, new findings can be expected for both autochthonous and invasive species. The present study is a part of several-year research of Auchenorrhyncha diversity and it aims to provide up-to-date records on alien species in Serbian entomofauna.

## MATERIAL AND METHODS

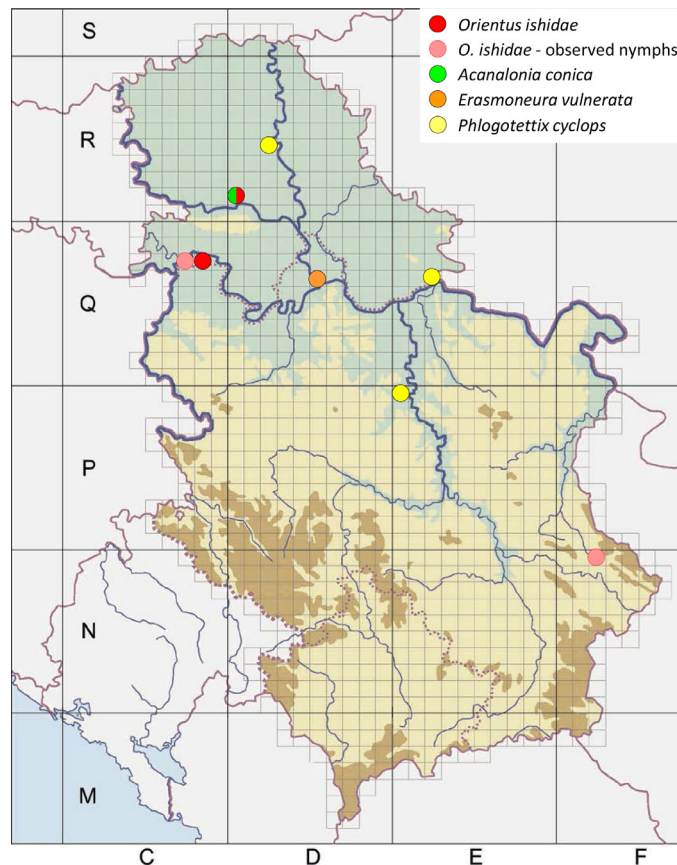
Insects were collected over a seven-month period (from March to September) in 2019. Three types of habitats were surveyed: (1) urban area of Novi Sad in order to detect species associated with ornamental plants and vegetation adapted to urban habitats; (2)

vegetation near the Belgrade Customs Office Terminal, and areas along the A1 motorway in Serbia (part of the European route E-75) as a front row barrier for species that can be spread by means of transport, and (3) undisturbed habitats of the Special Nature Reserve Zasavica (SNR Zasavica), SNR Deliblatska peščara, and Stara planina Nature Park (NP Stara planina), rich in diverse native vegetation. Sampling was performed by sweep nets and mouth aspirators for precise collecting from specific plant species and supplemented with insect light traps to enhance the sampling rate of species that are low in abundance, arboreal or hard to reach. Swept specimens were immediately placed into 2 ml plastic tubes (Sarstedt, Germany) filled with 96% ethanol, while specimens attracted by light traps were transferred the following morning. The insect material was then transported in a portable cooler (10°C) and stored in a laboratory refrigerator at 4°C until further analysis. Collected samples were examined under a stereomicroscope (Leica MZ7.5) and assigned to

specific species based on male genitalia. Photographs of the specimens were made using an iPhone 6S phone camera attached to a stereomicroscope, while photographs of specimens in the field were taken with a Canon SX160 IS Camera. The collected and examined insect material was stored with corresponding aedeagi in glycerol and deposited in Marko Šćiban's private entomological collection.

## RESULTS

The collected specimens were sorted based on the specific plants they were found on and/or collecting sites. They were assigned to specific species based on their outer morphological features and male genitalia. Of more than 200 Auchenorrhyncha species identified during this survey (data not shown), three alien species, previously unknown in the Serbian fauna, were discovered: *O. ishidae*, *A. conica* and *E. vulnerata* (Figures 1 and 2). Light traps set in Novi Sad and SNR



**Figure 1.** Sampling sites of *O. ishidae*, *A. conica*, *E. vulnerata* and *Ph. cyclops*. Colored circles represent specific species as given in legend

Zasavica sites successfully attracted a single *O. ishidae* specimen per site, while probable nymphs of this species were swept from shrubby vegetation in SNR Zasavica and NP Stara Planina. Specimens of *A. conica* were originally spotted at the entrance into an open market place in the Novi Sad city center. After surveying the local area, cca. 30 specimens were observed on ornamental Chinese plum (*Prunus salicina*) and a few more specimens were noticed on a nearby small-leaved lime (*Tilia cordata*). Another alien species, *E. vulnerata*, was detected in vegetation along a motorway in the vicinity of the Belgrade Customs Office Terminal. Specimens were collected by sweeping *Vitis* sp. plants permeating dense vegetation. The presence of *Ph. cyclops* in Serbia was confirmed on three sites. One specimen was previously collected in 2018 in a private rusty vineyard in Bačko Gradište, while a single male specimen was swept during 2019 from *Ulmus* sp. plants along the A1 motorway in Markovac, and several others were attracted by a light trap in SNR Deliblatska pešćara (Figures 1 and 2).

Along with these novel alien species in Serbia, the presence of all four previously recorded allochthonous Auchenorrhyncha species in local entomofauna was confirmed. Sweeping *Acer campestre* in SNR Zasavica [44°57'33.9"N 19°31'30.4"E] and Novi Sad [45°15'7.93"N 19°47'49.31"E] resulted in detecting very abundant populations of *J. hyalinus*. Encounters with *S. titanus* in July and August were frequent in neglected vineyards and on wild *Vitis vinifera* plants in Vojvodina (northern Serbia), while several specimens were attracted by the light trap in SNR Zasavica [44°57'27.4"N 19°31'34.9"E] and SNR Deliblatska pešćara [44°14'10.6"N 21°07'00.3"E]. Polyphagous *S. bisonia* and swarming populations of *M. pruinosa* were observed in Markovac and on several sites in SNR Zasavica.

## Examined material

### *Orientus ishidae* (Matsumura, 1902)

First record in Serbia: (1) Novi Sad (Vojvodina, northern Serbia), [45°15'7.93"N 19°47'49.31"E] (DR01), 1♂ collected on a light trap, 18.07.2019; (2) SNR Zasavica, Visitor center (Vojvodina, northern Serbia), [44°57'27.4"N 19°31'34.9"E] (CQ87), 1♀ collected on a light trap, 06.08.2019.

Distribution: Italy, Switzerland, Austria, Germany, France, Netherlands, Slovenia, Hungary, Czech Republic, Poland, Romania, USA, Japan. The closely related species

*Orientus amurensis* Guglielmino, 2005 was recently described after examination of specimens from Russia and China, theretofore treated as *O. ishidae*, leaving unreviewed records from Korea, Philippines and Taiwan (Guglielmino, 2005).

Remarks: Identity confirmed based on male genitalia according to Guglielmino, (2005). It was not possible to determine the host plant of the collected specimens due to the sampling method. Solitary nymphs of probable *O. ishidae* were observed on *Populus alba* on two sites: (1) SNR Zasavica, Ribnjača pond (Vojvodina, northern Serbia) [44°54'41.15"N 19°24'44.39"E] (CQ77), 06.07.2019, and (2) NP Stara planina, Temska (eastern Serbia) [43°16'2.16"N 22°33'32.14"E] (FN29), 20.07.2019 and 24.07.2019.

### *Acanalonia conica* (Say, 1830)

First record in Serbia: Novi Sad (Vojvodina, northern Serbia), [45°14'51.66"N 19°47'21.47"E] (DR01), 4♂ and 4♀ collected by hand on ornamental *P. salicina*, 20.07.2019.

Distribution: USA, Italy, Switzerland, Slovenia, Romania.

Remarks: *A. conica* is the only member of the fam. Acanaloniidae present in Europe with distinctive bright green coloring and conical vertex; male genitals were examined according Freund & Wilson (1995).

### *Erasmoneura vulnerata* (Fitch, 1851) (Syn: *Erythroneura vulnerata*)

First record in Serbia: Belgrade, along A1 motorway [44°49'38.5"N 20°23'11.8"E] (DQ56), 2♂ and 2♀ swept on *Vitis* sp. plants permeating bushy vegetation, 11.09.2019.

Distribution: USA, Canada, Mexico, Italy, Slovenia.

Remarks: Identity was confirmed based on male genitalia according to Seljak (2011); all specimens had typical red R1 vein characteristic for summer populations, but the collecting date indicated that they could belong to the second generation (Duso et al., 2019).

### *Phlogotettix cyclops* (Mulsant & Rey, 1855)

First and only record in Serbia: Niš (Horvath, 1903).

Collected material: (1) Bačko Gradište (Vojvodina, northern Serbia), rusty vineyard [45°30'55.04"N 20°3'30.75"E] (DR24), 1♀ hand collected on *Vitis vinifera*,





**Figure 2.** Photographs of alien Auchenorrhyncha species either recorded for the first time in Serbia or confirmed as present: (A) *O. ishidiae*, aedeagus-lateral view; (B) *A. conica*, aedeagus-lateral view; (C) *E. vulnerata*, aedeagus-ventral view and (D) *Ph. cyclops*, aedeagus-lateral view

12.08.2018; (2) Markovac, along A1 motorway (central Serbia), [44°14'10.6"N 21°07'00.3"E] (EP09), 1♂ swept on *Ulmus* sp., 27.07.2019; (3) SNR Deliblatska peščara, Kajtasovo, Ludvig polje (Vojvodina, north Serbia) [44°50'27.11"N 21°17'14.33"E] (EQ26), 1♂ 3♀ attracted by a light trap, 29.08.2019.

Distribution: France, Southern France, Hungary, Italy, Germany, Austria, Slovakia, Serbia, Romania, Belgium, Israel, Azerbaijan, Maritime territory, Moldavia, Ukraine, Siberia, China, Taiwan, Japan, Korea (Metcalf, 1967; Nast 1972) and Croatia (Plavec et al., 2019).

Remarks: Identity confirmed based on male genitals, according to Zahniser (2007).

## DISCUSSION

The current faunistic survey has revealed the presence of three alien Auchenorrhyncha species, so far unknown in Serbian entomofauna: *O. ishidiae*, *A. conica* and *E. vulnerata*, and confirmed the presence of *Ph. cyclops* in Serbia. Frequent findings of *J. hyalinus*, *S. titanus*, *S. bisonia* and *M. pruinosa* in SNR Zasavica and numerous other sites (data not shown) have indicated successful naturalization of these invasive species in Serbia. The newly detected species were attracted by light traps, already proven to be helpful in faunistic studies in urban and suburban areas (D'Urso & Uliana, 2006; Koczor et al., 2013). Arrival routes of the recorded allochthonous species are currently unclear, especially

knowing that alien Auchenorrhyncha benefit from agricultural and ornamental plant trade and can migrate significant distances after their initial intrusion (Mifsud et al., 2010). The occurrence site of *A. conica* in Novi Sad was in the vicinity of a city nursery garden, which could have been its entry point. Although the population of *E. vulnerata* was found in vegetation near the customs area in Belgrade, this finding does not necessarily imply that it was introduced by trading and transport.

Unlike the numerous and seemingly well-adjusted population of *A. conica* observed in Novi Sad, it is difficult to evaluate the accommodation level of *O. ishidiae* and *E. vulnerata* due to their fairly low number of detected individuals. All three species, along with *Ph. cyclops*, are more or less *Vitis*-related, whether as disease vectors or physical damage inducers (Zimmerman et al., 1996; Lessio et al., 2016; Duso et al., 2019; Plavec et al., 2019). Even though *O. ishidiae* was not detected in vineyards, and only one *Ph. cyclops* specimen was collected on grapevine, agro-economic losses that accompanied the emergence of *S. titanus* and FD in Europe should be considered when assessing the findings of these two leafhoppers in Serbia. Assumptions regarding plant associations of the polyphagous *O. ishidiae* in Serbia would be very speculative. The findings of solitary nymphs on *P. alba* that almost certainly belong to *O. ishidiae*, suggest that this plant could be preferred in Serbia as it is in Italy (Parise, 2017). A single specimen of *Ph. cyclops* was swept from *Ulmus* sp. in central Serbia, which would be

consistent with the reported plant preferences in China (Zheng et al., 2005). More individuals were attracted by light traps in SNR Deliblatska peščara, where local vegetation is mainly comprised of deciduous plants (*Populus alba*, *P. nigra*, *Robinia pseudoacacia*, *Fraxinus excelsior*, *Ulmus sp.*), afforested conifers (*Pinus sylvestris*, *P. nigra*) and densely overgrown wild creeping plants (*Vitis sp.*, *Clematis sp.*). Data on vector potential of *Ph. cyclops* originate from Taiwan, where this leafhopper was reported as a vector of Aster yellows phytoplasma (16SrI group) (Chen et al., 2011). This data should be taken with caution, given that the photograph of the specimen noted as "*Ph. cyclops*" in that paper is misleading (de Haas, M., personal communication). Therefore, examination of male genitalia should be essential for the genus *Phlogotettix*, as well as for other Auchenorrhyncha species in order to avoid vague data (Meshram et al., 2015).

The North American species *A. conica* and *E. vulnerata* are not known as plant disease vectors, but being associated with grapevine, they affect the plant's fitness. Interestingly, interactions between *E. vulnerata* and European grapevine cultivars were first investigated in the USA (Zimmerman et al., 1996). The first occurrence of this species in Europe was followed by leaf curling, discoloration and scorching of *V. vinifera* and *V. lambrusca*, which hosted insect populations (Duso et al., 2005). Grapevine plants from which *E. vulnerata* was collected in Belgrade did not show any of the aforementioned symptoms, which can be due to low abundance of the observed population or the phenological period of specimens transitioning toward their overwintering sites. The biology of polyphagous *A. conica* is very similar to the life traits of *M. pruinosa*, known to cause severe damage of wild and cultivated plants by producing large amounts of wax and honeydew, and enhancing the development of mildew and leading to fruit decay (D'Urso & Uliana, 2006). Although *A. conica* has not yet been recorded on grapevine in Serbia, progressing out of the urban area of Novi Sad may lead this species towards the vine-growing region of Fruška Gora in the vicinity.

First findings of *O. ishidiae*, *A. conica* and *E. vulnerata*, and confirmed presence of *Ph. cyclops*, come from urban and natural ecosystems, indicating their phenological independence from cultivated plants and crops. Nevertheless, all four species should be accounted for in the context of grapevine protection since their intrusion into vineyards can be erroneous

event or can lead to the establishment of populations inside crops which may cause plant physical damage and/or induce phytoplasma-borne diseases. We strongly encourage future research of this insect group to focus on interactions that Auchenorrhyncha species establish within natural ecosystems because invasive potential can be predicted only as a result of comprehensive knowledge of the species life traits.

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## Novi i ažurirani podaci o alohtonim vrstama Auchenorrhyncha u Srbiji

### REZIME

Tokom istraživanja faune Auchenorrhyncha u Srbiji sprovedenog 2019. godine, tri alohtone vrste su prvi put zabeležene na ovoj teritoriji: azijska vrsta *Orientus ishidae* (Cicadellidae, Deltocephalinae) i dve severnoameričke vrste, *Acanalonia conica* (Acanaloniidae) i *Erasmoneura vulnerata* (Cicadellidae, Typhlocybinae). Pored navedenih prvih nalaza, prisustvo vrste *Phlogotettix cyclops* (Cicadellidae, Deltocephalinae) u Srbiji je potvrđeno nakon više od 100 godina starog prvog nalaza. Invazivne vrste cikada prethodno prijavljene kao prisutne u lokalnoj fauni su takođe detektovane: *Japananus hyalinus* (Cicadellidae, Deltocephalinae), *Scaphoideus titanus* (Cicadellidae, Typhlocybinae), *Stictocephala bisonia* (Membracidae) i *Metcalfa pruinosa* (Flatidae). Alohtone vrste Auchenorrhyncha zabeležene prvi put u Srbiji: *O. ishidae*, *A. conica* i *E. vulnerata*, kao i *Ph. cyclops*, tretiraju se kao štetočine ili potencijalne štetočine vinove loze u Evropi, koje nanose fizičku štetu ili prenose fitoplazmatične bolesti. Prikazani nalazi ukazuju na potencijalni fitosanitarni rizik u vinogradima Srbije koji se može pojaviti u narednim godinama i podržavaju promenu pristupa u istraživanjima diverziteta grupe Auchenorrhyncha preusmeravanjem fokusa na prirodna staništa u odnosu na agroekosisteme i njihovo neposredno okruženje.

**Ključne reči:** Auchenorrhyncha, diverzitet, alohtone vrste, Srbija