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First detection of *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Aphidiinae) in Serbia: an introduced species invading Europe?

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Abstract. Fauna of aphidiine parasitic wasps has been intensively studied in the past 20 years in Serbia. *Lysiphlebus testaceipes*, a species introduced originally to France, has become broadly established in the Mediterranean area. Also, it has recently been categorized as an invasive species. It was found in the Niška kotlina valley and in a small gorge, the Sićevo gorge, both in southeast Serbia and under the influence of the Mediterranean climate. Twenty one trophic associations of *L. testaceipes* on 17 plant species and 16 aphid hosts have been identified.

Key words: *Lysiphlebus testaceipes*, introduction, invasion, parasitoids, biological control.

Introduction

There are many examples of insect introduction as biological control agents around the world, which, after being introduced, become invasive species and thus cause major ecological and economic damage to ecosystems, irrespective of whether they are predators or parasitoids. One of the best-known examples of practical usage is *Lysiphlebus testaceipes* (Cresson 1880), native in South America (Chile, Costa Rica, Cuba, Ecuador, Guatemala and Venezuela) (Starý et al. 2014). This species was introduced from Cuba to Europe in 1973 in the Mediterranean part of France as a biological control agent against two citrus aphids *Toxoptera aurantii* (Boyer de Fonscolombe 1841) and *Aphis spiraecola* Patch 1914 (Starý et al. 1988a). Gradually, *L. testaceipes* populated a narrow coastal part of the Mediterranean region, more or less competing with the local parasitoid species. Within a relatively short period, *L. testaceipes* had established itself over the whole Mediterranean area (Canary Islands, Croatia, France, Greece, Italy, Montenegro, Portugal, Sicily, Spain) (Starý et al. 1988b, Cecilio 1994, Suay & Michelena 1997, Kavallieratos et al. 2004, Laamari & Coeur d' Acier 2010, Havelka et al. 2011, Žikić et al. 2012, Satar et al. 2012, Yu et al. 2013), in Iran (Rakhshani et al. 2005), in Tunisia (Boukhris-Bouhachem 2011), Turkey, North Africa: Algeria and Libya, and West Africa: Benin (Tepa-Yotto et al. 2013, Hofsvang et al. 2014).

During the last decade, this species has been recorded in the central part of the Iberian Peninsula (Hiernaux et al. 2011) invading apparently even the foothills of the Pyrenees (Pons et al. 2004, Starý et al. 2004). Hughes et al. (2011) suggests that *L. testaceipes* may have expanded its area of invasion, making a breakthrough in the cooler territories of the continental part of Europe. This is one of the reasons why *L. testaceipes* was removed from the positive list of biological control agents of the European Plant Protection Organization (EPPO) in 2008. Further, America has been overrun by this species, including its Northern, Central and Southern regions (Havelka et al. 2011).

Until today the host-range of *L. testaceipes* exceeds 100 aphid species in many plant/aphid/parasitoid associations (Pike et al. 2000). Thirty years after its introduction in Europe, it has been noted that *L. testaceipes* parasitizes over 20 species of aphids, some of which are newly adopted hosts (Kavallieratos et al. 2005, 2010, Tomanović et al. 2009, Mitrović et al. 2013). Apart from laboratory experiments, there have been some attempts to explain its adaptability in the sense of being accepted by the very new aphid hosts (e.g. Mitrović et al. 2013).

Material and methods

The Niš basin (Nišavska kotlina) is located on the border of the oldest Rhodope massif in the Balkans from the Pa-

leozoic, and younger pleated mountains from the Jurassic period (Janković 1909). The valley begins with the Sićevo gorge in the East expanding through the city of Niš to the confluence of the Nišava River and the South Morava River to the West (43°15'-43°30' north latitude and 21°49'-22°13' east of Greenwich). Generally, in this area summers are hot and winters are snowy. Precipitation is typical in spring and autumn, influenced by the continental climate of the North (Rakićević 1980, Ducić & Radovanović 2005). On the other hand the Sićevo gorge is influenced, through the valleys of the rivers Vardar and Pčinja to the Niš basin, by the Mediterranean climate (the Aegean Sea) of the South (Žikić et al. 2000, 2010, 2013, Zlatković et al. 2011). The average temperature for each season measured for the investigated area in a period of 60 years ranged from 1.53°C in winter to 21.37 °C in summer (Ivanović et al. 2011).

Apart from the aforementioned area, *Lysiphlebus testaceipes* has been recorded on another locality 50 km to the South of the Niš basin near the small town of Lebane (42°56' North latitude and 21°45' East of Greenwich) about 270 m a.s.l. This locality is situated near the river Jablanica in the vicinity of Lebane, which also flows through a valley.

Samples were collected in urban and natural habitats in the investigated area (Fig. 1), from autochthonous and allochthonous plants infested with different aphid species. Cut plant material with aphid colonies was put separately into plastic boxes covered with thin textile to allow ventilation of the system and prevent aphids and parasitoids from escaping. Prior to the emerging time of adult parasitoids, we placed all samples into a growing cabinet under the daylight regime of light:dark=16:8h, relative humidity 65%, temperature 22.5° C. Identification was done using ZEISS Discovery V8 stereomicroscope.

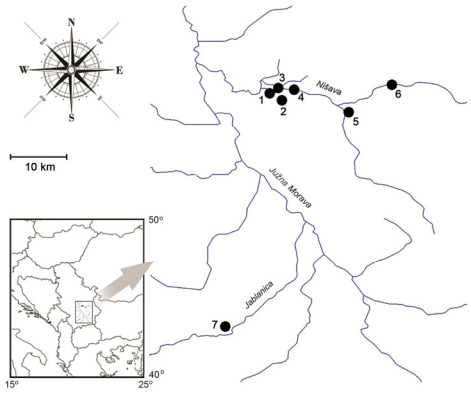

Figure 1. Sampling localities where L. *testaceipes* was recorded.

Researched localities

1. Niš, Tvrdjava: 43°19'35.9"N 21°53'41.8"E
2. Niš, Trošarina: 43°18'40.5"N 21°55'31.5"E
3. Niš, Pantelej: 43°20'21.9"N 21°54'26.2"E
4. Niš, Donji Matejevac: 43°21'38.0"N 21°57'12.2"E
5. Niš, Niška banja: 43°17'36.2"N 22°00'30.1"E
6. Sićevo gorge, Sićevo: 43°19'52.1"N 22°04'02.8"E
7. Lebane, Konjino: 42°56'08.4"N 21°45'49.9"E

Results

During the investigation period from May to November 2013, we collected 21 samples of *Lysiphlebus testaceipes*, with exactly 400 specimens of both sexes in 19 aphid/plant associations. Records of *L. testaceipes* are given by chronological order according to aphid hosts names and presented in Fig. 1. Legator abbreviations are as follows: MD = M. Djordjević, SS = S. Stanković, VŽ = V. Žikić.

The list of host species

Aphis balloticola Szelegiewicz 1968, Niš, Trošarina, 16.05.2013, *Ballota nigra*, 39♀ 19♂ (VŽ).

Aphis craccivora Koch 1854, Niš, Trošarina, 19.05.2013, *Robinia pseudoacacia*, 2♀ (MD); Niš, Niška banja, 06.06.2013, *Sophora japonica*, 1♀ 2♂ (VŽ).

Aphis fabae Scopoli 1763, Niš, Trošarina, 22.05.2013, *Bifora radians*, 6♀ 6♂ (MD); Lebane, Konjino, 01.06.2013, *Matricaria chamomilla*, 3♀ 10♂ (SS); Niš, Tvrdjava, 05.06.2013, *Papaver rhoeas*, 2♀ 3♂ (MD); Niš, Niška banja, 06.06.2013, *Tamarix* sp., 19♀ 10♂, (VŽ).

Aphis gossypii Glover 1877, Niš, Trošarina, 05.06.2013, *Hibiscus syriacus*, 5♀ 2♂ (SS).

Aphis salviae Walker 1852, Niš, Donji Matejevac, 05.06.2013, *Salvia aethiops*, 26♀ (VŽ).

Aphis sambuci Linnaeus 1758, Sićevo gorge, Sićevo, 12.05.2013, *Sambucus nigra*, 1♂ (VŽ).

Aphis sp. 1, Niš, Trošarina, 22.05.2013, *Fumaria officinalis*, 3♀ 1♂ (MD).

Aphis sp. 2, Niš, Donji Matejevac, 05.06.2013, *Sanguisorba minor*, 7♀ (VŽ).

Aphis spiraecola Linnaeus 1758, Niš, Trošarina, 21.05.2013, *Tecoma radicans*, 2♀ 1♂ (SS); Niš, Trošarina, 05.06.2013, *Tecoma radicans*, 8♀ 6♂ (SS).

Aphis umbrella (Börner 1950), Niš, Trošarina, 28.09.2013, *Malva sylvestris*, 4♀ (VŽ); Niš, Trošarina, 25.10.2013, *Malva sylvestris*, 3♀ (VŽ).

Brachycaudus cardui (Linnaeus 1758), 25.05.2013, Niš, Trošarina, *Hibiscus syriacus*, 2♀ 3♂ (MD).

Brachyunguis tamaricis (Lichtenstein 1885), Niš, Niška banja, 06.06.2013, *Tamarix* sp., 120♀ 51♂ (VŽ).

Capitophorus hippophaes Walker 1852, Niš, Pantelej, 13.10.3013, *Polygonum lapathifolium*, 2♀ 2♂ (VŽ).

Cavariella aegopodii (Scopoli 1763), Sićevo

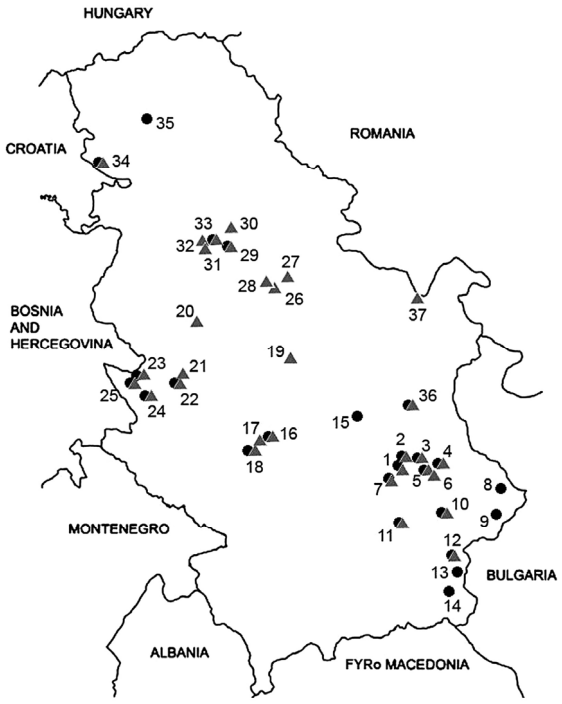

Figure 2. Survey of the investigated localities concerning Aphidiinae on the teritorry of Serbia for three continous years: [grey triangles] mark the records of 2011/2012, [black dots] mark the records of 2013.

george, Sićevo, 06.06.2013, *Foeniculum vulgare*, 20♀ 8♂ (VŽ).

Schizaphis scirpi (Passerini 1874), Niš, Niška banja, 23.07.2013, *Typha latifolia*, 1♂ (VŽ).

Apart from the localities where *L. testaceipes* was found, we provide the complete investigation conducted throughout Serbia for the past three years in order to point out the absence of this species. Beside the results concerning findings of *L. testaceipes* during 2013 given in Fig. 1, the results of the last three years of research of the territory of Serbia are shown in Fig. 2.

The list of the investigated localities during the period of 2011-2013 with altitudes in meters above sea level

[1] Niš with sourondings, 180-230 m; [2] Niš, Matejevac, 360-400 m; [3], Niška banja, 250 m; [4] Sićevo gorge, 250-1330 m; [5] Jelašnica gorge, 250-880 m; [6] Suva planina, Bojanine vode Mt, 880-900 m; [7] Niš, Lalinac, Salt Marsh, 180 m; [8] Pirot, Vlasi, 470 m; [9] Jerma canyon, 530-600 m; [10] Vlasinsko jezero Lake, 1200-1400 m; [11] Lebane, Konjino, 272 m; [12] Bosilegrad, Jarešnik, 1280-1400 m; [13] Bosilegrad, 750 m; [14] Dukat Mt, 1200-1400 m; [15] Kruševac, Bagdala 200 m; [16] Brzeće, 1000 m; [17] Metođe, 1300-1500 m; [18] Kopaonik Mt, 1600-1700 m; [19] Kragujevac, 180-200 m; [20] Valjevo, 190 m; [21] Užice, Ponikovica, 550; [22] Užice, 400 m; [23] Bajina Bašta, 245 m; [24] Tara Mt, Derventa, 300-450 m; [25] Zlatibor Mt, Čavlovac, 940-960 m; [26] Malo Orašje, 215 m; [27] Vodanj, 150 m; [28] Umčari, 140 m; [29] Avala, 450-500 m; [30] Kovilovo, 70 m; [31] Obedska bara 70 m; [32] Belgrade, Zemun 90; [33] Belgrade 120 m; [34] Plavna 80 m; [35] Kula, 100 m; [36] Bovansko jezero Lake, 250-300 m; [37] Donji Milanovac, 70-120 m.

Discussion

The assertion that the breakthrough of this introduced parasitoid in Serbia has happened since 2013 (the first detection date) is supported by the results of continuous investigation of aphidiines for more than 20 years (e.g. Kavalieratos et al. 2004, Žikić et al. 2012). Like in the previous years of exploring the parasitoid fauna (in Fig 2. we presented the last three years), we also collected samples from all over Serbia in 2013 (black dots: e.g., Western Serbia: Tara mt., Zlatibor mt., Užice; Central Serbia: Kopaonik mt., Južna Morava valley, Belgrade; Eastern Serbia: Dukat mt, Besna kobila

mt., Vlasina lake, Northern Serbia: Kovilovo, Obrenovac, Fruška gora mt.). Therefore, it can be stated with a great degree of confidence that *Lysiphlebus testaceipes* has invaded Serbia.

Although the average temperature in this part of Europe is about 12° C, with harsh winters, the winter of 2012/2013 was rather mild and relatively short. Finally, the next cold period with subzero temperatures started very late that year, exactly on 23.11.2013, extending the activity period of plants and aphids, thus allowing the parasitoids to reproduce. This could be one of the reasons why this foreign species has established itself on the territory which bears the typical characteristics of continental Europe. We found that in the Southern and Southeast Serbia *L. testaceipes* showed its competitive potential to an autochthonous parasitoid such as *Lysiphlebus fabarum*. This opportunistic pattern of acquiring new hosts of *L. testaceipes* is already known (e.g. Starý et al. 2004, Mitrović et al. 2013). In some of our samples we found both parasitoid species in the mixed populations. Beside *L. fabarum*, that is a common opponent of *L. testaceipes* that exploit the same aphid species, *L. testaceipes* encounters other native parasitoids as its competitors, and it is slowly establishing its place in the ecosystems. Our findings suggest that at least three more native parasitoid wasps are more or less successful in competing for the same aphid hosts; *Binodoxys acalephae* (Marshall 1896), *B. angelicae* (Haliday 1833) and *Lipolexis gracilis* Förster, 1862 (e.g. Kavallieratos et al. 2004, Žikić et al. 2012) making lots of associations which alternate during the season. At this time, with only 21 findings of *L. testaceipes*, we cannot be conclusive about its interaction with its hosts as well as with the native aphidiine species. Another question is how this species came to this part of the Balkan Peninsula, i.e. which corridor it could have used.

The most plausible way to explain how this parasitoid penetrated the territory of Serbia is from Greece through the river valley of Vardar, then through FYR of Macedonia continuing along the valley of the river Pčinja (tributary of the river Vardar). Then it followed the Južna Morava river to the Nišava river (with its source in Bulgaria, flowing through the Sićevo gorge, the city of Niš, flowing into the Južna Morava river). As a result, the parasitoid is now attempting to establish itself in a territory including the Valley of the Jablanica River, then through the city of Niš with its surroundings following the Valley of Nišava River via Niška banja to Sićevo gorge. In spite of the fact that Montenegro, where *L. testaceipes* was already recorded (e.g. Žikić et al. 2012), is geographically closer to Serbia than Greece, we think that this may not be the entry route due to the high mountain range which serves as a natural barrier.

The presence of *L. testaceipes* could be responsible of a partial change of the structure of the native parasitoid fauna in the form of a decrease in their relative abundance. On the other hand, *L. testaceipes* often parasitizes populations of common and often even exotic pest species such as *Toxoptera aurantii* and *Aphis spiraecola*. Moreover, the presence of *L. testaceipes* prevents newly invasive pests in the Mediterranean such as *Aphis illinoisensis* Shimer 1866 (Havelka et al. 2011), *Toxoptera citricida* (Kirkaldy 1906) and some others. Obviously, the role of *L. testaceipes* in the Mediterranean ecosystems needs to be re-classified both with respect to ecosystem changes as well as biological control of pests. A number of exotic aphids have invaded the Mediterranean for many years, mostly without parasitoids which have only recently adapted to these new immigrants. Thus, why not support the "local" parasitoids through new exotic elements which have substantially supplemented the "local" parasitoid guilds.

Our next goal is to compare the previous studies on *L. testaceipes* in the introduced areas across the Mediterranean with the first contact with the aphids, as well as the parasitoids in Serbia. We intend to observe whether this species will show its 'aggressive conquest' in the brand new habitat or its behavior will show a balanced exploitation of common hosts, thus becoming a recommended biological control agent in the temperate continental climate.

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