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Revision of the European *Lysiphlebus* species (Hymenoptera: Braconidae: Aphidiinae) on the basis of COI and 28SD2 molecular markers and morphology

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Abstract. Members of the genus *Lysiphlebus* Foerster are medium-sized endoparasitoid aphidiine wasps with over 30 species distributed worldwide. They attack numerous aphid hosts, and some species are important biological control agents. All European *Lysiphlebus* species are revised based on sequence information from the mitochondrial COI barcoding gene and the nuclear 28SD2 gene, morphological traits, and on information about their host specificity. The taxonomic status of 13 European *Lysiphlebus* species is confirmed, while 11 are redescribed and illustrated. The following four *Lysiphlebus* species are synonymized: *L. melandriicola* Starý, 1961 [= *L. fabarum* (Marshall, 1896)], *L. hirtus* Starý, 1985 (= *L. confusus* Tremblay & Eady, 1978), *L. hispanus* Starý, 1973 (= *L. desertorum* Starý, 1965), and *L. safavii* Starý, 1985 (= *L. hirticornis* Mackauer, 1960). Two new *Lysiphlebus* species are described: *L. volkli* **sp.n.** and *L. brachycaudi* **sp.n.**. Our results, based on both COI and 28SD2, confirm the existence of three monophyletic species groups within the genus: (1) the 'fabarum' group, which includes *L. fabarum*, *L. cardui* (Marshall, 1896), *L. confusus*, *L. hirticornis*, *L. brachycaudi* **sp.n.**, and *L. volkli* **sp.n.**; (2) the '*testaceipes*' group, which includes *L. testaceipes* (Cresson, 1880), *L. orientalis* Starý & Rakhshani, 2010, *L. fritzmuelleri* Mackauer, 1960, *L. balcanicus* Starý, 1971. Geometric morphometrics of fore wing shape proved to be a powerful tool to delineate sibling species within the 'fabarum' group. We were able to confirm for the first time sexual lines of *L. cardui* and *L. volkli* **sp.n.** in Iran. An illustrated key for identification of the European Lysiphlebus species is provided.

Key words. Lysiphlebus, Europe, phylogeny, barcoding, revision, L. volkli sp.n., L. brachycaudi sp.n.

1. Introduction

With more than 30 species around the world, *Lysiphle*bus Foerster, 1862, represents a moderately species-rich genus of solitary endoparasitoid aphidiine wasps (STARÝ 1961, 1975). The diversity of this genus has been best explored in Europe, where 14 species have been described (KAVALLIERATOS et al. 2004; STARÝ 2006; VAN ACHTERBERG



2013); in Asia, where eight species have been described (STARÝ 1965, 1979; TAKADA 1968); and in America (North and South), where five species have been described (PIKE et al. 2000). In contrast, the African fauna has been poorly explored, and only two imported species are known in Australia (Carver & Franzmann 2001). Lysiphlebus spp. attack mostly small aphid hosts from various genera (e.g., Aphis L., 1758, Brachycaudus van der Goot, 1913). Interestingly, most of the European species are strictly monophagous or exhibit narrow oligophagous host specificity (i.e., L. fritzmuelleri Mackauer, 1960, L. hirticornis Mackauer, 1960, L. balcanicus Starý, 1998, L. hispanus Starý, 1973, L. safavii Starý, 1985, L. hirtus Starý, 1985, L. melandriicola Starý, 1961, L. alpinus Starý, 1971), while the host specificity of members of the L. fabarum group ranges from oligophagous to broadly oligophagous [i.e., L. fabarum (Marshall, 1896), L. confusus Tremblay & Eady, 1978, L. cardui (Marshall, 1896)]. For example, L. fabarum is recorded from over 150 aphid species (KAVALLIERATOS et al. 2004; STARÝ 2006; RAKHSHANI et al. 2013). As in the case of native European species, in North and South America some species such as L. flavidus (Gahan, 1911) and L. utahensis (Smith, 1944) have a very narrow host range (PIKE et al. 2000), while others like L. testaceipes (Cresson, 1880) are broadly oligophagous. Meanwhile, L. testaceipes has become a cosmopolitan species and parasitizes approximately 100 aphid species (PIKE et al. 2000).

Lysiphlebus fabarum and L. testaceipes parasitize many pest aphids in vegetable crops, orchards, leguminous plants, cereal crops and ornamental plants (KAV-ALLIERATOS et al. 2004, 2010, 2013; BENELLI et al. 2016; KAVALLIERATOS et al. 2016; Yu et al. 2016). Also, it is well known that L. testaceipes was introduced from Cuba to the Mediterranean part of France for the control of citrus aphids (Starý 1988a). In addition, L. testaceipes parasitizes some new exotic immigrants in Europe, viz., the grapevine aphid, Aphis illinoisensis Shimer, 1866 (HAVELKA et al. 2011) and Siphonatrophia cupressi (Swain, 1918) on plants of the family Cupressaceae (RA-BASSE et al. 2005). The recently described L. orientalis from China represents a good candidate for mass releases on soybean crops in the USA against the soybean aphid, Aphis glycines Matsumura, 1917 (RAGSDALE et al. 2011). Lysiphlebus orientalis has been recently recorded in Europe (Serbia), where it is involved in several new aphidplant associations and has adopted new aphid hosts in comparison with its native area in China (PETROVIĆ et al. 2013). It is known that several species of the L. fabarum group (L. fabarum, L. cardui, and L. confusus) have polymorphic populations which contain sexual and asexual lines (BELSHAW et al. 1999; SANDROCK & VORBURGER 2011; SANDROCK et al. 2011). More recently we found asexual reproduction within the L. testaceipes species group as well (L. orientalis and L. balcanicus) (PETROVIĆ et al. 2015). SANDROCK & VORBURGER (2011) found that asexual reproduction in Lysiphlebus fabarum has a simple genetic basis in that homozygosity for a single recessive allele determines thelytoky.

There are serious doubts about the taxonomic status of some Lysiphlebus species inhabiting Europe. This primarily applies to the L. fabarum species group (i.e., L. fabarum, L. confusus, L. cardui) and other European Lysiphlebus spp. (i.e., L. hirtus, L. safavii, L. melandrii*cola*) because they show little genetic differentiation when analysed using several molecular markers (BELSHAW et al. 1999; SANDROCK et al. 2011) and also exhibit weak and inconsistent morphological differentiation (STARY 1961, 1985). For some species (e.g., L. safavii and L. hirtus), only the type material is available, without any additional records for many years after the original description (VAN ACHTERBERG 2013). The restricted number of diagnostic morphological characters in Lysiphlebus taxonomy combined with existing sympatric asexual and sexual lines in several species (SANDROCK et al. 2011; PETROVIĆ et al. 2015) make Lysiphlebus a taxonomically and biologically very complex group. There is a long history of different opinions concerning the taxonomic position of the genus Adialytus Foerster and its relationships with Lysiphlebus. Some authors treated Adialytus as a subgenus of Lysiphlebus (Starý 1975; TREMBLAY & EADY 1978) or a separate genus, due to the more reduced fore wing venation and host range pattern mainly restricted to Chaitophorinae and Thelaxinae aphid hosts (MACKAUER 1968; MARSH 1971; MESCHELOFF & ROSEN 1990; STARÝ 2005; RAKHSHANI et al. 2012). Lysiphlebus species are common model organisms in evolutionary and ecological research (VÖLKL 1994; NYABUGA et al. 2010; ROUCHET & VORBUR-GER 2014), with apparent biocontrol importance and good prospects for mass production and use in biological control programs (STARÝ et al. 1988a,b; HAGVAR & HOFSVANG 1991; BENELLI et al. 2016), but they have surprisingly rarely been taxonomically investigated. We agree with the assertion of the "unsatisfactory nature of the current classification" by BELSHAW et al. (1999), since there are only a few old revisionary and taxonomic studies devoted to Lysiphlebus (MACKAUER 1960; STARÝ 1961, 1975). Over the last decades, only a few studies have contained descriptions of new species (Starý 1971, 1985; Starý & REMAUDIÈRE 1973; STARÝ et al. 1998; STARÝ et al. 2010) or dealt with species groups within the genus Lysiphlebus (RAKHSHANI et al. 2013; STARÝ et al. 2014; PETROVIĆ et al. 2015; PARREÑO et al. 2017).

The objectives of the present study were as follows: (i) to resolve the taxonomic status of several species that belong to the *L. fabarum* group; (ii) to reveal cryptic species within the polyphagous *L. fabarum*; (iii) to evaluate the *Lysiphlebus* subgeneric classification on the basis of molecular markers and morphology, thereby resolving phylogenetic relationships within the genus; and (iv) to provide the necessary redescriptions of existing European species with a reliable key for the identification of species.

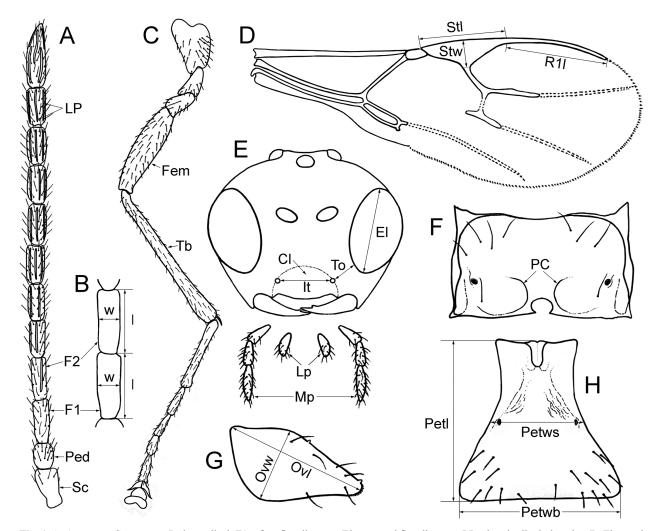


Fig. 1. A: Antennae: Sc – scape, Ped – pedicel, F1 – first flagellomere, F2 – second flagellomere, LP – longitudinal placodes. B: First and second flagellomere: 1 - length, w – width. C: Hind leg: Fem – hind femur, Tb – hind tibia. D: Fore wing: Stl – stigma length, Stw – stigma width, R11 – length of anterior branch of radial vein (= metacarpal vein). E: Head: Cl – clypeus, It – intertentorial line, To – tentoriocular line (proportion between To and It, considered as tentorial index), El – eye length, Mp – maxillary palpomeres, Lp – labial palpomeres. F: Propodeum: PC – propodeal carinae. G: Ovipositor sheath: Ovl – length of ovipositor sheath, Ovw – width of ovipositor sheath. H: Petiole (= tergite 1): Petl – length of T1, Petws – width of T1 at the spiracles, Petwb – width of T1 at the base. Measurements are shown on the drawings.

2. Material and methods

2.1. Insect material

Lysiphlebus specimens were collected across Europe by the authors, while some of them were obtained on loan from other scientists. In this study, we also analysed material collected outside of Europe belonging to species with broad geographical distribution in order to compare it with that of the European populations. Samples with plants bearing aphid colonies consisting of both live and mummified aphids were collected in Europe at many localities ranging from lowland to high mountain areas in 20 European countries (Austria, Belgium, Bulgaria, Croatia, Czech Republic, Finland, France, Germany, Greece, Italy, Lithuania, Malta, Montenegro, Serbia, Slovenia, Spain, Russia, Sweden, Switzerland and Turkey) China, Costa Rica, Iran, Libya, USA, and Uzbekistan). Plant material was carefully herbarized for later identification. Live aphids (5-10 adults, sometimes wingless)oviparous specimens and some alataes from each sample/colony) were put in 90% ethanol and 75% lactic acid in a ratio of 2:1 (EASTOP & VAN EMDEN 1972) and later identified. Remaining parts of the aphid colony (which contained mummified and non-mummified aphids) with plant parts were placed in plastic boxes covered with muslin until the emergence of parasitoids. When more than one aphid species occurred on certain plant species, we isolated some mummies from samples to be able to assign parasitoids to the appropriate hosts. These prepared samples were kept inside a growth cabinet (22.5°C, 65% relative humidity, 16:8 L:D photoperiod). Emerged parasitoids were identified and kept in 80% alcohol. In order to take measurements for the purpose of redescrip-

and nine non-European countries (Algeria, Benin, Chile,

tions and making an identification key, slides were made in Berlese fluid with dissected body parts of parasitoid specimens. For illustrations, we used scanning electron micrographs obtained with a Jeol JSM-6390 scanning electron microscope, line drawings, and slide photographs. External morphology of the specimens was studied using a ZEISS Discovery V8 stereomicroscope (Carl Zeiss MicroImaging GmbH, Göttingen, Germany) and a LEICA DM LS phase-contrast microscope (Leica Microsystems GmBH, Wetzlar, Germany). The examined specimens mostly represent material not described previously. However, additional data already published by the authors are reported as well. Available type specimens are also here examined.

Fig. 1 presents the morphological characters used in this study, including measurements. The terminology for morphological characters of parasitoids follows SHARKEY & WHARTON (1997). Specimens were deposited in the collection of the Institute of Zoology, Faculty of Biology, University of Belgrade, Serbia; Institute of Entomology, České Budějovice, Czech Republic; University of Zabol, Iran.

2.2. DNA extraction, PCR amplification and sequencing

The taxonomic status and phylogenetic relationships of parasitoid species of the genus *Lysiphlebus* were investigated using sequence data from the barcoding region of the mitochondrial cytochrome oxidase subunit I (COI) and the nuclear second expansion segment of 28S rRNA (28SD2). In total, 13 parasitoid species were subjected to molecular analyses, viz., *Lysiphlebus fabarum, L. cardui, L. confusus, L. hirticornis, L. 'melandriicola', L. brachy-caudi* sp.n., *L. testaceipes, L. orientalis, L. fritzmuelleri, L. balcanicus, L. desertorum, L. alpinus,* and *L. volkli* sp.n. (Electronic Supplement Table S1).

Genomic DNA was extracted from individual parasitoids using the QIAGEN DNeasy[®] Blood & Tissue Kit (Qiagen Inc., Valencia, CA) following the manufacturer's instructions. The mitochondrial COI fragments were amplified using the LCO1490 and HCO2198 primers (FOLMER et al. 1994). Each PCR reaction was carried out in a volume of 20 μ l, containing 1 μ l of extracted DNA, 11.8 μ l of H₂0, 2 μ l of high-yield reaction buffer A (with 1 × Mg), 1.8 μ l of MgCl₂ (2.25 mM), 1.2 μ l of dNTP (0.6 mM), 1 μ l of each primer (0.5 μ M), and 0.2 μ l of KAPA-*Taq* DNA polymerase (0.1 U/ μ l) (Kapa Biosystems Inc., USA). The PCR protocol consisted of initial denaturation at 95°C for 5 min, 35 cycles of 1 min at 95°C, 1 min at 54°C, and 2 min at 72°C, and a final extension at 72°C for 10 min.

The nuclear 28SD2 fragments were amplified using the forward primer 28SD2f (5'-AGAGAGAGATTCAA GAGTACGTG-3') (BELSHAW & QUICKE 1997) and the reverse primer 28SD2r (5'-TTGGTCCGTGTTTCAA GACGGG-3') (CAMPBELL et al. 1993). Amplification was carried out in a volume of 20 µl, with 1 µl of extracted DNA, 14.35 μ l of H₂0, 2 μ l of high-yield reaction buffer A (with 1 × Mg), 1.5 μ l of MgCl₂ (2.25 mM), 0.5 μ l of dNTP (0.25 mM), 1 μ l of each primer (0.5 μ M), and 0.15 μ l of KAPA*Taq* DNA polymerase (0.0375 U/ μ l) (Kapa Biosystems Inc., USA). The amplification protocol included initial denaturation at 95°C for 3 min, 30 cycles consisting of 30 s at 95°C, 30 s at 48°C, and 2 min at 72°C, and a final extension at 72°C for 10 min. The obtained products were run on 1% agarose gel, stained with ethidium bromide, and visualized under a UV transilluminator.

Mitochondrial and nuclear fragments were sequenced using automated equipment (Macrogen Inc. Seoul, Korea). As outgroups to root the phylogenetic tree, we used the reference sequences of *Praon abjectum* COI mitochondrial fragment (Acc. No. KC128669) and the 28SD2 nuclear region (Acc. No. KC128680) (www.ncbi.nlm. nih.gov).

Sequences were manually edited in FinchTV v.1.4.0 (Geospiza, Inc., Seattle, USA; www.geospiza.com) and aligned using the ClustalW program integrated in MEGA5 (TAMURA et al. 2011). Mitochondrial COI fragments were submitted to best fit model analysis using the MEGA5 program, which identified the Tamura 3-parameter model (TAMURA 1992) as the best model for estimation of evolutionary divergence. The Neighbor-Joining method (NJ) was used to construct a phylogenetic tree using the MEGA5 software, with 500 bootstrap replicates (Felsen-STEIN 1985; SAITOU & NEI 1987). The DnaSP5.10 program was used to identify haplotype diversity (LIBRADO & Rozas 2009). The NETWORK ver. 4.6.1.2 program (www.fluxus-engineering.com) was used to construct a median-joining haplotype network (BANDELT et al. 1999) with maximum parsimony calculation.

In order to evaluate the suitability of the barcoding region of COI for identification of species from the genus *Lysiphlebus*, the Maximum within species distance (Max-WSD) was plotted versus the Minimum between species distance (Min-BSD) for each species pair (HAJIB-ABAEI et al. 2007; DEROCLES et al. 2012; YE et al. 2017). The species-pairs with the Max-WSD higher than the Min-BSD were considered as difficult to be discriminated using COI sequences.

2.3. Geometric morphometrics

The analysis of fore wing shape is a useful additional tool for the taxonomy of aphid parasitoids, including closely related species such as those of the *Lysiphlebus fabarum* group (PARREÑO et al. 2017). Here, the landmark configuration (Fig. 2) described in PETROVIĆ et al. (2015) and PARREÑO et al. (2017) was used to explore divergence of fore wing shape in the samples from the *L. fabarum* group for which genetic differences were determined based on the COI barcoding mitochondrial gene. The geometric morphometrics analysis of wing shape was performed on the sample of 186 *Lysiphlebus* individuals (*L. brachycaudi*, n = 45; *L. cardui*, n = 39; *L. volkli*, n = 26; *L. fabarum*, n = 76).

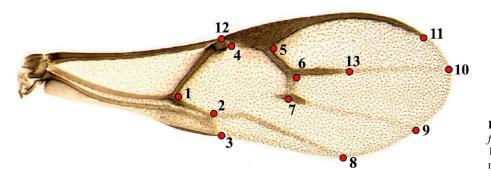


Fig. 2. Fore wing of *Lysiphlebus fabarum* with configuration of 13 landmarks used for geometric morphometric analyses.

Fore wing slides were prepared and photographed as described by MITROVSKI-BOGDANOVIĆ et al. (2013).

A generalized Procrustes analysis was performed to remove variation in scale, position, and orientation of wings and obtain the matrix of shape variables (Procrustes coordinates). To explore and visualize variation in wing shape, a principal component analysis (PCA) was performed. Mean shapes were calculated for each species, and Procrustes distances between these mean shapes were calculated. The statistical significance of Procrustes distances was determined using a permutation test with 10.000 iterations. To obtain information for distinguishing groups or species and the probability of correct classification, we performed a discriminant function analysis (DFA). Since the discriminant function tends to over-estimate the separation between species, reliability of the discrimination was also assessed by the crossvalidation test (LACHENBRUCH 1967). All statistical analyses and visualization of divergences in wing shape were done using the MorphoJ software (KLINGENBERG 2011).

2.4. Abbreviations

Initials of collectors. AA – Ammar Alhmedi, AMB – Ana Mitrovski Bogdanović, AP – Andjeljko Petrović, AS – Alkasim Shukshuk, CV – Christoph Vorburger, DM – David Misfud, DZM – Daniel Zamora Mejias, ER – Ehsan Rakhshani, GR – George Remaudière, HZ – H. Zwolfer, IJ – Ivana Jovičić, JH – Jan Havelka, KH – Kim Hoelmer, KK – Katarina Kos, MGS – May-Guri Sæthre, MJ – Marina Janković, ML – Malik Laamari, MM – Milana Mitrović, MS – M. Safavi, MY – Maria Yovkova, ND – Nicolas Dassonville, NK – Nickolas G. Kavallieratos, OPO – Olivera Petrović-Obradović, PS – Petr Starý, SK – Sahin Kok, SS – Saša Stanković, ST – Snežana Tomanović, VŽ – Vladimir Žikić, XP – Xavier Pons, ŽT – Željko Tomanović.

Countries. ALG – Algeria, AUS – Austria, BEL – Belgium, BEN – Benin, CR – Costa Rica, CZ – Czech Republic, ESP – Spain, FR – France, GR – Greece, IR – Iran, IT – Italy, LIB – Libya, LTV – Lithuania, MNE – Montenegro, SLO – Slovenia, SRB – Serbia, SWIS – Switzerland, USA – United States of America.

Morphology. Cl – clypeus, El – eye length, F_1 – first flagellomere, F_2 – second flagellomere, Fem – hind fe-

mur, It – intertentorial line, LP – longitudinal placodes, Lp – labial palpomeres, Mp – maxillary palpomeres, Ovl – length of ovipositor sheath, Ovw – width of ovipositor sheath, PC – propodeal carinae, Ped – pedicel, Petl – length of T1, Petws – width of T1 at the spiracles, Petwb – width of T1 at the base, R11 – length of anterior branch of radial vein (= metacarpal vein), Sc – scape, Stl – stigma length, Stw – stigma width, Tentorial index – tentoriocular line / intertentorial line, Tib – hind tibia, To – tentoriocular line.

The number of antennomeres is scape + pedicel + number of flagellomeres. Antennomere numbers in brackets indicate a rare character state.

Genes. 28SD2 – the nuclear second expansion segment of 28S rRNA, COI – mitochondrial cytochrome oxidase subunit I.

3. Results

3.1. Analyses of COI barcoding region

In total, 183 COI sequences were aligned, trimmed to equal length of 552 bp, and compared. Haplotype diversity (Hd) was 0.9010, with 36 identified haplotypes (Table 1). Most of the Lysiphlebus species had one to three COI haplotypes registered: L. hirticornis (LH1), L. fritzmuelleri (LFR1), L. balcanicus (LBA1), L. brachycaudi sp.n. (LB1), L. cardui (LC1), L. dessertorum (LD1), L. alpinus (LA1, LA2), L. orientalis (LO1, LO2, LO3), and L. confusus (LCN1, LCN2, LCN3). A higher diversity of haplotypes was identified for L. testaceipes (LT1-LT6), L. fabarum (LF1-LF7), and L. volkli sp.n. (LV1-LV7). Two specimens of L. cardui and five of L. fabarum share the same LCF1 haplotype. The most dominant mitochondrial COI haplotype in the analysed material was LFG1, shared by 37 specimens of L. fabarum, seven of L. 'melandriicola', three of L. confusus, and three of L. cardui (Table 1).

The neighbor-joining tree shows a clear separation of three groups of COI haplotypes, i.e., the 'fabarum' group (L. fabarum, L. 'melandriicola', L. cardui, L. confusus, L. hirticornis, L. brachycaudi, L. volkli); the 'testaceipes' group (L. testaceipes, L. orientalis, L. fritzmuelleri,

НрТ	n	Sequences	Species	Accession numbers
LA1	3	La5, La6, La7	L. alpinus	KY887524
LA2	3	La8, La9, La10	L. alpinus	KY887525
LFR1	9	Lfm7, Lfm8, Lfm9, Lfm10, Lfm11, Lfm4, OP8, OP7, OP3	L. fritzmuelleri	KY887526
LT1	1	809-01	L. testaceipes	KY887527
LT2	1	ST36-2	L. testaceipes	KY887528
LT3	7	B10-81, B10-84, B10-114, B10-115, ST35-1, ST35-2, B10-66	L. testaceipes	KY887529
LT4	6	B06-01, B10-55, B10-111, B10-112, Z06-24, Z06-43	L. testaceipes	KY887530
LT5	16	B09-02, B09-03, B09-04, B09-13, ST2-1, ST2-2, ST3-1, ST3-2, ST19, B10-63, B10-59, B10-60, B10-91, B10-94, B10-95, B10-99	L. testaceipes	KY887531
LT6	1	OP22	L. testaceipes	KY887532
L01	7	S11-22, S11-3-1, S11-3-2, B08-04, B10-13, B10-21, B10-22	L. orientalis	KY887533
L02	10	OP15, S11-20-1, S11-23, S11-27-1, S11-27-2, S11-37, B10-04, B10-08, B08-02, B10-46	L. orientalis	KY887534
L03	1	S11-2-1	L. orientalis	KY887535
LBA1	2	OP2, B10-56	L. balcanicus	KY887536
LD1	4	Ld1, Ld2, Ld3, Ld4	L. desertorum	KY887537
LH1	6	R2-1, R2-2, S11-08-1, S11-08-2, S11-30-1, B06-03	L. hirticornis	KY887538
LB1	11	OP20, OP10, OP9, B10-26, B10-39, S11-752-1, S11-752-2, BEL15-225-1, BEL15-225-2, S11-468-1, S11-468-2	<i>L. brachycaudi</i> sp.n.	KY887539
LV1	6	B07-01, B10-02, B10-23, B10-41, S11-6-2, B10-44	<i>L. volkli</i> sp.n.	KY887540
LV2	3	LT2-1, LT2-2, LT2-4	<i>L. volkli</i> sp.n.	KY887541
LV3	1	LT3-1	<i>L. volkli</i> sp.n.	KY887542
LV4	2	S11-31-1, S11-31-2	<i>L. volkli</i> sp.n.	KY887543
LV5	1	CF2	<i>L. volkli</i> sp.n.	KY887544
LV6	4	R3-1, R3-2, B07-02, B07-04	<i>L. volkli</i> sp.n.	KY887545
LV7	2	\$14-202-1, \$14-202-2	<i>L. volkli</i> sp.n.	KY887546
LFG1	50	*Lm1, Lm4, Lm7, ST20-2, ST20-1, S11-24-1, S11-24-2; * B10-102, R9-2, R9-3; * S11-506-1, S11-506-2, B10-28; * B10-32, Bc2, Bc3, Bc4, Bc5, Bc6, Bc7, Bc8, Bc9, Bc11, Bc12, 55, 60, 111, 143, 158, 168, 206, 242, B07-05, B10-01, B10-18, B10-25, B10-29, Lb1, Lb2, Lb3, Lb5, Lb6, R5-1, S11-19-1, S11-19-2, S11-26-1, S11-26-2, S11-32, BEL14-180-1, BEL14-180-2	^a L. 'melandriicola' ^b L. confusus ^c L. cardui ^d L. fabarum	KY887547
LCF1	7	*B10-11, B10-33; *R5-2, R5-3, S11-21-1, S11-35-1, S11-35-2	^e L. cardui ^f L. fabarum	KY887548
LCN1	2	B08-01, B08-10	L. confusus	KY887549
LCN2	3	R8-1, R8-2, R8-3	L. confusus	KY887550
LCN3	2	S11-14-1, S11-14-2	L. confusus	KY887551
LF1	1	LF1	L. fabarum	KY887552
LF2	3	B10-10, B10-15, B10-47	L. fabarum	KY887553
LF3	1	B10-20	L. fabarum	KY887554
LF4	1	B10-38	L. fabarum	KY887555
LF5	1	R6-2	L. fabarum	KY887556
LF6	2	S11-16-1, S11-16-2	L. fabarum	KY887557
LF7	1	\$11-29	L. fabarum	KY887558
LC1	2	S11-40-1, S11-40-2	L. cardui	KY887559

Table 1. List of COI barcoding haplotypes detected for 13 analysed *Lysiphlebus* species. — *Abbreviations*: HpT = haplotype; n = number of sequences.

L. balcanicus, and L. desertorum); and the 'alpinus' group (L. alpinus) (Fig. 3). Within the 'fabarum' group, seven haplotypes of sexual wasps morphologically resembling L. cardui (newly described as L. volkli) clustered with L. hirticornis and L. brachycaudi within one lineage with 100% bootstrap support, while haplotypes of L. fabarum, L. confusus, L. 'melandriicola', and L. cardui clustered within a second lineage with 99% support. Evolutionary distances based on the Tamura 3-parameter model showed a divergence of 4.0-4.7% between the two lineages (Table 2). On the other hand, the COI marker could not distinguish four species within the haplotype complex of L. fabarum + confusus + cardui + 'melandriicola' with an average distance of only 0.3%. Within the second lineage, L. volkli and L. hirti-

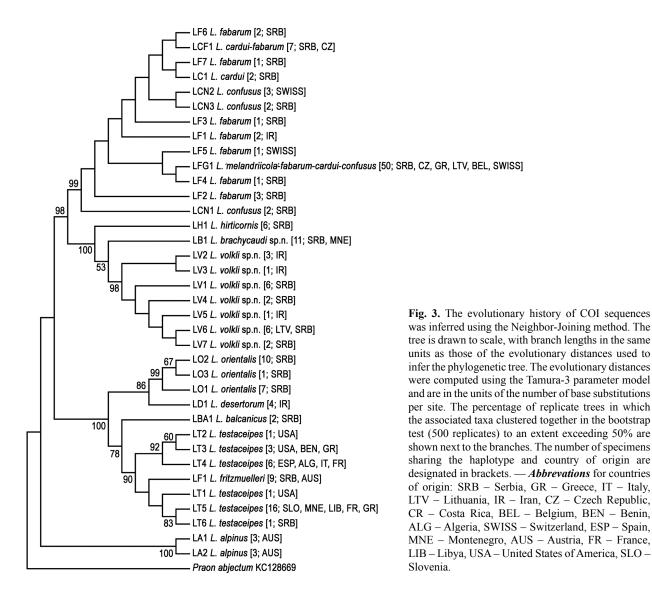
cornis showed 2.2-2.6% sequence divergence, while *L. brachycaudi* is positioned as an intermediary entity 1.3% distant from *L. hirticornis* and 1.7-2% distant from *L. volkli*. The divergence between the haplotypes of *L. volkli* was 0.2-0.5%.

Lysiphlebus alpinus clustered as a separate species with 100% support. The genetic distance between the two haplotypes LA1 and LA2 was 0.1%, and these haplotypes were separated from the '*fabarum*' group by 6.6-7.7% sequence divergence and from the '*testaceipes*' group by 8.2-9.4% (Table 2).

Within the third mitochondrial lineage (the 'testaceipes' group), L. desertorum clustered as the sister species to L. balcanicus, and L. orientalis (Fig. 3). The distances between these three species was 2.5-4.4%

COI hereleture group	Evolutionary distance between haplotype groups (%)							
COI haplotype group	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]
[1] L. alpinus								
[2] L. testaceipes + fritzmuelleri	8.2							
[3] L. orientalis	8.9	3.8						
[4] L. balcanicus	9.4	3.0	4.4					
[5] L. desertorum	8.2	3.1	2.5	3.8				
[6] L. fabarum + confusus + cardui + melandriicola	6.6	7.7	7.6	8.0	7.8			
[7] L. hirticornis	6.9	8.1	8.5	8.2	8.7	4.0		
[8] <i>L. brachycaudi</i> sp.n.	7.4	8.5	8.5	8.5	8.7	4.4	1.3	
[9] <i>L. volkli</i> sp.n.	7.7	8.8	8.8	9.2	9.0	4.7	2.3	1.8

Table 2. Estimates of average evolutionary divergence between the mitochondrial COI haplotype groups according to their clustering on the ML phylogenetic tree using the Tamura 3-parameter model.



(Table 2). Only one haplotype was identified for the first two species, while three very similar COI haplotypes (0.1% divergence) were identified for *L. orientalis*. *L. testaceipes* and *L. fritzmuelleri* haplotypes grouped within the same lineage, with a divergence from *L. desertorum*, *L. balcanicus*, and *L. orientalis* of 3.0-3.8% (Fig. 3, Table 2). The average divergence among the six *L. testaceipes* haplotypes was 0.7% (0.2-1.3%), and the divergence between the single *L. fritzmuelleri* haplotype and the *L. testaceipes* haplotypes was 1.1-1.5%. Divergence between the '*fabarum*' and '*testaceipes*' groups of mitochondrial haplotypes was 7.7-9.2% (Table 2).

A comparison of the maximum within species divergence (Max-WSD) compared with the minimum between species divergence (Min-BSD) showed that most species can be readily identified based on the COI barcoding

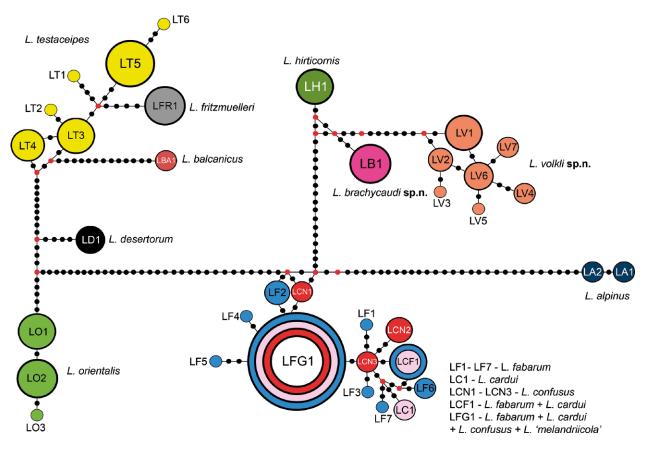


Fig. 4. Median-joining network of mitochondrial COI haplotypes obtained for *Lysiphlebus* species. — *Haplotype codes*: LA1, LA2 – *L. alpinus* (dark-blue circles); LFR1 – *L. fritzmuelleri* (grey circle); LF1–LF7 – *L. fabarum* (blue circles); LT1–LT6 – *L. testaceipes* (yellow circles); LB1 – *L. brachycaudi* **sp.n.** (purple circle); LH1 – *L. hirticornis* (dark green circle); LV1–LV7 – *L. volkli* **sp.n.** (orange circles); LO1–LO3 – *L. orientalis* (light green circles); LD1 – *L. desertorum* (black circle); LC1 – *L. cardui* (light purple circle); LBA1 – *L. balcanicus* (brown circle); LCN1–LCN3 – *L. confusus* (red circles); LFG1 – haplotype shared by *L. fabarum*, *L. confusus*, *L. cardui*, *L. 'melandriicola'* (multicoloured circle, the white circle is *L. 'melandriicola'*); LCF1 – haplotype shared by *L. fabarum* and *L. cardui* (bicoloured blue + light purple circle). — *Symbols*: Circle size reflects the number of individuals with that haplotype (not to scale); red dots are median vectors, black dots mutational steps.

marker. There was an evident delineation of *L. alpinus*, *L. desertorum*, *L. balcanicus*, *L. orientalis*, *L. volkli*, *L. hirticornis* and *L. brachycaudi* from other congeners. On the other hand, barcoding failed to distinguish *L. fabarum*, *L. cardui*, *L. confusus* and *L. 'melandriicola'*. In the case of *L. testaceipes* the Max-WSD is 1.3% while the Min-BSD in comparison with *L. fritzmuelleri* is 1.1%, indicating that also these two species cannot be discriminated based solely on the barcoding analysis.

The median-joining network recognized the same three groups of mitochondrial barcoding haplotypes with a confidence limit of 95%: group 1 - L. *alpinus*; group 2 - L. *fritzmuelleri*, *L*. *testaceipes*, *L*. *orientalis*, *L*. *desertorum* and *L*. *balcanicus*; group 3 - L. *fabarum*, *L*. *brachycaudi*, *L*. *hirticornis*, *L*. *volkli*, *L*. *cardui*, *L*. *confusus*, and *L*. *'melandriicola*' (Fig. 4). *Lysiphlebus alpinus* as an independent taxon is connected with the '*fabarum*' group by 31 mutations. Within the '*fabarum*' group, two lineages are clearly established with a minimum of 16 mutations dividing them, i.e., *L*. *cardui*, *L*. *confusus*, *L*. *'melandriicola'*, and *L*. *fabarum* separated within the first group, while *L*. *brachycaudi*, *L*. *hirticornis*, and *L*. *volkli* form the second. The haplotypes of *L*. *desertorum*,

L. orientalis and *L. balcanicus* are all connected but recognized as separate taxa within the '*testaceipes*' group, while the *L. testaceipes* and *L. fritzmuelleri* haplotypes group together. The analysis resulted in the detection of a minimum of 31 mutational steps between the '*fabarum*' and '*testaceipes*' groups (Fig. 4).

3.2. Analysis of the nuclear second expansion segment of 28S rRNA

In total, 102 28SD2 fragments 655 bp in length were subjected to sequence analysis. Alignment showed that the nuclear sequences of *L. alpinus* differ in length from those of the '*fabarum*' and '*testaceipes*' groups in the insertion of one nucleotide. Species from the '*testaceipes*' group (*L. fritzmuelleri*, *L. desertorum*, *L. balcanicus*, *L. testaceipes*, *L. orientalis*) differ from those of the '*fabarum*' group and *L. alpinus* in two insertions of a single nucleotide and three nucleotides, respectively. In addition, *L. orientalis* and *L. desertorum* have one deletion of a single nucleotide compared to all other analysed sequences.

НрТ	n	Sequences	Species	Accession number
Lys1	2	La5, La8	L. alpinus	KY887508
Lys2	10	*OP3, OP7, Lfm4, OP8; *OP22, B10-55, B09-06, ST31, ST32, ST21	^a L. fritzmuelleri ^b L. testaceipes	KY887509
Lys3	16	°S11-37, S11-23, OP15, S11-20-1, S11-02-1, B10-08, S11-03-1, S11-03-2, B10-13, B10-22, B10-21, B08-04, B08-02, B10-04; ªLd1, Ld2	° L. orientalis ª L. desertorum	KY887510
Lys4	1	B10-56	L. balcanicus	KY887511
Lys5	3	S11-08-1, S11-30-1, B06-03	L. hirticornis	KY887512
Lys6	10	* OP9, OP20, OP10; ¹ B10-39, B10-26, Bc2, Bc10, Bc4; *S11-31-1, S11-31-2	° <i>L. brachycaudi</i> sp.n. † <i>L. fabarum</i> 9 <i>L. volkli</i> sp.n.	KY887513
Lys7	21	▶S11-19-2, S11-19-1, B10-01,B10-15, B10-18, B10-20, B10-47, S11-16-2, S11-16-1, S11-26-1, S11-21-1, S11-26-2, R6-2; iS11-06-2, B10-33; iLm7, Lm4; *B08-10; 'LT2-2, B07-02, B10-41	 b.L. fabarum i.L. cardui i.L. 'melandriicola' k.L. confusus i.L. volkli sp.n. 	KY887514
Lys8	24	■ S11-14-2, S11-14-1, R8-3, R8-2, R8-1, R9-3, R9-2; ■ S11-24-1, S11-24-2; ■ Bc1, Bc3, S11-35-2, S11-32, R5-3, R5-2, B10-38, B10-25, B10-29; ■ R3-2, R3-1, B10-28, B07-04, B07-01, B10-23	^m L. confusus ⁿ L. 'melandriicola' ^o L. fabarum PL. volkli sp.n.	KY887515
Lys9	1	S11-40-2	L. cardui	KY887516
Lys10	5	Bc5, Bc6, Bc7, Bc8, Bc9	L. fabarum	KY887517
Lys11	1	LF1	L. fabarum	KY887518
Lys12	2	Lb3, Lb1	L. fabarum	KY887519
Lys13	1	CF2	<i>L. volkli</i> sp.n.	KY887520
Lys14	1	B08-01	L. confusus	KY887521
Lys15	1	807-05	L. fabarum	KY887522
Lys16	3	168, 206, 158	L. fabarum	KY887523

Table 3. List of haplotypes of nuclear 28SD2 fragments identified within the genus *Lysiphlebus*. — *Abbreviations*: HpT = haplotype; n = number of sequences.

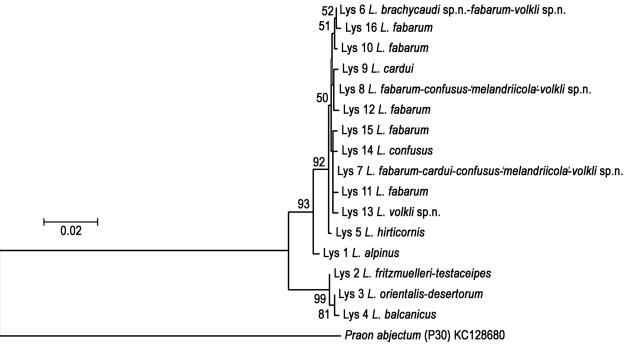


Fig. 5. The evolutionary history of 28S haplotypes was inferred using the Neighbor-Joining method. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Tamura-3 parameter model and are in the units of the number of base substitutions per site. The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analysed. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Only branch supports exceeding > 50% are shown. — *Haplotype codes*: Lys1 – *L. alpinus*; Lys2 – *L. testaceipes*, *L. fritzmuelleri*; Lys3 – *L. orientalis*, *L. desertorum*; Lys4 – *L. balcanicus*; Lys5 – *L. hirticornis*; Lys6 – *L. brachycaudi* **sp.n.**, *L. fabarum*, *L. volkli* **sp.n.**; Lys7 – *L. fabarum*, *L. confusus*, *L. cardui*, L, volkli **sp.n.**; Lys1 – *L. alpinus*; Lys12, Lys15, Lys16 – *L. fabarum*; Lys13 – *L. volkli* **sp.n.**; Lys14 – *L. confusus*.

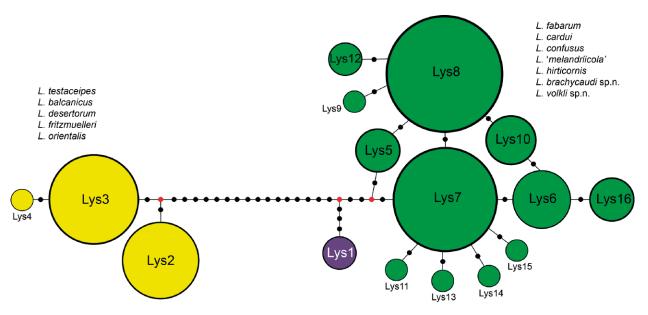


Fig. 6. Median-joining network of nuclear 28S haplotypes obtained for *Lysiphlebus* species. — *Symbols and haplotypes*: Red dots are median vectors, black dots mutational steps. Purple circle – haplotype Lys1 (*L. alpinus*). Yellow circles – haplotypes Lys2 (*L. testaceipes*, *L. fritzmuelleri*, *L. balcanicus*), Lys3 (*L. orientalis*, *L. desertorum*), and Lys4 (*L. balcanicus*). Green circles – haplotypes Lys5 (*L. hirticornis*), Lys6 (*L. brachycaudi* **sp.n.**, *L. fabarum*, *L. volkli* **sp.n.**), Lys7 (*L. fabarum*, *L. confusus*, *L. cardui*, *L. 'melandriicola'*, *L. volkli* **sp.n.**), Lys8 (*L. cardui*), Lys10, Lys11, Lys12, Lys15, Lys16 (*L. fabarum*), Lys13 (*L. volkli* **sp.n.**), and Lys14 (*L. confusus*).

The total number of sites was 649, but due to insertions and deletions, 643 sites were analysed. Sites with alignment gaps were not considered. Haplotype diversity (Hd) was 0.8614, with 16 haplotypes detected (Lys1-Lys16) and 32 variable sites overall (Table 3).

Although haplotype diversity was lower, the neighbor-joining tree constructed from nuclear 28SD2 sequences shows separation into the same three lineages as for mitochondrial COI, i.e., the 'alpinus' (Lys1), 'testaceipes' (Lys2-Lys4), and 'fabarum' (Lys5-Lys16) groups (Fig. 5). The 'alpinus' lineage has a single haplotype, while the number of base substitutions per site obtained by averaging all 28S sequence pairs shows that the average divergence rate within both the 'fabarum' and 'testaceipes' groups was 0.2%. Estimation of the average evolutionary divergence between species groups using the Tamura 3-parameter model shows that the 'alpinus' group differs from the 'fabarum' group on average by 1.1% (range: 0.9-1.3%) and from the 'testa*ceipes*' group on average by 3.1% (range: 3.0-3.3%), while the 'fabarum' and 'testaceipes' groups differ by 3.2-3.7%.

The median-joining network reconstructed on the basis of maximum parsimony calculation with a confidence limit of 95% confirms the clustering of nuclear haplotypes into the same three lineages (Fig. 6). Within the '*fabarum*' group, 12 similar nuclear haplotypes connected by just 1–4 mutational steps were distinguished, and species commonly shared identical haplotypes. The *Lysiphlebus alpinus* haplotype Lys1 is positioned separately in the network, evidently being more closely related to the '*fabarum*' group. Clear separation of the '*testaceipes*' group is evident, with three haplotypes shared by

the species and with one or two mutational steps connecting them (Fig. 6).

3.3. Geometric morphometrics

The first two principal components, PC1 and PC2, accounted for 44.53% of the total variation in wing shape. The positions of individuals in the morphospace described by the first and second PCs are shown in Fig. 7. Individuals of *L. brachycaudi* and *L. volkli*, which have a narrower distal part of the wing, narrower stigma, and a relatively elongated metacarpal vein, separated along the first PC from individuals of *L. fabarum* and *L. cardui. Lysiphlebus brachycaudi* and *L. volkli* separated clearly along the second PC axis. *Lysiphlebus volkli* has longer and narrower wings and a narrower stigma than *L. brachycaudi*, but these two species have the same stigma to metacarpal vein ratio (Fig. 7).

Species differed significantly in wing shape, with very high percentages of correct classification (Table 4). Histograms with values of the discriminant scores obtained from a discriminant function analysis and the differences of shape between species shown as the change from the first to the second species are presented in Fig. 8. From these pairwise comparisons, it is clear that *L. volkli* differs from all other species in having a narrower stigma and a less convex, almost flat proximal margin of the fore wing. Compared to *L. cardui*, individuals of *L. volkli* have a longer radial vein and a more proximally positioned radio-medial vein.

Compared species	Procrustes distance	Correct classification	Cross-validation
L. brachycaudi sp.n. vs. L. cardui	0.041 ***	98%	89%
L. brachycaudi sp.n. vs. L. volkli sp.n.	0.034 ***	99%	94%
L. brachycaudi sp.n. vs. L. fabarum	0.030 ***	94%	92%
<i>L. cardui</i> vs. <i>L. volkli</i> sp.n.	0.048 ***	98%	94%
L. cardui vs. L. fabarum	0.025 ***	96%	89%
L. volkli sp.n. vs. L. fabarum	0.033 ***	97%	93%

Table 4. Procrustes distances between average wing shapes of four Lysiphlebus species and percentages of correct classification obtainedfrom discriminant function analysis and the cross-validation test. *** P < 0.0001.

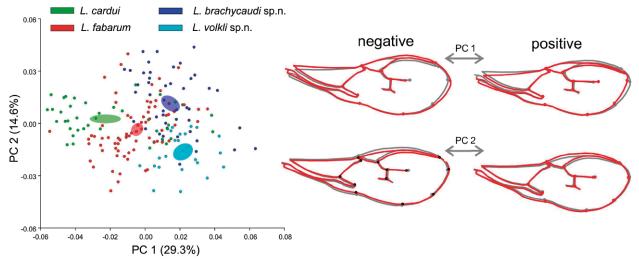


Fig. 7. Positions of *Lysiphlebus* individuals relative to the first and second principal component axes (PC1 and PC2). Values of the 90% variance of mean wing shape were calculated for each species separately and are presented as shaded ellipses. Shape changes associated with the first (PC1) and second axis (PC2) are visualized as warped outline drawings. The mean wing shape is in grey and the extreme wing shapes representing the positive and negative end of each axis are in red.

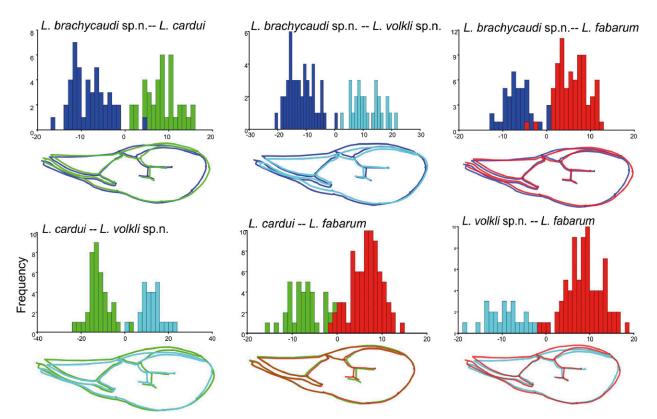


Fig. 8. Distribution of discriminant scores obtained from discriminant function analysis and differences between the average shape of two compared species. Species (phylogenetic clades) are colour-coded as in Fig. 7. All changes are enlarged $2 \times$.

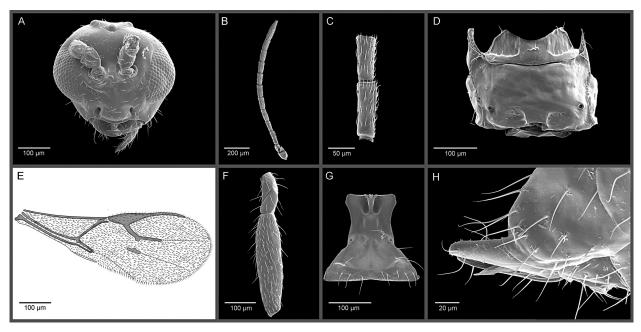


Fig. 9. Lysiphlebus volkli sp.n. A: Head, frontal view. B: Antennae. C: Flagellomeres 1 and 2. D: Propodeum, dorsal view. E: Fore wing. F: Hind femur, lateral view. G: Petiole, dorsal view. H: Ovipositor sheath, lateral view.

3.4. European *Lysiphlebus* species: descriptions of new species and redescriptions

Lysiphlebus volkli Tomanović & Kavallieratos sp.n. (Fig. 9A–H)

Diagnosis. *Lysiphlebus volkli* sp.n. morphologically resembles *L. cardui* in having long semi-erect setae on the hind femur (Fig. 9F) and in shape of the petiole (Fig. 9G), in addition to which it has a similar host range pattern (e.g., it parasitizes the *A. fabae* complex – *A. fabae fabae* and *A. fabae cirsiiacanthoidis* Scopoli). *Lysiphlebus volkli* differs from *L. cardui* in having a more elongated stigma (length/width of stigma 3.80–4.00 in *L. volkli* but 3.20–3.60 in *L. cardui*). Additionally, *L. volkli* has a somewhat shorter metacarpal vein and slightly longer F_1 than F_2 , which is not the case in most *L. cardui* populations.

Description. *FEMALE: Head* (Fig. 9A) transverse, wider than mesosoma at tegulae, bearing sparse setae. Eyes oval, medium-sized. Tentorial index 0.55-0.65. Clypeus with 5-6 long setae. Labrum distinct, with 3-5 short setae. Malar space $0.33-0.40 \times \text{longitudinal}$ eye diameter. Mandible bidentate, with 13-14 setae on outer surface. Maxillary palpi with three palpomeres, labial palpi with one palpomere. Antenna 12-segmented, slightly thickened at apex (Fig. 9B). Pedicel subspherical. F₁ (Fig. 9C) slightly longer than F₂ and $2.7-3.0 \times \text{as}$ long as its maximum width at middle. F₁ without and F₂ without or exceptionally with 1-2 longitudinal placodes (Fig. 9C). Flagellomeres covered uniformly with short appressed and semi-erect setae. *Mesosoma*: Mesoscutum smooth, notaulices distinct in very short ascendent portion of anterolateral margin, effaced dorsally, with usually two rows setae along dorsolateral part of mesoscutum. Scutellum elongated, bearing five long setae in central part. Propodeum (Fig. 9D) smooth, sometimes with two short divergent carinae at base. Upper and lower parts of propodeum with 2-5 and 1-3 long setae on each side. Fore wing (Fig. 9E) densely pubescent, with short lower marginal setae, equal to those on fore wing surface in examined European populations; some Iranian populations (Aphis verbasci Schrank/Verbascum sp.) possess somewhat longer fore wing marginal setae. Stigma elongate, $3.8-4.0 \times$ as long as its width, $0.65-0.80 \times$ as long as metacarpal vein (Fig. 9E). Hind femur with semi-erect setae (Fig. 9F). Metasoma: Petiole (Fig. 9G) smooth, slightly convex dorsally, with lateral depression after prominent spiracular tubercles, its length $1.50-1.80 \times$ its width at spiracles, $1.00-1.15 \times$ its width at base; 4-5 setae positioned on posterior dorsolateral margin on each side, one to three long setae posterior to spiracles (Fig. 9G). Ovipositor sheath (Fig. 9H) elongate, wide at base, dorsally slightly concave, narrowed toward tip, bearing 11-15 short setae at tip and 1-2 long setae at tip and 1-2 long setae on ventral and dorsal surface, respectively. Length of ovipositor sheath 2.40-2.60 × its maximum width. Body length: 1.5-2.0 mm. Colouration: General body colour brown. Scape and pedicel light-brown to brown, and F₁ with narrow yellow ring at the base. Mouthparts yellow to light brown. Pronotum brown; mesoscutum and mesopleuron dark brown. Propodeum light brown. Legs yellow to light brown. Wings hyaline, venation yellowish brown. Petiole yellow, other metasomal terga light brown, and gradually darker at end of metasoma. Ovipositor sheath dark brown. - MALE: Antenna 13-14-segmented. Maxillary palpi with three palpomeres, labial palpi with one palpomere. Fore wing venation as in female except shorter metacarpal vein. Fore wing: lower marginal setae distinctly longer than those on surface (the usual sexual dimorphism in *Lysiphlebus*). Body darker than female, generally brown. Petiole and first half of metasoma light brown, remaining part of metasoma brown. Legs and mouthparts light brown. Body length: 1.5-2.0 mm.

Etymology. The new species is named in honour of the late Prof. Dr. Wolfgang Völkl, who made an important contribution to knowledge of the population and community ecology of aphid parasitoids. A common model species in his research was *L. cardui*.

Remarks. Lysiphlebus volkli is morphologically similar to L. cardui, and this is the reason why it was unrecognized despite frequent sampling of parasitoids of A. fabae colonies in Europe. Only the elongated stigma and flagellomere 1 longer than flagellomere 2 differentiates it from L. cardui and most other members of the L. fabarum group. Therefore, measurements should be taken from slide-mounted specimens. Lysiphlebus volkli was previously considered as an L. cardui phenotype, but our field evidence showed it is a sexual species in Europe, although females were dominant in some samples (see examined material). Lysiphlebus volkli parasitizes aphid hosts from the A. fabae complex and other Aphis spp. in Europe. Although we found this species only in two European countries (Serbia and Lithuania), we suspect it is present in more regions of Europe since we recorded L. volkli also outside of Europe (in Iran), associated with B. tragopogonis (Kaltenbach) and A. verbasci aphid hosts. Interestingly, all sampled populations from A. verbasci were asexual. All examined European populations are sexual, although this statement needs to be confirmed by further sampling and examination of available material.

Examined material. Holotype 12, slide-mounted, SERBIA, Belgrade-Batajnica, A. fabae cirsiiacanthoidis on Cirsium arvense (L.) Scopoli, 1772, 6.vii.2010 (MM). Holotype deposited in the collection of the Institute of Zoology, Faculty of Biology, University of Belgrade. Paratypes 2[♀], slide-mounted, SERBIA, Belgrade-Slanci, A. fabae on Chenopodium album L., 1753, 29.v.2007 (ŽT); 4♀ 1 \Diamond , slide-mounted, 8 \downarrow 5 \Diamond , alcohol-preserved, Vodanj, A. fabae on Ch. album, 8.vi.2014 (MJ). Paratypes deposited in the collection of the Institute of Zoology, Faculty of Biology, University of Belgrade. Other material excluded from type series (material from Lithuania deposited in collection of Institute of Entomology, České Budějovice; material from Serbia deposited in collection of Institute of Zoology, Faculty of Biology and material from Iran deposited in collection of University of Zabol): LITHUANIA: 5∂. Vilnius, Aphis farinosa Gmelin, 1790 on Salix fragilis L., 1753, 3.vii.2011 (JH); 15^Q, Vilnius, A. farinosa on S. fragilis, 5.vii.2011 (JH). SERBIA: 2[⊖]₊, Belgrade-Slanci, A. fabae fabae on Rumex sp., 29.v.2007 (ŽT); 3♀, Vodanj, A. fabae on Ch. album, 8.vi.2014 (MJ); 17^{\bigcirc}_{+} 32^{\bigcirc}_{-} , Zemun-Tempo, A. fabae cirsiiacanthoidis on C. arvense, 22.vi.2010 (MM); 19 40, Ostružnica, A. fabae on Carduus acanthoides L., 1753, 17.vi.2010 (MM); 5 4 3, Smederevo, Aphis sp. on Rumex sp., 27.v.2011 (OPO); 50♀ ♂, Kalna, Aphis sp. on Sanguisorba minor Scopoli, 1771, 11.vi.2011 (SS). IRAN: 86[♀] 144[♂], Esfahan, Brachycaudus tragopogonis (Kaltenbach, 1843) on Tragopogon pratensis L., 1753, 5.vii.2005 (ER); 20° 21³, Hamadan, B. tragopogonis on T. pratensis, 25.vi.2005 (ER); 12[♀], Ardebil, Aphis verbasci Schrank, 1801 on Verbascum sp., 1.vii.2005 (ER).

Lysiphlebus brachycaudi Starý & Tomanović sp.n. (Fig. 10A–H)

Lysiphlebus brachycaudi in Starý 2006 (nomen nudum)

Diagnosis. Lysiphlebus brachycaudi sp.n. morphologically resembles *L. fabarum* by the presence of appressed setae on the hind femur (Fig. 10F), metacarpal vein length (Fig. 10E), and shape of the petiole (Fig. 10G). Lysiphlebus brachycaudi differs from L. fabarum in having a more elongated stigma (length/width of stigma is 3.40-3.80 in L. brachycaudi instead of 2.80-3.40 in L. fabarum) (Fig. 10E). After examination of numerous L. fabarum populations, we found great intraspecific variability of stigma shape. Some biotypes have a more elongated stigma, close to that of L. brachycaudi (e.g., L. fabarum / Aphis origani Passerini, 1860: 3.15-3.40; L. fabarum / A. epilobii Kaltenbach, 1843: 3.00-3.40; L. fabarum / A. urticata Gmelin, 1790: 3.00-3.40). We therefore recommend careful examination of series of slide-mounted specimens, since it is clear that the majority of L. brachycaudi specimens tend to have a more elongated stigma with higher values (3.70-3.80), while in the majority of L. fabarum specimens the value of the stigma length/width ratio is around 3.00-3.20. Lysiphlebus brachycaudi is a specific parasitoid of Brachycaudus cardui L., 1758.

Description. FEMALE: Head (Fig. 10A) transverse, wider than mesosoma at tegulae, with sparse setae. Eyes medium-sized, oval, laterally prominent. Tentorial index 0.50-0.58. Clypeus with 7-10 long setae. Labrum distinct, with 4–6 short setae. Malar space $0.34-0.37 \times$ longitudinal eye diameter. Mandible bidentate, with 14-15 setae on outer surface. Maxillary palpi with three palpomeres, labial palpi with one palpomere. Antenna 12(13)-segmented, slightly thickened at apex (Fig. 10B). Pedicel subspherical. F₁ slightly longer or subequal to F₂ and $2.8-3.1 \times$ as long as its maximum width at middle. F_1 without and F_2 without or with one longitudinal placode (Fig. 10C). Flagellomeres covered uniformly with short appressed and semi-erect setae. Mesosoma: Mesoscutum smooth, notaulices distinct in very short ascendent portion of anterolateral margin, effaced dorsally, with usually two rows of dense setae along dorsolateral part of mesoscutum. Scutellum elongate, with $5-6 \log$ setae in central part. Propodeum (Fig. 10D) smooth, sometimes with two short divergent carinae at base. Upper and lower parts of propodeum with 4-5 and 1-3 long setae on each side. Fore wing (Fig. 10E) densely pubescent, with short lower marginal setae equal to those on fore wing surface. Stigma elongate, $3.4-3.8 \times$ as long as its width, $0.70-0.80 \times$ as long as metacarpal vein (Fig. 10E). Hind femur with short appressed setae (Fig. 10F). Metasoma: Petiole (Fig. 10G) smooth, slightly convex dorsally, with lateral depression after prominent spiracular tubercles, its length $1.70-1.90 \times$ its width at spiracles, $1.15-1.25 \times$ its width at base; 5-6 setae positioned on posterior dorsolateral margin on each side. Ovipositor sheath (Fig. 10H) elongate, wide at base, dorsally slightly concave, narrowed toward tip, bearing 2-3 long setae at tip, 2-3

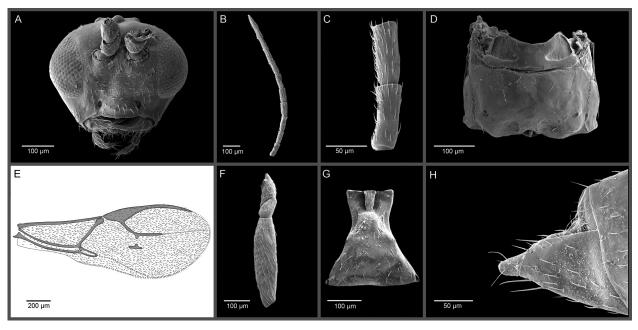


Fig. 10. Lysiphlebus brachycaudi sp.n. A: Head, frontal view. B: Antennae. C: Flagellomeres 1 and 2. D: Propodeum, dorsal view. E: Fore wing. F: Hind femur, lateral view. G: Petiole, dorsal view. H: Ovipositor sheath, lateral view.

long setae on ventral surface, and 3-4 on dorsal surface. Length of ovipositor sheath $2.40-2.80 \times$ its maximum width. Body length: 1.5-2.0 mm. Colouration: General body colour brown to dark brown. Antennae brown, annelus and narrow ring of flagellomere 1 yellow. Mouthparts yellow. Pronotum light brown. Mesosoma brown. Legs yellow to light brown, with dark apices. Wings hyaline, venation yellowish brown. Petiole yellow, other metasomal terga light brown to brown. Ovipositor sheath dark brown. - MALE: Antenna 13-14-segmented. Maxillary palpi with three palpomeres, labial palpi with one palpomere. Fore wing venation as in female except for shorter metacarpal vein which is sometimes subequal to stigma length. Stigma less elongate than in female (length/width of stigma 3.20-3.40). Petiole stouter than in females (length/width of petiole 1.40-1.60 at spiracle level and 1.00-1.10 at base). Fore wing lower marginal setae longer than those on surface. Body darker than female, generally brown. Petiole brown, sometimes light brown, remaining part of metasoma brown. Legs and mouthparts light brown. Body length: 1.5–2.0 mm.

Etymology. The name of the new species is derived from its host aphid.

Remarks. On the basis of the mentioned records that are sequenced, we determined that *L. brachycaudi* is a sexual species, although in some samples females were dominant (see examined material). Apparently, the specialized *L. brachycaudi* shares *B. cardui* on *Carduus* spp. and *Cirsium* spp. with the broadly oligophagous *L. fabarum*. It should be noted that *Carduus* and *Cirsium* plants very often are colonized by mixed colonies of *A. fabae* and *B. cardui*, which are both parasitized by *L. fabarum*. SCHAR & VORBURGER (2013) found no overlap in host use in mixed colonies of *A. fabae cirsiiacanthoidis* and *B. cardui* in Switzerland. From *A. fabae cirsiiacanthoidis*

only asexual wasps with a *L. cardui* phenotype were obtained, and from *B. cardui* only sexual wasps, with a *L. fabarum* phenotype were obtained, even when they occurred on the very same plant. Interestingly, all analyzed sexual wasps with a *L. fabarum* phenotype possessed a *L. fabarum* s.str. haplotype (LFG1). We have confirmed the presence of *L. brachycaudi* only in Serbia and Montenegro until now, but we assume a broader distribution in Europe, following the distribution of its host aphid. We also recommend revisiting and thorough examination of all *Lysiphlebus* specimens emerged from *B. cardui* / *Carduus* spp. and *Cirsium* spp., which would probably confirm a broader distribution of *L. brachycaudi* in Europe.

Examined material. Holotype 1^Q, slide-mounted, SERBIA, Mt. Vlasina-Čemernik, 1400 m, B. cardui on Carduus crispus L., 1753, 4.v.2011 (VŽ). Holotype deposited in the collection of the Institute of Zoology, Faculty of Biology, University of Belgrade. Paratypes 11♀ 1♂, SERBIA, slide-mounted and preserved in alcohol, Mt. Vlasina-Čemernik, 1400 m, B. cardui on C. crispus, 04.v.2011 (VŽ); 238♀ 31♂, Mt. Vlasina, 1200 m, B. cardui on C. acanthoides, 6.viii.2010 (ŽT). Paratypes deposited in the collection of Institute of Zoology, Faculty of Biology, University of Belgrade. Other material excluded from type series (material from Serbia deposited in collection of Institute of Zoology, Faculty of Biology): **MONTENEGRO**: >500 \bigcirc \bigcirc ; Andrijevica, *B. cardui* on C. acanthoides, 22.vii.2012 (ŽT). SERBIA: 31♀ 17♂, Belgrade, *B. cardui* on *Carduus* sp., 14.vi.2015 (AP); $2 \stackrel{\circ}{\downarrow} 5_{\circ}^{\circ}$, Bosilegrad, *B.* cardui on Cirsium vulgare (Savi) Tenore, 1835, 22.vii.2013 (SS); 8^Q, Mt.Vlasina, 1200 m, B. cardui on Cirsium eriophorum (L.) Scopoli, 1772, 21.vii.2013 (SS); 13, Zemun, B. cardui on C. acanthoides, 18.vi.2010 (AP); 3 4 d, Dobanovci, B. cardui on C. acanthoides, 7.vi.2010 (AP).

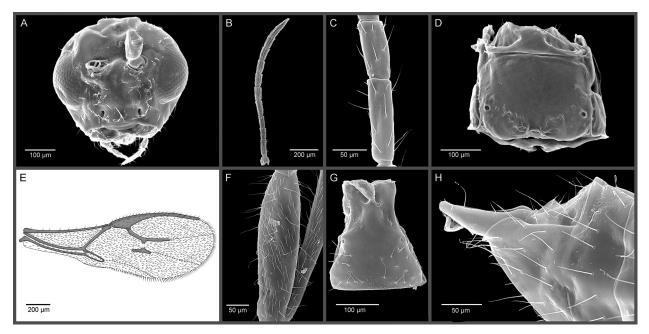


Fig. 11. Lysiphlebus hirticornis. A: Head, frontal view. B: Antennae. C: Flagellomeres 1 and 2. D: Propodeum, dorsal view. E: Fore wing. F: Hind femur, lateral view. G: Petiole, dorsal view. H: Ovipositor sheath, lateral view.

Lysiphlebus hirticornis Mackauer, 1960

(Fig. 11A-H)

Lysiphlebus safavii Starý, 1985 syn.n.

Diagnosis. On the basis of the wing venation pattern (a very long metacarpal vein reaching the fore wing edge) (Fig. 11E) and the number of palpomeres (three maxillary palpomeres and one labial palpomere), this species belongs to the '*fabarum*' group. However, it differs clearly from other members of the '*fabarum*' group in having long and erect setae on femurs and tibiae of the hind legs (Fig. 11F), a F₁ that is clearly longer than F₂ (Fig. 11C), and an elongate and clearly concave ovipositor sheath (Fig. 11H). It is a specialized parasitoid of the pink tansy aphid, *Metopeurum fuscoviride* Stroyan, 1950.

Description. FEMALE: Head (Fig. 11A) rounded with sparse setae. Eyes medium-sized, oval. Tentorial index 0.50-0.60. Clypeus with 8-12 long setae. Labrum distinct, with 3–4 short setae. Malar space $0.37-0.42 \times$ longitudinal eye diameter. Mandible bidentate, with 14-15 setae on the outer surface. Maxillary palpi with three palpomeres, labial palpi with one palpomere. Antenna 12(13)-segmented, moderately thickened at the apex (Fig. 11B). F₁ (Fig. 11C) clearly longer than F₂ (F₁ length / F_2 length is 1.20–1.40) and 2.9–3.1 × as long as its maximum width at the middle (Fig. 11C). F_1 and F_2 without longitudinal placodes (Fig. 11C). Flagellomeres covered uniformly with short semi-erect setae. Mesosoma: Mesoscutum smooth, notaulices distinct in very short ascendent portion of anterolateral margin, effaced dorsally, with usually two rows of setae along dorsolateral parts of the mesoscutum. Scutellum elongate, with eight long setae in the central part. Propodeum (Fig. 11D) smooth with irregular rugosities laterally from the base. Upper and lower parts of propodeum with 4-5 and 2-4

long setae on each side. Fore wing (Fig. 11E) with long marginal setae around it. Stigma elongated, $3.3-3.7 \times as$ long as its width, and clearly shorter than the metacarpal vein $(0.51-0.68 \times \text{as long as it})$. Hind femora and tibiae with long erect setae (Fig. 11F). Metasoma: Petiole (Fig. 11G) smooth, convex dorsally, with lateral depression after prominent spiracular tubercles, its length $1.60-2.00 \times$ its width at the spiracles and $1.05 - 1.17 \times$ its width at the base; setous with 7-10 setae positioned on posterior part of each side of petiole. Ovipositor sheath (Fig. 11H) elongate, dorsally clearly concave, narrowed toward tip with 2-3 long setae and 10-15 short setae. Length of ovipositor sheath $2.70-3.00 \times$ its maximum width. **Body** length: 1.7-2.3 mm. Colouration: General body colour yellow to light-brown. Scape and pedicel yellow and F1 yellow at the base. Remaining part of antenna lightbrown to brown. Eyes black. Mouthparts and face yellow. Mesoscutum and mesopleuron light-brown. Propodeum light-brown. Legs vellow with dark apices. Wings hyalinized, venation brown. Petiole yellow, other metasomal terga yellow to light-brown, gradually darker at the end of metasoma. Ovipositor sheath black. — MALE: Antenna (13)14-segmented. Maxillary palpi with three palpomeres, labial palpi with one palpomere. Fore wing venation as in female, except for a somewhat shorter metacarpal vein. Body gradually darker than female, generally light-brown to brown. Mouthparts yellow to light-brown. Petiole yellow, remaining part of metasoma brown. Legs light-brown. Body length: 1.5–2.1 mm.

Remarks. It has been recorded in several European countries from north (Finland) to south (Spain), so we suppose it to be distributed throughout Europe, since the *M. fuscoviride / T. vulgare* association is very common in Europe. It is a sexual species. After examination of the slide-mounted type specimens of *L. safavii* we concluded

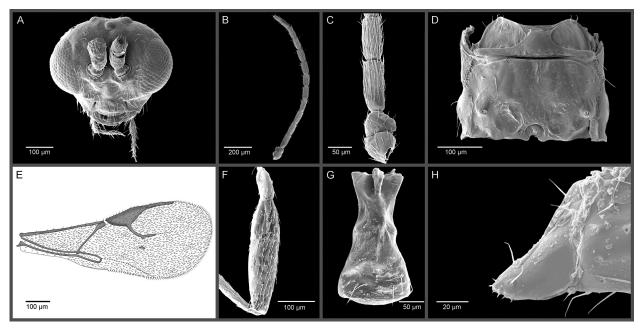


Fig. 12. Lysiphlebus fritzmuelleri. A: Head, frontal view. B: Antennae. C: Flagellomeres 1 and 2. D: Propodeum, dorsal view. E: Fore wing. F: Hind femur, lateral view. G: Petiole, dorsal view. H: Ovipositor sheath, lateral view.

that its main diagnostic characters (pubescence of flagellomeres, long F_1 , narrowly pointed and dorsally concave ovipositor sheath) are related to an already known species, *L. hirticornis*. After comparing several specimens of both taxa, we found no consistent morphological differences among them.

Examined material. BELGIUM: 99 163, Gingelom, Metopeurum fuscoviride on Tanacetum vulgare L., 1753, 7.vii.2015 (AA); 55♀ 28♂, Brustem, *M. fuscoviride* on *T. vulgare*, 23.vii.2015 (AA). CZECH REPUBLIC: Neveklov, M. fuscoviride on T. vulgare, 26.vii.2003 (PS); Hulin, M. fuscoviride on T. vulgare, 15.vii.2004 (PS); Češke Budějovice, *M. fuscoviride* on *T. vulgare*, 29.vii.2003 (PS). **FINLAND**: $2 \oplus 8 \Im$, Turku, *M. fuscoviride* on *T. vulgare*, 22.vi.2016 (ŽT). SERBIA: 16♀ 2♂, Valjevo, M. fuscoviride on T. vulgare, 19.vi.2011 (ŽT); 17♀ 13♂, Tamnič, M. fuscoviride on *T. vulgare*, 11.vi.2011 (VŽ); >100 \bigcirc \circlearrowleft , Mt. Vlasina, 1200 m, *M*. fuscoviride on T. vulgare, 6.viii.2010 (VŽ); >1000 \bigcirc Å, Lebane-Konjino, M. fuscoviride on T. vulgare, 6.vii.2010 (SS); 16^{\bigcirc}_{+} 7 $^{\diamond}_{-}$, Mt. Dukat, 1300 m, 7.viii.2011 (VŽ); 1182 883, Mt. Vlasina, 1200 m, M. fuscoviride on T. vulgare, 3.viii.2011 (VŽ); $21 \stackrel{\circ}{\downarrow} 6\stackrel{\circ}{\triangleleft}$, Jerma Gorge, M. fuscoviride on T. vulgare, 19.vii.2013 (SS); 15 4d, Mt. Vlasina, 1200 m, M. fuscoviride on T. vulgare, 15.vi.2013 (SS); 11 ♀ 32 ♂, Mt. Vlasina, 1200 m, M. fuscoviride on T. vulgare, 21.vii.2013 (SS); 12♀ 7♂, Vladičin Han, M. fuscoviride on T. vulgare, 17.vi.2011 (VŽ); 11♀ 2♂, Mt.Vlasina, 1200 m, M. fuscoviride on T. vulgare, 13.viii.2006 (ŽT); 8♀ 8♂, Plavna, M. fuscoviride on T. vulgare, 27.vi.2011 (VŽ).

Examined type specimens of *L. safavii.* Holotype 1, **GER-MANY**, Cologne, 1.viii.1963 (MS). – Paratypes 4, **GERMANY**, Cologne, 1.viii.1963 (MS).

For additional records see KAVALLIERATOS et al. (2004) and STARÝ (2006).

Lysiphlebus fritzmuelleri Mackauer, 1960 (Fig. 12A–H)

Diagnosis. Lysiphlebus fritzmuelleri belongs to the 'testaceipes' group and is morphologically similar to L. testaceipes (with a similar wing venation pattern, strong triangular stigma and large number of longitudinal placodes on the flagellomeres) (Fig. 12C,E). It differs from L. testaceipes in having a broader petiole (the ratio between petiole length and width at the base is 1.50-1.70 in L. fritzmuelleri instead of 1.70-2.00 in L. testaceipes) (Fig. 12G). Also, L. fritzmuelleri is a specialized parasitoid of the tufted vetch aphid, Aphis craccae L., 1758, in Europe.

Description. FEMALE: Head (Fig. 12A) transverse, with sparse setae. Eyes medium-sized, oval. Tentorial index 0.61-0.67. Clypeus with 7-12 long setae. Labrum distinct, with 4-5 short setae. Malar space $0.34-0.41 \times$ longitudinal eve diameter. Mandible bidentate, with 13–14 setae on outer surface. Maxillary palpi with three palpomeres, labial palpi with two palpomeres. Antenna 13(14)-segmented, moderately thickened at the apex (Fig. 12B), almost flagellate. F_1 subequal to F_2 (Fig. 12C). F₁ relatively short and $2.2-2.5 \times$ as long as its maximum width at the middle. F_1 and F_2 with 4-5(6) and 5-6(7) longitudinal placodes, respectively (Fig. 12C). Flagellomeres covered uniformly with short appressed and semi-erect setae. Mesosoma: Mesoscutum smooth, notaulices distinct in very short ascendent portion of anterolateral margin, effaced dorsally, with usually two rows of setae along the dorsolateral part of mesoscutum. Scutellum elongate, with 6-7 long setae in the central part. Propodeum (Fig. 12D) smooth, with small rugosities at the base. Upper and lower parts of propodeum with 3-5 and 2-3 long setae on each side. Fore wing

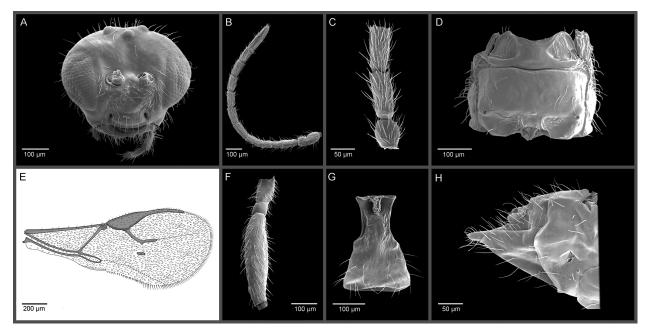


Fig. 13. Lysiphlebus alpinus. A: Head, frontal view. B: Antennae. C: Flagellomeres 1 and 2. D: Propodeum, dorsal view. E: Fore wing. F: Hind femur, lateral view. G: Petiole, dorsal view. H: Ovipositor sheath, lateral view.

(Fig. 12E) with marginal setae, longer than on wing surface. Stigma $2.6-2.8 \times$ as long as its width and longer than metacarpal vein (stigma length / metacarpal vein length is 1.20-1.50). Hind femur with short, appressed setae (Fig. 12F). Metasoma: Petiole (Fig. 12G) smooth, convex dorsally, with lateral depression after prominent spiracular tubercles, elongated with length $2.30-2.70 \times$ its width at the spiracles and $1.50-1.70 \times$ its width at the base; 3-5 setae positioned on each side of posterior dorsolateral margins. Ovipositor sheath (Fig. 12H) elongate, wide at base, dorsally slightly concave, with $1-2 \log 1$ setae at the tip and 2-3 long setae on the ventral and dorsal surface. Length of ovipositor sheath $2.00-2.30 \times$ its maximum width. Body length: 1.6-2.2 mm. Colouration: General body colour dark brown. Scape, pedicel and flagellum brown. Mouthparts yellow to light-brown, remaining part of head dark-brown. Eyes black. Pronotum light-brown; mesoscutum and mesopleuron darkbrown. Propodeum light-brown to brown. Legs yellow to light-brown with dark apices. Wings hyaline, venation brown. Petiole yellow to light-brown, other metasomal terga light-brown and gradually darker at the end of the metasoma. Ovipositor sheath black. - MALE: Antenna (14)15-segmented. Maxillary palpi with three palpomeres, labial palpi with two palpomeres. Body darker than female. Petiole light-brown, remaining part of metasoma dark-brown. Legs and mouthparts light-brown. Body length: 1.5-2.0 mm.

Remarks. Although it is a strictly specialized parasitoid of *A. craccae* on *Vicia cracca* L., 1753 and has been recorded in several European countries, it has never been found in the Mediterranean parts of Europe. It is a sexual species.

Examined material. AUSTRIA: 6♀ 5♂, Solden, *A. craccae* on *V. cracca* 25.vii.2015 (ŽT). **CZECH REPUBLIC**: Chlumee n. Cidl.,

A. craccae on V. cracca, 29.vi.2004 (PS); Dobrejov, A. craccae on V. cracca, 06.viii.2004 (PS); České Budějovice, A. craccae on V. cracca, 24.vi.1998 (PS). FINLAND: 2♀ 5♂, Turku, A. craccae on V. cracca, 22.vii.2016 (ŽT). MONTENEGRO: 2♀, Mt. Durmitor-Jezerska gora, 1500 m, A. craccae on V. cracca, 16.vii.2006 (ŽT). SERBIA: $1 \ 1 \ 3$, Mt. Vlasina, 1200 m, A. craccae on V. cracca, 11.viii.2006 (ŽT); 49♀ 21♂, Niš-Popovac, A. craccae on V. cracca, 4.vii.2010 (VŽ); 15♀ 6♂, Mt. Vlasina-Čemernik, 1450 m, A. craccae on V. cracca, 4.viii.2011 (ŽT); 3♀ 36♂, Mt. Vlasina, 1200 m, A. craccae on V. cracca, 21.vii.2013 (VŽ); >200♀ ♂, Mt. Vlasina, 1200 m, A. craccae on V. cracca, 6.viii.2010 (ŽT); 17♀ 133, Mt. Kopaonik, 1700 m, A. craccae on V. cracca, 27.vii.2010 (ŽT); $177 \stackrel{\circ}{_{\pm}} 122 \stackrel{\circ}{_{\odot}}$, Mt. Dukat, 1400 m, A. craccae on V. cracca, 7.viii.2011 (VŽ); 3♀ 3♂, Mt. Tara-Derventa, 800 m, A. craccae on V. cracca, 25.vi.2015 (VŽ); 2♀ 1♂, Mt. Zlatibor, 1000 m, A. craccae on V. cracca, 22.vii. 1998 (ŽT).

For additional records see Kavallieratos et al. (2004) and Starý (1965, 2006).

Lysiphlebus alpinus Starý, 1971

(Fig. 13A-H)

Diagnosis. *Lysiphlebus alpinus* is the only known member of the '*alpinus*' group, which is characterized by having a short metacarpal vein (13E), a one-segmented labial palpomere, and a generally densely setose body (Fig. 13A–H). Also, *L. alpinus* differs from the other congeneric species in possessing the combination of a short F_1 (Fig. 13C), long setae along the fore wing margin (Fig. 13E), and semi-erect setae on the hind femur (Fig. 13F). Although it was previously known to parasitize only *Semiaphis* spp., it is now confirmed that this species also parasitizes *Aphis* spp. and *Cavariella* spp., as new host records reported here.

Description. *FEMALE: Head* (Fig. 13A) transverse, with numerous sparse long setae. Eyes medium-sized, slightly oval. Tentorial index 0.50–0.57. Clypeus with 9–13

long setae. Labrum distinct, with 10-12 short setae. Malar space $0.30-0.32 \times$ longitudinal eye diameter. Mandible bidentate, with 11-14 setae on the outer surface. Maxillary palpi with three palpomeres, labial palpi with one palpomere. Antenna 12-segmented, slightly thickened at the apex (Fig. 13B). F_1 equal or subequal to F_2 (sometimes longer than F_2) (Fig. 13C). The F_1 length / F_2 length is 1.00–1.13. F_1 relatively short and 2.00–2.25 × as long as its maximum width at the middle. F_1 and F_2 bearing 1-2(3) and (2)3-4 longitudinal placodes, respectively (Fig. 13C). Flagellomeres covered uniformly with short semi-erect and erect setae, which are usually shorter than diameter of the segments. Mesosoma: Mesoscutum smooth, notaulices distinct in very short ascendent portion of anterolateral margin, effaced dorsally, with usually two rows of setae along dorsolateral parts of the mesoscutum. Scutellum elongate, bearing 8-9 long setae in the central part. Propodeum (Fig. 13D) smooth and sometimes with very short carinae and rugosities at the base. Upper and lower parts of propodeum with 3-4 and 2-3 long setae on each side. Fore wing (Fig. 13E) with long marginal setae, longer than on wing surface. Stigma triangular, $2.70-3.20 \times$ as long as its width, and equal to or about one quarter longer than metacarpal vein (stigma length / metacarpal vein length is 1.00-1.26). Hind femur with short semi-erect setae (Fig. 13F). *Metasoma*: Petiole (Fig. 13G) smooth, convex dorsally, with lateral depression after prominent spiracular tubercles, elongated with length $1.75-2.00 \times$ its width at spiracles and $1.24 - 1.40 \times$ its width at base; 11 - 12 setae positioned on posterior dorsolateral margins. Ovipositor sheath (Fig. 13H) elongate, wide at base, dorsally slightly concave, with two long setae at the tip and 4-5 long setae on the ventral and dorsal surface, respectively. Length of ovipositor sheath $2.43-2.50 \times$ its maximum width. **Body** *length*: 1.6–2.2 mm. *Colouration*: Body colour dark. Head dark with mouthparts yellow to light-brown. Antenna brown. Mesosoma dark. Legs yellow with dark apices. Petiole light-brown with yellow bands. Mesosoma light-brown to brown. Ovipositor sheath brown. - MALE: Antenna 13-14-segmented. Maxillary palpi with three palpomeres, labial palpi with two palpomeres. Body a little darker than female. Petiole light-brown, remaining part of metasoma brown with small light-brown bands. Legs light-brown. Body length: 1.6-2.0 mm. Remarks. Lysiphlebus alpinus has not been often record-

ed in Europe. According to Fauna Europea, it was known in Austria and France, but we recorded this species in Serbia as well. Although it was originally described from the Alps, it also parasitizes aphids in lowlands. *Lysiphlebus alpinus* is a sexual species.

Examined material. Holotype 1, slide-mounted, **AUSTRIA**, Zirmkogl, Ötztal Alps, Tirol, *Semiaphis* sp. on *Lonicera caerulea* L., 1753, 25.viii.1968 (PS). — Paratypes 2, slide-mounted, **AUSTRIA**, Zirmkogl, Ötztal Alps, Tirol, *Semiaphis* sp. on *Lonicera caerulea* L., 25.viii.1968 (PS). — **AUSTRIA**: 5, 4, Obergurgl, *Semiaphis* sp. on *Lonicera nigra* L., 1753, 24.vii.2015 (ŽT); 50 17, Vent, *Semiaphis* sp. on *Chaerophyllum* sp., 25.vii.2015 (ŽT); 3, Vent, *Cavariella pastinacae* (L., 1758) on *Pastinaca* sp., 25.vii.2015 (ŽT). **SERBIA**: 77♀ 48♂, Bački monoštor, *Semiaphis dauci* (Fabricius, 1775) on *Daucus carota* L., 1753, 16.vii. 1996 (OPO); 19♀ 26♂, Niš-Trošarina, *Aphis podagrariae* Schrank 1801 on *Aegopodium podagraria* L., 1753, 01.vi.2016 (VŽ).

For additional records see Starý (1971) and KAVALLIERATOS et al. (2004).

Lysiphlebus fabarum group

(L. fabarum, L. confusus, L. cardui)

Diagnosis. The whole group is characterized by having a long metacarpal vein which is longer than the stigma and reaches the fore wing margin (Fig. 14E). Setae on the fore wing edge are shorter or longer than setae on the fore wing surface, with a regular or irregular pattern along the fore wing edge (Figs. 14E, 15E). The antennae are thickened (Fig. 14B), and the hind femur has appressed or semi-erect setae (Figs. 14F, 15F).

Remarks. We here recognize several phenotypes with still unclear taxonomic status: *L. fabarum*, *L. confusus* and *L. cardui*. Although there is no genetic support for the species status of any of these phenotypes (SANDROCK et al. 2011; STARÝ et al. 2014; PETROVIĆ et al. 2015), we still keep them as separate morphospecies but within the *L. fabarum* s.str. group. All these morphospecies are affected by the existence of many asexual populations and apart from morphological differences, we also noted several ecological peculiarities (see below). We do not know how widespread asexuality within the *L. fabarum* s.str. group affected resolution of the standard molecular markers used. Short redescriptions of all morphospecies are given below.

Lysiphlebus fabarum (Marshall, 1896) (Fig. 14A–H)

Lysiphlebus melandriicola Starý, 1961 syn.n. Lysiphlebus monilicornis Thomson, 1895 Lysiphlebus ivanovi Mackauer, 1967

Diagnosis. *Lysiphlebus fabarum* differs from the other congeneric species in having a long metacarpal vein (Fig. 14E), short setae along the fore wing margin which are shorter than those on the fore wing surface (Fig. 14E), appressed setae on the hind femur and tibia (Fig. 14F), no longitudinal placodes on flagellomere 1 (Fig. 14C), and thickened 12(13)-segmented antennae (Fig. 14B). It is a polyphagous species (Yu et al. 2016).

Description. *FEMALE: Head* (Fig. 14A) transverse and wide with sparse setae. Eyes medium-sized. Tentorial index 0.50-0.60. Malar space $0.34-0.40 \times \text{longitudinal}$ eye diameter. Maxillary palpi with three palpomeres, labial palpi with one palpomere. Antenna 12(13)-segmented, thickened at the apex (Fig. 14B). F₁ usually equal to F₂ (Fig. 14C) and of variable length, $2.5-3.2 \times \text{as long}$ as its maximum width at the middle ($2.50-2.80 \times \text{as long}$ in the population originating from *A. fabae / Solanum* spp., $3.00-3.20 \times \text{as long}$ in the population originating

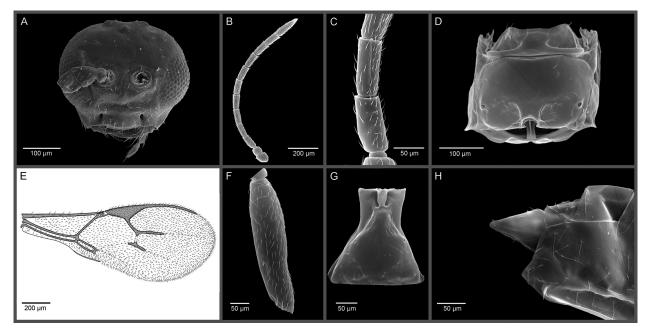


Fig. 14. Lysiphlebus fabarum. A: Head, frontal view. B: Antennae. C: Flagellomeres 1 and 2. D: Propodeum, dorsal view. E: Fore wing. F: Hind femur, lateral view. G: Petiole, dorsal view. H: Ovipositor sheath, lateral view.

from A. origani / Nepeta nuda L., 1753). F₁ without and F₂ without or with one longitudinal placode (Fig. 14C). Flagellomeres uniformly covered with short semi-erect setae. Mesosoma: Propodeum (Fig. 14D) smooth, usually with two short divergent carinae at the base. Upper and lower parts of propodeum with 2-6 and 1-3 long setae on each side. Fore wing (Fig. 14E) densely pubescent, with very short lower marginal setae, equal to those on fore wing surface. Stigma elongated, $2.8-3.4 \times as \log 100$ as its width $(2.80-3.15 \times \text{ in the population originating})$ from Aphis oenotherae Oestlund, 1887 / Oenothera bien*nis* L., 1753, $3.10-3.40 \times$ in the population originating from A. origani / N. nuda) and $0.50-0.80 \times$ as long as the metacarpal vein (Fig. 14E). Hind femur with short appressed setae (Fig. 14F). Metasoma: Petiole (Fig. 14G) of variable shape and length, length of the petiole $1.50-2.10 \times$ its width at spiracles $(1.50-2.10 \times \text{ in})$ the population emerged from A. fabae / Solanum spp., $1.90-2.10 \times$ in the population emerged from A. origani / N. nuda) and $1.00-1.35 \times$ its width at base $(1.00-1.15 \times$ in the population emerged from A. fabae / Solanum sp., $1.20-1.35 \times$ in the populations emerged from A. epilobii / Epilobium spp. and A. origami / N. nuda). Petiole with 10-13 long setae on each side of lower dorsolateral part. Ovipositor sheath slightly concave (Fig. 14H) and of variable length – elongated $(2.46-2.58 \times \text{ its maxi-}$ mum width) in populations emerged from A. craccivora / M. sativa L., 1753 or short and pointed $(2.14-2.20 \times its)$ maximum width) in populations emerged from A. fabae cirsiiacanthoidis / Cirsium spp. **Body length**: 1.5-2.0 mm. Colouration: General body colour brown to black. Scape, pedicel and base of flagellomere 1 light-brown to brown. Mouthparts yellow to light-brown, except for dark apices of mandibles. Pronotum brown to black; mesoscutum and mesopleuron dark-brown. Propodeum

apices. Wings subhyaline, venation brown. Petiole and base of tergite 2 yellow to light-brown, other metasomal terga light-brown to brown and gradually darker at end of metasoma. Ovipositor sheath dark-brown. - MALE: Antenna 13-14-segmented. Maxillary palpi with three palpomeres, labial palpi with one palpomere. Fore wing venation as in female, except for shorter metacarpal vein, which is sometimes subequal to stigma length. Stigma less elongated than in female (length/width of stigma is 3.20-3.40). Petiole stouter than in females (length/width of petiole is 1.40-1.60 at the spiracle level, 1.00-1.10at its base). Fore wing marginal setae distinctly longer than those on surface. Body colour darker than in female. Legs and mouthparts brown. Body length: 1.5-2.0 mm. Remarks. Lysiphlebus fabarum is the most common European Lysiphlebus species, distributed throughout Europe. It consists of both sexual and asexual populations across the Palaearctic. We synonymize L. 'melandriicola' with L. fabarum as a junior synonym, since they have neither consistent morphological differences nor genetic differentiation. Although L. monilicornis and L. ivanovi are sometimes considered as valid species (most authors considered them as synonyms), on the basis of their descriptions and known variability of L. fabarum, we support opinions that both these species should be considered as synonyms of L. fabarum.

light-brown to brown. Legs yellow to brown with dark

Examined material. AUSTRIA: $8 \ \ 2 \ \ 3$, Obergurgl, *A. f. cirsiia-canthoidis* on *C. arvense*, 28.vii.2015 (ŽT); $1 \ \ 9$, Obergurgl, *B. cardui* on *Matricaria* sp., 28.vii.2015 (ŽT); $1 \ \ 9$, Vent, *C. pastinacae* on *Pastinaca* sp., 25.vii.2015 (ŽT). **BELGIUM**: $5 \ \ 10 \ \ 3$, Vegi, *B. cardui* on *Matricaria maritima* L., 1753, 19.xi.2014 (AA); >100 \ \ 3, Vegi, *B. cardui* on *M. maritima*, 19.xi.2014 (AA); 60 \ 9, Vegi, *A. fabae* on *Vicia faba* L., 1753, 12.vi.2015 (AA); 69 \ 9, Noduwez, *A. fabae* on *Rumex obtusifolius* L., 1753, 14.vii.2015 (AA); 75 \ 9, \ 10 \ 100 \ 1

Brustem, A. nasturtii Kaltenbach, 1843 on R. obtusifolius, 05.x.2015 (AA); 2^Q, Vegi, Aphis pomi DeGeer, 1773 on Malus domestica L., 1753, 27.viii.2015 (AA); 12, Sint-Lambrechts-Herk, A. urticata on U. dioica L., 1753, 23.vii.2015 (AA); 2♀ 1♂, St.Truiden – PCFruit, B. helichrysi Kaltenbach, 1843 on Achillea millefolium L., 1753, 30.vi.2015 (AA); $28^{\bigcirc}_{+} 3^{\triangleleft}_{\circ}$, Vegi, Brachycaudus lychnidis (L., 1758) on Silene latifolia Poiret, 1789, 12.v.2015 (AA); 2^Q, Vegi, Dysaphis plantaginea (Passerini, 1860) on M. domestica, 12.vi.2015 (AA); 1^Q, Neerlanden, Pterocallis alni DeGeer, 1773 on Alnus glutinosa (L., 1753), 10.vii.2015 (AA); 38♀ 11♂, Gingelom, Sitobion avenae (Fabricius, 1775) on Dactylis glomerata L., 1753, 26.vi.2015 (AA). BULGARIA: 1♀1♂, Blagoevgrad, Aphis intybi Koch, 1855 on Cichorium intybus L., 1753, 27.vi.2012 (MY). CROATIA: 12, Korana village, Aphis affinis de Guercio, 1911 on Mentha longifo*lia* (L., 1756), 22.vi.2015 (ŽT); 69 518, Korana village, A. fabae on *Ch. album*, 22.vi.2015 (ŽT); 28♀ 8♂, Plitvice-Čujića krčevina, A. fabae on Rumex sp., 22.vi.2015 (ŽT); 46♀ 17♂, Plitvice-Homoljačko polje, A. fabae on Peucedanum sp., 22.vi.2015 (ŽT); >200 \bigcirc Koreničko vrelo. A. fabae cirsiiacanthoidis on C. arvense, 22.vi.2015 (ŽT). CZECH REPUBLIC: 45♀ 5♂, Hrabětice, A. fabae on Ch. album, vi.2011 (PS); 12♀ 1♂, Česke Budějovice, B. cardui on Carduus sp., 6.vii.1998 (PS). GREECE: 14°_{\pm} 11 $^{\circ}_{\odot}$, Kyparissia, Aphis cytisorum Hartig, 1841 on Spartium junceum L., 1753, 2.v.2010 (NK); 2^Q, Kyparissia, Aphis parietariae Theobald, 1922 on Parietaria difussa Mert. & W.D.J.Koch, 1823, 1.v.2010 (NK); 91 $\stackrel{\circ}{\downarrow}$, Kyparissia, A. fabae on Ch. album, 02.v.2010 (ŽT); 19[♀], Kalamata, A. fabae on Galium aparine L., 1753, 2.v.2010 (ZT); 18 $\stackrel{\circ}{\downarrow}$ 9 $\stackrel{\circ}{\supset}$, Kalamata, A. fabae on Pittosporum tobira (Thunb.) W.T. Aiton, 1811, 2.v.2010 (AP); 57^Q, Kyparissia, A. fabae cirsiiacanthoidis on C. arvense, 01.v.2010 (AP); 12, Kyparissia, A. gossypii on Schefflera arboricola (Hayata) Kanehira, 1936, 1.v.2010 (ŽT). MALTA: 2[♀], Sigeiewi, Aphis euphorbiae Kaltebach, 1843, on Euphorbia sp., 08.v.2012 (DM). MONTENEGRO: 53[♀], Bar, Brachyunguis tamaricis (Lichtenstein, 1885) on Tamarix sp., 24.v.2011 (AP); 8^o, Sutomore, A. fabae on Opuntia sp., 25.v.2011 (VŽ); 24 \bigcirc , Virpazar, A. fabae on Ch. album, 26.v.2011 (VŽ); 25 \bigcirc , Petrovac, A. fabae cirsiiacanthoidis on C. arvense, 24.v.2011 (AP); >500^Q, Kruče, A. nasturtii on Malva silvestris L., 1753, 23.v.2011 (AP); 21 \bigcirc , Bar, A. fabae on G. aparine, 24.v.2011 (VŽ); 8 \bigcirc , Kotor, Aphis hederae Kaltenbach, 1843, on Hedera helix L., 1753, 25.v.2011 (VŽ); 74 $^{\circ}_{\pm}$, Sutomore, A. craccivora on Tecoma alata DC. 1838, 25.v.2011 (AP); 62^Q, Kruče, Aphis balloticola Szelegiewicz, 1968 on Ballota nigra L., 1753, 23.v.2011 (VŽ); 184♀ 105♂, Sutomore, A. cytisorum on S. junceum, 25.v.2011 (AP); 69[♀], Tivat, Aphis crepidis (Börner, 1940) on Crepis sp., 25.v.2011 (VŽ); 63[⊖]₊, Petrovac, A. davletsinae Hille Ris Lambers, 1966 on Malva neglecta Wallroth, 1824, 24.v.2011 (VŽ); 95 24 d, Mt. Durmitor-Sušica, 1200 m, A. praeterita Walker, 1849 on Epilobium palus*tre* L., 1753, 27.vii.2012 (ŽT); 92♀ 76♂, Mt. Durmitor-Ledena pećina, 1700 m, A. craccivora on Onobrychis alba (Waldst & Kit) Desv. 1814, 26.vii.2012 (ŽT); 2♀, Mt. Durmitor-Sušica, 1000 m, Anuraphis subterranea (Walker, 1852) on Heracleum orsinii Gussone, 1826, 27.vii.2012 (ŽT); 13[♀], Šasko jezero, A. craccivora on *Amorpha fruticosa* L., 1753, 23.v.2011 (VŽ). **SERBIA**: 1♀ 114∂, Mt. Kopaonik-Brzeće, A. fabae cirsiiacanthoidis on C. arvense, 29.vii.2010 (VŽ); 86[♀], Sićevo Gorge, Aphis ruborum (Börner and Schilder, 1932) on Rubus sp., 29.v.2010, (VŽ); 98 103, Niš-Popovac, A. urticata on U. dioica, 22.v.2010 (VŽ); 17 203, Lebane-Konjino, A. fabae on Amaranthus retroflexus L., 1753, 6.vii.2010 (SS); 50♀, Brestovik, Aphis sp. on Rubus sp., 27.v.2011 (ŽT); 8^Q, Radmilovac, A. affinis on Mentha aquatica L., 1753, 9.vi.2011 (OPO); 22♀ 14♂, Mt. Vlasina, 1200 m, A. fabae cirsiiacanthoidis on C. arvense, 6.viii.2010 (VŽ); 500[♀], Bešujaja, Aphis sp. on Rubus sp., 14.vi.2011 (VŽ); 29♀ 3♂, Preševo, A. craccivora on Salvia pratensis L., 1753, 5.vi.2011 (VŽ); 13♀, Slankamen, A. fabae on C. acanthoides, 24.vi.2011 (AP); 40°_{\pm} 5Å, Bešnjaja, A. craccivora on M. sativa, 14.vi.2011 (VŽ); 63[♀], Kragujevac, A. in*tybi* on *C. intybus*, 5.vii.2011 (AMB); 118^Q, Mt. Dukat, 1000 m, Aphis frangulae Kaltenbach, 1843 on N. nuda, 6.viii.2011 (VŽ); 92[⊖], Mt. Vlasina, 1200 m, A. verbasci on Verbascum nigrum L.,

1753, 5.viii.2011 (VŽ); 127♀ 51♂, Mt. Vlasina, 1200 m, Aphis sp. on Pastinaca sativa L., 1753, 5.viii.2011 (VŽ); 8♀ 10♂, Progar, A. craccivora on M. sativa, 19.vii.2012 (OPO); 378, Mt. Dukat, 1000 m, Aphis salviae Walker, 1852 on Salvia verticillata L., 1753, 29.vi.2012 (VŽ); 10^o₊, Mt. Tara-Derventa, 1000 m, A. fabae on *Rumex* sp., 3.vii.2012 (ŽT); 5♀ 8♂, Zemun-Galenika, Aphis polygonata (Nevsky, 1929) on Polygonum aviculare L., 1753, 05.vi.2011 (MM); >50♀ ♂, Novi Beograd, A. craccivora on M. sativa, 13.vii.2010 (ŽT); 3 2 2, Grocka, A. craccivora on M. sativa, 20.vii.2010 (ŽT); 20 \bigcirc 16 \bigcirc , Zemun-Banatska, A. craccivora on M. sativa, 18.vi.2010 (AP). SLOVENIA: 16^Q, Bovec, A. urticata on U. dioica, 16.vi.2009 (ŽT); 14°_{\downarrow} 11Å, Koper, A. polygonata on Polygonum arenastrum Boreau, 1857, 17.vi.2009 (KK); 20^Q, Zalog, A. fabae on Impatiens glandulifera Royle, 1834, 26.ix.2012 (KK); 2^{\bigcirc}_+ , Slanci, A. fabae fabae on Ch. album, 29.v.2007 (ŽT); $9^{\bigcirc}_+ 4^{\land}_-$, Koper, A. intybi on C. intybus, 17.vi.2009 (KK); 5^Q, Bohinj, Aphis vitalbae Ferrari, 1872 on Clematis vitalba L., 1753, 14.vii.2009 (KK); 5^O₊, Bohinjska bistrica, Aphis ulmariae Schrank, 1801 on Filipendula ulmaria (L.) Maximowicz, 1879, 20.vii.2014 (KK); 256^Q, Zalog, A. hederae on H. helix, 29.x.2012 (KK); 3^Q, Bohinj, A. epipactis Theobald, 1927 on Epipactis sp., 16.vii.2014 (KK). SWITZERLAND: 10°_{\pm} 2 $^{\circ}_{\odot}$, Rudolfstetten-Friedlisberg, B. cardui on Cirsium vulgare, 16.vii.2009 (CV); 6♀ 1♂, Sophyères, B. cardui on Cirsium vulgare, 20.vii.2009 (CV); 11 4 4, Dübendorf, B. cardui on Cirsium vulgare, 17.vii.2009 (CV); 4^{\bigcirc}_{\pm} , Dietikon, B. cardui on Cirsium vulgare, 16.vii.2009 (CV); 4♀ 1♂, Liesberg, B. cardui on Cirsium vulgare, 20.vii.2009 (CV); 3♀ 5♂, Laufen, B. cardui on Cirsium vulgare, 20.vii.2009 (CV); 30♀ 34♂, Niederweningen, A. salviae on Salvia pratensis, 14.v.2011 (CV); 10^o/₊, Steinmaur, A. fabae on Sonchus oleraceus L., 1753, 05.vii.2010 (CV); TURKEY: 43[♀], Canakkale-Kepez, A. fabae on Phaseolus vulgaris L., 1753, 05.vi.2015 (SK); 10^Q, Canakkale-Kepez, A. umbrella (Börner, 1950) on Malva sp., 25.iv.2014 (SK); 10°_{+} , Canakkale-center, A. gossypii on Abelmoschus esculentus (L., 1753), 06.vi.2015 (SK); 4^Q, Canakkale-University campus, A. fabae on S. junceum, 23.v.2014 (SK). CHINA: $12 \stackrel{\circ}{\downarrow} 2 \stackrel{\circ}{\circlearrowleft}$, Xiuan, Liaoning Province, A. glycines on Glycine max (L.) Merrill, 1917, 29.vii.2006 (KH).

Examined type specimens of *L. melandriicola.* Holotype 1 \bigcirc , **CZECH REPUBLIC**, Raná, *Brachycaudus lychnidis* on *Silene latifolia* Poir., 1789, 18.vi.1957 (PS). — Paratypes 3 \bigcirc , Raná, *Brachycaudus lychnidis* on *S. latifolia*, 18.vi.1957 (PS).

For additional records see Starý (1961, 1965, 2006), Starý et al. (1971, 1975), KAVALLIERATOS et al. (2001, 2004), EL-MALI et al. (2004), Starý & HAVELKA (2008), and KAVALLIERATOS et al. (2016).

Lysiphlebus confusus Tremblay & Eady, 1978 (Fig. 15A–H)

Lysiphlebus hirtus Starý, 1985 syn.n.

Diagnosis. *Lysiphlebus confusus* possesses a long metacarpal vein that reaches the tip of the fore wing (Fig. 15E), setae along the fore wing margin that are longer than setae on the fore wing surface (Fig. 15E) (and have different distribution patterns), and a hind femur with semi-erect setae (Fig. 15F), although some Mediterranean biotypes (e.g., *Aphis passeriniana* (Del Guercio, 1900) / *Salvia officinalis* L., 1753, *A. ruborum / Rubus* spp., *Cavariella* sp. / *Tordylium apulum* L., 1753) have appressed setae on the hind femur.

Description. *FEMALE: Head* (Fig. 15A) transverse and wide, with sparse setae. Eyes medium-sized. Tentorial index 0.50-0.60. Malar space $0.30-0.40 \times$ longitudinal eye diameter. Maxillary palpi with three palpomeres, labial palpi with one palpomere. Antenna 12(13)-seg-

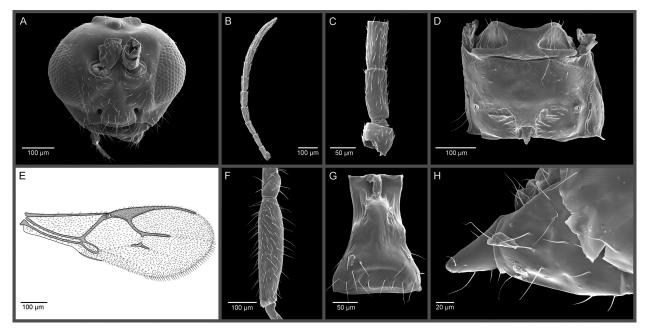


Fig. 15. Lysiphlebus confusus. A: Head, frontal view. B: Antennae. C: Flagellomeres 1 and 2. D: Propodeum, dorsal view. E: Fore wing. F: Hind femur, lateral view. G: Petiole, dorsal view. H: Ovipositor sheath, lateral view.

mented, thickened at the apex (Fig. 15B). F_1 generally equal to F₂ (Fig. 15C) and of unusually variable length in the range of $2.3-3.3 \times$ as long as its maximum width at the middle (e.g., specimens emerged from Cavariella spp. / Tordylium sp. have a short $F_1 2.30-2.70 \times as long$ as its maximum width, while those emerged from A. fa*rinosa / Salix* spp. have F_1 that is $3.00-3.30 \times as$ long as its maximum width). F_1 without and F_2 without or with one longitudinal placode (Fig. 15C). Flagellomeres uniformly covered with short semi-erect setae. Mesosoma: Propodeum (Fig. 15D) smooth, usually with two short divergent carinae at the base. Upper and lower parts of propodeum with 2-5 and 1-2 long setae on each side. Fore wing (Fig. 15E) densely pubescent, with marginal setae which are longer than those on the fore wing surface and have a variable distributional pattern. Stigma elongated, $3.00-3.60 \times$ as long as its width (members of the population originating from Aphis passeriniana / Salvia officinalis have a more elongated stigma $3.40-3.60 \times$ as long as its width). Metacarpal vein about one third or one quarter longer than stigma (stigma lenght / metacarpal vein lenght is 0.65-0.75) (Fig. 15E). Hind femur with semi-erect setae in the continental population (Fig. 15F), appressed setae in the Mediterranean population. Metasoma: Petiole (Fig. 15G) with length about $1.60-1.90 \times$ its width at spiracles and $1.00-1.40 \times$ its width at the base $(1.00-1.10 \times \text{in populations originating from Aphis pas-}$ seriniana / Salvia officinalis and A. fabae / Solanum sp.; and $1.20-1.40 \times$ in populations emerged from A. schneideri (Börner, 1940) / Ribes spp. and Cavariella spp. / Tordylium sp.). Ovipositor sheath almost straight dorsally (Fig. 15H), usually pointed $(2.10-2.20 \times \text{as long as its})$ maximum width). Body length: 1.5-2.0 mm. Colouration: General body colour brown. Antennae brown with narrow yellow ring at the base of F₁. Mouthparts yellow

to light-brown. Pronotum brown; mesoscutum and mesopleuron dark-brown. Propodeum light-brown to brown. Legs light-brown. Wings subhyaline, venation brown. Petiole yellow, other metasomal terga light-brown, gradually darker at end of metasoma. Ovipositor sheath dark-brown. - MALE: Antenna 13-14-segmented. Maxillary palpi with three palpomeres, labial palpi with one palpomere. Fore wing venation as in female, except for a slightly shorter metacarpal vein, which is sometimes subequal to stigma length. Stigma more triangular than in female (length/width of stigma is 2.90-3.30). Petiole stouter than in females (length/width of petiole is 1.40-1.70 at spiracle level, and 1.00-1.20 at its base). Lower marginal fore wing setae distinctly longer than those on surface. Body darker than female, generally brown. Petiole and first half of metasomal terga lightbrown, remaining part of metasoma brown. Legs and mouthparts light-brown. Body length: 1.5–2.0 mm.

Remarks. This morphospecies is distributed in many European countries. It mainly consists of asexual populations, although our field records confirmed the existence of sexual biotypes in Greece (e.g., A. cytisorum on S. junceum and A. craccivora on Medicago arborea L., 1753) and Finland (A. fabae cirsiiacanthoidis on C. arvense). According to our evidence, L. confusus is morphologically the most variable Lysiphlebus species, with an unusually wide range of the ratio between length and width of F_1 ($F_1L/W = 2.30 - 3.30$), variable shape of the petiole (length/width of petiole are 1.60-1.90 and 1.00-1.40 at spiracle level and base, respectively), and variable shape of the stigma (length/width of stigma = 3.00-3.60). Based on our evidence, it is the most variable Lysiphlebus morphospecies of the species we examined. After careful examination of the type specimens of L. hirtus reared from the Brachycaudus populi / Silene

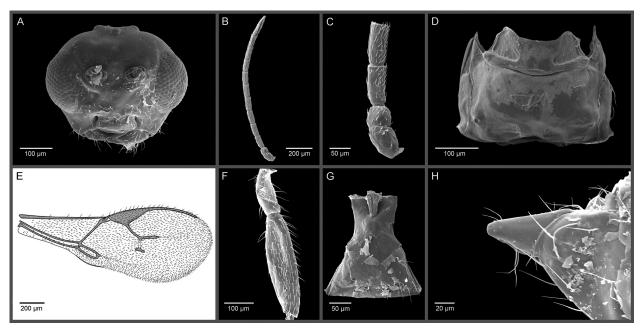


Fig. 16. *Lysiphlebus cardui*. A: Head, frontal view. B: Antennae. C: Flagellomeres 1 and 2. D: Propodeum, dorsal view. E: Fore wing. F: Hind femur, lateral view. G: Petiole, dorsal view. H: Ovipositor sheath, lateral view.

sp. association, we concluded that they are morphologically closely related to phenotypes of *L. confusus* (long setae along wing margins, obtusely pointed ovipositor sheaths, shape of the petiole, F_1 without rhinaria), which is part of the *L. fabarum* s. str. group, so we synonymized *L. hirtus* with *L. confusus*.

Examined material. BELGIUM: 6^o, Gingelom, Hyalopterus pruni (Geoffroy, 1762) on Prunus domestica L., 1753, 8.vi.2015 (AA); 11°_{\pm} 5 $^{\circ}_{\odot}$, St. Truiden, A. fabae on Ribes rubrum (L., 1753), 18.v.2015 (AA); 35♀ 15♂, Wellen, A. schneideri (Börner, 1940) on Ribes nigrum L., 1753, 15.vi.2015 (AA); 2♀ 2♂, Mettekoren, *Cryptomyzus ribis* L., 1758, on *R. rubrum*, 12.vi.2015 (AA); 85^o₊, Hannut, Aphis sp. on Salix udensis Trautvetter & C.A.Meyer, 1856 7.vi.2015 (ND); 40°_{\pm} 28 $^{\circ}_{\odot}$, St. Truiden, A. schneideri on R. nigrum, 02.vii.2015 (AA). **CROATIA**: $104 \stackrel{\circ}{\downarrow} 4 \stackrel{\circ}{\circ}$, Plitvice-Čujića krčevina, A. farinosa on Salix caprea L., 1753, 22.vi.2015 (ŽT); 2, Plitvički Ljeskovac, A. fabae on Anthriscus sylvestris (L.) Hoffmann, 1814, 22.vi.2015 (ŽT); 28^Q, Plitvice-Milanovac, A. farinosa on Salix am*plexicaulis* Bory, 1832, 20.vi.2015 (ST). FINLAND: 237♀ 18∂, Turku, Aphis sp. on Anthriscus sp., 22.vii.2016 (ŽT); 102♀ 91♂, Turku, A. fabae cirsiiacanthoidis on C. arvense, 22.vii.2016 (ŽT). GREECE: 56^Q, Kalamata, A. fabae on G. aparine, 2.v.2010 (ŻT); 238[♀] 108[♂], Kyparissia-Messinia, A. cytisorum on S. junceum, 21.v.1997 (NK); 2^Q, Kalamata, A. fabae on G. aparine, 2.v.2010 (NK); 17[♀], Kyparissia, A. fabae on Papaver sp., 2.v.2010 (AP); 12[♀] 7[♂], Kyparissia, A. cytisorum on S. junceum, 7.v.2011 (NK); 6^Q, Kyparissia, A. ruborum on Rubus sp., 2.v.2010 (NK). LITHU-ANIA: 3^Q, Vilnius-Kairenai, Bot. Garden, A. farinosa on S. fragilis, 03.vii.2011 (JH). MONTENEGRO: 4^o₊, Tivat, Aphis spiraecola Patch, 1914 on *P. tobira*, 19.v.2010 (VŽ); 6^Q₊, Budva, *A. ruborum* on Rubus sp., 17.v.2010 (VŽ); 3^Q, Sutomore, A. fabae on Opuntia sp., 25.v.2011 (VŽ); 10[♀], Kotor, A. hederae on H. helix, 22.v.2011 (AP); 94^Q 1∂, Bar, Aphis davletshinae Hille Ris Lambers, 1966 on M. silvestris, 24.v.2011 (VŽ); 61♀, Mt.Durmitor-Sušica, 1200 m, A. farinosa on S. caprea, 27.vii.2012 (ŽT); 41°_{+} , Budva, A. ruborum on Rubus sp., 17.v.2010 (SS); 5^Q, Mt.Prokletije-Hridsko jezero, 2000 m, A. farinosa on S. caprea, 23.vii.2012 (ŽT). SER-**BIA**: 18[°], Sićevo Gorge, A. ruborum on Rubus caesius L., 1753, 4.vi.2011 (VŽ); 1^Q, Vlasina lake, 1200 m, Aphis crepidis on Crepis foetida L., 1753, 21.vii.2013 (VŽ); 2^Q, Vrbovski, A. fabae fabae on

G. aparine, 20.v.2008 (ŽT); $5 \ 13$, Kotraža, *A. farinosa* on *Salix* sp., 19.vi.2011 (IJ); $26 \ Q$, Mt.Tara-Derventa, *A. farinosa* on *Salix alba* L., 1753, 3.vii.2012 (VŽ). **SLOVENIA**: $7 \ Q$, Šempeter, *Aphis* sp. on *Plantago* sp., 18.vi.2009 (KK); $19 \ Q \ 23$, Bohinjska bistrica, *Aphis* sp. on *Plantago* major L., 14.viii.2012 (KK); $43 \ Q$, Bohinjska bistrica, *A. farinosa* on *S. caprea*, 20.vii.2014 (KK); $29 \ Q$, Bohinjska bistrica, *A. ruborum* on *Rubus* sp., 20.vii.2014 (KK); $90 \ Q$, Bohinjska bistrica, *A. ulmariae* on *F. ulmaria*, 20.vii.2014 (KK); $5 \ Q$, Bohinjska bistrica, *A. fabae* on *A. podagraria*, 16.vii.2014 (KK); $5 \ Q$, Bohinjska bistrica, *A. fabae* on *Cirsium erisithales* (Jacques) Scopoli, 1769, 20.vii.2014 (KK); $124 \ Q$, Bohinj, *A. fabae* on *Anthericum ramosum* L., 1753, 14.vii.2009 (KK). **SWEDEN**: $10 \ Q$, Skaltsa, *A. fabae* on *Antherica*, 2.vii.2014 (ŽT); $5 \ 13$, Upsala, *A. fabae* on *Salix* sp., 2.vii.2014 (ŽT).

Examined type specimens of *L. hirtus.* Holotype 1, SWIT-ZERLAND, Devoggio, *Brachycaudus populi* (Del Guercio, 1911) on *Silene* sp., 21.viii.1962 (GR). — Paratypes 4 2 1, SWITZER-LAND, Devoggio, *Brachycaudus populi* (Del Guercio, 1911) on *Silene* sp., 21.viii.1962 (GR).

For additional records see T_{REMBLAY} & EADY (1978), STARÝ (1985, 2006), KAVALLIERATOS et al. (2001, 2004), EL-MALI et al. (2004), and STARÝ & HAVELKA (2008).

Lysiphlebus cardui (Marshall, 1896)

(Fig. 16A-H)

Diagnosis. *Lysiphlebus cardui* is characterized by the combination of a long metacarpal vein that reaches the fore wing margin (Fig. 16E), semi-erect setae on the hind femur (Fig. 16F), and setae on the fore wing margin equal to those on the fore wing surface (Fig. 16E).

Description. *FEMALE: Head* (Fig. 16A) wide, with sparse setae. Eyes medium-sized. Tentorial index 0.50-0.60. Malar space $0.30-0.40 \times$ longitudinal eye diameter. Maxillary palpi with three palpomeres, labial palpi with one palpomere. Antenna 12(13)-segmented, thickened at the apex (Fig. 16B). F₁ equal to F₂ (Fig. 16C), $2.5-3.0 \times$

as long as its maximum width at the middle. F_1 without and F_2 without or with one longitudinal placode (Fig. 16C). Flagellomeres covered uniformly with short appressed and semi-erect setae. Mesosoma: Propodeum (Fig. 16D) smooth, sometimes with two short divergent carinae at the base. Upper and lower parts of propodeum with 2-5 and 1-2 long setae on each side. Fore wing (Fig. 16E) densely pubescent, with marginal setae which are shorter than those on the fore wing surface. Stigma elongated, $3.20-3.60 \times$ as long as its width. Metacarpal vein one third or one quarter longer than stigma (stigma lenght / metacarpal vein lenght is 0.65-0.75) (Fig. 16E). Hind femur with semi-erect setae (Fig. 16F). Metasoma: Petiole (Fig. 16G) with length $1.60-1.80 \times$ its width at spiracles and $1.00-1.20 \times$ its width at the base. Ovipositor sheath almost straight dorsally (Fig. 16H), usually pointed (length $2.10-2.20 \times$ its maximum width). **Body** length: 1.5-2.0 mm. Colouration: General body colour brown. Scape and pedicel light-brown to brown, F_1 with narrow yellow ring at the base. Mouthparts yellow to light-brown. Thorax generally brown to dark-brown with some small light-brown parts usually present in the Mediterranean populations. Pronotum brown; mesoscutum and mesopleuron dark-brown. Propodeum lightbrown. Legs yellow to light-brown. Wings subhyaline, venation yellowish brown. Petiole yellow, other metasomal terga light-brown, gradually darker at the end of the metasoma. Ovipositor sheath dark-brown. - MALE: Antenna 13-14-segmented. Maxillary palpi with three palpomeres, labial palpi with one palpomere. Fore wing venation as in female, except for a shorter metacarpal vein, which is sometimes subequal to stigma length. Stigma less elongated than in female (length/width of stigma is 3.20-3.40). Petiole stouter than in females (length/width of petiole is 1.40-1.70 at spiracle level, and 1.00-1.15 at its base). Lower fore wing marginal setae distinctly longer than those on the surface. Body darker than female, generally brown to dark-brown. Petiole and first half of metasomal terga light-brown, remaining part of metasoma brown. Legs and mouthparts lightbrown. Body length: 1.5–2.0 mm.

Remarks. *Lysiphlebus cardui* was considered to occur only in asexual populations (BELSHAW et al. 1999), but we confirm for the first time that it occurs in both asexual and sexual populations. It shares a similar host range pattern with *L. fabarum*, parasitizing various *Aphis* spp. but preferably the *A. fabae* complex (e.g.. *A. fabae cirsiia-canthoidis*) (KAVALLIERATOS et al. 2004; STARÝ 2006).

Examined material. AUSTRIA: 367, Zwieselstain, *A. fabae cirsiiacanthoidis* on *C. arvense*, 25.vii.2015 (ŽT); >200, Gries, *Aphis* sp. on *Peucedanum* sp., 27.vii.2015 (ŽT). **BELGIUM**: 20 2δ , Jodoigne, *A. fabae* on *G. aparine*, 18.vi.2015 (AA); 45, Gingelom, *A. fabae cirsiiacanthoidis* on *C. arvense*, 29.vi.2015 (AA). **CZECH REPUBLIC**: 5φ , Stráž n/N., *A. fabae cirsiiacanthoidis* on *Cirsium* sp., 12.vii.2005 (PS). **MONTENEGRO**: 35φ , Bar, *A. fabae* on *Yucca* sp., 24.v.2011 (VŽ); >1000 φ , Kruče, *A. fabae* on *G. aparine*, 24.v.2011 (VŽ); 1 φ , Bar, *A. fabae* on *G. aparine*, 24.v.2011 (VŽ); 1 φ , Nirpazar, *A. fabae* on *C. album*, 26.v.2011 (VŽ); 8φ 3δ , Mt. Vizitor, 1400 m, *A. fabae* on *Rumex* sp., 22.vii.2006 (OPO). **RUSSIA**: 3ϕ , Moscow-Leninskye gory, A. fabae on Apiaceae, 8.viii.1968 (PS). SLOVENIA: 32, Slap-Vipava, Aphis sp. on Tussilago farfara L., 1753, 4.vi.2009 (ŽT); 2[♀], Zelenci, B. cardui on Cirsium oleraceum (L.) Scopoli, 1769, 18.vii.2014 (KK); 3^Q, Koper, A. davletshinae on Althaea cannabina L., 1753, 17.vi.2009 (KK). SERBIA: 92 13, Slankamen, A. fabae cirsiiacanthoidis on C. arvense, 24.vi.2011 (MM); 3^Q, Surčin, Brachycaudus sp. on Silene vulgaris (Moench) Garcke, 1863, 15.vi.2011 (ŽT); 6♀, Mt.Tara-Derventa, A. fabae on Rumex sp., 03.vii.2012 (VŽ); 82, Mt.Tara-Derventa, A. fabae on Digitalis ambigua Murray, 1770, 3.vii.2012 (VŽ); 75♀ 34♂, Sićevo Gorge, A. fabae on Arctium lappa L., 1753, 28.v.2013 (VZ); 18 \bigcirc 3 \bigcirc , Sićevo Gorge, A. fabae on Ch. album, 17.vii.2013 (VZ); >100 \bigcirc \circlearrowleft , Niš-Popovac, A. urticata on U. dioica, 25.v.2010 (VŽ); 21♀ 7∂, Niš-Pantelej, A. fabae on Ch. album, 4.vi.2011 (VZ); 15 $\stackrel{\frown}{\downarrow}$ 11 $\stackrel{\frown}{\circ}$, Niš-Pantelej, A. fabae on Euonymus europaeus L., 1753, 4.vi.2011 (VŽ); 1^Q, Vladičin Han, A. fabae on Ch. album, 12.vi.2011 (VŽ); 52♀ 11♂, Surčin-Galovica, Brachycaudus klugkisti (Börner, 1942) on M. album, 22.v.2011 (ŽT); SWEDEN: 31^Q, Uppsala-Botanical Garden, A. fabae cirsiiacanthoidis on Cirsium sp., 1.vii.2014 (ŽT).

For additional records see KAVALLIERATOS et al. (2001, 2004) and STARY (2006).

Lysiphlebus dissolutus (Nees, 1811) (Fig. 17A–H)

Diagnosis. The type species for the genus *Lysiphlebus* (see Discussion), it is easy to diagnose and differentiate from the other congeneric species by virtue of having 15-16-segmented antennae and subsquare or square flagellomeres (Fig. 17B).

Description. FEMALE: Head (Fig. 17A) transverse, as wide as thorax, sparsely setaceous. Eyes medium-sized, rounded. Clypeus smooth, with sparse setae and deep tentorial pits. Maxillary palpi with three palpomeres, labial palpi with one palpomere. Antenna 15-16-segmented, filiform, with square or subsquare flagellomeres (Fig. 17B). F_1 slightly longer than F_2 (Fig. 17C) (F_1 length / F_2 length is 1.10–1.20). F_1 relatively short, $1.70-1.80 \times as$ long as its maximum width at the middle. F_1 and F_2 with 1-2 and 2-3 longitudinal placodes, respectively (Fig. 17C). Flagellomeres densely setaceous, covered uniformly with appressed and short semi-erect setae. Mesosoma: Mesoscutum smooth with poorly visible notaulices. Propodeum (Fig. 17D) smooth, with two divergent carinae at the base. Upper and lower parts of propodeum with 5-8 and 1-3 long setae on each side. Fore wing (Fig. 17E) with marginal setae longer than setae on wing surface. Stigma $4.00-5.00 \times$ as long as its width and twice as long the metacarpal vein. Hind femur with short semierect setae (Fig. 17F). Metasoma: Petiole (Fig. 17G) smooth, convex dorsally, with lateral depression after prominent spiracular tubercles and length $1.30-1.40 \times$ its width at the spiracles, $1.10-1.20 \times$ its width at the base. Ovipositor sheath short (Fig. 17H). Body length: 1.7–1.9 mm. *Colouration*: General body colour brown. Antennae, legs, and metasoma light-brown. Base of antennae, mouthparts, and F_1 and F_2 light-brown to yellow. Ovipositor sheath black. — MALE: Antenna 17-segmented. Petiole with very prominent central and spiracular tubercles.

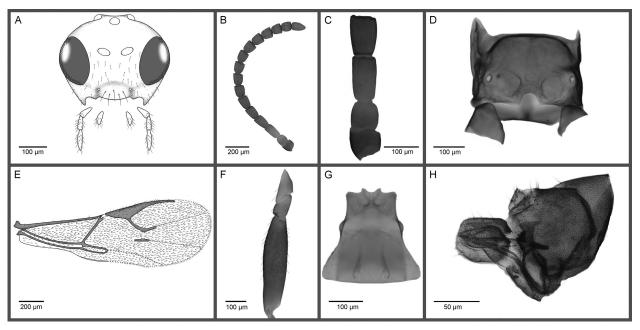


Fig. 17. Lysiphlebus dissolutus. A: Head, frontal view. B: Antennae. C: Flagellomeres 1 and 2. D: Propodeum, dorsal view. E: Fore wing. F: Hind femur, lateral view. G: Petiole, dorsal view. H: Ovipositor sheath, lateral view.

Remarks. *Lysiphlebus dissolutus* is a root aphid parasitoid with poorly known biology. It is known from several European countries but has not been frequently sampled.

Examined material. Neotype, 1♀, **GERMANY**, dry-mounted, Aachen, without host records. — **GERMANY**: 3♀, Erlangen, North Bayern, *Anoecia corni* (Fabricius, 1775) on *Poa nemoralis* L., 1753, 29.ix.1954 (H. Zwolfer).

Lysiphlebus orientalis Starý & Rakhshani, 2010 (Fig. 18A–H)

Diagnosis and description. See STARÝ et al. (2010). **Remarks.** Introduced from China to Europe, it is there known only in Serbia until now. We suppose a broader distribution of this species in Europe given that it was first recorded near the Hungarian border in 1995. More intesive sampling efforts should confirm this opinion. *Lysiphlebus orientalis* is an asexual species.

Examined material. Holotype 1^o, CHINA, Harbin, Aphis glycines on Glycine max, viii 2006, (K. Hoelmer) (Collection of United States National Museum of Natural History). - Paratypes 4[°] CHINA, Harbin, Aphis glycines on Glycine max, viii 2006, deposited in the collection of the Institute of Zoology, Faculty of Biology, University of Belgrade (Serbia). — SERBIA: 13♀, Belgrade-Konjarnik, A. schneideri on Ribes sp., 6.vi.2011 (AP); 2^Q, Zemun, A. hederae on H. helix, 14.vi.2011 (ŽT); 2♀, Zemun-Galenika, A. gossypii on Althaea officinalis L., 1753, 7.vi.2011 (MM); 12[♀], Zemun-Galenika, Aphis sp. on Geranium sp., 7.vi.2011 (MM); 28^o₊, Zemun-Galenika, A. fabae on Ch. album, 7.vi.2011 (MM); 4^Q, Belgrade-Botanical Garden, A. fabae on Papaver sp., 6.xii.2013 (AP); 5^Q, Belgrade, A. craccivora on Robinia pseudoacacia L., 1753, 28.vi.2014 (AP); 4^o₊, Radmilovac, A. fabae on G. aparine, 20.v.2015, (OPO); 1^o, Belgrade, A. gossypii on Hibiscus sp., 19.v.2015 (AP); 2[⊖]₊, Niš-Pantelej, A. hederae on H. he*lix*, 22.iv.2014 (VŽ); 3♀ 1♂, Niš-Trošarina, *Aphis* sp. on *Fumaria*

officinalis L., 1753, 22.v.2013 (VŽ); 15 \bigcirc , Belgrade, A. nasturtii on Malva sp., 29.vi.2015 (KK); 8 \bigcirc , Pančevački rit, A. craccivora on M. sativa, 27.vi.2015 (AP); 30 \bigcirc , Belgrade, A. nasturtii on M. silvestris, 6.vii.2015 (AP); 11 \bigcirc , Palić, Aphis sp. on Picris hieracioides L., 1753, 6.ix.1995 (ŽT).

For additional records see PETROVIĆ et al. (2013) and KAVALLIERATOS et al. (2016).

Lysiphlebus testaceipes (Cresson, 1880) (Fig. 19A–H)

Diagnosis. *Lysiphlebus testaceipes* is characterized by having a short metacarpal vein (Fig. 19E) and two-segmented labial palpomeres. It is morphologically similar to *L. fritzmuelleri* (see Diagnosis of *L. fritzmuelleri*).

Description. FEMALE: Head (Fig. 19A) transverse, bearing dense setae. Eyes medium-sized, rounded. Tentorial index 0.60–0.68. Clypeus with 8–12 long setae. Labrum distinct, with 4–5 short setae. Malar space $0.25-0.30 \times$ longitudinal eye diameter. Mandible bidentate, with 15-16 setae on the outer surface. Maxillary palpi with three palpomeres, labial palpi with two palpomeres. Antenna 13-14-segmented, slightly thickened at the apex (Fig. 19B). F_1 longer than F_2 (F1 length / F2 length is about 1.20) (Fig. 19C). F_1 relatively short, $2.3-2.6 \times as$ long as its maximum width at the middle. F_1 and F_2 with 3-5(6) and 4-5(6) longitudinal placodes, respectively (Fig. 19C). Flagellomeres covered uniformly with short appressed and semi-erect setae. Mesosoma: Mesoscutum smooth, notaulices distinct in very short ascendent portion of anterolateral margin, effaced dorsally, with usually two rows of setae along the dorsolateral part of the mesoscutum. Scutellum elongate, bearing 7-8 long setae in the central part. Propodeum (Fig. 19D) smooth, with two short divergent carinae at the base. Upper and lower parts

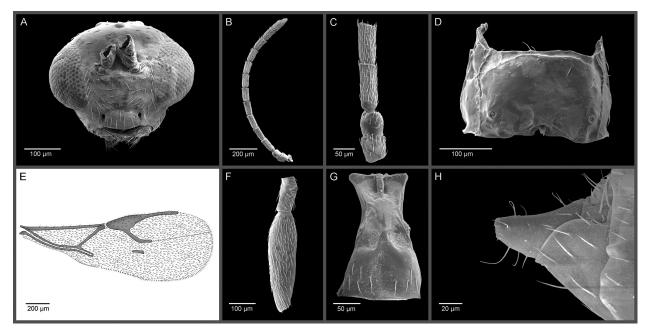


Fig. 18. Lysiphlebus orientalis. A: Head, frontal view. B: Antennae. C: Flagellomeres 1 and 2. D: Propodeum, dorsal view. E: Fore wing. F: Hind femur, lateral view. G: Petiole, dorsal view. H: Ovipositor sheath, lateral view.

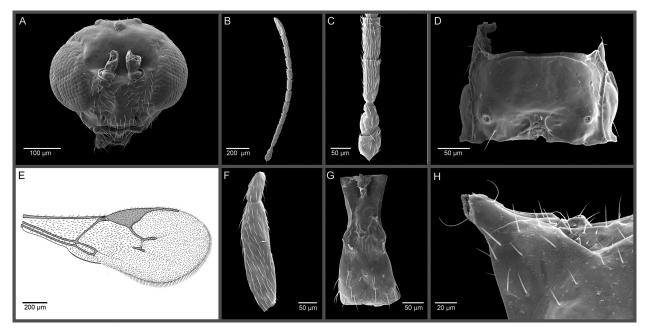


Fig. 19. Lysiphlebus testaceipes. A: Head, frontal view. B: Antennae. C: Flagellomeres 1 and 2. D: Propodeum, dorsal view. E: Fore wing. F: Hind femur, lateral view. G: Petiole, dorsal view. H: Ovipositor sheath, lateral view.

of propodeum with 3-4 and 1-2 long setae on each side. Fore wing (Fig. 19E) with marginal setae longer than setae on wing surface. Stigma $2.5-2.8 \times$ as long as its width, and about one quarter longer than metacarpal vein (stigma length / metacarpal vein length is 1.20-1.35) (Fig. 19E). Hind femur with short appressed setae (Fig. 19F). *Metasoma:* Petiole (Fig. 19G) smooth, convex dorsally, with lateral depression after prominent spiracular tubercles, elongated with length $2.20-2.60 \times$ its width at spiracles and $1.70-2.00 \times$ its width at base; 5-8 setae positioned on posterior dorsolateral margins. Ovipositor sheath (Fig. 19H) elongated, wide at base, dorsally slightly concave, with two long setae at the tip and two long setae on the ventral and dorsal surfaces, respectively. Length of ovipositor sheath $2.20-2.30 \times$ its maximum width. *Body length:* 1.6-2.2 mm. *Colouration:* General body colour dark-brown. Scape, pedicel, and flagellum brown. Mouthparts yellow to light-brown, remaining part of head dark-brown. Eye black. Pronotum light-brown; mesoscutum and mesopleuron dark-brown. Propodeum light-brown to brown. Legs yellow to light-brown with dark apices. Wings hyaline, venation brown. Petiole yel-

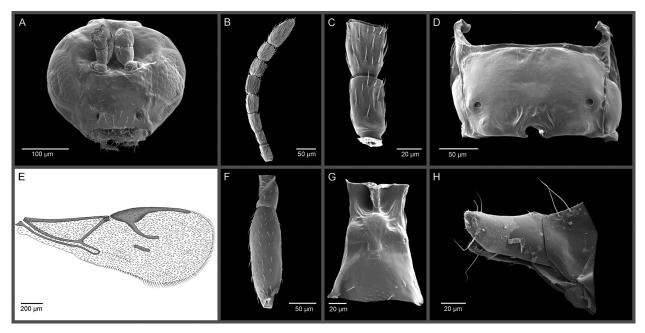


Fig. 20. Lysiphlebus balcanicus. A: Head, frontal view. B: Antennae. C: Flagellomeres 1 and 2. D: Propodeum, dorsal view. E: Fore wing. F: Hind femur, lateral view. G: Petiole, dorsal view. H: Ovipositor sheath, lateral view.

low to light-brown, other metasomal terga light-brown, gradually darker at the end of the metasoma. Ovipositor sheath black. — *MALE:* Antenna (14)15-segmented. Maxillary palpi with three palpomeres, labial palpi with two palpomeres. Body darker than female. Petiole lightbrown, remaining part of metasoma dark-brown. Legs and mouthparts light-brown. Body length: 1.5-2.0 mm. **Remarks.** *Lysiphlebus testaceipes* is endemic to the Nearctic Region. Although a morphologically uniform species, it has shown differentiation in some mitochondrial lineages (SHUFFRAN et al. 2004; MITROVIĆ et al. 2013). It is a sexual species.

Examined material. CROATIA: 32, Pula, Aphis sp. on Tunica sp., 12.ix.1990 (OPO); 17♀ 6♂, Plitvice-Kozjak, Aphis clematidis Koch, 1854 on *Clematis recta* L., 1753, 21.vi.2015 (ŽT); 7♀ 3♂, Plitvički Ljeskovac, A. fabae on A. sylvestris, 22.vi.2015 (ŽT); 33, Plitvice-Gavanovac, A. fabae on Laserpitium siler L., 1753, 20.vi.2015 (ŽT). FRANCE: 3♀ 14♂, Antibes, A. nerii on Nerium oleander L., 1753, v.2006 (VŽ); >100 \mathcal{Q} , Parc Residential de l'Esterel, Aphis arbuti Ferrari, 1872 on Arbutus sp., 1.i.1986 (PS); 29^{\bigcirc}_{\pm} 13 $^{\bigcirc}_{\odot}$, Antibes, A. fabae on Carduus sp., 1.i.1986 (PS). GREECE: 143♀ 93♂, Kalamata, A. fabae on P. tobira, 2.v.2010 (NK); 168 $\stackrel{\bigcirc}{_{\sim}}$ 124 $\stackrel{\bigcirc}{_{\sim}}$, Kalamata, A. fabae on G. aparine, 2.v.2010 (NK). MONTENEGRO: 114♀ 95♂, Bar, B. tamaricis on Tamarix sp., 24.v.2011 (AP); 14^Q 21^A, Tivat, A. gossypii on Citrus aurantium L., 1753, 25.v.2011 (VŽ); 59 5 3 d, Bar, A. gossypii on Citrus deliciosa, 24.v.2011 (AP); 20 21 , Bar, A. fabae cirsiiacanthoidis on Cirsium sp., 24.v.2011 (VŽ); 53♀ 42∂, Bar, Aphis punicae on Punica granatum L., 1753, 24.v.2011 (SS); 28° 30°, Bar, A. nerii on N. oleander, 24.v.2011 (AP); 16^{\bigcirc}_{+} 13 $^{\land}_{-}$, Bar, A. fabae on Magnolia grandiflora L., 1753, 24.v.2011 (VŽ); 3 2 3, Bar, A. fabae on Yucca sp., 24.v.2011 (VŽ); 56♀ 49♂, Bar, A. fabae on Hibiscus syriacus L., 1753, 24.v.2011 (VŽ); 3[♀], Bar, S. avenae on Festuca arundinacea Schreber, 1771, 24.v.2011 (VŽ); 1918. Bar. *A. craccivora* on *Cichorium endivia* L., 1753, 24.v.2011 (VŽ); 3♀1 J, Bar, A. galiiscabri Schrank, 1801 on Galium firmum Tausch, 1831, 24.v.2011 (VŽ); 17♀ 7♂, Bar, A. parietariae on Parietaria officinalis. 1753, 24.v.2011 (VŽ); 2913, Petrovac, A. davletshinae on *M. neglecta*, 24.v.2011 (AP). **ITALY**: 1[♀], Romagna, Cesena, *A*.

hederae on H. helix, 9.v.2006 (CV). SERBIA: 3^Q, Niš, A. craccivora on R. pseudoacacia, 19.v.2013 (VŽ); 1∂, Sićevo Gorge, A. sambuci L., 1758, on Sambucus nigra L., 1753, 12.v.2013 (VŽ); 24♀ 24♂, Niš-PMF, A. spiraecola on Tecoma radicans (L., 1753), 6.v.2013 (VŽ); 5♀ 3♂, Niš-Niška banja, Rhopalosiphum nymphaeae (L., 1761) on Typha latifolia L., 1753, 23.vii.2013 (VŽ); 70² 24³, Niš-Niška banja, *B. tamaricis* on *Tamarix* sp., 6.vi.2013 (VŽ); 3[⊖], Niš, A. craccivora on R. pseudoacacia, 19.v.2013 (VŽ); 2[♀], Niš-Pantelej, A. hederae on H. helix, 22.iv.2014 (VŽ). SLO-**VENIA**: >100 \bigcirc \bigcirc , Portorož, *A. nerii* on *N. oleander*, 17.vi.2009 (ZT); >200 \bigcirc \bigcirc , Portorož, A. fabae on P. tobira, 17.vi.2009 (ZT). SPAIN: 9[♀] 7[∧], LIeida, Siphonatrophia cupressi (Swain, 1918) on Cupressus sp., vi.2014 (XP); 23 \bigcirc 21 \bigcirc , Lleida, A. nerii on N. oleander, 7.vi.2010 (XP); $9 \bigcirc$, Madrid-La Grania, A. fabae on Ch. album, 27.xi.2006 (ŽT). LIBYA: 1♀, Derna, Aphis illinoisensis Shimer, 1866, on Vitis sp., 8.vii.2010 (AS); 6♀ 9♂, Derna, A. nerii on N. oleander, 7.viii.2010 (AS). USA: 12^Q, Florida, Winter Haven, Lake Alfred, A. fabae on S. nigrum, 01-20.vii.2010 (AT). CHILE: 13♀ 19♂, Santiago, A. fabae on Ligustrum sp., 1992 (PS); 13♀ 20♂, LaCruz, *B. cardui* on *Prunus* sp., 1992 (PS); 34♀ 213, Rinconada, Diuraphis noxia Kurdjumov, 1913, on Triticum sp., 1992 (PS). COSTA RICA: 25♀ 11♂, San Jose, A. gossypii on *Piper* sp., 1.i.2000 (DZM); $>100^{\circ}$, San Jose, *T. aurantii* on C. aurantium, i.2007 (DZM); $11^{\circ}_{\sim}22^{\circ}_{\circ}$, San Jose, A. nerii on N. oleander, i.2007 (DZM); 7[⊖] 12[°], San Jose, Toxoptera aurantii (Boyer de Fonscolombe, 1841) on Syzygium wilsonii (F. Mueller) B. Hyland, 1983, 10.i.2007 (DZM). BENIN: 5♀ 7♂, Hla Avame, A. gossypii on Capsicum annuum L., 1753, 29.v.2010 (MGS); 4♀ 53, Benin, A. gossypii on Phaseolus sp., 12.v.2011 (MGS). AL-**GERIA**: $4^{\bigcirc}_{\rightarrow}$ 5Å, Algeria, *A. nerii* on *N. oleander*, 11.v.2008 (ML); $2 \ 3 \ 3 \ 3$, Algeria, D. plantaginea on M. domestica, 14.v.2008 (ML). For additional records see Starý et al. (1988b), KAVALLIERATOS et al. (2001, 2004, 2016), and PIKE et al. (2000).

Lysiphlebus balcanicus Starý, 1998 (Fig. 20A–H)

Diagnosis and description. See STARÝ et al. (1998). **Remarks.** *Lysiphlebus balcanicus* is a highly specialized root aphid parasitoid species associated with *Aphis psam*-

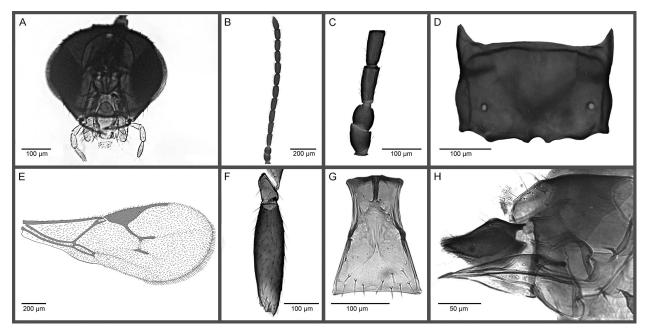


Fig. 21. Lysiphlebus desertorum. A: Head, frontal view. B: Antennae. C: Flagellomeres 1 and 2. D: Propodeum, dorsal view. E: Fore wing. F: Hind femur, lateral view. G: Petiole, dorsal view. H: Ovipositor sheath, lateral view.

mophila Szelegiewicz, 1967. It possesses the antennae within the smallest number of flagellomeres within the subfamily Aphidiinae (STARÝ et al. 1998), with a tendency of F_2 reduction (Fig. 20C). Apart from reared specimens in Serbia, it has been collected with a Malaise trap in Spain (SANCHIS et al. 1999). DAVIDIAN (2016) found this species in southern Russia. We suppose a southern European / Palaearctic distribution of *L. balcanicus* that follows the distribution of its aphid hosts. Given that all sampled specimens from Serbia, Spain, and Russia are females, we believe that it is an asexual species.

Examined material. Holotype 1, **SERBIA**, Mt. Vlasina, 1400 m, *Aphis psammophila* Szelegiewicz, 1967 on *Jasione dentata*, 21.vii.1990 (OP). — Paratypes 3, Mt.Vlasina – Čemernik, 1500 m, *A. psammophila* on *J. dentata*, 30.vii.1996 (ŽT). Holotype and paratypes deposited in the collection of the Institute of Zoology, Faculty of Biology, University of Belgrade (Serbia). — **SERBIA**: 8, Mt. Vlasina, 1200 m, *Aphis psammophila* on *Jasione heldreichii* Boissier & Orphanides, 1859, 3.vi.2012 (VŽ); 3, Mt. Vlasina, 1500 m, *A. psammophila* on *J. heldreichii*, 7.viii.2010 (ŽT). For additional records see STARÝ et al. (1998), SANCHIS et al. (1999) and DAVIDIAN (2016).

Lysiphlebus desertorum Starý, 1965 (Fig. 21A–H)

Lysiphlebus hispanus Starý, 1973 syn.n.

Diagnosis. The wing venation pattern and number of labial palpomeres place it in the '*testaceipes*' group. *Lysiphlebus desertorum* is similar to *L. fritzmuelleri* and *L. testaceipes* in the large number of longitudinal placodes on the first (2-4) and second (3-5) flagellomeres (Fig. 21C). However, it differs from both species in having a shorter and stouter petiole (Fig. 21G) and a generally somewhat shorter F₁ (Fig. 21C). It is a specialized

parasitoid of *Protaphis* spp. on *Achillea* or *Artemisia* plants in desert or semidesert areas of the Palaearctic (STARÝ & REMAUDIERE 1973).

Description. FEMALE: Head (Fig. 21A) transverse, wider than thorax, with sparse setae. Eyes large, oval, sparsely haired. Tentorial index 0.40-0.50. Clypeus transverse, with $4-6 \log$ setae. Mandible bidentate. Maxillary palpi with three palpomeres, labial palpi with two palpomeres. Antenna 12(13)-segmented, filiform (Fig. 21B), reaching half of petiole. F_1 equal to F_2 and $2.0-2.3 \times$ as long as its maximum width at the middle (Fig. 21C). F_1 and F_2 with 2-4 and 3-5 longitudinal placodes, respectively (Fig. 21C). Flagellomeres uniformly covered with short semi-erect setae. Mesosoma: Mesoscutum sparsely haired, notaulices feebly visible and rugose in very short ascendent portion of anterolateral margin, effaced dorsally, with usually two rows of setae along the dorsolateral parts of the mesoscutum. Propodeum (Fig. 21D) smooth, sometimes rugose in the area of petiole insertion. Upper and lower parts of propodeum with 3-4 and 1-2 long setae on each side. Fore wing (Fig. 21E) with long marginal setae, which are longer than those on the wing surface. Stigma triangular, $2.5-2.9 \times$ as long as its width and longer than the metacarpal vein $(1.50-1.90 \times$ as long as the metacarpal vein). Hind femur and tibia with long semi-erect setae (Fig. 21F). Metasoma: Petiole (Fig. 21G) smooth, with longitudinal central impression, sparsely haired, its length $1.90-2.10 \times$ its width at spiracles and $1.00-1.30 \times$ its width at the base. Ovipositor sheath (Fig. 21H) pointed. Body length: 1.0-1.3 mm. Colouration: General body colour brown to light-brown. Antennae brown, F_1 yellow at the base. Eye black. Face and clypeus light-brown, Mouthparts and face yellow to light-brown. Mesoscutum and mesopleuron brown till to light-brown. Propodeum light-brown in lower parts.

Legs yellow except for brown basal parts, with dark apices. Wings hyaline, venation brown. Petiole yellow, remaining parts of metasoma dark-brown. Ovipositor sheath black. — *MALE*: Unknown.

Remarks. After comparison of the type specimens of *L. hispanus* and *L. desertorum*, we found that they are identical, so we synonymized *L. hispanus* as a junior synonym of *L. desertorum*. We used fresh material from Iran for molecular analysis and established that *L. desertorum* falls within the '*testaceipes*' group. It is distributed in southern parts of the Palaearctic in *Protaphis* spp. / *Achillea* spp. and *Artemisia* spp. associations. On the basis of published data and our own sampling efforts, we conclude that *L. desertorum* is an asexual species within the '*testaceipes*' group.

Examined material. Holotype 1, **UZBEKISTAN**, Yangi-Yulski District, Tashkent Reg., on *Achillea* sp. / unknown aphid, vi.1962 (PS). — Paratypes 87, Yangi-Yulski District, Tashkent Reg., vi.1962 (PS). — **IRAN**: 6, Ardebil, *Protaphis* sp. on *Artemisia* sp., 1.vii.2005 (ER); 11, Kordestan, Sanandaj, *Protaphis* sp. on *A. millefolium*, 16.v.2005 (ER).

Examined type material of *L. hispanus.* Holotype 1, **SPAIN**, Aranjuez, *Protaphis* sp. on *Artemisia campestris* L., 1753, 7.vi.1965, (GR). – Paratypes 6, **SPAIN**, Aranjuez, *Protaphis* sp. on *A. campestris*, 7.vi.1965 (GR).

For additional records see Starý (1965), Starý & Remaudière (1973), and Barahoei et al. (2013).

3.5. Identification key for European *Lysiphle- bus* species based on females

- **3'** Metacarpal vein clearly longer than stigma (Figs. 9E-11E, 14E-16E). F₁ without and F₂ without or with 1(2) longitudinal placodes (Figs. 14C-16C) ... **8**
- 4 Labial palp with one palpomere L. alpinus Starý
- 4' Labial palp with two palpomeres 5
- 5 Fore wing with very short lower marginal setae, equal to those on fore wing surface (Fig. 18E). Fore wing stigma elongate, 2.9-3.0 × as long as wide (Fig. 18E) *L. orientalis* Starý & Rakhshani

- 6 Petiole wide at base, 1.0−1.3 × as long as wide at base (Fig. 21G). Hind femur with semi-erect setae (Fig. 21F) *L. desertorum* Starý
- 7 Petiole 1.5–1.7 × as long as width at base (Fig. 12G)*L. fritzmuelleri* Mackauer
- 7' Petiole 1.7–2.0 × as long as width at base (Fig. 19G) *L. testaceipes* (Cresson)
- 8' Hind femur with semi-erect or appressed setae. F₁ subequal to F₂. Ovipositor sheath dorsally slightly concave or almost straight (Figs. 9H, 10H, 14H-16H)
 9
- **9** Fore wing with very short lower marginal setae, equal to those on surface (Figs. 9E, 14E) **10**
- **9'** Fore wing with marginal setae longer than those on surface (Fig. 15E)
 - L. confusus Tremblay & Eady
- 10 Hind femur with semi-erect setae (Figs. 9F, 16F) ... 11
- 10' Hind femur with appressed setae (Figs. 10F, 14F) ... 12
- Fore wing stigma 3.8-4.0 × as long as wide (Fig. 9E). F₂ with 1-2 longitudinal placodes (Fig. 9C) *L. volkli* Tomanović & Kavallieratos sp.n.
- 11' Fore wing stigma $3.2-3.6 \times$ as long as wide (Fig. 16E). F₂ usually without or exceptionally with one longitudinal placode (Fig. 16C)

..... *L. cardui* (Marshall)

- 12 Fore wing stigma $3.4-3.8 \times$ as long as wide (Fig. 10E). Specialized parasitoid of *Brachycaudus cardui* on *Carduus* spp. and *Cirsium* spp.
- L. brachycaudi Starý & Tomanović sp.n.
 12' Fore wing stigma 2.8-3.4 × as long as wide (Fig. 14E). Polyphagous species

..... L. fabarum (Marshall)

4. Discussion

4.1. General issues

Molecular analyses based on nuclear 28SD2 and mitochondrial COI sequences recognized three separate groups of species within the genus *Lysiphlebus*, i.e., '*fabarum*', '*testaceipes*', and '*alpinus*'. The distinction of the '*testaceipes*' and '*fabarum*' groups within the genus *Lysiphlebus* was also recognized previously by investigators using other molecular markers with slow rates of evolution, e.g., 18S rRNA (SANCHIS et al. 2000) and 16S rRNA (KAMBHAMPATI et al. 2000). The limited variability seen in our nuclear 28SD2 sequences was insufficient for clear delineation of species within subclades. A single 28SD2 distinct haplotype identified *L. alpinus* as a separate lineage more closely related to species in the 'fabarum' group than the 'testaceipes' group, but within the 'fabarum' group, specimens of L. fabarum, L. cardui, L. confusus, L. 'melandriicola', L. hirticornis, L. brachycaudi sp.n. and L. volkli sp.n. showed diversity of only 12 nuclear haplotypes with low divergence and some overlap between the species. Likewise, in the 'testaceipes' group, specimens of L. testaceipes, L. fritzmuelleri, L. balcanicus, L. desertorum, and L. orientalis comprised only three very similar haplotypes.

Better resolution was provided by the barcoding region of the mitochondrial COI gene, which has proven a reliable molecular marker in resolving the species status and revealing cryptic species of many parasitoids from the subfamily Aphidiinae (Kos et al. 2011; MITROVSKI-BOGDANOVIĆ et al. 2013, 2014; TOMANOVIĆ et al. 2014; STANKOVIĆ et al. 2015; JAMHOUR et al. 2016). Here it clearly confirmed the species status of L. alpinus, placing it closer to the 'fabarum' than the 'testaceipes' group, and it unambiguously supported the distinction of L. orientalis, L. balcanicus, and L. desertorum within the 'testaceipes' group. Within the 'fabarum' group, COI sequences clustered into two subclades, splitting 'L. fabarum s.str.' from the other species with maximum support and evolutionary distances in excess of 4%, indicating that the estimated time of this subclade's divergence was over two million years ago (DeSalle et al. 1987; BROWER 1994). In the other "hirticornis" subclade, plotting of the Max-WSD and Min-BSD of COI sequences provided strong support for the distinction of the two newly described species L. volkli and L. brachycaudi, as well as L. hirticornis.

These distinctions are also supported by biological information. The analysed L. hirticornis specimens reproduce through arrhenotoky and are strictly associated with the aphid host Metopeurum fuscoviride. Lysiphlebus brachycaudi is also an arrhenotokous species and known only as a specialized parasitoid of B. cardui. We assume that L. brachycaudi represents a young lineage that has recently acquired B. cardui as a new host. Several apomorphies (e.g. elongated stigma and ovipositor sheaths) (QUICKE & VAN ACHTERBERG 1991) distinguish it from the presumably ancestral L. fabarum s.str. group. Our results corroborate a previous report by BELSHAW et al. (1999) indicating genetic isolation of a L. fabarum ex Brachycaudus cardui biotype (newly described as L. brachycaudi) from other L. fabarum biotypes. Also, LIEPERT & DETTNER (1996) reported a specific cuticular hydrocarbon profile for the L. fabarum ex B. cardui biotype, and SANDROCK et al. (2011) used microsatellite markers to show that sexual wasps from the 'fabarum' group collected from B. cardui were strongly differentiated from all other morphs, either sexual or asexual. The newly described L. volkli is less host-specific and has been found to parasitize A. fabae, A. farinosa, B. cardui, and B. tragopogonis. It appears to be arrhenotokous in Europe, but there is clear indication of a thelytokous Iranian population that emerged from A. verbasci. The distinct status of L. volkli has been hinted at before. PETROVIĆ et al. (2015) reported clear

molecular separation of *L. cardui* morphs with sexual reproduction (here described as *L. volkli*) from all other species/morphs in the '*fabarum* s.str.' group, suggesting that these sexual parasitoids represent an undescribed species.

While the analysis of mitochondrial COI sequences clearly aided in clarifying the evolutionary relationships among European species of the genus and thus supported this taxonomic revision, there were also two groups where the status of taxa could not be satisfactorily resolved: the single haplotype of the European species *L. fritzmuelleri* grouped within the cluster of highly diverse haplotypes of the American species *L. testaceipes*, and in '*L. fabarum* s.str.' there was little correspondence between the closely related haplotypes and morphologically defined taxa. We discuss each of these clades in more detail below.

4.2. Lysiphlebus fabarum s.str.: a mixed bag

The 13 COI haplotypes associated with the 'L. fabarum s.str.' subgroup had 0.2–1.1% sequence divergence and showed little correlation with the morphologically determined species they were collected from, i.e., L. fabarum, L. cardui, L. confusus, and L. 'melandriicola' (here synonymized with L. fabarum). Characteristically, the dominant mitochondrial haplotype (LFG1) was shared by all four species, i.e., 50 specimens of different morphology, geographic origin and aphid host associations.

This finding is in an agreement with various molecular studies showing little or no genetic divergence between taxa from the L. fabarum s.str. group. BELSHAW et al. (1999) reported a low level of divergence between L. fabarum, L. cardui, L. confusus, and L. 'melandriicola' in the mitochondrial genome. Using barcoding sequences, as well as analysis of ATP6 data, SANDROCK et al. (2011) discovered that there is no clear separation between sexual and asexual L. cardui, L. confusus and L. fabarum, suggesting that these three species should be treated as one. Using the mitochondrial COI gene and a nuclear long-wavelength rhodopsin, DEROCLES et al. (2012) found no differences between L. confusus and L. fabarum. PETROVIĆ et al. (2015) reported the absence of genetic differences in mitochondrial barcoding fragments among L. fabarum, L. cardui, L. confusus, and L. 'melandriicola'. Also, STARÝ et al. (2014) presented ecological and morphological evidence for discrimination between L. cardui, L. fabarum and L. confusus but without molecular data to support the species delineation. Later, DEROCLES et al. (2016) used seven mitochondrial and nuclear gene fragments to analyse genetic differentiation among seven Aphidiinae forms, including members of the L. fabarum s.str. group. They reported this group to consist of paraphyletic morphospecies that separated according to trophic association with the aphid host species but with no clear evidence as to whether it is attributable to intraspecific variation or to the existence of reproductively isolated cryptic species. We thus have a situation

where heritable morphological differences are clearly recognizable and ecologically informative but do not show a clear phylogenetic signal. For example, L. cardui is reliably collected from A. fabae cirsiiacanthoidis on thistles (Cirsium sp.), L. fabarum from A. hederae on ivy (Hedera helix), and L. confusus from A. ruborum on blackberries (Rubus sp.), yet they may share the same mitochondrial haplotypes (SANDROCK et al. 2011). How can this discrepancy be explained? We believe that the frequent occurrence of thelytoky within L. fabarum s.str. contributes importantly to this complex situation. SANDROCK & VORBURGER (2011) described the 'fabarum' group as an evolutionarily young sexual-asexual complex with incomplete reproductive isolation, and they identified the genetic basis of reproductive mode variation in L. fabarum. Thelytoky appears to be inherited as a single-locus recessive trait, and because thelytokous lines may still produce fertile males occasionally (at very low frequency), the yet unknown genetic factor determining thelytoky can be introgressed into sexual populations and result in the formation of new asexual lines (SANDROCK & VORBURGER 2011). This mechanism is referred to as contagious parthenogenesis (SIMON et al. 2003). It implies that asexual wasps should have close relatives that are sexual, which is consistent with our data, and it could explain the high genotypic diversity observed in asexual Lysiphlebus (SANDROCK et al. 2011; VORBURGER & ROUCHET 2016). Alternative mechanisms of genetic exchange might include mating between sexual males and thelytokous females or even 'cryptic sex' within asexuals (SANDROCK et al. 2011). BELSHAW et al. (1999) also emphasized the importance of rare sex in predominantly asexual wasps with members of closely related sexual populations and its influence on the persistence of asexual lineages.

The co-occurrence of sexual and asexual lineages that do not show full reproductive isolation will inevitably lead to taxonomic challenges. Mitochondrial haplotypes and nuclear alleles will move between lineages, and ecological (e.g. host specialization) as well as morphological variation present in sexuals can become 'frozen' and amplified in asexual lines. A good example might be the asexual L. cardui reliably associated with A. f. cirsiiacanthoidis on thistles throughout Europe. It clearly seems to be a host-associated lineage with particular morphological traits identifying it as L. cardui (STARÝ et al. 2014). These traits may well be adaptive in its ecological niche, but they may also just represent 'frozen' morphological variation from a polymorphic source population that is inherited along with the host specialization. Lysiphlebus cardui has long been considered a purely asexual taxon, but in the present study we also found several sexual L. cardui lines, something that was previously unknown.

Given that they exhibit reproducible ecological differences (e.g. STARÝ et al. 2014), we maintain that the distinction of *L. fabarum*, *L. cardui*, and *L. confusus* is useful. However, it is important to recognize that they represent distinctive morphospecies with distinctive ecological attributes (e.g. host ranges) but little phylogenetic justification till now.

4.3. *Lysiphlebus testaceipes*: high haplotype diversity and close relation to *L. fritzmuelleri*

High diversity of haplotypes and substantial intraspecific variation of mitochondrial sequences was detected for *L. testaceipes* specimens, confirming previous reports by MITROVIĆ et al. (2013) and SHUFRAN et al. (2004). Diversity of sequenced mitochondrial haplotypes was expected, in view of the sexual mode of reproduction, sampled material originating from a wide distribution area, and diverse aphid/plant associations. More unexpected was that the single mitochondrial haplotype of *L. fritzmuelleri* was positioned among the haplotypes of *L. testaceipes*, with genetic distances at the same level as the intraspecific variation of *L. testaceipes* haplotypes. Low evolutionary distances between the mitochondrial haplotypes and identical 28SD2 nuclear sequences indicate close relatedness between *L. testaceipes* and *L. fritzmuelleri*.

These two species are morphologically distinguishable, they exhibit different host specificity, and they are considered to be of different geographic origin. Lysiphlebus fritzmuelleri is described from Europe and strictly associated with Aphis craccae (MACKAUER 1960), while the North- and South American species L. testaceipes exhibits polyphagous behaviour (PIKE et al. 2000). Although L. fritzmuelleri has been recorded in several European countries (Kavallieratos et al. 2004; Starý 2006; Starý & LUKÁŠ 2009; VAN ACHTERBERG 2013), it is here analysed for the first time using molecular markers. Surprisingly, we found weak differences between L. fritzmuelleri and L. testaceipes for mitochondrial COI with an average distance of about 1.1%, which corresponds to the range of intraspecific genetic variation (0.2-1.3%) reported for various populations of L. testaceipes (MITROVIĆ et al. 2013). Populations of L. fritzmuelleri collected from the same association of A. craccae / Vicia cracca from Serbia and high altitudes of Austria shared the same mitochondrial haplotype, indicating that they may have originated from the same refuges after the last ice age and suggesting that the pattern of range expansion through Europe is linked with a particular aphid host/plant association. On the other hand, L. testaceipes is oligophagous in its native range and was characterized by high generalism and invasive propagation across Europe, encountering and appropriating a wide range of new aphid hosts after its introduction as a biological control agent in the Mediterranean region during the 1970s (STARÝ 1988b).

The current knowledge thus suggests that *L. fritzmuelleri* is a monophagous Old World sister species to the oligophagous *L. testaceipes* from the New World, but their relatively small genetic divergence is surprising. It should not be ruled out, therefore, that *L. testaceipes* might have been present in Europe much earlier than its official introduction and prior to description of *L. fritzmuelleri*. As a polyphagous parasitoid, *L. testaceipes* might have been accidentally introduced hitchhiking on some aphid host and become established in a restricted area from which lineages adapted to the *A. craccae / V.* *cracca* association. ANTOLIN et al. (2006) documented adaptation to new hosts on the example of the generalist parasitoid *Diaeretiella rapae* (M'Intosh, 1855) (Hymenoptera: Braconidae: Aphidiinae). If specialization on *A. craccae* reduced fitness on other hosts (e.g. HENRY et al. 2008), this could have prevented genetic exchange with later-introduced *L. testaceipes*.

A more conclusive statement on the status of L. fritzmuelleri and L. testaceipes has to be adjourned for the moment. Similar COI haplotypes may be indicative of ongoing hybridization and introgression between closely related species (HARRISON & LARSON 2014), but they could also represent incomplete lineage sorting (PAMILO & NEI 1988). In the absence of clear evidence for gene exchange between them, it remains the case that L. testaceipes and L. fritzmuelleri have to be treated as two separate species. This imposes the need to re-evaluate the status of these two species, taking into account morphological and behavioural peculiarities, results of multilocus genotyping, and patterns of population genetic divergence. Experimental work testing whether they are reproductively isolated would further help to clarify the issue in the future.

4.4. Lysiphlebus classification

The genus Lysiphlebus was classified within the tribe Aphidiini (MACKAUER 1961) and subtribe Lysiphlebina (Adialytus + Lysiphlebus + Lysiphlebia) which are usually considered as monophyletic (SANCHIS et al. 2000; SMITH et al. 1999). However, SANCHIS (2000) found Adialytus to be paraphyletic, due to A. ambiguus falling within Lysiphlebus on the basis of the 18S nuclear gene (only two Adialytus species were used in this study). KAMBHAMPATI (2000), on the basis of the 16S mitochondrial gene, supported the generic status of Adialytus. However, there is little biological information about the genus Adialytus, which includes seven species with Holarctic distribution (RAKHSHANI et al. 2012), from which just few molecular results exist for three species, namely A. ambiguus, A. salicaphis and A. thelaxis (Starý) (STANKOVIĆ et al. 2015), while some species are recorded only as few type specimens (e.g. A. balticus Starý & Rakauskas, 1979, and A. veronicaecola (Starý, 1978)). Although some results indicate that Lysiphlebus represents a paraphyletic group, due to controversal data and lack of sufficient knowledge about Adialytus, further studies are necessary to resolve the phylogenetic status of the genus Lysiphlebus.

Lysiphlebus dissolutus is the type species for the genus Lysiphlebus and was described as Bracon dissolutus Nees, 1811 based on a male specimen. It was redescribed later by the same author in 1834. However, both descriptions were unsatisfactory because they concealed several similar species (STARÝ 1975). Additionally, the holotype was lost, which further complicated the problem of solving the taxonomic status of L. dissolutus (STARÝ 1961, 1975), more so because L. dissolutus was assigned the status of type species of the new genus Lysiphlebus in FOERSTER (1862). This caused misinterpretation of the status of the species and gave rise to descriptions which led to a synonymization (Starý 1961, 1975). MACKAUER (1960) considered L. dissolutus (Nees, 1811 and Nees, 1834) to be a dubious species and described Lysiphlebus (Platycyphus) macrocornis Mackauer, 1960 as a new subgenus and species. However, we agree with the opinion that the name L. dissolutus should be considered valid and keep L. (Platycyphus) macrocornis as a junior synonym of L. dissolutus. Lysiphlebus dissolutus is a parasitoid specialized on various root aphids. STARY (1975) established two subgenera: Lysiphlebus Foerster and Phlebus Starý. The subgenus Lysiphlebus, which includes L. dissolutus, is characterized by having square flagellomeres, 16-17-segmented antennae, and two divergent carinae on the propodeum. STARÝ et al. (1998) described the new species L. balcanicus, a root aphid parasitoid, as an additional species, classifying it as belonging to the subgenus Lysiphlebus. The description of L. balcanicus somewhat changed the diagnostic characters of this subgenus, since it possesses 10-segmented antennae (the smallest number within the Aphidiinae) with square flagellomeres and two divergent carinae on the propodeum. Unfortunately, L. dissolutus was unavailable for our molecular analysis, and we had at our disposal only microscope slides of prepared specimens. We presume that the square flagellomeres and small eyes are consequences of fossorial life in the case of obligatory root aphid parasitoids. Although these characters have an adaptive significance, they probably are of no phylogenetic importance. Species which belong to the subgenus Phlebus (all other Lysiphlebus species) usually possess a smooth propodeum or one that sometimes has short carinae on its basal part. However, in some specimens we found the presence of long divergent carinae on the propodeum. We are not sure about the phylogenetic position of L. dissolutus within the genus Lysiphlebus because our knowledge comes from morphological characters only. For possessing a short metacarpal vein, we classify L. dissolutus in the 'testaceipes' group. Furthermore, the analysed molecular markers put L. balcanicus very clearly within the 'testaceipes' group. Although there are very consistent differences of nuclear and mitochondrial genes among members of the 'fabarum', 'testaceipes', and 'alpinus' groups, for the time being we prefer to consider them as species groups rather than subgenera. This is due to the relatively low genetic divergences obtained in the COI mitochondrial gene

Our results do not support the current subgeneric classification of the genus *Lysiphlebus*.

4.5. Taxonomy

Lysiphlebus safavii is a taxon which is known only from Germany and is represented by a few type specimens emerged from the *Aphis fabae / Amaranthus* sp. association (STARÝ 1985). Despite the numerous available records across Europe, no *L. safavii* phenotype has

(6.6-9.4%) and the 28SD2 nuclear gene (1.1-3.7%).

ever been recorded from this very common trophic association. We carefully examined the slide-mounted type specimens of L. safavii and reached the conclusion that their main diagnostic characters (pubescence of flagellomeres, long F₁, narrowly pointed and dorsally concave ovipositor sheath) apply to an already known species, L. hirticornis. After comparing several specimens of both taxa, we found no consistent morphological differences among them. Since L. safavii was reared from the A. fabae / Amaranthus sp. association, while L. hirticornis is a specialized parasitoid of Metopeurum fuscoviride / Tanacetum sp., we assume that parasitized individuals of M. fuscoviride probably moved to adjacent Amaranthus plants with an A. fabae colony or (less likely) that L. hirticornis has a slightly broader host range pattern and parasitizes A. fabae. In accordance with these findings, we synonymize L. safavii with L. hirticornis.

We analysed several *Lysiphlebus* phenotypes that fit the description of *L. hirtus* (long setae along wing margins, obtusely pointed ovipositor sheaths, shape of the petiole, F_1 without rhinaria) (STARÝ 1985). After careful examination of the type specimens of *L. hirtus* reared from the *Brachycaudus populi / Silene* sp. association, we concluded that they are morphologically closely related to phenotypes of *L. confusus*, which is part of the *L. fabarum* s.str. group (see re-description), so we synonymized *L. hirtus* with *L. confusus*.

According to the original description of L. melandriicola as a very specific parasitoid of Brachycaudus lychnidis on Melandrium album (=Silene latifolia Poir.), it possesses 13-14 antennal segments, which is the only morphological difference between this species and L. fabarum, whose antennae are 12-13-segmented (STARY 1961). However, in our reared material, most of the L. melandriicola specimens that emerged from B. lychnidis / M. album had 13-segmented antennae with an undivided or semi-divided last apical flagellar segment. We argue that this variation is a consequence of some developmental instability. Furthermore, the sexual L. melandriicola phenotypes do not differ genetically from several L. fabarum populations in the analysis based on the COI barcoding gene. Accordingly, we synonymize L. melandriicola with L. fabarum as a junior synonym, since they show neither consistent morphological differences nor genetic differentiation.

STARÝ (1965) described *L. desertorum* from Uzbekistan as a specialized parasitoid of aphids on *Achillea* plants in desert or semi-desert areas of Central Asia. Later, STARÝ & REMAUDIÈRE (1973) described a new *Lysiphlebus* species from Spain, *L. hispanus*, associated with *Protaphis* on *Artemisia campestris*. Both *Lysiphlebus* species belong to the '*testaceipes*' group, both parasitize aphids in similar habitats, and both are characterized by asexual reproduction (only females existed in all available samples). However, after comparing the types of both species and analysing additional material from Central Asia (Iran) associated with *Protaphis* sp. / *Achillea* sp., we conclude that *L. desertorum* and *L. hispanus* are conspecific and propose *L. hispanus* as a junior synonym of *L. deserto*- *rum*. Our results indicate that *L. desertorum* is distributed in southern areas of the Palaearctic, including Europe, in conjunction with the specific associations *Protaphis* sp. / *Achillea* spp. and *Protaphis* sp. / *Artemisia* spp.

Lysiphlebus fritzmuelleri was described from Europe (MACKAUER 1960) as a specialized monophagous parasitoid of tufted vetch aphid, *A. craccae*. On the other hand, *L. testaceipes* is a polyphagous species in North and South America (PIKE et al. 2000; STARÝ et al. 1993). After being introduced in Europe, it acquired many new aphid hosts there (STARÝ 1988b; STARÝ et al. 2004; TOMANOVIĆ et al. 2009; ŽIKIĆ et al. 2015). We found some morphological differences between these two species, e.g., a more elongated petiole, which is considered an apomorphic character state, in *L. testaceipes* (see description and the key for identification of species).

We decided to keep L. fabarum, L. confusus, and L. cardui together as taxonomic entities within the L. fabarum s.str. group and have here for the first time defined and diagnosed L. fabarum s.str. as a group (see descriptions). As previously indicated, these three taxa are not clearly discriminated based on nuclear and mitochondrial molecular markers, but they show morphological peculiarities and differences in host use. Also, the map of the distribution of these taxa in southeastern Europe shows that L. confusus prefers warmer Mediterranean and northern lowland areas, whereas L. fabarum is dominant in central mountainous (colder) areas (KAVALLIERATOS et al. 2004). In addition, all three species consist of asexual and sexual populations which are sympatric. It was not previously known that L. cardui comprises sexual strains as well (BELSHAW et al. 1999). The sexual strains of L. cardui are morphologically similar to the newly described L. volkli, but they show clear genetic differentiation, which is accompanied by weak but consistent morphological differences in the shape of the stigma. The newly described species L. volkli and L. brachycaudi form a subclade within the L. fabarum clade and are phylogenetically related to L. hirticornis. The reliable separation of L. volkli and L. brachycaudi is difficult without using molecular markers or host range patterns and is based only on the hind femur setation (see Key). Both species exhibit clear synapomorphy, with an elongated stigma and a broad petiole as plesiomorphic character states.

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6. References

- ACHTERBERG C. VAN 2013. Fauna Europaea: Hymenoptera: Braconidae. – Fauna Europaea version 2.6. at http://www.fauna-eu.org [Accessed 30 May, 2017]
- ANTOLIN M.F., BJORKSTEN T.A., VAUGHN T.T. 2006. Host-related fitness trade-offs in a presumed generalist parasitoid, *Diaeretiella rapae* (Hymenoptera: Aphidiidae). – Ecological Entomology 31: 242–254.
- BANDELT H.J., FORSTER P., RÖHL A. 1999. Median-joining networks for inferring intraspecific phylogenies. – Molecular Biology and Evolution **16**: 37–48.
- BARAHOEI H., RAKHSHANI E., MADJDZADEH S.M., ALIPOUR A., TAHERI S., NADER E., MITROVSKI BOGDANOVIĆ A., PETROVIĆ-OBRADOVIĆ O., STARÝ P., KAVALLIERATOS N.G., TOMANOVIĆ Ž. 2013. Aphid parasitoid species (Hymenoptera: Braconidae: Aphidiinae) of central submountains of Iran. – North-Western Journal of Zoology 9: 70–93.
- BELSHAW R., QUICKE D.L. 1997. A molecular phylogeny of the Aphidiinae (Hymenoptera: Braconidae). – Molecular Phylogenetics and Evolution 7: 281–293.
- BELSHAW R., QUICKE D.L., VOLKL W., GODFRAY H.C.J. 1999. Molecular markers indicate rare sex in a predominantly asexual parasitoid wasp. – Evolution 53: 1189–1199.
- BENELLI N.G., KAVALLIERATOS N.G., DONATI E., GIUNTI G., STEFANINI C., CANALE A. 2016. Singing on the wings! Male wing fanning performances affect female willingness to copulate in the aphid parasitoid *Lysiphlebus testaceipes* (Hymenoptera: Braconidae: Aphidiinae). – Insect Science 23: 603–611.
- BROWER A.V. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. – Proceedings of the National Academy of Sciences **91**: 6491–6495.
- CAMPBELL B.C., STEFFEN-CAMPBELL J.D., WERREN J.H. 1993. Phylogeny of the *Nasonia* species complex (Hymenoptera: Pteromalidae) inferred from an internal transcribed spacer (ITS2) and 28S rDNA sequences. – Insect Molecular Biology 2: 225– 237.
- CARVER M., FRANZMANN B. 2001. Lysiphlebus Förster (Hymenoptera: Braconidae: Aphidiinae) in Australia. – Australian Journal of Entomology 40: 198–201.
- DAVIDIAN E.M. 2016. Parasitoid wasps of subgenus *Lysiphlebus*s. str. of the genus *Lysiphlebus* Foerster, 1862 (Hymenoptera: Aphidiidae) of the Russian fauna. Eurasian Entomological Journal 15: 24–28. (In Russian with English abstract)
- DESALLE R., FREEDMAN T., PRAGER E.M., WILSON A.C. 1987. Tempo and mode of sequence evolution in mitochondrial DNA of Hawaiian *Drosophila*. – Journal of Molecular Evolution 26: 157– 164.
- DEROCLES S.A., LE RALEC A., PLANTEGENEST M., CHAUBET B., CRU-AUD C., CRUAUD A., RASPLUS J.Y. 2012. Identification of molecular markers for DNA barcoding in the Aphidiinae (Hym. Braconidae). – Molecular Ecology Resources 12: 197–208.
- DEROCLES S.A., PLANTEGENEST M., RASPLUS J.Y., MARIE A., EVANS D.M., LUNT D.H., LE RALEC A. 2016. Are generalist Aphidiinae (Hym. Braconidae) mostly cryptic species complexes? – Systematic Entomology 41: 379–391.
- EASTOP V.F., VAN EMDEN H.F. 1972. The insect material. Pp. 1–45 in: VAN EMDEN H.F. (ed.), Aphid Technology. – Academic Press, London.
- EL-MALI M.U., STARÝ P., SAHBAZ A., OZSERMECI F. 2004. A review of aphid parasitoids (Hym., Braconidae, Aphidiinae) of Turkey. – Egyptian Journal of Biological Pest Control 14: 355–370.
- FELSENSTEIN J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791.
- FOLMER O., BLACK M., HOEH W., LUTZ R., VRIJENHOEK R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. – Molecular Marine Biology and Biotechnology 3: 294–297.

- HAJIBABAEI M., SINGER G.A.C., HEBERT P.D.N., HICKEY D.A. 2007. DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. – Trends in Genetics 23(4): 167–172.
- HARRISON R.G., LARSON E.L. 2014. Hybridization, introgression, and the nature of species boundaries. – Journal of Heredity **105** (Special Issue): 795–809.
- HÁGVAR E.B., HOFSVANG T. 1991. Aphid parasitoids (Hymenoptera, Aphidiidae): biology, host selection and use in biological control. Biocontrol News and Information **12**: 13–42.
- HAVELKA J., SHUKSHUK A.H., GHALIOW M.E., LAAMARI M., KAVAL-LIERATOS N.G., TOMANOVIĆ Ž., RAKHSHANI E., PONS X., STARÝ P. 2011. Review of invasive grapevine aphid, *Aphis illinoisensis* Shimer, and native parasitoids in the Mediterranean (Hemiptera, Aphididae; Hymenoptera, Braconidae, Aphidiinae). – Archives of Biological Sciences **63**: 269–274.
- JAMHOUR A., MITROVIĆ M., PETROVIĆ A., STARÝ P., TOMANOVIĆ Ž. 2016. Re-visiting the *Aphidius urticae* s. str. group: re-description of *Aphidius rubi* Starý and *A. silvaticus* Starý (Hymenoptera: Braconidae: Aphidiinae). – Zootaxa **4178**: 278–288.
- KAMBHAMPATI S., VÖLKL W., MACKAUER M. 2000. Phylogenetic relationships among genera of Aphidiinae (Hymenoptera: Braconidae) based on DNA sequence of the mitochondrial 16S rRNA gene. – Systematic Entomology 25: 437–445.
- KLINGENBERG C.P. 2011. MorphoJ: an integrated software package for geometric morphometrics. – Molecular Ecology Resources 11: 353–357.
- KAVALLIERATOS N.G., LYKOURESSIS D.P., SARLIS G.P., STATHAS G.J., SANCHIS SEGOVIA A., ATHANASSIOU C.G. 2001. The Aphidiinae (Hymenoptera: Ichneumonoidea: Braconidae) of Greece. – Phytoparasitica 29: 306–340.
- KAVALLIERATOS N.G., TOMANOVIĆ Ž., STARÝ P., ATHANASSIOU C.G., SARLIS G.P., PETROVIĆ O., NIKETIĆ M., VERONIKI M.A. 2004. A survey of aphid parasitoids (Hymenoptera: Braconidae: Aphidiinae) of southeastern Europe and their aphid-plant associations. – Applied Entomology and Zoology 39: 527–563.
- KAVALLERATOS N.G., TOMANOVIĆ Ž., ATHANASSIOU CG., STARÝ P., ŽIKIĆ V., SARLIS G.P., FASSEAS C. 2005. Aphid parasitoids infesting cotton, citrus, tobacco and cereal crops in southeastern Europe: aphid-plant associations and keys. – The Canadian Entomologist 137: 516–531.
- KAVALLIERATOS N.G., TOMANOVIĆ Ž., STARÝ P., ŽIKIĆ V., PETROVIĆ-OBRADOVIĆ O. 2010. Parasitoids (Hymenoptera: Braconidae: Aphidiinae) attacking aphids feeding on Solanaceae and Cucurbitaceae crops in southeastern Europe: aphidiine-aphid-plant associations and key. – Annals of the Entomological Society of America 103: 153–164.
- KAVALLIERATOS N.G., TOMANOVIĆ Ž., PETROVIĆ A., JANKOVIĆ M., STARÝ P., YOVKOVA M., ATHANASSIOU C.G. 2013. Review and key for the identification of parasitoids (Hymenoptera: Braconidae: Aphidiinae) of aphids infesting herbaceous and shrubby ornamental plants in southeastern Europe. – Annals of the Entomological Society of America 106: 294–309.
- KAVALLIERATOS N.G., TOMANOVIĆ Ž., PETROVIĆ A., KOCIĆ K., JAN-KOVIĆ M., STARÝ P. 2016. Parasitoids (Hymenoptera: Braconidae: Aphidiinae) of aphids feeding on ornamental trees in southeastern Europe: key for identification and tritrophic associations. – Annals of the Entomological Society of America 109: 473– 487.
- Kos K., PETROVIĆ A., STARÝ P., KAVALLIERATOS N.G., IVANOVIĆ A., TOŠEVSKI I., JAKŠE J., TRDAN S., TOMANOVIĆ Ž. 2011. On the identity of the cereal aphid parasitoid wasps *Aphidius uzbekistanicus*, *Aphidius rhopalosiphi* and *Aphidius avenaphis* (Hymenoptera: Braconidae: Aphidiinae) by examination of the COI mitochondrial gene, geometric morphometrics and morphology. – Annals of the Entomological Society of America 104: 1221–1232.
- LACHENBRUCH P.A. 1967. An almost unbiased method of obtaining confidence intervals for the probability of misclassification in discriminant analysis. Biometrics **23**: 639–645.

- LIBRADO P., ROZAS J. 2009. DnaSP v5: A software for comprehensive analysis of DNA plumorphism data. – Bioinformatics **25**: 1451–1452.
- LIEPERT C., DETTNER K. 1996. Role of cuticular hydrocarbons of aphid parasitoids in their relationship to aphid-attending ants. Journal of Chemical Ecology **22**: 695–707.
- MACKAUER M. 1960. Die europäischen Arten der Gattung Lysiphlebus Förster (Hymenoptera: Braconidae, Aphidiinae). Eine monographische Revision. – Beiträge zur Entomologie 10: 582–623.
- MACKAUER M. 1968. Hymenopterorum Catalogus. Part 3. Aphidiidae. – Dr. W Junk, The Hague.
- MARSH P.M. 1971. Keys to the Nearctic genera of the families Braconidae, Aphidiidae, and Hybrizontidae (Hymenoptera). – Annals of the Entomological Society of America 64: 841–850.
- MESCHELOFF E., ROSEN D. 1990. Biosystematic studies on the Aphidiidae of Israel (Hymenoptera: Ichneumonoidea). 3. The genera *Adialytus* and *Lysiphlebus*. – Israel Journal of Entomology 24: 35–50.
- MITROVSKI BOGDANOVIĆ A., PETROVIĆ A., MITROVIĆ M., IVANOVIĆ A., ŽIKIĆ V., STARÝ P., VORBURGER C., TOMANOVIĆ Ž. 2013. Identification of two cryptic species within the *Praon abjectum* group (Hymenoptera: Braconidae: Aphidiinae) using molecular markers and geometric morphometrics. – Annals of the Entomological Society of America **106**: 170–180.
- MITROVIĆ M., PETROVIĆ A., KAVALLIERATOS N.G., STARÝ P., PETROVIĆ-OBRADOVIĆ O., TOMANOVIĆ Ž., VORBURGER C. 2013. Geographic structure with no evidence for host-associated lineages in European populations of *Lysiphlebus testaceipes*, an introduced biological control agent. – Biological Control **66**: 150–158.
- NYABUGA F.N., LOXDALE H.D., HECKEL D.G., WEISSER W.W. 2010. Spatial population dynamics of a specialist aphid parasitoid, *Lysiphlebus hirticornis* Mackauer (Hymenoptera: Braconidae: Aphidiinae): evidence for philopatry and restricted dispersal. – Heredity **105**: 433–442.
- PAMILO P., NEI M. 1988. Relationships between gene trees and species trees. – Molecular Biology and Evolution 5: 568–583.
- PARREÑO A., IVANOVIĆ A., PETROVIĆ A., ŽIKIĆ V., TOMANOVIĆ Ž., VOR-BURGER C. 2017. Wing shape as a taxonomic trait: Separating genetic variation from host-induced plasticity in aphid parasitoids. – Zoological Journal of the Linnean Society 180: 288–297.
- PETROVIĆ A., MITROVIĆ M., STARÝ P., PETROVIĆ-OBRADOVIĆ O., ŽIKIĆ V., TOMANOVIĆ Ž., VORBURGER C. 2013. Lysiphlebus orientalis (Hymenoptera, Braconidae), a new invasive aphid parasitoid in Europe – evidence from molecular markers. – Bulletin of Entomological Research 103: 451–457.
- PETROVIĆ A., MITROVIĆ M., IVANOVIĆ A., ŽIKIĆ V., KAVALLIERATOS N.G., STARÝ P, MITROVSKI-BOGDANOVIĆ A., TOMANOVIĆ Ž., VOR-BURGER C. 2015. Genetic and morphological variation in sexual and asexual parasitoids of the genus Lysiphlebus – an apparent link between wing shape and reproductive mode. – BMC Evolutionary Biology 15: 5.
- PIKE K.S., STARÝ P., MILLER T., GRAF G., ALLISON D., BOYDSTON L., MILLER R. 2000. Aphid parasitoids (Hymenoptera: Braconidae: Aphidiinae) of northwest USA. – Proceedings of the Entomological Society of Washington 102: 688–740.
- QUICKE D.L., VAN ACHTERBERG C. 1990. Phylogeny of the subfamilies of the family Braconidae (Hymenoptera: Ichneumonoidea). – Zoologische Verhandelingen Leiden **258**: 1–95.
- RABASSE J.M., COCEANO P.G., BARBAGALLO S. 2005. On the presence in France and North Italy of *Siphonatrophia cupressi* (Homoptera, Aphididae), a new aphid of North American origin living on Cupressaceae. – Bollettino di Zoologia Agraria e di Bachicoltura **37**: 77–83.
- RAGSDALE D.W., LANDIS D.A., BRODEUR J., HEIMPEL G.E., DESNEUX N. 2011. Ecology and management of the soybean aphid in North America. – Annual Review of Entomology 56: 375–399.
- RAKHSHANI E., STARÝ P., TOMANOVIĆ Ž. 2012. Species of *Adialytus* Förster, 1862 (Hymenoptera, Braconidae, Aphidiinae) in Iran: taxonomic notes and tritrophic associations. – ZooKeys **221**: 81–95.

- RAKHSHANI E., STARÝ P., TOMANOVIĆ Ž. 2013. Tritrophic associations and taxonomic notes on *Lysiphlebus fabarum* (Marshall) (Hymenoptera: Braconidae: Aphidiinae), a keystone aphid parasitoid in Iran. – Archives of Biological Sciences 65: 667–680.
- ROUCHET R., VORBURGER C. 2014. Experimental evolution of parasitoid infectivity on symbiont-protected hosts leads to the emergence of genotype-specificity. – Evolution 68: 1607–1616.
- SAITOU N., NEI M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. – Molecular Biology and Evolution 4: 406–425.
- SANCHIS A., MICHELENA J.M., PUJADE-VILLAR J. 1999. Afidiinos (Hymenoptera, Braconidae) del Pirineo Andorrano. Boletín de la Asociación Española de Entomología **23**: 239–247.
- SANCHIS A., LATORRE A., GONZÁLEZ-CANDELAS F., MICHELENA J.M. 2000. An 18S rDNA-based molecular phylogeny of Aphidiinae (Hymenoptera: Braconidae). – Molecular Phylogenetics and Evolution 14: 180–194.
- SANDROCK C., VORBURGER C. 2011. Single-locus recessive inheritance of asexual reproduction in a parasitoid wasp. – Current Biology **21**: 433–437.
- SANDROCK C., SCHIRRMEISTER B.E., VORBURGER C. 2011. Evolution of reproductive mode variation and host associations in a sexualasexual complex of aphid parasitoids. – BMC Evolutionary Biology 11: 348.
- SHARKEY M.J., WHARTON R.A. 1997. Morphology and terminology. Pp. 19–37 in: WHARTON R.A., MARSH P.M., SHARKEY M.J. (eds), Manual of the New World Genera of the Family Braconidae (Hymenoptera). Special Publication 1. – International Society of Hymenopterists, Washington, DC.
- SCHÄR S., VORBURGER C. 2013. Host specialization of parasitoids and their hyperparasitoids on a pair of syntopic aphid species. – Bulletin of Entomological Research 103: 530–537.
- SHUFRAN K.A., WEATHERSBEE A.A., JONES D.B., ELLIOTT N.C. 2004. Genetic similarities among geographic isolates of *Lysiphlebus testaceipes* (Hymenoptera: Aphidiidae) differing in cold temperature tolerances. – Environmental Entomology 33: 776– 778.
- SIMON J.C., DELMOTTE F., RISPE C., CREASE T. 2003. Phylogenetic relationships between parthenogens and their sexual relatives: the possible routes to parthenogenesis in animals. – Biological Journal Linnean Society **79**: 151–163.
- STANKOVIĆ S.S., PETROVIĆ A., ILIĆ MILOŠEVIĆ M., STARÝ P., KAVAL-LIERATOS N.G., ŽIKIĆ V., TOMANOVIĆ Ž. 2015. Morphological and molecular characterization of the common European *Adialytus* species (Hymenoptera: Braconidae: Aphidiinae) based on the mtCOI barcoding gene and geometric morphometrics of fore wings. – European Journal of Entomology **112**: 165–174.
- STARÝ P. 1961. Faunistic survey of Czechoslovak species of the genera Lysiphlebus Förster and Trioxys Haliday:(Hymenoptera: Aphidiidae). – Acta Faunistica Entomologica Musei Nationalis Pragae 7: 131–149.
- STARÝ P. 1965. Aphidiid parasites of aphids in the USSR (Hymenoptera: Aphidiidae). – Acta Faunistica Musei Nationalis Pragae 96: 187–227.
- STARÝ P. 1971. Lysiphlebus alpinus n. sp., a new aphid parasite from the Austrian Alps (Hymenoptera, Aphidiidae). – Mitteilungen der Schweizerischen Entomologischen Gesellschaft 44: 299–302.
- STARÝ P. 1975. The subgeneric classification of *Lysiphlebus* Förster, 1862 (Hymenoptera, Aphidiidae). Annotationes Zoologicae et Botanicae, Bratislava **105**: 1–9.
- STARÝ P. 1979. Aphid Parasites (Hymenoptera, Aphidiidae) of the Central Asian Area. – Springer Science & Business Media, Dordrecht. 114 pp.
- STARÝ P. 1985. Two new *Lysiphlebus* species from Europe. Acta Entomologica Bohemoslovaca **82**: 426–430.
- STARÝ P. 2006. Aphid parasitoids of the Czech Republic (Hymenoptera: Braconidae, Aphidiinae). – Academia, Praha. 430 pp.
- STARÝ P., REMAUDIÈRE G., LECLANT F. 1971. Les Aphidiidae (Hym.) de France et leurs Hôtes (Hom. Aphididae): description d'es-

pèces nouvelles écologie lutte biologique. – Mémoire Hors-série 5. 72 pp.

- STARÝ P., REMAUDIÈRE G. 1973. Some aphid parasites [Hym., Aphidiidae] from Spain. – Entomophaga 18: 287–290.
- STARÝ P., LECLANT F., LYON J.P. 1975. Aphidiides (Hym.) et aphides (Rom.) de Corse I. Les Aphidiides. – Annales de la Société Entomologique de France 11: 745–762.
- STARÝ P., LYON J.P., LECLANT F. 1988a. Biocontrol of aphids by the introduced *Lysiphlebus testaceipes* (Cress.) (Hym., Aphidiidae) in Mediterranean France. – Journal of Applied Entomology 105: 74–87.
- STARÝ P., LYON J.P., LECLANT F. 1988b. Post-colonisation host range of *Lysiphlebus testaceipes* in the Mediterranean area (Hymenoptera, Aphididae). – Acta Entomologica Bohemoslovaca 85: 1–11.
- STARÝ P., GERDING I., NORAMBUENA I., REMAUDIÈRE G. 1993. Environmental research on aphid parasitoid biocontrol agents in Chile (Hym., Aphidiidae; Hom., Aphidoidea). – Journal of Applied Entomology 115: 292–306.
- STARÝ P., TOMANOVIĆ Ž., PETROVIĆ O. 1998. A new parasitoid of root-feeding aphids from the Balkan mountains (Hymenoptera, Braconidae, Aphidiinae). – Deutsche Entomologische Zeitschrift 45: 175–179.
- STARÝ P., LUMBIERRES B., PONS X. 2004. Opportunistic changes in the host range of *Lysiphlebus testaceipes* (Cr.), an exotic aphid parasitoid expanding in the Iberian Peninsula. – Journal of Pest Science 77: 139–144.
- STARÝ P., HAVELKA J. 2008. Fauna and associations of aphid parasitoids in an up-dated farmland area (Czech Republic). – Bulletin of Insectology 61: 251–276.
- STARÝ P., LUKÁŠ J. 2009. Aphid parasitoids and their tritrophic associations in Slovakia (Hymenoptera: Braconidae, Aphidiinae). – Folia Hymenopterologica Slovaca, Bratislava, 1, 63 pp.
- STARÝ P., ŘAKHSHANI E., TOMANOVIĆ Ž., HOELMER K., KAVALLIERATOS N.G., YU M., WANG J., HEIMPEL G.E. 2010. A new species of Lysiphlebus Förster 1862 (Hymenoptera: Braconidae: Aphidiinae) attacking soybean aphid, Aphis glycines Matsumura (Hemiptera: Aphididae) from China. – Journal of Hymenoptera Research 19: 179–186.
- STARÝ P., KAVALLIERATOS N.G., PETROVIĆ A., ŽIKIĆ V., RAKHSHANI E., TOMANOVIĆ S., TOMANOVIĆ Ž., HAVELKA J. 2014. Interference of field evidence, morphology, and DNA analyses of three related *Lysiphlebus* aphid parasitoids (Hymenoptera: Braconidae: Aphidiinae). – Journal of Insect Science 14: 1–6.
- TAKADA H. 1968. Aphidiidae of Japan (Hymenoptera). Insecta Matsumurana 30: 67–124.

- TAMURA K., PETERSON D., PETERSON N., STECHER G., NEI M., KUMAR S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. – Molecular Biology and Evolution 28: 2731–2739.
- TAMURA K. 1992. Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + Ccontent biases. – Molecular Biology and Evolution 9: 678–687.
- TOMANOVIĆ Ž., KAVALLIERATOS N.G., STARÝ P., STANISAVLJEVIĆ LJ., ĆETKOVIĆ A., STAMENKOVIĆ S., JOVANOVIĆ S., ATHANASSIOU C.G. 2009. Regional tritrophic relationship patterns of five aphid parasitoid species (Hymenoptera: Braconidae: Aphidiinae) in agroecosystem-dominated landscapes of southeastern Europe. – Journal of Economic Entomology **102**: 836–854.
- TOMANOVIĆ Ž., PETROVIĆ A., MITROVIĆ M., KAVALLIERATOS N.G., STARÝ P., RAKHSHANI E., RAKHSHANIPOUR M., POPOVIĆ A., SHUK-SHUK A.H., IVANOVIĆ A. 2014. Molecular and morphological variability within the *Aphidius colemani* group with redescription of *Aphidius platensis* Brethes (Hymenoptera: Braconidae: Aphidiinae). – Bulletin of Entomological Research 104: 552–565.
- TREMBLAY E., EADY R.D. 1978. Lysiphlebus confusus n. sp. per Lysiphlebus ambiguus sensu Auct. nec Haliday (1834) (Hymenoptera Ichneumonoidea). – Bollettino del Laboratorio di Entomologia Agraria di Portici 35: 180–184.
- YE Z., VOLLHARDT I.M.G., TOMANOVIĆ Ž., TRAUGOTT M. 2017. Evaluation of three molecular markers for identification of European primary parasitoids of cereal aphids and their hyperparasitoids. PLoS ONE 12(5): e0177376. https://doi.org/10.1371/journal. pone.0177376
- YU D.S., ACHTERBERG C. VAN, HORSTMANN K. 2016. World Ichneumonoidea 2015: taxonomy, biology, morphology and distribution. – Taxapad [database]; http://www.taxapad.com/
- VORBURGER C., ROUCHET R. 2016. Are aphid parasitoids locally adapted to the prevalence of defensive symbionts in their hosts? – BMC Evolutionary Biology 16: 271.
- VÖLKL W. 1994. The effect of ant-attendance on the foraging behaviour of the aphid parasitoid *Lysiphlebus cardui*. – Oikos **70**: 149–155.
- ŽIKIĆ V., STANKOVIĆ S.S., ILIĆ MILOŠEVIĆ M., PETROVIĆ-OBRADOVIĆ O., PETROVIĆ A., STARÝ P., TOMANOVIĆ Ž. 2015. First detection of *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Aphidiinae) in Serbia: an introduced species invading Europe? – North-Western Journal of Zoology 11: 97–101.

Electronic Supplement File

at http://www.senckenberg.de/arthropod-systematics

File 1: tomanovic&al-europeanlysiphlebus-asp2018-electronicsupplement-1.doc — **Table S1.** List of analyzed species from the genus *Lysiphlebus* submitted to molecular analyses of mitochondrial COI and nuclear 28S fragments.

Zoobank Registrations

at http://zoobank.org

Present article: http://zoobank.org/urn:lsid:zoobank. org:pub:6356F7BC-BSC2-43BF-AB02-F4381575AS20

Lysiphlebus volkli Tomanović & Kavallieratos, 2018: http://zoobank.org/urn:lsid:zoobank.org:act:AE5328E8-2161-446E-953A-AD4472DE98E8

Lysiphlebus brachycaudi Starý & Tomanović, 2018: http://zoobank.org/urn:lsid:zoobank.org:act:0277D5CE-7753-41E9-DA7F-30558B38DB6A

ZOBODAT - www.zobodat.at

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