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Molecular characterization and phylogenetic relationships among European *Aphidius* Nees (Hymenoptera, Braconidae, Aphidiinae)

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УНИВЕРЗИТЕТ У БЕОГРАДУ
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Молекуларна карактеризација и филогенетски односи европских врста рода *Aphidius* Nees (Hymenoptera, Braconidae, Aphidiinae)

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Author

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ABSTRACT

The genus *Aphidius* includes many species of economic importance that are used as biocontrol agents against numerous pest aphids in greenhouses and under open field conditions. However, classification within this genus is constantly revisited, in view of the fact that the biology, ecology and taxonomic status of many species are still understudied. Partial sequences of the cytochrome oxidase subunit I mitochondrial gene (mtCOI) and Elongation factor 1-α nuclear gene (EF1-α) were used to explore the taxonomic status and phylogenetic relationships of 33 European species from the genus *Aphidius* in different aphid /plant host associations over a wide area of distribution. Phylogenetic analyses clarified that *Aphidius* is in fact a paraphyletic group. Topology of the maximum likelihood tree showed separation of 22 taxa as independent species: *A. erysimi, A. sonchi, A. linosiphonis, A. hieraciorum, A. arvensis, A. balcanicus, A. phalangomyzi, A. banksae, A. uzbekistanicus, A. sussi, A. silvaticus, A. avenae, A. rosae, A. ericaphidis, A. eadyi, A. viaticus, A. schimitscheki, A. ribis, A. setiger, A. asteris, A. matricariae* and *A. urticae.* Besides "good" species, five more clades were distinguished: i) *A. salicis* and *A. aquilus*; ii) *A. funebris, A. tanacetarius, A. absinthii*; iii) *A. ervi* and *A. microlophii*; iv) *A. chaetosiphonis* and *A. hortensis*; v) *A. rubi* and *A. rhopalosiphi*. Taxa within five clades could not be clearly discriminated as separate species based on either mtCOI or EF1-α. Failure of the two markers to delimit these taxa could be attributed either to adaptive divergence due to host and/or habitat range expansion and speciation or to mitochondrial introgression via hybridization of sibling species. In any event, it is suggested that their taxonomic status be re-visited using an integrative approach. Molecular characterization revealed cryptic taxa associated with different hosts within the *A. urticae* group. Re-descriptions of *A. urticae* s. str., *A. rubi* and *A. silvaticus* are given. Also, mtDNA barcoding identified the presence of *A. ericaphidis* for the first time in Europe.

Key words: *Aphidius*, mtDNA barcoding, speciation, elongation factor 1-α, paraphyletic group

Scientific field: Biology

Scientific subfield: Morphology, systematics and phylogeny of animals

UDK number: [575.8+577.21]:595.79(4)(043.3).

Молекуларна карактеризација и филогенетски односи европских врста рода *Aphidius* Nees (Hymenoptera, Braconidae, Aphidiinae)

САЖЕТАК

У род *Aphidius* класификоване су многе врсте које су економски значајне као биолошки агенти за контролу штетних врста биљних вашију у стакленицима и на отвореном пољу. Обзиром да биологија, екологија и таксономски статус многих врста нису довољно истражени, класификација врста унутар рода се континуирано ревидира. Делимичне секвенце гена митохондријске ДНК цитохром оксидазе субјединица I (mtCOI) и једарног гена за фактор елонгације 1-α а (EF1-α) су маркери коришћени у истраживању таксономског статуса и филогенетиских односа 33 врсте рода *Aphidius* у асоцијацији са раличитим врстама ваши и биљака домаћина сакупљених са ширег арела у Европи. Филогенетске анализе су потврдиле да је род *Aphidius* парафилетички. На филогенетском стаблу конструисаном *maximum likelihood* методом јасно је издвајање следећа 22 таксона као засебних врста: *A. erysimi, A. sonchi, A. linosiphonis, A. hieraciorum, A. arvensis, A. balcanicus, A. phalangomyzi, A. banksae, A. uzbekistanicus, A. sussi, A. silvaticus, A. avenae, A. rosae, A. ericaphidis, A. eadyi, A. viaticus, A. schimitscheki, A. ribis, A. setiger, A. asteris, A. matricariae* и *A. urticae* Поред ових „добрих“ врста, издовјило се још пет клада: i) *A. salicis* и *A. aquilus*; ii) *A. funebris, A. tanacetarius, A. absinthii*; iii) *A. ervi* и *A. microlophii*; iv) *A. chaetosiphonis* и *A. hortensis*; v) *A. rubi* и *A. rhopalosiphi*. Врсте у овим кладама се не могу јасно идентификовати на основу митохондријског и једарног маркера. Неуспех у идентификацији врста применом ових маркера може се приписати адаптивној дивергенцији услед ширења круга домаћина или станишта и специјацији, или митохондријалној интрогресији приликом хибридизације примерака сродних врста. За поједине врсте сугерише се ревизија таксономског статуса, примењујући савремени интегративни приступ. Молекуларна идентификација mtCOI открила је у оквиру комплекса врста *A. urticae* три криптичне врсте *A. urticae* s. str., *A. rubi* и *A. silvaticus*. Такође, ДНК баркодинг метод је потврдио први пут у Европи присуство врсте *A. ericaphidis.*

Кључне речи: *Aphidius,* ДНК баркодинг, специјација, фактор елонгације 1-α, парафилетичка група

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1. INTRODUCTION

1.1. General introduction to the subfamily Aphidiinae and its economic importance

General introduction. Aphidiinae is a subfamily of aphid parasitoids within the family Braconidae (Hymenoptera). It consists of over 500 described species, belonging to more than 60 genera (Yu et al. 2012), and four tribes Aclitini, Aphidiini, Ephedrini and Praini (Smith & Kambhampati, 2000). They are distributed worldwide, especially in the temperate and subtropical belts of the northern hemisphere, closely following distribution of their aphid hosts (Starý, 1988b). Members of this subfamily are obligatory and solitary endoparasitoids of aphids, with only a single specimen completing its development inside the host. They belong to the group of koinobionts, which means that the parasitoid larva develops in a living host until it reaches the stage of maturity (Starý, 1970).

The host specialization of aphidiine parasitoids ranges from monophagous forms to generalists (Starý, 1970). The monophagous species are restricted to a single host (e.g. *Pseudopraon mindariphagum* Starý, 1975 which parasitizes only *Mindarus abietinus* Koch, 1857 while the generalists parasitize from two or more aphid genera of the same or more subfamilies, to more than a hundred aphid hosts (e.g. *Ephedrus persicae* Frogatt, 1904) (Gärdenfors, 1986; Starý, 1988b; Tremblay & Pennacchio, 1988).

Economic importance. Aphids (Hemiptera: Aphidoidea) are among the economically most important pests threatening the well-being of cultivated and wild-growing plants in different ecosystems. They cause direct damage to most crops by feeding or inflict indirect damage as vectors of over 200 plant viruses (Hogenhout et al. 2008). Furthermore, aphids have a high reproductive capability, some of the species producing more than 10 generations in one year. They also are capable of developing resistance to many insecticides, which makes pest management rather difficult to maintain (Iversen & Harding, 2007).

Interest in alternative methods of pest management such as biological control has increased after the development of aphid resistance to insecticides, which led to stricter regulation of pesticide use. One of the most promising techniques in biological control programs is using their natural enemies for the regulation of pests. As solitary endoparasitoids, Aphidiinae are one of the most important natural enemies of aphids and can

effectively regulate their populations (Hagvar & Hofsvang, 1991). Endoparasitoids attack essentially all life stages of aphid hosts except the eggs. For this reason, they play an important role in keeping aphid populations below the economic threshold and preventing serious outbreaks in agricultural landscapes of different geographical regions (Hagvar & Hofsvang, 1991; Brewer & Elliott, 2004; Kavallieratos et al. 2010; Boivin et al. 2012).

Aphidiine parasitoids have been commercially produced and released as classical biological control agents of aphids in many regions and have achieved significant results. The most important genera used in biological control are *Aphidius* Nees, 1818; *Diaeretiella* Starý, 1960; *Ephedrus* Haliday, 1833 and *Praon* Haliday, 1833 (Vollhardt et al. 2008; Boivin et al. 2012).

According to Hagvar & Hofsvang (1991) the most successful species for controlling *Myzus persicae* Sulzer, 1776 on cucumber in the Netherlands, sweet pepper in Britain and Russia, different vegetables in Germany, and tomato in Canada were *Aphidius colemani* Viereck, 1912, and *A. matricariae* Haliday, 1834. In the United States good results in controlling the blue alfalfa aphid *Acyrthosiphon kondoi* Shinji, 1938 and pea aphid *A. pisum* Harris, 1776 were obtained with the introduced parasitoid species *Aphidius eadyi* Starý, Gonzalez & Hall, 1980, *A. smithi* Subba Rao and Sharma, 1959, *A. stary* Chen & Luhman, 1991, *Ephedrus plagiator* Nees, 1811 and *Praon barbatum* Mackauer, 1967 (Gonzalez.et al. 1995). Furthermore, *A. colemani*, *A. matricariae*, *A. rhopalosiphi* de Stefani-Perez, 1902, *E. plagiator* and *Praon gallicum* Stary, 1971 were used in biological control of the Russian wheat aphid *Diuraphis noxia* Kurdjumov, 1913 in North America (Gonzalez et al. 1995; Boivin et al. 2012).

According to Teulon et al. (2008) introductions in New Zealand included *Aphidius eadyi* and *A. ervi* Haliday, 1834 to control the pea aphid (*Acyrthosiphon pisum*) and the blue alfalfa aphid *Acyrthosiphon kondoi*; *Trioxys complanatus* Quilis, 1931 to control the spotted alfalfa aphid *Therioaphis trifollii* Monell, 1882; *A. rhopalosiphi* to control the rose-grain aphid *Metopolophium dirhodum* Walker, 1849 on cereals etc. Additionally, *A. smithi*, *Ephedrus plagiator* and *Praon barbatum* were introduced to New Zealand in 1977 for biocontrol of *A. kondoi* and *A. pisum*.

Biological control currently applied in greenhouses gives the best results with *Aphidius colemani* to control *Aphis gossypii* Glover, 1877 and *A. ervi* to control the potato aphid *Macrosiphum euphorbiae* Thomas, 1878 (van Lenteren, 2012).

1.2. Biological traits, modes of reproduction and host associated behaviour of Aphidiinae

Biological traits. Females of aphidiine wasps oviposit a single microscopic egg into the bodies of their aphid hosts, after which the larva hatches and completes its development consuming tissues within the host. The parasitoids go through four larval instars before pupating. The aphid dies before pupation of the parasitoid, forming a 'mummy' that consists of the hardened exoskeleton of the host. The parasitoid larva pupates inside the mummy in the majority of parasitoid species (e.g. *Aphidius*, *Lysiphlebus* Förster, 1862, *Trioxys* Haliday, 1833), while in species of the genera *Praon* and *Dyscritulus* Hincks, 1943 the larvae eat their way out of the aphid and spin a cocoon underneath the dead aphid. The adult parasitoids emerge from the aphid mummy by biting a circular hole.

Males often emerge before females and upon emergence need a short time to mature and mate. The females release pheromones to attract males and copulation usually takes between 15 and 80 seconds. Females copulate only once and start searching for a suitable host for oviposition, while males can copulate several times. The female fecundity is about 300 - 1800 eggs (Mackauer & Chow 1986), and the whole life cycle lasts 9 to 15 days (approx. 11 days at 23ºC) (Starý, 1970).

Modes of reproduction. Two modes of reproduction have been demonstrated in the Aphidiinae. The first is arrhenotoky (sexual reproduction), when unfertilized eggs develop as haploid males and fertilized eggs give females. This mode of reproduction is most common for *Aphidius* species. The second mode of reproduction is thelytokous (asexual) reproduction, when unfertilized eggs produce diploid females (e.g. *Lysiphlebus* species).

***Oviposition behaviour*.** According to Hofsvang & Hagvar (1991), the oviposition behaviour of aphidiinae wasps has been classified into six groups. The first type is unspecialized oviposition behaviour with long oviposition time varying between 5 and 10 seconds (e.g., Ephedrini). The second and third types have various morphological and behavioural adaptations like immobilization of the potential host for oviposition (e.g. Trioxini). The fourth type is characterized by females with a highly flexible abdomen and very long oviposition time (e.g., *Pauesia* Quilis, 1931). In the fifth type, females usually avoid direct contact with a potential host by administering a quick sting that ensures a short oviposition time (less than 5 sec), as in the case of *Aphidius* species. Species with behaviour

of the sixth type such as *Adialytus* Förster, 1862 and *Lysiphlebus*, have evolved chemical strategies to counter the guarding behaviour of trophobiotic ants.

***Host selection by Aphidiinae*.** The larvae of Aphidiinae are obligatory parasitoids, which means they entirely depend on the host for their development, feeding exclusively on its tissues until they reach the stage of maturity. For this reason, the reproductive success of parasitoids is strongly dependent on the fitness of females and their ability to select a suitable aphid host for development of their progeny (Godfray, 1994). The process of host location and selection is rather complex, and can be classified into the following five general steps which sometimes can be overlapping: (1) host habitat location, (2) host location, (3) host acceptance, (4) host suitability, and (5) host regulation (Hagvar & Hofsvang, 1991; Rehman & Powell, 2010).

1.3. Molecular tools in taxonomy of parasitoids

Traditional methods of taxonomy and species identification based on comparison of morphological traits have been shown to be influenced by environmental conditions, trophic interactions, and the intraspecific morphological plasticity of individuals (Pfenninger & Schwenk, 2007). With many parasitoids, identification based on morphology has often been shown to be inadequate in distinguishing taxa and misleading in the case of cryptic species complexes (Pungerl, 1986; Landry et al. 1993).

Over the second half of the past century, molecular techniques have become increasingly important as a supplement to traditional methods of species identification (Hebert et al. 2003, Savolainen et al. 2005, Witt et al. 2006). Development of the polymerase chain reaction (PCR) and various universal primer sequences for numerous genomic regions has increased the sensitivity and resolution of genetic analyses of even very small tissue samples (Mullis & Faloona, 1987; Simon et al., 1994). Sensitivity of specific PCR and the possibility to design species-specific primers have been used for identification of many hymenopteran parasitoid species, such as *Trichogramma australicum* Girault, 1912 (Amornsak et al., 1998), *Anaphes iole* Girault, 1911 (Hymenoptera: Mymaridae) (Zhu & Williams, 2002), *Peristenus stygicus* Loan, 1973 (Braconidae) (Zhu et al. 2004), etc.

Molecular markers have been used to provide valuable information about insect speciation by distinguishing sympatric from allopatric species (Ballinger-Crabtree et al. 1992;

Apostol et al. 1996; Banuls et al. 1999; Ayres et al. 2003; Margonari et al. 2004). These tools were used to study gene flow and genetic variations among and within the populations in efforts to explain the population structure and dynamics (Cervera et al. 2000), and infer the phylogeny of insect populations in order to understand their modes of evolution (Luque et al. 2002; Chatterjee & Tanushree, 2004).

Application of molecular techniques in diverse domains of parasitoids biology and ecological studies has significantly contributed to understanding their fundamental biology, genetic diversity and evolution. To avoid false interpretation, it is crucial to choose an appropriate molecular marker for a particular analysis with a rate of sequences substitution adequate for the level of divergence under study. Different types of mitochondrial and nuclear DNA markers have been widely applied in population genetics and phylogenetic studies that reveal valuable information regarding genetic diversity.

Nuclear genes represent thousands of highly conserved genetic loci. They evolve more slowly than mitochondrial genes, making them better markers to study the relationships among classes and phyla (Cho et al. 1995; Kelly & Palumbi, 2009). Nuclear gene sequences have two regions, slowly evolving exons and more rapidly evolving introns (Brower & DeSalle, 1994; Lina & Danforth, 2004). Multiple copy nuclear genes, represented by nuclear ribosomal RNA genes (rRNA) consisting of 18S, 5.8S and 28S subunits, ITS1 and ITS2 regions have been widely applied and have proved valuable in resolving relationships mostly at higher taxonomic levels (Malafronte et al. 2007). Among rRNA markers, the 18S subunit is the standard gene used to study relationships among arthropod classes (Turbeville et al. 1991; Wheeler et al. 1993a), among insect orders (Carmean et al. 1992), and within orders (Martin & Pashley, 1992; Wheeler et al. 1993b).

Single-copy nuclear genes, which are known as protein-coding regions, include: elongation factor 1-α (EF1-α), alcohol dehydrogenase (adh), 6-phosphogluconate dehydrogenase (g6pdh), Wingless (wg), PEPCK, DDC, white, opsin, hunchback (hb), and period (Brower & DeSalle, 1994; Caterino et al. 2000).

Elongation factor 1-α is a nuclear coding gene involved in the GTP-dependent binding of charged tRNAs to the acceptor site of the ribosome during translation (Sanchis et al. 2001). EF1-α is highly conserved nuclear coding gene useful for phylogenetic studies of relationships among species and genera within subfamilies (Cho et al. 1995; Belshaw & Quicke, 1997). It can be used to investigate recent divergences due to the presence of rapidly evolving introns

(Sanchis et al. 2001). However, a universal feature of intron sequences is that even closely related species exhibit insertion and deletion events, which cause variation in the lengths of the sequences. Indels are frequently rich in evolutionary information, but most investigators ignore sites that fall within these variable regions, largely because the analytical tools and theory are not well developed.

Mitochondrial markers (mtDNA) are the most widely used genes especially for determining phylogenetic differences between closely related species. Their maternal inheritance almost without recombination makes them valuable for population genetic studies, and they have a high rate of evolution, which is a useful feature for species delineation (Roehrdanz, 1993; Kambhampati & Smith, 1995; Zhang & Hewitt, 1997). Mitochondrial genes also do not have introns and rarely undergo recombination, thus making them useful for barcoding of species (Saccone et al. 1999). In general, animal mitochondrial genomes contain 37 genes: 13 protein-coding genes, 22 transfer RNA genes (tRNA) and two ribosomal RNA genes (rRNA) (Boore, 1999), some of which are applied repeatedly in phylogenetic studies. For example, the 16S rRNA gene is a useful molecular marker at the family and generic levels, while 12S rRNA is useful for studying genetic diversity in phyla and subphyla. The mitochondrial noncoding region, known as the control region responsible for regulation of transcription and replication (Vila & Bjorklund, 2004) is used for population genetics or phylogenetic analysis of closely related species. Mitochondrial protein-coding markers, such as cytochrome b, cytochrome oxidase subunit I and subunit II, ATPase 6, ND3 and ATPase 8, have evolutionary rates faster than those of rRNA genes, and for this reason they are used for evolutionary studies of families, genera and species.

Termed DNA barcoding, the most suitable approach for species discrimination is based on analysis of small gene segments of mitochondrial DNA (Hebert et al. 2003; Stoeckle, 2003). There are several studies on barcoding focused on mitochondrial ribosomal genes (12S, 16S, Cyt b), but their sequence alignments were complicated because of the insertions and deletions which are common in these genes (Doyle & Gaut, 2000). DNA barcoding as proposed by Hebert et al. (2003), is a process of species identification using a short standardized gene sequence of the mtDNA cytochrome c oxidase subunit I gene (COI). The concept of DNA barcoding based on 650- to 750-bp segments of the COI gene has proven to be an effective tool in diverse systematic studies of animal species. Among protein-coding genes, COI has been found to be the best molecular marker for evolutionary studies,

one with a high rate of nucleotide substitution and high frequency of insertion-deletion events. On the other hand, the COI gene has areas with relatively conserved sequences, which allowed the designing of universal primers for this gene (Folmer et al. 1994; Hebert et al. 2003). The Consortium for the Barcode of Life has selected the COI gene as the standard molecular barcode for animals (CBOL, http://www.barcodeoflife.org). The world-open Barcode of Life Database has over 4.4 million barcodes from different animal species (BOLD, http://www.boldsystems.org).

The barcoding method based on COI sequence analysis is well established. Now applied routinely, it has proven to be a powerful tool facilitating biodiversity research, phylogeny studies, and discrimination of cryptic species (Hebert et al. 2003; Hebert & Gregory, 2005). The subfamily Aphidiinae is a diverse group with many cryptic species (Žikić et al. 2009; Derocles et al. 2012). For this reason, reliable identification of parasitoids is particularly crucial in aphid biological control programs. Moreover, the identification of species complexes that share similar morphological characters based on taxonomic keys alone is a difficult task (Kavallieratos et al. 2001; Tomanović et al. 2003, 2007). Furthermore, molecular tools enable parasitoid species to be identified from their immature stages instead of relying solely on the morphological features of adult specimens.

Mitochondrial COI sequences analysis has been used to reconstruct the phylogenetic relationships within genera (Ahmadabadi et al. 2011), and to examine the phylogenetic affinity and diversity of Aphidiinae from different geographical regions (Lenin, 2015). In addition, it has successfully detected immature stages of Aphidiinae inside their aphid hosts, for example *Lysiphlebus testaceipes* Cresson, 1880 inside its host *Aphis fabae* Scopoli, 1763 (Traugott & Symondson 2008), parasitoids inside the grain aphid *Sitobion avenae* Fabricius, 1775 including *Aphidius ervi*, *A. picipes* Nees, 1811, *A. rhopalosiphi*, *A. uzbekistanicus* Luzhetzki, 1960, *Ephedrus plagiator*, *Praon volucre*, *P. gallicum* and *Toxares deltiger* Haliday, 1833 (Traugott et al. 2008). The COI barcode region has been employed as well to differentiate species inside *Praon abjectum* Haliday, 1833 (Mitrovski Bogdanović et al. 2013) and *Praon dorsale–yomenae* s. str. groups (Mitrovski Bogdanović et al. 2014). Besides species identification, it was recently used to discover new species that were introduced accidentally in new habitats, such as the invasive species *Lysiphlebus orientalis* Stary & Rakhshani, 2010 reported from Serbia (Petrović et al. 2013).

1.4. Systematics and phylogeny of the Aphidiinae

The classification within the subfamily Aphidiinae is extremely controversial. Generally, aphidiines are small elongate black or brown wasps, their size ranging from one to several mm. Head width is about equal to thorax width, antennae are filiform and rarely moniliform, the transverse clypeus is with long setae, the prothorax is hidden by the gibbous mesoscutum, and the propodeum is connected by a narrow petiole to the lanceolate gaster.

The phylogenetic hypotheses that have been proposed for this economically important group are based on: morphology (Mackauer, 1968; Chou, 1984; Gardenfors, 1986; O'Donnell, 1989; Finlayson, 1990), embryology (Tremblay & Calvert, 1971), and (more recently) DNA sequences (Belshaw & Quicke, 1997; Smith et al. 1999; Belshaw et al. 2000; Kambhampati et al. 2000; Sanchis et al. 2000; Shi & Chen, 2005).

The first monograph on the Aphidiinae group was written by Nees (1818), while the first author to describe and propose the classification of these wasps as a subfamily within the Braconidae was Haliday (1833) (Raychaudhuri, 1990). In a long series of papers published since 1833, there is disagreement about the taxonomic status of this group. Some authors treated the Aphidiinae group as a subfamily within Braconidae (Smith, 1944; Tremblay & Calvert, 1971; Van Achterberg, 1993), while others placed it in a separate family Aphidiidae within Ichneumonoidea Latreille, 1802 (Takada, 1968; Chow & Mackauer, 1992). There are several reasons for this disagreement. Takada (1968) distinguishes this group as a separate family within the superfamily Ichneumonoidea fact of specific parasitism and presence of a flexible suture between metasomal tergites two and three. Wharton et al. (1997) classified them as a separate family on the basis of the presence of other morphological features such as a short ovipositor, weakly sclerotized metasoma, smooth scutellar sulcus, hind wing lacking cross vein cu-a, and lateral occipital carina. Additionally, Short in 1952 called attention to significant differences in structure of the respiratory openings at the first larval stage, the existence of which also supports the opinion as to the family status of Aphidiidae.

The monophyletic status of Aphidiinae has been demonstrated in many studies, but there is disagreement concerning the phylogenetic place of certain Aphidiinae taxa. Some studies considered *Lysiphlebus* and *Aphidius* as members of subtribe Aphidiina within the tribe Aphidiini (Mackaur, 1961), while others consider *Lysiphlebus* as a separate clade (Smith et al. 1999; Sanchis et al. 2000; Chen et al. 2002; Ahmadi et al. 2011).

Edson & Vinson (1979) observed that Aphidiinae arose from two independent lines in the course of evolution and placed them as a subfamily within the family Braconidae. Also, phylogenetic studies of Ichneumonoidea using molecular data confirmed that Aphidiinae is a lineage within Braconidae (Quicke & van Achterberg, 1990; Whitfield, 1992; Belshaw & Quicke, 1997; Smith et al. 1999; Kambhampati et al. 2000). In addition, Aphidiinae were used in studies attempting to illustrate the phylogenetic connections among subfamilies within the family Braconidae (Dowton et al. 1998; Dowton, 1999; Shi et al. 2005) and others presenting phylogenetic reconstructions of the superfamily Ichneumonoidea (Belshaw et al. 1998).

The subfamily Aphidiinae has been divided into four tribes (Mackauer, 1961): Ephedrini, Praini, Aclitini and Aphidiini. The tribe Aphidiini includes the majority of known genera and species of parasitoids (Belshaw & Quicke, 1997; Smith et al. 1999). The main difference between these tribes lies in innervation of the front wings. Ephedrini and Praini have a complex wing venation, while Aphidiini tend to exhibit reduction in wing venation. Another difference is related to their specialization, Ephedrini and Praini are unspecialized; in contrast to Aphidiini, which have evolved in the direction of higher specialization. In fact, according to the literature Ephedrini and Praini are the most ancient clades of the Aphidiinae, because they possess many primitive braconid features in both larval and adult morphology (Mackauer, 1961; Starý, 1970; Tremblay & Calvert, 1971; Gärdenfors, 1986; O'Donnell, 1989; Finlayson, 1990). On the other hand, members of the tribe Aclitini shows several plesiomorphic characters (ancestral traits) and share a number of synapomorphies with Aphidiini, such as their distinct specialization for parasitizing root aphids. Accordingly, Takada & Shiga (1974) maintained that the tribe Aclitini is an intermediate form between the subtribes Aphidiina and Trioxina.

1.5. General information, economic importance and biological traits of *Aphidius* species

General information. Parasitoids of the genus *Aphidius* represent the largest group with over 70 species described worldwide, \nd this number is increasing continually (Tomanović et al. 2003; Kavallieratos et al. 2004). They are biologically and ecologically the most diverse group of parasitoids, but in terms of their taxonomic status, biology and ecology they have still been understudied (Sequeira & Mackauer, 1992; Brodeur & McNeil, 1994; Wei et al. 2003; Tahriri et al. 2007; Prado et al. 2015).

Aphidius species are cosmopolitan and able to adapt to different climatic conditions, inhabiting continental Europe, high plateaus, coasts and deserts. The area of their distribution includes continental North and South America, Europe, North Africa, the Middle East, Australia, New Zealand, and Asia (Starý, 1974; Marsh, 1977; Cameron et al. 1981; Raychaudhuri 1990; Tomanović et al. 2003; Kavallieratos et al. 2004: 2005). Their geographical distribution in fact follows that of their aphid hosts, with the result that they occur mostly in temperate regions.

Economic importance. The genus *Aphidius* consists of numerous species which are economically important natural enemies of aphids (*Myzus* Passerini, 1860; *Aphis* Linnaeus, 1758*; Brachycaudus* Van der Goot, 1913; *Acyrthosiphon* Mordvilko, 1914*; Macrosiphum* Passerini, 1860). Their high degree of specialization, great fecundity, and ability to become established in different environmental conditions make them good candidates for use in biological control programs. For this reason, *Aphidius* species have been utilized successfully worldwide in aphid management through releasing in open fields or augmentation in greenhouses. Good examples of successful application in biological control of aphids include the introduction of *A. ervi, A. eadyi* and *A. smithi* in South and North America, Australia and New Zealand to control the pea and blue alfalfa aphids (Cameron et al. 1981; Mackauer & Kambhampati, 1986; Cameron & Walker, 1989; Waterhouse & Sands, 2001); *Aphidius rhopalosiphi* was successfully introduced to New Zealand from England and France during 1985-1987 to control the aphid *Metopolophium dirhodum* on cereals. According to Grundy (1989) the use of *A. rhopalosiphi* provided annual benefits ranging between $300,000 and $5,000,000. It was recommended that *A. rhopalosiphi* be used as a bioagent to control *Sitobion avenae* on wheat crops in Belgium (Levie et al. 2005). Another successful release with reported fascinating results was that of *Aphidius gifuensis* Ashmead, 1906 to control *Myzus persicae* in tobacco fields in China (Wei et al. 2003; Yang et al. 2009).

In greenhouses the most commonly used and effective parasitoids of aphids are *A. colemani*, *A. matricariae* and *A. ervi*. Among those parasitoids, *A. colemani* is the one most generally employed as a commercial biological control agent: used in greenhouses and on open field crops, it has been mass-reared and sold commercially throughout the world since 1991 (Benelli et al. 2014). Although this wasp parasitizes more than 60 species of aphids (Ode et al. 2005), it is mainly used to control the economically important aphids *Myzus persicae* and *Aphis gossypii* on vegetable and ornamental crops grown in greenhouses, crops such as

peppers, cucumbers, tomatoes, bedding plants, foliage plants, and cut flowers (Bilu et al. 2006; Vásquez et al. 2006). It is capable of controlling pesticide-resistant strains of the aforementioned aphid species (Boivin et al. 2012). *Aphidius ervi* is another cosmopolitan species that parasitizes numerous aphid species in many different crops. It has been reared commercially in Europe for control of *Macrosiphum euphorbiae* on tomato and *Aulacorthum solani* Kaltenbach, 1843 on sweet pepper. In addition, *A. matricariae* with a host range of about 40 aphid species, is also produced commercially and is used in particular to control green peach aphid and closely related species in greenhouses.

Biological traits, reproductive mode and oviposition behaviour. The biology and behaviour of *Aphidius* species has been extensively studied (Volkl, 1994; Takada & Tada, 2000; Ode et al. 2005; Baaren et al. 2009). As in most aphidiinae species, the foraging females of this genus usually encounter hosts of different ages and sizes, then select the one most likely to increase their reproductive fitness. In order to locate potential hosts, in the guise of host plant characteristics or volatiles induced by aphid feeding to locate a promising habitat (Wickremasinghe & van Emden, 1992; Du et al. 1998; Guerrieri et al. 1999; Battaglia et al. 2000). For host recognition and acceptance, short range cues including host cuticle and cornicle secretion and honeydew [which act as contact kairomones (Powell & Zhi-LI, 1983; Bouchard & Cloutier, 1984; 1985)] play an important role, as do visual cues as well (Powell et al. 1998; Battaglia et al. 2000). The aphid host species and host-instar preferences are complex and variable (Barrette et al. 2009). These preferences of *Aphidius* species are based on quality of the host as a medium for development of the parasitoid's larva (Sequeira & Mackauer, 1992); defensive behaviour of the aphid, which affects the handling time for oviposition (Volkl, 1994; De Farias & Hopper, 1999); and host size, larger hosts containing more resources for the parasitoid's offspring (Mackauer & Kambhampati, 1988; Sequeira &. Mackauer, 1992). Host age may affect the sex allocation in parasitoids (Godfray, 1994), but the parasitoids for the most part prefer the second and third instar, even though all aphid instars are parasitized. Generalist species that have a broad host range, for example species such as *A. ervi*, are capable of switching their host preference behaviour according to which hosts are available (Cameron & Walker, 1989).

Most *Aphidius* species are characterized by arrhenotoky (sexual reproduction), which means that unfertilized eggs develop into males and fertilized eggs give rise to females. Oviposition behaviour has been studied for several species in detail (Hagvar & Hofsvang,

1991; Chow & Mackauer, 1992; Prinsloo et al. 1993; Takada & Tada, 2000; Ode et al. 2005). According to the studies, females approach the aphid host from an angle and probe by palpating it with their antennae. If the aphid is accepted, the parasitoid female bends her abdomen forward beneath the thorax and pierces the aphid with her ovipositor. In most species, oviposition occurs within one second (Hagvar & Hofsvang, 1991). Each female lays a single egg in each suitable host and the hatched larva lives inside the aphid host, where it consumes all of the body's contents, leaving only the exoskeleton, which will form a mummy later on. The larva does not kill the host until it becomes ready to pupate. The last larval instar pupates inside the empty aphid's exoskeleton by spinning a loose cocoon around itself. In species such as *A. rhopalosiphi* and *A. ervi*, the larva during the season with low temperatures spins a thicker cocoon around itself and undergoes a diapause or quiescence (Langer & Hance, 2000). Adult wasps emerge from the mummies by cutting a circular exit hole on the top with their mandibles, and the empty mummy remains on the leaf surface. Following emergence and within 1-2 hours, both sexes are capable of mating. A life cycle takes 10–14 days at temperatures of 21-25ºC (Takada & Tada, 2000).

1.6. Taxonomic status of different *Aphidius* species

Classification of the genus *Aphidius* is as follows:

Order: Hymenoptera
Family: Braconidae
Subfamily: Aphidiinae
Tribe: Aphidiini
Genera: *Aphidius*, *Lysaphidus* Smith, 1944, *Euaphidius* Mackauer, 1961, *Lysiphlebus*, *Paralipsis* Förster 1862, *Diaeretiella*, *Diaeretellus* Starý, 1960

Relationships between *Aphidius* and the other closely related genera *Lysaphidus*, *Euaphidius* and *Diaeretiella* are poorly understood and under constant reconsideration (Kambhampati et al. 2000; Sanchis et al. 2000; Tomanović et al. 2007; Ilić-Milošević et al. 2015). Many modern hymenopterists are in agreement as to the paraphyletic position of the genus *Aphidius* in relation to *Lysaphidus* (Smith et al. 1999; Kambhampati et al. 2000; Sanchis et al. 2000). On the other hand, Chen et al. (2002) maintained that all *Aphidius*

species are a monophyletic group, which was later supported by Ahmadabadi et al. (2011) and Lenin (2015).

Chen et al. (2002) stated that *Aphidius* can be treated as a paraphyletic group along with the genus *Diaeretiella*. In 1944, Smith described *Lysaphidus* as a subgenus of *Aphidius*, but later Starý (1960a) raised *Lysaphidus* to generic status. Recent studies using several molecular markers found no support for its generic position (Smith et al. 1999; Kambhampati et al. 2000; Sanchis et al. 2000; Shi & Chen, 2005; Tomanović et al. 2007). Based on phylogenetic analyses, Tomanović et al. (2007) concluded that *Lysaphidus* is a synonym of *Aphidius*. The other genus (*Euaphidius*) also considered to be a synonym of *Aphidius* (Starý, 1973). To the contrary, however, Kambhampati et al. (2000) supported the generic status of *Euaphidius* on the basis of its mitochondrial 16S rRNA. Later, van Achterberg (2006) described *Euaphidius* as a subgenus of *Aphidius* consisting of *Aphidius cingulatus* Ruthe, 1859.

Reliable identification of *Aphidius* species is of key importance for their use as biological control agents in aphid management programs and as model organisms in fundamental research. However, the taxonomic status of many *Aphidius* species is uncertain due both to the limited number of morphological characters valid for taxon discrimination and to their high variation on an intraspecific level (e.g. *A. matricariae, A. rhopalosiphi, A. urticae* Haliday, 1834 etc.).

The list of diagnostic characters used for species identification within the genus *Aphidius* includes wing venation and sculpturation of the anterolateral area of the petiolus (Eady, 1969; Marsh, 1977); the nature of antennal segments (Marsh, 1977; Tomanović et al. 2003; Kavallieratos et al. 2006); shape and setation of the ovipositor sheath; the tentorial index; and the angle of the ocelli (Smith, 1944; Pungerl, 1983;1986); maxillary and labial palps (Tomanović et al. 2003), body color (Garantonakis et al. 2009). As mentioned above, those characters are difficult to estimate precisely, particularly in the case of closely related species. It was earlier suggested that morphological features be combined with knowledge of host range patterns in order to separate species (Pungerl, 1983).

However, many papers have been recently published that deal with taxonomy, tritrophic associations, and species complexes, and several keys have been generated for species identification (Marsh, 1977; Powell, 1982; Pungerl, 1983; 1986; O'Donnell, 1989; Finlayson, 1990; Kavallieratos et al. 2004; Muratori et al. 2004; Tomanović et al. 2004; Garantonakis et

al. 2009; Petrović et al. 2010; Kos et al. 2011; Rakhshani et al. 2012; Tomanović et al. 2013). In fact, over the past century, *Aphidius* species have been constantly rearranged on the basis of new morphological characters and generic revisions. Thus, the exact number of species is unclear. Moreover, several species have confusing taxonomic histories and are in need of revision.

The uncertainty surrounding the taxonomic status of *A. colemani* and the closely related *A. platensis* Brèthes, 1913 and *A. transcaspicus* Telenga, 1958 has been the subject of several articles that used different approaches and reported different or similar results. *Aphidius transcaspicus* was first described from Uzbekistan, while *A. platensis* was originally described in South America. Both species were synonomized as *A. colemani* based on morphology in the revision given by Stary (1975). In 1995 Messing & Rabasse reported the existence of a reproductively isolated sibling species with a different host range within *A. colemani*. Kavallieratos & Lykouressis (1999) subsequently separated *A. transcaspicus* from *A. colemani* on the basis of morphological traits, i.e., characteristics of the antenna and labial palps. After analysing the 5.8S, ITS2, and 28S regions, Garantonakis et al. in 2009 indicated that the populations of *A. colemani* and *A. transcaspicus* are compatible and genetically very similar. In addition, Lozier and Mills (2009) used COI mitochondrial data and discovered geographic variability between *A. transcaspicus* populations. By analysing the ITS2 region, Ahmadabadi et al. (2011) confirmed the separation of *A. colemani* and *A. transcaspicus*. More recently, combining molecular analyses of COI with geometric morphometrics, Tomanović et al. (2014) clarified that the three mentioned species are distinct species and that *A. platensis* and *A. colemani* share a common host range pattern and the same origin. The authors also noted that the use of COI is a reliable approach for species identification within the *A. colemani* group, whereas forewing shape and wing venation are less informative for species discrimination.

The case of *A. ervi*, *A. microlophii* Pennacchio & Tremblay, 1987 and *A. pisivorus* Smith, 1941, which are known as the *A. ervi* complex is another common example of uncertain taxonomic position. These species are morphologically similar, but they are completely separated ecologically; *A. microlophii* is restricted to *Microlophium carnosum* Buckton, 1876 (Pennacchio & Tremblay, 1986) and *Wahlgreniella ossiannilssoni* Hille Ris Lambers, 1949 (Petrović et al. 2006). In contrast *A. ervi* has a host range that is broad but does not include the two indicated species. A large area of distribution and wide host range can lead to great

genetic variability of populations, which was reported in the case of *A. ervi*, and such populations are referred to as biotypes (Nemec & Starý, 1983). In 1986, Pennacchio & Tremblay described one of the *A. ervi* biotypes which parasitizes *Microlophium carnosum* as a distinct new species and named it *A. microlophii*, while Unruh et al. (1989) presented *A. pisivorus* as belonging to a complex of sibling species that differ little in morphology and behaviour to judge from analyses of polymorphic enzymes in 10 strains of the *A. ervi* complex from different places (Western Europe, the Mediterranean region, Israel, Pakistan, Japan and North America). According to Starý (1974), *A. pisivorus* may represent a past extension of the range of *A. ervi*, which could have spread from the eastern Palaearctic region to North America. Although, Atanassova et al. (1998) confirmed the reproductive isolation of *A. ervi* and *A. microlophii* populations based on enzyme analysis, the COI sequences showed no differences between these two parasitoid species (Derocles et al. 2012).

Further updated studies with integration of morphological, biological, and DNA analyses are needed to throw light on the species, species complexes, and precise geographic ranges of members of the genus *Aphidius* in order to improve the effectiveness of their use in aphid management programs.

2. MAIN OBJECTIVES

This thesis is focused on implementing the molecular tools with major objectives as follows:

- to conduct molecular characterization and determine genetic divergence of the parasitoid species from the genus *Aphidius* originating from wide area in Europe in association with diverse aphid/plant host associations
- to investigate the cryptic speciation in *Aphidius* species
- to evaluate the known morphological characters in identification of the *Aphidius* species, in particular discrimination of cryptic species
- to investigate phylogenetic relationships between the *Aphidius* species
- to recognize new phyletic groups unknown from prior classifications.

3. MATERIAL AND METHODS

3.1. Sampling the insect material

In total, 33 parasitoid species from the genus *Aphidius* have been submitted to molecular analyses (Table 1). Specimens were collected between 2001 and 2015 from 48 localities from the following 15 countries: Belgium, Iran, Germany, Czech Republic, Croatia, Montenegro, Serbia, Slovenia, Bulgaria, Japan, Greece, Lithuania, Malta, Switzerland and Sweden. Samples of leaves with mummies were collected and kept under laboratory conditions until parasitoid emergence. After emergence, parasitoids were immersed in 96% ethanol and preserved for later examination. Some specimens which were dry have been obtained from collection of the Institute of Zoology, Faculty of Biology, University of Belgrade. External morphology of the specimens was studied using a ZEISS Discovery V8 stereomicroscope. Scanning electron micrographs were obtained using a Jeol JSM-6390 scanning electron microscope.

In addition, several of *Aphidius* species were also included in the phylogenetic study as a reference. Their barcoding sequences were obtained from the GeneBank (Table 2).

Table 1. The list of *Aphidius* species submitted to molecular analyzes

Parasitoid species	Country of origin	Sampling locality	Sampling date	Host plant	Aphid host	Old PCR code
A. tanacetarius Mackauer, 1962	Serbia	Valjevo	19.06.2011.	*Tanacetum vulgare* L., 1753	*Metopeurum fuscoviridae* Stroyan, 1950	IM1, IM2, IM3
A. tanacetarius	Serbia	Bosilegrad, Jarešnik	22.07.2013.	*Tanacetum vulgare*	*Metopeurum fuscoviride*	Atan1, Atan2
A. tanacetarius	Belgium	Brusten	23.07.2015.	*Tanacetum vulgare*	*Metopeurum fuscoviride*	IM121, IM122
A. sussi Pennachio & Tremblay, 1989	Montenegro	Crno jezero	11.08.2005.	*Aconitum toxicum* Rchb.	*Delphiniobium junackianum* Karsch, 1887	IM4
A. sussi	Montenegro	Durmitor - Crno jezero	27.08.2013.	*Aconitum toxicum*	*Delphiniobium junackianum*	S12/833
A. sussi	Slovenia	Bohinj	14.07.2009.	*Aconitum maximum*	*Delphiniobium sp* Mordvilko, 1914	Sl09/86_1
A. asteris Haliday, 1834	Bulgaria	Sofia	26.11.2009.	*Dendrathema* spp. des Moulins 1860	*Macrosiphoniella sambornii* Gillette, 1908	IM5, IM7
A. sonchi Marshall, 1896	Serbia	Niš-Popovac	20.06.2010.	*Sonchus arvensis* L., 1753	*Hyperomyzus lactucae* Linnaeus, 1758	IM9, IM10
A. linosiphonis	Montenegro	Crno jezero	31.07.2011.	*Galium* sp. L., 1753	*Linosiphon* sp. Börner, 1950	IM11
A. ribis	Montenegro	Crno jezero	31.07.2011.	*Ribes petreum* Wulfen, 1781	*Cryptomyzus* sp. Oestlund, 1923	IM12, IM13
A. ribis	Belgium	Mettekoren		*Ribes rubrum* L., 1753	*Cryptomyzus ribis* Linnaeus, 1758	IM107, IM108, IM109
A. schimitscheki Stary, 1960	Serbia	Kopaonik-Mala greda	08.08.2011.	*Abies* sp Mill., 1754		IM14, IM139
A. viaticus Sedlag, 1968	Serbia	Valjevo	01.05.2011.	*Filago germanica* L., 1763	*Pleotrichophorus filaginis* Schouteden, 1906	IM15, IM16, IM17
A. viaticus	Serbia	Valjevo	18.06.2011.	*Filago germanica*	*Pleotrichophorus filaginis*	IM24, IM25, S11/435_1
A. erysimi Stary, 1960	Czech Republic	Rana, Louny, Boh.c.		*Erisymum* L., 1753	*Pseudobrevicoryne erysimi* Holman, 1961	IM135, IM136
A. arvensis Stary, 1960	Iran	Gorgan	28.05.2010.	*Inula* sp. L., 1753	*Aphis sargasi*	IM145, IM146
A. banksae Kittel,	Serbia	Zemun-Metro	09.05.2011.	*Artemisia vulgaris* L., 1753		IM18, IM19

2016						
A. banksae	Serbia	Vlasina	03.08.2011.	*Lathyrus pratensis* L., 1753	*Megoura viciae* Buckton, 1876	IM27
A. banksae	Montenegro	Tivat	25.05.2011.	*Vicia cracca* L., 1753	*Acyrthosiphon pisum*	IM151, IM152
A. phalangomyzi Starý, 1963	Belgium	PCF (Vegi)		*Artemisia vulgaris*	*Macrosiphoniella* sp.	IM49
A. hortensis Marshall, 1896	Serbia	Petnica	12.06.2011.	*Mahonia aquilegifolium* Pursh, 1814	*Liosomaphis berbeidis* Kaltenbach, 1843	IM21, IM22, IM23
A. hortensis	Serbia	Tara-Perućac	03.07.2012.	*Berberis vulgaris* L., 1753	*Liosomaphis* sp.	IM31, IM32
A. hieraciorum Starý, 1962	Slovenia	Zelenci	18.07.2014.	*Berbeis vulgaris*	*Liosomaphis berberidis*	IM105
A. hieraciorum	Serbia	Kopaonik	17.07.2013.	*Hieracium* sp L., 1753	*Nasonovia* sp. Mordvilko, 1914	IM102
A. hieraciorum	Serbia	Kopaonik	17.07.2013.	*Hieracium pilosum* Froel, 1838	*Nasonovia ribisnigri* Mosley, 1841	IM103
A. hieraciorum	Sweden	Uppsala	07.01.2014.	*Pilosella aurantiaca* (L.) F.W.Schultz & Sch.Bip., 1862	*Nasonovia ribisnigri*	IM104
A. rosae Haliday, 1834	Belgium	Jodoigne		*Rosa canina* L., 1753	*Macrosiphum rosae* Linnaeus, 1758	IM110, IM111,IM112
A. rosae	Slovenia	Zelenci	18.07.2014.	*Knautia brymeia*	*Macrosiphum rosae*	IM113, IM114
A. rosae	Serbia	Topčider	30.05.2008.	*Rosa* sp L., 1753	*Macrosiphum* sp.	Ar/1
A. rosae	Iran	Jiroft	07.05.2008.	*Rosa* sp.	*Macrosiphum rosae*	Ar 2
A. rosae	Croatia	Koreničko vrelo	22.06.2015.	*Knautia* sp. L., 1753	*Macrosiphum rosae*	IM87, IM88, IM89
A. uzbekistanicus	Belgium	PCF (Vegi)		*Poa annua* L., 1753	*Sitobion avenae*	IM48
A. avenae Haliday, 1834	Montenegro	Škrka	07.08.2005.	*Salix retusa* L., 1759	*Acyrthosiphon malvae* Mosley, 1841	A-1
A. avenae	Germany	Jena				A-2
A. microlophii	Belgium	PCF (Vegi)		*Urtica dioica* L., 1753		IM45, IM46, IM47
A. ericaphidis Pike&Stary, 2011	Scotland		19.06.2014.	*Vaccinium corymbosum* L., 1753	*Ericaphis scammelli* Mason, 1940	IM50, IM51, IM52
A. chaetosiphonis Tomanović & Petrović 2011	Montenegro	Durmitor - Sedlena greda	08.08.2013.	*Potentilla clusiana* Jacq. 1774	*Chaetosiphon* sp. Mordvilko, 1914	IM53
A. rhopalosiphi	Serbia	Niš - Niška banja	23.07.2013.	*Typha latifolia* L., 1753	*Rhopalosiphum nymphaeae*	IM55, IM133,

					Linnaeus, 1761	IM134
A. balcanicus Tomanović & Petrović 2011	Croatia	Vidikovci	20.06.2015.	*Geranium rober* L., 1753	*Acyrthosiphon malvae*	IM86
A. salicis Haliday, 1834	Serbia	Niš	25.06.2010.	*Pimpinella anisum* L., 1753	*Cavariella* sp. Del Guercio, 1911	IM57, IM60, IM61
A. salicis	Croatia	Homoljačko polje	22.06.2015.	*Apiacae* Lindl., 1836	*Cavariella aegopodii* Scopoli, 1763	IM77, IM78, IM79
A. salicis	Slovenia	Zalog	27.06.2011.	*Daucus carota* L., 1753	*Cavariella* sp.	IM116, IM117, IM118
A. salicis	Montenegro	Žabljak		*Daucus* sp. L., 1753	*Cavariella* sp.	Lya1, Lya4, Lya5
A. funebris Mackauer, 1961	Serbia	Kragujevac - Adžine livade,	01.06.2011.	*Crepis biennis* L., 1753	*Uroleucon cichorii* Koch, 1855	IM59, I8, I9, I10, I15, I16
A. funebris	Serbia	Vlasinsko jezero	15.06.2013.	*Centaurea* sp. L., 1753	*Macrosiphonella sp* Del Guercio, 1911	Aa94, Aa95, Aa98, Aa99, Aa101, Aa103, Aa104, Aa105, Aa106, Aa108
A. absinthii Marshall, 1896	Croatia	Čujića krčevina	22.06.2015.	*Artemisia vulgaris*	*Macrosiphoniella artemisiae* Boyer de Fonscolombe, 1841	IM80, IM81, IM82
A. absinthii	Serbia	Beograd – Bežanija	12.05.2001.	*Artemisia vulgaris*	*Macrosiphoniella sp.*	IM141, IM142
A. absinthii	Montenegro	Vranjina	26.05.2011.	*Artemisia vulgaris*	*Macrosiphonella artemisiae*, *Pleotrichophorus glandulosus* Kaltenbach, 1846	S11/250_1
A. absinthii	Belgium	Gingelom	26.06.2015.	*Artemisia vularis*	*Macrosiphoniella artemisiae*	IM94, IM95, IM96
A. absinthii	Serbia	Zemun	02.06.2011.	*Artemisia vularis*	*Macrosiphoniella* sp.	S11-345-1, S11-345-2
A. absinthii	Malta		05.04.2014.	*Dittrichia viscosa* (L.) Greuter, 1973		S11-884-1, S11-884-2, S11-884-3
A. absinthii	Serbia	Vlasina	28.06.2012.	*Achilea millefolium* L., 1753	*Macrosiphoniella* sp.	S12-55-1
A. silvaticus Stary, 1962	Croatia	Kozjak	21.06.2015.	*Rubus* sp. L., 1753	*Amphorophora rubi* Kaltenbach, 1843	IM90, IM91, IM92
A. aquilus	Serbia	Vlasina	28.06.2012	*Betula* sp. L., 1753	*Betulaphis quadrituberculata*	IM28, IM29,

Mackauer, 1961					Kaltenbach, 1843	IM30
A. urticae	Serbia	Krčedin	29.05.2008.	*Urtica dioica*	*Microlophium carnosum*	Ud4
A. urticae	Russia	Western Caucasus	2008		*Microlophium carnosum*	Mc2
A. urticae	Serbia	Kruševac, Pakašnica	11.05.2013.	*Urtica dioica*	*Microlophium carnosum*	IM137, IM138
A. rubi Stary, 1962	Austria	Obergurgl	28.07.2015.	*Vaccinium uliginosum*	*Aulacorthum vaccinia*	IM149, IM150
A. rubi	Croatia	Kozjak	22.06.2015.	*Rubus sp.*	*Macrospihum funestum* Macchiati, 1885	IM84, IM85
A. setiger Mackauer, 1961	Belgium	Jodoigne		*Acer campestre* L., 1753	*Peryphyllus testudinaceus* Fernie, 1852	IM119, IM120
A. ervi	Slovenia	Nova Gorica	04.06.2009.	*Triticum aestivum* L., 1753	*Sitobion avenae*	IM123, IM124
A. ervi	Serbia	Beograd	06.11.2012.	*Medicago sativa* L., 1753	*Acyrthosiphon pisum*	IM131, IM132
A. eadyi	Sweden	Skaltsa	07.02.2014.	*Pisum sativum* L., 1753	*Acyrthosiphon pisum*	IM99
A. eadyi	Serbia		31.05.2011.	*Medicago sativa*	*Acyrthosiphon pisum*	AE 1/1
A. eadyi	Serbia	Umčari	08.06.2012.	*Medicago sativa*	*Acyrthosiphon pisum*	AE 1/3
A. eadyi	Serbia	Malo Orašje	08.06.2012.	*Medicago sativa*	*Acyrthosiphon pisum*	AE 2/2, AE 2/3
A. eadyi	Slovenia	Strujan	20.11.2008.	*Medicago sativa*	*Acyrthosiphon pisum*	Sl08/26_2
A. matricariae	Serbia	Beograd - Vukov spomenik	06.11.2013.	*Tilia sp.* L., 1753	*Eucallipterus tiliae* Linnaeus, 1758	IM54
A. matricariae	Austria	Obergurgl	28.07.2015.	*Vaccinium uliginosum* L., 1753	*Aulacorthum vaccinii* Hille Ris Lambers, 1952	IM147, IM148
A. matricariae	Serbia	Radmilovac	31.05.2013.	*Prunus persica* (L.) Batsch, 1801	*Myzus persicae*	IM125

Table 2. The list of *Aphidius* specimens from the GeneBank included in the phylogenetic analysis with designated geographic origin and aphid host/plant associations

Acc. No	species	aphid host	plant	origin
JN164785	*A. avenae*	*S. avenae*	*T. aestivum*	Germany (Jena)
JN164752	*A. uzbekistanicus*	*S. avenae*	*Hordeum vulgare* L., 1753	Sweden (Bessinge)
JN164751	*A. uzbekistanicus*	*S. avenae, R. padi* Linnaeus, 1758, *D. noxia*	*H. vulgare*	Czech Republic (J. Moravia)
KJ615375	*A. transcaspicus*	*Hyalopterus pruni* Geoffroy, 1762	*Phragmites australis* (Cav.) Trin Ex Steud. (1841)	Greece
KJ615374	*A. transcaspicus*	*A. fabae*	Solanum *lycopersicum* L., 1753	Iran
KJ615373	*A. colemani*	*Aphis* sp.	*C. limon* L., 1766	Iran
KJ615365	*A. platensis*	*Brachycaudus tragopogonis* Kaltenbach, 1843	*Tragopogon graminifolius DC*	Iran
KP698106	*A. cingulatus*	*Pterocomma pilosum* Buckton, 1879	*Salix fragilis* Linnaeus 1753	Lithuania (Vilnius)
KP698108	*A. cingulatus*	*Pterocomma sp* Buckton, 1879	*Salix sp* L., 1753	Montenegro (Plav)
KP698111	*A. setiger*.	*Periphyllus sp* van der Hoeven, 1863	*Acer platanoides* L., 1753	Serbia (Nis)
KP698112	*A. setiger*	*Periphyllus sp.*	*A. pseudoplatanus* L., 1753	Switzerland (Steinmaur)
JN164779	*A. rhopalosiphi*	*Schizaphis scirpi*	*Typha sp* L., 1753	Serbia
JN164773	*A. rhopalosiphi*	*Sitobion avenae*	*Hordeum vulgare*	Slovenia
JN164765	*A. rhopalosiphi*	*S. avenae R. padi D. noxia*	*Hordeum vulgare*	Czech Republic
JN164764	*A. rhopalosiphi*	*S. avenae R. padi D. noxia*	*Hordeum vulgare*	Czech Republic
JN164762	*A. rhopalosiphi*	*S. avenae R. padi D. noxia*	*Hordeum vulgare*	Czech Republic
JN164763	*A. rhopalosiphi*	*S. avenae R. padi D. noxia*	*Hordeum vulgare*	Czech Republic
JN164761	*A. rhopalosiphi*	*S. avenae R. padi D. noxia*	*Hordeum vulgare*	Czech Republic
JN164778	*A. rhopalosiphi*	*Schizaphis scirpi*	*Typha sp.*	Serbia
JN164777	*A. rhopalosiphi*	*Sitobion avenae*	*Triticum aestivum*	Serbia
JN164759	*A. rhopalosiphi*	*Metopolophium dirhodum*	*Triticum aestivum*	Germany
JN164776	*A. rhopalosiphi*	*Sitobion avenae*	*Triticum aestivum*	Serbia
JN164757	*A. rhopalosiphi*	*Rhopalosiphum padi*	*Triticum aestivum*	Germany
JN164756	*A. rhopalosiphi*	*Metopolophium dirhodum*	*Triticum aestivum*	Germany
JN164755	*A. rhopalosiphi*	*Rhopalosiphum padi*	*Triticum aestivum*	Germany
JN164753	*A. rhopalosiphi*	*Rhopalosiphum padi*	*Triticum aestivum*	Germany
JN164754	*A. rhopalosiphi*	*Rhopalosiphum padi*	*Triticum aestivum*	Germany
JN164775	*A. rhopalosiphi*	*Sitobion avenae*	*Triticum aestivum*	Serbia
JN164774	*A. rhopalosiphi*	*Rhopalosiphum padi*	*Hordeum vulgare*	Sweden
JN164772	*A. rhopalosiphi*	*Sitobion avenae*	*Hordeum vulgare*	Slovenia
JN164771	*A. rhopalosiphi*	*Sitobion avenae*	*Hordeum vulgare*	Slovenia

JN164770	*A. rhopalosiphi*	*Rhopalosiphum padi*	*Hordeum vulgare*	Sweden
JN164769	*A. rhopalosiphi*	*Rhopalosiphum padi*	*Hordeum vulgare*	Sweden
JN164768	*A. rhopalosiphi*	*Metopolophium dirhodum*	*Triticum aestivum*	Poland
JN164767	*A. rhopalosiphi*	*Sitobion avenae*	*Triticum aestivum*	Poland
JN164766	*A. rhopalosiphi*	*S. avenae R. padi D. noxia*	*Hordeum vulgare*	Czech Republic
JN164760	*A. rhopalosiphi*	*Metopolophium dirhodum*	*Triticum aestivum*	Germany
JN164758	*A. rhopalosiphi*	*Metopolophium dirhodum*	*Triticum aestivum*	Germany

3.2. DNA extraction

Genomic DNA was extracted from individual parasitoids using the QIAGEN Dneasy® Blood & Tissue Kit. In 1,5ml tubes 20 µl of proteinase K and 180 µl of ATL buffer were added. Individual insects were punctured with a sterile needle and placed into the tubes with proteinase and buffer. After vortexing the tubes with specimens for 10 minutes, they were placed in a water bath for incubation overnight at 56°C. In order to preserve all parasitoids for potential morphological analyses, the specimens were removed from the buffer the following day. In the remaining solution a 200 µl of AL buffer and 200 µl of ethanol were added and again vortexed for 10 minutes. The solution from the tubes was transfered into DNeasy Mini spin columns with a filter and centrifuged for a minute at the speed of 8000 rpm. Collector tubes were removed from the bottom of the spin columns and replaced with new ones. Than 500 µl of AW1 buffer were added in each spin column and centrifuged again for a minute at 8000 rpm. Collector tubes were replaced with new ones, 500 µl of AW2 buffer added in mini spin columns and thereafter centrifuged for 3 min at 14000 rpm. Minispin columns were placed in 1.5 ml tubes, 50 µl of AE buffer was added and left for 10 minutes at room temperature. Than the tubes with columns were centrifuged for 1 min at speed of 8000 rpm. Afterwards, the minispin columns were removed from the tubes now containing the extracted DNA.

3.3. PCR amplification and sequencing

A barcoding region of mitochondrial gene cytochrome oxidase subunit I (mtCOI) has been used to analyse the populations differences and to elucidate phylogenetic relationships between the separated taxa. A barcoding region of the mtCOI gene was amplified using the primer pair:

1. forward primer LCO1490 (5' GGTCAACAAATCATAAAGATATTGG 3'),
2. reverse primer HCO2198 (5' TAAACTTCAGGCTGACCAAAAAATCA 3') (Folmer et al. 1994).

Each PCR reaction was carried out in a volume of 20µl following the receipt:

- 1µl extracted DNA
- 11.8 µl H_20
- 2 µl High Yield Reaction Buffer A with 1xMg
- 1.8 µl of $MgCl_2$ 2.25 mM
- 1.2 µl of dNTP 0.6 mM
- 1µl LCO1490 0.5 µM
- 1µl HCO2198 0.5 µM
- 0.2 µl DNA polymerase 0.05U/µl.

The amplification protocol included three steps as follows:

1. initial denaturation at 95°C for 5 min,
2. I 1 min at 94°C
II 1 min at 54°C
III 30 sec at 72°C } 35 cycles
3. final extension at 72°C for 7 min.

Several of the parasitoid specimens submitted to the molecular analyses were dry mounted and amplification of a barcoding region with standard LCO1490/HCO2198 primers had no success due to DNA defragmentation. For this reason, degenerative primers were designed to amplify short fragments of the barcoding region through direct and nested PCR, and thereafter alligned to a complete sequence (Table 3, Fig. 1).

Table 3. The list of primers designed for dry specimens to amplify a barcoding region through direct and nested PCR analyses

primer name	5 ’→ 3’ primer sequence*	primer direction	PCR reaction
Aph1Rd	GRGGRAAAGCYATATCAGGAG	reverse	direct
Aph1Fn	TAAGWTTATTAATTCGWATRGA	forward	nested
Aph1Rn	CAATTWCCAAATCCWCCAATTAT	reverse	nested
Aph2Fd	ATAATTGGWGGATTTGGWAATTG	forward	direct
Aph2Rd	GTWCTAATAAAATTAATWGCWCC	reverse	direct
Aph2Fn	CTCCTGATATRGCTTTYCCYC	forward	nested
Aph2Rn	GADGAAATHCCTGCTAAATG	reverse	nested
Aph3Fd	CATTTAGCWGGDATTTCYTC	forward	direct
Aph3Fn	GGAGCWATTAATTTTATTAGWAC	reverse	nested
Aph3Rn	GTAGTATTTAARTTWCGATC	forward	nested

*degenerative base designation/actual base coded: R/A or G, Y/C or T, W/A or T.

Following protocol has been developed for direct and nested PCR reaction for amplification of mtCOI short fragments:

1. initial denaturation at 95°C for 5 min,
2. I 1 min at 95°C
   II 1 min at 54°C } 37 cycles
   III 30 sec at 72°C
3. final extension at 72°C for 7 min.

For ampification of the nuclear gene coding Elongation factor 1 alpha (EF1-α), following primers were used:

- forward EF1-Bf (5' AGAACGTGAACGTGGTATCA 3')
- reverse EF1-Br (5' CTTGGAGTCACCAGCTACATAACC 3').

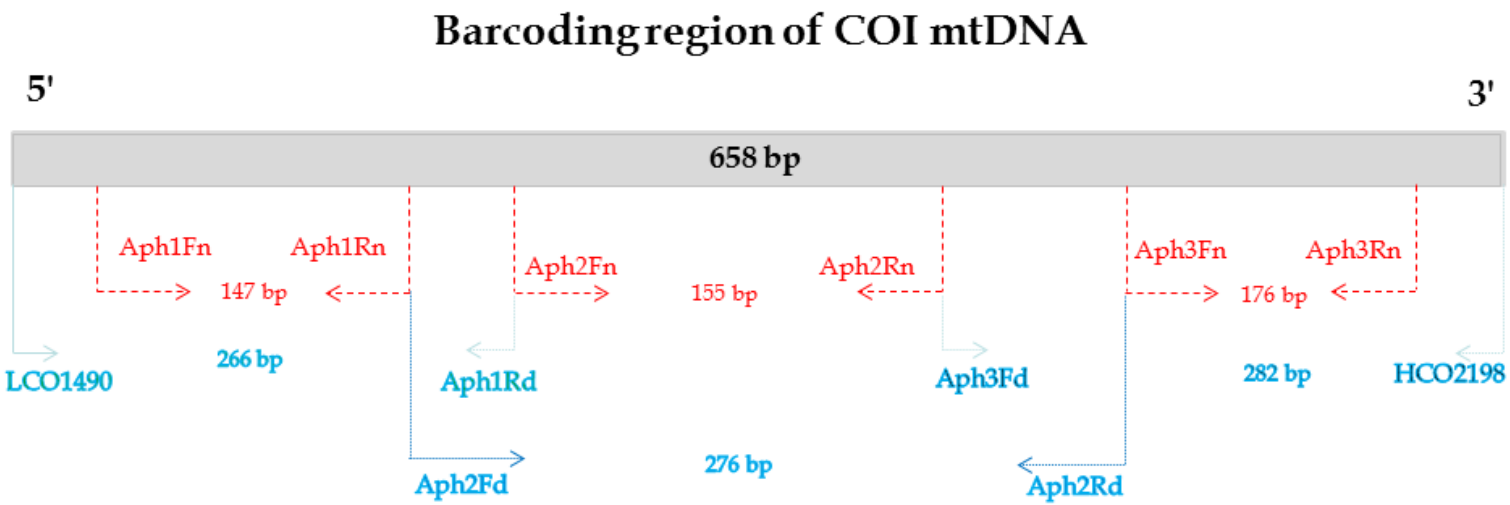

Fig. 1 Scheme of positions for internal degenerative primers within the barcoding region of COI mtDNA. Blue color refers to forward and reverse primers used in direct PCR reactions (LCO1490, Aph1Rd, Aph2Fd, Aph2Rd, Aph3Fd, HCO2198). Red colour represents the primers used in nested PCR (Aph1Fn, Aph1Rn, Aph2Fn, Aph2Rn, Aph3Fn, Aph2Rn). Arrows refer to the primers direction, forward or reverse. The length of amplified short fragments are designated between the primer pairs.

PCR mix for EF1- α is the same as for the set of primers LCO1490/HCO2198 in volume of 20 µl.

The amplification protocol for EF1- α is as follows:

1. initial denaturation at 95°C for 2 min,
2. I 45 sec at 94°C
   II 1 min at 50°C
   III 1:30 min at 72°C
   } 40 cycles
3. final extension at 72°C for 7 min.

Amplified products of both, mitochondrial and nuclear gene, were run on 1% agarose gel, stained with ethidium bromide and visualized under a UV transiluminator. PCR products have been shipped to Macrogen Inc. in Korea for sequencing using an automated equipment. All barcoding products amplified with the LCO1490/HCO2198 primer pair were sequenced using the forward primer LCO1490. Products obtained with designed degenerative primers in direct/nested PCR reactions were sequenced with combination of forward and reverse primers for each part of the barcoding region (for/rev combinations were as follows: LCO1490/ Aph1Rd; Aph1Fn/ Aph1Rn; Aph2Fd/ Aph2Rd; Aph2Fn/ Aph2Rn; Aph3Fd/ HCO2198; Aph3Fn/ Aph3Rn). For nuclear gene, both primers were used for sequencing, forward EF1 Bf and reverse EF1 Br.

3.4. Data analyses

Sequences of mtCOI and EF1-α were manually edited in FinchTV ver.1.4.0 (www.geospiza.com) (Fig.2) and aligned using the Clustal*W* program integrated in MEGA5 (Tamura et al. 2011) (Fig. 3). Sequenced mitochondrial and nuclear fragments were submitted to maximum likelihood best fit model analysis using the MEGA5 program (Tamura et al. 2011). Short fragments of the barcoding regions were concatenated in the MEGA5 program to obtain long sequence for further analyses. According to the obtained Akaike Information Criterion scores, the best fit model for estimation of evolutionary divergence was a Tamura-Nei model (Tamura & Nei, 1993).

In order to evaluate the suitability of the barcoding region of COI for identification of species from the genus *Aphidius*, the Maximum within species distance (Max-WSD) was plotted versus the Minimum between species distance (Min-BSD) of the barcoding region gene for each species pair (Hajibabaei et al. 2006; 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 the COI sequences.

A Maximum likelihood (ML) and Maximum parsimony (MP) trees were constructed using the MEGA5 software, with 500 bootstrap replicates performed to assess the branch support (Felsenstein, 1985). Mitochondrial COI sequence was amplified and sequenced for other parasitoid, belonging to the same subfamily (Aphidiinae) *Ephedrus niger* Gautier, Bonnamour & Gaumont, 1929 which was used as an outgroup to root the tree. A median-joining network (Bandelt *et al.* 1999) was constructed with the NETWORK ver. 4.6.1.2 program (http://www.fluxus-engineering.com), using a maximum parsimony calculation.

Fig 2. Mitochondrial COI barcoding fragments edited in Finch TV and analysed for sequences quality and length.

Fig. 3. Mitochodrial COI sequences of *Aphidius* species alligned in MEGA software and compared for nucleotide differences.

4. RESULTS

4.1. PCR amplification and sequencing of the barcoding region of mitochondrial COI gene and Elongation factor 1-α nuclear gene

A barcoding region of mitochondrial COI gene has been succesfully amplified for 144 samples of all 33 parasitoid species (Table 1). The number of sequenced specimens differed between the species as a result of an unequl sample size. Mitochondrial DNA product has also been amplified for one specimen of *Ephedrus niger* (Aphidiinae) which was used as an outgroup to root the phylogeny trees.

Elongaton factor 1-α (EF1-α) has been amplified and sequenced for *A. absinthii* (IM80, IM82, IM94, S12-884-1), *A. funebris* (I8, I9, I10, Aa94, Aa95, Aa98, Aa99, Aa101, A103, Aa106), *A. tanacetarius* (IM1, IM3, IM121, IM122), *A. salicis* (IM77, IM79, IM116, IM117), *A. aquilus* (IM28, IM29, IM30), *A. microlophii* (IM45, IM46, IM47), *A. ervi* (IM123, IM131, IM132), *A. hieraciorum* (IM102, IM103, IM104), *A. urticae* (IM137, IM138), *A. matricariae* (IM54, IM125), *A. rhopalosiphi* (IM55, IM133), *A. rubi* (IM84, IM85), *A. hortensis* (IM31) and *A. chaetosiphonis* (IM53).

Results are presented individually for each species and overall phylogenetic analysis including all taxa.

4.2. *Aphidius urticae* s. str., *A. rubi, A. silvaticus*

The specimens of *Aphidius urticae* that were subjected to molecular analyses originated from five localities in Austria, Croatia, Serbia and Russia (Table 4). In total, 11 parasitoids were sampled associated with *Amphorophora rubi, Aulacorthum vaccinii, Macrosiphum funestum* and *Microlophium carnosum.*

Mitochondrial COI fragments were amplified and sequenced for all 11 specimens of different "*A. urticae"* host-associated lineages. Aligned barcoding sequences were indel-free and trimmed to equal size of 596 bp in length. Comparison of the barcoding sequences revealed 57 variable sites, 54 of which were parsimony-informative. Mutations produced a total of eight amino acid substitutions, seven of which are parsimony informative. Using the Tamura–Nei parameter method, the average divergence rate computed between the *A. urticae* specimens was 4.6%, with the range of distances being from 0.2 to 9.4% (Table 5).

Table 4. The list of *Aphidius urticae* samples subjected to molecular analyses of barcoding fragments with their geographical origin and host associations

Country	locality	aphid host	plant	sampling date	Sample code
Croatia	Kozjak	*Macrospihum funestum*	*Rubus* sp.	22.06.2015	IM84, IM85
Croatia	Kozjak	*Amphorophora rubi*	*Rubus* sp.	21.06.2015.	IM90, IM91, IM92
Serbia	Kruševac, Pakašnica	*Microlophium carnosum*	*Urtica dioica*	11.05.2013.	IM137, IM138
Austria	Obergurgl	*Aulacorthum vaccinii*	*Vaccinium uliginosum*	28.07.2015.	IM149, IM150
Serbia	Krčedin	*Microlophium carnosum*	*Urtica dioica*	29.05.2008.	Ud4
Russia	Western Caucasus	*Microlophium carnosum*	*Urtica dioica*	2008	Mc2

Topology of the Maximum parsimony tree shows separation of three mitochondrial lineages (Fig. 4). Parasitoids associated with *Aulacorthum vaccinii* and *Macrosiphum funestum* aphid hosts grouped together with 97% bootstrap support. With the same support, specimens of *A. urticae* parasitizing *Microlophium carnosum* clustered within the second lineage. The first two mitochondrial lineages form a group with 99% support. The third lineage included *A. urticae* associated with the aphid host *Amphorophora rubi*, which separated from the first two lineages with bootstrap support of 99%.

Table 5. Evolutionary distances between the *Aphidius urticae* specimens estimated using the Tamura-Nei model.

	IM84	IM85	IM90	IM91	IM92	IM137	IM138	IM149	IM150	Mc2
IM84	-									
IM85	0.000	-								
IM90	0.085	0.085	-							
IM91	0.083	0.083	0.002	-						
IM92	0.085	0.085	0.000	0.002	-					
IM137	0.022	0.022	0.092	0.089	0.092	-				
IM138	0.022	0.022	0.092	0.089	0.092	0.000	-			
IM149	0.005	0.005	0.092	0.090	0.092	0.020	0.020	-		
IM150	0.005	0.005	0.092	0.090	0.092	0.020	0.020	0.000	-	
Mc2	0.022	0.022	0.094	0.092	0.094	0.004	0.004	0.020	0.020	-
Ud4	0.022	0.022	0.092	0.089	0.092	0.000	0.000	0.020	0.020	0.004

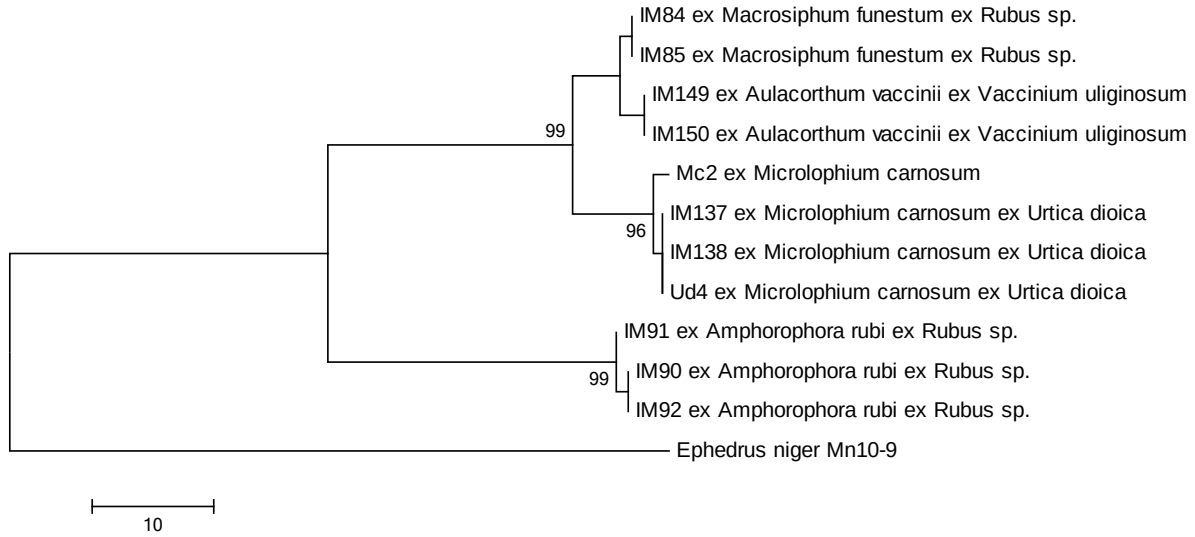

Fig. 4. Maximum Parsimony tree obtained from partial sequences of the mtCOI gene. The tree is drawn to scale, with branch lengths calculated using the average pathway method and are in units of the number of changes over the whole sequence. The scale bar indicates the number of substitutions per site. Bootstrap values >90% are indicated above/below the branches.

The Maximum Likelihood tree confirmed separation of the three described mitochondrial lineages (Fig. 5). Within the second lineage, there is a grouping of *A. urticae* associated with *Aulacorthum vaccinii* and *Macrosiphum funestum* with 91 and 97% support, respectively, while other lineages clustered with minimal differences in bootstrap support compared to the maximum parsimony tree.

Genetic divergences within each group estimated with the Tamura–Nei model were as follows: 0.3% within the first lineage associated with *Aulacorthum vaccinii* and *Macrosiphum funestum*, 0.2% within the group parasitizing *Microlophium carnosum* and 0.1% within the lineage of specimens originating from *Amphorophora rubi*.

The average evolutionary divergence between *Aulacorthum vaccinii/Macrosiphum funestum* and *Microlophium carnosum* lineages of *A. urticae* is 2.3%, which corresponds to their clustering in the same group with 99% bootstrap support. On the other hand, parasitoids originating from *Amphorophora rubi* differ on average by 8.8% from specimens associated with *Aulacorthum vaccinii* and *Macrosiphum funestum* (range: 8.3–9.2%) and by 8.9% from specimens parasitizing *Microlophium carnosum* (range: 8.9–9.4%) (Table 5).

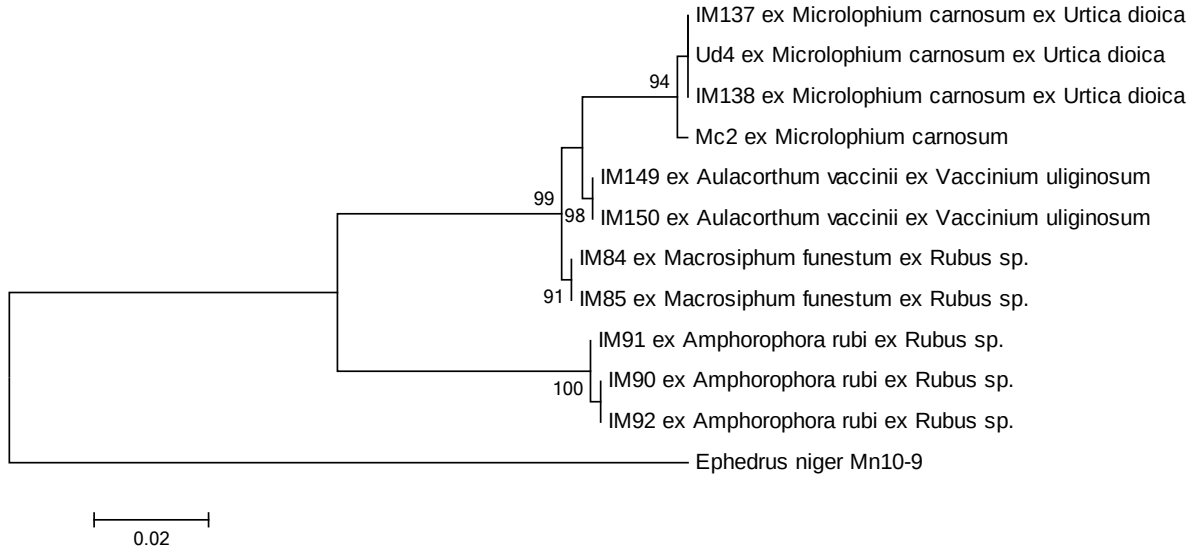

Fig. 5. Maximum Likelihood bootstrap consensus tree constructed from barcoding fragments of *A. urticae* mtCOI gene. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Bootstrap support exceeding 90% is presented above branches.

Five haplotypes were detected within the *A. urticae* s.str. group, with assigned codes ARU1 (IM84, IM85), ARU2 (IM149, IM150), ASL1 (IM90, IM92, IM91), AUR1 (IM137, IM138, Ud4) and AUR2 (Mc2). The median-joining network recognized three groups of mitochondrial haplotypes with a confidence limit of 95%: group 1 – haplotypes AUR1 and AUR2 associated with *Microlophium carnosum*; group 2 –ARU1 associated with *Macrosiphum funestum* and ARU2 with *Aulacorthum vaccinii*; and group 3 – ASL1 associated with *Amphorophora rubi* (Fig. 6). In total, 11 mutational steps were detected connecting groups 1 and 2, while the third group of haplotypes was connected with 44 mutational steps with group 2 with no ambiguities (Fig. 6).

Based on these results and after careful morphological examination of analysed specimens, as well as other specimens of the *Aphidius urticae* s. str. group (see below), three separate species were identified. Specimens associated with *Microlophium carnosum* represent *Aphidius urticae*, those associated with *Amphorophora* spp. represent *Aphidius silvaticus*, while specimens associated with *Macrosiphum funestum* and *Aulacorthum* spp. represent *Aphidius rubi*. The latter two were previously synonymized with *A. urticae*. In Appendix 1, re-description of *A. silvaticus* and *A. rubi* is presented.

Maximum within species distances (Max-WSD) were as follows: *A. rubi* – 0.5%, *A. silvaticus* – 0%, *A. urticae* – 0.4%. Minimum between species distances (Min-BSD) for each

pair were: *A. rubi/A. silvaticus* – 8.3%, *A. rubi/A. urticae* – 2.0%, *A. silvaticus/A.urticae* – 8.9 %. Clearly in case of all three species designated within the *A. urticae* s.str. group, the values of Min-BSD significantly exceeded Max-WSD, confirming the suitability of the barcoding COI fragments in species delineation.

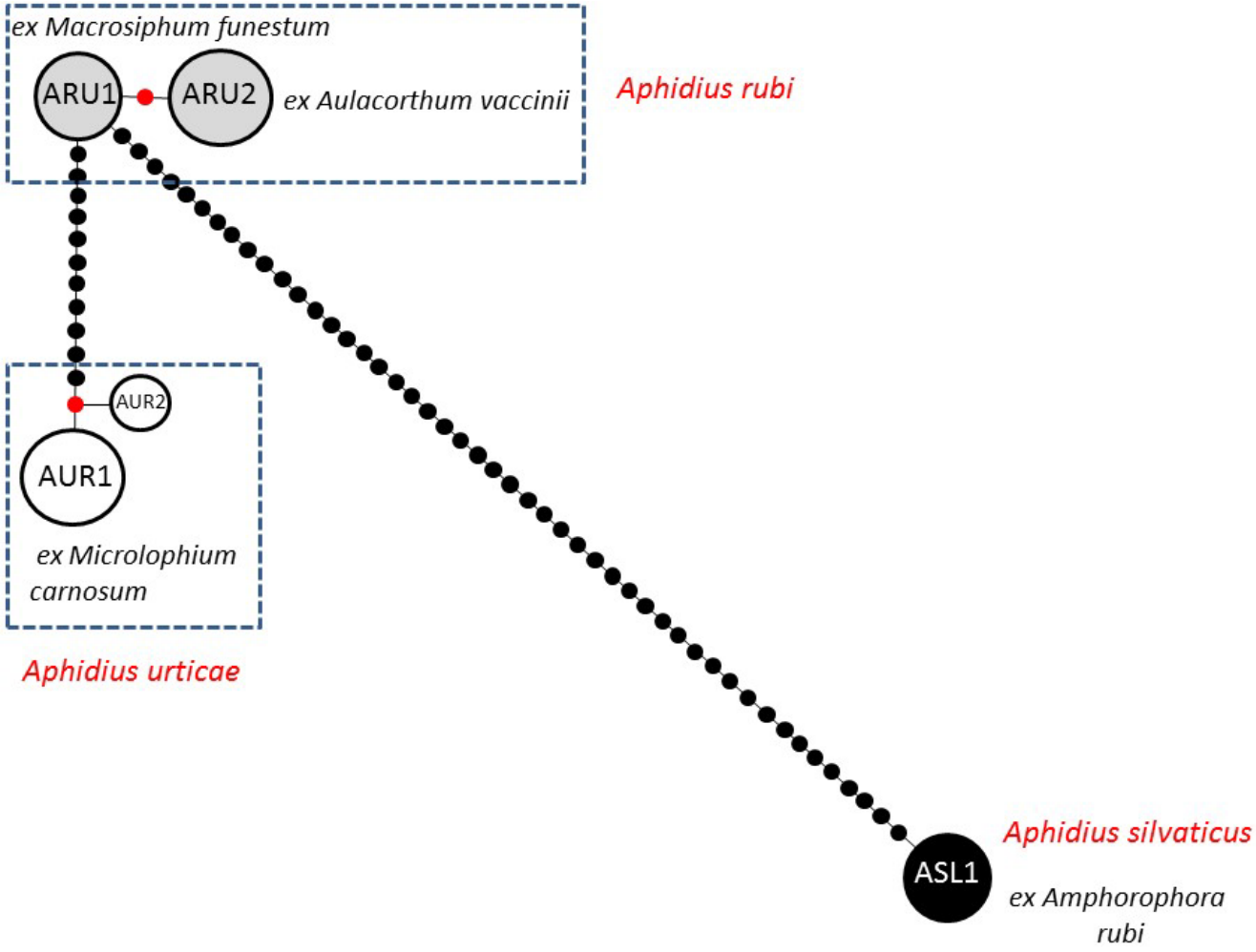

Fig. 6. Median-joining network of mtCOI haplotypes obtained for 11 *Aphidius urticae* specimens. White circles represent group 1 – haplotypes AUR1 and AUR2 from *Microlophium carnosum*; grey circles represent group 2 – ARU1 from *Macrosiphum funestum* and ARU2 from *Aulacorthum vaccinii*; and black circles represent group 3 - ASL1 from *Amphorophora rubi*. Circle size reflects the number of individuals with that haplotype (not to scale). Red circles are median vectors. Black dots are mutational steps.

4.3. *Aphidius matricariae*

Specimens of *A. matricariae* collected from Austria and Serbia were submitted to molecular characterization (Table 6). In total four barcoding sequences were aligned and trimmed to length of 613 bp. Two haplotypes were detected, AM1 (IM54, IM147, IM125) and AM2 (IM148). Divergence rate of 0.3% was determined, with two variable sites discriminating the two haplotypes. However, qualitative analysis showed that both mutations were synonymous with no amino acid substitutions.

Table 6. The list of *Aphidius matricariae* specimens analysed for mtCOI differences

Country of origin	Sampling locality	Sampling date	Host plant	Aphid host	PCR code
Serbia	Beograd - Vukov spomenik	06.11.2013	*Tilia* sp.	*Eucallipterus tiliae*	IM54
Austria	Obergurgl	28.07.2015	*Vaccinium uliginosum*	*Aulacorthum vaccinii*	IM147, IM148
Serbia	Radmilovac	31.05.2013	*Prunus persica*	*Myzus persicae*	IM125

Comparison of the barcoding haplotypes of *A. matricariae* with other *Aphidius* species, interestingly showed closest relatedness with *A. urticae.* Haplotype AM1 differs from the *A. urticae* haplotype AUR1 – 1% and AUR2 – 0.7%. Evolutionary distance between the *A. matricariae* haplotype AM2 and AUR1 is 1.4%. i.e. between AM2 and AUR2 - 1%. If we plot the Maximum within species distances (*A. maticariae*-0.3%, *A. urticae* – 0.4%) versus Minimum between species distances for this pair of species (0.7%), withdrawn conclusion is that the barcoding marker could not be excluded as non informative in taxonomic study od these two species.

Elongation factor. Partial sequences of elongation factor 1-α were obtained for two *A. matricariae* specimens (IM54, IM125) and two of the *A. urticae* (IM137, IM138). Nuclear fragments, 498bp in length, covering the front part prior to intron, were compared within and between the two species. All four EF1- α sequences were determined to be identical.

4.4. *Aphidius absinthii*

Overall 15 specimens of *Aphidius absinthii* originated from Croatia, Serbia, Montenegro, Belgium and Malta were included in the molecular characterization of the barcoding sequences (Table 7).

Table 7. The list of *Aphidius absinthii* analysed for barcoding sequences divergence

Country of origin	Sampling locality	Sampling date	Host plant	Aphid host	PCR code
Croatia	Čujića krč.	22.06.2015.	*Artemisia vulgaris*	*Macrosiphoniella artemisiae*	IM80, IM81, IM82
Serbia	Beograd Bežanija	12.05.2001.	*Artemisia vulgaris*	*Macrosiphoniella* sp.	IM141, IM142
Montenegro	Vranjina	26.05.2011	*Artemisisa vulgaris*	*Macrosiphoniella artemisiae*, *Pleotrichophorus glandulosus*	S11-250-1
Belgium	Gingelom	26.06.2015.	*Artemisia vularis*	*Macrosiphoniella artemisiae*	IM94, IM95, IM96
Serbia	Zemun	02.06.2011.	*Artemisia vularis*	*Macrosiphoniella* sp.	S11-345-1, S11-345-2
Malta		05.04.2014.	*Dittrichia viscosa*		S11-884-1, S11-884-2, S11-884-3
Serbia	Vlasina	28.06.2012.	*Achilea millefolium*	*Macrosiphoniella* sp.	S12-55-1

Analyses of 613bp long COI fragments determined eight haplotypes (AB1-AB8; Table 8). In total, 11 variable sites were identified, out of which three were parsimony informative. Qualitative analysis showed that all mutations were synonymous resulting in no amino acid susbstitutions. Tamura-Nei model determined the evolutionary distances between the *A. absinthii* haplotypes to range from 0.3% to 1.2% (Table 9).

Table 8. The list of COI mitochondrial haplotypes identified among analysed specimens of *A. absinthii*

Haplotype code	No of sequences	Sequences
AB1	2	IM80, IM81
AB2	1	IM82
AB3	4	IM94, IM95, IM96, S12-55-1
AB4	1	IM141
AB5	1	IM142
AB6	1	S11-250-1
AB7	3	S12-884-1, S12-884-2, S12-884-3
AB8	2	S11-345-1, S11-345-2

Table 9. Evolutionary distances between the haplotypes of *Aphidius absinthii*

	AB1	AB2	AB3	AB4	AB5	AB6	AB7
AB1							
AB2	0.003						
AB3	0.002	0.002					
AB4	0.005	0.005	0.003				
AB5	0.012	0.012	0.010	0.007			
AB6	0.003	0.003	0.002	0.005	0.012		
AB7	0.005	0.002	0.003	0.007	0.013	0.005	
AB8	0.005	0.005	0.003	0.003	0.010	0.005	0.007

In Fig. 7 a median joining network constructed for eight barcoding mtCOI haplotypes of *A. absinthii* is presented.

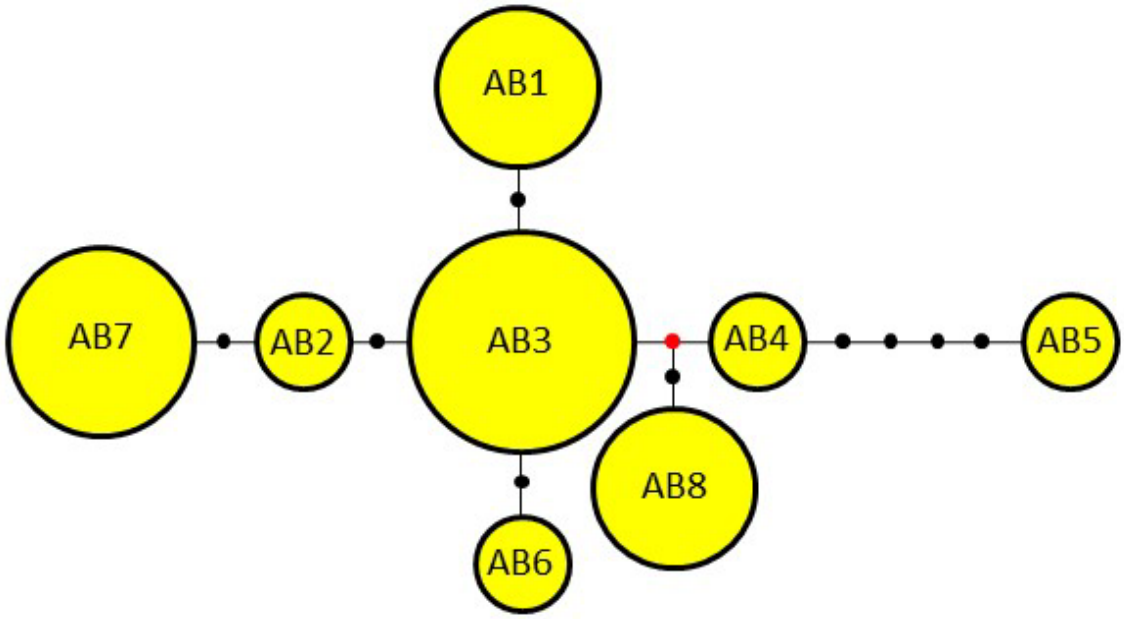

Fig. 7. Median-joining network for mtCOI haplotypes of *Aphidius absinthii* specimens. Circle size reflects the number of individuals with that haplotype (not to scale). Red small circles are median vectors. Black dots represent the mutational steps.

4.5. *Aphidius funebris*

In total sixteen specimens of *A. funebris* have been submitted to the barcoding fragments analyses (Table 10). Four haplotypes were identified: AF1 (IM59, I8, I9, I10, I15, I16), AF2 (Aa94, Aa95, Aa101, Aa103, Aa108), AF3 (Aa98, Aa105, Aa106) and AF4 (Aa99, Aa104). Evolutionary distances between the *A. funebris* haplotypes ranged from 0.2 to 1.6%. Comparison of 590bp long distances revealed ten variable sites, five being parsimony informative. However, qualititative analysis showed synonymous character of all mutations with no difference in amino acid content.

Comparison of the *A. funebris* mitochondrial haplotypes with other parasitoids revealed close relatedness with *A. absinthii* and *A. tanacetarius*. In addition, haplotype AF4 of *A. funebris* was identical with the barcoding haplotype AB3 of *A. absinthii*. Tamura-Nei distances ranged from 0.2 to 1.6% when *A. funebris* haplotypes were paired with *A. absinthii* i.e. 0.2 to 1.4% if paired with *A. tanacetarius*.

Table 10. The list of *A. funebris* specimens analysed molecularly

Country of origin	Sampling locality	Sampling date	Host plant	Aphid host	PCR code
Serbia	Vlasinsko jezero	15.06.2013.	*Centaurea* sp.	*Macrosiphoniella* sp.	Aa94, Aa95, Aa98, Aa99, Aa101, Aa103, Aa104, Aa105, Aa106, Aa108
Serbia	Kragujevac - Adžine livade,	01.06.2011.	*Crepis biennis*	*Uroleucon cichorii*	IM59, I8, I9, I10, I15, I16

4.6. *Aphidius tanacetarius*

Seven sequences of the barcoding region of the mtCOI gene of *Aphidius tanacetarius* were aligned and analysed (Table 11). All specimens were collected from the aphid host *Metopeurum fuscoviridae* associated with *Tanacetum vulgare*, in two countries, Serbia and Belgium. Two haplotypes were determined, AT1 (IM121, IM122) and AT2 (IM1, IM2, IM3, Atan1, Atan2). Divergence rate between the two haplotypes was 0.5%. Comparison of 613 bp long sequences determined three variable sites. Qualitative analysis showed that all mutations were synonymous, with no amino acid substitution.

Table 11. The list of *Aphidius tanacetarius* specimens submitted to the molecular analysis of COI mtDNA fragments

Country of origin	Sampling locality	Sampling date	Host plant	Aphid host	PCR code
Serbia	Valjevo	19.06.2011.	*Tanacetum vulgare*	*Metopeurum fuscoviridae*	IM1, IM2, IM3
Serbia	Bosilegrad, Jarešnik	22.07.2013.	*Tanacetum vulgare*	*Metopeurum fuscoviride*	Atan1, Atan2
Belgium	Brusten	23.07.2015.	*Tanacetum vulgare*	*Metopeurum fuscoviride*	IM121, IM122

Analysis of all available barcoding sequences of *Aphidius* species determined that *A. tanacetarius* haplotype AT2, shared by the two specimens from Belgium has identical sequence as the *A. absinthii* haplotype AB7, shared by three specimens originating from Malta (S12-884-1, S12-884-2, S12-884-3). Moreover, *A. tanacetarius* haplotype AT1 is

closely related to another *A. absinthii* haplotype AB3, from which it is distinguished by one mutation which was non-synonymous.

Median joining network constructed with haplotypes of *A. absinthii, A. funebris* and *A. tanacetarius* shows close relatedness with no clear separation between the species (Fig. 8). A maximum parsimony tree reconstructed from all barcoding sequences also confirms that *A. absinthii, A. tanacetarius* and *A. funebris* form a monophyletic group without clear delineation on a species level (Fig. 9). The values of the Maximum within species distances were for *A. funebris* – 1.6%, *A. absinthii* – 1.2% and *A. tanacetarius* – 0.5%. Minimum between species distances (Min-BSD) for each pair were: *A. absinthii/A. funebris* – 0.2%, *A. absinthii/A. tanacetarius* – 0.3%, *A. tanacetarius/A. funebris* – 0.2%. The values of Min-BSD and Max-WSD clearly indicate that the barcoding COI marker could not be used in discrimination between *A. absinthii*, *A. tanacetarius* and *A. funebris*.

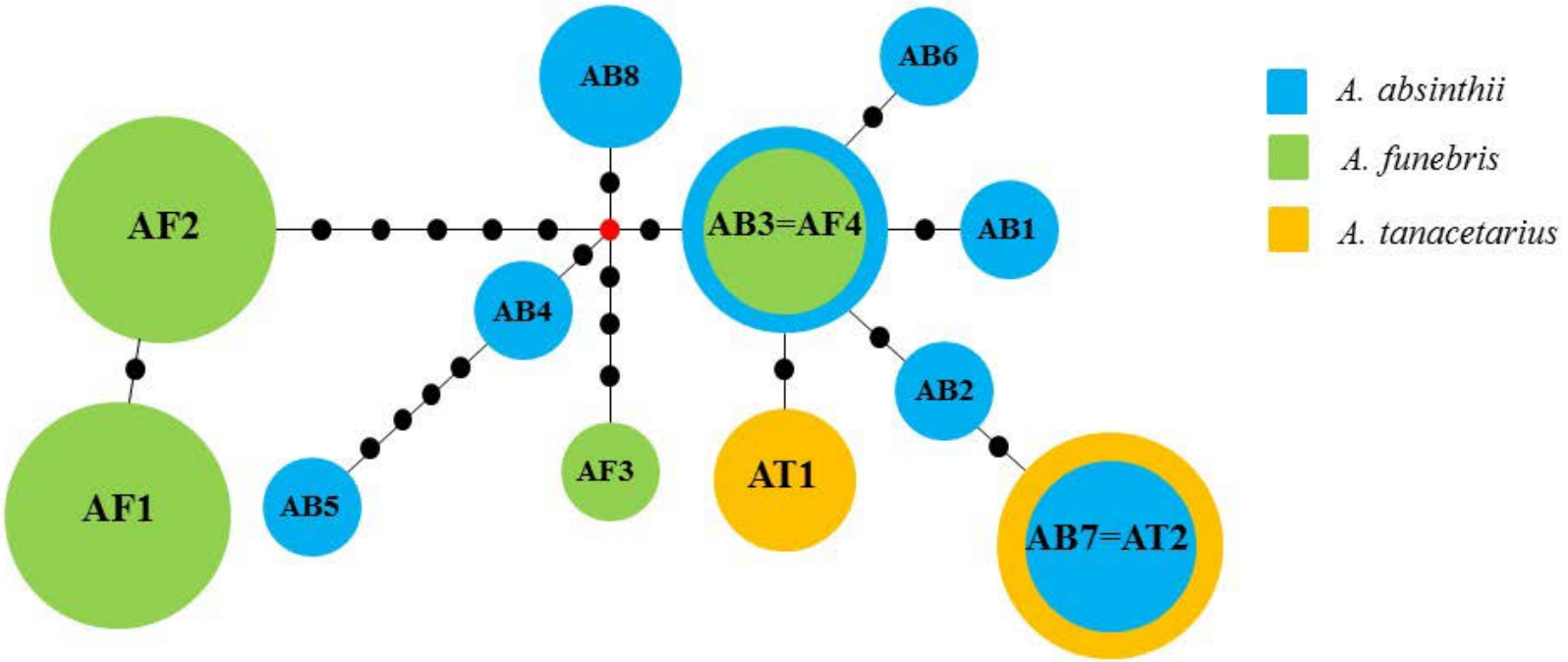

Fig. 8. Median joining network designed for mitochondrial COI haplotypes of *A. absinthii*, *A. funebris* and *A. tanacetarius.* Circle size reflects the number of individuals with that haplotype. Red small circles are median vectors. Black dots represent the mutational steps. Blue circles represent the *A. absinthii* haplotypes AB1-AB8, green – *A. funebris* haplotypes AF1-AF4 and orange - *A. tanacetarius* haplotypes AT1 and AT2.

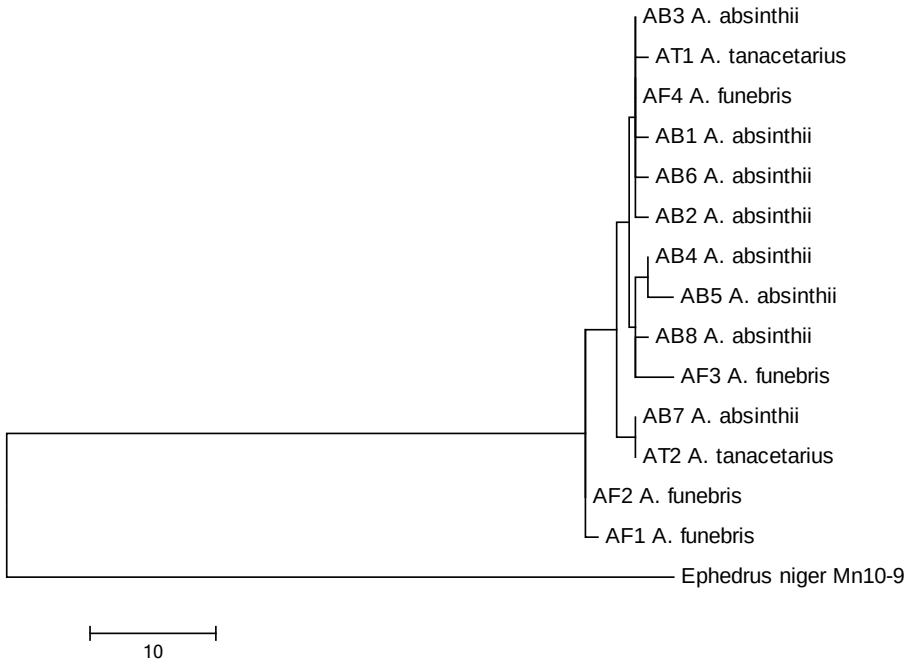

Fig. 9. Maximum parsimony tree for mitochondrial COI haplotypes of *A. absinthii, A. funebris* and *A. tanacetarius.*

Elongation factor. In total four sequences of elongation factor 1-α were obtained for *A. absinthii* (IM80, IM82, IM94, S12-884-1), ten of *A. funebris* (I8, I9, I10, Aa94, Aa95, Aa98, Aa99, Aa101, A103, Aa106) and four of *A. tanacetarius* (IM1, IM3, IM121, IM122). Complete sequences of the nuclear gene 633 bp in length, were aligned and compared for variable sites.

Determined haplotypes diversity was Hd=0.6797, with the following five haplotypes identified:

Hap_1: IM1, IM3, IM121, IM122, IM94, Aa99, Aa98, Aa106

Hap_2: I8, I9, I10, Aa101, Aa94, Aa95, Aa103

Hap_3: IM80

Hap_4: IM82

Hap_5: S11-884-1.

Aphidius tanacetarius and *A. funebris* share the same nuclear haplotype Hap_1. According to the Tamura-Nei model, evolutionary distances between the EF1- α haplotypes ranged from 0.2 to 0.8% (Table 12). Six variable sites were detected between the nuclear haplotypes. Qualitative analysis of the variable sites showed that all nucleotide substitutions

were synonymous, i.e. there are no differences in the protein coded by the EF1- α gene between *A. absinthii*, *A. funebris* and *A. tanacetarius*.

Table 12. Evolutionary distances between the nuclear gene EF1-α haplotypes of *A. absinthii*, *A. funebris* and *A. tanacetarius*

	Hap1	Hap2	Hap3	Hap4
Hap1				
Hap2	0.005			
Hap3	0.002	0.006		
Hap4	0.002	0.003	0.003	
Hap5	0.003	0.008	0.005	0.005

Median-joining network constructed with 95% confidence shows close relatedness with all five nuclear haplotypes grouped together, connected with 1-2 mutational steps (Fig. 10).

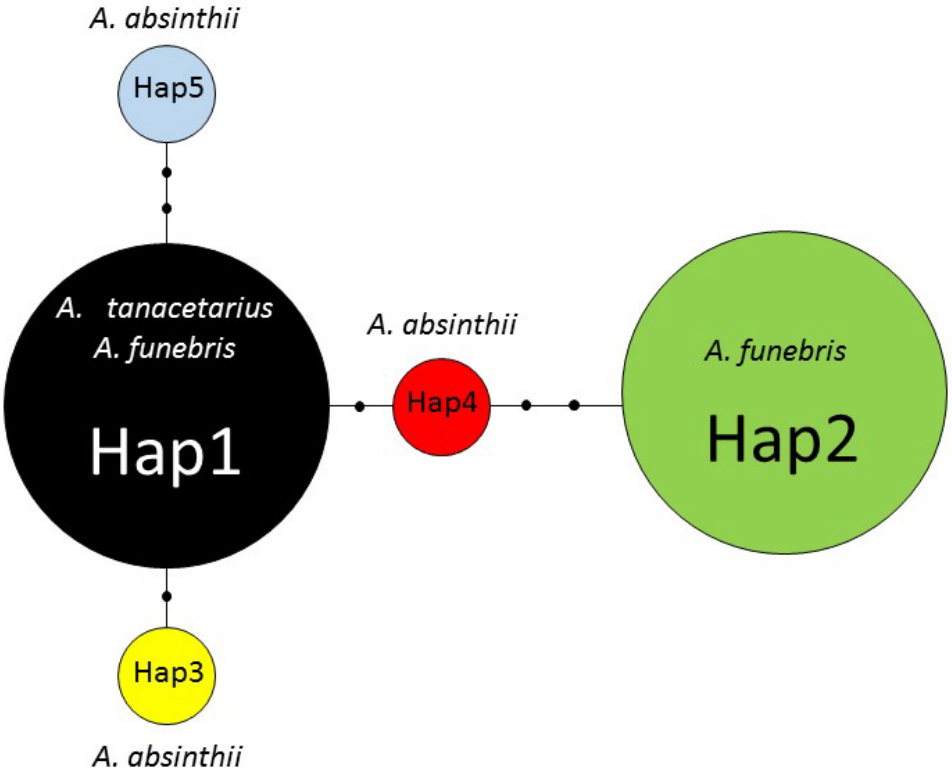

Fig. 10. Median joining network designed for nuclear EF1- α haplotypes of *A. absinthii*, *A. funebris* and *A. tanacetarius.* Circle size reflects the number of individuals with that haplotype (not to scale). Black dots represent the mutational steps.

4.7. *Aphidius microlophii*

Barcoding region of mtCOI gene was analysed for three specimens of *A. microlophii* (IM45, IM46, IM47) collected in Belgium from aphid hosts associated with *Urtica dioica* (Table 1). No difference between the three sequences has been determined. Their haplotype was given a code AMC1 and used in phylogeny study.

4.8. *Aphidius ervi*

In total two specimens of *A. ervi* originating from *Sitobion avenae/Triticum aestivum* (IM123, IM124) and two from *Acyrthosiphon pisum/Medicago sativa* associations (IM131, IM132), were analysed molecularly (Table 13). Comparison of the barcoding sequences determined that all four sequences belong to the same haplotype AE1.

Table 13. The list of analysed *Aphidius ervi* specimens

Country of origin	Sampling locality	Sampling date	Host plant	Aphid host	PCR code
Slovenia	Nova Gorica	04-06-09	*Triticum aestivum*	*Sitobion avenae*	IM123, IM124
Serbia	Beograd	06-11-12	*Medicago sativa*	*Acyrthosiphon pisum*	IM131, IM132

Comparison of *A. ervi* barcoding haplotype AE1 with sequences of other *Aphidius* species determined that it shares 100% identity with the AMC1 haplotype of *A. microlophii*.

Elongation factor. Complete 631 bp long sequences of the elongation factor 1-α have been amplified and aligned for three specimens of *A. ervi* (IM123, IM131, IM132) and three of *A. microlophii* (IM45, IM46, IM47). Comparison showed no differences in the nuclear gene's sequences between the two *Aphidius* species.

4.9. *Aphidius hieraciorum*

Specimens of *A. hieraciorum* parasitising the *Nasonovia ribisnigri* aphid host associated with *Hieracium pilosum*. or *Pilosella aurantiaca* were collected in Serbia and Sweden and submitted to molecular characterization based on the barcoding sequences of

COI gene (Table 14). Three mitochondrial sequences 566 bp in length (IM102, IM103, IM104) were identical, and grouped within the same haplotype AH1.

Table 14. The list of A. *hieraciorum* analysed for mitochondrial COI fragments

Country of origin	Sampling locality	Sampling date	Host plant	Aphid host	PCR code
Serbia	Kopaonik	17.07.2013.	*Hieracium pilosum*	*Nasonovia ribisnigri*	IM102, IM103
Sweden	Uppsala	07.01.2014.	*Pilosella aurantiaca*	*Nasonovia ribisnigri*	IM104

In comparison with other *Aphidius* species, haplotype AH1 of *A. hieraciorum* is closest with the haplotypes AE1 (*A. ervi*) and AMC1 (*A. microlophii*). Tamura-Nei model calculated 1.9% distance between the haplotypes. Using a maximum parsimony calculation, it was determined that minimum 15 mutational steps is connecting the AH1 haplotype with other two AE1 and AMC1, with three median vectors in between representing either unsampled or extinct haplotypes. Minimum between species distance (Min-BSD) in this case is 1.9% either the *A. hieraciorum* is paired with *A. ervi* or *A. microlophii* since the two latter species share identical COI sequence. Considering that Maximum within species divergence in all three cases equals zero, mitochondrial marker is a suitable for delineation of *A. hieraciorum* from *A. microlophii* and *A. ervi*.

Elongation factor. In addition to the barcoding fragments of mtCOI, complete sequences of the elongation factor 1-α have been compared for these parasitoid species. All three *A. hieraciorum* specimens (IM102, IM103, IM104) shared identical EF1- α sequences. In comparison with *A. ervi* and *A. microlophii* which share the same nuclear haplotype, *A. hieraciorum* differed in three nucleotide substitions in the 631 bp long EF1-α sequences (0.5%). Qualitative analysis showed synonymous character of all three nucleotide substitions, confirming that all three *Aphidius* share the same coding protein.

4.10. *Aphidius banksae*

In total five barcoding sequences were obtained and analysed for the species *A. banksae* (Table 15). Comparions of aligned sequences detected three haplotypes ABN1 (IM18), ABN2 (IM19, IM152) and ABN3 (IM27, IM151). Sequences 590 bp long were

compared between the haplotypes and 10 variables sites were detected, all being parsimony informative. Qualitative analyses showed that all mutations were synonymous with no amino acid substitutions. Haplotyes ABN1 and ABN2 are 0.2% distant, while the third haplotype ABN3 differs from ABN1 and ABN2, 1.6 and 1.7%, respectively.

Table 15. The list of *A. banksae* specimens analysed molecularly

Country of origin	Sampling locality	Sampling date	Host plant	Aphid host	PCR code
Serbia	Zemun-Metro	09.05.2011	*Artemisia vulgaris*		IM18, IM19
Serbia	Vlasina	03.08.2011	*Lathyrus pratensis*	*Megoura viciae*	IM27
Montenegro	Tivat	25.05.2011	*Vicia cracca*	*Acyrthosiphon pisum*	IM151, IM152

4.11. *Aphidius (Lysaphidus) erysimi*

Two *A. erysimi* (IM135, IM136) collected in Czech Republic parasitising *Pseudobrevicoryne erysimi* in association with *Erisymum* sp. were subjected to barcoding region analysis (Table 1). Mutations were not detected in 617 bp long mtCOI sequences, and a single identified haplotype was assigned a code ALE1.

4.12. *Aphidius rhopalosiphi*

Material has been sampled in Niška banja from the association of *Rhopalosiphum nymphaeae*/*Typha latifolia* (Table 1). Two haplotypes were identified in the analysed material, AR1 (IM55) and AR2 (IM133, IM134). Comparison of the barcoding sequences 590bp long confirmed eight variables sites, none being parsimony informative. Tamura-Nei model calculated 1.4% distance between the haplotypes. Seven nucleotide substitutions were synonymous, while only one produced amino acid substitution.

Comparison of *A. rhopalosiphi* barcoding sequences with other *Apidius* species determined close relatedness with *A. rubi*. Distances between the *A. rhopalosiphi* haplotype AR1 and two *A. rubi* haplotypes (ARU1, ARU2) were 0.7 and 0.9%, respectively (Table 16). Haplotype AR2 diverged from *A. rubi* haplotypes in range from 1 to 1.2%. Maximum within species distances for *A. rhopalosiphi* is 1.4%, and for *A. rubi* - 0.5%, while Minimum between species distance for this pair is 0.7%.

Table 16. Estimates of evolutionary divergence between *A. rhopalosiphi* and *A. rubi* mtCOI sequences

	IM55	IM133	IM134	IM149	IM150	IM84
IM55 A.rhopalosiphi	-					
IM133 A.rhopalosiphi	0.014					
IM134 A.rhopalosiphi	0.014	0.000				
IM149 A.rubi	0.007	0.010	0.010			
IM150 A.rubi	0.007	0.010	0.010	0.000		
IM84 A.rubi	0.009	0.012	0.012	0.005	0.005	
IM85 A.rubi	0.009	0.012	0.012	0.005	0.005	0.000

The phylogeny tree constructed using the maximum likelihood method showed that the barcoding sequences of *A. rubi* clustered within the *A. rhopalosiphi* clade with 90% bootstrap support (Fig. 11). The referent barcoding sequences of *A. rhopalosiphi* obtained from the open data base in the Genebak were also included in the analysis (Table 2).

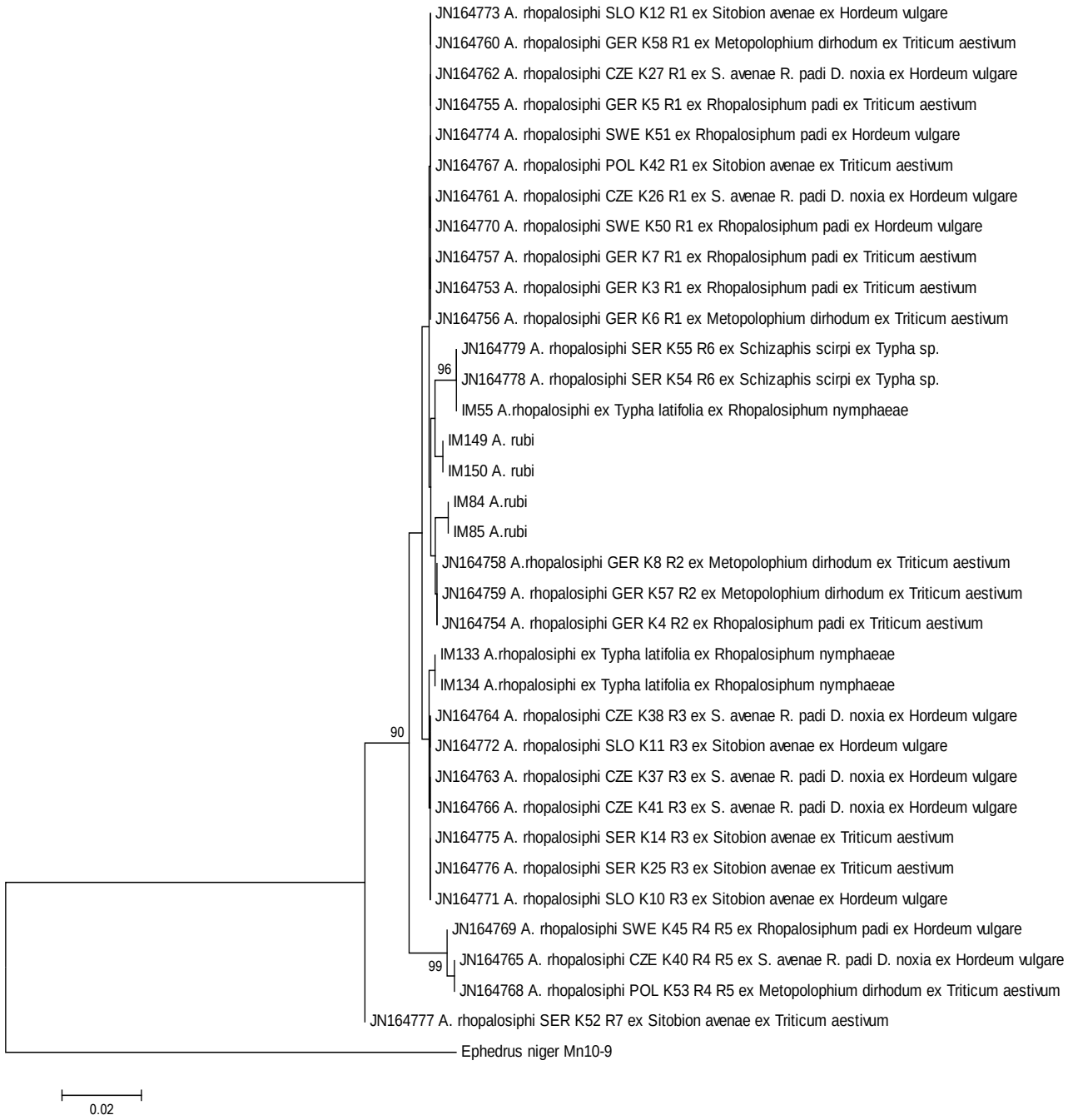

Fig. 11. Maximum likelihood tree of *A. rhopalosiphi* and *A. rubi* COI mtDNA sequences. Besides the analysed sequences (IM55, IM133, IM134), referent barcoding sequences of *A. rhopalosiphi* associated with different aphid/plant host associations were obtained from the Genebank for comparison (details in Table 2).

Median joining network was recounstructed for the barcoding seuqences of *A. rhopalosiphi* and *A. rubi* (Fig. 12). In total, 34 mtCOI sequences were compared and ten following haplotypes detected:

AR1: IM55, JN164779, JN164778

AR2: IM133, IM134

ARU2: IM149, IM150

ARU1: IM84, IM85

Rop1: JN164773, JN164762, JN164761, JN164757, JN164756, JN164755, JN164753, JN164774, JN164770, JN164767, JN164760

Rop2: JN164765, JN164768

Rop3: JN164764, JN164763, JN164776, JN164775, JN164772, JN164771, JN164766

Rop4: JN164777

Rop5: JN164759, JN164754, JN164758

Rop6: JN164769.

Haplotypes ARU1 and ARU2 assigned to *A. rubi* are connected with haplotypes of *A. rhopalosiphi* with two median vectors and several mutational steps (Fig. 12). Twenty-six variable sites were identified in analysed barcoding sequences 590 bp in lentgh. Evolutionary distances between the barcoding COI haplotypes of *A. rhopalosiphi* and *A. rubi* ranged from 0.5 to 2.6% (Table 17).

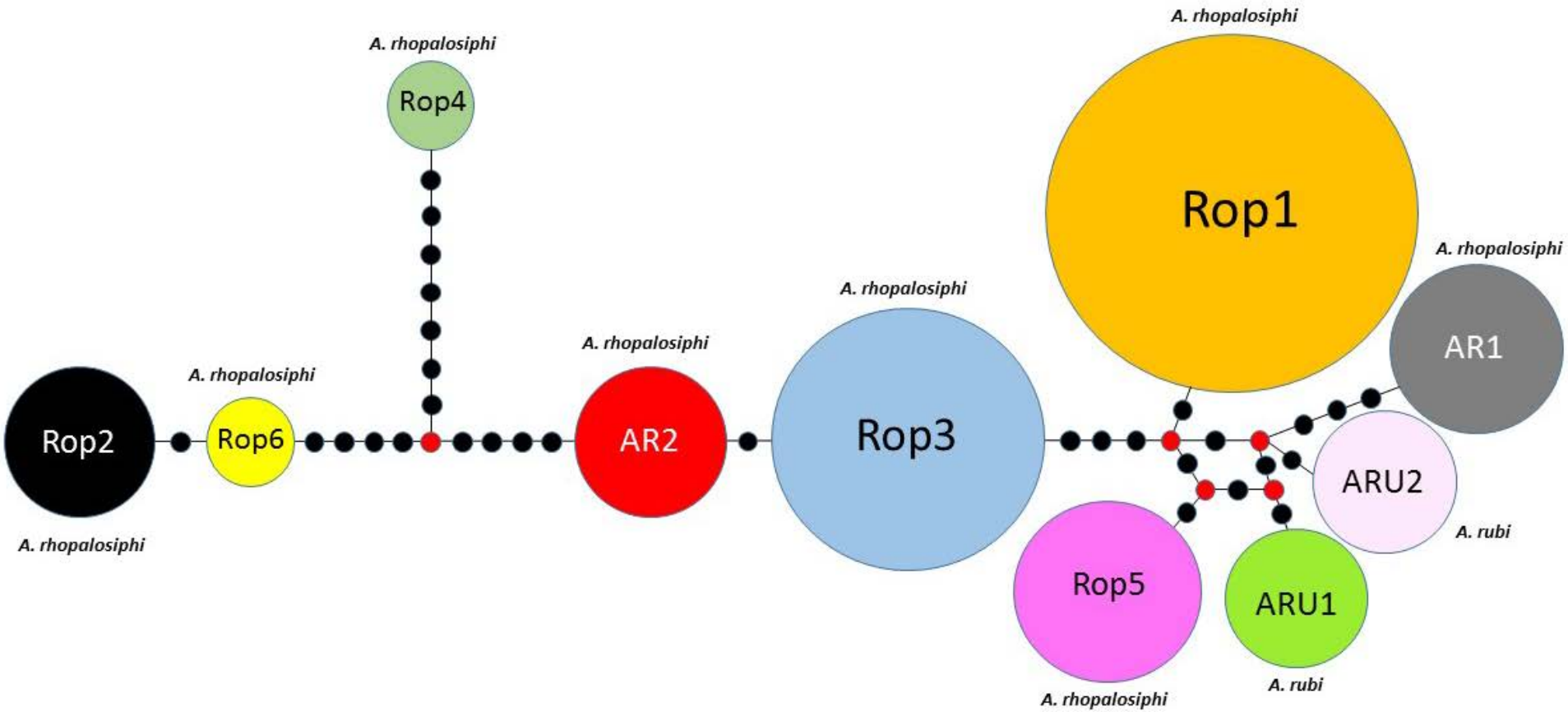

Fig. 12. Median joining network designed for mitochondrial COI haplotypes of *A. rhopalosiphi* and *A. rubi*. Circle size reflects the number of individuals with that haplotype (not to scale). Red circles represent the median vectors. Black dots represent the mutational steps.

Table 17. Evolutionary distances between the barcoding COI haplotypes of *A. rhopalosiphi* and *A. rubi*

	AR1	AR2	ARU2	ARU1	Rop 1	Rop 2	Rop 3	Rop 4	Rop 5
AR 1									
AR 2	0.014								
ARU 2	0.007	0.010							
ARU1	0.009	0.012	0.005						
Rop 1	0.009	0.009	0.005	0.007					
Rop 2	0.026	0.016	0.023	0.025	0.021				
Rop 3	0.012	0.002	0.009	0.010	0.007	0.018			
Rop 4	0.026	0.019	0.026	0.028	0.024	0.021	0.021		
Rop 5	0.010	0.010	0.007	0.005	0.005	0.019	0.009	0.023	
Rop 6	0.025	0.014	0.021	0.023	0.019	0.002	0.016	0.019	0.017

Elongation factor. Complete sequences of the elongation factor were amplified for two specimens of *A. rhopalosiphi* (IM55, M133) and two of *A. rubi* (IM84, IM85) for confirmation of their relatedness. Within each species, specimens shared the same nuclear sequences. On the other hand, comparison of nuclear sequences between the two species, showed divergence rate of 0.6%. In total four variable sites were identified in 633 bp long EF1-α sequences, however all nucleotide substitions were synonymous in character.

4.13. *Aphidius salicis*

Twelve specimens of *A. salicis* were analysed molecularly for the barcoding region divergence (Table 18). Comparison of sequences trimmed to equal size of 590 bp determined seven variable sites, four of which are parsimony informative. All seven mutations were synonymous not producing amino acid substitutions.

Five haplotypes were determined ASC1 (IM78), ASC2 (IM77), ASC3 (IM61, IM116), ASC4 (IM57, IM118, Lya4), ASC5 (IM60, IM79, IM117, Lya1, Lya5). Overall evolutionary distance between haplotypes was 0.3% (range 0.2 - 1%).

Table 18. The list of *A. salicis* specimens analysed for mtCOI sequences variation

Country of origin	Sampling locality	Sampling date	Host plant	Aphid host	PCR code
Serbia	Niš	25.06.2010	*Pimpinella anisum*	*Cavariella* sp.	IM57, IM60, IM61
Croatia	Homoljačka	22.06.2015.	*Apiacae*	*Cavariella aegopodii*	IM77, IM78, IM79
Slovenia	Zalog	27.06.2011.	*Daucus carota*	*Cavariella* sp.	IM116, IM117, IM118
Montenegro	Žabljak		*Daucus* sp.	*Cavariella* sp.	Lya1, Lya4, Lya5

4.14. *Aphidius aquilus*

In total three specimens of *A. aquilus* (IM28, IM29, IM30) were collected from the locality Vlasina in Serbia, parasitising the host *Betulaphis quadrituberculata* associated with *Betula* sp. Barcoding sequences 590 bp long were 100% identical for all three analysed parasitoids, and their joint haplotype was assigned a code AA1.

Comparison of the haplotype AA1 with other *Aphidius* species identified low evolutionary distance between *A. aquilus* and *A. salicis* haplotypes. An overall divergence rate between the haplotypes AA1, ASC1, ASC2, ASC3, ASC4 and ASC5 was 0.6% (range of genetic distances was 0.5 to 1%) (Table 19).

Barcoding region of COI did not show as a relilable marker in delineation of these two parasitoid species. Minimum between species distance was 0.5%, Max-WSD in *A. salicis* was 1%, while in case of *A. aquilus* the value of Max-WSD=0 because all sequences were identical.

Table 19. Evolutionary distances between *A. aquilus* and *A. salicis* mtCOI haplotypes based on Tamura-Nei model

Haplotype	ASC1	ASC2	ASC3	ASC4	ASC5
ASC1					
ASC2	0.005				
ASC3	0.005	0.010			
ASC4	0.005	0.010	0.003		
ASC5	0.003	0.009	0.002	0.002	
AA1	0.009	0.010	0.007	0.007	0.005

Median joining network only confirms low divergence rate between the haplotypes of *A. salicis* and *A. aquilus* (Fig. 13). All haplotypes are connected and the position of the haplotype AA1 and the number of mutational steps connecting it with haplotypes ASC1-ASC5 show no delineation between the two species as separate taxa.

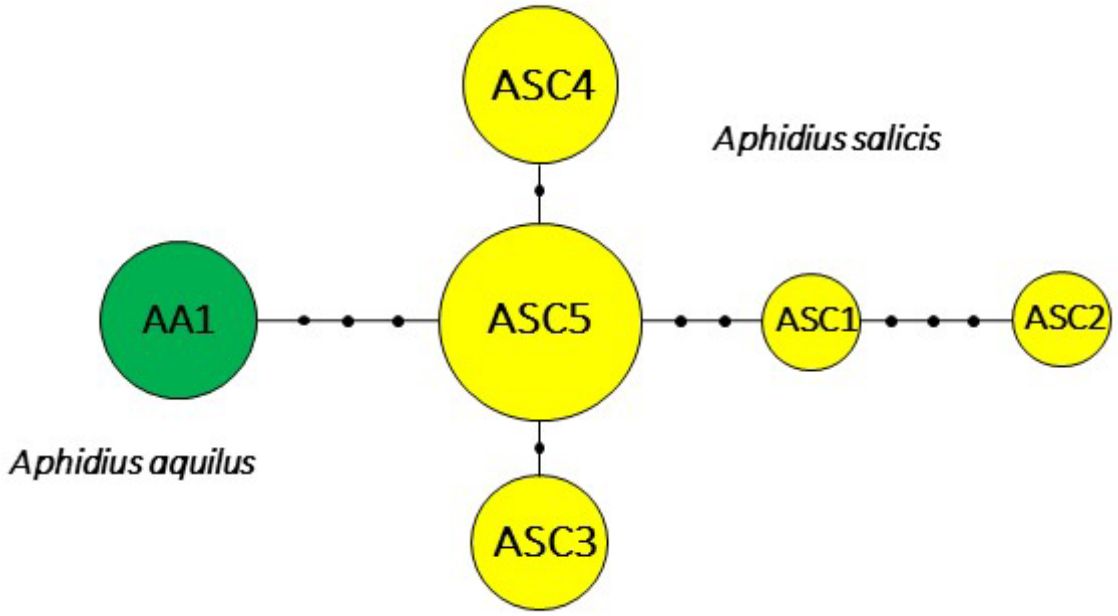

Fig. 13. Median joining network designed for mitochondrial COI haplotypes of *A. salicis* and *A. aquilus*. Circle size reflects the number of individuals with that haplotype. Yellow circles are *A. salicis* haplotypes ASC1 – ASC5. Green circle is the *A. aquilus* haplotype AA1. Black dots represent the mutational steps.

Elongation factor. Complete sequences of the elongation factor 1-α have been succesfully amplified and aligned for four specimens of *A. salicis* (IM57, IM79, IM116, IM117) and three of *A. aquilus* (IM28, IM29, IM30). Comparison of 631 bp long EF1-α sequences showed no differences within the two *Aphidius* species, as well as between them.

4.15. *Aphidius sonchi*

Two specimens of *A. sonchi* were available for molecular characterization (IM9, IM10). Both were collected in Serbia parasitising *Hyperomyzus lactucae* associated with *Sonchus arvensis* (Table 1). One haplotype was determined (AS1), with both specimens sharing the same barcoding sequence.

4.16. *Aphidius asteris*

Two specimens of *Aphidius asteris* (IM5, IM7) collected in Bulgaria from the *Macrosiphoniella sambornii/ Dendrathemum hybridum* association have been submitted to molecular analysis (Table 1). Both sequences of barcoding region have been succesfully amplified. Comparison determined only one haplotype named AAS1, shared by the two specimens.

4.17. *Aphidius linosiphonis*

A single specimen of *A. linosiphonis* collected from Montenegro was available (IM11) for molecular analysis (Table 1). It was assigned a haplotype code AL1 and used in phylogeny study.

4.18. *Aphidius schimitscheki*

Two specimens of *A. schimitscheki* were captured from *Abies* sp. on the mountain Kopaonik in Serbia (IM14, IM139) and used for molecular characterization and phylogenetic analysis (Table 1). Barcoding sequences were determined identical and their haplotype was given a code ASH1.

4.19. *Aphidius balcanicus*

A single specimen of *A. balcanicus* (IM86) collected in Croatia from *Acyrthosiphon malvae* associated with *Geranium rober* was used in phylogeny study (Table 1). Barcoding region was succesfully sequenced and it was assigned a haplotype code ABL1.

4.20. *Aphidius phalangomyzi*

In phylogeny study one specimen of *A. phalangomyzi* (IM49) was included. The parasitoid was collected in Belgium from the association *Macrosiphoniella* sp./ *Artemisia vulgaris* (Table 1). Barcoding region was succesfully sequenced and it was assigned a haplotype code AP1.

4.21. *Aphidius avenae*

Two specimens of *A. avenae* originating from Montenegro (A1) and Germany (A2) were submitted to molecular analysis (Table 1). Comparison of the two barcoding sequences 613 bp in length showed existence of a single haplotype AAV1.

4.22. *Aphidius sussi*

In total, three specimens of *A. sussi* were available for molecular characterization and the barcoding sequences analysis (Table 20). Alligment of mitochondrial sequences showed no difference between the analysed *A. sussi* (haplotype ASU1).

Table 20. The list of analysed *A. sussi*

Country of origin	Sampling locality	Sampling date	Host plant	Aphid host	PCR code
Montenegro	Crno jezero	11.08.2005.	*Aconitum toxicum*	*Delphiniobium junackianum*	IM4
Montenegro	Durmitor - Crno jezero	27.08.2013.	*Aconitum toxicum*	*Delphiniobium junackianum*	S12-833
Slovenia	Bohinj	14.07.2009.	*Aconitum maximum*	*Delphiniobium* sp.	Sl09-86-1

4.23. *Aphidius rosae*

Barcoding sequences analysis and molecular characterizaton have been conducted for ten specimens of *A. rosae* (Table 21). Three haplotypes were identified, ARS1 (IM110, IM111, IM112), ARS2 (IM113, IM114, Ar1, IM87, IM88, IM89) and ARS3 (Ar2). Alignment of 590 bp long barcoding sequences detected only four variable sites, one being parsimony informative. No amino acid substitutions were identified because all four mutations were synonymous. Evolutionary distances between the three halotypes based on Tamura-Nei model were in range of 0.2 to 0.7%.

Table 21. The list of *A. rosae* specimens analysed for mtCOI fragments difference

Country of origin	Sampling locality	Sampling date	Host plant	Aphid host	PCR code
Belgium	Jodoigne		*Rosa canina*	*Macrosiphum rosae*	IM110, IM111,IM112
Slovenia	Zelenci	18.07.2014.	*Knautia brymeia*	*Macrosiphum rosae*	IM113, IM114
Serbia	Topčider	30.05.2008.	*Rosa* sp.		Ar1
Iran	Jiroft	07.05.2008.	*Rosa* sp.	*Macrosiphum rosae*	Ar2
Croatia	Koreničko	22.06.2015.	*Knautia* sp.	*Macrosiphum rosae*	IM87, IM88, IM89

4.24. *Aphidius eadyi*

Six specimens of *A. eadyi* were available for molecular studies (Table 22). Alignment of the barcoding sequences 590bp in length determined two haplotypes, AEA1 (IM99) and AEA2 (AE1/1, AE1/3, AE2/2, AE2/3, Sl08-26-2). Comparison of haplotypes detected five variable sites, none parsimony informative. Qualitative analysis showed no amino acid substitutions. Evolutionary distance based on Tamura-Nei model determined between AEA1 and AEA2 was 0.9%.

Table 22. The list of *A. eadyi* specimens analysed for mtCOI fragments difference

Country of origin	Sampling locality	Sampling date	Host plant	Aphid host	PCR code
Sweden	Skaltsa	07.02.2014.	*Pisum sativum*	*Acyrthosiphon pisum*	IM99
Serbia	L-CL3	31.05.2011.	*Medicago sativa*	*Acyrthosiphon pisum*	AE 1/1
Serbia	Umčari	8.6.2012.	*Medicago sativa*	*Acyrthosiphon pisum*	AE 1/3
Serbia	Malo Orašje	8.6.2012.	*Medicago sativa*	*Acyrthosiphon pisum*	AE 2/2, AE 2/3
Slovenia	Strujan	20.11.2008.	*Medicago sativa*	*Acyrthosiphon pisum*	Sl08-26-2

4.25. *Aphidius ericaphidis*

Three specimens of *A. ericaphidis* (IM50, IM51, IM52) reared from *Ericaphis scammelli* on *Vaccinium corymbosum* were submitted to mitochondrial COI fragments characterization. All three barcoding sequences shared the same haplotype AER1. This is the first record of *A. ericaphidis* in Europe and morphological description of analysed material is presented in the Appendix 2.

4.26. *Aphidius (Euaphidius) setiger*

Two specimens of *Aphidius setiger* (IM119, IM120) collected in Belgium from the association *Peryphyllus testudinaceus/Acer campestre* were subjected to molecular characterization of the barcoding region of mitochondrial COI gene (Table 1). No difference was detected in the nucleotide content between the two analysed sequences and haplotype was assigned a code AES1.

4.27. *Aphidius uzbekistanicus*

A single parasitoid (IM48) parasitising *Sitobion avenae* associated with *Poa annua* was available for phylogeny study (Table 1). The barcoding haplotype was named AU1.

4.28. *Aphidius (Lysaphidus) arvensis*

In total two specimens of *Aphidius arvensis* (IM145, IM146) associated with *A. sargai/Inula sp.* from Iran were available for molecular analysis (Table 1). Comparison of 615 bp long barcoding sequences showed no difference and their haplotype was named ALA1 and used for phylogeny study.

4.29. *Aphidius (Lysaphidus) viaticus*

Barcoding sequence analysis included six specimens of *A. viaticus* all originating from the same locality in Serbia, associated with *Pleotrichophorus filaginis*/ *Filago germanica* (Table 23). Two haplotypes were determined, ALV1 (IM15, IM16, IM17, IM25, S11-435-1) and ALV2 (IM24). One mutation was detected discriminating the haplotypes, with synonymous character in terms of protein sequence alterations.

Table 23. The list of analysed *A. viaticus*

Country of origin	Sampling locality	Sampling date	Host plant	Aphid host	PCR code
Serbia	Valjevo	01.05.2011.	*Filago germanica*	*Pleotrichophorus filaginis*	IM15, IM16, IM17
Serbia	Valjevo	18.06.2011.	*Filago germanica*	*Pleotrichophorus filaginis*	IM24, IM25, S11-435-1

4.30. *Aphidius ribis*

Five specimens of *A. ribis* (IM12, IM13, IM107, IM108, IM109) were subjected to analyses of the barcoding region divergence (Table 1). Comparison of 590 bp long sequences detected three haplotypes ARI1 (IM12, IM13), ARI2 (IM107) and ARI3 (IM108, IM109). Divergence rate between the haplotypes ranged from 0.2 to 0.7%. Four mutations were identified, none however parsimony informative. Substitutions had a synonymous character not producing amino acid substitutions in the protein sequence.

4.31. *Aphidius chaetosiphonis*

A single parasitoid was available for molecular studies (IM53) and used for phylogeny tree reconstruction (Table 1). *Aphidius chaetosiphonis* was collected in Montenegro parasitising *Chaetosiphon* sp. associated with *Potentilla clusiana*. Haplotype of *A. chaetosiphonis* was named ACH1.

4.32. *Aphidius hortensis*

Six specimens of *A. hortensis* were included in molecular characterization of the barcoding genetic divergence (Table 24). Four haplotypes were identified: AHI1 (IM21, IM23), AHI2 (IM22), AHI3 (IM31, IM32) and AHI4 (IM105). Evolutionary distances between haplotypes varied from 0.2 to 0.7%. In total four variable sites were determined, all synonymous and only one parsimony informative.

Table 24. The list of *A. hortensis* specimens analysed for mtCOI fragments difference

Country of origin	Sampling locality	Sampling date	Host plant	Aphid host	PCR code
Serbia	Petnica	12.06.2011	*Mahonia aquilegifolia*	*Liosomaphis berberidis*	IM21, IM22, IM23
Serbia	Tara-Perućac	03.07.2012	*Berberis vulgaris*	*Liosomaphis berberidis*	IM31, IM32
Slovenia	Zelenci	18.07.2014.	*Berbeis vulgaris*	*Liosomaphis berberidis*	IM105

In comparison with other *Aphidius*, barcoding haplotypes of *A. hortensis* (AHI1-AHI4) are closely related with the haplotype of *A. chaetosiphonis* (ACH1). Evolutionary distances between the haplotypes are below 1% (Table 25). The maximum WSD for *A. hortensis* is 0.7%, while *A. chaetosiphonis* had only one sequence available thus the Max-WSD could not be calculated. However, if the Max-WSD of *A. hortensis* is plotted versus Minimum between species distance which is 0.5%, it is evident that these two species cannot be identified based solely on the barcoding analyses.

Table 25. Evolutionary distances between the mtCOI haplotypes of *A. hortensis* and *A. chaetosiphonis*

haplotype	AHI1	AHI2	AHI3	AHI4
AHI1				
AHI2	0.002			
AHI3	0.005	0.007		
AHI4	0.002	0.003	0.003	
ACH1	0.007	0.009	0.005	0.005

Median joining network shows no separation of lineages or clear species delineation (Fig. 14). It groups *A. chaetosiphonis* haplotype with other four of *A. hortensis*, connected with two mutational steps and one median vector representing the missing unsampled intermediary haplotype(s).

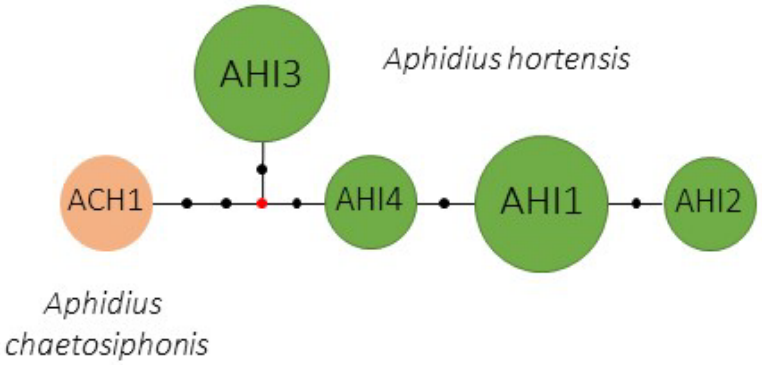

Fig. 14. Median joining network designed for mitochondrial COI haplotypes of *A. hortensis* and *A. chaetosiphonis*. Circle size reflects the number of individuals with that haplotype. Green circles are *A. hortensis* haplotypes AHI1–AHI4. Orange circle is the *A. chaetosiphonis* haplotype ACH1. Red dot is a median vector. Black dots represent the mutational steps.

Elongation factor. Complete sequences of elongation factor 1-α were amplified for one *A. chaetosiphonis* (IM53) and one *A. hortensis* (IM31). Comparison of 633 bp long nuclear sequences showed that these two parasitoid species share the same haplotype of EF1-α.

4.33. Phylogenetic analyses of *Aphidius* species

A Maximum likelihood phylogeny tree has been constructed including all 62 barcoding COI haplotypes identified for 33 *Aphidius* species (Table 26). Along with our haplotypes, referent mitochondrial sequences of *A. avenae, A. colemani, A. transcaspicus, A. rhopalosiphi, A. platensis, A. uzbekistanicus, A. cingulatus, A. setiger* obtained from the Genebank were also included for comparison (Table 2).

The topology of ML tree shows species and species groups' delineation with different bootstrapp support (Fig. 15). Parasitoid species which were clearly recognized as separate taxa were: *A. erysimi, A. sonchi, A. linosiphonis, A. hieraciorum, A. arvensis, A. balcanicus, A. phalangomyzi, A. banksae, A. uzbekistanicus, A. sussi, A. silvaticus, A. avenae, A. rosae, A. ericaphidis, A. eadyi, A. viaticus, A. schimitscheki, A. ribis, A. setiger, A. asteris, A. matricariae* and *A. urticae.*

Individual analyses of the COI barcoding fragments showed close relatedness of several species, which is confirmed by their joint clustering on the phylogeny tree as well. Thus, following five groups of species were identified on the phylogeny tree:

- *A. rhopalosiphi* and *A. rubi*
- *A. microlophii* and *A. ervi*
- *A. absinthii, A. funebris* and *A. tanacetarius*
- *A. salicis* and *A. aquilus*
- *A. chaetosiphonis* and *A. hortensis.*

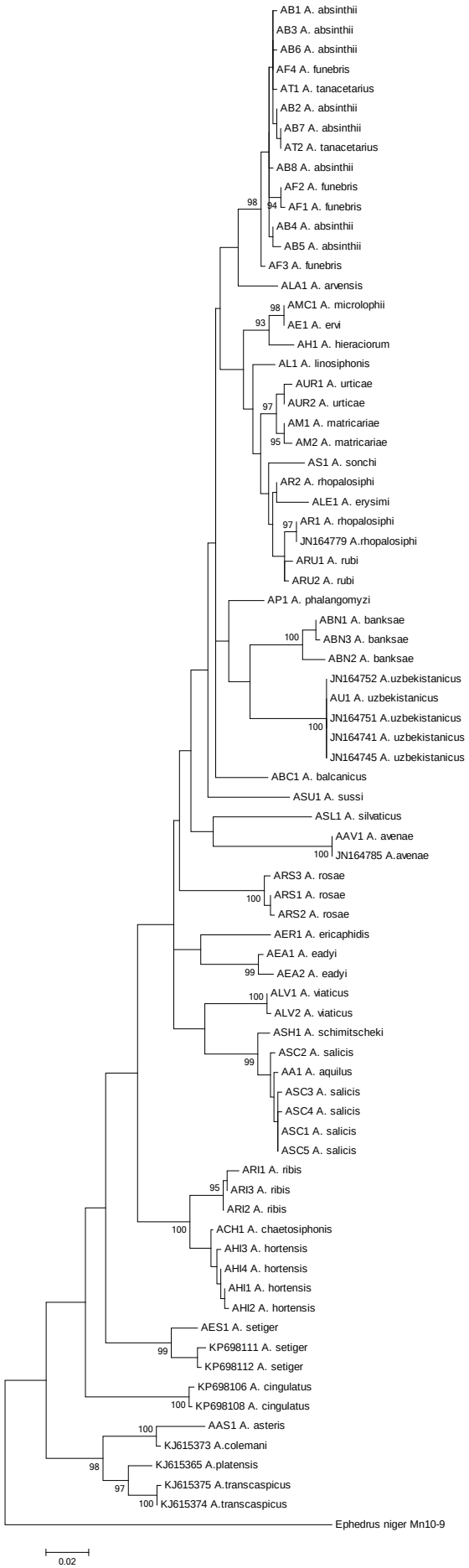

Fig. 15. Maximum likelihood phylogeny tree with the barcoding sequences of COI gene for 33 *Aphidius* species. Bootstrapp support exceeding 90% was presented above the branches.

Table 26. The list of all DNA barcoding haplotypes detected within the 33 *Aphidius* species

species	Haplotypes	sequences
A. absinthii	AB1	IM80, IM81
	AB2	IM82
	AB3	IM94, IM95, IM96, S12-55-1
	AB4	IM141
	AB5	IM142
	AB6	S11-250-1
	AB7	S11-884-1, S11-884-2, S11-884-3
	AB8	S11-345-1, S11-345-2
A. funebris	AF1	IM59, I8, I9, I10, I15, I16
	AF2	Aa94, Aa95, Aa101, Aa103, Aa108
	AF3	Aa98, Aa105, Aa106
	AF4	Aa99, Aa104
A. tanacetarius	AT1	IM121, IM122
	AT2	IM1, IM2, IM3, Atan1, Atan2
A. sussi	ASU1	IM4, S12-833, Sl09-86-1
A. asteris	AAS1	IM5, IM7
A. sonchi	AS1	IM9, IM10
A. linosiphonis	AL1	IM11
A. ribis	ARI1	IM12, IM13
	ARI2	IM107
	ARI3	IM108, IM109
A. schimitscheki	ASH1	IM14, IM139
A. viaticus	ALV1	IM15, IM16, IM17, IM25, S11-435-1
	ALV2	IM24
A. banksae	ABN1	IM18
	ABN2	IM19, IM152
	ABN3	IM27, IM151
A. phalangomyzi	AP1	IM49
A. hortensis	AHI1	IM21, IM23
	AHI2	IM22
	AHI3	IM31, IM32
	AHI4	IM105
A. hieraciorum	AH1	IM102, IM103, IM104
A. rosae	ARS1	IM110, IM111, IM112
	ARS2	IM113, IM114, AR1, IM87, IM88, IM89
	ARS3	AR2
A. uzbekistanicus	AU1	IM48
A. avenae	AAV1	A1, A2
A. microlophii	AMC1	IM45, IM46, IM47
A. ervi	AE1	IM123, IM124, IM131, IM132
A. ericaphidis	AER1	IM50, IM51, IM52
A. chaetosiphonis	ACH1	IM53
A. rhopalosiphi	AR1	IM55
	AR2	IM133, IM134
A. salicis	ASC1	IM78
	ASC2	IM77
	ASC3	IM116, IM61
	ASC4	IM118, IM57, Lya4
	ASC5	IM117, Lya1, IM79, Lya5, IM60

A. balcanicus	ABC1	IM86
A. silvaticus	ASL1	IM90, IM91, IM92
A. aquilus	AA1	IM28, IM29, IM30
A. urticae	AUR1	Ud4, IM137, IM138
	AUR2	Mc2
A. matricariae	AM1	IM54, IM125, IM147
	AM2	IM148
A. rubi	ARU1	IM84, IM85
	ARU2	IM149, IM150
A. setiger	AES1	IM119, IM120
A. erysimi	ALE1	IM135, IM136
A. eadyi	AEA1	IM99
	AEA2	AE1/1, AE1/3, AE2/2, AE2/3, Sl08/26-2
A. arvensis	ALA1	IM145, IM146

4.34. Suitability of the barcoding region of COI mtDNA in *Aphidius* species identification

Aphidius linosiphonis, A. phalangomyzi, A. uzbekistanicus, A. chaetosiphonis and *A. balcanicus* had been excluded from the analysis due to a single barcoding sequence available. Other species are paired to each other and maximum within species divergence (Max-WSD) compared with the minimum between species divergence (Min-BSD).

Results show that species which had Max-WSD exceeding the Min-BSD in all paired combinations and can be easily identified based on the COI barcoding marker are: *A. sussi, A. asteris, A. sonchi, A. ribis, A. schimitscheki, A. viaticus, A. banksae, A. hieraciorum, A. rosae, A. avenae, A. ericaphidis, A. silvaticus, A. setiger, A. erysimi, A. eadyi, A. arvensis A. matricariae* and *A. urticae*.

On the other hand, barcoding has failed to distunguish *A. rhopalosiphi* from *A. rubi, A. microlophii* from *A. ervi, A. absinthii* from *A. funebris* and *A. tanacetarius* and vice versa, *A. salicis* from *A. aquilus, A. chaetosiphonis* from *A. hortensis.* In case of these species, Max-WSD exceeded or was close in values to the Min-BSD.

5. DISCUSSION

In Aphidiinae classification, the use of morphological characteristics has been the standard method of identifying species. The morphological characteristics most commonly used as diagnostic features are wing venation, number of antennal segments, general shape and carination of the propodeum, shape of the petiole and ovipositor sheaths, number of maxillary or labial palpomeres, and colouration of the mummy. In addition, pupation under or within the aphid mummies is also used as a discriminatory feature.

The combination of molecular studies and geometric morphometrics has proved to be efficient in detecting morphological variation even in cryptic species of aphidiineparasitoids. Molecular analyses of Aphidiinae using different molecular markers have yielded similar results on the problem of monophyly of different parasitoid groups. For example, the analyses of Belshaw and Quicke (1997) included 11 genera of Aphidiinae and 13 genera of other subfamilies of Braconidae. These analyses were conducted using three molecular markers, viz., mitochondrial cytochrome b, nuclear EF1-α, and 28S rRNA. All three markers questioned the monophyly of Aphiidiinae. Smith et al. (1999) used sequences of the mitochondrial NADH1 dehydrogenase gene to analyse the phylogeny of aphid parasitoids. They determined that three of the seven genera which include multiple species are not monophyletic: *Pauesia, Aphidius,* and *Trioxys.*

Sanchis et al. (2000) conducted a phylogenetic study of the subfamily Aphidiinae by sequencing the 18S rDNA in 37 aphidiine taxa. The results of their analysis favour either the hypothesis as to the existence of three tribes (Ephedrini, Praini and Aphidiini) or a new classification with at least five tribes (Ephedrini, Praini, Monoctonini, Trioxini and Aphidiini).

The results obtained by Shi and Chen (2005) also indicate that the genus *Aphidius* can be considered as a paraphyletic group. They used three genes, the mitochondrial large ribosomal subunit 16S, 18S ribosomal DNA and mitochondrial ATPase 6, to investigate phylogenetic relationships among 16 genera of the subfamily Aphidiinae. Their results support the existence of three tribes, viz., Ephedrini, Praini, and Aphidiini, with Ephedrini occupying the basal position. They also show that Aphidiini can be further subdivided into three subtribes, viz., Monoctonina, Trioxina, and Aphidiina.

Tomanović et al. (2007) analysed the phylogenetic relationships among eight *Aphidius* and six *Lysaphidus* species on the basis of 12 morphological characters. Although *Lysaphidus*

parasitoids have been considered as a possible subgenus within the genus *Aphidius* due to certain apomorphies (e.g. reduced wing venation pattern), Tomanović et al. (2007) did not find evidence to support any subgeneric status for *Lysaphidus*, and several new combinations were proposed (*Aphidius adelocarinus* Smith, 1944, comb. rev., *A. ramythirus* Smith, 1944, comb. rev., *A. rosaphidis* Smith, 1944, comb. rev., *A. viaticus* (Sedlag) comb. nov., *A. arvensis* (Starý) comb.nov., and *A. erysimi* (Starý) comb. nov.). Elsewhere, the taxonomic position of the subgenus *Tremblayia* Tizado and Núñez-Pérez, 1994 was also reconsidered and newly classified as a synonym of *Aphidius* (Ilić-Milošević et al. 2015).

Our molecular analyses confirmed the revision by Tomanović et al. (2007) based on morphological data. On the phylogeny tree based on the obtained barcoding COI sequences *A. arvensis*, *A. erysimi* and *A. viaticus* clustered within the genus *Aphidius*. Moreovoer, these three species were dispersed on the tree and belonged to different *Aphidius* clades very far apart from each other, so it can be assumed that *Lysaphidus* is a paraphyletic group. Surprisingly, the *A. colemani* group, consisting of *A. colemani, A. transcaspicus, A. platensis*, and *A. asterias*, is basal within the genus *Aphidius*, existing as a sister group to the remaining *Aphidius* species.

Ilić-Milošević et al. (2015) analysed the taxonomic position and phylogenetic relationships of species of the genera *Euaphidius, Remaudierea* Starý, 1973 and *Aphidius* using the mitochondrial cytochrome oxidase subunit I and nuclear 28SD2 genes and geometric morphometrics. Molecular markers showed small genetic differences between the selected *Euaphidius, Remaudierea* and *Aphidius* species. On the basis of molecular data and shape of the wing, they redescribed *Euaphidius cingulatus, E. setiger*, and *Remaudierea plocamaphidis* Starý, 1973 by placing them within the genus *Aphidius*. Finally, that group of authors proposed placing of species *E. cingulatus* and *R. plocamaphidis* in the subgenus *Euaphidius* within the genus *Aphidius*. Our molecular characterization and phylogeny tree of COI sequences also corroborated the position of *A. setiger* and *A. cingulatus,* which clustered as separate lineages within the genus *Aphidius,* thereby confirming that *Euaphidius* cannot be treated as a subgenus.

Two molecular markers were employed in the present study to evaluate the taxonomic status and phylogenetic relationships among 33 species from the genus *Aphidius*, viz., a barcoding region of the mitochondrial COI gene and nuclear elongation factor 1-α. Overall, mtDNA barcoding showed itself to be a reliable method for identifying most of the species

included in this study, while EF1-α was too conservative and thus failed to be informative in delimiting species.

Based on mtDNA sequence analysis, a total of 22 species were successfully distinguished: *A. erysimi, A. sonchi, A. linosiphonis, A. hieraciorum, A. arvensis, A. balcanicus, A. phalangomyzi, A. banksae, A. uzbekistanicus, A. sussi, A. silvaticus, A. avenae, A. rosae, A. ericaphidis, A. eadyi, A. viaticus, A. schimitscheki, A. ribis, A. setiger, A. asteris, A. matricariae* and *A. urticae.* On the phylogeny tree, five clades were separated with bootstrap support exceeding 90%: i) *A. salicis* and *A. aquilus*; ii) *A. funebris, A. tanacetarius, A. absinthii*; iii) *A. ervi* and *A. microlophii*; iv) *A. chaetosiphonis* and *A. hortensis*; and v) *A. rubi* and *A. rhopalosiphi*. When the minimum between species distance was plotted versus the maximum within species distance, species within the five clades could not be distinguished.

The results obtained from combined morphological and molecular analyses using the barcoding region revealed three cryptic lineages within the *A. urticae* s. str. group (Jamhour et al. 2016). Significant evolutionary distances ranging from 2.3 to 9.2% between *A. silvaticus, A. rubi* and *A. urticae* were not accompanied by clear morphological differences among these sibling species. All three species share the same synapomorphies: elongated stigma, shape of flagellomere 1 and the shape of petiole. The shorter metacarpus in *A. rubi* can be treated as a reduction of this vein and as an apomorphic character state, opposed to a very long metacarpus (a plesiomorphic character state) in other two species. It would seem that length of the metacarpus is useful in *Aphidius* taxonomy, where it is commonly employed (Starý, 1973; Pennacchio, 1989). Recently, Tomanović et al. (2014) demonstrated the usefulness of this character in delimitation of species of the *A. colemani* group. Large body size and retention of four maxillary and three labial palpomeres in all three species are considered as plesiomorphic character states, since smaller body size accompanied by reduction in the number of maxillary and labial palpomeres is a clear trend within the genus *Aphidius* (Starý, 1973; Tomanović et al. 2003; Rakhshani et al. 2008).

Although species of the *A. urticae* s. str. group have no great economic impact, growing interest in cultivation of blackberry and raspberry in many countries during the last decades increases the need to resolve the taxonomic status of parasitoids associated with their aphids (Mitchell et al. 2010; Dassonville et al. 2013). There is also growing interest in control of the large raspberry aphid, *Amphorophora idaei* Börner, 1939, because of virus transmission and common cases of insecticide resistance (McMenemy et al. 2009). In addition to *A.*

silvaticus as a specialized parasitoid of *A. idaei*, it is known that the parasitoid complex of *Amphorophora* aphids in Europe also includes *Aphidius ervi*, *A. geranii* Tomanović & Kavallieratos, 2010, *Ephedrus lacertosus* Haliday, 1833, *Praon grossum* Starý, 1971, *P. volucre* and *P. longicorne* Marshall, 1896, while that of *Macrosiphum* aphid hosts (in addition to *A. rubi*) includes several other species, as follows: *A. ervi*, *A. avenae*, *A. rosae*, *E. plagiator*, *E. lacertosus*, *P. volucre*, *P. longicorne*, *P. rosaecola* Starý, 1961, *P. orpheusi* Kavallieratos, Athanassiou & Tomanović, 2003, *P. exsoletum* Nees, 1811 and *Toxares deltiger* Haliday, 1833 (Kavallieratos et al. 2004; Starý, 2006; Žikić et al. 2012).

We presume that speciation in the case of the *A. urticae* group is driven by parasitoid specialization to different aphid host lineages, which is a common way of speciation in aphidiine parasitoids (Tremblay & Pennacchio, 1988; Mitrovski Bogdanović et al. 2013). Over 20 Macrosiphini aphid hosts in Europe are known to be parasitized by members of the *A. urticae* s. str. group (Starý, 1973; Tomanović et al. 2003; Starý, 2006). There are also discrepancies in the host range of re-described species compared with the original description. While in the case of *A. rubi* we determined a wider host range (*Aulacorthum* spp. and probably *Macrosiphum* spp. in addition to *M. funestum* Macchiati, 1885 from the original description), for *A. silvaticus* we determined a narrower host range restricted to *Amphorophora* spp. (excluding some *Macrosiphum* species from the original description) (Starý, 1962). As mentioned above, Müller et al. (1999) found significant differences between *A. rubi* and *A. urticae* at isocitrate dehydrogenase. In that study they analysed *"A. rubi"* originating from *Amphorophora rubi* and *Macrosiphum funestum* and obtained results which suggest that both, *A. rubi* and *A. silvaticus* differ from *A. urticae*. Recently, Derocles et al. (2016) also analysed genetic differentiation of *A. urticae* specimens originating from different aphid hosts and found significant differences between specimens from *Acyrthosiphon pisum* and *Microlophium carnosum*.

Specimens reared from the aphid *Ericaphis scammelli* in association with *Vaccinium corymbosum* were molecularly identified as *A. ericaphidis*, which represents the first record of this parasitoid's presence in Europe (Petrović et al. 2017). Increased international trade of highbush blueberry planting material was inevitably accompanied by the spread of the pests such as *E. scammelli* into new areas as well. Although *E. scammelli* has been present in Europe for more than a half a century (Coeur d'acier et al. 2010), *A. ericaphidis* is its first parasitoid detected in Europe. Unlike other Aphidiinae species introduced to Europe as

biocontrol agents (Roy et al. 2011, Petrović et al. 2013), *A. ericaphidis* was more likely accidentally introduced from the North America as a parasitoid of *E. scammelli* infesting *V. corymbosum* plant material. Also, another explanation could lie in a possibility that the European populations of *A. ericaphidis* adapted to this introduced aphid pest. The current distribution of *A. ericaphidis* and *E. scammelli* in the *Vaccinium* growing areas in Europe is understudied and probably much broader than had been reported. Pike et al. (2011) report rare occurrences of parasitization of *Macrosiphum parvifolii* Richards, 1967 by *A. ericaphidis*, which implies its potential to parasitize other species as well. Potential of this parasitoid in biological control requires host preference testing, in particular since diverse parasitoid species which were introduced as biocontrol agents became widespread in non-native areas and broadened their host range (Roy et al. 2011; Mitrović et al. 2013, Petrović et al. 2013).

Our results with the COI barcoding gene indicate that *Aphidius matricariae* is closely related to *A. urticae*, with divergence between the two species ranging from 0.7 to 1.4%. Furthermore, they share identical partial sequences of EF1-α. Although COI divergence between the two species was below the level of 2%, most often used as the threshold for species delineation in parasitoids, the maximum within species distances were lower than the minimum between species distance, enabling the COI marker to clearly distinguish *A. matricariae* from *A. urticae*.

Aphidius matricariae is a well known biocontrol agent that is commercialy produced by many companies (Hagvar and Hofsvang, 1991). It is also morphologically very well defined by 14 to 15-segmented antennae, a long metacarpus, subequal to stigma length and three segmented maxillary and two segmented labial palps, which is rare within the genus *Aphidius* (Starý, 1973; Pennacchio, 1989; Tomanović et al. 2003). *Aphidius matricariae* is a widely specialized parasitoid, its hosts being mainly the Aphidine and Myzine aphids (Starý, 1973),

Aphidius urticae shows a different pattern of palpomeres numbers (four segmented maxillary and three segmented labial palps), a greater number of antennal segments (17-19-segmented antennae) and different ecological specialization on Macrosiphini aphid hosts lineages (Kavallieratos et al. 2004).

Results similar to ours were obtained by Derocles et al. (2012) who presented a phylogeny tree based on COI analyses showing *A. urticae* and *A. matricariae* clustered together within the same clade with 80% bootstrap support. In a later study, Derocles et al. (2016) used seven molecular markers (COI, cytochrome *b*, 16S rRNA, 28S rRNA, long wavelength rhodopsin,

arginine kinase and elongation factor 1-*α*) to investigate are the generalist Aphidiinae mostly cryptic species. Their analyses revealed for both, *A. matricariae* and *A. urticae,* genetic structuring of populations with respect to aphid host specialization, as well as paraphyly in relation to other species in the genus *Aphidius.* Samples of *A. matricariae* were strongly separated in four paraphyletic subgroups, while *A. urticae* in two paraphyletic clades. In both cases it's evident that intraspecific differentiation in the morphospecies was influenced by the host specialization and geographical structuring. On the other hand, no morphological traits could be defined to distinguish these populations belonging to different subgroups (Derocles et al. 2016). It is unclear whether genetic substructure within generalist morphospecies reflects intraspecific variation or represents reproductively isolated cryptic species. Genetic structuring in populations of *A. matricariae* and *A. urticae* is one of many reported examples indicating that the generalist life strategy is unstable in aphidiine parasitoid-aphid host interactions, manifesting a tendency of generalists to diversify into host specialized taxa (Derocles et al. 2016). Generalism may provide benefits to the parasitoid populations in a variable environment, such as agroecosystems, but under more stable conditions selection may favor specialization (Raymond et al. 2016).

DNA barcoding did not produce accurate identification of all species included in the analyses. Three specimens of *A. microlophii* sampled from Belgium in association with aphids of *Urtica dioica,* two specimens of *A. ervi* associated with *Sitobion avenae/Triticum aestivum* and two of *A. ervi* from the *Acyrthosiphon pisum/Medicago sativa* association, shared identical COI sequences. Moreover, EF1-α sequences were the same for both species.

Aphidius microlophii was earlier separated from *A. ervi* by Pennacchio and Tremblay (1987) on the basis of host preference, the former parasitoid being specialized on *Microlophium carnosum* associated with the stinging nettle *Urtica dioica,* the latter predominantly attacking *Acyrthosiphon pisum*. Petrović et al. (2006) also reported that samples of *A. microlophii* from the Mt Durmitor Biosphere Reserve develop on association of *Wahlgreniella ossiannilssoni*/*Arctostaphylos uva ursii* (L.) Spreng., 1825.

The two species can also be distinguished morphologically based on a wing vein ratios, the outline of the third valvulae and colour patterns (Pennacchio & Tremblay, 1987). Later, Tremblay and Pennacchio (1988) discussed the relatedness of these two species, indicating *A. microlophii* to have arisen from separate populations of *A. ervi* specialized on *Microlophium carnosum* associated with the stinging nettle *Urtica dioica*. It was hypothesized

that reproductive isolation developed over time, based on the ecological and behavioural factors like host preference and mating behaviour, such isolation consequently leading to host-adapted divergence and speciation. According to Tremblay and Pennacchio (1988), *A. microlophii* and *A. ervi* can coexist in sympatry on their respective host aphids and host plants due to high host specialization. This might be the case as a result of pre-zygotic reproductive isolation defined by restrictive female choice of a suitable host.

Atanassova et al. (1998) also supported the status of *A. microlophii* and *A. ervi* as separate species, based on differences detected after conducting an electrophoretic study of four polymorphic enzymes (PEP, PGI, PGM and IDH).

On the other hand, our findings correspond with previous results of molecular analyses obtained by Derocles et al. (2012), who likewise reported no success in discriminating *A. microlophii* from *A. ervi* based on the barcoding fragments of COI gene. However, they were distinguished from one another by the same authors based on the nuclear long wavelength rhodopsin sequences (LWRh). Ye et al. (2017) used COI and 16S and had no results in delineation of the two parasitoid species with either of the two markers.

Shared COI barcodes do not represent a substantial taxonomic problem, presumably representing a result of ongoing hybridization and introgression among closely related species (Hebert & Gregory, 2005). Introgression is described as the introduction of genes from one species into the gene pool of another species via hybridization, when matings between the two produce fertile hybrids (Harrison & Larson, 2014).

Derocles et al. (2012) discussed that *A. ervi* and *A. microlophii* might have been indistinguishable on the basis of their COI sequences because of mitochondrial introgression. Such a scenario can be the case with two species, because mitochondrial introgression i.e. transfer of mtDNA variants between closely related species or lineages influences the physiological properties of parasitoids. Although initially considered as a rare event in nature, introgression of mtDNA has been found to be widespread in different animals, including insects [e.g. introgression of mtDNA between *Drosophila simulans* Sturtevant, 1919 and *D. mauritiana* Tsacas & David, 1974 - William & Ballard, 2000; or between the two mosquito species *Aedes mariae* Sergent & Sergent, 1903 and *A. zammitii* Theobald, 1903 - Mastrantonio et al. 2016].

Neither the barcoding region of COI nor the EF1-α gene showed itself to be a relilable marker in delineation of *A. aquilus* and *A. salicis*. The two species share identical nuclear

haplotypes, while mitochondrial haplotypes diverge in the range of 0.5 to 1%. Derocles et al. (2012) previously reported failure of the LWRh marker to discriminate between *A. aquilus* and *A. salicis*. This is an interesting finding because up till now no studies questioned taxonomic status of these species. Starý (1973) described *A. aquilus* as similar to *A. salicis*, differing from the latter in width of the temples, length of the metacarpus, coloration and host range. Both species parasitize arboricolous aphids on *Salix* spp. and *Populus* spp. L., 1753. (*A. salicis*) or on *Betula* spp. trees (*A. aquilus*) (Starý, 1973). *A. salicis* and *A. aquilus* share some important diagnostic characters as well as forewing venation patterns with some veins reduced (not common case within the genus *Aphidius*) and 13 to 14-segmented antennae.

Low evolutionary distances between the mitochondrial haplotypes and identical nuclear sequences indicate close relatedness and evident gene exchange between *A. salicis* and *A. aquilus*. This imposes the need to re-evaluate the status of these two species, taking into account morphological and behavioural pecularities, results of multi-locus genotyping, and patterns of population genetics divergence.

Barcoding fragments solely cannot discriminate *A. chaetosiphonis* from *A. hortensis* with evolutionary rates below 1%. In addition, their EF1-α sequences were identical. *Aphidius hortensis* is a strictly specialized parasite of *Liosomaphis berberidis* on *Berberis* spp. in the Holarctic (Starý, 1973). It inhabits parks, deciduous and mixed forests, thickets and shrubs. According to Starý (1973) *A. hortensis* is biparental parasitoid, belonging to the group of congeners characterized by having a tentorial index equal to 0.4-0.5 and it differing from the related species in the number of antennal segments (female 14-15, male 16-17 segmented) and host range.

Petrović et al. (2011) described the new species *Aphidius chaetosiphonis* sp. n. as a specialist associated with *Chaetosiphon* sp./ *Potentilla clusiana*. This whole aphid-parasitoid association seems to be new to science and subendemic in high montane areas of southeastern Europe. According to wing venation and the number of antennal segments *A. chaetosiphonis* is related to *Aphidius salicis* (Petrović et al. 2011). Both species have a low number of antennal segments in females, *A. chaetosiphonis* has 12–13 and *A. salicis* (12) 13-segmented antennae, while the male of *A. chaetosiphonis* has 14 antennal segments compared to the 16–17-segmented antennae of *A. salicis* male. *Aphidius chaetosiphonis* has shorter F1, shorter petiole and labial palps with two palpomeres (*A. salicis* with three palpomeres). *Aphidius*

chaetosiphonis parasitizes *Chaetosiphon* aphid hosts, while *A. salicis* mainly parasitizes *Cavariella* aphids (Petrović et al. 2011).

The mitochondrial COI marker clearly separated *A. salicis* from *A. chaetosiphonis* in two distinct clusters, while on the other hand grouping the latter species with *A. hortensis* within the same clade with high bootstrap support. Plotting of maximum within-species distances versus minimum between-species distances showed that *A. chaetosiphonis* and *A. hortensis* cannot be identified on the basis of barcoding fragments. The nuclear marker also confirmed an evident gene flow between these two specialists associated with phylogenetically distinct aphid hosts and originating from different habitats.

Starý and Nemec (1985) studied population diversity in monophagous parasitoids using electrophoretic techniques (isoesterases on polyacrylamide gels) and determined that *A. hortensis* exhibits narrowly oligomorphic pattern of sexual differences in the electromorphs. In discussing the phenomenon of monophagy in parasitoid species, the authors describe it as a specialized branch derived from the main developmental trend of the genus, which may be either regressive or possibly even relatively progressive in preserving the ability to develop oligophagous patterns again and becoming a center of subsequent speciation (e.g., *Aphidius hortensis*). Thus, monophagy can be attributed to a phylogenetically ancient, regressive, or surviving group that has adapted to an ancient and regressive group of aphids or in other words is a case of ancient parasitoid-host coincidence (Starý and Nemec, 1985). They also claim that phylogenetically younger species tend to manifest sexual differences in the electromorphs (narrow oligomorphism) i.e. slight variation in the electromorphs per sex tends to indicate the relatively young monophagy that either has resulted from a narrow oligophagy or, on the contrary, tends to develop into narrow oligophagy. In the light of this, further studies are required to investigate the origin and mechanism(s) underlying the low interspecific divergence rate, not excluding possible introgression nor emergence of the young monophagy in *A. hortensis* or *A. chaetosiphonis*.

Both the mitochondrial and the nuclear marker failed to distinguish *A. absinthii, A. tanacetarius* and *A. funebris* as separate species. In case of all three parasitoids, maximum within species distances exceeded the minimum between species distance between the COI sequences. Furthermore, we found that *A. absinthii* shared one mitochondrial haplotype with *A. tanacetarius,* as well as another with *A. funebris*. Analysis of E1-α sequences determined five haplotypes, with one shared by *A. funebris* and *A. tanacetarius*. The median joining

network showed connection with 1-2 mutational steps between the nuclear haplotypes and all mutations were synonymous.

All three species have been described on the basis of morphological differences and ecological prefferences (host range pattern). The morphological description (Starý, 1973; Tomanović et al. 2003) indicated no clear differentiation among these species, except in case of *A. funebris* which is characterized by a larger number of antennal segments. Interestingly, some species show an unstable number of palpomeres (2-3 labial palpomeres in *A. funebris*, 3-4 maxillary palpomeres in *A. tanacetarius*) which is not a common case within the genus *Aphidius*. In terms of host specificity, *A. funebris* is specialized to parasitize *Uroleucon* spp., *A. tanacetarius* parasitizes *Metopeurum* ssp. and closely related groups (*Microsiphium*) and *A. absinthii* parasitizes *Macrosiphoniella* aphid hosts (Starý, 1973).

Interestingly, all three of the mentioned aphid hosts are phylogenetically closely related and belong to the tribe Macrosiphini. Parasitoids are often restricted to develop in certain aphid host species, genera or higher taxa, in other words, a host range of such parasitoids is often shaped by phylogenetic distances among the aphid hosts. In the absence of substantial molecular and morphological evidence to discriminate *A. funebris, A. absinthii* and *A. tanacetarius* as separate taxa, we might consider that in case of these parasitoids the trade-off and cost of adaptation of independent lineages to phylogenetically more related aphid hosts may be lower, as such hosts are often more similar in ecological, behavioural, physiological or biochemical traits (Gagić et al. 2016). These findings clearly indicate the need to re-visit the taxonomic status of *A. absinthii, A. tanacetarius* and *A. funebris*, taking into account morphological, ecological and molecular data.

Aphidius rhopalosiphi is an important species in the control of cereal aphid populations in the Western Palaearctic. However, we found mitochondrial haplotypes differing by 1.4%, obviously indicating the existence of strains or biotypes.

Starý (1973) initially classified *A. rhopalosiphi* as an unclear species. He noted that it is known to parasitize *Rhopalosiphum* sp. on *Potamogeton pectinatum* L. 1753, and that the 17-segmented antennae and coloration indicate the species to be close to *A. urticae, A. uzbekistanicus* or *A. equiseticola*. However, *A. rhopalosiphi* has a broader host range that includes *Sitobion* spp., *Metopolophium* spp., *Diuraphis* spp., *Schizaphis* spp. Börner, 1931, and *Rhopalosiphum* spp Koch, 1854 (cited in Tomanović et al. 2013).

Pungerl (1986) conducted a morphometric and electrophoretic study of *Aphidius* species reared from a variety of aphid hosts and determined that *A. rhopalosiphi* appears to be polymorphic giving a variable number of banding patterns obtained for esterase and malic enzyme at the same mobility. However, studies by Pungerl (1983, 1986) have shown that *A. rhopalosiphi* as a nominal species cannot be reliably separated, since there is no additional bionomical character to justify further separation.

Interestingly, Powell and Wright (1988) presented experimental evidence indicating the ability of the aphid parasitoid *A. rhopalosiphi* to transfer between different known host species. Moreover, Stilmant et al. (2008) claimed that *A. rhopalosiphi* is habitat-specific and it can parasitize most species available in its habitat, not manifesting any host preference.

Contrary to this, Holler (1991) conducted transfer trials with different strains of *A. rhopalosiphi* and revealed the presence of two groups of strains differing in host range: three strains exclusively parasitized *Sitobion avenae* and *S. fragariae*, Walker, 1848 while the other strains successfully developed in both *Sitobion* species, *Metopolophium dirhodum, M. festucae* Theobald, 1917, *Rhopalosiphum padi* and *Diuraphis muehlei* Börner, 1950. Crossing experiments with laboratory-bred and field-collected material showed that reproductive isolation between the two *A. rhopalosiphi* groups was clear but not complete. Holler (1991) stated that the two host range groups were poorly distinguishable on the basis of morphological characters. In the group restricted to *Sitobion* spp. associated with cereals and grasses, he observed that males had no more than three hair rows between MPS on the first flagellar segment, while females were with none to two MPS on the first flagellar segment, carina emerging approximately between the spiracles on the dorsal surface of the petiole always present, female derived sex pheromones specific. In a second group, one with an extended host range, males had two to six hair rows between MPS on first flagellar segment, while females were without MPS on the first flagellar segment, a carina emerging approximately between the spiracles on the dorsal surface of the petiole was not always present in them, and specific female-derived sex pheromones were observed.

Tomanović et al. (2013) investigated the relationship between molecular divergence and variation in the wing shape of several *Aphidius* species, including *A. rhopalosiphi*. Their study showed that populations of *A. rhopalosiphi* clustered as a separate clade with a high level of within-clade genetic divergence. However no correlation with the level of the wing shape variation was detected.

Our results correspond with the data reported by Kos et al. (2011) who determined seven mtCOI haplotypes (R1-R7) among the analysed specimens of *A. rhopalosiphi*, with a mean nucleotide distance of 1.5% (max. 2.4%). Most of the *A. rhopalosiphi* specimens had four maxillary palpomeres and three labial palpomeres, but in specimens reared from the *Typha* sp./*Schizaphis scirpi* association (R6 haplotype), the terminal labial palpomere was undivided, resulting in a labial palp with two palpomeres. Kos et al. (2011) also found that haplotype R6 was characterized by a yellow F1 and a prevailingly yellow F2. It is interesting to note that the R3 haplotype showed an almost uniform yellow color pattern of F1. The pattern of F1 and F2 coloration in haplotypes R1, R2, R4 and R5 was variable, with F1 ranging one third yellow to entirely yellow and F2 partially yellow. The morphological and genetic diversity found by Kos et al. (2011) in *A. rhopalosiphi* support host specialization and may suggest the existence of cryptic species within the COI mitochondrial lineages, especially for lineages that have considerable degree of mtCOI diversity and are characterized by sympatric occurrence.

Although the host range of *A. rhopalosiphi* includes a wide spectrum of cereal aphids, it seems that some host specialization occurs. The existence of such cryptic species complexes raises questions about the limits between intra-specific genetic structuring and speciation, and drives an attention to the taxonomic uncertainty that exists within the parasitoid group (Raymond et al. 2016). Identification of clearly distinct mitochondrial lineages in the material analysed herein indicates that the taxonomic position of all specimens that are morphologically close to the *A. rhopalosiphi* group should be carefully re-examined and their status revised.

Interestingly, mitochondrial haplotypes of *A. rubi* clustered with *A. rhopalosiphi* haplotypes with 90% support. Genetic distances between the two parasitoids ranged from 0.7 to 1.2%, which is lower than the maximum within species distance between the two haplotypes of *A. rhopalosiphi* (1.4%). However, the two species are morphologically clearly different (*A. rubi* has a shorter metacarpus and larger number of longitudinal placodes on flagellomere 1 than in *A. rhopalosiphi*) and ecologically different (*A. rubi* parasitizes *Macrosiphum* aphid hosts on shrubs in forest and semiforest habitats, while *A. rhopalosiphi* parasitizes various cereal aphids in steppe habitats (Kavallieratos et al. 2004).

Mitochondrial introgression has been recorded as often occurring in instances of species (host or habitat) range expansion, which might be the case with *A. rhopalosiphi* and *A.*

rubi. It seems that the nuclear marker better separated these species, since we found 0.6% differences, which is not surprising inasmuch as a case where mitochondrial DNA introgresses more frequently than the nuclear genome was previously reported in animals, together with observation of complete mtDNA replacement in the presence of little or no nuclear introgression (cited in Harrison & Larson, 2014). Introgression is often described to occur in sympatry across species boundaries that are maintained by different barriers to gene exchange, which however may not be spatially and temporally uniform (Harrison & Larson, 2014). Gene flow between closely related taxa may be a product of a neutral event(s) that had no effect on an individual's phenotype or fitness (Boratynski et al. 2014).

Adaptive divergence is probably one of the dominant modes of genetic differentiation leading to speciation in the Aphidiinae (Tremblay & Pennacchio, 1988). It would seem that adaptive radiation determines population splitting into two or more subpopulations by some barrier to gene flow (e.g. geographical, ecological etc.) and divergence leading these gene pools to evolve independently (Templeton, 1981).

Host ranges of parasitoid wasps are influenced by behavioural responses to hosts and their environment, localized genetic variation and whether or not natural selection leads to specialization on particular hosts (Antolin et al. 2006). Local adaptation to other hosts or habitats can lead to trade-offs in parasitoids and limited gene flow between populations. Evolution towards specialization on a single host will be even more rapid if fitness trade-offs favour the evolution of host preferences and host-associated mating preferences. In this way, genetic differences between sub-populations can arise in parasitoids that exploit numerous host species in patchy environments (cited in Antolin et al. 2006).

From an applied point of view, host ranges of parasitoids are of great importance, since they determine effectiveness of the latter as biological control agents. This refers to their capacity to switch between different hosts, to persist in agroecosystems in the absence of the pest, and to regulate pest outbreaks in a rapidly changing system (Raymond et al., 2016). Differences in host use among populations of biological control agents are often attributable to the existence of cryptic species unrecognized prior to their introduction. As a result, populations of non-target aphid hosts can be affected following parasitoid introduction.

According to the Biological Species Concept (BSC), new species are formed when populations are reproductively isolated. However, this concept does not offer a universal tool in species delineation because mechanisms of reproductive isolation differ among taxa. Lack

of systematic studies leaves many questions open, such as whether cryptic species are more common in particular habitats, latitudes or taxonomic groups (Bickford et al. 2006).

Development of molecular tools enabled researchers to identify failures in determination by detecting and differentiating morphologically similar species (Hebert & Gregory, 2005). Aphidiinae are mostly composed of specialist parasitoids and the few species described as generalist are suspected to be composed of cryptic specialists, almost indistinguishable based on morphological characteristics. DNA barcoding has been helpful in case of presumed parasitoid generalists which in fact hide complexes of morphologically cryptic host specialized taxa (Derocles et al. 2016). In our study, successful identification of 22 *Aphidius* species based on their barcoding COI fragments is encouraging, considering all the challenges encountered by taxonomists in accurate identification of most of the aphidiine species.

DNA barcoding is an additional tool of aid in delimiting species, but barcodes by themselves are never sufficient to describe new species (Hebert & Gregory, 2005). Although many examples have shown that the barcoding fragment of the gene encoding COI is reliable for accurate species identification, some studies indicated that a unique region of mitochondrial DNA does not supply ample resolution and can be misleading (cited in Derocles et al. 2012). For this reason, a nuclear locus is sometimes required to improve species identification. Complementary use of mitochondrial and nuclear genes was the most relevant approach to reliably identify cryptic genetic clades in the Aphidiinae (Derocles et al. 2016). Overall, most of the analysed generalist morphospecies were shown to be composed of subgroups related to the aphid host, some of them revealed as cryptic species by the species delimitation analysis.

Multi-locus analysis employing two or more different genes is more and more often becoming the method of choice in phylogeny studies because it is less sensitive to specific gene genealogies that might result in faulse interpretation of the population’s history. However, this approach also grouped specimens of different morphospecies in the same species, thus demonstrating mismatches between morphological and molecular identification (Derocles et al., 2016).

There are many concerns about the widespread integration of DNA barcoding in molecular taxonomy, related to the use of a single gene in delineating and identification of species and to separate between intra- and interspecies variations. One of the concerns is that

the use of individual sequences may not provide sufficient discrimination for cryptic species complexes, and its maternally inherited characters may reduce barcode diversity. Although the use of COI barcoding has become more prevalent in resolving relationships within aphidiinae species complexes, it has failed in some cases.

In light of the issues involved in attempting to obtain clear separation of several *Aphidius* species based on the barcoding region and in view of the fact that introgression is one of the mechanisms triggering such events, it is necessary to assess the prevalence of introgression in mtDNA before using the barcoding method in classification. For example, females could maintain mtDNA divergence by means of oviposition choice, while males on the contrary could homogenize genetic variation by mating indiscriminately between different host-associated females, in which case the barcoding method would fail to realistically estimate the number of species within the species complex.

Thus, DNA barcoding does not hold unlimited promise for identification in taxonomically understudied groups. Consequently, there is clearly a need for an alternative additional way to resolve ambiguous identification and phylogenetic relationships, and one such way is by resorting to an integrated taxonomic approach taking into account different types of characteristics for species delimitation, including phenotypic distinctiveness, ecological niche divergence, and molecular data. Integrated taxonomy approach has been proven as a efficient tool in Aphidiinae species delimination (Žikić et al., 2009; Kos et al., 2011; Mitrovski-Bogdanović et al., 2013; 2014; Tomanović et al. 2014, Ilić-Milošević et al. 2015, Petrović et al. 2015, Stanković et al. 2015).

6. CONCLUSIONS

The following conslusions can be withdrawn on the basis of our results obtained in studying the taxonomic status and phylogenetic relationships of European species of the genus *Aphidius* using molecular techniques:

1. The barcoding fragment of the cytochrome oxidase subunit I mitochondrial gene has shown to be a reliable marker for identifying most of the species included in this study. The nuclear gene Elongation factor 1-α was too conservative for delimitation of species.
2. Newly designed *Aphidius* - specific primers and direct and nested PCR protocols succesfully amplified short fragments of the barcoding region of the mtCOI gene extracted from dry specimens. Concatenated short fragments enabled recovery of complete barcoding sequences which were subsequently used in the phylogenetic study.
3. Phylogenetic analyses based on mtCOI fragments showed that *Aphidius* is in fact a paraphyletic group, consisting of several distinct monophyletic clades.
4. Topology of the maximum likelihood tree showed separation of 22 taxa as independent species: *A. erysimi, A. sonchi, A. linosiphonis, A. hieraciorum, A. arvensis, A. balcanicus, A. phalangomyzi, A. banksae, A. uzbekistanicus, A. sussi, A. silvaticus, A. avenae, A. rosae, A. ericaphidis, A. eadyi, A. viaticus, A. schimitscheki, A. ribis, A. setiger, A. asteris, A. matricariae* and *A. urticae.*
5. Scattered clustering of *A. arvensis, A. erysimi* and *A. viaticus* within the genus *Aphidius* on the phylogeny tree confirms synonymization of the genus *Lysaphidus*.
6. The positioning of *A. setiger* and *A. cingulatus* among other species within the genus *Aphidius* confirms re-description of *Euaphidius* as a subgenus of *Aphidius.*
7. *Aphidius colemani, A. transcaspicus, A. platensis* and *A. asteris* act as sister groups to the remaining *Aphidius* species and have a basal position on the phylogeny tree.
8. Molecular characterization revelaed cryptic taxa within the *A. urticae* group associated with different hosts. Significant evolutionary distances among the lineages ranged from 2.3 to 9.2% and were not substantiated by clear morphological differences.
9. Speciation within the *A. urticae* group is driven by specialization of parasitoids to different aphid host lineages. The lineage associated with *Aulacorthum* spp. and *Macrosiphum* spp. is re-described as *A. rubi, A. silvaticus* as the species in association with *Amphorophora*

spp. and *A. urticae* s.str. as that associated with *Microlophium carnosum*. New key for determination of these three parasitoid species has been presented.

10. Parasitoid samples reared from the aphid *Ericaphis scammelli* infesting *Vaccinium corymbosum* were molecularly identified as *A. ericaphidis,* which represents the first record of this parasitoid's presence in Europe.
11. Five distinct clades were distinguished based on mtCOI: 1) *A. rubi* and *A. rhopalosiphi*; 2) *A. funebris, A. tanacetarius* and *A. absinthii*; 3) *A. ervi* and *A. microlophii*; 4) *A. chaetosiphonis* and *A. hortensis* and 5) *A. salicis* and *A. aquilus*. The taxonomic status of these clades should be re-considered using an integrative approach.
12. In spite of low divergence rate, *A. matricariae* could be delimited from *A. urticae* using the mtCOI barcode. They are morphologically and biologically distinct species, however with similar behavioural patterns in terms of host shift, manifesting a tendency to diversify into host specialized taxa.
13. Neither the mitochondrial nor the nuclear marker could distinguish *A. microlophii* from *A. ervi*, which indicates incomplete reproductive isolation with possible introgression through the transfer of mtDNA variants between the sibling species. Different host preferences and morphological characters distuinguish the taxa as separate entities, but with no clear molecular support.
14. *Aphidius funebris, A. tanacetarius* and *A. absinthii* clustered within a separate clade, sharing the same barcoding haplotypes. Divergence of mitochondrial haplotypes within the species equals the between species divergence, with no substantial distinctive morphological characters. Nuclear haplotypes confirm the mtDNA grouping.
15. *Aphidius funebris, A. tanacetarius* and *A. absinthii* are indistinguishable as separate species, which could be attributed to adaptive divergence of independent lineages associated with host and/or habitat range expansion and speciation.
16. Barcoding fragments solely could not discriminate *A. chaetosiphonis* from *A. hortensis* with evolutionary divergence rates below 1%, while their EF1-α sequences were identical. They are specialists associated with phylogenetically distant aphid hosts. *Aphidius hortensis* is strictly specialized to *Liosomaphis berberidis* on *Berberis* spp., while *A. chaetosiphonis* is a specialist associated with *Chaetosiphon* sp./ *Potentilla clusiana.*

17. The monophagy of *A. chaetosiphonis* and *A. hortensis* might have originated from a common ancestor, as separate lineages independently adapted to ancient and regressive groups of aphids.
18. Molecular markers indicate that *A. rhopalosiphi* is a complex of several polymorphic lineages exhibiting different behavioural patterns in regard to host and habitat preferences and range expansion. These data point to the possibility of cryptic speciation within the *A. rhopalosiphi* group, but the taxonomic status of such lineages is uncertain in the absence of bionomical and morphological evidence for further separation.
19. *Aphidius rubi* clustered within the *A. rhopalosiphi* group, with distances between the mitochondrial haplotypes in the range of 0.7 to 1.2%. High distances between the nuclear haplotypes of 0.6% indicate ocassional and neutral hybridization between the two morphologically and biologically distinct species, inducing mtDNA exchange events via intogression, which is evidenced by low divergence rates of the barcode sequences.

7. REFERENCES

1. Ahmadabadi N. J., Karimi J., Modarres M., Rakhshani E. 2011. Morphological and molecular methods in identification of *Aphidius transcaspicus* Telenga (Hym: Braconidae: Aphidiinae) parasitoid of *Hyalopterus* spp. (Hom: Aphididae) with additional data on Aphidiinae phylogeny. J. Entomo. Res. Soc., 13(2), 91- 103.

2. Amornsak W., Gordh G., Graham G. 1998. Detecting parasitized eggs with polymerase chain reaction and DNA sequence of *Trichogramma australicum* Girault (Hymenoptera: Trichogrammtidae). Aust. J. Entomol.37: 174–179.

3. Antolin M. F., Bjorksten T. A., Vaughn T.T. 2006. Host-related fitness trade-offs in a presumed generalist parasitoid, *Diaeretiella rapae* (Hymenoptera: Aphidiidae). Ecol. Entomol. 31, 242–254.

4. Apostol B. L., Black W. C., Reiter P., Miller B. R. 1996. Population genetics with RAPD-PCR markers: the breeding structure of *Aedes aegypti* in Puerto Rico. Heredity 76: 325–334.

5. Atanassova P., Brooks C.P., Loxdale H.D., Powel W. 1998. Electrophoretic study of five aphid parasitoid species of the genus *Aphidius* (Hymenoptera: Braconidae), including evidence for reproductively isolated sympatric populations and a cryptic species. Bull. Entomol. Res. 88, 3-13.

6. Ayres C. F. J., Melo-Santos M. A. V., Solé-Cava A. M., Furtado A. F. 2003. Genetic differentiation of *Aedes aegypti* (Diptera, Culicidae), the major dengue vector in Brazil. J. Med. Entomol. 40: 430–435.

7. Baaren J. van, Lann C. L., Pichenot J., Pierre J. S., Krespi L., Outreman Y. 2009. How could host discrimination abilities influence the structure of a parasitoid community? Bull. Entomol. Res. 99, 299–306.

8. Ballinger-Crabtree M. E., Black W. C., Miller B.R. 1992. Use of genetic polymorphisms detected by the random-amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) for differentiation and identification of *Aedes aegypti* subspecies and populations. Am. J. Trop. Med. Hyg. 47(6):893-901.

9. Bandelt H. J., Forster P., Röhl A. (1999). Median-joining networks for inferring intraspecific phylogenies. Molec. Biol. Evol. 16, 37–48.

10. Banuls A. L., Jonquiere R., Guerrini F., Lepont F., Barrera C., Espinel I., Guderian R., Echeverria R,. Tibayrenc M. 1999. Genetic analysis of *Leishmania* parasites in Ecuador: Are *Leishmania* (Viannia) *panamensi*s and *Leishmania* (V) *guyanensis* distinct taxa? Am. J. Trop. Med. Hyg. 61 (5):838-845.

11. Barrette M., Wu G-M., Brodeur J., Giraldeau L-A., Boivin G. 2009. Testing competing measures of profitability for mobile resources. Oecologia, 158:757–764.

12. Battaglia D., Poppy G., Powell W., Romano A., Tranfaglia A., Pennacchio F. 2000. Physical and chemical cues influencing the oviposition behaviour of *Aphidius ervi*. Entomol. Exper. Appl. 94: 219–227.

13. Belshaw R., Quicke D.L.J. 1997. A molecular phylogeny of Aphidiinae (Hymenoptera: Braconidae). Mol. Phylog. Evol. 5: 423–428.

14. Belshaw R., Fitton M., Herniou E., Gimeno C., Quicke D. L. J. 1998. A phylogenetic reconstruction of the Ichneumonoidea (Hymenoptera) based on the D2 variable region of 28S ribosomal RNA. Syst. Entomol. 23: 109- 123.

15. Belshaw R., Dowton M., Quicke D.L.J., Austin A. D. 2000. Estimating ancestral geographical distributions: a Gondwanan origin for Aphid parasitoids? Proc. R. Soc. Lond. (B) 267: 491–496.

16. Benelli G., Messing R. H., Wright M. G., Giunti G., Kavallieratos N., Grand Canale A. 2014. Cues Triggering Mating and Host-Seeking Behaviour in the Aphid Parasitoid *Aphidius colemani* (Hymenoptera: Braconidae: Aphidiinae): Implications for Biological Control. J. Econ. Entomol. 43(6): 2005-2022.

17. Bickford D., Lohman D. J., Sodhi N., N.,P.,K., L., Meier R., Winker K., Ingram K.K., Das, I. (2006). Cryptic species as a window on diversity and conservation. Trend. Ecol. Evol., Vol.22 No.3.

18. Bilu E., Hopper K. R., Coll M. 2006. Host choice by *Aphidius colemani*: effects of plants, plant– aphid combinations and the presence of intra-guild predators. Ecol. Entomol. 31, 331–336.

19. Boivin G., Hance T., Brodeur J. 2012. Aphid parasitoids in biological control. Can. J. Plant. Sci. 92(1): 1-12.

20. Boore, J. (1999) Animal mitochondrial genomes. Nucleic Acids Res., 27, 1767–1780.

21. Boratynski V., Melo-Ferreira J., Alves P.C., Berto S., Koskela E., Pentikainen O., Tarroso P., Ylilauri M., Mappes T. 2014. Molecular and ecological signs of mitochondrial adaptation: consequences for introgression? Heredity 113, 277–286.

22. Bouchard Y., Cloutier C. 1984. Honeydew as a source of host-searching kairomones for the aphid parasitoid *Aphidius nigripes* (Hymenoptera: Aphidiidae). Can. J. Zool. 62:1513-1520.

23. Bouchard Y., Cloutier, C. 1985. Role of olfaction in host finding by aphid parasitoid *Aphidius nigripes* (Hymenoptera: Aphidiidae). J. Che. Ecol. 11(6): 801- 808.

24. Brewer M. J., Elliott N. C. 2004: Biological control of cereal aphids in North America and mediating effects of host plant and habitat manipulations. Ann. Rev. Entomol. 49: 219–242.

25. Brodeur J., Mcneil J. N. 1994. Seasonal Ecology of *Aphidius nigripes* (Hymenoptera: Aphidiidae), a Parasitoid of *Macrosiphum euphorbiae* (Homoptera: Aphididae). Environ. Entomol. 23(2): 292-298.

26. Brower A.V.Z., DeSalle R. 1994. Practical and theoretical considerations for choice of a DNA sequence region in insect molecular systematics, with a short review of published studies using nuclear gene regions. Ann. Entomol. Soc. Am. 87 (6), 702–716.

27. Cameron P. J., Walker G. P., Allan, D. J. 1981. Establishment and dispersal of the introduced parasite *Aphidius eadyi* (Hymenoptera: Aphidiidae) in the North Island of New Zealand, and its initial effect on pea aphid. New Zeal. J. Zoo. 8 (1): 105-112.

28. Cameron P. J., Walker G.P. 1989. Release and establishment of *Aphidius* spp. (Hymenoptera: Aphidiidae) parasitoids of pea aphid and blue green aphid in New Zealand. New Zeal. J. Agr. Res. 32: 281-290.

29. Carmean D., Kimsey L.S., Berbee M.L. 1992.18S rDNA sequences and holometabolous insects. Mol. Phylogenet. Evol. 1:270–278.

30. Caterino M. S., Cho S., Sperling F. A. H. 2000. The current state of insect molecular systematics: a thriving tower of babel. Ann. Rev. Entomol. 45: 1–54.

31. Cervera M. T., Cabezas J. A., Simon B., Martínez-Zapater J. M., Beitia F., Cenis J. L. 2000. Genetic relationships among biotypes of *Bemisia tabaci* (Hemiptera, Aleyrodidae) based on AFLP analysis. Bull. Entomol. Res. 90(5):391-396.

32. Chatterjee S. N., Tanushree T. 2004. Molecular Profiling of Silkworm Biodiversity in India: An Overview. Rus. J. Gen. 40 (12): 1339–1347.

33. Chen Y., Gilesy K. L., Greenstonez M. H. 2002. Molecular evidence for a species complex in the genus *Aphelinus* (Hymenoptera, Aphelinidae), with additional data on Aphidiine phylogeny (Hym, Braconidae). Ann. Entomol. Soc. Am., 95: 29- 34.

34. Cho S., Mitchell A., Regier J. C., Mitter C., Poole R. W., Friedlander T. P., Zhao S. 1995. A highly conserved nuclear gene for low-level phylogenetics: elongation factor-1 alpha recovers morphology-based tree for heliothine moths. Mol. Biol. Evol. 12(4): 650-656.

35. Chou L.Y. 1984. The phylogeny of Aphidiidae (Hymenoptera). J. Agric. Res. (China) 33: 97–177.

36. Chow, A. Mackauer M. 1992. The influence of prior ovipositional experience on host selection in four species of aphidiid wasps. J. Insect. Beh, 5, 99–108.

37. Coeur d'acier A., Pérez Hidalgo N., Petrović-Obradović O. 2010. Aphids (Hemiptera, Aphididae). In Roques A, Kenis M, Lees D, Lopez-Vaamonde C, Rabitsch W, Rasplus JY, Roy D (Eds.) Alien terrestrial arthropods of Europe. Biorisk 4(1): 435–474.

38. Dassonville N., Thiellemans T., Gosset V. 2013. FresaProtect and BerryProtect: mixes of parasitoids to control all common aphid species on protected soft fruit crops: Product development and case studies from three years of experience. Aspec. Appl. Biol., 119: 79–81.

39. De Farias A. M. I., Hopper K. R. 1999. Oviposition Behaviour of *Aphelinus asychis* (Hymenoptera: Aphelinidae) and *Aphidius matricariae* (Hymenoptera: Aphidiidae) and Defense Behaviour of Their Host *Diuraphis noxia* (Homoptera: Aphididae). Envi. Entomol. 28 (5): 858- 862.

40. Derocles S. A., Le Ralec A., Plantegenest M., Chaubet B., Cruaud C., Cruaud, A., Rasplus, J. Y. 2012. Identification of molecular markers for DNA barcoding in the Aphidiinae (Hym. Braconidae). Molec. Ecol. Res., 12, 197-208.

41. 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? System. Entom., 41, 379-391.

42. Dowton M., Austin A.D., Antolin M. F. 1998. Evolutionary relationships among the Braconidae (Hymenoptera: Ichneumonoidea) inferred from partial 16S rDNA gene sequences. Insect Mol. Biol. 7: 129–150.

43. Dowton M. 1999. Relationships among the Cyclostome Braconid (Hymenoptera: Braconidae) subfamilies inferred from a mitochondrial tRNA gene rearrangement. Mol. Phylogt. Evol. 11: 283- 287.

44. Doyle J. J., Gaut B. S. 2000. Evolution of genes and taxa: a primer. Plant. Mol. Biol. 42 (1): 1-23.

45. Du Y., Poppy G.M., Powell W., Pickett J. A., Wadhams L. J., Woodcock C. M. 1998. Identification of semiochemicals released during Aphid Feeding That Attract Parasitoid *Aphidius ervi*. J. Chem. Ecol. 24 (8): 1355- 1368.

46. Eady R. D. 1969. A new diagnostic character in *Aphidius* (Hymenoptera: Braconidae) of special significance in species on pea aphid. Proc. R. Entomol. Soc. Lond. (B). 38. 165-173.

47. Edson K.M., Vinson S. B. 1979. A comparative morphology of the venom apparatus of the female braconids (Hymenoptera: Braconidae). Can. Entomol. 111: 1013–1024.

48. Felsenstein J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783-791.

49. Finlayson T. 1990. The systematics and taxonomy of final instar larvae of the family Aphidiinae (Hymenoptera). Mem. Entomol. Soc. Can. 152: 3–74.

50. 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. Mol. Mar. Biol. Biot. 3, 294–299.

51. Gagić V., Petrović-Obradović O., Fründ J., Kavallieratos N.G., Athanassiou C.G., Starý (2016) The Effects of Aphid Traits on Parasitoid Host Use and Specialist Advantage. PLoS ONE 11(6): e0157674. doi:10.1371/journal.pone.0157674

52. Garantonakis N., Perdikis D., Lykouressis D., Kourti A., Gkouvitsas T. 2009. Studies on the identity of the parasitoids *Aphidius colemani* and *Aphidius transcaspicus* (Hymenoptera: Braconidae). Eur. J. Entomol. 106 (4): 491-498.

53. Gärdenfors U. 1986. Taxonomic and biological revision of palearctic *Ephedrus* Haliday (Hymenoptera: Braconidae, Ahpidiinae). Entomol. Scand. (Suppl.) 27: 1–95.

54. Godfray H.C.J. 1994: Parasitoids: Behavioural and Evolutionary Ecology. Princeton University Press, Princeton, NJ, 473 pp.

55. Gonzalez D., Hagen K. S., Starý P., Bishop G. W., Davis D. W., Pike K. S. 1995. Pea aphid and blue alfalfa aphid. In: Nechols, J.R. (Ed), Biological Control in the Western United States: accomplishments and benefits of Regional Research Project W-84, 1964-1989. UCANR Publications.129- 136.

56. Grundy T. P. 1989. An economic evaluation of biological control of rose- grain aphid in New Zealand. Aqribusiness and Economics Research; Unit Lincoln Colleqe, Canterbury, New Zealand. Research Report No. 200.

57. Guerrieri E., Poppy G. M., Powell W., Tremblay, E., Pennacchio F. 1999. Induction and Systemic Release of Herbivore-Induced Plant Volatiles Mediating In-Flight Orientation of *Aphidius ervi.* J. Chem. Ecol. 25 (6): 1247–1261.

58. Hagvar E. B., Hofsvang T. 1991 Aphid parasitoids (Hymenoptera, Aphidiidae): biology, host selection and use in biological control. Bioc. New. Inform., 12:13- 42.

59. 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. Trend. Genet., Vol. 23, No. 4.

60. Harrison R.G., Larson E.L. 2014. Hybridization, Introgression, and the Nature of Species Boundaries. J. Hered., 105(Special Issue): 795–809.

61. Hebert P.D.N., Cywinska A., Ball S.L., Dewaard J.R. 2003. Biological identifications through DNA barcodes. Proc. R. Soc. Lond. (B). 270:313–321.

62. Hebert P. D. N., Gregory T. R. 2005. The Promise of DNA Barcoding for Taxonomy. Syst. Biol. 54(5):852–859.

63. Hogenhout S. A., Ammar E-D., Whitfield A. E., Redinbaugh M. G. 2008. Insect Vector Interactions with Persistently Transmitted Viruses. Ann. Rev. Phyt. 46: 327-359.

64. Holler C. 1991 Evidence for the existence of a species closely related to the cereal aphid parasitoid *Aphidius rhopalosiphi* De Stefani-Perez based on host ranges, morphological

characters, isoelectric focusing banding patterns, cross-breeding experiments and sex pheromone specificities (Hymenoptera, Braconidae, Aphidiinae). System. Entom. 16, 15-28.

65. Ilić-Milošević M., Petrović A., Stanković S., Čkrkić J., Starý P., Žikić V., Tomanović Ž. 2015. Taxonomic Position and Phylogenetic Relationships of the Genera and Species *Euaphidius* and *Remaudierea* (Hymenoptera: Braconidae: Aphidiinae) Analysed Using Molecular Markers and Geometric Morphometrics. Ann. Entomol. Soc. Am. 1–11.

66. Iversen T., Harding S. 2007. Life table parameters affecting the population development of the woolly beech aphid, *Phyllaphis fagi*. Entomol. Exp. Appl., 123: 109-117.

67. Jamhour, A., Mitrovic, M., Petrovic, A., Starý, P., Tomanovic, Z. (2016): Re-visiting the *Aphidius urticae* s. str. group: re-description of *Aphidius rubi* Starý and *A. silvaticus* Starý (Hymenoptera: Braconidae: Aphidiinae). Zootaxa, vol. 4178 No. 2, pp. 278-288.

68. Kambhampati, S; Smith, P. T. 1995. PCR primers for the amplification of four insect mitochondrial gene fragments. Insect. Mol. Biol. 4 (4): 233–236.

69. Kambhampati S., Volkl W., Mackauer M. 2000. Phylogenetic relationships among genera of Aphidiinae (Hymenoptera: Braconidae) based on DNA sequence of the mitochondrial 16S rDNA gene. Syst. Entomol. 25, 437–445.

70. 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.

71. 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. Appl. Entomol. Zoo. 39, 527–563.

72. Kavallieratos N. G., Tomanović Ž., Athanassiou C.G., 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. Canad. Entomol., 137: 516-531.

73. Kavallieratos N. G., Tomanović Ž., Starý P., Žikić V., Petrović-Obradvic O. 2010. Parasitoids (Hymenoptera: Braconidae: Aphidiinae) attacking aphids feeding on Solanaceae and Cucurbitaceae crops in southeastern Europe: aphidiine-aphid-plant associations and key. Ann. Entomol. Soc. Am. 103: 153-164.

74. Kelly R. P., Palumbi S.R. 2009. General-use polymerase chain reaction primers for amplification and direct sequencing of enolase, a single-copy nuclear gene, from different animal phyla. Mol. Eco. Reso. 9, 144–147.

75. Kos K., Petrović A., Starý P., Kavallieratos N. G., Ivanović A., Toševski I., Jakše J., Trdan S., Tomanović Ž.. On the Identity of Cereal Aphid Parasitoid Wasps *Aphidius uzbekistanicus*, *Aphidius rhopalosiphi*, and *Aphidius avenaphis* (Hymenoptera: Braconidae: Aphidiinae) by Examination of COI Mitochondrial Gene, Geometric Morphometrics, and Morphology. Ann. Entom. Soci. Amer., 104(6):1221-1232. 2011

76. Landry B. S., Dextraze L., Boivin G. 1993. Random amplified polymorphic DNA markers for DNA fingerprinting and genetic variability assessment of minute parasitic wasp species (Hymenoptera: Mymaridae and Trichogrammatidae) used in biological control programs of phytophagous insects. Genome. 36 (3): 580-587.

77. Langer A., Hance T. 2000. Overwintering strategies and cold hardiness of two aphid parasitoid species (Hymenoptera: Braconidae: Aphidiinae). J. Insect. Phys. 46; 671–676.

78. Lenin E. A. 2015. Intergeneric affinity of sixty-two Aphidiine parasitoids (Hymenoptera: Braconidae: Aphidiinae) from different geographical regions. J. E. Z. S. 3(6): 405-410.

79. Levie A., Legrand M-A., Dogot P., Pels C., Baret P.V., Hance T. 2005. Mass releases of *Aphidius rhopalosiphi* (Hymenoptera: Aphidiinae), and strip management to control of wheat aphids. Agr. Ecos. Envi. 105, 17–21.

80. Lina, C.P., Danforth B.N. 2004. How do insect nuclear and mitochondrial gene substitution patterns differ? Insights from Bayesian analyses of combined datasets. Mol. Phylo. Evol. 30, 686–702.

81. Lozier J. D., Mills N. J. 2009. Ecological Niche Models and Coalescent Analysis of Gene Flow Support Recent Allopatric Isolation of Parasitoid Wasp Populations in the Mediterranean. PLoS One 4: e5901.

82. Luque I., Contreras A., Zabulon G., Herrero A., Houmard J. 2002. Expression of the glutamyl-tRNA synthetase gene from the cyanobacterium *Synechococcus* sp. PCC 7942 depends on nitrogen availability and the global regulator NtcA. Mol. Mic. 46 (4): 1157–1167.

83. Mackauer M. 1961. Die Gattungen der Familie Aphidiidae und ihre verwandsschaftliche Zuordung. Beitr. Entomol. 11, 792–803.

84. Mackauer M. 1968. ‘‘Hymenopterorum Catalogus. Pars 3, Aphidiidae,’’ Junk, Gravenhage.

85. Mackauer M., Chow F. J. 1986. Parasites and parasite impact on aphid populations. In: Plant Virus Epidemics: Monitering, Modeling and Predicting Outbreaks (eds G.D. McLean, R.G. Garret and W.G. Ruesink), Academic Press, Sydney. 95-117.

86. Mackauer M., Kambhampati S. 1986. Structural changes in the parasite guild attacking the pea aphid in North America. In: I. Hodek (ed.) Ecology of Aphidophaga. Academia Press, Prague and Dr. W. Junk, Dordrecht. pp. 347-356.

87. Mackauer M., Kambhampati S. 1988: Parasitism of aphid embryos by *Aphidius smithi*: some effects of extremely small host size. Entomol. Exp. Appl. 49: 167-173

88. Malafronte R.S., Marrelli M.T., Ramirez C.C., Nassar M.N., Marinotti O. 2007. Intraspecific variation of second internal transcribed spacer of nuclear ribosomal DNA among

populations of *Anopheles* (Kerteszia) *cruzii* (Diptera: Culicidae). J. Med. Entomol. Vol. 44, pp. 538-542.

89. Margonari C. S., Dias-Fortes C. L., Dias E.S. 2004. Genetic variability of *Lutzomyia whitmani* (Antunes & Coutinho) (Diptera: Psychodidae: Phlebotominae) in four geographical Brazilian populations by RAPD-PCR. J. Med. Entomol. *41*: 187-192.

90. Marsh P.M. 1977. Notes on the taxonomy and nomenclature of *Aphidius* species (Hym: Aphidiidae) parasitic on the pea aphid in North America. Entomophaga, 22 (4), 365-372.

91. Martin J.A., Pashley D.P. 1992. Molecular systematic analysis of butterflies family and some subfamily relationships (Lepidoptera: Papilionoidea). Ann. Entomol. Soc. Am. 85: 127-139.

92. Mastrantonio V., D. Porretta, S. Urbanelli, G. Crasta, G. Nascetti. Dynamics of mtDNA introgression during species range expansion: insights from an experimental longitudinal study. Sci Rep. 2016; 6: 30355.

93. McMenemy L. S., Mitchell C., Johnson S. N. (2009) Biology of the European large raspberry aphid (*Amphorophora idaei*): its role in virus transmission and resistance breakdown in red raspberry. Agric. Fore. Entom., 11, 61–71.

94. Messing R. H., Rabasse J. M. 1995. Oviposition behaviour of the polyphagous aphid parasitoid *Aphidius colemani* Viereck (Hymenoptera: Aphidiidae). Agr. Eco. Sys. Env. 52: 13-17.

95. Mitchell C., Johnson S. J., Gordon S. C., Birch A. N. E., Hubbard S. F. (2010) Combining plant resistance and a natural enemy to control *Amphorophora idaei*. BioControl 55, 321–327.

96. 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. Ann. Entomol. Soc. Am. 106: 170–180.

97. Mitrovski-Bogdanović A., Tomanović Ž., Mitrović M., Petrović A., Ivanović A., Žikić V., Starý P., Vorburger C. 2014. The *Praon dorsale–yomenae* s.str. complex (Hymenoptera, Braconidae, Aphidiinae): Species discrimination using geometric morphometrics and molecular markers with description of a new species. Zoolog. Anz., 253, 270–282

98. Müller C. B., Adriaanse I. C. T., Belshaw R., Godfray H. C. J. (1999) The structure of an aphid-parasitoid community. J. Anim. Ecol., 68, 346–370.

99. Mullis K.B., Faloona F.A. 1987. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. Meth. Enzymol. 155:335-50.

100. Muratori F., Lannic J. L., Nénon J-P., Hance T. 2004. Larval morphology and development of *Aphidius rhopalosiphi* (Hymenoptera: Braconidae: Aphidiinae). Can. Entomol.136: 169 – 180.

101. Nemec V., Starý P. 1983. Genetic polymorphism in *Aphidius ervi* Hal. (Hym: Aphidiidae), an aphid parasitoid on *Microlophiurn carnosum* (Bckt.). Z. ang. Ent. 95, 345-350.

102. Pennacchio F., Tremblay E. 1987. Biosystematic and morphological study of two *Aphidius ervi* Haliday (Hymenoptera, Braconidae) „biotypes“with the description of a new species. Boll. Lab. Ent. Agr. „F. Silvestri“, 43:105-117.

103. Pennacchio F. (1989) The Italian species of the genus *Aphidius* Nees (Hymenoptera, Braconidae, Aphidiinae). Boll. del Labor. di Entom. Agrar. 'Fil. Sil.', 46, 75–106.

104. Petrović A., Tomanović Ž., Žikić V. 2006. Wahlgreniella ossiannilssoni Hille Ris Lambers, A new host for A*phidius microlophii* Pennacchio and Tremblay (Hymenoptera, Braconidae, Aphidiinae). Arch. Biol. Sci., Belgrade, 58 (4), 41P-42P.

105. Petrović A.,Tomanović Ž., Kavallieratos N. G., P. Starý, 2010. *Aphidius geranii new species* (Hymenoptera: Braconidae: Aphidiinae) from southeast Europe – a new member of the *Aphidius urticae* sensu stricto group. Entomol. Fenn. 20, (4), 233-238.

106. Petrović A., Žikić, V, Petrović-Obradovic, O, Mitrovski Bogdanović, A., Kavallieratos, N. G., Starý, P., Tomanović Ž. 2011. Two new species of aphid parasitoids (Hymenoptera, Braconidae, Aphidiinae) from the Balkan Peninsula. *Zootaxa* 2895: 58–64.

107. 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. Bull. Entomol. Res.103, 451–457.

108. Petrović A., Mitrović M., Ivanović A., Žikić V., Kavallieratos N. G., Starý P., Mitrovski-Bogdanović A., Tomanović Ž., Vorburger 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 Evo. Bio. 15:5.

109. Petrović. A, Čkrkić. J, Jamhour A., Obradović O.P., Mitrović M., Starý P., Nedstam B., Tomanović Ž. (2017) First record of *Aphidius ericaphidis* (Hymenoptera, Braconidae) in Europe: North American hitchhiker or overlooked Holarctic citizen? J. Hymen. https://doi.org/10.3897/jhr.@@.12517.

110. Pfenninger M., Schwenk K. 2007. Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. BMC Evo. Bio. 7:121.

111. Pike K.S., Starý P., Graf G., Raworth D.A., Mathur S., Tanigoshi L.K., Murray T. 2011. A new *Aphidius* Nees (Hymenoptera, Braconidae, Aphidiinae) of *Ericaphis fimbriata* (Richards) (Hemiptera, Aphididae) and key to parasitoids of blueberry aphid in the Pacific Northwest. Zootaxa 2802: 58–62.

112. Powell W., Zhi-Li Z. 1983.The reactions of two cereal aphid parasitoids, *Aphidius uzbekistanicus* and *A. ervi* to host aphids and their food plants. Physiol. Entomol. 8:439-443.

113. Powell W., Wright A. F. 1988. The abilities of the aphid parasitoids *Aphidius ervi* Haliday and *A. rhopalosiphi* De Stefani Perez (Hymenoptera: Braconidae) to transfer between different known host species and the implications for the use of alternative hosts in pest control strategies. Bull. ent. Res. 78, 683-693.

114. Powell W., Pennacchio F., Poppy G.M., Tremblay E. 1998. Strategies involved in the location of hosts by the aphid parasitoid *Aphidius ervi* Haliday (Hymenoptera: Braconidae: Aphidiidae). Biol. Contr. 11: 104-112.

115. Prado S. G., Jandricic S. E., Frank S. D. 2015. Ecological Interactions Affecting the Efficacy of *Aphidius colemani* in Greenhouse Crops. Insects. 6(2): 538–575.

116. Prinsloo G.J., Hewitt P. H., van der Westhuizen M.E. 1993. The effect of temperature on oviposition behaviour and success of two parasitoids of the Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Hemiptera: Aphididae) Afric. Entom., 1 (2): 189-193

117. Pungerl N. B. 1983. Variability in characters commonly used to distinguish *Aphidius* species (Hymenoptera: Aphidiidae). Sys. Entomol. 8,425-430.

118. Pungerl N. B. 1986. Morphometric and electrophoretic study of *Aphidius* species (Hymenoptera: Aphidiidae) reared from a variety of aphid hosts. Sys. Entomol.11, 327-354.

119. Quicke D.L.J., Van Achterberg C. 1990. Phylogeny of the subfamilies of the family Braconidae (Hymenoptera: Ichneumonoidea). Zool. Verhand. 258: 1–95.

120. Rakhshani E., Talebi A. A., Starý P., Tomanović Ž., Kavallieratos N. G., Manzari S. 2008. A review of *Aphidius* Nees (Hymenoptera: Braconidae: Aphidiinae) in Iran: host associations, distribution and taxonomic notes. Zootaxa, 1767, 37–54.

121. Rakhshani E., Barahoei H., Ahmed Z., Tomanović Ž., Janković M., Petrović A., Bodlah I., Starý, P. 2012. New species and additional evidence of aphid parasitoids (Hymenoptera: Braconidae: Aphidiinae) from India. Zootaxa 3397: 45–54.

122. Raychaudhuri D. 1990. Aphidiids (Hymenoptera) of Northeast India. Indra Publ. House, India, pp. 152.

123. Raymond L., Plantegenest M., Gagić V., Navasse Y., Lavandero B. 2016. Aphid parasitoid generalism: development, assessment, and implications for biocontrol. J. Pest. Sci., 89:7–20.

124. Rehman A., Powell W. 2010. Host selection behaviour of aphid parasitoids (Aphidiidae: Hymenoptera). J. Pla. Bree. Cro. Sci., 2: 299–311.

125. Roehrdanz R. L. 1993. An improved primer for PCR amplification of mitochondrial DNA in a variety of insect species. Inse. Molec. Biol. 2(2): 89-91.

126. Roy H.E., Roy D.B., Roques A. 2011. Inventory of terrestrial alien arthropod predators and parasites established in Europe. BioControl 56: 477–504.

127. Saccone C., De Giorgi C., Gissi C., Pesole G., Reyes A. 1999. Evolutionary genomics in Metazoa: the mitochondrial DNA as a model system. Gene. 238:195–209.

128. Sanchis A., Latorre A., Gonzalez-Candelas F., Michelena J.M. 2000. An 18S rDNA-Based Molecular Phylogeny of Aphidiinae (Hymenoptera: Braconidae). Mol. Phylog. Evol., Vol. 14, No. 2, February, pp. 180–194.

129. Sanchis A., Michelena J.M., Latorre A., Quicke D.L.J., Gardenfors U., Belshaw R. 2001. The Phylogenetic Analysis of Variable-Length Sequence Data: Elongation Factor–1a Introns in European Populations of the Parasitoid Wasp Genus *Pauesia* (Hymenoptera: Braconidae: Aphidiinae). Mol. Biol. Evol., 18(6):1117–1131.

130. Savolainen, V., Cowan R.S., Vogler A.P., Roderick G.K., Lane R. 2005. Towards writing the encyclopedia of life: an introduction to DNA barcoding. Phil. Trans. R. Soc. Lond. B. 360: 1850-1811.

131. Sequeira R., Mackauer M. 1992. Covariance of adult size and development time in the parasitoid wasp *Aphidius ervi* in relation to the size of its host, *Acyrthosiphon pisum*. Evol. Ecol. 6(1): 34–44.

132. Sequeira R., Mackauer M. 1992. Nutritional Ecology of an Insect Host-Parasitoid Association: The Pea Aphid-*Aphidius ervi* System. Ecology. 73 (1): 183-189. Sharkey & Wharton (1997)

133. Shi M., Chen X.X. 2005. Molecular phylogeny of the Aphidiinae (Hymenoptera: Braconidae) based on DNA sequences of 16S rRNA, 18S rDNA and ATPase 6 genes. Eur. J. Entomol. 102: 133- 138.

134. Shi M., Chen X.X., Van Achterberg C. 2005. Phylogenetic relationships among the Braconidae (Hymenoptera: Ichneumonoidea) inferred from partial 16S rDNA, 28S rDNA D2, 18S rDNA gene sequences and morphological characters. Mol. Phylog. Evol., 37: 104– 116.

135. Short J. R. T. 1952. The morphology of the head of larval Hymenoptera with special reference to the head of Ichneumonoidea, including a classification of the final instar larvae of the Braconidae. Trans. R. Entomol. Soc. Lond. 103: 27- 84.

136. Simon C., Frati F., Beckenbach A., Crespi B., Liu H., Flook P. 1994. Evolution, Weighting, and Phylogenetic Utility of Mitochondrial Gene Sequences and a Compilation of Conserved Polymerase Chain Reaction Primers. Ann. Entomol. Soc. Am. 87(6):651-701.

137. Smith C.F. 1944. The Aphidiinae of North America (Braconidae: Hymenoptera). The Ohio State University, Columbus. 52 – 72.

138. Smith P., Kambhampati S., Volkl W., Mackauer M. 1999. A phylogeny of aphid parasitoids (Hymenoptera: Braconidae: Aphidiinae) inferred from mitochondrial NADH 1 dehydrogenase gene sequence. Mol. Phylog. Evol. 11, 236–245.

139. Smith, P., Kambhampati, S. 2000. Evolutionary transitions in Aphidiinae (Hymenoptera: Braconidae). In: Hymenoptera: Evolution, Biodiversity and Biological Control (A. D. Austin & M. Dowton, eds), pp. 106-113. Collingwood, Victoria: CSIRO.

140. Stanković S., Petrović A., Ilić-Milošević M., Starý P., Kavallieratos 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 forewings. Eur. J. Entomol.112, 165–174.

141. Starý, P. 1960. The generic classification of the family Aphidiidae (Hymenoptera). Act. Soc. Ent. Cec., 57: 239-52.

142. Starý P. 1962. New aphid parasites of the genus *Aphidius* Nees from Europe (Hymenoptera, Aphidiidae). Bull. Entom. Pol., 32, 109–122.

143. Starý P. 1970. Biology of Aphid Parasites (Hymenoptera: Aphidiidae) with Respect to Integrated Control. Dr. W. Junk, b.v., The Hague. 643 pp.

144. Starý P. 1973. A review of the Aphidius species (Hymenoptera: Aphidiidae) of Europe. Annot. Zool. Bot. Bratislava. 84:1-85.

145. Starý P. 1974. Taxonomy, origin, distribution and host range of *Aphidius* species (Hym: Aphidiidae) in relation to biological control of the pea aphid in Europe and North America. Z. ang. Entomo., 77 (1974), 141-171.

146. Starý P. 1975. Aphidius colemani Viereck: its taxonomy, distribution and host range (Hymenoptera: Aphidiidae). Acta Entomol. Bohemo. 72: 156–163.

147. Starý P., Nemec V. 1985. Population diversity in monophagous aphid parasitoids (Hym., Aphidiidae). Z. ang. Entomo., 100, 265-271

148. Starý P. 1988. Aphidiidae, pp: 171–184, In: Minks, A.K. and Harrewijn, P. (Eds.), Aphids: Their Biology, Natural Enemies and Control. Volume B. Elsevier, Amsterdam.

149. Starý P. 2006. Aphid parasitoids of the Czech Republic (Hymenoptera: Braconidae, Aphidiinae). Academia, Praha, 430 pp.

150. Stilmant D., van Bellinghen C., Hance T., Boivin G. 2008. Host specialization in habitat specialists and generalists. Oecologia 156:905–912.

151. Stoeckle M. 2003. Taxonomy, DNA and the bar code of life. BioScience.53:2–3.

152. Tahriri S., Talebi A. A., Fathipour Y., Zamani A. A. 2007. Host stage preference, functional response and mutual interference of *Aphidius matricariae* (Hym: Braconidae: Aphidiinae) on *Aphis fabae* (Hom: Aphididae). Entomol. Sci. 10, 323–331

153. Takada H. 1968. Aphidiidae of Japan (Hymenoptera). Insec. Matsum., 30(2): 67-124.

154. Takada H., Shiga M. 1974: Description of a new species and notes on the systematic position of the genus *Aclitus* (Hymenoptera: Aphidiindae). Kontyu 42: 283–292.

155. Takada H., Tada E. 2000. A comparison between two strains from Japan and Europe of *Aphidius ervi*. Entomol. Exp. App. 97, 11-20.

156. Tamura K., Nei M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol., 10: 512–526.

157. 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. Mol. Biol. Evol., 28: 2731–2739.

158. Templeton A. R. 1981. Mechanisms of speciation-A population genetic approach. Ann. Rev. Eco. Syst. 12: 23-48.

159. Teulon D.A.J., Drayton G.M., Scott I.A.W. 2008. Exotic introductions of primary parasitoids of aphids in New Zealand: the good and the bad. In: Mason PG, Gillespie DR, Vincent C eds. Proceedings of the Third International Symposium on Biological Control of Arthropods, Christchurch NZ. Morgantown, WV, USDA Forest Service. Pp. 421- 430.

160. Tomanović Ž., Kavallieratos N. G., Starý P., Athanassiou C. G., Žikić, V., Petrović-Obradović O., Sarlis G.P. 2003. *Aphidius* Nees (Hymenoptera: Braconidae: Aphidiinae) in Serbia and Montenegro: tritrophic associations and keys. Act. Entomol. Serb. 8, 15 –39.

161. Tomanović Ž., Kavallieratos N. G., Athanassiou C.G., Petrović O., Stanisavljević Lj. 2004. A new *Aphidius* species (Hymenoptera: Braconidae: Aphidiinae) from high-montane areas of southeastern Europe. Phytoparasitica, 32, 221-225.

162. Tomanović Ž., Rakhshani E., Starý P., Kavallieratos N.G., Stanisavljević Lj., Žikić V., Athanassiou C.G. 2007. Phylogenetic relationships between the genera *Aphidius* and *Lysaphidus* (Hymenoptera: Braconidae: Aphidiinae) with description of *Aphidius iranicus* sp. nov. Can. Entomol. 139, 297-307

163. Tomanović Ž., Kos K., Petrović A., Starý P., Kavallieratos N.G., Žikić V., Jakše J., Trdan S., Ivanović A. 2013. The relationship between molecular variation and variation in the wing shape of three aphid parasitoid species: *Aphidius uzbekistanicus* Luzhetzki, *Aphidius rhopalosiphi* De Stefani Perez and *Aphidius avenaphis* (Fitch) (Hymenoptera: Braconidae: Aphidiinae). Zool. Anz., 252, 41-47.

164. Tomanović Ž., Petrović A., Mitrović M., Kavallieratos N.G., Starý P., Rakhshani E., Rakhshanipour M., Popović A., Shukshuk A.H., Ivanović A. 2014. Molecular and morphological variability within the Aphidius colemani group with redescription of *Aphidius platensis* Brethes (Hymenoptera: Braconidae: Aphidiinae). Bull. Entom. Res., 104, 552–565.

165. Traugott M., Symondson W.O.C. 2008. Molecular analysis of predation on parasitized hosts. Bull. Entomol. Res. 98, 223– 231.

166. Traugott M., Bell J.R., Broad G.R., Powell W., Van Veen F.J.F., Vollhardt I.M.G., Symondson W.O.C. 2008. Endoparasitism in cereal aphids: molecular analysis of a whole parasitoid community. Mol. Ecol. 17, 3928–3938.

167. Tremblay E., Calvert D. 1971. Embryosystematics in the Aphidiinae (Hymenoptera: Braconidae). Boll. Lab. Entomol. Agr. Filippo Silvestri. 29: 223–249.

168. Tremblay E., Pennacchio F. 1988. Speciation in Aphidiinae Hymenoptera. Adva. Paras. Hymen. Rese., 139-146.

169. Turbeville J. McC., Pfeifer D. M., Field K.G., Rafl R.A. 1991. The phylogenetic status of arthropods, as inferred from 18s rRNA sequences. Mol. Biol. Evol. 8:669- 686.

170. Unruh T. R., White W., Gonzalez D., Woolley J. B. 1989. Genetic Relationships Among Seventeen *Aphidius* (Hymenoptera: Aphidiidae) Populations, Including Six Species. Ann. Entomol. Soc. Ame. 82, 754-768.

171. Van Achterberg C. 1993. Illustrated key to the subfamilies (Hymenoptera: Ichneumonoidea). Zoolog. Verhand., 283: 1-189.

172. Van Achterberg C. 2006. The Braconidae (Hymenoptera) of Greenland. Zoolog. Verhand., 80, 13–62.

173. Van Lenteren J. C. 2012. The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake. BioControl. 57:1–20

174. Vila, M., Bjorklund M. 2004. Testing biennialism in the butterfly *Erebia palarica* (Nymphalidae: Satyrinae) by mtDNA sequencing. Insect Mol. Biol.13 (2), 213 – 217.

175. Volkl W. 1994. Searching at different spatial scales: the foraging behaviour of the aphid parasitoid *Aphidius rosae* in rose bushes. Oecologia.100:177-183.

176. Vollhardt I. M.G., Tscharntke T., Wäckers F. L., Bianchi F. J.J. A., Thies C. 2008. Diversity of cereal aphid parasitoids in simple and complex landscapes. Agr. Ecos. Envi. 126, 289–292

177. Waterhouse D.F., Sands D.P.A. 2001. Classical Biological Control of Arthropods in Australia. CSIRO Entomology. Australian Centre for International Agricultural Research. Canberra, Australia.

178. Wei J., Li T., Kuang R., Wang Y., Yin T., Wu X., Zou L., Zhao W., Cao J., Deng J. 2003. Mass Rearing of *Aphidius gifuensis* (Hymenoptera: Aphidiidae) for Biological Control of *Myzus persicae* (Homoptera: Aphididae). Bioc. Sci. Tech.13, 87- 97.

179. Wharton R. A., Marsh P. M., Sharkey M. J. 1997. Manual of the New World Genera of the Family Braconidae (Hymenoptera). No. 1, Spec. Public. Intern. Soc. Hymenop., 439 p.

180. Wheeler W. C., Cartwright P., Hayashi C.Y. 1993a. Arthropod phylogeny: A combined approach. Cladistics 9:1-39.

181. Wheeler W. C., Schuh R.T., Bang R. 1993b. Cladistic relationships among higher groups of Heteroptera: Congruence between morphological and molecular data sets. Entomol. Scand., 24: 121– 137.

182. Wickremasinghe M. G. V., Van Emden H. F. 1992. Reactions of adult female parasitoids, particularly *Aphidius rhopalosiphi*, to volatile chemical cues from the host plants of their aphid prey. Phys. Entomol. 17, 297-304.

183. William J., Ballard O. 2000. When One Is Not Enough: Introgression of Mitochondrial DNA in Drosophila Mol. Biol. Evol. 17(7):1126–1130. 2000

184. Witt J. D. S., Threloff D.L., Hebert P.D.N. 2006. DNA barcoding reveals extraordinary cryptic diversity in an amphipod genus: implications for desert spring conservation. Mol. Ecol., 15:3073 -3082.

185. Yang S., Yang S.Y., Zhang C.P., Wei J-N., Kuang R.P. 2009. Population dynamics of *Myzus persicae* on tobacco in Yunnan Province, China, before and after augmentative releases of *Aphidius gifuensis.* Bioco. Sci. Techn. 19 (2): 219- 228.

186. 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

187. Yu D.S., Van Achterberg C., Horstmann K. 2012. World Ichneumonoidea 2011. Taxonomy, biology, Morphology and distribution (Braconidae). Taxapad (Scientific names for information management) Interactive catalogue on DVD/CDROM. Vancouver.

188. Zhang D. X., Hewitt G. M. 1997. Assessment of the universality and utility of a set of conserved mitochondrial COI primers in insects. Insect. Mol. Bio., 6(2):143-150.

189. Zhu Y.C., Williams L. 2002. Detecting the Egg Parasitoid *Anaphes iole* (Hymenoptera: Mymaridae) in Tarnished Plant Bug (Heteroptera: Miridae) Eggs by Using a Molecular Approach. Ann. Entomol. Soc. Am., 95(3): 359- 365

190. Zhu Y. C., Riddick E. W., Williams L., Schotzko D. J., Logarzo G. A., Jackson C.G. 2004. Potential of Detection and Identification of Nymphal Parasitoids (Hymenoptera: Braconidae) of Lygus Bugs (Heteroptera: Miridae) by Using Polymerase Chain Reaction and ITS2 Sequence Analysis Techniques. Ann. Entomol. Soc. Am., 97(4): 743-752

191. Žikić V., Tomanović Ž., Ivanović A., Kavallieratos N.G., Starý P., Stanisavljević Lj., Rakhshani E. 2009. Morphological characterization of *Ephedrus persicae* biotypes (Hymenoptera: Braconidae: Aphidiinae) in the palaearctic. Ann. Entomol. Soc. Am., 102, 223–231.

192. Žikić V., Ilić-Milošević M., Stanković S., Petrović A., Petrović-Obradović O., Kavallieratos N., Starý P., Tomanović Ž. 2012. Aphidiinae (Hymenoptera: Braconidae) of Serbia and Montenegro – tritrophic interactions. Act. Entom. Serb., 17(1/2): 83–105.

8. APPENDIX 1

Re-description of *A. silvaticus* and *A. rubi*

The nomenclature of parasitoids follows Sharkey & Wharton (1997). The nomenclature of aphids is based on Remaudière & Remaudière (1997).

Aphidius rubi Starý (Fig. 16)

Diagnosis

In its host range pattern, long R1 vein and light body colour, *Aphidius rubi* belongs to the *A. urticae* s. str. group, from which it differs with respect to a combination of several characters [wing venation pattern, shape and carination of the dorsal side of petiole, number of longitudinal placodes on flagellomere 1 (F1) and the tentorial index]. *Aphidius rubi* differs from the sibling species *A. silvaticus* in having a prominent carina on petiole (Fig. 16f), which is not clearly defined in *A. silvaticus* (Fig. 16f). Moreover, the R1 vein (= metacarpus) is shorter than the stigma in *A. rubi* (the ratio between the stigma and the metacarpus is 1.11–1.34 in *A. rubi*), in contrast to *A. silvaticus*, where the metacarpus is equal to or longer than the stigma (the ratio between the stigma and the metacarpus is 0.91–1.00 in *A. silvaticus*). Also, *A. rubi* is a parasitoid of the blackberry aphid (*Macrosiphum funestum*) and probably some other *Macrosiphum* species, as well as *Aulacorthum* spp., while *A. silvaticus* parasitizes different *Amphorophora* aphid hosts. *Aphidius rubi* differs from *A. urticae* in having more longitudinal placodes on F2 (2–3 longitudinal placodes in *A. rubi* instead of one placode in *A. urticae*) and a higher value of the tentorial index (the tentorial index in *A. rubi* is 0.48–0.55, instead of 0.35–0.40 in *A. urticae*). It differs from *A. urticae* in its host range pattern, since *A. urticae* is a specialized parasitoid of the stinging nettle aphid (*Microlophium carnosum*).

Description

Female. **Head:** Head wider than mesoscutum (the ratio between head width and mesoscutum width is 1.25–1.41). Eyes oval, with sparse setae (Fig. 16a). Clypeus oval, with 10–17 long setae. Tentorial index (tentoriocular line/intertentorial line) equal to 0.48–0.55. Antennae 18–19 (20)segmented, filiform, reaching about half of the metasoma, with semi-

erect and adpressed setae shorter than diameter of the segments. Flagellomere 1 (F1) (Fig. 16b) 3.40–3.70 × as long as median width, usually without longitudinal placodes. Flagellomere 2 (F2) (Fig. 16b) is 2.70–3.30 × as long as median width, with 3 (2) longitudinal placodes. F1 subequal to F2 (F1/F2 = 0.93–1.06) (Fig. 16b). Maxillary palp with four palpomeres, labial palp with three palpomeres.

Mesosoma: Mesonotum with notaulices distinct in the ascendent part, deep, crenulated, sparsely setaceous, with setae positioned in two rows along the mesonotum. Notaulices effaced on the disc (Fig. 16c). Propodeum areolated with narrow central pentagonal areola (Fig. 16d). Upper lateral areolae with 8–13 setae each, lower lateral areolae with 4–6 setae. The ratio between hind leg tibia length and first tarsomere length is 3.10–3.35, while that between hind leg tibia length and second tarsomere length is 7.20–8.45. The ratio between lengths of the first and second tarsomere of the hind leg is 2.30–2.60.

Fore wing: Stigma moderately elongated, 3.60–4.10 × as long as wide and 1.11–1.34 × as long as distal abscissa of R1 (= metacarpus) (Fig. 16e). The ratio between length and width of the fore wing is 2.50–2.66.

Metasoma: Petiole elongate, slender, 3.30–3.60 × as long as wide at spiracles (Fig. 16f), with 10–12 costulae on its anterolateral area (Fig. 16g) and with prominent central mediodorsal carina (Fig. 1f). Ovipositor sheath slightly concave on its dorsal margin (Fig. 16h).

Colour: Head yellow with occiput and frons brown to light brown. Mouthparts yellow. Scape light brown to yellow, pedicel brown. F1 with yellow ring at the base, remaining part of the antennae brown. Mesosoma and metasoma yellow to light brown. Legs yellow with brown apices. Propodeum brown to light brown. Ovipositor sheath black.

Body length: 2.4–4.0 mm.

Male: Antennae 22-segmented. F1 short, 1.60-1.90 × as long as wide. F1 and F2 with 9–10 and 11–12 longitudinal placodes, respectively. Stigma about 3.30-3.50 × as long as wide, metacarpus about a quarter shorter than stigma length (the ratio between stigma length and metacarpus length is 1.15–1.30). Petiole 3.10–3.30 × as long as wide at spiracle level. Body generally darker than female, with brown and black body parts.

Examined material: We re-examined type specimens (for collection details see Starý, 1962); ***Aulacorthum solani* Kaltenbach** on *Filipendula ulmaria* 1m#, Plitvice–Okrugljak, 20-VI-2015 (CRO); on *Fuchsia magellanica* 2f#, Wellen, 15-V-2015 (BEL); 1f#, Wellen, 15-

VI-2015 (BEL); on *Lathyrus tuberosus* 3f# 3m#, Prokuplje–Bresničić, 16-VI-2014 (SRB); on *Myosotis aquatica* 3m#, Sremska Mitrovica, 24-V-1998 (SER), on *Raphanus sativus* 3f#, Tivat, 25-V-2011 (MNE); on *Sanguisorba minor* 1f# 1m#, Durmitor–Crno jezero lake, 01-VII-1998 (MNE); ***Aulacorthum vaccinii* Hille Ris Lambers** on *Vaccinium uliginosum* 3f#, Obergurgl, 28-VII-2015 (AUT); ***Macrosiphum funestum* (Macchiati)** on *Rubus armeniacus* 3f# 1m#, Tara–Mitrovac, 03-VII-2012 (SRB); on *Rubus fruticosus* 12m#, Rudolfov, 26-VI-2004 (CZE); on *Rubus hirsutum* 4f# 8m#, Komovi, 07-X-2013 (MNE); on *Rubus idaeus* 1m#, Vlasina, 10-VI-2010 (SRB); on *Rubus* sp. 1f#, Roskoff, 02-V-1974 (FRA); 1m#, Roskoff, 26-V-1974 (FRA); 1m#, Gérardmer, 19-VI-1974 (FRA); 6f# 2m# Soto de Sajambre, 09-VII-1982 (ESP); 1m# 5f#, Šara, 17-VII-1995 (SRB); 1f#, Užice–Sredići, 10-VI-2011 (SRB); 12f# 9m#, Golija–Bele vode, 21-VII-2011 (SRB); 1f# 4m#, Vasojevća Komovi, 22-VII-2012 (MNE); 11f# 13m# Prokletije–Pepići, 24-VII-2012 (MNE); 17f# 7m#, Andrijevica–Komovi, 10-VII-2013 (MNE); 29f# 10m#, Murino, 11-VII-2013 (MNE); 3f# 1m# Plitvice–Vidikovci, 20-VI-2013 (CRO); 1f# 5m# Plitvice–Kozjak, 22-VI-2013 (CRO).

Aphidius silvaticus Starý (Fig. 17)

Diagnosis

For differentiation of *A. silvaticus* from *A. rubi*, see *A. rubi* diagnosis above. *Aphidius silvaticus* differs from *A. urticae* in having more longitudinal placodes on F2 (2–3 longitudinal placodes in *A. silvaticus*, one placode in *A. urticae*) and a higher tentorial index (*A. silvaticus* is 0.45–0.55, 0.35–0.40 in *A. urticae*). It differs from *A. urticae* in its host range pattern, since *A. urticae* is a specialized parasitoid of the stinging nettle aphid (*Microlophium carnosum*), while *A. silvaticus* parasitizes *Amphorophora* aphid hosts.

Description

Female. **Head:** Head wider than mesoscutum (the ratio between head width and mesoscutum width is 1.30–1.39). Eyes oval, with sparse setae (Fig. 17a). Clypeus oval, with 10–16 long setae. Tentorial index (tentoriocular line/intertentorial line) equal to 0.45–0.55. Antennae (18) 19–20-segmented, filiform, reaching about half of metasoma with semierect

and adpressed setae shorter than diameter of the segments. F1 (Fig. 17b) 3.30–3.80 × as long as median width, without longitudinal placodes. F2 (Fig. 17b) is 3.00–3.50 × as long as median width, with (2)–3 longitudinal placodes. F1 subequal to F2 (F1/F2 = 0.94–1.03) (Fig. 17b). Maxillary palp with four palpomeres, labial palp with three palpomeres.

Mesosoma: Mesonotum with notaulices distinct in the ascendent part, deep, crenulated, sparsely setaceous, with two rows of setae along the mesonotum. Notaulices effaced on the disc (Fig. 17c). Propodeum areolated with central pentagonal areola (Fig. 17d). Upper lateral areolae with 10–13 setae each, and lower lateral areolae with 4–5 setae. The ratio between hind leg tibia length and first tarsomere length is 3.35–3.42, while that between hind leg tibia length and second tarsomere length is 7.45–8.15. The ratio between lengths of the first and second tarsomeres of the hind leg is 2.18–2.45.

Fore wing: Stigma moderately elongate, 3.50–4.00 × as long as wide and 0.91–1.00 × as long as distal abscissa of R1 (= metacarpus). The ratio between length and width of the fore wing is 2.44–2.60.

Metasoma: Petiole elongate, slender, 3.30–3.60 × as long as wide at spiracles (Fig. 17f), with 10–12 costulae on its anterolateral area and with a short mediodorsal carina that is not clearly defined. Ovipositor sheath slightly concave on its dorsal margin (Fig. 17g).

Color: Head black-brown with yellow face, frons, genae, clypeus and mouthparts. Scape yellow, pedicel brown. F1 yellow at the base, and sometimes F2 and F3 also yellow. Remaining part of antennae brown. Mesosoma and metasoma mostly yellow or light brown. Legs yellow with brown apices. Propodeum light brown. Ovipositor sheath black.

Body length: 2.9–3.6 mm.

Male: Antennae 21–22 segmented. F1 2.00–2.10 × as long as wide. F1 and F2 with 6–8 and 7–9 longitudinal placodes, respectively. Stigma about 3.50 × as long as wide, metacarpus subequal to stigma length. Petiole subsquare, about 2.80–2.90 × as long as wide at spiracle level. Body generally darker than female.

Examined material: We re-examined type specimens (for collection details, see Starý, 1962); ***Amphorophora ampullata* Buckton** on *Dryopteris austriaca* 2f#, Rusava, 20-VII-1963 (CZE); ***Amphorophora idaei* (Börner)** on *Rubus idaeus* 4f# 5m#, Sint–Truiden, 09-VII-2015 (BEL); on *Rubus* sp. 1f# 1m#, Sint–Truiden, 16-IX-2014 (BEL); ***Amphorophora rubi* (Kaltenbach)** on *Rubus idaeus* 1m#, Kopaonik, 17-VII-2013 (SER); on *Rubus* sp. 1f# 2m#, Brestovik, 27-V-2011 (SRB); 2f# 3m# Helsinki 15-28-VII-1963 (FIN); 23f# 15m#,

Plitvice–Kozjak, 21-VI-2015 (CRO); ***Amphorophora* sp.** on *Dryopteris* sp. 3f# 1m#, Divčibare, 05-VIII-1999 (SRB); on *Rubus* sp. 1f# 1m# Petnica, 18-V-1998 (SRB); 2m#, Tara–Derventa, 31-V-1998 (SRB); 5f# 2m#, Tara–Derventa, 20-V-2000 (SRB); 4f# 1m#, Sint–Truiden, 16-IX-2014 (BEL), 1f#, Praha–Košíře, VI-1978 (CZE).

Key for identification of female parasitoids belonging to the *Aphidius urticae* s. str. species group

1 Metacarpus shorter than stigma; mediodorsal carina on petiole prominent and clearly defined; parasitoid of *Macrosiphum funestum* (large blackberry aphid) ..... ***Aphidius rubi*** Starý

- Metacarpus equal to or slightly longer than stigma; mediodorsal carina on petiole short and not clearly defined ……..... 2

2 Three or two longitudinal placodes on F2; tentorial index 0.45–0.55; parasitoid of *Amphorophora* aphids..... ***Aphidius silvaticus*** Starý

- One longitudinal placode on F2; tentorial index 0.35–0.40; parasitoid of *Microlophium carnosum* (common nettle aphid) ..... ***Aphidius urticae*** Haliday.

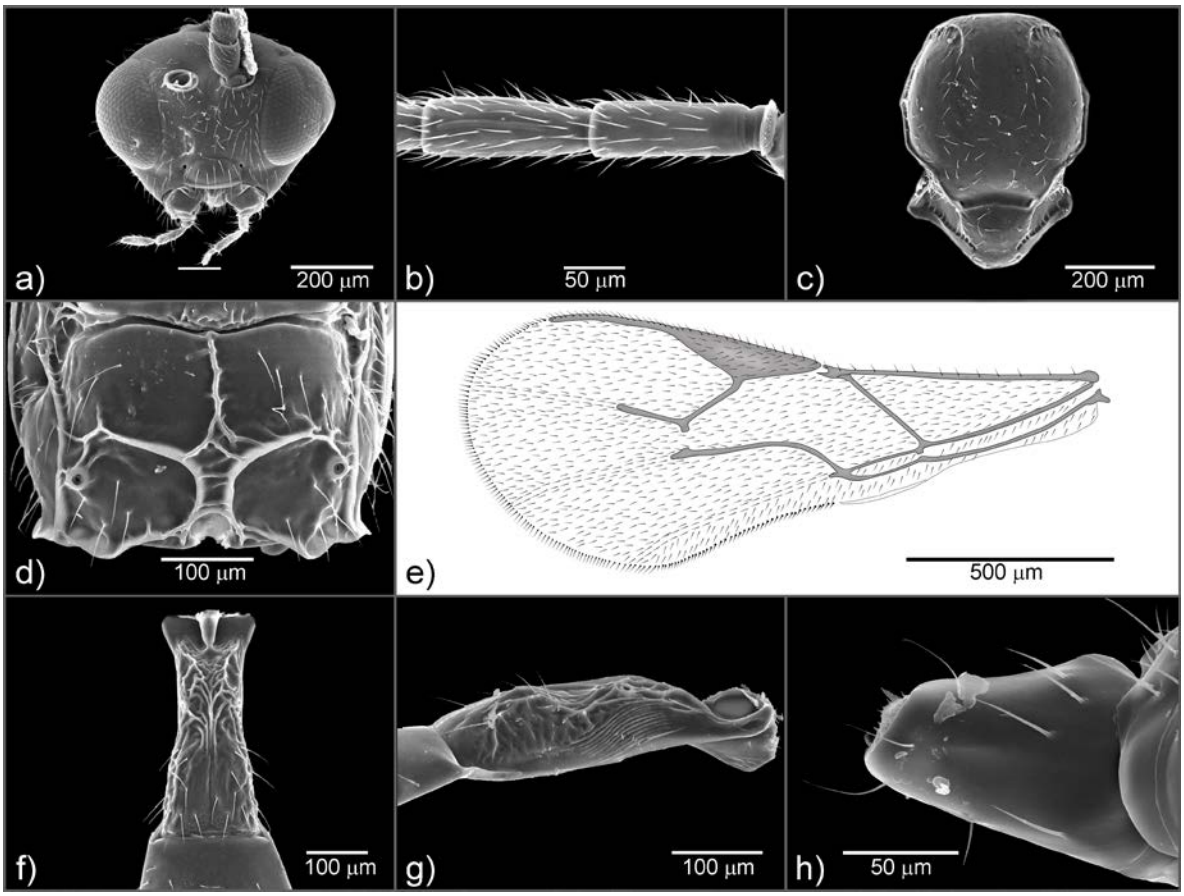

Fig. 16. *Aphidius rubi*. female: a) head; b) flagellomere 1 and 2; c) mesonotum–dorsal view; d) propodeum–dorsal view; e) fore wing; f) petiole–dorsal view; g) petiole–lateral view; h) ovipositor–lateral view.

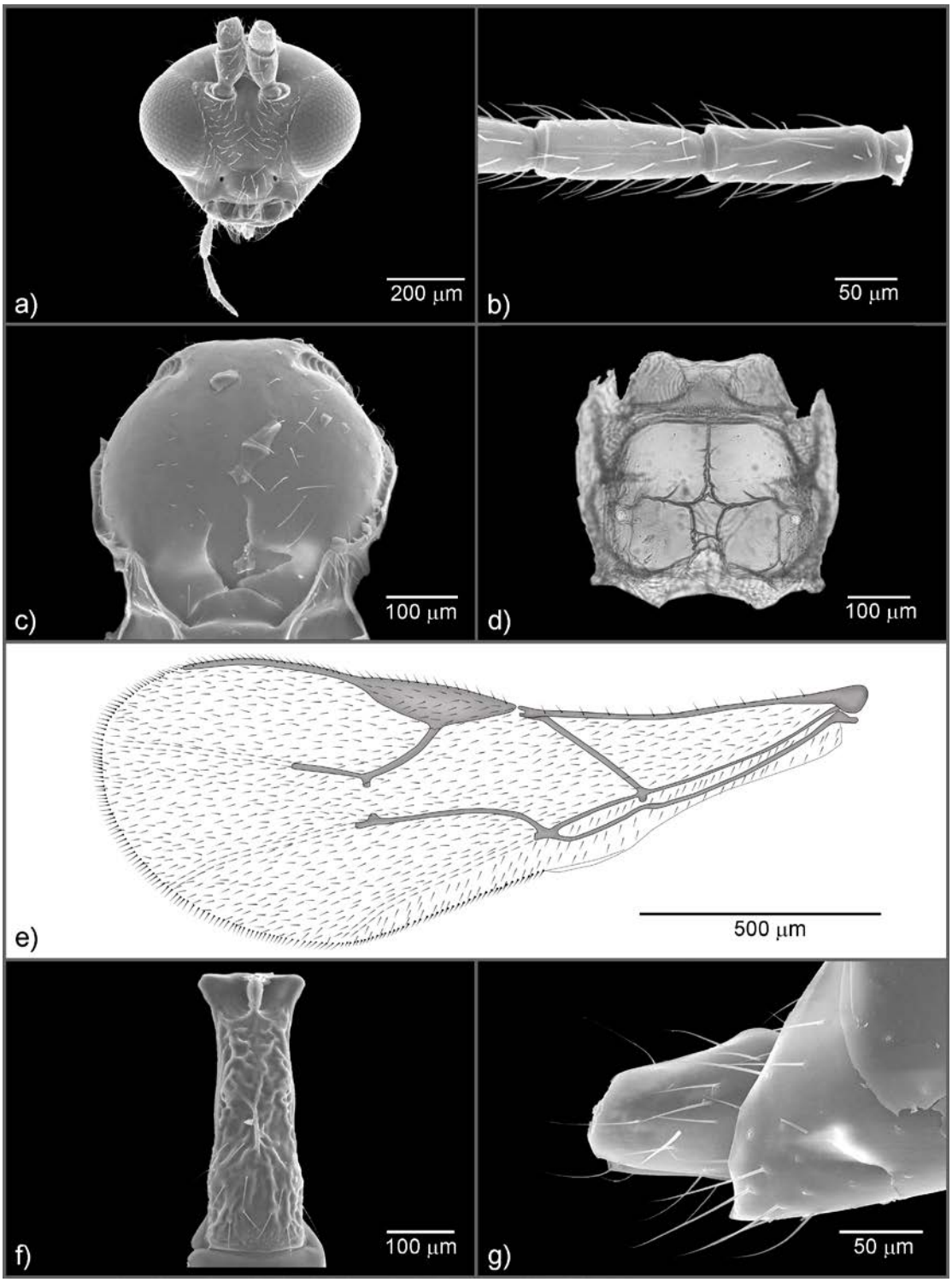

Fig. 17. *Aphidius silvaticus*. female: a) head; b) flagellomere 1 and 2; c) mesonotum–dorsal view; d) propodeum–dorsal view; e) fore wing; f) petiole–dorsal view; g) ovipositor–lateral view.

9. APPENDIX 2

Aphidius ericaphidis

Aphidius ericaphidis (Fig. 18) is most similar to *A. matricariae*. It can be easily distinguished from the latter by the number of maxillary and labial palpomeres. *A. matricariae* has 3-segmented maxillary palps and 2-segmented labial palps while *A. ericaphidis* has 4-segmented maxillary palps and 3-segmented labial palps. *A. matricariae* has a ratio of pterostigma length / R1 forewing vein in range of 1 to 1.2, while *A. ericaphidis* 1.7–2.7.

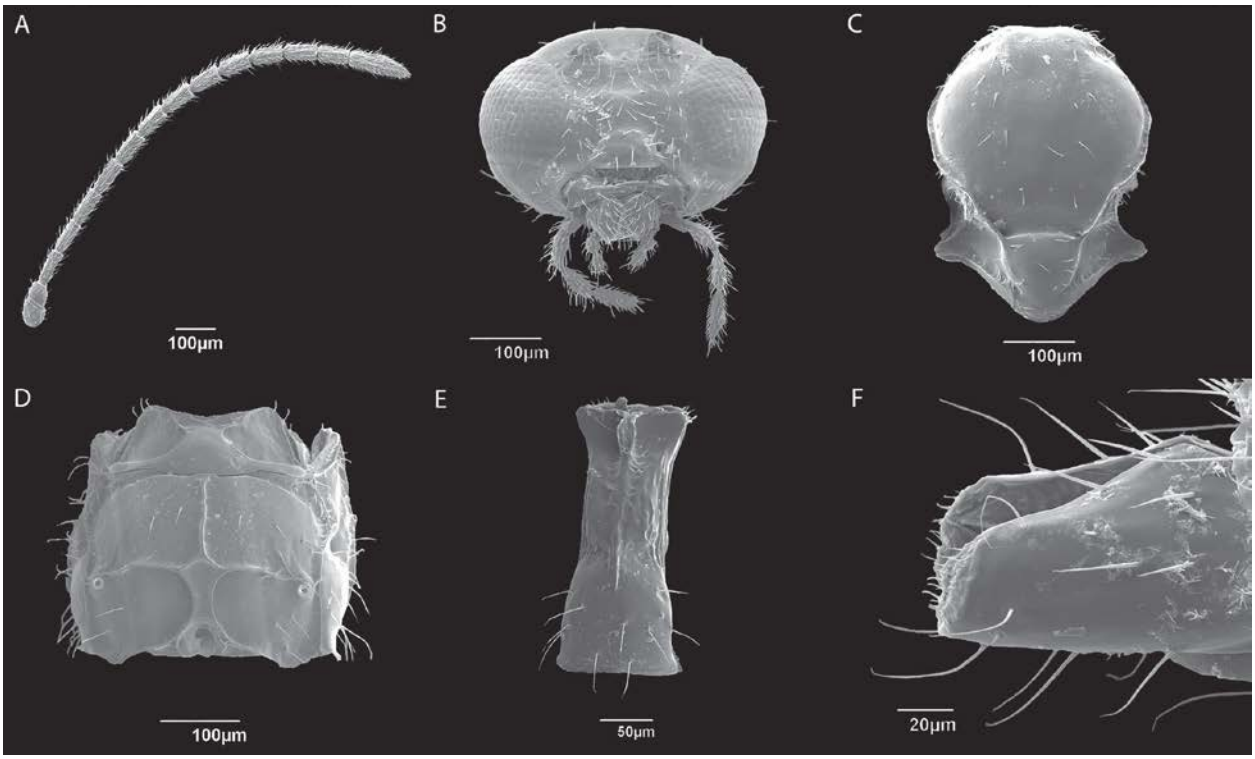

Fig. 18. *Aphidius ericaphidis* female; **A** antenna **B** head **C** mesonotum – dorsal aspect **D** propodeum – dorsal aspect **E** petiole – dorsal aspect **F** ovipositor – lateral aspect.

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Aiman (Mohamed) Jamhour was born on January 15th, 1974 in Tripoli, Lybia. She studied at the Plant Protection Department, at the Faculty of Agriculture, University of Tripoli and graduated in 1999. Between 2001 and 2005 she completed a postgraduate programme at the same Faculty of Agriculture.

Since August 2000 till April 2005 she has had an experience as a teacher assistance at the Department of Plant Protection at the Faculty of Agriculture in Tripoli. Later on, between May 2005 till March 2014, she was engaged as a lecturer at the Department of Plant Protection at the Faculty of Agriculture in Tripoli.

During her lecturer career at the Faculty of Agriculture in Tripoli, she held many classes such as: General Entomology, Insects of field crops and stored grains, Insects of horticulture crops, Insects Control, Insects physiology, Fundamentals of Plant Protection.
Her research interests include biology and taxonomy of natural enemies.

In 2014, she started PhD studies at the Department of Invertebrate Zoology and Entomology at the Faculty of Biology, University of Belgrade.
She has published in journals such as Zootaxa, Journal of Hymenopteran Research, The 3rd Biotechnical Conference, Sebha, Libya, The 9th Arab Plant Protection Symposium, etc.

Her goal is to become a better researcher and teacher, and stand at multiple panels in conferences related to topics of her interest.

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Изјава о ауторству

Потписана Aiman M. Jamhour

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