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Morphological and molecular characterization of *Aphidius eadyi* species complex (Hymenoptera, Braconidae, Aphidiinae), parasitoids of pea aphid – *Acyrthosiphon pisum* Harr. (Hemiptera, Aphididae)

Doctoral Dissertation

Belgrade, 2018

УНИВЕРЗИТЕТ У БЕОГРАДУ
БИОЛОШКИ ФАКУЛТЕТ

Mustafa E. Ghaliow

Морфолошка и молекуларна карактеризација врста *Aphidius eadyi* комплекса (Hymenoptera, Braconidae, Aphidiinae), паразитоида зелене луцеркине ваши – *Acyrthosiphon pisum* Harr. (Hemiptera, Aphididae)

докторска дисертација

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Acknowledgments

Foremost, I would like to express my sincere gratitude to my main supervisor and mentor ***Professor Željko Tomanović*** *for his academic support of my Ph.D. study and research, for his patience, motivation, enthusiasm, and immense knowledge. Thank you for all your efforts and all the time invested in planning this dissertation as well as for all the support you have provided me during the research.*

I am grateful to my second supervisor ***Dr. Andjeljko Petrović*** *on all the suggestions and corrections to improve and complete this research, thank you for having all the patience and help with my research. His guidance helped me in all the time of research and writing of this dissertation.*

Thank you very much to ***Dr. Ana Ivanović*** *for the helping in morphometrics, for all the knowledge that she gave me in the statistical data processing, and thank her for all useful suggestions when writing the dissertation.*

I owe a great gratitude to ***Dr. Milana Mitrović*** *for enormous help in molecular methods, on all constructive suggestions and suggestions, on friendly support and genuine cooperation.*

To all the ***members of the Department of Invertebrate Zoology and Entomology****, I owe gratitude for the collegial and friendly attitude.*

I owe special gratitude to my ***parents*** *and my* ***brothers*** *and* ***sisters*** *for always believing in me and encouraging me and supporting me throughout my life and for unconditional love, understanding, and optimism.*

Last, but not the least, I owe most to my ***wife*** *and my* ***daughters*** *without whose support, love, patience, and understanding I would not be able to persevere to the end.*

Thank you all.

Morphological and molecular characterization of *Aphidius eadyi* species complex (Hymenoptera, Braconidae, Aphidiinae), parasitoids of pea aphid – *Acyrthosiphon pisum* Harr. (Hemiptera, Aphididae)

ABSTRACT

Acyrthosiphon pisum Harris is an aphid species of the greatest agricultural importance. It is a major pest on several plants of the family Fabacae, and there have been numerous programs involving biological control of *Acyrthosiphon pisum* worldwide. Species belonging to the *Aphidius eadyi* group have been used as biocontrol agents in those programs, but knowledge about their taxonomy and distribution has remained scarce with big gaps. Here we identify all aphidiine parasitoid species that have parasitized *A. pisum* in Europe, including three species within the *Aphidius eadyi* species group, using both molecular (mtDNA COI sequences) and morphological analyses. The *Aphidius eadyi* species group consists of the following species: *Aphidius smithi*, *A. eadyi*, and *A. banksae*. Morphological characterization showed that the most important morphological characters for separation of species of the *Aphidius eadyi* group are: shape of costulae on the anterolateral part of the petiole; shape of the central areola on the propodeum; and shape and venation of the forewings. Forewing shape was analysed using geometric morphometrics, and it is demonstrated that all three species differ in wing shape with some overlap. Morphological differences were confirmed by molecular data, mean genetic distances between the species varying from 5 to 7.4%. Identification of *Aphidius banksae* as a widely distributed pea aphid parasitoid whose range covers most of the western Palaearctic (from the United Kingdom to Israel) is the most interesting finding of this study. In addition, *Aphidius banksae* is diagnosed and redescribed. A key for identification of all aphidiine species attacking *Acyrthosiphon pisum* in Europe is provided.

Keywords: *Aphidius eadyi* species complex, mtDNA barcoding, geometric morphometrics, integrative taxonomy
Scientific field: Biology
Scientific subfield: Morphology, systematics, and phylogeny of animals
UDC number: [595.79:591.557.1]:595.7(4)(043.3).

Морфолошка и молекуларна карактеризација врста *Aphidius eadyi* комплекса (Hymenoptera, Braconidae, Aphidiinae), паразитоида зелене луцеркине ваши – *Acyrthosiphon pisum* Harr. (Hemiptera, Aphididae)

САЖЕТАК

Acyrthosiphon pisum Harris је једна од економски најзначајнијих биљних ваши у пољопривреди, првенствено на културама из фамилије Fabacae. Из тог разлога су и бројни програми биолошке контроле реализовани широм света. У овим програмима су веома често као агенти коришћене врсте *Aphidius eadyi* комплекса. И поред честе употребе и значаја, број врста унутар комплекса, њихова таксономија и распрострањење су углавном непознати. У овој студији извршена је идентификација свих паразитоида из потфамилије Aphidiinae који паразитирају *A. pisum* на простору Европе. Међу њима су, употребом морфолошких и молекуларних анализа (секвенци mtCOI гена) идентификоване три врсте које припадају *Aphidius eadyi* комплексу: *Aphidius smithi*, *A. eadyi* и *A. banksae*. Морфолошком карактеризацијом је утврђено да су за разликовање ових врста најзначајнији следећи морфолошки карактери: облик бразди на антеролатералном региону петиолуса, облик централне ареоле на проподеуму, облик и нерватура предњих крила. Облик предњих крила је анализиран употребом геометријске морфометрије и утврђено је да се све три врсте разликују и поред мањег преклапања. Морфолошке разлике су потврђене и молекуларним анализама којима је утврђено да се генетичке дистанце између врста *A. eadyi* комплекса крећу у распону од 5% до 7,4%. Идентификација врсте *Aphidius banksae*, као широко распрострањеног паразитоида зелене луцеркине ваши представља најинтересантнији налаз ове студије. Утврђено је да распрострањење врсте *A. banksae* обухвата највећи део западног Палеарктика, од Уједињеног Краљевства до Израела. Додатно, дат је и поновни опис врсте *Aphidius banksae* као и кључ за идентификацију свих паразитоида потфамилије Aphidiinae који паразитирају *Acyrthosiphon pisum* у Европи.

Кључне речи: *Aphidius eadyi* коплекс врста, ДНК баркодинг, геометријска морфометрија, интегративна таксономија

Научна област: Биологија

Ужа научна област: Морфологија, систематика и филогенија животиња

УДК број: [595.79:591.557.1]:595.7(4)(043.3).

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1. INTRODUCTION

Alfalfa (*Medicago sativa* L.) is the most widely used forage crop (Walton, 1983). It has been grown for centuries as valuable feed for livestock. In ancient times, it was called al–fac–facach, "the father of all food", and nowadays it is often referred to as "the queen of forages" because it has the highest food value of all commonly grown hay crops (Castelman, 1991). Alfalfa produces more protein per hectare than any other crop used as feed for livestock, and it also can improve soil quality, which eventually enhances agricultural profitability (Hanson *et al*., 1988).

It has been utilized in the form of green feed, hay, or (more recently) dried pellets (AntogioVanni and Bruni, 1994; Marten *et al*., 1990). Alfalfa is an important forage crop that has large amounts of protein, calcium, phosphorus, and vitamins A and D (Nuernberg *et al*., 1990). The high nutritional quality of alfalfa hay is determined by the high content of good-quality protein and carbohydrates. The aerial parts of alfalfa are one of the richest sources of chlorophyll and vitamin C, E, B1, B2, B6, B12, niacin, folic acid, biotin, inositol, choline, some digestive enzymes and β–carotene (Gnasiak and Lesisns, 1975) (Table 1).

Table 1. Essential amino acid composition of protein from different livestock food (FAO/WHO/UNU, 2007).

	Amino acid						
	Lysine	Phenylalanine	Methionine	Threonine	Isoleucine	Valine	Tyrosine
Alfalfa leaf	6.3	6.0	2.1	5.2	9.8	6.3	1.6
Soybean	6.4	4.8	0.6	3.7	3.5	5.0	1.2
Mixed grass	4.8	5.8	2.3	4.7	5.7	6.8	2.1

Alfalfa is also used in human nutrition as a garnish, leaf protein concentrate, or nutritional supplement in the guise of products such as tablets or drinks containing alfalfa juice that improve digestion (Anonymous, 1937; Story *et al*., 1984). Experiments performed on animals showed that alfalfa can be used for treatment of hypercholesterolemia (Colodny *et al*., 2001; Sharma, 1987).

Alfalfa is attacked by several insect groups (Kalvelage, 1992), among which aphids are one of the most important (Conti, 1985).

1.1. Pea aphid – *Acyrthosiphon pisum* Harr.

Aphids (Hemiptera: Aphidoidea) are economically important agricultural pests throughout the world. Their economic importance is a result of direct damage caused by feeding on plants (Eastop, 1977; Carter *et al*., 1980; Conti, 1985; Kennedy *et al*., 1962), which can seriously harm shoots, shrink crop size, and reduce yields (Mamontova, 1987; Petrukha *et al*., 1989; Gorbach *et al*., 1989); and indirect damage stemming from their role as vectors of plant viruses (Eastop, 1977; Carter *et al*., 1980; Conti, 1985; Kennedy *et al*., 1962).

The pea aphid, *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae), is an important pest of alfalfa throughout the world (Harper *et al*., 1978). Originally a Palaearctic species, *A. pisum* now has an almost worldwide distribution (Van Emden and Harrington, 2007), and it is one of the major agronomic pests in alfalfa fields in Europe (Bournoville, 1976). With its virtually worldwide distribution, the pea aphid is now a major pest of alfalfa (Bommarco, 1991).

Acyrthosiphon pisum forms colonies on young growth and developing pods of many plants of the family Fabaceae from the tribes Genistae (*Cytisus*, *Genista*, *Sarothamnus*, *Spartium*), Trifolieae (*Medicago*, *Melilotus*, *Ononis*, *Trifolium*, *Trigonella*), Fabeae (*Lathyrus*, *Lens*, *Pisum*, *Vicia*), and Hedysareae (*Hippocrepis*, *Onobrychis*), and it also colonizes a few members of other tribes, e.g., *Lotus* (Loteae) and *Glycine* (Phaseolae) (Van Emden and Harrington, 2007).

Acyrthosiphon pisum is a rather large green or pink aphid with appendages that are long and slender (Figure 1) (Van Emden and Harrington, 2007).

Figure 1. Pea aphid (*Acyrthosiphon pisum*) (foto by Dr. Chun-Che Chang's lab)

Generally, aphids are small soft–bodied insects that feed exclusively on plant phloem sap by inserting their slender mouthparts into sieve elements (Blackman and Eastop, 2000; Morrison and Peairs, 1998; Oerke *et al*., 1994).

Usually, sexual and parthenogenic types of reproduction alternate in the life cycle, with sexual forms typically appearing in autumn to oviposit overwintering eggs on the primary host (Komazaki, 1993). Eggs hatch in spring, and each hatched larva develops into a female that reproduces parthenogenically (Komazaki, 1993). Adult females can be wingless or winged, with the presence of wings indicating a decline in food quality or overcrowding (Broughton, 2007).

The typical annual life cycle of aphids is cyclical parthenogenesis in which several apomictic parthenogenetic (clonal) generations in spring and summer are followed by a single sexual generation in autumn, with overwintering as eggs (Figure 2) (Simon *et al*., 2002).

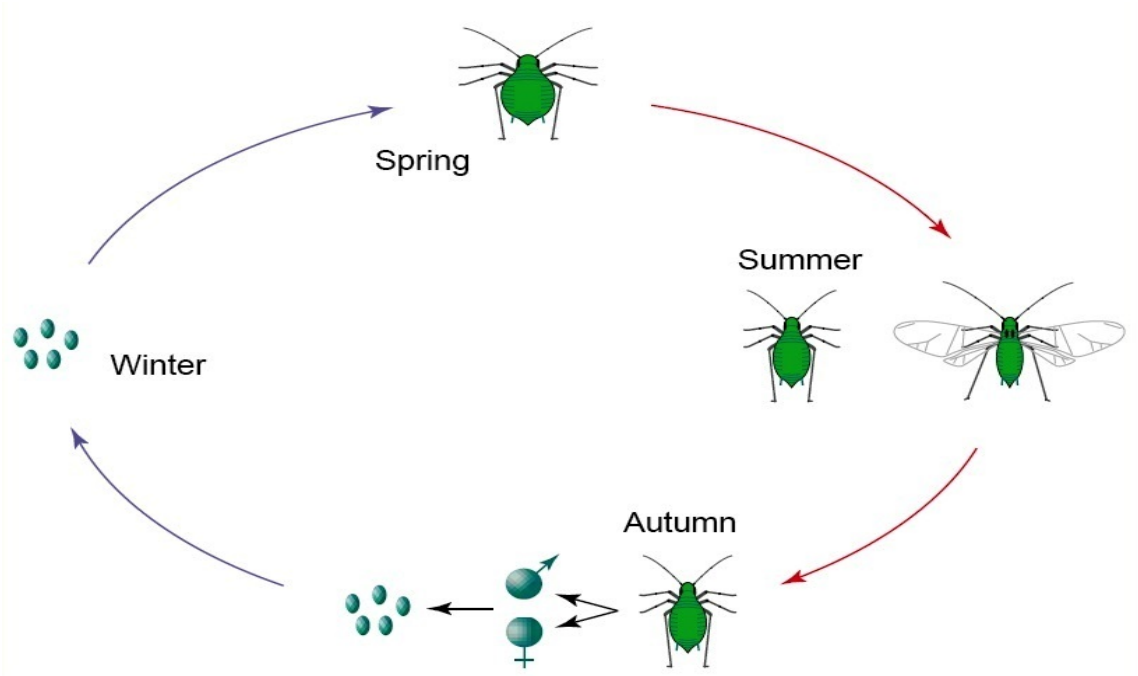


Figure 2. Typical annual life cycle of aphids (Simon *et al*., 2002).

The pea aphid's life cycle is very similar to the typical aphid life cycle (Figure 3). During spring and summer, asexual females of *A. pisum* give birth to clonal offspring (Figure 3, left). The offspring after four larval molts can become wingless or winged asexually reproducing adults. Wingless adults are more common, while winged

individuals are produced in cases of crowding or stress during prenatal stages (Figure 3) (The International Aphid Genomics Consortium, 2010). Following repeated cycles of asexual reproduction, the shorter length of autumn days triggers the production of sexual females and males, which can be winged or wingless in pea aphids, depending on the genotype (Figure 3). After mating, oviparous sexual females deposit overwintering eggs, which hatch in spring to produce wingless asexual females. In some populations in locations without a cold winter, *A. pisum* individuals have continuous cycles of asexual reproduction without sexual and egg–producing periods (The International Aphid Genomics Consortium, 2010).

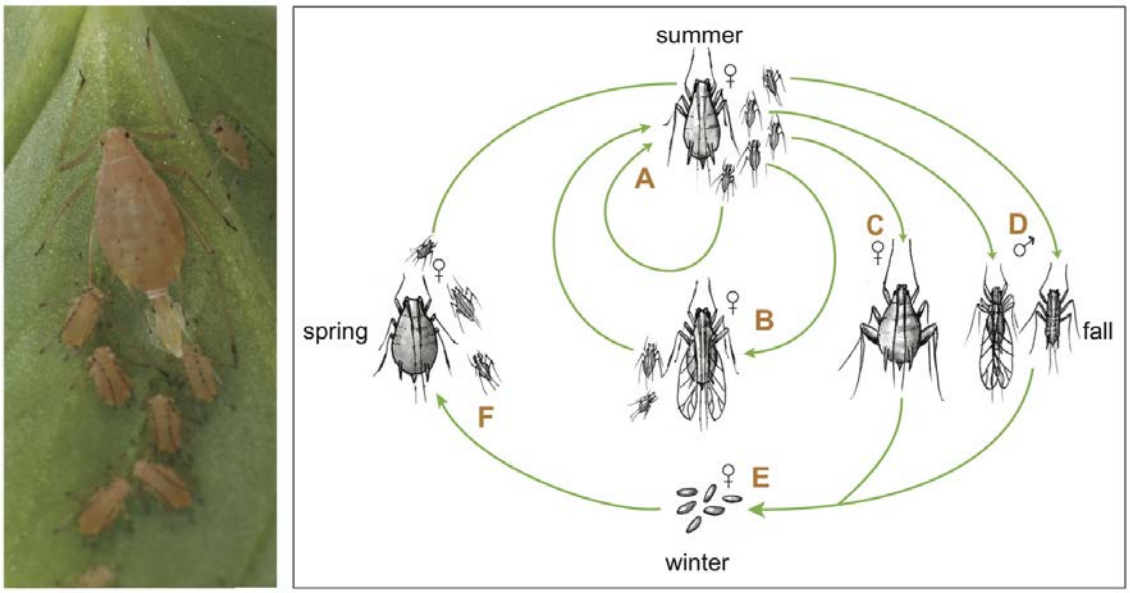


Figure 3. The pea aphid's life cycle: A – wingless asexually reproducing adults, B - winged asexually reproducing adults, C – wingless sexual females, D - males, E - overwintering eggs, F - wingless asexual females. (The International Aphid Genomics Consortium, 2010).

Crop destruction and disease transmission by insects have a notable impact on the human economy and health. Nearly 20% of annual crop production is destroyed by insects (Oerke and Dehne, 2004). Many of the 5,000 aphid species attack agricultural plants and inflict damage both through the direct effects of feeding and indirectly by vectoring debilitating plant viruses. Annual worldwide crop losses due to aphids are estimated at hundreds of millions of dollars (Blackman and Eastop, 2000; Morrison and Peairs, 1998; Oerke *et al*., 1994).

As obligate parasites, plant viruses need to move from infected to healthy plants in order to survive. This is achieved either by mechanical means or, in the case of most plant viruses, by exploiting biological vectors (Van Regenmortel *et al*., 2000). Efficient virus transmission from the host plant to another plant by vectors is very important. Arthropods can transmit most plant viruses, and particularly important vectors are hemipteran insects, which transmit the majority of vectored viruses (55%) (Nault, 1997; Van Emden and Harrington, 2007; Hogenhout *et al*., 2008). Insects are the most common of vectors, and aphids account for the transmission of 50% of all insect–vectored viruses (Brunt *et al*., 1996; Nault, 1997). The list of aphid–borne virus groups are summarized in Table 2 (Raccah and Fereres, 2009).

Table 2. Groups of viruses transmitted by aphids, adapted after Raccah and Fereres (2009).

Virus groups	Mode	Persistance	Presence in vector
Alfamovirus	N	few hours	external
Carlavirus	N	few hours	external
Caulimovirus	N	many hours	external
Cucumovirus	S	few hours	external
Enamovirus	C	weeks	internal
Fabavirus	N	few hours	external
Luteovirus	C	weeks	internal
Polerovirus	C	weeks	internal
Potyvirus	N	few hours	external
Sequivirus	SP	few hours	external

C – circulative, N – nonpersistent, SP - semipersistent

The aphids (Aphididae) are by far the most important family among plant virus vectors, transmitting many more viruses than whiteflies (Aleyrodidae), leafhoppers (Cicadellidae), or planthoppers (Delphacidae) (Figure 4) (Van Emden and Harrington, 2007).

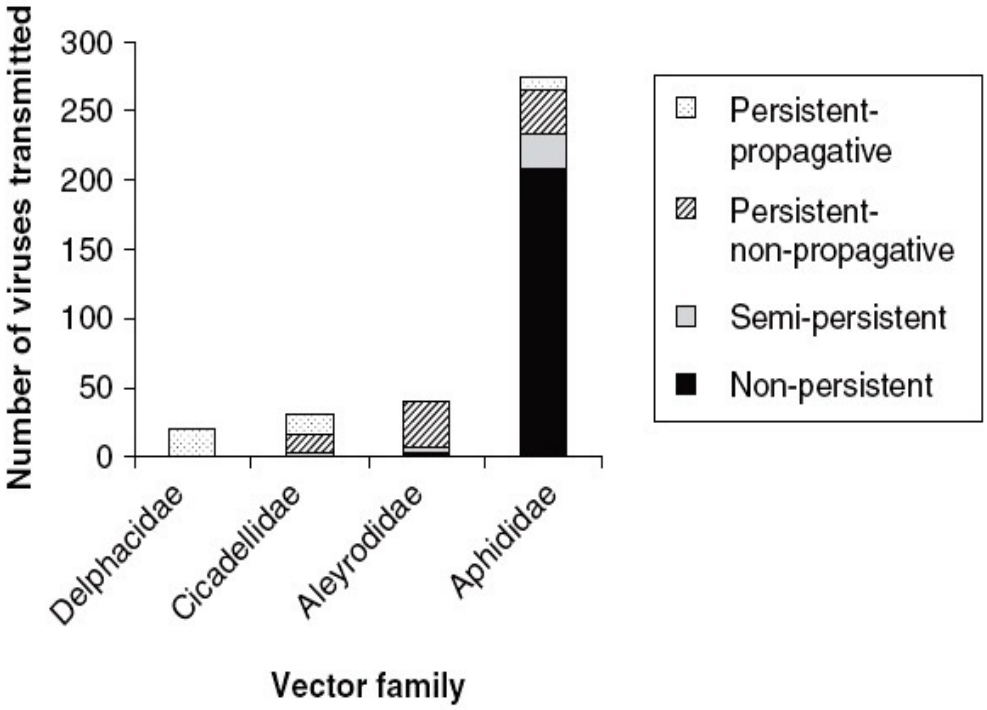


Figure 4. Number of viruses transmitted by the four major hemipteran vector families, divided into four transmission categories (Van Emden and Harrington, 2007).

Pea aphid infestations have been shown to reduce growth and dry–mass yields (Franklin, 1953; Harvey *et al.*, 1971; Kindler *et al.*, 1971; Cuperus *et al*., 1982; Harper and Kaldy, 1982). In addition, *Acyrthosiphon pisum* is a vector of more than 30 disease-causing viruses, including non–persistent viruses of beans, peas, beet, clover, cucurbits, narcissus, and plants of the family Brassicaceae, as well as the persistent viruses pea enation mosaic virus (PEMV) and bean leaf roll virus (BLRV) (Van Emden and Harrington, 2007). Damage from pea aphid infestation and symptoms of viral infection are shown in Figure 5.

Figure 5. Field peas exhibiting symptoms of viral infection (Clement, 2006).

1.2. Aphid parasitoids

Aphids have many natural enemies, including hymenopteran parasitoids, which can play a significant role in reducing aphid populations (Van Emden, 1995; Starý, 1988). Aphid parasitoids are grouped into two subfamilies, the Aphelininae (Hymenoptera: Aphelinidae) and the Aphidiinae (Hymenoptera: Braconidae), the second one including the largest number of species of aphid parasitoids (Mackauer and Starý, 1967). In the case of the subfamily Aphidiinae (Hymenoptera: Braconidae), all species are exclusively solitary endoparasitoids of aphids, and they can have a great impact in control of pest aphids (Starý, 1970, 2006; Adashkevich, 1972; Hågvar and Hofsvang, 1991; Shyiko *et al*., 1991; Kavallieratos *et al*., 2004; Kavallieratos *et al*., 2010).

Aphidiinae are sometimes considered as an independent group within the family Braconidae. Because of their importance as agents for biological pest control, much attention has been paid to this relatively small group (Mackauer and Starý, 1967; Mackauer, 1968; Starý, 1970, 1976, 1979, 1988).

There are approximately 50 genera and 500 species of aphidiine wasps (Braconidae: Aphidiinae) around the world (Mackauer and Starý, 1967; Starý, 1970, 1988; Chow and Mackauer, 1986; Yu *et al.*, 2012). They are small wasps (Figure 6), with an adult size ranging from 1 mm to several mm. They are all solitary endophagous parasitoids with different levels of specialization to aphid hosts (Kavallieratos *et al.*, 2001; Mackauer and Starý, 1967). Most aphidiine wasps can attack a range of instars of a given host, although a few specialize on winged adults (Quicke, 2015).

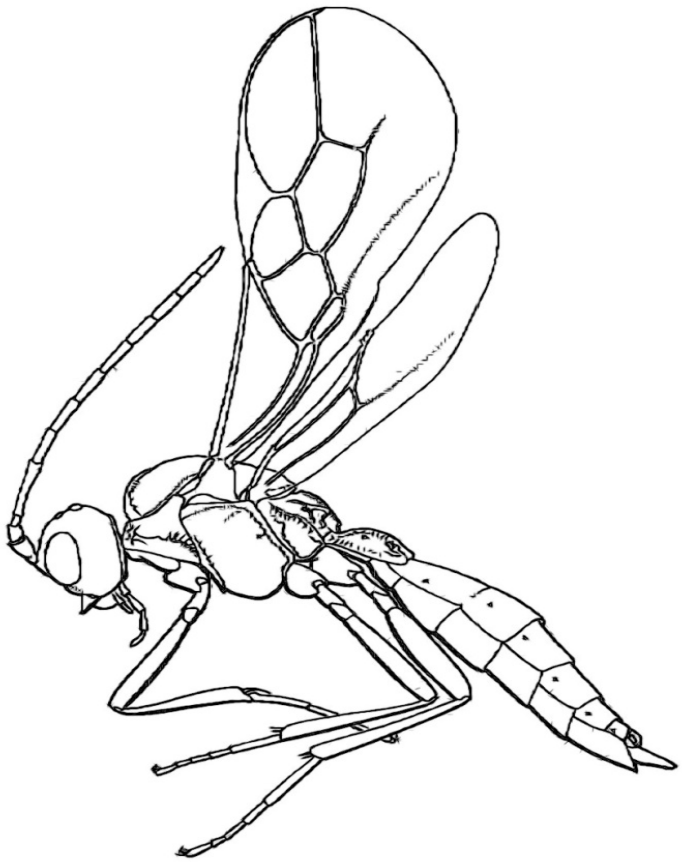


Figure 6. General body plan of Braconidae: Aphidiinae
(Goulet and Huber, 1993).

The taxonomic status and phylogeny of aphidiines are not always clear. Figure 7 shows relationships between the tribes of Aphidiinae recovered from various studies (Quicke, 2015).

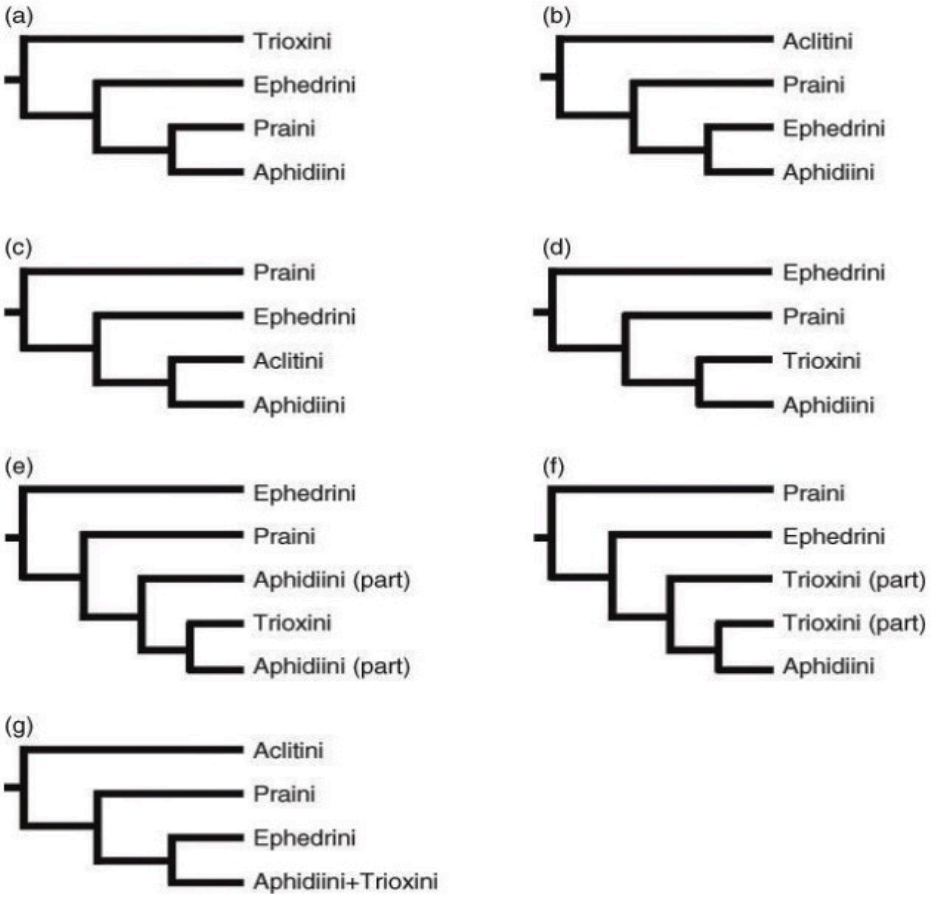


Figure 7. Relationships between the tribes of Aphidiinae recovered from various studies: (a) from Finlayson (1990), based on characters of the final larval instar; (b) from Chou (1984), based on morphology and behaviour; (c) from Tobias (1967) and Edson and Vinson (1979), based on pupation habit and venom apparatus, respectively; (d–g) from Belshaw and Quicke (1997), Sanchis *et al.* (2000), P.T. Smith *et al*. (1999), and Kambhampati *et al*. (2000), respectively, based on various combinations of molecular markers. Shi and Chen's (2005) tree was essentially the same as that of Sanchis *et al*. (2000) (Quicke, 2015).

Aphidiinae, like the majority of Hymenoptera, have a haplodiploidy sex-determination system, which means that females are developed from fertilized eggs, while males develop from unfertilized eggs. Females of most species are monandrous, although males often mate multiple times. Females of several species release sex pheromones that attract males (Quicke, 2015). Adults feed on aphid honeydew and

extrafloral nectaries. The majority of species have several generations per year. Exceptions are *Monoctonia pistaciaecola* and *Pseudopauesia prunicola*, which apparently are obligatorily monovoltine (Halme, 1986; Starý, 1988).

Host finding starts with the selection of a suitable habitat, with food plants of the host aphids playing an important role because the parasitoids are attracted to odours released from aphid–infested plants (Du *et al*., 1998; Powell *et al*., 1998). Aphidiines parasitize all aphid instars except eggs, but oviposition mostly occurs in larval instar II or III (Shaw & Huddleston, 1991).

During oviposition, the female bends its abdomen under the thorax with the tip of the abdomen protruding between the front legs and under the head (Figures 8 and 9). Oviposition is a rather swift process with no specific place on the host's body, the only exception being species of the genus *Monoctonus*, which lay their eggs in the mass of ganglia in the thoraco–abdominal part of the host's body by inserting the ovipositor through the ventral suture of the thorax (Griffiths, 1960, 1961).

Females normally deposit a single egg in an aphid, although superparasitism may occur when unparasitized hosts are scarce or not available (Mackauer, 1990).

Figure 8. *Aphidius ervi* attacking a pea aphid (Zepeda–Paulo *et al.*, 2015).

Figure 9. Female of *Aphidius* sp. in a colony of *Aphis fabae* (Starý *et al*., 2014).

After eclosion from the egg, the larva feeds first on the aphid's haemolymph (Couchman and King, 1977; Van Emden and Harrington, 2007), but later on other tissues, which leads to the aphid's death (Polaszek, 1986; Van Emden and Harrington, 2007). The number of larval instars is unclear because there are different data in the literature. Various authors stated three, four, or five instars (Pennacchio and Digilio, 1990; Hoek, 1971; Quicke, 2015).

Whatever the number of instars, the last larval instar gains mandibles and begins to feed on tissues and organs of the host, starting from the reproductive system and other nonvital organs. Thus, the host lives almost until the parasitoid's pupation. Aphidiinae larvae attach the host's exoskeleton to the plant and spin their cocoon inside (most species) (Figure 10) or under the host's exoskeleton (some species of the tribe Praini) (Figure 11). In this stage, the chitinous shell of the host is called the 'mummy' (Starý *et al*., 2014).

Figure 10. Colony of *Aphis nerii* with mummies made by *Lysiphlebus testaceipes* (photo by A. Petrović)

Figure 11. Aphid mummies made by *Praon* sp. (Photo by A. Petrović).

The morphology and anatomy of representatives of the subfamily Aphidiinae are shown in Figure 12. The head has a transverse to somewhat sub–square shape and a hypognathous position (Figure 12a). There are two large compound eyes and three ocelli (Figure 12b and 12c). On the head is positioned a pair of antennae. As in other insects, the antenna is built of a base (scapus), a stem (pedicel) (Figure 12d), and a flagellum. The flagellum consists of from eight flagellomeres (females of the species *Lysiphlebus balcanicus* Starý) to up to 30 flagellomeres (in some species of the genus *Pauesia*). In most species, males have more flagellar segments than females. The only exceptions are species of the genus *Ephedrus*, where both males and females have nine segments (Gardenfors, 1986). The clypeus is concave and covered with few or over 20 hairs. The number and position of the clypeus hairs represent an important taxonomical character. Along both sides of the clypeus are positioned two tentorial pits, one on each side. The ratio of the distance between the tentorial pit and the eye margin to that between the two tentorial pits represents the tentorial index, which is also used for identification of some species (Starý, 1973, 1981).

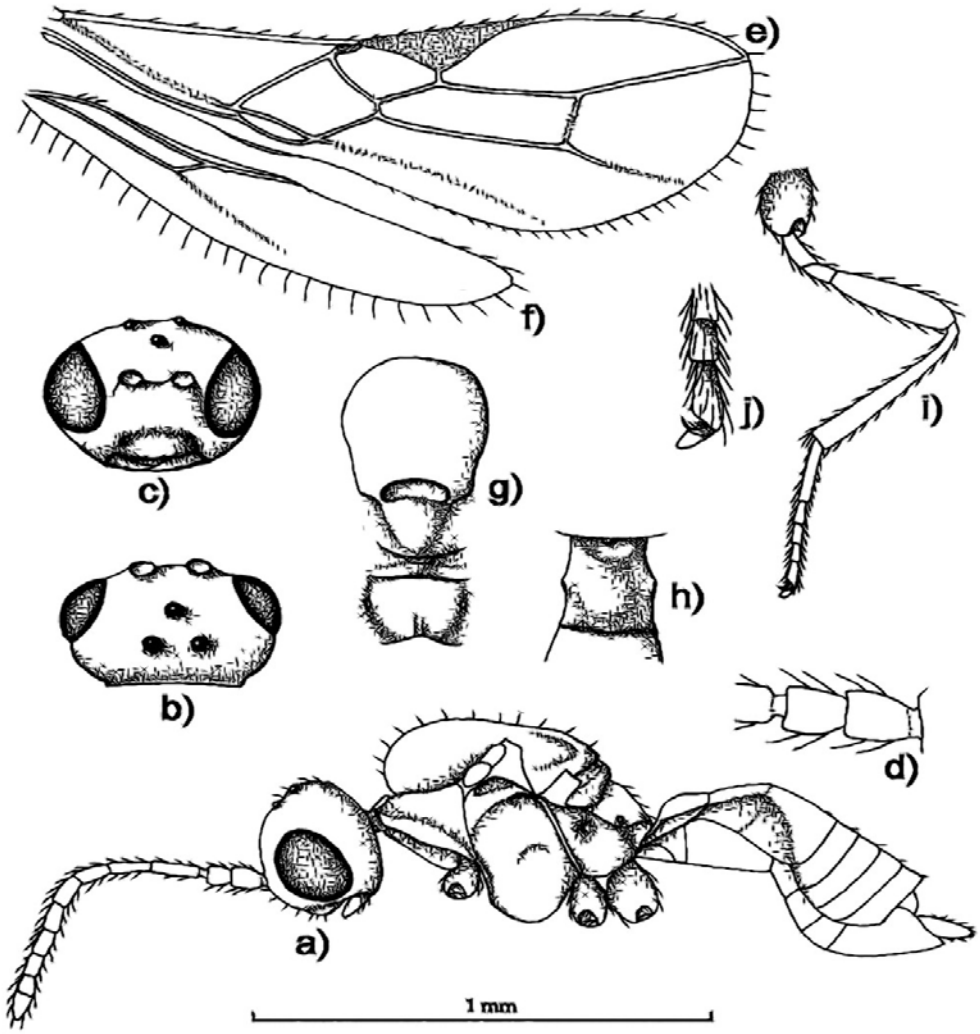


Figure 12. General body plan of Aphidiinae (Goulet and Huber, 1993)

Mouthparts are adapted for sipping, with bidentate mandibles and a variable number of labial and maxillar segments. The thorax is very stiff and compact. The pronotum is usually smooth or sculptured. The mesonotum is mostly smooth, but sometimes can be slightly granulated, covered with hairs. The mesoscutum can be smooth or with grooves and/or with one pit (fovea) (Figure 12g). The propodeum represents one of the most important taxonomic characters (Starý, 1973). It has various sculptures (grooves and ridges) and a variable number and position of hairs. It is usually

divided by sutures into a small number of surfaces with different shape and size. Most species of this subfamily have two pairs of wings (Figure 12e and 12f). Very few species are apterous (without wings) or brachipterous (with rudimentary wings). *Diaeretellus svalbardicum* and females of the species *Autriquella aptera* (Starý) and *Trioxys apterus* are known to be brachipterous or micropterous, whereas females of *Diaeretellus ephippium* are the only apterous forms.

In the subfamily Aphidiinae, there is a trend toward wing nerve reduction. For instance, species of the genus *Ephedrus* have typically braconoid wing venation, which is very similar to venation of species from the subfamily Euphorinae, while species of the genera *Trioxys*, *Binodoxys*, and *Lipolexis* have almost entirely reduced wing venation. Legs are well developed, long, and slender (Figures 12i and 12j). Females have a lancet-shaped abdomen, while males have a more round abdomen shape. The second and third abdominal segments are fused together, but in contrast to other braconids, there is a flexible suture between the two segments (Starý, 1970; Sharkey, 1993). All other metasomal segments are connected by membranes.

The genital apparatus is located on the end of the abdomen. The female genitalia are built out of the eighth and the ninth abdominal segment. They consist of a quadrate plate; valvifera I and II; and the first, second, and third valvulae. All parts have uniform structure among the genera of aphidiines, with the exception of the third valvula. Depending on the genera, the third valvulas are broad and short or narrow and elongated (Starý, 1976). The male genitalia are composed of parts of the ninth abdominal segment and aedeagus.

Aphid parasitoids (Hymenoptera: Braconidae and Aphelinidae) have been used in biological control and integrated pest management (IPM) programs much more often than other natural enemies of aphids because they prey exclusively on aphids. Many parasitoid species are oligophagous or polyphagous and will attack a wide range of species (Van Emden and Harrington, 2007). Several parasitoid species are produced commercially as biocontrol agents, particularly for use in greenhouses, but several species have also been used in classical introductions to control major aphid pests of outdoor crops (Table 3) (Van Emden and Harrington, 2007).

Table 3. Successful introductions of Aphidiinae parasitoids for biological control of aphids (adapted after Van Emden and Harrington, 2007).

Parasitoid	Aphid	Crop	Origin	Introduced
Aphidius colemani	*Diuraphis noxia*	Cereals	Chile	Czech Republic
			Eurasia, Morocco	USA
	Pentalonia nigronervosa	Banana	Australia	Tonga
Aphidius eadyi	*Acyrthosiphon pisum*	Lucerne;peas	USA, Canada	Australia,New Zealand
Aphidius ervi	*Acyrthosiphon pisum*	Lucerne;peas	India, Europe	USA, Canada
	A. pisum, A. kondoi	Legumes	Europe	Argentina
	Sitobion avenae	Cereals	France, Iran	Chile
	Acyrthosiphon kondoi	Lucerne	Europe	Australia,New Zealand
Aphidius matricariae	*Range of species*		France	Brazil, Chile
Aphidius pisivorus	*Acyrthosiphon pisum*	Lucerne;peas	USA, Canada	Australia,New Zealand
Aphidius rhopalosiphi	*Metopolophium dirhodum*	Cereals	France, Iran	Chile
			England, France	New Zealand
Aphidius salicis	*Cavariella aegopodii*	Carrot	USA	Australia
Aphidius smithi	*Acyrthosiphon pisum*	Lucerne;peas	India, Europe	USA, Canada
		Legumes	USA	Chile
		Lucerne;peas	USA, Canada	Australia,New Zealand
Aphidius sonchi	*Hyperomyzus lactucae*	Lettuce	Mediterranean, Japan	Australia
Aphidius uzbekistanicus	*Metopolophium dirhodum*	Cereals	France, Iran	Chile
Binodoxys indicus	*Aphis craccivora*	Lupin	India	Australia
Diaeretiella rapae	*Diuraphis noxia*	Cereals	Czech Republic	USA
Ephedrus plagiator	*Acyrthosiphon kondoi*	Lucerne	Europe	Australia,New Zealand
Lysiphlebus testaceipes	*Toxoptera aurantii*	Citrus	Cuba	France
	Schizaphis graminum	Cereals	USA	Chile
	Aphis craccivora	Beans	USA	Australia
	Schizaphis graminum	Cereals	USA	Argentina
Pauesia bicolor	*Cinara cronartii*	Pine trees	USA	S. Africa, Kenya,Malawi
Pauesia cedrobii	*Cinara laportei*	Cedar trees	Morocco	France
Praon barbatum	*Acyrthosiphon kondoi*	Lucerne	Europe	Australia,New Zealand
Praon exsoletum	*Therioaphis trifolii*	Lucerne	Middle East, Europe	USA
			USA, Iran, Cyprus, Pakistan, France	Australia
Praon gallicum	*Metopolophium dirhodum*	Cereals	France, Iran	Chile
Praon volucre	*Metopolophium dirhodum*	Cereals	France, Iran	Chile
	Hyperomyzus lactucae	Lettuce	Mediterranean	Australia
Trioxys complanatus	*Therioaphis trifolii*	Lucerne	Middle East, Europe	USA
			USA, Iran, Cyprus, Pakistan, France	Australia
Trioxys curvicaudus	*Eucallipterus tiliae*	trees	Europe	USA
Trioxys pallidus	*Chromaphis juglandicola*	Walnut	France, Iran	USA
	Myzocallis coryli	Hazel	Europe	USA
Trioxys tenuicaudus	*Tinocallis platani*	trees	Europe	USA

More than 20 Aphidiinae species have been deliberately released to help control exotic pests in classical biological control programs (Hågvar and Hofsvang, 1991) throughout the world (Table 3) (Carver, 1989; Hughes, 1989). The rate of success of those programs is approximately 20% (Hirose, 2006).

Several aphidiine species are commercially produced to control pest aphids in greenhouses (e.g., *Aphidius ervi*, *A. colemani*, *Praon volucre*, *Ephedrus plagiator*) (Van Lenteren, 2012).

1.3. The *Aphidius eadyi* species complex

Species belonging to the *Aphidius eadyi* species group are among those most commonly used in biological control programs against *Acyrthosphon pisum*. The *Aphidius eadyi* species group can also be treated as a subgroup within the *Aphidius urticae sensu lato* group (Eady, 1969; Starý, 1979) and is defined as a group of species with costulate anterolateral area of the petiole which parasitize *Acyrthosiphon pisum*. It consists of three species: *Aphidius smithi* Sharma & Subba Rao, 1959; *Aphidius eadyi* Starý, González & Hall; and *Aphidius banksae* Kittel (= *A. staryi* sens. auct. - Kittel 2016).

The first described species from this group was *Aphidius smithi*. It was identified as one of the main factors responsible for natural control of the pea aphid in India (Hagen and Shlinger, 1960) and was introduced to California even prior to its description. After just one year, it became well established and accomplished considerable control of pea aphid (Hagen and Shlinger, 1960). Mass releases of *A. smithi* continued in the USA and Canada during the 1960's and 1970's. Also, there were a few experimental releases of *A. smithi* in Poland (Wi¥ckowski, 1962), the Czech Republic (Starý, 1970, 1974) and Moldavia (Starý, 1974). Contrary to the situation in North America, introduction in Central Europe was unsuccessful (Starý, 1974). Further efforts to find additional biocontrol agents (BCA) against *A. pisum* as well as *A. kondoi* Suhnji resulted in description of the species *Aphidius eadyi*, which is widely distributed throughout Europe as far as Western Siberia and also in Central Asia and North Africa (Starý *et al.*, 1980). In the same paper, Starý *et al.* (1980) concluded that most parasitoid specimens attacking pea aphid identified as *A. urticae* Haliday or members of the *A.*

urticae group were actually *A. eadyi*, which somewhat clarified the problem of *A. urticae* (Starý *et al.*, 1980). Soon after its description, *A. eadyi* was introduced as a BCA in New Zealand and Burundi (Cameron *et al.*, 1981; Autrique *et al.*, 1989; Cameron & Walker, 1989), where it established stable populations and also reduced pea aphid populations (Cameron *et al.*, 1981). The last species from the *A. eadyi* group to be described was *A. banksae*. It was first described as *Aphidius staryi* Chen & Luhman (Chen *et al.*, 1990). However, it turned out that *A. staryi* Chen & Luhman is a primary junior homonym of *Aphidius staryi* Das & Chakrabarti described in the same year (Das & Chakrabarti, 1990), and in 2016 its name was changed to *A. banksae* (Kittel, 2016). The discovery of *A. banksae* was a result of research projects on biological control of the pea aphid in North America. It was initially introduced to the USA as *A. smithi* from Israel and Turkey *(González et al*. 1995), but it was later shown that those specimens differ from other *A. smithi* specimens in morphology, biology, and isozyme patterns (Unruh *et al.*, 1989; Chen *et al.*, 1990). After its description, the species was mentioned only three times in the literature: 1) it was listed as a member of the aphidiine fauna from Bulgaria (Atanassova, 1997); 2) Atanassova *et al.*, (1998) determined the possible existence of a cryptic species which resembles *A. eadyi* based on isozyme patterns, but stated that it is unlikely that *A. banksae* can be distributed in Bulgaria; and 3) Akar & Çetin Erdoğan (2017) listed *A. banksae* as a member of the aphidiine fauna from Turkey.

2. OBJECTIVES

Species belonging to the *Aphidius eadyi* group are among the most important natural enemies of the pea aphid, Acyrthosiphon *pisum*, and also the blue alfalfa aphid, *A. kondoi*, which has been designated as a species possibly invasive in Europe and potentially a future pest. Defining the taxonomic status of these parasitoids is crucial to any fundamental or applied research on economically important aphid species. It is necessary to determine which of the listed species are found in Europe, and also determine phylogenetic relationships between them. Accordingly, we used samples from across the ranges of the species in question to achieve the following major objectives:

• To determine the taxonomic status of the species *A. eadyi, A. banksae*, and *A. smithi* and resolve their phylogenetic relationships;

• To evaluate morphological characters significant for species identification;

• To obtain a molecular characterization and analyse morphological variability of *A. eadyi, A. banksae*, and *A. smithi*.

• To detect the presence and distribution of *A.banksae* and *A. smithi* in Europe and determine potential routes of their introduction.

3. MATERIAL AND METHODS

3.1. Parasitoid spectrum of *Acyrthosiphon pisum*

In order to identified presence and distribution of *Aphidius eadyi* species group, we performed detailed literature survey, as well as examination of aphid parasitoid collections from University of Belgrade - Faculty of Biology and collection of Dr Petr Starý - Laboratory of Aphidology, Institute of Entomology, Academy of Sciences of the Czech Republic. Additionally, all parasitoids of *Acyrthosiphon pisum* in Europe are identified by critical use of following references: Van den Bosch (1957), Starý (1974), Bańkowska *et al*. (1975), Kierych (1975), Aeschlimann (1981), Tomanović *et al*. (1996), Atanassova *et al*. (1998), Kavallieratos *et al*. (2001), Tomanović & Brajković (2001), Tomanović & Kavallieratos (2002), Ölmez & Ulusoy (2003), Tomanović *et al*. (2003a, 2003b), Aslan *et al*. (2004), Uysal *et al*. (2004), Starý & Havelka (2008), Kos *et al*. (2009), Tomanović *et al*. (2009), Pons *et al*. (2011), Ferrer-Suay *et al*. (2013), Kaliuzhna & Zubenko (2013) and Zubenko (2014). We used only data where both plant and aphid species were known.

Adult Aphidiinae parasitoids were dissected and slide-mounted for detailed examination (dissection protocol explained later). External morphology was studied using a LEICA DMLB (Leica Mycrosystems, Wetzlar, Germany), a ZEISS Discovery V8 (Carl Zeiss MicroImaging GmbH, Göttingen, Germany), or an Olympus SZX9 (Olympus Corporation, Tokyo, Japan) stereomicroscope. An identification key for parasitoids parasitizing *Acyrthosiphon pisum* in Europe is constructed based on the measurements taken from slide-mounted specimens using an ocular micrometer. Several specimens were gold-coated with sputter coaters and examined using JSM 6460 LV, JSM 6390 or JSM 6360 (JEOL, Tokyo, Japan) scanning electron microscopes.

The following characters were used for construction of the identification key: number of antennomeres (numbers in parentheses in the key indicate character states which are not common); number of cells in the forewing; length of the forewing stigma; length of the forewing R1 (metacarpus); existence and development of forewing 3RSbr & RS, m-cu, and RS + M, veins; setation of the face; sculpture of the propodeum, sculpture the petiole; shape of the ovipositor sheath; colour of mummy; place of pupation.

Morphological terminology of parasitoids follows Sharkey & Wharton (1997).

3.2. Collection and preparation of parasitoids belonging to *Aphidius eadyi* species group

Furthermore, we analyzed parasitoid specimens belonging to *Aphidius eadyi*, *Aphidius smithi* and *Aphidius banksae* collected over the period 1976–2012. The collection was performed using standard methods. All specimens were obtained by rearing. Some of the specimens were obtained from field sampling of plant parts infested by both live and mummified aphids and reared under laboratory conditions until emergence of parasitoids. Insect material was collected in the field and placed into plastic containers covered with nylon mesh. Caged samples were held at 22.5 °C, 65% relative humidity, 16:8 L:D photoperiod for three weeks (Kavallieratos *et al*., 2001). Plant samples were collected as herbarium specimens for later identification. Few aphids from every sample were preserved in solution containing two parts of 90% ethyl–alcohol and one part of 75% lactic acid (Eastop and Van Emden, 1972).
Other specimens were collected by Prof. Dan Gonzalez during his field trips in Asia and reared in insectaries for programs of biological control of alfalfa aphids in the USA.

In order to measure and count selected characters microscope slides were made using Canada balsam or Swann solution. Regardless of medium following procedure of microdissection was applied:

- Forewings were removed by fine forceps and needle and then submerged in 70% ethanol.
- The rest of the body was submerged in 10% KOH for 30 minutes and afterward boiled in 10% KOH for 6 minutes
- The following body parts were removed and placed in 70% ethanol: antennae, head, mesoscutum, propodeum, petiole and genitals. (Figure 13)
- After dissection body parts were dehydrated in series of ethanol solutions of ascending concentrations: 80%, 96% and 99% ethanol (10 minutes in each)
- Dehydrated body parts were then mounted on microscope slides in a drop of Canada balsam or Swann solution.
- After 24–48 h more medium is applied and covered.
- Prepared slides had been dried for 30 days at 36 °C.

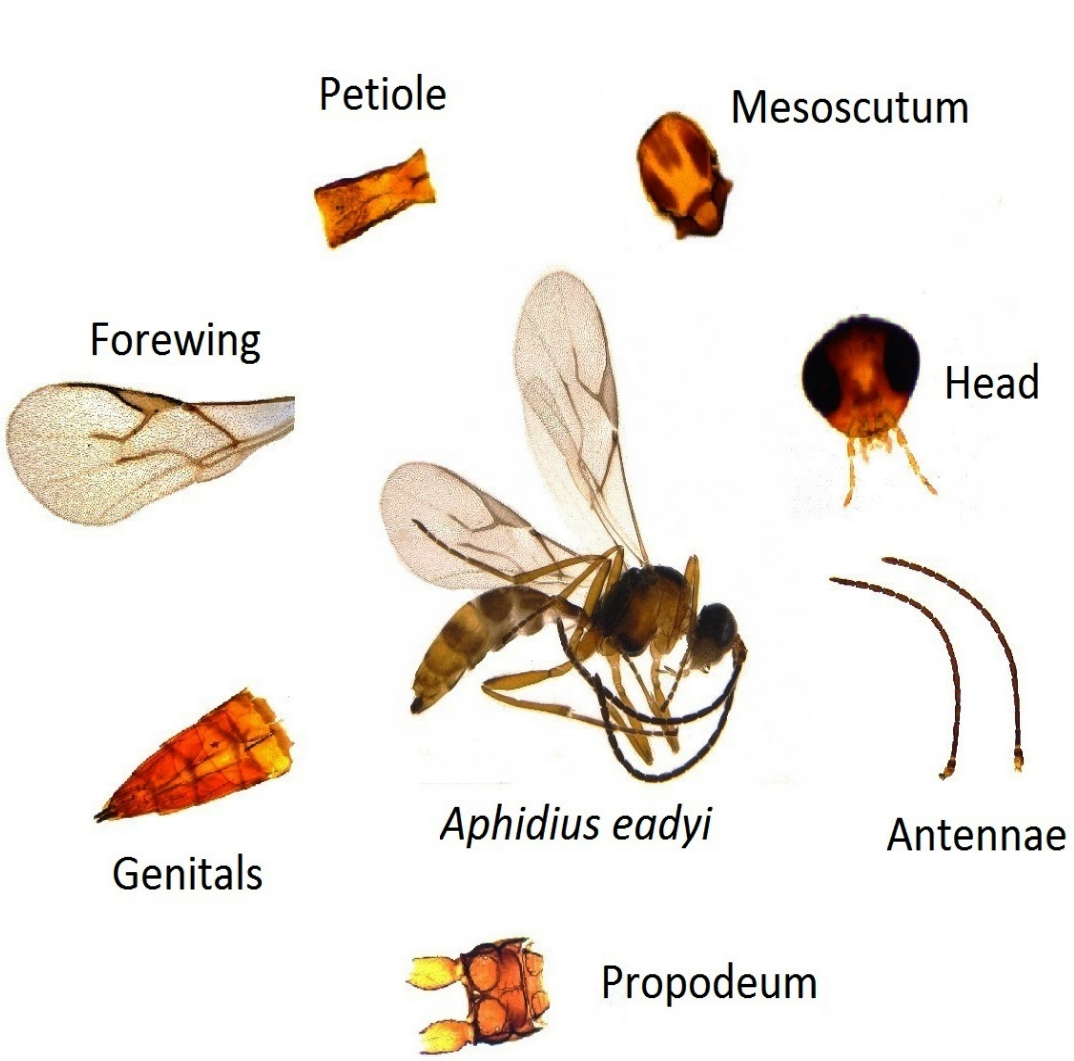


Figure 13. Body parts of *Aphidius eadyi* that are separated during dissection.

3.3. Molecular analyses

3.3.1. Material used in molecular analyses

A total of 51 specimens belonging to the *Aphidius eadyi* group were used for molecular analyses. The parasitoid specimens belonging to *A. eadyi* (14 specimens), *A. banksae* (29 specimens) and *A. smithi* (8 specimens) were collected from 16 countries: Afghanistan (AF), Czech Republic (CZ), India (IN), Iran (IR), Israel (IS), Serbia (SE), Spain (SP), USA (US), Uzbekistan (UZ), Turkey (TU), Slovenia (SLO), Montenegro

(MO), Brazil (BRA), France (FRA), England (GBR) and Belgium (BEL) on four continents (Europe, Asia, North America and South America). (Table 4.).

Table 4. List of specimens belonging to *Aphidius eadyi* group submitted to molecular analysis. All specimens were reared from *Acyrthosiphon pisum*.

Parasitoid	Code	Country	Year	Plant	Haplotype	Acc. Number
A. smithi	AE TU16	Turkey	1984	*Medicago sativa*	Asmit1	MG987145
A. smithi	AE BR11	Brasil	1989	*Medicago sativa*	Asmit2	MG987146
A. smithi	AE SP13	Spain	1981	*Medicago sativa*	Asmit3	MG987147
A. smithi	AE AF07	Afghanistan	/	*Medicago sativa*	Asmit4	MG987148
A. smithi	AE UZ15	Uzbekistan	1976	*Medicago sativa*	Asmit5	MG987149
A. smithi	AE US06	United States	1977	*Medicago sativa*	Asmit6	MG987150
A. smithi	AE IN10	India	1978	*Medicago sativa*	Asmit7	MG987151
A. smithi	AE IN19	India [*]	1982	*Medicago sativa*	Asmit8	MG987152
A. eadyi	AE2/2[a]	Serbia	2012	*Medicago sativa*	Aeady1	MG987153
A. eadyi	AE2/3 [a]	Serbia	2012	*Medicago sativa*	Aeady1	MG987153
A. eadyi	AE1/1[b]	Serbia	2012	*Medicago sativa*	Aeady1	MG987153
A. eadyi	AE1/3 [b]	Serbia	2012	*Medicago sativa*	Aeady1	MG987153
A. eadyi	1AE1/2	Serbia	2011	*Medicago sativa*	Aeady1	MG987153
A. eadyi	S11/610	Serbia	2012	*Medicago sativa*	Aeady1	MG987153
A. eadyi	SI08/26_2[c]	Slovenia	2008	*Medicago sativa*	Aeady1	MG987153
A. eadyi	SL08/06	Slovenia	2008	*Pisum sativum*	Aeady1	MG987153
A. eadyi	/	France	2009	/	Aeady1	JN620550[#]
A. eadyi	SI08/12	Slovenia	2008	*Pisum sativum*	Aeady2	MG987154
A. eadyi	AE CZ14	Czech Republic	1982	*Medicago sativa*	Aeady3	MG987155
A. eadyi	AE CZ12	Czech Republic	1984	*Medicago sativa*	Aeady4	MG987156
A. eadyi	AE IR09	Iran[*]	1977	*Medicago sativa*	Aeady5	MG987157
A. eadyi	AE CZ21	Czech Republic [*]	/	*Medicago sativa*	Aeady6	MG987158
A. banksae	AE1/2 [b]	Serbia	2012	*Medicago sativa*	Abank1	MG987159
A. banksae	BE14/496	Belgium	2014	*Lotus corniculatus*	Abank2	MG987160
A. banksae	AE 2/1 [a]	Serbia	2012	*Medicago sativa*	Abank3	MG987161
A. banksae	S11/672	Montenegro	2011	*Vicia cracca*	Abank3	MG987161
A. banksae	AE3/2[d]	Serbia	2012	*Medicago sativa*	Abank3	MG987161
A. banksae	S11/316	Serbia	2011	*Lotus corniculatus*	Abank3	MG987161
A. banksae	SI08/26_1[c]	Slovenia	2008	*Medicago sativa*	Abank3	MG987161
A. banksae	AuS3	Slovenia	2008	*Medicago sativa*	Abank3	MG987161
A. banksae	/	United Kingdom	/	/	Abank4	MG987162
A. banksae	BE154	Belgium	2014	*Trifolium* sp.	Abank5	MG987163
A. banksae	BE14/171	Belgium	2014	*Trifolium repens*	Abank6	MG987164
A. banksae	/	United Kingdom	/	*Pisum sativum*	Abank6	KP983663[#]
A. banksae	/	United Kingdom	/	*Pisum sativum*	Abank6	KP983664[#]

A. banksae	/	United Kingdom	/	*Pisum sativum*	Abank6	KP983665[#]
A. banksae	/	France	/	*Vicia faba*	Abank6	KP983656[#]
A. banksae	/	France	/	*Vicia faba*	Abank6	KP983657[#]
A. banksae	/	France	/	*Trifolium* sp.	Abank6	KP983658[#]
A. banksae	/	France	/	*Trifolium* sp.	Abank6	KP983659[#]
A. banksae	AE IS 05	Israel[*]	1979	*Medicago sativa*	Abank7	MG987165
A. banksae	AE3/1 [d]	Serbia	2012	*Medicago sativa*	Abank8	MG987166
A. banksae	1AE 2/1	Serbia	2010	*Medicago sativa*	Abank9	MG987167
A. banksae	S11/672	Montenegro	2011	*Vicia cracca*	Abank9	MG987167
A. banksae	AE 4/2	Serbia	2012	*Medicago sativa*	Abank9	MG987167
A. banksae	S11/672	Montenegro	2011	*Vicia cracca*	Abank9	MG987167
A. banksae	S11/316	Serbia	2011	*Lotus corniculatus*	Abank9	MG987167
A. banksae	S11/233	Montenegro	2011	*Vicia cracca*	Abank10	MG987168
A. banksae	1AE 2/2	Serbia	2010	*Medicago sativa*	Abank11	MG987169
A. banksae	AE3/3 [d]	Serbia	2012	*Medicago sativa*	Abank11	MG987169
A. banksae	AE IS 18	Israel[*]	/	*Medicago sativa*	Abank12	MG987170

[*] -origin of populations reared in insectaries at the University of California, Riverside, CA, USA; [a], [b], [c], [d] - specimens designated with same sign are reared from same aphid population - sample; [#]- sequences retrieved from GenBank

3.3.2. DNA extraction, PCR amplification, and sequencing

DNA was extracted from each individual wasp using the KAPA Express Extract kit (Kapa Biosystems, Inc. Boston, USA) or the Dneasy® Blood & Tissue Kit (Qiagen Inc., Valencia, CA) following the manufacturer's instructions. Mitochondrial marker used for the species delineation was the barcoding region of the cytochrome c oxidase subunit I gene (COI mtDNA). DNA extracted from recently collected specimens was amplified using the standard barcoding primers

LCO1490 (5' GGTCAACAAATCATAAAGATATTGG 3') and

HCO2198 (5' TAAACTTCAGGCTGACCAAAAAATCA 3') (Folmer *et al.*, 1994). In order to retrieve the COI mtDNA from specimens collected few decades ago, a set of degenerative primers was used to amplify the short overlapping fragments:

Aph1Rd (5' GRGGRAAAGCYATATCAGGAG 3'),

Aph2Fd (5' ATAATTGGWGGATTTGGWAATTG 3'),

Aph2Rd (5' GTWCTAATAAAATTAATWGCWCC 3'), and

Aph3Fd (5' CATTTAGCWGGDATTTCYTC 3') (Jamhour 2017; Mitrović & Tomanović 2018) (Figure 14).

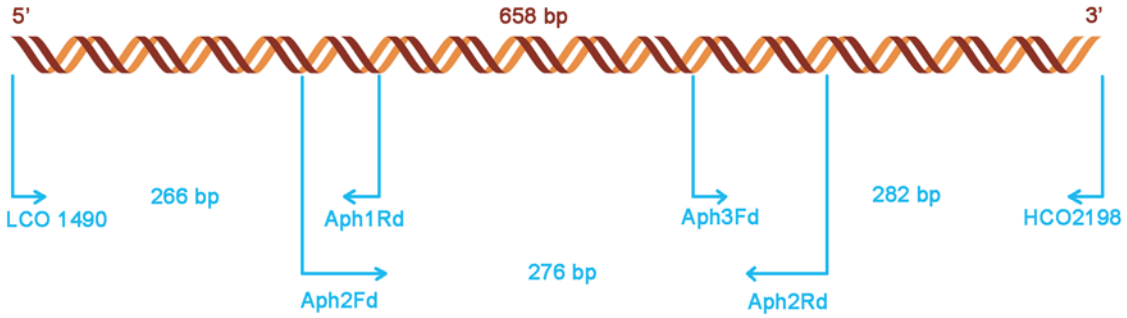


Figure 14. Scheme of positions for internal degenerative primers within the barcoding region of COI mtDNA. Arrows refer to the primers direction, forward or reverse. The length of amplified short fragments are designated between the primer pairs (modified after Jamhour 2017 and Mitrović & Tomanović, 2018).

DNA amplification was performed in a final volume of 20µl. The reaction mixture contained:

- 1µl of the extracted DNA as the template,
- 11.8 µl H_20
- 2 µl High Yield Reaction Buffer A with 1xMg
- 1.8 µl of $MgCl_2$ (final concentration: 2.25 mM)
- 1.2 µl of dNTP (final concentration: 0.6 mM)
- 1µl LCO1490 (final concentration: 0.5 µM)
- 1µl HCO2198 (final concentration: 0.5 µM)
- 0.2 µl DNA polymerase (final concentration: 0.05U/µl).

All PCR reactions were conducted in an Eppendorf Mastercycler® (Hamburg, Germany) ® using the following thermal profile (Petrović *et al*. 2013):

Initial denaturation at 95°C for 5 min,

I 1 min at 94°C
II 1 min at 54°C } 35 cycles
III 30 sec at 72°C

Final extension at 72°C for 7 min.

Amplification of mtCOI short fragments were performed using following protocol by Jamhour (2017) and Mitrović & Tomanović (2018):

Initial denaturation at 95°C for 5 min,

I 1 min at 95°C
II 1 min at 54°C
III 30 sec at 72°C
} 37 cycles

Final extension at 72°C for 7 min.

Amplified products were run on 1% agarose gel, stained with Midori green and visualized under a UV transiluminator. The PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. DNA sequencing was performed by Macrogen Inc. (Seoul, Korea). All barcoding products amplified with the LCO1490/HCO2198 primer pair were sequenced using the forward primer LCO1490. Products obtained with designed degenerative primers were sequenced with combination of forward and reverse primers for each part of the barcoding region (for/rev combinations were as follows: LCO1490/ Aph1Rd; Aph2Fd/ Aph2Rd; Aph3Fd/ HCO2198). Short fragments of barcodes were aligned and concatenated to complete sequences for further analyses.

3.3.3. Genetic analysis

Sequence editing was performed using FinchTV (www.geospiza.com). Sequence alignment was performed using CLUSTALW (Thompson *et al*. 1994) integrated in MEGA6 software (Tamura *et al*., 2013). Kimura's two-parameter method (K2P) of base substitution (Kimura, 1980) was used to calculate average genetic distances between sequences within each group and between the groups. Three different methods were used to reconstruct phylogenetic relationships: maximum likelihood (ML), maximum parsimony (MP), and neighbour joining (NJ). All analyses were performed using MEGA6 software. For all methods, 1000 bootstrap replicates were performed to assess the branch support. In the case of ML phylogenetic reconstruction, Hasegawa-Kishino-Yano model (Hasegawa *et al*., 1985) with in-variant sites (HKY+I) was identified as the best-fitting model of sequence evolution based on the Bayesian Information Criterion

and Akaike Information Criterion corrected (Nei & Kumar, 2000). Identification of best-fitting model of sequence evolution was determined by Modeltest (Posada & Crandall, 1998). The sequence of *Areopraon chaitophori* (GenBank Acc. No. KC128679) was used as an outgroup for phylogenetic analyses. An *A. banksae* haplotype network based on statistical parsimony with a confidence limit of 95% was created using the TCS program, ver. 1.21 (Clement *et al*., 2000). Same program was used for construction of haplotype networks for *A. smithi* and *A. eadyi* with a confidence limit of 90%.

Two different methods of DNA taxonomy were used to identify species/ entities from COI sequence data:

1) Poisson Tree Process (PTP) was developed by Zhang *et al*. (2013) as a tool for delimiting species/ entities in single-locus molecular phylogenies. It identified genetic clusters representing independently evolving entities, optimizing differences in branching patterns within and between taxa (Zhang *et al*. 2013). PTP was applied on MP tree using its online tool (http://species.h-its.org/ptp/) with default settings.

2) Automatic Barcode Gap Discovery (ABGD) tests the existence of a barcode gap in genetic distances and then identifies species as groups of individuals united by shorter genetic distances than the gap (Puillandre *et al*. 2012). Groups identified like this were considered to be equivalent to species (Puillandre *et al*. 2012). ABGD was used to test all previous methods including PTP which could overestimate the number of recognized speciesn in data sets with uneven sampling of individuals per species. ABGD was applied on COI alignment through its online tool (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html) using the Kimura two-parameter model of pairwise distances (Kimura 1980).

3.4. Morphometric analysis

A total of 233 females were used for morphometric analysis and were collected from 13 different localities from 9 countries: Serbia (SE), Afghanistan (AF), Czech Republic (CZ), Iran (IR), Israel (IS), Turkey(TU), Spain (SP), USA (US) and Uzbekistan (UZ) on

three continents (Europe, Asia and North America) (Table 5, Appendix A.). Specimens were *a priori* assigned to three species:

Aphidius eadyi: 87 specimens from 5 different localities in Serbia, Iran and Czech Republic (3 localities);

Aphidius banksae: 46 specimens from 3 different localities in Serbia (2 localities) and Israel;

Aphidius smithi: 90 specimens from 5 different localities in Afghanistan, Spain, Turkey, USA, and Uzbekistan.

Table 5. List of specimens belonging to *Aphidius eadyi* group, reared from *A. pisum*, submitted to morphometric analyses (* -origin of populations reared in insectaries at the University of California, Riverside, CA, USA).

Morphometrics code (Number of specimens)	Country	Year	Plant	Parasitoid
TU16 (17)	Turkey	1984	*Medicago sativa*	*A. smithi*
SP13 (18)	Spain	1981	*Medicago sativa*	*A. smithi*
AF07 (20)	Afghanistan	/	*Medicago sativa*	*A. smithi*
UZ15 (17)	Uzbekistan	1976	*Medicago sativa*	*A. smithi*
US06 (18)	United States	1977	*Medicago sativa*	*A. smithi*
SE01SE02 (28)	Serbia	2012	*Medicago sativa*	*A. eadyi*
CZ14 (16)	Czech Republic	1982	*Medicago sativa*	*A. eadyi*
CZ12 (16)	Czech Republic	1984	*Medicago sativa*	*A. eadyi*
IR09 (14)	Iran*	1977	*Medicago sativa*	*A. eadyi*
CZ21 (13)	Czech Republic *	/	*Medicago sativa*	*A. eadyi*
SE03 (14)	Serbia	2012	*Medicago sativa*	*A. banksae*
IS05 (15)	Israel*	1979	*Medicago sativa*	*A. banksae*
SE04 (17)	Serbia	2012	*Medicago sativa*	*A. banksae*

The geometric morphometric analyses were carried out on the right forewing. Microscopic slides were photographed using a Leica System Microscope DM2500 with a Leica DFC490 Digital Camera (Leica Microsystems©, Wetzlar, Germany). Thirteen homologous landmarks were positioned using the TPSDIG2 software package to explore and quantify the variation of the wing size and shape (Rohlf, 2005) (Figure 15)

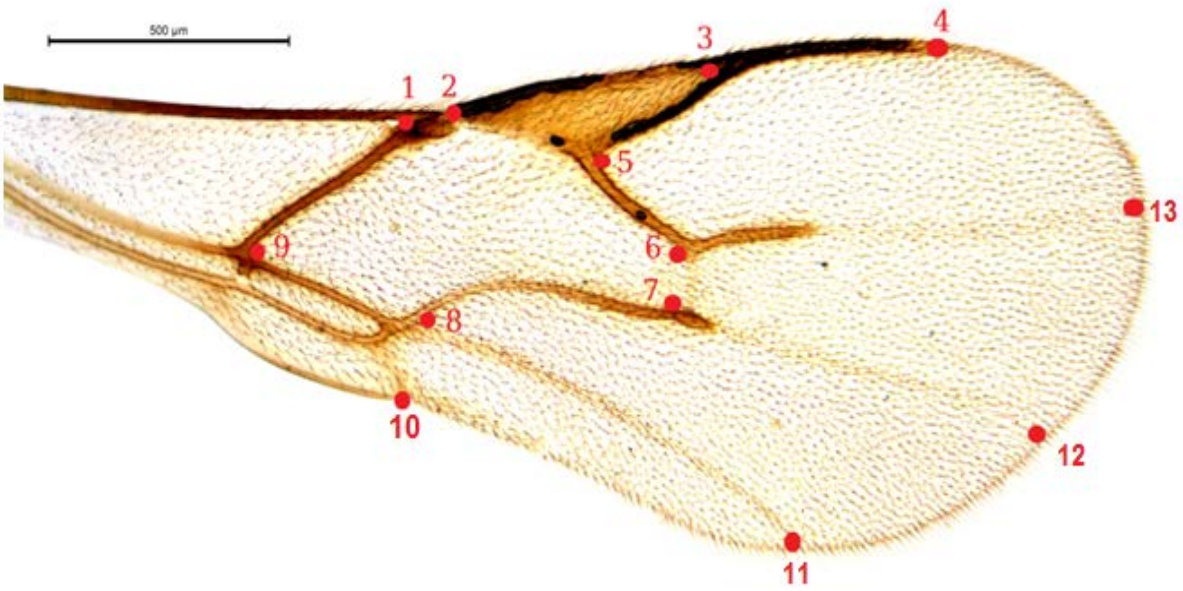


Figure 15. *Aphidius smithi* forewing with 13 selected landmarks.

The landmarks 1, 2, 3, 5, 6, 7, 8, 9 and 10 define the proximal part of the forewing; the distal part of the wing is defined by the landmarks 4, 11, 12 and 13. The landmarks 11, 12 and 13 are projections of the three veins on the wing edge. Stigma and radial abscissa 1 (R1) were defined by the landmarks 2, 3 and 4 (2 is the very apex of the stigma, 4 is the end of R1 vein); the landmarks 5 and 6 marks the first sector of the radial vein; and the vein between the landmarks 6 and 7 is defined as 2SR. The terminology used in this study regarding the forewing venation of the aphidiines follows Sharkey and Wharton (Sharkey and Wharton, 1997) on Figure 16 is presented forewing with marked venation.

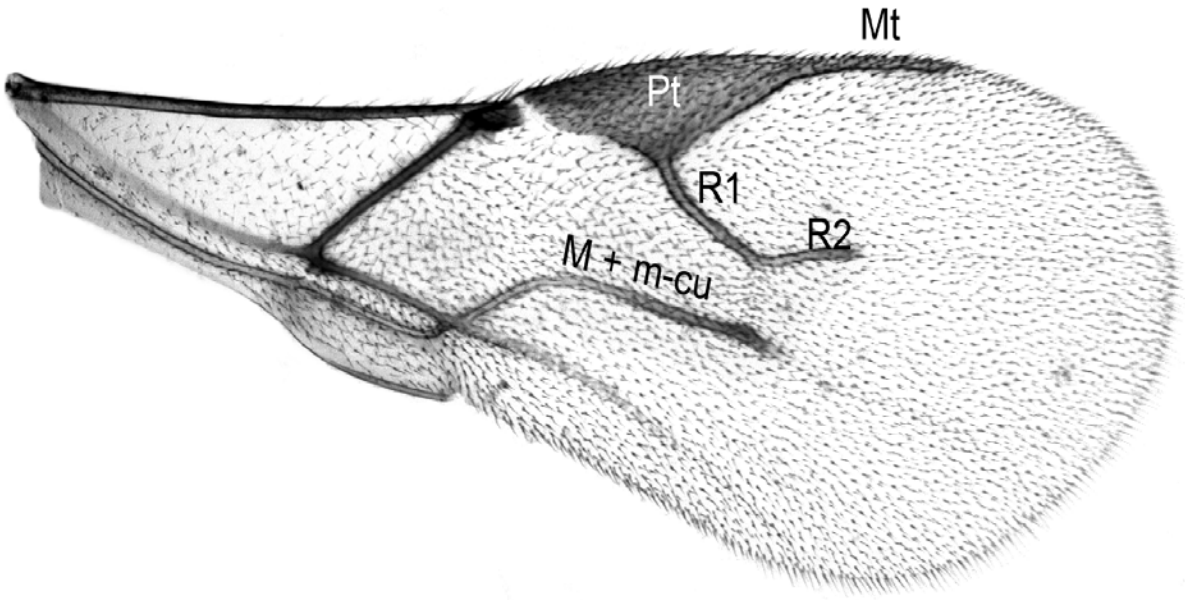


Figure 16. Forewing venation pattern of *Aphidius eadyi* parasitoid wasp.

Generalized Procrustes Analysis (GPA) (Zelditch *et al*., 2012) was applied to obtain a matrix of the wing shape coordinates (Procrustes coordinates) from which the differences due to position, scale and orientation had been discarded (Rohlf and Slice, 1990; Dryden and Mardia, 1998). We computed centroid size (CS) as measure of the wing size. CS in geometric morphometrics reflects the amount of dispersion around the centroid of the landmark configuration. Variation in wing shape was explored by principal component analysis (PCA) based on the covariance matrix. The differences between phylogenetic lineages on the size (CS) and the wing shape were tested with ANOVA and MANOVA, respectively.

To reconstruct and visualize evolutionary shape changes, we mapped the PC scores onto the phylogeny obtained using a partial sequence of mitochondrial cytochrome c oxidase subunit 1 (Chapter 3.2). Shapes corresponding to the internal nodes were reconstructed using the weighted squared-change parsimony (Maddison, 1991; Klingenberg and Gidaszewski, 2010).

In order to test whether species of *Aphidius eadyi* group can be distinguished on the basis of wing morphology, we conducted a discriminant analysis of pairwise Procrustes distances between forewings of the phylogenetic lineages/species studied. The

reliability of species identification was assessed by Discrimination function analysis and cross-validation (Lachenbruch, 1967). The wing shapes changes were visualised by outline-wrapped graphs.

Analyses were all performed using MorphoJ software (Klingenberg, 2011), except for ANOVA and Tukey HSD test, which were done with SAS (SAS Institute Inc., Cary, NC, version 9.1.3).

4. RESULTS

4.1. Parasitoid spectrum of *Acyrthosiphon pisum* (Harris) in Europe

A detailed critical survey of the literature and inspection of insect collections resulted in identification of nine parasitoid species parasitizing *Acyrthosiphon pisum* in Europe. The following species were identified: *Aphidius avenae* Haliday; *Aphidius eadyi* Stary, Gonzalez & Hall; *Aphidius ervi* Haliday; *Aphidius smithi* Sharma & Subba Rao; *Ephedrus plagiator* (Nees); *Monoctonus nervosus* (Haliday); *Praon barbatum* Mackauer; *Praon volucre* (Haliday); and *Aphidius banksae* Kittel. *Aphidius banksae* was previously overlooked in Europe. Additionally, we found some minor morphological departures from the original description of *A. banksae* (*A. staryi* sens. auct.) (Chen *et al.*, 1990) and re-describe it below.

Redescription of *Aphidius banksae* Kittel

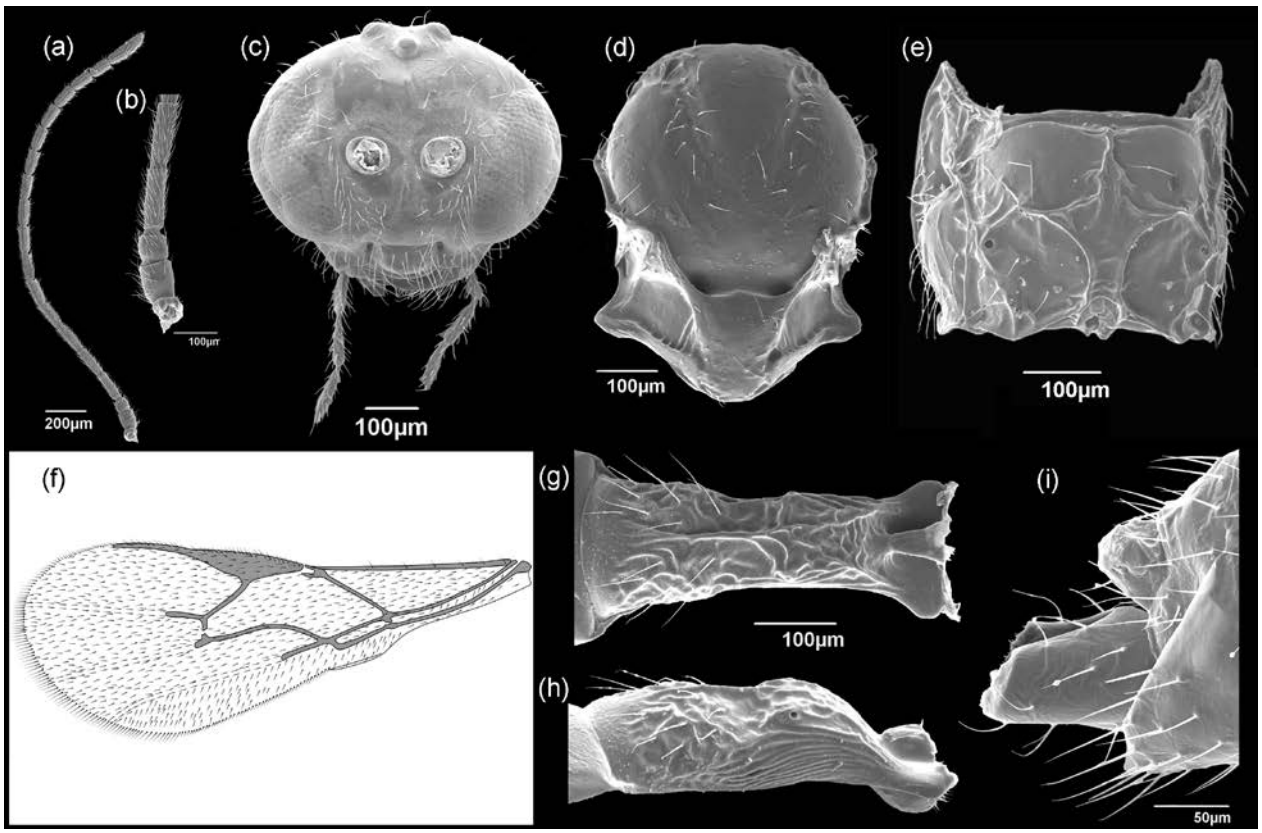


Figure 17. *Aphidius banksae*, female. (a) antenna, (b) first antennal segments, (c) frontal view of head, (d) dorsal aspect of mesonotum, (f) forewing, (e) propodeum, (g) dorsal

aspect of petiole, (h) anterolateral area of petiole, (i) last genital segment and ovipositor sheath

Diagnosis:

Aphidius banksae belongs to the *A. eadyi* group, by the host range pattern and wing venation. *A. banksae* differs from *A. eadyi* by having a longer R1, which is subequal to one-third shorter than the pterostigma length (Figure 17f) (proportion between the pterostigma length and length of R1 in *A. banksae* is 1.1–1.35 vs. 1.5-2.2 in *A. eadyi*) and having propodeum with pentagonal areola wide anteriorly (Figure 17e) while it is narrow in *A. eadyi*. *A. banksae* differs from *A. smithi* by having 7-14 irregular curved costule on the anterolateral area of the petiole (Figure 17h) while there are 4-6 almost straight costule in *A. smithi*.

Description:

Female: Head (Figure 17c) wider than mesosoma at the tegulae (proportion between width of head and width of mesoscutum, 1.31–1.44). Frons, vertex, and occipital area with dense setae. Face moderately setose (Figure 17c). Tentorial index 0.45–0.55. Malar space equal to 0.25-0.35 of longitudinal eye diameter. Eyes oval, converging toward clypeus. Clypeus rounded, with 7–13 long setae. Antennae 19-segmented, very rarely 20-segmented uniformly filiform (Figure 17a), with semi-erected and adpressed setae, which are for 1/4 shorter than segment diameter. Scape and pedicel subglobular. Flagellomere 1 (= F1), 3.00–4.00 times as long as its maximum width (Figure 17b). F2, 3.00–4.30 times as long as its maximum width. F1 somewhat shorter to subequal to F2 (F1l/F2l = 0.85-0.93). F1 and F2 without and with 3 longitudinal placodes, respectively. Maxillary palps with 4 palpomeres. Labial palps with 3 palpomeres.

Mesosoma: Mesonotum with notaulices in the ascendant portion of its anterolateral area, erased dorsally and outlined by two rows of long sparse setae (Figure 17d). Scutellum with 5–6 short setae, mostly in lateral parts. Forewing (Figure 17f) stigma moderately elongated, 3.00–3.55 times as long as its width, for one-third longer than R1 (the proportion between stigma length and R1 is 1.10–1.35). Propodeum (Figure 17e) areolated with a pentagonal central areola, wide anteriorly and narrow posteriorly. Upper areolae with 2-3 long setae laterally and lower areolae with 2-4 setae. Hind femur and tibia with semi-erected sparse setae.

Metasoma: Petiole almost parallel-sided (Figure 17g), 3.10–3.70 times as long as its width at the spiracles, anterolateral area with 7-14 irregular curved costule (Figure 17h). Dorsal surface of the petiole with fine rugosities and with moderately prominent mediodorsal carina, and 15 long semi-erected lateromedial setae on its lower half (Figure 17g).

Genitalia: Ovipositor sheath (Figure 17i) slightly concave at the dorsal margin.

Coloration: Head brown with black eyes, face and genae yellow to light brown, mouthparts yellow; scapus and pedicel light brown to yellowish, annellus yellow; except for a narrow yellow ring at the base of F1, remaining parts of flagellum uniformly brown. Pronotum yellow. Mesonotum light brown to brown with a light brown metapleuron. Legs yellow with dark apices. Wings hyaline. Metasoma (including petiole) light brown to yellowish with a dark brown ovipositor sheath. According to the original description (Chen *et al*., 1990) there can be a variation in colouration due to season (temperature).

Body length: ~ 3 mm.

Male: Antennae 20–21-segmented. Generally darker than the female. Scapus and pedicel yellow to light brown. Face and mouthparts light brown. Pronotum light brown. Legs yellow with dark apices. Remaining body parts brown.

Host: *Acyrthosiphon pisum*

Material examined:

Belgium: 1♀, Sint-Truiden (PCF) *Acyrthosiphon pisum* on *Lotus corniculatus*, 14.x.2014. (AA); 1♀, Sint-Truiden (PCF) *A. pisum* on *Trifolium repens*, 07.x.2014. (AA); 1♀, Sint-Truiden (PCF) *A. pisum* on *Trifolium* sp., 16.ix.2014. (AA). **Israel*:** 48♀ 90♂, Beirut Sheian (Insectary Riverside), *A. pisum* on *M. sativa*, 1979 (DG); 1♀ 14♂, Afigim (Insectary Riverside), *A. pisum* on *M. sativa* (DG). **Montenegro:** 4♀ 3♂, Tivat, *A. pisum* on *Vicia cracca*, 25.v.2011. (AP); 1♂, Tivat, *A. pisum* on *V. cracca*, 25.v.2011. (VŽ). **Serbia:** 2♀ 2♂, Zemun, *A. pisum* on *L. corniculatus*, 12.v.2011. (AP); 21♀ 10♂, Živkovac, *A. pisum* on *M. sativa*, 3.vi.2012. (MJ); 18♀ 2♂, Reka, *A. pisum* on *M. sativa,* 6.vi.2012. (MJ); 2♀, Pančevački rit, *A. pisum* on *M. sativa,* 7.vi.2010. (MJ); 1♂, Umčari, *A. pisum* on *M. sativa*, 8.vi.2012. (MJ); 1♂, Malo Orašje, *A. pisum* on *M. sativa*, 8.vi.2012. (MJ). **Slovenia:** 1♀, Strujan, *A. pisum* on *M. sativa*, 20.xi.2008.

(KK); 1♀, Strujan, *A. pisum* on *M. sativa*, 20.xi.2008. (KK); 1♀, Nova Gorica, *A. pisum* on *M. sativa*, 30.ix.2008. (KK).

* - This Riverside population (which originated from Israel, Beirut Sheian) is the same one which was used for original description of *A. banksae* (= A. staryi) by Chen *et al*. (1990).

Unfortunately, the holotype and paratypes from the NMNH Smithsonian (Washington, D. C.) were not available to us for re-examination.

4.2. Key for identification of female aphidiines attacking *Acyrthosiphon pisum* (Harris) in Europe

1 Forewing venation with eight cells; forewing 3RSb reaching the wing margin (Figure 18A); mummy black (Figure 18B) ……. *Ephedrus plagiator* (Nees)

- Forewing venation with fewer than eight cells; forewing r&RS vein (Figure 18C–D) or RS vein (Figure 18E–I) not reaching the wing margin; mummy not black (Figure 18J-K) …..... 2

2 Forewing RS + M vein present (Figure 18C–D); pupation under aphid's empty skin (mummy) (Figure 18J) …….……..... 3

- Forewing RS + M vein absent (Figure 18E–I); pupation inside mummy (Figure 18K) …..... 4

3 Antenna 20–21 segmented; forewing m-cu vein colourless throughout (Figure 18C); face densely setaceous …….…… *Praon barbatum* (Mackauer)

- Antenna 17–18 (19)-segmented; forewing m-cu vein coloured throughout (Figure 18D); face moderately setaceous …….. *Praon volucre* (Haliday)

4 Ovipositor sheath widened ventrally, ploughshare-shaped (Figure 18L) ……..…. …….*Monoctonus nervosus* Haliday

- Ovipositor sheath not widened ventrally, short (Figure 18M) ……. 5

5 Anterolateral area of petiole rugose (Figure 18N) ……. *Aphidius ervi* Haliday

- Anterolateral area of petiole costate (Figure 18O) or costulate (Figure 19A - C) ….. 6

6 Anterolateral area of petiole costate (Figure 18O) ……. *Aphidius avenae* Haliday

- Anterolateral area of petiole costulate (Figure 19A - C) ……. 7

8 Anterolateral area of petiole with 4–6 almost straight costulae (Figure 19A) ……...
…….*Aphidius smithi* Sharma & Subba Rao
- Anterolateral area of petiole with 7–14 irregular curved costulae (Figure 19B - C) ... 9
9 Forewing stigma 1.5–2.2 times as long as forewing R1 vein (Figure 18H); propodeum with narrow pentagonal areola (Figure 19D); body generally dark-brown...
……...*Aphidius eadyi* Starý, Gonzales & Hall
- Forewing stigma 1.1–1.35 times as long as forewing R1 vein (Figure 18I); propodeum with wide pentagonal areola (Figure 19E); body generally yellow ……..
……...*Aphidius banksae* Kittel

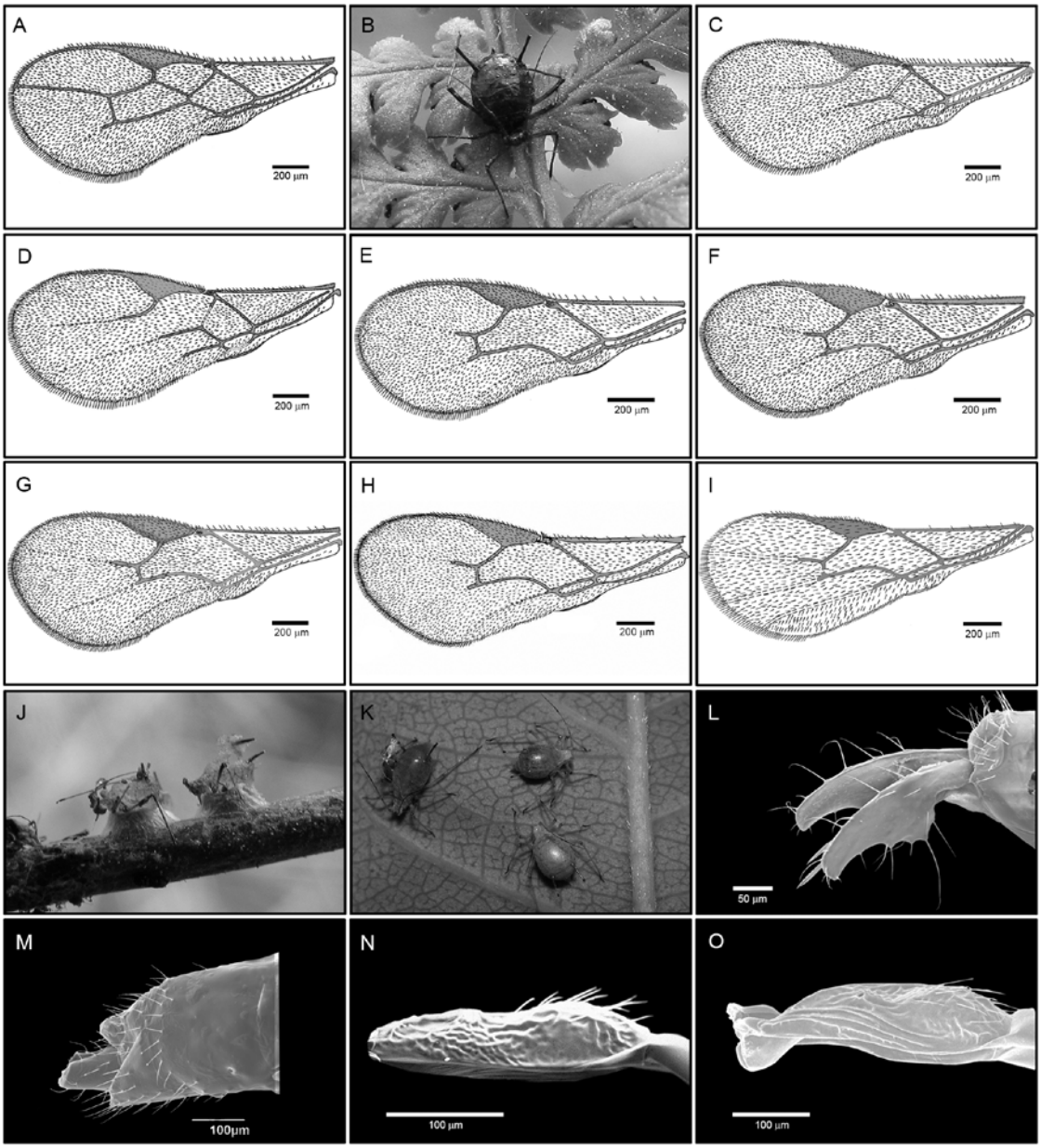


Figure 18. A - forewing of *Ephedrus plagiator* (Nees). B - *Ephedrus* spp. mummy. C - *Praon barbatum* Mackauer. D - *Praon volucre* (Haliday). E - forewing of *Aphidius ervi* Haliday. F - forewing of *Aphidius avenae* Haliday. G - forewing of *Aphidius smithi* Sharma & Subba Rao. H - forewing of *Aphidius eadyi* Stary, Gonzalez & Hall. I - forewing of *Aphidius banksae* Kittel. J - *Praon* spp. mummy. K - *Aphidius* spp. mummy. L - lateral view of ovipositor sheath of *Monoctonus nervosus* Haliday. M - lateral view of ovipositor sheath of *Aphidius banksae* Kittel. N - lateral view of ovipositor sheath of *Aphidius ervi* Haliday. O - lateral view of ovipositor sheath of *Aphidius avenae* Haliday.

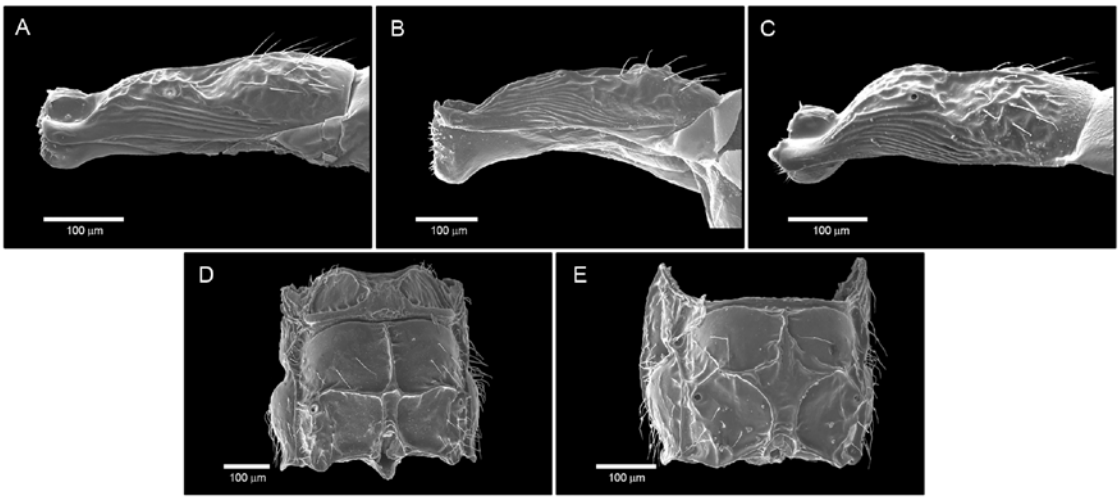


Figure 19. A - lateral view of ovipositor sheath of *Aphidius smithi* Sharma & Subba Rao. B - lateral view of ovipositor sheath of *Aphidius eadyi* Stary, Gonzalez & Hall. C - lateral view of ovipositor sheath of *Aphidius banksae* Kittel. D - dorsal view of propodeum of *Aphidius eadyi* Stary, Gonzalez & Hall. E - dorsal view of propodeum of *Aphidius banksae* Kittel

4.3. Molecular analyses

In total, we used 51 partial COI sequences to reconstruct phylogenetic relationships of species belonging to *Aphidius eadyi* group. Obtained phylogenetic trees showed same topology, clustering *A. banksae*, *A. eadyi* and *A. smithi* as separate taxa no matter what method (ML, MP and NJ) was applied (Figure 20).

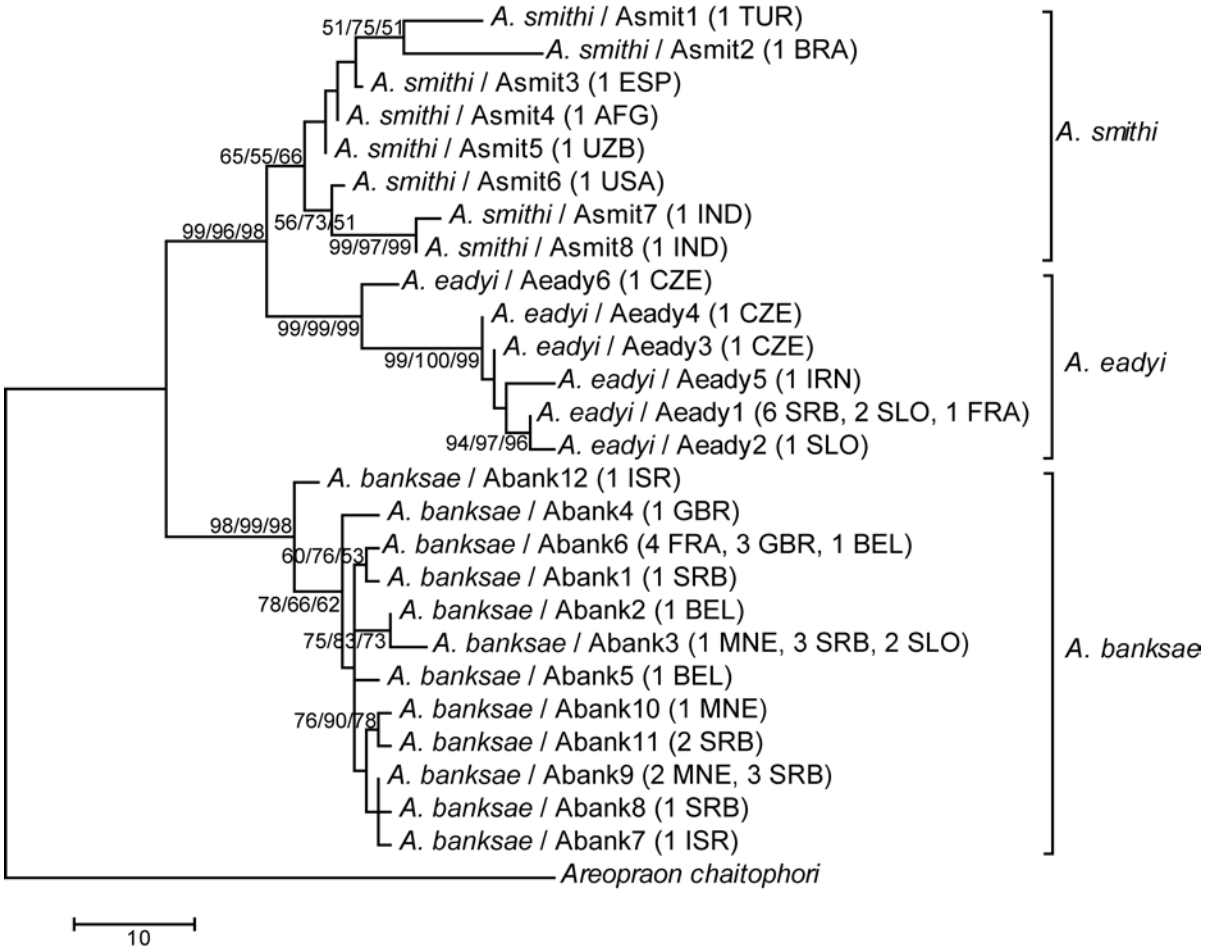


Figure 20. Phylogenetic tree of *Aphidius eadyi* species group based on partial mtCOI sequences obtained using maximum likelihood (ML), maximum parsimony (MP) and neighbor joining (NJ) methods. Bootstrap values are indicated above/below branches in the following order ML/MP/NJ. Numbers and letters between parentheses refer to the number of sequences for each haplotype and geographic origin of sequences, respectively.

Species *A. banksae* and *A. eadyi* were clustered as separate taxa with very high bootstrap support (>95%), while a bit lower support was determined for *A. smithi* (~ 60%). *Aphidius eadyi* and *A. smithi* clustered together forming one clade with >96% bootstrap supports. This clustering corresponds with a lower genetic distance between these two taxa in comparison with *A. banksae* (Table 6). The mean genetic distance between *A. banksae* and *A. eadyi* was 7.4%, between *A. banksae* and *A. smithi* was 5.5%, while between *Aphidius eadyi* and *A. smithi* was 5%. Within group genetic

divergence varied among analysed species from 1% for *A. banksae* to 2.1% for *A. smithi* (Table 6).

Table 6. Mean genetic distances (K2P) between (bold) and within the groups of parasitoids belonging to the *Aphidius eadyi* group

	A. banksae	*A. eadyi*	*A. smithi*
A. banksae	0.010		
A. eadyi	**0.074**	0.015	
A. smithi	**0.055**	**0.050**	0.021

In total existence of 26 different haplotypes was determined, 12 of which belongs to *A. banksae* (Abank1-12) six to *A. eadyi* (Aeady1-6), and eight to *A. smithi* (Asmit1-8).
All eight haplotypes of *A. smithi* were determined within single specimen. Genetic divergence between *A. smithi* haplotypes were surprisingly high and ranging from 0.2% between Asmit4 (from Afghanistan) and Asmit5 (Uzbekistan), up to 4.3% between Asmit1 and Asmit7 from Turkey and India, respectively (Table 7).

Table 7. K2P genetic distances between haplotypes of *Aphidius smithi*

	Asmit1	Asmit2	Asmit3	Asmit4	Asmit5	Asmit6	Asmit7	Asmit8
Asmit1								
Asmit2	0.035							
Asmit3	0.019	0.031						
Asmit4	0.023	0.031	0.004					
Asmit5	0.025	0.033	0.006	0.002				
Asmit6	0.035	0.039	0.015	0.011	0.010			
Asmit7	0.047	0.037	0.027	0.023	0.025	0.019		
Asmit8	0.043	0.037	0.023	0.019	0.021	0.015	0.004	

Haplotype network based on statistical parsimony also confirmed high divergence of haplotype Asmit1 which is connected to network when confidence limit is 90% while it is separate from the network at confidence limit 95%. Haplotype Asmit2 (Brazil) is even more diverged and it is not connected to the network (Figure 21).

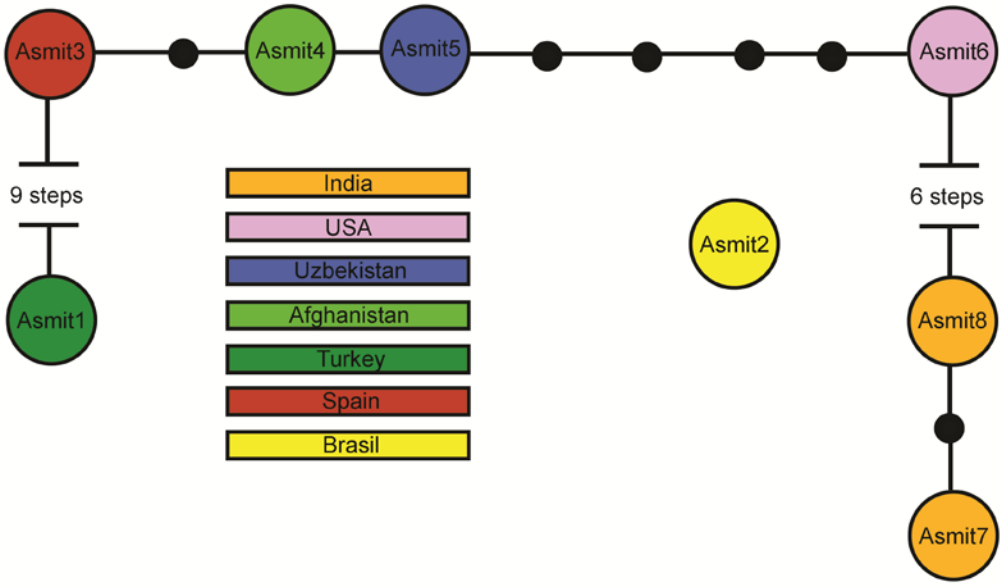


Figure 21. Haplotype network obtained from eight *Aphidius smithi* specimens using a statistical parsimony (TCS). Circles represent specific haplotypes, colour represents geographic distribution. Smaller filled circles represent missing haplotypes; lines between circles are mutational steps.

Six haplotypes (Aeady1-6) were identified within 14 analyzed specimens of *A. eadyi*. Mean divergence rate between haplotypes was 1.5%. The most diverged haplotype is Aeady6 which is identified within one specimen from Czech Republic. Genetic distances between Aeady6 and other *A. eadyi* haplotypes range from 2.5% to 3.7%. Divergence of haplotype Aeady6 could also be seen on phylogenetic trees where it forms its own phylogenetic clade (Figure 19) and on haplotype network where it is connected only with confidence level of 90% (Figure 21). Haplotypes Aeady1-5 differs from each other in range of 0.2% - 1.4% (Table 8). The most common *A. eadyi* haplotype was Aeady1 which is identified within 9 specimens originated from France, Serbia and Slovenia. All other haplotypes (Aeady2-5) were identified within single specimen (Figure 22).

Table 8. K2P genetic distances between haplotypes of *Aphidius eadyi*

	Aeady1	Aeady2	Aeady3	Aeady4	Aeady5	Aeady6
Aeady1						
Aeady2	0.004					
Aeady3	0.006	0.010				
Aeady4	0.008	0.011	0.002			
Aeady5	0.010	0.013	0.008	0.010		
Aeady6	0.033	0.037	0.027	0.025	0.033	

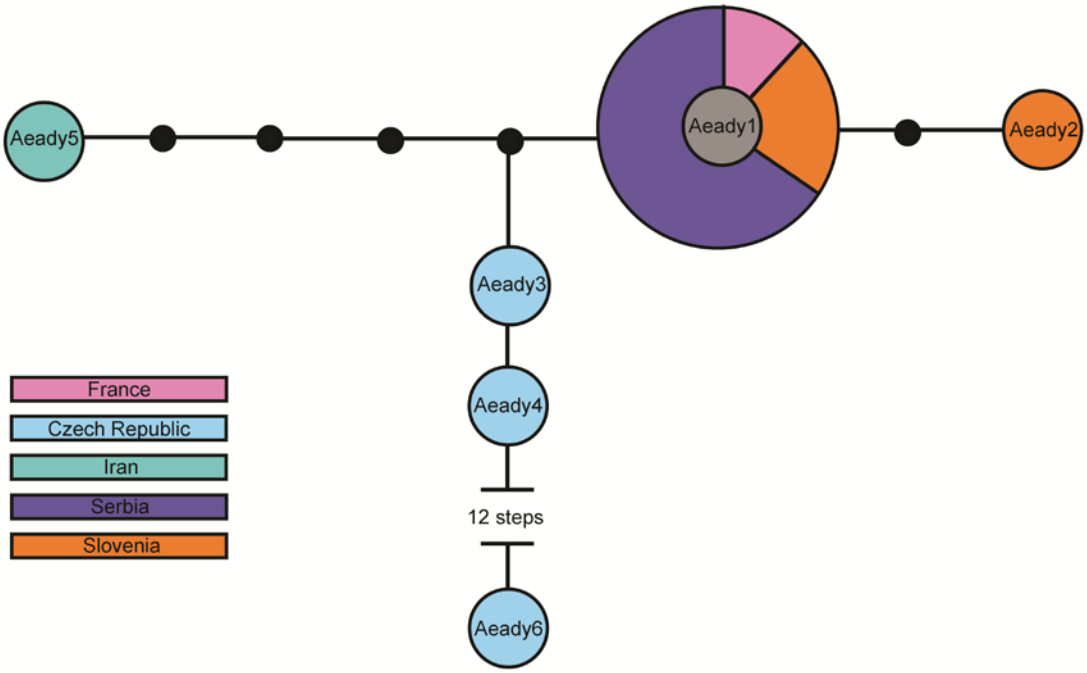


Figure 22. Haplotype network obtained from 14 *Aphidius eadyi* specimens using a statistical parsimony (TCS). Circles represent specific haplotypes, size of circle reflects the number of individuals with that haplotype (not to scale), colour represents geographic distribution. Smaller filled circles represent missing haplotypes; lines between circles are mutational steps.

The highest number of haplotypes was detected within *A. banksae*. In total 12 different haplotypes were identified within 30 analysed specimens (Abank1-12). The mean genetic distance between *A. banksae* haplotypes was 1%. All haplotypes were genetical very close to each other with genetic distances in range of 0.2% - 2.1% (Table 9). The most common haplotype was Abank6 which is determined within eight specimens.

Haplotypes Abank3 and Abank9 were identified in six and five specimens, respectively, while haplotype Abank11 was identified within 2 specimens. All other haplotypes (Abank1, Abank2, Abank4, Abank5, Abank7, Abank8, Abank10, Abank12) were represented with single specimen (Table 1, Figure 23).

Table 9. K2P genetic distances between haplotypes of *Aphidius banksae*

	Abank1	Abank2	Abank3	Abank4	Abank5	Abank6	Abank7	Abank8	Abank9	Abank10	Abank11	Abank12
Abank1												
Abank2	0.010											
Abank3	0.015	0.006										
Abank4	0.011	0.010	0.015									
Abank5	0.008	0.010	0.011	0.011								
Abank6	0.004	0.010	0.015	0.011	0.008							
Abank7	0.008	0.010	0.015	0.008	0.008	0.008						
Abank8	0.008	0.010	0.015	0.011	0.008	0.008	0.004					
Abank9	0.006	0.008	0.013	0.010	0.006	0.006	0.002	0.002				
Abank10	0.010	0.011	0.017	0.013	0.010	0.010	0.006	0.006	0.004			
Abank11	0.010	0.011	0.013	0.013	0.010	0.010	0.006	0.006	0.004	0.004		
Abank12	0.010	0.015	0.021	0.013	0.013	0.013	0.006	0.010	0.008	0.011	0.011	

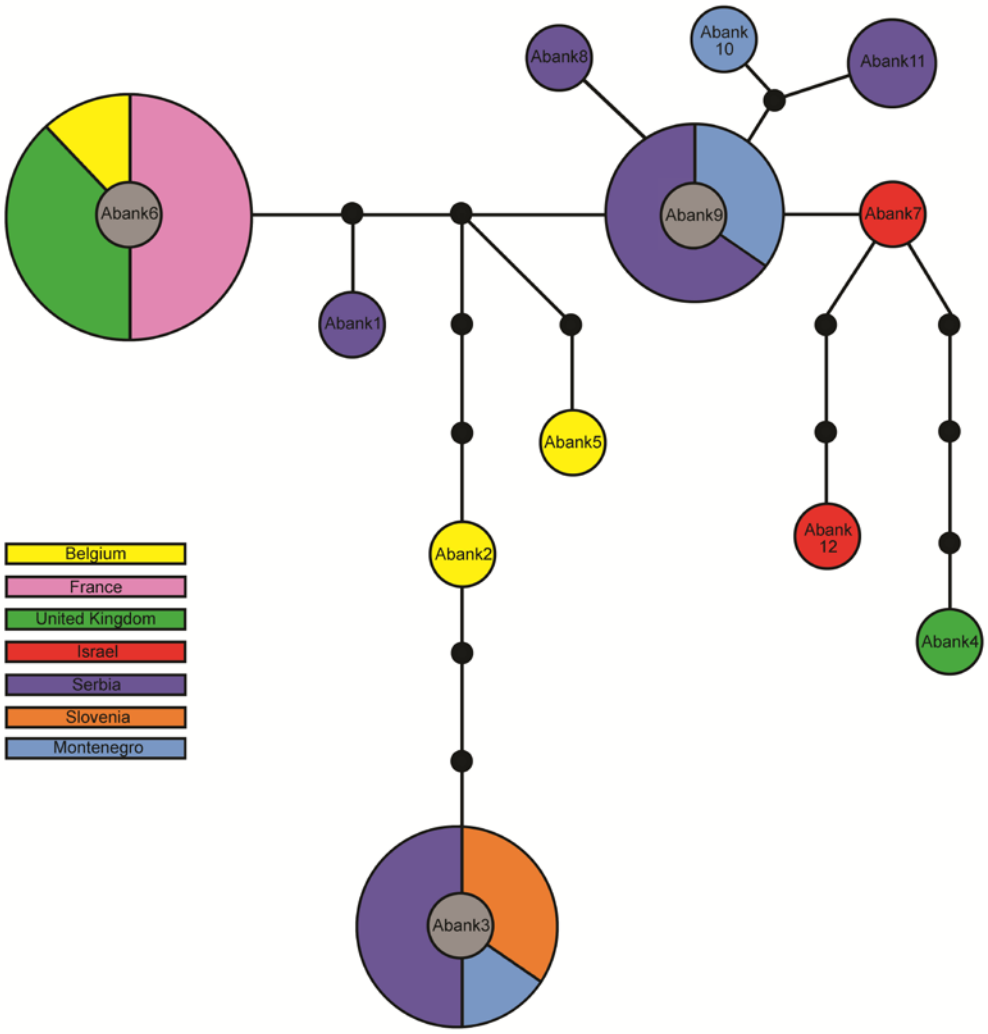


Figure 23. Haplotype network obtained from 30 *Aphidius banksae* specimens using a statistical parsimony (TCS). Circles represent specific haplotypes, size of circle reflects the number of individuals with that haplotype (not to scale), colour represents geographic distribution. Smaller filled circles represent missing haplotypes; lines between circles are mutational steps.

Based on literature and molecular data geographical distribution of species belonging to *Aphidius eadyi* group was determined for Europe (Figures 24 and 25).

We determined Mediterranean distribution of *Aphidius smithi* beside some literature data for central Europe. All those data are suspicious and most likely there are no stable populations of *A. smithi* in non-Mediterranean Europe.

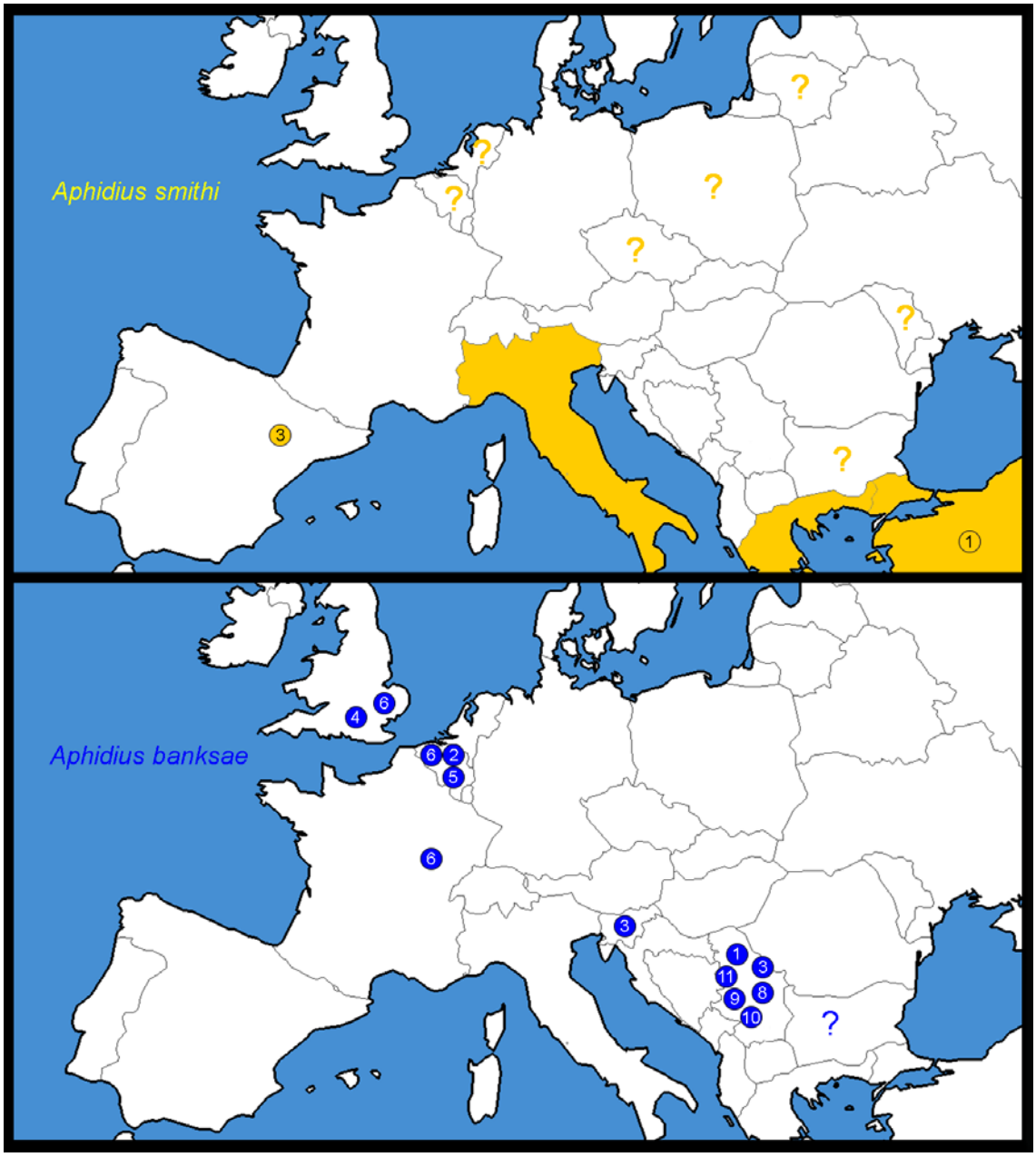


Figure 24. Distribution of *A. eadyi* group in Europe. Circles - different haplotypes; colour on the map - species distribution adapted after van Achterberg (2013). Colour code: Yellow - *A. smithi*, Blue - *A. banksae*;? - Suspicious literature finding.

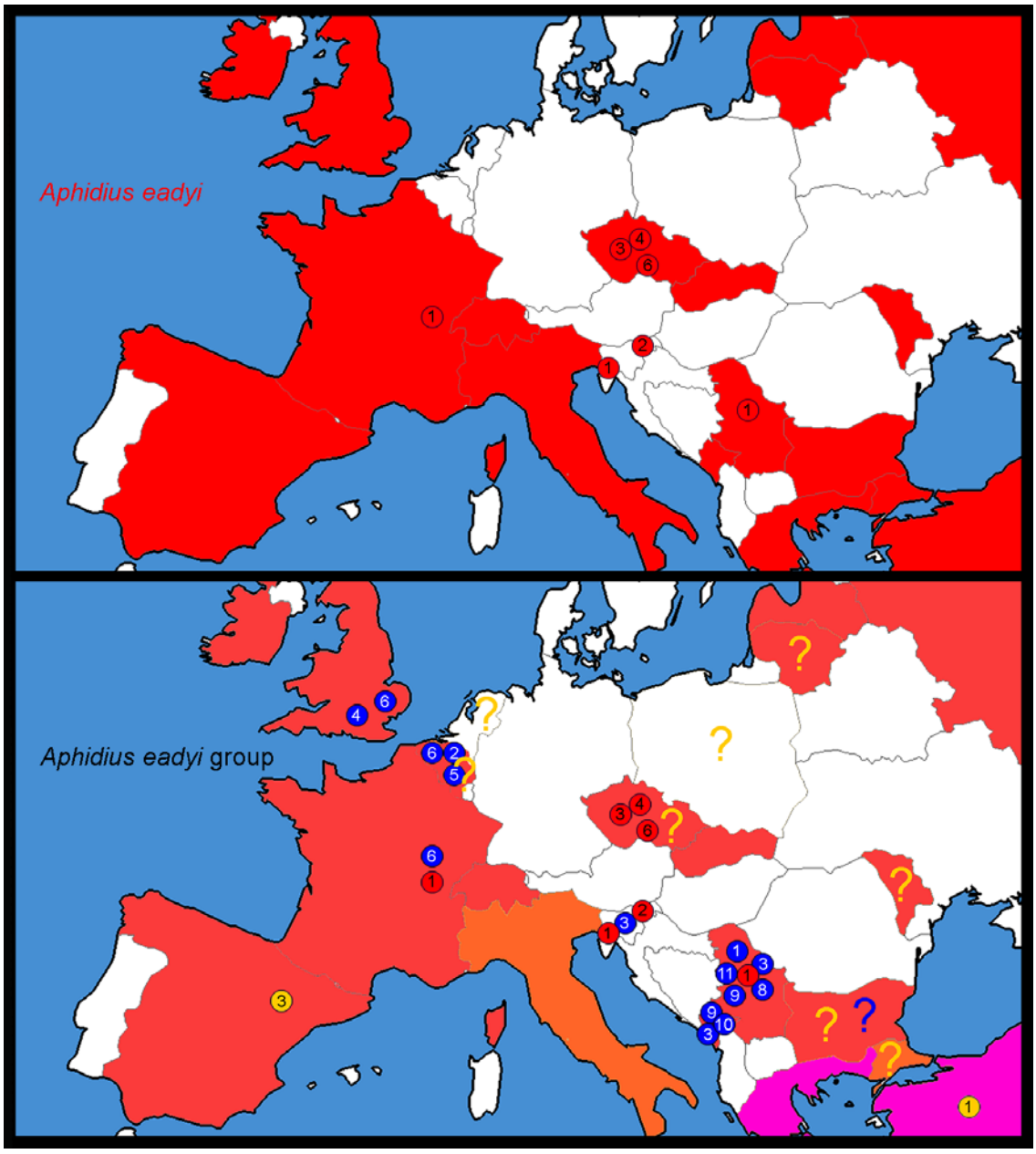


Figure 25. Distribution of *A. eadyi* group in Europe. Circles - different haplotypes; colour on the map - species distribution adapted after van Achterberg (2013). Colour code: Red - *A. eadyi*, Yellow - *A. smithi*, Blue - *A. banksae*, Orange - *A. eadyi* and *A. smithi* co-occurring, Pink - *A. banksae*, *A. eadyi* and *A. smithi* co-occurring;? - Suspicious literature finding.

Aphidius eadyi is distributed all over Europe. Although there is no data for north Europe most likely it can be found there too.

As it is already stated, *Aphidius banksae* was previously overlooked in Europe and here we determine that it is present and widely distributed from United Kingdom on the west to the Balkan on the east (Figure 24).

Both species discovery methods revealed genetic discontinuities that might indicate independently evolving lineages within species of *A. eadyi* group. Poisson Tree Process method based on Maximum Likelihood solutions (PTP ML) identified 11 taxa in total. There were 7 taxa within *A. smithi* where only to haplotypes from India grouped together. Within *A. eadyi* and *A. banksae* PTP ML identified two taxa in each, separating haplotypes Aeady6 and Abank12 as separate entities (Figure 26).

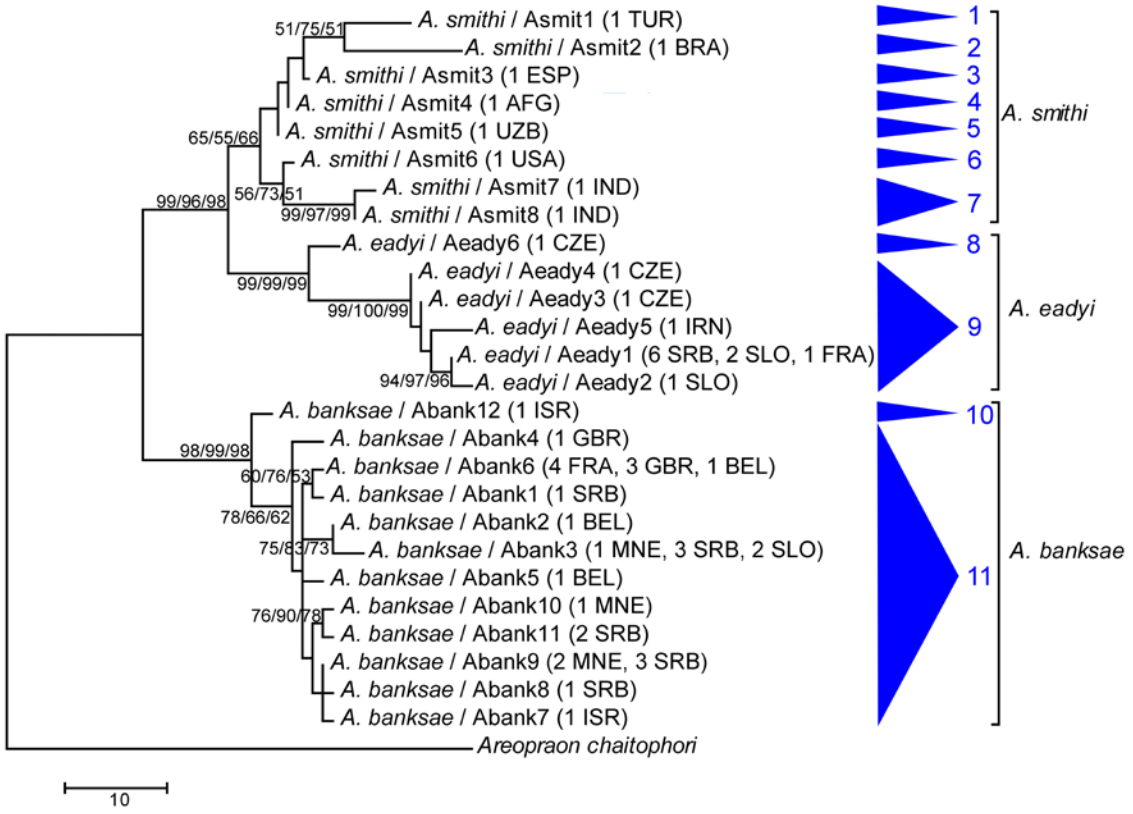


Figure 26. Results of the Poisson Tree Process method based on Maximum Likelihood solutions applied on MP phylogenetic tree of *Aphidius eadyi* group. Blue triangles and numbers represents independently evolving entities.

On the other hand, PTP method based on Bayesian solutions (PTP BI) identified 18 taxa/entities. PTP BI identified highest number (seven) of hidden taxa within *Aphidius*

smithi, same as PTP ML. In addition PTP BI identified five taxa within *A. eadyi* and six taxa within *A. banksae* (Figure 27).

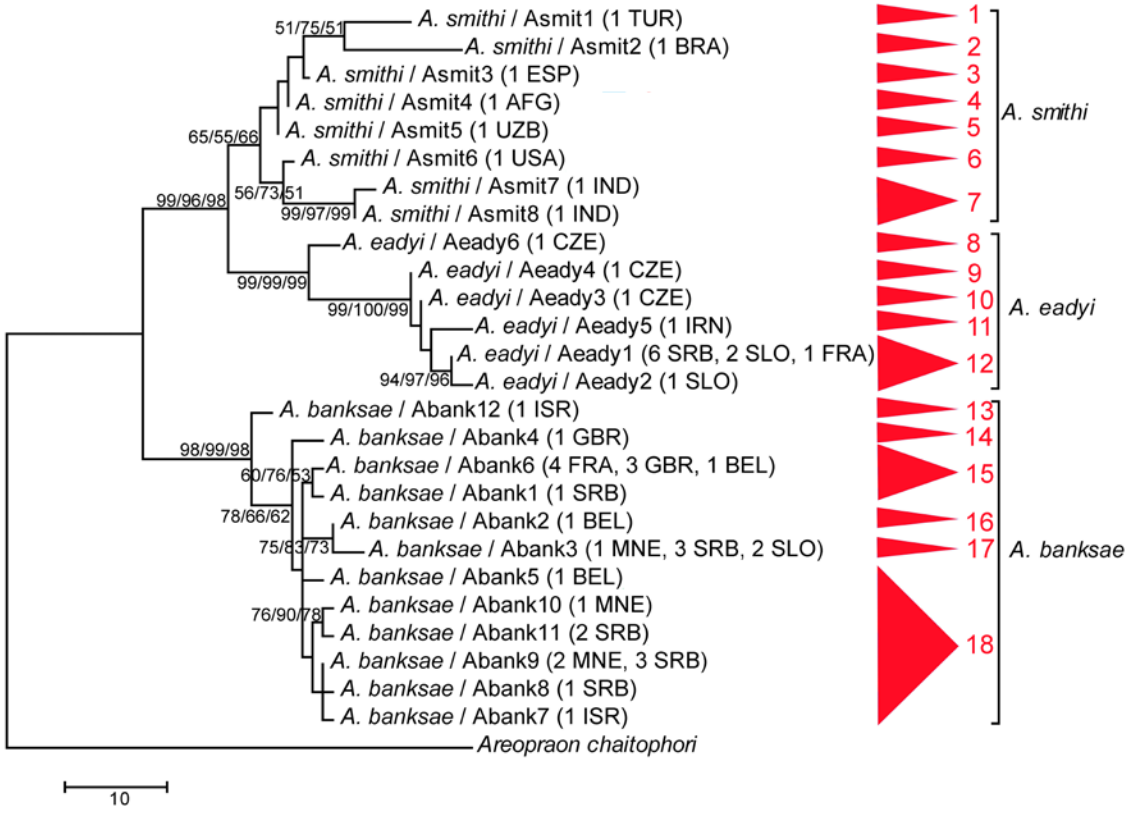


Figure 27. Results of the Poisson Tree Process method based on Bayesian solutions applied on MP phylogenetic tree of *Aphidius eadyi* group. Red triangles and numbers represents independently evolving entities.

Automatic Barcode Gap Discovery (ABGD) method provided estimate of five taxa in total, separating only haplotypes Asmit2 (within *A. smithi*) and Aeady6 (within *A. eadyi*) as independently evolving lineages (Figure 28). Haplotypes Asmit2 and Aeady6 were recognized as separate entities by both species discovery methods as well as by genetic distances, phylogeny and haplotypes networks (Tables 6-7; Figures 21-22, 26-28).

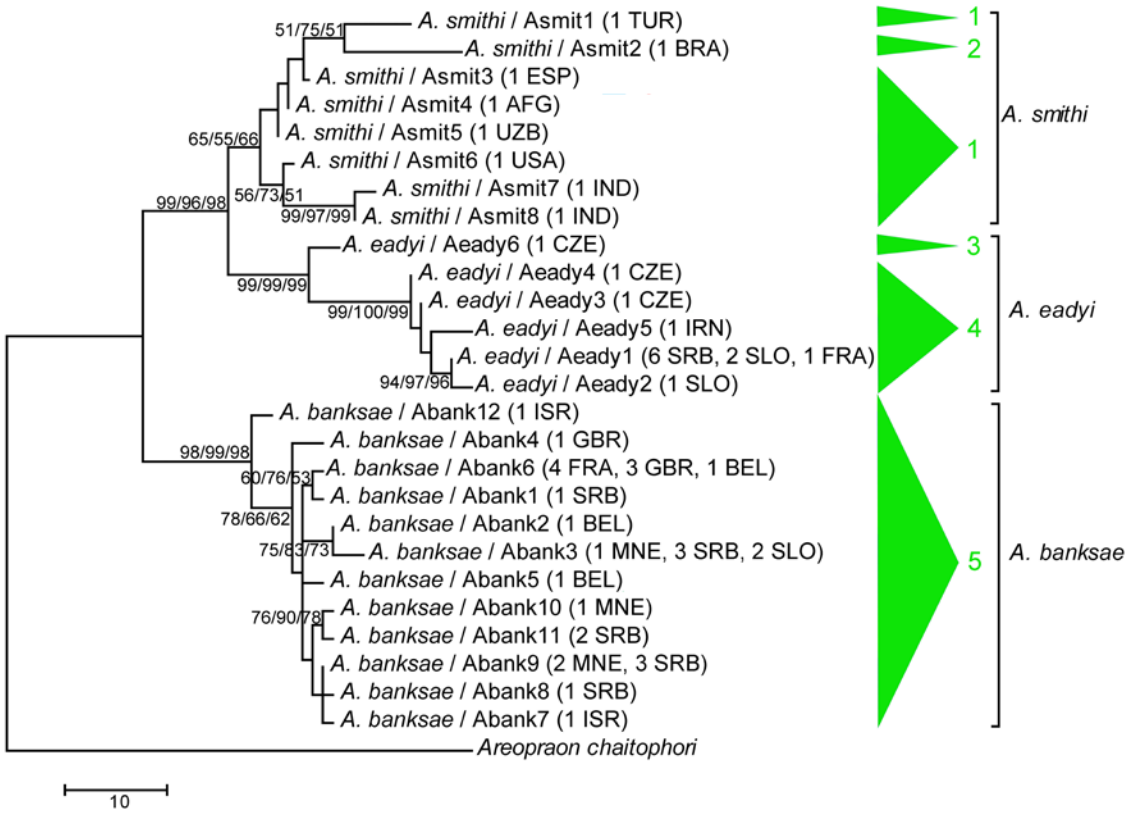


Figure 28. Results of Automatic Barcode Gap Discovery method applied on COI sequences alignment of *Aphidius eadyi* group. Green triangles and numbers represents independently evolving entities.

4.4. Geometric morphometrics

Morphological differentiation of species belonging to *Aphidius eadyi* group was tested by analysing forewing size and shape using geometric morphometrics. Analysis of forewing size showed that analysed species (*A. smithi*, *A. eadyi* and *A. banksae*) do not differ (one-way ANOVA, $F_{2, 220} = 0.903$; $p = 0.407$). On contrary all three species differ significantly in the forewing shape (MANOVA, Wilks' lambda = 0.25413, $F_{44, 398} = 8.90$; $p < 0.0001$). Principal Component Analysis (PCA) showed that total variance is 0.00178943. Total variance is described with 22 PC axis among which first three axes describe 54.3 % of total variance in wing shape (PC1 describes 24.4%, PC2 describes 17.7% and PC3 12.2%) (Table 10).

Table 10. Principal Component Analysis (PCA) of forewing shape variables (Procrustes coordinates)

PC	Eigenvalues	Variance %	Cumulative %
1.	0.00043745	24.446	24.446
2.	0.00031592	17.655	42.101
3.	0.00021855	12.213	54.314
4.	0.00015708	8.778	63.092
5.	0.00014786	8.263	71.355
6.	0.00011895	6.647	78.003
7.	0.00008486	4.742	82.745
8.	0.00005302	2.963	85.708
9.	0.00004495	2.512	88.220
10.	0.00003686	2.060	90.280
11.	0.00003029	1.693	91.973
12.	0.00002326	1.300	93.272
13.	0.00002100	1.174	94.446
14.	0.00002044	1.142	95.588
15.	0.00001773	0.991	96.579
16.	0.00001367	0.764	97.343
17.	0.00001224	0.684	98.027
18.	0.00000951	0.531	98.558
19.	0.00000876	0.490	99.048
20.	0.00000751	0.420	99.467
21.	0.00000608	0.340	99.807
22.	0.00000345	0.193	100.000

PCA analysis plots of the studied lineages/species along the first axis showed discrimination of *A. banksae* and *A. smithi* (Figure 29) while *A. eadyi* slightly separate from other two lineages along second axis.

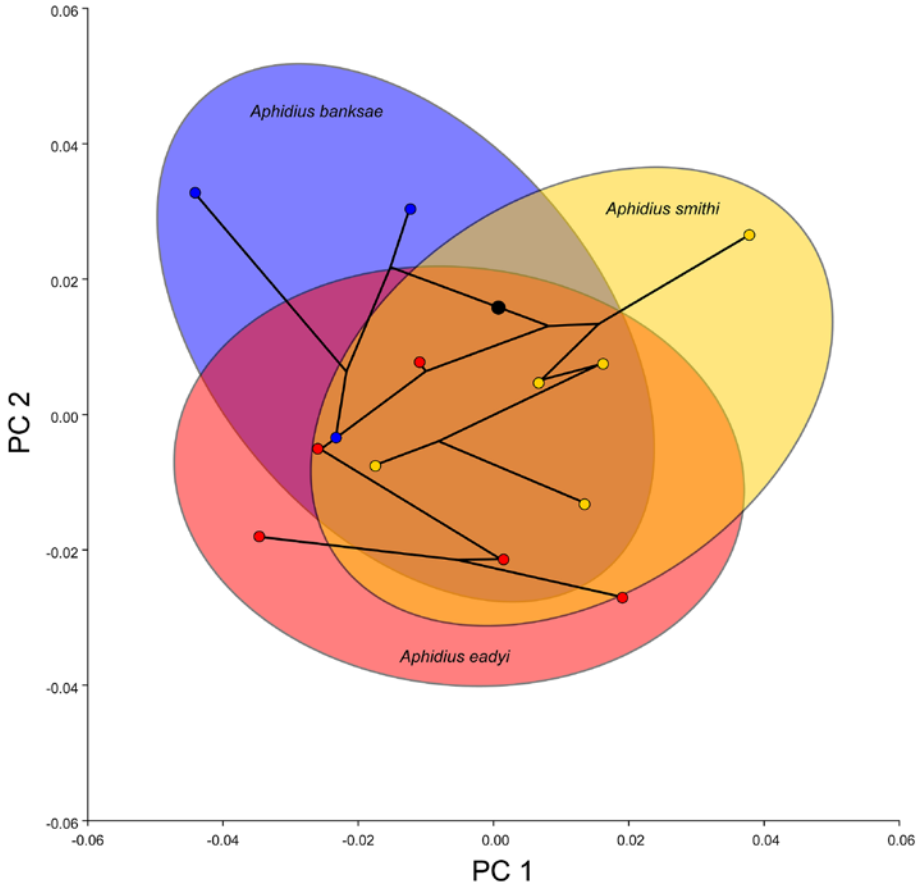


Figure 29. Bivariate plot of mean PC-scores for the PC1 and PC2 axes of the forewing shape along with the superimposed phylogeny. The ellipses are sized as to comprise 90% of the observations belonging to three phylogenetic lineages/species. Colour code: Red - *A. eadyi*, Yellow - *A. smithi*, Blue - *A. banksae*

In the morphospace defined by second (PC2) and third (PC3) axes, *A. banksae* slightly separate, with the populations from Israel having the most positive scores along PC3 (Figure 30)

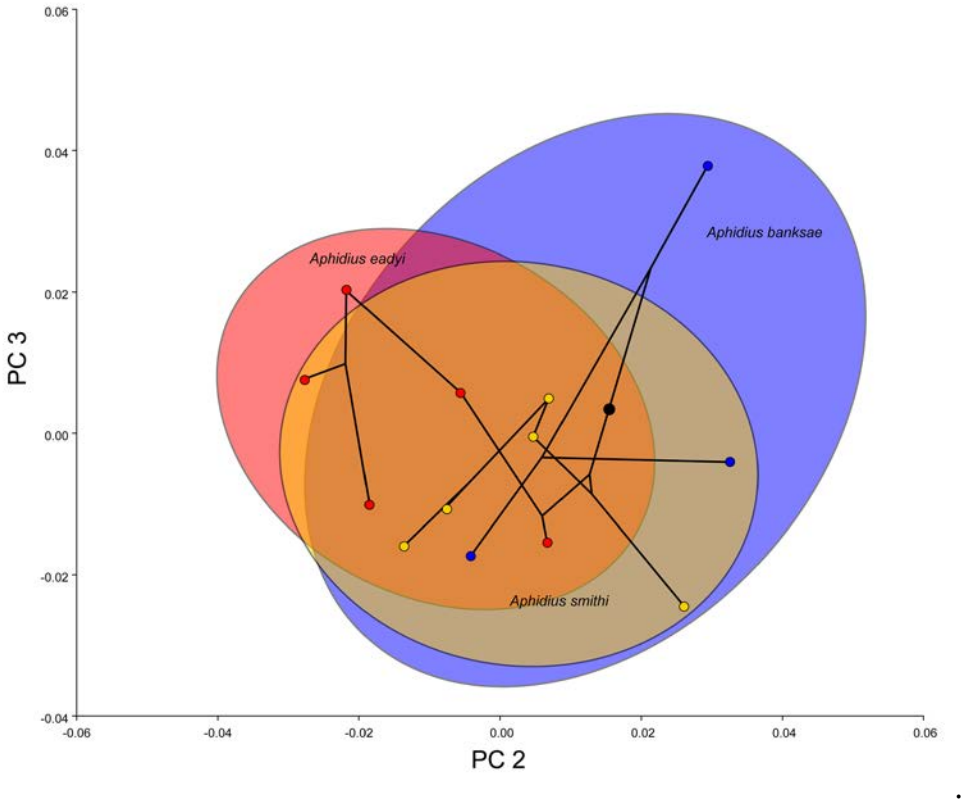


Figure 30. Bivariate plot of mean PC-scores for the PC2 and PC3 axes of the forewing shape along with the superimposed phylogeny. The ellipses are sized as to comprise 90% of the observations belonging to three phylogenetic lineages/species. Colour code: Red - *A. eadyi*, Yellow - *A. smithi*, Blue - *A. banksae*

Shape changes along PC1 are related to changes of the proximal part of the wing described by landmarks 2, 5, 6, 7, 8 and 9 and shape of the stigma (landmarks 2, 3, 4 and 5).The PC1 separated relatively shorter and wider wings with the wider proximal part and more robust stigma and longer radial vein, from wings with narrower proximal part of the wing, narrower stigma and relatively shorter radial vein (Figure 31).

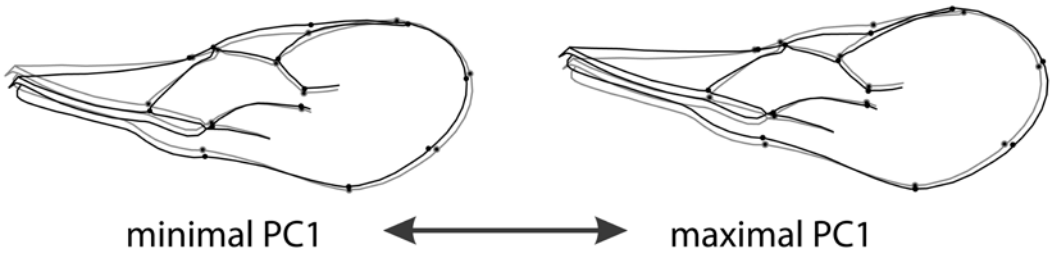


Figure 31. Forewing shape changes associated with the first PC. Black outline representing the shape at maximal positive and negative score of each axis comparing to the mean shape for the sample (grey).

The PC2 separated the relative wider wings with concave anterior margin defined by stigma and radial nerve (landmarks 1, 2, 3 and 4) from wings with more or less flattened anterior margin of the forewing such as in populations of *A. banksae* from Serbia and Israel (Figure 32).

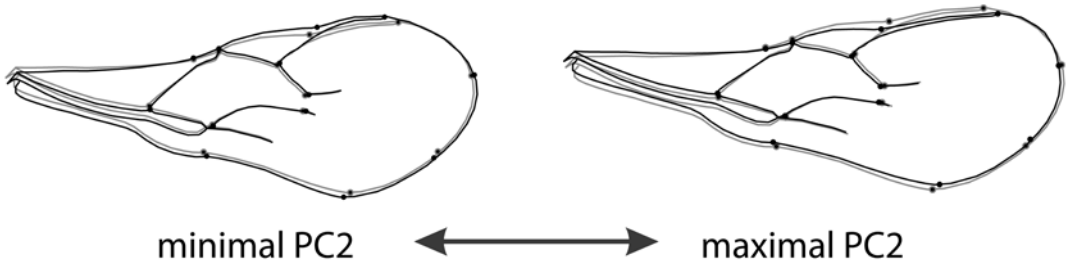


Figure 32. Forewing shape changes associated with the second PC. Black outline representing the shape at maximal positive and negative score of each axis comparing to the mean shape for the sample (grey).

The third PC separated relatively shorter and wider forewings with shorter radial vein relative to stigma (negative end of PC3 axis) from more elongated wings with longer radial vein (Figure 33).

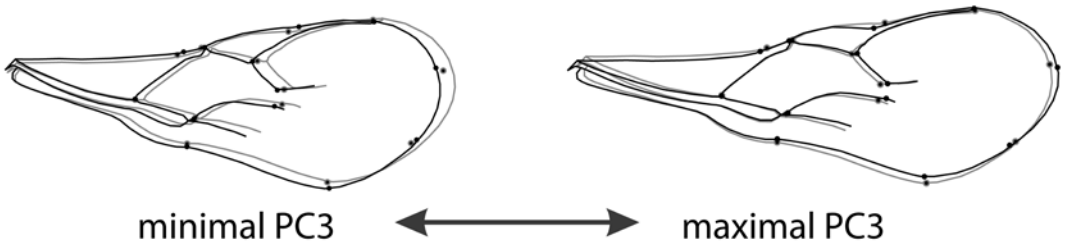
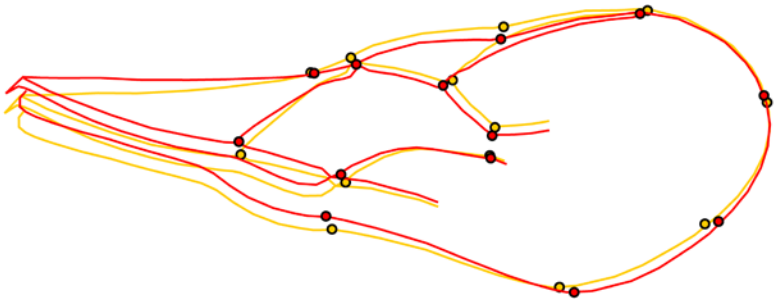
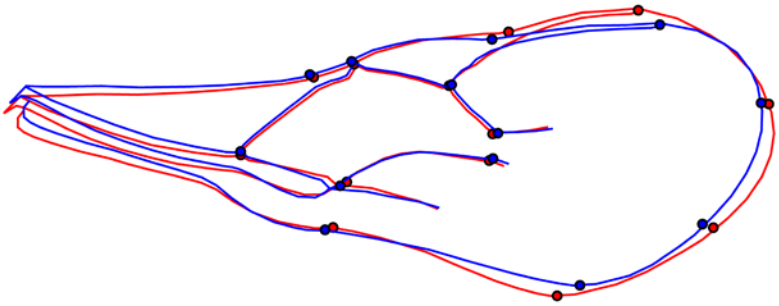


Figure 33. Forewing shape changes associated with the third PC. Black outline representing the shape at maximal positive and negative score of each axis comparing to the mean shape for the sample (grey).

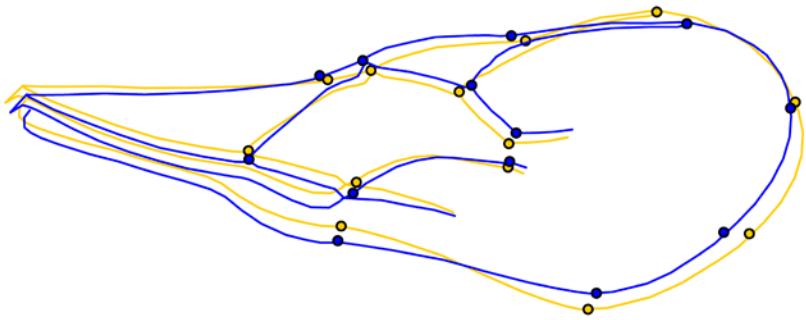
The species average shape and visualisation of shape differences between species were presented in Figure 34. Procrusted distances between *A. eadyi* and *A. smithi* was 0.022, between *A. eadyi* and *A. banksae* was 0.026 and between *A. smithi* and *A. banksae* was 0.030. All distances were statistically significant ($P < 0.0001$ in all comparisons).



Aphidius eadyi -- Aphidius smithi



Aphidius banksae -- Aphidius eadyi



Aphidius banksae -- Aphidius smithi

Figure 34. Illustration of wing shape differences between the three analysed Aphidius species. The shape changes are shown as the difference between the average shape of species compared. Colour code: Red - *A. eadyi*, Yellow - *A. smithi*, Blue - *A. banksae*. All changes are exaggerated 3 times.

Assignment of individual specimens (forewings) to species

Discriminant function analysis shows that based on the forewing shape, a large proportion (>75%) of individual specimens in the confusion matrix is assigned to the correct species (Table 11).

Table 11. Assignment of individual specimens (forewings) to species as misclassified / number of specimens investigated. Values from the Discrimination function analysis were given below diagonal and those obtained through cross-validation were given above of the diagonal. All species combinations ware above 75% of correct classification.

	A. eadyi	*A. smithi*	*A. banksae*
A. eadyi		39/177	22/133
A. smithi	28/177		22/136
A. banksae	11/133	12/136	

5. DISCUSSION

Correct identification of natural enemies is essential to the success of biological control programs (Rosen, 1986; Moraes, 1987), and identification of the primary parasitoids of aphids is thus highly important for successful biological control of economically significant aphids like *Acyrthosiphon pisum* (Desneux & Ramirez-Romero, 2009; Pons *et al*., 2011). We identified nine species of aphidiine parasitoids of *A. pisum* in Europe. The parasitoid complexes of *A. pisum* in Asia and North Africa are almost identical to that of the same aphid in Europe (González *et al.* 1978; Starý, 1979; Rakhshani *et al*., 2006; Laamari *et al*., 2012), and our results are applicable to those regions as well.

We employed the approach of integrative taxonomy to resolve the taxonomic status of members of the *Aphidius eadyi* species complex. Combining molecular characterization, geometric morphometrics, and morphology has already been shown to be a very good integrative approach in taxonomic studies of the subfamily Aphidiinae (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; Petrović *et al*., 2017). At the same time, there are strong suggestions that the only method that can be treated as reliable taxonomy is an integrative one which goes beyond the naming of species and gives priority to species delineation and processes underlying it (Dayrat, 2005; Schlick-Steiner *et al*., 2010).

Our study of the *Aphidius eadyi* species group resulted in clear separation of three species, viz., *A. smithi*, *A. eadyi*, and *A. banksae*. Species separation was determined on the basis of both morphology and molecular data, specifically the barcoding region of mtDNA COI sequences. All three speceis of the *A. eadyi* group can be distinguished by considering the following morphological characters: number and shape of costulae on the anterolateral area of the petiole; shape of the central areola on the propodeum; and shape and venation of the forewings. With respect to wing shape, species belonging to the *A. eadyi* group form a kind of gradient with some overlap, but still with statistically significant differences between all three species. On the one hand, there is *A. banksae*, having relatively shorter and wider wings with a wider proximal part, more robust stigma, and longer radial vein. On the other hand, there are the wings

of *A. smithi* with a narrower proximal part, narrower stigma, and relatively shorter radial vein, while shape of the *A. eadyi* wing was found to be in between. Those results are very similar to the ones obtained by Tomanović *et al.* (2014) for the *Aphidius colemani* species group. In that study, they determined a similar pattern of wing differences between the species *Aphidius colemani* Viereck, 1912, *A. transcaspicus* Telenga, 1958, and *A. platensis* Brethes, 1913. It is evident that wing shape in *Aphidius* species sometimes evolves in similar ways within different groups. Tomanović *et al.* (2014) also concluded that wing shape is not a good trait for identification of species when used solely, which is partially confirmed by results of the present study.

Genetic separaton of species belonging to the *A. eadyi* group was analysed on the basis of the barcoding region of the mtCOI gene. The obtained results were congruent with the differences of forewing shape, but more pronounced with clear separation of all three species (*A. eadyi*, *A. smithi*, and *A. banksae*). The mean genetic distances between species were above the rate common for between-species divergence in the genus *Aphidius* (Kos *et al.*, 2011; Tomanović et al., 2014; Yu *et al*., 2017) and ranged from 5 to 7.4%. Genetic relationships between species were similar to those obtained using geometric morphometrics. *Aphidius eadyi* and *A. smithi* are genetically closer to each other than to *A. banksae*, which also had the most divergent wing shape. Although high intraspecific genetic variation was recorded in *A. smithi* and *A. eadyi*, all phylogenetic analyses resulted in phylogenetic trees having the same topology, with haplotypes of all three species clustered separately.

With eight detected haplotypes differing from each other in the range of 0.2-4.3% (mean 2.1%), *Aphidius smithi* represents the species with the highest intraspecific genetic divergence within the *A. eadyi* group. Some of those differences exceed the intraspecific genetic variation previously reported in *Aphidius* (Kos *et al.*, 2011; Tomanović *et al.*, 2014; Derocles *et al.*, 2016) and could possibly represent some cryptic species, but additional research is needed to confirm this. The most distinct haplotypes were from Turkey (Asmit1) and Brazil (Asmit2). Those haplotypes differ by more than 2% from all other haplotypes and also from each other. Both the PTP and ABGD methods also suggested that Asmit1 and Asmit2 (among others) represent independently evolving lineages. It is usually considered that speciation in aphid parasitoids is driven by the aphid hosts or by geography (Tremblay & Pennacchio,

1988; Kos *et al.,* 2011; Mitrovski Bogdanović *et al*., 2013; Tomanović *et al*., 2014; Jamhour *et al*., 2016), but neither scenario can account for the high genetic differences within *A. smithi*. That is because *A. smithi* is a specific parasitoid of *Acyrthosiphon pisum* and all analysed specimens originated from the same host, which disqualifies the aphid host as a factor driving genetic variation. The geographic origin of specimens can also be excluded because the genetically closest relatives were from Afghanistan (Asmit3), Uzbekistan (Asmit4), and Spain (Asmit5). Although we are dealing with limited data (eight analysed specimens), there is one possible explanation for such genetic diversity of *A. smithi*. Most likely, high genetic diversity occurs within and/or between populations from the native range of *A. smithi* - India. Haplotypes Asmit8 and Asmit6, which differ by 1.5%, represent circumstantial evidence for this statement. The Asmit8 haplotype was initially collected from India and reared for mass release in an insectarium in Riverside, while Asmit6 was collected in Lakeview (CA, USA) as the initial establishment recovery sample, which means that Asmit6's ancestors (most likely parents) also originated from India. The majority, if not all, *A. smithi* specimens in North America originate from a few long-term biocontrol projects targeting *A. pisum* in the USA. Those projects resulted in numerous references and data about the biology and ecology of *A. smithi* (Starý, 1974; Angalet and Fuster, 1977). Other than those data, there is still a very big gap in knowledge about the current status and distribution of *A. smithi* in North America. The reason for this can be found in the fact that almost all biocontrol projects were focused on *Aphidius ervi,* which at least partially displaced *A. smithi* (Angalet and Fuster, 1977). Moreover, it has been determined that *A. smithi* was displaced by *A. ervi* all over the USA and became almost eliminated in North America (McBrien and Mackauer, 1990). Wylie *et al.* (2005) stated that *A. smithi* potentially still exists in North America in low densities populations with no useful agricultural effect on *Acyrthosiphon pisum*. Although *A. smithi* may not have an economic effect at the moment, a detailed survey is necessary to prove the current existence and determine the status of *A. smithi* in North America. *Aphidius smithi* has been present in Europe for decades (Pennacchio, 1989; Rasplus *et al.,* 2010; Yu *et al.,* 2012; van Achterberg, 2013), but its origin and data on its current distribution are questionable and scarce. According to the literature, there have been three attempts to introduce *A. smithi* in Europe (in Poland, the Czech Republic, and Moldova), and in all cases parasitoid

populations failed to establish themselves (Starý, 1974). Starý (1974) expressed the opinion that *A. smithi* was introduced and established in hot and dry areas of Europe prior to official releases in Central Europe. We analysed only one available European population of *A. smithi* and cannot draw any conclusion about its origin in Europe based on these sparse data. To judge from the analysed mtCOI sequences, it can be stated that the Spanish population of *A. smithi* (Asmit3) is closely related to populations from Afghanistan and Uzbekistan, with genetic distances of 0.4 and 0.6%, respectively. According to Rasplus *et al.* (2010) and Yu *et al.* (2012), *A. smithi* is widely distributed in Europe and is present in more than 25 countries. After a critical review of all relevant literature (summarized in Yu *et al.*, 2012), we found that the distribution of *A. smithi* is greatly overestimated. Bearing in mind the Oriental origin of *A. smithi* as well as its specific climatic requirements (Campbell and Mackauer, 1973; Starý, 1974), we conclude that *A. smithi* is distributed in the Mediterranean part of Europe. The only European findings of *A. smithi* that can be treated as relevant are from Spain (herein), Italy (Pennacchio, 1989), and Greece (Kavalliratos *et al.*, 2004), which is in agreement with our conclusion about its Mediterranean distribution. Records from Bulgaria (Atanassova, 1997) and Turkey (Akar and Çetin Erdoğan, 2017) should be taken with caution, especially in the light of our results. The presence of *A. smithi* in Turkey is very likely, but there is no evidence to confirm this assumption because the analysed sample of *A. smithi* from Turkey (Asmit1) was collected in central Anatolia, so it cannot be treated as Europe. Also, all other records of *A. smithi* should be reevaluated.

In total, six different haplotypes with mean genetic divergence of 1.5% were recorded among the analysed specimens of *Aphidius eadyi*. This genetic variabilty of *A. eadyi* can be considered very high when compared with other *Aphidius* species. Two recent studies determined intraspecific genetic variability of ≤ 0.5% for both the *Aphidius colemani* group (Tomanović *et al.*, 2014) and the *Aphidius urtice* s. str. group (Jamhour *et al.*, 2016). However, most of the detected divergence is caused by one haplotype (Aeady6) from the Czech Republic, which differs from all others by ≥ 2.3%. Haplotype Aeady6 is also recognized as an independent entity by the PTP and ABGD methods. This specific haplotype needs to be further examined for two reasons: a) it might represent some unknown cryptic species, but also may be a mitochondrial heteroplasmy (Magnacca and Brown, 2010); and b) this haplotype was detected from a

population which was reared as *Aphidius smithi* in insectaries of the University of California for mass release in North America. All other haplotypes are closely related to each other. The distinctiveness of haplotype Aeady6 can be illustrated by the fact that haplotypes Aeady3 and Aeady4, also originally from the Czech Republic, are genetically closer to haplotypes from other parts of Europe (Aeady1 and Aeady2) and Iran (Aeady5) than to Aeady6. Starý *et al.* (1980) postulated a West Palaearctic distribution of *A. eadyi*, a view which receives molecular confirmation by the results presented here. *Aphidius eadyi* was used as a biocontrol agent in order to control populations of *Acyrthosiphon pisum* in New Zealand and Burundi (Autrique *et al.*, 1989). The last published data about *Aphidius eadyi* in introduced areas were given by Cameron & Walker (1989), who concluded that *Aphidius eadyi* has been displaced by *A. ervi* in New Zealand. Considering this, we can say that the current status of *Aphidius eadyi* in introduced areas (Burundi and New Zealand) is unknown.

Our use of an integrative taxonomic approach resulted in identification of *Aphidius banksae* as a common and widely distributed parasitoid of the pea aphid in the Western Palaearctic, which is the most interesting finding of this study. Analysing both mtCOI sequences and forewing shape, we were able to determine that *A. banksae* is unambiguously a separate species. Specimens belonging to the species *A. banksae* were previously treated as *A. urticae* (Todorov, 2002; Tomanović *et al*., 2003b; Kavallieratos *et al*., 2004; Alhmedi *et al.*, 2009; Žikić *et al*., 2012; Derocles *et al.*, 2016) or as *A. eadyi* (Elias *et al*., 2013). Additional molecular conformation of our results can be found in the paper of Derocles *et al.* (2016), who analysed six genes and showed that *A. urticae* specimens that originally came from the pea aphid are genetically divergent from those that came from the common nettle aphid. The "*A. urticae*" specimens from the pea aphid are actually *A. banksae*.

We identified the greatest number of haplotypes within the species *Aphidius banksae.* Such a high haplotype diversity within this species could be a result of its invading new areas and selection pressure. The 12 identified haplotypes showed the lowest mean intraspecific genetic variation (1%) within the *A. eadyi* species group. Also, no evident association with any specific geographic region was determined for *A. banksae* haplotypes. Prior to this study, *A. banksae* was considered as allopatric to *A. eadyi* and distributed in Asia Minor (Israel and Turkey) (Chen *et al.*, 1990). Moreover,

there are no data about results of its introduction in the USA. Our results showed a much broader distribution (from the United Kingdom to Israel) of *A. banksae*, as well as its sympatry with *A. eadyi*. *Aphidius banksae* and *A. eadyi* have almost identical geographic distribution, and both species exclusively parasitize *Acyrthosiphon pisum* on a variety of plants belonging to the family Fabaceae (see Tables 4 and 5; and Starý *et al.*, 1980). Sympatric speciation is common in Aphidiinae and mostly driven by parasitoid specialization to different aphid host lineages (Tremblay & Pennacchio, 1988; Kos *et al.*, 2011; Mitrovski Bogdanović *et al.*, 2013; Tomanović *et al.*, 2014; Jamhour *et al.*, 2016). For the reasons mentioned above, that cannot be case with the *A. eadyi* group. Although pea aphid is a complex of host-specialized races and species (Peccoud *et al.*, 2009a) with one of the fastest evolutionary diversifications ever recorded (Peccoud *et al.*, 2009b), we found no correlation between host lineage and speciation of the *A. eadyi* group. There are several cases where *A. banksae* and *A. eadyi* were collected from the same locality and same aphid colony (Table 4). For example, haplotypes Aeady1 and Abank1 were collected from the same pea aphid colony in Serbia, at the Umčari locality (SE 01 in Table 4). Most aphid colonies are formed by a single female aphid (or a few related aphids), and thus the vast majority of colonies consist of specimens belonging to one host-specialized race or species. Similarly, three different *A. banksae* haplotypes (Abank3, Abank8, and Abank11) were collected from the same aphid colony at the Živkovac locality, also in Serbia (SE 03 in Table 4). Those examples, as well as the fact that in previous years it was common to find *A. banksae* and *A. eadyi* in the same sample (where *A. banksae* was erroneously identified as *A. urticae* or as a light form of *A. eadyi*) (Tomanović and Petrović, personal communication), represent hard evidence indicating that there is no correlation between host lineage and speciation of the *A. eadyi* group. *Aphidius banksae* and *A. eadyi* evolved independently for a relatively long time (genetic divergence of 7.4%), which together with the obvious sympatry leads us to the conclusion that those two species acquired the pea aphid independently, as in the case of all other *Aphidius* parasitoids (*A. avenae* Haliday, *A. ervi*, and *A. smithi*). The geographic origin of *Aphidius banksae* is unknown, but some assumptions can be made. Based on the fact that it was originally described from Asia Minor (Chen *et al.*, 1990) and is genetically more closely related to *A. smithi* than to *A. eadyi*, it can be assumed that it originated from Asia (probably Asia

Minor). This assumption is speculative but seems justified because Asia Minor is the centre of diversity of the *A. eadyi* species group, and all three species most likely cohabit naturally there.

The economic importance of species belonging to the *Aphidius eadyi* species group has been considerably reduced after the 1980's because programs for biocontrol of *Acyrthosiphon pisum* concentrated almost exclusively on *A. ervi*. Although *A. ervi* has been shown to be a better competitor than *A. eadyi* and *A. smithi* (Angalet and Fuster, 1977; Cameron and Walker, 1989; McBrien and Mackauer, 1990), the discovery of symbiont-conferred resistance to parasitoids in pea aphid (Oliver *et al.*, 2003) has the potential to compromise the effectiveness of biological control (Vorburger, 2018). Defensive symbionts of the pea aphid can protect the pest from *A. ervi*, an assertion which is confirmed by the results of numerous studies (see Vorburger, 2018). On the other hand, only one study showed possible symbiont-conferred resistance to *A. eadyi* (Ferrari *et al.*, 2004). Results of the present study can serve to clarify the taxonomic status of species belonging to the *A. eadyi* group. They also provide insight into genetic diversity of the three analysed species, something which could be very useful in future biological control strategies. Maintaining high genetic diversity of stock parasitoids is one of the recommendations for future successful biocontrol strategies. High genetic diversity can overcome symbiont-conferred resistance of aphid pests (Vorburger, 2018). *Aphidius banksae*, *A. eadyi*, and *A. smithi* are good candidates for such an approach in biocontrol because they possess relatively high intraspecific genetic diversity.

5. CONCLUSIONS

The spectrum of parasitoids of *Acyrthosiphon pisum* in Europe consists of nine Aphidiinae parasitoids: *Aphidius avenae* Haliday, *A. eadyi*, *A. ervi*, *A. smithi*, *Ephedrus plagiator* (Nees), *Monoctonus nervosus* (Haliday), *P. barbatum* Mackauer, *P. volucre* (Haliday), and *A. banksae*. Among those parasitoids, *Aphidius banksae* was previously overlooked in Europe, and the present study represents the first record of this species in Europe.

Analysing sequences of the COI barcoding region, we determined the existence of three independent taxa within the *Aphidius eadyi* species complex. Clustering of *A. banksae*, *A. eadyi*, and *A. smithi* as separate taxa was confirmed using three different methods of phylogenetic reconstruction (ML, MP, and NJ).

The mean genetic distances between the three species were above the common rate for between-species divergence in the genus *Aphidius* and ranged from 5 to 7.4%. *Aphidius eadyi* and *A. smithi* are genetically closer to each other than to *A. banksae*. Twenty-six different haplotypes were determined within the *Aphidius eadyi* species group, 12 of which belong to *A. banksae* (Abank1-12), six to *A. eadyi* (Aeady1-6), and eight to *A. smithi* (Asmit1-8).

Species discovery methods (the Poisson Tree Process and Automatic Barcode Gap Discovery) revealed genetic discontinuities that might indicate independently evolving lineages within species of the *A. eadyi* group. Both methods labeled haplotypes Asmit2 (within *A. smithi*) and Aeady6 (within *A. eadyi*) as separate entities that could represent hidden cryptic species.

Geometric morphometric analysis applied on the right forewings showed that none of the three species (*A. smithi*, *A. eadyi*, and *A. banksae*) differ in wing size, while all three species differ significantly in shape of the forewing. *Aphidius banksae* is characterized by having relatively shorter and wider wings with a wider proximal part, a more robust stigma, and a longer radial vein, while *A. smithi* has longer wings with a narrower proximal part, a narrower stigma, and a relatively shorter radial vein. Shape of the *A. eadyi* wing is in between.

The geographic distribution of species belonging to the *Aphidius eadyi* group in Europe is determined. *Aphidius smithi* has a Mediterranean distribution, while both *Aphidius eadyi* and *Aphidius banksae* are distributed all over Europe.

The presented results raise questions about the current distribution of biocontrol agents belonging to the *A. eadyi* group in the areas of its introduction (especially in North America). They can be answered by conducting a detailed survey of pea aphid parasitoids. The origin of *A. banksae* and to some extent that of *A. eady* are also questions opened with this study, ones that could be resolved by performing a phylogeographic analysis covering the whole area of distribution of these species.

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8. APPENDIX A

Table of specimens used for geometric morphometric analyses.

ID	Country	Locality	Date	Plant	Aphid	Parasitoid
AF 07-64	Afghanistan	(AF) Kabul		*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
AF 07-65	Afghanistan	(AF) Kabul		*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
AF 07-66	Afghanistan	(AF) Kabul		*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
AF 07-67	Afghanistan	(AF) Kabul		*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
AF 07-69	Afghanistan	(AF) Kabul		*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
AF 07-70	Afghanistan	(AF) Kabul		*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
AF 07-71	Afghanistan	(AF) Kabul		*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
AF 07-72	Afghanistan	(AF) Kabul		*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
AF 07-73	Afghanistan	(AF) Kabul		*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
AF 07-74	Afghanistan	(AF) Kabul		*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
AF 07-75	Afghanistan	(AF) Kabul		*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
AF 07-76	Afghanistan	(AF) Kabul		*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
AF 07-77	Afghanistan	(AF) Kabul		*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
AF 07-78	Afghanistan	(AF) Kabul		*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
AF 07-79	Afghanistan	(AF) Kabul		*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
AF 07-80	Afghanistan	(AF) Kabul		*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
AF 07-81	Afghanistan	(AF) Kabul		*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
AF 07-82	Afghanistan	(AF) Kabul		*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
AF 07-83	Afghanistan	(AF) Kabul		*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
AF 07-84	Afghanistan	(AF) Kabul		*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
CZ 12-111	Czech Republic	(CZ) Kroměříž, Mor. C.	1984	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 12-112	Czech Republic	(CZ) Kroměříž, Mor. C.	1984	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 12-113	Czech Republic	(CZ) Kroměříž, Mor. C.	1984	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*

ID	Country	Locality	Date	Plant	Aphid	Parasitoid
CZ 12-114	Czech Republic	(CZ) Kroměříž, Mor. C.	1984	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 12-115	Czech Republic	(CZ) Kroměříž, Mor. C.	1984	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 12-116	Czech Republic	(CZ) Kroměříž, Mor. C.	1984	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 12-117	Czech Republic	(CZ) Kroměříž, Mor. C.	1984	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 12-118	Czech Republic	(CZ) Kroměříž, Mor. C.	1984	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 12-119	Czech Republic	(CZ) Kroměříž, Mor. C.	1984	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 12-120	Czech Republic	(CZ) Kroměříž, Mor. C.	1984	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 12-121	Czech Republic	(CZ) Kroměříž, Mor. C.	1984	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 12-122	Czech Republic	(CZ) Kroměříž, Mor. C.	1984	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 12-123	Czech Republic	(CZ) Kroměříž, Mor. C.	1984	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 12-124	Czech Republic	(CZ) Kroměříž, Mor. C.	1984	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 12-125	Czech Republic	(CZ) Kroměříž, Mor. C.	1984	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 12-126	Czech Republic	(CZ) Kroměříž, Mor. C.	1984	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 14-143	Czech Republic	(CZ) Průhonice, Boh.	1982	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 14-144	Czech Republic	(CZ) Průhonice, Boh.	1982	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 14-145	Czech Republic	(CZ) Průhonice, Boh.	1982	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 14-146	Czech Republic	(CZ) Průhonice, Boh.	1982	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*

ID	Country	Locality	Date	Plant	Aphid	Parasitoid
CZ 14-147	Czech Republic	(CZ) Průhonice, Boh.	1982	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 14-148	Czech Republic	(CZ) Průhonice, Boh.	1982	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 14-150	Czech Republic	(CZ) Průhonice, Boh.	1982	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 14-151	Czech Republic	(CZ) Průhonice, Boh.	1982	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 14-152	Czech Republic	(CZ) Průhonice, Boh.	1982	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 14-153	Czech Republic	(CZ) Průhonice, Boh.	1982	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 14-154	Czech Republic	(CZ) Průhonice, Boh.	1982	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 14-155	Czech Republic	(CZ) Průhonice, Boh.	1982	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 14-156	Czech Republic	(CZ) Průhonice, Boh.	1982	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 14-157	Czech Republic	(CZ) Průhonice, Boh.	1982	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 14-158	Czech Republic	(CZ) Průhonice, Boh.	1982	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 14-159	Czech Republic	(CZ) Průhonice, Boh.	1982	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 21- 274	Czech Republic	(CZ) Insectary Riverside		*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 21- 275	Czech Republic	(CZ) Insectary Riverside		*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 21- 276	Czech Republic	(CZ) Insectary Riverside		*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 21- 277	Czech Republic	(CZ) Insectary Riverside		*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 21- 278	Czech Republic	(CZ) Insectary Riverside		*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*

ID	Country	Locality	Date	Plant	Aphid	Parasitoid
CZ 21- 279	Czech Republic	(CZ) Insectary Riverside		*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 21- 280	Czech Republic	(CZ) Insectary Riverside		*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 21- 281	Czech Republic	(CZ) Insectary Riverside		*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 21- 282	Czech Republic	(CZ) Insectary Riverside		*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 21- 283	Czech Republic	(CZ) Insectary Riverside		*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 21- 284	Czech Republic	(CZ) Insectary Riverside		*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 21- 285	Czech Republic	(CZ) Insectary Riverside		*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
CZ 21- 286	Czech Republic	(CZ) Insectary Riverside		*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
IR 09-100	Iran	(IR) karaj, lab culture	1977	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
IR 09-244	Iran	(IR) karaj, lab culture	1977	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
IR 09-245	Iran	(IR) karaj, lab culture	1977	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
IR 09-246	Iran	(IR) karaj, lab culture	1977	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
IR 09-247	Iran	(IR) karaj, lab culture	1977	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
IR 09-248	Iran	(IR) karaj, lab culture	1977	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
IR 09-249	Iran	(IR) karaj, lab culture	1977	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
IR 09-250	Iran	(IR) karaj, lab culture	1977	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
IR 09-251	Iran	(IR) karaj, lab culture	1977	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
IR 09-252	Iran	(IR) karaj, lab culture	1977	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
IR 09-253	Iran	(IR) karaj, lab culture	1977	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
IR 09-96	Iran	(IR) karaj, lab culture	1977	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
IR 09-97	Iran	(IR) karaj, lab culture	1977	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
IR 09-98	Iran	(IR) karaj, lab culture	1977	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
IS 05-200	Israel	(IS) Beir She 'an, insectary Riverside	1979	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*

ID	Country	Locality	Date	Plant	Aphid	Parasitoid
IS 05-36	Israel	(IS) Beir She 'an, insectary Riverside	1979	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
IS 05-37	Israel	(IS) Beir She 'an, insectary Riverside	1979	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
IS 05-38	Israel	(IS) Beir She 'an, insectary Riverside	1979	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
IS 05-39	Israel	(IS) Beir She 'an, insectary Riverside	1979	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
IS 05-40	Israel	(IS) Beir She 'an, insectary Riverside	1979	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
IS 05-41	Israel	(IS) Beir She 'an, insectary Riverside	1979	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
IS 05-42	Israel	(IS) Beir She 'an, insectary Riverside	1979	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
IS 05-43	Israel	(IS) Beir She 'an, insectary Riverside	1979	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
IS 05-44	Israel	(IS) Beir She 'an, insectary Riverside	1979	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
IS 05-45	Israel	(IS) Beir She 'an, insectary Riverside	1979	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
IS 05-46	Israel	(IS) Beir She 'an, insectary Riverside	1979	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
IS 05-47	Israel	(IS) Beir She 'an, insectary Riverside	1979	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
IS 05-48	Israel	(IS) Beir She 'an, insectary Riverside	1979	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
IS 05-49	Israel	(IS) Beir She 'an, insectary Riverside	1979	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
SE 01-01	Serbia	(SE) Umčari	8.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 01-03	Serbia	(SE) Umčari	8.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 01-05	Serbia	(SE) Umčari	8.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 01-06	Serbia	(SE) Umčari	8.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 01-07	Serbia	(SE) Umčari	8.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*

ID	Country	Locality	Date	Plant	Aphid	Parasitoid
SE 01-201	Serbia	(SE) Umčari	8.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 01-203	Serbia	(SE) Umčari	8.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 01-204	Serbia	(SE) Umčari	8.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 01-205	Serbia	(SE) Umčari	8.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 01-206	Serbia	(SE) Umčari	8.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 01-207	Serbia	(SE) Umčari	8.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 01-208	Serbia	(SE) Umčari	8.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 01-209	Serbia	(SE) Umčari	8.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 01-255	Serbia	(SE) Umčari	8.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 02-09	Serbia	(SE) Malo Orašje	7.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 02-10	Serbia	(SE) Malo Orašje	7.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 02-11	Serbia	(SE) Malo Orašje	7.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 02-13	Serbia	(SE) Malo Orašje	7.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 02-14	Serbia	(SE) Malo Orašje	7.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 02-16	Serbia	(SE) Malo Orašje	7.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 02-210	Serbia	(SE) Malo Orašje	7.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 02-211	Serbia	(SE) Malo Orašje	7.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 02-212	Serbia	(SE) Malo Orašje	7.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 02-213	Serbia	(SE) Malo Orašje	7.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 02-214	Serbia	(SE) Malo Orašje	7.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 02-215	Serbia	(SE) Malo Orašje	7.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 02-216	Serbia	(SE) Malo Orašje	7.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 02-217	Serbia	(SE) Malo Orašje	7.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 03-18	Serbia	(SE) Živkovac	3.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
SE 03-19	Serbia	(SE) Živkovac	3.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
SE 03-20	Serbia	(SE) Živkovac	3.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
SE 03-21	Serbia	(SE) Živkovac	3.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*

ID	Country	Locality	Date	Plant	Aphid	Parasitoid
SE 03-218	Serbia	(SE) Živkovac	3.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
SE 03-219	Serbia	(SE) Živkovac	3.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
SE 03-220	Serbia	(SE) Živkovac	3.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
SE 03-221	Serbia	(SE) Živkovac	3.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
SE 03-222	Serbia	(SE) Živkovac	3.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
SE 03-223	Serbia	(SE) Živkovac	3.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
SE 03-224	Serbia	(SE) Živkovac	3.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
SE 03-225	Serbia	(SE) Živkovac	3.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
SE 03-23	Serbia	(SE) Živkovac	3.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
SE 03-24	Serbia	(SE) Živkovac	3.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
SE 04-226	Serbia	(SE) Reka	6.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 04-227	Serbia	(SE) Reka	6.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 04-228	Serbia	(SE) Reka	6.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 04-229	Serbia	(SE) Reka	6.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 04-230	Serbia	(SE) Reka	6.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 04-231	Serbia	(SE) Reka	6.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 04-232	Serbia	(SE) Reka	6.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 04-233	Serbia	(SE) Reka	6.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 04-234	Serbia	(SE) Reka	6.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius eadyi*
SE 05-25	Serbia	(SE) Reka	6.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
SE 05-26	Serbia	(SE) Reka	6.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*

ID	Country	Locality	Date	Plant	Aphid	Parasitoid
SE 05-27	Serbia	(SE) Reka	6.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
SE 05-28	Serbia	(SE) Reka	6.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
SE 05-288	Serbia	(SE) Reka	6.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
SE 05-29	Serbia	(SE) Reka	6.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
SE 05-30	Serbia	(SE) Reka	6.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
SE 05-32	Serbia	(SE) Reka	6.VI.2012	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius banksae*
SP 13-128	Spain	(SP) Cordoba, Gonzalez	3.VII.1981	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
SP 13-129	Spain	(SP) Cordoba, Gonzalez	3.VII.1981	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
SP 13-130	Spain	(SP) Cordoba, Gonzalez	3.VII.1981	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
SP 13-131	Spain	(SP) Cordoba, Gonzalez	3.VII.1981	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
SP 13-132	Spain	(SP) Cordoba, Gonzalez	3.VII.1981	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
SP 13-133	Spain	(SP) Cordoba, Gonzalez	3.VII.1981	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
SP 13-134	Spain	(SP) Cordoba, Gonzalez	3.VII.1981	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
SP 13-135	Spain	(SP) Cordoba, Gonzalez	3.VII.1981	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
SP 13-136	Spain	(SP) Cordoba, Gonzalez	3.VII.1981	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
SP 13-137	Spain	(SP) Cordoba, Gonzalez	3.VII.1981	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
SP 13-138	Spain	(SP) Cordoba, Gonzalez	3.VII.1981	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
SP 13-139	Spain	(SP) Cordoba, Gonzalez	3.VII.1981	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
SP 13-140	Spain	(SP) Cordoba, Gonzalez	3.VII.1981	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
SP 13-141	Spain	(SP) Cordoba, Gonzalez	3.VII.1981	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
SP 13-190	Spain	(SP) Cordoba, Gonzalez	3.VII.1981	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
SP 13-191	Spain	(SP) Cordoba, Gonzalez	3.VII.1981	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
SP 13-192	Spain	(SP) Cordoba, Gonzalez	3.VII.1981	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
SP 13-193	Spain	(SP) Cordoba, Gonzalez	3.VII.1981	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
TU 16-260	Turkey	(TU) Kayseri et Erzinran	1984	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*

ID	Country	Locality	Date	Plant	Aphid	Parasitoid
TU 16-261	Turkey	(TU) Kayseri et Erzinran	1984	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
TU 16-262	Turkey	(TU) Kayseri et Erzinran	1984	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
TU 16-263	Turkey	(TU) Kayseri et Erzinran	1984	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
TU 16-264	Turkey	(TU) Kayseri et Erzinran	1984	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
TU 16-265	Turkey	(TU) Kayseri et Erzinran	1984	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
TU 16-266	Turkey	(TU) Kayseri et Erzinran	1984	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
TU 16-267	Turkey	(TU) Kayseri et Erzinran	1984	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
TU 16-268	Turkey	(TU) Kayseri et Erzinran	1984	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
TU 16-269	Turkey	(TU) Kayseri et Erzinran	1984	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
TU 16-270	Turkey	(TU) Kayseri et Erzinran	1984	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
TU 16-271	Turkey	(TU) Kayseri et Erzinran	1984	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
TU 16-272	Turkey	(TU) Kayseri et Erzinran	1984	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
TU 16-273	Turkey	(TU) Kayseri et Erzinran	1984	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
TU 16-292	Turkey	(TU) Kayseri et Erzinran	1984	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
TU 16-293	Turkey	(TU) Kayseri et Erzinran	1984	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
TU 16-294	Turkey	(TU) Kayseri et Erzinran	1984	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
US 06-235	USA	(US) Lakeview, CA	1977	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
US 06-236	USA	(US) Lakeview, CA	1977	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
US 06-289	USA	(US) Lakeview, CA	1977	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
US 06-290	USA	(US) Lakeview, CA	1977	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
US 06-291	USA	(US) Lakeview, CA	1977	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
US 06-50	USA	(US) Lakeview, CA	1977	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
US 06-51	USA	(US) Lakeview, CA	1977	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
US 06-52	USA	(US) Lakeview, CA	1977	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
US 06-53	USA	(US) Lakeview, CA	1977	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
US 06-54	USA	(US) Lakeview, CA	1977	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
US 06-55	USA	(US) Lakeview, CA	1977	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
US 06-56	USA	(US) Lakeview, CA	1977	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
US 06-57	USA	(US) Lakeview, CA	1977	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
US 06-58	USA	(US) Lakeview, CA	1977	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*

ID	Country	Locality	Date	Plant	Aphid	Parasitoid
US 06-59	USA	(US) Lakeview, CA	1977	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
US 06-60	USA	(US) Lakeview, CA	1977	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
US 06-61	USA	(US) Lakeview, CA	1977	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
US 06-62	USA	(US) Lakeview, CA	1977	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
UZ 15-160	Uzbekistan	(UZ) Chumsan, lab. Cult.	1976	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
UZ 15-161	Uzbekistan	(UZ) Chumsan, lab. Cult.	1976	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
UZ 15-162	Uzbekistan	(UZ) Chumsan, lab. Cult.	1976	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
UZ 15-163	Uzbekistan	(UZ) Chumsan, lab. Cult.	1976	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
UZ 15-164	Uzbekistan	(UZ) Chumsan, lab. Cult.	1976	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
UZ 15-165	Uzbekistan	(UZ) Chumsan, lab. Cult.	1976	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
UZ 15-166	Uzbekistan	(UZ) Chumsan, lab. Cult.	1976	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
UZ 15-167	Uzbekistan	(UZ) Chumsan, lab. Cult.	1976	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
UZ 15-168	Uzbekistan	(UZ) Chumsan, lab. Cult.	1976	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
UZ 15-169	Uzbekistan	(UZ) Chumsan, lab. Cult.	1976	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
UZ 15-170	Uzbekistan	(UZ) Chumsan, lab. Cult.	1976	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
UZ 15-172	Uzbekistan	(UZ) Chumsan, lab. Cult.	1976	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
UZ 15-173	Uzbekistan	(UZ) Chumsan, lab. Cult.	1976	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
UZ 15-174	Uzbekistan	(UZ) Chumsan, lab. Cult.	1976	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
UZ 15-175	Uzbekistan	(UZ) Chumsan, lab. Cult.	1976	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
UZ 15-176	Uzbekistan	(UZ) Chumsan, lab. Cult.	1976	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*
UZ 15-177	Uzbekistan	(UZ) Chumsan, lab. Cult.	1976	*Medicago sativa*	*Acyrthosiphon pisum*	*Aphidius smithi*

BIOGRAPHY OF THE AUTHOR

Mustafa (Elhadi) Ghaliow was born on May 1st, 1970 in Cairo, Egypt. He studied at the Faculty of Veterinary Medicine, Omar Almokhtar University, Bayda, Libya, and graduated in 1996. Mustafa completed his postgraduate program in 2006 at the October 7th University, Misurata, Libya.

Since July 1998 till August 2003 he has was employed as a veterinary doctor in Taworgha complex for cattle and poultry, Misurata, Libya. Later on, in period January 2004 to July 2006 he has worked as a veterinary doctor in Environment affairs office, General People's Committee for Housing Facilities and Environment, Misurata, Libya.

From 2006 he become a staff member in Biology Department, College of Education at October 7th University, Misurata, Libya, where he get expiriance as lecturer.

In 2011, he started PhD studies at the Department of Invertebrate Zoology and Entomology at the Faculty of Biology, University of Belgrade.

He has published in journals such as Zootaxa, Archives of Biological Sciences, Bulletin of Entomological Research.

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

Since 2018 he becomes lecturer Staff member in Biology Department, Faculty of Biology, Misurata University, Misurata, Libya.

He can be contacted at: m.ghaliow@sci.misuratau.edu.ly

Прилог 1.

Изјава о ауторству

Потписани-а Mustafa (Elhadi) Ghaliow

број уписа B3065/2011

Изјављујем

да је докторска дисертација под насловом

„Морфолошка и молекуларна карактеризација врста *Aphidius eadyi* комплекса (Hymenoptera, Braconidae, Aphidiinae), паразитоида зелене луцеркине ваши – *Acyrthosiphon pisum* Harr. (Hemiptera, Aphididae)“

- резултат сопственог истраживачког рада,
- да предложена дисертација у целини ни у деловима није била предложена за добијање било које дипломе према студијским програмима других високошколских установа,
- да су резултати коректно наведени и
- да нисам кршио/ла ауторска права и користио интелектуалну својину других лица.

Потпис докторанда

У Београду, 20.8.2018.

_____

Прилог 2.

Изјава о истоветности штампане и електронске верзије докторског рада

Име и презиме аутора Mustafa E. Ghaliow

Број уписа B3065/2011

Студијски програм Биологија

Наслов рада Морфолошка и молекуларна карактеризација врста *Aphidius eadyi* комплекса (Hymenoptera, Braconidae, Aphidiinae), паразитоида зелене луцеркине ваши – *Acyrthosiphon pisum* Harr. (Hemiptera, Aphididae)

Ментор проф. Др Жељко Томановић

Потписани Mustafa E. Ghaliow

изјављујем да је штампана верзија мог докторског рада истоветна електронској верзији коју сам предао/ла за објављивање на порталу **Дигиталног репозиторијума Универзитета у Београду.**

Дозвољавам да се објаве моји лични подаци везани за добијање академског звања доктора наука, као што су име и презиме, година и место рођења и датум одбране рада.

Ови лични подаци могу се објавити на мрежним страницама дигиталне библиотеке, у електронском каталогу и у публикацијама Универзитета у Београду.

Потпис докторанда

У Београду, 20.8.2018.

_____

Прилог 3.

Изјава о коришћењу

Овлашћујем Универзитетску библиотеку „Светозар Марковић“ да у Дигитални репозиторијум Универзитета у Београду унесе моју докторску дисертацију под насловом:

Морфолошка и молекуларна карактеризација врста *Aphidius eadyi* комплекса (Hymenoptera, Braconidae, Aphidiinae), паразитоида зелене луцеркине ваши – *Acyrthosiphon pisum* Harr. (Hemiptera, Aphididae)

која је моје ауторско дело.

Дисертацију са свим прилозима предао/ла сам у електронском формату погодном за трајно архивирање.

Моју докторску дисертацију похрањену у Дигитални репозиторијум Универзитета у Београду могу да користе сви који поштују одредбе садржане у одабраном типу лиценце Креативне заједнице (Creative Commons) за коју сам се одлучио/ла.

1. Ауторство

2. Ауторство - некомерцијално

3. Ауторство – некомерцијално – без прераде

4. Ауторство – некомерцијално – делити под истим условима

5. Ауторство – без прераде

6. Ауторство – делити под истим условима

(Молимо да заокружите само једну од шест понуђених лиценци, кратак опис лиценци дат је на полеђини листа).

Потпис докторанда

У Београду, 20.8.2018.

_____

1. Ауторство - Дозвољавате умножавање, дистрибуцију и јавно саопштавање дела, и прераде, ако се наведе име аутора на начин одређен од стране аутора или даваоца лиценце, чак и у комерцијалне сврхе. Ово је најслободнија од свих лиценци.

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3. Ауторство - некомерцијално – без прераде. Дозвољавате умножавање, дистрибуцију и јавно саопштавање дела, без промена, преобликовања или употребе дела у свом делу, ако се наведе име аутора на начин одређен од стране аутора или даваоца лиценце. Ова лиценца не дозвољава комерцијалну употребу дела. У односу на све остале лиценце, овом лиценцом се ограничава највећи обим права коришћења дела.

4. Ауторство - некомерцијално – делити под истим условима. Дозвољавате умножавање, дистрибуцију и јавно саопштавање дела, и прераде, ако се наведе име аутора на начин одређен од стране аутора или даваоца лиценце и ако се прерада дистрибуира под истом или сличном лиценцом. Ова лиценца не дозвољава комерцијалну употребу дела и прерада.

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6. Ауторство - делити под истим условима. Дозвољавате умножавање, дистрибуцију и јавно саопштавање дела, и прераде, ако се наведе име аутора на начин одређен од стране аутора или даваоца лиценце и ако се прерада дистрибуира под истом или сличном лиценцом. Ова лиценца дозвољава комерцијалну употребу дела и прерада. Слична је софтверским лиценцама, односно лиценцама отвореног кода.