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Dezene Huber / 20 Mar 2017

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Morphological variation of *Aphidius ervi* Haliday (Hymenoptera: Braconidae) associated to different aphid hosts

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Background. Parasitoids are frequently used in biological control due to the fact that they are considered host specific and highly efficient attacking their hosts. As they spend a significant part of their life cycle within their hosts, feeding habits and life history of their host can promote specialization via host-race formation (sequential radiation). The specialized host races from different hosts can vary morphologically, behaviorally and genetically. However, these variations are sometimes inconspicuous and require more powerful tools in order to detect variation such as geometric morphometrics analysis.

Methods. We examined the case of *Aphidius ervi*, an important introduced biological control agent in Chile associated to a great number of aphid species which are exploiting different plant hosts and habitats. Several combinations (biotypes) of aphid/host plant originated parasitoids were analyzed in order to obtain measures of forewing shape and size. To show the differences among defined biotypes we chose 13 homologous landmarks on each individual parasitoid wing. The analysis of allometric variation calculated in wing shape and size over centroid size (CS) revealed the allometric changes among biotypes collected from different hosts. To show all differences in shape of forewings we made seven biotype pairs using the outline-based geometric morphometrics comparison.

Results. The biotype *A. pis_pea* (*Acyrthosiphon pisum* on pea) was the extreme wing size in this study compared to the other analyzed biotypes. Aphid hosts have a significant influence in the morphological differentiation of the forewing, splitting biotypes in two groups. The first group consisted of biotypes connected with *Acyrthosiphon pisum* on legumes, while the second group is composed of biotypes connected with aphids attacking cereals with an exception of the *R. pad_wheat* (*Rhopalosiphum padi* on wheat) biotype. There were no significant plant species effect on wing size and shape.

Discussion. Although there are indications that suggest that the genotype of parasitoids is of greater significance on the morphological variations of size and shape of wings, this study indicates that the aphid host for *A. ervi* alter in a significant way the structure of forewings, excluding variation between genotypes, due largely to the low genetic variability of *A. ervi* populations in Chile when comparing between geographical areas and aphid hosts. Bigger aphid host implied shape difference in the forewing explained as longer and broader wings of *A. ervi*, as well as the size differences.

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Abstract

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Introduction

Parasitoids are frequently used in biological control as they are considered ~~as~~ highly specialized natural enemies (Godfray, 1994). By being highly specialized, released parasitoids will be the most efficient at attacking the target pest species, reducing the possibility of environmental harm through spillover of rapidly-growing parasitoid populations from crops into adjacent natural habitats (Rand et al., 2006), as has been observed for generalist predators (Duelli et al., 1990; French et al., 2001). Although~~,~~ many highly specialized parasitoid species have a great host range (Mackauer and Starý, 1967) exploiting many hosts may not be consistent across an entire species, and different biotypes may be specialized to different host/environments (Stireman et al., 2006; Forbes et al., 2009). Previous studies have shown that host-associated biotypes of parasitoids from different ~~host~~/environments can vary morphologically, behaviorally and genetically (Žikić et al., 2009; Feder and Forbes, 2010; Kos et al., 2012; Zepeda-Paulo et al., 2013). In terms of morphological features, the shape and size of their ~~appendices~~ have shown great promise for separating host-associated races of parasitoids. Among these, insect wings have been especially relevant as they are two dimensional structures with important characteristics, in terms of adaptation and function (Wootton, 2002; Žikić et al., 2009). ~~This is how~~ previous studies have shown that the size, shape and venation of the wings can be important features to separate species and characterize populations within a single species (Sadeghi et al., 2009). ~~Geometric~~ morphometrics approach is very useful for detecting minute variations ~~on~~ morphology of different parasitoid populations which otherwise cannot be identified easily (Villemant et al., 2007; Žikić et al., 2009; Kos et al., 2011). This can be of high importance because these morphological variations in wing shape could be associated ~~to~~ a specific environment or host-associated population of a parasitoid species.

The Chilean populations of *Aphidius ervi* H. may be a good example where different host association and environment could have had ~~some~~ influence on morphology. This species is an oligophagous parasitoid associated with several aphid~~s~~ species, such as *Acyrthosiphon pisum* H.

on legumes, *Acyrthosiphon kondoi* Shinji on legumes, *Macrosiphum euphorbiae* T., *Aulacorthum solani* K. on Solanaceae (Takada and Tada, 2000) and cereal aphids such are *Sitobion avenae* F., *Rhopalosiphum padi* L., *Schizaphis graminum* R. and *Metopolophium dirhodum* W. (Starý, 1993). *Aphidius ervi* was introduced in Chile in the 1970's as part of a classical biological control in order to minimize the damage provoked by the grain aphid (*S. avenae*) on cereals and maintain the pest population under low densities in the field (Zúñiga et al., 1986). Nowadays, *A. ervi* is the ~~most~~ predominant parasitoid species controlling *A. pisum* and *S. avenae* (more than 94% of prevalence on *A. pisum* on legumes and 38% of prevalence on *S. avenae* on cereals) and considered a highly efficient biological control example of aphids on both crops (Gerding et al., 1989; Starý et al., 1994; Zepeda-Paulo et al., 2013). The main goal of the present study is to analyze the shape and size of forewings of *A. ervi* collected in different plant/host~~/parasitoid~~ associations, on legumes and cereals.

Materials & Methods

Sampled material

Aphids were collected from fields of legumes and cereals in two different geographic regions of central Chile: "Región de los Rios" (S 39° 51´, W 73° 7´) and "Región del Maule" (S 35° 24´, W 71° 40´). Parasitoids were obtained from presumably parasitized aphids collected in the field, and after ~~the~~ emergence carefully examined and identified. Reared samples were transferred in the growing laboratory and treated under following conditions: 20°C, 50-60 RH, D16:N8 of photoperiod. Parasitoid wasps were put in plastic microtubes with 96% ~~of~~ ethyl alcohol. The identification was done using ~~adequate~~ taxonomic keys (Starý, 1995).

A total of 131 females of *Aphidius ervi* were analyzed. All parasitoids are divided into eight biotypes according to their aphid hosts and to the plant species where the aphids were found (Table 1). The alfalfa biotype was reared from *Acyrthosiphon pisum* and sampled on alfalfa fields (*Medicago sativa* L.), the pea biotype from pea (*Pisum sativum* L.), and the clover biotype from red clover (*Trifolium pratense* L.). Biotypes reared on cereals ~~are~~ the bird cherry-oat aphid (*Rhopalosiphum padi*), the rose grain aphid (*Metopolophium dirhodum*) the green-bug (*Schizaphis graminum*), and the grain aphid (*Sitobion avenae* F.) sampled from wheat (*Triticum* *aestivum* L.). Another cereal biotype is also the grain aphid (*Sitobion avenae* F.) which was collected from oat (*Avena sativa* L.) (Table 1).

Geometric morphometrics

To conduct the ~~G~~eometric morphometrics analysis, we applied two dimensional landmark-based methods (Bookstein, 1986; 1991). Right forewings of each female parasitoid ~~was~~ removed and mounted in ~~the~~ Neo Mount (Merck) following procedure ~~as~~ described in Žikić et al. (2009). ~~Such~~ prepared, forewings were recorded using OPTIKA SZN (45x) stereoscopic compound microscope with a mounted 5 ~~M~~pixel photographic camera using software Optika Vision Pro v2.7. Using Geometric morphometrics method (Zelditch et al., 2004) we ~~tried to~~ ~~determine~~ and ~~quantify~~ morphological variation ~~of~~ different *Aphidius ervi* biotypes ~~such as wing size and shape~~.

Eight different aphid-host~~s~~/plant-host associations were used for morphological characterization of *A. ervi* biotypes (Table 1). To analyze the variation in wing shape on parasitoids, 13 homologous landmarks were scored for each analyzed forewing. Positioned landmarks were digitized using software TpsDig v2.16 (Rohlf, 2010) (Figure 1, Table 2). In order to analyze and visualize the variations, software MorphoJ v1.06b was used (Klingenberg, 2011).

Results

To test variability of the forewings from the different *A. ervi* biotypes a Principal Component Analysis (PCA) was carried out. According to PCA the variability explained by the first three axes was rather low; all three explain 50.6% of the total variability. However, after testing for variance, the results were statistically significant (Procrustes ANOVA: $F = 17.30$; $df = 7$; $P < 0.000001$). Beside the forewing size, even multivariate analysis of shape using PC scores showed statistical significance (MANOVA: Wilks' $\lambda = 0.112737$; $F= 1.74$; $df =154$; $P < 0.000001$). Considering that all statistical tests of variance were statistically significant, we performed Canonical ~~Variant~~ Analysis (CVA) in order to observe the variability among the *A. ervi* biotypes. However, still there was no conspicuous grouping of the biotypes. The first canonical axis (CV1) explains 38.4%, while the second axis (CV2) explains only 23% of the total variability. To see if there was some correlation between the wing size and shape we performed the regression test between the Centroid size and PC scores. ~~From the graph (Fig. 2) it is evident that the wing~~ shape is clearly correlated with the wing size (P-value: < 0.0001). The percentage of the variability explained by this regression test is 6.78 % (% predicted: 6.7783%). ~~On the graph (Fig. 2) it seems obvious that the~~ biggest wings were of the specimens from the biotype *A. pis_pea*.

Considering that the regression result was statistically significant (P-value: <0.0001) we performed Discriminant Analysis (DA) using the residuals. This particular analysis showed that none of the Procrustes distances were statistically significant (P-value: >0.05), and therefore the changes of the wing shape are due to the change of the wing size. Therefore, we wanted to visualize how the wings of all other *A. ervi* biotypes change in relation to this particular biotype (*A. pis_pea*) using outline-based geometric morphometric methods (Fig. 3). The changes between the biotype *A. pis_pea* and the other six can be ~~tracked form the~~ Figure 3.

The least changes of the wing shape and size are in the following relations: *A. pis_pea*/*A. pis_alfalfa*, *A. pis_pea*/*A. pis_clover* and *A. pis_pea*/*R. pad_wheat*. More conspicuous changes are visible in the relation *A. pis_pea*/*S. ave_oat*, ~~than~~ *A. pis_pea*/*S. ave_wheat*. These changes are due to the narrowing of the wing in the two biotypes (*S. ave_oat* and *S. ave_wheat*). The greatest difference is between the biotype *A. pis_pea* and *S. gra_wheat* where this biotype has the narrowest wing in relation to *A. pis_pea*.

Discussion

Aphidius ervi is known to attack economically important pests in the Chilean agricultural landscapes and is considered a successful example of classical biological control of legume and cereal aphids (Starý, 1993; Starý et al., 1993; Rojas, 2005). Although it is very efficient in parasitizing target aphid pests, it has little or no effect in attacking native aphid species in shared environments (e.g: *Uroleucon* species developing on native plants in and around agricultural valleys in Chile) (Zúñiga et al., 1986; Starý, 1993). ~~However,~~ many studies have shown heritable host fidelity and have hypothesized the possibility of different host associated biotypes. Recent studies of Bilodeau et al. (2013) and Zepeda-Paulo et al. (2013) using population genetics suggest~~s~~ that in both North America and Chile there are no specialized races or biotypes on different aphid-host species, revealing high gene flow between aphid-host originated parasitoid populations.

~~However, in~~ a recent study it has been shown that the parasitoid genotype can have a stronger influence on wing shape compared to the effect of developing on different parasitoid host species (Parreño et al. 2016). These authors used five asexual lines of *Lysiphlebus fabarum* and four aphid hosts, and using the Procrustes Coordinates on wings, found that the lineages were the better grouping factor compared to the parasitoid aphid-host variable. In this study we did not discover any drastic morphological features which could conspicuously differentiate the Chilean population of *A. ervi*. This is probably due to the very short period i.e. insufficient number of generations among these particular Chilean populations. ~~This is obvious from the fact that analyzed specimens of *A. ervi* from various aphid hosts and different localities throughout Chile exhibit a rather low genetic variability~~ (Zepeda-Paulo et al., 2016).

One of the most conspicuous differences among the eight biotypes of *A. ervi* analyzed is in the shape and size of forewings from the biotype *A. pis_pea*. Specimens of this particular biotype have generally larger forewings than other biotypes and somewhat broader in the middle and the distal part (Figures 2 and 3). Of all investigated biotypes the most similar are those which were reared from *Acyrthosiphon pisum* (*A. pis_alfalfa*, *A. pis_clover* and *A. pis_pea*) independent of the aphid clone (host-plant), ~~which is at the same time the~~ biggest aphid, when compared to all other aphid species mentioned here. The differences of the wing size and shape begin to appear in those biotypes reared on cereals, with the exception of the *R. pad_wheat* biotype where the differences were small (Fig. 3). This could be probably the effect of the aphid host size, because the *Acyrthosiphon pisum* is rather a large aphid when comparing to *R. padi.* Analyzing the relation of wing shape and size between the biotypes *A. pis_pea* and *Sc. gra_wheat* (Fig. 3), there are clear differences. At the same time, the differences in the body size are probably the greatest between *Acyrthosiphon pisum* and *Schizaphis graminum* which is rather a small aphid host for *Aphidius ervi*. This suggests that beside the host plant, the aphid host has ~~primal mayor~~ influence in the morphological differentiation of the forewing among the analyzed biotypes, as it has been shown in other studies with individuals of the genus *Eubazus*, a parasitoids of the conifer bark weevil (Villemant et al., 2007) and with the parasitoid *Lysiphlebus fabarum* (Parreño et al. 2016). Additionally, the effect of plant species should not be neglected because there are clear preference of *A. ervi* biotypes toward particular plant species i.e. its volatiles which will eventually lead them to the adequate aphid host (Daza-Bustamante et al., 2002).

Variations of the shape of insect wings are known to affect flight ability, which in turn could alter the host and mate allocation (Kölliker-Ott et al., 2003). Betts and Wootton (1988) studied the effects of wing structure on the flight of six butterfly species and showed that there was a correlation between flight performance and wing shape. Additionally, studies have described how the wing shape can alter predation success by dragonflies (Combes et a., 2010) and also, the ability of damselflies to avoid predation by passerine birds (Outomuro and Johansson, 2015). More specifically, parasitoids are also affected by the changes in wing size and shape. The wing size and shape of *Trichogramma brassicae* and *T. pretiosum* as egg parasitoids, increase the ability to locate host eggs. Differences in wing size and shape were found between parasitoid obtained from field conditions compared to those parasitoids that were reared in laboratory (Kölliker-Ott et al., 2003). Authors suggest that wing shape and wing size can be reliable predictors of field fitness for these parasitoid species. In the present study, the biotypes of *A. ervi* emerged from *A. pisum* ~~encounter~~ larger and broader forewings compared to the other studied biotypes. These differences of wing shape and size could be reflected on the fitness of *A. ervi* to control pest increasing the ability to find aphid host as size and shape of forewings increased. Further research to determine most suitable aphid host for *A. ervi* to increase its fitness will lead to enhance rearing conditions for *A. ervi* and consequently, ~~to~~ improve any inundative biological control strategies with this parasitoid.

Conclusion

Given that there is a low genetic variability of *A. ervi* in Chile and that there was little effect of plant species on morphological features in this study, the main factor affecting morphological variations of *A. ervi* forewings is the size of their aphid host.

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FIGURES

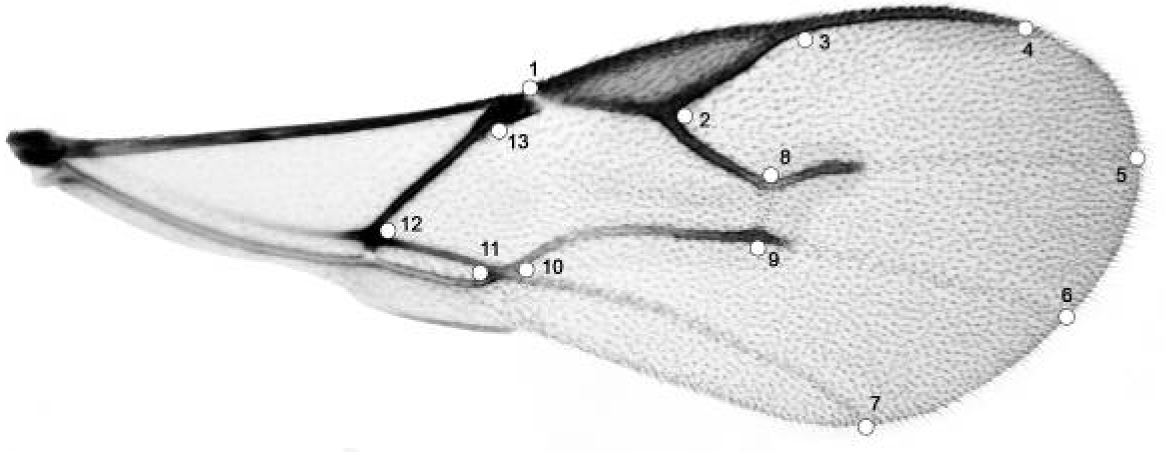

Figure 1. Right forewing of *Aphidius ervi*; set of 13 homologous landmarks.

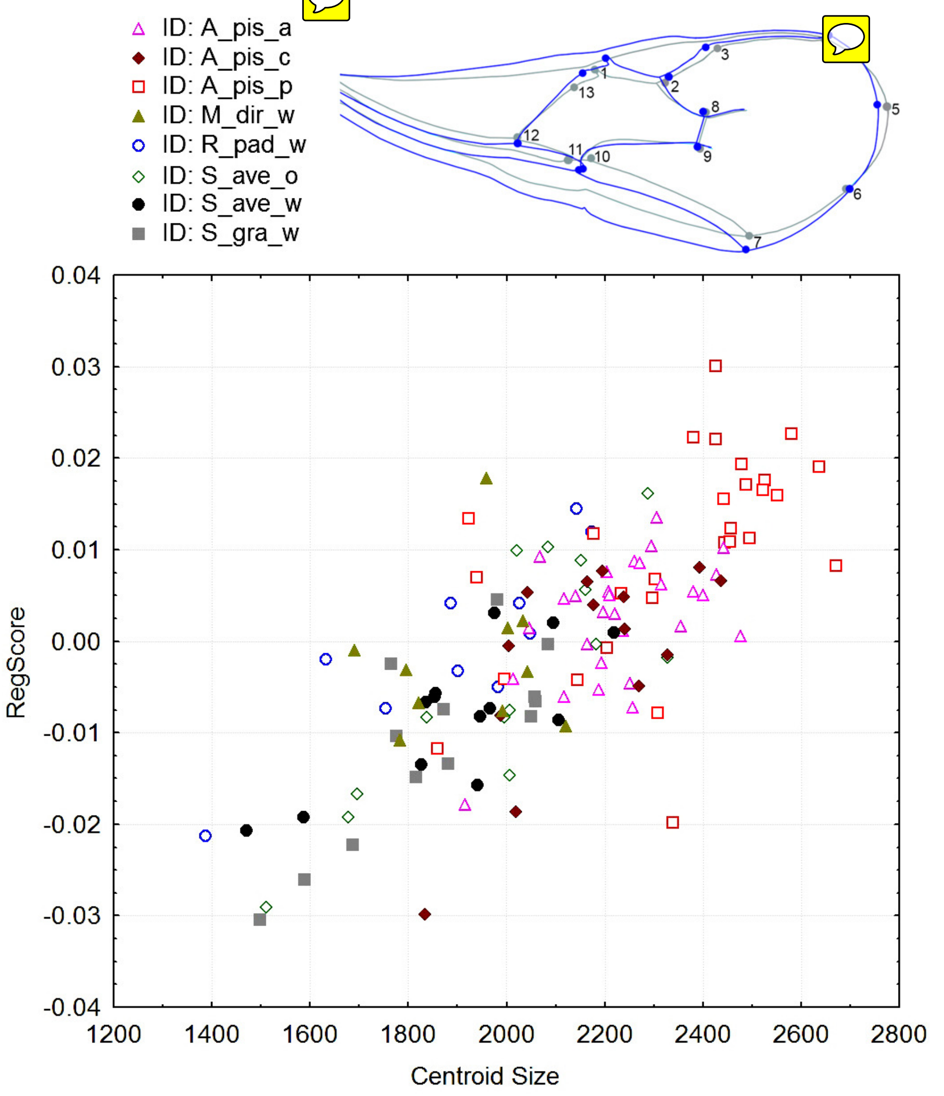

Figure 2. The regression results of the Centroid Size (CS) and PC scores (Permutation test against the null hypothesis of independence, P-value: <0.0001).

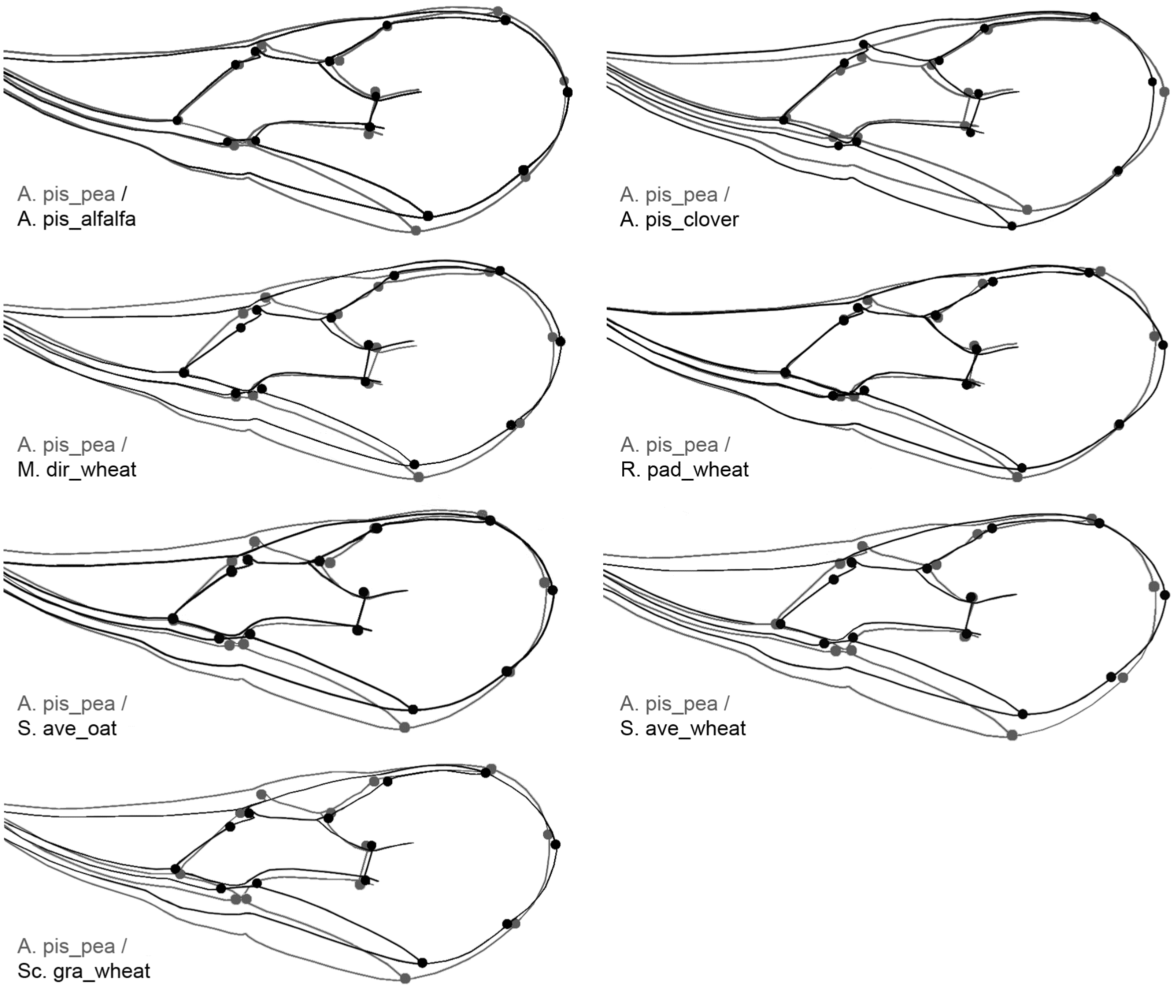

Figure 3. Outline-based comparison of the wing shape between the biotype *A. pis_pea* and the rest seven biotypes. Shape differences are the results of Discriminant Analysis (DA). The scale factor is increased by 5. Grey color of outline represents the biotype *A. pis_pea*; black color of outline represent compared biotypes.

TABLES

Table 1. Sampled material of *Aphidius ervi* and defined biotypes.

Aphid host	**Host-plant**	**N° of specimens**	**Biotype**
Acyrthosiphon pisum	alfalfa	29	*A. pis_alfalfa*
Acyrthosiphon pisum	pea	28	*A. pis_pea*
Acyrthosiphon pisum	red clover	14	*A. pis_clover*
Metopolophium dirhodum	wheat	10	*M. dir_wheat*
Rhopalosiphum padi	wheat	10	*R. pad_wheat*
Schizaphis graminum	wheat	13	*Sc. gra_wheat*

Table 2. Description of homologous landmarks of forewing. Wing veins terminology follows Wharton et al. (1997).

Landmark number	**Landmark definition**
1	beginning of stigma
2	corner at the middle of stigma and r vein
3	end of stigma
4	end of metacarpus
5	projection of RS vein on the edge of wing
6	projection of M vein on the edge of wing
7	projection of CU vein on the edge of wing
8	corner of RS and r-m veins
9	corner of M and r-m veins
10	corner of m-cu and 1CU veins
11	corner of 1CU and 1A veins
12	corner of 1M and 1CU
13	beginning of parastigma