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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18   **Abstract**

19

20   **Background.** Parasitoids are frequently used in biological control due to the fact that they are

21   considered host specific and highly efficient attacking their hosts. As they spend a significant

22   part of their life cycle within their hosts, feeding habits and life history of their host can promote

23   specialization via host-race formation (sequential radiation). The specialized host races from

24   different hosts can vary morphologically, behaviorally and genetically. However, these

25   variations are sometimes inconspicuous and require more powerful tools in order to detect

26   variation such as geometric morphometrics analysis.

27   **Methods.** We examined the case of *Aphidius ervi*, an important introduced biological control

28   agent in Chile associated to a great number of aphid species which are exploiting different plant

29   hosts and habitats. Several combinations (biotypes) of aphid/host plant originated parasitoids

30   were analyzed in order to obtain measures of forewing shape and size. To show the differences

31   among defined biotypes we chose 13 homologous landmarks on each individual parasitoid wing.

32   The analysis of allometric variation calculated in wing shape and size over centroid size (CS)

33   revealed the allometric changes among biotypes collected from different hosts. To show all

34   differences in shape of forewings we made seven biotype pairs using the outline-based geometric

35   morphometrics comparison.

36   **Results.** The biotype *A. pis_pea* (*Acyrthosiphon pisum* on pea) was the extreme wing size in this

37   study compared to the other analyzed biotypes. Aphid hosts have a significant influence in the

38   morphological differentiation of the forewing, splitting biotypes in two groups. The first group

39   consisted of biotypes connected with *Acyrthosiphon pisum* on legumes, while the second group

40   is composed of biotypes connected with aphids attacking cereals with an exception of the *R.*

41   *pad_wheat* (*Rhopalosiphum padi* on wheat) biotype. There were no significant plant species

42   effect on wing size and shape.

43   **Discussion.** Although there are indications that suggest that the genotype of parasitoids is of

44   greater significance on the morphological variations of size and shape of wings, this study

45   indicates that the aphid host for *A. ervi* alter in a significant way the structure of forewings,

46   excluding variation between genotypes, due largely to the low genetic variability of *A. ervi*

47   populations in Chile when comparing between geographical areas and aphid hosts. Bigger aphid

48   host implied shape difference in the forewing explained as longer and broader wings of *A. ervi*,

49   as well as the size differences.

50

51   **Introduction**

52

53   Parasitoids are frequently used in biological control as they are considered as highly specialized

54   natural enemies (Godfray, 1994). By being highly specialized, released parasitoids will be the

55   most efficient at attacking the target pest species, reducing the possibility of environmental harm

56   through spillover of rapidly-growing parasitoid populations from crops into adjacent natural

57   habitats (Rand et al., 2006), as has been observed for generalist predators (Duelli et al., 1990;

58   French et al., 2001). Although, many highly specialized parasitoid species have a great host

59   range (Mackauer and Starý, 1967) exploiting many hosts may not be consistent across an entire

60   species, and different biotypes may be specialized to different host/environments (Stireman et

61   al., 2006; Forbes et al., 2009). Previous studies have shown that host-associated biotypes of

62   parasitoids from different host/environments can vary morphologically, behaviorally and

63   genetically (Žikić et al., 2009; Feder and Forbes, 2010; Kos et al., 2012; Zepeda-Paulo et al.,

64   2013). In terms of morphological features, the shape and size of their appendices have shown

65   great promise for separating host-associated races of parasitoids. Among these, insect wings have

66   been especially relevant as they are two dimensional structures with important characteristics, in

67   terms of adaptation and function (Wootton, 2002; Žikić et al., 2009). This is how previous

68   studies have shown that the size, shape and venation of the wings can be important features to

69   separate species and characterize populations within a single species (Sadeghi et al., 2009).

70   Geometric morphometrics approach is very useful for detecting minute variations on

71   morphology of different parasitoid populations which otherwise cannot be identified easily

72   (Villemant et al., 2007; Žikić et al., 2009; Kos et al., 2011). This can be of high importance

73   because these morphological variations in wing shape could be associated to a specific

74   environment or host-associated population of a parasitoid species.

75   The Chilean populations of *Aphidius ervi* H. may be a good example where different host

76   association and environment could have had some influence on morphology. This species is an

77   oligophagous parasitoid associated with several aphids species, such as *Acyrthosiphon pisum* H.

78   on legumes, *Acyrthosiphon kondoi* Shinji on legumes, *Macrosiphum euphorbiae* T.,

79   *Aulacorthum solani* K. on Solanaceae (Takada and Tada, 2000) and cereal aphids such are

80   *Sitobion avenae* F., *Rhopalosiphum padi* L., *Schizaphis graminum* R. and *Metopolophium*

81   *dirhodum* W. (Starý, 1993). *Aphidius ervi* was introduced in Chile in the 1970's as part of a

82   classical biological control in order to minimize the damage provoked by the grain aphid (*S.*

83   *avenae*) on cereals and maintain the pest population under low densities in the field (Zúñiga et

84   al., 1986). Nowadays, *A. ervi* is the most predominant parasitoid species controlling *A. pisum* and

85   *S. avenae* (more than 94% of prevalence on *A. pisum* on legumes and 38% of prevalence on *S.*

86   *avenae* on cereals) and considered a highly efficient biological control example of aphids on both

87   crops (Gerding et al., 1989; Starý et al., 1994; Zepeda-Paulo et al., 2013). The main goal of the

88   present study is to analyze the shape and size of forewings of *A. ervi* collected in different

89   plant/host/parasitoid associations, on legumes and cereals.

90

91   **Materials & Methods**

92   *Sampled material*

93   Aphids were collected from fields of legumes and cereals in two different geographic regions of

94   central Chile: "Región de los Rios" (S 39° 51´, W 73° 7´) and "Región del Maule" (S 35° 24´, W

95   71° 40´). Parasitoids were obtained from presumably parasitized aphids collected in the field,

96   and after the emergence carefully examined and identified. Reared samples were transferred in

97   the growing laboratory and treated under following conditions: 20°C, 50-60 RH, D16:N8 of

98   photoperiod. Parasitoid wasps were put in plastic microtubes with 96% of ethyl alcohol. The

99   identification was done using adequate taxonomic keys (Starý, 1995).

100   A total of 131 females of *Aphidius ervi* were analyzed. All parasitoids are divided into eight

101   biotypes according to their aphid hosts and to the plant species where the aphids were found

102   (Table 1). The alfalfa biotype was reared from *Acyrthosiphon pisum* and sampled on alfalfa

103   fields (*Medicago sativa* L.), the pea biotype from pea (*Pisum sativum* L.), and the clover biotype

104   from red clover (*Trifolium pratense* L.). Biotypes reared on cereals are the bird cherry-oat aphid

105   (*Rhopalosiphum padi*), the rose grain aphid (*Metopolophium dirhodum*) the green-bug

106   (*Schizaphis graminum*), and the grain aphid (*Sitobion avenae* F.) sampled from wheat (*Triticum*

107   *aestivum* L.). Another cereal biotype is also the grain aphid (*Sitobion avenae* F.) which was

108   collected from oat (*Avena sativa* L.) (Table 1).

109

110   *Geometric morphometrics*

111   To conduct the Geometric morphometrics analysis, we applied two dimensional landmark-based

112   methods (Bookstein, 1986; 1991). Right forewings of each female parasitoid was removed and

113   mounted in the Neo Mount (Merck) following procedure as described in Žikić et al. (2009). Such

114   prepared, forewings were recorded using OPTIKA SZN (45x) stereoscopic compound

115   microscope with a mounted 5 Mpixel photographic camera using software Optika Vision Pro

116   v2.7. Using Geometric morphometrics method (Zelditch et al., 2004) we tried to determine and

117   quantify morphological variation of different *Aphidius ervi* biotypes such as wing size and shape.

118   Eight different aphid-hosts/plant-host associations were used for morphological characterization

119   of *A. ervi* biotypes (Table 1). To analyze the variation in wing shape on parasitoids, 13

120   homologous landmarks were scored for each analyzed forewing. Positioned landmarks were

121   digitized using software TpsDig v2.16 (Rohlf, 2010) (Figure 1, Table 2). In order to analyze and

122   visualize the variations, software MorphoJ v1.06b was used (Klingenberg, 2011).

123

124   **Results**

125   To test variability of the forewings from the different *A. ervi* biotypes a Principal Component

126   Analysis (PCA) was carried out. According to PCA the variability explained by the first three

127   axes was rather low; all three explain 50.6% of the total variability. However, after testing for

128   variance, the results were statistically significant (Procrustes ANOVA: $F = 17.30$; $df = 7$; $P <$

129   0.000001). Beside the forewing size, even multivariate analysis of shape using PC scores showed

130   statistical significance (MANOVA: Wilks' $\lambda = 0.112737$; $F = 1.74$; $df = 154$; $P < 0.000001$).

131   Considering that all statistical tests of variance were statistically significant, we performed

132   Canonical Variant Analysis (CVA) in order to observe the variability among the *A. ervi* biotypes.

133   However, still there was no conspicuous grouping of the biotypes. The first canonical axis (CV1)

134   explains 38.4%, while the second axis (CV2) explains only 23% of the total variability. To see if

135 there was some correlation between the wing size and shape we performed the regression test

136 between the Centroid size and PC scores. From the graph (Fig. 2) it is evident that the wing

137 shape is clearly correlated with the wing size (P-value: < 0.0001). The percentage of the

138 variability explained by this regression test is 6.78 % (% predicted: 6.7783%). On the graph (Fig.

139 2) it seems obvious that the biggest wings were of the specimens from the biotype *A. pis_pea*.

140 Considering that the regression result was statistically significant (P-value: <0.0001) we

141 performed Discriminant Analysis (DA) using the residuals. This particular analysis showed that

142 none of the Procrustes distances were statistically significant (P-value: >0.05), and therefore the

143 changes of the wing shape are due to the change of the wing size. Therefore, we wanted to

144 visualize how the wings of all other *A. ervi* biotypes change in relation to this particular biotype

145 (*A. pis_pea*) using outline-based geometric morphometric methods (Fig. 3). The changes

146 between the biotype *A. pis_pea* and the other six can be tracked form the Figure 3.

147 The least changes of the wing shape and size are in the following relations: *A. pis_pea*/*A.*

148 *pis_alfalfa*, *A. pis_pea*/*A. pis_clover* and *A. pis_pea*/*R. pad_wheat*. More conspicuous changes

149 are visible in the relation *A. pis_pea*/*S. ave_oat*, than *A. pis_pea*/*S. ave_wheat*. These changes are

150 due to the narrowing of the wing in the two biotypes (*S. ave_oat* and *S. ave_wheat*). The greatest

151 difference is between the biotype *A. pis_pea* and *S. gra_wheat* where this biotype has the

152 narrowest wing in relation to *A. pis_pea*.

153

Discussion

155 *Aphidius ervi* is known to attack economically important pests in the Chilean agricultural

156 landscapes and is considered a successful example of classical biological control of legume and

157 cereal aphids (Starý, 1993; Starý et al., 1993; Rojas, 2005). Although it is very efficient in

158 parasitizing target aphid pests, it has little or no effect in attacking native aphid species in shared

159 environments (e.g: *Uroleucon* species developing on native plants in and around agricultural

160 valleys in Chile) (Zúñiga et al., 1986; Starý, 1993). However, many studies have shown heritable

161 host fidelity and have hypothesized the possibility of different host associated biotypes. Recent

162 studies of Bilodeau et al. (2013) and Zepeda-Paulo et al. (2013) using population genetics

163 suggests that in both North America and Chile there are no specialized races or biotypes on

164   different aphid-host species, revealing high gene flow between aphid-host originated parasitoid

165   populations.

166   However, in a recent study it has been shown that the parasitoid genotype can have a stronger

167   influence on wing shape compared to the effect of developing on different parasitoid host species

168   (Parreño et al. 2016). These authors used five asexual lines of *Lysiphlebus fabarum* and four

169   aphid hosts, and using the Procrustes Coordinates on wings, found that the lineages were the

170   better grouping factor compared to the parasitoid aphid-host variable. In this study we did not

171   discover any drastic morphological features which could conspicuously differentiate the Chilean

172   population of *A. ervi*. This is probably due to the very short period i.e. insufficient number of

173   generations among these particular Chilean populations. This is obvious from the fact that

174   analyzed specimens of *A. ervi* from various aphid hosts and different localities throughout Chile

175   exhibit a rather low genetic variability (Zepeda-Paulo et al., 2016).

176   One of the most conspicuous differences among the eight biotypes of *A. ervi* analyzed is in the

177   shape and size of forewings from the biotype *A. pis_pea*. Specimens of this particular biotype

178   have generally larger forewings than other biotypes and somewhat broader in the middle and the

179   distal part (Figures 2 and 3). Of all investigated biotypes the most similar are those which were

180   reared from *Acyrthosiphon pisum* (*A. pis_alfalfa*, *A. pis_clover* and *A. pis_pea*) independent of

181   the aphid clone (host-plant), which is at the same time the biggest aphid, when compared to all

182   other aphid species mentioned here. The differences of the wing size and shape begin to appear

183   in those biotypes reared on cereals, with the exception of the *R. pad_wheat* biotype where the

184   differences were small (Fig. 3). This could be probably the effect of the aphid host size, because

185   the *Acyrthosiphon pisum* is rather a large aphid when comparing to *R. padi*. Analyzing the

186   relation of wing shape and size between the biotypes *A. pis_pea* and *Sc. gra_wheat* (Fig. 3), there

187   are clear differences. At the same time, the differences in the body size are probably the greatest

188   between *Acyrthosiphon pisum* and *Schizaphis graminum* which is rather a small aphid host for

189   *Aphidius ervi*. This suggests that beside the host plant, the aphid host has primal mayor influence

190   in the morphological differentiation of the forewing among the analyzed biotypes, as it has been

191   shown in other studies with individuals of the genus *Eubazus*, a parasitoids of the conifer bark

192   weevil (Villemant et al., 2007) and with the parasitoid *Lysiphlebus fabarum* (Parreño et al.

193   2016). Additionally, the effect of plant species should not be neglected because there are clear

194   preference of *A. ervi* biotypes toward particular plant species i.e. its volatiles which will

195   eventually lead them to the adequate aphid host (Daza-Bustamante et al., 2002).

196   Variations of the shape of insect wings are known to affect flight ability, which in turn could

197   alter the host and mate allocation (Kölliker-Ott et al., 2003). Betts and Wootton (1988) studied

198   the effects of wing structure on the flight of six butterfly species and showed that there was a

199   correlation between flight performance and wing shape. Additionally, studies have described

200   how the wing shape can alter predation success by dragonflies (Combes et a., 2010) and also, the

201   ability of damselflies to avoid predation by passerine birds (Outomuro and Johansson, 2015).

202   More specifically, parasitoids are also affected by the changes in wing size and shape. The wing

203   size and shape of *Trichogramma brassicae* and *T. pretiosum* as egg parasitoids, increase the

204   ability to locate host eggs. Differences in wing size and shape were found between parasitoid

205   obtained from field conditions compared to those parasitoids that were reared in laboratory

206   (Kölliker-Ott et al., 2003). Authors suggest that wing shape and wing size can be reliable

207   predictors of field fitness for these parasitoid species. In the present study, the biotypes of *A. ervi*

208   emerged from *A. pisum* encounter larger and broader forewings compared to the other studied

209   biotypes. These differences of wing shape and size could be reflected on the fitness of *A. ervi* to

210   control pest increasing the ability to find aphid host as size and shape of forewings increased.

211   Further research to determine most suitable aphid host for *A. ervi* to increase its fitness will lead

212   to enhance rearing conditions for *A. ervi* and consequently, to improve any inundative biological

213   control strategies with this parasitoid.

214

215   **Conclusion**

216   Given that there is a low genetic variability of *A. ervi* in Chile and that there was little effect of

217   plant species on morphological features in this study, the main factor affecting morphological

218   variations of *A. ervi* forewings is the size of their aphid host.

219

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223

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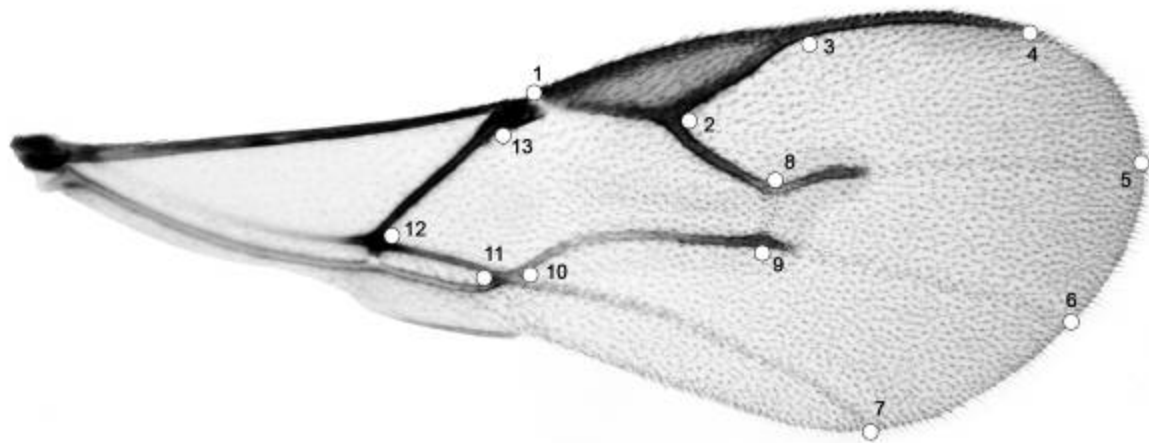
333 **FIGURES**

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

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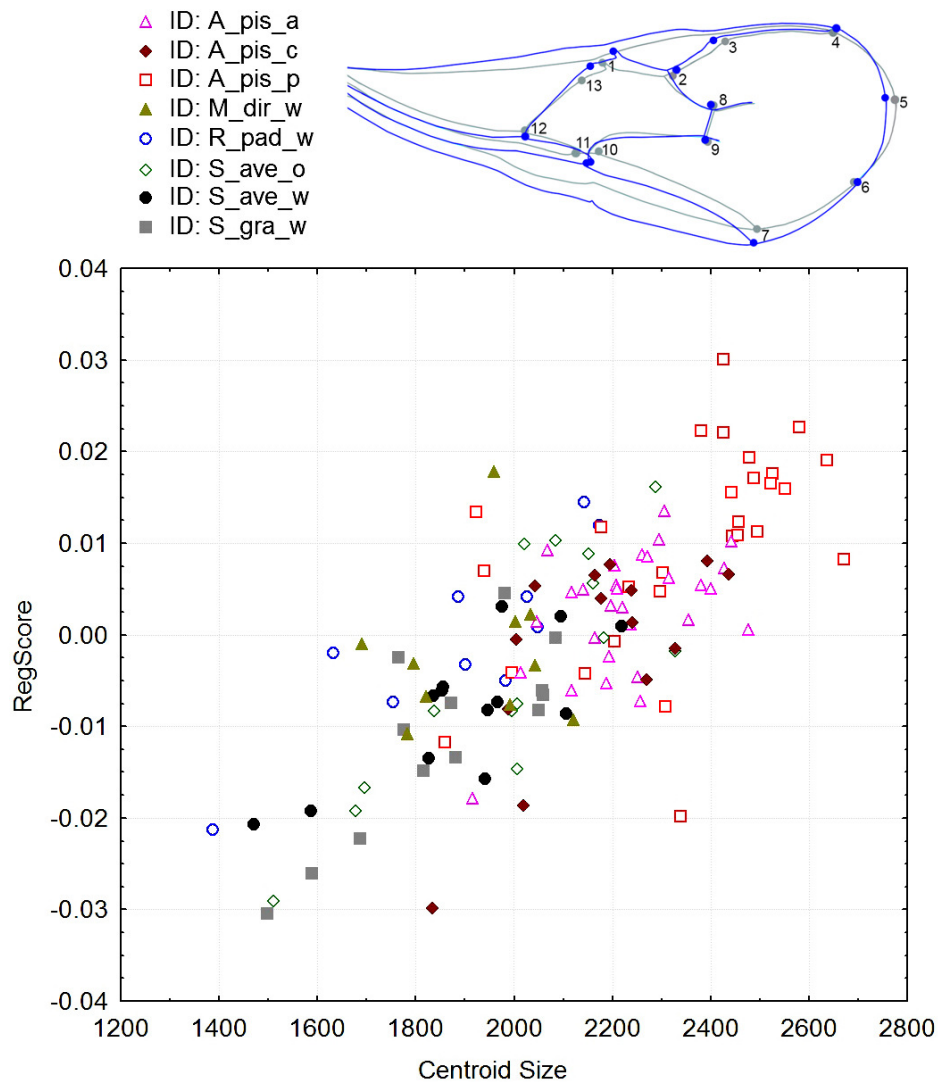
Figure 2. The regression results of the Centroid Size (CS) and PC scores (Permutation test against the null hypothesis of independence, P-value: <0.0001).

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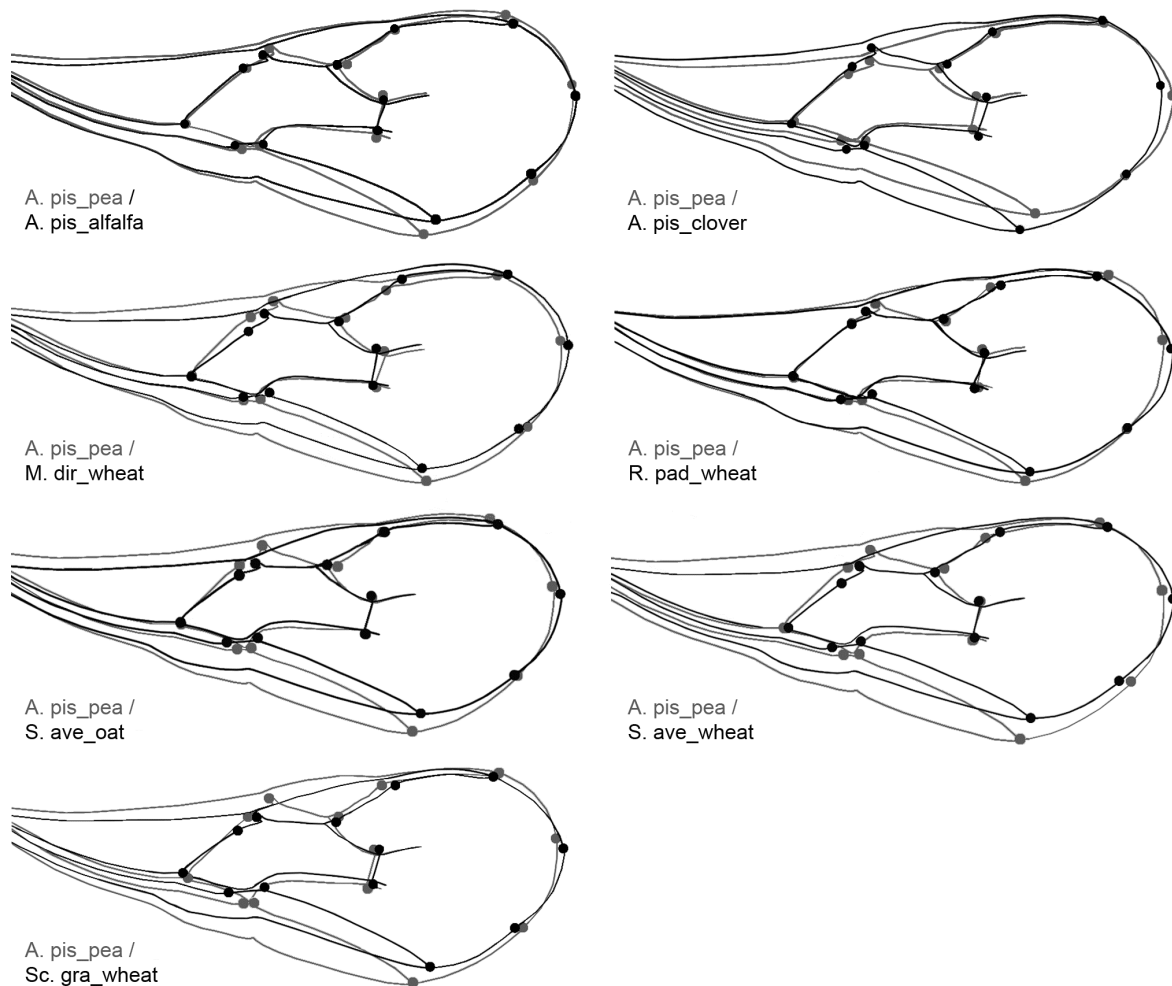
343 **Figure 3.** Outline-based comparison of the wing shape between the biotype *A. pis_pea* and the

344 rest seven biotypes. Shape differences are the results of Discriminant Analysis (DA). The scale

345 factor is increased by 5. Grey color of outline represents the biotype *A. pis_pea*; black color of

346 outline represent compared biotypes.

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351 **TABLES**

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

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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*

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371   **Table 2.** Description of homologous landmarks of forewing. Wing veins terminology follows

372   Wharton et al. (1997).

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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