

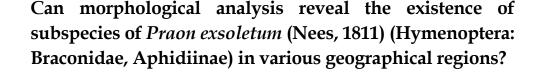
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Research Article

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ABSTRACT. In this study, 13 different biotypes of *Praon exsoletum* were investigated based on selected morphological characteristics. The analysis of variance showed that *P. exsoletum* biotypes differ in many morphometric traits. Moreover, all biotypes displayed their maximum differences in six morphological characters as follows: (1) number of antennal segments; (2) tentorio-ocular distance/inter-tentorial; (3) length/width of ovipositor sheath; (4) length/width of second flagellar segment; (5) length/width of third flagellar segment and (6) number of longitudinal placodes on second flagellar segment. The discriminant function analysis indicated 45.23% of correct assignment of specimens to the *a priori* designated groups of specimens. The results indicated an overlapping of analyzed biotypes according to selected morphological characters. On the other hand, geometric morphometrics analysis applied on forewings, the size and the shape revealed statistically significant differences. Statistical analyses showed that there were significant differences in size and shape of forewings. Our results showed lack of subspecies for *P. exsoletum*.

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Introduction

Praon Haliday, 1833 (Braconidae, Aphidiinae) is the largest genus within the tribe Praini with over 50 species described worldwide, occurring throughout the Palaearctic and Nearctic regions (Johnson 1987; Kavallieratos et al. 2005). Species of this genus are solitary endoparasitoids, which attack several host aphids (Mackauer 1959; Starý 1971; Mescheloff

and Rosen 1988; Kavallieratos and Tomanović 2001). The systematic position of the genus *Praon* has slightly been confused. From a taxonomic viewpoint, intraspecific variation is often as great as the interspecific range. From an evolutionary point of view, generally this variation is more developed in phylogenetically younger

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taxa than in primitive aphidiines such as the genus *Ephedrus*, which is positioned as basal on the phylogenetic tree in the case for debates. However, the *Praon's* pupation is a derived adaptation and not an inherited form of behavior (Mackauer 1959; Tobias and Kyriak 1971; Johnson 1987; Mescheloff and Rosen 1988).

The genus *Praon* has been treated taxonomically by several researchers (Smith 1944; Mackauer 1959; Starý and Schlinger 1967). The use of various taxonomic characters in previous reviews concerning *Praon*, has led to confusion and misunderstanding in separation of some species. This could be the consequence of a large degree of intraspecific variability. Also, some species have been described on the basis of a single male or a small number of specimens (Johnson 1987).

In his work, Johnson (1987) offered the relative length and width of wing veins as a main character key for the identification of Praon species. Starý (1976) used the coloration of the first flagellar segment as a criterion to divide this genus into two large sub-groups. Moreover, Starý (1983) described the genus Parapraon completely based on differences in wing venation. Johnson (1987) treated Parapraon as a new synonym of Praon. He noted that Stary's new genus disagreed with the real variation existing in the species given to Paraparon. After the description of several new Praon species from southeastern Europe (Kavallieratos and Lykouressis 2000; Tomanović and Kavallieratos 2002; Tomanović et al. 2003a, b; Kavallieratos et al. 2003), phylogenetic relationships within the genus Praon were reconstructed, and three species groups were recognized with weak bootstrap support including: "Parapraon", "dorsaleyomenae", and "rosaecola" (Kavallieratos et al. 2005). The following publications: Kavallieratos et al. (2005), Tomanović et al. (2007) and Takada (2014) pointed out that Parapraon group included six species: Praon exsoletum (Nees, 1811), Praon necans (Mackauer, 1959), Praon spinosum (Mackauer, 1959), Praon gallicum (Starý, 1971), Praon retusae (Tomanović and Kavallieratos, 2002) and Praon kurisakiae (Takada, 2014).

Aphids that attack alfalfa are wellknown in Iran and many other countries (Rakhshani et al. 2006). Among the aphids, the spotted alfalfa aphid (SAA), Therioaphis trifolii (Monell, 1882) forma maculata Buckton, is an important pest for agriculture. Praon exsoletum (Nees, 1811), is a monophagous and thermophile species representing an effective natural enemy of Therioaphis trifolii maculata. This parasitoid was primarily limited to the Old world, latter being transferred from Italy to Western America for biological control of T. trifolii in 1955-1956 (van den Bosch et al. 1959). In 1987, P. exsoletum was collected from USA and Canada, stating that with true identifications, it showed Holarctic distribution (Johnson 1987). Before dividing P. exsoletum to two subspecies, several studies had been done considering T. trifolii maculata and its parasitoids. Hagen et al. (1958) mentioned P. palitans as a parasite of T. trifolii. Thereafter, Mackauer (1959) distinguished two subspecies: P. exsoletum exsoletum, reportedly distributed in Europe, exclusive of the Mediterranean area; and exsoletum palitans distributed Mediterranean region.

(1976)that Starý cited some morphological characters, and also the host range and distribution of P. exsoletum may not be used in separation in two subspecies. Therefore, Starý (1979) considered P. exsoletum exsoletum as synonym of P. exsoletum palitans, which has been also verified by Johnson (1987). However, several researchers in their considered Р. exsoletum palitans biological control agent for Therioaphis trifolii (van den Bosch 1957; van den Bosch

et al. 1959; Schlinger and Hall 1960; Monajemi and Esmaili 1981; Vail et al. 2001; Gutierrez and Ponti 2013). But, in many other investigations *P. exsoletum* is cited as cosmopolitan parasitoid of *T. trifolii* (Kavallieratos et al. 2005; Tomanović et al. 2006; Rakhshani et al. 2006; Nazari et al. 2012).

The definition of biotype says: it is a group of organisms with the same or nearly the same genotype which distinguishes it from other similar group(s). Biotypes are usually differentiated with criteria other than morphology (ecological behavior or feeding or oviposition) (Diehl and Bush 1984). Due to the existence of sibling species and intraspecific variability among parasitoid wasps, possibilities of being biotypes should be considered. Therefore, classical morphological principles often lack to explain their taxonomy, and other investigations are required (Gauld 1986; Kenis and Mills 1998; Kimani-Njogu *et al.* 2001; Tomić *et al.* 2005).

To create a comparative taxonomic station, morphometric analysis is used. Multivariate morphometric studies have been carried out on different insect taxa to analyze biotype variation because insect exoskeletons retain their form throughout adult life and can be easily measured (Sneath and Sokal 1973). The powerful tool of geometric morphometrics can resolve problematic species complexes, even in cases where it is not possible to use more than a few landmark points (Baylac and Daufresne 1996). Several morphometric studies in Braconid wasps (Kimani-Njogu et al. 1997; Kenis and Mills 1998; Kimani-Njogu et al. 2001; Villemant et al. 2007; Billah et al. 2008), especially aphid parasitoids (Tomić et al. 2005; Žikić et al. 2009, 2010; Mitrovski-Bogdanović et al. 2013, 2014; Tomanović et al. 2013; Stanković et al. 2015; Alejandra Parreno et al. 2016) have been carried out to date but no morphometric study has been done on P. exsoletum.

Notwithstanding studies declare doubts about situation of *P. exsoletum* (or existence of two subspecies). Moreover, because of the importance of *P. exsoletum* in biological control of SAA and for being cosmopolitan, it is essential to study biotypes of *P. exsoletum* by geometric morphometrics as well as traditional morphometric analysis.

Materials and methods

Examined materials: Parasitoid specimens were collected from different geographic regions (Table 1). Plant samples with already mummified aphid hosts were collected and transferred to the laboratory emerging of parasitoids. Samples of adult aphids were conserved in 90% ethanol and 75% lactic acid in a ratio of 2:1 (Eastop and van Emden 1972) for later identification. The aphids were identified according to Rezwani (2001). The parasitoid specimens were boiled in 10% KOH, dissected, and mounted in Hoyer.

Traditional morphometric analysis: The external morphology of the parasitoid specimens was studied using a Blue Light stereomicroscope (Aran TajhizTM Co) and OlympusTM microscope BX51. Three meristic and 15 continuous characters were used for morphological characterization (Table 2, Fig. 1). Some of the continuous characters were presented in terms of a ratio of two characters (Table 2). The terminology used in this paper is based on Kavallieratos et al. (2005) and Tomanović et al. (2006). Analysis of variance (ANOVA) was used to test statistical significance of differences in the variation of morphological characters. Canonical Variate Analysis (CVA) was done on characters that showed statistically significant variation between biotypes. Percentages of correct identification were Discriminant calculated by **Function** Analysis (DFA).

Host aphid	Host plant	Number of specimens	Dates	Origin
		8	2014-15	Shush
		6	2014-15	Gilan-Gharb
		14	2014-15	Kangavar
Therioaphis trifolii forma maculata		15	2014-15	Bistun
		13	2013	Songhor
	Medicago sativa	26	2015	Firozan
		30	2015-16	Bam
		9	2015-16	Jiroft
		25	2015-16	Nikshahr
		23	2015-16	Sarbaz
		9	2012	Spain
		5	2012	Czech Republic
		7	2012	USA

Table 1. Sampling data for specimens used in morphometric analyses.

Geometric morphometrics: To explore and quantify morphological variation, the wing size and shape of *P. exsoletum* females were analyzed. The forewing of each specimen was photographed using Dino-capture 2.0 digital camera mounted on an Olympus microscope BX51 at 10× magnification, and 11 landmarks were used (positions and definitions are given in Fig. 2). All landmarks were digitized using Tps-Dig software (Rohlf 2005). The landmarks were superimposed by the Generalized Procrustes analysis (GPA), estimating the scaling factor applied to respondent scale usage, thus eliminating variation in the size of the wings (Rohlf and Slice 1990; Bookstein 1991). For determining the variation in the size of the wings among the specimens of P. exsoletum, we performed an analysis of variance (ANOVA) on the centroid size. In addition, to analyze variation in wing shape (Zelditch et al. 2012) multivariate analysis of variance (MANOVA) was done on the full set of shape variables.

To estimate the wing size, we computed the centroid size (CS) (Bookstein 1991). To explore the variation in wing shape within biotypes, we performed principal component analysis (PCA). The measurement of variation in wing shape is visualized by

Canonical Variate Analysis (CVA). The software MorphoJ (Klingenberg 2011) was used to analyze and visualize shape changes described by canonical axes (Klingenberg 2011).

All standard statistical analyzes were performed with the SPSS 16.0 (SPSS 2007) and STATISTICA 10.0 (StatSoft 2011) software package. All the material examined in this study is deposited in the archives of the Department of Plant Protection, Razi University, Kermanshah (Iran).

Results

Morphometric Analysis

The material obtained from morphometric analysis consisted of 190 female specimens of P. exsoletum. The result of DFA [(N of variables in the model = 12; $Wilks' \lambda = 0.133$; F = 2.558; $P \le 0.000$] showed a median discrimination among the biotypes (P. exsoletum) based on the first two canonical axes (CV1 versus CV2), which explain 62.89 % variability (Fig.3). Eigenvalues for first three canonical axes were: CV1 = 1.252, CV2 = 0.381, and CV3 = 0.339, respectively. Table 4 shows standardized coefficients and class means for canonical variables.

Table 2 . List of characters used for morphometric analyses.

Character code	Type	Description		
F1L/F1W	Ratio	Length/width of first flagellar segment		
F2L/F2W	Ratio	Length/width of second flagellar segment		
F3L/F3W	Ratio	Length/width of third flagellar segment		
F1L/F2L	Ratio	Length of first flagellar segment/length of second flagellar segment		
F1L/F3L	Ratio	Length of first flagellar segment/length of third flagellar segment		
F1W/F2W	Ratio	Width of first flagellar segment/width of second flagellar segment		
F1W/F3W	Ratio	Width of first flagellar segment/width of third flagellar segment		
TO/IT	Ratio	Distance of compound eyes to tentorial/internal tentorial		
Ovip Sheath L/W	Ratio	Length/width of ovipositor sheath		
STL/STW	Ratio	Length/width of stigma		
LW/WW	Ratio	Length/width of wing		
PTL/PTW	Ratio	Length/width of petiol		
LW/LR1	Ratio	Length of wing/length of R1		
LW/STL	Ratio	Length of wing/length of stigma		
STL/LR1	Ratio	Length of stigma/length of R1		
F	Meristic	Number of flagellar segments		
LP2	Meristic	Number of longitudinal placodes on second flagellar segment		
LP3	Meristic	Number of longitudinal placodes on third flagellar segment		

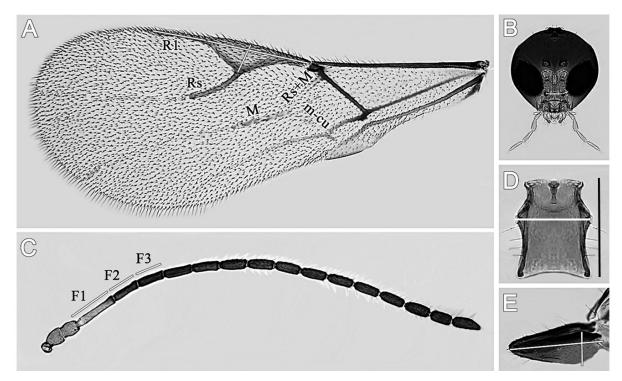


Figure 1. The morphological characters of *Praon exsoletum* (Nees, 1811). **A.** Forewing; **B.** Head (frontal view); **C.** Antenna; **D.** Petiole (dorsal view); **E.** Ovipositor sheath (lateral view).

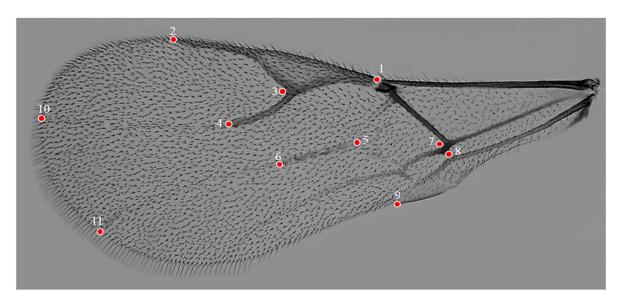


Figure 2. Forewing and landmarks in *Praon exsoletum* (Nees, 1811).

The total correct percentage of classification matrix of all biotypes was 45.226%. The taxonomic characters that largely contribute to the discrimination of different P. exsoletum biotypes along the first canonical axis are length of wing/length of stigma (LW/STL), distance of compound eves tentorial/internal tentorial (TO/IT), length of (STL/LR1) stigma/length of R1 length/width of third flagellar segment (F3L/F3W). Along the second canonical axis, of antennal segments number length/width of second flagellar segment (F2L/F2W) and number of longitudinal placodes on the second flagellar segment (LP2) and the third canonical axis in addition to LW/STL and F2L/F2W, length/width of stigma (STL/STW) are the most important characters discriminating exsoletum Р. biotypes (Table 4).

Regarding canonical variate analysis with morphological characters, CV1 showed that more biotypes of Iran except Shush could be separated from USA, Spain and Czech Republic (Fig. 3). In CV2 the two biotypes Czech Republic and USA were separated from another biotypes. However, CV1 and

CV2 cannot separate other biotypes such as Firozan and Jiroft; also, there were few distances between them. Two adjacent cities of the same province (Bisotun and Kangavar) which were near each other in CV1xCV2, were well separated by CV3. The maximum discrimination by the CV3 occurred between Bam and Shush (Fig. 3).

The analysis of variance (ANOVA) displayed that the *P. exsoletum*'s biotypes we different in many morphometric traits analyzed, except for width of first flagellar segment/width of second flagellar segment (F1W/F2W) and length of first flagellar segment/length of third flagellar segment (F1L/F3L). Besides, all biotypes in six characters including: number of antennal segments (F), distance of tentorial/internal compound eyes to tentorial (TO/IT), length/width of second flagellar segment (F2L/F2W), number of longitudinal placodes on second flagellar segment (LP2), Length/width of third flagellar segment (F3L/F3W) and length/width of sheath ovipositor (Ovip sheath L/W) showed maximum difference (Table 5).

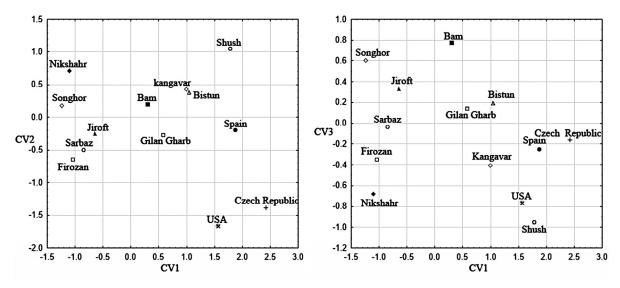


Figure 3. Canonical Discriminant Functions of the various geographic biotypes of *Praon exsoletum*.

Geometric morphometrics

We tested ANOVA (F = 20.25, df = 105, $P \le 0.0001$) for significant variation in wing size among the P. exsoletum biotypes analyzed. Then we applied multivariate analysis of shape using PC scores, MANOVA, ($Wilks' \lambda = 0.081$, F = 1.922, df1 = 130, df2 = 736, P = 0.000). PCA applied on original data, without a priory formed groups for variation in the shape of the forewing. Ordination of the specimens in morphospace defined by the first two principal axes is shown in Fig. 4. It is obvious that biotypes obtained by the aphid T. trifolli maculata do not form separate groups, but they actually overlap.

The divergence in wing shape among different biotypes of *P. exsoletum* was visualized by canonical variate analysis (CVA), which decreases within group variances and increases the between group divergences in the shape of the wing. First two canonical axes explained 31.88% of the total variability in wing shape (Fig. 5). The biotypes from other countries such as USA, Czech Republic and Spain did not yield group, especially for the biotype from USA where *P*.

palitans was introduced. In PCA and CVA, geographically different biotypes, are not discriminated; however, we expected that the biotypes from similar climate conditions will form similar groups, but there were a strong overlap between biotypes of Praon exsoletum. Conversely, individual biotypes along the axis of CV1 and CV2 could be separated. Individual biotypes such as Songhor, Firozan along CV1 and individual biotypes such as Bistun, Nikshahr, Sarhaz in CV2 separated each other. Based on Mahalanobis distances, maximum discrimination was shown between several biotypes of Iran and USA. Also, two cities Bistun and Songhor exhibited maximum Mahalanobis distances to Spain. Also biotype of Gilan-Gharb had maximum distance to Czech Republic (Table 3).

Discussion

According to our results, notwithstanding have similar host range, biotypes of *Praon exsoletum* with geographically similar ranges did not make similar groups, because they have strong overlapping in morphology and geometric of forewing.

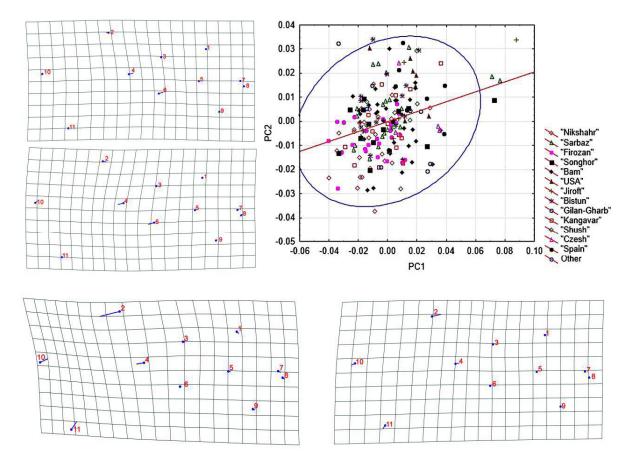


Figure 4. Principal Component analysis scatterplot of the results for the shape of the forewing. The PC1 axis accounts for 54.491% and PC2 axis for 15.718% of total variability. The confidence interval of the ellipses is 99%.

Hence, we showed that other factors may be effective in wing shape, like genetic structure. As such species often exploit different hosts, it is useful to know that even in different genotypes of the same species, wing shape is a relatively rigid (Alejandra Parreno et al. 2016). The recent study showed that wing shape genetically determined and relatively invariant different across host environments, which is an important requirement for its use in the taxonomy of aphid parasitoids. It is an empirical observation that in aphid parasitoids, wing shape often differs significantly cryptic that amongst species distinguishable by molecular methods

(Stanković et al. 2015). There is also evidence from Drosophila melanogaster that wing shape is remarkably robust to environmental perturbations (Birdsall et al. 2000). However, this does not imply that wing shape is generally useful as a taxonomic trait (Alejandra Parreno et al. 2016). In other groups of insects would have to be established independently because it is unlikely to be universal. For example, a recent study on bumblebees has shown that wing shape is virtually useless for species delimitations (Lecocq et al. 2015), whereas another study on hornets found wing shape to be a very reliable predictor of species identity (Perrard et al. 2014).

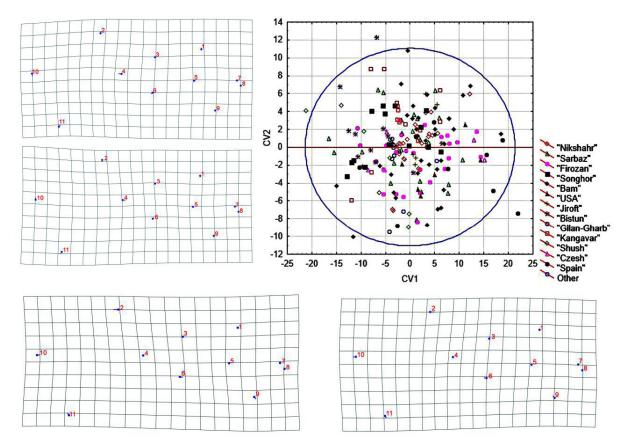


Figure 5. Ordination of the *Praon exsoletum* specimens in the space of two canonical.

Table 3. Mahalanobis distances among groups. Bold numbers are minimum and maximum Mahalanobis distance.

	Bam	Bistun	Czech R.	Firozan	Gilan- Gharb	Jiroft	Kangavar	Shush	Spain	USA	Nikshahr	Sarbaz
Bistun	2.638											
Czech R.	3.311	3.186										
Firozan	1.878	2.524	3.480									
Gilan-Gharb	3.551	2.850	4.814	3.574								
Jiroft	2.253	2.527	2.934	2.624	3.388							
Kangavar	2.855	2.235	3.622	3.148	2.675	2.852						
Shush	3.305	2.815	3.703	3.300	2.732	3.198	2.813					
Spain	3.205	4.027	3.061	3.371	4.737	3.232	4.399	3.374				
ŪSA	3.464	3.701	3.077	3.585	4.744	3.340	4.666	4.175	2.951			
Nikshahr	1.318	2.910	3.499	2.110	4.101	2.893	3.114	3.901	3.627	3.891		
Sarbaz	1.518	2.006	2.944	1.697	3.518	1.911	3.159	3.180	3.167	2.931	1.914	
Songhor	2.216	2.332	3.427	1.834	3.750	2.999	2.895	3.406	4.071	3.974	2.334	1.942

We cannot split *P. exsoletum* in true subspecies, because of overlapping measured character even when they are far from another regarding its geography. The differences in wing shape amongst biotypes of *P. exsoletum* were mainly related to the length of the R1, and the

length of the radial vein; so along the CV1xCV2 axis the individual of biotypes distributed from each other. However, Mackauer (1959) pointed out two subspecies of *P. exsoletum* explaining this by the broad dispersion of populations (and two characters: host range and

morphological characters), of biotypes. The biotypes coming from USA considered as *P. palitans* were not different from Iranian biotypes, but were smaller on body size.

Regarding our results, the most important landmarks that showed R1 and Rs veins and stigma (e.g. 2, 3 and 4 landmarks) were different in individual of biotypes; as well as length of R1 in individual of biotype like as Jiroft was longer than other individual (or biotypes), so their wings were long. Moreover, these difference were not to the extent that can be separated biotypes. The shape of stigma was different, so we measured the length/width of stigma. However, it seems necessary to use other methods such as semi-landmark. As, Starý (1976) indicated that variability of morphological characters, host-range and the species dispersion are not so variable with regards to *P. exsoletum* in subspecies.

Although no morphometric studies have been done on this species to date, the morphological characters selected here were based on morphometric studies on other braconid taxa; for example, the morphometric characters of antennal segments and forewing were used in Aphidius species associated with various aphid hosts (Pungerl 1986; Tomić et al. 2005). Same series of characters were used also for discrimination of biotypes of Ephedrus persicae Froggatt (Žikić et al. 2009) and for dorsale-yomenaes.str. complex (Mitrovski-Bogdanović et al. 2014), as well. These morphometric analyses were applied on other members of very large family Braconidae: the ovipositor length was used in Cotesia flavipes (Cameron) species complex (Microgastrinae) (Kimani-jogu et al. 1997), and the forewing length was used in sibling species of Eubazus spp. (Brachistinae) (Kenis and Mills 1998). However, some characters such as length/width of third flagellar segment, length and width of first flagellar segment to third flagellar segment, length of wing to stigma and R1 were first to be used in morphological analysis for Praon species especially P. exsoletum. The number of longitudinal placodes was not difference character in *P. exsoletum* biotypes, but several lines of evidence show that the number of longitudinal placodes is а reliable morphological character in **Ephedrus** (Gardenfors 1986). However, the length of ovipositor sheaths has been regarded as an evolutionarily stable character (Starý 1976); an adaptation to increase the chances of successful parasitism.

Studying allometric relations of several morphological characters of Ephedrus persicae complex, Žikić et al. (2010) also revealed that the ovipositor was very conservative, not depending on the size of the body. Our results support both studies that the character (Ovipositor sheath L/W) is stable among the biotypes analyzed. The number of antennal segments in two biotypes of Czech Republic and USA were 18 segments that had maximum segments in P. exsoletum. However, a few of individual of biotypes (Firozan) had 18 segments. However, canonical analysis of morphological characters can separate some of the biotypes (e.g. USA, Czech-Republic), but they did not have significant distance from each other, because characters overlapped.

The differences in biotypes of *P. exsoletum* could reasonably be related to flight ability; hence, substantial intraspecific variability as observed here does not suggest that wing shape is under strong selection (Mousseau and Roff 1987). This study we can conclude that *P. exsoletum* really has a broad dispersal range, which in turn can be used in general integrated biological control programs.

Table 4 . A standardized canonical discriminant function coefficients for <i>Praon exsoletum</i> .
The most effective characters in CV1, CV2 and CV3 shown with Bold number.

Characters	CV1	CV2	CV3
TO/IT	-0.541	-0.170	-0.082
F	-0.389	0.477	-0.007
F3L/F3W	0.452	0.135	-0.136
LW/STL	0.578	-0.130	-0.645
STL/LR1	0.463	-0.283	0.261
STL/STW	0.146	-0.128	-0.848
Ovip Sheath L/W	-0.345	-0.398	0.059
LP2	0.242	0.641	-0.002
F2L/F2W	0.124	-0.401	0.508
LW/WW	-0.074	-0.039	-0.145
F1L/F2L	-0.134	-0.230	-0.241
LP3	-0.057	-0.179	0.280
Eigenvalue	1.252	0.381	0.339
Cum.Prop	0.482	0.629	0.760

Table 5. Analysis of variance for the morphological characters (Character abbreviations are listed in Table 2).

Character	df	MS	F	P
F	13	0.826	4.670	0.000
LP2	13	0.556	3.143	0.000
LP3	13	0.136	1.186	0.293
F1L/F1W	13	0.841	1.393	0.166
F2L/F2W	13	0.387	3.352	0.000
F3L/F3W	13	0.256	3.631	0.000
F1L/F2L	13	0.011	1.129	0.337
F1W/F2W	13	0.008	0.772	0.689
F1L/F3L	13	0.013	0.539	0.898
F1W/F3W	13	0.014	1.446	0.142
LW/WW	13	.•061	1.184	0.294
LW/STL	13	0.112	2.544	0.003
LW/LR1	13	1.341	1.875	0.035
STL/STW	13	0.139	1.998	0.023
STL/LR1	13	0.079	2.470	0.004
PTL/PTW	13	0.009	1.460	0.136
TO/IT	13	0.013	4.849	0.000
Ovip Sheath L/W	13	0.254	3.111	0.000

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آیا آنالیز ریخـتشناسـی مـی توانـد وجـود زیرگونـههـای (Nees, 1811) (Hymenoptera, Braconidae, Aphidiinae)

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چکیده: در این بررسی ۱۳ بیوتیپ مختلف Praon exsoletum براساس صفات ریختشناسی انتخاب شده مورد بررسی قرار گرفتند. آنالیز واریانس نشان داد که بیوتیپهای حداکثر اختلاف را در بیشتر ویژگیهای مرفومتریک اختلاف دارند. همچنین، تمام بیوتیپها حداکثر اختلاف را در شش صفت ریختشناسی (۱) تعداد بندهای شاخک؛ (۲) فاصلهی چشم مرکب تا تنتوریال فاصلهی داخلی تنتوریال؛ (۳) طول/ عرض غلاف تخمریز؛ (۴) طول/ عرض بند دوم فلاژلوم و (۶) تعداد پلاکودهای طولی بند دوم شاخک نشان دادند. (۵) طول/ عرض بند سوم فلاژلوم و (۶) تعداد پلاکودهای طولی بند دوم شاخک نشان دادندی تعلیل تابع تشخیص، نسبت دادن صحیح ۴۵/۲۳ درصد نمونهها را به نمونهی طراحی شده قبلی نشان داد. این نتایج همپوشانی بیوتیپهای آنالیز شده را براساس صفات ریختشناسی انتخاب شده نشان داد. از طرفی، آنالیز مرفومتریک هندسی با استفاده از بال جلو، اختلافات معنی دار آماری را برای اندازه و شکل بالهای جلو وجود دارد. نتایج ما نبود دو زیرگونه را برای معنی داری در اندازه و شکل بالهای جلو وجود دارد. نتایج ما نبود دو زیرگونه را برای داد.

واژگان کلیدی: مرفومتریک هندسی، لندمارک، تحلیل تابع تشخیص