Multivariate analysis techniques in the study of the male genitalia of *Pyrgus bellieri* (Oberthür 1910) and *P. alveus* (Hübner 1803) (Lepidoptera: Hesperiidae): species discrimination and distribution in the Iberian Peninsula

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Abstract. We used statistical multivariate analyses to study two morphologically similar species: *Pyrgus bellieri* and *P. alveus*. Ten different variables were measured in the male genitalia, and the results tested with Principal Component Analysis which showed a clear cut separation between the two species later confirmed by Discriminant Analysis. The discerning variables were the length of cuiller and the length of uncus that are inversely proportional in the two species. The classification functions for each species have been worked out resulting in the possibility to identify any individual just using some parameters in the male genitalia. The presence of *P. bellieri* in the Iberian Peninsula is confirmed and 18 literature citations of the species are rejected while 13 new ones are given. The resulting distribution in the Peninsula is restricted to 32 UTM squares (10 km) in the NE, all of them in Catalonia and in the Province of Huesca (Aragón). The study highlights the validity of statistical multivariate analysis techniques, using variables taken from the male genitalia, to discriminate species with identification problems.

Résumé. Etude par analyses multivariées des génitalia mâles de *Pyrgus bellieri* (Oberthür 1910) et *P. alveus* (Hübner 1803) (Lepidoptera : Hesperiidae) : discrimination spécifique et distribution dans la Péninsule Ibérique. Nous avons utilisé des analyses statistiques multivariées pour étudier deux espèces morphologiquement similaires, *Pyrgus bellieri* et *P. alveus*. Dix variables distinctes ont été mesurées sur les organes génitaux mâles, et les résultats ont été examinés au moyen de l'Analyse en Composantes Principales (ACP). Celle-ci a montré une séparation nette entre les deux espèces. Cette séparation a été confirmée par l'Analyse Discriminante. Les variables de classification sont la longueur de la cuillère et la longueur de l'uncus, qui sont inversement proportionnelles chez les deux espèces. Les fonctions de classification pour chacune des espèces ont été établies, avec pour résultat la possibilité d'identifier tout individu à partir de quelques paramètres génitaux mâles. La présence de *P. bellieri* dans la Péninsule Ibérique est confirmée et 18 citations bibliographiques sont rejetées, tandis que 13 nouvelles sont livrées. La distribution qui en résulte dans la Péninsule est limitée à 32 mailles UTM de 10 km dans la zone nord-est, toutes situées en Catalogne et dans la province de Huesca (Aragón). L'étude met en évidence la validité des techniques d'analyse statistique multivariée pour distinguer des espèces proches en utilisant des variables prises sur les organes génitaux mâles.

Keywords: *Pyrgus bellieri*, *Pyrgus alveus*, multivariate morphometric analysis, genitalia, Iberian Peninsula.

Morphometric studies based on measurements taken from the male genitalia have been used in taxonomic studies and have experienced an important increase in the last years, showing that they are useful and valid for such studies (Velásquez de Ríos & Colmenares 1999; Wakehan-Dawson *et al.* 2003, 2004; Kolev 2005; Mutanen *et al.* 2006). Among the currently used statistical methods, the principal component analysis (PCA) has proved to be an adequate tool in the differentiation of species within the Hymenoptera (Velásquez De Ríos & Colmenares 1999) and has also been used together with other methods such as discriminant analysis in Lepidoptera from the families Nymphalidae (Wakehan-Dawson *et al.* 2003), Lycaenidae (Kolev 2005) and Noctuidae (Mutanen *et al.* 2006). In the butterfly family Hesperiidae, statistical multivariate analysis methods were used to compare data taken from the male genitalia, such as the Mahalanobis distance (D^2), that was useful in the discrimination of the species *Pyrgus carlinae* (Rambur [1840]) and *P. cirsii* (Rambur [1840]) made by Guillaumin (1972a, 1972b, 1973, 1974) and Guillaumin & Lefebvre (1974).

The Hesperiidae are one of the less studied butterfly families because entomologists show less interest for the species in this family, particularly those in the genus *Pyrgus* Hübner 1819 that comprises poorly coloured species that cannot be identified properly by the exclusive use of external characters (Guillaumin 1964).

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Oberthür (1910) described the forms *bellieri* and *foulquieri* within the species *Pyrgus alveus* (Hübner [1803]) considering the differences in the extension of the ocre-yellowish bands in the underside of the posterior wings. Reverdin (1913) used the study of the male genitalia to consider *P. bellieri* (Oberthür 1910) as a different species from *P. alveus*, and *P. foulquieri* Oberthür 1910 as just a form of variation within *P. bellieri*. In the literature, *P. bellieri* has been named sometimes as such (Picard 1949; Guillaumin 1964; Jong 1972; Manley & Allcard 1970; Gómez-Bustillo & Fernández-Rubio 1974; Vives-Moreno 1994; García-Barros *et al.* 2004) while other authors have used the name *foulquieri* (Warren 1926; Nel 1985a, 1985b; Fernández-Rubio 1991; Viader 1992; Lafranchis 2000).

P. bellieri and *P. alveus* are very similar externally (Agenjo 1963) and besides, show a remarkable geographic variation (Jong 1972), which altogether make extremely difficult the attempt to identify the specimens without the use of an analysis of the genital structures. The main distinctive characters have been described in the male genitalia (Warren 1926; Guillaumin 1964; Jong 1972), although Guillaumin (1966) also pointed out differences in the female genitalia. *P. alveus* shows a good deal of variation as far as the form and dimensions of the male genitalia are concerned (Warren 1926; Agenjo 1963; Jong 1972). Some specimens, particularly from the Pyrenees, have the so-called cuiller (see explanation below) with a morphology that is very close to that of *P. bellieri* (Warren 1926; Agenjo 1963; Jong 1972). Although morphologic differences are not clear cut, the specialists in the family Hesperiidae Latreille 1809 are coincident in considering *P. bellieri* to be a distinct species (Picard 1949; Jong 1972; Nel 1985a). Differences in the biology and ecology of both species also support this consideration (Nel 1985a).

The records for *P. bellieri* are restricted to the Southwestern region of the Mediterranean area (Jong 1972; Kudrna 2002). In the Iberian Peninsula, it has been recorded in the North of the Peninsula in a strip ranging from the Catalonian Prepyrenean mountains to the Cantabrian Mountains (García-Barros *et al.* 2004). *P. alveus* is a more widespread species in the Palaearctic zone and is found from Northwest Africa and Spain to the region of Amur, in Eastern Russia (Jong 1972; Kudrna 2002). In the Iberian Peninsula it is present in mountain areas in the North of the Peninsula, in the Iberian and Central Mountain Systems and in some mountain ranges in Murcia and Andalusia Provinces (García-Barros *et al.* 2004). Some authors think that the presence of *P. bellieri* in the Iberian Peninsula is doubtful (Jong 1972,1977; Viader 1992) while others strongly reject its presence in the mentioned area (Fernández-Vidal 2002). They consider that the Iberian records are a result of identification errors due to the confusion with *P. alveus*, and that therefore every record should be carefully reviewed (García-Barros *et al.* 2004).

The precise knowledge of a species is vital for its conservation, necessary for the design of adequate conservation strategies and for the evaluation of the species status in Red Data Lists (Viedma & Gómez-Bustillo 1985; Munguira 1989). In the case of *P. bellieri*, it is a priority to produce relevant and reliable information for the species in the Iberian area (García-Barros *et al.* 2004) in order to implement successful measures towards its conservation. In this paper we aim to reach the following objectives: (i) Show the interest of comparative morphometric studies as tools in the differentiation of species. (ii) Confirm the specific separation between *Pyrgus bellieri* and *P. alveus* based on a morphometric analysis of the male genitalia using multivariate analysis techniques. (iii) Point out the differences in the morphology and dimensions between the male genitalia of *P. bellieri* and *P. alveus* and design better morphological criteria for their correct identification. And (iv) confirm or reject the presence of *P. bellieri* in the Iberian Peninsula and review its actual distribution that is different from the current available records in the literature due to identification errors in the latter.

Material and methods

1. Origin of specimens

The published records and location of the citations were extracted from the database ATLAMAR compiled by García-Barros *et al.* (2004). All the records in the database were reviewed, together with the recently published that came from the study area. In all the cases in which we could identify the public or private collections from which the records of *Pyrgus bellieri* came, we contacted the person in charge asking for loan of specimens.

The male genitalia of specimens identified initially as *P. bellieri* from 20 of the 34 localities (59%) from which the species had been cited in the Iberian Peninsula was studied. The sample covered all the main recorded areas (Cantabrian Mountains, Vasque Country, Aragón and Catalonia) and 24 new localities. Specimens from Southern France collected in localities close to the Type Locality of *P. bellieri* were also included in the analysis. The male genitalia of *P. alveus* individuals were also studied from regions of Spain, Morocco, France, Switzerland, Italy, The Netherlands, Serbia and Russia. A total of 213 male specimens from both species were studied, that came from the collections of the following institutions or entomologists: Museo Nacional de Ciencias Naturales of Madrid (MNCN), Museo de Ciencias Naturales de Álava, Basque Country (MCNA), Biology Department in the Universidad Autónoma de Madrid (UAM), Ángel Blázquez (Plasencia), Dr Fidel Fernández-Rubio

(Madrid), Javier Gastón (Bilbao), Juan Hernández-Roldán (Madrid), Hugo Mortera (Gijón), Jesús Requejo (Pontevedra) and Dr Josep Yllla (Gurb, Barcelona).

2. Male genitalia preparation

In 69 specimens the male genitalia were already extracted and mounted in the collection from which they came. In the remaining 144 specimens, the genitalia were processed by cutting the last segments of the abdomen and macerating them in a 20% KOH solution for 24 hours. The hard structures were then cleaned in distilled water and 70% ethanol with an Olympus stereomicroscope. The genitalia were then mounted using DMHF (DiMethyl Hydantoin Formaldehyde). The resulting slides were labelled and kept in the collection from which the specimens came. When the genitalia were mounted, eleven specimens had to be excluded from the study because they were erroneously identified and did not belong to *P. bellieri* or *P. alveus*, but to other *Pyrgus* species. The correct identification of each specimen and the list of all the individuals used in this study can be obtained upon request from J. Hernández-Roldán.

3. Morphometric study

The variables included in the multivariate analysis come from measurements taken over the male genitalia of 202 specimens, initially assigned to *P. alveus* (159) and *P. bellieri* (43). Variable selection took into account differences pointed out by other specialists in relation to the cuiller and valve dimensions (Warren 1926; Guillaumin 1964; Jong 1972) and the antestyle or lateral apophysis (Warren 1926). Other variables were also included, such as measurements of the uncus, tegumen, gnathos and penis. The latter were previously used by Guillaumin (1972b, 1974) in other species of the genus *Pyrgus*. The ten selected variables are shown in fig. 1. The male genitalia nomenclature follows Jong (1972).

Images of the male genitalia slides were captured and digitalized with the same protocol for all specimens, using a video camera attached to a Leica MZ125 stereomicroscope. Image capture was performed with the image analysis software LEICA QWIN, that was also used to measure the variables from the digitalized images. In some individuals certain variables could not be measured because the involved structure was absent (this frequently happened with the penis) or because it was damaged or some part of the genitalia showed a distortion in the preparation.

A preliminary analysis was made to verify whether the average of each variable was different in each species. This was performed with the T test using the Bonferroni correction for multiple testing. The contrast parameter used for each variable depended on the similarity of the variances, and this was verified through the Levene test.

All the statistical analyses were performed using SPSS 12.0 (2003) for Windows.

3.1. Principal Component Factorial Analysis

Factors were extracted through the principal components method (Kim & Mueller 1978), using a selection criterion that consisted in retaining only those factors with values above the unit. By this criterion the loss of predictive capacity of the model was not significant. The total variance explained by the principal components was calculated and this parameter showed the percentage of the variance explained by each factor. Then the factors were rotated, with the orthogonal Varimax rotation, to obtain the expected weights for each extracted factor. The rotation is oriented towards the maximization of the factor variance that resulted in a matrix that showed the rotated components.

3.2. Discriminant Analysis

The discriminant analysis (Huberty 1994) was used for the correct identification of the two species, through the use of discriminant functions that predict to which species each individual should be assigned. We previously took into account the results obtained in the principal component analysis and reassigned the individuals that were erroneously identified to the species suggested by the PCA results.

In order to perform the discriminant analysis the stepwise method was used, adding to the discriminant functions in each step the variable that minimizes the value of the selected statistical parameter. For this the lambda of Wilks was used, so that lower lambda values indicated that a lower proportion of the total variability was caused by error (Catena *et al.* 2003). To confirm the obtained results and test whether the classification was correctly made, a cross validation was made. To test the validity of each variable in the species discrimination, the canonic values from the structure matrix were used, i.e. the correlations between the variables and the discriminant functions.

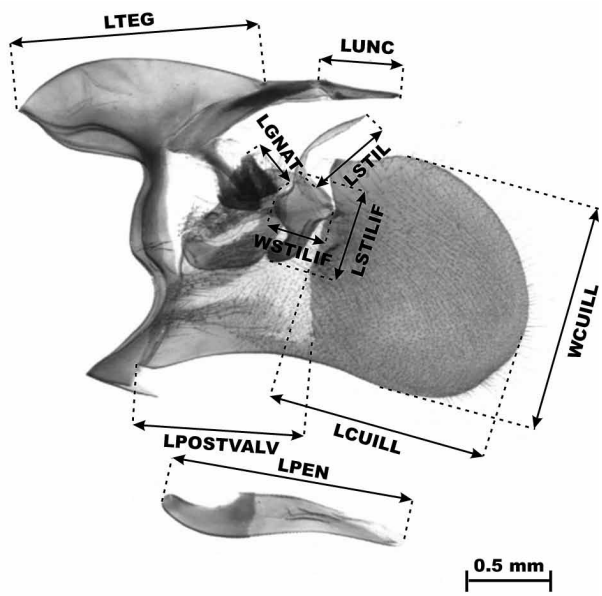

Figure 1
Selected variables for the male genitalia analysis of *Pyrgus bellieri* and *P. alveus*. The photograph represents the male genitalia of a specimen of *P. bellieri* collected in the Province of Tarragona: Sierra de La Musara, 31TCF36, 850 m, 22.VII.1979, J. Gastón *leg.* **WCUILL**, width of the cuiller in the valve; **LCUILL**, length of the cuiller; **LPOSTVALV**, length of the posterior part of the valve; **LUNC**, length of the uncus; **LSTIL**, length of the style; **LSTILIF**, length of the stylifer; **WSTILIF**, width of the stylifer; **LTEG**, length of the tegumen; **LGNAT**, length of gnathos; **LPEN**, length of penis or aedeagus.

Table 1. Values and percentages of the total variance that was explained by each of the ten components extracted through the principal component analysis method (PCA), out of ten variables measured in the male genitalia in *Pyrgus bellieri* and *P. alveus* (N=202).

Component	Value	% of variance	Accumulated %
1	3.649	36.5	36.5
2	1.834	18.3	54.8
3	1.040	10.4	65.2
4	0.751	7.5	72.7
5	0.693	6.9	79.7
6	0.627	6.3	85.9
7	0.481	4.8	90.7
8	0.360	3.6	94.3
9	0.336	3.4	97.7
10	0.230	2.3	100

3.3. Linear Classification Functions

The coefficients of Fisher's linear discriminant functions were obtained and used to develop the linear classification functions for each species. These functions were used to identify the specimen for which genitalia are photographed in Fernandez-Rubio (1981), where it is classified as *P. bellieri.* This publication, with photographs of male genitalia from Iberian butterflies, is widely used by European entomologists. In order to perform this identification the linear classification function was calculated for each species and the specimen was assigned to the species that resulted in a higher value of the function (Catena *et al.* 2003).

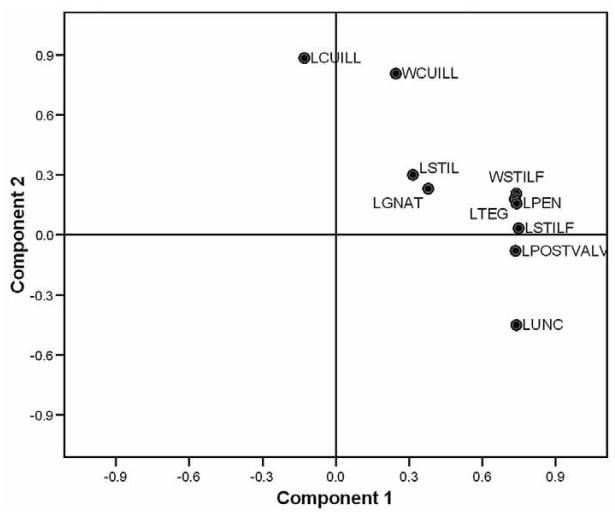

Figure 2
Graph in rotated space of the two components that were extracted from the ten variables in the male genitalia of *Pyrgus bellieri* and *P. alveus* (N=202). The factors were extracted with the principal component method using the Varimax rotation. The variable codes are those shown in fig. 1.

4. Distribution map in the Iberian Peninsula

The map representing the geographic distribution of *P. bellieri*, in 10 km UTM squares, was drawn with the automatic cartography software Mapinfo 3.0 for Windows.

Literature references were taken from the database ATLAMAR (García-Barros *et al.* 2004) to which some new records were added. The data for the revised geographic distribution were obtained from the records resulting from the analysis in this paper. In the localities for which the collector did not provide the UTM coordinates, the digital map Carta Digital version 2.0 (Servicio Geográfico del Ejército 2000) was used to assign the 10 × 10 km square to the record.

Results

1. Role of the variables in the PCA

The principal component analysis of the variables studied in the male genitalia of 202 *Pyrgus bellieri* and *P. alveus* specimens is sumarized in tab. 1. The results show that the three first components of the PCA together explain 65.2% of the total variance. These 3 components only can thus be retained in the analyses. From the figure showing the rotated components (fig. 2), we could infer that the variables with a higher weight in the first component are the length of the posterior part of the valve (LPOSTVALV), the uncus length (LUNC), the length of the stylifer (LSTILIF), the stylifer width (WSTILIF), and the length of the tegumen (LTEG) and the penis (LPEN). Important for the second component were the width and length of the cuiller (WCUILL and LCUILL) and for the third component the length of the style (LSTIL) and the gnathos (LGNAT).

2. Specimen classification

2.1. Previous classification

The graph with the first two dimensions from the principal component analysis (fig. 3) shows two groups of specimens neatly separated. In the further statistical analyses the results from the principal component analysis were taken into account, and this classification was used as null hypothesis for the discriminant analysis. We therefore reassigned to *P. alveus* the eleven specimens that were erroneously identified as *P. bellieri* in the collections they came from. These specimens are from the collections in the Museo Nacional de Ciencias Naturales in Madrid (four specimens collected in Andorra, Cantabrian Mountains and Catalonia) and in the Museo de Ciencias Naturales de Álava (seven specimens from Burgos and the Vasque Country).

Based on the measurements made in each specimen, we obtained the average and standard deviation for

Table 2. Average and standard deviation (SD) of the number of specimens (N) measured for each of the ten selected variables in the male genitalia of *Pyrgus bellieri* and *P. alveus* (values in mm). The variable codes correspond to those used in fig. 1.

Variable	Species	N	Average	SD
WCUILL	*Pyrgus alveus*	170	1.15	0.07
	Pyrgus bellieri	32	1.22	0.05
LCUILL	*Pyrgus alveus*	170	1.05	0.08
	Pyrgus bellieri	32	1.34	0.08
LPOSTVALV	*Pyrgus alveus*	169	1.06	0.08
	Pyrgus bellieri	32	0.98	0.06
LUNC	*Pyrgus alveus*	170	0.62	0.04
	Pyrgus bellieri	31	0.49	0.03
LSTIL	*Pyrgus alveus*	170	0.51	0.08
	Pyrgus bellieri	32	0.52	0.08
LSTILIF	*Pyrgus alveus*	170	0.61	0.05
	Pyrgus bellieri	32	0.55	0.04
WSTILIF	*Pyrgus alveus*	170	0.39	0.04
	Pyrgus bellieri	32	0.38	0.02
LTEG	*Pyrgus alveus*	160	1.50	0.09
	Pyrgus bellieri	31	1.43	0.07
LGNAT	*Pyrgus alveus*	168	0.30	0.03
	Pyrgus bellieri	31	0.29	0.03
LPEN	*Pyrgus alveus*	157	1.58	0.07
	Pyrgus bellieri	30	1.52	0.07

Table 3. Results of the Levene test for variance similarity and the T test for average similarity with independent samples of ten variables taken from the male genitalia of *Pyrgus bellieri* and *P. alveus*. The asterisk represents the significant *p* values considering the Bonferroni correction for ten comparisons ($\alpha = 0.005$). Regarding the WSTILIF variable, the T test values were calculated not assuming similar variance, following the result of the Levene test that was significant. The variable codes correspond to those explained in fig. 1.

	Levene test for variance similarity		T test for average similarity		
Variable	F	p	t	DF	p (Bilateral)
WCUILL	3.000	0.085	–5.416	200	0.0001*
LCUILL	0.268	0.606	–19.130	200	0.0001*
LPOSTVALV	2.332	0.128	5.399	199	0.0001*
LUNC	4.350	0.038	15.628	199	0.0001*
LSTIL	1.212	0.272	–0.579	200	0.563
LSTILIF	6.590	0.011	5.824	200	0.0001*
WSTILIF	10.260	0.002*	3.633	77.2	0.001*
LTEG	3.099	0.080	3.492	189	0.001*
LGNAT	0.027	0.869	0.357	197	0.721
LPEN	0.040	0.842	4.444	185	0.0001*

each variable and group, considering the species as aggregation variable (tab. 2). The Levene test (tab. 3) shows that all the variables have a homogeneous dispersion except the stylifer width (WSTILIF). The T test showed significant differences between the two species for all the variables measured in the male genitalia, except for the length of the style (LSTIL) and the gnathos (LGNAT) (tab. 3). For the signification of the p-values we used the Bonferroni correction for multiple testing (ten tests in our analysis, $\alpha = 0.005$).

2.2. Discriminant Analysis

The variables introduced in the prediction equation with the stepwise method and using the Wilks Lambda were by order of introduction: the length of cuiller (LCUILL), length of uncus (LUNC), length of penis (LPEN), length of stylifer (LSTILIF) and the width of the cuiller (WCUILL). The excluded variables were the length of the posterior part of the valve (LPOSTVALV), the length of the style (LSTIL), the width of the stylifer (WSTILIF) and the length of the tegumen (LTEG) and gnathos (LGNAT). The structure matrix (tab. 4) shows the canonic weight of each variable that is an indication of its discriminant power. The selected discriminant variables were those used in the analysis.

The results confirmed that 100% of the specimens belonged to the predicted species and were therefore correctly identified. The cross validation classification further supported these results. The dispersion graph of the two most discriminant variables (length of the cuiller, LCUILL and length of uncus, LUNC) (fig. 4)

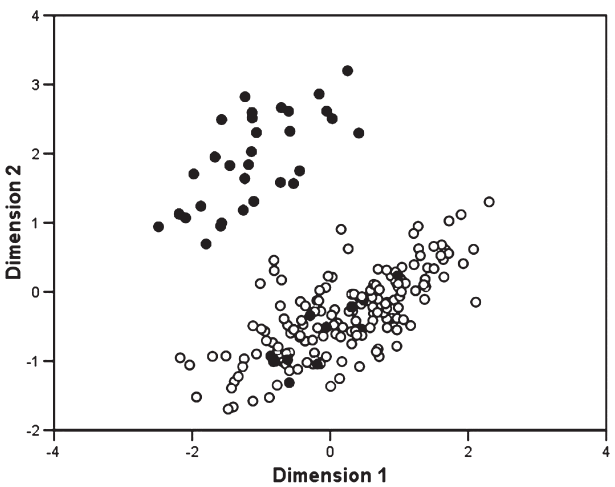

Figure 3
Results from the total studied individuals (N=202) in the two first axes of the principal component analysis from ten variables in the male genitalia. The solid circles show the specimens previously identified as *Pyrgus bellieri* and the white circles those that were identified as *P. alveus*.

Table 4. Correlations between the discriminant variables and the typified canonical discriminant function in the analysis of the male genitalia variables of *Pyrgus bellieri* and *P. alveus*. The variables were ordered according to their correlation with the discriminant function. The variables labelled with (a) were not used in the analysis. The variable codes correspond to those described in fig. 1.

Variable	Discriminant function
LCUILL	–0.490
LUNC	0.417
LSTILIF	0.162
WCUILL	–0.142
LGNAT(a)	0.141
LPEN	0.123
LSTIL(a)	–0.123
LPOSTVALV(a)	0.079
LTEG(a)	0.051
WSTILIF(a)	0.012

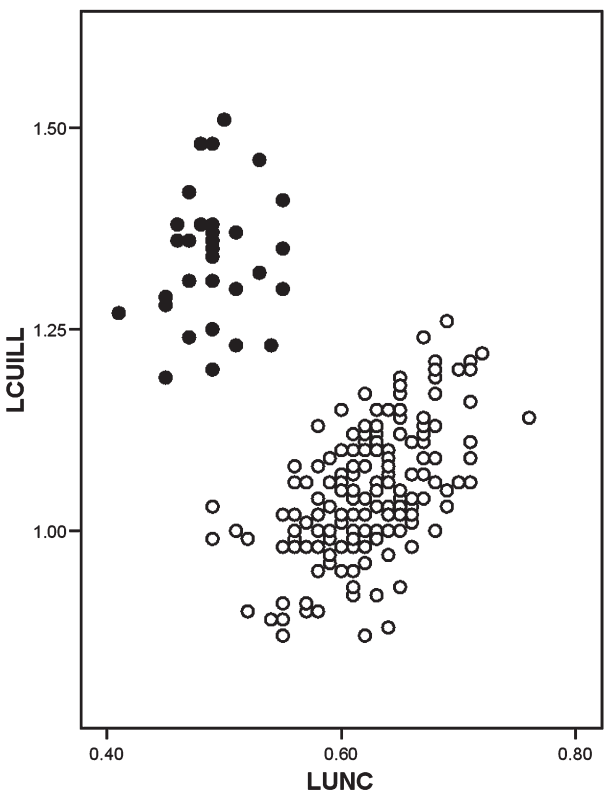

Figure 4
Dispersion graph obtained for the variables length of cuiller (LCUILL) and length of uncus (LUNC) from the male genitalia of the studied individuals of *Pyrgus bellieri* (solid circles) and *P. alveus* (white circles). Values are in mm.

shows two clear cut specimen aggregations, which confirms the differences for these two variables in the two studied species.

2.3. Linear Classification Functions

Based on the equation coefficients, the following linear classification functions (LCF) were obtained for each species:

LCF_{ALVEUS} = –314.66 + 52.12 WCUILL – 18.89 LCUILL + 185.51 LUNC + 41.77 LSTILIF + 281.75 LPEN + ln (150/180)(Equation 1)

$LCF_{BELLIERI}$= –295.17 + 81.94 WCUILL + 79.16 LCUILL + 52.71 LUNC – 12.70 LSTILIF + 239.29 LPEN + ln (30/180)(Equation 2)

These linear classification functions of the studied *Pyrgus* species were used to confirm if the specimen assigned to *P. bellieri* by Fernández-Rubio (1981) was correctly identified. The male genitalia of this specimen are photographed in the mentioned paper, but we were unable to study the specimen, and therefore used it as an application of the LCF method. The variables were measured over the photograph with the following results (expressed in cm): WCUILL (5.6), LCUILL (6.8), LUNC (3.2), LSTILF (3.0), LPEN (7.4). Changing the values in equations 1 and 2 we obtained the following values for the linear classification functions in the specimen: LCF_{ALVEUS}= 2652; $LCF_{BELLIERI}$ = 2601. This result clearly supports that the specimen should be assigned to *P. alveus* and not to *P. bellieri.*

3. Geographic distribution of *Pyrgus bellieri* in the Iberian Peninsula

The distribution of the species *P. bellieri* in the Iberian Peninsula is limited to Catalonia and the Province of Huesca in Aragón (fig. 5). 56% of the 32 UTM squares from which the species had been recorded (i.e. 18 records) are due to misidentifications. Taking this into account, the species is now considered to be present in 27 10 km UTM squares from which 13 (48%) correspond to new records (tab. 5).

Discussion

1. Nomenclature of *Pyrgus bellieri*

Although some authors still name the species *Pyrgus foulquieri* (Warren 1926; Nel 1985a, 1985b; Fernández-Rubio 1991; Viader 1992; Lafranchis 2000), the name that should be considered valid by the application of the priority principle (International Commission on Zoological Nomenclature, 1999) is *Pyrgus bellieri* (Oberthür 1910). The taxa *bellieri* and *foulquieri* belong to the same species and,

although the descriptions for both taxa are made in the same publication and page, *bellieri* is mentioned before (Oberthür 1910: 404) and is therefore the former available name for the species.

2. Genital differences in *Pyrgus bellieri* and *P. alveus*

The male genitalia of the species *P. bellieri* and *P. alveus* present a good deal of variation, mainly in the form and dimensions of the cuiller in the valve (fig. 6). Some specimens of the species *P. alveus* collected in the Pyrenees were considered by Agenjo (1963) as transition forms to *P. bellieri* (an example is given in fig. 6b). In these specimens the cuiller length (LCUILL) is larger than the average of the species leading to errors if only this variable is taken into account for identification. The multivariate analysis that considers the variables length of cuiller (LCUILL), length of uncus (LUNC), length of penis (LPEN), length of stylifer (LSTILIF) and width of cuiller (WCUILL) provides the correct assignment of these specimens. Based on the linear classification functions it is possible to undertake the correct identification of any specimen with a high reliability, having measurements of the variable dimensions. It is for example possible to identify specimens with a photograph of the genitalia, even if it is not scaled.

The principal component analysis (PCA) made with ten variables of the male genitalia in 202 specimens of *P. bellieri* and *P. alveus* shows a dispersal graph for the two first dimensions (fig. 3), with two neatly separated groups. These two groups are separated through the extracted factors and we could thus state that the differences in the male genitalia support the validity of *P. bellieri* and *P. alveus* as different species. The pattern of results in the figure of rotated components (fig. 2) implies, from a theoretical point of view, that the three groups of variables with high weight in each

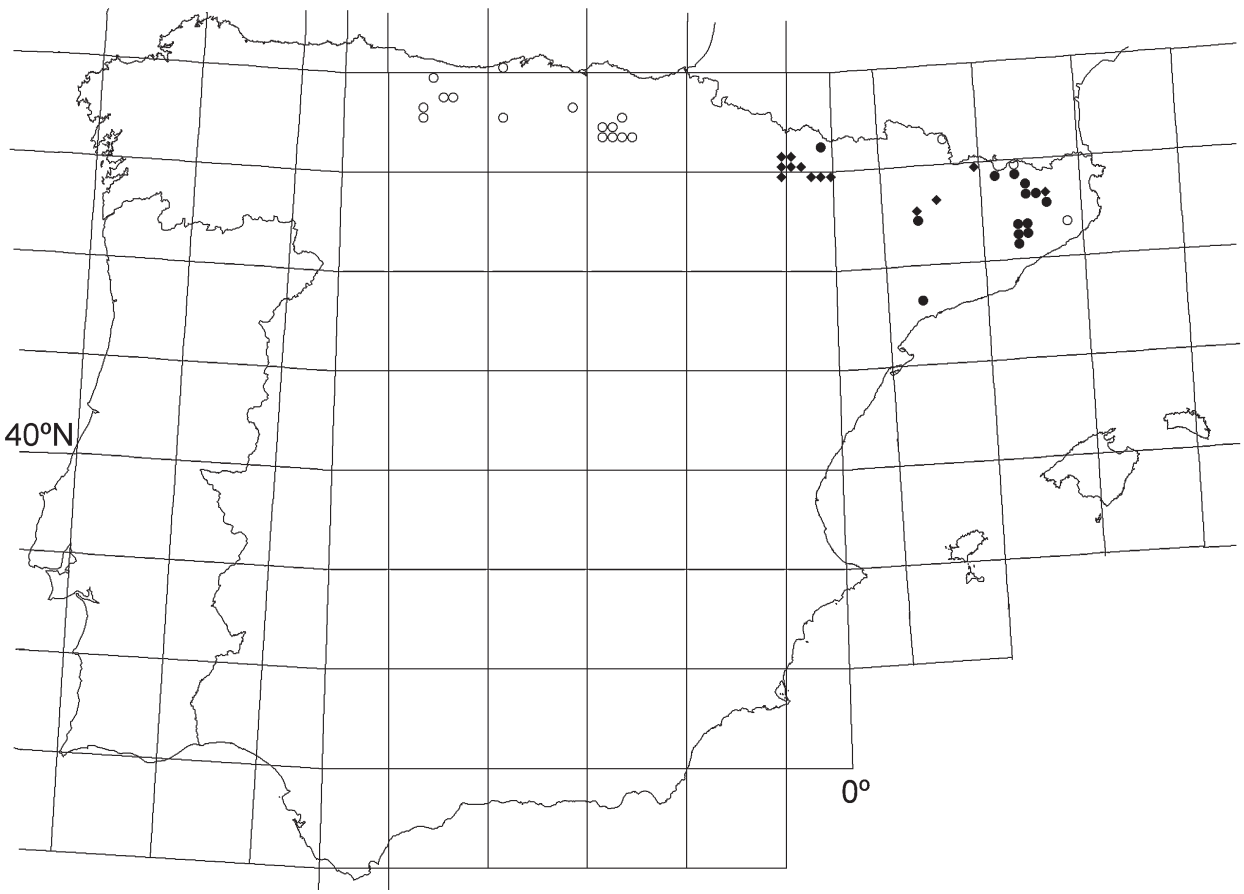

Figure 5
Revised distribution map of *Pyrgus bellieri* in the Iberian Peninsula using 10 km U.T.M. squares (○ squares representing erroneous records from the literature, ● squares representing records from the literature that were verified or are reliable, ◆ squares representing new records). The map was produced with Mapinfo (see methods). Grid square size is 100 km, symbols size is 10 km.

component (dimensions in the stylifer, the posterior part of the valve, the uncus, tegumen and penis for the first component, dimensions of the cuiller in the second component and lengths of the style and gnathos for the third component) are dependent on different mechanisms. The results of the T test show significant differences between the two species for eight out of ten of the selected variables. The similarity of the averages in the two species in the variables length of the style (LSTIL) and length of gnathos (LGNAT) could be explained by errors in the measurement process. The errors could be caused by the difficulty in measuring the frequently curved style and by the superposition of elements above the gnathos and the poor definition of this structure. However, it could also be attributed to the fact that these two variables are not relevant in the differentiation of the two species.

The canonic variable weights obtained from the structure matrix (tab. 4) highlight what was suggested by Warren (1926), Guillaumin (1964) and Jong (1972) regarding the length of the cuiller (LCUILL) as the best discriminant character between the two species and also shows that the uncus length (LUNC) is of great identification importance. It is also noteworthy that the two variables LCUILL and LUNC have different sign, which implies that they have an opposite influence in the discriminant equation. The data in tab. 2 also support this idea: LCUILL and LUNC are inversely proportional in *P. alveus* and *P. bellieri*.

3. *Pyrgus bellieri* records in the Iberian Peninsula

In Catalonia and Aragón, some individuals previously identified as *P. alveus* were proved by both PCA and discriminant analysis to actually belong *P. bellieri*. This confirms without doubt the presence of the former species in the Iberian Peninsula. Conversely, the records of *P. bellieri* from Nuria and Caldas de Malavella (Girona Province), Vall Ferrera (Lleida Province), Lloroza and Camaleño (Cantabrian Mountains), the Vasque Country, Burgos Province and Andorra were proved to be erroneous, due to identifications errors. Only *P. alveus* occurs in these localities. The *P. bellieri* records in the region of Cantabria (Northern Spain, Sanz & Marcos 2004) have not been studied for this work, but their authors only mention external morphology differences, suggesting that they did not use characters in the male genitalia for identification and that the records are almost certainly erroneous. Simi-

Table 5. New records of *Pyrgus bellieri* in the Iberian Peninsula: (*) have been considered in the morphometric analysis of male genitalia, (**) were studied by the authors, but not included in the morphometric analysis, (***) personal communications from collectors. Provinces are shown in brackets, Alt.= altitude of locations in m.

Location	Alt.	UTM 10 × 10	Date	No. & Sex	Collector
Aiguafreda (Barcelona)	450	31TDG32	2.VIII.1978	1 ♂	J. Ylla (*)
El Sallent (Gerona)	350	31TDG66	9.VIII.1999	1 ♂, 1 ♀	J. Requejo (*)
Coll de Sentigosa (Gerona)	1060	31TDG47	2.VIII.1999	1 ♂	M. Taymans (*)
Anzánigo (Huesca)	675	30TXM99	28.VII.1991	1 ♂	M. Taymans (***)
Bernues (Huesca)	1080	30TXN90	12.VII.1990	1 ♂	Ch. Taymans (*)
Broto (Huesca)	950	30TYN32	20.VII.1991	1 ♂	M. Taymans (***)
Pto. de Serrablo (Huesca)	1150	30TYM49	12.VIII.1988	2 ♂♂, 1 ♀	P. Taymans (*)
Pto. de Serrablo (Huesca)	1295	30TYM49	22.VII.2005	2 ♂♂, 2 ♀♀	J. Hernández & A. Blázquez (**)
Javierrelatre (Huesca)	675	30TXM99	29.VII.1991	1 ♂	M. Taymans (***)
Laguarta (Huesca)	950	30TYM49	11.VIII.1988	2 ♂♂	P. Taymans (*)
Laguarta (Huesca)	1216	30TYM49	22.VII.2005	4 ♂♂, 2 ♀♀	J. Hernández & A. Blázquez (**)
Laguarta (Huesca)	1140	30TYM39	23.VII.2005	2 ♂♂, 2 ♀♀	J. Hernández & A. Blázquez (**)
Laguarta (Huesca)	1100	30TYM39	31.VII.1991	1 ♀	M. Taymans (***)
Laguarta (Huesca)	1065	30TYM39	23.VII.2005	3 ♂♂, 2 ♀♀	J. Hernández & A. Blázquez (**)
Oroel (Huesca)	--	30TYN01	26.VII.1996	1 ♂	M. L. Munguira (*)
Osia (Huesca)	850	30TXN90	4.VIII.1991	2 ♂♂, 2 ♀♀	M. Taymans (***)
Puerto de Oroel (Huesca)	850	30TYN00	15.VII.1994	1 ♂	P. Vincens (***)
Sabiñanigo-Villobas (Huesca)	--	30TYM29	15.VII.1985	1 ♂	M. Taymans (*)
Sabiñanigo-Villobas (Huesca)	--	30TYN10	15.VII.1985	1 ♂	M. Taymans (*)
Sta. Cruz de la Seros (Huesca)	740	30TXN91	22.VII.1990	1 ♂	P. Vincens (***)
Sta. Cruz de la Seros (Huesca)	1010	30TXN91	6.VIII.1998	1 ♂	P. Vincens (***)
Coll de Coseu (Lérida)	1250	31TCG56	19.VII.1999	1 ♂	M. Taymans (*)
Vilanova de Meia (Lérida)	1000	31TCG35	14.VII.1998	1 ♂	M. Taymans (*)
Montella i Martinet (Lérida)	1000	31TCG99	3.VIII.1997	2 ♂♂	J. Ylla (*)

larly, the *P. bellieri* record from Riaño (León Province, Cantabrian Mountains) could not be confirmed in this paper. We also suggest that this record is probably an error, because it is geographically isolated and is only based on a single specimen collected in 1961 (Manley & Allcard 1970). This record was later mentioned in Gómez-Bustillo & Fernández-Rubio (1974), but all the further records from the locality, including six specimens studied for this work, were classified as *P. alveus.*

4. Morphometric studies as tools in species identification

In this paper the utility for the identification of

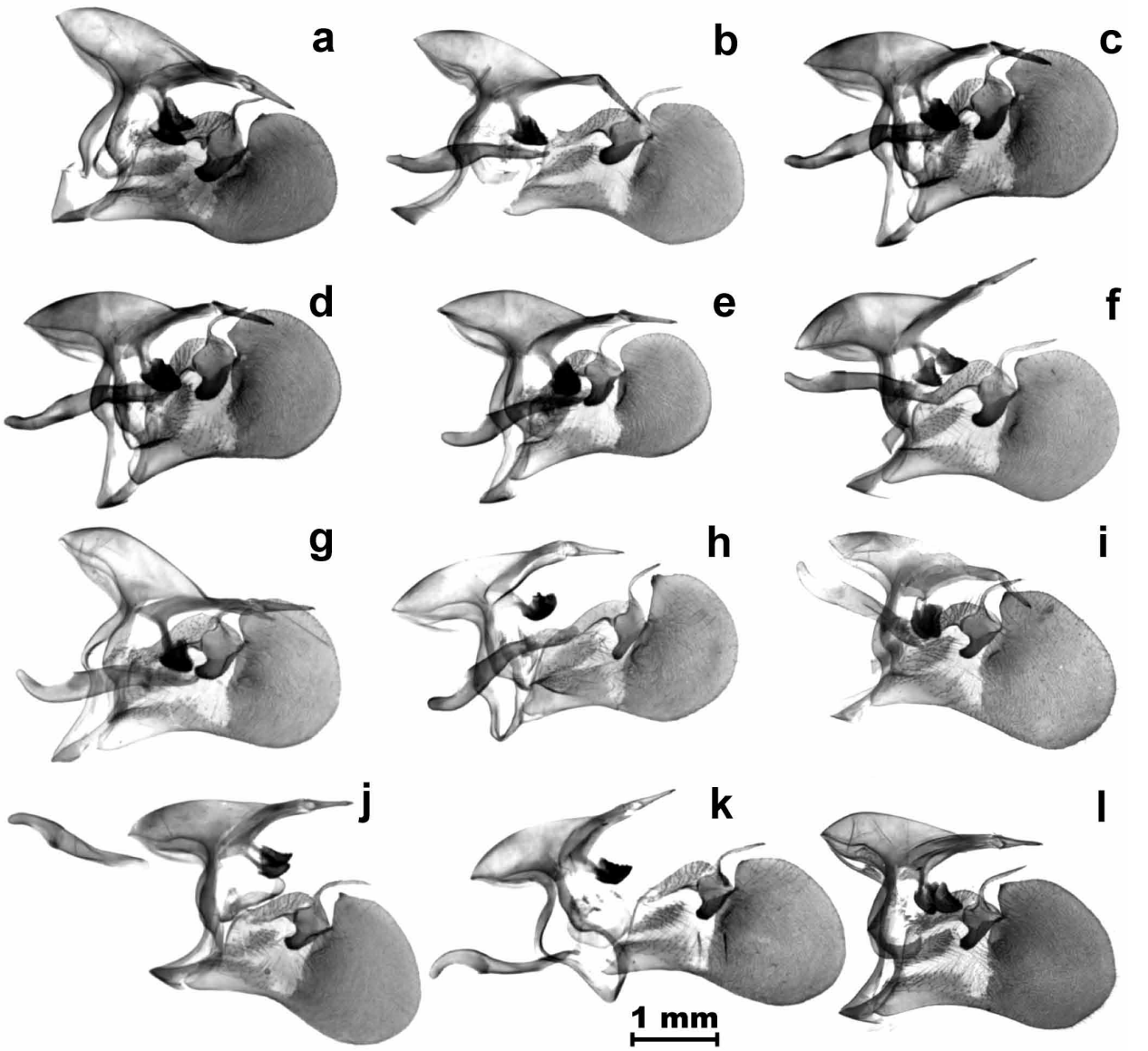

Figure 6
Digitalised microphotographs of the male genitalia of *Pyrgus alveus* (a-h) and *P. bellieri* (i-l) collected in different locations. **a,** *P. alveus*, Kabardino-Balkaria, North Caucasus, Russia (J. Hernández-Roldán *collection*); **b,** *P. alveus,* Setcasas, Gerona, Spain (M.N.C.N. *col.*); **c,** *P. alveus*, Puerto de Okina, Álava, Spain (M.C.N.A. *col.*); **d,** *P. alveus*, Camaleño, Cantabria, Spain (M.N.C.N. *col.*); **e,** *P. alveus*, Cercedilla, Madrid, Spain (M.N.C.N. *col.*); **f,** *P. alveus*, Sierra de Albarracín, Teruel, Spain (F. Fernández-Rubio *col.*); **g,** *P. alveus*, Peñones, Granada, Spain (F. Fernández-Rubio *col.*); **h,** *P. alveus*, Mischliffen, Middle Atlas, Marocco (F. Fernández-Rubio *col.*); **i,** *P. bellieri*, Digne, Lower Alps, France (M.N.C.N. *col.*); **j,** *P. bellieri*, Laguarta, Huesca, Spain (A. Blázquez *col.*); **k,** *P. bellieri*, Gavarra, Lérida, Spain (A. Blázquez *col.*); **l,** *P. bellieri*, Sierra de la Musara, Tarragona, Spain (J. Gastón *col.*). M.N.C.N.: Museo Nacional de Ciencias Naturales (Spanish National Natural History Museum), M.C.N.A.: Museo Ciencias Naturales de Álava.

species of morphometric studies based on statistical analyses of measurements in the male genitalia is clearly supported. Such analyses were also successfully used for morphometric studies of other insects (Velásquez de Ríos & Colmenares 1999; Wakehan-Dawson *et al.* 2004). Moreover, Martín *et al*. (2003) and Ford and Hutchings (2005) have similarly used multivariate analysis techniques in the taxonomic studies in the Polychaeta (Annelida). Morphometric analyses can also be used as complementary tools in studies using DNA or other molecular techniques (Nice & Shapiro 1999; Wahlberg *et al.* 2005), as they require only easily available material and equipments, and allow to use museum specimens.

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