

Contribution to the knowledge
on the Iberian endemic subgenus *Platyzabrus*
Jeanne, 1968 (Coleoptera, Carabidae)

Vicente M. ORTUÑO, Óscar ARRIBAS & Elena ANDRÉS



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COUVERTURE / COVER:

Habitus of *Zabrus (Platyzabrus) constrictus* Gräells, 1858 (male) and *Zabrus (Platyzabrus) pecoudi* Colas, 1942 (male).

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ABSTRACT

The endemic subgenus *Platyzabrus* Jeanne, 1968 has two species with restricted distributions in the Iberian Central System Mountains. Both species are morphologically very close, being the aedeagus the only unequivocal character for differentiation. A total of 244 specimens have been studied, providing a detailed chorology. A morphometric study has been developed measuring 54 specimens, and multivariate (CDA, ANOSIM) and univariate (ANOVA/ANCOVA) analyses have been used to detect subtle differences in body proportions among three geographical units. The only characters that morphometrically discriminate *Zabrus* (*Platyzabrus*) *constrictus* Gräells, 1858 (Béjar) from *Zabrus* (*Platyzabrus*) *pecoudi* Colas, 1942 (Gredos and La Serrota), apart from the size, are MEW (maximum elytral width) and HL (head length). Also, presence/absence of pronotal setae has been evaluated as a distinctive character between species. The specimens from the Sierra de Gredos and La Serrota (*Z. (P.) pecoudi*) have a bilateral pronotal setigerous pore (sometimes unilateral), that appears also in a few specimens from the Sierra de Béjar (*Z. (P.) constrictus*). Any *Platyzabrus* specimen with bilateral absence of these pores belongs to *Z. (P.) constrictus* (Béjar), but when the pore is present it is necessary to study the aedeagus, and in the case of females to carry out a morphometric analysis and take into account chorological data. Both species are allopatric nowadays. This study suggests that original populations became isolated and underwent rapid speciation and aedeagus differentiation, probably due to founder effect and genetic drift; body size reduction of Sierra de Gredos population was possibly posterior to the speciation of both taxa.

KEY WORDS

Zabrini,
morphometrics,
Iberian Peninsula,
Spain,
Central System
mountains.

RÉSUMÉ

Contribution à la connaissance du sous-genre endémique ibérique Platyzabrus Jeanne, 1968 (Coleoptera, Carabidae).

Le sous-genre endémique *Platyzabrus* Jeanne, 1968 compte deux espèces à répartition restreinte dans les montagnes du système central ibérique. Les deux espèces sont morphologiquement très proches, l'aedeagus étant le seul caractère non équivoque permettant de les différencier. Au total, 244 spécimens ont été étudiés, fournissant une chorologie détaillée. Une étude morphométrique a été réalisée sur 54 spécimens et des analyses multivariées (CDA, ANOSIM) et univariées (ANOVA/ANCOVA) ont été utilisées pour détecter des différences subtiles dans les proportions corporelles entre trois unités géographiques. Les seuls caractères qui distinguent morphométriquement *Zabrus* (*Platyzabrus*) *constrictus* Gräells, 1858 (Béjar) de *Zabrus* (*Platyzabrus*) *pecoudi* Colas, 1942 (Gredos et La Serrota), outre la taille, sont MEW (largeur maximale des élytres) et HL (longueur de la tête). La présence/absence de soies pronotales a également été évaluée en tant que caractère distinctif entre les espèces. Les spécimens de la Sierra de Gredos et de La Serrota (*Z. (P.) pecoudi*) présentent un pore sétigère pronotal bilatéral (parfois unilatéral), qui apparaît également chez quelques spécimens de la Sierra de Béjar (*Z. (P.) constrictus*). Tout spécimen de *Platyzabrus* présentant une absence bilatérale de ces pores appartient à *Z. (P.) constrictus* (Béjar), mais lorsque le pore est présent, il est nécessaire d'étudier l'aedeagus et, dans le cas des femelles, de réaliser une analyse morphométrique et de prendre en compte les données chorologiques. Les deux espèces sont aujourd'hui totalement allopatriques. Cette étude suggère que les populations d'origine se sont retrouvées isolées et ont subi une spéciation rapide et une différenciation de l'aedeagus, probablement en raison de l'effet fondateur et de la dérive génétique, la réduction de la taille corporelle de la population de Sierra de Gredos étant peut-être postérieure à la spéciation des deux taxons.

MOTS CLÉS
Zabrini,
morphométrie,
péninsule ibérique,
Espagne,
montagnes du système
central.

INTRODUCTION

The genus *Zabrus* Clairville, 1806 comprises medium and large species that typically exhibit a distinctive, robust, and highly convex appearance, commonly referred to as “zabroid habitus” (Machado 1992). The genus includes more than a hundred species (Andújar & Serrano 2001) that are into more than a dozen subgenera, many of which are stenotopic and predominantly orophilous. The genus is naturally distributed from the Canary Islands (subgenus *Macarozabrus* Ganglbauer, 1915), to the Mediterranean region (part of Europe, North Africa, and the Middle East) reaching eastwards the Himalayas, where the subgenus *Himalayozabrus* Andújar & Serrano, 2000 is found. Species of the nominal subgenus are the only ones with large distribution areas (Machado 1992; Andújar & Serrano 2000, 2001).

Regarding the Iberian Peninsula, the genus is represented by six subgenera, five of which are included in the first catalogues of Ibero-Balearic carabids (Jeanne & Zaballos 1986; Zaballos & Jeanne 1994; Serrano 2003) and the monograph on Iberian species (Andújar & Serrano 2001). The sixth and last subgenus is *Cantabrozabrus* Anichtchenko & Ruiz-Tapiador, 2008, separated from *Iberozabrus* Ganglbauer, 1915 as a monotypic subgenus (Anichtchenko & Ruiz-Tapiador 2008; Serrano 2013, 2020).

Many Iberian *Zabrus* are found in the northern half of the Iberian Peninsula. This area has a series of mountain ranges arranged in the form of a discontinuous ring that surrounds the wide plain called Northern Subplateau (“Submeseta norte”). A wide variety of organisms have evolved on these reliefs and are now Iberian endemics (Ortuño 2002), many of them “microendemics” in the sense of Rapoport (1975).

Most of the Iberian species of *Zabrus* are good examples of this sort of endemism. The southern part of this orographic ring is formed by the Sistema Central, where the subgenus *Platyzabrus* Jeanne, 1968, is found. *Platyzabrus* species have a sub-depressed body and cordiform pronotum, showing a “nebrioid” rather than a “zabroid” appearance (Jeanne 1968; Andújar & Serrano 2001). There are two species both adapted to live in oromediterranean environments: *Zabrus* (*Platyzabrus*) *constrictus* Gräells, 1858 (Fig. 1) in Sierra de Béjar, and *Zabrus* (*Platyzabrus*) *pecoudi* Colas, 1942 (Fig. 2) in Sierra de Gredos (and now discovered in La Serrota massif). The reduction of wings (to become non-functional structures), that also occurs in other orophilous *Zabrus* species, limits their dispersal potential. The external morphology does not offer features that allow to distinguish them unequivocally, as described by Andújar & Serrano (2001). Although Colas (1942) proposed several external differentiating characters, the only unequivocal character to distinguish the two species is the shape of the median lobe of the aedeagus. However, Freude (1990) did not consider these differences strong enough and proposed *Z. (P.) pecoudi* as a junior synonym of *Z. (P.) constrictus*. Other entomologists dismiss this synonymy because of the obvious differences in the male genitalia, and their correspondence with two geographically distinct population groups (Jeanne & Zaballos 1986; Zaballos 1994; Zaballos & Jeanne 1994; Andújar & Serrano 2001; Serrano 2003, 2013, 2020; Ortuño *et al.* 2003; Sánchez-Gea *et al.* 2004; Anichtchenko & Ruiz-Tapiador 2008).

Based on current knowledge of *Platyzabrus*, the aims of this work are: 1) to outline the known distribution of both species with UTM 1 × 1 km grids, and to add some new records that extend their already known ranges; 2) to explore



FIG. 1. — Habitus of *Zabrus (Platyzabrus) constrictus* Gräells, 1858 (male) from Travieso/Candelario (Sierra de Béjar, Salamanca). Scale bar: 3 mm.



FIG. 2. — Habitus of *Zabrus (Platyzabrus) pecoudi* Colas, 1942 (male) from La Mira (Sierra de Gredos, Ávila). Scale bar: 3 mm.

morphological characteristics in *Platyzabrus* species to evaluate their taxonomic statuses and facilitate their identifications; 3) to determine the degree of diagnostic reliability of the trait presence/absence of pronotal seta; and 4) to check if a morphometric analysis is able to detect differences between the two species and in which characters they rely.

MATERIAL AND METHODS

SAMPLED AREA AND STUDIED SPECIMENS

The studied specimens come from three mountainous areas of the Sistema Central: Sierra de Béjar, Sierra de Gredos and La Serrota. The geology of these three reliefs is similar and is dominated by Palaeozoic granitoids (Sánchez-Muñoz 1999; Bellido 2004, 2006), accompanied by some metamorphic outcrops. From a geomorphological point of view, the Pal-

aeozoic materials were uplifted by the Alpine orogeny and then long erosive processes rounded their summits. These reliefs show around 40 glacial traces, thus constituting the best set of Quaternary glaciers in the interior of the Iberian Peninsula (Arenillas & Martínez de Pisón 1976; Pedraza & Carrasco 2005; Carrasco *et al.* 2008).

The distribution of the studied species has been mapped using bibliographic records, as well as data from the scientific collections of the Museo Nacional de Ciencias Naturales de Madrid (MNCN), Vicente M. Ortuño (Universidad de Alcalá) (coll. VMO) and Oscar Arribas (Soria) (coll. OA) (Table 1).

MORPHOLOGICAL STUDY

A total of 244 specimens of *Platyzabrus* (98 exx *Z. (P.) constrictus* and 146 exx *Z. (P.) pecoudi*) were studied and qualitative features were checked, especially genital anatomy and pronotal setation, recording the presence or absence of pronotal setae.

TABLE 1. — Known localities of *Zabrus (Platyzabrus) constrictus* Gräells, 1858 and *Zabrus (Platyzabrus) pecoudi* Colas, 1942 used to build up the distribution map of *Platyzabrus* Jeanne, 1968 of Figure 3. Repository: **MNCN**, Museo Nacional de Ciencias Naturales de Madrid; **coll. VMO**, Vicente M. Ortuño (Universidad de Alcalá); **coll. OA**, Oscar Arribas, Soria.

Province	Location	Coordinates (UTM)	Collection	References
<i>Zabrus (Platyzabrus) constrictus</i> Gräells, 1858				
Ávila	El Trampal, Sierra de Béjar	30TTK7066	–	Zaballos (1987)
	Laguna del Trampal, Sierra de Béjar	30TTK6965	–	Anichtchenko & Ruiz-Tapiador (2008)
Cáceres	Hervás	30TTK6362	–	Anichtchenko & Ruiz-Tapiador (2008)
Salamanca	Candelario, 1800 m	30TTK6868	MNCN	Zaballos (1987)
	Candelario, 1850 m	30TTK6868	MNCN	–
	El Calvitero, 1800-2200 m	30TTK6867	coll. OA	–
	El Calvitero, 2400 m	30TTK6866	coll. OA	Anichtchenko & Ruiz-Tapiador (2008)
	Sierra de Béjar, 1800-2400 m	30TTK6867	–	Anichtchenko & Ruiz-Tapiador (2008)
	Sierra de Béjar, 1900-2200 m	30TTK6865	–	Zaballos (1987)
	Sierra de Béjar, 2400 m	30TTK6764	–	Zaballos (1987)
	Sierra de Béjar, 2400 m	30TTK6866	coll. OA	–
	Sierra de Candelario, 2400 m	30TTK6866	–	Anichtchenko & Ruiz-Tapiador (2008)
	Travieso, Candelario, 1700-1800 m	30TTK6868	coll. OA	–
	Travieso, Candelario, 1800 m	30TTK6868	–	Anichtchenko & Ruiz-Tapiador (2008)
	<i>Zabrus (Platyzabrus) pecoudi</i> Colas, 1942			
Ávila	Camino de la Laguna Grande de Gredos, 2100 m	30TUK0759	coll. VMO	Jeanne (1968)
	Circo de Gredos, 2100 m	30TUK0658	coll. VMO, OA	–
	Garganta Cerradillas, Navalguijo, 1900 m	30TTK8654	–	Zaballos (1994)
	Garganta de las Pozas, 2100 m	30TUK0859	MNCN	–
	Hoya de los Lobos	30TUK1160	coll. OA	–
	Hoya de los Lobos, San Juan de Gredos, 1900 m	30TUK1260	–	Zaballos (1994)
	Hoya del Belesar, Sierra de Gredos	30TUK0260	MNCN	–
	La Mira, 2240 m	30TUK1459	coll. VMO	–
	La Serrota, 2290 m	30TUK2385	coll. VMO	–
	Laguna del Barco, Puerto Castilla, 1850 m	30TTK7857	–	Zaballos (1994)
	Laguna Grande de Gredos, Navalperal de Tormes, 2000 m	30TUK0658	–	Zaballos (1994)
	Los Barrerones, Navalperal de Tormes, 2200 m	30TUK0959	–	Zaballos (1994)
	Navarredonda de Gredos, 1700 m	30TUK2069	MNCN	Colas (1942)
	Navarredonda de Gredos (Parador), 1600 m	30TUK2069	–	Zaballos (1994)
	Plataforma, San Juan de Gredos, 1650 m	30TUK1060	–	Zaballos (1994)
	Plataforma, San Juan de Gredos, 1780 m	30TUK1060	coll. VMO	–
	Puerto de Candeleda, Macizo Central, 2000 m	30TUK0957	–	Zaballos (1994)
	Refugio Club Alpino, 2000 m	30TUK0961	coll. OA	–
	Refugio del Rey, San Juan de Gredos, 2175 m	30TUK0858	–	Zaballos (1994)
	Refugio del Rey, Sierra de Gredos	30TUK0858	–	Anichtchenko & Ruiz-Tapiador (2008)
Cáceres	Sierra de la Vera	not specified	coll. OA	–

A priori species attribution was assigned by the geographic distribution (Béjar and Gredos) and by the aedeagus shape in the case of La Serrota specimens.

The localities of the studied specimens and the citations already published with sufficient accuracy were listed in Table 1 and then projected on a map using points corresponding to UTM 1 × 1 km coordinates (Fig. 3). The projection of coordinates was made using QGIS (Quantum Gis Development Team 2018).

The morphometric study was performed on 54 specimens and both sexes were studied together (Table 2). Specimens were measured with a stereomicroscope (Nikon SMZ 1000[®]) with an ocular micrometer. For the morphometric characters, the following measurements were taken (Fig. 4): **Head**. Maximum head width, including eyes (MHW); postocular head width (poHW); head length between the anterior margin of the clypeus and the posterior contour of the eyes (HL). **Pronotum**. Pronotum width between anterior angles (aaPW); maximum width of the pronotum (PMW); pronotum width between posterior angles (paPW); maximum length of the pronotum between the anterior and posterior angles (MPL);

minimum length of the pronotum between the middle of the anterior and posterior margins (mPL). **Elytra**. Maximum width of the elytra (MEW); elytral width between humeral angles (EWha); maximum elytral length (MEL) (from vertex of the scutellum to apex of elytra).

Statistical analyses used in the morphological study included univariate techniques (ANOVA/ANCOVA, with post-hoc Tukey-Kramer tests at $p < 0.05$ *, $p < 0.01$ ** and $p < 0.001$ *** to detect differences among the samples) as well as multivariate techniques (Canonical Discriminant Analysis, CDA). For univariate statistics we ran analyses with transformed (log [x+1] for linear measurements) and untransformed data, and the results were equivalent, meeting normality assumptions (Sokal & Rohlf 1995). Finally, we used the Log [x+1] transformations for subsequent calculations. The Modified-Levene Equal-Variance Test (Levene 1960; Conover *et al.* 1981) was used to assure the equality of variances (homoscedasticity) that was not rejected for any of the measurements. For the multivariate analysis, Chi-square and Wilks' Lambda tests were used to determine the significance of each axis of the CDA. If groups scored

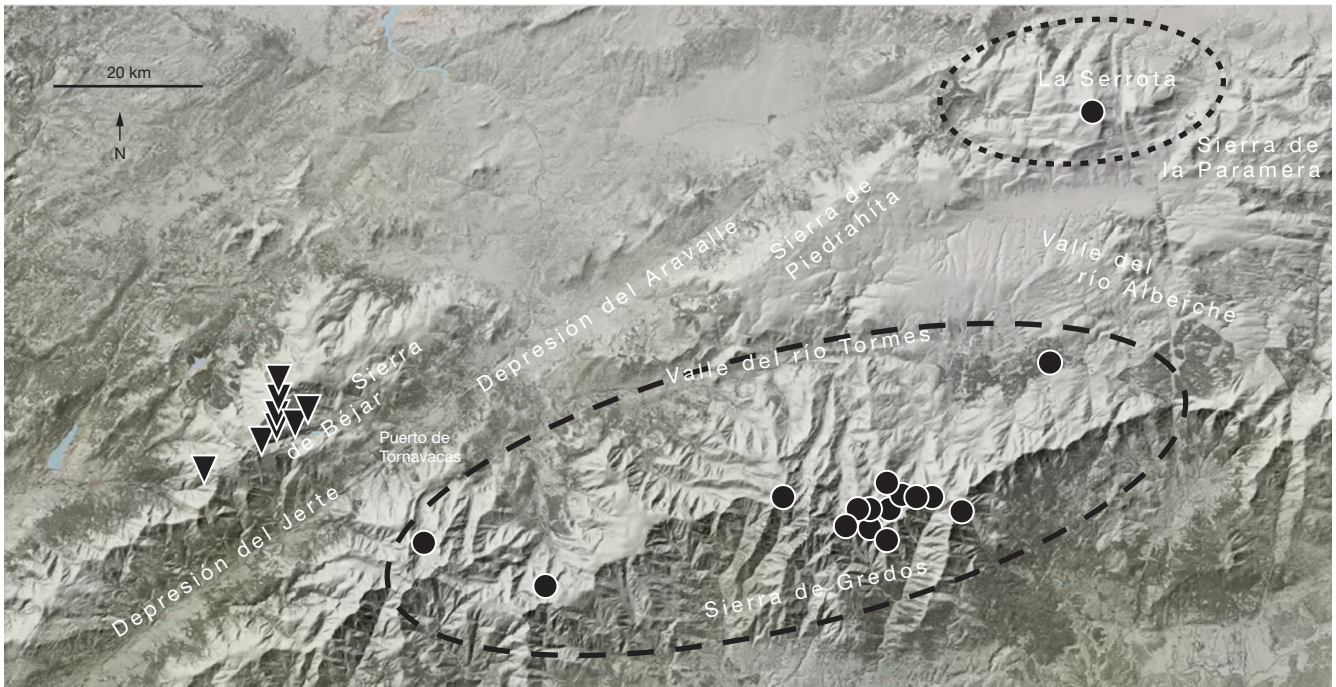


FIG. 3. — Distribution of *Platyzabrus* Jeanne, 1968 species: *Zabrus constrictus* Gräells, 1858 (triangles) and *Zabrus pecoudi* Colas, 1942 (circles). Already known distribution of *Z. (P.) pecoudi* Colas, 1942 is shown with a dashed line and the distribution of the new population with a dotted line.

differently, the discriminant patterns between groups and axes were significant. Bartlett's Sphericity Tests confirmed whether the variables were uncorrelated, a prerequisite for a successful discriminant analysis (Blackith & Reymont 1971; Sokal & Rohlf 1995; Legendre & Legendre 1998; and online help in the statistic programs utilized; see below). A Principal Components Analysis (PCA) was previously run to detect the existence of allometric differences in the *Platyzabrus* imagoes depending on their size. All individuals are treated equally by PCA analysis, and population assignment shown in the graphic is only informative and *a posteriori*.

As all the signs of the first axis were the same, it was interpreted as overall size representing the main source of variation. Differences in coefficients in the second axis were allometric if the sample was homogeneous (same population or species) or taxon-specific, as in our case (Blackith & Reymont 1971; Sokal & Rohlf 1995; Legendre & Legendre 1998). We tested the significance of the differences among pre-established groups by conducting an Analysis of Similarity (ANOSIM) (Clarke 1988, 1993), which tests whether the assigned groups are meaningful (i.e., whether they are more similar within groups than with samples from different groups; see more details and examples of its use in taxonomy in Ortuño & Arribas 2018). We checked for significance by running resampling tests (1000 randomizations) to test whether the given results could occur by chance. If the R value was significant this was evidence that the samples within groups were more similar than would be expected by random chance. Furthermore, pair-wise tests among populations allowed testing of the significance of the differences among the concerned groups and the detec-

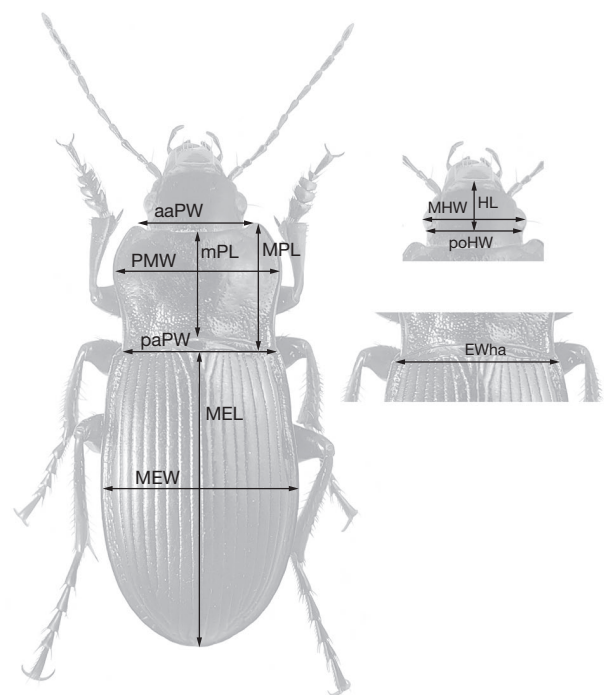


FIG. 4. — Measurements used for morphometric analysis of subgenus *Platyzabrus* Jeanne, 1968. Abbreviations: [Head] HL, head length; MHW, maximum head width; poHW, postocular head width; [Pronotum] MPL, maximum length of the pronotum; mPL, minimum length of the pronotum; PMW, maximum width of the pronotum; aaPW, pronotum width between anterior angles; paPW, pronotum width between posterior angles; [Elytra] MEL, maximum elytral length (apex – vertex of the scutellum); MEW, maximum width of the elytra; EWha, elytral width between humeral angles.

RESULTS

The anatomical character that better allows differentiation between the two *Platyzabrus* species is the aedeagus, especially the shape of the median lobe (Figs 5; 6), while the female genitalia does not show discriminating characters (see Ortuño *et al.* 2003).

Of the 146 specimens of *Z. (P.) pecoudi* studied, 99% show the pronotal setigerous pore bilaterally, while the setigerous pore is only present on one side in the remaining 1% (Fig. 7). This asymmetry is common in many other Carabidae species, but dominant situations can be of diagnostic value. On the other hand, of the 98 specimens of *Z. (P.) constrictus* studied, 88% lack both setigerous pores; 8% have them one unilateral and 4% bilateral (Fig. 7).

Regarding the appearance of the prosternum and the projection of the posterior angles of the pronotum, among other characters (see the characters cited by Andújar & Serrano 2001: 45), it is not possible through ocular inspection, to detect differences between both species. However, a morphometric examination can reveal subtle differences between them, and to discriminate the population of *Z. (P.) pecoudi* discovered in La Serrota.

PRINCIPAL COMPONENTS ANALYSIS

PCA provides two main axes. The first axis explained 87.37% of total variance whereas axis 2 only explained 5.23%. The first axis showed all the coefficients positive, so was interpreted as overall size representing the main source of variation, while the second axis with some positive and other negative values, likely represents positive and negative allometries paralleling the overall increase in size factor.

Specimens from the Sierra de Béjar are larger than those from the Gredos Massif (Fig. 8), but this character clearly overlaps between them; La Serrota specimens showed intermediate scores. The variable that presents the highest coefficient on the first axis (and therefore best represents the increase in size) is EW_{ha} (0.314), which appears in the PCA plot as the longest vector. All the variables that are on the positive side of the second axis (and above the line of the first axis) such as HL (0.953), and MPL (0.061) have positive allometry during growth (thus, the larger the animal, the larger the pronotum and head, increasing the robustness in these two traits), while all other characters (with negative coefficients and on the negative side of the second axis, under the line of the first) have negative allometry. Overall, HL has independent growth concerning all other characters, that more clustered on the graph near the first axis.

EW_{ha} is the best size descriptor (0.314) and differs among the different populations [$F_{2,51} = 30.57$, $P = 0.000000$], so it was taken as a covariate for the statistic comparisons in ANCOVA (see below).

CANONICAL DISCRIMINANT ANALYSIS

The first axis had an Eigenvalue 3.60651 and explained 79.72% of variability ($\text{Chi-Sq.} = 99.11$, 22 d.f., $P = 9.56 \cdot 10^{-7}$); the second axis had an Eigenvalue 0.917344 (20.27% of vari-



FIG. 5. — Aedeagus of a *Zabrus (Platyzabrus) constrictus* Gräells, 1858 individual from Travieso/Candelario (Sierra de Béjar, Salamanca): **A**, middle lobe in lateral view; **B**, right paramere in lateral view; **C**, left paramere in lateral view; **D**, middle lobe in dorsal view. Scale bar: 1 mm.

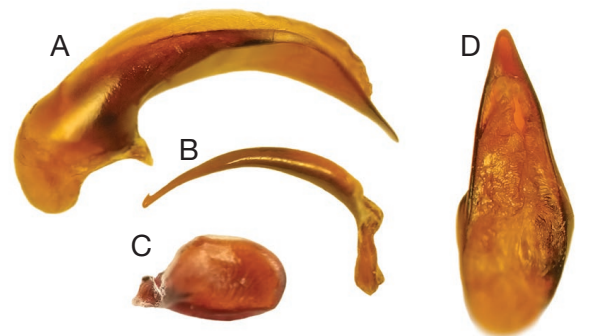


FIG. 6. — Aedeagus of a *Zabrus (Platyzabrus) pecoudi* Colas, 1942 individual from La Mira (Sierra de Gredos, Ávila): **A**, middle lobe in lateral view; **B**, right paramere in lateral view; **C**, left paramere in lateral view; **D**, middle lobe in dorsal view. Scale bar: 1 mm.

ability explained; $\text{Chi-Sq.} = 29.61$, 10 d.f., $P = 0.00098933$). The plot is shown in Figure 9.

Axis 1 separates towards its negative part the population of Gredos that is characterized by the smaller values of MPL (1.743), aaPW (1.121) and, to a lesser extent, paPW (0.569); and, by the comparatively greater scores for mPL (-1.499), MEL (-0.760), PMW (-0.584) and poHW (-0.541). Populations from Béjar and La Serrota showed the opposite scores. In brief, Gredos specimens had comparatively smaller Maximum-pronotum-length, a greater Minimum-pronotum-length (a different shape or proportions of pronotum), a smaller width between the anterior angles and, to a lesser extent, a shorter distance between the posterior angles. Also, Gredos specimens appeared in the plot with greater Maximum elytral length and less marked Maximum pronotal width and postocular head width. La Serrota and Béjar showed opposite tendencies in these characters.

The second axis showed the overlapping of Gredos beetles with those from Béjar and La Serrota, but not between the

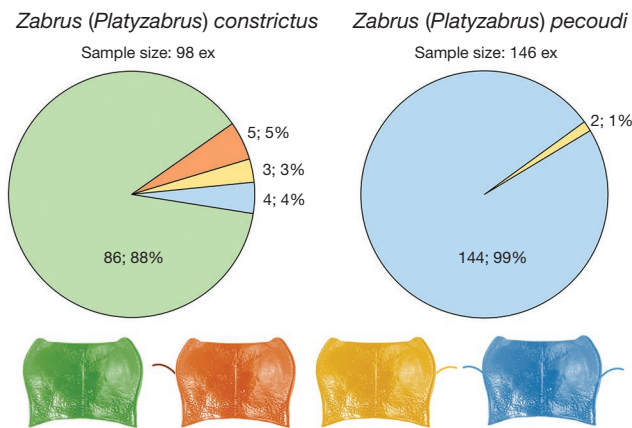


Fig. 7. — Prevalence of the presence of pronotal setae in *Platyzabrus* Jeanne, 1968. The colors are related with the number of pronotal setae (green for zero seta, red for one left seta, yellow for one right seta, blue for two setae).

latter two. La Serrota individuals were found towards the most negative side of the axis and were characterized by higher values of poHW (−1.784), lower values of MEW (1.722), MHW (1.38) and less markedly of MEL (0.866). In contrast, opposite values were found in Béjar specimens. This means that La Serrota specimens, being as large as most of those from Béjar, had comparatively greater postocular width, smaller maximum elytral width, and smaller maximum head width.

There was hardly any overlap between the clouds of the three populations, although there was contact between them (Fig. 9). Both axes together explained almost all the variability (99.9%). The most differentiated population is that from Gredos, while those from La Serrota and Béjar are barely differentiated by external morphology.

The ANOSIM of the sample data showed a fairly good group assignment as the test value was positive, showing more relation between specimens inside each group than with other groups (R-statistic = 0.41676, P = 0.001; 1000 randomizations). The result was significant indicating that the specimens of the involved populations were more similar among them than with other of different samples (only one test greater, by chance, among the 1000 randomizations): Gredos vs Béjar (partial R-statistic = 0.55857, P = 0.001, Significant); Gredos vs La Serrota (partial R-statistic = 0.291005, P = 0.005, Significant), and finally Béjar vs La Serrota (partial R-statistic = 0.0688217, P = 0.228, Not Significant). In summary, specimens from Béjar and La Serrota were not so different from each others based on external morphology but both significantly differed from beetles from Gredos.

As the best descriptor of the increase in body size in the PCA was EWha that presented significant differences between populations in the ANOVA (specifically between Gredos, smaller, and larger ones from Béjar and La Serrota), we performed an ANCOVA with EWha as a covariate, to compare the populations extracting the effect of the size (it is a way to compare the specimens that have the same size but that may differ in other proportions).

The ANCOVA showed differences ($p < 0.001$) between Gredos and Béjar in HL and aaPW as Béjar specimens had comparatively greater head length and pronotum width between anterior angles. When comparing beetles from Gredos and La Serrota the first showed a greater maximum width of the elytra (MEW) and to a lesser extent, a greater maximum elytral length (MEL). Comparison between Béjar and La Serrota populations showed that beetles from the last one showed greater width between the anterior angles and smaller maximum width of the elytra.

However, as we hypothesized that individuals from La Serrota and Gredos belong to the same species, *Z. pecoudi*, according to the aedeagus characters, we repeated the ANOVA/ANCOVA but now merging data from both populations into a single one. We found that EWha again significantly differed between *Z. pecoudi* and *Z. constrictus* in MEW and HL measures, that were comparatively larger (at equal size) in the Béjar specimens (*Z. constrictus*) than in La Serrota plus Gredos ones (*Z. pecoudi*).

DISCUSSION

The morphological similarity between the two *Platyzabrus* species does not facilitate their discrimination, except by examining the middle lobe of the aedeagus. Species assignment is not possible according to female genitalia as these are similar in both taxa that includes a complex spermathecal model, characteristic of this subgenus in the Iberian area (Ortuño *et al.* 2003). The state “presence of pronotal setigerous pore” should be interpreted as a plesiomorphic manifestation, although, in some species of *Zabrus* where the setigerous pore has disappeared it occurs sporadically as an atavistic character (as in Kataev 1996). Pronotal setation had not been used as a differentiating character between *Platyzabrus* species until Andújar & Serrano (2001) indicated that “its presence is exceptional in *Z. constrictus*, while it is common in *Z. pecoudi*” [sic]. Through the evaluation of differences in the pronotum setation, the lack of taxonomic identification of *Platyzabrus* females could be alleviated. But how reliable is the presence/absence of the “pronotal seta” character? According to Andújar & Serrano (2001), we observed the generalized presence of the setigerous pore in the populations of *Z. (P.) pecoudi*, while is absent in almost 90% of *Z. (P.) constrictus*. So, we can infer with a high degree of certainty that any *Platyzabrus* specimen with a bilateral absence of these pores belongs to *Z. (P.) constrictus* (Sierra de Béjar). It is important to remember that both species are allopatric and their distribution limits seem to be well-defined, even between the populations of the Sierra de Béjar (*Z. constrictus*) and the westernmost populations of *Z. pecoudi* in the vicinity of the Puerto de Tornavacas, the higher pass across the “Jerte-Aravalles” depression separating the Sierra de Gredos and Béjar (Fig. 3).

From our morphological study, it is inferred that external morphology and male genitalia do not always match in *Platyzabrus*. Both species (*Z. constrictus* and *Z. pecoudi*)

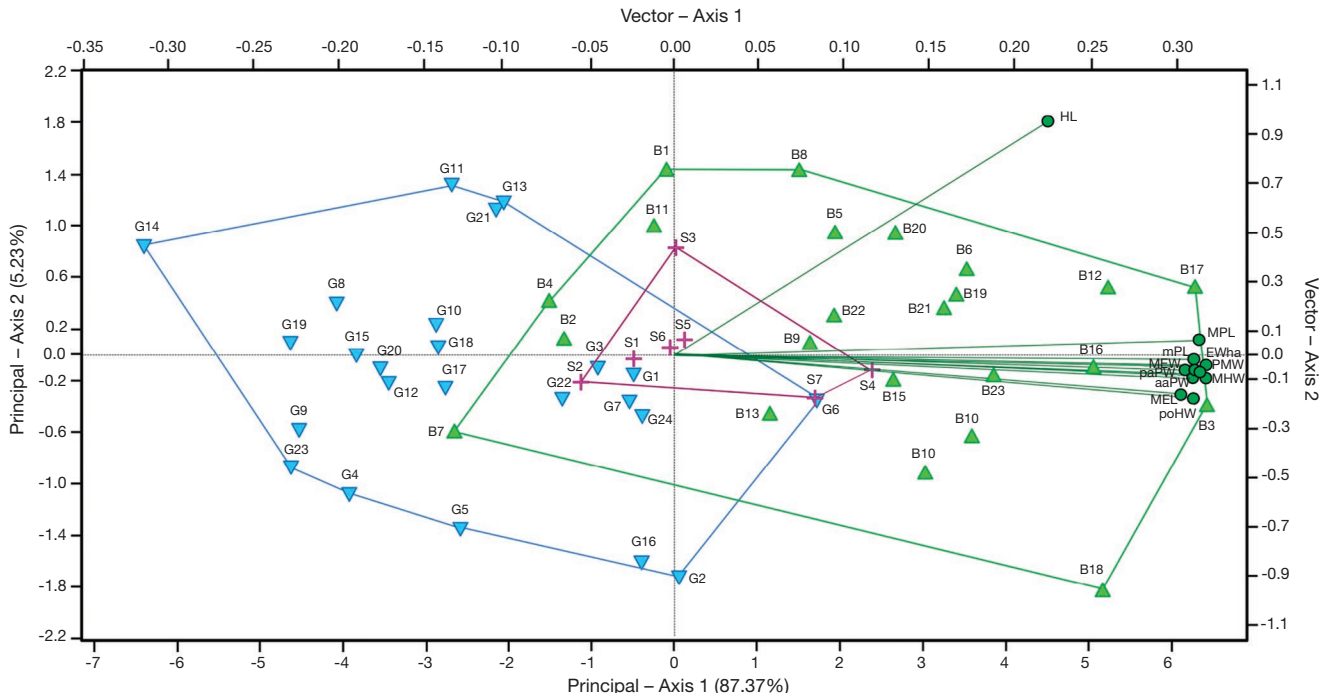


Fig. 8. — PCA plot showing the *Zabrus* (*Platyzabrus*) specimens studied (inverted blue triangle, Gredos Massif; green triangle, Sierra de Béjar; purple cross, La Serrota Massif). All the specimens are ordered by their size in the first axis (87.37 % of variance explained, all variable coefficients positive), whereas the second axis (5.23% of variance, not significant) likely expresses shape difference. Polygons of locality attribution are *a posteriori* and do not intervene in the calculations.

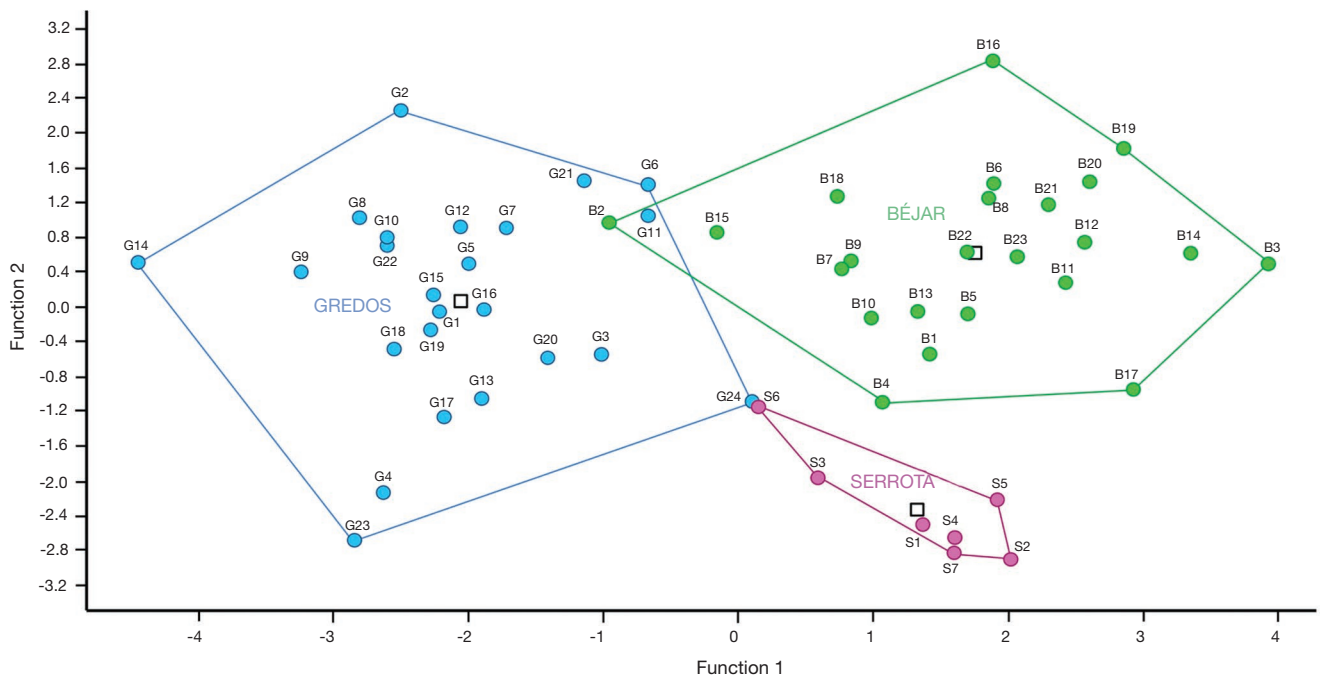


Fig. 9. — CDA plot showing the differentiation of the three populations studied. There is hardly any overlap between the point clouds of the three or populations, although they do contact each other.

have very different aedeagi (Figs 5; 6) but external characters adduced so far to distinguish them (Colas 1942; Andújar & Serrano 2001) are difficult to be appreciate and are not constant. The problem became more complex

after considering results on a third population (La Serrota), from a mountainous area now isolated from the other two (Fig. 3). The specimens from La Serrota are quite large but their size overlaps with the largest specimens from Gredos

and the smallest from Béjar (see PCA, Fig. 8). According to aedeagus characters, specimens from La Serrota are unequivocally conspecific with those from Gredos (*Z. pecoudi*), although they generally have a significantly larger size, a comparatively smaller maximum elytral width, and a shorter maximum elytral length. The only characters that morphometrically discriminate *Z. constrictus* (Béjar) from *Z. pecoudi* (Gredos and La Serrota), apart from the size, were MEW (maximum elytral width) and HL (head length), both larger (independently of size) in the specimens from Béjar (*Z. constrictus*) than in those from La Serrota and Gredos (*Z. pecoudi*).

The subgenus *Platyzabrus* is a sister group to the subgenera *Cantabrozabrus* and *Epomidozabrus*, and the three subgenera have *Euryzabrus* as an external sister group (Sánchez-Gea *et al.* 2004; Anichtchenko & Ruiz-Tapiador 2008). The four subgenera form a basal clade sister to the *Iberozabrus* clade. The two *Z. constrictus* sequences from Sánchez-Gea *et al.* (2004) in GENBANK®, of 772 and 754 bp (AY551850 and AY551851), obtained by concatenation of part of the cytochrome oxidase subunit I gene, tRNA-Leu gene -complete sequence-; and part of the cytochrome oxidase subunit II gene; all they mitochondrial) from Béjar, are identical, once aligned and checked their common parts. By other side, these *Z. constrictus* sequences once aligned with the 555 bp sequence from *Z. pecoudi* from Gredos (AY551879), show six mutations of difference: five in the COI-I segment, and one in the small fragment in common of tRNA-Leu. This small difference suggests that both species could have been separated in relatively recent times, probably during Pleistocene climatic fluctuations. This small genetic distance is striking compared to the notable difference in the aedeagus between both taxa. This fact suggests that the three studied populations diverged during the Pleistocene from a common ancestor of relatively large size, as that found in La Serrota population (see PCA, Fig. 8), whose nowadays intermediate size overlaps with the smallest specimens from Béjar and the largest from Gredos. The Béjar population would have been isolated from this common ancestor, conserving, or even increasing its general size and modifying the aedeagus to evolve into *Z. constrictus*; while the ancestor of the populations of La Serrota and Gredos would have conserved or (moderately) modified the aedeagus found in present *Z. pecoudi* beetles. Later, perhaps due to the harsher conditions in the Gredos area (more heavily glaciated during several phases of the Upper Pleistocene), this population would have decreased in size. The reduction in body size when increasing altitude is a well-known constant, not only in Carabidae (see Rensch 1943 examples in *Carabus* Linnaeus, 1758, and Thiele & Kirchner 1958 with examples and comments on other Carabidae) but also in other beetles and insect groups (Mani 1968). Temperature seems to be a more important factor than food resources (Desender 1989). The rapid speciation and pronounced differentiation of the aedeagus must have occurred due to a strong founder effect and genetic drift in original populations with a low number of individuals.

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