

ORIGINAL ARTICLE

Morphometrics confirms the conspecific between *Blaptica dubia* (Serville) and *B. interior* Hebard (Blattodea: Blaberidae)

María Marta González^{1 *}, Alejandra del Carmen Valverde¹, Mónica Sandra Iglesias¹,
Francisco Antonio Crespo²

¹Laboratorio de Artrópodos, Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón II, Ciudad Universitaria, Intendente Güiraldes 2160, Capital Federal, Buenos Aires, Argentina. E-mails: mariagonzalezch@gmail.com; bichitos.ale@gmail.com; iglesias.fcen@gmail.com

²Centro de diagnóstico e investigación en endemo-epidemias. Administración nacional de laboratorios e institutos de salud “Dr. Carlos G. Malbrán”. E-mail: paco.crespo@gmail.com

*Corresponding author, E-mail: mariagonzalezch@gmail.com

Abstract *Blaptica dubia* (Serville, 1838) and *B. interior* Hebard, 1921 were considered to be cryptic species. The characteristics originally proposed for their identification are not reliable. Thus the relationship between the two species should be re-considered. As a comparative approach and to analyze intraspecific variability, morphological and morphometric methods were performed. Traditional and new characters such as wing shape and pronotum contour were utilized. We found significant differences between both species ($p < 0.05$) in the shape of the wing, but there was an overlap of values. We did not find differences in the body measurements or in the shape and size of the pronotum between the species. The variability gradient does not allow an accurate identification of species neither with the male genitalia or the pronotal macula. Based on the obtained results, *B. interior* Hebard 1921 is considered to be a junior synonym of *B. dubia* (Serville, 1838).

Key words Cockroaches, pronotum, genitalia, geometric morphometric analysis, synonymy.

1 Introduction

The cockroach genus *Blaptica* Stål, 1874 included 17 Neotropical species, 11 recorded in the center, northwest and northeast of Argentina (Hebard, 1921; Princis, 1963; Crespo & Valverde, 2008; Crespo *et al.*, 2010). Hebard (1921) described *Blaptica interior* (Fig. 1) and considered it close to the type species, *Blaptica dubia* (Serville, 1838); meanwhile, he carried out some criteria for the identification of the two species. Roth (1970) studied the male genitalia of Blaberinae. He noticed that the medium phallomere prepuce of *B. dubia* shows numerous spines arranged in several rows while in *B. interior* fewer spines dispose in a single row. However, he only studied a single specimen for each species. Roth (1970) mentioned the intraspecific variation in the number of the preputial phallomere spines and their disposition species of *Blaberus* and suggested that probable intraspecific variation in the number of the preputial phallomere spines and their disposition may be also found in specimens of *B. dubia*. Later, Lopes & Oliveira (2005) studied the genus used the pronotal and macula shapes and the medium phallomere prepuce s as diagnostic characters to identify new species.

Geometric morphometrics is defined as the fusion between geometry and biology, where the biological shape information is extracted for comparative purposes such as the study of taxonomic differences (Jaramillo *et al.* 2015). The use of techniques based on geometric morphometrics applied to the wings and the contour of the pronotum can provide the

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possibility of perceiving small variations that are difficult to see with the naked eye (Bookstein, 1982). In other groups of insects, the shape of the wing is a conserved character that is successfully used in the discrimination of closely related species (i.e. Su *et al.*, 2015; Natero *et al.*, 2017)

The aim of this study is to figure out the relationship between two cryptic species by traditional morphological and morphometric techniques.

2 Materials and methods

2.1 Materials

The materials were clearly divided into two groups as the prior *B. dubia* and *B. interior*, based on the following characteristics: external characters, genital sclerotization and preputial spine arrangements, leaving apart those specimens with intermediate phenotypes. The shape analysis was used to test the validity of the determination.

Morphological terminology follows Hebard (1921), genitalia terms follow Roth (1970).

The 96 adult specimens, *B. dubia* (53) and *B. interior* (43), are deposited in the following institutions: Facultad de Ciencias Exactas y Naturales - Ciudad Autónoma de Buenos Aires, Argentina (FCEN); Museo Argentino de Ciencias Naturales “Bernardino Rivadavia” - Ciudad Autónoma de Buenos Aires, Argentina (MACN); Museo de Ciencias Naturales de La Plata, Argentina (MLP); Instituto Fundación Miguel Ángel Lillo - Tucumán, Argentina (IFML) and the National Museum of Natural History of France- Paris, France (MNHN, examined by photographs). The type specimen of *B. dubia* is missing and the type specimen of *B. interior* is deposited in the Entomology Type Collection at Academy of Natural Sciences of Drexel University (ANSI).

The specimens of *B. dubia* were collected in the Provinces of Buenos Aires, Entre Ríos, La Rioja, Misiones and Montevideo Uruguay; while those of *B. interior* were collected from the Provinces of Buenos Aires, Córdoba, Corrientes, Formosa, Jujuy, La Rioja, Salta, San Luis, Santiago del Estero and Tucumán (Fig. 2).

The specimens studied are from Argentina, except one male from Uruguay (Tables 1, 2).

2.2 Preparation of male genitalia and pronotal macula

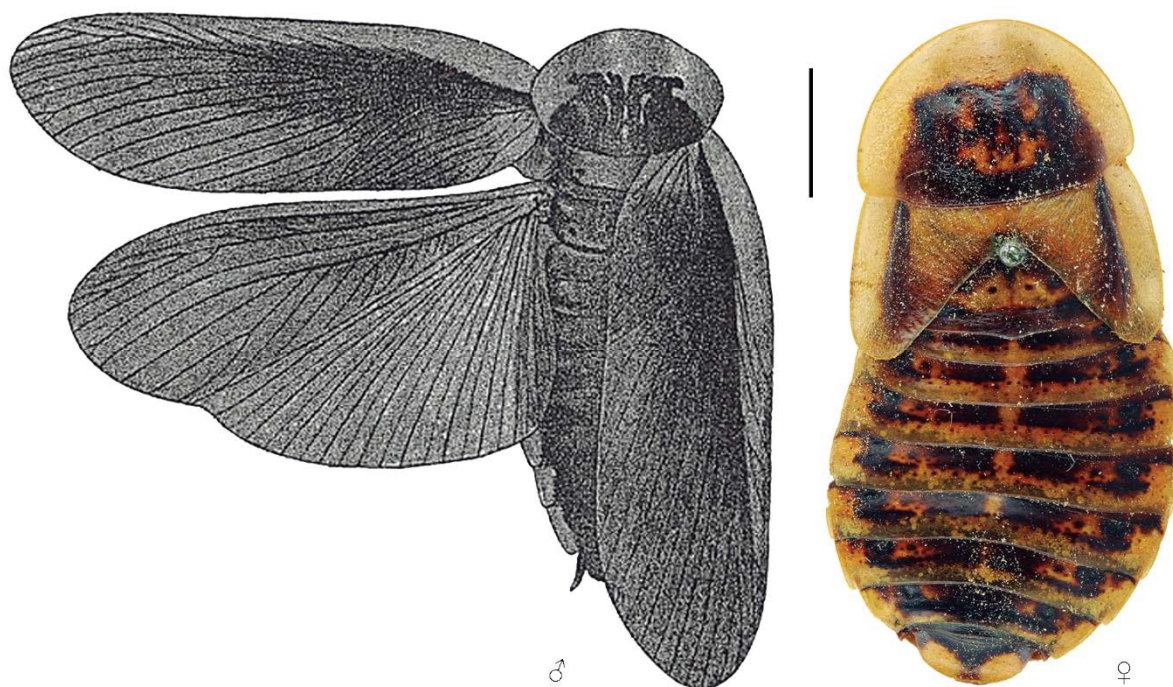


Figure 1. *Blaptica interior*, dorsal habitus. Male (after Hebard, 1921) and female (deposited in MNHN). Scale bar = 1 cm.

Ten specimens of *B. interior* and 16 specimens of *B. dubia* were submerged in warm water to be softened before dissected. Specimens were photographed using a stereoscopic microscope Leica MZ8 (magnifications 6.3–50x), and a digital camera Nikon Coolpix S630 12MP mounted on the microscope. To study and photograph genitalia, the sclerites were treated in a 10% NaOH solution to remove remaining of soft tissue, rinsed with water and finally placed on a microscopic ring slide with glycerin to avoid structural deformation and allow proper description.

Pronotum of both species (*B. dubia* (30 males and 23 females), *B. interior* (16 males and 17 females)) were photographed with a Panasonic DMC-SZ8 digital camera coupled to the stereoscopic microscope Leica MZ8.

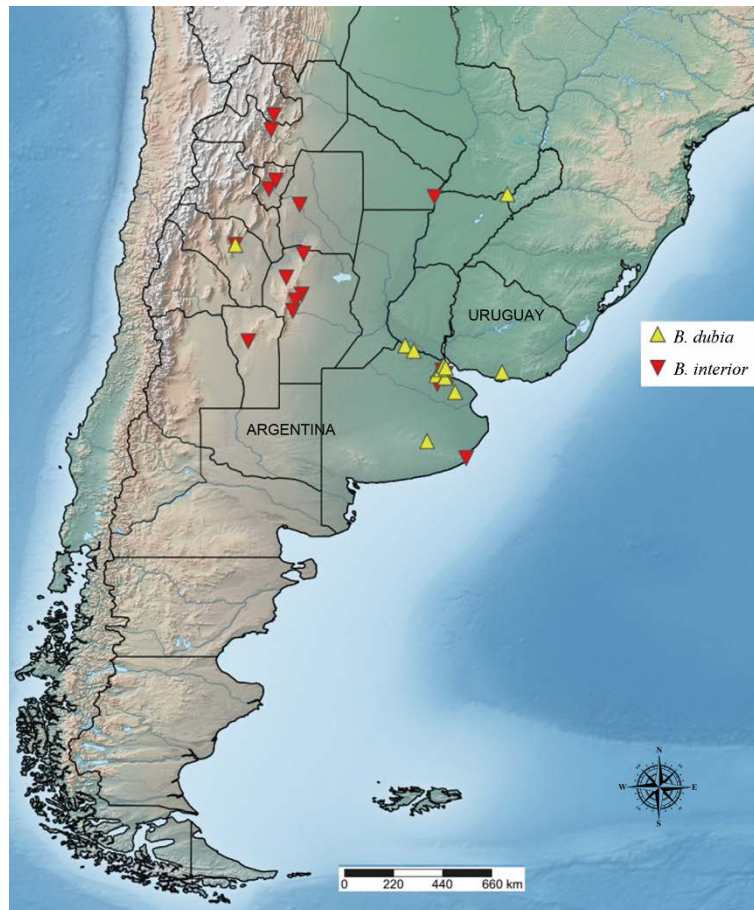


Figure 2. Distribution map of the phenotypes treated as *Blaptica dubia* and *B. interior*.

2.3 Traditional morphometry

Twenty-seven females and 55 males (*B. dubia* (30 males and 10 females), *B. interior* (25 males and 17 females)) were used in this study. The following measurements in millimeters (mm) were taken with a linear scale caliber and by means of a millimeter eyepiece: total body length (TL); pronotum length (PL); pronotum maximum width (PW); interocular distance (IO); interantennal distance (IA) and interocelar distance (IOc). The width of interval between the tegmina, mentioned by Hebard (1921), was not considered because it is not a reliable measure, due to the great variability in the posture after dying.

A Permanova test was performed with all the measures between species/sexes to detect among the four groups as well as Bonferroni test with *p* corrected values using the PAST 3.1 program (Hammer, 2011).

2.4 Geometric Morphometrics

2.4.1 Pronotum

Outline analysis. Forty-five male and 40 female images (*B. dubia* (30 males and 23 females), *B. interior* (15 males and 17 females)) were used in order to analyze the pronotum shapes. Each image was transformed to binary colors (black and

white) to detect pronotum contour. The parameters that best defined the pronotum shape were calculated using Elliptical Fourier Analysis (EFA) and analyzed using SHAPE software (Iwata & Ukay, 2002). The protocol included the extraction of the contour, the calculation and derivation of the normalized elliptical Fourier coefficient and determination of the principal component, which summarizes the information contained by the coefficients. Finally, the Fourier transformed inverse was applied, to obtain the graphs of the mean shapes of pronotum. The contours were processed and transcribed to a chain code that contains the geometrical information of the shape. The shape was then reconstituted by 10 harmonics (37 Fourier coefficients). The standardization is based on the ellipse of the first harmonica. The Fourier coefficients considered as a set of transformed variables can be used in Principal Component Analysis (PCA). In addition, the pronotum size (estimated as area of the contour) information offered by the program was extracted, to compare between species / sexes (4 groups) by means of a Kruskal Wallis test followed by a Tukey pairwise. To show pronotum shapes between species, two separate PCA were performed with the shape coefficients for each sex. These two main principal components were used as variables for Permanova with 10000 permutations and Bonferroni p-value corrected, is a test used to make a comparison between two or more groups (Boroni *et al.*, 2017).

Table 1. Specimens identified as *Blaptica interior* Hebard, 1921.

Institution	Locality	Date & collector	Identifier	♂	♀
MNHN	Argentina, Córdoba, Cruz del Eje	coll. M. Hebard	Hebard, 1921		1
Paratype					
MNHN	Argentina, Santiago del Estero, Río Salado	coll. Wagner	Hebard, 1922	1	
MACN	Argentina, Jujuy	Missing	Hepper, 1965	1	
MACN	Argentina, La Rioja	1920	Hepper, 1965		1
MACN	Argentina, La Rioja, Illiar	XI-1934, coll. M. Gómez	Valverde & González, 2015	1	
MACN	Argentina, Salta	VI-1933, coll. J. Daguerre	Valverde & González, 2015	1	
MACN	Argentina, Córdoba, Calamuchita	1938, coll. M. Viana	Valverde & González, 2015	5	
MACN	Argentina, Córdoba, Sobremonte	I-1964, coll. Gallardo	Valverde & González, 2015	3	
MACN	Argentina, Córdoba	Missing	Hepper, 1965	1	2
MACN	Argentina, Córdoba, Alta Gracia	XI-1922, coll. C. Bruch	Valverde & González, 2015	1	
MACN	Argentina, Buenos Aires, Capital Federal	Missing	Hepper, 1965		1
MACN	Argentina, Buenos Aires, Capital Federal	Missing	Valverde & González, 2015	1	
MLP	Argentina, Santiago de Estero	coll. Wagner	Valverde & González, 2015	1	
FCEN	Argentina, San Luis	Missing	Valverde & González, 2015	4	
FCEN	Argentina, Buenos Aires, Beccar	Missing	Valverde & González, 2015	1	
FCEN	Argentina, Buenos Aires, Cañuelas	Missing	Valverde & González, 2015		1
FCEN	Argentina, Buenos Aires, Mar del Plata	Missing	Valverde & González, 2015		1
FCEN	Argentina, Buenos Aires, Guillón	Missing	Valverde & González, 2015		1
FCEN	Argentina, Córdoba	Missing	Valverde & González, 2015		1
IFML	Argentina, Tucumán, Cañete	Missing	Rocha e Silva*	2	
IFML	Argentina, Córdoba	I-1948, coll. P. López	Crespo & Valverde, 2005	1	
IFML	Argentina, Corrientes	I-1947, coll. T. De Apostol	Crespo & Valverde, 2005	2	
IFML	Argentina, Tucumán	Missing	Crespo & Valverde, 2005		2
IFML	Argentina, Córdoba, Colón Salsipuedes	I-1956, coll. H. Giorgetta	Crespo & Valverde, 2005		3
IFML	Argentina, Córdoba, Calamuchita	17-I-1957, colls. Willink & Tonsic	Crespo & Valverde, 2005		1
IFML	Argentina, San Luis	Missing	Crespo & Valverde, 2005		2

*Not available

2.4.2 Wings

The male right wings (23 *B. dubia*, 12 *B. interior*) were mounted on a slide and photographed with Canon EOS Rebel T3i camera with scale in millimeter. Five anatomical reference points or landmarks were placed on the wing images, which generate the geometric configuration analyzed (Fig. 3): Landmark 1, Radial 1 and Radial 2 intersection (LM₁); Landmark 2, intersection between Medial and Cubital, proximal to the Medial (LM₂); Landmark 3, intersection between Medial and

Cubital, distal to Medial (LM₃); Landmark 4, maximum width distal edge and intersection with anal lobe (LM₄); and Landmark 5, maximum distal edge of the wing (LM₅). LM₁, LM₂ and LM₃ located at the intersection of ribs are type I, and LM₄ and LM₅ defined as distant points in the structure of interest correspond to type III (Bookstein, 1991). The reference points used do not completely reflect the shape of wing, however, the points chosen were the only ones that corresponded in all the specimens (homologous points). The landmarks were analyzed only in the right wing to avoid problems due to asymmetry (Fink, 1990). The TpsDig2 program (Rohlf, 2010) was used for the digitization of landmarks. PCA was performed to study wing shapes and permutation tests were performed with Procrustes and Mahalanobis distances. In addition, a cross-validation discriminant analysis was performed. Deformation grids were obtained from the consensus shape on the first principal component (PC1) axis. All these analyses were carried out through the Morpho J program (Klingenberg, 2011). The wing sizes of each specimen were estimated from the wing size-centroid. The CS (Centroid Size) consists of the square root of the sum of the Euclidean distances squared from each landmark to the centroid (Bookstein, 1991). These sizes were analyzed by Student's t test with the PAST 3.1 program (Hammer, 2011).

Geometric morphology for female wings could not be carried out because it was impossible to establish landmarks. In all cases a p-value <0.05 was considered significant.

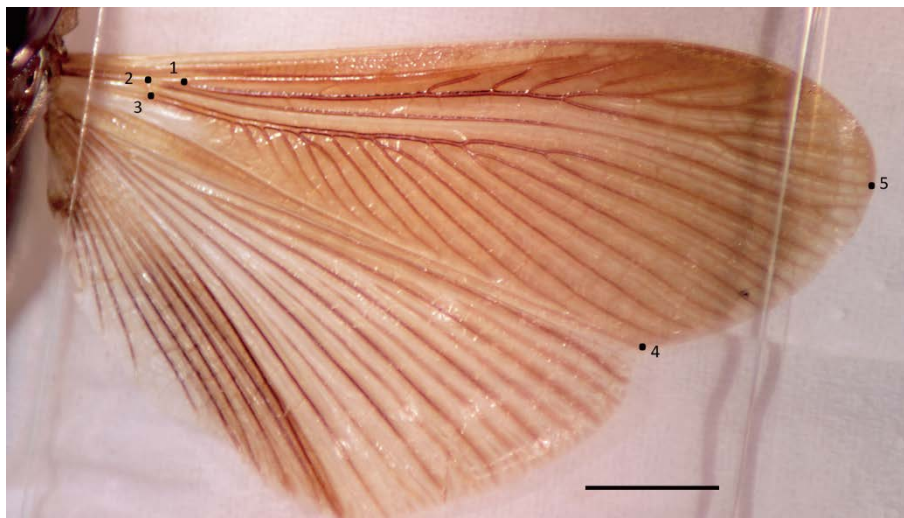


Figure 3. *Blaptica dubia* (male), position of landmarks on the right wing (dorsal view). Scale bar = 0.5 cm.

3 Results

3.1 Male genitalia

The genital sclerite shapes of both species are very similar. While in *B. dubia*, the phallomeres are more sclerotized than that in *B. interior*, especially the cleft (clf) of the left phallomere (L1) and the hook of the right phallomere (R) (Fig. 4). Moreover, there is a gradient between both species. The L2 average number of spines was analyzed, having 23–49 in *B. dubia* and 12–27 spines in *B. interior*. Therefore, species identification is blurred.

3.2 Pronotal macula

Intraspecific variability in the central macula shape was detected in males and females of both species. And an interspecific continuous gradient of shapes was noticed too (Fig. 5).

3.3 Traditional morphometry

The intervals of linear body measurements (TL, PL, PW, IO, IA, IOc) overlap in males and females of both species, with *B. interior* presenting values slightly below those of *B. dubia* (Table 3). The Permanova test (with 9.999 permutations) was significant among the four species/sexes-groups, whereas the Bonferroni test detected sexual dimorphism in both species. Significant interspecific differences were not detected (females p: 0.252 and males p: 0.364).

Table 2. Specimens identified as *Blaptica dubia* (Serville, 1838).

Institution	Locality	Date & collector	Identifier	♂	♀
MACN	Argentina, Tandil	15-III-1962	Hepper, 1965	1	
MACN	Argentina, La Rioja, Illiar	Missing	Hepper, 1965	1	1
MACN	Argentina, Buenos Aires	Missing	Hepper, 1965	1	
MACN	Argentina, Capital Federal	Missing	Hepper, 1965	1	
MACN	Argentina, Buenos Aires, Isla Martín García	Missing	Hepper, 1965	1	1
MACN	Argentina, Buenos Aires, Tandil	Missing	Hepper, 1965		1
MACN	Argentina, Buenos Aires	Missing	Valverde & González, 2015		1
MACN	Uruguay, Montevideo	V-1906	Valverde & González, 2015	1	
MLP	Argentina, Buenos Aires, Ramallo	18-III-1974	Valverde & González, 2015	1	
FCEN	Argentina, Misiones, Posadas	Missing	Valverde & González, 2015	1	
FCEN	Argentina, Capital Federal	3-XII-1975, coll. D. Nahabedian	Valverde & González, 2015	1	
FCEN	Argentina, Capital Federal	4-XII-1977, coll. D. Aljanati	Valverde & González, 2015	1	
FCEN	Argentina, Capital Federal	coll. Muñiz Saavedra	Valverde & González, 2015	1	
FCEN	Argentina, Capital Federal	XII-1972, coll. Puricelli	Valverde & González, 2015	1	
FCEN	Argentina, Capital Federal	18-X-1978, coll. N. Baro	Valverde & González, 2015	1	1
FCEN	Argentina, Buenos Aires, Tandil	Missing	Valverde & González, 2015	2	
FCEN	Argentina, Buenos Aires, San Martín	Missing	Valverde & González, 2015	1	
FCEN	Argentina, Buenos Aires, Villa Martelli	Missing	Valverde & González, 2015	1	
FCEN	Argentina, Buenos Aires, Merlo	9-V-1968, coll. Comas	Valverde & González, 2015	1	
FCEN	Argentina, Buenos Aires, Merlo	Missing	Valverde & González, 2015	1	
FCEN	Argentina, Buenos Aires, Lanús	1-XII-1977, coll. Pelaez	Valverde & González, 2015	1	
FCEN	Argentina, Buenos Aires, Merlo	4-XII-1977, coll. Fadel	Valverde & González, 2015	1	
FCEN	Argentina, Buenos Aires, Lomas de Zamora	9-XI-1981, coll. Palmieri	Valverde & González, 2015	1	
FCEN	Argentina, Buenos Aires	6-X-1977, coll. Donadio	Valverde & González, 2015	1	
FCEN	Argentina, Buenos Aires	15-XI-1978, coll. Mazzuchelli	Valverde & González, 2015	1	
FCEN	Argentina, Buenos Aires	XI-1978, coll. Alberdi	Valverde & González, 2015	1	
FCEN	Argentina, Buenos Aires	7-XII-1977, coll. M. Pepa	Valverde & González, 2015	1	
FCEN	Argentina, Buenos Aires	20-X-1977, coll. Ramaglio	Valverde & González, 2015	1	
FCEN	Argentina, Buenos Aires	II-1970, coll. Avanzati	Valverde & González, 2015	2	
FCEN	Argentina, Capital Federal	1971, coll. Avanzati	Valverde & González, 2015		1
FCEN	Argentina, Capital Federal	1980, coll. Cei	Valverde & González, 2015		1
FCEN	Argentina, Capital Federal	III-1966, coll. Micastronuovo	Valverde & González, 2015		1
FCEN	Argentina, Capital Federal	1968, coll. Mistchelf	Valverde & González, 2015		1
FCEN	Argentina, Buenos Aires, San Pedro	1971, coll. Fiocchi,	Valverde & González, 2015		1
FCEN	Argentina, Buenos Aires, Hurlingham	IX-1967, coll. Prenski	Valverde & González, 2015		2
FCEN	Argentina, Capital Federal	Missing	Valverde & González, 2015		5
FCEN	Argentina, Buenos Aires, Chascomús	11-IV-1971, coll. Lacorte	Valverde & González, 2015		1
FCEN	Argentina, Buenos Aires, Tigre	Missing	Valverde & González, 2015		1
FCEN	Argentina, Buenos Aires, Lanús	Missing	Valverde & González, 2015		1
FCEN	Argentina, Buenos Aires, Nogués	Missing	Valverde & González, 2015		2
FCEN	Argentina, Buenos Aires, Isla Martín García	Missing	Valverde & González, 2015		1

3.4 Geometric morphometry

3.4.1 Pronotum

Fig. 6A shows that the male pronotum contours of both species occupy overlapping morphospaces. It can be observed

on the Principal Component 1 (PC1) that there are specimens of *B. dubia* at each extreme, while the specimens of *B. interior* occupy mainly the middle zone of the morphospace. The 69.62% variation is explained by the PC1 and the 30.38% by the Principal Component 2 (PC2). The male pronotum outline in specimens of both species graduates from a quadrangular to a triangular shape, although the intermediate shape is mainly found in *B. interior* specimens. In females, the first two principal components explain 99.99% of the total variance; PC1 explains 87.58% of the variability between species. PC2 explains 12.41%, no differences of the pronotum shapes are observed from one end to the other. The pronotum shape graph shows for both species the variability from a more elliptical shape and with the posterior margin convex to a more triangular shape with the straight posterior edge (Fig. 6B). Unlike males, females of both species display all pronotal shapes. The males showed a greater variability in pronotum shapes than females (Fig. 6).

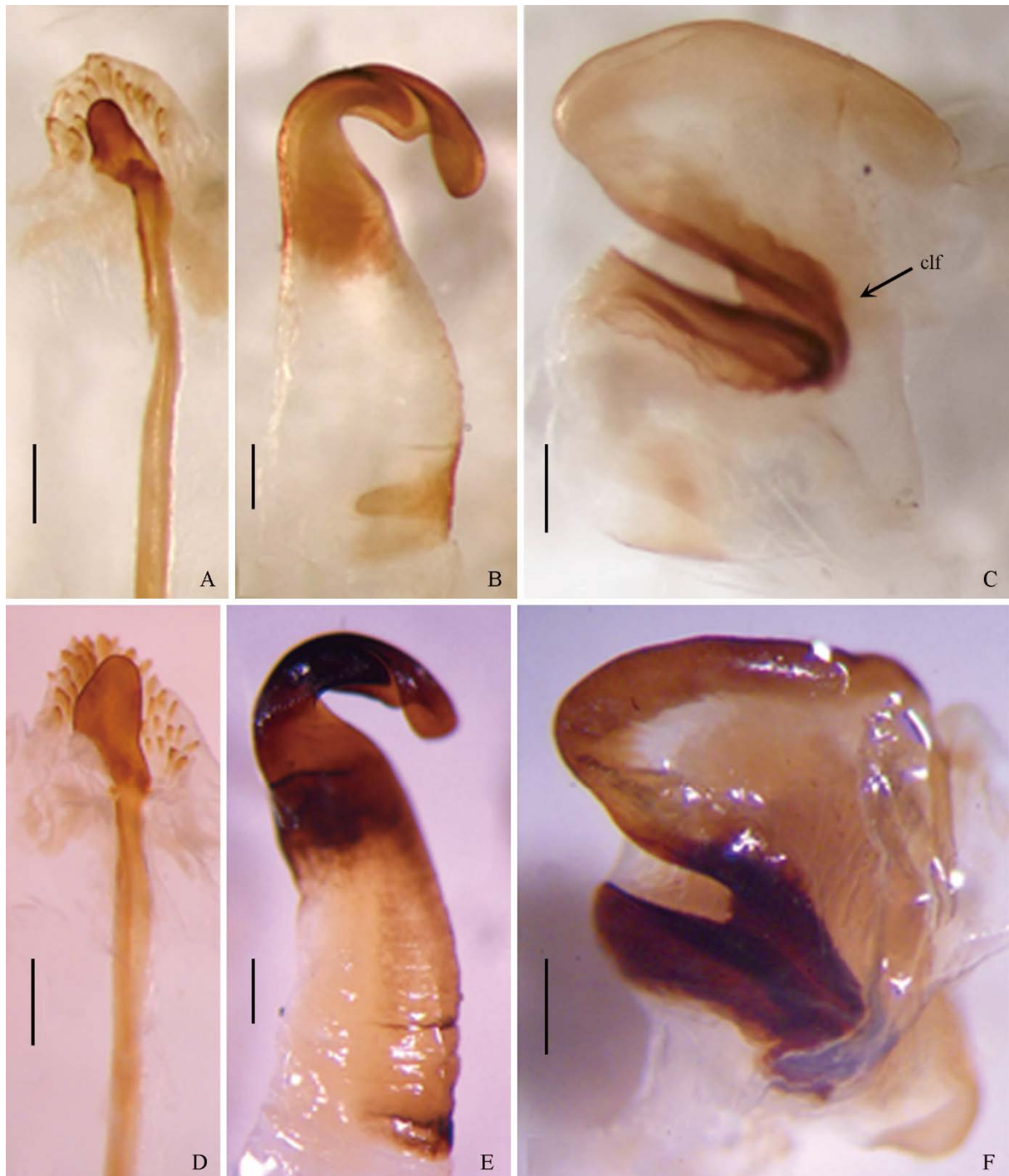


Figure 4. Male genitalia. A–C. *Blaptica interior*. D–F. *B. dubia*. From left to right, the phallomeres are presented: L2, dorsal view; R, ventral view; L1, dorsal view and clf, dorsal view. Scale bars = 1 mm.

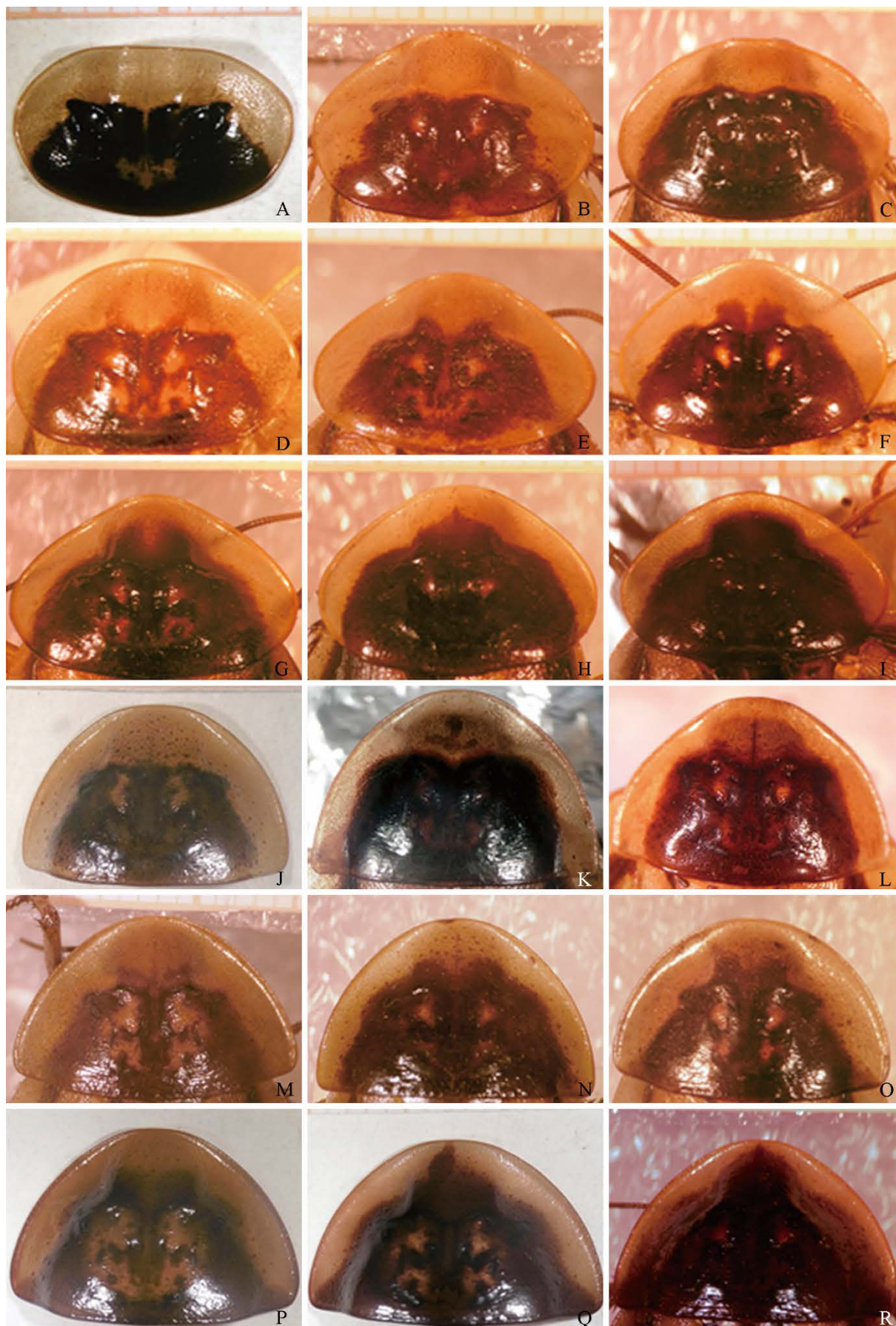


Figure 5. Male (A–I) and female (J–R) macula pattern variations. A–D, J–M. *Blaptica interior*. E–I, N–R. *Blaptica dubia*.

Sexual dimorphism was detected for both species when pronotum outline in pair comparisons was analyzed. The shapes between species of the same sex, showed that differences were not significant (Permanova, females: $p = 0.83$ and males: $p = 0.72$).

Male area of the pronotum contour ranges *B. dubia* (117.23–175.12) and *B. interior* (127.22–163.14). Female ranges *B. dubia* (91.97–164.14), and *B. interior* (125.27–153.63). The values (in square millimeters) of *B. interior* are contained within the range values of *B. dubia*. Significant differences were detected only between females and males of *B. dubia* ($p = 0.043$).

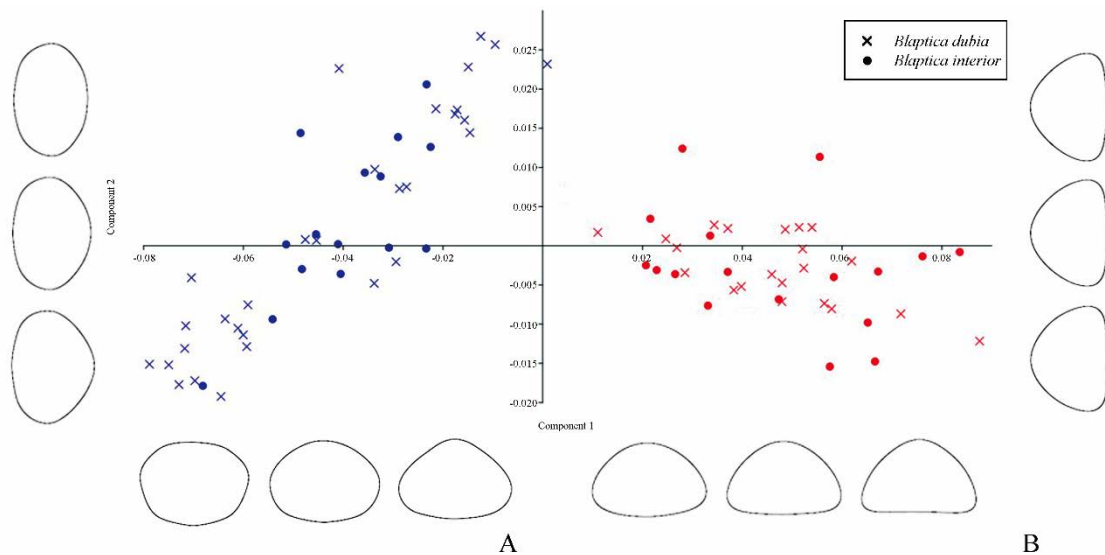


Figure 6. PCA scatterplot, *Blaptica dubia* and *B. interior* pronotum shape variables. A. Males (blue). B. Females (red). Visualization of the pronotal shape variation for each PC1 and PC2 next to the graph.

3.4.2 Wings

The PCA of the wing shape shows that morphospaces of both species overlap (Fig. 7A). PC1 represents 62.28% of the variation, PC2 represents 26.87%, and both explain 89.15% of the total variance. Deformation grids associated with PC1 show that landmarks 4 and 5 are affected in opposite ways in the two species, but this unique result is not enough to discriminate between species (Figs 7B–C).

The discriminant function analysis with the cross-validation classified correctly 74% specimen of *B. dubia*, and 83% of *B. interior*. The permutation test between Mahalanobis and Procrustes distances of both species shows significant differences ($p < 0.05$).

Male of both species were compared, and no significant differences between the wing centroid sizes were found ($p = 0.4686$).

4 Discussion

Hebard (1921) proposed IO as a character to identify species. However, by examining a greater number of specimens and analyzing more morphometric characters (TL, PL, PW, IA, IOc), our results did not show significant differences between species.

Genital characters are of great importance in morphology-based taxonomy because they are involved in reproduction. They are an important clue for specific identification and contribute to genus diagnosis. In this study, the inter- and intraspecific variability observed in male genitalia do not show obvious differences. For example, the number of preputial spines was variable in both species, overlapping the minimum and maximum intervals (*B. interior* 12–27 and *B. dubia* 23–49). Coincidentally, Roth (1970) postulated that *Blaptica* would present similar variation in the number of preputial spines as occur in the genus *Blaberus*. Regarding the disposition of preputial spines, we found that *B. interior* presents 1 to 2 rows

and the *B. dubia* presents from 2 to 3 rows. Another example of the variability is the sclerotized degree of the phallomere sclerites in both species, although there is a tendency for more sclerotized in *B. dubia* (Fig. 4).

Unfortunately, the variability cannot be confirmed in other *Blaptica* species present in Argentina described by Hepper (1965, 1966a, b, 1967, 1968a, b), because of the missing types. Lopes & Oliveira (2005) described five species and compared them with *B. dubia*, of which illustrations seem to be *B. interior*. It is also important to stand out that *B. confusa*, *B. formosa*, *B. gaucha*, *B. rothi* and *B. sulina* were based on a single specimen, respectively. Therefore, their intraspecific variability could not be observed.

The morphs of both species present an evident wing dimorphism. Wings are well-developed in males but reduced in females. The geometric morphometrics analysis separates species by the male wing shape through landmarks (Fig. 7), but is not reliable as it is very variable in cockroaches. Pronotum contour analysis was performed for both sexes (Fig. 6). Although this character differentiates sexes, it was useless to differentiate species of the same sex because they shared similarities in the pronotum conformation and size (Fig. 6) (Table 3).

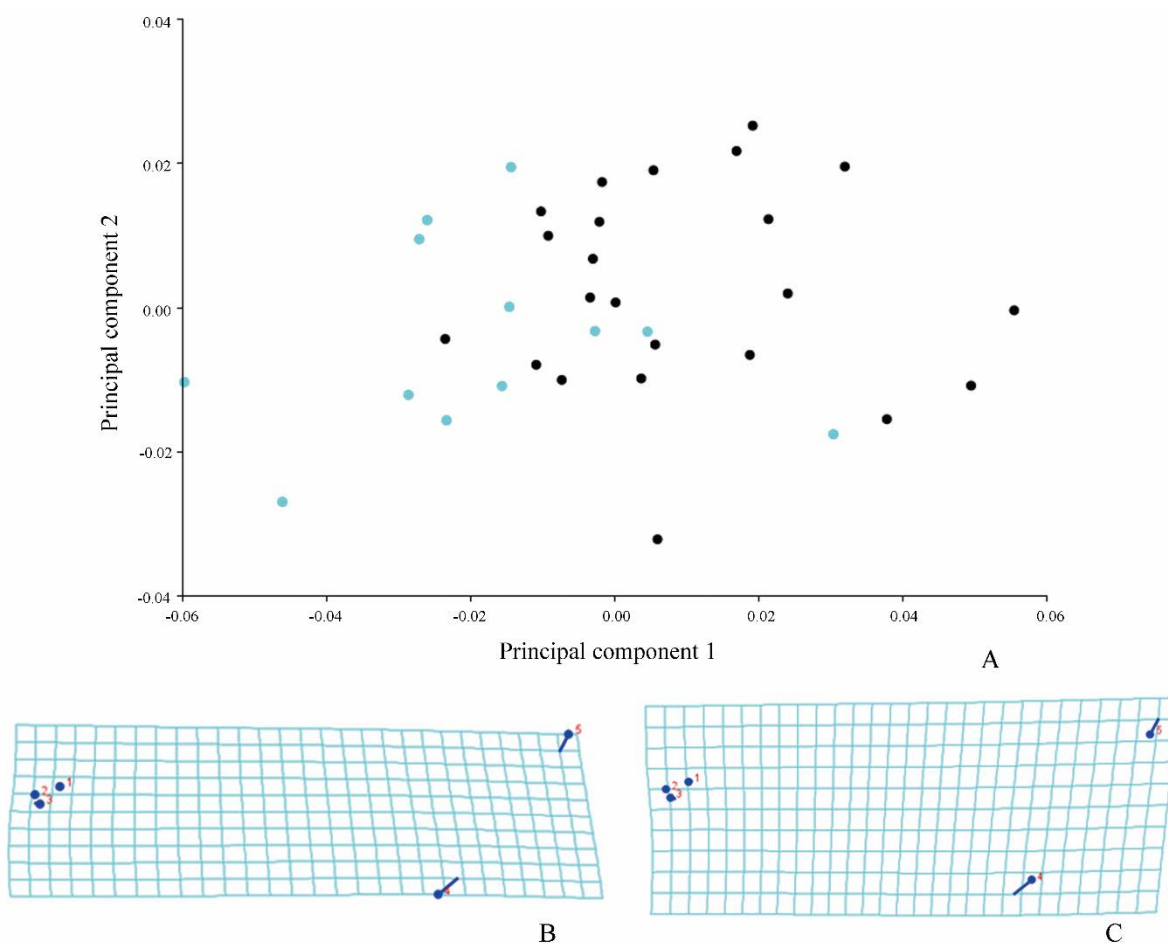


Figure 7. A. PCA scatterplot of *Blaptica interior* (turquoise) and *B. dubia* (black) wing shape variables. B–C. Deformation grids from the consensus shape for PC1. B. *Blaptica interior*. C. *Blaptica dubia*.

Table 3. Linear body measurements in millimeters.

	TL	PL	PW	IO	IA	IOc
Males						
<i>Blaptica dubia</i>	32.9–48.0	7.7–11.1	11.2–16.1	0.9–2.0	2.3–2.9	1.2–1.6
<i>Blaptica interior</i>	31.5–47.4	7.2–10.8	10.2–15.1	0.3–2.0	2.2–2.7	1.1–1.7
Females						
<i>Blaptica dubia</i>	32.7–38.5	9.9–11.2	14.2–16.4	1.8–2.0	2.7–3.0	1.7–1.9
<i>Blaptica interior</i>	30.3–38.0	8.6–11.1	12.7–18.2	1.2–2.0	2.5–3.0	1.5–1.9

The pronotal macula is a qualitative character which can lead to an erroneous identification (Adams *et al.*, 2004). In this study, the geometric morphometrics was used as an effective tool to discriminate small variations, which are difficult to see and to increase the reliability of the research. According to the present work, the characters are not reliable due to large variation in the examined materials.

During the study, we found the materials examined in the study were confused identification between *B. dubia* and *B. interior* by the external characters. The identification of *B. dubia* and *B. interior* is based on variable characters such as the pronotal macula and the sclerites of the genitalia. Its validity is under discussion as the characters are of a variability gradient. Difficulties for identification arise from the underestimate of intraspecific variability in morphological characters. In addition, the two species show a sympatric distribution. The study of a large number of individuals shows that there is a single species that presents two morphs with a great variability of intermediate forms. Therefore, we propose that *B. interior* Hebard 1921 is a junior synonym of *B. dubia* (Serville, 1838).

Consequently, the taxonomic status of the species under these new studies is:

***Blaptica dubia* (Serville, 1838)**

= *B. claraziana* (Saussure, 1864)

= *B. ligata* (Brunner von Wattenwyl, 1865)

= *B. interior* Hebard, 1921 **syn. nov.**

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