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Patterns of morphometric variation among species of the genus *Cicada* (Hemiptera: Cicadidae) in the Mediterranean area

PAULA CRISTINA SIMÕES and JOSÉ ALBERTO QUARTAU

Centro de Biologia Ambiental/Departamento de Biologia Animal, Bloco C2, 3º Piso, Faculdade de Ciências de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal; e-mails: pcsimoes@fc.ul.pt, jaquartau@fc.ul.pt

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Abstract. Selected populations of five closely related species of the genus *Cicada* L. were collected mainly on the Portuguese, Greek and Turkish mainland, as well as on several Aegean islands. Ten morphometric traits of external structures and seven of male genitalia were analysed and the results revealed patterns in morphometric variation for each species. Only *C. lodosi* was always completely discriminated by both character sets and *C. barbara* by the male genitalia analysis. For the remaining species there was great overlap between the clusters. Body length, of the external morphological structures, and measurements of the pygophore, of the male genitalia, were the best variables for identifying *C. lodosi* and *C. barbara*. The present morphometric analyses revealed that divergence in morphology is much less pronounced than the divergence in acoustic signals and DNA. Thus, the congruence between morphological divergence, namely at the level of the external structures, and both behavioural (acoustic) and genetic divergence is quite low.

INTRODUCTION

Cicadas are Hemipteran insects usually recognised by the ability of males to produce loud airborne acoustic signals during pairing and courtship (e.g. Claridge, 1985; Boulard & Mondon, 1995; Quartau, 1995). Females are only attracted to the calls of conspecific males, thus cicada acoustic signals are species specific (e.g. Claridge, 1985) and can be used, like taxonomic characters, to identify most species of cicada.

In practice it is usually not always possible to have live specimens and thus difficulties may arise in the identification of cicadas. On the other hand, in many instances, like in the genus *Cicada* Linnaeus, where the male calls of the different species are quite distinct, it is difficult to separate specimens only on the basis of their morphology. In fact, these cicadas form a complex of sibling species looking very similar on the basis of the external morphology and even the male genitalia. However, a few differences in structure and colour can be found, especially when large series of specimens are analysed (Quartau, 1988; Simões et al., 2000; Quartau & Simões, 2006).

Five species occurring mainly on the Portuguese, Greek and Turkish mainland, as well as on several Aegean islands, were analysed in this study: *Cicada barbara* Stål 1866 (with the two subspecies *C. b. barbara* Stål 1866 and *C. b. lusitanica* Boulard 1982), *C. cretensis* Quartau & Simões 2005, *C. lodosi* Boulard 1979, *C. mordoganensis* Boulard 1979 and *C. orni* Linnaeus 1755.

The objective of the present paper is to use a set of measurements of the external morphology and male genitalia to identify and quantify subtle differences between the five species in this complex and determine whether they are reproductively isolated (e.g. Claridge et al., 1997; Simon, 1992) by studying considerably more individuals of each of the five species than Quartau (1988), who only studied *C. orni* and *C. barbara*. In particular, the phenetic (morphological) divergence will be assessed and compared with acoustic (Simões et al., 2000; Quartau & Simões, 2006) and genetic data (Seabra et al., 2000; Quartau et al., 2000, 2001; Pinto-Juma, 2009).

MATERIAL AND METHODS

Males of the *Cicada* species were identified in the field by their songs, located and collected during the summers of 1996 to 2006 in the Mediterranean area. Specimens collected from populations of five species on the mainland as well as several islands were analysed (*C. barbara, C. cretensis, C. lodosi, C. mordoganensis* and *C. orni*) (Fig. 1; Table 1).

Based on previous results (e.g. Quartau & Simões, 2006) it was decided to study two areas in particular: the first is located in the western part of the Mediterranean area (Iberian Peninsula and north-western Africa), where *C. barbara* and *C. orni* coexist. The second encompasses a larger assemblage of closely related species – *C. cretensis, C. lodosi, C. orni* and *C. mordoganensis* – and is located in the eastern part of the Mediterranean basin, and includes mainly the Balkans, the Aegean islands and Turkish mainland. *Cicada permagna* Haupt 1917 and *C. cerisyi* Guérin-Méneville 1844, two nominal species of doubtful affinities, which are reported from Turkey, and Egypt and Libya, respectively, were not considered.

In order to determine whether there are patterns in the variation in the size of the 10 morphological structures listed in Fig. 2 the measurements for a total of 316 males of five species (Table 1) were analyzed. The measurements were of external morphological structures (head and thorax including wings, and tymbals). In addition, a submatrix of measurements of seven traits of the male genitalia of 48 specimens was subjected to a morphometric analysis (Table 2 and Fig. 3). This analysis is of particular interest since some of the structures of the male genitalia are thought to be phylogenetically informative (e.g. Quartau, 1988; Claridge et al., 1997). Using a ruler the lengths of the body and wings were measured to the nearest 0.5 mm. All the

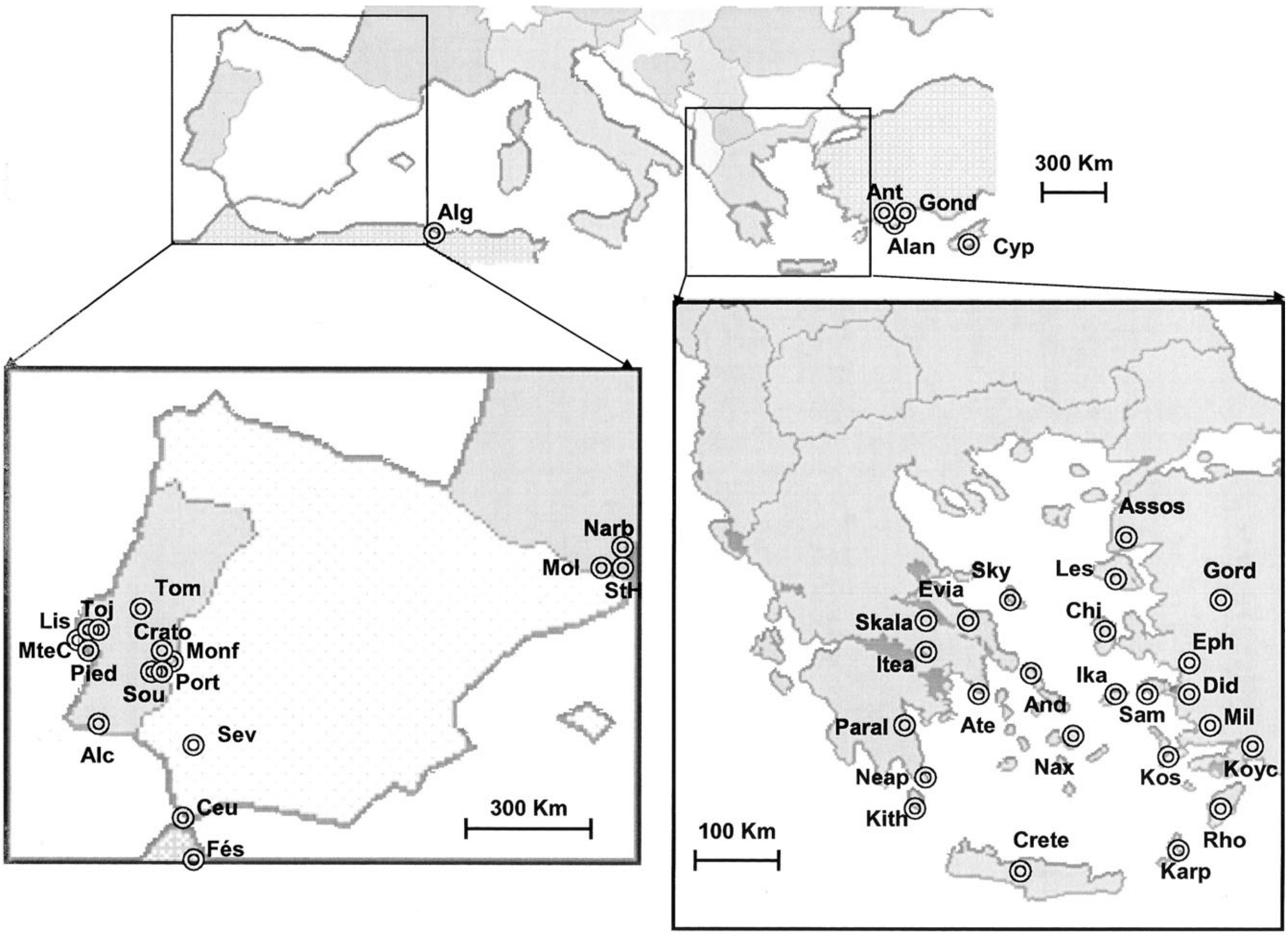

Fig. 1. Localities where the specimens of *Cicada* analysed were collected (for full names of abbreviations see Tables 1 and 2).

other measurements were made using a Wild M5 binocular microscope fitted with an ocular micrometer. Except for damaged specimens all measurements were made on the left side of each specimen. Each trait was measured twice and the measurements averaged.

Univariate analyses were used to estimate means and standard deviations, and minimum and maximum values. Nonparametric Kruskal-Wallis (KW) tests were used to compare the results for several independent samples of each trait, in addition to two-sample comparisons using nonparametric Mann-Whitney U (MW) tests. The significance level of the multiple tests was assessed by changing the critical P value obtained using the Dunn-Sidak method from $p < 0.05$ to $1-(0.951)^{1/k}$, where k is the number of tests (Dytham, 2003).

Discriminant Function Analysis (DFA) of both the measurements of external morphological structures and male genitalia was used to identify the statistically significant discriminant functions that separate the groups and determine how each discriminant function contributes to the discrimination between groups. Moreover, a R-type principal component analysis (PCA) was also performed on both data sets, and the Kaiser criterion used to determine the components with eigenvalues greater than one. Ultimately, the results chosen for presentation were those selected on the basis of the results of the above statistical tests.

Statistical procedures were performed using Statistica 8.0 software (Statsoft inc., Tulsa, USA).

RESULTS

The present paper is a comparative morphometric study of selected populations of five closely related species of the genus *Cicada* in the Mediterranean area. A total of 316 males were studied. Figs 4 and 6, and Tables 3 and 9 give a summary of the descriptive statistics of the morphometric features measured.

External morphological traits

As expected, *C. lodosi* proved to be the biggest species (male body length: 48–51 mm) in all the morphometric features measured (cf. Fig. 4 and Table 3). In contrast, in terms of most of these features, *C. orni* and *C. barbara* were the smallest species (cf. Table 3). In addition, *C. orni* and *C. mordoganensis* were the most variable of the species (Fig. 4), which can be related to the fact that there were larger samples of these species.

Nonparametric Kruskal-Wallis tests showed that all variables differed significantly among the species ($p > 0.05$).

In fact, the results of the Mann-Whitney U tests, after applying the Dunn-Sidak correction, indicate that *C. lodosi* differs in all morphometric traits, with the exception of the antenna-eye distance, from the other species, and *C. orni* differs from *C. cretensis* in all traits except the length of rostrum, tymbal width and wing width. When compared with *C. mordoganensis*, it differed in

TABLE 1. Number of specimens of each *Cicada* species used in the analysis of the variation in external morphology, with abbreviations for the localities from which they were collected (abbrev.).

Locality	Abbrev.	*Cicada barbara barbara*	*Cicada barbara lusitanica*	*Cicada cretensis*	*Cicada lodosi*	*Cicada mordoganensis*	*Cicada orni*
ALGERIA (Ziana)	Alg	2					
CYPRUS	Cyp						5
PORTUGAL							
Monforte (Alto Alentejo)	Monf						1
Sousel (Alto Alentejo)	Sou		6				15
Crato (Alto Alentejo)	Crato		3				1
Portel (Alto Alentejo)	Port						1
Lisboa (Estremadura)	Lis						1
Piedade (Arrábida, Estremadura)	Pied						1
Monte-da-Caparica (Estremadura)	MteC						11
S. Julião-do-Tojal (Estremadura)	Toj						15
SPAIN							
Seville (Andalusia)	Sev		1				
Ceuta	Ceu		3				
FRANCE							
Molitg-les-Bains (Languedoc-Roussillon)	Mol						2
St Hippolyte (Languedoc-Roussillon)	StH						10
Narbonne (Languedoc-Roussillon)	Narb						6
GREECE							
Itea (Athika)	Itea						18
Skala (Athika)	Skala						1
Evia (Athika)	Evia						9
Athens (Athika)	Ate						6
Paralio (Peloponnese)	Paral						12
Neapolis (Peloponnese)	Neap						16
Skyros (Sporades)	Sky						14
Naxos (Cyclades)	Nax						14
Andros (Cyclades)	And						9
Lesbos (Northeastern Aegean sea)	Les						8
Kos (Dodecanese)	Kos					14	
Rhodes (Dodecanese)	Rho					2	
Chios (North Aegean sea)	Chi					1	
Ikaria (eastern Aegean sea)	Ika					11	
Samos (Eastern Aegean sea)	Sam					7	
Crete (Southern Aegean sea)	Crete			28			
Karpathos (Dodecanese)	Karp			4			
Kithira (Ionian)	Kith			19			
MOROCCO (Fés)	Fés	5					
TURKEY							
Assos (Ayvacyk)	Assos						2
Alanya (Antalya)	Alan				1		
Gundogmus (Antalya)	Gond				1		
Koycegiz (Mugla)	Koyc				1	8	
Gordes (Manisa)	Gord				2		
Antalya (Antalya)	Ant					3	
Milas (Mugla)	Mil					3	
Didim (Aydim)	Did					9	
Ephesus (Izmir)	Eph					4	
TOTAL				316			

every trait except front length, tymbal width and tymbal length. It had fewer significant differences when compared with *C. barbara* (differed in head width, pronotum width, front length, antenna-eye distance, length of rostrum and tymbal length). Likewise, *C. mordoganensis* differed in few traits when compared with *C. cretensis* (front length and length of rostrum). Moreover, in terms of all the traits, *Cicada barbara barbara* and *Cicada barbara lusitanica* did not differ significantly.

When comparing specimens from different geographical areas (Portugal, France, Greece, Turkey and Cyprus) after applying the Dunn-Sidak correction, males of *C. orni* from some pairs of areas differed. Nonetheless, the eastern Mediterranean populations studied (from

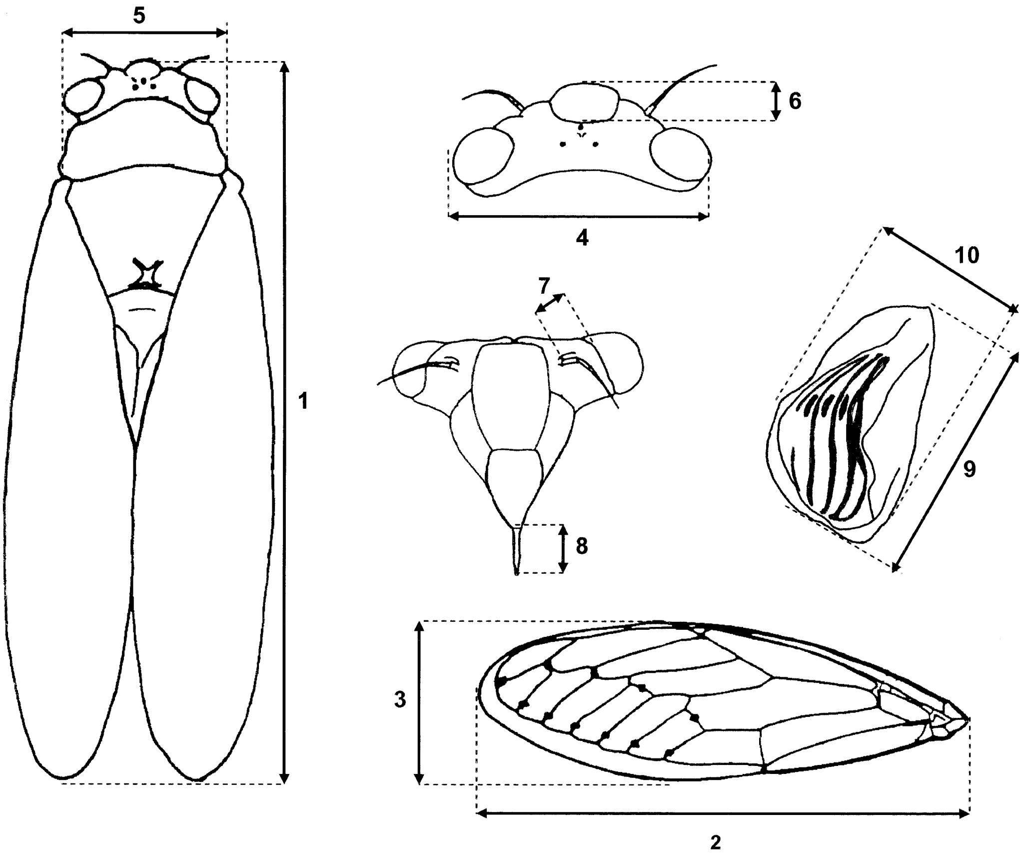

Fig. 2. Measurements of external morphology of the five species of *Cicada* studied (different scales). 1 – body length (overall body length from tip of the head to the end of the wings in resting position); 2 – forewing length (distance from base of left forewing articulation to tip of wing); 3 – forewing width (greatest width of left forewing); 4 – head width (maximum head width measured between exterior eye margins); 5 – pronotum width (maximum width of pronotal collar); 6 – front length (length of the front measured along the dorsal median line); 7 – antenna-eye distance (distance between the base of the left antenna and the left ocular suture); 8 – rostrum length; 9 – tymbal length (overall length of left tymbal); 10 – tymbal width (greatest width of left tymbal).

TABLE 2. Number of specimens of each *Cicada* species used in the analysis of the variation in male genitalia, with abbreviations for the localities where they were collected (Abbrev.).

Locality	Abbrev.	*Cicada barbara barbara*	*Cicada barbara lusitanica*	*Cicada cretensis*	*Cicada lodosi*	*Cicada mordoganensis*	*Cicada orni*
PORTUGAL							
Monforte (Alto Alentejo)	Monf						3
Crato (Alto Alentejo)	Crato		3				
Alcalar (Alarve)	Alc		3				
Tomar (Estremadura)	Tom						3
SPAIN							
Ceuta	Ceu		3				
GREECE							
Athens (Athika)	Ate						3
Skyros (Sporades)	Sky						3
Lesbos (Northeastern Aegean sea)	Les						3
Kos (Dodecanese)	Kos					3	
Rhodes (Dodecanese)	Rho					3	
Samos (Eastern Aegean sea)	Sam					3	
Crete (Southern Aegean sea)	Crete			3			
Kithira (Ionian)	Kith			3			
MOROCCO (Fés)	Fés	3					
TURKEY							
Gondogmus (Antalya)	Gond				1		
Gordes (Manisa)	Gord				2		
Milas (Mugla)	Mil					1	
Didim (Aydim)	Did					2	
TOTAL				48			

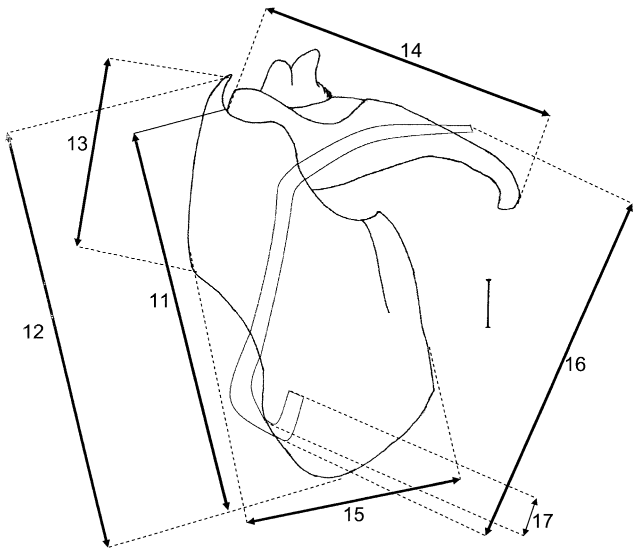

Fig. 3. Measurements of male genitalia of the five species of *Cicada* studied (illustrated *C. cretensis*; scale = 0.05 mm). 11 – pygophore length without spine (length of pygophore from the anterior side to the base of the small apical spine in left lateral view); 12 – overall pygophore length (overall length of pygophore in left lateral view); 13 – pygophore posterior length (length of dorsal posterior side of pygophore); 14 – 10th abdominal segment length (overall length of 10th abdominal segment in left lateral view); 15 – pygophore width (maximum width of pygophore in left lateral view); 16 – aedeagus length; 17 – aedeagus apodeme length (length of basal apodeme of aedeagus).

Cyprus, Turkey and Greece) did not differ significantly from each other. In contrast, specimens from Portugal differed more from those from all the other areas, particularly the Greek specimens (differed in all morphometric traits except forewing width and antenna-eye distance). Portuguese specimens also differed from the French ones in body length, wing width, wing length and tymbal length, and the French specimens from the Greek ones in front length, tymbal length and tymbal width.

Discriminant function analysis of the ten morphometric traits of the species studied revealed that they differed statistically significantly in more of the traits than revealed by PCA (except for forewing length and tymbal width) (Table 4). Body length, front length and length of rostrum were the variables with the lowest partial Wilks' lambda values (0.819, 0.817 and 0.681, respectively), and therefore the best characters for discriminating between groups (Table 4).

Classification functions were significant for *C. lodosi* (p = 0.016), *C. b. barbara* (p = 0.022) and *C. b. lusitanica* (p = 0.041) (Table 5). *C. lodosi* clustered mainly on the basis of its body length, while rostrum length, head width and body length were the most important traits for the clustering of the two subspecies of *C. barbara.*

Overall 81% of the classifications were correctly attributed (Table 6). All *C. lodosi* specimens were correctly attributed and for the remaining species it was 88% for *C. orni*, 76% for *C. mordoganensis*, 68% for *C. cretensis*, 69% for *C. b. lusitanica* and 43% for *C. b. barbara*.

The first and second roots of the canonical variables extracted eigenvalues of 1.58 and of 0.66, respectively (Table 7) and were mainly marked by negative coefficients for body length (–1.300) and rostrum length (–0.951). Root 1 discriminated mainly *C. lodosi* from all the remaining species, while root 2 was not particularly helpful in discriminating any species (Fig. 5, Table 8).

For most species except *C. lodosi* (Fig. 5), there were no clear-cut clusters, in spite of some clear trends in variation. *C. barbara* specimens are in the up-right quadrant but overlap some *C. orni* specimens (identified as from Portel, Molitg and Narbonne but not shown). On the other hand, most specimens of *C. cretensis* form a uniform cluster partially overlapping, however, *C. orni* and *C. mordoganensis*.

Moreover, *C. mordoganensis* and *C. orni* tended to cluster separately, overlapping in the middle. Noteworthy is that the specimens of *C. mordoganensis* from Turkey (not shown in Fig. 5) form a quite homogeneous group with little overlap with the remaining species.

In contrast, males of *C. orni* from several of the localities in western and south-eastern Europe clustered as a quite heterogeneous group (not shown in 5), with the French males forming a more or less consistent group closer to those of *C. barbara* (not represented) than those of *C. cretensis* and *C. mordoganensis*.

TABLE 3. Descriptive statistics of the measurements (in mm) of 10 external morphological traits of the species of *Cicada* studied.

	C. barbara lusitanica (N = 13)				*C. barbara barbara* (N = 7)				*C. cretensis* (N = 51)				*C. lodosi* (N = 5)				*C. mordoganensis* (N = 62)				*C. orni* (N = 178)			
Trait	Mean	Min	Max	S. Dev.	Mean	Min	Max	S. Dev.	Mean	Min	Max	S. Dev.	Mean	Min	Max	S. Dev.	Mean	Min	Max	S. Dev.	Mean	Min	Max	S. Dev.
Body length	39.9	38.0	42.0	1.3	38.9	38.5	39.0	0.2	41.8	37.0	46.0	2.0	49.2	48.0	51.0	1.3	42.1	35.5	46.0	2.2	38.9	32.0	46.0	2.4
Forewing length	32.9	31.5	35.0	1.2	31.9	31.5	32.0	0.2	34.1	31.0	37.0	1.5	39.4	38.0	41.0	1.5	34.4	29.5	38.0	1.8	31.7	22.0	39.0	2.2
Forewing width	11.1	10.0	12.0	0.5	10.4	9.0	11.0	0.8	11.0	9.0	12.0	0.7	12.3	12.0	13.0	0.4	11.2	9.5	13.0	0.7	10.6	8.5	12.0	0.8
Head width	9.1	8.3	9.4	0.3	8.9	8.7	9.0	0.1	8.7	7.8	9.6	0.4	9.4	8.9	9.7	0.3	8.6	4.1	10.1	0.7	8.4	7.3	9.5	0.5
Pronotum width	9.9	9.2	10.4	0.5	9.7	9.3	10.2	0.3	10.1	8.8	11.3	0.5	10.8	10.0	11.1	0.4	9.9	8.1	11.0	0.6	9.5	7.7	10.8	0.6
Front length	1.2	1.1	1.5	0.1	1.1	1.1	1.3	0.1	1.2	0.9	1.5	0.1	1.5	1.5	1.6	0.1	1.1	0.8	1.3	0.1	1.1	0.8	1.4	0.1
Antenna-eye distance	1.3	1.2	1.4	0.1	1.2	1.2	1.3	0.0	1.2	1.0	1.3	0.1	1.2	1.1	1.4	0.1	1.2	1.0	1.4	0.1	1.2	1.0	1.4	0.1
Rostrum length	9.1	8.5	9.6	0.4	8.5	8.2	9.0	0.2	9.4	8.4	10.4	0.4	11.0	10.6	11.4	0.3	10.0	8.0	11.1	0.6	9.2	6.3	10.4	0.6
Tymbal length	4.5	3.9	4.8	0.3	4.7	4.6	4.9	0.1	5.0	3.2	5.7	0.4	5.3	5.2	5.5	0.2	4.9	4.2	5.4	0.3	4.8	4.0	5.4	0.3
Tymbal width	2.5	2.2	2.7	0.1	2.4	2.3	2.6	0.1	2.5	2.3	2.9	0.1	2.7	2.6	2.7	0.1	2.5	2.1	2.7	0.1	2.4	2.0	3.3	0.2

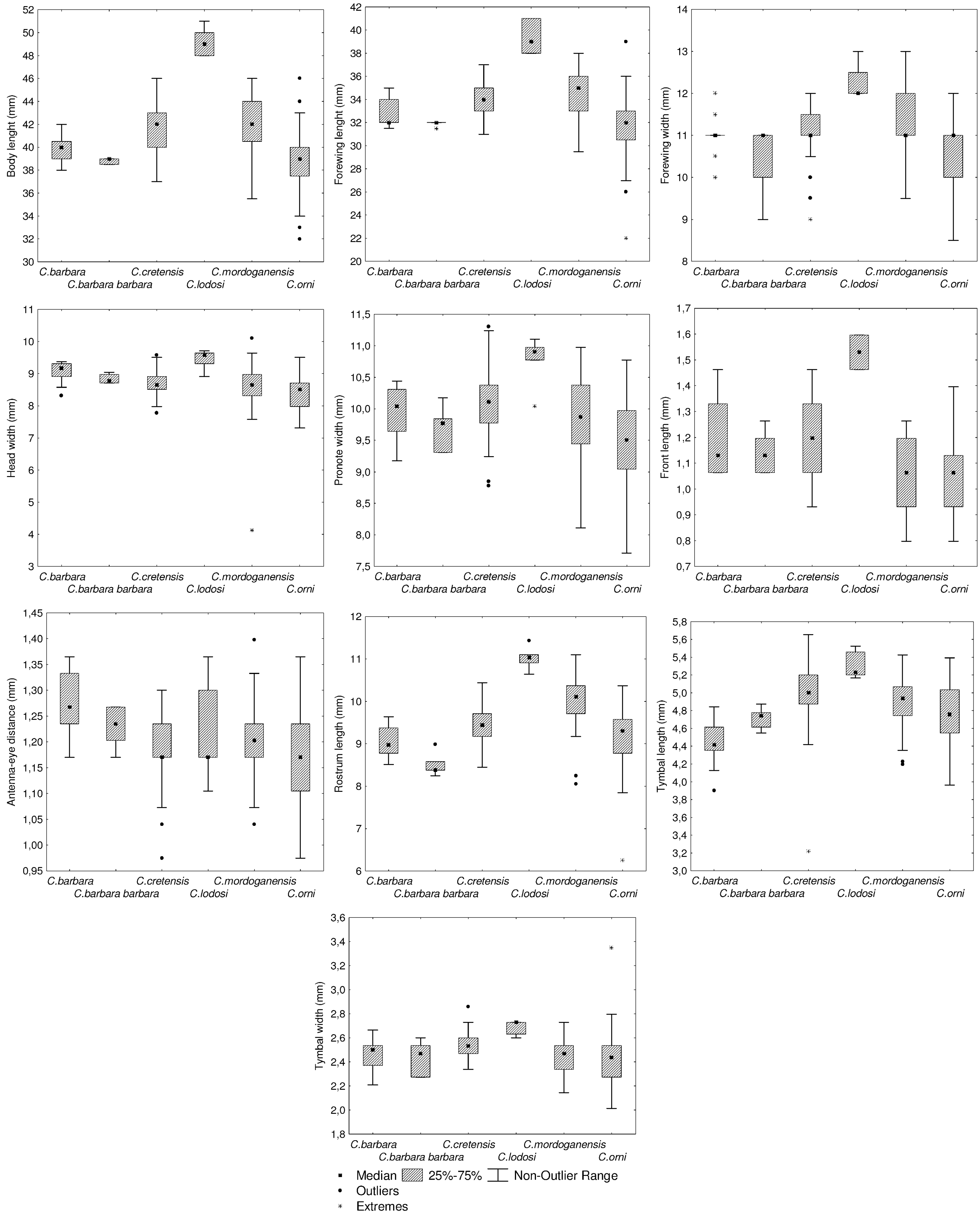

Fig. 4. Box and whisker plots comparing the measurements of 10 external morphological features for each of the species of *Cicada* studied. (Non-outlier range – values below the upper outlier limit and above the lower outlier limit; Ouliers – values outside 1.5 box length range from the upper and lower value of the box; Extremes - values outside 3 box length range from the upper and lower value of the box).

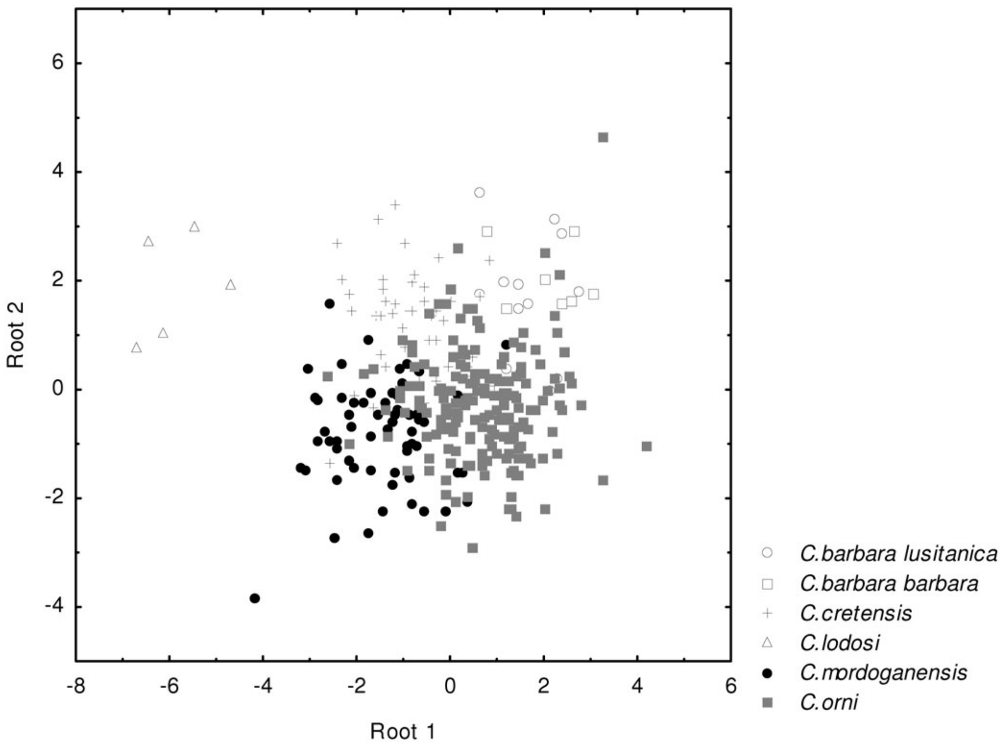

Fig. 5. Scatter-plot of the results of a canonical analysis based on discriminant functions of the 10 external morphological features of the species of *Cicada* studied (316 OTUs).

Male genitalia

The analyses of the male genitalia, Fig. 6 and Table 9, compared the seven variables for each of the species investigated. The specimens of *C. barbara* had lower values for the majority of the traits considered. In contrast, males of *C. lodosi* had higher values. The remaining three species had similar values.

MW tests performed with the Dunn-Sidak correction did not reveal any significant differences between pairs of species.

Principal component analysis gave better results than DFA and the resulting two dimensional diagram of the relationships between species is shown in Fig. 7. The first two components accounted for 77.9% of the total variation and more than half (65.3%) of the variation was explained by the 1st component. When plotting components 1 and 2, the distribution of most of the species along the 1st axis is noteworthy. *C. lodosi* forms a clearly separated cluster in the upper left quadrant, similarly to what happened with the two subspecies of *C. barbara* in the right quadrant (Fig. 7). In this plot there is some separation of *C. cretensis* and *C. orni*, which overlap considerably with *C. mordoganensis*.

Factor loadings were high (Table 10) for the majority of the variables and as component 1 had the highest loadings for most of pygophore related traits, this was the structure that contributed most to the discrimination among the *Cicada* species studied.

DISCUSSION AND CONCLUSIONS

Previous studies on external morphology have shown that it is possible to recognise some general trends in each

TABLE 4. Summary of the Discriminant Function Analysis of the measurements of 10 external morphological traits of the species of *Cicada* studied (Wilks' Lambda = 0.15973, F (50.1376) = 13.626 $p < 0.0001$).

Trait	Wilks'	Partial	F-remove	p-level	Toler.	1-Toler.
Body length	0.195	0.819	13.273	0.000	0.166	0.834
Forewing length	0.166	0.965	2.183	0.056	0.240	0.760
Forewing width	0.182	0.878	8.371	0.000	0.329	0.671
Head width	0.179	0.891	7.402	0.000	0.367	0.633
Pronotum width	0.172	0.928	4.680	0.000	0.262	0.738
Front length	0.196	0.817	13.487	0.000	0.857	0.143
Antenna-eye distance	0.181	0.881	8.161	0.000	0.600	0.400
Rostrum lengh	0.234	0.681	28.171	0.000	0.509	0.491
Tymbal length	0.183	0.875	8.605	0.000	0.560	0.440
Tymbal width	0.162	0.988	0.760	0.580	0.627	0.373

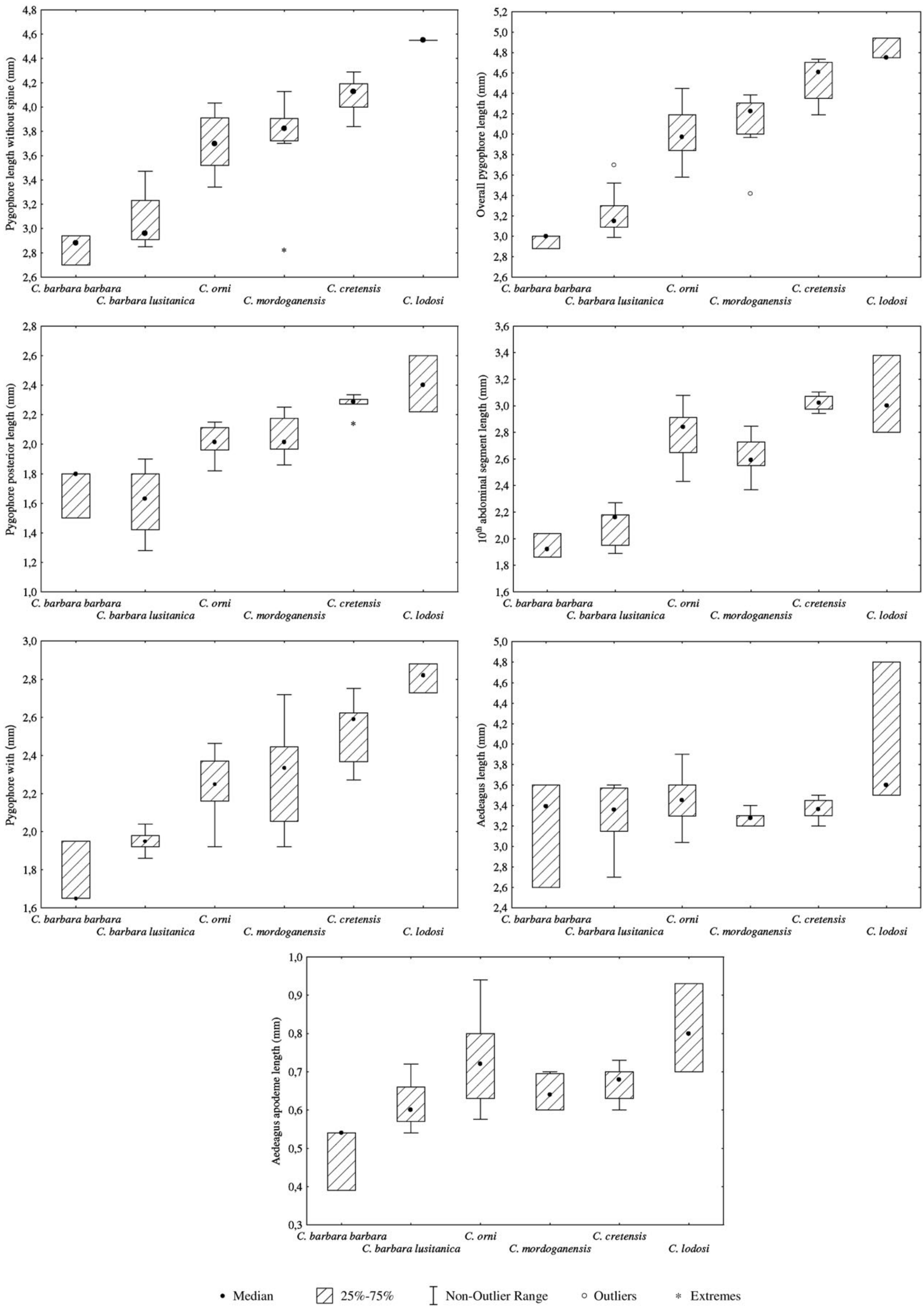

Fig. 6. Box and whisker plots comparing the measurements of seven structures of the male genitalia of each of the species of *Cicada* studied (Non-outlier range – values below the upper outlier limit and above the lower outlier limit; Ouliers –values outside 1.5 box length range from the upper and lower value of the box; Extremes – values outside 3 box length range from the upper and lower value of the box).

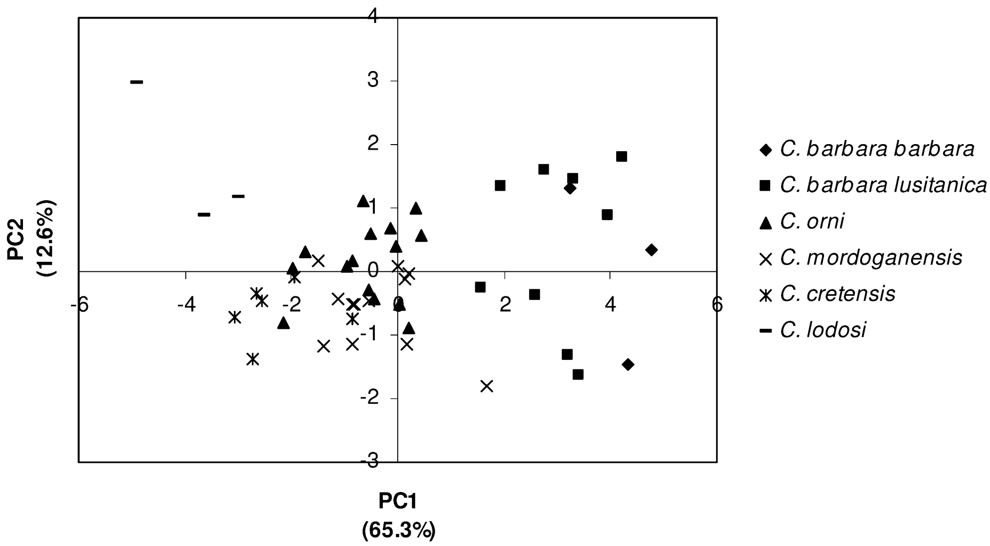

Fig. 7. Scatter-plot of the results of a principal component analysis based on a correlation matrix of the measurements of seven structures of the male genitalia of the species of *Cicada* studied (48 OTUs).

of the *Cicada* species, however there is considerable overlap between species. Thus, when considering individual specimens it is difficult to make a correct identification (e.g. Quartau & Simões, 2006). Our previous experience indicated that is particularly difficult to discriminate between *C. orni*, *C. cretensis* and *C. mordoganensis*.

The present results strongly support the impression based on intuitive analyses (Quartau & Simões, 2006). In fact, all species showed a typical pattern of morphometric variation. However, with the exception of *C. lodosi*, the analyses based on external morphology did not completely discriminate between any species or populations, with a general overlap among species being the rule. Body length, representing size, was the most important morphometric trait allowing some discrimination and classification, namely for *C. lodosi*, the biggest species. Moreover, *C. b. barbara* and *C. b. lusitanica* were also fairly well discriminated on the bases of their body and rostrum lengths.

The analysis based on male genitalia traits discriminated the species better than that based on external mor-

TABLE 5. Classification functions of the discriminant analysis conducted on the measurements of 10 external morphological traits of the species of *Cicada* studied.

Trait	*C. barbara lusitanica* $p = 0.04114$	*C. barbara barbara* $p = 0.02215$	*C. cretensis* $p = 0.14873$	*C. lodosi* $p = 0.01582$	*C. mordoganensis* $p = 0.19620$	*C. orni* $p = 0.57595$
Body length	–2.634	–2.347	1.573	11.825	2.095	–1.166
Forewing length	0.739	0.910	0.977	0.174	0.380	–0.474
Forewing width	1.336	–0.596	–1.523	–3.698	–0.413	0.563
Head width	2.427	2.582	–0.705	–1.025	–0.831	0.221
Pronotum width	0.245	0.626	1.126	–3.286	–0.723	0.004
Front length	0.592	0.427	0.890	4.105	–0.455	–0.246
Antenna-eye distance	2.018	1.770	–0.421	–2.796	–0.511	0.148
Rostrum length	–2.423	–4.231	–1.092	3.057	2.113	–0.186
Tymbal length	–2.464	–0.150	0.359	0.084	–0.395	0.221
Tymbal width	–0.014	0.050	0.345	–0.332	-0.238	0.000

TABLE 6. Classification matrix of the discriminant analysis of the measurements of 10 external morphological traits of the species of *Cicada* studied. Rows: observed classification. Columns: predicted classification.

	Percentage	*C. barbara lusitanica*	*C. barbara barbara*	*C. cretensis*	*C. lodosi*	*C. mordoganensis*	*C. orni*
C. barbara lusitanica	69.231	9.000	1.000	0.000	0.000	0.000	3.000
C. barbara barbara	42.857	2.000	3.000	1.000	0.000	0.000	1.000
C. cretensis	68.085	1.000	0.000	32.000	0.000	3.000	11.000
C. lodosi	100.000	0.000	0.000	0.000	5.000	0.000	0.000
C. mordoganensis	75.806	0.000	0.000	1.000	0.000	47.000	14.000
C. orni	88.462	3.000	1.000	6.000	0.000	11.000	161.000
Total	81.329	15.000	5.000	40.000	5.000	61.000	190.000

TABLE 7. Standardized coefficients for canonical variables based on discriminant functions of the measurements of 10 external morphological traits of the species of *Cicada* studied.

	Root 1	Root 2	Root 3	Root 4	Root 5
Body length	–1.300	0.255	–0.053	0.439	0.666
Forewing length	–0.173	0.351	0.160	–0.891	0.092
Forewing width	0.561	–0.502	0.446	0.444	–0.840
Head width	0.477	0.310	0.583	0.530	0.911
Pronotum width	0.248	0.366	–0.502	–1.065	–1.030
Front length	–0.220	0.602	–0.081	0.633	–0.244
Antenna-eye distance	0.457	0.164	0.478	–0.271	0.244
Rostrum length	–0.626	–0.951	0.270	0.309	–0.193
Tymbal length	–0.016	–0.110	–0.952	0.228	0.516
Tymbal width	0.036	0.133	–0.183	–0.141	–0.348
Eigenvalue	1.580	0.664	0.298	0.101	0.021
Cum. Prop.	0.593	0.842	0.954	0.992	1.000

TABLE 8. Means of canonical variables based on discriminant functions of the measurements of 10 external morphological traits of the species of *Cicada* studied.

	Root 1	Root 2	Root 3	Root 4	Root 5
C. barbara lusitanica	1.650	1.620	2.055	0.006	–0.251
C. barbara barbara	2.103	2.046	0.262	–0.275	0.837
C. cretensis	0.829	1.211	–0.616	–0.349	0.101
C. lodosi	–5.887	1.907	0.303	1.807	0.142
C. mordoganensis	–1.470	–0.807	0.468	–0.280	0.058
C. orni	0.678	–0.285	–0.165	0.146	–0.012

phology. In fact, it gave a good separation of both *C. lodosi* and *C. barbara* and to a lesser extent of *C. cretensis*. As indicated above, the pygophore proved to be the structure that contributed most to this discrimination.

It should be noted that the calling songs of *C. lodosi* and *C. barbara* are continuous while those of the other species are discontinuous, consisting of echemes of different duration, separated by silent intervals. In fact, the species that show the greatest morphological divergence (*C. lodosi* and *C. barbara*) are the ones with the greatest acoustic and genetic divergences (Quartau et al., 2000, 2001; Quartau & Simões, 2006; Pinto-Juma, 2009). Therefore, the present results resemble those obtained from acoustic and genetic analyses.

On the other hand, the pronounced morphometric differences between the populations of *C. orni* from the eastern and the western Mediterranean (MW tests and results not shown in Fig. 5) correlate with the acoustic data (Pinto-Juma et al., 2005). In the absence of samples from intermediate areas other than a few localities in France it is not possible to establish whether there is a cline from west to east.

The present results clearly corroborate previous studies (Quartau & Simões, 2006), showing that in this complex of species the acoustic divergence observed in the calling songs is associated with low levels of morphological differentiation, especially in external morphology. Recent bioacoustic investigations have shown a similar pattern of divergence in other groups of cicadas. This is the case for *Cicadetta montana* Scopoli 1772, a complex of morphologically similar European species, which are also best characterized by their calling songs (e.g., Gogala & Trilar, 2004; Hertach, 2007; Sueur & Puissant, 2007; Gogala et al., 2008).

However, it is interesting to note that this trend is not general for cicadas. For instance, in the case of the genus *Tibicina* Amyot, conspicuous morphological divergence is associated with very subtle acoustic differentiation (Quartau & Simões, 2003; Sueur & Aubin, 2003).

Hence, the calling song of the genus *Cicada* not only plays a role in long range attraction but also in short range communication, which is important in specific mate recognition (Paterson, 1985), as previous data suggested. This is possibly the reason why the divergence in morphology in these cicadas is less pronounced than the divergence in their acoustic signals, the latter being greater in sympatric species. In fact, *C. lodosi* can be sympatric with *C. mordoganensis*, while *C. barbara lusitanica* can either be allopatric or sympatric with *C. orni* (Sueur et al., 2004; Quartau & Simões, 2006). On the other hand *C. orni*, *C. mordoganensis* and *C. cretensis* do not occur sympatrically and they are the three most similar and closely related species. Furthermore, no character displacement in acoustic characters between sympatric species of genus *Cicada* is recorded (Seabra et al., 2008).

Summing up, the present morphometric analyses revealed that divergence in morphology is much less pronounced than the divergence in acoustic signals and DNA. Thus, the congruence between the morphological

TABLE 9. Descriptive statistics for the measurements (in mm) of seven structures of the male genitalia of thespecies of *Cicada* studied.

	C. barbara lusitanica (N = 3)				*C. barbara barbara* (N = 9)				*C. cretensis* (N = 6)				*C. lodosi* (N = 5)				*C. mordoganensis* (N = 12)				*C. orni* (N = 15)			
Trait	Mean	Min	Max	S. Dev.	Mean	Min	Max	S. Dev.	Mean	Min	Max	S. Dev.	Mean	Min	Max	S. Dev.	Mean	Min	Max	S. Dev.	Mea n	Min	Max	S. Dev.
Pygophore length without spine	3.1	2.9	3.5	0.2	2.8	2.7	2.9	0.1	4.1	3.8	4.3	0.2	4.6	4.6	4.6	0.0	3.8	2.8	4.1	0.3	3.7	3.3	4.0	0.2
Overall pygophore length	3.2	3.0	3.7	0.2	3.0	2.9	3.0	0.1	4.5	4.2	4.7	0.2	4.8	4.8	4.9	0.1	4.1	3.4	4.4	0.3	4.0	3.6	4.4	0.3
Pygophore posterior length	1.6	1.3	1.9	0.2	1.7	1.5	1.8	0.2	2.3	2.1	2.3	0.1	2.4	2.2	2.6	0.2	2.1	1.9	2.3	0.1	2.0	1.8	2.2	0.1
10th abdomonal segment length	2.1	1.9	2.3	0.1	1.9	1.9	2.0	0.1	3.0	2.9	3.1	0.1	3.1	2.8	3.4	0.3	2.6	2.4	2.8	0.1	2.8	2.4	3.1	0.2
Pygophore width	2.0	1.9	2.0	0.1	1.8	1.7	2.0	0.2	2.5	2.3	2.8	0.2	2.8	2.7	2.9	0.1	2.3	1.9	2.7	0.3	2.2	1.9	2.5	0.1
Aedeagus length	3.3	2.7	3.6	0.4	3.2	2.6	3.6	0.5	3.4	3.2	3.5	0.1	4.0	3.5	4.8	0.7	3.3	3.2	3.4	0.1	3.5	3.0	3.9	0.2
Aedeagus apodeme length	0.6	0.5	0.7	0.1	0.5	0.4	0.5	0.1	0.7	0.6	0.7	0.0	0.8	0.7	0.9	0.1	0.6	0.6	0.7	0.0	0.7	0.6	0.9	0.1

TABLE 10. Factor loadings of the principal component analysis (PCA) based on a correlation matrix of the seven measurements made on structures of male genitalia of the species of *Cicada* studied.

	Component 1	Component 2
Pygophore length without spine	–0.953	0.112
Overall pygophore length	–0.980	–0.042
Pygophore posterior length	–0.897	–0.216
10^{th} abdominal segment length	–0.940	0.030
Pygophore width	–0.856	0.090
Aedeagus length	–0.415	0.791
Aedeagus apodeme length	–0.610	0.068

divergence in external structures, and either behavioural (acoustic) or genetic divergence is quite low.

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