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Separation of *Aspidiotes* species using morphometric analysis (Coleoptera: Curculionidae)

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Abstract. The efficacy of morphometric characters for separating the species of the genus *Aspidiotes* Schoenherr, 1847, was evaluated. Thirty characters were analyzed. Multivariate and univariate analyses of variance, and discriminant function analysis, all demonstrated that each species is morphometrically distinguishable. The lengths of rostrum, scape, onychium, pronotum, and width and length of elytra have the maximum discriminatory power. Males and females are also morphometrically distinguishable, mainly due to differences in the widths of rostrum between pterigia and at base of pronotum, and width and length of elytra. The classification functions provided by discriminant gave the correct identification of every single specimen by sex and species. Mahalanobis' distances between species were calculated and subjected to UPGMA clustering, to construct a dendrogram reflecting the morphometric relationships between species. This dendrogram did not correspond to the phylogenetic relationships depicted by a cladogram based on discrete characters (Sánchez-Ruiz & Alonso-Zarazaga, 1994). Some hypotheses are reviewed, which might explain this discrepancy.

INTRODUCTION

The systematics of the genus *Aspidiotes* Schoenherr, 1847 was recently revised by Sánchez-Ruiz & Alonso-Zarazaga (1994), who found the genus was made up of six species (*A. anatolicus*, *A. cottyi*, *A. gonzalezi*, *A. larbii*, *A. thalassinus* and *A westringii*). Their cladistic analysis, based on qualitative characters of the external morphology and genitalia, suggested the existence of two subgenera within the genus, *Aspidiotes* and *Phaenognathus*. The genus *Aspidiotes*, as presently delimited, shows an East-West Mediterranean disjunction, well known for other taxa of plants and animals (Oosterbroek & Arntzen, 1992).

Previously, Alonso-Zarazaga & Sánchez-Ruiz (1990) used multivariate morphometric analysis to divide the Iberian species *A. westringii* into two allopatric species: *A. westringii*, which occurs in southeastern Spain, and a northeastern population, later described as *A. gonzalezi* Sanchez-Ruiz & Alonso-Zarazaga, 1994. Differences in the external morphology and genitalia of these species were corroborated by metric characters, thus indicating the usefulness of these characters in the taxonomy of *Aspidiotes*. In this paper we propose a new approach to the taxonomy of the genus based on an analysis of morphometric characters. This analysis reveals new taxonomic characters.

Morphometric analysis has been successfully used in other groups of insects such as aphids (Foottit, 1992; Tizado & Nieto-Nafría, 1994), bees (Daly, 1992), planthoppers (Claridge & Gillham, 1992), or beetles (Sanmartín & Martín-Piera, 1999). There are, however, few studies on the systematics of Curculionidae using these techniques (Godwin et al., 1982; Horng & Peng, 1983).

Multivariate morphometrics has proved useful not only in resolving taxonomic problems but also in coevolution studies (Houck, 1992), and even for phylogenetic inference. Sorensen & Foottit (1992) argue that multivariate morphometric methods, in particular discriminant analysis, can be used for estimating phylogeny because, "as with cladistic methods, they evaluate partitioned variance and reflect polarity or apomorphic character states" (Sites & Willing, 1994).

Unlike in other groups of insects, there is usually no striking (visible) sexual dimorphism in shortnosed Curculionidae. Differences between sexes in *Aspidiotes* involve the entire form of the insect and are thus difficult to define in terms of qualitative characters. Multivariate morphometric analysis considers simultaneously many different intercorrelated characters, thus it might help to visualize these latent, non obvious differences.

Aims of this work is: (i) evaluate morphometric characters for separating *Aspidiotes* species; (ii) determine the discriminatory characters for (a) every pair of species, and (b) males and females; (iii) to compare the classification of species based on this morphometric analysis with that of Sanchez-Ruiz & Alonso-Zarazaga (1994), based on a cladistic analysis of qualitative characters.

MATERIAL AND METHODS

Table 1 shows the geographic distribution and number of specimens measured for each of the six species of genus *Aspidiotes*. In all, 177 specimens were measured.

Thirty characters from all parts of the body of male and female adults were measured (Table 2), measurements were made with the aid of an ocular micrometer attached to a binocular microscope. Ratios or indices were not included in the analysis because of the difficulties created by using of ratios in morphometric analysis (Albretch et al., 1993).

TABLE 1. Distribution and sample size of each species of *Aspidiotes*.

Species	Author & date	N	Distribution	Males / Females
A. anatolicus	(Colonnelli, 1978)	26	Turkey	14 / 12
A. cottyi	(Lucas, 1858)	51	Morocco, Algeria	24 / 27
A. gonzalezi	Sanchez-Ruiz & Alonso-Zarazaga, 1994	20	Northeastern Spain	10 / 10
A. larbii	(Escalera, 1914)	8	Morocco	7 / 1
A. thalassinus	(Schoenherr, 1847)	31	Greece, Turkey	17 / 14
A. westringii	Schoenherr, 1847	41	Southeastern Spain	19 / 22

Statistical analyses were performed using STATISTICA 5.1 for WINDOWS (Statsoft, 1996). The basic principles of the analyses used may be found in Sneath & Sokal (1973).

(i) We used an analysis of variance to evaluate the efficacy of morphometric characters in the separation of *Aspidiotes* species. First, a one-way multivariate analysis of variance (MANOVA) was performed on the six species to determine whether statistically significant differences existed between species based on the entire set of characters. One-way analyses of variance (ANOVAs) were then computed for each character to evaluate whether it contributed significantly to species differences. However, before ascribing statistical significance, we applied the sequential Bonferroni correction to each character to avoid overestimating the significance of particular characters in a large suite of attributes (Rice, 1989).

(ii) (a) The most important characters for discriminating between species in species pairs were determined by multiple discriminant function analysis (DFA). DFA demonstrated the degree of separation in multivariate space defined by the main patterns of morphological variation among species (the discriminant functions). It also showed which characters contribute more to the discrimination between species. The standardized discriminant function coefficients (coefficients of the original variables in the discriminant functions) represent the contributions of every variable to the discriminatory power of the function; the larger the standardized coefficient, the larger the weight of the variable in the function.

In addition, DFA allows for the predictive classification of specimens. The attribution of specimens to species was checked by computing the classification functions: an individual was allocated to the species for which it had the highest classification score. The percentage of specimens properly classified is a measure of the diagnostic value of the set of characters (Foottit & Sorensen, 1992).

(ii) (b) A similar methodology was followed to determine the existence of sexual morphometric dimorphism in the genus *Aspidiotes*. First, MANOVA was used to determine if significant differences existed between males and females within the genus, regardless of species. A MANOVA test was then performed on each analysed species to test whether it presented sexual dimorphism (Planned Comparisons). An exception was made for *A. larbii*, where statistical comparison was not possible (7 males/ 1 female). Finally, discriminant analysis was used to determine the most important characters for identifying males and females within the genus.

(iii) Finally, Mahalanobis' generalized distances (D^2) were computed between all pairs of species. The square root of Mahalanobis' distance (D) for any two species represents the length of the line between the centroids of the two species in the discriminant space (Sneath & Sokal, 1973). Mahalanobis' distances were then subjected to the clustering method UPGMA (Unweighted Pair-Group Method Arithmetic average) to construct a dendrogram reflecting the morphometric relationships between *Aspidiotes* species. This dendrogram was then compared with the cladogram based on qualitative characters given by Sánchez-Ruiz & Alonso-Zarazaga (1994).

TABLE 2. List of characters used in this study.

Character	Description
WRP	Width of rostrum on pterigia
WRS	Width of rostrum between scrobes
LR	Length of rostrum
WF	Width of frons (between eyes)
LSC	Length of scape
WSC	Width of scape
LF1	Length of 1st funicular joint
LF2	Length of 2nd funicular joint
WF1	Width of 1st funicular joint
WF2	Width of 2nd funicular joint
LC	Length of antenal club
WC	Width of antenal club
LP	Length of pronotum (on midline)
WP	Width of pronotum (maximum)
WPB	Width of pronotum at base
WPA	Width of pronotum at apex
WE	Width of elytra (maximum)
LE	Length of elytra
LPF	Length of profemur
WPF	Width of profemur (maximum)
LPT	Length of protibia
WPT	Width of protibia (not including mucro)
LT1	Length of 1st protarsomere
LT2	Length of 2nd protarsomere
LT3	Length of 3rd protarsomere
LON	Length of onychium
WT1	Width of 1st protarsomere
WT2	Width of 2nd protarsomere
WT3	Width of 3rd protarsomere
WON	Width of onychium

RESULTS

The mean value, standard deviation, and range of variation for each of the thirty characters in the six species are listed in Table 3.

(i) MANOVA showed highly significant differences (Wilks' $lambda_{150,702} = 0.00004$; $p < 0.001$) between the six species based on the entire set of characters. Moreover, subsequent ANOVAs resulted in highly significant differences ($p < 0.00001$) between the species in each character, even after applying sequential Bonferroni correction. Thus, all characters contributed significantly to the separation of species.

(ii) (a) Discriminant function analysis provided five significant functions ($\chi^2 = 1585.089$; $p < 0.000001$). About 94% of the variability in the data is attributable to between-species differences when all thirty variables were considered ($R^2 = 0.94$). The first four functions explain 97% of the total variation in the data, which is sufficient for the analysis (Table 4).

TABLE 3. Mean value, standard deviation and range of variation of the characters measured in each species.

Character		*A. anatolicus*	*A. cottyi*	*A. gonzalezi*	*A. larbii*	*A. thalassinus*	*A. westringii*
WRP	Mean	0.735	0.836	0.974	0.934	1.117	0.818
	StdDev.	0.046	0.064	0.085	0.057	0.093	0.073
	Range	0.665–0.875	0.7–0.962	0.822–1.137	0.822–0.997	0.944–1.259	0.682–0.944
WRS	Mean	0.536	0.615	0.683	0.715	0.811	0.609
	StdDev.	0.042	0.053	0.058	0.060	0.072	0.056
	Range	0.472–0.647	0.507–0.735	0.577–0.805	0.612–0.787	0.680–0.962	0.505–0.700
LR	Mean	1.154	1.119	1.363	1.174	1.542	0.998
	StdDev.	0.088	0.082	0.108	0.084	0.129	0.094
	Range	1.01–1.361	0.922–1.317	1.098–1.580	1.054–1.295	1.273–1.712	0.812–1.207
WF	Mean	0.722	0.720	0.851	0.813	1.029	0.752
	StdDev.	0.084	0.084	0.102	0.081	0.121	0.091
	Range	0.595–0.997	0.525–0.909	0.647–1.067	0.665–0.909	0.840–1.278	0.560–0.910
LSC	Mean	0.713	0.734	1.007	1.052	1.174	0.828
	StdDev.	0.047	0.058	0.080	0.064	0.087	0.070
	Range	0.630–0.805	0.595–0.909	0.805–1.154	0.944–1.137	0.962–1.312	0.700–0.962
WSC	Mean	0.155	0.183	0.210	0.222	0.206	0.156
	StdDev.	0.011	0.016	0.024	0.017	0.012	0.013
	Range	0.139–0.181	0.139–0.222	0.167–0.250	0.194–0.236	0.181–0.222	0.139–0.194
LF1	Mean	0.291	0.315	0.423	0.368	0.417	0.336
	StdDev.	0.021	0.027	0.044	0.020	0.027	0.054
	Range	0.250–0.347	0.250–0.347	0.347–0.486	0.347–0.403	0.361–0.458	0.278–0.617
LF2	Mean	0.196	0.240	0.312	0.212	0.228	0.184
	StdDev.	0.016	0.023	0.026	0.022	0.021	0.024
	Range	0.167–0.222	0.181–0.292	0.264–0.347	0.181–0.236	0.194–0.278	0.139–0.236
WF1	Mean	0.136	0.147	0.163	0.168	0.167	0.125
	StdDev.	0.011	0.017	0.024	0.012	0.018	0.010
	Range	0.111–0.153	0.111–0.181	0.111–0.208	0.153–0.181	0.109–0.208	0.097–0.139
WF2	Mean	0.115	0.136	0.152	0.149	0.148	0.111
	StdDev.	0.014	0.016	0.022	0.010	0.014	0.010
	Range	0.097–0.139	0.097–0.167	0.111–0.194	0.139–0.167	0.125–0.181	0.097–0.125
LC	Mean	0.474	0.547	0.538	0.583	0.574	0.493
	StdDev.	0.030	0.035	0.042	0.031	0.042	0.031
	Range	0.431–0.542	0.431–0.625	0.444–0.597	0.542–0.625	0.486–0.667	0.431–0.556
WC	Mean	0.216	0.259	0.242	0.292	0.274	0.220
	StdDev.	0.012	0.025	0.030	0.013	0.018	0.020
	Range	0.194–0.236	0.150–0.306	0.153–0.278	0.278–0.319	0.250–0.306	0.139–0.264
LP	Mean	1.623	1.914	1.958	1.918	1.991	1.509
	StdDev.	0.106	0.145	0.162	0.143	0.166	0.138
	Range	1.471–1.866	1.602–2.195	1.574–2.265	1.646–2.129	1.580–2.292	1.185–1.778
WP	Mean	2.026	2.135	2.370	2.151	2.649	1.798
	StdDev.	0.177	0.202	0.232	0.185	0.240	0.181
	Range	1.778–2.513	1.756–2.569	1.795–2.762	1.866–2.403	2.173–3.042	1.405–2.151
WPB	Mean	1.800	2.042	2.219	1.945	2.514	1.732
	StdDev.	0.173	0.243	0.271	0.207	0.264	0.207
	Range	1.537–2.237	1.602–2.596	1.630–2.734	1.668–2.348	2.072–2.972	1.383–2.129
WPA	Mean	1.485	1.634	1.796	1.669	2.003	1.465
	StdDev.	0.110	0.147	0.165	0.115	0.167	0.133
	Range	1.317–1.712	1.317–1.93	1.409–2.072	1.471–1.851	1.685–2.273	1.207–1.668
WE	Mean	2.795	2.944	3.168	2.972	3.802	2.757
	StdDev.	0.405	0.376	0.401	0.280	0.453	0.381
	Range	2.292–3.948	2.320–3.640	2.486–3.991	2.541–3.427	3.007–4.633	2.098–3.462
LE	Mean	4.479	5.284	5.323	4.997	6.295	4.657
	StdDev.	0.580	0.658	0.680	0.536	0.661	0.656
	Range	3.728–5.942	4.254–6.739	4.079–6.666	4.298–6.087	5.123–7.392	3.509–5.870
LPF	Mean	1.975	1.966	2.208	2.142	2.459	1.763
	StdDev.	0.121	0.142	0.211	0.145	0.184	0.167
	Range	1.822–2.348	1.712–2.458	1.795–2.569	1.888–2.320	2.129–2.797	1.383–2.085
WPF	Mean	0.482	0.519	0.596	0.634	0.660	0.472
	StdDev.	0.034	0.052	0.046	0.074	0.071	0.036
	Range	0.417–0.569	0.258–0.611	0.500–0.667	0.477–0.708	0.542–0.965	0.389–0.583

(continued on next page)

TABLE 3 (continued).

Character		*A. anatolicus*	*A. cottyi*	*A. gonzalezi*	*A. larbii*	*A. thalassinus*	*A. westringii*
LPT	Mean	2.248	2.223	2.446	2.196	2.661	1.841
	StdDev.	0.194	0.160	0.166	0.129	0.187	0.150
	Range	1.932–2.623	1.756–2.541	2.044–2.734	1.976–2.320	2.265–2.972	1.558–2.173
WPT	Mean	0.339	0.390	0.412	0.448	0.468	0.337
	StdDev.	0.023	0.038	0.044	0.042	0.040	0.031
	Range	0.306–0.403	0.319–0.472	0.319–0.500	0.389–0.528	0.389–0.569	0.264–0.417
LT1	Mean	0.510	0.451	0.547	0.477	0.595	0.447
	StdDev.	0.042	0.041	0.058	0.043	0.042	0.038
	Range	0.417–0.611	0.361–0.583	0.486–0.695	0.417–0.528	0.542–0.695	0.375–0.556
LT2	Mean	0.384	0.364	0.357	0.340	0.420	0.302
	StdDev.	0.034	0.030	0.041	0.022	0.048	0.027
	Range	0.306–0.444	0.306–0.444	0.292–0.431	0.306–0.375	0.319–0.514	0.250–0.361
LT3	Mean	0.292	0.317	0.315	0.328	0.372	0.247
	StdDev.	0.025	0.028	0.042	0.023	0.040	0.024
	Range	0.250–0.347	0.250–0.375	0.222–0.375	0.292–0.361	0.297–0.472	0.208–0.319
LON	Mean	0.563	0.557	0.609	0.601	0.651	0.544
	StdDev.	0.036	0.047	0.046	0.028	0.035	0.039
	Range	0.486–0.639	0.444–0.681	0.528–0.722	0.556–0.639	0.569–0.708	0.486–0.625
WT1	Mean	0.280	0.322	0.310	0.323	0.361	0.235
	StdDev.	0.019	0.033	0.035	0.031	0.028	0.017
	Range	0.250–0.333	0.250–0.403	0.250–0.375	0.292–0.375	0.296–0.417	0.208–0.278
WT2	Mean	0.263	0.305	0.295	0.307	0.352	0.217
	StdDev.	0.018	0.034	0.039	0.032	0.030	0.017
	Range	0.236–0.306	0.222–0.397	0.236–0.375	0.250–0.347	0.278–0.403	0.181–0.264
WT3	Mean	0.391	0.460	0.453	0.467	0.524	0.354
	StdDev.	0.032	0.041	0.052	0.026	0.046	0.026
	Range	0.347–0.472	0.361–0.569	0.361–0.569	0.428–0.514	0.417–0.625	0.292–0.417
WON	Mean	0.139	0.159	0.160	0.160	0.190	0.138
	StdDev.	0.011	0.013	0.015	0.010	0.010	0.011
	Range	0.125–0.167	0.139–0.181	0.139–0.181	0.139–0.167	0.181–0.208	0.125–0.167

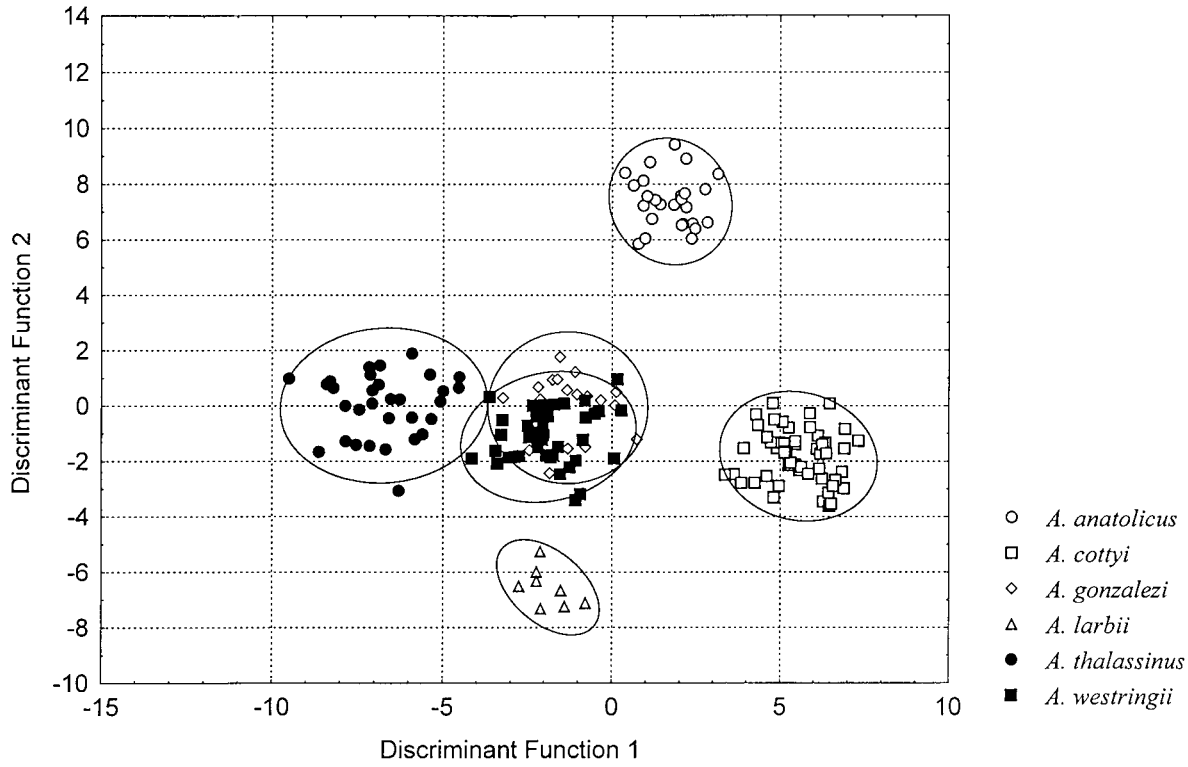

Fig. 1. Plot of all *Aspidiotes* specimens onto the first and second discriminant functions based on a set of 30 morphometric characters. Circles include 95% of specimens in each species.

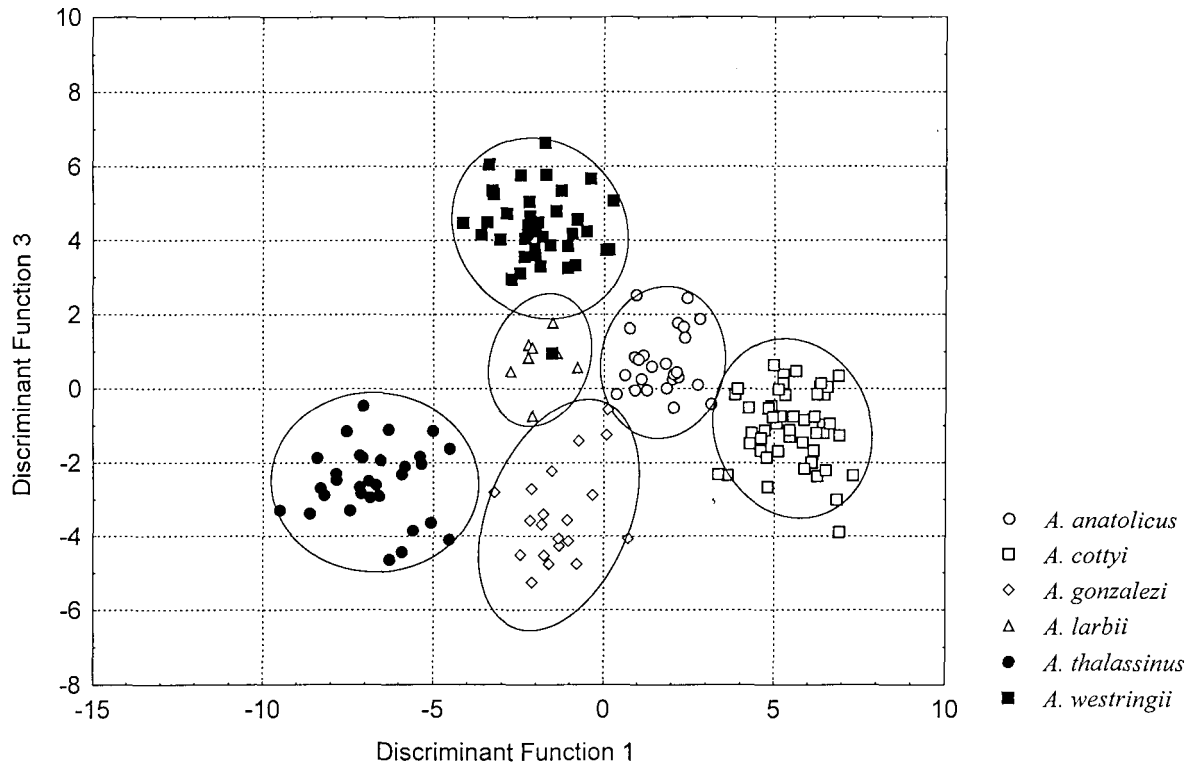

Fig. 2. Plot of all *Aspidiotes* specimens onto the first and third discriminant functions based on a set of 30 morphometric characters. Circles include 95% of the specimens in each species.

Individual specimens are projected onto the first two discriminant functions in Fig. 1, and onto the first and third functions in Fig. 2. Because all species were clearly separated in the discriminant space defined by the first three functions, the fourth function was not used.

The first discriminant function explains 44% of total variation (Table 4), and mainly separates *A. thalassinus* from *A. cottyi*. The other four species, clustered in the middle, cannot be discriminated by this function. From the standardized coefficients (Table 4), the characters that have the greatest weight on this function (characters most discriminatory) are the lengths of scape (LSC) and pronotum (LP) and, to a lesser extent, the elytral length (LE). LSC is opposite in sign to LP and LE. In general *A. thalassinus* is larger than *A. cottyi*. The length of the pronotum (LP), however, is very similar in both species: average 1. 99 mm in *A. thalassinus* and 1.91 mm in *A. cottyi* (Table 3). Likewise, the differences between the elytral length (LE) in both species (5.284–6.295 mm) do not correspond to the general differences in size. The scape, however, is much longer in *A. thalassinus* (1.174) than in *A. cottyi* (0.734). That is *A. thalassinus* is characterized by a proportionally shorter pronotum, shorter elytra, and a very long antennal scape, whereas *A. cottyi* has a longer pronotum, elongated elytra, and a shorter scape.

The second discriminant function accounts for 27% of total variation. *A. larbii* and *A. anatolicus* are clearly discriminated by this function while the other four species are clustered in the middle (Fig. 1). The contrast between the length of rostrum (LR) and length of elytra (LE) is responsible for this discrimination. *A. larbii* is larger than *A. anatolicus*, but in proportion *A. anatolicus* has a longer

TABLE 4. Standardized coefficients of the first four discriminant functions separating the six species of *Aspidiotes*. In bold, characters with the greatest weight in the function.

Character	DF1	DF2	DF3	DF4
WRP	–0.451	–0.568	–0.036	–0.160
WRS	–0.644	–0.600	–0.258	–0.372
LR	–0.341	**1.169**	**0.940**	–0.168
WF	0.031	**0.743**	–0.591	–0.289
LSC	**–1.179**	–0.638	–0.024	0.252
WSC	0.169	–0.281	0.149	0.352
LF1	0.038	–0.101	0.036	0.168
LF2	0.281	0.046	0.374	0.632
WF1	0.025	0.127	–0.244	–0.136
WF2	–0.141	–0.312	0.082	0.152
LC	0.429	–0.316	0.048	–0.124
WC	–0.002	–0.350	–0.138	–0.153
LP	**1.489**	**–0.787**	0.227	0.442
WP	–0.047	0.511	0.219	0.407
WPB	–0.639	0.136	–0.187	–0.705
WPA	0.103	–0.192	0.433	0.254
WE	0.076	**0.731**	0.067	0.577
LE	**0.860**	**–1.428**	–0.274	**–0.965**
LPF	0.057	0.443	0.022	0.032
WPF	–0.103	–0.037	0.123	0.198
LPT	0.068	0.492	0.501	0.068
WPT	0.126	–0.246	–0.432	0.437
LT1	–0.300	0.380	–0.222	0.258
LT2	0.128	0.571	–0.234	–0.360
LT3	–0.003	0.102	0.211	–0.440
LON	–0.180	0.189	**–0.734**	0.510
WT1	0.428	–0.120	–0.320	–0.352
WT2	–0.179	0.035	0.575	–0.027
WT3	–0.171	–0.178	0.328	–0.320
WON	–0.031	–0.118	0.085	–0.461
Percentage of explained variance	44%	27%	17%	9%
Eigenvalue	19.01	11.55	7.47	3.73
Cumulative variance	0.44	0.71	0.88	0.97

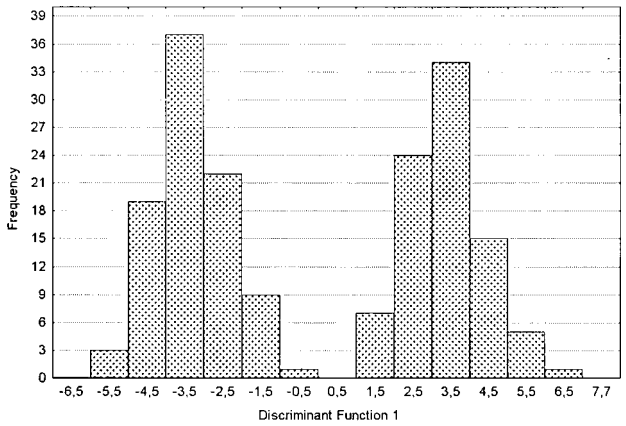

Fig. 3. Frequency distribution of *Aspidiotes* specimens along the first discriminant function. Left – females; right – males.

rostrum and shorter elytra than *A. larbii*. This shape difference is reinforced by contrasts in three other variables: frons width between the eyes (WF) and elytral width (WE) against length of pronotum (LP).

As seen in Fig. 1, the discriminant space defined by the first two functions allows us to clearly separate four species of the genus: *A. thalassinus*, *A. cottyi*, *A. anatolicus*, and *A. larbii*.

TABLE 5. Standardized coefficients of the Fisher's linear discriminant function separating specimens of *Aspidiotes* by sex. In bold, characters with the greatest weight in the function.

Character	DF1
WRP	**1.126**
WRS	0.481
LR	–0.014
WF	–0.145
LSC	0.561
WSC	–0.011
LF1	–0.101
LF2	–0.306
WF1	–0.207
WF2	0.808
LC	0.101
WCM	0.224
LP	–0.376
WP	0.713
WPB	**–1.186**
WPA	–0.327
WE	**–1.751**
LE	**–1.574**
LPF	–0.304
WPF	0.090
LPT	–0.145
WPT	–0.072
LT1	0.371
LT2	0.653
LT3	0.175
LON	–0.066
WT1	0.564
WT2	0.046
WT3	0.683
WON	0.341
Percentage of variance explained	100%
Eigenvalue	11.30
Cumulative variance	1.00

The third discriminant function explains 17% of total variation. This function morphologically separates the two remaining species (Fig. 2): *A. gonzalezi* from *A. westringii*, by an increase in the length of rostrum (LR) and decrease in the length of onychium (LON).

The DFA classification functions, based on linear combinations of the original variables, correctly identified all specimens, thus demonstrating the efficacy of this set of morphometric characters for identifying species of *Aspidiotes*.

(ii) (b) MANOVA revealed highly significant differences (Wilks' lambda$_{30,191}$ = 0.081765; $p < 0.001$) between males and females within the genus *Aspidiotes*. Subsequent MANOVAs revealed sexual dimorphism in five species: *A. thalassinus*, *A. cottyi*, *A. anatolicus*, *A. westringii* and *A. gonzalezi* ($p < 0.001$). A discriminant analysis, to separate specimens by sex, provided one discriminant function, allowing for complete discrimination of males and females within the genus (Fig. 3). Based on the standardized coefficients, the weight of this function is mainly dependent on: width of pronotum at base (WPB), width of elytra (WE), length of elytra (LE), and width of rostrum on pterigia (WRP) (Table 5). Females have wider, longer elytra and a wider pronotum than males whereas males have a wider rostrum.

Table 6 shows classification functions that separate specimens by sex and the percentage of correct attributions (100%). These classification functions can serve as an additional diagnostic tool for determining the sex of specimens. This may substitute the study of genitalia, which has been often necessary in order to distinguish males from females in *Aspidiotes*.

(iii) The dendrogram (Fig. 4) constructed with UPGMA based on Mahalanobis' distance values, shows the morphometric relationships between the six species of the genus. This dendrogram shows the same patterns of association as found previously in the plots of the first three discriminant functions. At the base of the dendrogram there is the first separation, which separates *A. anatolicus* and *A. cottyi* from the other four species; within the latter there are two subclusters: *A. gonzalezi–A. thalassinus* and *A. larbii–A.westringii*. It should be noted

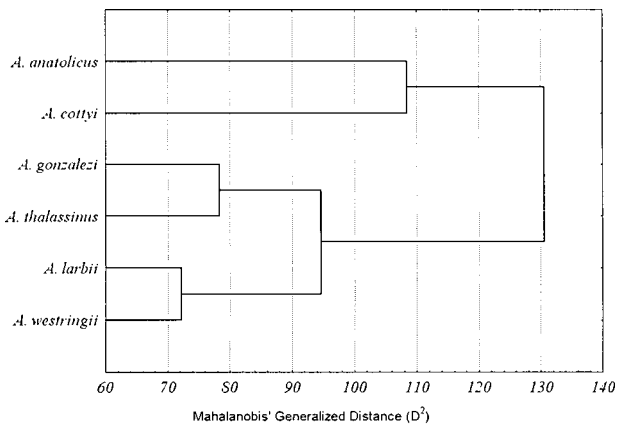

Fig. 4. UPGMA dendrogram of Mahalanobis' generalized distances showing the morphometric relationships between the species of *Aspidiotes*.

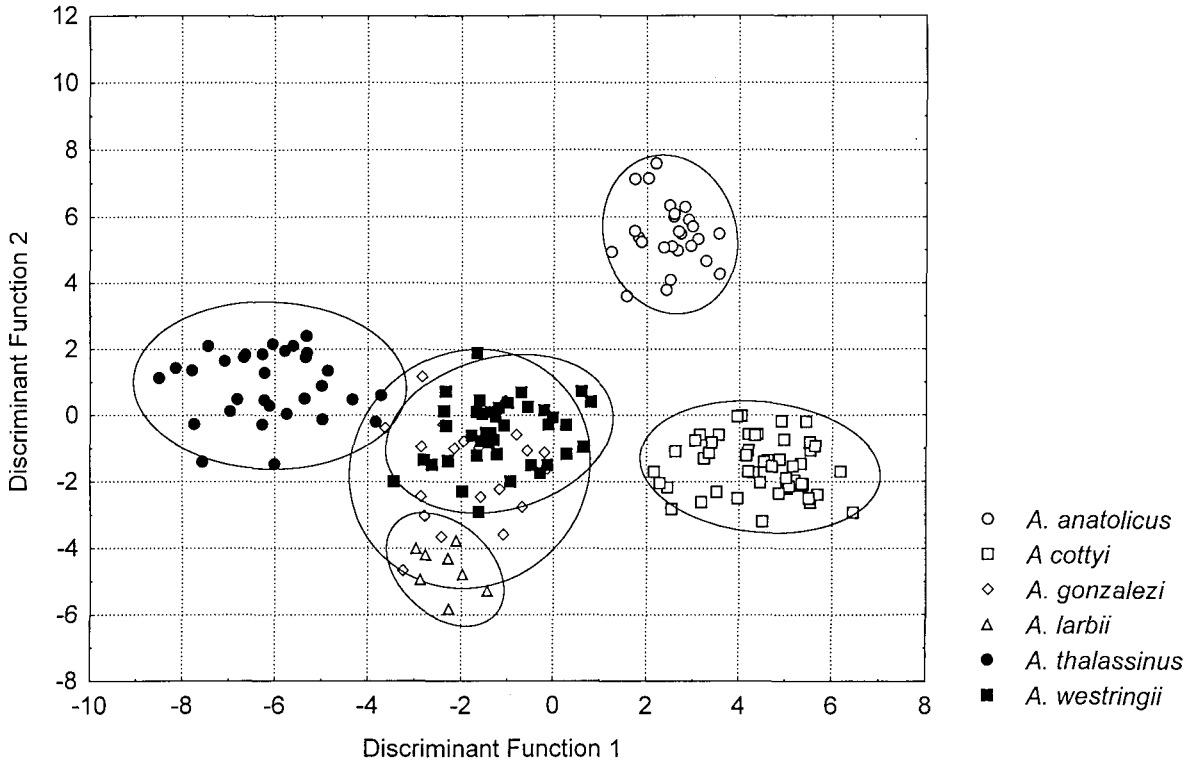

Fig. 5. Plot of all *Aspidiotes* specimens onto the first and second discriminant functions based on a reduced set of 15 morphometric characters (see text).

TABLE 6. Classification functions used to determine the sex of specimens of *Aspidiotes* and the percentage of correct attributions.

Character	Females	Males
WRP	-98.926	-45.082
WRS	38.139	70.357
LR	-21.259	-21.709
WF	52.455	45.455
LSC	-111.112	-90.813
WSC	65.916	63.205
LF1	33.175	22.047
LF2	30.260	-15.716
WF1	-221.049	-284.883
WF2	-9.307	257.394
LC	224.056	237.767
WC	0.256	0.304
LP	39.486	28.799
WP	-35.206	-20.911
WPB	-28.523	-53.362
WPA	99.006	89.232
WE	25.330	-0.660
LE	-4.935	-19.474
LPF	-11.546	-18.736
WPF	-9.285	-2.383
LPT	33.674	30.613
WPT	12.988	4.899
LT1	98.715	133.364
LT2	7.074	92.942
LT3	-270.805	-247.107
LON	289.669	281.752
WT1	-191.796	-117.017
WT2	-153.107	-147.196
WT3	-44.464	22.541
WON	298.142	403.545
Constant	-156.870	-156.691
% of correctly classified	100%	100%

that a considerable distance ($D^2 = 130$) separates the first two clusters, indicating that *A. anatolicus* and *A. cottyi* are very different morphometrically from the other species, and also very different from each other ($D^2 = 108$). Even within the second cluster the four species are significantly separated (D^2 ranges from 72 to 78). In short, there are marked morphometric differences between the species.

Statistical reliability of our results

Because the specimen to variable ratio is relatively low (≅6), our results may be statistically unreliable. Typically, a minimum ratio of 10–20 is considered necessary for reliability. The number of specimens was limited and any a priori selection among the 30 characters, obtained after a preliminary study, was not feasible, as all of them differed significantly between species ($p < 0.00001$).

As a check of the accuracy of our results, we performed a multivariate analysis using a reduced set of 15 characters. This included those characters that proved the best discriminators among species in the previous analysis; we also excluded those characters that were redundant (as judged by the tolerance values), and summed others into a single character (e.g., LT1 + LT2 + LT3). In addition, results from this analysis (having greater reliability but poorer resolution), if consistent with the first 30-characters study, would provide strong support for our conclusions. The results of this analysis conformed with those of the previous analysis. Although there was a loss of resolution (i.e., morphometric differences between species were lower), both the characters and the species discriminated were the same (Fig. 5). We believe that this

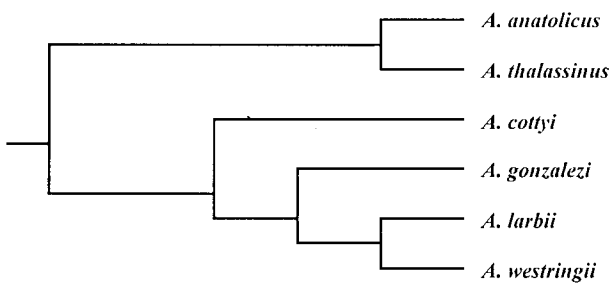

Fig. 6. Cladogram depicting the phylogenetic relationships between the species of *Aspidiotes* as proposed by Sánchez-Ruiz & Alonso-Zarazaga (1994).

two-step procedure gets over the problem of limited numbers of specimens.

DISCUSSION

Character efficacy

On the basis of this study it is evident that morphometric characters can be used to separate species within the genus *Aspidiotes*. The set of morphometric characters herein analysed has proved effective in species discrimination, in particular, lengths of rostrum, scape, onychium and pronotum, and width and length of elytra.

Despite differences in their average values, the ranges in morphometric characters overlapped to some extent between species (Table 3). No character alone can be used for full discrimination. That is, species can only be separated on the basis of all the characters. Thus, taxonomic discrimination requires a multivariate approach: species overlap when characters are used individually but become distinct entities when many characters are considered jointly (Foottit & Sorensen, 1992). This is a consequence of the intercorrelation of characters, itself derived from the epistatic or pleiotropic interactions among genes coding them (Leamy & Sustarsic, 1978; Atchley et al., 1982).

The genus *Aspidiotes* shows clear sexual morphometric dimorphism. Differences between sexes occur in the width of rostrum on pterigia, width of pronotum at base, and width and length of elytra. The last three characters differ in size between males and females, as in other genera of Curculionidae. Females are generally larger than males. This size difference, however, is more difficult to appreciate in *Aspidiotes* without the help of multivariate analysis.

In addition, the classification functions provided by discriminant analysis can be used as a diagnostic tool when discrete characters are unreliable. They have the advantage of identifying "problematic" specimens objectively, and their efficacy can be evaluated by the percentage of correct identifications.

Systematic implications

The comparison of the dendrogram in Fig. 4, portraying the morphometric relationships between species, with the cladogram in Fig. 6, representing their phylogenetic relationships (Sánchez-Ruiz & Alonso-Zarazaga, 1994), show notable differences. The species closely clustered in the dendrogram, such as *A. anatolicus-A. cottyi* or *A. thalassinus-A. gonzalezi*, are very far apart in the cladogram, and are even included in different subgenera, *Phaenognathus* and *Aspidiotes* s. str., respectively (Sánchez-Ruiz & Alonso-Zarazaga, 1994). Conversely, the species that are closely related in the cladogram and belong to the same subgenus (*A. anatolicus-A. thalassinus*) showed the greatest morphometric differences in the phenogram, even greater than in species from different subgenera. The only exception occurs in the pair *A. larbii-A. westringii*, which appear closely related in both figures.

This tends to contradict the claim of Sorensen & Foottit (1992) about the potential use of discriminant function analysis in phylogenetic inference. These authors claim that the DFA-based dendrogram reflects not only morphometric similarity but also the phylogenetic relationships between species. Using Lande's (1979) phenotypic model for multivariate evolution, discriminant functions would represent "historical gradients of selective pressure that the species have been exposed to during their common evolutionary history" (Sorensen & Foottit, 1992). This approach has been criticized, however, because of the lack of biological meaning of discriminant functions (Crespi & Bookstein, 1989; Crespi, 1992). To date, the DFA model has been used several times (Schluter, 1984; Sorensen, 1987; Wood & Pesek, 1992; Simon, 1992). In these studies, the DFA-based dendrograms largely corroborated previous cladograms for the same groups. In our case, there was no congruence between the relationships depicted by the cladogram and the phenogram. Because phylogenetic arguments have a more sound theoretical base than phenetics, it is more likely that the cladogram reflects the phylogenetic relationships between species of *Aspidiotes* whereas the phenogram only depicts their morphometric relationships.

The comparison of the distributions of the species in both figures revealed other important differences. The species that are closely related in the cladogram have similar geographic distributions. This suggests that the species could have originated from a succesion of vicariance events on a widespread ancestor, with every vicariance being followed by a speciation event (Sánchez-Ruiz & Alonso-Zarazaga, 1994). In the phenogram, in constrast, those species occurring in the same or adjacent geographical areas are separated. This is the case for the species pairs *A. anatolicus* (Turkey)-*A. thalassinus* (Greece + Turkey), and *A. cottyi* (Morocco)-*A. larbii* (Morocco). In each pair, both species belong to the same subgenus, *Phaenognathus* or *Aspidiotes*, respectively (Fig. 6). The only apparent exception to this pattern is the species-pair *A. larbii-A. westringii*, which appears closely clustered both in the cladogram and in the phenogram. They have allopatric geographic distributions: *A. westringii* occurs in southeastern Spain whereas *A. larbii* is apparently restricted to the westernmost foothills of the High Atlas (Mogador) (Sánchez-Ruiz & Alonso-Zarazaga, 1994).

Therefore, two conclusions can be drawn from a comparison of the DFA-phenogram and the cladogram of *Aspidiotes*: (1) The contradictory form of the species

relationships depicted by both methods. The most similar species based on discrete characters are in terms of morphometric characters the most different. (2) The largest morphometric differences were between closely related species of *Aspidiotes* (i.e., belonging to the same subgenus), which share a similar geographic distribution (sympatric species).

Morphometric changes are evolutionarily less expensive than discrete changes, which imply the modification or disappearance of a structure. The latter necessarily require disassociations or rearrangements of genetic linkages so discrete changes are more expensive from an energetic viewpoint, and evolutionarily constrained (Sorensen & Foottit, 1992). Morphometric changes only require arrangements of a few pleiotropically correlated genes. Thus, a large morphometric divergence could simply arise from a small number of genetic divergences (Atchley et al., 1982).

For species belonging to the same subgenus, that share very similar genetic covariance matrices and have the same geographic distribution (i.e., subjected to similar selection pressures), "morphometric variance may be the last and easiest way to diverge during evolution because of lower evolutionary energy constraints" (Sorensen, 1991). Therefore, morphometric differences have proved to be more important for closely related and sympatric species of *Aspidiotes* whereas divergence in discrete characters is more important in the case of distantly related species (belonging to different subgenera). Thus, the phenogram in Fig. 4 mainly reflects the recent morphometric divergence between closely related, sympatric species of *Aspidiotes* whereas the cladogram in Fig. 6 would actually reflect the evolution of the genus.

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