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The *Calosoma* species (Coleoptera, Carabidae) of the Galápagos archipelago. II. Discriminant analyses and species identification key

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Abstract

Continuous and meristic morphometric data on the *Calosoma* species of Galápagos are analyzed by means of multivariate statistics. Multiple canonical discriminant functions, derived in a stepwise procedure, for male genitalia size measurements and for a large number of external body measurements (standardized by means of Analysis of Covariance) classify specimens accurately into their a-priori species. A more limited number of external morphometric characters are identified as pertinent discriminators between the *Calosoma* species of Galápagos. *C. linelli* is discriminated by its small size, the relatively broader and deeper prothorax and the more ovoid shape of the elytra, with maximal elytral width situated closer to the basis of the elytra. *C. leleuporum* possesses a distinctly wider forebody. *C. galapageium* can, besides the size of male genitalia, be distinguished by means of the relatively narrow elytra, long head and broad profemur. Discriminant functions for male and female external morphometrics yield very similar results, although a number of characters shows a pronounced sexual dimorphism. Functionally, the observed sexual dimorphism seems to be related to reproductive characters. Discriminant functions based on dispersal power characters yield a small number of misclassifications, whereas those derived from meristic counts or female genitalia characters produce a large number of misclassifications. An identification key is added for these *Calosoma* species from Galápagos.
Key-words : Galápagos - *Calosoma* - discriminant analysis - identification. Contribution n° 443 of the Charles Darwin Research Foundation.

Résumé

Des données morphométriques continues et méristiques concernant les espèces du genre *Calosoma* des îles Galápagos ont été analysées par des méthodes statistiques multivariées. Des fonctions discriminantes canoniques multiples, dérivées à partir de mesures des génitaux mâles d'une part et d'un grand nombre de mesures externes (standardisées par Analyse de Covariance) d'autre part, classifient les spécimens de manière exacte selon les espèces définies « a priori ».

Un nombre plus limité de caractères morphométriques externes sont identifiés comme des discriminateurs pertinents entre ces espèces du genre *Calosoma* de Galápagos. *C. linelli* est discriminé par sa faible taille, le prothorax relativement large et épais et la forme plus ovoïde des élytres, sa largeur maximale se situant plus proche de la base de l'élytre. *C. leleuporum* possède l'avant-partie distinctement plus large. *C. galapageium* peut être identifié, à côté de mesures faites sur les génitaux mâles, par les élytres relativement étroites, la tête longue et le profemur robuste. Les fonctions discriminantes à partir des mesures externes de mâles ou de femelles sont similaires. Néanmoins, certains caractères démontrent un dimorphisme sexuel prononcé, qui d'aspect fonctionnel, semble être en rapport avec des caractères reproductifs. Les fonctions discriminantes, dérivées de données en rapport avec le pouvoir de dispersion, montrent peu d'erreurs de classification, tandis que ceux à base d'énumérations méristiques ou de caractères ayant trait aux génitaux femelles, produisent un grand nombre d'erreurs de classification. Une clé d'identification est présentée pour les espèces de *Calosoma* des îles Galápagos.
Mots-clés : Galápagos - *Calosoma* - analyse discriminante - identification. Contribution n° 443 de la Fondation de Recherche Charles Darwin.

Introduction

In a former paper on the beetles of Galápagos belonging to the genus *Calosoma* WEBER, 1801 (Coleoptera, Carabidae), the four distinguished species were redescribed in detail (DESENDER & DE DIJN, 1989). One of these species, *C. granatense* GEHIN, 1885, occurs on virtually every island of the archipelago, especially in the dry arid vegetation belt. The remaining three species are true endemics and limited to the higher parts of one island each, namely *C. galapageium* HOPE, 1838 on Isla Santiago, *C. leleuporum* (BASILEWSKY, 1968) on Isla Santa Cruz and *C. linelli* MUTCHLER, 1925 on Isla San Cristóbal. Our descriptive results will be extended here, based on a large number of morphometrics and meristic counts. We will analyze our biometric and meristic data set by means of multivariate statistical techniques in order to define the most pertinent species discriminators in the different character sets, mainly by means of stepwise multiple canonical discriminant function analysis. Infraspecific data, a hypothesis on the speciation sequence in these beetles as well as population aspects and ecology will be dealt with in future papers.

Material and Methods

A. *Material*

Adult specimens for all recognized phena (on species level) within the *Calosoma* group of Galápagos (cfr. DESENDER & DE DIJN, 1989) are included in the analyses in this paper. Up to ten individuals per population were investigated for (1°) standard total length, (2°) 23 external morphometrics, (3°) 5 (male) and 2 (female) genitalia morphometrics, (4°) 7 meristic characters and (5°) wing

length, wing width and 4 related variables describing the metepisternal size and its punctuation. On the whole, data were gathered on 376 males/367 females of *C. granatense* from 73 populations on 14 different islands or volcanoes of the archipelago, on 19 males/13 females of *C. galapageium* from 5 populations on Isla Santiago, on 38 males/27 females of *C. leleuproum* from 10 populations on Isla Santa Cruz and on 8 males/1 female of *C. linelli* from 3 populations on Isla San Cristóbal.

Some of our population samples of Isla Santiago presumably contain cases of introgressive hybridization between *C. galapageium* and *C. granatense*. For the purpose of clarity, we have omitted these tentative hybrids from the present analysis. They will be dealt with in detail in another paper (DESENDER, in prep.). These possible cases of hybridization however strengthen our vieuws on speciation sequences in the Galápagos archipelago (DESENDER, in prep.).

All measurements were taken on body parts held horizontal in the field of view at maximal power magnification (6 × to 50 × according to the size of the different characters) using a calibrated ocular on a WILD M5 binocular microscope. The measurements are depicted and defined in figs. 1-10, whereas Tables 1-4 provide a list with abbreviations (as used further in this paper) for the same characters as well as for the meristic counts. With the exception of standard total length, all measurements were taken independently of each other to avoid redundancy and statistical problems associated with part/total measures. This large number of characters was investigated in an attempt to describe the size and especially also the shape of most different body parts. We have employed measurements and counts that have been commonly used to study morphological variation in beetles (e.g. LIEBHERR, 1986), as well as characters less frequently used, such as genitalia morphometrics and meristic counts on setation frequencies.

Table 1
List of external morphological measurements (cfr. figs. 1-5)

	code
Standard total length	STL
Head width between the eyes	HWE
Head width behind the eyes	HWB
Head length	HL
Eye width	EYW
Eye length	EYL
Length third antennomere	AL3
Apical prothoracic width	APW
Maximum prothoracic width	MPW
Basal prothoracic width	BPW
Prothoracic length part 1	PL1
Prothoracic length part 2	PL2
Humeral elytral width	HUW
Maximum elytral width	MEW
Elytral length part 1	EL1
Elytral length part 2	EL2
Profemur width	PFW
Profemur length	PFL
Metafemur width	MFW
Metafemur length	MFL
Trochanter length	TRL
Metatibia length	MTL
Prothoracic depth	PD
Lateral length of abdominal sternites 1-3	L3ST

Table 2
List of genitalia measurements (cfr. figs. 8-10)

Males :	*code*
Penis length	PEL
Penis width chitinous parts (at orificium)	PEWC
Penis width membraneous part (at orificium)	PEWM
Penistip length	PETL
Minimum penis width at basis	PEBW
Females :	
Length of terminal process gonapophysus	GOL
Width of terminal process gonaphysys	GOW

Table 3
List of hind wing and metepisternal morphometrics and counts (cfr. figs. 6-7)

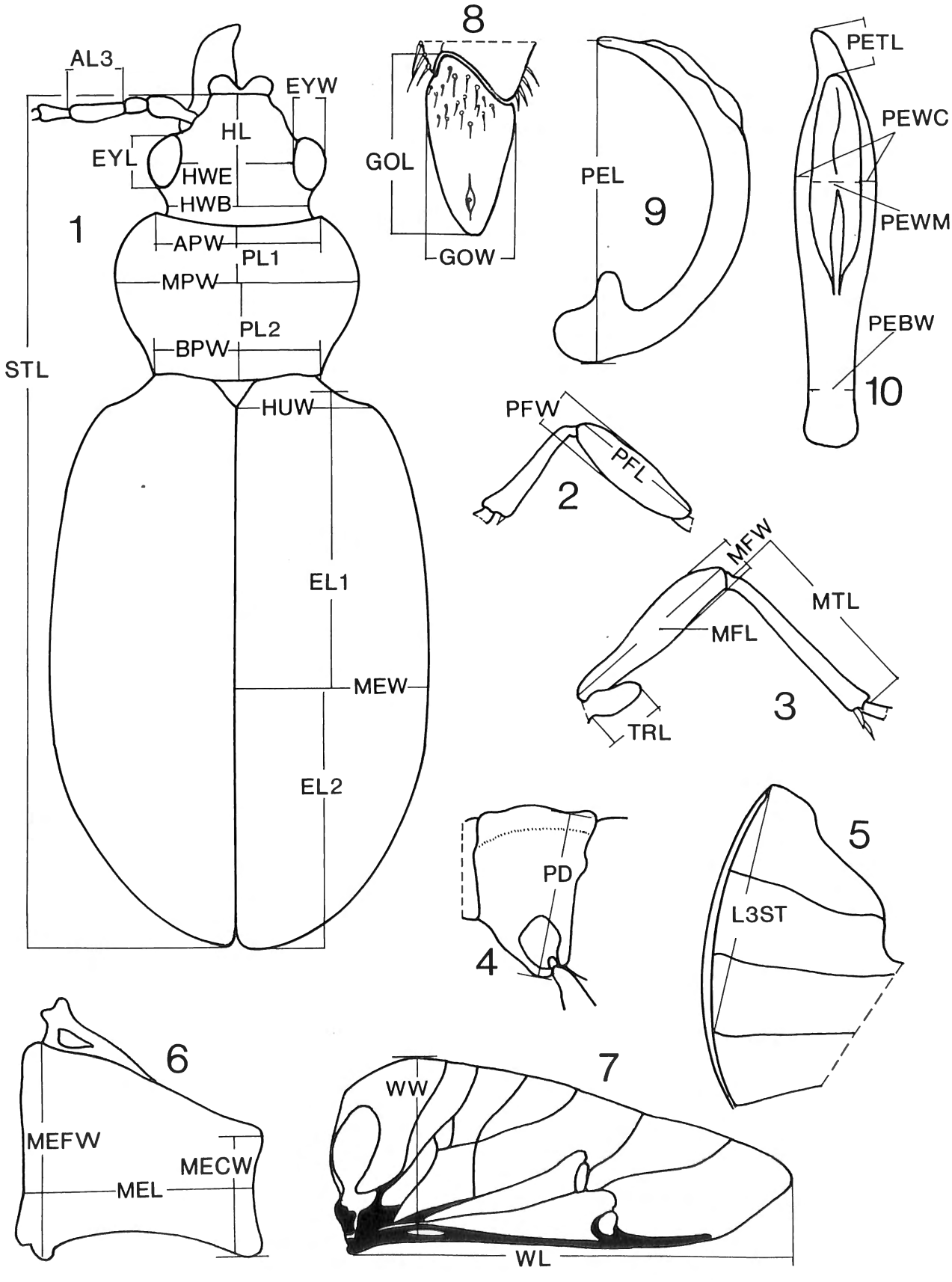
	code
Wing length	WL
Wing width	WW
Metepisternal frontal width	MEFW
Metepisternal caudal width	MECW
Metepisternal length	MEL
Number of punctures on metepisternum	NPME

Table 4
List of meristic counts (number of setae on :)

	code
Mentum	NSME
Left + right labial palp	NSLP
Pronotum	NSP
Mesofemur (external vertral row of setae)	NSMSF
Metafemur (external ventral row of setae)	NSMTF
Trochanter	NSTR
Apical part of last abdominal sternite	NSST

B. *Treatment of the raw data prior to analysis*

Because of a prevailing sexual size dimorphism in most carabid species, and also in these *Calosoma* species (DESENDER & DE DIJN, 1989), we will analyse our data on males and females separatedly.



Figs. 1-10 - *Illustration of the measurements : figs. 1-5 : external morphometrics; fig. 6-7 : hind wing and metepisternal morphometrics; figs. 8-10 : genitalia measurements; see Tables 1-3 for list of abbreviations.*

The morphological measurements were first tested for allometry by regressing them against standard total length. Scatterplots for most external morphometrics against standard total length were best fitted by a linear (isometric) regression. An important exception are the wing development characters : in a previous paper (DESENDER *et al.*, 1986) we were able to show that wing size in Carabid beetles has a negative allometric relationship to body size. To avoid this problem and to make these data independent of individual body size differences, wing measurements were transformed to an index value (% MAX ALL) which corrects for the allometric relationship and enables to compare in a justified way the wing development in beetles of different size (see DESENDER *et al.*, 1986 for more details).

A number of characters, especially those of continuous nature, are expected to be largely dependent on individual body size. Body size (expressed here as standard total length) in itself poses the problem of being influenced to a certain (but mostly unknown) degree by environmental circumstances during (postnatal) ontogeny as well as by prenatal maternal effects (cfr. DESENDER, 1989). Shape characteristics are expected to be less influenced by such effects.

In order to calculate and evaluate the influence of individual body size on the other variables, Ancova (ANalysis of COVAriance) was applied. Table 5 summarizes for all variables in the different sets the mean percentage of the variation (derived from the multiple r^2 values in the Ancova) which is accounted for by standard total length. The continuous measurements (external morphometrics) are indeed strongly dependent on body size, about 70 % of their variation being accounted for by individual differences in standard total length.

Table 5

Summary on Ancova results : mean amount (%) of variation in the different character sets explained by standard total length for males (A) and females (B) (% calculated from the mean multiple r^2 values)

Character set	Number of variables	(A)	(B)
External morphometrics	23	72.4	70.1
Genitalia measurements	5	12.2	
	2		12.6
Meristic counts	7	6.5	7.9
Dispersal power			
MEFW, MECW, MEL	3	75.7	74.1
NPME	1	14.5	12.7

These measurements were then corrected for differences in standard total length. This was not done by expressing the measurements as ratios of body length for several reasons (see also MISRA & NI, 1983) : (1 °) ratios have unusual distributions and are subject to various statistical errors; (2°) the argument against the appropriateness of analyzing ratio data holds even for the case when two variables (say X = STL and Y) are correlated, even if related in an isometric way (as here). A simple linear regression Y = a + b. X (as in our case) would lead to the equation Y/X = a/X + b which shows that the ratio would still be dependent on X. Instead, analysis of covariance was used (see e.g. IHSSEN *et al.*, 1981; MISRA & NI, 1983), adjusting each of the morphometric characters to standard total length according to the formula :

$AM = OM - (RC . (STL - \bar{x}STL))$

where AM is the measurement adjusted for the covariate,

OM is the original measurement,

RC is the overall regression coefficient (common slope) between character and standard total length,

STL is the individual standard total length and

$\bar{x}STL$ is the overall mean standard total length (in our data set = 18.888 mm for males and 19.736 mm for females).

The common slope values, along with their multiple r^2 values (from the Ancova) are given in Table 6 for males and females separatedly.

Table 6

Common slopes (Ancova) and multiple r^2 values for external morphometrics strongly dependent on STL in the male and female data sets (all r^2 values significant at $p<0.001$)

	Males		Females	
	Common slope	Multiple r^2	Common slope	Multiple r^2
HWE	0.0876	0.613	0.0833	0.605
HWB	0.1389	0.797	0.1267	0.733
HL	0.0808	0.736	0.0774	0.716
EYW	0.0279	0.448	0.0241	0.378
EYL	0.0379	0.627	0.0384	0.582
AL3	0.0780	0.766	0.0730	0.779
APW	0.1500	0.721	0.1254	0.626
MPW	0.2540	0.788	0.2335	0.742
BPW	0.1974	0.756	0.1837	0.709
PL1	0.0691	0.473	0.0556	0.353
PL2	0.1097	0.654	0.1123	0.665
HUW	0.2011	0.764	0.1923	0.731
MEW	0.2411	0.882	0.2406	0.824
EL1	0.3592	0.705	0.3780	0.731
EL2	0.2971	0.586	0.3068	0.592
PFW	0.0576	0.581	0.0507	0.642
PFL	0.2195	0.841	0.2156	0.847
MFW	0.0565	0.636	0.0488	0.643
MFL	0.3130	0.879	0.3019	0.849
TRL	0.0966	0.817	0.0951	0.820
MTL	0.3233	0.829	0.3218	0.830
PD	0.2661	0.882	0.2584	0.851
L3ST	0.2979	0.886	03132	0.872
MEFW	0.1468	0.801	0.1511	0.799
MECW	0.0730	0.722	0.0776	0.697
MEL	0.1830	0.747	0.1866	0.726

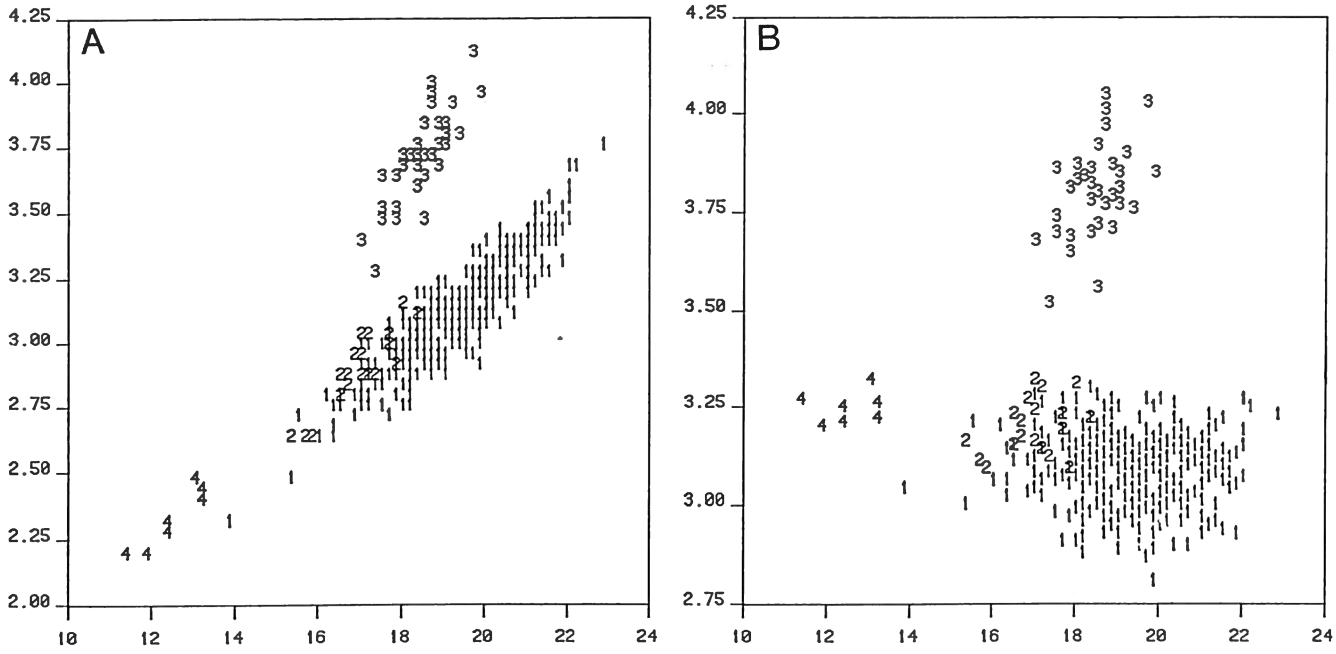


Fig. 11 - *Plot of HWB (in mm) versus STL in mm) : A. raw HWB data; B. Ancova-transformed HWB data; 1* = Calosoma granatense, *2* = C. galapageium, *3* = C. leleuporum, *4* = C. linelli.

Using the above-mentioned fomula transforms the raw data as to make them independent of body size differences. The effectiveness of this transformation is illustrated in fig. 11 for an example character (HWB). The raw data are clearly dependent on standard total length (fig. 11A) and one can see that species 3, although possessing a relatively wider head, cannot be distinguished on the basis of this character alone. The transformed head width measurements plotted against the original standard total length data (fig. 11B) clearly show the appropriateness of the transformation : species 3 possesses a distinctly larger head width (Ancova-transformed) as compared to the other species. As already mentioned, meristic characters, as well as genitalia morphometrics, were nearly completely independent of standard total length (cfr. Table 5) and were thus used in the analyses without prior transformations. Test runs with these characters Ancova-transformed indeed show these do not change the interpretation of the derived discriminant functions. Mean values (and their standard deviations) are tabulated for each species (males and females separatedly) and for all variables as used in the discriminant analyses (i.e. raw data or Ancova-transformed values according to the set) in Table 7.

Rather surprisingly, male genitalia size characteristics were almost independent of individual body size. This could imply that, due to their possible role in species recognition (sexual selection), they are severely constrained by selection as to their individual variation in size.

C. *Stepwise discriminant function analysis*

Discriminant analysis is a statistical technique which allows the researcher to study differences between two or more groups of objects with respect to several variables simultaneously (KLECKA, 1980). The basic prerequisites are that two or more groups exist which we presume differ on several variables and that those variables can be measured at the interval or ratio level. Discriminant analysis will then help us analyze the differences between the groups ("interpretation") and/or provide us with a means to assign any case into the group which it most closely resembles ("classification").

The first part of the analysis answers the question whether the groups can be discriminated on the basis of some set of characteristics, how well these discriminate and which characteristics are the most powerful discriminators. Mathematically, a canonical discriminant function is a linear combination of the discriminating variables with their associated discriminant function coefficients and a constant term. These coefficients are derived so that the group means on the function are as different as possible. In the second part of the analysis one or more mathematical equations can be derived for the purpose of classification. These equations combine the groups characteristics in a way that will allow one to identify the group which a case most closely resembles. In some research settings, we may also have cases which are not identified a-priori. These can then be included in the classification phase of the analysis and be assigned to the most probable group.

The basic assumptions of discriminant analysis (KLECKA, 1980) can be stated as :

(1°) at least two a-priori groups,
(2°) at least two cases per group,
(3°) any number of discriminating variables, provided that it is less than the total number of cases minus two,
(4°) no discriminating variable may be a linear combination of other discriminating variables,
(5°) approximately equal covariance matrices for each group,

Table 7
Mean and Standard Deviation ($\bar{x}$(SD)) for all variables in the different sets as used in the discriminant function analyses for males and females in the different species : 1 : Calosoma granatense, *2 :* C. galapageium, *3 :* C. leleuporum, *4 :* C. linelli*; 1st set in mm; 2nd set, in mm, Ancova-transformed; 3rd set : % MAX ALL : percentage; MEFW, MECW, MEL : in mm, Ancova-transformed; NPME : number of punctures; 4th set : number of setae.*

Species Number of ind. Character	1 ♂♂ 376	1 ♀♀ 367	2 ♂♂ 19	2 ♀♀ 13	3 ♂♂ 38	3 ♀♀ 27	4 ♂♂ 8	4 ♀♀ 1
PEL	4.06(.14)		4.14(.10)		4.54(.18)		3.15(.11)	
PEWC	.20(.03)		.22(.03)		.23(.03)		.12(.01)	
PEWM	.42(.03)		.49(.02)		.55(.03)		.30(.02)	
PETL	.42(.04)		.24(.03)		.34(.03)		.39(.02)	
PEBW	.39(.02)		.38(.01)		.41(.02)		.30(.01)	
GOL		.96(.06)		1.00(.04)		1.05(.05)		.66(–)
GOW		.50(.03)		.45(.02)		.50(.02)		.38(–)
HWE	2.46(.09)	2.49(.08)	2.61(.05)	2.65(.06)	3.12(.10)	3.24(.03)	2.38(.03)	2.31(–)
HWB	3.10(.09)	3.13(.09)	3.20(.07)	3.25(.08)	3.80(.12)	3.97(.03)	3.24(.04)	3.18(–)
HL	2.22(.06)	2.25(.06)	2.39(.03)	2.42(.05)	2.41(.07)	2.42(.05)	2.08(.05)	2.07(–)
EYW	.73(.04)	.72(.04)	.78(.02)	.77(.03)	.79(.04)	.78(.04)	.70(.02)	.67(–)
EYL	1.22(.04)	1.21(.04)	1.23(.02)	1.24(.03)	1.32(.04)	1.33(.04)	1.13(.02)	1.11(–)
AL3	1.43(.06)	1.43(.05)	1.44(.03)	1.41(.03)	1.30(.05)	1.27(.04)	1.36(.03)	1.34(–)
APW	3.54(.12)	3.53(.11)	3.65(.09)	3.65(.12)	4.20(.12)	4.33(.17)	3.59(.07)	3.45(–)
MPW	5.40(.16)	5.44(.17)	5.46(.14)	5.53(.14)	5.70(.13)	5.74(.18)	5.90(.20)	5.82(–)
BPW	3.46(.15)	3.53(.14)	3.42(.10)	3.51(.10)	3.48(.12)	3.53(.12)	4.00(.12)	4.02(–)
PL1	1.38(.09)	1.36(.09)	1.35(.06)	1.28(.05)	1.23(.11)	1.27(.07)	1.57(.10)	1.47(–)
PL2	2.24(.10)	2.26(.10)	2.30(.07)	2.36(.08)	2.25(.11)	2.23(.09)	2.52(.08)	2.55(–)
HUW	3.22(.15)	3.34(.14)	3.03(.08)	3.16(.11)	3.02(.12)	3.09(.08)	3.57(.18)	3.53(–)
MEW	4.23(.11)	4.49(.14)	3.96(.08)	4.26(.12)	4.04(.11)	4.26(.12)	4.37(.07)	4.63(–)
EL1	6.60(.29)	7.01(.28)	6.19(.34)	6.73(.16)	6.06(.24)	6.38(.24)	5.17(.18)	6.01(–)
EL2	5.43(.32)	5.80(.31)	5.53(.37)	5.85(.19)	5.43(.16)	5.79(.26)	6.46(.18)	6.50(–)
PFW	1.33(.06)	1.18(.05)	1.54(.06)	1.28(.05)	1.54(.06)	1.29(.05)	1.46(.07)	1.30(–)
PFL	4.33(.13)	4.29(.11)	4.36(.07)	4.29(.09)	4.27(.10)	4.13(.09)	4.34(.07)	4.19(–)
MFW	1.28(.06)	1.20(.04)	1.37(.05)	1.25(.05)	1.38(.05)	1.27(.03)	1.38(.05)	1.23(–)
MFL	5.94(.15)	6.06(.16)	6.10(.10)	6.16(.09)	5.89(.15)	5.91(.13)	6.14(.10)	6.05(–)
TRL	1.80(.06)	1.82(.05)	1.86(.04)	1.87(.05)	1.84(.05)	1.82(.05)	1.89(.04)	1.95(–)
MTL	6.01(.19)	6.13(.18)	6.18(.14)	6.24(.12)	5.96(.17)	5.96(.13)	6.30(.11)	6.27(–)
PD	5.13(.13)	5.23(.13)	5.12(.12)	5.22(.09)	5.09(.11)	5.17(.09)	5.56(.05)	5.57(–)
L3ST	5.22(.14)	5.60(.15)	5.21(.08)	5.59(.10)	5.03(.12)	5.32(.11)	5.33(.09)	5.80(–)
% MAX ALL	107.88(24.61)	106.37(24.54)	15.74(3.11)	20.22(10.44)	13.50(4.52)	14.40(5.68)	1.50(.47)	2.32(–)
MEFW	2.36(.10)	2.47(.09)	2.25(.08)	2.40(.09)	2.20(.07)	2.28(.07)	2.47(.05)	2.71(–)
MECW	1.34(.06)	1.43(.06)	1.31(.05)	1.42(.06)	1.34(.06)	1.46(.05)	1.37(.03)	1.47(–)
MEL	2.39(.14)	2.46(.14)	2.08(.08)	2.24(.22)	1.90(.09)	1.91(.09)	2.27(.10)	2.40(–)
NPME	18.85(8.84)	18.74(9.21)	2.00(3.65)	5.85(5.97)	10.87(7.04)	10.82(8.03)	.00(.00)	.00(–)
NSME	1.42(.85)	1.41(.88)	.89(.88)	.85(.90)	.87(1.02)	.56(.80)	.00(.00)	.00(–)
NSLP	7.49(1.11)	7.49(1.09)	7.41(1.04)	7.46(1.14)	8.50(1.13)	7.67(1.27)	4.75(.87)	6.00(–)
NSP	3.16(1.46)	3.12(1.52)	4.26(.76)	4.00(.00)	4.26(.86)	4.18(.89)	.00(.00)	1.00(–)
NSMSF	14.65(1.99)	14.96(2.05)	10.95(1.27)	11.39(1.33)	12.21(1.36)	11.85(1.26)	8.25(1.17)	9.00(–)
NSMTF	9.52(1.70)	9.39(1.75)	7.26(1.10)	7.54(1.39)	7.63(1.28)	7.26(1.16)	6.00(.93)	4.00(–)
NSTR	.05(.27)	.14(.47)	.11(.32)	.23(.60)	.00(.00)	.22(.58)	.00(.00)	.00(–)
NSST	4.14(1.05)	5.44(1.50)	4.21(.54)	6.23(1.42)	5.24(1.70)	6.93(1.86)	3.75(.71)	6.00(–)

(6°) each group has been drawn randomly from a "population" with a multivariate normal distribution on the discriminating variables.

In practice the technique is very robust and the two last-mentioned assumptions need not be strongly adhered to (KLECKA, 1975).

Initial species recognition for a-priori designations in our analyses was based on our previous redescriptions, along with ecological (habitat preference) and distributional data. The technique used in this paper derives the canonical discriminant functions (which are a linear combination of the discriminating variables) in a stepwise procedure based on Rao's V, a generalized distance measure. Each variable selected is the one which contributes the largest increase in V when added to the previously selected variables. This amounts to the greatest overall separation of the groups (which are the different species in our data set). Variables which do not further improve the separation are then disregarded from the analysis. In our analyses all or nearly all variables were however used by the stepwise procedure. I.o.w., our results will be very comparable to those obtained from procedures with direct entry of all variables in the analysis. For statistical and interpretative reasons the number of discriminant functions was set to a maximum of three. Test runs, in which the derivation of more functions was allowed, had shown that these additional functions, although sometimes significant, did not improve much the interpretation or the classification and that they possessed only low canonical correlation coefficients. All analyses were performed on a Siemens 7570-C mainframe computer of the State University Ghent, using SPSS.X statistics software to process the data (ANONYMOUS, 1988). Standard results given in this paper will be limited to the significant discriminant function(s) (with associated eigenvalue(s) and relative discriminating power (%) and their canonical correlation coefficients). The canonical correlation coefficients are a measure of association summarizing the relatedness between the groups (species) and the discriminant function. Their squared values give the proportion of variation in the discriminant function explained by the groups. Furthermore we will show the structure matrix composed of the total structure coefficients. These are the product-moment correlation values between each variable and the discriminant functions. On the basis of this matrix the most pertinent discriminators can be identified for the separation of the a-priori groups (species). We can "name" a function on the basis of the structure coefficients by noting the variables having the highest coefficients. If those variables seem to measure a similar characteristic, we could name the function after that characteristic. Graphical results (two-function plots) will portray the discriminant scores for each individual case on the most important and significant discriminant functions. The discriminant score for a given case represents the position of that case along the continuum (axis) defined by that function. Classification results will tabulate the predicted species to which the cases most likely belong and are obtained by comparing the case's position to each of the group centroids in order to locate the "closest" one. Centroids (added on the two-function plots) summarize the position of a species on the derived discriminant functions and have coordinates that are the species' mean on each of the variables. Because our main goal is to identify the a-priori species exactly, a classification will be perfect only when all cases are assigned to their a-priori species. For more information on canonical discriminant function analyses we refer to KLECKA (1980).

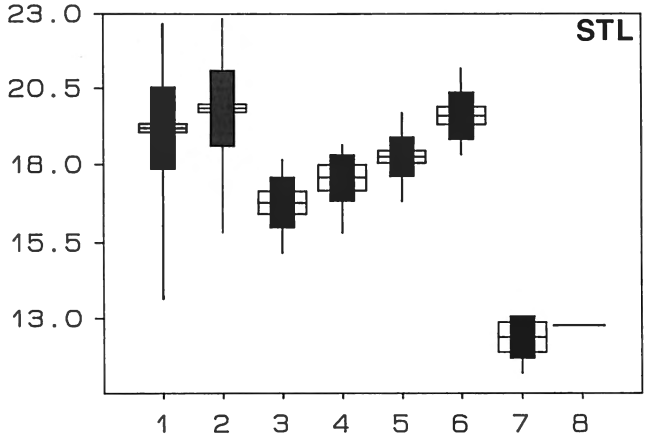


Fig. 12 - *Dice-Leraas diagram for STL in the 4* Calosoma *species : 1,2 = males, resp. females* Calosoma granatense, *3, 4* = C. galapageium, *5, 6* = C. leleuporum, *7, 8* = C. linelli*; range, mean, 95 % c.l., and one standard deviation-limits (black column) added.*

Results and Discussion

1. *Standard total length*

As already mentioned STL was mainly used as a character in order to standardize other morphometrics for an easier comparison of shape characteristics. Fig. 12 portrays for each species and for males and females separatedly the obtained values for STL in Dice-Leraas diagrams. Obviously, there is a high individual variability in STL, especially in *Calosoma granatense*. By means of STL, only *C. linelli* can be distinguished with certainty from the other species : STL ranges in that species from 11.17 to 13.00 mm, whereas minimum STL in the other species is 13.67 mm. A sexual dimorphism in STL can be observed in each species, males in the mean being smaller than females.

2. *Genitalia morphometrics*

Results of the discriminant analysis based on the five male genitalia metrics are summarized in Table 8, whereas individual discriminant scores and species centroids are plotted for the first two functions in Fig. 13. From the analysis (Table 8A) we can deduce that only the first two derived functions are important, accounting for most of the variation between species. Moreover, the canonical correlation value for the third function is low. The structure matrix

(Table 8B) indicates that the first function can be defined as the width of the penis at the orificium (PEWM), whereas the second function corresponds to PETL, PEL and PEWB. The two-function plot of the individual discriminant scores (Fig. 13) shows a good separation between the species, Species 2 and 3 load positively and species 4 negatively on the first function, whereas species 2 and 4 load negatively on the second function. In view of the obtained structure matrix and the measurements (depicted along with their range in Dice-Leraas diagrams in Fig. 14) the discriminant functions are easily interpreted. Species 2 and 3 have wider, species 4 narrower genitalia at the orificium; the separation of species 2 on the second axis is mainly due to a very low PETL, whereas species 4 shows a combination of a low PEL and PEBW. The classification results (Table 8c) show that nearly all individuals are classified within their a-priori species based on this analysis. The few misclassifications between species 1, 2 and 3 disappear when only comparing species co-occurring on separate islands. This is an indication of reproductive character displacement which will be more elaborated in a future paper (DESENDER, in prep.).

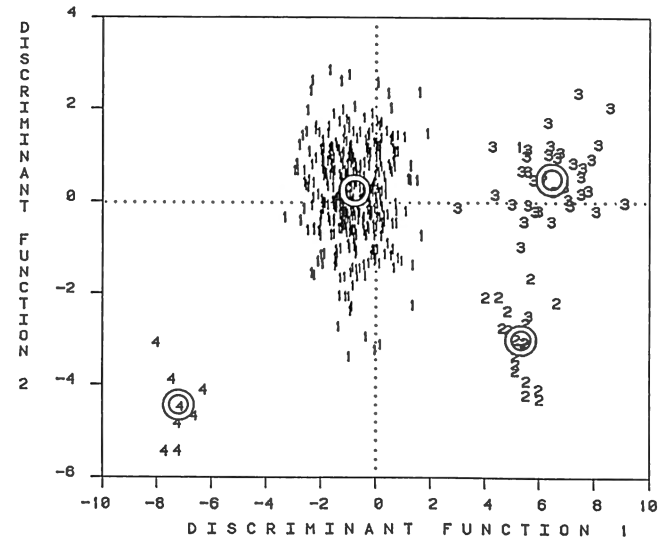


Fig. 13 - *Two function plot of discriminant scores for all individual cases based on male genitalia metrics. Centroid location for each species indicated by circles; species codes : cfr. legend fig. 11.*

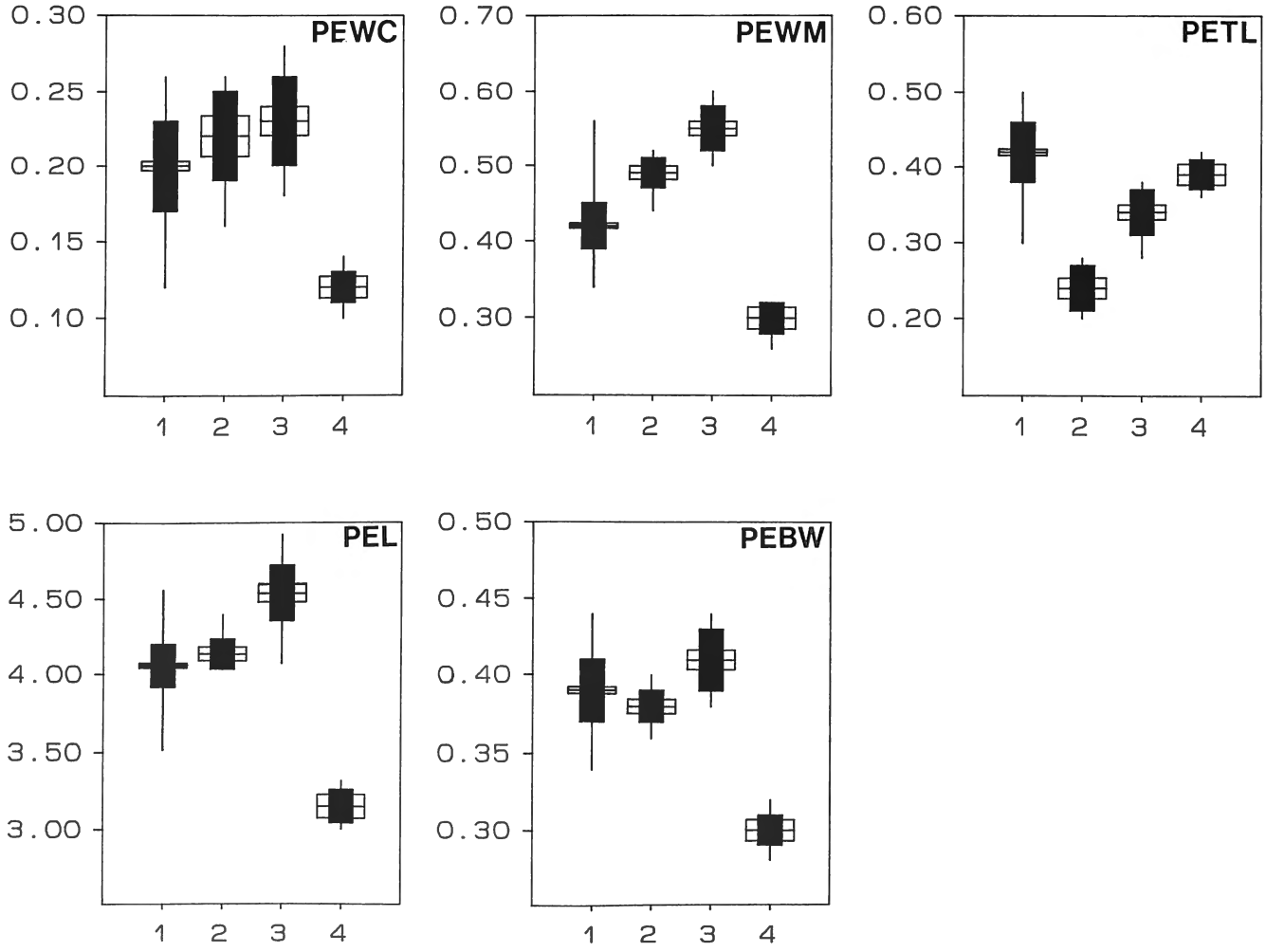


Fig. 14 - *Dice-Leraas diagrams for male genitalia metrics (see legend fig. 11 for species codes); range, mean, 95 % c.l., and one standard deviation-limits (black collumn) added.*

Diagnostic male genitalia metrics are thus a small PETL for *C. galapageium*, a small PEL, PEWM as well as PEBW for *C. linelli* and the combination of a high PEWM and PEWC to distinguish *C. leleuporum* from *C. granatense*.

Table 8
Results of canonical discriminant analyses on MALE GENITALIA metrics : (A) Analysis : significant discriminant functions, associated eigenvalues and canonical correlation, (B) Structure matrix = pooled within-species correlations between discriminating variables and canonical discriminant functions (variables ordered by size of correlation), (C) Classification results.

(A) Function	Eigenvalue	% of variance	Canonical correlation
1	6.327	87.41	0.929
2	0.823	11.37	0.672
3	0.088	1.22	0.285

(B) Variable	Function 1	Function 2	Function 3
PEWM	0.568 *	0.266	0.418
PETL	−0.333	0.758 *	0.398
PEL	0.449	0.692 *	−0.090
PEBW	0.118	0.581 *	−0.498
PEWC	0.215	0.252	−0.443

(C)	Predicted species membership :			
	Species 1	Species 2	Species 3	Species 4
Actual group :				
Species 1	*375*	0	1	0
Species 2	0	*18*	1	0
Species 3	1	1	*36*	0
Species 4	0	0	0	*8*

Percent of "grouped" cases correctly classified : 99.09 %.

Table 9
Results of canonical discriminant analyses on FEMALE GENITALIA metrics : (A) Analysis, (B) Structure matrix, (C) Classification results (see legend Table 8 for further explanation)

(A) Function	Eigenvalue	% of variance	Canonical correlation
1	0.309	66.76	0.486
2	0.154	33.24	0.365

(B) Variable	Function 1	Function 2
GOL	0.708 *	0.706
GOW	−0.318	0.948 *

(C)	Predicted species membership :			
	Species 1	Species 2	Species 3	Species 4
Actual group :				
Species 1	*365*	1	1	0
Species 2	7	*6*	0	0
Species 3	20	2	*5*	0
Species 4	0	0	0	*1*

Percent of "grouped" cases correctly classified : 92.40 %.

Results of the discriminant analysis based on the two female genitalia metrics are summarized in Table 9. Obviously the species are not very well discriminated by these characters, the only exception being *C. linelli* (but with only one female at hand). The classification results show a large number of misclassifications. The mean values for GOL and GOW (Table 7) show indeed a large degree of overlap between species 1, 2 and 3. Female genitalia metrics thus do not aid very much in species recognition.

3. *External morphometrics*

Results of the discriminant analysis based on the 23 external morphometrics (Ancova-transformed) for males are summarized in Table 10, whereas individual discriminant scores and species centroids are plotted for the first two functions and for function 1 and 3 in Fig. 15. From the analysis (Table 10A) we can deduce that the three derived functions are important, with high canonical correlation values. The structure matrix (Table 10B) indicates that the first function can be defined as the relative width of the forebody (HWB, HWE, APW), whereas the second function corresponds in a positive way to EL1 and in a negative way to MPW, BPW,PD. The third function corresponds positively to HL and PFW, and negatively to MEW. The two-function plots of the individual discriminant scores (Fig. 15) show a good separation between the species. Species 3 is separated on function 1, species 4 on function 2 and species 2 on function 3. In view of the obtained structure matrix and the measurements (depicted for males and for females in Dice-Leraas diagrams in Fig. 16) the discriminant functions are again rather easily interpreted. *C. leleuporum* has a relatively wider forebody, *C. linelli* a relatively broader and deeper prothorax and more ovoid elytra, with maximal elytral width situated closer to the basis of the elytra (smaller EL1). The separation of *C. galapageium* on the third function is mainly due to its narrower elytra, longer head and broader profemur (as compared to *C. granatense* and *C. linelli*) in combination with a narrow forebody (as compared to *C. leleuporum*). The classification results (Table 10C) show a perfect classification. Results for the female data set were comparable and also yielded a completely exact classification table. Therefore, we restrict the presentation of female results to the Ancova-transformed range diagrams (Fig. 16).

As can be observed from these diagrams several characters show a distinct sexual dimorphism, which means that they are differently shaped in males as in females (these values are indeed made independent of STL prior to analysis): females possess a relatively larger (longer: EL1, EL2, L3ST and wider: HUW, MEW) hind body, whereas males possess relatively wider legs (especially PFW) than females. Both sets of characters most probably are related in some way to different reproductive needs: a larger hind body in females could enhance the possibilities for egg production and accomodation, whereas stouter forelegs in males could be functionally related to copulation behaviour and/or enhaced locomotory activity in the search of a copulation partner.

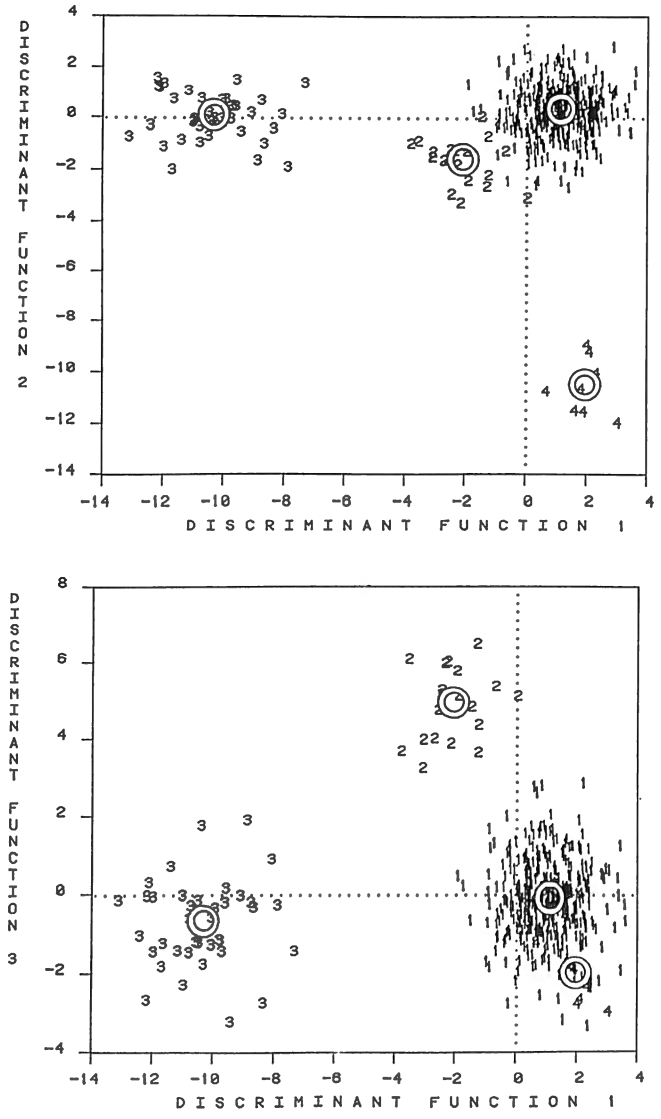


Fig. 15 - *Two function plot of discriminant scores for all individual cases on function 1 and 2 (upper figure) and function 1 and 3 (lower figure) based on 23 external morphometrics (see legend fig. 13 for further explanation).*

Table 10
Results of canonical discriminant analyses on male EXTERNAL morphometrics (Ancova-transformed): (A) Analysis, (B) Structure matrix, (C) Classification results (see legend Table 8 for further explanation)

(A) Function	Eigenvalue	% of variance	Canonical correlation
1	10.559	75.54	0.956
2	2.257	16.15	0.832
3	1.162	8.31	0.733

(B) Variable	Function 1	Function 2	Function 3
HWB	– 0.677 *	– 0.159	– 0.267
HWE	– 0.668 *	0.035	– 0.046
APW	– 0.482 *	– 0.054	– 0.128
EYL	– 0.262 *	0.185	0.006
L3ST	0.124 *	– 0.070	0.031
EL1	0.152	0.462 *	– 0.001
MPW	– 0.156	– 0.332 *	– 0.144
BPW	0.005	– 0.319 *	– 0.179
PD	0.039	– 0.300 *	– 0.110
EL2	0.009	– 0.293 *	– 0.047
PL2	– 0.004	– 0.259 *	0.014
MFW	– 0.173	– 0.218 *	0.159
TRL	– 0.055	– 0.167 *	0.117
PL1	0.154	– 0.166 *	– 0.049
MTL	0.022	– 0.166 *	0.132
HL	– 0.296	0.122	0.415 *
MEW	0.171	– 0.036	– 0.398 *
PFW	– 0.309	– 0.271	0.381 *
HUW	0.144	– 0.168	– 0.251 *
AL3	0.190	0.094	0.197 *
MFL	0.025	– 0.157	0.179 *
EYW	– 0.144	0.023	0.151 *
PFL	0.031	– 0.065	0.088 *

(C)	Predicted species membership:			
	Species 1	Species 2	Species 3	Species 4
Actual group:				
Species 1	*376*	0	0	0
Species 2	0	*19*	0	0
Species 3	0	0	*38*	0
Species 4	0	0	0	*8*

Percent of "grouped" cases correctly classified: 100 %.

4. *Dispersal power characters*

Results of the discriminant analysis based on dispersal power characters for males are summarized in Table 11, whereas individual discriminant scores and species centroids are plotted in Fig. 17. From the analysis (Table 1A)

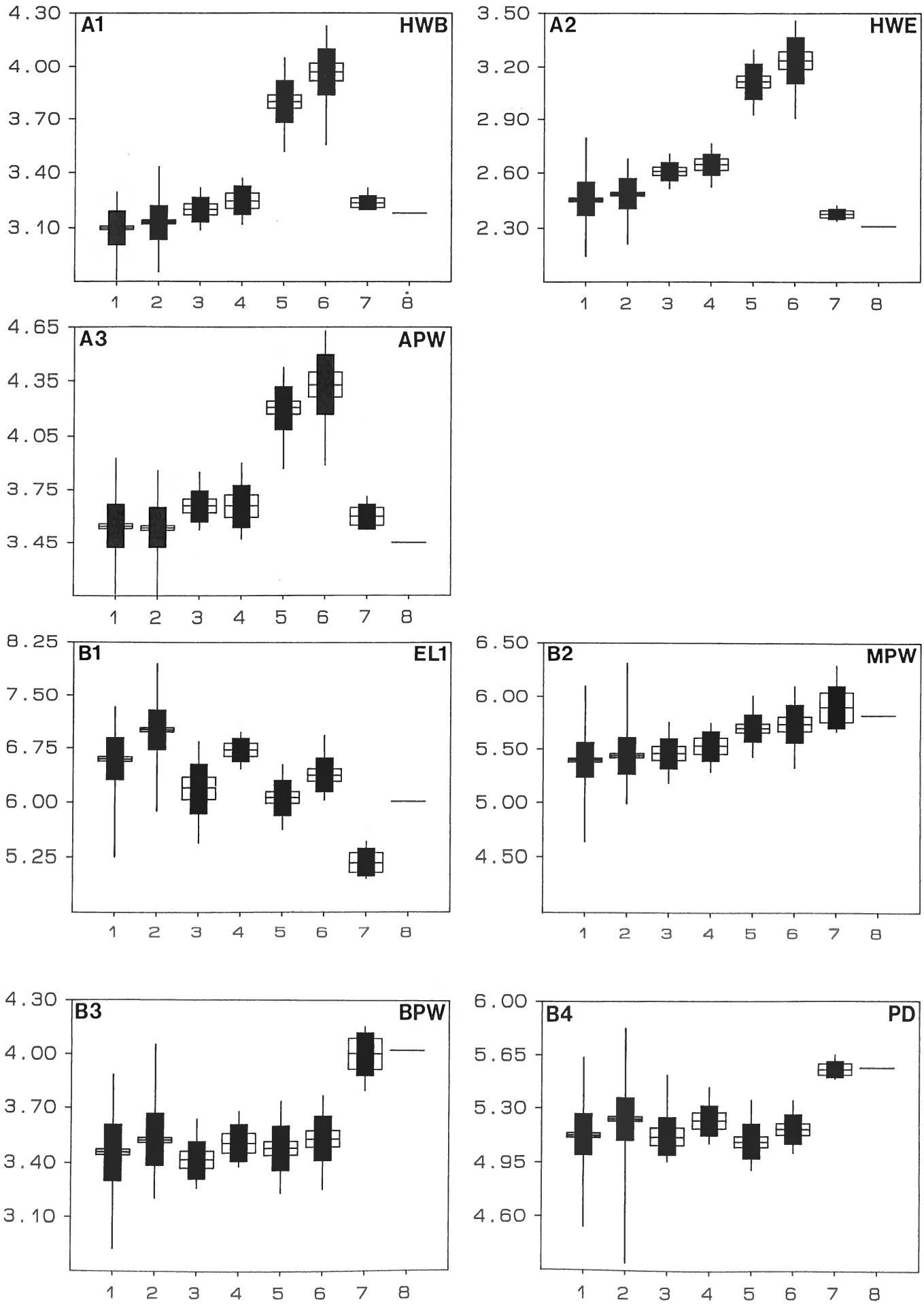
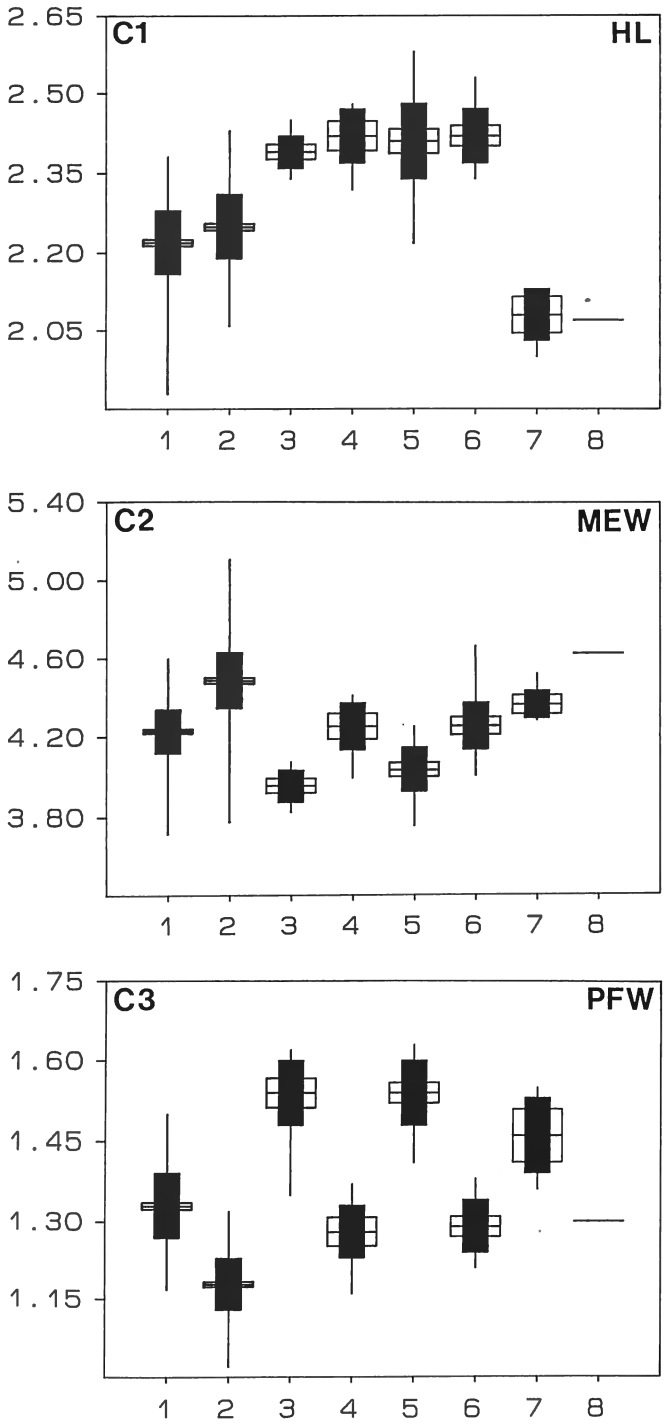


Fig. 16 - *Dice-Leraas diagrams for selected Ancova-transformed external morphometrics (see legend fig. 12 for further explanation) : A, B or C : characters related to respectively discriminant function 1, 2 or 3.*



we can deduce that the first derived function is very important with a high canonical correlation value. The structure matrix (Table 11B) shows this function to be defined by % MAX ALL, which is the index describing relative wing development. The plot of individual discriminant scores shows that especially species 1 is well separated from the others. Dice-Leraas diagrams (Fig. 18) show the prevalence of reduced wing development in *C. galapageium*, *C. leleuporum* and *C. linelli* as compared to the wing polymorphic *C. granatense*. Female results (also portrayed on Fig. 18) are again very comparable to those of males. The classification results (Table 11C) show a small number of misclassifications, especially between *C. galapageium* and *C. leleuporum*.

Table 11
Results of canonical discriminant analyses on the male DISPERSAL POWER data set : (A) Analysis, (B) Structure matrix, (C) Classification results (see legend Table 8 for further explanation)

(A) Function	Eigenvalue	% of variance	Canonical correlation
1	2.587	87.86	0.849
2	0.319	10.86	0.492
3	0.038	1.29	0.191

(B) Variable	Function 1	Function 2	Function 3
% MAX ALL	0.923 *	– 0.031	0.319
MEL	0.637	0.687 *	0.257
MEFW	0.265	0.613	0.711 *
NPME	0.309	– 0.327	0.639 *
MECW	– 0.004	0.036	0.558 *

(C)	Predicted species membership :			
	Species 1	Species 2	Species 3	Species 4
Actual group :				
Species 1	*371*	3	2	0
Species 2	0	*15*	3	1
Species 3	0	3	*35*	0
Species 4	0	0	0	*8*

Percent of "grouped" cases correctly classified : 97.28 %.

5. *Meristic counts*

Results of the discriminant analysis based on meristic counts for males are summarized in Table 12, whereas individual discriminant scores and species centroids are plotted in Fig. 19. Only the first and second derived functions yield more or less high canonical correlation values (Table 12A). The first function is correlated to an increasing setation of the legs (Table 12B) being higher for species 1 as compared to the other species (cfr. Table 7). *C. linelli* shows the lowest values for these characters and indeed loads negatively on the first discriminant function (Fig. 19). The second function is positively related to the number of setae on the labial palp and pronotum (Table 12B) : *C. linelli* shows reduction in the number of these setae (cfr. Table 7) and indeed again loads negatively on the function. *C. galapageium* and *C. leleuporum* load positively because they possess in the mean a higher number of these setae as compared to *C. granatense*. Results for the female data set are again very similar to those of the males. The classification results (Table 12C) show a large number of misclassifications, especially between species 1, 2 and 3. This can also be deduced from Fig. 19 and means that the meristic counts which were used here do not aid very much in species identification. In our detailed

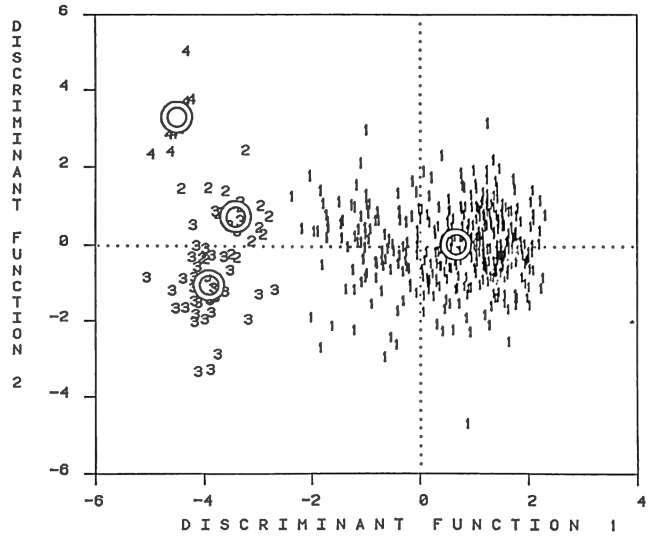


Fig. 17 - *Two function plot of discriminant scores for all individual cases based on male dispersal power metrics (see legend fig. 13 for further explanation).*

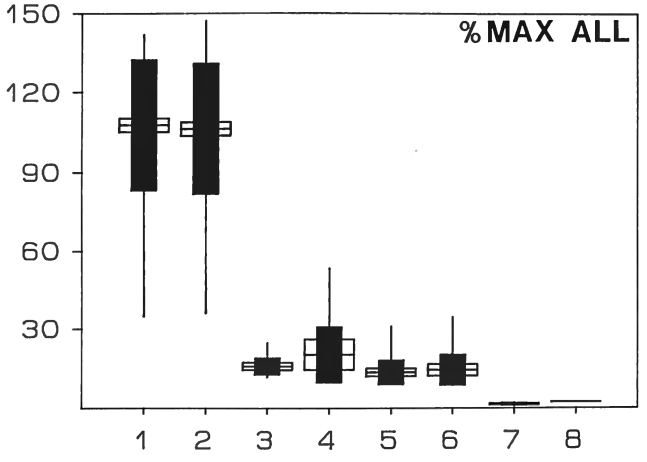


Fig. 18 - *Dice-Leraas diagram for % MAX ALL (see legend fig. 12 for further explanation).*

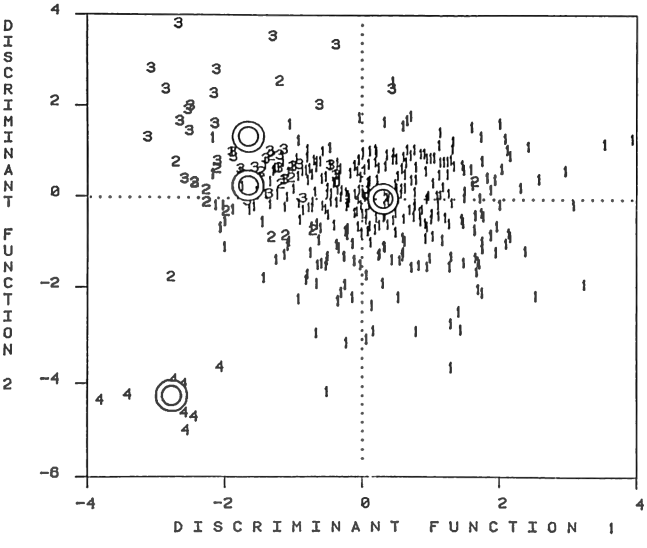


Fig. 19 - *Two function plot of discriminant scores for all individual cases based on male meristics; centroids from top to bottom : species 3, 2, 1 and 4 (see legend fig. 13 for further explanation).*

redescriptions of the species (Desender & De Dijn, 1989) we came already to the same conclusion. Moreover, we recognized a lot of cases of asymmetry in these characteristics, added to the already pronounced individual variation in setation.

Table 12
Results of canonical discriminant analyses on MERISTIC COUNTS for males : (A) Analysis, (B) Structure matrix, (C) Classification results (see legend Table 8 for further explanation)

(A) Function	Eigenvalue	% of variance	Canonical correlation
1	0.579	52.19	0.606
2	0.490	44.18	0.574
3	0.040	3.63	0.197

(B) Variable	Function 1	Function 2	Function 3
NSMSF	0.850 *	0.202	– 0.389
NSMTF	0.645 *	0.050	– 0.149
NSME	0.377 *	0.105	0.126
NSLP	– 0.001	0.695 *	– 0.331
NSP	– 0.107	0.637 *	0.595
NSST	– 0.222	0.287	– 0.549 *
NSTR	0.035	– 0.008	0.351 *

(C)	Predicted species membership :			
	Species 1	Species 2	Species 3	Species 4
Actual group :				
Species 1	*370*	0	5	1
Species 2	12	*4*	3	0
Species 3	19	0	*19*	0
Species 4	0	0	0	*8*

Percent of "grouped" cases correctly classified : 90.93 %.

6. *Identification key for the* Calosoma *species of Galápagos*

The following key is designed to identify males as well as females of the different *Calosoma* species, occurring in the Galápagos archipelago, and uses a minimal number of reliable characters. Where necessary, parsimonious discriminant functions are given for aid in the identification. These steps in the dichotomous key were obtained after separate discriminant analysis runs based on a minimal number of pertinent discriminating morphometrics (as deduced from our detailed analysis above) between species groups or pairs. The characters were moreover now selected for their ease and precision of measurement and therefore also used as raw (untransformed) morphometrics (in mm). This should augment their utility in identification with a minimum of necessary calculations. In these cases

we will mention the formula to be used to obtain the discriminant function score (d.f. score) for any individual case as well as the decision cut level for species or species group designations (for males and females separatedly).

1. – Forebody relatively larger : d.f. score A (for males = – 9.156 + 10.686. (head width between the eyes) – 0.939. (standard total length)) always larger than 3.50 (mean = 6.47); d.f. score B (for females = – 11.394 + 11.693. (HWE) – 0.931. (STL)) always larger than 3.50 (mean = 8.12); restricted in its occurrence to the highlands and top of isla Santa Cruz . . . . . *C. leleuporum*
   – Forebody narrower : d.f. score A less than 3.50 (mean = – 0.61); d.f. score B less than 3.50 (mean = – 0.58) 2
2. – Standard total length less than 13.5 mm; hind wings extremely reduced to a small rudiment (always shorter than 3 mm) without venation; restricted in its occurrence to the highlands of Isla San Cristóbal . . . . . *C. linelli*
   – Always longer than 13.5 mm; hind wings sometimes reduced but always with a relatively long rudiment (> 3 mm) with distinct venation . . . 3
3. – Males with penistip length less than 0.30 mm (mean = 0.24 mm); externally only distinguishable by a combination of characters : d.f. score C (for males = 4.103 + 5.524. (maximal elytral width) – 6.730. (head length) – 9.260. (profemur width)) always less than – 3.00 (mean = – 4.96); d.f. score D (for females = 2.901 + 5.496. (MEW) – 7.578. (HL) – 8.819. (PFW)) less than – 3.00 (mean = – 3.90); restricted in its occurrence to the highlands and top zone of Isla Santiago, but probably showing some introgressive hybridization with *C. granatense* . *C. galapageium*
   – Males with PETL always longer than 0.30 mm (mean = 0.42 mm); d.f. score C larger than – 3.00 (mean = 0.25); d.f. score D larger than – 3.00 (mean = 0.14); widely distributed, especially occurring in the dry arid and lower transition zone of nearly all islands of the Galápagos archipelago; sometimes also at higher altitude (Fernandina and all separate volcanoes of Isabela) . . . . . *C. granatense*

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