

# Identification and discrimination of *Electrogena* species by numerical methods (Ephemeroptera: Heptageniidae)

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**Abstract.** Nine species of *Electrogena* from thirty-one populations were investigated on the basis of five meristic and five ratio characters of the larvae. The attribution of populations to species was checked by a cluster analysis based on the generalized distances between pairs. Groups representing species were then subjected to a multiple discriminant analysis; discriminant functions and loadings were calculated. The correct attribution of individuals to species by the discriminant functions ranged from 97% to 100%. The analysis of discriminant loadings shows which characters contribute more to the discrimination of species. Although many species could be identified with some confidence by a sequential monothetic key based on meristic and qualitative characters, the discriminant analysis improves the effectiveness of identification of all species.

## Introduction

The palaearctic genus *Electrogena* Zurwerra & Tomka includes about thirty species. In recent descriptions the species are poorly defined, and relationships between them have been little investigated. Some trials to construct a system of relationships within the genus were based on arbitrary and, sometimes, contradictory elements, owing to the insufficiency of comparable taxonomic data. The main difficulty is in the poor definition and standardization of characters: every author makes reference to a different set of characters, and reports character states from a subjective point of view. Furthermore, the taxonomy of *Electrogena* is based mostly on adult morphology and coloration, which are very variable within species or contains little useful information for species characterization. Consequently there is great confusion in the nomenclature. It affects the stability of taxa, and makes the identification of most species difficult or quite impossible. At the same time, the increasing ecological study of rivers and the growth of interest in species of naturalistic relevance demand correct identification of taxa, a stable nomenclature and easy recognition of species.

The aim of this work is to present an approach to the taxonomy of the genus *Electrogena*, based on the choice of unequivocally defined characters, which share a high content of diagnostic information, and on numerical elaborations which discriminate between species. Numerical methods can help also to point out the characters which most contribute to the diagnosis, in spite of geographic variability and overlapping of character states. Larvae are preferred for this kind of study over adults. They share a greater number of meristic and measurement characters, and can be collected more easily.

The first problem in the present study was to find measurements

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which gave maximum information for separating taxa. In choosing the more promising set of diagnostic characters an inescapable tool is long experience in the practice of taxonomy. In some cases the ratio of two linear measurements proved to be a useful way to represent the taxonomic differences. The attribution of populations to species was checked with hierarchic agglomerative methods, based on the generalized distances between each pair of populations.

In another step, species were considered as Operational Taxonomic Units (OTUs). A multiple discriminant analysis demonstrated the separation of species and showed which characters contributed with a greater information content to the discrimination.

## Materials and Methods

Ten quantitative characters (Table 1) from a set of sixteen diagnostic ones (Belfiore, 1994, 1995, 1996) were measured in 356 specimens from thirty-one populations belonging to nine species of the genus *Electrogena* (Table 2). The populations were tentatively attributed to species using the traditional taxonomic methodology. Larvae and images of *E.calabra*, *E.fallax*, *E.grandiae*, *E.gridellii*, *E.hyblaea* and *E.zebrata* were directly compared with the type material.

The quantitative characters were of two kinds: meristic (alphanumeric code N\_) and ratios (code R\_, Fig. 1). The use of ratios in studies of numerical taxonomy has been criticized by some authors (Atchley *et al.*, 1976). Hills (1978) and Dodson (1978) refuted this opinion, pointing out the value of ratios as measures of shape in many real biological cases. The use of logarithmic transformations of raw data was recommended by Hills (1978). Numeric elaborations in the present study were performed on the  $\log_{10}(x + 1)$  transformation of both ratio and meristic characters.

**Table 1.** List of characters.

Char code	Type	Description	Notes
1 N_PLP	Meristic	No of hairs on first segment of maxillary palpus	Mean between left and right palpus, ventral view
2 N_OUT	Meristic	No of hairs near outer edge of galea-lacinia, from the papuli to fore margin	Mean between left and right maxilla, ventral view
3 N_CBS	Meristic	No of comb shaped bristles on fore edge of galea lacinia	Mean between left and right maxilla, ventral view
4 N_TCB	Meristic	No of teeth of fifth (from medial corner) comb-shaped bnstle of galea-lacinia	Mean between left and right maxilla
5 N_CLW	Meristic	No of teeth on tarsal claw	Modal number between the six claws
6 N_1GI	Ratio	First gill length/width	See Fig 1(a)
7 R_7GI	Ratio	Seventh gill length/width	See Fig 1(b)
8 R_LBR	Ratio	Labrum width/mean length of left/right lateral projections	See Fig 1(c), ventral view
9 R_GLA	Ratio	Distance between outer corners/distance between inner corners of glossae	See Fig 1(d), ventral view
10 R_GLB	Ratio	Distance between outer corners of glossae/mean width between left/right glossae	See Fig 1(d), ventral view

**Table 2.** List of species, populations, and localities of material examined

Species code	Species	Author	Pop code	<i>n</i>	Locality	
CAL	<i>n</i> = 43	<i>E.calabra</i>	Belfiore, 1995	CAL1	14	Calabria, Mesoraca (Catanzaro)
				CAL2	14	Calabria, Alello Calabro (Cosenza)
				CAL3	15	Campania, Mongerati (Salerno)
FAL	<i>n</i> = 37	<i>E.fallax</i>	(Hagen, 1864)	FAL1	11	Corsica, Vizzavona
				FAL2	15	Corsica, Zonza
				FAL3	11	Sardegna, Tempio Pausama (Sassan)
GRA	<i>n</i> = 46	<i>E.grandiae</i>	(Belfiore, 1981)	GRA1	14	Marche, Serra S Abbondio (Urbmo)
				GRA2	11	Lazio, Canale Monterano (Roma)
				GRA3	10	Calabria, S Luca (Reggio Calabna)
				GRA4	11	Toscana, Roccastrada (Grosseto)
GRI	<i>n</i> = 57	<i>E.gridellii</i>	(Grandi, 1953)	GRI1	11	Friuli Venezia Giulia, Trieste
				GRI2	11	Friuli Venezia Giulia, Stregna (Udine)
				GRI3	11	Veneto, Riese Pio X (Treviso)
				GRI4	13	Lombardia, Rovagnate (Como)
				GRI5	11	Friuli Venezia Giulia, Attimis (Udine)
HYB	<i>n</i> = 39	<i>E.hyblaea</i>	Belfiore, 1994	HYB1	18	Sicilia, Camcattini Bagni (Siracusa)
				HYB2	10	Sicilia, Vizzini (Catania)
				HYB3	11	Sicilia, Giarratana (Ragusa)
LAT	<i>n</i> = 53	<i>E.laterahs</i>	(Curtis, 1834)	LAT1	11	Sicilia, Collesano (Palermo)
				LAT2	11	Lazio, Spigno Saturnia (Latina)
				LAT3	11	Lazio, Accumoli (Rieti)
				LAT4	10	Friuli Venezia Giulia, Stregna (Udine)
				LAT5	10	Marche, Pennabilli (Urbino)
MAL	<i>n</i> = 32	<i>E.malickyi</i>	(Braasch, 1983)	MAL1	11	Crete, Nea Roumata (Hania)
				MAL2	11	Crete, Zaros
				MAL3	10	Crete, Episkopi (Rethymno)
UJH	<i>n</i> = 18	<i>E.ujhelyii</i>	(Sowa, 1981)	UJH1	12	Austria, Amstetten
				UJH2	6	Austria, Krems
ZEB	<i>n</i> = 31	<i>E.zebrata</i>	(Hagen, 1864)	ZEB1	11	Corsica, Ghisoni
				ZEB2	9	Sardegna, Montresta (Nuoro)
				ZEB3	11	Sardegna, S Vito (Caglian)

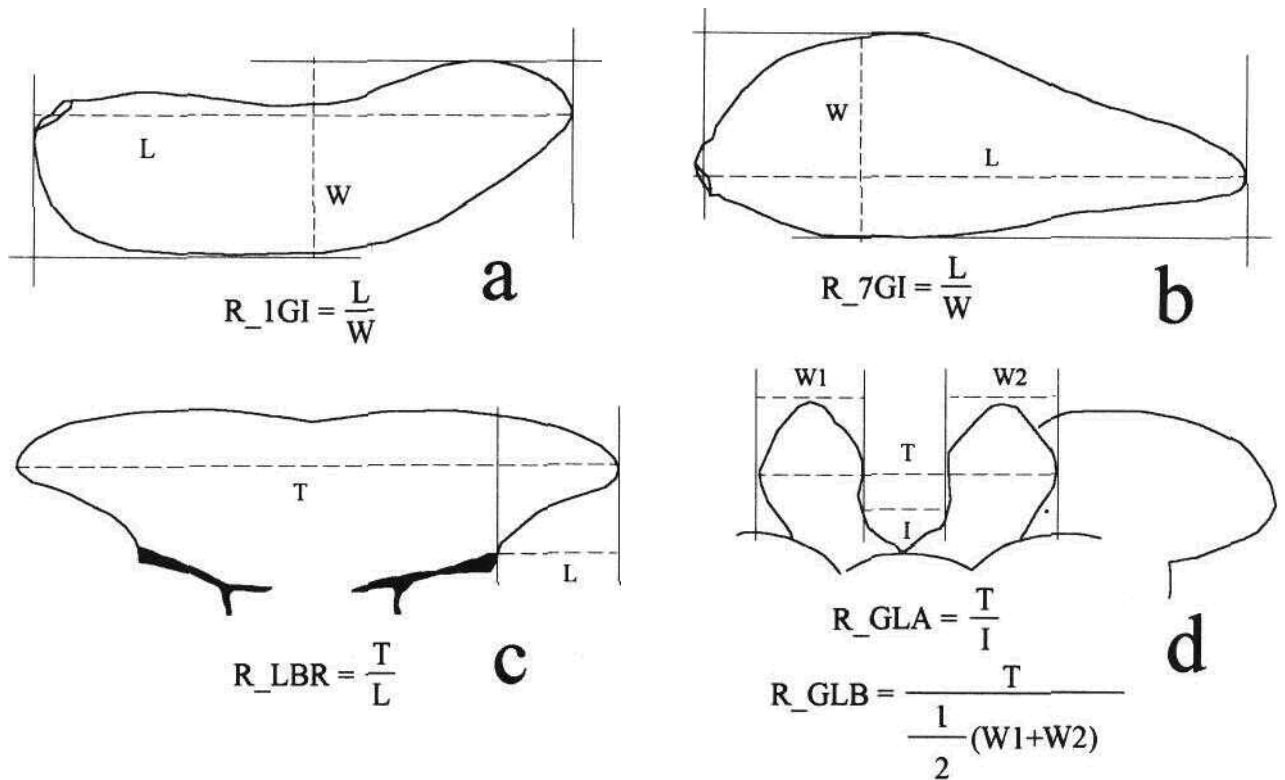


Fig. 1. Ratio characters: a, first gill-plate (R\_1GI); b, seventh gill-plate (R\_7GI); c, labrum (R\_LBR); D, glossae (R\_GLA and R\_GLB).

Measures of labrum, glossae and gills were taken from drawings made with a drawing tube mounted on a high-magnification microscope. Other characters were checked directly from slide mounts of maxillae and legs. In the case of paired parts (i.e. left and right maxillae), the mean was used. Canonical variate analysis was performed with SYSTAT package, version 4.0 (Wilkinson, 1987), and generalized distances were computed with programs developed by the author. Numerical methods here used are described in several books on multivariate analysis, following mainly Krzanowski (1990) and Dillon & Goldstein (1984). First, Mahalanobis' Distances (generalized distances) were computed between all pairs of populations, and a dendrogram with UPGMA (Unweighted Pair-Group Method Arithmetic average) method was constructed to verify the tentative attribution to species. A multiple discriminant analysis (Canonical Variate Analysis: CVA) was then performed on species, plotting centroids and discriminant scores of individuals onto the canonical axes. The attribution of individuals to species was checked by computing discriminant functions: the transformed character values for each individual are multiplied by the respective coefficients and a sum of these products, added to a constant, is obtained for each species. An individual is attributed to the species for which this value is highest (Wilkinson, 1987). Finally, discriminant loadings (correlations of characters with canonical variates) were computed to evaluate the contribution of each character to the discrimination of species.

**Classification of the populations**

The mean value, variance, and range of each of the ten characters

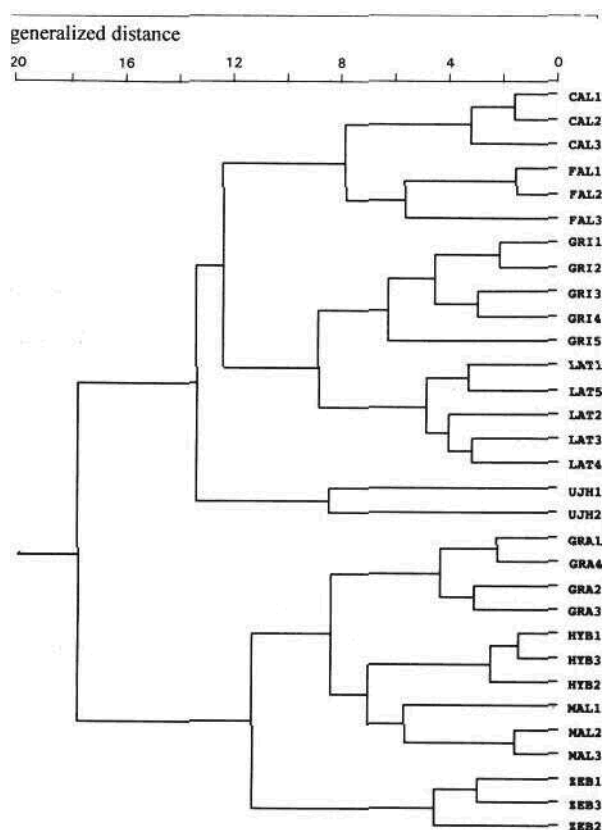


Fig. 2. UPGMA dendrogram of generalized distances between populations.



Table 3 (continued).

HYBI	HYB2	HYB3	LAT1	LAT2	LAT3	LAT4	LAT5	MALI	MAL2	MAL3	UJH1	UJH2	ZEB2	ZEB3
N_PLP	16.1111	15.2000	12.0000	13.5455	12.3636	13.7500	13.8000	5.6818	7.9091	7.9000	22.2917	15.4167	10.5455	9.3333
$x^2$	9.4869	4.4000	24.7727	10.9500	5.8045	9.2917	4.6778	3.7636	4.4409	1.8222	14.1117	5.8417	9.6227	19.6409
$x^2$	10.0-22.5	11.0-18.0	10.5-27.0	7.0-21.0	10.0-16.0	9.0-19.0	10.5-17.5	4.5-10.5	5.0-10.0	5.0-10.0	15.0-27.0	13.5-20.0	7.0-18.0	10.0-25.0
$x'$	1.2266	1.2059	1.1143	1.1028	1.1198	1.1603	1.1661	0.8082	0.9379	0.9444	1.3615	1.2117	1.0500	1.2454
$s^2$	0.0004	0.0001	0.0007	0.0009	0.0005	0.0007	0.0003	0.0007	0.0005	0.0015	0.0012	0.0022	0.0003	0.0010
N_OUT	0.0833	0.1000	0.0455	2.5455	3.4545	2.3500	4.9500	0.0000	0.0000	0.0000	33.1667	35.1667	0.0000	0.0000
$x^2$	0.0662	0.1000	0.0227	3.8227	4.6727	3.7806	5.4139	0.0000	0.0000	0.0000	60.8788	136.5667	0.0000	0.0000
$x^2$	0.0-1.0	0.0-1.0	0.0-0.5	0.0-3.0	0.0-8.0	0.0-5.0	2.0-10.0	0.0-0.0	0.0-0.0	0.0-0.0	18.0-43.0	20.0-46.0	0.0-0.0	0.0-0.0
$x'$	0.0265	0.0301	0.0160	0.4840	0.1255	0.4414	0.7453	0.0000	0.0000	0.0000	1.5219	1.5372	0.0000	0.0000
$s^2$	0.0064	0.0091	0.0028	0.0670	0.0508	0.0918	0.0285	0.0000	0.0000	0.0000	0.0119	0.0233	0.0000	0.0000
N_CBS	17.6667	18.1000	17.0000	19.1364	17.4091	16.5000	16.8000	21.9091	20.5909	19.8000	20.6250	19.9167	22.6818	21.3333
$x^2$	16.0-19.0	17.5-19.0	15.0-20.0	16.0-21.0	16.0-19.0	15.0-18.0	15.5-18.0	20.0-24.0	19.0-22.0	18.0-24.5	18.0-23.0	17.5-23.5	21.0-24.0	19.0-23.5
$x^2$	1.2706	1.2809	1.2541	1.2545	1.3031	1.2645	1.2501	1.3593	1.3338	1.3164	1.3337	1.3184	1.3741	1.3704
$s^2$	0.0004	0.0001	0.0011	0.0007	0.0009	0.0005	0.0003	0.0007	0.0005	0.0015	0.0012	0.0022	0.0003	0.0010
N_TCB	11.3889	11.9000	10.5909	7.5455	9.4091	9.5455	7.7000	14.3636	14.5455	12.9500	13.6667	13.2500	12.7273	13.8889
$x^2$	1.7810	1.2667	1.1409	0.2727	1.3409	3.0727	0.4556	0.6250	0.2727	0.4694	0.8788	3.9750	1.9182	0.6736
$x^2$	8.0-13.0	10.5-14.0	9.5-13.0	7.0-8.0	8.0-12.0	8.0-13.0	7.0-9.0	13.0-16.0	12.5-18.0	12.0-14.0	12.0-15.0	11.0-16.0	10.0-15.0	13.0-15.0
$x'$	1.0904	1.1091	1.0625	0.9310	1.0151	1.0180	0.9384	0.9405	1.1856	1.1441	1.1655	1.1502	1.1355	1.1723
$s^2$	0.0025	0.0014	0.0015	0.0007	0.0022	0.0047	0.0011	0.0008	0.0017	0.0005	0.0008	0.0038	0.0006	0.0003
N_CLW	2.0000	2.0000	2.0000	1.0000	1.0000	1.0000	1.0000	2.0000	2.0000	2.0000	2.5000	2.8333	3.3636	3.3636
$x^2$	2.2	2.2	2.2	1-1	1-1	1-1	1-1	2-2	2-2	2-2	2-3	3-4	3-4	3-4
$x^2$	0.4771	0.4771	0.4771	0.3010	0.3010	0.3010	0.3010	0.4771	0.4771	0.4771	0.5396	0.5766	0.6344	0.6373
$s^2$	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0043	0.0073	0.0024	0.0024
R_IGI	2.7231	2.6018	2.8404	2.1228	2.0941	2.2876	2.1784	2.4578	2.6032	2.6740	1.7510	2.1099	1.8581	2.2678
$x^2$	0.0630	0.0653	0.0792	0.0244	0.0283	0.0510	0.0193	0.0756	0.0308	0.0940	0.0336	0.1107	0.0574	0.0332
$x^2$	2.25-3.18	2.29-3.03	2.41-3.28	1.95-2.38	1.84-2.36	2.09-2.67	1.97-2.34	1.80-2.52	2.12-2.87	2.25-3.26	1.53-2.23	1.83-2.67	1.35-2.13	2.01-2.59
$x^2$	0.5700	0.5556	0.5833	0.4941	0.4900	0.5160	0.5018	0.4844	0.5376	0.5658	0.4387	0.4908	0.4546	0.5137
$s^2$	0.0008	0.0009	0.0010	0.0005	0.0006	0.0009	0.0004	0.0014	0.0012	0.0011	0.0008	0.0020	0.0014	0.0005
$x'$	2.6827	2.4073	2.8637	1.9228	1.9784	2.2936	2.1326	2.1330	2.5428	2.3237	2.4014	2.1685	2.5113	2.6093
$s^2$	0.0571	0.0553	0.0625	0.0211	0.0295	0.0342	0.0351	0.0827	0.0209	0.0454	0.0597	0.0792	0.0178	0.0255
$x^2$	2.21-3.08	2.09-2.84	2.33-3.21	1.65-2.15	1.76-2.23	2.03-2.75	1.84-2.37	1.78-2.62	2.36-2.79	2.07-2.65	1.80-2.57	2.15-2.85	1.99-2.39	2.38-2.82
$x^2$	0.5653	0.5315	0.5862	0.4653	0.4733	0.5171	0.4952	0.5490	0.5202	0.5309	0.4997	0.5443	0.4953	0.5570
$s^2$	0.0008	0.0009	0.0008	0.0005	0.0006	0.0006	0.0007	0.0015	0.0013	0.0007	0.0011	0.0012	0.0003	0.0004
R_LBR	4.4589	4.6804	4.5938	5.6685	5.1958	5.5583	5.3485	5.7116	4.2475	4.3789	4.8274	5.0099	4.6128	4.8476
$x^2$	0.0506	0.0138	0.0311	0.2646	0.0680	0.1374	0.0717	0.4826	0.0308	0.0415	0.0250	0.0388	0.0109	0.0341
$x^2$	4.15-4.87	4.46-4.88	4.34-4.84	5.18-6.81	4.88-5.73	4.87-5.91	4.97-5.91	5.00-7.40	4.15-4.68	4.11-4.79	4.67-5.15	4.75-5.33	4.37-5.00	4.62-4.44-5.07
$x^2$	0.7368	0.7543	0.7475	0.8229	0.7918	0.8161	0.8023	0.8157	0.7196	0.7304	0.7653	0.7786	0.7489	0.7669
$s^2$	0.0003	0.0002	0.0002	0.0010	0.0003	0.0006	0.0003	0.0018	0.0003	0.0002	0.0003	0.0003	0.0002	0.0002
R_GLA	3.2223	3.0859	3.3591	3.1715	3.0190	2.9513	3.6448	2.8862	3.2229	3.1036	2.8214	3.2173	2.9699	2.7810
$x^2$	0.0377	0.0562	0.0371	0.0131	0.0568	0.0227	0.0373	0.3434	0.0069	0.0400	0.0330	0.0071	0.0323	0.0515
$x^2$	2.91-3.63	2.75-3.50	3.16-3.80	2.93-3.37	2.82-3.28	2.96-3.75	2.70-3.27	2.84-4.63	2.78-3.05	2.88-3.45	2.70-2.99	3.00-3.46	2.47-3.38	2.51-3.01
$x^2$	0.6251	0.6106	0.6390	0.6168	0.6038	0.5963	0.6639	0.5894	0.6252	0.6128	0.5821	0.6247	0.5981	0.5773
$s^2$	0.0004	0.0006	0.0004	0.0001	0.0006	0.0003	0.0004	0.0030	0.0004	0.0003	0.0001	0.0003	0.0006	0.0004
R_GLB	2.7200	2.7651	2.6927	2.6547	2.7213	2.8158	2.4828	2.8119	2.5818	2.6270	2.8899	2.7818	2.6708	2.8137
$x^2$	0.5704	0.5755	0.5671	0.5626	0.5703	0.5812	0.5851	0.5411	0.5539	0.5594	0.5898	0.5774	0.5646	0.5798
$x^2$	2.58-3.00	2.51-3.10	2.56-2.95	2.49-2.83	2.37-2.89	2.54-3.03	2.50-3.09	2.22-2.92	2.44-2.73	2.51-2.83	2.76-3.01	2.58-2.98	2.53-2.89	2.53-3.11
$x^2$	0.0002	0.0003	0.0002	0.0002	0.0004	0.0003	0.0003	0.0008	0.0002	0.0002	0.0001	0.0003	0.0001	0.0006
$s^2$	0.0120	0.0226	0.0184	0.0140	0.0250	0.0254	0.0248	0.0537	0.0121	0.0117	0.0088	0.0201	0.0108	0.0473

**Table 4.** Species: mean ( $\bar{x}$ ), variance ( $s^2$ ) and range of raw data; mean ( $\bar{x}'$ ) and variance ( $s'^2$ ) of transformed data ( $x' = \log_{10}(x + 1)$ ).

		CAL	FAL	GRA	GRI	HYB	LAT	MAL	UJH	ZEB
N_PLP	$\bar{x}$	9.9651	4.6000	5.7037	16.2281	15.8333	12.9364	7.1406	20.2632	12.5161
	$s^2$	3.7785	2.5795	6.1558	59.5453	11.9518	10.0653	4.3264	22.0658	22.6081
	range	6-14	1-8.5	2-14.5	5-34	10-27	7-21	3-10.5	13.5-27	7-25
	$\bar{x}'$	1.0332	0.7287	0.7986	1.1908	1.2178	1.1331	0.8954	1.3170	1.1077
N_OUT	$s'^2$	0.0062	0.0188	0.0246	0.0414	0.0073	0.0098	0.0146	0.0100	0.0198
	$\bar{x}$	8.2093	8.4425	0.4722	12.0175	0.0769	2.7455	0.0000	33.5000	0.0000
	$s^2$	8.1456	14.4579	0.4851	34.7318	0.0597	5.3322	0.0000	78.1389	0.0000
	range	3-16.5	3-22	0-2.5	2-25.5	0-1	0-10	0-0	18-46	0-0
N_CBS	$\bar{x}'$	0.9446	0.9422	0.1288	1.0691	0.0245	0.4776	0.0000	1.5232	0.0000
	$s'^2$	0.0175	0.0296	0.0314	0.0421	0.0058	0.0962	0.0000	0.0141	0.0000
	$\bar{x}$	19.1163	21.7625	18.3519	18.9123	17.5897	17.4818	20.7969	20.4211	22.2258
	$s^2$	1.6647	3.4229	1.6380	6.5904	1.1299	2.0645	2.8848	3.3406	1.9640
N_TCB	range	17-23	18-26	16-21	14-25	15-20	15-21	18-24.5	17.5-23.5	19-25
	$\bar{x}'$	1.3027	1.3558	1.2858	1.2956	1.2686	1.2655	1.3371	1.3293	1.3652
	$s'^2$	0.0007	0.0013	0.0008	0.0032	0.0006	0.0011	0.0011	0.0014	0.0007
	$\bar{x}$	12.1860	13.1500	8.5833	10.1754	11.2949	8.4364	13.9844	13.3158	13.2258
N_CLW	$s^2$	1.3336	2.9128	1.4127	2.5669	1.6410	1.8894	1.7175	2.5336	1.1306
	range	10-14.5	10-17.5	6.5-11.5	7-13	8-14	7-13	12-18	9.5-16	10-15
	$\bar{x}'$	1.1185	1.1478	0.9783	1.0437	1.0874	0.9707	1.1741	1.1531	1.1518
	$s'^2$	0.0014	0.0026	0.0028	0.0041	0.0022	0.0035	0.0014	0.0026	0.0011
R_1GI	$\bar{x}$	2.0233	2.0250	2.0000	2.0351	2.0000	1.0000	2.0000	2.5789	3.3548
	$s^2$	0.0233	0.0250	0.0000	0.0345	0.0000	0.0000	0.0000	0.3684	0.2366
	range	2-3	2-3	2-2	2-3	2-2	1-1	2-2	2-4	3-4
	$\bar{x}'$	0.4800	0.4802	0.4771	0.4815	0.4771	0.3010	0.4771	0.5480	0.6364
R_7GI	$s'^2$	0.0004	0.0004	0.0000	0.0005	0.0000	0.0000	0.0000	0.0052	0.0022
	$\bar{x}$	2.8058	2.8642	2.7772	2.3192	2.7251	2.1460	2.5753	1.8936	1.9924
	$s^2$	0.1001	0.0706	0.1020	0.0497	0.0723	0.0410	0.0867	0.0899	0.0843
	range	2.28-3.87	2.23-3.43	2.01-3.87	1.88-2.87	2.25-3.28	1.80-2.67	2.12-3.26	1.53-2.67	1.35-2.59
R_LBR	$\bar{x}'$	0.5790	0.5860	0.5757	0.5201	0.5700	0.4969	0.5519	0.4594	0.4740
	$s'^2$	0.0012	0.0009	0.0013	0.0008	0.0010	0.0008	0.0012	0.0018	0.0018
	$\bar{x}$	2.7812	3.5063	2.6221	2.3134	2.6631	2.0884	2.4233	2.3149	2.2434
	$s^2$	0.0824	0.1535	0.0510	0.0938	0.0841	0.0532	0.0543	0.1042	0.0827
R_GLA	range	2.16-3.69	2.69-4.38	2.00-3.16	1.58-3.22	2.09-3.21	1.65-2.75	1.87-2.89	1.80-2.89	1.70-2.82
	$\bar{x}'$	0.5764	0.6523	0.5581	0.5185	0.5625	0.4886	0.5335	0.5185	0.5094
	$s'^2$	0.0011	0.0014	0.0007	0.0016	0.0012	0.0010	0.0009	0.0018	0.0014
	$\bar{x}$	4.5421	4.5649	4.8665	4.8316	4.5538	5.4631	4.3313	4.8792	4.7221
R_GLB	$s^2$	0.0496	0.1272	0.1688	0.0699	0.0431	0.2127	0.0394	0.0387	0.0363
	range	3.88-4.89	4.07-6.00	3.04-6.05	4.14-5.41	4.15-4.88	4.87-7.40	3.92-4.79	4.66-5.33	4.37-5.07
	$\bar{x}'$	0.7433	0.7446	0.7673	0.7653	0.7443	0.8094	0.7265	0.7691	0.7573
	$s'^2$	0.0003	0.0007	0.0010	0.0004	0.0003	0.0009	0.0003	0.0002	0.0002
R_GLB	$\bar{x}$	3.1155	2.9105	2.7527	3.3724	3.2259	3.1752	3.0699	2.9702	2.8496
	$s^2$	0.0241	0.0290	0.0226	0.0826	0.0502	0.1374	0.0454	0.0535	0.0365
	range	2.88-3.54	2.57-3.19	2.39-3.17	2.93-4.16	2.75-3.80	2.70-4.63	2.78-3.54	2.70-3.46	2.47-3.38
	$\bar{x}'$	0.6141	0.5918	0.5740	0.6398	0.6253	0.6192	0.6090	0.5981	0.5849
R_GLB	$s'^2$	0.0003	0.0004	0.0003	0.0008	0.0005	0.0013	0.0005	0.0006	0.0005
	$\bar{x}$	2.6427	2.7836	2.9586	2.6757	2.7239	2.7079	2.6750	2.8382	2.7607
	$s^2$	0.0184	0.0218	0.0201	0.0261	0.0163	0.0413	0.0216	0.0182	0.0328
	range	2.23-2.97	2.49-3.12	2.65-3.35	2.35-3.07	2.51-3.10	2.22-3.09	2.44-2.95	2.56-3.01	2.45-3.17
R_GLB	$\bar{x}'$	0.5611	0.5776	0.5973	0.5649	0.5708	0.5685	0.5649	0.5839	0.5748
	$s'^2$	0.0003	0.0003	0.0002	0.0004	0.0002	0.0006	0.0003	0.0002	0.0004

in the thirty-one populations and in the nine species are listed in Tables 3 and 4. The generalized distances between all pairs of populations are reported in Table 5. The dendrogram (Fig. 2), constructed with UPGMA method on the distance values, shows nine clusters corresponding to the classification of the populations by traditional methods. In some species (HYB, CAL and GRA)

the populations are closely clustered. A considerable distance (8.44) divides the two populations of UJH. It should, however, be noted that the number of individuals of UJH2 examined is low (six) and many characters in this population have high variance. However, the conspecificity between the two populations can be corroborated by considering other qualitative



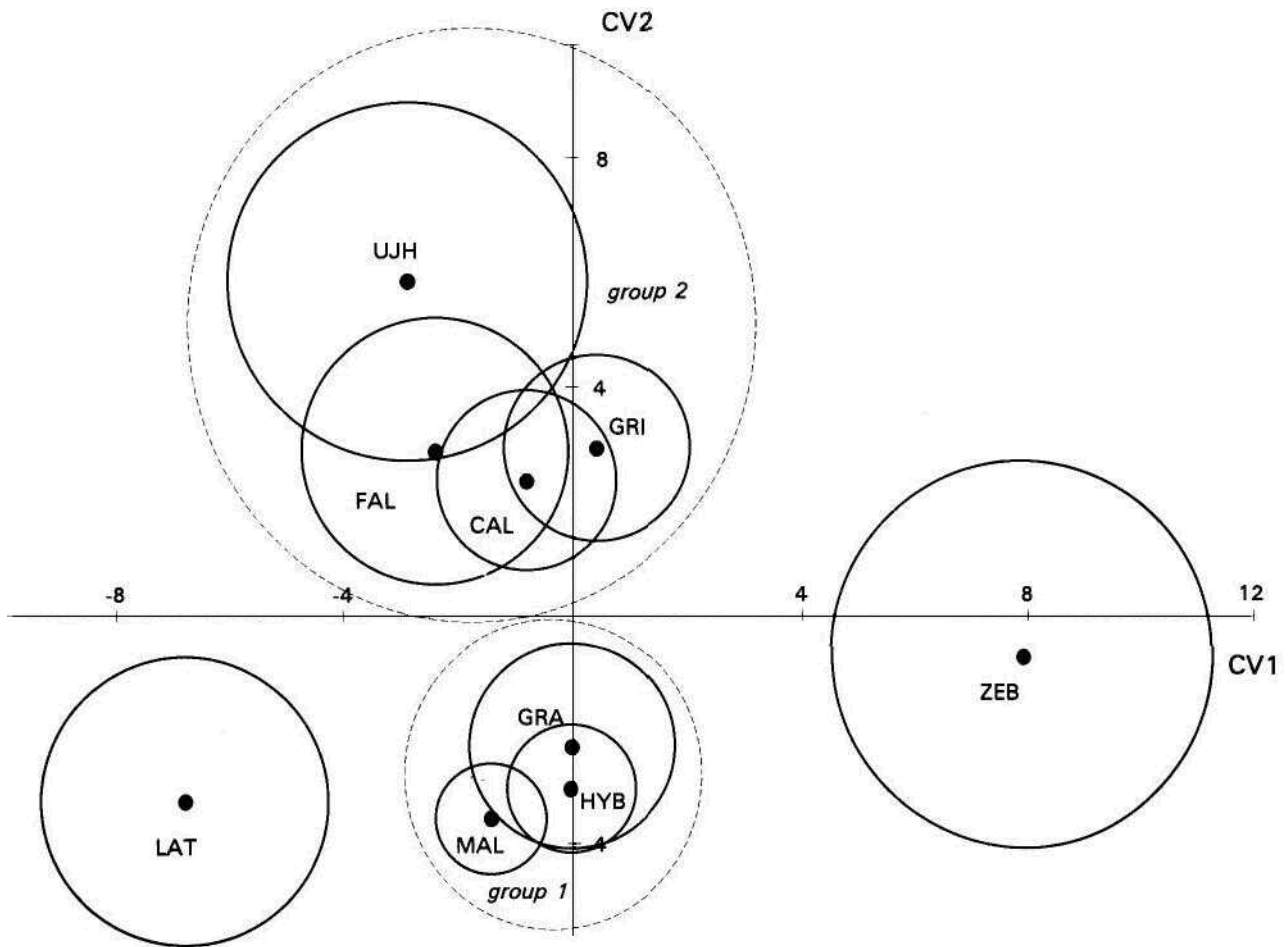


Fig. 3. Plot of species centroids on the first two canonical variates (circles include empirically 95% of cases).

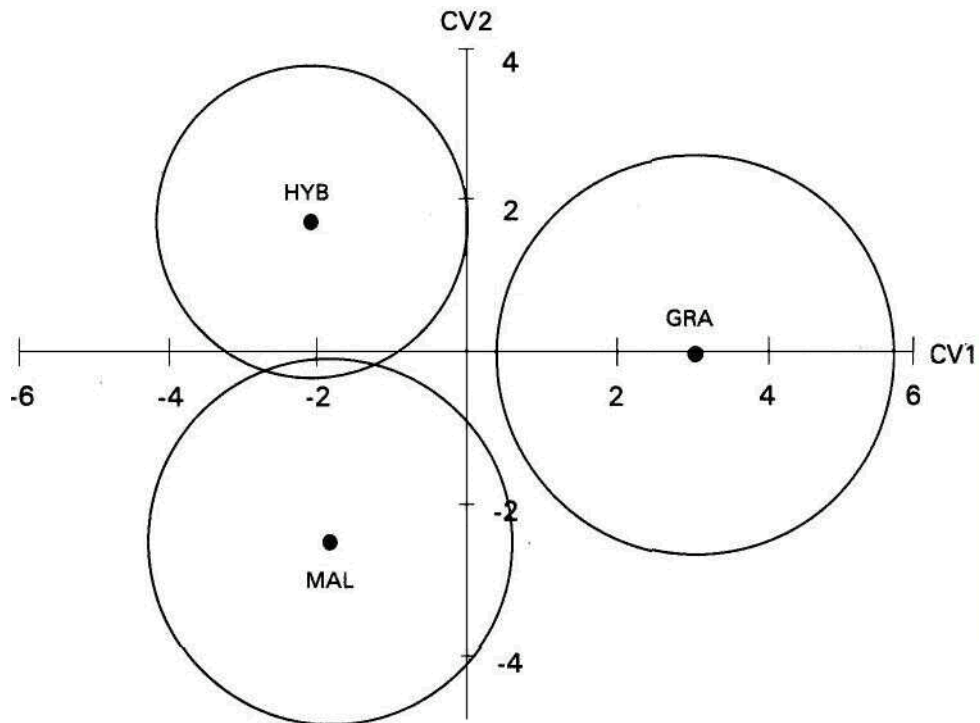


Fig. 4. Plot of 'group 1' species centroids on the first two canonical variates (circles include empirically 95% of cases).



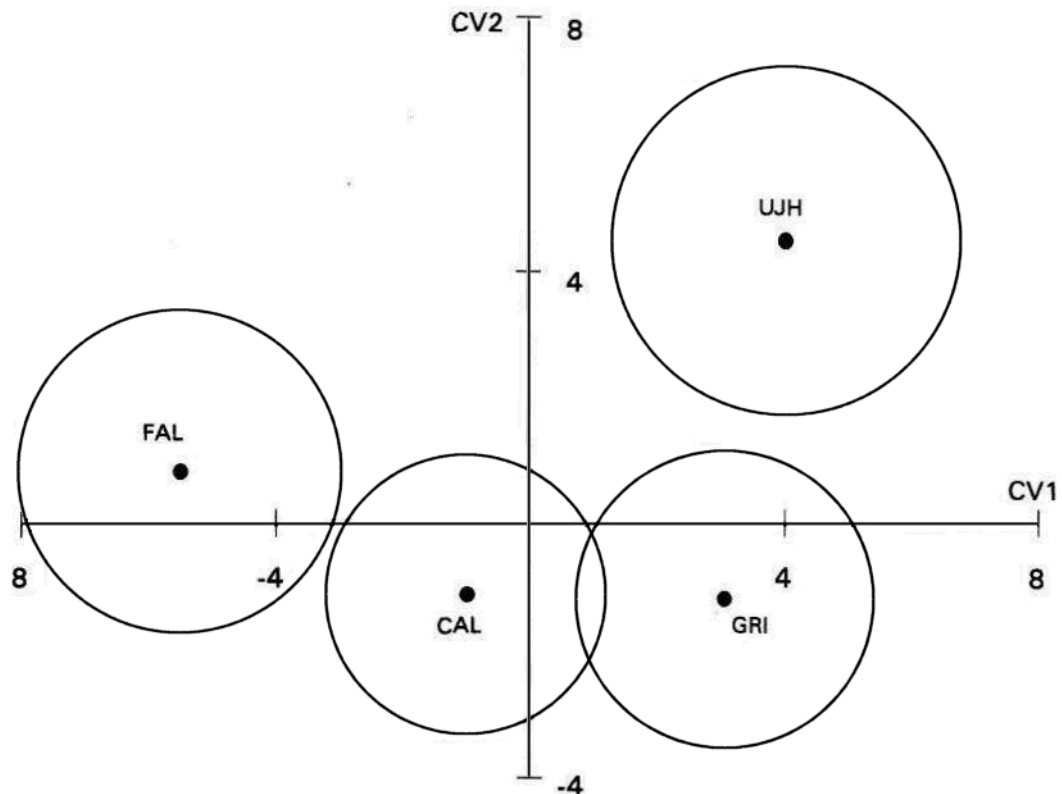


Fig. 5. Plot of 'group 2' species centroids on the first and second canonical variates (circles include empirically 95% of cases).

Table 6. Coefficients of multiple discriminant equations from transformed data ( $x' = -\log_{10}(x + 1)$ ).

species	CONSTANT	N..PLP	NOUT	NCBS	NTCB	NCLW	R1GI	R7GI	R.LBR	RGLA	R GLB
CAL	-5198.470	18.017	-12.568	1974.621	208.991	956.105	165.342	884.139	2776.279	3112.890	4526.530
FAL	-5388.220	-5.469	-11.166	2085.211	217.357	955.623	154.926	978.680	2778.056	3099.565	4539.378
GRA	-5257.400	12.831	-38.107	2035.528	155.382	948.248	174.893	835.416	2823.540	3106.386	4610.663
GRI	-5276.320	30.037	-9.309	1949.689	174.440	974.677	110.459	842.690	2859.628	3195.647	4633.304
HYB	-5222.640	39.309	-45.871	1954.013	203.328	949.000	152.266	854.777	2779.018	3177.739	4587.475
LAT	-5117.460	30.827	-30.061	1929.139	161.078	668.443	109.291	798.639	2959.234	3173.027	4588.053
MAL	-5216.780	9.687	-47.594	2023.197	234.863	945.118	161.332	842.105	2738.361	3129.909	4521.960
UJH	-5403.920	35.416	6.646	1941.248	215.529	1107.810	31.052	878.040	2902.047	3129.375	4678.598
ZEB	-5473.610	30.264	-47.905	2063.779	206.348	1232.879	59.246	859.011	2848.762	3103.407	4577.749

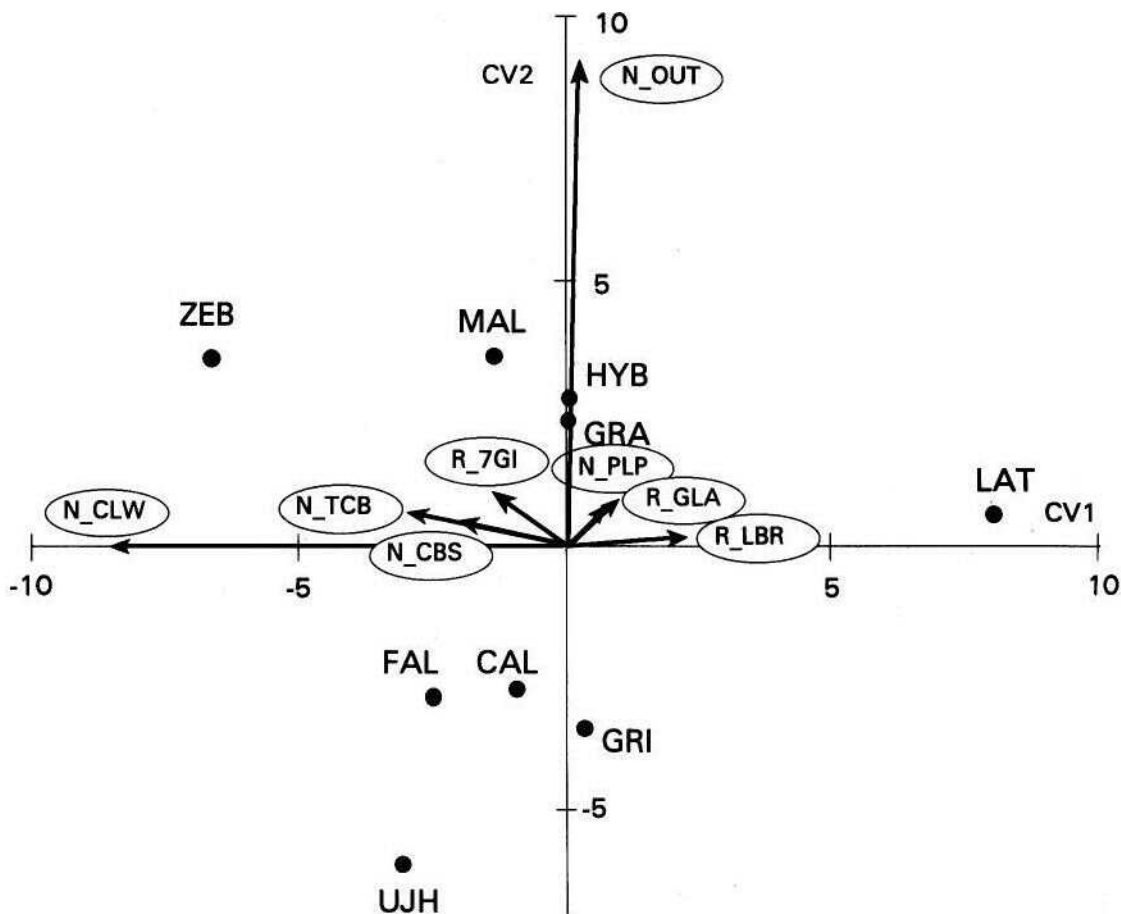
Table 7. Number of specimens correctly placed into species by discriminant equations.

species	n	correctly placed	%
CAL	43	42	97.67%
FAL	37	37	100.00%
GRA	46	45	97.83%
GRI	57	56	98.25%
HYB	39	38	97.44%
LAT	53	53	100.00%
MAL	32	32	100.00%
UJH	18	18	100.00%
ZEB	31	31	100.00%

populations; larvae belonging to GRI5 were collected near Udine, not far from GRI2. Possibly, further and more thorough studies might assign these groups to different species. *E. malickyi* is a species very close indeed to *E. hyblaea*, considering both larvae and adults, the outlying population MAL1 comes from the west of Crete, the end nearest to Sicily, and is the most distant population of MAL from HYB. This supports the attribution of all three MAL samples to the same cluster. In summary, it should be stressed that the nine identified species can be satisfactorily considered as natural groups, even if the status remains uncertain in some cases. These groups have undoubtedly some inner homogeneity, and are sufficiently separated from each other.

**Table 8.** Discriminant loadings with the first two canonical variates: CVA of all species (left), CVA of GRA, HYB and MAL (centre), CVA of CAL, FAL, GRI and UJH (right). Highest absolute values are in bold.

	CV1	CV2	CV1	CV2	CV1	CV2
N_PLP	0.075	0.076	<b>-0.483</b>	<b>0.609</b>	<b>0.366</b>	0.033
N_OUT	0.028	<b>0.933</b>	0.188	0.062	0.183	<b>0.441</b>
N_CBS	-0.202	0.044	-0.081	<b>-0.564</b>	-0.129	0.196
N_TCB	-0.299	0.064	<b>0.597</b>	<b>-0.477</b>	-0.161	<b>0.345</b>
N_CLW	<b>-0.856</b>	0.001	-	-	0.085	<b>0.352</b>
R_1GI	-0.035	-0.040	0.097	0.131	<b>-0.312</b>	<b>-0.345</b>
R_7GI	-0.138	0.100	0.053	0.225	<b>-0.397</b>	0.057
R_LBR	0.227	0.017	<b>0.335</b>	0.229	0.162	0.064
R_GLA	0.096	0.087	<b>-0.430</b>	0.160	0.168	<b>-0.323</b>
R_GLB	-0.030	-0.041	<b>0.382</b>	0.111	-0.023	0.251



**Fig. 6.** Plot of discriminant loading vectors (x10) and species centroids onto first and second CV.

**Discrimination of species**

The centroids of species clusters, with circles including empirically about 95% of individuals, are plotted in the discriminant space, against the first two axes, in Fig. 3. In this

step four groups are clearly discriminated, two of which are composed of a single species, ZEB and LAT. The other groups (groups 1 and 2 of Fig. 3), separated mainly along the second axis, are composed respectively of MAL-HYB-GRA and UJH-FAL-CAL-GRI. Within the latter group, UJH and FAL, FAL and

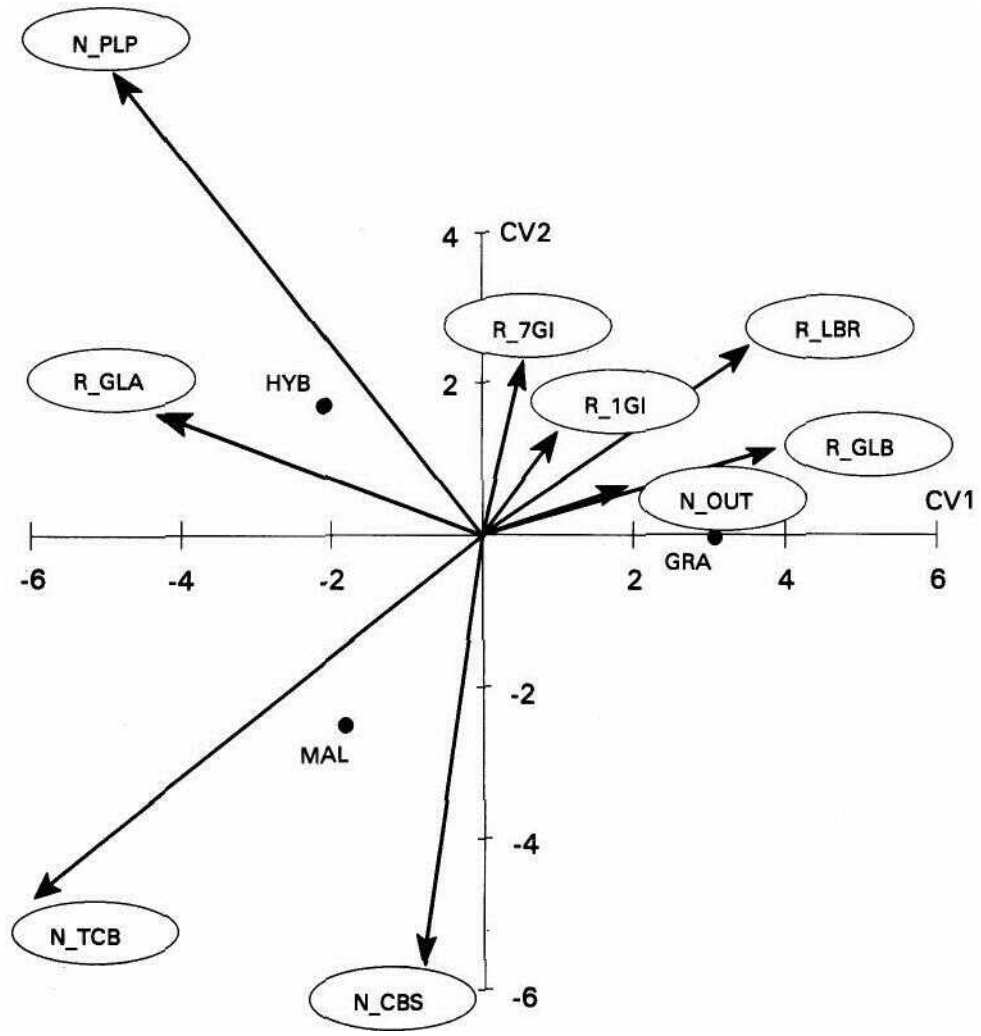


Fig. 7. Plot of discriminant loading vectors (x10) and species centroids of 'group 1' CVA onto first and second CV.

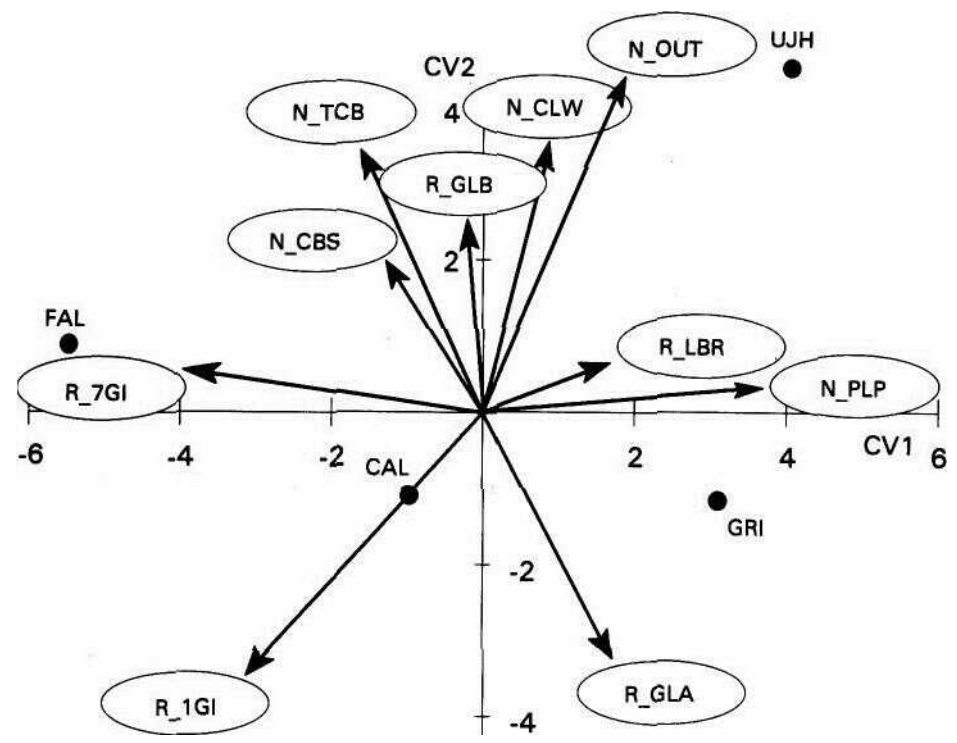


Fig. 8. Plot of discriminant loading vectors (x10) and species centroids of 'group 2' CVA onto first and second CV.

CAL, CAL and GRI overlap widely. A further CVA was performed separately on these two groups. Figs 4 and 5 show the centroids of species for groups 1 and 2 respectively, plotted against the first two canonical variates with circles including about 95% of individuals. All species are completely discriminated, except for narrow overlap areas between MAL and HYB, and CAL and GRI. In Table 6 are listed constants and coefficients of the multiple discriminant equations for the nine species (referred to the total discriminant analysis). Table 7 shows the percentage of correct attributions by discriminant equations. In five cases (FAL, LAT, MAL, UJH and ZEB) all individuals were correctly placed. In the other cases correct placements were not less than 97%. The further partial CVAs did not improve these results. The number of misclassified individuals remaining was four.

Analysing the discriminant loadings of the total CVA (Table 8, on the left), the first two axes are each highly correlated with one character, N\_CLW for CV1 and N\_OUT for CV2. The discriminant loading vectors (x10) are plotted onto the first two CVs, together with species centroids, in Fig. 6. In fact, the number of teeth on tarsal claw (N\_CLW) is sufficient by itself to identify *E.lateralis* from the other species considered here, and this character, together with the number of hairs on the outer edge of galea-lacinia (N\_OUT), are sufficient to identify *E.zebrata*. The species of group 1 and group 2 are also strongly discriminated along the second axis, the former with few or no hairs on the outer edge of galea-lacinia (GRA, HYB and MAL); the latter with a high number of such hairs (UJH, GRI, CAL and FAL). The discriminant loadings of the partial analyses (first and second canonical variates) are given in Table 8 (middle and right columns); respective vectors (x10) are plotted in Figs 7 and 8. In these cases there are many characters which contribute at about the same level to the discrimination of species. For group 1 they are mainly N\_TCB, N\_PLP, R\_GLA, R\_GLB and R\_LBR along the first axis, and N\_PLP, N\_CBS, N\_TCB along the second one. Thus, GRA is discriminated by the number of teeth on the fifth comb-shaped bristle (low N\_TCB), number of hairs on the first segment of maxillary palpus (low N\_PLP), glossae spread apart (low R\_GLA) and narrower (high R\_GLB), and labrum with shorter projections (high R\_LBR). HYB and MAL are discriminated mainly along the second axis by N\_PLP (higher in HYB), N\_CBS and N\_TCB (both higher in MAL). Within group 2, the first canonical variate is correlated mainly with R\_7GI (negative), N\_PLP and R\_1GI (negative); the second one with N\_OUT, N\_CLW, N\_TCB, R\_1GI (negative), R\_GLA (negative). Three clusters are discriminated along the first axis (FAL, CAL and GRI-UJH) by a decrease in the value of R\_7GI and R\_1GI (which in fact represent the slenderness of the gills) while N\_PLP increases. Along the second axis UJH is separated from GRI by increasing values of N\_OUT, N\_CLW, N\_TCB, while those of R\_1GI and GLA diminish.

## Discussion

All continuous (ratio) characters overlap to some extent among the species here dealt with. They are, however, essential to the discrimination of some species. After an inspection of the ranges of the characters (Table 4; Belfiore, 1996), and considering also

a two-state character (number of bristles on the ventral surface of femora, near hind edge) discussed in Belfiore & Desio (1995), the following monothetic sequential key can be constructed (the percentages of the 356 individuals identified with this key are indicated in parentheses).

1. N\_CLW = 1 ..... *E.lateralis* (100%)
- N\_CLW = 2 ..... 2.
2. N\_OUT ≤ 2 ..... 3.
- N\_OUT > 2 ..... 6.
3. N\_CLW < 3 ..... 4.
- N\_CLW = 3 ..... *E.zebrata* (100%)
4. N\_PLP > 10 ..... *E.hyblaea* (94.9%)
- N\_PLP ≤ 10 ..... 5.
5. N\_TCB ≤ 11 ..... *E.grandiae* (95.7%)
- N\_TCB > 11 ..... *E.malickyi* (96.9%)
6. A row of several bristles on the ventral surface of femora, near hind edge ..... 8.
- At most three ventral bristles near hind edge of femora ..... 7.
7. R\_1GI < 2.60 ..... *E.gridellii* (89.5%)
- R\_1GI > 2.61 ..... *E.calabra* (79.1%)
8. N\_PLP > 12 ..... *E.ujhelyii* (100%)
- N\_PLP < 10 ..... *E.fallax* (100%)

One hundred percent of individuals are assigned to the correct species for FAL, LAT, UJH and ZEB. Considering the group GRA-HYB-MAL (couples 4 and 5 of the key), where the dichotomy was optimized to achieve maximum discrimination, the percentages of individuals correctly assigned drop below 97%. poorer result is obtained for GRI and CAL, the latter with only 79% of correct identifications. Although performing less well than CVA (Table 7), the sequential method produces results which could be considered satisfactory. But it is worthwhile noting that the percentages of correct identifications, both in the sequential key and CVA, refer to the same data set from which the discriminating values are drawn out, and therefore may be overestimated. There is also the problem that some other sources of error can creep into the sequential method. If a mistake is made in the first couplets of the key, there is no way to recover. Some dichotomies are based on very faint differences. A slight error in measurement can falsify identifications. On the other hand, canonical variate analysis takes into account all characters simultaneously and is more robust to slight errors of measurement, giving the best possible representation of the unknown specimens. relevant by-product of this kind of analysis is the focus on character definition and standardization, particularly needed in taxa like *Electrogena*. In the present study the use of quantitative characters was stressed and the multiple discriminant analysis proved to be very useful for characterization of species, both for discrimination capability and diagnostic character evaluation. Some inner homogeneity and heterogeneity of taxonomic units can be revealed by this kind of analysis. There are also some limits to the use of only quantitative characters. Many diagnostic

features, which contribute to species discrimination, cannot be expressed by measurements. Further studies can conveniently introduce these characters into numerical analyses.

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