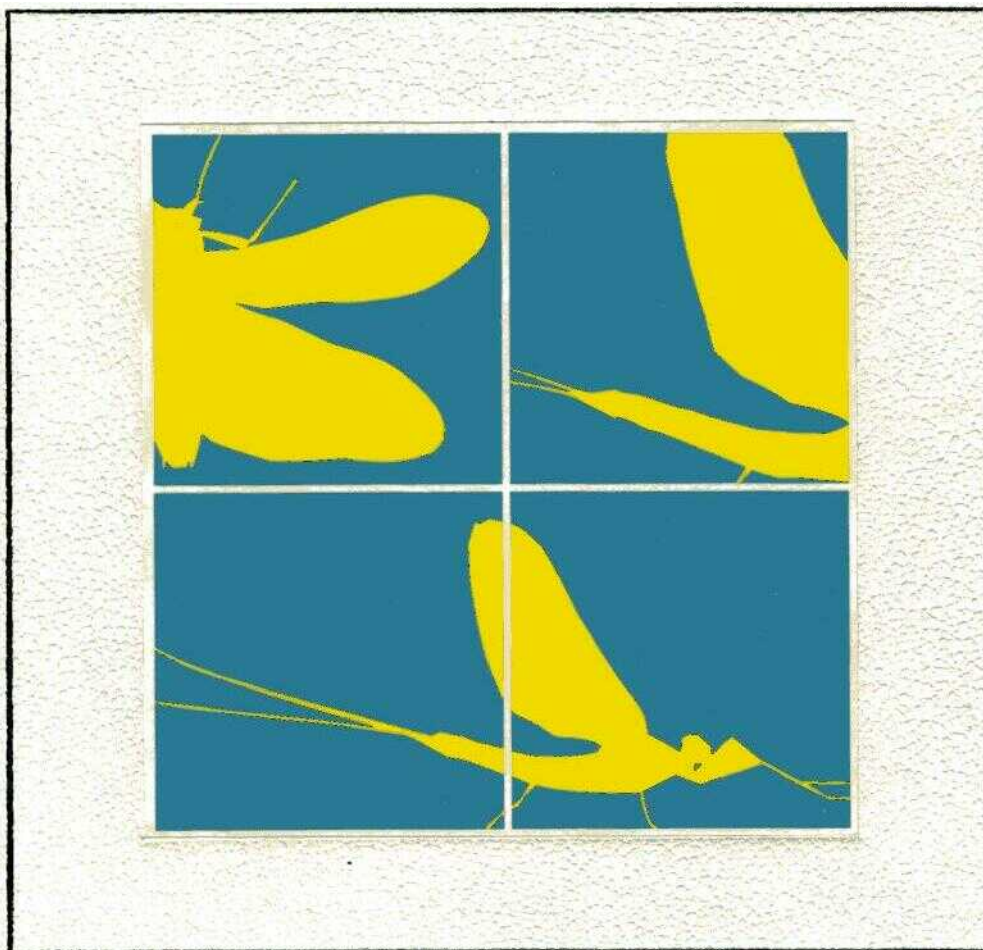


EPHEMEROPTERA & PLECOPTERA

BIOLOGY-ECOLOGY-SYSTEMATICS



PETER LANDOLT AND MICHEL SARTORI
EDITORS

**TAXONOMIC CHARACTERS AND DISCRIMINATION OF SPECIES
IN THE GENUS *ELECTROGENA* ZURWERRA & TOMKA, 1985
(EPHEMEROPTERA, HEPTAGENIIDAE)**

CARLO BELFIORE

Dipartimento di Zoologia dell'Università di Napoli «Federico II», via Mezzocannone 8,I-80134 Napoli, Italy

A new approach to the taxonomy of the genus *Electrogena* ZURWERRA & TOMKA is presented, based on a set of diagnostic characters of larvae, univocally defined and coded: the set was selected after a sample study on 356 individuals from 31 populations belonging to 9 species. 5 characters are meristic (N₋), 5 ratios (R₋), and 6 descriptions of shape or multistate characters (S₋). N₋ and R₋ characters (as well as S₋, with suitable tools) can be worked out with numerical methods. Characters are described in detail, variation among species is shown, role and weight of characters in discriminating species are discussed. A clean definition of characters and the standardization of taxonomic practice, with additional use of numerical methods, proved to be very useful for the solution of most current problems on the taxonomy of the genus *Electrogena*.

INTRODUCTION

The genus *Electrogena* ZURWERRA & TOMKA, 1985 includes about 40 species, distributed over the Palaearctic and Oriental regions. About 20 species were described within Europe (s.str.). The characterization of the genus from a morphological point of view is problematic at any developmental stages. Males have an obovate penis, larvae have subtriangular gill plates. Both these characters are shared with many other genera.

KLUGE (1988) synonymized *Electrogena* and other 10 genera with *Ecdyonurus* EATON, 1865. But *Ecdyonurus* s.str. is a well characterized genus both in adults and larvae. The other genera considered by Kluge could be monophyletic: they have been distinguished mainly on the basis of male imago features. The larvae are more or less alike, all with subtriangular gills. A revision of larvae and adults of these genera is needed, for a reliable attribution of species to genera.

Also the taxonomic status of several species is problematic. The diagnosis is uncertain in most cases. The main problem is the lack of standard definitions of the diagnostic characters. The descriptions of species were based primarily on male imagines. The main characters used by authors to discriminate species are the body colouration and the penis shape. The former is a good diagnostic character, with relatively little variation within the species, but it is really useful only if observed on fresh material. The penis shape is very variable within the species, and preservation can alter the basic elements

for the diagnosis. Dealing with larvae, the number of characters useful for species identification is higher, and most of them are quantitative (continuous or meristic), allowing some numeric elaborations for discriminating species (BELFIORE, 1994, 1995, 1996; BELFIORE & DESIO, 1995).

The main goal of the present study is to give a first set of diagnostic characters for the larvae of *Electrogena*, coded and unambiguously defined, to allow a standard reference in descriptions. The focus on these characters can improve the characterization of species and make their identification and discrimination easier, both by traditional and numerical methods. Descriptions from different authors could be actually compared and the discussion on the status of species could be more effective. As an example of the usefulness of this approach, I will briefly discuss methods and results of a sample study on the numerical discrimination of nine species of *Electrogena* based on the new set of characters.

MATERIAL AND METHODS

Populations and species

The set of characters was established by a preliminary analysis on 356 larvae of 31 populations, most by from Italy, belonging to 9 species: *E. calabra* BELFIORE, 1995, *E. fallax* (HAGEN 1864), *E. grandiae* (BELFIORE, 1981), *E. gridellii* (GRANDI, 1953), *E. hyblaea* BELFIORE, 1994, *E. lateralis* (CURTIS, 1834), *E. malickyi* (BRAASCH, 1983), *E. ujhelyii* (SOWA, 1981) and *E. zebrata* (HAGEN, 1864). HEFTI & TOMKA (1989) referred *Baetis zebrata* HAGEN, 1864 to the genus *Afronurus* LESTAGE, 1924, on the basis of

the genetic identity with some species of Heptageniidae, without an actual comparison with the true African *Afronurus*. As I have discussed elsewhere (BELFIORE, 1994), *B. zebra*, from a morphological point of view, is closer to *Electrogena* than to *Afronurus*. Although it may belong to a further genus, I keep, for the moment, the name *Electrogena zebra*. Species, number of individuals (n), populations and localities are shown in Table 1. A map of the locations of the populations is presented in Fig. 1.

Morphological analysis

Characters were examined both from larvae and exuviae. Larvae were of both sexes and of different ages, from young (with small wing-pads) to fully grown (with dark wing-pads). The analysis of character variation for sex and age will be the object of a future work. Individuals, identified by population and individual code, were dissected, and characters were checked by observation at 100-400x. Meristic characters were counted directly, measurements of continuous characters were taken on drawings made with a drawing tube. Mouthparts were examined from ventral view. Values from a paired pieces were averaged.

Numerical methods

An UPGMA dendrogram was constructed on the basis of Mahalanobis distances (see, for reference to numerical

Table 1. Names, codes, number of individuals (n) and populations (Pop.), localities of the mine *Electrogena* species.

Species	Code	n	Pop.	Localities	
<i>E. calabra</i>	BELFIORE, 1995	CAL	43	3	Southern Italy
<i>E. fallax</i>	(HAGEN, 1864)	FAL	37	3	Corsica, Sardinia;
<i>E. grandiae</i>	BELFIORE, 1981	GRA	46	4	Southern and Central Italy
<i>E. sndellu</i>	(GRANDI, 1953)	ORI	57	5	Northern Italy
<i>E. hyblaea</i>	BELFIORE, 1994	HYB	39	3	Sicily
<i>E. lateralis</i>	(CURTIS, 1834)	LAT	53	5	Italy
<i>E. malickyi</i>	(BRAASCH, 1983)	MAL	32	3	Crete
<i>E. ujhelyii</i>	(SOWA, 1981)	UJH	18	2	Austria
<i>E. zebra</i>	(HAGEN, 1864)	ZEB	31	3	Corsica, Sardinia;
			356	31	

methods here used: DILLON & GOLDSTEIN, 1984; KRZANOWSKI, 1990), calculated between all pairs of populations. Clustered populations were grouped into species. On species, considered as OTUs, was performed a Canonical Variate Analysis. A further CVA was performed on the two groups not entirely resolved by the plot on the first two canonical axes. The correlations between characters and canonical variates (discriminant loadings) were calculated, to assess the discriminant weight of each character. The coefficients of the discriminant equations were also calculated, and each individual was referred to a species by these equations.

THE SET OF CHARACTERS

Meristic characters (code: N_, Fig. 2);

N_PLP - maxilla: number of hairs on fore margin and ventral surface of the first segment of maxillary palpus; N_OUT - maxilla: number of long bristles on outer margin of galea-lacinia; N_CBS - maxilla: number of comb-shaped bristles of galea-lacinia; N_TCB - maxilla: number of pointed teeth on the 5th (starting from inner side) comb-shaped bristle of galea-lacinia; N_CLW - legs: number of teeth on tarsal claw (modal number among the legs); N_BVF - legs: number of bristles on ventral surface of femora, near the hind edge (right fore leg).

Ratio characters (code: R_; refer to Fig. 3 for the location of landmarks):

R_LBR - labrum: total length/length of lateral projection: the higher the values, the stouter the labrum; R_GLA - labium: distance between outer margins of glossae/distance between



Fig. 1. Map of locations of the examined populations: C = *E. calabra*; F = *E. fallax*; G = *E. grandiae*; I = *E. gridellii*; H = *E. hyblaea*; L = *E. lateralis*; M = *E. malickyi*; U = *E. ujhelyii*; Z = *E. zebra*.

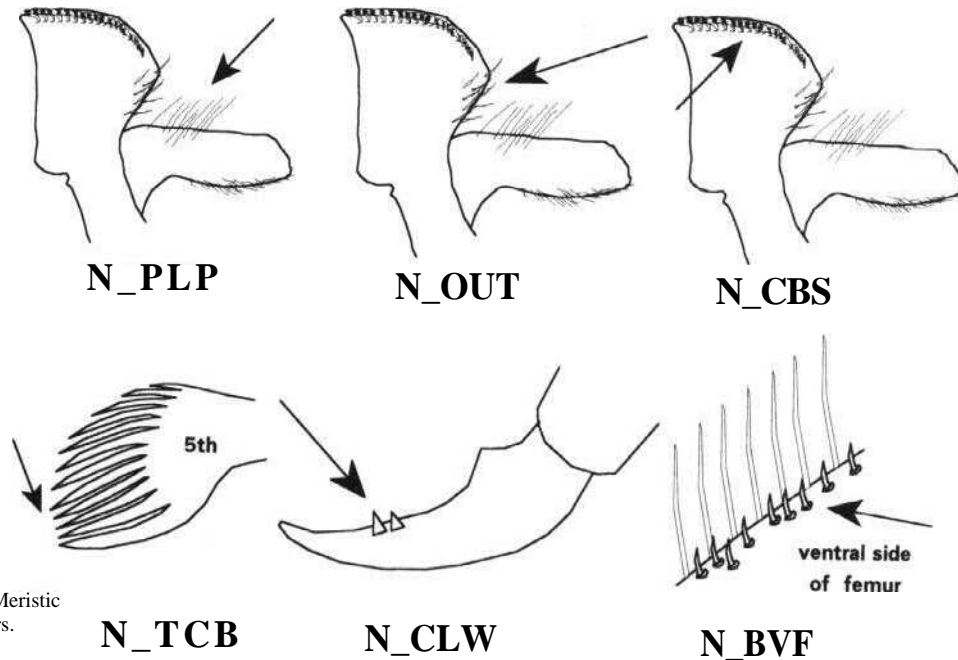


Fig. 2. Meristic characters.

inner margins of glossae: closer the glossae, higher the values of this ratio; R_GLB - labium: distance between outer margins of glossae/width of glossa: wider the glossae, the lower the ratio; R_1GI - gills: 1st gill length/width: the lower is the ratio, the stouter the gill; R_7GI - gills: 7th gill length/width.; the lower the ratio, the stouter the gill.

Other characters (code: S_, Fig. 4):

S_HLB - hypopharynx: extension of pilosity on lateral lobes; two character states: long hairs

extending over the apex of lobes/apex of lobes with very short hairs only; S_PGL - labium: shape of paraglossae; descriptive, more or less pointed; S_PNT - pronotum shape; two states: hind corners with a blunt step/smoothly rounded; S_BFE - legs: shape of distal bristles on upper surface of femora; three states: sharply pointed/long and blunt/short and rounded; S_TAR - legs: marking on tarsus; two states: basis of tarsus darkened/tarsus darkened at apex only; S_7GI - 7th gill shape; states: elliptical/gradually narrowing at apex/steeply narrowing before apex.

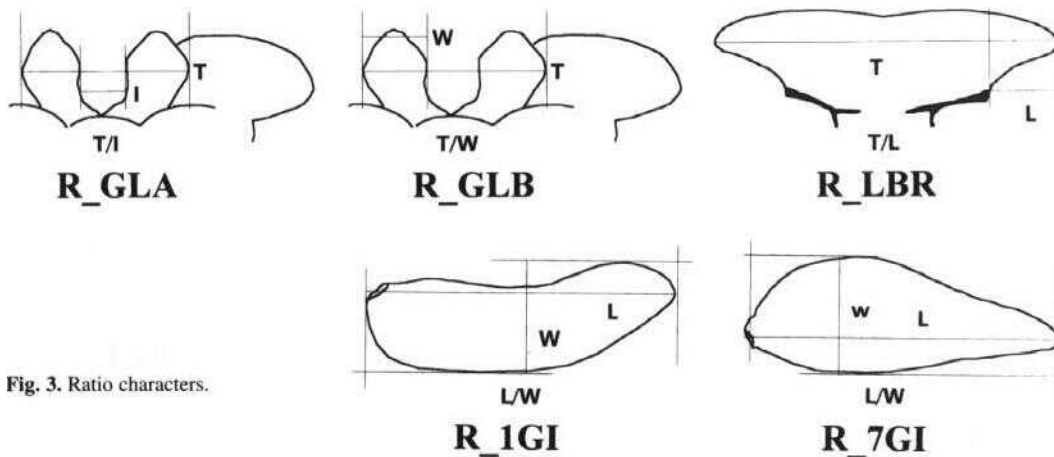


Fig. 3. Ratio characters.

VARIATION WITHIN AND BETWEEN SPECIES

The distribution of quantitative characters within species is often skewed to right. In some cases is evident a bimodal distribution.

Ratio characters (Figs. 5 partim; 7)

The mean values of species increase gradually; the minimum mean value is clearly separated from the maximum, but, in most cases, species cannot be grouped in classes at sight.

R_LBR: the labrum is very slender in MAL, FAL, CAL and HYB; lateral lobes are short in LAT, with wider within-species variation; other species are intermediate.

R_GLA: the mean values increase gradually from GRI (glossae very close each other) to GRA (glossae spread apart). Maximum range of within-species variation is in LAT and GRI.

R_GLB: from CAL to UJH the width of glossae decreases very gradually; GRA has the narrowest glossae. There is a bimodal distribution of this character in GRI and GRA.

R_1GI: first gill is very broad in UJH and ZEB, slender in HYB, CAL, GRA and FAL. CAL and GRA have the widest range of variation.

R_7GI: the mean values increase gradually from LAT to CAL; FAL has seventh gill very long and slender,

Meristic characters (Figs 5 partim; 6)

N_PLP: mean values increase gradually from FAL (4.5) to UJH (20). Bimodal distribution in LAT and GRI. Species in the upper range of values have a wider within-species variation.

N_OUT: the species can be divided into four groups: with no hairs on outer margin of galea lacinia (MAL, ZEB, HYB, GRA), with few hairs (LAT), with several hairs (CAL, FAL, GRI) and with numerous hairs (UJH). FAL, GRI and UJH have a wider range of within-species variation.

N_CBS: the mean values of the number of comb-shaped bristles on maxilla increase gradually from LAT (17.4) to ZEB (22.2). The distribution of this character within GRI, the species with the widest range of variation, is bimodal.

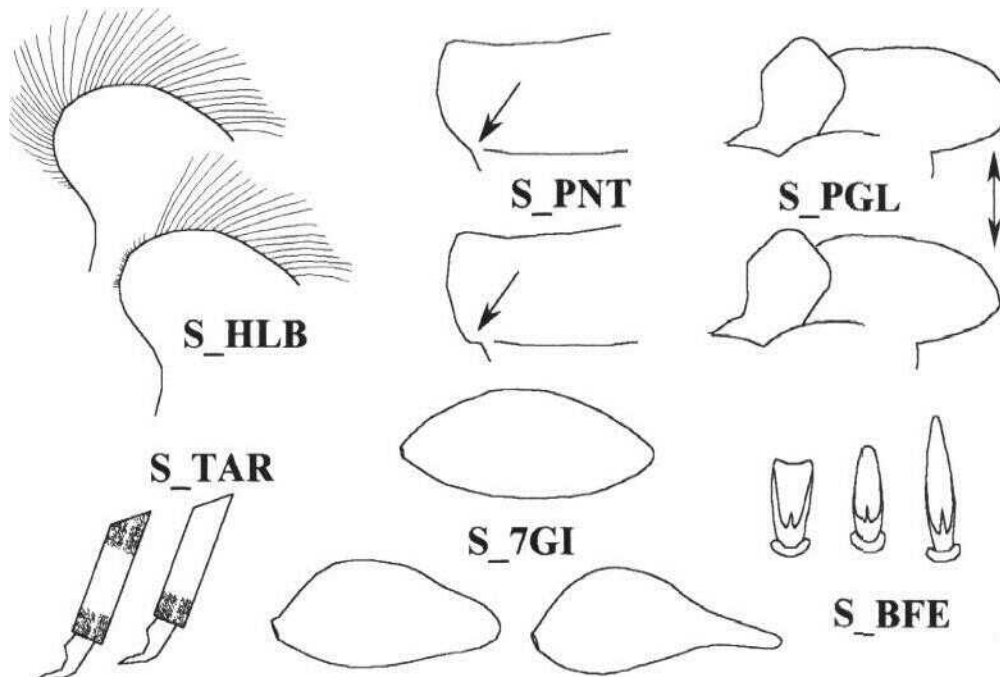


Fig. 4. Shape and multistate characters.

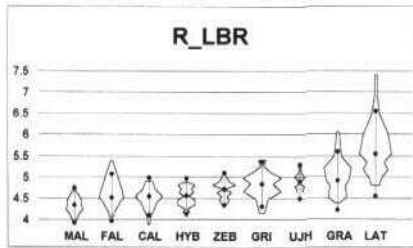
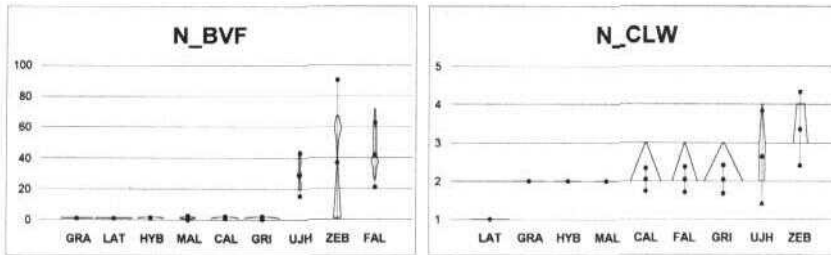


Fig. 5. Graphs of character variation within and between species. Relative size of graphs is proportional to the number of individuals in each class. Black circles indicate the mean value and the \pm double standard deviation for each species. For species and characters codes see text.

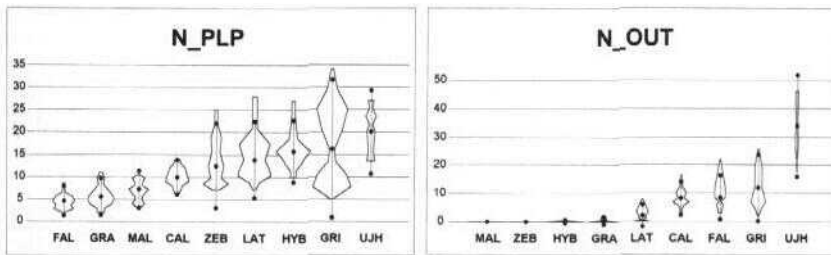


Fig. 6. Figure legend see Fig. 5.

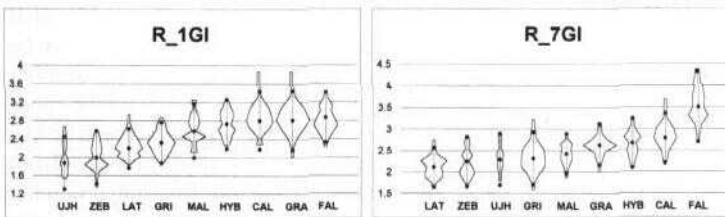
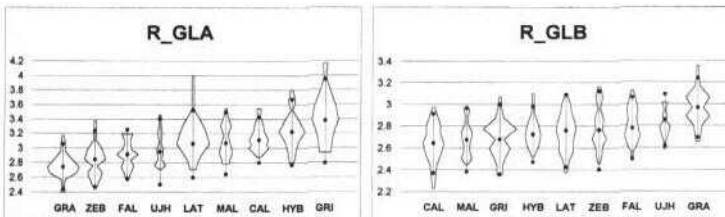
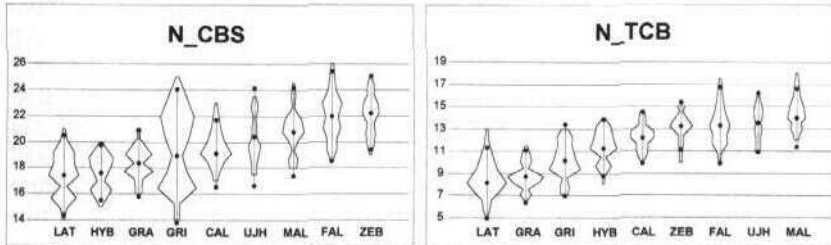


Fig. 7. Figure legend see Fig. 5.

N_TCB: mean values increase gradually from LAT (8.1) to MAL (14).

N_BVF: species can be divided in two groups, with or without bristles on hind-ventral margin of femora. To the former group belong UJH, ZEB and FAL. One population of ZEB has no bristles (see BELFIORE, 1996 for a more detailed discussion), making the distribution of this species strongly bimodal. This character could be alternatively considered a two-state one. It was not included in the numerical analysis.

N_CLW: several groups can be identified. LAT has always one denticle on claws; GRA, HYB and MAL have always two denticles; CAL, FAL and GRI have two denticles but occasionally they can have three. UJH ranges from two to four denticles; ZEB may have three or four denticles.

Other characters

S_HLB - long hairs do not extend over the apex of lobes only in LAT and UJH.

S_PGL - paraglossae are widely rounded in GRA, asymmetrically pointed in HYB, more or less symmetrically pointed in the other species.

S_PNT - FAL is the only species which has very frequently stepped hind comers of pronotum.

S_BFE - very distinctive are the short and rounded distal bristles on the upper side of fore femora in GRA; most of other species have bluntly pointed bristles.

S_TAR - this character was included in the set although it was invariant among the species considered (tarsi darkened at apex only). Its diagnostic value will be checked when more species are examined in the study.

S_7GI - only ZEB has elliptical seventh gill: this could be a character which allows the generic distinction of this species from *Electrogena*, considering also the peculiar shape of penis in the male imago. Very distinctive is also the seventh gill of UJH, steeply narrowing before apex.

NUMERICAL TAXONOMY

Details of numerical elaborations on the nine species of *Electrogena* are in BELFIORE (1996).

Population grouping

The dendrogram built on Mahalanobis distances (BELFIORE, 1996: Fig. 2) shows clusters which are assumed to be species. An analysis of the distances and of the distribution of characters within populations, evidences some problematic cases which need further studies (see BELFIORE, 1996 for more details).

Species discrimination

Multiple discriminant functions allow the correct identification of about 99% of individuals examined (352 out of 356: BELFIORE, 1996), giving a better performance than sequential key (BELFIORE, 1996). Minimum percentages of correct identification are in HYB, CAL, GRA and GRI (all between 97% and 98%). Other species are correctly identified at 100%. The better performance of the simultaneous key is due to the fact that in multiple discriminant analysis all diagnostic characters are considered together, and also that characters which widely overlap can contribute to the discrimination of species.

Considering the canonical variate analysis of all nine species, the plot of individuals onto the first two canonical variates (BELFIORE, 1996) evidences four distinct groups: ZEB and LAT at the two extremes of the first axis, a former cluster which includes MAL, HYB and GRA, and a second cluster with CAL, FAL, GRI and UJH. The two clusters are clearly separated along the second axis. The analysis of the third and fourth axis, or the plot onto the first two variates after a further canonical variate analysis on each cluster (BELFIORE, 1996), resolve satisfactorily the within-cluster discrimination. The discriminant loadings give us a way to estimate the contribution of each character to discrimination. Considering the analysis on all species, two characters are highly correlated with the first two canonical variates, N_CLW with the first axis, N_OUT with the second one (BELFIORE, 1996). This implies that LAT, ZEB and the group including the other species can be discriminated simply on the basis of the number of denticles on the claws. The two above-mentioned clusters (MAL, HYB, GRA and CAL, FAL, GRI, UJH) can be discriminated from each other simply by the number of hairs on the outer margin of galea-lacinia. More complex is

the situation of character weight in the within-cluster discrimination (BELFIORE, 1996). MAL and HYB are discriminated from GRA along the first axis (CVA of the cluster 1), mainly by N_TCB, N_PLP, R_GLA, R_GLB and R_LBR (in weight order). MAL and HYB are discriminated along the second axis by N_PLP, N_CBS and N_TCB. FAL, CAL and GRI-UJH are discriminated along the first axis (CVA of the cluster 2) by R_7GI, N_PLP and R_1GI. UJH is discriminated from other species along the second axis mainly by N_OUT and, in second order, by N_CLW, N_TCB, R_1GI and R_GLA.

CONCLUSIONS

From the data here synthetically presented the following conclusions can be drawn, possibly valid for all *Electrogena* species:

- 1) Larvae are richer than adults in quantitative taxonomic characters, their study is of great relevance in the taxonomy of Heptageniidae;
- 2) A thorough examination of several specimens from different populations is necessary to assess the within-species variability and find characters diagnostic to species;
- 3) These characters must be carefully defined and standardized;
- 4) Numerical analysis on quantitative characters may prove the discriminatory power of each character and give a global picture of phenetic separation between taxa;
- 5) The whole of data obtained in this way, jointly with adult and egg morphology, biochemical and biological data, is the basic tool to take taxonomic decisions, which can be extended also to some doubtful cases of generic attribution.

To set up a powerful tool for the identification of all *Electrogena* species, the set of characters here proposed should be tested on more populations, with the inclusion of more standard characters selected and defined in the way of the present work. Further data for a stable and substantiated taxonomy at genus level could be obtained from cladistic methods, applied to the same set of characters, after a polarity analysis of the character states.

ACKNOWLEDGMENTS

This kind of work, which needs abundant material from many different localities, can be accomplished only with the friendly help of many colleagues. I wish here to express how grateful I am to the following Ephemeropterists: Y. J. Bae, Seoul, Korea; E. Bauernfeind, Wien, Austria; J. Fischer, Mainz, Germany; A. Haybach, Mainz, Germany; S.C. Kang, Taiwan; P. Weichselbaumer, Tulfes, Austria.

REFERENCES

- BELFIORE, C. 1994. Taxonomic characters for species identification in the genus *Electrogena* ZURWERRA & TOMKA, with a description of *Electrogena hyblaea* sp. n. from Sicily (Ephemeroptera, Heptageniidae). *Aquatic Insects* 16(4): 193-199.
- BELFIORE, C. 1995. Description of *Electrogena calabra* n. sp., a new species from Southern Italy (Ephemeroptera, Heptageniidae). *Annls Limnol.* 31(1): 29-34.
- BELFIORE, C. 1996. Identification and discrimination of *Electrogena* species by numerical methods (Ephemeroptera, Heptageniidae). *Syst. Entomol.* 21: 1-13.
- BELFIORE, C. & DESIO, F. 1995. Taxonomy and distribution of *Electrogena ujhelyii* (SOWA, 1981). *Ann. Naturhist. Mus. Wien* 97 B: 151-154.
- DILLON, W.R. & GOLDSTEIN, M. 1984. *Multivariate analysis. Methods and Applications.* John Wiley & Sons, 587 p.
- HEFTI, D. & I. TOMKA 1989. Comparative morphological and electrophoretic studies on *Afronurus zebratus* (HAGEN, 1864) comb. n. and other European Heptageniidae (Ephemeroptera), including a key to the European genera of Heptageniidae. *Aquatic Insects* 11(2): 115-124.
- KLUGE, N.Y. 1988. Revision of genera of the family Heptageniidae (Ephemeroptera). I. Diagnoses of tribes, genera and subgenera of the subfamily Heptageniinae. *Entomol. Obozr.* 67(2): 291-313.
- KRZANOWSKI, W.J. 1990. *Principles of Multivariate Analysis. A User's Perspective.* Clarendon Press, Oxford, 563 p.