Taxonomy and phenetic relationships of *Electrogena affinis* (Eaton, 1883) (Ephemeroptera : Heptageniidae)

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*Electrogena affinis* (Eaton, 1883) is redescribed in all developmental stages. Larvae were analysed, in comparison with other congeneric european species, by a standard set of diagnostic characters which, in previous works, proved to be very useful in the taxonomy of *Electrogena*. The status of *E. fasciocolata* (Sowa, 1974) is discussed and the synonymy *E. fasciocolata = E. affinis* is stated. Finally, phenetic relationships between *E. affinis* and some other related european species are represented by cluster analysis, on the basis of quantitative characters of larvae.

**1. Introduction**

*Electrogena affinis* (Eaton, 1883) was described more than a century ago, based on a male imago collected in the Netherlands (Arnheim) (Eaton 1887). In past decades some authors dealt with this species (Mikulski 1934, 1936, Kimmins 1960, Keffermüller 1967, Landa 1969, Landa & Soldán 1985, 1989, Sowa 1974, 1975, Malzacher 1996) and gave also some summary descriptions of larvae. Notwithstanding the accuracy of some of these works (i.e. the very detailed description of male genitalia by Malzacher (1996)), they do not allow a definite characterisation of the species, especially in the larval stage.

Recently, some species of the genus *Electrogena* (*E. calabra* Belfiore, *E. fallax* (Hagen), *E. grandiae* (Belfiore), *E. gridellii* (Grandi), *E. lateralis* (Curtis), *E. lunaris* Belfiore, *E. malickyi* (Braasch), *E. ujhelyii* (Sowa), *E. zebrata* (Hagen), *E. galileae* (Demoulin)) have been investigated with a numerical approach, which proved to be a useful tool for the identification of larvae and for outlining phenetic relationships between species (Belfiore 1996). That study was based mainly on a standard set of diagnostic characters, both quantitative and qualitative, proposed for the first time by Belfiore (1994) and subsequently utilized for descriptions and redescriptions of several species of *Electrogena* (Belfiore 1995, 1996, 1997, Belfiore & Desio 1995, Belfiore et al. 1997, Belfiore & Sartori, in press).

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A supplementary recent approach to the taxonomy of Ephemeroptera, which proved to be very useful also for species identification, is based on the description of chorionic structures of eggs (Koss 1968, Koss & Edmunds 1974, Malzacher 1982, Gaino et al. 1993, Studemann et al. 1995). The eggs of several Electrogena species have been recently described in detail (Gaino & Mazzini 1987, Gaino et al. 1987, Mazzini & Gaino 1990).

On the basis of abundant material of *E. affinis* collected throughout Eastern Europe, we were able to perform a detailed analysis of diagnostic characters and thus characterize the species in all its developmental stages. In the present paper we give the results of a thorough analysis of the larvae using the characters defined in the above mentioned works. The number of diagnostic characters is raised to 22, adding two more characters useful for the diagnosis of *E. affinis*.

We reconsider also the taxonomic status of *E. fascioculata* (Sowa 1974), proposing the synonymy of the latter species with *E. affinis*. Finally, we analyse the phenetic relationships of *E. affinis* amongst allied congenic species, using numerical methods.

The specimens of *E. affinis* here considered were identified by the comparison of male imago characters (some male imagines were reared from nymphs) with those evidenced by Malzacher (1996), after a study on the lectotype preserved in the British Museum, and on the basis of proximity of some collection sites with the type locality (especially locality No.1, which is only about 70 Km from Arnheim).

2. Descriptions

*Electrogena affinis* (Eaton, 1883)

**Material**

(M: male imago ; F: female imago ; SM, SF: male and female subimago ; L: larva; a map of localities is in Fig. 1. If not differently specified, material is in C. Belfiore collection)

**GERMANY**

3a. Hutzdorf, R. Fulda, 9°34'E 50°42'N, M. Siebert leg., 19.VII.1996 2 L ;
3b. Friedlos, R. Fulda, 9°44'E 50°54'N, M. Siebert leg., 20.VII.1996 2 L ;
3c. Hartershausen, R. Fulda, 9°34'E 50°38'N, M. Siebert leg., 21.VII.1996 2 L ;
4a. Kordel, R. Kyll, 6°38'E 49°51'N, A. Haybach leg., 11.VII.1996 6 L + 1 M in Haybach collection ;
12.VIII.1995 1 F, 1 SM, 1 SF in Haybach collection ;
4b. R. Kyll at Mouth to R. Mosel, 6°41'E 49°49'N 11.VII.97 A. Haybach leg., 1 F in Haybach collection ;
5a. R. Alfbach, mouth to R. Mosel, 7°08'E 50°04'N, 18.VII.96 A. Haybach leg. 2 M, 2 SM, 2 SF in Haybach collection ;
5b. R. Alfbach, at Hólental, 7°06'E 50°02'N, 22.VIII.95, A. Haybach leg. 3 F in Haybach collection ;
6. R. Main, at Lohr, 9°35'E 50°01'N, 31.VII.1998 4 M, 3 F, 4 L.

**POD**

10a. Želków, R. Łupawa, M. Kostiów leg., 22.VIII.1964 10 L + 8 L in Klonowska collection ;

**AUSTRIA**

11. Jennersdorf, R.Raab, 242 m, E. Bauernfeind leg. 20.VIII.1991 1 L ;

**CZECH REPUBLIC**

Fig. 1. Map of collection sites.
Fig. 1. Carte des localités de récolte.
17. Klenci, Trhanovský stream, V. Landa leg., 29.VIII.1956 1 M, 3 F, 1 SF in Klonowska collection;
19. Roztoky, Klucná brook, M. Putz leg., 15.VII.1997 2 L in Klonowska collection (E. cf. affinis M. Klonowska det.);
GREECE
SWEDEN
22b. Vramsan, uppstroms Tollarp, B. S. Svensson leg., 22.VII.1981 1 L.

Description

MALE IMAGO

Body 10 mm, fore wing 10.5 mm, cerci 21.5 mm

Eyes relatively large and globular, dorsally separated by small gap or slightly touching at their inner border. In fresh specimens, dorsum of the eyes with wine-red to ferrugineous patch in form of cap followed by four stripes of different colours, from dorsum to base: whitish, black, yellowish, black (see also Sowa (1974), in the description of E. fascioculata). After some years in alcohol eyes uniformly grey.

Thorax yellowish brown, with reddish brown markings. Fore legs yellowish brown with darker spots on middle of femur and at basis and apex of tibia, mid and hind legs yellowish with reddish brown spots on middle of femur and near base of tarsal segments. Fore wings slightly opaque, milky white. Distal part of costal area opaque. First cross veins of costal area bordered with red. Other cross veins not evident, especially those in hind part of wing. Abdomen light, yellowish brown, with contrasting reddish brown markings (Fig. 2). Sternites II-VIII with two reddish U-shaped spots at sides of median line. Tergites with median subtriangular spot and two paramedian large spots converging towards median line. Cerci whitish with reddish brown rings, at least in their basal half.

Genitalia as in Figs 3-4 (see also : Kimmins 1960, Sowa 1974, Malzacher 1996). Penis lobes with paired elevations near middle of inner border (in ventral view), described as missing by Sowa (1974). Projections of styli ger generally large. However, size of these projections shows some variation, overlapping basis of gonopodes or not, sometimes in the same specimen (Fig. 3).

Male imago diagnosis

Abdominal markings and genital characters are the main diagnostic tools for the identification of Electrogena imagines. Genital characters of E. affinis were exhaustively treated by Malzacher (1996), who pointed out the large projections of the hind margin of the styli ger, the strongly diverging dorsal ridges of the penis and the large spines on the lateral sclerite. The markings of abdomen are also very characteristic, even though they tend to fade after a long time in alcohol.

FEMALE IMAGO

General colour and markings as in male imago. Eyes lighter, with more evident lateral stripes. Wings hyaline, cross veins evident, brownish. Subanal plate without slight median elevation in form of rib, strongly elongated caudally and distinctly bent below.

SUBIMAGINES

General coloration patterns, especially markings on abdomen, similar to imagines in both sexes. Wings uniformly grey, lighter with yellowish hue in freshly emerged individuals, darker, with greenish hue, at end of subimaginal life. After some time in alcohol wings always lighter. Styli ger of male subimago with slight central projection between two large lateral ones.

EGG

Length 125-168 μm; width 100-120 μm.

Chorionic surface covered with large protuberances and attachment structures, characterized by knob-terminated coiled threads (KCTs) of differentiated size (Fig. 5). Shape of protuberances (diameter : 1.7-2.9 μm) variable. Protuberances usually rounded or irregularly polygonal and covering whole surface of egg (Figs. 6, 7). At high magnification, chorionic surface with very delicate granular ground matrix, also covering protuberances (Figs. 7, 9). Large KCTs densely (1.1-3.0 μm of distance between them) concentrated at one pole of egg (Fig. 8). Size of the attachment structures decreasing gradually towards opposite pole, and their distribution looser (3-10 μm). 4-7 micropyles are found in subequatorial area. Sperm guide ovoidal, 5.2-6.8 μm long and 3.6-4.4 μm wide, micropylar opening adjacent to sperm guide. Micropylar rim appearing thick, because of concentration of many large protuberances (Fig. 9).
Figs. 2-4. *E. affinis*, male imago: abdominal segments III-V (2) (from a slide); genitalia from ventral view (3); penis from dorsal view (4).

Figs. 2-4. *E. affinis*, image mâle: segments abdominaux III-V (2) (montage sur lame); genitalia en vue centrale (3); pénis en vue dorsale (4).
Figs 5-9. *E. affinis*, egg: general view, 518x (5); chorionic surface, 1740x (6); chorionic surface at high magnification, 2775x (7); large KCTs, 2960x (8); micropyle, 3588x (9).

Figs 5-9. *E. affinis*, egg: vue d'ensemble, 518x (5); surface du chorion, 1740x (6); surface du chorion à fort grossissement, 2775x (7); grands filaments spirales, 2960x (8); micropyle, 3588x (9).
Egg diagnosis

The chorionic structure of *E. affinis* is quite different from that of the other species of *Electrogena* whose eggs have been described. Main distinctive characters are the very large and numerous protuberances, the concentration of large KCTs at one egg pole, and the thick micropylar rim surrounded by protuberances.

**LARVA**

Body: 9.5 mm (male) ; 11 mm (female). General colour brown with yellowish markings. Head brown with light spots (see below: character 22, S_HEM; Fig. 12). Thorax brown with light markings, pleurae yellowish with dark brown stripes. Legs yellowish. Dark stripes on coxa and distal part of trochanther. An evident brown marking on femora: two large V-shaped spots and distal smaller spot (Fig. 13). Tarsi slightly darkened at basis and apex. Surface of central tergites of abdomen smooth, small denticles (i.e. microdenticleation of other authors) visible only in small stripe near hind margins of tergites.

Diagnostic characters

The following list of diagnostic character is based on the set used by Belfiore & Sartori (in press: 20 characters) to which were added two more characters: a meristic one (N_PLH) and a qualitative one (S_HEM). A more detailed description and tips for the measurements can be found in previous works (e.g. Belfiore 1997). Mean, range and variance are reported for each quantitative character. Means of character states of *E. affinis* are compared with mean values of other species examined by the standard set of diagnostic characters.

**Quantitative characters** (N=meristic characters; R=ratios):

1. N_PLP: 34.72, 19.0-54.0, 49.9145. Number of hairs on the fore part of the first segment of maxillary palpus is very high. Mean value is the highest among all species. Closest species is *E. ujhelyii* (20.17). Non overlapping species are *E. fallax*, *E. grandiae*, *E. ma lickedyi* and *E. calabra*: all these species share N_PLLP<15.
2. N_OUT : 0.02, 0.0-1.0, 0.0089. Generally hairs are not present on the outer margin of galea-lacinia, near the fore corner. This character state is shared with E. zebrata, E. malickyi, E. hyblaea and E. grandiae.

3. N_CBS : 17.31, 13.5-20.0, 1.9953. The mean number of comb-shaped bristles on the fore margin of galea-lacinia is very low. The only species with a lesser value is E. lateralis (17.30).

4. N_TCB (Fig. 14) : 12.03, 10.0-15.0, 1.1798. The number of teeth on the fifth comb-shaped bristle is intermediate among the species considered. Closest species are E. hyblaea (11.22) and E. calabra (12.19). The only non-overlapping species is E. lunaris (N_TCB<8).

5. N_CLW : 3.02, 2.0-4.0, 0.0923. Most specimens have three denticles on the tarsal claw. Closest species are E. ujhelyii (2.52) and E. zebrata (3.35). E. lateralis and E. lunaris always have only one denticle; other species generally have two ones.

6. N_BVF : 1.13, 1.0-3.0, 0.1502. In most cases only one bristle is present on the ventral side of femora, near the hind margin. This character state is shared with E. lateralis, E. lunaris, E. hyblaea, E. grandiae, E. malickyi, E. calabra and E. gridellii. In E. ujhelyii, E. zebrata and E. fallax N_BVF is greater than 10.

7. N_HFF : 27.18, 8.0-58.0, 135.2256. Several hairs on fore side of fore femur (opposite to the side bearing the row of very long bristles) are always present (Fig. 13). Hairs counted for this character are those at least
twice as long as the bluntly pointed bristles along the fore side of femur. All other species have only very short hairs.

8. N_PLH - number of long and tiny hairs on the hind side of the first segment of maxillary palpus, near the basis: 4.57, 0.0-10.0, 5.9487. *E. affinis* is the only species with some such hairs (Fig. 19).

9. R_1GI (Fig. 15): 1.795, 1.372-2.341, 0.0438. Length/width of the first gill-plate is the lowest among considered species (gill is very short and wide). Closest species is *E. ujhelyii* (1.933).

10. R_7GI (Fig. 16): 1.936, 1.522-2.619, 0.0331. Also length/width of the seventh gill-plate is the lowest among all species. Closest species is *E. lunaris* (2.101).

11. R_LBR: 4.691, 4.069-5.523, 0.0993. Total width of labrum/mean width of lateral projections is intermediate between considered species. Closest species are *E. hyblaea* (4.544) and *E. zebrata* (4.722).

12. R_GLA: 2.955, 2.588-3.392, 0.0292. Outer distance/inner distance between glossae is generally low: glossae are spread apart. Only *E. grandiae* (2.746), *E. zebrata* (2.850) and *E. fallax* (2.915) have a lesser R_GLA. This character is not very constant throughout the distribution range of *E. affinis*. For example, populations from localities 9, 21, 22 have glossae close to each other.

13. R_GLB: 2.806, 2.449-3.178, 0.0265. Outer distance between glossae/mean width of glossae is high (glossae are narrow). Species with narrower glossae are *E. ujhelyii* (2.837) and *E. grandiae* (2.967).

**Qualitative characters:**

14. S_HLB (Fig. 20): Long hairs cover the tip of the hypopharynx lobes. Only short hairs are present in *E. lateralis, E. lunaris* and *E. ujhelyii*.

15. S_PGL: Paraglossae are tapering toward apex, which is symmetrical and rounded.
Fig. 21. Unweighted pair group average dendrogram based on Mahalanobis distance between pairs of species (all quantitative characters; co-
phenetic correlation=0.871).

Fig. 21. Dendrogramme de classification par moyennes de paires non pondérées sur la base de la distance de Mahalanobis entre couples d’espèces
(sur tous les caractères quantitatifs; corrélation cophénétique = 0.871).

Fig. 22. Unweighted pair group average dendrogram based on Mahalanobis distance between pairs of species (without N_HFF; cophenic cor-
relation=0.873).

Fig. 22. Dendrogramme de classification par moyennes de paires non pondérées sur la base de la distance de Mahalanobis entre couples d’espèces
(sans N_HFF; corrélation cophénétique = 0.873).
16. **S_PNT** : Hind corners of pronotum are smoothly rounded, without evident step.

17. **S_BFE** (Fig. 17) : Bristles on upper surface of fore femur moderately long, bluntly pointed.

18. **S_TAR** : Tarsus is pigmented at basis and apex. In all other species tarsus is coloured only at apex.

19. **S_7GI** (Fig. 16) : Seventh gill has fore margin straight, in some case slightly concave. Apex is rounded.

20. **S_PLB** (Fig. 19) : Bristles on hind margin of the first segment of maxillary palpus are very long, about 0.5x of the segment width. Other species have bristles shorter than 0.45x of the segment width.

21. **S_LBB** (Fig. 18) : The paramedian bristles of labrum, near the fore margin, are arranged in one regular row, like all European species.

One more qualitative character was considered :

22. **S_HEM** (Fig. 12) : Markings on head : six light spots are along the fore and lateral border. In specimens with more contrasted pigmentation some other light spots are evident : a narrow stripe anterior to the median ocellum and small rounded spots near the basis of antennae. This arrangement of spots is unique in *E. affinis*.

### Larva diagnosis

The outstanding characters for the diagnosis of the larva of *E. affinis* are the presence of a row of moderately long hairs along the whole fore margin of femora (*N_HFF>*7), and the long hairs on the hind side of the first segment of maxillary palp (N_PLH>1), both of which are not found in any other European species. Other relevant characters are the tip of the hypopharynx lobes covered with long hairs (*S_HLB*), three denticles on tarsal claw (*N_CLW=3*) and the peculiar markings of head (*S_HEM*).

### 3. The status of *Electrogena fascioculata* (Sowa, 1974)

*Electrogena fascioculata* was described from Poland and considered closely related to *E. affinis* (Sowa, 1974). On the basis of the original description the two species cannot be distinguished. We could not find any type material, and some attempts to collect that species at the type locality (Raba stream) were unsuccessful. Possibly the species has disappeared from that locality, which is now heavily damaged by a large reservoir. The junior author (M. Klonowska-Olejnik) examined several specimens (reported as "E. cf. affinis M. Klonowska det." in the above listed material) whose eggs were different from those described in the present paper. Their chorion is thinner and covered with small and rounded tubercles (Figs 10, 11). Large KCTs are concentrated at one egg pole and smaller KCTs cover the whole chorion, but not densely. The micropylar rim is thin, surrounded by many small tubercles. Some of these differences were evidenced by Sowa (1974), in the comparison between *E. fascioculata* and *E. affinis* : he referred the eggs with thin chorion to the latter species and eggs with thick chorion to the former one. However, the assignment of specimens with thin chorion to *E. affinis* was arbitrary. Very possibly, on the basis of the results of the present work, if more than one species are involved, the specimens with thick chorion would be correctly identified as *E. affinis*. Therefore we establish the synonymy *Electrogena affinis* (Eaton, 1883) = *E. fascioculata* (Sowa, 1974) syn. n.

Furthermore, the analysis of larvae and imagines belonging to the populations with the two different kinds of eggs did not indicate any constant and clear difference between the two entities. In this paper we consider them conspecific, waiting for further investigations with different methods (i.e. : electrophoretic analysis).

### 4. Phenetic relationships

One of the most straightforward methods for evidencing phenetic relationships based on multivariate similarity or dissimilarity is the hierarchic agglomerative cluster-analysis. This method is very simple but the results may vary widely, depending from the choice of parameters (i.e. : type of distance measurement, type of agglomeration, weighting of characters, etc.). In the case of present work, for measuring dissimilarities we utilised the Mahalanobis distances obtained from a discriminant analysis on every pair of species. All quantitative characters (*N*_ and *R*_, with the exception of *N_PLH* (not recorded from all species), were included in the analysis. Raw data were log-transformed (*x*=log(*x*+1) : see Belfiore 1996). From the comparison were excluded species with no long hairs on the apex of hypopharynx lobes (*E. lateralis, E. lunaris* and *E. ujhelyii*) and *E. zebrata*. They belong to groups phenetically very distinct from other species.

The first dendrogram (Fig. 21), constructed by the Unweighted Pair Group Average method, shows the separation of *E. fallax* and *E. affinis* from other species. In this case the unique arrangement of *N_BVF* (for *E. fallax*) and *N_HFF* (for *E. affinis*) contribute most to the separation of these species. If the character *N_HFF*, peculiar to *E. affinis*, is removed from analysis, three clusters are evidenced (Fig. 22) : the first one including only *E. fallax*, the second one with *E. gridelli* and *E. calabra*, and the third with *E. affinis, E.
grandiae, E. malickyii and E. hyblaea. Considering directly the distance matrix (Table 1), reporting the distances calculated without N_HFF, the closest species to E. affinis is E. hyblaea (squared distance 79). Characters contributing to the proximity between the latter two species are N_OUT (0), N_CBS (respective mean values: 17.3, 17.6), N_TCB (12.03, 11.22), R_LBR (4.69, 4.55), R_GLB (2.81, 2.72).

**Table 1. Squared Mahalanobis distances between species; characters included in analysis:**

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**Tableau 1. Distances de Mahalanobis entre espèces sur la base des caractères pris en compte dans l'analyse.**

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