

**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**

by

Daniel HEFTI and Ivan TOMKA

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Specimens of *Baetis fallax* (Hagen, 1864) and *B. zebrata* (Hagen, 1864) were collected on the Island of Corsica. Imagines were obtained by breeding and were investigated morphologically and biochemically. Our results show that *B. fallax* belongs to the genus *Electrogena* Zurwerra & Tomka, 1985 (= *E. pseudograndiae* Zurwerra & Tomka, 1986, syn. n.), but that *B. zebrata* belongs to the genus *Afronurus* Lestage, 1924. The genus *Afronurus* is new to Europe. Using isoenzyme electrophoresis we could show that the genera *Afronurus*, *Electrogena* and *Ecdyonurus* Eaton, 1868, are mutually separated at a low level of the genetic identity index that is characteristic of the genera of this family (Zurwerra et al., 1987). A key for the larval and imaginal stages of the European genera of the family Heptageniidae is presented.

D. HEFTI, I. TOMKA, Zoological Institute of the University of Fribourg, Department of Entomology, P  rolles, CH-1700 Fribourg, Switzerland.

INTRODUCTION

Hagen (1864) described two new *Baetis* species from the Island of Corsica: *Baetis fallax* and *B. zebrata*. The author did not produce any drawings of the holotype specimens but the most important morphological characteristics were clearly described for both species. Eaton (1871) placed the two species in synonymy under the name *Heptagenia zebrata* and drew the genitalia of this presumed single species. Nevertheless the description did not correspond to the original one of *B. zebrata* and Eaton again recognized them as separate species in his monograph (1887) under the genus *Ecdyonurus* Eaton, 1968. Ulmer (1921) investigated specimens of the Selys Collection and he described the two species more precisely. He confirmed the validity of Hagen's original characters but he included the two species in the genus *Ecdyonurus* Eaton, 1868. Grandi (1952) re-examined some of Eaton's specimens from the Island of Sardinia and she concluded that they belonged to the species *Heptagenia fallax*. The author drew parts of the male imago which corresponded to the original description of the species *Baetis zebrata* (abdominal pattern, penis morphology, legs and wings venation). In the Limnofauna Europaea, Puthz (1978) consigned both species to the genus *Ecdyonurus*. Zurwerra & Tomka (1986) rejected

the determination of specimens of *Heptagenia fallax* deposited in the British Museum (Corsica, Nord Region Upper Golo Valley, VII-VIII, 1961, A. Meats, Trib. R. Erco, CA 28, 31.VIII, Durham Univ. Explor., Soc. Brit. Mus. 1962, 258) and proposed the new species *Electrogena pseudograndiae*. Finally, Gaino & Belfiore (1987) included both species in the genus *Electrogena* Zurwerra & Tomka, 1985.

The confused taxonomical status of the two species motivated us to re-examine the situation in the light of new material. During expeditions to Corsica, we had the opportunity to sample mayflies belonging to the family Heptageniidae. The comparison of this material with the original types from the Hagen Collection (Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, USA) confirmed the presence of both species in our material.

The aim of this study is to define the generic affiliation of these species with the following European genera of the family Heptageniidae: *Ecdyonurus*; *Electrogena*; *Epeorus* Eaton, 1881; *Heptagenia* Walsh, 1862; *Nixe* Flowers, 1980 and *Rhithrogena* Eaton, 1881 (here we consider that the species *Arthroplea congener* (Bengtsson, 1908) belongs to the family Arthropleidae (Landa, 1973; Studemann et al., 1987)).

METHODS

The field collected last instar larvae were reared to obtain the subimaginal and imaginal stages. For morphological investigations, the wings, the forelegs and the genitalia of the male imagines were dissected and preserved in 80% alcohol together with the exuviae of the last larval instar. The rest of the body of each imago was put into a separate, identified plastic tube, placed in liquid nitrogen for transport and stored at -70°C until biochemical analysis could be carried out. As the method for the electrophoretic procedure has already been described in detail by Zurwerra et al. (1986), only a brief account will be given here.

The electromorph mobilities of 15 enzyme-loci were investigated using starch gel electrophoresis. Table 1 lists the enzyme-loci used in this study along with the relevant electrophoresis buffers. After homogenisation in 0.1 M Tris HCl buffer (pH 8) and centrifugation, 20 μl of the protein supernatant were placed in a slot of the gel. Vertical electrophoresis was then carried out at 12 V cm^{-1} at 4°C for 5 to 8 hours, depending on the buffer used. The following staining systems were applied to visualize the position of the enzyme-loci (electromorphs):

- the tetrazolium salt system for the enzyme-loci ALD, AK, APK, GPDH, HK-1, HK-2, IPO-1, IPO-2, MDH-1, MDH-2, MPI, PGM and RDH;
- the fast blue dye for the enzyme-loci GOT-1 and GOT-2.

The mobilities of the electromorphs were assessed in relation to individuals from a reference population (*Epeorus sylvicola*, Châtel-St.-Denis, CH) and were expressed as a relative mobility index (RMI; Zurwerra et al., 1986). The frequencies of the electromorphs were calculated for each enzyme-locus and were finally used to evaluate a pairwise correlation of the populations. The correlation coefficients

Table 1: List of the enzyme-loci investigated (and their abbreviations) with the buffer systems used.

Tris borate EDTA pH 9 (Ayala et al., 1972)

- α -Glycerophosphate dehydrogenase (GPDH)
- Mannose phosphate isomerase (MPI)
- Retinol dehydrogenase (RDH)

N-(3-amino-propyl)-morpholine citrate pH 7 (Clayton and Tretiak, 1972)

- Aldolase (ALD)
- Glutamate-oxaloacetate transaminase (GOT-1 and GOT-2)
- Hexokinase (HK-1 and HK-2)
- Indophenol oxidase (IPO-1 and IPO-2)
- Malate dehydrogenase (MDH-1 and MDH-2)
- Phosphoglucomutase (PGM)

N-(3-amino-propyl)-morpholine citrate pH 6 (Zurwerra et al., 1986)

- Adenylate kinase (AK)
- Arginine kinase (APK)

are an expression of the genetic distance between populations (Nei, 1972).

For scanning electron microscopy, the male genitalia were dehydrated in an alcohol-acetone series, critical-point dried (Tomka & Hasler, 1978), mounted on stubs and sputtered with a 75 nm Au-Pd layer.

RESULTS

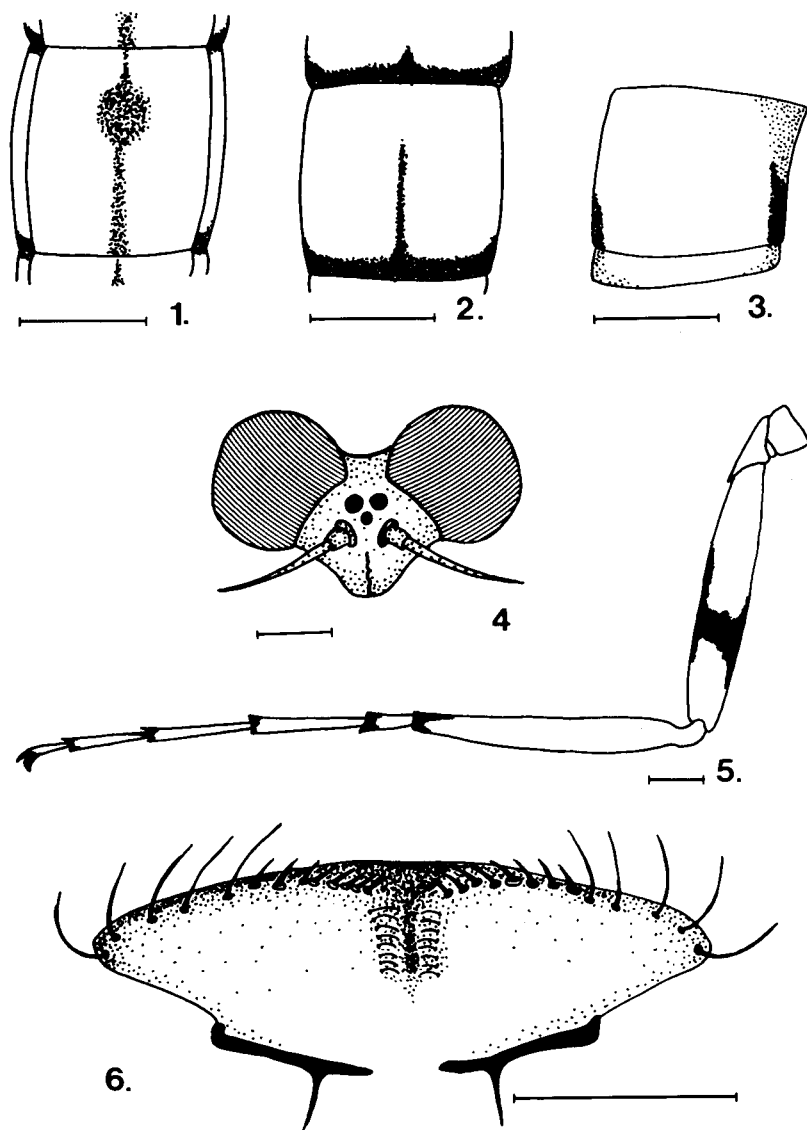
Electrogena fallax (Hagen) (Figs. 1-6 and 13)

- =*Baetis fallax* Hagen, 1864
- =*Heptagenia zebrata* Eaton, 1871, pro parte
- =*Ecdyurus fallax* Eaton, 1887
- =*Ecdyonurus fallax* Ulmer, 1921
- =*Electrogena pseudograndiae* Zurwerra and Tomka, 1986, syn. n.
- =*Electrogena fallax* Gaino and Belfiore, 1987

Material: Holotype of *B. fallax*, Corsica, 16. Ch. Type 14549 (MCZ). Fresh specimens came from the following localities: River Figarella/Moncale, Corsica, France, 155 m, 24.5.1982 (Leg. Zurwerra), - Tributary of the Stabiacco/Forêt de l'Ospedale, Corsica, France, 1100 m, 1.6.1982 (Leg. Zurwerra), - River Fango/Galeria, Corsica, France, 16 m, 23.5.1986 (Leg. Hefti, Landolt, Studemann), - River Fango/Montrestrema, Corsica, France, 200 m, 23.5.1986 (Leg. Landolt, Studemann).

Our studies confirm the assignment of this species to the genus *Electrogena*. The penis-lobes and the forceps of the male imago are of the same shape as those of the other species of the genus *Electrogena* (Fig. 13). Furthermore, the colouration patterns on the abdominal segments of the male imago (Figs. 1-3) show that *E. fallax* and *E. pseudograndiae* are, in fact, the same taxon. In the larval stage, the shape of the gill plates and the morphology of the anterior margin of the labrum (Fig. 6) also places the species in the genus *Electrogena*.

Biochemically, the distribution of the electromorphs for the species *E. fallax* (Table 2) does not show any interspecific difference from the species *E. pseudograndiae* (cf. table 2 in Zurwerra et al., 1987). The 2 taxa share the same genetic pool and, as a consequence, they cannot be regarded as separate species.



Figs. 1-6 *Electrogena fallax*. Male imago: third abdominal segment ventrally (1), dorsally (2) and laterally (3), head (4) and foreleg (5). Nymph: labrum (6). Scale line: 500 μ m.

***Afronurus zebratus* (Hagen) comb. n. (Figs. 7-12 and 14)**

=*Baetis zebrata* Hagen, 1864

=*Heptagenia zebrata* Eaton, 1871, pro parte

=*Ecdyurus zebrata* Eaton, 1887

=*Ecdyonurus zebratus* Ulmer, 1921

=*Heptagenia fallax* Grandi, 1952

=*Electrogena zebrata* Gaino and Belfiore, 1987

Material: Holotype of *B. zebratus*, Corsica, B. Ch. Type 14550-128 (MCZ). Fresh material was from the following localities: River Fango/Galéria, Corsica, France, 16 m, 23.5.1986 (Leg. Hefti, Landolt, Studemann), - River Tavignano/Corte, Corsica, France, 250 m, 27.5.1986 (Leg. Landolt, Studemann).

The pointed penis-lobes alone suggest membership of this species in the genus *Afronurus* Lestage, 1924 (Fig. 14); other Heptageniidae never show this shape of penis. The same applies to the distinctive notch in the larval labrum (Fig. 12).

Afronurus zebratus is the first European representative of the genus and is widespread on the Islands of Corsica and Sardinia, allowing the first biochemical study of this genus.

Biochemically, the species *A. zebratus* exhibits some specific monomorphic enzyme-loci (MDH-1, ALD, GOT-1, IPO-1, MDH-2, MPI, PGM and RDH (Table 2)), which demonstrate genetic isolation from *E. fallax*.

DISCUSSION

The combination of morphological and biochemical methods to solve taxonomical problems has already provided useful information in the study of mayfly systematics (Zurwerra and Tomka, 1985; Söderström and Nilsson, 1986; Zurwerra et al., 1986, 1987; Hefti et al., 1988). A correlation between the morphological and the biochemical results was also found in the present study.

The specific morphological characters of the species *A. zebratus* recorded for the different developmental stages establish a distinct generic status that deviates from that of the genus *Electrogena*. In the adult stage, the medio-apically expanded penis-lobes of the male genitalia (Fig. 14) provide a diagnostic characteristic. In the larval stage, the notched anterior labral margin (Fig. 12) also places the species in the genus *Afronurus*; no other known genus of the family shares this feature. In addition, recent scanning electron microscopic investigations of the egg surface of this species (Gaino et al., 1987) revealed significant differences in the arrangement and size of the knob-terminated coiled threads (KCTs) (Koss and Edmunds, 1974) between *A. zebratus* and the members of the genus *Electrogena*.

The use of an electrophoretic method allows the determination of the genetic distance between taxa based on the identity of genes shared by various populations (Nei, 1972). The coefficient of identity (\bar{I}) is directly calculated from the differential electrophoretic mobilities of the enzyme-loci investigated (Table 2). Zurwerra et al. (1987) published a biochemical revision for 55 species of the European Heptageniidae. This survey includes the following genera: *Ecdyonurus*, *Electrogena*, *Epeorus*, *Heptagenia*, *Nixe* and *Rhithrogena*. The authors found \bar{I} values ranging from 0.2 to 0.3 between these genera (cf. Fig. 1 in Zurwerra et al., 1987). The elements of the correlation matrix found are presented in Table 3. It can be seen that the coefficient of identity of the species *A. zebratus* and the other European genera of the Heptageniidae lies between 0.07 to 0.31. These low values of genetic affinity demonstrate a particular generic status for the species *A. zebratus*. This situation and the medio-apically expanded penis-lobes motivated us to change the generic

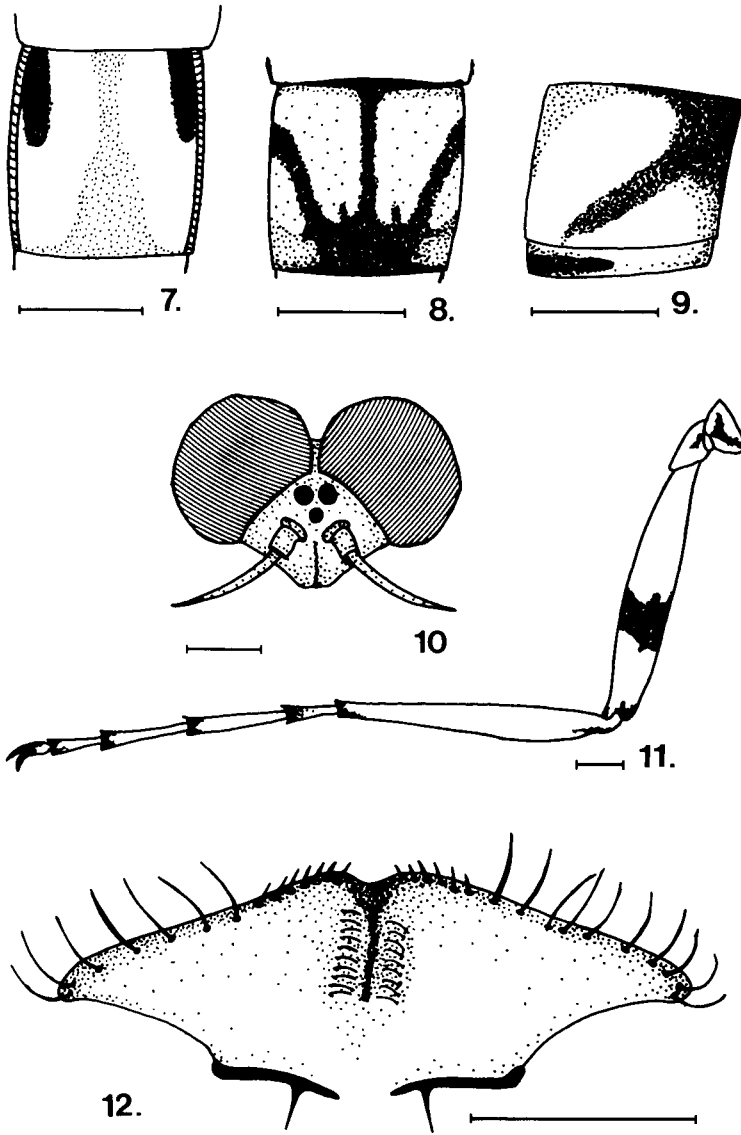
Table 2: Electromorph frequencies of the 15 different enzyme-loci used (for abbreviations, see Table 1), showing the enzyme-loci used (Enzyme), the relative mobility index of the electromorphs (Electromorph) with their frequencies (in %) for the species *Electrogena fallax* and *Afronurus zebratus*. n = number of specimens analysed for each enzyme-locus.

Enzyme	Electromorph	<i>Electrogena fallax</i> (n = 16)	<i>Afronurus zebratus</i> (n = 14)	Enzyme	Electromorph	<i>Electrogena fallax</i> (n = 17)	<i>Afronurus zebratus</i> (n = 34)								
APK	100	100	100	ALD	95	0	100								
					96	100	0								
GPDH	98	(n = 14) 100	(n = 31) 100	GOT-1	104 108	(n = 17) 0 100	(n = 35) 100 0								
								IPO-2	102	(n = 17) 100	(n = 33) 100	IPO-1	98 99	(n = 17) 0 100	(n = 34) 100 0
GOT-2	99 100	(n = 20) 20 80	(n = 34) 0 100	MDH-2	98 100	(n = 17) 100 0	(n = 34) 0 100								
								HK-1	101 102	(n = 16) 0 100	(n = 39) 69 31	MPI	100 102	(n = 16) 0 100	(n = 34) 100 0
HK-2	107 108 110	(n = 17) 94 6 0	(n = 20) 85 0 15	PGM	97 99	(n = 17) 100 0	(n = 34) 0 100								
								AK	100 104 106 107	(n = 16) 0 0 0 100	(n = 44) 25 20 41 0	RDH	99 100	(n = 16) 100 0	(n = 20) 0 100

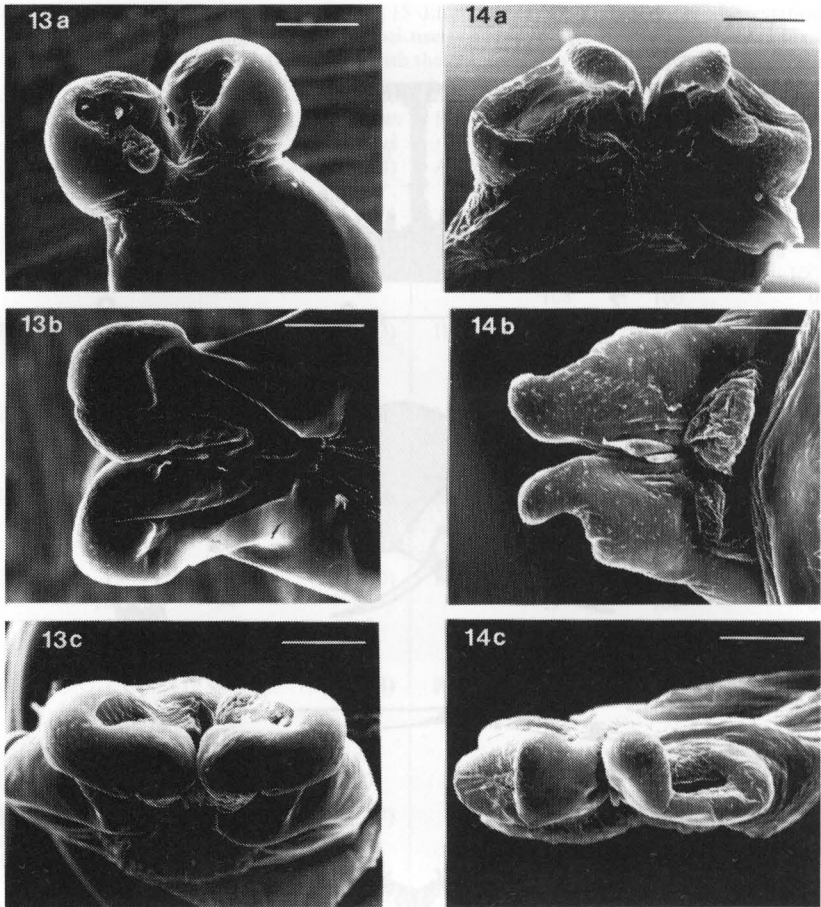
affiliation of this species and to include it in the African genus *Afronurus*.

The original definition of the genus *Afronurus* by Lestage (1924) is based on the tibia-tarsus ratio of the type *A. peringueyi* (Esben-Petersen, 1913). The validity of the initial characterization was rejected by Barnard (1932), Ulmer (1939), Schoonbee (1968) and Demoulin (1973). The latter redefined the genus *Afronurus* on the basis of the male genitalia. The ratio of the length of femora, tibia, tarsi is 1.00:1.04:1.21.

All the recent changes of the systematics at the generic level of the European Heptageniidae (1 new genus: *Electrogena*, 2 new genera for Europe: *Nixe* and *Afronurus* and a new sub-genus for Europe: *Ironopsis* Traver, 1935) make it necessary to present an up-to-date key for the determination of the larval and imaginal stages (p. 123):



Figs. 7-12. *Afronurus zebratus*. Male imago: third abdominal segment ventrally (7), dorsally (8) and laterally (9), head (10) and foreleg (11). Nymph: labrum (12). Scale line: 500 μ m.



Figs. 13-14. SEM photographs of the male genitalia (penes) of: *Electrogena fallax* (fig. 13), dorsally (a), ventrally (b), apically (c) and *Afronurus zebratus* (fig. 14), dorsally (a), ventrally (b) and apically (c). Critical-point dried, Au-Pd coated, 25 kV. Scale line: 100 μm (Photo by Müller, Institute for Cell Biology, ETH Zürich, Switzerland).

Table 3: Correlation matrix of the relative mobilities of 15 enzyme-loci for each genus of the family Heptageniidae.

Genus	(1)	(2)	(3)	(4)	(5)	(6)	(7)
<i>Afronurus zebratus</i> (1)	—	.31	.24	.30	.07	.30	.19
<i>Ecdyonurus</i> (2)		—	.19	.25	.16	.22	.26
<i>Electrogena</i> (3)			—	.17	.28	.28	.17
<i>Epeorus</i> (4)				—	.18	.17	.23
<i>Heptagenia</i> (5)					—	.19	.13
<i>Nixe</i> (6)						—	.18
<i>Rhithrogena</i> (7)							—

A. Larvae:

1. Larvae with two cerci but no terminal filament present	<i>Epeorus</i> (2)
Larvae with two cerci and one terminal filament	3
2. The first gill lamellae touching each other on the ventral side	subgenus <i>Ironopsis</i>
The first gill lamellae not touching each other on the ventral side	subgenus <i>Epeorus</i>
3. External borders of pronotum elongated caudally	<i>Ecdyonurus</i>
External borders of pronotum not elongated caudally	4
4. The first gill lamellae are expanded ventrally and are bigger than the other ones	<i>Rhithrogena</i>
The first gill lamellae are not expanded ventrally and are shorter than the other ones	5
5. Galea and lacinia with a row of setae on the ventral side	<i>Heptagenia</i>
Galea and lacinia with scattered hairs on the ventral side	6
6. Fine setae on the cerci	<i>Nixe</i>
Cerci without fine setae	7
7. Border of labrum with a median notch (Fig. 12)	<i>Afronurus</i>
Border of labrum straight or concave but without a median notch (Fig. 6)	<i>Electrogena</i>

B. Male Imago:

1. Base of the penis stem sclerotized	2
Base of the penis stem not sclerotized	6
2. Penis-lobes elongated laterally	3
Penis-lobes not elongated laterally	5
3. The outer lateral sclerites on the penis-lobes are separated from the ventral sclerites by a deep incision (Tshernova, 1974)	<i>Heptagenia</i>
The outer lateral sclerites on the penis-lobes are not separated from the ventral sclerites	4
4. The segments 3 and 4 of the genital forceps are each longer than 1/4 of the length of the second segment	<i>Nixe</i>
The segments 3 and 4 of the genital forceps are each shorter than 1/4 of the length of the second segment	<i>Ecdyonurus</i>
5. Each penis-lobe with a prominent medio-apical sclerite (Fig. 14)	<i>Afronurus</i>
Each penis-lobe without prominent medio-apical sclerite (Fig. 13)	<i>Electrogena</i>
6. First segment of the fore tarsus longer than the second	<i>Epeorus</i> (7)
First segment of the fore tarsus shorter than the second	<i>Rhithrogena</i>
7. Penes with laterally expanded lobes, apices blunt	subgenus <i>Epeorus</i>
Penes rod-like	subgenus <i>Ironopsis</i>

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