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Morphological and enzyme electrophoretic studies on the relationships of the European *Epeorus* species (Ephemeroptera, Heptageniidae)

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ABSTRACT. Forty-two enzyme–substrate systems were tested on starch gels in order to characterize the Ephemeroptera biochemically. Of these, twelve systems were useful and enabled the evaluation of sixteen loci. This biochemical method correlated well with the results of the morphological characterization of the four European *Epeorus* taxa, which are subdivided into two groups: *E.sylvicola–E.torrentium* and *E.alpicola–E.yougoslavicus*. Our biochemical comparison with the type species of *Iron* (*I.longimanus*) from North America clearly shows that all four European taxa belong to the genus *Epeorus* and that *Iron* is a distinct genus. Keys to larvae and imagines are provided.

Introduction

To clarify taxonomic problems in the family Heptageniidae we used scanning electron microscopy and enzyme electrophoresis (Zurwerra *et al.*, 1984) in addition to the usual morphological methods. In the present paper we investigate the usefulness of enzyme electrophoresis for solving taxonomic problems in Ephemeroptera. A further aim was to resolve taxonomic relationships within the European *Epeorus* species. We have chosen the genus *Epeorus* Eaton, 1881 as our initial group because of its rather simple external morphology.

In Europe *Epeorus* is represented by the following species: *E.alpicola* (Eaton, 1871), *E.sylvicola* (Pictet, 1865), *E.torrentium* Eaton, 1881, *E.yougoslavicus* (Šamal, 1935) and *E.zajtzevi* (Tshernova, 1981). We could not investigate *E.zajtzevi* (Ashtarak, Caucasus) as it is represented by the type specimen only, in the collection of Tshernova. Braasch (1980) suggested transferring *E.alpicola* and *E. yougoslavicus* into the genus *Iron*. However there is no proper definition for the genus *Iron*: if one considers larval characteristics, *E.alpicola* and *E.yougoslavicus* clearly belong to *Iron*; in contrast to this the imaginal characters of these two species give evidence for *Epeorus*. Eaton gives the differential diagnosis of the larval stages in his original descriptions of the genera *Epeorus*, 1881, and *Iron*, 1883:

> Laminae of the foremost abdominal tracheal branchiae convergent, coming into contact beneath the metasternum ..... *Iron*
>
> Laminae of the foremost abdominal tracheal branchiae widely remote from each other *Epeorus*

Sinitshenkova (1978) reinvestigated the imaginal characters given by Eaton for the differentiation of the two genera and found them

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inadequate. She defines the two genera as follows:

> The outer corners of the lobes of the penes elongated to a well-defined point ..... *Iron*
>
> The lobes of the penes rounded ..... *Epeorus*

A further characteristic, namely the presence (*Iron*) or absence (*Epeorus*) of titillators on the genital apparatus is not, in our opinion, monophyletic (see the genus *Rhithrogena*: species with and without titillators).

The roots of the controversy surrounding the classification of *E.alpicola* and *E.yougoslavicus* can be found in the fact that neither Eaton nor Sinitshenkova were able to investigate the larval stages of these two species. We could not reinvestigate the differential diagnostic characters for the larvae stages of the two genera: *Iron* and *Epeorus,* because most representatives are widely distributed in the Far East and in the Nearctic Region. Consequently, we obtained material of the type species of *Iron* from North America in order to clarify the generic ranks and interrelationships of the European *Epeorus* species.

Material and Methods

The material of the four European *Epeorus* species was collected by us in the following localities (the data refer to about ten samples of the populations in larval and imaginal stage; exceptions are indicated. Abbreviations: He, Hefti; St, Studemann; To, Tomka; Zu, Zurwerra). Deep-frozen specimens of *I.longimanus* from North America were generously provided by Dr Thomas Fink.

E.sylvicola

FRANCE: Doubs; Dessoubre/Orgeans, 408 m, 26.vi.1983 (larvae only) (*He/To*). Jura; Lemme/Syam, 532 m, 21.vii.1979 (*St/Zu*); Saine/Syam, 530 m, 30.vi.1983 (*He*). Haute-Savoie; Aire/Présilly, St Julien, 550 m, 24.v.1981 (*St/Zu*). Isère; Furon/Gorges d'Engins, 900 m, 24.v.1981; Vence/Quaix-en-Chartreuse, Grenoble, 560 m, 24.v.1981; (*St/Zu*). Savoie; Leysse/Pont des Callets, Chambéry, 590 m, 23.v.1981 (*St/Zu*).

GERMANY: Baden-Württemberg; Wiese/Mambach, 470 m, 12.v.1983 (*He/To/Zu*); Wutach/St Grimmelshofen, 510 m, 28.viii.1981 (*St/To*).

GREECE: Pindos; Kastaniotis/Joanina, 5.iv.1983 (larvae only); Vojzomatis/Melisopetra, 680 m, 3.iv.1983 (larvae only); (*He/To/Zu*).

ITALY: Marche; Ambro/Amandola, 530 m, 17.vii.1983 (larvae only); Fiastrone/Acquacanina, 700 m, 10.vii.1983; Ambro/Madonna dell'Ambro, 690 m, 9.vii.1983; (*Zu*). Emilia-Romagna; Setta/Baragazza-Castiglione, 700 m, 8.iv.1982 (*To/Zu*).

ROMANIA: Harghita; Kis Madaras/Csik Madaras, 1100 m, 23.vii.1982; Nagy Madaras/Csik Madaras, 1140 m, 23.vii.1982; Köves/Barot, 940 m, 26.vii.1982; Kormos/Barot, 660 m, 26.vii.1982 (*To/Zu*).

SWITZERLAND: (see Zurwerra & Tomka, 1984).

YUGOSLAVIA: Montenegro; Lešnica/Ivangrad, 740 m, 11.iv.1983 (larvae only); Zeta/Nikšić, 620 m, 12.iv.1983 (larvae only); Županica/Rožaj, 1090 m, 11.iv.1983 (larvae only) (*He/To/Zu*). Makedonija; Radika/Razvalina Strazimir, 1100 m, 7.iv.1983 (larvae only); Straška Reka/Kačanik, 1200 m, 9.iv.1983 (larvae only) (*He/To/Zu*); Zlidowska Reka/Kažani, Resan, 885 m, 6.iv.1983 (larvae only); Koseljska Reka/Kosel, 740 m, 7.iv.1983 (larvae only) (*He/To/Zu*). Slovenia; Krka/Krka, Novo Mesto, 290 m, 18.v.1976 (*To*).

E.torrentium

FRANCE: Lozère; Tarn/Florac, 540 m, 19.vii.1981; Tarn/Ispagnac, 520 m, 9.vii.1981; Esclancide/above Mende, 870 m, 18.vii.1981 (larvae only) (*St/To*).

E.alpicola

FRANCE: Isère; brooklet into Vence/Sarcenas, 850 m, 23.v.1981 (*St/Zu*). Haute-Savoie; Barberine/Barberine, 1140 m, 18.vi.1983 (larvae only); Eau de Bérard/Le Couteray-Vallorcine, 1400 m, 5.vii.1981, 18.vi.1983 (larvae only); Eau Noire/Barberine, 1220 m, 18.vi.1983 (larvae only) (*He/To/Zu*). Savoie; brooklet into Arc/Epierre, 400 m, 24.v.1981 (*St/Zu*).

SWITZERLAND: see Zurwerra & Tomka (1984).

E.yougoslavicus

ITALY: Marche; Fiastrone/Acquacanina,

700 m, 10.vii.1983; Ambro/Amandola, 530 m, 11.vii.1983; Ambro/Madonna dell'Ambro, 690 m, 8.vii.1983; Ambro/Piedivalle, 590 m, 10.vii.1983 (larvae only) (*Zu*).

YUGOSLAVIA: Makedonija; Straška Reka/Kačanik, 1200 m, 9.iv.1983 (larvae only) (*He/To/Zu*). Montenegro; Lešnica/Ivangrad, 740 m, 11.iv.1983 (larvae only); Lepešnica/Bojište, Mojkovac, 1000 m (larvae only); (*He/To/Zu*). Kosovo; Dečanska Bistrica/Dečani, Peć, 610 m, 10.iv.1983 (larvae only) (*He/To/Zu*).

Iron longimanus

U.S.A.: Utah; Salt Lake Co., Mt Dell Canyon Ck, off Rt 65 0.85 miles NE of junction with Emigration Canyon Rd, elev. approx. 1804 m, 23.vii.1984, leg. and det. (as *Epeorus longimanus*) T. Fink.

We collected mature larvae at each location and were able to transport living specimens to the laboratory for rearing to the adult stage. In this way, we could produce sufficient material, e.g. larvae, cast larval skins, subimagines and imagines from most localities. We cut off the wings and the terminal abdominal segments of most female and male imagines. The remainder of each imago was stored separately at 70°C until it was used for enzyme electrophoresis. The detached parts of the imagines and sometimes whole specimens were preserved in 80% alcohol and were used later for taxonomical determination.

The larval mouthparts, tracheal gills, legs and cast skins, and the wings and other parts of the subimagines and imagines were mounted on microscope slides in a polyvinylalcohol–lactophenol mixture. Penes were photographed with a scanning electron microscope (SEM) after critical point drying. Further technical details are given by Tomka & Hasler (1978).

Biochemical taxonomy using gel electrophoresis

The theory of the method used is given by Ferguson (1980) and Harris & Hopkinson (1976). The enzymatic investigations have been carried out according to standard methods (Ayala *et al.*, 1972; Brewer, 1970; Scholl *et al.*, 1978; Shaw & Prasad, 1970).

We investigated the following enzymes: Adenylate kinase (*AK), Arginine kinase (*APK), Aldolase (*ALD), Glutamate-oxaloacetate transaminase (*GOT-1 and *GOT-2), α-Glycerophosphate dehydrogenase (*α-GPDH), Indophenol oxydase (*IPO-1 and *IPO-2), Hexokinase (*HK-1 and *HK-2), Leucine amino peptidase (**LAP), Malate dehydrogenase (*MDH-1 and *MDH-2), Mannose phosphate isomerase (**MPI), Phosphoglucomutase (*PGM) and Retinol dehydrogenase (**RDH). Some enzyme stains were used with minor modifications. APK was stained with an agar overlay containaing 100 mg $MgCl_2$ H_2O, 15 mg NADP, 15 mg ADP, 150 mg glucose, 4 mg PMS, 6.6 mg MTT, 40 i.u. glucose-6-phosphate dehydrogenase, 10 i.u. hexokinase, 10 mg arginine phosphate (A. Scholl, personal communication). The separation of the enzymes was carried out at 12 V/cm on vertical starch gels for 5½–8 h depending on the buffers used. We used the following buffers: *N-(3-Aminopropyl)-morpholine-citrate (Clayton & Tretiak, 1972); **Tris borate EDTA buffer, pH 9.0 (Ayala *et al.*, 1972). The first buffer was modified and used at two different pH values: pH 6.0 (for AK and APK only) and pH 7.0 of the same concentration (Gel: 1 mM citric acid, electrode: 20 mM citric acid; buffers were adjusted to the correct pH with N-(3-Aminopropyl)-morpholine).

The homogenate of one individual was sufficient for the investigation of all enzymes considered. About ten individuals per population and per taxon were needed for each comparison. After the electrophoretic run at 4°C the starch gels were cut horizontally and stained by means of a generally very selective enzyme–substrate reaction. The distances of the positions of the different electromorphs from the starting slots were measured and the mobilities relative to a reference electromorph were determined (Fig. 8). We chose arbitrarily a population of *E.sylvicola* as reference. Among the electrophoretic variants of each enzyme we assigned the most frequent variant to the relative mobility index 100.

Evaluation of the data

Contrary to the conventional methods of measurement (Ayala *et al.*, 1972; Geiger, 1980) we defined the relative mobility index ($RMI_{x,i}$)

for the electromorph of the population i (subscript i) as follows:

$$RMI_{x,i}=\frac{(m_{x,i}-m_{x,ref})}{m_{x,ref}}\times 10+100$$

where $m_{x,i}$ and $m_{x,ref}$ are the distances (measured from the origin) of the sample and reference electromorphs in millimetres. using this definition we could eliminate the influences of some arbitrary changes on the mobilities of the electromorphs. In this way we could compare the mobilities of electromorphs from one electrophoretic run to the other. Thus we were able to reduce the source of the scatter of the data due to the electrophoretic separation on starch (starch gels show frequent inhomogeneities with regard to their composition).

The relative mobility indexes of the alleles with their frequencies (see Table 1) can be compared in pairs (correlation analysis, see Nei, 1972) over all loci. The results are given in the correlation matrix (Table 2) which forms the basis for the construction of the dendrogram (Fig. 9) according to the unweighted pair-group arithmetic average (UPGMA) clustering method (Ferguson, 1980). Genetic similarities (Nei's coefficient of genetic identity ($\bar{I}$)) between populations and taxa can be seen in both the correlation matrix and in the dendrogram. An $\bar{I}$-value of 1.0 means that all alleles identified are identical between the two compared populations. If no common alleles are found the $\bar{I}$-value is 0.

The correct genetic interpretation of the enzymes phenotypes with polymorphic loci could not be checked by cross breeding experiments. Our results could only be interpreted by comparing them with other enzymatically separated groups on the basis of analogy (Geiger, 1980; Scholl *et al.*, 1980).

Results

The results of the morphological investigations by light microscopy are given in Figs. 1, 2 and 7. The penis-lobes were photographed with the SEM (Figs. 3–6). We investigated about half a dozen penes from different localities by SEM. The characteristics given in the key are very constant. The orifice of the ejaculatory duct of the penis-lobes is circular or oval in *E.sylvicola* and *E.torrentium,* the two others species have rift-shaped orifices. However, the orifice is very

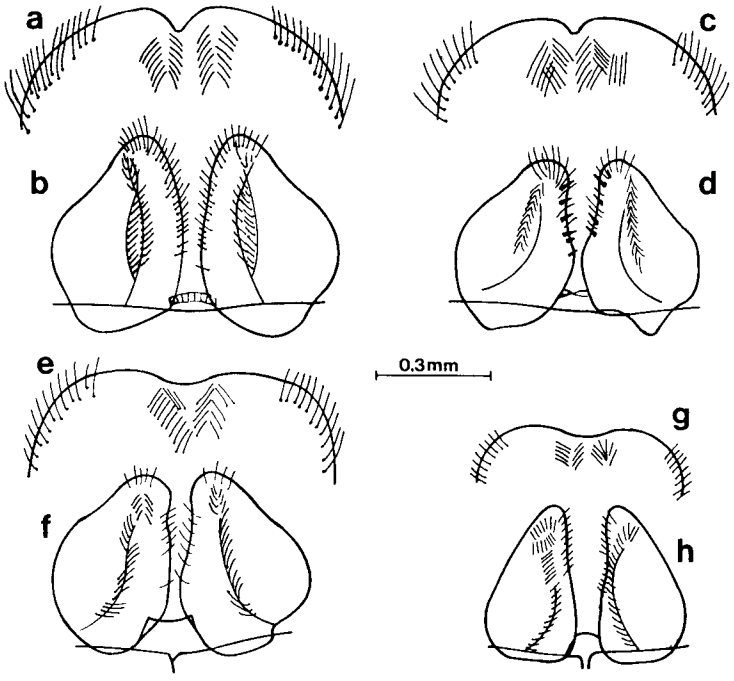


FIG. 1. Part of the labrum (above) and larval glossae (below). a–b, *E.sylvicola*; c–d, *E.torrentium*; e–f, *E.alpicola*; g–h, *E.yougoslavicus*. (a, b, c, f after Eaton (1883–88), Steinmann (1907) and Belfiore (1983).)

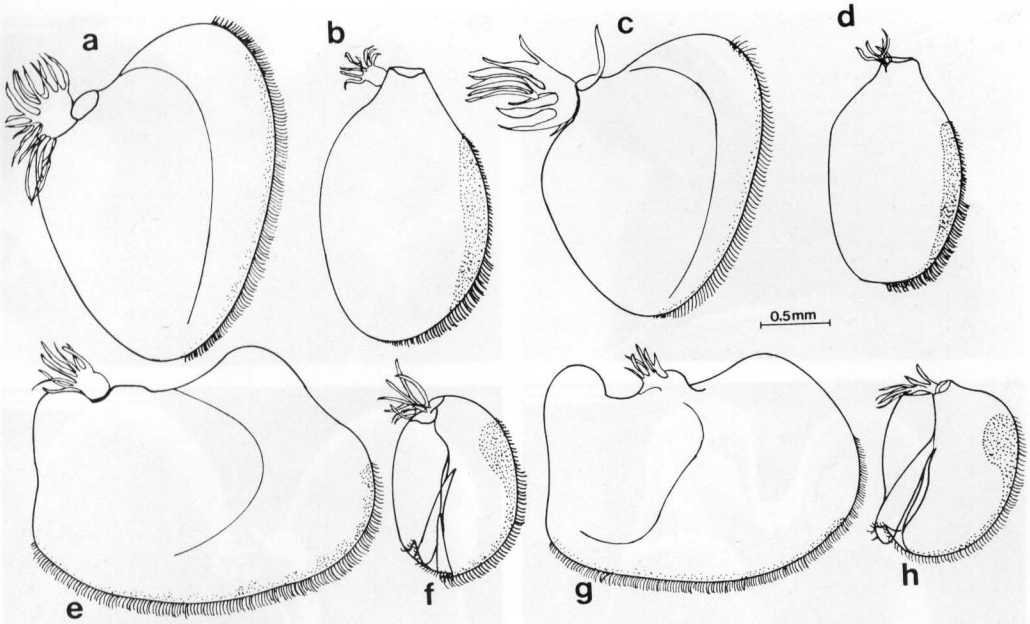
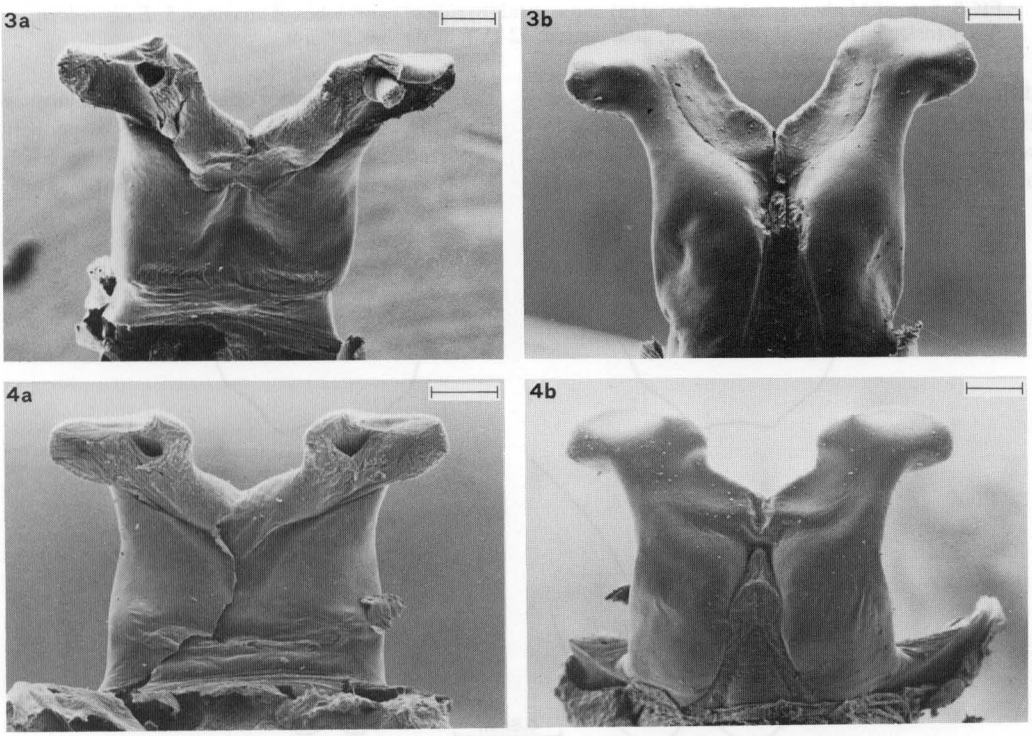
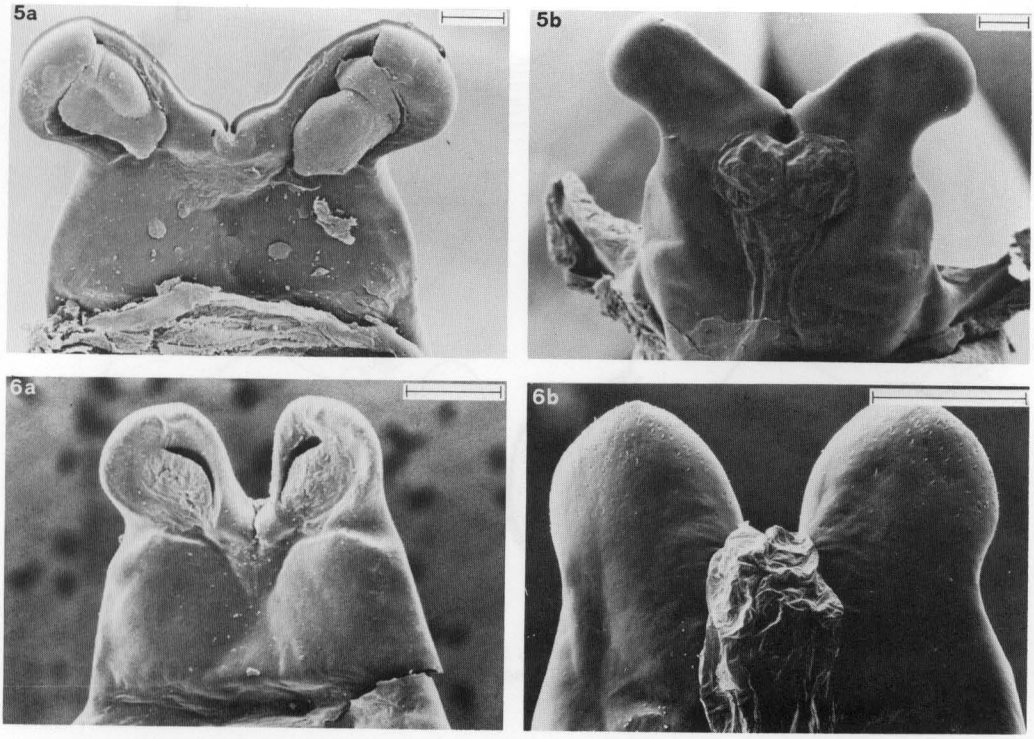


FIG. 2. First (left) and seventh (right) tracheal gill. a–b, *E.sylvicola*; c–d, *E.torrentium*; e–f, *E.alpicola*; g–h. *E.yougoslavicus*. (a–f after Eaton (1883–88) and Belfiore (1983).)



FIGS. 3–4. SEM photographs of dorsal (a) and ventral (b) side of penis-lobes of imagines: 3, *E.sylvicola*; 4, *E.torrentium* (critical point dried, gold coated, 12 kV). scale line: 100 μm.



FIGS. 5–6. SEM photographs of dorsal (a) and ventral (b) side of penis-lobes of imagines: 5, *E.alpicola*; 6, *E.yougoslavicus* (critical point dried, gold coated, 12 kV). Scale line: 100 μm.

difficult to see with an optical microscope. Berthélemy & Thomas (1967) were not able to separate the larvae of *E.sylvicola* and *E.torrentium*. The difference between the two species is the pilosity of the inner edge of the larval glossae (see Fig. 1b, d). We include here previously reported results (Grandi, 1960; Tshernova, 1974) and give the following detemination keys.

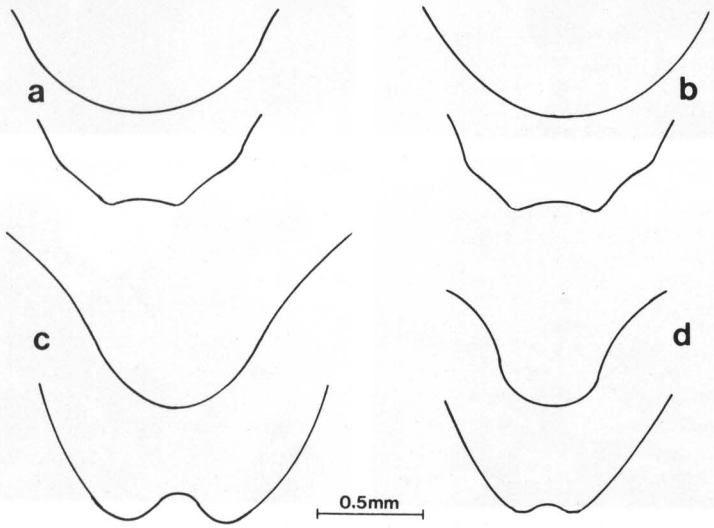


FIG. 7. Subgenital plate (top) and pygidium of female imagines. a, *E.sylvicola*; b, *E.torrentium*; c, *E.alpicola*; d, *E.yougoslavicus*.

Key to larvae

(*Microscopic preparation necessary; ventral markings on the abdominal segments not included, see key to imagines).

1 (4) First pair of tracheal gills heart-shaped (Fig. 2a, c), does not cover the first abdominal segment ventrally. First tracheal gill smaller than fourth; seventh gill simple and not folded lengthways (Fig. 2b, d); a clearly defined V-shaped depression in apical part of labrum* (Fig. 1a, c); apex of glossae very hairy.

2 (3) Inner edge of larval glossae with long and short thin hairs* (Fig. 1b), which cover the inner edge up to two-thirds ..... *sylvicola*

3 (2) Inner edge of larval glossae with long thin and short thick hairs* (Fig. 1d), which cover the inner edge about half ..... *torrentium*

4 (1) First pair of tracheal gills kidney-shaped (Fig. 2e, g) and overlaps on ventral side of first abdominal segment; first tracheal gill larger than fourth; the seventh folded lengthways (Fig. 2f, h); a very flat U-shaped depression in apical part of labrum* (Fig. 1e, g); apex of glossae with few to no hairs.

5 (6) Inner edges of larval glossae convex (Fig. 1f); apex of glossae with few hairs ..... *alpicola*

(5) Inner edges of larval glossae nearly straight (Fig. 1h); no hairs on apex of glossae; in contrast to other three *Epeorus*-species it does not have a bulbous form but is flat (index maximal length : width>1.5) ..... *yougoslavicus*

Key to imagines (females and males)

1 (4) Penis-lobes boot-shaped with a triangular incision between them; apex of penis-lobes with a horizontal transverse edge (Figs. 3, 4); subgenital plate in females largely rounded, pygidium with four points (Fig. 7a, b); distal margin of tergites dark except at junction with sternites and in close proximity to them. The dark stripe there turns proximally (Schoenemund, 1930: 19, Fig. 20).

2 (3) Notch between penis-lobes V-shaped (Fig. 3); each sternite with a dark conical spot (Berthélemy & Thomas, 1967: photographs) *sylvicola*

3 (2) Notch between penis-lobes not simple V-shaped but distally parallel (Fig. 4); sternites with semicircular, spatulate or sublinear (chiefly terminal segments) dark spots (Berthélemy & Thomas, 1967: photographs) ..... *torrentium*

4 (1) Penis-lobes thumb-shaped, their apices rounded (Figs. 5, 6); subgenital plate of females converging into a blunt tip, pygidium with deep or small incision (Fig. 7c, d); each tergite with completely dark distal margin (Ulmer, 1929: III, 34, Fig. 127).

5 (6) Penis-lobes separated by a broad V-shaped incision (Fig. 5); each sternite with a medium dark stripe dilated in the middle of each segment (Schoenemund, 1930: 19, Fig. 21) .... *alpicola*

6 (5) Penis-lobes separated by a narrow V-shaped incision. Dark spots on sternites cover terminal margin and form also a large band along the median line (Ikonomov, 1954: 2, Fig.) *E.yougoslavicus*

The characteristics in Figs. 1–7 illustrate the close relationship between *E.sylvicola* and *E.torrentium*. These two species are clearly distinct from *E.alpicola* and *E.yougoslavicus*.

The results of the biochemical investigations are given in Tables 1 and 2 and in Figs. 8 and 9. The Ephemeroptera turn out to be a group which is difficult to differentiate by enzyme electrophoresis. We were able to use only twelve enzyme–substrate systems for the biochemical identification of the Heptageniidae species although we had investigated forty-two enzyme–substrate systems in various buffers. With these twelve systems we could establish sixteen different enzyme loci for the five Heptageniidae taxa. The relative mobilities of the proteins obtained by these sixteen independent enzyme loci were compared within the twelve populations. For the sake of clarity, only the main frequencies of the alleles (electromorphs) are listed in Table 1. This does not affect the conclusions of the present work. APK, α-GPDH, MDH-2 and MPI were identical in their mobility within the four European taxa. Other monomorph enzymes clearly indicated interspecific differences in their mobility, e.g. AK, GOT-2, IPO-2, HK-2, LAP and RDH. ALD, GOT-1, IPO-1, HK-1, MDH-1 and PGM were polymorphic enzymes. Some electrophoretic variants, however, were rare. There are little intraspecific differences (i.e. GOT-1, IPO-1, HK-1, PGM).

The dendrogram (Fig. 9) shows the eleven European populations and their arrangement in four species. Populations of the same species show an $\bar{I}$-value of about 1, because their alleles, with the corresponding frequencies, are similar (see populations 1–5, 7–9, 10–11). The eleven populations split up into two distinct groups: *E.sylvicola–E.torrentium* and *E.alpicola–E.yougoslavicus*; the first group of an $\bar{I}$-value of 0.85, the second with an $\bar{I}$-value of 0.66. Both groups are clearly separated at a level of 0.36. For the diagnostic enzymes AK, GOT-2, IPO-2 and RDH we obtained only one allele for each

TABLE 1. Mean allelic frequencies of sixteen different enzymes (abbreviations see p. 257); 1–5, *Epeorus sylvicola*; 6, *E.torrentium*; 7–9, *E.alpicola*; 10–11, *E.yougoslavicus*; 12, *Iron longimanus*.

Pop.	1	2	3	4	5	6	7	8	9	10	11	12
AK												
100	1.0	1.0	1.0	1.0	1.0	1.0	---	---	---	---	---	---
105	---	---	---	---	---	---	1.0	1.0	1.0	1.0	1.0	---
112	---	---	---	---	---	---	---	---	---	---	---	1.0
APK												
99	---	---	---	---	---	---	---	---	---	---	---	1.0
100	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	---
ALD												
98	---	---	---	---	---	1.0	---	---	---	---	---	---
99	---	---	---	---	---	---	1.0	1.0	1.0	1.0	1.0	---
100	1.0	1.0	.94	1.0	1.0	---	---	---	---	---	---	---
101	---	---	---	---	---	---	---	---	---	---	---	1.0
GOT-1												
96	---	---	---	---	---	---	1.0	1.0	1.0	---	---	---
99	---	---	---	---	---	.89	---	---	---	---	---	---
100	1.0	1.0	1.0	1.0	1.0	---	---	---	---	---	---	---
104	---	---	---	---	---	---	---	---	---	---	---	1.0
105	---	---	---	---	---	---	---	---	---	1.0	.83	---
GOT-2												
100	1.0	1.0	1.0	1.0	1.0	1.0	---	---	---	---	---	---
101	---	---	---	---	---	---	---	---	---	---	---	.92
102	---	---	---	---	---	---	1.0	1.0	1.0	1.0	1.0	---
GPDH												
100	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
IPO-1												
92	---	---	---	---	---	---	---	---	---	---	---	1.0
100	1.0	1.0	.94	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	---
IPO-2												
96	---	---	---	---	---	---	1.0	1.0	1.0	1.0	1.0	1.0
100	1.0	1.0	1.0	1.0	1.0	1.0	---	---	---	---	---	---
HK-1												
98	---	---	---	---	---	---	---	---	---	---	---	1.0
100	1.0	1.0	1.0	1.0	.76	1.0	---	---	---	---	---	---
101	---	---	---	---	---	---	1.0	1.0	1.0	---	---	---
104	---	---	---	---	---	---	---	---	---	1.0	1.0	---
HK-2												
96	---	---	---	---	---	---	---	---	---	---	---	1.0
100	1.0	1.0	1.0	1.0	1.0	1.0	---	---	---	---	---	---
103	---	---	---	---	---	---	1.0	1.0	1.0	---	---	---
113	---	---	---	---	---	---	---	---	---	1.0	1.0	---
LAP												
98	---	---	---	---	---	---	---	---	---	1.0	1.0	---
99	---	---	---	---	---	---	1.0	1.0	1.0	---	---	---
100	1.0	1.0	1.0	1.0	1.0	1.0	---	---	---	---	---	.73
MDH-1												
98	---	---	---	---	---	---	.65	.67	.44	---	---	.67
99	---	---	---	---	---	.76	---	---	---	---	---	---
100	.56	.50	.60	.58	.64	---	---	---	---	.64	---	---
102	---	---	---	---	---	---	---	---	---	---	.60	---
MDH-2												
99	---	---	---	---	---	---	---	---	---	---	---	1.0
100	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	---
MPI												
100	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	---
102	---	---	---	---	---	---	---	---	---	---	---	1.0
PGM												
97	---	---	---	---	---	---	1.0	1.0	1.0	---	---	---
100	1.0	1.0	1.0	.85	.54	.82	---	---	---	---	---	---
102	---	---	---	---	---	---	---	---	---	1.0	1.0	.71
RDH												
98	---	---	---	---	---	---	---	---	---	---	---	1.0
99	---	---	---	---	---	---	1.0	1.0	1.0	1.0	1.0	---
100	1.0	1.0	1.0	1.0	1.0	1.0	---	---	---	---	---	---

group (Table 1). There are different enzymes, e.g. GOT-1 (see Fig. 8) which allow the separation of species. *Iron longimanus*, however, clusters with the low $\bar{I}$-value of 0.16. There are fewer common alleles in comparison with the European taxa (α-GPDH, LAP, MDH-1 and IPO-2); the highest genetic similarity is to populations of *E. alpicola* and *E.yougoslavicus* ($19 \leq \bar{I} \leq 21$), whilst the lowest is to *E.torrentium* ($\bar{I}=14$), the type species for *Epeorus* (Table 2).

Discussion

We have chosen the four morphologically well-defined European *Epeorus* species to test forty-

TABLE 2. Correlation matrix of the relative mobilities of sixteen different enzymes for European *Epeorus* populations (1–11) and *Iron longimanus* (12) from North America. Abbreviations: N, number of biochemically separated animals; *tor.*, *E.torrentium*; *you.*, *E.yougoslavicus*.

Pop.	*sylvicola*					*tor.*	*alpicola*			*you.*		*Iron*	*N*
	1	2	3	4	5	6	7	8	9	10	11	12	
1		1.0	0.98	0.98	0.97	0.85	0.35	0.35	0.34	0.37	0.34	0.16	14
2		–	0.99	0.99	0.97	0.85	0.36	0.37	0.35	0.36	0.34	0.17	6
3			–	1.0	0.98	0.82	0.38	0.38	0.35	0.37	0.34	0.18	15
4				–	0.97	0.82	0.37	0.38	0.35	0.37	0.35	0.19	11
5					–	0.83	0.42	0.42	0.41	0.37	0.35	0.15	9
6						–	0.32	0.32	0.34	0.33	0.33	0.14	8
7							–	1.0	0.98	0.66	0.66	0.21	15
8								–	0.98	0.65	0.65	0.21	10
9									–	0.65	0.66	0.19	11
10										–	0.99	0.21	18
11											—	0.19	5
12												–	12

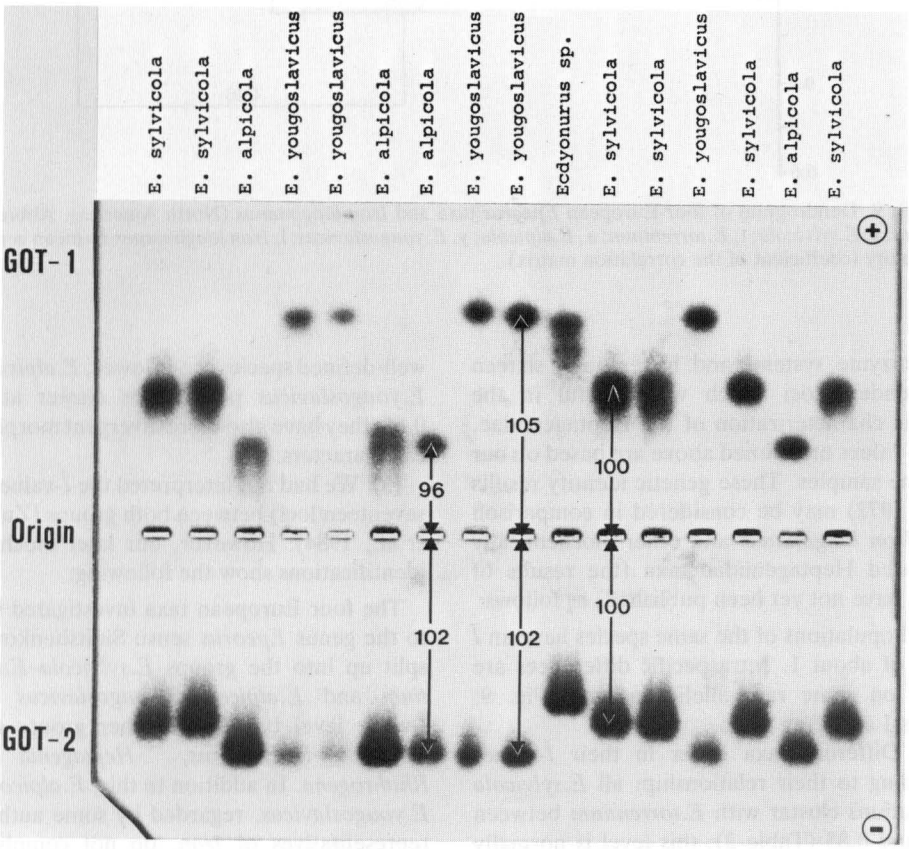


FIG. 8. Enzyme-phenotypes (electromorphs) for two independent loci of the glutamate-oxaloacetate transaminase (starch gel, tris borate EDTA buffer pH 9.0, 5½ h at 12 V/cm). Full details about calculation for the designation of the alleles are given in the text.

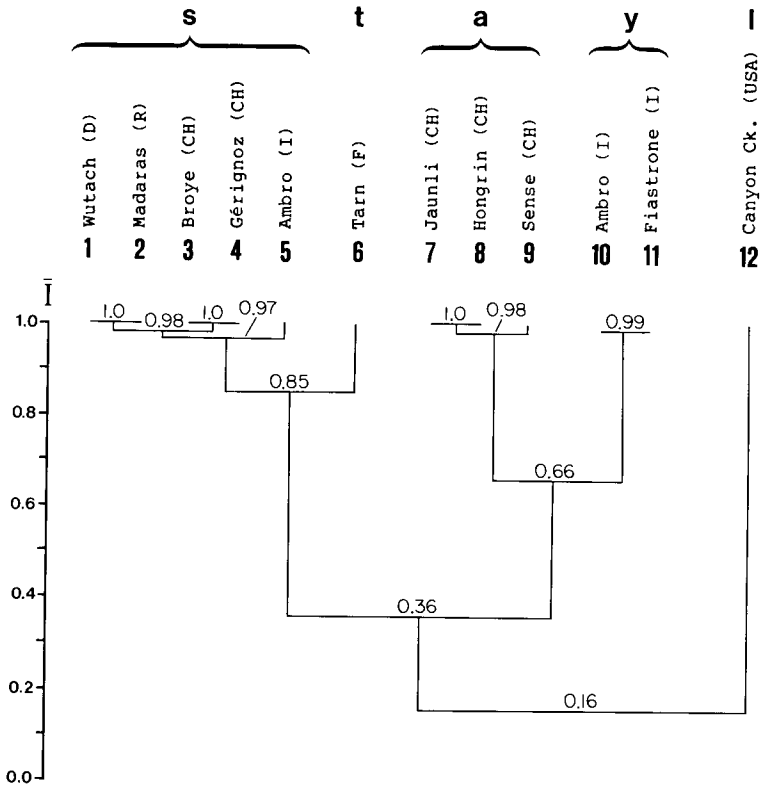


FIG. 9. Dendrogram of four European *Epeorus* taxa and *Iron longimanus* (North America). Abbreviations: s, *E.sylvicola*; t, *E.torrentium*; a, *E.alpicola*; y, *E.yougoslavicus*; l, *Iron longimanus*; $\bar{I}$=mean genetic identity (coefficient of the correlation matrix).

two enzyme systems and have found sixteen independent loci which were useful in the routine characterization of the Heptageniidae. The $\bar{I}$-values mentioned above are based on our enzyme samples. These genetic identity results (Nei, 1972) may be considered in comparison with *Iron longimanus* and other biochemically separated Heptageniidae taxa (the results of which have not yet been published) as follows:

(1) Populations of the same species have an $\bar{I}$ value of about 1. Intraspecific differences are based on some rare allelic variants (Fig. 9; Tables 1 and 2).

(2) Different taxa differ in their $\bar{I}$-values according to their relationship: all *E.sylvicola* populations cluster with *E.torrentium* between 0.82 and 0.85 (Table 2); this level is normally found with closely related taxa of the same species group, e.g. representatives of the *Rhithrogena hybrida*-group. $\bar{I}$-values between well-defined species range lower; *E.alpicola* and *E.yougoslavicus* populations cluster at 0.65–0.66; they have also more divergent morphological characters.

(3) We had not interpreted the $\bar{I}$-value (0.33; seventeen loci) between both groups (Zurwerra *et al.*, 1984). However, our later biochemical identifications show the following:

The four European taxa investigated belong to the genus *Epeorus* sensu Sinitshenkova and split up into the groups *E.sylvicola–E.torrentium* and *E.alpicola–E.yougoslavicus* at an $\bar{I}$-value level typical for other groups of the genera *Ecdyonurus, Heptagenia* and *Rhithrogena*. In addition to this, *E.alpicola* and *E.yougoslavicus*, regarded by some authors as representatives of *Iron*, do not comply with adult characteristic of *Iron* (see Introduction).

Iron longimanus ($\bar{I}$=0.16) does not belong to the genus *Epeorus*. *Iron* is generically distinct

(Eaton, 1883, and Sinitshenkova, 1978, as opposed to Edmunds *et al.*, 1979). Relying on the differential diagnostic characters of the imaginal stages (Sinitshenkova, 1978, see Introduction) and on our biochemical results we can state confidently that none of the four European taxa under investigation belong to the genus *Iron*.

Our results for the genus *Epeorus* show a high correlation of data obtained with different methods. The usefulness of the biochemical method encourages us to attempt the differentiation of more problematic genera, where morphological characters are difficult to interpret. Biochemical analysis necessitates the use of large numbers of deep-frozen specimens which is an advantage in that conclusions are not drawn from single individuals or from any particular development stage, but from whole populations.

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