EUROPEAN SIPHLONURIDAE (EPHEMEROPTERA): A PHYLOGENETIC SYSTEM FOR THE FOUR GENERA

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ABSTRACT

The four genera of the European Siphlonuridae have been studied on the morphological and biochemical level. We applied the cladistic method of Hennig (1950) to develop a phylogenetic system. The morphology of the larvae and the adults, the structures of the eggs (scanning electron microscopy) and the isoenzyme mobilities detected by starch gel electrophoresis clearly demonstrated a cleavage between two groups: the *Siphlonurus-Parameletus* complex and the *Ameletus-Metreletus* complex.

INTRODUCTION

Usually one tries to find *differences* between the species or the genera studied. That was the purpose of the papers by Malzacher (1981), Soederstroem and Nilsson (1986) and Studemann *et al.* (1988) for the family Siphlonuridae. In order to elaborate a phylogenetic relationship between the representatives of this family, we looked for *similitudes* between two or more taxa. This work presents the results of our investigations, being restricted to the four European genera of the Siphlonuridae.

MATERIAL AND METHODS

All the species studied here were collected in Europe. The complete list of the stations is given in Studemann *et al.* (1988). The eggs were dissected from frozen females in a buffer medium of 50 mM sodium cacodylate, pH 7.4, 2.5% glutaraldehyde and fixed for two hours at room temperature. After washing twice with 50 mM sodium cacodylate, pH 7.4, the eggs were treated during four hours with 1% osmium tetraoxyde-sodium cacodylate buffer, pH 7.4. The steps for dehydration, critical point drying as well as the preparation for the scanning electron micrographs were given in Studemann *et al.* (1987). Details of the method of the enzyme electrophoresis on starch gel and evaluation of the data are fully described in Zurwerra *et al.* (1987). The phylogenetic system has been developed using the cladistic definitions of Ax (1984) and Hennig (1950).

RESULTS

The four European genera of Siphlonuridae are compared morphologically and biochemically:

- *Siphlonurus* Eaton, 1868,
- *Parameletus* Bengtsson, 1908,
- *Ameletus* Bengtsson, 1885,
- *Metreletus* Demoulin, 1951.

The investigations are made on all species given in Table 1. For the morphology, we include *Parameletus minor* Bengtsson, 1909, as well. The wings and the tarsal claws of the imagines are constant within a genus, so that we do not give the drawings for each species. The labium and the maxillae of the larvae show some differences between the five species of *Siphlonurus* concerning the spines. However the characters used here are constant within the genus (distal margin of the maxillae, penultimate segment of the labial palpus). The hind margin of the abdominal tergites of the larva and the form of the gills present the same general aspect in the five *Siphlonurus* species. *S. aestivalis* is drawn as representive of the genus. The chorionic structure of the eggs is very similar in all species of *Siphlonurus* (Fig. 5). The mouthparts, the hind margin of the abdominal tergites and the gills of the larvae of Parameletus minor are very similar to those of *P. chelifer*.

The combination of morphological structures of the larvae, the imagines and the eggs, as well as the electromorphs at two enzyme-loci, gives us the cladogramm shown in Fig.1. For each separation, the apomorphic (new) characters are given.

(1) Synapomorphy for *Ameletus* and *Metreletus*:

- In the larva: maxillae distally broadened, truncate at the apical margin and provided with a row of comb-like bristles (Fig. 2),
- In the imago: tarsal claws dissimilar, one pointed, the other blunt (Fig. 3),
- In the imago male: penis attached to the styliger plate by a membrane (Fig. 4) which plays a part during ejaculation,
- In the egg: exochorion covered with a raised net-like structure (Fig. 6).

(2) Synapomorphy for *Parameletus* and *Siphlonurus*:

- In the larva: partial reduction of the sclerotised band along the fore margin of the gills (Fig. 7),
- In the imago: enzyme MDH-2 with the electromorph 97 or 99 (Table 1).

(3) Autapomorphy for *Parameletus*:

- In the larva: labial palpus with inner apical process on the penultimate segment (Fig. 8),
- In the larva: hind margin of the abdominal tergites without spines (Fig. 9),
- In the imago: vein MP of hind wing simple, unforked (Fig. 10).
- In the imago: enzyme MDH-2 with the electromorph 97 (Table 1).

(4) Autapomorphy for *Siphlonurus*:
- In the imago: enzyme MDH-2 with the electromorph 99 (Table 1).

(5) Autapomorphy for *Ameletus*:
- In the imago: enzyme GPDH with the electromorph 99 (Table 1).

(6) Autapomorphy for *Metreletus*:
- In the imago: cubital sector of the forewings with transversal veins but no intercalaries (Fig. 11).

DISCUSSION

The choice between the apomorphic character and the plesiomorphic one is based on the special feature of the character, on the trend of reduction during the evolution and on the comparison with other families of Ephemeroptera. A character is autapomorph when it is present only in one of the four genera (e.g. unforked MP in hind wings of *Parameletus* or electromorph 99 of GPDH by *Ameletus*). Similar tarsal claws on the imago and complete sclerotised bands along fore and hind margin of the gills are considered as primitive characters. In the mayflies, the larval maxillae mostly present a pointed distal margin, and the truncate form of *Metreletus* and *Ameletus* is apomorph. The enzyme loci of MDH-2 have been compared with the results of Zurwerra *et al.* (1987). This highly conservative enzyme often presents the same electromorph within a genus and most of the Heptageniidae show the electromorph 100. This explains the choice of the electromorph 97 and 99 as apomorphic characters.

All the characters used here let us establish the cladogramm without any convergences, which presents an ideal case. The numerous synapomorphic characters found to separate *Metreletus* and *Ameletus* from the two other genera (1) indicate a close relationship between *Ameletus* and *Metreletus*, both distant from *Siphlonurus* and *Parameletus*. More enzymes are now being tested in our laboratory and results should help develop a cladogramm for all species of the European Siphlonuridae.

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Table 1. List of species investigated, with number of tested animals and populations and with the main electromorphs at two enzyme-loci. GPDH = glycerophosphate dehydrogenase, E.C.1.1.1.8.. MDH-2 = malate dehy drogenase, E.C.1.1.1.37.

Species investigated	Number of specimens	Number of populations	Main electromorph at GPDH-locus	MDH-2-locus
S. aestivalis (EATON, 1908)	20	4	101	99
S. alternatus (SAY, 1824)	9	1	101	99
S. armatus EATON, 1870	6	1	101	99
S. croaticus ULMER, 1919	18	1	101	99
S. lacustris EATON, 1870	32	5	101	99
P. chelifer BENGTSSON, 1908	11	1	101	97
A. inopinatus EATON, 1887	14	2	99	100
M. balcanicus (ULMER, 1919)	11	1	101	100

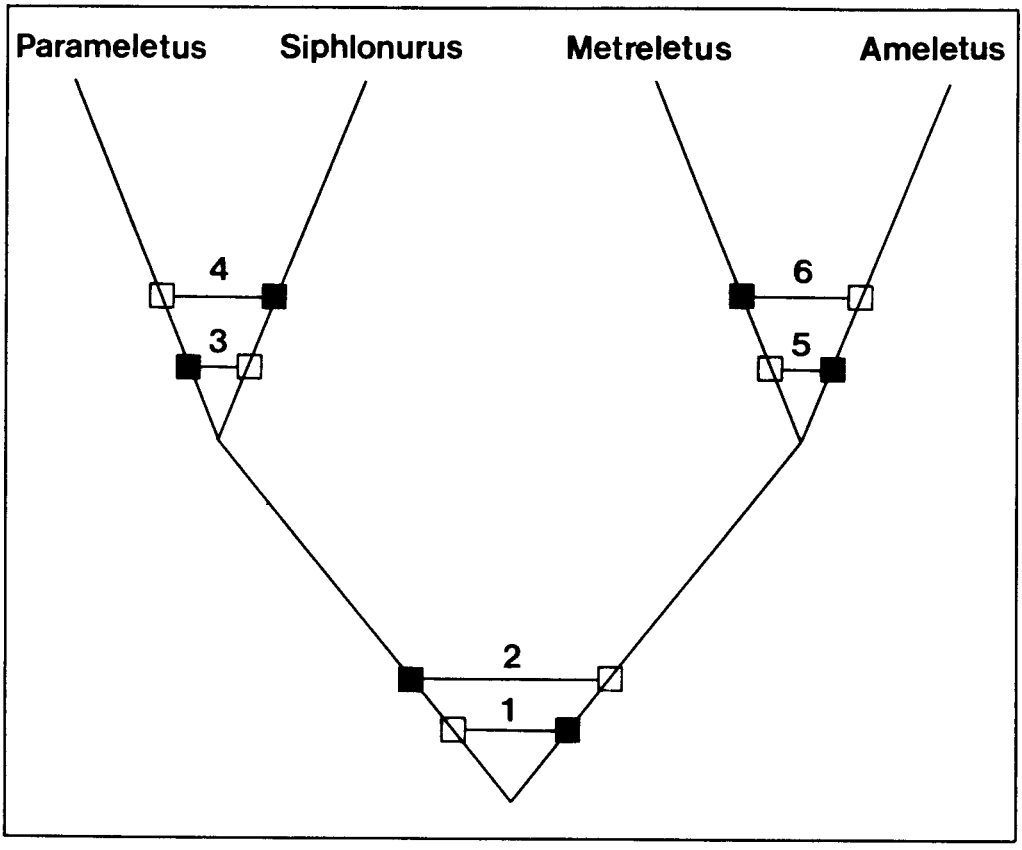

Fig. 1. Cladogramm of the four European genera of Siphlonuridae. Solid square = apomorphic character; open square = plesiomorphic character.

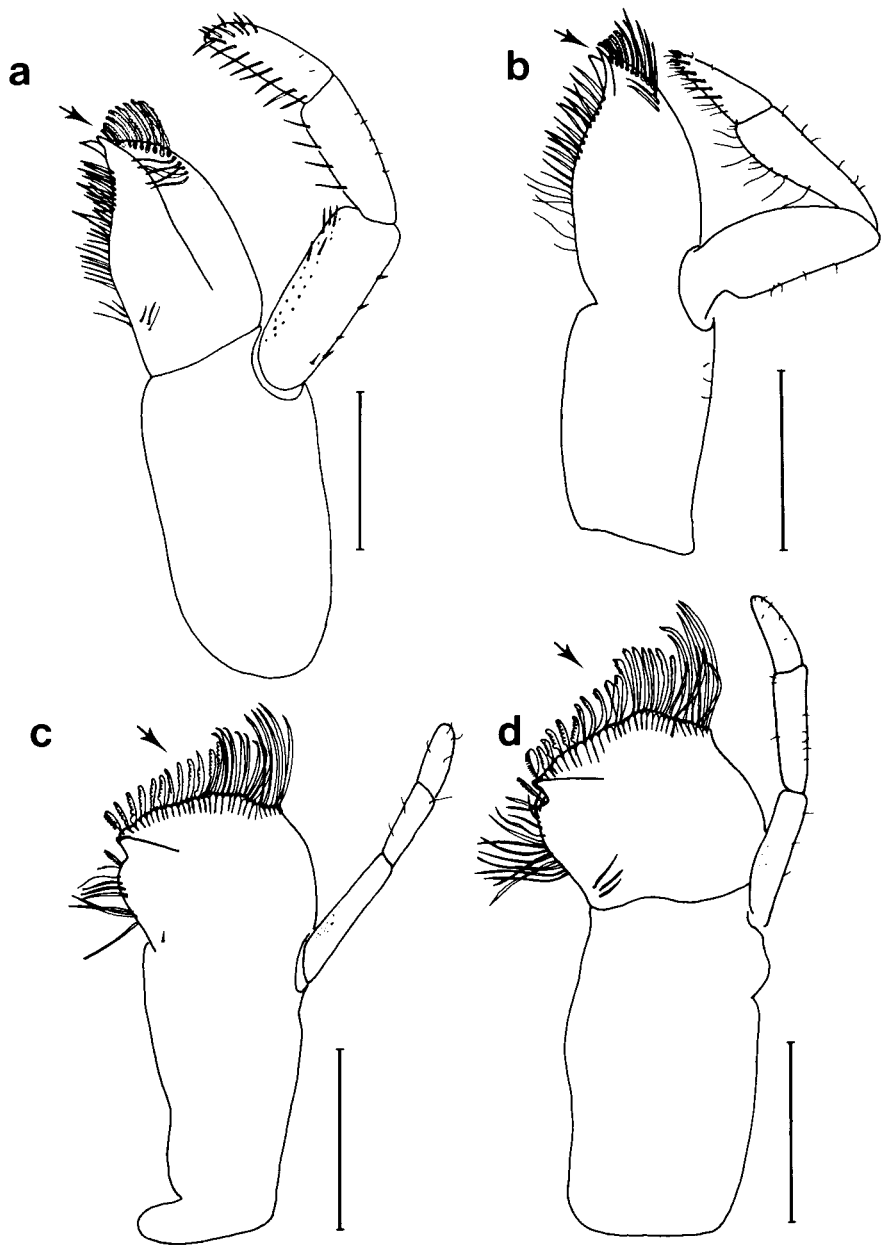

Fig. 2. Left maxilla, ventral view. a = *S. aestivalis*. b = *P. chelifer*. c = *A. inopinatus*. d = *M. balcanicus*. (scale line = 0,25 mm)

Fig. 3. Tarsal claw of mid and hind legs of an imago. a = *Siphlonurus* sp. and *Parameletus* sp. b = *Ameletus* sp. and *Metreletus* sp.

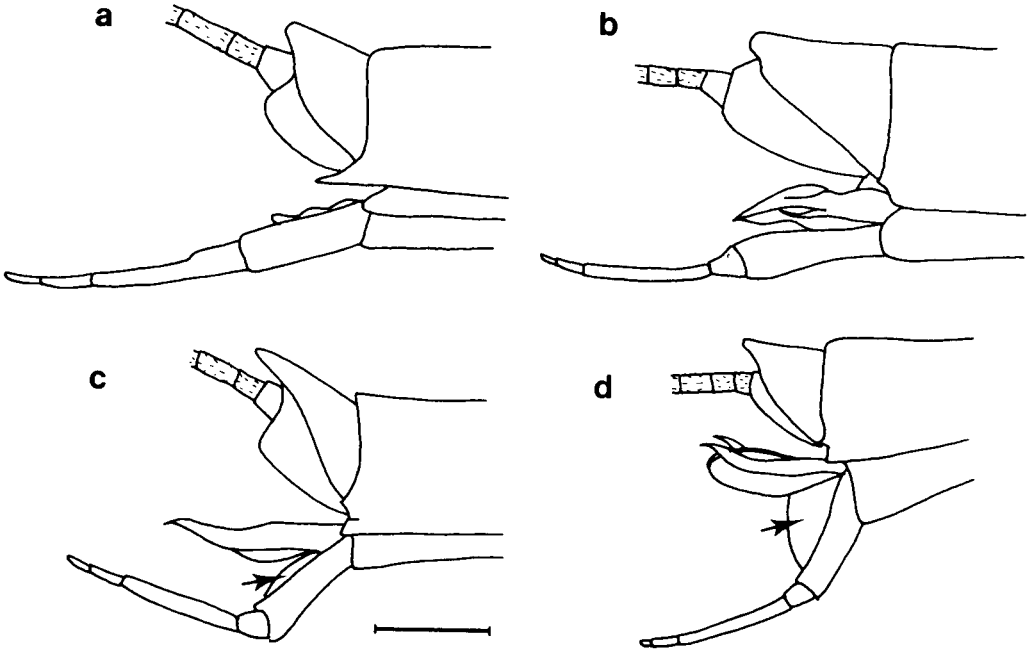

Fig. 4. Terminal abdominal segments of the imago male. a = *S. aestivalis*. b = *P. chelifer*. c = *A. inopinatus*. d = *M. balcanicus*. (scale line = 0,5 mm)

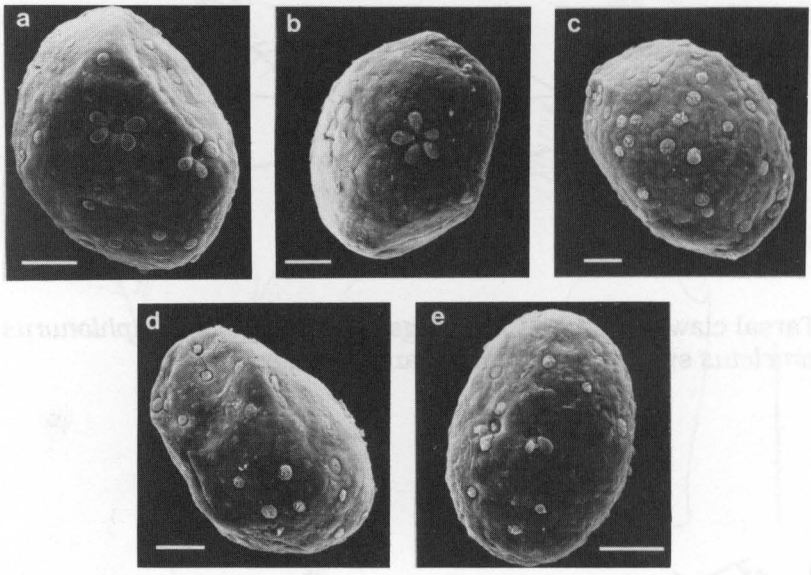

Fig. 5. Eggs of the *Siphlonurus* species: a = *S. aestivalis*. b = *S. alternatus*. c = *S. armatus*. d = *S. croaticus*. e = *S. lacustris*. (scale line = 50 um)

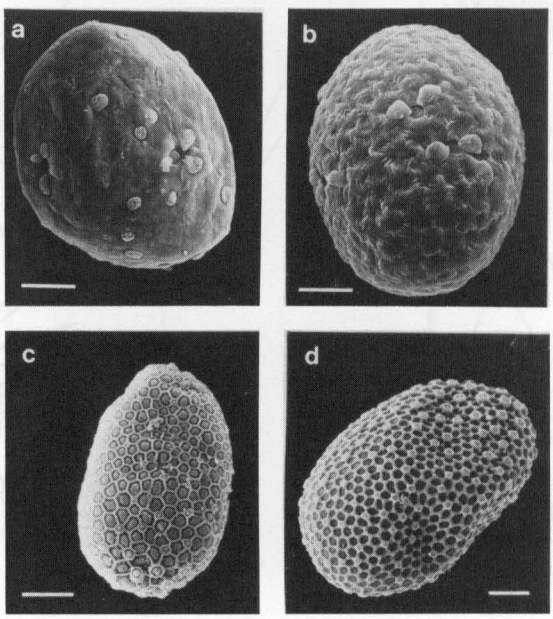

Fig. 6. Eggs of the Siphlonuridae genera. a = *S. aestivalis*. b = *P. chelifer*. c = *A. inopinatus*. d = *M. balcanicus*. (scale line 50 um)

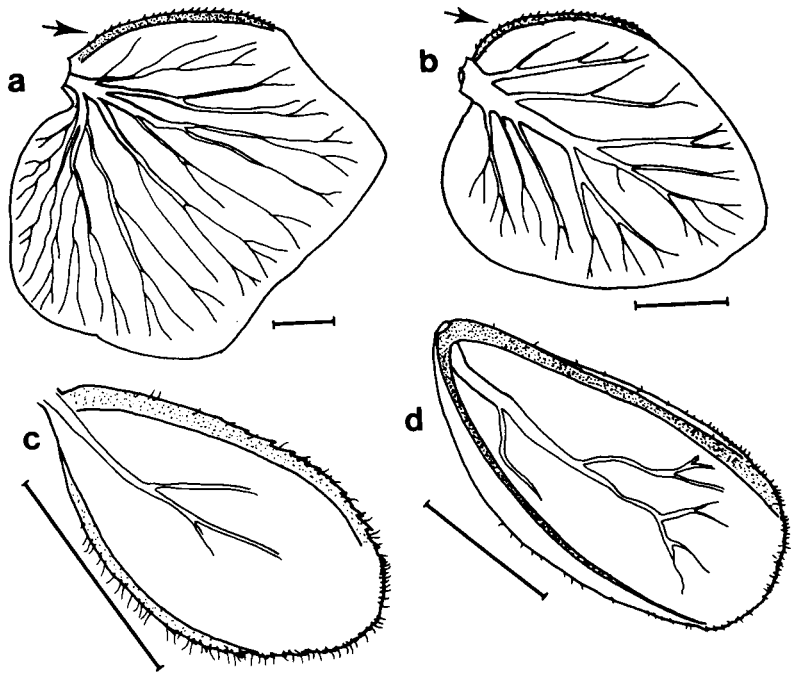

Fig. 7. Third gill of the right side. a = *S. aestivalis*. b = *P. chelifer*. c = *A. inopinatus*. d = *M. balcanicus*. (scale line = 0.5 mm)

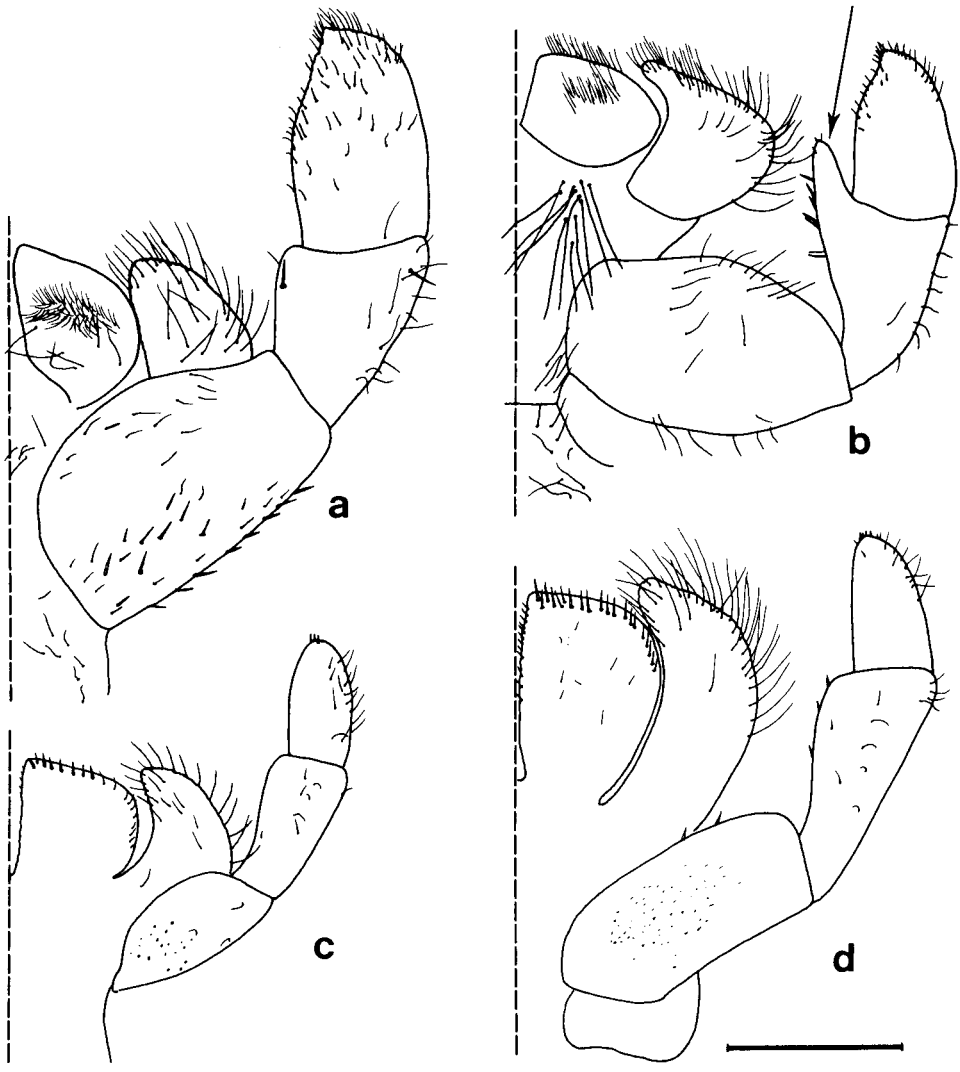

Fig. 8. Left part of labium, ventral view. a = *S. aestivalis*. b = *P. chelifer*. c = *A. inopinatus*. d = *M. balcanicus*. (scale line = 0,25 mm)

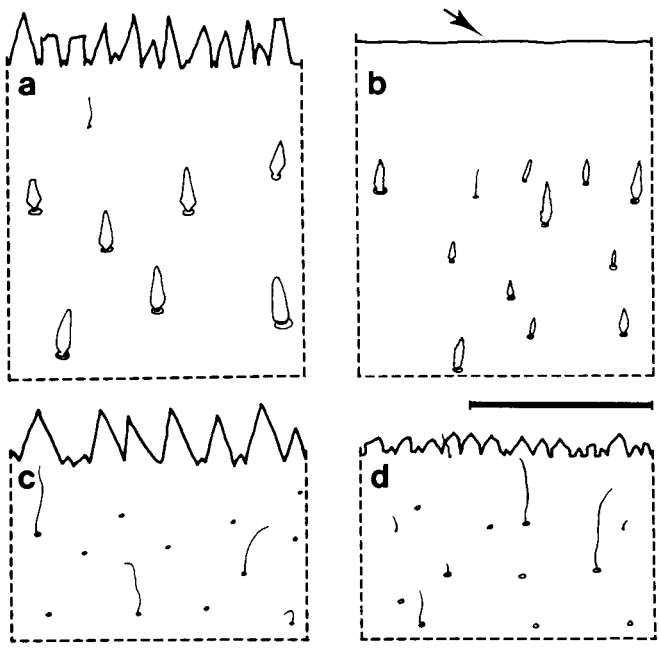

Fig. 9. Hind margin of the 5th abdominal tergite of the nymph. a= *S. aestivalis*. b = *P. chelifer*. c = *A. inopinatus*. d = *M. balcanicus*. (scale line = 0.1 mm)

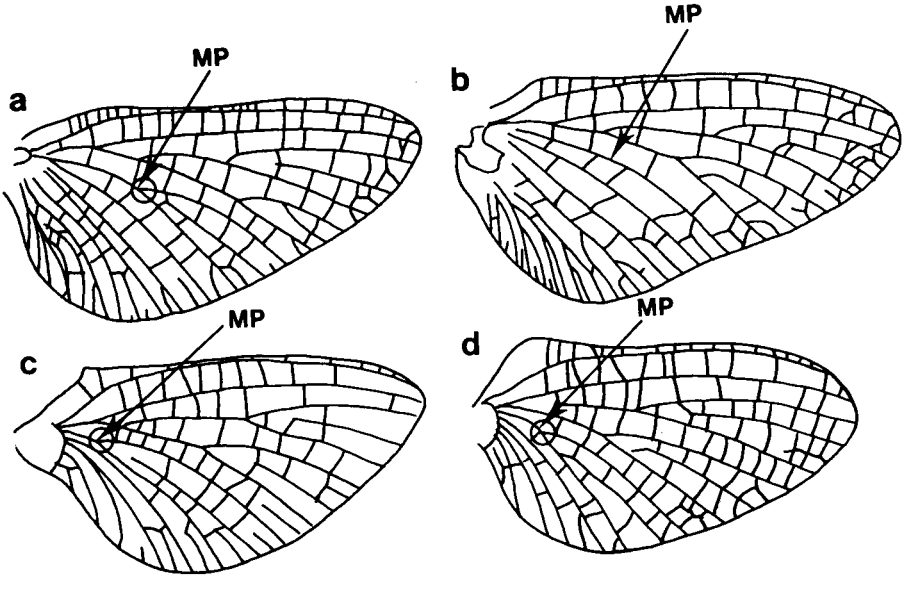

Fig. 10. Hind wing. a = *Siphlonurus* sp. (after Edmunds *et al.*, 1976). b = *P. chelifer* (after Söderström and Nilsson, 1986). c = *A. inopinatus*. d = *M. balcanicus*.

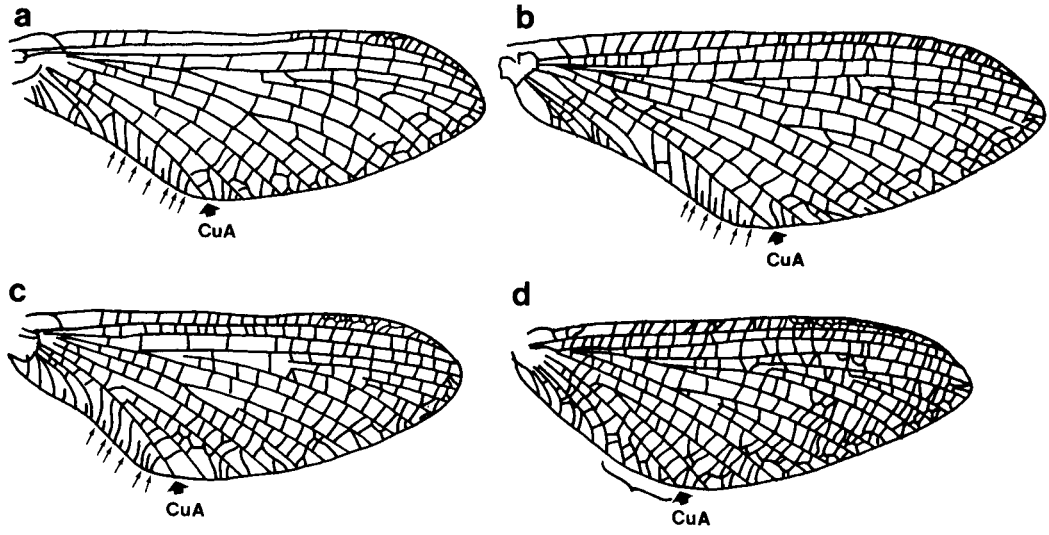

Fig. 11. Forewing. a = *Siphlonurus* sp. (after Edmunds *et al.* 1976). b = *P. chelifer* (after Söderström and Nilsson, 1986). c = *A. inopinatus*. d = *M. balcanicus*.