

A PHYLOGENETIC SYSTEM FOR THE EUROPEAN SPECIES OF *SIPHONURUS* (EPHEMEROPTERA, SIPHLONURIDAE)

DENISE STUEDEMANN & PETER LANDOLT

Entomological Department, Institute of Zoology, University of Fribourg, Pérolles, CH-1700 Fribourg, Switzerland

Eleven European species of *Siphonurus* are investigated on a morphological and biochemical level. Cladistic methods are applied to develop a phylogenetic system for this genus. The three usual groups of species, *S. alternatus*-, *S. lacustris*- and *S. aestivalis*-groups are confirmed. Problems of these methods are discussed, specially the different positions of *S. flavidus*, when studied on a morphological level (chaetotaxy) or biochemical level (electrophoreses).

INTRODUCTION

The status of the Siphonuroidea worldwide has been defined by KLUGE *et al.* (1995) and morphological characteristics have been given for the four genera of Siphonuridae *s. str.*: *Siphonurus* EATON, 1868; *Edmundsius* DAY, 1953; *Parameletus* BENGTTSSON, 1908, and *Siphonisca* NEEDHAM, 1909. The European genera *Siphonurus* and *Parameletus* have been investigated biochemically by STUEDEMANN *et al.* (1994). A first phylogenetic system was presented by STUEDEMANN & TOMKA (1991) for *Siphonurus* and *Parameletus*. In most cases, phylogenetic studies have been applied on high taxons. Few works present phylogenetic diagrams on species level, e. g. HEFTI *et al.*, 1989; BAE & MCCAFFERTY, 1991; PESCADOR & PETERS, 1991; MCCAFFERTY & WANG, 1994;. In the present work, we establish a phylogenetic system for the eleven known European species of *Siphonurus*, using both morphological and biochemical characteristics.

MATERIAL AND METHODS

The species studied are listed in Table 1 with their distribution in Europe. The collecting localities are given in STUEDEMANN *et al.* (1988, 1992, 1994). The Nearctic species *S. occidentalis* EATON, 1885 has been included in the analyses because it is the single species of *S. lacustris*-group investigated biochemically (beside *S. lacustris*). Five further species of *Siphonurus* have been described for Palaearctic: *S. chankae* TSCHERNOVA, 1952 (*S. aestivalis*-group, Far East), *S. immanis* KLUGE, 1985 (*S. aestivalis*-group, Far East), *S. muchei* BRAASCH, 1983 (*S. aestivalis*-group, Turkey), *S. palaearticus* TSCHERNOVA, 1930 (*S. lacustris*-group, Russia), *S. zhelochovtsevi* TSCHERNOVA, 1952 (*S. aestivalis*-group, Far East). However we do not include them in this work, because some characters used here could not be tested on the base of descriptions and

drawings only (e.g. chaetotaxy of larvae) and no electrophoreses could be undertaken.

The biochemical investigations are described in STUEDEMANN *et al.* (1994). Imagines of all the species listed in Table 1 have been investigated biochemically, except *Siphonurus abraxas* and *S. ireneae*. The electromorph frequencies are given in STUEDEMANN *et al.* (1994, Table 3). The main electromorphs at 16 enzymes loci for the species concerned are listed in Table 2, with the explanation of the abbreviations. The word *partim* after an electromorph means that the species presents more than one electromorph for this enzyme locus.

The morphological preparations and observations have been made by the usual methods explained in KLUGE *et al.* (1995).

Standard cladistic methods are essential according to AX (1984). The polarization of the various character states was derived by out-group comparison with the Heptageniidae for the biochemical data (ZURWERRA *et al.* 1987) and specially with the other Siphonuroidea for the morphological characteristics (KLUGE *et al.*, 1995).

Table 1. Species of *Siphonurus* studied with their distribution in Europe.

Species of <i>Siphonurus</i>	Distribution in Europe
<i>abraxas</i> Jacob, 1886	East Mediterranean regions
<i>aestivalis</i> (Eaton, 1903)	all Europe except Great Britain (Altai also)
<i>alternatus</i> (Say, 1824)	North Europe, incl. Great Britain (North America and Far East also)
<i>armatus</i> Eaton, 1870	all Europe except Fennoscandinavia
<i>croaticus</i> Ulmer, 1919-1920	Central Europe
<i>flavidus</i> (Pictet, 1865)	Iberian Peninsula
<i>hispanicus</i> (Demoulin, 1958)	Iberian Peninsula
<i>ireneae</i> Alba-Tercedor, 1990	Iberian Peninsula
<i>lacustris</i> Eaton, 1870	all Europe (Far East also)
<i>lusoenis</i> Puthz, 1977	Iberian Peninsula
<i>montanus</i> Studemann, 1992	Iberian Peninsula

RESULTS

The genus *Siphonurus* is characterized by four electromorphs, typical and common for all species studied, incl. *S. occidentalis*: APK =

100, G3PDH = 102, GOT-1 = 103, MDH-2 = 99, *partim* (Table 2). Fig. 1 depicts the phylogenetic relationships of the eleven known European *Siphonurus* species, obtained with the combination of biochemical and morphological investigations. The following characteristics are presumed to be apomorphies:

1. – male imago: one long lanceolate sclerite on the inner side of each penis lobe (Figs 8 and 10 in STUDEMANN *et al.*, 1988),
 - electromorphs: HK-2 = 105, ME = 106, CK = 97, 6-PGDH = 71, IPO-2 = 95.
2. – larva: loss of one tergalia lamella on abdominal segments 3-7,
 - electromorph: ALD = 97.
3. – larva: one row of bristles on the inner margin of the second segment of maxillary palp atrophied in short setae (MACAN, 1951),
 - two electromorphs common for *S. lacustris* and *S. occidentalis*: CK = 94, IPO-1 = 94 (unknown for *S. abraxas*).
4. – larva: – surface of abdominal tergites provided with big spines (ratio width : length ≥ 0.25) (Table 3)
 - abdominal tergite 10 provided with big spines near the caudolateral concavity (MALZACHER, 1981),
 - male imago: ventral part of the penis composed of two distinct tubes (Figs 2-3),
 - electromorph: CK = 93.
5. – three electromorphs typical for *S. lacustris*: LAP = 99; 6-PGDH = 100; IPO-2 = 96 (unknown for *S. abraxas*).
6. – imago: blackish areas around crossveins in forewing (JACOBS, 1986).
7. – male imago: lateral parts of dorsotransversal sclerite of the penis very narrow (Fig. 5),
 - electromorph: PK = 107.
8. – larva: surface of abdominal sternites provided with big spines (ratio width : length ≥ 0.22) (Table 3).
9. – not yet found.
10. – larva: surface of mesothoracic sternites provided with big spines (ratio width : length ≥ 0.17) (Table 3).
11. – electromorph: PK = 106.

Table 2. Main electromorphs at 16 enzyme loci for 10 species of *Siphonurus*. The apomorphies are bold. (The allele frequencies are given in STUDEMANN *et al.*, 1994).

Abbreviation	Name	<i>alternatus</i>	<i>lacustris</i>	<i>flavidus</i>	<i>hispanicus</i>	<i>lusoensis</i>	<i>montanus</i>	<i>aestivalis</i>	<i>croaticus</i>	<i>armatus</i>
ALD	aldolase	100	97	97	97	97	97	97	97	97
APK	arginine phosphokinase	100	100	100	100	100	100	100	100	100
CK	creatine kinase	97	94	93	93	93	93	93	93	93
GOT-1	glutamate-oxaloacetate transaminase-1	103	103	103	103	103	103	103	103	103
GOT-2	glutamate-oxaloacetate transaminase-2	102	101 102	101	101	101	101	101	101	101
GPDH	glyceraldehyde-3-P-dehydrogenase	102	102	102	102	102	102	102	102	102
HK-2	hexokinase-2	105	106 108	106 104	106 104	106 104	106 104	106 108	106	106 108
IPO-1	indophenol oxidase-1	97	94	97	97		97	97	97	97
IPO-2	indophenol oxidase-2	95	96	93	93	93	93	93	93	93
LAP	leucine aminopeptidase	100	99	100	100	100	100	100	100	100
MDH-2	malate dehydrogenase	99	99	99	99	99	99	99	99	99
ME	malic enzyme	106	100	100	100	100	100	100	100	100
MPI	mannose phosphate isomerase	100	99	100	99	99	98	100	100	100
6PGDH	6-phosphogluconate dehydrogenase	71	100	96	96	96	96	96	96	96
PGM	phosphoglucomutase	102	102	104	103	103	103	103	103	101
		103	103		104			104	104	
		104							105	
PK	pyruvate kinase	105	105	107	106	106	106	105	108	108

Table 3. Spines and bristles on sternites and tergites of *Siphonurus* larvae.

	<i>S. aestivalis, armatus, croaticus, montanus, lusoensis, hispanicus</i>	<i>S. ireneae</i>	<i>S. flavicus</i>	<i>S. alternatus, lacustris</i>
abdominal tergites				
spines: ratio width / length		0.3 (0.25 - 0.33)		0.19 (0.16 - 0.20)
abdominal sternites				
spines: ratio width / length		0.27 (0.22 - 0.30)		0.14 (0.10 - 0.18)
mesothoracic sternites				
spines: ratio width / length		0.19 (0.17-0.22)		0.08 (0.07 - 0.10)

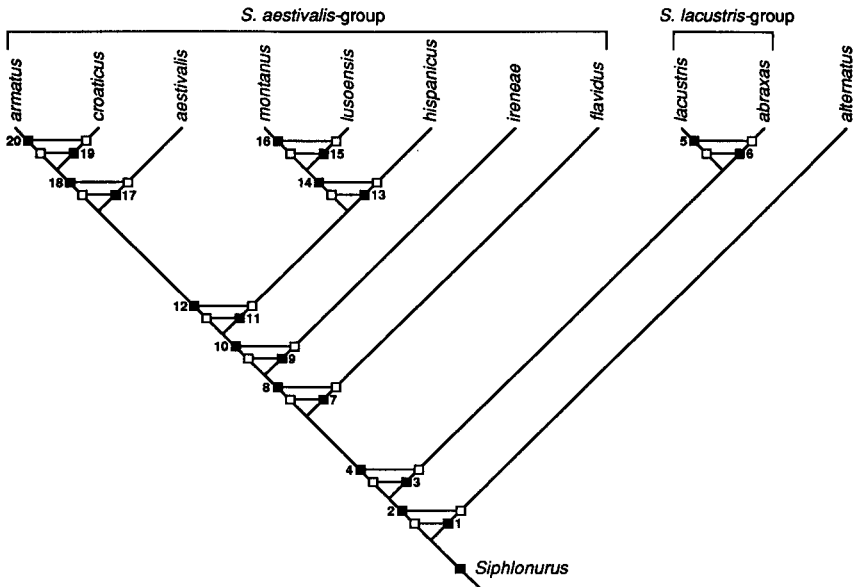
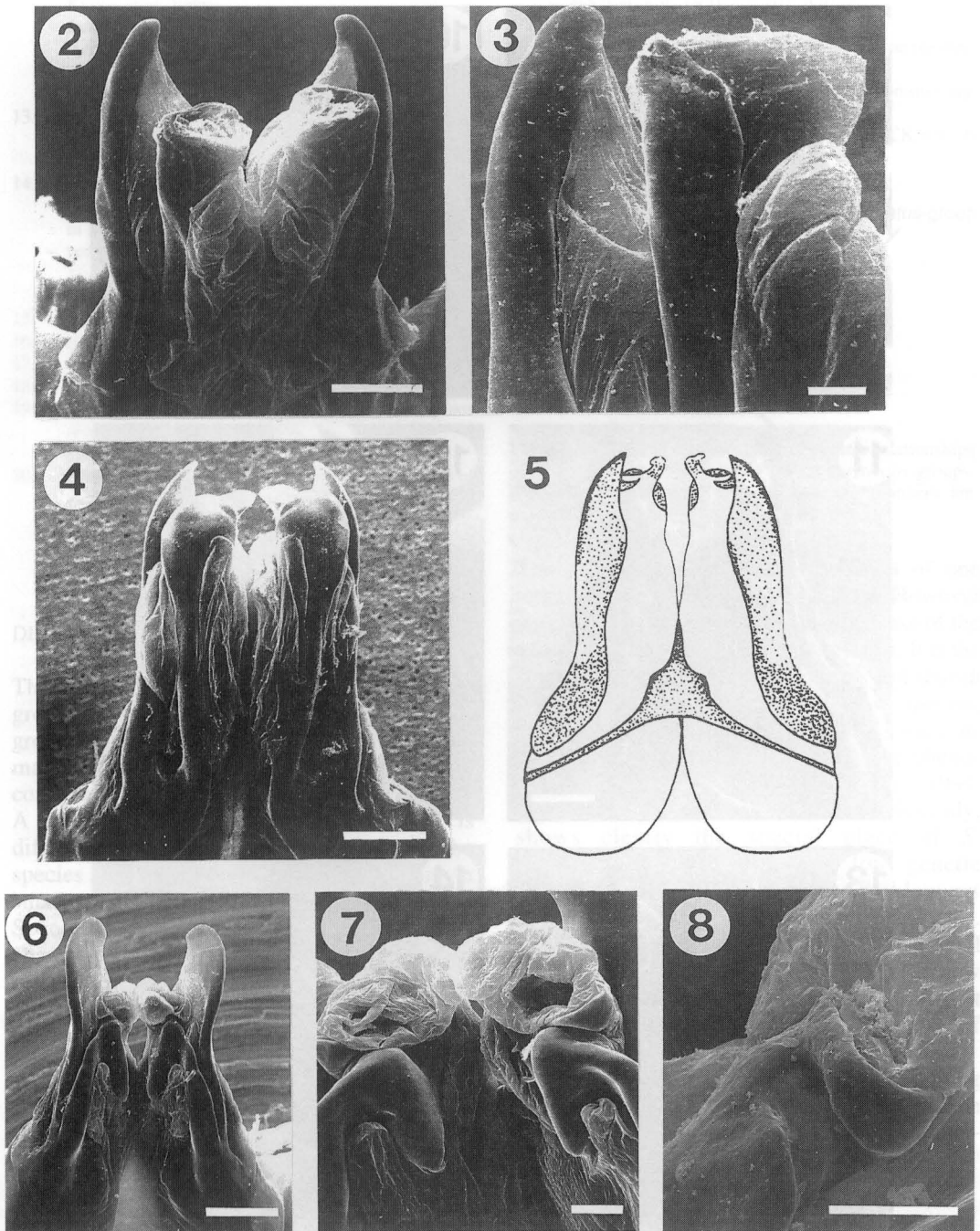
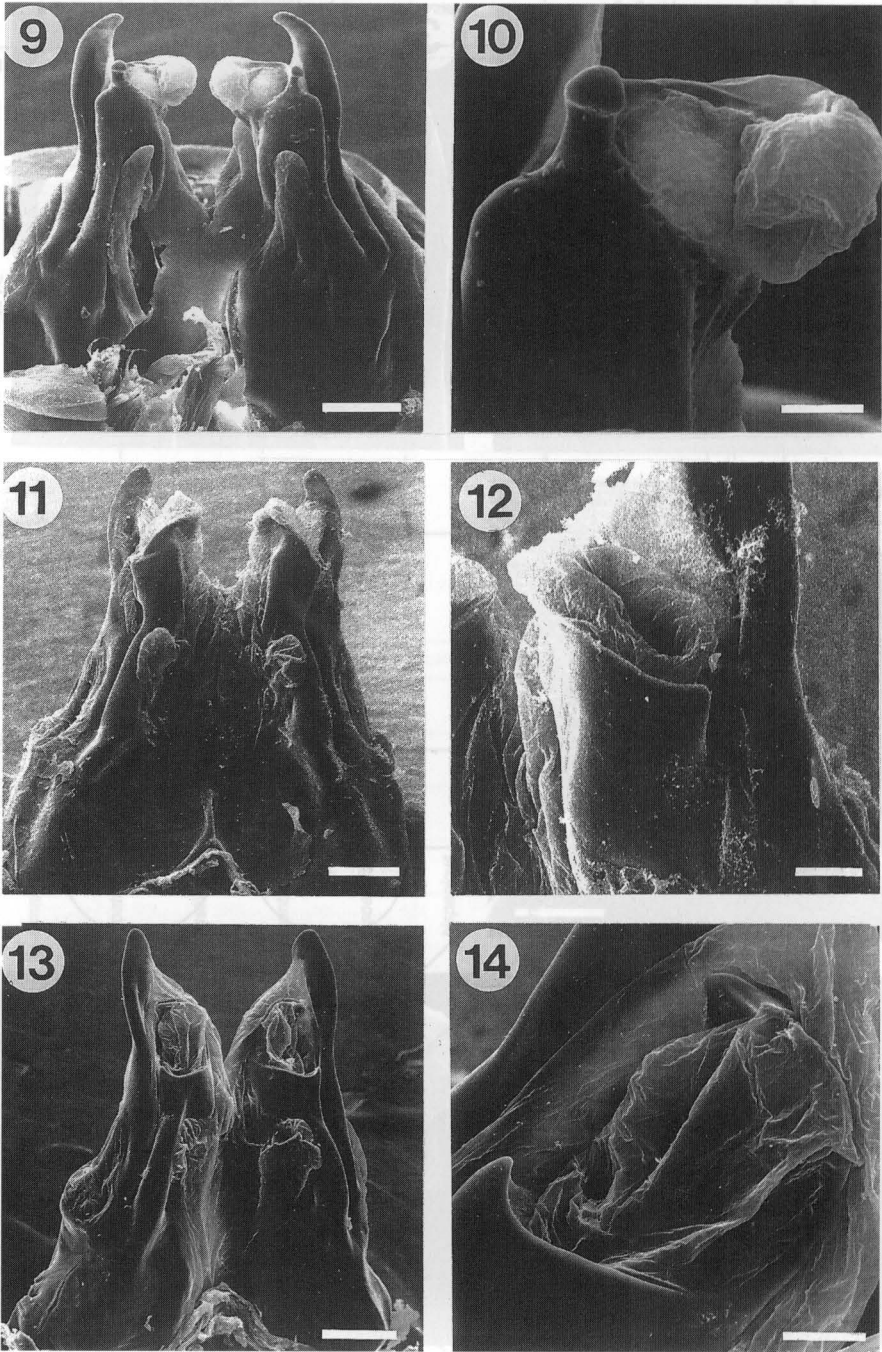


Fig. 1. Diagram of phylogenetic relationships of the European *Siphonurus* species. Black squares represent apomorphies, the numbers are explained in the text.



Figs 2-8. Male genitalia of *Siphonurus* species. 2, 3, 4, 6, 7, 8: ventral view, 5: dorsal view; 2-3: *S. ireneae*; 4-5: *S. flavidus*; 6: *S. armatus*; 7: *S. croaticus*; 8: *S. aestivalis*; (scale lines 2, 4, 6: 200 μm ; scale lines 3, 7, 8: 50 μm).

Table 1. Species and habitats of stemites and tergites of *Siphonurus* larvae.



Figs 9-14. Male genitalia of *Siphonurus* species, ventral view. 9, 10: *S. hispanicus*; 11, 12: *S. lusoensis*; 13, 14: *S. montanus* (scale lines 9, 11, 13: 200 µm; scale lines 10, 12, 14: 50 µm).

12. – female imago: copulatory pouch composed of two distinct strongly sclerotized rounded lobes (Fig. 9 in KLUGE *et al.* 1995),
 - male imago: apicolateral part of ventral sclerite of the penis split transversally (Figs 6-8),
 - electromorph: MDH-2 = 98, *partim* (convergence with *S. alternatus*).
13. – male imago: penis provided with a pad on the gonoporus (Figs 9-10)
 - electromorph: CK = 96, *partim*.
14. – male imago: gonoporus nearly completely bordered with two strongly sclerotized bows (Figs. 6 and 33 in STUDEMANN *et al.*, 1992),
 - ventral sclerite of the penis without projection (Figs 11-14)
 - electromorph: ALD = 99, *partim*.
15. – electromorph: 6-PGDH = 99, *partim*.
16. – electromorph: MPI = 98, *partim*.
17. – not yet found.
18. – electromorph: PK = 108.
19. – larva: mentum provided with stout and broad spines (Fig. 13 in MALZACHER, 1981),
 - electromorph: 6-PGDH = 104, *partim*.
20. – male and female imago: posterolateral projections of tergites 8 and 9 enlarged (Fig. 13 in STUDEMANN *et al.*, 1988),
 - electromorph: PGM = 101.

21. – male imago: posterolateral projections of tergites 9 lost
 - electromorph: GOT-2 = 102, *partim*.
4. – unchanged.
22. – male imago: one long lanceolate sclerite on the inner side of each penis lobe,
 - larva: double tergalia lamella on abdominal segments 1 to 7,
 - electromorphs: HK-2 = 105, ME = 106, CK = 97, 6-PGDH = 71, IPO-2 = 95.
3. – unchanged.

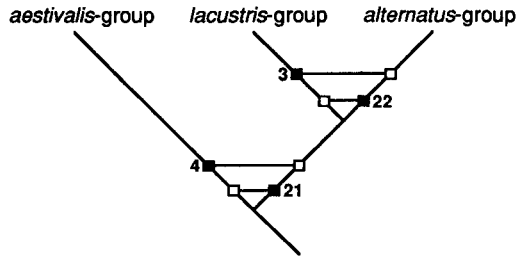


Fig. 15. Alternative diagram of phylogenetic relationships of *S. alternatus*-, *S. lacustris*- and *S. aestivalis*-groups. Black squares represent apomorphies, the numbers are explained in the text.

DISCUSSION

The three usual groups of species, *S. alternatus*-group, *S. lacustris*-group and *S. aestivalis*-group (MALZACHER, 1981; JACOB, 1986) are maintained in the present phylogenetic configuration of relationships (Fig. 1).

A phylogenetic study at species level is difficult. Firstly, because in most cases the species are alike and consequently have only a small number of different characteristics. Secondly, the polarization of the various character states (apomorphy or plesiomorphy) can be sometimes hazardous, for example if out-group comparison cannot be applied. The difficulty increases with electrophoretical data. As often as not, there are more than two electromorphs for one enzyme locus, so that one common electromorph for a group of species can be a good distinctive character but not always an apomorphy. That problem is illustrated by the two following examples, for which alternative diagrams are discussed.

Fig. 15 depicts the variant grouping *S. lacustris*- and *S. alternatus*-groups, with the following apomorphies:

The probabilities of loss or addition of one tergalia lamella are genetically equal. However we chose the first variant (Fig. 1) because of the geographic distribution of *S. alternatus*. It is the single species present in Palaearctis and also in Nearctis. It is probably the oldest species, carrying plesiomorphic characters, such as double tergalia lamellae. Moreover the phenogram presented by STUDEMANN *et al.* (1994, Fig. 1), using biochemical characteristics only, shows clearly the special place of *S. alternatus*, which presents a low genetic identity value with all other *Siphonurus* species (Table 4 in STUDEMANN *et al.*, 1994). BENTSSON (1909) mentioned the special place of *S. alternatus* by establishing the new genus *Siphurella* for this species. At present *Siphurella* is held to be a subgenus of *Siphonurus* (HUBBARD, 1990).

The second example concerns the grouping inside the *S. aestivalis*-group, specially the position of *S. flavidus*. Fig. 16 depicts an alternative diagram with following apomorphies:

4. – unchanged (*S. aestivalis*-group).
12. – unchanged.
31. – endemic in Iberian Peninsula,
 - male and female imago: forewing of living animal with red spot in C and Sc proximal areas (STUDEMANN *et al.*, 1992),

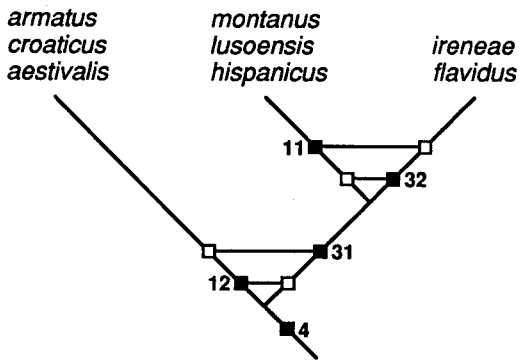


Fig. 16. Alternative diagram of phylogenetic relationships inside *S. aestivalis*-group. Black squares represent apomorphies, the numbers are explained in the text.

- electromorph: HK-2 = 104, partim.
- 11. – unchanged.
- 32. – larva: surface of mesothoracic sternites provided with long and thin setae (convergence with *S. alternatus* and *S. lacustris*).

In this case, the five endemic species in the Iberian Peninsula would have the same origin. However the long, thin setae found on the mesothoracic sternites of *S. flavidus* and *S. ireneae* seem to be rather plesiomorphic, because they are found in *S. alternatus*, *S. lacustris*, *S. abraxas* and many other mayflies. Besides, in the phenogram presented by STUEDEMANN *et al.* (1994, Fig. 1), using biochemical data only, *S. flavidus* is closely related to *S. croaticus* and *S. armatus*. In this situation, biochemical, morphological and geographical data give different results.

It would be useful to examine more internal morphological characters and to investigate DNA sequences of these species to clarify uncertain phylogenetic positions.

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