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Biochemical investigations of Siphlonuridae and Ameletidae (Ephemeroptera)

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With 1 figure and 5 tables in the text

Abstract

Thirteen European and four North American species of Siphlonuridae and Ameletidae were investigated biochemically using starch gel electrophoresis. Twenty enzyme loci were examined in 28 populations. In comparison with *Siphlonurus aestivalis, S. croaticus* exhibits different electromorphs for two enzymes, and thus maintains the species status. Two common electromorphs and high identity values with the other species of the *S. aestivalis*-group allow one to place *S. flavidus* in the *S. aestivalis*-group. For three enzyme loci the *Ameletus* species share common electromorphs, different from those of *Metreletus balcanicus.* This fact allows one to consider *Metreletus* as a valid genus. Because of the few common enzyme electromorphs and the low identity values, the *Ameletus-Metreletus*-complex should be separated from the *Siphlonurus-Parameletus*-complex and included in the Family Ameletidae.

Introduction

The Families Siphlonuridae and Ameletidae include fifteen European species split into four genera. Recent revisions of these families consist of morphological investigations (Puthz 1977, Malzacher 1981, Söderström & Nilsson 1986, Studemann et al. 1988, 1992 a, 1992 b).

The following taxonomical problems have not yet been solved by morphological investigations. *Siphlonurus croaticus* seems to be very closely related to *S. aestivalis* (Jacob 1986). *S. lusoensis* and *S. montanus* present morphological differences at the imaginal stage only (Studemann et al. 1992 b). *S. flavidus* possesses too few morphological characteristics to be included in the *S. aestivalis*-group (Jacob 1986, Studemann et al. 1992 b). The genus *Metreletus* Demoulin, 1951 was synonymised with *Ameletus* Bengtsson, 1885 by Jacob (1984). *Metreletus* and *Ameletus* differ so much from *Siphlonurus* Eaton, 1868 and *Parameletus* Bengtsson, 1908 that they could belong to a separate family (McCafferty 1991, Studemann & Tomka 1991, Studemann et al. 1992 a, Tomka & Elpers 1991).

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Biochemical methods assayed on Ephemeroptera were used successfully to solve taxonomical problems in the Family Heptageniidae (Belfiore et al. 1992, Hefti et al. 1988, 1989, Hefti & Tomka 1989, Imhof et al. 1988, Zurwerra & Tomka 1985, Zurwerra et al. 1986, 1987), in the Family Ephemerellidae (Funk et al. 1988, Sweeney et al. 1986) and in some genera of the Siphlonuridae (Söderström & Nilsson 1986, Studemann & Tomka 1991). We used starch gel electrophoresis of isozymes to solve taxonomical problems of the European Siphlonuridae and Ameletidae.

Materials and methods

The species that were investigated biochemically are listed in Table 1 with their localities and the number of specimens tested. Amongst the 15 European species of Siphlonuridae and Ameletidae, 13 are involved in this study. *Siphlonurus abraxas* Jacob, 1986 and *Siphlonurus ireneae* Alba-Tercedor, 1990 could not be tested, because we did not have fresh or frozen imagines. Most of the specimens were collected by the authors and the supplementary informations such as date of collection, flight-period, and ecological data of the rivers are given in Studemann et al. (1988, 1992 b). *Parameletus minor* was provided by Dr. Olle Söderström. The American species (*Siphlonurus occidentalis, Ameletus* sp., *A. subnotatus* and *A. validus*) were collected by Dr. Thomas Fink. Most of the specimens were collected as larvae and reared to the imaginal stage (methods described in Studemann et al. 1992 a). Fresh or frozen adults were used for the biochemical investigations.

Isozymes were separated by vertical starch gel electrophoresis using methods similar to those described by Ayala et al. (1972), with recipes from Harris & Hopkinson (1976) and Richardson et al. (1986). We assayed 17 enzymes including 20 presumed loci. The enzymes and the buffers are listed in Table 2.

For each gene locus, the position of the electromorph of a particular sample was compared with that of the reference *Epeorus sylvicola* (Pictet 1865) (index 100). The relative mobility index defining the electromorphs (Table 3) was evaluated as described by Zurwerra et al. (1986) and the allelic frequencies for each taxon were calculated (Ferguson 1980). The correlation of the allelic frequencies for pairs of taxa (Table 4) followed Nei (1972); the identity values between two taxa are indicated as I-values; the identity values calculated between more than two taxa are called mean identity values (= Ī-values). The identity values obtained were compared with genetic distances (D) given in the literature with the conversion formula $D = -\ln I$. The phenogram (Fig. 1) was constructed by the unweighted pair-group method of cluster analysis (UPGMA, Sneath & Sokal 1973). The five populations of *Siphlonurus lacustris* were compared by heterozygosity analysis (Ferguson 1980), based on the theory of Nei (1978).

Results

The allelic frequencies of the 20 enzyme-loci for the 28 populations are given in Table 3. The taxa are arranged according to their affinities and the enzymes alphabetically according to the abbreviations given in Table 2. The enzymes GOT-2, IPO-2 and MDH-2 migrated cathodally, 6PGDH both anodally and cathodally, while the other enzymes migrated anodally. The relatively conservative enzymes are those for which only three or four electro-

Table 1. Species studied electrophoretically with collecting place and number of specimens tested (= n).

Species	Collecting place (river, county and country)	Abbreviation	n
Siphlonurus aestivalis (EATON)	Fichtenbergerrot, Baden-Württemberg, Germany	aes G	9
Siphlonurus aestivalis (EATON)	Bievjavaejjokka, Finnmark, Norway	aes N	25
Siphlonurus alternatus SAY	Vindelälven, Västerbotten, Sweden	alt	24
Siphlonurus armatus EATON	Sperbersbach (pool), Baden-Württemberg, Germany	arm p	18
Siphlonurus armatus EATON	Sperbersbach (brooklet), Baden-Württemberg, Germany	arm b	4
Siphlonurus croaticus ULMER	Obrh, Slovenia, former Yugoslavia	cro Yu	36
Siphlonurus croaticus ULMER	Zwiefalter, Baden-Württ., Germany	cro G	6
Siphlonurus flavidus (PICTET)	Roduelos, Segovia, Spain	fla	6
Siphlonurus hispanicus DEMOULIN	Tenebrilla, Salamanca, Spain	his SS	10
Siphlonurus hispanicus DEMOULIN	Guadalupejo, Caceres, Spain	his SC	9
Siphlonurus lacustris EATON	Bregenzer Aach, Vorarlberg, Austria	lac AV	33
Siphlonurus lacustris EATON	Grünsee, Steiermark, Austria	lac AS	8
Siphlonurus lacustris EATON	Fätschbach, Uri, Switzerland	lac CH	23
Siphlonurus lacustris EATON	Manzanares, Madrid, Spain	lac S	9
Siphlonurus lacustris EATON	Navaccia, Corsica, France	lac F	10
Siphlonurus lusoensis PUTHZ	Liria, Beira Baixa, Portugal	lus PL	6
Siphlonurus lusoensis PUTHZ	affl. of Tejo, Beira Baixa, Portugal	lus PT	20
Siphlonurus montanus STUDEMANN	Manzanares, Madrid, Spain	mon SM	3
Siphlonurus montanus STUDEMANN	Sangusin, Salamanca, Spain	mon SS	15
Siphlonurus occidentalis MCDUN.	USA	occ	3
Parameletus chelifer BENGSSTON	Bievjavaejjokka, Finnmark, Norway	che N	30
Parameletus chelifer BENGSSTON	Vindelälven, Västerbotten, Sweden	che S	11
Parameletus minor BENGSSTON	Vindelälven, Västerbotten, Sweden	min	15
Ameletus inopinatus EATON	Sjodalen, Oppland, Norway	inop	29
Ameletus subnotatus EATON	USA	sub	6
Ameletus validus EATON	USA	val	3
Ameletus sp.	USA	sp	6
Metreletus balcanicus (ULMER)	Sperbersbach, Baden-Württemberg, Germany	balc	36

morphs were observed for all the populations tested: GOT-2, αGPDH, G3PDH, HK-1, IPO-1, LAP, MPI, RDH, XDH. The polymorphic enzymes contained many different electromorphs (e.g. CK, HK-2, 6PGDH, PGM, PK).

Only one electromorph was common for all the populations tested: allele 100 for the enzyme LAP. The four genera investigated can be separated into two groups: the *Siphlonurus-Parameletus*-complex, having the same electromorph for the enzyme APK (100), and the group formed by *Ameletus* and *Metreletus*, being identical for the MDH-2 locus (100). Each genus exhibited at least one typical electromorph; *Siphlonurus* for the loci GOT-1 (103), G3PDH

Table 2. Enzymes assayed with buffers used. PB = Phosphate buffer, pH 7.0 (RICHARDSON et al., 1986); AC7 = N-(3-aminopropyl)-morpholine-citrate, pH 7.0 (CLAYTON & TRETIAK, 1972); TBE = Tris-EDTA-borate, pH 9.0 (AYALA et al., 1972); TBMg = Tris-borate-$MgCl_2$, pH 9.0: the same as TBE but $MgCl_2$ replaces EDTA; E.C.N. = Enzyme Commission Number.

Abbreviation	Name	E.C.N.	Buffer
ALD	aldolase = fructose diphosphate aldolase	4.1.2.13	PB
APK	arginine phosphokinase	2.7.3.3	AC7
CK	creatine kinase	2.7.3.2	AC7
GOT-1 and GOT-2	glutamate-oxaloacetate transaminase	2.6.1.1	AC7
αGPDH	glycerol-3-phosphate dehydrogenase	1.1.1.8	TBE
G3PDH	glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	TBE
HK-1 and HK-2	hexokinase	2.7.1.1	AC7
IPO-1 and IPO-2	indophenol oxidase = superoxide dismutase	1.15.1.1	AC7
LAP	leucine aminopeptidase	3.4.11.1	TBMg
MDH-2	malate dehydrogenase	1.1.1.37	AC7
ME	malic enzyme	1.1.1.40	TBE
MPI	mannose phosphate isomerase	5.3.1.8	TBE
6PGDH	6-phosphogluconate dehydrogenase	1.1.1.44	AC7
PGM	phosphoglucomutase	2.7.5.1	AC7
PK	pyruvate kinase	2.7.1.40	TBMg
RDH	retinol dehydrogenase	1.1.1.105	TBE
XDH	xanthin dehydrogenase	1.2.1.37	TBE

(102), MDH-2 (99) and ALD (97, except *S. alternatus*), *Parameletus* for MDH-2 (97) and PK (103), *Ameletus* for αGPDH (99), and *Metreletus* for ALD (102), APK (99), GOT-1 (106 and 110), HK-2 (107) and IPO-2 (94 and 100).

Within the genus *Siphlonurus* three groups are recognized by MALZACHER (1981) and JACOB (1986) based on morphological characteristics. Our electrophoretic results confirm the presence of two groups, *S. alternatus* and the other *Siphlonurus* species, which are separated into two subgroups, *S. lacustris*-group and *S. aestivalis*-group. The *S. alternatus*-group, containing the single species *S. alternatus*, is distinguished from the other groups by the enzyme-loci CK (97), HK-2 (105), IPO-2 (95), ME (106) and 6PGDH (71). The *S. lacustris*-group, including *S. lacustris* and *S. occidentalis*, presents typical electromorphs for CK (94) and IPO-1 (94). The other *Siphlonurus* species belong to the *S. aestivalis*-group and share common electromorphs for two loci, CK (93) and 6PGDH (96).

Except in *S. aestivalis* and *S. accidentalis*, we found at least one electromorph distinguishing each species of *Siphlonurus* from the other species in the genus. At least one electromorph is completely different in *S. armatus* (PGM = 101), *S. flavidus* (PK = 107) and *S. alternatus* (CK = 97, HK-2 = 105, IPO-2 = 95, ME = 106, 6PGDH = 71). The other European species reveal partial differences in their electromorphs: *S. croaticus* for 6PGDH (104), *S. lusoensis*

Table 3. Electromorph frequencies for 20 enzyme loci in 28 populations of Siphlonuridae and Ameletidae (for abbreviations, see Tables 1 and 2).

		arm p	arm b	cro Yu	cro G	fla	aes G	aes N	lus PT	lus PL	mon SM	mon SS	his SC	his SS	lac AV	lac CH	lac AS	lac F	lac S	occ	alt	che N	che S	min	sub	val	sp	inop	balc
ALD	97	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.68	1.00	0.67	0.63	0.67	0.90	1.00	1.00	1.00	1.00	1.00	1.00	0	0	0	0	1.00	1.00	0	0	0
	99	0	0	0	0	0	0	0	0.32	0	0.33	0.37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.00	1.00	0
	100	0	0	0	0	0	0	0	0	0	0	0	0.33	0.10	0	0	0	0	0	0	1.00	1.00	1.00	0	0	0	0	0	0
	101	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.00	0	0	0	0	0
	102	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.00
APK	97	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.67	0	1.00	0	0
	98	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.33	1.00	0	1.00	0
	99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.00
	100	0.76	0.80	0.69	0.75	0.50	0.50	0.73	0.54	1.00	0.67	0.69	0.73	0.67	0.63	0.56	0.63	0.80	0	1.00	0.57	1.00	1.00	0	0	0	0	0	0
	101	0.24	0.20	0.31	0.25	0.50	0.50	0.27	0.46	0	0.33	0.31	0.27	0.33	0.37	0.44	0.37	0.20	1.00	0	0.43	0	0	0	0	0	0	0	0
CK	93	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0	0.10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	94	0	0	0	0	0	0	0	0	0	0	0	0	0	1.00	1.00	1.00	1.00	1.00	1.00	0	0	0	0	0	0	0	0	0
	96	0	0	0	0	0	0	0	0	0	0	0	1.00	0.90	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	97	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.00	0	0	0	0	0	0	0	0
	102	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.00	1.00	0	0	0	0	0	0
	103	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.00	0	0	0	0
	105	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0	0	0	0	0
	106	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.00	0.29	1.00	0
	108	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.71	0	1.00
GOT-1	98	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	103	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0	0	0	0	0	0	0	0
	106	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.50
	107	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.68	0.50	0	0	0	1.00	0	0
	108	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.32	0.50	0	0	0	0	0	0
	109	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.40	0
	110	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.50
	111	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.60	0

Table 3. (Continued).

		arm p	arm b	cro Yu	cro G	fla	aes G	aes N	lus PT	lus PL	mon SM	mon SS	his SC	his SS	lac AV	lac CH	lac AS	lac F	lac S	occ	alt	che N	che S	min	sub	val	sp	inop	balc
GOT-2	99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.85	0
	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.00	1.00	1.00	1.00	1.00	0	0.15	0
	101	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.31	0.11	0.45	0.56	0.50	0	0	0	0	0	0	0	1.00	0	1.00
	102	0	0	0	0	0	0	0	0	0	0	0	0	0	0.69	0.89	0.55	0.44	0.50	1.00	1.00	0	0	0	0	0	0	0	0
αGPDH	98	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.36	0
	99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.00	1.00	1.00	0.64	0
	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.19	0	0	0	0	0	0	0.82
	101	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.81	1.00	0	0	0	0	0	0.18
G3PDH	102	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0	0	0	0	0	0	0	0
	103	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.00	0	0	0	0	1.00
	104	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.00	1.00	0	1.00	1.00	1.00	1.00	0
HK-1	101	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.50	0	1.00	0
	102	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.40	0.40	0	0.33	0.50	1.00	0	1.00
	103	0.48	0.43	1.00	1.00	1.00	0.47	0.41	1.00	1.00	1.00	1.00	1.00	1.00	0.50	0.40	0.67	0	0.75	1.00	0	0.60	0.60	0	0.67	0	0	0	0
	104	0.52	0.57	0	0	0	0.53	0.59	0	0	0	0	0	0	0.50	0.60	0.33	1.00	0.25	0	1.00	0	0	1.00	0	0	0	0	0
HK-2	103	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.38	0
	104	0	0	0	0	0.45	0	0	0.13	0	0.50	0.42	0.40	0.47	0	0	0	0	0	0	0	0.34	0.50	0.50	0	0	0	0.62	0
	105	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.00	0	0	0	0	0	0	0	0
	106	0.48	0.43	1.00	1.00	0.55	0.50	0.35	0.87	1.00	0.50	0.58	0.60	0.53	0.47	0.45	0.47	0.46	0.36	0.40	0	0.66	0.50	0.50	0	0	0	0	0
	107	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.00
	108	0.52	0.57	0	0	0	0.50	0.65	0	0	0	0	0	0	0.53	0.55	0.53	0.54	0.64	0.60	0	0	0	0	0	0	0	0	0
IPO-1	92	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.37	0
	94	0	0	0	0	0	0	0	0	0	0	0	0	0	1.00	1.00	1.00	1.00	1.00	1.00	0	1.00	1.00	0	0.40	0	0.67	0.63	0
	96	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.37
	97	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0	0.00	1.00	1.00	1.00	1.00	0	0	0	0	0	0	1.00	0	0	1.00	0.60	1.00	0.33	0	0.63

Table 3. (Continued).

		arm p	arm b	cro Yu	cro G	fla	aes G	aes N	lus PT	lus PL	mon SM	mon SS	his SC	his SS	lac AV	lac CH	lac AS	lac F	lac S	occ	alt	che N	che S	min	sub	val	sp	inop	balc
IPO-2	93	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0	0	0	0	0	1.00	0	0	0	1.00	1.00	1.00	1.00	1.00	0
	94	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.51
	95	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.00	1.00	1.00	0	0	0	0	0	0
	96	0	0	0	0	0	0	0	0	0	0	0	0	0	1.00	1.00	1.00	1.00	1.00	0	0	0	0	0	0	0	0	0	0
	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.49
LAP	99	0	0	0	0	0	0	0	0	0	0	0	0	0	0.52	0.50	0.50	0.43	0	0	0	0	0	0	0	0	0	0	0
	100	0.91	1.00	0.28	0.60	0.75	0.80	0.50	0.47	0.33	0.50	0.48	0.56	0.58	0.48	0.50	0.50	0.57	1.00	1.00	1.00	0.23	0.38	0.50	0.67	0.60	1.00	0.40	0.07
	101	0.09	0	0.72	0.40	0.25	0.20	0.50	0.53	0.67	0.50	0.52	0.44	0.42	0	0	0	0	0	0	0	0.77	0.62	0.50	0.33	0.40	0	0.60	0.93
MDH-2	96	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.35	0	0	0	0	0	0	0
	97	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.65	1.00	1.00	0	0	0	0	0
	98	0.39	0	0.43	0.25	0	0.13	0.28	0	0	0	0	0	0	0	0	0	0	0	0	0.47	0	0	0	0	0	0	0	0
	99	0.61	1.00	0.57	0.75	0.75	0.88	0.72	1.00	1.00	1.00	1.00	1.00	1.00	0.55	0.64	0.90	1.00	1.00	1.00	0.53	0	0	0	0	0	0	0	0
	100	0	0	0	0	0.25	0	0	0	0	0	0	0	0	0.45	0.36	0.10	0	0	0	0	0	0	0	1.00	1.00	1.00	1.00	1.00
ME	98	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.00	0	0	0	0	0
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0	0	0	0	1.00	0	0	0	0
	103	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.00	1.00	1.00
	104	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.00	0	0	0
	106	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.00	1.00	1.00	0	0	0	0	0	0
MPI	98	0	0	0	0	0	0	0	0	0	0.25	0.21	0	0	0	0	0	0	0	0.67	0	0	0	0	0	0	0	0.81	0
	99	0	0	0	0	0	0	0	0.38	0.86	0.75	0.79	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.33	0	0	0	0	0	0	0.63	0.19	0.88
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.62	0.14	0	0	0	0	0	0	0	0	0	0	1.00	0.83	1.00	1.00	1.00	0	0.37	0	0.12
	101	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.17	0	0	0	0	0	0	0
6PGDH	71	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	1.00	0	0	0	0	0	0	0	0
	86	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.61	0.60	0	0.00	0	0	0	0.31
	96	1.00	1.00	0.45	0.14	1.00	1.00	1.00	0.91	0.57	1.00	1.00	1.00	1.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.69
	99	0	0	0	0	0	0	0	0.09	0.43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 3. (Continued).

		arm p	arm b	cro Yu	cro G	fla	aes G	aes N	lus PT	lus PL	mon SM	mon SS	his SC	his SS	lac AV	lac CH	lac AS	lac F	lac S	occ	alt	che N	che S	min	sub	val	sp	inop	balc
6PGDH	100	0	0	0	0	0	0	0	0	0	0	0	0	0	1.00	1.00	1.00	1.00	1.00	0	0	0	0	0	0	0	0	0	0
	104	0	0	0.55	0.86	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.39	0.40	0	0.00	0	0	0	0
	108	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.17	0
	114	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.83	0
PGM	99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.55
	101	1.00	1.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.00	0	0	0	0
	102	0	0	0	0	0	0	0	0	0	0	0	0	0	0.79	0.82	1.00	1.00	0.80	0	0.37	0	0	0	0	0	0	0	0
	103	0	0	0.08	0.50	0	0.64	0.68	1.00	1.00	1.00	1.00	0.90	0.90	0.21	0.18	0	0	0.20	1.00	0.52	0.48	0.33	0.08	0	0	1.00	0.50	0.45
	104	0	0	0.56	0.50	1.00	0.36	0.32	0	0	0	0	0.10	0.10	0	0	0	0	0	0	0.11	0.52	0.67	0.92	0	1.00	0	0.50	0
	105	0	0	0.36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PK	95	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.00	0
	97	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.00	0	0	0
	99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.83	0	1.00
	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.00	0	0.17	0	0
	103	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.00	1.00	1.00	0	0	0	0	0
	105	0	0	0	0	0	1.00	1.00	0	0	0	0	0	0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0	0	0	0	0	0	0	0
	106	0	0	0	0	0	0	0	1.00	1.00	1.00	1.00	1.00	1.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	107	0	0	0	0	1.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	108	1.00	1.00	1.00	1.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RDH	98	0	0	0	0	0	0	0	0	0	0	0	0	0	0.58	0.35	0	0	0	0	0	0	0	0	0	0	0	0	0
	99	1.00	1.00	1.00	1.00	1.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.00	0	0	0	0	0	0	0	0
	100	0	0	0	0	0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.42	0.65	1.00	1.00	1.00	1.00	0	0	0	0	0	0	1.00	1.00	1.00
	101	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.00	1.00	0	0	0	0	0	0
XDH	103	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.11	0.20	0	0	0	0	0	0
	104	0.81	0.75	0.17	0.40	1.00	0.90	0.19	0	0	1.00	1.00	0.75	1.00	0.83	1.00	1.00	0.67	1.00	0	1.00	0.89	0.80	0	0	0	0	0	0
	105	0.19	0.25	0.83	0.60	0	0.10	0.81	1.00	1.00	0	0	0.25	0	0.17	0	0	0.33	0	0	0	0	0	0	0	0	0	1.00	1.00

for 6PGDH (99), *S. montanus* for MPI (98), *S. lacustris* for LAP (99) and 6PGDH (100), *S. hispanicus* for ALD (100, convergence with *S. alternatus*). *Parameletus chelifer* and *P. minor* are distinguishable with the enzymes ALD, CK, G3PDH, HK-1, IPO-1, IPO-2 and ME.

Table 4 gives the identity matrix between the 28 populations tested. An I-value of 1.00 indicates that the two populations compared are identical in their electromorph frequencies, whereas an I-value of 0.00 means that the two populations compared are completely different. For nearly all the species of *Siphlonurus* and *Parameletus*, we investigated at least two populations, if possible geographically distant from each other (Table 1). The I-values obtained between two populations (Table 4) are very high for *Siphlonurus montanus* (1.00), *S. hispanicus* (0.99), *S. armatus* (0.99), *S. croaticus* (0.97), *S. aestivalis* (0.96), *S. lusoensis* (0.96) and *Parameletus chelifer* (0.98). The five populations of *Siphlonurus lacustris* present I-values from 0.91 between the populations from Corsica (lac F) and that from Spain (lac S), to 0.99 between the populations from Austria (lac AV) and Switzerland (lac CH) which are separated by 100 km.

The $\bar{I}$-value between the species of *Siphlonurus* is 0.64 (range 0.28–0.95). In the *S. aestivalis*-group, the I-values between the species range from 0.65 (between one population of *S. armatus* and one population of *S. lusoensis*) to 0.95 (between one population of *S. hispanicus* and one population of *S. montanus*) with an average of 0.78. *S. alternatus* is distinguished from the other *Siphlonurus* species by low I-values (0.28–0.54). *Parameletus chelifer* and *P. minor* are quite distinct from each other (I-values of 0.35–0.38). The $\bar{I}$-value obtained between the species of *Ameletus* is 0.47 (range 0.33–0.58). *Metreletus balcanicus* exhibits low I-values with all the other species studied: $\bar{I}$-value = 0.21 (range 0.07–0.49).

In Fig. 1, the correlations between the populations are presented graphically. The four genera are distinguished from each other by low I-values (0.25–0.28). The three groups of *Siphlonurus* are quite distinct. In the *S. aestivalis*-group, *S. hispanicus, S. montanus* and *S. lusoensis* (all from the Iberian Peninsula) are separated from the other species. *S. flavidus* is similar to *S. croaticus* and *S. armatus*. *S. croaticus* is clearly distinct from *S. aestivalis*.

Heterozygosity (H) was analysed for the five populations of *S. lacustris* (Table 5). The 11 monomorphic loci (where H = 0) are not listed but they are included in the calculation of the average heterozygosity. For most of the loci, the difference between the heterozygosity calculated H_{calc} and the heterozygosity observed H_{obs} is low. For some loci (APK, HK-1, PGM, XDH), it happens that all the specimens tested in one population are homozygous (H_{obs} = 0) but they present various electromorphs. The differences between the average H_{calc} and the average H_{obs} in each population are smaller than the corresponding standard error of 0.04–0.07. The differences of the average heterozygosities between the five populations are not significant. Only the Spanish population

Table 4. Genetic identity-values (I) among 28 populations of Siphlonuridae and Ameletidae (for abbreviations, see Table 1).

	arm p	arm b	cro Yu	cro G	fla	aes G	aes N	lus PT	lus PL	mon SM	mon SS	his SC	his SS	lac AV	lac CH	lac AS	lac F	lac S	occ	alt	che N	che S	min	sub	val	sp	inop	balc
arm p	1.00	0.99	0.86	0.87	0.85	0.83	0.81	0.69	0.65	0.73	0.73	0.67	0.69	0.50	0.48	0.50	0.51	0.50	0.53	0.51	0.24	0.25	0.28	0.43	0.24	0.22	0.09	0.17
arm b		1.00	0.85	0.86	0.84	0.84	0.81	0.70	0.66	0.73	0.73	0.68	0.69	0.50	0.49	0.51	0.53	0.51	0.55	0.51	0.23	0.24	0.28	0.42	0.23	0.22	0.10	0.17
croYu			1.00	0.97	0.85	0.77	0.80	0.77	0.75	0.71	0.71	0.68	0.67	0.45	0.42	0.46	0.44	0.45	0.52	0.42	0.29	0.30	0.30	0.38	0.27	0.19	0.16	0.22
cro G				1.00	0.85	0.79	0.79	0.77	0.75	0.74	0.74	0.70	0.70	0.48	0.45	0.49	0.47	0.49	0.58	0.47	0.31	0.32	0.30	0.38	0.27	0.23	0.15	0.19
fla					1.00	0.83	0.77	0.71	0.66	0.77	0.77	0.71	0.74	0.48	0.47	0.50	0.45	0.52	0.52	0.48	0.29	0.31	0.32	0.39	0.31	0.22	0.14	0.18
aes G						1.00	0.96	0.81	0.75	0.84	0.84	0.78	0.80	0.60	0.60	0.63	0.63	0.66	0.69	0.54	0.26	0.27	0.31	0.36	0.26	0.31	0.18	0.25
aes N							1.00	0.84	0.80	0.80	0.80	0.76	0.76	0.57	0.56	0.59	0.62	0.58	0.68	0.49	0.24	0.25	0.31	0.36	0.26	0.30	0.23	0.31
lus PT								1.00	0.96	0.89	0.89	0.84	0.83	0.51	0.49	0.54	0.53	0.56	0.66	0.32	0.25	0.24	0.18	0.30	0.15	0.33	0.27	0.31
lus PL									1.00	0.87	0.88	0.85	0.84	0.55	0.52	0.58	0.57	0.55	0.69	0.28	0.25	0.24	0.15	0.28	0.16	0.30	0.25	0.31
mon SM										1.00	1.00	0.93	0.95	0.55	0.55	0.60	0.55	0.60	0.66	0.39	0.26	0.25	0.20	0.29	0.21	0.34	0.23	0.29
mon SS											1.00	0.93	0.94	0.55	0.55	0.60	0.55	0.60	0.66	0.39	0.27	0.26	0.20	0.29	0.21	0.34	0.23	0.30
his SC												1.00	0.99	0.56	0.55	0.60	0.57	0.61	0.66	0.40	0.27	0.27	0.21	0.29	0.22	0.33	0.22	0.32
his SS													1.00	0.58	0.57	0.62	0.58	0.63	0.66	0.40	0.27	0.26	0.21	0.30	0.23	0.33	0.20	0.30
lac AV														1.00	0.99	0.97	0.94	0.92	0.74	0.45	0.25	0.25	0.07	0.24	0.12	0.20	0.13	0.15
lac CH															1.00	0.98	0.95	0.94	0.75	0.47	0.24	0.24	0.07	0.23	0.11	0.19	0.13	0.14
lac AS																1.00	0.97	0.96	0.76	0.43	0.25	0.25	0.05	0.22	0.09	0.20	0.13	0.15
lac F																	1.00	0.91	0.73	0.45	0.22	0.22	0.10	0.18	0.09	0.21	0.14	0.17
lac S																		1.00	0.75	0.44	0.22	0.22	0.06	0.23	0.10	0.24	0.13	0.15
occ																			1.00	0.43	0.26	0.27	0.12	0.32	0.17	0.30	0.25	0.12
alt																				1.00	0.39	0.40	0.24	0.15	0.11	0.13	0.04	0.07
che N																					1.00	0.98	0.35	0.29	0.22	0.24	0.19	0.12
che S																						1.00	0.38	0.30	0.23	0.23	0.19	0.10
min																							1.00	0.31	0.34	0.16	0.17	0.15
sub																								1.00	0.58	0.47	0.33	0.15
val																									1.00	0.40	0.50	0.17
sp																										1.00	0.53	0.49
inop																											1.00	0.30
balc																												1.00

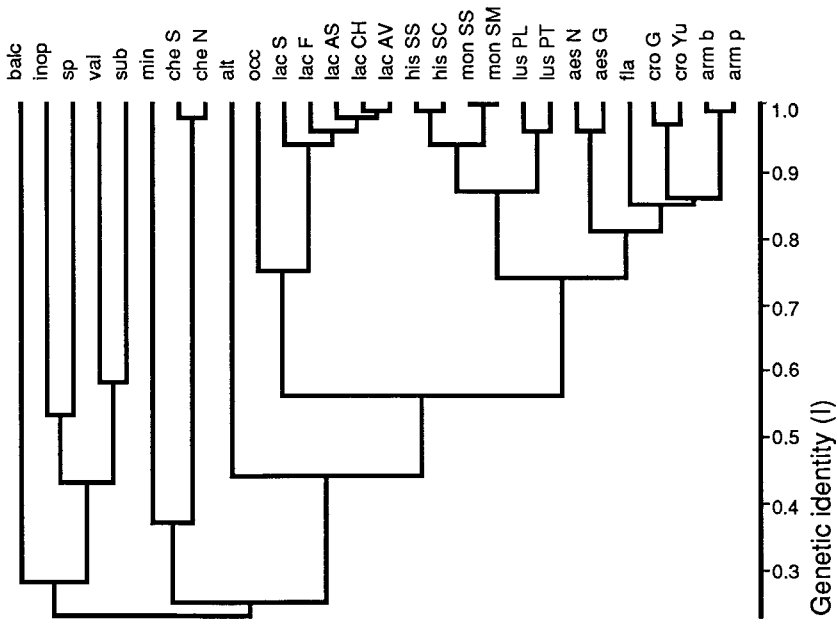

Fig. 1. Phenogram of 28 populations of Siphlonuridae and Ameletidae based on a cluster analysis (UPGMA) of genetic identity values.

Table 5. Heterozygosity for five populations of *Siphlonurus lacustris*, calculated following FERGUSON (1980). *The monomorphic loci (where H = 0) are not listed but they are included in the calculation of the average heterozygosity. H_{calc} = heterozygosity calculated, H_{obs} = heterozygosity observed, n = number of specimens tested.

	Austria, Vorarlberg (lac AV) n = 33		Austria, Steiermark (lac AS) n = 8		Switzerland (lac CH) n = 23		Spain (lac S) n = 9		Corsica (lac F) n = 10	
	Hcalc.	Hobs.	Hcalc.	Hobs.	Hcalc.	Hobs.	Hcalc.	Hobs.	Hcalc.	Hobs.
APK	0.46	0.58	0.46	0.60	0.49	0.57	0	0	0.32	0
GOT-2	0.43	0.37	0.49	0.83	0.20	0.13	0.50	1.00	0.49	0.50
HK-1	0.50	0.19	0.44	0	0.48	0.25	0.37	0	0	0
HK-2	0.50	0.90	0.50	0.67	0.49	0.88	0.46	0.43	0.50	0.86
LAP	0.50	0.57	0.50	0.83	0.50	0.57	0	0	0.49	1.00
MDH-2	0.49	0.56	0.18	0.13	0.46	0.45	0	0	0	0
PGM	0.39	0.25	0	0	0.30	0.11	0.32	0	0	0
RDH	0.49	0.20	0	0	0.45	0.55	0	0	0	0
XDH	0.28	0	0	0	0	0	0	0	0.44	0.50
Average*	0.20 ± 0.05	0.18 ± 0.07	0.13 ± 0.05	0.15 ± 0.07	0.17 ± 0.05	0.18 ± 0.05	0.08 ± 0.04	0.07 ± 0.05	0.11 ± 0.05	0.14 ± 0.07

presents few heterozygous specimens and the average heterozygosity is lower than in the other populations.

Discussion

In most of the *Siphlonurus* and *Parameletus* species, at least two populations were investigated biochemically. Even if two populations of one species are geographically very distant, high I-values can be observed (Table 4). One population of *Siphlonurus croaticus* was collected in southern Germany (cro G) and the other one in former Yugoslavia (cro Yu); the Ī-value between them is 0.97. A similar I-value (0.96) was observed between two populations of *S. aestivalis,* one from southern Germany (aes G) and the other one from northern Norway (aes N). The two populations of *Parameletus chelifer* were collected respectively in northern Norway (che N) and central Sweden (che S); their I-value amounts to 0.98. From Austria (lac AV) to Spain (lac S), the five populations of *Siphlonurus lacustris* do not reveal geographic clines in allelic frequencies. However the I-values are higher between neighbouring populations (e.g. lac AV and lac CH). The population of Spain (lac S) is the most geographically distant and exhibits the lowest I-values among the *S. lacustris*-group. The average heterozygosity for populations of *S. lacustris* (average H = 0.14 ± 0.05, Table 5) is in good agreement with the values of 0.16 given by POWELL (1975) for the Insecta, 0.12 ± 0.01 measured by BELFIORE et al. (1992) in four populations of *Rhithrogena insularis* ESBEN-PETERSEN, 1913 (Heptageniidae), and 0.12 obtained by FUNK et al. (1988) for sixteen populations of *Eurylophella verisimilis* MCDUNNOUGH, 1931 (Ephemerellidae). Even if the number of specimens is low in some populations, the average heterozygosity can be adequately estimated because the number of loci is sufficiently high (GORMAN & RENZI 1979, NEI 1978).

THORPE (1982) postulates that 97% of I-values among species are below 0.85, but he does not indicate for which organisms. More recent studies present the interspecific relationships for different families of mayflies. The Ī-value between fifteen species of *Eurylophella* (Ephemerellidae) amounts to 0.48 (range 0.15–0.90) (FUNK et al. 1988). In the Family Heptageniidae (ZURWERRA et al. 1987), the Ī-values amount to 0.49 (range 0.34–0.87) between four species of *Epeorus,* to 0.47 (range 0.10–0.91) between sixteen species of *Rhithrogena,* to 0.55 (range 0.22–0.89) between nineteen species of *Ecdyonurus,* to 0.60 (range 0.33–0.87) between eight species of *Electrogena* and to 0.60 (range 0.54–0.62) between four species of *Heptagenia.* The high Ī-value obtained between the species of *Siphlonurus* (0.64) can be compared with that obtained in *Electrogena* and *Heptagenia,* whereas the low Ī-value found for *Ameletus* (0.47) approximates that of *Epeorus, Rhithrogena* and *Eurylophella.* The lower Ī-values obtained in *Ameletus* (0.47) and *Parameletus* (0.37) can be explained by the lack of some electromorphs.

S. croaticus is a valid species, although it has high Ī-values with both *S. armatus* (0.86) and *S. flavidus* (0.85). Many morphological differences between *S.*

croaticus and the two species mentioned (STUDEMANN et al. 1988) support the difference at the specific level. Morphologically, the closest species to *S. croaticus* is *S. aestivalis.* The electrophoretic results reveal two enzymes (PK and RDH, Table 3) which differ completely between these species, meaning that no gene flow occurs between these taxa.

S. montanus shows high I-values with two other Iberian species, *S. lusoensis* ($\bar{I}$ = 0.88) and *S. hispanicus* ($\bar{I}$ = 0.94). However, *S. montanus* differs from *S. lusoensis* by its completely different electromorphs at XDH and partially different electromorphs for MPI and 6PGDH (Table 3), as well as the shape of the penis (STUDEMANN et al. 1992 b). *S. montanus* differs from *S. hispanicus* with partially different electromorphs for the enzymes ALD, CK and MPI (Table 3) and the absence of spines on the penis (STUDEMANN et al. 1992 b). When two species are very similar to each other, it would be useful to compare still more biochemical and morphological characteristics.

The $\bar{I}$-value levels obtained between the groups of *Siphlonurus* (0.44–0.56, Fig. 1) agree very well with those between the groups of *Rhithrogena* or *Ecdyonurus* (0.40–0.50) obtained by ZURWERRA et al. (1987).

From morphological characteristics, *Siphlonurus flavidus* could not be assigned to a *Siphlonurus*-group (JACOB 1986, STUDEMANN et al. 1992 b). Electrophoresis allows us to include *S. flavidus* in the *S. aestivalis*-group thanks to the common typical electromorphs for CK (93) and 6PGDH (96) (Table 3) and its location in the phenogram (Fig. 1).

According to THORPE (1982), the critical level for I-values distinguishing between species and genera would appear to be about 0.35. The four genera studied are separated from each other at $\bar{I}$-values between 0.25 and 0.28 (Fig. 1). This level is nearly the same as that revealed for the genera of the Heptageniidae by ZURWERRA et al. (1987).

Based on morphological characteristics, JACOB (1984) proposed the synonymy of the genus *Metreletus* with the genus *Ameletus.* The present study reveals low I-values (Table 4) between *M. balcanicus* and the four species of *Ameletus* ($\bar{I}$ = 0.28). For three enzyme loci (G3PDH, αGPDH and IPO-2, Table 3), the four species of *Ameletus* share a common electromorph while *M. balcanicus* shows a different one. The electromorph 99 for the αGPDH enzyme locus is synapomorphic for all the species of *Ameletus* tested; *Metreletus* does not exhibit it (Table 3). Additionally, the morphological analysis showed differences between *Metreletus* and *Ameletus* in the larva (tarsal claws dented, terminal filaments longer than the abdomen in *Metreletus*) and in the imago (cubito-anal sector of the forewing provided with many transversal veins in *Metreletus*). The biochemical and morphological characteristics allow us to consider *Metreletus* as a valid genus.

Our biochemical results confirm the separation between the *Siphlonurus-Parameletus*-complex and the *Ameletus-Metreletus*-complex observed previously

with morphological characteristics. As suggested by some authors (McCafferty 1991, Tomka & Elpers 1991), *Ameletus* and *Metreletus* should be removed from the Family Siphlonuridae. Tomka & Elpers (1991) proposed a new family called Rallidentidae, based on an apomorphy observed in the mandible, including *Ameletus, Metreletus, Ameletoides* Tillyard, 1933, *Metamonius* Eaton, 1885, *Nesameletus* Tillyard, 1933 and *Rallidens* Penniket, 1966. However, there are a number of reasons to separate *Rallidens* from the other genera cited and consequently to refute the name of Rallidentidae: (i) the characteristic given by Tomka & Elpers (1991) is not sufficient to prove a close relationship between *Rallidens* and the other genera; (ii) the abdominal gills of *Rallidens* are composed of plates and fibrilliform tufts instead of simple plates in the other genera; (iii) *Rallidens* carries filamentous gills on the base of the maxillae which seems to be a common characteristic for some Setisura (Coloburiscidae, Isonychiidae, Oligoneuriidae). The family name Ameletidae suggested by McCafferty (1991) seems to be more adequate. However, a complementary study of all the genera of Siphlonuridae sensu lato is already in preparation (N. J. Kluge et al., unpubl.).

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