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Revision of morphological and biochemical characters of the European species of the *Ecdyonurus helveticus*-group (Ephemeroptera, Heptageniidae)

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The European species of the *Ecdyonurus helveticus*-group are revised and a key of determination is provided to the nymphal and imaginal stages. The differential electrophoretical mobility of fifteen enzyme-loci was examined in all species apart from *E. epeorides*. The morphological and biochemical characters are used to construct a cladogram.

INTRODUCTION

The systematic status of *Ecdyonurus* EATON, 1868 of the Heptageniidae has been clearly defined by ZURWERRA & TOMKA (1985) and the European genera of the family were diagnosed with larval and imaginal characters by HEFTI & TOMKA (1989). *Ecdyonurus* is actually subdivided into the *E. helveticus*-group and the *E. venosus*-group. Species of the *E. helveticus*-group differ morphologically from members of the *E. venosus*-group by the distal part of the larval hypopharynx which lacks a long and dense pilosity (HEFTI & TOMKA, 1986; BELFIORE, 1987) and by presence of laterally elongated apical sclerites of the penis lobes. JACOB & BRAASCH (1984) included the following nine taxa in the *E. helveticus*-group: *E. carpathicus carpathicus* SOWA, 1973; *E. carpathicus vitoshensis* JACOB & BRAASCH, 1984; *E. epeorides* DEMOULIN, 1955; *E. helveticus* (EATON, 1885); *E. krueperi* (STEIN, 1863); *E. picteti* (MEYER-DÜR, 1864); *E. siveci* JACOB & BRAASCH, 1984; *E. subalpinus* (KLAPALEK, 1907) and *E. zelleri* (EATON, 1885).

Subsequently, two additionnal species were described from the Alps: *E. alpinus* HEFTI, TOMKA & ZURWERRA, 1987 and *E. parahelveticus* HEFTI, TOMKA & ZURWERRA, 1986. *E. austriacus* KIMMINS, 1958, which was incorrectly synonymised with *E. picteti* by PUTHZ (1975), was reestablished as distinct taxon (HEFTI & TOMKA, 1986).

Eighty-two populations of the *E. helveticus*-group were sampled in Austria, France, Germany, Greece, Italy, Rumania, Switzerland and Yugoslavia. Due to the wide geographical area covered phenotypic variability became apparent. This necessitated further characterization by biochemical means in order to separate the various species. For that aim thirty-six populations were analysed biochemically using starch gel electrophoresis.

The present study revises the morphology and provides, with the exception of *E. epeorides*, a biochemical characterization of the European species of the *E. helveticus*-group. A key to the larval and imaginal stages is included. The morphological and electrophoretical data are used to construct a cladogram.

MATERIAL AND METHOD

A complete and detailed list of the stations prospected in Europe is given in ZURWERRA & TOMKA (1984), HEFTI *et al.* (1986), HEFTI & TOMKA (1986), HEFTI *et al.* (1987) and HEFTI & TOMKA (1988) for the investigated taxa.

Mature larvae were reared in the field or in the laboratory to subimaginal stages. This procedure allowed a reliable determination of the larvae. For morphological investigations, the wings, the legs and the male genitalia of the imagines were preserved in 80% alcohol and the exuviae of the last larval instar were embedded in a mixture of polyvinylalcohol-lactophenol after HEINZE (1952). For scanning electron microscopy (SEM), the male genitalia and the larval abdominal sternites 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.

The rest of the body of each imago was stored individually deep frozen at −70 °C until biochemical analysis. Starch gel electrophoresis was used to investigate the specific electromorph mobilities of fifteen enzyme-loci with the following buffer systems:

- *Tris-borate-EDTA pH 9* (AYALA *et al.*, 1972) for the enzyme-loci α-glycerophosphate dehydrogenase (α-GPDH), mannose phosphate isomerase (MPI) and retinol dehydrogenase (RDH),

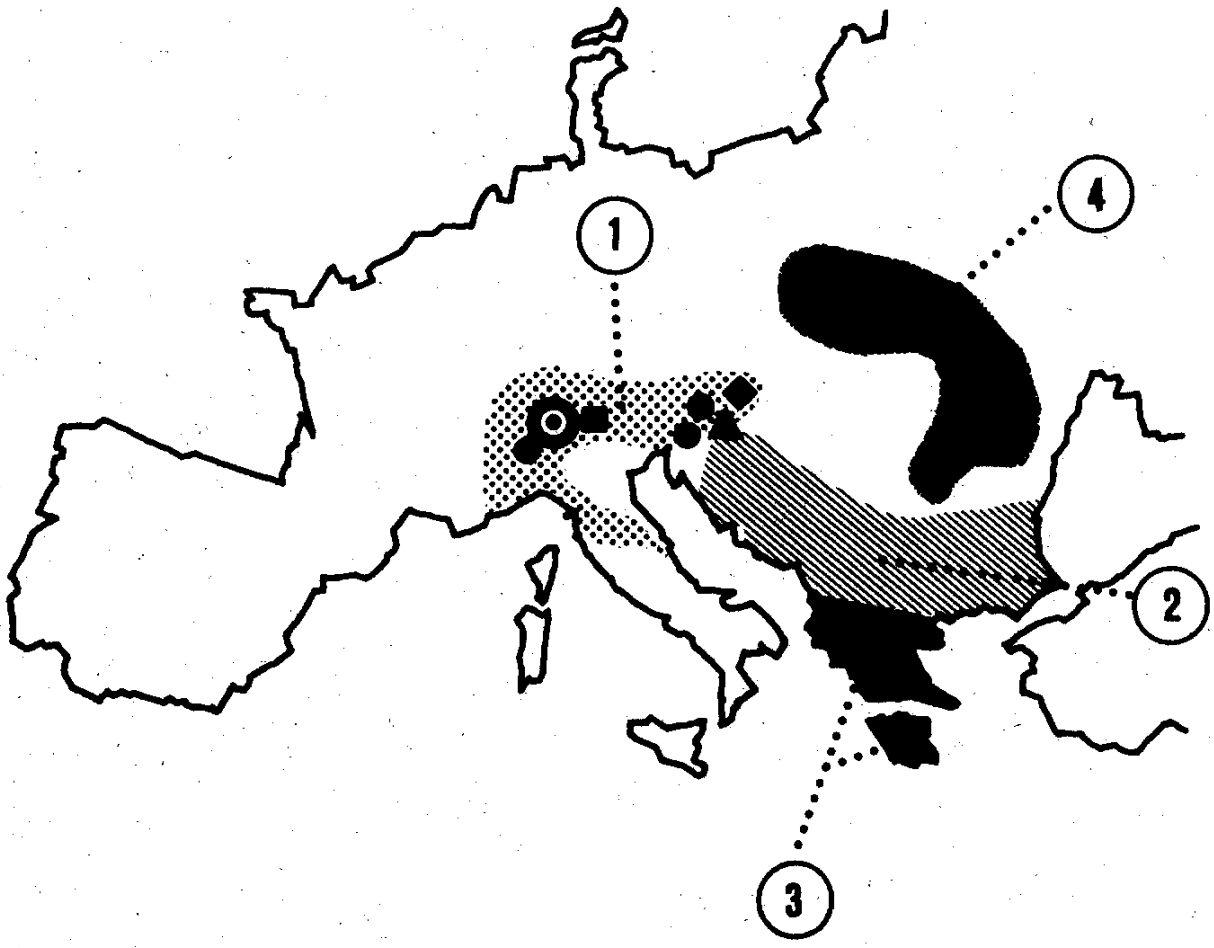


Fig. 1. Distribution of the species of the *E. helveticus*-group: *E. helveticus* and *E. picteti* (1), *E. carpathicus vitoshensis* (2), *E. krueperi* and *E. epeorides* (3), *E. subalpinus* and *E. carpathicus carpathicus* (4), *E. parahelveticus* (⊙) *E. austriacus* (◆), *E. alpinus* (■), *E. zelleri* (●) and *E. siveci* (▲).

- *N-(3-amino-propyl)-morpholine citrate pH 7* (CLAYTON & TRETIAK, 1972) for the enzyme-loci 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) and phosphoglucomutase (PGM). The same buffer system has been used with slight modifications (ZURWERRA *et al.*, 1986) at pH 6 for the enzyme-loci adenylate kinase (AK) and arginine phosphokinase (APK).

In contrast to the standardized method of measurement by AYALA *et al.* (1972), the electromorph mobilities of each enzyme-locus were assessed in relation to individuals from a reference population (*Epeorus sylvicola:* Switzerland, canton of Fribourg, La Broye/Châtel-St-Denis, 705 m) and expressed as a relative mobility index (RMI: ZURWERRA *et al.*, 1986). The frequences of the calculated RMI-values are presented for each enzyme-locus as a percentage (0 to 100%) in Table 2. The pairwise correlations of populations lead to the identity matrix (Table 3), which defines genetic distance ($\bar{I}$) between two populations (NEI, 1972). The matrix was clustered according to the unweighted pair group arithmetic average (UPGMA) method (FERGUSON, 1980) to obtain the dendrogram (Fig. 12). A cladogram is proposed for the species investigated using morphological and electrophoretical data.

Details on starch gel electrophoresis and the evaluation of data are fully described in ZURWERRA *et al.* (1986) and HEFTI *et al.* (1988).

RESULTS

Morphology

At imaginal stage, the species of the *E. helveticus*-group are characterized by the existence of laterally elongated apical sclerites of the penis lobes. The determination of species is essentially based on the complex structure of the dorsal sclerites of the penis (Figs. 10–11), in *E. parahelveticus* and *E. austriacus* also on the forceps base (Fig. 9). At larval stage, species of the group are characterized by the presence of pronotal expansions and hypopharynx without pilosity on its distal margin. Larval identification is difficult and requires the determination of several different characters (Table 1 and Figs. 2 to 8). Larvae and imagines can be separated with the following keys:

Key to nymphs

1 Posterior margin of abdominal tergites with pointed spines only (Fig. 2a) 2
– Posterior margin of abdominal tergites with small pointed and large distally rounded spines (Figs. 2b, c, d) 10
2 Length: width ratio of the fourth gill plate ≥ 2.20 (DEMOULIN, 1955) . . . . . *E. epeorides*
– Length: width ratio of the fourth gill plate < 2.20 3
3 Posterior margin of the metafemora distally with regular short spines (Fig. 7c) 4
– Posterior margin of the metafemora distally without regular short spines 7
4 Outer margin of pronotum distinctly concave distally (Fig. 3a) . . . . . *E. siveci*
– Outer margin of pronotum not concave distally 5
5 Femora slender spindle-shaped; length: width ratio of metafemora > 2.40 (Fig. 7b) . *E. picteti*
– Femora broad, divergent distally; length: width ratio of metafemora ≤ 2.40 (Fig. 7a) 6
6 Profemoral spines divergent toward apex (Fig. 5a) (SOWA, 1973); lateral expansion of pronotum pointed distally (Fig. 3b) . . . . . *E. subalpinus*
– Profemoral spines convergent toward apex (Fig. 5b) (SOWA, 1973); lateral expansion of pronotum rounded distally (Fig. 3d) . . . . . *E. carpathicus carpathicus*

7 Glossa laterally elongated (Fig. 6a) 8
– Glossa longitudinally elongated (Fig. 6b) . . . . . *E. krueperi*
8 Length: width ratio of fourth gill plate ≥ 2.00; lateral pronotal expansion flattened distally with a curved outer margin (Fig. 3c) . . . . . *E. zelleri*
– Length: width ratio of fourth gill plate < 2.00; lateral pronotal expansion rounded or ± pointed distally (Fig. 3e) 9
9 Femora broad (Fig. 7a); length: width ratio of metafemora ≤ 2.40 . . *E. carpathicus vitoshensis*
– Femora slender, spindle-shaped (Fig. 7b); length: width ratio of metafemora > 2.40 . . . . . *E. helveticus*
10 Tarsal claws on all legs twofold, asymmetrical (Fig. 4) . . . . . *E. austriacus*
– Tarsal claws absent or if present, not twofold and asymmetrical 11
11 Procoxa with small tuft or row of spines dorsally (Fig. 8) . . . . . *E. parahelveticus*
– Procoxa without dorsal tuft or row of spines, or sometimes with some isolated spines . *E. alpinus*

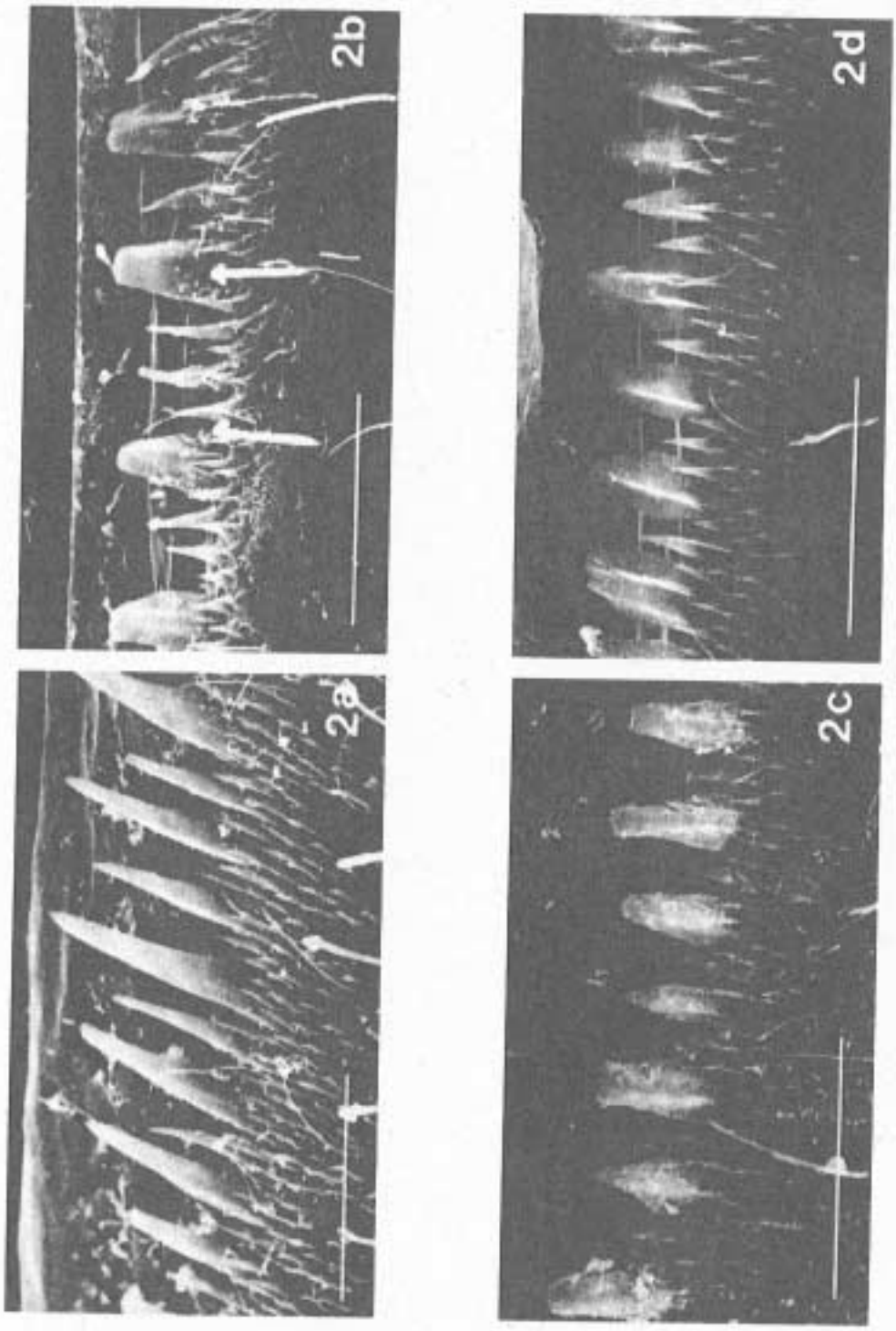
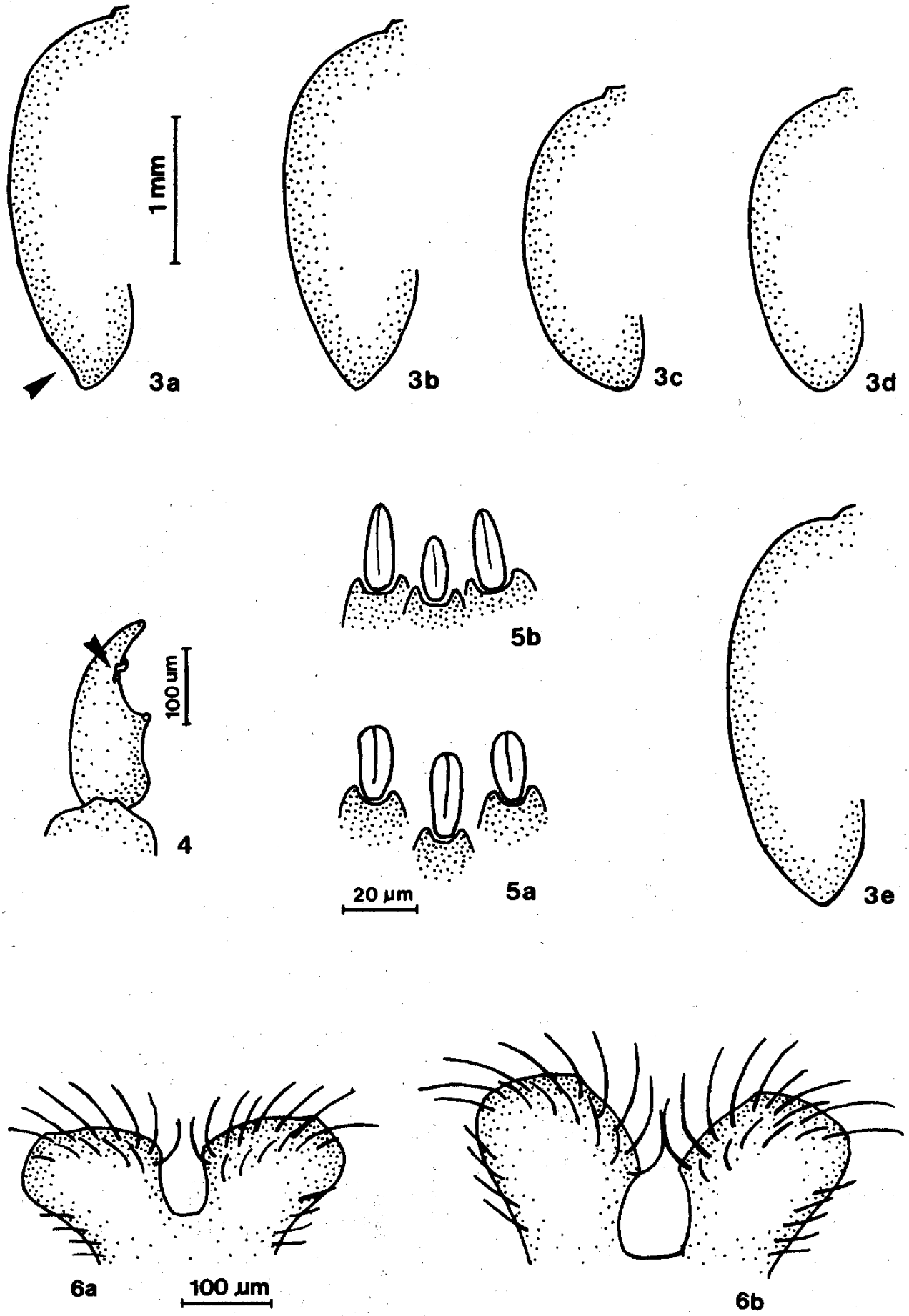


Fig. 2. SEM photographs of the posterior margin of the larval abdominal tergites for the species *E. picreti* (a), *E. austriacus* (b), *E. parahelveticus* (c) and *E. alpinus* (d). Scale line = 50 μm.



Figs. 3–6. Nymph: Lateral pronotal expansions of *E. siveci* (3a), *E. subalpinus* (3b), *E. zelleri* (3c), *E. carpathicus carpathicus* (3d) and *E. helveticus*. (3e) Tarsus of *E. austriacus* with 2 claws (4). Profemoral spines of *E. subalpinus* (5a) and *E. carpathicus carpathicus* (5b). Glossa of *E. helveticus* (6a) and *E. krueperi* (6b).

Key to male imagines

1 Outer margin of apical sclerite of the penis lobe formed by two more or less straight lines; apical sclerite divergent and rounded distally (Fig. 11) 2
– Outer margin of apical sclerite regularly curved; apical sclerite convergent and more or less pointed distally (Fig. 10) 7
2 Straight lines of the apical sclerite ± forming an obtuse angle (Figs. 11a, b) 3
– Straight lines of apical sclerite perpendicular (Figs. 11c, d, e, f) 4
3 Forceps base with slight or no protuberance (Fig. 9b); penis lobe truncated laterally; superior border of lateral sclerite sinuous and strongly curved (Fig. 11a) . . . . . *E. parahelveticus*
– Forceps base with two strong protuberances (Fig. 9a); penis lobe rounded laterally; superior border of lateral sclerite regularly curved (Fig. 11b) . . . . . *E. austriacus*
4 Basal sclerite with teeth projected obliquely to axis of symmetry of the penis lobe (Fig. 11c) . . . . . *E. picteti*
– Basal sclerite with teeth projected perpendicularly to axis of symmetry of the penis lobe or without teeth (Figs. 11d, e, f) 5
5 Penis lobe strongly elongated laterally with narrow lateral sclerite which is situated in the basal half of the penis lobe (Fig. 11d) . . . . . *E. helveticus*
– Penis lobe not strongly elongated laterally; the lateral sclerite is broad or is situated in the middle of the penis lobe (Figs. 11e, f) 6
6 Lateral sclerite typically enlarged covering a large portion of the penis lobe; apical sclerite with a slight inner projection (Fig. 11e) . . . . . *E. alpinus*
– Lateral sclerite moderately enlarged; apical sclerite with a well visible inner projection (Fig. 11f) . . . . . *E. zelleri*
7 Penis lobe almost triangular with square lateral sclerite which does not reach outer distal edge of the penis lobe (Fig. 10b) . . . . . *E. krueperi*
– Penis lobe rounded or laterally elongated with narrow lateral sclerite which reaches outer distal edge of the penis lobe (Figs. 10a, c, d, e, f) 8
8 Apical sclerite with no or slight denticulation (Figs. 10a, c) 9
– Apical sclerite with strong denticulation (Figs. 10d, e, f) 10
9 Basal sclerite with strong tooth overlapping the lateral sclerite (Fig. 10a) . . . . . *E. epeorides*
– Basal sclerite not overlapping the lateral sclerite (Fig. 10c) . . . . . *E. carpathicus carpathicus*
10 Penis lobe spherically shaped (Fig. 10e) . . . . . *E. subalpinus*
– Penis lobe elongated laterally (Fig. 10d, f) 11
11 Lateral outline of the penis lobe rounded (Fig. 10d) . . . . . *E. carpathicus vitoshensis*
– Lateral outline of the penis lobe straight (Fig. 10f) . . . . . *E. siveci*

Biochemistry

The mobility pattern of the electromorphs is presented in Table 2. The α-GPDH, MDH-2, AK, APK, IPO-1 and RDH loci have single electromorphs. The α-GPDH and MDH-2 loci are the most conservative ones with common RMI-values for all the species of the *E. helveticus*-group investigated biochemically. The AK, APK, IPO-1 and RDH loci exhibit specific patterns of mobilities which indicate genetic isolation between species or groups of species with no common RMI-values. The enzyme-loci ALD, GOT-1, GOT-2, IPO-2, HK-1, HK-2, MDH-1, MPI and PGM are polymorphic.

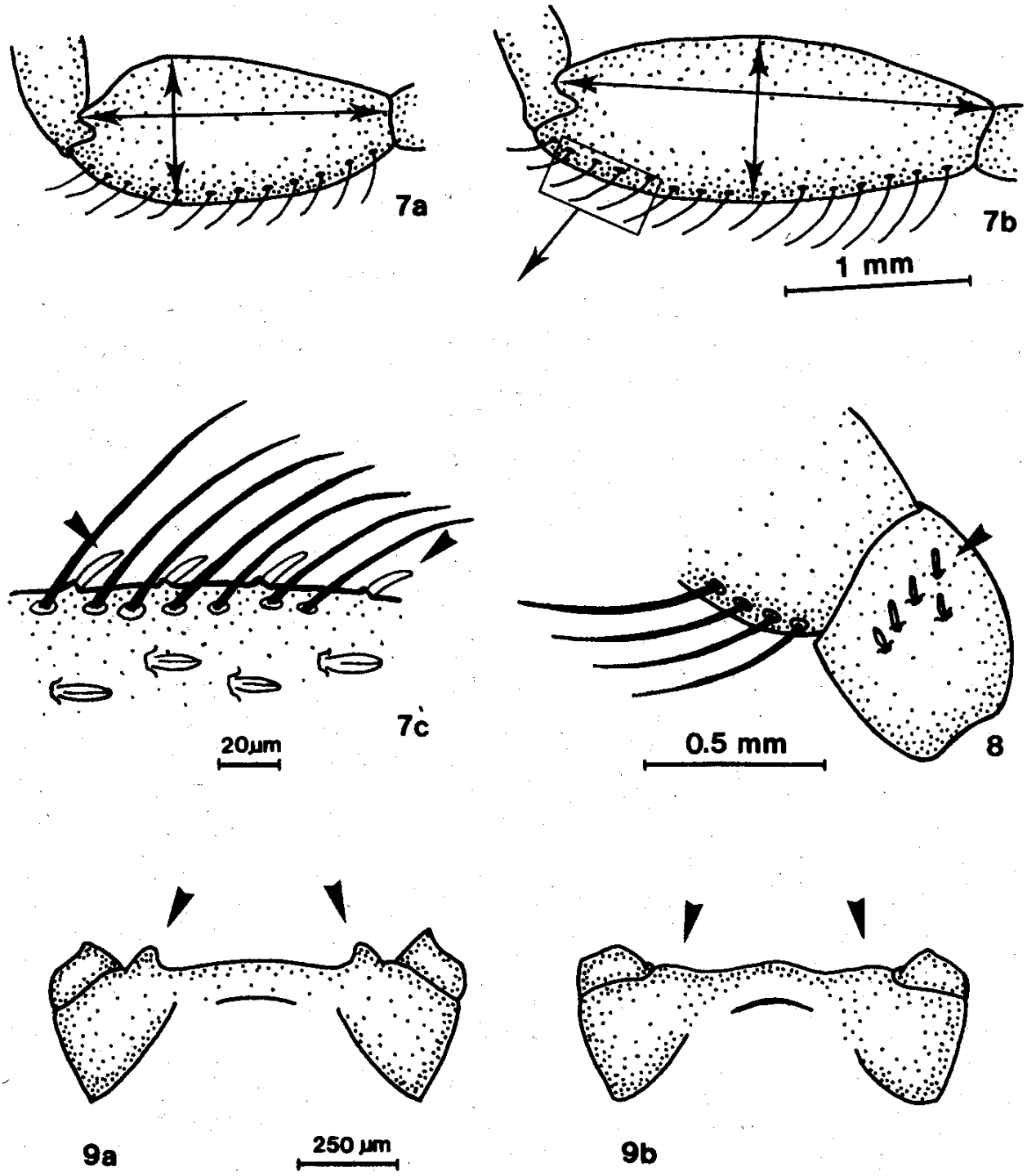
The pairwise coefficients of genetic identity ($\bar{I}$) recorded between all the different species of the *E. helveticus*-group are summarized in the identity matrix (Table 3).

	Glossa	Pronotum	Spines on the femur	Spines on the posterior margin of the hind leg	Number of tarsal claws	Length / width ratio of the fourth gill plates	Tergo-abdominal spines	Gill colouration
E. epeorides	-	rounded distally	rounded distally	-	**2**, 3	> 2.20*	pointed	-
E. c. carpathicus	rounded but elongated laterally	rounded distally	rounded distally ± dark at the base	yes	**2**, 3	< 2.00	pointed	sometimes with a violet tinge
E. c. vitoshensis	rounded	sligthly pointed distally	rounded distally ± dark at the base	yes	**2**, 3	< 2.00	pointed	sometimes with a violet tinge **
E. subalpinus	rounded	pointed distally ***	slightly divergent ± dark at the base	yes	**2**, 3	< 2.00	pointed	no
E. alveci	± squared	pointed distally	flattened distally ± dark at the base	yes	**2**, 3	< 2.00	pointed	sometimes with a violet tinge **
E. krueperi	quadrangular ****	pointed distally	pointed distally	no	**0**, 1, **2**, 3	< 2.00	pointed	no
E. helveticus	rounded	rounded to ± pointed	rounded or slightly concave distally	no	0, 1, **2**, 3	< 2.00	pointed	no
E. paraheiveticus	rounded	rounded distally	rounded or concave distally	yes	**0**, 1, 2	< 2.00	rounded	no
E. alpinus	rounded	rounded distally	rounded or concave distally	yes	**0**, 1, **2**	< 2.00	rounded distally	no
E. zelleri	rounded	rounded distally with concave outer margin	rounded distally	no	0, **1**, 2	= 2.00	pointed	no
E. austriacus	rounded	rounded distally with concave outer margin	rounded	yes	**2** assymetrical	< 2.00	rounded	no
E. picteti	rounded	rounded distally	rounded or slightly concave distally	yes	**0**, 1, **2**, 3	< 2.00	pointed	no

Tab. 1. Summary of morphological characters to separate larvae of members of the *E. helveticus*-group. *DEMOULIN (1955); **JACOB & BRAASCH (1984); ***SOWA (1973); ****HEFTI & TOMKA (1988).

Tab. 2. Electromorph frequencies of fifteen enzyme-loci (for abbreviations see text): enzyme-loci (Enz.), relative mobility index of the electromorphs (Elect.) with their frequencies (%); n = number of specimens analysed.

Taxa / Enz.	Elect.	ca.c.	ca.v.	suba.	sive.	krue.	helv.	para.	alpi.	zell.	aust.	pict.
	n	6	4	5	32	14	72	28	50	31	15	71
GPDH	100	100	100	100	100	100	100	100	100	100	100	100
	n	6	4	5	36	14	72	28	50	31	15	71
MDH-2	100	100	100	100	100	100	100	100	100	100	100	100
	n	6	4	5	24	14	71	28	50	30	15	71
AK	100	0	0	0	0	0	0	0	0	100	0	0
	106	100	100	100	100	100	100	100	100	0	100	100
	n	6	4	5	24	14	71	28	50	30	15	71
APK	98	100	100	100	100	0	100	100	100	0	100	100
	100	0	0	0	0	100	0	0	0	100	0	0
	n	6	4	5	20	14	71	28	48	29	15	69
IPO-1	96	0	0	0	0	0	100	100	100	100	100	100
	99	100	100	100	100	100	0	0	0	0	0	0
	n	6	4	5	30	14	72	28	50	30	15	71
RDH	100	100	100	100	0	0	0	0	0	0	0	0
	101	0	0	0	100	100	100	100	100	100	100	100
	n	6	4	5	24	14	67	28	47	31	15	71
ALD	95	0	0	100	0	0	100	100	100	100	100	100
	96	33	0	0	100	0	0	0	0	0	0	0
	97	67	100	0	0	0	0	0	0	0	0	0
	100	0	0	0	0	100	0	0	0	0	0	0
	n	6	4	5	22	15	72	28	50	31	16	72
GOT-2	97	0	0	0	0	0	0	0	4	0	0	2
	98	0	0	100	0	0	0	0	0	0	0	0
	99	100	100	0	100	100	0	0	96	100	100	98
	101	0	0	0	0	0	100	100	0	0	0	0
	n	6	4	5	32	14	71	26	38	32	11	77
HK-1	101	0	0	0	0	60	0	0	5	0	0	0
	102	100	100	100	100	40	95	100	95	97	0	0
	103	0	0	0	0	0	5	0	0	3	100	92
	104	0	0	0	0	0	0	0	0	0	0	8
	n	6	4	5	30	14	71	28	51	31	16	71
IPO-2	93	0	0	0	0	0	0	0	4	0	0	0
	97	0	0	0	0	100	96	96	92	100	100	100
	99	0	0	0	0	0	0	4	0	0	0	0
	101	100	100	100	100	0	4	0	4	0	0	0
	n	6	4	5	22	15	62	29	45	31	14	69
MPI	99	0	0	0	0	0	0	0	60	0	0	2
	100	0	0	100	0	0	100	100	36	100	100	98
	101	100	100	0	0	100	0	0	4	0	0	0
	102	0	0	0	100	0	0	0	0	0	0	0
	n	6	4	5	23	14	71	30	48	29	11	69
HK-2	102	0	0	0	0	0	0	7	0	0	0	0
	107	0	0	0	0	57	0	0	0	0	0	0
	108	100	100	100	100	43	100	93	100	100	0	0
	112	0	0	0	0	0	0	0	0	0	33	0
	113	0	0	0	0	0	0	0	0	0	67	100
	n	6	4	5	23	14	76	25	49	29	13	73
GOT-1	100	0	0	0	0	0	5	0	0	0	0	0
	101	0	0	0	0	0	0	0	4	0	0	0
	105	100	100	100	0	100	95	0	96	100	20	0
	106	0	0	0	0	0	0	96	0	0	0	80
	108	0	0	0	0	0	0	4	0	0	80	20
	110	0	0	0	100	0	0	0	0	0	0	0
	n	11	4	10	36	21	105	50	65	37	16	113
MDH-1	96	0	0	0	0	0	0	29	0	0	0	0
	97	0	0	0	0	0	0	4	0	0	0	0
	98	0	0	50	0	50	44	56	25	35	4	46
	100	55	100	50	0	50	45	21	40	40	48	54
	101	0	0	0	0	0	11	0	35	20	48	0
	102	45	0	0	100	0	0	0	0	5	0	0
	n	6	4	5	33	14	74	28	49	43	13	74
PGM	98	0	0	0	0	0	0	0	2	19	0	0
	99	0	0	0	0	0	0	0	0	21	0	0
	100	0	0	0	0	0	0	0	2	0	0	0
	101	0	0	100	0	100	94	100	96	60	100	0
	102	100	100	0	0	0	6	0	0	0	0	96
	103	0	0	0	100	0	0	0	0	0	0	4



Figs. 7–9. Nymph: Metafemur of *E. carpathicus vitoshensis (7a), E. picteti* (7b) and detail of the posterior margin of the metafemur (7c). Procoxa of *E. parahelveticus* (8). Imago: forceps base of *E. austriacus* (9a) and *E. parahelveticus* (9b).

DISCUSSION

Starch gel electrophoresis is a highly specific technique, which detects changes in the genetic pool of populations.

ZURWERRA *et al.* (1987) analysed eight species of the *E. helveticus*-group electrophoretically illustrating their specific status. The present study adds a biochemical characterization of *E. austriacus, E. siveci* and *E. carpathicus vitoshensis. E. austriacus* exhibits high biochemical affinities with *E. picteti* ($\bar{I}$ = 0.87) but

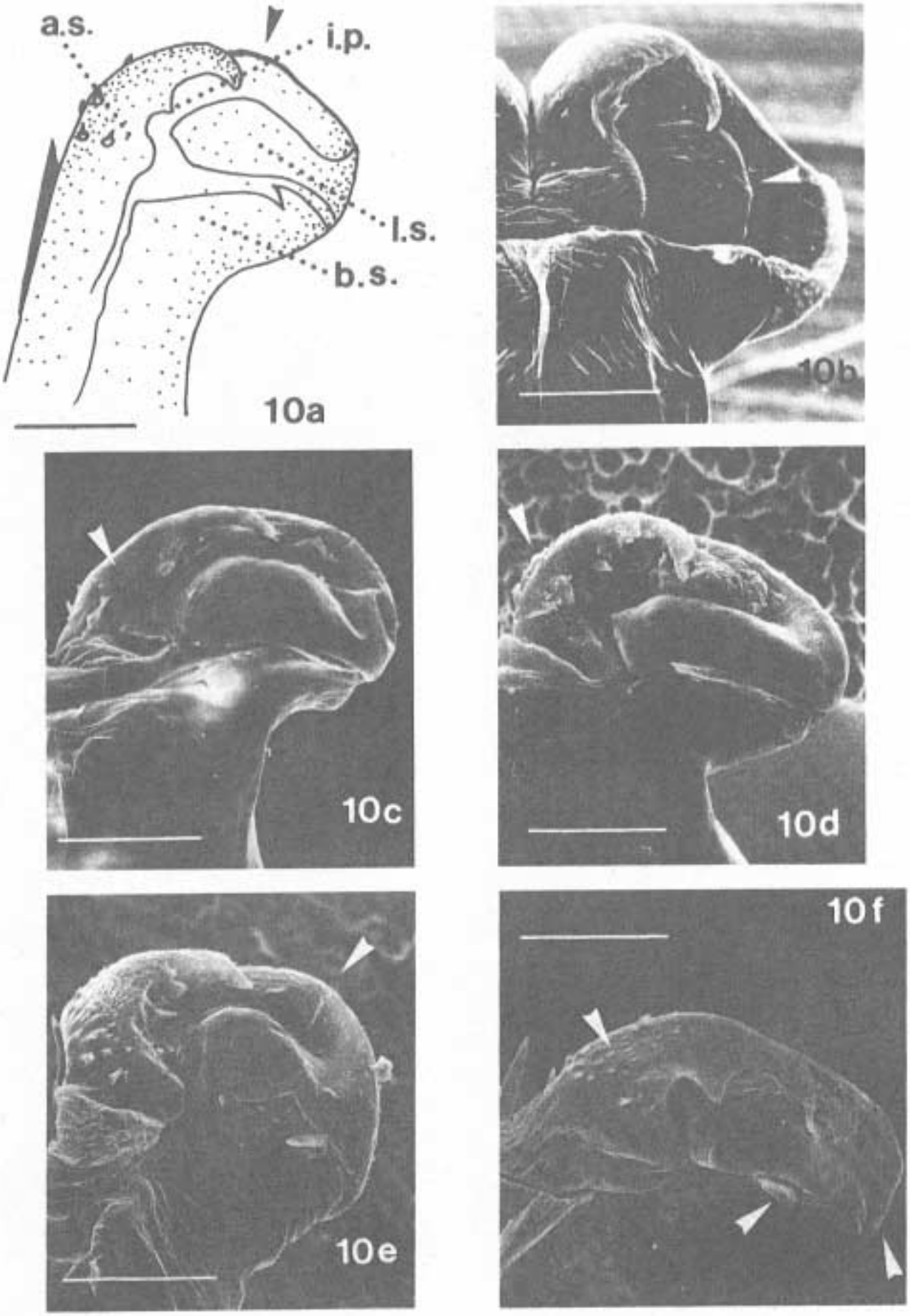


Fig. 10. SEM photographs of dorsal face of the penis lobe of: *E. epeorides* (10a), *E. krueperi* (10b), *E. carpathicus carpathicus* (10c), *E. carpathicus vitoshensis* (10d), *E. subalpinus* (10e), and *E. siveci* (10f). a. s. = apical sclerite; l. s. = lateral sclerite; b. s. = basal sclerite; i. p. = inner projection. Scale lines = 100 μm.

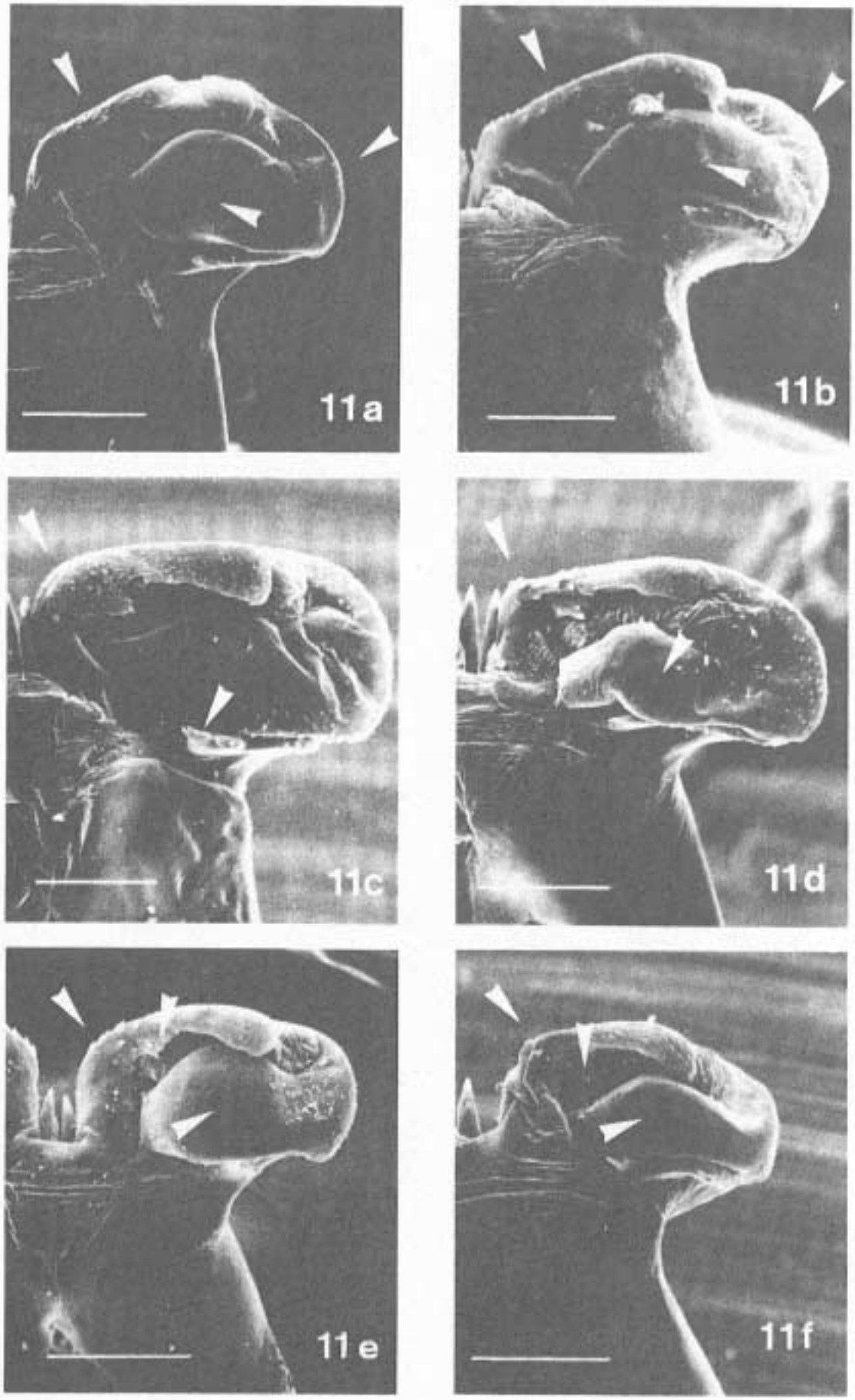


Fig. 11. SEM photographs of dorsal face of the penis lobe of: *E. parahelveticus* (11a), *E. austriacus* (11b), *E. picteti* (11c), *E. helveticus* (11d), *E. alpinus* (11e) and *E. zelleri* (11f), specimen with no typical teeth on the b. s.. Scale lines = 100 μm.

Tab. 3. Identity matrix of relative mobilities of fifteen enzyme-loci between the taxa of the *E. helveticus*-group analysed electrophoretically showing the coefficient of genetic identity ($\bar{I}$) between two taxa.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
E. para. (1)	-	.93	.82	.73	.66	.70	.43	.42	.52	.65	.47
E. helv. (2)		-	.90	.75	.67	.77	.51	.51	.59	.72	.48
E. alpi. (3)			-	.79	.70	.81	.59	.59	.67	.69	.56
E. aust. (4)				-	.87	.67	.39	.40	.55	.53	.42
E. pict. (5)					-	.61	.45	.45	.47	.46	.42
E. zell. (6)						-	.45	.44	.64	.56	.42
E. ca.c. (7)							-	.98	.59	.72	.67
E. ca.v. (8)								-	.58	.71	.60
E. krue. (9)									-	.52	.48
E. sub. (10)										-	.54
E. siv. (11)											-

both taxa are biochemically differentiable at the PGM locus. *E. siveci* is clearly separable from other members of the *E. helveticus*-group (Table 2). In contrast, the electromorph distribution of *E. carpathicus vitoshensis* does not present any interspecific difference from *E. carpathicus carpathicus* (Table 2). On the basis of the enzyme-loci investigated, both taxa share the same genetic pool and no sign

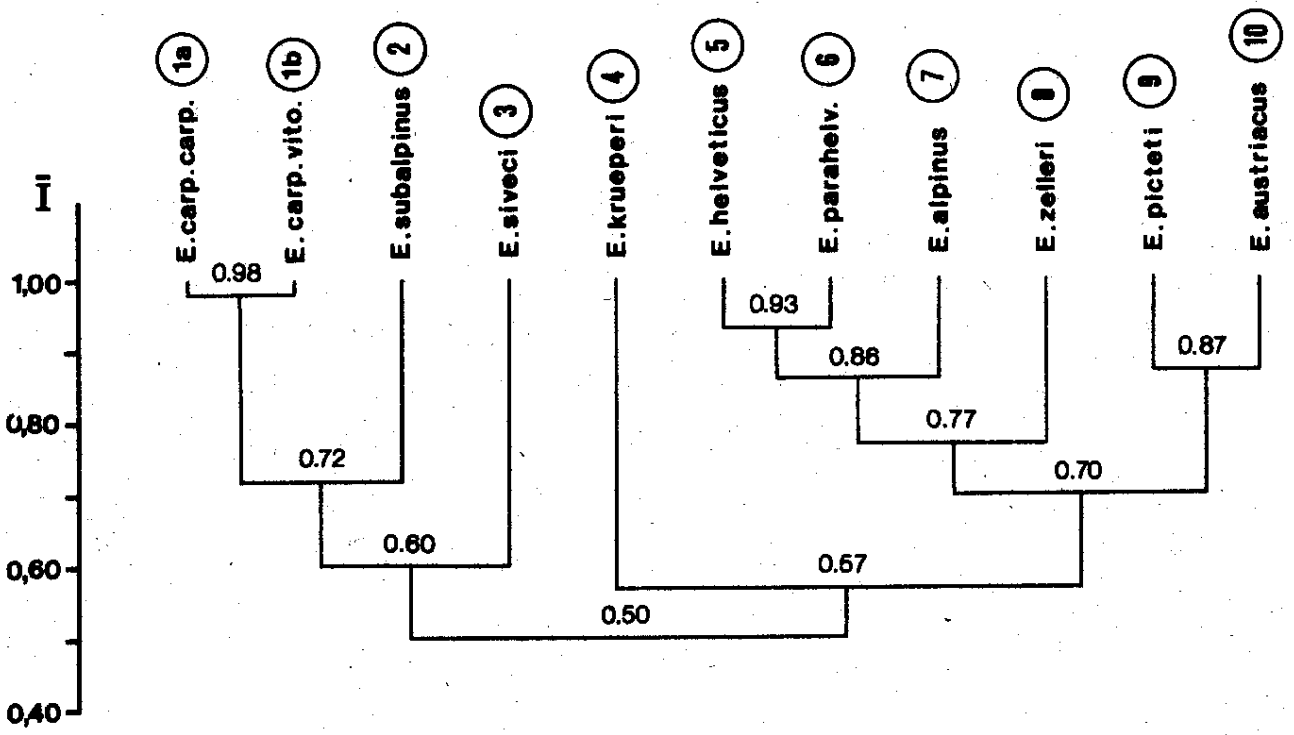


Fig. 12. Dendrogram of biochemical affinities within the *E. helveticus*-group (except for *E. epeorides*) using the unweighted pair group arithmetic average (UPGMA) clustering method, where $\bar{I}$ expresses the mean genetic identity.

of genetic isolation was found. The Ī-value of 0.98 recorded indicates intraspecific differences between the two taxa, which are due to the presence of polymorphic enzyme-loci in *E. carpathicus carpathicus* (ALD, MDH-1). This is not in contradiction to their subspecific status.

All values of the correlation matrix were used in constructing the dendrogram (Fig. 12) where plesio- and apomorphic character states are not distinguished. The dendogram expresses genetic similarities. A phylogenetic tree is achieved when both convergencies and synapomorphies are recognized. The

Tab. 4. Characters used to construct the cladogram (Fig. 13); only apomorphic character states are listed.

0	-	Enzyme IPO-2 (97 or 101) if α-GPDH (100). The nearest sister-group is the *E. venosus*-group.
1	-	Outer margin of apical sclerite of penis lobe regularly curved and convergent and more or less pointed distally.
2	-	IPO-2 (101).
3	-	ALD (100).
4	-	APK (100). Convergence between *E. zelleri* and *E. krueperi*.
5	-	MPI (102).
6	-	GOT-1 (110).
7	-	RDH (100).
8	-	MPI (101). Convergence between *E. krueperi* and *E. carpathicus*.
9	-	GOT-2 (98).
10	-	IPO-1 (96).
11	-	AK (100).
12	-	Enzyme HK-1 (103/104).
13	-	Enzyme HK-2 (112/113).
14	-	Enzyme PGM (102). Convergence between *E. carpathicus* and *E. picteti*.
15	-	Enzyme GOT-2 (101).
16	-	Lateral sclerite situated in the basal half of penis lobe.
17	-	GOT-1 (106). Convergence between *E. parahelveticus* and *E. picteti*.

cladogram was constructed in such a way as to minimize possible convergencies (Fig. 13). The *E. helveticus*-group is divided into two clades, the East-European species: *E. carpathicus, E. subalpinus, E. siveci, E. krueperi* form one, the remaining species the other. No character could be found to define the sister-group relationships of *E. zelleri, E. alpinus* and the two clades *E. picteti-E. austriacus, E. helveticus-E. parahelveticus.* The polarity of both morphological characters (1 and 16 of Table 4) were tested by out-group comparison.

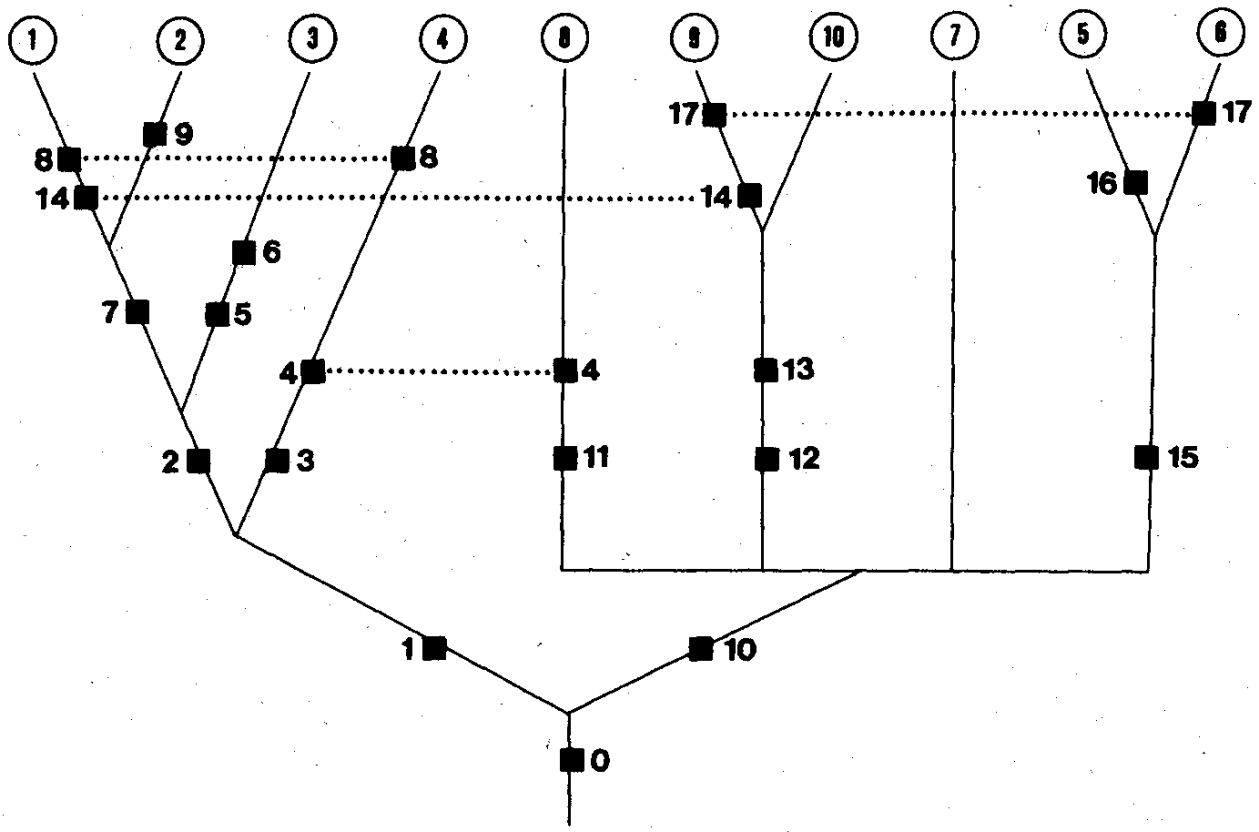


Fig. 13. Cladogram illustrating the phylogeny of the *E. helveticus*-group (for characters see Table 4) investigated biochemically. Apomorphic character: ■, convergence: .....

BIOGEOGRAPHICAL CONSIDERATIONS

The members of the *E. helveticus*-group are exclusively restricted to Europe. The geographical distribution of each taxon is illustrated in Fig. 1 and shows that the species are mainly related to mountain and submountain areas of the Alps, the Balkans and the Carpathians.

The Alps

E. helveticus and *E. picteti* are species widely distributed throughout the Alps and are associated with cold streams of low productivity. These species have nevertheless extended their initial alpine distribution to other mountain regions (Jura, Italian Apennine) and even to submountaneous zones, where both species can be considered as bioindicators for cold unpolluted streams.

The occurrence of *E. alpinus* and *E. parahelveticus* is more limited. The fi species is a typical "high mountain" stenotherm organism, colonizing col poorly buffered rivers of the epirhithron. *E. alpinus* is restricted to granitic ge logical formations, whereas *E. parahelveticus* has been recorded only in cold a calcareous prealpine rivers of the epi-metarhithron.

E. zelleri is rare and shows a discontinuous distribution. The species is oft associated with isolated populations of *E. picteti.* One hypothesis which might e plain the geographical distribution of *E. zelleri* may be its progressive disappea ance from the Alps.

Until now, the species *E. austriacus* has been recorded only from its ty locality (near Lunz) and in a few stations in the north-east part of the Austri Alps.

The Balkans

The species *E. siveci* has been collected only at its type locality in Sloven and is therefore considered as a part of the Balkan fauna (Dr. I. Sivec, person communication).

The subspecies *E. carpathicus vitoshensis* is a mountain and submountai species from the Yugoslavian, Bulgarian (JACOB & BRAASCH, 1984) and Helleni Balkans.

E. krueperi and *E. epeorides* are more adapted to low altitudinal and war streams of the hyporhithral or epipotamal stages of the Hellenic Balkans.

The Carpathians

E. carpathicus carpathicus and *E. subalpinus* are typical components of th Varian and Carpathian fauna (LANDA & SOLDAN, 1980). The former is know from Poland and Rumania (SOWA, 1973) and it probably occurs throughout th Carpathian range (SOWA, 1975a). *E. subalpinus* is distributed from souther Czechoslovakia to the Sudeten Mountains (SOWA, 1975b; LANDA, 1969). It taxonomical status is still in doubt as two forms have been found by Dr. R. SOWA one in Czechoslavakia and another in Poland (personal communication). *E. sub alpinus* is mainly a mountain species whereas *E. carpathicus carpathicus* present a lower altitudinal distribution (SOWA, 1975b).

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RÉSUMÉ

Les espèces européennes appartenant au genre *Ecdyonurus* du groupe *helveticus* sont révisées, et une clé de détermination est proposée pour les larves et les imagines. La mobilité électrophorétique de quinze loci enzymatiques a été examinée pour toutes les espèces du groupe à l'exception de *E. epeorides*. Des caractéristiques morphologiques et biochimiques ont permis d'élaborer un cladogramme.

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