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Morphology and Evolution of *Cypria cavernae* n. sp. (Ostracoda, Crustacea) [1]

by

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Key words: Ostracoda, *Cypria*, troglobiont species, morphology, evolution, taxonomy.

Abstract: *Cypria cavernae* n. sp. is the first exclusively cavernicolous dwelling species of the genus *Cypria* and displays typical troglomorphic characteristics, i.e. reduction of eyes; lack of body and occular pigments; elongation of distal setae, claws of antennae and of walking legs; and enlargement of the distal chemosensorial region of the antennal aesthetasc Y. *C. cavernae* n. sp. occurs only in the Karst region near Trieste (Italy). *C. cavernae* n. sp. originates most probably from the cosmopolitan species *C. ophthalmica* (Jurine, 1820), which commonly colonizes karstic habitats. *C. ophthalmica* is a highly polymorphic species and can not be distinguished from *C. lacustris* Sars, 1890.

Zusammenfassung: *Cypria cavernae* n. sp. ist die erste ausschließlich in Höhlen vorkommende Art der Gattung *Cypria* und weist typische troglomorphe Merkmale auf, wie: die Reduktion der Augen; den Verlust der Pigmente; eine Verlängerung der distalen Borsten, Klauen der Antennen und der Schreitbeine; und eine Vergrößerung des chemosensorischen Abschnittes des Aesthetasken Y der zweiten Antenne. *C. cavernae* n. sp. kann nur im Karstgebiet von Triest (Italien) nachgewiesen werden und hat sehr wahrscheinlich ihren Ursprung in der kosmopolitischen Art *C. ophthalmica* (Jurine, 1820), die gewöhnlich auch Karstgebiete besiedelt. *C. ophthalmica* ist eine äußerst polymorphe Art und kann nicht von *C. lacustris* Sars, 1890 unterschieden werden.

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Introduction

Cypria cavernae n. sp. lives in caves of the Karst region of Trieste (Italy). It is the only blind and unpigmented *Cypria* species and it lives exclusively in subterranean waters. In Europe only a few species of the genus *Cypria* can be found, but these are wide-spread (Löffler & Danielopol 1978). The most important ones are *C. ophthalmica* (Jurine, 1820), *C. lacustris* Sars, 1890, *C. exsculpta* Fischer, 1855. *C. cavernae* n. sp. belongs to the species-group *C. ophthalmica* (as subsequently defined here, see below). It has long natatory antennal setae, which exceed the extremities of the distal antennal claws and differs from the other species of this group only through slight, mainly quantitative, morphological differences.

As *C. lacustris* and *C. ophthalmica* look very similar, since the first description of *C. lacustris* by Sars (1890) the validity of the two species has not always been admitted. Alm (1915) considered *C. lacustris* a local race. In response Sars (1925) made a new description and again defined *C. lacustris* as an independent species. Klie (1938a) expressed his scepticism about Sars' opinion. In his further papers he often recorded *C. ophthalmica* (e.g. 1938b), but never *C. lacustris*. He favoured the idea of a unique polymorphic species, *C. ophthalmica* (considering *C. lacustris* just a morphotype of *C. ophthalmica*). In the sixties, discussion about the differenciation of the two species was resumed.

Petkovski (1960) distinguished *C. lacustris* and *C. ophthalmica* by the female genital processes (see below). Löffler (1964) confirmed Klie's supposition when he found, within a population of *Cypria*, animals with transitional characters between *C. lacustris* and *C. ophthalmica*. Finally Petkovski (1976) tried to save the validity of *C. lacustris* (Sars) and used two taxonomic characters, the male copulatory organ and the female genital process, to distinguish the two species, viz.: *C. ophthalmica* - female with two genital processes; male with distal process of inner lobe of hemipenis shorter than outer lobe; *C. lacustris* - female with one finger-like genital process; male with distal process of inner lobe of hemipenis rising beyond outer lobe.

For our investigation this problem is of special importance, because in the literature there are indications that *C. lacustris* has different ecological preferences, e.g. it prefers cold and deep lake waters and/or it commonly colonizes interstitial habitats (Nüchterlein, 1969). *C. lacustris* (Danielopol, 1965, 1978) and the closely related *C. sketi* Petkovski, 1976, also colonize karstic habitats. *C. ophthalmica* inhabits running waters (Mertiny, 1980) and interstitial groundwaters (Klie, 1938b), but less frequently than *C. lacustris* (Petkovski, 1960, 1962).

In this paper we examine the morphological variability of *C. ophthalmica* and the validity of the related species *C. lacustris* and *C. sketi*. This will help us to achieve a further understanding of the origin and evolution of the new cave species *C. cavernae*.

Material and methods

Of *C. cavernae* n. sp. 2 males, 3 females and 1 juvenile were found near the sealevel in a 50 m deep karst cave near S. Giovanni di Duino, VG 226 (VG = land register number of the caves of Venezia Giulia), 20 km NW of Trieste (leg. F. Gasparo, 11. Dec. 1983). One male was found in the cave of Comarie, VG 4221, 3 km north of the previous sampling place (leg. F. Gasparo, 24. Mar. 1984). The comparative morphology of several populations belonging to what we have called the *Cypria ophthalmica*-group (see below) was investigated: (1) A population from an uncovered well of the village Agios Georghios in the island of Euboea, Greece (fig. 1), depth to the groundwater table 1.8 m, water depth 0.5 m, 58 specimens (sampled with a Cvetkov net, see location sample number 10 in Danielopol, 1981; the taxonomic identification was C. lacustris). (2) A sample from a well in Artas near Lyon, France (fig.1), at a depth of 35 m, six specimens (sampled with a Cvetkov net, leg. P. Marmonier, identifed by the latter (pers. comm.) as *C. ophthalmica*). (3) A sample from a lake, the Neusiedlersee, in the reed belt at Purbach, Austria (fig. 2) at a depth of 1 m, 75 specimens (see details M. C. Mendez, 1987). (4) A sample from a lake, the Mondsee, Austria (fig. 2) in the profundal zone (40-50 m), 58 specimens (Kajak corer) at the site MO-7 (see location in Danielopol et al., 1985). (5) From the littoral zone, (in front of the Limnological Institute) depth 1-3 m, 85 specimens (leg. D.L. Danielopol and H. Wagenleitner); (6) A sample from a fish pond in Mayerwies near Salzburg, depth 1 m, 56 specimens (fig. 2) (leg. D.L. Danielopol); (7) Lake of Halleswies, Upper Austria (fig. 2), collected in the macrophyte belt, depth 1 m, 51 specimens (leg. M. Handl and H. Wagenleitner).

For the description of *Cypria cavernae* n. sp. the author is following the description model of Broodbakker & Danielopol (1982).

For comparative morphology of the various *Cypria* species we studied the following character traits: height/length ratio of the carapace, the length of the distal claw of the walking leg, the length of the chemosensorial part of the aesthetasc Y.

Measurements: The height and the length of the carapace were measured (see fig. 3). The means of the height/length ratio of the carapace for adult males and females were compared by variance analysis, using the Scheffe procedure (Zöfel, 1988). The length of the distal (chemosensorial) part of the antennal aesthetasc Y (Ld) (figs 5, 16, 17) as well as the length from the point of insertion of the aesthetasc Y to the end of the first segment of the antennal endopodite (Len) (fig. 5), and the length of the distal claw (LG) of the walking legs (fig.4) were measured. The means of the ratios Ld/Len and LG/Len were compared using a Student *t*-test.

Scanning electron microphotographs were made with a "Cambridge Stereoscan Mark 2 A" at the Institute of Zoology, University of Salzburg.

The *Cypria ophthalmica* problem

C. ophthalmica and *C. lacustris* look very similiar, therefore the validity of *C. lacustris* was questioned by several authors, as mentioned above. In this section the range of the morphological variability of *C. ophthalmica* and *C. lacustris* will be studied and the validity of the taxonomic characters used by Petkovski (1976) will be reappraised.

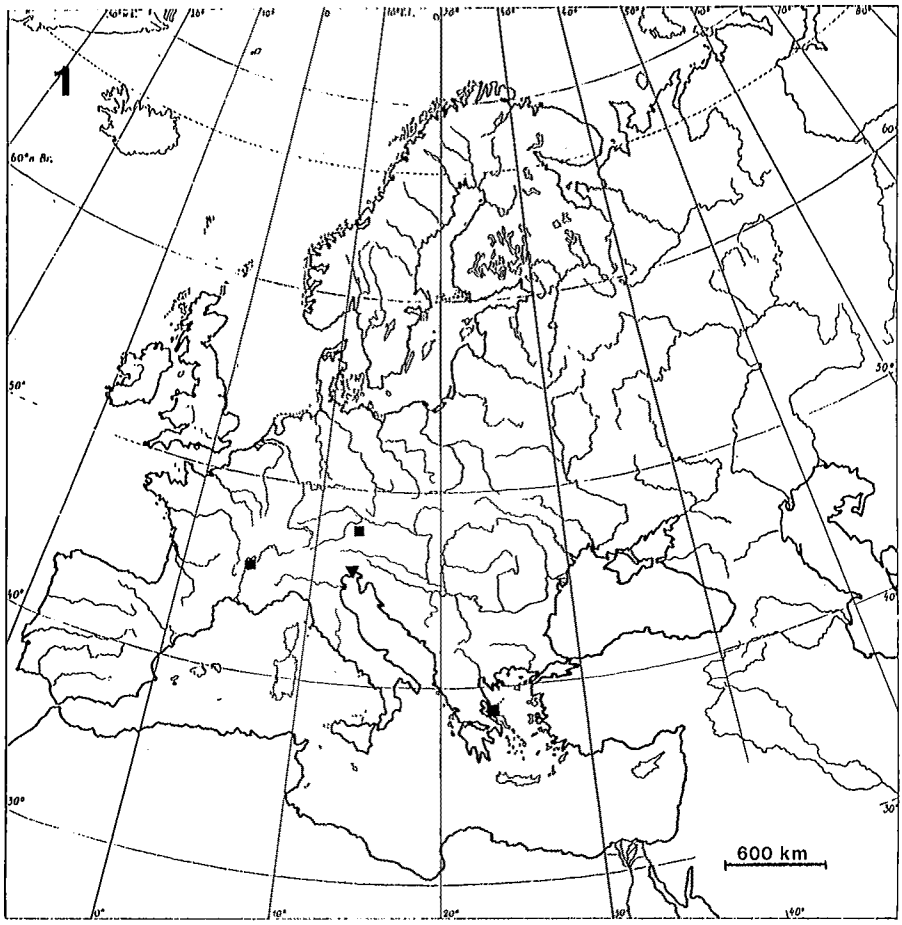


Fig. 1: The sampling sites for the populations of *C. ophthalmica* and *C. cavernae*: squares = *C. ophthalmica* in Austria, Artas (France), Euboea (Greece); triangle = *C. cavernae* near Trieste (Italy).

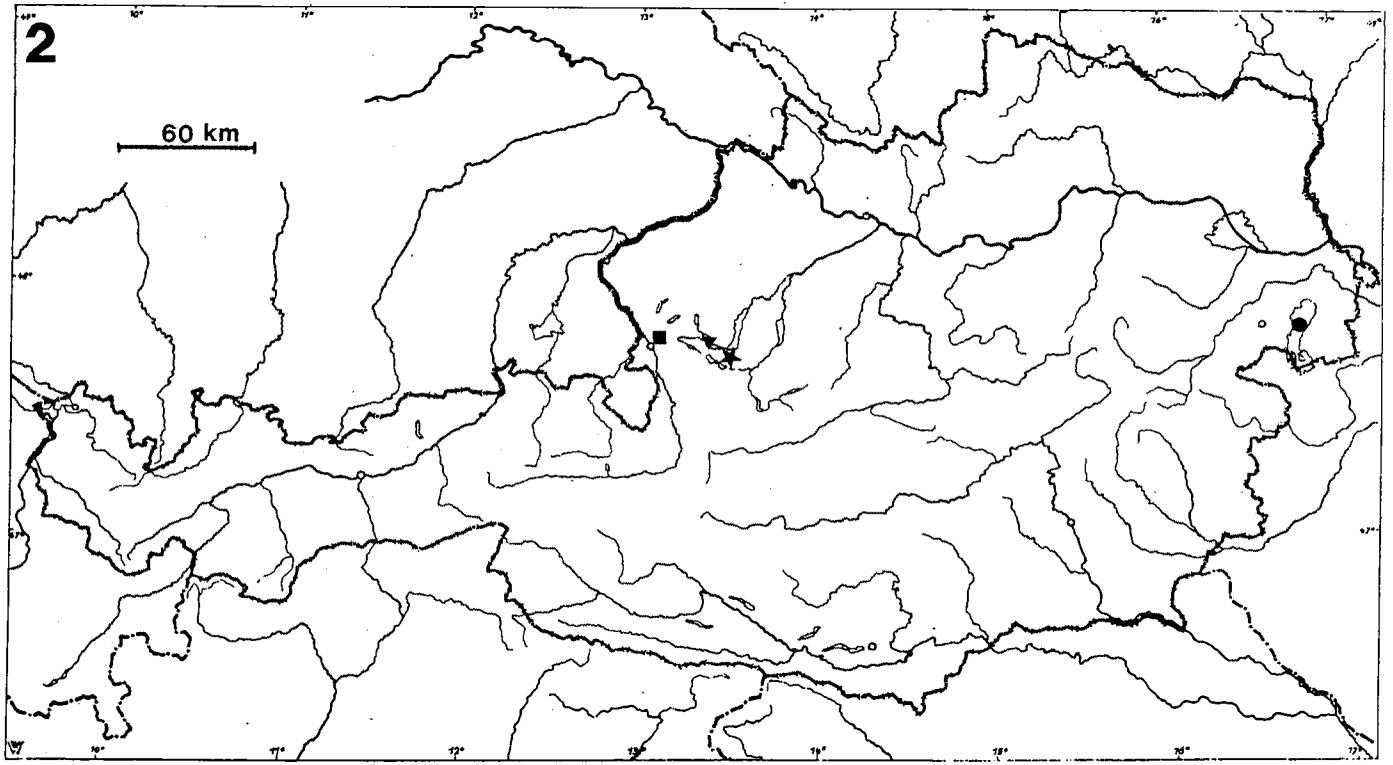
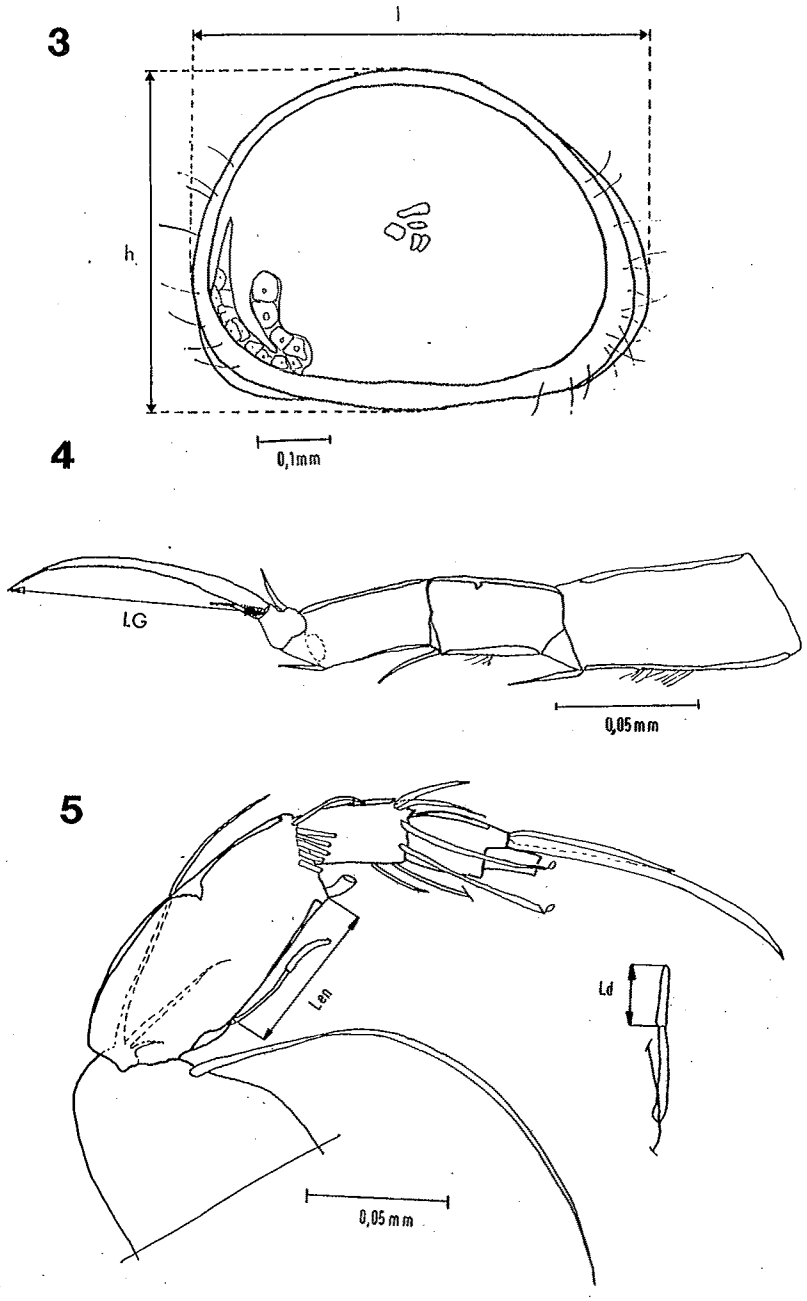
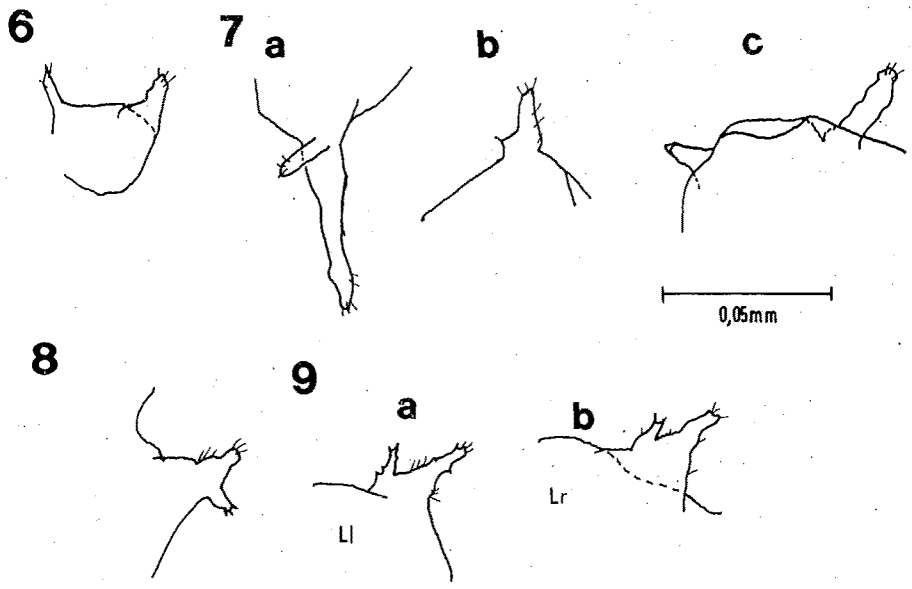


Fig. 2: The sampling sites for *C. ophthalmica* in Austria: dot = lake of Neusiedl, square = Mayerwies, closed tringle = lake of Mondsee, star = lake of Halleswies.



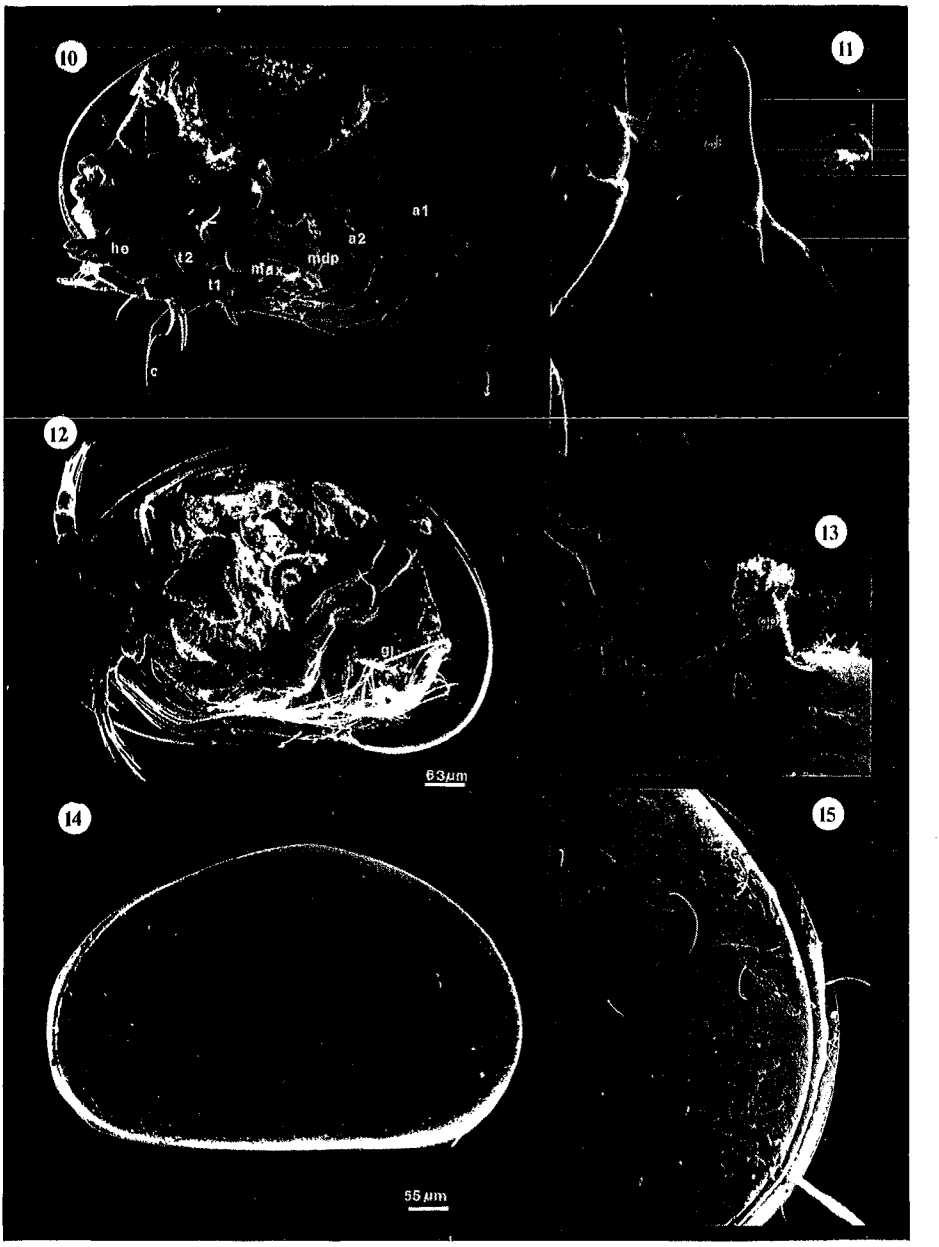
Figs 3 - 5: *Cypria ophthalmica*: 3 - female, left valve; l = length and h = height of the carapace. 4 - walking leg; LG = length of the distal claw. 5 - male 2nd antenna; Len = measured length of the first endopodite, Ld = length of the distal part of the aesthetasc Y.



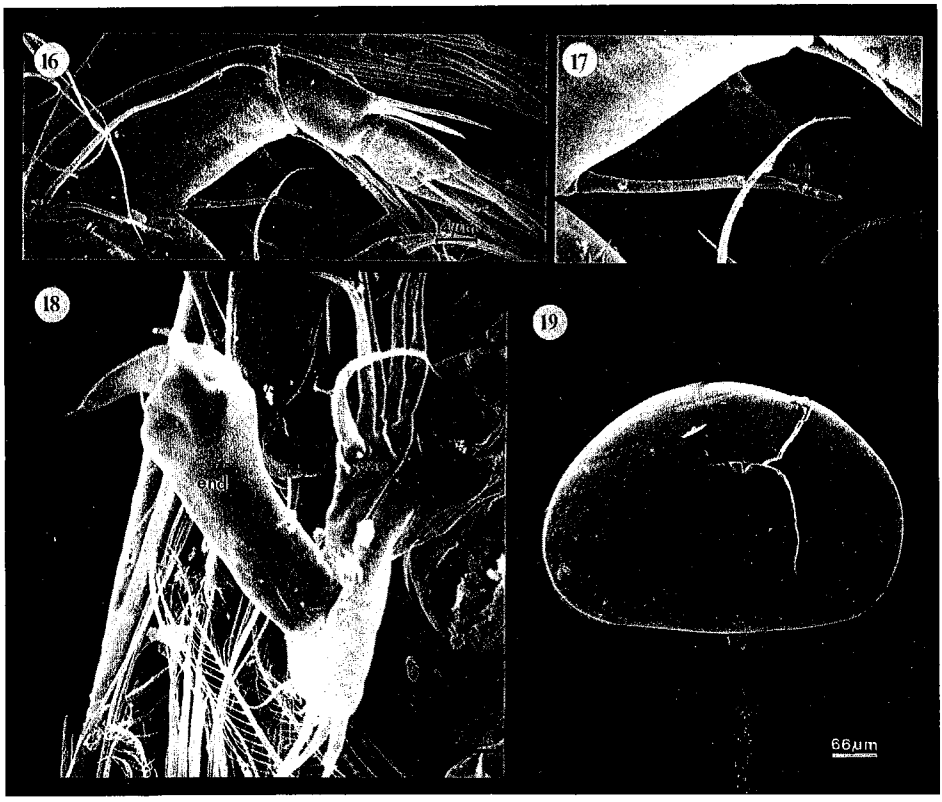
Figs 6 - 9: *C. ophthalmica*, female, shape of the genital processes: 6 - Neusiedlersee; 7 - Halleswiessee, different morphotypes; 8, 9 - Artas, different morphotypes, Ll = left lobe, Lr = right lobe.

Two populations, those of Neusiedlersee and of Mayerwies, show *C. ophthalmica* characters, according to Petkovski's (1976) criteria, i.e. females with two genital processes (figs 6, 12, 13). For the population of the Neusiedlersee we found within one sample of 75 animals only seven males (sex ratio 91 : 9). In all the other populations the sex ratio is about 50 : 50. Two samples, that of the profundal zone of the Mondsee and that of the well in Euboea, display *C. lacustris* characters, i.e. female genitalia with one finger-like process. In two populations we found females with transitional characters between *C. ophthalmica* and *C. lacustris* as described by Löffler (1964): those of the Artas well and those from the Halleswiessee. These populations show a wide variation in the shape of the female genital lobe. In Halleswiessee we found females having one finger-like genital process as well as females having a genital lobe with one long and one small lateral process (fig. 7a), having one process with a small peaked protuberance (fig. 7b) or having two processes (fig. 7c). In Artas we observed specimens with one process and a small lateral process (figs 8, 9a,b).

The sample of the littoral of lake Mondsee is the only one in which we found female specimens displaying typical characters of *C. ophthalmica* mixed with *C. lacustris* - like morphotypes. No transitional characters between the two morphological types occur. In this population the females with two genital processes were clearly in the minority, the ratio being 74 : 26.



Figs 10 - 15: SEM microphotographs: 10-13 *Cypria ophthalmica*; 10 - male (Mondsee profundal zone), right valve removed, a1 = 1st antenna, a2 = 2nd antenna, mdp = mandibular palp, max = maxilla with endo- and exopodite, t1 = 1st thoracopod, t2 = 2nd thoracopod, c = distal claw, he = hemipenis; 11 - detail of hemipenis, il = inner lobe, ol = outer lobe; 12 - female (Neusiedlersee), left valve removed, gl = genital lobe; 13 - genital processes (= gp); 14, 15 - *Cypria cavernae* n. sp. carapace; 14 - left valve external view; 15 - anterior selvage (= se).



Figs 16 - 19: *Cypria ophthalmica*, SEM microphotographs: 16 - male 2nd antenna (Mondsee profundal zone), Y = aesthatasc Y; 17 - enlargement of aesthetasc Y in fig. 16, Ld = distal part of the aesthetasc Y; 18 - detail of the maxilla in fig.10, end = endopodite, exo = exopodite; 19 - left valve of *C. ophthalmica* (Mondsee profundal zone).

The morphology of the hemipenis in males shows an interesting variability within each population. One can find copulatory organs, displaying exactly the taxonomic characters given by Petkovski (1976), i.e.: for *C. ophthalmica* the inner lobe of the hemipenis shorter than the outer lobe and for *C. lacustris* the inner lobe rising beyond the outer lobe. On the other hand we found a lot of material which is very hard to classify, because of the intermediate position of the inner lobe. An example of this is provided by the specimens of the population of the profundal zone of the Mondsee. As shown by the SEM microphotographs (figs 10, 11), the distal process of the inner lobe of the hemipenis does not rise beyond the outer lobe. (This is an important finding, because by the SEM method one can distinguish between true structures and artefacts due to the mounting of specimen for transmitted light microscopy). In addition it can be said, that the variability of the male copulatory organ is comparable to that of the female genital processes described above.

population	sex	mean h/l %	s.e.	n
Neu	m	73.67	1.163	7
Neu	f	72.90	0.242	68
May	m	68.09	0.472	23
May	f	69.95	0.468	33
Eub	m	66.41	0.321	24
Eub	f	67.50	0.308	34
Mo.Li	m	66.62	0.311	33
Mo.Li	f	67.41	0.320	52
Mo.Pro	m	68.39	0.420	26
Mo.Pro	f	69.17	0.263	32

Table I: The mean values of the height/length ratios of the carapace.
Neu = Neusiedlersee, May = Mayerwies, Eub = Euboea, Mo.Li = Mondsee littoral zone, Mo.Pro = Mondsee profundal zone.

	significance level			
populations	n.s.	<0.05	<0.01	<0.001
May-Neu				m,f
May-Eub	m	f		
May-Mo.Li	m		f	
May-Mo.Pro	m,f			
Neu-Eub				m,f
Neu-Mo.Li				m,f
Neu-Mo.Pro				m,f
Mo.Li-Eub	m,f			
Mo.Pro-Eub	m,f			
Mo.Pro-Mo.Li	m,f			

Table II: Analysis of variance of the mean values of the height/length ratios of the carapace. Multiple range test (Scheffe procedure).
Neu = Neusiedlersee, May = Mayerwies, Eub = Euboea, Mo.Li = Mondsee littoral zone, Mo.Pro = Mondsee profundal zone, m = male, f = female.

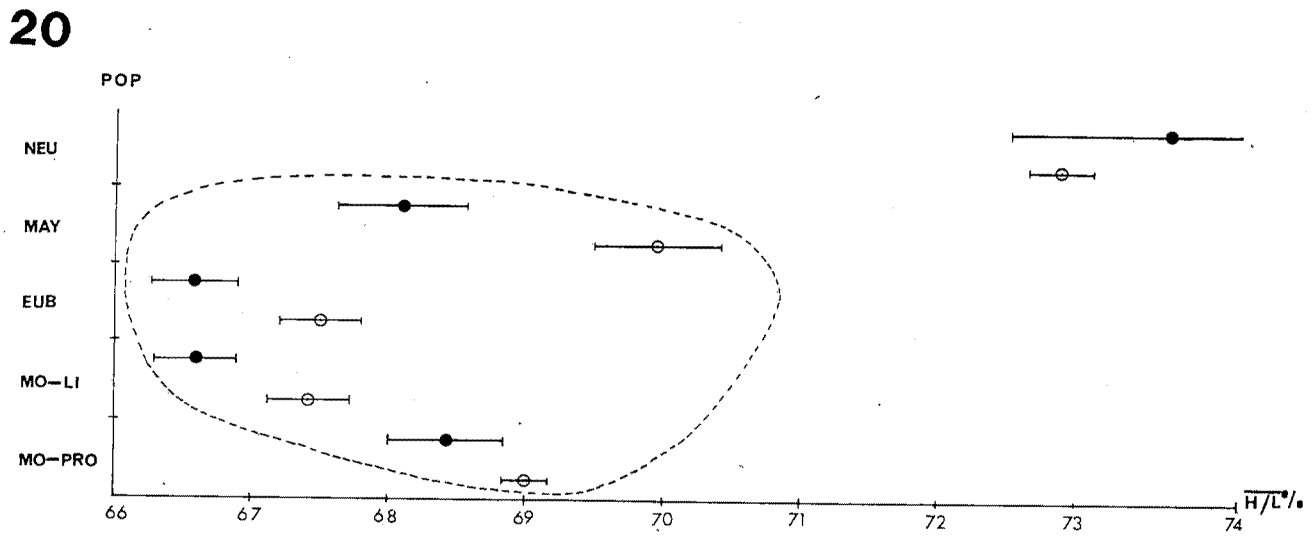


Fig. 20: Cluster diagram of the mean values of the height/length ratios (H/L %), POP = populations, NEU = Neusiedlersee, MAY = Mayerwies, EUB = Euboea, MO-LI = Mondsee littoral zone, MO-PRO = Mondsee profundal zone; dots = males, open symbols = females.

pop.LG/Len %	n_1	n_2	mean$_1$ %	mean$_2$ %
C.cav : Mo.Pro	4	12	261.24	189.56
C.cav : Mo.Li	4	12	261.24	183.70
C.cav : Neu	4	12	261.24	158.23
Mo.Li : Neu	12	12	183.70	158.23
Mo.Pro : Neu	12	12	189.25	158.23
Mo.Pro : Mo.Li	12	12	189.25	183.70
pop.Ld/Len %	**n_1**	**n_2**	**mean$_1$ %**	**mean$_2$ %**
C.cav : Neu	6	12	51.65	38.94
C.cav : Mo.Pro	6	12	51.65	36.09
C.cav : Mo.Li	6	12	51.65	41.17
Neu : Mo.Pro	12	12	38.94	36.09
Neu : Mo.Li	12	12	38.94	41.17
Mo.Pro : Mo.Li	12	12	36.09	41.17

Table III: The length of the distal claw and the distal part of the aesthetasc Y compared by a Student *t*-test.
pop.LG/Len = compared populations concerning the length of the claw (LG)/length of the first enopodite (Len) ratio, pop.Ld/Len = compared populations concerning the length of the distal part of the aesthetasc Y (Ld)/Len ratio, C.cav = *Cypria cavernae* n.sp., Neu = Neusiedlersee, Mo.Li = Mondsee littoral zone, Mo.Pro = Mondsee profundal zone, $n_{1,2}$ = number, s.d.$_{1,2}$ = standard deviation, P = probability.

The other limbs of *C. ophthalmica* and *C. lacustris* were also compared. No differences in the shape or the structure were found. The chaetotaxy is identical for both taxa. Sars (1925) was of the opinion that the prehensile palp of the male's maxillae (fig. 18) is different in the two species. In the present investigation we did not find any differences.

The results of the measurements, the height/length ratio, of the carapace for five populations (fig. 3) gives us range values of the mean for *C. ophthalmica*: males 68.09 - 73.67 %, females 69.95 - 72.9 % and for *C. lacustris*: males 66.41 - 68.39 %, females 67.41 - 69.17 % (table I, fig. 19). The analysis of variances shows that only the means of the height/length ratio from the population of the Neusiedlersee are very significantly ($P < 0.001$) different from the other populations. Only the females from the Mayerwies population are significantly different ($P < 0.05$) from the Euboea population and significantly different ($P < 0.01$) from the population of the Mondsee littoral. All other compaired pairs and even the males of the latter two populations are not significantly different (table II, fig. 20).

$s.d._1$	$s.d._2$	P	t-value
18.253	9.703	0.001	7.508***
18.253	11.459	0.001	7.988***
18.253	6.434	0.001	11.060***
11.459	6.434	0.001	6.713***
9.703	6.434	0.001	9.323***
9.703	11.459	0.05	1.353n.s.
$s.d._1$	$s.d._2$	P	t-value
3.063	2.074	0.001	9.166***
3.063	3.913	0.001	9.227***
3.063	4.421	0.001	5.862***
2.074	3.913	0.05	2.221*
2.074	4.421	0.05	1.586n.s.
3.913	4.421	0.05	2.905*

Table III: (continued)

Discussion

In the literature the status of *Cypria lacustris* as a clearly distinct species always was questionable and repeatedly new taxonomic characters, such as the colour of pigment, the shape of the furca, the shape of the prehensile palp, the height/length ratio of the carapace, the length and shape of the distal lobes of the hemipenis, and the number and shape of the female genital processes, were used in a try to save the validity of this species. The latter two characters have been discussed very often (Löffler, 1964; Petkovski, 1964, 1966, 1976). The height/length ratio of the carapace of *C. ophthalmica* and *C. lacustris* are rather variable. Sars (1925) illustrated individuals which have 65 % for *C. lacustris* and 72-73 % for *C. ophthalmica*. Nüchterlein (1969) has measured for *C. ophthalmica* males 69.5 - 75.5 % and for females 73 - 77 %, for *C. lacustris* males 69 - 71 % and for females 71 - 75 %. The calculated height/length ratio from data based on Petkovski (1960) for females *C. ophthalmica* is 72 - 75 % and for females *C. lacustris* 65 - 72 %. This agrees with the data in the present paper. The height/length ratios of the carapace of *C. ophthalmica* and *C. lacustris* overlap as the following data demonstrate. We have found specimens from different populations with exactly the same dimensions and therefore with the same height/length ratio (70 % and 67.8 %). Females from the populations of the Neusiedlersee and Mondsee (profundal), for example, with a length from

646.8 µm and a height of 454.3 µm, or females from Mondsee (profundal) and Mayerwies with 646.8 µm length and 438.9 µm height. Consequently the height/length ratio of the valves is not a valid taxonomic character.

Furthermore, it should also be noticed that Meisch (1987) presented SEM photos of the valves of *C. lacustris* and *C. ophthalmica* collected in southwestern France: on these pictures it can be seen that the structure of the marginal zones of the valves - an important taxonomic character - does not show any significant difference in the two 'species'.

The results in the present paper concerning the genital region also show a wide variation. The shape of the female genital processes in the left or right lobe for the same animal can be different. Löffler (1964) shows a specimen which has a tubercle on one side only and a finger-like process on the other side. Sywula (personal communication) has found specimens with one process on one side and two processes on the other. Petkovski (1964) points to the high diversity in the female genital processes and later (1966) found *Cypria* specimens in Slovenia that could be integrated neither within *C. ophthalmica* nor within *C. lacustris*. The great number of male specimens with an intermediate inner lobe on the hemipenis, as shown in fig. 11, also document that it is not possible to distinguish between different species with the taxonomic characters given by Petkovski.

In table III one can see that the largest *C. ophthalmica* sample from the Neusiedlersee has shorter distal claws on the walking legs than that of the Mondsee. The differences are very highly significant. In table III one can also see differences concerning the distal part of the aesthetasc Y, between Neusiedlersee and Mondsee profundal and littoral zones. The distal part of the aesthetasc Y in the Mondsee littoral population is larger than in the profundal and the Neusiedlersee populations. The differences are significant and could likewise be taken as an indication of the polymorphism of *C. ophthalmica*.

From all these data we suggest that only one species, *Cypria ophthalmica* (Jurine, 1820), should be recognized, of which "*C. lacustris*" is a local variation without taxonomic value. *Cypria ophthalmica* is therefore a very polymorphic species.

The results in the present paper suggest that microevolutionary processes have a dissimiliar effect on the morphology of different populations. In the Neusiedlersee a morphotype evolved which has a large carapace shape (as expressed by the height/length ratio), in the Mondsee the local population has evolved towards a morphotype with long distal claws on the walking legs. The morphological polymorphism is paralleled by the broad ecological valence and wide zoogeographical distribution. *C. ophthalmica* can be found in all kinds of waters, even in sulphurous springs (Mertiny, 1980), with a low oxygen content. It can be found even in sewage waters where this species shows a very high tolerance to pH values from 4.9 to 11.1 (Nüchterlein, 1969; Mertiny, 1980). *C. ophthalmica* is distributed worldwide with the exception of Australia (Hartmann & Hiller, 1977; Klie, 1938a; Löffler & Danielopol, 1978). In our case it is important that it has been found on the Yugoslavian Adriatic coast in both wells and caves with mixohyaline water (Sket, 1986).

Further investigations are needed for a better understanding of the impact of various ecological factors on the change in shape and size of the carapace and on the genital structure.

Cypria cavernae n. sp.

Holotype: one female; allotype: one male; paratypes: two males and two females deposited at the Limnological Institute of Mondsee, Austria. Locus typicus: Cave near S. Giovanni di Duino, Italy, VG 226.

Description

Carapace (figs 14, 15, 21-25): Unpigmented, smooth and sparsely setose (fig. 14). Greatest height in the middle third of the carapace. Height/length ratio from 63 to 67 % (table IV). Dorsal margin of left valve nearly regulary arched, passing smoothly into anterior and posterior margins (fig. 21). Ventral margin straight. At point of greatest height, dorsal margin of right valve overhanging left valve and exhibiting nearly obtuse angle (fig. 22). Broadly rounded anterior and posterior margins passing smoothly into dorsal margin. Ventral margin nearly straight. Both valves with well-developed anterior and posterior selvages, anterior is larger than posterior one (figs 15, 23-25). Length of anterior margin up to 4 % of carapace length. Anterior vestibules about twice larger than posterior ones.

Sexual dimorphism: females larger than males. Length: males 562-616 µm, females 631 µm. Height: males 354-400 µm, females 408-415 µm (table IV).

Limbs: Antennula (fig. 26): E I: A-1*l*, P-2*l* /E II: A-1*s* /E III: A-1*m*, P-1*s* / E IV: A-2*l*, P-2*s* /E V: A-2*l*, P-1*m*-1*s* / E VI: A-2*l*-1*l* (G) P-2*l* / E VII: D-ya-3*l*. Chaetotaxy identical for males and females. Ratios between length of the segments III to VII: 2.2 : 1.2 : 1.4 : 1.2 : 1.0.

Aesthetasc ya very long. Ratio of total length of aesthetasc ya to length of the seventh endopodite segment 600 %.

Second antenna (figs 31-34): Chaetotaxy different for males and females. Female: Pr: P-1*l* / Exo: 1*l*-1*m*-1*s* / E I: In-5*l*-1*s*, P-Y-1*l* (pl) / E (II+III):A-2*s*, P-y_1-4*m* ($t_{1,2,3,4}$)-y_2, D-2(z_2,z_3)-1(z_1: ser)-3*l*($G_{1,2,3}$: ser) / E IV: D-y_3+1-1*l* (G_m: ser)-1*l*(G_M: ser).

Male: Pr: P-1*l* / Exo: 1*l*-1*m*-1*s* / E I: In-5 *l*-1*s*, P-Y-1*l* (pl) / E II: P-y_1-1*m*(t_1), In-2*m*($t_{2,3}$)-1*s*(t_4), A-2*s* / E III: P-y_2, D-3($z_{1,2,3}$)-1*m* (G_2: ser) - 2*l*($G_{1,3}$: ser) / E IV: D-y_3+1-1*l* (G_m:ser)-1*l*(G_M: ser).

The main differences compared to the female second antenna are the subdivided penultimate joint and that the two t_2, t_3 setae are well-marked sensory setae. In female all t-setae undifferenciated. Relative length of segments I,(II+III) to IV: 5.7 : 3.4 : 1.0. Natatory setae very long, more than 3.5 times longer than first endopodite segment. Male G_2 shorter than homologous claw of female. In both sexes G_m longer than first endopodite segment. G_M about 25 % longer than G_m.

Mandibular palp (figs 35-37): No sexual dimorphism visible. E I: In-1*m*-2*l*(S1,2: pl)-alpha / E II: In-1*l*-3*l* (pl)-beta, Ex-1*s*-1*m* / E III: In-1*l*, Ex-4*m*, A-3*s*-gamma / E IV: D-2*m*-2*m*(pl)-1*s*. Alpha-seta short, plumed and not reaching 50 % of length of third enopodite segment. Beta-seta short and plumed too, wider and twice longer than alpha-seta. Gamma-seta thin, more than 1.5 times longer than third endopodite segment, not plumed. Ratio of third to fourth segment 76 %.

male			female		
l (µm)	h (µm)	h/l (%)	l (µm)	h (µm)	h/l (%)
616	400.40	65	631.40	415.80	65.85
600.60	385	64.10	631.40	408.10	64.63
562.10	354.20	63.01	631.40	408.10	67.04
			(592.90)	(392.70)	(66.23)
mean					
592.8	379.87	64.03	631.4	410.7	65.8
s.e.					
16.05	13.58	0.56	0	2.54	0.69

Table IV: Measurements of the carapace of *Cypria cavernae* n. sp.
l = length, h = height, h/l = height/length ratio of the carapace, s.e. = standard error, in brackets = data of juvenile.

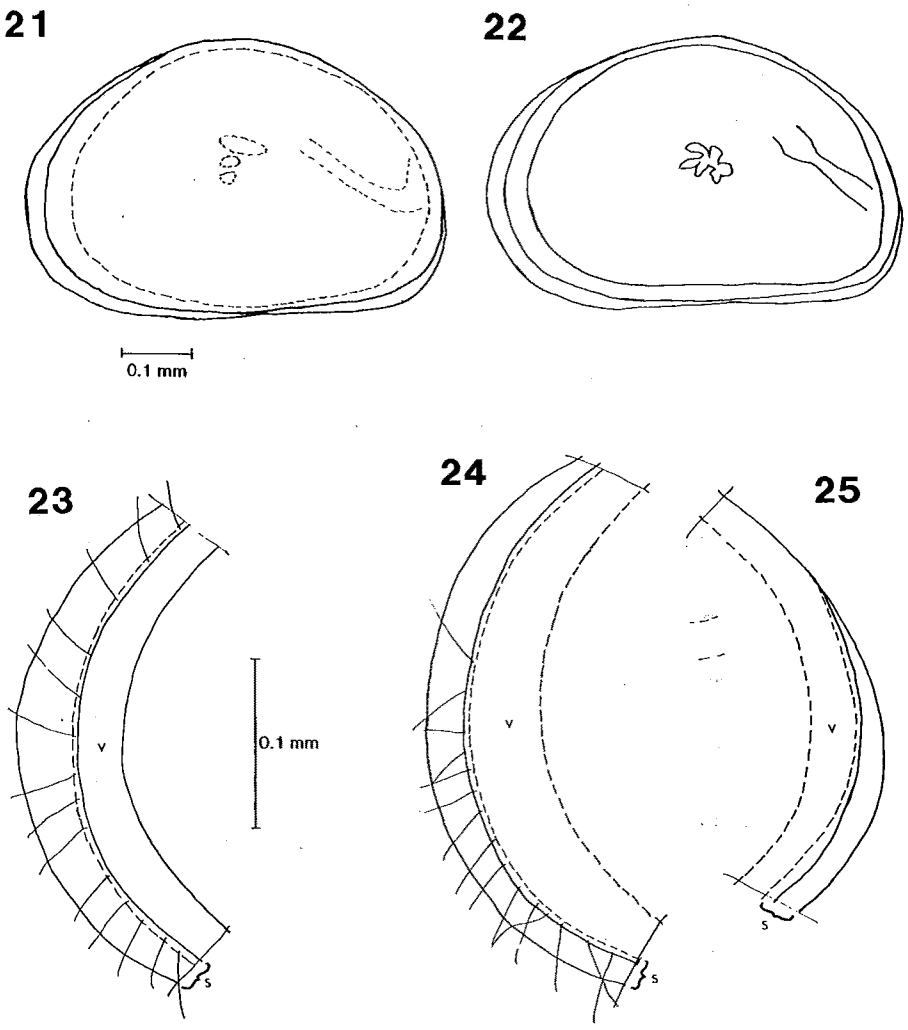
Maxillula (fig. 27): Maxillular palp (Mxup) 2-segmented. Mxup I: Ex-3*m*-2*s* /Mxup II: D-3*l*-3*s*. Setae of masticatory processes I,II,III less than twice second segment of maxillular palp, but setae of masticatory processes III relativly enlarged. Maxillula identical in males and females.

Maxilla (figs 18, 28-30): Endopodite dimorphic. In female endopodite distally with 3 small setae. Male endopodite transformed into prehensile organ, with distal claw, different on right and left side. Exopodite with 6 setae.

Thoracopod 1 (fig. 38): E I: A-1*s* /E II: A-1*s* / E III: A-1*s* / E IV: D-2*s*-1*l* (G). Length ratio 1.0 : 13, showing enormous length of distal claw, more than 3.5 times as long as second endopodite segment.

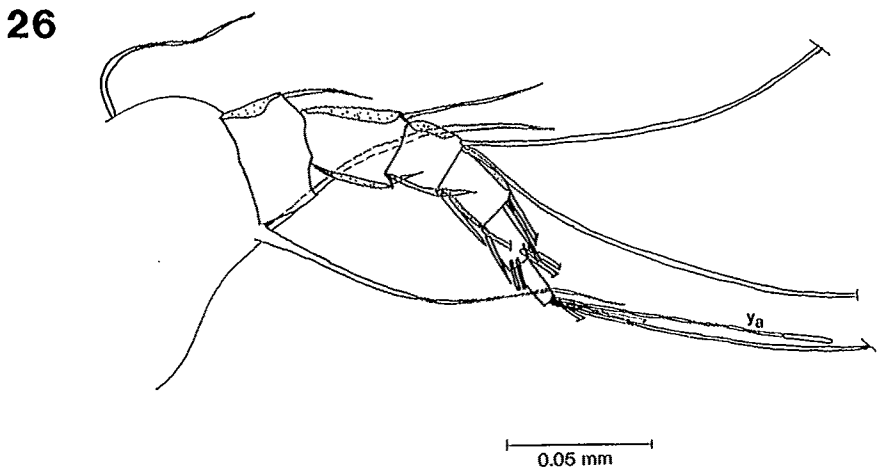
Thoracopod 2 (fig. 39): E I: P-1*m* / E (II+III): P-1*m*, D-Lo-pz_1 / E IV: CL-pz_2-1*s*(ser). Ratio of segments E I, (E II + E III) to E IV: 2,6 : 3,2 : 1.

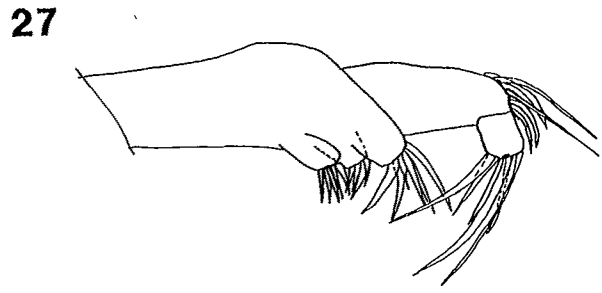
Furca (fig. 40): No sexual dimorphism. R: s_a-G_a-G_p-s_p. The length ratios of the setae G_a/R : G_p/G_a : s_a/G_a : s_p/G_p are 44 : 63 : 30 : 52 (%).

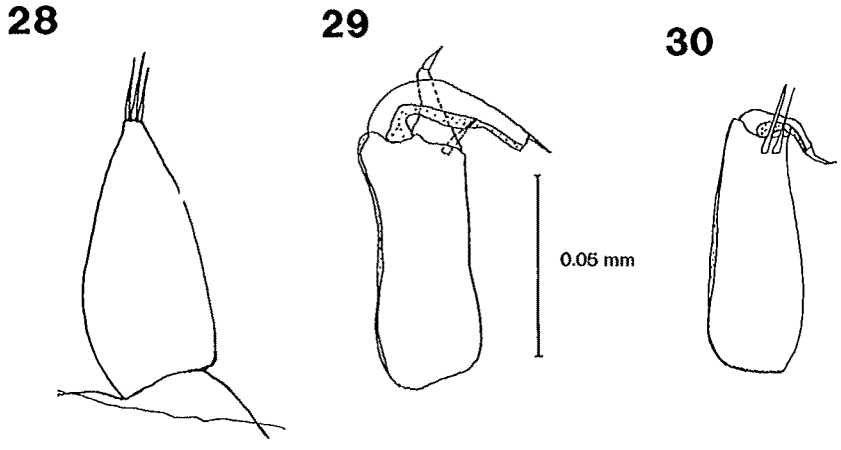


Figs 21 - 25: *Cypria cavernae* n.sp.: 21 - left valve; 22 - right valve; 23 - inner view of the anterior margin of the right valve; 24 - outer view of the anterior margin of the left valve; 25 - outer view of the posterior margin of the left valve, s = selvage, v = vestibule;

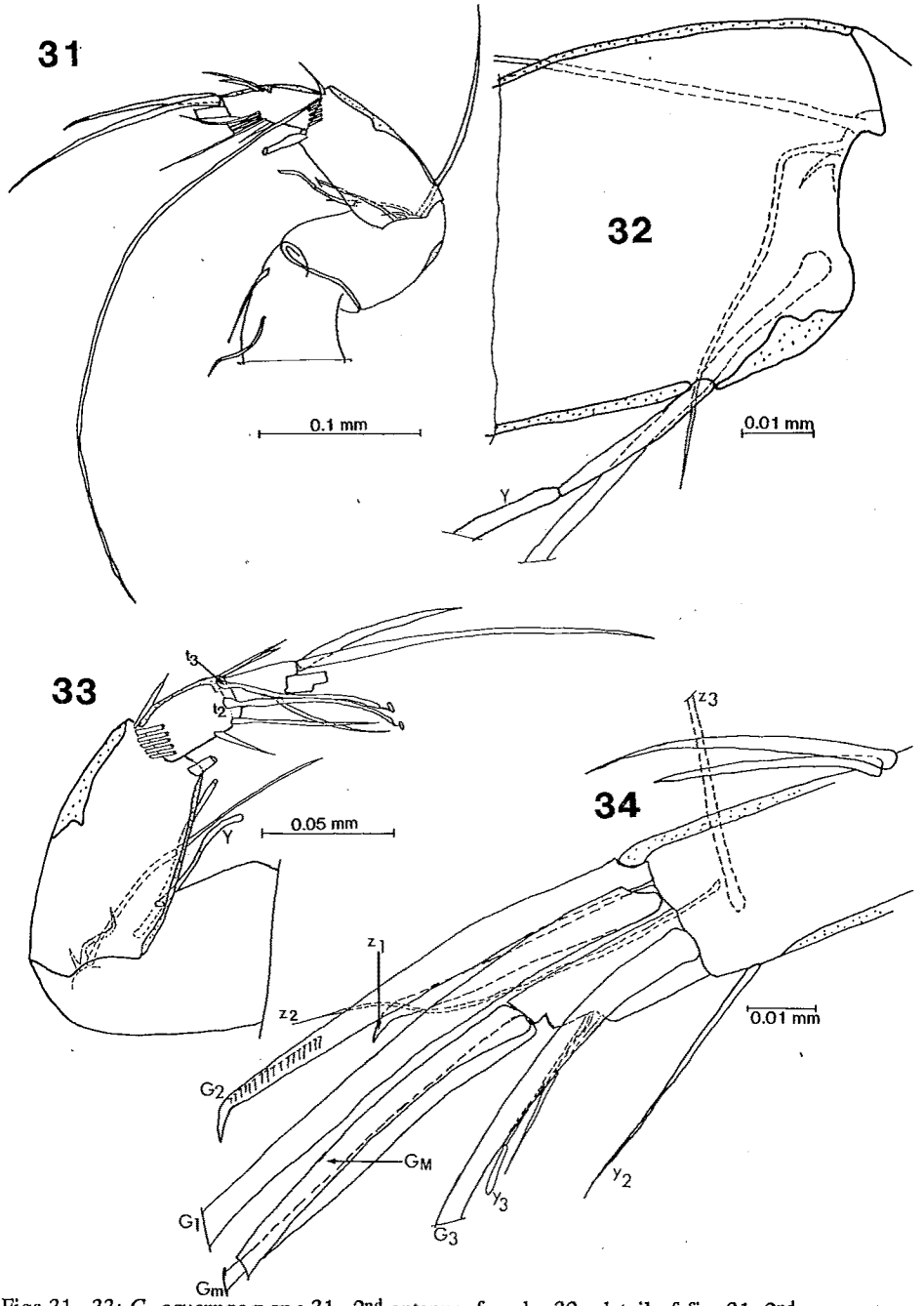
Genital organs (figs 41, 42): Outline of female genital lobe shown in fig.41; one small genital process. Shape of hemipenis resembling that of *C. ophthalmica*. Inner lobe also shorter than outer, but distal process of inner lobe thinner and exterior margin with gentle hump before rounding of proximal part of peniferum. At base of peniferum, seminal tube (vas deferens) covered by sclerotized wall forms labyrinth. Labyrinth and copulatory tube typical for *C. ophthalmica*-group.



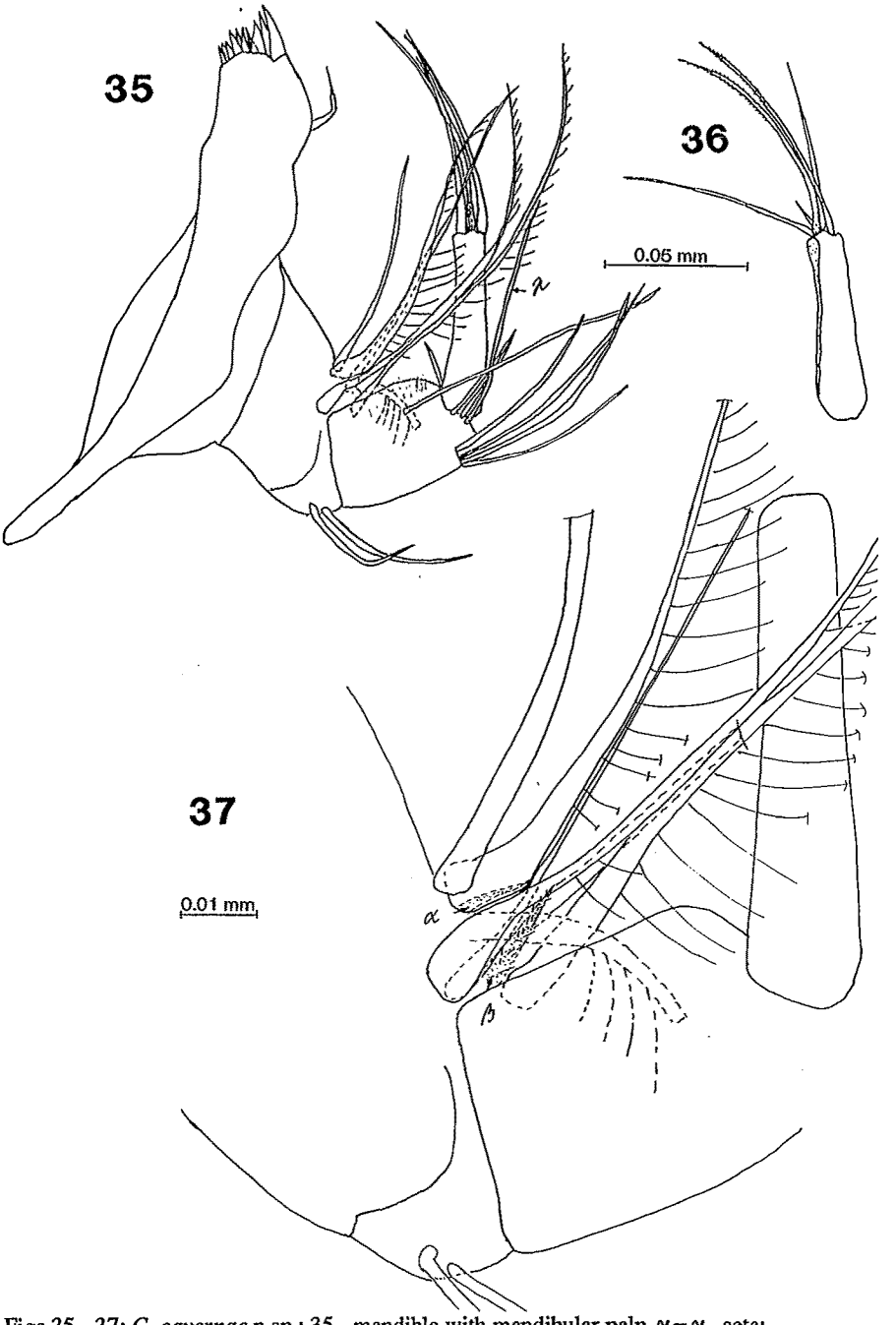




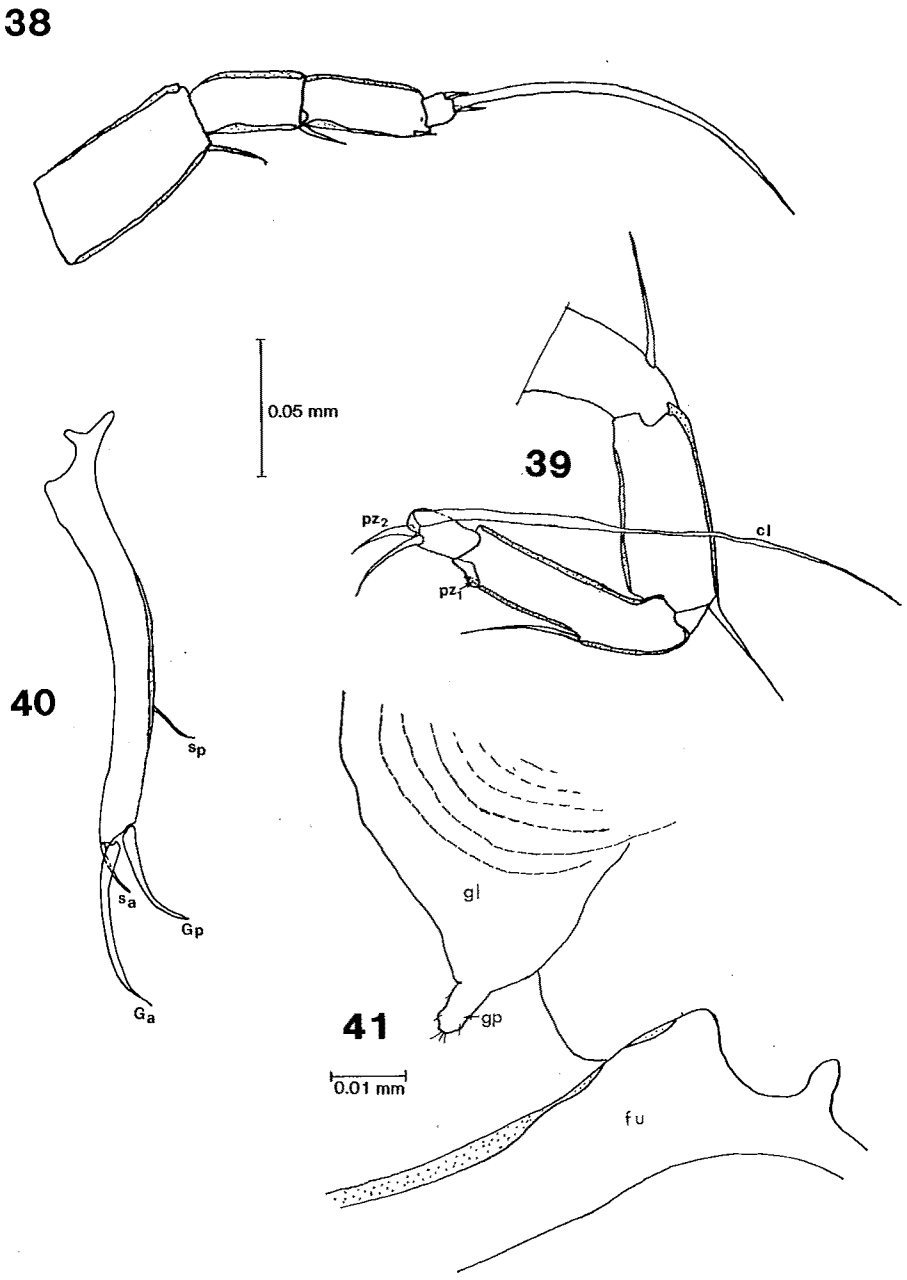
Figs 26 - 30: *C. cavernae* n. sp.: 26 - 1st antenna, ya = aesthetasc ya; 27 - maxillula; 28 - endopodite of the maxilla, female; 29 - right endopodite of the maxilla, male; 30 - left endopodite of the maxilla, male.



Figs 31 - 33: *C. cavernae* n.sp.: 31 - 2nd antenna, female; 32 - detail of fig. 31, 2nd segment; 33 - 2nd antenna, male, t_2 and t_3 = sensorial t-setae.
Fig. 34: The chaetotaxy of the distal part of the second antenna: *Cypria ophthalmica*, female (Neusiedlersee), z_1-z_3 = z-setae, y_2,y_3 = aesthetascs, G_1 = interior - anterior claw, G_2 = exterior-anterior claw, G_3 = exterior-posterior claw, G_M,G_m = major and minor claw.



Figs 35 - 37: *C. cavernae* n.sp.: 35 - mandible with mandibular palp, γ = γ - seta; 36 - 4th endopodite segment of the mandibular palp; 37 - detail of fig.35, α,β = α - and β - seta.



Figs 38 - 41: *C. cavernae* n.sp.: 38 - 1st thoracopod; 39 - 2nd thoracopod, cl = beak-like claw, pz_1 = small seta at the third endopodite segment, pz_2 = small distal setae at the 4th endopodite segment; 40 - furca; s_a,s_p = anterior and posterior setae, G_a,G_p = anterior and posterior claw; 41 - shape of the female genital lobe (= gl), gp = genital process, fu = furca.

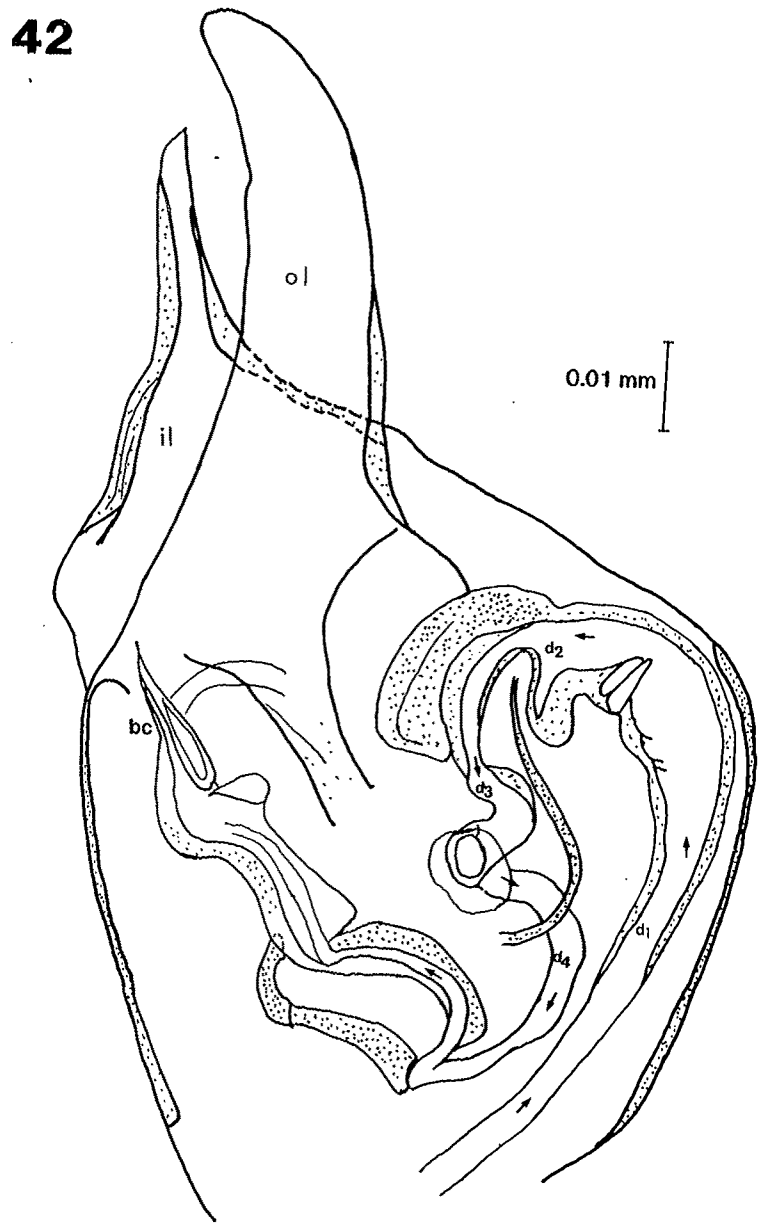


Fig. 42: *C. cavernae* n. sp., hemipenis, d_1 - d_4 = sleeves of the labyrinth, bc = bursa copulatorix, il = inner lobe, oi = outer lobe, arrowed = vas deferens.

Affinities and differences

Cypria cavernae n. sp. is the first exclusively cavernicolous dwelling species of the genus *Cypria*. No eyes are visible and the carapace is unpigmented. The chaetotaxy is nearly identical to that of *C. ophthalmica*. (Therefore, for better demonstrating the chaetotaxy of the second antenna of *C. cavernae* n. sp., fig. 34 showing the distal part of the second antenna of *C. ophthalmica* can be used). Only the length of the setae diplays differences, e.g. the ratio of the aesthetasc ya (fig. 26) to the length of E VII is 227 % for *C. ophthalmica* and 600 % for *C. cavernae* n. sp.

In some waters in northern Dalmatia e.g. a marsh and a well near Zadar, a spring near the lake Vrana (Petkovski, 1976) occurs *C. sketi* which resembles *C. ophthalmica*. The former species also inhabits the Karst region near Trieste (Italy). We have found it in a cave sample (leg. F. Gasparo, 22. July 1984) from SE Trieste. The smaller size with a maximum length of 0.60 mm is a questionable taxonomic character, because the minimum length of *C. ophthalmica* individuals (present paper) reaches from 0.50 to 0.62 mm. Petkovski (1976) records a minimum length for *C. ophthalmica* of 0.65 mm. The main difference which distinguishes *C. sketi* from *C. ophthalmica* is the subtriangular right valve of the former. In *C. ophthalmica* the shape of the right valve is broadly arched. Compared to the right valve of *C. sketi* the right valve of *C. cavernae* n. sp. forms an obtuse angle. The height/length ratio of the carapace of *C. sketi* ranges from 70 to 74 % and this corresponds with the upper level of the height/length ratios of the *ophthalmica* populations in the present investigation. The height/length ratio of *C. cavernae* n. sp. ranges from 63 - 65 % for males and from 64 - 67 % for females (table IV) and this corresponds with the lower level of our *C. ophthalmica* measurements. This means that the shape of the carapace of *C. cavernae* n. sp. is flatter than that of *C. sketi*. A further taxonomic character of the carapace to distinguish *C. ophthalmica* and *C. sketi* from *C. cavernae* n. sp. is the size of the anterior selvage of the valves, which is twice as large in *C. cavernae* n. sp. The anterior selvages of *C. ophthalmica* and *C. sketi* have nearly the same size. In *C. ophthalmica* the anterior selvage attains 2 % of the carapace length and in *C. cavernae* n. sp. 4 %. The ratios of the segments E III - E VII/E VII of the antennula are: *C. sketi* 1.9 : 1.3 : 1.1 : 0.9 : 1 (Petkovski, 1976); *C. ophthalmica* 1.9 : 0.9 : 1 : 0.9 : 1; and *C. cavernae* n. sp. 2.2 : 1.2 : 1.4 : 1.2 : 1. The ratio (E IV : E VII) shows the greatest variability between the three species, the other segment ratios are nearly identical for *C. sketi* and *C. ophthalmica* and clearly distinct in *C. cavernae* n. sp. The ratio of the fourth segment and the G_2 claw (E IV/G_2) of the second antenna is 2.9 for *C. sketi* according to the figure in Petkovski (1976). (Because what Petkovski defined as the shortest claw of the penultimate segment, is called the z_1 setae in the present paper.) In *C. ophthalmica* it is 2.7-2.9, in *C. cavernae* n. sp. 4.3. This means that the distal claws of the second antenna are clearly longer in *C. cavernae* n. sp. as compared with the other species. The distal claw of the walking legs of *C. sketi* are slightly longer than the last three leg segments. The ratio G/E II - IV is 108 % (Petkovski, 1976). For *C. ophthalmica* this ratio is about 100 %. The variability of this trait is shown in table III; again *C. cavernae* n. sp. shows a G/E II - IV ratio of 148 % and that is clearly longer in comparison with *C. sketi* and *C. ophthalmica*. The length of the distal claw and the length of the distal part of the aesthetasc Y are very highly significantly different (table III) when one compares the specimens from the Neusiedlersee and Mondsee, both the littoral and the profundal zones, with *C.*

cavernae n. sp. The shape of the hemipenis in *C. sketi* (Petkovski, 1976) also resembles that of *C. ophthalmica*, the inner lobe being shorter than the outer lobe, but the shape of the inner lobe is different from that of *C. cavernae* n. sp. In the latter species the shape of the inner lobe is more triangular than in *C. sketi* or *C. ophthalmica* and the interior margin of this lobe is concave. In *C. sketi* the interior margin of the inner lobe is convex.

A further species which resembles *C. ophthalmica* and also inhabits wells is *C. karamani* Petkovski, 1976. In this species the whole left valve is overhanging the right valve and therefore *C. karamani* can be clearly distinguished from *C. cavernae* n. sp.

All these characters, i.e. the shape of the carapace, the shape of the hemipenis, the size of the selvages, the length of the segments, length of the claws and the longer chemosensorial part of the aesthetasc Y show that *C. cavernae* n. sp. is a valid species.

We suggest here that the genus *Cypria* should be divided into three species groups. (1) The *C. ophthalmica*-group, with long antennal natatory setae, which exceed the extremities of the distal claws represented by *C. ophthalmica* (Jurine, 1820), *C. sketi* Petkovski, 1976, *C. subsalsa* Redeke, 1936, *C. cavernae* n. sp. and *C. karamani* Petkovski, 1976. (2) The *C. reptans*-group, with short antennal natatory setae, which do not exceed the extremities of the distal claws contains *C. reptans reptans* Bronstein, 1828, *C. reptans stygia* Klie, 1935, *C. reptans helokrenica* Kantorek & Absolon, 1975, *C. obliqua* Klie, 1939. (3) The *C. exsculpta*-group, containing species of larger size (longer than 0.75 mm) (Petkovski, 1976), containing *C. exsculpta* Fischer, 1855, *C. curvifurcata* Klie, 1923.

Origin and evolution of *Cypria cavernae* n. sp.

The close morphological similiarities between *Cypria cavernae* n. sp., *C. ophthalmica* and *C. sketi* suggest that the former descended from one of those two species. The carapace shape of *C. cavernae* n. sp. resembles the last postembryonal stage of *C. ophthalmica* while the slightly developed dorsal protuberance of *C. cavernae* n. sp. suggests some affinity with the right valve of *C. sketi*. Both *C. ophthalmica* and *C.* sketi are generalist species occuring in epigean and subterranean environments also along the Adriatic karstic coast, as mentioned above. It is plausible that *C. ophthalmica* or *C. sketi* living in karstic epigean habitats such as doline lakes or karstic springs (Petkovski, 1976) were able to penetrate into the subsurface habitats via karstic accesses to subterranean systems. We know for instance that *C. ophthalmica* lives in such a doline lake, the Halleswiessee in Austria (fig. 2) - and this lake has a karstic spring which during a part of the year is an insurgence and is at other times an exsurgence. It is very likely that *C. ophthalmica* colonizes the karstic system of the Halleswiessee surroundings.

The age of *C. cavernae* n. sp. is not known. Sket (1971) supposes that many troglobionts inhabiting the Karst area originated during the Quaternary. The geographical distribution of *C. cavernae* n. sp. is restricted to a narrow karstic area around Trieste (fig. 1). We did not find this species in samples from other zones located in the northwestern part of this area (such as in Lombardy around Brescia and in Friuli - Venezia Giulia - where Dr. Gasparo sampled a rich troglobitic fauna,

including monolistrinid isopods, which could be of an older age e.g. Upper Tertiary (Sbordoni & al., 1980, Danielopol & Hart, 1985).

C. cavernae n. sp. is the first troglobite Cyclocypridinae which displays the classic set of troglomorphic characters, viz.: lack of body and occular pigments, lack of eyes, elongation of the chemosensorial setae such as the antennal aesthetascs, and elongation of the distal part (the claws) of the ambulatory limbs (i.e. second antenna and the first thoracic leg). Such troglomorphic characters have been discussed for other crustaceans inter alia by De Lattin (1939), Kosswig & Kosswig (1940), Kosswig (1960), Culver & Fong (1986) and specifically for ostracods by Danielopol (1973) and Marmonier et al. (1989).

Considering the pigmentation and the eye reduction of *C. cavernae* n. sp. one can assume that this is the result of a regressive evolutionary mechanism, if one considers that the epigean ancestor of *C. cavernae* n. sp. looked like *C. ophthalmica* or *C. sketi*, which are pigmented and occulated. Genetic drift in small populations has produced such effects in the case of the cavernicolous fauna as demonstrated by the studies of Kosswig & Kosswig (1940) for *Asellus aquaticus* and by Wilkens and his associates (see review in Wilkens, 1988) for *Astyanax fasciatus* (Characidae, Pisces).

The elongation of the distal part of the antennal aesthetascs of *C. cavernae* n. sp. and of the distal ambulatory claws as compared to those of *C. ophthalmica* and *C. sketi* has to be considered as progressive evolutionary characters, most probably specialisations to the life in subterranean, karstic environments. The compensatory developement of sensory organs has been discussed by Schemmel (1984) for cave fishes and for ostracods by Danielopol (1973). Considering this latter group, one has to note that the most elongated aesthetascs are found in ostracod groups which nowadays have no epigean represantatives (e.g. *Mixtacandona* and *Phreatocandona* species), therefore it is difficult to decide on their degree of specialisation to subterranean habitats. In other cases where direct comparisons between populations of the same species inhabiting either the surface or the subsurface habitats were possible no difference could be observed in aesthetasc elongation. This is the case for *Nannocandona faba* studied by Marmonier & Danielopol (1988) and *Cavernocypris subterranea* (cf. Marmonier & al., 1989).

The elongation of the ambulatory distal claws in *C. cavernae* n. sp. could have an adaptive value. They are useful for the animal when moving either on clay or within crevicular systems, for example. For similar explanations dealing with cave ostracods see Hartmann (1973) and for Collembola see discussion in Culver (1982), based on Christiansen's data. Considering the Ostracoda once again one can see that there are also epigean species which display such elongated claws e.g. *Nannocandona faba* (vide supra) and *Cavernocypris subterranea* (vide supra). In the case under consideration, because the closely related species *C. ophthalmica* and *C. sketi*, exhibit short claws, we postulate that the elongation in *C. cavernae* n. sp. took place during the life in the subterranean environment.

One of the exciting problems for which we cannot formulate an answer is, whether the evolutionary changes here discussed occured gradually or in a sharply discontinuous way during a short period of time as postulated by the punctuated equilibria model of Eldredge & Gould (1972). The quantitative analysis of the various *C. ophthalmica* populations suggest the occurrence of microevolutionary processes without sharp saltational changes, at least within this species. It would be

interesting therefore to continue to study the Adriatic coastal zone in order to see if one can find transitional populations between *C. ophthalmica*, *C. sketi* or *C. cavernae* n. sp.

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