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CRYPTOCANDONA KIEFERI (CRUSTACEA, OSTRACODA): REDESCRIPTION, MORPHOLOGICAL VARIABILITY, GEOGRAPHICAL DISTRIBUTION

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CRUSTACEA
OSTRACODA
MORPHOLOGY
BIOGEOGRAPHY
MICROEVOLUTION

ABSTRACT. – *Cryptocandona kieferi* (Klie), a stygobitic ostracod species widely distributed in Europe, was redescribed based on the type material and specimens from interstitial waters of the Upper Rhine near the type locality. Furthermore, morphology of specimens collected from other four areas was investigated and compared. Those areas are: the Rhône and Ain at Lyon, the Saône near Dijon, the Upper Danube around Vienna and Lumina, a new site within the Danube drainage basin near Constanța (Romania). The quantitative study of intra- and interpopulational variability of the valve and limb traits allowed to follow the microevolutionary changes within the *C. kieferi* lineage. In a first step one can discriminate between the Rhône-Saône-Rhine populations and two Danubian ones, followed by a separation between the Austrian and Romanian populations. The revealed morphological differentiation and geographical segregation validated the description of two new subspecies: *C. kieferi danubialis* n. ssp. from Austria and *C. kieferi dobrogensis* n. ssp. from Romania. The Rhône-Saône-Rhine populations were left as the nominate subspecies. Finally, it is shown that *C. kieferi* can be used as an environmental sentinel which could inform on groundwater with a moderately load of the organic matter and circulating through sediments with the reduced colmation.

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RÉSUMÉ. – *Cryptocandona kieferi* (Klie) est une espèce stygobie largement répandue en Europe. Cette espèce est redécrite à partir du matériel type provenant de la vallée du Haut Rhin, en Allemagne ainsi que des exemplaires trouvés dans cinq autres régions géographiques: le long du Rhône et de l'Ain près de Lyon, de la Saône près de Dijon, le Haut Rhin près de Strasbourg, le Haut Danube aux alentours de Vienne et dans une nouvelle localité, Lumina, dans la Dobrogea (Roumanie). L'étude quantitative de la variabilité intra- et inter-populations des traits morphologiques des valves et des appendices permet de suivre les changements microévolutifs apparus à l'intérieur de la lignée *C. kieferi*. On remarque dans un premier temps l'existence d'une ségrégation entre les populations du Rhône-Saône-Rhin et celles du bassin du Danube, suivie par la différentiation de la population de la Dobrogea par rapport à celles des environs de Vienne, en Autriche. Les différences morphologiques observées, ainsi que les répartitions géographiques justifient la description de deux nouvelles sous-espèces: *C. kieferi danubialis* n. ssp. d'Autriche et *C. kieferi dobrogensis* n. ssp. de Roumanie. Enfin, *C. kieferi* semble pouvoir être utilisée comme "sentinelle environnementale". Elle signale la présence d'eau souterraine modérément chargée en matières organiques circulant dans des milieux poreux faiblement colmatés.

INTRODUCTION

Groundwater ecologists are often confronted with stygobitic species, which nowadays have locally discontinuous distributions (i.e. on the metre and/or kilometre scales populations are patchily dispersed). Additionally, such species display on the regional scale a wide geographical distribution. Ostracods like *Cryptocandona vavrai* Kaufmann discussed by Baltanás *et al.* (2000) and copepods like *Acanthocyclops sensitivus* (Graeter et Chappuis) (review in Pospisil 1999) are such examples. Even if the groundwater environment is perceived by many ecologists and biogeographers (e.g. Illies 1971) as more stable and persistent than many surface water systems, one could still ask if populations geographically separated one from the other could not during the time evolve locally new morphological traits and ecological specialisations. Answering this latter question is important not only for basic research topics, e.g. for a better perception of the ways groundwater fauna evolves within the subterranean realm (Gibert *et al.* 1994, Culver *et al.* 1995) but also for practical purposes. For instance, for monitoring water quality one always seeks species with wide geographical distributions and nearly constant ecological responses over their whole distribution range (Hellawell 1978). However, one could argue if such kind of stygobitic species really exists given that the subterranean fauna is often presumed to be made up of specialised taxa locally distributed, i.e. having a high degree of endemism (Botosaneanu 1986, Griebler & Mösslacher 2003).

This paper looks into that topic through the study of the morphological variability of the widely distributed stygobitic ostracod *Cryptocandona kieferi* (Klie) and its fine geographical distribution. The morphology of this species was shortly described by Klie (1938) from several localities of the Upper Rhine Valley. However, the peculiar morphological traits of the valves and the male's hemipenis allowed during the years identifying the species in many areas of Western and Central Europe (for the geographical distribution of this species see Rogulj *et al.* (1993), Baltanás *et al.* (2000), Meisch (2000) and additional data in the present paper).

Here we redescribe the morphology of this species and assess its intraspecific morphological variability as inferred from the study of five geographically isolated populations. Two new subspecies of *C. kieferi* will be proposed and described. This will lead us further to discuss the dispersal ability and the likely geographical and evolutionary pathways followed by this groundwater dwelling species.

Finally, we will offer some observations, which allow one to use *C. kieferi* for the assessment of environmental quality.

MATERIAL AND METHODS

Redescription was based on both the type material and new collections. The type material (W Klie's collection) is deposited in the Zoologisches Museum und Zoologisches Institut der Universität Hamburg (ZMH). We used in this study: a) one dissected female (ZMH 1396), leg. R Lais, April 1936, Teningen (Germany), considered here lectotype and b) one dissected female (ZMH 1395), leg. R Lais, Oct 1935, Ringsheim 138 (Germany), considered here paralectotype. Since there were no female valves and males in this collection, we examined for the redescription also specimens collected from the Rhine River floodplain near Benfeld (details below), ca. 30 km NW of Teningen (the type locality).

To assess the morphological variability within the geographical range of the species, besides the Klie's material, we investigated in this study specimens collected with the Bou-Rouch pump or the Cvetkov net from groundwater of alluvial sediments within five geographically distant areas (Fig. 1)

1. The Rhine River floodplain (RHI), ca. 30 km S of Strasbourg (France). Sampling stations: a) a drainage canal close to the Rhine main channel (3 km upstream of Rhinau) and b) the Lutter spring close to the Ill River, a tributary of the Rhine River (near Benfeld), ca. 13 km away from the former station. Samples were taken at 50 and 100 cm depth below the soil surface. From this area, 8 males and 7 females were analysed.

2. The interstitial habitats of the Rhône River (RHO), ca. 15 km NE of Lyon (France). Sampling stations: a) the abandoned Puits-Novet channel of the Ain River close to the confluence with the Rhône River (near St Maurice de Gourdan), b) the regulated channel Miribel of the Rhône River (near Nievroz) and c) an abandoned gravel pit (near La Valbonne) (details in Marmonier 1988 and Marmonier *et al.* 1992). All these stations are located less than 10 km each from the other. Groundwater and alluvial samples were taken at 50-200 cm depth below the soil surface. From this area, we studied 8 males and 6 females.

3. Sediments of the alluvial plain of the Saône River (SAO), ca. 30 km N of Dijon (France). Sampling site: the Tilles wetland (near Orgeux and Arcelot) (details in Idbennacer 1985). Specimens were collected at 50 cm depth below the soil surface. Five males and 20 females were examined.

4. Groundwater habitats in the Danube Wetland (DAV) around Vienna (Austria). Sampling stations: a) Lobau, left side of the Danube River (details in Danielopol 1983, Pospisil 1994): a_1) the Eberschüttwasser (Lobau A area, the piezometre C120 and A1/-0.5 m; Lobau C area – the wells D15 and D120); a_2) Lobau B – the well A81 (details in Pospisil 1994) and b) the Regelsbrunn backwaters, right side of the Danube River, ca. 20 km downstream of Vienna, the piezometre D/4 m and SW/3 m (Pospisil & Danielopol 2000). At the Lobau A site A1, C120 and Lobau C site D120 animals were collected at 50 and 120 cm depth below the surface. From this area 11 males, 8 females and 3 juveniles (8th stage) and 2 juveniles (7th stage) were subjected to the examination.

5. A well in the village Lumina (DAC), 15 km NW from Constanța and ca. 10 km W from Navodari in the geographical region of Dobrogea (SE Romania). The well, 1.5 m in diameter and 4 m deep (the water table at about 2.5 m depth), not covered at the top in order to protect the water quality from intrusion of allochthonous material was used by local people for obtaining drinking water. From this site 8 males, 14 females and 15 juveniles of the 8th stage were examined.

Sampled individuals were fixed with 5-10% formalin or 96% ethanol. Appendages of the dissected specimens were mounted in glycerine on glass slides whereas valves stored dry. The morphology of both valves and appendages were investigated with transmitted light microscopy. Line drawings were made using a microscope fitted with a drawing tube. Description of appendages follows the model proposed by Broodbakker & Danielopol (1982) modified for the second antenna by Martens (1987) and for thoracopods by Meisch (1996).

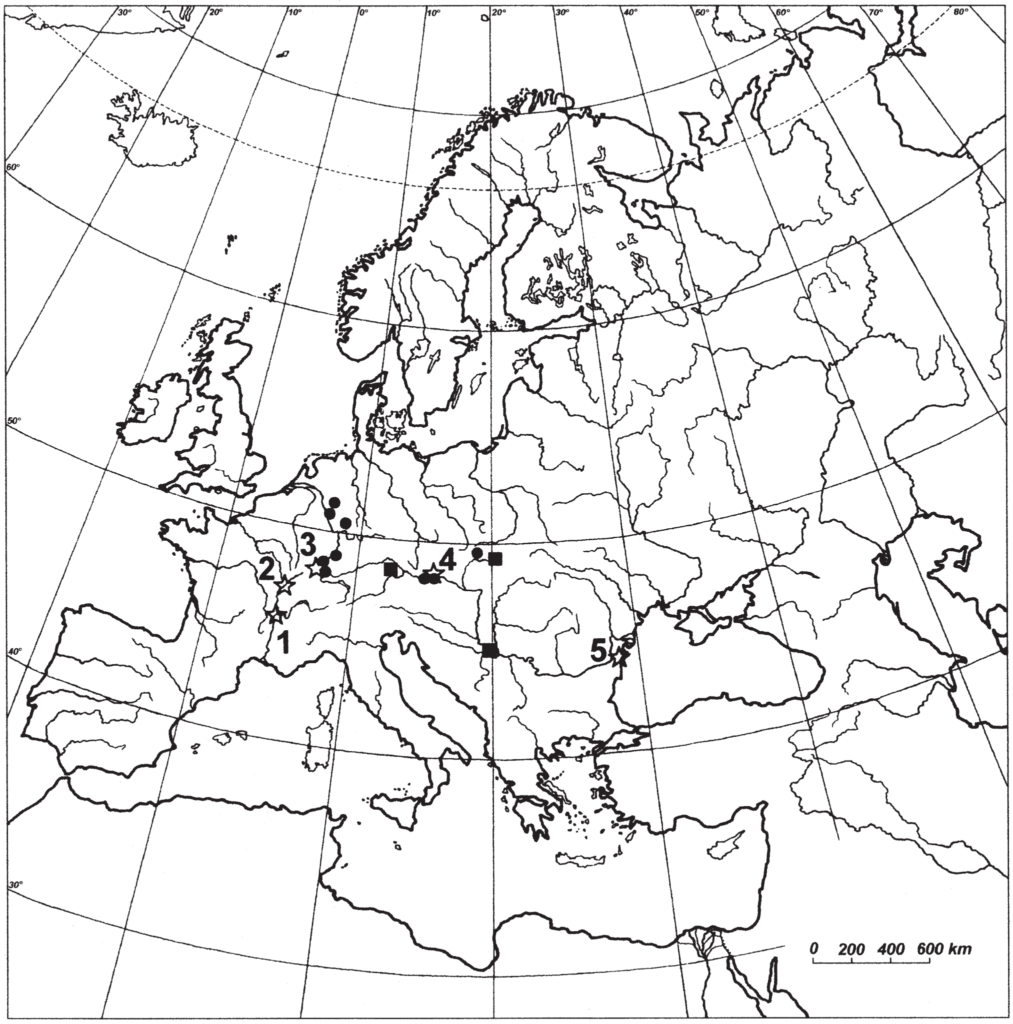

Fig. 1. – Recent (filled circles) and fossil Quaternary (filled squares) distribution of *Cryptocandona kieferi* (Klie) in Europe with localities from which material was obtained for the present study (open stars) situated in the drainage basins of: 1 = the Rhône River near Lyon (RHO) in France, 2 = the Saône River near Dijon (SAO) in France, 3 = the Rhine River (RHI) near Strasbourg in France and Freiburg in Germany, 4 = the Danube River near Vienna (DAV) in Austria, 5 = the Danube River near Constanța (DAC) in Romania.

Abbreviations and names for limbs were used after Meisch (2000): A1 – antennule; A2 – antenna; Md – mandible (3rd limb); Mdp – mandibular palp; Mx1 – maxillule (4th limb); L5 – first thoracopod (5th limb); L6 – walking leg (6th limb); L7 – cleaning leg (7th limb); Fu – furca (= uropod in Meisch 2000). Hemipenis terminology was used according to Danielopol (1969), as reviewed by Meisch (2000). All other symbols and their combinations used in the descriptive part as in Broodbakker & Danielopol (1982).

The measured and illustrated specimens, except the Klie's type material, were deposited at the University of Rennes 1 (in the collection of PM) and the specimens from the stations in the Danube area at Vienna and those from Romania in the collection of the Natural History Museum in Vienna (NHMV).

Various morphological traits for both the carapace and limbs were selected to assess intraspecific variability of the species. A total of 16 different linear distances was taken on limbs using a phase contrast microscope after dissection. The morphological measurements used (acronyms are given here to assist in interpreting the results) were as follow: 1 – length of intero-distal claw on the penultimate podomere of A2 (G_1); 2 – length of antero-distal claw on the penultimate podomere of A2 (G_2); 3 (females only) – length of intero-distal claw on the penultimate podomere of A2 (G_3); 4 (males only) – length of extero-distal large claw on the penultimate podomere of A2 (z_1); 5 (males only) – length of extero-distal large to medium claw or seta on the penultimate podomere of A2 (z_2); 6 – length of major claw on the terminal podomere of A2 (G_M); 7 – length of minor claw on the terminal podomere of A2 (G_m); 8 – length of apical claw on the terminal podomere of L6 (h_2L6); 9 – length of short seta on the terminal podomere of L7 (h_1L7); 10 – length of medium seta on the terminal podomere of L7 (h_2L7); 11 – length of large seta on the terminal podomere of L7 (h_3L7); 12 – length of anterior seta on Fu (s_a); 13 – length of posterior seta on Fu (s_p); 14 – length of anterior claw on Fu (G_a); 15 – length of posterior claw on Fu (G_p); 16 – distance between the insertion points of the posterior seta and the posterior claw on Fu (s_p-G_p).

From each sampling area 15 measurements (all but character 3) were taken on four adult males whereas 14 (all but characters 4 and 5) were gathered from four adult females. To reduce further the influence of individual size, all the data obtained were converted to the appropriate ratios and expressed as percentages. Podomeres of a given limb used as references were as follows: A2 – 1st endopodial podomere, L6 – 2nd endopodial podomere, L7 – 1st endopodial podomere, Fu – anterior margin of the ramus.

In addition, carapaces of all available individuals were measured under a stereo-microscope (magnification 500×) to the nearest 0.01 mm. For each ostracod carapace both the maximum length (L) and height (H) were recorded and the length to height ratio (H/L) calculated.

For each variable the mean, standard deviation (S.D.) and range were computed too.

To determine if individuals could be accurately classified by area and, if so, which of the measurements were important in distinguishing between areas, a discriminant function analysis (Manly 1998) was carried out for each sex separately with Statistica for Windows v. 5.1. – StatSoft (1997) programme. In the analysis we used characters that showed promising variation among specimens from five studied areas and that were not too redundant with the other characters (their practical contributions to the discriminatory power seemed to be unambiguous), so that the variance/covariance matrix of variables included in the model could be inverted and the discriminant function analysis could be performed. The seven measurements chosen for females were: G_3, h_2L6, h_1L7, h_2L7, h_3L7, G_a, s_p-G_p, whereas those seven used for males: z_1, z_2, G_M, h_2L6, h_2L7, G_a and G_p.

RESULTS

Redescription of Cryptocandona kieferi (Klie, 1938)

Candona kieferi Klie, 1938: 21, figs 24-32.
Cryptocandona kieferi – Danielopol, 1978: 14.

Carapace

Female (Fig. 2D-F). The carapace is elongated with smooth and pearly white surface. The greatest H represents 47-50% of the carapace L and is situated behind mid-length and behind the central muscle scars (at 54-58% of the total L). Dorsal margin widely arched, cardinal angles not marked, posterior margin sloping more steeply than anterior one, which is more regularly curved. Ventral margin almost straight. Shape of both valves similar, left valve (LV) slightly longer and higher than the right one (RV), which has more concave ventral margin. Calcified part of the inner lamella anteriorly represents 12% and on the posterior end 6% of the carapace length. Anterior vestibule distinctly larger ventrally than dorsally. Marginal zone narrow with simple pore canals. Central muscle scar arrangement typical for the subfamily Candoninae. Carapace viewed dorsally slender, LV overlaps RV at both pointed ends, the greatest width (W) about at mid-length and amounting to 35-36% of the carapace L. Measurements in Table I.

Male (Fig. 2A-C). Male carapace similar in outline to that of female but distinctly larger. The greatest H located just behind the muscle scars (at 52-55% of the total L) and represents 46-49% of the total L. Dorsally the greatest W (located behind mid-length) equalling ca. 1/3 of the carapace L. LV

Table I. – Carapace measurements of *Cryptocandona kieferi* from the five areas studied (N = number of specimens, S.D. = standard deviation).

		RHI	SAO	RHO	DAV	DAC
				Males		
	N	8	5	4	11	8
L (mm)	Mean	1.13	1.07	1.12	1.12	0.87
	Range	1.08-1.20	1.06-1.08	1.08-1.14	1.08-1.16	0.83-0.92
	S.D.	0.04	0.01	0.03	0.03	0.03
H (mm)	Mean	0.54	0.52	0.55	0.55	0.42
	Range	0.52-0.58	0.50-0.52	0.53-0.56	0.52-0.58	0.39-0.44
	S.D.	0.02	0.01	0.02	0.02	0.02
H/L	Mean	0.48	0.48	0.49	0.49	0.48
	Range	0.46-0.49	0.47-0.49	0.48-0.50	0.46-0.52	0.47-0.49
	S.D.	0.01	0.01	0.01	0.01	0.01
				Females		
	N	7	20	6	8	14
L (mm)	Mean	1.02	0.99	1.00	1.02	0.79
	Range	0.98-1.06	0.94-1.04	0.94-1.02	0.98-1.04	0.76-0.82
	S.D.	0.02	0.03	0.03	0.02	0.02
H (mm)	Mean	0.50	0.46	0.48	0.48	0.37
	Range	0.48-0.52	0.44-0.50	0.47-0.50	0.46-0.52	0.36-0.38
	S.D.	0.02	0.01	0.01	0.02	0.01
	Mean	0.49	0.47	0.48	0.47	0.47
H/L	Range	0.47-0.50	0.46-0.49	0.47-0.50	0.46-0.50	0.46-0.48
	S.D.	0.01	0.01	0.01	0.01	0.01

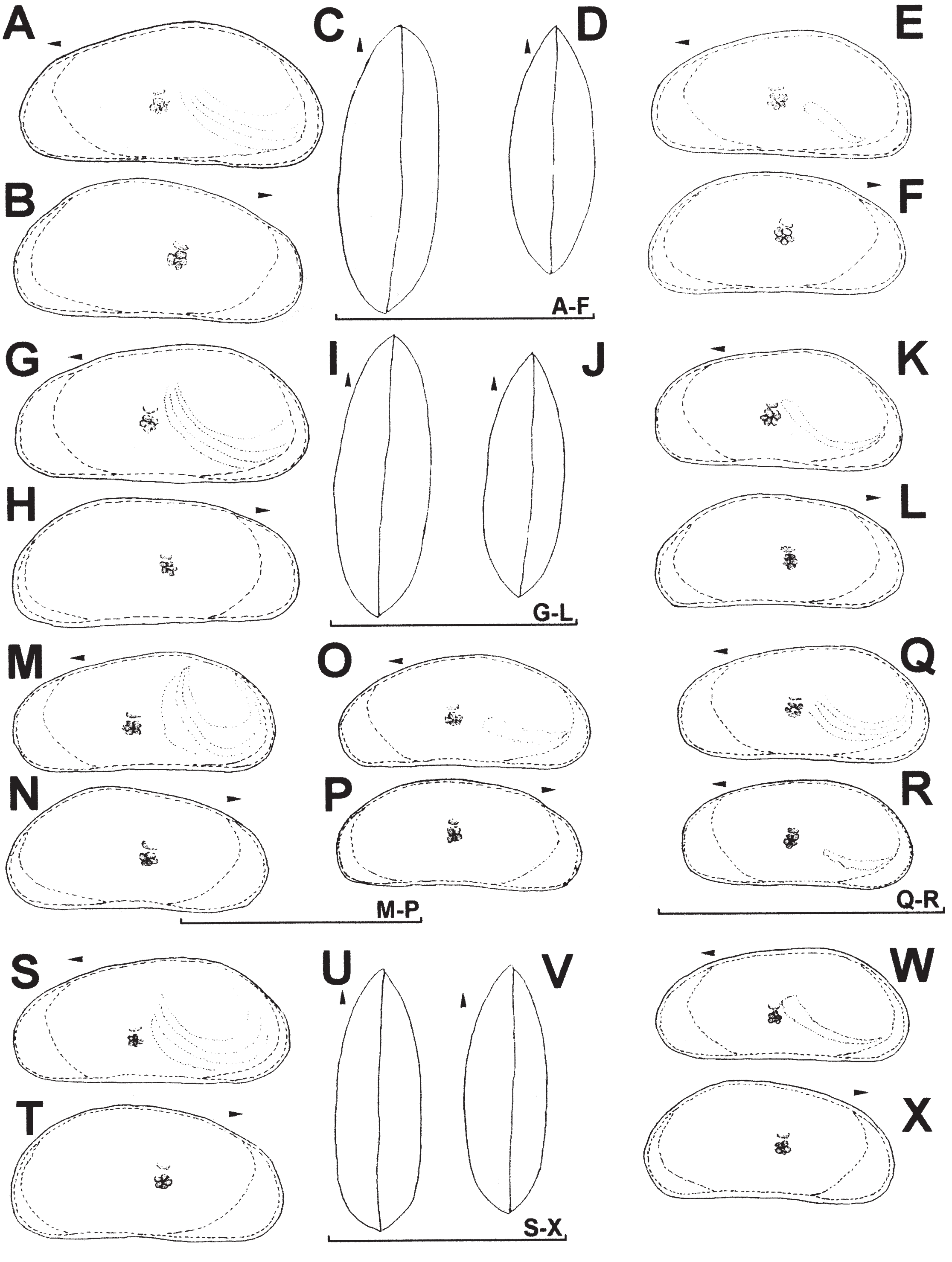

Fig. 2. – Carapace shapes of *Cryptocandona kieferi*. A-F, specimens of *C. kieferi kieferi* (Klie) from RHI (A-B, male valves, external view; C, male carapace, dorsal view; D, female carapace, dorsal view; E-F, female valves, external view); G-L, specimens of *C. kieferi kieferi* (Klie) from RHO (G-H, male valves, external view; I, male carapace, dorsal view; J, female carapace, dorsal view; K-L, female valves, external view); M-P, specimens of *C. kieferi kieferi* (Klie) from SAO (M-N, male valves, external view; O-P, female valves, external view); Q-R, specimens of *C. kieferi dobrogensis* n. ssp. from DAC (Q, male valve, external view; R, female valve, external view); S-X, specimens of *C. kieferi danubialis* n. ssp. from DAV (S-T, male valves, external view; U, male carapace, dorsal view; V, female carapace, dorsal view; W-X, female valves, external view). Scale bars = 1 mm.

slightly overlapping RV (the difference represents 5% of the height of LV). Anterior and posterior vestibules are larger ventrally than dorsally, their widths represent 10-14% and 5-6% of the carapace L, respectively. Selvage reduced, equalling only 9% of the anterior vestibule. Measurements in Table I.

Naupliar eye reduced, not visible on the examined living specimens of the DAV. Note that as in the Candoninae subfamily, the eye pigment is absent and the crystalline structure becomes invisible once the specimens die.

Appendages (A1, Md, Mx1, L6 and L7 without sexual dimorphism).

A1 (Fig. 3A): I+II: A-2*l*(pu), P-2*l*(pu) / III: A-1*m*(pu) / IV: A-1*m*(pu), P-1*m*(pu) / V: A-2*l*, P-1*m*(pu) / VI: A– 2*l*, P-1*m* / VII: A-1*m*(α)-3*l*, P-*ml* / VIII: D-1*l*(G)-2*l*-y_a. Seventh podomere bears anteriorly two extremely large setae, one distinctly shorter but also large seta and one small to medium (α) seta. Relative L of the posterior seta of this podomere is medium to large. L ratios of the last five podomeres (IV-VIII) from proximal to distal as: 1.0 : 0.8-1.0 : 0.8-0.9 : 1.2-1.3 : 1.6. The aesthetasc y_a 2.0-2.6 times the length of the terminal podomere.

A2 (Fig. 3D-F): Pr: P-1*m* / Exo: 2*s*-1*m* / EI: P-Y-1*s*-1*m*(pu).

Female (Fig. 3E): EII+III: A-2*s*, P-y_1-1*s*(t_4)-3*m*(pu, t_{1-3}), D-y_2-2*s*($z_{2,3}$)-1*s*(z_1: G)-1*m*(G_2: ser)-2*l*(G_1, G_3: ser) / EIV: D-y_3-2*m*-1*m*(G_m: ser)-1*l*(G_M: ser). Aesthetasc Y represents 44-46% of the EI length. EII and EIII undivided in females. Internal t_{1-3} setae of this compound podomere amounting up to 73% of EI length. Amongst three external 'z' bristles of EII+III, z_1 is claw-like (ca. 0.4 times the L of EI). The G_1 and G_3 claws well developed, subequal, 1.3-1.4 times as long as EI; G_2 claw is reduced (ca. 0.7 of EI length). The aesthetasc y_3 is medium in length (it represents 72% of EI length). The G_m and G_M claws equalling ca. 0.7 and 1.1 of EI length, respectively; G_m claw has thus similar L as that of the aesthetasc y_3. Two medium setae on EIV, one associated with y_3, the other one alone (so-called seta g), not always visible (seta g not visible on the lectotype female no. ZMH 1396).

Male (Fig. 3D-F): EII: A-2*s*, P-y_1, D-1*s*(t_4)-3*m*(t_{1-3}) / EIII: D-y_2-2*s*(z_3, G_1: ser)-2*m*(z_2: G, G_3: ser)-2*l*(z_1: G, G_2: ser) / EIV: D-y_3-1*s*(G_m)-2*m*-1*l*(G_M: ser). Setae t_2 and t_3 are transformed into male-bristles and they represent 61% and 72% of EI length, respectively. z_1 and z_2 are developed as claws (the relative L of z_2 is medium to large), whereas z_3 remains as a short seta. Aesthetasc y_3 represents 64% of EI length. The G_2 claw is well developed (130% of EI length), G_1 claw reduced (only 43% of EI length) and G_3 claw-like seta is medium in L (60% of EI length). G_M claw well developed, its L represents 104% of EI length. The minor claw G_m is relatively short compared with that in females and equals only ca. 0.4-0.5 of EI length, thus it is shorter than the aesthetasc y_3.

Mdp (Fig. 3C): I: In-1*s*(α)-1*s*(S_2: pu)-1*m*-1*l*(S_1: pu) / II: Ex-2*m*, In-1*s*(β)-4*m* / III: Ex-3*m*, In-4*m*, D-2*m*(γ) / IV: D-4*s*-2*m*(G: ser). Setae α and β subequal. In some specimens (e.g. the lectotype) the relative L of external setae on the 2nd podomere and of three internal setae on same podomere (so-called setal group) could be assigned as large. The terminal podomere with two claws that represent 130% of the 3rd podomere L, the central one fused and pectinated, the external one movable.

Mx1 palp (Fig. 3B): I: Ex-4*l* (pu) / II: In-3*m*(pu), D-1*m*(pu)-2*l*(G).

L5 (Fig. 3G-I): Pr: A-1*s*(a)-1*m*(a), Ex-2*m*(b, d) / Mastic: D-14*s*(pu) / Exo: 3*s*(pu) / E: D-3*s*. Male prehensile palps asymmetrical, the left one (Fig. 3H) cylindrical and slender, the right one (Fig. 3G) arched and slightly longer than the left one.

L6 (Fig. 3K): Pr: A-1*s*(d) / EI: A-1*s*(e) / EII: A-1*s*(f) / EIII: A-2*s*(g) / EIV: P-1*s*(h_3), D-1*s*(h_1)-1*l*(h_2: G, ser). Terminal claw 2.3-2.5 times the length of EII.

L7 (Fig. 3J): Pr: Ex-2*m*(d_{1-2}), In-1*l*(d_p) / EI: P-1*s*(e) / EII: P-1*s*(f) / EIII: P-1*m*(g) / EIV: D-1*s*(h_1)-1*m*(h_2)-1*l*(h_3). All setae plumose. Relative lengths of three apical setae (L of EI used as reference) as: h_1 = 0.3-0.4, h_2 = 0.7-0.9, h_3 = 1.7-2.0.

Fu (Fig. 3L, M). Posterior margin almost straight, anterior one proximally regularly weakly rounded. Ratio of the lengths of anterior margin, anterior claw (G_a), posterior claw (G_p), anterior seta (s_a) and posterior seta (s_p) in males as: 100: 56: 46: 8: 12. Female distal claws relatively larger than those in males, they represent 61% (G_a) and 50% (G_p) of the anterior margin. Furcal attachment long and distally bifurcating as in other species of the genus *Cryptocandona*.

Copulatory organs. The female genital lobe (Fig. 3L) distinctly protruding and regularly rounded. The ovocyte diameter represents 11-13% of the carapace L. Male hemipenis (Fig. 3N) is moderately sclerotised with dorsally rectangular outer lobe (a), distally widely rounded inner lobe (b) and medial lobe (h) of laminar shape and slightly acuminated. Labyrinth with four successive sections (d_1, d_2, d_3, d_4), from which the last one is poorly developed and poorly reticulated. Distal part of the copulatory tube is simple, bursa copulatrix (e) has a complex shape, with a dorsal sclerotised lobe (x). Process M is well sclerotised, with a rounded median part, which dorsally bears a central sclerotised bar (y). The distal part of the M process is sword shaped. Zenker's organ represents 38% of the carapace length and contains seven whorls of spines.

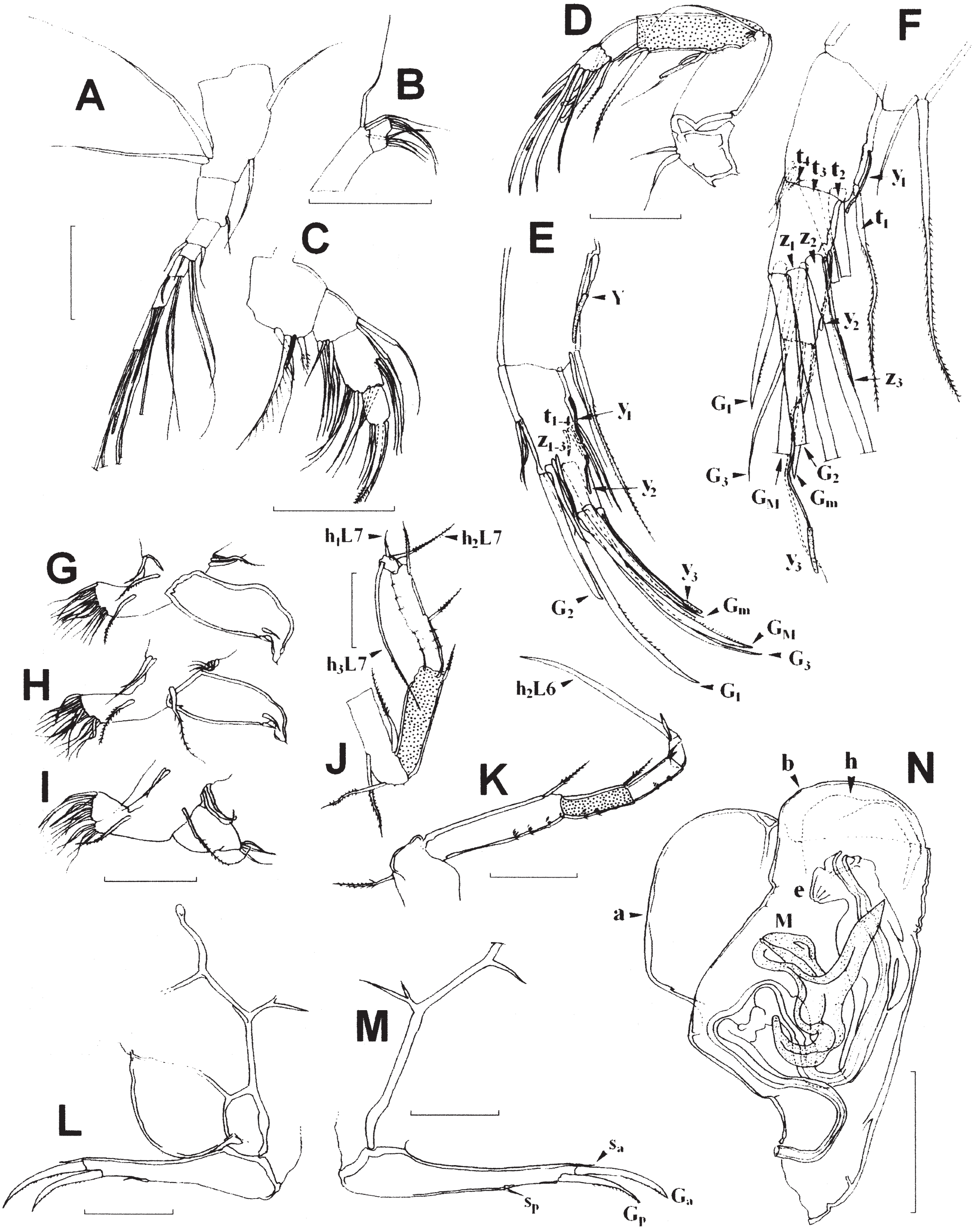

Fig. 3. – Appendages of *Cryptocandona kieferi kieferi* (Klie) from RHI: A, male A1; B, male Mx1 palp; C, male Mdp; D, male A2; E, left female A2 in external view; F, left male A2 in external view (detail of D); G, right male L5; H, left male L5; I, female L5; J, male L7; K, male L6; L, female Fu and genital lobe; M, male Fu; N, hemipenis. A-D, F-H, J-K and M-N, appendages of a male from the Lutter spring; E, I, and L appendages of the female lectotype (ZHM 1396). Podomeres of A2, L6 and L7 used as references for the assessment of the relative length of various setae and claws are dotted. Scale bars = 0.1 mm.

Morphological variability

In the following, we present information on the degree of variability (including the range of variation) for the valves and different limbs, at the intra- and interpopulational levels. Interesting enough, the structure of the male hemipenis (compare Fig. 3N, Fig. 4A-C and Fig. 5C) and the shape of the female genital lobe (compare Fig. 3L, Fig. 4D-F and Fig. 5B) do not differ substantially between the populations when inspected visually.

Measurements of the carapace length (L) and high (H) as well as the H/L ratio of the adult specimens from five studied areas are listed in Table I. Considerably smaller carapaces of both males and females were recorded in DAC. Mean length and height are, respectively, below 0.9 and 0.5 mm for the males, and below 0.8 mm (L) and 0.4 mm (H) for the females. The specimens from the remaining areas were of the comparable size with the mean carapace L around 1.1 mm for the males and 1.0 mm for the females, and with the mean carapace H equalling 0.52-0.55 mm and 0.46-0.50 mm for the males and females, respectively. It appears that specimens of DAC have the paedomorphic carapace size. The length of the adult females of DAC (0.76-0.82 mm, N = 14) corresponds to that of the 8th stage juveniles of DAV (0.76-0.82 mm, N = 3), and the difference is not statistically significant when tested by the Student t-test (t = 0.559, P = 0.584). Almost the same pattern is seen when the carapace length of the 8th stage juveniles of DAC (0.67-0.74 mm, N = 15) is compared with that of 7th stage juveniles of DAV (0.66 mm, N = 2).

Tables II and III which provide biometric data on 14 morphological traits for both males and females from the five areas considered both intra- and interpopulational variability. While the S.D. values around the arithmetic mean remain low, there is in most of cases some overlap of values (note the range between the maximal and minimal values).

Table II. – Relative length of various claws, setae and linear distances measured on the appendages of *Cryptocandona kieferi* (males) from the five areas studied. N = 4 specimens per locality (S.D. = standard deviation).

Trait		RHI	SAO	RHO	DAV	DAC
G_1	Mean	41	49	44	42	44
	Range	37-46	39-66	42-48	36-49	41-45
	S.D.	4	12	3	6	2
G_2	Mean	131	134	122	134	127
	Range	123-140	127-139	119-126	128-137	117-136
	S.D.	7	6	3	4	8
z_1	Mean	125	125	112	116	115
	Range	117-136	116-136	108-116	112-122	105-125
	S.D.	9	9	4	5	8
z_2	Mean	102	104	97	51	48
	Range	95-106	84-119	92-102	45-60	45-51
	S.D.	5	15	5	7	3
G_M	Mean	109	106	99	103	96
	Range	103-116	97-115	93-103	101-105	95-97
	S.D.	5	8	4	2	1
G_m	Mean	45	49	49	43	43
	Range	37-51	43-57	45-50	36-50	38-46
	S.D.	6	6	2	6	3
h_2L6	Mean	244	257	227	234	228
	Range	237-252	243-276	222-234	223-242	215-234
	S.D.	6	14	5	10	9
h_1L7	Mean	32	33	30	31	26
	Range	28-36	31-35	28-32	24-35	24-28
	S.D.	3	2	2	5	2
h_2L7	Mean	85	80	67	82	62
	Range	80-91	78-81	61-77	77-86	54-67
	S.D.	6	1	7	5	6
h_3L7	Mean	183	182	175	188	169
	Range	172-193	145-202	156-185	186-190	156-176
	S.D.	9	26	13	2	9
s_a	Mean	9	12	10	11	10
	Range	7-11	9-14	8-12	9-12	10
	S.D.	2	2	2	1	0
s_p	Mean	10	14	11	11	10
	Range	7-12	9-17	9-14	8-14	9-12
	S.D.	2	4	2	3	2
G_a	Mean	54	60	59	61	57
	Range	52-56	55-62	55-62	59-63	55-58
	S.D.	2	3	3	2	1
G_p	Mean	45	52	50	52	47
	Range	41-48	48-54	45-52	48-55	43-51
	S.D.	4	3	3	3	3
s_p-G_p	Mean	31	28	28	28	27
	Range	30-31	24-32	25-31	25-30	24-32
	S.D.	1	3	3	2	3

Table III. – Relative length of various claws, setae and linear distances measured on the appendages of *Cryptocandona kieferi* (females) from the five areas studied. N = 4 specimens per locality (S.D. = standard deviation).

Trait		RHI	SAO	RHO	DAV	DAC
G_1	Mean	137	145	136	132	138
	Range	135-140	143-147	125-142	124-144	132-143
	S.D.	2	2	8	9	5
G_2	Mean	71	85	76	72	77
	Range	67-79	76-88	71-83	60-87	69-86
	S.D.	5	6	5	12	8
G_3	Mean	128	136	122	122	135
	Range	126-129	132-141	112-131	115-132	131-140
	S.D.	1	4	9	8	4
G_M	Mean	107	115	109	104	108
	Range	100-109	111-118	102-115	95-112	104-111
	S.D.	4	3	5	7	3
G_m	Mean	72	77	71	69	70
	Range	66-78	75-79	64-77	64-76	62-79
	S.D.	5	2	7	5	7
h_2L6	Mean	238	261	243	236	226
	Range	226-247	252-269	208-268	218-251	219-233
	S.D.	9	8	25	14	7
h_1L7	Mean	30	33	34	29	26
	Range	25-33	31-36	29-37	26-33	24-28
	S.D.	3	2	3	3	2
h_2L7	Mean	79	77	71	69	61
	Range	74-85	67-86	67-78	59-81	59-63
	S.D.	5	8	5	10	2
h_3L7	Mean	188	193	199	190	164
	Range	180-198	183-207	186-204	173-204	156-170
	S.D.	9	10	9	13	6
s_a	Mean	15	14	11	12	12
	Range	13-17	11-16	8-12	9-15	10-13
	S.D.	2	2	2	3	1
s_p	Mean	15	16	14	15	10
	Range	14-15	14-20	11-15	12-16	9-13
	S.D.	1	3	2	2	2
G_a	Mean	63	66	71	63	65
	Range	59-67	63-69	66-75	61-64	62-67
	S.D.	3	3	4	1	2
G_p	Mean	52	58	57	54	55
	Range	47-55	55-59	53-64	52-57	50-58
	S.D.	3	2	5	2	3
s_p-G_p	Mean	31	28	29	26	24
	Range	29-33	27-29	27-30	24-28	22-29
	S.D.	2	1	1	2	3

A discriminant function analysis was therefore carried out for each sex separately using the biometric data collected in order to discover whether the populations of the five geographical areas could be distinguished morphometrically. Results of the male data grouped into the five areas (RHI, RHO, SAO, DAV and DAC) are shown in Fig. 6, whereas those for the females are plotted in Fig. 7. Differences between localities were highly significant for both male (Wilks' lambda = 0.001; F = 11.655, P < 0.001) and female (Wilks' lambda = 0.010; F = 5.586; P < 0.001) data sets. Discriminant functions coefficients are listed in Table IV with the variables ranked in the order they entered the model. A plot of the first (Root 1) and the second (Root 2) discriminant functions for the analysis of males shows that DAC and DAV are clearly separated from each other and from the remaining three populations along Root 1 (Fig. 6). That first function accounted for 67% of the variance explained and was heavily loaded by z_1, G_M and z_2 (Table IV). It should be noticed that the males of RHI are also well separated and plotted far to the left. The Root 2 (25% of the explained variance) discriminates between males in DAV and in RHO, which occupy the most extreme positions (further to the bottom and the uppermost part of the plot, respectively).

Considering the females, the discrimination is not nearly as clear as that provided for the males. In the scatterplot (Fig. 7) only females of DAC are plotted clearly further to the left. Thus, first discriminant function, which accounted for 51% of the explained variability, discriminates mostly between the females of this population and those of the four other. This function is marked mainly by G_3, h_2L6 and h_3L7. Despite the significant differences between the statistical populations of the data representing five geographical areas, there is still some overlap of the individual females of RHI, RHO, SAO and DAV. Thus, no individual female can be assigned unambiguously to a particular geographical area on the basis of any of the used linear measurements alone. However, it could be more possible when employing a combination of these measurements.

Table IV. – Standardised coefficients for the first two discriminant functions (roots) of the multiple group discriminant analysis of biometric data for the males and females of *Cryptocandona kieferi* grouped into five areas (RHI, RHO, SAO, DAV and DAC).

Males			**Females**		
Variable	Root 1	Root 2	Variable	Root 1	Root 2
z_2	-1.040	0.821	h_3L7	0.582	-0.164
h_2L7	-0.969	-0.928	G_3	-1.096	0.395
G_a	0.823	0.715	G_a	0.018	-1.204
G_M	-1.381	-1.906	s_p-G_p	0.253	1.109
z_1	1.577	0.985	h_2L6	0.922	0.435
h_2L6	0.161	-0.085			
Eigenvalue	26.915	10.060	Eigenvalue	5.871	4.292
Cumulative proportion	0.672	0.923	Cumulative proportion	0.512	0.886

To summarise the results, it appears that the first most significant and clear discrimination is possible for two groups of populations (DAV and DAC versus RHI, RHO and SAO) by the length and degree of development of the z_2 seta/claw on the A2 of males. The males of two Danubian populations (DAV and DAC) have z_2 clearly shorter, poorly sclerotised and developed as a seta when compared with the large z_2 and developed as a claw in the males of RHI, RHO and SAO (compare Fig. 3F, 4G-H and 5A). The ratio of z_1/z_2 in the former group equals 2.0-2.7, whereas this ratio in the males of the non-Danubian populations does not exceed 1.4 (1.1-1.4). In all the studied populations the 8th male instar bears a reduced z_2 like in the adult females (Fig. 4G-J). During the last moult, in the cypridoid ostracods this z_2 bristle is transformed into a claw in males (Martens 1987, Meisch 2000). In both Danubian populations the reduction of the z_2 to a simple seta is a derived character which could be considered a paedomorphic and non-adaptive feature because the energetic expense connected with the reinforcement of that seta into a claw would be minimal. This stop in the post-embryonic development could be genetically fixed in the males of DAV and DAC where it occurs in all dissected males.

In the next step, the separation between DAV and DAC is apparent by a combination of several traits. Specimens of DAC could be separated from those of DAV mainly by the male G_M claw and h_2L7 seta due to their relative shortness in the former population as well as by the relatively shorter h_3L7 seta and the relatively longer G_3 claw in the females of DAC.

Even if the range of variation of these traits for the DAC population partly overlaps with those measured for RHI, RHO and SAO (cf. Tables II, III) we consider that the combination of those traits is useful for the extended taxonomy of *C. kieferi* (cf. next section).

Infraspecific systematics

The morphological data presented above support our decision to split the monotypic *C. kieferi* (Klie) species into three subspecies, *C. kieferi kieferi* (Klie), *C. kieferi danubialis* n. ssp. and *C. kieferi dobrogensis* n. ssp. Details for the availability of the new subspecies conforming the International Code of Zoological Nomenclature (1999) are here presented.

Cryptocandona kieferi danubialis n. ssp. (Figs 2S-X, 4C,F-J)

Derivation of name. From Danubius, the Latin name of the river Danube.

Diagnosis**.** A *C. kieferi* characterised by a large carapace, i.e. male L > 1 mm, female L around 1 mm and male H > 0.5 mm, female H around 0.5 mm (cf. Table 1). The z_2 on the male A2 short, poorly sclerotised and developed as a seta (Fig. 4G). The ratio z_1/z_2 on the male A2 amounting to 2.0-2.7. Male G_M and h_3L7 as well as female h_3L7 relatively long (cf. Table II, III).

C. kieferi danubialis n. ssp. differs from the nominate subspecies *C. kieferi kieferi* (Klie) mainly by the peculiar shape of the short seta z_2 instead of a long claw. The new subspecies differs from *C. kieferi dobrogensis* n. ssp. by its larger carapace size and the longer male claw G_M on A2, longer both setae h_2 and h_3 on L7 as well as the longer female seta h_3 on L7. Hence, the new subspecies can be recognised through combination of the characters, which are only parly shared with the other two subspecies.

Type material. Holotype: a male deposited in the collections of Natural History Museum in Vienna, Department of Crustacea (= NHMW) under no NHMW 18802. Allotype: a female NHMW 18803. Paratypes: five males and five females NHMW 18804-18808.

Type locality. Lobau, Eberschüttwasser at Vienna, collected from an interstitial habitat saturated in groundwater at the depth of 1.2 m below the surface sediment of the backwater Eberschüttwasser (the site Lobau A in Danielopol 1983). Leg DL Danielopol on 24 November 1978.

Cryptocandona kieferi dobrogensis n. ssp. (Figs 2Q-R, 5A-F)

Derivation of name. From Dobrogea, with the Latin transcription for the Eastern Romanian re-

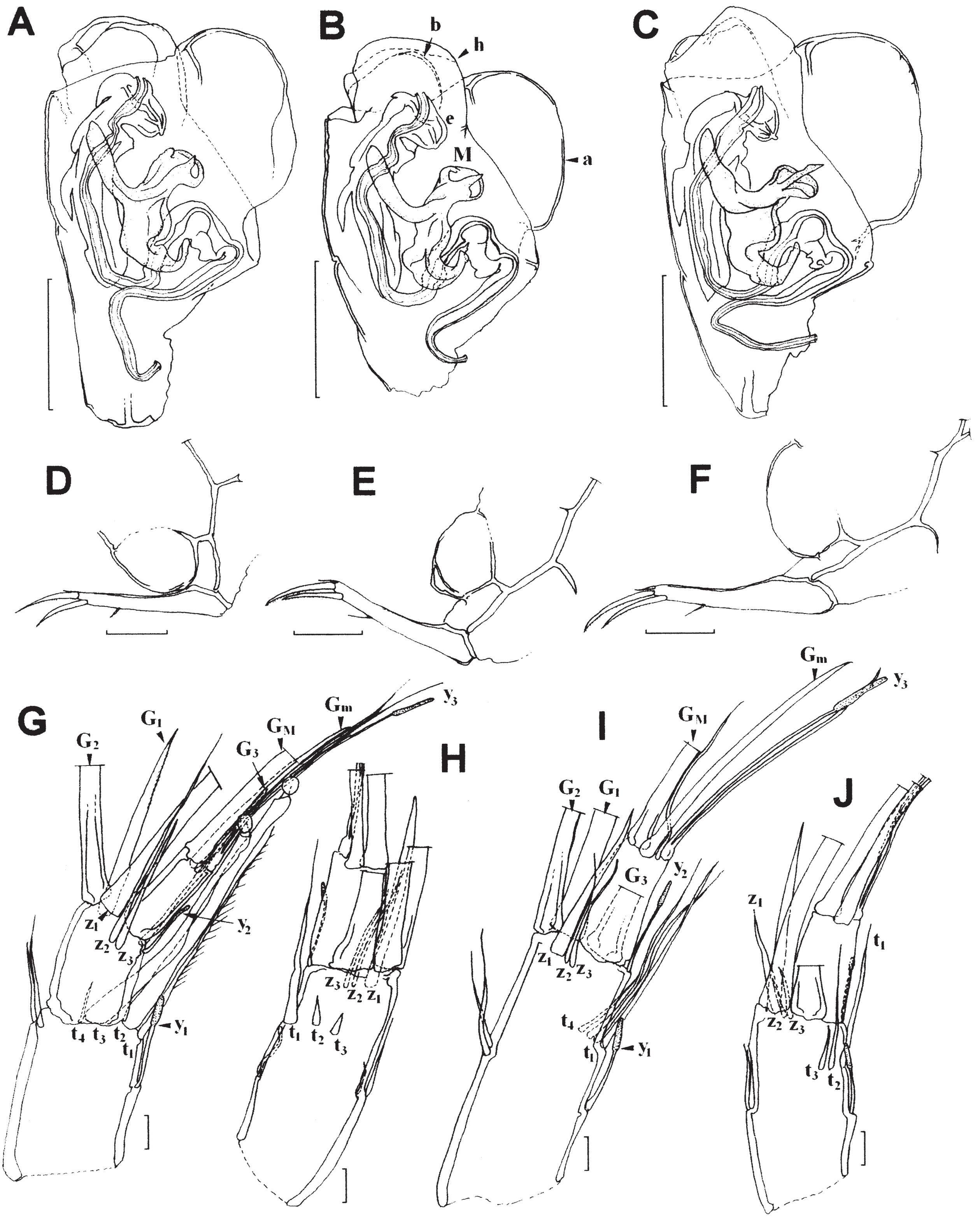

Fig. 4. – Appendages of *Cryptocandona kieferi* (Klie). A-C, hemipenes (A, *C. k. kieferi* from RHO; B, *C. k. kieferi* from SAO; C, *C. k. danubialis* n. ssp. from DAV – holotype NHMW 18802); D-F, female furcae and genital lobes (D, *C. k. kieferi* from RHO; E, *C. k. kieferi* from SAO; F, *C. k. danubialis* n. ssp. from DAV – allotype NHMW 18803); G-J, details of the A2 endopodial chaetotaxy of *C. k. danubialis* n. ssp. from DAV (G, adult male; H, the last male juvenile stage; I, adult female; J, the last female juvenile stage). Scale bars: A-C = 0.1 mm; D-E = 0.08 mm; G-J = 0.01 mm.

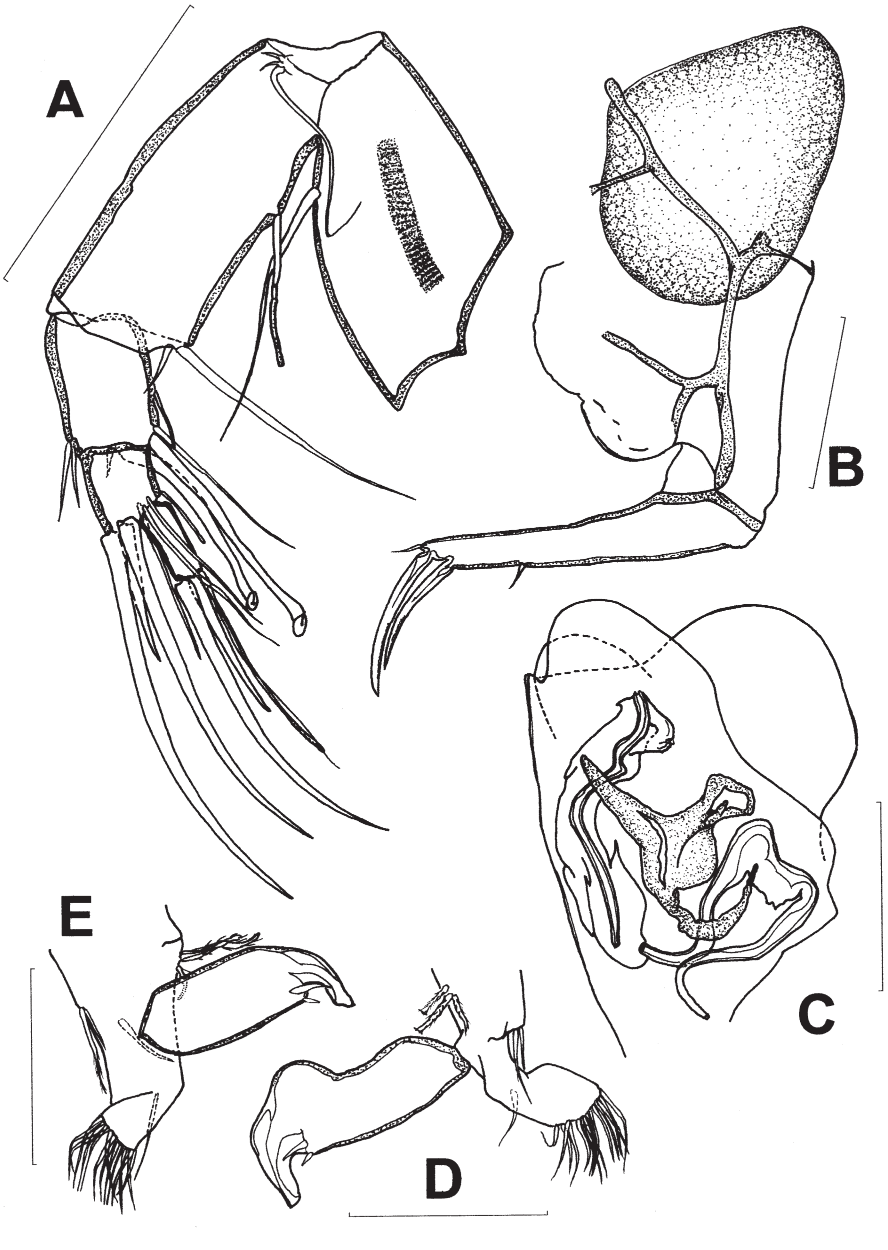

Fig. 5. – Appendages of *Cryptocandona kieferi dobrogensis* n. ssp from DAC. A, left male A2 in external view; B, female Fu and genital lobe; C, left hemipenis in internal view; D, right male L5; E, left male L5. A, C-E – appendages of the male holotype (NHMW 18809), B, appendage of the female allotype (NHMW 188011). Scale bars = 0.1 mm.

gion located between the Danube and the Black Sea coast.

Diagnosis. A *C. kieferi* characterised by the small carapace, i.e. male L < 1 mm, female L < 0.9 mm and male H < 0.5 mm, female H < 0.4 mm (cf. Table I). The z_2 on the male A2 of juvenile stage, a relatively short, poorly sclerotised seta not transformed into a claw (Fig. 5A). The z_1/z_2 ratio on the male A2 equals 2.1-2.5. Male G_M and h_2L7 as well as female h_3L7 relatively short (cf Table II, III).

C. kieferi dobrogensis n. ssp. differs from *C. kieferi kieferi* (Klie) in the degree of the development of the male z_2 (a short seta versus a long claw in the nominate subspecies) as well as in the carapace size (distinctly smaller than that in *C. kieferi kieferi*). The short, poorly sclerotised seta z_2 is shared with *C. kieferi danubialis* n. ssp., however z_3 seta is only imperceptibly shorter than z_2 in *C. kieferi dobrogensis* thus the ratio z_2/z_3 is only 1.1, whereas this ratio in *C. kieferi danubialis* equals 1.5. The new subspecies differs from *C. kieferi danubialis* n. ssp. also by its smaller carapace size, shorter male claw G_M on A2, shorter both setae h_2 and h_3 on L7 as well as the shorter female seta h_3 on L7 (cf Table II, III). *C. kieferi dobrogensis* n.

ssp. has also the relatively long aesthetasc y_a on A1, which is on average 4.1 times longer that the L of the IV podomere of this limb (3.2 in *C. kieferi kieferi* and 3.4 in *C. kieferi danubialis* n. ssp.). Hence, the new subspecies can be recognised through combination of characters, which are only partly shared with the other two subspecies.

Type material. Holotype: a male with the limbs dissected and mounted in glycerine on a permanent slide (NHMW 18809) and the left valve stored dry in a micropalaeontological cell (NHMW 18810). Allotype: a female dissected and stored as the holotype (NHMW 18811, 18812). Paratypes: four males and three females either dissected or in toto preserved in ethanol (NHMW 18813-18816).

Type locality. The village Lumina at about 15 km west from Constanţa, collected from the garden well of the house Lumina no 78 (property of M Stefan) accessing to an interstitial habitat saturated in groundwater at the depth of about 4 m below the surface. Leg DL Danielopol & D Dancau on 7 November 1968.

Geographical Distribution

Cryptocandona kieferi is known to live within the alluvial valleys and/or plains of large rivers the Rhine, the Rhône, the Saône, the Danube and within areas of the drainage basins of these rivers (cf. Fig. 1 and details below). Initially the species was caught in alluvial sediments at Breuschwickersheim on the left side of the Upper Rhine near Strasbourg (Klie 1938). The sediments were drained for water exploitation. From the same area the species was sampled from wells in Graffenstaden and Illkirch. Klie (op. cit.) obtained samples also from the right side of the Rhine from various wells located around Freiburg (at Bucholz, Kems, Lehen, Ringsheim, Teningen and Vörstetten), Offenburg (at Ichenheim and Meissenheim) and Karlsruhe (at Elchesheim). It is well known that the aquifer of the alluvial plain is well recharged and further strongly drained by the Rhine (Steenken 1998). Haine (1946) collected *C. kieferi* in wells on the Rhine plain around Bonn (at Siegburg and Seligenthal). Dittmar (1955) mentioned the species in exfiltrating groundwater which discharged into a stream Aabach (south of Albaum). This latter stream is a tributary of the Rhine. Furthermore, the species was sampled at Rhinau (France) in the alluvial sediments of a drainage channel located closely to a regulated channel of the Rhine River. It was also collected in exfiltrating groundwater at Huttenheim, in the spring of the Lutter River closely located to the Ill, a tributary of the Rhine river (cf here Material and methods section).

The groundwater fauna of the Rhône and the Ain rivers (closely to the confluence with the Rhône) in the Lyon area were intensively studied by one of us (PM) and colleagues from the University of Lyon. In the alluvial plain of these rivers one can recognise beside the main channel three types of side arms, i.e. those closely located to the main channel and still in connection with this latter, those being located more inside of the plain and isolated from the main channel but still having an active subsurface water circulation and finally the far way backwaters located inland, more or less clogged and in a process of terrestrialisation (Dole-Olivier *et al.* 1994). *Cryptocandona kieferi* occurs mainly in the areas of intermediary backwaters closely located to the main channel (Marmonier 1988, Dole-Olivier & Marmonier 1992, Dole-Olivier *et al.* 1994) in sediments at the depth of 0.5 m to 1-2 m (Creuzé des Châtelliers & Marmonier 1993 and here cf Material and methods section). For instance in the Rhône plain at Jons this species was found in the main channel, in the area of the “Lone des Pêcheurs” – a backwater still connected to the main channel, and in a gravel pit located at 2 km far from the river but in unclogged coarse sand and gravel (Marmonier 1988).

In the Ain plain *C. kieferi* occurs at two sites: the backwaters of Puits Novet and the Brotteaux which display similar ecological situation with that mentioned for the “Lone des Pêcheurs”. Idbennacer (1985) found *C. kieferi* in the Saône plain near Dijon (cf Marmonier 1988 and here Material and methods section) when studying the groundwater fauna at sites located closely to a surface drainage channel.

On the Danube Löffler (1963) identified *C. kieferi* in samples collected from wells located on the margin of the river at Melk and Kagran (Lowerustria) and mentioned previous record of the species from wells at Vienna (Fig. 1). Danielopol (1976, 1984, 1991) and Danielopol & Baltanás (1996) rediscovered the species in Lower Austria and added the following new localities: riverbed sediments at Tulln (Danube R-km 1963.5) and March (Danube L-km 180.2). Long-term ecological investigations in the Vienna area (Lobau and Regelsbrunn) allowed the collection of a rich material of *C. kieferi*. Details about these sites were presented in the section Material and methods. Here one should mention that generally the most abundant sites harbouring this species were located in the Lobau in areas with an active subsurface water circulation like in the Eberschüttwasser area (Danielopol 1983, 1989, Pospisil 1994, Danielopol *et al.* 1997, 2000). The species occurs also in the wetland aquifer of the Danube called Lobau B (Rogulj *et al.* 1993). This aquifer is seasonally recharged by the Danube river (Pospisil 1994, Danielopol *et al.* 2001).

Furthermore, Absolon (1978) illustrated the valves of the species recorded as Recent from Blatnica in Slovakia, close to the Vah river, a tributary of the Danube (Fig. 1).

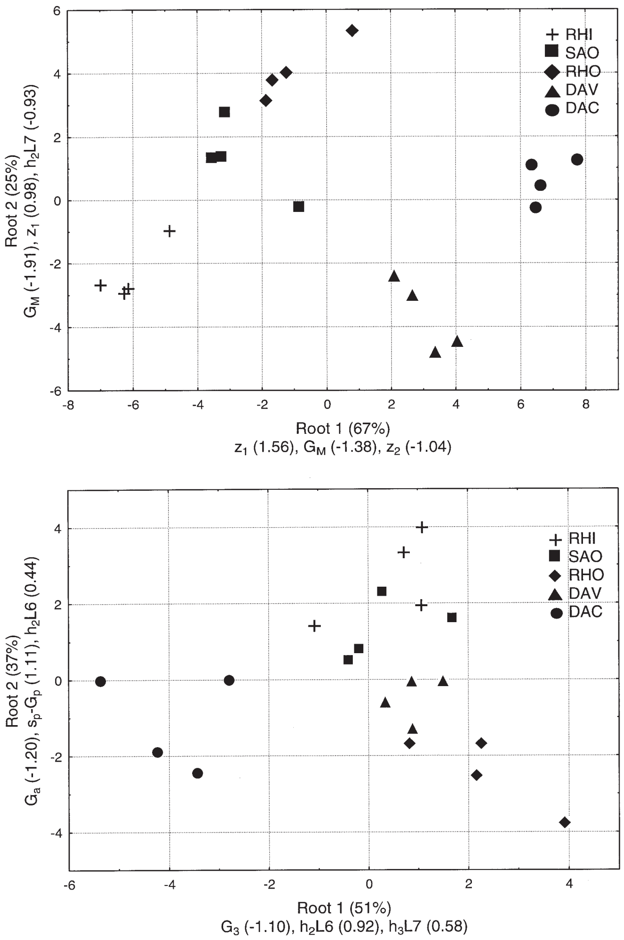

Fig. 6. – Top, a plot of the first discriminant function (Root 1) against the second (Root 2) depicting any discrimination by biometric measurements of *Cryptocandona kieferi* males grouped into five areas. Bottom, a plot of the first discriminant function (Root 1) against the second (Root 2) depicting any discrimination by biometric measurements of *Cryptocandona kieferi* females grouped into five areas.

Finally, *C. kieferi* occurs in the Lower Danube area at Lumina in Romania (cf Material and methods section).

Some fossils were also assigned to *C. kieferi*. Absolon (1973) mentioned this species from Holocene in areas closely located to the tributaries of the Danube: at Ismaning and Lochhausen nearby the Isar river in Bavaria and at Horka Bolek adjacent to the Hron and Torysa rivers in Slovakia (cf Fig 1). From the Absolon's (op. cit.) information this material seems to belong to stygobitic populations with exfiltrated individuals on the surface of various water bodies. Sokač (1978) and Sokač *et al.* (1982) also mentioned and illustrated Quaternary fossil individuals, which look like juveniles of *C. kieferi*. The material comes from boreholes at Erdut (Lower Pleistocene), Dalj (Middle Pleistocene) and Vinkovci (Upper Pleistocene), all in the surroundings of Osijek near the Danube river in Croatia (Fig. 1). Moreover, Bilan (1992) mentioned *C. kieferi* among ostracods of Holocene calcareous sediments of the Sąspówka river (a tributary of the Vistula river) at Ojców in southern Poland. However, he (op. cit.) did not offer any illustrations and description of these specimens, thus the identification is dubious and was not indicated in Fig. 1.

Finally, Pipik (2000) mentioned *C. kieferi* in the lacustrine sediments of the Upper Miocene (? Pliocene) in the Turiec depression in Slovakia (not indicated in Fig. 1). This is the oldest record of the *C. kieferi* lineage. Pipik (pers comm 7 October 2004 to DLD) found only one valve that we identified as *C.* aff. *vavrai*.

DISCUSSION

Comments on the systematics of Cryptocandona kieferi

Löffler (1963) comparing the shape of the male clasping organs of *C. kieferi* from the Rhine and the Danube areas suggested for the first time that populations from Lower Austria along the Danube valley (at Melk and Vienna) belong to a separate subspecies as compared to those existing in the upper Rhine. The arguments of Löffler (op. cit.) are in our opinion based on an artefact of the limbs preparation (i.e. the clasping organs when compressed under the cover glass may lay on the slide in various positions from horizontal to more oblique giving the impression of being differently shaped). However, using more solid arguments the present study confirms Löffler's hypothesis.

The infraspecific systematics used for *C. kieferi* is based on the following considerations. We decided to use a conservative solution for the species concept, respectively the classic Linnean one based on morphological criteria. This is operative as far as one can document distinct gaps between the range of variability of various morphological traits belonging to different populations (Martens *et al.* 1998). We combined the principle of the morphological species concept with those of the evolutionary species (lineages formed by ancestor-descendant populations, which display their own evolutionary tendencies and historical fate).

Because the morphological differences between the various populations are subtle and useful for identification of the various taxa only through comparative studies we decided to give a priority to the taxonomic stability of *C. kieferi* as an evolutionary lineage. Therefore we used the infraspecific level of the taxonomic hierarchy and proposed here two new subspecies. The advantage of this solution is on one side the possibility to recognise the species also when we have at hand only the valves (the case of the fossil material), and on the other side it allows to continue the study of various populations and to try to characterise using genetic and molecular criteria (cf Mallet 1995) in order to further detect microevolutionary changes. The other alternative would be to use the phenetic concepts for taxonomic definition of species, e.g. the multidimensional concept of Sbordoni (1993). Using the results of the discriminant analysis presented above one could clearly define the DAC population as a new species. However, when we need to classify each individual as it happens in quantitative ecological surveys, statistical criteria for the species identification are useless.

Phylogenetic criteria, i.e. the smallest biological entities that are diagnosable and help to recognise a monophyletic lineage (for the definition of so-called phylogenetic species cf Martens *et al.* 1998), would discriminate in a first step the *C. kieferi* populations Saône-Rhône-Rhine from those of the Danube domain (DAV and DAC) because of the apomorphic state of the z_2 on the male second antenna in the Danubian populations. Note that this trait is a synapomorphy, uniting the DAV and the DAC taxa. Further on, the Austrian population can be separated from the Romanian one by the apomorphic state of the carapace size, which in the case of this latter represent a neotenous state as previously explained as well as by the relative length of the antennal G_M claw and the h_3 seta on L7 (which in the case of DAC are shorter as compared to DAV specimens). Applying this phylogenetic concept one could create a new stem species *C. danubialis* with two crown taxa: *C. danubialis danubialis* and *C. danubialis dobrogensis*. This taxonomical solution is inoperative for the fossil material as it can not discriminate *C. kieferi* and *C. danubialis* using only the valves. Hence, the solution of compromise we proposed above, i.e. to separate *C. kieferi* in three subspecies.

Dispersal pathways of **Cryptocandona Kieferi**

The documentation of both the Recent and fossil geographical distribution of *C. kieferi* presented above suggests that the dispersal of this ostracod took place mainly along the valleys of the large rivers like the Rhône, Rhine and Danube during the extended time-span (the Pliocene-Pleistocene). This biogeographical scenario was already proposed for both surface dwelling animals (fishes) and for stygobitic ones like the crustacean amphipod *Niphargopsis casparyi* (Pratz) by Thienemann (1950).

During the Upper Pliocene the Rhine was related to the Saône through the Doubs valley (Henry 1976). Intermittent connections between the Rhine and the Danube existed through the Aar valley during the Upper Pliocene (Fink 1966, Henry 1976). Considering these connections Henry (1976) explains the presence of the hypogean isopod *Proasellus strouhali* Karaman in both the Rhône (*P. strouhali puteanus* Henry) and the Danube (*P. strouhali strouhali* Karaman). The present Danube course in Romania is rather recent. The Iron Gates were definitively cut and eroded by the river during the last stages of the Pliocene, beginning of the Pleistocene (Fink 1966, Jipa pers com to DLD). The arrival of the modern Danube close to the Dobrogea block is more recent, i.e. during Riss-Würm Quaternary stages about 120,000 yr BP (Jipa 1996, and 2002 pers com to DLD). A strong regressive event of the Black Sea in front of the Dobrogea block at about 7.5 kyr BP (Ryan *et al.* 1997) seems to have strongly drained groundwater from the vicinity of the Danube toward east through the karstic system located south from Constanța. These connections and historical events seem to explain also the actual distribution of the subterranean dwelling amphipod *Niphargus gallicus* Schellenberg known from the Rhône valley as well as from a tributary of the Danube in Romania and the karst block of Dobrogea (Dancau 1963). Considering *C. kieferi* we hypothesise that the dispersal followed from West (Rhône, Saône, Rhine) towards the Upper Danube continuing further to the Lower Danube, down to the Dobrogea. The assumption that *C. kieferi dobrogensis* n. ssp. at Lumina has its origin in the lower Danubian populations may be supported by the fact that this ostracod co-occurred in the well at Lumina with *Mixtacandona pietrosani* Danielopol, an ostracod known from the lower terrace of the Danube at Giurgiu and Oltenița (Danielopol & Cvetkov

1979). It is therefore possible that subterranean dwelling *C. kieferi* from the Danube infiltrated first in the Dobrogea karst and further migrated toward the alluvial porous sediments which exist in the area north-west from Constanța (viz. at Lumina).

The alternative scenario could be that the ancestor species of *C. kieferi* was an epigean dweller widely distributed in Europe which colonised repeatedly and independently local subsurface habitats around the places where we found this species today. This latter scenario applies for instance to *Cavernocypris subterranea* (Wolf). As documented in Marmonier *et al.* (1989) this ostracod species colonised groundwater habitats which belong to various hydrographical basins in Europe and Asia as well sites which are not closely located to running-water systems. Obviously this is not the case of *C. kieferi* since all the sites where this species was identified with certainty lay closely to systems related to the large rivers the Rhône, Rhine and Danube.

Microevolutionary changes within the **Cryptocandona kieferi** *lineage*

One of the surprising facts is the low morphological difference we could document when comparing the populations of *C. kieferi* being hundreds and/or thousand of kilometres distant from each other (cf Fig. 1). Neither the various mechanosensorial structures of the hemipenes nor the prehensile palps, so important in the process of sexual recognition (cf Horne *et al.* 1998) helped to discriminate between the five populations (compare these structures in Fig. 3, 4, 5). This is in opposition with our observations on some more evolved Candoninae lineages (Danielopol 1969, 1978) and could be explained by the poor development and weak sclerotisation of the internal structures within the hemipenis complex in *Cryptocandona*.

A minor morphological differentiation (remember e.g. the shape and the length of the male z_2 trait) is noticeable between the populations of the Saône-Rhône-Rhine and those of the Danube areas. Additionally, one should observe the differences existing between the morphological traits of the Austrian and Romanian populations related to the length of the carapace and the A2 and L7 setae. The smaller size and/or the poorly developed state of these characters in the case of the Danube specimens are more probably due to a perturbation of the normal development and represent neotenous states as compared with the fully developed structures existing in the case of the western populations of the Saône-Rhône-Rhine (cf Fig. 3, 4, 5).

The present study shows a west-east morphological trend dichotomously punctuated. A first step is the separation between the large western stock of *C. kieferi* and the Danubian populations, followed by a second morphological segregation within the Danube catchment area of the Viennese and the Dobrogean stocks. This serial morphological diversification seems to be related to the temporal dispersion scenario presented above, i.e. the oldest stock is related to the Saône-Rhône-Rhine riverine systems and their Pliocene connections, followed by eastward dispersion through the Danube. The most derived morphology belongs to the Romanian population. The palaeogeographical information suggests that the aquifer at Lumina had closer connection with the Danube waters during the end of the Quaternary as described above.

Relevance of **Cryptocandona kieferi** *for environmental research*

Dole-Olivier *et al.* (1993) showed that a series of stygobitic species, especially crustaceans, could be used as environmental descriptors (called "sentinels" or "indicator species") if we have enough information about their ecological requirements. *C. kieferi* in the Rhône and the Ain areas as well in the Danube valley in Lower Austria displays similar ecological preferences, it colonises alluvial sediments with high permeability (i.e. high subsurface water circulation) and/or low accumulation of particulate organic matter. It prefers well oxygenated sediments but survives also during periods when groundwater habitats become hypoxic and/or support high temperature fluctuations (Danielopol 1983, Marmonier 1988, Danielopol 1989, 1991, Marmonier *et al.* 1992, Creuzé des Châtelliers & Marmonier 1993, Rogulj *et al.* 1993, Danielopol & Marmonier 1994, Pospisil 1994, Danielopol *et al.* 1997, 2000). In organically polluted sediments with low permeability like the site Reichsbrücke at Vienna described by Danielopol (1976), *C. kieferi* could not survive (only valves and carapaces, rests of a living population were found there).

Hence, *C. kieferi* in our opinion can be used as an indicator species for unpolluted sediments with an active groundwater circulation.

CONCLUSIONS

1. Thorough morphologic analysis of five populations of *Cryptocandona kieferi* combined with the (palaeo-)biogeographical data pertaining to the areas where this species occurs provide enough evidence to consider this taxon a distinct phylogenetic lineage within the *Cryptocandona* clade.

2. *Cryptocandona kieferi* undertook minor evolutionary changes during its temporal and spatial expansion from the western part of its distribution

area (the Saône-Rhône-Rhine valleys) towards the eastern part of the geographical domain (the central and lower part of the Danube).

3. Subtle morphological peculiarities of the limb traits belonging to the Austrian and Romanian populations form meaningful diagnostic characters on which we erected two new subspecies. This systematic solution reflects partly an evolutionary situation and at the same time is a convenient compromise for neontologist and palaeontologist taxonomists.

4. Preliminary observations on the distribution of *C. kieferi* from the Rhône and the Danube alluvial aquifers with different degrees of pollution suggest that this taxon can be used as environmental marker within its wide geographical area.

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