Hadodiaptomus dumonti n. gen., n. sp., a new freshwater stygobitic calanoid (Crustacea: Copepoda: Calanoida) from Vietnam (South Asia) and a new member of the subfamily ....

1 author:

Brancelj Anton

National Institute of Biology - Nacionalni inštitut za biologijo

172 PUBLICATIONS   **2,595** CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:

Project   A new species of Pseudomoraria Brancelj, 1994 from a spring in the Cantabrian Mountains, North of Spain View project

Project   Discovery of indigenous species in Korea View project

Hadodiaptomus dumonti n. gen., n. sp., a new freshwater stygobitic calanoid (Crustacea: Copepoda: Calanoida) from Vietnam (South Asia) and a new member of the subfamily Speodiaptominae Borutzky, 1962

Anton Brancelj

National Institute of Biology, Večna pot 111, 1000 Ljubljana, Slovenia
E-mail: anton.brancelj@nib.si

Abstract

A new species of freshwater stygobitic calanoid, *Hadodiaptomus dumonti* n. gen., n. sp., from a cave in Vietnam (South Asia) is described. It is the ninth taxon from the order Calanoida described from freshwater subterranean (i.e. cave) environments. It is the fourth member of the subfamily Speodiaptominae Borutzky, 1962. It differs from stygobitic taxa in Europe and Mexico by numerous characters, especially in the armature of P1–P5, antenullas, mouth parts and genital segments in both sexes. Arthrodial membrane development from CII to CV is different from epigean as well as from subterranean taxa. These differences suffice to raise a new genus – *Hadodiaptomus*, characterised by two-segmented exopodites and endopodites of P1–P4 and reduced P5 endopodites in both sexes. Males are without widened segments 13–18 on right A1. Relations with other stygobitic taxa of the subfamily Speodiaptominae are discussed.

Introduction

Freshwater stygobitic Calanoida are rare and most were discovered only recently. So far, only eight stygobitic taxa are known. *Microdiaptomus cokeri* Osorio-Tafall, 1942, was described from caves in Mexico about 60 years ago (Elías-Gutiérez & Suárez-Morales, 1998). *Speodiaptomus birsteini* Borutzky, 1962, was described from one cave on the Crimean Peninsula. *Argyrodiaptomus cavernicolax* Shen & Tai, 1965 (Shen & Song, 1979) was found in a cave in South China. The remaining five species are concentrated in Europe. *Spelaeodiaptomus rouchi* Dussart, 1970 lives in France and *Troglodiaptomus sketi* Petkovski, 1978, *Stygodiaptomus kieferi* Petkovski, 1981, *S. petkovski* Brancelj, 1991 and *S. ferus* Karanovic, 1999, are known from the Dinaric Balkans (Petkovski, 1978,

1981; Brancelj, 1991; Karanovic, 1999). Most of these stygobionts are known from one or few locations but *T. sketi*, the most common and widespread member of stygobitic Calanoida, occurs in Slovenia, Italy and the Balkans (Stoch, 1984, 1985; Brancelj, 1991, 2001).

Some more members of the order Calanoida have been collected from caves (Madagascar, Cuba, Barbados) and some were described as new subspecies, but their ecological status is not clear (see Petkovski, 1981).

Freshwater stygobitic Calanoida have been little studied (Bowman, 1986) and so far only the species from Mexico and China were known outside Europe. With the new taxon, *Hadodiaptomus dumonti* n. gen., n. sp., the number of species outside Europe is increased. The area occupied by freshwater stygobitic Calanoida expands at the

moment from Mexico to Europe and to the Far East (Vietnam and South China).

The scattered distribution of freshwater stygobitic taxa raises a question on the phylogenetic relationship among them and between epigean and subterranean taxa. Elías-Gutiérez & Suárez-Morales (1998) suggested that *Microdiaptomus* and *Troglodiaptomus* should be placed in the subfamily Microdiaptominae (Elías-Gutiérez & Suárez-Morales, 1998), apart from the subfamily Speodiaptominae Borutzky, 1962 (which should contains only genus *Speodiaptomus*). Both subfamilies are different from other taxa within the family Diaptomidae Baird, 1850 by a number of characters, mainly a result of life in a subterranean environment. Morphologically these are expressed by a reduction in the number of segments and setation. According to Camacho (2003) oligomerisation and simplification in stygobionts should be considered as an apomorphic character, i.e. as a result of progressive evolution in the subterranean environment. On the other hand, colonisation of the hypogean environment does not necessarily require morphological changes (Romero, 2004) but it is important that any particular feature of an organism as an adaptation to (hypogean) environment is passed on to succeeding generation (Culver, 2004).

In the rest of the article we describe the new taxon and discuss its relation to other freshwater stygobitic taxa.

Material and methods

Material was collected by means of 100 μm mesh plankton net. Samples were preserved in 4% formalin. In the laboratory, specimens were sorted and stored in 70% alcohol before further analyses.

One female (designated as holotype) and one male (paratype) were completely dissected and mounted on a slide in glycerol and sealed with nail-polisher. Two slides, one with two intact males and one with two intact females, were mounted as permanent slides in the same way (all designated as paratypes). Some more observations were made on partly dissected material (mounted as permanent slides) (including copepodites CII–CV) and whole specimens, stored in plastic vials in 70% alcohol with a drop of glycerol. Drawings

were made using a camera lucida at 1000 or 400-times magnification. The final version of the drawings was made using CorelDRAW®9 graphic programme.

Location: siphon lake; Dang Water Cave (GPS: 48Q 0570603; UTM: 2243835); Cuc Phuong National Park; 100 km SSW from Ha Noi; Vietnam.

Date of collection: 20 June 2003.

Material examined: 25 juveniles; 21 females (two with attached spermatophore) and 16 males.

Repository: the holotype: (adult female, completely dissected) under the catalogue number I.G.30.112 COP 4651 and 5 paratypes (one completely dissected male) under the catalogue number I.G.30.112 COP 4652 and 4 intact specimens (two males and two females) mounted as permanent slides (each sex on separate slide) in glycerol under the catalogue number I.G.30.112 COP 4653 and sealed with nail polisher as well as 2 females and 2 males stored in a plastic vial in 70% alcohol with a drop of glycerol under the catalogue number I.G.30.112 COP 4655 (all designed as paratypes) are deposited in Royal Museum in Brussels (Belgium). The rest of material (permanent mounts as well as material in alcohol) is deposited in the author's collection at the National Institute of Biology (Ljubljana).

Type locality: Dang Water Cave (GPS: 48Q 0570603; UTM: 2243835); Cuc Phuong National Park; 100 km SSW from Ha Noi; Vietnam.

To compare onthogenetic development, i.e. formation of arthrodial membrane and setae development, specimens of *Troglodiaptomus sketi* Petkovski, 1985 and *Eudiaptomus transylvanicus* (Daday, 1890) of different copepodite stages from the author's collection were analysed, too.

Results

Genus Hadodiaptomus *n. gen.*

Female
Small-sized (less than 1 mm); shape of body typical for Diaptomidae. P1–P4 with 2 segments in exopodites and endopodites; with no inner seta on exopodite 1 of P1–P4. Pediger 4 wider than pediger 5. Fifth pediger slightly asymmetrical, with a sensillum on each side. Urosome symmetrical, with

three segments. Antennula 25-segmented; number and position of setae characteristic for Speodiaptominae. Number of apical and inner setae on exopodite 2 of P1–P4 reduced as well as on terminal segment of endopodites P1–P4. Exopodite 3 of P5 fused with exopodite 2. Endopodite reduced. Inner attenuation of the middle exopodite segment ('claw') long and robust.

Male

Small-sized (less than 1 mm); shape of body typical for Diaptomidae. P1–P4 with 2 segments in exopodites and endopodites; with no inner seta on exopodite 1 of P1–P4. Pediger 4 wider than pediger 5. Fifth pediger slightly asymmetrical, with a sensillum on each side. Urosome 5-segmented, urosomite 1 asymmetrical. Right antennula 22 segmented. Segments 14–16 only slightly widened; number and position of setae characteristic for Speodiaptominae. Number of apical and inner setae on exopodite 2 of P1–P4 reduced as well as on terminal segment of endopodites of P1–P4. Endopodite of right P5 small or reduced. Terminal claw well developed. Endopodite on left P5 1-segmented.

Copepodites CII–CV

CI: not found;

CII: 2-segmented exopodite and endopodite on P1;

CIII: 2-segmented exopodite and endopodite on P1–P3;

CIV: 2-segmented exopodite and endopodite on P1–P4;

CV: 2-segmented exopodite and endopodite on P1–P4.

Hadodiaptomus dumonti *n.gen., n.sp.*

Description

Female: Length (*without furcal setae*): 784–910 μm (average: 849 ± 35 μm; *n* = 14) (Fig. 1). Colourless and eyeless; transparent and soft.

Shape of body: length of cephalothorax 590 ± 25 μm and typical for Diaptomidae. Rostral filaments absent, rostrum presented by two finger-like projections with rounded tips (Fig. 2).

Fourth and fifth pedigers partly fused, fourth one slightly wider than fifth one. Fifth pediger slightly asymmetrical with small postero-lateral sensillum on each side (Figs 1 and 3b). Urosome

with three segments. Proximal one (genital segment) slightly oval and about two times as long as wide, with two sensilae laterally (Figs 1, 3a and b). In lateral view, anterior part of genital segment well separated from distal one by deep groove (Fig. 3c). Second and third urosomal segments similar in length but anal somite wider. Furcal ramus about twice as long as wide (average = 1.8:1). Inner and outer margins smooth, with five finely plumose furcal setae. Length of setae increasing from outer one to inner one; outer one about 70% of length of innermost one. Dorsal seta inserted on inner distal corner of furcal ramus, about as long as longest terminal seta, smooth (Fig. 3b). Operculum well shaped, semicircular.

Antennula 25-segmented (Fig. 4), reaching posterior margins of furcal rami. Seta on segment 1 short, not reaching distal margin of segment 2. Largest setae on segments 7, 9, 14, 18, 21, 23 (the longest of all), 24 and 25. Armature per segment as follows (Roman numbers – segment; Arabic numbers – number of setae; a = aesthetasc; sp = spine):

I(1 + **a**), II (3 + a), III(1 + **a**), IV(1), V(1 + a), VI(1), VII(1 + **A**), VIII(1 + sp), IX(2 + **a**), X(1), XI(1), XII(1 + sp + a), XIII(1), XIV(1 + a), XV(1), XVI(1 + **A**), XVII(1), XVIII(1), XIX(1 + **a**), XX(1), XXI(1), XXII(2), XXIII(2), XXIV(2), XXV (5 + a)

Letters in bold and underline indicates extra easthetascs not present in other Speodiaptominae; capitals, bold, italic and underline not present in *Stygodiaptomus* (Diaptominae).

Antenna (Fig. 5) with exopodite as long as endopodite, coxa with one seta, basis with two setae, sub-equal in length. Endopodite with two segments; distal one with two lobes. Outer one with six long setae and one inserted sub-terminally; inner one with one short, one medium-sized and 5 long setae. Exopodite 7-segmented; first segment with one, second with three and 3–7 with one lateral seta. The 7th with three additional apical setae.

Mandible (Fig. 6a and b) with 6–7 small teeth on gnathobasis, one stronger and outermost ventral tooth strong and robust. Basis with 4 subequal setae, one of them plumose. Endopodite with 2 segments. Proximal one with protuberance
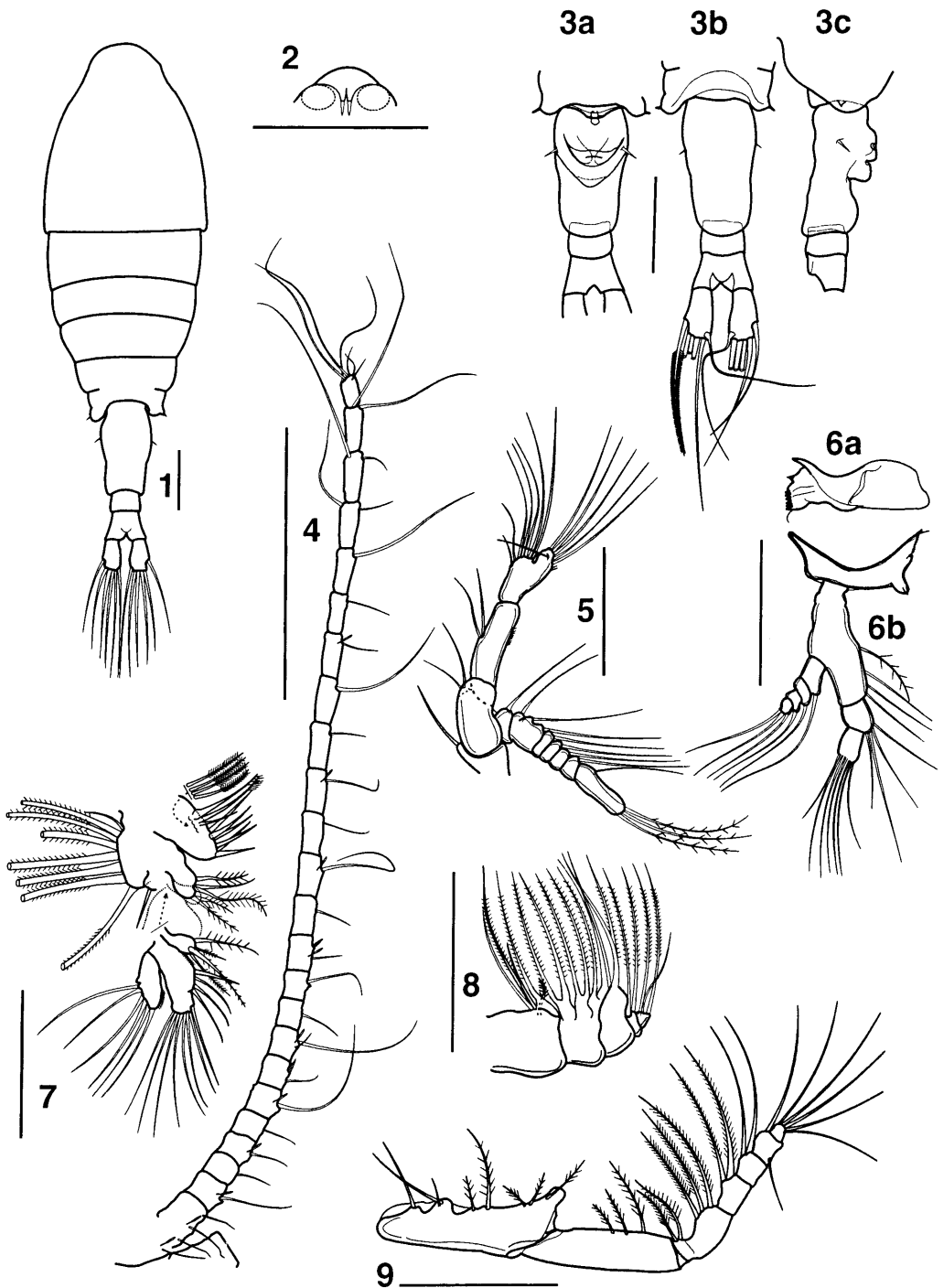
Figures 1–9. Hadodiaptomus dumonti n. gen., n. sp., (female). 1: Habitus; 2: Rostrum; 3a: Urosome, ventrally; 3b: Urosome, dorsally; 3c: Urosome, laterally (right side); 4: Antennula; 5: Antenna; 6a: Mandible, detail of gnathobasis; 6b: Mandible; 7: Maxillula; 8: Maxilla; 9: Maxilliped; Scale bar: 0.1 mm.
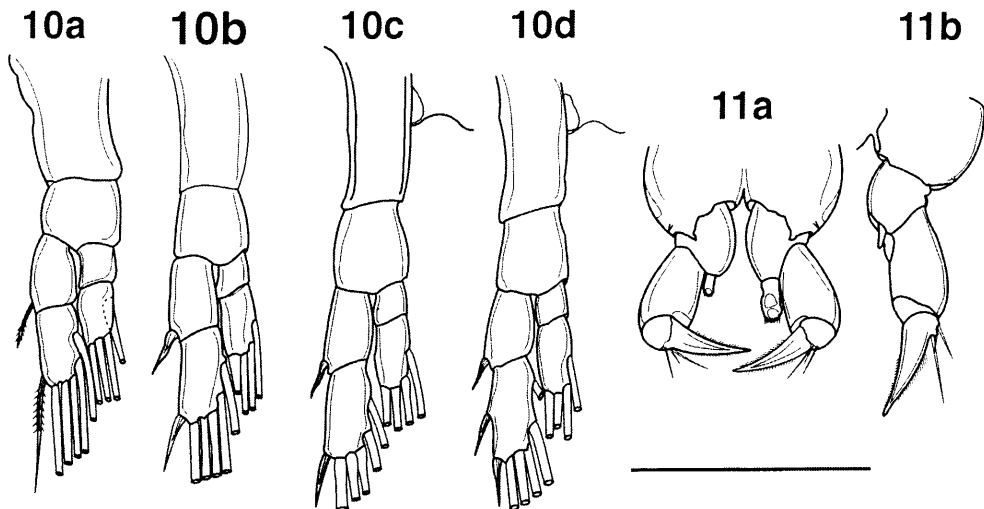
Figures 10–11. Hadodiaptomus dumonti n. gen., n. sp., (female). 10a–d: P1–P4; 11a–b: P5. Scale bar: 0.1 mm.

on inner side with 4 setae – 3 sub-equal in length and one long. Terminal segment as long as wide with seven terminal setae. Exopodite 4-segmented with 1, 1, 1 and 3 setae.

Maxillula (Fig. 7) with nine apical spiniform setae (proximalmost two with few small setulae on one side at top) and posteriorly with four plumosae setae on praecoxal arthrite. Coxal epipodite with seven long setae with short spines and proximalmost short and bare. Two and three setae on coxal and first basal endites, respectively. Endopodite one segmented with one group of four setae laterally and eight setae apically. Exopodite with six long setae.

Maxilla (Fig. 8) with two praecoxal (3 and 2 setae) and three coxal lobes each with two setae and well developed basal lobe with three long setae. Endopodite 2-segmented with two and three setae respectively.

Maxilliped (Fig. 9) well developed. Syncoxa (fused praecoxa and coxa) with four lobes (according Ferrari & Ivanenko, 2001) with 1, 2, 2 and 3 unequal setae each. Fourth lobe (corresponds to lobe on coxa) with well developed protuberance projecting over next segment. Basis elongated, with three plumose setae increasing in length, inserted on distal half of inner margin. Endopodite 6-segmented, with first and second segment partly fused. First segment with two and second with three sub-equal plumosae setae. Third to fifth segment with two setae each, terminal one with five sub-equal setae.

Legs 1–4 (P1–P4) with 2-segmented exopodite and 2-segmented endopodite (Table 1), coxa and basis without setae (Fig. 10a–d). Armament formula of swimming legs as (S = spine):

	Coxa	Basis	Exopodite	Endopodite
P1	0-0	0-0	S-0; S-3-2	0-0; 3-1
P2	0-0	0-0	S-0; S-3-2	0-0; 3-1
P3	0-0	0-0	S-0; S-3-2	0-0; 3-1
P4	0-0	0-0	S-0; SS-3-2	0-0; 3-1

No Schmeil's organ on P2 endopodite.

Leg 5 (P5) (Fig. 11a and b). Coxa simplified and rounded, with small spine on distal outer corner. Basis almost triangular, with inner margin rounded. Left and right endopodite unequally reduced (Fig. 4a and b). Maximum length of left endopodite up to 1/3 of length of exopodite, on the opposite side reduced to the shape of a hemisphere or even simple hyaline spine. Each endopodite with few short spines at rounded tip but the most reduced one. First segment of exopodite about 1.8 times as long as wide, with smooth margins. Inner attenuation of the middle exopodite segment ('claw') as long as first exopodite segment; its margins armed with a row of small hairs on both sides. Outer proximal corner armed with two long and one short seta.

Male: Length (*without furcal setae*): 701–856 μm (average: 775 ± 41 μm; *n* = 12) (Fig. 12). Colourless and eyeless; transparent and soft.
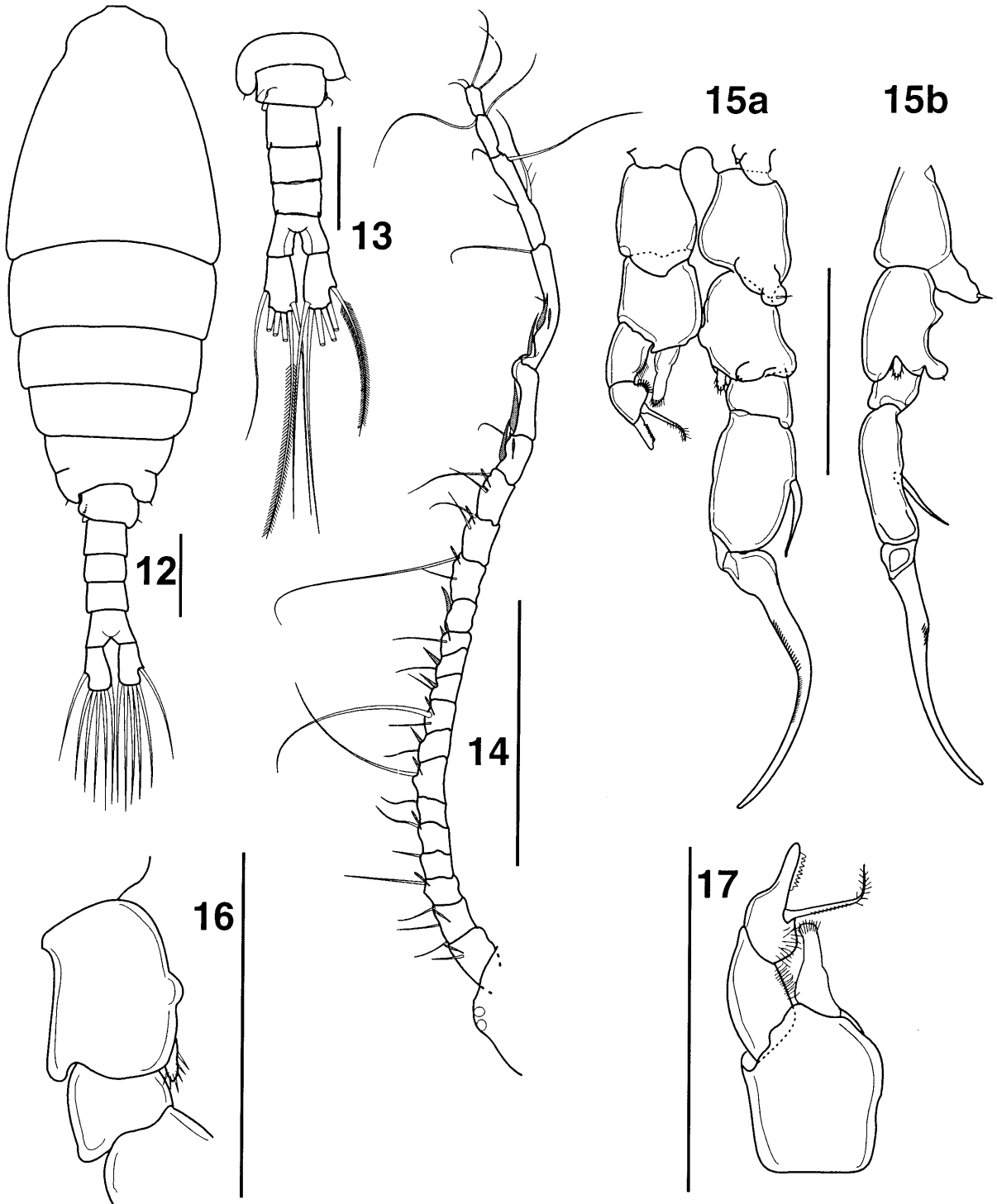
Figures 12–17. Hadodiaptomus dumonti n. gen., n. sp., (male). 12: Habitus; 13: Urosome, dorsally; 14: Antennula; 15a: P5; 15b: P5, right leg (lateral view; anterior part on the left); 16: P5, left leg (detail of basis and endopodite); 17: P5, right leg (detail of basis, exopodite and endopodite). Scale bar: 0.1 mm

Table 1. Comparison of stygobitic diaptomid species: *Argyrodiaptomus cavernicolax* Shen & Tai, 1965; *Spelaeodiaptomus rouchi* Dussart, 1970; *Stygodiaptomus kiefferi* Petkovski, 1981; *Speodiaptomus birsteini* Borutzky, 1962; *Hadodiaptomus dumonti* n. gen, n. sp.; *Microdiaptomus cokeri* Osorio-Tafall, 1942 and *Troglodiaptomus sketi* Petkovski, 1978

Character	Plesiomorphic characters	A. cavernicolax	S. rouchi	S. kiefferi	S. birsteini*	H. dumonti*	M. cokeri*	T. sketi*
Segments on P1–P4 (exp/end)	2/3; 3/3; 3/3; 3/3	(2/3; 3/3; 3/3; 3/3)	2/3; 3/3; 3/3; 3/3	2/2-3; 3/3; 3/3; 3/3	2/1; 2/2; 2/2; 3/2	2/2; 2/2; 2/2; 2/2	2/1; 2/1; 2/1; 2/1	2/1; 2/1; 2/1; 2/1
Inner setae on exopodite 1: P1–P4	1; 1; 1; 1	(1; 1; 1; 1)	1; 1; 1; 1	1; 1; 1; 1	0; 0; 0; 0	0; 0; 0; 0	1; 1; 1; 1	0; 0; 0; 0
Outer spines on exopodite 1: P1–P4	1; 1; 1; 1	(1; 1; 1; 1)	1; 1; 1; 1	0-1; 1; 1; 1	1; 1; 1; 1	1; 1; 1; 1	1; 0; 0; 0	1; 0; 0; 0
Setae on exopodite 2: P1–P4 (inner/apical)	1/0; 1/0; 1/0; 1/0	(1/0; 1/0; 1/0; 1/0)	1/0; 1/0; 1/0; 1/0	2/3; 1/0; 1/0; 1/0	2/3; 2/3; 2/3; 1/0	2/3; 2/3; 2/3; 2/3	1/3; 2/2; 2/2; 2/2	2/3; 2/3; 2/3; 2/3
Outer spines on exopodite 2: P2–P4	1; 1; 1	(0; 1; 1)	0; 1; 1	0; 1; 1	1; 1; 1	1; 1; 2/1	1; 1; 1	1; 1; 1
Setae on terminal segment of endopodite P1–P4 (inner/apical)	3/3; 2/3; 2/3; 2/3	(3/3; 2/3; 2/3; 2/3)	3/3; 2/3; 2/3; 1/3	2/2; 2/2; 2/2; 2/2	1/3; 2/3; 2/3; 2/3	1/3; 1/3; 1/3; 1/3	1/3; 2/2; 2/2; 2/2	0/3; 0/3; 0/3; 0/3
No of segments on left endopodite P5 in male	2	1	1	2	1	1	1	2
No of segments on endopodite P5 in female	2	1	2	2	2	1	1	1
Reduced right endopodite P5 in male	No	Yes	No	Yes	No	Yes	No	Yes (absent)
Reduced endopodite P5 in female	No	Yes	No	No	No	Yes	No	Yes
Sensilae on pediger 5 and genital segment (in female)	Yes	Yes	Yes	Yes	No	Yes	No	No
Sensilae on pediger 5 (in male)	Yes	Yes	No	No	No	Yes	No	No
Setae on basipodite P5 in male	Yes	Yes	No	No	No	No	No	No
Dilatation of segments 13–18 on right A1 of male	Yes	Yes	No	No	No	No	No	No
Schmeil's organ on P2 end 2 present	Yes	?	No	Yes	No	No	No	No

Asterisk indicates members of Speodiaptominae.
(Data are collected from the literature and personal observation; data in bracket – as general characteristics of fam. Diaptomidae).

Shape of body: length of cephalothorax 545 ± 26 μm, typical for Diaptomidae. Rostral filaments absent, rostrum presented by two finger-like chitinised projections. Pediger 4 and 5 partly fused; pediger 4 wider than pediger 5. Pediger 5 slightly asymmetrical, with right posteriolateral edge rounded and left side angular; with sensory seta on each side (Figs 12 and 13).

Urosome 5-segmented; segments equal in length (Fig. 13). Urosomite 1 asymmetrical. Right side with well-formed opening for spermatophore, left side with small and short sensila. Furcal ramus almost two times as long as wide (average = 1.8:1) with outer terminal seta about 70% of length of inner one. Length of terminal setae increase from outermost to innermost. Dorsal seta as long as innermost terminal one, inserted at inner distal corner of ramus, smooth. Furcal setae finely plumose, almost as long as urosome. Inner and outer margin of furcal ramus without hair-setae.

Antennules reaching distal part of furcal rami. Right antennula 22-segmented (Fig. 14) with long seta on 7th, 9th, 14th and 20th segment. Segments 8 and 10–13 each with short spine. Segments 14–16 only slightly widened. Spine on segment 13 small. Aesthetascs on same segments as in female, with additional two on segments 15 and 17. Aesthetasc on segment 14 larger than others.

Mouth parts and swimming legs P1–P4 as in female. In some males only one spine on distal segment of P4 exopodite.

P5 biramous. Right P5 (Fig. 15a and b): Coxa with big outgrowth on postero-lateral margin, tipped with short sensor. Basis as long as coxa, with small lobe at middle of inner margin. On distal margin of basis a small outgrowth with a small knob on the tip in the middle of its posterior side. Endopodite reduced, with few spine-like hair-setae on its tip (Fig. 16). Exopodite 1 short and trapezoidal. Exopodite 2 about 3-times as long as exopodite 1, oval in shape. Lateral spine curved and inserted at about middle of outer margin and not extending over distal margin of exopodite 2. Terminal claw relatively robust, with blunt tip and curved. Inner margin adorned with two series of shallow transversal grooves. Claw about two times longer than exopodite 2.

Left P5: Coxa as long as wide with slightly convex lateral margins. Basis as long as wide, sub-rectangular. Endopodite 1-segmented, with rim of short hair-setae at tip. Proximal part wide but second part narrow. Slightly longer than exopodite 1. Exopodite 1 about 1.5 times as long as wide with row of hairs on inner margin (Fig. 17). Exopodite 2 triangular with outer margin convex and inner tip rounded and covered with short hair-setae. Distal part of exopodite 2 with digitiform process, as long as exopodite 2, with small serrated membrane on inner side. Lateral spine slim and long with bent tip, overreaching tip of digitiform process. Some short hair-setae on its tip and several small spines along proximal part.

Spermatophore sausage-like, with slightly conical distal tip. Not different from epigean taxa.

Transformation of swimming legs P1–P4 from CII to CVI (adult female)

Number of segment and armature of the first three pairs of swimming legs (P1–P3) becomes constant from stage C III onward (Table 2) and it is identical to the armature in adult female. P4 get the final number of segments in stage CIV but the final number of setae and spines is added during the last moult (i.e. transformation from CV to CVI stage) when two (sometimes one) additional spines are added on proximal and distal segment of P4.

Differential diagnosis

According to the differential diagnosis between Diaptominae and Speodiaptominae (see Petkovski, 1983; Dussart & Defaye, 1995) the new monospecific taxon, *Hadodiaptomus dumonti* n. gen., n. sp., belongs to the subfamily Speodiaptominae (Borutzky, 1962). Differences between both subfamilies are based primarily on the reduced number of segments in P1–P4 in Speodiaptominae (see Borutzky, 1962) where number of segments on P2–P3 exopodites is 2 and P2–P4 endopodites is 2 or 1. The common feature of stygobitic Calanoida is reduction and simplification of different morphological characters as a reflection of specific environment, poor in food (i.e. energy). Lack of food reflects in reduction of organs/organic systems (Culver, 2004) which are not necessary for survival in subterranean environment and at the same time require a lot of energy. Simplifications and reduc-

Table 2. Structure of legs P1–P5 in copepodites CII–CVI in *Hadodiaptomus dumonti* n.gen., n.sp. (female)

Copepodite stage	P1	P2	P3	P4	P5
C I	Not known	Not known	Not known	Not known	Not known
C II					
A	2/2	2/1	1/1	Absent	Absent
B	1,6/0,4	1,6/4	6/4		
C		/+	+/+		
C III					
A	2/2	2/2	2/2	1/1	Absent
B	1,6/0,4	1,6/0,4	1,6/0,4	5/4	
C				+/+	
C IV					
A	2/2	2/2	2/2	2/2	1/1
B	1,6/0,4	1,6/0,4	1,6/0,4	0,6/0,4	3/0
C					+/
C V					
A	2/2	2/2	2/2	2/2	2/1(reduced)
B	1,6/0,4	1,6/0,4	1,6/0,4	0,6/0,4	3/0
C					
C VI (adult female)					
A	2/2	2/2	2/2	2/2	2/1(reduced)
B	1,6/0,4	1,6/0,4	1,6/0,4	1,6–7/0,4	3/0
C					

A = number of segments on exopodite and endopodite; B = number of spines and setae on exopodite/endopodite on proximal and distal segment; C = formation of arthrodial membrane in the next step of development on exopodite/endopodite.

tions as a result of adaptive (and also convergent) evolution (Sket, 1985), make differentiation between taxa difficult. Thus even the smallest morphological difference must be reconsidered in the analyses as reduced segmentation has evolved independently several times. Whenever it is possible, developmental stages in copepodites, especially mouth parts and proximal segments of swimming legs and their setae development (see Ferrari & Bonforado, 1998), should be included into differential diagnosis, too.

There are several taxonomic characters distinguishing genus *Hadodiaptomus* from other genera of Speodiaptominae. Morphological differences are presented in the Table 1, together with characters specific for members of subfamily Diaptominae. Plesiomorphic characters of ancestor of Speodiaptominae are added and which should be shared with ancestors of both subfamilies. In the article genera *Microdiaptomus* and *Troglodiapto-*

mus are considered as members of Speodiaptominae – following definition of Petkovski (1983) and Dussart & Defaye (1995).

From other three monospecific genera of the subfamily Speodiaptominae (*Speodiaptomus, Microdiaptomus, Troglodiaptomus*) the new genus differs in:

(a) *Specific number of segments in P1–P4 exo- and endopodites.* In *Hadodiaptomus* there are two segmented exopodites and endopodites on P1–P4. In closely related *Speodiaptomus* there is one segment on P1 endopodite and three segments on P4 exopodite. In *Microdiaptomus* and *Troglodiaptomus* number of segments is uniform on P1–P4 – two exopodites and one endopodite.

(b) *Armature of antennula.* In *Hadodiaptomus* there are more aesthetascs on antennula than in other members of Speodiaptominae. In com-

Table 3. Structure of legs P1–P5 in copepodites CII–CVI in *Troglodiaptomus sketi* Petkovski, 1978 (female)

Copepodite stage	P1	P2	P3	P4	P5
C I	Not known	Not known	Not known	Not known	Not known
C II					
A	2/1	1/1	1/1	Absent	Absent
B	1,6/3	6/3	5/3		
C		+/	+/		
C III					
A	2/1	2/1	2/1	1/1	Absent
B	1,6/3	0,6/3	0,6/3	5/3	
C				+/	
C IV					
A	2/1	2/1	2/1	2/1	1/1(reduced)
B	1,6/3	0,6/3	0,6/3	0,6/3	1/0
C					+/
C V					
A	2/1	2/1	2/1	2/1	2/1(reduced)
B	1,6/3	0,6/3	0,6/3	0,6/3	0,1/0
C					
C VI (adult female)					
A	2/1	2/1	2/1	2/1	2/1(reduced)
B	1,6/3	0,6/3	0,6/3	0,6/3	0,1/0
C					

A = number of segments on exopodite and endopodite; B = number of spines and setae on exopodite/endopodite on proximal and distal segment; C = formation of arthrodial membrane in the next step of development on exopodite/endopodite.

parison with *Microdiaptomus* and *Troglodiaptomus* additional aesthetascs are on 1st, 3rd, 7th, (9th – in *Microdiaptomus*), 16th and 19th segment. Male of *Hadodiaptomus* has an additional aesthetasc on 15th and 17th segment. This makes genus *Hadodiaptomus* distinct from other members of Speodiaptomine which express reduced number of aesthetascs on antennula in comparison with *Hadodiaptomus*.

(c) *Reduced numbers of setae on maxilla.* In *Hadodiaptomus* three coxal lobes bears each only two setae, while in other genera there are three, usually one of them smaller than other two.

(d) *Number of setae on lobes of syncoxa of maxilliped.* In *Hadodiaptomus* four lobes, corresponding praecoxa and coxa combined, bear 1, 2, 2 and 3 setae. In *Troglodiaptomus* number of setae is 1, 1, 3, 3 and in *Microdiaptomus* 1, 2, 3, 4. In *Speodiaptomus* number of setae on syncoxa is not known.

(e) *Sensilae on pediger 5 in both sexes.* In *Hadodiaptomus* they are present in both sexes while in other stygobitic genera (of both subfamilies) are absent (incl. *Troglodiaptomus*; Brancelj, personal observation).

(f) *Sensilae on genital segment in female.* They are present in female of *Hadodiaptomus* but absent in *Troglodiaptomus* (Brancelj, personal observation). In other genera of Stygodiaptominae are also absent (actually not reported in species description).

(g) *Formation of arthrodial membrane and setation* (see Ferrari & Benforado, 1998) is unique so far for at least two stygobitic genera of subfamily Speodiaptominae (see Tables 2 and 3). In *Hadodiaptomus* specific number of segments on P1–P3 (2-segmented exopodite and endopodite) and their armature (setatiton) on P1–P3 (1,6/04) is established as early as in stage CIII. The same early final specific seg-

mentation (2/1 segments on P1–P3 exopodite/endopodite) and armature (1,6/3; 0,6/3; 0,6/3 on P1–P3) in stage CIII was observed also in *Troglodiaptomus sketi* (comp. Tables 2 and 3).

Based on listed differences, the new taxon is a member of a new, mono-specific genus *Hadodiaptomus*. According to the present state of the knowledge, considering morphology of the adults, the closest genus to *Hadodiaptomus* is *Speodiaptomus* from the Crimean peninsula (the Black sea area).

Discussion

Taxonomic relations between epigean and stygobitic taxa of Calanoida

It is rather unusual that freshwater epigean eu-planktonic group occupies subterranean environment. Most (actually all) other stygobitic taxa are related to benthic way of life (see Botosaneanu, 1986). From this point of view freshwater Calanoida are an exception. Ancestors of inland stygobitic Calanoida are beyond any doubt freshwater epigean planktonic Calanoida. Structure of antennules (number of segments) and armature of segments (setae, aestheratscs, spines) are similar in epigean and stygobitic taxa (for details see this article; Damian-Georgescu, 1966; Petkovski, 1983) but in principle in stygobitic taxa number of setae is reduced. In Diaptominae (representatives of epigean freshwater Calanoida) there is quite often duplication of setae on segments 11–19 (Dussart & Defaye, 1995), while in marine Calanoida (i.e. genus *Calanus*) higher number of seta is a rule (see Huys & Boxshall, 1991, p. 60).

Not only setae on antennules, some other structures express a tendency of reduction (eyes, segments on P1–P5, setae on mouth parts, etc.), too. The most obvious link, which indicates derivation of stygobitic Calanoida from epigean freshwater ancestors is development and setation of P1 exopodite. P1 has in stage copepodite III (CIII) in epigean *Eudiaptomus transylvanicus* and stygobitic *Troglodiaptomus sketi* and *Hadodiaptomus dumonti* identical number of setae/spines on two-segmented exopodite (proximal segment one

spine; distal segment six spines/setae). In stygobitic taxa this armature remains to the adulthood (CVI) (in *M. cokeri* there is only five spines/setae on distal segment of P1–P4), while in epigean taxa (i.e. *Eudiaptomus transylvanicus*) an additional segment with two more setae is added in stage CV. The final formula of setae on P1 exopodite in adult epigean freshwater member of Diaptominae is 2, 1, 6. Reduced segmentation and armature of legs P1–P4 in subterranean freshwater Calanoida is modification which appears to be neotenic (Bowman, 1986).

Armature of other legs (P2–P4 exopodite) in epigean and stygobitic taxa differs significantly at least from stage CIII onward. This indicates that differences between subfamilies as well as between genera within Speodiaptominae are significant not only in the adults but also in stages of copepodites. Differences in legs' armature start early in ontogenetic development.

Much less differences between epigean and stygobitic taxa could be observed in armature of maxillipeds, precisely on syncoxa. In three members of Speodiaptominae armature of syncoxa differs. When we compare some epigean taxa (members of subfamily Diaptominae) with stygobionts from subfamily Speodiaptominae we can see no differences. For example, *Hemidiaptomus hungaricus* (in Damian-Georgescu, 1966) and *Hemidiaptomus gurneyi* (in Stella, 1984) have identical armature of syncoxa as *Microdiptomus* (1, 2, 3, 4) and *Arctodiaptomus salinus* (in Dussart & Defaye, 1995) have identical armature of syncoxa as *Troglodiaptomus* (1, 1, 3, 3). This agree with Ferrari & Ivanenko (2001) who found 'that the distal arthrodial membrane of a segment (in Cyclopoida) has been more labile during the evolutionary history of the maxilliped than have the seta which inserts on a protopodal segment'... or, in other words, number of setae (in Cyclopoida) differs randomly between genera and subfamilies and the same can be valid for Calanoida, too.

Taxonomic relations among stygobitic Calanoida

It is unlikely that the four currently known, monospecific stygobitic genera (*Troglodiaptomus*, *Microdiptomus*, *Speodiaptomus* and *Hadodiaptomus*), having distinct morphologies, being geographically disjunct (by at least 1500 km) and relatively well isolated, would share a common

epigean ancestor, because that would entail that troglobitic calanoids would only have invaded the subterranean space once.

Petkovski (1978) mentioned that *Troglodiaptomus sketi* could be a member of a new subfamily (Troglodiaptomidae?) but he left it in subfamily Speodiaptominae. Later on Elías-Gutiérez & Suárez-Morales (1998) established a new subfamily which includes *T. sketi* and *M. cookeri*. Authors at the same time cast doubt on a common ancestor for European *Troglodiaptomus* and American *Microdiaptomus*. Nevertheless, the authors finally put both genera in the same subfamily, different from Speodiaptominae. A very detail analysis of differences between both taxa show that there are not only differences on armature of P1–P4 in both taxa but there are differences also in number of setae on syncoxal lobes. At this point of knowledge on freshwater stygobitic Calanoida we can not support their idea on separate subfamily. Members of Speodiaptominae exhibit different degrees of adaptation (i.e. reductions) to the subterranean environment which results in very diverse armature of appendages in different taxa. The only character in common of four freshwater stygobitic taxa is a reduction of segments on P1–P4 and their reduced armature. With the new taxon, *Hadodiaptomus*, the idea of several independent invasions and/or ancestors of stygobitic Calanoida is put on firmer footing. But at the same time all four taxa share enough common characters to be placed in a common subfamily – Speodiaptominae.

Reductions of organs in subterranean environment tend to produce similar morphologies, and such homoplastic events blur the phylogenetic relations between epigean and subterranean taxa as well as relations among stygobionts themselves. On the other hand onthogenetic development supports a hypothesis on several independent ancestors. From this point, morphology of adults, supported by development of copepodites, gets in favour an idea on four different (monospecific) genera within subfamily Speodiptominae.

When Elías-Gutiérez & Suárez-Morales (1998) propose to establish the new subfamily Microdiaptomidae, they made extensive comments on the nature of subfamilies within Diaptomidae. They followed Borutzky (1962) and Petkovski (1978) in subscribing to the point of view that a

phenomenon of reduction of the swimming legs and of some other characters (no inner setae on the first exopodite of P1 and no dilated segments 13–18 on male right antennule) are adaptations to a stygobitic way of life, and that these reductions represent good characters for distinction between different subfamilies, too.

The new analyses suggest that evolution in subterranean environment is, in general, relatively predictable (reductions) but very specific on local level (the pattern and intensity of reductions). At this point we should accept that 'if a stygobitic way of life evolves through adaptive reduction in the external morphology of different taxa' (Sket, 1985), with simplification of certain body parts, including segmentation of legs and their armature, tracing relationships from morphology only may become tricky. A stygobitic way of life thereby results in '...the highly repeated nature of the morphological changes in many taxonomical groups in many areas...' (Culver et al., 1995). Another characteristic of cave organisms, apart from 'a bizarre morphology, including reduced eyes or an absence of eyes' is 'a highly restricted geographical range with no closely related surface-dwelling relatives' (Culver et al., *loc. cit.*).

Distribution and sampling techniques

Freshwater stygobitic Calanoida are a relatively rare ecological group, limited to few locations scattered widely over the world: South Europe (France, Balkan and Crimean Peninsula), Central America (Mexico) and South Asia (Vietnam, South China). So far, their distribution within certain areas suggests a local occurrence. Most taxa are known from one or few localities; only *T. sketi* is known from many caves across the Balkan, Slovenia and NE Italy. We presume that sampling techniques, using too coarse nets (>200 μm) are the main reason for our limited knowledge on the distribution of stygobitic Calanoida. Most of them are relatively small (<1 mm) and they can easily escape the plankton or hand nets with mesh size 200 μm normally used by speleobiologists. In siphon lakes or rivers in the saturated zone such mesh size is quite commonly used by speleobiologists. When the author started to use nets with mesh size of 100 μm in siphons or pools in Slovenia, it appeared that *T. sketi* (body size be-

tween 0.8 and 0.9 mm) was one of the most common stygobionts in Slovenia (Brancelj, 2001). Systematic use of hand nets or plankton nets with mesh size of 100 μm is thus recommended in search for stygobitic Calanoida all over the globe.

Ecology

The new species is beyond any doubt a stygobiont. Specimens were collected in a small siphon lake, about 3 m in diameter, several tens of metres from the entrance. During sampling, there was no running water in the cave. Hydrographic connections with other water bodies are not known but access to phreatic water is likely (Sket, personal communication).

Specimens are small-bodied, blind and transparent – all well-known characteristics of stygobionts. An additional adaptation to the subterranean environment is the reduction of the integument, which makes animals soft and delicate to handle. They have also reduced number of segments or reduction of size of some segments on P1–P5, an additional character for stygobionts (for more details see Table 1). On the other hand some body parts, especially maxillipeds, and some setae (on mouth parts) are much longer than in epigean taxa, an additional indication for a stygobitic way of life. Actually, the maxillipeds are the longest appendage, about 1.5 times longer that swimming legs.

The digestive tract was filled with a mixture of clay and small mineral particles, a common feeding habit in stygobionst, especially bottom-dwellers. This type of feeding was noticed also in benthic stygobitic Cladocera (*Alona hercegovinae* and *A. sketi*) (Brancelj, 1992). The feeding habit of the new calanoid species is thus somewhat different from its pelagic relatives. Clay and mineral particles, along with organic material, including bacteria and fungi, probably originate from the bottom and from the walls of the siphon and are re-suspended by the long maxillipeds.

Etymology

Had, hades is Ancient Greece for underworld; the specific name 'dumonti' is dedicated to Prof Henri J. Dumont from Ghent with whom I made several expeditions to remote places in Asia and who supported me during my taxonomic work on Copepoda and Cladocera.

References

Borutzky, E. V., 1962. First discovery of a troglobiontic calanoid (Crustacea, Copepoda) in underground waters. Dokladie Akademii nauki SSSR 147: 1499–1502 (in Russian with English summary).

Botosaneanu, L. (ed.), 1986. Stygofauna Mundi. Brill, Leiden.

Bowman, T. E., 1986. Copepoda: Calanoida. In Botosaneanu, L. (ed.), Stygofauna Mundi. Brill, Leiden: 295–298.

Brancelj, A., 1991. Stygobitic Calanoida (Crustacea: Copepoda) from Yugoslavia with the description of a new species – Stygodiaptomus petkovskii from Bosnia and Hercegovina. Stygologia 6: 165–176.

Brancelj, A., 1992. Alona sketi sp.n. (Cladocera: Chydoridae), the second cave-inhabiting Cladoceran from former Yugoslavia. Hydrobiologia 248: 105–114.

Brancelj, A., 2001. Male of Moraria radovnae Brancelj, 1988 (Copepoda: Crustacea), and notes on endemic and rare copepod species from Slovenia and neighbouring countries. Hydrobiologia 453/454: 513–524.

Camacho, A. I., 2003. Historical biogeography of Hexabathynella, a cosmopolitan genus of groundwater Syncarida (Crustacea, Bathynellaceae, Parabathynellidae). Biological Journal of the Linnean Society 78: 457–466.

Culver, D. C., 2004. Adaptation: genetics. In Gunn, J. (ed.), Encyclopedia of Caves and Karst Science. Fitzroy Dearborn, New York.

Culver, D., T. C. Kane & D. W. Fong, 1995. Adaptation and Natural Selection in Caves. The Evolution of Gammarus Minutus. Harvard University Press, Cambridge.

Damian-Georgescu, A., 1966. Crustacea: Copepoda: Calanoida (forme de apă dulce). Fauna republici socialiste Romănia, Vol. IV, fasc. 8. Academia republici socialiste Romănia, Bucureşti.

Dussart, B. H. & D. Defaye, 1995. Introduction to the Copepoda. In Dumont, H. J. F. (ed.), Guides to the Identification of the Microinvertebrates of the Continental Waters of the World. 7. SPB Academic Publishing, Amsterdam.

Elías-Gutiérez, M. & E. Suárez-Morales, 1998. Redescription of Microdiaptomus cokeri (Crustacea: Copepoda: Diaptomidae) from caves in central Mexico, with the description of a new diaptomid subfamily. Proceedings of the Biological Society of Washington 111: 199–208.

Ferrari, F. D. & A. Benforado, 1998. Relationships between arthrodial membrane formation and addition of setae to swimming legs 1–4 during development of Diothona oculata, Rodgewayia klausruetzleri, Pleuromamma xiphias, and Temora longicornis (Copepoda). Crustaceana 71: 545–564.

Ferrari, F. D. & V. N. Ivanenko, 2001. Interpreting segment homologies of the maxilliped of cyclopoid copepods by compring stage-specific changes during development. Organisms, Diversity & Evolution 1: 113–131.

Huys, R. & G. A. Boxshall, 1991. Copepod Evolution. The Ray Society, London.

Karanovic, T., 1999. A new stygobitic Calanoida (Crustacea: Copepoda) of the genus Stygodiaptomus Petkovski, 1981 from the Balkan Peninsula. Proceedings of the Biological Society of Washington 112: 682–686.

Petkovski, T. K., 1978. Troglodiaptomus sketi n. gen., n. sp., Ein neuer Höhlen-Calanoide vom Karstgelände Istriens (Crustacea, Copepoda). Acta Musei Macedonici Scientiarum naturalium 15: 151–165.

Petkovski, T. K., 1981. Stygodiaptomus kieferi n. gen. et n. sp., Zweiter Höhlen-Calanoide vom Dinarischen Karstgebiet (Crustacea, Copepoda). Fragmenta Balcanica 11: 63–74.

Petkovski, T. K., 1983. Faune de Macedoine. Calanoida (Crustacea-Copepoda). Musée d'histoire naturelle de la Macédoine, Skopje.

Romero, A., 2004. Evolution of hypogean fauna. In Gunn, J. (ed.), Encyclopedia of Caves and Karst Science. Fitzroy Dearborn, New York.

Shen, C. & D. Song, 1979. Fauna Sinica, Crustacea, Freshwater Copepoda. Science Press, Peking.

Sket, B., 1985. Why all cave animals do not look alike – a discussion on adaptive value of reduction processes. Journal of Caves and Karst Studies 47: 78–85.

Stella, E., 1984. Fauna d'Italia. Crustacea: Copepoda: Calanoida (d'acqua dolce). Calderini, Bologa.

Stoch, F., 1984. Sulla presenza di Troglodiaptomus sketi Petkovski, 1978 (Copepoda, Calanoida) in una grotta del Carso Triestio (Italia Nordorientale). Atti e Memmorie della Comm. Grotte 'E. Boegan' 23: 65–67.

Stoch, F., 1985. Calanoidi e Ciclopoidi (Crustacea, Copepoda) delle acque carsiche sotterrranee della Venezia Giulia (Itaia Nordorietale). Atti e Memmorie della Comm. Grotte 'E. Boegan' 24: 61–72.