

# Fauna of an unsaturated karstic zone in Central Slovenia: two new species of Harpacticoida (Crustacea: Copepoda), *Elaphoidella millennii* n. sp. and *E. tarmani* n. sp., their ecology and morphological adaptations

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**Abstract** The unsaturated zone in fissured (= karstic) aquifers continues to be a source of new species of Harpacticoida (Crustacea: Copepoda). The first species were discovered about 70 years ago in the Škocjanske Jame Cave in Slovenia. Intensive sampling of percolating water in caves there over the last 20 years has yielded several new species, some of them well adapted to that environment. The most recent studies revealed that such a specialised fauna is also present in other regions of Europe, South and North America, and Asia. In Europe, three genera belonging to the order Harpacticoida are characteristic of the unsaturated karstic zone: *Morariopsis*, *Paramorariopsis* and *Elaphoidella*. In this article, two highly specialised species of *Elaphoidella* are described. A detailed analysis of their ecology and morphological adaptations along with other species of the genus *Elaphoidella* from Slovenia is included, and comparisons are made with the epikarstic genera *Morariopsis* and *Paramorariopsis*.

**Keywords** *Elaphoidella* · New species · Unsaturated zone · Karst · Slovenia · Taxonomy

## Introduction

About 200 species and subspecies of the genus *Elaphoidella* (Crustacea: Copepoda: Harpacticoida) are known from different habitats all over the world (for a complete list, see: Wells 2007; [http://invertebrates.si.edu/copepod/species/species\\_Result.cfm](http://invertebrates.si.edu/copepod/species/species_Result.cfm); <http://www.geocities.com/~mediaq/elaph.html>). They inhabit different habitats, from epigeal to hypogean water bodies and from standing to running waters (more details in Galassi, 2001; Mori & Brancelj, 2008).

Seventy-five species have been recorded from Europe (Fauna Europaea Service—<http://www.faunaeur.org>). In most countries, the number of known species varies between 0 and 5, and only in a few countries does the number exceed 10 (Bulgaria: 18, France: 14, Italian peninsula: 21, Romania: 10, Slovenia: 13).

In Slovenia, the list of *Elaphoidella* species increased significantly after 1983, when Petkovski described *E. cvetkai* and *E. franci* from percolating water in the Postojna-Planina Cave System (PPCS) (Petkovski, 1983). Two years later, a species from percolating water in the Škocjanske Jame caves was described as *E. kieferi* (Petkovski & Brancelj, 1985) but later renamed as *E. karstica* by Dussart & Defaye

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(1990). In 2000, several specimens of two new species of *Elaphoidella* were found in a small karstic cave, Velika Pasjica, about 20 km south of Ljubljana (Brancelj, 2002). They were reported in Brancelj (2002) as *E. millennii* n. sp. and *E. tarmani* n. sp., but no taxonomic description was given and the species names were *nomina nuda*. During the PASCALIS project (2004), many specimens of *E. millennii* n. sp. were found in different habitats in the vicinity of the cave Velika Pasjica. In a study of habitat preferences of different species of *Elaphoidella* from Slovenia, they were cited as *Elaphoidella* sp. 1 (= *E. millennii* n. sp.) and *Elaphoidella* sp. 2 (= *E. tarmani* n. sp.) (Mori & Brancelj, 2008). The latest *Elaphoidella* species to be added to the fauna list of Slovenia is *E. boui* Rouch, 1988, found in a littoral zone of the lake Bohinj Jezero (Brancelj unpublished).

In this paper, two species, *E. millennii* and *E. tarmani* are described, with comments on their habitat, ecology and morphological adaptations. They are compared with other *Elaphoidella* species found in Slovenia, to test the hypothesis that habitat, especially unsaturated karstic zone with its topmost part (= epikarst), plays an important role in shaping the morphological adaptations of its inhabitants. Morphological data from *Elaphoidella* species are in addition compared with adaptations in genera *Morariopsis* and *Paramorariopsis*, two endemic and highly specialised genera from unsaturated karstic zone in the Dinaric mountains (*Morariopsis*) and the Alps (*Paramorariopsis*).

## Materials and methods

During the year 2000, pools filled with dripping water in the cave Velika Pasjica in central Slovenia were sampled intensively (Brancelj, 2002) and about 2,000 specimens of Copepoda were collected. Among them were specimens of three species of *Elaphoidella*. One male specimen was identified as *E. cvetkae* Petkovski, 1983 and the others belonged to two unknown taxa, described herein.

In addition, between 2002 and 2004, water bodies in unsaturated and saturated zones of porous and fissured subterranean aquifers south of Ljubljana were sampled intensively under the PASCALIS project (2004). Different sampling techniques were used: filtering of water dripping from the roof of the caves

and water from pools on the cave floor, drift and kick sampling in springs, and use of a Bou-Rouch pump in porous aquifers. The samples were filtered with a plankton net with mesh size 60  $\mu\text{m}$ . As a result, many specimens belonging to several species of the genus *Elaphoidella* were collected (Mori & Brancelj, 2008), including two species that were unknown but identical to those found in the cave Velika Pasjica. All the sampling stations where the new taxa were collected were within a radius of about 10 km from the cave, both in fissured and porous aquifers.

Samples were stored in plastic bottles immediately after sampling, and formaldehyde was added to a final concentration of about 4%. Animals were sorted under a stereomicroscope and stored in 70% alcohol. Before dissection, specimens were placed in a mixture of glycerol and 70% alcohol (ratio ~1:10 v/v), which was replaced within 1 h by pure glycerol. They were dissected at 100 $\times$  magnification under an Olympus SZH 2 stereomicroscope. Examination of all appendages and body ornamentation was done under a magnification of 1,000 $\times$ . All drawings, except for the female/male habitus, were made at the same magnification (1,000 $\times$ ) with a drawing tube mounted on an Olympus microscope (BHS 40). The final version of the drawings was made using the CorelDRAW<sup>®</sup>12 graphic program. For permanent slides, all body parts were put in a drop of glycerol on a microscope slide, covered by a cover glass and sealed by nail polish.

We used PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b1 (Swofford, 2003) to generate a small cladogram illustrating position of *E. millennii* and *E. tarmani* within Slovenian representatives of *Elaphoidella*. Branch-swapping algorithm: TBR with random addition sequence (1,000 replicates) was applied. Characters, states and data matrix are available in the Supplementary Electronic Material. We performed a heuristic search with 100 replicates and parsimony as optimality criterion. A total of 17 morphological characters of females were used for the analysis. Lang's (1948) groups are not included in these characters. All characters were unordered and of equal weight, starting tree obtained via stepwise additions, default settings. We chose 14 taxa, of which 2 form the out-group (*Morariopsis* and *Paramorariopsis*). They were chosen as specialised representatives of habitats also occupied by some *Elaphoidella* species from Slovenia. Data for

morphology was based on Petkovski (1983, 1984), Brancelj (1986, 2000, 2006), Rouch (1988), material collected by author and this study. All characters were found parsimony informative by PAUP.

The following abbreviations are used, when required, throughout the text and figures: Endp = endopod; Exp = exopod; Exp/Endp-1 = proximal segment; Exp/Endp-2 = middle segment; Exp/Endp-3 = distal segment; P1–P5 = thoracic appendages. The nomenclature and descriptive terminology follow Huys & Boxshall (1991).

#### *Loc. typ.* site description

Cave Velika Pasjica is located about 20 km south of Ljubljana (capital of Slovenia), near the village of Gornji Ig, on a plateau called the Krim massif. The entrance of the cave is at an elevation of 660 m. The cave is a 75-m-long horizontal gallery, which is formed in thinly bedded Norian-Retian dolomite from the Upper Triassic period (Pleničar, 1970). The cave is near the surface, with the roof in some places only 3–5 m thick. Its only source of water is what drips from the ceiling. There are four permanent drips with a maximum rate of  $1\text{--}2\text{ l s}^{-2}$  immediately after heavy rain; most of the time there are only a few drops per minute but they never dry out completely. For more details, see Brancelj (2002).

#### Taxonomic account

##### *Elaphoidella millennii* n. sp.

(Figures 1: A–J; 2: A–E: female; Fig. 3: A–H: male).

##### *Material*

*Holotype* Adult female (total length: 432  $\mu\text{m}$ ), completely dissected and mounted on a slide in glycerol and sealed with nail polish; collected on March 03 2000 in the cave Velika Pasjica (= *loc. typ.*); deposited in the Natural History Museum (London), registration No.: 2008.994.

*Allotype* Adult male (total length: 412  $\mu\text{m}$ ), completely dissected and mounted on a slide in glycerol and sealed with nail polish; collected on 3 March 2000 in the cave Velika Pasjica; deposited in

the Natural History Museum (London), registration no.: 2008.995.

*Paratypes* 5♀♀ and 5♂♂ (stored in 60% alcohol) collected in the cave Velika Pasjica on 3 March (2♂♂), 6 October (1♂), 16 November (3♀♀, 2♂♂) and 5 December 2000 (2♀♀), deposited in the Natural History Museum (London), registration no.: 2008.16–25. 2♂♂ and 2♀♀ (completely dissected and mounted on a slide in glycerol and sealed with nail polish) collected on 5 December 2000 in the cave Velika Pasjica—in author's collection. 16 specimens (stored in 60% alcohol) (2♂♂, 12♀♀, 1♂ and 1♀ in copula) collected in the cave Velika Pasjica on 13 April (1♀), 8 May (1♂, 2♀♀), 6 June (1♂, 3♀♀), 14 August (1♀) and 15 December 2006 (3♀♀), and 16 February (2♀♀) and 13 November 2007 (1♂ and 1♀ in copula)—in author's collection.

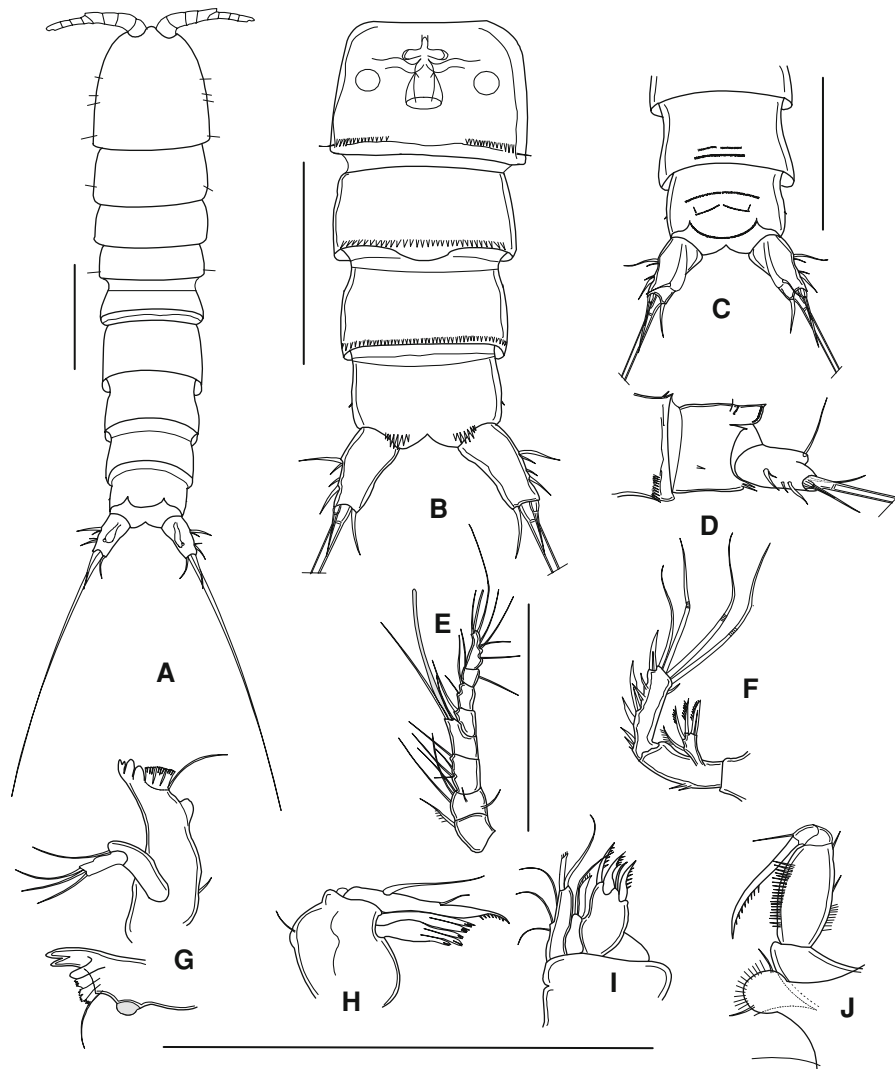
*Additional material* Ten specimens of both sexes from six small springs and one cave close to the cave Velika Pasjica (within a radius of 10 km) (springs: Povšečka, Dobra Voda, Gabrovica, Kalašca, Izber, Šumik; cave: Jelenska Jama) collected between 28 August and 4 September 2002; several tens of specimens of both sexes collected by Bou-Rouch sampling method in a hyporheic/phreatic zone of the river Iška (c. 300 m below the cave Velika Pasjica) between 15 and 30 July 2002.

All material collected by A. Brancelj.

##### *Description*

Female: body length 423–444  $\mu\text{m}$  ( $x = 435$ ;  $n = 10$ ); elongated, cylindrical, colourless (Fig. 1A). Naupliar eye absent. Cephalosome with four pairs of sensilla, second and fourth thoracic somites laterally with one pair each (Fig. 1A). Posterior margins of thoracic and abdominal somites dorsally smooth. Fourth abdominal somite with two dorsal rows of small spinules just above posterior margin (Fig. 1C).

Genital double-somite with distal row of small spinules on ventral side, interrupted in the middle (Fig. 1B). Posterior margin of somite with pair of sensilla ventro-laterally. Ventral distal margin of third and fourth abdominal somites with continuous row of small spinules. Anal somite ventrally with group of 6–7 strong spinules above base of caudal



**Fig. 1** *Elaphoidella millennii* n. sp., female; **A** habitus, dorsal view; **B** double genital somite, abdominal somites, and anal somite, ventral view; **C** abdominal somites and anal somite,

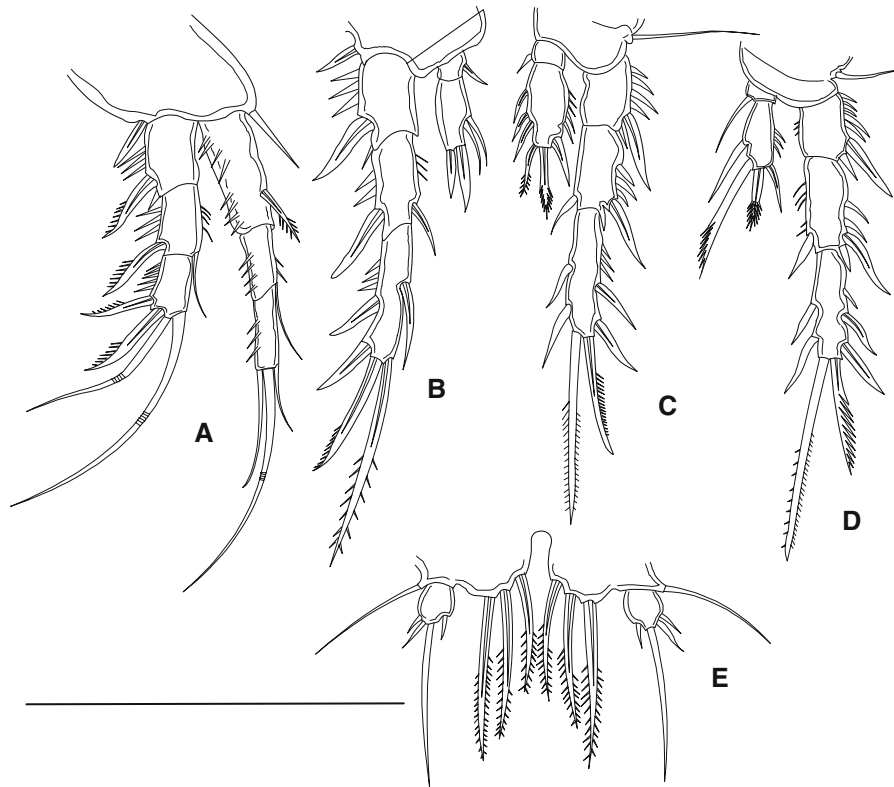
dorsal view; **D** anal somite and caudal ramus, lateral outer view; **E** antennule; **F** antenna; **G** mandible; **H** maxillule; **I** maxilla; **J** maxilliped. Scale bar 100  $\mu$ m

ramus, 2 middle ones strongest (Fig. 1B, D). Laterally one small spinule on each side, positioned on middle of segment. Dorsally with two longitudinal rows of small dorsal spinules, distal row slightly oblique and interrupted in middle (Fig. 1C), with small sensillum at each end (Fig. 1C, D).

Anal operculum large, rounded, not reaching to distal end of anal somite, with several small spinules along distal margin (Fig. 1C). Receptaculum seminis (Fig. 1B) characteristic for genus *Elaphoidella*.

Caudal rami divergent, slightly arched, each about 1.5 times as long as wide, tapering posteriorly, with

dorsal keel (Fig. 1A–C). Inner margin smooth. Anterolateral seta (II) inserted at about the middle of caudal ramus; anterolateral accessory seta (I) short and thin. Posterolateral seta (III) shorter than anterolateral (I) and terminal accessory (VI) setae, with one spinule near its insertion. Terminal accessory (VI) seta short and strong, spiniform, curved inward. Dorsal seta (VII) 0.8 times as long as caudal ramus, inserted at inner distal corner of keel. Outer terminal seta (IV) short and spiniform, as long as caudal ramus, with breaking plane. Inner terminal seta (V) bare, with breaking plane.



**Fig. 2** *Elaphoidella millennii* n. sp., female; **A** P1; **B** P2; **C** P3; **D** P4; **E** P5. Scale bar 100  $\mu$ m

#### Rostrum small

Antennule (Fig. 1E) short and moderately stout, eight-segmented. Aesthetasc on fourth segment cylindrical, slightly curved, with rounded tip and reaching past end of antennule. Second aesthetasc on terminal segment slightly overreaching tip of first aesthetasc.

Antenna (Fig. 1F) with allobasis, and robust one-segmented Exp and Endp. Two strong spines on outer margin of Endp accompanied by several strong shorter spinules of different lengths; terminal armature consisting of one short spine, one normal and three geniculate setae. Exp with four spiniform setae, armed on one margin with coarse spinules.

Mandible (Fig. 1G) short and robust, with three strongly chitinised teeth on gnathobase. One dorsal seta near gnathobase. Mandibular palp relatively long, two-segmented, with one seta on proximal segment (basis) and three setae, sub-equal in length, on distal segment (=endopod).

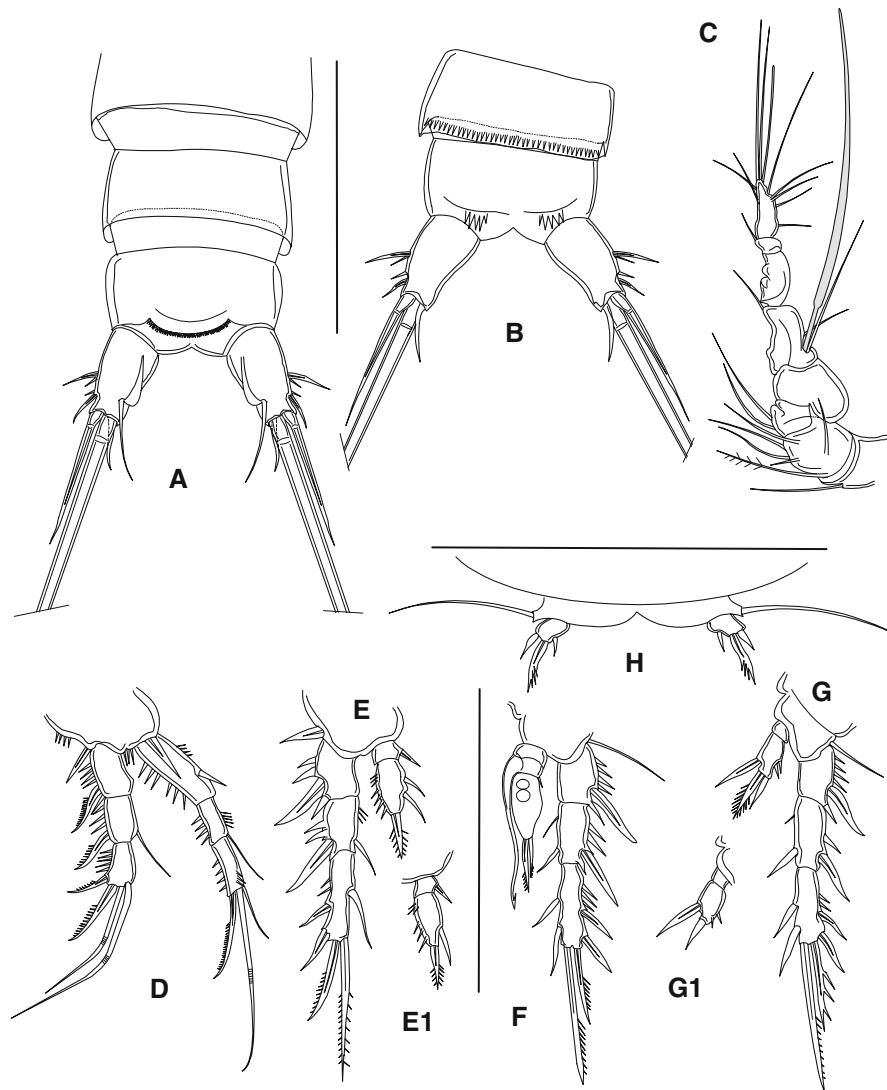
Maxillule (Fig. 1H) with strong and robust spine on praecoxal arthrite. Coxa with strong chitinised spines.

Basis with one strong, beak-like outgrowths, with one long seta proximally and shorter one distally.

Maxilla two-segmented (Fig. 1I) syncoxa with 4 + 1 short and robust endites. Basis apically with strong spine and long seta. Endp reduced to three setae.

Maxilliped (Fig. 1J) comprising syncoxa, basis and one-segmented Endp. Syncoxa with no spinules or setae. Basis twice as long as wide with about 20 spinules positioned near palmar margin, increasing in length distally. Endp drawn out into strong, acutely curved claw; slightly longer than basis and armed with several spinules in distal half; accessory armature represented by short seta.

P1 (Fig. 2A) with three-segmented Exp and three-segmented Endp. Endp longer than Exp. Basis with strong and slender inner seta and stout outer basal spine. Exp-1 and Exp-2 with one strong outer spine. Exp-3 with one strong spine laterally. One spine and two long geniculate setae terminally. Inner terminal seta as long as Exp. Endp-1 with long seta at two-thirds length of inner margin. Endp-2 with long



**Fig. 3** *Elaphoidella millennii* n. sp., male; **A** abdominal somites 2–3, and anal somite, dorsal view; **B** last abdominal somite and anal somite, ventral view; **C** antennule; **D** P1; **E** P2;

**F** P3; **G** P4; **H** P5. **E1** P2 endopod, variability; **G1** P4 endopod, variability. Scale bar 100  $\mu\text{m}$

bristle at inner distal corner. Endp-3 with three setae; innermost small, terminal seta long and geniculate, outer one spiniform and bare.

P2 (Fig. 2B) basis with spiniform outer seta. Three-segmented Exp and two-segmented Endp. Endp slightly longer than Exp-1. Exp-1 1.5 times as long as wide, with one strong pointed spine at distal corner. Exp-2 with one strong outer spine with rounded tip and one short inner spiniform seta. Exp-3 with two outer spines, two terminal setae and one spiniform seta inserted midway along inner margin. Outer terminal seta transformed, shorter than inner,

inner one shorter than Exp-3. Endp-1 shorter than wide, with one spine on inner margin. Endp-2 twice as long as wide, with two sub-apical spiniform setae on inner margin, and two blade-like terminal spines, sub-equal in length. Longer one slightly shorter than terminal segment.

P3 (Fig. 2C) outer basal seta long and very thin. Exp similar to that of P2 but with additional seta on inner margin of terminal segment. Endp two-segmented; proximal segment shorter than wide, with short inner seta. Terminal segment with two apical setae; unequal in length; one strong, transformed, as

long as segment and pinnate; second one shorter and spiniform seta on outer distal corner; inner margin with three strong spines.

P4 (Fig. 2D) outer basal seta thin. Three-segmented Exp and two-segmented Endp. Exp-1 and Exp-2 similar to those of P3. Exp-3 with two strong spines on outer and two spines on inner margin. Two terminal spines; outer one as long as terminal segment, with strong teeth along one margin; inner terminal spine twice as long as outer one, robust and pinnate. Proximal segment of Endp very short, with long inner seta. Endp-2 with one strong spine on inner margin, and three terminal setae; outer one spiniform, terminal seta strong, slightly longer than outer one with strong teeth on tip, inner seta longer than Endp, with unilaterally serrated tip.

Additional ornamentation of P1–P4 as in Fig. 2A–D

P5 (Fig. 2E) with separate Exp and baseoendopod; baseoendopodal lobe small, with three long, strong pinnate spines of unequal length, decreasing in length from outer margin. Exp small, sub-oval, with three apical setae and one seta on the inner corner. On outer corner two and on inner corner one short seta. One terminal seta, as long as longest one on baseoendopod, bare. Outer lateral seta on baseoendopod long and bare.

### Male

Male: slightly smaller than female; body length 398–418  $\mu\text{m}$  (mean = 408,  $n = 7$ ). Body shape similar to that of female (Fig. 3A). Anal operculum, antenna, mouthparts, P1 (Fig. 3D), P2–P3 exopods similar to those of female. Caudal rami similar to female, but outer terminal seta twice as long as in female.

Antennule (Fig. 3C) eight-segmented. Aesthetasc on fourth segment cylindrical, slightly curved, with rounded tip and slightly longer than antennule. Second aesthetasc on terminal segment slightly overreaching tip of first aesthetasc.

P2 (Fig. 3E) Endp slightly past Exp-2. Endp-1 shorter than wide, with seta on inner margin. Endp-2 twice as long as wide, with two short, robust seta on inner margin, positioned sub-terminally and one strong terminal pinnate spine, as long as segment.

P3 (Fig. 3F) Endp three-segmented; proximal segment shorter than wide. Second segment with

long inner spine, with harpoon-like tip and small spine on inner distal corner. Terminal segment twice as long as wide with two terminal unilaterally pinnate spines; inner spine about 0.75 times and outer one about 0.6 times length of Exp-2.

P4 (Fig. 3G) Exp as in female, but outer terminal seta robust, with 2–3 strong teeth on outer side—resembling deer antlers. Endp-1 very short, without inner seta. Distal segment with two small spines on outer margin of Endp and one strong spine at inner distal corner. Two terminal setae, outer one spiniform, inner one as long as Endp.

Additional ornamentation of P1–P4 as in Fig. 3D–G

P5 (Fig. 3H) with separate Exp and baseoendopod; baseoendopodal lobe small, without any armament. Exp small, pyriform. One short sub-distal seta on inner margin. Two short terminal setae; outer one as long as segment, inner one strong, twice as long as outer one, unipinnate.

P6: not found.

### Variability

In two males, variability in armature of endopods was observed. On P2 End-2 there was a reduction of number of short setae on inner margin: one seta instead of two (Fig. 3E1). On P4 End-2 there was a reduction of apical setae: one seta instead of two (Fig. 3G1). No such variability was observed in females.

### Etymology

The new species is named after the year of discovery (2000), just at the start of a new millennium.

### Relation to other taxa

With two segmented Endp-P1 and two-segmented Endp-P4, *E. millennii* n. sp. clearly fits into the genus *Elaphoidella* s. str. Chappuis, 1929. The armature of the P4 Exp-3 of the male (Fig. 3G) and P5 of the female (Fig. 2E) puts the species into III (–*jeanneli*) group sensu Lang (1948). Detailed analysis of characters in male and female of *E. millennii* n. sp. confirmed that the closest species are *E. jeanneli* (Chappuis, 1928) and *E. charon* Chappuis, 1936, both described from caves

in Slovenia (Chappuis, 1928, 1936) and also belonging to the III group sensu Lang (1948).

*E. jeanneli* was described from the cave Črna Jama (part of the PPCS), c. 40 km southwest from the cave Velika Pasjica. In 1985, one male and one female were found in the nearby cave Planinska Jama, which is close to *loc. typ.* (Brancelj, 1986). Later on, several new locations with *E. jeanneli* were found in caves in southwest Slovenia and also during the PASCALIS project in a hyporheic/phreatic zone of three rivers draining from the Krim massif (Brancelj, unpublished). *E. charon* was described from the cave Krška Jama, about 40 km southeast from the cave Velika Pasjica. Petkovski (1984) re-described it from the spring Tominčev Izvir, c. 10 km from *loc. typ.* Later, a few new locations with individual specimens of *E. charon* (all of them females) were found in southeast and central Slovenia, which includes locations collected during the PASCALIS project (together with *E. jeanneli*) (Brancelj, unpublished).

On the species level, *E. jeanneli* and *E. charon* differ only slightly (Table 1). Petkovski (1984) listed only four differences between them. The main ones are the length of spines on distal segments of Exp and Endp P2–P4 and P5, which are longer in *E. charon*, and the number of spines on terminal segments of Endp-2 of P2 and P4. At the same time, Apostolov

(1985) mentioned that some species of the genus *Elaphoidella* (specifically *E. elaphoides*) show great variability in the number of setae and also in their length. Based on those facts, Brancelj (1986), supported by the paper of Petkovski (1984), concluded that there are two populations of *E. jeanneli* that differ only in the length of setae; one in the watershed of the Krka River and one in the watersheds of Ljubljanica and Reka Rivers. However, the present knowledge on ecology of subterranean Harpacticoida from unsaturated zone and their morphological adaptations, along with a finding of a new species (i.e. *E. millennii*) geographically positioned between the *E. jeanneli* and *E. charon*, supports the original idea of two “good” species (i.e. *E. jeanneli* and *E. charon*). From original papers describing *E. jeanneli* and *E. charon*, it is not evident from which micro-locations specimens were collected. Based on personal observations of the author, both species were most probably collected from pools filled by percolating water.

The new species from the Krim massif, *E. millennii* n. sp., has short and robust spines on all appendages, a character also present in *E. jeanneli* and *E. charon* but not in an extreme form (Table 1). Some other characters distinguish these three species, as revealed by examination of specimens of the three taxa collected

**Table 1** Differential diagnosis of species within a complex of *Elaphoidella millennii* n. sp.: *E. jeanneli* (Chappuis, 1928): *E. charon* Chappuis, 1936, and within a complex of *E. tarmani* n. sp.: *E. gordani* Karanovic, 1998: *E. franci* Petkovski, 1983

Character	<i>E. millennii</i>	<i>E. jeanneli</i>	<i>E. charon</i>	<i>E. tarmani</i>	<i>E. gordani</i>	<i>E. franci</i>
F: P3—length of Endp-2: outer apical seta	1:1	1:1.5	1:2.1	1:2	1:2.2	1:1.6
F: P4—length of Endp-2: outer apical seta	1:0.8	1:1	1:2	1:1.2	1:1	1:1
F: P4—length of Exp-3: inner apical seta	1:2	1:1.9	1:2.5	1:2	1:2	1:2
F: P4—shape of inner apical seta on Exp-3	lanceolate, robust	long, soft	long, soft	long, soft	long, soft	long, soft
F: P5—length of Exp: outer apical seta	1:1	1:1.2	1:2.1	1:2.1	1:1.5	1:1.5
F: P5—armature (Exp/baseoendopod)	4/3	4/3	4/3	2/2	3/4	3/3
F: anal operculum—no. of denticles on free margin	c. 20	c. 30	many	c. 20	c. 25	4
F: Fu—spinules on base of caudal ramus	6–7	2	2	4	4	4
F: shape of caudal ramus	arcuated	conical	pear-like	elongated 1:3.3	elongated 1:2	elongated 1:3.5
M: P4 Exp-3—length and shape of “antler-like” spines	short, robust	long, soft	short, robust	long, robust	short, robust	short, robust
M: P5 Exp—longest apical seta	1:2	1:4	1:1	1:8	1:1	1:4
M: P5 armature (Exp/baseoendopod)	3/0	4/0	4/0	2/0	2/0	3/0

F female, M male



from locations in the Krim massif (females only) and from the literature. The most characteristic is the shape of caudal rami—longer and arcuated in *E. millennii*, shorter and conical in *E. jeanneli* and pear-shaped in *E. charon*. The number of spines at the base of caudal rami is greater in both sexes in *E. millennii* n. sp. (6–7) than in *E. charon* and *E. jeanneli* (2). Abdominal somites in *E. jeanneli* and *E. charon* are irregularly serrated but smooth in *E. millennii* n. sp. Outer terminal seta on the P4 Exp-3 of the female in *E. millennii* n. sp. have very strong teeth but weak ones in *E. jeanneli* and *E. charon*. In *E. jeanneli* and *E. charon* the two spines on the P4 Exp-3 of males have strong teeth (deer antler form) but there is only one spine modified in such a way in *E. millennii* n. sp. The distal spine on outer margin of P4 Exp-3, which is normally “antler like” in males of III group, is short and bare in *E. millennii* n. sp. This is probably a result of general reduction of length of spines and setae as adaptation to specific environment.

The three species differ markedly in the length of the middle apical seta on the female P5 Exp: about 40% of the length of the inner apical seta in *E. charon*, about 25% in *E. jeanneli* and >20%, and spiniform, in *E. millennii* n. sp. The male P5 Exp in *E. jeanneli* and *E. charon* have one long and slender terminal spine but a robust one in *E. millennii* n. sp. There are two setae on the outer margin of male P5 Exp in *E. jeanneli* and *E. charon* but only one in *E. millennii* n. sp. These new observations support the idea that, in a rather limited area of the southern part of Slovenia, there are three closely related species: *E. charon* in the southeast; *E. jeanneli* in the southwest and *E. millennii* n. sp. in the central part of Slovenia, between the areas of the aforementioned taxa. (For more information see “Discussion”.)

*Elaphoidella tarmani* n. sp.

(Figure 4: A–H; 5: A–E: female; Fig. 6: A–I: male).

### Material

**Holotype** Adult female (total length: 382  $\mu\text{m}$ ), completely dissected and mounted on a slide in glycerol and sealed with nail polish; collected on 3 March 2000 in the cave Velika Pasjica (= *loc. typ.*); deposited in the Natural History Museum (London), registration no.: 2008.996.

**Allotype** Adult male (total length: 375  $\mu\text{m}$ ), completely dissected and mounted on a slide in glycerol and sealed with nail polish; collected on 3 March 2000 in the cave Velika Pasjica; deposited in the Natural History Museum (London), registration no.: 2008.997.

**Paratypes** 3♀♀ (stored in 60% alcohol) collected in the cave Velika Pasjica on 28 January, 16 November and 5 December 5 2000 (one female per date); deposited in the Natural History Museum (London), registration no.: 2008.26–28. 1♂ (completely dissected and mounted on a slide in glycerol and sealed with nail polish) collected on 6 October 2000 in the cave Velika Pasjica—in author’s collection. 2♀♀ (stored in 60% alcohol) collected from a small spring Šumik, about 10 km north of the cave Velika Pasjica, on 4 September 2002—in author’s collection. 2♀♀ (stored in 60% alcohol) collected in the cave Velika Pasjica on 16 April and 6 June 2000 (one female per date)—in author’s collection.

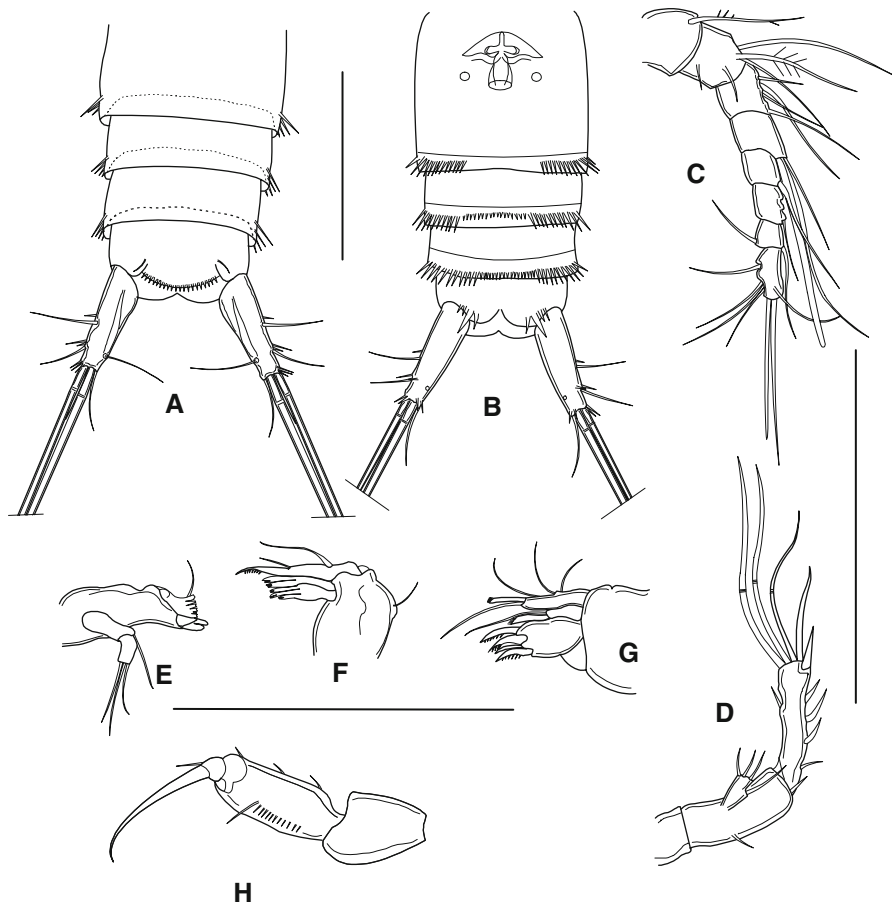
All the material collected by A. Brancelj.

### Description

Female: body length 380–408  $\mu\text{m}$  (mean = 398,  $n = 4$ ); elongated, cylindrical, colourless (Fig. 4A, B). Naupliar eye absent. Cephalosome with three pairs of sensilla. Distal margins of thoracic and abdominal somites dorsally smooth. Distal margins of all abdominal somites ventrally smooth. Genital double-somite with row of rather strong spinules on ventral side of distal margin, interrupted in middle. Ventral distal margin of third and fourth somites with row of rather strong spinules shorter in middle. Anal somite ventrally with group of 4 strong spinules above base of caudal ramus, 2 innermost strongest (Fig. 4B). Anal somite dorsally with one sensillum on each side of anal operculum.

Anal operculum large, convex, with about 20 strong spinules around free margin, not reaching to distal end of anal somite (Fig. 4A). Receptaculum seminis (Fig. 4B) characteristic of genus *Elaphoidella*.

Caudal rami divergent, slim, each about 3.5 times as long as wide, slightly tapering posteriorly, with dorsal keel along 2/3 of ramus length (Fig. 4A, B). Inner margin smooth. Anterolateral seta (II) long, inserted at about middle of caudal ramus; anterolateral accessory seta (I) short and thin. Posterolateral



**Fig. 4** *Elaphoidella tarmani* n. sp., female; **A** last two abdominal somites and anal somite, dorsal view; **B** double genital somite, abdominal somites, and anal somite, ventral

view; **C** antennule; **D** antenna; **E** mandible; **F** maxillule; **G** maxilla; **H** maxilliped. Scale bar 100 µm

seta (III) shorter than anterolateral and terminal accessory (VI) setae, with two spinules of different lengths near insertion. Terminal accessory (VI) seta thin, as long as dorsal seta, curved inward. Dorsal seta (VII) 0.9 times as long as caudal ramus, inserted at inner distal corner of keel. Outer terminal seta (IV) about 4 times as long as ramus, straight, with breaking plane. Inner terminal seta (V) long, bare, with breaking plane.

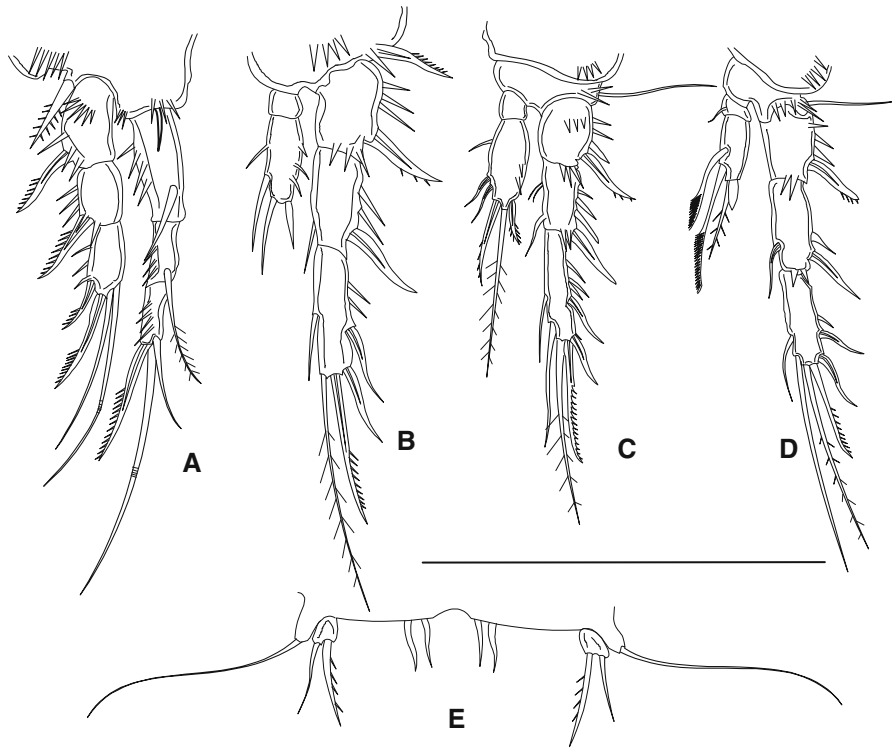
Rostrum small

Antennule (Fig. 4C) short and moderately stout, eight-segmented. Aesthetasc on fourth segment cylindrical, straight, with rounded tip reaching past end of antennule. Second aesthetasc on terminal segment well overreaching tip of first aesthetasc.

Antenna (Fig. 4D) with allobasis, and one-segmented Exp and Endp; rather elongated and robust. Several strong spines on outer margin of Endp; terminal armature consisting of one spine, one normal and three geniculate setae. Exp with three apical and one sub-apical short, bare setae.

Mandible (Fig. 4E) short and robust, with three strongly chitinised teeth on gnathobase. One dorsal seta near gnathobase. Mandibular palp relatively long, two-segmented, with one seta on proximal segment (basis) and three setae, sub-equal in length, on distal segment (endopod).

Maxillule (Fig. 4F) with strong and robust spine on praecoxal arthrite. Coxa with strong chitinised spines. Basis with one strong, beak-like outgrowth; with one long seta proximally and shorter one distally.



**Fig. 5** *Elaphoidella tarmani* n. sp., female; **A** P1; **B** P2; **C** P3; **D** P4; **E** P5. Scale bar 100  $\mu$ m

Maxilla two-segmented (Fig. 1I) syncoxa with 4 + 1 short and robust endites. Basis apically with strong spine and long seta. Endp reduced to three setae.

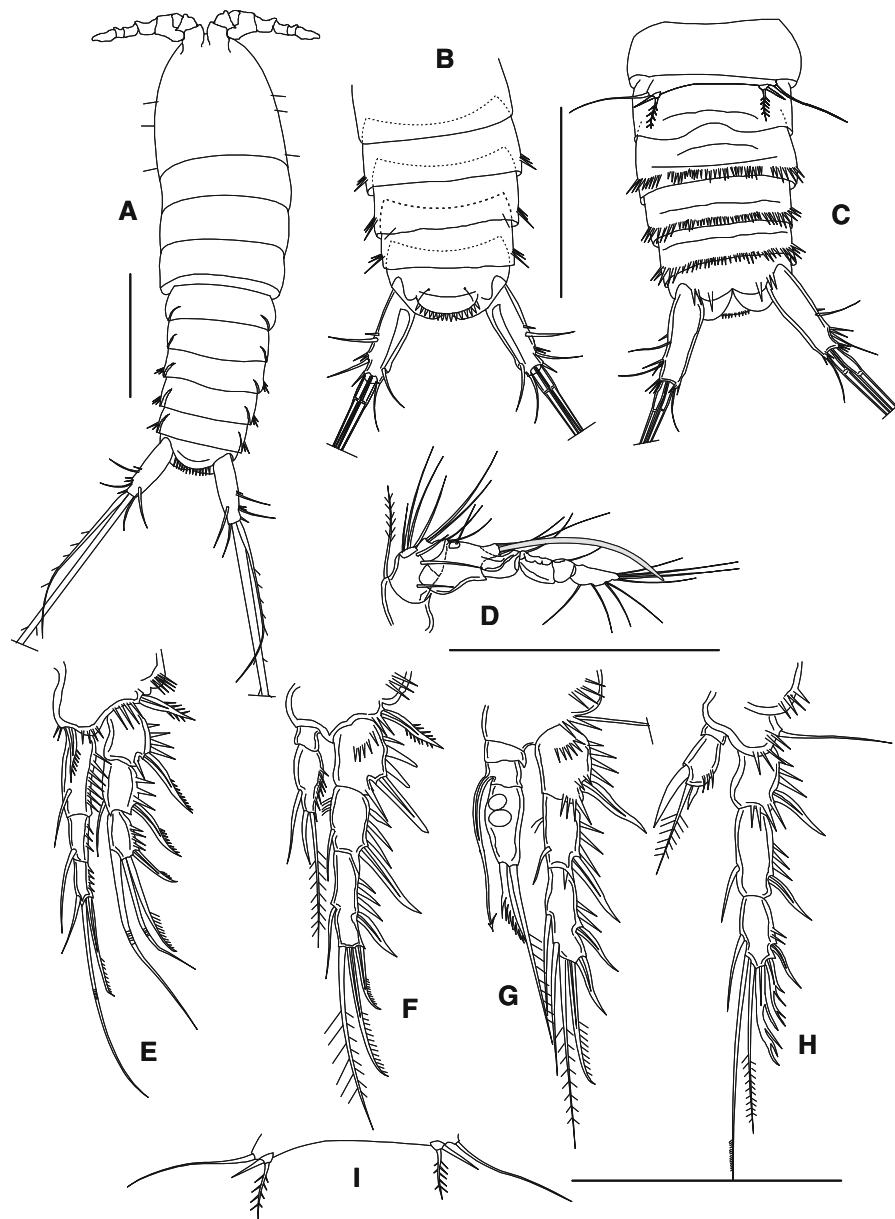
Maxilliped (Fig. 1J): comprising syncoxa, basis and one-segmented Endp. Syncoxa with no spinules or setae. Basis twice as long as wide with about 20 spinules positioned near palmar margin. Spinules small, equal in length but distalmost, being long and strong. Endp drawn out into strong, acutely curved claw; slightly longer than basis, bare; accessory armature represented by short seta.

P1 (Fig. 5A) with three-segmented Exp and Endp. Endp longer than Exp. Basis with a strong and slender inner seta and robust outer basal spine. Exp-1 and Exp-2 with strong distal outer unipinnate spine. Exp-3 with strong sub-distal outer unipinnate spine. One unipinnate spine and two long geniculate setae terminally. Inner terminal seta as long as Exp. Endp-1 with long seta at two-thirds of inner margin. Endp-2 with long pinnate seta on distal corner. Distal segment with three setae; innermost slightly longer than Endp-3, median seta long and geniculate, outer one spiniform, distally pinnate.

P2 (Fig. 5B) outer basal seta spiniform; with three-segmented Exp and two-segmented Endp. Endp as long as half of Exp. Exp-1 1.5 times as long as wide; with one strong outer spine, with sharp tip; Exp-2 with one strong outer spine with sharp tip and one short, inner, spiniform seta; with two short and robust spinules along outer margin. Exp-3 with two outer spines, one terminal and one inner unipinnate spiniform seta shorter than Exp and spiniform seta on inner distal corner. Endp-1 shorter than wide, without seta on inner margin. Endp-2 2.5 times as long as wide, with two setae on inner margin: one on the middle of the margin, one sub-terminally; two terminal setae, sub-equal in length.

P3 (Fig. 5C) outer basal seta long and thin; Exp similar to that of P2 with additional seta on inner margin of terminal segment. Endp two-segmented. Endp-1 shorter than wide, with no inner seta. Endp-2 with two pinnate spines of different lengths and one pinnate seta on the apex. Inner margin with two short, sub-apical, and one proximal setae.

P4 (Fig. 5D) outer basal seta long and thin; with three-segmented Exp and two-segmented Endp.



**Fig. 6** *Elaphoidella tarmani* n. sp., male; **A** habitus, dorsal view; **B** last three abdominal somites, and anal somite, dorsal view; **C** P5, genital somite, abdominal somites 1–3, and anal somite, ventral view; **D** antennule; **E** P1; **F** P2; **G** P3; **H** P4; **I** P5. Scale bar 100  $\mu\text{m}$

Exp-1 and Exp-2 similar to those in P3. Exp-3 with two strong spines on outer and one spine on inner margin. Apically one outer unipinnate spiniform seta, one middle pinnate seta, one inner bare seta. Endp-1 very short, with short inner seta. Endp-2 with two strong, modified brush-like spines sub-apically on inner margin and at inner corner, respectively. One pinnate apical seta as long as Enpd-2, one outer very short blade-like transformed spine.

Additional ornamentation of P1–P4 as in Fig. 5A–D

P5 (Fig. 5E) with separate Exp and baseoendopod; baseoendopodal lobe very small, with two normal, slightly curving setae of same length. Exp small, pyriform, with two terminal setae: outer one bare, inner one somewhat longer and unipinnate. Outer lateral seta on baseoendopod long and bare.

## Male

Male: slightly smaller than female; body shape similar to that of female, including shape of anal operculum and caudal rami (Fig. 6A–C), except terminal accessory (VI) seta of caudal rami slightly shorter than in female. Antenna, mouth parts, P1 (Fig. 6E), P2–P3 exopod as in female.

Antennule (Fig. 6D) eight-segmented. Aesthetasc on fourth segment cylindrical, slightly curved, with rounded tip and reaching past end of antennule. Second aesthetasc on terminal segment slightly overreaching tip of first aesthetasc.

P2 (Fig. 6F) Endp reaching middle of Exp-2. Endp-1 as long as wide, with no seta on inner margin. Endp-2 twice as long as wide, with one sub-apical spiniform seta on inner margin. One outer pinnate seta twice length of Endp-2 and one spine as long as Endp-2 apically.

P3 (Fig. 6G) Endp three-segmented; proximal segment shorter than wide. Endp-2 with long inner spine, with hook-like tip. Endp-3 2.5 times as long as wide; on apex: one inner transformed spine and one outer unipinnate seta twice as long as inner spine.

P4 (Fig. 6H) Exp similar to that of female, but apicalmost outer spine and spine at distal outer corner transformed, with 3–4 strong teeth on outer side—resembling deer antlers. Endp-1 very short, with no inner seta. Endp-2 with three small spines on outer margin and one very strong spine on inner margin. Apically: one outer very short spine and one inner pinnate seta, as long as Endp.

Additional ornamentation of P1–P4 as in Fig. 6E–H

P5 (Fig. 6I) with separate Exp and baseoendopod; baseoendopodal lobe small, without any armament. Exp small, rectangular. Two short terminal setae; outer one short and bare, inner one pinnate, twice as long as outer one. Outer lateral seta on baseoendopod long and bare.

P6: not found.

## Variability

No variability in armature of P1–P4 or any other body part was observed in males or females.

## Etymology

The new species is dedicated to Professor Kazimir TARMAN at the University of Ljubljana, who gave me my very first insight into the field of ecology.

## Relation to other taxa

Based on the shape of the male's Endp. P4, the new species *E. tarmani* n. sp. belongs to the III (–*jeanneli*) group sensu Lang (1948). In Slovenia, *E. charon* Chappuis, 1936, *E. jeanneli* (Chappuis, 1928), *E. stammeri* Chappuis, 1936, *E. franci* Petkovski, 1983 and *E. millennii* n. sp. belong to the same group. However, the armature of the female's P5 (with only two spines on Exp and baseoendopod instead of 3–4) does not fit exactly into the above mentioned group.

*Elaphoidella tarmani* n. sp. is most similar to *E. gordani* Karanovic, 1998 from the cave Golubija in Montenegro (Karanovic, 1998) and to *E. franci* Petkovski, 1983 from the cave Planinska Jama in Slovenia (Petkovski, 1983), especially when males are compared, but there are several differences. Caudal rami of both sexes of *E. tarmani* n. sp. are longer than those of *E. gordani* but slightly shorter than in *E. franci* (Table 1). On End-1 of the female P2 and P3, the inner seta is absent in *E. tarmani* n. sp. but present in *E. gordani* and *E. franci*. P4 Endp-3 of female of *E. tarmani* n. sp. has a finger-like seta, which is longer and sharper in *E. gordani* and *E. franci*. In *E. tarmani* n. sp., Endp-1 of male P2 lacks the inner seta and the terminal segment has 3 spines/setae only, but in *E. gordani* the basal segment has an inner seta and a terminal segment with 5 spines/setae. In *E. franci* there is also an inner spine on the basal segment but 3 spines/setae on the terminal segment. P4 Endp-2 of male in *E. tarmani* n. sp. carries a strong inner spine and a long terminal seta and an additional small outer spine, whereas in *E. gordani* it carries three spines almost identical in length. P4 Endp-2 of *E. franci* is very similar in ornamentation to that of *E. tarmani* n. sp. The male's P4 Exp show additional differences: (1) the inner spine on Exp-2 of *E. gordani* is strong and pinnate, but it is rather weak and bare in *E. tarmani* n. sp. and *E. franci*, and (2) two spines on the inner margin of the same segment are short and strong in *E. gordani* but they are long and thin in *E. tarmani* n. sp. and *E. franci*. P5 Exp of the male has a blade-like outer spine and a short and

slim inner spine in *E. gordani*, whereas in *E. tarmani* n. sp. the inner spine is long, slim, pinnate, and the outer one is about 50% shorter and bare. P5 of *E. tarmani* n. sp. is similar to those in *E. franci* but without inner spine. The anal operculum of *E. franci* has three (male) and four (female) strong spines but has numerous fine denticles in *E. tarmani* n. sp. and *E. gordani*.

Based on the similarity in the main morphological features, setation/spinulation pattern and regardless of differences in the armature of the female's P5, we can consider *E. tarmani* n. sp. and *E. franci* from the northern area of the Dinaric region and *E. gordani* from the southern part as allopatric species descending from a common ancestor. For more information see "Discussion".

## Discussion

### Ecology of epikarst and zoogeography of *Elaphoidella* in Slovenia

Both new species of *Elaphoidella* were first collected in a small cave with no connection with running water (= in the unsaturated zone) (Brancelj, 2002). The most recent observations and sampling revealed that they are indeed inhabitants of the very thin subsoil part of the karst, called the epikarst (Brancelj & Culver, 2005). Epikarst was first defined by Rouch (1968) (see also Bakalowitz, 2005) as an abode of some specialised organisms in a karstic environment, and acts "as a skin of karst" with a thickness of only few metres. It is characterised by a honeycomb system of small fractures, partly filled with (rain)-water and with a predominantly vertical direction of flow. "This supports the hypothesis that communication between voids in the horizontal direction is limited and thus distribution/migration of individuals, including genetic material, are limited. Each hydrological unit within a certain geological block (an equivalent to watershed) thus functions as an island with its own fauna" (Brancelj & Culver, 2005). This is important in understanding the evolution and distribution of epikarst aquatic taxa (= stygobionts). The hydrological conditions are very different from those in porous aquifers, where horizontal connections are much better developed in the gravel bars along the rivers and alluvial deposits in the lowlands.

*E. tarmani* n. sp. has been found so far only in the epikarst (the spring Šumnik belongs to this type of karstic zone) whilst *E. millennii* n. sp., having also been collected in hyporheic/phreatic habitats, has much wider colonisation potential—from unsaturated karstic zone to saturated alluvium (Mori & Brancelj, 2008). Both species are limited to the Krim massif in central Slovenia. Intensive sampling in surrounding areas did not reveal their presence outside the Krim massif (Brancelj, personal observation). Krim massif is a ca. 22-km-long and 10-km-wide outcrop between the southeast and northwest Dinaric regions. The position of the massif appears to be important biogeographically and from the point of view of species evolution. The two new *Elaphoidella* species, along with *Morariopsis dumonti* Brancelj, 2000 described from the same cave (Brancelj, 2000), appear to be isolated species living in a transition zone between two mentioned Dinaric biogeographic regions where intensive speciation of some groups living in unsaturated karstic zone has taken place [e.g. in troglobitic beetles (Vrezec, personal communication) as well as in copepods]. Two groups of aquatic inhabitants living there, including those in the epikarst, should be considered. In the first group there are taxa that are common in the unsaturated zone and widespread over broader areas. Typical representatives are *Speocyclops infernus* (Kiefer, 1930), *Bryocamptus typhlops* (Mrazek, 1893) and *Elaphoidella cvetkae* Petkovski, 1983. In contrast, the second group includes specialised taxa, living in the epikarst zone within a limited area. In the Krim massif, this group is represented by three species: *Morariopsis dumonti*, *Elaphoidella tarmani* n. sp., and *Elaphoidella millennii* n. sp. In a wider area of Slovenia, there are two additional species of *Paramorariopsis*, *P. anae* Brancelj, 1991 and *P. irenae* Brancelj, 2006. All were found in dripping water from epikarst and they share some peculiar morphological characters (Brancelj, 1991, 2006) (for details, see below). Abdominal somites are usually equipped with strong spinules ventrally and laterally, including spinules at the base of the caudal ramus. Their caudal rami are divergent and terminal setae are stiff and wide apart. They have short antennules, only slightly exceeding the width (i.e. cross-section) of the cephalosoma. The most obvious adaptations are the short and strong setae on terminal segments of P2–P5, usually transformed into spines. Robust setae are probably an

adaptation for moving through small spaces in fractured rock and a mechanical protection, along with a possible positive rheotaxis which prevents the animals from being washed out from the epikarst zone. Measurements of dripping water indicate that fluxes in the cave Velika Pasjica can increase by a magnitude of  $10^2$ – $10^3$  within 1 h after heavy rain (Brancelj, unpublished). Even such a strong increase of flow does not significantly affect the drift of epikarstic taxa and only a few specimens are washed down from time to time. Within 1.5 years of systematic sampling of drippings, less than five individuals of *S. infernus*, *M. dumonti*, *E. tarmani* n. sp., *E. millennii* n. sp. or *Bryocamptus typhlops* per catch (with about 1 month sampling intervals) were collected, even during stormy weather. This indicates strong adaptation to epikarstic conditions, and most probably only random drift transports animals from the voids in the epikarst into the cave. Even their naupliar stages (not determined to the species level) were randomly collected in filtering bottles (Brancelj & Culver, 2005) set with a mesh size of 60  $\mu$ m.

Weak horizontal water flow in unsaturated zone prevents dispersal of stygobitic taxa laterally but not vertically. Most specialised epikarstic taxa are weak competitors and sooner or later disappear when they enter the cave environment (= sinking populations) (Brancelj & Culver, 2005). However, some have a wider dispersal potential—like *E. millennii* n. sp. In fact, during intensive sampling conducted for the PASCALIS project (2004), this species was found in both karstic and adjacent porous aquifers, where it had a rather wide distribution in three small rivers interconnected by common alluvial deposits. So far, *E. millennii* n. sp. has never been collected out of the reported range.

Limited horizontal dispersion of specimens in the unsaturated zone facilitates speciation. The pattern already observed in genus *Morariopsis* (Brancelj, 1999) can also be applied to *Elaphoidella*. Each of the two new species has on each side of its range two closely related species: for *E. tarmani* n. sp. they are *E. franci* to the west and *E. gordani* to the southeast, while for *E. millennii* n. sp. they are *E. jeanneli* to the west and *E. charon* to the southwest. Actually, the ranges of the first three species overlap slightly in the area of the Krim massif. Such an intermediate and very limited distribution of the new species can be explained by the tectonic uplift of a massif.

A common ancestor of each set of three species was distributed on the whole area of Dinaric karst, probably well before glaciation. When tectonic movements (uplift) separated the Krim massif from the surrounding area, speciation resulted in three closely related species. This model can only explain speciation in an unsaturated zone, where downward drift is a normal phenomenon, but upward colonisation from a saturated fissured or porous aquifer is unlikely to occur.

It is already known that Copepoda in a subterranean environment exhibit some specific adaptations, including reduction of segments or setae (for a complete list, see Brancelj & Dumont, 2007). 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 (i.e. oligomerisation) of certain body parts, including segmentation of legs and their armature, tracing relationships from morphology only may become difficult. A stygobitic way of life thereby results in “...the highly repeated nature of the morphological changes in many taxonomic 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., 1995). Those statements could, in part, be applied to the two new taxa described here. There is an evident reduction in length of setae, an adaptation to reduced space in a fissured environment and prevention against downward drift, but there is no reduction in the numbers of segments, common in taxa like *Speocyclops* and *Morariopsis* & *Paramorariopsis*. Testing of the relationship between *Elaphoidella* species (in central Slovenia) would require a biochemical/genetic analysis. The question is yet to be answered, as all the taxa were not appropriately preserved for such analyses (mainly preserved in formaldehyde) and collecting them from their very specific environment cannot always be achieved, given their low abundances and patchy distribution.

Morphological adaptations as a reflection of habitat

Animals in different habitats express specific morphological features as a result of adaptations driven

by abiotic environment as well as by inter- and intraspecific competition (Odum, 1971). Morphological adaptations (= differences), in parallel with physiological and behavioural ones, are more obvious in closely related species living in similar and/or partly overlapping habitats. They could originate from allopatric speciation as a result of physical separation of populations or from sympatric speciation, as a result of intraspecific competition, with an extreme expression in an adaptive radiation. Classical examples of adaptive radiation are Darwin's finches on Galapagos Archipelago (Grant, 1999) or cichlid fish in the African rift valley lakes (Salzburger et al., 2005). In contrast, there is convergent evolution, which is a result of similar selection pressures on different organisms and can be manifested in similar morphology but it is not a result of a common ancestry (Calow, 1998).

In the freshwater genus *Elaphoidella*, with its numerous species (c. 200 known) extending geographically from the tropics to the temperate zone and ecologically from benthic to phytohelmic and from epigeal to hypogean, all the above mentioned morphological adaptations could be found. In the study of Mori & Brancelj (2008), 13 *Elaphoidella* species were analysed regarding their correlations with environmental parameters. The taxa included species collected in the benthos, the hyporheic/phreatic zone of the rivers and lakes (alluvium, i.e. porous aquifers), as well as from localities in saturated and unsaturated zone in karstic areas (= fissured aquifers). Taxa are listed in Table 2, where some of morphological characteristics of females are given and compared with genera *Paramorariopsis* and *Morariopsis*. Mori & Brancelj (2008) concluded that the most important parameter in the distribution of *Elaphoidella* taxa is habitat ( $\approx$  physical environment; explaining 28.0% of variance), followed by region ( $\approx$  climate; 25.8% of variance). There is a clear distinction between taxa inhabiting exclusively benthic/hyporheic/phreatic zone in porous aquifers and taxa from the unsaturated zone in karst. This supports a hypothesis that habitat plays an important role in determining morphology within the genus *Elaphoidella*.

Taxa inhabiting exclusively benthos, hyporheic, phreatic and saturated karstic zone habitats (*E. bidens*, *E. boui*, *E. gracilis*, *E. elaphoides*, *E. phreatica*), which are characterised by relatively slow horizontal flow,

normally possess long and soft (exceptionally strong) setae on the baseoendopodite of P5, which is normally longer or equal in length with Exp. Caudal rami are normally parallel or there is a small angle between their longitudinal axes and setae I–VI are normally long and soft, including seta V, which is the longest.

In contrast to benthic/hyporheic/phreatic species, which are limited to those saturated habitats, some species from the unsaturated karstic zone can also build a viable population in other types of habitats (Table 2). The most extreme case is *E. millennii* n. sp. which occupies both unsaturated and saturated fissured aquifers as well as porous ones. Taxa from unsaturated karstic zone share some morphological characteristics. The most common are short and strong or (more commonly) robust setae on the baseoendopodite of P5 and short setae I–VI on caudal rami. Exp P5 is normally shorter than length of baseoendopodite and in some taxa very small (reduced). The angle between longitudinal axes of caudal rami varies between 22.5 and 45° (the largest is in *E. franci*, *E. tarmani* and *E. millennii* n. sp.). Most of *Elaphoidella* taxa from unsaturated fissured aquifers belong to the III (*-jeanneli*) group sensu Lang (1948) except for *E. cvetkae* (group I sensu Lang 1948) and *E. karstica* (group X sensu Lang 1948). Their body lengths are small compared to taxa inhabiting benthos, hyporheic, phreatic and saturated karstic zone habitats.

In the case of stygobitic *Elaphoidella* species in unsaturated zones in Slovenia, the most likely scenario is allopatric speciation (rather than adaptive radiation), based on a theory of geological fractionation of limestone/dolomite blocks which act as islands with rather limited horizontal hydrological, and consequently genetic, communication.

#### Convergent evolution in caves

The unsaturated karstic zone is a place where convergent evolution also took place. In three genera from Slovenia, *Morariopsis* (with representatives: *M. scotenophila*, *M. dumonti*), *Paramorariopsis* (representatives: *P. anae*, *P. irenae*) and *Elaphoidella* (*E. millennii*, *E. tarmani*), morphological similarities could be observed (Fig. 7). Apart from having short and robust spines on P5, decreasing in size from *Elaphoidella* to *Morariopsis* to *Paramorariopsis*,



**Table 2** Morphological characteristics of females of 13 *Elaphoidella* species inhabiting different habitats in Slovenia

Females species	Habitat	P5: setae on baseoendopod	P5: Exp. vs. baseoendopod	P4: length of Endp vs. outer terminal seta	Fu: width of base vs. length	Fu: angle between rami	Fu: form of setae I–VI	Group (after Lang, 1948)	Body length (mm)
<i>Elaphoidella bidens</i>	H	long, soft	longer	c. 1:4–5	c. 1:1	parallel	long, soft	II	0.48–0.80
<i>Elaphoidella boui</i>	H, B	short, strong	shorter	c. 1:3	c. 1:2	small	short, strong	I	0.65–0.82
<i>Elaphoidella gracilis</i>	H, P, B	long, strong	longer	c. 1:2–3	c. 1:2	parallel	long, soft	II	0.60–0.74
<i>Elaphoidella elaphoides</i>	cSZ, sSZ, H, P	long, strong	equal	c. 1:1.5	c. 1:1.5	small	short, soft	IV	0.58–0.64
<i>Elaphoidella phreatica</i>	sUZ, H	long, strong	equal	c. 1:2	c. 1:3	big	long, soft	V	0.60–0.65
<i>Elaphoidella charon</i>	cUZ, sSZ, H, P	long, strong	shorter	c. 1:2	c. 1:1.5	small	short, soft	III	0.55–0.63
<i>Elaphoidella stammeri</i>	cUZ, sUZ	short, strong	reduced	c. 1:2	c. 1:1.5	small	short, soft	III	0.58–0.64
<i>Elaphoidella cvetkae</i>	cUZ, sUZ	short, robust	reduced	c. 1:3	c. 1:2	small	short, soft	I	0.70–0.73
<i>Elaphoidella franci</i>	cUZ	short, robust	shorter	c. 1:1	c. 1:3.5	big	short, soft	III	0.73
<i>Elaphoidella tarmani</i> n. sp.	cUZ, sUZ	short, robust	reduced	c. 1:1	c. 1:3.5	big	short, soft	III	0.38–0.41
<i>Elaphoidella jeanneli</i>	cUZ, cSZ, H	short, robust	shorter	c. 1:1	c. 1:1.5	small	short, strong	III	0.45–0.51
<i>Elaphoidella millemii</i> n. sp.	cUZ, sSZ, H, P	short, robust	shorter	c. 1:1	c. 1:2	big	short, robust	III	0.42–0.44
<i>Elaphoidella karstica</i>	cUZ	no data	no data	no data	no data	no data	no data	X	no data
<i>Paramorariopsis irenae</i>	cUZ	short, robust	reduced	c. 1:1.5	c. 1:1.5	big	short, robust		0.40–0.42
<i>Morarariopsis dumonti</i>	cUZ	short, robust	equal	c. 1:1.5	c. 1:2	big	short, robust		0.35–0.44

Two specialised species from unsaturated karstic zone (= epikarst) are added for comparison (*P. irenae* and *M. dumonti*)

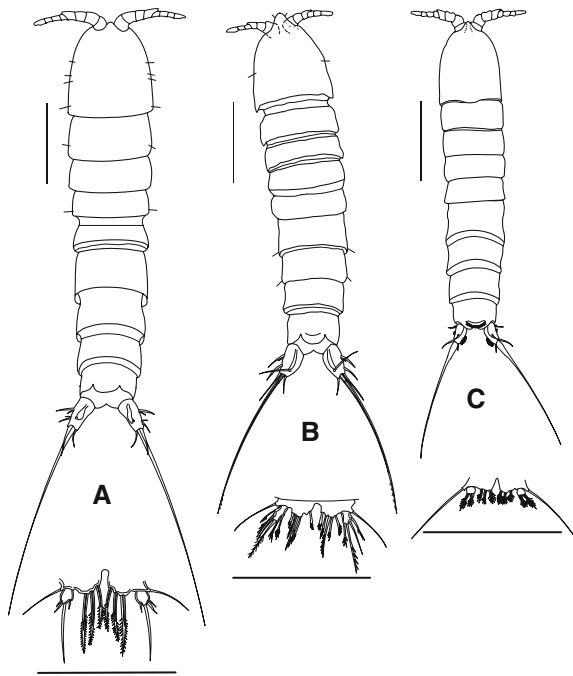
*Habitat*: B benthos, H hyporheic, P phreatic, cSZ saturated zone in cave, cUZ unsaturated zone in cave, sSZ spring on the upper level of saturated karstic zone, sUZ spring in unsaturated karstic zone

*P5: setae on baseoendopod*: long: longer than genital segment; short: shorter than genital segment; soft: plumose-like setae; strong: spiniform and barbed; robust: pinnate spines

*P5: length of Exp. vs. baseoendopod*: longer: Exp. < baseoendopod; equal: Exp. ≈ baseoendopod; shorter: Exp. > baseoendopod; reduced: Exp. small

*Fu: angle between rami*: parallel: no angle; small: angle between rami > 22.5°; big: angle between rami < 22.5° (up to 45°)

*Fu: form of setae I–VI*: long: longer than width of base of caudal ramus; short: shorter than width of base of caudal ramus; soft: seta-like; strong: spine-like; robust: spine-like & arcuated (esp. outer ones)



**Fig. 7** Habitus and shape of P5 in three genera of epikarstic Harpacticoida: an example of convergent evolution in caves. **A** *Elaphoidella millennii* n. sp.; **B** *Morariopsis dumonti*, Brancelj, 2000; **C** *Paramorariopsis irenae*, Brancelj, 2006. Scale bar 100  $\mu$ m

there is a great similarity in body habitus (elongated with short antennules) especially in a region of anal somite and armature of caudal rami. Caudal rami in mentioned taxa are short (but *E. tarmani*), robust and very divergent (up to  $45^\circ$ ), with the inner terminal seta (V) relatively short, strong and wide apart. Outer lateral setae (I–(III)–IV) are short, robust and arcuated, while terminal accessory seta (VI) is normally short, spiniform and arcuated.

The above-mentioned species share an identical habitat, i.e. epikarst, situated just below the soil layer. In the cave Velika Pasjica, specimens of *E. millennii* and *M. dumonti* (sporadically also *E. tarmani*) are regularly collected in dripping water from epikarst. For other caves and species such intimate co-existence of taxa has not yet been observed. The epikarst is characterised by a honeycomb of small fractures, filled by rainwater, and there is also capillary water. Narrow fissures are linked to the general, vermiform habitus of inhabitants, with short antennules. It is of vital importance for animals inhabiting such small narrow spaces that setae on legs are short and robust, to enable them to move

efficiently and to prevent accidental drifting in a vertical direction, especially during heavy rain.

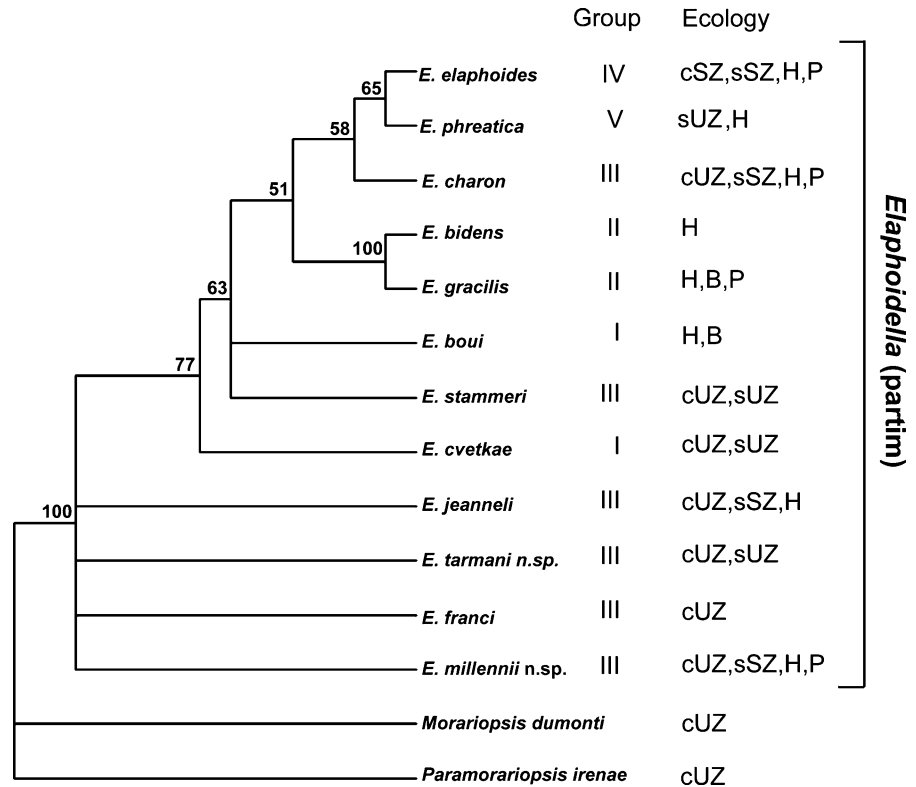
Highly specialised morphological adaptations to narrow spaces in fissured aquifers do not prevent some species from also surviving in other habitats. An example is *E. millennii* which has also been recorded in habitats which are in direct or indirect contact with the epikarst. Rainwater from Krim massif is directly drained, via fissures, into porous aquifers just at the foothills of the massif. Specimens of *E. millennii* have a good morphological predisposition for life in porous aquifers (and potentially also specimens of *Morariopsis* and *Paramorariopsis*). But so far, only *E. millennii* has been found in both aquifers which indicates: (1) high competitiveness of the species in different habitats or (2) high reproduction in fissured aquifers from where a constant drift of population is assured. In any case, both options indicate a high competitive/reproduction fitness of *E. millennii*.

#### Cladistic analysis on *Elaphoidella* inhabiting different habitats in Slovenia

Heuristic search yielded 426 trees with tree length 50, consistency index (CI) = 0.5400, homoplasy index (HI) = 0.4600, retention index (RI) = 0.5741 and rescaled consistency index (RC) = 0.3100. The strict consensus tree (not shown here) resulted in polytomy for genus *Elaphoidella* except for the pair *E. bidens* and *E. gracilis*. We show the 50% majority rule tree. The 50% majority rule consensus tree (Fig. 8) shows relatively basal position of *E. tarmani* n. sp. and *E. millennii* n. sp. The latter two taxa are in polytomy with *E. jeanelli* and *E. franci*, and basal to the rest of the taxa. The cladogram shows relatively moderate support (bootstrap 77) for grouping of the other *Elaphoidella* species, and support is relatively low to very low for all branches in this clade, except for species pair *E. bidens*–*E. gracilis*. The presented cladogram (Fig. 8) thus represents primarily an illustration of morphological (di)similarities, in this case interpreted as convergences due to similar pressures of the environment and evolutionary restraints of the organisms, and does not reflect real phylogenetic relationships.

It is clear from Fig. 8 that taxa belonging to Group III (Lang, 1948), to which the two new species belong, do not form a monophyletic group. *E. cvetkae* for

**Fig. 8** Morphology of 12 *Elaphoidella* species known so far from Slovenia and *Morariopsis dumonti* and *Paramorariopsis irenae* as out-group. Characters for dendrogram were based on female morphology only. Additional columns refer to habitats and groups according to Lang (1948). These groupings are not included in the analysis. Abbreviations for habitat: *B* benthos, *H* hyporheic, *P* phreatic, *cSZ* saturated zone in cave, *cUZ* unsaturated zone in cave, *sSZ* spring on the upper level of saturated karstic zone, *sUZ* spring in unsaturated karstic zone



example belongs to Group I, but appears between Group III species (*E. jeanneli* and *E. stammeri*). The same applies to *E. charon* which appears close to *E. elaphoides* and *E. phreatica*. However, bootstrap value there is low (i.e. 58), so the branches with such a support can be interpreted as almost polytomic. The small dendrogram presented in Fig. 8 is based on females' characters only, whereas Lang's characters are based on a combination of characters on P5 of both sexes. The shape and armature of P5 in both sexes are conservative within each group; thus from a cladistic point of view they are a parsimony, and the information were added after analysis such as on the habitats occupied by each taxon. The cladogram shows very low support for most branches in this clade, especially those connected with highly endemic and specialised taxa in the unsaturated zone. This supports an idea that allopatric speciation of stygobitic *Elaphoidella* in the unsaturated zone leads to convergent evolution (= parallelisms) but at the same time some conservative characters remain unchanged. From this point of view we can also explain the outstanding position of *E. charon* (belonging to group III), which is in ecology as well as in many morphological characters

closer to the *E. jeanneli*/*E. millennii* complex than to *E. elaphoides*/*E. phreatica*. As mentioned before, for a final answer on phylogenetic relationships we will have to wait for biochemical analyses and not rely only on morphology.

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