*Hydrobiologia* **412:** 165–176, 1999.



# Two new species of the *Diacyclops languidoides*-group (Copepoda, Cyclopoida) from groundwaters of Austria

Peter Pospisil[1] & Fabio Stoch[2]*
[1]*Zoological Institute, University of Vienna, A-1090 Vienna, Austria*
[2]*Museum of Natural History, Trieste, Italy. Current address: Viale XXV Aprile 24, I-34015 Muggia (Trieste), Italy*
*E-mail: fabiocop@tin.it*

Received 24 November 1998. in revised form 29 June 1999; accepted 7 July 1999

*Key words:* copepods, cyclopoids, *Diacyclops*, groundwaters

## Abstract

Two new syntopic species of the *Diacyclops languidoides*-group (*D. danielopoli* n. sp. and *D. felix* n. sp.) are described from interstitial groundwaters of Lobau wetlands, Austria. Both species lack the exopodal seta on antenna basipod, but differ in several minute morphological details of antennules, antennae, maxillulary palp, coxal plates, P4 basipod and caudal rami. Some of these characters are present in *D. danielopoli* n. sp. in an apomorphic state (reduction of setation patterns, longer aesthetascs on male and female antennules, absence of exopodal seta on maxillulary palp, longer dorsal caudal seta) suggesting a higher degree of specialization to the subterranean environment than in *D. felix* n. sp. The relationship between the reduction of setation patterns on mouthparts and the degree of stygobitization in the *Diacyclops languidoides*-species group is critically discussed.

## Introduction

The *Diacyclops languidoides*-group of species includes several poorly known taxa (usually reported as subspecies of *Diacyclops languidoides* Lilljeborg, 1901) and is in urgent need of revision. Einsle (1993) raised some doubts about the validity of certain taxa, which are poorly described even in recent literature (Pesce & Galassi, 1987) and supposed that the observed differences may be due to environmental variability.

In view of the ecological importance of this species group, which includes several stygophilic and stygobiont species which are sometimes very common in groundwater samples, a revision of the whole species complex was carried out by one of us (Fabio Stoch) after a careful examination of all existing type material. The first results (Stoch, in press) suggest that several valid species are included in the group, but unfortunately their distinction is not easy and is based on minute morphological details. The use of these details may be properly evaluated when more species are syntopic and one of these events is taken in account in the present paper.

During an ecological and faunistic survey of alluvial groundwaters in the Lobau wetlands (Pospisil, 1994a,b), several samples collected in wells yielded specimens of cyclopoids which were ascribed to some unnamed species of the *Diacyclops languidoides*-group (Pospisil, 1994b). Two of these species are described herein as new to science and the differential characters used in this study are evaluated in relation to their degree of adaptation to the subterranean environment.

## Material and methods

The specimens were collected from groundwater piezometers with a diameter of about 5 cm using a double-packer-sampler (Danielopol & Niederreiter, 1987). The sample was filtered through a 100 $\mu$m plankton-net and stored in 4% formaldehyde.

* Author for correspondence

Copepods were extracted from detritus under a stereomicroscope; selected specimens were dissected in glycerine and permanently mounted on slides in glycerine sealed with Caedax. A Zeiss Axioskop microscope fitted with a drawing tube was used to study the details at 1000× using an oil immersion lens.

The terminology related to the external morphology follows Huys & Boxshall (1991).

## Taxonomic account

Family Cyclopidae Burmeister, 1834
Subfamily Cyclopinae Dana, 1853 emend. Kiefer, 1927
Genus *Diacyclops* Kiefer, 1927

*Diacyclops danielopoli* n. sp. (Figures 1–3)

Synonymy – *Diacyclops languidoides* 'D': Pospisil, 1994b: 88.

*Material examined*: Twenty one ♀♀ and six ♂♂ were collected between June and July, 1988 from groundwater observation wells A66, A84, A87, A89 and A90 in the 'Lobau' riverine wetland, Danubian basin, Vienna, Austria (map of locations in Pospisil, 1994). One ♀ and one ♂ were collected in October, 1992 from groundwater observation well A89, same area. Four ♀♀ collected on 24/09/95 in groundwater observation well 2/4 in the 'Regelsbrunn' riverine wetland near Haslau, Lower Austria.

*Type series*: Holotype ♀ (slide no. 521), allotype ♂ (slide no. 295), paratypes two ♂♂, two ♀♀, completely dissected and mounted on slides in glycerine, deposited in the Museum of Natural History, Vienna. Other paratypes in the authors' collections.

*Type locality*: Lobau riverine wetland, 'Hanslgrund' region, Austria.

*Etymology*: The new species is dedicated to Prof. Dan Danielopol (Mondsee) for his contribution to the knowledge of the groundwater fauna of the Danube near Vienna.

*Female*: Length, excluding caudal setae, 442–578 $\mu$m (average 497 $\mu$m, $n$=22). Habitus as in Figures 1a,b. Hyaline fringes of posterior margins of urosomites not crenulate (Figure 1a); urosomites with dorsal and ventral sides smooth. Genital double somite as in Figure 1d, broadest in anterior half, tapering posteriorly, greatest breadth about 1.08 times length. Seminal receptacle with broader anterior part, posterior expansion little produced, as in Figure 1d. Anal somite with lateral rows of spinules on distal margin (Figure 1c), bearing two sensilla on dorsal surface; anal operculum broad, slightly convex and weakly sclerotized, dorsal surface bearing two rows of hairs. Caudal ramus about 2.5–2.8 times longer than wide (Figure 1c), average length 46 $\mu$m, without ornamentation. Anterolateral caudal seta shorter than width of caudal ramus (average length 16.4 $\mu$m); terminal accessory seta 1.2–1.8 times longer than posterolateral seta (average ratio 1.43), their average lengths respectively 25.6 and 36.5 $\mu$m; average values of outer and inner terminal setae length 127.3 and 239.2 $\mu$m; dorsal seta very long (average length 63.2 $\mu$m), approximately 1.3 times longer than caudal ramus.

Antennule (Figure 1e) eleven segmented, its length aproximately 37% length of body; segment one, with a short comb of spinules, surfaces of the other segments smooth; segment eight (Figure 1f) with one long aesthetasc of Cyclopinae-type (sensu Pospisil & Stoch, 1997), extending beyond the first half of segment 10. Segments (with number of setae and aesthetascs in brackets): 1[8], 2[4], 3[8], 4[4], 5[2], 6[2], 7[3], 8[2 + 1 aesth.], 9[2], 10[3], 11[8]. Antenna (Figure 2a): coxa unarmed; basis with simple spinulation pattern, as in Figure 2b, bearing two setae; exopod absent. Endopod segment one with one seta; endopod segment two typically with seven setae, one female with six setae (on both antennae); endopod segment three with seven distal setae.

Labrum typical of the genus. Mandible (Figure 1g): coxa without spinules, gnathobase with four stout teeth, a comb of spinules and one spinulose seta; mandibular palp represented by one short and two long plumose setae. Maxillule (Figure 2c) comprising praecoxa and maxillulary palp; praecoxa with a short proximal spine; praecoxal endite typical of the genus *Diacyclops*, bearing six spinulose setae and three naked spines; palp segment one (derived from coxa and basis) without spinules, bearing three setae; palp segment two (endopod) bearing three setae; exopodal seta absent (Figure 2c). Maxillary syncoxa (Figure 2d) typical of subfamily, bearing three endites; basis with one claw and two setae; endopod segment one bearing two setae, segment two with three setae. Maxilliped (Figure 2e) four segmented as usual in family; second endopodal segment with one spinulose seta and two short naked setae.

Swimming legs: P1 exopod two segmented, P2–P4 exopods three segmented (Figures 2f–g and 3a–b); P1–P2 endopods two segmented, P3–P4 endo-

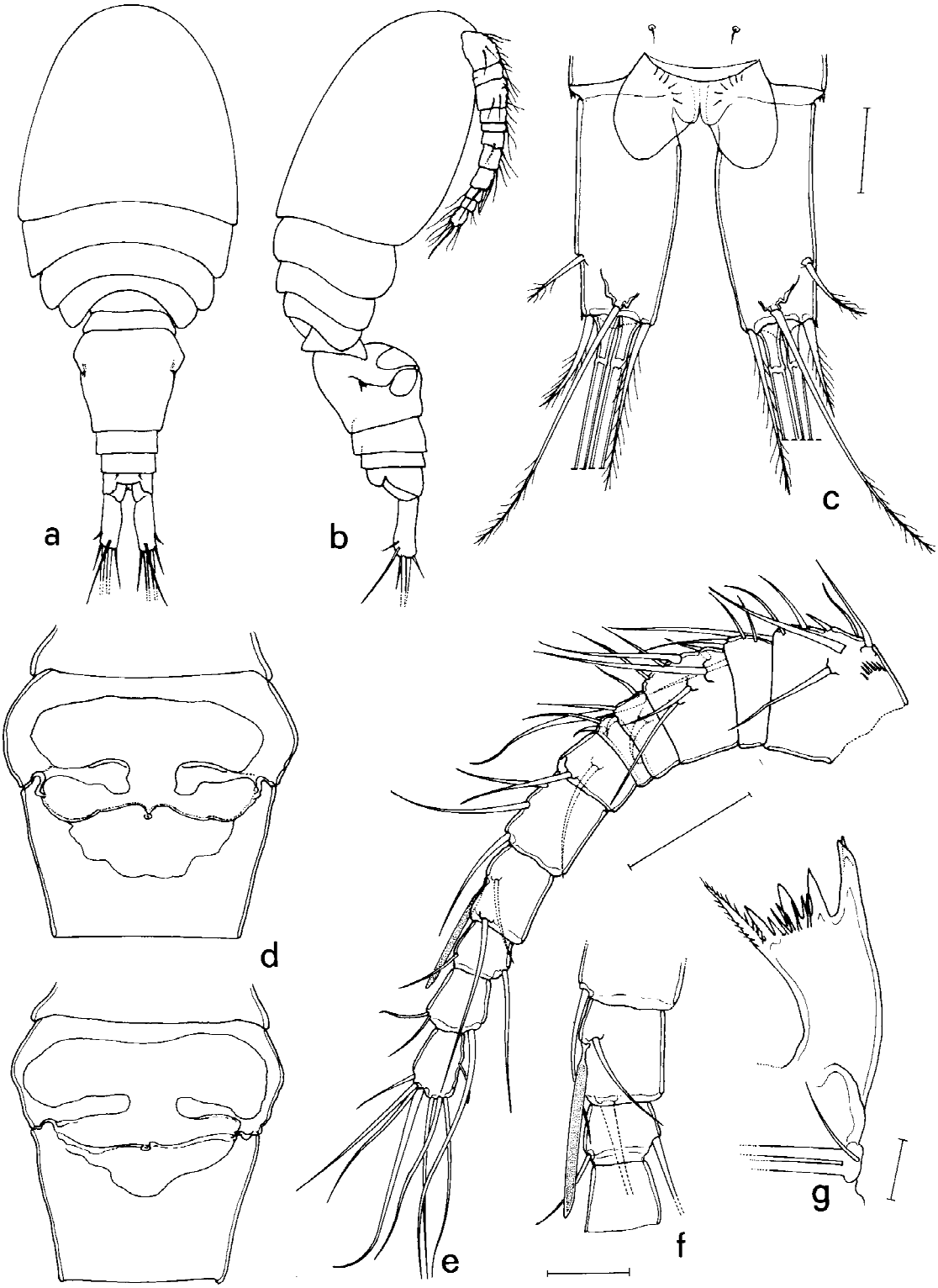


*Figure 1. Diacyclops danielopoli* n. sp. ♀: (a) habitus, dorsal view; (b) habitus, lateral view; (c) caudal rami, dorsal view; (d) genital double somite and seminal receptacle, ventral view; (e) antennule; (f) detail of antennulary segments 8–10; (g) mandible. Scale bars: 10 $\mu$m (g), 20 $\mu$m (c, f) and 50 $\mu$m (e).

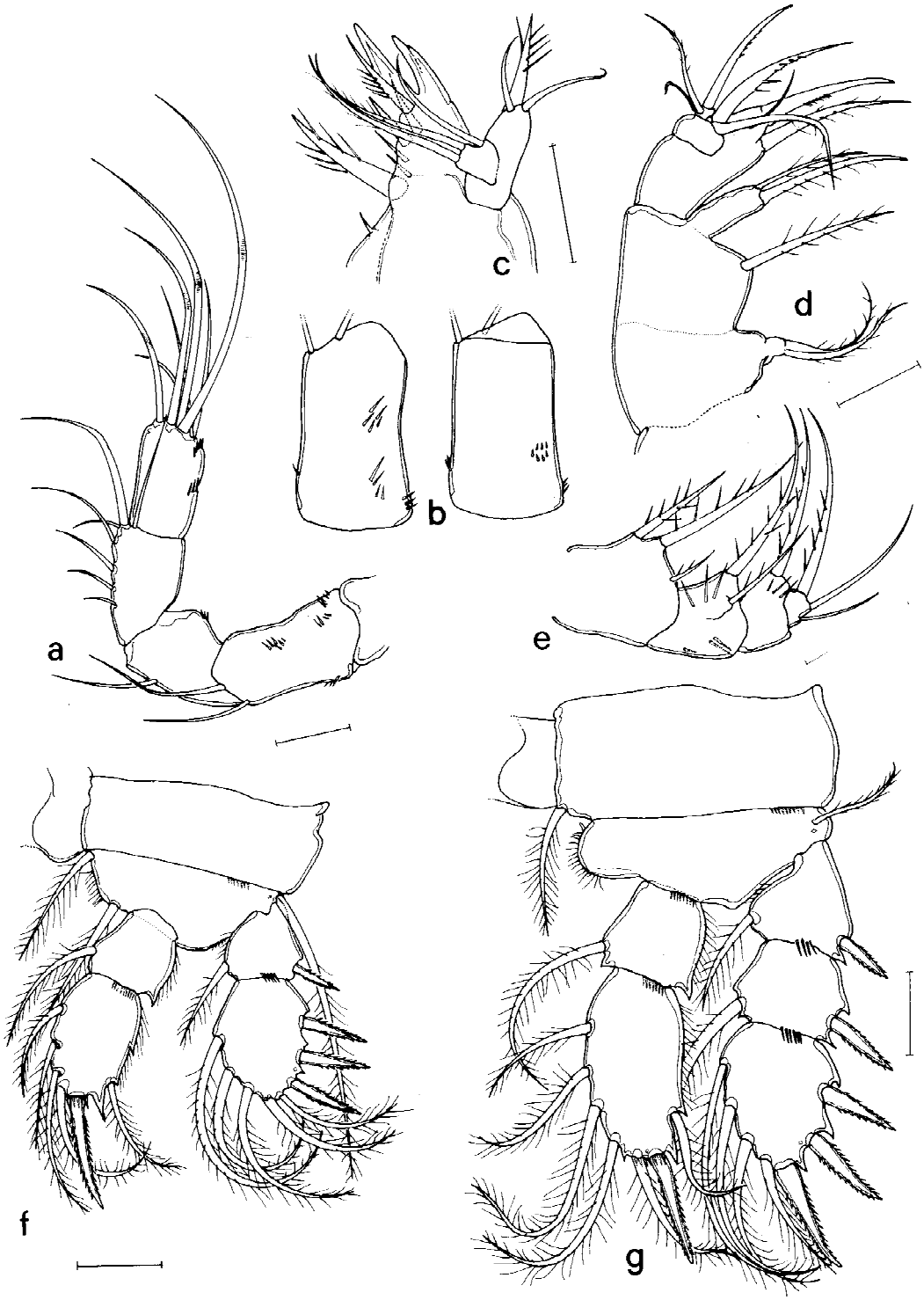


*Figure 2. Diacyclops danielopoli* n. sp. ♀: (a) antenna; (b) antennary basis with spinule pattern on caudal (left) and frontal (right) sides; (c) maxillule; (d) maxilla; (e) maxilliped; (f) P1; (g) P2. Scale bars: 20 $\mu$m.

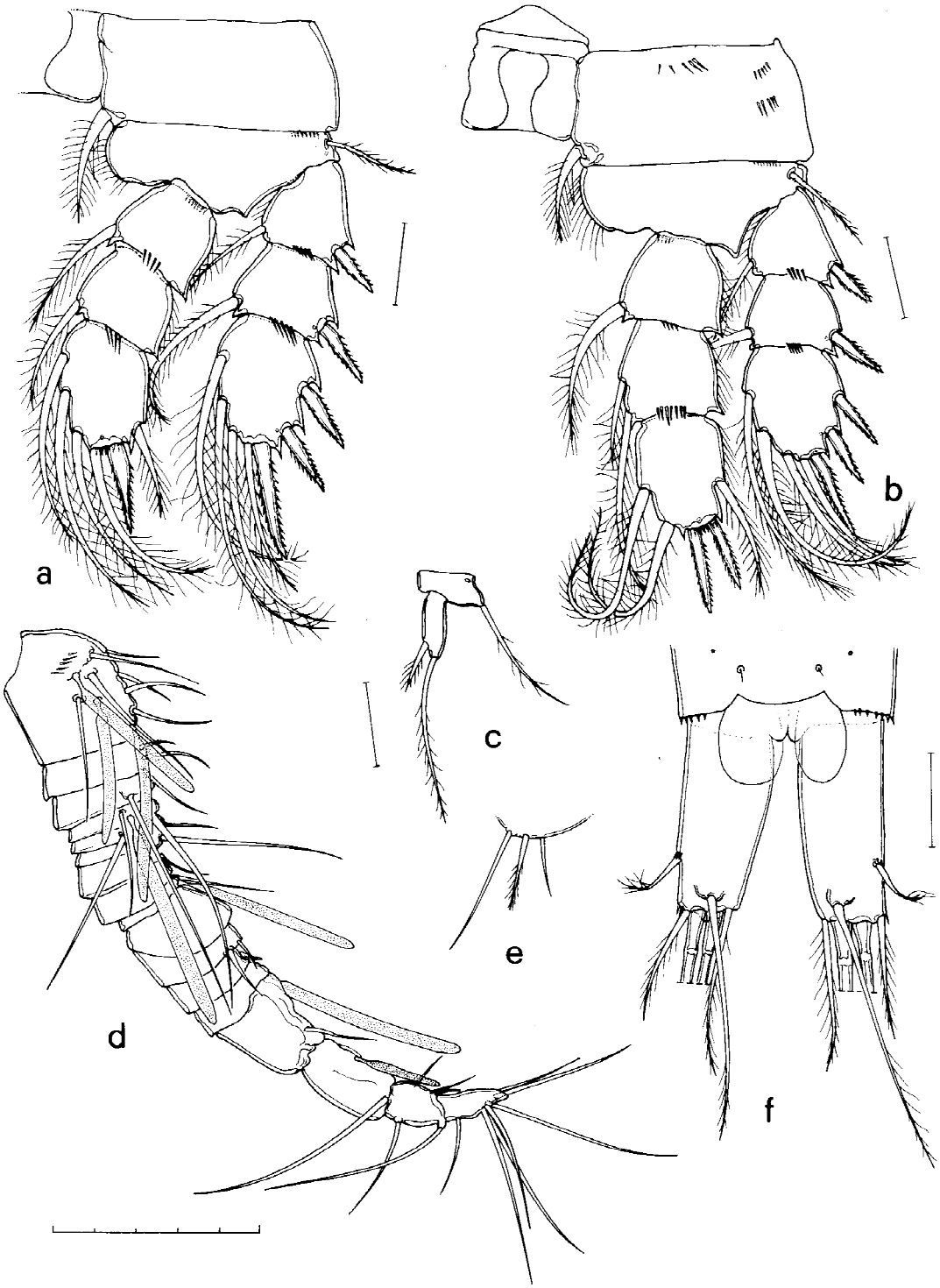


*Figure 3. Diacyclops danielopoli* n. sp. ♀: (a) P3; (b) P4; (c) P5. ♂: (d) antennule; (e) P6; (f) caudal rami, dorsal view. Scale bars: 20 $\mu$m (a, b, c, f) and 50 $\mu$m (d).

pods three segmented, as usual in the *Diacyclops languidoides*-group; pores as in Figures 2f–g and 3a–b. Distal segments of exopods one to four with 3,3,3,3 spines and 5,4,4,4 setae respectively. Intercoxal sclerites without ornamentation. P4 (Figure 3b): coxa with spinulation pattern as in figure; basis inner margin rounded, with a distinct notch and without inner sclerified tooth; third endopodal segment 1.17–1.46 times longer than wide (average value 1.33); inner terminal spine 0.68–0.93 times longer than segment (average value 0.79), slightly longer than outer terminal spine. P5 (Figure 3c): protopodal segment with one outer plumose seta and one pore; exopodal segment about twice as long as wide, armed with one seta and one inner spine shorter than segment. P6 (Figure 1b) bearing three short spinous processes.

*Male*: Mean length, excluding caudal setae, 452 $\mu$m. Hyaline fringes of posterior margins of urosomites smooth. Caudal ramus about 2.6 times longer than wide (Figure 3f), without ornamentation. Proportion of lengths of setae much as in female.

Antennule (Figure 3d): digeniculate, 17-segmented; neocopepodan distal geniculation between segments 14 and 15. 6 long aesthetascs, typical of subfamily (Pospisil & Stoch, 1997), are located on segments one (three aesthetascs), four, nine and 13; a smaller one is located on segment 15.

Antenna: as in female, endopod segment two with six setae. Other mouthparts and legs one to five as in female.

P6 (Figure 3e): forming opercular plate and bearing three slender setae.

*Remarks*: *Diacyclops danielopoli* n. sp. resembles several species of the *Diacyclops languidoides* - group e.g. *D. insularis* Monchenko, 1982 from Ukrain, *D. paolae* Pesce & Galassi, 1987 from Italy, Turkey and Albania, *D. clandestinus* Kiefer, 1926 widely distributed in Europe and Turkey (but probably confused in several localities with *D. paolae*), *D. sardous* Pesce & Galassi, 1987 from Sardinia, *D. eriophori* Gurney 1927 from Great Britain and some undescribed species from North America (Stoch & Reid, in prep.). All the above mentioned species share the absence of the exopodal seta on the antenna. One of us (Fabio Stoch) had the opportunity to re-examine the type material of these species with the exception of *D. insularis*; they clearly differ from *D. danielopoli* n. sp. by the following features: spinulation pattern of antennary basis, proportions of caudal setae, shape of endopodal segment three of P4, proportions of terminal spines of endopodal segment three of P4, shape of basis of P4; moreover *D. paolae* differs in the number of setae on endopodal segment two of maxilliped and other minor details (Stoch, in press).

*D. insularis* shares with *D. danielopoli* n. sp. the absence of exopodal seta on maxillulary palp (a feature shared with *D. paolae* and some other undescribed taxa: Stoch, in prep; Stoch & Reid, in prep.), but has longer caudal rami, shorter dorsal caudal seta, nine setae on endopod segment two of antenna (plate 3 of Monchenko (1982) erroneously shows eight setae), different shape of endopod segment two of P4 and the inner margin of P4 basis with a strong tooth.

*Distribution and habitat*: *D. danielopoli* n. sp. was only found in interstitial groundwaters several hundred meters away from permanently flooded surface waters over a research period of 7 years, and seems to be a species with a rather restricted ecological tolerance (Pospisil, 1994b and in prep.). Up to now, the species is known only from the Lobau riverine wetland near Vienna (Danubian basin). Closely related populations were recently discovered in Italy (Stoch, unpublished) and France (Galassi, pers. comm.), but their taxonomic status is still under study.

*Diacyclops felix* n. sp.

Synonymy
*Diacyclops languidoides* ‘B’: Pospisil, 1994b: 86.

*Material examined*: 28 ♀♀ and 17 ♂♂ were collected between January and July, 1988 from groundwater observation wells A63, A66, A81, A84, A85, A89 and A90 in the ‘Lobau’ riverine wetland near Vienna, Austria (map of locations in Pospisil, 1994). Twenty two ♀♀ and 15 ♂♂ were collected between May, 1991 and January, 1993 from groundwater observation wells T3, D3, D4, D10, D15 and D17 in Lobau (map of locations in Pospisil, 1994). Four ♀♀ sampled on 24/09/95 in groundwater observation wells 1/7 and 4/5 in the ‘Regelsbrunn’ riverine wetland near Haslau, Lower Austria.

*Type series*: Holotype ♀ (slide no. 195), allotype ♂ (slide no. 307), paratypes five ♀♀ and two ♂♂, all dissected and mounted on slides in glycerine, deposited in the Museum of Natural History, Vienna. Other material in the Authors‘ collections.

*Type locality*: Lobau riverine wetland, region around the ‘Kreuzgrundtraverse’, Austria.

*Etymology*: Name dedicated to Felix Gemeinhardt, godchild of Peter Pospisil.

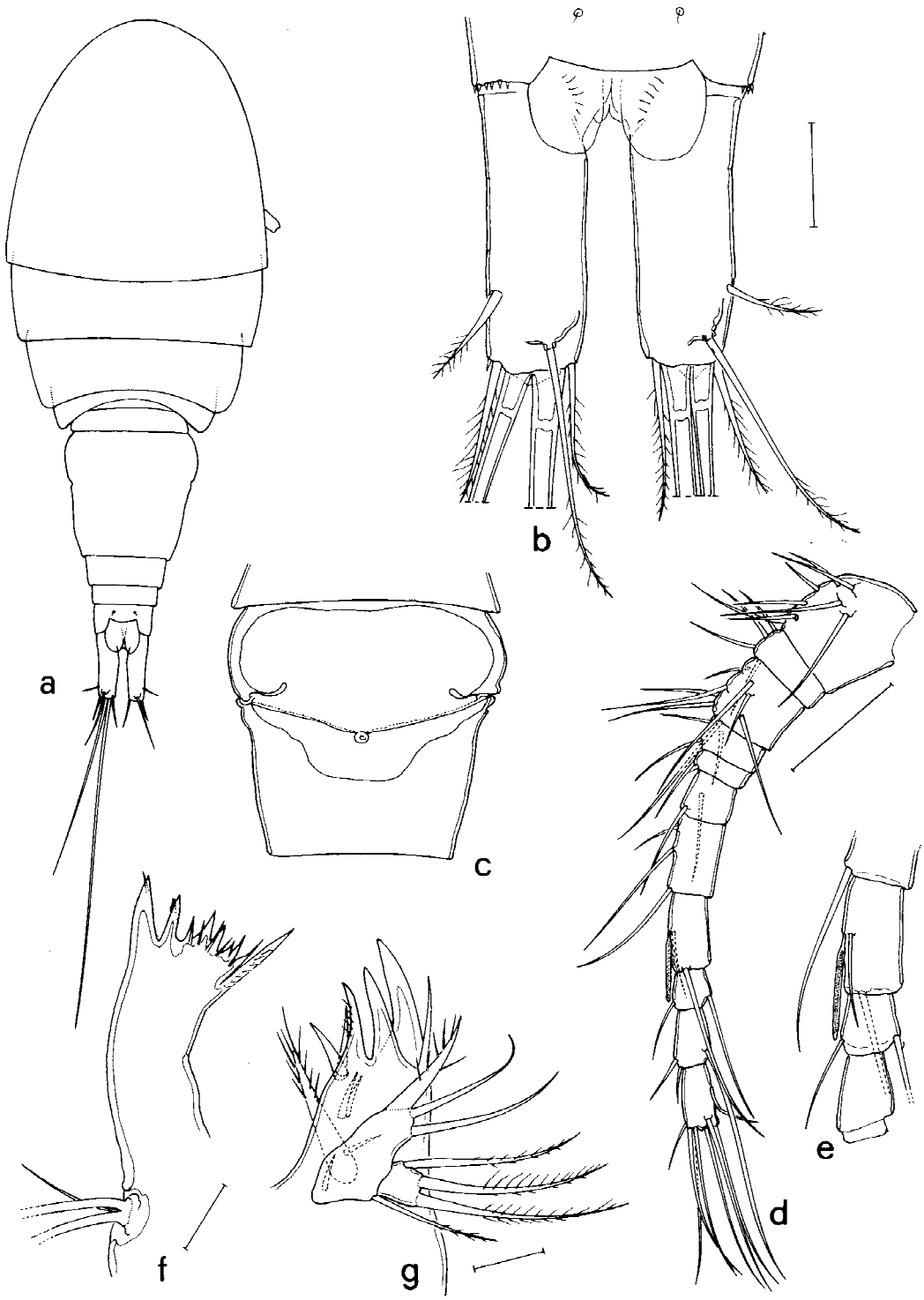


*Figure 4. Diacyclops felix* n. sp. ♀: (a) habitus, dorsal view; (b) caudal rami, dorsal view; (c) genital double somite and seminal receptacle, ventral view; (d) antennule; (e) detail of antennulary segments 8–10; (f) mandible; (g) maxillule. Scale bars: 10 $\mu$m (f, g), 20 $\mu$m (b) and 50 $\mu$m (d).

*Female*: Length, excluding caudal setae, 429–816 $\mu$m (average length 613 $\mu$m, $n$=53). Habitus as in Figure 4a. Hyaline fringes of posterior margins of urosomites not crenulate (Figure 4a); urosomites with dorsal and ventral sides smooth. Genital double somite as in Figure 4c, broadest in anterior half, tapering posteriorly, greatest breadth about 1.16 times length. Seminal receptacle with broader anterior part, posterior expansion little produced, as in Figure 4c. Anal somite with lateral rows of spinules on distal margin (Figure 4b), bearing two sensilla on dorsal surface; anal operculum broad, slightly convex and weakly sclerotized, dorsal surface bearing two rows of hairs. Caudal ramus about 2.9 times longer than wide (Figure 4b), average length 60 $\mu$m, without ornamentation. Anterolateral caudal seta as long as width of caudal ramus (average length 21 $\mu$m); terminal accessory seta 0.98–1.17 times longer than posterolateral seta (average ratio 1.08), their average lengths respectively 35 and 32 $\mu$m; average values of outer and inner terminal setae length 157 and 330 $\mu$m; dorsal seta slightly shorter (90%) than caudal ramus (average length 64 $\mu$m).

Antennule (Figure 4d) eleven-segmented, its length aproximately 40% length of body; segment one with a short comb of spinules, surfaces of the other segments smooth; segment eight (Figure 4e) with one aesthetasc of Cyclopinae-type (sensu Pospisil & Stoch, 1997), not reaching segment 10. Segments with number of setae and aesthetascs in brackets 1[8], 2[4], 3[8], 4[4], 5[2], 6[2], 7[3], 8[2 + 1 aesth.), 9[2], 10[3], 11[8].

Antenna (Figure 5b): coxa unarmed; basis with spinulation pattern simple, as in Figure 5c, bearing two setae; exopod absent. Endopod segment one with one seta; endopod segment two with nine setae; endopod segment three with seven distal setae.

Labrum typical of the genus. Mandible (Figure 4f): coxa without spinules, gnathobase with four stout teeth, a comb of spinules and one spinulose seta; mandibular palp represented by one short and two long plumose setae. Maxillule (Figure 4g) comprising praecoxa and maxillulary palp; praecoxal endite bearing eight setae and three spines; surface of palp segment one (derived from coxa and basis) smooth; palp segment two (endopod) bearing three setae; exopodal seta present. Maxillary syncoxa (Figure 5a) typical of the subfamily, bearing three endites; basis with one claw and two setae; endopod segment one bearing two setae, segment two with three setae. Maxilliped (Figure 5f) four segmented as usual in family; second endopodal segment with one spinulose seta and two short naked setae.

Swimming legs: P1 exopod two segmented, P2–P4 exopods three segmented (Figures 5d–e and 6a–b); P1–P2 endopods two segmented, P3–P4 endopods three-segmented, as usual in the *Diacyclops languidoides* – group; pores as in Figures 5d–e and 6a–b. Spines on endopods and exopods of P1–P4 stout, strongly sclerotized. Distal segments of exopods 1–4 with 3,3,3,3 spines and 5,4,4,4 setae respectively. Couplers of P2–P3 ornamented with a row of spinules; coupler of P4 (Figure 6c) ornamented with two rows of spinules on posterior surface and with one row of setules on anterior surface. P4 (Figure 6b): coxa with spinulation pattern on posterior surface as in figure; basis inner margin with a distinct notch accompanied by one inner and one outer teeth; third endopodal segment 1.26–1.78 times longer than wide (average value 1.48); inner terminal spine 0.72–0.98 times longer than segment (average value 0.83), slightly longer than outer terminal spine. P5 (Figure 6d): protopodal segment with one outer plumose seta and one pore; exopodal segment about twice as long as wide, armed with one seta and one inner spine shorter than segment. P6 (Figure 6e) bearing three short spinous processes.

*Male*: Mean length, excluding caudal setae, 537 $\mu$m. Hyaline fringes of posterior margins of urosomites smooth. Caudal ramus about 2.7 times longer than wide (Figure 6g), without ornamentation. Proportions of setae much as in female.

Antennule (Figure 6f) digeniculate, 17-segmented; neocopepodan distal geniculation between segments 14 and 15. Six aesthetascs shorter than in *Diacyclops danielopoli* n.sp., typical of subfamily (Pospisil & Stoch, 1997), are located on segments one (three aesth.), four, nine and 13; segment 15 lacking aesthetasc.

Antenna as in female, endopod segment two with seven setae. Mouthparts and legs one to five as in female.

P6 (Figure 6h) forming opercular plate and bearing two short and one long setae.

*Remarks*: *Diacyclops felix* n. sp. is closely related to the sympatric *Diacyclops danielopoli* n. sp. from which it can be differentiated on the basis of the following characters:

1. caudal ramus with terminal accessory seta 1.08 times longer than posterolateral seta (1.43 in *D. danielopoli* n. sp.)

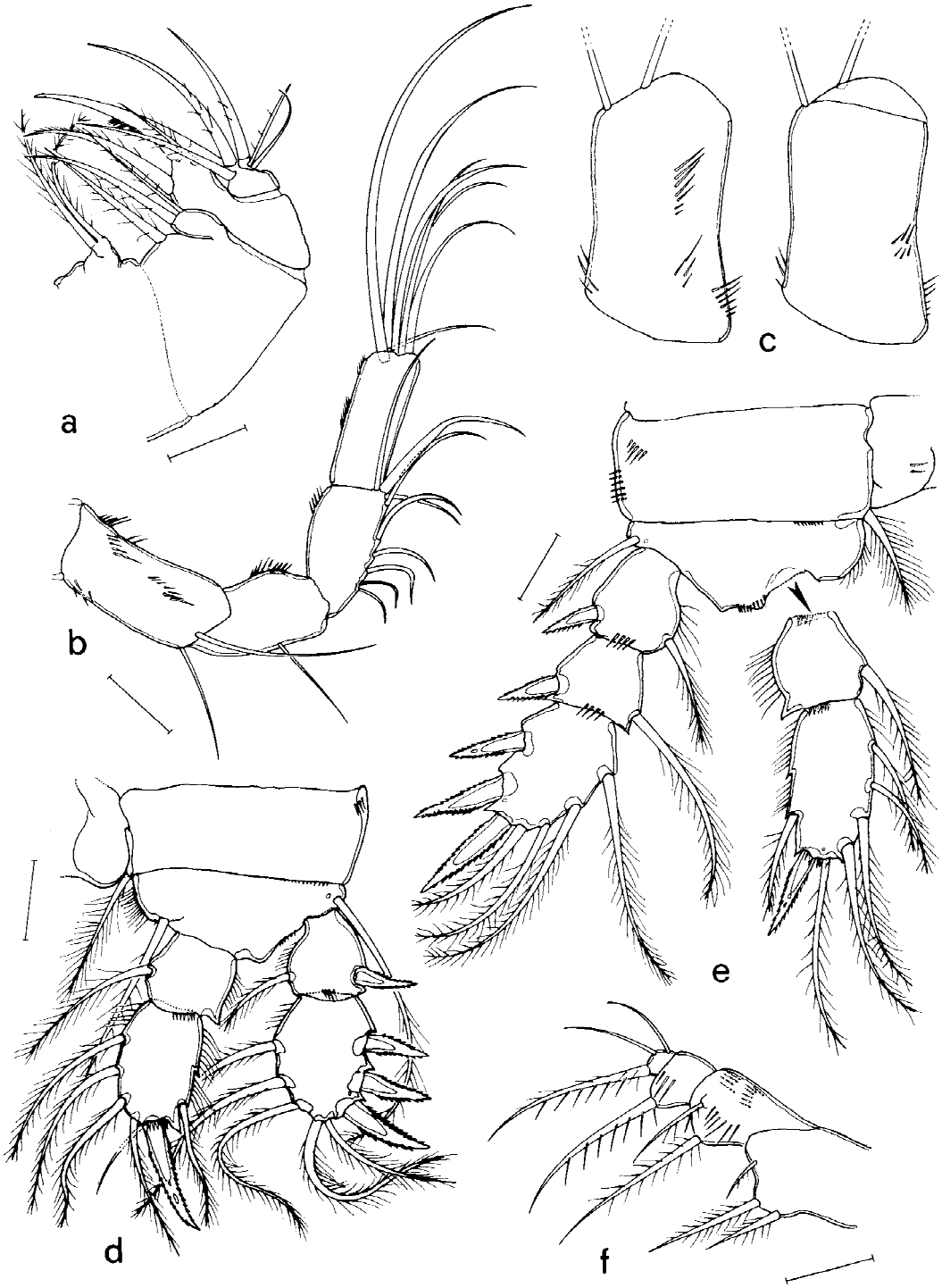


*Figure 5. Diacyclops felix* n. sp. ♀: (a) maxilla; (b) antenna; (c) antennary basis with spinule pattern on caudal (left) and frontal (right) sides; (d) P1; (e) P2; (f) maxilliped. Scale bars: 20 $\mu$m.

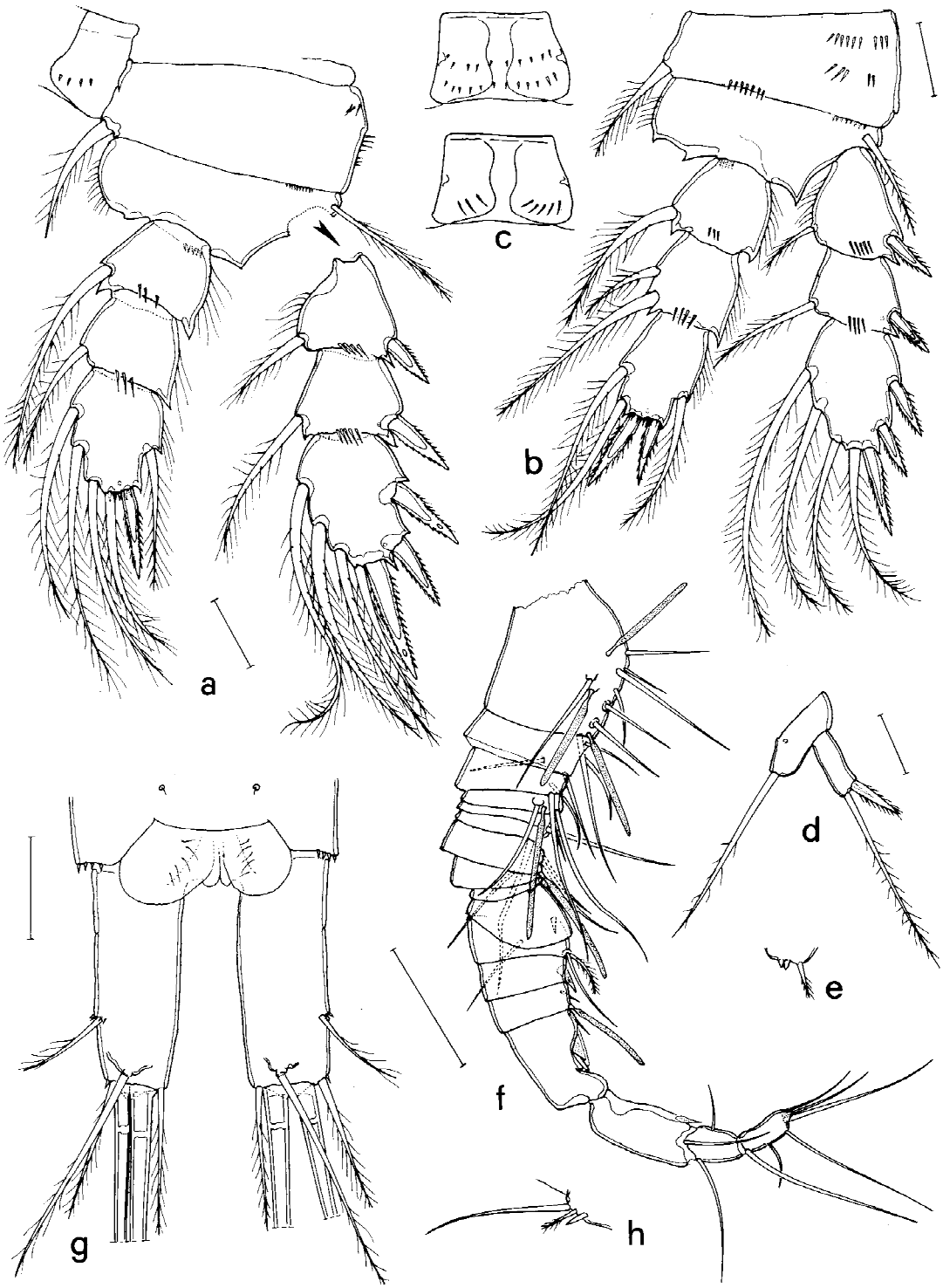


*Figure 6. Diacyclops felix* n. sp. ♀: (a) P3; (b) P4; (c) intercoxal sclerite of P4, posterior (above) and anterior (below) surfaces; (d) P5; (e) P6. ♂: (f) antennule; (g) caudal rami, dorsal view; (h) P6. Scale bars: 20 $\mu$m (a, b, d, g) and 50 $\mu$m (f).

2. dorsal seta slightly shorter than caudal ramus (1.3 times longer than caudal ramus in *D. danielopoli* n. sp.)
3. antennule of female with segments 7–11 more slender
4. antennule of female with shorter aesthetasc on segment eight
5. antennary endopod segment one of female bearing nine setae
6. slender spinules on antennary basis
7. maxillulary palp bearing exopodal seta
8. intercoxal sclerites of P1–P4 ornamented with rows of spinules and setules
9. inner margin of P4 basis with a large notch delimited by two sclerotized teeth
10. male antennule bearing shorter aesthetascs
11. male antennule segment 15 lacking aesthetasc
12. stouter, more sclerotized spines on P1–P4
13. setation of male P6.

This unique combination of features allows a distinction of *Diacyclops felix* n. sp. from all other members of the *Diacyclops languidoides*-group.

*Distribution and habitat*: *D. felix* n. sp. was found in river bed sediments and in groundwaters close to old arms of the Danube river, as well as in sampling wells located several hundred meters away from surface waters. *D. felix* n. sp. was found to co-exist with *D. danielopoli* n. sp. from four wells (A66, A84, A89, A90).

## Discussion

The detailed morphological analysis of the *Diacyclops* populations inhabiting groundwaters in the Lobau area clearly demonstrate that several, closely related species of the *Diacyclops languidoides*-group may coexist in the same habitat, supporting the observations of Stoch (1995) and Pesce & Galassi (1987). Unfortunately, the discrimination of the two new species is based on minute characters of antennules, antennae, mouthparts and swimming legs which require a complete dissection of the specimens and careful examination. In several modern taxonomic accounts of cyclopid species, there is often no description or illustration of the mouthparts. Moreover, the setation pattern of male and female antennules and antennae is often omitted or described erroneously, and aesthetascs are usually overlooked. This may explain the alledged circumboreal distribution of *Diacyclops languidoides* (Lilljeborg), being reported in papers dealing with groundwater ecology in Europe, Asia and North America, while this species is probably confined to epigean waters of Scandinavia (Stoch, unpublished observations). Moreover, groundwater biodiversity of the sampling sites is frequently underestimated, considering that up to five closely related species (Pospisil, 1994b; Stoch, unpublished) may be concealed under this name in the same location.

The careful examination of the morphology of the two new species of *Diacyclops* described above revealed some interesting differences in minute morphological details, suggesting a different degree of adaptation to the subterranean environment. *Diacyclops danielopoli* n. sp. shows several characters in the apomorphic state (female antennule with shorter segments 7–11; reduction in the number of setae on antennal endopod segment two; absence of exopodal seta on maxillulary palp; longer aesthetascs in male and female antennules; longer dorsal caudal seta; absence of ornamentation on intercoxal sclerites) which could suggest a higher degree of adaptation to the subterranean habitat in comparison to *Diacyclops felix* n. sp. This observation is in agreement with its more restricted distribution in the Lobau area pointed out by Pospisil (1994b).

Nevertheless, the relationship between the reduction in the number of setae on antenna and the degree of stygobitization of the species of the *Diacyclops languidoides*-group, suggested by some authors (Monchenko, 1982; Pesce & Galassi, 1985), should be treated with caution. It is noteworthy that the antennary exopodal seta is absent in both *Diacyclops danielopoli* n. sp. and *Diacyclops felix* n. sp.; this feature was considered typical of stygobiont species by Pesce & Galassi (1985), but recent observations (Stoch, unpublished) demonstrated that some epigean species also lack the antennary exopod, while some specialized stygobionts retain this feature. Moreover, this seta is lacking also in some species related to *Diacyclops langvidus* Sars, 1863 (Stoch, unpublished; Fiers, in litt.), and this fact is probably due to convergence or parallel evolution.

## Acknowledgements

The authors are indebted to Dan Danielopol (Mondsee) for supporting this work in every possible way, to Ilse Wenzel for supplying the Regelsbrunn material, and to the staff of the ‘Lobau’ forest admin-

istration, R. Schreckeneder, G. Haubenberger, A. Placho and H. Tomsic (Gross-Enzersdorf). The Austrian Science Foundation 'Fonds zur Förderung der wissenschaftlichen Forschung' (FWF-projects 7881-Bio and 11149-Bio) supported this work financially.

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