



How many species of *Diacyclops*? New taxonomic characters and species richness in a freshwater cyclopoid genus (Copepoda, Cyclopoida)

Fabio Stoch

Museum of Natural History, Verona, Italy.

Address for correspondence: Viale XXV Aprile 24, I-34015, Muggia (Trieste), Italy

E-mail: fabiocop@tin.it

Key words: copepod taxonomy, *Diacyclops*, species richness

Abstract

The genus *Diacyclops* Kiefer, 1927 emend. Morton (1985) includes more than 100 species, widely distributed in all kinds of freshwater environments, and is the richest genus in the family Cyclopidae. Traditionally, *Diacyclops* species were defined according to differences in a few morphological characters; some characters (number of antennular segments, segmentation pattern of swimming legs) are useful only in the separation of species groups, others (length of caudal rami and caudal setae) are highly variable even within the same population. During the study of the *D. languidoides*-group, minute morphological characters were used to differentiate between species (spinulation pattern and setation of antennary basis, setation and aesthetasc shape of male antennule, setation of mandibular palp and maxilliped, shape of leg 4 basis) which allow to identify several valid species up to now concealed under the name '*Diacyclops languidoides*' (Lilljeborg, 1901). The coexistence of up to six congeneric species in the same sampling area: (a) supports the validity of the proposed taxonomic characters, (b) demonstrates that species richness may be highly underestimated in freshwater cyclopoid assemblages in absence of good taxonomic practice; and (c) requires an ecological explanation of species coexistence. The role of morphologically based taxonomy in order to solve general problems of distributional ecology and theoretical biology is explored.

Introduction

The genus *Diacyclops* Kiefer, 1927, emend. Morton (1985) is the richest genus within the family Cyclopidae. Up to now, more than 100 species (listed in: Reid & Strayer, 1994; Pesce, 1994; Dussart & Defaye, 1995; additions in: Fiers et al., 1996; Pospisil & Stoch, 1999; Stoch & Pospisil, 2000a) are known from almost all kinds of freshwater environments, and may be very abundant in local assemblages inhabiting temporary pools (Champeau, 1966), alluvial groundwaters (Pospisil, 1994), littoral areas of lakes (Sarvala, 1986) and sphagnum bogs (Fryer, 1993).

Unfortunately, the lack of sufficient detail in the original descriptions of most species creates many problems in species identification, and consequently in the estimation of species richness in local assemblages. Herein, I suggest the possibility of using new microcharacters important for species discrimination, exploring the influence that this practice could

have on the total number of *Diacyclops* species and estimates of species richness in local and regional ecological surveys. Finally, I examine the possible explanations of the high species richness found in some *Diacyclops* assemblages.

Materials and methods

Several hundred specimens from Europe, Africa, Asia and North America were examined, including representatives and the type material of all the species and subspecies described within the *D. languidus* and *D. languidoides* groups (see Pesce, 1994; Dussart & Defaye, 1995; Pospisil & Stoch, 1999; Stoch & Pospisil, 2000a, 2000b), as well as most of the slides deposited in the Kiefer collection, Staatliches Museum für Naturkunde, Karlsruhe (see Franke, 1989 for a complete list).

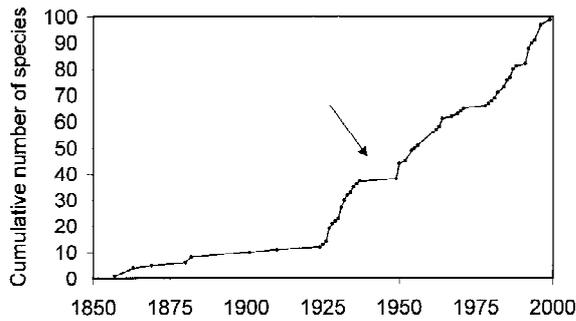


Figure 1. Steyskal's curve of the genus *Diacyclops*, representing the rate of increase of species description. The arrow indicates the stasis in species description during World War II.

Whenever possible, whole males and females were examined in temporary lactophenol or glycerine mounts using the technique of the 'sandwich-slide' recommended by Huys & Boxshall (1991). Selected specimens were dissected in glycerine, and permanently mounted on slides in glycerine sealed with epoxids. A Zeiss Axioskop microscope fitted with a drawing tube was used to study the details at 1000 \times , using an oil immersion lens. Terminology related to the external morphology follows Huys & Boxshall (1991).

Results

How many species of *Diacyclops*?

The rate of increase in species number of *Diacyclops* can be visualized using Steyskal's curves (Fig. 1). A careful examination of this graph reveals that the rate of increase after 1925 was high and constant over time, apart from a short stasis during World War II (arrowed in Fig. 1). The description of new species may increase in certain situations, for example when new taxonomists begin to work, new taxonomic characters are discovered, new geographical regions and habitats are studied, and novel sampling methods are employed. Taking into account the fact that some regions are virtually unexplored (wide areas of Asia, Central and South America, Australia and North America: Reid, 1992), and that new taxonomic characters are proposed herein, we can expect a dramatic increase in species number in the near future.

Taxonomic characters in the genus *Diacyclops*

The genus *Diacyclops* is one of the several genera currently recognised in the family Cyclopidae (Dussart

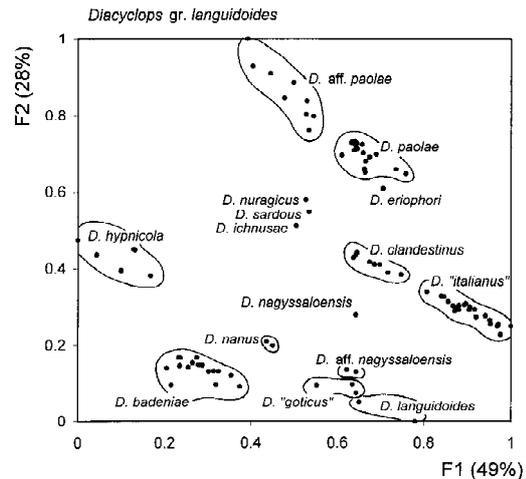


Figure 2. Ordination of European specimens (mainly from the Kiefer Collection) of the *Diacyclops languidoides*-group using principal component analysis. Total variance explained by the first two axes: 77%. The characters used are (1) the presence of an exopodal seta on antennary basis, and the following ratios: (2) length/width of caudal ramus; (3) terminal accessory seta *versus* posterolateral seta; (4) dorsal seta *versus* length of caudal ramus; (5) posterolateral seta *versus* length of caudal ramus; (6) distance between the insertion of anterolateral seta and posterolateral seta *versus* length of caudal ramus; (7) length/width of third segment of leg 4 endopod; (8) inner/outer terminal spine of third segment of leg 4 endopod; (9) inner terminal spine of third segment of leg 4 endopod *versus* length of segment.

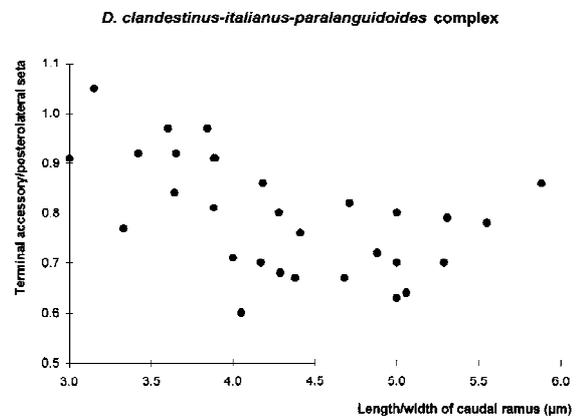


Figure 3. Ordination of a cluster of specimens (including type material) of *D. italianus* (Kiefer, 1931), *D. clandestinus* (Kiefer, 1926) and *D. paralanguidoides* (Pesce & Galassi, 1987) from Italy and Germany, using the two differential characters used in the original diagnoses. All the specimens examined lacked the antennary exopod.

& Defaye, 1995), notwithstanding that some authors (Plesa, 1969; Fryer, 1993) consider *Diacyclops* as synonym of *Acanthocyclops* (*Megacyclops* in Plesa's opinion). Unfortunately, the publication of many incompletely described species and subspecies that are closely related to *Diacyclops languidooides* (Lilljeborg, 1901) has created considerable taxonomic confusion. For this reason, Einsle (1993) raised some doubts about the validity of certain taxa, and supposed that the observed differences might result from environmental variability.

Most of the easily recognisable characters, such as the number of antennular segments and the segmentation pattern of legs 1–4, are useful only in discriminating species groups. The taxonomic confusion has been exacerbated by the use of another limited set of traditional characters for differentiating between *Diacyclops* species, such as the morphology of the caudal rami and third endopod of leg 4. Only recently, some additional characters (presence of exopodal seta on antennary basis and ornamentation of frontal and caudal surfaces of the same segment) were introduced (Pesce & Galassi, 1985, 1987), but no descriptions of the other appendages were reported even in recent publications.

A morphometric analysis was carried out on several species of the *Diacyclops languidooides*-group to test the validity of the traditional characters cited above. Some of the most interesting results for closely related species are reported in Figures 2 and 3. The results of the phenetic analysis clearly suggest (Fig. 2) that several taxonomic entities are represented in the samples, and that covariation of traditional characters may help in distinguishing between them. Nevertheless, some characters such as the length/width ratio of the caudal ramus or the terminal accessory seta/posterolateral caudal seta ratio showed a continuous variation within clusters (Fig. 3). Some of them varied so much even within the same population that they have no value as diagnostic characters in the genus *Diacyclops*; the same conclusion was reached by Karaytug & Boxshall (1998) during the revision of the genus *Paracyclops*.

Careful examination of the morphology of the species belonging to the *Diacyclops languidooides*-group revealed several useful new or rarely mentioned morphological characters, described herein:

(1) Ornamentation of antennular segments: the number and position of setae and aesthetascs are very good taxonomic characters both in males and in females; the length of aesthetascs on the male antennule

is particularly noteworthy (Fig. 4) and may be related to the degree of adaptation to groundwater life (Pospisil & Stoch, 1999).

(2) Absence of exopodal seta from the antennary basis: this character was considered typical of the stygobiont species within the *Diacyclops languidooides*-group (Pesce & Galassi, 1985); however, the exopodal seta is lacking in some epigeal species as well, for example in *Diacyclops eriophori* (Gurney, 1927). Moreover, the absence of the exopodal seta in species belonging to other groups (*Diacyclops stygius*-group, *Diacyclops crassicaudis*-group) is obviously due to convergence.

(3) Ornamentation of antennary basis: the pattern allows an easy discrimination of species groups, and may be important also in differentiating species (Fig. 5a–c); unfortunately, there are some cases of valid, coexisting species with identical spinulation patterns.

(4) Number of setae on antennary endopod segment 2: there are usually seven or nine setae on this segment, with some exceptions; this character may be sexually dimorphic (Pospisil & Stoch, 1999).

(5) Absence of exopodal seta on maxillary palp: this seta is lacking only in some stygobiont species (Pospisil & Stoch, 1999).

(6) Absence of a seta on endopodal segment 2 of maxilliped: this seta is lacking in *Diacyclops paolae* (Pesce & Galassi, 1987) and an unnamed North American species (Reid, in litt.).

(7) Presence of a notch delimited by two sclerotized teeth on inner margin of P4 basis: this is a very constant character in several species of the *Diacyclops languidus*- and *D. languidooides*-groups (Fig. 5d–f). Its function remains unknown; perhaps it could play a role during copulation.

The characters proposed herein are surely difficult to visualize, but have the advantage of being constant within the *Diacyclops languidooides*-group, and will be tested in other species groups. Using the new taxonomic characters discussed above, several valid species (see Pospisil & Stoch, 1999) have been identified within the *Diacyclops languidooides*-group, and several others are probably concealed under the name *Diacyclops languidus* (Sars, 1863) (see Stoch & Pospisil, 2000a). For this reason, I suggest that most early records of these two species of *Diacyclops*, as well as of their subspecies, are unreliable.

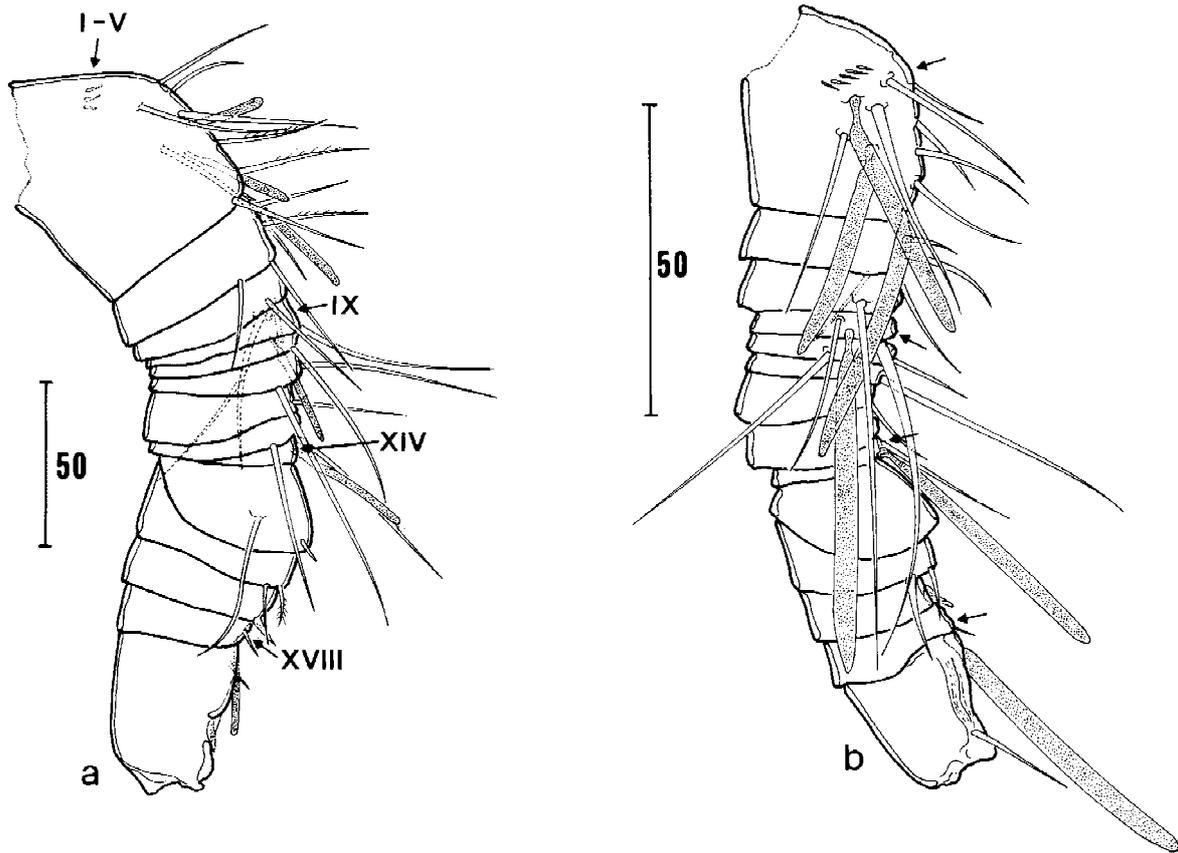


Figure 4. Male antennule of (a) *Diacyclops hiberniae* (Gurney, 1927) and (b) *D. danielopoli* (Pospisil & Stoch, 1999), showing the different lengths of aesthetascs. Arrows indicate the segments bearing the aesthetascs; Roman numerals refer to ancestral segments (following Huys & Boxshall, 1991). Scale bars in μm .

Taxonomic practice and species richness in *Diacyclops* assemblages

Taxonomic practice may influence species richness estimations at local and regional scales, and some examples are discussed below.

Local diversity

In the Danube, Pospisil (1994) recognized five closely related *Diacyclops* species co-existing in the groundwaters of the Lobau area; they belong to the *Diacyclops languidus* and *D. languidoideus* species groups, and can be distinguished only using minute morphological structures (Pospisil & Stoch, 1999). An additional groundwater species from the Danube near Vienna, closely related to *Diacyclops languidoideus*, was discovered later (Stoch & Pospisil, 2000b). The coexistence of up to six species of the *Diacyclops languidus* and *D. languidoideus* species groups in the same local area confirms that local species richness may be

severely underestimated in absence of good taxonomic practice (compare Reid, 1992).

Regional diversity

In some areas intensively sampled in northern Italy and Slovenia (Stoch, 1995), *Diacyclops* species richness in a 10×10 km grid area varied from two to seven species, significantly contributing to the definition of the hotspots of species richness, especially in karstic areas. Careful taxonomic analysis of *Diacyclops* specimens during these regional surveys revealed more than ten species in regions smaller than 100 km^2 (for example the Venezia Giulia area in northeastern Italy). In this area, *Diacyclops* species colonized almost all kinds of freshwater environments (temporary pools, ponds, lakes, tree-holes, springs, marshes, streams and rivers, and alluvial and karstic groundwaters). This number exceeds that of any other copepod genus (the closely related genus *Acanthocyclops* is represented

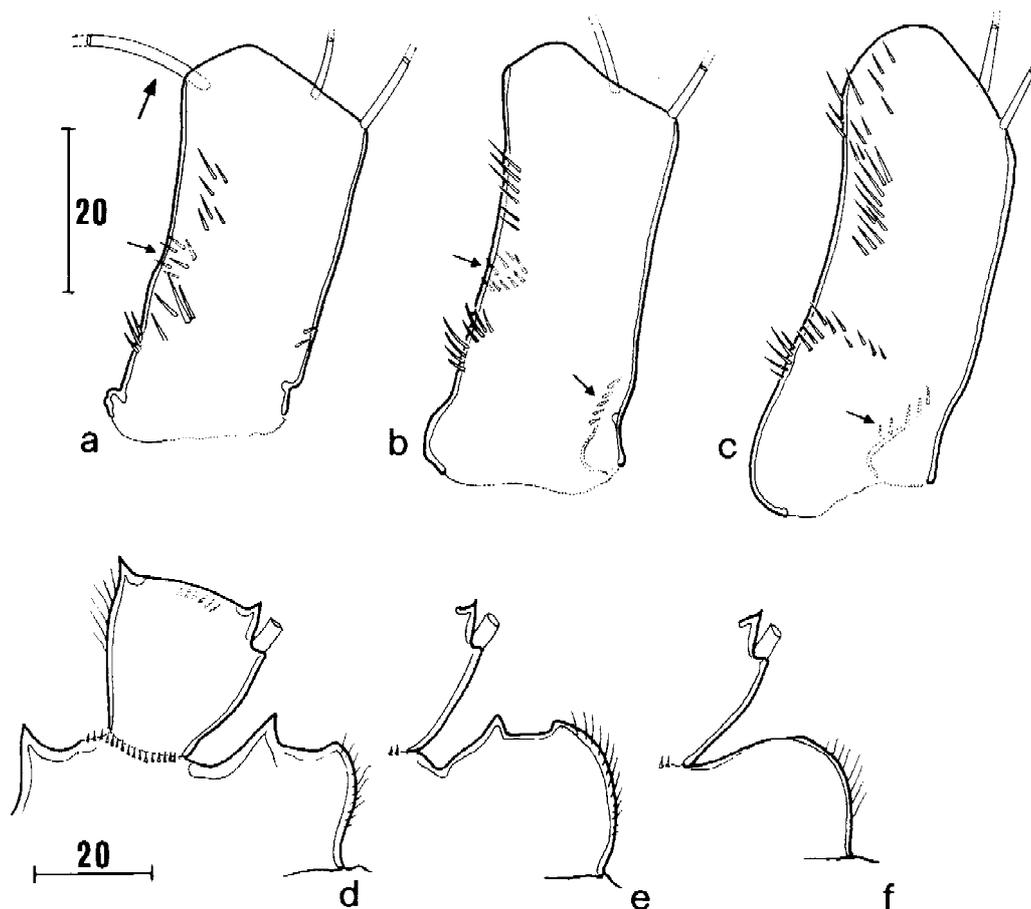


Figure 5. Some morphological characters useful in *Diacyclops* taxonomy. Spinulation pattern of caudal side of antennary basis in: (a) *D. nanus* (Sars, 1863) (Germany); (b) *D. clandestinus* (Kiefer, 1926) (Germany); (c) *Diacyclops* n. sp. (Julian Pre-Alps, northern Italy); small arrows indicate spinulation on frontal side; the large arrow in (a) indicates the exopodal seta. Inner margin of P4 basis in: (d) *D. languidoides* (Lilljeborg, 1901) (Sweden); (e) *D. badeniae* (Kiefer, 1933) (Germany); (f) *D. paolae* Pesce & Galassi, 1987 (Italy). Scale bars in μm .

by six species, all the other genera by three or fewer species).

Species coexistence

Diacyclops species assemblages are under study to test the adaptive zone theory (Stoch, 1995), as well as the multiple colonization process, in explaining colonization, adaptive radiation and species coexistence in groundwaters. Of the various explanations suggested by Stoch (1995) for species coexistence in groundwaters, the age of colonization seems to be unimportant in explaining *Diacyclops* diversity: as a matter of fact, some of the richest species assemblages are found in alluvial groundwaters which are probably of post-glacial origin (Pospisil, 1994). The same consideration applies to food resources partitioning: the closely related, coexisting *Diacyclops* species in the Danube

area showed little variation in the mouthparts (Stoch, 1995; Pospisil & Stoch, 1999), indicating low food specialization. This is not a general rule, because other species groups can display a certain degree of variability in mouth appendages (Reid & Strayer, 1994): in epigeic species 'flocks', as the '*Acanthocyclops-Diacyclops* complex' (Boxshall & Evstigneeva, 1994) in Lake Baikal, there may be a 'hypervariation' of mouthpart structure. This fact suggests that mouthpart structure is not the key feature which indicates resources partitioning (Stoch, 1995): coexisting species may differ in size, feeding and swimming behaviour, and hence the use of spatial resources. These are general rules for congeneric benthic copepods (Marcotte, 1983), but their importance in *Diacyclops* assemblages is unknown. A different degree of adaptation to groundwater life in co-existing *Diacyclops*

species revealed by careful taxonomic analysis (Pospisil, 1994; Pospisil & Stoch, 1999) may be a further key factor in interpreting species coexistence and the use of spatial resources.

However, species coexistence is not the only important factor in explaining the distributional ecology of *Diacyclops*. The abundance of certain species in temporary ponds and pools and the production of resting stages are important topics of study (Champeau, 1966, 1971), and may explain the wide distribution and wide range of habitats of some species, such as *Diacyclops bicuspidatus* (Claus, 1857) and *Diacyclops bisetosus* (Rehberg, 1880). Usually, these species, being good colonizers and tolerant of harsh environmental conditions, do not coexist with other congeners, and are sometimes the only cyclopoids found in certain habitat types (Fryer, 1993). Even in these simple cases, more than one species could be included within each of these 'widespread' or 'cosmopolitan' species (Reid, Fiers, pers. comm.), and more detailed taxonomic work is needed in this field as well.

Discussion

The title of this paper asks how many species of *Diacyclops* are there on earth; can we answer this question? In my opinion we cannot, at least for two reasons.

First, the rate of increase of species description is so high that the genus could even double its species in the near future. Moreover, detailed morphological analysis of *Diacyclops* populations inhabiting groundwaters clearly demonstrated that several, closely related species of *Diacyclops* may coexist in the same habitat, and their discrimination is based on minute characters which require a complete dissection of the specimens and careful examination. In many taxonomic accounts of cyclopid species, there is often no description or illustration of the mouthparts and antennules, and this fact increases the degree of uncertainty regarding species numbers. This probably will be a serious impediment for the compilation of a *Diacyclops* guide in the near future (Stoch & Reid, unpubl.).

The second reason is that increasing taxonomic practice may demonstrate that the genus *Diacyclops* is not monophyletic. For example, Pandourski (1997) included in the same group some *Diacyclops* and *Acanthocyclops* species, suggesting that the 'aberrant' *Diacyclops stygius*-group belongs to a different genus.

Moreover, the swimming leg segmentation pattern and the homologies discovered within the *D. languidus* and *D. languidoides* groups suggest that these two species groups could be ascribed to a separate genus. Further taxonomic studies and a cladistic analysis are required to support both hypotheses. The recent description of *Rheocyclops* (Reid et al., 1999) goes towards the direction of separating from the genus *Diacyclops* some smaller monophyletic units.

The impossibility of a correct estimate of *Diacyclops* species number has consequences in other fields besides taxonomy. The examination of *Diacyclops* species assemblages demonstrated that weak taxonomic practice is a serious impediment to ecological research (Reid, 1992; Stoch, 1995), and may lead to severe underestimation of local and regional species richness. Moreover, the results of careful taxonomic analysis of the genus *Diacyclops* suggested further questions relevant to ecology and evolutionary biology: why are there so many species of *Diacyclops* compared with other cyclopid genera? And why are there so many of them in the *Diacyclops languidoides*-group? These questions require an answer involving ecological concepts such as habitat heterogeneity, environmental stability and biotic interactions, as well as evolutionary concepts such as multiple colonization and diversification via niche differentiation (Stoch, 1995). Unfortunately, the importance of these factors in assembling freshwater – and particularly groundwater – communities is little known. I suggest that improved copepod taxonomy may have a basic role in solving these general problems of theoretical biology.

Acknowledgements

I am indebted to: J. Reid (Washington), for supporting my work by sending me material from the United States and drawings of *Diacyclops* appendages, and for her kind advice; C.E.F. Rocha (São Paulo) for his kind encouragement and suggestions on the antennule structure; P. Pospisil (Vienna), for the loan of material, data on aesthetasc length, and discussions on *Diacyclops* taxonomy. Special thanks are due to R. Lopes (Curitiba; currently in Ilhéus); without his kind help and economic support, my participation in the 7th ICOC would have been impossible.

References

- Boxshall, G. A. & T. D. Evstigneeva, 1994. The evolution of species flocks of copepods in Lake Baikal: a preliminary analysis. In Martens, K., B. Goddeeris & G. Coulter (eds), Speciation in Ancient Lakes. Archiv Hydrobiol. Beih. Ergebn. Limnol. 44: 235–245.
- Champeau, A., 1966. Contribution à l'étude écologique de la faune des eaux temporaires de la haute Camargue. Arch. Oceanogr. Limnol. 14: 309–357.
- Champeau, A., 1971. Recherches sur l'adaptation à la vie latente des Copépodes Cyclopoïdes et Harpacticoides des eaux temporaires Provençales. Bull. Soc. Écol. 2 (2–3): 151–167.
- Dussart, B. & D. Defaye, 1995. Répertoire Mondial des Copépodes Cyclopoïdes. C.N.R.S., Paris: 236 pp.
- Einsle, U., 1993. Crustacea, Copepoda, Calanoida und Cyclopoida. In Schwoerbel, J. & P. Zwick (eds), Süßwasserfauna von Mitteleuropa. G. Fischer, Stuttgart, 8/4-1: 1–206.
- Fiers, F., J. W. Reid, T. M. Iliffe & E. Suárez-Morales, 1996. New hypogean cyclopoid copepods (Crustacea) from the Yucatán Peninsula, Mexico. Contrib. Zool. 66 (2): 65–102.
- Franke, U., 1989. Katalog zur Sammlung limnischer Copepoden von Prof. Dr. Friedrich Kiefer. Carolea 5: 1–433.
- Fryer, G., 1993. The freshwater Crustacea of Yorkshire. A Faunistic and Ecological Survey. Yorkshire Naturalists' Union & Leeds Philosophical and Literary Society, Leeds: 312 pp.
- Huys, R. & G. A. Boxshall, 1991. Copepod Evolution. The Ray Society, London: 468 pp.
- Karaytug, S. & G. A. Boxshall, 1998. The *Paracyclops fimbriatus*-complex (Copepoda, Cyclopoida): a revision. Zoosystema 20: 563–602.
- Marcotte, B. M., 1983. The imperatives of copepod diversity; perception, cognition, competition and predation. In Schram, F. R. (ed.), Crustacean Phylogeny. Crustacean Issues 1: 47–72.
- Morton, D. W., 1985. Revision of the Australian Cyclopidae (Copepoda: Cyclopoida). I. *Acanthocyclops* Kiefer, *Diacyclops* Kiefer and *Australocyclops*, gen. nov. Aust. J. mar. Freshwat. Res. 36: 615–634.
- Pandourski, I. S., 1997. Composition, origine et formation de la faune cyclopidienne stygobie de Bulgarie et définition du groupe d'espèces '*kieferi*' du genre *Acanthocyclops* (Crustacea, Copepoda, Cyclopoida). Boll. Mus. Reg. Sci. Nat., Torino 15: 279–297.
- Pesce, G. L., 1994. The genus *Diacyclops* Kiefer in Italy: a taxonomic, ecological and biogeographical up-to-date review (Crustacea Copepoda Cyclopidae). Arthropoda Selecta 3 (34): 13–19.
- Pesce, G. L. & D. P. Galassi, 1985. Due nuovi *Diacyclops* del complesso '*languidoïdes*' (Copepoda: Cyclopoida) di acque sotterranee di Sardegna e considerazioni sul significato evolutivo dell'antenna nei copepodi stigobionti. Boll. Mus. civ. Stor. nat. Verona 12: 411–418.
- Pesce, G. L. & D. P. Galassi, 1987. New or rare species of *Diacyclops* Kiefer, 1927 (Copepoda: Cyclopoida) from different groundwater habitats in Italy. Hydrobiologia 148: 103–114.
- Plesa, C., 1969. Nota critica asupra unor ciclopide (Crustacea, Copepoda) din apele subterane ale României. Lucr. Inst. Speol. 'E. Racovitza' 8: 81–89.
- Pospisil, P., 1994. Die Grundwassercyclopiden (Crustacea, Copepoda) der Lobau in Wien (Österreich): faunistische, taxonomische und ökologische Untersuchungen. Ph. D. Thesis Univ. Vienna: 222 pp.
- Pospisil, P. & F. Stoch, 1999. Two new species of the *Diacyclops languidoïdes*-group (Copepoda, Cyclopoida) from groundwaters of Austria. Hydrobiologia 412: 165–176.
- Reid, J. W., 1992. Taxonomic problems: a serious impediment to groundwater ecological research in North America. First Int. Conf. Ground Water Ecol.: 133–142.
- Reid, J. W. & D. L. Strayer, 1994. *Diacyclops dimorphus*, a new species of copepod from Florida, with comments on morphology of interstitial cyclopine cyclopoids. J. n. am. Benthol. Soc. 13: 250–265.
- Reid, J. W., D. L. Strayer, J. V. McArthur, S. E. Stibbe & J. J. Lewis, 1999. *Rheocyclops*, a new genus of copepods from the southeastern and central U.S.A. (Copepoda: Cyclopoida: Cyclopidae). J. crust. Biol. 19: 384–396.
- Sarvala, J., 1986. Patterns of benthic copepod assemblages in an oligotrophic lake. Anns. Zool. fennici 23: 101–130.
- Stoch, F., 1995. The ecological and historical determinants of crustacean diversity in groundwaters, or: why are there so many species? Mém. Biospéol. 22: 139–160.
- Stoch, F. & P. Pospisil, 2000a. Redescription of *Diacyclops disjunctus* (Thalwitzer, 1927) from Austria, with remarks on the *Diacyclops languidus*-group in Europe (Copepoda, Cyclopoida, Cyclopidae). Crustaceana, 73(4): 469–478.
- Stoch, F. & P. Pospisil, 2000b. The *Diacyclops languidoïdes*-group (Copepoda, Cyclopoida) in Austria, with redescription of *Diacyclops cohabitatus* Monchenko, 1980. Ann. Limnol., 36(1): 21–29.