

Diversity, ecology and evolution of groundwater copepods

DIANA M. P. GALASSI*, RONY HUYS[†] AND JANET W. REID[‡]

*Dipartimento di Scienze Ambientali, University of L'Aquila, L'Aquila, Italy

[†]Department of Zoology, Natural History Museum, London, U.K.

[‡]Virginia Museum of Natural History, Martinsville, VA, U.S.A.

SUMMARY

1. With few exceptions, copepods dominate over other crustacean and non-crustacean invertebrate groups in ground water. They have colonised a vast array of habitats in continental ground waters, where they are represented by over 1000 species in six orders: Platycopioidea, Misophrioida, Calanoida, Cyclopoida, Harpacticoida, Gelyelloida. However, members of only the last four orders entered genuine fresh ground water.
2. Stygobiotic copepods show a wide range of morphological and physiological adaptations to different groundwater habitats. They frequently exhibit simplifications in body plans, including reductions in appendage morphology, which is regarded as a result of paedomorphic heterochronic events.
3. Copepod distributions at small spatial scales are most strongly affected by habitat type and heterogeneity, with sediment grain size and availability of organic matter being important habitat characteristics. Large-scale spatial distributions (biogeographical) are mainly related to past geological, climatic and geographical processes which occurred over medium to long time scales.
4. Such processes have affected colonisation patterns and diversification of copepods in ground water, leading to a number of phylogenetic and distributional relicts and a high degree of endemism at different taxonomic levels. This is reflected in the composition of groundwater copepod communities characterised by distantly related species in the phylogenetic tree.
5. Copepods dominate the species richness of groundwater fauna in all regions and on all continents where more than cursory surveys have been carried out, i.e. in Europe, North and Latin America as well as in Australia.
6. Species-specific microhabitat preferences, high proportions of local endemics, high proportions of phylogenetic and distributional relicts, and higher-level taxonomic diversity are all factors suggesting that copepods are a useful indicator group of overall species richness for defining conservation priorities in ground water.

Keywords: biodiversity, conservation, copepods, ground water, stygobiont

Introduction

Crustaceans represent about 10% of the total number of invertebrate species known from fresh water

world-wide (Balian *et al.*, 2008). Copepoda with 2814 described species (Boxshall & Defaye, 2008), Ostracoda with about 1936 species (Martens *et al.*, 2008) and Amphipoda with some 1870 species (Väinölä *et al.*, 2008) are by far the most species-rich crustacean groups. The extensive taxonomic diversification of crustaceans in surface fresh water is paralleled in ground water, where copepods are

Correspondence: Diana M. P. Galassi, Dipartimento di Scienze Ambientali, University of L'Aquila, Via Vetoio, Coppito, I-67100 L'Aquila, Italy. E-mail: dianamariapaola.galassi@univaq.it

represented by around 1000 species and subspecies, amphipods by about 950 species and ostracods by approximately 300 species, collectively outnumbering all other invertebrate groups living in this environment. As a result, some 40% of the European crustacean fauna is represented by stygobiotic species (Danielopol, Pospisil & Rouch, 2000), i.e. species that are strictly confined to ground water and hence complete their life cycle in this environment and have evolved specific morphological and physiological adaptations to cope with the environmental conditions in groundwater habitats.

Copepods have colonised many different subterranean habitats. Their substantial diversity in body morphology and degree of adaptation to life in groundwater suggests that copepods may be good indicators of habitat heterogeneity. They show marked differences in microhabitat preference (Galassi, 2001; and references therein) and sensitivity to anthropogenic disturbance, including human-induced alterations in water chemistry and hydrological regime of ground water (Dole-Olivier *et al.*, 1994; Dole-Olivier, Marmonier & Beffy, 1997; Rundle & Ramsay, 1997; Malard, Reygrobellet & Laurent, 1998; Paran *et al.*, 2005). Additionally, copepods may serve as useful biological indicators of subsurface-surface water connectivity (Malard *et al.*, 1994; Di Lorenzo *et al.*, 2005b) and provide information on past geological, climatic and geographical events (Galassi, 2001; Castellarini *et al.*, 2007). Although their ecological functions in ground water are imperfectly known, copepods may play significant roles in groundwater food webs and ecosystems (Gibert & Deharveng, 2002). These include (i) decomposition of organic matter through feeding on particulate organic matter (POM) and bacterial biofilms, (ii) provision of food for macroinvertebrates and meiofauna (i.e. early-instar chironomid larvae, hydrachnidians, *Hydra* spp., turbellarians), and (iii) promotion of nutrient flow between surface and subterranean environments.

The aim of this contribution is to summarise information on (i) copepod adaptations to life in ground water, (ii) their distribution in various groundwater habitats, (iii) the multiple evolutionary origins of groundwater copepod lineages, (iv) their high taxonomic diversity in ground water, and (v) their value as indicators in setting conservation priorities in ground water.

Adaptations to life in ground water

The colonisation of subterranean environments by the generally small-sized copepods did not always require drastic alterations of their ancestors' body plans. This pre-adaptation probably explains in part the success of copepods in ground water. However, their great morphological diversification observed in contrasting groundwater habitats and illustrated in Figs 1 & 2 is also partly attributable to heterochrony, i.e. changes in the timing and/or rates of processes underlying the ontogenetic formation of morphological traits.

Heterochrony in groundwater copepods

Appendage reductions, character losses and miniaturisation (i.e. the evolution of extremely small adults) are common denominators of the 'darkness syndrome' of many stygobionts. Stygobiotic copepods frequently exhibit simplifications in body plans, including reductions in appendage morphology, which can be regarded as the result of paedomorphic heterochronic events, i.e. truncated development or underdevelopment in descendant adults: post-displacement (i.e. delayed onset of growth or other developmental processes), progenesis (i.e. early cessation of development) and neoteny (slowed or delayed development resulting in the retention of traits by adults that are normally restricted to juveniles) (Valentine, 2004).

Post-displacement is possibly the primary mechanism in the regressive evolution of groundwater copepods, affecting body and limb segmentation and sexual dimorphism. For instance, in some groundwater lineages, post-displacement has caused the loss of entire appendages that are usually expressed in advanced stages of ontogeny. In the canthocamptid *Stygepactophanes jurassicus* Moeschler & Rouch and the parastenocaridid genus *Simplicaris* the development of the fifth legs is suppressed in both sexes (Galassi & De Laurentiis, 2004a). In the two described species of the Gelyelloidea, post-displacement is even more extreme, resulting in the complete loss of legs 4–6 (Huys, 1988). In several groundwater Ameiridae, adult females lack the genital double-somite typically expressed in other copepods. Fusion of the genital and first abdominal somite normally occurs at the moult from copepodid V

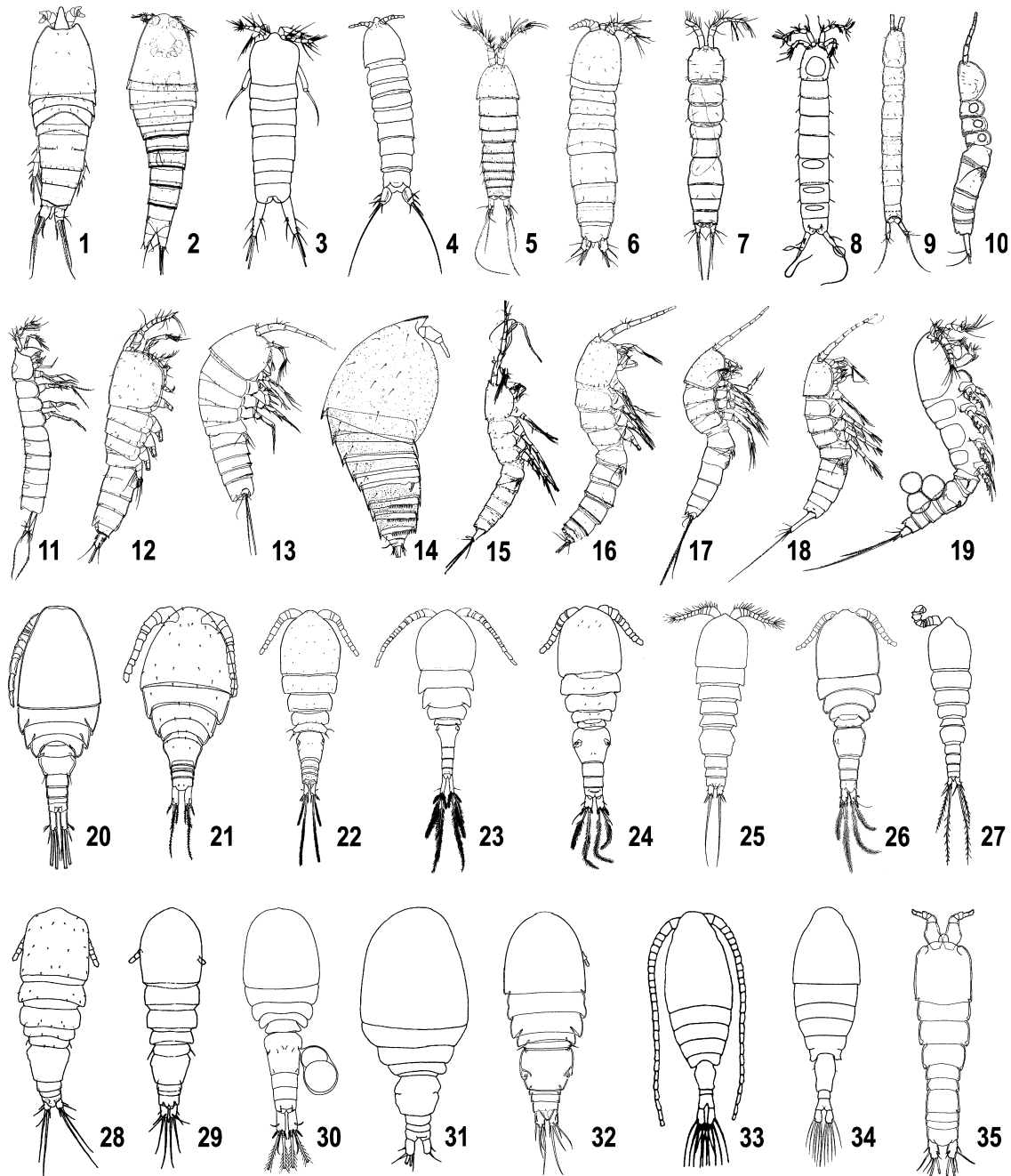


Fig. 1 Body plans of groundwater copepods (Harpacticoida: 1. *Neomiscegenus indicus* Karanovic & Reddy, 2. *Rangabradya indica* Karanovic & Pesce, 3. *Stygepactophanes jurassicus* Moeschler & Rouch, 4. *Morariopsis dumonti* Brancelj, 5. *Nitocrellopsis rouchi* Galassi, De Laurentiis & Dole-Olivier, 6. *Phyllognathopus bassoti* Rouch, 7. *Inermipes humphreysi* Lee & Huys, 8. *Parastenocaris fontinalis* Schnitter & Chappuis, 9. *Remaneicaris analuizae* Corgosinho & Martínez Arbizu, 10. *Stygonitocrella sequoyahi* Reid, Hunt & Stanley, 11. *Simplicaris lethaea* Galassi & De Laurentiis, 12. *Schizopera depotspringsi* Karanovic, 13. *Nitocrella obesa* Karanovic, 14. *Pseudectinosoma janinae* Galassi, Dole-Olivier & De Laurentiis, 15. *Parapseudoleptomesochra karamani* Karanovic, 16. *Nitocrella trajani* Karanovic, 17. *Hirtaleptomesochra bispinosa* Karanovic, 18. *Haifameira pori* Karanovic. Cyclopoida: 19. *Itocyclops yezoensis* (Ito), 20. *Acanthocyclops rebecca* Fiers & Ghene, 21. *Acanthocyclops agamus* Kiefer, 22. *Metacyclops kimberleyi* Karanovic, 23. *Diacyclops lewisi* Reid, 24. *Diacyclops dimorphus* Reid & Strayer, 25. *Graeteriella unisetigera* (Graeter), 26. *Rheocyclops carolinianus* Reid, 27. *Reidcyclops imparilis* (Monchenko), 28. *Allocyclops consensus* Karanovic, 29. *Gonicyclops uniarticulatus* Karanovic, 30. *Diacyclops biceri* Boxshall, Evstigneeva & Clark, 31. *Teratocyclops cubensis* Plesa, 32. *Haplocyclops gudrunae* Kiefer. Calanoida: 33. *Stygodiaptomus petkovskii* Brancelj, 34. *Hadodiaptomus dumonti* Brancelj. Gelyelloida: 35. *Gelyella droguei* Rouch & Lescher-Moutoué.



Fig. 2 Scanning electron micrographs of stygobiotic harpacticoids. (a) *Phyllognathopus bassoti* Rouch, (b) *Pseudectinosoma* sp., (c) Ectinosomatidae gen. 1., sp. 1.; (d) *Nitocrella pescei* Galassi & De Laurentiis, (e) *Elaphoidella* sp., (f) *Parastenocaris amalasuntae* Bruno & Cottarelli.

to adult, but as a result of post-displacement it is delayed beyond the final moult in the genera *Psammoneitocrella*, *Neonitocrella* and several species of *Nitocrella* and *Nitocrellopsis* (Galassi, De Laurentiis & Dole-

Olivier, 1999a; Galassi, Dole-Olivier & De Laurentiis, 1999b; Lee & Huys, 2002). The functional significance of this paedomorphic change is unknown, but it is conceivable that the extra somitic articulation enhances

the wriggling ability in at least some female ground-water copepods, either in their search for food or as part of mate location.

The great majority of segmental and setal reductions in appendages of groundwater copepods are attributable to progenesis or neoteny. Examples include the segmental reductions encountered in the swimming legs of many stygobiotic Ameiridae and Canthocamptidae in the Harpacticoida, the Speodiaptominae in the Calanoida, various cyclopid genera in the Cyclopoida and both species of the Gelyelloida (legs 1–3). It should be noted, however, that without knowledge about the ontogenetic trajectories of the descendants and the ancestors (Ferrari & Dahms, 2007), it is impossible to determine whether progenesis or neoteny (or a combination of both) is responsible for the expression of juvenile traits in the adult descendant.

Miniaturisation is widespread in groundwater copepods and likely to be the result of sequential progenesis during which a number of (or all) stages in a multiphasic ontogeny are affected in the same way, i.e. by shortening the period spent in each stage. The moulting sequence and morphological expression of the moult in arthropods is under endocrine control. Changes in the timing of induction of the deposition of the new cuticle will affect the degree of intermoult morphological development. Thus, if cuticle deposition occurs precociously (i.e. soon after the previous moult), body size increase will be significantly reduced and certain morphological features will appear in an intermediate stage of development (such as incomplete boundaries between swimming leg segments in some groundwater cyclopoids, e.g. *Acanthocyclops agamus* Kiefer). The incomplete expression of urosomal boundaries in some marine interstitial genera (e.g. *Leptopsyllus*, *Apodopsyllus*) and the flaccid nature of somatic cuticles in many groundwater lineages (Parastenocarididae, various ameirid genera, Gelyelloida) can be interpreted as the result of perturbations of the moulting cycle, enhancing their 'worm-like' locomotory ability in interstitial environments.

Competition and heterochrony

Marcotte's (1983) study on two salt-marsh harpacticoids provided evidence that heterochrony can minimise competition between closely related species,

determining changes in mouthpart morphology and allowing trophic niche partitioning in sympatric, closely related species. Among groundwater copepods, *Nitocrella pescei* Galassi & De Laurentiis and *N. kunzi* Galassi & De Laurentiis occur sympatrically in the Presciano Spring (Italy) (Galassi, 2001) but differ in mouthpart morphology, suggesting that heterochrony facilitated partitioning of food resources.

Preadaptation versus adaptation

The widely accepted notion that stygobiotic copepods originated from surface ancestors living either in marine, freshwater or semiterrestrial environments raises the question whether paedomorphosis preceded the transition from epigeal to groundwater habitats (pre-adaptation) or evolved as colonisation of the latter occurred (adaptation) (cf. Westheide, 1987). It is clear that different evolutionary pathways lie at the base of the diversification of the stygobiotic fauna (Galassi, 2001), and even at family level different scenarios may apply to different phyletic lineages. For example, many of the paedomorphic traits displayed by the hyper-speciose Parastenocarididae are morphological attributes inherited from the common epigeal ancestor shared with the marine interstitial Leptopontiidae. A similar pre-adaptation to groundwater life is shown by the stygobiont *Diacyclops charon* (Kiefer), which, except for depigmentation and anophthalmy (i.e. absence of eyes), does not differ markedly from its epigeal relative, *D. bicuspidatus* (Claus). However, *D. charon* (and also other cyclopoids, see Lescher-Moutoué, 1973; Fiers *et al.*, 1996) exploited only karstic pools and subterranean lakes that lack significant spatial constraints, requiring minimal adaptation to small spaces (Galassi, 2001), although adaptation to darkness still must have occurred. Conversely, some harpacticoid genera such as *Pseudectinosoma* and *Rangabradya* (Ectinosomatidae) colonised minute fractures of karstic aquifers without further reduction in size or morphological complexity relative to their marine allies.

Several stygobiotic lineages show dissociated progenesis, retaining the adult design in mouthpart structure but undergoing considerable juvenilisation in the postcephalic appendages. The harpacticoid family Chappuisiidae displays primitive mandibles, maxillae and maxillipeds but also numerous reductions in the swimming legs. Huys & Iliffe (1998)

indicated a possible relationship between the Chappuisiidae and the marine Tisboidea, noting that this family may represent a specialised lineage of relicts that diverged early in the evolution of the superfamily and subsequently radiated in groundwater habitats in Central Europe. Within the Calanoida, the stygobiotic genera belonging to the Speodiaptominae show profound reductions in the segmentation of the swimming legs compared to their epigeal diaptomid relatives. These changes are the result of progenetic development, not neoteny as claimed by Bowman (1986) and Brancelj (2005). The enigmatic order Gelyelloida presents another stygofaunal example of adaptation via progenesis and post-displacement. Although the precise origin of this lineage is as yet unknown, the primitive facies of some cephalic appendages suggests that colonisation of groundwater habitats by the Gelyelloida preceded that of the Cyclopoida. Gelyelloids probably descended from a misophrioid-like ancestral marine stock but their small size, their reduced somatic chitinisation and genital-field morphology, and their truncated swimming-leg development (offset of legs 1–3, late onset of legs 4–6) may be paedomorphic traits for adaptation to a very special environment.

Spatial distribution of copepods in ground water

The subclass Copepoda currently includes eight orders (Huys & Boxshall, 1991; Boxshall & Halsey, 2004; Huys *et al.*, 2007), six of which (Platycopioidea, Calanoida, Misophrioida, Cyclopoida, Harpacticoida, Gelyelloida) contain stygobiotic representatives. Current knowledge suggests that platycopioids and misophrioids were unable to cross the salinity boundary and never entered fresh ground water, being restricted to anchialine and marine subterranean habitats. Gelyelloida are exclusively known from fresh ground water, comprising only two European species, both being confined to the saturated karst; no published records are available from surface habitats. Calanoida are rare in fresh ground water, represented only by 10 species in the family Diaptomidae, all of which lead a planktonic mode of life in subterranean lakes (Brancelj & Dumont, 2007).

Cyclopoida and Harpacticoida are the groundwater copepods *par excellence*, the former being represented by over 330 stygobiotic species in the family Cyclopidae, and the latter containing some 640 truly

freshwater stygobionts belonging to at least five families. Within the Harpacticoida, the families Canthocamptidae, Parastenocarididae and Ameiridae have radiated very successfully in groundwater habitats. Sporadically, members of the predominantly marine Ectinosomatidae and Miraciidae (formerly Diosaccidae) are recorded from fresh ground water. The Chappuisiidae are only known from Europe, being exclusively found in alluvial aquifers and in the hyporheic habitat. The Phyllognathopodidae are occasionally found in ground water.

Although some degree of habitat partitioning can be detected at the ordinal level, i.e. between calanoids, cyclopoids and harpacticoids, habitat preferences in groundwater copepods are frequently species-specific. Calanoids are predominantly planktonic. Cyclopoids can be planktonic in subterranean lakes and pools, benthic or reside in interstitial habitats. In the last case, they generally prefer sediment of medium-coarse grain size, in slow-current sectors of subterranean and surface streams, in springs, or in pools and trickles in the karst. Harpacticoids dominate the benthos, and no records are as yet available from plankton in ground water. They can either be benthic, or, more frequently, interstitial, irrespective of grain size composition. Gravel and sand are the preferred substrata for most species, with diversity being higher in well-sorted sediments, whereas poorly sorted or clogged sediments show a drastic decrease in species diversity.

The environmental features influencing copepod distributions have received significant attention during the last two decades. Given their prevalence in freshwater meiofauna, most of the pertinent studies dealing with 'microcrustaceans' specifically focussed on copepods (Dole-Olivier *et al.*, 2000; Galassi, 2001; Galassi *et al.*, 2002; Malard *et al.*, 2003; Paran *et al.*, 2005; and references therein), and results indicated that copepods are sensitive to the hydraulic habitat conditions and geomorphological features of the environment (Robertson, 2002). In the hyporheic habitat, spatial patterns of copepod diversity have been analysed at spatial scales ranging from river floodplains to microhabitats. Because hyporheic communities are composed of both epigeal and stygobiotic species, different species-specific responses to habitat patchiness are expected as a result of differences in microhabitat preferences, life-history and dispersal ability. Although ecological information is

becoming available for epigeal cyclopoids and harpacticoids in the hyporheic habitat (Ward & Voelz, 1994, 1997), very little is known about how stygobiotic species of the hyporheic communities respond to variation in environmental factors (Galassi, 2001 and references therein). A longitudinal gradient in copepod diversity was observed by Malard *et al.* (2003) in the hyporheic zone of a glacial stream in the Alps, where *Parastenocaris glacialis* Noodt was uniformly distributed along the longitudinal gradient, being more abundant in the upwelling sectors of the streambed. This observation can be explained by the broad ecological tolerance of this species and its dispersal ability, as reflected by its wide geographical distribution (Enckell, 1969; Pesce, Galassi & Cottarelli, 1995). At the gravel-bar scale, Dole-Olivier & Marmonier (1992a,b) observed a greater abundance of *Parastenocaris fontinalis* Schnitter & Chappuis in upwelling sectors of the River Rhône, France. At the floodplain scale, *Pseudectinosoma janineae* Galassi, Dole-Olivier & De Laurentiis was restricted to the floodplain margins and to deep phreatic habitats along this river (Galassi *et al.*, 1999a,b), whereas *Nitocrellopsis rouchi* Galassi, De Laurentiis & Dole-Olivier preferred the phreatic zone, in the transition zone from coarse to fine sediments, a storage zone for organic matter and site of greatest bacterial activity (Galassi *et al.*, 1999a,b). The abundance of *Elaphoidella leruthi leruthi* Chappuis varied along an environmental gradient defined primarily by hydrogeological features (aquifer permeability and thickness of the vadose zone) in the alluvial aquifer of the Forez plain (Loire River catchment, France), whereas *Parastenocaris meridionalis* Rouch was uniformly distributed along the same gradient (Paran *et al.*, 2005). At the microspatial scale, stygobionts show marked differences in microhabitat preferences, as observed for *Elaphoidella bouilloni* Rouch, which prefers sites dominated by fine sand, whereas *Parapseudoleptomesochra subterranea* (Chappuis) and *Parastenocaris palmerae* Reid prefer coarse sediments (Rouch, 1991; Palmer *et al.*, 1995).

Another vast groundwater habitat is the karstic environment, where the environmental factors affecting copepod distribution are less well known. Two hydrological zones of the karst are distinguishable: the unsaturated karst (i.e. the epikarstic and vadose zones) and the saturated karst, the latter being macroscopically divided into capacitive (where water

percolates in medium- and small-sized fractures in branching anastomoses, often adjacent to the main drainage system) and conductive (i.e. the main drainage system of a karstic aquifer, with great hydraulic conductivity and high-water velocity) subsystems (Gibert *et al.*, 1994). In general, the spatial constraint imposed by karst is much less than in interstitial habitats, but the higher water flow, especially in the conductive subsystem, is potentially detrimental for most invertebrates, including copepods. Copepods avoid the conductive subsystem and are mostly spread out in the adjacent capacitive zone, where water flow is lower and retention of organic matter higher. Moreover, microhabitat diversity is noticeable in the large network of interconnected fractures, where pools and puddles contain silt, clay, sand and organic material percolating from the surface. Habitats characterised by low water velocities are colonised by free-swimming cyclopoids (e.g. *Acanthocyclops agamus*), and interstitial or crevicular (i.e. species living in small fractures of fissured aquifers) harpacticoids (*Ceuthonectes gallicus* Chappuis, *Nitocrella gracilis* Chappuis, *Pseudectinosoma* sp., *Parastenocaris orcina* Chappuis) (Gibert *et al.*, 2000; Galassi & De Laurentiis, 2004b; Di Lorenzo *et al.*, 2005a, and references therein).

A peculiar groundwater system exists in the Preciano spring in Italy, where limestone is partially covered by Quaternary alluvial deposits. In this spring, a clear gradient was observed in stygobiotic copepod distribution along both horizontal and vertical profiles (Fiasca *et al.*, 2005). Diversity varied with hydraulic conductivity and grain-size composition of the sediments at the site scale: truly karstic sites harboured virtually monospecific communities (up to 90% *Nitocrella pescei*), whereas porous sites had more species and a rather even distribution of *N. pescei*, *Elaphoidella mabelae* Galassi & Pesce, *Parastenocaris lorenzae* Pesce, Galassi & Cottarelli, and *Diacyclops paolae* Pesce & Galassi.

A growing body of evidence suggests that epikarst is the groundwater habitat showing the highest diversity of stygobiotic copepods (Brancelj, 1991; Stoch, 1997, 2000; Pipan & Brancelj, 2004; Pipan, 2005; Pipan & Culver, 2005). The network of fractures that characterises epikarst provides a multitude of microhabitats. Fractures are not always hydrologically interconnected, leading to a high degree of isolation among microhabitats. Together, habitat heterogeneity

and isolation of microhabitats thus appear to be the major determinants of copepod diversity in terms of species richness, endemism and higher-level taxonomic diversity. Typical stygobiotic taxa of the epikarst include most species of the harpacticoid genera *Lessinocamptus* and *Paramorariopsis*, some *Parastenocaris* spp. and the cyclopid *Speocyclops infernus* (Kiefer).

Origin and evolution of groundwater copepods

According to Purvis *et al.* (2000), the 'hierarchical nature of phylogenies means that random extinction of species affects a smaller fraction of higher taxa, and so the total amount of evolutionary history lost may be comparatively slight'. Analysis of phylogenetic diversity (i.e. how distantly related species are in the phylogenetic tree) versus relatedness (how closely related species are in the phylogenetic tree) may generate clues as to whether this generalisation can be extended to groundwater fauna, and in particular to stygobiotic copepods.

Copepods originated in the marine environment, and apparently entered fresh water through multiple colonisation waves. Boxshall & Jaume (2000) identified 22 independent colonisations of inland waters within six orders, only four of which were further able to enter ground water. The order Calanoida is represented in subterranean habitats by members of the Diaptomidae, the largest and most widespread family in fresh water. Seven of the 60 genera are known to inhabit groundwater habitats, but their phylogenetic relationships are still under debate. Pending a phylogenetic analysis of the family, an independent colonisation of ground water has been postulated for at least the subfamily Speodiaptominae (Brancelj, 2005). The order Cyclopoida is represented in fresh ground water only by the Cyclopidae, which supposedly colonised fresh water through a single colonisation event. Individual lineages descending from surface ancestors subsequently and independently entered ground water.

Colonisation of subterranean habitats is often the result of multiple invasions, frequently followed by radiation through niche diversification (e.g. for the genera *Diacyclops* and *Acanthocyclops*). This evolutionary trajectory has typically led to cryptic biodiversity and the sympatric occurrence of morphologically very similar congeners at a single groundwater site (e.g. Galassi, 2001; Stoch, 2001; Reid, 2004). Harpacticoida

entered fresh water on multiple occasions and invaded ground water via different pathways. Most taxa crossed the saline boundary during an initial surface freshwater phase before entering ground water; examples include most Canthocamptidae and some Ameiridae, Miraciidae, Phyllognathopodidae and Ectinosomatidae (Rouch, 1986; Galassi, 2001). Alternatively, some harpacticoid lineages bypassed the surface water phase and may have colonised ground water directly (e.g. the ectinosomatid genus *Pseudectinosoma*, and the ameirid genera *Nitocrella*, *Parapseudoleptomesochra*, *Nitocrellopsis*, *Stygonitocrella*, *Psammonitocrella*, *Inermipes* and *Neonitocrella*). The family Parastenocarididae, currently accommodating over 250 species, is primarily restricted to interstitial freshwater habitats, although it has also been recorded from semi-terrestrial and surface freshwater habitats, especially in the Southern Hemisphere. However, parastenocaridids most often occur, world-wide, in unconsolidated sediments (i.e. hyporheic and phreatic habitats). The Canthocamptidae are very speciose in ground water, rivalling the Parastenocarididae. Some canthocamptid genera are found in both surface and groundwater environments, while others are strictly confined to ground water (e.g. *Antrocamptonus*, *Paramorariopsis*, *Lessinocamptus*, *Australocamptonus*).

Species of some copepod genera show different habitat preferences along latitudinal gradients. They are predominantly hypogean in the Northern Hemisphere and mostly epigean in the Southern Hemisphere, particularly in tropical and subtropical areas. This difference may be the legacy of Quaternary glaciation, which led to massive extinction of surface faunas in northern countries, whereas in the tropics they were able to survive in their ancestral surface habitats. Accordingly, geographical areas strongly affected by Pleistocene glaciations show fewer endemic stygobiotic copepods than non-glaciated areas (Strayer & Reid, 1999; Rundle *et al.*, 2002; Lewis & Reid, 2007). Fully glaciated areas were probably subjected to massive extinctions, not only affecting epigean species but also stygobiotic taxa. Generalist species, such as *Speocyclops demetiensis* (Scourfield), *Elaphoidella bidens* (Schmeil) and *E. gracilis* (Sars), may have reinvaded surface waters during post-glacial periods; however, the greater isolation of groundwater habitats in conjunction with their low dispersal ability prevented most stygobiotic copepods from

recolonising ground water in previously fully glaciated areas. There are a few remarkable exceptions, such as *Elaphoidella elaphoides* (Chappuis) and *Graeteriella unisetigera* (Graeter), which assume a wide distribution in European ground waters, possibly resulting from both active and passive dispersal. In this light, the hypothesis that stygobionts are more wide-ranging than troglobionts (i.e. cave-dwelling organisms), because of the hydrological continuum of the groundwater environment (Lamoreaux, 2004), should be reconsidered for at least some taxonomic groups.

A striking feature of copepods in ground water is their higher-level taxonomic diversity. Taxa are frequently only distantly related to each other, and most of them can be considered phylogenetic relicts (i.e. taxa with no close living relatives, unique remnants of formerly diversified taxonomic groups) or distributional relicts (i.e. taxa with close counterparts traceable in disjunct geographical areas) (Holsinger, 1988; Humphreys, 2000). An example of such distributional relicts is the order Gelyelloida, until recently known only from karstic systems and springs in southern France and Switzerland. The recent discovery of a new gelyelloid in North American ground water (J. W. Reid, unpubl. data) lends credence to the ancient colonisation of ground water by this group, which probably pre-dated the opening of the Atlantic Ocean in the Late Cretaceous or Early Tertiary. Numerous copepod genera, known exclusively from

ground water, occur in restricted geographical areas (strict endemics). Others can show wide disjunct distributions (e.g. Galassi, 2001), with groundwater localities occurring in the Caribbean, the Mediterranean, and sometimes the Australasian region, fitting the 'Tethyan track' of distribution, which runs along major Tertiary orogenic belts formed during the disappearance of the Tethys Sea (Michaux, 1989).

Diversity of groundwater copepods

Europe

The PASCALIS project was aimed at analysing and comparing the stygobiotic fauna at the European scale (Malard *et al.*, 2009). The Copepoda were the dominant group of stygobionts in the PASCALIS data set (Table 1). A total of 110 copepod species was collected, with Harpacticoida and Cyclopoida almost equally represented (Table 1). Within individual regions, copepods also consistently outnumbered all or most other taxonomic groups, although there were some regional differences. The relative importance of Cyclopoida and Harpacticoida also varied between regions (Table 1). At the extreme, only seven stygobiotic copepods were found in the Walloon Karst, all belonging to the order Cyclopoida (Table 1). In the Lessinian Mountains and the Krim Massif, harpacticoids were more diverse than cyclopoids, whereas in the remaining regions the number

Table 1 Species richness of stygobiotic copepods in comparison to species richness of other stygobiotic invertebrates in six different European regions. Data were derived from the PASCALIS database (Dole-Olivier *et al.*, 2009). Species whose classification as stygobionts was uncertain were omitted

Taxon	Cantabria (Spain)	French Jura (France)	Krim Massif (Slovenia)	Lessinian Massif (Italy)	Roussillon (France)	Walloon Karst (Belgium)	All
Copepoda, Calanoida	0	0	1	0	0	0	1
Copepoda, Cyclopoida	10	11	13	12	11	7	50
Copepoda, Harpacticoida	10	9	18	24	7	0	59
Polychaeta	0	0	0	1	0	0	1
Oligochaeta	12	4	21	15	8	3	58
Gastropoda	2	6	14	2	5	1	29
Cladocera	0	1	0	0	1	1	2
Ostracoda	5	10	11	7	4	5	33
Isopoda	3	7	4	2	10	3	26
Amphipoda	6	10	9	12	10	9	45
Syncarida, Bathynellacea	13	2	5	6	7	0	33
Thermosbaenacea	0	0	0	1	0	0	1
Acari	6	0	8	7	4	5	23
Coleoptera	0	1	0	0	0	0	1

of species was almost evenly distributed between the two orders.

At the level of different kinds of aquifers (karstic versus porous), copepod species richness in karstic aquifers ranged from 5 in the Walloon Karst to 26 in the Lessinian Massif, and accounted for 20–42% of the total stygobiotic species richness (Fig. 3). In porous aquifers, copepod species richness varied from 5 to 24, accounting for 17–44% of total species richness. The same situation was observed when evaluating copepod species richness in saturated aquifers (7–20 species, representing 22–50% of the total richness) and upper karstic and porous aquifers (3–31 species, representing 17–41% of the total richness) among regions. The higher copepod diversity observed in unsaturated karst and in hyporheic habitats of Italy and Slovenia (Fig. 3) is probably the result of high habitat heterogeneity, and also reflects the ancient age of the areas in which these aquifers are located. Moreover, the Quaternary glaciation in the southernmost parts of the PASCALIS study area was less important than farther north, allowing for the survival of more species than in the glaciated areas of the French Jura or the Walloon Karst. The number of copepods was lower in the saturated karstic and porous aquifers compared to hyporheic and unsaturated karstic habitats (Fig. 3), most probably due to sampling bias and lower organic matter availability (Dole-Olivier *et al.*, 2009; Stoch *et al.*, 2009).

On the Italian peninsula and nearby islands, 353 stygobiotic species have been recorded. The stygobiotic Copepoda are represented by 160 species and greatly outnumber other crustacean and non-crustacean taxa (Ruffo & Stoch, 2005). The stygobiotic copepod distribution reflects that observed for surface freshwater, from which some 160 species have been recorded in Italy. Consequently, 50% of the Italian copepod fauna is represented by stygobiotic species, suggesting that speciation in ground water accounts for a significant proportion of the total copepod species richness (Table 2). In France, Ferreira *et al.* (2007) listed 380 stygobiotic species and subspecies, 65% of which are crustaceans. Copepods amounted to 43% of the recorded crustacean species, of which 33% belonged to Cyclopoida (35 species) and 63% to Harpacticoida (68 species). Subterranean waters of Slovenia have been the subject of intensive sampling for a long time. Here, the Copepoda represent some 19% of the total number of stygobiotic species, being

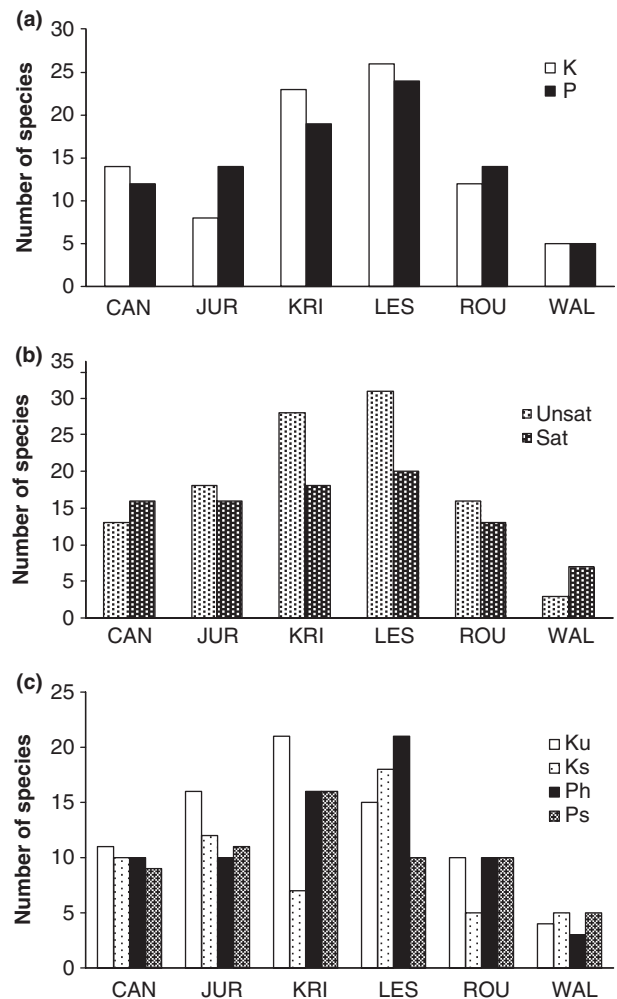


Fig. 3 Copepod species richness in different habitats of six European regions: (a) karstic and porous upper aquifers versus saturated aquifers; (b) karstic versus porous aquifers; (c) saturated porous versus hyporheic versus saturated karst versus unsaturated karst. Can = Picos de Europa, Cantabria, Spain; Jur = Jura Massif, France; Krim = Krim Massif, Slovenia; Les = Lessinian Massif, Italy; Rous = Têt Massif, Roussillon, France; Wal = Walloon Karst, Belgium. UpPKA = porous and karstic upper aquifers, Sat = saturated aquifers, K = karstic aquifers, P = porous aquifers; Ku = unsaturated karst, Ks = saturated karst, Ph = hyporheic, Ps = saturated porous.

outnumbered only by the Malacostraca (Sket, 1999a,b).

To evaluate the intrageneric diversity of stygobiotic copepods in ground water, further analyses were carried out at the regional scale, as exemplified in the Lessinian Massif of Italy. Stygobiotic copepods were unevenly distributed among genera, with the cyclopoid *Diacyclops* (nine species) and the harpacticoid genera *Parastenocaris* (six species), *Lessinocamptus* (five

Table 2 Number of described freshwater invertebrate species in surface water and ground water of Italy. The species listed for ground water are genuine stygobionts (n.a. = data not available, undescribed species not included). Data were derived from Ruffo & Stoch (2005)

Taxon	Surface water	Ground water
Platyhelminthes		
Turbellaria	190	4
Annelida		
Polychaeta	0	1
Oligochaeta	93	16
Hirudinea	23	0
Mollusca		
Gastropoda	101	34
Bivalvia	25	0
Crustacea		
Cladocera	111	1
Ostracoda	n.a.	9
Copepoda		
Calanoida	26	1
Cyclopoida	99	53
Harpacticoida	195	106
Isopoda	54	32
Amphipoda	94	79
Mysidacea	0	2
Thermosbaenacea	0	4
Syncarida	0	7
Acari		
Hydrachnidia	380	5
Insecta	~2500	0

species) and *Elaphoidella* (four species) being the most speciose. All the other genera were represented by only one or two species. *Diacyclops* and *Parastenocaris* were mostly represented in the hyporheic habitat, with a high incidence of sympatric species. This suggests that microhabitat diversity might have been the primary factor promoting radiation, generating a high level of cryptic diversity in these genera (Reid, 1992; Boxshall & Evstigneeva, 1994; Rouch, 1995; Pospisil & Stoch, 1999; Galassi, 2001). The highest species richness was found again in the hyporheic habitat and in the epikarst, with 21 and 15 stygobiotic copepod species, respectively. The epikarst also had the highest ratio of exclusive copepod species (eight of 15 species), whereas only seven of 21 stygobiotic copepods were exclusive to the hyporheic habitat.

The Americas and Australia

In North America, very few regional inventories of subterranean aquatic invertebrates are available, and

most of these either do not include copepods or the records listed do not reflect targeted sampling for copepods (e.g. Reddell, 1965, 1970; Holsinger & Peck, 1972; Holsinger & Culver, 1988; Culver *et al.*, 2000; Lewis *et al.*, 2003). Exceptions are the reports by Reeves (2000) for caves of a small karst region within the Great Smoky Mountains National Park, eastern Tennessee; by Bruno, Reid & Perry (2005) for the Everglades; and by Lewis & Reid (2007) for karstic regions in the Interior Low Plateaus. In Mexico, the best-known stygobiotic fauna is that of karstic habitats on the Yucatan Peninsula (Suárez-Morales & Reid, 2003) (Table 3). Most copepod species found in these studies are widespread and common stygoxenes (i.e. epigeal species which enter ground water accidentally) or stygophiles (i.e. species with incipient adaptation to groundwater life, being able to live in both surface and subsurface environments) (Table 3).

Recent estimates of stygobiotic species richness in cave waters of the U.S.A. returned surprisingly low numbers of copepods (Culver *et al.*, 2000; Pipan, 2004; Pipan & Culver, 2005; Lewis & Reid, 2007), a result possibly biased by undersampling of meiofauna in North American caves and unconsolidated sediments. Despite the low number of copepods found in cave waters, sampling in other habitats has led to remarkable new discoveries. These include the ameirid genus *Psammonitocrella*, the cyclopoid genus *Rheocyclops*, a large number of stygobiotic *Diacyclops*, *Elaphoidella* and *Parastenocaris* species, and the Gelyelloida (Strayer & Reid, 1999; Reid, 2004). In general, comparisons between copepod distribution in surface *versus* groundwater habitats in the U.S.A., Canada (McLaughlin *et al.*, 2005) and Mexico (Suárez-Morales & Reid, 1998, 2003) did not follow the trend observed in Europe: copepod diversity in surface waters was significantly higher than in ground water (Table 4).

In Central and South America, only very few cave systems have been thoroughly sampled for copepods. Except for a few records of canthocamptid harpacticoids, which may be stygophiles, the known true stygobiotic fauna consists mainly of the *Parastenocarididae*. Clearly, the stygobiotic copepods of Central and South America are so incompletely known that it is difficult to perceive general patterns. Recent sampling in Australia has revealed an 'unfolding plethora of stygal biodiversity' (Humphreys, 2001), and the number of known stygobiotic copepod species on this

Table 3 Copepod species richness in groundwater habitats in several regions of the U.S.A. and Mexico (SX = stygoxene, SP = stygophilous; SB = stygobiont). Data were derived from Lewis & Reid (2007), Reeves (2000), Bruno *et al.* (2005) and Suárez-Morales & Reid (2003)

Taxon	Interior Low Plateaus, U.S.A.		Great Smoky Mountains National Park, TN, U.S.A.		Everglades, FL, U.S.A.		Yucatan Peninsula, Mexico	
	SX-SP	SB	SX-SP	SB	SX-SP	SB	SX-SP	SB
Calanoida	2	0	0	0	1	0	8	0
Cyclopoida	25	14	1	0	27	0	26	13
Harpacticoida	8	1	1	0		2	10	2
Total	35	15	2	0	28	2	44	15

Table 4 Species richness of freshwater copepods in surface water (SW) and ground water (GW) in Europe, Mexico and North America (SW = surface water, GW = ground water). The species listed for ground water are genuine stygobionts. Data were derived from Sket (1999b), Ferreira *et al.* (2007), Ruffo & Stoch (2005), Suárez-Morales & Reid (1998, 2003) and McLaughlin *et al.* (2005)

Taxon	Slovenia		France		Italy		Mexico		U.S.A. and Canada	
	SW	GW	SW	GW	SW	GW	SW	GW	SW	GW
Calanoida	10	1	24	1	26	1	21	1	94	0
Cyclopoida	33	19	31	35	99	53	54	13	105	27
Harpacticoida	29	18	35	68	195	106	15	4	72	16
Gelyelloida	0	0	0	1	0	0	0	0	0	1
Total	72	38	90	105	320	160	90	18	271	44

continent has exponentially increased in recent years (Karanovic, 2004, 2006; Eberhard, Halse & Humphreys, 2006). This suggests that even moderate sampling efforts on other continents and in other regions are very likely to result in the discovery of many more species.

Copepods and groundwater biodiversity assessment and conservation

Efforts towards biodiversity conservation start with an assessment of species richness and population sizes. However, comprehensive assessments are time-consuming, especially when large areas are surveyed, and almost always economically and practically unattainable (Balmford *et al.*, 2005). As a result, biodiversity assessments rarely consider the entire assemblage at a given site. Several alternative approaches for measuring biodiversity are currently in use (Magurran, 2004). They are often based on subsets of taxa that have been shown or are assumed to be surrogates that capture biodiversity as a whole

(Magurran, 2004). The most common and feasible procedure involves selection of a focal group. This requires reliable species identification, adequate representation of the chosen group in samples, and a certain degree of taxonomic and functional diversity, including feeding habits and species-specific microhabitat preferences. In practice, these prerequisites make it difficult to identify a single subtaxon that would be fully satisfactory as a focal group. Faced with this obstacle, Hammond (1995) recommended the use of what he called a 'shopping basket' of subtaxa that together serve as composite focal group, covering a wider range of niches and functional roles than single groups and thus ensuring better performance as surrogates for entire assemblages.

Rank-order multiple-regression analyses have proved useful to extract combinations of such composite focal group in European ground water (Stoch *et al.*, 2009). These analyses showed in all cases that copepod species richness, variously combined with that of other taxonomic groups, led to accurate prediction of the residual species richness in different

European regions (Stoch *et al.*, 2009). This predictive power may be due, to a large extent, to the regular dominance of copepods in ground water in terms of both abundance and species richness. Recent estimates of copepod species richness in the Americas and Australia support this hypothesis (Strayer & Reid, 1999; Reeves, 2000; Karanovic, 2004, 2006; Reid, 2004; Eberhard *et al.*, 2006).

Communities sharing the same number of species and pattern of species abundances but differing in the phylogenetic relatedness of the constituent species may be considered different in their conservation priorities, if higher-level taxonomic diversity is assigned greater value (Faith, 1992; Clarke & Warwick, 1999; Faith & Baker, 2006). The protection of 'evolutionarily significant lineages' (i.e. phylogenetic or distributional relicts; see Posadas, Miranda Esquivel & Crisci, 2001; Faith, Reid & Hunter, 2004; and references therein), which, once lost, cannot be recovered, is a central question in conservation biology, although its relevance for establishing priorities has been questioned (Purvis *et al.*, 2000; Woodruff, 2001).

The extinction risk of taxonomically isolated lineages is extreme in groundwater environments. Even if species richness and abundances in ground water are relatively low, especially in deep ground water, the phylogenetic diversity may be very high, as observed at different spatial scales in various biogeographical regions (Rouch & Danielopol, 1997; Galassi, 2001; Danielopol, Rouch & Baltanás, 2002). Effective temporal isolation in ground water facilitated establishment and survival of a large number of independent phylogenetic lineages, while their surface counterparts were more likely to become extinct during drastic climatic changes in the Tertiary (e.g. Messinian salinity crisis in Mediterranean, and other marine regressive/transgressive phases) and the Quaternary glaciations in the Northern Hemisphere. Although Nee & May (1997) postulated that losing 90% of the species on Earth would reduce global phylogenetic diversity by less than 20%, it is debatable whether this assumption holds for groundwater communities. Ground water is, in this respect, a reliquary, its communities being frequently composed of strictly endemic taxa, confined to single sites and representing the only survivors of groups once distributed more widely.

The value of a taxon as an indicator group for defining conservation priorities is likely to depend not

only on its higher-level taxonomic diversity, but also on high proportion of local endemics and biogeographical concordance with co-occurring species (Moritz *et al.*, 2001). Copepods meet this criterion (Rouch, 1986; Stoch, 1995; Galassi, 2001). They can serve as indicators of microhabitat conditions, since their species-specific requirements are reflected in clear microhabitat preferences, and they can serve as 'historical' indicators of both ancient and recent evolutionary events. Such events can be 'frozen' in the primitive body plan of some taxa, which entered ground water in ancient times (e.g. Gelyelloida, Chappuisiidae, *Pseudectinosoma*, *Rangabradya*, *Nitocrella*, *Nitocrellopsis*, *Parapseudoleptomesochra*). Alternatively, as a result of more recent events, they may represent the sole remnants of lineages surviving exclusively in ground water within limited geographical areas (e.g. the cyclopoids *Speocyclops*, *Graeteriella* and *Rheocyclops*, and the harpacticoids *Paramorariopsis*, *Lessinocamptus*, *Australocamptus* and *Antrocamptus*).

Taken together, the widespread occurrence and high abundance of groundwater copepods in different biogeographical regions, their high diversity, including at higher taxonomic levels, high proportions of local endemics and of phylogenetic and distributional relicts, and species-specific preference for different microhabitats suggest that the Copepoda, in combination with other invertebrate groups such as the Amphipoda (Stoch *et al.*, 2009), are likely to be useful groups for Hammond's (1995) 'shopping basket' to assess groundwater biodiversity as a whole.

Acknowledgments

This review benefited greatly from unpublished data kindly provided by F. Stoch, S. Eberhard and D. Ferreira. The PASCALIS members granted permission to use the lists of Copepoda collated within Work Package 6 of the project. M.-J. Dole-Olivier, D. Ferreira, D. Danielopol, A. Boulton and W. Humphreys greatly assisted D.M.P.G. with recent literature. The image of *P. amalasuntae* was kindly provided by R. Berera and V. Cottarelli. Two anonymous reviewers made constructive criticisms on an early version of the manuscript. We are also much indebted to J. Gibert, D. Culver and M. Gessner for useful comments on the final draft of the manuscript. This contribution was supported by PASCALIS (EVK2-CT-2001-00121) and by MIUR-PRIN.

References

- Balian E.V., Segers H., Lévêque C. & Martens K. (2008) The freshwater animal diversity assessment: an overview of the results. *Hydrobiologia*, **595**, 627–637.
- Balmford A., Bennun L., ten Brink B. *et al.* (2005) The convention on biological diversity's 2010 target. *Science*, **307**, 212–213.
- Bowman T.E. (1986) Copepoda: Calanoida. In: *Stygofauna Mundi – A Faunistic, Distributional and Ecological Synthesis of the World Fauna Inhabiting Subterranean Waters (Including the Marine Interstitial)* (Ed. L. Botosaneanu), pp. 295–298, E. J. Brill, Leiden, The Netherlands.
- Boxshall G.A. & Defaye D. (2008) Global diversity of copepods (Crustacea: Copepoda) in freshwater. *Hydrobiologia*, **595**, 195–207.
- Boxshall G.A. & Evstigneeva T.D. (1994) The evolution of species flocks of copepods in Lake Baikal: a preliminary analysis. *Archiv für Hydrobiologie Beihefte Ergebnisse der Limnologie*, **44**, 235–245.
- Boxshall G.A. & Halsey S.H. (2004) *An Introduction to Copepod Diversity*. The Ray Society, London.
- Boxshall G.A. & Jaume D. (2000) Making waves: the repeated colonization of fresh water by copepod crustaceans. *Advances in Ecological Research*, **31**, 61–79.
- Brancelj A. (1991) *Paramorariopsis anae* gen. n., sp. n. and the female of *Ceuthonectes rouchi* Petkovski, 1984 – two interesting harpacticoids (Copepoda: Crustacea) from caves in Slovenia (NW Yugoslavia). *Stygologia*, **6**, 193–200.
- Brancelj A. (2005) *Hadodiaptomus dumonti* n. gen., n. sp., a new freshwater stygobiotic calanoid (Crustacea: Copepoda: Calanoida) from Vietnam (South Asia) and a new member of the subfamily Speodiaptominae Borutzky, 1962. *Hydrobiologia*, **534**, 57–70.
- Brancelj A. & Dumont H.J. (2007) A review of the diversity, adaptations and groundwater colonization pathways in Cladocera and Calanoida (Crustacea), two rare and contrasting groups of stygobionts. *Fundamental and Applied Limnology*, **168**, 3–17.
- Bruno C., Reid J.W. & Perry S. (2005) A list and identification key for the freshwater, free-living copepods (Crustacea) of Florida (U.S.A.). *Journal of Crustacean Biology*, **25**, 384–400.
- Castellarini F., Malard F., Dole-Olivier M.-J. & Gibert J. (2007) Modelling the distribution of stygobionts in the Jura Mountains (eastern France). Implications for the protection of ground waters. *Diversity and Distributions*, **13**, 213–224.
- Clarke K.R. & Warwick R.M. (1999) A taxonomic distinctness index and its statistical properties. *Journal of Applied Ecology*, **35**, 523–531.
- Culver D.C., Master L.L., Christman M.C. & Hobbs H.H. III (2000) Obligate cave fauna of the 48 contiguous United States. *Conservation Biology*, **14**, 386–401.
- Danielopol D.L., Pospisil P. & Rouch R. (2000) Biodiversity in groundwater: a large-scale view. *Trends in Ecology and Evolution*, **15**, 223–224.
- Danielopol D.L., Rouch R. & Baltanás A. (2002) Taxonomic diversity of groundwater Harpacticoida (Copepoda, Crustacea) in southern France. A contribution to characterise hotspot diversity sites. *Vie et Milieu*, **52**, 1–15.
- Di Lorenzo T., Cipriani D., Bono P., Rossini L., De Laurentiis P., Fiasca B., Pantani P. & Galassi D.M.P. (2005a) a) Dynamics of groundwater copepod assemblages from Mazzoccolo karstic spring (central Italy). *Meiofauna Marina*, **14**, 97–103.
- Di Lorenzo T., Stoch F., Fiasca B., Gattone E., De Laurentiis P., Ranalli F. & Galassi D.M.P. (2005b) Environmental quality of deep groundwater in the Lessinian Massif (Italy): signposts for sustainability. In: *World Subterranean Biodiversity, Proceedings of an International Symposium* (Ed. J. Gibert), pp. 115–125, Université Claude Bernard, Lyon 1, France.
- Dole-Olivier M.-J. & Marmonier P. (1992a) Ecological requirements of stygofauna in an active channel of the Rhône River. *Stygologia*, **7**, 65–75.
- Dole-Olivier M.-J. & Marmonier P. (1992b) Patch distribution of interstitial communities: prevailing factors. *Freshwater Biology*, **27**, 177–191.
- Dole-Olivier M.-J., Marmonier P., Creuzé des Châtelliers M. & Martin D. (1994) Interstitial fauna associated with the alluvial floodplains of the Rhône river (France). In: *Groundwater Ecology* (Eds J. Gibert, D.L. Danielopol & J.A. Stanford), pp. 313–346, Academic Press, San Diego, CA.
- Dole-Olivier M.-J., Marmonier P. & Beffy J.L. (1997) Response of invertebrates to lotic disturbance: is the hyporheic zone a patchy refugium? *Freshwater Biology*, **37**, 257–276.
- Dole-Olivier M.-J., Galassi D.M.P., Marmonier P. & Creuzé des Châtelliers M. (2000) The biology and ecology of lotic microcrustaceans. *Freshwater Biology*, **44**, 63–91.
- Dole-Olivier M.-J., Castellarini F., Coineau N., Galassi D.M.P., Martin P., Mori N., Valdecasas A. & Gibert J. (2009) Towards an optimal sampling strategy to assess groundwater biodiversity: comparison across six European regions. *Freshwater Biology*, **54**, 777–796.
- Eberhard S.M., Halse S.A. & Humphreys W.F. (2006) Stygofauna in the Pilbara region, north-west Western Australia: a review. *Journal of the Royal Society of Western Australia*, **88**, 167–176.

- Enckell P.H. (1969) Distribution and dispersal of Parastenocarididae (Copepoda) in northern Europe. *Oikos*, **20**, 493–507.
- Faith D.P. (1992) Systematics and conservation: on predicting the feature diversity of subsets of taxa. *Cladistics*, **8**, 361–373.
- Faith D.P. & Baker A.M. (2006) Phylogenetic diversity (PD) and biodiversity conservation: some bioinformatics challenges. *Evolutionary Bioinformatics Online*, **2**, 70–77.
- Faith D.P., Reid C.A.M. & Hunter J. (2004) Integrating phylogenetic diversity, complementarity, and endemism for conservation assessment. *Conservation Biology*, **18**, 255–261.
- Ferrari F.D. & Dahms H.U. (2007) *Post-embryonic Development of the Copepoda*. Crustaceana Monographs, 8, Brill, Leiden, The Netherlands.
- Ferreira D., Malard F., Dole-Olivier M.-J. & Gibert J. (2007) Obligate groundwater fauna of France: diversity patterns and conservation implications. *Conservation Biology*, **16**, 567–596.
- Fiasca B., Di Lorenzo T., De Laurentiis P., Pantani P. & Galassi D.M.P. (2005) Biodiversità acquatica sotterranea nel Parco Nazionale del Gran Sasso-Monti della Laga: analisi taxocenotica e proposte di conservazione. *Studi Trentini di Scienze Naturali, Acta Biologica*, **81**, 157–166.
- Fiers F., Reid J.W., Iliffe T.M. & Suárez-Morales E. (1996) New hypogean cyclopoid copepods (Crustacea) from the Yucatán Peninsula, Mexico. *Contributions to Zoology*, **66**, 65–102.
- Galassi D.M.P. (2001) Groundwater copepods: diversity patterns over ecological and evolutionary scales. *Hydrobiologia*, **454/453**, 227–253.
- Galassi D.M.P. & De Laurentiis P. (2004a) Towards a revision of the genus *Parastenocaris* Kessler, 1913: establishment of *Simplicaris* gen. nov. from groundwaters in central Italy and review of the *P. brevipes*-group (Copepoda, Harpacticoida, Parastenocarididae). *Zoological Journal of the Linnean Society*, **140**, 417–436.
- Galassi D.M.P. & De Laurentiis P. (2004b) Little-known cyclopoids from groundwaters in Italy: re-validation of *Acanthocyclops agamus* and redescription of *Speocyclops italicus* (Crustacea, Copepoda, Cyclopoida). *Vie et Milieu*, **54**, 203–222.
- Galassi D.M.P., De Laurentiis P. & Dole-Olivier M.-J. (1999a) *Nitocrellopsis rouchi* sp. n., a new ameirid harpacticoid from phreatic waters of France (Copepoda: Harpacticoida: Ameiridae). *Hydrobiologia*, **412**, 177–189.
- Galassi D.M.P., Dole-Olivier M.-J. & De Laurentiis P. (1999b) Phylogeny and biogeography of the genus *Pseudectinosoma*, and description of *P. janineae* sp. n. (Crustacea, Copepoda, Ectinosomatidae). *Zoologica Scripta*, **28**, 289–303.
- Galassi D.M.P., Marmonier P., Dole-Olivier M.-J. & Rundle S. (2002) Microcrustacea. In: *Freshwater Meiofauna: Biology and Ecology* (Eds S.D. Rundle, A.L. Robertson & J.M. Schmid-Araya), pp. 133–175, Backhuys Publishers, Leiden, The Netherlands.
- Gibert J. & Deharveng L. (2002) Subterranean ecosystems: a truncated functional biodiversity. *BioScience*, **52**, 473–481.
- Gibert J., Vervier P., Malard F., Laurent R. & Reygrobellet J.-L. (1994) Dynamics of communities and ecology of karst ecosystems: examples of three karsts in eastern and southern France. In: *Groundwater Ecology* (Eds J. Gibert, D.L. Danielopol & J.A. Stanford), pp. 425–450, Academic Press, San Diego, CA.
- Gibert J., Malard F., Turquin M.J. & Laurent R. (2000) Karst ecosystems in the Rhône River basin. In: *Ecosystems of the World, 30. Subterranean Ecosystems* (Eds H.D. Wilkens, D.C. Culver & W.F. Humphreys), pp. 533–558, Elsevier, Amsterdam, The Netherlands.
- Hammond P.M. (1995) Practical approaches to the estimation of the extent of biodiversity in speciose groups. In: *Biodiversity, Measurement and Estimation* (Ed. D.L. Hawksworth), pp. 119–136. Chapman & Hall, London.
- Holsinger J.R. (1988) Trogllobites: the evolution of cave-dwelling organisms. *American Scientist*, **76**, 147–153.
- Holsinger J.R. & Culver D.C. (1988) The invertebrate cave fauna of Virginia and a part of eastern Tennessee: Zoogeography and ecology. *Brimleyana*, **14**, 1–160.
- Holsinger J.R. & Peck S.B. (1972) The invertebrate cave fauna of Georgia. *Bulletin of the National Speleological Society*, **33**, 23–44.
- Humphreys W.F. (2000) Relict faunas and their derivation. In: *Ecosystems of the World, 30. Subterranean Ecosystems* (Eds H.D. Wilkens, D.C. Culver & W.F. Humphreys), pp. 417–432. Elsevier, Amsterdam, The Netherlands.
- Humphreys W.F. (2001) Groundwater calcrite aquifers in the Australian arid zone: the context to an unfolding plethora of stygal biodiversity. *Records of the Western Australian Museum*, **64**, 63–83.
- Huys R. (1988) Gelyelloida, a new order of stygiobont copepods from European karstic systems. *Hydrobiologia*, **167/168**, 485–495.
- Huys R. & Boxshall G.A. (1991) *Copepod Evolution*. Ray Society, London.
- Huys R. & Iliffe T. (1998) Novocriiniidae, a new family of harpacticoid copepods from anchihaline caves in Belize. *Zoologica Scripta*, **27**, 1–15.
- Huys R., Llewellyn-Hughes J., Conroy-Dalton S., Olson P.D., Spinks J.N. & Johnston D.A. (2007) Extraordinary

- host switching in siphonostomatoid copepods and the demise of the Monstrilloidea: integrating molecular data, ontogeny and antennular morphology. *Molecular Phylogenetics and Evolution*, **43**, 368–378.
- Karanovic T. (2004) Subterranean Copepoda from arid Western Australia. *Crustaceana Monographs*, **3**, 1–366.
- Karanovic T. (2006) Subterranean copepods (Crustacea, Copepoda) from the Pilbara Region in Western Australia. *Records of the Western Australian Museum Supplement*, **70**, 1–239.
- Lamoreaux J. (2004) Stygobites are more wide-ranging than troglobites. *Journal of Cave and Karst Studies*, **66**, 18–19.
- Lee W. & Huys R. (2002) A new genus of groundwater Ameiridae (Copepoda: Harpacticoida) from boreholes in Western Australia and the artificial status of *Stygonitocrella* Petkovski, 1976. *Bulletin of the Natural History Museum (Zoology)*, **68**, 39–50.
- Lescher-Moutoué F. (1973) Sur la biologie et l'écologie des copépodes cyclopidés hypogés (Crustacés). *Annales de Spéléologie*, **28**, 429–502.
- Lewis J.J. & Reid J.W. (2007) Patterns and processes of groundwater invasion by copepods in the Interior Low Plateaus of the United States. *Acta Carsologica*, **36**, 279–289.
- Lewis J.J., Moss P., Tecic D. & Nelson M.E. (2003) A conservation focused inventory of subterranean invertebrates of the southwestern Illinois karst. *Journal of Cave and Karst Studies*, **65**, 9–21.
- Magurran A.E. (2004) *Measuring Biological Diversity*. Blackwell Science Ltd, Oxford.
- Malard F., Reygrobellet J.-L., Mathieu J. & Lafont M. (1994) The use of invertebrate communities to describe groundwater flow and contaminant transport in a fractured rock aquifer. *Archiv für Hydrobiologie*, **131**, 93–110.
- Malard F., Reygrobellet J.-L. & Laurent R. (1998) Spatial distribution of epigeal invertebrates in an alluvial aquifer polluted by iron and manganese, Rhône River, France. *Verhandlungen der Internationalen Vereinigung für Limnologie*, **26**, 1590–1594.
- Malard F., Galassi D.M.P., Lafont M., Dolédec S. & Ward J.V. (2003) Longitudinal patterns of invertebrates in the hyporheic zone of a glacial river. *Freshwater Biology*, **48**, 1709–1725.
- Malard F., Boutin C., Camacho A.I., Ferreira D., Michel G., Sket B. & Stoch F. (2009) Diversity patterns of stygobiotic crustaceans across multiple spatial scales in Europe. *Freshwater Biology*, **54**, 756–776.
- Marcotte B.M. (1983) The imperatives of copepod diversity: perception, cognition, competition and predation. In: *Crustacean Phylogeny. Crustacean Issues, Vol. 1*, (Ed. F.R. Schram), pp. 47–72. Balkema, Rotterdam, The Netherlands.
- Martens K., Schön I., Meisch C. & Horne D.J. (2008) Global diversity of ostracods (Ostracoda, Crustacea) in freshwater. *Hydrobiologia*, **595**, 185–193.
- McLaughlin P.A., Camp D.K., Angel M.V. et al. (2005) Common and scientific names of aquatic invertebrates from the United States and Canada: crustaceans. *American Fisheries Society, Special Publication*, **31**, 1–545.
- Michaux B. (1989) Generalized tracks and geology. *Systematic Zoology*, **38**, 390–398.
- Moritz C., Richardson K.S., Ferrier S., Monteith G.B., Stanistic J., Williams S.E. & Whiffin T. (2001) Biogeographical concordance and efficiency of taxon indicators for establishing conservation priority in a tropical rainforest biota. *Proceedings of the Royal Society of London, Series B*, **268**, 1875–1881.
- Nee S. & May R.M. (1997) Extinction and the loss of evolutionary history. *Science*, **278**, 692–694.
- Palmer M.A., Arensburger P., Botts P.S., Hakenkamp C.C. & Reid J.W. (1995) Disturbance and the community structure of stream invertebrates: patch-specific effects and the role of refugia. *Freshwater Biology*, **34**, 343–356.
- Paran F., Malard F., Mathieu J., Lafont M., Galassi D.M.P. & Marmonier P. (2005) Distribution of groundwater invertebrates along an environmental gradient in a shallow water-table aquifer. In: *World Subterranean Biodiversity, Proceedings of an International Symposium* (Ed. J. Gibert), pp. 99–105. University Claude Bernard, Lyon, France.
- Pesce G.L., Galassi D.P. & Cottarelli V. (1995) *Parastenocaris lorenzae* n. sp., and first record of *Parastenocaris glacialis* Noodt (Copepoda, Harpacticoida) from Italy. *Hydrobiologia*, **302**, 97–101.
- Pipan T. (2004) Ecological and microgeographical study of an epikarst in West Virginia (USA). *Acta Carsologica*, **33**, 269–275.
- Pipan T. (2005) Epikarst – a promising habitat. Copepod fauna, its diversity and ecology: a case study from Slovenia (Europe). *Carsologica*, **5**, 1–101.
- Pipan T. & Brancelj A. (2004) Distribution patterns of copepods (Crustacea: Copepoda) in percolation water of the Postojnska Jama Cave system (Slovenia). *Zoological Studies*, **43**, 206–210.
- Pipan T. & Culver D.C. (2005) Estimating biodiversity in the epikarstic zone of a West Virginia cave. *Journal of Cave and Karst Studies*, **67**, 103–109.
- Posadas P.D., Miranda Esquivel D.R. & Crisci J.V. (2001) Using phylogenetic diversity measures to set priorities in conservation: an example from southern South America. *Conservation Biology*, **15**, 1325–1334.

- Pospisil P. & Stoch F. (1999) Two new species of the *Diacyclops languidoides* – group (Copepoda, Cyclopoida) from groundwaters of Austria. *Hydrobiologia*, **412**, 165–176.
- Purvis A., Agapow P.-M., Gittleman J.L. & Mace G.M. (2000) Nonrandom extinction and the loss of evolutionary history. *Science*, **288**, 328–330.
- Reddell J.R. (1965) A checklist of the cave fauna of Texas. 1. The Invertebrata (exclusive of Insecta). *Texas Journal of Science*, **17**, 143–187.
- Reddell J.R. (1970) A checklist of the cave fauna of Texas. 4. Additional records of Invertebrata (exclusive of Insecta). *Texas Journal of Science*, **21**, 389–415.
- Reeves W.K. (2000) Invertebrate cavernicoles of the Great Smoky Mountains National Park, USA. *Journal of the Elisha Mitchell Scientific Society*, **116**, 334–343.
- Reid J.W. (1992) Taxonomic problems: a serious impediment to groundwater ecological research in North America. In: *Proceedings of the First International Conference on Ground Water Ecology* (Eds J.A. Stanford & J.J. Simons), pp. 133–142, American Water Research Association, Bethesda, MD.
- Reid J.W. (2004) New records and new species of the genus *Diacyclops* (Crustacea; Copepoda) from subterranean habitats in southern Indiana, USA. *Jeffersoniana*, **12**, 1–65.
- Robertson A.L. (2002) Changing times: the temporal dynamics of freshwater benthic microcrustacea. In: *Freshwater Meiofauna: Biology and Ecology* (Eds S.D. Rundle, A.L. Robertson & J.M. Schmid-Araya), pp. 261–278, Backhuys Publishers, Leiden, The Netherlands.
- Rouch R. (1986) Copepoda: les Harpacticoides souterrains des eaux douces continentales. In: *Stygofauna Mundi – A Faunistic, Distributional and Ecological Synthesis of the World Fauna Inhabiting Subterranean Waters (Including the Marine Interstitial)* (Ed. L. Botosaneanu), pp. 321–335, E. J. Brill, Leiden, The Netherlands.
- Rouch R. (1991) Structure du peuplement des harpacticides dans le milieu hyporhéique d'un ruisseau des Pyrénées. *Annales de Limnologie*, **27**, 227–241.
- Rouch R. (1995) Peuplement des Crustacés dans la zone hyporhéique d'un ruisseau des Pyrénées. Implications écologiques. *Annales de Limnologie*, **31**, 9–28.
- Rouch R. & Danielopol D. (1997) Species richness of microcrustacea in subterranean freshwater habitats. Comparative analysis and approximate evaluation. *Internationale Revue der gesamten Hydrobiologie*, **82**, 121–145.
- Ruffo S. & Stoch F. (2005) *Checklist e distribuzione della fauna italiana*. Memorie del Museo Civico di Storia Naturale di Verona, 2. Serie, Sezione Scienze della Vita, 16, Verona, 307 pp.
- Rundle S.D. & Ramsay P. (1997) Microcrustacean communities in streams from two physiographically contrasting regions of Britain. *Journal of Biogeography*, **24**, 101–111.
- Rundle S., Bilton D.T., Galassi D. & Shiozawa D.K. (2002) The geographical ecology of freshwater meiofauna. In: *Freshwater Meiofauna: Biology and Ecology* (Eds S.D. Rundle, A.L. Robertson & J.M. Schmid-Araya), pp. 279–294, Backhuys Publishers, Leiden, The Netherlands.
- Sket B. (1999a) The nature of biodiversity in hypogean waters and how it is endangered. *Biodiversity and Conservation*, **8**, 1319–1338.
- Sket B. (1999b) High biodiversity in hypogean waters and its endangerment – the situation in Slovenia, the Dinaric Karst and Europe. *Crustaceana*, **72**, 767–780.
- Stoch F. (1995) The ecological and historical determinants of crustacean diversity in groundwaters, or: why are there so many species? *Mémoires de Biospéologie*, **22**, 139–160.
- Stoch F. (1997) A new genus and two new species of Canthocamptidae (Copepoda, Harpacticoida) from caves in northern Italy. *Hydrobiologia*, **350**, 49–61.
- Stoch F. (2000) New and little known *Parastenocaris* (Copepoda, Harpacticoida, Parastenocarididae) from cave waters in Northeastern Italy. *Bollettino del Museo Civico di Storia Naturale di Verona*, **24**, 195–206.
- Stoch F. (2001) How many species of *Diacyclops*? New taxonomic characters and species richness in a freshwater cyclopid genus (Copepoda, Cyclopoida). *Hydrobiologia*, **453/454**, 525–531.
- Stoch F., Artheau M., Brancelj A., Galassi D.M.P. & Malard F. (2009) Biodiversity indicators in European ground waters: towards a predictive model of stygobiotic species richness. *Freshwater Biology*, **54**, 745–755.
- Strayer D.L. & Reid J.W. (1999) Distribution of hyporheic cyclopoids (Crustacea: Copepoda) in the eastern United States. *Archiv für Hydrobiologie*, **145**, 79–92.
- Suárez-Morales E. & Reid J.W. (1998) An updated list of the free-living freshwater copepods (Crustacea) of Mexico. *Southwestern Naturalist*, **43**, 256–265.
- Suárez-Morales E. & Reid J.W. (2003) An updated checklist of the continental copepod fauna of the Yucatan Peninsula, Mexico, with notes on its regional associations. *Crustaceana*, **76**, 977–991.
- Väinölä R., Witt J.D.S., Grabowski M., Bradbury J.H., Jazdzewski K. & Sket B. (2008) Global diversity of amphipods (Amphipoda; Crustacea) in freshwater. *Hydrobiologia*, **595**, 241–255.
- Valentine J.W. (2004) *On the Origin of Phyla*. The University of Chicago Press, Chicago, IL.

- Ward J.V. & Voelz N.J. (1994) Groundwater fauna of the South Platte River system, Colorado. In: *Groundwater/Surface Water Ecotones: Biological Interactions and Management Options* (Eds J. Gibert, J. Mathieu & F. Fournier), pp. 37–41, International Hydrology Series, Cambridge University Press, Cambridge.
- Ward J.V. & Voelz N.J. (1997) Interstitial fauna along an epigeal-hypogean gradient in a Rocky Mountain river. In: *Groundwater Ecology* (Eds J. Gibert, D.L. Danielopol & J.A. Stanford), pp. 391–423, Academic Press, San Diego, CA.
- Westheide W. (1987) Progenesis as a principle in meiofauna evolution. *Journal of Natural History*, **21**, 843–854.
- Woodruff D.S. (2001) Declines of biomes and biotas and the future of evolution. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 5471–5476.

(Manuscript accepted 25 December 2008)