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Testing dispersal and cryptic diversity in a widely distributed groundwater amphipod (*Niphargus rhenorhodanensis*)

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Abstract

Theories about colonization and evolution in groundwater have assumed that the fragmented structure of groundwater strongly limits dispersal. The high number of endemic and allopatric species in groundwater supports this hypothesis, but the occurrence of widely distributed groundwater taxa calls into question its universality. These widely distributed taxa might also be sets of cryptic species because extreme conditions of life in groundwater promote cryptic diversity by inducing convergent morphological evolution. *Niphargus rhenorhodanensis* is a widely distributed and ubiquitous groundwater amphipod which supposedly colonized the Alps after Quaternary glaciations. We tested the dispersal and the cryptic species hypotheses within this species using a phylogeographic approach based on two mitochondrial genes (COI and 16S) and a nuclear gene (28S). Results support the view that poor dispersal is a main evolutionary factor in groundwater. All genes independently supported the existence of numerous cryptic and mostly allopatric units within *N. rhenorhodanensis*, indicating that its apparently wide distribution range is an artefact generated by cryptic diversity. We reject the hypothesis of a recent and global colonization of the Alps and argue that some *N. rhenorhodanensis* lineages probably survived glaciations near or within the Alps.

Keywords: Cryptic diversity; Dispersal; Glaciation; Subterranean; Subsurface; Crustacea; Phylogeography

1. Introduction

Groundwater (GW) forms a huge ocean under our feet (Danielopol et al., 2000) inhabited by a diversified fauna sharing traits like depigmentation, anophtalmy, long life cycle and resistance to anoxia and starvation (e.g., Poulson and White, 1969; Culver, 1982; Hervant et al., 1999; Malard and Hervant, 1999; Hüppop, 2000). Darkness, the relative stability of environmental conditions compared to surface water and the fragmented structure of small hydrological units are basic attributes of GW (Gibert et al., 1994). These attributes supposedly favour long-therm evolution in stable conditions and result in the occurrence of a high number of endemics and allopatric species (Gibert and Deharveng, 2002). Therefore, groundwater organisms are thought to form stable populations over geological time that cannot disperse over long distances. However, this evolutionary scenario hardly applies to widely distributed groundwater taxa or organisms living in habitats that were recently created (fresh alluvium) or made available (newly deglaciated terrain) (Danielopol et al., 1994; Ward and Palmer, 1994). The present-day distributional ranges of these taxa suggest that dispersal has occurred, although this possibility has long been debated among subterranean biologists (e.g., Rouch and Danielopol, 1987; Botosaneanu and Holsinger, 1991).

Amphipods of the family Niphargidae, and especially the genus *Niphargus*, are often considered as the most representative group of groundwater organisms. This genus comprises more than 250 GW species with a palearctic distribution mostly limited to Europe (Karaman and Ruffo, 1986; Holsinger, 1994). Most of these species are endemic to

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one or a few locations, but notable exceptions include species with ranges spanning several hundreds of kilometers (e.g. *Niphargus tauri*—1900 km, *Niphargus longicaudatus*—1500 km, and *N. rhenorhodanensis*—500 km). Interestingly, some of these widely distributed niphargids colonize areas that were covered by ice and presumably permafrosted during the last glaciations (e.g., Jura, Alps, United Kingdom).

Niphargus rhenorhodanensis (Schellenberg, 1937) is a typical example of a groundwater species with a wide distribution apparently owing to dispersal (Ginet, 1971). This amphipod is distributed throughout the Rhône River basin and has colonized multiple habitats ranging from GW in alluvial sediments (hereafter called porous GW) to GW in consolidated rocks, including calcareous rocks (karstic GW) and metamorphic rocks (fissured GW). Many of these habitats are recent (modern alluvium, glaciofluvial deposits, and glacial tills) or were covered by Quaternary glaciers (Fig. 1). *N. rhenorhodanensis* could have colonized the Alps and Jura after the last glaciation by dispersing along alluvial corridors (Ward and Palmer, 1994; Fig. 1). Earlier analyses based on enzyme electrophoresis revealed gene flow among populations of *N. rhenorhodanensis* along a 100-km alluvial corridor (Mathieu et al., 1997; Berettoni et al., 1998). This ability to disperse could have given rise to distinct lineages representing different refuges and colonization routes following Quaternary glacial periods. The resulting phylogeographic pattern should resemble those described for many epigean organisms in Europe (reviews in Taberlet et al., 1998; Hewitt, 2004) including some freshwater peracarids (Cristescu et al., 2003, 2004; Verovnik et al., 2005).

Niphargus rhenorhodanensis may also be composed of geographical and/or ecological (e.g., karstic versus porous) cryptic species with restricted dispersal abilities and reduced distributions that diverged a long time before Quaternary glaciations. Cryptic species were reported from many crustacean groups (e.g., Burton and Lee, 1994; King and Hanner, 1998; Jarman and Elliott, 2000; Lee, 2000; Edmands, 2001; Rocha-Olivares et al., 2001; Wares, 2001; Williams et al., 2001; Mathews et al., 2002; Daniels et al., 2003; Rawson et al., 2003; de Bruyn et al., 2004; Penton et al., 2004), including a number of freshwater amphipods (Müller, 2000; Witt and Hebert, 2000; Hogg et al., 2006). The widespread occurrence of cryptic diversity suggests that morphological and molecular evolution are uncoupled. Under convergent conditions, cryptic species are often found, while under adaptive radiation, morphological characters are likely to evolve quickly and cryptic species was not be observed (Witt et al., 2003; Macdonald et al., 2005). Recent molecular studies of GW animals suggested that morphological taxonomy might critically under-estimate groundwater diversity (Proudlove and Wood, 2003; Lefébure et al., 2006a) because extreme conditions of life promoted cryptic diversity by inducing convergent morphological evolution (Caccone and Sbordoni, 2001; Dowling et al., 2002; Wiens et al., 2003). Thus, *N. rhenorhodanensis* could be an example of a widely distributed crustacean that actually consists of a series of allopatric and sibling species, each with its own restricted distribution. This scenario would also imply that some *N. rhenorhodanensis* groups could have survived Quaternary glaciations within the Alps.

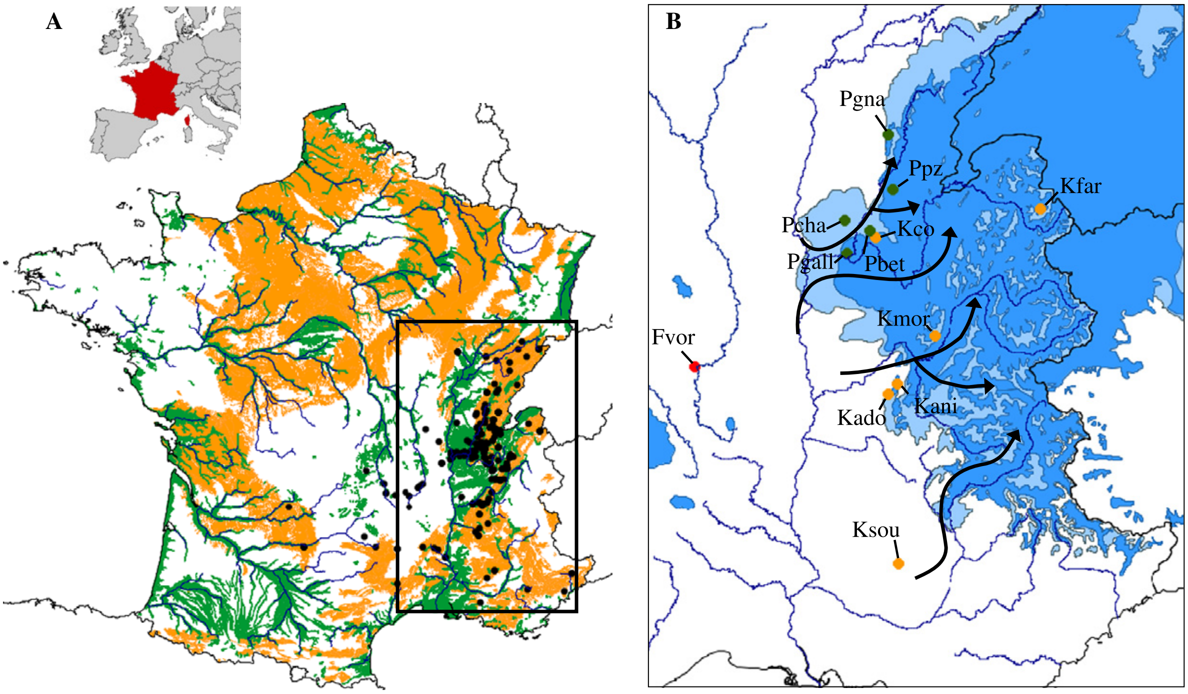

Fig. 1. Distribution of *Niphargus rhenorhodanensis* and sample locations. (A) Distribution of karstic (orange) and porous (green) groundwater and the occurrence of *N. rhenorhodanensis* (Ginet, pers. comm.) (black dots). (B) Sample sites in karstic (orange dots), porous (green dots) and fissured (red dots) GW. Last glacial maximum extension (Würm period) is represented in dark blue, and maximum glacial extension of Pleistocene glaciations in light blue from Buoncristani and Campy (2004). Arrows indicate hypothetical post-glacial colonization routes.