



## Identifying species of *Bythinella* (Caenogastropoda: Rissoidae): A plea for an integrative approach

MARTIN HAASE<sup>1,4</sup>, THOMAS WILKE<sup>2</sup> & PAUL MILDNER<sup>3</sup>

<sup>1</sup>Zoologisches Forschungsmuseum Alexander Koenig, Adenauerallee 160, D-53113 Bonn, Germany

<sup>2</sup>Institut für Allgemeine und Spezielle Zoologie, Justus-Liebig-Universität Gießen, D-35392 Gießen, Germany.

E-mail: Tom.Wilke@allzool.bio.uni-giessen.de

<sup>3</sup>Landesmuseum Kärnten, Museumgasse 2, A-9021 Klagenfurt, Austria. E-mail: Paul.Mildner@landesmuseum-ktn.at

<sup>4</sup>Corresponding author, present address: Vogelwarte Hiddensee, Zoologisches Institut und Museum, Universität Greifswald, Soldmannstraße 16, D-17489 Greifswald, Germany. E-mail: martin.haase@uni-greifswald.de

### Abstract

The genus *Bythinella* comprises many species throughout Europe, but species delimitation, traditionally based on shell morphology and genital anatomy, is often a matter of debate. Out of an ongoing large-scale project on the phylogeny of the genus, we analyzed the relationships of species occurring in the south Austrian province Carinthia and in neighboring Slovenia as a model for similar cases of systematic and taxonomic ambiguity. Our analyses based on sequence data of a fragment of COI comprising 638 bp, morphological and anatomical investigations confirmed the presence of three species, *B. opaca* (Gallenstein, 1848), *B. robiciana* (Clessin, 1890) and *B. angelitae* nom. nov. for *B. opaca* (Frauenfeld, 1857). The latter, while genetically distinct, is morphologically and anatomically cryptic in that it can only be distinguished from *B. opaca* by the denticulation of the radular marginal teeth. *B. robiciana*, on the other hand, is morphologically well defined, but genetically not separable from *B. opaca*, its stem species. Thus, taxonomy in *Bythinella* has to be based on the integration of morphology, anatomy and genetics. Our phylogenetic analyses suggest that *B. opaca* has colonized Carinthia, which has largely been covered by glaciers during the last ice age, along two routes, one from the south and a second one from the southeast.

**Key words:** *Bythinella*, Carinthia, cryptic species, DNA-barcoding, DNA-taxonomy, paraphyly, post-glacial colonization, punctuated equilibrium, Slovenia

### Introduction

The genus *Bythinella* Moquin-Tandon, 1856 occurs throughout southern, central and eastern Europe and western Asia, from the Iberian Peninsula in the southwest (Boeters 1988) to the Ukraine in the east (Son 2006) and Turkey in the southeast (Radoman 1976; Boeters & Falkner 2001). Typically, species are less than 3 mm in shell height and they inhabit springs from the lowlands up to 1465 m (Boeters 2006). While it is generally agreed that the genus comprises many species, delimitation of species based on shell shape and anatomy is difficult and often controversial. Because of intergradation in practically all characters among populations investigated throughout Italy, Giusti and Pezzoli (1977) concluded that there is only a single taxon present, viz. *B. schmidtii* (Küster, 1852), whereas Alzona (1971) has previously recognized eight species. Based on a similar variability among Polish *Bythinella* Falniowski (1987, 1992) discriminated six species. However, evidence from subsequent population genetic investigations using allozymes pointed at the existence of only a single species (Falniowski *et al.* 1998, 1999). Lately, two projects have been set up aiming at clarification of the taxonomy of *Bythinella* on larger geographic scales based on DNA sequence data (Bichain *et al.* 2007;