THE GENUS **MARSTONIOPSIS** (GASTROPODA: RISSOOIDEA): INTRA- AND INTERGENERIC PHYLOGENETIC RELATIONSHIPS

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(Received 24 November 2000; accepted 26 April 2001)

ABSTRACT

Marstoniopsis scholtzi (A. Schmidt, 1856) and *M. insubrica* (Küster, 1853), have been commonly accepted as distinct species, partly because of their allopatric distribution patterns, but there appear to be no morphological differences between them. Also the phylogenetic relationships of the genus *Marstoniopsis* van Regteren Altena, 1936 remain enigmatic. In the present study the authors sequenced a fragment of the mitochondrial cytochrome oxidase I gene in representatives of three populations of *M. scholtzi* and one population of *M. insubrica* to access the degree of genetic differentiation between those taxa. A phylogenetic analysis was performed with representatives of the families Hydrobiidae, Amnicolidae and Cochliopidae in order to test the taxonomic position of *Marstoniopsis*.

The specimens from the three populations of *Marstoniopsis scholtzi* differ from each other in only one or two observed substitutions in 638 bp (= 0.152–0.304%). Each one of them also differs from the specimen of *M. insubrica* in one or two observed substitutions (= 0.152–0.304%). Based on the extremely low genetic divergence and the lack of morphological differentiation we consider all populations to belong to one species, *Marstoniopsis insubrica*. Maximum likelihood analysis places the genus *Marstoniopsis* in the family Amnicolidae, close to the genera *Erhaia* and *Amnicola*.

INTRODUCTION

The genus *Marstoniopsis* van Regteren Altena, 1936 is represented by two species distinguished for a long time: *M. scholtzi* (A. Schmidt, 1856) and *M. insubrica* (Küster, 1853). *Marstoniopsis scholtzi* is distributed in lakes and stagnant parts of big rivers in the northern, western, central, and eastern parts of Europe (Ehrmann, 1956; Jaeckel, 1962; Boeters, 1973; Willmann & Pieper, 1978). Its distribution range covers Europe from northeastern France through Belgium, the Netherlands, Germany, and southern Denmark (East Jutland, Fünen, the islands), northern and central Poland to central Sweden (up to 60°40’ N), southern Finland, Lithuania, Latvia, Estonia to the Ladoga Lake, and Russia (near Kaliningrad, the Dnieper catchment area, the north part of the Volga drainage area, Seliger Lake, Zabolotskoye Lake). Isolated occurrences are found in western France (Loire-Atlantique) and in Great Britain (the vicinities of Manchester and Stirling). According to Taylor (1966) ‘The species was introduced from continental Europe to England, not from America as stated by Fretter & Graham.’ The southernmost localities are known from the Rhine drainage basin in Germany, and the vicinities of Wroclaw and Kraków in Poland (Fig. 1). *Marstoniopsis insubrica* is known only from a few localities in southern Switzerland (Lago di Mazzano) and neighboring northern Italy (the subalpine lakes Lago Maggiore, Lago di Garda, Lago di Levico, Lago Piano, etc.; Boeters, 1973; Giusti & Pezzoli, 1980) (Fig. 1).

Boeters (1973) studied *Marstoniopsis* in western Europe and indicated the possibility that *M. scholtzi* is a ‘geographic race’ of *M. insubrica*. However, in 1998 Boeters treated *M. scholtzi* as a good species without further references to *M. insubrica*. He also erroneously attributed the lack of the bursa to *M. scholtzi*. Falniowski (1983, 1987) studied the shell, radula and soft part anatomy of Polish and Dutch *Marstoniopsis* and found practically no differences from the morphology described in the literature for *M. insubrica*. However, the allopatric distribution of the two taxa still suggested they might be distinct.

The taxonomic position of the genus *Marstoniopsis* is enigmatic as well. Giusti & Pezzoli (1980) included it in the family Bythinellidae sensu Radoman (1973); this assignment was followed by Falniowski (1987). A review of opinions on hydrobioid systematics, including *Marstoniopsis*, is given in Falniowski & Szarowska (1995). It must be stressed that each data set, e.g.
anatomy, shell structure, or egg capsule morphology, results in a different inferred phylogeny of *Marstoniopsis* (Falniowski & Szarowska, 1995, Szarowska, 1996).

In the present paper we use partial sequences of the mitochondrial cytochrome c oxidase subunit I (COI) gene from three populations of *M. scholtzi* and one population of *M. insubrica* to test the following questions: (1) Are the two known species of *Marstoniopsis*, *M. insubrica* and *M. scholtzi* conspecific? (2) Does *Marstoniopsis* belong to the family Amnicolidae (= Bythinellidae) as previously suggested?

**MATERIALS AND METHODS**

*Materials studied*

We studied methanol preserved specimens of *Marstoniopsis scholtzi* from two localities in Germany: the Warnow River near the feeder of the Rostock waterworks, Rostock, Mecklenburg-Vorpommern (DNA isolation # 2599; GenBank accession # AF322408; ANSP collection # A19638) and the Nausdorfer Canal near Nausdorf, Prignitz, Mecklenburg-Vorpommern (DNA isolation # 2600; GenBank accession # AF322409; ANSP collection # A19639) and from one locality in Poland: the Narew River near Lomza (DNA isolation # 1994; GenBank accession # AY027813; ANSP collection # A19640). *Marstoniopsis insubrica* was collected in the Mincio River near Goito, Mantova, Lombardy, Italy (DNA isolation # 2558; GenBank accession # AF322707; ANSP collection # A19641) (Fig. 1).

**DNA isolation, amplification, and sequencing**

The methods of Spolsky et al. (1996) and Davis et al. (1998) were used for isolating DNA from individual snails (two from Germany, one from Poland, one from Italy). For amplification and sequencing of a 658 base pair-long fragment of the COI gene we used the methods of Wilke et al. (2000). The first and last ten base pairs of our sequences are uniformly excluded from subsequent analyses as they often can only be read in one direction, leaving a 638 bp fragment.

**Data analyses**

In order to test whether *Marstoniopsis* belongs to the family Amnicolidae or the Hydrobiidae, we compared our sequences for *Marstoniopsis* with published sequences available from GenBank of three hydrobiid taxa and three amnicolid taxa (Table 1). The cochliopid taxon *Heleobops carrikeri* was used as an outgroup (for the phylogenetic relationships of the

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**Figure 1.** *Marstoniopsis insubrica* and *M. scholtzi*. Distribution areas for the two nominal species and locations of the populations studied.
The specimens from the three populations of *Marstoniopsis scholtzi* differ from each other in only one or two observed substitutions in 638 bp (≈ 0.152–0.304%). Each of them also differs from the specimen of *M. insubrica* in one or two observed substitutions (≈ 0.152–0.304%). The very low genetic differentiation clearly indicates intraspecific relationships.

The phylogenetic analysis resulted in one ‘best tree’. The tree consists of three main clades, an amnicolid (containing *Amnicola limosa*, *Bythinella austriaca* and *Erhaia jianouensis*), a hydrobid (containing *Hydrobia acuta*, *Ventrosia ventrosa* and *Peringia ulvae*), and a cochliopid (containing *Heleobops carrikeri*) (Fig. 2). *Marstoniopsis* securely fits into the Amnicolidae, clustering together with the type species of the Amnicolidae, *Amnicola limosa*. An empirical analysis of 100 bootstrap pseudo-trees indicates that *Marstoniopsis* alternatively clusters with the amnicolid *Erhaia jianouensis*, resulting in a relative low bootstrap support for the *Amnicola/Marstoniopsis* and *Erhaia* clades. However, the large genetic distance (both observed substitution rate and branch lengths in the phylogenetic tree) among *Erhaia*, *Amnicola* and *Marstoniopsis* indicate the distinctness of these genera.

**DISCUSSION**

The COI gene has been extensively used for population- and species-level studies in rissooidean snails because it differentiates well between taxa from the population to family level and it provides a good phylogeographic signal (Hershler et al., 1999; Wilke & Davis, 2000; Wilke et al., 2000 a,b). Although there is no uniform molecular clock in animal mtDNA (e.g. Avise, 2000), and the molecular clock has to be scaled for each gene and each taxon, a difference of no more than one or two substitutions in the rather variable COI gene clearly suggests conspecificity. So far we have studied the COI gene in more than 100 rissooidean species, in which the smallest uncorrected genetic distances found between two putative species was 3.0% or 19 substitutions (Wilke et al., 2000a).

Our mtDNA data for *Marstoniopsis* are in concordance with the morphological data presented by Falniowski (1983, 1987): we conclude that all the studied populations belong to the same species, whose proper name, because of the priority rule, is *M. insubrica* (Küster, 1853).

Our data on the mtDNA COI sequence variation in *Marstoniopsis* are derived from only four populations.

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**THE GENUS MARSTONIOPSIS**

**Table 1.** Genbank accession # and references for the sequences of taxa of the Amnicolidae, Hydrobiidae, and Cochliopidae obtained from GenBank.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>GenBank accession #, references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrobiidae Troschel, 1857</td>
<td></td>
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<tr>
<td><em>Hydrobia acuta</em> (Draparnaud, 1805)</td>
<td>AF213344, Wilke et al., 2000a,b</td>
</tr>
<tr>
<td><em>Ventrosia ventrosa</em> (Montagu, 1803)</td>
<td>AF118333, Wilke &amp; Davis, 2000</td>
</tr>
<tr>
<td><em>Peringia ulvae</em> (Pennant, 1777)</td>
<td>AF118288, Wilke &amp; Davis, 2000</td>
</tr>
<tr>
<td>Amnicolidae Tryon, 1862</td>
<td></td>
</tr>
<tr>
<td><em>Amnicola limosa</em> (Say, 1817)</td>
<td>AF213348, Wilke et al., 2000b</td>
</tr>
<tr>
<td><em>Bythinella austriaca</em> (Frauenfeld, 1856)</td>
<td>AF213349, Wilke et al., 2000b</td>
</tr>
<tr>
<td><em>Erhaia jianouensis</em> (Liu &amp; Zhang, 1979)</td>
<td>AF213340, Wilke et al., 2000b</td>
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<tr>
<td>Cochliopidae Tryon, 1866</td>
<td></td>
</tr>
<tr>
<td><em>Heleobops carrikeri</em> Davis &amp; McKee, 1989</td>
<td>AF213347, Wilke et al., 2000b</td>
</tr>
</tbody>
</table>

families Hydrobiidae, Amnicolidae and Cochliopidae see Wilke et al., 2000b).

Prior to the phylogenetic analysis we used the computer program Modeltest 3.0 (Posada and Crandall 1998) in order to find the optimal model of DNA substitution for our data. It performs hierarchical likelihood ratio tests among 56 possible models. The model selected was GTR (general time reversible model) with a rate matrix of \([A–C]/H11005 = 2.3947, [A–G]/H11005 = 44.9647, [A–T]/H11005 = 29.6641, [C–G]/H11005 = 16.4952, [C–T]/H11005 = 140.8773, and [G–T]/H11005 = 1.0000; equal base frequencies of A/H11005 = 0.2761, C/H11005 = 0.1747, G/H11005 = 0.1804, and T/H11005 = 0.3688; a proportion of invariable sites of I/H11005 = 0; as well as a gamma distribution shape parameter of G/H11005 = 0.

A maximum likelihood phylogram was constructed from all sequences utilizing the computer program PAUP 4.0b3 (Swofford, 1998). The analysis (heuristic search) was performed with the parameter of sequence substitution suggested by Modeltest (except for the proportion of invariable sites that was set to I = 0.000001 as PAUP does not accept 0 as input). Bootstrap replicates (100) were generated with a stepwise-addition search.

**RESULTS**

The COI gene has been extensively used for population- and species-level studies in rissooidean snails because it differentiates well between taxa from the population to family level and it provides a good phylogeographic signal (Hershler et al., 1999; Wilke & Davis, 2000; Wilke et al., 2000 a,b). Although there is no uniform molecular clock in animal mtDNA (e.g. Avise, 2000), and the molecular clock has to be scaled for each gene and each taxon, a difference of no more than one or two substitutions in the rather variable COI gene clearly suggests conspecificity. So far we have studied the COI gene in more than 100 rissooidean species, in which the smallest uncorrected genetic distances found between two putative species was 3.0% or 19 substitutions (Wilke et al., 2000a).

Our mtDNA data for *Marstoniopsis* are in concordance with the morphological data presented by Falniowski (1983, 1987): we conclude that all the studied populations belong to the same species, whose proper name, because of the priority rule, is *M. insubrica* (Küster, 1853).

Our data on the mtDNA COI sequence variation in *Marstoniopsis* are derived from only four populations.
Nonetheless the extremely low genetic differentiation between populations that are separated by the Alps and a straight line distance of up to 1,220 km is remarkable. It suggests that there is no or very little concordance between genetic differentiation and geographic distribution.

Due to the limited number of populations and specimens studied and due to the lack of Pleistocene fossil or subfossil deposits it is difficult to reconstruct population history and historic pathways of dispersal. There are at least three possible scenarios that could explain the habitat range of *Marstoniopsis*:

1. The species originated south of the Alps and then spread throughout the northern part of central Europe, perhaps after the last glaciation;
2. *Marstoniopsis* survived the glaciations in refuges in both northern Europe and northern Italy;
3. The snails survived the Pleistocene glaciations in refugia on the northern side of the Alps with a subsequent dispersal to southern Switzerland and northern Italy.

If the species originated south of the Alps and then spread throughout the northern part of central Europe then one would expect DNA polymorphism to the south of the Alps and polymorphism or the lack of it (depending on single vs. multiple invasion) to the north of the Alps. As we have studied only one southern specimen, we are unable to access the degree of genetic variability in that area. However, considering the present-day ecology and habitat range of *Marstoniopsis scholtzi*, e.g. its strong preference for lowland rivers and lakes and its widespread occurrence in northern localities with cold climate vs. the restricted range in northern Italy with much milder climate, makes a southern origin in mountainous areas of northern Italy and southern Switzerland unlikely. The present day occurrence of *Marstoniopsis* in areas like the Ladoga Lake in Russia, only 550 km south of the polar circle, raises questions relative to the survival of Pleistocene glaciations. Climate and vegetation in these northern areas resembles very much areas in southern Poland and southern Germany that remained ice-free during the Pleistocene and which are known to be refugia for numerous invertebrate species (Skompski, 1991). We therefore speculate that *Marstoniopsis* could have survived the Pleistocene glaciation events on the northern side of the Alps. If it survived in the north, the Italian populations are possibly secondary, peripheral isolates, much like the isolated occurrences in western France and northern England.

The question whether the Italian *Marstoniopsis* populations are recently founded or if *Marstoniopsis* already occurred in Italy during the Pleistocene glacia-

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**Figure 2.** Maximum likelihood tree based on partial sequences of the COI gene. The scale bar indicates the expected substitution rate. Bootstrap values are indicated at the branches.
vations remains open. Data from more population and more sensitive genetic marker (e.g. microsatellites) would be necessary to answer this question.

The examination of maps does not suggest any possible way of migration along waterways or other corridors between the northern European and the Italian populations. It seems therefore that the only explanation is the passive transportation by birds. Several hydrobioids are known to be able to pass the gut of a bird still alive and in a good condition (e.g. Drake & Arias, 1995). This way of dispersal, although in our opinion overused in the literature to explain the distribution of hydrobioids, seems the only reasonable explanation for the observed distribution.

Today the northern populations of Marstoniopsis are widely distributed in lowland areas. In fact, recent studies have shown that in some habitats, like the deeper parts of the littoral zone of big lakes, the populations may be quite dense (Falniowski, 1987; Zettler, 1999). Interestingly, Marstoniopsis populations are never or rarely long lasting and they show unusual fluctuations in population density. Thus, it is often impossible to find the species at the same place again after some years (Falniowski, unpublished data). These fluctuations, together with a relatively low effective population size, may explain the observed low level of the mtDNA polymorphism, as the result of multiple bottle-necks instead of postulating one severe bottleneck accompanying the glaciations. The frequent ‘disappearance’ of Marstoniopsis populations also may have contributed to the fact that Marstoniopsis is considered to be a rare and endangered species.

Molecular data confirmed that, despite the doubts in the literature (see Falniowski & Szarowska, 1995), Marstoniopsis belongs to the family Amnicolidae (Fig. 2). Interestingly, this monotypic genus seems to be closely related to the genus Erhia, a taxon involved in the transmission of the lung fluke Paragonimus in Asia (Wilke et al., 2000b).

REFERENCES


ACKNOWLEDGMENTS

The study was in part supported by a grant of the German Science Foundation (WI 19021–1) to Thomas Wilke and in part by a grant of the Polish Committee of Scientific Research (PB 0775/P0499/17) to Andrzej Falniowski. We would like to thank Dr Michael Zettler for providing specimens of M. scholtzi from Germany, and Dr Marco Bodon for providing specimens of M. insubrica from Italy. We also thank Professor Folco Giusti, Dr Robert Hershler and Dr David Brown for their comments on a former version of the manuscript.


