Evolutionary biogeography of water shrews (Neomys spp.) in the western Palaearctic Region

B. Kryštufek, A. Davison, and H.I. Griffiths

Abstract: We studied the morphology, DNA sequence, and Recent and Pleistocene distributions of three species of the water shrew genus Neomys (N. fodiens, N. anomalus, and N. teres) represented by samples from the Balkans and Asia Minor. Adaptations to semi-aquatic life (large body size, fringes of stiff hairs bordering the hind foot, and a tail keel) were most developed in N. fodiens and N. teres and least developed in N. anomalus. However, sympatric N. fodiens and N. anomalus did not differ significantly in relative braincase size. The three Neomys species clearly differed in glans penis morphology, N. teres being the most distinct, with a longer glans (length = 10.8–14.6 mm) than N. anomalus (7.0–8.0 mm) or N. fodiens (7.5–8.5 mm). Phylogenetic analysis placed N. fodiens as a sister-group to the anomalus–teres clade, based on both cytochrome b and 12S rRNA fragments. Palaeodistribution maps are presented for the three Recent taxa and the palaeo-species N. newtoni and N. browni. Possible evolutionary scenarios are proposed.

Résumé : Nous avons étudié la morphologie, la séquence de l’ADN ainsi que la répartition Récente et la répartition au Pleistocène de trois espèces de Neomys (N. fodiens, N. anomalus et N. teres) représentées par des échantillons des Balkans et d’Asie mineure. Les adaptations à la vie semi-aquatique (grande taille, franges de poils drus le long de la patte arrière et de l’arète de la queue) se sont révélées apparentes surtout chez N. fodiens et N. teres, un peu moins chez N. anomalus. Cependant, la taille de la boîte crânienne ne différait pas significativement chez des N. fodiens et des N. anomalus symaptiques. Les trois espèces de Neomys sont très différentes par la morphologie du gland du pénis, celui de N. teres étant plus long (longueur = 10.8–14.6 mm) que celui de N. anomalus (7.0–8.0 mm) et celui de N. fodiens (7.5–8.5 mm). Une analyse phylogénétique place N. fodiens comme groupe soeur du clade anomalus–teres, aussi bien au moyen de fragments de cytochrome b que de fragments de 12S ARN. Des cartes illustrent la paléorépartition des trois taxons Récents de même que des paléo-espèces N. newtoni et N. browni. Des scénarios possibles de l’évolution du groupe sont élaborés.

[Traduit par la Rédaction]

Introduction

The Balkan peninsula is one of the major foci of biodiversity in the western Palaearctic Region (Gaston and Davies 1994). This in part reflects its unique geological history (Steininger and Rögl 1996), the large number of endemics present (e.g., Stanković 1960; Sket 1999), and the area’s role as a glacial refugium (Willis 1994; Hewitt 1999). These effects are enhanced by periodic connections with Asia Minor through the formation of Pleistocene land bridges across the Bosphorus and the Straits of Marmara (Hosey 1982). Authors of several recent studies (e.g., Filippucci et al. 1995, 1996; Kryštufek and Macholán 1998) have therefore sought to investigate the contribution of Pleistocene small-mammal migrations across Balkano-Anatolian land bridges to the genomic diversity of the area, i.e., of European populations to the evolutionary biogeography of Anatolian Turkey and vice-versa. We here report the results of investigations into the phylogeography of the Palaearctic water shrew genus Neomys, the three extant species of which inhabit the Balkano-Anatolian region.

The three extant Neomys species (Corbet 1978; Spitzberger 1990a; Hutterer 1993) are N. fodiens (Pennant, 1771), N. anomalus Cabrera, 1907, and N. teres Miller, 1908. The last of these is more widely known as N. schelkovnikovi Satunin, 1913, a junior synonym of N. teres (see Kryštufek et al. 1998), and was long considered conspecific with N. fodiens (e.g., Bobrinskii et al. 1965; Ellerman and Morrison-Scott 1966). Although N. fodiens ranges from the Atlantic to the Pacific coast of the Palaearctic Region, the other two species are restricted to the western Palaearctic Region, the range of N. teres being particularly small.

Neomys spp. are the only semi-aquatic shrews inhabiting the western Palaearctic Region. Of the six different lifestyles (sensu Hutterer 1985) adopted by Soricidae, semi-aquatic life has resulted in the most obvious morphological adaptations: the hind feet are larger than those of comparably sized terrestrial shrews, and fringes of stiff hairs along the sides of the toes and the lateral edges of the foot increase surface area and add propellant power. In addition, short stiff hairs form a keel along the ventral side of the tail, increasing stability during swimming. Semi-aquatic shrews are also unique amongst Soricidae in having reduced olfactory lobes. As prey are detected by the vibrissae rather than by smell, the...
trigeminal nerves are enlarged, and consequently the mass of the brain has also increased; semi-aquatic shrews have larger braincases than their terrestrial counterparts (Hutterer 1985). Despite these adaptations, the water shrews are considered to be morphologically and physiologically ill-adapted to diving, because aquatic shrews should be larger, so that their thermal inertia is increased and the aquatic heat loss reduced (Churchfield 1990).

It is well known that aquatic adaptations are more evident in *N. fodiens* and *N. teres* than in *N. anomalus* (e.g., Spitzenberger 1990a). Because of this, and because of its mosaic-like geographical distribution, *N. anomalus* has been considered ancestral within the genus (Spitzenberger 1990a). In truth, the phylogenetic relationships of the three species are poorly understood, not least because the differences in morphological characters are slight (Spitzenberger 1990a), the karyotype is stable (2n = 52; Zima and Kral 1984; Sokolov and Tembotov 1989), and, unlike some other shrews, the three *Neomys* species have not been subjected to molecular phylogenetic analysis (Ohdachi et al. 1997; Fumagalli et al. 1999). The only categorical data available refer to the morphology of the male genitalia (Pucek 1964; Yudin 1970), but this character has often been ignored. Furthermore, the unreliability of many palaeontological determinations, a consequence of poor morphological differentiation within the genus, complicates efforts to reconstruct the phylogenetic history of these species (Kryštufek and Griffiths 2000).

The availability of new material and field data collected recently in the Balkans and Asia Minor have allowed resolution of the phyletic relationships between the three *Neomys* species. We here report the results of analyses of molecular, phenetic, and (palaearc)-zoogeographic data sets that permit a revised view of the evolutionary history of *Neomys*.

**Material and methods**

**Morphology**

From over 500 *Neomys* voucher specimens taken from southeastern Europe and Asia Minor, 336 ascribable to five homogeneous geographic samples were selected for further analyses (Fig. 1). European specimens were identified on the basis of diagnostic characteristics proposed by Tvrkovič et al. (1985), Kryštufek and Petkovski (1989), and Spitzenberger (1990a), whilst Kryštufek et al. (1998) was used for Anatolian material. Finally, nine samples were defined according to geographical homogeneity and previous subspecific designation: three for *N. fodiens* (FS, Slovenia; FB, western Bosnia; FM, Macedonia) and five for *N. anomalus* (AS, Slovenia; AB, western Bosnia; AM, Macedonia; AT, Turkish Thrace; AA, Anatolia), whilst the ninth sample was made up of all the specimens of *N. teres* (T). This approach avoided problems posed by a lack of data on geographic variation, which is particularly evident for *N. anomalus* (Spitzenberger 1990b). The study material is held in the collections of the Slovenian Museum of Natural History, Ljubljana (all samples), and the Naturhistorisches Museum Wien, Vienna (part of sample T).

Phenetic analyses focused on the degree of adaptation to semi-aquatic life, and on assessing differentiation in the shape of the glands penis. Metrical analyses were based on two external measurements taken from specimen tags: HB, head and body length (snout to anus), and HF, hind-foot length (excluding claws).
Cranial and mandibular characteristics were also evaluated but found to be unhelpful in elucidating phyletic relations between taxa (see Kryštufek and Griffiths 2000). Body and braincase size and peculiarities of the hind foot and tail were considered to be the main indicators of the degree of adaptation to diving. Since body mass shows significant seasonal oscillations in soricid shrews (Pucek 1970), we considered HB to represent the less biased of the two measures indicating overall size. Three skull parameters were measured with dial callipers (accurate to the nearest 0.05 mm) to evaluate braincase size: condylobasal length, braincase breadth, and neurocranium height. As neurocranial height oscillates seasonally in Soricinae (Dehnel’s phenomenon; Pucek 1970), we considered only specimens taken during the same season (i.e., collected between June and October 15). This greatly reduced the samples suitable for analysis to 24 N. fodiens and 44 N. anomalus (all from Slovenia), and excluded N. teres. These dimensions were regressed against condylobasal length to eliminate general skull size from the analysis. Regression against HB was used to estimate the relative size of HF.

Phalli were preserved in 70% alcohol and examined under a dissecting microscope. Species were represented by the following samples (number and sample are in parentheses): N. teres (6), N. anomalus (1 from AS, 1 from AT, 3 from AA), and N. fodiens (1 from FS, 3 from FM). Our specimens were compared with illustrations by Pucek (1964) and Yudin (1970): nomenclature follows Pucek (1964).

**Zoogeography**

Distributional ranges are summarised from different sources (see Fig. 1). The sizes of distributional areas (expressed in million kilometres squared; Mkm²) were estimated by superimposing individual ranges on a 300×300 km grid, the presence of each species being scored for each grid. Palaeontological data were taken primarily from Rzebik-Kowalska (1991, 1998) with some additions (see Figs. 2 and 3), and mapped to assess palaeozoogeographic occurrence. Only records cited to species level were considered, and uncertain, unclear, or questionable determinations were excluded.

Field data were taken from the collector’s (usually B.K.) protocols. Habitat types were divided into two main categories: (1) “horizontal” and (2) “vertical.” The former refers to flat country with dense, lush sedge to tall forb vegetation along slow-running or stagnant waters and (or) swamps. Vertical habitats include running streams and small rivers in hilly country; streams were frequently rapid (even torrential), with rocky banks that lacked dense vegetation. Dominance (percentage of the total number of water shrews of a given species) was assessed by means of a N. anomalus that were

**Results**

**Phenetics**

**Semi-aquatic adaptations**

One-way ANOVA demonstrated significant differences in HB between the nine samples (F test = 15.59, P < 0.0001). N. teres being the largest species (Fig. 4). Neither the three N. fodiens samples or the five N. anomalus samples formed a single, homogeneous species set. In spite of this, N. fodiens was always larger than sympatric N. anomalus; this was clear in samples from Slovenia and Bosnia (differences within a sympatric species tandem were significant at P < 0.05), but not significant in Macedonian water shrews. The largest N. anomalus were those from the Anatolian plateau, where they are nearly exclusively allopatric (Kryštufek et al. 1998).

Differences in the degree of development of the stiff hairs on the hind feet and the ventral tail keel are summarised in Table 1. Of 36 pairwise comparisons, 20 differed significantly when HF character states were considered and 18 when tail data were included (Table 2). The degree of development of the stiff hairs on the hind foot provided better interspecific differentiation than the tail. N. anomalus having significantly less well developed marginal hairs. The tail keel was always present in N. fodiens, but absent in 43.8% of N. teres and 25.0% (AS) to 100% (AT) of N. anomalus. Interlocality variation within N. anomalus was due mainly to the Slovenian animals (AS), which had strong keel. The tail of N. teres differed from that of N. fodiens, more closely resembling that of N. anomalus.

One-way ANOVA of HF residuals (from regression on HB) demonstrated highly significant between-sample differences (F ratio = 54.29, P < 0.0001): all five N. anomalus samples were significantly smaller than both the N. teres and the three N. fodiens samples (Fig. 5). Neomys teres and the three N. fodiens samples formed a homogeneous group in terms of relative hind-foot length. The only significant pairwise comparison within N. anomalus was between AS and AM.

Two individuals of the two braincase parameters on condylobasal length had lower values in N. anomalus (mean braincase breadth = −0.038; mean neurocranial height = −0.038) than in N. fodiens (mean = 0.069 and 0.043, respectively), but MANOVA revealed no significant differences between the two species (Wilks’ λ = 0.916, ns).
Glans penis

The three *Neomys* species clearly differed in the morphology of the glans penis, that of *N. teres* being the most distinct (Fig. 6). The glans is longer in *N. teres* (length 10.8–14.6 mm) than in *N. anomalus* (length 7.0–8.0 mm) or *N. fodiens* (length 7.5–8.5 mm) (Pucek 1964; B. Kryštufek, unpublished data). This is due to distal prolongation (i.e., of the anterior part to the lobular processes) in *N. teres*, resulting in a pointed apex (the apex is blunt in the other species). In addition, the apical process was narrow and elongate in *N. teres*, but broad with a T-shaped anterior expansion in the other species. Fleshy lateral folds were more expanded in *N. teres*, but *N. anomalus* lacked the small lateral flap seen in *N. fodiens* and *N. teres*. The glans penis of *N. teres* also had dense, hornified areas that were more extensive than those in *N. anomalus* and *N. fodiens*.

Zoogeography

*Neomys fodiens* has the most extensive modern distribution, covering ca. 16.65 Mkm², followed by *N. anomalus* (3.33 Mkm²) and *N. teres* (0.68 Mkm²). Latitudinal range coverage is also greatest in *N. fodiens* (40°–70°N, 30° span), followed by *N. anomalus* (ca. 36°30’–55°30’N, 19° span) and *N. teres* (ca. 38°30’–45°N, 6°30’ span). *Neomys fodiens* therefore inhabits the greatest diversity of habitats, populating inland waters from the Mediterranean coast to the Arctic Ocean and from sea level to 2500 m asl (Spitzenberger 1990c). A similar vertical range is also known for *N. anomalus*, which ranges from the shores of the Caspian Sea to 2440 m asl (Sokolov and Tembotov 1989). *Neomys anomalus*, however, does not inhabit high-altitude areas, although it has been recorded up to 1850 m asl (Spitzenberger 1990b).

*Neomys fodiens* is allopatric over approximately 89.6% of its extensive range; elsewhere it is sympatric with *N. anomalus*. *Neomys teres* is almost entirely allopatric, only one case of sympatric occurrence (with *N. anomalus*) being known (Kryštufek et al. 1998), although further data from Turkey and northern Iran may provide additional evidence of sympathy. Much of *N. anomalus’ range overlaps that of *N. fodiens*, so the former is allopatric in less than half of its range (ca. 43%). There are extensive areas of allopatry in Iberia, the southern and eastern Balkans, and western Anatolia. No range overlap has been reported between *N. fodiens* and *N. teres*, so the two most specialised aquatic shrews are strictly allopatric. In general, allopatry seems to be a prevalent feature of *Neomys* zoogeography. Thus, of ca. 18.77 Mkm² occupied by the genus, 17.06 Mkm² (i.e., 90.9%) is inhabited by a single species.

In areas where *N. fodiens* and *N. anomalus* are broadly sympatric (Slovenia, Bosnia, and Macedonia in this study), they show clear differences in habitat selection and are rarely syntopic (Table 3). Indeed, the occurrence of the two water shrew species is significantly different from that predicted by chance for both major habitat classes: of 63 localities in Slovenia, Bosnia, and Macedonia, 48 (76.2%) were inhabited by a single species. *Neomys anomalus* nearly always inhabits inland waters from the Mediterranean coast to the Arctic Ocean and from sea level to 2500 m asl (Spitzenberger 1990c).

© 2000 NRC Canada
horizontal habitats (23/24 localities), but *N. fodiens* lives in vertical habitat types. In Turkish Thrace, where *N. anomalus* is allopatric, it was the only water shrew in vertical habitats. In Anatolia, where an equal number of vertical habitats had only *N. anomalus* or *N. teres*, there was no significant departure from random distribution (Tables 3 and 4). However, this was due to their allopatric occurrence in Anatolia; all Anatolian vertical habitats with only *N. anomalus* were in the western Anatolian mountains, where the species does not compete with *N. teres*.

**Molecular analysis**

A 355 base pair fragment of the mitochondrial cytochrome *b* gene and a 375–378 base pair 12S rRNA fragment were sequenced from nine *Neomys* individuals (two *N. fodiens*, four *N. anomalus*, three *N. teres*; GenBank reference Nos. AF182172–AF182187). The sequences were aligned with suitable outgroups and a separate tree was constructed for each gene. For all three phylogenetic methods, and using either gene fragment, *N. teres* grouped with *N. anomalus*, and the bootstrapping support for this clade was significant. A minimum-evolution tree (neighbour-joining) based on the cytochrome *b* sequences and rooted with *Sorex mirabilis* (Ognev, 1937) and *Crocidura dsinezumi* (Temminck, 1843) is shown in Fig. 7. Maximum-likelihood and parsimony analyses resulted in trees of the same topology. Also, the use of different outgroups did not alter the topology (not shown).

**Palaeontology of Neomys**

The Pleistocene evolutionary history of *Neomys* is relatively poorly understood. A small number of Pleistocene taxa have been described: *N. bohlini* Young, 1934 from China, *N. castellarini* Pasa, 1947 from the Middle Pleistocene of Italy, *N. intermedius* Brunner, 1952 from (mainly) the Late Pleistocene of Germany, and *N. newtoni* Hinton, 1911 and *N. browni* Hinton, 1911 from the early Middle and Late Pleistocene of the U.K., respectively (see Rzebik-Kowalska 1991, 1998). Of these, *N. bohlini* and *N. castellarini* have since been transferred to the genera *Chodisgoa* and *Episoriculus*, respectively (Repennning 1967; Rzebik-Kowalska 1991), whilst *N. intermedius* (intermediate in size between *N. anomalus* and *N. fodiens*) is considered invalid by some authorities (see Rzebik-Kowalska 1991, 1998). This leaves only *N. newtoni* and *N. browni* as European palaeospecies. From the frag-
mentary material known, *N. newtoni* appears to be more akin to *N. anomalus* than to *N. fodiens* (D. Schreve, personal communication).

The fossil distributions of *Neomys* spp. are shown in Figs. 2 and 3. *Neomys newtoni* is known from the Early and (or) Middle Pleistocene in England, France, Poland, Germany, Korea, and Japan. The fossil distributions of *Neomys* spp. are shown in Figs. 2 and 3. *Neomys newtoni* is known from the Early and (or) Middle Pleistocene in England, France, Poland, Germany, Korea, and Japan.

**Fig. 4.** Variation in body and head length among nine samples of three *Neomys* species (mean with 95% confidence intervals; see Fig. 1 for species designations). Samples are arranged along an east–west transect and sympatric species tandems are plotted next to each other (*, significant pairwise comparison (*P* < 0.05); ns, not significant). S, Slovenia; B, Bosnia; M, Macedonia; T, Turkish Thrace; A, Anatolia.

**Fig. 5.** Variation in relative hind-foot length (residuals resulting from regression on head and body length) among nine samples of three *Neomys* species (see Fig. 1 for species designations). S, Slovenia; B, Bosnia; M, Macedonia; T, Turkish Thrace; A, Anatolia.

<table>
<thead>
<tr>
<th>Hind foot</th>
<th>T</th>
<th>AS</th>
<th>AB</th>
<th>AM</th>
<th>AT</th>
<th>AA</th>
<th>FS</th>
<th>FB</th>
<th>FM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
<td>23.6</td>
<td>9.5</td>
<td>9.1</td>
<td>33.3</td>
<td>0</td>
<td>100</td>
<td>92.3</td>
<td>95.8</td>
</tr>
<tr>
<td></td>
<td>(12 )</td>
<td>(89 )</td>
<td>(42 )</td>
<td>(22 )</td>
<td>(9 )</td>
<td>(8 )</td>
<td>(40 )</td>
<td>(13 )</td>
<td>(24 )</td>
</tr>
<tr>
<td>Tail</td>
<td>56.3</td>
<td>80.0</td>
<td>14.6</td>
<td>38.9</td>
<td>0</td>
<td>12.5</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>(16 )</td>
<td>(85 )</td>
<td>(41 )</td>
<td>(18 )</td>
<td>(9 )</td>
<td>(8 )</td>
<td>(38 )</td>
<td>(13 )</td>
<td>(24 )</td>
</tr>
</tbody>
</table>

**Table 1.** Frequencies (%) of aquatic adaptation of the hind foot and tail in nine *Neomys* samples (see the text for sample designations).

<table>
<thead>
<tr>
<th>Fishes</th>
<th>T</th>
<th>AS</th>
<th>AB</th>
<th>AM</th>
<th>AT</th>
<th>AA</th>
<th>FS</th>
<th>FB</th>
<th>FM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11.2</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>17.2</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>17.2</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>FS</td>
<td>64.6</td>
<td>67.5</td>
<td>53.7</td>
<td>30.4</td>
<td>48.0</td>
<td>39.2</td>
<td>17.1</td>
<td>26.9</td>
<td></td>
</tr>
<tr>
<td>FB</td>
<td>24.5</td>
<td>33.0</td>
<td>23.6</td>
<td>17.2</td>
<td>17.2</td>
<td>39.2</td>
<td>17.1</td>
<td>26.9</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2.** Chi-squared (χ²) values resulting from pairwise comparisons in hind-foot (below the diagonal) and tail (above the diagonal) character states (see the text for sample identities).

<table>
<thead>
<tr>
<th>Fishes</th>
<th>T</th>
<th>AS</th>
<th>AB</th>
<th>AM</th>
<th>AT</th>
<th>AA</th>
<th>FS</th>
<th>FB</th>
<th>FM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11.2</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>17.2</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>17.2</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>FS</td>
<td>64.6</td>
<td>67.5</td>
<td>53.7</td>
<td>30.4</td>
<td>48.0</td>
<td>39.2</td>
<td>17.1</td>
<td>26.9</td>
<td></td>
</tr>
<tr>
<td>FB</td>
<td>24.5</td>
<td>33.0</td>
<td>23.6</td>
<td>17.2</td>
<td>17.2</td>
<td>39.2</td>
<td>17.1</td>
<td>26.9</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** Bonferroni adjustment was applied in order to correct critical values. Only significant values are given. ns, not significant.

*Pairwise comparison contains identical rows of zeros, so χ² cannot be estimated.
Fig. 6. Glans penis of three *Neomys* species in ventral and lateral view. 1, apical process; 2, three lobular processes; 3, lateral fold; 4, lateral flap.

Table 3. Numbers of water shrew localities according to main habitat type.

<table>
<thead>
<tr>
<th>Horizontal habitat</th>
<th>Vertical habitat</th>
<th>A</th>
<th>F/T</th>
<th>A–F/T</th>
<th>$\chi^2$</th>
<th>A</th>
<th>F/T</th>
<th>A–F/T</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slovenia</td>
<td></td>
<td>12</td>
<td>1</td>
<td>5</td>
<td>12.8**</td>
<td>0</td>
<td>12</td>
<td>4</td>
<td>15.0**</td>
</tr>
<tr>
<td>Bosnia</td>
<td></td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>7.0**</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>Macedonia</td>
<td></td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>12.0**</td>
<td>0</td>
<td>11</td>
<td>5</td>
<td>12.2**</td>
</tr>
<tr>
<td>Thrace</td>
<td></td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>Anatolia</td>
<td></td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>6</td>
<td>1</td>
<td>ns</td>
<td>—</td>
</tr>
</tbody>
</table>

Note: A, *N. anomalus* only; F/T, only *N. fodiens* or *N. teres*; A–F/T, sympatric occurrence of *N. anomalus* with *N. fodiens* or *N. teres* (see the text for definitions of habitat types). A $\chi^2$ value denotes deviations from a random distribution of observations. Statistics were not calculated for samples with <6 observations. Significance levels are as follows: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.001$; ns, not significant.

Table 4. Dominance of *N. anomalus* (as a percentage of all *Neomys* spp.) in two main habitat types according to geographic region.

<table>
<thead>
<tr>
<th>Horizontal</th>
<th>Vertical</th>
<th>Total Neomys</th>
<th>Percentage of <em>N. anomalus</em></th>
<th>$\chi^2$</th>
<th>Total Neomys</th>
<th>Percentage of <em>N. anomalus</em></th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slovenia</td>
<td></td>
<td>193</td>
<td>88.6</td>
<td>51.1***</td>
<td>33</td>
<td>15.5</td>
<td>12.4***</td>
</tr>
<tr>
<td>Bosnia</td>
<td></td>
<td>142</td>
<td>82.4</td>
<td>28.8***</td>
<td>9</td>
<td>0.0</td>
<td>8.3**</td>
</tr>
<tr>
<td>Macedonia</td>
<td></td>
<td>22</td>
<td>100</td>
<td>18.6***</td>
<td>45</td>
<td>17.8</td>
<td>13.4***</td>
</tr>
<tr>
<td>Thrace</td>
<td></td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>11</td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td>Anatolia</td>
<td></td>
<td>9</td>
<td>100</td>
<td>8.3**</td>
<td>25</td>
<td>52.0</td>
<td>ns</td>
</tr>
</tbody>
</table>

Note: A $\chi^2$ value denotes deviation from 50% occurrence of *N. anomalus*. Statistics were not calculated for Thrace, where *N. anomalus* is the only water shrew species present (see Table 3 for probability designations).
and the Czech Republic, but *N. browni* is known only from the lower Middle Pleistocene of the U.K. (Schreve 1997; D. Schreve, personal communication). The earliest fossil record of *N. anomalus* is from the Middle Pleistocene of Austria (Rabeder 1972), but it is known from later deposits in Germany, Italy, and Romania (Rzebik-Kowalska 1991, 1998). *Neomys fodiens* appears to be widely distributed throughout the Late Pleistocene and Holocene in much of Europe, with a small number of Middle Pleistocene records. There appear to be no fossil records of *N. teres*. Vereshchagin (1959) does record *N. fodiens* s.l. from a site in the Middle Pleistocene of the Caucasus (an area that should be occupied by *N. teres*); however, it is clear that he does not distinguish between the two taxa. It should be emphasised that the palaeontological criteria for recognising all these taxa are difficult, and misidentifications are possible (see Kryštufek and Griffiths 2000).

**Discussion**

Of the 312 shrew species presently known (Hutterer 1993), only 11 (in four genera) are adapted to semi-aquatic life (Hutterer 1985). This makes aquatic adaptation one of the rarest shrew specialisations; it is outnumbered by both semi-fossorial and scansorial life-styles (29 and 15 species, respectively); only psammophily (known in a single species) is rarer (Hutterer 1985). With the exception of three semi-aquatic *Sorex* species, the remaining water shrew species from the genera *Neomys*, *Chimarrogale*, and *Nectogale* are in tribe Neomyini, and all are exclusively semi-aquatic. As well as being restricted taxonomically to subfamily Soricinae, aquatic shrews are also restricted geographically to the northern hemisphere. One of the main possible reasons for their low species number is their predominant allopatry. Of the three semi-aquatic (Nearctic) *Sorex* species, one (*S. alaskanus* Merriam, 1900) is an island form and the ranges of the other two (*S. palustris* Richardson, 1828 and *S. bendirii* Merriam, 1884) overlap only marginally (Hall 1981). Within Neomyini, the genus *Neomys* is entirely allopatric, whilst there is very marginal overlap between the monotypic genus *Nectogale* and the four species of *Chimarrogale* (Hutterer 1993). All *Chimarrogale* species are allopatric (Corbet and Hill 1992), so the relatively broad sympathy seen in *N. fodiens* and *N. anomalus* is exceptional, and seems to be possible only because of their ecological segregation.

The phylogenetic analysis of the mitochondrial sequences suggests that *N. teres* and *N. anomalus* diverged relatively recently from a common ancestor, compared with the earlier split between *N. fodiens* and *N. teres + N. anomalus* (Fig. 7). Assuming that the tree does reflect actual between-species relationships and is not affected by mitochondrial introgression between *N. teres* and *N. anomalus* or “long-branch attraction” (Hillis et al. 1996), what were the main events driving the evolution of *Neomys* in southeastern Europe and Asia Minor? The phylogenetic history of the three species is far from clear, and several palaeobiogeographic scenarios are plausible.

Firstly, if *N. anomalus* and *N. teres* share ancestry, it seems likely that *N. anomalus* underwent range extension in a Pleistocene interglacial period, crossed the Bosphorus in a period of marine drawdown, and then colonised Anatolia. Later, as climatic conditions cooled, population isolates ancestral to *N. teres* could have remained on the southern Black Sea coast, here undergoing simple allopatric speciation to *N. teres*. The main problem is that *N. anomalus* is not strongly adapted to semi-aquatic life, so any morphological similarities between *N. teres* and *N. fodiens* may be the result of *N. teres* retaining these adaptations, whilst *N. anomalus* lost them but retained “deeper” characters, e.g., braincase size.

The oldest fossil species appears to be *N. newtoni*, with *N. browni* somewhat intermediate in age between it and *N. fodiens* (D. Schreve, personal communication). Assuming that the semi-aquatic *N. fodiens* is the oldest extant species, a second plausible scenario is that during a climatic cold phase, *N. fodiens* spread southwards across the Balkano-Anatolian land bridge. Later the climate ameliorated and *N. fodiens* retreated northwards, leaving an isolated population in the Pontic Mountains, where local conditions still favoured its existence. This “stranded” population underwent allopatric speciation to *N. teres*. However, as the ancestral form receded northwards, populations across Balkano-Anatolia become progressively more and more adapted to xeric/seasonal habitats, eventually becoming the form we know as *N. anomalus*. This hypothesis is not necessarily inconsistent with the molecular phylogeny. If the Greco-Anatolian *N. fodiens* became *N. anomalus*, it would be the closest current-day taxon to *N. teres*. Depending upon the time scale over which these events occurred, either of the above hypotheses could account for paraphyletic taxa in the phylogeny (Fig. 7). That this is not the case may be due to inadequate sampling or to events occurring close to one another.

A third hypothesis is that *N. fodiens* underwent a 500-km range expansion around the northern coast of the Black Sea into Caucasia, eventually reaching the Pontic Mountains. Later,
as its range contracted, the small population in the Pontic Mountains became isolated and evolved allopatrically into *N. teres*. However, this hypothesis is congruent with the morphological data only, and conflicts with the molecular phylogeny.

Because of the closer molecular phylogenetic affinity of *N. anomalus* with *N. teres* than with *N. fodiens*, the first presumed divergence is that of the ancestor of *N. fodiens* from the common ancestor of *N. anomalus* and *N. teres*. Considering the isolated distribution of *N. teres* and the occurrence of *N. anomalus* in Anatolia, it is reasonable to assume that vicariance followed the disappearance of the Bosphorous land bridge. That is, the Black Sea (which was a freshwater lake until the Middle Pleistocene) became connected with the Mediterranean at the Post-Tschauda Black Sea Stage (Hosey 1982). This caused the first of several vicariance events, which presumably resulted also in the divergences of previously widespread species and in speciation on both sides of the Bosphorous and Straits of Marmara (e.g., Kryštufek and Macholán 1998). More recent evidence shows rapid change in Late Pleistocene sea levels in the Sea of Marmara and the Dardanelles, which would have facilitated further faunal interchange (Stanley and Blanpied 1980; Ryan et al. 1997).

Further divergence between *N. anomalus* and *N. teres* could have followed environmental changes in the Anatolian plateau, particularly an increase in seasonality, which resulted in permanent waters becoming temporary. The available data suggest that *N. anomalus* is particularly successful in areas with seasonal waters. For example, in the western Balkans, *N. anomalus* was the dominant small mammal along temporary rivers in karstic fields. Its dominance (percentage of all small mammals collected that were *N. anomalus*) on three karstic fields in western Bosnia was 28.5% at the Kupres field (*n* = 165), 48.6% at the Glomac field (*n* = 74), and 52.7% at the Duvno field (*n* = 55) (Kryštufek and Tvrtković 1988). We therefore speculate that temporary waters also favoured *N. anomalus* on the Anatolian plateau, whilst *N. teres* evolved along streams in the Pontic Mountains, and consequently retained the semi-aquatic habit of the ancestral form. The Quaternary history of much of Anatolia is still poorly understood, although it is known that much of central Anatolia was covered by vast Pleistocene palaeolakes until the Middle Pleistocene (Lemcke 1996; Roberts et al. 1999). In the latter case, a large palaeolake system is known to have existed in the last glacial period (e.g., Roberts 1983; Roberts et al. 1999). Because of the changing regional climates of the period, lake areas would have fluctuated, as would the large marshy areas associated with them. In contrast, the more mountainous landscape of the Pontic Mountains would have been comparatively stable and hydrological conditions would (presumably) have remained relatively constant. This would have produced a landscape favouring *N. anomalus* in the west and *N. teres* in the northeast.

*Neomys anomalus* may have colonised Europe via the Bosphorous land bridge during a Late Pleistocene phase of marine drawdown, when the Bosphorous land bridge was an important land route for faunal movements between Europe and Asia Minor (Hosey 1982).

**Acknowledgements**

Examination of additional specimens of *N. teres* from Turkey and the Caucasus was made possible by Dr. Friederike Spitzengerber (Vienna) and Dr. Richard Kraft (Munich). Dr. Barbara Rzebić–Kowalska (Kraków) provided generous assistance with palaeontological data, Dr. Danielle Schreve (London and Durham) kindly shared information on fossil *Neomys* from the U.K. and Dr. Robert Hoffmann (Washington) donated further specimens for sequence analysis. The Swiss Topographic Service assisted in locating fossil–bearing sites in Switzerland. Two anonymous reviewers are thanked for their helpful comments.

**References**


© 2000 NRC Canada
Kryštufek et al. 1625


Kumar, S., Tamura, K., and Nei, M. 1993. MEGA: Molecular evolutionary genetics analysis, version 1.01. Pennsylvania State University, Philadelphia.


© 2000 NRC Canada