

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 palaeospecies *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'arête 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* sympatriques. Les trois espèces de *Neomys* sont très différentes par la morphologie du gland de leur 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 phylogénique place *N. fodiens* comme groupe soeur du clade *anomalus-teres*, aussi bien au moyen de fragments de cytochrome *b* que de fragments de 12S ARNr. Des cartes illustrent la paléorépartition des trois taxons Récents de même que des paléoespèces *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; Spitzenberger 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

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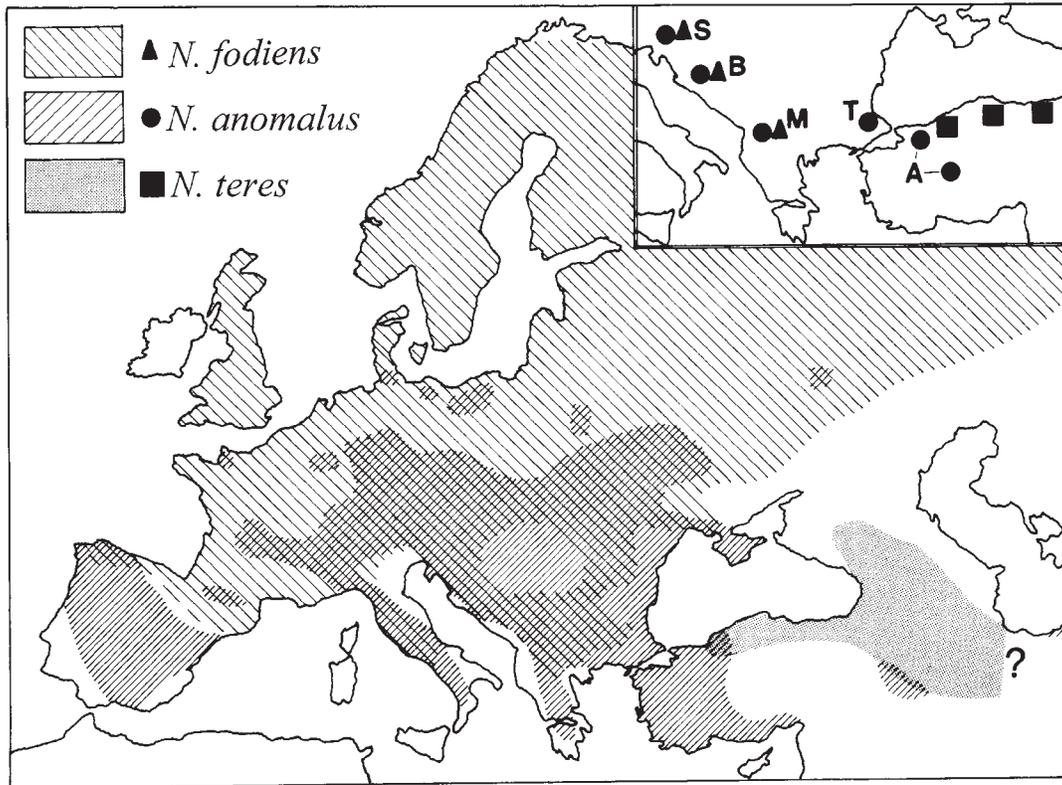
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Fig. 1. Approximate distributions of three *Neomys* species in the western Palaearctic Region, based on data from Spitzenberger (1990b, 1990c), Petrov (1992), Bobrinskii et al. (1965), Abelentsev et al. (1956), Sokolov and Tembotov (1989), Leuge et al. (1994), and Kryštufek et al. (1998). The insert shows study-sample origins: S, Slovenia; B, Bosnia; M, Macedonia; T, Turkish Thrace; A, Anatolia.



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 resolu-

tion of the phyletic relationships between the three *Neomys* species. We here report the results of analyses of molecular, phenetic, and (palaeo)-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 south-eastern 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 Tvrtković 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 sub-specific 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 glans 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 soricine 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 neurocranial 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 that were *N. anomalus*) was used to express the structure of the sympatric assemblage. Contingency between the two categorical variables was assessed by means of a χ^2 test. The Bonferroni adjustment was applied in order to correct critical values in the case of multiple comparisons.

Molecular genetic analysis

Genomic DNA was extracted from 1- to 2-year-old *Neomys* skin specimens alongside an extraction blank using Qiagen blood/tissue purification kits. Two separate mitochondrial DNA fragments were amplified by the polymerase chain reaction with the same cycling parameters of a 5-min denaturing step at 96°C followed by 35 cycles of 60 s at 94°C, 60 s at 55°C, and 60 s at 72°C with 0.25U Thermo-prime taq polymerase (Advanced Biotechnologies) and 1.5 mM magnesium. A cytochrome *b* fragment was amplified using primers L14771 (5'-CAACATTCGTAACCCACC-3') and H15149 (Irwin et al. 1991), and a 12S rRNA fragment was amplified using L1091 and H1478 (Kocher et al. 1989). Approximately 50 ng of DNA was sequenced in both directions, including 3.5 pmol of the relevant primer.

All sequences were aligned by eye against outgroup sequences from GenBank. Phylogenetic trees were constructed using three

methods. A minimum-evolution method (neighbour-joining, Kimura two-parameter) was performed in PHYLIP (Felsenstein 1993), with a 2:1 transition:transversion ratio. Latterly, transition:transversion ratios of 1:1 and 10:1 were also used to test for any effect on overall tree topology, and a Tamura-Nei distance was calculated in MEGA (α was set to 0.5; Kumar et al. 1993) to allow rate variation among sites. Maximum-likelihood analysis included five global rearrangements, which helps ensure that the global optimum is found, and was also performed in PHYLIP. PAUP version 3.1.1 (Sinauer Associates, Sunderland, Mass.) was used to identify the most parsimonious tree, using the exhaustive-search option; otherwise a heuristic search with the branch-swapping (tree bisection reconnection) option was performed. Analyses were bootstrapped 1000 times, except when the maximum-likelihood method was used. The computer program TREEVIEW (Page 1996) was used to help draw trees.

Results

Phenetics

Semi-aquatic adaptations

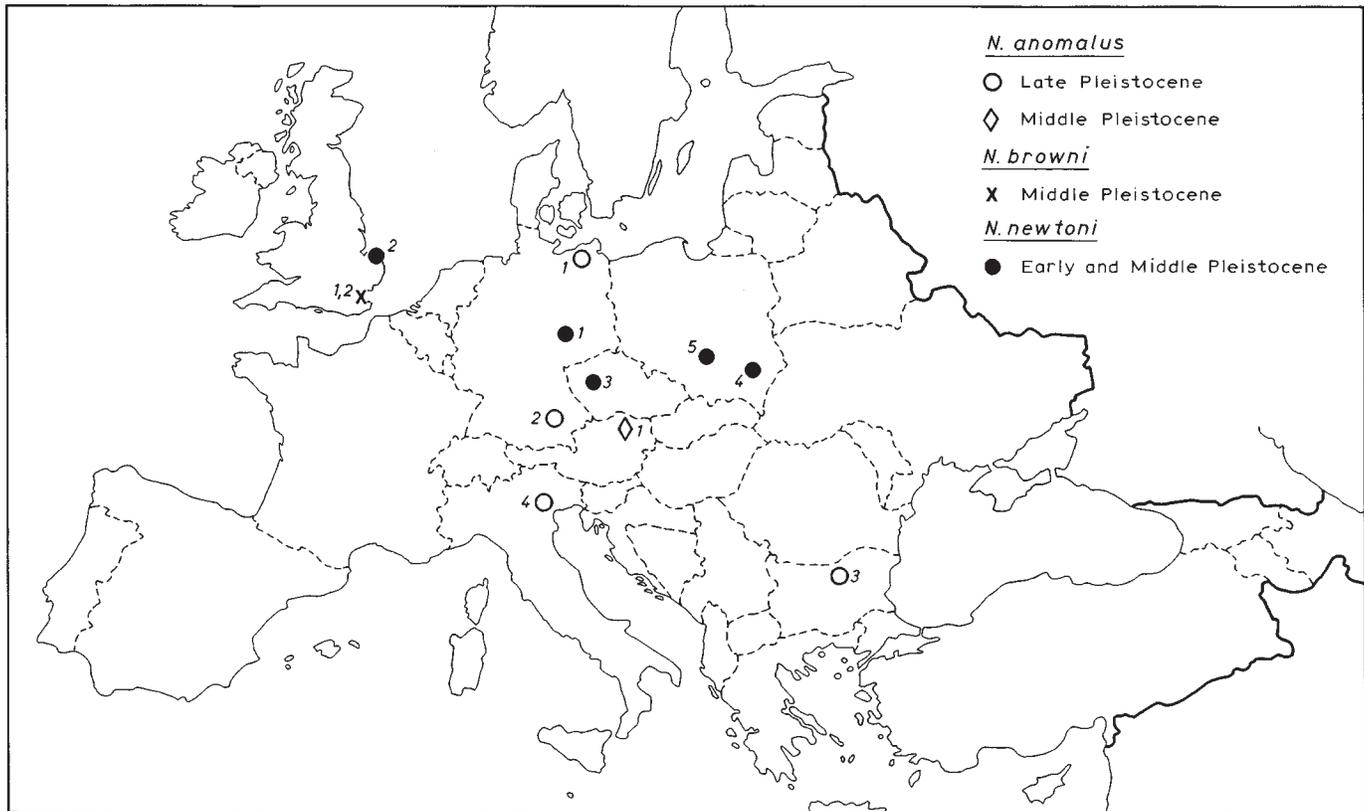
One-way ANOVA demonstrated significant differences in HB between the nine samples (F test = 15.59, $P < 0.0001$), *N. teres* being the largest species (Fig. 4). Neither the three *N. fodiens* samples or the five *N. anomalus* samples formed a single, homogeneous species set. In spite of this, *N. fodiens* 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.

Residuals 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' $\lambda = 0.916$, ns).

Fig. 2. Palaeontological records of *N. newtoni*, *N. browni*, and *N. anomalus* from Rzebik–Kowalska (1991, 1998), Schreve (1997), and Bon et al. (1991). Sites are as follows: *N. newtoni*: 1, Voigstedt; 2, West Runton; 3, Koneprusy; 4, Kozi Grzbiet; 5, Zalesiaki IA; *N. browni*: 1, Grays Thurrock; 2, Cudmore Grove; *N. anomalus*: Middle Pleistocene: 1, Hundesheim; Late Pleistocene and Holocene: 1, Piseide; 2, Schmiedberg-Abri; 3, Backo Kiro Cave; 4, Grotta Averla.



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, horny-spined areas that were more extensive than those in *N. anomalus* and *N. fodiens*.

Zoogeography

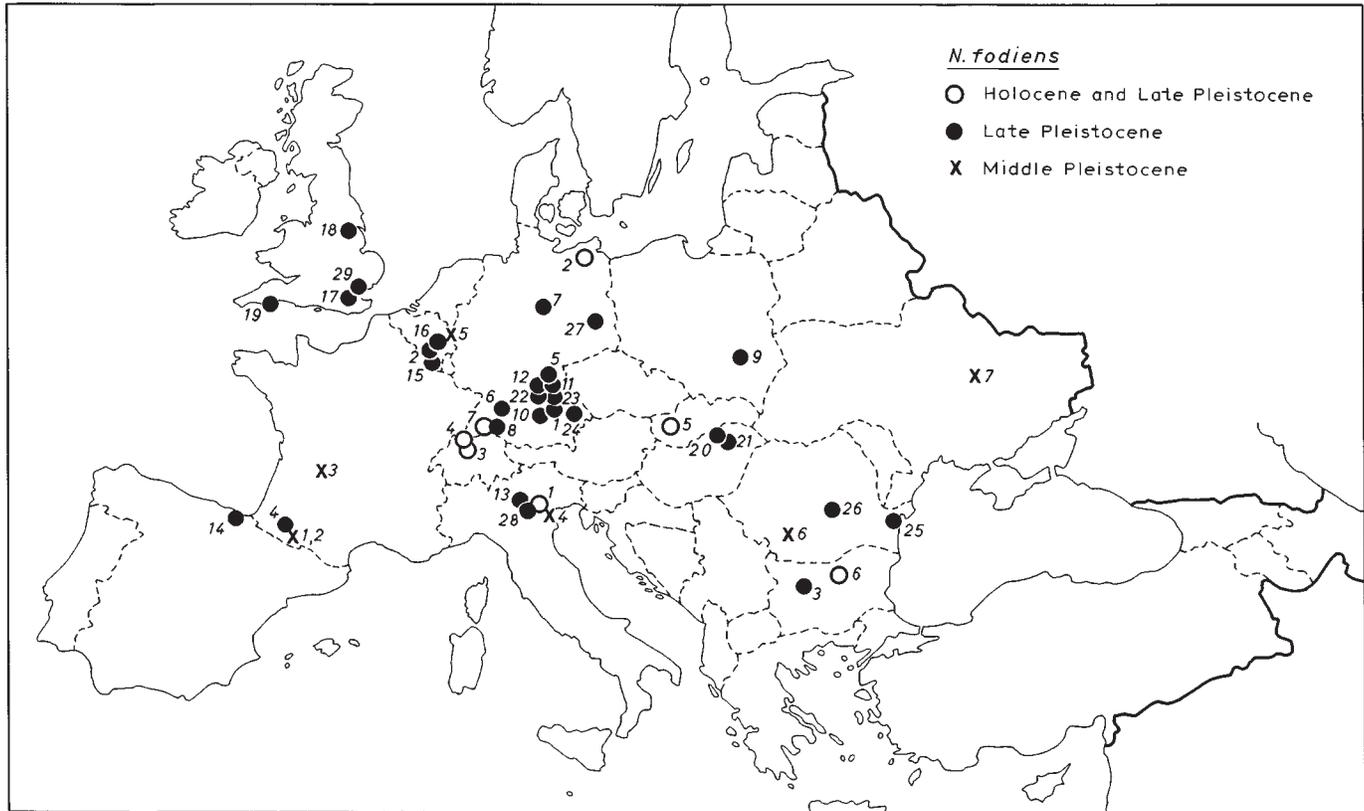
Neomys fodiens has the most extensive modern distribution, covering ca. 16.65 Mkm², followed by *N. anomalus* (3.33 Mkm²) and *N. teres* (0.68 Mkm²). Latitudinal range coverage is also greatest in *N. fodiens* (40–70°N, 30° span), followed by *N. anomalus* (ca. 36°30′–55°30′N, 19° span) and *N. teres* (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. teres*,

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 sympatry. 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

Fig. 3. Palaeontological records of *N. fodiens* from Rzebik-Kowalska (1991, 1998) and Heinrich (1991). Sites are as follows: Middle Pleistocene: 1, Grotte de la Carrière; 2, Grotte du Cappe de la Bielle; 3, La Fage; 4, Grotta Minore di San Bernadino; 5, Maastricht-Belvédère 3 and 4; 6, cave in Parului Valley; 7, Middle Dniepr alluvium. Late Pleistocene: 1, Weinberghöhle 1; 2, Marie-Jeanne Cave; 3, Mecha Dupka Cave; 4, Grotte Noëlle; 5, Dohlenloch, Markgrabenhöhle, Grubenloch; 6, Erkenbrechtsweiler; 7, Füchsloch; 8, Niklaushöhle; 9, Kielce; 10, Kemathenhöhle; 11, Reichentalloch, Schmeidberg-Abri; 12, Raumgrotte; 13, Veia (Cave A); 14, Erralla; 15, Trou de Frontal; 16, Goyet Cave; 17, Ightham fissures; 18, Dog Hole; 19, Tornewton Cave; 20, Istállósko; 21, Puskaparos; 22, Skythenloch; 23, Helmloch; 24, Sesselfels; 25, Pestera Bursucilor; 26, Pestera din Valea Coacazei; 27, Schönfeld; 28, Cengelle 2; 29, Sutton. Late Pleistocene-Holocene: 1, Broion Cave; 2, Piseide; 3, Solothurn (Kastelhöhle); 4, Ettingen; 5, Palfy Cave (Deravá Skala); 6, Backo Kiro Cave; 7, Petersfels.



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 cyto-

chrome *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 *Chodsigoa* and *Episoriculus*, respectively (Repenning 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-

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.

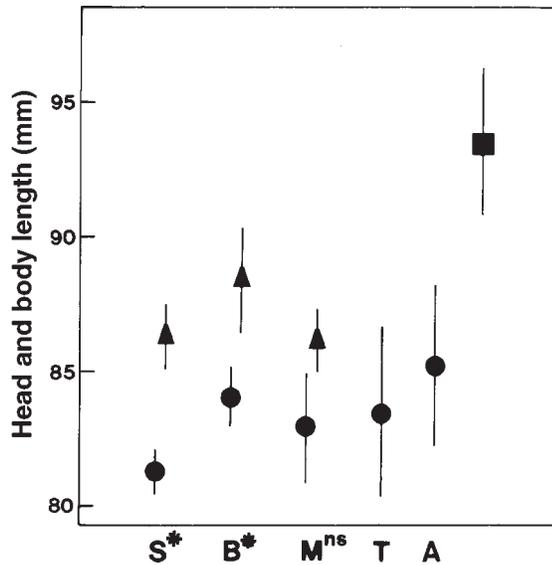


Fig. 5. Variation in relative hind-foot length (residuals resulting from regression on head and body length) among nine samples of three *Neomys* species (see Fig. 1 for species designations). S, Slovenia; B, Bosnia; M, Macedonia; T, Turkish Thrace; A, Anatolia.

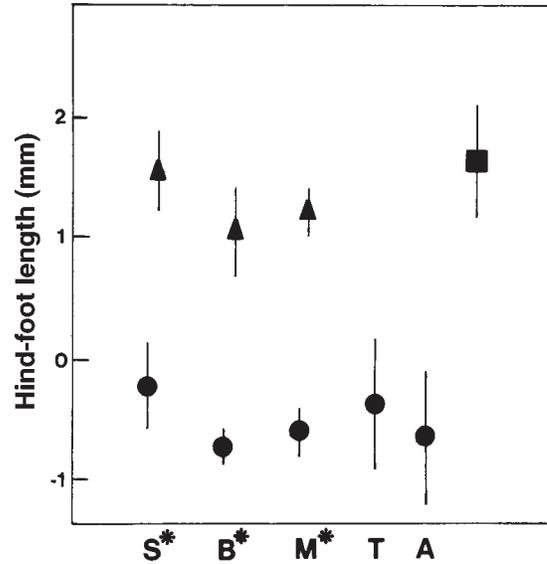


Table 1. Frequencies (%) of aquatic adaptation of the hind foot and tail in nine *Neomys* samples (see the text for sample designations).

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

Note: Long stiff hairs forming a fringe on the sides of toes and along the lateral edge of the hind foot are classified as an aquatic adaptation; hind feet with short stiff hairs are excluded. A prominent keel of stiff hairs along the ventral side of the tail is classified as an aquatic adaptation; tails with no keel are excluded. Numbers in parentheses are numbers of specimens examined.

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

	T	AS	AB	AM	AT	AA	FS	FB	FM
T	—	ns	ns	ns	ns	ns	19.1	ns	12.7
AS	28.6	—	48.8	12.7	26.0	17.4	ns	ns	ns
AB	36.6	ns	—	ns	ns	ns	58.2	31.5	44.4
AM	26.5	ns	ns	—	ns	ns	28.9	12.3	19.9
AT	11.2	ns	ns	ns	—	ns	47.0	22.0	33.0
AA	20.0	ns	ns	ns	30.4	—	39.2	17.1	26.9
FS	— ^a	64.6	67.5	53.7	30.4	48.0	—	— ^a	— ^a
FB	ns	24.5	33.0	23.6	ns	17.2	ns	—	— ^a
FM	ns	41.5	47.1	34.8	15.3	27.3	ns	ns	—

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

^aPairwise comparison contains identical rows of zeros, so χ^2 cannot be estimated.

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,

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.

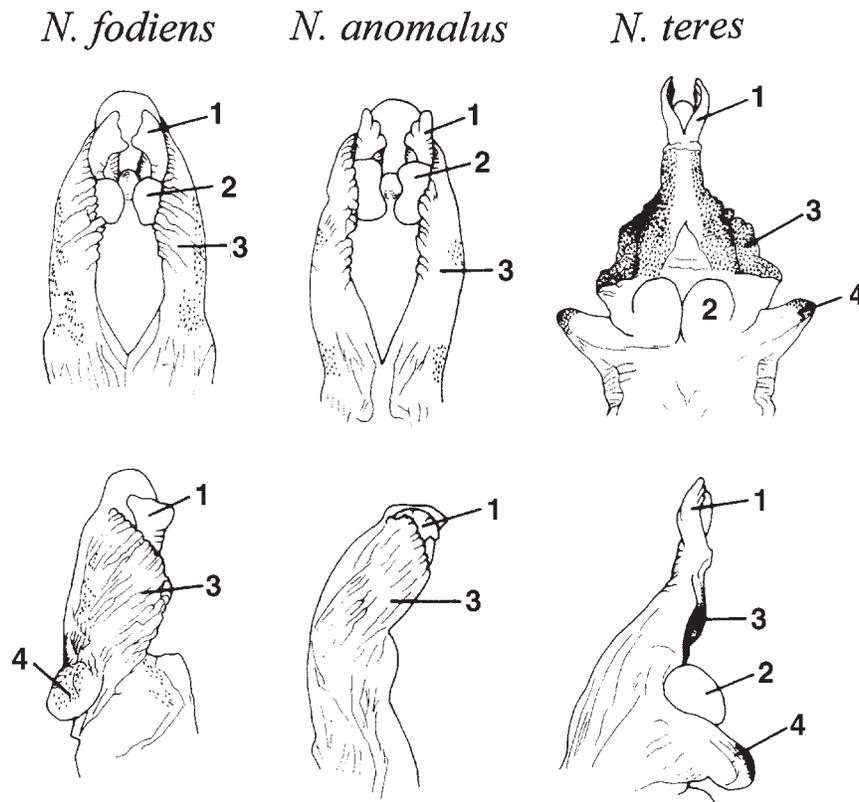


Table 3. Numbers of water shrew localities according to main habitat type.

Horizontal habitat	Vertical habitat				A	F/T	A-F/T	χ^2
	A	F/T	A-F/T	χ^2				
Slovenia	12	1	5	12.8**	0	12	4	15.0**
Bosnia	5	0	1	7.0**	0	1	0	—
Macedonia	6	0	0	12.0**	0	11	5	12.2**
Thrace	—	—	—	—	3	0	0	—
Anatolia	2	0	0	—	6	6	1	ns

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 χ^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.

	Horizontal			Vertical		
	Total <i>Neomys</i>	Percentage of <i>N. anomalus</i>	χ^2	Total <i>Neomys</i>	Percentage of <i>N. anomalus</i>	χ^2
Slovenia	193	88.6	51.1***	33	15.5	12.4***
Bosnia	142	82.4	28.8***	9	0.0	8.3**
Macedonia	22	100	18.6***	45	17.8	13.4***
Thrace	—	—	—	11	100	—
Anatolia	9	100	8.3**	25	52.0	ns

Note: A χ^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).

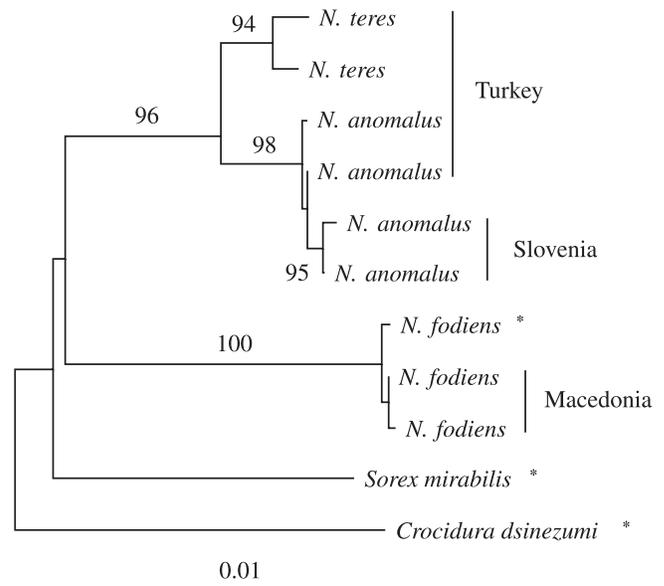
Discussion

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 sympatry 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

Fig. 7. Minimum-evolution (neighbour-joining) tree based upon Tamura–Nei distances with α set to 0.5. Numerals show branch support from 1000 bootstrap replicates. An asterisk denotes a sequence from GenBank (Ohdachi et al. 1997).



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 become 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) become 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 Glamoc 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 in the terminal Pleistocene, and that these included the areas around modern Lake Van and the Konya Basin (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).

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