Supplementary Methods, Tables and Figures

Multilocus species trees and species delimitation in a temporal context: application to the water shrews of the genus *Neomys*

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Supplementary Methods

Bayesian phylogenetic analyses of cytochrome *b*

Using BEAST version 1.8, the Markov chain was run for 50 million generations and 10% of the generations were discarded as burn-in. The program Tracer of the BEAST package was used to check that the effective sample sizes of all the parameters of interest were above 200 and convergence had been reached. TreeAnnotator of the same package was used to obtain the maximum clade credibility tree and the corresponding posterior probabilities of each clade.

Tree based on average genomic divergence

We calculated pairwise distances between all specimens using the formula 8.2 in Freedman et al. [1]. Basically, for each position, the average of the four possible matches between two individuals (one with nucleotides a and b and the other with nucleotides c and d) is computed as: $1 - (\delta_{ac} + \delta_{bd} + \delta_{ad} + \delta_{bc})/4$, where $\delta$ equals 1 if both nucleotides in the comparison are identical and 0 otherwise. Using a custom-made Perl script, this value was computed for all positions of all concatenated introns, summed, and divided by the total length to obtain the
distance between two individuals. A similar tree was obtained from distances calculated with formula 8.1 in Freedman et al. [1], where a more conservative estimate of the differences at each position is computed (not shown).

**Structurama MCMC chain parameters**

In each run, the Markov chain was run for 10 million generations, sampling every 100th cycle and with the initial 10,000 samples discarded as burn-in. Population assignment of each specimen was based on the mean partition or partition that minimizes the squared distance to all of the sampled partitions.

**BEAST priors and MCMC chain parameters**

Several mammalian fossil dates were used as hard bound minimum and soft bound maximum constraints in key nodes in order to calibrate the phylogenetic tree (Table S6). Specifically, we set lognormal prior distributions as follows: the offset was defined by the hard minimum, the mean in real space was adjusted so that the upper 95th percentile of the probability density distribution was coincident with the soft maximum, and the standard deviation parameter was set to 1 (Table S6). All calibrated nodes were older than 10 Myr, and therefore well above the time at which the difference between estimated gene tree and species tree divergences is minimal for nuclear genes [2]. A Yule speciation model was used as tree prior. 75 million generations were run and 10% of the generations were discarded as burn-in. The program Tracer of the BEAST package was used to check that the effective sample sizes of all the parameters of interest were above 200 and convergence had been reached.

For the soricid mitochondrial DNA analysis, the tree was calibrated using a set of fossil constraints available for soricids (Table S7), setting lognormal prior distributions as before.
All calibrated nodes were older than 3 Myr, and therefore well above the time at which the difference between estimated gene tree and species tree divergences is minimal for mitochondrial genes; this time is smaller than for nuclear genes due the reduced population size of mitochondrial genes [2]. To improve convergence, the priors of the substitution rate parameters of the GTR model and relative rate parameters of the codon positions were changed to uniform distribution between 0 and 100. 50 million generations were run, 10% of the generations were discarded as burn-in, and convergence was checked with Tracer.

*BEAST priors and MCMC chain parameters*

For each partition, HKY was selected as the substitution model. This model was used to match the model available in the program IMa2, which was used in a subsequent step to estimate additional parameters. However, it was checked that the use of more complex substitution models did not affect the results (not shown). The corresponding ploidy type of each marker (nuclear or mitochondrial) was set. In addition, a strict molecular clock was used for all partitions, a Yule process was set as species tree prior and the population size model was set as piecewise constant. All analyses were run for 50 million generations, 10% samples were discarded as burn-in and convergence was checked as before. The maximum clade credibility tree was constructed using median node heights.

**IMa2 priors and MCMC chain parameters**

The HKY model was used as substitution model. Maximum split time priors were set to 8, population size priors to 15 and migration rate priors to 2. Similar results were obtained when setting exponential migration rate priors with mean = 1 (not shown). When using cytochrome b, the heredity scalar for this locus was set as 0.25. Heating parameters were set as: hfg, hn15, ha0.96 and hb0.9. The final analyses consisted of a total of 50,000 sampled genealogies after
100,000 burn-in steps. As summary statistics of the posterior distributions, the bin with the highest value (after smoothing when this value was available in the IM output) and 95% confidence intervals were taken.

In IMa2, absolute mutation rates are not sampled in the MCMC chain. Rather, mutation rate scalars (the relative values of mutation rates) are estimated. The geometric mean of the externally estimated mutation rate of all loci is then used to scale demographic parameters, including divergence time. Therefore, unlike in *BEAST, it is not possible to introduce the variability of the rates that had been previously calculated in the mammalian multilocus analysis. However, it is possible to set ranges on mutation rates. The ratios of these limits are used as limits on the ratios of the mutation rate scalars. In order to test the effect of these limits, we used as mutation rate ranges the 95% confidence intervals of the mutation rates estimated in the previous mammalian multilocus analysis. In the introns-only analysis, the means and 95% confidence limits of the divergence times estimated by IMa2 were very similar than in the main analysis. When cytochrome b was included, the results with mutation rate ranges were more altered. However, these estimations were very similar again when the upper range of the cytochrome b was increased (10 times the estimated upper limit) to account for the possibility that the mutation rate previously calculated was saturated, similarly as we did in *BEAST (not shown).

**BPP priors, MCMC chain parameters and additional tests**

Mutation rates were set to be variable among loci and relative rates were generated from a Dirichlet distribution. When using cytochrome b, the heredity scalar for this locus was set as 0.25. As priors for \( \theta \) (population size parameter) and \( \tau \) (age of the root) we initially used values estimated from IMa2, after scaling them to reflect mutations per site. Two different
Gamma distributions were constructed for each of these parameters with \( \alpha = 2 \) (fairly diffused) and \( \alpha = 20 \) (more informative), respectively. The \( \beta \) of the Gamma distribution for \( \theta \) and \( \tau \) was obtained by dividing the \( \alpha \) value by the corresponding mean of the parameter.

Other divergence time parameters were assigned a Dirichlet prior. Additionally, we ran BPP with \( \theta \) and \( \tau \) priors that were respectively one order of magnitude lower and higher than the initial ones. Each prior set was analyzed using the two described reversible-jump Markov Chain Monte Carlo algorithms (rjMCMC) using default options. Each analysis consisted of 20,000 samples taken after 2000 burn-in steps.

References

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Table S1. *Neomys* specimens used, locations and number of genes sequenced for the species tree.

| Specimen Code | Species          | Subspecies | Sample type | Locality (and map number) | Country | Lat. | Long. | Genes used in the species tree |
|---------------|------------------|------------|-------------|---------------------------|---------|------|-------|--------------------------------|
| IBE-C1529     | *N. anomalus*    | anomalus   | Tissue (a)  | Tielve (1)                | Spain   | 43.3 | -4.8  | 14                             |
| IBE-C1789     | *N. anomalus*    | anomalus   | Tissue (b)  | Navalgujo (2)             | Spain   | 40.3 | -5.5  | 14                             |
| IBE-C2895     | *N. anomalus*    | anomalus   | Tissue (c)  | Picos de Europa (3)       | Spain   | 43.2 | -4.9  | 14                             |
| IBE-C1435     | *N. anomalus*    | anomalus   | Skull       | Peñaflor de Hornija (4)   | Spain   | 41.7 | -5.0  | 1                              |
| IBE-C1683     | *N. anomalus*    | anomalus   | Faeces      | Trefacio (5)              | Spain   | 42.2 | -6.7  | 1                              |
| IBE-C1662     | *N. anomalus*    | anomalus   | Faeces      | Molinos de Razón (6)      | Spain   | 42.0 | -2.6  | 1                              |
| IBE-C1144     | *N. anomalus*    | anomalus   | Faeces      | Vega de Hórreo (7)        | Spain   | 43.1 | -6.6  | 1                              |
| IBE-C2664     | *N. anomalus*    | anomalus   | Faeces      | Bergantes (8)             | Spain   | 40.7 | -0.2  | 1                              |
| IBE-C1808     | *N. anomalus*    | milleri    | Tissue (b)  | La Pobla de Segur (9)     | Spain   | 42.3 | 1.0   | 14                             |
| IBE-C3786     | *N. anomalus*    | milleri    | Tissue (d)  | Guardiola de Berguedà (10)| Spain   | 42.3 | 1.9   | 14                             |
| IBE-C4115     | *N. anomalus*    | milleri    | Tissue (e)  | Vitebsk (11)              | Belarus | 55.2 | 30.2  | 9                              |
| IBE-C4116     | *N. anomalus*    | milleri    | Tissue (e)  | Belgorod (12)             | Russia  | 50.6 | 36.6  | 14                             |
| IBE-S1926     | *N. anomalus*    | milleri    | Faeces      | Osor (13)                 | Spain   | 41.9 | 2.5   | 1                              |
| IBE-C4120     | *N. teres*       |            | Tissue (e)  | North Caucasus (14)       | Russia  | 43.9 | 40.1  | 14                             |
| IBE-C4122     | *N. teres*       |            | Tissue (e)  | North Caucasus (14)       | Russia  | 43.9 | 40.1  | 14                             |
| IBE-C1914     | *N. fodiens*     |            | Tissue (f)  | Coll                      | Spain   | 42.5 | 0.8   | 14                             |
| IBE-C101      | *N. fodiens*     |            | Tissue (b)  | Queralbs                  | Spain   | 42.4 | 2.1   | 14                             |
| IBE-S1915     | *N. fodiens*     |            | Faeces      | Zalduondo                 | Spain   | 42.9 | -2.3  | 1                              |

a, Capture permit CO/09/0004/2010, National Park Picos de Europa  
b, Found dead in the field  
c, Collection National Park Picos de Europa  
d, Capture permit SF/209 (2012), Generalitat de Catalunya  
e, Previous work [1]  
f, Capture permit SF/238 (2010), Generalitat de Catalunya
[1] Bannikova, A. A., and D. A. Kramerov. 2005. Molecular phylogeny of Palearctic shrews inferred from RFLP and IS-PCR data. Advances in the biology of shrews II (eds. J. F. Merritt, S. Churchfield, R. Hutterer, and B. I. Sheftel). Special Publication of the International Society of Shrew Biologists 87–98.
Table S2. Cytochrome b sequences downloaded from GenBank.

| GenBank Accession and reference | Species                  | Subspecies  | Country (and map number) | Genes used in the species tree |
|--------------------------------|--------------------------|-------------|--------------------------|-------------------------------|
| DQ991052 [1]                   | *Neomys anomalus*         | milleri     | Italy (15)               | 1                             |
| DQ991049 [1]                   | *Neomys anomalus*         | milleri     | Italy (16)               | 1                             |
| DQ630409 [2]                   | *Neomys anomalus*         | milleri     | Macedonia (17)           |                               |
| AF182182 [3]                   | *Neomys anomalus*         | milleri     | Turkey (18)              |                               |
| AB175099 [4]                   | *Neomys anomalus*         | milleri     | Switzerland (19)         | 1                             |
| HQ621861 [6]                   | *Neomys teres*            |             | Armenia (20)             | 1                             |
| HQ621860 [6]                   | *Neomys teres*            |             | Armenia (20)             | 1                             |
| HQ621859 [6]                   | *Neomys teres*            |             | Armenia (20)             | 1                             |
| HQ621858 [6]                   | *Neomys teres*            |             | Armenia (20)             | 1                             |
| DQ991062 [1]                   | *Neomys fodiens*          |             | Italy                    | 1                             |
| AB175098 [4]                   | *Neomys fodiens*          |             | Switzerland              | 1                             |
| AB175097 [4]                   | *Neomys fodiens*          |             | Finland                  | 1                             |
| AB175096 [4]                   | *Neomys fodiens*          |             | Russia                   | 1                             |
| AB175071 [5]                   | *Neomys fodiens*          |             | China                    | 1                             |
| GU981264 [7]                   | *Chimarrogale himalayica* |             | China                    |                               |
| GU981263 [7]                   | *Chimarrogale himalayica* |             | China                    |                               |
| AB108768 [8]                   | *Chimarrogale platycephala|             | Japan                    |                               |
| AB108766 [8]                   | *Chimarrogale platycephala|             | Japan                    |                               |

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**Table S3.** Primers used for the amplification of three overlapping fragments of the mitochondrial cytochrome b gene.

| Primer               | Sequence                        | Fragment |
|----------------------|---------------------------------|----------|
| Neomys_tRNA{Glu}     | ATCGTTGTTATTCAACTATAAGAAC       | First    |
| Neomys_cy{b}403R     | YCCYCARAAATGATATTTGYCCTCA       | First    |
| Neomys_cy{b}389F     | GTTATAGCCACTGCTTTTATAG          | Second   |
| Neomys_cy{b}746R     | TAATTGTCGGGTCTCCGAGTA           | Second   |
| Neomys_cy{b}614F     | TWTTCCTYCATGAAACAGGATC          | Third    |
| Neomys_tRNA{Thr}     | TTTGTTTACAAGACCAGTGAT           | Third    |
Table S4. Nuclear intron markers and primers used in this study. TD: touchdown PCR in which the annealing temperature was lowered from 65 °C to 50 °C at 1 °C decrease per cycle.

| Marker | Primer sequences | T (°C) | Length |
|--------|------------------|--------|--------|
| ALAD-10 | AGAGTTYGCRYATGYTGATGGCA / GGYGTGATGAGGRATGATGA | TD | 455 |
| ASB6-2 | TGYTGAAGATGGCYAGCTG / TCCACATGTCAAGCTGGTT | TD | 319 |
| CSF2-2 | RAAACAGTARAWGRGCTCTTCTG / TNCAGACNGTCTGAGGA | TD | 673 |
| CST6-1 | RYTACACATGGGAGCAACA / KGCMAGSGGGGCGAGRGTGA | 65 | 267 |
| GALNT5-4 | ATYTTAGATTCTCAYGTGGAATG / ACRTCYGGAATKGTCTGC | 60 | 727 |
| GDAP1-1 | ACDCAATTTCATCASYCBAAGAATTCGGACTTC | TD | 688 |
| HIF1AN-5 | TACGAGAGGTTYCCYATTTCCA / CTCTACCAGAAAGTGTTCTC | TD | 389 |
| JMJD-2 | ACCABTGGCVTGCATGMAGARGT / TGATGAATCRYTGACCGTACAGTGAT | TD | 450 |
| MCM3-2 | GGAATTTATCAGAGCAAGGTC / RTAGAAYTCTCRTACTGCTT | TD | 335 |
| MYCBPAP-11 | AAYAAYGGCAGVGTGYYCATTT / CAGCATYCRVAGAYTTRAAGAA | TD | 341 |
| PRPF31-3 | GTCTYGTRGAGYCAACAAC / BTTSACNGTGCAGTGAATC | TD | 481 |
| SLA-2 | AGGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGCTGTGGC | 685 |
Table S5. GenBank accession numbers

| Specimen Code | Species                  | Subspecies | Cytochrome b | ALAD-10 | ASBD-2 | CSF2-2 | CST6-1 | GALNTS-4 | GAPA1-1 | HIF1AN-5 | JMDD-2 | MCM3-2 | MYCBPAP-11 | PRPF31-3 | SLA-2 | TRAK-8 |
|---------------|--------------------------|------------|--------------|---------|---------|---------|---------|-----------|----------|----------|---------|---------|-----------|-----------|--------|---------|
| BE-C1529      | Neomys anomalus          | milleri    | LK936659    | LK936777 | LK936778 | LK936898 | LK936870 | LK936872 | LK936741 | LK936742 | LK936761 | LK936762 | LK936781 | LK936782 | LK936801 | LK936802 |
| BE-C1789      | Neomys anomalus          | milleri    | LK936860    | LK936979 | LK936980 | LK936977 | LK936972 | LK936974 | LK936974 | LK936974 | LK936975 | LK936974 | LK936974 | LK936974 | LK936974 | LK936974 |
| BE-C2895      | Neomys anomalus          | milleri    | LK936861    | LK936901 | LK936902 | LK936870 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 |
| BE-C1445      | Neomys anomalus          | milleri    | LK936863    | LK936901 | LK936902 | LK936870 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 |
| BE-C1982      | Neomys anomalus          | milleri    | LK936864    | LK936901 | LK936902 | LK936870 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 |
| BE-C1983      | Neomys anomalus          | milleri    | LK936865    | LK936901 | LK936902 | LK936870 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 |
| BE-C2964      | Neomys anomalus          | milleri    | LK936866    | LK936901 | LK936902 | LK936870 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 |
| BE-C3909      | Neomys anomalus          | milleri    | LK936867    | LK936901 | LK936902 | LK936870 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 |
| BE-C7996      | Neomys anomalus          | milleri    | LK936868    | LK936901 | LK936902 | LK936870 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 |
| BE-C1155      | Neomys anomalus          | milleri    | LK936869    | LK936901 | LK936902 | LK936870 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 |
| BE-C4116      | Neomys anomalus          | milleri    | LK936870    | LK936901 | LK936902 | LK936870 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 |
| BE-D1026      | Neomys anomalus          | milleri    | LK936871    | LK936901 | LK936902 | LK936870 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 |
| BE-C1911      | Neomys fletcheri         | milleri    | LK936872    | LK936901 | LK936902 | LK936870 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 |
| BE-C1914      | Neomys fletcheri         | milleri    | LK936873    | LK936901 | LK936902 | LK936870 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 |
| BE-D1015      | Neomys fletcheri         | milleri    | LK936874    | LK936901 | LK936902 | LK936870 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 |
| BE-C1220      | Neomys fletcheri         | milleri    | LK936875    | LK936901 | LK936902 | LK936870 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 |
| BE-C1232      | Neomys fletcheri         | milleri    | LK936876    | LK936901 | LK936902 | LK936870 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 | LK936874 |
| BE-C1233      | Crocidura russula        | milleri    | LK936854    | LK936865  | LK936858  | LK936860  | LK936862  | LK936864  | LK936866  | LK936867  | LK936868  | LK936869  | LK936870  | LK936871  | LK936872  | LK936873  | LK936874  |
**Table S6.** Calibration constraints (in Myr) used as priors in the BEAST analysis of mammalian introns. Node numbers correspond to numbers in figure 4.

| Clade (Node number) | Minimum hard bound | Maximum soft bound | Lognormal parameters |
|---------------------|---------------------|---------------------|----------------------|
|                     |                     |                     | Mean    | Offset |
| Boreoeutheria (1)   | 61.50               | 131.50              | 22.28   | 61.50  |
| Laurasiatheria (2)  | 62.50               | 131.50              | 21.95   | 62.50  |
| Eulipotyphla (3)    | 61.50               | 131.50              | 22.28   | 61.50  |
| Ferungulata (4)     | 62.50               | 131.50              | 21.95   | 62.50  |
| Zooamata (5)        | 62.50               | 131.50              | 21.95   | 62.50  |
| Cetartiodactyla (6) | 52.40               | 65.80               | 4.27    | 52.40  |
| Carnivora (7)       | 39.68               | 65.80               | 8.28    | 39.68  |
| Catarrhini (8)      | 23.5                | 34.00               | 3.35    | 23.50  |
Table S7. Calibration constraints (in Myr) used as priors in the BEAST analysis of cytochrome $b$ of soricids. Node numbers correspond to numbers in figure S1.

| Clade (Node number)       | Minimum hard bound | Maximum soft bound | Lognormal parameters |
|---------------------------|--------------------|--------------------|---------------------|
| Soricinae–Crocidurinae (1) | 20                 | 25                 | 1.59                |
| Blarinini (2)             | 15                 | 20                 | 1.59                |
| *Otisorex* (3)            | 3.5                | 5                  | 0.48                |

Node numbers correspond to numbers in figure S1.
Figure S1. Maximum-likelihood trees reconstructed from each individual intron of Neomys. The two alleles of each specimen are indicated with the letters A and B. The trees were rooted at the midpoint. The scale is in substitutions/site.
Figure S2. Maximum-likelihood trees reconstructed from different concatenations of *Neomys* introns. In each concatenation, the order of each allele pair was randomly changed. Names include specimen code and locality data. The trees were rooted at the midpoint. The scale bar represents 0.002 substitutions/site in all trees.
**Figure S3.** Species tree obtained by *BEAST* with branch lengths in relative units.
Figure S4. Bayesian relaxed clock tree reconstructed with cytochrome $b$ sequences of soricids. Calibration nodes are shown with a white circle and the corresponding constraints are given in Table S7. The *Neomys fodiens* branch from which the mutations rate was estimated is shown with a thicker line.