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1 Did the Quaternary climatic fluctuations really influence the tempo and mode of
2 diversification in European rodents?

3

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5

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10

11 **Key words:** Ancestral biogeographic areas - molecular dating – phylogeny – *Microtus*
12 (*Terricola*) - speciation

13

1 **Abstract**

2 The objective of the present study is to establish if the Quaternary climatic fluctuations
3 influenced the tempo and mode of diversification in European rodents. Our case study is the
4 subgenus *Microtus (Terricola)* distributed from Western Europe to the Caucasus.
5 Mitochondrial cytochrome *b* gene sequences from several representatives of all the species
6 were used to generate maximum-likelihood and Bayesian phylogenetic trees, to estimate
7 divergence times, to identify biogeographic ancestral areas and to study the rate of
8 diversification. Results showed that phylogenetic tree topologies were similar to previous
9 published studies but with a better resolution at some nodes. The origin of *Microtus*
10 (*Terricola*) is dated back to approximately 4.05 Myr in the Early Pliocene, and molecular
11 dating for most *Terricola* species corresponds to several glacial periods of the Pleistocene.
12 Results of the biogeographic ancestral area reconstruction suggest that *Microtus (Terricola)*
13 diversified from the Caucasus/Turkey/Iran area through Western Europe. Several periods of
14 diversity variation were highlighted: two period of diversity increase, between 3 and 2 Myr,
15 and after 1 Myr; two periods of diversity decrease, before 3 Myr, and between 2 and 1 Myr.
16 The diversification rate of *Microtus (Terricola)* was 0.353 ± 0.004 event/Myr, a rate similar to
17 that of the Muridae family. To conclude, although the Pleistocene glacial conditions had an
18 impact on the speciation events, the Quaternary does not appear however as a period with an
19 exceptional rate of diversification for European rodents.

20

1 **Introduction**

2 Estimating diversification rates appears essential in ecology and evolutionary biology to
3 understand how the biodiversity varies across space and time (Ricklefs 2007; Morlon 2014).
4 Because speciation and extinction processes require thousands to millions of years to happen,
5 diversification has long been studied from fossil data. Since the 1990's, a phylogenetic
6 alternative is commonly used to estimate speciation, extinction, and thus diversification rates
7 because phylogenies contain information about evolutionary relationships among species with
8 a temporal dimension (Hey 1992; Nee et al. 1994; Sanderson and Donoghue 1996; Paradis
9 1997; Ricklefs 2007; Morlon 2014) and a characteristic signature left by extinction events
10 (Nee 2001; Rabosky 2009). Molecular phylogenies were used to infer diversification rates,
11 for instance, in plants (Magallón and Sanderson 2001; Hughes and Eastwood 2006), insects
12 (Barraclough and Vogler 2002), amphibians (Kozak et al. 2006) and birds (Zink et al. 2004).
13 In mammals, a fluctuating diversification rate since the Cretaceous/Tertiary boundary was
14 recently highlighted (Stadler 2011). Several shifts in diversification rates were even identified
15 in the most diversified mammalian clade, the Rodentia (Steppan et al. 2004; Fabre et al.
16 2012). Several hypotheses were proposed to explain these shifts as key innovations,
17 biogeographic events, absence of competition or predation, chromosomal rearrangements as
18 well as environmental changes (Rowe et al. 2011; Fabre et al. 2012).

19 Quaternary climatic fluctuations (during the last 2.6 Myr, Cohen and Gibbard 2012)
20 are thought to have influenced plant and animal distribution through repeated range
21 contraction (population isolation) and expansion (colonization by tracking favourable climatic
22 space). For this reason, they are often considered as a major driving force of allopatric
23 diversification. In particular, the role of Quaternary glacial cycles is often considered to
24 explain the extraordinary diversification of the rodent genus *Microtus* Schrank, 1798 from the
25 family Cricetidae (Chaline 1987; Chaline et al. 1999; Jaarola et al. 2004). The ancestor of the
26 *Microtus* species is apparently within species of the genus *Allophaiomys* Kormos, 1930
27 (Chaline et al. 1999). Early radiation and diversification about 2.4 – 2 Myr ago (Early
28 Pleistocene; Chaline and Graf 1988; Chaline et al. 1999; Zheng and Zhang 2000) would have
29 generated *Microtus* subgenera that subsequently would have led to the appearance of the
30 diverse extant species. One of the most studied radiations from systematic, paleontological,
31 odontometric, cytogenetic, ethological, morphometric or genetic standpoints (*e.g.* Bastos-
32 Silveira et al. 2012; Brunet-Lecomte 1988, 1989, 1990; Brunet-Lecomte and Chaline 1990,
33 1991, 1992; Castiglia et al. 2008; Chaline and Graf 1988; Chaline et al. 1988, 1999; Giannoni

1 et al. 1993; Haring et al. 2000; Jaarola et al. 2004; Kryštufek et al. 1996; Macholán et al.
2 2001; Martínková and Dudich 2003; Martínková and Moravec 2012; Martínková et al. 2004,
3 2007; Meylan 1972; Mitsainas et al. 2009; Rovastos and Giagia-Athanasopoulou 2012;
4 Santos et al. 2009; Storch and Winking 1977; Thanou et al. 2005; Tougard et al. 2008a;
5 Zagorodnyuk 1990) is the radiation of the subgenus *Terricola* Fatio, 1867 (European ground
6 voles). The oldest fossil remains of *Microtus (Terricola)* are known from the end of Early
7 Pleistocene (around 0.9 Myr) in Italy and they were attributed to *M. (T.) arvalidens* Kretzoi,
8 1958 (Masini and Sala 2007).

9 The subgenus *Microtus (Terricola)* is characterized by a “pitymyan rhombus” on the
10 first lower molar, a primitive character found in evolved species of *Allophaiomys* (Chaline et
11 al. 1999). First grouped with some Nearctic voles under the subgenus *M. (Pitymys)*
12 McMurtrie, 1831, evolutionary studies and phylogenetic analyses underlined that the
13 European ground voles are closer to European *M. (Microtus)* than to the American subgenera
14 (Graf, 1982; Chaline et al. 1988; Jaarola et al. 2004; Martínková and Moravec 2012;
15 Robovský et al. 2008). Indeed, although Palearctic and Nearctic ground voles seem to share
16 the same *Allophaiomys* ancestor, they evolved independently leading to two monophyletic
17 subgenera, respectively *M. (Terricola)* and *M. (Pitymys)* (Chaline et al. 1988, 1999). If the
18 phylogenetic position of the European ground voles within the Arvicolinae is now clearly
19 established (Jaarola et al. 2004; Martínková and Moravec 2012; Robovský et al. 2008),
20 attempts to reconstruct phylogenetic relationships among *M. (Terricola)* from molecular
21 markers have failed maybe because of the dataset incompleteness: mitochondrial cytochrome
22 *b* gene (cytb) sequences of one or several individuals but not all species represented (Jaarola
23 et al. 2004; Robovský et al. 2008); concatenated supermatrix (72.8% of missing data) of
24 mitochondrial (cytb, control region, cytochrome *c* oxidase subunit I and NADH
25 dehydrogenase subunit 4) and nuclear (interphotoreceptor retinoid-binding protein, growth
26 hormone receptor, exon 10, sex-determining region Y and lecithin cholesterol acyl
27 transferase, exons 2-5) markers for one individual of each species (Martínková and Moravec
28 2012).

29 According to Ricklefs (2007), using phylogenetic information to estimate
30 diversification rates depends on three assumptions: the completeness of the phylogenetic data,
31 a reliable time scale and the constancy of speciation and extinction rates through all clades.
32 Because of their high specific diversity (14 extant and 11 extinct species; Brunet-Lecomte
33 1990; Brunet-Lecomte et al. 1992; Gil 1996; Kowalski 2001; Musser and Carleton 2005),
34 their geographic distribution (from the Caucasus Mountains to the Iberian Peninsula; Musser

1 and Carleton 2005), the knowledge of their fossil record and their evolutionary history rooted
2 in the Quaternary (Chaline and Graf 1988; Chaline et al. 1999; Zheng and Zhang 2000), the
3 European ground voles are a species group particularly suitable to understand if and how the
4 climatic fluctuations of the Quaternary could have promoted the diversification of rapid
5 evolving small mammals such as rodents in temperate zones. In other words, does the level of
6 diversification during this period exceed other mammal rates? In this context, the aim of the
7 present study is as follows: to investigate the phylogenetic framework of the diversification
8 from sequences of multiple representatives of all the *Microtus (Terricola)* species, and
9 subspecies when sequences were available; to identify the origin of the diversification; to
10 estimate the rate of diversification. For simplicity, the present study follows the systematic
11 designation of Musser and Carleton (2005), and when dealing with the species, it refers to the
12 genus (*Microtus* or *M.*) rather than the subgenus name (*Microtus* or *Terricola*), and to
13 *Terricola* rather than *Microtus (Terricola)* when dealing with the subgenus.

14

15 **Material and Methods**

16

17 **Data**

18 Our dataset included original (15) and GenBank (85) DNA sequences for 100 specimens
19 representing all the *Microtus (Terricola)* species (according to Musser and Carleton 2005)
20 and some *Microtus (Microtus)* species, these latter being used as outgroup (Table 1). For
21 original data, total DNA was extracted from 96% ethanol-preserved tissue pieces (skin or
22 foot) following standard procedures (Sambrook et al. 1989). The complete cytochrome *b* gene
23 (*cytb*) was PCR-amplified ($T_m = 50^\circ\text{C}$) with universal primers located in the flanking tRNAs
24 (L7 5'-ACCAATGACATGAAAATCATCGTT-3' and H6 5'-
25 TCTCCATTTCTGGTTTACAAGAC-3'). Direct sequencing was performed in both
26 directions to confirm polymorphic sites by Macrogen Inc (Seoul, Korea). Original and
27 GenBank sequences were aligned by hand using MEGA v5.2.2 (Tamura et al. 2011). Details
28 about the sampling and sequences are in Fig. 1 and Table 1.

29

30 **Phylogenetic inference**

31 Phylogenetic trees were reconstructed using a maximum-likelihood approach (ML) and a
32 Bayesian inference (BI) through the technical facilities of the Platform Montpellier
33 Bioinformatics Biodiversity (MBB) of the "Institut des Sciences de l'Evolution de

1 Montpellier” (Centre Méditerranéen de l’Environnement et de la Biodiversité, Montpellier,
2 France; ISEM and CeMEB). Best-fitting models of nucleotide substitution were determined
3 using MrModeltest v2.3 (Nylander 2004). The optimal fitted model in both cases is identified
4 by the minimum value of the Akaike Information Criterion.

5 ML reconstruction was conducted using PhyML v3.0 (Guindon et al. 2010) under the
6 GTR model (Yang 1994) with a proportion of invariable sites (I) and a gamma distribution
7 (G). Nodal robustness was estimated by bootstrap percentage values (BP) after 1000 pseudo-
8 replicates. In BI, a mixed model analysis was applied according to the cytb codon positions:
9 (1) and (2) HKY (Hasegawa et al. 1985) +I+G; (3) GTR+I+G. Five independent runs of five
10 Markov chain Monte Carlo (MCMC) chains were simultaneously carried out for 5,000,000
11 generations using MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003). Bayesian posterior
12 probabilities (PP) were obtained from the 50% majority rule consensus of trees sampled every
13 100th generation after a burn-in stage of 25,000.

14 Alternative hypotheses of intra-*Terricola* relationships were compared with the test of
15 Shimodaira and Hasegawa (1999) as implemented in PAUP*4.010b (Swofford 2002).

16 Intra- and intergroup genetic distances were estimated by the Kimura-2-parameter
17 distance (K2P; Kimura 1980) with MEGA. An internal branch test was performed also with
18 MEGA to determine whether short internal branches in the phylogeny are solved relationships
19 or polytomies. A neighbour-joining distance-based method was used to build a tree under the
20 K2P nucleotide substitution model.

21

22 **Divergence time estimates**

23 Time to the most recent common ancestor (TMRCA) was estimated for several clades, and
24 especially each *Terricola* species, by a Bayesian coalescent analysis under the GTR+I+G
25 model with BEAST, v2.3.1 (Bouckaert et al. 2014). Through the technical facilities of the
26 Platform MBB of ISEM (CeMEB), divergence time estimates were achieved under the
27 recently introduced “fossilized birth-death” (FBD) process (Heath et al. 2014). The FBD
28 model considers the diversification of extant and fossil species are part of the same macro-
29 evolutionary process. Four parameters (speciation, extinction and fossilization rates as well as
30 proportion of sampled extant species) are taken into account to inform the amount of temporal
31 uncertainty associated with speciation events on the tree (Heath et al. 2014). Three molecular
32 clocks (strict, relaxed uncorrelated lognormal or relaxed uncorrelated exponential) were
33 compared using Bayes factor values (BF) to test which of them best fit our data. BF
34 significance was then determined from 2lnBF results (Brandley et al. 2005). BEAST analyses

1 consisted of five independent runs of 20 million generations with the first 10% discarded as
2 burn-in. Results of these five independent runs were combined with LogCombiner v2.3.1
3 (Bouckaert et al. 2014) to estimate TMRCAs and 95% confidence intervals. A consensus tree
4 was generated using TreeAnnotator v2.3.1 (Bouckaert et al. 2014) with mean node heights as
5 node heights option and maximum clade credibility as target tree type option.

6 The literature regarding the evolutionary history of the subgenus *Terricola* is rich but
7 full of temporal inaccuracies (a time period such as, for instance, the Cromerian or the Middle
8 Pleistocene in Brunet-Lecomte 1990, Kowalski 2001; a relative dating as, for instance, in
9 Cuenca- Bescós et al. 2010, Masini et al. 2005, Masini and Sala 2007; rarely an absolute
10 dating as in Bonfiglio et al. 2008) or contradictory relationships (notably for the phylogenetic
11 position of *Allophaiomys* or for the relationships between extinct and extant species as in
12 Chaline, 1987, Chaline et al., 1999). For these reasons, only the dates of 0.800 ± 0.100 Myr
13 for the occurrence of the ancestor of *M. subterraneus*, *i.e.* *M. arvalidens* (Kowalski 2001;
14 Masini et al. 2005; Masini and Sala 2007; Cuenca- Bescós et al. 2010), as well as the date of
15 0.475 ± 0.025 Myr for the origin of *M. arvalis* (Miesenheimer I, Germany; Kowalski 2001)
16 were used as fossil node and tip calibration points, respectively.

17

18 **Identification of the ancestral area and diversification rate**

19 Present-day distribution range (Fig. 1) of *Terricola* was divided into 11 biogeographic areas,
20 based on the occurrence of one or more *Terricola* species (Austria, the Balkans, the
21 Carpathians, the Caucasus/Turkey/Iran area, France, Germany/Bavaria, Italy, Portugal, Spain,
22 Switzerland and eastern Europe). The possible ancestral areas of the *Terricola* main clades
23 were then reconstructed using the Statistical Dispersal-Vicariance Analysis method (S-DIVA;
24 Yu et al. 2010) implemented in RASP v2.1b (Yu et al. 2012) from 2,000 trees obtained from
25 the five combined BEAST runs.

26 Diversification rates were estimated using BayesRate v1.63b (Silvestro et al. 2011)
27 and BEAST. With BEAST, the analysis was performed simultaneously with the divergence
28 time estimates as previously mentioned on a dataset including the outgroup. To evaluate the
29 accumulation of lineages through time (LTT), a LTT plot was constructed with Tracer1.6
30 (Rambaut et al. 2014). With BayesRate, marginal likelihoods via the thermodynamic
31 integration were calculated to select the best-fitting model of diversification between the
32 pure-birth or birth-death processes, under the following parameters: 100,000 MCMC
33 iterations per five chains for 1,000 randomly subsampled posterior BEAST trees excluding

1 the outgroup. Marginal likelihoods were then compared using Bayes factor tests as previously
2 mentioned. Speciation (λ), extinction (μ) and diversification ($\lambda - \mu$) rates through time were
3 finally estimated with the selected model and previously mentioned parameters. The results
4 were checked using Tracer.

5

6 **Results**

7

8 **Sequence analysis and phylogenetic reconstructions**

9 The alignment of the complete cytb was 1143 nucleotides long with 348 phylogenetically
10 informative sites (368 with the outgroup). The new sequences were deposited in the EMBL
11 Nucleotide Sequence Database under the accession numbers LT222298-LT222312. Base
12 composition (A = 31%, C = 29%, G = 13%, T = 27%) was quite similar to that of other
13 rodents (Martin et al. 2000; Lecompte et al. 2002; Montgelard et al. 2002), and especially
14 *Microtus* rodents (Conroy and Cook 1999; Tougaard et al. 2008). This indicated that no
15 artificial cluster occurred due to a misleading compositional signal in the dataset.

16 Phylogenetic reconstructions provided similar tree topologies in ML (Fig. 2 with BP
17 and PP robustness values) and BI (data not shown). The monophyly of the subgenus *Terricola*
18 is highly supported in both approaches (BP = 98%; PP = 1.00). Among *Terricola*, all the
19 species are also supported with high values (88% < BP < 100%; 0.96 < PP < 1.00). Four
20 internal nodes characterized by short branches remain unsolved as in previously published
21 studies (Jaarola et al. 2004; Martínková and Moravec 2012). The internal branch test indicates
22 that these branches are significantly different from zero with length confidence probabilities
23 higher than 95% (data not shown; Nei and Kumar 2000; Tamura et al. 2011). On the other
24 hand, some nodes, not supported in previously published studies (Jaarola et al. 2004;
25 Martínková and Moravec 2012; Robovský et al. 2008), are here moderately or highly
26 supported: the position of *M. majori* as the first offshoot of the *Terricola* species (BP = 98%;
27 PP = 1.00); the cluster of *M. gerbei* with *M. duodecimcostatus* and *M. lusitanicus* (BP = 60%;
28 PP = 0.87). As in Castiglia et al. (2008), *M. savii* appeared paraphyletic because of the
29 internal position of *M. brachycercus* in this group (BP = 100%; PP = 1.00). The present study
30 also confirmed that *M. tatricus* belongs to the subgenus *Terricola* as suggested by several
31 authors (Chaline et al. 1988; Jaarola et al. 2004; Martínková and Moravec 2012), and that *M.*
32 *schelkovnikovi* does not seem to be a *Terricola* species.

1 The intra-*Terricola* relationships from the present ML tree topology (Fig. 2) were
2 compared with the intra-*Terricola* relationships presented in Martínková and Moravec (2012)
3 with the test of Shimodaira and Hasegawa (1999). The best ML tree is the tree presented in
4 Fig. 2. However, the tree topology from Martínková and Moravec (2012) is not significantly
5 worse at the 5% confidence level ($P = 0.076$). These trees differ by the position of *M. majori*
6 as the sister group of all the *Terricola* species (present study) or of the *daghestanicus* /
7 *subterraneus* clade (Martínková and Moravec 2012), as well as the position of *M. thomasi*
8 and *M. tatricus* as the sister group of either *M. felteni* or the *multiplex* complex.

9 Genetic distances were estimated within and between *Terricola* species or subspecies
10 (Table S1). Intragroup distances are from $0.1 \pm 0.1\%$ for *M. thomasi atticus* to $2.8 \pm 0.4\%$ for
11 *M. daghestanicus*, while intergroup distances are from $0.8 \pm 0.3\%$ (*M. thomasi atticus*/*M.*
12 *thomasi evia*) to $12.6 \pm 1.5\%$ (*M. brachycercus*+*M. savii niethammericus*/*M. daghestanicus*).
13

14 **Molecular dating, biogeographic history and diversification rates**

15 Likelihood differences suggested that the relaxed uncorrelated lognormal clock is
16 significantly more adapted to our dataset ($2\ln BF > 10$). For this reason, divergence times for
17 TMRCA in the subgenus *Terricola* were estimated under a lognormal molecular clock and the
18 fossilized birth-death process. Convergence to stable values was checked with Tracer 1.6,
19 obtaining an effective sample size (ESS) greater than 200 for all parameters. Divergence
20 times are provided in Fig. 3. The origin of this subgenus is dated back to approximately 4.05
21 Myr in the Early Pliocene, and molecular dating for most *Terricola* species corresponds to
22 several glacial periods of the Pleistocene: from 1.81 Myr for the *savii* complex to 0.08 Myr
23 for *M. majori*. In this context, the substitution rate is estimated at 0.033 ± 0.004 substitution /
24 site / Myr which seems lower than the rate (0.08) estimated for the genus *Microtus*
25 (represented by *M. levis* and *M. kikuchii* Kuroda, 1920) but closer to that of others rodent
26 genera such as *Mus* Linnaeus, 1758, *Rattus* Fisher, 1903, *Ctenomys* Blainville, 1826 and
27 *Chaetopidus* Merriam, 1889 (Triant and DeWoody 2006).

28 Results of the ancestral area reconstructions with RASP are presented in Fig. 3.
29 S-DIVA suggests that the subgenus *Terricola* most likely diversified early in the
30 Caucasus/Turkey/Iran area (CTI in Fig. 3; frequency of occurrence = 100%). Subsequently,
31 multiple dispersal (7) and vicariance (5) events occurred. Within the CTI area, two clades
32 diverged giving rise to *M. majori* and the clade containing all the other *Terricola* species with
33 an ancestor distribution including the CTI area and Southern Europe (100%). With an
34 occurrence frequency of 100%, possible ancestor ranges should be: the CTI area for *M.*

1 *daghestanicus* and *M. subterraneus*; Italy for the *savii* complex; Spain + France for *M. gerbei*,
2 *M. lusitanicus* and *M. duodecimcostatus*; the Balkans for *M. felteni* and *M. thomasi*; the
3 Carpathians for *M. tatricus*. The biogeographic history of the *multiplex* complex (*M.*
4 *multiplex*, *M. liechtensteini* and *M. bavaricus*) is much more difficult to interpret with Italy,
5 the Balkans, France, Switzerland and/or Austria as possible ancestral area.

6 In diversification estimates, BF values provided a strong support ($2\ln\text{BF} > 10$) for the
7 birth-death process. The diversification rate detected for this model was 0.353 ± 0.004 ($\lambda =$
8 5.767 ± 0.033 and $\mu = 5.414 \pm 0.031$) and 0.483 ± 0.051 event/Myr with BayesRate and
9 BEAST, respectively. The LTT plot (Fig. 4) reflects two periods of diversity increase
10 (between 3 and 2 Myr, and after 1 Myr) and decrease (before 3 Myr, and between 2 and 1
11 Myr).

12

13 **Discussion**

14

15 **Phylogenetic framework of the diversification**

16 From a molecular standpoint, the phylogeny of the subgenus *Terricola* was never studied for
17 itself but it was always studied in a larger context, *i.e.* included in the phylogeny of the
18 Arvicolinae or of the genus *Microtus* (Jaarola et al. 2004; Martínková and Moravec 2012;
19 Robovský et al. 2008). For this reason, its evolutionary history was never deeply investigated.
20 The use of only *cytb* sequences should be seen as a limitation of the study. Nevertheless, our
21 phylogeny based on a single mitochondrial marker but including several representatives of all
22 the *Terricola* species appears better resolved.

23 As in previously published studies, the present study confirms the monophyly of the
24 subgenus *Terricola*. Several clusters are not questionable, notably the *multiplex* complex
25 (Haring et al. 2000; Jaarola et al. 2004; Martínková and Moravec 2012; Robovský et al.
26 2008). The cluster composed of *M. subterraneus* and *M. daghestanicus* is also always highly
27 supported, although Macholán et al. (2001) suggested the paraphyly of *M. subterraneus* with
28 *M. majori* and *M. daghestanicus* from karyotypic and allozymic data. Regarding the *savii*
29 complex, three clades (*M. s. savii*, *M. s. nebrodensis* and *M. brachycercus*+*M. s.*
30 *niethammericus*) were identified as in Castiglia et al. (2008). These latter authors suggested first
31 the paraphyly of *M. savii* because of the internal position of *M. brachycercus*, and they then
32 proposed a specific status for both species in considering *M. s. niethammericus* and *M.*
33 *brachycercus* as conspecific. As for *M. s. nebrodensis*, some authors supported the hypothesis

1 that it is an endemic species of Sicily because of its phylogenetic position in the *savii*
2 complex and its high morphological, cytogenetic, mitochondrial and nuclear genetic
3 divergence (Castiglia et al. 2008; Bezerra et al. 2015). The cluster of *M. gerbei* with the clade
4 formed by *M. lusitanicus* and *M. duodecimcostatus* is also highly supported, as in Robovský
5 et al. (2008), although Chaline et al. (1988, 1999) considered *M. gerbei* as a species of the
6 *savii* complex from paleontological and morphological data. Lastly, *M. schelkovnikovi* does
7 not seem to be a *Terricola* species as proposed by morphological, karyological and molecular
8 studies (Nadachowski 2007; Martínková and Moravec 2012). Nadachowski (2007) even
9 considered this species as the sole member of the subgenus *Microtus* (*Hyrceanicola*).

10 By contrast, the present study strongly supports the position of *M. majori* at the basis
11 of the *Terricola* clade, what is in opposition with previous published works (Jaarola et al.
12 2004; Martínková and Moravec 2012; Robovský et al. 2008) where *M. majori* is clustered
13 with *M. subterraneus* and *M. daghestanicus* but with low supports. Based on its karyotype,
14 Zagorodnyuk (1990) considered besides *M. majori* as the sole member of its own species
15 complex. An other point of divergence between the present and previously published studies
16 (Jaarola et al. 2004; Martínková and Moravec 2012; Robovský et al. 2008) is the phylogenetic
17 position of *M. thomasi* and *M. tatricus*. Regarding *M. thomasi*, three groups are identified in
18 the present study and they are attributed to three (*thomasi*, *atticus* and *evia*) out of the five *M.*
19 *thomasi* subspecies, as in Rovatsos and Giagia-Athanasopoulou (2012) but here with better
20 supports. Based on reproductive, cytogenetic and cyt b data, these latter authors proposed to
21 consider *M. thomasi* and *M. atticus* (including the two “chromosome races” *atticus* and *evia*)
22 as separate biological species. In the present study, *M. thomasi* would be the sister group of
23 the *multiplex* complex, while *M. tatricus* would be the sister species of *M. felteni*. It is the
24 opposite situation in previously published studies (Jaarola et al. 2004; Martínková and
25 Moravec 2012; Robovský et al. 2008), but, in any case, relationships are poorly supported. No
26 information in the literature can support a hypothesis rather than the other, except maybe the
27 geographic proximity of *M. thomasi* and *M. felteni* in the Balkans (Mitchell-Jones et al. 1999;
28 Musser and Carleton 2005; Shenbrot and Krasnov 2005).

29 Unfortunately, two internal clusters remain not supported among the *Terricola* species
30 (Fig. 2) and are characterized by short branches. Several studies at generic level recovered
31 polytomies among species, leading some authors to the conclusion of a burst of species
32 diversification with no time left for the accumulation of synapomorphies in mitochondrial
33 DNA (Conroy and Cook 1999; Galewski et al. 2006; Jaarola et al. 2004; Lessa and Cook

1 1998). In the present case, the internal branches not supported appear not significantly short
2 that suggests a lack of phylogenetic resolution rather than fast lineage differentiations.

3 4 **Origin of the diversification**

5 From the fossil record, the evolutionary history of the genus *Microtus*, and thus of the
6 subgenus *Terricola*, seems rooted in the Quaternary because the oldest fossil remains
7 attributed to the ancestor of *Microtus* (*i. e.* *Allophaiomys*) are from the Early Pleistocene (2.4
8 - 2 Myr; Chaline and Graf 1988; Chaline et al. 1999; Zheng and Zhang 2000). However, our
9 molecular dating and ancestral biogeographic reconstruction place the origin of *Terricola* at
10 the end of the Early Pliocene (\approx 4 Myr) in a region including the Caucasus, Turkey and Iran
11 (CTI; Fig. 3). Indeed, this subgenus shares a common ancestor with the subgenus *Microtus*
12 (Jaarola et al. 2004; Martínková and Moravec 2012; Robovský et al. 2008) of which most
13 species are distributed in Eastern Europe, Asia Minor and Western Central Asia, including the
14 CTI area (Musser and Carleton 2005; Shenbrot and Krasnov 2005). In this region, the
15 orogenic activity linked to the collision between the Arabian and Eurasian plates was at its
16 acme during the late Miocene-Pliocene and it was accompanied by a rapid mountain uplift
17 that could potentially lead to the isolation of ancestral *Terricola* populations (Mosar et al.
18 2010; Ruban et al. 2007). A vicariant event (\approx 3.6 Myr) seems then at the origin of the split
19 between two ancestral lineages: one including the species of the *subterraneus* complex and
20 the other leading to all endemic European species. After 3 Myr, the progressive global
21 cooling leading to a more suitable composition of the vegetation (from sub-tropical forested
22 environments to temperate broad-leaved deciduous or coniferous forests and boreal
23 vegetation; Fauquette and Bertini 2003; Pross and Klotz 2002; Thompson and Fleming, 1996;
24 Willis et al. 1999) for *Terricola* species would have allowed the colonization of Europe
25 westwards.

26 From the Pleistocene climatic records, glacial conditions dominated between the
27 Praetiglian (2.6 – 2.4 Myr) to the Menapian (1.20 – 1.07 Myr) stages (Cohen and Gibbard
28 2012; De Jong 1988; Vandenberghe 2001). They should be at the origin of the differentiation
29 of ancestral evolutionary lineages in Mediterranean peninsulas (Italy, the Balkans and the
30 Iberian Peninsula; Fig. 3). Indeed, these Mediterranean areas are often considered as glacial
31 refugia and sources of northwards postglacial colonization (Hewitt 1996, 2000, 2004;
32 Taberlet et al. 1998) or as a hotspot of endemism for small mammals (Bilton et al. 1998). In
33 both cases, geographic isolation of small populations in these peninsulas during Pleistocene
34 glaciations could have served as “speciation traps”, thus promoting allopatric speciation

1 events (Chaline 1987). This was notably exemplified by the *in situ* differentiation of the *savii*
2 or *multiplex* complex ancestral lineages in Italy or in the Alps, respectively (Fig. 3). Even if
3 Italy is recognized as a potential glacial refugium, few Italian populations would have
4 participated to postglacial (re)colonization of northern Europe because of the Alps (Hewitt
5 2000; Taberlet et al. 1998), although the Alps are considered either as a barrier to postglacial
6 expansion as for the *savii* complex species or as a glacial refugium (in southwestern,
7 southeastern and/or northern marginal areas) as for the *multiplex* complex species (Haring et
8 al. 2000; Hewitt 2000, 2004; Schmitt and Varga 2012).

9 From the present molecular dating, most extant *Terricola* species have their origin
10 after the end of the Cromerian interglacial (≈ 0.5 Myr; Fig. 3), *i. e.* during a period
11 characterized by the development of regular and vast North European ice sheets and mountain
12 caps (Böse et al. 2012). This results combined with the evolutionary hypotheses emitted on
13 the ancestral lineages, the knowledge on the origin and present-day restricted geographic
14 distribution (Fig. 1) of these *Terricola* species suggests that their speciation occurred after a
15 long-term isolation in Mediterranean (Italy, the Balkans and the Iberian Peninsula including
16 Southern France) and extra-Mediterranean (marginal areas of the Alps and the Carpathians,
17 and the Caucasus) glacial refugia (Hewitt 1996, 2000, 2004; Schmitt and Varga 2012;
18 Taberlet et al. 1998) according to an allopatric model. Only *M. subterraneus* and *M.*
19 *daghestanicus* seem to have an older origin (respectively, 0.79 and 0.84 Myr). They probably
20 evolved *in situ* as suggested by our reconstruction of the biogeographic ancestral areas (Fig.
21 3) and by Baskevich (1997) for *M. daghestanicus*. As mentioned previously, *M. arvalidens* is
22 considered as being the ancestor of *M. subterraneus*. These two species, the former extinct
23 and the latter extant, were and are the only widespread *Terricola* species in Europe with a
24 nearly similar geographic distribution. Moreover, the distribution of *M. subterraneus* seems
25 limited by the interspecific competition (Quéré and Le Louarn 2011), and, for this reason, it
26 could have colonized Europe from the CTI area by replacing *M. arvalidens* by competitive
27 exclusion (Krause 1986; Futuyma 1997).

28 Discrepancies exist between molecules and fossils on the origin of the subgenus
29 *Terricola* and *Terricola* species. Indeed, the fossil record documents at best the first
30 appearance of a morphologically recognizable group (*Allophaiomys* or *Terricola*
31 morphotypes) and not the time when species became genetically isolated (Douzery et al.
32 2004; Yang 2014). In a way, this situation is comparable with what it is observed with the
33 cryptic species: they are morphologically similar but genetically distinct, and for this reason,

1 they can have diverged thousand to millions of years ago (for instance, Hulva et al. 2004;
2 Irwin et al. 2001; Šlapeta et al. 2006; Tougaard et al. 2013).

3

4 **Rate of diversification**

5 The Quaternary climatic fluctuations, and especially the glacial periods, are thought to have
6 an impact on species diversity and distribution of plants and animals. In the present context,
7 most extinct *Terricola* species originated from the Cromerian interglacial or other later and
8 shorter interglacials but it seems, from the fossil record, they did not survived these periods
9 (Brunet-Lecomte 1989; Cuenca- Bescós et al. 2010; Gil 1996; Kowalski 2001). According to
10 several authors (Dalén et al. 2007; Hewitt 1996; Provan and Bennett 2008; Stewart et al.
11 2010), populations, with a dispersal ability that does not allow to track retreating habitats
12 when climatic conditions became harsher, went extinct. There is no evidence (no fossil
13 remain) allowing to say that the extinct *Terricola* species, mostly endemic to some restricted
14 continental or insular localities, migrated southwards when colder climatic conditions
15 occurred nor if there is an ancestor-descendant relation between some of these extinct and
16 extant *Terricola* species, except for *M. arvalidens* and *M. subterraneus*. On the other hand,
17 most extant *Terricola* species found their origin during glacial periods because of a long-term
18 divergence of ancestral lineages in Mediterranean and extra-Mediterranean glacial refugia
19 thus seen as allopatric speciation traps (Chaline 1987; Haring et al. 2011; Hewitt 2004;
20 Martínková and Moravec 2012). All the extant *Terricola* species have a relatively recent
21 origin (between 0.84 and 0.08 Myr; Fig. 3), while the “lifespan” for some extinct *Terricola*
22 species is estimated between 0.2 and 0.4 Myr (Brunet-Lecomte 1988). According to Avise et
23 al. (1998), speciation in mammals requires a time frame of 2.2 ± 1.0 Myr, and thus the fossil
24 record and our molecular dating could suggest that the time frame may be too short in voles to
25 achieve complete speciation. Chaline et al. (1999) considered however that the speciation
26 duration in vole species is relatively short, from 0.3 to less than 1.5 Myr, compared to other
27 mammal species.

28 From the present study, it seems that the Pleistocene glacial conditions had an impact
29 on the speciation and extinction events, but does that mean that the rate of diversification
30 during the Quaternary was heightened? The LTT plot (Fig. 4) underlined several shifts in the
31 *Terricola* diversification rate with two phases of increase (between 3 and 2 Myr and after 1
32 Myr), more or less corresponding to periods dominated by cool or cold climate conditions.
33 The estimated rate of diversification for the *Terricola* species was 0.353 ± 0.004 events / Myr.
34 Diversification rates were also estimated for numerous plant and animal groups but rarely at

1 the subgenus level. In rodents, several shifts in diversification rates were identified (Steppan
2 et al. 2004; Fabre et al. 2012). Seven of these shifts were notably underlined in the Cricetidae
3 (Fabre et al. 2012). Unfortunately, no study focused specifically on diversification rates of
4 this rodent family. With 1.36 events / Myr, the cricetid *Akodon* of the subfamily
5 Sigmodontinae presents a higher diversification rate than the subgenus *Terricola* (Leite et al.
6 2014), whereas *Akodon* originated at the beginning of the Pleistocene (between 2.65 and 2
7 Myr; Smith and Patton 2007). It thus presents a diversification rate among the highest
8 reported for vertebrates, that is to say four times higher than that of the subgenus *Terricola*.
9 This rate for *Terricola* is rather comparable to the average diversification rate of another
10 rodent family, the Muridae (around 0.36 events / Myr; Stanley 1998). Consequently, the
11 Quaternary does not appear as a period with an exceptional rate of diversification compared to
12 other animal groups, although some periods in the Quaternary appear more favourable for the
13 diversification of this subgenus.

14

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30

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30

31 **Figure Legends**

32

1 Figure 1. Map showing the geographic distribution (1) of the *Microtus (Terricola)* species
2 according to the IUCN Red List, and the sampling localities (2) for the specimens used in the
3 present study. Details about the localities and/or the samples are in Table 1.

4

5 Figure 2. Maximum-likelihood tree topology reconstructed from cytochrome *b* gene
6 sequences of *Microtus (Terricola)* species as well as *M. (Microtus) arvalis* and *M. (M.) levis*
7 used as outgroup. Numbers at node are for maximum-likelihood bootstrap values (BP \geq 50%)
8 and Bayesian posterior probabilities (PP \geq 0.85). Black crosses indicate nodes with BP =
9 100% and PP = 1.00, while grey crosses are for nodes with BP < 50% and PP = < 0.85. The
10 species names are indicated on the right and are followed by the symbols used in Fig. 1.
11 Details about specimen numbers are given in Table 1.

12

13 Figure 3. Chronogram showing the divergence time estimates and the reconstruction of the
14 biogeographic ancestral areas. Values in bold and under species names (at right) reflect the
15 time to the most recent common ancestor and, in brackets, the 95% confidence interval, while
16 letters (biogeographic areas) and values (occurrence frequencies) in regular are related to the
17 biogeographic ancestral area analysis.

18

19 Figure 4. Lineages through time plot of all the *Microtus* species (in- and outgroup included)
20 taken into account in the present study.

21

22 **List of supporting information**

23

24 Table S1. Genetic distance within and between *Microtus (Terricola)* species and the
25 outgroup.

26

1 Tables

2 Table 1. List of taxa used in the present analyses. Systematic arrangement is from Musser and
 3 Carleton (2005). Sample localities, GenBank accession numbers and author references are
 4 indicated for each cytochrome *b* gene sequence. Locality numbers are for sample numbers of
 5 the Fig. 2. Letters in regular are for published data (see the footnote below the table for
 6 details), whereas letters in bold are the initials of the tissue providers (see details in
 7 acknowledgements).

8

Species (Common Name)	Sample Locality	Accession Number	Source
Order Rodentia			
Family Cricetidae			
Subfamily Arvicolinae			
Genus <i>Microtus</i>			
Subgenus <i>Microtus</i>			
<i>M. arvalis</i> (Common vole)	1	AM991045	I ^a
	2	AM991075	I
	3	AY220766	D
	4	AY220788	D
<i>M. levis</i> (East European vole)	1	DQ015676	J
	2	AY513820	E
<i>M. tatricus</i> (Tatra vole)	1. Tretie Rohácske pleso Lake, Western Tatra Mts, Slovakia	LT222298	NM
	2. Tretie Rohácske pleso Lake, Western Tatra Mts, Slovakia	AY513837	E
	3. Smutná dolina Valley, Western Tatra Mts, Slovakia	AY513838	E
	4. Velka studena dolina Valley, Slovakia	AY513839 DQ841699	E F
	5. Rakytovská dolina Valley, Veľká Fatra Mts, Slovakia	DQ841700	F
	6. Dolný Harmanec, Veľká Fatra Mts, Slovakia		
Subgenus <i>Terricola</i>			
<i>M. (T.) bavaricus</i> (Bavarian pine vole)	1. Steinberg am Rofan, Northern Tyrol, Austria	LT222299 DQ841693	NM F
	2. Steinberg am Rofan, Northern Tyrol, Austria	DQ841694 GQ243218	F K
	3. Garmisch-Partenkirchen, Bavaria, Germany		
	4. Papuk Mountain, Croatia		
<i>M. (T.) brachycercus</i> (Calabria pine vole)	1. Fiumefreddo, Sicily, Italy	AY513827	E
	2. Lago di Cecita, Calabria, Italy	EU158784	B
	3. Muro Lucano, Basilicata, Italy	EU158785	B
	4. Pollinello, Calabria, Italy	EU158787	B
	5. Muro Lucano, Basilicata, Italy	EU158776	B
<i>M. (T.) daghestanicus</i> (Caucasus pine vole)	1. Cew Valley, Central Caucasus, Russia	LT222300 AY513790	FC E
	2. Beniani, Georgia	AY513791	E

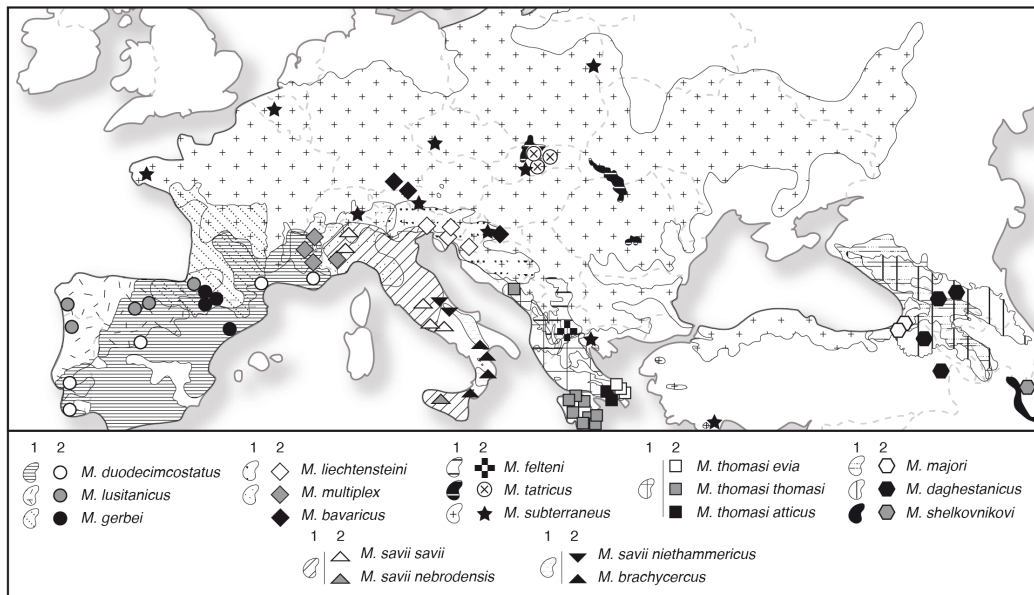
	3. Bagdasan, Turkey	AY513792	E
	4. Handere, Turkey		
<i>M. (T.) duodecimcostatus</i> (Mediterranean pine vole)	1. Jardin des Gardes, Hautes-Alpes, France	LT222301 AJ717744	JPQ I
	2. Montferrier, Hérault, France	AY513796	E
	3. Setubal, Portugal	AY513797	E
	4. Algarve, Portugal	JX424211	A
	5. Segovia, Spain		
<i>M. (T.) felteni</i> (Balkan pine vole)	1. Mt Pelister, Begova Cesma, Macedonia	AY513798	E
	2	DQ663661	C
<i>M. (T.) gerbei</i> (Pyrenean pine vole)	1. Vall d'Aran, Catalogna, Spain	AJ717748	I
	2. Armendarits, Pays Basque, France	LT222302	JPQ
	3. Vall d'Aran, Catalogna, Spain	AY513799	E
	4. Riba, Spain	AY513800	E
	5. Hecho, Spain	AY513801	E
<i>M. (T.) liechtensteini</i> (Liechtenstein's pine vole)	1. Brikini, Slovenia	LT222303	FS
	2. Campo di Mezzo, Pian del Cansiglio, Veneto, Italy	LT222304	LN
	3. Anhovo, Slovenia	AY513811	E
	4. Croatia	EF379100	F
<i>M. (T.) lusitanicus</i> (Lusitanian pine vole)	1. Saint-Pée-sur-Nivelle, Pays Basque, France	AJ717746 LT222305	I JPQ
	2. Saint-Pée-sur-Nivelle, Pays Basque, France	AY513812 AY513813	E E
	3. Burgos, Spain	JX424207	A
	4. Melgar de Fernamental, Spain	JX424208	A
	5. Rebordelo, Portugal		
	6. Grijó da Para, Portugal		
<i>M. (T.) majori</i> (Major's pine vole)	1. Damar, Turkey	AY513814	E
	2. Damar, Turkey	DQ841703	F
	3. Hopa, Turkey	DQ841704	F
<i>M. (T.) multiplex</i> (Alpine pine vole)	1. Saint Jean de Vaulx, Isère, France	LT222306	PBL
	2. Carmagnola, Piedmont, Italy	LT222307	GB
	3. Saint Martin de la Cluze, Isère, France	AJ717747 AY513817	I E
	4. Lillaz, Italy	AY513818	E
	5. Méribel, France		
<i>M. (T.) savii</i> (Savi's pine vole)	6. Palumbara, Sabina, Latium, Italy	LT222308	NM
<i>M. (T.) s. savii</i>	7. Viterbo, Lazio, Italy	AY513824	E
	8. Torino, Piedmont, Italy	AY513825	E
	9. Cerano, Piedmont, Italy	AY513826	E
	10. Velletri, Italy	EU158777	B
	11. Circeo, Italy	EU158778	B
	12. Capracotta, Molise, Italy	EU158780	B
<i>M. (T.) s. niethammericus</i>	13. Capracotta, Molise, Italy	EU158781	B
	14. Capracotta, Molise, Italy	EU158782	B
	15. Farindola, Italy	EU158790	B
	16. Farindola, Italy	EU158791	B

<i>M. (T.) s. nebrodensis</i>	17. Ficuzza, Sicily, Italy	EU158802	B	
	18. Ficuzza, Sicily, Italy	EU158804	B	
	19. Ficuzza, Sicily, Italy	EU158803	B	
	20. Ficuzza, Sicily, Italy	EU158779	B	
<i>M. (T.) schelkovnikovi</i> (Schelkovnikov's pine vole)	Talysh, Hyrkanian reserve, Azerbaijan	LT222309	FC	
<i>M. (T.) subterraneus</i> (Common pine vole)	1. Val Piora Ticino, Switzerland	AJ717745	I	
	2. Úzka dolina Valley, Western Tatra Mts., Slovakia	LT222310	NM	
	3. Tournay, Finistère, France	LT222311	JPQ	
	4. Seli, Greece	AY513832	E	
	5. Glocknerhaus, Austria	AY513833	E	
	6. Ciglikara, Turkey	AY513834	E	
	7. Nova Kapela, Croatia	FR869858	G	
	8. Brussels, Waterloo, Belgium	FR869862	G	
	9. Kasperske hory Mts, Czech Republic	FR869878	G	
	10. Bialowieza, Poland	FR869884	G	
<i>M. (T.) thomasi</i> (Thomas's pine vole)	1. Trebinje, Bosnia and Herzegovina	LT222312	NM	
<i>M. (T.) t. thomasi</i>	2. Kalavryta, Greece	JN019756	H	
	3. Kyparissia, Greece	AY513842	E	
	4. Trebinje, Bosnia and Herzegovina	AY513844	E	
	5. Ano Kastritsi, Greece	JN019765	H	
	6. Ano Kastritsi, Greece	JN019766	H	
	7. Vrodamas, Greece	JN019773	H	
	8. Strofylia, Greece	JN019775	H	
	9. Peleta, Greece	JN019778	H	
	10. Strofylia, Greece	JN019780	H	
	11. Aigies, Greece	JN019762	H	
	12. Voutianoï, Greece	JN019763	H	
	13. Agios Stefanos, Greece	AY513840	E	
	14. Afidnes, Greece	JN019760	H	
	<i>M. (T.) t. atticus</i>	15. Afidnes, Greece	JN019761	H
		16. Agios Stefanos, Greece	JN019767	H
		17. Eretria, Greece	JN019764	H
	<i>M. (T.) t. evia</i>	18. Vasiliko, Greece	JN019768	H
		19. Kimassi, Greece	JN019771	H

1 ^a A: Bastos-Silveira et al. (2012); B: Castiglia et al. (2008); C: Fink et al. (2006); D: Haynes et
2 al. (2003); E: Jaarola et al. (2004); F: Martínková et al. (2007); G: Martínková et al. (unpubl.);
3 H: Rovastos and Giagia-Athanasopoulou (2012); I: Tougard et al. (2008b); J: Triant and
4 DeWoody (2006); K: Tvrtkovic et al. (2010).

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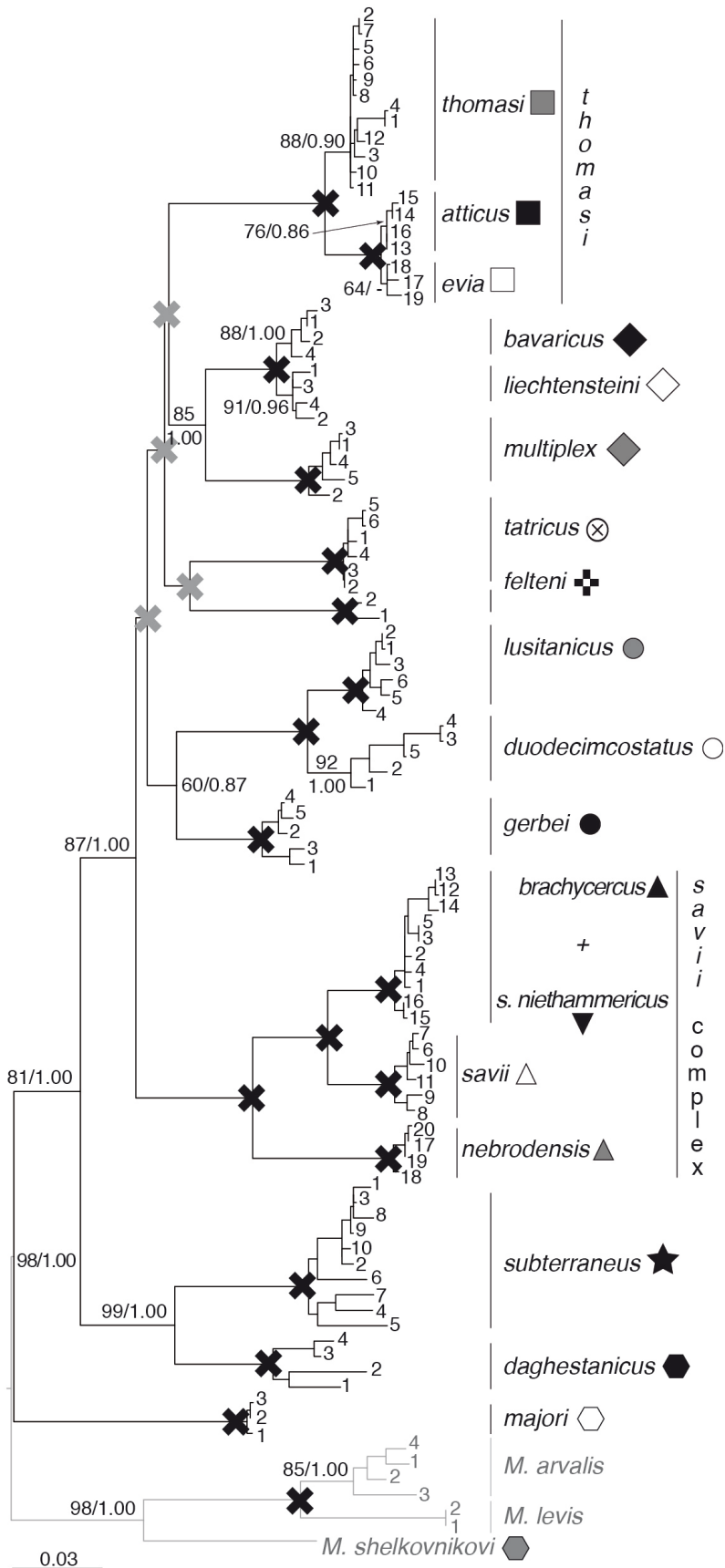
1 Fig. 1



2

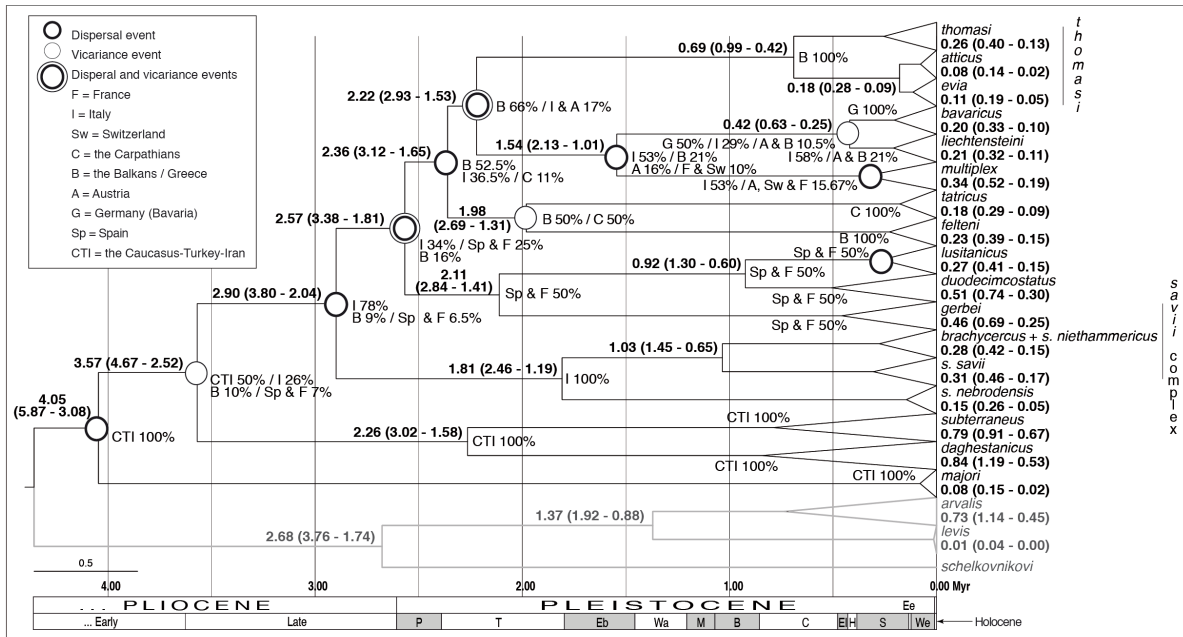
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1 Fig. 2



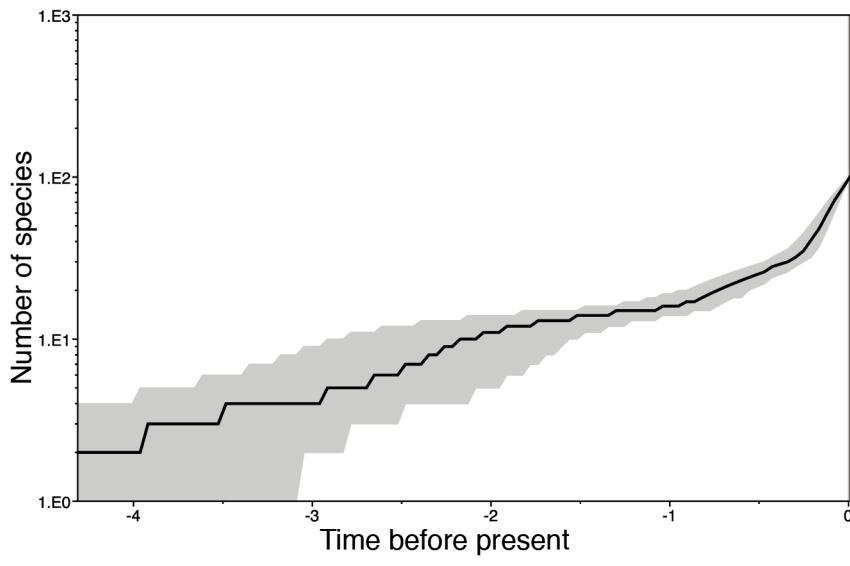
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1 Fig. 3



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1 Fig. 4



2

Tableau S1 of C. TOUGARD, Did the Quaternary climatic fluctuations really influence the tempo and mode of diversification in European rodents?

Genetic distance within and between *Microtus (Terricola)* species and the outgroup

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
1. <i>majori</i>	0.002 (0.001)^a	0,015 ^c	0,015	0,016	0,017	0,016	0,014	0,013	0,013	0,013	0,014	0,015	0,016	0,016	0,014	0,013	0,013	0,012	
2. <i>daghestanicus</i>	0,103 ^b	0.028 (0.004)	0,010	0,014	0,015	0,015	0,012	0,014	0,014	0,012	0,012	0,013	0,013	0,013	0,012	0,012	0,012	0,013	
3. <i>subterraneus</i>	0,106	0,080	0.022 (0.003)	0,014	0,015	0,014	0,013	0,014	0,014	0,013	0,013	0,013	0,013	0,013	0,010	0,012	0,011	0,013	
savii group	4. <i>s. savii</i>	0,113	0,118	0,115	0.011 (0.002)	0,007	0,012	0,013	0,012	0,011	0,012	0,013	0,013	0,014	0,015	0,013	0,010	0,012	0,015
	5. <i>brachycercus</i> + <i>s. niethamericus</i>	0,125	0,126	0,120	0,046	0.011 (0.002)	0,011	0,013	0,013	0,013	0,013	0,013	0,013	0,015	0,015	0,014	0,012	0,013	0,016
	6. <i>nebrodensis</i>	0,121	0,124	0,112	0,071	0,077	0.005 (0.002)	0,015	0,014	0,013	0,014	0,012	0,013	0,014	0,014	0,013	0,013	0,014	0,015
7. <i>gerbei</i>	0,082	0,093	0,094	0,090	0,095	0,106	0.013 (0.003)	0,011	0,012	0,011	0,012	0,012	0,012	0,012	0,011	0,008	0,009	0,012	
8. <i>duodecimcostatus</i>	0,088	0,113	0,112	0,095	0,101	0,109	0,080	0.025 (0.005)	0,007	0,012	0,012	0,013	0,014	0,014	0,011	0,012	0,012	0,014	
9. <i>lusitanicus</i>	0,084	0,112	0,105	0,087	0,102	0,102	0,079	0,049	0.009 (0.002)	0,012	0,012	0,012	0,013	0,014	0,011	0,011	0,012	0,014	
10. <i>felteni</i>	0,095	0,094	0,094	0,096	0,097	0,109	0,071	0,084	0,079	0.010 (0.004)	0,012	0,011	0,011	0,012	0,011	0,010	0,009	0,013	
11. <i>tatricus</i>	0,097	0,104	0,104	0,099	0,106	0,101	0,075	0,097	0,092	0,079	0.005 (0.002)	0,013	0,013	0,014	0,011	0,010	0,011	0,013	
thomasi	12. <i>thomasi</i>	0,106	0,105	0,099	0,090	0,094	0,094	0,076	0,101	0,089	0,077	0,090	0.008 (0.002)	0,007	0,007	0,012	0,011	0,011	0,012
	13. <i>atticus</i>	0,100	0,098	0,096	0,090	0,093	0,094	0,073	0,091	0,090	0,071	0,080	0,030	0.001 (0.001)	0,003	0,012	0,013	0,013	0,014
	14. <i>evia</i>	0,099	0,095	0,094	0,097	0,100	0,102	0,071	0,094	0,094	0,074	0,088	0,034	0,008	0.005 (0.002)	0,012	0,013	0,013	0,013
15. <i>multiplex</i>	0,085	0,090	0,086	0,091	0,099	0,102	0,071	0,079	0,074	0,071	0,075	0,078	0,078	0,080	0.009 (0.002)	0,009	0,010	0,013	
16. <i>liechtensteini</i>	0,081	0,097	0,087	0,082	0,093	0,094	0,057	0,073	0,072	0,072	0,076	0,073	0,078	0,079	0,056	0.009 (0.003)	0,004	0,013	
17. <i>bavaricus</i>	0,086	0,089	0,080	0,088	0,096	0,099	0,059	0,077	0,075	0,065	0,079	0,072	0,082	0,083	0,057	0,021	0.008 (0.002)	0,013	
18. outgroup	0,101	0,115	0,116	0,138	0,147	0,146	0,102	0,119	0,115	0,112	0,107	0,109	0,112	0,113	0,097	0,108	0,101	0.047 (0.006)	

^a Genetic distance within species (standard error)

^b Genetic distance between lineages (below the diagonal)

^c Standard error (above the diagonal)