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# Molecular systematics of dormice (Rodentia: Gliridae) and the radiation of *Graphiurus* in Africa

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The phylogenetic relationships among the Gliridae (order Rodentia) were assessed using 3430 nucleotides derived from three nuclear fragments (β-spectrin non-erythrocytic 1, thyrotropin and lecithin cholesterol acyl transferase) and one mitochondrial gene (12S rRNA). We included 14 glirid species, representative of seven genera of the three recognized subfamilies (Graphiurinae, Glirinae and Leithiinae) in our analysis. The molecular data identified three evolutionary lineages that broadly correspond to the three extant subfamilies. However, the data suggest that the genus *Muscardinus*, previously regarded as falling within the Glirinae, should be included in the Leithiinae. Molecular dating using local molecular clocks and partitioned datasets allowed an estimate of the timing of cladogenesis within the glirids. *Graphiurus* probably diverged early in the group's evolution (40–50 Myr ago) and the three subfamilies diverged contemporaneously, probably in Europe. The radiation within *Graphiurus* is more recent, with the colonization of Africa by this lineage estimated at *ca*. 8–10 Myr ago.

**Keywords:** Gliridae; phylogeny; molecular clock; taxonomy; nuclear DNA; mitochondrial DNA

## 1. INTRODUCTION

The family Gliridae includes 27 extant dormice species (Holden 1993) distributed among three subfamilies: the Glirinae, comprising *Glis*, *Glirulus* and *Muscardinus*, the Leithiinae, which is subdivided into four genera, *Dryomys*, *Eliomys*, *Myomimus* and *Selevinia*, and the monotypic Graphiurinae containing *Graphiurus*. Recently, the taxonomy has been expanded to include a new genus, *Chaetocauda* (Wang 1985), although *C. sichuanensis* is considered to be nested within the genus *Dryomys* by Holden (1993). Uncertainty also characterizes the positions of the genera *Graphiurus* and *Selevinia*. On the basis of a morpho-palaeontological interpretation, Vianey-Liaud & Jaeger (1996) proposed *Graphiurus* to be closely related to representatives of the Anomaluridae. The genus *Selevinia*, in turn, is sometimes placed in its own family, the Seleveniidae (see Bashanov & Belosludov 1941). However, owing to its endangered status and restricted distribution (the species is endemic to the area around Lake Balkhash, eastern Kazakhstan), none of the recent morphological or molecular studies has included this species (see Wahlert *et al*. 1993; Daams & De Bruijn 1995), and consequently support for the inclusion of *Selevinia* within the Gliridae is largely anecdotal (Holden 1993). However, the inclusion of *Graphiurus* within the Gliridae is strongly underpinned by molecular evidence (Bentz & Montgelard 1999; Montgelard *et al.* 2002). Members of the Glirinae and Leithiinae are found in the palaearctic and central and southwestern Asia, as well as in Japan (*Glirulus japonicus*) and North Africa (*Eliomys melanurus*). Within the Gliridae, however, *Graphiurus* is unique in having a strictly African distribution that extends from south of the Sahara to the Cape Province of South Africa. In comparison with other glirids, *Graphiurus* is also the most species rich genus (14 species are currently described, whereas other glirid genera consist of a maximum of three species; Holden 1993).

However, the rather low glirid species diversity contrasts sharply with the fossil record, where more than 30 extinct genera have been described since the Eocene (Daams & De Bruijn 1995). This leads to the view that extant dormice represent a relict rodent group that is probably in decline (Hartenberger 1994; Daams & De Bruijn 1995), *Graphiurus* being the only apparent exception. The earliest records date back to the Lower Eocene (50 Myr ago) with the family representing a derived offshoot of the fossil ischyromyoids (Hartenberger 1998). The two fossil genera, *Eogliravus* and *Gliravus* (subfamily Gliravinae according to Daams & De Bruijn (1995)), are dated from the Lower and Upper Eocene, respectively, and are thought to represent the primitive glirids that gave rise to the modern forms. The first evidence of extant genera is from the Upper Oligocene for *Glis* (*ca*. 28 Myr ago) and the Lower Miocene for *Glirulus* and *Myomimus* (*ca*. 24 Myr ago), whereas the first appearances of *Muscardinus*, *Eliomys* and *Dryomys* date from the Middle Miocene (*ca*. 18 Myr ago; Daams & De Bruijn 1995; Daams 1999). Most fossils have been recorded in Europe, suggesting a European origin for the family. By contrast, the earliest known *Graphiurus* is described in the Pliocene of South Africa (Pocock 1976; Hendey 1981). The marked differences in the ages of the fossils and the absence of chronological transitional forms between *Graphiurus* and the European taxa render the evolution of the genus problematic and the timing of the African graphiurine radiation controversial (Bachmayer & Wilson 1980; Hartenberger 1994; Daams & De Bruijn 1995).

The aims of this study were twofold. First, by studying the sequence relationships among genera representative of

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Table 1. Samples included in this study.

| genus | species | family or subfamily | specimen number[a] | geographical origin | collector/donator |
|---|---|---|---|---|---|
| *Glis* | *glis* | Glirinae | T-1453 | Switzerland | A. Meylan |
| *Glirulus* | *japonicus* | Glirinae | T-1715 | Japan | H. Suzuki |
| *Muscardinus* | *avellanarius* | Glirinae | T-1451 | Switzerland | P. Vogel |
| *Dryomys* | *laniger* | Leithiinae | T-2132 | Turkey | C. Kurtonur |
| *Dryomys* | *nitedula* | Leithiinae | T-768 | Georgia | M. Baskevitch |
| *Eliomys* | *quercinus* | Leithiinae | T-361 | Switzerland | F. Catzeflis |
| *Eliomys* | *melanurus* | Leithiinae | T-1271 | Israel | D. Graur |
| *Myomimus* | *roachi* | Leithiinae | T-2131 | Turkey | C. Kurtonur |
| *Graphiurus* | *murinus* | Graphiurinae | SUN 754 | South Africa | T. Robinson |
| *Graphiurus* | *microtis* | Graphiurinae | 4471 | Tanzania | E. Verheyen |
| *Graphiurus* | *platyops* | Graphiurinae | 46377 | South Africa | D. MacFayden |
| *Graphiurus* | *ocularis* | Graphiurinae | DM3281 | South Africa | P. Taylor |
| *Graphiurus* | *parvus* | Graphiurinae | 158120 | Tanzania | B. Stanley |
| *Graphiurus* | *lorraineus* | Graphiurinae | SMF 11476 | Cameroon | H. Burda |
| *Sciurus* or *Tamiasciurus* | *aestuans* | Sciuridae | T-1484 | French Guiana | J. C. Vié |
| | sp. | Sciuridae | 5813 | USA | R. Honeycutt |
| *Glaucomys* | *volans* | Sciuridae | T-1110 | USA | Zink, Reynolds |
| *Glaucomys* | sp. | Sciuridae | 4660 | USA | R. Honeycutt |
| *Aplodontia* | *rufa* | Aplodontidae | T-1458 | USA | D. Nolte |

[a] T-prefixed numbers are those from the collection of mammalian tissue of Montpellier (Catzeflis 1991); other numbers are references from collectors or donators.

the three glirid subfamilies we hoped to resolve some of the conflicting evolutionary relationships suggested by earlier morphological, palaeontological (e.g. Wahlert *et al.* 1993; Daams & De Bruijn 1995) and molecular studies (Bentz & Montgelard 1999). In the molecular studies, the sequence analysis of two mitochondrial genes from six glirid genera strongly supported a monophyletic Leithiinae clade (consisting of *Dryomys* and *Eliomys*) but failed to resolve relationships between other members of the family. Herein we extend this analysis to include sequences derived from three nuclear fragments (β-spectrin non-erythrocytic 1 (SPTBN), thyrotropin (TH) and lecithin cholesterol acyl transferase (LCAT)) and one mitochondrial gene (12S rRNA) from a more comprehensive glirid taxonomic sample. Second, we were particularly interested in producing accurate dates for the different glirid divergences by using partitioned data and local molecular-clock models. Based on the interpretation of these data we propose a hypothesis to explain the patterns of colonization of Africa, and the subsequent diversification of *Graphiurus* through a process of adaptive radiation.

## 2. MATERIAL AND METHODS

### (a) *Samples and DNA sequencing*

Our study includes 14 species of the Gliridae, which, with the exception of *Selevinia*, comprises representatives of all glirid genera (table 1). The more species rich *Graphiurus* is represented by six out of the 14 species described by Holden (1993). Two genera of the Sciuridae (*Sciurus* or *Tamiasciurus* and *Glaucomys*) and one of the Aplodontidae (*Aplodontia rufa*) have been used as outgroups because they are thought to be the closest relatives of the Gliridae (Huchon *et al.* 2002; Montgelard *et al.* 2002). The present study contributed 51 new sequences, which have been deposited with the EMBL data bank (accession numbers AJ536348–AJ536398). Although the dataset consists of sequences from three nuclear fragments (SPTBN, TH and LCAT) and one mitochondrial gene (12S rRNA) from each specimen analysed, the *Graphiurus lorraineus* DNA was badly degraded and we failed to produce sequence from two (SPTBN and TH) out of the three nuclear genes screened.

DNA sequencing was carried out from PCR products. The nuclear SPTBN and TH fragments were amplified using primers published by Matthee *et al.* (2001): FA and RA for SPTBN and FA and RB for TH. These fragments consist largely of introns. Exons 2–5, including the LCAT gene introns, were amplified using primers U221 and L666 (Robinson *et al.* 1997), whereas R1 and S2 (Douzery & Catzeflis 1995) were used to amplify the 12S rRNA mitochondrial gene. Standard PCR cycling procedures were followed (see Douzery & Catzeflis 1995; Matthee *et al.* 2001).

PCR products were purified from 1% TAE agarose gels using Amicon UltrafreeDNA columns (Millipore) or the NucleoSpin Kit (Macherey–Nagel). These products were cycle sequenced and analysed on an ABI 3100 automatic sequencer using BigDye Terminator chemistry.

### (b) *Sequence alignment and saturation analysis*

Sequences were aligned manually using the ED editor of the program MUST (Philippe 1993). No indels were detected in the exonic regions of the three nuclear genes, and gaps were introduced only in introns. For the mitochondrial 12S rRNA gene, indels were introduced in loops using the model of Springer & Douzery (1996). Saturation of nucleotide substitutions was evaluated graphically according to the procedure of Philippe & Forterre (1999). This procedure is based on plotting the number of substitutions inferred by maximum-likelihood (ML) analysis (GTR + Γ8 distance) against the observed distance (*p*-distance) between each pair of taxa. This was performed using the programs TREEPLOT and COMP_MAT as

Table 2. Characteristics of the four genes under study and parameters of the best model of evolution given by MODELTEST.

| | SPTBN | TH | LCAT | 12S | total |
|---|---|---|---|---|---|
| total length | 1332 | 985 | 701 | 1007 | 4025 |
| excluded | 290 | 250 | 0 | 55 | 595 |
| exons or stems | 68 | 268 | 410 | 457 | 746 |
| introns or loops | 974 | 467 | 291 | 495 | 2684 |
| best model | TrN + $\Gamma$ | TVM + $\Gamma$ | TrN $\Gamma$ | GTR + $\Gamma$ | GTR + $\Gamma$ + $I$ |
| A | 0.25 | 0.30 | 0.19 | 0.36 | 0.28 |
| C | 0.22 | 0.20 | 0.28 | 0.21 | 0.23 |
| G | 0.22 | 0.17 | 0.28 | 0.17 | 0.21 |
| T | 0.31 | 0.33 | 0.25 | 0.26 | 0.28 |
| AC | 1 | 1.206 | 1 | 8.746 | 1.794 |
| AG | 4.238 | 5.219 | 3.754 | 19.622 | 4.931 |
| AT | 1 | 0.563 | 1 | 13.085 | 1.178 |
| CG | 1 | 1.201 | 1 | 1.0345 | 1.08 |
| CT | 3.162 | 5.219 | 5.847 | 53.214 | 7.173 |
| GT | 1 | 1 | 1 | 1 | 1 |
| alpha | 2.347 | 1.234 | 0.688 | 0.246 | 0.731 |
| invariable | — | — | — | — | 0.172 |

implemented in MUST (Philippe 1993). The slope of the regression between the inferred and observed substitutions gives an inverse evaluation of the saturation level (i.e. the steeper the slope, the lower the level of saturation).

### (c) *Phylogenetic analysis*

Phylogenetic trees were reconstructed using maximum parsimony (MP), ML and Bayesian inference (BI). MP reconstructions were performed in PAUP (Swofford 1999) using equal weighting of characters with the random stepwise addition of taxa (10 replications) and tree bissection–reconnection branch swapping. Prior to ML phylogenetic reconstruction, the program MODELTEST v. 3.06 (Posada & Crandall 1998) was used to determine the best fitting model of sequence evolution for each gene separately and in combination (see table 2). ML analysis using the model and the parameters suggested by MODELTEST was performed in PAUP. Gaps were included and treated as missing characters in both the MP and ML analyses. The reliability of nodes was tested by bootstrap percentage (BP) after 1000 replications.

To use the information provided by insertion–deletion events, trees were constructed using the program MAC5 (McGuire & Agapow 2001; McGuire *et al.* 2001), which relies on Bayesian analysis of the *a posteriori* probability of a tree being accepted once given the data (Huelsenbeck *et al.* 2001). With this program, gaps are treated as a fifth character with the model F84, which considers two different substitution rates for transversions and transitions, as well as unequal base frequencies. The Markov chain Monte Carlo algorithm was run using the default settings (and repeated more than once for stability).

Alternative topologies to that portrayed in the optimal ML tree were tested using the Shimodaira & Hasegawa (1999) test implemented in the PAML software (Yang 1997). Several gene partitions were tested and log-likelihood scores were compared under different models using independent parameters for the different partitions tested (option G with Mgene = 4; that is separate analysis of the different partitions) under the REV + $\Gamma$ 8 model.

Differences in evolutionary rates between taxa were evaluated from the combined dataset using the relative-rate test as implemented in the program RRTREE (Robinson-Rechavi & Huchon 2000). The dating of cladogenic events was estimated using a local molecular-clock model (Yoder & Yang 2000) in which lineages previously identified as slower or faster evolving were assigned a special rate in the BASEML program of the PAML software (Yang 1997). The optimal ML topology was used as the input each time.

## 3. RESULTS

### (a) *Analysis of each gene*

#### (i) *TH*

The TH intron varies from 129 to 692 base pairs (bp) in the different glirid species. The two *Eliomys* species and the two *Dryomys* species were characterized by long insertions (250 bp in *Eliomys* and 190 bp in *Dryomys*); given the autapomorphic nature of these insertions, they were excluded from subsequent analyses. The resulting alignment consisted of 735 bp, of which 268 positions are situated in the exon and 467 in the intron of the gene. The saturation analysis showed that the inferred and observed differences increase linearly (slope of 0.82), indicating that the TH gene is not saturated in either the Gliridae ingroup analysis, or in the outgroups. The absence of saturation is supported by a high consistency index of 0.83 for 129 parsimony-informative sites.

#### (ii) *SPTBN*

The intronic region of the SPTBN gene varies in length from 590 to 1154 bp. Two autapomorphic indels and one synapomorphic indel were found. The two autapomorphies, one of 254 bp defining the genus *Graphiurus* and a second of 36 bp restricted to *Myomimus*, were omitted from our analyses. An insertion of 238 bp, shared by *Eliomys*, *Dryomys* and *Graphiurus*, was retained in the analyses. The final alignment therefore consisted of 1042 bp of which 68 bp were in exonic regions. Although more scattered than in TH, the plots show no evidence of saturation in the whole dataset (slope of 0.83); this was supported by a high consistency index of 0.79 (314 parsimony-informative positions).

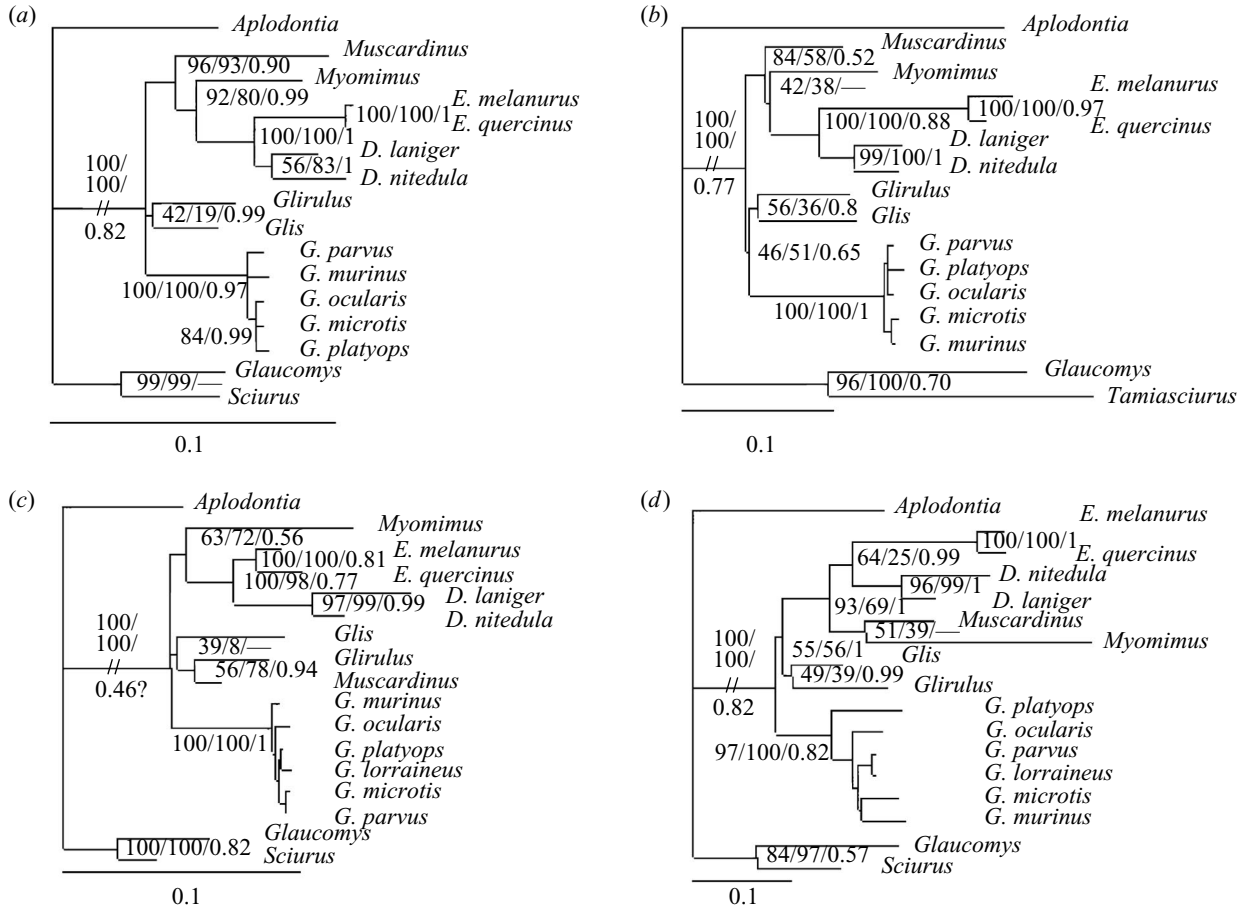


Figure 1. ML tree for each data partition. Bootstrap proportions for ML and MP and Bayesian posterior probabilities are given from left to right at each node. The lengths of the branches uniting the ingroup are drawn proportionally; the branch leading to the outgroups has been reduced twice. (*a*) TH, (*b*) SPTBN, (*c*) LCAT and (*d*) 12S.

(iii) *LCAT*

The LCAT sequences span five exons and three introns (Robinson *et al.* 1997). In our analysis of this gene no indels were excluded, resulting in an alignment of 701 bp including 232 intron positions and 405 exon positions. The slope of saturation ($S = 0.78$) and the consistency index of 0.81 (135 parsimony-informative positions) indicated that intra-Gliridae comparisons were not saturated.

(iv) *12S rRNA*

This marker consists of 1007 bp of which 55 bp reside in the terminal loop; they have been removed owing to alignment ambiguities. The remaining 952 bp included 455 stem positions and 433 loop characters. The saturation plot ($S = 0.36$) and consistency index of 0.57 for 278 parsimony-informative sites suggest that this mitochondrial gene is saturated when compared with the three nuclear genes. As expected, the loop characters were more saturated ($S = 0.26$) than the stems ($S = 0.52$). All positions were included in subsequent analyses following suggestions by Voelker & Edwards (1998).

### (b) *Phylogenetic trees*

Different models of sequence evolution were identified for each gene fragment by MODELTEST (table 2). All genes required a gamma distribution to account for rate heterogeneity between sites. The values of the $\Gamma$ parameter vary from 0.25 for the 12S rRNA (strong heterogeneity) to 2.35 for SPTBN (low heterogeneity). Figure 1 shows the ML trees obtained for the four independent partitions (SPTBN, TH, LCAT and 12S rRNA). Bootstrap support for ML and MP analyses and BI posterior probabilities are shown for each node. The three genera represented by more than one species (*Eliomys*, *Dryomys* and *Graphiurus*) as well as the *Eliomys* + *Dryomys* clade are consistently supported by all genes and methods of analysis. The clustering of the genus *Myomimus* with the *Dryomys–Eliomys* clade, representing the Leithiinae subfamily, is consistent for three genes (SPTBN, TH and 12S rRNA), but with low to moderate support. In MP and ML, the association between *Glis* and *Glirulus* appears weakly supported by the 12S rRNA, SPTBN and TH topologies, but is not supported by LCAT, which suggests a *Glis–Muscardinus* association. Contrasting with this low support, the posterior probabilities for the *Glis–Glirulus* clade are rather high (more than 0.8). Discrepancies between BP proportions and Bayesian probabilities have been noted (Huelsenbeck *et al.* 2002) and it is also evident that posterior probabilities can be 'excessively liberal' (Suzuki *et al.* 2002).

### (c) *Combined analysis*

The combined sequences from all four genes resulted in a dataset consisting of 3430 characters. Out of these, 746 were in exons, 1730 were in introns and the remaining 952 bp were contributed by the mitochondrial 12S rRNA gene. MODELTEST selected the GTR + $\Gamma$ 8 + $I$ as the best fit for our data and the optimal ML tree is shown in figure

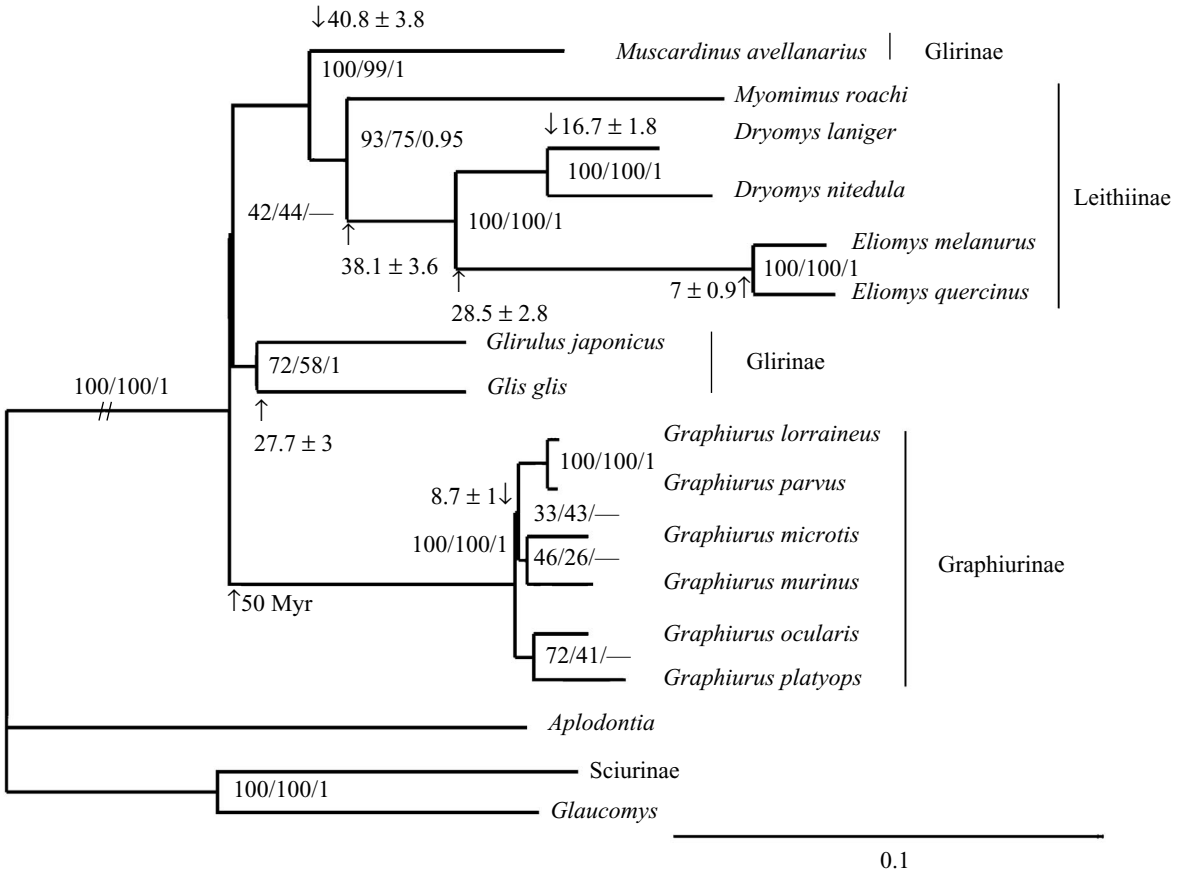


Figure 2. ML phylogram of Gliridae relationships using the concatenation of SPTBN, TH, LCAT and 12S rRNA genes. Bootstrap proportions for ML and MP and Bayesian posterior probabilities are given from left to right at each node. The lengths of the branches uniting the ingroup are drawn proportionally; the branch leading to the outgroups has been reduced twice. Branching dates with standard errors (in Myr ago) are provided at each node (arrows).

2. Six glirid clades are supported by high BP support in ML (greater than 93%); moreover, the posterior probabilities of these clades were all greater than 0.95. The monophyly of *Glis* + *Glirulus* is also supported by ML (BP = 72%) and BI (1.00). The intrageneric relationships of the species belonging to *Graphiurus* were generally not well defined. Apart from supporting *G. lorraineus* + *G. parvus* as a monophyletic lineage, no other relationships were clearly resolved. *Graphiurus* appears to be basal within the Gliridae, but this is not supported by ML or by MP bootstrap analysis (BP = 42% and 44%, respectively). The BI method supports an association, albeit a non-significant one ($p = 0.53$), between *Glis* + *Glirulus* and *Graphiurus*.

### (**d**) *Test of alternative topologies*

To evaluate the stability of the optimal ML tree, alternative topologies were tested using two approaches. First, we compared all 15 trees (see table 3) that retrieved *Graphiurus*, Leithiinae + *Muscardinus*, *Glirulus* and *Glis* as distinct evolutionary lineages (the two latter genera were treated as separate lineages because their clustering is moderately supported, see figure 2). To account for the different evolutionary processes in different genes, and in different regions of the same gene, we used the partitioned likelihood approach developed by Yang (1996). We tested four partition models: complete homogeneity among the four genes (i.e. only one partition is considered); coding (exons + 12S, of 1698 characters) versus non-coding (introns, 1730) (i.e. two partitions); exon (746 characters), intron (1732) and 12S (952) (i.e. three partitions); and four partitions that included exon characters (746), intron characters (1732) and the 12S data partitioned into stems (457) and loops (495). Out of the 15 topologies tested, the most likely topology is the same as the ML tree presented in figure 2. Additionally, the log-likelihood values were compared using the Akaike information criterion (AIC) (Cao *et al.* 2000) with AIC = $-2(\mathrm{LnL}) + 2n$ (*n*, number of parameters). When one partition was considered, the AIC value was 34 457.2 ($n = 39$), with two partitions it was reduced to 34 034.7 ($n = 156$), with three partitions it was reduced to 33 811.9 ($n = 234$) and with four partitions the AIC was 33 723.4 ($n = 312$). When the model with the lowest AIC is chosen as the most appropriate, our results show that treating exons, introns, stems of the 12S and loops of the 12S as separate partitions better approximates the underlying evolutionary processes.

Irrespective of the number of partitions used, however, the log-likelihood scores of the 15 alternative topologies were not significantly different (see table 3). Interestingly, the two topologies (2 and 3 in table 3) in which *Glis* and *Glirulus* are sister taxa have approximately the same log-likelihood scores as the optimal ML tree (number 1); the remaining topologies (4–15 in table 3) display much lower probabilities. We believe that this result, although not stat-

Table 3. Differences in log-likelihood scores and SH probabilities for the 15 possible trees of the four most supported glirid clades, under different partitions of the combined dataset.

| | one partition[a] | | two partitions[b] | | three partitions[c] | | four partitions[d] | |
|---|---|---|---|---|---|---|---|---|
| tree topology | ΔLnL | $P_{SH}$ | ΔLnL | $P_{SH}$ | ΔLnL | $P_{SH}$ | ΔLnL | $P_{SH}$ |
| 1 ((Leit,(*Glis*–*Glir*)),Graph) | <−17 189.61> | best tree | <−16 939.34> | best tree | <−16 788.9> | best tree | <−16 705.71> | best tree |
| 2 (Leit,((*Glis*–*Glir*),Graph)) | −0.14 ± 0.58 | 0.793 | −0.03 ± 0.39 | 0.872 | −0.10 ± 0.23 | 0.904 | −0.045 ± 0.26 | 0.886 |
| 3 ((Leit,Graph),(*Glis*–*Glir*)) | −0.15 ± 0.56 | 0.791 | −0.049 ± 0.31 | 0.874 | −0.039 ± 0.208 | 0.870 | −0.072 ± 0.28 | 0.857 |
| 4 (((Leit,*Glir*),*Glis*),Graph) | −5.28 ± 3.86 | 0.116 | −4.37 ± 3.60 | 0.182 | −4.483 ± 3.62 | 0.184 | −4.62 ± 3.70 | 0.199 |
| 5 (((Leit,*Glir*),Graph),*Glis*) | −5.58 ± 3.97 | 0.097 | −4.45 ± 3.65 | 0.174 | −4.07 ± 3.85 | 0.255 | −4.34 ± 3.93 | 0.249 |
| 6 ((Leit,*Glir*),(*Glis*,Graph)) | −4.23 ± 4.52 | 0.228 | −2.59 ± 4.40 | 0.423 | −2.67 ± 4.39 | 0.461 | −3.04 ± 4.42 | 0.415 |
| 7 (((Leit,*Glis*),*Glir*),Graph) | −4.47 ± 4.25 | 0.197 | −3.30 ± 4.14 | 0.324 | −3.36 ± 4.23 | 0.362 | −3.60 ± 4.24 | 0.352 |
| 8 (((Leit,*Glis*),Graph),*Glir*) | −4.72 ± 4.34 | 0.177 | −3.36 ± 4.17 | 0.314 | −3.37 ± 4.23 | 0.361 | −3.70 ± 4.26 | 0.334 |
| 9 ((Leit,*Glis*),(*Glir*,Graph)) | −4.72 ± 4.34 | 0.177 | −3.35 ± 4.18 | 0.315 | −3.07 ± 4.32 | 0.383 | −3.48 ± 4.36 | 0.362 |
| 10 ((Leit,(*Glis*,Graph)),*Glir*) | −4.22 ± 4.52 | 0.228 | −2.59 ± 4.40 | 0.423 | −2.67 ± 4.39 | 0.459 | −4.09 ± 4.69 | 0.308 |
| 11 (((Leit,Graph),*Glir*),*Glis*) | −5.58 ± 3.96 | 0.098 | −4.47 ± 3.63 | 0.174 | −4.08 ± 3.85 | 0.255 | −4.36 ± 3.94 | 0.246 |
| 12 (((Leit,Graph),*Glis*),*Glir*) | −5.58 ± 3.96 | 0.098 | −4.47 ± 3.63 | 0.173 | −4.52 ± 3.63 | 0.179 | −4.76 ± 3.73 | 0.178 |
| 13 ((Leit,(Graph,*Glir*)),*Glis*) | −5.38 ± 4.08 | 0.115 | −4.16 ± 3.80 | 0.206 | −3.17 ± 4.23 | 0.370 | −3.61 ± 4.26 | 0.342 |
| 14 (Leit,((Graph,*Glir*),*Glis*)) | −5.28 ± 4.05 | 0.121 | −4.05 ± 3.78 | 0.218 | −3.45 ± 4.03 | 0.327 | −3.86 ± 4.07 | 0.298 |
| 15 (Leit,((Graph,*Glis*),*Glir*)) | −4.15 ± 4.49 | 0.231 | −2.51 ± 4.38 | 0.434 | −2.57 ± 4.37 | 0.476 | −2.94 ± 4.41 | 0.437 |

[a] 3428 characters, $\alpha = 0.50$.

[b] Coding (1698 characters, $\alpha = 0.39$) and non-coding (1730 characters, $\alpha = 0.60$) regions.

[c] Exons (746 characters, $\alpha = 0.39$), introns (1730 characters, $\alpha = 2.67$) and 12S rRNA (952 characters, $\alpha = 0.23$).

[d] Exons (746 characters, $\alpha = 0.39$), introns (1730 characters, $\alpha = 2.24$), 12S stems (457 characters, $\alpha = 0.29$) and 12S loops (495 characters, $\alpha = 0.24$).

istically significant, is supportive of the monophyletic status of *Glis* and *Glirulus* because topologies in which *Glis* and *Glirulus* do not form a clade are less likely than those in which they do.

Our second approach focused on the remaining four glirid genera (*Eliomys*, *Dryomys*, *Myomimus* and *Muscardinus*). For each genus, 10 alternative hypotheses were tested (one partition only owing to computational time) in which each was placed as the sister taxon to the remaining genera and suprageneric groups. In the case of *Eliomys* and *Dryomys*, all topologies were significantly worse than the best ML tree ($p_{SH} < 0.011$). In fact, only with *Myomimus* and *Muscardinus* were the alternatives not rejected: (i) *Myomimus* and *Muscardinus* formed a sister clade to *Dryomys* + *Eliomys* ($p_{SH} > 0.5$); and (ii) *Myomimus* is most basal in the Leithiinae + *Muscardinus* clade ($p_{SH} > 0.5$). All other topologies in which *Muscardinus* or *Myomimus* is not grouped with *Eliomys* and *Dryomys* are significantly worse ($p_{SH} < 0.038$) than the best ML solution.

### (e) *Rate of evolution and molecular dating*

The molecular-clock estimates were based on the combined genes using four data partitions (see § 3d). The assumption of a global molecular clock was rejected by a likelihood ratio test ($2\Delta\text{LnL} = 175.05$, $p < 10^{-4}$ for d.f. = 60). Differences in substitution rates between lineages were determined using the relative rate test (program RRT). In this analysis the six *Graphiurus* species were considered to comprise a single lineage and were therefore not tested separately. All other species and supraspecific clades were tested relative to the remaining glirid lineages resulting in 15 pairwise comparisons between taxa. *Myomimus* ($p = 0.000\,28$), *Eliomys quercinus* ($p = 0.006$), the two *Eliomys* species ($p = 0.025$) and the Leithiinae clade ($p = 0.028$) were characterized by faster rates of evolution, whereas the clade *Glis* + *Glirulus* was identified as slower evolving ($p = 0.000\,94$). The hypothesis of three local molecular clocks was accepted ($2\Delta\text{LnL} = 51.6$, $p = 0.49$ for d.f. = 52, with rates $r1 = 1.56$ (for fast-evolving lineages), $r2 = 0.11$ (for *Glis* + *Glirulus*) and $r3 = 0.67$ (for the outgroups). Molecular dates of divergences were estimated using the 50 Myr ago fossil origin for the Gliridae as a calibration point (Hartenberger 1998). The molecular-based estimates of the dates of glirid cladogenesis and their respective standard errors are indicated by arrows in figure 2.

## 4. DISCUSSION

### (a) *Intra-Gliridae phylogenetic relationships and taxonomy*

Our study is based on comprehensive taxonomic sampling within the Gliridae, and the phylogenetic affinities of the dormice are largely consistent with previous morphological, palaeontological and molecular data (Koenigswald 1992; Wahlert *et al.* 1993; Daams & De Bruijn 1995; Bentz & Montgelard 1999; Montgelard *et al.* 2001). Similarities include the sister-taxon relationship between *Dryomys* and *Eliomys*, which is strongly supported by the present study. Alternative topologies to that portrayed in the optimal ML tree were tested using the SH-test (Shimodaira & Hasegawa 1999) implemented in the PAML software (Yang 1997). Using our clock calibration, the divergence between *Dryomys* and *Eliomys* is placed in the Late Oligocene (*ca*. 28 Myr ago), an estimate that predates their fossil appearance in the Middle Miocene (11–18 Myr ago). Interestingly, the divergence between the two *Dryomys* species appears much older (17 Myr ago) than that between the two *Eliomys* species (7 Myr ago).

The genus *Myomimus* groups as the sister taxon to the *Dryomys–Eliomys* clade, together defining the subfamily Leithiinae (supported by BP, posterior probabilities and the SH-test). This result is consistent with that of Wahlert *et al.* (1993) in which Leithiinae is supported by four morphological synapomorphic characters. Importantly, our study places *Muscardinus* basal to the Leithiinae. This novel finding was not previously suggested by either the morphological or the palaeontological studies (Wahlert *et al.* 1993; Daams & De Bruijn 1995), which included *Muscardinus* in the Glirinae as the sister to *Glis*. In our study, the divergences of *Muscardinus* and *Myomimus* from the rest of the Leithiinae are the oldest within the extant Gliridae, being dated at *ca*. 40 and 38 Myr ago (the Upper Eocene), respectively.

The analysis of the combined data retrieved the two Glirinae genera, *Glis* and *Glirulus*, as a moderately well-supported clade (BP = 72% in ML). Although not significant, the hypothesis of a monophyletic *Glis–Glirulus* clade appears more likely than topologies in which *Glis* and *Glirulus* do not form a clade (see table 3). However, the Glirinae subfamily, as proposed by Wahlert *et al.* (1993), is not supported by bootstrap analyses. Moreover, the SH-test indicates that the three possible topologies supporting a *Glis*, *Glirulus* and *Muscardinus* assemblage are significantly worse than the optimal topology at the 5% level. Based on our results, the Glirinae subfamily should be reduced to include only *Glis* and *Glirulus*. *Muscardinus* should be included in the Leithiinae. The divergence between the two former lineages is estimated to be at *ca*. 28 Myr ago, which is in agreement with the first appearance of *Glis* in the Middle Oligocene.

The six species of *Graphiurus* constitute a clade that is consistently retrieved using sequences from all four genes independently or in combination. However, except for the grouping of *G. lorraineus* and *G. parvus* as sister species, the resolution was weak and this is probably indicative of a rapid radiation that did not allow for sufficient synapomorphies to accumulate. Nevertheless, *Graphiurus* constitutes one of the three major lineages identified among the Gliridae, justifying its subfamilial rank. The optimum ML tree places *Graphiurus* as basal within the Gliridae, a finding that is in agreement with Wahlert *et al*'s (1993) morphologically based analysis. However, other alternatives such as *Graphiurus* being a sister taxon to the *Glis–Glirulus* clade or, alternatively, a sister taxon to the Leithiinae–*Muscardinus* clade are equally likely (trees 2 and 3 in table 3). In summary, except for the position of *Muscardinus* (which should not be included in the Glirinae subfamily), the identification of three major glirid clades (*Graphiurus*, *Muscardinus* + Leithiinae and *Glis* + *Glirulus*) on molecular grounds confirms and extends the classification proposed by Wahlert *et al.* (1993) on the basis of morphological data.

### (b) *Fossil data and the colonization of Africa by* Graphiurus

The fossil record is rather good for the European members of the Gliridae. By contrast, however, fossils attributable to the graphiurine lineage are rare, confounding attempts to shed light on the group's African evolution. The oldest fossil that can unequivocally be assigned to *Graphiurus* is described from the Langebaanweg deposits in South Africa (Pocock 1976; Hendey 1981) and is dated at 4.5 Myr ago (Early Pliocene). Younger fossils have been recorded from the Plio-Pleistocene of Angola, Botswana, Namibia, Tanzania and South Africa (Pocock 1976; Denys 1987; Pickford *et al.* 1992, 1994). The oldest graphiurine fossil, *Otaviglis*, thought to be the direct ancestor of *Graphiurus*, was discovered in Namibia and dates back to the Late Miocene 10–11 Myr ago (Mein *et al.* 2000). According to these authors, *Otaviglis daamsi* may be ancestral to all extant African dormice.

Our molecular results indicate that *Graphiurus* constitutes one of the three major evolutionary lineages comprising the family Gliridae. This lineage diverged early during glirid evolution, although there are currently no palaeontological data to underpin this hypothesis. The European fossil that most closely resembles the African graphiurine species is *Graphiurops austriacus* (Bachmayer & Wilson 1980), which has been described from deposits in Kohfidisch, Austria (Upper Miocene, MN10 = 10 Myr ago). However, this is controversial (Bachmayer & Wilson 1980; Daams & De Bruijn 1995) and at least three alternative hypotheses have been proposed to account for the presumed phylogenetic affinities between *Graphiurus* and *Graphiurops* (Bachmayer & Wilson 1980): first, that the dental modifications are convergent; second, that *Graphiurops* is really a member of the Graphiurinae; and, third, that a primitive branch of the Gliridae was isolated south of the Sahara and that *Graphiurops* represents a recent derivative that extended its range back into Europe during the Vallesian (*ca*. 11.5–10 Myr ago). The third hypothesis is not corroborated by our results indicating a recent diversification of *Graphiurus* (see below) and there is no fossil evidence to support an early isolate of the graphiurine lineage in Africa. Our conclusions are broadly consistent with proposals that *Graphiurops* falls within the graphiurine lineage, or, alternatively, that the dental characters used in their delimitation are convergent.

Using our molecular-clock calibrations, we estimate that *Graphiurus* radiated *ca*. 8–10 Myr ago. This predates the oldest *Graphiurus* fossil recorded (*ca*. 5 Myr ago) but is remarkably consistent with the occurrence of the graphiurine *Otaviglis* at 10–11 Myr ago. Therefore it seems plausible that the colonization of Africa by *Graphiurus* took place relatively recently (10–11 Myr ago). This corresponds to the lower Vallesian of Europe (MN9; Mein 1999), which was characterized by low sea levels (Haq *et al.* 1987) and extensive faunal interchange between Europe and Africa (Pickford & Morales 1994). It also coincides with the appearance of murids in the Mediterranean area (Hartenberger & Thaler 1963).

The opening up of new niches that followed the colonization of Africa by *Graphiurus* prompted diversification through adaptive radiation. The latter can be defined as a burst of evolution with rapid divergence from a single ancestral form, and this proliferation of species is driven by novel ecological opportunities (Skelton 1993). *Graphiurus* provides a case in point. The genus is more species rich than extant Eurasiatic glirid genera and the short internal branches resulting in unresolved nodes between the different species also indicate rapid speciation events. It is difficult to differentiate between extrinsic (environmental) and intrinsic (pre-adapted) factors driving the speciation process. In Africa, *Graphiurus* species colonized most habitats from dense forests to rocky areas but the genus appears very homogeneous from a morphological perspective (Genest-Villard 1978; Holden 1996). This suggests that environmental factors were probably decisive in driving the speciation process in *Graphiurus*.

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