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Exhaustive sample set among Viverridae reveals the sister-group of felids: the linsangs as a case of extreme morphological convergence within Feliformia

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Although molecular studies have helped to clarify the phylogeny of the problematic family Viverridae, a recent phylogenetic investigation based on cytochrome *b* (cyt *b*) has excluded the Asiatic linsangs (genus *Prionodon*) from the family. To assess the phylogenetic position of the Asiatic linsangs within the Feliformia, we analysed an exhaustive taxonomic sample set with cyt *b* and newly produced transthyretin intron I sequences (TR-I-I). TR-I-I alone and cyt *b*+TR-I-I combined (maximum-likelihood analysis) highly support the position of Asiatic linsangs as sister-group of the Felidae. The estimation of minimum divergence dates from molecular data suggests a splitting event *ca*. 33.3 million years (Myr) ago, which lends support to historical assertions that the Asiatic linsangs are 'living fossils' that share a plesiomorphic morphotype with the Oligocene feliform *Paleoprionodon*. The African linsang is estimated to appear more than 20 Myr later and represents the sister-group of the genus *Genetta*. Our phylogenetic results illustrate numerous morphological convergences of 'diagnostic' characters among Feliformia that might be problematic for the identification of fossil taxa. The morphotype reappearance from the Asiatic to the African linsangs suggests that the genome of the Feliformia conserved its potential ability of expression for a peculiar adaptive phenotype throughout evolution, in this case arboreality and hypercarnivory in tropical forest.

Keywords: Viverridae; *Prionodon*; Felidae; transthyretin intron I; phylogeny; morphological convergence

1. INTRODUCTION

The phylogeny of the problematic family Viverridae has been clarified by recent molecular studies (Veron & Catzeflis 1993; Flynn & Nedbal 1998; Veron & Heard 2000; Yoder *et al.* 2003). The family, as traditionally delimited (Simpson 1945; Wozencraft 1989), is polyphyletic, with *Nandinia* as a sister-group of the other Feliformia, and Malagasy 'viverrids' and 'mongooses' forming a clade that is the sister-group of Herpestidae. A preliminary phylogenetic investigation based on cytochrome *b* (cyt *b*) and focused on African taxa has excluded the Asiatic linsangs (genus *Prionodon*) from the Viverridae, thus questioning again the monophyly of the family (Gaubert *et al.* 2003). However, the phylogenetic position of *Prionodon* within Feliformia remains unresolved.

The Asiatic linsangs are traditionally included under the 'genet-like taxa' in the subfamily Viverrinae. The latter consists of an assemblage of dissimilar mesopredator morphotypes from the large, digitigrade and mainly terrestrial civets, to the slender and semi-digitigrade genets and genet-like taxa (Taylor 1988; Veron 1999), distributed in the Afro-Asian intertropical zone and adapted to almost all terrestrial habitats (Nowak 1999). Recent phylogenetic investigations based either on morphology or DNA do not support the monophyly of the Viverrinae (Veron 1995; Veron & Heard 2000; Gaubert *et al.* 2002). The genus *Prionodon* shares a global morphological similarity with the African linsangs (genus *Poiana*), including a very similar spotted coat pattern, a small and rounded skull (figure 1), a hypercarnivorous dentition and an absence of second upper molar (M^2) (Wozencraft 1984). Phylogenetic relationships between genets (genus *Genetta*) and linsangs are poorly understood, and have not been questioned in an exhaustive evolutionary framework since the work of Gregory & Hellman (1939). Asiatic and African linsangs were first placed in the tribe Prionodontini (Gray 1864; Mivart 1882), but subsequent authors considered *Poiana* a 'primitive' genet, which had acquired a morphotype similar to *Prionodon* by convergence due to their common way of life in tropical rainforests of both continents (Gregory & Hellman 1939; Simpson 1945; Rosevear 1974; Crawford-Cabral 1981, 1993). In addition, *Prionodon* shares similarities with both Viverrinae and Felidae (Horsfield 1821; Veron 1995; Hunt 2001) that several authors have also considered to be evolutionary convergences (Gray 1864; Pocock 1933; Gregory & Hellman 1939). Furthermore, *Prionodon* shares a similar morphology of the basicranial region with Oligocene fossils of Feliformia like *Paleoprionodon*, but also *Proailurus* (Teilhard de Chardin 1915; Gregory & Hellman 1939; Veron 1995; Hunt 2001). Recently, Hunt (2001) suggested that the transformation of bullar elements observed from *Paleoprionodon* to the linsangs may constitute a morphocline and thus grouped them in the subfamily Prionodontinae together with *Genetta*. However, the fossil data are not helpful because both Asiatic and African linsangs have no fossil records (McKenna & Bell 1997). The major classifications currently in use include *Prionodon* within the

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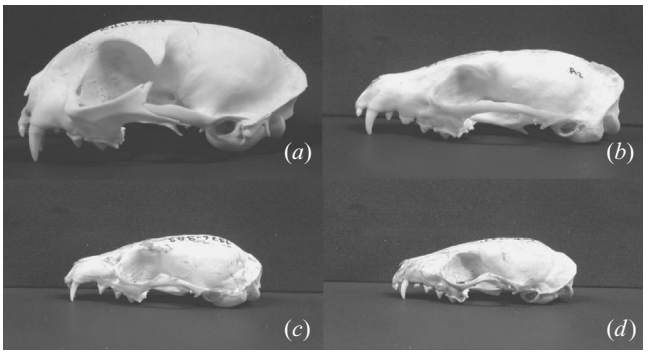

Figure 1. Skulls in lateral view of (*a*) *Felis silvestris* (MNHN 1995-443), (*b*) *Genetta genetta* (MNHN 1995-417), (*c*) *Poiana richardsonii* (MNHN 1976-389) and (*d*) *Prionodon pardicolor* (MNHN 1929-424). The greatest condylo-basal length of *P. pardicolor* (*b*) is 669 mm. MNHN, Muséum National d'Histoire Naturelle, Paris.

Viverrinae under the monogeneric tribe Prionodontini (Gray 1864; Simpson 1945; Wozencraft 1989).

Although material for DNA investigations is difficult to obtain in the case of the rare genet-like taxa, we managed to sample an exhaustive taxonomic sample set to investigate the phylogenetic relationship of the Asiatic linsangs within the Feliformia. We enriched our dataset with new sequences of the transthyretin intron I (TR-I-I), a nuclear gene already used successfully for questioning the deep relationships within the order Carnivora (Flynn & Nedbal 1998; Flynn *et al.* 2000; Yoder *et al.* 2003). On the basis of the obtained phylogeny and estimations of divergence times, the high morphological convergence evidenced among Feliformia and its implications for interpretation of the fossil record are discussed.

2. MATERIAL AND METHODS

(a) *Taxonomic sampling*

The TR-I-I data matrix represents 29 species of Carnivora comprising 13 species of Viverrinae (all seven genera are represented) and three species representing the two other subfamilies of Viverridae, namely Paradoxurinae (*Paradoxurus*, *Paguma*) and Hemigalinae (*Chrotogale*). Species traditionally included in the family Viverridae and known to be taxonomically problematic, such as *Nandinia binotata* and *Cryptoprocta ferox*, were also considered. At least one species belonging to the three other families of Feliformia were included (two Herpestidae, one Hyaenidae and two Felidae). All four families of Caniformia were represented and used as outgroups. DNA extracts were obtained from fresh tissue, hair samples or tooth material (in the case of *Genetta piscivora*).

(b) *DNA extraction and sequencing*

Total genomic DNA was prepared following standard methods (Kocher *et al.* 1989). The amplification of TR-I-I was performed with a set of 16 new specific primers designed from available sequences of both Viverridae and Herpestidae (Flynn & Nedbal 1998) using Oligo, v. 4.0 (Rychlik 1992). Oligonucleotide primers are listed with U and L referring to upper and lower strands. The numbers correspond to the 5′ first position of the primer on the aligned sequences of Viverridae and Herpestidae. Squared brackets indicate pairs: [VIVN1U 5′-GAT GAA GTA GAA GTG CCT-3′; VIVN504L 5′-CAC AGG ACC AAA TAG TT-3′] [VIVN504U 5′-ACA AAC TAT TTG GTC CTG TG-3′; VIVN807L 5′-CAG TGA GAG GTC AAC GAA-3′] [VIVN28U 5′-TCT GCC AGY CAG CTT GTG T-3′; VIVN30U 5′-GCC AGC CAG CTT GTG TAM T-3′; VIVN543L 5′-GCC TTG CCA TTT GAG TGG A-3′] [VIVN486U 5′-AAA WGT TCT CAG GAA AAA CA-3′; VIVN750L 5′-AAC AAG GCA ARA AGT CCA T-3′] [VIVN2U 5′-GAC GAA GGA GAA GTG-3′; VIVN812L 5′-CCA GTG ACA GGT CAA-3′] [OSB175U 5′-CAC TGR GTT TMC CGT GCC T-3′; OSB341L 5′-GTW GTC CRT CCA GCC TTT C-3′] [OSB339U 5′-TTG AAA GGC TGG AYG GAC-3′; OSB508L 5′-TGC CAC ARG ACC AAR TAG-3′] [HERP5U 5′-AAG TAG CAR TGY CTT MCT C-3′; HERP459L 5′-ACT GCY GCT RTA GTA ATT C-3′]. Reaction parameters for amplification are described in Veron & Heard (2000). We used variable temperatures of annealing (48–52 °C) and variable numbers of cycles (40–50), depending on the primer pairs. The reaction products were visualized in a 1.5% agarose gel, then either purified directly from the PCR mixture or from agarose gel (MinElute PCR Kit), and sequenced directly in both forward and reverse directions with an automated DNA sequencer (CEQ 2000 DNA Analysis System, Beckman). Nucleotidic sequences were obtained manually in the case of *G. piscivora*. The 17 new sequences of TR-I-I have been deposited at GenBank with the following accession numbers: *Prionodon pardicolor* AY23261; *Herpestes ichneumon* AY232611; *Cryptoprocta ferox* AY232612; *Nandinia binotata* AY232613; *Chrotogale owstoni* AY232614; *Paguma larvata* AY232615; *Viverra zibetha* AY232616; *V. megaspila* AY232617; *Viverricula indica* AY232618; *Civettictis civetta* AY232619; *Poiana richardsonii* AY232620; *Genetta piscivora* AY232621, AY232622 and AY232623; *G. johnstoni* AY232624; *G. thierryi* AY232625; *G. servalina* AY232626; *G. maculata* AY232627; and *G. genetta* AY232628.

(c) *Phylogenetic analyses and data partition*

TR-I-I sequences were first aligned with ClustalX (Thompson *et al.* 1997) using default settings. Sequences were then manually adjusted through BioEdit v. 5.0.6 (Hall 1999). Phylogenetic analyses were run under PAUP* Beta v. 4.0b2 (Swofford 2001), using maximum parsimony (MP) and maximum likelihood (ML) with heuristic search (tree bisection–reconnection branch-swapping—random stepwise addition: 10 replicates). Likelihood models and parameters were estimated using ModelTest v. 3.06 (Posada & Crandall 1998). The general time reversible (GTR) model (Yang 1994) was selected from ModelTest with among-site substitution rate heterogeneity described by a gamma distribution for both TR-I-I alone and the combined analysis (cyt *b*+TR-I-I), and a fraction of sites constrained to be invariable for cyt *b* (Gaubert *et al.* 2003). Saturation tests (Hassanin *et al.* 1998; Vidal & Lecointre 1998) were performed using PAUP*. Indels—following the definition of Simmons & Ochoterena (2000)—resulting from the alignment of TR-I-I were either excluded or included (as fifth state) in the analyses. Phylogenetic signal value (g1; 100 000 random trees) was used as an estimation of the structuring of the signal versus random noise (Hillis 1991; Hillis & Huelsenbeck 1992).

The combination of TR-I-I and cyt *b* resulted in a matrix of 23 taxa as we considered the species in common between the two datasets only. However, sequences were also combined between representatives of the same genus, namely *Canis familiaris* and *C. lupus*, *Mustela erminea* and *M. frenata*, *Felis catus* and *F. silvestris*, and *Panthera tigris* and *P. leo*. There has been

a long debate about estimating heterogeneity among data partitions and whether or not to combine datasets in a single analysis (Kishino & Hasegawa 1989; Kluge 1989; De Queiroz *et al.* 1995; Miyamoto & Fitch 1995; Huelsenbeck *et al.* 1996; Page 1996; Cunningham 1997). We estimated the conflicts in phylogenetic signals between the two datasets through several methods. The incongruence length difference (ILD) (Farris *et al.* 1995) was measured in order not to justify data combination, but to estimate the extent of conflict among datasets (Liu & Miyamoto 1999). Tree comparisons were made to statistically test the difference between alternative phylogenetic hypotheses using the test of Kishino & Hasegawa (1989), as implemented in PAUP*. We also measured data partition incongruence by the values of nodal support between conflicting topologies, using bootstrap values (bp; 100 and 1000 replicates in ML and MP analyses, respectively) together with the likelihood ratio test of branch length in ML trees (Felsenstein 1993). We assumed that node support reflects phylogenetic signal (Mason-Gamer & Kellogg 1996; Flynn & Nedbal 1998) and considered values superior to 70% as strongly supported (Hillis & Bull 1993; Mason-Gamer & Kellogg 1996). The repeatability of clades observed throughout the different phylogenetic analyses was an additional criterion of node reliability (Springer *et al.* 1999).

(d) *Estimation of divergence time*

We used the test of Kishino & Hasegawa (1989) to test for the significance ($p < 0.05$) in branch length differences between enforced (molecular clock) and non-enforced ML trees. To estimate divergence times within a ML framework, we used RHINO, a modified version of the software QDATE (Rambaut & Bromham 1998), allowing our analyses to take into account more than four taxa (Cooper *et al.* 2001; Paxinos *et al.* 2002). RHINO provides an estimation of confidence interval for each node that reflects uncertainties occurring in the phylogenetic reconstruction process and stochasticity of the molecular clock (Rambaut & Bromham 1998; Paxinos *et al.* 2002). We used the divergence *Felis–Panthera*, well documented in the fossil record, as the calibration point of our analysis. It is estimated from clearly diagnosable fossil remains at 3 million years (Myr) ago (first occurrence of genus *Panthera*; see Turner (1987) and Wayne *et al.* (1991)), which corresponds to the minimal divergence date (MDD).

(e) *Estimation of morphological convergence*

To assess the morphological convergence between the Asiatic and African linsangs, we compared pairwise ML genetic distances (from combined analysis) with morphological 'distances'. The latter were estimated as the number of different morphological character states between two taxa divided by the total number of characters, on the basis of the morphological matrix of 69 characters produced by Gaubert *et al.* (2002), to which an Asiatic civet (*Viverra zibetha*) was added. The taxonomic sample set consisted of the spotted Asiatic linsang (*P. pardicolor*), the Central African linsang (*P. richardsonii*), the aquatic genet (*G. piscivora*), the genus *Felis* (Felidae) and three representatives each of terrestrial civets (*C. civetta*, *V. zibetha*, *V. indica*) and the genus *Genetta* (*G. johnstoni*, *G. thierryi*, *G. genetta*). The correlation between genetic and morphological distances was tested by using the coefficient of correlation of Bravais–Pearson (r).

3. RESULTS

(a) *Phylogenetic estimates*

No amplification of putative nuclear-translocated cyt *b* genes was detected following standard recommended criteria (Lopez *et al.* 1994, 1997; Arctander 1995; Zhang & Hewitt 1996; Cracraft *et al.* 1998; Hassanin 2002). Sequences from 'sensitive' material (i.e. DNA extracts from collection specimens) were compared with sequences from fresh material obtained later in the course of our investigations (*P. richardsonii*, *P. pardicolor*), and did not reveal any aberrant pattern (Gaubert *et al.* 2003). The TR-I-I sequence length varied from 802 bp (*Chrotogale owstoni*) to 1060 bp (*Canis lupus*), and the data matrix after alignment was constituted by 1096 characters. Complete sequences were difficult to obtain for two taxa, namely *G. piscivora* (tooth material) and *V. indica*, which are represented by nearly 55% of the total bp number. The large 266 bp indel reported to characterize Caniformia (Flynn & Nedbal 1998) is only 228 bp in our alignment and was included in the analysis. The χ^2-test run by PAUP* detected no significant heterogeneity among base frequencies ($p > 0.999$): A = 0.280 78, C = 0.218 81, $G = 0.196\ 67$, $T = 0.303\ 74$. The data matrix with indel excluded is 775 bp long. Relationships between patristic and observed distances are linear for transitions (not saturated). Transversions are not saturated but pairwise distances show a more heteroclite distribution (data not shown). A strong structuring of the signal is present in the TR-I-I dataset, indels included or excluded, because the shape of tree-length distribution is clearly left-skewed ($g1 = -1.837\ 535$ and $-1.017\ 820$, respectively; $p < 0.01$). The ML analysis used a shape parameter of gamma distribution of 1.9145, indicating a low heterogeneity of rate variation among sites.

The MP and ML analyses of TR-I-I yielded very similar topologies and node supports. Figure 2 shows one of the three equiprobable trees obtained using the ML criteria. As previously reported (Flynn & Nedbal 1998), *Nandinia* appears basal to the other Feliformia. *Cryptoprocta* is a sister-taxon to Herpestidae (bp = 100%), whereas *Crocuta* (Hyaenidae) is a sister-taxon to the latter clade (bp < 70%). The most striking result of our analysis resides in the highly supported phylogenetic position of *Prionodon* as a sister-taxon of Felidae (bp = 98%). The African linsangs (*Poiana*) share no close affinities with *Prionodon*, and are nested in a derived clade as a sister-taxon of *Genetta* (bp = 100%). The node defining terrestrial civets as a sister-group of the clade *Poiana–Genetta* is highly supported (bp = 78–95%). The MP analyses strongly support (bp = 76–81%) a sister relationship between *Viverricula* and *Viverra* (Asiatic terrestrial civets), with *Civettictis* (the African terrestrial civet) as a sister-taxon (bp = 99–100%). However, the missing data in the sequence of *V. indica* must temper the support of this hypothesis, especially as relationships are unresolved in the ML strict consensus. The monophyly of Viverridae as newly defined (i.e. excluding *Nandinia*, *Cryptoprocta* and *Prionodon*) is strongly supported (bp = 90–100%).

The phylogenetic hypotheses resulting from the analysis of cyt *b* had low resolutions at basal nodes mainly due to saturation at the third codon position (see Zhang & Ryder 1993; Meyer 1994; Honeycutt *et al.* 1995), and *Prionodon*

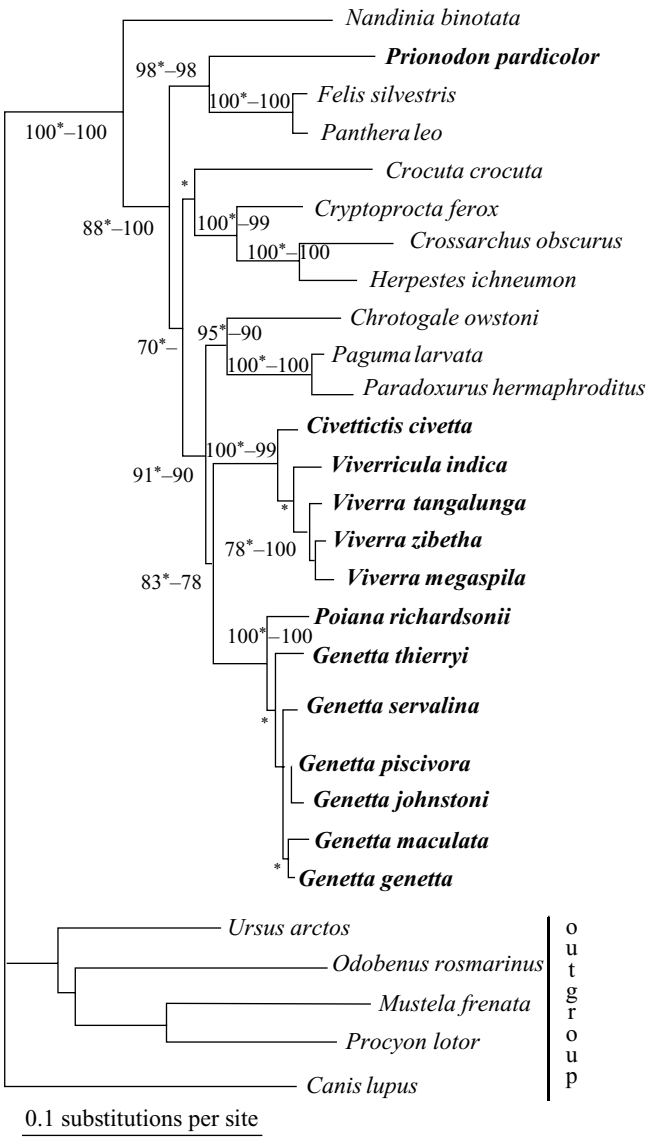

Figure 2. One of the three equiprobable ML trees (GTR model with Γ rate distribution = 1.9145) illustrating the phylogenetic relationships of the subfamily Viverrinae and based on 775 bp of TR-I-I sequences (indels excluded; −ln likelihood = 4991.794 77). Bootstrap values above 70% are shown at nodes for ML (together with an asterisk when branch length is significant) and MP analyses, respectively. Scale bar corresponds to 10% sequence divergence. Taxa traditionally included in Viverrinae appear in bold.

occupied an unresolved position among Feliformia (Gaubert *et al.* 2003). The ILD test revealed incongruence ($p < 0.05$) between TR-I-I and cyt *b*, but the null hypothesis was not rejected (although the *p*-value neared the rejection threshold) when excluding indels and the two incomplete sequences of *G. piscivora* and *V. indica* ($p = 0.058$). We then chose to combine the two datasets (indels excluded), all the more because the phylogenetic hypotheses based on the analysis of the separate matrices were the same or did not significantly conflict (low node supports). The signal structuring of the combined matrix was significant and the shape of tree-length distribution left-skewed ($g1 = -0.694\,42$; $p < 0.01$). The tree topology based on TR-I-I alone was recovered by the combined analysis using ML criteria (figure 3). Combination of datasets increased the support at the node linking Hyaenidae to Herpestidae + *Cryptoprocta* (bp = 86%), whereas it reduced bootstrap values at the node supporting terrestrial civets as a sister-group of the clade *Poiana–Genetta* (less than 50%). Although the latter case is isolated, it exemplifies that saturated positions, base composition biases and heterogeneity in evolutionary rates—characterizing cyt *b* in this case—might cause a lower resolution within the combined analysis tree, even if one of the dataset supplies a 'clear' phylogenetic signal (Hillis & Bull 1993; De Queiroz *et al.* 1995; Cunningham 1997; Prychitko & Moore 2000). The sister relationship of the Asiatic linsangs with felids is recovered (bp = 77%) and strongly supported by five strict synapomorphies (TR-I-I: three transitions and one transversion; cyt *b*: one transversion). Moreover, the test of Kishino–Hasegawa significantly rejected ($p < 0.05$) the phylogenetic hypotheses of *Prionodon* as (i) a sister-taxon of *Poiana* or (ii) a sister-taxon of Viverridae, for both TR-I-I and combined trees (data not shown). However, in the latter case, although the *p*-value was very low it did not reach the rejection threshold for the second hypothesis (0.0741).

(b) *Divergence times*

Estimations of divergence times were made with the genera *Canis* and *Procyon* as outgroup taxa. The Kishino–Hasegawa test rejected the hypothesis of a global molecular clock for the ML trees resulting from the separated analyses ($p < 0.0001$) but did not detect significant branch length differences for the combined analysis ($p = 0.8795$). The ML tree resulting from the combined dataset was then used for estimating divergence times with RHINO, using the GTR model along with a discrete gamma distribution. As a criterion of reliability, our estimates of MDD for the age of Malagasian taxa (*Cryptoprocta*) were 19.4 (16.5–22.7) Myr ago, which is very congruent with the estimates of Yoder *et al.* (2003). MDD between taxa of the Viverridae clade (Paradoxurinae, Hemigalinae and Viverrinae without *Prionodon*) is estimated *ca*. 23.4 (20.8–26.3) Myr ago, in agreement with the fossil record (*Herpestides*; 21–23 Myr ago; see Hunt 1991, 1996). One of the most striking results concerns the MDD estimation between the Asiatic linsang and Felidae lineages, predicting a split *ca*. 33.3 (31.9–35.2) Myr ago, which is antecedent to the dates of appearance of the first fossils representing the Felidae (*Haplogale*, *Proailurus*: 24–30 Myr ago; see Ginsburg 1983; Hunt & Tedford 1993) and Viverridae. The African linsangs are estimated to have diverged 11.2 (9.5–13.3) Myr ago, *ca*. 20 Myr later than the split between the Asiatic linsangs and Felidae.

(c) *Morphological convergence*

The correlation between morphological and ML genetic distances was highly significant for the datasets constituted by terrestrial civets/*Felis*, and *Genetta*/*Felis* (respectively, $r = 0.966\,41$ and $0.920\,64$; $p < 0.01$), indicating strongly correlated distances among terrestrial civets and *Genetta* as well as among these two groups and *Felis*. The extreme morphological convergence between *Poiana* and *Prionodon* was evidenced by a non-significant correlation between morphological and molecular distances when *Poiana* was compared with the entire sample set, i.e. including

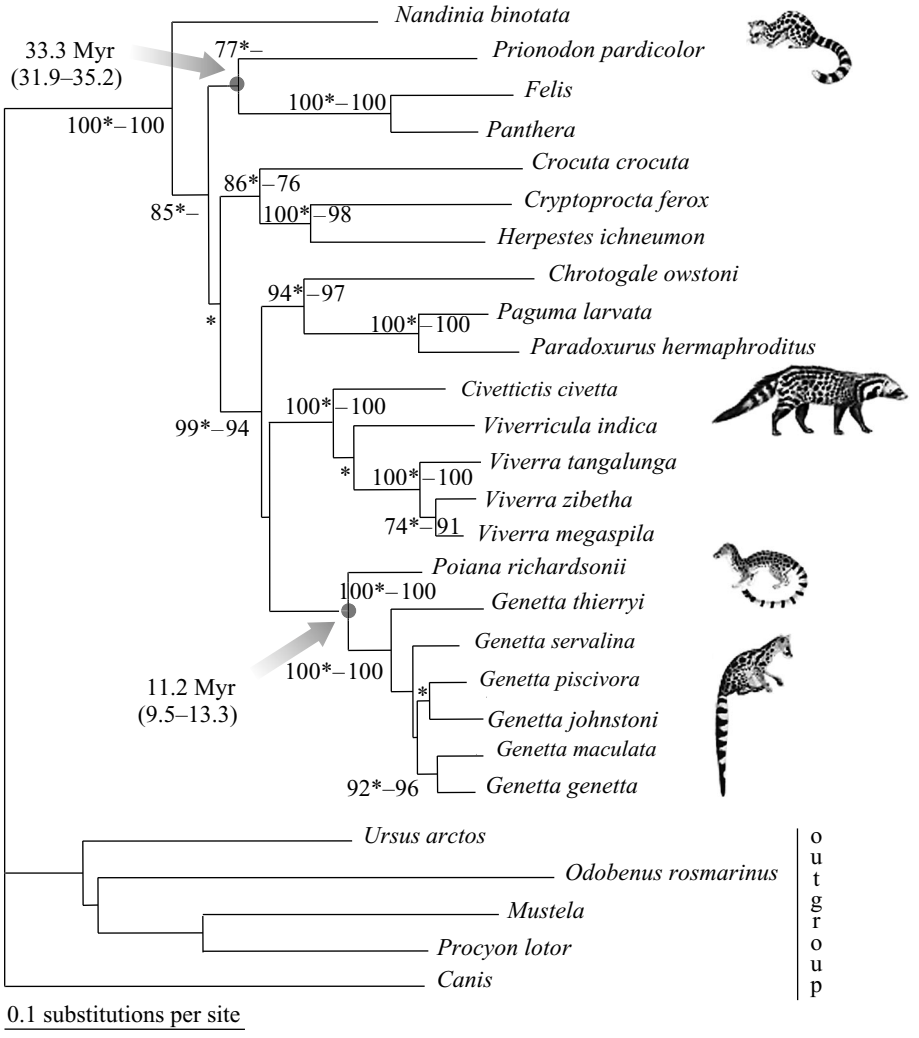

Figure 3. ML tree (GTR model with Γ rate distribution = 0.3201) based on the 1915 bp combined dataset of TR-I-I and cyt *b* sequences (−ln likelihood = 16 514.205 06). Bootstrap values above 70% are shown at nodes for ML (together with an asterisk when branch length is significant) and MP analyses, respectively. Scale bar corresponds to 10% sequence divergence. Grey circles and arrows indicate nodes and estimates of minimum divergence date (with confidence interval) between (i) *Prionodon* and Felidae, and (ii) *Poiana* and genets (genus *Genetta*). Genera *Felis*, *Panthera*, *Mustela* and *Canis* represent combined sequences from two different species (see § 2). Drawings modified from Dorst & Dandelot (1976), Kingdon (1997) and Nowak (1999).

Prionodon ($r = 0.444\ 65$; $p > 0.05$), whereas the correlation was highly significant after the exclusion of the Asiatic linsang ($r = 0.931\ 73$; $p < 0.001$).

4. DISCUSSION

(a) *The Asiatic linsangs as the sister-group of felids*

This is the first time, to our knowledge, that the phylogenetic relationships of the enigmatic Asiatic linsangs have been established. The placement of *Prionodon* as the sister-group of the family Felidae is strongly supported, both by TR-I-I alone and the combined dataset (ML). Strong evidence is provided by the repeatability of clades observed throughout using different methods of analysis, high bootstrap support and unique synapomorphies at node. Horsfield (1821) placed the Asiatic linsangs within the Felidae as 'section Prionodontidae' (dixit the author), but all subsequent authors considered morphological similarities between the Asiatic linsangs and felids as acquired by convergence and adaptation to hypercarnivory (Gray 1864; Pocock 1933; Gregory & Hellman 1939; Rosevear 1974; Veron 1995). Probably for this reason, molecular investigations questioning the phylogenetic relationships between families of Carnivora have never included the Asiatic linsangs, and have suffered from very few family representatives (Wayne *et al.* 1989; Zhang & Ryder 1993; Flynn & Nedbal 1998). The uncertainty among previous hypotheses proposed for the sister-group of felids is here solved by using a large taxonomic sample set among Viverridae and sequences of TR-I-I, which is an appropriate marker for questioning the deep phylogenetic relationships within Feliformia (Flynn & Nedbal 1998; Yoder *et al.* 2003).

Several differences between the morphotypes of the Asiatic and African linsangs were previously reported (Gregory & Hellman 1939; Rosevear 1974; Veron 1995) but the Asiatic linsangs were not suspected of being excluded from the Viverridae since Horsfield's publication. Our results support a new definition of the

Viverridae as excluding *Prionodon*, and we propose that the Asiatic linsangs be placed in the monogeneric family Prionodontidae (Horsfield 1821). Our investigations also clarify the controversial issue of the boundaries of the subfamily Viverrinae (Veron 1995; Veron & Heard 2000; Gaubert *et al.* 2002) in that it supports Viverrinae as being constituted by two monophyletic groups, namely the terrestrial civets (*Civettictis*, *Viverra* and *Viverricula*) and *Poiana* + *Genetta*.

(b) *Divergence dates and biogeography*

Our estimations of divergence dates fit with previous assertions that the Asiatic linsangs are 'living fossils' that have shown little morphological changes in 25–30 Myr (Gregory & Hellman 1939; Hunt 2001). Although the study of auditory bulla characteristics revealed a light evolution in *Prionodon* from the plesiomorphic morphotype found in *Paleoprionodon* (Hunt 2001), the estimated dates of divergence suggest that the Asiatic linsangs and *Paleoprionodon* are part of a same and very ancient Eurasian lineage. This is in disagreement with the recent hypothesis considering the genus *Prionodon* as a critical link between *Paleoprionodon* and the African linsangs and genets (Hunt 2001), because the two latter groups share no close phylogenetic affinities with the Asiatic linsangs and are estimated to diverge more than 20 Myr later.

Area mapping using MacClade (Maddison & Maddison 2000) and Bremer's method (Bremer 1992) from the combined ML tree congruently argues for an Asiatic origin of the newly defined Viverridae at *ca.* 23.4 Myr ago (see electronic Appendix A available on The Royal Society's Publications Web site). The hypothesis of a migration from the Asiatic continent leading to a subsequent diversification into Africa (Hunt 1987; Schmidt-Kittler 1987) then becomes plausible. However, the geographical origin of the subfamily Viverrinae is uncertain, as the presence of an extant African taxon (*Civettictis*) within the terrestrial civets, as well as the recent discovery of a civet-like fossil from the Middle Miocene of Africa (Morales *et al.* 2000), makes the reconstruction of ancestral area somewhat ambiguous.

(c) *Morphotype convergence and implications for fossil taxonomy*

The Asiatic linsangs share a high morphological similarity with the African linsangs, but, given our phylogenetic results, this similarity was achieved by a phenomenon of convergence characterizing the whole morphotype. The phylogenetic position of *Prionodon* confirms the homoplasy of dental characters (Hunt & Tedford 1993; Veron 1995) and hair ultrastructure (Gaubert *et al.* 2003) within Feliformia. It also suggests that morphological characters previously considered as diagnostic and 'reliable', such as the structure of the auditory bulla (Hunt 1987, 1991, 2001) and the relative length of metacarpal bones (Taylor 1988) are, on the contrary, subject to convergence. The general shape of the skull and dentition might be highly adaptive traits, as exemplified by the morphotypes of the linsangs, whereas perineal gland and pad characteristics are likely to be less homoplastic within Feliformia. Given the high potential for morphological homoplasy characterizing this group, we urge caution before assigning taxonomic affinities between fossil and extant taxa, particularly when based on a very reduced number of available characteristics (for instance, see material in Hunt 1996; Morales *et al.* 2000).

Our study points out an extreme case of morphological convergence at least partly due to ecological constraints, which is a unique phenomenon among the order Carnivora. The morphotype resurgence from *Prionodon* to *Poiana* suggests that the genome of the Feliformia conserved its potential ability of expression for a peculiar adaptive phenotype (i.e. hypercarnivory and arboreality in tropical forest) throughout evolution. Our results also dramatically exemplify the need for taking into account exhaustive taxonomic sample sets when questioning large-scale phylogenetic relationships.

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