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Molecular phylogeny of the Herpestidae (Mammalia, Carnivora) with a special emphasis on the Asian *Herpestes*

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ABSTRACT

Until now, phylogenetic studies of the mongooses (Carnivora, Herpestidae) have not included an exhaustive sampling of the Asian members of this family. In this study, we used mitochondrial (Cytochrome *b* and ND2), nuclear (β-fibrinogen intron 7 and Transthyretin intron 1) sequences from almost all of the recognized mongoose species to produce a well-resolved phylogeny of the Herpestidae. We also performed molecular dating analyses to infer divergence dates of the different lineages within the Herpestidae. Our results confirmed the paraphyly of the *Herpestes* genus and other phylogenetic relationships, which previously had only been moderately supported. The Asian herpestid species were found to form a monophyletic group within the Herpestidae. Within the Asian species, a cyto-nuclear conflict was discovered between the small Indian mongoose (*Herpestes auropunctatus*), the Indian gray mongoose (*Herpestes edwardsii*) and the Javan mongoose (*Herpestes javanicus*), which may have occurred through interspecific hybridization. This study inferred an Early Miocene origin for the Herpestidae and a Middle Miocene origin for the Asian mongooses.

1. Introduction

The mongooses (Carnivora, Herpestidae) are small-bodied carnivores (total length ranging from 34 to 151 cm, and body weight from 200 g to 5 kg; Gilchrist et al., 2009). The majority are mainly carnivorous, feeding on invertebrates or small vertebrates, while some species are omnivorous (Gilchrist et al., 2009). They are terrestrial and mostly diurnal, although some species, such as the marsh mongoose (*Atilax paludinosus*), white-tailed mongoose (*Ichneumia albicauda*) and Meller's mongoose (*Rhynchogale melleri*), are crepuscular or nocturnal (Ray, 1997; Kingdon, 1997; Gilchrist et al., 2009). Mongooses occupy a wide range of habitats, from deserts to tropical forests, across their natural range in Africa and Asia (Corbet and Hill, 1992; Kingdon, 1997). This distribution extends to the West Indies, Mauritius, Fiji, Okinawa and Adriatic Islands, where the small Indian mongoose (*Herpestes auropunctatus*) was introduced for biological control of rodents and snakes (Simberloff et al., 2000; Veron et al., 2007); this is now considered an invasive species in these islands (Morley, 2004; Yamada and Sugimura, 2004; Hays and Conant, 2007). The Egyptian mongoose (*Herpestes ichneumon*) is found in southern Europe (Wozencraft, 2005) and was also probably introduced in this area (Riquelme-Cantal et al., 2008).

The monophyly of the Herpestidae is supported by morphological (Pocock, 1919; Gregory and Hellman, 1939; Wozencraft, 1989), karyological (Fredga, 1972; Couturier and Dutrillaux, 1985) and molecular characters (Veron et al., 2004; Yu et al., 2004; Flynn et al., 2005; Perez et al., 2006). According to Wozencraft (2005), the Herpestidae comprises 33 species from 14 genera, with only one genus (*Herpestes*) occurring in Asia.

Previous molecular studies on the Herpestidae (Veron et al., 2004; Perez et al., 2006 and see also Flynn et al., 2005) revealed the existence of two main clades: (i) the true social mongooses (*Crossarchus*, *Helogale*, *Liberiictis*, *Mungos*, and *Suricata*), and (ii) the solitary mongooses (including the yellow mongoose *Cynictis penicillata*, which exhibits some social but not 'true' social behaviors; see Veron et al., 2004 for details). These results suggested a single origin for eusociality.

The preliminary results of these molecular studies also highlighted that *Herpestes* is not monophyletic as the two African species (*H. ichneumon* and *Herpestes naso*) are paraphyletic with regards to the Asian *Herpestes* species (Veron et al., 2004; Perez et al., 2006). This is also supported by karyological data as the Egyptian mongoose (*H. ichneumon*) displays important differences

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from the Asian *Herpestes* species (Fredga, 1972). This result was unexpected because the morphological literature consistently refers to *Herpestes* as a monophyletic unit (e.g., Taylor and Matheson, 1999). The marsh mongoose (*A. paludinosus*) and *Galerella* spp. were also often included in this genus (Fredga, 1972; Taylor, 1975; Wozencraft, 1989; Taylor and Goldman, 1993; Kingdon, 1997).

Veron et al. (2007) showed that the systematics of the Asian mongooses was unclear. This study, using mitochondrial DNA, revealed that the Javan mongoose (*Herpestes javanicus*) and small Indian mongoose (*H. auropunctatus*) are two distinct species, whereas previously, using morphological features, they were considered as one species (see Veron et al., 2007 for details). Moreover, the Javan mongoose was found to be the sister-taxon to the Indian gray mongoose (*Herpestes edwardsii*), rather than to the small Indian mongoose.

The Asian members of the herpestid family were not exhaustively sampled in these previous molecular studies, which only included the Javan mongoose (*H. javanicus*, *sensu* Veron et al., 2007), small Indian mongoose (*H. auropunctatus*, *sensu* Veron et al., 2007), Indian gray mongoose (*H. edwardsii,* Veron et al., 2004), and crab-eating mongoose (*Herpestes urva,* Perez et al., 2006). For this study we used samples from animals captured in the field and from museum specimens from almost all of the recognized mongoose species. For many of the Asian species, this is the first time they have been included in a molecular study of the Herpestidae. The main objective was to unravel the phylogeny of the Asian species and determine their systematic position within the mongoose family. We also aimed to estimate the divergence times within the Herpestidae. For this purpose, we used sequence data from mitochondrial DNA (mtDNA: Cytochrome *b* [Cytb] and NADH2 [ND2]) as well as nuclear DNA (nDNA: intron 7 of the β-fibrinogen [FGBi7]). Previously published sequences of a nuclear marker (Transthyretin intron 1 [TTRi1]) were also used to support the deepest nodes of the phylogeny.

2. Material and methods

2.1. Taxonomic sampling

Both fresh (hairs or tissue) and museum samples were used in this study (Table 1). Ten out of the eleven recognized species of *Herpestes* were included. The missing species was the collared mongoose (*Herpestes semitorquatus*); despite several attempts to obtain DNA from museum samples, we were unable to obtain any sequences for this species. Phylogenetic analyses were rooted by the fossa (*Cryptoprocta ferox*) and the Malagasy civet (*Fossa fossana*), two representatives of the Eupleridae, the Herpestidae's closest relatives (Yoder et al., 2003).

2.2. DNA extraction and sequencing

Total genomic DNA was isolated from fresh biological samples following a CTAB-based protocol (Winnepenninckx et al., 1993) and from museum specimen samples with a QIAamp DNA microkit (Qiagen). Yu et al.'s (2006) protocol was used prior to extraction to soften and pre-digest the samples. Dithiothreitol (DTT) was also added during the tissue lyses to increase the amount of DNA collected during extraction.

We used Cytb primers from Veron et al. (2004) and Gaubert et al. (2004), and ND2 primers from Perez et al. (2006). A new ND2 internal primer (ND2 IntR1: 5′-TTATTATGGTTGATGCTGC-3′) was especially designed to amplify a short 5′ end fragment (*ca.* 250 bp) from museum specimen extracts. The nuclear locus FGBi7, which allowed the resolution of the Asian Viverridae phylogeny (Patou et al., 2008), was amplified using primers from Yu and Zhang (2005).

PCRs were carried out in a 30 μl reaction volume containing 2.5 mM MgCl2, 0.26 mM dNTPs, 10 pmol of each primer, and 1.5 U Taq polymerase (QBiogene). The initial PCR cycle used for DNA amplification was 94 °C for 4 min. The following cycle was then repeated 35 times: 94 °C for 30 s, 49–55 °C (mtDNA) or 58–60 °C (nDNA) for 40–45 s, 72 °C for 30 s. The last extension step was 72 °C for 7 min. For the DNA from museum specimens, we performed two-step PCRs (Debruyne et al., 2003). After 94 °C for 3 min, the first step was 10 cycles as follows: 94 °C for 1 min, 52 °C (mtDNA) or 57 °C (nDNA) for 40 s, 72 °C for 40 s. The second step was repeated 25 times: 94 °C for 40 s, 54 °C (mtDNA) or 59 °C (nDNA) for 40 s, 72 °C for 40 s. The final extension was 7 min at 72 °C. PCR products were visualized on a 1.5% agarose gel and successfully amplified fragments were purified using ExoSap (GENOSCOPE Evry, France). PCR products were sequenced in both directions and run on an automated DNA sequencer (Applied Biosystems 3100) by GENOSCOPE. Sequences were edited and assembled using SEQUENCHER 4.14 (Gene Codes Corporation) and were queried with the BLASTN 2.2.18 program (Altschul et al., 1997) to check for putative contamination. Sequences were then aligned by eye in BIOEDIT (Hall, 1999).

2.3. Phylogenetic analyses

Phylogenetic analyses were performed using probabilistic reconstruction methods: Maximum Likelihood (ML) and Bayesian Inference. MRMODELTEST v2 (Nylander, 2004) was used to select the best-fitting evolutionary model (by gene and by codon position, when applicable), according to AIC criterion. Each gene (Cytb: 1140 bp; ND2: 1044 bp; FGBi7: 598 bp) was evaluated individually and checked for putative incongruences. The sequences of TTRi1 available on GenBank were also used. The datasets were compiled as follows: mitochondrial (Cytb + ND2), nuclear (FGBi7), and combined (Cytb + ND2 + FGBi7 + TTRi1). Taxa not represented for some parts of the dataset were coded as missing data. All the available samples were included in the mitochondrial analyses. In the combined analyses, we included only the individuals with the most complete sequence for each species (in bold in Table 1). PHYML 2.4 (Guindon and Gascuel, 2003) was used to perform the ML analyses and 1000 bootstrap (BP) replicates were run to assess the robustness of nodes. MRBAYES 3b4 (Huelsenbeck and Ronquist, 2001) was used to perform Bayesian analyses. Analyses were run for datasets partitioned into genes (Cytb, ND2, FGBi7, TTRi1) and into codon positions for coding genes (Cytb, ND2), to improve both estimation and accuracy of the phylogeny (Nylander et al., 2004; Brandley et al., 2005). Five Metropolis-coupled Markov chains Monte Carlo (MCMCMC) were run for 5×10^6 generations, with one tree sampled every 100 generations. Output parameters were explored using TRACER v 1.4 (Rambaut and Drummond, 2004) to make sure a sufficient number of generations were run and to settle the length of the "burn-in" period according to the Effective Sample Size (ESS) and Auto-Correlation Time (ACT) values. Analyses were conducted twice, independently, to check for convergence of the results.

2.4. Dating of divergence events

The fossil record of the Herpestidae is scarce and fragmentary (Peigné et al., 2005). The oldest fossil attributed to the Herpestidae is *Leptoplesictis* from the Early–Middle Miocene of Europe (*ca.* 18 millions years ago MYA; Roth, 1988) and from the Middle Miocene of Africa (Schmidt-Kittler, 1987; Hunt, 1996). The genera *Atilax* (Hill et al., 1985), *Ichneumia* and *Herpestes* (Morales et al., 2004) are documented from Late Miocene-dated sites in Africa, while

Table 1
List of the samples used in this study with their collection number, origin and GenBank Accession Numbers for Cytb, ND2, FGBi7 and TTRi1 genes. In bold are reported the samples included in the combined analyses. Samples from the AMNH and FMNH were from museum specimens. References linked to the sequences: (∗) this study; (a): Veron et al. (2004); (b) Perez et al. (2006); (c) Veron et al. (2007); (d) Gaubert et al. (2004); (e) Morley et al. (2007); (f) Yoder et al. (2003); (g) Flynn et al. (2005).

	Cytb	ND2	FGBi7	TTRi1
Atilax paludinosus	**AF522324[a] (Kenya)**	**AY974021[b] (Central Africa)**	**MNHN TC-138 FJ391185[*] (Liberia)**	**AY750604[g]**
Bdeogale crassicauda	**AY950654[b] (Tanzania)**	**–**	**MNHN TC-318 FJ391186[*] (Unknown)**	**AY950642[b]**
Bdeogale nigripes	**AY950655[b] (Gabon)**	**AY974022[b] (Gabon)**	**FMNH 167685 FJ391187[*] (Gabon)**	**AY950643[b]**
Crossarchus alexandri	**AF522326[a] (Captivity)**	**AY974024[b] (Captivity)**	**–**	**–**
Crossarchus obscurus	**AF522327[a] (Ivory Coast)**	**AY170041[f] (Unknown)**	**MNHN TC-140 FJ391189[*] (Liberia)**	**–**
Cynictis penicillata	**AF511060[a] (South Africa)**	**AY974025[b] (South Africa)**	**MNHN TC-35 FJ391190[*] (Sth Africa)**	**AY170024[f]**
Galerella pulverulenta	**AF522329[a] (South Africa)**	**AY974026[b] (South Africa)**	**MNHN TC-109 FJ391192[*] (Sth Africa)**	**AY950645[b]**
Galerella sanguinea	**AF522331[a] (South Africa)**	**AY974027[b] (South Africa)**	**MNHN TC-113 FJ391193[*] (Captivity)**	**AY950646[b]**
Helogale hirtula	**AF522335[a] (Captivity)**	**–**	**TAX 2339 FJ391199[*] (Captivity)**	**AY950647[b]**
Helogale parvula	**AF522333[a] (Captivity)**	**AY974028[b] (Captivity)**	**MNHN TC-30 FJ391205[*] (Captivity)**	**AY950650[b]**
Herpestes auropunctatus	**DQ519057[c] (Myanmar)**	**AY974031[b] (Guyana)**	**MNHN TC-340** FJ391194[*] **(Croatia)**	**–**
Herpestes auropunctatus	FMNH 129495 FJ391219[*] (Captivity)	FMNH 129495 FJ391226[*] (Captivity)	MNHN TC-221 FJ391195[*] (Myanmar)	–
Herpestes brachyurus	MNHN TC-480 **FJ391212**[*] (Malaysia)	MNHN TC-480 **FJ391228**[*] (Malaysia)	–	–
Herpestes brachyurus	**MNHN TC-493** FJ391213[*] **(Malaysia)**	**MNHN TC-493 FJ391229[*] (Malaysia)**	**MNHN TC-493 FJ391196[*] (Malaysia)**	**–**
Herpestes brachyurus	MNHN TC-429 FJ391211[*] (Malaysia)	MNHN TC-429 FJ391227[*] (Malaysia)	–	–
Herpestes brachyurus	FMNH 85936 FJ391223[*] (Borneo)	–	–	–
Herpestes brachyurus	FMNH 85604 FJ391224[*] (Borneo)	–	–	–
Herpestes brachyurus	FMNH 43343 FJ391225[*] (Sumatra)	–	–	–
Herpestes edwardsii	**AF522336[a] (Bahrain)**	**AY974029[b] (Bahrain)**	**MNHN TC-144 FJ391197[*] (Bahrain)**	**AY170025[f]**
Herpestes edwardsii	DQ519055[c] (Bangladesh)	MNHN TC-295 FJ391230[*] (Bangladesh)	MNHN TC-295 FJ391198[*] (Bangladesh)	–
Herpestes fuscus	**AMNH 217678 FJ391214[*] (Sri-Lanka)**	**–**	**–**	**–**
Herpestes fuscus	**FMNH 95035 FJ391215[*] (Sri-Lanka)**	**FMNH 95035 FJ391232[*] (Sri-Lanka)**	**–**	**–**
Herpestes ichneumon	**AF511059[d] (Spain)**	**AY974030[b] (Spain)**	**MNHN C-21 FJ391200[*] (Spain)**	**AY950648[b]**
Herpestes javanicus	**DQ519058[c] (Thailand)**	**MNHN 99-510 FJ391233[*] (Thailand)**	**MNHN 99-510 FJ391201[*] (Thailand)**	**AY170026[f]**
Herpestes javanicus	DQ519057[c] (Thailand)	MNHN TC-258 FJ391234[*] (Thailand)	MNHN R3406 FJ391202[*] (Thailand)	–
Herpestes javanicus	DQ519060[c] (Vietnam)	–	MNHN TC-472 FJ391203[*] (Vietnam)	–
Herpestes javanicus	AMNH 101655 FJ391216[*] (Java)	–	–	–
Herpestes naso	**AF522339[a] (Gabon)**	**AY974032[b] (Gabon)**	**MNHN TC-226 FJ391204[*] (Gabon)**	**AY950649[b]**
Herpestes smithii	**AMNH 171168 FJ391217[*] (India)**	**–**	**–**	**–**
Herpestes smithii	**AMNH 163179 FJ391218[*] (India)**	**–**	**–**	**–**
Herpestes sp. (Fiji)	**DQ391277[e] (Fiji is.)**	**FJ391231[*] (Fiji is.)**	**FJ391191[*] (Fiji is.)**	**–**
Herpestes urva	**DQ519074[c] (Vietnam)**	**AY974033[b] (Taiwan)**	**–**	**–**
Herpestes urva	FMNH 39355 FJ391220[*] (China)	FMNH 39355 FJ391235[*] (China)	–	–
Herpestes urva	FMNH 39352 FJ391221[*] (China)	FMNH 39352 FJ391236[*] (China)	–	–
Herpestes vitticollis	**AMNH 163180 FJ391222[*] (India)**	**–**	**–**	**–**
Ichneumia albicauda	**AF511058[d] (Saudi Arabia)**	**AY974034[b] (Uganda)**	**MNHN C-79 FJ391206[*] (Saudi Arabia)**	**AY950651[b]**
Liberiictis kuhni	**AF522342[a] (Ivory Coast)**	**AY974035[b] (Liberia)**	**FN 28732 FJ391207[*] (Liberia)**	**AY950652[b]**
Mungos mungo	**AF522348[a] (Captivity)**	**AY170035[f] (Unknown)**	**ISEM 4678 FJ391208[*] (Captivity)**	**AY170017[f]**
Paracynictis selousi	**–**	**AY750651[g] (Unknown)**	**–**	**AY750606[g]**
Rhynchogale melleri	**AF522344[a] (South Africa)**	**AY974036[b] (South Africa)**	**MNHN TC-224 FJ391209[*] (Sth Africa)**	**AY950653[b]**
Suricata suricatta	**AF522345[a] (South Africa)**	**AY170054[f] (Unknown)**	**MNHN TC-46 FJ391210[*] (Sth Africa)**	**AY170028[f]**
Cryptoprocta ferox	**AF125139[d]**	**AY170036[f]**	**ISEM 4613 FJ391188[*]**	**AY170018[f]**
Fossa fossana	**AF511062[d]**	**AY170037[f]**	**–**	**AY170019[f]**

the genus *Galerella* is known to be present in the Late Miocene of Africa (*ca.* 7 MYA; Peigné et al., 2005). Fossils attributed to *Herpestes* are also found in the Late Miocene of Pakistan (9.5–7 MYA; Barry, 1983). However, the attribution of *Herpestes* fossils remains ambiguous as this genus is suspected to be paraphyletic, so no *Herpestes* calibration point was used in the analysis. Given the datasets compiled here and the reliability of fossil attribution, we chose to use only two calibration points: one as the minimum boundary for the Herpestidae, *Leptoplesictis* (18 MYA), and one as the minimum boundary for the *Galerella* genus (7 MYA).

Divergence ages were estimated using a relaxed molecular clock approach, as implemented in the PAML/MULTIDIVTIME package (Thorne et al., 1998; Kishino et al., 2001; Thorne and Kishino, 2002) and following Rutschmann (2005) instructions. BASEML (PAML 3.14b Yang, 1997) was used to estimate model parameters, and ESTBRANCHES was then used to estimate branch lengths of the rooted tree and to generate a variance–covariance matrix of branch lengths from the dataset. Finally, we performed a Bayesian estimation of divergence times, via MCMC, using MULTIDIVTIME. The Markov chain was run for 1×10^5 generations with one tree sampled every 100 generations and a burn-in period of 1×10^4 generations (10%). Analyses were performed twice to ensure convergence of the results. To thoroughly explore our data and determine the impact of the calibration points on the divergence time estimates, analyses were completed on each marker and a combination of markers, with the two calibration points considered separately or together. The combined analyses included the taxa with the markers Cytb, ND2 and FGBi7 (not TTRi1, since this was missing for too many taxa; see Table 1).

3. Results

3.1. Phylogenetic results

The models used for each analysis, with their associated parameters, are available in Appendix 1. Datasets examined individually did not show strong incongruence relating to the Herpestidae phylogeny, except cyto-nuclear conflicts concerning (i) the species *H. edwardsii*, *H. javanicus* and *H. auropunctatus*, (ii) the position of *H. naso*. In the mitochondrial tree (Fig. 1a), (i) *H. edwardsii* and *H. javanicus* were sister-species and *H. auropunctatus* was sister to these two species and (ii) *H. naso* is the sister-species to *A. paludinosus*. Whereas, in the tree obtained from the FGBi7 analysis (Fig. 1b), (i) *H. auropunctatus* and *H. javanicus* formed a monophyletic group, with a strong support and (ii) *H. naso* is included within

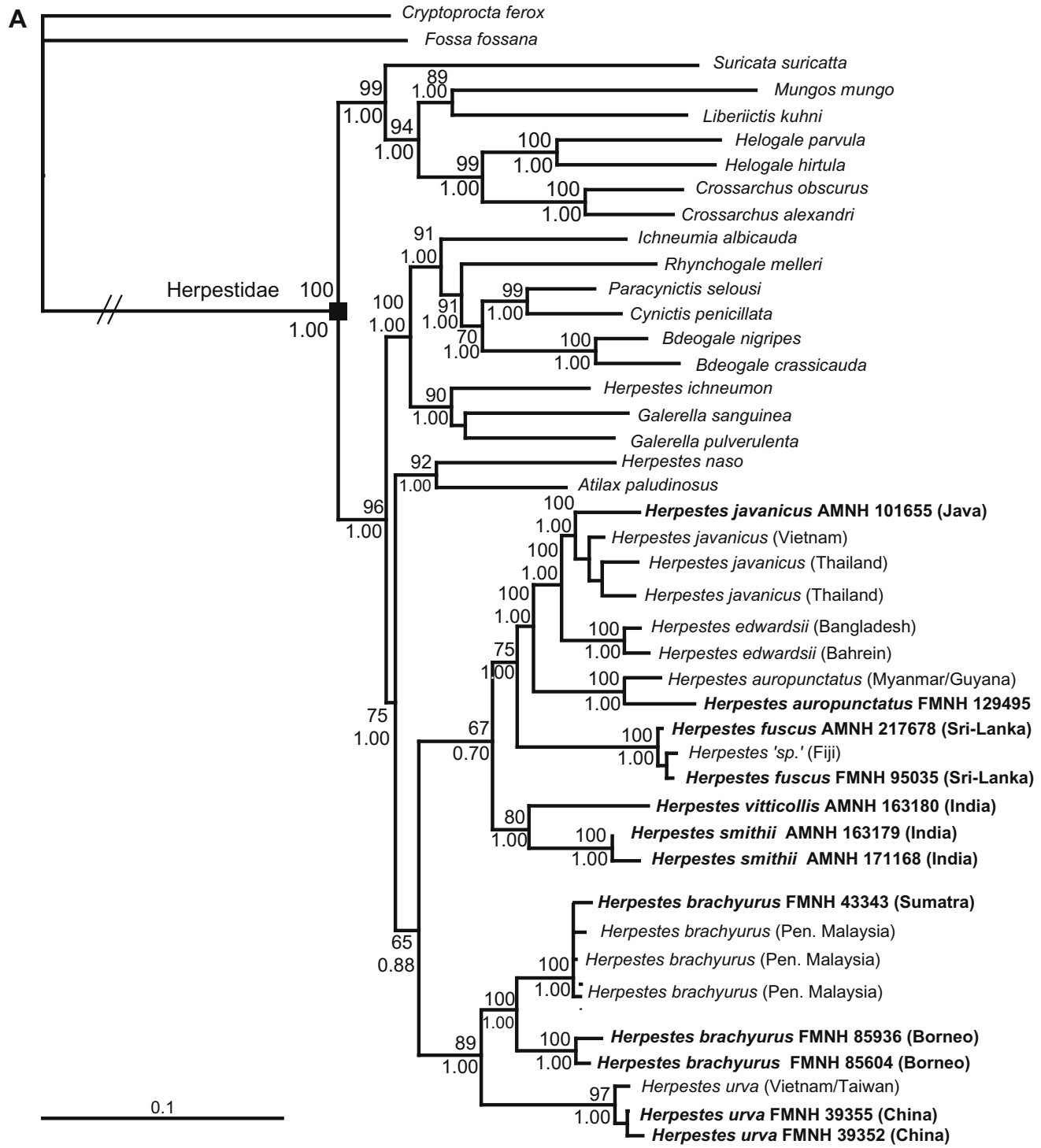

Fig. 1. Phylogenetic tree obtained from Bayesian analyses of: (A) the mitochondrial (Cytb + ND2) dataset and (B) the nuclear (FGBi7) dataset. BP_{ML} and BPP values are reported for each node. Localities of Asian samples are reported. Sequences obtained from museum specimens (with their number) are in bold.

the Asian *Herpestes* clade while *A. paludinosus* is the sister-group to this clade.

The tree resulting from the Bayesian analyses of the combined matrix (3550bp) was well resolved with high nodal supports (Fig. 2). Monophyly of the Herpestidae was strongly supported, as in all previous molecular studies. The major split within the Herpestidae was also retrieved and highly supported with: (i) the eusocial mongoose clade (*Suricata, Helogale, Crossarchus, Liberiictis*, and *Mungos*) and (ii) the solitary mongoose clade (*Ichneumia*, *Cynictis*, *Paracynictis*, *Rhynchogale*, *Bdeogale*, *Herpestes*, *Galerella*, and *Atilax*).

The eusocial mongooses formed a monophyletic clade, with the meerkat (*Suricata suricatta*) a sister-species to all other genera (*Crossarchus, Helogale*, *Liberiictis*, and *Mungos*). The sister-relationship between *Liberiictis kuhni* and *Mungos mungo* was strongly supported and together they constituted the sister-group to

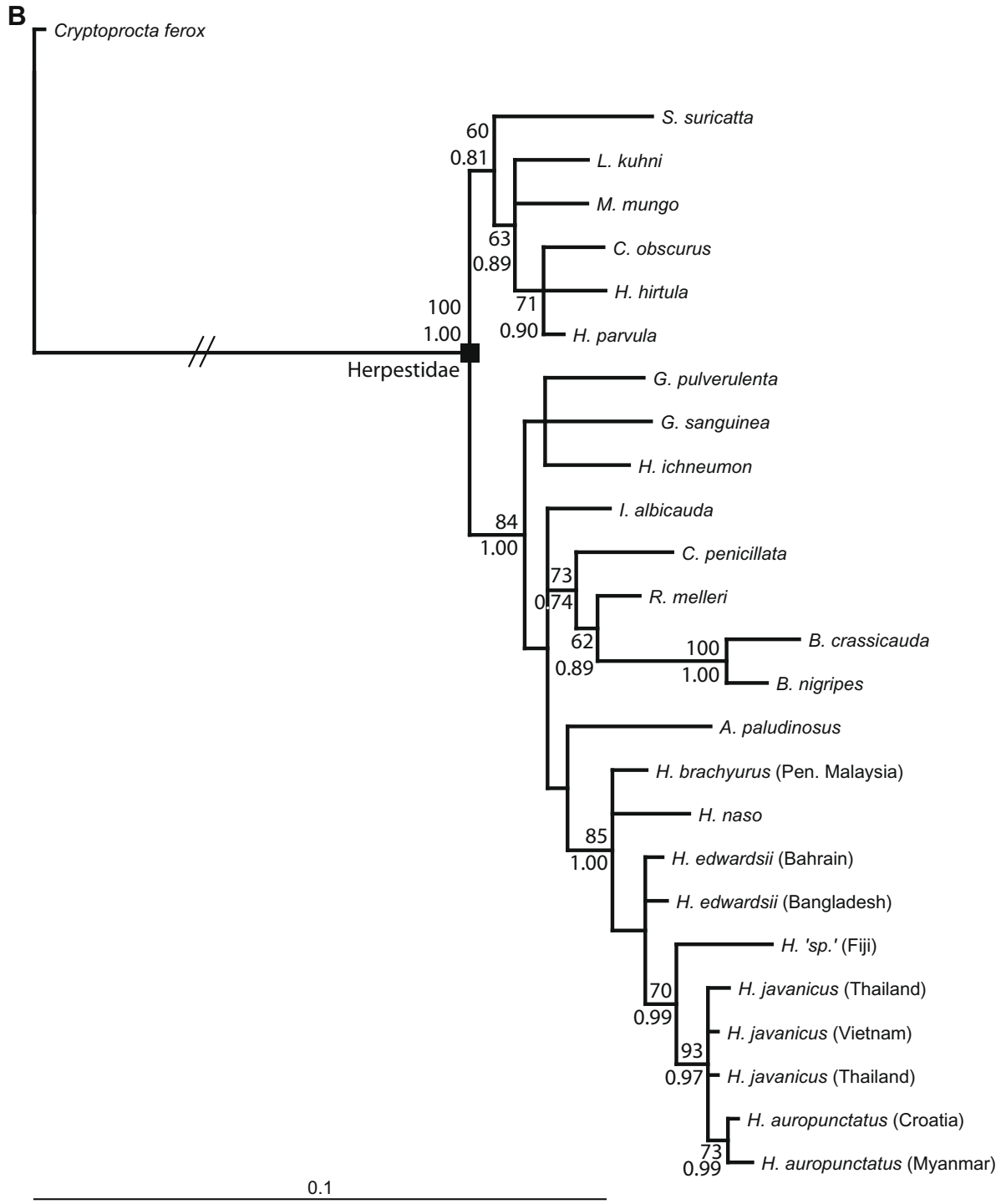

Fig 1. (*continued*)

Crossarchus and *Helogale*. The monophylies of *Crossarchus* and *Helogale* were strongly supported, as well as their sister-relationship.

Within the solitary mongooses, two strongly supported clades were revealed: one containing African mongooses (*Ichneumia, Cynictis, Paracynictis, Rhynchogale, Bdeogale, Galerella*, and *H. ichneumon*) and the other comprising the Asian mongooses (*Herpestes*) and the African species *A. paludinosus* and *H. naso*. As already shown in previous studies, and here confirmed with an exhaustive sampling, the *Herpestes* genus is paraphyletic.

The Egyptian mongoose (*H. ichneumon*) and the *Galerella* species formed a monophyletic group (the '*Herpestes–Galerella* group'), sister-group to the monophyletic clade that included *Ichneumia*, *Cynictis*, *Paracynictis*, *Rhynchogale*, and *Bdeogale* (the '*Ichneumia* group'). Within the '*Herpestes–Galerella* group', the monophyly of *Galerella* was only moderately supported. Within the well-supported monophyletic '*Ichneumia* group', the white-tailed mongoose (*I. albicauda*) was the sister-species to all others. The yellow mongoose (*C. penicillata*) and Selous' mongoose (*Paracynictis selousi*) were sister-species, and together they were a sister-group

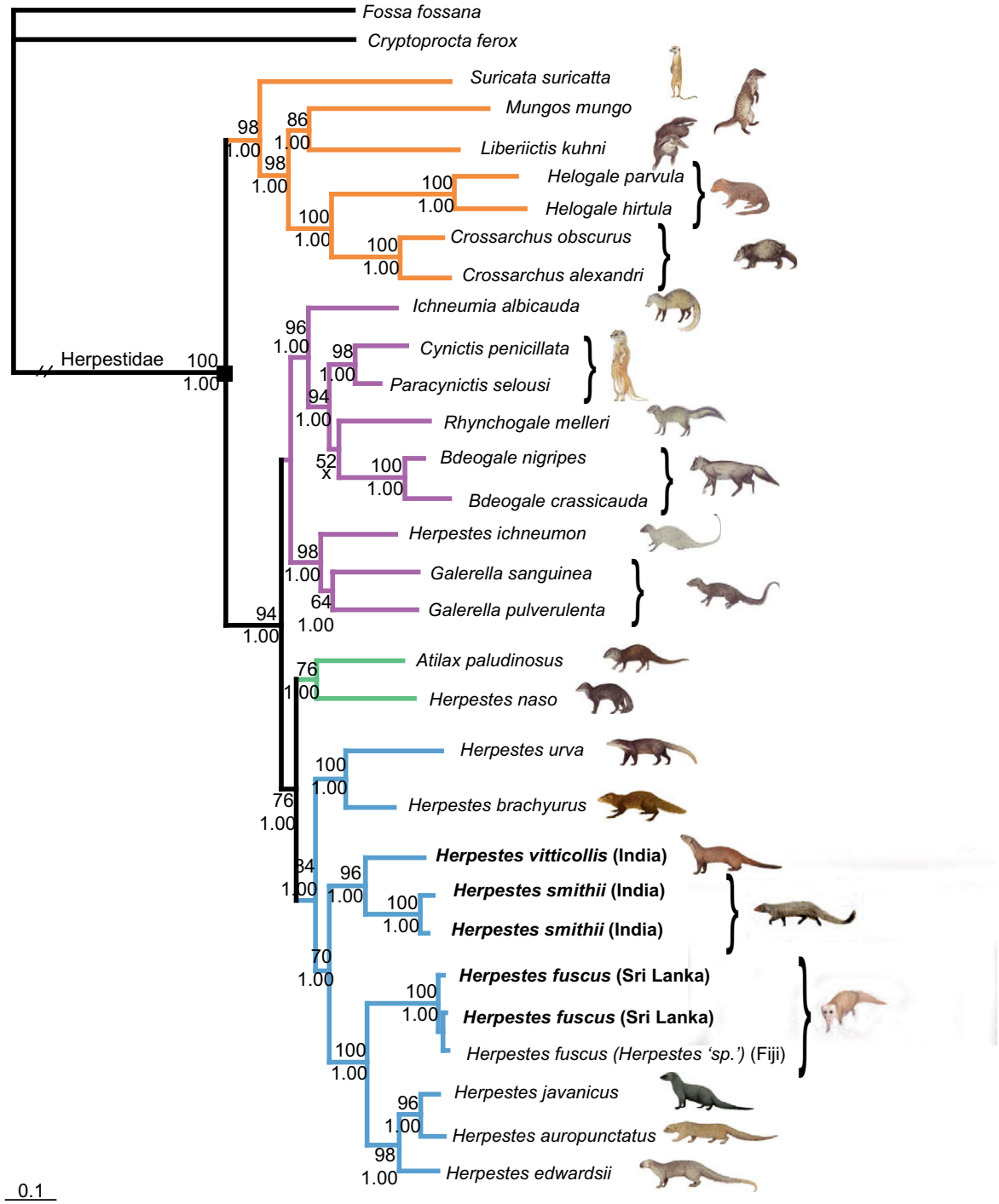

Fig. 2. Phylogenetic tree obtained from Bayesian analyses of the combined dataset: Cytb, ND2, FGBi7 and TTRi1 (3350bp). BP_{ML} and BPP values are reported for each node. (×) indicates BPP values inferior to 0.70. Sequences obtained from collection specimens are in bold (with their geographic origin). Illustrations modified from Kingdon (1997) and Prater (1971).

to the *Rhynchogale* and *Bdeogale* genera. Meller's mongoose (*R. melleri*) was suggested to be the closest relative to the monophyletic *Bdeogale* genus, with moderate supports.

There was strong support for the monophyly of the clade containing the Asian *Herpestes* species, *A. paludinosus* and *H. naso*. There was also strong support for the sister-relationship of *A. paludinosus* and *H. naso* and their position as a sister-group to the Asian species. The short-tailed mongoose (*Herpestes brachyurus*) and the crab-eating mongoose (*H. urva*) were retrieved as sister-species and as a sister-group to all the other Asian *Herpestes* species. Concerning *H. brachyurus*, for which several individuals were sampled (Fig. 1a), individuals from Borneo (FMNH 85936 and FMNH 85604) proved to be distinct from all the others sampled in Peninsular Malaysia and Sumatra.

The stripe-necked mongoose (*Herpestes vitticollis*) and the ruddy mongoose (*Herpestes smithii*) were sister-species, with strong supports. An undetermined species from the Fiji islands clustered unambiguously with two Indian brown mongoose individuals (*Herpestes fuscus*), allowing its identification as a *H. fuscus* representative. All the *H. fuscus* representatives, both from the original

distribution range and from an introduced area (Fiji), were monophyletic and were the sister-species to a species complex composed of *H. auropunctatus*, *H. javanicus* and *H. edwardsii*. The monophylies of *H. auropunctatus* and of *H. javanicus* were each strongly supported (Fig. 1a).

3.2. Dating analyses results

Dating analyses, performed using the two calibration points either alone or combined, provided overlapping divergence date estimates, although the analyses calibrated using only the *Galerella* fossil retrieved slightly more recent estimates. Whatever the dataset used (mitochondrial, nuclear or combined), dating analyses also provided overlapping estimates, although the nuclear dataset retrieved slightly older estimates, with larger standard deviations. Since the results were congruent, we chose to discuss only the results obtained from the combined dataset calibrated by the two available fossils, except for certain nodes estimated only from the mitochondrial data (Fig. 3).

The Herpestidae are inferred to have an Early Miocene origin (21.8 MYA ± 3.6 standard deviation). The two main lineages (solitary and social mongooses) diverged almost synchronously in the Early Miocene (18.5 MYA ± 3.3 and 19.1 MYA ± 3.5, respectively). Within the solitary mongooses, the two clades (*Ichneumia, Cynictis, Paracynictis, Rhynchogale, Bdeogale, Galerella*, and *H. ichneumon*) and (Asian *Herpestes*, *A. paludinosus*, and *H. naso*) both diverged soon after this main split within the Herpestidae (17.4 MYA ± 3.2 and 18.5 MYA ± 3.3, respectively). The Asian *Herpestes* diverged in the Early to Middle Miocene (15.0 MYA ± 3.0).

4. Discussion

4.1. The phylogeny of the Herpestidae

Results from previous molecular studies concerning the Herpestidae are here supported and consolidated by the addition of new molecular data. The monophyly of the social mongooses clade is confirmed by our analyses, which is also supported by a putative synapomorphy: the situation of the *foramen rotundum* alongside the anterior orifice of the alisphenoid canal and close to the sphenoidal fissure (Pocock, 1919). Within this clade, the use of the nuclear gene FGBi7 brought additional support for the sister-relationship between the Liberian mongoose (*L. kuhni*) and the banded mongoose (*M. mungo*), as was suggested by previous studies (Veron et al., 2004; Perez et al., 2006).

The major division within the solitary mongooses was also recovered by this study: the (*Ichneumia*, *Cynictis*, *Paracynictis*, *Rhynchogale*, *Bdeogale*, *Galerella*, and *H. ichneumon*) clade and the (Asian *Herpestes*, *A. paludinosus*, and *H. naso*) clade. The sister-relationship between the '*Ichneumia* group' and the '*Herpestes-Galerella* group' was strongly supported in this study, whereas previously this relationship only had weak support. Within the '*Ichneumia* group', there were still only moderate supports to designate *R. melleri* as the closest relative to the *Bdeogale* genus. The nuclear gene FGBi7 alone retrieved a polytomy *Cynictis/Rhynchogale/Bdeogale*, which probably results from its poor variability. The monophyly of the Asian *Herpestes*, and the sister-relationship between the clade (*A. paludinosus* + *H. naso*) and the Asian *Herpestes*, was also strongly supported in the combined analyses.

4.2. The Herpestes genus

4.2.1. Paraphyly of Herpestes

Our study confirmed that the genus *Herpestes* is paraphyletic (see Veron et al., 2004; Perez et al., 2006). The two African species (*H. naso* and *H. ichneumon*) and the monophyletic Asian *Herpestes* clade belong to three distinct lineages.

The long-nosed mongoose (*H. naso*) was retrieved as the closest relative to the marsh mongoose (*A. paludinosus*). Allen (1939) actually placed *H. naso* in the *Atilax* genus. However, although Orts (1970) and Ray (1997) found morphological similarities between these two species, several morphological differences have been described (in the webbing on the digits, dental formula, and skeleton; Orts, 1970; Rosevear, 1974; Ray, 1995). We have also noticed an important difference in the shape of the tympanic bulla, resulting from differences in the relative development of the ectotympanic compared to the entotympanic (pers. obs.). Another important distinctive feature between these two species is ecological: *H. naso* is diurnal and *A. paludinosus* is crepuscular (Ray, 1997). These morphological and ecological differences, a genetic distance value of *ca.* 10% (Cytb) between these two species, and a fairly ancient last common ancestor (*ca.* 13.6 MYA), argue in favor of keeping these two species in two separate genera. However, the phylogenetic position of *H. naso* needs to be confirmed since the nuclear result suggested a closer relationship to the Asian *Herpestes* clade rather than to *A. paludinosus*.

The inclusion of the Egyptian mongoose (*H. ichneumon*) in the same clade as the *Galerella* genus was strongly supported. This clade is also supported by chromosomal evidence, with both genera having 19–21 pairs of autosomes (*versus* 17 pairs in all other species; Fredga, 1972). According to the molecular dating analyses, the last common ancestor of these two genera occurred *ca.* 15.4 MYA. Our results suggest that the *Galerella* genus is monophyletic, but this is only moderately supported by the combined dataset, and all the recognized *Galerella* species were not included. The molecular dating analyses revealed that the last common ancestor of *G. sanguinea* and *G. pulverulenta* was quite ancient (*ca.* 13.5 MYA). The relationships within this clade have always been unclear, and support for the monophyly of the *Galerella* genus has been lacking in previous molecular studies (Veron et al., 2004; Perez et al., 2006). Some of the results from the study of Taylor et al. (1991), based on allozymes, suggested a close relationship between *H. ichneumon* and *G. pulverulenta.* The cytogenetic study of Fredga (1972) showed a greater similarity between *H. ichneumon* and *G. sanguinea* than between *G. sanguinea* and *G. pulverulenta*.

From a karyological viewpoint, the '*Herpestes-Galerella* group' shares the presence of a translocated Y chromosome with the Asian *Herpestes* + *A. paludinosus* + *H. naso* clade (Wurster and Bernischke, 1968; Fredga, 1972). According to the phylogeny retrieved here, there are two equally parsimonious ways for this chromosomal evolution to have occurred within the Herpestidae. The first scenario implies a translocation event of the Y chromosome to an autosome in the common ancestor of the solitary mongooses. This event would have been followed by a fission of the Y chromosome in the '*Ichneumia* group'. A second scenario would imply two independent translocation events of the Y chromosome to an autosome in: (i) the '*Herpestes–Galerella* group' and (ii) the Asian *Herpestes* + *A. paludinosus* + *H. naso* clade. In this latter case, the observation of a translocated Y chromosome in these two distinct groups would be a convergence.

4.2.2. The Asian Herpestes

The Asian *Herpestes* mongooses form a monophyletic group, suggesting a unique Asian ancestor for all these species. A well-supported sister-relationship unites the short-tailed mongoose (*H. brachyurus*) to the crab-eating mongoose (*H. urva*), which are two species that are often found close to water bodies (pers. obs.). This phylogenetic affinity has already been suggested by craniometrical analyses (Taylor and Matheson, 1999), anatomical characters, (such as the posterior development of the ectotympanic; B. Li pers. com. and pers. obs.), and by chromosomal evidence

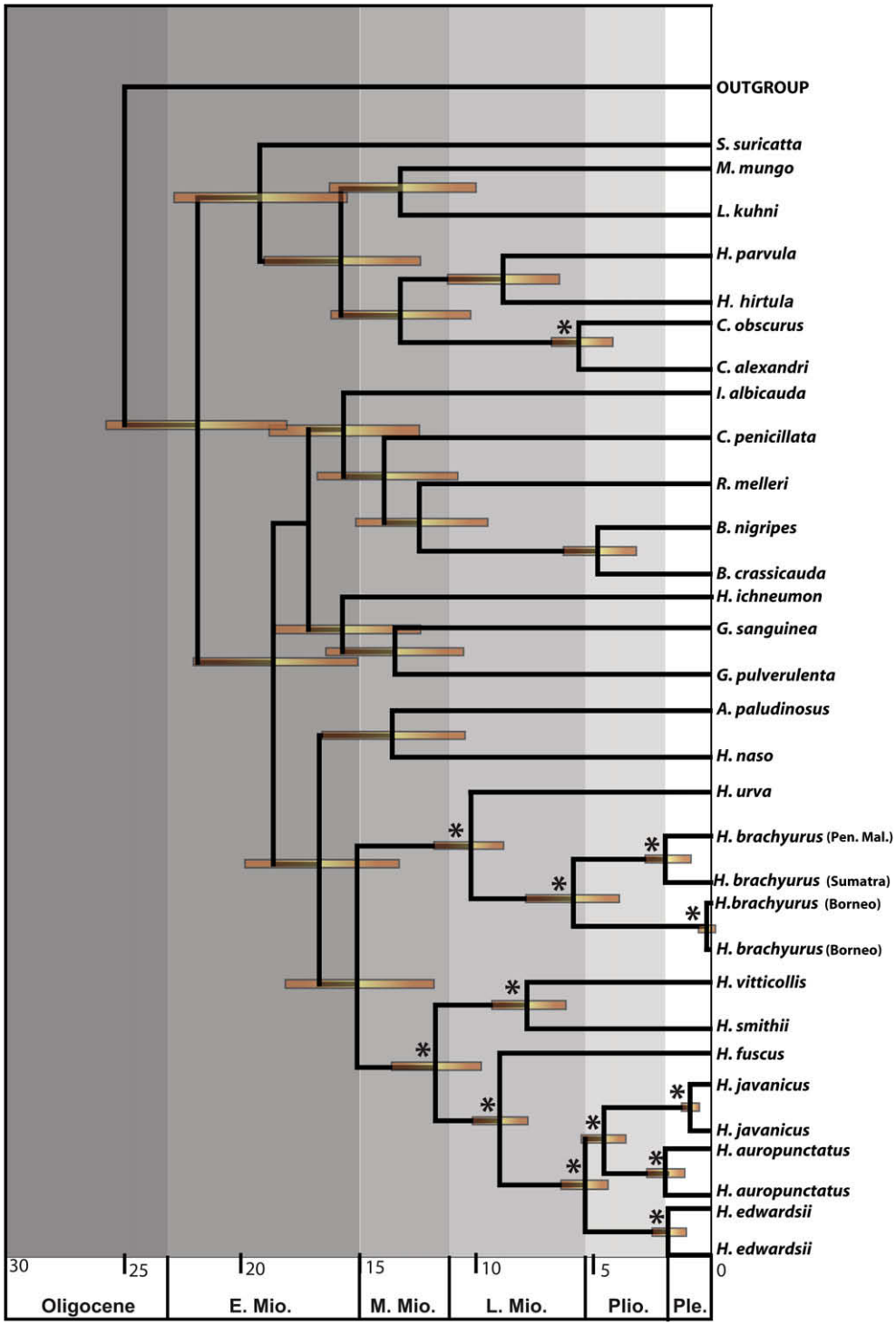

Fig. 3. Chronogram of the Herpestidae family. Bars correspond to the standard deviations of the divergence date estimated from the combined dataset. (∗) mark the estimates obtained from the mitochondrial dataset only, owing to the absence of nuclear sequences for these taxa. E. Mio. = Early Miocene (23–15 MYA); M. Mio. = Middle Miocene (15–11 MYA); L. Mio. = Late Miocene (11–5 MYA); Plio. = Pliocene (5–1.8 MYA); Ple. = Pleistocene (1.8–0.01 MYA).

(Fredga, 1972). Their distribution is allopatric, except in Peninsular Malaysia where they both occur (Wozencraft, 2005; Gilchrist et al., 2009), and where it is likely that these two species are ecologically separated.

Several individuals of *H. brachyurus* from Peninsular Malaysia, Sumatra and Borneo were included in our analyses. They correspond to different described subspecies: *H. b. brachyurus* Gray, 1836 (Peninsular Malaysia), *H .b. sumatrius* Thomas, 1921 (Sumatra), and *H. b. rajah* Thomas, 1921 (Borneo). The subspecies *H. b. palawanus* (Allen, 1910), from Palawan Island (Philippines), remains to be tested. Our results clearly showed that the individual from Sumatra groups within the Peninsular Malaysia clade, while the two individuals from Borneo are distinct from all the others (with *ca.* 6% of genetic divergence). The taxonomic status of the Bornean individuals requires further studies, as only mitochondrial data were available in our analyses.

The number of *Herpestes* taxa present on Borneo is unclear in the literature (e.g., Payne et al., 1985; Corbet and Hill, 1992). Currently, two species, the short-tailed mongoose (*H. brachyurus*) and collared mongoose (*H. semitorquatus* Gray, 1846), are recognized to occur on Borneo (Wozencraft, 2005). Thomas (1921) divided *H. brachyurus* into two subspecies on Borneo, *H. b. rajah* and *H. b. dyacorum*, corresponding to a lowland and a mountain form. Jentink (1903) described another Borneo mongoose species, Hose's mongoose (*Herpestes hosei*), based on an adult female collected from Sarawak in 1893, which is the only specimen that has been attributed to this species. This specimen is very similar to *H. brachyurus*, but was treated as a separate species on the basis of distinctive coloration and a less rounded coronoid process of the lower mandible (Jentink, 1903; Payne et al., 1985). The type specimen of *H. hosei* (RMNH 34987) and several specimens of *H. semitorquatus* (AMNH 108803, AMNH 103746, BMNH 1892.2.7.2 and RMNH 20803) were sampled in this study but they all yielded no DNA, so we were unable to assess the systematic status of these species using molecular data. Nonetheless, comparison of morphological characters of *H. brachyurus* specimens from Borneo and the type specimen of *H. hosei* did not reveal any marked differences (pers. obs.) to support a specific status for Hose's mongoose (in agreement with Corbet and Hill, 1992 and Wozencraft, 2005). Although *H. semitorquatus* has been seen as a subspecies of *H. brachyurus* by Schwarz (1947), specimens observed in RMNH and BMNH collections exhibited several marked differences in pelage color (much more red in *H. semitorquatus*), size of the M^2 (smaller in *H. semitorquatus* than in *H. brachyurus*), length of the tail (shorter in *H. brachyurus*), and in the presence of a conspicuous stripe on the neck in *H. semitorquatus* (Gray, 1837; pers. obs.), supporting their attribution to two distinct species.

The occurrence of *H. semitorquatus* on Sumatra is currently unclear. There are only two confirmed records: one specimen from the Ophir District, West Sumatra (the holotype *H. s. uniformis* Robinson and Kloss, 1919) and one specimen from Soekadana, South Sumatra (Jentink, 1894). The type specimen *H. s. uniformis* (RMNH 20803) unfortunately did not provide DNA; however, based on its morphology (pers. obs.), it is clearly a red morph of *H. semitorquatus* (and not a red morph of *H. brachyurus*, as suggested by Schwarz, 1947). The other specimen is missing in the RMNH, and its locality name exists both on Borneo and Sumatra (see Jentink, 1894).

The stripe-necked mongoose (*H. vitticollis*) and the ruddy mongoose (*H. smithii*) are here retrieved as sister-taxa. These two species are restricted in distribution to India and Sri Lanka. They are both forest species and diurnal, but it is suggested that *H. vitticollis* is only found near water bodies, while *H. smithii* occupies a wider variety of habitats (Santiapillai et al., 2000). Their sister-relationship has never been proposed and these results contradict Pocock's (1937) hypothesis that *H. smithii* is very close to *H. edwardsii* and may even be a 'jungle form' of the latter.

Herpestes javanicus and *H. auropunctatus* formed two distinct and well-supported clades, as was previously found by Veron et al. (2007). The Javan mongoose (*H. javanicus*) is sister-taxon to the Indian gray mongoose (*H. edwardsii*) in the mitochondrial tree, but in the nuclear (FGBi7) tree, it is more closely related to *H. auropunctatus* than to *H. edwardsii*. This discrepancy may have resulted from an exchange of genetic material between these species (see below). The Indian brown mongoose (*H. fuscus*) was found to be the closest relative to the clade *H. edwardsii*/*H. auropunctatus*/*H. javanicus*. This phylogenetic arrangement has already been suggested by Fredga (1972), based on observations of karyotypes, and invalidates the proposition of Bechthold (1939), who considered *H. fuscus* and *H. brachyurus* to be conspecific.

An interesting result of this study was the identification of a second mongoose species present in the Fiji islands (in addition to the introduced small Indian mongoose), as was suggested by Morley et al. (2007). Here, we are able to clearly identify this second species as the Indian brown mongoose (*H. fuscus*). This means that there has been a second recent and undocumented introduction of a mongoose species since that of a single pair of *H. auropunctatus* from Calcutta in 1883 (Simberloff et al., 2000).

4.3. A nucleo-cytoplasmic conflict

An interesting conflict was revealed between mitochondrial and nuclear signals concerning the *H. auropunctatus*/*H. edwardsii*/*H. javanicus* species complex. In a previous study, we showed a clear genetic differentiation between *H. auropunctatus* and *H. javanicus*, which were usually recognized as a single species (see Veron et al., 2007). The natural range of *H. auropunctatus* is across Central Asia, whereas *H. javanicus* occurs throughout Southeast Asia. The Salween River in Myanmar may act as a potential geographical barrier, with *H. auropunctatus* occurring west and *H. javanicus* east of it. *H. edwardsii* occurs largely in sympatry with *H. auropunctatus*, but is not sympatric with *H. javanicus* (Veron et al., 2007). Using mitochondrial data, a well-supported relationship (*H. auropunctatus*, (*H. edwardsii, H. javanicus*)) was retrieved. Using nuclear data (FGBi7), a different phylogenetic pattern was recovered, with *H. javanicus* more closely related to *H. auropunctatus* than to *H. edwardsii*. *H. auropunctatus* and *H. javanicus* remain distinct species whether mitochondrial or nuclear datasets were used.

How can this cyto-nuclear conflict be explained? Apart from a potential lack of resolution of the nuclear marker, three different hypotheses could explain this conflicting pattern: inclusion of paralogues or pseudogenes in the phylogenetic analysis; incomplete lineage sorting; and interspecific hybridization (see Funk and Omland, 2003 for review). The inclusion of paralogues or pseudogenes can be *a priori* dismissed for the nuclear gene (FGBi7 since another nuclear marker (IRBP intron 1) was tested for a subset of individuals and this provided the same topology (i.e., (*H. edwardsii*, (*H. auropunctatus, H. javanicus*)); unpubl. data). This can also be dismissed for mitochondrial sequences since only coding genes were used and the translation of the nucleotide sequences into protein sequences did not show any changes in the amino acids or any stop-codons. Also, there are no shifts in the reading frames of the genes and the sequences are similar to those previously published on GenBank and obtained with different and independent primer sets (Fernandes et al., 2008).

Incomplete lineage sorting could be hypothesized to account for the mitochondrial pattern, whereby *H. javanicus* and *H. edwardsii* share mitochondrial allelic copies of their last common ancestor. However, given the differential coalescence times in mitochondrial and nuclear genes (much shorter in mitochondrial genes, Curole and Kocher, 1999), incomplete lineage sorting would be expected to occur in the nuclear tree rather than in the mitochondrial tree. As the opposite was observed, we reject this hypothesis.

Interspecific hybridization is the most likely scenario. The ancestor of *H. javanicus* may have been introgressed by the mitochondrial genome of *H. edwardsii* through hybridization, which would then account for the conflicting mitochondrial topology. This introgression event may be reflected in the mitochondrial dating results, which yielded a more ancient origin (*ca.* 2 MYA) for *H. edwardsii* and *H. auropunctatus* than for *H. javanicus* (*ca.* 0.5 MYA). However, this introgression needs to be confirmed, as *H. javanicus* and *H. edwardsii* are allopatric. On the other hand, evidence of interspecific hybridization between *H. auropunctatus* and *H. edwardsii* has been documented, based on microsatellite analyses (Thulin et al., 2006). These results suggest that gene flow may occur between these three species, but a more comprehensive study is required to evaluate this further. This would involve exploration of several nuclear and Y-linked loci to better understand the

underlying mechanisms of hybridization (as, for example, in Hassanin and Ropiquet, 2007).

4.4. Biogeographical considerations

Our study estimated that the Herpestidae diverged from their sister-group (Eupleridae) in the Late Oligocene/Early Miocene, around 22 MYA. This is older than the estimate obtained by Koepfli et al. (2006), who retrieved a divergence date of 16.4 MYA. However, they did not include an exhaustive sample set of herpestid species, which may have biased their dating results.

The place of origin of the Herpestidae remains unclear. While the phylogenetic arrangement yielded by this study may argue for an African origin of the Herpestidae, the oldest known herpestid fossil was described from an European site (Roth, 1988). An African origin would imply a single migration event from Africa to Asia, probably through the Arabian microplate, allowing for the differentiation of the extant Asian *Herpestes* clade. The divergence date obtained for the Asian *Herpestes* (*ca.* 15 MYA) and the age of the most ancient fossil attributed to the Herpestidae, *Leptoplesictis* from Europe (18 MYA), are congruent with this scenario. An Eurasian-origin hypothesis would imply a migration event of a common ancestor of the extant Herpestidae from Eurasia to Africa, and then a migration from Africa to Asia, leading to the further diversification of Asian mongooses. Such retro-migration events have been inferred, for example, in the Viverridae, according to the fossil record (Gaubert and Cordeiro-Estrela, 2006).

Soon after the split of the Asian *Herpestes* in the Middle Miocene (*ca.* 15 MYA), many divergence events occurred within the Asian mongooses during the period 11–8 MYA (Late Miocene), with one branch leading to extant (*H. brachyurus* + *H. urva*) and another leading to the remaining Asian *Herpestes* species. Ancestors of the clade (*H. fuscus*, *H. edwardsii*, *H. javanicus* and *H. auropunctatus*) and of the clade (*H. smithii* and *H. vitticollis*) also diverged and diversified within this timeframe. Many divergence events took place in the Middle–Late Miocene in Asia in other mammal groups, as shown for the Felidae (Johnson et al., 2006), Hemigalinae (Viverridae) (Patou et al., 2008), Sciuridae (Mercer and Roth, 2003), and Muntiacinae (Cervidae) (Gilbert et al., 2006). This time period corresponds to the lowest sea-level observed before the Plio-Pleistocene (Haq et al., 1987), and may have allowed for dispersal across the Sunda shelf and between India and Sri Lanka. The uplift of the Tibetan plateau may have had a major impact on climate and vegetation in the Miocene throughout Asia, by modifying monsoon cycles and causing shifts in vegetation from forest to grassland (Zhisheng et al., 2001). These drastic changes may have generated vicariance events and allowed for the diversification of open habitat-dwelling mongoose species in Central Asia (such as the ancestor of *H. edwardsii*/*H. javanicus*/*H. auropunctatus*). Sri Lanka and the Western Ghats (Southwest India) may have played a refuge role for forest mongooses, such as *H. fuscus*, since this region retained tropical rainforest throughout this period.

Another wave of diversification occurred almost simultaneously within *H. brachyurus* (*ca.* 6 MYA) and within the *H. edwardsii*/*H. javanicus*/*H. auropunctatus* species complex (*ca.* 5 MYA). During the Late Miocene/Early Pliocene, seasonality and dryness increased on the Asian mainland, whereas Sundaland remained primarily tropical rainforest, resulting in divergences between Indochinese and Sundaic mammal groups (Meijaard, 2004). The Isthmus of Kra also flooded between 4.5 and 5.5 MYA (Woodruff, 2003), physically isolating the Indochinese and Sundaic regions.

The last divergence events occurred in the Plio-Pleistocene (0–2 MYA) (*H. auropunctatus*, *H. javanicus*, *H. edwardsii, H. fuscus*, and within *H. brachyurus*). However, Ho and Larson (2006) suggested that during this period the mutation rate was higher, which may result in an overestimation of divergence dates.

4.5. Taxonomic implications

As *H. ichneumon* is the type species of the genus *Herpestes* (see Wozencraft, 2005) and this genus has been shown to be paraphyletic, all the Asian *Herpestes* species and *H. naso* should be placed in other genera. A second option would be to place the entire clade of solitary mongooses (including *Atilax, Bdeogale, Cynictis, Ichneumia, Galerella*, and *Rhynchogale*) within the *Herpestes* genus. Given the unequivocal morphological heterogeneity within this clade, we favor the first proposition.

The Asian group exhibits a relative morphological homogeneity, a geographical unity, and strong molecular evidence for their monophyly, suggesting the placement of all Asian *Herpestes* species into one newly defined genus. The most ancient available generic name for Asian mongooses is *Urva* Hodgson, 1837, first applied to the crab-eating mongoose (*H. urva*), and then used as a subgenus of *Herpestes* for *H. brachyurus*, *H. hosei* (now a synonym of *H. brachyurus*), *H. semitorquatus*, *H. urva,* and *H. vitticollis* (Bechthold, 1939). Thus, all Asian mongooses, which were placed in *Herpestes*, should hence be placed in *Urva*. The taxonomic changes for the Asian mongooses would be as follows: *Urva auropunctata* (Hodgson, 1836); *Urva brachyura* (Gray, 1836); *Urva edwardsii* (Geoffroy Saint-Hilaire, 1818); *Urva fusca* (Waterhouse, 1838); *Urva javanica* (Geoffroy Saint-Hilaire, 1818); *Urva semitorquata* (Gray, 1846); *Urva smithii* (Gray, 1837); *Urva urva* (Hodgson, 1836); *Urva vitticolla* (Bennett, 1835).

The long-nosed mongoose (*Herpestes naso*) appears to be morphologically, ecologically and genetically distinct from its sister-species the marsh mongoose (*A. paludinosus*), thus, we suggest its exclusion from the genus *Herpestes* and its placement back as *Xenogale naso* Allen, 1919, following Gregory and Hellman (1939), Rosevear (1974), Ansell (1978), Colyn and Van Rompaey (1994), and Gilchrist et al. (2009).

The *Galerella* species are closely related to the Egyptian mongoose (*H. ichneumon*), but according to our molecular results and to the morphological differences between *H. ichneumon* and *Galerella*, the latter genus should be maintained. However, further confirmation of the monophyly of the *Galerella* genus is required.

5. Conclusion

Our work underlined the crucial role of museum collections for molecular systematic studies. Using museum specimens also enabled us to determine the identity of another introduced mongoose species from Fiji.

Using the nuclear gene FGBi7 for the first time in Herpestidae phylogeny proved useful for consolidating certain nodal supports within African taxa, and for resolving the relationships of the Asian members of this family. Our molecular data confirmed that the *Herpestes* genus is paraphyletic, with three distinct lineages. However, their morphological similarities remain striking, raising the question of whether these species converged to this morphotype or retained some ancestral characteristics. The evolution of the Y chromosome within the solitary mongooses is a very interesting evolutionary question and further investigations are needed.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2009.05.038.

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