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Systematic relationships of the bushy-tailed and black-footed mongooses (genus *Bdeogale*, Herpestidae, Carnivora) based on molecular, chromosomal and morphological evidence

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Abstract

The relationships within the mongooses (Herpestidae) have been recently reconsidered on the basis of molecular data. However, these studies failed to completely resolve the relationships within the subfamily Herpestinae. Moreover, the species of the genus *Bdeogale* have not been included in previous studies. Three genes were sequenced, Cytochrome b, ND2 and Transthyretin intron I, for 20 species of Herpestidae. The results show that the Herpestidae form two clades, corresponding to the traditional Herpestinae and Mungotinae, but with Cynictis included in the former rather than the latter. Within the Herpestinae, the genus *Herpestes* is not monophyletic. A newly proposed clade groups *Bdeogale*, *Cynictis*, *Ichneumia* and *Rynchogale*. Some morphological and karyological characters were mapped on the trees so as to characterize the newly defined molecular groups.

Key words: phylogeny – Herpestidae – *Bdeogale* – Cytochrome *b* – ND2 – Transthyretin intron I

Introduction

The mongooses (Herpestidae) are small-sized carnivores with terrestrial habits, which can be roughly divided into two groups: small-sized, social, diurnal, invertebrate eater species, and solitary, large-sized and small vertebrate eaters (Veron et al. 2004). Gray (1865) and later Wozencraft (1989), on the basis of dentition and the shape of auditory bullae, separated the mongooses into three subfamilies: Galidiinae (Malagasy mongooses), Herpestinae (*Atilax*, *Bdeogale*, *Herpestes*, *Ichneumia*and *Rhynchogale*) and Mungotinae which includes the social mongooses. Wozencraft (1989) also suggested two clusters within the Herpestinae: the *Herpestes–Atilax* clade and the *Ichneumia–Bdeogale–Rhynchogale* clade. Later, Wozencraft (1993) separated the family into only two subfamilies, the Malagasy Galidiinae and the African and Asian Herpestinae. Recent molecular analyses have demonstrated the monophyly of the mongooses *sensu stricto* (without Malagasy taxa) and the monophyly of the Malagasy carnivores (Galidiinae and three species previously placed in the Viverridae: *Cryptoprocta*, *Fossa* and *Eupleres*) (Yoder et al. 2003; Veron et al. 2004; Flynn et al. 2005). Veron et al. (2004) and Flynn et al. (2005) also demonstrated that the social mongooses form a monophyletic clade and that the yellow mongoose (*Cynictis penicillata*), believed to be close to the social mongooses, should be included in the solitary mongooses clade. These results force us to reconsider the family systematics. The Malagasy taxa have been placed in a separate family (Eupleridae; Wozencraft 2005). The Herpestidae *sensu stricto* are split into two clades (Veron et al. 2004; Flynn et al. 2005), which roughly correspond to the Herpestinae and Mungotinae of Wozencraft (1989), but with *Cynictis* included in the former rather than the latter. However, these previous studies failed to fully resolve intra-herpestine relationships.

The aim of this study is to contribute to our understanding of the phylogenetic relationships among the genera within the Herpestinae and particularly the systematic relationship of the problematic and the less-studied *Bdeogale* to other mongooses. This genus comprises two or three species according to previous authors (see review in Wozencraft 2005), commonly called the bushy-tailed mongooses. *Bdeogale nigripes* and *Bdeogale jacksoni*, which occur in west central Africa, have been placed in the genus *Galeriscus* by Hill and Carter (1941), Schoutenden (1945) and Rosevear (1974), and are considered conspecific by Kingdon (1977). *Bdeogale crassicauda* is an East African species.

The study provides a new or expanded data set of Cytochrome *b*, ND2 and Transthyretin intron I sequences for 20 species belonging to 12 different genera of Herpestidae. Cytochrome *b* and ND2 are highly variable genes which have proved to be useful for resolving carnivoran intra-familial relationships (Yoder et al. 2003; Veron et al. 2004; Flynn et al. 2005). Transthyretin intron I has been proved to be suitable for more ancient divergences within feliform carnivorans and Mammalia more generally (Flynn and Nedbal 1998; Gaubert and Veron 2003; Yoder et al. 2003; Flynn et al. 2005). Morphological and karyological features have been studied to assess their evolution relative to a molecular phylogenetic framework and to characterize the groups newly defined by the molecular evidence.

Materials and Methods

Sample collection

Tissue and hair samples were obtained from various sources (see Acknowledgments and Veron et al. 2004) from 26 Feliformia, of which 20 species are Herpestidae (Table 1 and Veron et al. 2004), represented 12 of the 14 living genera. One species of Felidae, one of Hyaenidae and four Malagasy taxa have been chosen as out-groups, according to previous results (Veron and Catzeflis 1993; Flynn and Nedbal 1998; Yoder et al. 2003; Veron et al. 2004).

DNA extraction, amplification and sequencing

For this study, two mitochondrial genes (Cytochrome *b* and ND2) and one nuclear gene (Transthyretin intron I) were sequenced, and combined with previously sequenced data from sources cited below. The data set of Cytochrome *b* gene was previously obtained by G.V. (Veron et al. 2004) and completed for this study by G.V. for *Bdeogale*.

Table 1. List of samples

Species	Tissue/DNA sample – Genbank accession number	Gene	References	Locality
Atilax paludinosus	TC-89/C-158	ND2	This study	Central African Republic
Atilax paludinosus	FMNH-160388/M-2	Transthyretin	This study	Uganda
Bdeogale crassicauda	TC-320/C-377	Transthyretin	This study	Tanzania
Bdeogale crassicauda	TC-318/C-376	Cytochrome *b*	This study	Tanzania
Bdeogale nigripes	FMNH-167685/M-3	ND2/Cytochrome b /transthyretin	This study	Gabon
Crossarchus alexandri	TC-72 (C-109)	ND2	This study	Captivity
Crossarchus obscurus	AY170041	ND2	Yoder et al. 2003	
Crossarchus obscurus	AF039726	Transthyretin	Flynn and Nedbal 1998	
Cynictis penicillata	TC-35/C-211	ND2	This study	South Africa
Cynictis penicillata	AY170024	Transthyretin	Yoder et al. 2003	Unknown
Galerella pulverulenta	TC-110/C-165	ND2	This study	South Africa
Galerella pulverulenta	TC-109/C-164	Transthyretin	This study	South Africa
Galerella sanguinea	TC-112/C-167	ND2	This study	South Africa
Galerella sanguinea	FMNH-145231/M-5	Transthyretin	This study	Uganda
Helogale hirtula	TAX-2339/C-134	Transthyretin	This study	Captivity
Helogale parvula	TC-30/C-140	ND2/Transthyretin	This study	Captivity
Herpestes edwardsii	TC-144/C-232	ND2	This study	Bahrain
Herpestes edwardsii	AY170025	Transthyretin	Unpubl. data	
Herpestes ichneumon	C-21	ND2	This study	Spain
Herpestes ichneumon	TC-154/C-246	Transthyretin	This study	South Africa
Herpestes javanicus	TC-141/C-228	ND2	This study	Guyana
Herpestes javanicus	AY170026	Transthyretin	Yoder et al. 2003	
Herpestes naso	TC-226/C-242	ND2/Transthyretin	This study	Gabon
Herpestes urva	TC-176/M-12	ND2	This study	Taiwan
Ichneumia albicauda	FMNH-157991/M-1	ND2/Transthyretin	This study	Uganda
Liberiicis kuhni	TC-143/C-230	ND2/Transthyretin	This study	Liberia
Mungos mungo	AY170035	ND2	Yoder et al. 2003	
Mungos mungo	AY170017	Transthyretin	Yoder et al. 2003	
Rhynchogale melleri	TC-224/C-243	ND2/Transthyretin	This study	South Africa
Suricata suricatta	AY170054	ND2	Yoder et al. 2003	
Suricata suricatta	AY170028	Transthyretin	Yoder et al. 2003	
Cryptoprocta ferox	AY170036	ND2	Yoder et al. 2003	Madagascar
Cryptoprocta ferox	AY170018	Transthyretin	Yoder et al. 2003	Unknown
Fossa fossana	AY170037	ND2	Yoder et al. 2003	Madagascar
Fossa fossana	AY170019	Transthyretin	Yoder et al. 2003	Madagascar
Galidictis fasciata	AY170039	ND2	Yoder et al. 2003	Madagascar
Galidictis fasciata	AY170022	Transthyretin	Yoder et al. 2003	Madagascar
Mungotictis decemlineata	AY170034	ND2	Yoder et al. 2003	Madagascar
Mungotictis decemlineata	AY170013	Transthyretin	Yoder et al. 2003	Madagascar
Crocuta crocuta	C-70	ND2	This study	Captivity
Crocuta crocuta	AF039728	Transthyretin	Flynn and Nedbal 1998	
Panthera leo	AY170043	ND2	Yoder et al. 2003	
Panthera leo	AF039725	Transthyretin	Unpubl. data	

Herpestidae (*sensu stricto*, see Veron et al. 2004) in bold.

The ND2 gene was sequenced in its entirety by M.P. and the new Transthyretin sequences have been obtained by B.L. The molecular work was executed at the Service de Systématique Moléculaire, M.N.H.N., Paris.

Total genomic DNA was prepared according to Kocher et al. (1989), with an extended digestion time. The fragments were amplified in a reaction volume of 25 µl using 1 µl of DNA and HiTaq polymerase (MP Biomedical) or TaqDNApol and QBioTaq (Illkirch, France). Amplifications were run in a thermal cycler with a typical profile of 35 cycles, with each cycle consisting of 30 s at 93°C, 40 s at 54°C and 40 s at 72°C, for the denaturation, annealing, and extension steps respectively. Four primers were used for amplification and sequencing of the ND2 gene and seven primers for the Transthyretin gene (Table 2). The reaction products were visualized in a 1.5% agarose gel and then purified directly from the PCR mixture, or in some cases, from the agarose gel (MinElute PCR kit, Quiagen, Hilden, Germany). Purified fragments were sequenced in both forward and reverse directions, with an automated DNA sequencer (CEQ 2000 DNA Analysis system, Beckman Coulter, Inc., Fullerton, CA, USA). All the sequences were aligned with Bioedit version 5.0.6 (Hall 1999) and adjusted manually.

Phylogenetic analyses

The data set was analysed with maximum parsimony (MP) and maximum likelihood (ML) methods using PAUP* 4.0b10 (Swofford 2001). Likelihood models and parameters were estimated using MODELTEST version 3.06 (Posada and Crandall 1998). The cladistic analysis used heuristic search with random stepwise addition and TBR branch-swapping. To assess statistical support for hypothesized clades, bootstrap analysis (Felsenstein 1985) was done with 100 (ML) and 1000 (MP) bootstrap replicates. The amount of homoplasy was measured through the consistency index (Kluge and Farris 1969) and the retention index (Farris 1989). The support of each gene in the combined analyses was calculated with the Partitioned Bremer Support

Table 2. List of primer used in this study

	Primer identification	Position	Nucleotide sequence (5′-3′)	Source
ND2	tRNA-Met L	External	5′-CCCATACCCCGAAAATGATG-3′	Sorenson et al. 1999
	tRNA-Trp H	External	5′-CTCTTATTTAAGGCTTTGAAGGC-3′	Sorenson et al. 1999
	ND2intF L	Internal	5′-CTHAAYCARACMCAACTACG-3′	This study (MP)
	ND2intR H	Internal	5′-GCYAKRATTTTHCGTAGTTG-3′	This study (MP)
Transthyretin	Herp5L	External	5′-AAGTAGCARTGYCTTMCTC-3′	Gaubert and Veron 2003
	Herp459H	Internal	5′-ACTGCYGCTRTAGTAATTC-3′	Gaubert and Veron 2003
	Vivn504L	Internal	5′-ACAAACTATTTGGTCCTGTG-3′	Gaubert and Veron 2003
	Vivn807H	External	5′-CAGTGAGAGGTCAACGAA-3′	Gaubert and Veron 2003
	Herp35L	External	5′-GCCACCTTGTGTTACTAGGAC-3′	This study (BL)
	Herp296L	Internal	5′-GTTTATTTCCCTTCAGCTAATC-3′	This study (BL)
	Herp854H	External	5′-AAARGATCTGTTCTTTATGGTG-3′	This study (BL)

The letters L and H refer to light and heavy strands respectively.

(PBS; Baker and DeSalle 1997). The PBS was estimated for the tree codon positions using TREEROT version 2 (Sorenson 1999).

Character mapping

A set of 11 morphological characters was recorded by A.C., B.L. and G.V. (see Table 3) on specimens from the Museum National d'Histoire Naturelle, Paris. They were mapped together with caryological data (from Fredga 1972; Couturier and Dutrillaux 1985) on the molecular tree. Ancestral state was provided by MacClade (version 4.0) (Maddison and Maddison 2000).

Results

Thirty-four new sequences were obtained for the three genes (Genbank accession number: AY950641 to AY950653 for Transthyretin, AY950654 to AY950655 for Cytochrome b, and AY974021 to AY974036 for ND2). For some taxa, only incomplete sequences were obtained because of DNA condition and amount available (*Bdeogale crassicauda* and *Helogale hirtula* for ND2; *Chrossarchus alexandri* and *Herpestes urva* for Transthyretin). The three genes, ND2, Cytochrome *b* and Transthyretin, yielded alignments of 1044 base pairs (bp), 1140 bp and 841 bp respectively. The sequences of the three genes were combined and the data matrix used for the analyses consisted of a total of 3025 bp which included 1266 variable sites and 853 informative characters. The ML analysis yielded one tree ($-\ln L = 19\,568.33727$) represented in Fig. 1. The ML tree was generated using the GTR model (Rodriguez et al. 1990) with a proportion of invariable sites ($I = 0.3875$) and a Gamma distribution shape parameter ($G = 0.6831$). Fig. 2 shows the most parsimonious tree of 3875 steps (CI = 0.4470 and RI = 0.4352) realized with the MP analysis of the data matrix, including the four taxa with incomplete sequences.

Phylogenetic analyses performed with the three gene sequences confirmed that the family of Herpestidae is separated into two clades:

(1)The first clusters the social mongooses: *Crossarchus*, *Helogale*, *Liberiictis*, *Mungos*, and *Suricata*. The latter genus appears in a basal position within the social mongooses (in both MP and ML trees).

(2)The second groups, with a strong support (bootstrap value of 100% in both the MP and ML trees), the genera *Atilax*, *Bdeogale*, *Cynictis*, *Galerella*, *Herpestes*, *Ichneumia* and *Rhynchogale*.

Table 3. Morphological and chromosomal characters used in this study

Number	Character	Coded States
1	Sagittal crest	absent:0; strong:1
2	Postorbital processes	open:0; close:1
3	Inflation of anterior part of entotympanic, covering posterior part of ectotympanic	absent:0; present:1
4	P^1	present:0; absent:1
5	p_3: posterior cusp	absent or very small:0; present, large:1
6	m_1: trigonid	cusps well separated:0; metaconid close to paraconid:1; metaconid small then other cusps and close to paraconid:2; reduction of metaconid, not close to paraconid:3
7	Claws of digits of fore foot	short: 0; long and curved: 1
8	Hind foot metatarse (plantar surface)	hairy: 0; naked only in posterior part:1; completely naked:2
9	Digits: fore foot	5 : 0; 4 : 1
10	Digits: hind foot	5 : 0; 4 : 1
11	Tail	thin:0; very wide and bushy:1
12	Translocation of the sexual chromosome Y	absent:0; present:1

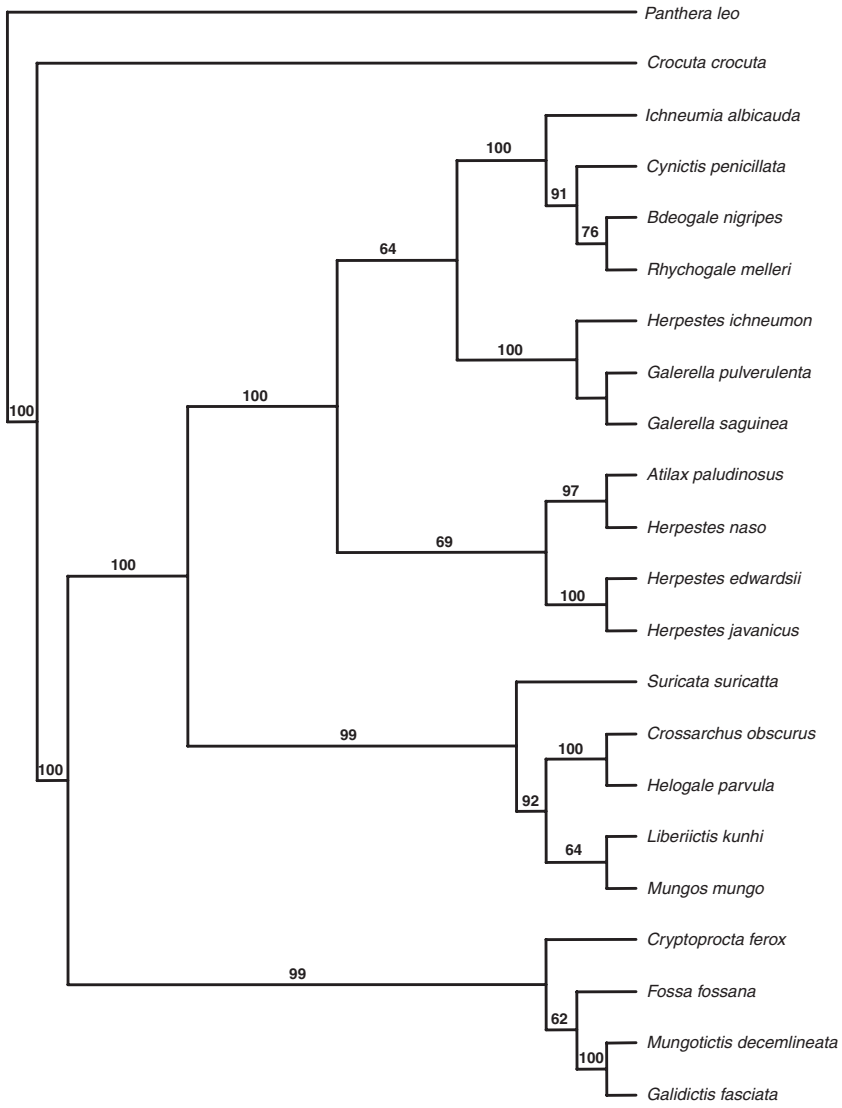

Fig. 1. Maximum likelihood tree (GTR model with gamma rate distribution = 0.6831) based on the combined matrix of ND2, Cytochrome b and Transtyretin sequences without uncompleted taxa (−ln likelihood = 19 568.33727). Bootstrap values (over the lines) above 50% are shown at node

However, in this second clade grouping the solitary mongooses (Herpestinae), most of the basal relationships were poorly supported. Nevertheless, the genus *Herpestes* clearly appeared not to be monophyletic. The grouping of *Herpestes naso* and *Atilax paludinosus* was highly supported by all methods (97% in the ML tree and 85% in the MP tree). All analyses strongly supported the grouping of *Herpestes ichneumon* with *Galerella* species, but the monophyly of the latter genus was weakly supported.

A clade clusters the genus *Bdeogale*, *Cynictis*, *Ichneumia* and *Rhynchogale* (100% in the ML tree and 82% in the MP tree). *Ichneumia* is placed at the base of this clade. ML analysis highly supports *Cynictis* as the sister-taxon of *Rhynchogale* + *Bdeogale* whereas MP analysis places *Rhynchogale* sister-taxon of *Cynictis* + *Bdeogale*. However, the latter grouping was not supported by Cytochrome *b* data (as shown by PBS values, see Fig. 2).

The MP analysis with the incomplete molecular data set of *B. crassicauda* showed that this species clusters with *B. nigripes* (Fig. 2) indicating that the genus *Bdeogale* is monophyletic.

Twelve characters (chromosomic and morphologic; see Tables 3 and 4) were mapped on the MP tree. The ancestral condition of these characters for the mongoose family estimated by MacClade (version 4.0) (Maddison and Maddison 2000) is strong sagittal crest, presence of first superior premolar, absence or very small posterior cusp on third inferior premolar; short claws on the fore foot, hind foot metatarse completely naked, five digits on the fore and hind feet, thin tail and no translocation of Y onto an autosome. The result is equivocal for the postorbital processes, the tympanic bullae inflation and for trigonid cusps of the first inferior molar. Some characters which provided synapomorphies for the newly proposed clades are shown in Fig. 2. The presence of long and curved claws on the fore feet appears at the basis of the social mongooses group. The group *Bdeogale-Cynictis-Ichneumia- Rhynchogale*, is characterized by a very wide and bushy tail and a large posterior cusp on P_3.

Discussion

These results show that the combined results incorporating ND2 and Transthyretin sequences provide better resolution for the internal nodes within herpestids than analyses with Cytochrome *b* sequences alone. The monophyly of the social mongooses (*Crossarchus*, *Helogale*, *Liberiictis*, *Mungos* and *Suricata*; Veron et al. 2004; Flynn et al. 2005) is confirmed

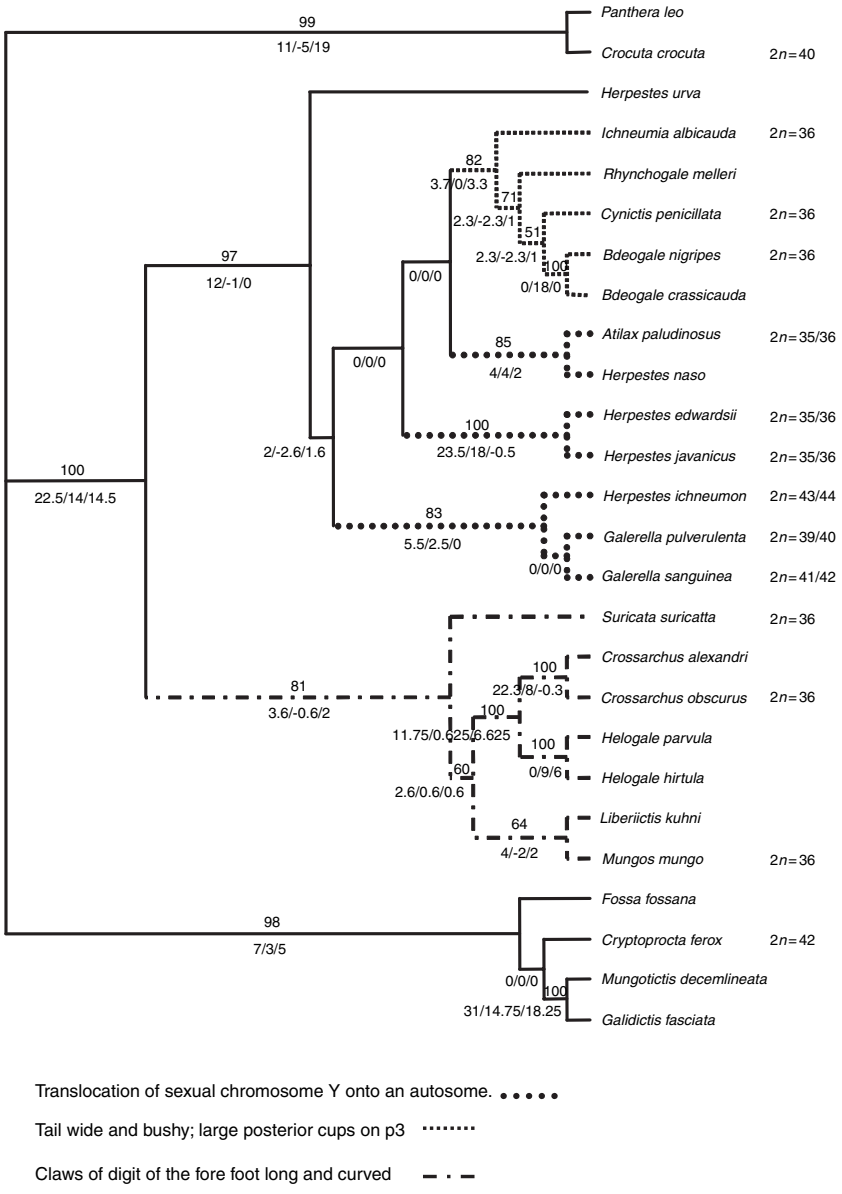

Fig. 2. Tree obtained by the MP method with the total data matrix (1 tree at 3875 steps; CI = 0.4470; RI = 0.4352). Bootstrap values (over the lines) above 50% are shown at node and Partitioned Bremer Supports (ND2/Cytb/Trans) are given under the lines

here. The relationships of these genera also are similar to the results obtained by Veron et al. (2004) with Cytochrome *b* only. The social mongooses are characterized by their small size and the presence of long claws on the fore feet and this clade corresponds to the Mungotinae subfamily suggested by some authors (Gray 1865; Wozencraft 1989) but from which *Cynictis* must be excluded. The systematic position of one genus, *Dologale*, remains to be studied; no sample being so far available. It is believed to be close to *Crossarchus* (in which genus it has been previously included by Pousargue 1893), or to *Helogale* (Hayman 1936). There is no data on its behaviour (Kingdon 1977) and the social organization of this species remains to be studied. However, *Dologale* bears the other morphological features characterizing the social mongoose clade (notably small size, general body shape, tooth formula, and shape and size of the claws of the fore feet; Kingdon 1977).

The second clade includes the solitary mongooses and corresponds to the Herpestinae, in which *Cynictis* must now be included.

Phylogenetic relationships of *Herpestes* species

The study confirms that the genus *Herpestes* is not monophyletic (see Veron et al. 2004). It supports the association between *H. ichneumon* and the species of the genus *Galerella*, in agreement with Baker (1987) – morphological and behavioural data – and Taylor et al. (1991) – allozyme analyses. This relationship also is supported by the existence of a higher number of chromosomes in this group: 19–21 pairs of autosomes rather than the 17 pairs observed in other Herpestidae (Fredga 1972; Couturier and Dutrillaux 1985). The trees weakly support monophyly of *Galerella* (*G. pulverulenta* and *G. sanguinea*). The number of species in this genus is still debated (see Taylor and Goldman 1993 for review). Samples from the two other species included in this genus (*G. flavescens* and *G. swalius*; following Allen 1924; Wozencraft 1993, 2005) were not obtained for this study and need to be sequenced to ascertain the validity of including those species within this genus. The species of *Galerella* are included by some authors in the genus *Herpestes* (see Wozencraft 2005 for review).

Table 4. Data matrix of morphological and cytogenetic characters

	1	2	3	4	5	6	7	8	9	10	11	12
Atilax paludinosus (G.Cuvier, 1829)	1	1	1	0/1	0	0	0	2	0	0	0	1
Bdeogale crassicauda Peters, 1852	1	0	1	0	1	1	0	1	1	1	1	?
Bdeogale nigripes Pucheran, 1855	?	?	?	?	?	?	0	2	1	1	1	0
Crossarchus alexandri Thomas & Wroughton, 1907	0	0	0	0	?	?	1	?	0	0	0	0
Crossarchus obscurus F. Cuvier, 1825	0	0	0	0	0	0	1	2	0	0	0	0
Cynictis penicillata (G. Cuvier, 1829)	1	1	0	0	1	0	0	0	0	1	1	0
Galerella pulverulenta (Wagner, 1839)	0	1	0	0	0	0	0	0	0	0	0	1
Galerella sanguinea (Rüppell, 1836)	0	1	0	0	0	0	0	1	0	0	0	1
Helogale hirtula Thomas, 1904	0	0	0	1	0	2	1	2	0	0	0	0
Helogale parvula (Sundevall, 1847)	0	0	0	1	0	2	1	2	0	0	0	0
Herpestes edwardsii (E. Geoffroy Saint-Hilaire, 1818)	0	1	0	0	0	0	0	1	0	0	0	1
Herpestes ichneumon (Linnaeus, 1758)	1	1	0	0	0	0	0	1	0	0	0	1
Herpestes javanicus (E. Geoffroy Saint-Hilaire, 1818)	0	1	0	0	1	0	0	1	0	0	0	1
Herpestes naso de Winton, 1901	1	1	1	0	0	3	0	2	0	0	0	?
Herpestes urva (Hodgson, 1836)	0	1	1	0	0	0	0	2	0	0	0	?
Ichneumia albicauda (G. Cuvier, 1829)	1	1	1	0	1	0	0	1	0	0	1	0
Liberiictis kuhni Hayman, 1958	0	0	0	0	0	?	1	2	0	0	0	?
Mungos mungo (Gmelin, 1788)	0	0	0	1	0	1	1	2	0	0	0	0
Rhynchogale melleri (Gray, 1865)	1	1	1	0	1	1	0	2	0	0	1	0
Suricata suricatta (Schreber, 1776)	0	1	0	1	0	1	1	2	1	1	0	0

However, Allen (1924) reported proportional differences in skull and skeleton measurements between *Galerella* and *Herpestes*, but that author considered only *H. ichneumon*. Taylor et al. (1991) also argued for generic recognition of *Galerella* on the basis of allozyme analysis. However, neither study included Asian *Herpestes* and it has been shown that the morphological gap between *Herpestes* and *Galerella* no longer exists when Asian species are included in the analyses (see review in Wozencraft 1993).

More data are needed to determine the relationships of the Asian mongooses (all belonging to the genus *Herpestes*) and to test their monophyly. Most of the previous studies (morphometrics, cytogenetics, allozymes) did not include Asian species. The monophyly of the Asian subgenus *Urva* (which includes *H. urva*, *Herpestes semitorquatus*, *Herpestes vitticollis*, *Herpestes brachyurus* and *H. hosei*) needs also to be tested, but our results already show that *H. urva* does not cluster with the other Asian species. However, its phylogenetic position remains uncertain (see the low support for its basal position in the herpestine clade).

The association between *H. naso* and *A. paludinosus*, originally revealed through analyses of Cytochrome *b* sequences (Veron et al. 2004), was confirmed and supported by the ND2 and Transthyretin gene analyses. These species have a similar morphology and live in the same habitat (swamp and riverine forest) but differ in activity, *H. naso* being diurnal and *A. paludinosus* crepuscular (Ray 1997). Their close relationship has never been proposed before (see for review Bininda-Emonds et al. 1999; Veron et al. 2004). While some authors had observed their morphological resemblances, they were believed to be the results of convergences because of similar adaptation to their habitat (Orts 1970; Rosevear 1974; Ray 1997). *H. naso* has been previously placed in a separate genus, *Xenogale*, by Allen (1919) and this was followed by Gregory and Hellman (1939), Rosevear (1974), Ansell (1978) and Colyn and Van Rompaey (1994). It was generally regarded as the sister-taxon of *H. ichneumon* and then placed in the genus *Herpestes* (Coetzee 1977; Kingdon 1977; Happold 1987; Corbet and Hill 1991; Wozencraft 1993, 2005). Our results show that its placement in this genus has to be reconsidered.

Fredga (1972), Pathak and Stick (1976) and Wurster and Benirschke (1968) observed a different number of chromosomes in male and female, resulting from the translocation of the Y chromosome onto an autosome, in some Herpestinae (*Atilax*, *Galerella* and *Herpestes*, see Fig. 2). It could not be discerned if there were one or several chromosomal transformations, because of the weak support of the basal relationships of herpestines. It appears, considering the rarity of the translocation of a sexual chromosome onto an autosome (see for review Corin-Frederic 1969), that the most probable scenario was a single transformation.

Systematic relationships of the bushy-tailed mongooses

The group of herpestines which do not have the gonosome translocation comprises the following studied genera: *Bdeogale*, *Cynictis*, *Ichneumia* and *Rhynchogale*. This group can be characterized by a very wide and bushy tail, as well as a large posterior cusp on the third inferior premolar. The cytogenetic data from Fredga (1972) and Wurster and Benirschke (1968) are congruent with this grouping, *Bdeogale*, *Cynictis* and *Ichneumia* having $2n = 36$ chromosomes and sharing a reduced sexual Y chromosome.

The bushy-tailed mongooses (*Bdeogale*) have never been included in previous phylogenetic studies but their systematics has been debated. The genus *Bdeogale* is currently separated into three species (Honacki et al. 1982; Wozencraft 2005): *B. nigripes* inhabits the tropical belt from east Nigeria to north-east Zaire and to north Angola, *B. jacksoni* is restricted to south-west of Kenya, and *B. crassicauda* occurs in East Africa (Kenya, Malawi, Mozambique, Tanzania, Zambia and Zimbabwe) (Wozencraft 2005). Rosevear (1974) believed that the characters used to separate them could be intra- and interspecific colour variation. However, there was 4.7% of molecular divergence for the Cytochrome *b* gene between the two species included in this study (*B. crassicauda* and *B. nigripes*), which does not differ from that found between other mongoose species. The third species *B. jacksoni* has been considered as conspecific with *B. nigripes* by Kingdon (1977) but this remains to be tested.

According to Thomas (1882), Pocock (1919) and Gregory and Hellman (1939), *Bdeogale* shares many dental features with *Ichneumia*, as well as a similar external morphology. However, *Bdeogale* has many foot specializations (e.g. the suppression of the pollex and the hallux, the shortening of the four main digits, and symmetrical toes) as well as expanded molars which have been said to be related to an insectivorous diet, mainly ants and termites (Kingdon 1977; Smithers 1983). Their dense fur, their short, woolly ears and the plush muzzle and feet may be a protection against soldier ants or termites (Kingdon 1977). However, in *B. nigripes*, vertebrates (mainly rodents and insectivores) have also been reported as an important part of the diet (Ray and Sunquist 2001).

Rhynchogale was first believed to be associated with *Crossarchus* and *Suricata* by Gray (cited by Pocock 1919) and Thomas (1882). However, Pocock (1919) suggested that it is closest to *Ichneumia* and *Bdeogale*, notably on the basis of dental characters. The shape of the body of *Rhynchogale* is very similar to that of *Ichneumia* (Kingdon 1977). However, *Rhynchogale* differs from the other members of this clade by the absence of the groove on the upper lip (Pocock 1919), a distinctly snub nose, and by the flatness of its molars (Kingdon 1977). The last feature may result from a diet adaptation, but while some authors suggest that it may feed mostly on termites (see Smithers 1966), others consider it to be an adaptation to a frugivorous diet (see Pocock 1919 and Kingdon 1977). In fact, its biology is almost entirely unknown.

Cynictis, which possesses social traits, was allied to the social mongooses by Baker (1987). However, Gregory and Hellman (1939), Hendey (1974) and Taylor et al. (1991) had placed *Cynictis* among the solitary mongooses. Pocock (1919) also emphasized that the position of *Cynictis* is difficult to establish regarding its morphological specializations, but suggested close relationships with *Ichneumia* on the basis of ear and plantar pad characters. The molecular studies by Veron et al. (2004) and Flynn et al. (2005) revealed the exclusion of *Cynictis* from the social group and the present study shows its inclusion in the bushy-tailed mongoose clade. *Paracynictis* was considered as the sister-taxa of *Cynictis* (Pocock 1919) and this has been confirmed by molecular results (Flynn et al. 2005).

Conclusions

The phylogenetic relationships within the mongooses, as revealed by molecular data, showed that social, morphological and karyological characters are not highly homoplastic in the Herpestidae (Veron et al. 2004, and this study). Only the yellow mongoose, *Cynictis*, has been shown to possess some convergent morphological and social characters (Veron 1995; Veron et al. 2004), sharing some traits with *Suricata*. However, previous authors (Pocock 1919; Gregory and Hellman 1939) had already suspected these to be convergent adaptations, but more detailed studies (see review in Veron et al. 2004) showed that this resulted mainly from the lack of information notably for ethological characters.

In the supertree of Herpestidae by Bininda-Emonds et al. (1999), the genus *Herpestes* was monophyletic, and *Bdeogale* and *Cynictis* were placed close to the social mongooses, which differs from our results. However, the source data of the studies used by Bininda-Emonds et al. (1999) to build the supertree consist mainly of purportedly non-phylogenetic features. It is based on descriptive works in which authors do not detect synapomorphies and the resulting tree is then based on shared plesiomorphies.

The fossil record of mongooses is poor, but early Miocene sediments in East Africa have produced the oldest African carnivores (Schmidt-Kittler 1987). Among these, *Leptoplesictis* is recognized as a true herpestid, which occurs in middle Miocene of Europe as well. Plio-Pleistocene sites have yielded mongooses very close to the extant species in East and South Africa (Hunt 1996). Concerning the herpestines, *Cynictis* and *Herpestes* were recorded in the Plio-Pleistocene (Hunt 1996) and *Atilax*, *Ichneumia* and *Paracynictis* in the Pleistocene (Cooke 1964; Savage and Russell 1983). No fossils were assigned to the genus *Bdeogale* and *Rhynchogale* (Savage and Russell 1983) and then the date of their origin remains uncertain. However, according to the fossil records (Cooke 1964; Savage and Russell 1983; Schmidt-Kittler 1987; Hunt 1996) and our phylogenetic results, it is likely that the clade *Bdeogale–Cynictis–Ichneumia–Rhynchogale* originated in Africa.

One of the major problems of molecular phylogeny is the access to biological material and taxonomic sampling having proved to impact on the phylogenetic accuracy (see Zwickl and Hillis 2002). As many small carnivores are elusive species, the use of museum specimens is crucial in obtaining DNA for molecular studies. This could be done even for taxa represented in collections by very few specimens, using non-destructive DNA extraction method recently designed (Rohland et al. 2004). However, as the biology of many small carnivores remains to be studied, the need for field studies is underlined. These can provide biological samples (e.g. plucked hairs) for molecular studies as well as biological and ecological data, which are crucial in understanding their evolution and designing conservation strategies (see Ferguson and Larivière 2002), urgently needed for many small carnivore taxa (Schreiber et al. 1989).

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Résumé

Relations systématiques des mangoustes à queue touffue (Bdeogale, Herpestidae) à partir de données moléculaires, chromosomiques et morphologiques

Les relations phylogénétiques au sein des mangoustes (Herpestidae) ont récemment été revues sur la base de données moléculaires. Toutefois, ces études n'ont pas permis de résoudre les relations au sein de la sous-famille des herpestinés. De plus, les espèces du genre *Bdeogale* n'ont été considérées dans aucune de ces études. Trois gènes ont été séquencés–Cytochrome b, ND2 et l'intron I de la Transthyretine–pour 20 espèces d'Herpestidae. Les résultats montrent que les Herpestidae sont divisés en deux clades correspondant aux Herpestinae et Mungotinae, avec *Cynictis* inclus dans la première sous-famille plutôt que dans la seconde. Au sein des herpestinés, le genre *Herpestes* apparaît non monophylétique. Un nouveau clade regroupant *Bdeogale, Cynictis, Ichneumia* et *Rynchogale* est proposé. Des caractères morphologiques et cytogénétiques ont été plaqués sur les arbres et révèlent des synapomorphies pour les nouveaux regroupements proposés.

References

Allen JA (1919) Preliminary notes on African Carnivora. J Mamm **1:**23–31.

Allen JA (1924) Carnivora collected by the American Museum Congo Expedition. Bull Am Mus Nat Hist **47:**73–281.

Ansell WF (1978) The Mammals of Zambia. The National Parks and Wildlife Service, Chilanga.

Baker CM (1987) Biology and behaviour of the water mongoose (*Atilax paludinosus*). PhD dissertation, University of Natal, Durban.

Baker RH, DeSalle R (1997) Multiples sources of character information and the phylogeny of Hawaïan Drosophila. Syst Biol **46:**654–673.

Bininda-Emonds ORP, Gittleman JL, Purvis A (1999) Building large trees by combining phylogenetic information: a complete phylogeny of the extant Carnivora (Mammalia). Biol Rev **74:**143–175.

Coetzee CG (1977) Order Carnivora. In: Meesters JAJ, Setzer HW (eds), The Mammals of Africa: An Identification Manual. Smisonian Institution Press, Washington, DC.

Colyn M, Van Rompaey H (1994) Morphometric evidence to the monotypic status of the African long-nosed mongooses *Xenogale naso* (Carnivora, Herpestidae). Belg J Zool **124:**175–192.

Cooke HBS (1964) The Pleistocene environment in southern Africa. In: Davis DHS (ed.), Ecological Studies in Southern Africa. Junk, The Hague, pp 1–23.

Corbet GB, Hill JE (1991) A World List of Mammals Species. British Museum (Natural History) Publications, London.

Corin-Frederic J (1969) Les formules gonosomiques dites aberrantes chez les Mammifères Euthériens. Chromosoma **27:**268–287.

Couturier J, Dutrillaux B (1985) Evolution chromosomique chez les Carnivores. Mammalia **50:**124–162.

Farris JS (1989) The retention index and the rescaled consistency index. Cladistics **5:**417–419.

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution **39:**783–791.

Ferguson SH, Larivière S (2002) Can comparing life histories help conserve carnivores? Anim Conserv **5:**1–12.

Flynn JJ, Nedbal MA (1998) Phylogeny of the Carnivora (Mammalia): congruence versus incompatibility among multiple data sets. Mol Phyl Evol **9:**414–426.

Flynn JJ, Finarelli JA, Zehr S, Hsu J, Nedbal MA (2005) Molecular phylogeny of the Carnivora (Mammalia): assessing the impact of increased sampling on resolving enigmatic relationships. Syst Biol **54:**317–337.

Fredga K (1972) Comparative chromosome studies in mongooses (Carnivora, Viverridea). Hereditas **71:**1–74.

Gaubert P, Veron G (2003) Exhaustive sample set among Viverridae reveals the sister-group of felids: the Linsangs as a case of extreme morphological convergence within Feliformia. Proc R Soc London B **270:**2523–2530.

Gray JE (1865) A revision of the genera and species of viverrine animals (Viverridae), founded on the collection in the British Museum. Proc Zool Soc London **1865:**502–579.

Gregory WK, Hellman M (1939) On the evolution and major classification of the civets (Viverridae) and allied fossil and recent Carnivora: a phylogenetic study of the skull and dentition. Proc Am Phil Soc **81:**309–392.

Hall TA (1999) BioEdit: a user-friendly biological sequences alignement editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Series **41:**95–98.

Happold DCD (1987) The Mammals of Nigeria. Clarendon Press, Oxford.

Hayman RW (1936) A note on *Dologale dybowskii*. Ann Mag Nat Hist **1018:**626–630.

Hendey QB (1974) Faunal dating of the late Cenozoic of Southern Africa, with special reference to the Carnivora. Q Res (N Y) **4:**149–161.

Hill JE, Carter TD (1941) The mammals of Angola, Africa. Bull Am Mus Nat Hist **78:**1–212.

Honacki JH, Kiman KE, Koeppl JW (1982) Mammal Species of the World: A Taxonomic and Geographic Review. Association of Systematics Collections, Lawrence, KS.

Hunt RM (1996) Biogeography of the Order Carnivora. In: Gittleman JL (ed.), Carnivore, Behavior, Ecology and Evolution. Comstock Publishing Association, Cornell University Press, Ithaca, London, pp 485–541.

Kingdon J (1977) East African Mammals. Academic Press, London and New York.

Kluge AG, Farris JS (1969) Quantitative phylogenetics and the evolution of anurans. Syst Zool **18:**1–32.

Kocher TD, Thomas WK, Meyer A, Edwards SV, Paabo S, Villablanca FX, Wilson AC (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc Natl Acad Sci USA **86:**6196–6200.

Maddison WP, Maddison DR (2000) MacClade: Analysis of Phylogeny and Character Evolution, version 4.0. Sinauer Associates, Sunderland.

Orts SG (1970) Le *Xenogale* de J. A. Allen (Carnivora, Viverridae) au sujet d'une capture effectuée au Kivu. Rev Zool Bot Afr **82:**174–186.

Pathak S, Stick AD (1976) Giemsa-banding and the identification of the Y/autosome translocation in the African marsh mongoose, *Atilax paludinosus* (Carnivora, Viverridae). Cytogenet Cell Genet **10:**487–494.

Pocock RI (1919) The classification of the mongooses (Mungotidae). Ann Mag Nat Hist **3:**515–524.

Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. Bioinf Appl Note **14:**817–818.

Pousargue E (1893) Note sur deux espèces nouvelles de mammifères rapportés par M. Jean Dybowski de la région de l'Oubangui. Bull Soc Zool France **1893:**51–54.

Ray JC (1997) Comparative ecology of two African forest mongooses, *Herpestes naso* and *Atilax paludinosus*. Afr J Ecol **35:**237–253.

Ray JC, Sunquist ME (2001) Trophic relations in a community of African rainforest carnivores. Oecologia **127:**395–408.

Rodriguez F, Oliver JF, Marin A, Medina JR (1990) The general stochastic model of nucleotide substitution. J Theor Biol **142:**485–501.

Rohland N, Siedel H, Hofreiter M (2004) Nondestructive DNA extraction method for mitochondrial DNA analyzes of museum specimens. Biotechniques **36:**814–821.

Rosevear DR (1974) The Carnivores of West Africa. British Museum (Natural History), London.

Savage DE, Russell DE (1983) Mammalian Paleofaunas of the world. Addison-Wesley Publishing Company, London.

Schmidt-Kittler N (1987) The Carnivora (Fissipedia) from the Lower Miocene of East Africa. Palaeontographica **197:**8–126.

Schoutenden H (1945) De Zoogdieren van belgish Congo en van Ruanda-Urundi. Ann Mus R Congo Belge Zool **2:**1–576.

Schreiber AR, Wirth M, Riffel H, Van Rompaey H (1989) Weasels, Civets, Mongooses, and their Relatives. An Action Plan for the Conservation of Mustelids and Viverrids. IUCN/SSC Mustelid and Viverrid Specialist Group, IUCN, Gland.

Smithers RHN (1966) The Mammals of Rhodesia, Zambia and Malaysia. Collins, London.
Smithers RHN (1983) The Mammals of Southern African Subregion. University of Pretoria, Pretoria.
Sorenson MD (1999) TreeRot, version 2. Boston University, Boston.
Sorenson MD, Ast JC, Dimcheff DE, Yuri T, Mindell DP (1999) Primers for PCR-based approach to mitochondrial genome sequencing in birds and other vertebrates. Mol Phyl Evol **12:**105–114.
Swofford DL (2001) PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods), Beta version 4.0b2. Sinauer Associates, Sunderland.
Taylor ME, Goldman CA (1993) The taxonomic status of the African mongooses, *Herpestes sanguineus*, *H. nigratus*, *H. pulverulentus* and *H. ochraceus* (Carnivora: Viverridae). Mammalia **57:**375–391.
Taylor PJ, Campbell GK, Meester JAJ, van Dyck D (1991) A study of allozyme evolution in African mongooses (Viverridae: Herpestinae). Z Säugetierk **5:**135–145.
Thomas O (1882) On African mongooses. Proc Zool Soc London **1882:**59–93.
Veron G (1995) La position systématique de *Cryptoprocta ferox* (Carnivora). Analyse cladistique des caractères morphologiques de carnivores Aeluroidea actuels et fossiles. Mammalia **59:**551–582.
Veron G, Catzeflis F (1993) Phylogenetic relationships of the endemic Malagasy Carnivore *Cryptoprocta ferox* (Aeluroidea): DNA/DNA hybridization experiments. J Mammal Evol **1:**169–185.
Veron G, Colyn M, Dunham AE, Taylor P, Gaubert P (2004) Molecular systematics and origin of sociality in mongooses (Herpestidae, Carnivora). Mol Phyl Evol **30:**582–598.
Wozencraft WC (1989) Classification of the recent carnivora. In: Gittleman JL (ed.), Carnivore Behaviour, Ecology and Evolution. Cornell University Press, Ithaca, NY, pp 569–593.
Wozencraft WC (1993) Carnivora: Herpestidae. In: Wilson DE, Reeder DM (eds), Mammal Species of the World a Taxonomic and Geographic Reference. Smithsonian Institution Press, Washington, DC, pp 279–348.
Wozencraft WC (2005) Carnivora: Herpestidae. In: Wilson DE, Reeder DM (eds), Mammal Species of the World a Taxonomic and Geographic Reference. Smithsonian Institution Press, Washington, DC. 531–628.
Wurster DH, Benirschke K (1968) Comparative cytogenetic studies in the Order Carnivora. Chromosoma **24:**336–382.
Yoder AD, Burns MM, Zehr S, Delefosse T, Veron G, Goodman SM, Flynn JJ (2003) Single origin of Malagasy Carnivora from an African ancestor. Nature **421:**734–737.
Zwickl DJ, Hillis DM (2002) Increased taxon sampling greatly reduces phylogenetic error. Syst Biol **51:**588–598.

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