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Use of phylogeny to resolve the taxonomy of the widespread and highly polymorphic African giant shrews (*Crocidura olivieri* group, Crocidurinae, Mammalia)

Sylvain Dubey[a,*], Maxence Antonin[a], Christiane Denys[b], Peter Vogel[a]

[a]*Department of Ecology and Evolution, University of Lausanne, CH-1015 Lausanne, Switzerland*
[b]*UMR CNRS 5202: OSEB. CP51-USM601, Département Systématique et Evolution, Musée National d'Histoire Naturelle, 55, rue Buffon, F-75005 Paris, France*

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Abstract

The aim of this study is to provide a better understanding of the genetic relationships within the widespread and highly polymorphic group of African giant shrews (*Crocidura olivieri* group). We sequenced 769 base pairs (bp) of the mitochondrial cytochrome *b* gene and 472 bp of the mitochondrial control region over the entire geographic range from South Africa to Morocco. The analyses reveal four main clades associated with different biomes. The largest clade occurs over a range covering Northwest and Central Africa and includes samples of *C. fulvastra*, *C. olivieri*, and *C. viaria*. The second clade is composed of *C. goliath* from Gabon, while South African *C. flavescens*, and *C. hirta* form two additional clades. On the basis of these results, the validity of some taxa in the *C. olivieri* group should be re-evaluated.

Keywords: Africa; Control region; *Crocidura olivieri*; Cytochrome *b*

Introduction

The family Soricidae, within the order Eulipotyphla (former Insectivora), is divided into three sub-families, the Soricinae, the Myosoricinae, and the Crocidurinae, of which *Crocidura* is the main genus with 172 species (Hutterer 2005). The Crocidurinae have a rather tropical distribution, with, in terms of genera, a higher diversity in Africa (Quérouil et al. 2001) where its morphology-based taxonomy presents many uncertainties (Butler et al. 1989). This problem was first noted more than 15 years ago, but has remained significant until today. Quérouil et al. (2005) compared molecular data in some of the problematic taxa with classical morphological assignment. The authors demonstrate that some morphologically distinguished species are not confirmed by molecular genetics and, in turn, strongly differentiated genetical clades do not correspond to morphological species.

The focus of our investigation are the African giant shrews, here named the '*Crocidura olivieri* group', because of the diversity of this species within this complex, and because of its large distribution. This group was previously named the *C. flavescens* group by Heim de Balsac and Barloy (1966). Members of this group are widespread throughout Africa and express an ecological plasticity seldom achieved by other *Crocidura*

*Corresponding author.
E-mail address: sylvain.dubey@unil.ch (S. Dubey).

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species (Heim de Balsac and Aellen 1958) but found in the ecologically equivalent Asian house shrew *Suncus murinus* (Lekagul and McNeely 1988; Hutterer and Tranier 1990). The species of this group are adapted to a variety of habitats such as savannah, mountain, hygrophile forest, steppe, and marsh, which provide diverse habitats for the species (Heim de Balsac and Barloy 1966). The present distribution and population density are strongly influenced by anthropogenic factors. Indeed, this group is synanthropic, and its current distribution may reflect human activities as well as natural processes. This is the case in African villages, and they may even reach higher densities in towns such as Abidjan and Dakar where they seem to replace rats as the dominant urban species (Heim de Balsac and Barloy 1966).

The main morphological character regrouping taxa in the African giant shrews group is their large size. Thus, the term 'group' for the African giant shrews is not well defined by synapomorphic characters, as a large size could be due to similar selective pressure and convergent evolution. It is therefore possibly not a monophyletic clade. Consequently, it is difficult to delineate the different species and sub-species. Heim de Balsac and Barloy (1966) regrouped a variety of local forms under the name *C. flavescens*, including for example *kivu*, *manni*, *nyansae*, *occidentalis*, and *sururae*. According to Heim de Balsac and Barloy (1966), the name of the *C. flavescens* group was first assigned on the basis that within the African giant shrews, *C. flavescens* (Geoffroy, 1827) was the oldest available name. They considered another giant shrew described in the same year, *C. olivieri* (Lesson, 1827), based on embalmed shrews from ancient Egypt (Hutterer 1994), to be invalid. When an analysis of chromosomes of South African *C. flavescens* (Maddalena et al. 1987) showed a significant difference to *C. olivieri* from Egypt (De Hondt 1974), it turned out that *C. flavescens* was a separate species confined to South Africa, whereas *C. olivieri* is a species with a wide range, including most of the broadly distributed polymorphic giant shrews (Meylan and Vogel 1982). The conclusions based on chromosome studies were confirmed by a subsequent study of allozymes (Maddalena 1990a). As the same karyotype may include several related species (Vogel et al. 2003), the taxonomic status of these different forms remained problematic.

A particular problem concerns the black forms that were originally described as different species, *C. odorata* and *C. giffardi*, or even as a different genus, *Praesorex goliath* Thomas, 1913. In a synthesis, Heim de Balsac and Meester (1977), followed by Hutterer et al. (1982), concluded that the black forms must be assigned to the same species, *C. odorata*, with a wide range from Burkina Faso (BF) to the Congo. Unexpectedly, the black *giffardi* morph turned out to show the same karyotype as *C. olivieri* (Meylan and Vogel 1982), and to pool genetically with *C. olivieri manni* (Maddalena 1990a, b). However, Hutterer (1993) redefined the previously named *Praesorex goliath* as *Crocidura goliath* because it apparently represented a distinct species that lives in sympatry with *C. olivieri* in the Central African forest. Molecular data for the *goliath* form were interpreted accordingly (Quérouil et al. 2005).

A further chromosomal study by Vogel et al. (1988) that focussed on the Moroccan *C. bolivari* showed once again the same karyotype as *C. olivieri*, and the authors concluded that this form also belongs to the African giant shrews. According to Hutterer (1986), *C. bolivari* had to be assigned to *C. viaria* from the south of the Sahara: a shrew also called *C. hindei* and *C. suahelae* by other authors (Hutterer and Happold 1983). The synonymy of *C. bolivari* and *C. viaria* was confirmed by Maddalena (1990a).

As the study of Quérouil et al. (2005) was based on 16s rRNA, with a weak resolution, we reinvestigated the African giant shrews using two mitochondrial sequences: the cytochrome *b* gene (*cyt-b*) and a non-coding sequence, the control region (*ctr*). As mtDNA has a high degree of variability, this polymorphism has often been used in previous phylogenetic and phylogeographical studies (Fumagalli et al. 1999; Quérouil et al. 2001; Vogel et al. 2003; Fink et al. 2004).

The aim of the present study is to provide a better resolution of the relationships among the species of the *C. olivieri* group throughout its range in Africa and thus to increase our understanding of their phylogenetic relationship by using a comprehensive molecular analysis within this group.

Materials and methods

Sampling and DNA extractions

Eighty-two individuals of the *C. olivieri* group were analysed, with two *C. poensis* and one *C. theresae* chosen as outgroups. Forty-nine specimens originated from West Africa (Fig. 1), 20 from Central Africa, and 13 from Southern Africa (Fig. 1). The set of samples (Table 1) includes material from the following collections: Lausanne (IZEA), Switzerland; Paris (MNHN: Musée National d'Histoire Naturelle de Paris), France; and some specimens issued from earlier collaborations, e.g., G. Bronner (Maddalena 1990b). DNA extraction was carried out using the QIA Amp DNA Mini Kit (Qiagen) either from part of the ethanol-preserved liver or the toes of the sample.

DNA amplification and sequencing

Double-stranded DNA amplifications (PCR) of *cyt-b* were performed with the primer pairs 231F/683r or

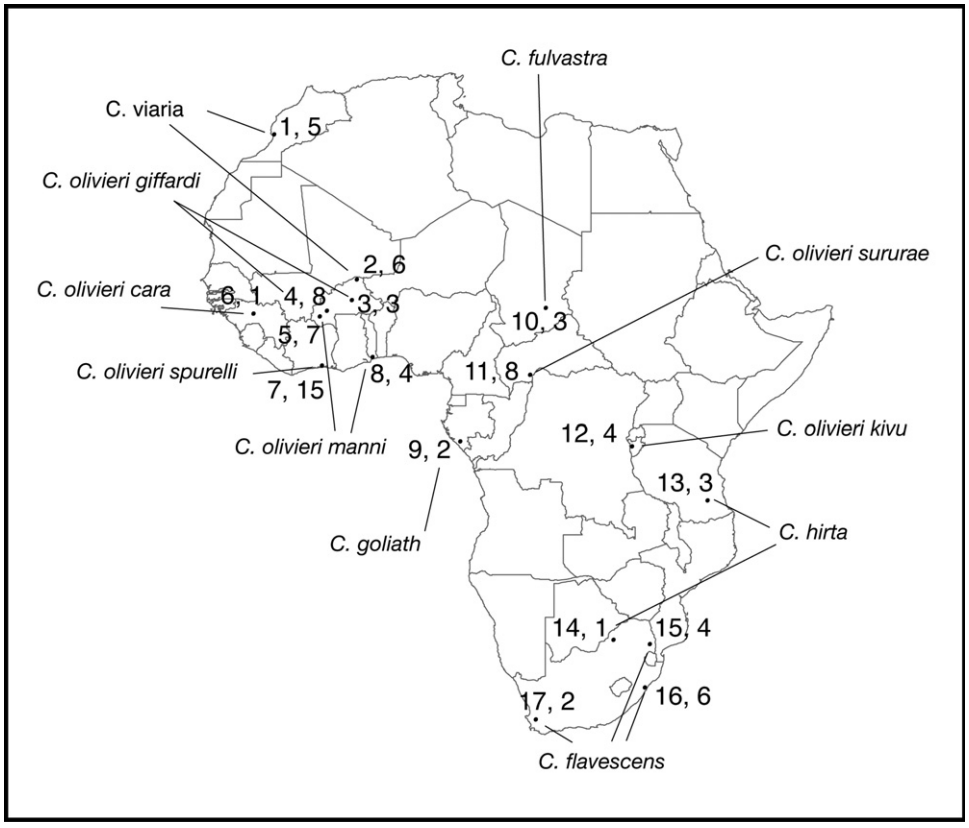

Fig. 1. Distribution of samples throughout Africa (locality number, number of samples). 1: Morocco, Massa; 2: Burkina Faso, Oursi; 3: Burkina Faso, Ouagadougou; 4: Burkina Faso, Bobo Dioulasso; 5: Burkina Faso, Banfora; 6: Guinea, Kazaouma; 7: Ivory Coast, Abidjan; 8: Togo, Lomé; 9: Gabon, Moueva; 10: Chad, Zakouma; 11: Republic of Central Africa, Bangui; 12: Burundi, Kigwena; 13: Tanzania, Ngaremba; 14: South Africa, Ellisras dist.; 15: Graskop dist.; 16: South Africa, Durban and 17: South Africa, Jonkershoek.

C6/683r, and C3/H15915 or 580F/H15915 (see Table 2, Irwin et al. 1991; Dubey et al. 2006), and *ctr* with the primer pairs L16517/H00651 or L16517/RCR2. PCR amplifications were carried out in a 25-µl reaction volume containing 1.25 µl of each of the primers, 2.5 µl of Qiagen PCR buffer 10 ×, 0.25 µl of $MgCl_2$ 25 mM, 2 µl dNTP 2.5 mM, 0.2 µl Quiagen Taq DNA polymerase 5 units/µl, and 2 µl of DNA from the extraction. Amplification conditions for the *cyt-b* consisted of 35 thermal cycles of 30 s denaturing at 94 °C, 45 s annealing at 50 °C (45 °C for the set 580F/H15915), and 1 min extension at 72 °C. Concerning the *ctr*, amplifications were made following the method described by Ehinger et al. (2002).

PCR products were then electrophoresed on a 1% agarose gel, visualized with ethidium bromide staining to verify PCR quality, and purified by centrifugal dialysis using the QIAquick PCR Purification Kit (Qiagen) according to manufacturer's instructions.

Cycle sequencing was performed in 10 µl total volume containing 1–3 µl of amplified DNA, 1 µl of 10 µM primer, and 4 µl of ABI PRISM^{TM} Dye Terminator 1 (Perkin-Elmer). Sequence reactions were visualized on an ABI 3100 genetic analyser (Applied Biosystems).

Sequence analysis

Nucleotide sequences of *cyt-b* and *ctr* sequences were edited with Sequence Navigator (Parker 1997) and aligned by eye. Three methods of phylogenetic analyses were carried out for the two genes using PAUP*version 4.0b10 PPC (Swofford 2001), and trees were rooted using sequences of two *C. poensis* (DQ305209 and DQ305210 for *ctr*, DQ305276 and DQ305277 for *cyt-b*) and one *C. theresae* (DQ305211 and DQ305278), which are genetically distant to the *C. olivieri* group (Maddalena 1990a). Tests were conducted on the total fragments (1241 base pairs (bp)). A neighbour joining (NJ) tree was constructed using Kimura two-parameter genetic distances (K2P; Kimura 1980). The maximum parsimony analyses (MP) were performed using the following options: heuristic search, 10 replicates of random addition of taxa, TBR branch swapping (Swofford 2001), all codon positions were equally

Table 1. Descriptions of samples analyzed in this study, including species, sub-species, country of origin (ISO code), locality, collection number, and sample number

Species	Sub-species	Country	Locality	Collection number	Sample number
Crocidura flavescens		ZA	Durban	IZEA-2349	1
Crocidura flavescens		ZA	Durban	IZEA-2546	2
Crocidura flavescens		ZA	Durban	IZEA-2547	3
Crocidura flavescens		ZA	Durban	IZEA-2549	4
Crocidura flavescens		ZA	Durban	IZEA-2582	5
Crocidura flavescens		ZA	Durban	IZEA-2586	6
Crocidura flavescens		ZA	Graskop dist.	GB-40552	7
Crocidura flavescens		ZA	Graskop dist.	GB-40785	8
Crocidura flavescens		ZA	Graskop dist.	GB-40786	9
Crocidura flavescens		ZA	Graskop dist.	GB-40787	10
Crocidura flavescens		ZA	Jonkershoek	GB-40564	11
Crocidura flavescens		ZA	Jonkershoek	GB-40565	12
Crocidura fulvastra		TD	Zakouma	MNHN-2000069	13
Crocidura fulvastra		TD	Zakouma	MNHN-2000070	14
Crocidura fulvastra		TD	Zakouma	MNHN-200065	68
Crocidura goliath		GA	Moueva	MNHN-2001067	15
Crocidura goliath		GA	Moueva	MNHN-2001096	16
Crocidura hirta		ZA	Ellisras dist.	GB-40208	17
Crocidura hirta		TZ	Ngarembe	MNHN-KP106	69
Crocidura hirta		TZ	Ngarembe	MNHN-KP122	70
Crocidura hirta		TZ	Ngarembe	MNHN-KP159	71
Crocidura olivieri	*cara*	GN	Kazaouma	MNHN-20030922	18
Crocidura olivieri	*giffardi*	BF	Bobo diulasso	IZEA-2503	19
Crocidura olivieri	*giffardi*	BF	Bobo diulasso	IZEA-2504	20
Crocidura olivieri	*giffardi*	BF	Bobo diulasso	IZEA-2505	21
Crocidura olivieri	*giffardi*	BF	Bobo diulasso	IZEA-2506	22
Crocidura olivieri	*giffardi*	BF	Bobo diulasso	IZEA-2507	23
Crocidura olivieri	*giffardi*	BF	Bobo diulasso	IZEA-3100	24
Crocidura olivieri	*giffardi*	BF	Bobo diulasso	IZEA-3101	25
Crocidura olivieri	*giffardi*	BF	Bobo diulasso	IZEA-3102	26
Crocidura olivieri	*giffardi*	BF	Ouagadougou	IZEA-3085	27
Crocidura olivieri	*giffardi*	BF	Ouagadougou	IZEA-3086	28
Crocidura olivieri	*giffardi*	BF	Ouagadougou	IZEA-3087	29
Crocidura olivieri	*kivu*	BI	Kigwena	IZEA-2732	30
Crocidura olivieri	*kivu*	BI	Kigwena	IZEA-3076	31
Crocidura olivieri	*kivu*	BI	Kigwena	IZEA-3078	32
Crocidura olivieri	*kivu*	BI	Kigwena	IZEA-3079	33
Crocidura olivieri	*manni*	BF	Banfora	IZEA-2492	34
Crocidura olivieri	*manni*	BF	Banfora	IZEA-2494	35
Crocidura olivieri	*manni*	BF	Banfora	IZEA-2495	36
Crocidura olivieri	*manni*	BF	Banfora	IZEA-2496	37
Crocidura olivieri	*manni*	BF	Banfora	IZEA-2497	38
Crocidura olivieri	*manni*	BF	Banfora	IZEA-2499	39
Crocidura olivieri	*manni*	BF	Banfora	IZEA-2500	40
Crocidura olivieri	*manni*	TG	Lome	IZEA-2351	41
Crocidura olivieri	*manni*	TG	Lome	IZEA-2352	42
Crocidura olivieri	*manni*	TG	Lome	IZEA-2353	43
Crocidura olivieri	*manni*	TG	Lome	IZEA-2355	44
Crocidura olivieri	*spurelli*	CI	Abidjan	IZEA-2404	45
Crocidura olivieri	*spurelli*	CI	Abidjan	IZEA-2672	46
Crocidura olivieri	*spurelli*	CI	Abidjan	IZEA-2673	47
Crocidura olivieri	*spurelli*	CI	Abidjan	IZEA-2392	48
Crocidura olivieri	*spurelli*	CI	Abidjan	IZEA-2406	49
Crocidura olivieri	*spurelli*	CI	Abidjan	IZEA-2553	50
Crocidura olivieri	*spurelli*	CI	Abidjan	IZEA-2762	51
Crocidura olivieri	*spurelli*	CI	Abidjan	IZEA-2365	52

Table 1. (*continued*)

Species	Sub-species	Country	Locality	Collection number	Sample number
Crocidura olivieri	*spurelli*	CI	Abidjan	IZEA-2366	53
Crocidura olivieri	*spurelli*	CI	Abidjan	IZEA-2449	54
Crocidura olivieri	*spurelli*	CI	Abidjan	IZEA-2465	55
Crocidura olivieri	*spurelli*	CI	Abidjan	IZEA-2467	56
Crocidura olivieri	*spurelli*	CI	Abidjan	IZEA-2361	57
Crocidura olivieri	*spurelli*	CI	Abidjan	IZEA-2362	58
Crocidura olivieri	*spurelli*	CI	Abidjan	IZEA-2363	59
Crocidura olivieri	*sururae*	CF	Bangui	IZEA-2570	60
Crocidura olivieri	*sururae*	CF	Bangui	IZEA-2765	61
Crocidura olivieri	*sururae*	CF	Bangui	IZEA-2766	62
Crocidura olivieri	*sururae*	CF	Bangui	IZEA-2802	63
Crocidura olivieri	*sururae*	CF	Bangui	IZEA-2804	64
Crocidura olivieri	*sururae*	CF	Bangui	IZEA-2807	65
Crocidura olivieri	*sururae*	CF	Bangui	IZEA-2821	66
Crocidura olivieri	*sururae*	CF	Bangui	IZEA-3077	67
Crocidura viaria		MA	Massa	IZEA-2616	72
Crocidura viaria		MA	Massa	IZEA-2619	73
Crocidura viaria		MA	Massa	IZEA-2620	74
Crocidura viaria		MA	Massa	IZEA-5071	75
Crocidura viaria		MA	Massa	IZEA-7804	76
Crocidura viaria		BF	Oursi	IZEA-3108	77
Crocidura viaria		BF	Oursi	IZEA-3110	78
Crocidura viaria		BF	Oursi	IZEA-3111	79
Crocidura viaria		BF	Oursi	IZEA-3118	80
Crocidura viaria		BF	Oursi	IZEA-3327	81
Crocidura viaria		BF	Oursi	IZEA-3331	82

weighted. For maximum likelihood (ML) analyses, likelihood ratio tests, implemented with the computer program Modeltest 3.06, were first used to choose the mutation model that best fitted the data, according to the protocol of Posada and Crandall (1998).

The TVM + I + G model was selected with base frequencies (A = 0.3043, C = 0.2437, G = 0.1455, T = 0.3065) estimated from the data, an unequal distribution of rates at variable sites ($\gamma = 0.7560$), and five different substitution types (rate [A–C] = 0.5207, rate [A–G] = [C–T] = 14.8806, rate [A–T] = 2.1171, rate [C–G] = 1.4770, rate [G–T] = 1.0000). ML analyses were then performed assuming this model with the program PhyML version 2.4.4. (Guindon and Gascuel 2003), which enables a dramatic reduction of computing time and a greater likelihood maximization ability. Bootstrap support values were obtained with 1000 pseudoreplicates for MP, NJ analysis, and 10 random replicates for stepwise addition of sequences, using PAUP*version 4.0b10 PPC (Swofford 2001). Bootstrap supports for ML analysis were obtained with 1000 pseudoreplicates using the program PhyML version 2.4.4. (Guindon and Gascuel 2003).

Nucleotide diversity (π) was estimated using the DnaSP program version 4.10.3 (Rozas et al. 2005). The population genetic structure was determined by an analysis of molecular variance (AMOVA), available in Arlequin version 2.0 (Schneider et al. 2002). This analysis was performed at two different hierarchical levels: among clades and within clades. The K2P genetic distance between and within groups was calculated using the software Mega version 2.1 (Kumar et al. 2001).

Table 2. Primers designed in our laboratory

Primer name	Sequence (5′–3′ direction)
683R	CCA CCA CAT ATT AAA CCA GA
231F	ATT TGC AGT AAT GGC CAC CG
580F	ACT ATA TTA TCC TCA CTA G
RCr2	GTG AAC ACG TGT GTG AAC ACG

Results

Cyt-b and *ctr*

The 85 individuals analysed in this study exhibit 64 different haplotypes for the whole sequence (*cyt-b* + *ctr*: 1241 bp), of which 421 variable sites were found and 364 were parsimony informative. As the three phylogenetic methods gave identical arrangements of the main

branches, the relationship between haplotypes is given only for the ML analysis (Fig. 2). The GenBank accession numbers are DQ305145–DQ305211 for *ctr* and DQ305212–DQ305278 for *cyt-b*.

Four major clades were observed. The first, Clade A, is the most basal and includes the sample of *C. hirta* from South Africa and Tanzania (Fig. 3). The second, Clade B, includes all the samples of *C. flavescens* from South Africa, and is highly supported by bootstrap values (all values of 100%). Clade C (bootstrap values of 100%) includes two samples of *C. goliath* from Gabon, and is a close relative to Clade D (bootstrap values > 93%), which covers a large region from North and West Africa to Central Africa. Clade D is represented by six sub-species of *C. olivieri* from eight localities, *C. viaria* from BF and Morocco, and *C. fulvastra* from Chad.

This clade (D) is divided into eight sub-clades (I–VIII), supported by reasonable bootstrap values (for sub-clades II–IV and VI–VIII, bootstrap values > 81%; for sub-clades V, bootstrap values > 53%). The northern (VIII) sub-clade includes the samples of *C. viaria* from BF and Morocco as well as the samples of *C. fulvastra*. Sub-clades I and II from the Central African Republic are represented by samples of *C. olivieri sururae*. Sub-clade III in East Africa is represented by samples of *C. olivieri kivu*. In western Africa the situation is more complex. First, we have sub-clade V with samples of *C. olivieri cara* from Guinea, *C. olivieri spurelli* from the Ivory Coast, *C. olivieri manni* from Banfora, BF, and *C. olivieri giffardi* from Bobo Dioulasso (BF). Second, we have sub-clade IV, which includes samples of *C. olivieri manni* from Togo and *C. olivieri giffardi* from Bobo Dioulasso and Ouagadougou (BF). Finally, we have two sub-clades (VI and VII) from Bobo Dioulasso, represented by samples of *C. olivieri giffardi*. Consequently, the samples from Bobo Dioulasso belong to four different sub-clades.

Cyt-b genetic distances

Mean genetic distances (K2P) between clades vary from 4.97% (C vs. D) to 11.83 (B vs. D) (Table 3), while mean genetic distances within clades vary from 0.26% (C) to 4.12% (A). Mean genetic distances between sub-clades of Clade D vary from 0.99% (V vs. VI) to 3.93% (I vs. VIII), while mean genetic distances within sub-clades vary between 0% (VII) and 1.71% (VIII).

Discussion

Our investigation was based on sampling coverage of a large part of Africa except from Egypt to Kenya. The results revealed the monophyly of the *C. olivieri* group ($2N = 50$), as previously demonstrated by Maddalena (1990a) from allozyme studies, and a remarkable distinction between four major clades (mean K2P distances between clades: 4.97–11.83%). The clades are geographically distinct, representing different biomes, and appear to confirm the existence of at least three monophyletic taxa in the African giant shrew group: *C. hirta* Peters, 1852 (Clade A) from southern and eastern Africa (Miombo woodland); *C. flavescens* (Geoffroy, 1827) (Clade B) from southern Africa (southern savannas and fine bush), showing a different fundamental number of chromosomes (NF = 74 vs. 66); and *C. goliath* (Thomas, 1906) from equatorial rain forest regions (Clade C). Considering *C. olivieri* (Lesson, 1827), *C. viaria* (Geoffroy, 1834), and *C. fulvastra* (Sundevall, 1843) the situation is more complicated. In fact, these species all belong to Clade D, which is sub-divided into eight sub-clades (mean K2P distances between sub-clades: 1–3.9%) with undefined inter-relationships. All sub-clades are constituted by sub-species of *C. olivieri* except the last one (VIII), which includes only samples of *C. viaria* and *C. fulvastra*. The proximity of these two taxa is not surprising, as they share the same distribution in northern savannas (Sahelo-Sudanian region) and at the fringe of the Guineo-Congolese forest blocks, with *C. viaria* found in western areas and *C. fulvastra* in eastern areas.

We also note a genetic differentiation between the *C. viaria* populations of BF and Morocco (K2P: 2.36%) that reflects the isolation of these species from the rest of Africa due to the Sahara desert. Concerning the other sub-clades (I–VII), they are distributed from Guinea to Burundi, with a hotspot in western areas where several sub-species of *C. olivieri* are mixed in the same clade, as *manni* (from BF), *spurelli* (from Ivory Coast), *cara* (from Guinea), and *giffardi* (from Bobo Dioulasso, BF) for sub-clade V, and *giffardi* (from Bobo Dioulasso and Ouagadougou from BF) and *manni* (from Togo) for sub-clade IV, which share a large size as a common characteristic. We also note the presence of two other distinct sub-clades represented by the sub-species *giffardi* in Bobo Dioulasso. This unexpected diversity of sub-clades observed in western Africa, especially in BF, is probably due to the high level of human activity in this part of the continent and the behaviour of *C. olivieri*, which is very commensal and commonly found in houses, food stores, markets, banana plantations, and farmlands as well as riverine habitats (Hutterer and Happold 1983). It is therefore likely that human activities have played a role in its dispersion.

Several studies of Soricidae show that the mean intraspecific genetic distances (K2P) for *cyt-b* for the genus *Sorex* vary from 0% to 5.21% and interspecific distances from 1.32% to 21.7% (Fumagalli et al. 1999). Equivalent values for the genus *Crocidura* are 0.1–9% and 5.7–20.6%, respectively (Vogel et al. 2003; Moto-

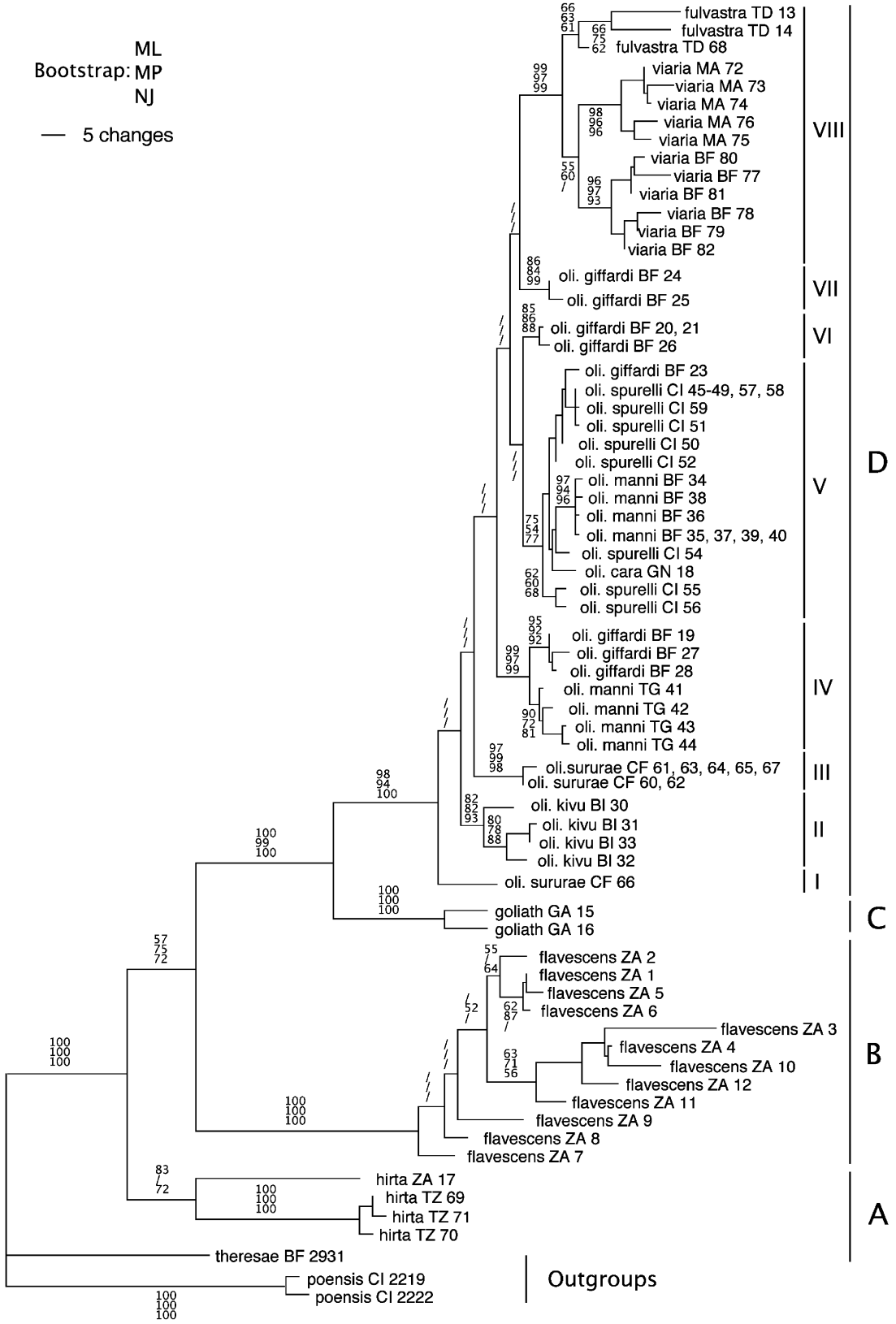

Fig. 2. Phylogeny of the *cyt-b* + *ctr* fragments (1241 bp) analysed with maximum likelihood (ML) using the TVM + I + G model of substitution and TBR branch swapping. Values in branches are bootstrap indices of support for the major branches for ML, maximum parsimony (MP), and distance (NJ) analyses (percentage of 1000 replications for each of the 10 random orders of stepwise addition of sequences). Codes are as in Table 1.

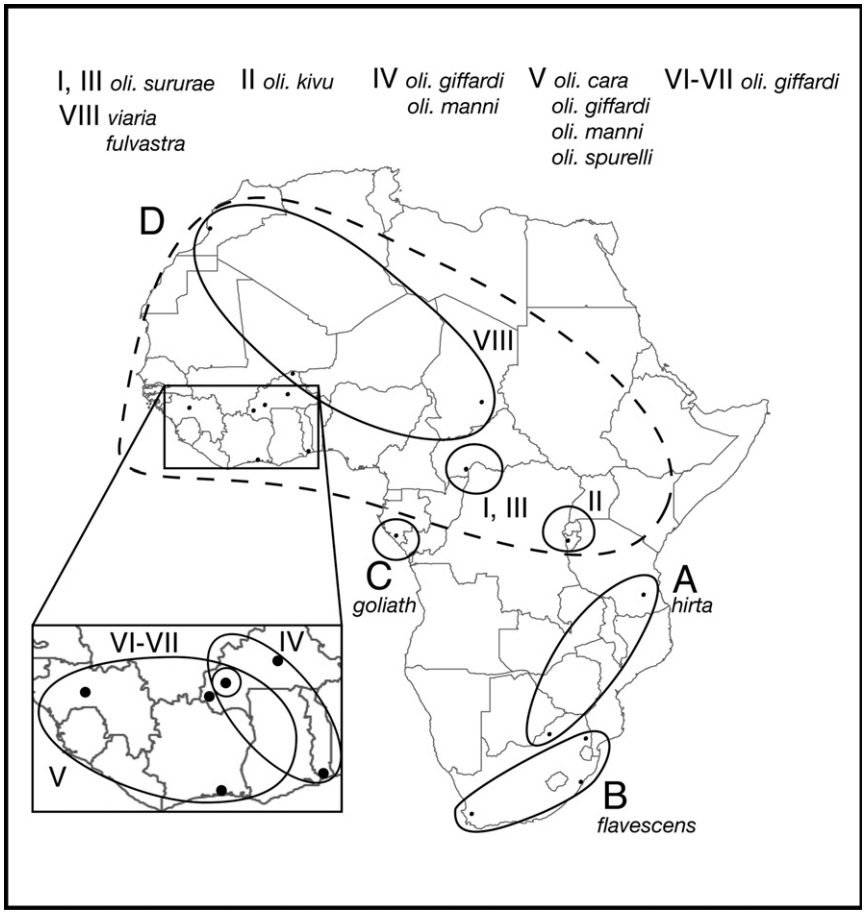

Fig. 3. Distributions of the different clades (A–D) and sub-clades (I–VIII).

kawa et al. 2000), and 0.48–3.51% and 7.73–10.7% for the genus *Blarina* (Brant and Orti 2002). In addition, based on the same gene and genetic distance, Bradley and Baker (2001) showed that genetic values between 2% and 11% had a high probability of being indicative of conspecific populations or valid species for rodents and bats, and therefore merit additional study concerning their specific status.

Thus, based on these previous studies and the large genetic distance (mean K2P between clades: 10.7–11.2%) between the monophyletic taxa of Clades A–C, i.e., *C. hirta*, *C. flavescens*, and *C. goliath*, we accept the species assignments of these previous authors. In contrast, regarding Clade D, i.e., *C. olivieri*, *C. fulvastra*, and *C. viaria*, the small genetic distances (1–3.9%) determined in our study do not enable us to state the level of differentiation. Consequently, we consider it premature to ascribe distinct species names to the different genetic populations of this clade. As in the Eurasian *C. suaveolens* group (Dubey et al. 2006), the only way to resolve this question is to study gene flow in the contact zones of the respective populations using both mitochondrial and nuclear markers.

Table 3. Mean *cyt-b* genetic distances (K2P) between clades (A–D) and outgroups (Out1 = *C. theresae*, Out2 = *C. poensis*)

	A	B	C	D	Out1	Out2
A	**0.0419**					
B	0.1096	**0.0251**				
C	0.1124	0.1142	**0.0026**			
D	0.1110	0.1183	0.0497	**0.0306**		
Out1	0.1647	0.1907	0.1873	0.1875	—	
Out2	0.1946	0.2038	0.1986	0.2004	0.1165	**0.0026**

Bold values in the diagonal column represent the genetic distance within clades.

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