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Phylogenetic relationships within the *cahirinus-dimidiatus* group of the genus *Acomys* (Rodentia: Muridae): new mitochondrial lineages from Sahara, Iran and the Arabian Peninsula

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Abstract

Spiny mice belonging to the *cahirinus-dimidiatus* group of the genus *Acomys* have become a widely used model in physiology and behaviour. To improve current knowledge concerning the phylogeny of this taxon, we analysed 24 samples from Libya, Chad, Egypt, Jordan, Cyprus, Crete, Turkey, Yemen and Iran. We sequenced the whole mitochondrial control region and part of the flanking tRNA genes for a total length of 986 to 996 bp and described 22 haplotypes. Our results confirmed that the Afro-Mediterranean and Asian clades are clearly distinct (p-distance = 6-8.1%). The former clade corresponds to *A. cahirinus sensu lato* (i.e. including also the Cretan *A. minous*, Cypriot *A. nesiotes* and Turkish *A. cilicicus*). Haplotypes of *A. cahirinus* from the E Sahara (S Egypt, SW Libya, N Chad) grouped with those of *A. cilicicus* and *A. minous* (p-distance $\leq$ 2.2%), while haplotypes of *A. nesiotes* grouped with one haplotype representing the commensal *A. cahirinus* from Cairo (p-distance = 1.2%). Close similarity among haplotypes from mainland Africa and NE Mediterranean (clade *A. cahirinus sensu stricto*) support the hypothesis that ancestors of *A. nesiotes*, *A. cilicicus* and *A. minous* dispersed most probably as commensal populations, thus questioning their status of valid species. The most surprising finding was the considerable genetic variation in Asia. In addition to a haplogroup from Sinai and Jordan (corresponding to *A. dimidiatus sensu stricto*), we detected two previously unknown haplogroups, from Yemen and Iran + United Arab Emirates. These clades are fairly distinct and separate species/subspecies status of these animals might be further considered.

Key words: spiny mice, mitochondrial DNA, mitochondrial control region, D-loop, phylogeography, commensalism, Yemen, Libya, Cyprus, Persian Gulf

Relazioni filogenetiche all'interno del gruppo *cahirinus-dimidiatus* nel genere *Acomys* (Rodentia: Muridae): nuove linee mitocondriali identificate nella regione del Sahara, in Iran e nella penisola araba

Sommario

I topi spinosi appartenenti al gruppo *cahirinus-dimidiatus* nel genere *Acomys* sono diventati animali modello ampiamente usati in studi fisiologici e comportamentali. Per migliorare le conoscenze attuali riguardanti la filogenesi di questo taxon, abbiamo analizzato 24 esemplari di topo spinoso provenienti da Libia, Chad, Egitto, Giordania, Cipro,

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Creta, Turchia, Yemen e Iran. Abbiamo sequenziato l'intera regione di controllo del mitocondrio e parte degli adiacenti geni per la sintesi di tRNA, per una lunghezza totale tra i 986 e i 996 bp, descrivendo 22 diversi aplotipi.

I nostri risultati hanno confermato la presenza di considerevoli differenze tra il clade afro-meditterraneo e il clade asiatico (distanza p = 6-8,1%). Il primo clade corrisponde ad *A. cahirinus sensu lato* (compresi *A. minous*, presente in Creta, e *A. nesiotes*, presente a Cipro). Gli aplotipi di esemplari di *A. cahirinus* provenienti dal Sahara orientale (Egitto meridionale, Libia sud-occidentale, Chad settentrionale) risultano simili a quelli di individui di *A. cilicicus* e *A. minous* (distanza p $\leq$ 2,2%). Mentre gli aplotipi di animali identificati come a *A. nesiotes* sono simili a quelli di topi commensali originari dal Cairo (*A. cahirinus* propriamente detto). La stretta somiglianza tra gli aplotipi proveneinti dall'Africa continentale e la regione meditterranea orientale (*A. cahirinus sensu strictu*) conferma l'ipotesi che gli antenati di *A. nesiotes, A. cilicicus* e *A. minous* si dispersero come popolazioni commensali, mettendo quindi in discussione la validità di questi taxa come specie.

Sorprendente è stata la scoperta di una notevole variabilità genetica presente in Asia. Oltre ad un chiaro raggruppamento di aplotipi, corrispondente ad esemplari della penisola del Sinai e della Giordania (appartenenti ad *A. dimidiatus sensu stricto*), abbiamo identificato due gruppi finora sconosciuti: un primo gruppo in Yemen e un secondo in Iran e negli Emirati Arabi. Questi due cladi sono chiaramente distinti, per essi drovrà essere preso in considerazione un possibile status di specie o subspecie.

Parole chiave: topo spinoso, DNA micondriale, regione mitocondriale di controllo, D-loop, filogeografia, commensalismo, Yemen, Libia, Cipro, Golfo persico

Introduction

Spiny mice belonging to the *cahirinus-dimidiatus* group of the genus *Acomys* have become a widely used model for physiological (e.g., Frynta *et al.* 2009), behavioural (Nováková *et al.* 2010 and references herein) and evolutionary (e.g., Krasnov *et al.* 2005) studies (also see Van der Straeten 1994). Although this complex is morphologically distinct from other *Acomys* species groups (Denys *et al.* 1994), there is no agreement among traditional taxonomists concerning the relationships among populations or species (Wilson & Reeder 2005). Consequently, nearly all experimental animals that come from somewhere within the region of the Fertile Crescent or the Levant were reported as *A. cahirinus* (Desmarest, 1819), irrespective of their precise taxonomic status (e.g., Carere *et al.* 1999; Hefner *et al.* 2001; Weber & Hohn 2005).

Barome *et al.* (1998; 2000; 2001), analysing variation in the cytochrome *b* (mtDNA) in 14 *Acomys* species, revealed the existence of two distinct subclades within the *cahirinus-dimidiatus* clade. One comprises *A. dimidiatus* (Cretzschmar, 1826) from Sinai, Israel, Jordan and Saudi Arabia, its sister branch including unnamed forms from Cameroon and Burkina Faso. The other one includes *A. cahirinus* (Desmarest, 1819) from Egypt and *A. airensis* Thomas et Hinton, 1921 from Niger and Mali. Currently, a thorough study examining samples from SW Sahara revealed that the populations from Mauretania, Mali and Niger form a distinct clade clearly separated from both the *cahirinus* and *dimidiatus* groups (Nicolas *et al.* 2009). This subclade includes not only *A. airensis* but also Mauretanian populations of *A. chudeaui* (Kollman, 1911), therefore the former species should be further considered as a junior synonym of the latter (Nicolas *et al.* 2009).

Surprisingly, two Mediterranean species, *A. nesiotes* Bate, 1903 from Cyprus, and *A. minous* Bate, 1906 from Crete, had cyt *b* sequences almost identical with those of *A. cahirinus* from the type locality (Cairo, Egypt). The remaining species of the Mediterranean area, *A. cilicicus* Spitzenberger, 1978 from Cilicia (SE coast of Anatolia, Turkey) in addition to some *A. minous*, shared a somewhat different haplotype lineage (labeled B) of unknown origin, but were still unequivocally closely related to those of Egyptian *A. cahirinus* (Barome *et al.* 2000; 2001). The above findings may suggest that these Mediterranean species are not endemic survivors from the Tertiary period, but rather are descendants from recent, most probably commensal colonists transferred to these areas by humans.

Recently, Volobouev *et al.* (2007) formally elevated *dimidiatus* to the rank of species and reviewed karyological (cf. Nevo 1985; Sokolov *et al.* 1993; Macholán *et al.* 1995; Volobouev *et al.* 1996a; b; 2002; Kivanc *et al.* 1997; Kunze *et al.* 1999; Zima *et al.* 1999), morphological and biogeographical evidence suggesting clear differences between *A. cahirinus* from Africa (including Egypt) and *A. dimidiatus* from the

Asian part of the range including Sinai. They also hypothesized a phylogeographic scenario including immigration of *dimidiatus* from Africa to south of the Arabian Peninsula through the Red Sea. Nevertheless, in spite of the extensive distribution of *A. dimidiatus* in Asia (Bates 1994), ranging from Sinai, throughout the Arabian Peninsula, the Iranian coast of the Persian Gulf region and the Gulf of Oman to W Pakistan (Bobrov & Neronov 1998), only the populations from Sinai, Israel and Jordan have been examined by molecular methods, so far.

Species rank of *A. dimidiatus* may be supported also by the fact that fertile hybrids between *A. dimidiatus* and *A. cahirinus*, neither natural nor artificial, have been described. Jordan (2000), however, reported sterile hybrids between the dark commensal population of *A. cahirinus* from Cairo and a large pale form from Giza (suburb of Cairo) referred to as *A. dimidiatus megalodus* Setzer, 1959. As the type locality of the latter taxon is the Suez region (Wadi Sayal), species identity of the latter population was not assessed via molecular methods, and according to Volobouev *et al.* (2007), not *A. dimidiatus* but rather *A. cahirinus* has to be expected at the locality situated in the African part of Egypt.

The aim of this paper is to sequence fragments of rapidly evolving mitochondrial genes providing high resolution for recent evolutionary history, to reconstruct the phylogeny of the *cahirinus-dimidiatus* group with a special focus on understudied regions including eastern Sahara, Cyprus, Yemen and the Persian Gulf. We then discuss our results in terms taxonomic and phylogeographic implications.

Material and methods

Specimens. For the present study, 24 individuals belonging to the *cahirinus-dimidiatus* group of the genus *Acomys* were analysed. Our specimens or their maternal ancestors were live-trapped from natural populations in Egypt (2 samples), Libya (1), Cyprus (2), Crete (1), Turkey (1), Sinai Peninsula (4), Jordan (3), Yemen (2), United Arab Emirates (1), and Iran (3). Other samples came from laboratory populations in Egypt (1), Chad (1), and zoological parks (2; original localities unknown). The tip of the tail or a finger were taken from sampled animals and stored in Eppendorf tubes with 96% ethanol. Alternatively, as concerned deceased animals, kidney or muscle tissues were used. Origins of the specimens are detailed in Table 1.

DNA extraction and sequencing. Total genomic DNA was isolated with DNAeasy Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer's guidelines.

The entire mitochondrial Control Region (CR) and the flanking tRNA genes were PCR-amplified in two overlapping segments for a total length of about 1000 bp, using primer pairs 5' ATAAACATTACTCTGGTCTTGTAAAC 3' – 5' CACAGTTATGTTGRTCATGG 3' and 5' CGTTCCCCTAAATAAGACA 3' – 5' TAATTATAAGGCCAGGACCA 3' (Bellinvia, 2004).

PCR reactions were carried out in 50 μl volume including 2.5 μl of each 10 μM primer, 5 μl of 10X PCR buffer (Fermentas), 5 μl of 10 mM dNTP, 2.5 μl of 50 mM $MgCl_2$, 0.5 μl of 5 U/ml Fermentas Taq DNA polymerase, 5 μl of DNA and 27 μl of ddH_2O. The PCR amplification protocol consisted of 31 cycles of denaturation at 95ºC for 30 s, annealing at 50ºC for 1 min, and extension at 72ºC for 1 min; a further 15 min elongation step at 72ºC followed the last cycle. Concentration and composition of the reaction mix were similar for both pairs of primers. The protocol used followed Bellinvia (2004). For some of the samples the temperature of annealing had to be decreased to 47ºC to obtain usable PCR products. All PCR products were purified with the Qiaquick® purification kit (Qiagen, Hilden, Germany) and directly sequenced using the same primers used for amplification.

Sequence and phylogenetic analyses. Sequences were aligned and manually checked using BioEdit (Hall 1999), Clustal X 1.81 (Thompson *et al.* 1997) and GENEDOC version 2.6.003 (Nicholas & Nicholas 1997). Three individuals of *A. russatus* were included as outgroup.

Neighbour-joining (NJ), maximum parsimony (MP) and maximum likelihood (ML) analyses were performed under PAUP* version 4.0b10 (Swofford 2002), and Bayesian analysis (BA) was conducted with MrBayes 3.1 (Huesenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003). For MP we conducted heuristic search analyses with 100,000 random taxon addition replicates using tree-bisection and reconnection (TBR)

branch swapping. The branch support was evaluated using 1000 bootstrap pseudoreplicates (Felsenstein 1985). All characters were equally weighted and unordered. Tree search with NJ algorithm was done with Jukes – Cantor distance and node support within the final topology was assessed through 1000 bootstrap pseudoreplicates.

TABLE 1. Sample set considered in the present study, with geographic origin of samples.

No.	Num. map	Species	Locality	Geographic origin
NES1	1	*A. nesiotes*	Ağirdağ, Cyprus (leg. M. Macholán)	35º 18' N, 33º 15' E
NES2	2	*A .nesiotes*	Cinarli 4km SE, NE Cyprus (leg. D. Frynta)	35º 19' 06 N, 33º 47' 26 E
CAIR	3	*A. cahirinus*	Cairo, Egypt, laboratory colony at Charles University, Praha (founders provided by P.-O. Barome)	30º 04' N, 31º 14' E
SIM1, SIM2	4	*A. cahirinus*	Abu Simbel archaeological site, Egypt, (founders captured by J. Borek)	22º 22' N, 31º 38' E
LIB	5	*A. cahirinus*	Mts Akakus, Libya (colony founders captured by D. Frynta and L. Schwarzová)	25º 44' 562 N, 12º 08' 211 E
CHAD	6	*A.cahirinus*	Tibesti, Chad, laboratory colony in ZOO Plzeň	–
CIL	7	*A. cilicicus*	E of Silifke, Turkey, 2 samples laboratory colony at Charles University, Praha (colony founders captured by J. Sádlová)	36º 26' N, 34º 06' E
MIN	8	*A. minous*	Crete, laboratory colony in ZOO Plzeň	–
EMIR	9	*A. cf. dimidiatus.*	Jabal Hafit, United Arab Emirates, laboratory colony in ZOO Plzeň (colony provided through Breeding centre for endangered Arabian wildlife, Sharjah, UAE, founders captured by Peter Arras, Al Ain)	24º 04' N, 55º 47' E
IRA1	10	*A. cf. dimidiatus*	Khos Hangan, N of Bandar Abbas, Iran; 500 m a.s.l. (colony founders captured by D. Frynta, L. Schwarzová and P. Kunzová)	27º 38' 362 N, 56º 13' 226 E
IRA2	11	*A. cf. dimidiatus*	Zagros, Iran (colony founders captured by D. Frynta, L. Schwarzová and P. Kunzová)	28º 55' 892 N, 52º 31' 770 E
IRA3	12	*A. cf. dimidiatus*	Dehbarez, Iran (leg. P. Benda and P. Nová)	27º 27' 745 N, 57º 19' 197 E
YEM1,YEM2	13	*A. cf. dimidiatus*	Hawf, Yemen (leg. P. Benda)	16º 39' N, 53º 03' E
SIN1,SIN2	14	*A. dimidiatus*	Wadi Gharandal, Sinai, Egypt (leg. R. Lučan)	29º 08' N, 31º 51' E
JOR3	15	*A.dimidiatus*	Wadi Ramm, Jordan (colony founders captured by D. Modrý)	29º 36' N, 35º 24' E
JOR4	15	*A.dimidiatus*	Wadi Ramm, Jordan, ZOO Plzeň (founders captured by D. Modrý a T. Peš)	–
JOR1	15	*A.dimidiatus*	Wadi Ramm, Harab Antar, Jordan (leg. D. Modrý)	29º 36' N, 35º 24' E
JOR2	15	*A.dimidiatus*	Wadi Ramm, Lawrence spring, Jordan (leg. D. Modrý)	29º 36' N, 35º 24' E
BRONX		*A. dimidiatus*	Lab. strains, 2 samples, ZOO Bronx and ZOO Prague	–
RUS1		*A. russatus*	Lab. strain, Charles University, Prague	–
RUS2		*A. russatus*	Wadi Ramm, Harab Antar, Jordan (leg. D. Modrý)	29º 36' N, 35º 24' E
LEW		*A.russatus „lewisi"*	laboratory colony, Al Wisad-Heber, Jordan (leg. D. Modrý)	31° 50' N, 38° 08' N

Optimal model of studied mtDNA sequence evolution was selected using the AIC criterion in Modeltest 3.7 (Posada & Crandall 1998). For ML analysis we used heuristic search with 300 random taxon addition replicates and TBR branch swapping. Node support within the ML tree topology was assessed by bootstrap analysis with 750 pseudoreplicates (in each 10 random addition replicates only).

For the Bayesian analysis, we partitioned our alignment into tree domains: (i) the Central domain (CD), which is the most conserved region of CR; (ii) the Extended terminal-associated sequence (ETAS) domain; and (iii) the Conserved sequence block (CSB) domain, adjacent to CD (see Larizza et *al*. 2002). Two

independent runs of analyses were conducted with a random starting tree and for $6x10^6$ generations, with trees sampled every 100 generations. The burn-in command was used to discard the first 15000 trees (1,500,000 generations).

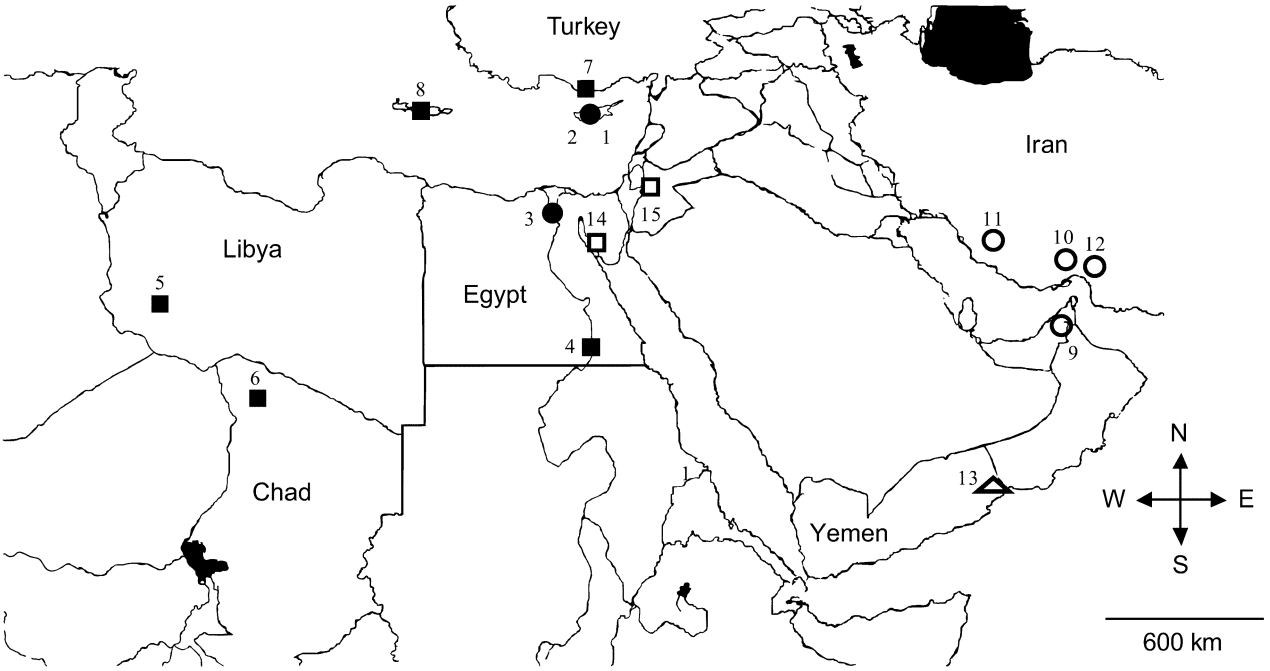

FIGURE 1. Map of sampled localities in North Africa, the Mediterranean, the Arabian Peninsula and Iran within the *cahirinus-dimidiatus* group of the genus *Acomys*. Black circles and squares show *cahirinus* lineages from Cairo-Cyprus and eastern Sahara-Turkey-Crete, respectively; white circles show *dimidiatus* lineages from Iran-Emirates; white triangle shows Yemen; white squares show the Sinai-Jordan lineage. Numbers refer to Table 1.

Results

We analyzed 24 ingroup samples representing 22 haplotypes. We obtained a nucleotide alignement of 1006 nucleotide positions, of which 219 were variable and 195 were parsimony-informative. The CR itself ranged in length from 837 to 839 bp.

Most of the recovered topologies agreed substantially (MP, NJ and BA), although ML somewhat differed by placing haplotypes from Iran and Emirates as basal offshoots of the *cahirunus-dimidiatus* group. MP, NJ and BA revealed revealed two clearly distinct clades. The first clade, further referred to as "Afro-Mediterranean", contained haplotypes from North Africa, Crete, Cyprus and Turkey. The second clade, further referred to as "Asian", contained haplotypes from Sinai, Jordan, the Arabian Peninsula as well as those from Iran (*dimidiatus*). Uncorrected p-distances between haplotypes belonging to the Afro-Mediterranean and Asian clades varied within the range of 6.0–8.1% (Table 2).

Haplotypes belonging to the Afro-Mediterranean clade were very similar to each other (uncorrected p-distances varied within the range of 0.2–2.2 %). Phylogenetic relationships within this clade were poorly supported. However, haplotypes from eastern Sahara (S Egypt, S Libya, N Chad), Turkey and Crete formed a distinct, monophyletic group.

The Asian clade was less homogenous than the Afro-Mediterranean one (within-group uncorrected p-distances = 0.3–5.6 %). It split into three distinct and geographically localised, well-supported lineages: (1) Sinai-Jordan, (2) Yemen, (3) Iran-Emirates. The relative position of these lineages in the tree was not resolved.

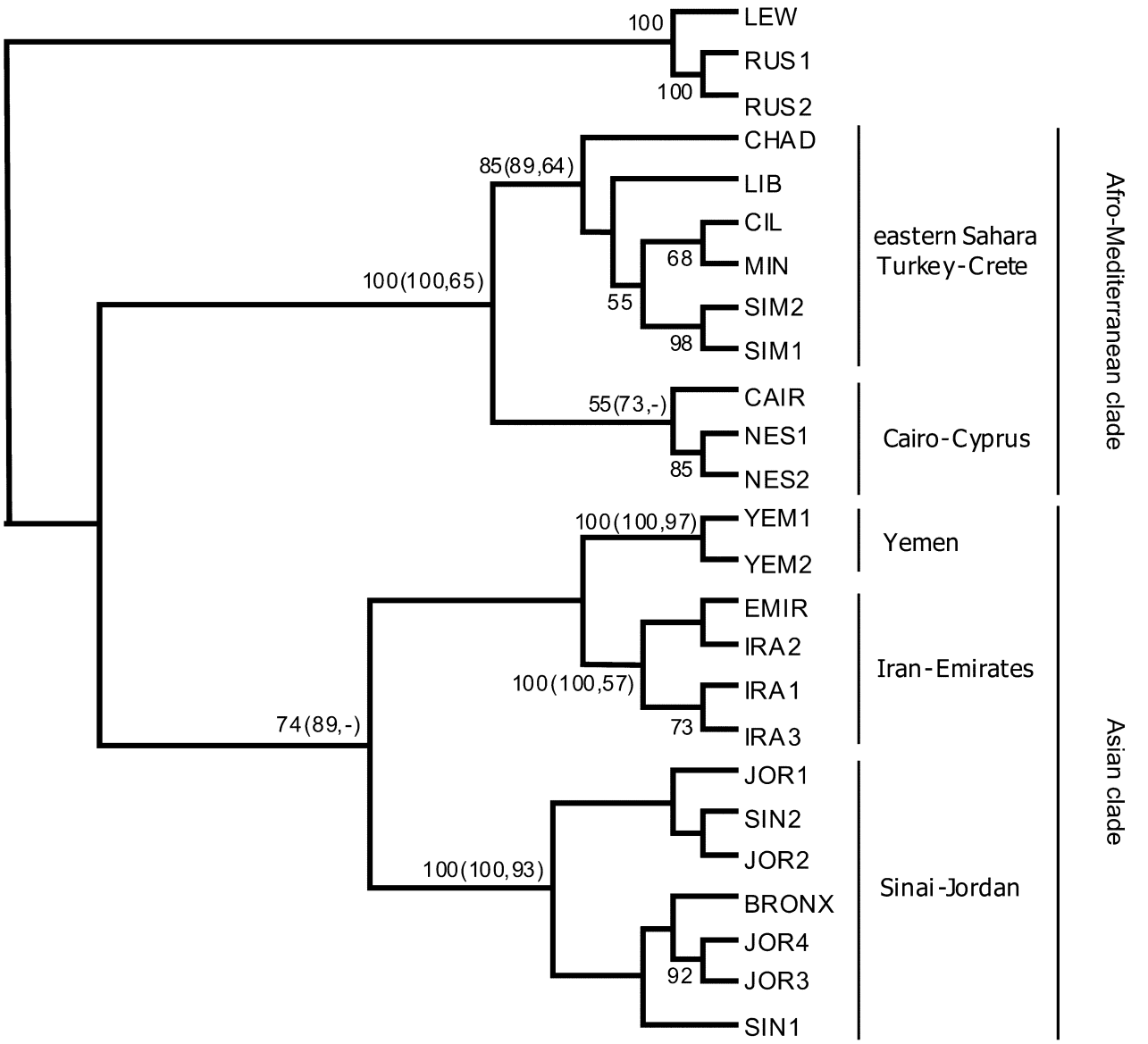

FIGURE 2. Strict consensus parsimony tree of the *A. cahirinus-dimidiatus* group (22 maximum parsimony trees). Tree length = 326, CI = 0.7761, RI = 0.9189, RC = 0.7131. Bootstrap values (in percentage) are indicated at nodes. Bootstrap supports of the main clusters obtained after Neighbour Joining and Maximum Likelihood analyses are provided in parentheses. Codes of samples refer to Table 1.

Discussion

Surprisingly, in spite of there being 2200 km distance between the Akakus Mts (Libya) and Abu Simbel (Egypt), the haplotypes that we found in these two sites and at Tibesti Mts (Chad) were nearly identical (p-distance = 0.3%). This may suggest that the range of the species *A. cahirinus* also encompasses most of the central Sahara region. Because the type locality of *A. airensis* in Air, another south Saharan mountain region that shares with our localities almost the same ecological conditions, is situated just 700 km from the Akakus Mts and 800 km from the Tibesti Mts, the validity of *A. airensis* as a species may be questioned (but see Denys *et al.* 1994; Sicard & Tranier 1996). The populations of *A. airensis* sequenced to date and that were sharply distinct from *A. cahirinus*, came from the lowlands of Mali and Niger (e.g., Barome *et al.* 1998; 2000; 2001, Nicolas *et al.* 2009).

TABLE 2. Uncorrected p-distances between studied haplotypes.

	SIN1	SIN2	JOR1	JOR2	BRONX	JOR4	JOR3	YEM1	YEM2	EMIR	IRA1	IRA2	IRA3	LIB	CHAD	CIL	MIN	SIM2	SIM1	NES1	NES2	CAIR	RUS1	LEW	RUS2
SIN1		0,012	0,009	0,008	0,008	0,007	0,010	0,051	0,051	0,052	0,048	0,047	0,048	0,070	0,068	0,069	0,070	0,069	0,073	0,065	0,067	0,069	0,140	0,137	0,138
SIN2	0,012		0,011	0,010	0,013	0,014	0,017	0,053	0,054	0,059	0,056	0,054	0,057	0,073	0,071	0,074	0,073	0,072	0,076	0,068	0,070	0,072	0,141	0,136	0,139
JOR1	0,009	0,011		0,009	0,013	0,011	0,014	0,051	0,051	0,053	0,049	0,048	0,049	0,069	0,067	0,068	0,069	0,066	0,070	0,066	0,068	0,070	0,138	0,138	0,135
JOR2	0,008	0,010	0,009		0,010	0,009	0,012	0,049	0,049	0,055	0,051	0,050	0,051	0,071	0,069	0,072	0,071	0,070	0,074	0,066	0,068	0,070	0,139	0,134	0,137
BRONX	0,008	0,013	0,013	0,010		0,008	0,011	0,052	0,052	0,055	0,053	0,050	0,053	0,069	0,067	0,070	0,069	0,068	0,072	0,064	0,066	0,068	0,141	0,136	0,139
JOR4	0,007	0,014	0,011	0,009	0,008		0,003	0,050	0,050	0,055	0,051	0,050	0,051	0,065	0,063	0,066	0,065	0,064	0,068	0,060	0,062	0,064	0,140	0,137	0,138
JOR3	0,010	0,017	0,014	0,012	0,011	0,003		0,053	0,053	0,056	0,052	0,051	0,052	0,068	0,066	0,069	0,068	0,067	0,071	0,063	0,065	0,067	0,143	0,140	0,141
YEM1	0,051	0,053	0,051	0,049	0,052	0,050	0,053		0,002	0,059	0,053	0,052	0,054	0,070	0,070	0,073	0,072	0,072	0,075	0,073	0,071	0,073	0,141	0,138	0,139
YEM2	0,051	0,054	0,051	0,049	0,052	0,050	0,053	0,002		0,059	0,053	0,052	0,054	0,070	0,070	0,073	0,072	0,072	0,075	0,073	0,071	0,073	0,141	0,138	0,139
EMIR	0,052	0,059	0,053	0,055	0,055	0,055	0,056	0,059	0,059		0,010	0,007	0,010	0,078	0,076	0,077	0,078	0,075	0,079	0,077	0,077	0,079	0,138	0,134	0,136
IRA1	0,048	0,056	0,049	0,051	0,053	0,051	0,052	0,053	0,053	0,010		0,003	0,002	0,080	0,078	0,079	0,080	0,077	0,079	0,077	0,077	0,081	0,135	0,131	0,133
IRA2	0,047	0,054	0,048	0,050	0,050	0,050	0,051	0,052	0,052	0,007	0,003		0,003	0,077	0,075	0,076	0,077	0,074	0,078	0,074	0,074	0,078	0,134	0,130	0,132
IRA3	0,048	0,057	0,049	0,051	0,053	0,051	0,052	0,054	0,054	0,010	0,002	0,003		0,080	0,078	0,079	0,080	0,077	0,079	0,077	0,077	0,081	0,136	0,132	0,134
LIB	0,070	0,073	0,069	0,071	0,069	0,065	0,068	0,070	0,070	0,078	0,080	0,077	0,080		0,004	0,008	0,006	0,008	0,009	0,019	0,017	0,017	0,136	0,135	0,136
CHAD	0,068	0,071	0,067	0,069	0,067	0,063	0,066	0,070	0,070	0,076	0,078	0,075	0,078	0,004		0,008	0,006	0,008	0,009	0,015	0,013	0,015	0,134	0,131	0,134
CIL	0,069	0,074	0,068	0,072	0,070	0,066	0,069	0,073	0,073	0,077	0,079	0,076	0,079	0,008	0,008		0,002	0,007	0,009	0,019	0,017	0,015	0,135	0,136	0,135
MIN	0,070	0,073	0,069	0,071	0,069	0,065	0,068	0,072	0,072	0,078	0,080	0,077	0,080	0,006	0,006	0,002		0,005	0,007	0,017	0,015	0,013	0,135	0,134	0,135
SIM2	0,069	0,072	0,066	0,070	0,068	0,064	0,067	0,072	0,072	0,075	0,077	0,074	0,077	0,008	0,008	0,007	0,005		0,002	0,018	0,016	0,016	0,135	0,135	0,134
SIM1	0,073	0,076	0,070	0,074	0,072	0,068	0,071	0,075	0,075	0,079	0,079	0,078	0,079	0,009	0,009	0,009	0,007	0,002		0,022	0,020	0,020	0,136	0,137	0,136
NES1	0,065	0,068	0,066	0,066	0,064	0,060	0,063	0,073	0,073	0,077	0,077	0,074	0,077	0,019	0,015	0,019	0,017	0,018	0,022		0,002	0,012	0,137	0,132	0,137
NES2	0,067	0,070	0,068	0,068	0,066	0,062	0,065	0,071	0,071	0,077	0,077	0,074	0,077	0,017	0,013	0,017	0,015	0,016	0,020	0,002		0,012	0,137	0,132	0,137
CAIR	0,069	0,072	0,070	0,070	0,068	0,064	0,067	0,073	0,073	0,079	0,081	0,078	0,081	0,017	0,015	0,015	0,013	0,016	0,020	0,012	0,012		0,140	0,137	0,140
RUS1	0,140	0,141	0,138	0,139	0,141	0,140	0,143	0,141	0,141	0,138	0,135	0,134	0,136	0,136	0,134	0,135	0,135	0,135	0,136	0,137	0,137	0,140		0,017	0,006
LEW	0,137	0,136	0,138	0,134	0,136	0,137	0,140	0,138	0,138	0,134	0,131	0,130	0,132	0,135	0,131	0,136	0,134	0,135	0,137	0,132	0,132	0,137	0,017		0,015
RUS2	0,138	0,139	0,135	0,137	0,139	0,138	0,141	0,139	0,139	0,136	0,133	0,132	0,134	0,136	0,134	0,135	0,135	0,134	0,136	0,137	0,137	0,140	0,006	0,015	

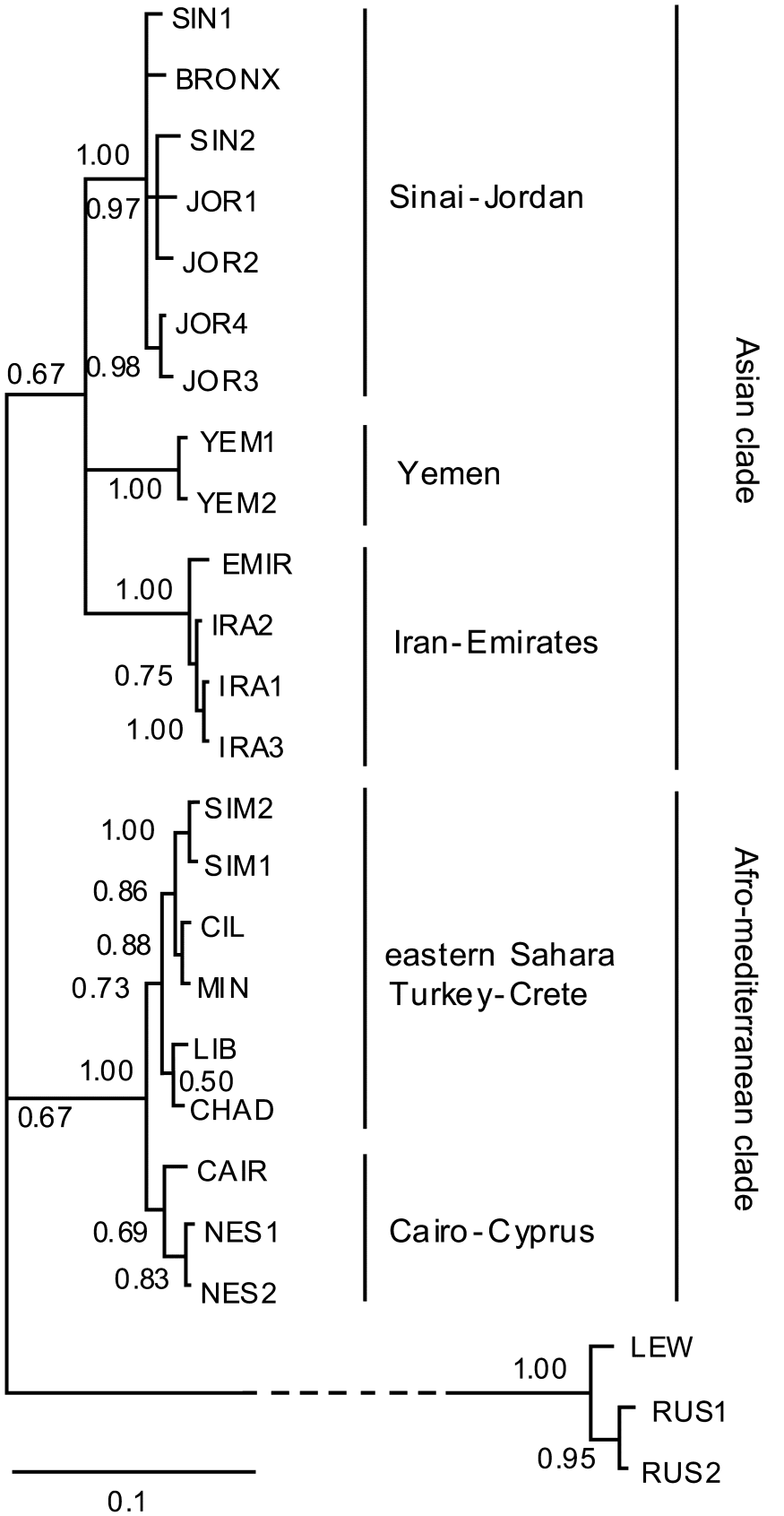

FIGURE 3. Bayesan phylogenetic tree of the *A. cahirinus-dimidiatus* group. Posterior probabilities are given at nodes. For visual convenience, the length of the branch leading to the outgroup has been divided by two. Codes of samples refer to Table 1.

As expected, our results also clearly supported previous studies' that suggested the paraphyly of the North African *A. cahirinus* with respect to other species of *Acomys* coming from the Mediterranean islands and northern coast (*A. nesiotes*, *A. cilicicus* and *A. minous*). Our data provide further evidence supporting the recent dispersal of spiny mice to the NE Mediterranean region (see Barome et al. 1998; 2000). As CR is non-coding sequence evolving even faster than cyt *b*, it provides a somewhat more sensitive test to examine the hypothesis supporting the anthropogenous origin of the Mediterranean species of spiny-mice. Close similarity among particular haplotypes from mainland Africa and those from the NE Mediterranean suggests that ancestors of *A. nesiotes*, *A. cilicicus* and *A. minous* dispersed most probably as commensal populations following ancient trade routes. Their status as valid species may thus be considered questionable.

Barome *et al.* (2001) identified two clearly distinct mitochondrial groups (A and B) within Cretan populations of *A. minous*. The authors reported that haplotypes of *A. nesiotes* from Cyprus and *A. cahirinus* from type locality (Cairo) belonged to group A, while those of *A. cilicicus* from Cilicia coast grouped with B. Given that we recovered the same haplotype attributions, we may tentatively conclude that our lineages from Cyprus and Cairo correspond to group A, while those from Crete and Cilicia belong to group B. We also found haplotypes belonging apparently to group B in the above-mentioned three localities from the eastern Sahara. We can speculate that bearers of group B haplotypes that colonized Crete and Cilicia in antiquity were transferred by Egyptian and/or Phoenician trade ships from southern Egypt. Nevertheless, sampling in North Africa is still too low to allow the exclusion of other mainland regions as potential geographical sources for the group B haplotypes found in NE Mediterranean.

Bearers of both A and B haplotype group contributed to contemporary *A. minous* (Barome *et al.* 2001), and both the *A. cilicicu*s from S Turkish coast and *A. cahirinus* populations from Abu Simbel (belonging to B group) hybridised in our laboratory with *A. nesiotes* and *A. cahirinus* from Cairo (belonging to A group; Frynta & Sádlová 1998 and Frynta et al., in prep.). Therefore we warn against premature taxonomic splitting of mainland populations of *A. cahirinus* according to haplotype group.

We found a close similarity between *A. dimidiatus* haplotypes from Sinai and Jordan, i.e., regions known to be inhabited by different chromosomal forms of this species (Nevo 1985), supporting the current view that 36 and 38 chromosome forms interbreed freely and thus obviously belong to a single species (Volobouev *et al.* 2007).

The similarity between Persian haplotypes and a haplotype from the Emirates on the opposite side of Persian Gulf and the Gulf of Oman may be explained by the presence of a land bridge that allowed free faunal dispersal across the Persian Gulf during the last glacial period, between the south of the Arabian Peninsula and Iran (Anderson 1999).

Considerable sequence divergence within the Asian clade is probably our most surprising finding. Obviously, the southern part of the Arabian Peninsula is a territory with high haplotype diversity and not just a peripheral area of the Asian clade expansion. Thus, it is unlikely that the Arabian Peninsula was colonized by *A. dimidiatus* from north-eastern Africa via Sinai and Jordan. More likely is that the colonisation event could result from prehistoric marine transgression through land bridge across the Red Sea, as suggested by Barome *et al.* (2000) and Volobouev *et al.* (2007). This southern route scenario was previously suggested for other mammals (Bailey 2009) including carnivores, hyrax, oryx (Harrison & Bates 1991), *Hamadryas* baboons (Wildman *et al.* 2004, Winney *et al.* 2004) and even humans (White *et al.* 2003). Nevertheless, recent geological surveys in the Bab al-Mandab revealed that the Red Sea never completely disappeared in this area during the Quaternary period (Fernandes *et al.* 2006). Thus, the occurrence of a land bridge between Africa and the Arabian Peninsula should be considered impossible since at least 2 millions years (Bailey 2009) or even the end of the Miocene (Fernandes *et al.* 2006).

Our results suggest that haplotypes from Iran and Emirates as well as those from Yemen are only distantly related to those from Sinai and Jordan. As *A. dimidiatus* was described from Sinai, the Sinaitic-Jordan lineage might correspond to the nominotypic subspecies *A. d. dimidiatus*. Scientific names for bearers of the Yemeni and Persian haplotype lineages (if their taxonomic distinctness is proven) should be searched among older geographically congruous descriptions, including *A. whitei* Harrison, 1980 from Oman—supposedly matching our Iran-Emirates lineage, *A. d. homericus* Thomas, 1923 from Yemen (type locality El Khaur, Aden Protectorate [= SW Yemen]), and *A. flavidus* Thomas, 1917 described from southern Pakistan. In addition, there are two older descriptions from an unspecified part of Arabia: *A. hispidus* (Brants, 1827) and

A. megalotis (Lichtenstein, 1829). Nevertheless, additional morphological, hybridization and behavioural data are necessary to clarify taxonomic statuses these populations. In addition, the consideration of CR sequences from their African relatives is needed to evaluate genetic variability and possibilities of additional spreading routes (from Africa to Asia, around Africa, etc).

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