



Evolutionary history of the *Paradoxurus* palm civets – a new model for Asian biogeography

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ABSTRACT

Aim Using molecular data and dental features, we investigated the genetic and morphological diversity among species of palm civets in the genus *Paradoxurus*, with a focus on the common palm civet, *Paradoxurus hermaphroditus* (Carnivora, Viverridae), in order to address biogeographic scenarios and provide recommendations for a taxonomic revision.

Location Asia: Pakistan to the Lesser Sunda Islands.

Methods We investigated the genetic diversity within *Paradoxurus* using two mitochondrial (cytochrome *b*, control region) and one nuclear (intron 7 of the β -fibrinogen) markers. We used samples from 85 individuals of *P. hermaphroditus* (including 20 museum specimens) and one representative of each of the other species in the genus *Paradoxurus*: *Paradoxurus jerdoni* and *Paradoxurus zeylonensis*. DNA sequences were analysed using phylogenetic and haplotype network methods, and divergence dates were estimated for the clades retrieved. Furthermore, we examined dental characters from a large series of specimens and compared the morphological variation with the molecular data.

Results Our phylogenetic analyses revealed that *P. hermaphroditus* is paraphyletic. We identified three major lineages distributed: (1) in the Indian subcontinent, south China, Hainan and in areas above 200 m in Indochina; (2) in Peninsular Malaysia, Java, Sumatra and in areas below 200 m in Indochina; and (3) in Borneo, the Philippines and the Mentawai archipelago. Our morphological observations were congruent with these three molecular lineages. Divergence date estimates inferred a Pliocene origin for *Paradoxurus* (2.8–5.7 Ma), with the three main clades diversifying from the mid–Early Pliocene to the end of the Pliocene. We suggest that the flooding of the Isthmus of Kra during the Pliocene was a major event shaping the diversification of *Paradoxurus* palm civets. We also hypothesize that the elevational segregation of the two lineages on the mainland could have resulted from the vegetational changes that were induced by Late Pliocene glacial episodes.

Main conclusions The Isthmus of Kra is a major boundary between two major lineages of *P. hermaphroditus*. There is a need for a taxonomic revision for *P. hermaphroditus*, and we suggest that this species should be split into at least three species.

Keywords

Asia, biogeography, glacial refugia, Isthmus of Kra, mammals, molecular systematics, palm civets, *Paradoxurus*, Viverridae.

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INTRODUCTION

The genus *Paradoxurus* Cuvier, 1821 (Mammalia, Carnivora, Viverridae) includes three species: the golden palm civet, *Paradoxurus zeylonensis* Schreber, 1778, endemic to Sri Lanka; the brown palm civet, *Paradoxurus jerdoni* Blanford, 1885, endemic to the Indian Western Ghats; and the common palm civet, *Paradoxurus hermaphroditus* (Pallas, 1777), which occurs across South and Southeast Asia (Corbet & Hill, 1992; Wozencraft, 2005). These species are largely frugivorous, although their diet includes small prey such as insects, earthworms, molluscs and small vertebrates (Jennings & Veron, 2009). They are solitary, nocturnal and mainly arboreal (Jennings & Veron, 2009), and play an important role as seed dispersers (Schreiber *et al.*, 1989; Gruèzo & Soligam, 1990; Mudappa, 2001). Furthermore, common palm civets are the

source of ‘Kopi Luwak’, one of the most sought-after and expensive coffees in the world (Marcone, 2004), whose seeds undergo chemical modifications in the civet’s digestive system. Like other carnivoran species occurring in tropical and subtropical forests, species in this genus are endangered by the destruction and fragmentation of their habitat, which has been particularly intense in Southeast Asia (Sodhi *et al.*, 2004; Papeş & Gaubert, 2007; Schipper *et al.*, 2008; IUCN, 2010).

This study focuses primarily on the common palm civet, *P. hermaphroditus*, which is widely distributed from Pakistan to the Lesser Sunda Islands (Fig. 1). This species is grey, greyish-brown or rusty-brown, with brown or black body spots and stripes. Its head pattern is very variable, but generally consists of a dark mask, with white or pale grey patches below the eyes, on the forehead, and at the bases of the ears. Substantial geographic variation in pelage pattern and dental

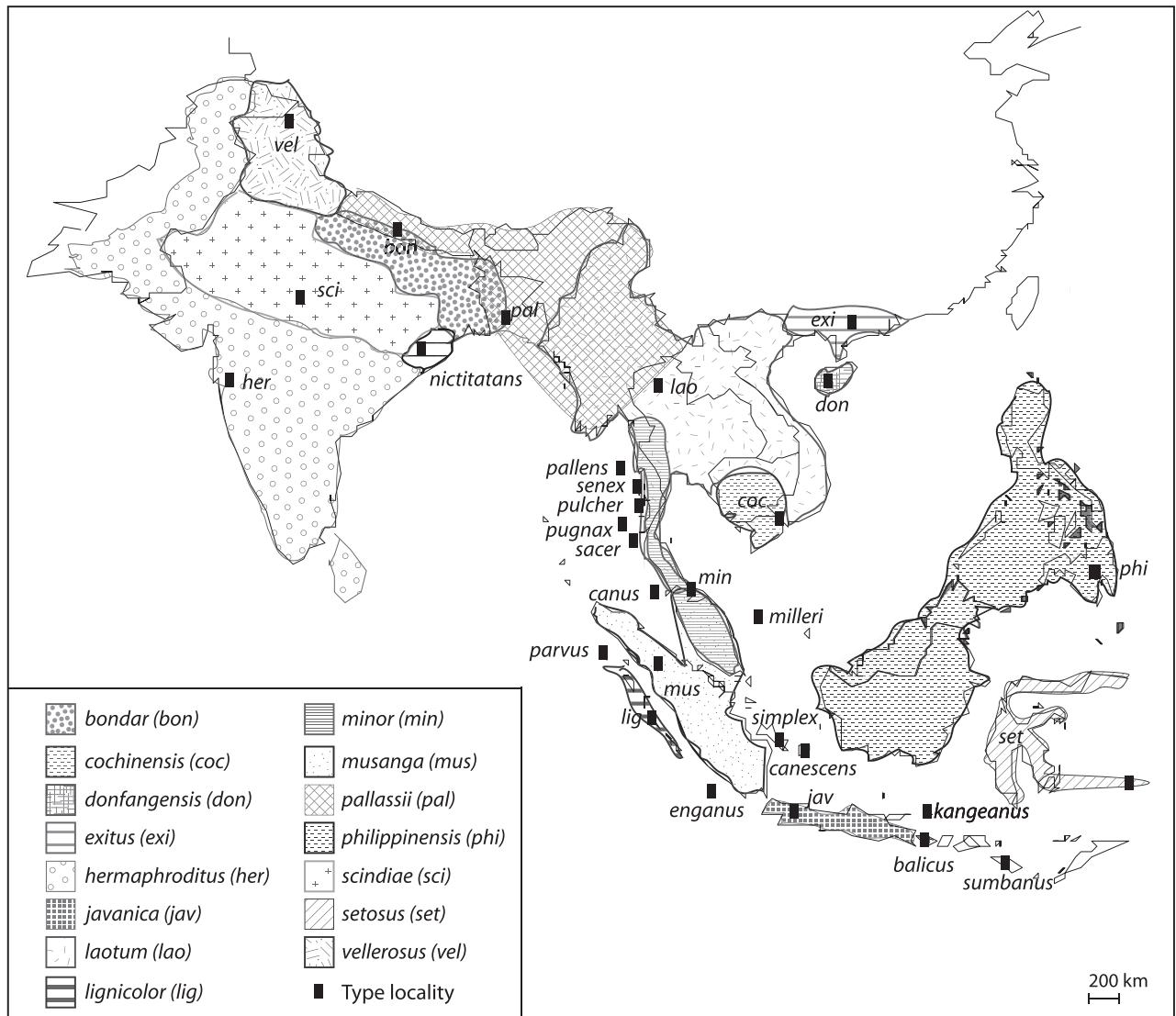


Figure 1 Distribution map of the 30 known subspecies of *Paradoxurus hermaphroditus* (Wozencraft, 2005). Subspecies distribution limits have been drawn following Pocock (1939). Solid rectangles denote the type locality of the subspecies.

morphology has been documented in the common palm civet throughout its range, and around 30 subspecies have been described (Corbet & Hill, 1992; Wozencraft, 2005; Fig. 1); however, their validity has not been recently reviewed or investigated using molecular data, and so the number and status of these subspecies is still debated. For example, the Mentawai palm civet, *Paradoxurus hermaphroditus lignicolor* Miller, 1903, which is endemic to the Mentawai Islands (Indonesia), is considered by some taxonomists as a subspecies (e.g. Chasen & Kloss, 1927; Pocock, 1934a,b), whereas others have suggested full species status (Schreiber *et al.*, 1989; Corbet & Hill, 1992).

The wide distribution of the common palm civet makes this species a good model with which to study Asian biogeography. However, nothing is known about the dispersal abilities of *Paradoxurus* civets, and humans may have transported the common palm civet to several areas, including the Lesser Sunda Islands and Sulawesi (Groves, 1976, 1984; Lekagul & McNeely, 1977; Schreiber *et al.*, 1989; Veron, 2001), and Palawan and the other Philippine islands (Reis & Garong, 2001), although Heaney *et al.* (2002) considered the common palm civet to be a native species in the Philippines.

The geological history of Asia was complex and dynamic during the Tertiary and the Plio-Pleistocene (Hall, 2001). The geographical distribution and intra-specific variation of Asian civet species were probably shaped by major Plio-Pleistocene events, including eustatic (Haq *et al.*, 1987; Miller *et al.*, 2005), climatic and vegetational (Gathorne-Hardy *et al.*, 2002; Bird *et al.*, 2005) changes that have been documented to have influenced the biogeography of other mammals (e.g. Heaney, 1986; Brandon-Jones, 1996; Meijaard, 2004a). For instance, within the Indian region, southern India and Sri Lanka were repeatedly connected when sea levels dropped at least 10 m below the present sea level (Worldbath, 2010). Other possible dispersal barriers include the Brahmaputra and Salween rivers and the mountain ranges in Myanmar, which may have prevented dispersal between the Indian and Southeast Asian regions (see e.g. Takacs *et al.*, 2005; Su *et al.*, 2006; Veron *et al.*, 2007).

The Isthmus of Kra, located just north of the Thai–Malaysian border, represents a limit between the Indochinese and Sundaic subregions (see e.g. Corbet & Hill, 1992; Hughes *et al.*, 2003; Woodruff & Turner, 2009). It has been suggested that during high-sea-level periods, the restricted land region around the Isthmus of Kra caused faunal compressions and local extinctions north and south of the isthmus (Woodruff & Turner, 2009), thus separating many Indochinese and Sundaic taxa. Alternatively, as suggested by Meijaard (2009), the long-term ecological differences that have existed between these two subregions may have strongly impacted the distribution of taxa. Either of these scenarios may explain the evolutionary history of a number of species (rodents, Gorog *et al.*, 2004; tiger, Luo *et al.*, 2004; binturong, Cosson *et al.*, 2007; *Macaca* spp., Ziegler *et al.*, 2007; masked palm civet, Patou *et al.*, 2009).

In the Sundaic region, eustatic variations created land bridges between Peninsular Malaysia, Sumatra, Java and

Borneo (Voris, 2000; Hall, 2001), allowing dispersal across the different landmasses. However, climatic changes also modified the distribution of savanna and rain forest across Southeast Asia (Morley, 2000; Bird *et al.*, 2005; Meijaard & Groves, 2006), which had an impact on the capacity of species associated with these habitats to utilize Pleistocene land bridges (Bird *et al.*, 2005).

Another interesting question of Asian biogeography is the origin of the Philippine fauna. The Philippine islands lie east of Borneo and are of volcanic origin. One of the Philippine islands, Palawan, was possibly connected to Borneo by a dry-land corridor (Heaney, 1986; Voris, 2000; Hall, 2001), but is thought to have never been connected by dry land to the other Philippine islands (Heaney, 1986; Reis & Garong, 2001; Esselstyn *et al.*, 2004; but see Harrison *et al.*, 2006). The Philippine fauna may have resulted from over-water colonization events (see review in Esselstyn *et al.*, 2009). However, human transport may also have been involved in the dispersal of the common palm civet to the Philippines.

The aims of our study were to investigate the genetic and morphological diversity among the *Paradoxurus* species, especially within *P. hermaphroditus*, using mitochondrial and nuclear markers and dental features. We used our phylogenetic results and molecular dating estimates to address alternative biogeographical scenarios for each of the *Paradoxurus* groups, and to propose provisional taxonomic classifications within *Paradoxurus*.

MATERIALS AND METHODS

Molecular sampling and extraction – PCR-sequencing protocols

We collected 65 fresh samples (hair or tissue) and 20 museum samples (skins, bone, and tissues taken from skulls) of *P. hermaphroditus*, one fresh tissue sample of *P. jerdoni*, and one dry skin sample of *P. zeylonensis*. The locations of samples ranged across Asia, from Pakistan to Indonesia (Table 1).

Total genomic DNA was isolated using a cetyl trimethyl ammonium bromide (CTAB)-based protocol (Winnepeninckx *et al.*, 1993) and a QIAamp DNA microkit (Qiagen, Illkirch, France) from fresh and museum samples, respectively. Museum samples were pre-processed following the protocol of Yu *et al.* (2006) to facilitate DNA isolation. We added dithiothreitol (DTT 1 M, 8 µL per extract) during tissue lysis to break up disulfide bonds. We also extracted DNA from bone fragments of one sample (VN07–178), following Poinar *et al.*'s (2006) protocol. DNAs were extracted from sensitive tissues in two independent laboratories (MNHN and IZW) in conditions that limited the risks of contamination by exogenous DNA.

We sequenced several mitochondrial fragments: the cytochrome *b* gene (cyt *b*), the flanking tRNAs (tRNA^{Thr} and tRNA^{Pro}), and the mitochondrial DNA (mtDNA) control region (CR). Cyt *b* primers used in this study were from Veron & Heard (2000; cyt *b* L14841) and Gaubert *et al.* (2004; all

Table 1 List of the samples included in this study. For each sample, we report the identification, the museum identification number (AMNH, American Museum of Natural History, New York City, USA; BMNH, British Museum of Natural History, London, UK; FMNH, Field Museum of Natural History, Chicago, USA; KU, Kansas University, Lawrence, USA; MNB, Museum für Naturkunde von Berlin, Germany; MNHN, Muséum National d'Histoire Naturelle, Paris, France; RMNH, Rijksmuseum van Natuurlijke Historie, Leiden, The Netherlands; SFN, Seckenberg Forschungsinstitut und Naturmuseum, Frankfurt, Germany; SMNS, Staatliches Museum für Naturkunde, Stuttgart, Germany), the DNA extract identification number, the locality and, when available, the GPS coordinates (longitude and latitude) and elevation, the genes [intron 7 of the β -fibrinogen (FGBI7); cytochrome *b* (cyt *b*); control region (CR)] included in this study, and the GenBank number (EF881493–EF881693: this study). Samples represented in both mitochondrial and nuclear datasets ($n = 43$) are shown in bold.

Species	Tissue ID	Sample ID	Locality	Long.	Lat.	Elev. (m)	FGBI7	Cyt <i>b</i>	CR
<i>Paradoxurus hermaphroditus</i>	FMNH-140476	Pk1*	Pakistan: Malakand Division, Swat District, Yakh Tangai	72.63	34.92	2080	–	FJ881654	FJ881545
<i>Paradoxurus hermaphroditus</i>	AMNH-163173	II*	India: Tamil Nadu State, Nilgiris District	76.47	11.05	853	–	FJ881651	FJ881537
<i>Paradoxurus hermaphroditus</i>	MNB-83096	I2*	India: Bengale	–	–	–	–	FJ881684	FJ881606
<i>Paradoxurus hermaphroditus</i>	MNHN-TC135	SL1	Sri Lanka: Galle District, Navina	80.14	6.05	–	–	FJ881632	FJ881560
<i>Paradoxurus hermaphroditus</i>	MNHN-TC216, TC217, TC218, TC219	My1, My2, My3, My4	Myanmar: Zoo	–	–	–	FJ881508, FJ881509, –, FJ881510	FJ881635, FJ881636, FJ881637, FJ881655	FJ881563, FJ881564, FJ881565, FJ881566
<i>Paradoxurus hermaphroditus</i>	MNHN-C128	T1	Thailand: Kapoe District	98.35	9.39	450	FJ881495	FJ881614	FJ881539
<i>Paradoxurus hermaphroditus</i>	MNHN-R3408, MNHN-R3416	T2, T3	Thailand: Loei (market)	101.50	17.42	–	–	FJ881629, FJ881624	FJ881552, FJ881553
<i>Paradoxurus hermaphroditus</i>	MNHN-TC379, MNHN-TC380	T4, T5	Thailand: Sakon Nakhon Province, Ban Chiang	104.09	17.10	–	–	FJ881657, FJ881658	FJ881578, FJ881579
<i>Paradoxurus hermaphroditus</i>	MNHN-TC116	C1	Pen Cambodia: Koh Khong Province, Thamar Bang Town (captive)	–	–	–	–	FJ881630	FJ881558
<i>Paradoxurus hermaphroditus</i>	MNHN-TC132	C2	Cambodia: Phnom Aural W.S.	104.10	11.50	192	FJ881506	FJ881631	FJ881559
<i>Paradoxurus hermaphroditus</i>	MNHN-TC357	C3	Cambodia: Siem Reap (captive)	–	–	–	–	FJ881656	FJ881577
<i>Paradoxurus hermaphroditus</i>	MNHN-TC500, TC501, TC502, TC503, TC504, TC505, TC506, TC507, TC508, TC509, TC510	C4, C5, C6, C7, C8, C9, C10, C11, C12, C13, C14	Cambodia: Zoo	–	–	–	FJ881524, FJ881525, FJ881526, FJ881527, FJ881528, FJ881529, FJ881530, FJ881531, –, FJ881532, –	FJ881667, FJ881668, FJ881669, FJ881670, FJ881671, FJ881672, FJ881673, FJ881674, FJ881675, FJ881677	FJ881588, FJ881589, FJ881590, FJ881591, FJ881592, FJ881593, FJ881594, FJ881595, FJ158896, FJ158897, FJ158898
<i>Paradoxurus hermaphroditus</i>	MNHN-AHCI	L1	Laos: Phongsaly Province, Nam Lan Conservation Area, Na Mack village	101.58E	21.26N	966	FJ881493	FJ881612	FJ881536

Table 1 Continued

Species	Tissue ID	Sample ID	Locality	Long.	Lat.	Elev. (m)	FGBI7	Cyt <i>b</i>	CR
<i>Paradoxurus hermaphroditus</i>		V1	Vietnam: Vinh Yen District, Tam Dao	105.64	21.45	664	–	AY928673	–
<i>Paradoxurus hermaphroditus</i>	MNHN-SH17, SH18, SH19	V2, V3, V4	Vietnam North (captive)	–	–	–	–, FJ881533, –	FJ881625, FJ881626, FJ881627	FJ881554, FJ881555, –
<i>Paradoxurus hermaphroditus</i>	MNHN-SH20	V5	Vietnam: Zoo Saigon	–	–	–	FJ881534	FJ881628	FJ881556
<i>Paradoxurus hermaphroditus</i>	MNHN-T2091	V6	Vietnam: North	–	–	–	FJ881505	AF511056	FJ881557
<i>Paradoxurus hermaphroditus</i>	MNHN-VN07-178	V7*	Vietnam: Kon Tum Province, Kon Tum vicinity	108.04	14.22	–	–	FJ881633	FJ881561
<i>Paradoxurus hermaphroditus</i>	MNHN-TC231	V8	Vietnam: Hanoi (captive)	–	–	–	FJ881513	FJ881640	FJ881569
<i>Paradoxurus hermaphroditus</i>	MNHN-TC264, TC265, TC266	V9, V10, V11	Vietnam	–	–	–	–, FJ881515, FJ881516	FJ881643, FJ881644, FJ1645	FJ881572, FJ881573, FJ881574
<i>Paradoxurus hermaphroditus</i>	MNHN-PL-120/L91	Ch1	China: Guangxi, Daxin County	107.10	21.58	–	FJ881499	FJ881653	FJ881544
<i>Paradoxurus hermaphroditus</i>	AMNH-27588	Ch2*	China: Hainan island, Mount Wuzhi	109.45	18.59	–	–	FJ881652	–
<i>Paradoxurus hermaphroditus</i>	MNHN-TC208	PM1	Peninsular Malaysia: Pahang State, Kuala Krau	102.22	3.42	–	FJ881507	FJ881634	FJ881562
<i>Paradoxurus hermaphroditus</i>	MNHN-TC225	PM2	Peninsular Malaysia: Pahang State, Jenderak	102.37	3.80	–	FJ881511	FJ881638	FJ881567
<i>Paradoxurus hermaphroditus</i>	MNHN-TC229	PM3	Peninsular Malaysia: Pahang State, Jenderak	102.24	3.41	–	FJ881512	FJ881639	FJ881568
<i>Paradoxurus hermaphroditus</i>	MNHN-TC260, MNHN-TC262	PM4, PM5	Peninsular Malaysia: Pahang State, Jenderak-Selatatan	102.24	3.41	–	FJ881514, –	FJ881641, FJ881642	FJ881570, FJ881571
<i>Paradoxurus hermaphroditus</i>	MNHN-TC412, TC423, TC424, TC425	PM6, PM7, PM8, PM9	Peninsular Malaysia: Zoo	–	–	–	–, –, FJ881519, FJ881520	FJ881659, FJ881660, FJ881661, FJ881662	FJ881580, FJ881581, FJ881582, FJ881583
<i>Paradoxurus hermaphroditus</i>	MNHN-TC481, MNHN-TC492	PM10, PM11	Peninsular Malaysia: Pahang State, Krau W.R.	102.37	3.72	–	–, FJ881522	FJ881663, FJ881665	FJ881584, FJ881586
<i>Paradoxurus hermaphroditus</i>	MNHN-TC482	PM12	Peninsular Malaysia: Pahang State, Temerloh	100.42	4.39	–	FJ881521	FJ881664	FJ881585

Table 1 Continued

Species	Tissue ID	Sample ID	Locality	Long.	Lat.	Elev. (m)	FGBI7	Cyt b	CR
<i>Paradoxurus hermaphroditus</i>	MNHN-TC499	PM13	Peninsular Malaysia: Pahang State, Jenderak	102.37	3.60	–	FJ881523	FJ881666	FJ881587
<i>Paradoxurus hermaphroditus</i>	MNHN-TC560	PM14	Peninsular Malaysia: Pahang State	–	–	–	–	FJ881678	FJ881599
<i>Paradoxurus hermaphroditus</i>	SMNS-6582	S1*	Indonesia: North Sumatra	–	–	–	–	FJ881683	FJ881604
<i>Paradoxurus hermaphroditus</i>	MNB-91298, ZMB-91297	S2*, S3*	Indonesia: Sumatra, Atjeh Province	–	–	–	–	FJ881691, FJ881692	–
<i>Paradoxurus hermaphroditus</i>	MNB-34026	S4*	Indonesia: Sumatra	–	–	–	–	FJ881685	–
<i>Paradoxurus hermaphroditus</i>	MNB-48414	N1*	Indonesia: Nias island	–	–	–	–	FJ881693	FJ881608
<i>Paradoxurus hermaphroditus</i>	RMNH-33950, RMNH-33917	Bk1*, Bk2*	Indonesia: Bangka island	–	–	–	–	FJ881687, FJ881688	FJ881609, FJ881610
<i>Paradoxurus hermaphroditus</i>	AMNH-101475, AMNH-101632, AMNH-101675, MNB-42831	J1*, J2*, J3*, J4*	Indonesia: Java, Cirebon	108.33	6.46S	22	–	FJ881648, FJ881649, FJ881650, FJ881682	FJ881605
<i>Paradoxurus hermaphroditus</i>	MNB-40986	J5*	Indonesia: Java, Cirebon	108.33	6.46S	n.a.	–	FJ881686	–
<i>Paradoxurus hermaphroditus</i>	MNHN-TC289	M1	Indonesia: Mentawai island.	–	–	–	FJ881517	FJ881646	FJ881575
<i>Paradoxurus hermaphroditus</i>	MNHN-C127	B1	Indonesia: Borneo, East Kalimantan, Bukit Soeharto	117.02	1.04	26	FJ881494	FJ881613	FJ881538
<i>Paradoxurus hermaphroditus</i>	MNHN-TC301	B2	Indonesia: Borneo, East Kalimantan, Long Tua	115.47	3.10	440	FJ881518	FJ881647	FJ881576
<i>Paradoxurus hermaphroditus</i>	MNHN-TC568, MNHN-TC569	B3, B4	Malaysia: Borneo, Sabah	–	–	–	–	FJ881679, FJ881680	FJ881600, FJ881601
<i>Paradoxurus hermaphroditus</i>	AB075975	Ph1	Philippines: Mindanao island, Surigao del Sur, Barobo	126.11	8.33	39	–	AB075975	–
<i>Paradoxurus hermaphroditus</i>	FMNH-145743	Ph2	Philippines: Sibuyan island, Romblon Province	122.71	12.33	–	FJ881496	FJ881615	FJ881540
<i>Paradoxurus hermaphroditus</i>	FMNH-EAR1355	Ph3	Philippines: Leyte island	124.83	10.83	–	FJ881497	FJ881616	FJ881541
<i>Paradoxurus hermaphroditus</i>	FMNH-EAR1818	Ph4	Philippines: Luzon island, Mount Isarog	123.22	13.38	–	FJ881498	FJ881542	FJ881617
<i>Paradoxurus hermaphroditus</i>	FMNH-LRH 3167	Ph5	Philippines: Leyte island	124.83	10.83	–	EF6805510	AY170113	FJ881543

Table 1 Continued

Species	Tissue ID	Sample ID	Locality	Long.	Lat.	Elev. (m)	FGBi7	Cyt <i>b</i>	CR
<i>Paradoxurus hermaphroditus</i>	FMNH-62846	Ph6*	Philippines: Palawan island, Puerto Princesa	118.73	9.73	0	–	FJ881618	FJ881546
<i>Paradoxurus hermaphroditus</i>	KU-165377, KU-165509, KU-165376	Ph7, Ph8, Ph9	Philippines: Palawan island	118.64	9.84	–	FJ881500, FJ881503, FJ881504	FJ881619, FJ881622, FJ881623	FJ881547, FJ881550, FJ881551
<i>Paradoxurus hermaphroditus</i>	KU-164764, KU-164763	Ph10, Ph11	Philippines: Camiguin Norte island	121.91	18.9	–	FJ881501, FJ881502	FJ881620, FJ881621	FJ881548, FJ881549
<i>Paradoxurus hermaphroditus</i>	RMNH-207925	R1*	Indonesia: Roti island	–	–	–	FJ881690	FJ881611	FJ881611
<i>Paradoxurus hermaphroditus</i>	SFN-20919	F1*	Indonesia: Flores island	–	–	–	FJ881689	FJ881607	FJ881607
<i>Paradoxurus jerdoni</i>	MNH-TC470	Pj	India: Western Ghats	75.00	14.00	–	EF680511	DQ683994	FJ881602
<i>Paradoxurus zeylonensis</i>	BMNH-1933.7.24.1	Pz-BMI*	Sri Lanka: Uva province, Badulla district, Koslanda, Lipton's Tea Estate	–	–	–	FJ881681	FJ881603	FJ881603
<i>Paguma larvata</i>	–	–	–	–	–	–	–	–	EU910455
<i>Paguma larvata</i>	–	–	–	–	–	–	–	–	EU910454

*DNA extracted from museum specimens. In column FGBi7 a dash (–) indicates that no sequence was available.

other cyt *b* primers). The contiguous region from the 3'-end of the cyt *b* to the Hyper Variable Region 2 (HVR2) of the CR was amplified using primers from Patou *et al.* (2009). Newly designed primers (cyt *b* and CR) were used for museum samples (see Appendix S1 in the Supporting Information). We amplified the nuclear marker β -fibrinogen intron 7 (FGBi7), using the primers of Yu & Zhang (2005), to provide an evolutionary assessment independent from mitochondrial markers. FGBi7 evolves neutrally and relatively rapidly among feliformians (Yu & Zhang, 2005).

Polymerase chain reactions (PCRs) were performed in 30- μ L reaction volumes with the following constituents: 0.5–8 μ L of DNA-extracts, 3 μ L of *Taq* polymerase buffer with MgCl₂ (2.5 mM), 1.2 μ L of dNTP mix (0.26 mM), 0.36 μ L of each primer (10 pmol μ L⁻¹), and 1.5 U of *Taq* Polymerase (QBiogene, Illkirch, France). PCR cycles for DNA amplification were 94 °C for 4 min; followed by 35 cycles of 94 °C for 30 s (denaturation), 49–56 °C (mtDNA) or 57–60 °C (nuclear DNA) for 30–45 s (annealing), 72 °C for 40 s (extension); and a final extension step at 72 °C for 7 min. PCR products were visualized in a 1.5% agarose gel; successfully amplified fragments were purified using ExoSAP (GENOSCOPE, Evry, France). Molecular sequences obtained from DNA extracted from sensitive tissues were amplified and sequenced twice to ensure the quality and authenticity of sequencing. Sequences obtained in the two independent laboratories were fully congruent. Amplicons were then sequenced bidirectionally using BigDye[®] Terminator 3.1 on an automated DNA sequencer A3100 (Applied Biosystems, Carlsbad, CA, USA). Sequences were edited and assembled using SEQUENCHER 4.14 (Gene Codes Corporation, Ann Arbor, MI, USA), and the BLASTN 2.2.18 program (Altschul *et al.*, 1997) was used to identify similarities with known sequences. We aligned sequences manually in BioEDIT 7.09 (Hall, 1999).

Phylogenetic and haplotypic network analyses

Phylogenetic analyses were performed using both maximum likelihood (ML) and Bayesian inference (BI). We rooted the phylogenetic analyses with the sister-group of *Paradoxurus*, *Paguma larvata* (Patou *et al.*, 2008). The best-fitting model was estimated prior to these analyses for each dataset and partitioned using MRMODELTEST 2.0 (Nylander, 2004), following the Akaike information criterion (AIC) (see Appendix S2).

Individual loci (cyt *b* and CR) were first analysed independently to detect any potential incongruence, before mtDNA sequences were combined. Mitochondrial and nuclear datasets were analysed separately given their potentially conflicting phylogenetic signals (see Knowles & Carstens, 2007; Kubatko & Degnan, 2007). The selected model was then implemented in PHYLML 2.4.4 (Guindon & Gascuel, 2003) to process the ML analyses, in which node robustness was assessed through 1000 bootstrap replicates (BP_{ML}). Partitioned Bayesian mtDNA (into codon positions for cyt *b* and loci) and nuclear DNA (nDNA) analyses were performed in MRBAYES 3b4 (Ronquist & Huelsenbeck, 2003). Five Metropolis-coupled Markov chain

Monte Carlo (MCMCMC) simulations were run for 5×10^6 generations, with one tree sampled every 100 generations. The 'burn-in' length was determined using TRACER 1.4 (Rambaut & Drummond, 2007), taking the effective sample size (ESS) and auto-correlation time (ACT) values into account. Eventually, the first 500,000 generations (5000 trees) were discarded. Bayesian analyses were run twice, independently, to ensure convergence.

We used NETWORK 4.5 (<http://www.fluxus-engineering.com>) to construct haplotype median-joining networks (Bandelt *et al.*, 1999). Networks were constructed for mtDNA and nDNA datasets separately (weights = 10 and $\epsilon = 0$), as with the phylogenetic analyses. Only individuals for which both mitochondrial and nuclear data were available ($n = 43$) were included. Given the higher level of mitochondrial divergence within *P. hermaphroditus* and the strong support retrieved for the deepest nodes of the phylogenies, networks were constructed separately for each major clade to further explore the underlying geographic distribution. We computed genetic diversity (haplotype and nucleotide diversity) within the different groups and estimated mismatch distributions for several widespread clades to test for sudden population expansion using ARLEQUIN 3.1 (Excoffier *et al.*, 2005).

Estimates of divergence times

Divergence times among lineages were estimated using fossil calibration points. Fossil records of *Paradoxurus* exist from the Late Pleistocene to the Holocene transition (Borneo, Medway, 1966; Piper *et al.*, 2008; Java, van den Bergh *et al.*, 2001; South China, Wang *et al.*, 2007; Thailand, Tougaard, 2001; Vietnam, Schwartz *et al.*, 1994). However, fossils from the Siwaliks, a Late Miocene site in Pakistan, have also been attributed to *Paradoxurus* (Flynn & Morgan, 2005; L.J. Flynn & J.C. Barry, Peabody Museum, pers. comm.). Although morphological comparisons of these fossils with extant taxa have suggested affinities to *Paradoxurus*, their taxonomic classification is arguable (G. Veron, pers. obs.). It is possible that these fossils represent a stem lineage on the branch leading to extant *Paradoxurus*, and so they are not considered here. Two fossils, originating from south China (*c.* 0.8 Ma; Wang *et al.*, 2007) and Borneo (*c.* 0.12 Ma; Medway, 1966; Piper *et al.*, 2008), were used as calibration points. The first subfossil was used to calibrate the origin of the clade that includes Chinese individuals, as the lower boundary, that is, as the minimum age for their diversification. The second was used as the lower boundary for the clade that includes the Bornean individuals. Other fossils were not included owing to their uncertain attribution to a specific clade and because different lineages occurred in sympatry in some areas (see Results).

Owing to the reduced number of variable sites, we did not include the nuclear dataset to estimate the divergence times. Molecular dating analyses were performed using the individuals that were included in the mitochondrial and nuclear phylogenetic analyses ($n = 43$) corresponding to the most complete dataset.

Molecular dating analyses were carried out using the PAML/MULTIDIVTIME package (Thorne *et al.*, 1998; Kishino *et al.*, 2001; Thorne & Kishino, 2002) following the instructions of Rutschmann (2005). This program produces estimations of divergence dates in a relaxed-clock framework and is suitable in the context of speciation (versus coalescence; Kishino *et al.*, 2001). Given the level of divergence observed here, we considered its use appropriate, although the results may be rough estimates and thus should be taken cautiously.

We estimated model parameters in BASEML (included in PAML 3.14b; Yang, 1997). We used ESTBRANCHES to estimate branch lengths of the rooted tree, and to generate a variance-covariance matrix of branch lengths from the dataset. Finally, we performed a Bayesian estimation of divergence times via MCMC simulation, using MULTIDIVTIME. The Markov chain was run for 10^6 generations with one tree sampled every 100 generations and a burn-in period of 10^3 trees (10%). For these analyses, we set the lower bound of the origins of the ancestor of extant clade I members at 0.8 Ma and the lower bound of the origins of the ancestor of extant clade N members at 0.12 Ma. The *rttm* (*a priori* expected number of time units between tip and root) was set in accordance with the results obtained in a previous study (Patou *et al.*, 2008), and thus we chose the most conservative dates obtained (3.1 Ma with a *rttm* standard deviation of 1.5 Myr).

Comparative morphology

In addition to the molecular analyses, we studied and compared the morphology of 799 specimens (skins and/or skulls) of *P. hermaphroditus* from 12 institutions (Appendix S3). We focused on the morphology of the most variable teeth in *Paradoxurus* – the fourth upper premolar (P^4) and upper molars – in adult specimens only (*c.* 78% of the observed specimens). We recorded their general shape, the relative development of the various cuspids, and the presence or absence of cingula (anterior and posterior).

Geography and biogeography

Global positioning system coordinates of sample and specimen localities were obtained directly from the collector, from museum information, or from Google Earth[®]. These coordinates were used to generate a map with PLANIGLOBE[®] (Kiel, Germany). DIVA-GIS (Hijmans *et al.*, 2001) was then used to analyse the distribution of the common palm civet clades with respect to elevation.

To depict the biogeographic history of the *Paradoxurus* genus, we performed a reconstruction of ancestral areas using MACCLADE 4.0 (Maddison & Maddison, 2000). We coded the distribution of extant clades by absence (0) or presence (1) in the 15 countries we identified in this study. The ancestral states of each character were then obtained for each node of interest.

RESULTS

Sequencing results

All new sequences were deposited in GenBank (Table 1). Owing to the degraded nature of DNA retrieved from samples taken from museum specimens and from some poorly preserved samples (especially hair samples), only parts of cyt *b* and/or the CR could be obtained from these specimens.

At the 3'-end of the CR fragment (HVR2), a region of a variable number of repetitions of a dinucleotide motif (CA) was discarded from the analyses. A concatenated sequence (642 bp) including tRNA^{Thr}, tRNA^{Pro} and the contiguous CR fragment (HVR1 to Central Conserved Domain) were then analysed. All *Paradoxurus* specimens carried an insertion between positions 16193 and 16194 (as referenced to the cat, *Felis catus*, mitochondrial genome, GenBank U20753; Lopez *et al.*, 1996): a single guanidine (G) in the majority of samples, and three nucleotides (AYA) in some individuals (IDs: Ch1, L1, T1, T3, V6, V7, V9 and V11).

Within *Paradoxurus*, the length of fragments/number of variable positions/number of parsimony-informative sites were as follows: cyt *b* = 1140 bp/272/146; CR = 642 bp/93/51 and FGBi7 = 642 bp/43/19.

Phylogenetic analyses

Mitochondrial DNA

We obtained mitochondrial DNA (mtDNA) sequences for 85 *P. hermaphroditus* samples, one *P. jerdoni* sample and one *P. zeylonensis* sample. Analyses of the two mitochondrial fragments yielded similar results and were thus combined into one matrix, which provided a well-resolved phylogenetic hypothesis (Fig. 2a), also showing a geographical structure.

The phylogenetic tree of *Paradoxurus* contains two major clades (clades A and B; Fig. 2a), and showed that *P. hermaphroditus* is paraphyletic. The first major clade of the mtDNA tree (clade A) grouped *P. jerdoni* and *P. zeylonensis* (clade C) with a well-differentiated lineage of *P. hermaphroditus* from Central, South and Southeast Asia (clade D). Within clade D, Sri Lankan and south-west Indian individuals (clade E) were a sister group to clade F. Clade F grouped three lineages: one represented by one individual from Hainan Island; one including individuals from Pakistan and Bengal (clade H); and one combining those from south Thailand, central and northern Vietnam, north Laos and south China (clade I). The latter clade (I) was supported by an insertion of three nucleotides (see above). The corresponding haplotype network (Fig. 2b) showed two groups differing by 14 mutations within clade I: (1) samples from south Thailand, north Laos and Vietnam, and (2) samples from south China and north Vietnam.

Paradoxurus hermaphroditus specimens in the second major clade (clade B) segregated into clade J (Southeast Asian mainland, Java, Sumatra, Flores, Roti, Nias and Bangka Islands) and clade K (Mentawai, Borneo and the Philippines).

Clade J was split into clade L (Myanmar, Thailand, Peninsular Malaysia, Cambodia, south Vietnam, Nias and Bangka Islands, and one sample from Sumatra), and clade M (Java, Flores and Roti Islands, and the other samples from Sumatra). The group L haplotype network (Fig. 2b) highlighted three subgroups separated by seven mutations: (1) Vietnam, plus one sample from Cambodia; (2) Cambodia, Myanmar and some samples from Peninsular Malaysia; and (3) remaining samples from Peninsular Malaysia. Within clade K, the Mentawai individual was sister to clade N, which encompassed all specimens from Borneo and the Philippines (Fig. 2a). The Mentawai sample differed from group N individuals by 51 substitutions (Fig. 2b). Group N segregated into two main lineages separated by 11 substitutions (Fig. 2b): (1) the Philippines (including Palawan) only and (2) Borneo, plus one specimen from Palawan and one from Luzon Island in the Philippines.

Some representatives of these two distinct lineages (A and B) were present in geographically proximate areas in Vietnam and Thailand, notably the two specimens from the Loie region, Thailand (T2, T3), which belonged to two well-differentiated clades (L and I, respectively).

Nuclear DNA

The analyses of nDNA were carried out with a reduced number of individuals ($n = 43$) because of the difficulty in obtaining nuclear sequences from museum specimens or poorly preserved samples. These 43 individuals represented haplotypes. Analyses yielded two major clades (O and K; Fig. 3a), of which the first (clade O) grouped all samples from mainland Asia (no corresponding clade in the mitochondrial tree), and the second (clade K) contained individuals from Mentawai, Borneo and the Philippines (corresponding to mitochondrial clade K), as well as *P. jerdoni*. Clade O consisted of clade L (corresponding to mitochondrial clade L) and clade I (corresponding to mitochondrial clade I). Haplotypes from group L exhibited a star-like structure (Fig. 3b), with one central haplotype shared by 16 individuals from Myanmar, Peninsular Malaysia, Cambodia and Vietnam, and four haplotypes differing by a single mutation. The most striking feature of this nDNA tree is the sister-relationship between clades I and L, which conflicts with the mtDNA tree topology. Within group K, the individuals from Mentawai and *P. jerdoni* were close to those from Borneo and the Philippines, and were both separated from Borneo and the Philippines by four mutations (Fig. 3b). Within group K we distinguished one haplotype shared by Philippine samples only, and differing from samples from Borneo and from the other samples from the Philippines.

Genetic diversity

Genetic diversity was explored in groups D, I, J, K, L, M and N for the mitochondrial dataset, and in groups I, K, L, N and O for the nuclear dataset. Nucleotide diversity (π) and the average nucleotide differences (κ) were at least 1.5 times higher in groups I and N than in group L (except for the CR, which

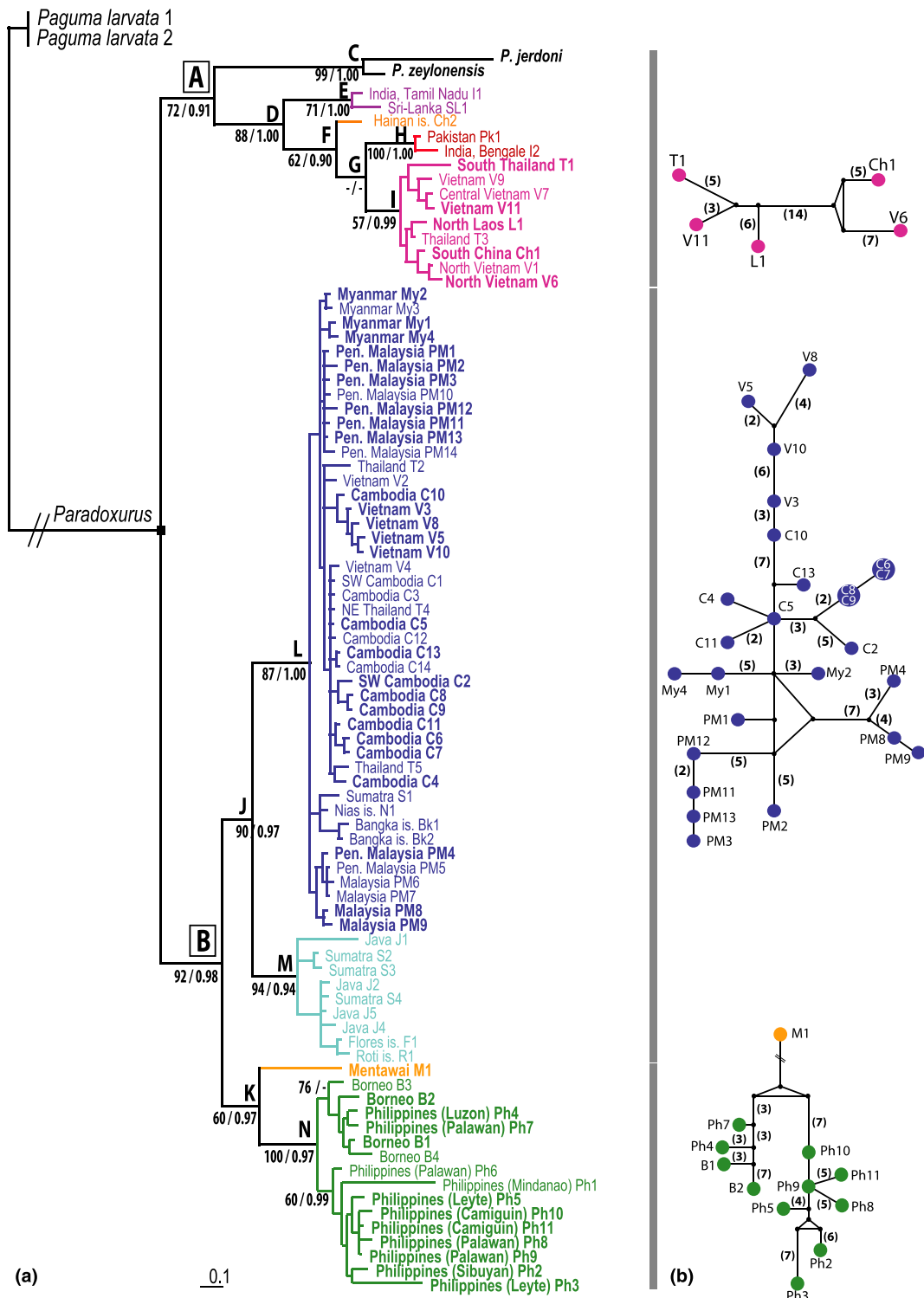


Figure 2 (a) Phylogenetic tree of *Paradoxurus hermaphroditus* obtained from the Bayesian analysis of the mitochondrial dataset (cytochrome *b* + control region). Maximum likelihood bootstrap (BP_{ML}) and Bayesian posterior probabilities (BPP) are reported for each node (A–N). ‘–’ means that the node is not supported by our analyses (BP_{ML} < 50; BPP < 0.90). Individuals included in the nuclear analysis and for which median joining networks were constructed are indicated in bold. Individuals are labelled according to their locations and sample ID. (b) Haplotype networks obtained from the analysis of the mtDNA datasets are shown beside the corresponding phylogenetic clade. Haplotype connecting lines represent single mutations unless indicated otherwise (in parentheses). Black circles are median vectors introduced by the NETWORK algorithm.

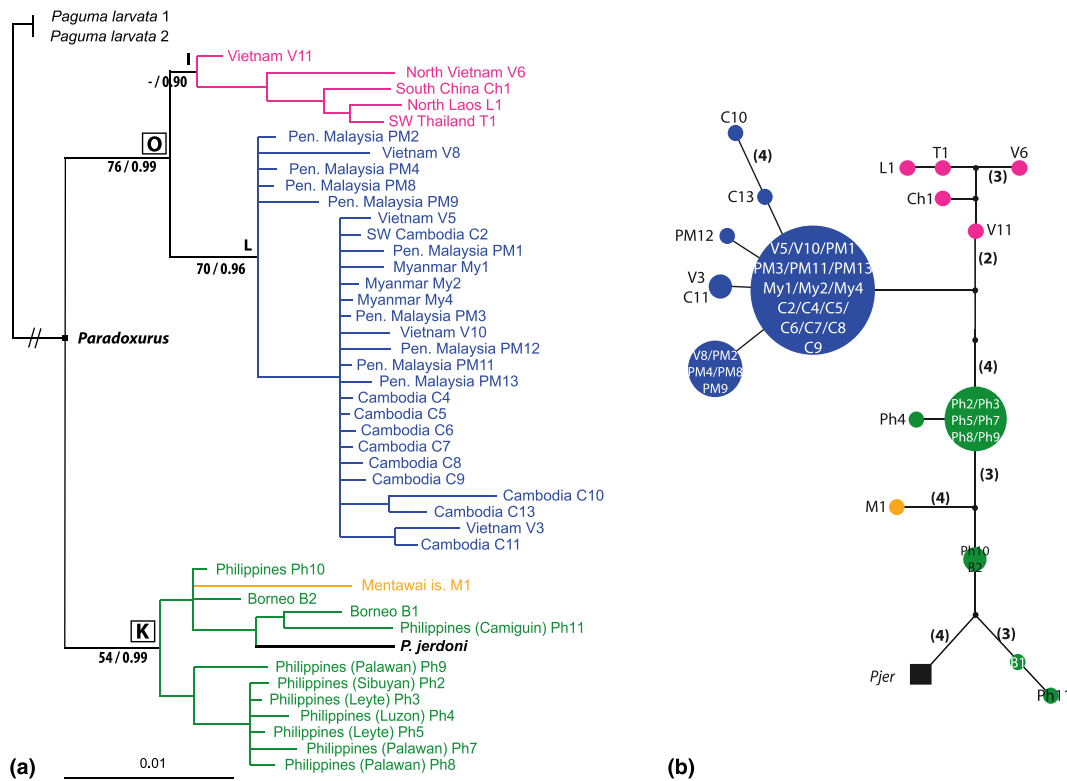


Figure 3 (a) Phylogenetic tree of *Paradoxurus hermaphroditus* obtained from the Bayesian analysis of the nuclear β -fibrinogen intron 7 (642 bp). Clade labelling corresponds to that in Fig. 2. (b) Haplotype network obtained from the analysis of the nuclear β -fibrinogen intron 7. Haplotype connecting lines represent single mutations unless indicated otherwise (in parentheses). Black circles are median vectors introduced by the NETWORK algorithm.

Table 2 Genetic diversity estimates within *Paradoxurus*, partitioned into genetic groups and molecular markers [cytochrome *b* (*cyt b*), control region and β -fibrinogen intron 7 (FGBi7)]. π , nucleotide diversity; κ , mean number of pairwise differences. – not applicable.

Group	D		I		J		L		M		K		N		O	
	π	κ	π	κ	π	κ	π	κ	π	κ	π	κ	π	κ	π	κ
<i>Cyt b</i>	0.019	13.49	0.0074	8.0	0.0046	1.5	0.0044	4.97	0.0041	4.64	0.015	17.6	0.013	14.11	–	–
CR	0.0098	6.32	0.0053	3.43	0.0087	5.6	0.0064	4.1	0.0047	3.0	0.0075	4.84	0.0053	3.4	–	–
FGBi7	–	–	0.005	3.5	–	–	0.0014	0.92	–	–	0.0048	3.1	0.004	2.6	0.0036	2.3

retrieved the same levels of variability) (Table 2). Mismatch distributions, generated for group L (Fig. 4), revealed a smooth and unimodal distribution for both mitochondrial and nuclear markers (raggedness index $r < 0.05$; $P > 0.05$), which did not differ significantly from the expected distribution in the case of an expanding population (Harpending, 1994). Mismatch distributions for groups I and K showed multimodal patterns, indicating stable or slowly declining populations (Rogers & Harpending, 1992).

Divergence time estimates

According to our estimates (Fig. 5), the genus *Paradoxurus* emerged at the end of the Late Miocene to the Mid–Late Pliocene (2.82–5.82 Ma). Clades A and B are estimated to have started to diversify (lineage sorting) almost synchronously

(3.95 \pm 1.4 Ma). Clades K, L, N and I diverged during the Pliocene at 2.3–4.9, 1.7–3.8, 1.4–3.4 and 1.1–2.9 Ma, respectively.

Comparative morphology

Our observations of teeth morphology support the existence of three major morphotypes within *P. hermaphroditus* (see Fig. 6 and Appendix S4). However, geographical variation was present within the distributional range of each of these morphotypes. The first group of individuals (corresponding to molecular groups E, H and I within clade D) had an antero-posteriorly elongated P⁴, without anterior or posterior cingula; the protocone of P⁴ was sometimes located in an anterior position to the paracone; and M² was well developed (> 1/3 of the P⁴). Individuals exhibiting this morphotype originated from

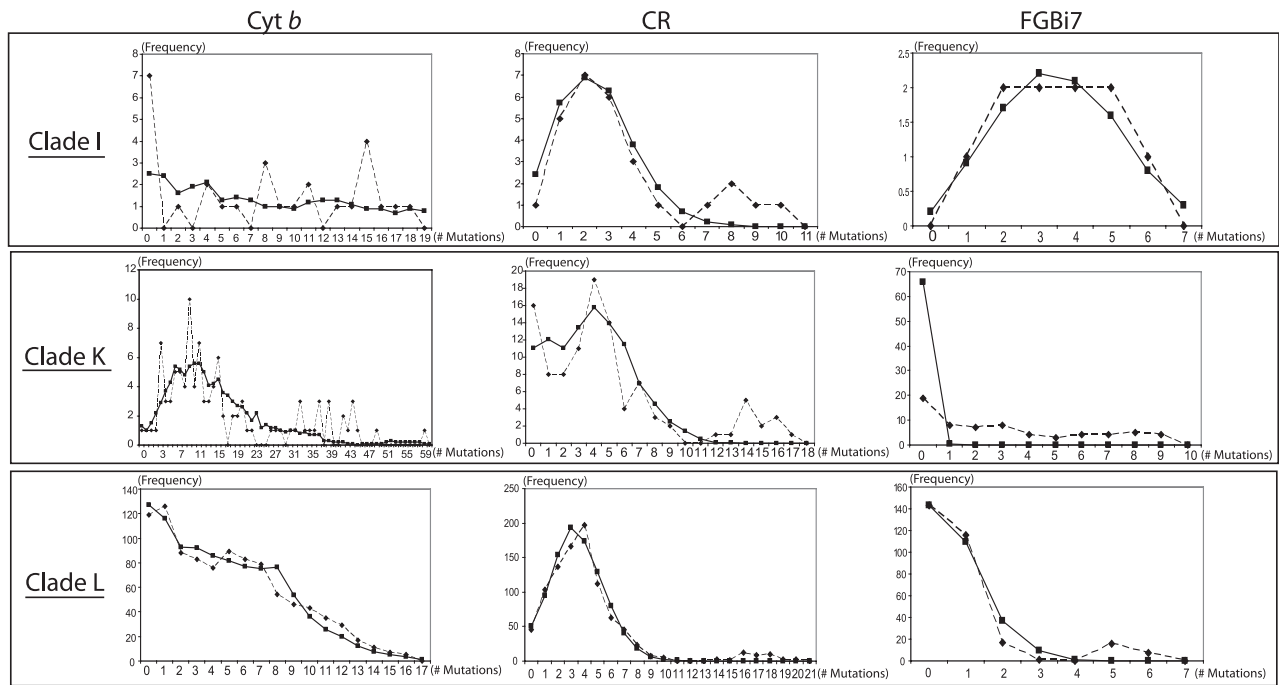


Figure 4 Mismatch distributions computed for cytochrome *b* (*cyt b*), control region (CR) and β -fibrinogen intron 7 (FGBi7) markers for clades I, K and L of *Paradoxurus hermaphroditus*. Dashed line, observed distribution of pairwise differences; solid line, expected distribution in the case of sudden demographic expansion.

Pakistan, Nepal, India, Sri Lanka, Myanmar (north-west and south-east), Thailand (north-west and central), Laos, Vietnam (north and central) and China (south and Hainan Island).

The second morphotype (groups L and M within clade J) had a more triangular P^4 with a posterior cingulum on the lingual surface, and a slightly developed anterior cingulum. The protocone and the paracone were almost aligned antero-posteriorly, and M^2 was reduced ($< 1/3$ of the P^4). Individuals exhibiting this morphotype originated from Myanmar (central: Irrawaddy valley), Thailand (north and south), Vietnam (south), Cambodia, Peninsular Malaysia, Sumatra, Java and the Lesser Sunda Islands (Bali, Lombok, Sumbawa, Flores, Roti and Timor).

The third group (clade N) also had a triangular P^4 with a posterior cingulum that was both lingual and labial, and a well-developed anterior cingulum. The protocone and the paracone were aligned antero-posteriorly. P^4 presented a strongly developed parastyle and M^2 was reduced ($< 1/3$ of the P^4). Individuals exhibiting this morphotype originated from Borneo and the Philippines. Individuals from the Mentawai Islands (*P. h. lignicolor*) possess a triangular P^4 with a thin posterior cingulum on both the lingual and labial surfaces. P^4 also has an anterior cingulum. The parastyle of P^4 was well developed, and M^2 was reduced ($< 1/3$ of the P^4).

Distribution and elevation

The geographical analysis of morphological and genetic variation in mainland Southeast Asia retrieved a complex

pattern, with the co-occurrence of individuals belonging to two distinct genetic clades or morphotypes (I and L) in Myanmar, Thailand and Vietnam. Considering that elevation might be an important factor for the separation of groups I and L, we found, using DIVA-GIS, that the best threshold segregating the distributions of these two groups was at an elevation of 200 m (see Fig. 6): individuals belonging to clade I were found above 200 m, and clade L individuals were distributed below 200 m. However, this should be considered a rough estimate of their elevational segregation. The reasons for this elevational segregation will have to be investigated more carefully through ecological field studies or ecological niche modeling.

Ancestral distribution reconstruction

The reconstruction of the ancestral areas of *Paradoxurus* using MACCLADE (Fig. 5) inferred an ancestral distribution of the *Paradoxurus* genus similar to that of clade A (Indian subcontinent, Myanmar, Thailand, Laos and Vietnam). The two species *P. jerdoni* and *P. zeylonensis* probably appeared through genetic isolation in the Western Ghats and Sri Lanka, respectively. Clade B ancestors were inferred to be absent from the Indian region and thus restricted to the east of the north-western range of Myanmar. Then, several dispersal events are likely to have occurred during diversification of this lineage: dispersal to Peninsular Malaysia, Sumatra, Java and surrounding islands (clade J); and dispersal to Mentawai, Borneo and the Philippine islands (clade K).

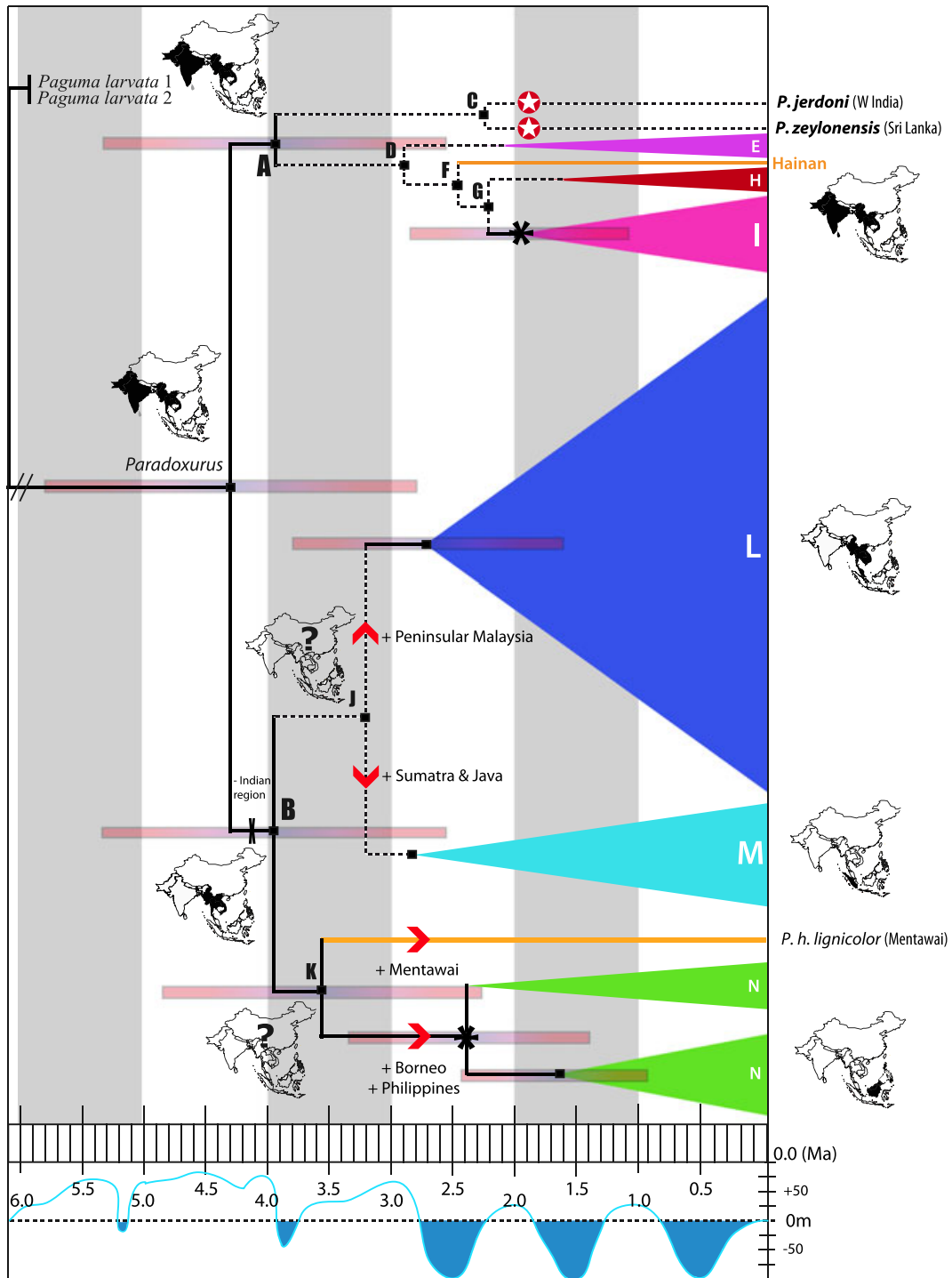


Figure 5 Mitochondrial chronogram of *Paradoxurus*, extending from 6.1 Ma to present. The two calibration points are shown by an asterisk (clades I and N). For the dated nodes, we also report the associated deviations, depicted by the horizontal bars. Parts of the phylogeny that were not dated are indicated by dashed lines. Maps represent the reconstructed ancestral areas for the different ancestors (countries in black are those where the ancestors were present; '?' means that the reconstruction was ambiguous) and for the extant representatives. Several biogeographic events are also depicted: ⊛ marks isolation with subsequent speciation; ➤ marks the dispersal of the ancestors to a new area; ✱ marks the disappearance of the ancestors from a given region. A rough picture of the eustatic sea levels (from Haq *et al.*, 1987) is presented below the chronogram. The dashed line represents the current sea level (0 m).

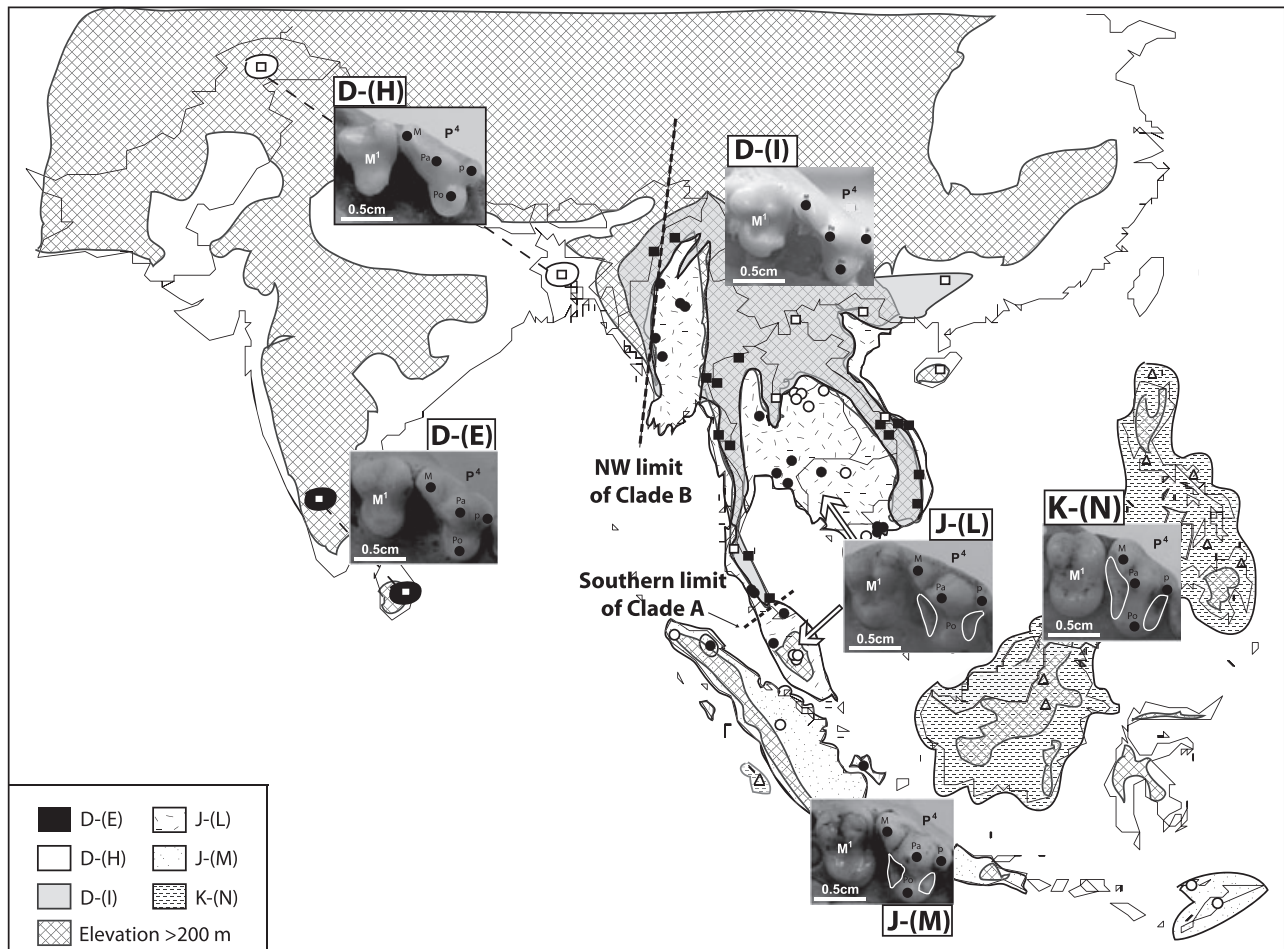


Figure 6 Schematic map of Asia illustrating the distribution of the genetic groups of *Paradoxurus hermaphroditus* (in different motifs), and the three major morphotypes corresponding to clade D (represented by squares: E, H and I), clade J (circles: L and M) and clade K (triangles: N). The areas exhibiting an elevation > 200 m are crosshatched. Molecular samples are illustrated as empty forms. Localities of specimens used for the morphological study (full forms) are shown only for groups I and L. The black dashed line in north-western Myanmar indicates the limit of the distribution of clade B; the line in southern Thailand corresponds to the Isthmus of Kra and marks the southern limit of distribution of clade A. Photographs are close-ups of the anterior part of the upper skull (left side, anterior on the left) showing M^1 (left) and P^4 (right) of common palm civets. Black circles mark the different cones of the P^4 (from left to right): metacone (M), paracone (Pa), protocone (Po) and parastyle (p): (H) FMNH-140476 (Pakistan); (E): AMNH-163173 (SE India); (I): MNHN-1988–165 (NW Myanmar); (L) AMNH-113771 (S Vietnam); (M) AMNH-101472 (Java); (N) FMNH-62861 (Palawan). Photographs by M.L.P., courtesy of the AMNH, FMNH and MNHN (higher-quality photographs illustrating the three major morphotypes are available in Appendix S4).

DISCUSSION

Evolutionary systematics of *Paradoxurus*

Both our mitochondrial and nuclear results agreed in the paraphyly of *P. hermaphroditus*, with *P. jerdoni* and *P. zeylonensis* nested within the *P. hermaphroditus* clade. Three distinct lineages, supported by both the mitochondrial and nuclear data, were identified within *P. hermaphroditus*: clade I (north-west Thailand, south-east Myanmar, north Laos, north and central Vietnam and south China); clade K (Mentawai, Borneo and the Philippine islands); and clade L (central Myanmar, central Thailand, Peninsular Malaysia, Cambodia and south Vietnam) (see Figs 2 & 3). Mitochondrial data (which included a larger dataset) supported the extension of clade I

to include representatives from Pakistan, India and Sri Lanka (mtDNA clade D), and the extension of clade L to include representatives from Sumatra, Java, Flores and Nias Island (mtDNA clade J).

The morphological variation of dental features (see Fig. 6 and Appendix S4) was in full agreement with the molecular results, revealing the existence of three main morphotypes that corresponded to the three major clades (D, J and K). Specimens from clade D (Indian subcontinent and Indochina) formed one morphotype that had a P^4 antero-posteriorly elongated and without cingula. It should be noted that specimens from Nepal and north-western Myanmar also exhibited this morphotype. All individuals from clade J (Indochina, Peninsular Malaysia, Sumatra and Java) exhibited a triangular P^4 , a postero-lingual cingulum and a reduced

anterior cingulum, and a reduced M^2 . Clade K (Borneo and the Philippines) individuals exhibited a triangular P^4 , with strongly developed posterior and anterior cingula, and a reduced M^2 .

In our mitochondrial phylogeny, *P. jerdoni* and *P. zeylonensis* grouped into a well-supported clade (clade C) that was sister to *P. hermaphroditus* clade D and nested within clade A (Fig. 2). The nuclear sequence of the one representative of *P. jerdoni* resulted in a conflicting position within clade K of the nDNA tree, but only with a moderately supported node (Fig. 3). Some discrepancies between mt and nDNA topologies were also observed in the relationships inferred for the major *P. hermaphroditus* lineages (Figs 2 & 3). Such incongruence may be a consequence of ancestral polymorphism with incomplete lineage sorting, putative ancient hybridization events within *Paradoxurus*, or simply of a paucity of informative characters of the nuclear marker that resulted in a random branching. Mitochondrial data were more informative for studying the evolution of *Paradoxurus* civets, and these results have thus been used in the discussion in the next sections.

Taxonomy of *Paradoxurus*

Our molecular and morphological evidence suggest that *P. hermaphroditus* should be split into at least three species, corresponding to the lineages D, J and K that were obtained in this study. However, in order to determine the exact taxonomic boundaries within *Paradoxurus*, additional investigations should be undertaken. A more extensive molecular sampling across the Indian subcontinent is needed in order to fill the taxonomic gap. More samples of *P. jerdoni* and *P. zeylonensis* will also have to be analysed: despite several attempts to amplify DNA from museum specimens, we were able to include only one representative of each species in our analyses. Finally, additional nuclear loci should be sequenced.

Our results revealed some discrepancies with the taxonomy of Pocock (1934a,b, 1939) and Wozencraft (2005). Four allopatric subspecies have been proposed for the Indochinese region and Peninsular Malaysia (Fig. 1). We found no evidence for the subspecies *P. h. cochinchensis* Schwarz, 1911 (south Vietnam and Cambodia). The geographical ranges of *P. h. laotum* Gyldenstolpe, 1917, *P. h. minor* Bonhote, 1903 and *P. h. musanga* (Raffles, 1821) did not match the ranges of our molecular lineages. Concerning Sumatra and Java, we found no genetic evidence to distinguish populations of these two islands, and thus our results do not agree with the definition of the two subspecies *P. h. musanga* (Raffles, 1821) (Sumatra) and *P. h. javanicus* Horsfield, 1824 (Java). The morphological distinctiveness of the Mentawai palm civet *P. h. lignicolor* was confirmed by our molecular data, and this civet was found to be close to the Borneo-Philippines *Paradoxurus*. Our results did confirm the validity of *P. h. philippinensis* Jourdan, 1837 from Borneo and the Philippines. Unfortunately, we did not have sufficient geographic coverage to either confirm or invalidate the subspecies proposed for the Indian region.

Concerning *P. jerdoni* and *P. zeylonensis*, the long branch leading to the clade that contained these two species, and the large pairwise genetic distance to *P. hermaphroditus* (> 10% in cyt *b*) support the species status for both these species. Morphological evidence also supports a specific status for *P. jerdoni* and *P. zeylonensis*. Both species have a reversed hair direction on the neck, which constitutes a synapomorphy within the genus *Paradoxurus*. In addition, *P. jerdoni* has a marked difference in the development of the prepalatine foramina, which distinguishes it from *P. hermaphroditus* (Blanford, 1885; Pocock, 1939), and *P. zeylonensis* has a narrow skull with small carnassials, relative to *P. hermaphroditus* (Phillips, 1984). However, additional molecular evidence is necessary to confirm this. Moreover, the taxonomy within *P. zeylonensis* has recently been questioned on the basis of morphology (Groves *et al.*, 2009) and should be investigated in a molecular framework.

Biogeography

According to our results, in the Pliocene, the ancestors of *Paradoxurus* were present in the Indian subcontinent, Myanmar, Thailand, Vietnam and Laos. The two major mitochondrial lineages (A and B) split during this period. The last common ancestor of clade A appears to have occupied the same distribution range as the ancestors of *Paradoxurus*. The ancestors of clade B were probably absent from the Indian subcontinent but were present in the Indochinese region and north of the Isthmus of Kra. North-western Myanmar may have acted as a biogeographic barrier to the westward spread of this clade. Many taxonomic groups have distribution boundaries in Myanmar (see e.g. Corbet & Hill, 1992; Shih *et al.*, 2009). In these cases, the north-western mountain ranges (Patkai Range, Chin Hills and Arakan Range; see below) or the Brahmaputra and Salween rivers may act as biogeographic barriers (Meijaard & Groves, 2006; Veron *et al.*, 2007). These barriers may also have restricted gene flow between Indian subcontinent and Southeast Asian groups (see Su *et al.*, 2006). The Isthmus of Kra has also been proposed as a biogeographic limit for many animals (mammals, Corbet & Hill, 1992; Woodruff, 2003; Woodruff & Turner, 2009; rodents, Gorog *et al.*, 2004; tiger, Luo *et al.*, 2004; binturong, Cosson *et al.*, 2007; primates, Ziegler *et al.*, 2007; birds, Hughes *et al.*, 2003; crustaceans, de Bruyn *et al.*, 2005). The repeated sea-level changes in the region of the Isthmus of Kra have been suggested to have had an area effect on mammal species ranges and limits (see Woodruff & Turner, 2009), and may have had a strong impact on the evolutionary history of the *Paradoxurus* lineages. Past changes in habitat and interspecific or ecological competition throughout this region could also have contributed to the distributional pattern that is observed (see Meijaard, 2009).

The biogeographic separation found for *P. jerdoni* (south-west India) and *P. zeylonensis* (Sri Lanka) has also occurred for *P. hermaphroditus* within clade E. Southern India and Sri Lanka were repeatedly connected when sea levels dropped

10 m under the present sea level (Worldbath, 2010), which may explain the common origin of south-west Indian and Sri Lankan *Paradoxurus*. Changes in sea levels would have led to isolation periods that may have resulted in local endemism within south-west India and Sri Lanka (Bossuyt *et al.*, 2004; Manamendra-Arachchi *et al.*, 2005). The contrasting genetic distances and morphological differences found between *P. hermaphroditus* from India and Sri Lanka and between *P. jerdoni* and *P. zeylonensis* suggest different isolation events.

On mainland Southeast Asia, two distinct genetic clades (I and L) both occur in Myanmar, Thailand and Vietnam. A similar overlapping phylogeographic pattern has been found in other Asian taxa, suggesting secondary contact zones between previously isolated populations (Iyengar *et al.*, 2005; in the dhole, *Cuon alpinus*; Fuchs *et al.*, 2008; in the white-browed piculet, *Sasia ochracea*). The genetic differences between these two clades were supported by marked morphological differences between individuals from geographically close localities. Our analyses showed that clade I members occurred in areas above 200 m, whereas clade L representatives were distributed in lower areas (below 200 m). We thus suggest that this dichotomic pattern of distribution in this region may result from ecological partitioning across elevational gradients (Outlaw & Voelker, 2008; see also biogeographic 'region 3' in amphibians, Inger, 1999), although other factors need to be investigated. We hypothesize that the continuous mountain ranges running through Myanmar, Thailand, Laos, south China and Vietnam served as a large, uninterrupted glacial refugial area for clade I ancestors (Brandon-Jones, 1996; Gorog *et al.*, 2004; Iyengar *et al.*, 2005). The glacial refuge zone may also have been restricted to southern China (Luo *et al.*, 2004). The split of clade I from other *Paradoxurus* groups occurred in the Late Pliocene–Early Pleistocene, during which a period of major cooling was followed by a drier and more seasonal climate (Heaney, 1991; Brandon-Jones, 1996; Meijaard, 2004b; Bird *et al.*, 2005). Within this region, the latter period resulted in a contraction of rain forests, which persisted only at higher elevations (Taylor *et al.*, 1999; Morley, 2000; Abegg & Thierry, 2002; Meijaard, 2004b; Meijaard & Groves, 2006). Forest-dwelling species, including representatives of clade I, may then have retreated to high-elevation refugia.

The ancestors of clade J (clades L and M) were present in the Indochinese region in the Late Pliocene–Early Pleistocene, and then dispersed south of the Isthmus of Kra (Fig. 5). Although clade L occurs today over a wide range (from central Myanmar and south Vietnam to Peninsular Malaysia), its low genetic variation and smooth mismatch distributions suggest a recent demographic expansion. We suggest that during the recent ice ages, populations corresponding to clade L retracted into glacial forest refugia. Once environmental conditions became favourable for range expansions, clade L populations recolonized what is now their current distribution. However, in the Indochinese region, they were probably restricted to low areas through ecological competition with clade I populations, which were already present in this region at higher elevations.

Our divergence time estimates yielded an Early–Late Pliocene period of diversification for clade K (Mentawai, Borneo and the Philippines). This corresponds to a period of low sea levels (3.5–4 Ma, Haq *et al.*, 1987; Miller *et al.*, 2005), during which land bridges emerged among Asian islands, allowing for faunal exchanges (Meijaard, 2004b; Outlaw & Voelker, 2008). The individual from Mentawai is included in clade K. Distance-wise, the Mentawai archipelago is very close to Sumatra, but is separated by a deep-sea channel, the Mentawai Strait. However, some studies focusing on primates (Cercopithecidae, *Macaca* spp.) have suggested a possible connection of the Mentawai Islands with the Batu Islands during periods of extremely low sea level (Abegg & Thierry, 2002; Roos *et al.*, 2003; Ziegler *et al.*, 2007). The divergence date estimated for clade K (2.3–4.5 Ma) is consistent with the timeframe inferred for the colonization of the Mentawai Islands (Roos *et al.*, 2003: 2.2 Ma; Ziegler *et al.*, 2007: 2.4–2.6 Ma). Affinities between groups from Mentawai and Borneo have already been shown in the Colobinae primates (*Simias concolor* from Mentawai and *Nasalis larvatus* from Borneo, Whittaker *et al.*, 2006). One hypothesis to explain the discontinuous distribution of clade K populations over the Sunda shelf is that high sea levels isolated populations, some of which then disappeared (except on Mentawai, Borneo and the Philippines), through either ecological competition or environmental changes (e.g. the Mount Toba eruption that occurred *c.* 74,000 years ago; Oppenheimer, 2002; e.g. Luo *et al.*, 2004; but see Louys, 2007). In those areas from which clade K populations disappeared, they were replaced by representatives of clade J. Thus, the Mentawai population is a relict group that was maintained because of its isolation. The estimation of divergence time between the Mentawai individual and clade N (Borneo and the Philippines) revealed that these two groups have been isolated for a long time (since the Pliocene).

Within clade N, our analyses identified two groups: 'Philippines' and 'Borneo + Philippines'. Borneo may have constituted a centre of origin of clade K, possibly acting as a refugial area during the Pleistocene (Brandon-Jones, 1998; Gathorne-Hardy *et al.*, 2002; Quek *et al.*, 2007). Although Borneo and the Philippines were isolated by high sea levels during the Pleistocene (Haq *et al.*, 1987), dispersals from Borneo to the Philippines were possible during periods of lower sea level (Reis & Garong, 2001; Bird *et al.*, 2005). However, it is thought that Palawan has never been connected by dry land to the other Philippines islands (Heaney, 1986; Reis & Garong, 2001; Esselstyn *et al.*, 2004; but see Harrison *et al.*, 2006, for a putative connection). Our results showed that some Palawan individuals had affinities with Philippine populations and others with Borneo populations, indicating that there were movements between these islands (see also the genetic pattern retrieved for Palawan populations in the fruit bat *Cynopterus brachyotis*; Campbell *et al.*, 2004). These colonization events of Palawan could have resulted from natural dispersal (Heaney, 1986; Heaney *et al.*, 2002) or/and were recently mediated by humans (Reis & Garong, 2001). Human transportation of palm civets may also explain why a

specimen from Luzon Island (the Philippines) was found within the Borneo clade rather than within the Philippine clade, and why several morphotypes of common palm civets were observed in the Philippine islands (J. A. Esselstyn, pers. obs.; L. R. Heaney, Field Museum of Natural History, Chicago, pers. comm.).

In conclusion, the genus *Paradoxurus* has revealed itself to be a good model with which to study Asian biogeography. Further studies in this region will give us new insights into how Asian biodiversity has emerged. We have also shown that there is a need for a taxonomic revision within *P. hermaphroditus* and we suggest that this species should be split into at least three species. However, further research, with additional samples, nuclear markers and microsatellite data, would help to resolve the exact taxonomic boundaries within *Paradoxurus*.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article:

Appendix S1 List of mitochondrial primers used for amplification and sequencing of cytochrome *b* and control region in museum specimens of *Paradoxurus hermaphroditus*.

Appendix S2 Model parameters retrieved by MRMODELTEST and used to perform phylogenetic analyses of the *Paradoxurus* dataset.

Appendix S3 List of the museum specimens of *Paradoxurus* studied.

Appendix S4 Morphological variation in dental features (upper teeth) of the various groups of *Paradoxurus hermaphroditus*.

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BIOSKETCH

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Author contributions: G.V. and M.L.P. conceived the ideas; A.P.J., A.W., G.V., J.A.E. and M.L.P. collected the data; M.L.P., A.W., C.C. and G.V. produced the DNA sequences; A.W. and M.L.P. analysed the data; G.V. and M.L.P. led the writing; A.J.P., A.W., J.A.E., J.F. and P.G. played a large part in the writing of the paper.

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Correction added after online publication: The species name was changed from *Paradoxurus erdoni* to *Paradoxurus jerdoni* in the Methods section of the Abstract and the species name was changed from *P. hermaphrodites* to *P. hermaphroditus* in the Main conclusions section of the Abstract, in August 2010.