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Sympatric Asian felid phylogeography reveals a major Indochinese–Sundaic divergence

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Abstract

The dynamic geological and climatological history of Southeast Asia has spawned a complex array of ecosystems and 12 of the 37 known cat species, making it the most felid-rich region in the world. To examine the evolutionary histories of these poorly studied fauna, we compared phylogeography of six species (leopard cat *Prionailurus bengalensis*, fishing cat *P. viverrinus*, Asiatic golden cat *Pardofelis temminckii*, marbled cat *P. marmorata*, tiger *Panthera tigris* and leopard *P. pardus*) by sequencing over 5 kb of DNA each from 445 specimens at multiple loci of mtDNA, Y and X chromosomes. All species except the leopard displayed significant phylogenetic partitions between Indochina and Sundaland, with the central Thai–Malay Peninsula serving as the biogeographic boundary. Concordant mtDNA and nuclear DNA genealogies revealed deep Indochinese–Sundaic divergences around 2 MYA in both *P. bengalensis* and *P. marmorata* comparable to previously described interspecific distances within Felidae. The divergence coincided with serial sea level rises during the late Pliocene and early Pleistocene, and was probably reinforced by repeated isolation events associated with environmental changes throughout the Pleistocene. Indochinese–Sundaic differentiations within *P. tigris* and *P. temminckii* were more recent at 72–108 and 250–1570 kya, respectively. Overall, these results illuminate unexpected, deep vicariance events in Southeast Asian felids and provide compelling evidence of species-level distinction between the Indochinese and Sundaic populations in the leopard cat and marbled cat. Broader sampling and further molecular and morphometric analyses of these species will be instrumental in defining conservation units and effectively preserving Southeast Asian biodiversity.

Keywords: Asian felids, central Thai–Malay Peninsula, Indochinese-Sundaic divergence, mtDNA, phylogeography, X chromosome, Y chromosome

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Introduction

Sundaland comprises the Thai–Malay Peninsula and islands on the Sunda Shelf including Java, Sumatra and Borneo and is considered one of the most important biodiversity hotspots in the world (Myers *et al.* 2000). To its north lies the Indochinese bioregion, encompassing east India, southwest China, Vietnam, Laos, Cambodia, Myanmar and Thailand. Genetic studies have distinguished assemblages of amphibians (Emerson *et al.* 2000), reptiles (Inger & Voris 2001), birds (Hughes *et al.* 2003), mammals (Corbet & Hill 1992;

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Tougard 2001; Tosi *et al.* 2002; Woodruff & Turner 2009), freshwater crustaceans (de Bruyn *et al.* 2005) and insects (Corbet & Pendlebury 1992) limited to varying degrees by the Isthmus of Kra (10°30′N), a narrow land bridge connecting the Thai–Malay Peninsula with mainland Southeast Asia (Fig. 1). Woodruff (2003) hypothesized that periods >1 MY of marine transgressions submerging the Isthmus (>100 m above present-day sea level) during the early/middle Miocene and the early Pliocene (24–13, 5.5–4 and 3 MYA) resulted in current biogeographical patterns. Although a refinement of the global glacioeustatic curve identified no sea level rise greater than 100 m above the present-day level in the last 5 MY, it alternatively proposed at least 58 rapid rises over 40 m, which while not transgressing the Isthmus, certainly reduced the width of the Thai–Malay Peninsula. Such frequent rises in sea level could have repeatedly compressed the area of habitat available on the narrow Peninsula, driving Indochinese–Sundaic faunal differentiation (Lisiecki & Raymo 2005; Miller *et al.* 2005; Woodruff & Turner 2009). The exact biogeographic boundaries vary across species and primarily cluster in the northern and central Thai–Malay Peninsula (5–10°N, Woodruff & Turner 2009).

During the Pleistocene, sea level fluctuations repeatedly exposed vast areas of the Sunda Shelf and formed land bridges among the islands and mainland. As recently as the last glacial maximum (LGM, c. 20 kya), Borneo, Sumatra, Java, the Thai–Malay Peninsula and Indochina formed a single land block (Wallace 1876; Molengraaff & Weber 1921). Although the Pleistocene land bridges may have enabled widespread faunal movement (Voris 2000; Meijaard & van der Zon 2003), recent studies indicate that effective dispersal of some

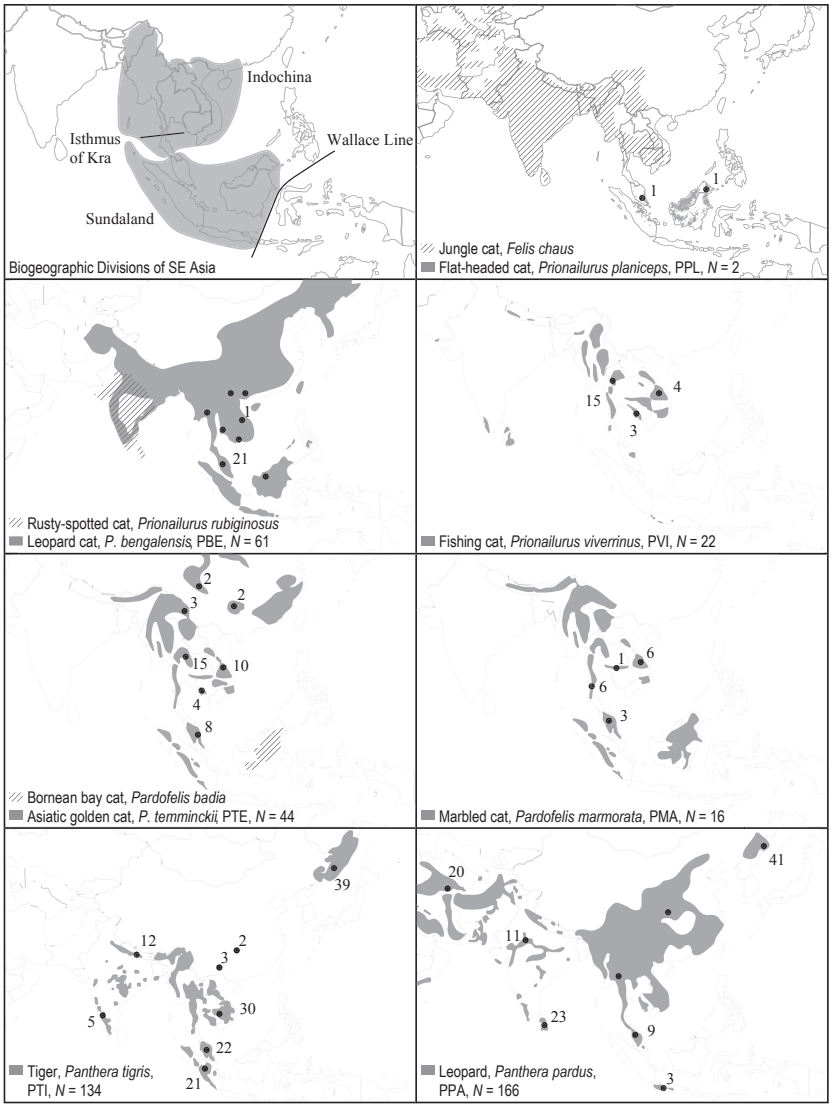

Fig. 1 Maps of major divisions of biogeography and ranges of felids (shaded areas) in Southeast Asia. Specimens and sample sizes from approximate geographic locations are indicated.

mammals was limited, since many species, including murine rodents (Gorog *et al.* 2004), leopards (Uphyrkina *et al.* 2001), colugos (Janecka *et al.* 2008), langurs (Roos *et al.* 2008; Meyer *et al.* 2011), clouded leopards (Buckley-Beason *et al.* 2006), and gymnures (Ruedi & Fumagalli 1996), display a much older time of vicariant isolation. Distinguishing historic vicariant population differentiation from recent migrations and dispersals associated with LGM land bridge formations is possible via a comparative phylogeography approach (Hewitt 2000; Moritz *et al.* 2000).

Fluctuating geological and climatological conditions in Southeast Asia coincided with the initial rapid radiation of major felid lineages around 10 MYA. The region is home to 12 of the 37 recognized modern Felidae species (Johnson *et al.* 2006), making it the most felid-rich region in the world. Each of these species is listed in CITES I or II as endangered, rare and protected over most of their remaining distribution (Table S2, Supporting information). Despite their high conservation priority, these wild cats remain poorly investigated and are threatened by habitat loss, fragmentation and illegal hunting (Nowell & Jackson 1996).

Many felid species in Southeast Asia have distribution patterns corresponding to major geographic divisions (Fig. 1). For example, the jungle cat (*Felis chaus*) occurs only north of the Thai–Malay Peninsula, while the flat-headed cat (*Prionailurus planiceps*) is restricted to the Thai–Malay Peninsula, Sumatra and Borneo south of the Thailand–Malaysia Boundary. The Asiatic golden cat (*Pardofelis temminckii*) occurs widely throughout Indochina, southwest China, the Thai–Malay Peninsula and Sumatra, but not Borneo. The bay cat (*Pardofelis badia*), a species closely related to *P. temminckii*, only occurs on Borneo and may be considered an island form of *P. temminckii*. The third species in the genus *Pardofelis*, the marbled cat (*P. marmorata*), overlaps the range of the other two and covers Indochina, the Thai–Malay Peninsula, Sumatra and Borneo. The fishing cat (*Prionailurus viverrinus*) has a discontinuous distribution in Indochina, Sumatra, Java, the northern Indian subcontinent and Sri Lanka. It is absent from most of the Indian subcontinent and the Thai–Malay Peninsula, although a few records from the southern Peninsula exist (Melisch 1995). The leopard cat (*P. bengalensis*), a sister species of *P. viverrinus* and the most common wild cat in Asia, ranges from the Russian Far East to Indonesian islands, and from India and Pakistan to the Philippines. The absence of *P. bengalensis* from Sri Lanka and central India is balanced by the presence of another *Prionailurus* species, the rusty-spotted cat (*P. rubiginosus*, Mukherjee *et al.* 2010).

The tiger (*Panthera tigris*), leopard (*P. pardus*) and clouded leopard (*Neofelis nebulosa*) have relatively continuous distributions in Southeast Asia. Significant population genetic structure has been discovered in tigers, leading to the recognition of tigers on the Thai–Malay Peninsula as a distinct subspecies, the Malayan tiger (*P. t. jacksoni*) (Luo *et al.* 2004). Further, the Sundaic clouded leopard is recognized as a new species of clouded leopard (*Neofelis diardi*) and has been distinct from the Indochinese clouded leopard (*N. nebulosa*) for 1–1.5 MY (Buckley-Beason *et al.* 2006; Kitchener *et al.* 2006; Wilting *et al.* 2007).

The sympatric occurrence and overall geographic structure of wild felids in Southeast Asia is an excellent model to test different biogeographic scenarios in Sundaland and Indochina. Here, we test the hypothesis that the central and northern Thai–Malay Peninsula (around southern Thailand and the Thailand–Malaysia border, and not confined to the Isthmus of Kra) serves as an important biogeographic barrier that has been influential in the diversification of regional fauna. We compared phylogeographical patterns and demographic histories of the tiger and leopard (*Panthera* spp.), leopard cat and fishing cat (*Prionailurus* spp.), and Asiatic golden cat and marbled cat (*Pardofelis* spp.) to capture the evolutionary history of the populations or species. Multiple sets of molecular genetic markers were examined: (i) mtDNA sequences that cover *CytB, 16S* and *ATP8*; (ii) Y-chromosome haplotyping system that includes four introns (*SMCY3*, *SMCY7*, *DBY7* and *UTY11*) and one Y-linked microsatellite; and (iii) X-chromosome nuclear introns in *PLP1*.

Methods

Samples

We obtained samples from 445 individuals (Fig. 1) across six focal species (134 tigers, 166 leopards, 61 leopard cats, 22 fishing cats, 44 Asiatic golden cats and 16 marbled cats) from their Asian ranges, with a particular emphasis on the northern Indochinese and southern Sundaic regions (Table 1, Table S1, Table S2 Supporting information). Two flat-headed cats (*Prionailurus planiceps*) were included in some of the analyses. Individuals were selected with known geographic origins and were presumed to be unrelated. Voucher samples from the tiger and leopard have been used in previous studies (Uphyrkina *et al.* 2001; Luo *et al.* 2004), and 53 leopard samples were collected from China and Southeast Asia in this study. Samples were collected in compliance with Federal Fish and Wildlife permits issued to SJO at the National Cancer Institute, NIH, by the US Fish and Wildlife Service. Genomic DNA was extracted using DNeasy Tissue Extraction Kits (QIA-

Table 1 Estimates of molecular genetic variation from combined mtDNA sequences

Species	Populations	*N*	Length (bp)	Number of haplotypes	Number of variable sites*				Percentage variable sites	Mean number of pairwise differences (±SD)	Nucleotide diversity (π±SD)
					Ti	Tv	I	Total			
Prionailurus bengalensis	All	61	1792	20	91	4	1	96	5.36	33.797 ± 14.930	0.0189 ± 0.00925
	Indochina	36	1792	14	29	0	0	29	1.62	3.765 ± 1.943	0.00210 ± 0.00121
	Sunda	25	1792	8	72	4	1	77	4.3	12.033 ± 5.630	0.00673 ± 0.00351
Prionailurus viverrinus	Indochina	17	1792	11	32	2	0	34	1.90	10.441 ± 5.010	0.00584 ± 0.00313
Pardofelis temminckii	All	38	1220	13	22	0	0	22	2.21	3.994 ± 2.042	0.00328 ± 0.00186
	Indochina	30	1220	11	13	0	0	13	1.07	1.497 ± 0.928	0.00123 ± 0.000847
	Sunda	8	1220	2	1	0	0	1	0.082	0.250 ± 0.311	0.000205 ± 0.000291
Pardofelis marmorata	All	11	1220	6	42	1	0	43	3.52	18.545 ± 8.918	0.0152 ± 0.00825
	Indochina	8	1220	5	4	0	0	4	0.33	1.500 ± 1.006	0.00123 ± 0.000939
	Sunda	3	1220	1	0	0	0	0	0	0	0
Panthera tigris†	All	100	4078	25	—	—	—	54	1.32	10.11 ± 4.66	0.00248 ± 0.00127
	Indochina	32	4078	4	3	0	0	3	0.074	0.54 ± 0.46	0.000132 ± 0.000125
	Malay	22	4078	5	9	1	0	10	0.25	4.83 ± 2.45	0.00118 ± 0.000670
Panthera pardus	All‡	69	727	33	—	—	—	50	6.88	8.67 ± 4.40	0.0121 ± 0.00620
	Indochina	36	613	6	6	1	0	7	1.14	1.529 ± 0.940	0.00250 ± 0.00170

*Ti, Transitions; Tv, Transversions; I, Indels.
†Data of the tiger from Table 6 in Luo *et al.* (2004).
‡Data of all leopards from Table 5 of Uphyrkina *et al.* (2001).

GEN) from whole blood, tissue or skin fibroblast cell culture, following the manufacturer's protocols.

Multilocus genetic marker system

Primer sets for amplifying over 5 kb of combined mtDNA, autosomal DNA, X- and Y-chromosome nucleotide sequences are summarized in Table S3 and Table S5 (Supporting information). The 1792 bp of mtDNA sequences spanned cytochrome *b* (*CytB*, 1240 bp), ATPase8 (*ATP8*, 180 bp) and 16S ribosomal DNA (*16S*, 370 bp). Primers CbM1 and CbMR2 were used to amplify the complete *CytB* gene (1240 bp) and three internal primers (CbM2, FBEN3 and FBEN2) for sequencing PCR products (Tamada *et al.* 2008). Sequences of mitochondrial NADH dehydrogenase subunit 5 (*ND5*) were obtained using primers and conditions described previously (Uphyrkina *et al.* 2001) to assess phylogenetic relationships between leopard populations from Indochina and the Thai–Malay Peninsula. The Y-chromosome haplotypes including 2154 bp of intronic regions of three Y-linked genes (*DBY7*, *SMCY3*, *SMCY7* and *UTY11*) and one Y-linked microsatellite *SMCY7*-STR were generated as described by Luo *et al.* (2007).

MtDNA and Y-chromosome haplotype analyses, phylogeny and coalescence dating

Phylogenetic analyses among mtDNA and Y-chromosome haplotypes were conducted using multiple approaches. A maximum parsimony (MP) analysis with a heuristic search, random addition of taxa and tree-bisection–reconnection branch-swapping (indels are treated as missing data), a minimum evolution (ME) heuristic search approach with neighbour-joining (NJ) trees constructed from Kimura 2-parameter distances followed by a branch-swapping procedure, and a maximum-likelihood (ML) analysis with the best evolutionary model selected with JMODELTEST v2.1.4 (Posada 2008) were performed with PAUP* v4.0b10 (Swofford 2001). The reliability of tree topologies was assessed by 1000 bootstrap iterations for the MP and NJ approaches and 100 for the ML approach. Construction of ML trees was also performed in RAxML executing 1000 rapid bootstrap inferences, with GTR substitution matrix and GAMMA or GAMMA+P-Invar model of rate heterogeneity. Bayesian inference was conducted with MRBAYES v3.2.0 (Ronquist & Huelsenbeck 2003), using both separate gene sets of mtDNA and Y chromosome concatenated haplotypes and a combined data set of mtDNA and Y sequences, with the best-fit evolutionary model estimated from JMODELTEST. The combined data set was partitioned into mitochondrial and Y-chromosome gene regions when analysed, with appropriate evolutionary models set for each partition and parameters unlinked. In MRBAYES, all analyses consisted of two simultaneous, independent Markov chain Monte Carlo (MCMC) runs starting from different random trees, each with three heated chains and one cold chain, for 2 000 000

generations sampled every 100 generations. Convergence of MCMC analyses was assessed in AWTY (Nylander *et al.* 2008), and reliability of parameters estimates was examined in the diagnostic program TRACER v1.5 (Rambaut & Drummond 2009), with the fraction of 25% burn-in samples based on a pilot study (Fig. S5, Fig. S6, Supporting information).

The time to the most recent common ancestor (TMRCA) for the mtDNA and Y haplotypes was estimated using BEAST v1.6.2 (Drummond & Rambaut 2007). The speciation times estimated previously (Johnson *et al.* 2006) of *Prionailurus bengalensis* and *P. viverrinus* (1.74–3.82 MYA), and of *Pardofelis temminckii* and *P. marmorata* (4.27–8.42 MYA) were used as calibrations. In addition, a 2.44–5.79 MYA divergence between *Panthera tigris* and *P. pardus* was used in Y-derived trees and the divergence time of *Prionailurus bengalensis*, *P. viverrinus* and *P. planiceps* (2.04–4.31 MYA) was used in trees inferred from mtDNA haplotypes. Nucleotide substitution and rate heterogeneity models were also estimated from JMODELTEST, and an uncorrelated lognormal relaxed clock model was implemented to allow for rate heterogeneity among lineages and also inspected as an indication of the extent to which our data accorded with the molecular clock model. A Yule speciation process that assumes a constant speciation rate per lineage was adopted for species-level phylogenies. With the combined data set, substitution models and clock models of the mtDNA and Y-chromosome sequences were independent. All BEAST MCMC analyses were performed with four independent runs simultaneously for 10 000 000 iterations. Samples were drawn every 10 000 steps and a burn-in of the first 10% was discarded. Validity of convergence and sample estimates were inspected in TRACER. All runs from one analysis produced the same topology and parameters distributions; thus, runs were combined to generate the final values of TMRCA and a consensus tree.

Signatures of past population dynamics were investigated for mtDNA haplotypes using Bayesian Skyline Plot model in BEAST. Site model parameters were estimated, respectively, for different species populations. For the Indochinese leopard cat, Indochinese Asiatic golden cat and Sundaic leopard cat, the number of groups (*m*) was set to six and for the Indochinese fishing cat population the group number was specified to four, with Piecewise-linear Skyline Model and randomly generated starting trees. For all populations, the strict-clock model was implemented after an individual population test and a mutation rate of 0.98% substitutions per site per million years was used as previously estimated. The MCMC chains were run for 10 000 000 generations and parameters sampled every 10 000 steps, the first 10% was discarded as burn-in. Output examination and Bayesian Skyline reconstruction were conducted in TRACER. Besides the Bayesian Skyline Plot, the BEAST time-aware Bayesian skyride method was also included in the test. All Bayesian analyses were run until the effective sample size (ESS) of each parameter was >200 to ensure a valid estimate of the Bayesian marginal posterior distribution. Pairwise mismatch distributions (Rogers & Harpending 1992) were also used to infer population dynamic histories with ARLEQUIN v3.5 (Excoffier & Lischer 2010). The goodness-of-fit of the observed data to a simulated model of expansion was tested with the raggedness (r) index and sum of squared deviation (SSD). Tajima's *D* and Fu's *Fs* were estimated in ARLEQUIN as additional measures for tracing population growth dynamics.

X-linked locus sequence analyses and population genetic indices

For the X-linked locus *PLP1,* the haplotype phase for a heterozygous female was inferred by affirming common haplotypes observed in the hemizygous males from the same population and identifying female haplotypes from likelihood frequencies. Statistical parsimony networks were then constructed using TCS v1.21 (Clement *et al.* 2000) to infer phylogeographic and potential ancestor–descendent relationships among haplotypes. Measures of population genetic variation such as the mean number of pairwise differences, haplotype diversity and nucleotide diversity (π) for mtDNA and nuclear DNA sequences were estimated using ARLEQUIN v3.5 (Excoffier & Lischer 2010). Slatkin's distances based on Kimura 2-parameter were used to estimate population pairwise F_{ST} (10 000 permutations for statistical significance tests). Exact tests of population differentiation based on haplotype frequencies were carried out using 100 000 steps in the Markov chain and 4000 dememorization steps. Observed phylogeographic partitions and estimated population genetic parameters were employed to define major subdivisions and draw inferences on the historic population history of Asian cat species.

Results

MtDNA phylogenetic analysis

MtDNA fragments were sequenced from three segments (*CytB, 16S* and *ATP8*) from three *Prionailurus* species and two segments (*CytB* and *16S*) from two *Pardofelis* species (Table 1). The *ATP8* primer set amplified both nuclear (*Numt*) and cytoplasmic mtDNA (*Cymt*) copies in *Pardofelis* species and was excluded from final analyses. The mtDNA fragments were con-

catenated into 1792-bp haplotypes for *Prionailurus* spp. and *Pardofelis* spp., with the *ATP8* region of the latter marked as missing data (Table 1, Table S4, Supporting information). Phylogenetic analysis of mtDNA haplotypes using maximum parsimony (MP), minimum evolution (ME), maximum-likelihood (ML) and Bayesian approaches produced congruent topologies corresponding to major geographic partitions in *Prionailurus* spp. and *Pardofelis* spp. (Fig. 2; Fig. S1, Fig. S3, Fig. S4, Supporting information).

MtDNA sequences from 61 leopard cats specified 95 variable sites and defined 20 haplotypes (Table 1). Consistent with previous results using fewer samples (Tamada *et al.* 2008), these haplotypes formed two deeply divergent haplogroups (Fig. 2A; Fig. S1, Fig. S3, Supporting information). The PBE mtDNA haplogroup S (South) consisted of six haplotypes from 23 leopard cats exclusively from the Thai–Malay Peninsula and Borneo. The PBE mtDNA haplogroup N (North) contained 14 haplotypes and occurred mainly in mainland Indochina (with the exception of two of the 38 individuals, or 5%, from the Thai-Malay Peninsula). MtDNA divergence between leopard cat N and S haplogroups was remarkably large and all phylogenetic methods (MP, ML and Bayesian) grouped the PBE mtDNA haplogroup S and fishing cat together, with relatively high support values (Fig. 2A). The deep divergence between leopard cat N and S mtDNA haplogroups is comparable to the species-level difference between the leopard cat (*Prionailurus bengalensis*) and fishing cat *(P. viverrinus)*.

Phylogeographic partitioning between the Thai–Malay Peninsula and mainland Indochina was also evident in the marbled cat (*Pardofelis marmorata*) and Asiatic golden cat (*P. temminckii*) (Fig. 2A; Fig. S1, Fig. S4, Supporting information). Three marbled cats from the Thai–Malay Peninsula shared a single haplotype that was highly divergent (PMA mtDNA haplogroup S) from a monophyletic group of eight Indochinese marbled cats (PMA mtDNA haplogroup N, five haplotypes). Similarly, Sundaic Asiatic golden cats (PTE mtDNA haplogroup S, N = 8) shared two haplotypes that formed a monophyletic haplogroup distinct from the 11 mainland Asian haplotypes (PTE mtDNA haplogroup N, N = 30). The difference between the two *P. temminckii* lineages is more shallow (8 bp changes) than that in *Prionailurus bengalensis* (64 bp changes) and *P. marmorata* (39 bp changes).

MtDNA sequences of *ND5* from 33 leopards from Indochina and Malaysia (the range of *P.p. delacouri*; DEL in Fig. 2B) were analysed with 69 leopards described previously (Miththapala *et al.* 1996; Uphyrkina *et al.* 2001). Eight individuals from the Thai–Malay Peninsula shared a single haplotype (DEL1) that was also common in 14 *P. p. delacouri* from mainland Indochina. This absence of phylogeographic differentiation in leopards is in marked contrast with tigers, which display very distinctive structure between the Malayan (*P. t. jacksoni*) and Indochinese tiger subspecies (*P. t. corbetti*) according to mitochondrial and nuclear markers (Luo *et al.* 2004).

Y-chromosome haplotype analysis

Alignment of the combined intronic regions from four Y-chromosome genes (*SMCY3*, *SMCY7*, *DBY7* and *UTY11*; 2154 bp in total) yielded 15 haplotypes across the six felid species. Intraspecific polymorphism was found in all species except *P. tigris* (Table 2). Seven SNPs and one indel that defined six Y-linked haplotypes were observed in 26 leopard cat males (Table 2A, Fig. 3; Fig. S2, Supporting information). One haplogroup with two haplotypes (PbeY-E and F) was found only in individuals from the Sundaland and was designated as PBE Y haplogroup S. The other four haplotypes formed a monophyletic haplogroup (PbeY-A to D) that mainly occurred in northern populations. PbeY-A, the most common haplotype was found in 64% of Indochina/China individuals (N = 14). Malayan leopard cats displayed the highest Y-haplotype diversity among the populations, with five of the six Y haplotypes. Three individuals (33% of the sampled population) shared haplotypes (PbeY-A, B and D) found in the N lineage, suggesting the occurrence of gene flow between the N and S haplogroups in this region. The Bornean leopard cat population (N = 3) was fixed with the unique haplotype PbeY-E.

The faster-evolving Y-STR markers may better capture recent expansion or founder demographic events. Y-linked microsatellite locus *SMCY7*-STR alleles exhibited geographic differences in the leopard cat (Table 2A). Seven alleles were found in 32 males, and allele sizes were continuous (269–283 bp), missing only the 275-bp allele. Alleles 277–283 bp were specific to the Sundaic populations on the Thai–Malay Peninsula and Borneo, while alleles 269–273 were mostly observed in Indochinese populations. Two of the 'northern' alleles (269 and 271 bp) were found in 9% (N = 1) and 18% (N = 2) of the Malayan population, respectively, similar to the pattern of the more slowly evolving Y biallelic markers.

In the marbled cat, Y-chromosome distinctions between the Sundaic and Indochinese populations were also detected (Table 2B, Fig. 3; Fig. S2, Supporting information). Three individuals with the unique PMA mtDNA haplogroup S (Fig. 2A) also shared the PMA Y haplogroup S. This haplotype is four mutations away from the PMA Y haplogroup N found exclusively in Indochinese marbled cats (Fig. 3). Despite limited sampling, it is striking that the alternative haplogroups of both Y and mtDNA were fixed

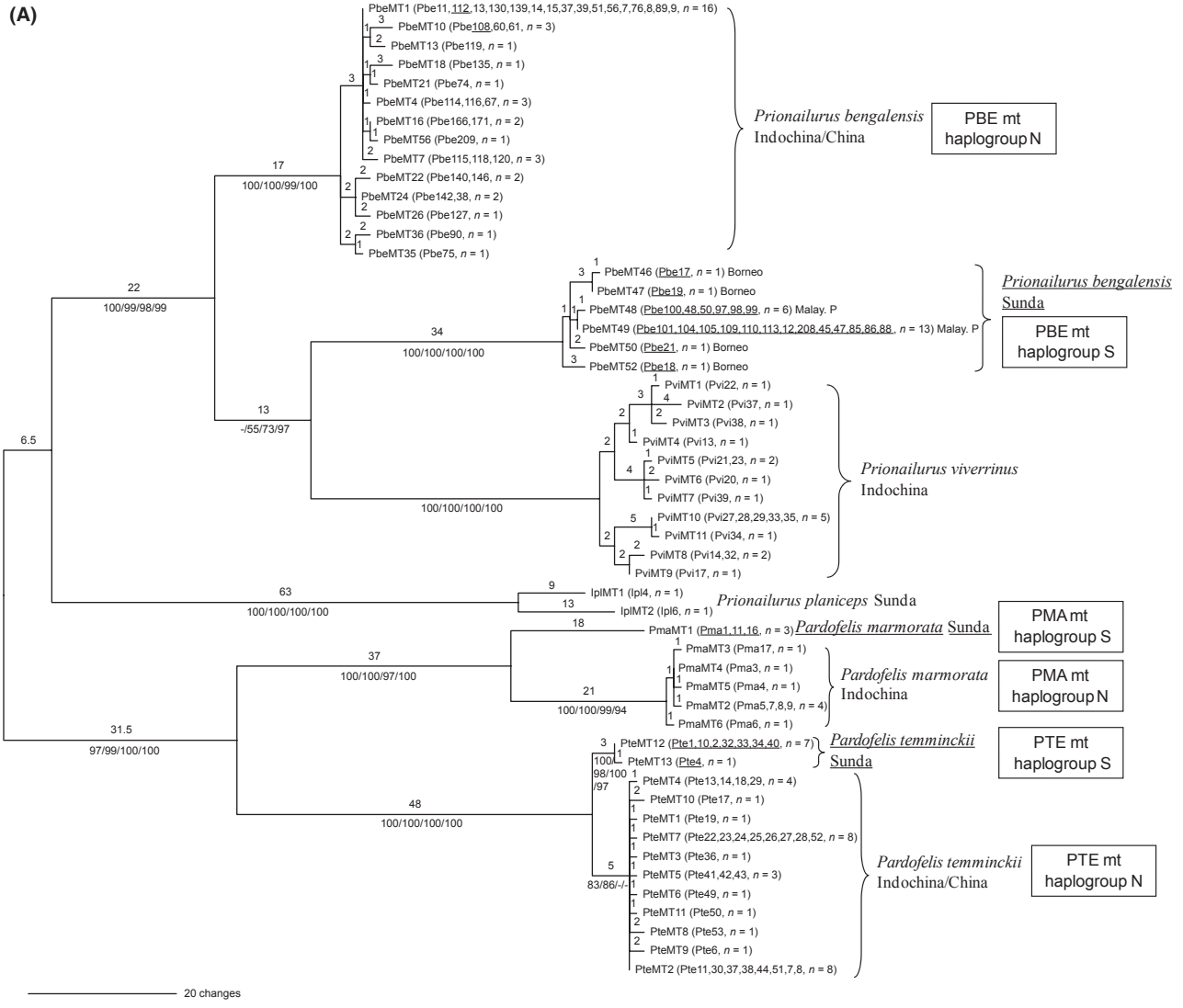

Fig. 2 MtDNA phylogenetic relationships among *Prionailurus* spp., *Pardofelis* spp. and *Panthera* spp. in Asia. (A) Phylogenetic relationships for *Prionailurus* spp. and *Pardofelis* spp. inferred with maximum parsimony (MP) from 1792 bp combined mtDNA sequences. Trees derived from minimum evolution (ME), maximum-likelihood (ML) and Bayesian analyses have identical topologies. Numbers below branches represent bootstrap support in percentage from 1000 replicates using the ME, MP and ML methods, followed by posterior probabilities using Bayesian analyses (only those over 50% are indicated). Numbers above branches show the number of changes. Codes following the branches indicate haplotype names, and text within parentheses refers to codes of the animals (Table S1, Supporting information) and the number of individuals with the same haplotype. Underlined animal codes represent Sundaic origins of the specimens. N = North, S = South. (B) Phylogenetic relationships for *Panthera pardus* for 613 bp *ND5* mtDNA sequences (N = 102) including 33 Southeast Asian leopards from this study marked in dashed box and four newly identified mtDNA haplotypes in bold.

between Sundaic versus Indochinese marbled cats, revealing a species-level divergence as observed between Indochinese and Sundaic leopard cats (Fig. 3).

Very modest Y-chromosome nucleotide diversity was observed in leopards, tigers and Asiatic golden cats, compared with the smaller leopard cat and marbled cat (Table 2C,D, Fig. 3; Fig. S2, Supporting information). Three Y-SNPs depicted three haplotypes (PpaY-A, B, C) among 75 male Asian leopards, and no Y-STR variation was found. Eastwest geographic structure of Y haplotypes was observed in leopards, probably reflecting historic isolation patterns. Except for two *SMCY7*-STR alleles, the tiger was invariant in Y-chromosome DNA (Table 2D), consistent with the overall low genetic variability seen with mtDNA, MHC and autosomal microsatellite markers (Luo *et al.* 2004). Two haplotypes differing at a single nucleotide site were detected in the Asiatic golden cat, and the major haplotype (PteY-A)

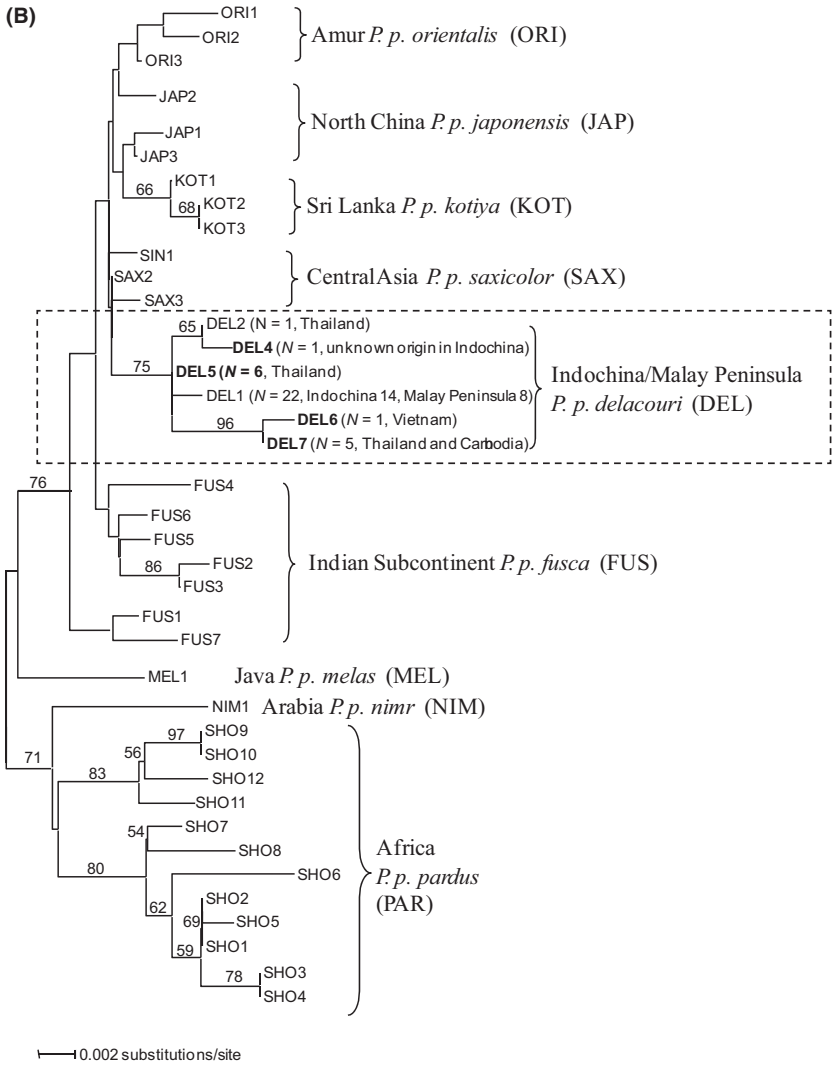

Fig. 2 *Continued*

was observed in both Indochina and the Thai–Malay Peninsula (Table 2C, Fig. 3). In contrast to *P. bengalensis* and *P. marmorata*, no support for Indochina–Sunda Y-chromosome haplogroup divergence along the Thai–Malay Peninsula was evident in the leopard, tiger and Asiatic golden cat (Fig. 3).

Nuclear DNA analyses

Statistical parsimony network based on *PLP1* haplotypes from six species provided additional support for phylogeographic divergence between Indochinese and Sundaic populations in both *Pardofelis marmorata* and *Prionailurus bengalensis* (Table 2, Fig. 4). The similar nonrandom frequency distribution of *PLP1* haplotypes corresponding to geographic range affirmed deep divergence between the northern and southern lineages in *P. bengalensis*. PbePLP-1 was found only in the Sundaic populations. It has a 26-bp insertion not seen in other *P. bengalensis* or felids. The other haplotype (PbePLP-5), a common allele found exclusively in the Indochina/China population, was also present in the Malayan population with a 42% frequency, supporting the hypothesis that this region is a transition zone between Indochinese and Sundaic populations. Likewise, the Sundaic *P. marmorata* individuals (N = 3) that differed from the Indochinese *P. marmorata* with both mtDNA and Y markers, also had a fixed distinct PmaPLP-1 haplotype, which was one nucleotide different from the Indochinese haplotype PmaPLP-2.

Population genetic structure

To further validate the Indochinese–Sundaic divergence, pairwise F_{ST} was calculated from multilocus haplotype frequency distributions with comparable population

Table 2 Haplotype and summary statistics of Y- and X-chromosome sequences

(A) *Prionailurus bengalensis* (Leopard cat)

	Y-Chromosome										X-Chromosome				
									SMCY7						
		SMCY3			*DBY7*				STR			*PLP1*			
Locus							*UTY11*	SNP							
		2	3	4		1	2		5					5	
Nucleotide position*		2	6	7	5	8	4		4			8	8	0	
Species	Haplotype	1	2	4	6	1	5	9	9	Allele size (bp)	*n*	Haplotype	0	2	0	*n*
Prionailurus bengalensis	PbeY-A-269	T	C	G	T	G	C	T	C	269	5	PbePLP-1	G	—	C	17
	PbeY-A-271	—	—	—	—	—	—	—	—	271	3	PbePLP-5	T	Δ26[†]	T	59
	PbeY-A-273	—	—	—	—	—	—	—	—	273	2					
	PbeY-B-269	—	—	—	C	—	—	—	—	269	3					
	PbeY-C-271	—	—	—	—	—	—	A	—	271	1					
	PbeY-D-271	—	—	—	—	—	—	—	T	271	2					
	PbeY-E-277	Δ1[‡]	T	—	—	T	T	A	—	277	2					
	PbeY-E-283	Δ1	T	—	—	T	T	A	—	283	2					
	PbeY-F-279	Δ1	T	A	—	T	T	A	—	279	4					
	PbeY-F-281	Δ1	T	A	—	T	T	A	—	281	1					
Number of chromosomes (*n*)	26										76				
Number of individuals (*N*)	26										53				
Number of haplotypes	10 (SNP + STR), 6 (SNP only)										2				
Nucleotide diversity π	0.00146 ± 0.000869										0.0150 ± 0.00768				
Mean number pairwise differences	3.117 ± 1.671										9.854 ± 4.560				

(B) *Pardofelis marmorata* (marbled cat)

	Y Chromosome							X Chromosome			
						SMCY7					
				UTY11		STR			*PLP1*		
Locus		*SMCY3*	*DBY7*								
				1	3				3		
Nucleotide position		3	8	5	5				9		
Species	Haplotype	9	7	1	1	Allele size (bp)	*n*	Haplotype	7	*n*	
Pardofelis marmorata	PmaY-A	A	G	C	G	257	5	PmaPLP-1	G	10	
	PmaY-B	G	A	T	C	259	3	PmaPLP-2	A	3	
Number of chromosomes (*n*)	8							13			
Number of individuals (*N*)	8							10			
Number of haplotypes	2							2			
Nucleotide diversity π	0.000999 ± 0.000705							0.000608 ± 0.000686			
Mean number pairwise differences	2.143 ± 1.327							0.385 ± 0.386			

Table 2 *Continued*

(C) *Pardofelis temminckii* (Asiatic golden cat)

	Y Chromosome				X Chromosome		
			SMCY7-STR		*PLP1*		
Locus		*SMCY3*					
		1				5	
Nucleotide position		6				4	
Species	Haplotype	8	Allele size (bp)	*n*	Haplotype	3	*n*
Pardofelis temminckii	PteY-A	A	266	24	PtePLP-1	T	37
	PteY-B	G	266	3	PtePLP-2	:	3
Number of chromosomes (*n*)	27				40		
Number of individuals (*N*)	27				33		
Number of haplotypes	2				2		
Nucleotide diversity π	0.000096 ± 0.000135				0.000225 ± 0.000369		
Mean number pairwise differences	0.205 ± 0.260				0.142 ± 0.210		

(D) *Panthera tigris* (tiger) and *Panthera pardus* (leopard)[§]

Species	*Panthera tigris*			*Panthera pardus*					
		SMCY7 STR			*SMCY3*		*SMCY7*		
Locus							SNP	STR	
					5	7	1		
Nucleotide position					2	7	7		
	Haplotype	Allele size (bp)	*n*	Haplotype	9	8	5	Allele size (bp)	*n*
	PtiY-251	251	44	PpaY-A	A	C	G	239	55
	PtiY-253	253	4	PpaY-B	—	T	A	239	18
	PtiY-unk	Unknown	7	PpaY-C	G	T	—	239	2
Number of chromosomes (*n*)	55			75					
Number of individuals (*N*)	55			75					
Number of haplotypes	2			3					
Nucleotide diversity π	0			0.000380 ± 0.000305					
Mean number pairwise differences	0			0.819 ± 0.593					

*Nucleotide position from the beginning of each nuclear DNA segment.

†*P. bengalensis* PLP1 haplotypes PbePLP-5 has a 26-bp deletion relative to PbePLP-1.

‡*P. bengalensis* Y-chromosome haplotypes PbeY-E and PbeY-F have a 1 bp deletion relative to PbeY-A, B, C and D.

§No variation was detected from the PLP1 segment in the tiger ($N = 66$, $n = 96$) or leopard ($N = 118$, $n = 167$).

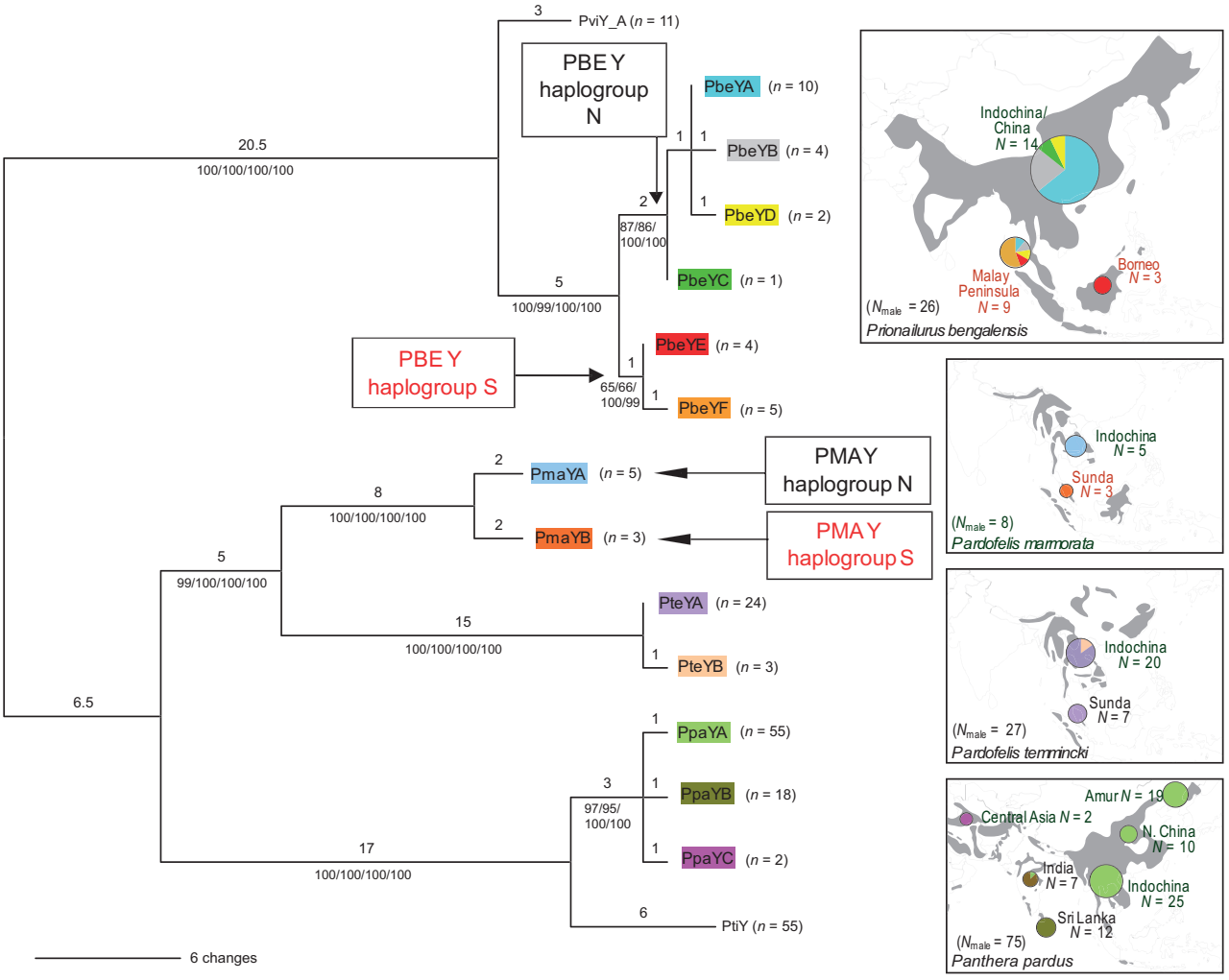

Fig. 3 Phylogenetic relationships based on maximum parsimony (MP) of Y-chromosome haplotypes defined among six Asian felid species based on 2154 bp combined Y-linked genes intronic sequences from *SMCY*, *DBY* and *UTY*. Trees derived from minimum evolution (ME), maximum-likelihood (ML) and Bayesian analyses have identical topologies. Numbers below branches represent bootstrap support in percentage from 1000 replicates using the ME, MP and ML methods, followed by posterior probabilities using Bayesian analyses (only those over 50% are indicated). Numbers above branches show the number of changes. N = North, S = South. The inset figures show the distributions and frequencies of Y haplotypes across the species range in different populations. Areas of pie charts are proportional to population sample sizes and haplotypes are colour-coded.

samples from Indochina and Sundaland (Table 3). Population structure analyses based on AMOVA with mtDNA haplotypes in *Panthera pardus* (Uphyrkina *et al.* 2001) and *P. tigris* (Luo *et al.* 2004) were compared with nuclear DNA data collected from this study and with the four other smaller felid species. The extent of population differentiation across markers with different inheritance modes was consistent with the estimated depth of divergence in various species. For example, in both *P. bengalensis* and *P. marmorata* where the Indochinese/Sundaic divergence seemed to be deep, pairwise population comparisons using mtDNA, Y- and X-chromosome markers all supported significant differentiation. In *P. temminckii* and *P. tigris*, divergence occurred more recently and north/south population distinctiveness was significant for mtDNA but not for nuclear DNA markers, which tended to have lower nucleotide diversity and generally reflected older vicariant events. No significant differentiation between leopard populations from northern Indochina and the Thai–Malay Peninsula was observed.

Estimation of TMRCA for the mtDNA and Y-chromosome haplotypes

Molecular dating was based on concatenated data sets of 1792 bp mtDNA (Fig. 5A), 2154 bp Y-chromosome DNA (Fig. 5B) and a combined mtDNA-Y data set of

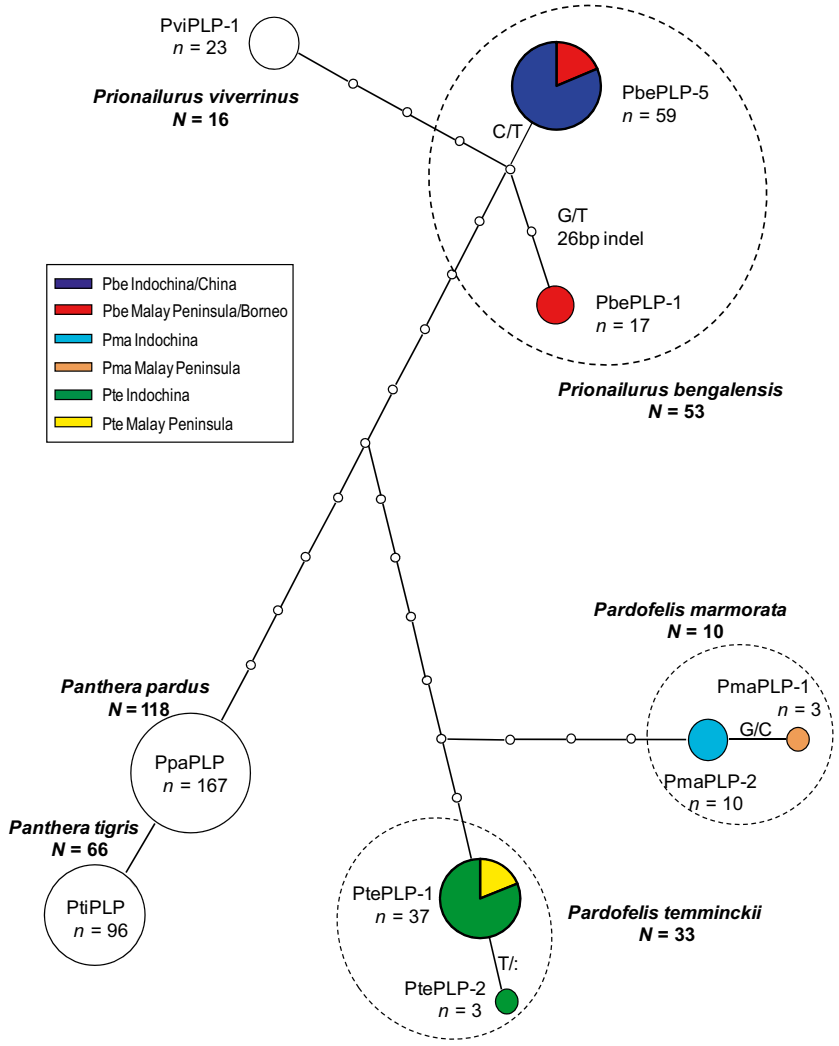

Fig. 4 Statistical parsimony network depicting relationship among nine haplotypes observed for the X-linked *PLP1* locus for six Asian felids. Each circle represents a distinct haplotype and the area is proportional to the number of individuals sharing the haplotype. The relative frequencies of each haplotype in different populations are colour-coded after adjusting different sample sizes across populations. The length of connecting lines is proportional to the exact nucleotide changes between haplotypes with each unit representing one nucleotide substitution or insertion/deletion. The 26 bp insertion in PbePLP-1 and 2 is considered one-step mutation. Missing haplotypes in the network are represented by small open circles.

3946 bp (Fig. S8B, Supporting information). When examined in TRACER, a ucld.stdev of 1.046 and a coefficient of variation of 1.202 from the four combined simultaneous runs of the mtDNA data set indicated that there was among-lineage rate heterogeneity within the mtDNA data. In contrast to Y-haplotype data, where these values were 0.222 and 0.210, respectively, depicting a clock-like data set. The estimated mean rates of the relaxed molecular clock were 0.98% and 0.097% substitutions per site per MY for mtDNA and Y-chromosome sequences. The estimated average net rates of lineage birth under a Yule speciation process were 1.151 and 0.295 per MY, respectively, showing a more rapid evolution of mtDNA compared with nuclear DNA sequences.

Estimated divergence between the Indochinese and Sundaic mtDNA haplogroups in *P. bengalensis* was 2.67 MY, while estimates of the time to the most recent common ancestor (TMRCA) for the Indochinese and Sundaic mtDNA haplogroups were 1.09 and 0.68 MY, respectively (Fig. 5A; Table 3). In the *Pardofelis* genus, the estimated TMRCA for the two divergent mtDNA lineages in marbled cats was 1.88 MY, an estimate similar to that in the leopard cat. Divergence between the Indochinese and Sundaic mtDNA haplogroups in Asiatic golden cats was more recent (1.19 MY), but nonetheless represented an event prior to the differentiation between Malayan (*P. t. jacksoni*) and northern Indochinese (*P. t. corbetti*) tigers (~72 000 years, Luo *et al.* 2004.

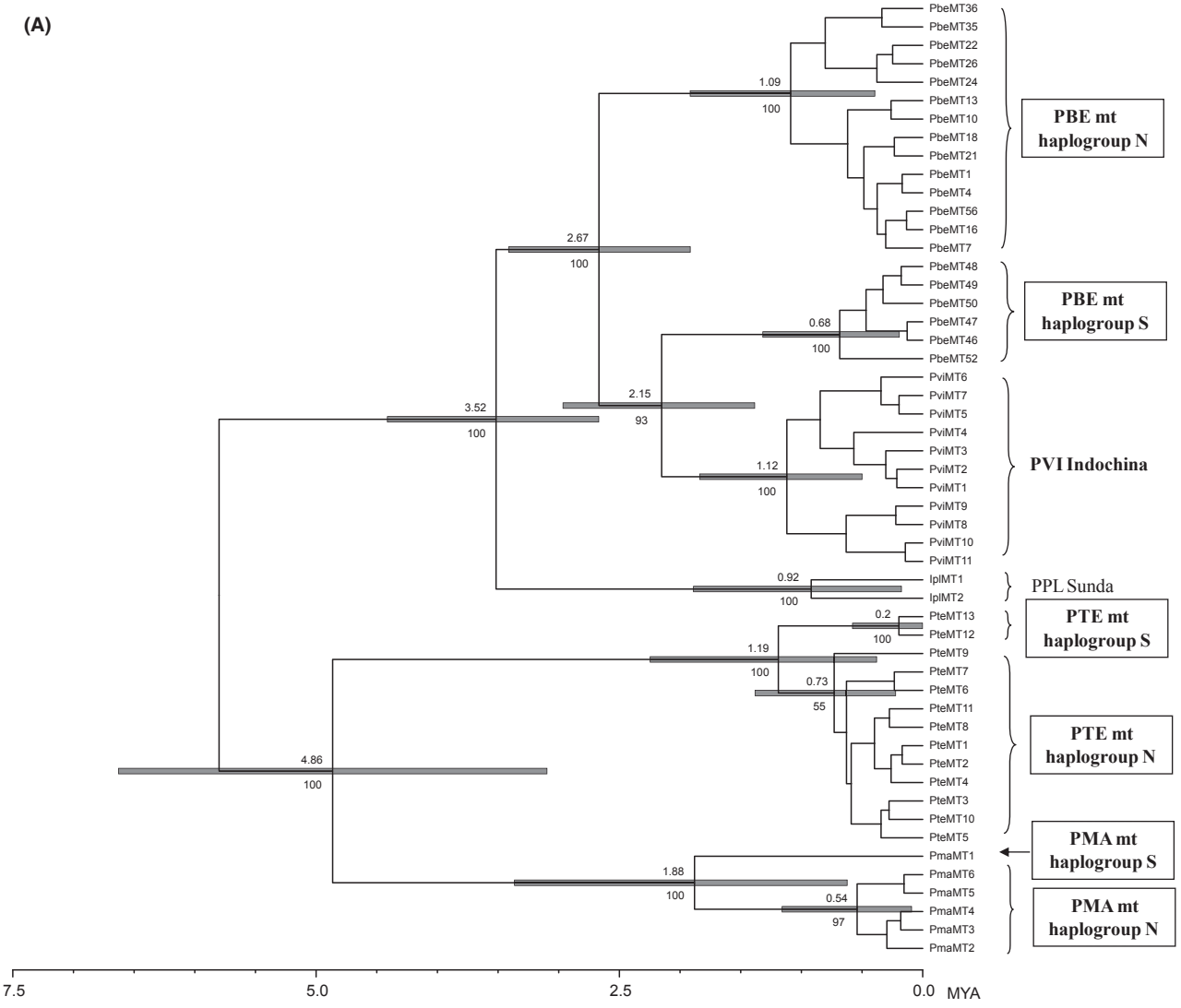

Fig. 5 Timescale of diversification among Asian felids estimated in BEAST, based on (A) mtDNA haplotypes (1792 bp) and (B) Y-chromosome haplotypes (2154 bp). Numbers below branches denote Bayesian posterior probabilities in percentage and numbers above branches are the estimated divergence in MYA, with 95% highest posterior density intervals (HPD, the shortest interval that contains 95% of the sampled values) indicated as blue node bars. The scale at the bottom represents the timescale in MYA. Haplotype names are colour-correlated to their geographic origins. A summary of time point estimates and corresponding 95% HPD intervals are presented in Table 3.

Estimates for Y haplotype TMRCA (Fig. 5B; Table 3) were more recent compared with mtDNA, likely due to selective sweeps and/or sex-biased migration patterns that reduce levels of Y-chromosome variability in mammals (Hellborg & Ellegren 2004). Consistent with matrilineal data, the estimated divergence time between the Indochinese and Sundaic Y-chromosome haplogroups was 1.36 MYA in *P. bengalensis* and 1.27 MYA in *P. marmorata*. These results imply that similar historic events may have caused vicariant divergence in both species.

The timeframe of the combined mitochondrial and Y-chromosome haplotypes was between those of the trees derived separately from mtDNA and Y sequences (Fig. S8, Supporting information; Table 3). In all cases, estimated divergence between the Indochinese and Sundaic groups of *P. bengalensis* and *P. marmorata* occurred 2 MYA, thus affirming the role of the northern and central Thai–Malay Peninsula in Indochinese–Sundaic faunal differentiation.

Test for scenarios of demographic expansions

To detect past population dynamics, mitochondrial DNA haplotypes from populations with sufficient samples (Indochinese *P. bengalensis*, Sundaic *P. bengalensis*, Indochinese *P. viverrinus* and Indochinese

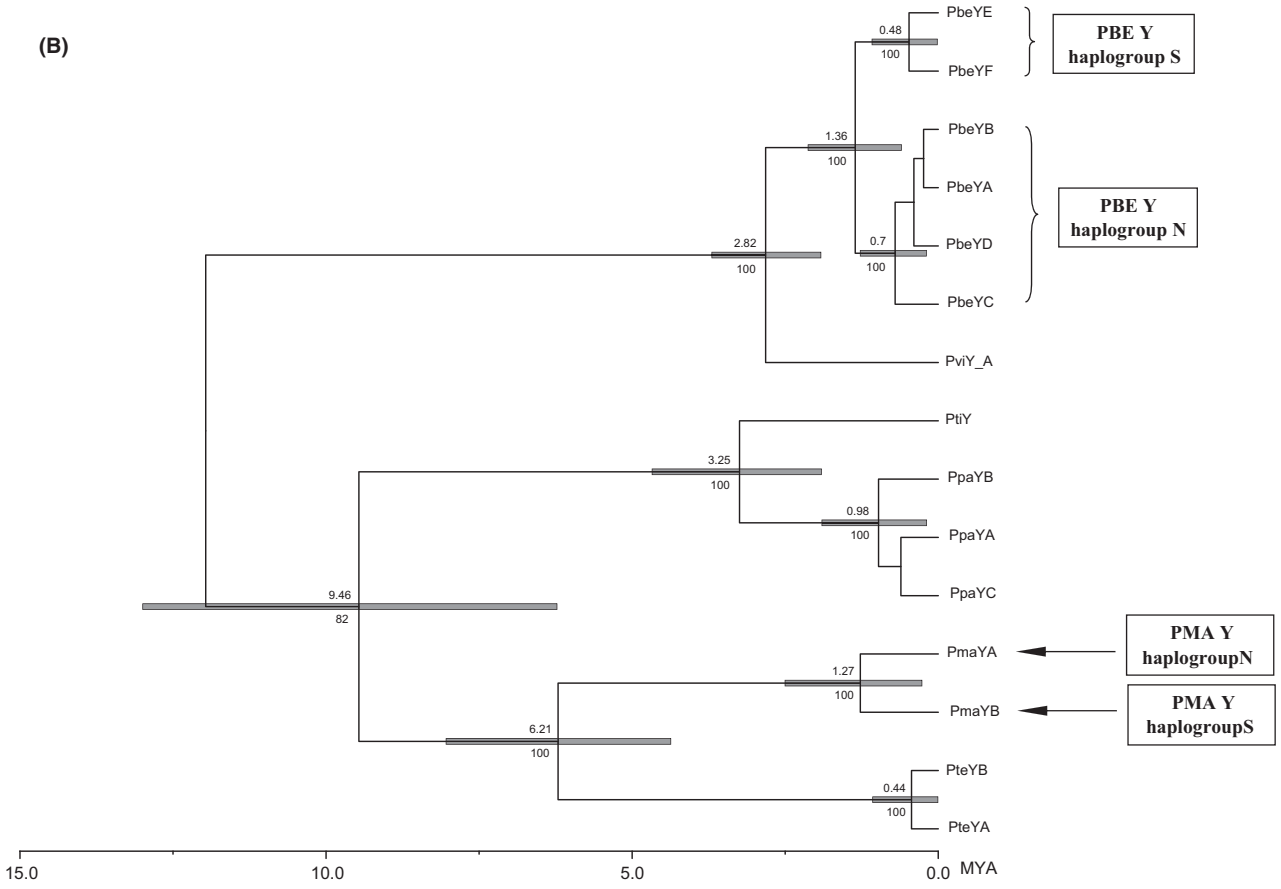

Fig. 5 *Continued*

P. temminckii) were used to construct Bayesian skyline plots (Fig. 6). The two Malayan leopard cats with northern mitochondrial haplotypes were excluded from this analysis due to species-level divergence from other Sundaic individuals. Among the four populations, Indochinese *P. temminckii* showed an apparent pattern of growth (Fig. 6C), and its mismatch distribution fitted a unimodal curve, indicating a population expansion event (Table S6, Fig. S7, Supporting information). The negative Tajima's *D* and Fu's *Fs* (Tajima's $D = -1.7962$, $p < 0.05$; Fu's $Fs = -5.9452$, $p < 0.02$) were concordant with the expansion model (Table S6, Supporting information). Similar to the Indochinese *P. temminckii* population, the skyline plot of Indochinese *P. bengalensis* showed a recent and moderate increase (Fig. 6A) but its Fu's *Fs* was not significant (Fu's $Fs = -3.146$, $p = 0.101$; Table S6, Supporting information). The population curve of Sundaic *P. bengalensis* trended downward, showing recent decline (Fig. 6B). The Indochinese *P. viverrinus* population probably maintained a relatively large and stable effective population size, judging from the plot and other estimators (Fig. 6D; Table S6, Fig. S7, Supporting information). BEAST time-aware Bayesian skyride analysis produced similar results as the Bayesian skyline plots.

Discussion

Patterns of genetic diversity in sympatric Southeast Asian felids

The four smaller cat species, *Prionailurus bengalensis*, *P. viverrinus*, *Pardofelis temminckii* and *P. marmorata*, display high nuclear and mtDNA genetic variation compared with the larger *Panthera tigris* and *P. pardus*. The tiger has very low nuclear DNA diversity, consistent with the idea that modern tigers are derived from a recent common ancestor after a genetic homogenization 72–108 kya. Only the more rapidly evolving mtDNA, microsatellites, and to a certain extent, genetic variation at the MHC, have been able to resolve tiger subspecies-level differentiation (Luo *et al.* 2004). Estimates of genetic variability in *P. bengalensis* are among the highest in Felidae, including South American small cats (Trigo *et al.* 2013), puma (Culver *et al.* 2000), jaguar (Eizirik *et al.* 2001), leopards (Uphyrkina *et al.* 2001), lions (Antunes *et al.* 2008) and tigers (Luo *et al.* 2004).

Table 3 Estimated time of divergence and coalescence of Asian cat species based on mtDNA and Y chromosome sequence variation

	Species					
	Asian leopard cat (PBE)	Fishing cat (PVI)	Asian golden cat (PTE)	Marbled cat (PMA)	Tiger (PTI)	Leopard (PPA)
MtDNA coalescent time MYA (95% HPD)						
(a) Indochinese-Sundaic pairwise F_{ST}	0.885, $P < 0.001$	n/a	0.866, $P < 0.001$	0.972, $P < 0.01$	0.797, $P < 0.001$‡	0.158, ns†
(b) Indochinese/Sundaic divergence	2.67 (1.92–3.41)	n/a*	1.19 (0.38–2.25)	1.88 (0.62–3.36)	0.072 (0.039–0.104)‡	n/a
(c) Indochinese population TMRCA	1.09 (0.39–1.92)	1.12 (0.50–1.84)	0.73 (0.22–1.38)	0.54 (0.09–1.16)	n/a	0.169 (0.120–0.218)§
(d) Sundaic population TMRCA	0.68 (0.19–1.32)	n/a	0.20 (0–0.58)	n/a		
Y-chromosome coalescent time MYA (95% HPD)						
(a) Indochinese-Sundaic pairwise F_{ST}	0.598, $P < 0.001$	n/a	0.0137, ns	1, $P < 0.05$	0, ns	0, ns
(b) Indochinese/Sundaic divergence	1.36 (0.60–2.13)	n/a	n/a	1.27 (0.27–2.50)	n/a	n/a
(c) Indochinese population TMRCA	0.70 (0.19–1.27)	n/a	n/a	n/a	n/a	0.74 (1–1.65)
(d) Sundaic population TMRCA	0.48 (0.02–1.08)					
Combined mtDNA and Y-chromosome coalescent time MYA (95% HPD)						
(b) Indochinese/Sundaic divergence	1.78 (0.95–2.67)	n/a	0.82 (0.25–1.57)	1.82 (0.60–3.19)	0.072 (0.039–0.104)‡	n/a
(c) Indochinese population TMRCA	0.74 (0.27–1.32)	0.95 (0.35–1.70)	0.51 (0.15–0.96)	0.35 (0.04–0.81)	n/a	0.169 (0.120–0.218)§
(d) Sundaic population TMRCA	0.72 (0.22–1.30)	n/a	0.13 (0–0.38)	n/a		
X chromosome						
(a) Indochinese-Sundaic pairwise F_{ST}	0.668, $P < 0.001$	n/a	0, ns	1, $P < 0.01$	0, ns	0, ns
Indochinese/Sundaic divergence?	Yes (ancient)	Yes? (empirical)	Yes (recent)	Yes (ancient)	Yes (most recent)	No

*The Indochinese–Sundaic divergence in the fishing cat is empirical because of the absence of the species from most of the Thai–Malay Peninsula but presence in Indochina, Sumatra, and Java.

†ns: not significant.

‡Data from Luo *et al.* (2004).

§Data from Uphyrkina *et al.* (2001).

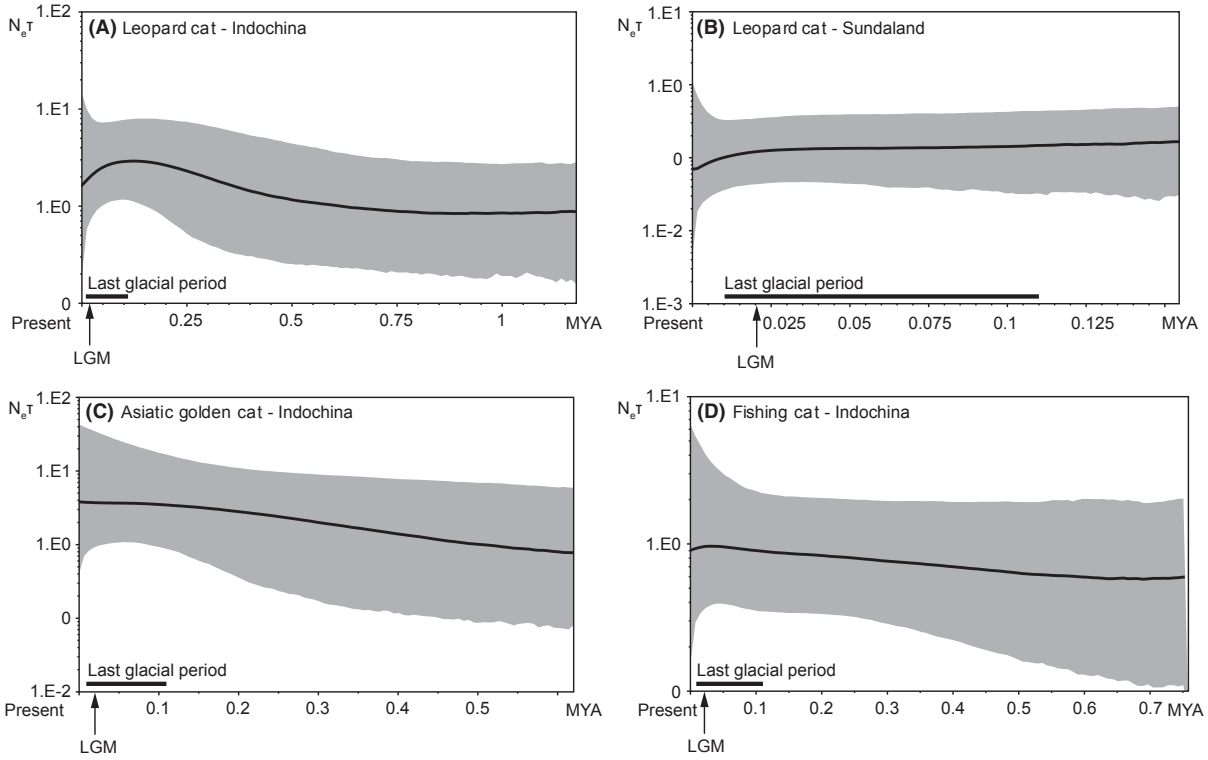

Fig. 6 Bayesian skyline plots of mtDNA sequences from Asian felids. The unit of the x axis is MYA, where the maximum time is the mean of the estimated root height. The last glacial period (c. 110–12 kya) and last glacial maximum (LGM) are marked. The y axis denotes $N_e\tau$, the product of effective population size and generation length in MY. The solid black line shows the posterior median estimate and the blue shading marks 95% highest posterior density limits. (A) Indochinese leopard cat population. (B) Sundaic leopard cat population (Thai-Malay Peninsula and Borneo combined). (C) Indochinese Asiatic golden cat population. (D) Indochinese fishing cat population.

Very large intraspecific Y-chromosome genetic variations and lineage divergence in *Prionailurus bengalensis* and *Pardofelis marmorata* have not been documented thus far in other wild felids. Levels of nucleotide diversity in mammalian Y chromosomes are generally low (20% of that in autosomes). In a survey of 3.5 kb of Y-linked gene intronic sequences in the Eurasian lynx (*Lynx lynx*), no variable sites were found (Hellborg & Ellegren 2004). Only one haplotype was observed from 1322 bp of *SRY* from 357 widely distributed African lions (Antunes *et al.* 2008). The abundant genetic variability in these Southeast Asian small cat species might reflect larger effective population sizes over a long period of time, which has allowed diversity within a population to accumulate and subsequent population substructure to develop over geographic locales or maybe due to the absence of recent selective sweeps.

Disentangling ancient vicariant divergence and recent introgression

Multiple independent loci provide a more complete understanding of the underlying evolutionary and demographic processes of conspecific populations. However, comparisons across paternal, maternal and biparental molecular lineages require careful considerations. The phylogeographical concordance of two highly distinct lineages in the leopard cat and marbled cat measured here in mtDNA, Y-chromosome and X-linked markers, is strong evidence of a significant and deep ancient divergence within each species.

The hybrid zone on the Thai–Malay Peninsula for the leopard cat following recent secondary contact is likely male-driven, as about 30% of the Y haplotypes (PbeY-A, B and D) are shared among Malayan and Indochinese populations (Fig. 3). To a lesser extent, two Sundaic leopard cats carry the 'Indochinese' mtDNA haplotypes (Pbe108 and Pbe112, Fig. 2A), but none from the Indochinese region carries any 'Sundaic' mtDNA haplotype. Evidence from both the maternal and paternal heritages is consistent with the hypothesis that introgression was more likely to be from north to south, reflecting recent expansion of the Indochinese population into central and southern parts of the Thai-Malay Peninsula.

Significant intraspecific Indochinese–Sundaic divergence

Of the six felids examined, all except the leopard have phylogeographical structures differentiated between Indochina and Sundaland (Table 3). Our sample size of the marbled cat was small (N = 16), but nevertheless revealed two highly divergent lineages (Figs 2A and 3) comparable to interspecific divergence between other Felidae species, indicating that the pattern is prominent and would very likely be confirmed with larger sampling. The depth of divergence is older in the leopard cat and marbled cat than in the Asiatic golden cat, and most recent in the tiger (Table 3).

In the leopard cat, if post-LGM (<10 000 years) isolation caused the divergence observed among modern populations, the Malayan and Indochinese populations would be more similar genetically, as a result of geographic connectivity between the regions throughout the Pleistocene and Holocene. By contrast, the majority of genetic markers support a closer relationship between Malayan and Bornean lineages than between Malayan and Indochinese groups. Similar patterns are also observed in gymnures (Ruedi & Fumagalli 1996), primates (Roos *et al.* 2008; Meyer *et al.* 2011) and rodents (Gorog *et al.* 2004). This concordance among multiple, unrelated taxa is best explained by an ancient vicariance event that separated the Indochinese and Sundaic fauna on the Thai–Malay Peninsula, followed by the differentiation of Sunda island populations.

In the upper Miocene (5–10 MYA), the Greater Sunda Islands were connected with Indochina by the Thai–Malay Peninsula during several periods of reduced eustatic sea levels (Woodruff 2003; Woodruff & Turner 2009). Palynological records from this period found a predominance of pollens from rain forest species and a perhumid or extremely moist climate (Hall 1998). This environment apparently prompted diversification and migration in many mammals, including radiation of the ancestors of modern felids from Southeast Asia into unoccupied niches across continents (Johnson *et al.* 2006). This tropical environment persisted until the early to mid-Pliocene.

Large-scale environmental alterations around the Pliocene–Pleistocene turnover (2.5–2.6 MYA) may have provided a mechanism of isolation facilitating the speciation of *Prionailurus bengalensis* and *P. viverrinus* as well as the intraspecific Indochinese–Sundaic lineage divergence in *P. bengalensis* (Fig. 5). Such speciation and lineage divergence events coincided with the onset of the northern hemisphere glacial cycles due to ice volume and Indian summer monsoon change (Zhisheng *et al.* 2011) and a marked increase in aridification as shown from sediment cores (Kashiwaya *et al.* 2001). Intriguingly, Indochinese–Sundaic lineage divergence in *Pardofelis marmorata* and coalescence times for some lineages within these species (i.e. Indochinese *P. bengalensis*, Indochinese *P. viverrinus* and *Pardofelis temminckii*; Fig. 5) were also associated with climate shifts of Indian summer monsoon at 1.8 and 1.1 MYA, respectively (Zhisheng *et al.* 2011), suggesting the potential influence of climate changes on Indochinese-Sundaic vacariance.

At a regional level, the numerous rapid sea level rises driven by climate dynamics could have directly contributed to the significant faunal differentiations between Indochina and Sundaland (Lisiecki & Raymo 2005; Miller *et al.* 2005; Woodruff & Turner 2009). Although the revised eustatic curve suggests the Isthmus of Kra along the Thai–Malay Peninsula was never submerged for notable periods of time during the Pliocene and Pleistocene as previously believed, a series of sea level fluctuations, or 10 rapid rises of >80 m and 48 rises of 40–80 m in the last 5 MY (Woodruff & Turner 2009), may have effectively isolated population. A 40 m rise from the present-day sea level would have reduced by half the width of the Peninsula and the area of habitat available. This occurred repeatedly, compressing the fauna on the narrow Peninsula and resulting in differentiation of the populations north and south of the impacted central Peninsula. As a consequence, the central Thai–Malay Peninsula may have acted as a biogeographic barrier shaping regional fauna diversification patterns in both *Prionailurus bengalensis* and *Pardofelis marmorata*. Such allopatric divergence was likely sustained by repeated isolation events associated with forest communities, river systems, habitat dynamics and/or sea level rises throughout the Pleistocene (Cannon *et al.* 2009).

Our data suggest that following the return of suitable climate after the last glacial maximum, the Indochinese leopard cat population expanded and formed a secondary contact zone with the Sundaic population on the Thai–Malay Peninsula. Dispersal across two bioregions is not surprising, as leopard cats are highly adaptable and commonly inhabit dense secondary forest, logged areas, rural agricultural land or the suburbs of cities (Nowell & Jackson 1996). The Thai–Malay Peninsula is likely a contemporary contact zone where the two previously diverged Indochinese and Sundaic lineages met, and a mixture of both 'northern' and 'southern' genetic signatures were observed. The direction of introgression may have been southbound, as Indochinese signature alleles from several loci are observed in the Malayan population, but Sundaic haplotypes or alleles are absent in the Indochinese population (Fig. 2A, Fig. 3, and Fig. 4). Similarly, a morphology study also confirmed co-occurrence in the Thai–Malay peninsula of a more ochre and 'Sumatran-colouring' pelage as well as

a less common 'light fawn' Indochinese pelage (Groves 1997). In the marbled cat, however, little gene flow was observed between the extant Indochinese and Sundaic lineages, indicating different evolutionary histories in responses to environmental shifts.

The Thai–Malay Peninsula is thought to represent a recent geographic break in tigers (Luo *et al.* 2004). Since population differentiation occurred at a much more recent timescale (72 000–108 000 years), it is probably unrelated with the above-mentioned ancient vicariance. A plausible explanation for tiger subspecies differentiation is ecological isolation of the ancestral population of the Malayan tiger from Indochina, reinforced by genetic drift in a relatively small population that has led to recognizable subdivisions among otherwise closely related populations (Luo *et al.* 2004).

Implications to felid taxonomy and conservation

The leopard cat (*P. bengalensis*) is considered the most common wild cat in Asia. Based on morphology, several distinct island subspecies have been described, including *P. b. iriomotensis* from Japan's Iriomote island (Masuda & Yoshida 1995), *P. b. borneoensis* in Borneo, *P. b. heaneyi* in Palawan, *P. b. javenensis* in Java and Bali, *P. b. rabori* in the Philippine islands of Negros, Cebu and Panay, and *P. b. sumatranus* in Sumatra and the offshore island of Tebingtinggi (Groves 1997). Although one mainland Asian subspecies is generally recognized, the nominotypical *P. b. bengalensis*, some mainland subspecies have been proposed, notably the Amur leopard cat (*P. b. euptilurus*) of the Korean Peninsula, Russian Far East and northeastern China (Groves 1997). In our study, genetic patterns between Indochinese and Sundaic leopard cats were distinct and supported a 2 MY divergence between the two groups, an estimate comparable to the speciation time between leopard cat and fishing cat (1.74–3.82 MYA; Johnson *et al.* 2006).

The marbled cat (*Pardofelis marmorata*) is classified by the IUCN Red List as vulnerable with a total population size below 10 000 mature breeding animals and in decline (Grassman *et al.* 2008). Two subspecies are currently recognized, *P. m. marmorata* in tropical Southeast Asia and *P. m. charltoni* in Nepal. Our study has revealed two monophyletic lineages within *P. m. marmorata* that diverged over one million years ago with extremely limited gene flow. As with the leopard cat, the genetic distance supports a differentiation of these two lineages into distinct species, and more samples originating from the Sundaland will be important to further elucidate this distinction.

While the most common coloration in the Asiatic golden cat is fox-red to golden brown, pelages, which are black, brown, grey or with a distinctive rosette-spotted pattern, have been reported (Nowell & Jackson 1996). Pocock (Pocock 1939) classified the spotted Asiatic golden cat, thus far reported only from southwest China, as the subspecies *P. t. tristis.* Two other subspecies, *P. t. temminckii* from Himalaya, mainland Southeast Asia and Sumatra, and *P. t. dominicanorum* from southeast China, have been proposed. The Asiatic golden cat samples in our study were collected across southwest China and Southeast Asia and included three distinct pelages (melanistic, rosette-spotted and golden brown) that showed no correlation to genetic distinctiveness as identified with neutral markers. For instance, the spotted Asiatic golden cat is not genetically distinguishable from other individuals in southwest China and thus such a coat pattern may represent merely a local variant rather than a fixed character for subspecies identification. Genetic distinction between populations from northern Indochina and the Thai–Malay Peninsula was evident in mtDNA lineages (1.19 MY), a recent divergence compared with that found in *P. bengalensis* and *P. marmorata,* but ancient compared with Malay–Indochina divergence in the tiger (<0.072 MY). However, such distinctiveness is not supported by nuclear DNA markers, which can be interpreted as male-biased migration that has facilitated contemporary gene flow. Based on these findings, there is modest evidence supporting subspecies differentiation within the examined range of *P. temminckii*.

Genetic investigation of leopards from Southeast Asia did not reveal population subdivision between northern Indochina and the Thai–Malay Peninsula, supporting the *P. p. delacouri* subspecies classification. The eight leopards of the Thai–Malay Peninsula showed reduced genetic variability and were not polymorphic across all nuclear or mtDNA markers examined.

The recent, rapid radiations in Felidae were accompanied by complex processes involving isolation, divergence, re-emergence and even hybridization among closely-related species (Trigo *et al.* 2013). Our study has uncovered a significant aspect of Indochinese–Sundaic divergence during Southeast Asian felid evolution, but questions remain regarding the extent of historic genetic connectivity within Sundaland. Based on morphological studies, Malayan leopard cats display more similarity with Sumatran individuals than with Bornean ones (Groves 1997). Biogeography studies even show Indochinese affinities in several Sumatran fauna, as the northern part of the island was contiguous with southern Thailand during times of low sea level (Woodruff & Turner 2009). More samples from Sundaland, particularly Sumatra, Java and Borneo, will be important to understand the complete biogeographic and evolutionary history of Southeast Asian fauna. Upon further validation with morphological evidence and large sampling, the

potential elevation of some subspecies in *Prionailurus bengalensis* and *Pardofelis marmorata* to species level would seem warranted, in light of the recent proposition of the new felid species *Leopardus guttulus* from Brazil (Trigo *et al.* 2013). We encourage the conservation community to consider geographic separation and genetic divergence in these wild felids when defining conservation units and establishing effective strategies to preserve biodiversity in Southeast Asia.

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References

Antunes A, Troyer JL, Roelke ME *et al.* (2008) The evolutionary dynamics of the lion *Panthera leo* revealed by host and viral population genomics. *Plos Genetics*, **4**, e1000251.

de Bruyn M, Nugroho E, Hossain MM, Wilson JC, Mather PB (2005) Phylogeographic evidence for the existence of an ancient biogeographic barrier: the Isthmus of Kra Seaway. *Heredity*, **94**, 370–378.

Buckley-Beason VA, Johnson WE, Nash WG *et al.* (2006) Molecular evidence for species-level distinctions in clouded leopards. *Current Biology*, **16**, 2371–2376.

Cannon CH, Morley RJ, Bush ABG (2009) The current refugial rainforests of Sundaland are unrepresentative of their biogeographic past and highly vulnerable to disturbance. *Proceedings of the National Academy of Sciences USA*, **106**, 11188–11193.

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9**, 1657–1659.

Corbet GB, Hill JE (1992) *The Mammals of the Indo-Malayan Region: A Systematic Review* Oxford University Press for British Museum (Natural History), Oxford.

Corbet AS, Pendlebury HM (1992) *The Butterflies of the Malay Peninsula (Revised By J. N. Eliot)*, 4th edn. Malay Nature Society, Kuala Lumpur.

Culver M, Johnson WE, Pecon-Slattery J, O'Brien SJ (2000) Genomic ancestry of the American puma (*Puma concolor*). *Journal of Heredity*, **91**, 186–197.

Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, 214.

Eizirik E, Kim JH, Menotti-Raymond M *et al.* (2001) Phylogeography, population history and conservation genetics of jaguars (*Panthera onca, Mammalia, Felidae*). *Molecular Ecology*, **10**, 65–79.

Emerson SB, Inger RF, Iskandar D (2000) Molecular systematics and biogeography of the fanged frogs of Southeast Asia. *Molecular Phylogenetics and Evolution*, **16**, 131–142.

Excoffier L, Lischer HE (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, **10**, 564–567.

Gorog AJ, Sinaga MH, Engstrom MD (2004) Vicariance or dispersal? Historical biogeography of three Sunda shelf murine rodents (*Maxomys surifer*, *Leopoldamys sabanus* and *Maxomys whiteheadi*). *Biological Journal of the Linnean Society*, **81**, 91–109.

Grassman L, Sanderson J, Hearn A *et al.* (2008) *Pardofelis marmorata*. In: IUCN 2013. IUCN Red List of Threatened Species. Version 2013.1.

Groves CP (1997) Leopard-cats, *Prionailurus bengalensis* (*Carnivora*: *Felidae*) from Indonesia and the Philippines, with the description of two new subspecies. *Zeitschrift Fur Saugetierkunde-International Journal of. Mammalian Biology*, **62**, 330–338.

Hall R (1998) The plate tectonics of Cenozoic SE Asia and the distribution of land and sea. In: *Biogeography and Geological Evolution of SE Asia*(eds Holloway JD & Hall R), pp. 99–131. Backhuys, Leiden.

Hellborg L, Ellegren H (2004) Low levels of nucleotide diversity in mammalian Y chromosomes. *Molecular Biology and Evolution*, **21**, 158–163.

Hewitt G (2000) The genetic legacy of the Quaternary ice ages. *Nature*, **405**, 907–913.

Hughes JB, Round PD, Woodruff DS (2003) The Indochinese-Sundaic faunal transition at the Isthmus of Kra: an analysis of resident forest bird species distributions. *Journal of Biogeography*, **30**, 569–580.

Inger RF, Voris HK (2001) The biogeographical relations of the frogs and snakes of Sundaland. *Journal of Biogeography*, **28**, 863–891.

Janecka JE, Helgen KM, Lim NTL *et al.* (2008) Evidence for multiple species of Sunda colugo. *Current Biology*, **18**, R1001–R1002.

Johnson WE, Eizirik E, Pecon-Slattery J *et al.* (2006) The late Miocene radiation of modern Felidae: a genetic assessment. *Science*, **311**, 73–77.

Kashiwaya K, Ochiai S, Sakai H, Kawai T (2001) Orbit-related long-term climate cycles revealed in a 12-Myr continental record from Lake Baikal. *Nature*, **410**, 71–74.

Kitchener AC, Beaumont MA, Richardson D (2006) Geographical variation in the clouded leopard, *Neofelis nebulosa*, reveals two species. *Current Biology*, **16**, 2377–2383.

Lisiecki LE, Raymo ME (2005) A Pliocene–Pleistocene stack of 57 globally distributed benthic $\delta^{18}O$ records. *Paleoceanography*, **20**, PA1003, doi:1010.1029/2004PA001071

Luo SJ, Kim JH, Johnson WE *et al.* (2004) Phylogeography and genetic ancestry of tigers (*Panthera tigris*). *Plos Biology*, **2**, e2442.

Luo SJ, Johnson WF, David VA *et al.* (2007) Development of Y chromosome intraspecific polymorphic markers in the Felidae. *Journal of Heredity*, **98**, 400–413.

Masuda R, Yoshida MC (1995) Two Japanese wildcats, the Tsushima cat and the Iriomote cat, show the same mitochondrial DNA lineage as the leopard cat *Felis bengalensis*. *Zoological Science*, **12**, 655–659.

Meijaard E, van der Zon APM (2003) Mammals of south-east Asian islands and their Late Pleistocene environments. *Journal of Biogeography*, **30**, 1245–1257.

Melisch R (1995) Early record of fishing cat in Peninsular Malaysia. *Cat News*, **22**, 19.

Meyer D, Rinaldi ID, Ramlee H *et al.* (2011) Mitochondrial phylogeny of leaf monkeys (genus *Presbytis*, Eschscholtz, 1821) with implications for taxonomy and conservation. *Molecular Phylogenetics and Evolution*, **59**, 311–319.

Miller KG, Kominz MA, Browning JV *et al.* (2005) The Phanerozoic record of global sea-level change. *Science*, **310**, 1293–1298.

Miththapala S, Seidensticker J, O'Brien SJ (1996) Phylogeographic subspecies recognition in leopards (*Panthera pardus*): molecular genetic variation. *Conservation Biology*, **10**, 1115–1132.

Molengraaff GAF, Weber M (1921) On the relation between the Pleistocene glacial period and the origin of the Sunda Sea (Java- and South China-Sea), and its influence on the distribution of coralreefs and on the land- and freshwater fauna. *Proceedings of the Section of Sciences*, **23**, 395–439. [English translation].

Moritz C, Patton JL, Schneider CJ, Smith TB (2000) Diversification of rainforest faunas: an integrated molecular approach. *Annual Review of Ecology and Systematics*, **31**, 533–563.

Mukherjee S, Krishnan A, Tamma K *et al.* (2010) Ecology driving genetic variation: a comparative phylogeography of jungle cat (*Felis chaus*) and leopard cat (*Prionailurus bengalensis*) in India. *PLoS ONE*, **5**, e13724.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GA, Kent J (2000) Biodiversity hotspots for conservation priorities. *Nature*, **403**, 853–858.

Nowell K, Jackson P (1996) *Wild Cats: Status Survey and Conservation Action Plan IUCN-World Conservation Union*. Gland, Switzerland.

Nylander JA, Wilgenbusch JC, Warren DL, Swofford DL (2008) AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. *Bioinformatics*, **24**, 581–583.

Pocock RI (1939) *The Fauna of British India Including Ceylon And Burma*. Taylor and Francis Ltd., London.

Posada D (2008) jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution*, **25**, 1253–1256.

Rambaut A, Drummond A (2009) *Tracer: MCMC Trace Analysis Tool*. University of Oxford, Oxford.

Rogers AR, Harpending H (1992) Population-growth makes waves in the distribution of pairwise genetic-differences. *Molecular Biology and Evolution*, **9**, 552–569.

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572–1574.

Roos C, Nadler T, Walter L (2008) Mitochondrial phylogeny, taxonomy and biogeography of the silvered langur species group (*Trachypithecus cristatus*). *Molecular Phylogenetics and Evolution*, **47**, 629–636.

Ruedi M, Fumagalli L (1996) Genetic structure of Gymnures (genus *Hylomys*; *Erinaceidae*) on continental islands of Southeast Asia: historical effects of fragmentation. *Journal of Zoological Systematics and Evolutionary Research*, **34**, 153–162.

Swofford DL (2001) *PAUP* Phylogenetic Analysis Using Parsimony and Other Methods*. Computer Program, Sinauer, Sunderland, MA.

Tamada T, Siriaroonrat B, Subramaniam V *et al.* (2008) Molecular diversity and phylogeography of the Asian leopard cat, *Felis bengalensis*, inferred from mitochondrial and Y-chromosomal DNA sequences. *Zoological Science*, **25**, 154–163.

Tosi AJ, Morales JC, Melnick DJ (2002) Y-chromosome and mitochondrial markers in *Macaca fascicularis* indicate introgression with Indochinese *M. mulatta* and a biogeographic barrier in the Isthmus of Kra. *International Journal of Primatology*, **23**, 161–178.

Tougard C (2001) Biogeography and migration routes of large mammal faunas in South-East Asia during the Late Middle Pleistocene: focus on the fossil and extant faunas from Thailand. *Palaeogeography Palaeoclimatology Palaeoecology*, **168**, 337–358.

Trigo TC, Schneider A, de Oliveira TG *et al.* (2013) Molecular data reveal complex hybridization and a cryptic species of Neotropical wild cat. *Current Biology*, **23**, 2528–2533.

Uphyrkina O, Johnson WE, Quigley H *et al.* (2001) Phylogenetics, genome diversity and origin of modern leopard, *Panthera pardus*. *Molecular Ecology*, **10**, 2617–2633.

Voris HK (2000) Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. *Journal of Biogeography*, **27**, 1153–1167.

Wallace AR (1876) *The Geographical Distribution of Animals*. Macmillan, London.

Wilting A, Buckley-Beason VA, Feldhaar H *et al.* (2007) Clouded leopard phylogeny revisited: support for species recognition and population division between Borneo and Sumatra. *Frontiers in Zoology*, **4**, 15.

Woodruff DS (2003) Neogene marine transgressions, palaeogeography and biogeographic transitions on the Thai-Malay Peninsula. *Journal of Biogeography*, **30**, 551–567.

Woodruff DS, Turner LM (2009) The Indochinese-Sundaic zoogeographic transition: a description and analysis of terrestrial mammal species distributions. *Journal of Biogeography*, **36**, 803–821.

Zhisheng A, Clemens SC, Shen J *et al.* (2011) Glacial-interglacial Indian summer monsoon dynamics. *Science*, **333**, 719–723.

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Data accessibility

DNA sequences: GenBank Accessions KF754862-KF755040; Final DNA sequence assemblies and tree files uploaded as online supplemental materials (Appendix S2–S8).

Supporting information

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

Appendix S1 Supplementary Methods, Tables and Figures.

Appendix S2 MtDNA sequence alignment for Fig. 2(A), Fig. 5 (A) and Fig S1.

Appendix S3 Y-chromosome sequence alignment for Fig. 3, Fig. 5(B) and Fig. S2.

Appendix S4 *PLP1* sequence alignment for Fig. 4.

Appendix S5 Combined mtDNA and Y chromosome sequence alignment for Fig. S8.

Appendix S6 Phylogenetic tree file for Fig. 5(A).

Appendix S7 Phylogenetic tree file for Fig 5(B).

Appendix S8 Phylogenetic tree file for Fig. S8(B).

Table S1 Samples included in this study, with geographic origins, sources, names and genetic information.

Table S2 Summary of wild cat specimens used in this study.

Table S3 Mitochondrial (mtDNA) and nuclear loci used in this study.

Table S4 Summary of mtDNA and Y haplotypes of the individuals used in the partitioned phylogenetic analyses.

Table S5 Summary of variable sites in the nuclear DNA segments within felid species.

Table S6 Results of mismatch distribution and estimates of Tajima's D and Fu's Fs of the mtDNA sequences in each felid population: Indochinese *Prionailurus bengalensis*, Sundaic *P. bengalensis,* Indochinese *P. viverrinus* and Indochinese *Pardofelis temminckii*.

Fig. S1 MtDNA phylogenetic relationships (1792 bp) among *Prionailurus* spp. and *Pardofelis* spp. estimated in PAUP with maximum-likelihood method.

Fig. S2 Phylogenetic relationships (2154 bp) among Y-chromosome haplotypes of *Prionailurus* spp., *Pardofelis* spp. and *Panthera* spp. estimated in PAUP with maximum-likelihood method.

Fig. S3 Phylogenetic relationships among mtDNA haplotype (1792 bp) of *Prionailurus* spp. estimated in PAUP with maximum parsimony (MP), minimum evolution (ME) and maximum-likelihood (ML) methods (the MP tree is shown).

Fig. S4 Phylogenetic relationships among mtDNA haplotypes (1220 bp) of *Pardofelis* spp. estimated in PAUP with maximum parsimony (MP), minimum evolution (ME) and maximum-likelihood (ML) methods (without missing data).

Fig. S5 Cumulative AWTY plots for diagnostics of output from MRBAYES analyses with mitochondrial haplotypes, with 25% as the fraction of burn-in.

Fig. S6 AWTY bivariate plot of the split frequencies for comparison between paired Bayesian MCMC simulations from MRBAYES analyses with mitochondrial haplotypes, with 25% as the fraction of burn-in.

Fig. S7 Mismatch distribution analyses of mtDNA sequences.

Fig. S8 Phylogenetic analysis and TMRCA estimates in *Prionailurus* spp. and *Pardofelis* spp. based on 3946-bp combined mtDNA and Y-chromosome haplotypes.