

Genetic diversity of captive binturongs (*Arctictis binturong*, Viverridae, Carnivora): implications for conservation

L. Cosson¹, L. L. Grassman Jr.², A. Zubaid³, S. Vellayan⁴, A. Tillier⁵ & G. Veron¹

1 Muséum National d'Histoire Naturelle, Département Systématique et Evolution, Paris, France

2 Caesar Kleberg Wildlife Research Institute, Texas A&M University-Kingsville, Kingsville, TX, USA

3 School of Environmental & Natural Resource Sciences, Faculty of Science & Technology, University Kebangsaan Malaysia, Bangi, Malaysia

4 Zoo Negara, Malaysia Zoological Society, Hulu Kelang, Selangor Darul Ehsan, Malaysia

5 Muséum National d'Histoire Naturelle, Département Systématique et Evolution, Paris, France

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Correspondence

Géraldine Veron, Muséum National d'Histoire Naturelle, Département Systématique et Evolution, CP 51, OSEB, USM 601-UMR 5202 CNRS, 57, rue Cuvier, 75231 Paris Cedex 05, France.
Email: veron@mnhn.fr

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Abstract

The binturong *Arctictis binturong* is a threatened carnivore (Mammalia) that ranges throughout the forests of South-east Asia. This study evaluates the genetic diversity of captive binturong populations in European zoos and attempts to assess their geographic origin. We sequenced the hypervariable region 1 of the mitochondrial control region of 56 binturongs, among which 20 had a known geographic origin. We showed that at least two distinct geographic clades exist and were able to assess the geographic clade to which captive individuals belong. Moreover, a low genetic diversity was observed among the captive population of European zoos. Although our results are preliminary, zoo managers should consider the evolutionary significant units identified by this study, and which correspond to recognized sub-species, when planning binturong reproduction programs.

Introduction

The binturong *Arctictis binturong* (Raffles, 1821) is the largest member of the family Viverridae (Pocock, 1933). Its black fur is long and coarse and its tail is particularly muscular at the base and prehensile at the tip. The only other carnivore with a truly prehensile tail is the kinkajou, which the binturong resembles in habits to some extent. The binturong lives in dense forests and is mainly arboreal and nocturnal. It is reported to dive, swim and catch fish. The diet also includes birds, carrion, leaves and principally fruits (Nowak, 1991). It was originally described by Raffles in 1821 and nine sub-species have been described on the basis of pelage color and body size variation (Table 1; Pocock, 1933).

In spite of its large distribution throughout South-east Asia (see Fig. 1), the binturong is uncommon on the mainland and is rare on the Indonesian islands of Java, Sumatra, Nias, Riau and the Bangka islands (Yossa *et al.*, 1991). The sub-species *Arctictis binturong whitei* (Palawan, Philippines) is listed as Vulnerable on the Red List of threatened species (IUCN, 2004). The destruction of primary rainforest represents the greatest threat for this highly arboreal species. Moreover, binturongs are targeted by hunting, notably in Palawan island (Quinnell & Balmford, 1988), and protection measures are often inappropriate for this species (Choudh-

ury, 2000). Binturongs are essential for the maintenance of forest ecosystems. They are keystone species as seed dispersers, with digestive enzymes capable of softening the seed coat of the strangler fig *Ficus* spp. and other fruiting species (CPT, 1997).

Binturongs are common in zoos and captive individuals represent a source of genetic diversity essential for long-term conservation, provided *ex situ* reproduction is managed taking this variation into consideration. The IUCN/SSC Mustelid and Viverrid Specialist Group recognizes that captive breeding is a powerful tool in ensuring the survival of endangered species, notably when the protection of wild animals and their natural habitats is not successful (Schreiber *et al.*, 1989). However, in captivity the geographic origin of animals is usually unknown for several reasons. Typically, zoo animals have unknown geographic origin or are the offspring of several generations of captive-bred animals with no information on the geographic origin of the founders.

We considered two points relevant to binturong conservation: (1) What is the genetic diversity of the captive population in Europe? (2) Can we identify geographic clades of which the genetic identity needs to be preserved?

This study evaluated the genetic diversity of captive binturongs, and attempted to identify their geographic origin, using a phylogeographic approach. For this purpose,

we sequenced a portion of mitochondrial DNA (the hyper-variable region 1 of the D-loop). Whereas the cytochrome *b* gene has been used extensively for previous intraspecific studies in carnivores (Veron *et al.*, 2003), the D-loop evolves three to five times faster in mammals than the average rate of mitochondrial DNA sequences (Vigilant *et al.*, 1989; Horai & Hayasaka, 1990) and has been used for intraspecific

systematics and phylogeography (Salguiero *et al.*, 2004; Durka *et al.*, 2005; Iyengar *et al.*, 2005).

This study provides a new data set of sequences of this mitochondrial gene for 56 captive-bred binturongs, including 20 individuals with known geographic origin and two wild-caught binturongs.

Materials and methods

Sample collection

Fifty-six binturong samples were obtained for this study (see Table 2), including two wild-caught individuals from Thailand. Twenty had a known geographic origin (information came from the ISIS database or was provided by zoos). The masked palm civet *Paguma larvata* was used as the outgroup according to Veron & Heard (2000).

DNA extraction and sequencing

DNA was extracted using the CTAB method (cetyltrimethylammonium bromide) (Winneppenninckx, Bäckeljau & De Wachter, 1993). Polymerase chain reactions (PCRs) were conducted in a reaction volume of 25 μ L using TAQ polymerase (Qbiogen, Illkirch, France). Amplifications were run in a thermal cycler with a typical profile of 35 cycles, with each cycle consisting of 30 s at 94 °C, 40 s at 51 °C and 40 s at 72 °C for the denaturation, annealing and extension steps, respectively. The last cycle was followed by a further 7 min extension at 72 °C.

Table 1 Binturong *Arctictis binturong* sub-species described since 1821 (Pocock, 1933; Wozencraft, 1993)

Sub-species	Type locality
<i>Arctictis binturong binturong</i> (Raffles, 1821)	Malacca (Malaysia)
<i>Arctictis binturong albifrons</i> (Cuvier, 1822)	Tonkin (north Vietnam)
<i>Arctictis binturong whitei</i> (Allen, 1910)	Palawan (Philippines)
<i>Arctictis binturong pageli</i> (Schwarz, 1911)	Sandakan (north Borneo)
<i>Arctictis binturong gairdneri</i> (Thomas, 1916)	Siam (north Thailand)
<i>Arctictis binturong niasensis</i> (Lyon, 1916)	Nias island (west Sumatra)
<i>Arctictis binturong penicillatus</i> (Pocock, 1933)	Java (Indonesia)
<i>Arctictis binturong kerkhoveni</i> Sody, 1936	Bangka island (east Sumatra)
<i>Arctictis binturong menglaensis</i> Wang & Li, 1937	Yunnan province (China)

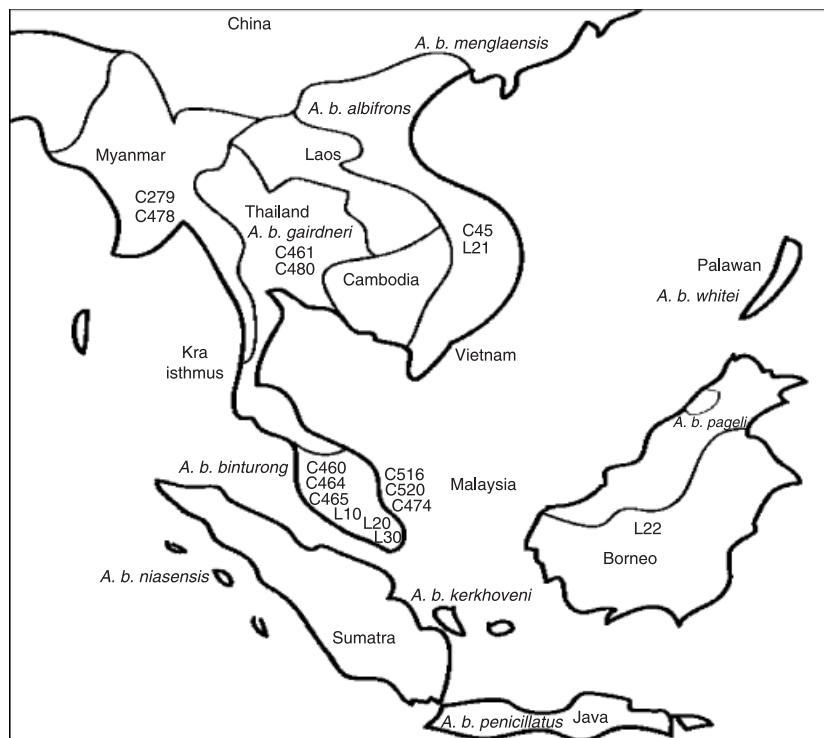


Figure 1 Map showing the distribution of the sub-species of *Arctictis binturong* and locality of samples.

Table 2 Binturong *Arctictis binturong* samples used for this study

Genus	Species	DNA number	Geographic origin	Sample	ISIS number
Arctictis	binturong	C-460	Malaysia (Singapore Zoological Garden)	Hairs	–
A.	b.	C-45	Vietnam (Saigon Zoo)	Hairs	367
A.	b.	C-151	Unknown (CPT, USA)	Tissue	01035
A.	b.	C-152	Unknown (CPT, USA)	Tissue	01027
A.	b.	C-461	Thailand (wild caught in Chaiyaphum province)	Hairs	–
A.	b.	C-480	Thailand (wild caught in Chaiyaphum province)	Hairs	–
A.	b.	C-279	Burma (Yangon Zoo)	Hairs	–
A.	b.	C-478	Burma (Yangon Zoo)	Hairs	–
A.	b.	C-429	Unknown (Servion Zoo, Switzerland)	Hairs	–
A.	b.	C-430	Unknown (Servion Zoo, Switzerland)	Hairs	–
A.	b.	C-431	Unknown (La Barben Zoo, France)	Hairs	–
A.	b.	C-432	Unknown (SMP Zoo, France)	Hairs	SA 0096
A.	b.	C-433	Unknown (SMP Zoo, France)	Hairs	S 96086
A.	b.	C-434	Unknown (SMP Zoo, France)	Hairs	SA 0095
A.	b.	C-436	Unknown (SMP Zoo, France)	Hairs	S 98003
A.	b.	C-437	Unknown (SMP Zoo, France)	Hairs	S 98111
A.	b.	C-438	Unknown (SMP Zoo, France)	Hairs	SA 0025
A.	b.	C-439	Unknown (SMP Zoo, France)	Hairs	S 91149
A.	b.	C-462	Unknown (Taipei Zoo, Taiwan)	Hairs	–
A.	b.	C-463	Unknown (Ostrava Zoo, Czech Republic)	Hairs	200145
A.	b.	C-464	Malaysia (Ostrava Zoo, Czech Republic)	Hairs	200293
A.	b.	C-465	Malaysia (Ostrava Zoo, Czech Republic)	Hairs	200291
A.	b.	C-466	Unknown (Ostrava Zoo, Czech Republic)	Hairs	202038
A.	b.	C-468	Unknown (Conservators' Centre, USA)	Hairs	01029
A.	b.	C-471	Unknown (Conservators' Centre, USA)	Hairs	–
A.	b.	C-472	Unknown (Linton Zoo, UK)	Hairs	74
A.	b.	C-474	Malaysia (Artis Zoo, Amsterdam)	Hairs	500363
A.	b.	C-475	Unknown (Paignton Zoo, UK)	Hairs	1376
A.	b.	C-476	Unknown (Colchester Zoo, UK)	Hairs	1728
A.	b.	C-488	Unknown (Lille Zoo, France)	Hairs	1531
A.	b.	C-489	Unknown (Lille Zoo, France)	Hairs	1533
A.	b.	C-490	Unknown (Lille Zoo, France)	Hairs	1822
A.	b.	C-491	Unknown (Lille Zoo, France)	Hairs	781
A.	b.	C-492	Unknown (Lille Zoo, France)	Hairs	782
A.	b.	C-493	Unknown (Lille Zoo, France)	Hairs	783
A.	b.	C-494	Unknown (Olomouc Zoo, Czech Republic)	Hairs	4990
A.	b.	C-495	Unknown (Olomouc Zoo, Czech Republic)	Hairs	4991
A.	b.	C-496	Unknown (Dierpark Wissel, Germany)	Hairs	5900
A.	b.	C-498	Unknown (Dierpark Wissel, Germany)	Hairs	101084
A.	b.	C-500	Unknown (Aqua Zoo, Friesland, NL)	Hairs	500217
A.	b.	C-501	Unknown (Dierpark Wissel, NL)	Hairs	100036
A.	b.	C-502	Unknown (Zoo Parc Overloon, NL)	Hairs	300039
A.	b.	C-503	Unknown (Zoo Parc Overloon, NL)	Hairs	–
A.	b.	C-516	Malaysia (Zoo Negara, KL)	Tissue	–
A.	b.	C-519	Malaysia (Zoo Negara, KL)	Tissue	–
A.	b.	C-520	Malaysia (Zoo Negara, KL)	Tissue	–
A.	b.	C-521	Malaysia (Zoo Negara, KL)	Tissue	–
A.	b.	C-522	Malaysia (Zoo Negara, KL)	Tissue	–
A.	b.	L-30	Malaysia (Zoo Negara, KL)	Tissue	–
A.	b.	C-505	Malaysia (Zoo Temerloh, Pahang)	Hairs	–
A.	b.	L-5	Unknown (Eberswalde Zoo, Germany)	Hairs	–
A.	b.	L-21	Vietnam (Servion Zoo, Switzerland)	Hairs	–
A.	b.	C-530	Unknown (Servion Zoo, Switzerland)	Hairs	–
A.	b.	L-22	Borneo (Artis Zoo, Amsterdam)	Hairs	MO 0139
A.	b.	L-10	Malaysia (Artis Zoo, Amsterdam)	Hairs	MO 4023
A.	b.	L-20	Malaysia (Zoo Plzen, Czech Republic)	Hairs	200291
Paguma	larvata	C-292	Vietnam (Hanoi)	Hairs	–

See acknowledgements for collectors; CPT, Carnivore Preservation Trust; SMP, Saint-Martin La Plaine. Animals with a known geographic origin are in bold.

We amplified the hypervariable region 1 of the D-loop using the following primers: L15513 (5'-CTAGGAGACC CAGACA ACTA-3'; modified from Irwin, Kocher & Wilson, 1991) and H16498 (5'-CATCTGGTTCTTACTTCAGG-3'; modified from Fumagalli *et al.*, 1996). Owing to difficulty in sequencing this fragment because of the presence of a series of repeated nucleotides, we modified the primers from Palomares *et al.* (2002) on the basis of a sequence obtained for the common palm civet *Paradoxurus hermaphroditus*. The sequences of these new primers were LCR1 (5'-CCACCAT CAGCACCCAAAGC-3') and HCR2 (5'-CCTCTTCTC GCTCCGGG-3').

The PCR products were purified using the MinElute PCR purification kit (Qiagen, Hilden, Germany) and sequenced directly with an automated sequencer (CEQ 2000 DNA Analysis system, Beckman Coulter, Fullerton, CA, USA).

Data analysis

Sequences were visually aligned using BioEdit version 5.0.6 (Hall, 1999). Phylogenetic relationships between individuals were reconstructed using PAUP 4.0 (Swofford, 2001) to perform maximum parsimony (MP) analysis with heuristic search using 100 random addition sequence and tree-bisection-reconnection branch swapping (Swofford *et al.*, 1996) with 1000 bootstrap replicates (Felsenstein, 1985). PhylML 2.3 (Guindon & Gascuel, 2003) was used for maximum likelihood (ML) analysis using an HKY model (Hasegawa, Kishino & Yano, 1985) and 1000 bootstrap replicates. The model that best fitted the data was identified by the Akaike information criterion and the hierarchical likelihood ratio test using the program MODELTEST 3.06 (Posada & Crandall, 1998; see Posada & Buckley, 2004). For Bayesian inference (BI) we used MrBayes 3.4 (Huelsenbeck *et al.*, 2001) with flat priors. Analysis parameters were four characters state and six substitutions type following an invgamma shape with the α parameter estimated from the data set. Four cold Metropolis-coupled Markov chains Monte Carlo (MCMCMC) for 1 000 000 generations were run and one tree was retained every 100 generations. The length of the 'burn-in' period was set at 2500 generations after plotting posterior probabilities against, or as a function of, the number of generations (from 9975 trees retained).

Arlequin version 2.000 (Schneider, Roessli & Excoffier, 2000) was used to determine genetic diversity and conduct population differentiation analysis. Genetic differentiation among samples from different geographic origins was assessed by comparing the average number of pairwise differences between populations (PiXY), the average number of pairwise differences within populations (PiX and PiY), and the corrected average pairwise difference $(PiXY - (PiX + PiY)/2)$. The same program was used to compute the minimum spanning network (MSN) obtained from a distance matrix calculated between all haplotype pairs. We also use the median-joining network (Bandelt, Forster & Röhl, 1999) to define the geographic assignment of all binturong mtDNA haplotypes.

Results

Phylogeography

The left domain of the mitochondrial DNA control region was obtained for all 56 individuals (Genbank accession numbers: DQ302034 to DQ302091). Its length varied from 319 to 390 base-pairs (bp). It covered the hypervariable region 1 (HVR1) flanking the tRNA^{Pro} gene and the extended terminal associated sequences (ETAS 1 and ETAS 2; Fumagalli *et al.*, 1996; Sbisà *et al.*, 1997). A deletion of 71 nucleotides occurred in two related individuals from Malaysia (DNA numbers C-474 and L-10). We observed 84 variable sites and 49 parsimony informative sites in the entire data set.

We identified 38 haplotypes within the 56 samples. Within the individuals with known geographic origin, four samples present identical haplotypes: C-516 and C-519 from Malaysia and C-45 and L-21 from Vietnam. Many individuals from European zoos were related and thus had identical haplotypes. C-430 has the same haplotype to all binturongs from Saint-Martin La Plaine, C-489, C-490 and C-492 from Lille, C-500, C-501 and C-502 from Netherlands zoos, C-475 from Paignton Zoo and C-471 from the Conservators' Centre. Individuals C-436 and C-476 have the same haplotype as C-495 and C-516 has the same haplotype as C-519.

The results of ML and MP analyses conducted with individuals with a known geographic origin are shown in Fig. 2. Both ML and MP analyses revealed two distinct geographic clades supported by high bootstrap values, one grouping individuals from the Indochinese zoogeographic sub-region, and the other grouping those from peninsular Malaysia (Sundaic sub-region). There is no strong support for the position of the individual from Borneo. The same analysis was conducted with the 38 haplotypes (Fig. 3). We could suggest to which geographic clade these binturongs are closer. For example, the haplotypes of two binturongs (C-429 and C-530) from Servion Zoo group with the haplotypes of binturongs from Thailand.

The MSN and the median-joining network both revealed three groups (Fig. 4). Similar to the ML tree, the networks separated a northern group from a southern one and isolated the individual from Borneo. Samples C-474 and L-10 are in a separate group because of the deletion of 71 nucleotides in the sequence. Moreover, these methods allowed us to assess the geographic clade of captive-bred individuals: C-151, C-152, C-429, C-430, C-431, C-463, C-466, C-472, C-495, C-488, C-491, C-493, C-494, C-498, C-503 and C-530 grouped with individuals from the Indochinese sub-region and C-462, C-468, C-496 and L-5 in the peninsular Malaysia clade.

Genetic diversity

Genetic differences were significant between the northern and peninsular Malaysia groups (see Table 3), but were not significant between individuals of the northern group.

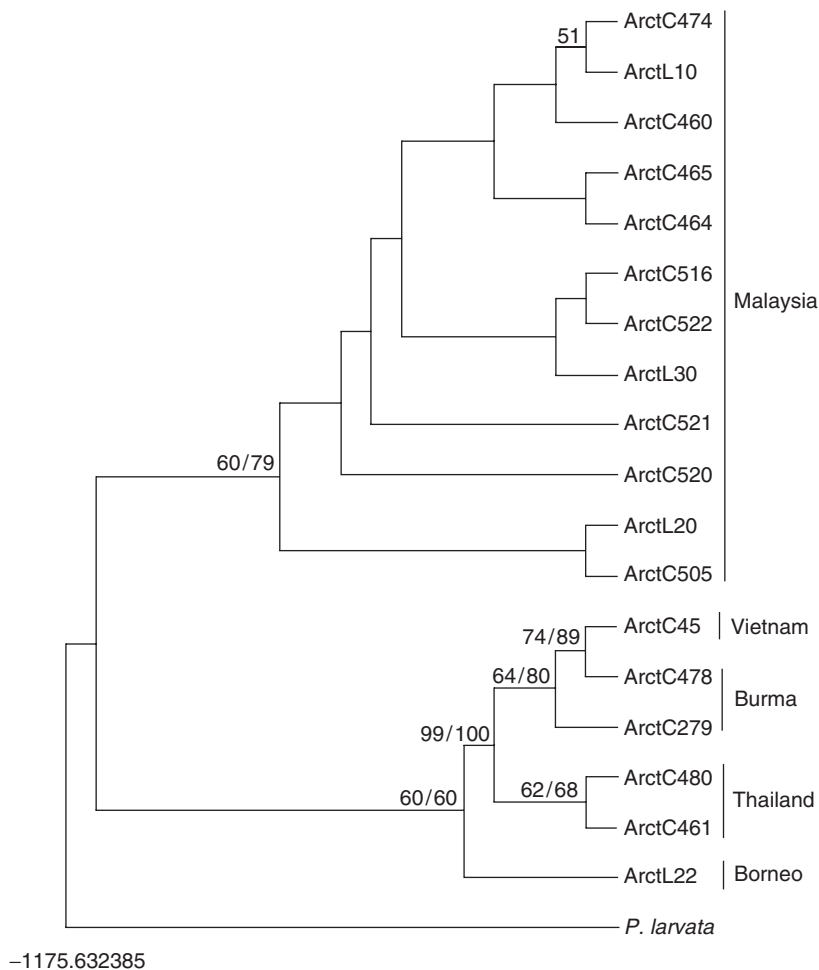


Figure 2 Phylogenetic relationships among binturongs *Arctictis binturong* with known geographic origin. Maximum likelihood analysis (ML) tree with HKY model. Numbers above branches represent bootstrap support from 1000 replicates with MP followed by bootstrap values with ML (only those over 50% are indicated). Arct, *Arctictis binturong*.

Binturongs from Asian zoos were either wild caught or descended from wild individuals less than two generations removed. In most European zoos (notably in France), binturongs have been captive bred for several generations. We divided the samples into two groups and compared their genetic diversity: one with individuals born in captivity for more than two generations, and the other from wild origins or bred for less than two generations. The results (Table 4) indicate that diversity was significantly lower for captive-bred binturongs than for wild binturongs.

Among the captive binturongs from European zoos, those from the Indochinese sub-region had low genetic diversity (Table 5). In European zoos, binturongs from the Indochinese sub-region have been bred for several generations, whereas captive binturongs from peninsular Malaysia have been bred for less than two generations.

Discussion

Although the phylogeography obtained is partial (missing representatives from northern India, southern China, Sumatra, Java and Palawan), the separation of Indochinese and peninsular Malaysia binturongs is congruent with a well-

recognized geographic barrier, the Isthmus of Kra in peninsular Thailand, separating the Indochinese and Sundaic sub-regions (Tougaard, 2001). The zoogeography of mammals in South-east Asia reveals that several species and sub-species boundaries lie at the Isthmus of Kra (Lekagul & McNeely, 1988; Corbet & Hill, 1992; Hughes, Round & Woodruff, 2003). This rugged area may have acted as a natural barrier to binturong dispersal as it has been proposed for the tiger *Panthera tigris* (Luo *et al.*, 2004). According to Woodruff (2003) and supported by De Bruyn *et al.* (2005), the Thai–Malay peninsula may have been flooded by marine seaways during both the mid-Miocene and early Pliocene high stand. More recently, during the Pleistocene much of the Isthmus of Kra was flooded during sea-level oscillations (Lekagul & McNeely, 1998). These sea-level fluctuations may have effectively isolated some binturong populations and prevented dispersal for extended periods of geologic time.

Our results aid in the identification of evolutionary significant units (ESUs) for binturong conservation (Moritz, 1994). According to Moritz's model, ESUs are characterized by reciprocally monophyletic mtDNA clades. Our data support at least two ESUs, one from the Indochinese

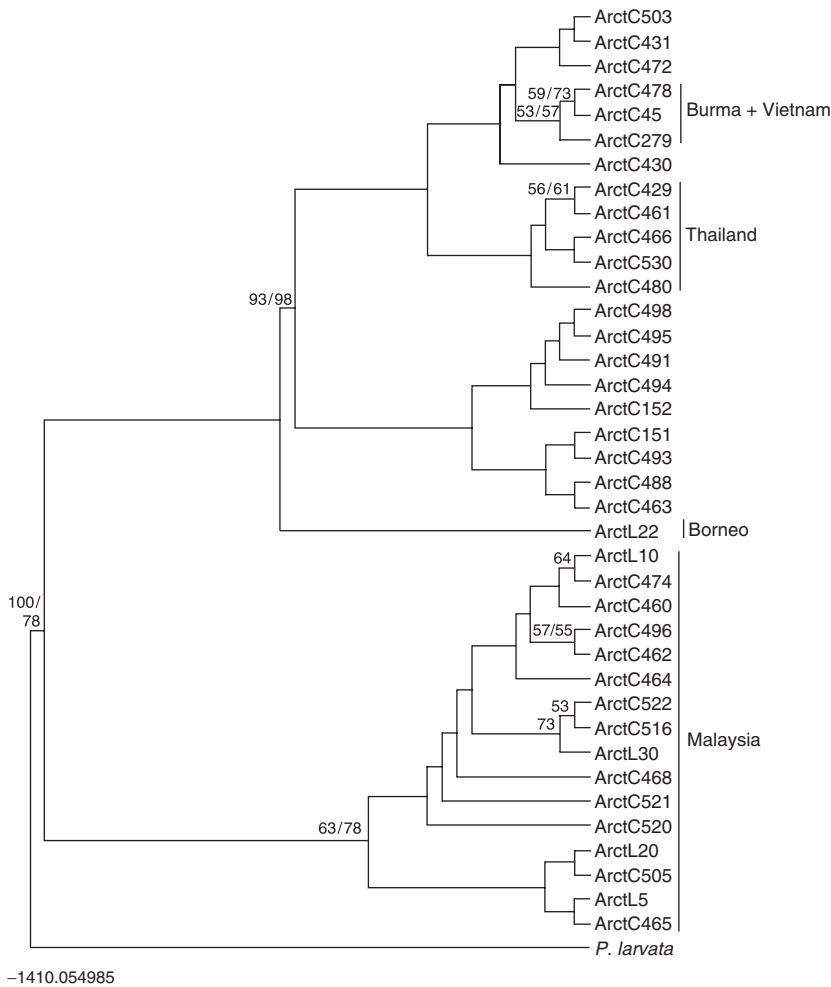


Figure 3 Phylogenetic relationships among all binturong *Arctictis binturong* mtDNA haplotypes. Maximum likelihood analysis (ML) tree with HKY model using Phylml. Numbers above branches represent bootstrap support from 1000 replicates with MP followed by bootstrap values with ML (only those over 50% are indicated). Arct, *Arctictis binturong*.

sub-region and one from the Sundaic sub-region. Pocock (1933) recognized at least four sub-species: *Arctictis binturong albifrons* (including *Arctictis binturong menglaensis* and *Arctictis binturong gairdneri*; see Table 1 and Fig. 1) in the northern regions (northern India, southern China, Laos, Vietnam, Burma and northern Thailand), *Arctictis binturong binturong* (Malay peninsula and Sumatra), *Arctictis binturong penicillatus* (Java and Borneo) and *A. b. whitei* (Palawan island). Our results are congruent with the existence of the sub-species *A. b. albifrons*, *A. b. binturong* and *A. b. penicillatus*. If confirmed by further studies, then these sub-species should be managed separately so that the evolutionary heritage within the species is maintained and genetic introgression is avoided.

There are several conceptual and practical problems, given the lack of ecological information and data, on the genetic structure and gene flow of wild binturong populations. According to Crandall *et al.* (2000), such studies are necessary to gain greater insight into conservation units. Given the distinctness of the two binturong clades, it would be of interest to look for adaptive differences in the different regions and its associated implications for reintro-

duction or supplement programs. The IUCN recommends that only individuals from a similar climatic or ecological zone (e.g. with a known geographic origin) should be used for reintroduction or restocking programs (IUCN, 1987).

Our results have allowed the determination of the geographic origin of captive-bred individuals. What does this imply for the conservation of the species? The American Zoo and Aquarium Association (AAZA) asserts that zoos should conserve the ESU of each species provided it does not threaten species survival, because stochastic events like genetic drift can endanger the ESU (Ryder, 1986). But can a reproduction management program take different binturong ESUs into consideration? As we have shown, genetic diversity among the European captive population is low, particularly among individuals originating from the Indo-chinese region. Managing ESUs could be possible provided that the captive population from Asian zoos is used to supplement the European zoos population through periodic immigration, thus preventing drift of the European zoos population from genetic characteristics of the wild population. As few as one migrant per two generations would be beneficial, and ≥ 5 migrants per generation would virtually

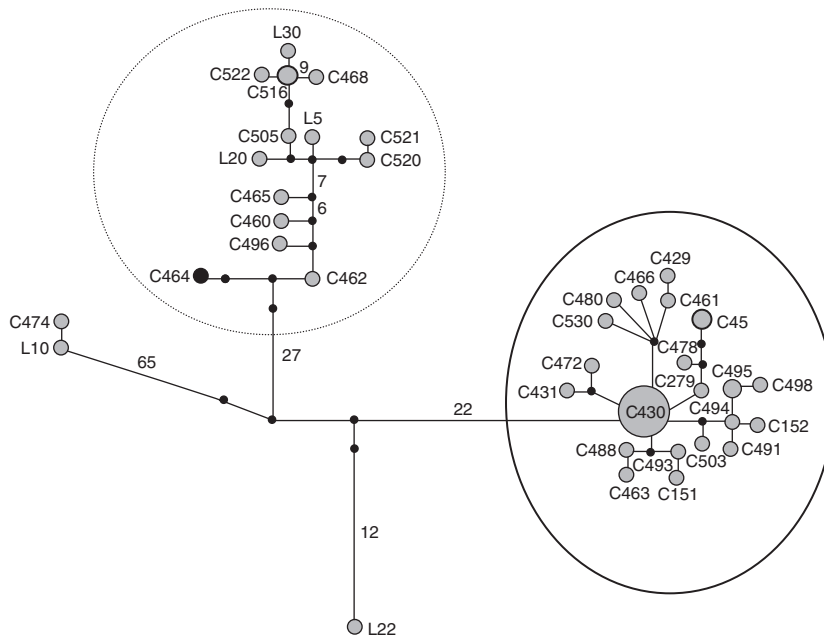


Figure 4 Median-joining network depicting the geographic assignment of all binturong *Arctictis binturong* mtDNA haplotypes. The size of each circle is proportional to the corresponding haplotype frequency. Only numbers over five mutational steps are indicated above branches. Missing intermediates are indicated by black circles. Individuals from Malaysia are circled with a dotted line whereas individuals from Indochina are circled with a continuous line.

Table 3 Pairwise population differentiation for control region sequences of binturongs *Arctictis binturong* with known geographic origin

	Thailand	Burma	Vietnam	Malaysia
Thailand	3.23	4.35	7.17	36.79
Burma	2.19	1.07	2.16	35.51
Vietnam	5.55	1.62	0.00	38.43
Malaysia	31.76**	31.55**	35.01**	6.84

Population average pairwise differences using the distance method Kimura two-parameter with $\gamma=0.7$. Above diagonal: average number of pairwise differences between populations (PiXY). Diagonal elements: average number of pairwise differences within populations (PiX and PiY). Below diagonal: corrected average pairwise difference $(PiXY - (PiX + PiY))/2$.

**Corrected average pairwise differences that are statistically different, $P < 0.01$.

halt genetic drift within the European captive population (Lacy, 1987).

The ultimate goal of captive breeding is to eventually reintroduce some animals back into their natural environment (AZA, 1992). According to Snyder *et al.* (1996), captive breeding should always be tightly coupled with recovery objectives of wild populations and should not be proposed as a long-term solution. Indeed, animals born in captivity through several generations should not be released in the wild because, for many species, long-term captive breeding, despite all efforts to slow changes, may result in domesticated forms with low reestablishment potentials (Lacy, 1987). However, if binturongs are threatened in their natural environment, there is no need to restock or reintroduce a binturong population in a degraded habitat if the original causes of extinction have not been removed. There-

Table 4 Diversity indices

Exact test of sample differentiation based on haplotype frequencies		
Populations	Wild origin (<2 g) (n=20)	Captive origin (>2 g) (n=25)
Haplotype diversity	0.99 ± 0.02	0.69 ± 0.10
Nucleotide diversity	0.03 ± 0.01	0.01 ± 0.008

Wild origin: individuals born in the wild or in captivity from less than two generations; captive origin: individuals born in captivity for more than two generations. We excluded individuals of uncertain origin.

Non-differentiation: exact P -value = 0.00000 ± 0.00000 .

fore, more information is needed on binturong ecology and short-term threats to survival before considering reintroductions.

Poaching throughout South-east Asia is a serious threat facing binturongs. When animals are seized from poachers, they are kept in captivity because, in most cases, their geographical origin is unknown. Compiling a database of sequences of binturongs with a known geographic origin will allow assessment of their geographic origin. Animals may then be released in the nearest natural habitat of their origin, avoiding risks of genetic introgression. Conservationists may participate in interactive zoo-wild metapopulation management. Zoo conservation efforts should focus on the preservation of wildlife communities and habitats rather than on captive propagation (Conway, 1995). Moreover, in order to decrease poaching, a successful conservation program should enhance public interest in the ecological importance of binturongs, particularly as agents of seed dispersal.

Table 5 Genetic diversity among the captive populations in European zoos

	European zoos (<i>n</i> = 34)	
Haplotype diversity	0.82 ± 0.07	
Nucleotide diversity	0.03 ± 0.02	
<i>Geographic clades</i>	<i>Indochina</i> (29)	<i>Malaysia</i> (5)
Haplotype diversity	0.75 ± 0.08	1.00 ± 0.12
Nucleotide diversity	0.008 ± 0.005	0.024 ± 0.016

Conclusion

This study has shown that the genetic diversity within the European zoo binturong population is low, and suggests that several ESUs should be considered for managing the breeding program. It also provides a tool to improve captive breeding management in assessing a captive animal's geographic origin. Such information should be required before any reintroduction or restocking program.

According to Schreiber *et al.* (1989), taxonomic revisions are a priority for such species, because sub-species with small ranges, if valid, must then be considered threatened. This study should be continued by the addition of more wild-caught animals from other regions.

Despite obvious difficulties in studying binturongs in the field because they are primarily nocturnal and arboreal, investigations evaluating their ecological requirements and status are essential to make informed decisions about ways of preserving binturongs. First results have been obtained on binturong ecology in Thailand (see Austin, 2002; Grassman, Michael & Nova, 2005), and we would like to underline the need for more field studies on this species in other parts of their range.

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