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# Mitochondrial data support an odd-nosed colobine clade

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**Abstract**

To obtain a more complete understanding of the evolutionary history of the leaf-eating monkeys we have examined the mitochondrial genome sequence of two African and six Asian colobines. Although taxonomists have proposed grouping the "odd-nosed" colobines (proboscis monkey, douc langur, and the snub-nosed monkey) together, phylogenetic support for such a clade has not been tested using molecular data. Phylogenetic analyses using parsimony, maximum likelihood, and Bayesian methods support a monophyletic clade of odd-nosed colobines consisting of *Nasalis*, *Pygathrix*, and *Rhinopithecus*, with tentative support for *Nasalis* occupying a basal position within this clade. The African and Asian colobine lineages are inferred to have diverged by 10.8 million years ago (mya or Ma). Within the Asian colobines the odd-nosed clade began to diversify by 6.7 Ma. These results augment our understanding of colobine evolution, particularly the nature and timing of the colobine expansion into Asia. This phylogenetic information will aid those developing conservation strategies for these highly endangered, diverse, and unique primates.


*Keywords:* Colobines; Leaf-eating monkeys; Odd-nosed monkeys; mtDNA genomes

## 1. Introduction

The colobines (subfamily Colobinae) are a diverse clade of Old World primates, with at least 30 species grouped into 4–10 genera (4 genera: 1 African genus, Szalay and Delson, 1979; 3 Asian genera, Groves, 1970; 10 genera: 3 African genera, Groves, 2001; 7 Asian genera, Groves, 2001; Brandon-Jones et al., 2004). Extant colobines are found in Africa and Asia in a wide range of forest and woodland habitats (Davies and Oates, 1994). Colobines are generally arboreal and are referred to as 'leaf-eating' monkeys because their diet is composed heavily of leafy plant material or hard fruits. A number of derived morphological traits distinguish the colobines from their sister group, the cercopithecines (subfamily Cercopithecinae; e.g., baboons, macaques, and guenons), including dental, skeletal, soft tissue, and physiological characters (e.g., Strasser and Delson, 1987). Many of the derived morphological traits found in the colobines—including extensive salivary glands and large, multi-chambered stomachs that contain a variety of microbes needed to process plant material—are adaptations to diets that are more folivorous than the diets of other Old World monkeys.

Most researchers support reciprocal monophyly of African and Asian colobines (morphological studies: Groves, 2001; Napier and Napier, 1970; Szalay and Delson, 1979; molecular studies: Collura et al., 1996; Messier and Stewart, 1997; Page et al., 1999; Xing et al., 2005; Zhang and Ryder, 1998; and chromosomal studies: Bigoni et al., 2003, 2004). However, the reciprocal monophyly hypothesis does not enjoy universal support as paraphyly

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of the Asian taxa has also been proposed (Groves, 1989; Jablonski, 1998).

Asian colobines have been divided into two groups, an odd-nosed group (consisting of *Nasalis, Simias, Rhinopithecus,* and *Pygathrix* species) and a langur group (consisting of *Presbytis, Semnopithecus,* and *Trachypithecus* species) (Groves, 1970; Jablonski, 1998; Jablonski and Peng, 1993). Within the odd-nosed group various relationships have been proposed including: (1) *Rhinopithecus* and *Pygathrix* are sister taxa (Delson, 1975; Groves, 1970; Jablonski and Peng, 1993; Li et al., 2004; Wang et al., 1997), (2) *Pygathrix* may be more closely related to *Nasalis*, than to *Rhinopithecus* (Jablonski, 1998); and (3) *Rhinopithecus* and *Nasalis* are sister taxa (Zhang and Ryder, 1998). Other research has suggested that the odd-nosed colobines are not monophyletic (Bigoni et al., 2003, 2004; Jablonski, 1998; Wang et al., 1995). The relationships among the langur group have proved even more difficult to resolve. In particular, *Trachypithecus* has variously been considered its own genus, a subgenus of *Presbytis* and a subgenus of *Semnopithecus* (Brandon-Jones, 1984, 1996; Delson, 2000; Groves, 1989; Strasser and Delson, 1987; Szalay and Delson, 1979).

Mitochondrial DNA (mtDNA) is commonly chosen for phylogenetic analyses of closely related species because the mitochondrial genome lacks recombination, has a lower effective population size, has a faster substitution rate, and is usually only inherited maternally (Ballard and Whitlock, 2004; Funk and Omland, 2003; Moore, 1995). Furthermore, a large comparative database of mitochondrial sequence data is available as a result of the frequent use of this locus for phylogenetic studies. However, use of mitochondrial DNA to infer phylogenetic relationships also carries several disadvantages that could produce incorrect or biased inferences, including the possible incorporation of nuclear-transferred mitochondrial fragments (numts) as has been demonstrated by Collura and Stewart (1995). By applying methods that mitigate the risk of amplifying numts (Raaum et al., 2005; Thalmann et al., 2004; see Section 2), it is possible to avoid this problem.

To obtain a more complete understanding of the evolutionary history of the colobines, we have sequenced the mitochondrial genomes of 1 African colobine monkey (*Procolobus* (*Piliocolobus*) *badius*) and 5 Asian colobine monkeys (*Nasalis larvatus*, *Presbytis melalophos*, *Semnopithecus entellus*, *Pygathrix nemaeus*, and *Rhinopithecus roxellana*). These sequences, in addition to the two previously published colobine sequences (*Colobus guereza and Trachypithecus obscurus*), represent 8 of the 9 colobine genera recognized in the most recent primate classifications (Brandon-Jones et al., 2004; Grubb et al., 2003). These data were used to re-evaluate the phylogenetic relationships among the colobines.

## 2. Materials and methods

### 2.1. Samples

The sequences collected for this study are presented (with GenBank accession nos.) in Table 1. The *Colobus guereza* and *Trachypithecus obscurus* sequences were taken from a previous study (Raaum et al., 2005; GenBank Accession Nos. AY863427 and AY863425). DNA was extracted from blood or tissue following the QIAamp DNA Blood Mini kit (Qiagen, cat. No. 51104) and DNeasy Tissue kit (Qiagen, cat. No. 69504) protocols.

### 2.2. Amplification and sequencing

Mitochondrial genomes were amplified in two overlapping segments of approximately 10,000 base pairs (bp) each following the Expand Long Template PCR system protocol (Roche, cat. No. 1681834). This procedure is an effective means of reducing the likelihood of amplifying nuclear pseudogenes of mtDNA (Raaum et al., 2005; Thalmann et al., 2004). Primers for long-range PCR were designed from catarrhine mtDNA sequences available in GenBank, and then modified if needed as more data were collected. All primer sequences and PCR conditions can be found in Supplementary materials (Table S1). PCR products were visualized on a 0.8% agarose gel and subsequently cleaned of excess nucleotides and primers by an exonuclease I, shrimp alkaline phosphatase method (Hanke and Wink, 1994).

Sequencing primers were designed from existing catarrhine mitochondrial genomes and by primer walking. Cycle-sequencing was preformed using the Big Dye kit (Big Dye v3.0 and 3.1, ABI, cat. No. 4337456) following the manufacturer's protocol for diluted reactions. Sequence products were

Table 1
Species sampled

| | Scientific name | Common name | GenBank Accession Nos. |
|---|---|---|---|
| Asian colobines[b] | *Nasalis larvatus*[a] | Proboscis monkey | DQ355298 |
| | *Pygathrix nemaeus*[a] | Red-shanked douc langur | DQ355302 |
| | *Rhinopithecus roxellana*[a] | Sichuan golden snub-nosed monkey | DQ355300 |
| | *Presbytis melalophos* | Mitered leaf monkey | DQ355299 |
| | *Trachypithecus obscurus* | Dusky or spectacled leaf monkey | AY863425 |
| | *Semnopithecus entellus* | Hanuman langur | DQ355297 |
| African Colobines[c] | *Colobus guereza* | Eastern black and white colobus | AY863427 |
| | *Procolobus (Piliocolobus) badius* | Western red colobus | DQ355301 |

[a] Odd-nosed colobines.
[b] Following Groves, 2001; Brandon-Jones et al., 2004.
[c] Following Oates et al., 1994; Grubb et al., 2003.

processed on an ABI PRISM 377 DNA Sequencer. Gel files were analyzed with the Sequencing Analysis software package (v3.4, ABI) and sequences were assembled with the Sequencher program (v4.1, Gene Codes Corp.). Genomes were assembled from multiple sequences derived from multiple PCR products to ensure that the amplification primers were consistently targeting the same mtDNA. Reliability of the base calls were also checked by examining regions where the two amplicons overlap as another test that a singular, thus presumably 'true,' mitochondrial sequence had been generated for each species. Sequence assemblies were always checked visually base-by-base following their initial assembly. All protein-coding genes were translated as an additional test to look for premature stop codons and frameshifts indicative of a numt sequence.

### 2.3. Alignments

The eight colobine mitochondrial genome sequences were aligned with those from the following additional primate species: *Papio hamadryas* (GenBank Accession No. NC_001992), *Macaca sylvanus* (GenBank Accession No. NC_002764), *Pan troglodytes* (GenBank Accession No. NC_001643), *Homo sapiens* (GenBank Accession No. NC_001807), and *Cebus albifrons* (GenBank Accession No. NC_002763). The last species, a New World monkey, was included for use as an outgroup.

Each individual gene within the mitochondrial genome was aligned using default settings in ClustalX (Thompson et al., 1994, 1997). Two alignments of all 12 heavy strand protein-coding genes were assembled. One alignment (**HeavyProteins**) is a concatenation of all 12 separate ClustalX alignments, and the second alignment (**HeavyProteinsP12**) is a concatenation that incorporates only first and second codon positions. Spurious insertions and deletions generated by the ClustalX alignments were then adjusted manually to ensure that protein-coding sequences conform to codon boundaries. We examined evolutionary rate variation across catarrhine primate mitochondrial genes and determined that the rRNAs and tRNAs appear to be evolving substantially slower than the protein-coding sequences, thus the protein-coding sequences were used in our phylogenetic analyses (see Supplementary material, Fig. S1). The control region was also not used because evolutionary rates in the mitochondrial control region are highly variable, with some portions evolving too fast to be phylogenetically useful for supra-specific phylogeny (Li, 1997). The **HeavyProteins** file is available in Supplemental material (Fig. S2).

### 2.4. Evolutionary model choice

To determine which model best fit the data, Model Test v3.5 (Posada and Crandall, 1998) was used to perform likelihood ratio tests on the **HeavyProteins** alignment. The **HeavyProteins** alignment was best fit by the general time reversible (GTR) model with invariant sites (I) and a gamma distribution (G) of site-specific rates as determined by the Hierarchical Likelihood Ratio Test (hLRT). When analyzed under the Akaike Information Criterion (AIC), this alignment was best fit by the Hasegawa, Kishino, and Yano (HKY) model with invariant sites (I) and a gamma distribution (G) of site-specific rates.

### 2.5. Phylogenetic analyses

Maximum likelihood bootstrap trees were inferred from the alignment of all 12 concatenated heavy strand protein-coding genes (**HeavyProteins**) and the alignment excluding the third codon position (**HeavyProteinsP12**) using PAUP* (version 4.0b10, Swofford, 2004). Each alignment was analyzed using the models of evolution suggested by the hLRT and the AIC in the ModelTest program. All missing and ambiguous data were excluded from the analyses. Trees were constructed from ML bootstrap results (1000 replicates, taxon addition set to random, heuristic searches). All other parameters were left as the default settings given in PAUP*. Only nodes with bootstrap support greater than 80 were considered strongly supported and retained in the tree (nodes less than 80 were considered tentatively supported).

Maximum parsimony bootstrap trees were also inferred from these two alignments using PAUP*. For these analyses, characters were unordered and equally weighted. As described above, all missing and ambiguous data were excluded and consensus trees were constructed from MP bootstrap results (1000 replicates, taxon addition set to random heuristic searches). Nodes with bootstrap support greater than 80 were retained in the tree (nodes less than 80 were considered tentatively supported).

In addition, a Bayesian analysis was employed using MrBayes version 3 (and Ronquist and Huelsenbeck, 2003) to infer phylogenetic relationships from the two heavy strand protein-coding genes alignments (**HeavyProteins** and **HeavyProteinsP12**). The general time reversal model with invariant sites (I) and a gamma distribution (G) of site-specific rates was used for these analyses. Markov Chain Monte Carlo (MCMC) chains were run for 150,000 generations sampled every 100 generations. The burn-in period was set to 200 after observing that the analysis stabilized after 20,000 generations. Nodes with clade credibility scores greater than 85 were retained in the tree (nodes less than 85 were considered tentatively supported).

### 2.6. Divergence date estimation

Divergence dates were estimated on a consensus tree representing only those nodes supported by each of the eight analyses (four ML bootstrap, two MP bootstrap, and two Bayesian). Divergence dates were anchored by two calibration points, 6 Ma for the human-chimpanzee divergence and 23 Ma for the hominoid-cercopithecoid divergence (for details on calibration point choice, see Raaum et al., 2005). To calculate dates with confidence intervals, the **HeavyProteinsP12** alignment was re-sampled 100 times and maximum likelihood branch lengths were calculated in the *PAML* software

package (Yang, 1997, 2004) using the GTR (REV) model with four gamma distributed rate categories. Divergence dates were then calculated using the truncated Newton algorithm for the penalized likelihood method in the *r8s* program (Sanderson, 2004). The sample of divergence dates for each node of interest was tested for normality (Shapiro-Wilks test for normality; Royston, 1995); all passed the normality test. To compensate for the unknown precision of the calibration dates, a fractional uncertainty of 10% was added to the 95% confidence interval.

## 3. Results

### 3.1. Phylogenetic analyses

Regardless of phylogenetic method employed or model used, all analyses supported the relationships shown in Fig. 1 (see Supplementary material, Figs. S3–S6 for individual trees). All analyses agree that the Colobinae can be divided into an African clade and an Asian clade. Among the Asian forms, there is strong support for an odd-nosed clade consisting of *Nasalis larvatus*, *Pygathrix nemaeus*, and *Rhinopithecus roxellana*, although relationships within this clade are unclear. All analyses also suggest *Presbytis* and *Trachypithecus* are sister taxa.

The main difference between the different analyses concerns the placement of *Semnopithecus entellus* (Fig. 2). The four ML bootstrap trees do not strongly resolve the placement of *Semnopithecus entellus* among the Asian genera. However, three of the four ML trees tentatively suggest *Semnopithecus entellus* is sister to the odd-nosed colobines (Fig. 2B). Similar to the ML analyses, Bayesian analyses also tentatively suggest that *Semnopithecus entellus* is the sister taxon to the odd-nosed clade (Fig. 2B). However, maximum parsimony analyses of the two alignments did not suggest this relationship and were unable to strongly

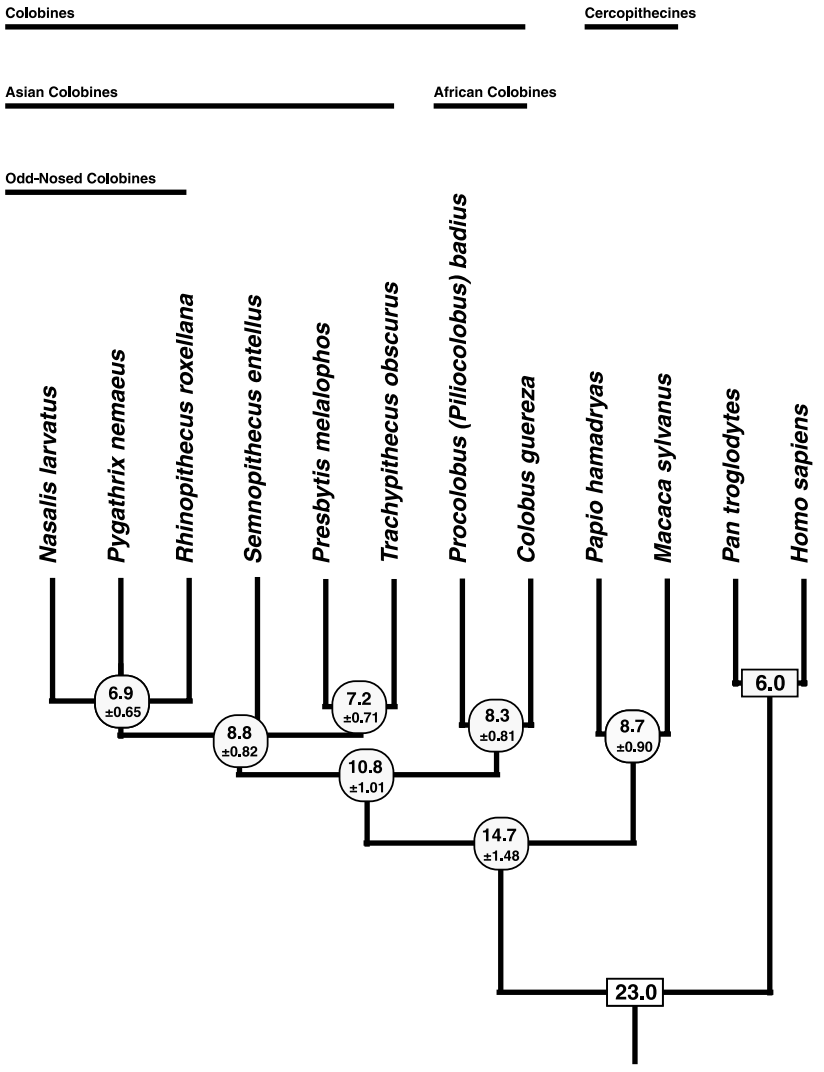


Fig. 1. Phylogenetic tree. Scaled phylogenetic tree inferred from both mitochondrial alignments (**HeavyProteins**, ~10,000 bases; and **HeavyProteinsP12**, ~7,000 bases) based upon likelihood, parsimony, and Bayesian analyses. Tree was rooted with *Cebus albifrons*. Nodes retained show >85 bootstrap (1000 replicates) or clade credibility values. Dates, in million years (Ma), were estimated from this tree with the **HeavyProteinsP12** alignment (see Section 2) and calibrated using both 6 Ma for the *Pan*/*Homo* divergence and 23 Ma for the cercopithecoid/hominoid divergence (following Raaum et al., 2005). Approximate 95% confidence intervals are given for each estimated date.

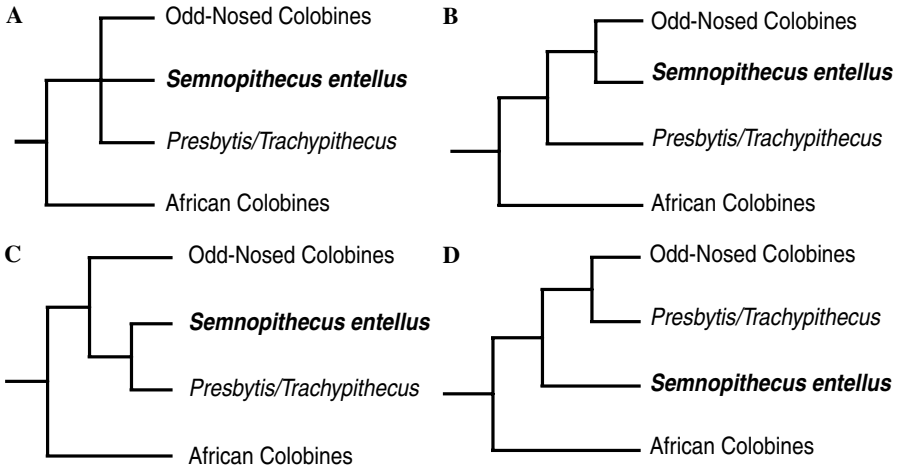


Fig. 2. Phylogenetic position of *Semnopithecus entellus*. (A) The authors' conservative interpretation based on multiple phylogenetic analyses. (B) Phylogenetic position of *S. entellus* suggested by Bayesian analyses of both alignments and likelihood analyses (both models, **HeavyProteinsP12**; and hLRT suggested model, **HeavyProteins**) if nodes with ~50–80 bootstrap/clade credibility are retained in the tree. (C and D) show the two alternative phylogenies each equally weakly supported by parsimony analyses of the two alignments (**HeavyProteins** and **HeavyProteinsP12**, respectively).

and consistently resolve the position of *Semnopithecus entellus* within the Asian clade (Figs. 2C and D).

In an attempt to further clarify the relationship between *Semnopithecus* and the other Asian colobines, we added the two mitochondrial rRNA sequences (12S and 16S) to our **HeavyProteins** alignment and analyzed it using maximum likelihood methods (see Supplementary material, Figs. S7 and S8). This analysis, however, yielded the same topology and very similar bootstrap values possibly suggesting that the addition of more sequence data to our alignment will not help resolve these relationships.

Additionally, the MP bootstrap and Bayesian analyses of the **HeavyProteins** alignment tentatively suggest *Rhinopithecus roxellana* and *Pygathrix nemaeus* are more closely related to each other than either is to *Nasalis larvatus*.

### 3.2. Divergence date estimates

Divergence date estimates (Fig. 1) suggest the colobines and cercopithecines were separated by 14.7 Ma (13.2–16.2 Ma). Within the colobines, the African and Asian clades diverged by 10.8 Ma (9.7–11.8 Ma). Within the Asian colobines the odd-nosed clade, *Presbytis*/*Trachypithecus* clade, and *Semnopithecus entellus* were separated by 8.8 Ma (8–9.6 Ma). The odd-nosed colobine taxa diverged by 6.9 Ma (6.3–7.6 Ma). *Presbytis melalophos* and *Trachypithecus obscurus* diverged by 7.2 Ma (6.5–7.9 Ma). Within the African colobines, *Colobus guereza* and *Piliocolobus badius* diverged by 8.3 Ma (7.5–9.2 Ma).

## 4. Discussion

Analysis of mitochondrial genome sequences strongly supports the existence of an 'odd-nosed' clade among the Asian colobines consisting of *Nasalis larvatus*, *Pygathrix nemaeus*, and *Rhinopithecus roxellana,* supporting previously proposed grouping of the odd-nosed taxa (Groves, 1970; Jablonski, 1998; Jablonski and Peng, 1993; Oates and Davies, 1994). Within this clade there is tentative support for a sister-taxon relationship between *Pygathrix* and *Rhinopithecus*. Molecular work by Whittaker et al. (in press) suggests *Nasalis* and *Simias* are sister taxa and tentatively supports the placement of *Simias* into the genus *Nasalis*. Therefore, although *Simias* was not included in this study, it is not unreasonable to suggest that all odd-nosed colobine genera cluster to the exclusion of the other Asian colobines.

The relationships among the remaining Asian taxa, often called the 'langur group,' proved more difficult to resolve. The mitochondrial data do suggest that *Presbytis* and *Trachypithecus* are sister taxa. However, our results do not strongly support a monophyletic langur group for two reasons. First, the precise position of *Semnopithecus entellus* within the Asian clade remains unresolved. Second, even when a relationship is tentatively suggested by these data, there is much stronger support for a sister-taxa relationship between the odd-nosed colobines and *Semnopithecus entellus* than there is for a langur clade.

Early colobine fossils are rare, but interpretations of them are consistent with the molecular divergence dates estimated here and may provide evidence for the geographic origin and subsequent dispersal of the leaf-eating monkeys. We estimate that the colobines and cercopithecines had diverged by 14.7 Ma (13.2–16.2 Ma), which is later than the date of 17.9 Ma (15.3–20.7 Ma) that we recently presented (Raaum et al., 2005); the cercopithecoid sample is considerably larger in the present analysis, which suggests that the later date (14.7 Ma) is probably more accurate. The earliest known fossil colobine is the African *Microcolobus*, most recently dated to 9–8.5 Ma (Kingston et al., 2002). In combination, these molecular and fossil data are consistent with an evolutionary scenario in which the colobines and

the cercopithecines diverged from their common ancestor in Africa in the Middle Miocene. After the origin of the colobine lineage, our mitochondrial analysis suggests that the African and Eurasian lineages became distinct by 10.8 Ma (9.7–11.8 Ma). The earliest fossil colobines found outside Africa are in Eurasia, with the appearance of *Mesopithecus pentelicus* at 8–7 Ma (Delson, 1994, 2000); the first fossil evidence in Asia proper appears around 7–6 Ma, possibly representing an extension of the genus *Mesopithecus* (Delson, 2000). Thus, the spatial and temporal distribution of fossil colobines suggests that they may have spread west to east from Africa through Eurasia during the Late Miocene.

European colobines were once a moderately diverse group of primates (including *Mesopithecus pentelicus*, *M. monspessulanus*, *M. delsoni*, and *Dolichopithecus ruscinensis*) that died out during the Pliocene (Delson, 2000). The African colobine species of the Late Miocene and Pliocene (including the following genera: *Microcolobus*, *Libypithecus*, *Cercopithecoides*, *Paracolobus*, and *Rhinocolobus*) were replaced by the African colobines species seen today (Delson, 2000). However, colobine variation increased in Asia where the majority of colobine species currently exist. More work is needed to reconstruct the radiation of the modern lineages of these taxa throughout Asia in the time period from 9–6 Ma. A more complete picture of this radiation, as well as clarification of phylogenetic relationships at a species level, may allow for new hypotheses concerning the adaptive pressures responsible for the diversification of the Asian colobines. The data presented here will encourage more interest in Asian colobine taxonomy and systematics and provide a springboard for future analyses on these highly endangered and unique primates.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2006.01.017.

## References

Ballard, J.W.O., Whitlock, M.C., 2004. The incomplete natural history of the mitochondria. Mol. Ecol. 13, 729–744.

Bigoni, F., Stanyon, R., Wimmer, R., Schempp, W., 2003. Chromosome painting shows that the proboscis monkey (*Nasalis larvatus*) has a derived karyotype and is phylogenetically nested within Asian colobines. Am. J. Primatol. 60 (3), 85–93.

Bigoni, F., Houck, M.L., Ryder, O.A., Wienberg, J., Stanyon, R., 2004. Chromosome painting shows that *Pygathrix nemaeus* has the most basal karyotype among Asian colobines. Int. J. Primatol. 25 (3), 679–688.

Brandon-Jones, D., 1984. Colobus and leaf monkeys. In: Macdonald, D. (Ed.), The Encyclopedia of Mammals, vol. 1. Allen & Unwin, London, pp. 398–403.

Brandon-Jones, D., 1996. The Asian Colobinae (mammalia: Cercopithecidae) as indicators of quaternary climatic change. Biol. J. Linn. Soc. 59, 327–350.

Brandon-Jones, D., Eudey, A.A., Geissmann, T., Groves, C.P., Melnick, D.L., Morales, J.C., Shekelle, M., Stewart, C.-B., 2004. Asian primate classification. Int. J. Primatol. 25 (1), 97–164.

Collura, R.V., Auerbach, M.R., Stewart, C.-B., 1996. A quick, direct method that can differentiate expressed mitochondrial genes from their nuclear pseudogenes. Curr. Biol. 6 (10), 1337–1339.

Collura, R.V., Stewart, C.-B., 1995. Insertions and duplications of mtDNA in the nuclear genomes of Old World monkeys and hominoids. Nature 378 (6556), 485–489.

Davies, A.G., Oates, J.F., 1994. Colobine Monkeys: Their Ecology, Behavior and Evolution. Cambridge University Press, Great Britain.

Delson, E., 1975. Evolutionary history of the Cercopithecidae. Contrib. Primatol. 5, 167–217.

Delson, E., 1994. Evolutionary history of the colobine monkeys in paleoenvironmental perspectives. In: Davies, A.G., Oates, J.F. (Eds.), Colobine Monkeys: Their Ecology, Behavior and Evolution. Cambridge University Press, Great Britain, pp. 11–44.

Delson, E., 2000. Colobinae. In: Delson, E., Tattersall, I., Van Couvering, J.A., Brooks, A.S. (Eds.), Encyclopedia of Human Evolution and Prehistory, second ed. Garland Publishing Inc., New York, pp. 186–189.

Funk, D.J., Omland, K.E., 2003. Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. Annu. Rev. Ecol. Evol. Syst. 34, 397–423.

Groves, C., 1970. The forgotten leaf-eaters, and the phylogeny of the Colobinae. In: Napier, J.R., Napier, P.H. (Eds.), Old World Monkeys: Evolution, Systematics and Behavior. Academic Press, New York, pp. 555–587.

Groves, C., 1989. A Theory of Human and Primate Evolution. Clarendon Press, Oxford.

Groves, C., 2001. Primate Taxonomy. Smithsonian Institution Press, Washington.

Grubb, P., Butynski, T.M., Oates, J.F., Bearder, S.K., Disotell, T.R., Groves, C.P., Struhsaker, T.T., 2003. Assessment of the diversity of African primates. Int. J. Primatol. 24, 1301–1357.

Hanke, M., Wink, M., 1994. Direct DNA sequencing of PCRr-amplified vector inserts following enzymatic degradation of primer and dNTPs. BioTechniques 17 (5), 858–860.

Jablonski, N., 1998. The Evolution of the Doucs and Snub-nosed monkeys and the question of the phyletic unity of the odd-nosed colobines. In: Jablonski, N. (Ed.), Natural History of the Doucs and Snub-Nosed Monkeys. World Scientific Publishing Company, New Jersey, pp. 13–52.

Jablonski, N.G., Peng, Y.-Z., 1993. The phylogenetic relationships and classification of the doucs and snub-nosed langurs of China and Vietnam. Folia Primatol. 60, 36–55.

Kingston, J.D., Jacobs, B.F., Hill, A., Deino, A., 2002. Stratigraphy, age and environment of the Late Miocene Mpesida Beds, Tugen Hills, Kenya. J. Hum. Evol. 42, 95–116.

Li, W.H., 1997. Molecular Evolution. Sinauer Associates, Inc., Sunderland, Massachusetts.

Li, M., Wei, F., Huang, C., Pan, R., de Ruiter, J., 2004. Phylogeny of snub-nosed monkeys inferred from mitochondrial DNA, cytochrome *b*, and 12S rRNA sequences. Int. J. Primatol. 25 (4), 861–873.

Messier, W., Stewart, C.-B., 1997. Episodic adaptive evolution of primate lysozymes. Nature 385, 151–154.

Moore, W.S., 1995. Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. Evolution 49, 718–726.

Napier, J.R., Napier, P.H., 1970. Old World Moneys: Evolution, Systematics, and Behavior. Academic Press, New York.

Oates, J.F., Davies, A.G., Delson, E., 1994. The diversity of the living colobines. In: Davies, A.G., Oates, J.F. (Eds.), Colobine Monkeys: Their

Ecology, Behavior and Evolution. Cambridge University Press, Great Britain, pp. 45–74.
Oates, J.F., Davies, A.G., 1994. What are the colobines? In: Davies, A.G., Oates, J.F. (Eds.), Colobine Monkeys: Their Ecology, Behavior and Evolution. Cambridge University Press, Great Britain, pp. 1–9.
Page, S.L., Chiu, C., Goodman, M., 1999. Molecular phylogeny of Old World monkeys (Cercopithecidae) as inferred from y-globin DNA sequences. Mol. Phylogenet. Evol. 13, 348–359.
Posada, D., Crandall, K.A., 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14, 817–818.
Raaum, R.L., Sterner, K.N., Noviello, C.M., Stewart, C.-B., Disotell, T.R., 2005. Catarrhine primate divergence dates estimated from complete mitochondrial genomes: concordance with fossil and nuclear DNA evidence. J. Hum. Evol. 48 (3), 237–257.
Ronquist, F., Huelsenbeck, J.P., 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
Royston, P., 1995. A Remark on algorithm AS 181: the W test for normality. Appl. Stat. 44, 547–551.
Sanderson, M.J., 2004. r8s version 1.70. Analysis of rates ("r8s") of evolution (and other stuff). <http://ginger.ucdavis.edu/r8s//>.
Strasser, E., Delson, E., 1987. Cladistic analysis of cercopithecid relationships. J. Hum. Evol. 16, 81–99.
Swofford, D.L. 2004. PAUP*: Phylogenetic Analysis Using Parsimony (* and other methods), version 4. Sinauer Associates, Sunderland, Massachusetts.
Szalay, F.S., Delson, E., 1979. Evolutionary History of the Primates. Academic Press, New York.
Thalmann, O., Hebler, J., Poinar, H.N., Pääbo, S., Vigilant, L., 2004. Unreliable mtDNA data due to nuclear insertions: a cautionary tale from analysis of humans and other great apes. Mol. Ecol. 13, 321–335.
Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.
Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25, 4876–4882.
Wang, W., Su, B., Lan, H., Zhang, Y., Lin, S., Liu, Al., Liu, R., Ji, W., Hu, H., Xie, Y., Wu, D., 1995. Phylogenetic relationships among two species of golden monkey and three species of leaf monkey inferred from rDNA variation. Folia Primatol. 65, 138–143.
Wang, W., Forstner, M.R.J., Zhang, Y., Liu, Z., Wei, Y., Huang, H., Hu, H., Xie, Y., Wu, D., Melnick, D.J., 1997. A phylogeny of Chinese leaf monkeys using mitochondrial ND3-ND4 gene sequences. Int. J. Primatol. 18, 305–320.
Whittaker, D.J., Ting, N., Melnick, D.J., in press. Molecular phylogenetic affinities of the Simakobu monkey (*Simias concolor*). Mol. Phylogenet. Evol., doi:10.1016/j.ympev.2005.12.013.
Xing, J., Wang, H., Han, K., Ray, D.A., Huang, C.H., Chemnick, L.G., Stewart, C.-B., Disotell, T.R., Ryder, O.A., Batzer, M.A., 2005. A mobile element based phylogeny of Old World monkeys. Mol. Phylogenet. Evol. 37 (3), 872–880.
Yang, Z., 1997. PAML: a program package for phylogenetic analysis by maximum likelihood. Comput. Appl. BioSci. 13, 555–556.
Yang, Z., 2004. Phylogenetic Analysis by Maximum Likelihood (PAML) version 3.14. <http://abacus.gene.ucl.ac.uk/software/paml.html/>.
Zhang, Y., Ryder, O.A., 1998. Mitochondrial cytochrome *b* gene sequences of Old World monkeys: with a special reference on evolution of Asian colobines. Primates 39, 39–49.