

Short communication

Molecular phylogenetic affinities of the simakobu monkey (*Simias concolor*)

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1. Introduction

The simakobu (*Simias concolor*), or pig-tailed snub-nosed langur, is one of four endemic primate species on the Mentawai Islands off the west coast of Sumatra, Indonesia, and is currently considered a monotypic genus (Brandon-Jones et al., 2004; Miller, 1903). This species, like many other colobines, is a specialized folivore found in tropical rainforests. The simakobu monkey is a member of the “odd-nosed” group of Asian colobines, an informal grouping that also includes the proboscis monkey of Borneo (*Nasalis larvatus*), the snub-nosed monkeys of China and Vietnam (*Rhinopithecus* spp.), and the douc langurs of Vietnam, Laos, and Cambodia (*Pygathrix* spp.). There is no agreement, however, on the relationships among these genera, nor whether they even form a natural taxonomic or phylogenetic group (Delson, 1975; Groves, 1970, 2001; Jablonski, 1998; though see Sterner et al., in press).

Geographically, the simakobu is found closest to the proboscis monkey, and some morphological data have indicated that these species are sister taxa. Both *Simias* and *Nasalis* exhibit narrow, long-faced skulls; long narrow nasal bones; and similar hair patterns (Groves, 1970). These two species appear to share more traits than do *Rhinopithecus* and *Pygathrix*, and some researchers have suggested that *Simias* should be subsumed into the genus *Nasalis* (Delson, 1975; Groves, 1970), perhaps as a subgenus (Delson, 1975); Groves (2001; Brandon-Jones, et al., 2004) appears to have retreated from the close linkage of these genera, though is not necessarily opposed to it (Groves, pers. comm.).

Alternative hypotheses suggest that there is no evidence that *Simias* and *Nasalis* are phylogenetically linked (Napier, 1985), and that the former may be a basal colobine unrelated to other members of the “odd-nosed” group (Jablonski, 1998). Other Mentawai primates have also been suggested as basal members of their respective lineages. While Brandon-Jones (1998) considers *Simias* a member of the genus *Nasalis*, he has suggested that the other three Mentawai primate species display primitive characteristics and are basal to Southeast Asian primate lineages. According to Brandon-Jones (1998), the primates of Sumatra went extinct during Pleistocene glaciations, and the Mentawai Islands may have provided a reservoir from which primates recolonized Sumatra during interglacials (Brandon-Jones, 1998). However, this scenario is not supported by more recent biogeographic analyses of *Presbytis* and genetic analyses of the Mentawai gibbon (*Hylobates klossii*) (Meijaard and Groves, 2004; Takacs et al., 2005; Whittaker, 2005).

To date, few behavioral or ecological studies have been conducted focusing on the simakobu monkey. The proboscis monkey was previously believed to be dependent on mangrove and coastal forests, while the simakobu is typically found in inland evergreen tropical rainforest. This habitat difference has led some researchers to conclude that these species are quite distinct ecologically and thus should be separated at the genus level (Napier, 1985; Oates et al., 1994). However, more recent data have shown that proboscis monkeys are more widely distributed than previously thought, occurring throughout Borneo (Meijaard and Nijman, 2000). The odd-nosed genera *Simias*, *Nasalis*, and *Pygathrix* seem to follow a similar adaptive strategy, including the exploitation of high-quality foods during part of the year with the ability to subsist on low-quality diets at

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other times (Bennett and Davies, 1994). These three genera also appear to have some flexibility in their social systems: in the simakobu monkey, social groups contain one male and either one or several females, plus their offspring (Fuentes and Tenaza, 1995; Tenaza and Fuentes, 1995; Watanabe, 1981), while proboscis monkeys are typically found in one-male multi-female groups, but with overlapping home ranges (Bennett and Sebastian, 1988). Neither the simakobu nor the proboscis monkey have been able to out-compete sympatric colobine species, in apparent contrast to members of the genus *Pygathrix* (Bennett and Davies, 1994). These ecological data suggest many similarities between *Simias* and *Nasalis*, though no firm conclusions can be drawn without further field studies of *Simias*.

We present here the results of the first molecular study to test hypotheses regarding the phylogenetic position and taxonomic status of the simakobu monkey.

2. Methods

Hairs were collected from a wild-caught infant simakobu pet in the village of Sikakap on North Pagai Island (Fig. 1) and stored dry in a tube. The monkey was likely caught in the Pagai Islands, but its origin could have been from another Mentawai Island.

Total genomic DNA was extracted from four hairs, using the Qiagen DNA Micro Kit and manufacturer-supplied protocols for hairs without roots. We amplified and sequenced 424 bp of mitochondrial DNA using universal cytochrome *b* primers (L14724, H15149) (Kocher et al., 1989). This region includes part of the tRNA^{thr} (24 bp) and cytochrome *b* genes (400 bp). Polymerase chain reaction (PCR) was performed in 50 μ l reactions containing 4 μ l of template DNA, 1 μ M each of primers L14724 and H15149, 0.25 mM dNTPs, 2.0 mM MgCl₂, and 1.25 U AmpliTaq DNA polymerase (Applied Biosystems). The PCR was run in a GeneAmp PCR System 9700 (Applied Biosystems) thermocycler with the following conditions: initial 2 min at 94 °C; followed by 45 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 2 min, and extension at 72 °C for 3 min; with a final extension at 72 °C for 7 min. A negative control containing no DNA was included in addition to the six replications of the *Simias* sample.

The PCR products were electrophoresed on an agarose gel, stained with ethidium bromide, and digitally photographed under UV light. The PCR products were purified using the QIAquick PCR Purification Kit (Qiagen) according to the manufacturer's instructions. Cycle sequencing was performed in 10 μ l volume reactions, containing 0.8 μ l of the purified PCR product and 0.5 μ l sequencing primer in a BigDye Terminator 3.1 Cycle Sequence reaction mixture (Applied Biosystems). Cycle sequence products were purified using the ethanol/EDTA/sodium acetate protocol specified by Applied Biosystems.

Sequencing was performed on an ABI 3730XL capillary sequencer. A consensus sequence from both strands and from six replications was generated using Sequencher 3.1. The *Simias* sequence has been submitted to GenBank (Accession No. DQ143883). Comparative sequence data from other cercopithecoid genera were obtained from GenBank (Table 1), including *Papio* and *Macaca* as outgroups. The sequences downloaded from GenBank were compared

Table 1

List of samples used in study. Taxonomy follows Brandon-Jones et al. (2004)

Taxon	GenBank Acquisition Number
<i>Simias concolor</i>	DQ143883
<i>Nasalis larvatus</i> (2)	AF020418, U62663
<i>Rhinopithecus roxellana</i>	AF020416
<i>Rhinopithecus avunculus</i>	AF020415
<i>Rhinopithecus bieti</i>	AF020413
<i>Pygathrix nemaes</i>	AF295582
<i>Semnopithecus entellus</i>	AF020417
<i>Semnopithecus johnii</i>	AF020419
<i>Semnopithecus vetulus</i>	AF020420
<i>Trachypithecus francoisi</i>	AF295578
<i>Trachypithecus obscurus</i>	AY863425
<i>Colobus guereza</i>	AY863427
<i>Colobus angolensis</i>	AF295583
<i>Colobus polykomos</i>	AF020411
<i>Procolobus badius</i>	AF295575
<i>Papio hamadryas</i>	Y18001
<i>Macaca mulatta</i>	NC005943

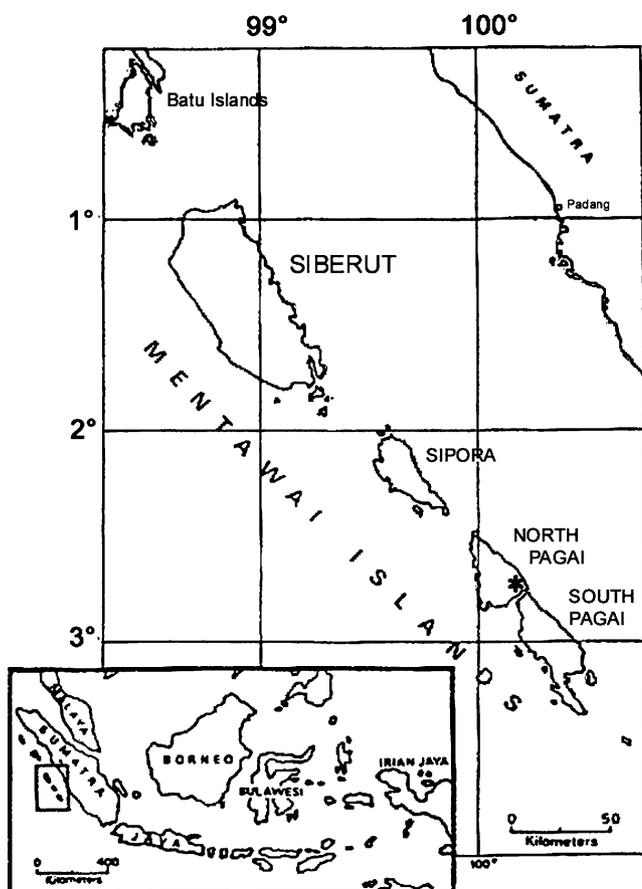


Fig. 1. Map showing location of the Mentawai Islands. Location of the village of Sikakap on North Pagai Island is indicated with an asterisk (*).

Table 2
Molecular pairwise distance matrix derived from the cytochrome *b* data

Taxon	<i>S. concolor</i>	<i>N. larvatus1</i>	<i>N. larvatus2</i>	<i>R. roxellana</i>	<i>R. avunculus</i>	<i>R. bieti</i>	<i>P. nemaesus</i>	<i>S. entellus</i>	<i>S. johinii</i>	<i>S. vetulus</i>	<i>T. francoisi</i>	<i>T. obscurus</i>	<i>C. guereza</i>	<i>C. angolensis</i>	<i>C. polykomos</i>	<i>P. badius</i>	<i>P. hamadryas</i>	<i>M. mulatta</i>
<i>S. concolor</i>	—	0.063	0.067	0.148	0.176	0.157	0.161	0.186	0.195	0.170	0.160	0.167	0.215	0.219	0.230	0.225	0.213	0.252
<i>N. larvatus1</i>	0.059	—	0.005	0.159	0.165	0.178	0.211	0.202	0.184	0.179	0.153	0.155	0.256	0.252	0.261	0.247	0.210	0.236
<i>N. larvatus2</i>	0.062	0.005	—	0.166	0.182	0.182	0.213	0.199	0.192	0.183	0.161	0.163	0.257	0.263	0.259	0.249	0.219	0.245
<i>R. roxellana</i>	0.125	0.135	0.139	—	0.060	0.050	0.134	0.192	0.168	0.143	0.142	0.168	0.251	0.233	0.279	0.216	0.209	0.272
<i>R. avunculus</i>	0.144	0.139	0.151	0.057	—	0.068	0.167	0.212	0.194	0.169	0.165	0.185	0.275	0.281	0.292	0.225	0.202	0.279
<i>R. bieti</i>	0.132	0.149	0.151	0.047	0.064	—	0.143	0.183	0.161	0.156	0.167	0.182	0.250	0.214	0.280	0.222	0.211	0.278
<i>P. nemaesus</i>	0.136	0.170	0.172	0.116	0.141	0.124	—	0.246	0.214	0.205	0.192	0.186	0.202	0.212	0.207	0.253	0.225	0.234
<i>S. entellus</i>	0.156	0.168	0.166	0.158	0.173	0.154	0.195	—	0.088	0.099	0.214	0.198	0.236	0.275	0.245	0.201	0.251	0.231
<i>S. johinii</i>	0.161	0.154	0.158	0.142	0.161	0.137	0.175	0.080	—	0.104	0.196	0.180	0.218	0.243	0.231	0.209	0.217	0.229
<i>S. vetulus</i>	0.147	0.154	0.156	0.125	0.145	0.135	0.170	0.090	0.095	—	0.191	0.201	0.242	0.237	0.258	0.216	0.222	0.246
<i>T. francoisi</i>	0.136	0.133	0.139	0.123	0.140	0.140	0.161	0.174	0.162	0.160	—	0.095	0.170	0.192	0.179	0.215	0.219	0.216
<i>T. obscurus</i>	0.140	0.133	0.138	0.140	0.152	0.149	0.155	0.164	0.152	0.164	0.086	—	0.225	0.204	0.233	0.249	0.209	0.205
<i>C. guereza</i>	0.172	0.198	0.199	0.193	0.208	0.193	0.167	0.188	0.179	0.193	0.145	0.180	—	0.067	0.010	0.187	0.198	0.231
<i>C. angolensis</i>	0.173	0.195	0.201	0.181	0.208	0.171	0.172	0.210	0.193	0.190	0.158	0.165	0.062	—	0.073	0.198	0.191	0.232
<i>C. polykomos</i>	0.182	0.200	0.200	0.213	0.220	0.213	0.172	0.194	0.189	0.206	0.152	0.185	0.010	0.068	—	0.199	0.212	0.232
<i>P. badius</i>	0.179	0.193	0.194	0.174	0.181	0.178	0.199	0.168	0.173	0.178	0.175	0.192	0.155	0.162	0.165	—	0.227	0.284
<i>P. hamadryas</i>	0.174	0.174	0.180	0.171	0.169	0.174	0.185	0.200	0.178	0.183	0.178	0.173	0.166	0.160	0.176	0.185	—	0.170
<i>M. mulatta</i>	0.200	0.193	0.198	0.212	0.217	0.217	0.193	0.189	0.190	0.199	0.180	0.173	0.189	0.188	0.190	0.220	0.145	—

Figures below the diagonal are uncorrected (*p*) distances; figures above the diagonal are Tamura–Nei distances. Note that individuals differing less than 10% (shaded cells) are generally considered congeners, with the exception of *Simias* and *Nasalis*.

4. Summary and discussion

An analysis of part of the mitochondrial cytochrome *b* gene strongly supports a sister taxon relationship between the simakobu and proboscis monkeys, and places this lineage within a monophyletic Asian colobine clade. This study is not inconsistent with the classification of the simakobu within the genus *Nasalis*, as suggested by earlier morphological analyses (Delson, 1975, 2000; Groves, 1970). Few studies have been conducted on the behavior and ecology of the simakobu, but some data indicate that *Simias* and *Nasalis* are following similar adaptive strategies (Bennett and Davies, 1994).

The hypothesis that *Simias* is a basal colobine (Jablonski, 1998) is not supported, nor is the corresponding suggestion for *Nasalis* (Groves, 1989). Recent genetic analyses of the Mentawai primates *H. klossii* and *Macaca pagensis* have consistently shown these species to be derived members of their respective clades with their closest relatives on nearby Sumatra (Roos et al., 2003; Takacs et al., 2005; Whittaker, 2005), and not primitive or ancestral as has sometimes been suggested. The phylogenetic tree presented here shows well-supported monophyletic Asian and African clades, but provides little resolution otherwise. *Nasalis* and *Simias* form a clade, but relationships among most of the Asian colobine genera appear as an unresolved polytomy. Further research is necessary to understand the phylogenetic and biogeographic history of the Asian colobines.

Currently, two subspecies are defined for *S. concolor* based on differences in pelage coloration: *S. concolor siberu* on the northernmost island of Siberut and *S. c. concolor* on the three southern islands. The same pattern is described in *P. potenziani* and *M. pagensis*, though not for *H. klossii* (Whittaker, 2005). Preliminary genetic analysis of *M. pagensis* suggests a paraphyletic origin (Roos et al., 2003). Future studies need to examine intraspecific patterns of divergence in Mentawai primates.

The simakobu monkey is among the least understood, but most endangered, of all the Asian primates. The IUCN Red List currently lists this species as “Endangered,” under criteria A1cd+2c (IUCN, 2004). However, a recent reassessment of the available evidence suggests that the status of *S. concolor* should be upgraded to “Critically Endangered” based on criteria A2cd (Whittaker, 2005). The simakobu is also included in Conservation International’s list of the 25 Most Endangered Primates (Mittermeier et al., 2002). Taxonomic uniqueness is sometimes used as a criterion for setting conservation priorities (Olson and Dinerstein, 1998). Should the simakobu monkey be reclassified as a member of the genus *Nasalis*, its conservation priority (or that of the Mentawai Islands as a whole) may be affected based on such methods. We believe that taxonomy should reflect phylogeny, and we do not mean to imply that this unique primate, or any of the Mentawai primates, requires any less attention or conservation action.

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