



Mitochondrial phylogeny of leaf monkeys (genus *Presbytis*, Eschscholtz, 1821) with implications for taxonomy and conservation

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ABSTRACT

The langurs of the genus *Presbytis* inhabit tropical rainforests of Sundaland, and with more than 50 color variants grouped in up to eleven species, *Presbytis* is one of the most diverse Old World monkey genera. The number of taxa and their phylogenetic relationships however remain controversial. To address these issues, we analyzed a 1.8 kb long fragment of the mitochondrial genome, including the cytochrome b gene, the hypervariable region I of the D-loop and the intermediate tRNAs, from individuals representing nine species. Based on our data, we obtained various well-supported terminal clades, which refer mainly to described taxa. Relationships among these clades are not fully resolved, suggesting at least two radiations in the evolutionary history of the genus. According to divergence age estimates, radiations occurred in the late Miocene and the early to middle Pleistocene. Our findings support the revision of the current classification of the genus *Presbytis* and enable us to discuss implications for conservation. However, further studies including nuclear sequence data are necessary to completely understand the evolutionary history of the genus, and to address possible hybridization events among taxa.

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1. Introduction

Langurs of the Asian colobine genus *Presbytis* are exclusively arboreal animals, which inhabit tropical rainforest habitats of Sundaland, i.e., the Malay Peninsula and the western Indo-Malay archipelago (Oates et al., 1994) (Fig. 1). Most *Presbytis* species live in unimale matrilineal or female bonded social systems, where males leave their natal troops at puberty (Bennett and Davies, 1994; Newton and Dunbar, 1994), but female dispersal is also reported (Sterck et al., 1997, 2005). Mainly driven by Sundaland's dramatic geological and climatic changes during the past million years, the genus has undergone an extensive radiation (Meijaard, 2004). With more than 50 described color variants, currently grouped into ten (Brandon-Jones et al., 2004) or eleven species

(Groves, 2001), *Presbytis* is one of the most diverse primate genera among Old World monkeys.

The classification of contemporary taxa and the evolutionary history of the genus, however, are poorly understood. Since the seminal work of Napier and Napier (1967), the genus *Presbytis* has been subject to a long history of frequent taxonomic revisions. Almost all proposed taxonomies and phylogenies for the genus are based on behavioral and anatomical features, largely coat coloration (Brandon-Jones, 1978, 1996a,b; Brandon-Jones et al., 2004; Chasen, 1940; Groves, 1989, 2001; Hooijer, 1962; Napier and Napier, 1967; Wilson and Wilson, 1977), while molecular genetic approaches are limited to a single study (Md Zain, 2001). Unfortunately, the conclusions arising from these studies are at best inconsistent and often contradictory. In particular, the taxonomic status of the Sumatran langurs of the *melalophos* group (Brandon-Jones et al., 2004; Groves, 2001; Md Zain, 2001; Md Zain et al., 2002; SAMD, 2006) and the Javanese *comata* group (Brandon-Jones, 1995, 1996c; Groves, 2001; Nijman, 1997, 2001) remains to be resolved. Similarly, the phylogenetic relationships among taxa and the biogeographic history of the genus have yet to be clarified.

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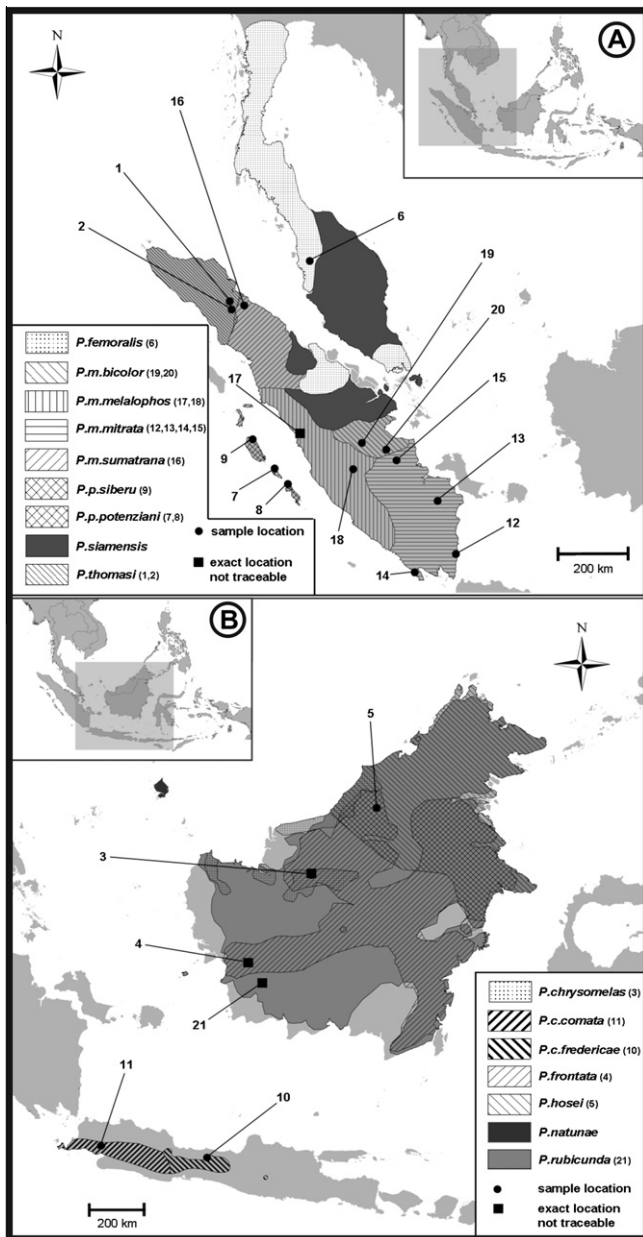


Fig. 1. Present distribution range of *Presbytis* on the Asian Mainland, Sumatra and the Mentawai Islands (A) and on Borneo, Java and the Natuna Islands (B). Numbers indicate the origin of samples (see Table 1).

For example, current reconstructions of the evolutionary history of the genus have yielded entirely conflicting scenarios, one proposing that either the Mentawai langur, *P. potenziiani* (Brandon-Jones, 1978, 1996a; Meijaard and Groves, 2004) off the west coast of Sumatra, or the Hose's langur, *P. hosei* (Md Zain, 2001), from Borneo is the sister to all *Presbytis* congeners. Unfortunately, Md Zain (2001) did not sample *P. potenziiani*.

In the present study, we analyze a 1.8 kb long fragment of the mitochondrial genome, including the cytochrome b (cytb) gene, the hypervariable region I (HVI) of the D-loop and the intermediate transfer RNAs (tRNA), from 31 individuals representing nine species. Based on analysis of an extensive range of samples derived predominantly from wild living animals of known location, our results enable us to (a) provide the most complete phylogeny of *Presbytis* available to date, (b) estimate divergence times between lineages, (c) provide a reliable basis for their taxonomic classification, and (d) discuss implications for conservation.

2. Material and methods

2.1. Sample collection

Fecal samples from 15 wild *Presbytis* populations were collected during two field surveys in September to October 2007 and June to November 2008. The localities spanned the range of *P. comata* (Java), *P. melalophos* (Sumatra), *P. thomasi* (Sumatra), *P. potenziiani* (Mentawai Islands) and *P. hosei* (Borneo) (Fig. 1). Animals were tracked in the early morning and followed until they defecated. Taxon identity of individuals was ascertained by pelage coloration, morphology, vocalization and geographic origin. The geographical position of the sampling sites was determined by GPS coordinates. Only fresh fecal samples were collected and preserved following the two-step ethanol-silica method described by Nsubuga et al. (2004). We additionally included two fecal samples from captive animals (*P. m. mitrata*, Schmutzer Primate Centre of the Ragunan Zoo, Jakarta; *Presbytis m. melalophos*, Howletts Wild Animal Park, Port Lympne) and four dry tissue samples from museum specimens preserved at the Bavarian State Collection of Zoology (*P. frontata*, coll. no. 1909/1394; *P. rubicunda*, coll. no. 1909/784; *P. chrysomelas*, coll. no. 1909/832; *P. m. sumatrana*, coll. no. 1921/226). The skins of the museum specimens were checked for their taxonomic affiliation by examining morphological features particularly pelage coloration and their origin. Tissue samples were stored in plastic bags without any additive. For details about sampling sites see Table 1 and Fig. 1.

2.2. Laboratory work

Genomic DNA from feces was extracted using the QIAamp™ Stool Mini Kit from Qiagen following the procedures recommended by the supplier, with the exception that the DNA was diluted in HPLC quality water and stored at -20°C before further processing. DNA from tissue material was extracted with the QIAamp™ DNA Mini Kit from Qiagen. The complete 1.8 kb fragment of the mitochondrial genome, which spans the complete cytb gene (1140 bp), the tRNAs (ca. 140 bp) for Threonine (tRNA-Thr) and Prolin (tRNA-Pro), and the HVI region of the D-loop (ca. 533 bp), was amplified via four overlapping fragments using primers listed in Table 2.

For all amplifications, standard wax-mediated hot-start PCRs were performed. The reactions were carried out in a total volume of 30 μl containing a final concentration of 0.33 μM of each primer, 3 mM MgCl_2 , 0166 mM dNTPs, 1 \times buffer and 1 U Taq DNA polymerase (Biotherm, Genecraft). PCR conditions for all amplifications were identical and consisted of a pre-denaturation step at 94°C for 2 min, followed by 40 cycles each with denaturation at 94°C for 1 min, annealing at variable temperatures (Table 2) for 1 min, and elongation at 72°C for 1 min. At the end, a final elongation step at 72°C for 5 min was added. Aliquots of all PCR amplifications were checked on 1% agarose gels and subsequently cleaned with the Qiagen PCR Purification Kit. Forward and reverse sequences of the PCR products were analyzed on an ABI 3730xl DNA Analyzer (Applied Biosystems) using the BigDye™ Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems). To prevent cross-species contamination, laboratory methods followed described standards (Osterholz et al., 2008; Roos et al., 2008; Thinh et al., 2010a,b). Generated sequences were assembled and edited using Geneious Pro 4.7 (Drummond et al., 2009) and manually checked by eye. The cytb sequences were further checked for their potential to be correctly transcribed. All newly generated sequences were deposited in GenBank and are available under the accession numbers JF295096–JF295125 (see also Table 1).

Table 1
Details on the *Presbytis* samples used in this study^a.

| Taxon | Location (number) | Source | Sample code | Accession number |
|---------------------------------|-----------------------------------|---------|-------------|------------------|
| <i>P. thomasi</i> | Bukit Lawang, Sumatra (1) | Wild | Blaw | JF295124 |
| <i>P. thomasi</i> | Tangkahan, Sumatra (2) | Wild | Tang | JF295125 |
| <i>P. chrysomelas</i> | Kuna, Borneo (3) | Museum | Kun | JF295112 |
| <i>P. frontata</i> | Paian, Borneo (4) | Museum | Pai | JF295113 |
| <i>P. hosei</i> | Bukit Nakan, Sarawak (5) | Wild | Msa | JF295114 |
| <i>P. femoralis robinsoni</i> | Redang Panjang, Malaysia (6) | GenBank | Mno | DQ355299 |
| <i>P. potenziani potenziani</i> | Sipora, Mentawai Islands (7) | Wild | Sip | JF295122 |
| <i>P. potenziani potenziani</i> | North Pagai, Mentawai Islands (8) | Wild | NPag | JF295123 |
| <i>P. potenziani siberu</i> | Pungut, Mentawai Islands (9) | Wild | Pun1 | JF295121 |
| <i>P. potenzinai siberu</i> | Pungut, Mentawai Islands (9) | Wild | Pun2 | JF295119 |
| <i>P. potenziani siberu</i> | Pungut, Mentawai Islands (9) | Wild | Pun3 | JF295120 |
| <i>P. comata fredericae</i> | Mt. Slamet, Java (10) | Wild | Sit1 | JF295115 |
| <i>P. comata fredericae</i> | Mt. Slamet, Java (10) | Wild | Sit2 | JF295116 |
| <i>P. comata comata</i> | TN Gunung Halimun, Java (11) | Wild | Hal1 | JF295118 |
| <i>P. comata comata</i> | TN Gunung Halimun, Java (11) | Wild | Hal2 | JF295117 |
| <i>P. melalophos mitrata</i> | TN Way Kambas, Sumatra (12) | Zoo | Rag | JF295098 |
| <i>P. melalophos mitrata</i> | TN Way Kambas, Sumatra (12) | Wild | Wk1 | JF295097 |
| <i>P. melalophos mitrata</i> | TN Way Kambas, Sumatra (12) | Wild | Wk2 | JF295096 |
| <i>P. melalophos mitrata</i> | Riding, Sumatra (13) | Wild | Rid | JF295099 |
| <i>P. melalophos mitrata</i> | Way Canguk, Sumatra (14) | Wild | Wc1 | JF295100 |
| <i>P. melalophos mitrata</i> | Way Canguk, Sumatra (14) | Wild | Wc2 | JF295101 |
| <i>P. melalophos mitrata</i> | Sungai Gelam, Sumatra (15) | Wild | Gel1 | JF295103 |
| <i>P. melalophos mitrata</i> | Sungai Gelam, Sumatra (15) | Wild | Gel2 | JF295102 |
| <i>P. melalophos sumatrana</i> | Deli (Medan), Sumatra (16) | Museum | Med | JF295110 |
| <i>P. melalophos melalophos</i> | West Sumatra, Sumatra (17) | Zoo | How | JF295105 |
| <i>P. melalophos melalophos</i> | Bangko, Sumatra (18) | Wild | Ban | JF295104 |
| <i>P. melalophos bicolor</i> | Bukit Tigapuluh, Sumatra (19) | Wild | Btp1 | JF295109 |
| <i>P. melalophos bicolor</i> | Bukit Tigapuluh, Sumatra (19) | Wild | Btp2 | JF295106 |
| <i>P. melalophos bicolor</i> | Bukit Tigapuluh, Sumatra (19) | Wild | Btp3 | JF295108 |
| <i>P. melalophos bicolor</i> | Sengeti, Sumatra (20) | Wild | Seng | JF295107 |
| <i>P. rubicunda</i> | Paun, Borneo (21) | Museum | Pau | JF295111 |

^a For locations see also Fig. 1.

2.3. Statistical analysis

To expand our dataset, we further incorporated orthologous sequences available at GenBank from one *P. melalophos* (DQ355299) which actually refers due to locality to *P. femoralis robinsoni* (Redang Panjang, Malaysia, pers. comm. Nelson Ting) and one *Trachypithecus obscurus* (AY863425), which was used as an out-group. Sequences were aligned with the ClustalW program as implemented in Geneious and manually checked by eye. A computerized method was applied to eliminate poorly aligned positions and divergent regions using Gblocks 0.91b (Castresana, 2000). Therefore, a relaxed selection of blocks was selected (Talavera and Castresana, 2007). Phylogenetic trees were constructed with neighbor-joining (NJ), maximum-likelihood (ML) and Bayesian approaches. Data were divided into three partitions

Table 2
Information about primers used in this study^a.

| Locus | Primer (ID) | Primer sequence 5'–3' | AT |
|----------|-------------------|--------------------------------|----|
| tRNA-Glu | H-45 uni F (6405) | AAT GAT ATG AAA ARY CAT CGT TG | 58 |
| tRNA-Thr | 15510 R (6232) | TGT CCG TTT CCA GTT TAC AAG | 60 |
| cytb | PresCytbR1 (6719) | TTR TCT GGG TCG CTY AAA AG | 58 |
| cytb | Pre945-F (6435) | TCG CCC AYT TAG CCA ATT CC | 58 |
| cytb | PresCytbF2 (6720) | CTR TTT CTA CAC GAA ACA GG | 58 |
| tRNA-Pro | PresCytbR2 (6721) | AAT ACA GAA AGT AGT TTA AAT AG | 54 |
| HVI | 2068R (2068) | ATT GAT TTC ACG GAG GAT GGT | 56 |
| tRNA-Pro | 2067 F (2067) | CTG GCA TTC TAT TTA AAC TAC TT | 58 |
| HVI | 16220 R (6234) | TGA TAG ACC CGT GAT CCA TC | 58 |
| tRNA-Thr | PresLoopF (6722) | AAA TAC ACC AGT CTT GTA AAC | 54 |
| HVI | PresLoopR (6723) | TTT AAG GGG AAC GTG TGA G | 52 |

^a The complete cytb was obtained by amplifying overlapping fragments (primer pairs 6405/6232 and 6435/2068 or 6405/6719 and 6720/6721). The HVI locus was amplified with primer pairs 2067/6234 and 6722/6723. AT: Annealing temperature.

(cytb, tRNAs, HVI). As the optimal nucleotide substitution model for each partition, the GTR+I+G model was chosen by the Bayesian Information Criterion (BIC) with jModelTest 0.1.1 (Posada, 2008). ML analyses were conducted using Garli v0.951 (Zwickl, 2006). In Garli, only the model specifications settings were adjusted, while all other settings were left at their default values. ML bootstrap percentages were estimated in Garli by performing 500 pseudoreplicate runs. 10 replicates were run to verify consistency in log likelihood scores and tree topologies. A 50% majority rule consensus tree was calculated with Paup* v4.0b10 (PPC) (Swofford, 2002). Phylogenetic relationships based on the NJ algorithm were calculated in Paup. Relative support of internal nodes was performed by bootstrap analyses with 10,000 replications. For the Bayesian analysis in MrBayes v3.1.2 (Huelsenbeck et al., 2001; Ronquist and Huelsenbeck, 2003), the dataset was partitioned into the three portions. Four Markov Chain Monte Carlo (MCMC) runs with a default temperature of 0.2 and a chain length of 10,000,000 generations were carried out. Trees and parameters were sampled every 100 generations. Flat priors were assumed for the model parameters including the proportion of invariable sites and the gamma shape parameter of rate variation among sites. The first 25% of samples were discarded as burnin, leaving 75,001 trees per run. The adequacy of this burnin and convergence of all parameters was assessed by examining the uncorrected potential scale reduction factor (PSRF) (Gelman and Rubin, 1992), which should approach 1 as runs converge, and by visually inspecting the trace of the parameters across generations using the software Tracer v1.3 (Rambaut and Drummond, 2005). Posterior probabilities for each split and a phylogram with mean branch lengths were calculated from the posterior density of trees. Phylogenetic trees were visualized with FigTree v1.3.1 (Rambaut, 2006).

To estimate divergence times, we included sequences from another 13 primate species, which derived from GenBank (*Pongo*

pygmaeus, NC001646; *Pan troglodytes*, D38113; *Homo sapiens*, AY339522; *Chlorocebus aethiops*, NC007009; *Macaca sylvanus*, AJ309865; *Papio hamadryas*, EU885446; *Theropithecus gelada*, EU885487; *Colobus guereza*, NC00690; *Semnopithecus entellus*, EU004478; *Rhinopithecus avunculus*, EU004480; *Nasalis larvatus*, EU004476). Due to the high mutation rate in the HVI region, the calculation was performed solely on the *cytb* sequence data. For the estimation, we applied a Bayesian MCMC method, which employs a relaxed molecular clock approach (Drummond et al., 2006), as implemented in the BEAST v1.5beta2 package (Drummond, 2007). A relaxed lognormal model of lineage variation and a Yule prior for branching rates was assumed. The alignment was partitioned according to 1 + 2 and 3 codon positions. The substitution model, rate heterogeneity and base frequencies were unlinked across codon positions ((1 + 2), 3). As calibration points, we selected the divergence between Hominoidea and Cercopithecoidea (C1), which was dated between 24 and 29 million years ago (Ma) (Zalmout et al., 2010), the divergence between Ponginae and Hominiinae (C2) ~14 Ma (Kelley, 2002; Raam et al., 2005), the split between *Homo* and *Pan* (C3) 6–7 Ma (Brunet et al., 2002; Steiper and Young, 2006; Vignaud et al., 2002), and the separation of *Theropithecus* from *Papio* (C4) ~4 Ma (Delson et al., 2000; Leakey, 1993; Ting, 2008). Instead of hardbounded calibration points, we

used the published dates as a normal distribution prior for the respective node. For C1 this translates into a normal distribution with a mean of 26.5 Ma and a standard deviation (SD) of 1.36 Ma (95% credibility interval [CI]: 24.0–29.0 Ma), for C2 into a mean of 14.0 Ma and a SD of 0.60 Ma (CI: 13.0–15.0 Ma), for C3 into a mean of 6.5 Ma and a SD of 0.31 Ma (CI: 6.0–7.0 Ma), and for C4 into a mean of 4.0 Ma and a SD of 0.31 Ma (CI: 3.5–4.5 Ma). For the analysis, two replicates were run for 25 million generations with tree and parameter sampling occurring every 2500 generations. The adequacy of a 10% burnin and convergence of all parameters were assessed by visual inspection of the trace of the parameters across generations using the software Tracer. Subsequently, the sampling distributions of multiple independent replicates were combined with the software LogCombiner v1.4.6 and then summarized and visualized with TreeAnnotator v1.4.6. Both programs are part of the Beast package (Drummond, 2007).

3. Results

In this study, we successfully sequenced a ca. 1.8 kb fragment of the mitochondrial genome from a total of 30 *Presbytis* individuals, of which 29 were from known origins. By including an additional

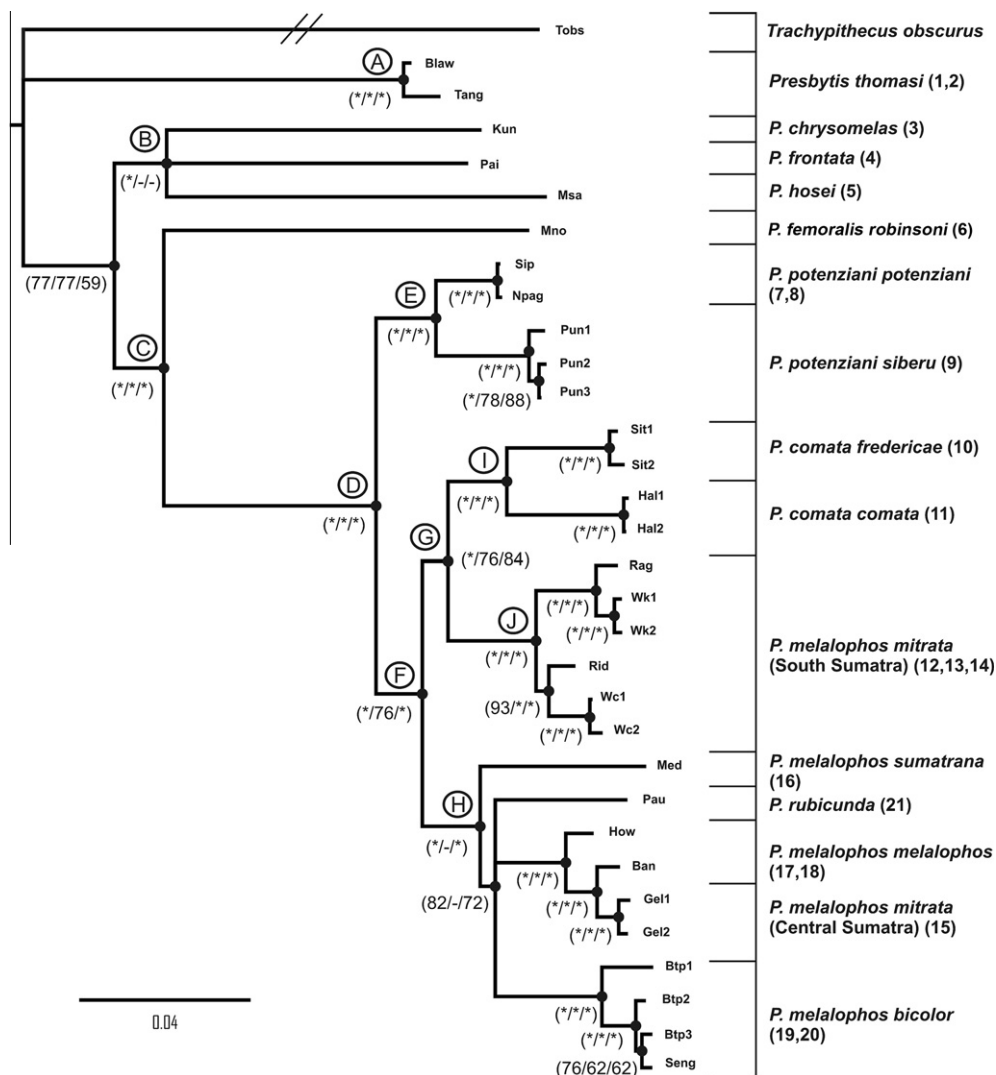


Fig. 2. Phylogenetic reconstruction derived from Bayesian, NJ and ML algorithms based on ~1.8 kb of the mitochondrial genome (for individual sequence codes see Table 1). Capital letters indicate clades mentioned in the text. Support values (posterior probabilities and bootstrap values) are given in brackets (Bayesian/NJ/ML). Asterisks indicate support values >96, hyphens indicate values <50.

sequence from *Presbytis femoralis* from GenBank and following the classification of Groves (2001), our data set comprises nine species and 14 subspecies. Nuclear pseudogenes could not be detected, given that overlapping fragments of different sequences from the same individual were identical, and that the cytb gene was correctly transcribed. Moreover, the amplification of nuclear pseudogenes (numts) is reduced since we used fecal and museum material in which nuclear DNA is highly degraded (Hofreiter et al., 2003; Thalmann et al., 2004).

The original alignment had a length of 1816 bp. Poorly aligned positions and divergent regions were solely detected within tRNAs and the HVI. After discarding these positions, the alignment was reduced to 1745 bp. Among them, 172 sites were parsimony-uninformative and 402 parsimony-informative. All sequences represented unique haplotypes.

Phylogenetic reconstructions based on NJ, ML and Bayesian algorithms (Fig. 2) revealed several strongly supported clades, which mainly referred to species and subspecies. However, although the branching pattern among various lineages gained only weak support, all algorithms showed similar tree topologies.

According to the phylogenetic reconstructions, the base of the tree indicates a separation of *P. thomasi* as the sister species to its congeners. However, since this initial split is only weakly supported (Bayesian: 77; NJ: 77; ML: 59), we interpret the base of the tree as a polytomy between *P. thomasi* (clade A), clade B, and the remaining *Presbytis* taxa (clade C). Clade B includes the Bornean species *P. chrysomelas*, *P. frontata* and *P. hosei*, but relationships among them remain unresolved. In clade C, *P. femoralis* represents the sister lineage to the remaining taxa (clade D), of which *P. potenziani* (clade E) is separated from the taxa on Sumatra and Java, and also from the Bornean *P. rubicunda* (clade F). Within *P. potenziani* (clade E), both subspecies, *P. p. potenziani* and *P. p. siberu*, form

reciprocally monophyletic clades. Clade F is further divided into subclades G and H. The former segregates into two strongly supported clades (I and J). The Javanese *P. comata* falls into clade I, which further diverges into two reciprocally monophyletic clades represented by the two subspecies, *P. c. comata* and *P. c. fredericae*. Clade J consists solely of South Sumatran *P. m. mitrata* individuals. Clade H contains an unresolved polytomy with four highly supported clades or lineages: *P. m. sumatrana*, *P. rubicunda*, *P. m. bicolor*, and a clade formed by *P. m. melalophos* and *P. m. mitrata* from Central Sumatra. Thus, *P. m. mitrata* is mitochondrially paraphyletic.

Based on divergence time estimates (Fig. 3, Table 3), Cercopithecidae separated from Hominidae (C1) 26.57 (24.26–29.09) Ma. Within Hominidae, *Pongo* branched off first (C2) 13.70 (12.54–14.79) Ma, followed by *Homo* and *Pan* (C3), which separated from each other 6.50 (5.92–7.09) Ma. The split between Colobinae and Cercopitheciinae (N1) occurred 19.41 (15.81–23.65) Ma. Within the latter, Papionini separated from Cercopitheciini (*C. aethiops*) 12.04 (8.97–15.30) Ma (N2). Among Papionini, *Macaca* diverged first (N3) 10.56 (7.71–13.46) Ma, followed by the differentiation of *Papio* and *Theropithecus* (C4) 4.02 (3.44–4.57) Ma. The African colobine genus *Colobus* separated from Asian colobines 15.39 (12.29–18.98) Ma (N4). Among the latter, the split between *Semnopithecus*/*Nasalis*/*Rhinopithecus* and *Trachypithecus*/*Presbytis* took place 12.31 (9.72–14.86) Ma (N5). *Semnopithecus* separated from *Nasalis* and *Rhinopithecus* 10.24 (7.56–12.93) Ma (N6) and latter two 7.51 (5.00–13.10) Ma (N7). The split between *Presbytis* and *Trachypithecus* (N8) occurred 11.46 (9.01–14.09) Ma. Within a relative short time period of only 1.43 (3.91–8.53) Ma (N9–N11), four major *Presbytis* lineages emerged. The first leads to *P. thomasi*, the second to a clade containing *P. chrysomelas*, *P. frontata* and *P. hosei*, the third to *P. femoralis*, and the fourth to a

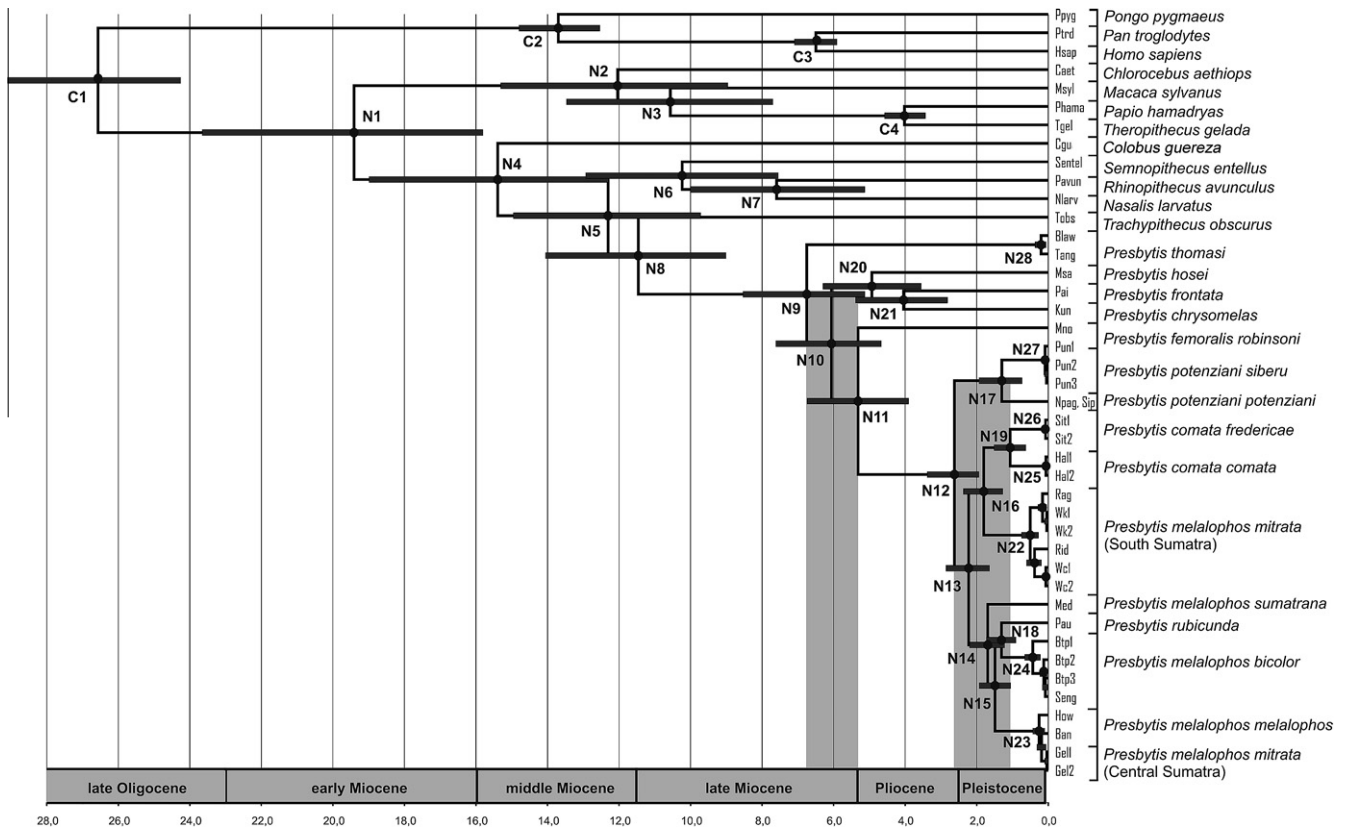


Fig. 3. Ultrametric tree showing phylogenetic relationships and estimated divergence ages among studied *Presbytis* individuals based on the complete mitochondrial cytb sequences (for individual codes see Table 1). A time scale in million years and the geological periods are given. Nodes of interest are arbitrarily numbered (N1–N28). C1–C4 refer to nodes used for calibration. Light gray bars indicate the two radiations.

Table 3
Bayesian divergence date estimates.

| Node | Mean (Ma) | 95% CI (Ma) |
|--|----------------------|---------------------------|
| C1 Hominoidea – Cercopithecoidea | 26.57 | 24.26–29.09 |
| C2 <i>Pongo</i> – <i>Pan</i> + <i>Homo</i> | 13.70 | 12.54–14.79 |
| C3 <i>Homo</i> – <i>Pan</i> | 6.50 | 5.92–7.09 |
| C4 <i>Papio</i> – <i>Theropithecus</i> | 4.02 | 3.44–4.57 |
| N1 Cercopithecinae – Colobinae | 19.41 | 15.81–23.65 |
| N2 Cercopithecini – Papionini | 12.04 | 8.97–15.30 |
| N3 <i>Macaca</i> – <i>Papio</i> + <i>Theropithecus</i> | 10.56 | 7.71–13.46 |
| N4 <i>Colobus</i> – Asian colobines | 15.39 | 12.29–18.98 |
| N5 <i>Semnopithecus</i> + <i>Nasalis</i> + <i>Pygathrix</i> – <i>Trachypithecus</i> + <i>Presbytis</i> | 12.31 | 9.72–14.86 |
| N6 <i>Semnopithecus</i> – <i>Nasalis</i> + <i>Rhinopithecus</i> | 10.24 | 7.56–12.93 |
| N7 <i>Nasalis</i> – <i>Pygathrix</i> | 7.51 | 5.00–13.10 |
| N8 <i>Trachypithecus</i> – <i>Presbytis</i> | 11.46 | 9.01–14.09 |
| N9 <i>P. thomasi</i> – remaining <i>Presbytis</i> taxa | 6.75 | 5.13–8.53 |
| N10 <i>P. hosei</i> + <i>P. chrysomelas</i> + <i>P. frontata</i> – remaining taxa | 6.06 | 4.67–7.61 |
| N11 <i>P. femoralis</i> – remaining taxa | 5.32 | 3.91–6.74 |
| N12 <i>P. potenziani</i> – remaining taxa | 2.62 | 1.94–3.38 |
| N13 <i>P. comata</i> + <i>P. m. mitrata</i> (South Sumatra) – remaining taxa | 2.22 | 1.65–2.85 |
| N14 <i>P. m. sumatrana</i> – <i>P. m. melalophos</i> + <i>P. m. mitrata</i> (Central Sumatra) + <i>P. m. melalophos</i> + <i>P. m. bicolor</i> + <i>P. rubicunda</i> | 1.69 | 1.22–2.20 |
| N15 <i>P. m. melalophos</i> + <i>P. m. mitrata</i> (Central Sumatra) – <i>P. rubicunda</i> + <i>P. m. bicolor</i> | 1.49 | 1.06–1.93 |
| | (1.40 ^a) | (0.91–1.93 ^a) |
| N16 <i>P. comata</i> – <i>P. m. mitrata</i> (South Sumatra) | 1.80 | 1.28–2.37 |
| N17 <i>P. p. siberu</i> – <i>P. p. potenziani</i> | 1.30 | 0.74–1.93 |
| N18 <i>P. rubicunda</i> – <i>P. m. bicolor</i> | 1.31 | 0.91–1.72 |
| N19 <i>P. c. comata</i> – <i>P. c. fredericae</i> | 1.06 | 0.63–1.51 |
| N20 <i>P. hosei</i> – <i>P. chrysomelas</i> + <i>P. frontata</i> | 4.93 | 3.56–6.29 |
| | (4.49 ^a) | (2.82–6.29 ^a) |
| N21 <i>P. chrysomelas</i> – <i>P. frontata</i> | 4.05 | 2.82–5.38 |
| N22 MRCA <i>P. m. mitrata</i> (South Sumatra) | 0.50 | 0.28–0.74 |
| N23 MRCA <i>P. m. melalophos</i> – <i>P. m. mitrata</i> (Central Sumatra) | 0.26 | 0.10–0.43 |
| N24 MRCA <i>P. m. bicolor</i> | 0.43 | 0.23–0.65 |
| N25 MRCA <i>P. c. comata</i> | 0.06 | 0.01–0.16 |
| N26 MRCA <i>P. c. fredericae</i> | 0.07 | 0.00–0.14 |
| N27 MRCA <i>P. p. siberu</i> | 0.09 | 0.02–0.17 |
| N28 MRCA <i>P. thomasi</i> | 0.20 | 0.07–0.36 |

Means and 95% credibility intervals (CI) are given for 31 nodes in Ma. Nodes used as calibration points are labeled with a “C”, all others with an “N”. MRCA denotes the most recent common ancestor.

^a Mean of divergence times of nodes that belong to unresolved polytomies (see also Fig. 3).

clade including all remaining taxa. In the latter, a subsequent radiation (N12–N19) leading to species and subspecies started with the separation of *P. potenziani* 2.62 (1.94–3.38) Ma (N12). Shortly afterwards, 2.22 (1.65–2.85) Ma (N13), South Sumatran *P. m. mitrata* and *P. comata* separated from Central Sumatran *P. m. mitrata*, *P. m. melalophos*, *P. m. sumatrana*, *P. m. bicolor* and *P. rubicunda*. In the former, *P. m. mitrata* diverged 1.8 (1.28–2.37) Ma (N16), before finally *P. comata* split into its two subspecies 1.06 (0.63–1.51) Ma (N19). In the latter clade, *P. m. sumatrana* diverged first (1.69 [1.22–2.20] Ma) (N14), followed by the *P. m. melalophos*/Central Sumatran *P. m. mitrata* clade 1.40 (0.91–1.93) Ma (N15), before finally *P. m. bicolor* separated from *P. rubicunda* 1.31 (0.91–1.72) Ma (N18).

4. Discussion

We report here on the analysis of mitochondrial sequences from 31 *Presbytis* individuals, representing nine of the eleven currently recognized species. Accordingly and since we mainly used only individuals from known locations, this study is the most complete and reliable one up to date. Moreover, calculated divergence ages

between various lineages are in similar ranges as earlier estimates (Chatterjee et al., 2009; Raaum et al., 2005; Sterner et al., 2006), although in general slightly older. According to our divergence age estimates, radiations within the genus occurred in two phases, one in the late Miocene and the other in the early to middle Pleistocene.

Interestingly, the tree topology depicted in the only other available molecular phylogeny (Md Zain, 2001) differs from our one, although both investigations are based on large segments of the mitochondrial genome, albeit with different samples and loci. In the study by Md Zain (2001), data rely on samples from wild populations from Malaysia, while samples from Indonesia came mainly from captive animals with unknown origin. Unfortunately, sequence data from the earlier study are not available for reanalysis and therefore a discussion of disagreements would remain speculative. However, due to our extensive sampling from clearly identified wild populations, we regard our data as more reliable.

Although *P. siamensis* and *P. natunae* are not represented, the results allow the most comprehensive evaluation of the evolutionary history of the genus to date including material from key taxa such as *P. potenziani* that have not been analyzed before and thus provide a sound basis for a revised taxonomic classification of the *Presbytis* genus.

4.1. Phylogenetic relationships

Based on the gray coat coloration, *P. thomasi*, *P. hosei* and *P. comata* have previously been combined in *P. comata* with a tripartite geographic distribution on West Java (*P. comata*), North Sumatra (*P. thomasi*) and North Borneo (*P. hosei*) (Brandon-Jones, 1978, 1996a,b; Chasen, 1940; Hooijer, 1962). Our present data, however, do not support a polytypic *P. comata* species, but instead indicate an early separation of *P. thomasi* and *P. hosei* in respective lineages and a late differentiation of *P. comata*.

Concerning *P. potenziani* our data reveal interesting results. Since the discovery of *potenziani* there have been conflicting statements on its taxonomic position relative to other Asian colobines (Tilson, 1976, p. 777). Based on postcranial data (Washburn, 1944) or infant coloration (Medway, 1970), *potenziani* was grouped together with *Trachypithecus*, while a morphological study by Groves (1970) proposed an intermediate position between *Trachypithecus* and *Presbytis*. Subsequent studies of infant coloration (Tilson, 1976) and male vocalization (Wilson and Wilson, 1975) grouped *potenziani* together with *Presbytis*. The similarity in the adult vocalization between *P. potenziani* and *P. thomasi* led Wilson and Wilson (1977) to conclude that both species are closely related and that a subspecific affiliation might be indicated. Nevertheless, *P. potenziani* is currently considered as a distinct species (Brandon-Jones et al., 2004; Groves, 2001). Based on its morphological and behavioral characters described above, *P. potenziani* is proposed to be the most “primitive” member of the genus and thus the sister species to all congeners (Brandon-Jones, 1978, 1993; Meijaard and Groves, 2004). Our molecular data support the monophyly of *P. potenziani*, but neither an early separation, nor a sister grouping with *P. thomasi*.

P. femoralis and *P. chrysomelas* were originally recognized as subspecies of *P. melalophos* (Napier and Napier, 1967; Oates et al., 1994), but later, *P. femoralis* was separated from *P. melalophos* at the species level, with *P. chrysomelas* being included as a subspecies (Brandon-Jones, 1984). More recently, Groves (2001) proposed species status for *P. chrysomelas* as well, while Brandon-Jones et al. (2004) kept *P. chrysomelas* as subspecies of *P. femoralis*. Our data do not support a conspecific relationship between any of these three taxa, since they descend from distinct lineages with ancient divergences.

Of the remaining species, *P. comata* and *P. rubicunda* are nested within *P. melalophos*. Additionally, the possible paraphyletic origin of *P. m. mitrata*, with the central Sumatran populations being closely related to *P. m. melalophos* and the South Sumatran populations forming a sister lineage to *P. comata*, reflects a puzzling situation of this polyphyletic group. We can not rule out that incomplete lineage sorting might have had an effect. But in the case of incomplete lineage sorting, paraphyletic relationships, like in *P. m. mitrata*, result from the failure of haplotypes to sort during speciation events and should be random with respect to geography (Avice, 2004). This is not the case in our phylogenetic reconstruction, where geographically close populations cluster together. Another explanation might be hybridization between *P. m. melalophos* and *P. m. mitrata*. Such a scenario is highly likely, since both taxa are found south of the Batang Hari river (Aimi and Bakar, 1992, 1996; Groves, 2001) eastwards from Bangko, where we identified *P. m. melalophos*. In addition to our mitochondrial data, the pale reddish coat coloration of the *P. m. mitrata* population from Central Sumatra seems to be intermediate between the red *P. m. melalophos* and the grayish-white Southern *P. m. mitrata* populations, which is in accordance with the observations of Aimi and Bakar (1996). Alternatively, the pale reddish Central Sumatran population might be a further color variant of *P. m. melalophos*. To test these hypotheses, the analysis of nuclear markers is mandatory.

Although the sample size ($n = 1$) of *P. rubicunda* is low, and incomplete lineage sorting might be possible, our data support previous hypotheses, proposing a close affiliation of *P. rubicunda* and *P. melalophos* based on the red coat coloration (Brandon-Jones, 1996b) or in some aspects of behavior and vocalization (Wilson and Wilson, 1975).

4.2. Taxonomic implications

The taxonomic confusion within the genus *Presbytis* – and also in other primate genera – is associated with much controversial discussion about the recognition on the degree or amount of certain characters, in particular pelage coloration, to recognize species or phylogenetic relatedness. To apply a more objective and falsifiable approach, Groves (2001, 2004) suggests the phylogenetic species concept (PSC) to delimit species. The PSC defines a species as the smallest cluster of individual organisms within there is a parental pattern of ancestry and descent and that is diagnosable distinct from other such clusters by a unique combination of fixed character states (Cracraft, 1983). At present, the PSC is widely applied in recent studies on primate taxonomy, for example in *Mico*

(Groves, 2001), *Callicebus* (van Roosmalen et al., 2002), *Saguinus* (Matauschek et al., in press), *Lepilemur* (Craul et al., 2007), *Microcebus* (Louis et al., 2008), *Trachypithecus* (Roos et al., 2008) or *Nomascus* (Thinh et al., 2010b).

Among *Presbytis*, we detect several highly supported terminal clades or lineages, mainly formed by taxa, which are all also clearly distinguishable in their pelage coloration, thus in agreement with the PSC. Moreover, most of the examined *Presbytis* taxa differentiated on a similar time scale as did other Asian primates. For example, Tosi et al. (2003) and Ziegler et al. (2007) estimated the divergence between Sundaic and Continental pig-tailed macaques at 1.4 (1.6–1.2) Ma, which afterwards differentiated into respective species. In *Trachypithecus*, Roos et al. (2008) calculated the divergence between *T. germaini* and *T. margarita* 0.95 (1.04–0.86) Ma. Considering estimated divergence ages and differences in mtDNA and pelage coloration, species status for all examined *Presbytis* taxa might be appropriate. An overview to the proposed revision in comparison with previous studies is given in Table 4.

4.3. Conservation implications

The rainforests of the Malay Peninsula and the western Indo-Malay archipelago belong to the biodiversity hotspots of our planet, but also to hotspots of environmental degradation. For instance, Indonesia is among the ten countries with the highest number of threatened species (World Bank, 2004; FWI/GFW, 2002). The langurs of the genus *Presbytis* also suffer from this ongoing situation and population sizes of all taxa are still decreasing (IUCN, 2010). Reasons for the decline of langurs are manifold, but habitat loss due to forest clearance for agricultural use or timber production, as well as illegal hunting for food, pet trade or traditional medicine, like bezoar stones (visceral secretions used in traditional medicine; Nijman, 2004), are major threats to wild *Presbytis* populations. In particular forest destruction often leads to isolated populations due to fragmentation and consequently to limited or disturbed gene flow. Recent evaluations of the conservation status rank various *Presbytis* taxa as vulnerable, endangered or even as critically endangered (Table 4).

For conservationists it is of great interest whether any population within a taxon is sufficiently differentiated genetically to warrant separate management and also to maintain genetic diversity. In our study we detected several monophyletic clades, which should all be regarded as independent management units (Moritz, 1994). Thus, conservation actions are needed for all *Presbytis* taxa, but especially for the critically endangered ones *P. chrysomelas* and *P. p. potenziani*.

Table 4

Proposed classification compared to earlier classifications along with information about type localities, authors and conservation status.

| Groves (2001) | Brandon-Jones et al. (2004) | Proposed classification | Conservation status (IUCN, 2010) | Author | Type locality |
|--------------------------------------|---------------------------------|-------------------------|----------------------------------|----------------------------|---|
| <i>P. p. potenziani</i> | <i>P. p. potenziani</i> | <i>P. potenziani</i> | CR | Bonaparte (1856) | Sipora Island, Mentawai Islands |
| <i>P. p. siberu</i> | <i>P. p. siberu</i> | <i>P. siberu</i> | EN | Chasen and Kloss (1927) | Siberut Island, Mentawai Islands |
| <i>P. c. comata</i> | <i>P. comata</i> | <i>P. comata</i> | EN | Desmarest (1822) | Java |
| <i>P. c. fredericae</i> ^a | <i>P. fredericae</i> | <i>P. fredericae</i> | EN | Sody (1930) | Mount Slamet, Central Java |
| <i>P. m. melalophos</i> | <i>P. m. melalophos</i> | <i>P. melalophos</i> | NT | Raffles (1821) | Bengkulu, Sumatra |
| <i>P. m. mitrata</i> | <i>P. m. mitrata</i> | <i>P. mitrata</i> | EN | Eschscholtz (1821) | Sumatran mainland opposite Zutphen Island |
| <i>P. m. bicolor</i> | <i>P. m. bicolor</i> | <i>P. bicolor</i> | DD | Aimi and Bakar (1992) | Batang Kering, Sumatra |
| <i>P. m. sumatrana</i> | <i>P. m. sumatrana</i> | <i>P. sumatrana</i> | EN | Müller and Schlegel (1841) | Mount Talamau, Sumatra |
| <i>P. chrysomelas</i> | <i>P. femoralis chrysomelas</i> | <i>P. chrysomelas</i> | CR | Müller (1838) | Pontianak, Borneo |
| <i>P. thomasi</i> | <i>P. thomasi</i> | <i>P. thomasi</i> | VU | Collett (1892) | Langkat, Aceh, Sumatra |
| <i>P. hosei</i> | <i>P. hosei</i> | <i>P. hosei</i> | VU | Thomas (1899) | Niah, Sarawak, Borneo |
| <i>P. rubicunda</i> | <i>P. rubicunda</i> | <i>P. rubicunda</i> | LC | Müller (1838) | Mount Sekumbang, South Kalimantan, Borneo |
| <i>P. frontata</i> | <i>P. frontata</i> | <i>P. frontata</i> | VU | Müller (1838) | Southeastern Borneo |
| <i>P. femoralis</i> | <i>P. femoralis</i> | <i>P. femoralis</i> | NT | Martin (1838) | Singapore |

^a According to IUCN (2010) *P. c. fredericae* is a synonym of *P. comata*; DD = data deficient, LC = least concern, NT = near threatened, VU = vulnerable, EN = endangered, CR = critically endangered.

Threats to both taxa are exemplary for most members of the genus. The main threat to *P. chrysomelas* has been conversion of habitat into agricultural land, resulting in its disappearance from most of its former range and leading to five isolated populations. In recent years, the species has in particular been affected by expanding plantations, especially oil palm (IUCN, 2010). The population decline of *P. p. potenziani* was estimated at more than 80% over the past 40 years due to hunting and loss of habitat (Whittaker, 2006). If *P. c. fredericae* is considered to be a separate species it undoubtedly can be ranked among the rarest and most endangered primate species in the world. It would be restricted to four isolated forest areas, none of which are adequately protected and two of them are situated on an active volcano (Nijman, 2001).

To protect langurs in the future, urgent actions are required to prevent ongoing habitat destruction and hunting activities. Often captive animals offer the opportunity to conserve and build up a viable gene pool for later release purposes, but in *Presbytis*, it is of particular interest to protect wild populations since they are difficult to be kept in captivity. However, it is also crucial to confirm the taxon identity and if possible the geographical origin of confiscated animals in general.

5. Conclusions

We showed that accurate taxonomic identification of *Presbytis* taxa based on behavioral or morphological data alone is sometimes contradictory or misleading. In this respect, mtDNA analysis is a promising tool, which additionally should be used to answer taxonomic affiliations. This is also important for practical issues in conservation management in nature (in situ) and in captivity (ex situ). As shown in our study, *Presbytis* taxa can be diagnosed through mtDNA, and, hence, a secure identification of the taxon, even the population, can easily be obtained. In our study for example, we were able to confirm Way Kambas as the origin of the *P. m. mitrata* sample from the Ragunan Zoo in Jakarta. Yet since mtDNA is only maternally inherited, possible hybrids can not be detected in such analysis. Thus, for the identification of captive hybrids and also to trace possible natural hybridization events as it might be the case between *P. m. melalophos* and the Northern population of *P. m. mitrata*, nuclear markers should be studied as well. Moreover, to fully understand the evolutionary history of the genus, further studies should also include *P. natunae* and *P. siamensis*, as well as a broader sampling set of the Bornean taxa.

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References

- Aimi, M., Bakar, A., 1992. Taxonomy and distribution of *Presbytis melalophos* group in Sumatera, Indonesia. *Primates* 33, 191–206.
- Aimi, M., Bakar, A., 1996. Distribution and deployment of *Presbytis melalophos* group in Sumatera, Indonesia. *Primates* 37, 399–409.
- Avise, J., 2004. *Molecular Markers, Natural History, and Evolution*. Sinauer Associates, Sunderland, MA.
- Bennett, E.L., Davies, A.G., 1994. The ecology of Asian colobines. In: Davies, J.F., Oates, J.F. (Eds.), *Colobine Monkeys: Their Ecology, Behaviour and Evolution*. Cambridge University Press, Cambridge, pp. 129–171.
- Brandon-Jones, D., 1978. The evolution of recent Asian colobines. In: Chivers, D.J., Joysey, K.A. (Eds.), *Recent Advances in Primatology*, vol. III. Academic Press, London, pp. 323–325.
- Brandon-Jones, D., 1984. Colobus and leaf monkeys. In: MacDonald, I.D. (Ed.), *Encyclopedia of Mammals*. George Allen and Unwin, London, pp. 398–410.
- Brandon-Jones, D., 1993. Taxonomic affinities of the Menawai islands surili (Bonaparte, 1856), *P. potenziani*. *Raffles Bull. Zool.* 41, 331–357.
- Brandon-Jones, D., 1995. *Presbytis fredericae* (Sody, 1930), an endangered Colobine species endemic to Central Java, Indonesia. *Primate Conserv.* 16, 68–70.
- Brandon-Jones, D., 1996a. The zoogeography of sexual dichromatism in the Bornean grizzled surili, *Presbytis comata* (Desmarest, 1822). *Sarawak Museum J.* 5, 177–200.
- Brandon-Jones, D., 1996b. *Presbytis* species in sympatry in Borneo versus allopatry in Sumatra: an interpretation. In: Edwards, D.S.E.A. (Ed.), *Tropical Rainforest Research – Current Issues*. Kluwer Academic Publishers, Dordrecht, pp. 71–76.
- Brandon-Jones, D., 1996c. The Asian Colobinae 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.J., Morales, J.C., Shekelle, M., Steward, C.-B., 2004. Asian primate classification. *Int. J. Primatol.* 25, 122–129.
- Brunet, M., Guy, F., Pilbeam, D., Mackaye, H.T., Likius, A., Ahounta, D., Beauvilain, A., Blondel, C., Bocherens, H., Boisserie, J.R., de Bonis, L., Coppens, Y., Dejax, J., Denys, C., Düringer, P., Eisenmann, V., Fanone, G., Fronty, P., Geraads, D., Lehmann, T., Lihoreau, F., Louchart, A., Mahamat, A., Merceron, G., Mouchelin, G., Otero, O., Pelaez Campomanes, P., Ponce de Leon, M., Rage, J.C., Sapanet, M., Schuster, M., Sudre, J., Tassy, P., Valentin, X., Vignaud, P., Viriot, L., Zazzo, A., Zollikofer, C., 2002. A new hominid from the upper Miocene of Chad, Central Africa. *Nature* 418, 145–151.
- Castresana, J., 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol. Biol. Evol.* 17, 540–552.
- Chasen, F.N., 1940. A handlist of Malaysian mammals. *Bull. Raffles Museum* 15, 1–209.
- Chatterjee, H.J., Ho, S.Y.W., Barnes, I., Groves, C., 2009. Estimating the phylogeny and divergence times of primates using a supermatrix approach. *BMC Evol. Biol.* 9, 259.
- Cracraft, J., 1983. Species concepts and speciation analysis. In: Johnson, R.F. (Ed.), *Current Ornithology*, vol 1, pp. 159–187.
- Craul, M., Zimmermann, E., Rasoloharjaona, S., Randrianambinina, B., Radespiel, U., 2007. Unexpected species diversity of Malagasy primates (*Lepilemur* spp.) in the same biogeographical zone: a morphological and molecular approach with the description of two new species. *BMC Evol. Biol.* 7, 83.
- Delson, E., Tattersall, I., van Couvering, J.A., Brooks, A.S., 2000. Cercopithecidae. In: Delson, E., Tattersall, I., van Couvering, J.A., Brooks, A.S. (Eds.), *Encyclopedia of Human Evolution and Prehistory*, second ed. Garland, New York, pp. 166–171.
- Drummond, A.J., 2007. Beast: Bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol.* 7, 214.
- Drummond, A.J., Ashton, B., Cheung, M., Heled, J., Kearse, M., Moir, R., Stones-Havas, S., Thierer, T., Wilson, A., 2009. Geneious. <http://www.geneious.com/>.
- Drummond, A.J., Ho, S.Y.W., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biol.* 4, e88.
- FWI/GFW 2002. The State of the Forest: Indonesia. Bogor, Indonesia: Forest Watch Indonesia, and Washington DC: Global Forest Watch. <<http://www.globalforestwatch.org/common/indonesia/sof.indonesia.english.low.pdf>> (10.31.10).

- Gelman, A., Rubin, D., 1992. Inference from iterative simulation using multiple sequences. *Stat. Sci.* 7, 457–511.
- Groves, C.P., 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. 557–587.
- Groves, C.P., 1989. *A Theory of Human and Primate Evolution*. Clarendon Press, Oxford and New York.
- Groves, C.P., 2001. *Primate Taxonomy*. Smithsonian Institution Press, Washington, DC.
- Groves, C.P., 2004. The what, why and how of primate taxonomy. *Int. J. Primatol.* 25, 1105–1126.
- Hofreiter, M., Siedel, H., van Neer, W., Vigilant, L., 2003. Mitochondrial DNA sequence from an enigmatic gorilla population (*Gorilla gorilla uellensis*). *Am. J. Phys. Anthropol.* 121, 361–368.
- Hooijer, D.A., 1962. Quaternary langurs and macaques from the Malay Archipelago. *Zool. Verhandelingen* 55, 1–64.
- Huelsenbeck, J.P., Ronquist, F., Nielsen, R., Bollback, J.P., 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. *Science* 294, 2310–2314.
- IUCN, 2010. IUCN Red List of Threatened Species. Version 2010.1. <www.iucnredlist.org> (20.6.10).
- Kelley, J., 2002. The hominoid radiation in Asia. In: Hartwig, W.C. (Ed.), *The Primate Fossil Record*. Cambridge University Press, Cambridge, pp. 369–384.
- Leakey, M.G., 1993. Evolution of *Theropithecus* in the Turkana Basin. In: Jablonski, N.G. (Ed.), *Theropithecus, the Rise and Fall of a Primate Genus*. Cambridge University Press, Cambridge, pp. 85–124.
- Louis Jr., E.E., Engberg, S.E., McGuire, S.M., McCormick, M.J., Randriamampionona, R., Ranaivoarisoa, J.F., Bailey, C.A., Mittermeier, R.A., Lei, R., 2008. Revision of the mouse lemurs, *Microcebus* (Primates, Lemuriformes), of Northern and North-western Madagascar with descriptions of two new species at Montagne d'Ambre National Park and Antafondro Classified Forest. *Primate Conserv.* 23, 19–38.
- Matauschek, C., Roos, C., Heymann, E.W., in press. Mitochondrial phylogeny of tamarins (*Saguinus*, Hoffmannsegg 1807) with taxonomic and biogeographic implications for the *S. nigricollis* species group. *Am. J. Phys. Anthropol.*, doi:10.1002/ajpa.21445.
- Md Zain, B.M., 2001. Molecular Systematics of the Genus *Presbytis*. Thesis, Columbia University, New York.
- Md Zain, B.M., Hasan, M.H., Melnick, D.J., 2002. Defining evolutionary significant units for *Presbytis melalophos* conservation using mitochondrial DNA sequences. In: Omar, R., Ali Rahman, Z., Latif, M.T., Lihan, T., Adam, J.H. (Eds.), *Proceedings of the Regional Symposium on Environment and Natural Resources 10–11th April 2002, Hotel Renaissance Kuala Lumpur, Malaysia*, vol. 1, pp. 279–286.
- Medway, Lord., 1970. The monkeys of Sundaland: ecology and systematics of the cercopithecids of a humid equatorial environment. In: Napier, J.R., Napier, P.H. (Eds.), *Old World Monkeys: Evolution*. Academic Press, New York, pp. 513–553.
- Meijaard, E., 2004. Solving Mammalian Riddles: A Reconstruction of Tertiary and Quaternary Distribution of Mammals and their Palaeoenvironments in Island South-East Asia. Thesis, School of Archaeology and Anthropology, The Australian National University, Canberra.
- Meijaard, E., Groves, C.P., 2004. The biogeographical evolution and phylogeny of the genus *Presbytis*. *Primate Rep.* 68, 71–90.
- Moritz, C., 1994. Defining 'evolutionarily significant units' for conservation. *Trends Ecol. Evol.* 9, 374–375.
- Napier, J.R., Napier, P.H., 1967. *Handbook of Living Primates*. Academic Press, London.
- Newton, P.N., Dunbar, R.I.M., 1994. Colobine monkey society. In: Davies, J.F., Oates, J.F. (Eds.), *Colobine Monkeys: Their Ecology, Behaviour and Evolution*. Cambridge University Press, Cambridge, pp. 311–346.
- Nijman, V., 1997. Geographic variation in pelage characteristics in *Presbytis comata* (Desmarest, 1822) (Mammalia: Primates, Cercopithecidae). *Z. Säugetierk.* 62, 257–264.
- Nijman, V., 2001. Forest (and) Primates: Conservation and Ecology of the Endemic Primates of Java and Borneo. Thesis, Amsterdam University, Wageningen, Tropenbos International.
- Nijman, V., 2004. Effects of habitat disturbance and hunting on the density and the biomass of the endemic Hose's leaf monkey *Presbytis hosei* (Thomas, 1889) (Mammalia: Primates: Cercopithecidae) in east Borneo. *Contrib. Zool.* 73, 283–291.
- Nsubuga, A.M., Robbins, M.M., Roeder, A.D., Morin, P.A., Boesch, C., Vigilant, L., 2004. Factors affecting the amount of genomic DNA extracted from ape faeces and the identification of an improved sample storage. *Mol. Ecol.* 13, 2089–2094.
- Oates, G.D., Davies, J.F., Delson, E., 1994. The diversity of living Colobines. In: Davies, J.F., Oates, J.F. (Eds.), *Colobine Monkeys: Their Ecology, Behaviour and Evolution*. Cambridge University Press, Cambridge, pp. 45–73.
- Osterholz, M., Walter, L., Roos, C., 2008. Phylogenetic position of the langur genera *Semnopithecus* and *Trachypithecus* among Asian colobines, and affiliations of their species groups. *BMC Evol. Biol.* 8, 58.
- Posada, D., 2008. JModelTest: phylogenetic model averaging. *Mol. Biol. Evol.* 25, 1253–1256.
- Raam, 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, 237–257.
- Rambaut, A., 2006. FigTree: Tree Figure Drawing Tool, Version 1.3.1. Institute of Evolutionary Biology, University of Edinburgh. <<http://tree.bio.ed.ac.uk/software/figtree/>>.
- Rambaut, A., Drummond, A., 2005. Tracer v1.3: MCMC Trace Analysis Tool. Institute of Evolutionary Biology, University of Edinburgh. <<http://tree.bio.ed.ac.uk/software/tracer/>>.
- Ronquist, F., Huelsenbeck, J.P., 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*). *Mol. Phylogenet. Evol.* 47, 629–636.
- SAMD, 2006. Southeast Asian Mammal Databank. <<http://www.ieaitaly.org/samd/>>.
- Steiper, M.E., Young, N.M., 2006. Primate molecular divergence dates. *Mol. Phylogenet. Evol.* 41, 384–394.
- Sterck, E.H.M., Watts, D.P., van Schaik, C.P., 1997. The evolution of female social relationships in nonhuman primates. *Behav. Ecol. Sociobiol.* 41, 291–309.
- Sterck, E.H.M., Willems, E.P., van Hoof, J.A.R.A.M., Wich, S.A., 2005. Female dispersal, inbreeding avoidance, and mate choice in Thomas langurs (*Presbytis thomasi*). *Behaviour* 142, 845–868.
- Sterner, K.N., Raam, R.L., Zhang, Y.-P., Stewart, C.-B., Disotell, T.R., 2006. Mitochondrial data support an odd-nosed colobine clade. *Mol. Phylogenet. Evol.* 40, 1–7.
- Swofford, D., 2002. PAUP* Phylogenetic Analysis using Parsimony (* and Other Methods). Sinauer Associates, Sunderland, MA.
- Talavera, G., Castresana, J., 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Syst. Biol.* 56, 564–577.
- 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.
- Thinh, V.N., Mootnick, A.R., Geissmann, T., Li, M., Ziegler, T., Agil, M., Moisson, P., Nadler, T., Walter, L., Roos, C., 2010a. Mitochondrial evidence for multiple radiations in the evolutionary history of small apes. *BMC Evol. Biol.* 10, 74.
- Thinh, V.N., Rawson, B., Hallam, C., Kenyon, M., Nadler, T., Walter, L., Roos, C., 2010b. Phylogeny and distribution of crested gibbons (genus *Nomascus*) based on mitochondrial cytochrome b gene sequence data. *Am. J. Primatol.* 72, 1047–1054.
- Tilson, R.L., 1976. Infant coloration and taxonomic affinity of the Mentawai Islands leaf monkey, *Presbytis potenzianni*. *J. Mammal.* 57, 766–769.
- Ting, N., 2008. Mitochondrial relationships and divergence dates of the African colobines: evidence of Miocene origins for the living colobus monkeys. *J. Hum. Evol.* 55, 312–325.
- Tosi, A.J., Morales, J.C., Melnick, D.J., 2003. Paternal, maternal, and biparental molecular markers provide unique windows onto the evolutionary history of macaque monkeys. *Evolution* 57, 1419–1435.
- van Roosmalen, M.G.M., van Roosmalen, T., Mittermeier, R.A., 2002. A taxonomic review of the titi monkeys, genus *Callicebus* Thomas, 1903, with the description of two new species, *Callicebus bernhardi* and *Callicebus stephennashi*, from Brazilian Amazonia. *Neotrop. Primates* 10, 1–52.
- Vignaud, P., Duringer, P., Mackaye, H.T., Likius, A., Blondel, C., Boisserie, J.R., de Bonis, L., Eisenmann, V., Etienne, M.E., Geraads, D., Guy, F., Lehmann, T., Lihoreau, F., Lopez-Martinez, N., Mourer-Chauvire, C., Otero, O., Rage, J.C., Schuster, M., Viriot, L., Zazzo, A., Brunet, M., 2002. Geology and palaeontology of the Upper Miocene Toros-Menalla hominid locality, Chad. *Nature* 418, 152–155.
- Washburn, S.L., 1944. The genera of Malayan langurs. *J. Mammal.* 25, 289–294.
- Whittaker, D., 2006. A conservation action plan for the Mentawai primates. *Primate Conserv.* 20, 95–105.
- Wilson, W.L., Wilson, C.C., 1975. Species-specific vocalisations and the determination of phylogenetic affinities of the *Presbytis aygala-melalophos* group in Sumatra. In: Kondo, S., Kawai, M., Ehara, A. (Eds.), *Contemporary Primatology, 5th International Congress of Primatology*, Nagoya, August 1974, Karger, Basel, pp. 459–463.
- Wilson, C.C., Wilson, W.L., 1977. Behavioral and morphological variations among primate population in Sumatra. *Yearb. Phys. Anthropol.* 20, 207–233.
- World Bank, 2004. Indonesia at Glance 2004. <<http://siteresources.worldbank.org/INTEEI/Data/20856180/Indonesia.pdf>> (10.31.10).
- Zalmout, I.S., Sanders, W.J., MacLachy, L.M., Gunnell, G.F., Al-Mufarreh, Y.A., Ali, M.A., Nasser, A.H., Al-Masari, A.M., Al-Sobhi, S.A., Nadhra, A.O., Matari, A.H., Wilson, J.A., Gingerich, P.D., 2010. New Oligocene primate from Saudi Arabia and the divergence of apes and Old World monkeys. *Nature* 466, 360–365.
- Ziegler, T., Abegg, C., Meijaard, E., Perwitasari-Farajallah, D., Walter, L., Hodges, J.K., Roos, C., 2007. Molecular phylogeny and evolutionary history of Southeast Asian macaques forming the *M. silenus* group. *Mol. Phylogenet. Evol.* 42, 807–816.
- Zwickl, D.J., 2006. Genetic Algorithm Approaches for the Phylogenetic Analysis of Large Biological Sequence Datasets under the Maximum Likelihood Criterion. Thesis, The University of Texas at Austin.