Research article

Phylogenetic position of the langur genera Semnopithecus and Trachypithecus among Asian colobines, and genus affiliations of their species groups

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Abstract

**Background:** The evolutionary history of the Asian colobines is less understood. Although monophyly of the odd-nosed monkeys was recently confirmed, the relationships among the langur genera Presbytis, Semnopithecus and Trachypithecus and their position among Asian colobines remained unclear. Moreover, in Trachypithecus various species groups are recognized, but their affiliations are still disputed. To address these issues, mitochondrial and Y chromosomal sequence data were phylogenetically related and combined with presence/absence analyses of retroposon integrations.

**Results:** The analysed 5 kb fragment of the mitochondrial genome allows no resolution of the phylogenetic relationships among langur genera, but five retroposon integrations were detected which link Trachypithecus and Semnopithecus. According to Y chromosomal data and a 573 bp fragment of the mitochondrial cytochrome b gene, a common origin of the species groups T. [cristatus], T. [obscurus] and T. [francoisi] and their reciprocal monophyly is supported, which is also underpinned by an orthologous retroposon insertion. T. [vetulus] clusters within Semnopithecus, which is confirmed by two retroposon integrations. Moreover, this species group is paraphyletic, with T. [vetulus] forming a clade with the Sri Lankan, and T. johnii with the South Indian form of S. entellus. Incongruence between gene trees was detected for T. [pileatus], in that Y chromosomal data link it with T. [cristatus], T. [obscurus] and T. [francoisi], whereas mitochondrial data affilates it with the Semnopithecus clade.

**Conclusion:** Neither relationships among the three langur genera nor their position within Asian colobines can be settled with 5 kb mitochondrial sequence data, but retroposon integrations confirm at least a common origin of Semnopithecus and Trachypithecus. According to Y chromosomal and 573 bp mitochondrial sequence data, T. [cristatus], T. [obscurus] and T. [francoisi] represent true members of the genus Trachypithecus, whereas T. [vetulus] clusters within Semnopithecus. Due to paraphyly of T. [vetulus] and polyphyly of Semnopithecus, a split of the genus into three species groups (S. entellus - North India, S. entellus - South India + T. johnii, S. entellus - Sri Lanka + T. vetulus) seems to be appropriate. T. [pileatus] posses an intermediate position between both genera, indicating that the species group might be the result of ancestral hybridization.

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Background
The Old World monkeys are traditionally divided into the two subfamilies Cercopithecinae and Colobinae, which differ from each other by numerous morphological, behavioural and ecological characteristics [1-4]. While detailed information on the evolutionary history of cercopithecines (baboons, mangabeys, macaques and guenons) is at hand, knowledge on colobines is still scarce. Although some molecular studies [5-13] exist, they mainly focus on relationships within genera or species groups and not on the general phylogeny of the subfamily. Recently, a first mitochondrial phylogeny of colobine genera was established [14], which confirmed some previous assumptions, but also led to confusions, calling for further research to definitively elucidate their evolutionary history.

Based on distribution and morphology, the colobines are traditionally divided into an African and an Asian clade [3,4], while Asian colobines are more diverse than their African relatives. Hence, the Asian forms are further split into the odd-nosed monkey (Pygathrix, Rhinopithecus, Nasalis, Simias) and langur (Presbytis, Trachypithecus, Semnopithecus) groups, which are both believed to be monophyletic. Accordingly, langurs were originally combined in the single genus Presbytis [15-17] or Semnopithecus [18], but based on neonatal colouration and cranial morphology, they were split into the three genera Semnopithecus, Trachypithecus and Presbytis [19], and a fourth genus (Kasi) was added [20]. Alternatively, Semnopithecus was separated from Presbytis, with Trachypithecus forming a subgenus of the former [21-23], but recent classifications use a subdivision of langurs into the three genera Presbytis, Trachypithecus and Semnopithecus [3,4,24-26].

Within the different langur genera, several species are recognized, which are lumped into species groups due to similar fur colouration, behaviour, ecology or distribution. With five species groups (T. [obscurus], T. [francoisi], T. [cristatus], T. [pileatus] and T. [vetulus]) [4], the genus Trachypithecus is the most diverse of all langurs and possesses also the widest distribution, ranging from South India and Sri Lanka through mainland Southeast Asia to the Sundaland (Fig. 1, Fig. 2). Although all of them are morphologically similar, T. [vetulus] was sometimes separated in its own genus Kasi [20], and recent mitochondrial sequence data indicate a closer affiliation of T. [vetulus] and T. [pileatus] to Semnopithecus than to Trachypithecus [7,9]. Accordingly, the two T. [vetulus] members were recognized as species of Semnopithecus [26]. In contrast to Trachypithecus, the genus Semnopithecus is restricted to the Indian subcontinent (Fig. 1) and traditionally regarded as monotypic with the only species S. entellus [3,27], although recently several subspecies were elevated to species status [4]. The third langur genus, Presbytis, includes several species,
which occur solely in the Sundaland, but are not lumped into distinct species groups [4].

The phylogenetic relationships among the different Asian colobine genera are disputed. Although a common origin of the odd-nosed monkeys was recently confirmed [14], evidence for monophyly of its putative sister clade, the langur group, is still lacking. Moreover, available data depict Trachypithecus and Presbytis as sister taxa to the exclusion of Semnopithecus [14], which contradicts with traditional classifications, in which Trachypithecus and Semnopithecus are believed to form a clade to the exclusion of Presbytis [4,21-23]. These findings raise the question of what positions the langur genera occupy among Asian colobines and whether the langurs form a monophyletic clade in general. Moreover, the affiliations of different Trachypithecus species groups, especially T. [setulus] and T. [pileatus], are disputed, and hence, led to different classifications. Currently, only few genetic data are available [7,9,28], so that further information from other markers is required to definitively establish their relationships.

To address all these issues, mitochondrial and Y chromosomal sequence data were phylogenetically related and combined with presence/absence analysis of retroposon integrations. This approach was used to simultaneously analyse paternal-, maternal- and biparental-inherited markers, which allow the detection of incongruence between different gene trees indicating possible hybridization or introgression events between different lineages [29-31]. To determine the phylogenetic position of the langur genera among Asian colobines, a 5 kb fragment of the mitochondrial genome was sequenced from eight colobine genera, and combined with presence/absence analysis of retroposon integrations. Retroposon insertion events are nearly homoplasys-free and precise excision of elements is highly unlikely [32,33]. Accordingly, retroposon insertions are powerful informative markers, which were already successfully applied to elucidate phylogenetic relationships in various primate lineages [34-38]. To study relationships among different langur species groups and their genus affiliations, a 573 bp fragment of the mitochondrial cytochrome b gene and a 777 bp portion of the SRY (sex-determining region, Y chromosome) gene was sequenced from at least one representative per species group, and complemented with retroposon analysis.

**Results and Discussion**

**Genus level phylogeny**

To elucidate the phylogenetic relationships among the different langur genera and their position among Asian colobines, mitochondrial sequence studies were combined with presence/absence analysis of retroposon insertions.

The herein analysed 5 kb fragment of the mitochondrial genome was assembled from sequences derived from 1–2 kb long and partly overlapping PCR products, whereby no inconsistencies in overlapping sequence fragments were detected. As template material, mainly DNA extracted from feces was used, which minimizes the amplification of nuclear pseudogenes [39], and comparisons of the data with sequences already deposited at GenBank revealed only intra-species or -generic variation, indicating that no nuclear pseudogenes were amplified.

To determine the phylogenetic relationships among analysed genera, tree reconstructions were conducted with different algorithms, which all led to the same tree topology (Fig. 3). Moreover, most relationships are significantly supported and congruent with previous classifications [1-4], indicating the reliability of the data set. In detail, the reconstructions confirm relationships among cercopithecine genera (Macaca, Papio, Chlorocebus), and reciprocal monophyly of cercopithecines and colobines, as well as of African and Asian colobines. Within the Asian clade, relationships are not resolved, although at least a common origin of the odd-nosed monkeys is depicted. Neither the assumed monophyly of the langur genera, nor the expected close affinity of Trachypithecus and Semnopithecus or the recently indicated sister grouping of Trachypithecus and Presbytis [14] can be verified with significance. Moreover, all alternative tree topologies, in which the three langur genera were regarded as monophyletic, or variously recognized as sister clades to each other, to the odd-nosed monkey clade, or even as basal among Asian colobines, were not rejected (P = 0.097 – 0.776). Accordingly, an unresolved polytomy among the three langur genera and the odd-nosed monkey clade may best reflect the relationships among Asian colobines based on the mitochondrial data.

Several retroposon insertions were detected, which provide insights into the branching pattern of colobines (Fig. 4). Together with seven already published integrations [38], now 14 loci confirm monophyly of the Asian colobines to the exclusion of the African genus Colobus. Among Asian colobines, one insertion indicates a common origin of the odd-nosed monkeys, which is verified by three further loci [38]. Most prominent are the five retroposon integrations, which confirm a sister grouping of Trachypithecus and Semnopithecus. No loci were detected, which link Presbytis either with the other two langur genera or the odd-nosed monkeys.

Although in general the mitochondrial data are suitable to elucidate relationships among the different genera, as indicated by the correct and significantly supported branching patterns among all other studied genera, the relationships among the langurs are unresolved, which is
concordant with previous results [14]. In contrast, the presence/absence analysis of retroposon integrations provides evidence for a monophyletic odd-nosed monkey clade and a common origin of *Trachypithecus* and *Semnopithecus*, which is in agreement with morphological hypotheses [1,3,4,21-23]. Regardless which markers were used, the phylogenetic position of *Presbytis* among Asian colobines and accordingly the unity of the langurs remains unclear and needs further investigations.

**Species group phylogeny**

In order to settle affiliations among the different *Trachypithecus* species groups and their members, mitochondrial and Y chromosomal sequence data were combined with information on retroposon integrations.

The mitochondrial phylogeny was established on the basis of 573 bp long cytochrome b gene sequences, generated from most species recognized in the genus and its sister genus *Semnopithecus*. In all tree reconstructions,
identical relationships were obtained, with most branches being significantly supported (Fig. 5a). Accordingly, the different species groups are divided into two major groups, with one including solely groups of the genus Trachypithecus, whereas the second one includes representatives of Trachypithecus and Semnopithecus. In the mixed clade, T. [vetulus] and T. [pileatus] members are lumped together with S. entellus. Whereby T. [pileatus] is monophyletic, the members of T. [vetulus] are paraphyletic, with T. vetulus forming a sister clade to S. entellus from Sri Lanka, and T. johnii with S. entellus from South India. Furthermore, a fourth lineage in the mixed clade was detected, which is represented by S. entellus from North India. In contrast, the three species groups (T. [obscurus], T. [francoisi], T. [cristatus]) in the clade comprising solely Trachypithecus groups are all monophyletic. Alternative relationships, in which T. [vetulus] is recognized as monophyletic, either T. [vetulus] or T. [pileatus] belongs to Trachypithecus or even both are members of Trachypithecus, were tested, but all of them were rejected (P < 0.05).

With some exceptions, the Y chromosomal data provide a similar picture (Fig. 5b), but due to the low number of polymorphic sites, support values are in general not as high as in the mitochondrial tree. According to the reconstructions, the species groups are divided into two major clades, with one comprising T. [obscurus], T. [cristatus], T. [francoisi] and T. [pileatus], and the other, T. [vetulus] and Semnopithecus. Relationships among the latter are not resolved. All alternative tree topologies, in which either T. [vetulus] belongs to Trachypithecus or T. pileatus groups with Semnopithecus, were rejected (P < 0.05).

Retroposon insertions further deepened our knowledge on the species group relationships. Altogether, three informative integrations were analysed (Fig. 5c), with one occurring in T. [obscurus], T. [cristatus] and T. [francoisi], and the other two in T. [vetulus] and Semnopithecus. Interestingly, all three integrations are absent in T. pileatus.

With the exception of the varying position of T. [pileatus], the affiliations of the remaining species groups are congruent among different gene trees. Accordingly, all analysed markers relate T. [vetulus] with Semnopithecus, indicating that this species group is a real member of the genus Semnopithecus and not of Trachypithecus as assumed by morphological similarities [4]. These similarities may be the results of adaptations to similar ecological conditions (Semnopithecus is semi-terrestrial and lives in deciduous forest, whereas Trachypithecus including T. [vetulus] is arboreal and occurs in wet evergreen forests). Although the Y chromosomal data allow no resolution within the Semnopithecus – T. [vetulus] clade, the mitochondrial data indicate paraphyly of the two T. [vetulus] species, with T. vetulus clustering with S. entellus from Sri Lanka and T. johnii with S. entellus from South India, which is concordant with their geographical distribution. These findings indicate paraphyly of S. entellus, whereby North Indian representatives form a further distinct lineage. Accordingly, the langurs of the Indian subcontinent should be split into three species groups, with one occurring solely on Sri Lanka, one in Southern India and a third one in Northern India, whereas the Gondavari river seems to be barrier between the latter two.

Monophyly of each of T. [obscurus], T. [cristatus] and T. [francoisi] and their close affiliation is depicted in all gene trees, so that all of them can be regarded as true members of Trachypithecus. These findings confirm previous molecular studies [8-11,13] and are in general agreement with recent classifications [4,26].

The only discrepancies between different gene trees were obtained for T. [pileatus]. Whereas the mitochondrial data link the species group with Semnopithecus and T. [vetulus], the Y chromosomal data affilites it with T. [obscurus], T. [cristatus] and T. [francoisi]. These findings might be explained by incomplete lineage sorting of ancestral montro-
Figure 5
Phylogenetic relationships among *Semnopithecus* and *Trachypithecus* species groups based on a) mitochondrial data, b) Y chromosomal data, and c) retroposon integrations. Numbers on nodes indicate support values (first: ML, second: NJ, third: MP), and boxed species belong to a species group, with species in bold giving the name of the group.
the varying position of *T. pileatus* in different gene trees might be explained by past hybridization between *Semnopithecus* and *Trachypithecus*. As depicted by the three retropon insertion, this putative hybridization event would have occurred between ancestral forms of *Semnopithecus* and *Trachypithecus*, before both genera diverged into distinct species groups. The hybridization hypothesis is also supported by some intermediate morphological characteristics [4] and the distribution of *T. pileatus*, which is sandwiched between those of *Semnopithecus* and other *Trachypithecus* species groups (Fig. 1, Fig. 2).

**Conclusion**

The present study provides detailed insights into the evolutionary history of Asian colobines and underpins the tremendous power of retropon integration as cladistic markers. Although mitochondrial data proved to be useful to elucidate and confirm several relationships among studied taxa, the data set failed to resolve the affiliations among the langur genera and to settle their position among Asian colobines. In contrast, retropon insertion provided clear evidence for a sister grouping of *Semnopithecus* and *Trachypithecus*, but no integrations were detected, which link *Presbytis* either with the other two langur genera or with the odd-nosed monkeys, so that further research is required to solve this issue. Moreover, to definitively explain the evolutionary history of colobines, further molecular markers should be analyzed, especially regarding possible discrepancies among gene trees due to hybridization or introgression, as such events are important speciation mechanisms in primates [31], and as it was possibly detected in the present study in the case of *T. pileatus*.

Although further investigations are necessary to fully understand the evolution of colobines, the present study provides sufficient data to revise the current classification of the genera *Trachypithecus* and *Semnopithecus* (Table 1). Accordingly, within *Trachypithecus*, three reciprocal monophyletic species groups (*T. obscurus*, *T. cristatus*, and *T. francoisi*) should be recognized, whereas *T. vetulus* should be included in *Semnopithecus*. Moreover, due to their paraphyletic origin, *T. vetulus* and *T. johnii* should not be lumped into a single species group, and *S. entellus* should not be regarded as monotopic. A classification into three species groups with one occurring in Northern India, one in Southern India and one in Sri Lanka may best reflect the evolutionary relationships among the langurs of the Indian subcontinent. However, this arrangement is tentative, because further research is required to confirm their distinctiveness not only on mitochondrial, but also on nuclear DNA level. *T. pileatus* might be the result of an ancient hybridization event between *Semnopithecus* and *Trachypithecus*, and hence, its classification is difficult. While a separation in its own distinct genus may be appropriate, we provisionally accept its traditional recognition as member of *Trachypithecus*.

**Methods**

**Sample collection, DNA extraction and preventing contaminations**

The species analyzed in this study are presented in Table 2. All study specimens were identified by fur colouration and other external characteristics. Hanuman langur (*S. entellus*) samples were collected only from founder animals, of which the area of capture was at least roughly known. Total genomic DNA was extracted from blood, tissue or faeces using the DNeasy or Stool Mini Kits from Qiagen. When hair follicle cells were used, 1–3 hairs were directly implemented into the PCR reaction after they were washed with sterile water and 95% ethanol. To prevent contaminations, sample collection and laboratory procedures followed described standard protocols [40–43]. In detail, all faecal and hair samples were collected with gloves and stored in sterile tubes or plastic bags before further processing. DNA extraction, PCR, gel extraction and sequencing was performed in separate laboratories and repeated after several months, while always only one individual per species was tested. Moreover, from most specimens two different sample types were available, which both were used as template material. Sequences from independent analyses were identical.
Finally, all PCR reactions were performed with negative (distilled water) controls.

**Mitochondrial sequence analysis**

To determine the phylogenetic position of langur genera among Asian colobines, a ~5 kb fragment of the mitochondrial genome was sequenced from all colobine genera with the exception of *Simias* and *Procolobus*. This region spans the cytochrome b gene, the control region, the 12S rDNA and 16S rDNA, and the intermediate tRNAs. To exclude contaminations of the dataset with nuclear pseudogenes, mainly DNA extracted from feces was used as template material, and ~1–2 kb long and ~200–400 bp overlapping fragments were amplified. PCR products were generated via hot-start technique using a set of 24 primers (Table 3) and PCR conditions comprising a pre-denaturation step at 94°C for 2 min, followed by 40 cycles at 94°C for 1 min, 60°C for 1 min and 72°C for 1–2 min, and a final extension step at 72°C for 5 min. The results of the PCR amplifications were checked on agarose gels. PCR products were cleaned with the Qiagen PCR Purification Kit and subsequently sequenced on an ABI 3100-Avant sequencer using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems). To obtain a comprehensive overview on the phylogeny of colobines, the dataset was expanded with further sequences from related taxa deposited at GenBank (Table 2). Accordingly, the final dataset comprised 15 taxa, including eight colobines, four cercopithecines, two hominoids and a New World monkey, which was used as outgroup taxon. Sequences were aligned with ClustalW [44] and subsequently checked by eye. Gaps and poorly aligned positions were removed with the G-blocks software [45], which reduced the final dataset to 4336 bp. Based on this alignment, phylogenetic trees were constructed with the maximum-parsimony (MP), neighbor-joining (NJ) and maximum-likelihood (ML) algorithms as implemented in PAUP 4.0b10 [46] and TREEPUZZLE 5.0 [47]. For MP analyses, all characters were treated as unordered and equally weighted throughout. A heuristic search was performed with the tree-bisection-reconnection (TBR) algorithm with random addition of sequences. The maximum
number of trees was set to 100. NJ and ML trees were constructed as described above. As best-fitting model, MODELTEST selected the TIM + I (= 0.4816) + Γ (= 2.3137) model, which was applied for NJ and ML reconstructions. As for the 5 kb fragment, several alternative tree topologies, in which T. [vetulus] is recognized as monophyletic, either T. [vetulus] or T. [pileatus] belongs to Trachypithecus, or even both are members of Trachypithecus, were tested.

Y chromosomal sequence analysis

The SRY gene was selected as it represents a single-copy gene and as it is proved to be informative in reconstructing the Y chromosomal evolutionary history of macaques [51]. PCR conditions and primers were applied as described [51]. To amplify the SRY gene from fecal material, two overlapping fragments were generated with published primers [51] and the newly generated internal primers 5′-TGGGCGGAGTTGAGAGGGGT-3′ and 5′-TAGCGGTCCCCTGTTGGCGG-3′. The final alignment of 300 bp comprised 15 taxa representing all species groups of the genera Semnopithecus and Trachypithecus as well as Presbytis malalophos, which was used as outgroup. To reconstruct NJ and ML trees, the K80 model of sequence evolution, determined with MODELTEST, was used. MP trees were generated as described above. The reliability of the depicted position of T. [vetulus] and T. [pileatus] was tested in PAUP by using alternative tree topologies, in which either T. [vetulus] belongs to Trachypithecus or T. pileatus groups with Semnopithecus.

Retroposon analysis

Due to their high copy number and relative small size (300 bp), the primate specific Alu elements were selected as cladistic markers. The presence or absence of Alu elements in different colobines at specific loci was tested via PCR using primers occupying the flanking region of the insertion site. Details on analysed loci, primers and studied species are listed in Table 4.

To detect new loci, a subtractive hybridization approach [52] was performed with some modifications. As tracer and driver, different colobine genera were selected. Genomic DNA of tracer and driver was digested with RSAI (Fermentas), and subsequently, the adapters AdapA1/AdapAA1 (5′-TGTAGCGTGACAGACAGACAGACAGACAGAGGGGCTGGTG-3′/5′-ACCCCTCCTCCG-3′) and AdapA2/AdapAA2 (5′-TGTAGCGTGACAGACAGACAGACAGACAGAGGGGCTGGTG-3′/5′-ACCCCTCCTCCG-3′) were ligated to the tracer fragments. Each of ~15 ng tracerA1 and tracerA2 were hybridized with ~1,500 ng driver for 20 h at 60°C. 2 μl of the hybridization result was used as template to amplify solely tracer fragments using the adapter primers A1 (5′-TGTAGCGTGACAGACAGACAGACAGACAGAGGGGCTGGTG-3′) and A2 (5′-TGTAGCGTGACAGACAGACAGACAGACAGAGGGGCTGGTG-3′). The PCR program consisted of a pre-
extension step at 72°C for 6 min to fill in adaptor ends, followed by 25 cycles, each with a denaturation step at 95°C for 1 min, annealing at 60°C for 1 min and extension at 72°C for 2 min. To enrich fragments with Alu insertions, a semi-nested PCR was added using either primer A1 or A2 and the Alu-specific AluY (5'-GGAGAAT- GGCGTGAACCCGGGA-3') oligonucleotide. The PCR products were separated on agarose gels and fragments over 500 bp were excised from the gel. After purification, the fragments were cloned into the pGEMTeasy vector (Promega) and transformed into electro-competent TOP10 cells (Invitrogen). Bacterial clones were collected in 96-well microtiter plates and re-screened via PCR with the primers A1 or A2 and AluY. Positive clones were sequenced and analysed with REPEATMASKER and BLAST as implemented in NCBI and EMBL. Based on the generated alignments, locus-specific primers were constructed, with the forward primer occupying a region 5’-end upstream of the insertion site, which is conserved among the colobine and human or chimp sequences. Due to the absence of the 3’-end downstream sequence of the tested colobine species, reverse primers were constructed solely on the basis of human or chimp sequences. Subsequently, the presence or absence of respective Alu insertions in different colobine species was tested via PCR. The orthology of insertions was confirmed by sequencing of at least one species per genus or species group. Sequences were deposited at GenBank and are available under the accession numbers EU004484-EU004537 and EU006662-EU006692.

Table 4: Presence/absence analysis of retroposon insertions.

| Locus | Primer (5’-3’) | Presence | Absence |
|-------|---------------|----------|---------|
| Asia1 | AAGAATCCAGGAAGAACACT TTGCGTCGCAAGTGACTCTCT | SENT, TOBS, PMEL, PNEM, NLAR | CGUE |
| Asia2 | CCTGCCGACCTCTGTGGGAC AGAACAAAAACAGAAAAAAC | SENT, TOBS, PMEL, PNEM, NLAR | CGUE |
| Asia3 | GCTTTGGCCATAGAAGAGCTGG GCTTAGGTGAAATGGGAAAC | SENT, TOBS, PMEL, PNEM, NLAR | CGUE |
| Asia4 | TCAATCTCCAGGGAAAAATAAAG GAATATGGTAGAAATATTAGGC | SENT, TOBS, PMEL, PNEM, NLAR | CGUE |
| Asia5 | GCCATGGTAGAGAATTGTTGG ACTCAGGTGGGAAAGACACC | SENT, TOBS, PMEL, PNEM, NLAR | CGUE |
| Asia6 | CACCAAGCAGACTGTTGGG, TCGTATACAGCCATAGGCA | TOBS, PMEL, PNEM, NLAR | CGUE |
| Asia7 | CTCTTGGGTTGGGTTAAGGCT TCACTAGTGGTGACTTACCTCT | SENT, TOBS, PMEL, PNEM, NLAR | CGUE |
| ST1 | TGATTTAAGCTCATAGAAGACCC GCTTAGGGGAAAGAACCAC | SENT, TOBS, PMEL, PNEM, NLAR | CGUE |
| ST2 | ATACCATAGCAGATGGGACAC CACGTTGGAGCGACACCGA | SENT, TOBS, PMEL, PNEM, NLAR | CGUE |
| ST3 | GGGATTGACCATCTCAATTAAAAGGA GTTACACCACAACTATACCT | SENT, TOBS, PMEL, PNEM, NLAR | CGUE |
| ST4 | TGTACCGAGGCAATTTTAAAGGA TGGATTGATTTAATAGCATGGTTTG | SENT, TOBS, PMEL, PNEM, NLAR | CGUE |
| ST5 | AGGATGGGACGGCCTCTCTTGGTTTACATATTTGCTTGTG | SENT, TOBS, PMEL, PNEM, NLAR | CGUE |
| odd1 | AGAAAGTTGGGGACGCTCTGGTAGTACAGCAGTGGGTTGAACGC | PNEM, NLAR, RAVU SENT, TOBS, PMEL, CGUE |
| T1 | GAAGATTAACAGTGAAATGACT TGGATATTGCACCATGTGG | TDEL, TOBS, TAUR TDEL, SENT, TOBS, PMEL, PNMEN |
| S1 | CAAATTGCATCCTCCTCAAGTGA GGAGAATATACAGTTCAGTTAG | SENT, TOBS, PMEL, PNEM, TAU |
| S2 | CCCATGTGCGTGTGGTGGTGGTAG TGGAAAGAAGTGGTAGTTGTTG | SENT, TOBS, PMEL, PNEM, TAU |

Abbreviations: CGUE – *Colobus guereza*, NLAR – *Nasalis larvatus*, PMEL – *Presbytis melalophos*, PNEM – *Pygathrix nemaeus*, RAVU – *Rhinopithecus avunculus*, SENT – *Semnopithecus entellus*, TAUR – *Trachypithecus auratus*, TDEL – *T. delacouri*, TJOH – *T. johnii*, TOBS – *T. obscurus*, TPIL – *T. pileatus*, TVET – *T. vetulus*.

Abbreviations: Species names in square brackets ([ ]) indicate species groups.

Authors’ contributions
MO designed primers, performed parts of the laboratory work and wrote parts of the manuscript. LW wrote parts of the manuscript and performed parts of the statistical analysis. CR collected samples, designed primers, performed
parts of the laboratory and statistical work and wrote parts of the manuscript. All authors read and approved the final manuscript.

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