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**Evolutionary History of Snub-nosed Monkeys
(*Rhinopithecus spp.*) and Population Genetic Structure of
the Guizhou Snub-nosed Monkey (*R. brelichi*)**

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Mouyu Yang

aus
Sichuan, China

Göttingen, November 2011

Referentin/Referent: Prof. Dr. Peter M. Kappeler

Korreferentin/Korreferent: Prof. Dr. Eckhard W. Heymann

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Summary

The colobines (leaf monkeys) represent a diverse group of Old World primates. They comprise ten genera with major radiations in Africa and Asia. As in most other primate radiations, recent molecular studies detected discordant gene trees depending on respective markers. Thus, the phylogeny among these genera remains debated. For the odd-nosed monkeys, one of the two major groups of Asian colobines and comprising ten species within four genera (*Nasalis*, *Simias*, *Rhinopithecus* and *Pygathrix*), the phylogenetic relationships among genera and species are also still largely unknown.

The genus *Rhinopithecus* represents one genus of the odd-nosed monkey group. It comprises five species, of which three are endemic to China (*R. roxellana*, *R. bieti*, and *R. brelichi*), one occurs in northern Vietnam (*R. avunculus*) and one in Myanmar (*R. strykeri*). All of them are classified as “Endangered” or “Critically Endangered” (IUCN, 2011). Among the three Chinese snub-nosed monkey species, *R. brelichi* shows the smallest wild population size and the most restricted distribution. Information about its population genetic structure is still missing.

In my thesis, I applied a top-down approach to clarify the phylogenetic relationships among all colobines genera, the phylogenetic relationships among the odd-nosed monkey species and the population genetic structure of one of the most endangered species of the colobines, the Guizhou snub-nosed monkey (*R. brelichi*).

The phylogenetic relationships among colobines genera are analyzed in this study by using maternal-, paternal- and biparental-inherited molecular markers (Chapter 1). The phylogenetic reconstructions from all these markers show similar results. However, a few discordances occurred, which are most likely caused by sex-specific introgression and hybridization among ancestral lineages. However, the monophyly of the odd-nosed monkeys and the relationships among genera appeared robust and was supported by the various topologies based on different genetic markers.

To further understand the phylogenetic relationships within the odd-nosed monkeys nine of the ten species were analyzed based on complete mitochondrial genome data (Chapter 2) providing the most comprehensive overview published so far. *R. strykeri* was not included in this part of my thesis, because this species was just

recently discovered and genetic material was not available. Among the odd-nosed monkeys, the genus *Rhinopithecus* is most basal, whereas *Pygathrix* forms a sister lineage to the *Nasalis* + *Simias* clade. Within *Rhinopithecus*, *R. roxellana* + *R. brelichi* form a sister clade to *R. bieti*, while *R. avunculus* appears as the most basal taxon. For *Pygathrix*, the analysis supported *P. nigripes* as the basal taxon to the *P. cinerea* + *P. nemaeus* clade. The diversification of odd-nosed monkeys into genera started roughly 6.85 mya, with the major speciation events occurring during the Pleistocene.

R. brelichi with a maximum of 800 individuals in the wild is one of the rarest Chinese primates. However, population genetic data is crucial for assessing the persistence of a population, especially for endangered species with a small population. Previous population genetic studies focused on *R. roxellana* and *R. bieti*, but no such information was available for *R. brelichi*. Therefore, the aim of chapter 3 was an assessment of the population genetic and demographic history of *R. brelichi* in comparison with respective data from the two other Chinese species (*R. roxellana*, *R. bieti*). The population genetic variation and the demographic history of this endangered species were assessed by using the hypervariable region I of the mitochondrial control region. All three Chinese snub-nosed monkey species showed rather low genetic diversity, while *R. brelichi* has the lowest. All tests for the demographic history proposed population equilibrium in *R. brelichi*. The low genetic diversity, the small population size and the restricted distribution imply that *R. brelichi* might be the most vulnerable species among the three Chinese snub-nosed monkeys.

Future studies should apply nuclear data to further understand the phylogenetic relationships among the odd-nosed monkeys' species and to answer questions about possible ancient hybridization events as depicted for colobines genera. For *R. strykeri* the phylogenetic position within the odd-nosed monkeys should be examined to complete the picture. Further detailed population genetic analyses for *R. brelichi* should be applied by using nuclear markers, e.g. microsatellites to get more detailed information about the genetic diversity and demographic history of *R. brelichi*. This information is also required for the two most endangered species of the genus *Rhinopithecus*, *R. avunculus* and *R. strykeri*.

1. General Introduction

"Nothing in biology makes sense except in the light of evolution" was famously claimed by Theodosius Dobzhansky (1973). It clearly indicates that the modern evolutionary synthesis is the cornerstone of biology. Evolution is a process of change on population-level over time and space. Evolutionary research includes two central goals: 1) describe the branching order of the life history on earth, and 2) explain why species differ from one another and how the evolutionary processes occur (Whelan et al., 2001). Nei (1975) gave a clear definition of the goal of studying evolution: *"understand all the processes of evolution quantitatively and be able to predict and control the future evolution of organisms"*.

Since Willi Hennig published his paper in 1965 "Phylogenetic Systematic", the phylogenetic tree gradually became the critical underpinning of research in all evolutionary studies (Soltis and Soltis, 2003). Phylogenetic research mainly aims to represent the evolutionary relationships among organisms (and genes) as a tree, which constitutes a phylogenetic hypothesis. Phylogenetic trees are constructed based on molecular or morphological data, or data on other traits (Edwards and Cavalli-Sforza, 1964; Stearns and Hoekstra, 2000). Phylogenies provide extremely useful information for many different fields within biology. Their paramount uses are testing hypotheses about the evolution of characters and tracking the history of any change in a specific character. Overall, phylogenies give scientists new perspectives for looking into the past (Losos, 2011). This important development has given rise to phylogenetic analyses as an instrument used for classification, identification, and naming of organisms (Edwards and Cavalli-Sforza, 1964; Wiley et al., 1991; Harvey et al., 1996). Phylogenetics is nowadays a critical important tool for investigating the evolutionary history of organisms, like J. M. Savage said *"nothing in evolutionary biology makes sense except in the light of a phylogeny"* (Savage, 1997 cited in Johnson et al., 2003).

Among our closest living relatives are the Old World monkeys (Cercopithecidae), one of the most diverse primate groups. Unfortunately, the evolutionary history and phylogenetic relationships among its members are not well resolved yet, thus, requiring additional phylogenetic studies. Old World monkeys comprise two subfamilies: Cercopithecinae and Colobinae (Groves, 2001). Each subfamily is clearly differentiated by distinctive morphological adaptations, mainly related to their digestive systems. The

cercopithecines - also called cheek pouch monkeys - are predominantly frugivorous and have cheek pouches, which they use for temporarily storing food. In contrast, colobines are predominantly folivorous and they have a multi-chambered ruminant-like stomach to digest food rich in cellulose (Groves, 1970; Brandon-Jones, 1984; Davies and Oates, 1994; Groves, 2001; Fashing, 2011, Kirkpatrick, 2011).

Cercopithecines have been extensively studied in many aspects of their natural history including molecular phylogenies (e.g., Jolly, 1966; Hill, 1974; Groves, 1978; Dutrillaux, 1979; Szalay and Delson, 1979; Groves, 1987; Strasser and Delson, 1987; Disotell et al., 1992; Jablonski and Peng, 1993; Benson et al., 1998; Harris and Disotell, 1998; Morales and Melnick, 1998; Evans et al., 1999; Jolly and Whitehead, 2000; McGraw and Fleagle, 2000; Frost et al., 2003; Roos et al., 2003; Tosi et al., 2003, Roos, 2004; Tosi et al., 2004, 2005; Roos et al., 2007; Xing et al., 2007; Ziegler et al., 2007; Moulin et al., 2008; Fabre et al., 2009; Li et al., 2009; Gilbert, 2011). Less emphasis was placed on the analysis of the phylogeny of the sister taxon of the cercopithecines, the colobines. Colobines build a diverse group of monkeys (Oates, 1994) with ongoing disputes about phylogenetic relationships among its members (Groves, 1989; Jablonski, 1998c; Jablonski, 1999; Stewart and Disotell, 1999; Zhang and Ryder, 1999) and their molecular phylogeny has only recently been studied (Zhang and Ryder, 1998; Roos, 2004; Sterner et al., 2006; Whittaker et al., 2006; Roos et al., 2007; Osterholz et al., 2008; Roos et al., 2008; Ting, 2008; Ting et al., 2008; Karanth, 2010; Md Zain et al., 2010; Meyer et al., 2011). One reason is probably that most of these species are difficult to keep in captivity and obtaining samples from them in the wild is due to their mainly arboreal life style more difficult than from cercopithecines (Zhang and Ryder, 1998; Xing et al., 2005).

Traditionally, colobines have been divided into an African and Asian group based on both morphological traits and geographical distribution (Delson, 1975). Both taxa are believed to form reciprocally monophyletic groups (Napier and Napier, 1967; Szalay and Delson, 1979; Davies and Oates, 1994; Groves, 2001), though paraphyly has also been proposed (Groves, 1989; Jablonski, 1998c). African colobines are distributed in many parts of Sub-Saharan Africa, but are predominantly found in the western and central rain humid forests. They are arboreal and share traits such as a reduced or missing pollex, mid-tarsal shortening, and some other postcranial and dental features (Szalay and Delson, 1979; Strasser and Delson, 1987; Falk, 2000;

Groves, 2001). Phylogenetic hypotheses for the African colobines have been developed based on pelage coloration and anatomical features (Kingdon, 1997; Groves, 2001; Grubb et al., 2003; Groves, 2007). Traditionally, all African colobines were classified as members of the genus *Colobus* (Szalay and Delson, 1979). Verheyen (1962) and Davies and Oates (1994) split the African colobines into two genera, *Procolobus* and *Colobus*, while some authors also separate red colobus monkeys from *Procolobus* as a distinct genus, *Piliocolobus* (Struhsaker, 1981; Groves, 2001; Grubb et al., 2003; Groves, 2007). The *Piliocolobus* + *Procolobus* clade is regarded as sister group of *Colobus* (Hill, 1952; Kuhn, 1972; Napier, 1985; Strasser and Delson, 1987; Ting, 2008).

Asian colobines are distributed throughout Southeast Asia, including Southern China, the Indian subcontinent, Indochina and Sundaland. They occupy a variety of habitats ranging from tropical rainforests to semi-desert conditions (Bennet and Davies, 1994). They are predominantly arboreal, but under certain conditions, such as in the semi-desert of north-western India, they are almost entirely terrestrial (Hanuman langurs of Jodhpur, Vogel, 1976). Traits such as a shorter face and the presence of a suborbital fossa distinguish them from the African species. Although the Asian colobines have somewhat larger pollexes than the African species, their pollexes are still reduced compared to those of the cercopithecines (Falk, 2000; Groves, 2001). Asian colobines are more diverse than the African colobines (Oates and Davies, 1994) and they comprise the langur/leaf monkey group with three genera (*Presbytis*, *Semnopithecus*, *Trachypithecus*) and the odd-nosed monkey group with four genera (*Nasalis*, *Simias*, *Rhinopithecus*, *Pygathrix*) (Jablonski and Peng, 1993; Jablonski, 1998c; Falk, 2000; Groves, 2001; Brandon-Jones et al., 2004; Groves, 2007; Osterholz et al., 2008).

The taxonomy of langurs and leaf monkeys is still disputed and contradicting. In the most recent overview (IUCN, 2011), *Trachypithecus* is acknowledged consisting of 16 species, *Semnopithecus* including eight species, and *Presbytis* comprising 11 species. Similarly to their taxonomy, the phylogenetic relationships among the langurs and leaf monkeys are also disputed. All langurs and leaf monkeys have been originally classified in the single genus *Semnopithecus* or *Presbytis* (Reichenbach, 1862; Napier and Napier, 1967; Groves, 1970; Delson, 1975). Brandon-Jones (1984) separated *Semnopithecus* from *Presbytis* and established *Trachypithecus* as a morphological

subgenus of the former based on the overall appearance. Based on neonatal coloration and cranial morphology, Pocock (1935) divided langurs and leaf monkeys into the three genera *Semnopithecus*, *Trachypithecus*, and *Presbytis*. Hill (1934) added *Kasi* as a fourth genus. However, recent classification of the langurs has been commonly accepted as consisting of three genera: *Presbytis*, *Trachypithecus* and *Semnopithecus* (Weitzel et al., 1988; Groves, 1989; Davies and Oates, 1994; Groves, 2001; Brandon-Jones et al., 2004; Osterholz et al., 2008; Karanth, 2010). Phylogenetic studies now arrange *Semnopithecus* with *Trachypithecus* to the exclusion of *Presbytis* (Brandon-Jones, 1984; Strasser and Delson, 1987; Brandon-Jones, 1995; Groves, 2001; Osterholz et al., 2008; Ting et al., 2008) or place *Trachypithecus* and *Presbytis* as sister taxa to the exclusion of *Semnopithecus* (Sternner et al., 2006).

Odd-nosed monkeys comprise the genera *Rhinopithecus*, *Pygathrix*, *Nasalis* and *Simias*. All are characterised by particular modifications to their noses (Groves 2001). The noses of the *Nasalis/Simias* group are entire. Their face is long and narrow and the inter-orbital pillar looks unusually narrow. Proboscis monkeys (*Nasalis*) have a long and narrow skull with elongated noses (in particular adult males) and strong supra-orbital ridges. This genus comprises only one species (*N. larvatus*), which lives mainly in mangrove and riverine forests on Borneo. Pig-tailed monkeys (*Simias*) have smaller noses than *Nasalis* and a very short upturned, curly and nearly hairless tail. The single species *S. concolor* is endemic to the Mentawai Islands, west of Sumatra. The *Pygathrix/Rhinopithecus* group shows flaps of skin on the upper margins of the nostrils and their nasal bones are reduced. Their face looks short and wide. Douc langurs (*Pygathrix*) have a short and wide facial skeleton, with strong supra-orbital ridges and a broad inter-orbital pillar. It comprises three species, red-shanked doucs (*P. nemaeus*), gray-shanked doucs (*P. cinerea*) and black-shanked doucs (*P. nigripes*). Doucs are distributed through parts of Vietnam, Laos and Cambodia, east of the Mekong River.

Snub-nosed monkeys (*Rhinopithecus*, Fig. 1) display a more prognathous and wider facial skeleton and stronger supra-orbital torus than *Pygathrix* and the palate is longer. This genus comprises five species: the Tonkin snub-nosed monkey (*R. avunculus*), the Myanmar snub-nosed monkey (*R. strykeri*), the golden or Sichuan snub-nosed monkey (*R. roxellana*), the gray or Guizhou snub-nosed monkey (*R. brelichi*), and the black or Yunnan snub-nosed monkey (*R. bieti*) (Groves, 2001; Roos et al., 2007; Geissmann et al., 2011; IUCN, 2011).

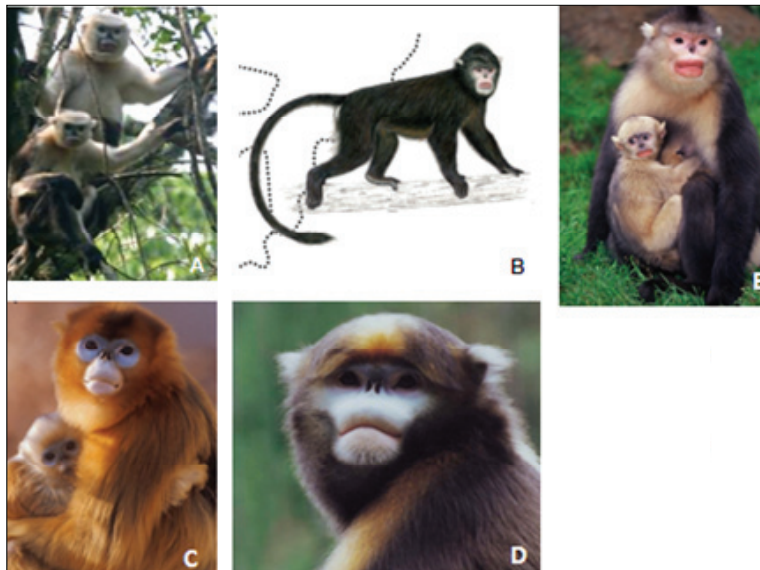


Figure 1: The five snub-nosed monkey species. A) *R. avunculus*; B) *R. strykeri*; C) *R. roxellana*; D) *R. brelichii*; E) *R. bieti* (photographs and drawings from Dong Thanh Hai, Primate Conservation INC.; Stephen Nash, Conservation International; Heather Angel, Naturalvisions.co.uk; Yeqin Yang, Fanjingshan National Nature Reserve; China Kunming animal institution).

Although a common origin of odd-nosed monkeys has been supported by recent genetic analyses (Sterner et al., 2006; Osterholz et al., 2008; Ting et al., 2008; Li et al., 2011; Perelman et al., 2011), debates on the phylogenetic relationships among genera and species still continue. Based on morphological or molecular data, previous studies proposed several phylogenetic hypotheses: (1) a sister taxon relationship between *Rhinopithecus* and *Pygathrix* (Delson, 1975; Napier, 1985; Jablonski and Peng, 1993; Oates et al., 1994; Wang et al., 1997; Groves, 2001; Li et al., 2004); (2) a sister taxon relationship between *Pygathrix* and *Nasalis* to the exclusion of *Rhinopithecus* (Jablonski, 1998c); (3) a sister taxon relationship between *Rhinopithecus* and *Nasalis* (Zhang and Ryder, 1998); (4) a sister taxon relationship between *Simias* and *Nasalis* (Whittaker et al., 2006); and (5) a basal position of *Simias* among Asian colobines (Jablonski, 1998c).

The monophyly of odd-nosed monkey genera is generally accepted, but Peng et al. (1993) suggested a paraphyly of *Rhinopithecus* and placed *R. avunculus* as sister taxon to *Pygathrix*. Within *Pygathrix*, *P. nigripes* is basal to a clade consisting of *P. cinerea* and *P. nemaeus* (Roos and Nadler, 2001; Roos, 2004; Roos et al., 2007). In contrast, the phylogenetic relationships among the five *Rhinopithecus* species are

debated. Jablonski and Peng (1993) pointed out that *R. roxellana* clusters together with the *R. bieti* + *R. brelichi* clade. A three-way relationship between *R. bieti*, *R. roxellana* and *R. avunculus* was proposed by Zhang and Ryder (1998). This hypothesis was supported by molecular studies of Roos (2004), Roos et al. (2007) and Li et al. (2004). In a recent study by Li et al. (2011), *R. avunculus* appeared as the sister lineage to the *R. bieti* + *R. roxellana* clade. Although *R. strykeri* was not included in all these studies, this species resembles *R. bieti* in many morphological attributes suggesting a sister taxon relationship of the two species (Geissmann et al., 2011).

Although the phylogenetic relationships within the Asian colobines became clearer due to a number of recent molecular studies, several questions remain especially in light of the observed incongruences found among various gene tree phylogenies (Sternler et al., 2006; Karanth et al., 2008; Osterholz et al., 2008; Ting et al., 2008). Therefore, the first aim of my study is to clarify the phylogenetic relationships among the four odd-nosed monkey genera and respective species.

Many of the Asian colobines are endangered including all snub-nosed monkey species (IUCN, 2011). The ultimate interest of conservation is the preservation of genetic diversity (Avice and Hamrick, 1995). The degree of genetic diversity within a population can have impacts on the long-term survival of the respective population, especially in case of rare and endangered species (Frankham et al., 2004). Molecular methods have been successfully applied in conservation biology, in particular on the population genetic level. Information about the genetic diversity within a population often provides hints for the management of such populations.

Fossils records of snub-nosed monkeys indicate that they were widely distributed in China and Vietnam during the Pleistocene (Li et al., 2002). The contemporary highly fragmented ranges of snub-nosed monkeys most likely result from environmental changes in the Holocene (Pan and Jablonski, 1987; Pan, 1995), and from increasing human activities during the last centuries. Suitable habitats for snub-nosed monkeys were heavily reduced and their populations survived only in restricted areas (Jablonski, 1998b; Li et al., 2002). Currently, *R. avunculus* occurs in tropical monsoon forests in Tuyen Quang, Bac Kan, Thai Nguyen and Ha Giang provinces of northern Vietnam at elevations of 200 - 1,200 m (Kirkpatrick, 1995; Nguyen, 2000; Groves, 2001; Nadler et al., 2003; Dong and Boontatana, 2006; IUCN, 2011) (Fig. 2). Its population comprises roughly 200 individuals (Covert et al., 2008; Mittermeier et al.,

2009; IUCN, 2011). *R. strykeri* has been recently discovered in Kachin state in north-eastern Myanmar where it inhabits mixed temperate and conifer forests at 1,720 - 3,190 m with an estimated population size of 260 - 330 individuals (Geissmann et al., 2011). The remaining three species are endemic to mountainous regions of south-western China. *R. roxellana* occupies deciduous broadleaf, conifer and mixed forests at an elevation of 1,200 - 3,000 m in the provinces of Sichuan, Hubei, Shaanxi and Gansu and its population size is approximately 15,000 individuals (IUCN, 2011). *R. bieti* inhabits evergreen forests composed primarily of conifers, evergreen oak in Yunnan and Tibet at 2,700 - 4,600 m. Its population comprises about 2,000 individuals (IUCN, 2011). *R. brelichii* lives in mixed deciduous and evergreen broadleaf forests in Guizhou at 1,500 - 2,200 m and its population comprises 750 - 800 individuals (Yang et al., 2002; IUCN, 2011). All species are today classified as “Endangered” or even “Critically Endangered” (IUCN, 2011).

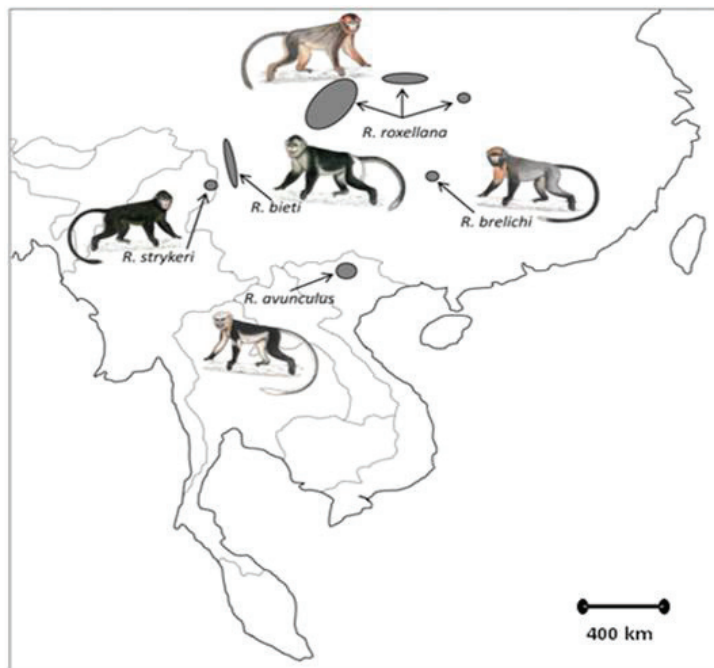


Figure 2: Map of the northern part of Southeast Asia indicating the approximate distributions of the five snub-nosed monkey species. Illustrations of monkeys by Stephen Nash, Conservation International.

The Guizhou snub-nosed monkey (*R. brelichii*) (Fig. 3) has the most restricted distribution and comprises the smallest population compared to the other two Chinese

snub-nosed monkey species. It occurs only in evergreen and deciduous broadleaf forests in a strictly protected core area of about 260 km² of the Fanjingshan National Nature Reserve, a small area of the Wuling Mountains in Guizhou province (Bleisch and Xie, 1998; Groves, 2001; Yang et al., 2002; Wu et al., 2004; MacKinnon, 2008; IUCN, 2011). Additionally, in a inter-specific comparison among the three Chinese snub-nosed monkey species indications were found that *R. brelichi* has the slowest population recovery capability due to a slower life-history (female age at first birth: *R. brelichi* 8-9 years, *R. roxellana* and *R. bieti* 5-6 years; longer inter-birth interval: *R. brelichi*: 3 years, *R. roxellana* and *R. bieti* 2 years (Ji et al., 1998; Zhang et al., 2000; Liang et al., 2001; Cui et al., 2006; Qi et al., 2008; Yang et al., 2009).

Population genetic data for *R. brelichi* are not available. Therefore, I did a detailed population genetic study of *R. brelichi* and compared it with already existing data for the other two Chinese snub-nosed monkey species (Li et al., 2001; Li et al., 2003; Li et al., 2007; Liu et al., 2007). The second aim of my thesis is to analyze the level of genetic diversity and demographic history of the wild population of *R. brelichi*.



Figure 3: Guizhou snub-nosed monkeys in the Fanjingshan National Nature Reserve (Photographs taken with camera traps).

Aim and content of this thesis

The first aim of my study was to elucidate the phylogenetic relationships among odd-nosed monkey genera and among respective species. The second aim was to analyze the population history and genetic diversity of *R. brelichi*. I organized my study in a top down manner, i.e. from the higher taxonomic levels to the species and population levels.

Chapter 1: On the first level we analyzed the evolutionary history of the subfamily Colobinae, in particular the phylogenetic relationships among the odd-nosed monkey genera. To obtain a better understanding of the evolutionary history of the odd-nosed monkey group, we analyzed sequence data from mitochondrial genomes and 12 nuclear loci (in total ~ 30,000 bp per genus), and combined it with the presence / absence patterns of mobile elements. (Roos et al. 2011. Nuclear versus mitochondrial DNA: evidence for hybridization in colobine monkeys. BMC Evolutionary Biology 11: 77).

Chapter 2: In a second step, we determined the phylogenetic relationships among nine of the ten odd-nosed monkey species using complete mitochondrial genome sequences. The obtained phylogeny is robust and provides new insights into the evolutionary history of the odd-nosed monkey group. (Yang et al. in press. The evolutionary history of odd-nosed monkeys. In: Tan C, Grueter C, Wright B, editors. Odd-nosed Monkeys: Recent Advances in the Study of the Forgotten Colobines).

Chapter 3: On the third level, my aim was to compare the genetic diversity of the three Chinese snub-nosed monkey species based on mitochondrial data (hypervariable region I of the control region). Analyses on level three are also designed to provide useful information for conservation management decisions. Here, I provide a comprehensive overview over the population genetic structure and the population history of the three Chinese *Rhinopithecus* species. (Yang et al. 2011. Population genetic structure of Guizhou snub-nosed monkeys (*Rhinopithecus brelichi*) as inferred from mitochondrial control region sequences, and comparison with *R. roxellana* and *R. bieti*. Doi 10.1002/ajpa.21618

Chapter 1

Nuclear Versus Mitochondrial DNA: Evidence for Hybridization in Colobine Monkeys

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Nuclear versus mitochondrial DNA: evidence for hybridization in colobine monkeys

Christian Roos^{1,2*}, Dietmar Zinner^{3†}, Laura S Kubatko⁴, Christiane Schwarz¹, Mouyu Yang¹, Dirk Meyer⁵, Stephen D Nash⁶, Jinchuan Xing⁷, Mark A Batzer⁸, Markus Brameier¹, Fabian H Leendertz⁹, Thomas Ziegler⁵, Dyah Perwitasari-Farajallah¹⁰, Tilo Nadler¹¹, Lutz Walter^{1,2}, Martin Osterholz^{1,12*}

Abstract

Background: Colobine monkeys constitute a diverse group of primates with major radiations in Africa and Asia. However, phylogenetic relationships among genera are under debate, and recent molecular studies with incomplete taxon-sampling revealed discordant gene trees. To solve the evolutionary history of colobine genera and to determine causes for possible gene tree incongruences, we combined presence/absence analysis of mobile elements with autosomal, X chromosomal, Y chromosomal and mitochondrial sequence data from all recognized colobine genera.

Results: Gene tree topologies and divergence age estimates derived from different markers were similar, but differed in placing *Piliocolobus/Procolobus* and langur genera among colobines. Although insufficient data, homoplasy and incomplete lineage sorting might all have contributed to the discordance among gene trees, hybridization is favored as the main cause of the observed discordance. We propose that African colobines are paraphyletic, but might later have experienced female introgression from *Piliocolobus/Procolobus* into *Colobus*. In the late Miocene, colobines invaded Eurasia and diversified into several lineages. Among Asian colobines, *Semnopithecus* diverged first, indicating langur paraphyly. However, unidirectional gene flow from *Semnopithecus* into *Trachypithecus* via male introgression followed by nuclear swamping might have occurred until the earliest Pleistocene.

Conclusions: Overall, our study provides the most comprehensive view on colobine evolution to date and emphasizes that analyses of various molecular markers, such as mobile elements and sequence data from multiple loci, are crucial to better understand evolutionary relationships and to trace hybridization events. Our results also suggest that sex-specific dispersal patterns, promoted by a respective social organization of the species involved, can result in different hybridization scenarios.

Background

With more than 50 species and due to some ecological adaptations, such as a ruminant-like chambered stomach to digest food rich in fiber, the Old World monkey subfamily Colobinae represents a diverse and enigmatic group of primates [1,2]. Colobines are predominantly arboreal and occur in forest and woodland habitats. They have experienced two major radiations, one in Africa with the genera *Procolobus*, *Piliocolobus* and

Colobus, and a second in South and Southeast Asia comprising the langur genera *Semnopithecus*, *Trachypithecus* and *Presbytis*, and the odd-nosed monkey genera *Rhinopithecus*, *Pygathrix*, *Nasalis* and *Simias* [2]. However, their phylogenetic relationships are disputed [3-7], and recent molecular studies detected substantial gene tree discordance [8-10].

Traditionally, African and Asian genera are believed to form reciprocally monophyletic groups [1,2,11,12], though paraphyly has also been proposed [3-5]. Molecular investigations clearly confirm a common origin of Asian colobines and the odd-nosed monkey group [8-10], but evidence for monophyly of the langur group as well as for African colobines is still lacking.

* Correspondence: croos@dpzeu; mosterh@gwdg.de

† Contributed equally

¹Primate Genetics Laboratory, German Primate Center, Kellnerweg 4, 37077 Göttingen, Germany

Full list of author information is available at the end of the article

Moreover, nuclear and mitochondrial data indicate conflicting relationships among langur genera, and between langurs and the odd-nosed monkeys [8-10]. While nuclear data consistently link *Semnopithecus* and *Trachypithecus* to the exclusion of all other Asian colobines [9,10], mitochondrial data either do not resolve these relationships [9] or suggest a clade consisting of *Presbytis* and *Trachypithecus* [8].

Incongruent phylogenetic relationships among genes, like those detected among colobines are common in phylogenetic studies and could be explained by homoplasy, insufficient data, nucleotide composition, differential lineage sorting, or hybridization [13-21]. To ascertain which of these possibilities are responsible for the incongruence, information from various independent molecular loci can be helpful [22]. To date, only mitochondrial and X chromosomal data as well as presence/absence information of mobile elements, all based on an incomplete taxon sampling, are available for comparative phylogenetic studies in colobines [8-10,23]. Among all marker systems, mobile element insertions are a promising tool to uncover phylogenetic relationships among colobine genera. Compared to sole sequence data, mobile elements such as Short Interspersed Elements (SINEs) and Long Interspersed Elements (LINEs) exhibit advantages which make them ideal markers for phylogenetic reconstructions (for review see [24-30]). Accordingly, mobile elements are successfully applied in numerous primate phylogenetic studies [9,28,31-39].

In our study, we examined the presence/absence pattern of mobile elements and compared the inferred phylogeny with those derived from mitochondrial and nuclear sequence data (in total ~30,000 bp per genus). We extended available X chromosomal and mitochondrial genome data, and sequenced de novo five autosomal loci that map to different human chromosomes, and six Y chromosomal loci from all ten colobine genera. By combining results from different marker systems, we provide detailed insights into the evolutionary and biogeographic history of colobine monkeys, and show that different hybridization mechanisms might have been involved during the colobine radiation.

Results

Nuclear phylogeny

Eighty-three mobile elements are phylogenetically informative for colobines (Figure 1A, Additional file 1). Each of the following clades is strongly supported by at least five integrations: all colobines (clade I [A-I]), Asian colobines (A-IV), odd-nosed monkeys (A-VII), *Trachypithecus* and *Semnopithecus* (A-V), and *Nasalis* and *Simias* (A-IX). Three integrations were found in *Ptilocolobus* and *Procolobus* and all Asian colobines (A-II), but not in *Colobus*. Two insertions suggested a sister grouping of *Procolobus*

and *Ptilocolobus* (A-III), *Presbytis* and the odd-nosed monkeys (A-VI), and a basal position of *Rhinopithecus* among the latter (A-VIII). Based on maximum-parsimony (MP) bootstrap analysis, most relationships were strongly supported ($\geq 95\%$). Only the *Ptilocolobus/Procolobus* (A-III), *Presbytis*/odd-nosed monkey (A-VI), and *Pygathrix/Nasalis/Simias* (A-VIII) clades gained relatively weak bootstrap values (86%). Based on alternative tree topology tests, different positions of the *Ptilocolobus/Procolobus* clade and *Presbytis* among colobines were not rejected ($P > 0.05$), while relationships other than the most likely one were significantly rejected for all other taxa ($P < 0.001$, $P < 0.05$) (Additional file 2).

Next, we performed phylogenetic analyses based on the concatenated nuclear sequence dataset, including five autosomal loci, six Y chromosomal loci and a fragment of the X chromosomal Xq13.3 region (see Methods for detailed locus description). We combined all nuclear sequence data, because heuristic search methods for individual loci produced no conflicting relationships (Additional file 3), and partition homogeneity tests revealed no significant difference in their evolutionary history (Y chromosomal loci combined: $P = 0.2939$; autosomal loci combined: $P = 0.1543$; all nuclear loci combined: $P = 0.3559$). Nucleotide composition of studied species was similar (Additional file 4). Phylogenetic reconstructions yielded identical and significantly supported branching patterns irrespectively of the applied algorithm (MP, neighbor-joining [NJ], maximum-likelihood [ML], Bayesian) (Figure 1B, for a phylogram see Additional file 5). Only the *Pygathrix/Nasalis/Simias* (B-VIII) clade had lower support values (MP: 93%, NJ: 80%, but ML: 98%, Bayesian posterior probabilities [PP]: 1.0). The resultant tree topology was mainly congruent with the mobile element-based phylogeny, but two cases of incongruence were obvious. First, in the nuclear sequence-based phylogeny, African (B-II) and Asian (B-IV) colobine genera formed reciprocally monophyletic clades and second, *Presbytis* represented a sister lineage to the other Asian genera (B-V). According to alternative tree topology tests (Additional file 2), paraphyly of African colobines with *Ptilocolobus/Procolobus* being closer related to Asian colobines than to *Colobus* as well as various alternative positions of *Presbytis* among Asian colobines were not rejected ($P > 0.05$). However, affiliations of *Presbytis* to either *Semnopithecus* or *Trachypithecus* were rejected ($P < 0.001$).

Estimated divergence ages from the combined nuclear dataset (Table 1) and single loci (Additional file 6), both based on an a-priori fixed tree topology as obtained from mobile elements, differed slightly, most likely due to the general low variability in the studied loci (Additional file 7). However, estimates were in the same range suggesting that loci evolve at similar evolutionary rates (Additional file 8).

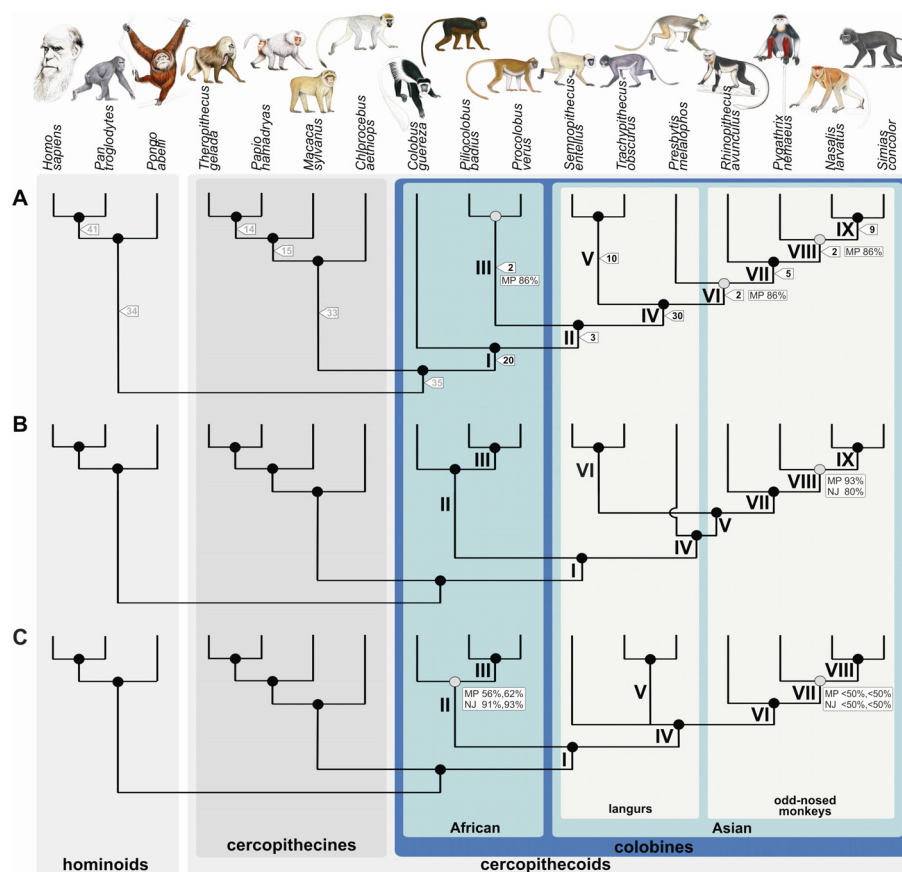


Figure 1 Phylogenetic relationships among colobine and outgroup genera as inferred from different datasets. Panels refer to insertions of mobile elements (A), combined nuclear sequence data (B), and mitochondrial genome data (C). Roman numerals are used as branch identifiers and are discussed in the text. In A, numbers in flags represent the number of available mobile elements (black: colobine markers, grey: non-colobine markers). In B and C, all nodes are significantly supported by ML and Bayesian reconstructions ($\geq 95\%$, 1.0). Black and grey dots on nodes indicate high ($\geq 95\%$) and lower ($< 95\%$) branch support as obtained from MP (in A-C) and NJ (in B and C) reconstructions, respectively. Bootstrap values $< 95\%$ are presented at respective nodes. In C, first and second values refer to those obtained from reconstructions using datasets mtDNA1 and mtDNA2, respectively.

According to our nuclear estimates, *Colobus* and *Piliocolobus/Procolobus* successively split off from Asian genera 10.93 million years ago (mya) and 10.73 mya, respectively (for 95% highest posterior densities see Table 1). The latter two separated 6.92 mya. In Asia, an initial split occurred 8.12 mya and led to a clade consisting of *Trachypithecus* and *Semnopithecus*, and a group containing *Presbytis* and the odd-nosed monkeys. Among the latter, *Presbytis* diverged 7.96 mya and the odd-nosed monkeys began differentiating 6.43 mya. The most recent splits among Asian genera occurred between *Trachypithecus* and *Semnopithecus* (2.56 mya) and between *Nasalis* and *Simias* (1.06 mya).

Mitochondrial phylogeny

Mitochondrial and nuclear datasets were not combined, because the partition homogeneity test suggested that both track different evolutionary histories ($P = 0.0002$). Thus, mitochondrial sequence data were analyzed separately. For

both alignments (mtDNA1, mtDNA2; for details about alignments see Methods), we observed a major shift in nucleotide composition between colobine and non-colobine representatives (Additional file 4). Both alignments produced identical and significantly supported branching patterns among genera (Figure 1C, for a phylogram see Additional file 5). Only the *Pygathrix/Nasalis/Simias* (C-VII) and African colobine (C-II) clades gained low MP ($< 50\%$, $< 50\%$, 56%, 62%) and NJ ($< 50\%$, $< 50\%$, 91%, 93%) bootstrap values, but ML and Bayesian reconstructions provided strong support for both nodes (96%, 100%; 1.0, 1.0). In principal, the tree topology was identical to those obtained from mobile elements and nuclear sequence data. However, as in the nuclear sequence tree, mitochondrial data suggested African (C-II) and Asian (C-IV) colobines as reciprocal monophyletic clades. Moreover, Asian colobines further diverged into a lineage leading to the odd-nosed monkeys (C-VI), a lineage comprising

Table 1 Estimation of divergence ages in mya (95% highest posterior density)

node	nuclear DNA	mitochondrial DNA
cercopithecoids - hominoids	24.39 (22.44-26.47)	23.73 (21.88-25.94)
<i>Pongo</i> - <i>Homo/Pan</i>	13.89 (12.80-14.95)	13.58 (12.51-14.64)
<i>Homo</i> - <i>Pan</i>	6.39 (5.85-7.01)	6.18 (5.62-6.70)
cercopithecines - colobines	15.50 (14.45-16.56)	15.92 (14.11-17.79)
<i>Chlorocebus</i> - other cercopithecines	9.47 (7.52-11.57)	10.56 (8.78-12.29)
<i>Macaca</i> - <i>Papio/Theropithecus</i>	6.59 (5.12-8.27)	8.55 (6.82-10.03)
<i>Papio</i> - <i>Theropithecus</i>	3.80 (3.20-4.38)	3.97 (3.39-4.46)
<i>Colobus</i> - other colobines (A-I)	10.93 (9.60-12.31)	-
<i>Piliocolobus/Procolobus</i> - Asian colobines (A-II)	10.73 (9.38-12.04)	-
African - Asian colobines (C-I)	-	10.90 (9.34-12.44)
<i>Colobus</i> - <i>Piliocolobus/Procolobus</i> (C-II)	-	8.47 (6.83-9.88)
<i>Piliocolobus</i> - <i>Procolobus</i> (A-III, C-III)	6.92 (4.38-9.35)	6.58 (4.99-8.04)
Asian colobines (A-IV, C-IV)	8.12 (7.14-9.16)	8.91 (7.43-10.23)
<i>Trachypithecus</i> - <i>Semnopithecus</i> (A-V)	2.56 (1.25-4.22)	-
<i>Presbytis</i> - odd-nosed monkeys (A-VI)	7.96 (6.93-8.95)	-
<i>Presbytis</i> - <i>Trachypithecus</i> (C-V)	-	7.45 (5.88-8.86)
odd-nosed monkeys (A-VII, C-VI)	6.43 (5.03-7.75)	6.91 (5.60-8.20)
<i>Pygathrix</i> - <i>Nasalis/Simias</i> (A-VIII, C-VII)	5.66 (4.22-7.01)	6.23 (5.11-7.38)
<i>Nasalis</i> - <i>Simias</i> (A-IX, C-VIII)	1.06 (0.44-1.81)	1.88 (1.21-2.45)

Trachypithecus and *Presbytis* (C-V), and finally a lineage with solely *Semnopithecus*, while the relationships among these three lineages remained unresolved.

According to alternative tree topology tests, paraphyly of African colobines with *Piliocolobus/Procolobus* being closer related to Asian colobines than to *Colobus* was rejected ($P < 0.001$, Additional file 2). Among Asian colobines, relationships in which *Trachypithecus* and *Presbytis* do not form a monophyletic clade were also rejected ($P < 0.001$, $P < 0.05$), as well as a close relationship of *Trachypithecus* and *Semnopithecus* ($P < 0.01$). In contrast, different positions of *Semnopithecus* among Asian colobines were similarly likely ($P > 0.05$).

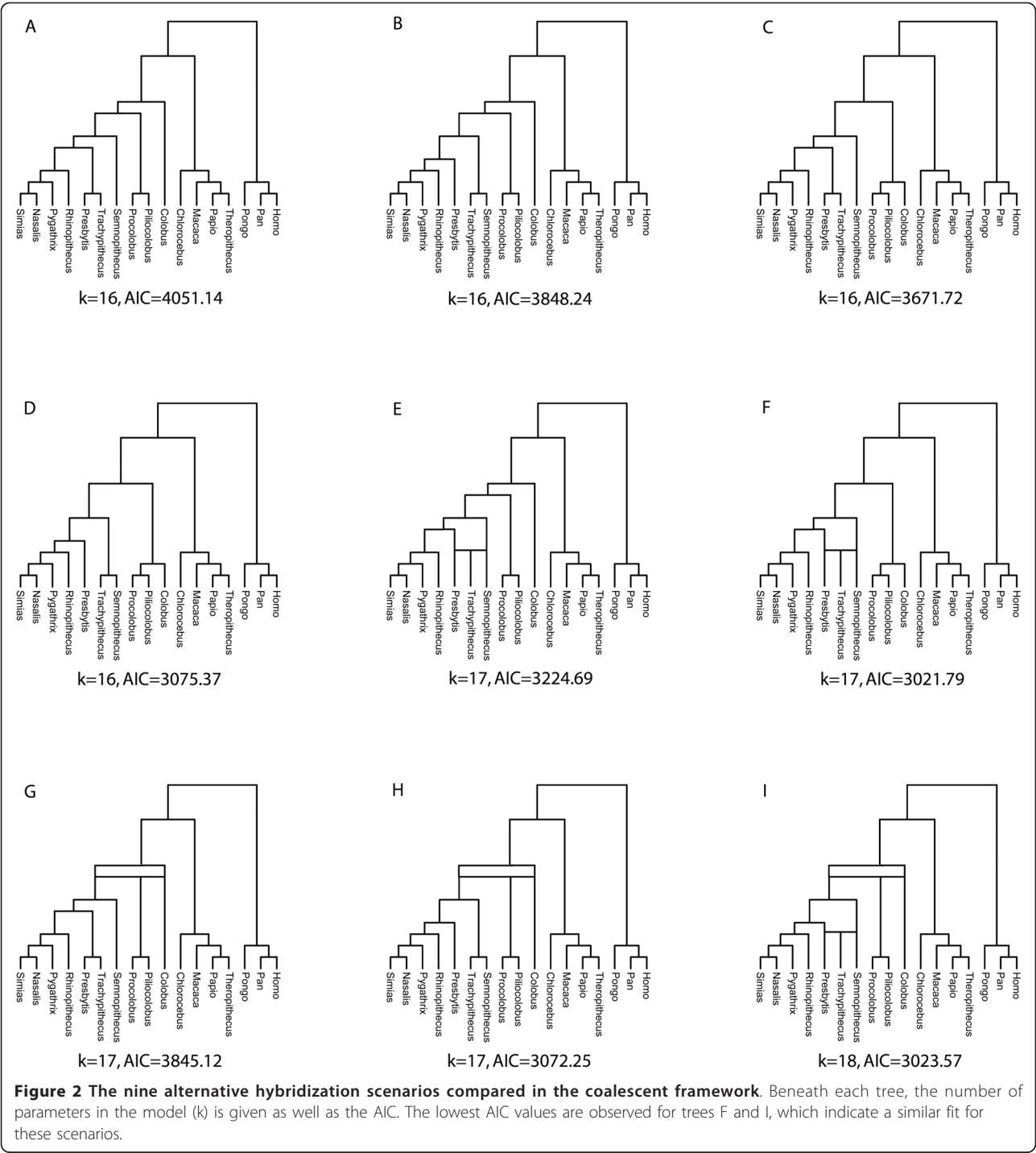
Divergence age estimates from mitochondrial data were similar to nuclear estimates in case where identical branching patterns were obtained (Table 1). According to mitochondrial data, African and Asian colobine lineages were separated 10.90 mya. In Africa, *Colobus* represents the first split (8.47 mya), followed by the divergence of *Piliocolobus* and *Procolobus* (6.58 mya). The major Asian split leading to the three lineages *Semnopithecus*, *Trachypithecus/Presbytis* and the odd-nosed monkeys occurred 8.91 mya. *Trachypithecus* diverged from *Presbytis* 7.45 mya. The diversification of odd-nosed monkeys into genera started 6.91 mya and ended with the split between *Nasalis* and *Simias* 1.88 mya.

Inferring hybridization in the presence of incomplete lineage sorting

To assess the possible reasons for the incongruence between the nuclear and mitochondrial trees, we applied

the method proposed by Kubatko [40]. The method assumes that incomplete lineage sorting (ILS) explains observed gene tree incongruence to some extent, and seeks to determine whether all variation in observed gene trees can be explained by ILS alone, as modeled by the coalescent process, or whether hybridization helps to explain significantly more the observed variation. Then, the Akaike information criterions (AIC) in each model (may or may not include hybridization scenarios) were compared to determine the best-fit model. For our data, two possible hybridization events were hypothesized. The first involved *Trachypithecus*, with parental taxa *Semnopithecus* and *Presbytis*, while the second involved the clade containing *Piliocolobus* and *Procolobus*, *Colobus* and the ancestor of Asian colobines.

By comparing the results from models with or without the hybridization events, the best-fit model (AIC = 3021.79, Figure 2F) was a tree in which *Trachypithecus* is the result of hybridization between *Presbytis* and *Semnopithecus*. The second best-fit model (AIC = 3023.57, Figure 2I) comprised the tree that includes both tested hybridization events. AIC values for all seven other models were considerably higher (3072.25 - 4051.14). Since AIC values for the scenarios presented in Figures 2F and 2I were the lowest and were within 2 of one another, both were considered plausible explanations for the observed gene tree discordances [41]. It is worth pointing out that the model used here to compute the AIC assumes that ILS is a possible source of gene trees incongruence. Since the two best-fit models include at least one hybridization event, it is clear that ILS alone



does not adequately describe the extent of incongruence in the observed gene trees.

Discussion

By combining presence/absence analysis of mobile elements with autosomal, X chromosomal, Y chromosomal and mitochondrial sequence data, the present study provides comprehensive insights into the evolutionary history

of colobines. Most relationships are resolved and strongly supported by mobile elements and sequence data. Moreover, relationships and estimated divergence ages as obtained from different datasets are mainly congruent and in agreement with earlier studies [8-10,23,42-45]. Our study, however, also reveals significant discrepancies among gene trees. First, mitochondrial and nuclear sequence data suggest a monophyletic African colobine

clade, while mobile elements provide evidence for a closer connection of the *Piliocolobus/Procolobus* clade to Asian genera than to *Colobus*. Second, mobile elements indicate close relationships between *Semnopithecus* and *Trachypithecus*, and between *Presbytis* and the odd-nosed monkeys. Nuclear sequence data support the former clade, but suggest *Presbytis* as basal among Asian colobines. In contrast, in the mitochondrial phylogeny, *Presbytis* and *Trachypithecus* are displayed as sister lineages, while the position of *Semnopithecus* remains ambiguous.

Possible explanations for gene tree discordance

Inadequate data, homoplasy, nucleotide composition, ILS or hybridization could be potential explanations for the observed differences [13-21]. For the mitochondrial dataset, at least for the African and *Presbytis/Trachypithecus* clades, incorrect branching patterns due to inadequate data or homoplasy are unlikely, since sufficient phylogenetic resolution with long internal branches is obtained. Likewise, a shift in nucleotide composition and differential sorting of ancestral mitochondrial lineages is implausible. Since the major shift in nucleotide composition was detected between colobines and non-colobines, it cannot be responsible for gene tree discordances among colobines. If the African and *Presbytis/Trachypithecus* clades are indeed the result of incomplete sorting of mitochondrial lineages, the mitochondrial divergence between respective genera should predate the nuclear splitting times, which is not the case (African colobines: 10.93 mya nuclear vs. 8.47 mya mitochondrial; *Presbytis* - *Trachypithecus*: 8.12 mya nuclear vs. 7.45 mya mitochondrial). However, the unresolved position of *Semnopithecus* among Asian colobines might have been affected by one or several of the above mentioned factors, or alternatively, might be the result of a true radiation-like divergence of lineages. For nuclear data, these factors are unlikely explanations as well for the branching of *Trachypithecus* and *Semnopithecus*, because ten independent insertions and sequence data from 12 nuclear loci clearly confirm their close relationship. More challenging are explanations for the discordant positions of *Presbytis* and the African genera among colobines in phylogenies revealed by mobile elements and nuclear sequence data. Mixed genomes due to differentially selected genes cannot be excluded, but interestingly, both mobile elements and nuclear sequence data (as revealed from single locus analysis) show no conflicting phylogenies themselves. Most prominent, however, the mobile element-based phylogeny is not rejected by nuclear sequence data, indicating that insufficient informative sites, as also suggested by the low resolution of phylogenetic relationships in single-locus analysis, in the latter dataset might display incorrect relationships. For the integration

of mobile elements, homoplasy is typically regarded as minimal [25,28,30], but ILS has been reported [36,39]. Only two and three integrations support the branching of *Presbytis* with odd-nosed monkeys and the paraphyly of African colobines, and alternative relationships cannot be rejected statistically. However, no inconsistent elements were detected and subtractive hybridizations specifically set up to screen for African colobine and *Trachypithecus/Presbytis* monophyly markers revealed no equivalent insertions. Accordingly, ILS seems to be an unreasonable explanation for our findings. Since the mobile element-based phylogeny is not rejected by nuclear sequence data and due to their reliability as molecular-cladistic markers, the phylogeny suggested by mobile elements is assumed to reflect the true nuclear phylogeny of colobines, although we explicitly note that mosaic genomes cannot be excluded.

Because all above-mentioned factors provide no sufficient explanation for the herein detected discordances between mitochondrial and nuclear phylogenies, we favor ancestral hybridization as the main reason for the discordant pattern. Furthermore, comparisons of models with and without hybridization in a model selection framework strongly support hybridization in the presence of ILS over models of ILS alone. In other words, even after ILS was taken into account as a factor in the observed incongruence among gene trees, we still found support for hybridization in the evolutionary history of these taxa. This refers at least to Asian colobines, but hybridization among African colobines cannot be excluded either by the method we applied here.

Hybridization hypothesis

Although bidirectional hybridization, which would be indicated by mixed genomes, cannot be excluded with our data, a female introgression event is hypothesized for African colobines. The direction of gene flow remains obscure due to the rapid diversification of the colobine ancestor in Africa, but female introgression from *Piliocolobus/Procolobus* into *Colobus* is indicated and gains further support by some biological data [1,2]. In contrast to *Colobus*, females in *Piliocolobus* and *Procolobus* tend to leave their natal groups, which was most likely also the case in their ancestor [1], and *Colobus* males are on average larger than *Piliocolobus* and *Procolobus* males [1], thus increasing the chance of hybridization between *Colobus* males and *Piliocolobus/Procolobus* females. Moreover, hybridization between both ancestral lineages is in principal possible, because (at least nowadays) they occur in sympatry over wide ranges of their distribution [1,2]. Accordingly, after the successive separation of *Colobus* and *Piliocolobus/Procolobus* from the Asian colobine ancestor, *Piliocolobus/Procolobus* females might have entered *Colobus* populations and

hybridized with their males. Backcrossing of hybrid females with resident *Colobus* males might have led to the fixation of the *Piliocolobus/Procolobus* mitochondrial lineage in the hybrid population, while the original nuclear genome of *Colobus* increased again in every generation.

For Asian langurs, we propose male introgression from *Semnopithecus* into *Trachypithecus* followed by nuclear swamping. Both genera are similar in their morphology and general appearance [2,46,47], but males in *Semnopithecus* are larger than in *Trachypithecus* [1]. Moreover, hybridization events due to (at least nowadays) partially overlapping ranges are generally possible [1,2]. Accordingly, after an initial separation, *Semnopithecus* males, which leave their natal group like most other primate males [1,48], might have invaded *Trachypithecus* populations and hybridized successfully with the resident females. By backcrossing with further invading *Semnopithecus* males over a longer period, the *Trachypithecus* population might have accumulated nuclear material of *Semnopithecus* (nuclear swamping), while the mitochondrial genome remained *Trachypithecus*-like.

Biogeographic implications

By combining the available information, we develop the following extended dispersal scenario for colobines (Figure 3). The origin of the subfamily is most likely in Africa, which is in agreement with earlier suggestions [1,49]. On the African continent, *Colobus* split off first from the main stem ~ 10.93 mya, followed shortly afterwards by the progenitor of *Piliocolobus* and *Procolobus*. After this initial separation, hybridization between both lineages might have lasted until finally both mitochondrial lineages diverged (~ 8.47 mya). Presumably, respective splitting and hybridization events took place in western Africa, because all three genera occur there in sympatry [1,2], and the most ancient splits among *Piliocolobus* and *Colobus* species are also found there [45]. The Asian colobine ancestor most likely invaded Eurasia via an emerging land bridge connecting Africa and the Arabian Peninsula in the late Miocene [49,50]. Whether a route into eastern Asia north or south of the Himalayas was chosen is a matter of speculation, but north of the Himalayas, on the Tibetan plateau, colobine fossils from the late Miocene were found, which is not the case south of the Himalayas [1]. Although not confirmed, the Hengduan Mountains in the border region of today's Burma, India

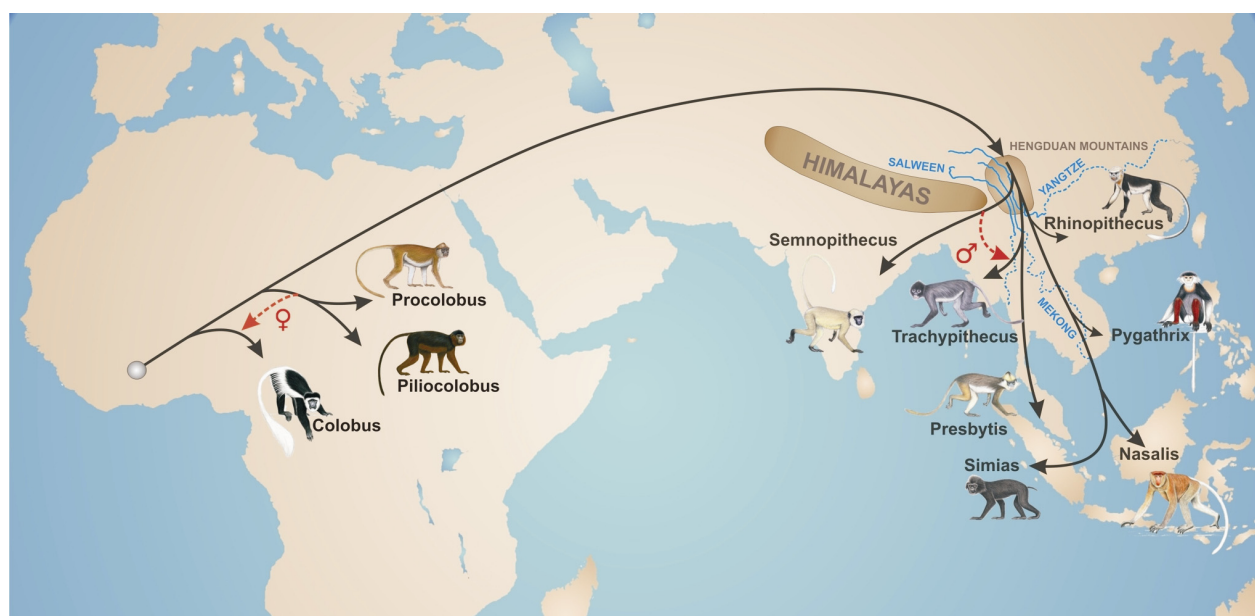


Figure 3 Dispersal scenario for colobine monkeys. Colobines most likely originated in western Africa. After the successive split of *Colobus* (~ 10.9 mya) and a progenitor of *Piliocolobus/Procolobus* (~ 10.7 mya) from the ancestor of Asian colobines, gene flow between both African lineages via female introgression from the *Piliocolobus/Procolobus* progenitor into *Colobus* occurred until ~ 8.5 mya (displayed by red-dashed arrow). During the late Miocene, colobines invaded eastern Asia most likely via a route north of the Himalayas. After their arrival at the Hengduan Mountains, Asian colobines diversified into a lineage comprising a progenitor of the odd-nosed monkeys and *Trachypithecus/Presbytis*, and of *Semnopithecus*, which later colonized the Indian subcontinent. Shortly afterwards, *Trachypithecus/Presbytis* split off from odd-nosed monkeys, and migrated to southern mainland Asia, before finally both genera diverged from each other. In the region of today's Burma, Bangladesh and India, *Semnopithecus* and *Trachypithecus* came into secondary contact and hybridized until ~ 2.6 mya (displayed by red-dashed arrow). In the latest Miocene, odd-nosed monkeys migrated from China to the south and expanded their range into Indochina and Sundaland. *Nasalis* and *Simias* finally separated from each other 1.1-1.9 mya.

and China might have been a possible diversification hot-spot [4,51,52]. In the region, all the larger Southeast Asian rivers (Mekong, Salween, Yangtze) rise, which are all well-known as barriers for arboreal primates [53] and are all known to exist since at least the early Miocene [54]. *Semnopithecus* might have diverged as first lineage and invaded the Indian subcontinent. Subsequently, the progenitor of *Presbytis* and *Trachypithecus* separated from the odd-nosed monkey ancestor and migrated into southern mainland Asia. Afterwards, *Presbytis* diverged from *Trachypithecus* and entered first the Malaysian peninsular and later on Sundaland during periods of lowered sea levels [55]. *Trachypithecus* and *Semnopithecus* came into secondary contact and might have hybridized until the earliest Pleistocene. A potential contact zone could be the region of today's Bangladesh, Burma and the northeast of India, which is suggested as hybridization area for several primate species [9,44,56]. On the Asian mainland, odd-nosed monkeys successively migrated from China to the south and expanded their range into Indochina and Sundaland in the latest Miocene. The migration into Sundaland was probably via land bridges connecting the mainland with Sundaland islands during periods of lowered sea levels [55]. Finally, *Nasalis* on Borneo and *Simias* on the Mentawai islands west of Sumatra diverged in the Pleistocene. Due to the dating discrepancy (mitochondrial data: 1.88 mya, nuclear data: 1.06 mya), further gene flow between both genera after the initial separation cannot be excluded, especially considering that migration was repeatedly possible via land bridge connections during the Pleistocene [55].

Conclusion

Our study gives new and most comprehensive insights into the evolutionary history of colobine monkeys, and suggests hybridization among ancestral lineages as the most likely cause for the observed phylogenetic incongruences. Only the combination of maternally, paternally and bi-parentally inherited markers as well as the combination of sequence data with presence/absence patterns of mobile elements proved to be an adequate and reliable phylogenetic approach, particularly in revealing hybridization events. However, data from additional nuclear loci and a broader taxonomic sampling is required to fully understand hybridization mechanisms in colobines.

Hybridization among taxa is traditionally recognized as a factor leading to limited diversification, reproductive isolation and lowered fitness [57,58], whereas our and earlier studies clearly indicate that hybridization played a prominent role in diversification and speciation of primates (for review see [59,60]). Hybridization events are genetically confirmed within all major primate lineages, mainly among species (e.g., [56,61-65]) but also between genera (e.g., [9,44,66]). Even for the human

lineage, hybridization has been suggested as an important evolutionary mechanism [67-69].

Since male dispersal and female philopatry predominates in primates [48], male introgression, and if intensive backcrossing of hybrids with more invading males occurs, followed by nuclear swamping would be the most likely hybridization scenario. In fact, the hybridization among Asian langur genera is most likely the result of such an event. However, as proposed for African colobines, alternative mechanisms (e.g. female introgression) could also occur, promoted by a respective social organization, where female migration predominates.

Methods

Sample collection and DNA extraction

Blood, tissue or fecal samples from representatives of all ten colobine genera (*Colobus*, *Piliocolobus*, *Procolobus*, *Presbytis*, *Trachypithecus*, *Semnopithecus*, *Rhinopithecus*, *Pygathrix*, *Nasalis*, *Simias*) and several non-colobine taxa (*Macaca*, *Papio*, *Theropithecus*, *Chlorocebus*, *Pongo*, *Pan*) were obtained from specimens kept in zoos or breeding facilities, or collected in the field (Table 2). Sample collection was conducted according to relevant German and international guidelines, including countries where we collected samples. Fecal samples were collected in a non-invasive way without disturbing, threatening or harming the animals. Blood samples were taken by veterinarians for diagnostic reasons to check

Table 2 Origin and sample type of studied species

species	origin	sample type
<i>Colobus guereza</i>	Cologne zoo, Germany	tissue
<i>Piliocolobus badius</i>	Taï National Park, Ivory Coast	tissue
<i>Procolobus verus</i>	Taï National Park, Ivory Coast	tissue
<i>Semnopithecus entellus</i>	Dresden zoo, Germany	blood
<i>Trachypithecus obscurus</i>	Wuppertal zoo, Germany	blood
<i>Presbytis melalophos</i>	Howletts Wild Animal Park, Great Britain	tissue
<i>Pygathrix nemaeus</i>	Cologne zoo, Germany	tissue
<i>Rhinopithecus avunculus</i>	Endangered Primate Rescue Center, Vietnam	tissue
<i>Nasalis larvatus</i>	Wilhelma Stuttgart, Germany	blood
<i>Simias concolor</i>	Siberut Conservation Programme, Indonesia	feces
<i>Macaca sylvanus</i>	Nuremberg zoo, Germany	blood
<i>Papio hamadryas</i>	Munich zoo, Germany	blood
<i>Theropithecus gelada</i>	Duisburg zoo, Germany	blood
<i>Chlorocebus aethiops</i>	Paul-Ehrlich-Institute, Germany	blood
<i>Pongo abelii</i>	Nuremberg zoo, Germany	blood
<i>Pan troglodytes</i>	Munich zoo, Germany	blood

the health status of the respective individuals, and tissue samples were obtained only from deceased specimens. Total genomic DNA was extracted with the DNeasy Blood & Tissue or QIAamp DNA Stool Mini kits from Qiagen following standard procedures.

Analysis of mobile elements

Due to their high copy number (~one million) and relatively small size (~300 bp), the primate specific *Alu* elements were selected as molecular-cladistic markers. The presence or absence of mobile elements in different colobines at specific loci was tested via PCR using primers occupying the flanking region of the insertion site. Details on analyzed loci, primers and presence/absence pattern of mobile elements in studied species are listed in Additional file 1. For most loci, sequencing was neglected, but in relevant cases the insertion orthology was confirmed by sequencing, and direct repeats flanking the insertion as well as the original target site prior to transposition were traced.

In our study, we included published markers [9,23,35], which were further examined in previously untested genera, and newly detected integration loci (Additional file 1). Therefore, we performed subtractive hybridizations following described methods [9]. To avoid biased hybridization results, various species combinations were used as tracer and driver (hybridization 1: tracer *Nasalis/pygathrix*, driver *Presbytis*; hybridization 2: tracer *Nasalis/pygathrix*, driver *Semnopithecus*; hybridization 3: tracer *Trachypithecus/presbytis*, driver *Pygathrix*; hybridization 4: tracer *Presbytis*, driver *Semnopithecus*; hybridization 5: tracer *Ptilocolobus/Colobus*, driver *Pygathrix*). Besides *Alu* insertions, a LINE present in *Ptilocolobus* and *Procolobus* in the studied Xq13.3 fragment was additionally applied as marker (Additional file 1).

Phylogenetic reconstructions using the MP algorithm were conducted in PAUP v4.0b10 [70]. Presence of an integration was coded as 1, its absence as 0, and missing data as '?'. Internal node support was obtained via a heuristic search with 10,000 bootstrap replications. To evaluate the reliability of the depicted relationships among colobines, various alternative tree topologies (Additional file 2) were assessed with the Kishino-Hasegawa test [71] with full optimization and 1,000 bootstrap replications in PAUP.

Amplification and sequencing of nuclear loci

Inter-exonic intron and exonic sequences were generated for six single-copy genes of the Y chromosome, five autosomal loci, and a fragment of the X chromosomal Xq13.3 region. With exception of the *SRY* gene (sex-reversal, Y chromosome), all other Y chromosomal loci (*DBY5*: Dead Box, intron 5; *SMCY7*: SMC mouse homologue, intron 7; *SMCY11*: SMC mouse homologue,

intron 11; *UTY18*: ubiquitous TPR motif, intron 18; *ZFYLI*: Zinc finger, last intron) have homologues on the X chromosome (X degenerate). As autosomal loci, we selected intron 11 of the von Willebrand Factor (*vWF11*), located on human chromosome 12, intron 3 of the serum albumin gene (*ALB3*, human chromosome 4), intron 3 of the interstitial retinol-binding protein (*IRBP3*, human chromosome 10), intron 1 of the transition protein 2 (*TNP2*, human chromosome 16) and intron 1 of the transthyretin gene (*TTR1*, human chromosome 18). *SRY*, *DBY5*, *SMCY7*, *SMCY11*, *UTY18*, *vWF11* and a ~4,300 bp fragment of the Xq13.3 region were amplified using primers and PCR conditions as described [10,72-75] (Additional file 9). For the amplification of *ZFYLI*, *ALB3*, *IRBP3*, *TNP2* and *TTR1*, new primers (Additional file 9) were designed on the basis of available primate sequences in GenBank. PCR conditions for the latter comprised 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 varying temperatures (Additional file 9) for 1 min, and extension at 72°C for 2 min. At the end, a final extension step at 72°C for 5 min was added. The results of all PCR amplifications were checked on 1% agarose gels. PCR products were cleaned with the Qiagen PCR Purification kit and subsequently sequenced on an ABI 3130 × 1 sequencer using the BigDye Terminator Cycle Sequencing kit. Alignments and sequences are available in TreeBASE (<http://purl.org/phylo/treebase/phyloWS/study/TB2:S11179>) and GenBank, respectively (for GenBank accession numbers see Additional file 10).

Amplification and sequencing of mitochondrial genomes

To reduce the likelihood of amplifying nuclear pseudogenes (numts), complete mitochondrial genomes from four colobine genera (*Rhinopithecus*, *Pygathrix*, *Nasalis*, *Procolobus*) were generated following an approach in which two overlapping ~10,000 bp long fragments were amplified via long-range PCR [8,43]. Due to degradation of DNA extracted from faeces, the mitochondrial genome of *Simias* was amplified via five overlapping fragments, each with a size of ~5,000 bp. All long-range PCRs were performed with the SuperTaq Plus polymerase from Ambion following protocols of the supplier and primers as described [8,43]. Long-range PCR amplicons were separated on 1% agarose gels, excised from the gel, purified with the Qiagen Gel Extraction kit and used as template for nested PCRs. PCR conditions for all nested PCR amplifications were identical and comprised a pre-denaturation step at 94°C for 2 min, followed by 30 cycles each with denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1.5 min. At the end, a final extension step at 72°C for 5 min was added. Nested PCR products (900-1,200

bp in length) were cleaned with the Qiagen PCR Purification kit and sequenced on an ABI 3130 × 1 sequencer. Sequences were assembled with Geneious v4.6.1 [76]. No inconsistent positions in overlapping regions were detected and all protein-coding genes were correctly translated. Annotation of mitochondrial genomes was conducted with the online program DOGMA [77] and manually inspected. Alignment and sequences are available in TreeBASE (<http://purl.org/phylo/treebase/phylogenies/study/TB2:S11179>) and GenBank, respectively (for GenBank accession numbers see Additional file 10).

Statistical analysis of sequence data

For phylogenetic reconstructions, all datasets comprised 17 sequences including each one representative of the ten colobine genera (*Colobus*, *Ptilocolobus*, *Procolobus*, *Trachypithecus*, *Semnopithecus*, *Presbytis*, *Rhinopithecus*, *Pygathrix*, *Nasalis*, *Simias*), four cercopithecine genera (*Papio*, *Theropithecus*, *Macaca*, *Chlorocebus*), and three hominoid genera (*Homo*, *Pan*, *Pongo*), which were used as outgroup taxa. To complete datasets, we partly implemented sequences from GenBank (Additional file 10). Alignments for individual loci were generated with MAFFT v6 [78] and corrected by eye. In all alignments, poorly aligned positions and indels were removed with Gblocks v0.91b [79] using default settings (Additional file 8). For the mitochondrial dataset, also the D-loop region was excluded (dataset mtDNA1) and a second alignment, generated in Mesquite v2.6 [80], included solely protein-coding genes (dataset mtDNA2). For all datasets, uncorrected pairwise differences were estimated in PAUP (Additional file 7). Nucleotide composition for all and only parsimony-informative positions for the combined nuclear and both mitochondrial alignments was also estimated in PAUP (Additional file 4). To test whether datasets can be combined, we performed partition homogeneity tests in PAUP with 10,000 replications.

Phylogenetic trees were constructed with MP and NJ algorithms as implemented in PAUP as well as with ML and Bayesian algorithms, using the programs GARLI v0.951 [81] and MrBayes v3.1.2 [82,83]. For MP analyses, all characters were treated as unordered and equally weighted throughout. A heuristic search was performed with the maximum number of trees set to 100. For NJ, ML and Bayesian reconstructions, the optimal nucleotide substitution models for each locus and concatenated datasets were chosen using AIC as implemented in MODELTEST v3.7 [84] (Additional file 8). Relative support of internal nodes was assessed by bootstrap analyses with 10,000 (MP, NJ) or 500 replications (ML). In GARLI, only the model specification settings were adjusted according to the respective concatenated dataset, while all other settings were left at their default

value. ML majority-rule consensus trees were calculated in PAUP. For Bayesian reconstructions, the datasets were partitioned treating each locus separately and each with its own substitution model. The solely protein-coding alignment of the mitochondrial genome (mtDNA2) was partitioned into codon positions. We used four independent Markov Chain Monte Carlo (MCMC) runs with the default temperature of 0.1. Four repetitions were run for 10,000,000 generations with tree and parameter sampling occurring every 100 generations. The first 25% of samples were discarded as burnin, leaving 75,001 trees per run. PPs for each split and a phylogram with mean branch lengths were calculated from the posterior density of trees.

To evaluate the reliability of obtained relationships among colobines, various alternative tree topologies (Additional file 2) were tested with the Shimodaira-Hasegawa test [85] with full optimization and 1,000 bootstrap replications in PAUP.

Divergence age estimation

A Bayesian MCMC method, which employs a relaxed molecular clock approach [86], as implemented in BEAST v1.4.8 [87], was used to estimate divergence times. Therefore, a relaxed lognormal model of lineage variation and a Yule prior for branching rates was assumed. Divergence times were calculated for each locus separately and for the combined nuclear dataset. The latter was partitioned treating each locus as distinct unit. The mitochondrial alignment comprising solely protein-coding genes (mtDNA2) was partitioned into codon positions and the substitution model, rate heterogeneity and base frequencies were unlinked across codon positions. Optimal nucleotide substitution models were chosen using AIC in MODELTEST.

As calibrations we used the fossil-based divergence between *Homo* and *Pan*, which has been dated at 6-7 mya [88-90], the separation of *Pongo* from the *Homo/Pan* lineage ~14 mya [91], the split between *Theropithecus* and *Papio* ~4 mya [92,93], and the divergence of hominoids and cercopithecoids ~24 mya [94-96]. Instead of hardbounded calibration points, we used the published dates as a normal distribution prior for the respective node. For the *Homo* - *Pan* divergence, this translates into a normal distribution with a mean of 6.5 mya and a standard deviation (SD) of 0.5 mya, for the separation of *Pongo* from the *Homo/Pan* clade into a mean of 14.0 mya and a SD of 1.0 mya, for the *Theropithecus* - *Papio* split into a mean of 4.0 mya and a SD of 0.5 mya, and for the hominoid - cercopithecoid divergence into a mean of 24 mya and a SD of 2 mya.

Since the estimation of phylogenetic relationships was not the main aim of this analysis, we used an a-priori fixed tree topology as obtained from mobile elements

(Figure 1A) for the calculation from nuclear sequence data. Four replicates were run for 10,000,000 generations with tree and parameter sampling occurring every 100 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 TRACER v1.4.1 [97]. Subsequently, the sampling distributions were combined (25% burnin) using the software LogCombiner v1.4.8 and a consensus chronogram with node height distribution was generated and visualized with TreeAnnotator v1.4.8 and FigTree v1.2.2 [98].

Inferring hybridization in the presence of incomplete lineage sorting

Statistical support for putative hybridization scenarios was assessed with the method proposed by Kubatko [40], in which statistical model selection techniques (e. g., AIC) are used to compare species trees that may or may not include hybridization scenarios. For our data, we hypothesized two possible hybridization events (for details see Results). The estimated gene trees used as input were those derived from single locus tree reconstructions (Additional file 3) and branch lengths as estimated in BEAST. To estimate evolutionary rates for individual loci, we followed the suggestion of Yang [99] (see also [100]) and computed for each gene the average pairwise sequence divergence of each ingroup (colobine) sequence to the outgroup (non-colobine) taxa. We then assigned to each locus a rate that was calculated by dividing the mean pairwise divergence for that locus by the median of the entire set of pairwise divergences (Additional file 8). To convert gene tree branch lengths to coalescent units, we considered two effective population sizes, 50,000 and 100,000, and used a generation time of 5 years. Since the results were identical in terms of the trees preferred, we show here the results only for effective population size 50,000. For haploid loci (mitochondrial genome, Y chromosomal loci), we additionally divided the rate by 2 (see [100]). We compared a total of nine species trees (four corresponding to no hybridization, four corresponding to single hybridization events, and one that included both hybridization scenarios, Figure 2). The AIC was computed for each tree using the STEM software [100]. Models with AIC values within 2 of one another were regarded as providing similar fit to the data [41].

Additional material

Additional file 1: Additional Table 1. Presence/absence pattern, location, primers and PCR product sizes of mobile elements

Additional file 2: Additional Table 2. Alternative tree topology tests

Additional file 3: Additional Figure 1. Single-locus phylogenetic trees (80% majority rule)

Additional file 4: Additional Figure 2. Nucleotide composition of both mitochondrial and the combined nuclear datasets

Additional file 5: Additional Figure 3. Phylograms based on the mitochondrial and combined nuclear datasets

Additional file 6: Additional Table 3. Divergence ages in mya estimated for each locus separately

Additional file 7: Additional Table 4. Uncorrected pairwise nucleotide differences for each locus

Additional file 8: Additional Table 5. Locus-specific information including alignment length, number of variable sites, selected substitution model and estimated evolutionary rates

Additional file 9: Additional Table 6. Primers and PCR conditions for the amplification of nuclear loci

Additional file 10: Additional Table 7. GenBank accession numbers

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Author details

¹Primate Genetics Laboratory, German Primate Center, Kellnerweg 4, 37077 Göttingen, Germany. ²Gene Bank of Primates, German Primate Center, Kellnerweg 4, 37077 Göttingen, Germany. ³Cognitive Ethology Laboratory, German Primate Center, Kellnerweg 4, 37077 Göttingen, Germany. ⁴Departments of Statistics and Evolution, Ecology and Organismal Biology, The Ohio State University, Columbus, Ohio 43210, USA. ⁵Reproductive Biology Unit, German Primate Center, Kellnerweg 4, 37077 Göttingen, Germany. ⁶Department of Anatomical Sciences, State University of New York, Stony Brook, New York 11794-8081, USA. ⁷Department of Human Genetics, University of Utah, 15 North 2030 East, Salt Lake City, Utah 84112, USA. ⁸Department of Biological Sciences, Louisiana State University, 202 Life Sciences Building, Baton Rouge, Louisiana 70803, USA. ⁹Research Group Emerging Zoonoses, Robert Koch Institute, Postfach 650261, 13302 Berlin, Germany. ¹⁰Primate Research Center and Department of Biology, Bogor Agricultural University, Jl. Lodaya II/5, Bogor 16151, Indonesia. ¹¹Frankfurt Zoological Society, Endangered Primate Rescue Center, Cuc Phuong National Park, Nho Quan District, Ninh Binh Province, Vietnam. ¹²Stem Cell Biology Unit, German Primate Center, Kellnerweg 4, 37077 Göttingen, Germany.

Authors' contributions

CR designed the study, collected samples, did laboratory work, analyzed data, and wrote the paper. DZ designed the study, analyzed data, and wrote the paper. LSK, JX, MAB and MB analyzed data and wrote the paper. CS, MY, DM did laboratory work. SDN and LW wrote the paper. FHL, TZ, DPF and TN provided valuable samples and wrote the paper. MO did laboratory work, analyzed data and wrote the paper. All authors read and approved the final manuscript.

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References

- Davies AG, Oates JF: *Colobine Monkeys - Their Ecology, Behaviour and Evolution* Cambridge: Cambridge University Press; 1994.
- Groves CP: *Primate Taxonomy* Washington DC: Smithsonian Institution Press; 2001.
- Groves CP: *A Theory of Human and Primate Evolution* Oxford: Clarendon Press; 1989.
- Jablonski NG: *Natural History of the Doucs and Snub-Nosed Monkeys* New Jersey: World Scientific Publishing Company; 1998.
- Jablonski NG: **Primate evolution - in and out of Africa.** *Curr Biol* 1999, **9**: R119-R122.
- Stewart CB, Disotell TR: **Primate evolution - in and out of Africa.** *Curr Biol* 1999, **9**: R120-R122.
- Zhang YP, Ryder OA: **Primate evolution - in and out of Africa.** *Curr Biol* 1999, **9**: R119-R120.
- Sternern KN, Raam RL, Zhang YP, Stewart CB, Disotell TR: **Mitochondrial data support an odd-nosed colobine clade.** *Mol Phylogenet Evol* 2006, **40**:1-7.
- Osterholz M, Walter L, Roos C: **Phylogenetic position of the langur genera *Semnopithecus* and *Trachypithecus* among Asian colobines, and genus affiliations of their species groups.** *BMC Evol Biol* 2008, **8**:58.
- Ting N, Tosi AJ, Li Y, Zhang YP, Disotell TR: **Phylogenetic incongruence between nuclear and mitochondrial markers in the Asian colobines and the evolution of the langurs and leaf monkeys.** *Mol Phylogenet Evol* 2008, **46**:466-474.
- Napier JR, Napier PH: *A Handbook of Living Primates* London: Academic Press; 1967.
- Szalay FS, Delson E: *Evolutionary History of the Primates* New York: Academic Press; 1979.
- Barton NH: **The role of hybridization on evolution.** *Mol Ecol* 2001, **10**:551-568.
- Nichols R: **Gene trees and species trees are not the same.** *Trends Ecol Evol* 2001, **16**:358-364.
- Funk DJ, Omland K: **Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA.** *Ann Rev Ecol Syst* 2003, **34**:397-423.
- Avise JC: *Molecular Markers, Natural History, and Evolution* Sunderland, MA: Sinauer Associates; 2004.
- Seehausen O: **Hybridization and adaptive radiation.** *Trends Ecol Evol* 2004, **19**:198-207.
- McCracken K, Sorenson M: **Is homoplasy or lineage sorting the source of incongruent mtDNA and nuclear gene trees in the stiff-tailed ducks (*Nomonyx-Oxyyura*)?** *Syst Biol* 2005, **54**:35-55.
- Pollard DA, Iyer VN, Moses AM, Eisen MB: **Widespread discordance of gene trees with species tree in *Drosophila*: evidence for incomplete lineage sorting.** *PLoS Genet* 2006, **2**:e173.
- Koblmueller S, Duftner N, Sefc KM, Aibara M, Stipacek M, Blanc M, Egger B, Sturmbauer C: **Reticulate phylogeny of gastropod-shell-breeding cichlids from Lake Tanganyika - the result of repeated introgressive hybridization.** *BMC Evol Biol* 2007, **7**:7.
- Philippe H, Laurent J: **How good are deep phylogenetic trees?** *Curr Opin Genetics Dev* 1998, **8**:616-623.
- Petit RJ, Excoffier L: **Gene flow and species delimitation.** *Trends Ecol Evol* 2009, **24**:386-393.
- Xing J, Wang H, Han K, Ray DA, Huang CH, Chernick LG, Stewart CB, Disotell TR, Ryder OA, Batzer MA: **A mobile element based phylogeny of Old World monkeys.** *Mol Phylogenet Evol* 2005, **37**:872-880.
- Batzer MA, Deininger PL: **A human-specific subfamily of Alu sequences.** *Genomics* 1991, **9**:481-487.
- Okada N: **SINES.** *Curr Opin Genet Dev* 1991, **1**:498-504.
- Shedlock AM, Okada N: **SINE insertions: powerful tools for molecular systematics.** *Bioessays* 2000, **22**:148-160.
- Valeim AH, Ray DA, Batzer MA: **Identity by descent and DNA sequence variation of human SINE and LINE elements.** *Cytogenet Genome Res* 2005, **108**:63-72.
- Schmitz J, Roos C, Zischler H: **Primate phylogeny: molecular evidence from retroposons.** *Cytogenet Genome Res* 2005, **108**:26-37.
- Van de Lagemat LN, Gagnier L, Medstrand P, Mager DL: **Genomic deletions and precise removal of transposable elements mediated by short identical DNA segments in primates.** *Genome Res* 2005, **15**:1243-1249.
- Ray DA, Xing J, Salem AH, Batzer MA: **SINES of a nearly perfect character.** *Syst Biol* 2006, **55**:928-935.
- Schmitz J, Ohme M, Zischler H: **SINE insertions in cladistic analyses and the phylogenetic affiliations of *Tarsius bancanus* to other primates.** *Genetics* 2001, **157**:777-784.
- Salem AH, Ray DA, Xing J, Callinan PA, Myers JS, Hedges DJ, Garber RK, Witherspoon DJ, Jorde LB, Batzer MA: **Alu elements and hominid phylogenetics.** *Proc Natl Acad Sci USA* 2003, **22**:12787-12791.
- Roos C, Schmitz J, Zischler H: **Primate jumping genes elucidate strepsirrhine phylogeny.** *Proc Natl Acad Sci USA* 2004, **101**:10650-10654.
- Ray DA, Xing J, Hedges DJ, Hall MA, Laborde ME, Anders BA, White BR, Stoilova N, Fowlkes JD, Landry KE, Chernick LG, Ryder OA, Batzer MA: **Alu insertion loci and platyrrhine primate phylogeny.** *Mol Phylogenet Evol* 2005, **35**:117-126.
- Herke SW, Xing J, Ray DA, Zimmermann JW, Cordaux R, Batzer MA: **A SINE-based dichotomous key for primate identification.** *Gene* 2007, **390**:39-51.
- Xing J, Wang H, Zhang Y, Ray DA, Tosi AJ, Disotell TR, Batzer MA: **A mobile element based evolutionary history of guenons (Tribe Cercopithecini).** *BMC Biol* 2007, **5**:5.
- Xing J, Witherspoon DJ, Ray DA, Batzer MA, Jorde LB: **Mobile DNA elements in primate and human evolution.** *Am J Phys Anthropol* 2007, **45**(Suppl):2-19.
- Osterholz M, Walter L, Roos C: **Retropositional events consolidate the branching order among New World monkey genera.** *Mol Phylogenet Evol* 2009, **50**:507-513.
- Li J, Han K, Xing J, Kim HS, Rogers J, Ryder OA, Disotell T, Yue B, Batzer MA: **Phylogeny of macaques (Cercopithecidae: *Macaca*) based on Alu elements.** *Gene* 2009, **448**:242-249.
- Kubatko LS: **Identifying hybridization events in the presence of coalescence via model selection.** *Syst Biol* 2009, **58**:478-488.
- Burnham KP, Anderson DR: *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach* New York: Springer; 2002.
- Goodman M, Porter CA, Czelusniak J, Page SL, Schneider H, Shoshani J, Gunnell G, Groves CP: **Toward a phylogenetic classification of primates based on DNA evidence complemented by fossil evidence.** *Mol Phylogenet Evol* 1998, **9**:585-598.
- Raam RL, Sternern KN, Noviello CM, Stewart CB, Disotell TR: **Catarrhine primate divergence dates estimated from complete mitochondrial genomes: concordance with fossil and nuclear DNA evidence.** *J Hum Evol* 2005, **48**:237-257.
- Karanth KP, Singh L, Collura RV, Stewart CB: **Molecular phylogeny and biogeography of langurs and leaf monkeys of South Asia (Primates: Colobinae).** *Mol Phylogenet Evol* 2008, **46**:683-694.
- Ting N: **Mitochondrial relationships and divergence dates of the African colobines: evidence of Miocene origins for the living colobus monkeys.** *J Hum Evol* 2008, **55**:312-325.
- Brandon-Jones D: **Colobus and leaf monkeys.** In *Encyclopedia of Mammals*. Edited by: MacDonald ID. London: George Allen and Unwin; 1984:398-408.
- Strasser E, Delson E: **Cladistic analysis of cercopithecoid relationships.** *J Hum Evol* 1987, **16**:81-99.
- Pusey AE, Packer C: **Dispersal and philopatry.** In *Primate Societies*. Edited by: Smuts BB, Cheney DL, Seyfarth RM, Wrangham RW, Struhsaker TT. Chicago: University of Chicago Press; 1987:250-266.
- Stewart CB, Disotell TR: **Primate evolution - in and out of Africa.** *Curr Biol* 1998, **8**:R582-R588.
- Whybrow PJ: **Land movements and species dispersal.** In *The Cambridge Encyclopedia of Human Evolution*. Edited by: Jones S, Martin RE, Pilbeam D. Cambridge: Cambridge University Press; 1992:169-173.
- Peng YZ, Pan RL, Jablonski N: **Classification and evolution of Asian colobines.** *Folia Primatol* 1993, **60**:106-117.
- Thinh VN, Mootnick AR, Geissmann T, Li M, Ziegler T, Agil M, Moisson P, Nadler T, Walter L, Roos C: **Mitochondrial evidence for multiple radiations in the evolutionary history of small apes.** *BMC Evol Biol* 2010, **10**:74.
- Meijaard E, Groves CP: **The geography of mammals and rivers in mainland Southeast Asia.** In *Primate Biogeography*. Edited by: Lehman SM, Fleagle JG. New York: Springer; 2006:305-329.
- Hallet B, Molnar P: **Distorted drainage basins as markers of crustal strain east of the Himalayas.** *J Geophys Res* 2001, **106**:13697-13709.
- Miller KG, Komazin MA, Browning JV, Wright JD, Mountain GS, Katz ME, Sugarman PJ, Cramer BS, Christie-Blick N, Pekar SF: **The Phanerozoic record of global sea-level change.** *Science* 2005, **310**:1293-1298.

56. Chakraborty D, Ramakrishnan U, Panor J, Mishra C, Sinha A: **Phylogenetic relationships and morphometric affinities of the Arunachal macaque *Macaca munzala*, a newly described primate from Arunachal Pradesh, northeastern India.** *Mol Phylogenet Evol* 2007, **44**:838-849.
57. Darwin C: *On the Origin of Species by Means of Natural Selection or the Preservation of Favoured Races in the Struggle of Life* London: John Murray; 1859.
58. Mayr E: *Animal Species and Evolution* Cambridge, MA: Belknap Press; 1963.
59. Arnold ML, Meyer A: **Natural hybridization in primates: one evolutionary mechanism.** *Zoology* 2006, **109**:261-276.
60. Zinner D, Arnold ML, Roos C: **The strange blood: natural hybridization in primates.** *Evol Anthropol* .
61. Cortés-Ortiz L, Duda TF Jr, Canales-Espinosa D, Garcia-Orduna F, Rodriguez-Luna E, Bermingham E: **Hybridization in large-bodied New World primates.** *Genetics* 2007, **176**:2421-2425.
62. Thalmann O, Fischer A, Lankester F, Pääbo S, Vigilant L: **The complex evolutionary history of gorillas: insights from genomic data.** *Mol Biol Evol* 2007, **24**:146-158.
63. Rumpler Y, Warter S, Hauwy M, Fausser JL, Roos C, Zinner D: **Comparing chromosomal and mitochondrial phylogenies of sportive lemurs (genus *Lepilemur*, Primates).** *Chromosome Res* 2008, **16**:1143-1158.
64. Merker S, Driller C, Pervitasari-Farajallah D, Pamungkas J, Zischler H: **Elucidating geological and biological processes underlying the diversification of Sulawesi tarsiers.** *Proc Natl Acad Sci USA* 2009, **106**:8459-8464.
65. Zinner D, Groeneveld LF, Keller C, Roos C: **Mitochondrial phylogeography of baboons (*Papio* spp.) - indication for introgressive hybridization?** *BMC Evol Biol* 2009, **9**:83.
66. Zinner D, Arnold ML, Roos C: **Is the new primate genus *Rungwecebus* a baboon?** *PLoS ONE* 2009, **4**:e4859.
67. Pääbo S: **The mosaic that is our genome.** *Nature* 2003, **421**:409-412.
68. Patterson N, Richter DJ, Gnerre S, Lander ES, Reich D: **Genetic evidence for complex speciation of humans and chimpanzees.** *Nature* 2006, **441**:1103-1108.
69. Green RE, Krause J, Briggs AW, Maricic T, Stenzel U, Kircher M, Patterson N, Li H, Zhai W, Fritz MH, Hansen NF, Durand EY, Malaspina AS, Jensen JD, Marques-Bonet T, Alkan C, Prüfer K, Meyer M, Burbano HA, Good JM, Schultz R, Aximu-Petri A, Butthof A, Höber B, Höffner B, Siegemund M, Weihmann A, Nusbaum C, Lander ES, Russ C, Novod N, Affourtit J, Egholm M, Verna C, Rudan P, Brajkovic D, Kucan Z, Gusic I, Doronichev VB, Golovanova LV, Lalueza-Fox C, de la Rasilla M, Fortea J, Rosas A, Schmitz RW, Johnson PL, Eichler EE, Falush D, Birney E, Mullikin JC, Slatkin M, Nielsen R, Kelso J, Lachmann M, Reich D, Pääbo S: **A draft sequence of the Neandertal genome.** *Science* 2010, **328**:710-722.
70. Swofford DL: *PAUP* Phylogenetic Analysis using Parsimony (*and other Methods)*, Version 4 Sunderland, MA: Sinauer Associates; 2003.
71. Kishino H, Hasegawa M: **Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea.** *J Mol Evol* 1989, **29**:170-179.
72. Whitfield LS, Lovell-Badge R, Goodfellow PN: **Rapid sequence evolution of the mammalian sex-determining gene SRY.** *Nature* 1993, **364**:713-715.
73. Chaves R, Sampaio I, Schneider MP, Schneider H, Page SL, Goodman M: **The place of *Callimico goeldii* in the callitrichine phylogenetic tree: evidence from von Willebrand factor gene intron 11 sequences.** *Mol Phylogenet Evol* 1999, **13**:392-404.
74. Hellborg L, Ellegren H: **Y chromosome conserved anchored tagged sequences (YCATS) for the analysis of mammalian male-specific DNA.** *Mol Ecol* 2003, **12**:283-291.
75. Tosi AJ, Detwiler KM, Disotell TR: **X-chromosomal window into the evolutionary history of the guenons (Primates: Cercopitheciini).** *Mol Phylogenet Evol* 2005, **36**:58-66.
76. Drummond AJ, Kearse M, Heled J, Moir R, Thierer T, Ashton B, Wilson A, Stones-Havas S: **Geneious, version 4.6.1.** 2009 [http://www.geneious.com].
77. Wyman SK, Jansen RK, Boore JL: **Automatic annotation of organellar genomes with DOGMA.** *Bioinformatics* 2004, **20**:3252-3255.
78. Katoh K, Kuma K, Toh H, Miyata T: **MAFFT version 5: improvement in accuracy of multiple sequence alignment.** *Nucleic Acids Res* 2005, **33**:511-518.
79. Castresana J: **Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis.** *Mol Biol Evol* 2000, **17**:540-552.
80. Maddison WP, Maddison DR: **Mesquite: a modular system for evolutionary analysis, version 2.6.** 2009 [http://mesquite.org].
81. Zwickl DJ: **Genetic algorithm approaches for the phylogenetic analysis of large biological sequence data sets under the maximum likelihood criterion.** *PhD thesis* Texas University, Austin; 2006.
82. Huelsenbeck JP, Ronquist F, Nielsen R, Bollback JP: **Bayesian inference of phylogeny and its impact on evolutionary biology.** *Science* 2001, **294**:2310-2314.
83. Ronquist F, Huelsenbeck JP: **MrBayes 3: Bayesian phylogenetic inference under mixed models.** *Bioinformatics* 2003, **19**:1572-1574.
84. Posada D, Crandall KA: **Modeltest: testing the model of DNA substitution.** *Bioinformatics* 1998, **14**:817-818.
85. Shimodaira H, Hasegawa M: **Multiple comparisons of log-likelihoods with applications to phylogenetic inference.** *Mol Biol Evol* 1999, **16**:1114-1116.
86. Drummond AJ, Ho SYW, Phillips MJ, Rambaut A: **Relaxed phylogenetics and dating with confidence.** *PLoS Biol* 2006, **4**:e88.
87. Drummond AJ, Rambaut A: **BEAST: Bayesian evolutionary analysis by sampling trees.** *BMC Evol Biol* 2007, **7**:214.
88. Vignaud P, Düringer P, Mackaye HT, Likies A, Blondel C, Boissiere JR, De Bonis L, Eisenmann V, Etienne ME, Geraads D, Guy F, Lehmann T, Lihoreau F, Lopez-Martinez N, Mourer-Chauviré C, Otero O, Rage JC, Schuster M, Viriot L, Zazzo A, Brunet M: **Geology and palaeontology of the Upper Miocene Toros-Menalla hominid locality, Chad.** *Nature* 2002, **418**:152-155.
89. Brunet M, Guy F, Pilbeam D, Lieberman DE, Likies A, Mackaye HT, Ponce de León MS, Zollikofer CP, Vignaud P: **New material of the earliest hominid from the Upper Miocene of Chad.** *Nature* 2005, **434**:752-755.
90. Lebatard AE, Bourlès DL, Düringer P, Jolivet M, Braucher R, Carcaillet J, Schuster M, Arnaud N, Monié P, Lihoreau F, Likies A, Mackaye HT, Vignaud P, Brunet M: **Cosmogenic nuclide dating of *Sahelanthropus tchadensis* and *Australopithecus bahrelghazali*: Mio-Pliocene hominids from Chad.** *Proc Natl Acad Sci USA* 2008, **105**:3226-3231.
91. Kelley J: **The hominoid radiation in Asia.** In *The Primate Fossil Record*. Edited by: Hartwig WC. Cambridge: Cambridge University Press; 2002:369-384.
92. Leakey MG: **Evolution of *Theropithecus* in the Turkana Basin.** In *Theropithecus, the Rise and Fall of a Primate Genus*. Edited by: Jablonski NG. Cambridge: Cambridge University Press; 1993:85-124.
93. Delson E: **Cercopitheciinae.** In *Encyclopedia of Human Evolution and Prehistory*. Edited by: Delson E, Tattersall I, Van Couvering JA, Brooks AS. New York: Garland Publishing Inc; 2000:166-171, 2000.
94. Benefit BR, McCrossin ML: **The Victoriapithecidae, Cercopithecoidea.** In *The Primate Fossil Record*. Edited by: Hartwig WC. Cambridge: Cambridge University Press; 2002:241-253.
95. Young NM, MacLatchy L: **The phylogenetic position of *Moropithecus*.** *J Hum Evol* 2004, **46**:163-184.
96. Zalmout IS, Sanders WJ, MacLatchy LM, Gunnell GF, Al-Mufarreah YA, Ali MA, Nasser AAH, Al-Masari AM, Al-Sobhi SA, Nadhra AO, Matari AH, Wilson JA, Gingerich PD: **New Oligocene primate from Saudi Arabia and the divergence of apes and Old World monkeys.** *Nature* 2010, **466**:360-365.
97. Rambaut A, Drummond AJ: **Tracer: MCMC trace analysis tool, version 1.4.1.** Institute of Evolutionary Biology, University of Edinburgh; 2007 [http://tree.bio.ed.ac.uk/software/tracer/].
98. Rambaut A: **FigTree: Tree figure drawing tool, version 1.2.2.** Institute of Evolutionary Biology, University of Edinburgh; 2008 [http://tree.bio.ed.ac.uk/software/figtree/].
99. Yang Z: **Likelihood and Bayes estimation of ancestral population sizes in hominoids using data from multiple loci.** *Genetics* 2002, **162**:1811-1823.
100. Kubatko LS, Carstens BC, Knowles LL: **STEM: species tree estimation using maximum likelihood for gene trees under coalescence.** *Bioinformatics* 2009, **25**:971-973.

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Additional file 1: Additional Table 1. Presence/absence pattern, location, primers and PCR product sizes of mobile elements.

Name	MM	PC	CG	PB	PV	SE	TO	PM	RA	PN	NL	SC	Forward	Reverse	Filled	Empty	AT	Locus position (Homo, hg18)	Reference
Colobinae (20)																			
DoucL_Yd_14Re	0	0*	1*	1	1*	1*	1*	1	1	1*	1*	1*	TTTAAATGATGCTCCTTGTC	TCACTGAACCTCTAAATCAGCA	468	158	58	chr11:88568822	1
DoucL_Yd_21	0	0*	1*	1	1*	1*	1*	1	1	1*	1*	1*	TGGGAAGTTTGAAGCCTGATA	TGAAGTTCAAAGGCTTAGTTTATTTT	792	482	58	chr14:52024695	1
DoucL_Yd_16	0	0*	1	1	1*	1*	1*	1	1	1	1*	1*	CCCTCTGAGCTCCTTCTGAAT	TGGGATCAGCTTTTGCAGCT	566	256	58	chr2:59962224	1
DoucL_Yd_28	0	0*	1*	1	1*	1*	1*	1	1	1*	1*	1*	TGAAAGAAACAGGGGAAATCA	TATCTGACAGCCCTTGACCTG	685	375	58	chr20:30348863	1
Kirk_Yd_2Re	0	0*	1*	1	1*	1*	1*	1	1	1*	1*	1*	GGCTCACATTTCATCTTCCAAAc	TCATAGTTTCTGGAGGTGCTT	530	220	58	chr4:7697083	1
Kirk_Yd_33Re	0	0*	1*	1	1*	1*	1*	1*	1*	1*	1*	1*	CTCACAAATTTTCAGTCTTGTGTA	GGCCTCAGAGAAGAGACTTTCC	464	154	58	chrX:127625240	1
SLL_Yd_1	0	0*	1*	1	1*	1*	1*	1	1	1*	1*	1*	TTTGGGGAACTTACGGTCTTT	GAGAAGCCACTCACCATTTTGA	554	244	58	chr15:24453270	1
SLL_Yd_8	0	0*	1	1	1	1*	1*	1	1	1	1*	1*	CCACCTTTCTCTAAATTTCCA	GTCTTGGCTTTCCCTCTACG	510	200	50	chr16:46418257	1,2
SLL_PY2_25	0	0*	1*	1	1*	1*	1*	1	1	1*	1*	1*	CCTGAGGCTGCTCAGAGAA	TGCTTATACGAGGCAACCTTTA	684	374	58	chr17:56469575	1,2
Nasalis_PY2_28	0	0*	1	1	1	1*	1*	1	1	1*	1*	1*	GTGCTAATCCTTGGCCACAT	GAATCTGTCTGCCAAAGTCACA	487	177	60	chr2:116383321	1
N21	0*	0*	1*	1	1*	1*	1*	1*	1*	1*	1*	1*	ACCACCTTTTGTCTGGTTCTCAT	TGTCAGACCAAGAGACATCTG	567	257	58	chr2:242112250	1,2
N16	0*	0*	1*	1	1*	1*	1*	1	1	1*	1*	1*	CAGATTCAGAGGATGATGCG	TAGGTGCTCAGGATGGTGCTA	540	230	58	chr11:56721243	1,2
2499	0	1	1	1	1*	1	1	1	1	1	1	1	CTTCTTTATCTCTGTATGCC	GTGGTCTAGCTATGCTG	740	410	58	chr1:43201455	this study
3133	0	1	1	1	1*	1*	1*	1*	1*	1	1	1	TGCTCTTCCCTCTGGAATCTA	GTGTCCCAATCTCCAAG	450	150	58	chr20:57502278	this study
3141	0	1*	1	1	1	1*	1*	1*	1*	1*	1*	1*	CCAGTGTTTGGTGTCAAAATG	GGGGTTTAAAGTTAATGATGACAG	550	250	58	chr12:4069255	this study
3249	0	1	1	1	1	1	1	1	1	1	1	1	ATGCTTTGAGAGTTCAAGGCG	CTTAAGATACACAATATAACAAGC	600	300	58	chr2:203941433	this study
3261	0	1	1	1	1	1	1	1	1	1	1	1	GTAATAGCTTGGAAAGATGC	TTATTCTGTGACTTGGAAATAGT	600	300	58	chr2:212208212	this study
3269	0	1	1	1	1	1	1	1	1	1	1	1	TGTAGCCAGGGAAGCCCTCT	TGGGATTTCTAATACTATGCTTTG	800	500/350	58	chr1:112743945	this study
3365	0	1	1	1	1	1	1	1	1	1	1	1	GTCCTTCCCTCTGGAATCT	AGTCCTGGATGAGACCT	500	200	58	chr20:57502300	this study
3369	0	1	1	1	1	1	1	1	1	1	1	1	TGGTTTCATGTGCCACTTAGG	CCAAGAATTTATTGAGCATCCA	900	600	58	chr17:35395755	this study
Ptilocolobus + Procolobus (2)																			
6169	0	0	0	1	1	0	0*	0	0	0	0	0	GACAAATCTGATAATCTTATAGG	CAGCAAGAACTCTTTGCAATC	720	400	58	chr15:57476905	this study
6023	0	0	0	1	1	0	0	0	0	0	0	0	ACAACCAAGCAACAAAGATAG	GTTCAAGATTCAAAACCTTTAAGTTG	1960	200	58	chrX:78092011	this study
Ptilocolobus/Procolobus + Asian colobines (3)																			
2474	0	0	0	1	1	1	1	1	1	1	1	1	TGGACAAGCTGAAGACATGG	CTGGATCTAGAGCTAGCTAG	500	200	58	chr6:4376475	this study
3371	0	0	0	1	1	1	1	1	1	1	1	1	CAGAGTGCTAAATTCATGCTTC	TGGCTGTTCCAAAGTCAGTTAG	600	300	58	chr9:111700546	this study
3373	0	0	0	1	1	1	1	1	1	1	1	1	TCGTTTGAAGATTTCAGTTGG	CTCTGTCTTGACAGCAGTAAC	600	300	58	chr1:8704528	this study

Name	MM	PC	CG	PB	PV	SE	TO	PM	RA	PN	NL	SC	Forward	Reverse	Filled	Empty	AT	Locus position (Homo. hg18)	Reference
Asian colobines (30)																			
DoucL_Py2_3	0	0*	0*	0	0	1*	1	1	1	1	1*		TGCGTATTCACATTTCTGAC	GGCAGACCAATGACTACGTGA	560	250	55	chr3:44778763	1
DoucL_Py2_12RE	0	0*	0*	0	0	1	1*	1	1	1	1		CCTTGATTCATCTATGGGCTTA	TGACAAGGGAATAGAAAGATTGA	743	433	60	chr3:32047343	1
DoucL_Py2_21a	0	0	0	0	0	1	1	1	1	1	1		CACATTAGGGGGCTAGAGTT	TCCTGTGGCTCTTTGGTAGTT	574	264	55	chr20:41704352	1
Nasalis_Py2_36	0*	0*	0*	0	0*	1*	1*	1*	1*	1*	1*		CAGAAATGTTTTGTGAAGCAG	ACCCATAATGGCAACATTCAGTT	706	396	60	chr3:194023457	1
DoucL_Yd_2RE	0	0*	0*	0	0	1	1	1	1	1	1		TCCCACTTCCTCTTTTCTGAG	CTTTATGGTTTTGGCGCTAAT	578	268	60	chr2:158707448	1
DoucL_Yd_10	0	0	0	0	0	1	1	1	1	1	1		ATGTGAAGACCTCTGCCAGTA	TCCTTTTGTCAAACCTGCTCTT	440	130	60	chr8:123230272	1,2
DoucL_Yd_20	0	0*	0*	0	0	1*	1	1	1	1	1*		TCAAGAGACGCGCTCAAAA	TGCAAAACTCATCTGTGCTGT	610	300	50	chr4:154132124	1
DoucL_Yd_22	0	0*	0*	0*	0*	1	1	1	1	1	1		TCAAACTTGCAATTTCTCACAA	CACITCAAATTCGATGAACA	680	370	58	chr11:24289704	1
DoucL_Yd_27	0	0*	0	0	0*	1*	1	1	1	1	1*		TTTCATTTAACCTCCAGCTATT	TAACCAACCTGGCCAGAAC	789	479	58	chr3:38767096	1
PFL_Py2_7	0	0*	0*	0	0	1	1	1	1	1	1*		CAAGGAGGATCTAGTCAGG	CAATACCTCTGAGAGCCAGTG	624	314	60	chr8:57979101	1
PFL_Py2_9	0	0*	0*	0	0	1	1	1	1	1	1		ATCCTGCGGTTCCACATTTCTAT	GCCAACTCTTAGAAACAACAGG	560	250	55	chr2:1049801	1
PFL_Yd_1	0	0*	0*	0	0	1	1	1	1	1	1		TTCTAAGGAGACCTAAAGCA	TCATTCAAGAGATTTGCTGATG	561	251	55	chr6:123355226	1,2
PFL_Yd_11	0	0*	0*	0	0	1*	1	1	1	1	1*		GTCAGACAAGGTGTGAACAA	AATGGTTATGTTTGATCTCTTTAACA	466	156	50	chr14:63230793	1
PFL_Yd_15	0	0*	0*	0	0	1*	1	1	1	1	1		CAAAATGCTAACCCCTTGTCAT	GCCCCATCAAAAATGTATTTTC	555	245	48	chr11:75528642	1
SLL_Yd_2	0*	0*	0*	0	0	1	1	1	1	1	1*		TTCAGTTTCATCTGGGAGTG	CATGTGGAGTGACCTGTGTG	407	97	50	chr15:63007801	1
SLL_Yd_20	0	0*	0*	0	0	1*	1	1	1	1	1*		AACACATCAACACATGCCCTCA	CCTTTGGTTACTCTCCAGGT	645	335	57	chr2:149525794	1
Nasalis_Py2_27	0	0*	0*	0	0	1*	1	1	1	1	1*		TGGTTAAGTAAAGGGGGTGCT	TCATGCTACACCAACAAGCTG	574	264	60	chr1:216592546	1
Nasalis_Py2_32	0	0*	0*	0	0	1	1	1	1	1	1		CAAAATGTTCCGTTGAGTCCA	GAGTCTTGGAAAGATGCAGTGA	688	378	60	chr6:43076825	1,2
3125	0	0*	0*	0	0	1*	1	1	1	1	1*		TTTTGTAAACAGCCAAAGCTCA	TTGTTGAAAATATGGCAACGC	624	314	55	chr10:50351825	1,2
3131	0	0	0	0	0	1	1	1	1	1	1		AAGAAATCCAGGGGAAGACACT	TTGCTGCAAAAGTGACTCCT	700	400	58	chr7:107109056	3
3149	0	0	0	0	0	1	1	1	1	1	1		CCTGCCACTTTGTGCCATCT	AGAACAACACCAAGACAACAGC	450	150	58	chr3:193991928	3
3247	0	0	0	0	0	1	1	1	1	1	1		GCTTTGCCACATAAAGAGCTG	GGTTAGTGCATAATGGGAAC	420	120	58	chr2:109178381	3
3253	0	0	0	0	0	1	1	1	1	1	1		TCAATCTTCCAGGGGAAAATAAAG	GAATATTAGTTGAAATATTTAGGC	600	300	58	chr15:48692337	3
3267	0	0	0	0	0	1	1	1	1	1	1		GACCATGGTAAGACAAATGTG	GACTCAGGCTTAATTTTAAGTC	500	200	58	chr4:39427060	3
3377	0	0*	0*	0	0	1	1	1	1	1	1		CACCAAGCACAACTGTGAGG	TCTGCCATAGCCATCAGTCA	600	300	58	chr1:217214400	3
2482	0	0	0	0	0	1	1	1	1	1	1		CTCTTGGTTGGGTGAAGC	GATGGTTGAACAGTGAGACTTGA	500	200	58	chr10:119562900	3
2490	0*	0	0	0*	0	1	1	1	1	1	1		ACTGAACTGGTTTCATGTGAC	GTTAGGGATCGTTTCTCCTCAG	490	160	58	chr3:180793781	this study
3379	0	0	0	0	0*	1	1	1	1	1	1		CATCTGTGATCTGAAGCAG	TAAGAACTACATGGAAAAGCC	540	194	58	chr12:25743239	this study
6580	0	0*	0*	0	0	1	1	1	1	1	1		AGCACCATCAGGCACCTCACT	GGGAGATTGGGAAATGGAGT	700	400	58	chr6:104107889	this study
Presbytis + odd-nosed monkeys (2)	0	0*	0*	0*	0*	1*	1*	1*	1*	1*	1*		GTTTGTTAAATGATGAGAAAAGA	CTCTAGAGATGGGCAGG	460	160	56	chr12:21646931	this study
DoucL_Py2_22	0	0*	0*	0	0	0	0	1	1	1	1		TGGTGTCTCACTATCAGCAATTT	TGCTTAAAGTCCATCAACATGC	524	214	58	chr12:18654751	2
3277	0	0	0	0	0	0	0	1	1*	1	1		ACCTTGATCTCAGGGATCCT	GGTCAAAGTCTCTACTTAAGGA	410	110	58	chr7:20757072	this study

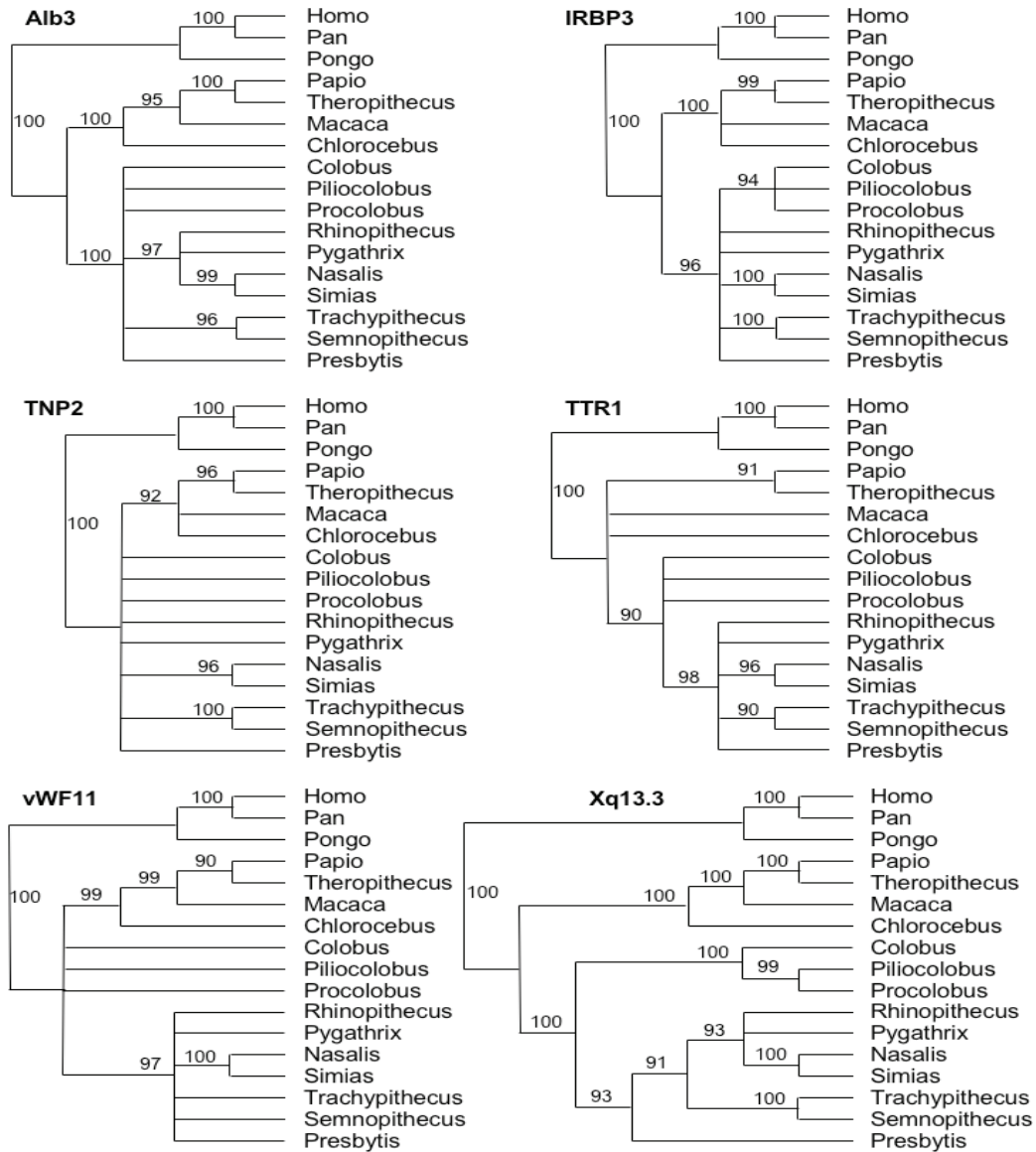
Name	MM	PC	CG	PB	PV	SE	TO	PM	RA	PN	NL	SC	Forward	Reverse	Filled	Empty	AT	Locus position (Homo. hg18)	Reference
odd-nosed monkeys (5)																			
Doucl_PY2_13RE	0	0*	0*			0	0	0	1	1	1	1	CAATGGGGACAGCTATTTTCT	CTTTGGGTGTAGTTCCTCATTT	451	141	58	chr13:104854570	1
Doucl_Yd_36	0	0*	0*			0	0	0	1	1	1	1	CTCAAGCTTCTCCCTCTCTTA	AAGGCACAGGATTCTGCTTTT	434	124	58	chr11:20035364	1
Nasalis_PY2_23	0	0*	0*			0*	0	0	1	1	1	1	GGGAAAAGATGAGTGAATCTG	TCCTTCATAGGATGCCAGTCA	700	351	58	chr4:86118446	1
3143	0	0	0			0	0	0	1	1	1	1	AGAAAGTCCTCCCAACAC	AAGTTGGCAAGTGGATTGC	550	250	58	chr1:201638132	3
6578	0	0*	0*			0*	0*	0*	1*	1*	1*	1*	CAATAAACTGCTTAAATTATC	ATTCTCAACCTTGGCCAG	430	130	56	chr15:40251990	this study
Pygathrix + Nasalis/Simias (2)																			
3381	0					0	0	0	0	1	1	1	GCATGATAAGAGTGGAAATCTGTG	TCAACTGATGCAGAAATGTC	500	200	58	chr18:3757704	this study
Doucl_Yd_33	0	0*	0*			0	0	0	0	1	1	1	CTGGCACCACCATTTGACTT	TTCACATTCATCTTCATGTC	610	300	58	chr13:56959102	1
Nasalis + Simias (9)																			
Nasalis_PY2_24	0	0*	0*			0*	0*	0	0	0*	0*	1*	TTATGGGCCCAATTTAAGTTT	ATAAAATGGACTTGCCAGATGC	650	340	58	chr7:89850261	1,2
Nasalis_PY2_25	0	0*	0*			0*	0*	0	0	0*	0*	1*	TGCTCCCATTTATAGGATTTT	ATGGGTTGTCTGCTGTCTCT	661	351	58	chr12:97767677	1,2
Nasalis_PY2_33	0	0*	0*			0*	0*	0	0	0*	0*	1*	CAGTTAGTGCGCTTAGGGA	TCCTATTGGCATTAAAGATGA	628	318	58	chr17:48297340	1,2
3127	0	0	0			0	0	0	0*	0	0	1*	TGCATTTATCTCCCTTCTTCC	TGGAGCTCCCTCTGTCCTCT	500	200	58	chr11:127217407	this study
3145	0*	0*	0*			0*	0*	0	0*	0*	0*	1*	CTTAGAGCTCAGATTGTTTATGACC	TGACAGCACACACTGCATA	623	323	58	chr4:152420536	this study
3153	0*	0*	0*			0*	0*	0*	0*	0*	0*	1*	GGACCAGAGGATATTAGCAG	CCTATATTAGAGCACCATTTGTGG	590	287	58	chr7:26371431	this study
3367	0	0	0			0	0	0	0*	0	0	1*	CCAGTGAGACAACATGTGGAA	CCAGAAATGTAATTTCTATTGATGAG	550	250	58	chr5:18542195	this study
3375	0	0	0			0	0	0	0*	0	0	1*	CCTGTGCATCTTGGGATAAC	AGCAGAAAGCCCTTGATTCAG	700	400	58	chr2:239017222	this study
6073-6022	0	0	0			0	0	0	0	0	0	1*	CTTCGCTCTCAATCTTCCCC	CAGGAAACAGCAGAGATTCC	717	390	58	chrX:78091960	this study
Trachypithecus + Semnopithecus (10)																			
SIL_Yd_18	0	0*	0*			1	1	0	0	0	0	0	GCAITCTGGACAGTGGTGATT	GACGAGATACAATGCTTCTGA	897	587	60	chr5:50538553	1
PFL_PY2_14RE	0	0*	0*			1	1	0	0*	0*	0*	0*	TGCCAAAACCTCAGTTAAGAGA	ATTTGGGGAAAACTGCTATC	563	253	60	chrX:14789579	1,2
SLL_PY2_23	0	0*	0*			1	1	0	0	0	0	0	ATAGCCTGCACGAAAAAGACCTA	CAGGAGTGTTCCTCATTGACCA	513	203	60	chr22:16311986	1
PFL_PY2_2	0	0*	0*			1	1	0	0	0	0	0	CATGCTACCTTTGTAATCCITG	TGAGGTACTGCTCTGGTGAGTT	664	354	55	chr11:111606847	1,2
SLL_PY2_17	0	0*	0*			1	1	0	0	0	0	0	TGATCCATCCCTCTTAGGAGTC	AGATCTCGGTGCCACAATAGT	694	384	50	chr12:118218700	1,2
2457	0	0*	0*			1	1	0	0	0	0	0	TGATTAAGTCAGATGAACACC	GTGTAATGGGATGAAGAACAC	540	240	58	chr15:52962566	3
2652	0	0*	0*			1	1	0	0	0	0	0	ATACATGACATTGACTTAACCT	GATCCTGAGCCCACTATTCT	520	220	58	chr5:25601752	3
2668	0	0*	0*			1	1	0	0	0	0	0	ACATCAGTGACATCAATAAGG	GAGGAAAAAGTACTTTCTCATG	470	170	58	chr8:95826066	3
3257	0	0	0			1	1	0	0	0	0	0	GGATTGAGAGCAATTTTAAAGGA	GTTCACTCCCAATTCATCTTC	940	640	58	chr15:63693377	3
3269	0	0	0			1	1	0	0	0	0	0	TGTAGCCAGGGAAGCCCTCT	TGGGATTTCTTAATACTATGCTTTG	800	500/350	58	chr11:12743945	3

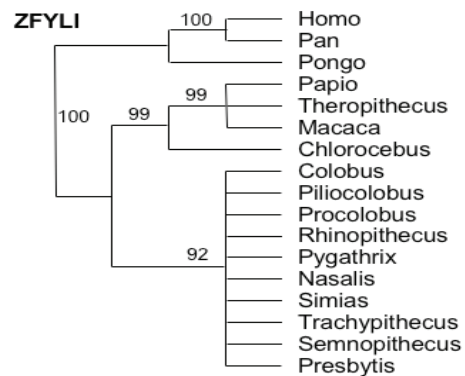
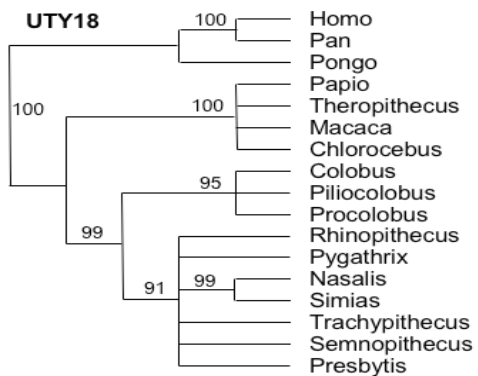
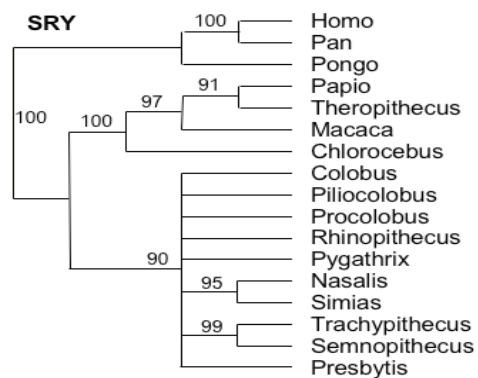
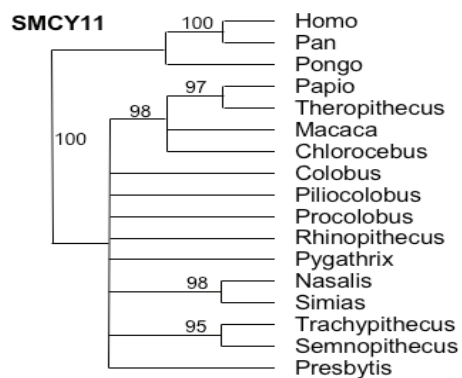
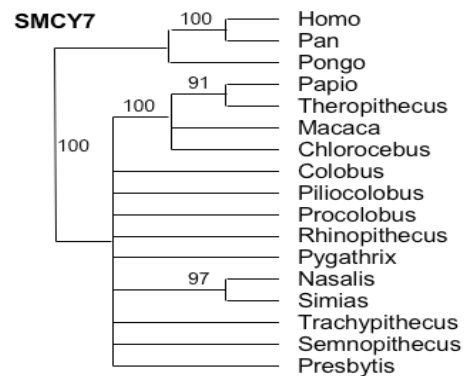
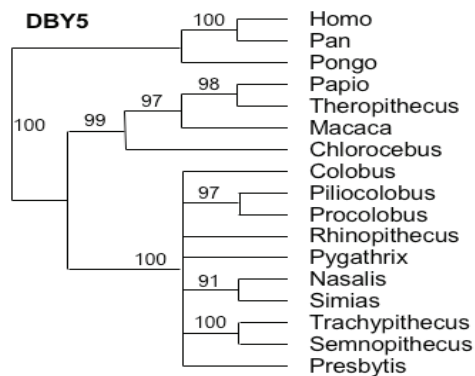
Abbreviations: MM, *Macaca mulatta*; PC, *Papio cynocephalus*; CG, *Colobus guereza*; PB, *Ptilocolobus badius*; PV, *Procolobus verus*; SE, *Semnopithecus entellus*; TO, *Trachypithecus obscurus*; PM, *Presbytis melalophos*; RA, *Rhinopithecus avunculus*; PN, *Pygathrix nemaeus*; NL, *Nasalis larvatus*; SC, *Simias concolor*; AT, Annealing temperature; 1, presence of integration; 0, absence of integration; 1/0*, not sequenced; Reference 1, Xing et al. 2005; Reference 2, Herke et al. 2007; Reference 3, Osterholz et al. 2008.

Additional file 2: Additional Table 2. Alternative tree topology tests.

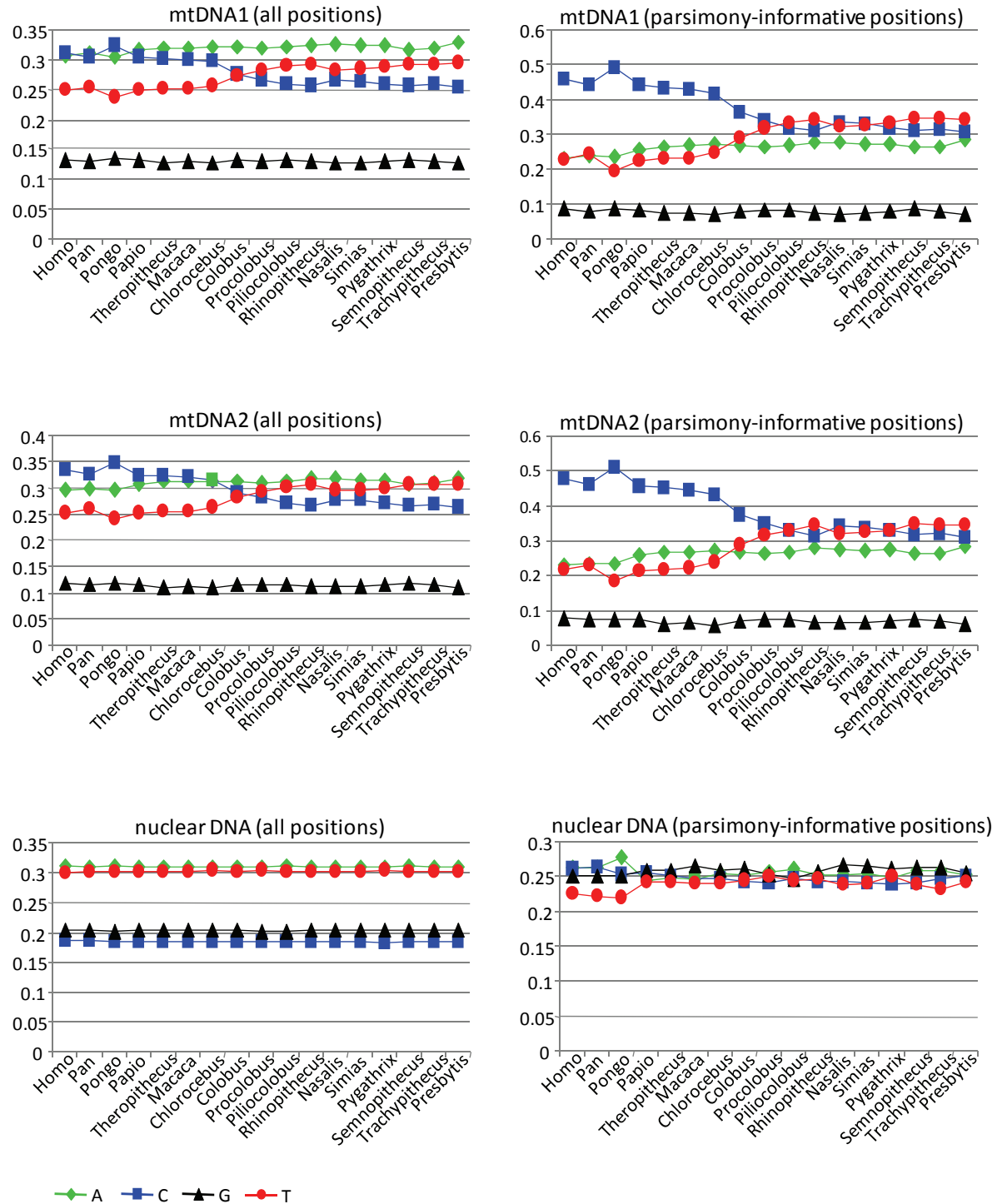
Nr	Tree Topology	mobile elements			autosomal loci combined			Xq13.3			Y chromosomal loci combined			nuclear loci combined			mitochondrial genome		
		length	diff.	KH	-InL	-InL	diff.	-InL	-InL	diff.	-InL	-InL	diff.	-InL	-InL	diff.	-InL	-InL	diff.
1	((SENT, TOBS, PMEL, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE))	239	5	P=0.001	13,549,635	99,516	P=0.001	10,399,644	124,455	P=0.001	10,538,999	117,079	P=0.001	34,606,316	335,356	P=0.001	99,620,443	37,591	P=0.001
2	((((SENT, TOBS), PMEL, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	249	5	P=0.025	13,451,996	1,877	P=0.880	10,282,614	7,426	P=0.674	10,441,529	19,609	P=0.314	34,285,420	24,461	P=0.444	99,616,149	35,286	P=0.001
3	((((SENT, TOBS), PMEL, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	239	5	P=0.025	13,451,689	1,971	P=0.888	10,281,568	6,380	P=0.716	10,441,529	19,609	P=0.314	34,294,864	33,926	P=0.457	99,616,789	33,926	P=0.001
4	((((SENT, TOBS), PMEL, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	237	3	P=0.083	13,451,940	0.922	P=0.910	10,282,614	7,426	P=0.674	10,441,529	19,609	P=0.314	34,295,193	24,233	P=0.452	99,614,178	31,315	P=0.001
5	((((SENT, TOBS), ((NLAR, SCON), PNEMRAVU)), PMEL, ((PBAD, PVER), CGUE)),	239	5	P=0.025	13,450,119		best	10,275,188		best	10,421,921		best	34,270,960		best	99,615,158	32,296	P=0.001
6	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	249	15	P=0.001	13,549,614	99,496	P=0.001	10,399,644	124,455	P=0.001	10,538,999	116,977	P=0.001	34,606,239	335,280	P=0.001	99,612,259	29,396	P=0.05
7	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	249	15	P=0.001	13,544,835	94,717	P=0.001	10,379,897	104,708	P=0.001	10,534,656	112,736	P=0.001	34,578,368	307,408	P=0.001	99,611,245	28,382	P=0.05
8	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	249	15	P=0.001	13,546,725	96,607	P=0.001	10,399,644	124,455	P=0.001	10,538,898	116,977	P=0.001	34,603,926	332,966	P=0.001	99,612,259	29,396	P=0.05
9	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	249	15	P=0.001	13,549,449	99,130	P=0.001	10,399,644	124,455	P=0.001	10,538,898	116,977	P=0.001	34,606,179	335,219	P=0.001	99,610,598	27,736	P=0.05
10	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	249	15	P=0.001	13,549,635	99,516	P=0.001	10,399,644	124,455	P=0.001	10,538,466	116,546	P=0.001	34,606,270	335,310	P=0.001	99,589,632	6,769	P=0.095
11	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	249	15	P=0.001	13,544,865	94,747	P=0.001	10,379,897	104,708	P=0.001	10,534,380	112,459	P=0.001	34,578,550	307,590	P=0.001	99,589,562	6,699	P=0.116
12	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	249	15	P=0.001	13,549,635	99,516	P=0.001	10,399,644	124,455	P=0.001	10,538,466	116,546	P=0.001	34,606,270	335,310	P=0.001	99,589,562	6,699	P=0.116
13	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	249	15	P=0.001	13,549,635	99,516	P=0.001	10,399,644	124,455	P=0.001	10,538,466	116,546	P=0.001	34,606,270	335,310	P=0.001	99,589,562	6,699	P=0.116
14	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	246	12	P=0.001	13,572,478	122,359	P=0.001	10,423,949	148,761	P=0.001	10,544,809	122,888	P=0.001	34,658,149	387,189	P=0.001	99,588,294	5,431	P=0.238
15	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	236	2	P=0.168	13,474,661	24,542	P=0.264	10,307,221	32,032	P=0.156	10,544,809	122,888	P=0.001	34,658,149	387,189	P=0.001	99,588,294	5,431	P=0.238
16	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	236	2	P=0.168	13,474,378	24,280	P=0.288	10,305,678	30,490	P=0.170	10,544,809	122,888	P=0.001	34,658,149	387,189	P=0.001	99,588,294	5,431	P=0.238
17	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	234	-	best	13,473,335	23,617	P=0.280	10,307,221	32,032	P=0.156	10,544,809	122,888	P=0.001	34,658,149	387,189	P=0.001	99,588,294	5,431	P=0.238
18	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	236	12	P=0.168	13,471,864	21,746	P=0.310	10,301,105	25,916	P=0.245	10,544,809	122,888	P=0.001	34,658,149	387,189	P=0.001	99,588,294	5,431	P=0.238
19	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	246	12	P=0.001	13,572,478	122,359	P=0.001	10,423,949	148,761	P=0.001	10,544,809	122,888	P=0.001	34,658,149	387,189	P=0.001	99,588,294	5,431	P=0.238
20	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	246	12	P=0.001	13,568,179	118,061	P=0.001	10,402,060	126,872	P=0.001	10,544,809	122,888	P=0.001	34,658,149	387,189	P=0.001	99,588,294	5,431	P=0.238
21	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	246	12	P=0.001	13,568,482	118,363	P=0.001	10,423,949	148,761	P=0.001	10,544,809	122,888	P=0.001	34,658,149	387,189	P=0.001	99,588,294	5,431	P=0.238
22	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	246	12	P=0.001	13,572,223	122,105	P=0.001	10,423,949	148,761	P=0.001	10,544,809	122,888	P=0.001	34,658,149	387,189	P=0.001	99,588,294	5,431	P=0.238
23	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	246	12	P=0.001	13,572,223	122,359	P=0.001	10,423,949	148,761	P=0.001	10,544,809	122,888	P=0.001	34,658,149	387,189	P=0.001	99,588,294	5,431	P=0.238
24	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	246	12	P=0.001	13,568,179	118,061	P=0.001	10,402,060	126,872	P=0.001	10,544,809	122,888	P=0.001	34,658,149	387,189	P=0.001	99,588,294	5,431	P=0.238
25	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	246	12	P=0.001	13,572,478	122,359	P=0.001	10,423,949	148,761	P=0.001	10,544,809	122,888	P=0.001	34,658,149	387,189	P=0.001	99,588,294	5,431	P=0.238
26	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	246	12	P=0.001	13,572,478	122,359	P=0.001	10,423,949	148,761	P=0.001	10,544,809	122,888	P=0.001	34,658,149	387,189	P=0.001	99,588,294	5,431	P=0.238
27	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	249	15	P=0.001	13,572,604	122,465	P=0.001	10,423,949	148,761	P=0.001	10,544,809	122,888	P=0.001	34,658,149	387,189	P=0.001	99,588,294	5,431	P=0.238
28	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	239	5	P=0.025	13,474,795	24,677	P=0.280	10,307,221	32,032	P=0.156	10,544,809	122,888	P=0.001	34,658,149	387,189	P=0.001	99,588,294	5,431	P=0.238
29	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	239	5	P=0.025	13,474,513	24,395	P=0.282	10,305,678	30,490	P=0.170	10,544,809	122,888	P=0.001	34,658,149	387,189	P=0.001	99,588,294	5,431	P=0.238
30	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	237	3	P=0.083	13,473,871	23,752	P=0.289	10,307,221	32,032	P=0.156	10,544,809	122,888	P=0.001	34,658,149	387,189	P=0.001	99,588,294	5,431	P=0.238
31	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	239	5	P=0.025	13,572,604	122,465	P=0.309	10,301,105	25,916	P=0.245	10,544,809	122,888	P=0.001	34,658,149	387,189	P=0.001	99,588,294	5,431	P=0.238
32	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	249	15	P=0.001	13,572,604	122,465	P=0.001	10,423,949	148,761	P=0.001	10,544,809	122,888	P=0.001	34,658,149	387,189	P=0.001	99,588,294	5,431	P=0.238
33	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	249	15	P=0.001	13,568,308	118,190	P=0.001	10,402,060	126,872	P=0.001	10,544,809	122,888	P=0.001	34,658,149	387,189	P=0.001	99,588,294	5,431	P=0.238
34	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	249	15	P=0.001	13,568,308	118,190	P=0.001	10,402,060	126,872	P=0.001	10,544,809	122,888	P=0.001	34,658,149	387,189	P=0.001	99,588,294	5,431	P=0.238
35	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	249	15	P=0.001	13,572,352	122,233	P=0.001	10,423,949	148,761	P=0.001	10,544,809	122,888	P=0.001	34,658,149	387,189	P=0.001	99,588,294	5,431	P=0.238
36	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	249	15	P=0.001	13,572,604	122,465	P=0.001	10,423,949	148,761	P=0.001	10,544,809	122,888	P=0.001	34,658,149	387,189	P=0.001	99,588,294	5,431	P=0.238
37	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	249	15	P=0.001	13,568,308	118,190	P=0.001	10,402,060	126,872	P=0.001	10,544,809	122,888	P=0.001	34,658,149	387,189	P=0.001	99,588,294	5,431	P=0.238
38	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	249	15	P=0.001	13,572,604	122,465	P=0.001	10,423,949	148,761	P=0.001	10,544,809	122,888	P=0.001	34,658,149	387,189	P=0.001	99,588,294	5,431	P=0.238
39	((((SENT, PMEL), TOBS, ((NLAR, SCON), PNEMRAVU)), (PBAD, PVER), CGUE)),	249	15	P=0.001	13,572,604	122,465	P=0.001	10,423,949	148,761	P=0.001	10,544,809	122,888	P=0.001	34,658,149	387,189	P=0.001	99,588,294	5,431	P=0.238

Abbreviations: KH, Kishino-Hasegawa test; SH, Shimodaira-Hasegawa test; CGUE, Colobus guereza; NLAR, Nasalis larvatus; PBAD, Ptilocolobus badius; PMEL, Presbytis melalophos; PNEM, Pygathrix nemaeus; PVER, Procolobus verus; RAVU, Rhinopithecus avunculus; SENT, Semnopithecus entellus; TOBS, Trachypithecus obscurus.

Additional file 3: Additional Figure 1. Single-locus phylogenetic trees (80% majority rule).

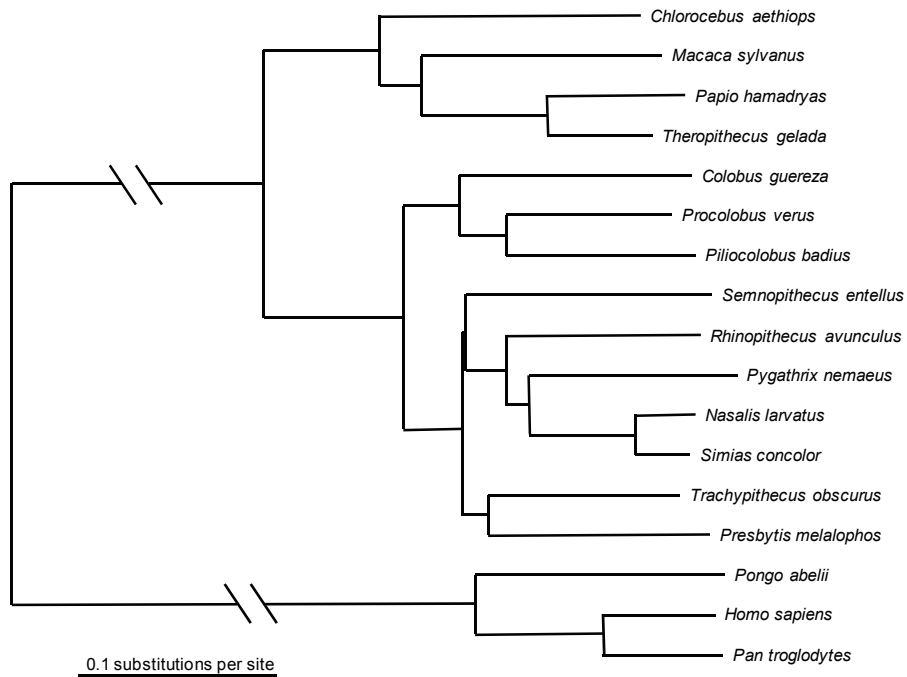


Additional file 4: Additional Figure 2. Nucleotide composition of both mitochondrial and the combined nuclear datasets.

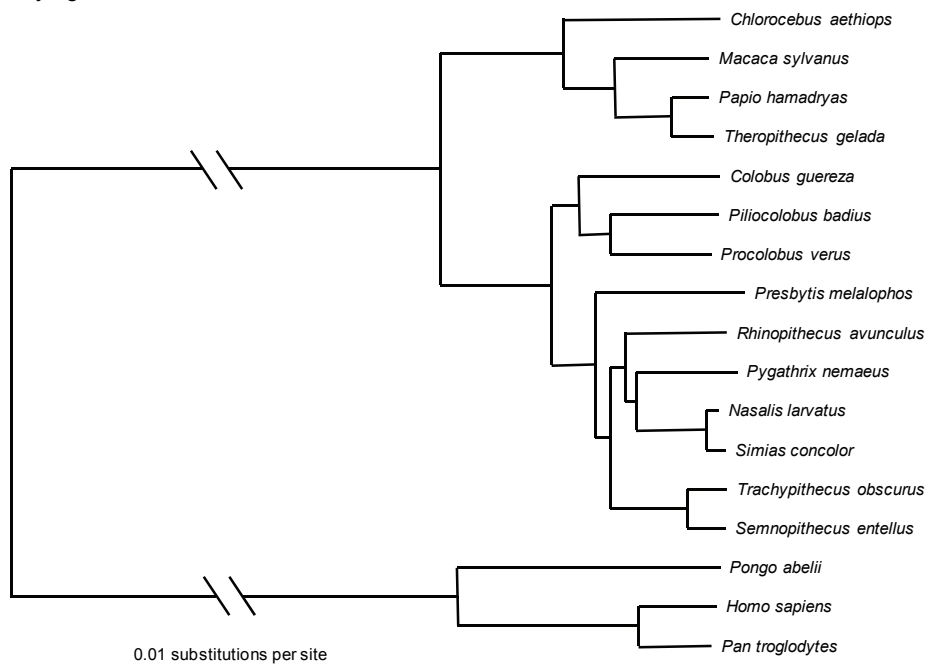


Additional file 5: Additional Figure 3. Phylograms based on the mitochondrial (mtDNA2) and combined nuclear datasets.

Phylogram based on the mitochondrial dataset (mtDNA2):



Phylogram based on the combined nuclear dataset:



Additional file 6: Additional Table 3. Divergence ages in mya estimated for each locus separately.

	Alb3	IRBP3	TNP2	TTR1
cercopithecoids-hominoids	23.45 (21.17-25.52)	24.09 (22.08-26.24)	23.57 (21.41-25.77)	23.49 (21.33-25.77)
<i>Pongo-Homo/Pan</i>	13.81 (12.57-14.91)	13.87 (12.74-14.97)	13.95 (12.79-15.09)	13.98 (12.87-15.15)
<i>Homo-Pan</i>	6.48 (5.87-7.04)	6.41 (5.83-6.99)	6.48 (5.92-7.07)	6.44 (5.87-7.03)
cercopithecines-colobines	15.45 (14.31-16.56)	15.15 (14.04-16.24)	15.03 (13.83-16.16)	15.37 (14.26-16.49)
<i>Chlorocebus</i> -other cercopithecines	8.93 (5.98-12.12)	8.49 (6.17-10.75)	9.42 (6.07-13.38)	11.76 (8.09-15.12)
<i>Macaca-Theropithecus/Papio</i>	7.12 (4.73-9.86)	6.03 (4.46-7.83)	7.27 (4.60-10.65)	9.54 (5.85-13.30)
<i>Theropithecus-Papio</i>	3.88 (3.28-4.47)	3.85 (3.28-4.41)	3.93 (3.37-4.51)	3.95 (3.36-4.54)
<i>Colobus</i> -other colobines	10.87 (8.86-12.95)	10.87 (9.28-12.67)	13.49 (10.97-15.56)	12.96 (10.67-15.10)
<i>Procolobus/Piliocolobus</i> -Asian colobines	10.26 (8.87-12.14)	10.41 (8.84-12.02)	12.14 (9.79-14.75)	12.26 (9.95-14.41)
<i>Procolobus-Piliocolobus</i>	7.67 (3.70-11.07)	7.62 (5.00-10.09)	6.19 (2.51-10.11)	7.52 (3.50-11.26)
Asian colobines	8.73 (7.61-9.81)	8.98 (7.88-9.95)	9.01 (7.93-10.10)	8.56 (7.42-9.70)
<i>Semnopithecus-Trachypithecus</i>	2.99 (0.61-5.81)	3.24 (1.58-4.94)	1.76 (0.21-3.74)	3.37 (0.67-6.67)
<i>Presbytis</i> -odd-nosed monkeys	8.04 (6.26-9.46)	8.09 (6.66-9.39)	7.94 (5.90-9.78)	7.22 (4.77-9.18)
odd-nosed monkeys	5.20 (2.96-7.43)	5.77 (4.10-7.45)	5.69 (3.21-8.16)	6.26 (3.75-8.50)
<i>Pygathrix-Nasalis/Simias</i>	3.13 (1.22-5.33)	5.41 (3.76-7.09)	4.63 (2.24-7.20)	5.41 (2.88-7.86)
<i>Nasalis-Simias</i>	0.36 (0.10-1.00)	2.43 (1.07-3.74)	1.10 (0.03-2.58)	0.51 (0.00-1.58)

	vWF11	Xq13.3	DBY5	SMCY7
cercopithecoids-hominoids	23.44 (21.24-25.73)	24.12 (22.04-26.28)	23.63 (21.44-25.80)	22.93 (20.53-25.23)
<i>Pongo-Homo/Pan</i>	13.93 (12.72-15.01)	13.82 (12.73-14.97)	13.94 (12.74-15.02)	14.02 (12.89-15.21)
<i>Homo-Pan</i>	6.41 (5.82-7.01)	6.43 (5.86-7.02)	6.40 (5.83-6.98)	6.44 (5.83-7.03)
cercopithecines-colobines	15.48 (14.32-16.56)	15.47 (14.40-16.56)	15.43 (14.32-16.51)	15.44 (14.35-16.63)
<i>Chlorocebus</i> -other cercopithecines	10.33 (7.16-13.40)	9.78 (7.37-12.50)	9.60 (6.72-12.90)	9.19 (5.25-13.01)
<i>Macaca-Theropithecus/Papio</i>	5.67 (3.87-7.78)	6.65 (4.86-8.84)	7.23 (4.82-10.04)	6.36 (4.07-9.45)
<i>Theropithecus-Papio</i>	3.96 (3.39-4.53)	3.83 (3.25-4.39)	3.88 (3.27-4.43)	3.90 (3.35-4.49)
<i>Colobus</i> -other colobines	13.46 (11.35-15.48)	10.80 (9.14-12.34)	10.15 (8.40-12.16)	11.77 (9.10-14.51)
<i>Procolobus/Piliocolobus</i> -Asian colobines	12.46 (10.35-14.67)	10.57 (9.01-12.14)	9.20 (7.79-10.66)	10.04 (8.01-12.48)
<i>Procolobus-Piliocolobus</i>	10.22 (7.56-13.02)	6.94 (4.17-9.63)	2.61 (0.27-3.13)	2.40 (0.02-6.34)
Asian colobines	8.56 (7.43-9.64)	8.35 (7.25-9.36)	8.67 (7.58-9.76)	8.74 (7.59-9.86)
<i>Semnopithecus-Trachypithecus</i>	6.64 (3.07-9.22)	2.02 (0.70-3.64)	1.76 (0.26-3.56)	3.21 (0.28-7.04)
<i>Presbytis</i> -odd-nosed monkeys	7.62 (5.72-9.33)	7.96 (6.71-9.27)	7.74 (5.92-9.49)	7.25 (4.31-9.84)
odd-nosed monkeys	6.75 (4.66-8.91)	6.28 (4.26-8.08)	6.06 (3.99-8.36)	5.53 (2.53-7.84)
<i>Pygathrix-Nasalis/Simias</i>	4.12 (1.87-6.37)	5.93 (3.87-7.83)	5.33 (3.07-7.55)	4.60 (1.59-7.90)
<i>Nasalis-Simias</i>	0.72 (0.01-1.81)	1.13 (0.28-2.23)	1.56 (0.27-3.13)	1.22 (0.02-3.13)

	SMCY11	SRY	UTY18	ZFYLI
cercopithecoids-hominoids	23.15 (21.04-25.45)	23.34 (21.27-25.63)	23.04 (20.85-25.35)	23.23 (21.11-25.43)
<i>Pongo-Homo/Pan</i>	13.94 (12.77-15.10)	13.93 (12.82-15.06)	14.00 (12.83-15.15)	14.13 (12.99-15.24)
<i>Homo-Pan</i>	6.50 (5.93-7.07)	6.47 (5.86-7.01)	6.46 (5.90-7.08)	6.39 (5.79-6.97)
cercopithecines-colobines	15.32 (14.20-16.46)	15.44 (14.35-16.56)	15.47 (14.26-16.54)	15.38 (14.28-16.51)
<i>Chlorocebus</i> -other cercopithecines	8.62 (5.16-12.41)	9.03 (6.20-12.07)	8.57 (4.96-12.29)	10.20 (6.86-13.25)
<i>Macaca-Theropithecus/Papio</i>	7.15 (4.22-10.40)	5.98 (4.18-8.23)	5.59 (3.64-8.22)	6.07 (3.96-8.61)
<i>Theropithecus-Papio</i>	3.91 (3.35-4.53)	3.84 (3.26-4.41)	3.92 (3.34-4.49)	3.92 (3.32-4.46)
<i>Colobus</i> -other colobines	11.37 (9.11-13.79)	10.97 (8.99-13.24)	11.46 (9.24-13.87)	11.96 (9.73-14.26)
<i>Procolobus/Piliocolobus</i> -Asian colobines	10.48 (8.54-12.61)	10.32 (8.40-12.28)	10.76 (8.72-13.02)	11.24 (9.19-13.52)
<i>Procolobus-Piliocolobus</i>	5.33 (1.75-8.90)	4.33 (1.09-8.11)	3.86 (0.35-8.61)	6.46 (2.72-10.35)
Asian colobines	8.80 (7.67-9.85)	8.66 (7.62-9.78)	8.58 (7.46-9.74)	8.67 (7.55-9.77)
<i>Semnopithecus-Trachypithecus</i>	3.10 (0.65-6.03)	3.37 (1.14-5.86)	3.03 (0.29-6.57)	2.66 (0.21-5.89)
<i>Presbytis</i> -odd-nosed monkeys	7.81 (5.74-9.60)	7.69 (5.71-9.38)	7.34 (4.96-9.41)	7.81 (6.02-9.52)
odd-nosed monkeys	6.43 (3.79-8.71)	6.12 (3.72-8.33)	5.92 (3.22-8.60)	6.53 (4.11-8.65)
<i>Pygathrix-Nasalis/Simias</i>	5.31 (2.61-7.87)	5.21 (2.57-7.78)	4.84 (2.03-7.68)	4.14 (1.84-6.49)
<i>Nasalis-Simias</i>	1.53 (0.15-3.31)	1.12 (0.03-2.67)	0.61 (0.00-1.91)	1.00 (0.02-2.37)

Additional file 7: Additional Table 4. Uncorrected pairwise nucleotide differences for each locus

Alb3	CGUE	PBAD	PVER	RAVU	PNEM	NLAR	SCON	PMEL	TOBS
PBAD	0.0156								
PVER	0.0147	0.0113							
RAVU	0.0233	0.0182	0.0190						
PNEM	0.0190	0.0138	0.0147	0.0112					
NLAR	0.0216	0.0165	0.0173	0.0138	0.0061				
SCON	0.0216	0.0165	0.0173	0.0138	0.0061	0.0000			
PMEL	0.0242	0.0208	0.0182	0.0233	0.0190	0.0216	0.0216		
TOBS	0.0190	0.0138	0.0147	0.0182	0.0138	0.0147	0.0147	0.0190	
SENT	0.0164	0.0130	0.0121	0.0156	0.0112	0.0138	0.0138	0.0164	0.0043
IRBP3	CGUE	PBAD	PVER	RAVU	PNEM	NLAR	SCON	PMEL	TOBS
PBAD	0.0137								
PVER	0.0189	0.0150							
RAVU	0.0207	0.0182	0.0214						
PNEM	0.0207	0.0182	0.0208	0.0117					
NLAR	0.0214	0.0202	0.0234	0.0110	0.0149				
SCON	0.0214	0.0202	0.0234	0.0110	0.0149	0.0065			
PMEL	0.0221	0.0209	0.0241	0.0188	0.0163	0.0208	0.0221		
TOBS	0.0233	0.0195	0.0240	0.0156	0.0195	0.0169	0.0169	0.0221	
SENT	0.0246	0.0208	0.0253	0.0182	0.0221	0.0195	0.0195	0.0234	0.0078
TNP2	CGUE	PBAD	PVER	RAVU	PNEM	NLAR	SCON	PMEL	TOBS
PBAD	0.0202								
PVER	0.0233	0.0124							
RAVU	0.0279	0.0202	0.0233						
PNEM	0.0264	0.0172	0.0202	0.0109					
NLAR	0.0264	0.0186	0.0217	0.0109	0.0093				
SCON	0.0279	0.0202	0.0234	0.0124	0.0109	0.0016			
PMEL	0.0326	0.0249	0.0279	0.0202	0.0186	0.0186	0.0202		
TOBS	0.0326	0.0249	0.0279	0.0202	0.0186	0.0186	0.0202	0.0248	
SENT	0.0326	0.0249	0.0279	0.0202	0.0186	0.0186	0.0202	0.0248	0.0031
TTR1	CGUE	PBAD	PVER	RAVU	PNEM	NLAR	SCON	PMEL	TOBS
PBAD	0.0214								
PVER	0.0248	0.0169							
RAVU	0.0281	0.0214	0.0293						
PNEM	0.0293	0.0225	0.0304	0.0146					
NLAR	0.0259	0.0214	0.0293	0.0113	0.0146				
SCON	0.0259	0.0214	0.0293	0.0113	0.0146	0.0000			
PMEL	0.0260	0.0192	0.0271	0.0090	0.0101	0.0090	0.0090		
TOBS	0.0281	0.0214	0.0293	0.0113	0.0146	0.0113	0.0113	0.0090	
SENT	0.0259	0.0192	0.0271	0.0090	0.0124	0.0090	0.0090	0.0068	0.0045

vWF11	CGUE	PBAD	PVER	RAVU	PNEM	NLAR	SCON	PMEL	TOBS
PBAD	0.0332								
PVER	0.0277	0.0332							
RAVU	0.0332	0.0354	0.0376						
PNEM	0.0322	0.0366	0.0388	0.0211					
NLAR	0.0366	0.0367	0.0366	0.0166	0.0134				
SCON	0.0376	0.0376	0.0376	0.0177	0.0144	0.0011			
PMEL	0.0300	0.0311	0.0299	0.0177	0.0167	0.0167	0.0155		
TOBS	0.0277	0.0265	0.0298	0.0144	0.0144	0.0133	0.0144	0.0100	
SENT	0.0321	0.0320	0.0320	0.0188	0.0166	0.0155	0.0166	0.0122	0.0111

Xq13.3	CGUE	PBAD	PVER	RAVU	PNEM	NLAR	SCON	PMEL	TOBS
PBAD	0.0157								
PVER	0.0166	0.0142							
RAVU	0.0180	0.0185	0.0199						
PNEM	0.0192	0.0202	0.0206	0.0126					
NLAR	0.0161	0.0171	0.0185	0.0100	0.0111				
SCON	0.0164	0.0173	0.0187	0.0102	0.0114	0.0017			
PMEL	0.0187	0.0197	0.0202	0.0149	0.0164	0.0133	0.0135		
TOBS	0.0195	0.0204	0.0218	0.0142	0.0149	0.0123	0.0126	0.0154	
SENT	0.0185	0.0195	0.0209	0.0133	0.0140	0.0114	0.0116	0.0145	0.0033

DBY5	CGUE	PBAD	PVER	RAVU	PNEM	NLAR	SCON	PMEL	TOBS
PBAD	0.0363								
PVER	0.0287	0.0076							
RAVU	0.0272	0.0242	0.0166						
PNEM	0.0257	0.0227	0.0181	0.0166					
NLAR	0.0302	0.0227	0.0151	0.0181	0.0196				
SCON	0.0287	0.0181	0.0106	0.0166	0.0181	0.0045			
PMEL	0.0317	0.0302	0.0242	0.0227	0.0212	0.0257	0.0242		
TOBS	0.0317	0.0257	0.0212	0.0227	0.0242	0.0257	0.0212	0.0272	
SENT	0.0332	0.0302	0.0227	0.0242	0.0257	0.0272	0.0227	0.0287	0.0045

SMCY7	CGUE	PBAD	PVER	RAVU	PNEM	NLAR	SCON	PMEL	TOBS
PBAD	0.0162								
PVER	0.0139	0.0023							
RAVU	0.0231	0.0208	0.0185						
PNEM	0.0139	0.0116	0.0092	0.0139					
NLAR	0.0208	0.0139	0.0116	0.0208	0.0116				
SCON	0.0231	0.0162	0.0139	0.0231	0.0139	0.0023			
PMEL	0.0162	0.0139	0.0116	0.0185	0.0116	0.0185	0.0208		
TOBS	0.0162	0.0139	0.0116	0.0162	0.0069	0.0139	0.0162	0.0139	
SENT	0.0162	0.0139	0.0116	0.0162	0.0069	0.0139	0.0162	0.0139	0.0046

SMCY11	CGUE	PBAD	PVER	RAVU	PNEM	NLAR	SCON	PMEL	TOBS
PBAD	0.0154								
PVER	0.0055	0.0166							
RAVU	0.0122	0.0351	0.0185						
PNEM	0.0147	0.0314	0.0148	0.0148					
NLAR	0.0169	0.0333	0.0166	0.0166	0.0129				
SCON	0.0193	0.0370	0.0203	0.0203	0.0166	0.0037			
PMEL	0.0227	0.0447	0.0296	0.0296	0.0296	0.0314	0.0351		
TOBS	0.0197	0.0388	0.0222	0.0222	0.0185	0.0203	0.0240	0.0333	
SENT	0.0197	0.0351	0.0185	0.0185	0.0148	0.0166	0.0203	0.0333	0.0074

SRY	CGUE	PBAD	PVER	RAVU	PNEM	NLAR	SCON	PMEL	TOBS
PBAD	0.0143								
PVER	0.0078	0.0065							
RAVU	0.0207	0.0220	0.0155						
PNEM	0.0168	0.0181	0.0117	0.0143					
NLAR	0.0155	0.0168	0.0104	0.0130	0.0091				
SCON	0.0168	0.0181	0.0117	0.0143	0.0104	0.0013			
PMEL	0.0194	0.0207	0.0143	0.0220	0.0181	0.0168	0.0181		
TOBS	0.0207	0.0220	0.0155	0.0181	0.0143	0.0130	0.0143	0.0220	
SENT	0.0233	0.0220	0.0181	0.0207	0.0168	0.0155	0.0168	0.0246	0.0078

UTY18	CGUE	PBAD	PVER	RAVU	PNEM	NLAR	SCON	PMLE	TOBS
PBAD	0.0121								
PVER	0.0073	0.0048							
RAVU	0.0145	0.0145	0.0097						
PNEM	0.0205	0.0205	0.0157	0.0109					
NLAR	0.0181	0.0181	0.0133	0.0085	0.0133				
SCON	0.0181	0.0181	0.0133	0.0085	0.0133	0.0000			
PMEL	0.0230	0.0230	0.0181	0.0133	0.0205	0.0169	0.0169		
TOBS	0.0169	0.0169	0.0121	0.0073	0.0121	0.0085	0.0085	0.0157	
SENT	0.0181	0.0181	0.0133	0.0060	0.0133	0.0097	0.0097	0.0133	0.0036

ZFYLI	CGUE	PBAD	PVER	RAVU	PNEM	NLAR	SCON	PMEL	TOBS
PBAD	0.0118								
PVER	0.0132	0.0118							
RAVU	0.0191	0.0206	0.0206						
PNEM	0.0221	0.0235	0.0235	0.0147					
NLAR	0.0191	0.0206	0.0206	0.0147	0.0088				
SCON	0.0177	0.0191	0.0191	0.0132	0.0103	0.0015			
PMEL	0.0206	0.0221	0.0221	0.0191	0.0221	0.0221	0.0206		
TOBS	0.0162	0.0177	0.0177	0.0088	0.0118	0.0117	0.0103	0.0162	
SENT	0.0162	0.0177	0.0177	0.0088	0.0118	0.0118	0.0103	0.0162	0.0029

mtDNA1	CGUE	PBAD	PVER	RAVU	PNEM	NLAR	SCON	PMEL	TOBS
PBAD	0.1361								
PVER	0.1318	0.1162							
RAVU	0.1476	0.1449	0.1402						
PNEM	0.1497	0.1497	0.1457	0.1210					
NLAR	0.1455	0.1465	0.1381	0.1174	0.1223				
SCON	0.1455	0.1418	0.1400	0.1164	0.1211	0.0458			
PMEL	0.1540	0.1501	0.1477	0.1329	0.1389	0.1337	0.1322		
TOBS	0.1464	0.1461	0.1420	0.1302	0.1367	0.1307	0.1323	0.1292	
SENT	0.1548	0.1505	0.1495	0.1350	0.1431	0.1347	0.1355	0.1405	0.1353

mtDNA2	CGUE	PBAD	PVER	RAVU	PNEM	NLAR	SCON	PMEL	TOBS
PBAD	0.1565								
PVER	0.1523	0.1363							
RAVU	0.1673	0.1659	0.1593						
PNEM	0.1689	0.1712	0.1664	0.1399					
NLAR	0.1660	0.1682	0.1576	0.1352	0.1429				
SCON	0.1656	0.1630	0.1601	0.1354	0.1415	0.0529			
PMEL	0.1758	0.1726	0.1694	0.1521	0.1592	0.1541	0.1524		
TOBS	0.1676	0.1684	0.1632	0.1494	0.1568	0.1480	0.1509	0.1486	
SENT	0.1760	0.1713	0.1712	0.1532	0.1648	0.1539	0.1560	0.1612	0.1537

Additional file 8: Additional Table 5. Locus-specific information including alignment length, number of variable sites, selected substitution model and estimated evolutionary rates.

Locus	Alignment with / without indels	Variable sites / parsimony- informative sites	Substitution model (AIC)	Estimated evolutionary rates
ALB3	1204 / 1157	182 / 93	TVM + I	0.902
IRBP3	1615 / 1543	263 / 114	K81uf + G	0.848
TNP2	894 / 645	120 / 56	GTR	1.000
TTR1	906 / 889	151 / 73	TVM + G	0.953
vWF11	938 / 905	181 / 98	HKY + G	1.156
autosomal loci combined	5557 / 5139	897 / 434	TVM + G	
Xq13.3	6634 / 4218	621 / 327	TVM + G	0.906
DBY5	1048 / 662	144 / 83	GTR + I	1.403
SMCY7	463 / 433	89 / 56	TVM	1.500
SMCY11	606 / 541	108 / 51	TVM	1.080
SRY	786 / 772	123 / 65	TVM	0.948
UTY18	881 / 828	131 / 73	HKY + G	1.082
ZFYLI	714 / 680	106 / 54	TVM	0.921
Y chromosomal loci combined	4498 / 3916	701 / 382	TVM + G	
nuclear loci combined	16689 / 13273	2154 / 1143	TVM + G	
mitochondrial genome	16860 / 15074* 16860 / 11316**	6998 / 5457* 5745 / 4592**	GTR + I + G GTR + I + G	3.511
all data combined	33549 / 28347* 33549 / 24589**	9152 / 6600* 7899 / 5735**	- -	

* mitochondrial alignment excluding indels, poorly aligned positions and D-loop

** mitochondrial alignment with only protein-coding genes

Additional file 9: Additional Table 6. Primers and PCR conditions for the amplification of nuclear loci.

Locus	Ref.	Forward primer	Reverse Primer	AT
ALB3	-	GCATTCAAAGTCAACCATG	ACGAAGAGTTGCAACTGTGC	56°C
IRBP3	-	CTCTGGACACACGCCAG	CACACTGCTGGTCAGAATGA	58°C
TNP2	-	GCAGGTGTACAAAACCAAG	GTCTCATTAGTTGGATTTCC	54°C
TTR1	-	GGCCCTACGGTGAGTGTT	ACTTTGACCATCAGAGGACA	56°C
vWF11	[73]	see ref.	see ref.	see ref.
Xq13.3	[10,75]	see ref.	see ref.	see ref.
DBY5	[74]	see ref.	see ref.	see ref.
SMCY7	[74]	see ref.	see ref.	see ref.
SMCY11	[74]	see ref.	see ref.	see ref.
UTY18	[74]	see ref.	see ref.	see ref.
SRY	[72]	see ref.	see ref.	see ref.
ZFYLI	-	CCTGATTCCAGGCAGTACC	ATCAGGGCCAATAATTATTGCT	58°C

Additional file 10: Additional Table 7. GenBank accession numbers*.

Species	ALB3	IRBP3	TNP2	TTR1	vWF11	DBY5	SMCY7	SMCY11	SRY	UTY18	ZFYLI	Xq13.3	mtDNA
<i>Homo sapiens</i>	EF649953	J05253	L03378	M11844	AC006576	AC004474	AF273841	AF273841	X53772	AF265575	U24118	AJ241091	X93334
<i>Pan troglodytes</i>	JF293097	JF293129	JF293193	JF293209	JF293241	JF293113	JF293145	JF293161	JF293177	JF293225	JF293262	AJ270088	D38113
<i>Pongo abelii</i>	JF293098	JF293130	JF293194	JF293210	JF293242	JF293114	JF293146	JF293162	JF293178	JF293226	JF293263	JF293257	X97707
<i>Papio hamadryas</i>	JF293100	JF293132	JF293197	JF293213	JF293243	JF293117	JF293149	JF293164	JF293180	JF293228	JF293265	AY899234	Y18001
<i>Theropithecus gelada</i>	JF293101	JF293133	JF293196	JF293212	JF293244	JF293116	JF293148	JF293165	JF293181	JF293229	JF293266	AY899236	FJ785426
<i>Macaca sylvanus</i>	JF293099	JF293131	JF293195	JF293211	JF293245	JF293115	JF293150	JF293163	JF293179	JF293227	JF293264	JF293258	AJ309865
<i>Chlorocebus aethiops</i>	JF293102	JF293134	JF293198	JF293214	JF293246	JF293118	JF293147	JF293166	JF293182	JF293230	JF293267	AY899216	AY863426
<i>Colobus guereza</i>	JF293103	JF293135	JF293199	JF293215	JF293247	JF293119	JF293151	JF293167	JF293183	JF293231	JF293268	AY899240	AY863427
<i>Ptilocolobus badius</i>	JF293104	JF293136	JF293200	JF293216	JF293248	JF293120	JF293152	JF293168	JF293184	JF293232	JF293269	EU342361	DQ355301
<i>Procolobus verus</i>	JF293105	JF293137	JF293201	JF293217	JF293249	JF293121	JF293153	JF293169	JF293185	JF293233	JF293270	JF293259	JF293092
<i>Trachypithecus obscurus</i>	JF293111	JF293144	JF293207	JF293223	JF293255	JF293127	JF293159	JF293175	JF293191	JF293239	JF293276	EU342365	AY863425
<i>Semnopithecus entellus</i>	JF293112	JF293143	JF293208	JF293224	JF293256	JF293128	JF293160	JF293176	JF293192	JF293240	JF293277	EU342364	DQ355297
<i>Presbytis melalophos</i>	JF293106	JF293138	JF293202	JF293218	JF293250	JF293122	JF293154	JF293170	JF293186	JF293234	JF293271	JF293260	DQ355299
<i>Nasalis larvatus</i>	JF293108	JF293140	JF293204	JF293220	JF293252	JF293124	JF293156	JF293172	JF293188	JF293236	JF293273	EU342359	JF293094
<i>Simias concolor</i>	JF293109	JF293141	JF293205	JF293221	JF293253	JF293125	JF293157	JF293173	JF293189	JF293237	JF293274	JF293261	JF293095
<i>Pygathrix nemaeus</i>	JF293107	JF293139	JF293203	JF293219	JF293251	JF293123	JF293155	JF293171	JF293187	JF293235	JF293272	EU342362	JF293096
<i>Rhinopithecus avunculus</i>	JF293110	JF293142	JF293206	JF293222	JF293254	JF293126	JF293158	JF293174	JF293190	JF293238	JF293275	EU342363	JF293093

*Sequences in italic are taken from GenBank

Chapter 2

The Evolutionary History of Odd-Nosed Monkeys

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The Evolutionary History of Odd-Nosed Monkeys

Mouyu Yang^{1,2}, Tilo Nadler³, Dietmar Zinner⁴, Christian Roos^{5,6}

¹Primate Genetics Laboratory, German Primate Center, Göttingen, Germany

²Fanjingshan National Nature Reserve, Jiangkou, Guizhou Province, China

³Frankfurt Zoological Society, Endangered Primate Rescue Center, Cuc Phuong National Park, Ninh Binh Province, Vietnam

⁴Cognitive Ethology Laboratory, German Primate Center, Göttingen, Germany

⁵Gene Bank of Primates, German Primate Center, Göttingen, Germany

Corresponding author: Christian Roos, Gene Bank of Primates, Primate Genetics Laboratory, German Primate Center, D-37077 Göttingen, Germany; phone: +49 (0)551 3851 300; croos@dpz.eu

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Figures: 2

Abstract

The odd-nosed monkeys represent one of the two major groups among Asian colobines. Although a common origin of the group was recently confirmed, the phylogenetic relationships among its genera and species, and the patterns leading to their current distribution are largely unknown. To address these issues, we analyzed complete mitochondrial genome sequence data from nine of the ten odd-nosed monkey species, and reconstructed phylogenetic relationships and estimated divergence ages. Based on our data, we can confirm a common origin of *Nasalis* and *Simias*, but not of *Rhinopithecus* and *Pygathrix*. In fact, *Rhinopithecus* forms a sister clade to all other genera in the group, indicating a northern origin of odd-nosed monkeys and a later invasion into Indochina and Sundaland. According to our divergence age estimates, the genera *Rhinopithecus*, *Pygathrix* and *Nasalis* + *Simias* originated in the late Miocene, while speciation events within genera and also the split between *Nasalis* and *Simias* occurred in the Pleistocene.

Keywords

Odd-nosed monkey, phylogeny, evolution, biogeography

Introduction

More than a decade ago, Nina Jablonski explained convincingly that odd-nosed monkeys represent a particular interesting group to study, and why they have been neglected scientifically for a long time (Jablonski 1998a). They are enigmatic and rare, and they show remarkable anatomical and behavioral adaptations to a range of, for primates, unusual habitats, such as mangrove swamps, and temperate and high altitude forests. The group consists of four genera, snub-nosed monkeys (*Rhinopithecus*), doucs langurs (*Pygathrix*), proboscis monkey (*Nasalis*) and pig-tailed monkey (*Simias*). Three of the five snub-nosed monkey species, *R. roxellana*, *R. brelichi* and *R. bieti* occur in a few isolated populations in China, while the fourth species, *R. avunculus* is restricted to the extreme North of Vietnam. A fifth species, *R. strykeri* was recently described from Myanmar (Geissmann et al. 2011). Douc langurs, represented by the three species *P. nemaeus*, *P. cinerea* and *P. nigripes*, are distributed through parts of Vietnam, Laos and Cambodia, east of the Mekong River. Both *Nasalis* and *Simias* are monotypic. *N. lavatus* is a pure Bornean species, whereas *S. concolor* is endemic to the Mentawai Islands, west of Sumatra. All species are endangered or even critically endangered (Geissmann et al. 2011; IUCN 2010). Since Jablonski's book, research has been intensified tremendously and a wealth of data was published concerning ecology, behavior and conservation of the Chinese species in particular (e.g. Grueter et al. 2009; Ren et al. 2008; Tan et al. 2007; Yang et al. 2009; Zhang et al. 2008).

Odd-nosed monkeys together with langurs, comprising the genera *Presbytis*, *Semnopithecus* and *Trachypithecus*, form the Asian colobines (Groves 2001; Jablonski 1998b; Jablonski and Peng 1993; Osterholz et al. 2008; Roos et al. 2011; Sterner et al. 2006; Ting et al. 2008). Although recent genetic investigations convincingly confirmed a common origin of the odd-nosed monkeys (Li et al. 2011; Osterholz et al. 2008; Perelman et al. 2011; Roos et al. 2011; Sterner et al. 2006; Ting et al. 2008), the phylogenetic relationships among genera and species are still debated. Several phylogenetic hypotheses based on morphological or molecular arguments have been proposed including: (1) *Rhinopithecus* and *Pygathrix* are sister taxa (Delson 1975; Groves 2001; Jablonski and Peng 1993; Li et al. 2004; Napier 1985; Oates et al. 1994; Wang et al. 1997), (2) *Pygathrix* is more closely related to *Nasalis* than to *Rhinopithecus* (Jablonski 1998b), (3) *Rhinopithecus* and *Nasalis* are sister taxa (Zhang

and Ryder 1998), (4) *Simias* is closely related to *Nasalis* (Whittaker et al. 2006), and (5) *Simias* is basal among Asian colobines (Jablonski 1998b).

In a study using complete mitochondrial genome sequences, Li et al. (2011) and Sterner et al. (2006) was not able to resolve relationships among the three studied genera *Rhinopithecus*, *Pygathrix* and *Nasalis*. Similar results were obtained from X chromosomal sequence data (Ting et al. 2008) and the presence/absence analysis of short interspersed elements (SINEs) (Osterholz et al. 2008). Unfortunately, none of these studies included *Simias*. However, based on a short fragment of the mitochondrial cytochrome b gene, Whittaker et al. (2006) suggested a sister grouping of *Nasalis* and *Simias* and a taxonomic placement of *Simias* within *Nasalis*. Latter arrangement was recently confirmed by a multi-maker approach using SINE integrations and sequence analysis of mitochondrial genomes as well as autosomal and gonosomal loci (Roos et al. 2011). Roos et al. (2011) also showed that *Rhinopithecus* is basal among odd-nosed monkeys.

Although relationships among odd-nosed monkey genera seem to be largely resolved now, this is not true for various relationships within them. Genetic data clearly resolve branching patterns within *Pygathrix* (Roos 2004; Roos and Nadler 2001; Roos et al. 2007), but relationships among the five *Rhinopithecus* species are still disputed. Jablonski and Peng (1993) found that *R. roxellana* grouped together with the *R. bieti* + *R. brelichi* clade and that *R. avunculus* was sister species to all other snub-nosed monkeys. Zhang and Ryder (1998), however, concluded that relationships between *R. bieti*, *R. roxellana* and *R. avunculus* are more or less three-way. Molecular studies by Roos (2004), Roos et al. (2007) and Li et al. (2004) support the view of Zhang and Ryder (1998). In a recent study by Li et al. (2011) using complete mitochondrial genome data, *R. avunculus* appeared as sister lineage to a *R. bieti* + *R. roxellana* clade. Unfortunately, *R. strykeri* was not included in all these studies, but the species resembles *R. bieti* in various characters (Geissmann et al. 2011).

Although our understanding of the phylogenetic relationships within the odd-nosed monkey group became clearer due to molecular studies, several questions remain. However, all molecular studies so far used only short mitochondrial fragments and/or did not include all species. To obtain a more complete picture about the evolutionary history of the odd-nosed monkeys, we have analyzed the complete mitochondrial genome from nine of the ten odd-nosed monkey species. Assuming that

the origin of the odd-nosed monkey group was in the North of its current range (Hengduan Mountains and southern Yunnan, Jablonski 1998b; Roos et al. 2011), we suppose a north-south migration of the group with corresponding successive divergences of the respective more southern taxa. As a result of our study, we present here the most complete and updated molecular phylogeny of odd-nosed monkeys and discuss their phylogeographic implications.

Methods

Sample Collection

Fecal samples from each one individual of *R. roxellana*, *R. brelichi*, *R. bieti*, *P. nigripes* and *P. cinerea* were obtained from the breeding station of Fanjingshan National Nature Reserve, China, Beijing Zoo, China, and the Endangered Primate Rescue Center, Vietnam, respectively. Fresh samples were preserved in tubes with 80% ethanol. Mitochondrial genome sequences from *R. avunculus*, *P. nemeus*, *N. larvatus* and *S. concolor* were recently published by our group (Roos et al. 2011).

Laboratory Methods

DNA from fecal material was extracted using the Qiagen Stool Mini kit following recommendations of the supplier. To reduce the likelihood of amplifying nuclear pseudogenes (numts), complete mitochondrial genomes were amplified via five overlapping fragments, each with a size of ~5,000 bp. All these long-range PCRs were performed with the SuperTaq Plus polymerase from Ambion following protocols of the supplier and primers as described (Raaum et al. 2005; Roos et al. 2011; Sterner et al. 2006). Long-range PCR amplicons were separated on 1% agarose gels, excised from the gel, purified with the Qiagen Gel Extraction kit and used as template for nested PCRs. PCR conditions for all nested PCR amplifications were identical and comprised a pre-denaturation step at 94°C for 2 min, followed by 30 cycles each with denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1.5 min. At the end, a final extension step at 72°C for 5 min was added. Nested PCR products (900 - 1,200 bp in length) were cleaned with the Qiagen PCR Purification kit and sequenced on an ABI 3130xl sequencer. Sequences were assembled with Geneious v4.6.1 (Drummond et al. 2009). No inconsistent positions in overlapping regions were detected and all protein-coding genes were correctly translated. Annotation of

mitochondrial genomes was conducted with the online program DOGMA (Wyman et al. 2004) and manually inspected.

Statistical Analysis

For phylogenetic reconstructions, we implemented further mitochondrial genome sequences deposited in GenBank. The final dataset comprised 22 sequences including each one representative of the nine studied odd-nosed monkey species and *Presbytis melalophos*, *Semnopithecus entellus*, *Trachypithecus obscurus*, *Colobus guereza*, *Ptilocolobus badius*, *Procolobus verus*, four cercopithecine species (*Papio hamadryas*, *Theropithecus gelada*, *Macaca sylvanus*, *Chlorocebus aethiops*), and three hominoid species (*Homo sapiens*, *Pan troglodytes*, *Pongo abelii*), which were used as outgroup taxa. The alignment was generated with MAFFT v6 (Katoh et al. 2005) and corrected by eye. Poorly aligned positions and indels were removed with Gblocks v0.91b (Castresana 2000) using default settings.

Phylogenetic trees were constructed with maximum-likelihood (ML) and Bayesian algorithms, using the programs GARLI v0.951 (Zwickl 2006) and MrBayes v3.1.2 (Huelsenbeck et al. 2001; Ronquist and Huelsenbeck 2003). For both reconstructions, the GTR + I + G model was applied as it was chosen as optimal nucleotide substitution model using the Bayesian information criterion (BIC) as implemented in jMODELTEST v0.1 (Posada 2008). Relative support of internal nodes for ML reconstructions was performed by bootstrap analyses with 500 replications. In GARLI, only the model specification settings were adjusted, while all other settings were left at their default value. ML majority-rule consensus trees were calculated in PAUP v4.0b10 (Swofford 2003). For Bayesian reconstructions, we used four Monte Carlo Markov Chains (MCMC) with the default temperature of 0.1. Four repetitions were run for 10,000,000 generations with tree and parameter sampling occurring every 100 generations. The first 25% of samples were discarded as burnin, leaving 75,001 trees per run. Posterior probabilities for each split and a phylogram with mean branch lengths were calculated from the posterior density of trees.

To estimate divergence ages, a Bayesian Markov Chain Monte Carlo (MCMC) method, which employs a relaxed molecular clock approach (Drummond et al. 2006), as implemented in BEAST v1.4.8 (Drummond and Rambaut 2007), was used. A

relaxed lognormal model of lineage variation and a Birth-Death Process prior for branching rates was assumed.

As calibrations we used the fossil-based divergence between *Homo* and *Pan*, which has been dated at 6 - 7 million years ago (mya) (Brunet et al. 2005; Lebartard et al. 2008; Vignaud et al. 2002), the separation of *Pongo* from the *Homo* + *Pan* lineage ~14 mya (Kelley 2002), the split between *Theropithecus* and *Papio* ~4 mya (Leakey 1993; Delson 2000), and the divergence of hominoids and cercopithecoids ~23 mya (Benefit and McCrossin 2002; Young and MacLatchy 2004; Zalmout et al. 2010). Instead of hardbounded calibration points, we used the published dates as a normal distribution prior for the respective node. For the *Homo* - *Pan* divergence, this translates into a normal distribution with a mean of 6.5 mya and a standard deviation (SD) of 0.5 mya, for the separation of *Pongo* from the *Homo* + *Pan* clade into a mean of 14.0 mya and a SD of 1.0 mya, for the *Theropithecus* - *Papio* split into a mean of 4.0 mya and a SD of 0.5 mya, and for the hominoid - cercopithecoid divergence into a mean of 23 mya and a SD of 2 mya.

Since the estimation of phylogenetic relationships was not the main aim of this analysis, we used an a-priori fixed tree topology as obtained from tree reconstructions mentioned above. Four replicates were run for 10,000,000 generations with tree and parameter sampling occurring every 100 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 TRACER v1.4.1 (Rambaut and Drummond 2007). Subsequently, the sampling distributions were combined (25% burnin) using the software LogCombiner v1.4.8 and a consensus chronogram with node height distribution was generated and visualized with TreeAnnotator v1.4.8 and FigTree v1.2.2 (Rambaut 2008).

Results

We successfully generated complete mitochondrial genome sequences from *R. roxellana*, *R. brelichi*, *R. bieti*, *P. nigripes* and *P. cinerea*. The complete alignment including nine of the ten odd-nosed monkey species and various other catarrhine taxa had a length of 16,910 bp. After the removal of indels and poorly aligned positions, the alignment was 15,608 bp in length. Among them, 7,352 sites were variable and 5,924 of them parsimony-informative.

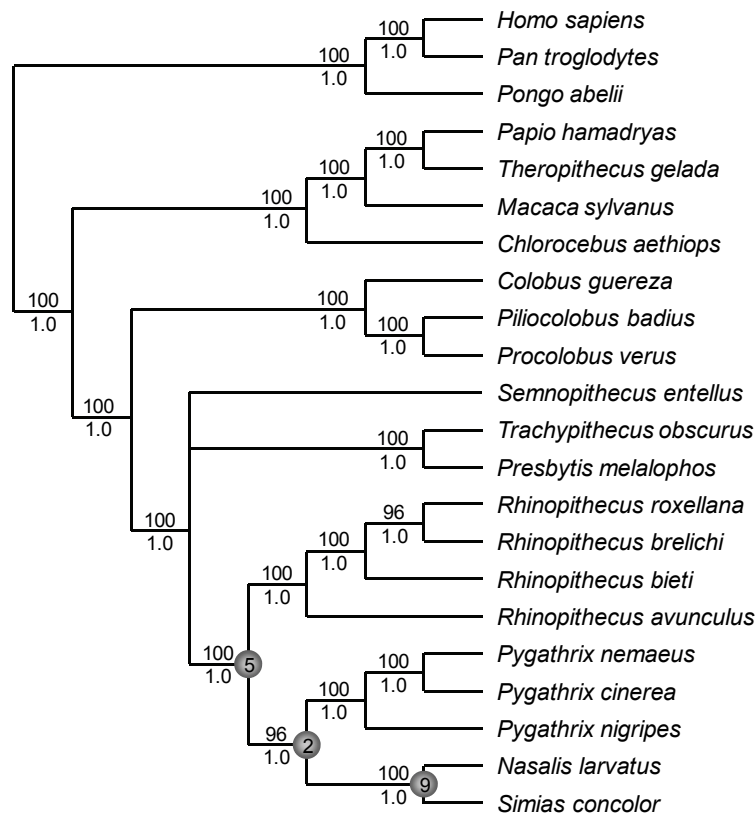


Fig. 1: Phylogenetic relationships among 22 catarrhine primates including nine of the ten odd-nosed monkey species. Numbers on branches indicate support values as obtained from ML and Bayesian reconstructions. Light grey circles and impeded numbers refer to number of SINE integrations supporting respective nodes (SINE data from Roos et al. 2011).

Phylogenetic reconstructions based on ML and Bayesian algorithms revealed identical tree topologies. With the exception of the unresolved relationship among the odd-nosed monkey clade, the *Trachypithecus* + *Presbytis* clade and *Semnopithecus*, all other branching patterns were well resolved and significantly supported (Fig. 1). According to our reconstructions and divergence age estimations (Fig. 2, Table 1), the initial split occurred between hominoids and cercopithecoids 23.99 mya (for 95% credibility intervals [CI] see Table 1). Among the former, *Pongo* branched off 13.44 mya, followed by the divergence of *Pan* and *Homo* 6.16 mya. 16.84 mya, cercopithecoids further segregated into two reciprocally monophyletic clades referring to Cercopithecinae and Colobinae. In the former, *Chlorocebus* represented the most basal split (10.91 mya), while *Macaca* was sister lineage to the *Papio* + *Theropithecus* clade. *Macaca* diverged from *Papio* + *Theropithecus* 9.11 mya and latter two separated

4.03 mya. Colobine monkeys further segregated into an African and an Asian clade 10.85 mya. Among African colobines, *Colobus* branched off first (8.48 mya), before finally also *Piliocolobus* and *Procolobus* separated (6.72 mya). Asian colobines initially diverged 8.60 mya into an odd-nosed monkey clade, a *Trachypithecus* + *Presbytis* clade and *Semnopithecus*, but the relationships among these three lineages remained unresolved. Within odd-nosed monkeys, *Rhinopithecus* was most basal and *Pygathrix* formed a sister lineage to the *Nasalis* + *Simias* clade. The last common ancestors of the odd-nosed monkeys and of the *Pygathrix* + *Nasalis* + *Simias* clade occurred 6.85 and 6.30 mya, respectively. *Nasalis* and *Simias* finally differentiated 1.98 mya. In *Rhinopithecus*, *R. avunculus* diverged first (2.38 mya), followed by *R. bieti* (1.93 mya), before finally also *R. brelichi* and *R. roxellana* separated (1.66 mya). Within *Pygathrix*, *P. nigripes* was basal to the *P. cinerea* + *P. nemaeus* clade. According to our estimates, the split between *P. nigripes* and the other two species occurred 2.51 mya, while latter two separated 0.69 mya.

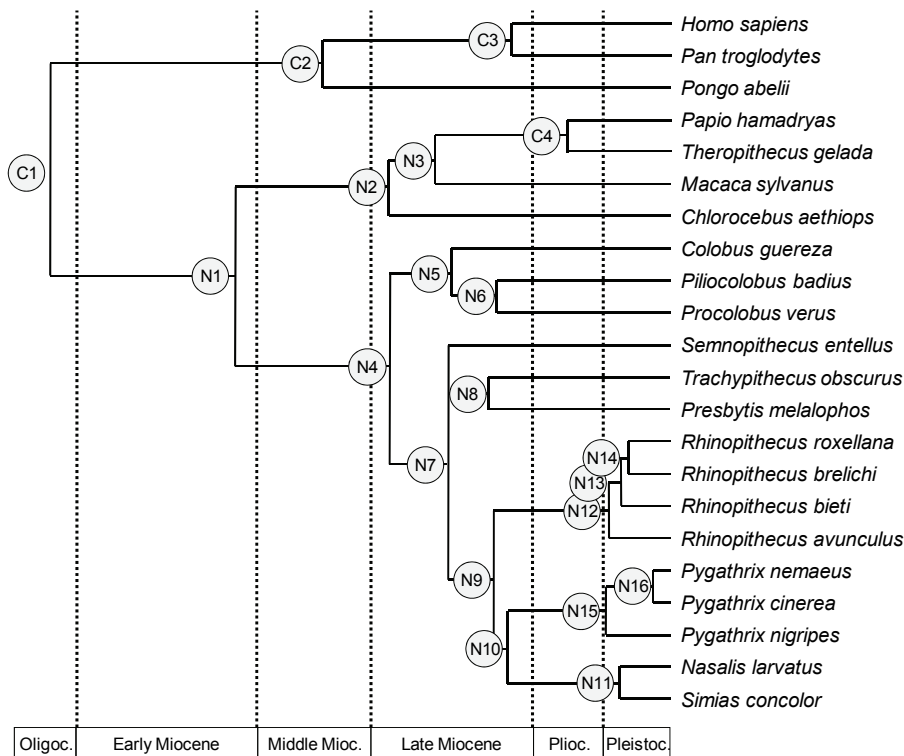


Fig. 2: Ultrametric tree showing phylogenetic relationships and estimated divergence ages among 22 catarrhine primates including nine of ten odd-nosed monkey species. Nodes labeled with C and N refer to fossil-based calibration points and nodes of interest, respectively. For full details of estimated divergence ages see Table 1.

Table 1: Divergence age estimations in mya (see also Fig. 2)

Node	Divergence	Mean (95% CI)
C1	Cercopithecoidea - Hominoidea	23.99 (22.18 - 26.10)
C2	<i>Pongo</i> - (<i>Homo</i> + <i>Pan</i>)	13.44 (12.43 - 14.56)
C3	<i>Homo</i> - <i>Pan</i>	6.16 (5.62 - 6.72)
C4	<i>Papio</i> - <i>Theropithecus</i>	4.03 (3.52 - 4.57)
N1	Cercopithecinae - Colobinae	16.84 (14.59 - 19.15)
N2	<i>Chlorocebus</i> - (<i>Macaca</i> + <i>Papio</i> + <i>Theropithecus</i>)	10.91 (9.08 - 12.66)
N3	<i>Macaca</i> - (<i>Papio</i> + <i>Theropithecus</i>)	9.11 (7.41 - 10.78)
N4	African Colobinae - Asian Colobinae	10.85 (9.65 - 12.08)
N5	<i>Colobus</i> - (<i>Piliocolobus</i> + <i>Procolobus</i>)	8.48 (7.09 - 9.82)
N6	<i>Piliocolobus</i> - <i>Procolobus</i>	6.72 (5.27 - 8.05)
N7	odd-nosed monkeys - <i>Semnopithecus</i> - (<i>Trachypithecus</i> + <i>Presbytis</i>)	8.60 (7.77 - 9.43)
N8	<i>Trachypithecus</i> - <i>Presbytis</i>	7.05 (5.96 - 8.02)
N9	<i>Rhinopithecus</i> - (<i>Pygathrix</i> + <i>Nasalis</i> + <i>Simias</i>)	6.85 (5.94 - 7.69)
N10	<i>Pygathrix</i> - (<i>Nasalis</i> + <i>Simias</i>)	6.30 (5.43 - 7.16)
N11	<i>Nasalis</i> - <i>Simias</i>	1.98 (1.36 - 2.56)
N12	<i>R. avunculus</i> - (<i>R. bieti</i> + <i>R. brelichi</i> + <i>R. roxellana</i>)	2.38 (1.91 - 2.85)
N13	<i>R. bieti</i> - (<i>R. brelichi</i> + <i>R. roxellana</i>)	1.93 (1.51 - 2.37)
N14	<i>R. brelichi</i> - <i>R. roxellana</i>	1.66 (1.24 - 2.09)
N15	<i>P. nigripes</i> - (<i>P. nemaus</i> + <i>P. cinerea</i>)	2.51 (1.87 - 3.15)
N16	<i>P. nemaus</i> - <i>P. cinerea</i>	0.69 (0.46 - 0.93)

Discussion

As in earlier molecular studies (Osterholz et al. 2008; Perelman et al. 2011; Roos et al. 2011; Sterner et al. 2006; Ting et al. 2008), the monophyly of the odd-nosed monkeys is strongly supported and further underpinned by five SINE integrations (Roos et al. 2011). Further, our data clearly suggest a basal position of *Rhinopithecus* and a sister grouping of *Pygathrix* and *Nasalis* + *Simias*, a pattern, which is also supported by two SINE integrations (Roos et al. 2011) and nuclear sequence data (Perelman et al. 2011; Roos et al. 2011). Moreover, our study provides clear information concerning the branching pattern within the two polytypic genera *Rhinopithecus* and *Pygathrix*. Since the overall relationships and estimated divergence ages among various lineages are in agreement with earlier studies (Goodman et al. 1998; Karanth et al. 2008; Osterholz et al. 2008; Perelman et al. 2011; Raaum et al.

2005; Roos et al. 2011; Sterner et al. 2006; Ting 2008; Ting et al. 2008; Xing et al. 2005), our data can be regarded as true and reliable.

Taxonomy of odd-nosed monkeys

Our study corroborates a three genera and ten species classification of the odd-nosed monkey group. Although *R. strykeri* was not studied, it is provisionally classified as distinct species here. All three clades, representing the three genera, diverged from a common ancestor in the latest Miocene. The divergence of *Simias* and *Nasalis* occurred in the early Pleistocene, a time frame in which most species of *Rhinopithecus* and *Pygathrix* emerged. Only the separation between *P. nemaeus* and *P. cinerea* occurred later, about 0.69 mya. Therefore, our data does not support the perpetuation of *Simias* as a genus or subgenus, but suggests its inclusion in *Nasalis*. Accordingly, the genus *Nasalis* contains two species, *N. larvatus* and *N. concolor*. Further, our data does not support the placement of *R. avunculus* in the subgenus *Presbyticus*, because the divergence time between *R. avunculus* and the other snub-nosed monkey species was estimated at roughly the same time as other species' splits in *Rhinopithecus* and *Pygathrix*. To avoid the erection of a subgenus for *P. nigripes*, we prefer the five species (*R. avunculus*, *R. bieti*, *R. brelichi*, *R. roxellana*, *R. strykeri*) classification of *Rhinopithecus* and the three species classification of *Pygathrix* (*P. cinerea*, *P. nemaeus*, *P. nigripes*). Although *P. cinerea* and *P. nemaeus* diverged relative recently, both split in the same range as various other Asian colobines (Roos, unpublished), and, thus, species level for both is proposed.

Evolutionary history and biogeography of odd-nosed monkeys

By combining the available information, we develop the following dispersal scenario for odd-nosed monkeys. The origin of Asian colobine monkeys and also of the odd-nosed monkeys might have been the Hengduan Mountains in the border region of today's Burma, India and China (Jablonski 1998b; Peng et al. 1993; Roos et al. 2011). In the region, all the larger Southeast Asian rivers (Mekong, Salween, Yangtze) rise, which are all well known as barriers for arboreal primates (Meijaard and Groves 2006) and which exist at least since the early Miocene (Hallet and Molnar 2001). After the separation of the langur progenitor from the odd-nosed monkey ancestor, members of the odd-nosed monkey group successively migrated from China to the south and

expanded their range into Indochina and Sundaland in the latest Miocene. The speciation within *Rhinopithecus* occurred in the early Pleistocene, most likely triggered by reduction and fragmentation of suitable habitat. For *Pygathrix*, our data suggest an origin in southern Vietnam and Cambodia. From there, the ancestor of *P. nemaeus* and *P. cinerea* migrated to the north in the early to middle Pleistocene. The separation of latter two occurred on a similar time scale as species splits among crested gibbons (Thinh et al. 2010) and limestone langurs (Perelman et al. 2011), suggesting tremendous environmental changes during that time. The migration of a *Nasalis* + *Simias* progenitor into Sundaland was probably via land bridges connecting the mainland with Sundaland islands during periods of lowered sea levels (Miller et al. 2005). Finally, *Nasalis* on Borneo and *Simias* on the Mentawai islands diverged in the early Pliocene. Most likely, *Simias* or at least a progenitor of *Simias* and *Nasalis* was also present on Sumatra but went extinct there. Due to the dating discrepancy (mitochondrial data: 1.98 mya, nuclear data: 1.1 mya, Roos et al. 2011), further gene flow between both genera after the initial separation until the end of the early Pleistocene cannot be excluded, especially considering that migration was repeatedly possible via land bridge connections during the Pleistocene (Miller et al. 2005).

Conclusions

By analyzing complete mitochondrial genome sequence data from nine of the ten odd-nosed monkey species, the present study provides comprehensive insight into the evolutionary and biogeographic history of this enigmatic primate group. Most importantly, our study shows that odd-nosed monkeys originated on the Asian mainland and migrated into Indochina and Sundaland during the late Miocene. Differentiation into species occurred during the Pleistocene. Although our study is the first, which allows such detailed information about phylogeny and phylogeography of odd-nosed monkeys, future studies should also include the newly described *R. strykeri* as well as nuclear sequence data which might allow to uncover possible ancient hybridization events as it was detected in the case of the Asian colobine genus *Trachypithecus* (Roos et al. 2011).

References

- Benefit BR, McCrossin KL (2002) Old World monkeys: the Miocene emergence. In: Hartwig W (ed) The primate fossil record. Cambridge University Press, Cambridge
- Brunet M, Guy F, Pilbeam D et al (2005) New material of the earliest hominid from the Upper Miocene of Chad. *Nature* 434:752–755
- Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol Biol Evol* 17:540–552
- Delson E (1975) Evolutionary history of the Cercopithecidae. *Contrib Primatol* 5:167–217
- Delson E (2000) Cercopithecinae. In: Delson E, Tattersall I, Van Couvering JA, Brooks AS (eds) Encyclopedia of human evolution and prehistory. Garland Publishing Inc, New York
- Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol Biol* 7:214
- Drummond AJ, Ho SYN, Phillips MJ et al (2006) Relaxed phylogenetics and dating with confidence. *PLoS Biol* 4:e88
- Drummond AJ, Ashton B, Cheung M et al (2009) Geneious v4.6. Available from <http://www.geneious.com/>
- Geissmann T, Lwin N, Aung SS et al (2011) A new species of snub-nosed monkey, genus *Rhinopithecus* Milne-Edwards, 1872 (Primates, Colobinae), from northern Kachin state, northeastern Myanmar. *Am J Primatol* 73:96–107
- Goodman M, Porter CA, Czelusniak J et al (1998) Toward a phylogenetic classification of primates based on DNA evidence complemented by fossil evidence. *Mol Phylogenet Evol* 9:585–598
- Groves CP (2001) Primate taxonomy. Smithsonian Institution Press, Washington, DC
- Grueter CC, Li D, Ren B et al (2009) Dietary profile of *Rhinopithecus bieti* and its socioecological implications. *Int J Primatol* 30:601–624
- Hallet B, Molnar P (2001) Distorted drainage basins as markers of crustal strain east of the Himalayas. *J Geophys Res* 106:13697–13709
- Huelsenbeck JP, Ronquist F, Nielsen R et al (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. Accessed 9 June 2010

Jablonski NG (1998a) The natural history of the doucs and snub-nosed monkeys. World Scientific, Singapore

Jablonski NG (1998b) The evolution of the doucs and snub-nosed monkey and the question of the phyletic unity of the odd-nosed colobines. In: Jablonski NG (ed) The natural history of the doucs and snub-nosed monkeys. World Scientific, Singapore

Jablonski NG, Peng YZ (1993) The phylogenetic relationships and classification of the doucs and snub-nosed langurs of China and Vietnam. *Folia Primatol* 60:36–55

Karanth KP, Singh L, Collura RV et al (2008) Molecular phylogeny and biogeography of langurs and leaf monkeys of South Asia (Primates: Colobinae). *Mol Phylogenet Evol* 46:683–694

Katoh K, Kuma K, Toh H et al (2005) MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Res* 33:511–518

Kelley J (2002) The hominoid radiation in Asia. In: Hartwig WC (ed) The primate fossil record. Cambridge University Press, Cambridge

Leakey MG (1993) Evolution of *Theropithecus* in the Turkana basin. In: Jablonski NG (ed) *Theropithecus*, the rise and fall of a primate genus. Cambridge University Press, Cambridge

Lebatard AE, Bourlès DL, Durringer P et al (2008) Cosmogenic nuclide dating of *Sahelanthropus tchadensis* and *Australopithecus bahrelghazali*: Mio-Pliocene hominids from Chad. *Proc Natl Acad Sci USA* 105:3226–3231

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

Li Y, Wang X, Ting N, Zhang YP (2011) Mitogenomic analysis of Chinese snub-nosed monkeys: Evidence of positive selection in NADH dehydrogenase genes in high-altitude adaptation. *Mitochondrion* 11:497–503

Meijaard E, Groves CP (2006) The geography of mammals and rivers in mainland South-East Asia. In: Lehman SM, Fleagle, JG (eds) *Primate biogeography*. Springer, New York

Miller KG, Kominz MA, Browning JV et al (2005) The phanerozoic record of global sea-level change. *Science* 310:1293–1298

Napier PH (1985) Catalogue of primates in the British Museum (Natural History) and elsewhere in the British Isles. Part III: Family Cercopithecidae, subfamily Colobinae. London, British Museum (Natural History)

Oates JF, Davies AG, Delson E (1994) The diversity of living colobines. In: Davies AG, Oates JF (eds) Colobine monkeys: their ecology, behaviour and evolution. Cambridge University Press, Cambridge

Osterholz M, Walter L, Roos C (2008) Phylogenetic position of the langur genera *Semnopithecus* and *Trachypithecus* among Asian colobines, and genus affiliations of their species groups. BMC Evol Biol 8:58

Peng YZ, Pan RL, Jablonski N (1993) Classification and evolution of Asian colobines. Folia Primatol 60:106–117

Perelman P, Johnson WE, Roos C et al (2011) A molecular phylogeny of living primates. PLoS Genet 7:e1001342

Posada D (2008) jModelTest: Phylogenetic model averaging. Mol Biol Evol 25:1253–1256

Raaum RL, Sterner KN, Noviello CM et al (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 (2008) FigTree: Tree figure drawing tool, version 1.2.2. Institute of Evolutionary Biology, University of Edinburgh. <http://tree.bio.ed.ac.uk/software/figtree/>

Rambaut A, Drummond AJ (2007) Tracer v1.4.1: MCMC trace analysis tool. Institute of Evolutionary Biology, University of Edinburgh. <http://tree.bio.ed.ac.uk/software/tracer/>

Ren B, Li M, Long Y et al (2008) Measuring daily ranging distances of *Rhinopithecus bieti* via a Global Positioning System collar at Jinsichang, China: a methodological consideration. Int J Primatol 29:783–794

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574

Roos C (2004) Molecular evolution and systematics of Vietnamese primates. In: Nadler T, Streicher U, Long HT (eds) Conservation of primates in Vietnam. Frankfurt Zoological Society, Frankfurt

Roos C, Nadler T (2001) Molecular evolution of the douc langurs. Zool Garten NF 71:1–6

Roos C, Thanh VN, Walter L et al (2007) Molecular systematics of Indochinese primates. *Vietn J Primatol* 1:41–53

Roos C, Zinner D, Kubatko LS et al (2011) Nuclear versus mitochondrial DNA: Evidence for hybridization in colobine monkeys. *BMC Evol Biol* 11:77

Sterner KN, Raaum RL, Zhang YP et al (2006) Mitochondrial data support an odd-nosed colobine clade. *Mol Phylogenet Evol* 40:1–7

Swofford DL (2003) PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4. Sinauer, Sunderland

Tan C, Guo S, Li B (2007) Population structure and ranging patterns of *Rhinopithecus roxellana* in Zhouzhi National Nature Reserve, Shaanxi, China. *Int J Primatol* 28:577–591

Thinh, VN, Mootnick A, Geissmann T et al (2010) Mitochondrial evidence for multiple radiations in the evolutionary history of small apes. *BMC Evol Biol* 10:74

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

Ting N, Tosi AJ, Li Y et al (2008) Phylogenetic incongruence between nuclear and mitochondrial markers in the Asian colobines and the evolution of the langurs and leaf monkeys. *Mol Phylogenet Evol* 46:466–474

Vignaud P, Durringer P, Mackaye HT et al (2002) Geology and palaeontology of the Upper Miocene Toros-Menalla hominid locality, Chad. *Nature* 418:152–155

Wang W, Forstner MRJ, Zhang YP et al (1997) A phylogeny of Chinese leaf monkeys using mitochondrial ND3-ND4 gene sequences. *Int J Primatol* 18:305–320

Whittaker DJ, Ting N, Melnick DJ (2006) Molecular phylogenetic affinities of the simakobu monkey (*Simias concolor*). *Mol Phylogenet Evol* 39:887–892

Wyman SK, Jansen RK, Boore JL (2004) Automatic annotation of organellar genomes with DOGMA. *Bioinformatics* 20:3252–3255

Xing J, Wang H, Han K et al (2005) A mobile element based phylogeny of Old World monkeys. *Mol Phylogenet Evol* 37:872–880

Yang M, Sun DY, Zinner D et al (2009) Reproductive parameters in Guizhou snub-nosed monkeys (*Rhinopithecus brelichi*). *Am J Primatol* 71:266–270

Young NM, MacLachy L (2004) The phylogenetic position of *Moropithecus*. *J Hum Evol* 46:163–184

Zalmout IS, Sanders WJ, MacLatchy LM et al (2010) New Oligocene primate fossil from Saudi Arabia and the divergence of apes and Old World monkeys. *Nature* 466:360–365

Zhang P, Watanabe K, Li B (2008) Female social dynamics in a provisioned free-ranging band of the Sichuan snub-nosed monkey (*Rhinopithecus roxellana*) in the Qinling mountains, China. *Am J Primatol* 70:1013–1022

Zhang YP, Ryder OA (1998) Mitochondrial cytochrome b gene sequences of Old World monkeys: with special reference on evolution of Asian colobines. *Primates* 39:39–49

Zwickl DJ (2006) Genetic algorithm approaches for the phylogenetic analysis of large biological sequence data sets under the maximum likelihood criterion. Ph.D. Dissertation. University of Texas, Austin

Chapter 3

Population Genetic Structure of Guizhou Snub-Nosed Monkeys (*Rhinopithecus brelichi*) as Inferred from Mitochondrial Control Region Sequences, and Comparison with *R. roxellana* and *R. bieti*

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Population Genetic Structure of Guizhou Snub-Nosed Monkeys (*Rhinopithecus brelichi*) as Inferred From Mitochondrial Control Region Sequences, and Comparison With *R. roxellana* and *R. bieti*

Mouyu Yang,^{1,2*} Yeqin Yang,² Duoying Cui,³ Gisela Fickenscher,⁴ Dietmar Zinner,⁴ Christian Roos,^{1,5} and Markus Brameier¹

¹Primate Genetics Laboratory, German Primate Center, Göttingen 37077, Germany

²Fanjingshan National Nature Reserve, Jiangkou 554400, Guizhou Province, China

³Beijing Zoo, Beijing 100044, China

⁴Cognitive Ethology Laboratory, German Primate Center, Göttingen 37077, Germany

⁵Gene Bank of Primates, German Primate Center, Göttingen 37077, Germany

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ABSTRACT The Guizhou snub-nosed monkey (*Rhinopithecus brelichi*) is a primate species endemic to the Wuling Mountains in southern China. With a maximum of 800 wild animals, the species is endangered and one of the rarest Chinese primates. To assess the genetic diversity within *R. brelichi* and to analyze its genetic population structure, we collected fecal samples from the wild *R. brelichi* population and sequenced the hypervariable region I of the mitochondrial control region from 141 individuals. We compared our data with those from the two other Chinese snub-nosed species (*R. roxellana*, *R. bieti*) and reconstructed their phylogenetic relationships and divergence times. With only five haplotypes and a maximum of 25 polymorphic sites, *R. brelichi* shows the lowest genetic diversity in terms of haplotype diversity (h), nucleotide diversity (π), and average number of pairwise nucleotide differences (Π). The most

recent common ancestor of *R. brelichi* lived ~0.36 million years ago (Ma), thus more recently than those of *R. roxellana* (~0.91 Ma) and *R. bieti* (~1.33 Ma). Phylogenetic analysis and analysis of molecular variance revealed a clear and significant differentiation among the three Chinese snub-nosed monkey species. Population genetic analyses (Tajima's D , Fu's F_s , and mismatch distribution) suggest a stable population size for *R. brelichi*. For the other two species, results point in the same direction, but population substructure possibly introduces some ambiguity. Because of the lower genetic variation, the smaller population size and the more restricted distribution, *R. brelichi* might be more vulnerable to environmental changes or climate oscillations than the other two Chinese snub-nosed monkey species. *Am J Phys Anthropol* 000:000–000, 2011. © 2011 Wiley-Liss, Inc.

Snub-nosed monkeys, genus *Rhinopithecus*, are enigmatic colobine monkeys, which inhabit subtropical and temperate zones of China, Vietnam and Burma (Fig. 1). The genus comprises five species (Groves, 2001; Roos et al., 2007; Geissmann et al., 2011), which diverged from each other in the early Pleistocene (Jablonski, 1998a; Li et al., 2004; Yang et al., in press). Three of these species are endemic to China (*R. brelichi*, Guizhou snub-nosed monkey; *R. roxellana*, Sichuan snub-nosed monkey; *R. bieti*, Yunnan snub-nosed monkey), while the fourth species occurs in northern Vietnam (*R. avunculus*, Tonkin snub-nosed monkey; Groves, 2001) and the fifth species in northern Burma (*R. strykeri*, Burmese snub-nosed monkey; Geissmann et al., 2011). Fossils indicate that snub-nosed monkeys were widely distributed in China and Vietnam during the Pleistocene. However, most likely due to environmental changes, their ranges became highly fragmented and populations survived only in restricted areas (Jablonski, 1998a,b). Human activities during the last centuries further reduced suitable snub-nosed monkey habitats (Li et al., 2002), and thus, species occur today only in isolated mountain regions: *R. roxellana* in the Chinese provinces of Sichuan, Hubei, Shaanxi, and Gansu, *R. bieti* in Yunnan and Tibet, *R. brelichi* in Guizhou, *R. avunculus* in Tuyen

Quang, Bac Kan, Thai Nguyen, and Ha Giang provinces of northern Vietnam, and *R. strykeri* in Kachin state of Burma (Groves, 2001; International Union for Conservation of Nature (IUCN), 2010; Geissmann et al., 2011). Among the five species, *R. roxellana* with ~15,000 individuals has the largest population (IUCN, 2010), followed by *R. bieti* with a maximum of 2,000 individuals (IUCN, 2010) and *R. brelichi* with 750–800 individuals (Yang et al., 2002; IUCN, 2010). For *R. avunculus* and *R. strykeri*, with only 200 and 260–330 individuals, respectively, the situation is even worse (Mittermeier et al., 2009; IUCN, 2010; Geissmann et al., 2011). Accord-

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*Correspondence to: Mouyu Yang, Primate Genetics Laboratory, German Primate Center, Kellnerweg 4, Göttingen 37077, Germany. E-mail: myang@dpz.eu

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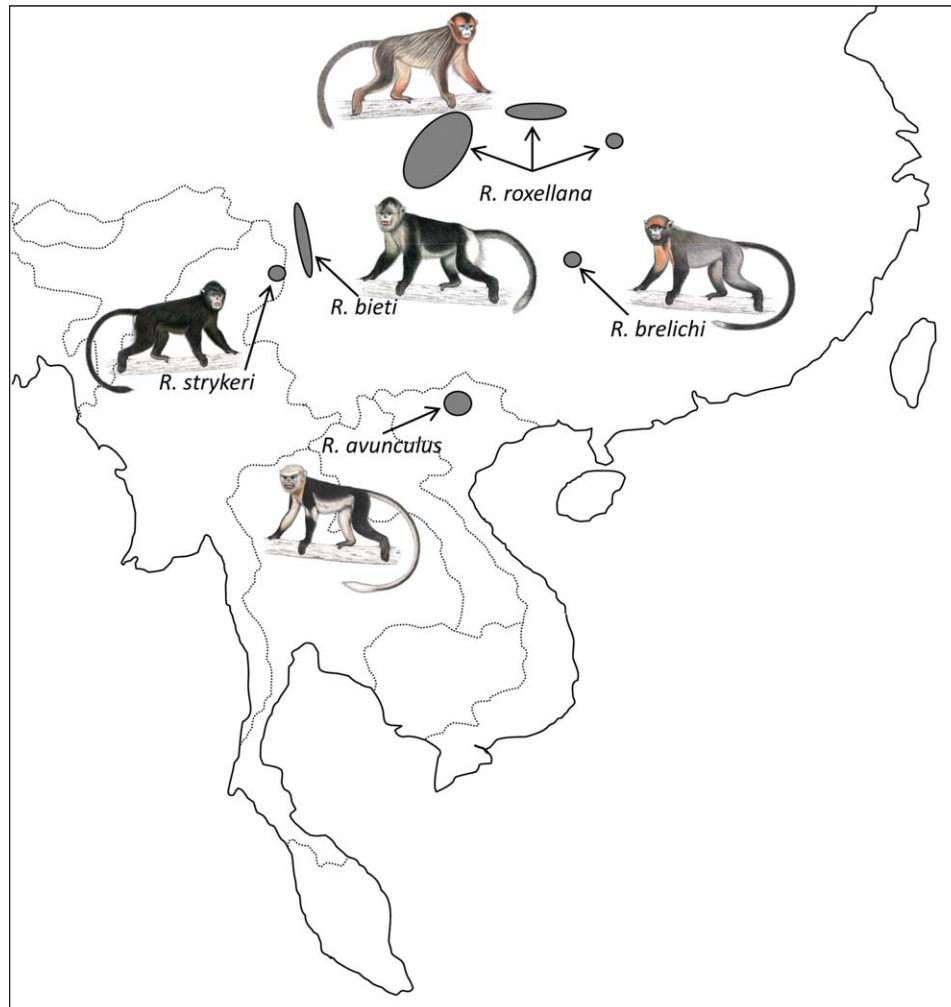


Fig. 1. Schematic distribution of the five snub-nosed monkey species. Dotted lines indicate country borders. Illustrations by Stephen Nash, Conservation International. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

ingly, the three Chinese species are classified as “Endangered” and *R. avunculus* as “Critically Endangered” (IUCN, 2010). *R. strykeri* is yet unclassified, but most likely deserves the status of a “Critically Endangered” species as well (Geissmann et al., 2011).

Of the Chinese snub-nosed monkey species, *R. brelichii* is the least studied and it exhibits the most restricted distribution. It occurs only in evergreen and deciduous broadleaf forests at altitudes of 1,400–2,300 m in the Fanjingshan National Nature Reserve (FNNR) in the Wuling Mountains, Guizhou province (Groves, 2001; Wu et al., 2004; MacKinnon, 2008; IUCN, 2010). Within the FNNR, the species inhabits a core area of about 260 km² (Bleisch and Xie, 1998; Yang et al., 2002). Thus, among the three Chinese species, *R. brelichii* is the only one, which comprises only one continuous population (Yang et al., 2002), while *R. bieti* consists of various geographically distinct subpopulations (Liu et al., 2007) and *R. roxellana* even of three subspecies (Li et al., 2007; IUCN, 2010). Furthermore, compared to its Chinese congeners, *R. brelichii* shows a longer interbirth interval (3 years compared to 2 years in *R. roxellana* and *R. bieti*; Ji et al., 1998; Zhang et al., 2000; Cui et al., 2006; Qi et al., 2008; Yang et al., 2009) and a later age at first reproduction in females (8–9 years compared to 5–6 years in *R.*

roxellana and *R. bieti*; Ji et al., 1998; Liang et al., 2001; Qi et al., 2008; Yang et al., 2009). Accordingly, among the three Chinese species, *R. brelichii* might hold the lowest population recovery potential.

Various studies on the phylogeny and phylogeography within the genus *Rhinopithecus* exist (Zhang and Ryder, 1998; Li et al., 2004; Roos et al., 2007; Yang et al., in press), but population genetic data are available only for *R. bieti* and *R. roxellana* (Li et al., 2001; Li et al., 2003; Li et al., 2007; Liu et al., 2007). As genetic diversity within a species is regarded, beside other factors such as population size, reproductive output, environmental changes, and human activities, as an important factor for the long-term survival of species, population genetic analyses contribute also to the assessment of a species’ conservation status (Dietz et al., 2000; Di Fiore, 2003; Spielman et al., 2004; Hoeglund, 2009).

In our study, we aimed to analyze and compare the genetic variation within and among the three Chinese snub-nosed species, their genetic population structure and their evolutionary history. Therefore, we sequenced the hypervariable region I (HVI) of the mitochondrial control region from ~20% of the total *R. brelichii* population and compared them to orthologous sequences of *R. roxellana* and *R. bieti* available from GenBank and the

literature (Li et al., 2007; Liu et al., 2007). As *R. brelichi* has the smallest population size and the most restricted distribution of the Chinese species, we expect a lower genetic diversity than reported from the other two species (hypothesis 1), and a population size reduction during its demographic history (hypothesis 2).

MATERIALS AND METHODS

Sample collection

In September/October 2007 and 2008, 146 fecal samples from *R. brelichi* individuals were collected in Yanggaping, an area of about 77 km² in the north of FNNR (N 127° 58'; E 108° 45'). In this region, roughly 600 individuals and thus the majority of the *R. brelichi* population come together twice a year (March–April, September–October). Accordingly, a division of the species into subpopulations is not indicated (Yang et al., 2002).

The animals were observed, and the samples were collected immediately after the monkeys left. To minimize the probability of collecting several samples from the same individuals, only samples with a distance of at least 2 m from each other were taken. Samples were preserved in plastic tubes with 70% ethanol and stored at room temperature before further processing.

Our study complied with protocols approved by the Forestry Ministry of Guizhou Province, China, and adhered to the legal requirements of the countries in which research was conducted. The study was carried out in compliance with respective animal care regulations and the principles of the American Society of Primatologists and the German Primate Center for the ethical treatment of nonhuman primates.

Individual identification

DNA from fecal samples was extracted using the QIAamp DNA Stool Mini Kit (Qiagen, Germany). From each sample, ~200 mg of feces was taken, pounded, and incubated overnight on a rotating wheel in argininosuccinate lyase (ASL) buffer. Extraction was further performed following the instructions of the manufacturer with the exception that, in the last step, the DNA was diluted in 100 μ l of high-performance liquid chromatography (HPLC) water instead of tris chloride elution buffer (AE) buffer. Extracted DNA was stored at -20°C before further processing. For each sample, two independent extractions were done.

To exclude the possibility that more than one sample per individual was included in the analysis, all samples were genotyped at eight polymorphic microsatellite loci (D1S533, D2S1326, D6S264, D6S501, D7S2204, D8S505, D10S1432, and D17S1290). Because of the low amount of DNA extracted from fecal samples, allelic dropout and null alleles tend to produce incorrect genotypes (Morin et al., 2001). Thus, we repeated genotyping multiple times depending on the DNA concentration of the sample following the approach of Morin et al. (2001). Accordingly, genotyping was repeated at least three times for heterozygous, and depending on the DNA amount three to seven times for homozygous samples.

No indications for null alleles, large allelic dropout or scoring errors due to stuttering were detected (MicroChecker 2.2.3; van Oosterhout et al., 2004). The probability of identity P_{ID} (Waits et al., 2001), i.e., the probability that two individuals drawn at random from a population have the same genotype at multiple loci, was

calculated with GenAlex 6.4 (Peakall and Smouse, 2006). In our estimation, P_{ID} was 6.9×10^{-6} over eight microsatellite loci. In wildlife forensic identification, $P_{ID} < 0.001$ – 0.0001 is required (Waits et al., 2001). On the basis of the direct observations and the calculation of the probability of identity, we were able to identify 141 individuals from 146 fecal samples.

Mitochondrial DNA amplification and sequencing

From the 141 individually assigned samples, we polymerase chain reaction (PCR)-amplified ~650 bp of the HVI using the primers 5'-AAATGAACCTGCCCTGTAGT-3' and 5'-GAGGATAGA ACCAGATGTCC-3'. PCR conditions consisted of a predenaturation step at 94°C for 5 min, followed by 40 cycles, each with a denaturation step at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min. At the end, a final extension step at 72°C for 5 min was added. The results of the PCR amplifications were checked on 1% agarose gels. Negative controls (reactions without DNA template) were used to check PCR performance and contamination. Subsequently, PCR products were cleaned with the Qia-gen PCR Purification Kit and sequenced on an ABI PRISM 3130xL Genetic Analyzer (Applied Biosystems, Germany) with the BigDye Cycle Sequencing Kit (Applied Biosystems, Germany). To further exclude contamination, we randomly repeated amplification and sequencing from the second DNA extract for 40 samples. Haplotype sequences were deposited to GenBank (HQ891105–HQ891109).

Statistical methods: Interspecies analysis

Sequences with a length of 603 bp were manually verified and aligned in BioEdit (Hall, 1999). For comparative purposes, orthologous sequences of *R. roxellana* (60 individuals) and *R. bieti* (157 individuals), published in earlier studies (Li et al., 2007; Liu et al., 2007), were taken from GenBank. Because of length differences, we trimmed the alignment to a length of 379 bp (positions 125–504 of the original *R. brelichi* alignment). Analysis of molecular variance (AMOVA) (Excoffier et al., 1992) was performed to examine the genetic differentiation and relationship among *R. brelichi*, *R. roxellana* and *R. bieti* populations with 10,000 permutations as implemented in Arlequin 3.5.1.2 (Excoffier and Lischer, 2010). Genetic relationships were estimated by comparing the average number of pairwise nucleotide differences between populations from two different species (π_{xy}), the average number of pairwise differences within each species (π_x), and the corrected average pairwise difference ($\pi_{xy} - (\pi_x + \pi_y)/2$). The genetic distance among species was determined by using the population's pairwise phiCT indices and P value (Wright, 1969, 1978). As a general rule, phiCT values above 0.25 indicate high genetic differentiation between populations (Nei 1977; Wright, 1978; de Jong et al., 1994; Hartl and Clark, 2007).

A median-joining network (Bandelt et al., 1999) was created with Network 4.6 to visualize the topology of the population structure based on frequencies and phylogenetic relationships among HVI haplotypes both at the intraspecific and interspecific level.

Phylogenetic relationships among haplotypes were constructed with maximum-likelihood (ML) and Bayesian algorithms, using the programs Garli 0.951 (Zwickl, 2006) and MrBayes 3.1.2 (Huelsenbeck et al., 2001; Ron-

quist and Huelsenbeck, 2003). Therefore, the orthologous sequence from *R. avunculus* (EU004480, Osterholz et al., 2008) was taken from GenBank and used as an outgroup. For both reconstructions, the HKY + G model was used as it was chosen as best-fit model by the Bayesian Information Criterion in jModeltest 0.1 (Posada, 2008). In Garli, only the model specification settings were adjusted according to the dataset, while all other settings were left at their default values. Relative support of internal nodes was assessed by bootstrap analyses with 500 replications. A majority-rule consensus tree was calculated in PAUP* 4.0b10 (Swofford, 2003). For Bayesian reconstructions, four Markov Chain Monte Carlo (MCMC) runs with the default temperature of 0.1 were used. Four repetitions were run for 10,000,000 generations with tree and parameter sampling occurring every 100 generations. The first 25% of samples were discarded as burnin, leaving 75,001 trees per run. Posterior probabilities for each split and a phylogram with mean branch lengths were calculated from the posterior density of trees.

A Bayesian MCMC method, which uses a relaxed molecular clock approach (Drummond et al., 2006), as implemented in BEAST 1.4.8 (Drummond and Rambaut, 2007), was used to estimate divergence times. Therefore, a relaxed lognormal model of lineage variation and a Birth–Death prior for branching rates was assumed. As no appropriate fossils are available as calibration points, we used a mean HVI substitution rate of 0.1643 substitutions per nucleotide per million years (Myr) (Soares et al., 2009). The 95% confidence interval for the normal distribution of substitution rates ranged from 0.06 to 0.25 substitutions per site per Myr (Santos et al., 2005). We analyzed four replicates for 25,000,000 generations with tree and parameter sampling occurring every 100 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 Tracer 1.4.1 (Rambaut and Drummond, 2007). Subsequently, the sampling distributions were combined (25% burnin) using the software LogCombiner 1.4.8 and a consensus chronogram with node height distribution was generated and visualized with TreeAnnotator 1.4.8 and FigTree 1.2.2 (Rambaut, 2008).

Statistical methods: Intraspecies analysis

Genetic diversity within species was estimated by using haplotype diversity (h), nucleotide diversity (π) (Nei and Li, 1979; Nei, 1987), and the average number of pairwise nucleotide differences (Π) (Wakeley, 1997) in Arlequin 3.5 (Excoffier and Lischer, 2010). To support hypothesis 1 of low genetic diversity in *R. brelichi*, all these values should be lower in this species than in the two congeners.

The haplotype network furthermore allows us to investigate whether any genetic population structure exists in *R. brelichi*. Within *R. roxellana* and *R. bieti* geographic substructures have been identified and have been shown to correspond partly to genetic substructures (Li et al., 2007; Liu et al., 2007). In *R. brelichi*, however, no geographically identifiable subpopulations exist (Yang et al., 2002). Therefore, we expect no comparable substructures in the current *R. brelichi* population, which would be reflected by distinct clusters in the median-joining network. The mutational distance between different haplotypes in the network is therefore expected to be clearly

smaller within *R. brelichi* than within the other two species. Population structure would be additionally supported by lower Π values. Note that Π equals the within-population parameter of AMOVA (π_x) and thus is a direct measure of the genetic variation within a population.

To explore the demographic history of the populations, we calculated Tajima's D statistic (Tajima, 1989) and Fu's F_S test (Fu, 1997) with 10,000 permutations as implemented in Arlequin 3.5. These statistics are applied to infer the population growth history under the null hypothesis of selective neutrality and population equilibrium. A significant Tajima's D value or Fu's F_S value indicates departure from the null hypothesis. In addition, we use the mismatch distribution analysis in Arlequin and DnaSP 5.10.01 (Librado and Rozas, 2009) using a demographic expansion model as a baseline. The sum of squared differences (SSD; Schneider and Excoffier, 1999) and the raggedness index (r) (Harpending, 1994) are indicators for how far the observed distribution deviates from the expected smooth and unimodal distribution of the model.

To support our second hypothesis, a reduction of population size in *R. brelichi*, we should find (1) significantly positive Tajima's D and Fu's F_S values, indicating a wider spectrum of allele frequencies in the population, and (2) an unimodal shape of the mismatch distribution with low parameter values SSD and r , indicating no (significant) deviation from the underlying model of expansion (or decline).

In contrast, both, nonsignificant D and F_S values implying no deviation from the neutral model, and a multimodal (or "ragged") shape of the mismatch distribution (Rogers and Harpending, 1992) with significant and high-index values, would indicate for populations that remained largely stable in size during their history.

It should be noted here that none of these statistics for investigating the demographic history of a population is very reliable by itself and may depend on additional factors. For instance, a positive Tajima's D may also be the result of other processes that promote maintaining many alleles not only in a population, in particular balancing selection, but also in population subdivision and migration (Schmidt and Pool, 2002). Only the combination of different indicators, if ever, can make reliable predictions about the demographic history.

RESULTS

We successfully PCR-amplified and sequenced the HVI from 141 *R. brelichi* samples, which were confirmed to originate from different individuals by microsatellite analysis. All amplifications produced a single band with a size of ~650 bp. Randomly repeated amplification and sequencing for 40 samples revealed identical sequences. Of the 603 bp, 26 sites were variable, including one transversion (tv) and 25 transitions (ts). Among the 141 individuals, we observed five haplotypes (see Table 1). To compare the population genetic parameters among *R. brelichi*, *R. roxellana* and *R. bieti*, we trimmed the alignment to a length of 379 bp. By doing so, only one variable site in the *R. brelichi* sequence (position 33 of the original 603 bp-alignment) was excluded.

Differentiation among the three Chinese species of *Rhinopithecus*

AMOVA results reveal a significant genetic differentiation among the three species (Table 2). The average

TABLE 1. Position of variable sites, and number (N) and frequency (f) of the five HVI haplotypes found in 141 *R. brelichi* individuals

Haplotype	Position of variable sites (bps)																										<i>N</i> (<i>f</i>)
	33	166	265	280	304	306	309	314	315	316	318	321	355	361	363	376	381	382	383	389	398	416	447	464	470	488	
ΞZ01	T	G	A	T	C	T	T	T	C	C	A	A	A	G	A	C	T	A	A	G	T	A	C	C	C	C	12 (0.085)
ΞZ02	G	2 (0.014)
ΞZ03	.	A	.	T	T	.	.	C	A	T	.	G	C	G	.	.	.	G	T	.	.	T	11 (0.078)
ΞZ04	.	A	G	C	A	.	.	.	A	.	.	.	G	A	G	.	C	.	G	A	.	G	14 (0.099)
ΞZ05	C	A	.	.	T	.	C	.	A	.	G	T	C	.	.	A	C	102 (0.723)

TABLE 2. Genetic differentiation analysis (AMOVA) between *R. brelichi*, *R. roxellana*, and *R. bieti*^a

	<i>R. brelichi</i> and <i>R. roxellana</i>	<i>R. brelichi</i> and <i>R. bieti</i>	<i>R. roxellana</i> and <i>R. bieti</i>
π_{xy}	40.8559	51.006	51.8529
$\pi_{xy} - (\pi_x + \pi_y)/2$	31.4625	41.4895	39.208
phiCT (P value)	0.803 (<0.001)	0.810 (<0.001)	0.741 (<0.001)

^a π_{xy} , average number of pairwise nucleotide differences between populations; $\pi_{xy} - (\pi_x + \pi_y)/2$, corrected average pairwise difference; π_x , within-population values ($\pi_x = \Pi$ in Table 3); phiCT, pairwise measure of genetic differentiation between populations.

number of pairwise nucleotide differences between populations (π_{xy}) results in the following estimates: *R. brelichi* and *R. roxellana* $\pi_{xy} = 40.9$; *R. brelichi* and *R. bieti* $\pi_{xy} = 51.0$; *R. roxellana* and *R. bieti* $\pi_{xy} = 51.9$. The corrected average pairwise difference ($\pi_{xy} - (\pi_x + \pi_y) / 2$) amounts to 31.5 for *R. brelichi* and *R. roxellana*, 41.5 for *R. brelichi* and *R. bieti*, and 39.2 for *R. roxellana* and *R. bieti*. Values for *R. brelichi* and *R. bieti*, and for *R. roxellana* and *R. bieti* are similar, but higher than the value for *R. brelichi* and *R. roxellana*. According to the population-specific phiCT, a large proportion of the genetic variation is triggered by disparities between the species (*R. brelichi* and *R. roxellana*: phiCT = 0.803, *R. brelichi* and *R. bieti*: phiCT = 0.810, *R. roxellana* and *R. bieti*: phiCT = 0.741; Table 2). All phiCT values are statistically significant ($P < 0.001$) indicating that all three species are distinct from each other. The lower phiCT value between *R. roxellana* and *R. bieti* is most likely triggered by their higher intraspecific diversity.

The topology of the median-joining network (Fig. 2) confirms a clear genetic differentiation between the three species by showing significantly larger haplotype distances between than within each species. The network corroborates that despite a similar number of individual samples, the diversity of haplotypes is much lower within *R. brelichi* than within *R. bieti* and *R. roxellana*. In *R. bieti* and *R. roxellana*, a division into the two described major clades, A and B, (Li et al., 2007; Liu et al., 2007) can be discerned. In contrast, there are no apparent substructures in *R. brelichi*.

The phylogenetic tree reconstructions based on ML and Bayesian algorithms resulted in almost identical tree topologies, but the relationships among the haplotypes within major clades differed slightly (Fig. 3). Accordingly, the three Chinese species form strongly supported monophyletic clades and, although only weakly supported, *R. roxellana* and *R. brelichi* are suggested as sister taxa. Based on divergence age estimations, *R. avunculus* separated from the Chinese species 2.34 (1.66–3.05) million years ago (Ma), and *R. bieti* from *R. roxellana* and *R. brelichi* 2.19 (1.62–2.80) Ma, before finally latter two diverged 1.77 (1.17–2.36) Ma. The most recent common ancestors (MRCA) of *R. bieti* and *R. roxellana* lived 1.33 (0.82–1.90) and 0.91 (0.53–1.31) Ma, while the MRCA of *R. brelichi* has a similar age (0.36 [0.17–0.56] Ma) as clades A and B of *R. bieti* (A: 0.24 [0.11–0.37] Ma, B: 0.10 [0.03–0.19] Ma) and *R. roxellana* (A: 0.46 [0.21–0.66] Ma, B: 0.36 [0.17–0.56] Ma).

Intraspecific variation

Haplotype diversity (h) and nucleotide diversity (π) for the three Chinese snub-nosed monkey species are sum-

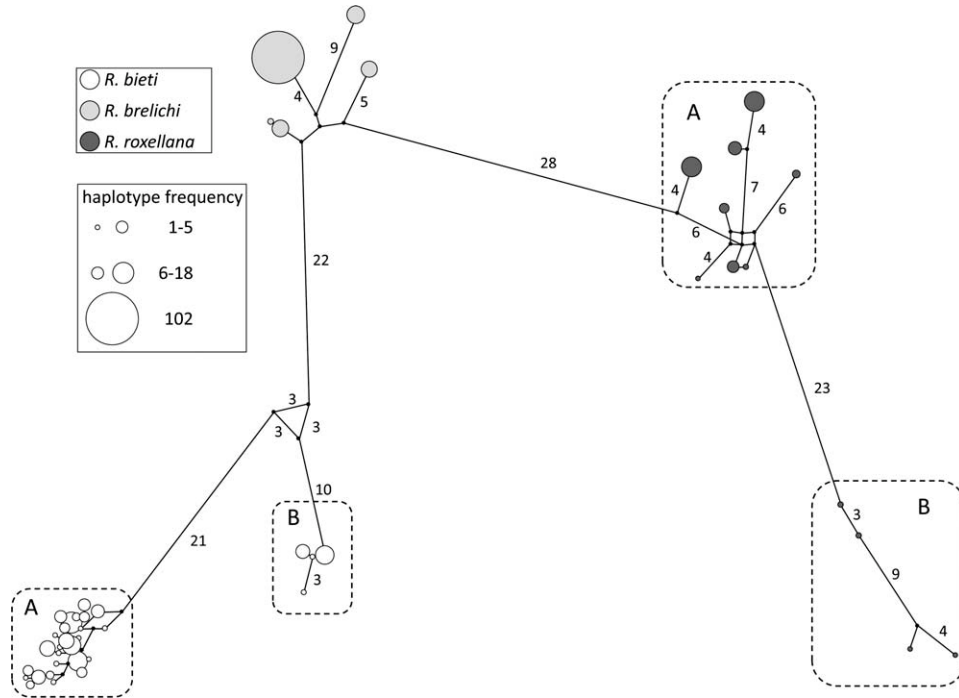


Fig. 2. Median-joining network of HVI haplotypes of the three Chinese *Rhinopithecus* species. Haplotype frequencies are reflected by node sizes. Links are proportional to number of mutational steps (also indicated by numbers for links with more than two mutational steps). **A** and **B** refer to respective subclades of *R. roxellana* and *R. bieti*.

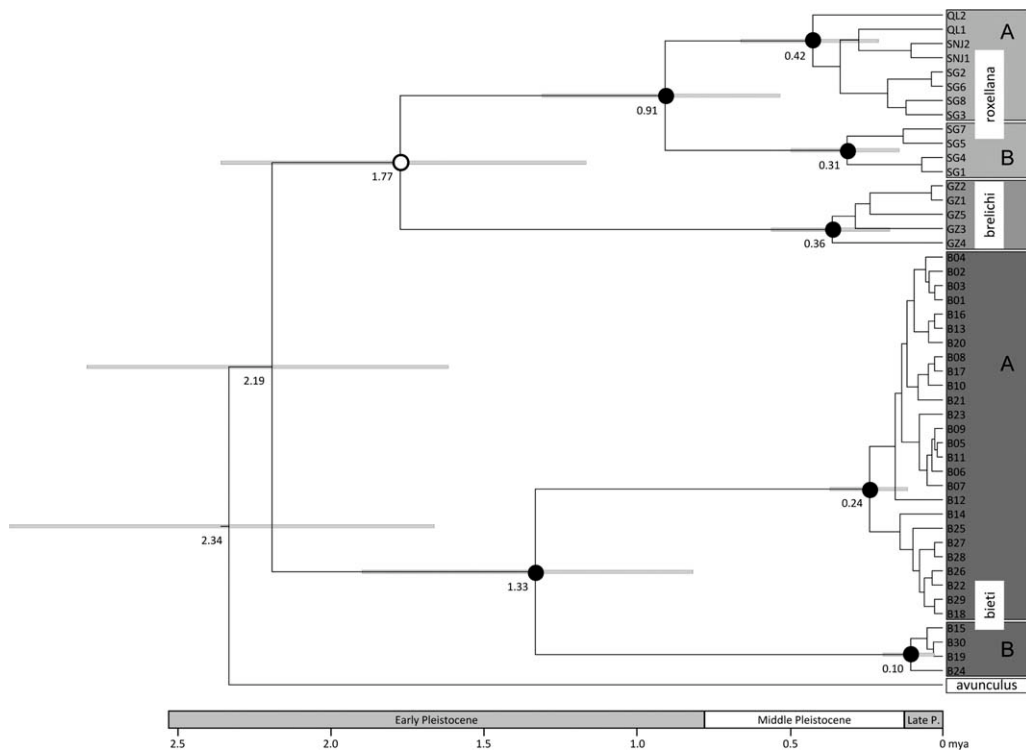


Fig. 3. Ultrametric tree showing phylogenetic relationships and estimated divergence ages among studied *Rhinopithecus* haplotypes. **A** and **B** refer to respective subclades in *R. roxellana* and *R. bieti*. Numbers on nodes indicate mean divergence ages and bars respective 95% highest posterior densities. Support values for ML and Bayesian reconstructions are indicated as circles (black circles: $\geq 97\%$, 1.0; open circle: $< 50\%$, 0.92).

TABLE 3. Population genetic variables for *R. brelichi*, *R. roxellana*, and *R. bieti*

	<i>R. brelichi</i>	<i>R. roxellana</i>	<i>R. bieti</i>
No. of individuals	141	60	157
No. of nucleotide sites	379	379	379
No. of polymorphic sites (<i>S</i>)	25	54	51
No. of haplotypes	5	12	30
Haplotype diversity (<i>h</i>)	0.457 ± 0.048	0.845 ± 0.026	0.945 ± 0.006
Mean no. of pairwise differences (Π) ^a	5.259 ± 2.557	13.029 ± 5.949	13.773 ± 6.216
Theta _s (θ_s) ^a	4.527 ± 1.350	11.580 ± 3.413	9.048 ± 2.354
Nucleotide diversity (π)	0.014 ± 0.007	0.034 ± 0.017	0.036 ± 0.018

^a Compared for Tajima's D test.

TABLE 4. Neutrality tests and mismatch distribution analyses for *R. brelichi*, *R. roxellana*, and *R. bieti*^a

	<i>R. brelichi</i>	<i>R. roxellana</i>	<i>R. bieti</i>
No. of individuals	141	60	157
Tajima's D (<i>P</i> value)	0.464 (0.739)	0.424 (0.742)	1.589 (0.955)
Fu's <i>F_s</i> (<i>P</i> value)	12.597 (0.988)	8.452 (0.976)	2.095 (0.770)
SSD (<i>P</i> value)	0.120 (0.080)	0.061 (0.030)	0.018 (0.100)
<i>r</i> (<i>P</i> value)	0.347 (0.447)	0.074 (0.008)	0.012 (0.590)
θ_0	<0.001	<0.001	2.589
θ_1	0.554	17.783	16.826
τ	3.250	35.113	4.207

^a SSD, sum of squared deviations; *r*, raggedness index; θ_0 , population size before expansion; θ_1 , population size after expansion; τ , age of expansion in units of mutational time.

marized in Table 3. The haplotype diversity for *R. brelichi* ($h = 0.457$) is roughly half of that estimated for *R. roxellana* ($h = 0.845$) and *R. bieti* ($h = 0.945$). For all three species, nucleotide diversities (*R. brelichi*: $\pi = 0.014$, *R. roxellana*: $\pi = 0.034$, *R. bieti*: $\pi = 0.036$) are relatively low, especially for *R. brelichi*. The average number of pairwise nucleotide differences (Π) between individuals amounts to 5.3 in *R. brelichi*, 13.0 in *R. roxellana* and 13.8 in *R. bieti*. The much lower Π in *R. brelichi* and the topology of the haplotype network support that there is no genetic population structure in this species.

Demographic history

The nonsignificant values of the neutrality tests, including both Tajima's D and Fu's *F_s*, indicate no departure from the null hypothesis of demographic stability within the 95% confidence interval (Table 4). This result is further supported by the multimodal patterns of the mismatch distribution of all three species pointing to a stable population size (Fig. 4). The distribution for *R. brelichi* with a very high initial peak (probability of identical haplotypes at around 0.55) may provide weak evidence for a population reduction (Rogers and Harpending, 1992). However, it should be noted that the mismatch distribution is highly influenced by population structure. The multimodal pattern in *R. roxellana* and *R. bieti*, with a prominent second peak for higher numbers of pairwise differences, is most likely caused by their respective population subdivision (discussed in detail in Li et al., 2007; Liu et al., 2007).

The statistical properties of the mismatch distribution under the population expansion model are compared in Table 4. The SSD values of all three species are found to be significant at least at the 90% confidence level. Only the SSD value for *R. brelichi* may indicate a departure from the estimated demographic model of a sudden pop-

ulation expansion, in contrast to the low SSD values for the other two species.

Similarly, the raggedness indices for *R. roxellana* and *R. bieti* are much lower than for *R. brelichi* and thus suggest a relatively good fit of the data to the model of population expansion (Harpending, 1994). However, it has to be noted here that only the raggedness index for *R. roxellana* is significant ($P = 0.008$).

DISCUSSION

The taxonomic classification and especially the phylogenetic relationships among *Rhinopithecus* taxa have been debated (Rowe, 1996; Jablonski, 1998a; Pan and Oxnard, 2001). Early studies suggested that the three Chinese species were subspecies of one species (*R. roxellana*; Ellerman and Morrison-Scott, 1951; Napier and Napier, 1967; Quan and Xie, 1981; Zhang et al., 1992). More recently, however, *R. bieti* and *R. brelichi* were separated from *R. roxellana* on species level (Peng et al., 1988; Jablonski and Peng, 1993; Zhang et al., 1997; Zhang and Ryder, 1998; Groves, 2001; Li et al., 2001; Pan and Oxnard, 2001). The results of our study clearly support the "three Chinese snub-nosed monkey species" hypothesis.

The phylogenetic relationships among *R. roxellana*, *R. bieti*, *R. brelichi*, and the Vietnamese *R. avunculus* are not well defined. Since *R. strykeri* was just recently described, its phylogenetic position within the genus is not explored in detail yet. For the four other species, Jablonski and Peng (1993) suggested a basal position of *R. avunculus* and a sister grouping of *R. roxellana* to a clade consisting of *R. bieti* and *R. brelichi*. Genetic studies based on relatively short mitochondrial DNA fragments allowed no significant resolution among these four species (Zhang and Ryder, 1998; Li et al., 2004; Roos et al., 2007), while recent work using complete mitochondrial genomes revealed a strongly supported branching pattern among them (Yang et al., in press). Accordingly, *R. avunculus* represents indeed the first lineage, but among the Chinese species, *R. bieti* and not *R. roxellana* is basal. Our results support the phylogenetic relationships as suggested by Yang et al. (in press), because the genetic distance between *R. brelichi* and *R. roxellana* is smaller than their distance to *R. bieti*, and also our phylogenetic reconstruction indicates *R. brelichi* and *R. roxellana* as sister taxa. Also in agreement with Yang et al. (in press), divergence age estimates suggest *Rhinopithecus* differentiation into species in the early Pleistocene. Although our estimates for the MRCAs of *R. bieti* and *R. roxellana* are slightly older, they are in a similar range as those obtained in earlier studies (Li et al., 2007; Liu et al., 2007). According to our estimate, the MRCA of the

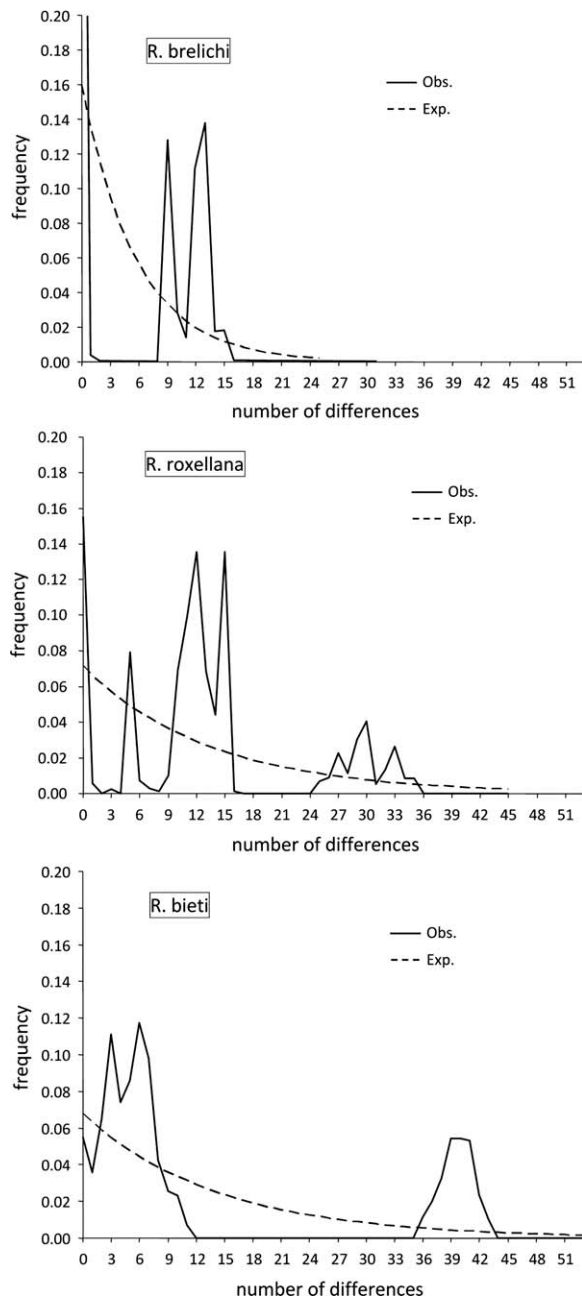


Fig. 4. Mismatch distribution of *R. brelichi*, *R. roxellana*, and *R. bieti* HVI sequences. Frequency of pairs of individuals (in percent) plotted over the number of nucleotide site differences. (*R. roxellana*: initial $\theta < 0.001$; final $\theta = 17.783$; $\tau = 35.113$; *R. bieti*: initial $\theta = 2.589$; final $\theta = 16.826$; $\tau = 4.207$; *R. brelichi*: initial $\theta < 0.001$; final $\theta = 0.554$; $\tau = 3.251$).

R. brelichi mitochondrial haplotypes occurred more recently than that of the other two species.

As shown in Table 3, the values for genetic diversity (e.g., polymorphic sites, number of haplotypes, haplotype diversity (h), mean number of pairwise differences, nucleotide diversity) for *R. brelichi* are roughly half of that for *R. roxellana* and *R. bieti*. Compared to other primate species, h and π for *R. roxellana* and *R. bieti* are similar to, for example, Barbary macaques (*Macaca sylvanus*, $h = 0.872$, $\pi = 0.026$, Modolo et al., 2005) and Arabian

hamadryas baboons (*Papio hamadryas*, $h = 0.856$, and $\pi = 0.023$, Winney et al., 2004), both with a restricted range and relatively small population. Even more extreme, h and π for *R. brelichi* are similar to a species which lives on a small island (Japanese macaque, *Macaca fuscata yakui*, $h = 0.305$, $\pi = 0.015$, Hayaishi and Kawamoto, 2006). Moreover, 102 of the 141 analyzed *R. brelichi* individuals share the same haplotype and the five haplotypes in total differ in 26 polymorphic sites only. All of these suggest that *R. brelichi* today has the lowest genetic variation among the three Chinese snub-nosed monkey species.

Both Tajima's D and Fu's F_S tests do not indicate population expansions or contractions in any of the three Chinese species. Furthermore, mismatch distribution analysis clearly reveals equilibrium in *R. brelichi*. For the other two species, the picture is more complicated, possibly due to their population structure. These results are consistent with earlier studies on the demographic history of *R. roxellana* and *R. bieti*, saying that although different subpopulations probably underwent different demographic changes, the total population remained stable (Li et al., 2007; Liu et al., 2007). In *R. brelichi*, there seems to be neither geographic nor genetic substructuring that could have influenced the analyses of demographic history. Overall, our data suggest population equilibrium also in *R. brelichi*.

Fossils suggest that snub-nosed monkeys were once widespread and experienced range reductions in the late Pleistocene or Holocene (Jablonski, 1998a,b; Quan and Xie, 2002). In the Qing Dynasty (1616–1911), however, snub-nosed monkeys still occurred in 11 provinces of China (Li et al., 2002). An additional reduction in population density and distribution occurred, particularly in *R. brelichi*, during the last 400 years (Li et al., 2002). During this period, the human population increased dramatically in southern China (Li et al., 2002) and with it, deforestation and hunting, which further reduced suitable monkey habitat and population sizes (Li et al., 2002). Both, the reduction of habitat and population size, might have led to the observed low genetic diversity of *R. brelichi*. However, the population size reduction is in contrast to our genetic data. One reason for this incongruence might be that the currently surviving population was isolated from others, now extinct *R. brelichi* populations, for a certain time period and might have kept its originally low genetic variability.

Researchers found extensive gene flow between the different subpopulations of *R. roxellana* and *R. bieti*, respectively (Liu et al., 2009; Pan et al., 2009), which might enhance survival under environmental changes or climate oscillations. In contrast, for *R. brelichi* genetic exchange with other subpopulations is impossible. Given the slow reproduction rate, the small total population size, and the low genetic diversity, *R. brelichi* is probably the most vulnerable snub-nosed monkey species in China. Therefore, it is of urgent management interest to collect more data on the genetics, ecology, particularly ranging of the species and about the mating system to assist in the protection of this enigmatic primate species.

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LITERATURE CITED

- Bandelt HJ, Forster P, Röhl A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Mol Biol Evol* 16:37–48.
- Bleisch W, Xie JH. 1998. Ecology and behavior of the Guizhou snub-nosed langur (*Rhinopithecus [Rhinopithecus] brelichi*), with a discussion of socioecology in the genus. In: Jablonski NG, editor. The natural history of the doucs and snub-nosed monkeys. Singapore: World Scientific Publishing. p 217–239.
- Cui LW, Sheng AH, He XC, Xiao W. 2006. Birth seasonality and interbirth interval of captive *Rhinopithecus bieti*. *Am J Primatol* 68:457–463.
- de Jong G, de Ruiter JR, Haring R. 1994. Genetic structure of a population with social structure and migration. In: Loeschcke V, Tomiuk J, Jain SK, editors. Conservation genetics. Basel: Birkhäuser Verlag. p 147–164.
- Dietz JM, Baker AJ, Ballou JD. 2000. Demographic evidence of inbreeding depression in wild golden lion tamarins. In: Yang A, Clark GM, editors. Genetics, demography, and viability of fragmented populations. Cambridge: Cambridge University Press. p 203–211.
- Di Fiore A. 2003. Molecular genetic approaches to the study of primate behavior, social organization, and reproduction. *Am J Phys Anthropol* 122:62–99.
- Drummond AJ, Ho SY, Phillips MJ, Rambaut A. 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biol* 4:e88.
- Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol Biol* 7:214.
- Ellerman JR, Morrison-Scott TCS. 1951. Checklist of Palaearctic and Indian mammals. London: Trustees of the British Museum (Natural History). p 22–89.
- Excoffier L, Lischer HL. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Res* 10:564–567.
- Excoffier L, Smouse PE, Quattro JM. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131:479–491.
- Fu YX. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* 147:915–925.
- Geissmann T, Lwin N, Aung SS, Aung TN, Aung MZ, Hla TH, Grindler M, Momberg F. 2011. A new species of snub-nosed monkey, genus *Rhinopithecus* Milne-Edwards, 1872 (Primates, Colobinae), from northern Kachin State, northeastern Myanmar. *Am J Primatol* 73:96–107.
- Groves CP. 2001. Primate taxonomy. Washington, DC: Smithsonian Institution Press.
- Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp* 41:95–98.
- Harpending HC. 1994. Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. *Hum Biol* 66:591–600.
- Hartl DL, Clark AG. 2007. Principles of population genetics. Sunderland, MA: Sinauer.
- Hayaishi S, Kawamoto Y. 2006. Low genetic diversity and biased distribution of mitochondrial DNA haplotypes in the Japanese macaque (*Macaca fuscata yakui*) on Yakushima Island. *Primates* 47:158–164.
- Hoeglund J. 2009. Evolutionary conservation genetics. Oxford: Oxford University Press.
- Huelsenbeck JP, Ronquist F, Nielsen R, Bollback JP. 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. *Science* 294:2310–2314.
- IUCN. 2010. The IUCN red list of threatened species. Available at: <http://www.iucnredlist.org>.
- Jablonski NG. 1998a. The response of catarrhine primates to Pleistocene environmental fluctuations in East Asia. *Primates* 39:29–37.
- Jablonski NG. 1998b. The evolution of the doucs and snub-nosed monkeys and the question of the phyletic unity of the odd-nosed colobines. In: Jablonski NG, editor. The natural history of the doucs and snub-nosed monkeys. Singapore: World Scientific Publishing. p 13–52.
- Jablonski NG, Peng YZ. 1993. The phylogenetic relationships and classification of the doucs and snub-nosed langurs of China and Vietnam. *Folia Primatol* 60:36–55.
- Ji WZ, Zou RJ, Shang EY, Zhou HW, Yang SC, Tian BP. 1998. Maintenance and breeding of Yunnan snub-nosed monkeys (*Rhinopithecus [Rhinopithecus] bieti*) in captivity. In: Jablonski NG, editor. The natural history of the doucs and snub-nosed monkeys. Singapore: World Scientific Publishing. p 323–335.
- Li BG, Pan RL, Oxnard CE. 2002. Extinction of snub-nosed monkeys in China during the past 400 years. *Int J Primatol* 23:1227–1244.
- Li HP, Meng SJ, Men ZM, Fu YX, Zhang YP. 2003. Genetic diversity and population history of golden monkeys (*Rhinopithecus roxellana*). *Genetics* 164:269–275.
- Li M, Liang B, Feng ZJ, Tamate HB. 2001. Molecular phylogenetic relationships among Sichuan snub-nosed monkeys (*Rhinopithecus roxellana*) inferred from mitochondrial cytochrome b gene sequences. *Primates* 42:153–160.
- Li M, Liu ZJ, Gou JX, Gou JX, Ren BP, Pan RL, Su YJ, Funk SM, Wei FW. 2007. Phylogeography and population structure of the golden monkeys (*Rhinopithecus roxellana*): inferred from mitochondrial DNA sequences. *Am J Primatol* 69:1195–1209.
- Li M, Wei FW, Huang CM, Pan RL, de Ruiter J. 2004. Phylogeny of snub-nosed monkeys inferred from mitochondrial DNA, cytochrome b, and 12S rRNA sequences. *Int J Primatol* 25:861–873.
- Liang B, Qi HJ, Zhang SY, Ren BP. 2001. Developmental traits of captive Sichuan snub-nosed monkeys (*Rhinopithecus roxellana*) at different age stages. *Acta Zool Sinica* 47:381–387.
- Librado P, Rozas J. 2009. DnaSP version 5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25:1451–1452.
- Liu ZJ, Ren BP, Wei FW, Long YC, Hao YL, Li M. 2007. Phylogeography and population structure of the Yunnan snub-nosed monkey (*Rhinopithecus bieti*) inferred from mitochondrial control region DNA sequence analysis. *Mol Ecol* 16:3334–3349.
- Liu ZJ, Ren BP, Wu RD, Zhao L, Hao YL, Wang BS, Wei FW, Long YC, Li M. 2009. The effect of landscape features on population genetic structure in Yunnan snub-nosed monkeys (*Rhinopithecus bieti*) implies an anthropogenic genetic discontinuity. *Mol Ecol* 18:3831–3846.
- MacKinnon J. 2008. Order primates. In: Smith AT, Xie Y, editors. A guide to the mammals of China. Princeton: Princeton University Press. p 158–172.
- Mittermeier RA, Wallis J, Rylands AB, Ganzhorn JU, Oates JF, Williamson EA, Palacios E, Heymann EW, Kierulff MCM, Long Y, Supriatna J, Roos C, Walker S, Cortés-Ortiz L, Schwitzer C. 2009. Primates in peril: the world's 25 most endangered primates 2008–2010. *Primate Conserv* 24:1–57.
- Modolo L, Salzburger W, Martin RD. 2005. Phylogeography of Barbary macaques (*Macaca sylvanus*) and the origin of the Gibraltar colony. *Proc Natl Acad Sci USA* 102:7392–7397.
- Morin PA, Chambers KE, Boesch C, Vigilant L. 2001. Quantitative polymerase chain reaction analysis of DNA from non-invasive samples for accurate microsatellite genotyping of wild chimpanzees (*Pan troglodytes verus*). *Mol Ecol* 10:1835–1844.
- Napier JR, Napier PH. 1967. A handbook of living primates. London: Academic Press.
- Nei M. 1977. F-statistics and analysis of gene diversity in subdivided populations. *Ann Hum Genet* 41:225–233.

- Nei M. 1987. Molecular evolutionary genetics. New York: Columbia University Press.
- Nei M, Li WH. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc Natl Acad Sci USA* 76:5269–5273.
- Osterholz M, Walter L, Roos C. 2008. Phylogenetic position of the langur genera *Semnopithecus* and *Trachypithecus* among Asian colobines, and genus affiliations of their species groups. *BMC Evol Biol* 8:58.
- Pan D, Hu HX, Meng SJ, Men ZM, Fu YX, Zhang YP. 2009. A high polymorphism level in *Rhinopithecus roxellana*. *Int J Primatol* 30:337–351.
- Pan RL, Oxnard CE. 2001. Cranial morphology of the golden monkey (*Rhinopithecus*) and douc langur (*Pygathrix nemaeus*). *Hum Evol* 16:199–223.
- Peakall R, Smouse PE. 2006. GENALEX 6: genetic analysis in Excel: population genetic software for teaching and research. *Mol Ecol Notes* 6:1288–1295.
- Peng YZ, Ye ZZ, Zhang YP, Pan RL. 1988. The classification and phylogeny of snub-nosed monkey *Rhinopithecus* spp. based on gross morphological characters. *Zool Res* 9:239–248.
- Posada D. 2008. jModelTest: phylogenetic model averaging. *Mol Biol Evol* 25:1253–1256.
- Qi XG, Li BG, Ji WH. 2008. Reproductive parameters of wild female *Rhinopithecus roxellana*. *Am J Primatol* 70:311–319.
- Quan GQ, Xie JH. 1981. Notes on *Rhinopithecus roxellanae brelichi* (Thomas). *Acta Theriol Sinica* 1:113–116.
- Quan GQ, Xie JY. 2002. Research on the golden monkey. Shanghai: Science and Education Publishing House.
- Rambaut A. 2008. FigTree: Tree figure drawing tool, version 1.2.2. Available at: <http://tree.bio.ed.ac.uk/software/figtree/>.
- Rambaut A, Drummond AJ. 2007. Tracer: MCMC trace analysis tool, version 1.4.1. Available at: <http://tree.bio.ed.ac.uk/software/tracer/>.
- Rogers AR, Harpending H. 1992. Population growth makes waves in the distribution of pairwise genetic differences. *Mol Biol Evol* 9:552–569.
- Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–1574.
- Roos C, Thanh VN, Walter L, Nadler T. 2007. Molecular systematics of Indochinese primates. *Vietn J Primatol* 1:41–53.
- Rowe N. 1996. The pictorial guide to the living primates. East Hampton: Pogonias Press.
- Santos C, Montiel R, Sierra B, Bettencourt C, Fernandez E, Alvarez L, Lima M, Abade A, Aluja MP. 2005. Understanding differences between phylogenetic and pedigree-derived mtDNA mutation rate: a model using families from the Azores islands (Portugal). *Mol Biol Evol* 22:1490–1505.
- Schmidt D, Pool J. 2002. The effect of population history on the distribution of the Tajima's D statistic. New York: Cornell University Press.
- Schneider S, Excoffier L. 1999. Estimation of demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. *Genetics* 152:1079–1089.
- Soares P, Ermini L, Thomson N, Mormina M, Rito T, Röhl A, Salas A, Oppenheimer S, Macaulay V, Richards MB. 2009. Correcting for purifying selection: an improved human mitochondrial molecular clock. *Am J Hum Genet* 84:740–759.
- Spielman D, Brook BW, Frankham R. 2004. Most species are not driven to extinction before genetic factors impact them. *Proc Natl Acad Sci USA* 101:15261–15264.
- Swofford, DL. 2003. PAUP*: phylogenetic analysis using parsimony (* and other methods), version 4.0b10. Sunderland, MA: Sinauer.
- Tajima F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123:585–595.
- van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. 2004. Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. *Mol Ecol Notes* 4:535–538.
- Wakeley J. 1997. Using the variance of pairwise differences to estimate the recombination rate. *Genet Res* 69:45–48.
- Waits LP, Luikart G, Taberlet P. 2001. Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. *Mol Ecol* 10:249–256.
- Winney BJ, Hammond RL, Macasero W, Flores B, Boug A, Biquand V, Biquand S, Bruford MW. 2004. Crossing the Red Sea: phylogeography of the hamadryas baboon, *Papio hamadryas hamadryas*. *Mol Ecol* 13:2819–2827.
- Wright S. 1969. Evolution and the genetics of populations. II: The theory of gene frequencies. Chicago: University of Chicago Press.
- Wright S. 1978. Evolution and the genetics of populations. IV: the variability within and among natural populations. Chicago: University of Chicago Press.
- Wu G, Wang HC, Fu HW, Zhao JZ, Yang YQ. 2004. Habitat selection of Guizhou golden monkey (*Rhinopithecus roxellanae brelichi*) in Fanjing Mountain Biosphere Reserve, China. *J Forest Res* 15:197–202.
- Yang M, Sun D, Zinner D, Roos C. 2009. Reproductive parameters in Guizhou snub-nosed monkeys (*Rhinopithecus brelichi*). *Am J Primatol* 71:266–270.
- Yang M, Nadler T, Zinner D, Roos C. The evolutionary history of odd-nosed monkeys. In: Tan C, Grüter C, Wright B, editors. Odd-nosed monkeys: recent advances in the study of the forgotten colobines (in press).
- Yang YQ, Lei XP, Sun DY, Yang CD, He RT, Zhang WY. 2002. Ecology of the wild Guizhou snub-nosed monkey. Guiyang: Guizhou Scientific Institute Press.
- Zhang SY, Liang B, Wang LX. 2000. Seasonality of mating and births in captive Sichuan golden monkeys (*Rhinopithecus roxellanae*). *Am J Primatol* 51:265–269.
- Zhang YP, Ryder OA, Fan Z, Zhang H. 1997. Sequence variation and genetic diversity in giant panda. *Sci China C Life Sci* 27:139–144.
- Zhang YP, Ryder OA. 1998. Mitochondrial cytochrome b gene sequences of Old World monkeys: with a special reference on evolution of Asian colobines. *Primates* 39:39–49.
- Zhang YZ, Quan GQ, Zhao TG, Southwick CH. 1992. Distribution of primates (except *Macaca*) in China. *Acta Theriol Sinica* 12:81–95.
- Zwickl DJ. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum-likelihood criterion. PhD thesis. The University of Texas at Austin.

2. General Discussion

My thesis is divided in three major parts which constitute a top-down approach of analyses of phylogenetic relationships and population genetic parameters in leaf monkeys (Colobinae). The first part of my thesis provides a comprehensive phylogeny of the colobines genera by combining different genetic markers. In the second part, my thesis focuses on the evolutionary history of one major radiation of the Colobinae, the odd-nosed monkeys. Based on complete mitochondrial genome sequence data from nine odd-nosed monkey species I reconstructed the phylogenetic relationships among genera and species and estimated respective divergence ages. Finally, a population genetic analysis was performed for one of the Chinese snub-nosed monkey species, the endangered Guizhou snub-nosed monkeys (*Rhinopithecus brelichi*). I assessed the population genetic variation and demographic history of this species in comparison with already existing data of its Chinese congeners.

Summary of the main findings

In the first part of my thesis (Chapter 1) a combination of mitochondrial genome data (ca. 16,600 bp), sequence information from five autosomal loci (ca. 5,300 bp), one X chromosomal locus (4,000 bp) and six Y chromosomal loci (ca. 4,000 bp), and the presence/absence pattern of the mobile element of the ten colobines genera were used to reconstruct the phylogenetic relationships among all Colobinae genera. Phylogenies based on different genetic markers show a similar topology, but several incongruences were also observed. First, the African colobines constitute a monophyletic clade based on the nuclear and mitochondrial sequence data, while according to the mobile elements data African colobines are paraphyletic with the *Piliocolobus/Procolobus* clade closer related to the Asian colobines than to *Colobus*. Second, nuclear sequence data put *Presbytis* as basal to all Asian colobines, whereas the mobile elements indicate a sister clade relationship between *Presbytis* and the odd-nosed monkeys. Third, the mitochondrial data reveal *Semnopithecus*, *Presbytis* + *Trachypithecus* and the odd-nosed monkeys as three-way lineages, while the nuclear data suggest a distinct *Presbytis* lineage (see above) and a *Semnopithecus* + *Trachypithecus* clade. Although various explanations are possible for the observed incongruences, ancient

hybridization events for at least two cases are most likely. For the odd-nosed monkey genera, identical branching patterns with *Rhinopithecus* as most basal lineage, followed by *Pygathrix* and a sister grouping of *Nasalis* and *Simias* was obtained from all data sets.

The second part of my thesis (Chapter 2) was a mitogenomic analysis of the phylogenetic relationships and divergence times within the odd-nosed monkey clade and included nine of the ten recognized species. *R. strykeri* was not included here because this species was only recently discovered in Myanmar and material for a genetic analysis was not available. My study strongly supports as in Chapter 1 a monophyly of the odd-nosed monkeys and indicates that *Rhinopithecus* occurs as basal, whereas *Pygathrix* forms a sister lineage to the *Nasalis* + *Simias* clade. The diversification of odd-nosed monkeys into genera started ca. 6.85 mya and ended 1.98 mya with the split between *Nasalis* and *Simias*. Subsequent radiations in *Rhinopithecus* and *Pygathrix* occurred during the Pleistocene. Within *Rhinopithecus*, my analysis reveals that *R. roxellana* + *R. brelichi* form a sister clade to *R. bieti*, while *R. avunculus* occurs as the most basal taxon. In *Pygathrix*, *P. nigripes* is basal to the *P. nemaeus* + *P. cinerea* clade.

In part 3 of my thesis (Chapter 3) I performed a population genetic study on *R. brelichi* for which I used the hypervariable region I of the mitochondrial control region. The data were compared with respective published information from the two other Chinese snub-nosed monkey species (*R. roxellana*, *R. bieti*). My results indicate significant genetic differences among the three species. Rather low nucleotide diversity was detected in all three Chinese snub-nosed species, with the lowest genetic diversity in *R. brelichi*. Among 141 *R. brelichi* individuals only five haplotypes were found and one occurred in 102 individuals. Tajima's D, Fu's F_s and mismatch distribution tests all showed an equilibrium population in the demographic history of the wild *R. brelichi* population. The MRCAs of *R. bieti*, *R. roxellana* and *R. brelichi* lived ca. 1.33, 0.91 and 0.36 mya, respectively.

The evolutionary history of colobines genera

In a comprehensive approach we investigated the evolutionary history of colobines by combining maternal-, paternal- and biparental-inherited molecular

markers from all ten colobines genera. Hence, this study represents the most detailed insight into the evolutionary history of colobines.

The phylogenetic trees derived from the analysis of different molecular markers are often incongruent (Grechko, 2002; Tosi et al., 2003). A few discordances have been found in the phylogenetic topologies in this study as well. Our mitochondrial and nuclear data both support a reciprocal monophyletic origin of the African and the Asian colobines, which is in agreement with morphological studies (Groves, 2001; Napier and Napier, 1967; Szalay and Delson, 1979) and earlier molecular studies (Collura et al., 1996; Messier and Stewart, 1997; Zhang and Ryder, 1998; Page et al., 1999; Bigoni et al., 2003, 2004; Xing et al., 2005). In contrast, the mobile elements propose a paraphyly of African colobines (Groves, 1989; Peng et al., 1993; Jablonski, 1998c; Osterholz et al., 2008).

Discordant phylogenetic relationships among genera based on different markers have been explained in past studies by using insufficient data, homoplasy, incomplete lineage sorting (ILS), or hybridization (Philippe and Laurent, 1998; Barton, 2001; Nichols, 2001; Funk and Omland, 2003; Avise, 2004; Seehausen, 2004; McCracken and Sorenson, 2005; Xing et al., 2005; Pollard et al., 2006; Koblmüller et al., 2007; Petit and Excoffier, 2009). For the mobile elements, we did not find any inconsistent elements; therefore, ILS is unlikely to be an explanation for our findings. Homoplasy is usually regarded as not relevant for the analyses of mobile elements (Okada, 1991; Schmitz et al., 2005; Ray et al., 2006). Overall, inadequate data, homoplasy, ILS could not provide sufficient explanations for the observed incongruence in this study. Moreover, mobile elements as molecular-cladistic marker are more reliable than pure sequence data (Kazazian, 2004; Osterholz et al., 2008), thus, the discordant pattern most likely is caused by ancestral hybridization between the ancestor of *Colobus* and the *Piliocolobus/Procolobus* lineage. This view is also supported by biological data (Davies and Oates, 1994; Groves, 2001). In contrast to *Colobus*, the females of *Piliocolobus/Procolobus* tend to leave their natal groups (Newton and Dunbar, 1994). *Colobus* males are normally bigger than *Piliocolobus/Procolobus* males, thus, *Colobus* males increase their chance to hybridize with *Piliocolobus/Procolobus* females. Moreover, the two ancestral genera occur over wide ranges of their distribution in sympatry (Davies and Oates, 1994; Newton and Dunbar, 1994; Groves, 2001); hence, it can be assumed that ancestral hybridization events were basically possible.

For the Asian colobines, the phylogenetic relationships among the odd-nosed monkeys were the same from all markers. Five mobile elements adduced sufficient evidence for the monophyly of the odd-nosed monkeys. The view on their monophyletic origin was already earlier supported by mitochondrial sequence data (Sternler et al., 2006). Among odd-nosed monkeys, mobile elements also support the basal position of *Rhinopithecus* and the grouping of *Nasalis* with *Simias*. However, the phylogenetic affiliations within the leaf monkeys and langurs are major parts of a debate. In our study, nuclear sequence data and mobile elements support a paraphyly of langurs and a close relationship of *Semnopithecus* and *Trachypithecus*. The morphological characteristics support the idea of a sister clade relationship between *Semnopithecus* and *Trachypithecus* (Brandon-Jones, 1984; Strasser and Delson, 1987; Groves, 2001). On the contrary, the mitochondrial sequence data provide a connection between *Presbytis* and *Trachypithecus*. Avise (2004) demonstrated that mitochondrial DNA was likely to sort faster than nuclear DNA because the effective population size of mitochondrial DNA was only a quarter of autosomal genes. If ILS was the reason for the observed incongruent gene trees, the mitochondrial divergence between respective genera should be earlier than the nuclear splitting times. However, the divergence time calculated in this study denied this case (*Presbytis* – *Trachypithecus*: 8.12 mya nuclear vs. 7.45 mya mitochondrial). Thus, ancestral hybridization is the most likely plausible explanation for the incongruent pattern. In general, hybridization is possible due to partial sympatry (Davies and Oates, 1994; Newton and Dunbar, 1994; Groves, 2001). *Semnopithecus* males are larger than *Trachypithecus* (Davies and Oates, 1994). *Semnopithecus* males leave their natal group and might hybridize with *Trachypithecus* females. By backcrossing with *Semnopithecus* males over a rather long time period, the *Trachypithecus* accumulated nuclear material of *Semnopithecus*, although the mitochondrial genome remained *Trachypithecus*-like.

The evolutionary history of odd-nosed monkey species

The second part of my thesis presents a detailed and updated view into the evolutionary history of odd-nosed monkeys on species level. Since previous studies on the evolutionary history of odd-nosed monkeys only used short mitochondrial fragments and/or did not include all species (Wang et al., 1997; Zhang and Ryder, 1998; Li et al., 2001; Li et al., 2004; Sternler et al., 2006; Whittaker et al., 2006; Li et al.,

2007; Liu et al., 2007; Osterholz et al., 2008; Ting et al., 2008; Li et al., 2011; Perelman et al., 2011), my study provides a phylogeny and respective divergence time estimates based on the complete mitochondrial genome from nine of ten odd-nosed monkey species. *R. strykeri* was not included in this study because this species was only recently described (Geissmann et al., 2011) and genetic material was not yet available.

The divergence time of *Simias* and *Nasalis* in the early Pleistocene occurred as most speciation events within *Rhinopithecus* and *Pygathrix*. My data points in the direction that *Simias* should not be recognized as distinct genus and respectively included in *Nasalis*. These results are in agreement with findings from several previous studies (Delson et al., 2000; Whittaker et al., 2006). Furthermore, my data suggest that the genus *Rhinopithecus* contains at least four species (*R. avunculus*, *R. roxellana*, *R. bieti*, *R. brelichi*) and that the genus *Pygathrix* comprises three species (*P. nigripes*, *P. cinerea*, *P. nemaeus*), because the estimated divergence times among them were similar. The hypothesis that *R. avunculus* represents a separate subgenus *Presbyticus* (Boonratana and Canh, 1998; Chaplin and Jablonski, 1998; Zhang and Ryder, 1998; Jablonski, 1998) is not supported by my analysis. Genetic data for the newly described *R. strykeri* is not available yet, but it clearly differs from its congeners by various morphological characters (Geissmann et al., 2011).

By combining our knowledge of phylogeny, divergence time and palaeo-environmental data, the following phylogeographic scenario for odd-nosed monkeys can be envisioned. Since fossil colobines were found in Eurasia at the end of the Miocene, the ancestor(s) of the Asian colobines probably invaded Eurasia via an emerging land bridge connecting Africa and the Arabian Peninsula and dispersed into Asia (Whybrow, 1992; Stewart and Disotell, 1998; Delson, 2000). The Hengduan Mountains in the border region of today's Myanmar, India and China have been identified as a possible diversification hotspot. Thus, the origin of the odd-nosed monkeys might have been here (Peng et al., 1993; Jablonski, 1998b). In this area, all larger south-eastern rivers (Mekong, Salween, and Yangtze) rise and exist here at least since the early Miocene (Hallet and Molnar, 2001). These rivers have been well known as barriers for arboreal primates (Meijaard and Groves, 2006). After the langur progenitor separated from the odd-nosed monkeys' ancestor in the latest Miocene, the odd-nosed monkeys successively separated and migrated from China to the south and expanded their range into Indochina and Sundaland via a land bridge during periods of

lower sea levels. Due to selective pressures in various environments, the odd-nosed monkeys chose a wide range of altitudinal and climatic zones and different types of vegetation (Peng et al., 1993). The radiation of *Rhinopithecus* started in the early Pleistocene probably caused by the reduction and the fragmentation of suitable habitat occurring during that time (Pan and Jablonski, 1987; Jablonski, 1992; Pan, 1995; Jablonski, 1998c; Hartwig, 2002). The divergence time between *P. nemaeus* + *P. cinerea* that I estimated is similar to the divergence time of the species splits among crested gibbons (Thinh et al. 2010) and among limestone langurs (Perelman et al., 2011), thus, the split of *Pygathrix* most likely was triggered by similar changes in habitat as shrinking forests due to climate changes. Finally, the ancestors of the *Nasalis* + *Simias* lineage immigrated into Sundaland most likely during periods of low sea levels (Miller et al., 2005). Although currently *Nasalis* lives on Borneo and *Simias* is distributed on the Mentawai Islands, *Simias* or at least an extinct progenitor of *Simias* and *Nasalis* might have existed also on Sumatra. This can be assumed because according to my analysis, gene flow between the two lineages was possible until the end of the early Pleistocene.

Limited genetic variation of Guizhou snub-nosed monkeys (*R. brelichi*)

Population genetic data were previously only available for *R. roxellana* and *R. bieti* (Li et al. 2007; Liu et al. 2007). My study presents the first comparative population genetic study on all three Chinese snub-nosed monkey species.

Theoretical considerations suggest that genetic diversity is related to population size because a small population harbours less genetic variation and loses genetic variation faster than larger ones (Wright, 1969, 1978; Frankham et al., 1995; Höglund, 2009). The fossil records indicated that the Chinese snub-nosed monkeys were once widespread in Asia (Li et al., 2002). They experienced a significant range reduction in the late Pleistocene or Holocene according to fossil records (Jablonski 1998c; Quan and Xie 2002). During the last 400 years, their ranges became highly fragmented due to a dramatic human population increase in southern China, which led to accelerating deforestation and hunting activities. The current populations of snub-nosed monkeys survived only in restricted areas (Li et al., 2002). Yang et al. (2002) reported that *R. brelichi* consists of only one population with roughly 800 individuals living in the core zone of Fanjingshan National Nature Reserve. In my study, the analysis of

mitochondrial DNA sequence data revealed rather low nucleotide diversities (π) for all three Chinese snub-nosed monkey species and the lowest genetic diversity for *R. brelichi*. A low π can also be found in other primates with a restricted range and a relatively small population size such as *Papio hamadryas* in Saudi Arabia ($\pi = 0.023$: Winney et al., 2004) and *Macaca sylvanus* in Morocco ($\pi = 0.026$: Modolo et al., 2005). Among the three Chinese snub-nosed monkey species, *R. brelichi* has the smallest population and the most restricted distribution (Bleisch and Xie, 1998; Groves, 2001; Yang et al., 2002; Wu et al., 2004; MacKinnon, 2008; IUCN, 2011). Therefore, the expected low genetic diversity in *R. brelichi* is not surprising. The genetic diversity indices of *R. brelichi* ($h = 0.457$; $\pi = 0.014$) were similar to those of Japanese macaques (*Macaca fuscata yakui*, $h = 0.305$; $\pi = 0.015$), which only lives on a small island (Hayaishi and Kawamoto, 2006). The isolation of the habitat due to historical environmental alterations and/or the recent human activities in the last centuries might provide an explanation for the low genetic diversity in all Chinese snub-nosed monkeys, in particular for *R. brelichi*.

However, analysis of mitochondrial data did not show that the population of *R. brelichi* experienced a recent bottleneck or contraction. The genetic impoverishment most likely was triggered by a population fragmentation and partial extinctions during the last centuries. The only existing *R. brelichi* population might have preserved its original low genetic variability for a certain time period. Additionally, a small population loses genetic variation not only because of a recent bottleneck but also due to genetic drift (Hartl and Clark, 2007; Hamilton, 2009). Genetic drift increases as the population size decreases (Hamilton, 2009). Therefore, genetic drift might cause the low genetic diversity in *R. brelichi* population as well.

Implications for conservation

All three Chinese snub-nosed monkeys (*R. roxellana*, *R. bieti* and *R. brelichi*) are classified as “Endangered” by the IUCN Red List (IUCN, 2011). *R. brelichi* has the lowest genetic diversity hitherto reported for Chinese snub-nosed monkeys. Gene flow among subpopulations via migration of individuals is one important way to preserve and support the recovery of the genetic diversity within a population (Usher, 1997; Fox and Wolf, 2006) and extensive gene flow was found among subpopulations of *R. roxellana* and *R. bieti*, respectively (Liu et al., 2009; Pan et al., 2009). However, current

knowledge indicates that for *R. brelichi* only one population exists (Yang et al., 2002). Therefore, an increase of genetic diversity through immigration of individuals from other populations in this species is not possible. The collected samples represented roughly 20% of the entire known population of *R. brelichi* and they revealed only five haplotypes. A population with low genetic diversity in general has lower adaptive potentials to follow possible environmental changes (Frankel and Soule, 1981; Simberloff, 1988; Barrett and Kohn, 1991; Ellstrand and Elam, 1993; Lande and Shannon, 1996). Furthermore, a study on reproductive pattern of *R. brelichi* disclosed that this species has the slowest reproduction (longest inter-birth interval and the latest age at the first birth) compared with the other two Chinese species (Ji et al., 1998; Zhang et al., 2000; Liang et al., 2001; Cui et al., 2006; Qi et al., 2008; Yang et al., 2009). Summarizing all these results, *R. brelichi* is probably the most vulnerable *Rhinopithecus* species of China. Accordingly, recovery of the population after suffering natural disasters, environmental changes or a disease can be expected to be rather slow. Any further loss of individuals due to hunting or habitat conversion would have even more dramatic effects than in the other two species. It most likely may not reach the necessary threshold for a sustainable population in time.

The current threats for *R. brelichi* are mainly caused by the habitat conversion induced by human economic activities. Accordingly, habitat protection and the elimination or the balancing of any further human economic activities is a major premise in protecting this species. Nonetheless, further research should collect more samples covering the whole distribution of *R. brelichi* to design effective conservation guidelines.

3. Outlook

The evolutionary relationships of the odd-nosed monkeys have been complemented and updated in my study. Future analyses should include *R. strykeri* to complete the phylogenetic relationships within the odd-nosed monkeys. Nuclear data should be applied as well to fully elucidate the phylogenetic relationships among odd-nosed monkey species, and probably to uncover possible ancient hybridization events among them.

In my study I was able to demonstrate a low level of genetic diversity in the wild population of *R. brelichi*, and a population equilibrium in this species based on mitochondrial data. Mitochondria are maternally inherited and therefore represent only one of many molecular tracings in the evolutionary histories of organisms. Therefore, nuclear DNA analyses (microsatellites, SNPs) are required to complement the analysis of genetic diversity and demographic history of *R. brelichi*. Furthermore, population genetic data are also required for *R. avunculus* and *R. strykeri*, and other members of the odd-nosed monkeys.

In the near future more samples from the southern part of the range of *R. brelichi* should be collected. These samples could be utilized to corroborate the results of my population genetic study and to test whether the remaining population of *R. brelichi* consists of more than one subpopulation.

4. Conclusion

My thesis shows that extended sequence data provide much better resolution of phylogenetic relationships than only short fragments. This is in particular true for the herein analysed mitochondrial genome data. Early studies using only short fragments of the mitochondrial genome (Zhang and Ryder, 1998; Li et al., 2004; Roos, 2004; Whittaker et al., 2006; Roos et al., 2007) revealed no or only low resolution for various relationships among odd-nosed monkeys and species of the genus *Rhinopithecus*. Another good example for the increased resolution for phylogenetic relationships among taxa are the gibbons. While Thinh et al. (2010) with analysing only one mitochondrial gene revealed only low support for relationships among genera and *Hylobates* species, Chan et al. (2010) by using complete mitochondrial genome data resolved respective relationships with significance. Moreover, the analysis of differently inherited marker systems has proven to be useful to uncover hybridization events, although the therefore required incongruent tree topologies might have been caused by alternatives. Accordingly, testing alternatives is necessary.

Also for population genetic analysis, mitochondrial DNA in particular variable regions as the hypervariable region I of the control region can provide detailed insights into the evolutionary history of a species. However, mitochondrial DNA is only

maternally inherited. Hence, to obtain a complete picture of the evolutionary history of a species, also paternally and biparentally inherited markers should be analyzed. These could shed light on male-mediated gene flow and socio-biological events in a species, which are otherwise not traceable by field observations. Finally, information about the evolutionary history and in particular the population genetic structure of a species or population provides helpful information for the conservation management of a species, which is of great importance of taxa with small habitat and small populations as in the case of the Guizhou snub-nosed monkey.

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References

- Arnold ML, Meyer A. 2006. Natural hybridization in primates: one evolutionary mechanism. *Zoology* 109:261-276.
- Awise JC. 2004. *Molecular Markers, Natural History, and Evolution*. Sunderland, MA: Sinauer Associates.
- Awise JC, Hamrick JL. 1995. *Conservation genetics, case histories from nature*. New York: Chapman and Hall.
- Bandelt HJ, Forster P, Röhl A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Mol Biol Evol* 16:37–48.
- Barrett SCH, Kohn JR. 1991. Genetic and evolutionary consequences of small population size in plants: implications for conservation. In: Falk DA, Holsinger KE, editors. *Genetics and Conservation of Rare Plants*. New York: Oxford University Press. p 3–30.
- Barton NH. 2001. The role of hybridization on evolution. *Mol Ecol* 10:551-568.
- Batzer MA, Deininger PL. 1991. A human-specific subfamily of Alu sequences. *Genomics* 9:481-487.
- Benefit BR, McCrossin ML. 2002. The Victoriapithecidae, Cercopithecoidea. In: Hartwig WC, editor. *The Primate Fossil Record*. Cambridge: Cambridge University Press. p 241-253.
- Bennett EL, Davies AG. 1994. The ecology of Asian colobines. In: Davies AG, Oates JF, editors. *Colobine monkeys: their ecology, behavior, and evolution*. Cambridge: Cambridge University Press. p 129–171.
- Benson DA, Boguski MS, Lipman DJ, Ostell J, Ouellette BF. 1998. Genbank. *Nucleic Acids Res* 26:1-7.
- Bigoni F, Stanyon R, Wimmer R, Schempp W. 2003. Chromosome painting shows that the proboscis monkey (*Nasalis larvatus*) has a derived karyotype and is phylogenetically nested within Asian colobines. *Am J Primatol* 60 (3):85–93.
- Bigoni F, Houck ML, Ryder OA, Wienberg J, Stanyon R. 2004. Chromosome painting shows that *Pygathrix nemaeus* has the most basal karyotype among Asian colobines. *Int J Primatol* 25 (3):679–688.
- Bleisch W, Xie JH. 1998. Ecology and behavior of the Guizhou snub-nosed langur (*Rhinopithecus [Rhinopithecus] brelichi*), with a discussion of socioecology in
-

the genus. In: Jablonski NG, editor. The natural history of the doucs and snub-nosed monkeys. Singapore: World Scientific Publishing. p 217–239.

Boonratana R, Canh LX. 1998. Preliminary observations of the ecology and behavior of the Tonkin snub-nosed monkey (*Rhinopithecus [Presbytiscus] avunculus*) in Northern Vietnam. In: Jablonski NG, editor. The Natural History of the Doucs and Snub-nosed Monkeys. Singapore: World Scientific Publishing. p 207-215.

Brandon-Jones D. 1984. Colobus and leaf monkeys. In: MacDonald ID, editor. Encyclopedia of Mammals. London: George Allen and Unwin. p:398-408.

Brandon-Jones D. 1995. A revision of the Asian pied leaf monkeys (Mammalia: Cercopithecidae: *Superspecies Semnopithecus auratus*), with a description of a new subspecies. Raffles Bull Zool 43:3-43.

Brandon-Jones D, Eudey AA, Geissmann T, Groves CP, Melnick DJ, Morales JC, Shekelle M, Stewart CB. 2004. Asian Primate Classification. Int J Primatol 25:97-164.

Brunet M, Guy F, Pilbeam D, Lieberman DE, Likius A, Mackaye HT, Ponce de León MS, Zollokofer CP, Vignaud P. 2005. New material of the earliest hominid from the Upper Miocene of Chad. Nature 434:752-755.

Burnham KP, Anderson DR. 2002. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. New York: Springer.

Castresana J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol Biol Evol 17:540-552.

Chakraborty D, Ramakrishnan U, Panor J, Mishra C, Sinha A. 2007. Phylogenetic relationships and morphometric affinities of the Arunachal macaque *Macaca munzala*, a newly described primate from Arunachal Pradesh, northeastern India. Mol Phylogenet Evol 44:838-849.

Chan YC, Roos C, Inoue-Murayama M, Inoue E, Shih CC, Pei KJC, Vigilant L. 2010. Mitochondrial genome sequence effectively reveal the phylogeny of *Hylobates* gibbons. Plos One 5 (12):e14419.

Chaplin G, Jablonski NG. 1998. The integument of the odd-nosed colobines. In: Jablonski NG, editor. The natural history of the doucs and snub-nosed monkeys. Recent advances in human biology. Singapore and London: World Scientific Publishing Co. p 79-104.

Chaves R, Sampaio I, Schneider MP, Schneider H, Page SL, Goodman M. 1999. The place of *Callimico goeldii* in the callithrichine phylogenetic tree: evidence from von Willebrand factor gene intron 11 sequences. *Mol Phylogenet Evol* 13:392-404.

Collura RV, Auerbach MR, Stewart CB. 1996. A quick, direct method that can differentiate expressed mitochondrial genes from their nuclear pseudogenes. *Curr Biol* 6 (10): 1337–1339.

Cortés-Ortiz L, Duda TF Jr, Canales-Espinosa D, Garcia-Orduna F, Rodriguez-Luna E, Bermingham E. 2007. Hybridization in large-bodied New World primates. *Genetics* 176:2421-2425.

Covert HH, Le KQ, Wright BW. 2008. On the Brink of Extinction: research for the conservation of the Tonkin snub-nosed monkey (*Rhinopithecus avunculus*), In: Fleagle JG, editor. *Elwyn Simons: A Search for Origins*. New York: NY, Kluwer Press. p 409–427.

Cui LW, Sheng AH, He XC, Xiao W. 2006. Birth seasonality and interbirth interval of captive *Rhinopithecus bieti*. *Am J Primatol* 68:457–463.

Darwin C. 1859. *On the origin of species by means of natural selection or the preservation of favoured races in the struggle of life*. London: John Murray.

Davies AG, Oates JF. 1994. *Colobine monkeys – their ecology, behaviour and evolution*. Cambridge: Cambridge University Press.

de Jong G, de Ruiter JR, Haring R. 1994. Genetic structure of a population with social structure and migration. In: Loeschcke V, Tomiuk J, Jain SK, editors. *Conservation genetics*. Basel: Birkhäuser Verlag. p 147–164.

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

Delson E. 2000. Cercopithecinae. In: Delson E, Tattersall I, Van Couvering JA, Brooks AS, editors. *Encyclopedia of human evolution and prehistory*. New York: Garland Publishing Inc. p 166-171.

Dietz JM, Baker AJ, Ballou JD. 2000. Demographic evidence of inbreeding depression in wild golden lion tamarins. In: Yang A, Clark GM, editors. *Genetics, demography, and viability of fragmented populations*. Cambridge: Cambridge University Press. p 203–211.

Di Fiore A. 2003. Molecular genetic approaches to the study of primate behavior, social organization, and reproduction. *Am J Phys Anthropol* 122:62–99.

Disotell TR, Honeycutt RL, Ruvolo M. 1992. Mitochondrial DNA phylogeny of the Old World Monkey Tribe *Papionini*. *Mole Biol Evol* 9(1):1-13.

Dobzhansky Th. 1973. Nothing in biology makes sense except in the light of evolution. *Am Biol Teach* 35:125-129.

Dong TH, Boonratana R. 2006. Further information on ecology and behavior of Tonkin snub-nosed monkey (*Rhinopithecus avunculus*) in Vietnam. Entebe, Uganda: paper presented at the the meeting of the international Primatological Society.

Drummond AJ, Ho SY, Phillips MJ, Rambaut A. 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biol* 4:e88

Drummond AJ, Kearse M, Heled J, Moir R, Thierer T, Ashton B, Wilson A, Stones-Havas S. 2009. Geneious, version 4.6.1. <http://www.geneious.com>

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol Biol* 7:214.

Dutrillaux B. 1979. Chromosomal evolution in Primate: tentative phylogeny from *Microcebus murinus* (*Prosimians*) to man. *Hum Genet* 48: 251-314.

Edwards AWF, Cavalli-Sforza LL. 1964. Reconstruction of evolutionary trees. In: Heywood VH, McNeill J, editors. *Phenetic and Phylogenetic Classification*. London: Systematics Association pub. p 67–76.

Ellerman JR, Morrison-Scott TCS. 1951. Checklist of Palaearctic and Indian mammals. London: Trustees of the British Museum (Natural History). p 22–89.

Ellstrand NC, Elam DR. 1993. Population genetic consequences of small population size: implications for plant conservation. *Annu Rev Ecol Syst* 24: 217–242.

Evans RC. 1999. Molecular, morphological, and ontogenetic evaluation of relationships and evolution in the Rosaceae. PhD diss. University of Toronto.

Excoffier L, Lischer HL. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Res* 10:564–567.

Excoffier L, Smouse PE, Quattro JM. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131:479–491.

Fabre V, Condemi S, Degioanni A. 2009. Genetic evidence of geographical groups among Neanderthals. *PLoS ONE* 4 (4): e5151.

Falk D. 2000. *Primate diversity*. New York: W.W. Norton and Company.

Fashing PJ. 2011. African colobine monkeys: Their behavior, ecology, and conservation. In: Campbell C, Fuentes A, MacKinnon K, Bearder S, Stumpf R, editors. *Primates in Perspective*. Oxford: Oxford University Press. p 203-229.

Fox CW, Wolf JB. 2006. *Evolutionary genetics: concepts and case studies*. New York: Oxford University Press.

Frankel OH, Soule ME. 1981. *Conservation and Evolution*. Cambridge: Cambridge University Press.

Frankham R. 1995. Conservation genetics. *Annu Rev Genet* 29:305-327

Frankham R, Ballou JD, Briscoe DA. 2004. *A primer of conservation genetics*. Cambridge: Cambridge University Press.

Frost SR, Marcus L, Bookstein F, Reddy C, Delson E. 2003. Cranial allometry, phylogeography, and systematics of large-bodied papionins (Primates: *Cercopithecinae*) inferred from geometric morphometric analysis of landmark data. *Anat. Rec.* 275A: 1048-1072.

Fu YX. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* 147:915–925.

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

Geissmann T, Lwin N, Aung SS, Aung TN, Aung MZ, Hla TH, Grindler M, Momberg F. 2011. A new species of snub-nosed monkey, genus *Rhinopithecus* Milne-Edwards, 1872 (Primates, Colobinae), from northern Kachin State, northeastern Myanmar. *Am J Primatol* 73:96–107.

Gilbert LB, Kasuga T, Glass NL, Taylor JW. 2011. Array CGH phylogeny: how accurate are comparative genomic hybridization-based trees? *BMC Genomics* 12:487-501.

Goodman M, Porter CA, Czelusniak J, Page SL, Schneider H, Shoshani J, Gunnell G, Groves CP. 1998. Toward a phylogenetic classification of primates based on DNA evidence complemented by fossil evidence. *Mol Phylogenet Evol* 9:585-598.

Grechko V. 2002. Using Molecular DNA Markers in Phylogeny and Systematics. *Russ J Genet* 38:1013-1033.

Green RE, Krause J, Briggs AW, Maricic T, Stenzel U, Kircher M, Patterson N, Li H, Zhai W, Fritz MH, Hansen NF, Durand EY, Malaspinas AS, Jensen JD, Marques-Bonet T, Alkan C, Prüfer K, Meyer M, Burbano HA, Good JM, Schultz R, Aximu-Petri A, Butthof A, Höber B, Höffner B, Siegemund M, Weihmann A, Nusbaum C, Lander ES, Russ C, Novod N, Affourtit J, Egholm M, Verna C, Rudan P, Brajkovic D, Kucan Z, Gusic I, Doronichev VB, Golovanova LV, Lalueza-Fox C, de la Rasilla M, Fortea J, Rosas A, Schmitz RW, Johnson PL, Eichler EE, Falush D, Birney E, Mullikin JC, Slatkin M, Nielsen R, Kelso J, Lachmann M, Reich D, Pääbo S. 2010. A draft sequence of the Neandertal genome. *Science* 328:710-722.

Groves CP. 1970. The forgotten Leaf-Eaters and the phylogeny of the Colobinae. In: Napier JR, Napier PH, editors. *Old World Monkeys*. New York: Academic Press p 555-587.

Groves CP. 1978. Phylogenetic and population systematics of the mangabeys (Primates: *Cercopithecoidea*). *Primates* 19:1-34.

Groves CP. 1987. *A theory of human and primate evolution*. Oxford: Oxford University Press.

Groves CP. 1989. *A Theory of Human and Primate Evolution*. Oxford: Clarendon Press.

Groves CP. 2001. *Primate taxonomy*. Washington, DC: Smithsonian Institution Press.

Groves CP. 2007. The taxonomic diversity of the Colobinae of Africa. *J Anthropol Sci* 85: 7-34.

Grueter CC, Li D, Ren B, Wei F, van Schaik CP. 2009. Dietary profile of *Rhinopithecus bieti* and its socioecological implications. *Int J Primatol* 30:601–624.

Grubb P, Butynski TM, Oates JF, Bearder SK, Disotell TR, Groves CP, Struhsaker TT. 2003. Assessment of the diversity of African primates. *Int J Primatol* 24 (6):1301-1357.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp* 41:95–98.

Hallet B, Molnar P. 2001. Distorted drainage basins as markers of crustal strain east of the Himalayas. *J Geophys Res* 106:13697-13709.

- Hamilton MB. 2009. Population genetics. United Kingdom: John Wiley & Sons Ltd.
- Harris EE, Disotell TR. 1998. Nuclear gene trees and the phylogenetic relationships of the mangabeys (Primates: Papionini). *Mol Biol Evol* 15:892-900.
- Harpending HC. 1994. Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. *Hum Biol* 66:591-600.
- Hartl DL, Clark AG. 2007. Principles of population genetics. Sunderland, MA: Sinauer.
- Hartwig WC, 2002. The primate fossil record. United Kingdom: Cambridge University Press.
- Harvey PH, Leigh Brown AJ, Maynard SJ, Nee S. 1996. New Uses for New Phylogenies. New York: Oxford University Press.
- Hayaishi S, Kawamoto Y. 2006. Low genetic diversity and biased distribution of mitochondrial DNA haplotypes in the Japanese macaque (*Macaca fuscata yakui*) on Yakushima Island. *Primates* 47:158-164.
- Hellborg L, Ellegren H. 2003. Y chromosome conserved anchored tagged sequences (YCATS) for the analysis of mammalian male-specific DNA. *Mol Ecol* 12:283-291.
- Hennig W. 1965. Phylogenetic systematic. *Annu. Rev. Entomol* 10:97-116.
- Herke SW, Xing J, Ray DA, Zimmermann JW, Cordaux R, Batzer MA. 2007. A SINE-based dichotomous key for primate identification. *Gene* 390:39-51.
- Hill WCO. 1934. A monography on the purple-faced leaf-monkeys (*Pithecus vetulus*). *Ceylon J Sci* 9:23-88.
- Hill WCO. 1952. The external and visceral anatomy of the Olive colobus monkey (*Procolobus verus*). *J Zool* 122 (1):127-186.
- Hill WCO. 1974. Primates: Comparative Anatomy and Taxonomy, Cynopithecinae (Cercopithecus, Macaca, Cynopithecus). Edinburgh: Edinburgh University Press.
- Höglund J. 2009. Evolutionary conservation genetics. Oxford: Oxford University Press.
- Huelsenbeck JP, Ronquist F, Nielsen R, Bollback JP. 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. *Science* 294:2310-2314.
- IUCN. 2011. The IUCN red list of threatened species. Available at: <http://www.iucnredlist.org>.
-

Jablonski NG. 1992. Dental agenesis as evidence of possible genetic differences isolation in the colobine monkey, *Rhinopithecus roxellana*. *Primates* 33: 371–376.

Jablonski NG. 1998a. The response of catarrhine primates to Pleistocene environmental fluctuations in East Asia. *Primates* 39:29–37.

Jablonski NG. 1998b. The evolution of the doucs and snubnosed monkeys and the question of the phyletic unity of the odd-nosed colobines. In: Jablonski NG, editor. *The natural history of the doucs and snub-nosed monkeys*. Singapore: World Scientific Publishing. p 13–52.

Jablonski NG. 1998c. *Natural History of the Doucs and Snub-Nosed Monkeys*. New Jersey: World Scientific Publishing Company.

Jablonski NG. 1999. Primate evolution – in and out of Africa. *Curr Biol* 9:R119,R122.

Jablonski NG, Peng YZ. 1993. The phylogenetic relationships and classification of the doucs and snub-nosed langurs of China and Vietnam. *Folia Primatol* 60:36–55.

Ji WZ, Zou RJ, Shang EY, Zhou HW, Yang SC, Tian BP. 1998. Maintenance and breeding of Yunnan snub-nosed monkeys (*Rhinopithecus [Rhinopithecus] bieti*) in captivity. In: Jablonski NG, editor. *The natural history of the doucs and snubnosed monkeys*. Singapore: World Scientific Publishing. p 323–335.

Johnson AD, Drum M, Bachvarova RF, Masi T, White ME, Crother BI. 2003. Evolution of predetermined germ cells in vertebrate embryos: implications for macroevolution. *Evol Dev* 5 (4):414-431.

Jolly CJ. 1966. Introduction to the Cercopithecoidea, with notes on their use as laboratory animals. *Symp Zool Soc London* 17:427-457.

Jolly CJ, Whitehead PF. 2000. Old world monkeys: three decades of development and change in the study of the Cercopithecoidea. In: Whitehead PF, Jolly CJ, editors, *Old world monkeys*. United Kingdom: Cambridge University Press. p 1-28.

Karanth KP, Singh L, Collura RV, Stewart CB. 2008. Molecular phylogeny and biogeography of langurs and leaf monkeys of South Asia (Primates: *Colobinae*). *Mol Phylogenet Evol* 46:683-694.

Karanth KP. 2010. Molecular systematic and conservation of the langurs and leaf monkeys of South Asia. *J Genet* 89 (4):393-399.

Katoh K, Kuma K, Toh H, Miyata T. 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Res* 33:511-518.

Kazazian HH. 2004. Mobile elements: drivers of genome evolution. *Science* 303 (5664):1626-1632.

Kelley J. 2002. The hominoid radiation in Asia. In: Hartwig WC, editor. *The Primate Fossil Record*. Cambridge: Cambridge University Press. p 369-384.

Kingdon J. 1997. *The Kingdon Field Guide to African Mammals*. San Diego, California, USA: Academic Press Natural World.

Kirkpatrick RC. 1995. The natural history and conservation of the snub-nosed monkeys (genus *Rhinopithecus*). *Biol Conserv* 72(3):363-9.

Kirkpatrick RC. 2011. The Asian colobines: Diversity among leaf-eating monkeys. In: Campbell C, Fuentes A, MacKinnon K, Bearder S, Stumpf R, editors. *Primates in Perspective*. Oxford: Oxford University Press. p 189-202.

Kishino H, Hasegawa M. 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. *J Mol Evol* 29:170-179.

Koblmüller S, Duftner N, Sefc KM, Aibara M, Stipacek M, Blanc M, Egger B, Sturmbauer C. 2007. Reticulate phylogeny of gastropod-shell-breeding cichlids from Lake Tanganyika - the result of repeated introgressive hybridization. *BMC Evol Biol* 7:7.

Kubatko LS, Carstens BC, Knowles LL. 2009. STEM: species tree estimation using maximum likelihood for gene trees under coalescence. *Bioinformatics* 25:971-973.

Kuhn HJ. 1972. On the perineal organ of male *Procolobus badius*. *J Hum Evol* 1:371-378.

Lande R, Shannon S. 1996. The role of genetic variation in adaptation and population persistence in a changing environment. *Evolution* 50: 434–437.

Leakey MG. 1993. Evolution of *Theropithecus* in the Turkana Basin. In: Jablonski NG, editor. *Theropithecus, the Rise and Fall of a Primate Genus*. Cambridge: Cambridge University Press. p 85-124.

Lebatard AE, Bourlès DL, Düringer P, Jolivet M, Braucher R, Carcaillet J, Schuster M, Arnaud N, Monié P, Lihoreau F, Likius A, Mackaye HT, Vignaud P, Brunet M. 2008. Cosmogenic nuclide dating of *Sahelanthropus tchadensis* and *Australopithecus bahrelghazali*: Mio-Pliocene hominids from Chad. *Proc Natl Acad Sci USA* 105:3226-3231.

Li BG, Pan RL, Oxnard CE. 2002. Extinction of snub-nosed monkeys in China during the past 400 years. *Int J Primatol* 23:1227–1244.

Li HP, Meng SJ, Men ZM, Fu YX, Zhang YP. 2003. Genetic diversity and population history of golden monkeys (*Rhinopithecus roxellana*). *Genetics* 164:269–275.

Li J, Han K, Xing J, Kim HS, Rogers J, Ryder OA, Disotell T, Yue B, Batzer MA. 2009. Phylogeny of macaques (Cercopithecidae: *Macaca*) based on Alu elements. *Gene* 448:242–249.

Li M, Liang B, Feng ZJ, Tamate HB. 2001. Molecular phylogenetic relationships among Sichuan snub-nosed monkeys (*Rhinopithecus roxellana*) inferred from mitochondrial cytochrome b gene sequences. *Primates* 42:153–160.

Li M, Liu ZJ, Gou JX, Ren BP, Pan RL, Su YJ, Funk SM, Wei FW. 2007. Phylogeography and population structure of the golden monkeys (*Rhinopithecus roxellana*): inferred from mitochondrial DNA sequences. *Am J Primatol* 69:1195–1209.

Li Y, Wang X, Ting N, Zhang YP. 2011. Mitogenomic analysis of Chinese snub-nosed monkeys: Evidence of positive selection in NADH dehydrogenase genes in high-altitude adaptation. *Mitochondrion* 11:497–503.

Li M, Wei FW, Huang CM, Pan RL, de Ruiter J. 2004. Phylogeny of snub-nosed monkeys inferred from mitochondrial DNA, cytochrome b, and 12S rRNA sequences. *Int J Primatol* 25:861–873.

Liang B, Qi HJ, Zhang SY, Ren BP. 2001. Developmental traits of captive Sichuan snub-nosed monkeys (*Rhinopithecus roxellana*) at different age stages. *Acta Zool Sinica* 47:381–387.

Librado P, Rozas J. 2009. DnaSP version 5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25:1451–1452.

Liu WT, Huang CM, Roos C, Zhou QH, Li YB, Wei FW. 2008. Identification of the species, origin and sex of smuggled douc langur (*Pygathrix sp.*) remains. *Vietnamese J Primatol* 2: 63–69.

Liu ZJ, Ren BP, Wei FW, Long YC, Hao YL, Li M. 2007. Phylogeography and population structure of the Yunnan snubnosed monkey (*Rhinopithecus bieti*) inferred from mitochondrial control region DNA sequence analysis. *Mol Ecol* 16:3334–3349.

Liu ZJ, Ren BP, Wu RD, Zhao L, Hao YL, Wang BS, Wei FW, Long YC, Li M. 2009. The effect of landscape features on population genetic structure in Yunnan snub-

nosed monkeys (*Rhinopithecus bieti*) implies an anthropogenic genetic discontinuity. *Mol Ecol* 18:3831–3846.

Losos JB. 2011. Convergence, adaptation, and constraint. *Evolution* 65 (7):1827-1840.

MacKinnon J. 2008. Order primates. In: Smith AT, Xie Y, editors. *A guide to the mammals of China*. Princeton: Princeton University Press. p 158–172.

Maddison WP, Maddison DR. 2009. Mesquite: a modular system for evolutionary analysis, version 2.6. <http://mesquite.org>

Mayr E. 1963. *Animal species and evolution*. Cambridge, MA: Belknap Press.

McCracken K, Sorenson M. 2005. Is homoplasy or lineage sorting the source of incongruent mtDNA and nuclear gene trees in the stiff-tailed ducks (*Nomonyx-Oxyyura*)? *Syst Biol* 54:35-55.

McGraw WS, Fleagle JG. 2000. Biogeography and evolution of the *Cercocebus* - *Mandrillus* clade. *Am J Phys Anthro Supp* 30:225.

Md-Zain BM, Lee SJ, Lakim M, Ampeng A, Mahani MC. 2010. Phylogenetic position of *Tarsius bancanus* based on partial Cytochrome b DNA sequences. *J. Biol Sci* 10 (4):348-354.

Meijaard E, Groves CP. 2006. The geography of mammals and rivers in mainland Southeast Asia. In: Lehman SM, Fleagle JG, editors. *Primate Biogeography*. New York: Springer. p 305-329.

Merker S, Driller C, Perwitasari-Farajallah D, Pamungkas J, Zischler H. 2009. Elucidating geological and biological processes underlying the diversification of Sulawesi tarsiers. *Proc Natl Acad Sci USA* 106:8459-8464.

Messier W, Stewart CB. 1997. Episodic adaptive evolution of primate lysozymes. *Nature* 385:151–154.

Meyer D, Rinaldi ID, Ramlee H, Perwitasari-Farajallah D, Hodges JK, Roos C. 2011. Mitochondrial phylogeny of leaf monkeys (genus *Presbytis*, *Eschscholtz, 1821*) with implications for taxonomy and conservation. *Mol Phylogenet Evol* 59:311-319.

Miller KG, Kominz MA, Browning JV, Wright JD, Mountain GS, Katz ME, Sugarman PJ, Cramer BS, Christie-Blick N, Pekar SF. 2005. The phanerozoic record of global sea-level change. *Science* 310:1293-1298.

Mittermeier RA, Wallis J, Rylands AB, Ganzhorn JU, Oates JF, Williamson EA, Palacios E, Heymann EW, Kierulff MCM, Long Y, Supriatna J, Roos C, Walker S,

Corte's-Ortiz L, Schwitzer C. 2009. Primates in peril: the world's 25 most endangered primates 2008–2010. *J Primate Conserv* 24:1–57.

Modolo L, Salzburger W, Martin RD. 2005. Phylogeography of Barbary macaques (*Macaca sylvanus*) and the origin of the Gibraltar colony. *Proc Natl Acad Sci USA* 102:7392–7397.

Morales JC, Melnick DJ. 1998. Phylogenetic relationships of the macaques (*Cercopithecoida: Macaca*), as revealed by high resolution restriction site mapping of mitochondrial ribosomal genes. *J Hum Evol* 34:1–23.

Morin PA, Chambers KE, Boesch C, Vigilant L. 2001. Quantitative polymerase chain reaction analysis of DNA from noninvasive samples for accurate microsatellite genotyping of wild chimpanzees (*Pan troglodytes verus*). *Mol Ecol* 10:1835–1844.

Moulin S, Gerbault-Seureau M, Dutrilliaux B, Richard FA. 2008. Phylogenomics of African guenons. *Chromosome Res* 16 (5): 783–99.

Nadler T, Momberg F, Nguyen XD, Lormée N. 2003. Vietnam Primate Conservation Status Review 2002. Part 2: Leaf Monkeys. Hanoi: Frankfurt Zoological Society and Fauna & Flora International-Indochina Programme.

Napier JR, Napier PH. 1967. A handbook of living primates. London: Academic Press.

Napier PH. 1985. Catalogue of primates in the British Museum (Natural History) and elsewhere in the British Isles. Part III: Family Cercopithecidae, subfamily Colobinae. London, British Museum (Natural History).

Nei M. 1975. Molecular population genetics and evolution. New York: American Elsevier.

Nei M. 1977. F-statistics and analysis of gene diversity in subdivided populations. *Ann Hum Genet* 41:225–233.

Nei M. 1987. Molecular evolutionary genetics. New York: Columbia University Press.

Nei M, Li WH. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc Natl Acad Sci. USA* 76:5269–5273.

Newton PN, Dunbar RIM. 1994. Colobine monkey society. In: Davies AG, Oates JF, editors. Colobine monkeys: their ecology, behaviour and evolution. Cambridge: Cambridge University Press p 311e346.

Nichols R. 2001. Gene trees and species trees are not the same. *Trends Ecol Evol* 16:358-364.

Nguyen N. 2000. A survey of Tonkin snub-nosed monkeys (*Rhinopithecus avunculus*) in Northern Vietnam. *Folia Primatol* 71:157-160.

Oates JF. 1994. The natural history of African colobines. In: Davies AG, Oates JF, editors. *Colobine Monkeys: Their Ecology, Behaviour and Evolution*. Cambridge: Cambridge University Press. p 75-128.

Oates JF, Davies AG, Delson E. 1994. The diversity of living colobines. In: Davies AG, Oates JF, editors. *Colobine monkeys: their ecology, behavior and evolution*. Cambridge: Cambridge University Press.

Okada N. 1991. SINEs. *Curr Opin Genet Dev* 1:498-504.

Osterholz M, Walter L, Roos C. 2008. Phylogenetic position of the langur genera *Semnopithecus* and *Trachypithecus* among Asian colobines, and genus affiliations of their species groups. *BMC Evol Biol* 8:58.

Osterholz M, Walter L, Roos C. 2009. Retropositional events consolidate the branching order among New World monkey genera. *Mol Phylogenet Evol* 50:507-513.

Page SL, Chiu C, Goodman M. 1999. Molecular phylogeny of Old World monkeys (*Cercopithecidae*) as inferred from γ -globin DNA sequences. *Mol Phylogenet Evol* 13:348–359.

Pan YR. 1995. Fossil primates discovered in China. In: Xia W, Zhang Y, editors. *Primate Research and Conservation*. Beijing: China Forestry Publishing House. p 99–105.

Pan D, Hu HX, Meng SJ, Men ZM, Fu YX, Zhang YP. 2009. A high polymorphism level in *Rhinopithecus roxellana*. *Int J Primatol* 30:337–351.

Pan YR, Jablonski NG. 1987. The age and geographical distribution of fossil cercopithecids in China. *Hum Evol* 2: 59-69.

Pan RL, Oxnard CE. 2001. Cranial morphology of the golden monkey (*Rhinopithecus*) and douc langur (*Pygathrix nemaeus*). *Hum Evol* 16:199–223.

Patterson N, Richter DJ, Gnerre S, Lander ES, Reich D. 2006. Genetic evidence for complex speciation of humans and chimpanzees. *Nature* 441:1103-1108.

Pääbo S. 2003. The mosaic that is our genome. *Nature* 421:409-412.

Peakall R, Smouse PE. 2006. GENALEX 6: genetic analysis in Excel: population genetic software for teaching and research. *Mol Ecol Notes* 6:1288–1295.

Peng YZ, Pan RL, Jablonski N. 1993. Classification and evolution of Asian colobines. *Folia Primatol* 60:106-117.

Peng YZ, Ye ZZ, Zhang YP, Pan RL. 1988. The classification and phylogeny of snub-nosed monkey *Rhinopithecus* spp. based on gross morphological characters. *Zool Res* 9:239–248.

Perelman P, Johnson WE, Roos C, Seuánez HN, Horvath JE, Moreira MAM, Kessing B, Pontius J, Roelke M, Rumpel Y, Schneider MPC, Silva A, O'Brien SJ, Pecon-Slattery J. 2011. A molecular phylogeny of living primates. *PLoS Genet* 7:e1001342.

Petit RJ, Excoffier L. 2009. Gene flow and species delimitation. *Trends Ecol Evol* 4:386-393.

Philippe H, Laurent J. 1998. How good are deep phylogenetic trees? *Curr Opin Genetics Dev* 8:616-623.

Pocock RI. 1935. The monkeys of the genera *Pithecus* (*Presbytis*) and *Pygathrix* found to the east of the Bay of Bengal. *Proc Zool Soc* 104:1895–1961.

Posada D. 2008. jModelTest: Phylogenetic model averaging. *Mol Biol Evol* 25:1253–1256.

Pollard DA, Iyer VN, Moses AM, Eisen MB. 2006. Widespread discordance of gene trees with species tree in *Drosophila*: evidence for incomplete lineage sorting. *PLoS Genet* 2:e173.

Posada D, Crandall KA. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14:817-818.

Posada D. 2008. Model Test: phylogenetic model averaging. *Mol Biol Evol* 25:1253–1256.

Pusey AE, Packer C. 1987. Dispersal and philopatry. In: Smuts BB, Cheney DL, Seyfarth RM, Wrangham RW, Struhsaker TT, editors. *Primate Societies*. Chicago: University of Chicago Press. p 250-266.

Qi XG, Li BG, Ji WH. 2008. Reproductive parameters of wild female *Rhinopithecus roxellana*. *Am J Primatol* 70:311–319.

Quan GQ, Xie JH. 1981. Notes on *Rhinopithecus roxellanae brelichi* (Thomas). *Acta Theriol Sinica* 1:113–116.

Quan GQ, Xie JY. 2002. Research on the golden monkey. Shanghai: Science and Education Publishing House.

Raaum RL, Sterner KN, Noviello CM, Stewart CB, Disotell TR. 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. 2008. FigTree: Tree figure drawing tool, version 1.2.2. Available at: <http://tree.bio.ed.ac.uk/software/figtree/>.

Rambaut A, Drummond AJ. 2007. Tracer: MCMC trace analysis tool, version 1.4.1. Available at: <http://tree.bio.ed.ac.uk/software/tracer/>.

Ray DA, Xing J, Salem AH, Batzer MA. 2006. SINEs of a nearly perfect character. *Syst Biol* 55:928-935. Ray DA, Xing J, Hedges DJ, Hall MA, Laborde ME, Anders BA, White BR, Stoilova N, Fowlkes JD, Landry KE, Chemnick LG, Ryder OA, Batzer MA. 2005. Alu insertion loci and platyrrhine primate phylogeny. *Mol Phylogenet Evol* 35:117-126.

Reichenbach HGL. 1862. Die Vollständige Naturgeschichte der Affen. In *Die Vollständige Naturgeschichte des in- und Auslandes* Dresden. Dresden and Leipzig, Germany: Central-Atlas für Zoologische Gärten.

Ren B, Li M, Long Y, Grüter CC, Wei F. 2008. Measuring daily ranging distances of *Rhinopithecus bieti* via a Global Positioning System collar at Jinsichang, China: a methodological consideration. *Int J Primatol* 29:783–794.

Rogers AR, Harpending H. 1992. Population growth makes waves in the distribution of pairwise genetic differences. *Mol Biol Evol* 9:552–569.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

Roos C, Nadler T. 2001. Molecular evolution of the douc langurs. *Zool Garten NF* 71:1–6.

Roos C, Ziegler T, Hodges K, Zischler H, Abegg C. 2003. Molecular phylogeny of Mentawai macaques: taxonomic and biogeographic implications. *Mol Phylogenet Evol* 29:139-150.

Roos C. 2004. Molecular evolution and systematics of Vietnamese primates. In: Nadler T, Streicher U, Ha Thang Long, editors. *Conservation of primates in Vietnam*. Hanoi: Haki Publishing. p 23-28.

Roos C, Schmitz J, Zischler H. 2004. Primate jumping genes elucidate strepsirrhine phylogeny. *Proc Natl Acad Sci USA* 101:10650-10654.

Roos C, Thanh VN, Walter L, Nadler T. 2007. Molecular systematic of Indochinese primates. *Vietn J Primatol* 1:41–53.

Roos C, Nadler T, Walter L. 2008. Mitochondrial phylogeny, taxonomy and biogeography of the silvered langur species group (*Trachypithecus cristatus*). *Mol Phylogenet Evol* 47 (2):629-636.

Rowe N. 1996. The pictorial guide to the living primates. East Hampton: Pogonias Press.

Rumpler Y, Warter S, Hauwy M, Fausser JL, Roos C, Zinner D. 2008. Comparing chromosomal and mitochondrial phylogenies of sportive lemurs (genus *Lepilemur*, Primates). *Chromosome Res* 16:1143-1158.

Salem AH, Ray DA, Xing J, Callinan PA, Myers JS, Hedges DJ, Garber RK, Witherspoon DJ, Jorde LB, Batzer MA. 2003. Alu elements and hominid phylogenetics. *Proc Natl Acad Sci USA* 100:12787-12791.

Salem AH, Ray DA, Batzer MA. 2005. Identity by descent and DNA sequence variation of human SINE and LINE elements. *Cytogenet Genome Res* 108:63-72.

Santos C, Montiel R, Sierra B, Bettencourt C, Fernandez E, Alvarez L, Lima M, Abade A, Aluja MP. 2005. Understanding differences between phylogenetic and pedigree-derived mtDNA mutation rate: a model using families from the Azores islands (Portugal). *Mol Biol Evol* 22:1490–1505.

Savage JM. 1997. Society of Systematic Biologists Presidential Address. Boulder: University of Colorado.

Seehausen O. 2004. Hybridization and adaptive radiation. *Trends Ecol Evol* 19:198-207.

Schmidt D, Pool J. 2002. The effect of population history on the distribution of the Tajima's D statistic. New York: Cornell University Press.

Schmitz J, Ohme M, Zischler H. 2001. SINE insertions in cladistic analyses and the phylogenetic affiliations of *Tarsius bancanus* to other primates. *Genetics* 157:777-784.

Schmitz J, Roos C, Zischler H. 2005. Primate phylogeny: molecular evidence from retroposons. *Cytogenet Genome Res* 108:26-37.

Schneider S, Excoffier L. 1999. Estimation of demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. *Genetics* 152:1079–1089.

Shedlock AM, Okada N. 2000. SINE insertions: powerful tools for molecular systematics. *Bioessays* 22:148-160.

Simberloff D. 1988. The contribution of population and community biology to conservation science. *Annu Rev Ecol Syst* 19:473–511.

Shimodaira H, Hasegawa M. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Mol Biol Evol* 16:1114-1116.

Soares P, Ermini L, Thomson N, Mormina M, Rito T, Roñhl A, Salas A, Oppenheimer S, Macaulay V, Richards MB. 2009. Correcting for purifying selection: an improved human mitochondrial molecular clock. *Am J Hum Genet* 84:740–759.

Soltis PS, Soltis DE. 2003. Applying the bootstrap in phylogeny reconstruction. *Statistical Sci* 18 (2):256-267.

Spielman D, Brook BW, Frankham R. 2004. Most species are not driven to extinction before genetic factors impact them. *Proc Natl Acad Sci USA* 101:15261–15264.

Stearns SC, Hoekatry RF. 2000. *Evolution: an introduction*. New York: Oxford University Press.

Sterner KR, Raaum RL, Zhang YP, Stewart CB, Disotell TR. 2006. Mitochondrial data support an odd-nosed colobine clade. *Mol Phylogenet Evol* 40:1-7.

Stewart CB, Disotell TR. 1998. Primate evolution – in and out of Africa. *Curr Biol* 8:R582-R588.

Stewart CB, Disotell TR. 1999. Primate evolution – in and out of Africa. *Curr Biol* 9:R120-R122.

Strasser E, Delson E. 1987. Cladistic analysis of cercopithecoid relationships. *J Hum Evol* 16:81-99.

Swofford, DL. 2003. *PAUP*: phylogenetic analysis using parsimony (* and other methods)*, version 4.0b10. Sunderland, MA: Sinauer.

Struhsaker TT. 1981. Census methods for estimating densities. In: Struhsaker TT, editor. *Techniques for the Study of Primate Population Ecology*. Washington DC: National Academy Press. p 36–80.

Szalay FS, Delson E. 1979. *Evolutionary history of the primates*. New York: Academic Press.

Tajima F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123:585–595.

Tan C, Guo S, Li B. 2007. Population structure and ranging patterns of *Rhinopithecus roxellana* in Zhouzhi National Nature Reserve, Shaanxi, China. *Int J Primatol* 28:577–591.

Thalmann O, Fischer A, Lankester F, Pääbo S, Vigilant L. 2007. The complex evolutionary history of gorillas: insights from genomic data. *Mol Biol Evol* 24:146-158.

Thinh VN, Mootnick AR, Geissmann T, Li M, Ziegler T, Agil M, Moisson P, Nadler T, Walter L, Roos C. 2010. Mitochondrial evidence for multiple radiations in the evolutionary history of small apes. *BMC Evol Biol* 10:74.

Ting N. 2005. Mitochondrial relationships and divergence dates of the African colobines: evidence of Miocene origins for the living colobus monkeys. *J Hum Evol* 55:312-325.

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.

Ting N, Tosi AJ, Li Y, Zhang YP, Disotell TR. 2008. Phylogenetic incongruence between nuclear and mitochondrial markers in the Asian colobines and the evolution of the langurs and leaf monkeys. *Mol Phylogenet Evol* 46:466-474.

Tosi AJ, Detwiler KM, Disotell TR. 2003. X-chromosomal window into the evolutionary history of the guenons (Primates: *Cercopithecini*). *Mol Phylogenet Evol* 36:58-66.

Tosi AJ, Melnick DJ, Disotell TR. 2004. Sex chromosome phylogenetics indicate a single transition to terrestriality in the guenons (tribe *Cercopithecini*). *J Hum Evol* 46 (2), 223–237.

Tosi AJ, Detwiler KM, Disotell TR. 2005. Y-chromosomal markers suitable for non-invasive studies of guenon hybridization. *Int J Primatol* 36:58-66.

Usher MB. 1997 . Small populations: Fragmentation, population dynamics and population genetics. In: Tew TE, Crawford TJ, Spencer JW, Stevens DP, Usher MB, Warren J, editors. *The role of genetics in conserving small populations*. United Kingdom: Joint Nature Conservation Committee, Peterborough. p 11 – 21.

Van de Lagemaat LN, Gagnier L, Medstrand P, Mager DL. 2005. Genomic deletions and precise removal of transposable elements mediated by short identical DNA segments in primates. *Genome Res* 15:1243-1249.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. 2004. Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. *Mol Ecol Notes* 4:535–538.

Verheyen WN. 1962. Contribution a la craniologie comparee des Primates: les genres *Colobus* Illiger 1811 et *Cercopithecus* Linne. 1758. *Ann. Mus. Roy. de l'Afrique Centrale, Ser. in 8°*, *Sci Zool* 105:1-255.

Vignaud P, Durringer P, Mackaye HT, Likies A, Blondel C, Boisserie JR, De Bonis L, Eisenmann V, Etienne ME, Geraads D, Guy F, Lehmann T, Lihoreau F, Lopez-Martinez N, Mourer-Chauviré C, Otero O, Rage JC, 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.

Vogel C. 1976. Ecology, lifestyle and social behavior of langur groups in differing Indian habitats. *Fort der Verhal Tensf* 17: 1-160.

Wakeley J. 1997. Using the variance of pairwise differences to estimate the recombination rate. *Genet Res* 69:45–48.

Waits LP, Luikart G, Taberlet P. 2001. Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. *Mol Ecol* 10:249–256.

Wang W, Forstner MRJ, Zhang YP, Liu ZM, Wei Y, Huang HQ, Hu HG, Xie YX, Wu DH, Melnick DJ. 1997. A phylogeny of Chinese leaf monkeys using mitochondrial ND3-ND4 gene sequences. *Int J Primatol* 18:305–320.

Weitzel V, Yang CM, Groves CP. 1988. A catalogue of primates in the Singapore Zoological Reference Collection. *Raffles Bull Zool* 36:1-166.

Whelan S, Lió P, Goldman N. 2001. Molecular phylogenetics: state-of-the-art methods for looking into the past. *Trends in Genetics* 17 (5):262-272

Whittaker DJ, Ting N, Melnick DJ. 2006. Molecular phylogenetic affinities of the simakobu monkey (*Simias concolor*). *Mol Phylogenet Evol* 39:887–892.

Whitfield LS, Lovell-Badge R, Goodfellow PN. 1993. Rapid sequence evolution of the mammalian sex-determining gene SRY. *Nature* 364:713-715.

Whybrow PJ. 1992. Land movements and species dispersal. In: Jones S, Martin RE, Pilbeam D, editors. *The Cambridge Encyclopedia of Human Evolution*. Cambridge: Cambridge University Press. p 169-173.

Wiley EQD, Siegel-Causey DRB, Funk DVA. 1991. The complete cladist. Univ Kans Mus Nat Hist Spec Publ 19:1-158.

Winney BJ, Hammond RL, Macasero W, Flores B, Boug A, Biquand V, Biquand S, Bruford MW. 2004. Crossing the Red Sea: phylogeography of the hamadryas baboon, *Papio hamadryas hamadryas*. Mol Ecol 13:2819–2827.

Wright S. 1969. Evolution and the genetics of populations. II: The theory of gene frequencies. Chicago: University of Chicago Press.

Wright S. 1978. Evolution and the genetics of populations. IV: the variability within and among natural populations. Chicago: University of Chicago Press.

Wu G, Wang HC, Fu HW, Zhao JZ, Yang YQ. 2004. Habitat selection of Guizhou golden monkey (*Rhinopithecus roxellanae brelichi*) in Fanjing Mountain Biosphere Reserve, China. J Forest Res 15:197–202.

Wyman SK, Jansen RK, Boore JL. 2004. Automatic annotation of organellar genomes with DOGMA. Bioinformatics 20:3252-3255.

Xing J, Wang H, Han K, Ray DA, Huang CH, Chemnick LG, Stewart CB, Disotell TR, Ryder OA, Batzer MA. 2005. A mobile element based phylogeny of Old World monkeys. Mol Phylogenet Evol 37:872-880.

Xing J, Wang H, Zhang Y, Ray DA, Tosi AJ, Disotell TR, Batzer MA. 2007. A mobile element based evolutionary history of guenons (Tribe Cercopithecini). BMC Biol 5:5.

Xing J, Witherspoon DJ, Ray DA, Batzer MA, Jorde LB. 2007. Mobile DNA elements in primate and human evolution. Am J Phys Anthropol 45(Suppl):2-19.

Yang M, Sun D, Zinner D, Roos C. 2009. Reproductive parameters in Guizhou snub-nosed monkeys (*Rhinopithecus brelichi*). Am J Primatol 71:266–270.

Yang YQ, Lei XP, Sun DY, Yang CD, He RT, Zhang WY. 2002. Ecology of the wild Guizhou snub-nosed monkey. Guiyang: Guizhou Scientific Institute Press.

Yang Z. 2002. Likelihood and Bayes estimation of ancestral population sizes in hominoids using data from multiple loci. Genetics 162:1811-1823.

Young NM, MacLachy L. 2004. The phylogenetic position of *Moropithecus*. J Hum Evol 46:163-184.

Zalmout IS, Sanders WJ, MacLachy LM, Gunnell GF, Al-Mufarreh YA, Ali MA, Nasser AAH, Al-Masari AM, Al-Sobhi SA, Nadhra AO, Matari AH, Wilson JA, Gingerich

PD. 2010. New Oligocene primate from Saudi Arabia and the divergence of apes and Old World monkeys. *Nature* 466:360-365.

Zhang SY, Liang B, Wang LX. 2000. Seasonality of mating and births in captive Sichuan golden monkeys (*Rhinopithecus roxellanae*). *Am J Primatol* 51:265–269.

Zhang YP, Ryder OA, Fan Z, Zhang H. 1997. Sequence variation and genetic diversity in giant panda. *Sci China C Life Sci* 27:139–144.

Zhang YP, Ryder OA. 1998. Mitochondrial cytochrome b gene sequences of Old World monkeys: with a special reference on evolution of Asian colobines. *Primates* 39:39–49.

Zhang YP, Ryder OA. 1999. Primate evolution – in and out of Africa. *Curr Biol* 9:R119-R120.

Zhang YZ, Quan GQ, Zhao TG, Southwick CH. 1992. Distribution of primates (except *Macaca*) in China. *Acta Theriol Sinica* 12:81–95.

Zhang P, Watanabe K, Li B. 2008. Female social dynamics in a provisioned free-ranging band of the Sichuan snub-nosed monkey (*Rhinopithecus roxellana*) in the Qinling mountains, China. *Am J Primatol* 70:1013–1022.

Ziegler T, Abegg C, Meijaard E, Perwitasari-Farajallah D, Walter L, Hodges JK, Roos C. 2007. Molecular phylogeny and evolutionary history of Southeast Asian macaques forming the *M. silenus* group. *Mol Phyl Evol* 42:807-816.

Zinner D, Arnold ML, Roos C. 2009. Is the new primate genus *Rungwecebus* a baboon? *PLoS ONE* 4:e4859.

Zinner D, Arnold ML, Roos C (in press). The strange blood: natural hybridization in primates. *Evol Anthropol*

Zinner D, Groeneveld LF, Keller C, Roos C. 2009. Mitochondrial phylogeography of baboons (*Papio spp.*) – indication for introgressive hybridization? *BMC Evol Biol* 9:83.

Zwickl DJ. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum-likelihood criterion. PhD thesis. The University of Texas at Austin.

Curriculum Vitae

PERSONAL

Date of Birth: January 22, 1973

Status: Married

Gender: Female

Nationality: P.R. China

EDUCATION

2007 – Present

Ph.D. in Biological Sciences - Georg-August-University Göttingen, Germany

Thesis:

Evolutionary History of Snub-nosed Monkeys (*Rhinopithecus spp.*) and Population Genetic Structure of the Guizhou Snub-nosed Monkey (*R. brelichi*)

Area of Specialization:

Phylogenetics and Population Genetics

Advisor:

Prof. Dr. Peter M. Kappeler

1994 – 1997

M.S. in Medicine Sciences - Guiyang Medical College, Guizhou, China,

Thesis:

The Analysis of Physiological and Biochemical Index in Guizhou Snub-nosed Monkey (*R. brelichi*)

Advisor:

Prof. Hanbin Chen

1990 – 1994

B.A. in Biological Sciences - South-West Forestry University, Yunnan, China.

Publications

Yang MY, Roos C, Nadler T, Zinner D. in press. The evolutionary history of Odd-nosed monkeys. In: Tan CL, Grueter C, Wright B, editors. Odd-nosed monkeys: Recent advances in the study of the forgotten Colobines. *Primateology: Progress and Prospects* series.

Roos C, Zinner D, Kubatko LS, Schwarz C, **Yang MY**, Meyer D, Dash SD, Xing JC, Batzer MA, Brameier M, Leendertz FH, Ziegler T, Perwitasari-Farajallah D, Nadler T, Walter L, Osterholz M. 2011. Nuclear versus mitochondrial DNA: evidence for hybridization in Colobine monkeys. *BMC Evolutionary Biology* 11: 1-13. DOI: 10.1186/1471-2148-11-77.

Yang MY, Yang YQ, Cui DY, Fickenscher G, Roos C, Brameier M. 2011. Population genetic structure of Guizhou snub-nosed monkeys (*Rhinopithecus brelichi*) as inferred from mitochondrial control region sequences, and comparison with *R.bieti* and *R.roxellana*. *Am J Phys Anthropol*. DOI: 10.1002/ajpa.21618

Yang MY, Sun DY, Zinner D, Roos C. 2009. Reproductive parameters in Guizhou snub-nosed monkeys (*Rhinopithecus brelichi*). *Am J Primatol* 71 (3): 266 – 270.

Yang MY, Pyörnilä A, Meyer-Rochow BV. 2004. UV-reflectivity of parafocal eyespot elements on butterfly wings in normal and abnormal specimens. *Entomol Fennica* 15 (1):34 - 40.

Yang MY, Meyer-Rochow BV. 2004. Fine-structural details of the photoreceptor membranes of the ocellus of the scale-insect parasite *Centrodora sp.* (*Hymenoptera, Apheneilidae*): A case of gene transfer between host and parasite? *Biocell* 28 (2):151-154.

Meyer-Rochow BV, **Yang MY**. 2004. Not just an empty cavity: the inter-rhabdomal space in the Jamaican cave fly *Neoditomyia farri* (*Diptera, Mycetophilidae*). *Zool Sci* 21:719-722.

Yang MY, Cheng HB. 2002. To determine and analysis E2 and TEST of Guizhou Golden Monkey by using radioimmunoassay. *Guizhou Science* 20 (3):56-58. (in Chinese)

Yang MY, Lei XP, Qiu Y. 1998. Test and analysis eight microelements from hair of wild and artificial breeding Guizhou Golden Monkey. J Guiyang Medical College 11(2):67-69. (in Chinese)

Wu FL, Tao BY, **Yang MY**, Sun DY. 1997. Test of T3, T4 and TSH blood concentration: compare and analysis the relation among Guizhou Golden Monkey, Yunnan Golden Monkey and Sichuan Golden Monkey. J Guiyang Medical College 22 (2):193-194. (in Chinese)

Wu FL, Tao BY, **Yang MY**, Sun DY. 1997. The analysis of serum estradiol content of Guizhou snub-nosed monkeys. Guizhou Sci 15 (2):118–120. (in Chinese)

Yang MY, Wu FL, Tao BY, Sun DY. 1996. To determine and analysis blood coagulation factor of Guizhou Golden Monkey. Guizhou Sci 14 (4):29-32. (in Chinese)

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Mouyu Yang

Bad Homburg, November 2011
