

Molecular phylogeny of Mentawai macaques: taxonomic and biogeographic implications

Christian Roos,^{a,*} Thomas Ziegler,^b J. Keith Hodges,^b Hans Zischler,^{a,1} and Christophe Abegg,^b

^a Primate Genetics, German Primate Centre, Kellnerweg 4, 37077 Göttingen, Germany

^b Department of Reproductive Biology, German Primate Centre, Kellnerweg 4, 37077 Göttingen, Germany

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Abstract

In order to elucidate the evolutionary history and taxonomy of the Mentawai macaques, we sequenced a 567 base pairs (bp) long fragment of the mitochondrial cytochrome b gene from 39 individuals representing pigtailed macaque populations from Siberut, Sipora, South Pagai, and Sumatra. Pairwise difference analyses carried out within and among populations have shown, that: (1) variation within populations is relative low, (2) variation among populations is increased, and (3) pairwise differences within and among the populations from Sipora and South Pagai are in the same range. From phylogenetic tree reconstructions including further macaque species, we detected a paraphyletic origin of Mentawai macaques with the Siberut population more closely related to *Macaca nemestrina* from Sumatra, than it is to populations from the Southern islands. Based on these results, we favour a scenario in that macaques entered the Mentawai islands by two independent colonisation events. Taking together the paraphyletic origin of Mentawai macaques and the genetic differences detected among pigtailed macaque populations, which are comparable with those observed among the seven Sulawesi macaque species, we propose to separate macaques from Siberut and Sipora, North and South Pagai into two distinct species, *Macaca siberu* and *Macaca pagensis*, respectively.

1. Introduction

The Mentawai islands off Sumatra's west coast, an archipelago that comprises the four major islands Siberut, Sipora, North Pagai, and South Pagai, are of considerable conservation significance due to their high incidence of endemic species. Among these, the geographic distribution of the four different primate taxa *Macaca pagensis*, *Simias concolor*, *Presbytis potenziani*, and *Hylobates klossii* is exclusively restricted to this hotspot of primate biodiversity.

Among the different Mentawai primates, information about the macaques is especially scarce. The phylogenetic relationships of Mentawai macaques to other members of the genus *Macaca*, the affiliations among them, and their taxonomic status are still controversially discussed, largely due to the extremely limited database on these remote and largely inaccessible primates. Concerning their higher level phylogenetic affiliations, several mutually exclusive alternatives are discussed. It is generally agreed that the Mentawai macaques, as close relatives of pigtailed macaques, belong to the *silenus*–*sylvanus* group (Fooden, 1976), which is distributed over a broad geographic range in Asia (Fig. 1), but also includes the only extant African macaque, *Macaca sylvanus*. Genetic data allow an

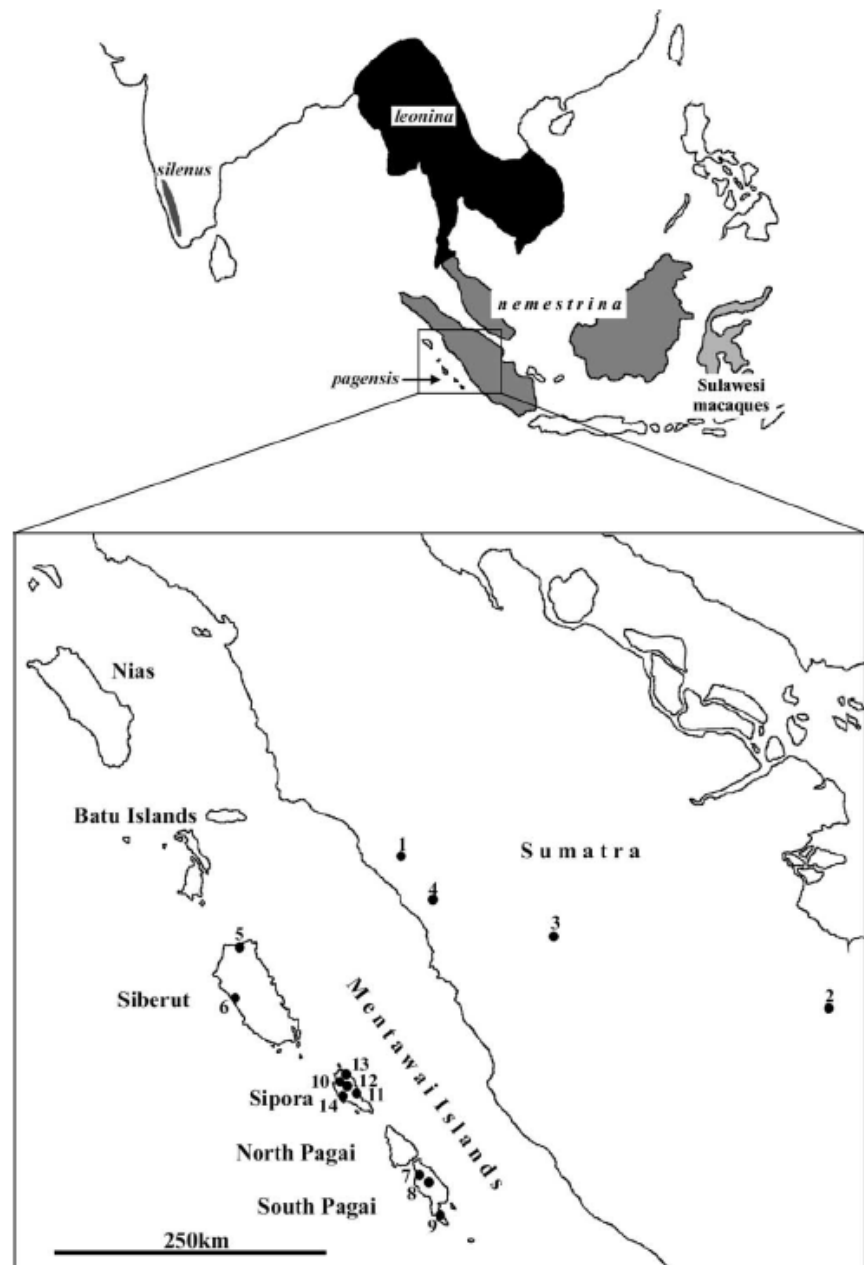


Fig. 1. Map displaying the geographic distribution of the *silenus* group. The more detailed map shows the origin of the Mentawai and Sumatra samples. Numbers refer to individual subpopulations analysed (see also Table 1).

interpretation with *M. sylvanus* representing the sister clade to all Asian macaques (Deinard and Smith, 2001; Hayasaka et al., 1996; Morales and Melnick, 1998), which justifies its separation into a separate, monotypic species group (Delson, 1980). The silenus group comprises several taxa, including *Macaca silenus*, seven species of Sulawesi macaques and pigtailed macaques, which are divided by Fooden (1975, 1980) into the Sunda form (*M. nemestrina nemestrina*), the Northern form (*M. n. leonina*), and the Mentawai form (*M. n. pagensis*). Groves (2001) recognises all three pigtailed macaques as distinct species, and we will adopt this model here. Fossil evidence suggests a scenario in which macaques colonised Asia about 5.5 million years ago (Mya) (Delson, 1996). Subsequently, a major split occurred which resulted in a splitting into two major lineages, the silenus group and a proto-fascicularis (Morales and Melnick, 1998) clade. Within the silenus group, it is now estimated that the Sulawesi macaques diverged from the silenus–nemestrina clade about 4.5 million years ago (Groves, 2001; Morales and Melnick, 1998), much earlier than previously suggested (1.0–0.7 Mya) (Delson, 1980; Fooden, 1969).

Most authorities now consider the Mentawai macaques as a distinct species (*M. pagensis*), resembling most closely the longtailed macaque (*Macaca fascicularis*) although possessing a short tail (Groves, 2001; Tenaza, 1975; Wilson and Wilson, 1977). Interpretations based on morphological and preliminary behavioural studies, however, conclude that they share features with *M. silenus* and the Sulawesi macaques (Abegg and Thierry, 2002a,b). Fooden's original arrangement (1975, 1980), which classified the taxon as a subspecies (*M. nemestrina pagensis*) of pigtailed macaque was based on colour, cranial and tail morphological features, and this interpretation—that, the Mentawai macaque is either a subspecies of *M. nemestrina* or, at very least, that this is its closest relative, is supported by ecological (Watanabe, 1979; Whitten and Whitten, 1982) and genetic data, indicating a close phylogenetic relationship of Mentawai macaques to other pigtailed macaques (*M. nemestrina* and *M. leonina*) (Evans et al., 1999; Rosenblum et al., 1997; Scheffrahn et al., 1996; Tosi et al., 2000).

From recent work of Fuentes and Olson (1995) and Groves (1996, 2001) it is becoming evident that differentiation between Mentawai macaques and other pigtailed macaques, as well as between the populations of the different islands of the archipelago, is more pronounced than formerly assumed. Thus, based on morphological studies, these authors proposed the existence of two different subspecies within *M. pagensis*: *M. p. pagensis* and *M. p. siberu* located on the three southern islands and Siberut, respectively. Extrapolating from the limited material available it has even recently been proposed that a separation of the two subspecies at the species level (Kitchener and Groves, 2002) would be justified, and this would also be supported by limited behavioural observations (Abegg and Thierry, 2002a). There has so far been no support for this classification from molecular studies, since in earlier analyses by Evans et al. (1999) and Rosenblum et al. (1997), only samples from Siberut macaques were included.

In order to get a more complete picture of the Mentawai macaque diversity, their evolutionary history and taxonomic status, we initiated a study including macaque individuals from Siberut, Sipora, and South Pagai. To this end, we used non-invasively obtained DNA from free ranging and zoo animals to PCR-amplify and sequence a portion of the mitochondrial cytochrome b gene. The resulting sequences were compared with the orthologs obtained from *M. nemestrina* from Sumatra, the seven Sulawesi macaque species and three other macaque species representing the sylvanus and the proto-fascicularis clade.

Our first aim was to clarify the phylogenetic relationships among the analysed taxa and their taxonomic position. Secondly, since it is obvious that the Mentawai islands can serve as a model for a recent radiation, a well established phylogeography of Mentawai macaques could therefore, represent a first platform to derive a model for the colonisation of the Mentawai islands by mammalian representatives.

2. Materials and methods

Faecal samples from 39 individuals representing pigtailed macaque populations from Sumatra and the Mentawai islands were collected from free ranging populations and zoo animals. To achieve the optimum geographic representation of the four islands, two different subpopulations on Siberut, five on Sipora, and three on South Pagai as well as four macaque subpopulations on the west coast of Sumatra were sampled (Fig. 1). Emphasis was given to a geographically broad taxonomic sampling of Mentawai macaques instead of a complete representation of the respective macaque group. In order to avoid repeated sampling of the same individuals, animals were observed and identified on the basis of characters pertaining to fur coloration, age, and gender. Hence, only fresh stool samples that could be individually assigned were collected and stored in 70% ethanol at ambient temperature for up to six months before further processing. To put the Mentawai macaque sequence information into context with other macaque taxa, DNA extracted from *M. sylvanus*, *Macaca thibetana*, and *M. fascicularis* blood samples was used to amplify the respective orthologs in these taxa. In addition, the data set was further expanded with homologous sequences from all seven currently recognised Sulawesi macaques and *Papio hamadryas* that are deposited in GenBank. Thus, a total of 50 individuals constitutes the initial data set. Further details are given in Table 1.

DNA was extracted from feces samples using the Qiagen Stool Kit as recommended by the supplier. DNA isolations from peripheral blood lymphocytes was done according to standard methods with an over-night digestion with Proteinase K followed by salting out of the proteins and subsequent ethanol precipitation of the DNA as outlined in Sambrook et al. (1989).

Species	Origin	Subpopulation (see Fig. 1)	Sample type	Abbreviation
<i>Papio hamadryas</i>	GenBank: Y16590	–	Sequence	–
<i>M. sylvanus</i>	Nuremberg zoo, Germany	–	Blood	–
<i>M. thibetana</i>	Primate Centre Strasbourg, France	–	Blood	–
<i>M. fascicularis</i>	German Primate Centre	–	Blood	–
<i>M. tonkeana</i>	GenBank: AF349041	–	Sequence	–
<i>M. maura</i>	GenBank: AF350379	–	Sequence	–
<i>M. hecki</i>	GenBank: AF350376	–	Sequence	–
<i>M. ochreata</i>	GenBank: AF350400	–	Sequence	–
<i>M. brunnescens</i>	GenBank: AF350407	–	Sequence	–
<i>M. nigra</i>	GenBank: AF350386	–	Sequence	–
<i>M. nigrescens</i>	GenBank: AF350383	–	Sequence	–
<i>M. n. nemestrina</i>	Pasaman, Sumatra	1	Feces	Sumatra 1, 2, 3, 4, 5, 6
<i>M. n. nemestrina</i>	Jambi, Sumatra	2	Feces	Sumatra 7
<i>M. n. nemestrina</i>	Pesisir selatan, Sumatra	3	Feces	Sumatra 8
<i>M. n. nemestrina</i>	Sicincin, Sumatra	4	Feces	Sumatra 9
<i>M. n. nemestrina</i>	Bukittinggi zoo, Sumatra	–	Feces	Sumatra 10
<i>M. n. nemestrina</i>	Bukittinggi zoo, Sumatra	–	Feces	Sumatra 11
<i>M. siberu</i>	Politioman, Siberut	5	Feces	Siberut 1, 2, 3
<i>M. siberu</i>	Sikapokna, Siberut	5	Feces	Siberut 4
<i>M. siberu</i>	Simatalu, Siberut	6	Feces	Siberut 5
<i>M. siberu</i>	Bukittinggi zoo, Sumatra	–	Feces	Siberut 6, 7
<i>M. siberu</i>	Padang, Sumatra	–	Feces	Siberut 8, 9, 10, 11, 12
<i>M. pagensis</i>	Malakopa, South Pagai	7	Feces	SPagai 1, 2, 3
<i>M. pagensis</i>	Kilo 37, South Pagai	8	Feces	SPagai 4, 5
<i>M. pagensis</i>	Matatonan, South Pagai	9	Feces	SPagai 6
<i>M. pagensis</i>	Bogor zoo, Java	–	Feces	SPagai 7, 8
<i>M. pagensis</i>	Tuapejat, Sipora	10	Feces	Sipora 1
<i>M. pagensis</i>	Sioban, Sipora	11	Feces	Sipora 2, 3
<i>M. pagensis</i>	Mara, Sipora	11	Feces	Sipora 4
<i>M. pagensis</i>	Sailoinan, Sipora	12	Feces	Sipora 5
<i>M. pagensis</i>	Saurenu, Sipora	12	Feces	Sipora 6
<i>M. pagensis</i>	Pogarik, Sipora	13	Feces	Sipora 7
<i>M. pagensis</i>	Betumonga, Sipora	14	Feces	Sipora 8

Table 1. Origin, sample type and abbreviation of analysed individuals

A 720 bp fragment of the mitochondrial cytochrome b gene was PCR-amplified (Saiki et al., 1988) using the oligonucleotide primers CYT-L: 5'-AATGATATGAA AAACCATCGTTTTA-3' and CYT-H: 5'-TAGTAGG GGTGGAAGGGGATTTT-3'. This fragment size represented the maximum size we could successfully amplify from all DNAs obtained from the feces samples (N = 39). Standard, wax-mediated hot-start PCRs were carried out for 35 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. A final extension for 5 min was added at the end of the reaction. To check the specificity of the reaction and the amount of DNA generated in the PCR amplifications, aliquots of the reaction were run on agarose gels and visualised by ethidium-bromide staining. Subsequently, about 100 ng of amplicate were further processed as template in the sequencing reaction using the Amersham Thermo Sequenase Primer Cycle Sequencing Kit according to the supplier's recommendations. All sequences were electrophoresed on an automated LICOR 4200L sequencer. The respective sequences have been deposited in Gen-Bank and are available under the Accession Nos. AY151088–AY151129.

All sequences were checked for their potential to be correctly transcribed. Due to the high copy number of mtDNA, and the amount and integrity of endogenous DNA obtained from stool samples, we consider the inadvertent amplification of single copy nuclear integrations of mtDNA as negligible.

Sequence alignments were carried out by CLUSTAL W (Thompson et al., 1994) and subsequently manually edited. The initial alignment comprises 50 individuals with 567 bp in length, which is available from the corresponding author upon request.

Observed sequence differences were calculated applying the PAUP package (Swofford, 1999). A maximum-likelihood estimate of the sequence distances was obtained with the PUZZLE software (Strimmer and von Haeseler, 1996) with transition:transversion ratios and base frequencies estimated from the data set.

The amount and pattern of DNA polymorphisms between and within populations of *M. nemestrina*, *M. siberu*, and *M. pagensis* were separately calculated for both synonymous and non-synonymous sites applying the DnaSP 3.52 program (Rozas and Rozas, 1999).

Base frequencies (26.1, 32.7, 14.4, and 26.8% for A, C, G, and T, respectively) as well as the transition:transversion ratio (8.81) were estimated from the data set using the PUZZLE software. Phylogenetic analyses were carried out applying the maximum-parsimony (MP) (Fitch, 1971), and neighbor-joining (NJ) (Saitou and Nei, 1987) algorithms both included in the PHYLIP package (version 3.5c) (Felsenstein, 1993). Maximum-likelihood (ML) reconstructions were done with PUZZLE (version 5.0) program (Strimmer and von Haeseler, 1996). Distance corrections for the NJ analysis were performed with the ML distance correction and transition:transversion ratios as estimated in PUZZLE. For ML reconstructions, the HKY (Hasegawa et al., 1985) and the TN (Tamura and Nei, 1993) model of sequence evolution were used.

Robustness of tree topologies was assessed by bootstrap analyses (MP and NJ) with 1000 replications. Support for a branching pattern in the ML trees is indicated by the ML quartet puzzling support values on the basis of 1000 puzzling steps.

A molecular clock likelihood ratio test as implemented in PUZZLE was performed to check whether the sequences evolve in a clock-like manner. The test was carried out for all codon positions as well as for third positions only. Divergence times ($\pm 95\%$ confidence interval) were calculated using the branch lengths as estimated in PUZZLE.

To examine whether there are significantly different lineage-specific evolutionary rates observable in the data set, we performed a relative rate test with the RRTree program (Robinson et al., 1998) for all possible pairwise comparisons and using the *Papio hamadryas* sequence as an outgroup.

3. Results

3.1. Within and between population differences

The Sumatran sample consisted of eleven individuals in which five different haplotypes could be detected. Pairwise difference analyses revealed that non-identical sequences differed by a mean value of 0.67%, whereas the five Siberut-specific haplotypes (obtained from twelve individuals), displayed an average difference of only 0.39%.

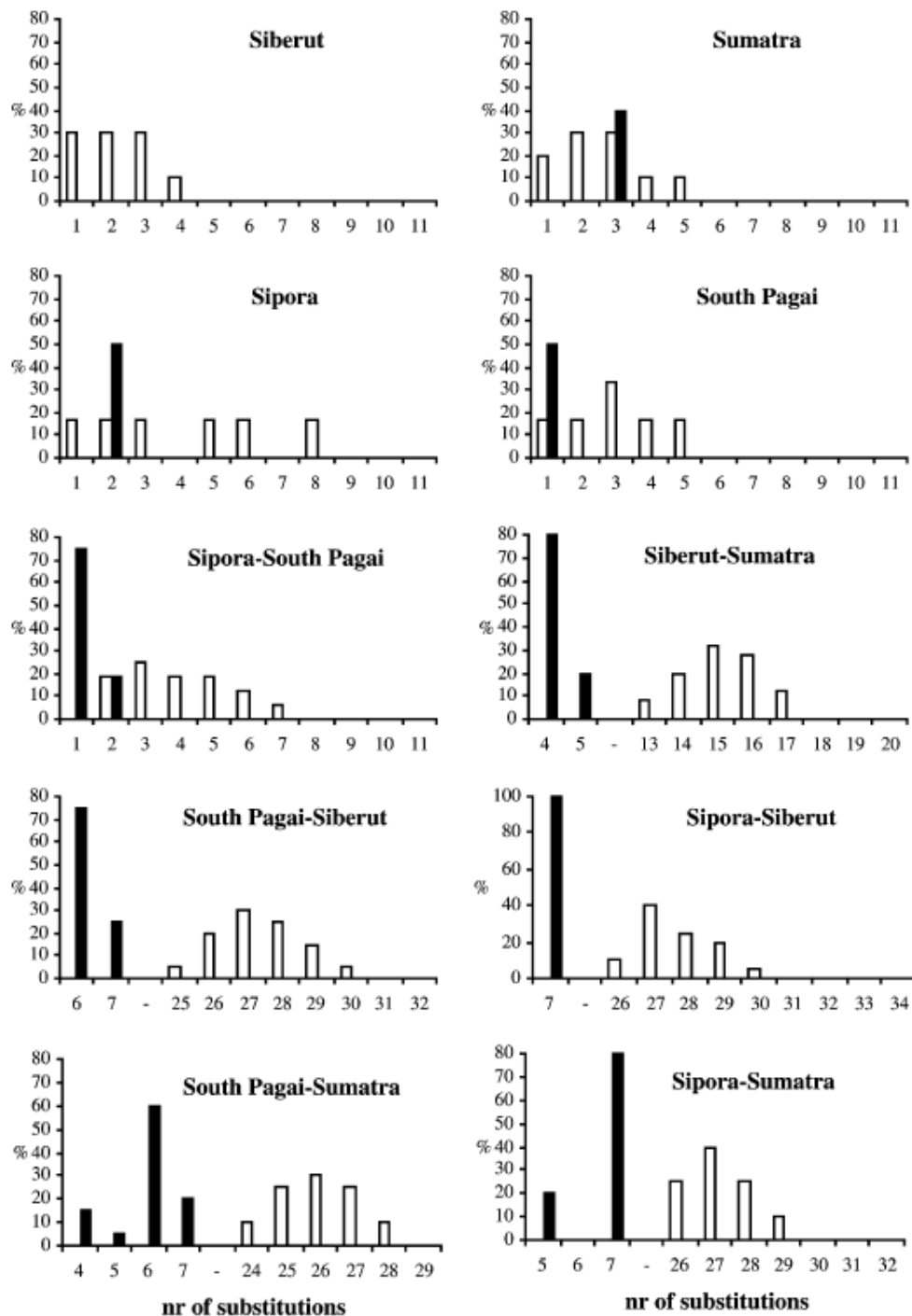


Fig. 2. Distribution of pairwise differences among non-identical haplotypes within and between populations, measured as %-pairwise comparisons for a specific number of substitutions (all comparisons for each synonymous and non-synonymous substitutions: 100%). Non-synonymous substitutions are indicated by black and synonymous ones by white columns (see also Table 2).

The highest within-population difference of 0.92% was detected between the four haplotypes from Sipora. From eight sampled individuals from South Pagai, four non-identical haplotypes were detected, which display an average difference of 0.62%.

Differences between the four populations are much higher than those within populations and display an increasing number of both synonymous and non-synonymous substitutions. This observation is reminiscent of mitochondrial sequence data obtained from other macaque taxa (Melnick and Hoelzer, 1993).

The lowest variation between populations was found between Sipora and South Pagai (0.90%). Siberut and Sumatran macaques differed by 3.42%, whereas the average difference between macaques from Sipora and South Pagai and those from Siberut and Sumatra is 5.88%. Further details to pairwise difference distributions are displayed in Fig. 2 and Table 2.

3.2. Between species differences

The average observed genetic difference among all analysed haplotypes is 7.88%. Within macaques, the highest difference was observed between *M. sylvanus* and all Asian macaques (8.99–12.35%). Lowest differences were detected within the pigtailed macaque populations (0.39–0.92%), as well as between the populations from Siberut and Sumatra (3.42%) and the Sulawesi macaque species *M. tonkeana*, *M. ochreata*, *M. brunnescens*, and *M. hecki* (2.82–3.70%). All other observed differences between analysed Asian macaque species are in the range from 4.06 to 11.29%. Further details on pairwise differences are given in Table 3.

3.3. Phylogeography

To investigate whether a clear geographical pattern in the spatial distribution of the Mentawai macaque haplotypes exists, the non-identical sequences were phylogenetically related applying the neighbor-joining, maximum-parsimony, and maximum-likelihood algorithms. Altogether 29 sequences which were identified as non-identical haplotypes on the DNA level were included in phylogenetic tree reconstructions, which were rooted using the *Papio hamadryas* sequence as an outgroup. As a result, all trees obtained on the basis of different algorithms and assumptions displayed the same tree topology and differed only in their support values for the different branches (Fig. 3).

To ascertain whether cyt b sequence evolution in the macaque sample follows a clock-like manner, the log likelihood for ML trees under the assumption of a molecular clock and non-clock were calculated using PUZZLE. Likelihood ratio tests with log likelihood values of -2496.80 and -2476.86 (all positions) and -1263.16 and -1245.00 (third positions), for trees reconstructed under a model of clock-like and non-clocklike sequence evolution, respectively showed that the clock-like tree cannot be rejected on a significance level of 5%. Additionally, no significant differences in the rates of

Comparison	Synonymous	Average synonymous	Non-synonymous	Average non-synonymous
Within Siberut	0.18–0.71	0.39	0.00	0.00
Within Sumatra	0.18–0.88	0.46	0.00–0.53	0.21
Within Sipora	0.18–1.41	0.74	0.00–0.35	0.18
Within South Pagai	0.18–0.88	0.53	0.00–0.18	0.09
Sipora–South Pagai	0.35–1.23	0.71	0.00–0.35	0.19
Siberut–Sumatra	2.29–3.00	2.68	0.71–0.88	0.74
South Pagai–Siberut	4.41–5.29	4.83	1.01–1.23	1.09
Sipora–Siberut	4.59–5.29	4.89	1.23	1.23
South Pagai–Sumatra	4.23–4.94	4.59	0.71–1.23	1.04
Sipora–Sumatra	4.59–5.11	4.80	0.88–1.23	1.16

Table 2. Synonymous and Non-synonymous substitutions in percentage within and between pigtailed macaque populations (see also Fig. 2)

	<i>P. hamadryas</i>	<i>M. sylvanus</i>	<i>M. fascicularis</i>	<i>M. thibetana</i>	<i>M. nigra</i>	<i>M. nigrescens</i>	<i>M. maura</i>	<i>M. hecki</i>	<i>M. brunnescens</i>	<i>M. ochreata</i>	<i>M. tonkeana</i>	<i>M. pagensis</i>	<i>M. siberu</i>	<i>M. nemestrina</i>
<i>P. hamadryas</i>	–													
<i>M. sylvanus</i>	0.1688	–												
<i>M. fascicularis</i>	0.1973	0.1307	–											
<i>M. thibetana</i>	0.1724	0.1111	0.0924	–										
<i>M. nigra</i>	0.1697	0.1092	0.1078	0.1034	–									
<i>M. nigrescens</i>	0.1745	0.1071	0.1275	0.1143	0.0459	–								
<i>M. maura</i>	0.1749	0.1154	0.1206	0.1033	0.0519	0.0577	–							
<i>M. hecki</i>	0.1533	0.0984	0.1163	0.1076	0.0442	0.0578	0.0419	–						
<i>M. brunnescens</i>	0.1693	0.1128	0.1008	0.1008	0.0421	0.0595	0.0478	0.0383	–					
<i>M. ochreata</i>	0.1647	0.0981	0.1073	0.1030	0.0441	0.0576	0.0536	0.0383	0.0289	–				
<i>M. tonkeana</i>	0.1578	0.1004	0.1053	0.0884	0.0460	0.0557	0.0460	0.0384	0.0363	0.0384	–			
<i>M. pagensis</i>	0.1814	0.1403	0.1205	0.1167	0.0981	0.0965	0.0953	0.0980	0.0710	0.0940	0.0370	–		
<i>M. siberu</i>	0.1751	0.1156	0.1155	0.1125	0.0849	0.0870	0.0807	0.0808	0.0663	0.0826	0.0778	0.0641	–	
<i>M. nemestrina</i>	0.1801	0.1344	0.1126	0.1126	0.0785	0.0909	0.0849	0.0825	0.0632	0.0815	0.0836	0.0614	0.0351	–

Table 3. Pairwise genetic differences among analysed taxa; Values represent substitutions per position. Above the diagonal are observed distances; below the diagonal are ML distances corrected with the HKY model and an estimated transition:transversion ratio of 8.81. Distances for *M. pagensis*, *M. siberu*, and *M. nemestrina* are an average for each taxon.

sequence evolution for the different lineages that were obtained in the PUZZLE tree could be detected in a relative rate test. Based on this information, we estimated divergence times, calibrating the molecular clock by using the fossil evidence attributed to the main split between African (*M. sylvanus*) and Asian macaques of about 5.5 Mya as reference (Delson, 1996). Using this calibration point, it can be deduced that this fragment of mitochondrial cytochrome b evolves at an average substitution rate of 0.0137 and 0.0397 substitutions/position per million years considering either all positions or third positions only.

In all trees, *M. sylvanus* branched off first, verifying the sister group relationship of African and Asian macaques. Within the Asian clade, *M. thibetana* and *M. fascicularis* represented a monophyletic sister clade to the silenus group, represented by Sulawesi and pigtailed macaques. Within the silenus group, Sulawesi macaques were the first to split off, followed by macaques from Sipora and South Pagai, which could not be separated from each other. Pigtailed macaques from Siberut and Sumatra were the last to diverge. All these main splits within the pigtailed macaques are well supported with bootstrap or quartet puzzling support values exceeding 89%. On the other hand the relationship within populations cannot be resolved with significance due to an insufficient number of informative sites.

4. Discussion

4.1. Variation within and among pigtailed macaque populations

Based on geographic location, individuals analysed in this study were initially regarded as belonging to five different populations. In all five populations, identical sequences could be traced. At first glance this could lead to the assumption that genetic variation within these populations is low, although another possible explanation is that this is related to the type of social system seen in macaques. Although Mentawai macaque behavioural data are scarce, transposing the observations from other macaque species would suggest a high degree of female philopatry and as a consequence, that the variability of mtDNA (a genetic

marker which is assumed to be exclusively maternally inherited) is low among members of a macaque social group and high between individuals belonging to different groups (Melnick and Hoelzer, 1993). However, restricted accessibility of the sampling sites makes it difficult to determine how many individual groups were sampled in the present study. Thus, the possible influence of female philopatry on mitochondrial DNA diversity can only be addressed when a more comprehensive sample set becomes available.

Pairwise difference distributions were computed separately for synonymous and non-synonymous substitutions and calculations were done between all possible pairs of populations and among the individuals of each population. From these different analyses it becomes evident that the variation observed among Sipora individuals falls within the South Pagai macaque variation, whereas other Mentawai populations do not exhibit this phenomenon. Further computations were therefore, carried out regarding both island populations as one entity.

The highest mean pairwise difference of non-identical sequences is found among individuals from Sipora and South Pagai, whereas the lowest value is within the Siberut macaque sample. These findings become clearer when the number of synonymous and non-synonymous substitutions are compared between the populations. Whereas in Siberut macaques, only synonymous substitutions were detected with a low frequency, the number of both synonymous and non-synonymous exchanges are higher in the other two populations.

Again, the possibility of sampling bias has to be taken into account when explaining the apparently low sequence diversity obtained, particularly for Siberut where sampling from only two locations was possible. Thus, confirmation of these results by inclusion of additional samples from other subpopulations on Siberut would be necessary before concluding that genetic variation is indeed low. Additionally, the influence of recent population history of these animals on their genetic make up can at present not be clarified for the same reason. The tree reconstructions presented below suggest the existence of recent mitochondrial coalescent events for Sumatra, Siberut, and Southern island populations. However, the number of sequences is too low to obtain statistical support for any interpretation of the population history as inferred from the pairwise mismatch distributions.

4.2. Branching pattern and dating

The results presented here have enabled us to resolve the phylogenetic affiliations among different Southeast-Asian macaque species at a high level of significance, and it is in accordance with interpretations of behavioural data, and with other molecular phylogenetic reconstructions that overlap with our sampling (Deinard and Smith, 2001; Delson, 1980; Morales and Melnick, 1998).

The observation that our data set evolves in a clocklike manner enabled us to estimate divergence times for the major splits occurring during the evolutionary history of Mentawai macaques. Though dating attempts for macaque branching events are difficult due to the sparseness of the Asian macaque fossil record, the splitting times computed from our data set fit very well with estimates from other molecular studies using mitochondrial 12S and 16S rRNA specifying DNA regions (Morales and Melnick, 1998) and γ -globin DNA sequences (Page et al., 1999).

A reasonable scenario might therefore, be that after macaques colonised Asia at about 5.5 Mya, they diverged into two main lineages, a proto-fascicularis clade, herein represented by *M. fascicularis* and *M. thibetana*, and the silenus group (*M. nemestrina*, *M. pagensis*, *M. siberu*, and Sulawesi macaques) about 4.5 Mya ($\pm 121,000$ years) to 4.7 Mya ($\pm 113,000$ years). Later on, within the range of about 3.5 Mya ($\pm 68,000$ years) to 3.8 Mya ($\pm 71,000$ years), the proto-fascicularis clade separated into the fascicularis and sinica groups. Within the silenus group, the monophyletic Sulawesi macaques were the first to split off at about 3.2 ($\pm 66,000$ years) to 3.4 ($\pm 68,000$ years) Mya, which is much earlier than initially suggested by Delson (1980) and Fooden (1975), although is in rough

agreement with other estimates (4.0–4.5 Mya) from molecular data (Morales and Melnick, 1998; Page et al., 1999).

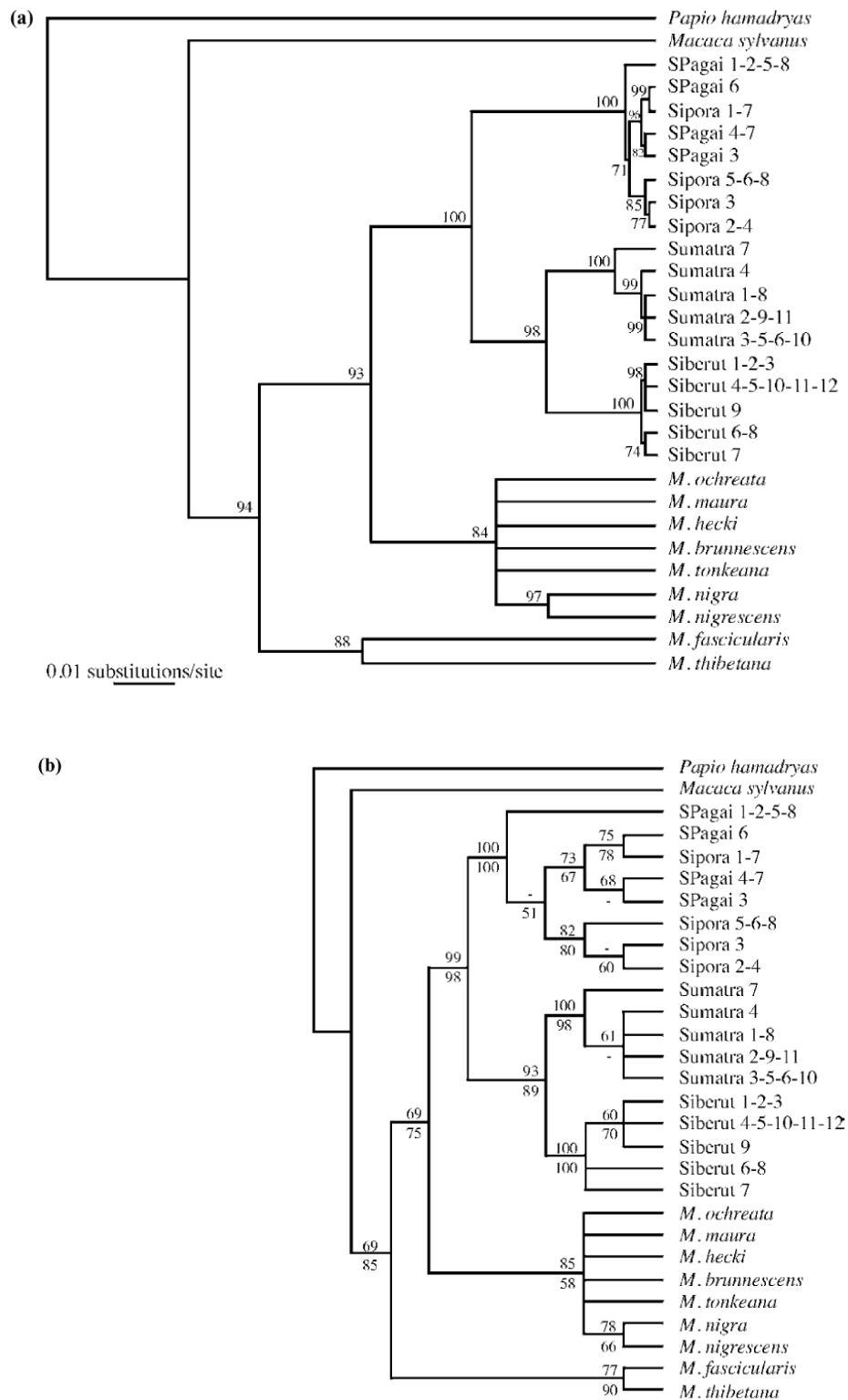


Fig 3. Fifty percentage majority rule consensus trees for the ML (a), NJ and MP (b) algorithms. The ML tree (a) is drawn under the assumption of a molecular clock-like sequence evolution; numbers in the tree indicate puzzling support values. In (b), numbers above and below branches indicate bootstrap support values for the NJ and MP method, respectively. Numbers displayed with the location names indicate the identical sequences sampled from other individuals and refer to the abbreviations listed in Table 1.

The phylogenetic relationships among pigtailed macaques, in which the Mentawai macaques are paraphyletic, are most unexpected. Macaques from Sipora and South Pagai, which cannot be separated from each other, represent the sister clade to the macaques from Siberut and the Sumatran *M. nemestrina*. This topology, which is supported at a high level of significance, is in stark contrast to previous assumptions that all Mentawai macaques share a common ancestor to the exclusion of all other macaques, including *M. nemestrina* (Groves, 2001; Fooden, 1975, 1980). The calculations presented herein suggest a separation of *M. pagensis* (Sipora, North Pagai, and South Pagai) from the clade consisting of *M. siberu* (Siberut) and *M. nemestrina* (Sumatra) at about 2.2 ($\pm 35,000$ years/ $\pm 33,000$ years) Mya, whereas *M. siberu* and *M. nemestrina* diverged more recently, about 1.1 ($\pm 14,000$ years) to 1.3 ($\pm 16,000$ years) Mya. The last mitochondrial common ancestor of the *M. nemestrina* population of Sumatra lived about 0.19 ($\pm 2,000$ years) to 0.47 ($\pm 3,000$ years) Mya, whereas those of the *M. siberu* and *M. pagensis* populations existed about 0.18 ($\pm 1,000$ years) to 0.19 ($\pm 1,000$ years) Mya and 0.28 ($\pm 18,000$ years) to 0.39 ($\pm 21,000$ years) Mya, respectively.

Though the data set presented herein represents a major step towards the description of the evolutionary history and taxonomy of the extant members of the *silenus* group, a final and complete history of its phylogeny cannot be established from our data set. To this end a more complete taxonomic sampling of pigtailed macaque populations is required, (although thus may be difficult due to the restricted accessibility of the different habitats) and the inclusion of a comprehensive *M. silenus* sample would also be necessary.

Previous molecular analyses, including pigtailed macaque populations over the entire geographic range, revealed different clades according to their geographic distribution, with one on Sumatra, Borneo, Siberut, Malaysia, and Thailand/China (Evans et al., 1999; Rosenblum et al., 1997). Although the monophyly of pigtailed macaques including the Mentawai populations is suggested, there is some molecular evidence that this might not be the case (Morales and Melnick, 1998). Hence, ideas about a close affiliation between Mentawai macaques and *M. silenus* based on morphological and behavioural data (Abegg and Thierry, 2002a) cannot be rejected at this stage, and approaching a solution for this problem on a genetic level requires a broader taxonomic sampling across the *silenus* group.

4.3. Taxonomic status of Mentawai macaques

The phylogenetic and systematic position of the Mentawai macaques within the *silenus* group is still an important issue in macaque evolution. Although macaques from the Pagai islands have been known since 1903 (Miller, 1903), little information is available on their ecology, behaviour, and evolution. Their systematic status has been changed several times, from subspecies (Fooden, 1975, 1980; Rosenblum et al., 1997) to full species (Abegg and Thierry, 2002a; Fuentes and Olson, 1995; Groves, 1996, 2001; Wilson and Wilson, 1977) and there have also been different views concerning their affiliations to other macaque species, such as *M. fascicularis* (Tenaza, 1975; Wilson and Wilson, 1977), *M. nemestrina* (Evans et al., 1999; Fooden, 1975, 1980; Rosenblum et al., 1997; Scheffrahn et al., 1996; Watanabe, 1979; Whitten and Whitten, 1982) or *M. silenus* and the Sulawesi macaques (Abegg and Thierry, 2002a). In 1995, the Siberut macaque was named as a new subspecies, *M. pagensis siberu* (Fuentes and Olson, 1995), thus reviving scientific interest in Mentawai macaques' phylogeny and systematics. Recent preliminary data on their ecology, behaviour, and morphology (Abegg and Thierry, 2002a; Kitchener and Groves, 2002) suggest that the two Mentawai macaque populations should be separated into different species.

The molecular data presented herein show that the Mentawai macaques form two distinct clades, one present on Siberut and the other on the southern islands, Sipora, and South Pagai.

Although no samples from North Pagai were available, it is reasonable to assume that macaques from North and South Pagai are genetically closely related, since no differentiation of individuals from Sipora and the geographically more distant South Pagai at the mtDNA level was detectable. Interestingly, an identical taxonomic distinction is made in two other Mentawai primates, *Presbytis potenziani* and *Simias concolor*, each of which has one subspecies on Siberut and another on the three southern islands (Groves, 2001). Whether this taxonomic distinction can be applied for all Mentawai primates or even other mammals, remains to be seen and has to await more data on the fourth Mentawai primate, *Hylobates klossii*, which is still regarded as monotypic. Furthermore, for any taxonomic proposal, it has to be taken into consideration that Mentawai macaques form a paraphyletic group, with Siberut macaques more closely related to pigtailed macaque populations from Sumatra than to *pagensis* and with estimated splitting times and genetic differences roughly resembling those calculated for Sulawesi macaques (Table 3). Taken together, these findings suggest a separation of macaques from Siberut and from the three Southern islands, Sipora, North, and South Pagai into two distinct species, *M. siberu* and *M. pagensis*, respectively.

4.4. Biogeographic implications for the colonisation of the Mentawai islands

Sea floor soundings suggest that there were landbridges between Sumatra and the Mentawai islands during extreme glacial periods, when sea levels were lowered by about 200m compared with present levels. In particular, since climatic fluctuations became more intensified from 5 to 1.6 Mya, primates could have entered the Mentawai islands on a landbridge from Sumatra via the Batu islands to the North within this period (Abegg and Thierry, 2002b).

However, the paraphyly of the Mentawai macaques was an unexpected result of this study which is at odds with previous formulated assumptions. We propose the following explanations for this finding:

1. From the available evidence, the most likely scenario would be that the colonisation of the Mentawai islands by macaques occurred not as a single, but rather as two independent events. There are a number of possible ways in which this could have happened:

- (1a) After a first colonisation of the Mentawai islands, a second invasion of Siberut may have been possible through the build up of another landbridge. The possibility of a second landbridge between Siberut and Sumatra, without a connection to the Southern islands seems unlikely, since there is - at least nowadays - no significant difference in the sea depth between Sipora and Siberut compared with that between Siberut and Sumatra. It seems therefore, more likely that if there was a second fall in sea level, the entire area around the Mentawai islands would have fallen dry allowing an unrestricted access to all islands, rather than just Siberut. On the other hand, it is also possible that there was a landbridge between Siberut and Sumatra at a time when the three Southern islands were already cut off. Such a scenario also seems to be improbable because, as we mentioned above, there are no known differences in sea floor depth between the various islands. Moreover, a land connection between Siberut and Sumatra would imply a continuous sea level of the required depth (about 200 m) for at least 1 million years, which is the estimated time span between the two colonisation events (2.2 and 1.1–1.3 Mya). This would in turn require constant climatic conditions over a long time period, and there is no indication of this.

- (1b) A further possibility for a second invasion would be rafting from Sumatra. However, the likelihood of rafting being successful would be at least partially dependent on whether or not Siberut was still inhabited by descendants of the first colonisation, information about which is unfortunately not available.

(1c) The shallow-water route between Sumatra and the Mentawai islands is to the north, so that dispersal into the Mentawais would be via Siberut. It is possible that, during the first dispersal event 2.2 million years ago, ancestral *M. pagensis* colonised the entire Mentawai group; during a second dispersal event, a million years later, the ancestor of *M. siberu* arrived, but populated Siberut not long before the next sea-level rise, so that, although it replaced *M. pagensis* on Siberut, it was unable to cross to Sipora and the Pagais.

(1d) It is also possible that there are undetected differences in habitat type between Siberut and the other Mentawai islands, so that during the second dispersal phase ancestral *M. siberu* was able to replace *M. pagensis* on Siberut, but was unable to dislodge it from Sipora and the Pagais. However, alternative explanations not necessarily requiring a second independent colonisation event are possible:

However, alternative explanations not necessarily requiring a second independent colonisation event are possible:

(2a) One of these could be an initial colonisation of Siberut by stocks containing multiple, divergent mitochondrial lineages. A single lineage may have expanded into the Southern islands, while a different lineage, more closely related to extant Sumatran mtDNA, went to fixation on Siberut.

(2b) Following an initial colonisation of Siberut, one or more females may have rafted from Sumatra to Siberut with a selectively advantageous mtDNA type, so that a subsequent “selective sweep” may have ensued on Siberut.

Although with limited data available we currently favour the first two hypotheses of part 1, it would be of the utmost interest to know how the other indigenous primate species colonised the islands; of special interest would be *Simias concolor* and *Presbytis potenziani*, both of which likewise have two subspecies with the same distribution pattern as observed in macaques, and it remains to be seen if, beside primates, other animals display the same phylogeography. In addition to these studies, further molecular work is urgently required to extend our understanding of the evolution and population history of the members of the silenus group, and of other primate species distributed on the Mentawai islands. We can predict that this will further deepen our knowledge on the origin and extent of Mentawai island biodiversity and the forces that have shaped it over evolutionary time.

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