The eventful history of a "living fossil"

Phylogeny and phylogeography
of Sulawesi tarsiers

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Abbreviations

ABCA1	ATP-binding cassette sub-family A member 1	MCMC	Markov chain Monte Carlo (method in statistical inference)
ADORA3	Adenosine receptor A3	µl	microliter
AXIN1	Axis inhibition protein 1	min	minute
bp	base pair	ML	maximum likelihood
°C	degree Celsius	mm	millimeter
CITES	the Convention on International Trade in Endangered Species of Wild Fauna and Flora	mt	mitochondrial
		MYA	Million years ago
		Na	Symbol for the element sodium
ca.	circa	no.	numero sign
cm	centimeter	PCR	Polymerase chain reaction
Cytb	cytochrome b	pH	potential of hydrogen
dd	double deionized	pmol	picomole
DNA	Deoxyribonucleic acid	pp	posterior probability
E. coli	*Escherichia coli*	RAG1	Recombination activating gene 1
EcoRI	restriction enzyme isolated from *Escherichia coli*		
		rpm	rounds per minute
Ed.	Editor	ROX	Carboxy-X-Rhodamine
EDTA	Ethylenediaminetetraacetic acid	SAP	Shrimp alkaline phosphatase
e.g.	exempli gratia (for example)	SDS	Sodium dodecyl sulfate
ESS	estimated sample size	sec	second
et al.	et alii (and others)	SRY	sex-determining gene on the Y chromosome
EXOI	Exonuclease I		
fig.	figure	STR	short tandem repeat
g	gram	tab.	table
HEX	hexachlorofluoresceine	TMRCA	time to the most recent common ancestor
HindIII	restriction enzyme isolated from *Haemophilus influenza*		
		TTR	Thyroxine-binding prealbumin
H_2O	hydrogen oxide	ucld.stdev	the standard deviation (σ) of the uncorrelated log-normal relaxed clock
HPD	highest posterior density		
HVRI	Hypervariable region I		
IBD	Isolation by distance	ucld.mean	the mean rate under the uncorrelated log-normal relaxed molecular clock
indel	insertion/delition		
km	kilometer		
l	liter	v.	version
LB	lysogeny broth	vs.	versus
m	meter	WGA	Whole genome amplification
		XML	Extensible Markup Language

Summary

In the 19th century, the British naturalist Alfred R. Wallace recognised the exceptional biogeography of the Malay Archipelago and the particularly interesting position of Sulawesi (Wallace 1863). Now, 150 years later, this region still casts its spell over scientists worldwide. Sulawesi represents the largest island belonging to Wallacea, a biogeographic transitional zone of Asian and Australian biota named after Wallace. Although having a rich fauna, only two primate taxa were able to colonize this island, macaques and tarsiers. The fossil record of tarsiers in Southeast Asia is assumed to go back to the Eocene epoch and also their arrival on Sulawesi clearly predates that of the macaque´s. Before Sulawesi took on its current shape and environmental conditions, tectonic processes and Pleistocene climate fluctuations formed today´s endemism patterns and thus also affected the diversification in tarsiers. However, even today, a well resolved phylogeny representing a wide range of Sulawesi tarsier taxa for concluding possible dispersal routes over the island is missing. Here a multifaceted investigation based on a comprehensive sample set (160 samples from 14 populations) and a variety of molecular tools (mitochondrial, Y-chromosomal, and autosomal DNA markers) complemented by acoustic data has been conducted to infer phylogenetic relationships among tarsier populations of different geographic origin. Species tree reconstructions derived from sequence data of five nuclear markers point to a common ancestor of Sulawesian tarsiers at approximately 20 MYA (95% confidence interval ranges from 13.71-28.15 MYA), going along with the split of crown tarsiers. The progenitor of Sulawesi tarsiers likely reached the island via dispersal and outlasted almost 10 millon years of the Neogene period on the palaeo-Sulawesi archipelago probably with limited expansion possibilities. Further speciation that separated two major lineages of Eastern tarsiers began around the Plio-Pleistocene border, ca. 2-2.5 MYA (95% confidence interval ranges from 1.34-3.54 MYA). Bayesian clustering applied on eight microsatellite loci partitioned populations into seven groups, whereby effects of male-mediated gene flow and isolation by distance biased the population structuring in some respects. Discordance between mtDNA and nuclear DNA strongly hint at female philopatry and male dispersal.

1   Introduction

1.1   Phylogeography and taxonomy of tarsiers

1.1.1   Tarsiers – A short overview

Taxonomic affiliation: Primata, Haplorhini, Tarsiiformes, Tarsiidae, *Tarsius*[1,2]

Activity pattern: nocturnal

Diet: insectivore/carnivore[3]

Sociality: monogamous, polygamous, noyau, solitary[4,5,6,7]

Habitat: dense vegetation, generally primary and secondary forests

Locomotion: clinging and leaping[8,9]

Body size: ca. 10-13 cm[10] (*T. pumilus*: 8 cm[11])

Body weight: ca. 90-150 g[10] (*T. pumilus*: 50 g[11])

Gestation: ca. 6 months[12]

Special features: elongated tarsus, head rotation 180°C in both directions, extremely enlarged orbits relative to brain size, lack a tapetum lucidum[13]

Figure 1.1: Sulawesi tarsiers in their natural habitat

Foto: C. Driller

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[1] Goodman *et al.* 1998

[2] Schmitz *et al.* 2001

[3] Niemitz 1984a

[4] Gursky 1995

[5] MacKinnon & MacKinnon 1980

[6] Crompton & Andau 1987

[7] Dagosto *et al.* 2003

[8] Napier & Walker 1967

[9] Niemitz 1984b

[10] This study

[11] Grow & Gursky 2010

[12] Izard *et al.* 1985

[13] Fleagle 1999

1.1.2 Past distribution

As sole survivors of one of the most ancient and independently evolved primate lineages, tarsiers and their strange appearance fascinated scientists for more than a century. Although extant Tarsiiformes are restricted to some few Southeast Asian islands (Hill 1955, Brandon-Jones *et al.* 2004, Groves & Shekelle 2010), fossil records from mainland Asia (fig. 1.2) and North Africa suggest a much broader distribution of their extinct relatives who lived in the Eocene-Miocene time (Beard *et al.* 1994, Rasmussen *et al.* 1998, Chaimanee *et al.* 2010). Fissure fillings discovered in southeastern China and northern Thailand revealed cranial fragments that resemble modern tarsier dentition and were dated to 45 and 13 MYA (Beard *et al.* 1994, Rossie *et al.* 2006, Chaimanee *et al.* 2010). Two of these fossil specimens, *Tarsius eocaenus* (Beard *et al.* 1994) and *T. sirindhornae* (Chaimanee *et al.* 2010), indicate that the considerable large orbits of present tarsiers may have already existed since the mid-Paleogene period, which in turn would be a prodigious feature. *Afrotarsius* is another possible representative of archaic Tarsiiformes based on anatomical characteristics of a lower leg bone found in Egypt and dated to the Oligocene epoch (Simons & Bown 1985). Rasmussen *et al.* (1998) emphasized strong evidence for an, in opposite to *Necrolemur* (omomyid, see below), more tarsier-like fused tibiofibular, among today´s primates a unique feature to the only living anthropoid sister taxon. A recent study on dental comparisons, however, link *Afrotarsius* to eosimiiform primates and therewith revise a phylogenetic position within the Tarsiiformes (Chaimanee *et al.* 2012). Fossilized remains from omomyids, an abundant paleogene small sized primate taxon once widespread over the northern hemisphere, show also similarities to living tarsiers, as for example enlarged orbits in *Shoshonius* (Beard *et al.* 1991) and skeletal specializations to arboreal living and leaping abilities in *Necrolemur* (Dagosto 1985). Though, no consensus has been reached on the phylogenetic relationship of extant tarsiers and extinct omomyids. Authors have placed omomyid primates as either ancestor to tarsiers (Szalay 1976) or sister group to haplorhine primates (Bajpai *et al.* 2008). Summing up it remains controversial which of those fragmentary fossils do best justify a close relationship or even an affiliation to the tarsiiform clade. However it seems to be reasonable that precursors of the smallest haplorhine primates inhabited a wider range than they do at present.

1.1.3 Present distribution

Modern tarsiers, *Tarsius* Storr 1780, can be subdivided into three biogeographic distinct taxa, namely Western, Philippine, and Eastern tarsiers or *Tarsius tarsier*-complex (fig. 1.2). The first group is distributed on southern Sumatra (*Tarsius bancanus bancanus*), Belitung (*T. b. saltator*), Borneo (*T. b. borneanus*), and the South Natuna Islands (*T. b. natunensis*). *Tarsius syrichta* occurs on some south Philippine islands, mainly Leyte and Samar (*Tarsius syrichta syrichta*), Mindanao (*T. s. carbonarius*) and Bohol (*T. s. fraterculus*, see Hill 1955 and Brandon-Jones *et al.* 2004). Eastern tarsiers inhabit Sulawesi and several off shore islands. They currently represent the most diverse taxon with nine recognized species: *Tarsius fuscus*, *T. pumilus*, *T. dentatus* (junior synonym *T. dianae*), *T. lariang* and *T. wallacei* from Sulawesi, and *Tarsius tarsier tarsier* (junior synonym *T. spectrum spectrum*), *T. pelengensis*, *T. sangirensis* and *T. tumpara* living on Selayar, Peleng, Sangihe and Siau Island (Hill 1955, Niemitz *et al.* 1991, Brandon-Jones *et al.* 2004, Shekelle *et al.* 2008a, Groves & Shekelle 2010, Merker & Groves 2006, Merker *et al.* 2010). Populations have also been recorded on Sulawesi´s north peninsula, in east

and southeastern parts of the island as well as on Buton, Banggai and Togian island (Brandon-Jones *et al.* 2004, Shekelle 2008a, Burton & Nietsch 2010, own surveys, see fig. 1.2).

Figure 1.2: Tarsiiform primates of Southeast Asia
Eocene and Miocene fossilized remains of ancient tarsiids from Shanguang, southeastern China (Beard *et al.* 1994), and from northern Thailand (Chaimanee *et al.* 2010), respectively, are marked by circles. Gray and black colored areas correspond roughly to the geographic range of living tarsiers (Hill 1955, Brandon-Jones *et al.* 2004, Groves & Shekelle 2010). Geographical map based on ARCMAP™ 10 (Esri).

1.1.4   Notes on the taxonomy of tarsiers

Contrary to the generally accepted taxonomy of extant tarsiers placing Western, Philippine and Eastern tarsiers in one genus, *Tarsius*, Groves & Shekelle (2010) proposed three tarsiid genera according to

their biogeographic distribution: *Cephalopachus* from Sundaland, *Carlito* from Greater Mindanao, and *Tarsius* from Sulawesi and surrounding islands. The revised classification is mainly based on selected morphological, behavioral and bioacoustic characteristics. Whether these justify nomenclatural changes or not cannot be discussed in detail here. A thorough examination integrating verifiable genetic data would certainly be very helpful in getting to the bottom of this issue. In this thesis the classical taxonomic concept will be used.

1.2   Ecology and behavior of Sulawesi tarsiers

At present the Eastern tarsier group is best documented among the three major tarsier clades. Sulawesi tarsiers occupy a wide range of habitat types, mainly being found in primary and secondary forests, but also inhabiting woodland affected by anthropogenic land use (MacKinnon & MacKinnon 1980, Merker *et al.* 2005, Merker 2006). Sufficient sleeping sites like strangler figs, bamboo or dense undergrowth, appropriate locomotion substrates, live animal prey (preferably arthropods), and, in case of habitat degradation, a limited degree of disturbance, are the minimal requirements for a suitable environment for tarsiers. Except for pygmy tarsiers (*Tarsius pumilus*), which appear to be restricted to altitudes above 1800 m (Shekelle 2008b, Grow & Gursky 2010), the island´s smallest primates are known as lowland species. Tarsiers from different subregions of Sulawesi share behavioral features like group living and sleeping site association. Grouping patterns vary from two adult individuals of opposite sex to multiple adult males and females, and associated offspring, whereby typically one adult male shares the territory with multiple adult females (MacKinnon & MacKinnon 1980, Niemitz *et al.* 1991, Merker *et al.* 2005, Driller *et al.* 2009, Grow & Gursky 2010, Merker *et al.* 2010). Especially lowland tarsiers emit sex specific duet calls, usually at dawn, when they return from nightly foraging. A variety of those vocalizations, which are –contrary to the ultrasonic communication of Philippine tarsiers (Ramsier *et al.* 2012) - audible to the human ear, have been recorded, with each distinct acoustic form being constrained to its respective geographical region and therefore hypothesized to be indicative of species identity (Nietsch & Kopp 1998, Shekelle 2008c, Burton & Nietsch 2010). Beside social interactions between group members Sulawesian lowland tarsiers are known to use their vocal repertoire for territorial defense. Territoriality of tarsiers is also expressed by scent marking involving either, urination and secretion of an epigastric and a circum-oral gland (Niemitz 1984c). Urinary scent marks of most Eastern lowland tarsiers have a distinctive smell, such that their presence can be easily recognized by humans.

1.3   Biogeography and geological evolution of Sulawesi

Covering an area of around 189,000 km² (Whitten *et al.* 2002) Sulawesi is the fourth largest island of Indonesia and ranks eleventh in the world. The island is surrounded by the Philippines to the north, the Lesser Sunda Islands to the south, by Borneo to the west, and the Maluku Islands to the east. Four long, narrow peninsulas, connected by the mountainous heartland, give Sulawesi its distinctive shape and over 6,000 km of coastline. No single spot on the island is further than 100 km from sea. Situated at the equator the climate is wet-tropical experiencing mean temperatures ranging from 25-27 °C (Schultz 2002) and an average annual rainfall of about 1,000 mm (l/m²), with regional peaks above 3,000 mm (Whitten *et al.* 2002).

Sulawesi is part of Wallacea, a biogeographic transition zone where Asian and Australian biota intermingle (Whitten *et al.* 2002). Geographically Wallace´s line delimits this region westward to a strict Asian biotic community, whereas Lydekker´s line separates the ecozones of Australia and Wallacea (fig. 1.3). As largest island of this biodiversity hotspot Sulawesi represents one of the richest reservoirs of plant and animal life on earth. The island is estimated to have 6045 species of higher plants, 12 % of which are endemic (Roos *et al.* 2004). A remarkable 62 % of 127 mammal species are restricted to Sulawesi, or 98 % if bats are excluded (Whitten *et al.* 2002). With exception of the dwarf cuscus (*Strigocuscus celebensis*) and the bear cuscus (*Ailurops ursinus*), both marsupials originating from Australia, Sulawesi´s non-flying mammals are derived from Asia (Whitten *et al.* 2002). Well-known native species are dwarf buffaloes, babirusas and the only two primate taxa that straddle the Wallace line, macaques and tarsiers. Sulawesi has also a rich avifauna including 332 species of which 27 % are endemic to the island (Whitten *et al.* 2002). The Sulawesi dwarf hornbill (*Penelopides exarhatus*) and the Maleo (*Macrocephalon maleo*) are perhaps best-known.

It is not only this outstanding biodiversity which attracts scientists, but also the eventful geological history reflected in recent faunal and floral distribution patterns on Sulawesi, albeit defined areas of endemism are more striking across the animal kingdom. Even within genera several species with limited geographical distributions are found, as observed in Sulawesi grasshoppers (*Chitaura*), toads (*Bufo celebensis*), fanged frogs (*Limnonectes*), flying lizards (*Draco*), and macaques (Butlin *et al.* 1998; Evans *et al.* 2003a, McGuire *et al.* 2007, Setiadi *et al.* 2011). The formation of Sulawesi was initiated approximately 45 MYA by the rapid northward movement of the Australian plate and the separation of today´s western Sulawesi from the Asian margin leading to the opening of the Makassar Strait (Hall 2001, 2009). It seems probable that at least small parts of west Sulawesi have been emergent from Eocene onwards (Hall 2001, Hall 2009, Stelbrink *et al.* 2012). In early Miocene (23-20 MYA) the Sula Spur collided with the volcanic arc that corresponds to present day northern Sulawesi (fig. 1.3), whereby ophiolite deposits within this subduction zone lifted the southeastern peninsula above sea level (Hall 2001, 2009, Stelbrink *et al.* 2012).

Although the direct land route from Borneo to Sulawesi was severed since mid-Eocene time (fig. 1.3), areas of shallow sea and small islands of the Sunda arc could have made transition over land to emergent parts of Sulawesi possible (Hall 2001, 2009). Periodic low sea levels and the ongoing convergence of the Australian and Eurasian plate at 10 MYA are thought to have resulted in an extension of subaerial regions in east and central Sulawesi (Haq *et al.* 1987, Hall 2009). From Pliocene orogenic processes led to the opening of deep basins between the arms of the Sulawesi Island (Lohman *et al.* 2011). Hall´s Cenozoic reconstructions (Hall 2001) of this complex geographic region suggest a significant expansion of land area and a final fusion of Sulawesi within the last 5 million years.
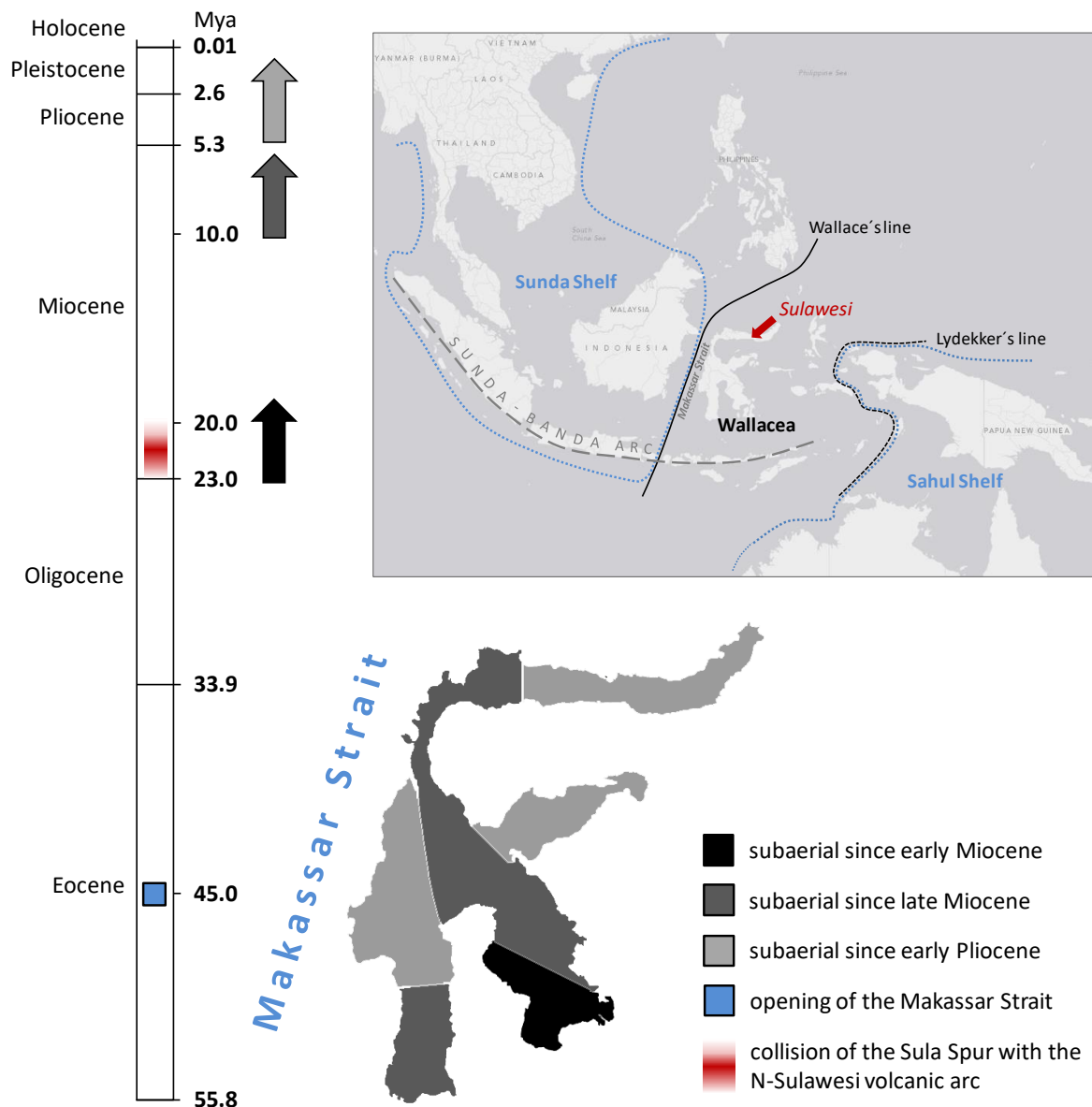
Figure 1.3: Subaerial regions of Sulawesi from Miocene onwards
Left: Geological time scale[14]. Right above: Map of the Indo-Malay Archipelago situated between mainland southeastern Asia and Australia. Biogeographical lines of Wallace (black continuous line) and Lydekker (black dashed line) bordering Wallacea, the Sunda and Sahul shelves (blue dotted lines), and the Sunda-Banda arc as the southern boundary of the archipelago are indicated. The northern margin of the Australian plate (Sahul Shelf) represents the Sula Spur that collided with proto-Sulawesi. Center below: Simplified illustration of land positive areas according to maps published by Hall (2001, 2009). Right below: caption to left and middle illustrations. Geographical map based on ARCMAP™ 10 (Esri).

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[14] Based on Walker *et al.* 2012

1.4   Research methodological approach

Research on the biodiversity of Sulawesi tarsiers was primarily driven by bioacoustics and morphological data. To date only few studies included molecular methods to resolve intra- and inter-population relatedness (Shekelle 2003, Driller *et al.* 2009, Merker *et al.* 2009, 2010, Shekelle *et al.* 2010). The study authored by Merker *et al.* (2009) presumably presents the most rigid investigation on the phylogeography of Sulawesi tarsiers and was the first to provide solid geographic and genetic results supporting a strong correlation between the island´s geological history and contemporary distribution patterns of it´s exciting residents. Furthermore, based on three parapatric Sulawesian species - *Tarsius dentatus*, *T. lariang* and *T. wallacei* - it has been illustrated that populations emitting distinct duet songs can also be distinguished on the molecular level (Merker *et al.* 2009, 2010). Nevertheless, a good resolved phylogeny comprising the Eastern tarsier diversity in a wide scope is still lacking. Shekelle (2003) and Shekelle *et al.* (2010) analyzed an extensive sample set with respect to the geographical range and the number of distinct tarsier populations. However, sequence data of a single and slowly evolving mitochondrial gene (12S rRNA) yielded not enough information to draw robust and fine-scaled phylogenetic inferences. The main purpose of this thesis is therefore to focus on a comprehensive sample set of Sulawesi tarsiers and molecular tools comprising autosomal and uniparental inherited markers with different sensitivities to historical processes, dispersal behavior and gene flow. Until recently, phylogeographic patterns of tarsiers have been inferred from single gene phylogenies alone. Gene trees are susceptible to incomplete lineage sorting (Heled & Drummond 2010), which might lead to false conclusions or incongruence in phylogenetic reconstructions depending on the gene analyzed (Knowles 2009, Heled & Drummond 2010). To address this problem multilocus sequence data will be combined to deduce species tree topologies using the approach of Heled & Drummond (2010). In order to trace speciation events in the light of glacial cycles and the geological formation that likely played a role in the evolution of Sulawesi tarsiers, divergence times among distinct populations will be estimated.

1.5   Research objectives, concepts and hypotheses

Many hypotheses have been proposed to explain how terrestrial biota came to Sulawesi. Like most mammals tarsiers colonized the island from continental Asia (Whitten *et al.* 2002). Though, a direct connection between Asia and Sulawesi did not exist since the formation of the Makassar Strait (see chapter 1.3). Furthermore distribution of land and sea was changeable up until the Pleistocene epoch. Therefore Sulawesi as center of tectonic activity and accreted terranes offers more than one scenario enabling biotic exchange and speciation. With their widespread distribution across insular Southeast Asia tarsiers provide a good model to explore the influence of tectonic and climatic history on contemporary patterns of endemism. In framework of this thesis the following concepts and hypotheses shall be tested:

- **Arrival by vicariance**

A taxon with a disjoint spatial distribution, e.g. spanning two continents, is often assumed to have been subject to vicariant speciation. Regarding paleogeographic reconstructions of Southeast Asia population isolation promoted by micro-continental drift therefore seems to be reasonable. Especially the splitting of west Sulawesi from the Sunda Shelf would perfectly match the fragmentary distribution

of modern tarsiers and other Sulawesian taxa with an Asian origin. The western peninsula may have been partially subaerial since Eocene time (Hall 2009, Lohmann *et al.* 2011). A founder population of the Eastern tarsier clade could have existed on little land until Sulawesi fully emerged. However there is poor evidence for vicariance as predominant colonization mechanism of Sulawesi´s terrestrial fauna (Stelbrink *et al.* 2012). As rare example divergence time estimates of mite harvestmen meet the geological time frame (Clouse & Giribet 2010). To maintain a vicariance scenario divergence time of Eastern tarsiers and their sister taxon inhabiting Sundaland must predate or coincide with the opening of the Makassar Strait at around 45 MYA.

- **Arrival by dispersal**

The Indo-Malay archipelago has undergone a number of tectonic movements and periodic sea level changes leading to varying exposure of land bridges and distances between land masses. Several dispersal routes to Sulawesi are conceivable (fig. 1.4) and apparently linked to climate induced sea level drop beginning in late Oligocene and followed by a negative trend with minima at 10.0 and 2.5 MYA (Haq *et al.* 1987). Low sea level periods expose more land. Continental shelves, carbonate platforms and volcanic arcs could have served as stepping stone islands for migration between Sundaland, the Philippines and Sulawesi. Successful dispersal crossing deep water barriers like the Makassar Strait became more likely with more land available in the target region (Stelbrink *et al.* 2012). If gradual sea level decline enforced dispersal to Sulawesi, the earliest possible divergence of Eastern tarsiers from Western and Philippine tarsiers should have been in the Oligo-Miocene period.

Figure 1.4: Conceivable dispersal routes to Sulawesi from continental Asia
Geographical map based on ARCMAP™ 10 (Esri). Tracks (arrows) adopted and modified from Whitten *et al.* (2002).

- **Single or multiple colonization of Sulawesi**

Even though opportunities to reach Sulawesi were according to geological reconstructions of Wallacea rather rare, routes out of continental Asia were comparatively numerous (fig. 1.4). Therefore it seems feasible that tarsiers could have colonized Sulawesi more than once, using different routes at different times. If ancestral tarsiers invaded Sulawesi just once, Eastern tarsiers have to be monophyletic with respect to their Western and Philippine congeners.

• **Speciation on Sulawesi**

Speciation mechanisms are generally categorized in allopatric, parapatric and sympatric which are understood in a spatial context. However, this geographical view merely superficially reflects the underlying processes driving species diversification. Population divergence mostly occurs over a geological period of time and involves forces like environmental changes, sexual selection, as well as prezygotic (e.g. assortative mating) and postzygotic isolation mechanisms (e.g. maladaptive gene combinations) that interact and finally evoke isolation in nascent species (Kirkpatrick & Ravigné 2002, Butlin *et al.* 2008). Vicariant events and variable dispersal possibilities, particularly during Pleistocene, certainly promoted speciation on Sulawesi and are mirrored by recent cross-taxon congruence in endemism (Evans *et al.* 2003a). As "old endemics" (Groves 2001) Eastern tarsiers are expected to have arrived on Sulawesi before the Plio-Pleistocene radiation of macaques took place. Hence, present distribution of tarsiers should correspond to known areas of endemism defined by more recent immigrants (Evans *et al.* 2003a), and to tectonic sutures that indicate possible boundaries to dispersal on proto-Sulawesi island, as was already shown for two central Sulawesi tarsier species (Merker *et al.* 2009). The goal of this thesis is to determine spatial genetic variation among parapatric and allopatric Sulawesi tarsier populations from which genetic structure, as well as historical dispersal and contemporary gene flow shall be deduced. The following questions should be addressed in particular:

- Have past tectonic configuration and glacial oscillations influenced tarsier radiation on Sulawesi?
- Can genetic relationships among populations be explained by shared ancestry or ongoing gene flow?
- Which evolutionary mechanisms have led to genetic differentiation among populations?
- Are phylogenies inferred from mitochondrial and nuclear DNA congruent?
- Do gender-specific differences in dispersal affect population structure?

2   Material and methods

2.1   Field methods

2.1.1   Choice of study areas

The sampling strategy was designed with the aim of reflecting the Sulawesi tarsier diversity in a wide scope. Criteria for selecting a study site were mainly based on tectonic origin of microplates (Hall 2001), possible environmental fluctuations during glacial periods (Whitten *et al.* 2002), published locations of distinct acoustic morphs of Sulawesi tarsiers (Shekelle *et al.* 1997, Nietsch & Burton 2002, Brandon-Jones *et al.* 2004, Merker & Groves 2006), and areas of endemism identified for other animal taxa, e.g. macaques (Evans *et al.* 2003a). Finally, accessibility and appropriate habitat conditions were decisive factors to choose a sample location. Figure 2.1 shows tectonical sutures (A), hybrid zones of Sulawesi macaques (B), distribution areas of tarsier taxa mainly based on vocal records (C), areas that have been sampled in past studies (Merker *et al.* 2009, 2010) and locations planned to be sampled during this project (D).
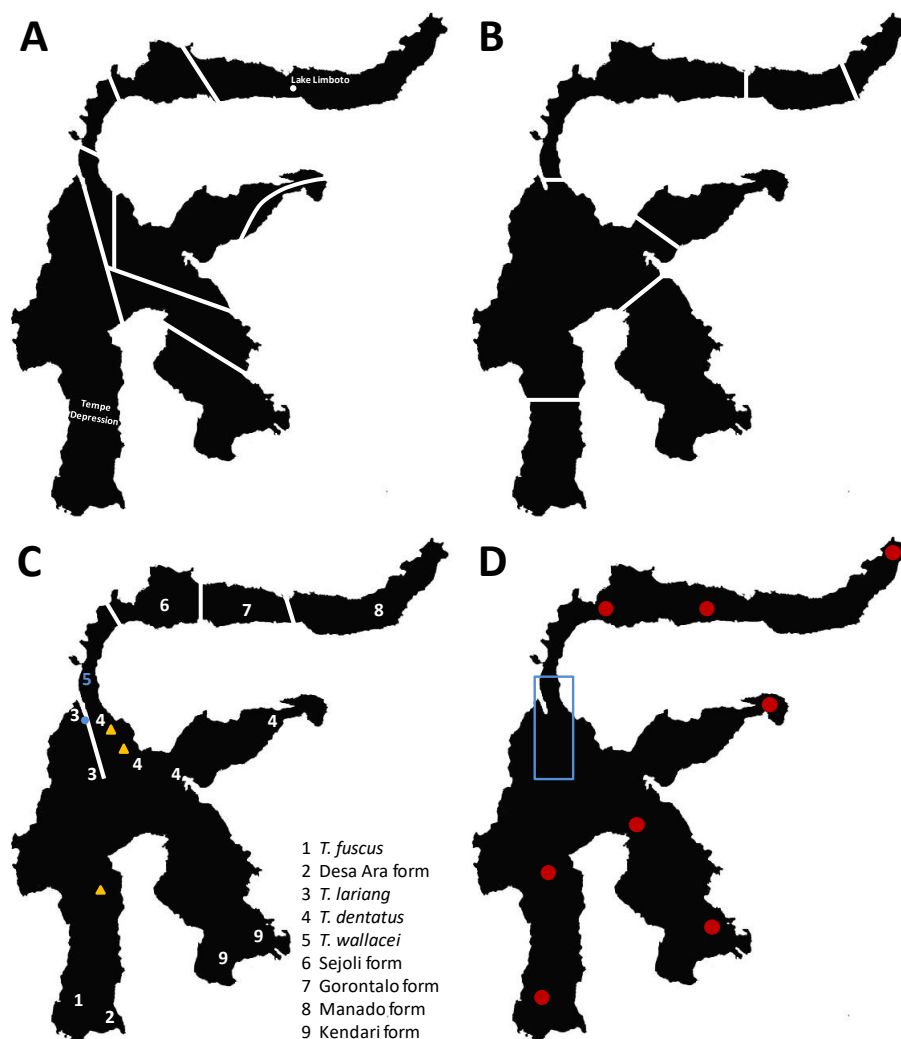
Figure 2.1: Selection criteria for study areas – a schematic overview
A) Tectonical sutures according to Hall (2001). Additionally Lake Limboto and the Tempe depression are indicated; B) Hybrid zones of Sulawesi macaques according to Evans *et al.* (2003a); C) Approximate distribution areas of tarsier acoustic forms are indicated with numbers and white lines. The blue dot points to the southern sub-population of *Tarsius wallacei* (5, Merker *et al.* 2010). Yellow triangles show locations of *T. pumilus*; D) Sample sites of former studies (blue rectangle), and sampling strategy to complement the existing sample set (red dots).

1 *T. fuscus*
2 Desa Ara form
3 *T. lariang*
4 *T. dentatus*
5 *T. wallacei*
6 Sejoli form
7 Gorontalo form
8 Manado form
9 Kendari form

2.1.2   Localizing, trapping, sampling and recording

Each possible study location was explored to ensure the presence of tarsiers. The animals were observed at dusk and dawn while leaving and returning to their sleeping site. Scent marks and the sex-specific loud calls most Sulawesi tarsiers emit were traced until the sleeping site or its vicinity could be located. Mist-nets of six, nine and twelve meter length and three meters in height (Vohwinkel) were positioned nearby to cross pathways of a selected tarsier group. At the beginning of the tarsier´s active phase nets were opened and monitored continuously. Captured individuals were sexed, aged and measured. Likewise earbiopsies of ca. 2x2 mm were taken and immediately stored in Urea-EDTA buffer. Shortly thereafter the animals were released at their capture site. Morning vocalizations of tarsiers were recorded with a RØDE NT3 microphone and a portable minidisc recorder (HI-MD Walkman MZ-NH900, Sony). Spectrograms were visualized with the Syrinx sound analysis program version 2.6h (developed by John Burt and available at www.syrinxpc.com).
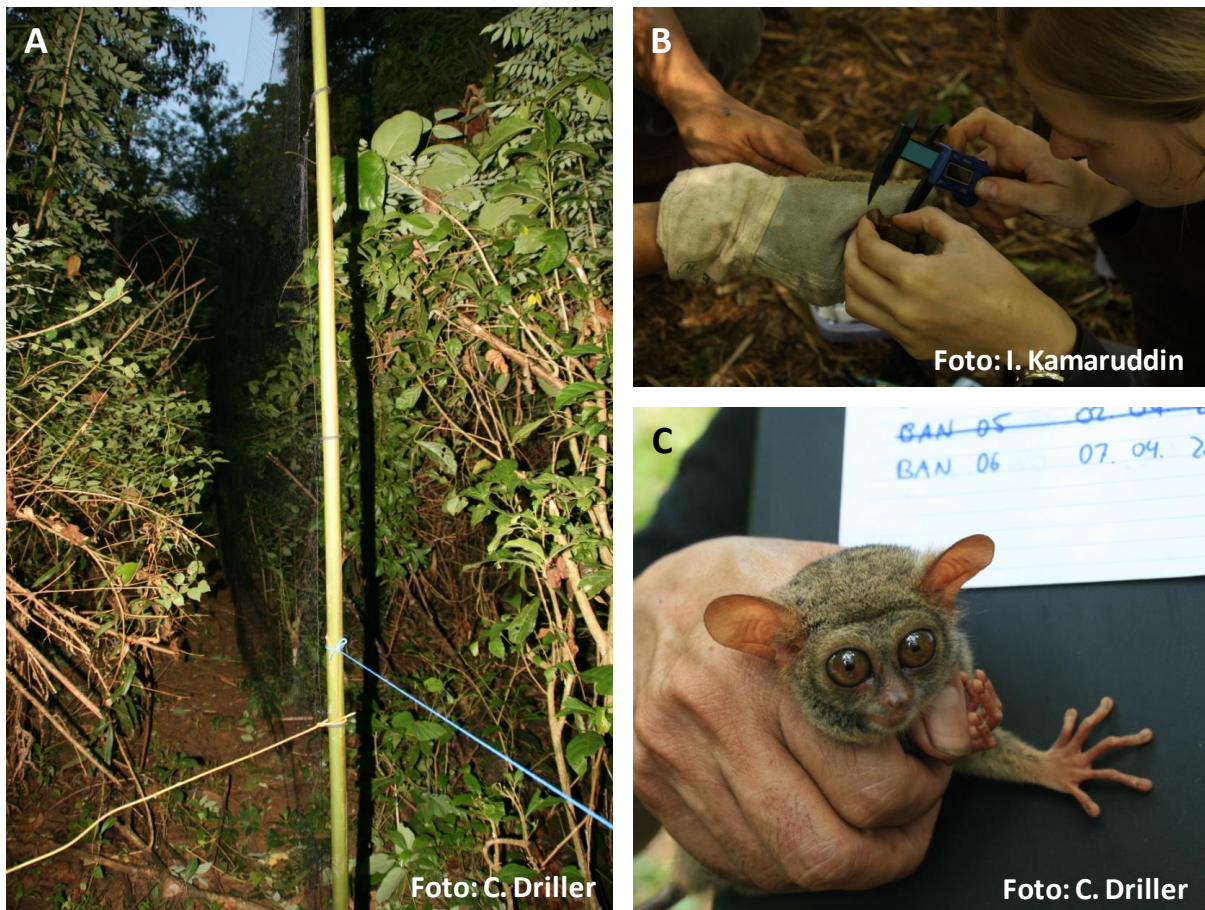
Figure 2.2: Field methods
A) Opened mist-net, B) measurements, C) documentation.

2.2   Laboratory methods

2.2.1   DNA-Extraction and whole genome amplification

Total DNA of 65 new tissue samples (see chapter 3.1) was extracted using *DNeasy Blood and Tissue Kit* (Qiagen) combining protocols of *DNeasy Blood and Tissue* and *QIAamp DNA Mini and Blood Mini* handbooks. Residual unlysed tissue particles were treated separately from lysed samples following the tissue protocol. DNA purification from Urea-EDTA buffer was conducted as outlined in the respective protocol for blood with an additional step after incubation at 56 °C. Before ethanol was added to the sample lysate/AL buffer mixture pH level was checked and, if necessary, brought to pH < 7 (~ 6.1-6.5) with Na-acetate (pH 5). All 65 DNA samples were WGA-amplified using the *GenomiPhi DNA Amplification Kit* (GE Healthcare) and applying the procedures as outlined by the manufacturer. Yield and size of DNA and WGA were estimated on ethidium bromide-stained 1 % and 1.5 % agarose gels together with Lambda DNA/EcoRI+HindIII Marker.

2.2.2   Samples and genetic markers

The whole sample examined comprised 14 populations and 160 individuals. Selected taxa included individuals from seven new locations (set 2, see chapter 3.1) and those originating from seven populations situated near the Palu-Koro fault sampled in former studies (set 1, Driller *et al.* 2009, Merker *et al.* 2009, 2010). A variety of different genetic markers has been applied to the two sample sets:

Uniparental inherited markers include the mitochondrial Cytochrome b gene and the D-Loop hypervariable region I as well as the Y-chromosomal SRY gene. PCR primers and conditions were adopted from Merker *et al.* (2009) and applied to the new sample set (set 2). Published sequences from previous studies (Driller *et al.* 2009, Merker *et al.* 2009, 2010) with Genbank accession no. FJ214312–FJ214337, FJ614263–614568, and HM115970-115991 have been incorporated into the new sequence alignments.

All individuals sampled between 2009 and 2010 were genotyped at nine ***polymorphic autosomal codominant microsatellite loci*** (Merker *et al.* 2007) by capillary electrophoresis on an ABI 3130xl genetic analyzer (Applied Biosystems). As genotypic data obtained from preliminary research (Driller *et al.* 2009, Merker *et al.* 2009, 2010) were generated on an ABI 377 automated sequencer (Applied Biosystems) allele sizes had to be adapted to the new data collection (see chapter 2.3.1.1).

The established gene marker set reported above has been extended by ***five nuclear loci of the Phylogenomic Toolkit*** developed by Horvath *et al.* (2008). Three exonic (ADORA3, AXIN1 and RAG1) and two intronic loci (ABCA1 and TTR) were analysed for a pruned sampling corresponding to the 14 populations in focus. Based on a preliminary cytochrome b maximum likelihood tree each population was represented by two terminal taxa. *Tarsius bancanus*[15] and *T. syrichta*[16] served as outgroups complementing the final set of 30 individuals (supplement tab. 8.1). Three exonic and two intronic loci

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[15] Courtesy of Prof. Dr. Yves Rumpler, Les Hôpitaux Universitaires de Strasbourg, France
[16] Courtesy of Prof. Dr. Jürgen Brosius, Institut für Experimentelle Pathologie,Westfälische Wilhelms-Universität Münster

combined with the two mitochondrial loci and the SRY gene yield around 5500 bp of sequence information.

2.2.3   Polymerase chain reaction (PCR)

PCR was performed for amplification of mitochondrial and nuclear loci with thermocyclers using standard and wax-mediated hot start methods. Basic reaction components were obtained from Qiagen Taq PCR Core Kit and mixed with locus-specific primers (Merker *et al.* 2009 and supplement tab. 8.5), ddH2O and target DNA to 30 µl (hot start PCR) and 20 µl (standard PCR) volumes. Composition of PCR reactions and thermocycler settings are specified in tables 8.2-8.4 (see supplement). Yield and size of nucleic acids were estimated on ethidium bromide-stained 1.5 % agarose gels together with 100 bp plus DNA Ladder (Fermentas). Before cycle sequencing PCR products were purified by ExoI/SAP (Fermentas) treatment. Incubation (30 min at 37 °C) and enzyme deactivation (15 min at 80 °C) were carried out in a thermocycler.

For microsatellite loci amplification was conducted in two PCR steps. First, each marker was amplified with the respective forward and reverse primers in a wax-mediated hot start PCR. Second, the PCR product was re-amplified by standard PCR using a HEX-labelled primer. The fluorescence tag was detected in subsequent fragment length analyses (chapter 2.3.1.1).

2.2.4   Cycle sequencing

After enzymatic cleanup PCR products were used as a template in a cycle sequencing reaction consisting of 1 µl Big Dye premix, 2 µl 5x Sequencing buffer (both components of the Big Dye® Terminator v. 3.1 Cycle Sequencing Kit), and 1 µl Primer (10 pmol/µl). The reaction was filled up with ddH2O to a final volume of 10 µl and placed in a thermocycler. Cycle sequencing was initiated by a 5 min denaturation step at 96 °C, followed by 30 cycles of alternating denaturation (10 sec at 96 °C) and annealing/elongation steps (4 min at primer specific temperatures).

2.2.5   Sequencing and fragment length analysis

To scavenge unincorporated dye terminators 1 µl 0.22 % SDS was added to the completed sequencing reaction and positioned in a thermocycler block for 5 min at 98 °C and for 10 min at 25 °C. Afterwards post-sequencing reactions were pipetted onto a Sephadex G-50 Fine column and centrifuged at 2750 rpm for 5 min. The flow-through was mixed with Hi-Di™ formamide (Applied Biosystems) to a total volume of 15 µl. Fluorescence tagged PCR products were prepared for fragment analysis by adding Hi-Di™ formamide/ROX™ standard mixture. Typically 11.7-12.0 µl Hi-Di™ formamide and 0.3-0.5 µl Genescan™ 350 ROX™ Standard (Applied Biosystems) were added to 0.5-1.0 µl PCR product for a final volume of 13 µl. Samples for sequencing and fragment length analysis were denatured at 95 °C for 3 min and subsequently processed on an ABI 3130xl genetic analyzer.

2.2.6   Cloning procedures

Sequence ambiguities from nuclear gene markers were taken as indication for a heterozygote genotype and therefore considered for cloning. As a next step the respective PCR product was purified by ethanol precipitation and ligated into pGEM®-T vector (Promega). Ligation reactions - composed of 3 µl PCR product, 1 µl pGEM®-T vector, 5 µl 10x T4 DNA ligase buffer and 1 µl T4 DNA ligase - were incubated at 4 °C overnight. Ligation products were then isolated by phenol/chloroform extraction, cleaned up with ethanol precipitation, and transformed into competent One Shot® TOP10 E. coli cells (Invitrogen) via electroporation. Transformed cells were grown at 37 °C for 1 hour on a shaker (~300 rpm) and plated on ampicillin-selective LB agar plates for incubation at 37 °C overnight. After blue/white screening at least eight colonies per individual were PCR amplified and sequenced.

2.3   Evaluating molecular data

2.3.1   Nuclear microsatellite loci

2.3.1.1 Genotyping

Raw data generated on an ABI 3130xl genetic analyzer were loaded into GENEMAPPER software version 4.0 (Applied Biosystems). Quality control and sizing were carried out following the instructions as outlined in the software manual. To incorporate genotypic data from previous projects where fragment analysis has been performed on an ABI 377 sequencer, a representative subset of individuals were PCR amplified for each microsatellite locus again and processed with the latest sampling simultaneously. Fragment lengths data produced on different instruments for the same individual were compared. Thus size differences between old and new data sets could consistently be adjusted to the most recently determined allele sizes.

2.3.1.2 Descriptive statistics

To characterize the genetic variability of microsatellite loci the number of alleles and allelic richness standardized for the smallest sample (R_S) were calculated in FSTAT v. 2.9.3.2 (Goudet 1995, 2002). Genotypic data were tested for Hardy-Weinberg equilibrium performing exact tests as implemented in GENEPOP 4.1.2 (Rousset 2008). For loci and/or populations with ≤4 alleles the complete enumeration method was applied. In cases where ≥5 alleles were present Markov chain algorithm was used (10,000 dememorizations, 1,000 batches, 1,000 iterations per batch). Weir and Cockerham´s F_{IS} served as estimator for heterozygote excess or deficiency. Pairwise independence of genotypes between different microsatellite loci was tested using the log-likelihood ratio test (G-test) in FSTAT v. 2.9.3.2. The level of significance was adjusted by standard Bonferroni correction for multiple testing.

2.3.1.3 Genetic distance

Genetic variation among sampled tarsier populations was evaluated using measures based on differences in allele frequencies of microsatellite loci. Assuming an infinite allele model pairwise F_{ST}, the proportion of shared alleles D_{ps} (Bowcock *et al.* 1994) over all loci, and the standard chord distance D_C (Cavalli-Sforza & Edwards 1967) were consulted for pairwise comparisons of populations. F_{ST} distances and their significance (alpha=0.05; 10,000 permutations) were calculated by ARLEQUIN v. 3.11 (Excoffier *et al.* 2005), D_{ps} (1-Ps) and D_C estimations were performed with MICROSATELLITE ANALYZER 4.05 (Dieringer & Schlötterer 2003). Distance matrices of D_{ps} and D_C were used for phylogenetic tree construction applying the neighbor-joining algorithm as implemented in PHYLIP 3.69 (Felsenstein 1989, 2005).

2.3.1.4 Bayesian cluster analysis

STRUCTURE 2.3.2 (Pritchard *et al.* 2000) was used to infer population structure of Sulawesi tarsiers. A total of 160 individuals from 14 sample locations were analyzed for eight microsatellite loci (one locus had to be excluded, see chapter 3.2.2) assuming an admixture model and using the independent allele frequency model. The burn-in period was set to 200,000 iterations followed by 200,000 MCMC repeats. Ten independent runs were carried out for K=1 to K=14 according to the number of sample locations. To determine the upper most hierarchical level of population structure informational pointers as outlined in the software manual (Pritchard *et al.* 2009) were checked and the method of Evanno *et al.* (2005) was applied. Membership coefficient (Q) matrices from ten runs were summarized for K=3 through K=9 in CLUMPP v. 1.1.2 (Jacobson & Rosenberg 2007) using the Greedy algorithm with random input order and 10,000 permutations.

2.3.1.5 Isolation by distance

Mantel tests were performed for groups of populations showing population genetic structure (suggested by Bayesian clustering, see 2.3.1.4) and geographical connectivity. Correlations were calculated based on Slatkin´s linearized F_{ST} and spatial distances as shortest over-land path between two study sites ignoring landscape features (for distance data see tab. 8.9 in the supplement data) Linearized F_{ST} values (i.e. $F_{ST}/(1-F_{ST})$ were estimated with ARLEQUIN v. 3.11 (Excoffier *et al.* 2005). Geographic distances between populations were measured in MAPSOURCE® v. 6.11.6 (Garmin). Because the spidery shape of Sulawesi did not allow straight line connections between each pair of populations, additional points were added to the map as link (see fig. 3.7, chapter 3.2.5). For purposes of illustration GPS-coordinates of study sites and links were extracted from MAPSOURCE® and transferred to ARCMAP™ 10 (Esri). Distance data were analysed with 10,000 randomizations applying the Isolation By Distance Web Service (IBDWS, Jensen *et al.* 2005).

2.3.2   Sequence processing

2.3.2.1 Multiple sequence alignment

All sequences were edited with BIOEDIT 7.0.9.0 (Hall 1999). A multiple sequence alignment was obtained using MUSCLE v. 3.8.31 (Edgar 2004). Identical haplotypes among 59 SRY, 151 Cytb and 154 D-loop sequences, respectively, were collapsed into unique haplotypes using FABOX 1.40 (Villesen 2007). Sequence alignments used for the species tree estimations were edited computationally by using GBLOCKS 0.91b (Castresana 2000) to remove phylogenetically uninformative sites and indels.

2.3.2.2 Phylogenetic tree reconstructions

2.3.2.2.1   Single locus gene trees

Single gene tree phylogenies were reconstructed for Y-chromosomal haplotypes applying maximum likelihood (ML) and Bayesian approaches. Prior to this the best-fit nucleotide substitution model (TN, Tamura & Nei 1993) was estimated based on Akaike´s information criterion corrected for small sample sizes (AICc) using TREEFINDER v. March 2011 (Jobb *et al.* 2004). ML trees were calculated in GARLI 2.0 (Zwickl 2006) with two search replicates, 100 bootstrap replicates and stepwise-addition starting trees. A majority rule consensus tree was generated from boostrap trees in CONSENSE, a program implemented in the PHYLIP package v. 3.69 (Felsenstein 1989, 2005). Bayesian gene trees were generated by MRBAYES 3.2 (Ronquist *et al.* 2011) running two independent analyses of 5×10^{6} generations with four Markov chains. Sample frequency was set to 1,000 resulting in 10,000 trees per run from which 25% were discarded before a majority rule tree (50 % consensus) was reconstructed.

2.3.2.2.2   Multilocus species trees

A subset of five nuclear loci (ABCA1, ADORA3, AXIN1, RAG1, and TTR) was subjected to *BEAST 1.6.2, a Bayesian program that enables the inference of species trees from multilocus sequences (Heled & Drummond 2010). As most of the newly sampled populations lack a certain taxonomic affiliation and data indicating on-going hybridization are rare (Merker *et al.* 2009), sampling locations were used as "species" trait. Following the author´s instructions (Drummond *et al.* 2007), the estimated ucld.stdev and coefficient of variation values obtained from preliminary runs under an uncorrelated relaxed lognormal clock were consulted to select proper clock models for the data set. For all loci a strict molecular clock could not be rejected. According to TREEFINDER results best fitting models of sequence evolution (see table 2.1) were incorporated into the XML input file. The remaining priors were kept at their default settings. Ten independent chains of length 1×10^{7} each were run sampling every 10,000 generations. Convergence of MCMC chains was assessed by screening estimated sample sizes (ESS) of log file parameters in TRACER 1.5 (Rambaut & Drummond 2009). MCMC samples of two runs were combined after a burn-in of 10 % each (1001 trees/run were discarded) and subsequently summarized onto a final species tree using LOGCOMBINER and TREEANNOTATOR, both programs implemented in the BEAST software package (Drummond & Rambaut 2007).

Table 2.1: Data set information of nuclear loci – *Tarsius*

Locus	N samples	bp	Substitution model	Clock model
ABCA1	60	536	HKY	Strict
ADORA3	60	370	HKY+I	Strict
AXIN1	60	809	HKY	Strict *
RAG1	60	745	TN+I	Strict
TTR	60	914	HKY+G (4 cat)	Strict

HKY (Hasegawa *et al*. 1985), TN (Tamura & Nei 1993), G: gamma distributed rate heterogeneity, I: invariable sites model, cat: rate categories, * clock rates will be estimated relative to AXIN1.

A species tree was also inferred from mtDNA sequences (table 2.2) applying the same procedure as outlined above using the following settings: uncorrelated relaxed lognormal clock; ucld.mean=gamma shape 0.001, scale 1000; chain length=2×10^7; sample frequency=every 5,000 generations. After tracing the log files six of ten independent runs were combined to reconstruct the final species tree.

Table 2.2: Data set information of mitochondrial loci – *Tarsius*

Locus	N samples	bp	Substitution model	Clock model
CYTB	153	1140	J3+G (4 cat)	uncorrelated relaxed lognormal *
HVRI	156	386	HKY+G (4 cat)	uncorrelated relaxed lognormal

HKY (Hasegawa *et al.* 1985), J3: Transition model (Posada 2008), G: gamma distributed rate heterogeneity, cat: rate categories, * clock rates will be estimated relative to CYTB.

2.4   Estimating divergence times

Divergence times of Sulawesi tarsier taxa and the Western/Philippine/Eastern tarsier split were estimated with a Bayesian approach as implemented in *BEAST v. 1.6.2 (Heled & Drummond 2010). Datasets used for the species tree analysis before (see 2.3.2.2.2) were complemented by sequence information of anthropoid and strepsirhine primates (see supplement tab. 8.6 and 8.7) obtained from former studies on primate evolution (Horvath *et al.* 2008, Perelman *et al.* 2011). Eight taxon sets were created: 1) Homininae, 2) Hominidae, 3) Hominoidea, 4) Catarrhini, 5) Anthropoidea, 6) Tarsiidae, 7) Haplorhini (monophyletic), and 8) Strepsirhini.

Selection of substitution models for each locus was done in TREEFINDER v. March 2011 (Jobb *et al.* 2004) using AICc. To allow rate variation among branches *BEAST analysis was performed under an uncorrelated lognormal relaxed molecular clock (table 2.3). A gamma distribution was used as prior on mean of branch length (ucld.mean: shape=0.001, scale=1000).

Table 2.3: Data set information of nuclear loci – Primates

Locus	N samples	bp	Substitution model	Clock model
ABCA1	74	556	TVM	uncorrelated relaxed lognormal
ADORA3	73	370	J1+G (4 cat)	uncorrelated relaxed lognormal
AXIN1	74	809	HKY+G (4 cat)	uncorrelated relaxed lognormal *
RAG1	74	745	J1+G (4 cat)	uncorrelated relaxed lognormal
TTR	74	927	TVM+G (4 cat)	uncorrelated relaxed lognormal

TVM: Transversion model (Rodriguez *et al.* 1990), J1: Transition model (Posada 2008), G: gamma distributed rate heterogeneity, cat: rate categories, * clock rates will be estimated relative to AXIN1.

Based on divergence time estimates recently published by Jameson *et al.* (2011) and Perelman *et al.* (2011), respectively seven (calibration 1) and eight nodes (calibration 2) were calibrated modelled as a normal distribution. First, mean time to the most recent ancestor (TMRCA) in million years ago (MYA) and standard deviation were set at the following values: 1) Homininae 6.45/0.68, 2) Hominidae 16.60/1.50, 3) Catarrhini 24.70/1.45, 4) Anthropoidea 38.40/2.30, 5) Haplorhini 68.80/1.95, 6) Strepsirhini 51.35/2.93, 7) Primates 72.90/2.00. Second, mean time to the most recent ancestor (TMRCA) in million years ago (MYA) and standard deviation were set at the following values: 1) Homininae 6.68/0.64, 2) Hominidae 18.07/0.81, 3) Hominoidea 20.41/1.91[17], 4) Catarrhini 31.77/3.06, 5) Anthropoidea 43.46/2.45, 6) Haplorhini 82.16/6.83, 7) Strepsirhini 67.69/4.46, 8) Primates 87.27/5.69. Other settings remained at their defaults. Ten independent chains of $2x10^7$ generations were run with sample frequency of 20,000. Convergence and mixing behavior of MCMC chains were evaluated in TRACER v. 1.5 (Rambaut & Drummond 2009). Finally, five trees were combined (burn-in 10 %) to a single tree as described above (see 2.3.2.2.2).

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[17] According to the results published by Jameson *et al.* (2011) divergence date estimates of Hominoidea were not available for calibration 1.

3   Results

3.1   Survey and sampling

In 2008 and 2009 putative study areas on Sulawesi were explored and evaluated for their suitability as capture sites. Tarsier populations of designated study sites were then sampled in two field studies between June 2009 and April 2010. A total of 65 individual tissue samples, five to twelve per study site, were acquired from seven populations (declared as set 2 in methods chapter 2.2.2) located in distinct geographical regions of Sulawesi. More detailed information on the number of sampled groups and individuals per population and the sex ratio can be found in table 3.1.

Table 3.1: Demographic composition of individuals sampled between 2009 and 2010

Study site	Province	Label	SUB$_{Loc}$	N$_G$	N$_{IND}$	N$_F$	N$_M$	GPS co-ordinates
TNBB	S-Sulawesi	BAN	-	5	10	8	2	5°04'48.3''S 119°42'22.2''E
Kendari	SE-Sulawesi	KEN	1*	3	3	2	1	3°57'01.2''S 122°31'12.2''E
			2	1	2	1	1	4°01'38.4''S 122°35'28.3''E
Korosule	C-Sulawesi	KOR	1	1	2	1	1	2°19'08.5''S 121°20'08.3''E
			2	2	4	3	1	2°11'58.4''S 121°18'21.5''E
Luwuk	C-Sulawesi	LUW	-	3	10	5	5	0°57'29.9''S 122°46'16.8''E
Ogatemuku	C-Sulawesi	OGA	-	6	12	7	5	0°31'57.4''N 120°34'13.6''E
Labanu	Gorontalo	LAB	-	5	10	7	3	0°43'54.3''N 122°50'31.5''E
Duasaudara	N-Sulawesi	DUA	-	6	12	7	5	1°29'17.6''N 125°07'42.6''E

TNBB: Taman Nasional Bantimurung Bulusaraung – National park; * Taman Hutan Raya – conservation area; SUB$_{Loc}$: Sub-location; N$_G$: Number of social groups sampled; N$_{IND}$: Number of individuals sampled; N$_{F/M}$: Number of females/males sampled

For ease of orientation the sites surveyed and sampled within the frame work of this thesis as well as sample locations of previous studies are shown in figure 3.1.
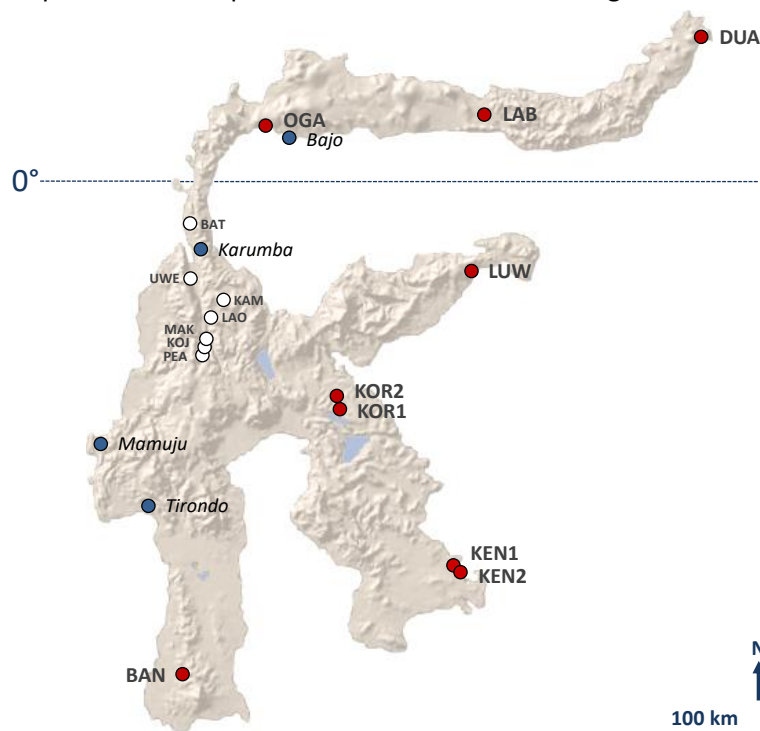
Figure 3.1: Origin of tissue samples and vocal recordings
Shown are study sites where either only tarsier vocalization (blue circles: 2008-2009, this study) or acoustic, morphological and genetic data are available (red circles: 2009-2010, this study; white circles: 2001-2008, previous studies). Topographical map based on ARCMAP™ 10 (Esri).

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Most study sites were influenced by anthropogenic land use. Tarsier sleeping sites were mainly located in secondary forest patches or succession areas that bordered plantations and the local infrastructure. Only populations of BAN and KEN1 inhabited protected forest areas that were exposed to a lower degree of human disturbance than those populations living in habitats outside conservation areas. KOR1 inhabited the most extreme environment. Small patches of secondary forests were highly fragmented by extensive palm oil plantations. Group sizes at the nine study sites varied from two to more than seven individuals. The social group composition pattern was generally one adult male, multiple adult females, and their offspring (see tab. 3.2).

Table 3.2: Group size and composition at study sites and description of habitat conditions

Study site	Label/SUB$_{LOC}$	Group size	Sex ratio adult individuals	Habitat description at capture site
TNBB	BAN	2 - 7	1 adult male, ≥1 female	Border of a conservation area, secondary forest, partially fragmented by rice fields. Sleeping sites primarily in bamboo.
Kendari	KEN1*	≥2	1 adult male, 1 female	Conservation area, secondary forest and succession areas. Sleeping sites in dense undergrowth and strangler figs.
	KEN2	≥2	1 adult male, 1 female	Secondary forest and succession areas. Sleeping sites primarily in dense undergrowth.
Korosule	KOR1	≥2	1 adult male, 1 female	Secondary forest surrounded by palm oil plantations. Sleeping sites in dense undergrowth and strangler figs.
	KOR2	≥2	1 adult male, ≥1 female	Secondary forest and succession areas bordering agricultural land. Sleeping sites primarily in dense undergrowth.
Luwuk	LUW	2 -≥ 7	1 adult male, ≥1 female	Secondary forest and succession areas bordering agricultural land. Sleeping sites in dense undergrowth and strangler figs.
Ogatemuku	OGA	2 -≥ 4	1 adult male, ≥1 female	Secondary forest and succession areas bordering agricultural land. Sleeping sites in dense undergrowth, bushes and strangler figs.
Labanu	LAB	2 - 5	1 adult male, ≥1 female	Secondary forest and succession areas bordering agricultural land. Sleeping sites in dense undergrowth, bushes and strangler figs.
Duasaudara	DUA	2 - 5	1 adult male, ≥1 female	Secondary forest and succession areas bordering agricultural land. Sleeping sites in dense undergrowth and strangler figs.

TNBB: Taman Nasional Bantimurung Bulusaraung – National park; * Taman Hutan Raya – conservation area; SUB$_{LOC}$: Sub-location.

3.2   Microsatellite data

3.2.1   Genetic variability

Overall 147 distinct alleles were detected at nine loci ranging from 35 to 61 among populations. The population BAN from southern Sulawesi outnumbered all other taxa in allelic richness, number of alleles and private alleles (R_S=46.099, N_A=61, N_{PA}=8; see fig. 3.2 and tab. 3.3). In all populations allelic richness did not exceed the observed number of alleles. The mean number of alleles per locus altered between 2.5 (T54) and 6.6 (D194). It was conspicuous that individuals of KEN and all northern populations had just a single allele at locus D238 (see tab. 3.3). Beside BAN populations of KOR, KEN, LAB and DUA had an above-average number of private alleles. No private alleles could be observed in KOJ and UWE.
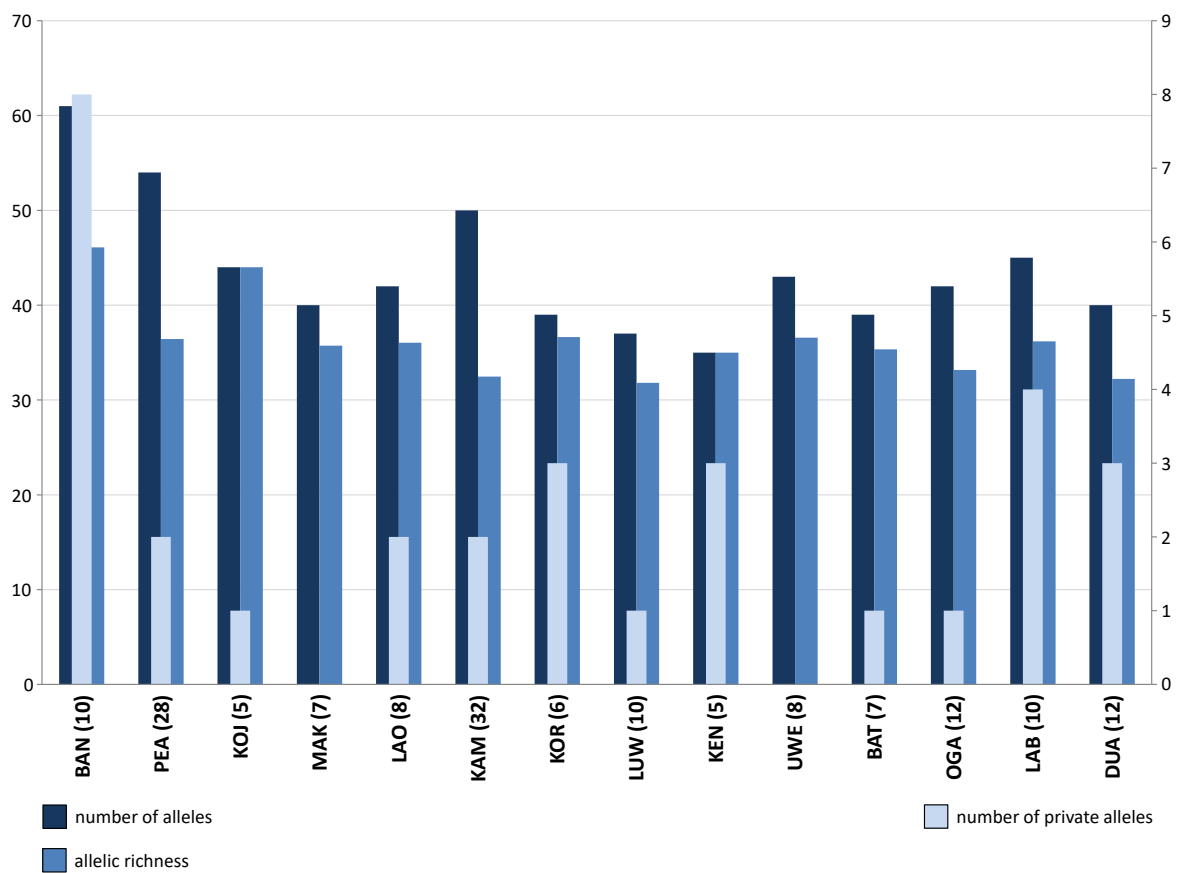
Figure 3.2: Allelic variation of nine microsatellite loci among 14 tarsier populations

Table 3.3: Genetic diversity of nuclear microsatellites

		Locus																		
		T42		T54		D157		D194		D220		D231		D238		D246		D251		
Pop	N_{IND}	N_A	R_S	N_A	R_S	N_A	R_S	N_A	R_S	N_A	R_S	N_A	R_S	N_A	R_S	N_A	R_S	N_A	R_S	$N_{A\,all\,loci}$	$R_{S\,all\,loci}$
BAN	10	6	5.139	2	1.957	9	6.310	8	6.229	8	5.683	7	5.019	6	4.702	7	5.447	8	5.613	61	46.099
PEA	28	5	3.124	2	1.712	4	2.995	7	5.128	6	4.654	10	5.679	6	3.701	8	5.450	6	3.986	54	36.429
KOJ	5	4	4.000	2	2.000	3	3.000	8	8.000	5	5.000	5	5.000	4	4.000	7	7.000	6	6.000	44	44.000
MAK	7	4	3.712	2	1.714	3	2.989	5	4.142	4	3.702	6	5.121	4	3.931	6	5.130	6	5.286	40	35.727
LAO	8	4	3.858	3	2.929	4	3.625	8	6.295	2	2.000	4	3.804	6	5.323	6	4.714	5	3.500	42	36.048
KAM	32	6	3.402	3	2.113	2	1.946	8	5.320	3	2.072	6	4.549	7	3.756	7	4.829	8	4.473	50	32.460
KOR	6	6	5.621	1	1.000	3	2.667	7	6.455	3	2.833	5	4.652	4	3.970	6	5.636	4	3.803	39	36.637
LUW	10	7	4.939	1	1.000	1	1.000	5	4.509	4	3.835	6	4.877	4	3.808	5	3.983	4	3.861	37	31.812
KEN	5	2	2.000	2	2.000	6	6.000	4	4.000	3	3.000	7	7.000	1	1.000	4	4.000	6	6.000	35	35.000
UWE	8	2	1.875	2	1.992	7	6.081	9	6.831	6	5.323	6	4.831	1	1.000	4	3.588	6	5.053	43	36.574
BAT	7	3	2.923	4	3.648	5	4.417	7	5.999	4	3.648	5	4.571	1	1.000	5	4.571	5	4.571	39	35.348
OGA	12	3	2.949	4	3.443	5	3.860	4	3.372	6	4.953	7	4.568	1	1.000	5	3.631	7	5.282	42	33.058
LAB	10	2	1.763	3	2.263	7	5.756	6	4.289	5	4.570	6	5.031	1	1.000	9	6.598	6	4.903	45	36.173
DUA	12	4	3.554	4	2.739	3	2.973	7	5.336	5	3.813	6	4.461	1	1.000	4	3.784	6	4.555	40	32.215
all Pop	160	17		9		18		25		14		15		12		19		18		147	

Population (Pop); Number of individuals (N_{IND}); Number of alleles (N_A); Allelic richness (R_S)

Table 3.4: Exact test for Hardy-Weinberg equilibrium.

	T42		T54		D157		D194		D220		D231		D238		D246		D251		
	F_{IS}	P	F_{IS}	P	F_{IS}	P	F_{IS}	P	F_{IS}	P	F_{IS}	P	F_{IS}	P	F_{IS}	P	F_{IS}	P	$P_{all\ loci}$
BAN	-0.0946	0.8794	-0.2000	1.0000	-0.0253	0.7612	0.1111	0.3192	-0.2000	1.0000	-0.1329	0.9095	-0.1329	0.9061	0.1486	0.1279	-0.2081	1.0000	**0.8863**
PEA	0.1591	0.2829	-0.1020	1.0000	-0.0246	0.6732	0.0906	0.0197	-0.0827	0.5693	-0.0506	0.7940	0.0275	0.5965	-0.0638	0.7300	-0.1836	0.9591	**0.6316**
KOJ	-0.1429	0.8730	NA	-	0.0769	0.6190	-0.0526	1.0000	-0.2121	1.0000	-0.0667	0.8708	-0.0323	0.7206	0.1351	0.4050	-0.2121	1.0000	**0.8437**
MAK	0.0625	0.5072	NA	-	-0.5000	1.0000	-0.0169	0.6857	0.4375	0.0709	-0.2353	1.0000	-0.2923	1.0000	0.3333	0.0765	-0.2174	1.0000	**0.6811**
LAO	-0.1529	0.6817	-0.3333	1.0000	-0.0448	0.7762	-0.1313	1.0000	0.0000	0.7762	0.1250	0.3343	-0.1789	1.0000	-0.2099	0.9805	-0.1200	1.0000	**0.9553**
KAM	0.1176	0.2938	0.1429	0.2412	0.2314	0.1970	0.2248	0.0120	0.2965	0.0844	0.0865	0.1217	-0.0586	0.7990	0.0062	0.8083	0.0008	0.7687	**0.0455**
KOR	0.0385	0.6158	NA	-	-0.0526	1.0000	0.2857	0.0961	0.3939	0.2727	-0.2766	1.0000	-0.0870	0.8153	-0.1538	1.0000	-0.0256	0.7576	**0.4500**
LUW	-0.2587	1.0000	NA	-	NA	-	0.1127	0.5243	0.7447	0.0002	-0.1408	0.9376	0.0455	0.4649	-0.2101	0.9699	-0.0435	0.6648	**0.2905**
KEN	-0.1429	1.0000	-0.6000	1.0000	-0.1429	1.0000	0.2500	0.2952	0.2381	0.3333	-0.1111	1.0000	NA	-	0.4074	0.1111	-0.1429	1.0000	**0.5796**
UWE	-0.0769	1.0000	-0.2727	1.0000	-0.1313	1.0000	0.0392	0.5617	-0.1789	1.0000	-0.2727	1.0000	NA	-	-0.0500	0.6308	-0.2444	1.0000	**0.9933**
BAT	0.2500	0.3287	0.2131	0.1715	-0.2923	1.0000	0.0270	0.6087	0.4194	0.1116	-0.1077	0.8770	NA	-	0.2836	0.2061	0.2836	0.2118	**0.1953**
OGA	0.1250	0.4559	-0.2222	0.9655	0.1299	0.3457	-0.0879	0.7279	0.1000	0.3545	0.1020	0.3833	NA	-	-0.0233	0.7163	0.2036	0.1421	**0.3536**
LAB	-0.0588	1.0000	-0.0800	1.0000	0.0886	0.3714	0.0609	0.5261	-0.1250	0.8974	0.0400	0.4955	NA	-	-0.1111	1.0000	-0.1172	0.2332	**0.6927**
DUA	0.1492	0.1967	0.0179	0.5606	0.1492	0.2438	0.2178	0.1411	-0.0539	0.7661	0.0526	0.4219	NA	-	0.0100	0.5213	0.0198	0.6033	**0.2036**

H1: Heterozygote deficiency (p <0.05, Bonferroni corrected significance level p<0.0004); Significant p-values are indicated in grey.
NA no data were available due to insufficient allele data

3.2.2   Hardy-Weinberg Equilibrium and Linkage Disequilibrium

Departure of microsatellite genotype frequencies from the Hardy-Weinberg proportions were tested using GENEPOP (Rousset 2008). Based on a 0.05 level of significance and standard Bonferroni corrections departures from Hardy-Weinberg equilibrium were observed for LUW at D220 with p=0.0002 (tab. 3.4). F_{IS} (0.7477) indicated clear heterozygote deficiency at this microsatellite locus. As homozygote excess does not necessarily imply presence of null alleles and this was a single finding, a technical artefact seems not to have caused heterozygote deficit. More striking is the complete absence of heterozygous individuals in six populations at D238 (see tab. 3.3 and 3.4). Though, in this case, lack of heterozygotes and the fact that all individuals were monomorphic for the same allele, sequence divergence in flanking sites of the repeat could be responsible for poor primer annealing. Since it remains ambiguous whether improper PCR conditions or identical monomorphism in closely related taxa affected this uniform allele pattern, locus D238 was excluded from further analyses. Genotypic disequilibrium was detected between several pairs of loci, but lost significance after standard Bonferroni adjustment for multiple comparisons (supplement tab. 8.8).

3.2.3   Genetic distance measures

3.2.3.1 Fixation index

Pairwise microsatellite F_{ST} values among populations ranged from 0.027 to 0.341 (tab. 3.5), whereby intraspecific genetic differentiation was far lower than differences among species or geographical distinct clusters. Populations belonging to *Tarsius lariang* (KOJ, MAK, PEA) had pairwise F_{ST} values of 0.029, 0.046 and 0.061. As observed in Lariang tarsiers, intraspecific comparisons of *T. dentatus* (KAM and LAO) and *T. wallacei* (BAT and UWE) sub-populations revealed small differences with F_{ST} values at 0.027 and 0.096, respectively. Moderate genetic distances are shown between *T. wallacei* and OGA (mean F_{ST}=0.116, p≤0.000), and among populations inhabiting the northern peninsula of Sulawesi (F_{ST} values of 0.136, 0.145, and 0.163; p≤0.0001). F_{ST} values for comparisons among BAN and all other Sulawesian taxa indicate high divergence (F_{ST} from 0.185 to 0.267, p≤0.0003), with largest genetic distances between BAN and populations from eastern parts of the island (KOR: FST=0.24; LUW: F_{ST}=0.267) and lowest level of divergence between BAN and Lariang tarsier populations (F_{ST} range from 0.149 to 0.185). On average Dian´s tarsiers were a little more distinct from KEN, *T. wallacei* and northern populations (OGA, LAB, DUA) than Lariang tarsiers (mean F_{ST} 0.293 and 0.257). *Tarsius dentatus* was genetically closer to KOR and LUW than any other taxon (F_{ST} range from 0.150 to 0.186, p≤0.0001).

Table 3.5: Pairwise F$_{ST}$ values among populations based on eight microsatellite loci

	BAN	PEA	KOJ	MAK	LAO	KAM	KOR	LUW	KEN	UWE	BAT	OGA	LAB	DUA
BAN		0.0000	0.0003	0.0000	0.0001	0.0000	0.0000	0.0000	0.0004	0.0000	0.0000	0.0000	0.0000	0.0000
PEA	0.1849		0.0397	0.0002	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000
KOJ	0.1491	0.0291		0.0436	0.0007	0.0000	0.0024	0.0002	0.0071	0.0006	0.0011	0.0001	0.0002	0.0006
MAK	0.1678	0.0611	0.0459		0.0002	0.0000	0.0007	0.0000	0.0005	0.0000	0.0010	0.0000	0.0001	0.0000
LAO	0.1779	0.2791	0.2533	0.2567		0.0476	0.0001	0.0001	0.0010	0.0001	0.0002	0.0000	0.0000	0.0001
KAM	0.2356	0.2997	0.2984	0.2841	0.0271		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
KOR	0.2444	0.2881	0.2672	0.2595	0.1500	0.1727		0.0001	0.0024	0.0006	0.0003	0.0002	0.0001	0.0001
LUW	0.2679	0.3181	0.3233	0.2912	0.1865	0.1558	0.1617		0.0005	0.0000	0.0000	0.0000	0.0000	0.0000
KEN	0.2188	0.2698	0.2384	0.2577	0.2862	0.3360	0.3264	0.3392		0.0011	0.0011	0.0000	0.0002	0.0001
UWE	0.2049	0.2770	0.2353	0.2664	0.2319	0.3005	0.2905	0.3337	0.2462		0.0005	0.0000	0.0000	0.0000
BAT	0.1872	0.2541	0.1992	0.2377	0.2407	0.2905	0.2611	0.2896	0.1775	0.0963		0.0001	0.0001	0.0000
OGA	0.2246	0.2453	0.2172	0.2381	0.2692	0.3260	0.2963	0.3385	0.1786	0.1122	0.1206		0.0000	0.0000
LAB	0.2294	0.2994	0.2728	0.2882	0.2843	0.3408	0.3268	0.3379	0.1794	0.2079	0.1784	0.1362		0.0000
DUA	0.2257	0.2942	0.2518	0.2767	0.2794	0.3335	0.3020	0.3143	0.2285	0.1787	0.1307	0.1625	0.1455	

Significance of pairwise comparisons is indicated by bold values.

3.2.3.2 Cavalli Sforza distance and proportion of shared alleles

Unrooted neighbor-joining trees constructed from microsatellite based distance matrices of Cavalli Sforza´s chord distance and the proportion of shared alleles (1,000 bootstraps each) produced very similar topologies (fig. 3.3). Both distance measures grouped north-northeastern populations and KEN, eastern populations (KOR, LUW) and *Tarsius dentatus* (KAM, LAO), and western regions inhabited by *T. tarsier* (BAN) and *T. lariang* (PEA, KOJ, MAK).
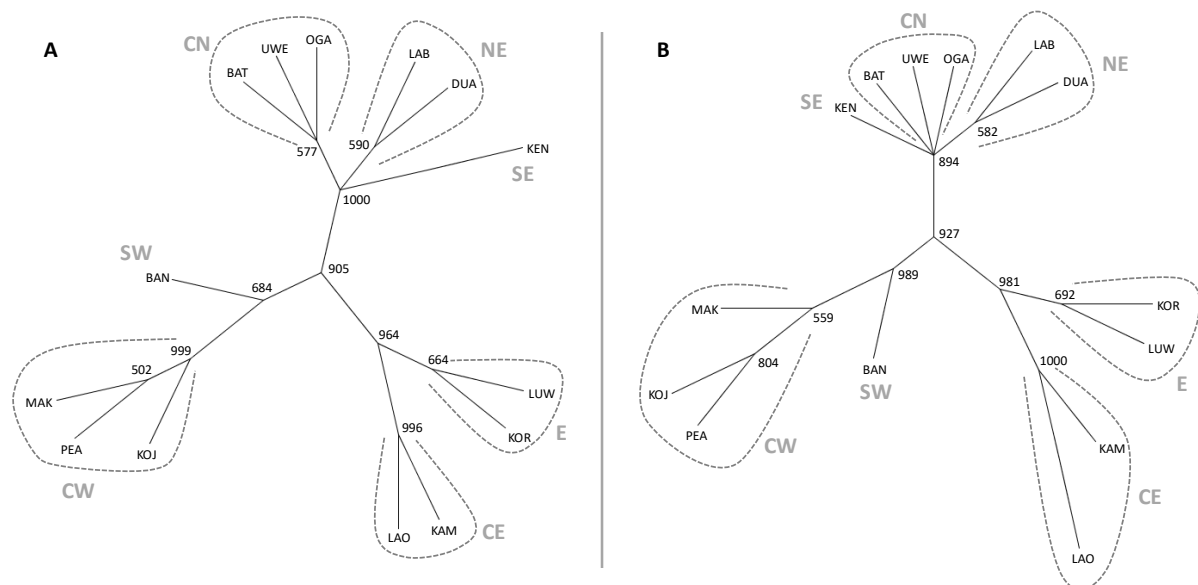
Figure 3.3: Unrooted neighbor-joining trees between tarsier populations
Trees are based on Cavalli-Sforza & Edwards (1967) chord distance (A) and the proportion of shared alleles (Bowock *et al.* 1994) (B) for eight microsatellite loci. Numbers at nodes represent bootstrap support after 1,000 replications. Geographic regions of populations are indicated in grey.

3.2.4 Population structure

Bayesian cluster analysis was conducted in STRUCTURE 2.3.2 (Pritchard *et al.* 2000). Ten independent simulations were run for K=1-14. Estimated log likelihood and log alpha values have shown low variance across replicate runs, confirming MCMC convergence. To find the optimal number of clusters, the mean log likelihood L(K) over ten runs was calculated for each K. The maximal value of L(K) was detected at K=7 (-4656.77). However, figure 3.4 shows that the difference of L(K) values between K=2 and K=3 is much higher (519.57) than between K=3 and K=4 (80.79), followed by a "more-or-less plateau" at larger Ks, which in turn would suggest that three clusters best fit the data. The method of Evanno *et al.* (2005) revealed the same result with ΔK highest at K=3.

K	L(K)	SD	ΔK
1	-5995.84	0.08	-
2	-5365.29	35.65	3.11
3	-4845.72	0.52	850.40
4	-4764.93	9.36	2.46
5	-4707.20	9.42	7.22
6	-4717.48	198.19	0.36
7	-4656.77	11.83	6.56
8	-4673.65	4.71	1.60
9	-4682.98	3.93	0.30
10	-4693.48	5.92	0.59
11	-4707.48	13.91	0.26
12	-4717.85	8.98	0.58
13	-4723.04	3.06	2.59
14	-4736.17	6.52	2.01
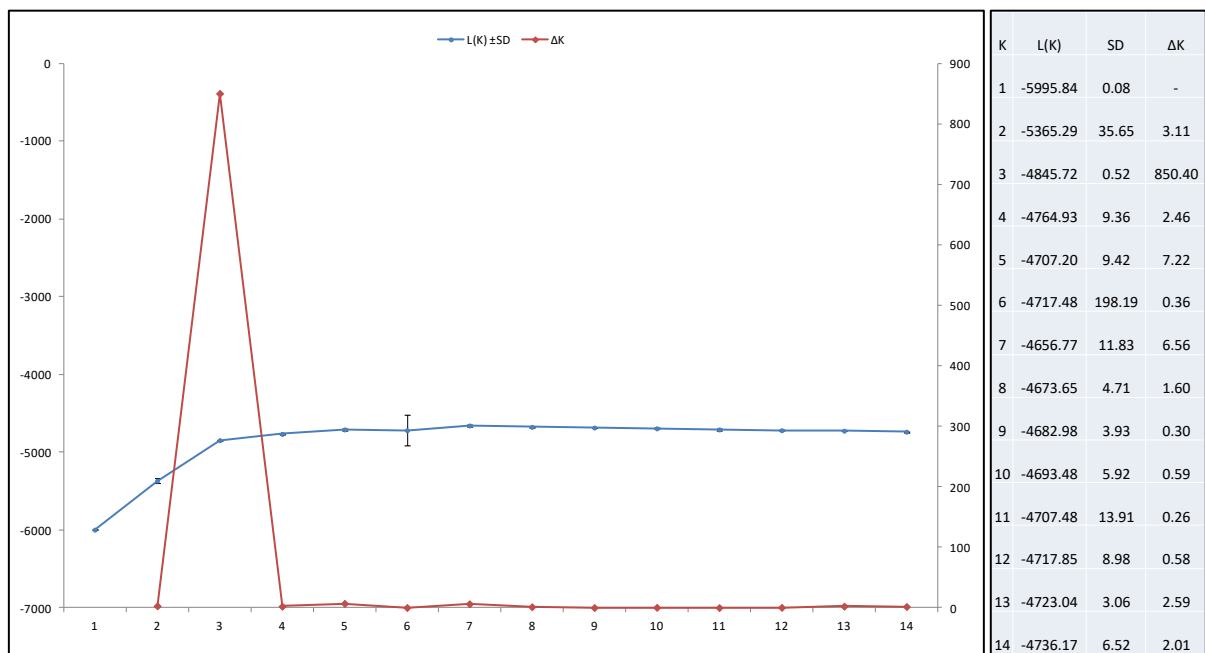
Figure 3.4: Mean posterior probabilities L(K) and ΔK of ten independent runs for K=1 to K=14

The three genetic distinct clusters correspond well with the geographic distribution and are grouped as follows: 1) western populations (BAN, KOJ, MAK, PEA), 2) eastern population (KAM, KOR, LAO, LUW) and 3) northern populations (BAT, DUA, LAB, OGA, UWE) together with KEN, a population located at the southeastern peninsula. With exception of BAN (average Q = 0.89), the mean membership coefficient Q of a population to one of the three clusters varied from 0.97 to 0.99.

Further structuring clearly separates BAN from the western population cluster at K=4 (fig. 3.5). Assuming seven distinct groups, which would be consistent with the highest likelihood observed after inspection of STRUCTURE results, the most eastern populations (KOR and LUW) diverged from Dian´s tarsiers (KAM and LAO), and the third cluster split into three groups, KEN, BAT/OGA/UWE, and DUA/LAB. With average Q values varying from 0.70 to 0.98 (tab. 3.6), the seven clusters can be readily distinguished, although few populations were admixed. At K=7 two of the seven newly sampled populations also represent a single cluster, BAN and KEN. OGA was grouped with *Tarsius* w*allacei* (BAT and UWE), and individuals of cluster 7 (DUA and LAB, see tab. 3.6) could not be differentiated further.

Table 3.6: Mean membership coefficients Q of tarsier populations to each of seven clusters

Pop	Cluster							N_{IND}
	1	2	3	4	5	6	7	
BAN	**0.944**	0.017	0.010	0.010	0.007	0.006	0.007	10
PEA	0.004	**0.981**	0.003	0.003	0.003	0.003	0.003	28
KOJ	0.008	**0.963**	0.006	0.007	0.006	0.005	0.005	5
MAK	0.005	**0.960**	0.010	0.014	0.004	0.004	0.003	7
LAO	0.004	0.003	**0.975**	0.005	0.004	0.005	0.004	8
KAM	0.004	0.004	**0.972**	0.010	0.003	0.004	0.003	32
KOR	0.005	0.006	0.114	**0.863**	0.005	0.004	0.003	6
LUW	0.003	0.003	0.087	**0.896**	0.004	0.003	0.004	10
KEN	0.005	0.008	0.008	0.004	**0.773**	0.037	0.164	5
UWE	0.007	0.005	0.014	0.005	0.006	**0.958**	0.005	8
BAT	0.005	0.004	0.006	0.005	0.100	**0.744**	0.136	7
OGA	0.003	0.004	0.003	0.003	0.037	**0.939**	0.011	12
LAB	0.008	0.003	0.012	0.010	0.101	0.165	**0.701**	10
DUA	0.003	0.003	0.005	0.005	0.009	0.014	**0.962**	12

Bold black: highest group membership; black: group membership > 0.05; grey: group membership < 0.05.

Three of ten individuals sampled in LAB possess mixed memberships, one of them could be a putative migrant with strongest membership proportion to cluster 6 (average Q value 0.83). Intermediate membership coefficients could also be detected for seven additional individuals from BAT (3), KEN (2), KOR (1), and LUW (1). The largest proportion of an individual´s genotype was generally allocated to that cluster where the individual´s sample population was assigned to (~ 98 % of all 160 individuals).
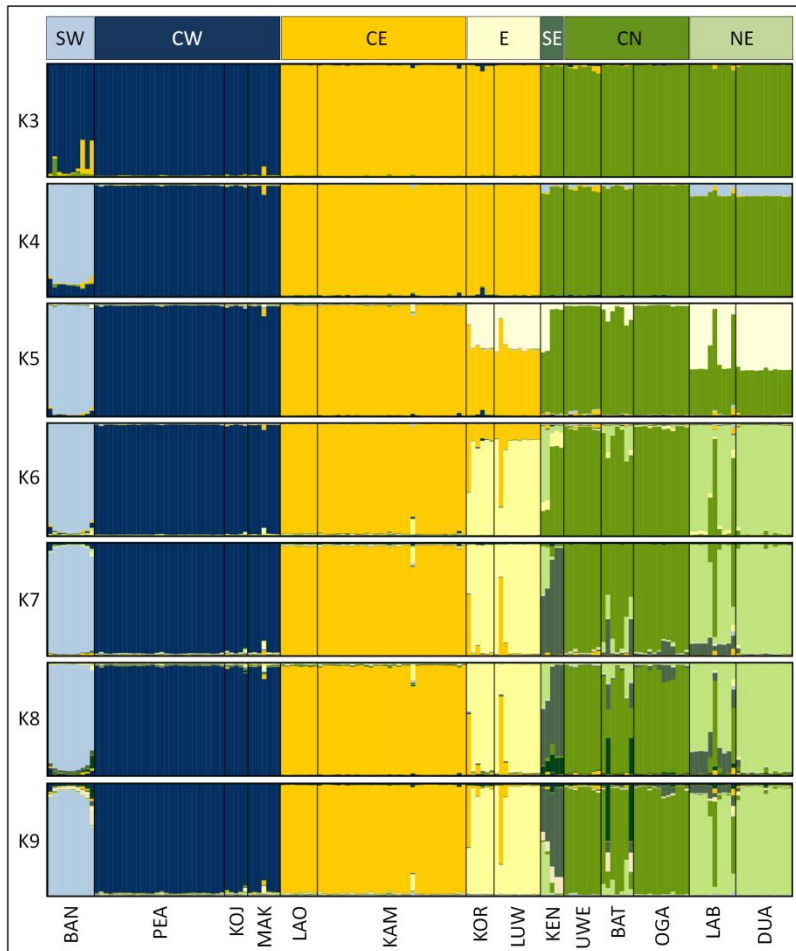
Figure 3.5: Estimated population structure of tarsier populations for microsatellite genotypes
Illustrated are clusters inferred by STRUCTURE (Pritchard *et al.* 2000) for K=3 to K=9. Each of 160 individuals is represented by a vertical colored bar, which is partitioned into K segments, each indicating the estimated population group membership.

3.2.5   Isolation by distance

Mantel tests were conducted for spatially connected sample localities where isolation by distance (IBD) could have caused population structure. Hence, populations of the northern peninsula and those located on central and eastern parts of Sulawesi were tested for IBD, see fig. 3.6 for details. No significant correlation between genetic and geographic distance among northern populations was found (p-values ranged from 0.164 to 0.293). However, among *Tarsius dentatus* (KAM and LAO) and most eastern populations (KOR and LUW) a significant effect of IBD could be revealed (Z=3.682, r=0.909, p-value=0.026). Scatter plots of genetic distance ($F_{ST}/1-F_{ST}$) vs. logarithmic geographic distance are displayed in fig. 3.6.

Northern Sulawesi	Z	r	$p_{one-sided}$
A BAT-OGA-LAB-DUA	2.623	0.357	0.293
B BAT-OGA-LAB	1.241	0.946	0.164
C OGA-LAB-DUA	1.323	0.968	0.167
Central-East Sulawesi			
D LAO-KAM-KOR1-KOR2-LUW	3.682	0.909	0.026
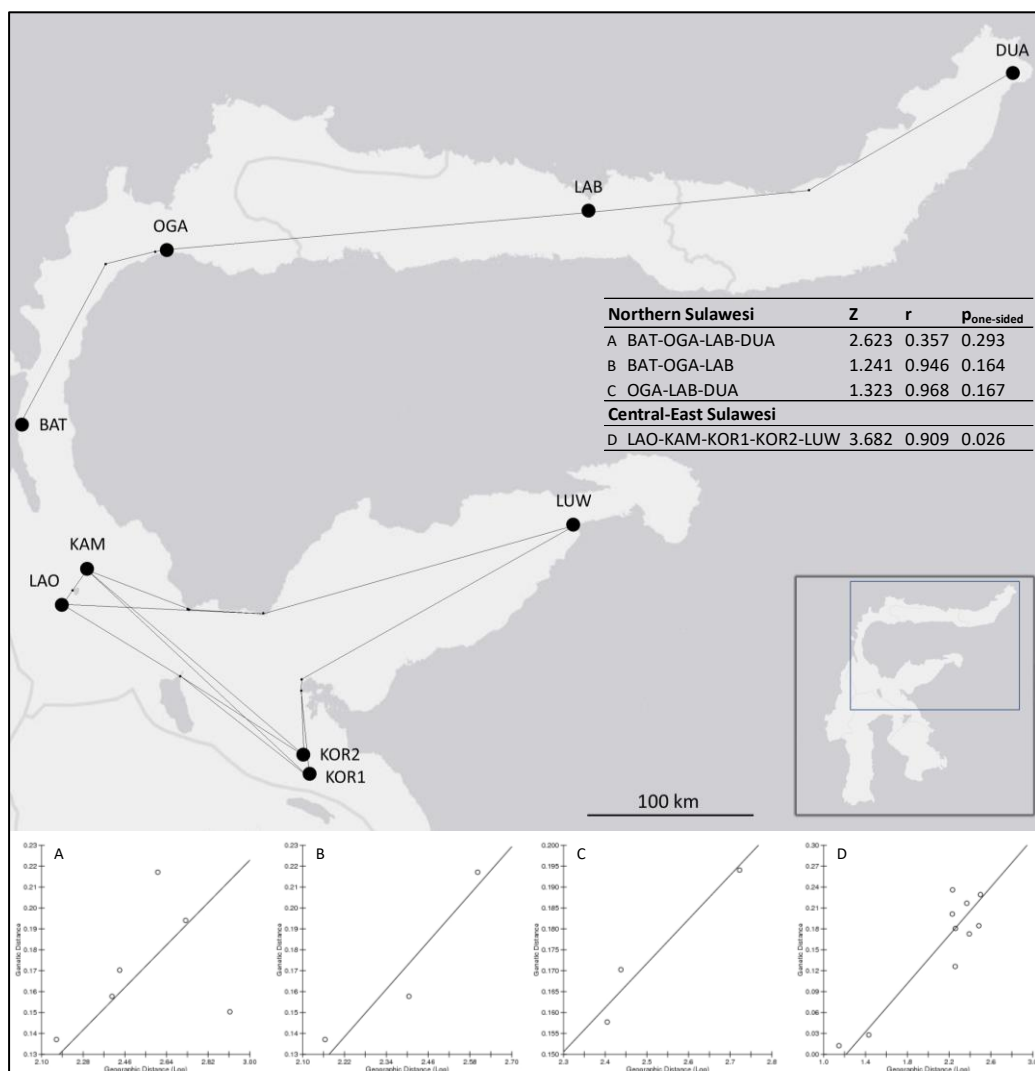
Figure 3.6: Isolation by distance analyses for northern and central-eastern populations
Above: Map of sample locations (large black circles). Black lines between study sites indicate the geographical distance. Small black circles are linker enabling the measurement of shortest over land path lengths between populations. Results of IBD analyses are displayed in the table. Below: Corresponding scatter plots of each of the four tested population combinations. The graphs show the genetic distance (on the y-axis) vs. geographic distance (on the x-axis).

3.3   Sequence data

3.3.1   Sex determining region of Y

A 630 bp fragment of the sex determining region Y gene (SRY) was PCR amplified and sequenced for the 24 males of the new sample set (set 2). Six novel haplotypes were detected. Two of the seven populations (LAB and LUW) have two haplotypes, each with one private haplotype and one they share with adjacent populations (OGA and KOR). The common haplotype in KOR and LUW has already been observed in *Tarsius dentatus*, a central Sulawesian species here represented by individuals of KAM and LAO. The populations BAN, DUA, and KEN carry one unique haplotype each. In total ten SRY haplotypes of Sulawesi tarsiers were used for gene tree reconstruction (fig. 3.7 A). Both, maximum likelihood and Bayesian analyses revealed that SRY haplotypes were derived from two distinct lineages (ML bootstrap value= 100, pp= 1.0). One lineage comprises all western, central, and eastern populations, while southeastern (KEN) and all central-northern and northern populations (BAT, UWE, DUA, LAB and OGA) were grouped together.

3.3.2   Mitochondrial sequence data

Cytochrome b: The complete cytochrome b gene (1140 bp) was amplified and sequenced for all 65 specimens sampled in 2009 and 2010. Integrity of sequences was verified by converting nucleic acids into protein sequences. In total 153 sequences, 44 non identical haplotypes, were included in phylogenetic analysis, with 26 novel haplotypes obtained from the seven new sample sites. Each new population possesses three to four haplotypes, all unique to its own.

Hypervariable Region 1: Partial sequences (380 bp) of the D-loop hypervariable region 1 (HVR1) were generated for the complete new sample set. In total 27 unique and population specific haplotypes were identified. The number of haplotypes per population varied from three to five. These data were complemented by 23 haplotypes of *Tarsius lariang*, *T. dentatus*, and *T. wallacei* from previous studies.

Species tree: 156 HVRI and 153 cytochrome b sequences were subjected to species tree reconstruction. Posterior effective sample size (ESS) of combined *Beast analyses was > 6,000, whereas most ESS values were observed between 5,000 and 10,000 (see supplement tab. 8.10). ESS values of all parameters of interest closely approximated or exceeded 200. In summary, the species tree generated by *BEAST supports two major mitochondrial lineages on Sulawesi (pp= 1.0). Southern populations (BAN and KEN, pp= 0.87) were distinguished from all other populations, which form their own lineage (pp= 0.85). Further structuring of the latter reveals four strongly supported monophyletic groups: *Tarsius wallacei* (pp= 1.0), the *T. lariang*/LUW clade (pp= 0.99), the northern clade (OGA/LAB/DUA, pp= 1.0), and the *T. dentatus*/KOR clade (pp= 1.0). The Bayesian species tree analysis however failed to resolve relationships between these clades with high confidence (fig. 3.8 C).

3.3.3   Nuclear DNA species tree

Two independent MCMC chains were combined to create the final species tree. Both analyses yielded ESS values generally above 1,000, the two chains converged and mixed well. Combined ESS values were greater than 200 throughout, and most exceeded 2,000 (supplement tab. 8.11). The resulting tree topology (fig. 3.7 B) has two peculiarities. First, Sulawesi tarsiers are recovered as monophyletic with a strongly supported sistergroup relationship to Western and Philippine tarsiers (pp=1.0), without having applied topological constraints to outgroup nodes.
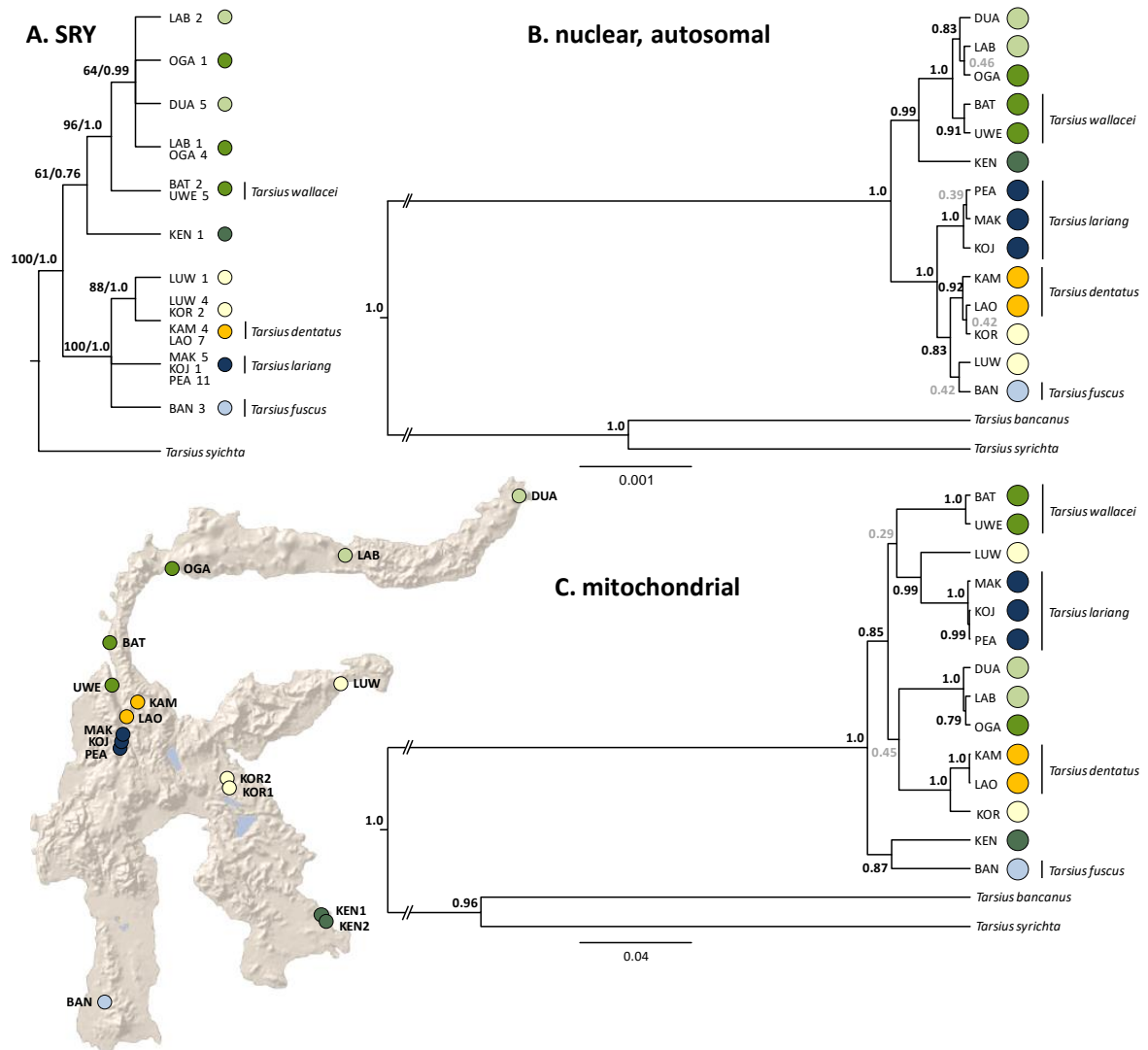
Figure 3.7: Gene tree and species tree phylogenies of Sulawesi tarsiers
A) Genealogy showing the relationship between ten SRY haplotypes using the Philippine tarsier as outgroup. Numbers above nodes represent bootstrap values (%) for maximum likelihood (ML) and posterior probabilities (pp) obtained from Bayesian analyses. Numbers behind population labels indicate the number of males with the respective haplotype. Multilocus species trees based on B) nuclear, autosomal, and C) mtDNA sequence data produced by Bayesian analysis using *BEAST. Node support values are posterior probabilities (pp). Black/grey values: pp above/below 0.5. Bottom left: Map of sample sites on Sulawesi. Colors in circles behind population labels correspond to colors used to discriminate clusters inferred from STRUCTURE analysis (see fig. 3.5).

Second, Sulawesi tarsiers are composed of two major clades (pp=1.0), both revealing further phylogeographic structuring on species/sub-species level. The first clade includes *Tarsius lariang* (KOJ, MAK, PEA), *T. tarsier* (BAN), *T. dentatus* (KAM, LAO) and the two most eastern populations (KOR, LUW), with Lariang tarsiers sister to all other groups. Populations from the central-northern and northern parts of Sulawesi (BAT and UWE, DUA, LAB, OGA) form a clade with KEN (pp=0.99), a population located on the southeastern peninsula and representing the deepest split of this lineage. The internal node of populations from northern Sulawesi splits up into two branches (pp=1.0), separating Wallace´s tarsier (BAT, UWE) from the most northern regions of the island (DUA, LAB, OGA).

3.3.4   Divergence times

Divergence times of Sulawesi tarsier populations were estimated based on a comprehensive DNA sequence set amplified from five nuclear genes and including ten genera representing both haplorhine (six anthropoid taxa and *Tarsius*) and strepsirhine (three genera) primates. Two different calibrations were applied to the species tree. Convergence of MCMC samples was verified in TRACER. MCMC chains from five independent *BEAST analyses of each calibration were combined to reconstruct a final species tree. All parameters of interest yielded ESS values above 200, most exceeded 1,000 (see supplement tab. 8.12 and tab. 8.13).

Table 3.7: Divergence times and posterior probabilities for haplorhine and strepsirhine primates

Node	#	Calibration 1				#	Calibration 2			
		Age (MYA	95% lower	95% upper	pp		Age (MYA	95% lower	95% upper	pp
Homininae	**1**	3.33	0.69	5.83	1.0	**1**	4.18	1.21	6.63	1.0
Hominidae	**2**	13.88	11.11	16.59	0.9	**2**	16.72	14.08	18.76	0.9
Hominoidea	3	15.55	12.77	18.41	1.0	3	18.81	16.44	21.31	1.0
Catarrhini	4	23.99	21.06	26.75	1.0	4	29.25	24.93	33.37	1.0
Anthropoidea	5	35.85	32.00	39.82	1.0	5	42.06	37.48	46.37	1.0
Haplorhini	6	68.53	64.71	72.15	0.9	6	84.20	76.01	92.25	0.9
Primates	7	73.04	69.79	76.27	1.0	7	90.74	83.58	98.03	1.0
Strepsirhini	8	51.67	46.46	56.94	1.0	8	67.05	59.71	74.05	1.0
Lemuriformes	9	42.67	36.29	49.64	1.0	9	55.44	46.79	64.38	1.0
Tarsius	1	18.28	13.71	23.23	1.0	1	22.16	16.58	28.15	1.0
Western-Philippine	1	8.02	4.79	11.27	1.0	1	9.82	5.92	13.96	1.0
Eastern tarsiers	1	2.05	1.34	2.91	1.0	1	2.49	1.62	3.54	1.0
	1	1.35	0.77	2.05	0.9	1	1.62	0.93	2.43	0.9
	1	0.43	0.25	0.66	1.0	1	0.51	0.29	0.78	1.0
Eastern tarsiers: Lineage 1	1	0.15	0.00	0.35	0.9	1	0.19	0.00	0.43	0.9
	1	0.25	0.09	0.46	0.8	1	0.30	0.10	0.56	0.8
	1	0.15	0.00	0.32	0.5	1	0.18	0.00	0.39	0.5
	1	0.77	0.41	1.26	1.0	1	0.95	0.50	1.53	1.0
	1	0.17	0.03	0.35	1.0	1	0.19	0.04	0.42	1.0
Eastern tarsiers: Lineage 2	2	0.49	0.23	0.80	0.7	2	0.59	0.27	0.99	0.7
	2	0.18	0.04	0.36	0.9	2	0.22	0.10	0.56	0.9

#: Numbers correspond to nodes in the species trees (fig. 3.8). Numbers written in bold represent calibrated nodes; Age (MYA): median node age in million years ago; HPD: Highest posterior density; pp: posterior probability.

Divergence times of both calibrated species trees support an initial diversification of crown tarsiers between late Oligocene and early Miocene (pp=1.0, see tab. 3.7 and fig. 3.8). The median node age was 18.28 MYA (95 % confidence interval: 13.71-23.23 MYA) using calibration 1 and 22.16 MYA (95 % confidence interval: 16.58-28.15 MYA) using calibration 2. During this period Eastern tarsiers split from their Western and Philippine sister taxa and represent a distinct evolutionary unit. The two major lineages within the Eastern tarsier complex (chapter 3.3.3) are most likely to have originated during Plio-Pleistocene (median node ages were 2.05 MYA and 2.49 MYA with confidence intervals ranging from 1.34-3.54 MYA) and split into several sublineages in Pleistocene, a period of intense glacial cycles and tectonic activity. Divergence of the Western and Philippine tarsier lineages took place at around 10 MYA (median node ages: 8.02/9.82 MYA, 95 % confidence interval: 4.79/5.92-11.27/13.96 MYA; pp=1.0/1.0).
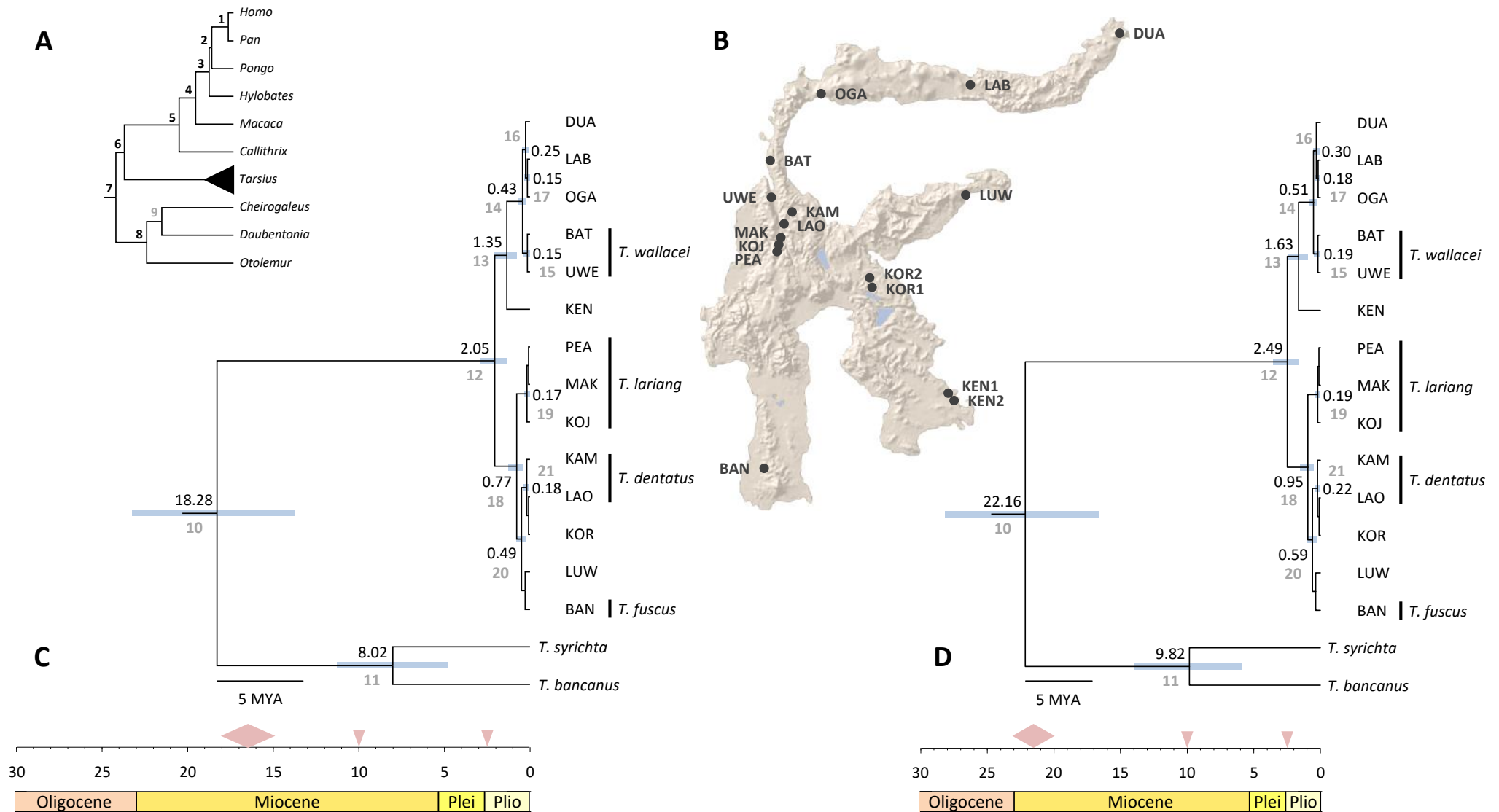
Figure 3.8: Calibrated species trees based on multilocus nuclear DNA markers

A) Primate phylogeny. Black/grey numbers at nodes indicate a calibration point/uncalibrated node; B) Map of sample sites on Sulawesi; C) and D) are enlarged sections of the primate phylogeny (A) showing branches for *Tarsius*. C) displays divergence times inferred from calibration1 and D) shows time estimates based on calibration 2. Black/grey numbers at nodes correspond to median node ages/uncalibrated nodes. Blue bars represent the 95% posterior credibility intervals for nodes. Light red diamond/triangles highlight the Sulawesi-Sula Spur collision (23-20 MYA)/glacial maxima (10 MYA and 2.5 MYA) on the time scale. Plei=Pleistocene, Plio=Pliocene.

4   Discussion

4.1   The origin of Sulawesi tarsiers

Molecular sequence data clearly show that Sulawesi tarsier populations share a common genetic ancestry. They are sister to Western and Philippine tarsiers and represent the oldest lineage of the genus *Tarsius*, with the split of crown tarsiers being estimated to 18.28 and 22.16 MYA, respectively. Philippine tarsiers presumably split from their western sister group about 10 MYA (8.02 and 9.82 MYA). A period which marks the lowest tertiary sea level (Haq *et al.* 1987) accompanied by an increase of dispersal possibilities at the Indo-Malay archipelago, including migration between Borneo and the Philippines (see paleogeographic maps in Hall 2001, Lohman *et al.* 2011). The term "old endemics" is gaining significance when considering that Eastern tarsiers possibly colonized Sulawesi between late Oligocene and early Miocene (95 % confidence intervals ranging from 13.71-28.15 MYA), what in turn falls well within the onset of sea level decrease (Haq *et al.* 1987). The midpoint of these age estimates at approximately 20 MYA is in line with the interval over which the Australian-Sunda plate collision and the resulting emergence of land at the paleo-Sulawesi archipelago are assumed to have occurred (Hall 2001, 2009, Stelbrink *et al.* 2012).

According to the divergence time estimation above no evidence for vicariance in terms of micro-continental drift is given. Eastern tarsiers seem to have diverged considerably later than West-Sulawesi separated from Borneo. Therefore tarsiers, as supposed for most Sulawesian fauna (Lohman *et al.* 2011, Stelbrink *et al.* 2012), very likely expanded their geographical range eastwards across Wallace´s line via dispersal. The mode of dispersal is open to speculation. Intermittent land connections as consequence of periodic sea level falls could have facilitated transition from Sundaland to Sulawesi´s precursors. There is no geological evidence for a land bridge through the Makassar Strait. Nevertheless, this hypothesis is not unrealistic considering the large expansion of continental shelves that could have exposed islets forming the basis of stepping stone dispersal (van den Bergh *et al.* 2001). Furthermore the traversal over the Sunda volcanic arc to Sulawesi maybe considered an option, even though some authors deem it unlikely given the lack of evidence for living tarsiers on Java (Whitten *et al.* 2002, Shekelle 2008a). An alternative theory is rafting dispersal. The majority of Malagasy mammals probably populated Madagascar from Africa by rafting (Yoder & Nowak 2006, Ali & Huber 2010). Sea rafting has also deemed to be the most likely dispersal mechanism of Sulawesi macaques (Abegg & Thierry 2002). Successful transoceanic rafting depends on longevity of the substrate (Thiel & Haye 2006) and the capability of the organism, especially of terrestrial vertebrates, to endure water deprivation (Houle 1998). Survival of small sized mammals on floating islands is not rendered impossible (Houle 1998). Yet resilient rafts are rare and long distance dispersal events thereby less frequently. This in turn would hamper migration over many generations and promote allopatric speciation (Thiel & Haye 2006), thus rafting dispersal is a plausible scenario for the progenitor of Sulawesi tarsiers.

There is still an open issue as to whether habitats suitable to tarsiers already existed in the period of tarsier radiation. Living tarsiids are often referred to as "living fossils" because of their morphological resemblance to their extinct ancestors. For this reason it is assumed that their ecological niche demands have not changed substantially over the past 45 million years (Jablonski 2003). Based on palynological analyses and climate models ever-wet rain forests became widespread in the Southeast

Asian region at about 20 MYA (Morley 1998) after a longer cooling event during Eocene that led to extinctions in tropical organisms (Prothero 1994). Indonesian rainforests have not been affected by this climate shift and persisted from Eocene onward (Jablonski 2005). The existence of rain forests on the Indo-Malay archipelago guaranteed refuge for organisms adapted to this specific environment. Given that ancient tarsiids inhabited mainland Asia before arrival on the Southeast Asian islands (see fossil record chapter 1.3), climatically mediated contraction of rain forests in Eocene could have enforced the southward movement of these highly specialized clingers and leapers.

4.2   Within-island diversification on Sulawesi

4.2.1   Geographical variation in vocalization

Sociality in non-human primates is thought to favour the evolution of complex vocal communication (McComb & Semple 2005). Sulawesi tarsiers live in groups and exhibit a wide repertoire of intra- and inter-specific variability in their vocalizations that have been hypothesized to serve several functions. Beside territorial defence, group cohesion and mate recognition, the conspicuous geographical variation of male-female duet songs have made some scientist to draw the conclusion that tarsier vocalization may be taken as species diagnostic criterion (MacKinnon & MacKinnon 1980, Nietsch 2003, Shekelle 2008a, Burton & Nietsch 2010,). Calls of diverse mammals were used as taxonomic determinants, including bats, rodents and monkeys (Parson & Jones 2000, Miller & Engstrom 2007, Meyer *et al.* 2012). However the questions that arise in this context relate to how reliable vocalisations as species identifier really are. An example of *Pan troglodytes* shows that vocal learning can affect the acoustic structure even within species. Differences in vocal behavior of contiguous chimpanzee communities did not correlate with genetic relationships, but rather depended on proximity of territories (Crockford *et al.* 2004). However, in Javanese gibbons and central Sulawesi tarsier species vocal similarity highly correlated with genetic relatedness, thus verifying loud calls as credible tool for taxon affiliation (Merker *et al.* 2009, Meyer *et al.* 2012).

Even if the above mentioned studies provide opposing results, published resources of tarsier vocalizations formed an important basis for the sampling strategy at early stages of this project. Therefore, duet songs have been recorded at every study location explored and at all capture sites. Because tarsier vocalizations were not the main purpose of this thesis, vocal data have not been subjected to detailed acoustic and statistical analyses, but have been visualized and compared to published spectrograms. Not surprisingly individuals of DUA, KEN, LAB and OGA could clearly be allocated to the Kendari form (KEN), Sejoli form (OGA) and the Manado form (DUA and LAB), respectively (fig. 4.1). Labanu (LAB) is situated at the boundary of two acoustic types (Gorontalo form and Manado form). Thus it was a coincidence which type of vocalization would be encountered there. At four additional points morning calls have been recorded to detect possible species boundaries. Given that tarsier vocalization indicates taxonomic affiliation three of them (Mamuju, Karumba and Tirondo) enabled the distribution areas of *T. lariang*, *T. dentatus*, and *T. wallacei* to be refined (compare fig. 4.1 A and B).

Altogether ten of eleven newly recorded vocalizations of tarsier populations - seven are documented by genetic data - could be assigned to five known acoustic forms without doubt. Spectrograms of BAN are in some respects similar to the duet pattern of the Bantimurung form/*T. fuscus* (Burton & Nietsch 2010, Shekelle *et al.* 2010). Unfortunately this acoustic form is barely documented in literature, such that there is no definitive assignment of BAN. In summary it can be said that populations performing highly similar duet songs seem to cluster genetically, as has been shown for *T. dentatus*. But with regard to tarsier populations inhabiting the northern peninsula of Sulawesi, unique acoustic characteristics are not inevitably reflected in genetic distinctiveness. Tarsier duet songs therefore can be seen as circumstantial evidence rather than as immediate proof for genetic relatedness.
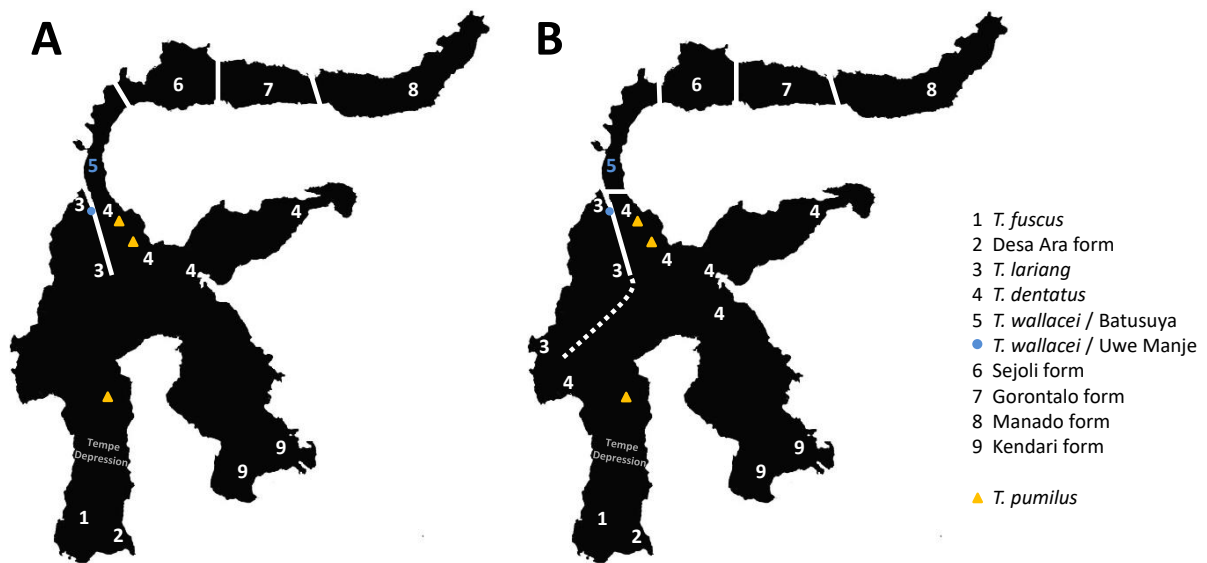
Figure 4.1: Distribution of distinct tarsier acoustic forms on Sulawesi
Source data (A) and redrawn ranges resulting from this study (B). Locations where *Tarsius pumilus* has been recorded are indicated with yellow triangles.

4.2.2   Nuclear markers

Analyses of nuclear DNA applying both microsatellite and sequence data lead to similar phylogeographic patterns. The SRY gene tree and the multilocus species tree support two major lineages on Sulawesi. Populations from the northern peninsula (*Tarsius wallacei*, DUA, LAB, OGA) are grouped with KEN from southeast Sulawesi. The second lineage includes southwestern (*T. fuscus*), western (*T. lariang*), and eastern populations (*T. dentatus*, KOR, LUW). Microsatellite analyses strongly support the monophyly of the north-southeast clade, the affiliation of KOR and LUW to *T. dentatus*, and the taxonomic independence of *T. fuscus* (BAN) and *T. lariang* (KOJ, MAK. PEA). Set out below are general remarks on each lineage.

Lineage 1: According to duet call characteristics previous publications have suggested that the northern peninsula hosts four species or subspecies (MacKinnon & MacKinnon 1980, Burton & Nietsch 2010, Merker *et al.* 2010, Shekelle *et al.* 2010). Although *T. wallacei* (BAT, UWE) is recovered as monophyletic, phylogenetic resolution among DUA, LAB, and OGA did not allow for a clear taxonomic

statement. Microsatellite data display that DUA and LAB cluster tightly, while OGA is allied to *T. wallacei*. On the contrary, Y-chromosomal and autosomal sequences suggest common ancestry of these three northern populations. DUA, LAB, and OGA live in spatial contiguity, the range of one population borders on the range of the next. Therefore geographically no apparent reproductive barrier exists between adjacent populations, which consequently provide an opportunity for gene flow. At this point the question arises as to whether gene flow emerged upon secondary contact of two formerly allopatric populations or if we seem to be witnessing parapatric speciation. Genetic similarity in rapidly evolving microsatellite genotypes among northern populations and short branch lengths in the nuclear species tree would indicate recent population divergence, thus findings seem to be more consistent with the second alternative. Otherwise, given that the northern peninsula was already populated by tarsiers in Pleistocene post glacial sea level rise could have induced range fragmentation, as has been the case at Lake Limboto in the Gorontalo region (Whitten *et al.* 2002). Here an oceanic inundation could have enforced allopatric speciation as assumed for *Macaca hecki* and *M. nigrescens* (Evans *et al.* 2003b). The period of isolation may have been too short to build impermeable interspecific reproductive barriers, but possibly was long enough to produce differences in mate recognition. The divergence in acoustic traits of northern tarsier populations and Sulawesi tarsiers in general could have led to vocal preferences for conspecifics. Hence, interspecific breeding will be reduced to a low level or, in the extreme, completely prevented. In the case of tarsier populations from the northern peninsula gene flow was detected so that it can be assumed that prezygotic isolation for example caused by divergence in acoustic mating signals is incomplete. Isolation by distance (IBD) could also explain genetic structure of a population occupying a linear geographic range. But even if there was indication for correlation of geographic and genetic distance between northern tarsier populations, no significant decline in genetic relationship with distance could be detected. Nevertheless, tarsiers from Sulawesi´s north arose in allopatry and/or distance-mediated divergence, presumably in the recent past, from the same ancestral population as KEN has evolved. The deep split between southeastern and northern tarsiers is reflected by a long geographic distance and a discontinuous range, obviously interrupted by *Tarsius dentatus*, a species with high dispersal capabilities (Merker *et al.* 2009, 2010). When Dian´s tarsiers invaded central and eastern parts of Sulawesi (see chapter 4.2.4), they ousted *T. wallacei* from the isthmus north of Palu and obviously limited the geographic range of KEN to southeast Sulawesi.

Despite the comparatively high divergence of nuclear sequence data and their spatial isolation from each other, northern and southeastern populations still share a large proportion of microsatellite alleles. Due to high mutation rates in short tandem repeats (STRs) these markers are especially useful to study closely related species and populations (Zhang & Hewitt 2003). The down side might be that replication-slippage based mutation together with an inefficient DNA mismatch repair system and a high mutation rate pander to size homoplasy (Schlötterer 2000, Estoup *et al.* 2002, Hey *et al.* 2004). However, the general consensus among applied nuclear markers is indicative for the reliability of microsatellite data. Genotypic similarity may have resulted from ongoing or recent gene flow. Although it seems paradoxical at first sight, a connection between northern and southeastern populations in the recent past is not necessarily impossible. The key to resolving this conflict possibly lies in the mountains. Pygmy tarsiers (*Tarsius pumilus*) today inhabit mossy rain-forests in the island´s mountainous heartland. Their morphology is well adapted to the special environmental conditions

they live in, and their behavioral traits seem to differ sharply from their lowland neighbors (Shekelle 2008b, Grow & Gursky 2010). But orogeny and therewith formation of this unique habitat began in Pliocene (Lohmann *et al.* 2011, see chapter 1.3). Here a cautious, hypothetical excursion may be dared. Lineage 1-tarsiers diversified from an ancestral species into their mountain and lowland phenotypes, respectively. Adaptive radiation in the context of orogeny is not an abrupt event but a continuous process. Reproductive barriers may have evolved progressively but moderately, thus gene flow did not stop suddenly. Fully isolated populations which have recently diverged and/or have had a relatively large effective population size could have either not yet experienced genetic drift or will suffer this effect more slowly (Bulgin *et al.* 2003). Thus, irrespective of whether *T. pumilus* maintained genomic exchange or not, ancestral allele variants were simply preserved due to little change in allele frequency. However, although the phylogenetic position of *T. pumilus* is still unknown, there is indication that pygmy tarsiers represent descendants of the lineage 1-stem population. They are significantly smaller than Sulawesi lowland tarsiers and seem to rarely scent-mark (Grow & Gursky 2010). Among lowland tarsiers the northern population of Wallace´s tarsiers is considerably smaller in size than other lowland tarsiers (Merker *et al.* 2010) and urine marks have less frequently been recognized (personal communication S. Merker, own observations). Inhabiting another ecological niche, pygmy tarsiers obviously could not be displaced from central Sulawesi by *T. dentatus* like their lowland relatives. Pursuing the probable dispersal route of lineage 1 on Sulawesi (fig. 4.2) it would be reasonable to expect that *T. pumilus* will phylogenetically be positioned between KEN and *T. wallacei*.

Lineage 2: This lineage comprises three annotated tarsier species - *Tarsius fuscus* (BAN), *T. lariang* (KOJ, MAK, PEA), and *T. dentatus* - and both eastern populations (KOR, LUW). The close relationship among *T. dentatus*, KOR and LUW is depicted by SRY and microsatellite data revealing genomic signatures of gene flow. Furthermore, population genetic structure of this assemblage is characterized by a pattern of isolation by distance. Based on acoustic records it has already been predicted that *T. dentatus* occupies an area ranging from east of the Palu-Koro fault to the edge of the eastern peninsula (Merker *et al.* 2010, Shekelle *et al.* 2010), but this thesis provided for the first time molecular evidence. A possible explanation for the slight discordance in Y-chromosomal and autosomal phylogenies could be incomplete lineage sorting. Following speciation genes of one lineage converge to the overall phylogeny of the respective species. The period of time to reach allelic fixation depends on the effective population size. Large populations generally need longer to become monophyletic (Maddison & Knowles 2006). Given that *T. dentatus* at minimum inhabits central and eastern parts of Sulawesi, own surveys predict a much broader range (see chapter 4.2.1 fig. 4.1), a relatively large population size can be assumed. Their recent divergence from *T. fuscus* and *T lariang*, as well as the geographical seclusion of LUW would support the protracted nature of the lineage sorting process in *T. dentatus*.

4.2.3   Discordant patterns of mitochondrial and nuclear markers

In vertebrates, nuclear DNA has a lower mutation rate than mtDNA (Vawter & Brown 1986). Due to the more rapid evolution, the matrilinear inheritance and the non-recombining nature of the mitochondrial genome, it is not surprising that differently transmitted genetic material leads to different phylogenies. While nuclear sequence data of tarsiers produced highly convergent results, the mtDNA based species tree is not compatible with the nuclear phylogeny. Incongruence between nuclear and mitochondrial inferred relationships is not uncommon and has already been revealed in many taxa, including mouse lemurs (Heckman *et al.* 2007) and macaques (Evans *et al.* 2003b). This phenomenon can have various causes that are often closely linked to historical and contemporary life history traits of the respective study organism. Sex bias in dispersal is certainly a widespread mechanism causing incongruent patterns between sex-linked and biparental inherited genetic markers (Melnick & Hoelzer 1992, Lyrholm *et al.* 1999, Evans *et al.* 2003b). Most tarsier populations examined in the framework of this thesis lived in single-male multi-female groups, which may already be an indication for female philopatry and male dispersal (Merker *et al.* 2009). Additionally mitochondrial haplotypes were almost entirely unique to a study locality, whereas nuclear genetic data hint at male-mediated gene flow. Contrasting mitochondrial sequence affiliations perhaps reopen the past and allow a view of ancestral distribution patterns. As is known Sulawesi was the centre of plate tectonic activity and attained its present form within the past five million years (Hall 2001). Fluctuating environmental conditions especially during Pleistocene alternately expanded and contracted habitats, presumably with the result that species boundaries shifted, vanished or emerged. Sulawesi tarsiers apparently have explosively diversified in this era, thus mitochondrial relationships perhaps testify to ancient hybridization of unrelated extant lineages.

Long term barriers to gene flow are usually considered to be the main cause of phylogeographic breaks, although such ruptures can also occur in species inhabiting a continuous range (Irwin 2002). Such a break was observed in *T. dentatus* and its newly affiliated populations, or more precisely, between central-east populations (KAM, KOR, LAO) and the population of the most eastern tip (LUW). Irwin (2002) simulated maternal genealogies and found that the likelihood for a phylogeographic break within an evenly distributed species increases with decreasing dispersal distances and population sizes. The remote location of LUW might hamper dispersal, thus slowing down the coalescence process of mitochondrial DNA in Dian´s tarsiers. However, the very well supported node suggesting common mitochondrial ancestry of LUW and *T. lariang* is opposing a phylogeographic break by chance in *T. dentatus*. It seems therefore more likely that Lariang tarsiers and the population of LUW carry similar ancestral mitochondrial haplotypes of lineage 2. Considering the sistergroup relationship of mitochondrial loci between BAN and KEN, these populations may represent another example for conserved ancestral haplotypes of today allopatric and genetically distinct (nuclear DNA) tarsier populations. Otherwise, their placement as sistergroup could also be the result of long-branch attraction. This phenomenon describes the erroneous grouping of distantly related species due to the accumulation of convergent changes in rapidly evolving sequences (Bergsten 2005). Considering the probable dispersal routes within Sulawesi BAN and KEN are putatively represented by more ancient sequences that behave as long branches which tend to attract each other (Felsenstein 1978).

Overall, the different geographic structure of mitochondrial and nuclear data can best be explained by 1) shared ancestral polymorphisms of the mitochondrial genome (causing phylogeographic breaks within extant lineages or species), 2) contemporary male dispersal (homogenization of nuclear gene pools), and 3) female philopatry (tight clustering of maternally inherited mtDNA). Interestingly, these findings are similar to phylogenetic patterns in Sulawesi macaques inferred from mitochondrial and autosomal DNA (Evans *et al.* 2003b). Furthermore, the mitochondrial species tree appears to be influenced by long-branch attraction, leading to false synapomorphy.

4.2.4   Time scale of divergence

Findings also enable the tracing of the biogeographic history of Sulawesi tarsiers. Divergence time estimates of the nuclear gene-based species tree suggest a Plio-Pleistocene cladogenesis event (median node ages calibration 1/2: 2.05/2.49 MYA), splitting crown Eastern tarsiers into two genetically distinct lineages (see chapter 3.3.3 and 3.3.4). The long time lag between arrival and first speciation, spanning a period of at least 10 million years, has likely been caused by a series of events. The progenitor of Sulawesi tarsiers reached land positive parts of the paleo-archipelago probably by dispersal sometime between Oligocene and Miocene, a period of falling sea levels and partial land elevation through tectonic uplift (see chapter 1.3). Successful dispersal was presumably rare and survivors ran the risk of experiencing a population bottleneck or local extinction, when considering the ongoing tectonic processes in Wallacea (Stelbrink *et al.* 2012). As land further expanded population growth and colonization of bordering areas became possible. Subsequently, Plio-Pleistocene climate changes and tectonic processes intensely shaped dispersal patterns of many taxa on Sulawesi (van den Bergh *et al.* 2001, Evans *et al.* 2003a, b, Stelbrink *et al.* 2012), thus also driving speciation of tarsiers. Although reconstructions of Wallacea have consistently been refined over the past 15 years (Moss & Wilson 1998, Hall 2001, Hall 2002, Hall & Smyth 2008, Hall 2009), detailed Quaternary scenarios are still deficient. Therefore redrawing tarsier radiation on Sulawesi represents a major challenge. Pleistocene eustatic curves (Haq *et al.* 1987), predicted distribution of land, and plate configuration (Hall 2001, Stelbrink *et al.* 2012) yield the following possible scenario:

•   According to Hall (2001) there is good evidence for land positive areas in southeast Sulawesi from early Miocene. Given that tarsiers arrived at proto-Sulawesi in early Miocene it seems obvious that they initially populated the southeast part of the island. Land expansion in late Miocene led to population growth. A glacial maximum at 2.5 MYA (Haq *et al.* 1987) may have facilitated dispersal from southeast to southwest Sulawesi across the Gulf of Bone, where glacial marine regression probably exposed shelf regions between the southern peninsulas (Moss & Wilson 1998, Hall 2001, Hope 2001, Evans *et al.* 2003b). This dispersal opportunity could have promoted the separation of lineage 1- and lineage 2-stem populations around the Plio-Pleistocene border (median node ages calibration 1/2: 2.05/2.49 MYA, 95% confidence intervals ranging from 1.34-3.54 MYA).

•   Starting from southeast Sulawesi lineage 1-tarsiers moved northward and inhabited the southeast, central-east and northern peninsula (Fig. 4.2). Orogeny beginning in Pliocene enforced divergence of southeastern and northern populations (median node ages calibration 1/2: 1.35/1.62

MYA) and probably the formation of a species adapted to the mountain environment, the pygmy tarsier.

• Further diversification of lineage 1 is estimated to have occurred in middle and late Pleistocene (median divergence dates ranging from 0.15 to 0.51 MYA). Tarsiers colonized the northern peninsula. Range fragmentation due to oceanic inundation of the isthmus near Tomini and low lying flat lands around Lake Limboto during interglacial periods could be responsible for population divergence.
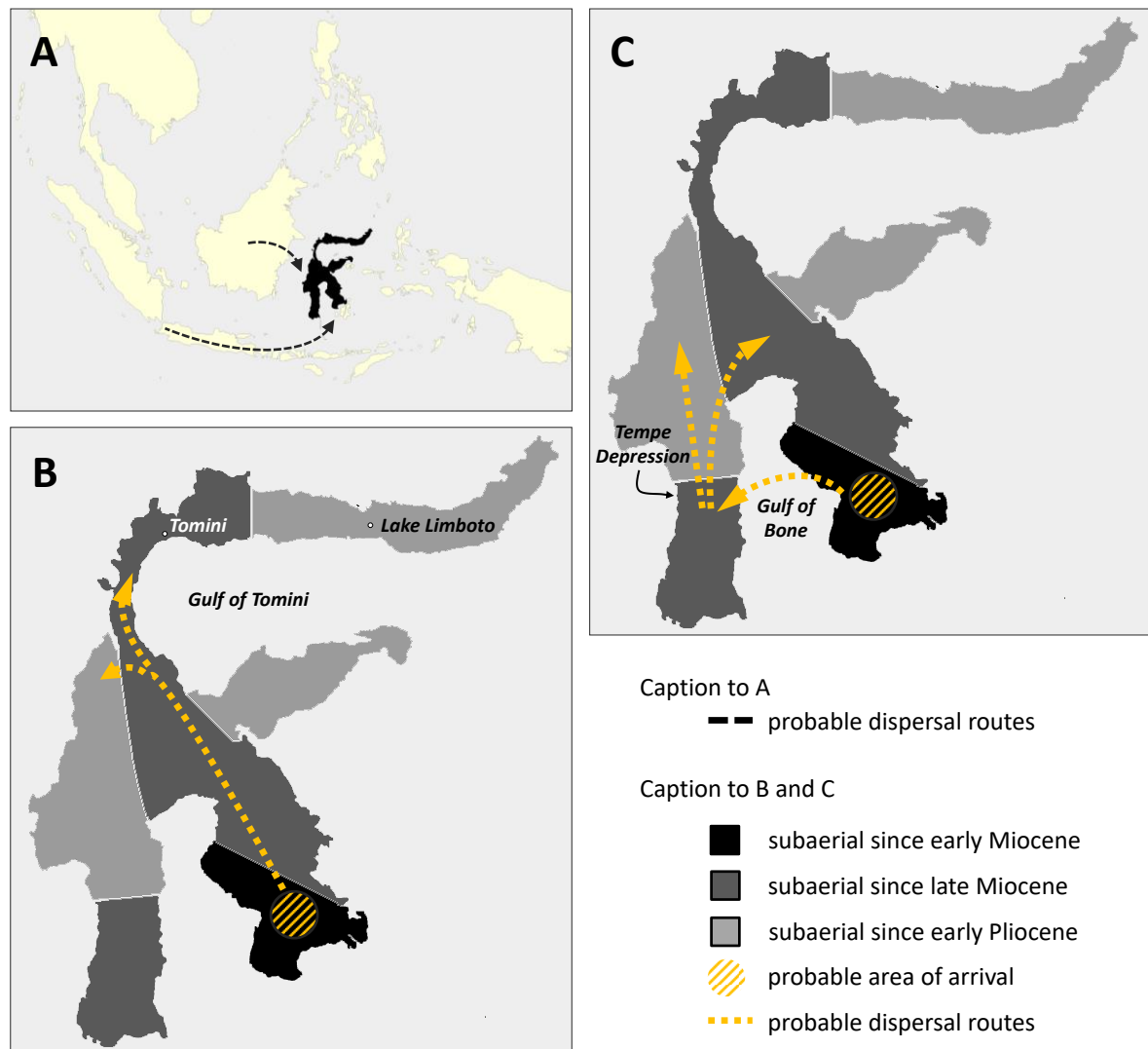
Figure 4.2: Dispersal and diversification of Sulawesi tarsiers
A) Geographical map of the Indo-Malay archipelago. Sulawesi is marked in black. Black arrows indicate putative dispersal routes to Sulawesi; B) and C) roughly show subaerial areas on proto-Sulawesi (Hall 2001, 2009) and probable dispersal routes of B) lineage 1 and C) lineage 2. Geographical maps based on ARCMAP™ 10 (Esri).

• The southwestern part of Sulawesi formed its own island, at least until mid-Pleistocene (Hall 2001, van den Bergh *et al.* 2001), only allowing for a late colonization of West-Sulawesi coming from the southwest. The progenitors of extant Lariang tarsiers seized upon this opportunity and entered the western peninsula in late Pleistocene (median node ages calibration 1/2: 0.77/0.95 MYA).

• Variation of climate conditions in central and southwest Sulawesi, the latter was affected by dry southeast trade winds, created an ecological gradient between these areas from moist to a more arid climate (Natus 2005). This might have induced the recent separation of *T. dentatus* and *T. fuscus* (median node ages calibration 1/2: 0.49/0.59 MYA). Furthermore, pollen remains around Lake Tempe account for a flooding of the Tempe depression between about 7,100 to 2,600 years ago (Whitten *et al.* 2002). This could have perpetuated isolation of *T. dentatus* and *T. fuscus*.

• Recurrent Pleistocene sea level low stand and orogenic uplift united islands of the Sulawesian archipelago (Fooden 1969, Hall 2001) and made migration across the Palu-Koro fault possible. *Tarsius dentatus* therewith invaded into the range of lineage 1 and expelled sub-populations, except for montane pygmy tarsiers, to the North and to the Southeast. Wallace tarsiers crossed the Palu-Koro fault north of Palu to the west. Merker *et al.* (2010) speculated that periodic droughts around Palu fragmented the range of *T. wallacei*. Further spread of invasive Dian´s tarsiers (*T. dentatus*) might have interrupted gene flow between northern and southern populations of *T. wallacei* (Merker *et al.* 2010) leading to the divergence in two sub-species about 150,000 to 190,000 years ago (median node ages calibration 1/2: 0.15/0.19 MYA, 95% confidence intervals ranging from 0.00-0.43 MYA).

4.2.5 Reliability of estimated divergence times

Dating divergence times relies on calibration points that have been incorporated into the analysis. Therefore calibration prior distributions used in this thesis based on the mean node age estimates of two recently published studies on primate evolution (Jameson *et al.* 2011, Perelman *et al.* 2011). Both studies employed comprehensive nuclear and mitochondrial sequence data and inferred divergence times from widely accepted fossil data. Additionally, a relaxed clock model rather than a global clock was applied to the nuclear sequence data set analysed in frame work of this thesis to allow variation in substitution rates among lineages and therewith to obtain more accurate estimates of ancestral nodes (Drummond *et al.* 2006).

Both calibrations yielded comparable TMRCA estimates of crown and Eastern tarsiers that postdate the opening of the Makassar Strait (45 MYA). The dating (median node age calibration 1/2: 18.28/22.16 MYA) does not concur with a scenario of micro-continental drift, thus excluding tarsiers´ arrival on Sulawesi by vicariance. More likely tarsiers dispersed to Sulawesi through rafting or island hopping during periods of low sea level in Miocene. The reconstructed tectonic and environmental setting of the archipelago during this epoch implies that habitable rainforests already existed (Morley 1998, Hall 2001, Jablonski 2005). In this regard the posterior confidence intervals for the divergence time of Eastern tarsiers do not contradict a colonization of Sulawesi by tarsiers as early as in Miocene and therewith appear to be credible. Furthermore, mean node ages of crown tarsiers (20.3 MYA and 18.64 MYA) and the Western/Philippine tarsier split (11.1 MYA) estimated by Shekelle *et al.* (2010) and Springer *et al.* (2012) are congruent with the respective divergence times of tarsiers obtained in this study.

The scenario of dispersal and subsequent diversification illustrated above (4.2.4) depends on plate tectonic reconstructions and implies that certain parts of Sulawesi offered suitable habitats to tarsiers at a given time. However, the overlap between geological events and the timing of speciation

processes is striking. The relatively narrow confidence intervals and well supported nodes provided here suggest that time estimates are comparatively precise and hence reliable.

4.3 Sample transfer according to CITES and Indonesian export requirements

According to CITES classification (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) there is a potential threat to Sulawesi tarsiers. Corresponding to CITES and Indonesian export requirements exporting original DNA of protected species is not allowed. Hence, DNA extraction of all tissue samples obtained from field studies between 2009 and 2010, subsequent WGA as well as PCR amplification of mitochondrial and Y chromosomal gene loci that already have been established at that time (Merker *et al.* 2009) were routinely conducted in the hosting laboratory at the Primate Research Center of the Agricultural University in Bogor, Indonesia. All further genetic analyses based on these WGA and PCR products, which were authorized to be transferred to Germany, were carried out at the laboratories at the Institute of Anthropology in Mainz.

5 Conclusions and prospects

Since over a century, the Wallacea region has offered a universe of unique animals and plants that attracts researchers from all over the world. Especially Sulawesi with its eventful geological history provides a valuable opportunity for discussing the speciation processes in the context of plate tectonics and glacial cycles. This study has shown that the biogeography of tarsiers has been significantly influenced by environmental changes and plate dynamics. Once widespread over the northern hemisphere, ancient tarsiiform primates were obliged to find refuge on insular Southeast Asia provoked probably by an Eocene cooling event that dramatically contracted the rain forest habitats. Deep oceanic trenches and rare dispersal possibilities seem to have enforced allopatric speciation among Sundaland and Sulawesi tarsier species. The long time lag between Oligo-Miocene colonization of Sulawesi and Plio-Pleistocene diversification of Eastern tarsiers supports the assumption that proto-Sulawesi has been an archipelago for a long time offering limited opportunities to dispersal and population growth for terrestrial fauna. The results of this study are partly in accord with previous divergence time estimates (Shekelle *et al.* 2010, Springer *et al.* 2012). However the molecular tool applied here to a comprehensive sample set, permitted a much more precise assessment of divergence times and allowed a more detailed view on internal nodes of the Eastern tarsier phylogeny. Moreover, findings concerning isolation by distance mechanisms, gene flow, and sex-specific dispersal behavior demonstrated the necessity to use different inherited and evolving gene markers. This study gained valuable insights to understand which factors possibly shaped contemporary genetic variation of the Eastern tarsier clade. But, as usual, there remain open issues. Firstly, populations inhabiting the northern peninsula cluster tightly. The question is whether this population structuring results from the geographical distance or ongoing hybridization between closely related species. Secondly, the phylogenetic position of the pygmy tarsier (*Tarsius pumilus*) within the Eastern tarsier complex is still unknown. It would be interesting to find out whether this taxon is affiliated to one of the two lineages (see chapter 4.2.2) or if pygmy tarsiers form a distinct monophyletic entity. Finally, a few words on tarsier conservation: although tarsiers can tolerate a certain degree of disturbance caused by anthropogenic land use - most study sites have been affected by this and still hosted numerous tarsier groups – continuing deforestation was a serious threat. At least one study site had to be relocated since forest disappeared within one year. It thus would be desirable if protection of these enigmatic primates with a long, independent evolutionary history and the habitats they live in is pursued further.

6   References

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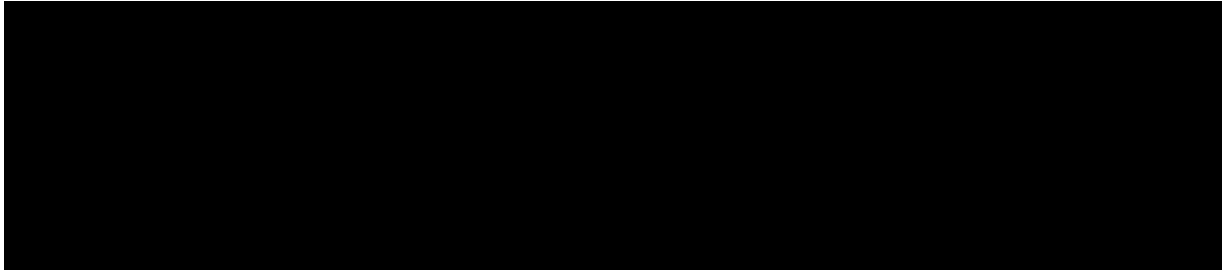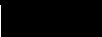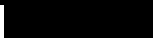
Special thanks are directed to the Primate Research Center at the Agricultural University (PSSP LPPM-IPB) in Indonesia.
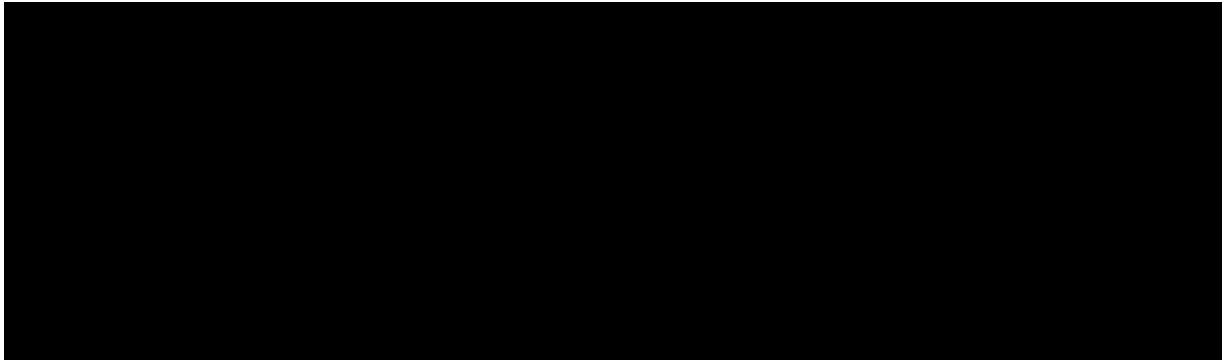
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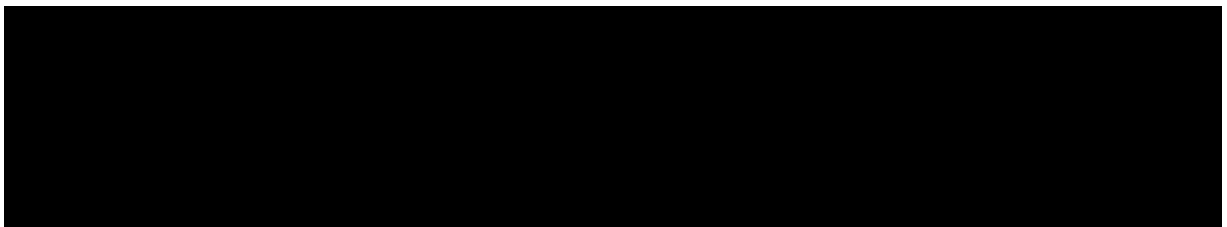
Special thanks are due   Taman Nasional Bantimurung Bulusaraung (TNBB)
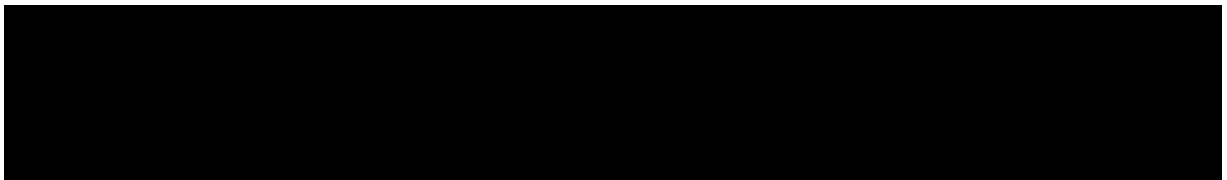
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8   Supplement

8.1   Solutions and buffers

Urea-EDTA tissue buffer

6 M	Urea
10 mM	Tris/HCl (pH 8)
10 mM	EDTA
125 mM	Sodium Chloride (NaCl)
1 %	SDS

LB medium (selective)

10 g	Tryptone
5 g	Yeast extract
5 g	Sodium Chloride (NaCl)
2 ml	Ampicillin (25 mg/µl)
	Ad 1 l ddH$_2$O

LB Agar (selective)

10 g	Tryptone
5 g	Yeast extract
5 g	Sodium Chloride (NaCl)
15 g	Agar
2 ml	Ampicillin (25 mg/µl)
2 ml	IPTG
2 ml	X-Gal (100 mg/2 ml DMSO)
	Ad 1 l ddH$_2$O

10 x TBE buffer

108 g	Tris(Hydroxymethyl)aminomethane
55 g	Boric acid
7.44 g	EDTA
	Ad 1 l ddH$_2$O

TE buffer

10 mM	TrisHCl (pH 7.4)
1 mM	EDTA (pH 8.0)

8.2   Samples of Sulawesi tarsiers

Table 8.1: Pruned sample set of Sulawesi tarsiers

Toolkit$_{IND}$	Population	Pop$_{LABEL}$	Sampled$_{IND}$	Sampled by
K02, K04	Kamarora	KAM	K01-K32	Merker 2001
T08, T09	Make	MAK	T06-T12	Merker 2005
T24, T39	Peana	PEA	T15-T42	Merker 2005
T46, T47	Koja	KOJ	T43-T47	Merker 2005
T111, T112	Laone	LAO	T105-T112	Merker 2006
SM26, SM28	Batusuya	BAT	SM24-30	Merker 2008
SM33, SM35	Uwemanje	UWE	SM31-38	Merker 2008
CD02, CD04	Ogatemuku	OGA	CD01-12	Driller 2009
CD13, CD16	Korosule	KOR	CD13-18	Driller 2009
CD19, CD24	Luwuk	LUW	CD19-28	Driller 2009
CD33, CD34	Labanu	LAB	CD29-38	Driller 2009
CD40, CD41	Kendari	KEN	CD39-43	Driller 2010
CD44, CD46	Duasaudara	DUA	CD44-55	Driller 2010
CD60, CD62	Bantimurung	BAN	CD56-65	Driller 2010

Listed are 28 Sulawesi tarsier samples which have been analyzed for five nuclear loci (Toolkit$_{IND}$) of the Phylogenomic Toolkit (Horvath *et al.* 2008) and their origin. Pop$_{LABEL}$: Population label; Sampled$_{IND}$: all individuals sampled.

8.3   PCR protocols and primer information

Table 8.2: Wax-mediated hot start PCR

Component	Volume (µl)	final concentration**	Partition
10x PCR Buffer*	2	1x	**upper**
Taq DNA Polymerase	0.15	0.75 units/reaction	
Template DNA#	variable	20-40 ng/reaction	
ddH2O	ad 20µl	-	
10x PCR Buffer*	1	1x	**lower**
Forward Primer	1	0.33 µM	
Reverse Primer	1	0.33 µM	
dNTPs	0.6	200 µM of each dNTP	
ddH2O	ad 10µl	-	

* contains 15 mM MgCl2
** related to a reaction volume of 30µl
# WGA

Table 8.3: Standard PCR

Component	Volume (µl)	final concentration
10x PCR Buffer *	2	1x
Forward Primer	0.67	0.33 µM
Reverse Primer	0.67	0.33 µM
dNTPs	0.4	200 µM of each dNTP
Taq DNA Polymerase	0.1	0.5 units/reaction
Template DNA#	variable	20-40 ng/reaction
ddH2O	ad 20µl	-

* contains 15 mM MgCl2
# WGA

Table 8.4: Thermocycler settings for PCR

Step	Time	°C	
1. Initial denaturation	3 min	94	
2. Denaturation	40 sec	94	
3. Annealing	1 min	***	**35 cycles**
4. Extension	***	72	
5. Final extension	5 min	72	

Table 8.5: Nuclear DNA loci and primer information

Locus	Gene	Target position[§]	Forward primer sequence 5'-3' / Reverse primer sequence 5'-3'	Amplicon size (bp)	T_A (°C)	Ext. (mm:ss)	Primer reference
ABCA1	ATP-binding cassette sub-family A member 1	intronic	CCTCCATCTTTTCAGCTCTACCTAC / ACAAGAGCCTGGAGATTGGATAAC	645-651	59	01:00	Horvath *et al.* 2008
ADORA3	Adenosine receptor A3	exonic	ACCCCCATGTTTGGCTGGAA / GATAGGGTTCATCATGGAGTT	411	58	00:45	Murphy *et al.* 2001
AXIN1	Axin 1 isoform a, Axis inhibition protein 1	exonic	CTCTGCCTTCGCTGTACCGTCTAC / CCCACCTTTCCTAATCCTTGTCCTC[#]	995	58	01:00	Horvath *et al.* 2008
RAG1	Recombination activating gene 1	exonic	AAGACATCCTGGAAGGCATGA* / AAAGTTGCCGTTCATCCTCA*	845	58	01:00	Murphy *et al.* 2001
TTR	Thyroxine-binding prealbumin	intronic	TGCCTTGCTGGACTGGTATT* / GACGGCATCTAGTACTTTGACCAT**	1005-1025	58	01:00	Flynn & Nedbal 1998

Primer modifications (based on *T. syrichta* sequences of the Ensembl Genome database):
* one mismatch to the published primer sequence
** two mismatches to the published primer sequence
# primer sequence modified from Horvath *et al.* 2008.

§ based on the human genome
T_A Annealing Temperature
Ext. Extension Time

8.4 Sources of anthropoid and strepsirhine primate sequence data

NCBI accession numbers and data bases of anthropid and strepsirhine primate sequences are listed in table 8.6 and table 8.7.

Table 8.6: NCBI accession nos. and ENSEMBL data location of anthropoid primates

Taxon	Label	Gene	Sequence a	Sequence b
Callithrix jacchus	CJA	ABCA1	HM765296	*chromosome:C_jacchus3.2.1:1:148170393:148191518:1*
		ADORA3	HM765164	*chromosome:C_jacchus3.2.1:7:147463921:147464931:-1*
		AXIN1	HM765306	*chromosome:C_jacchus3.2.1:12:351493:353038:-1*
		RAG1	HM759090	*chromosome:C_jacchus3.2.1:11:99861889:99868965:1*
		TTR	HM757710	AY434071 *
Homo sapiens	HSA	ABCA1	HM765327	NG_007981
		ADORA3	HM765141	NG_032119
		AXIN1	HM764284	NG_012267
		RAG1	HM759069	NG_007528
		TTR	HM757691	NG_009490
Hylobates lar	HLA	ABCA1	HM765324	-
		ADORA3	HM765143	-
		AXIN1	HM764286	-
		RAG1	HM759071	-
		TTR	HM757693	-
Macaca mulatta	MMA	ABCA1	HM765347	*chromosome:MMUL_1:15:31274263:31325078:-1*
		ADORA3	HM765108	*chromosome:MMUL_1:1:114504821:114508531:-1*
		AXIN1	HM764251	*chromosome:MMUL_1:20:334373:345620:1*
		RAG1	HM759037	AY011900 §
		TTR	HM757659	FJ846620 #
Pan troglodytes	PTR	ABCA1	HM765384	HM765385
		ADORA3	HM765152	HM765153
		AXIN1	HM764294	HM764295
		RAG1	HM759079	*chromosome:CHIMP2.1.4:11:36563611:36570686:1*
		TTR	HM757700	HM757701
Pongo pygmaeus	PPY	ABCA1	HM765381	HM765382
		ADORA3	HM765154	HM765155
		AXIN1	HM764296	HM764297
		RAG1	HM759081	HM759082
		TTR	HM757702	HM757703

Accession nos. of previously published sequences: blue=Perelman *et al.* 2011, *=Yoder & Yang 2004, §=Murphy *et al.* 2001, #=Stevison & Kohn 2009; Sequences obtained from online data bases: green=NCBI RefSeqGene, italics=ENSEMBL data base location.

Table 8.7: NCBI accession nos. and ENSEMBL data location of strepsirhine primates

Taxon	Label	Gene	Sequence a
Cheirogaleus medius	CHE	ABCA1	EU057428
		ADORA3	EU342218
		AXIN1	HM764359
		RAG1	HM759144
Cheirogaleus major	CHE	TTR	AY434064 *
Daubentonia madegascariensis	DMA	ABCA1	EU057429
		ADORA3	EU342219
		AXIN1	EU057284
		RAG1	EU342306
		TTR	EU342331
Otolemur garnetti	OGR	ABCA1	EU057451
		ADORA3	EU342237
		AXIN1	HM764378
		RAG1	HM759164
		TTR	*scaffold:OtoGar3:GL873531.1:19802889:19810218:-1*

Accession nos. of previously published sequences: blue=Perelman *et al.* 2011, red=Horvath *et al.* 2008, *=Yoder & Yang 2004; Sequences obtained from online data bases: Italics=ENSEMBL data base location

8.5 Genotypic linkage disequilibrium

Table 8.8: G- test for genotypic linkage disequlibrium

	Pop														
	BAN	KEN	KOR	LUW	PEA	KOJ	MAK	LAO	KAM	UWE	BAT	OGA	LAB	DUA	All
T42 X T54	0.57183	1.00000	NA	NA	0.36071	1.00000	0.57183	0.74792	0.04474	1.00000	0.57748	0.09673	0.22351	0.23879	**0.02708**
T42 X D157	0.00496	0.39752	1.00000	NA	0.44266	1.00000	0.77321	0.49702	0.21627	0.57808	0.08472	0.18591	0.64732	1.00000	**0.02272**
T42 X D194	0.08651	1.00000	1.00000	1.00000	0.31012	1.00000	0.50516	0.14315	0.14613	1.00000	1.00000	0.02222	1.00000	0.20685	**0.01220**
T42 X D220	0.17500	1.00000	1.00000	1.00000	0.72312	0.09613	1.00000	0.87282	0.79395	1.00000	0.08909	0.09524	0.91329	1.00000	**0.52599**
T42 X D231	0.25119	1.00000	1.00000	1.00000	0.27927	1.00000	0.28710	1.00000	0.36081	0.78482	0.34246	0.03641	0.65278	0.38026	**0.01954**
T42 X D238	0.25248	NA	1.00000	0.46438	0.22034	1.00000	1.00000	1.00000	0.82788	NA	NA	NA	NA	NA	**0.41885**
T42 X D246	0.16845	0.39603	1.00000	0.45000	0.03909	1.00000	1.00000	1.00000	0.11746	0.78899	0.33353	0.01974	1.00000	1.00000	**0.00278**
T42 X D251	1.00000	0.39444	1.00000	1.00000	0.01597	1.00000	1.00000	1.00000	0.76349	0.85546	0.33244	0.35813	0.47034	0.38155	**0.12768**
T54 X D157	0.70427	0.60456	NA	NA	0.68284	1.00000	1.00000	0.21558	0.97708	1.00000	0.17907	0.90694	0.48929	1.00000	**0.88929**
T54 X D194	1.00000	1.00000	NA	NA	0.70952	1.00000	0.57490	1.00000	0.19831	1.00000	1.00000	0.03036	0.08056	1.00000	**0.25476**
T54 X D220	0.21458	0.39841	NA	NA	0.12718	1.00000	0.70724	1.00000	0.13403	1.00000	0.17827	0.10159	0.20506	0.08433	**0.00317**
T54 X D231	1.00000	1.00000	NA	NA	0.26647	1.00000	1.00000	0.43075	0.75972	1.00000	0.09732	0.14663	0.49038	0.86994	**0.25377**
T54 X D238	1.00000	NA	NA	NA	0.55040	1.00000	1.00000	0.43472	0.45546	NA	NA	NA	NA	NA	**0.53819**
T54 X D246	1.00000	0.60565	NA	NA	0.89821	1.00000	1.00000	1.00000	0.08532	1.00000	0.09544	0.22371	0.48770	0.81885	**0.28363**
T54 X D251	0.09762	0.59524	NA	NA	0.28036	0.60179	0.70407	1.00000	0.84772	0.66032	0.09514	0.38512	0.09633	0.38026	**0.04196**
D157 X D194	1.00000	1.00000	1.00000	NA	0.39048	1.00000	0.30565	0.14474	0.77887	1.00000	1.00000	0.74841	0.17698	1.00000	**0.30387**
D157 X D220	0.08978	0.30357	1.00000	NA	0.22480	1.00000	1.00000	0.87321	0.79544	1.00000	0.00933	0.03155	1.00000	0.77609	**0.09306**
D157 X D231	0.13462	1.00000	0.39226	NA	0.13492	1.00000	0.08333	1.00000	0.37004	1.00000	0.09673	0.41607	0.02024	0.43333	**0.00327**
D157 X D238	0.12927	NA	1.00000	NA	0.99504	1.00000	1.00000	1.00000	0.87510	NA	NA	NA	NA	NA	**0.99772**
D157 X D246	0.08780	0.10089	1.00000	NA	0.81131	1.00000	1.00000	1.00000	0.45268	0.14315	0.09167	0.29058	1.00000	0.62361	**0.06806**
D157 X D251	1.00000	0.10060	1.00000	NA	0.17192	1.00000	0.34315	1.00000	0.73958	1.00000	0.09504	1.00000	1.00000	1.00000	**0.39008**
D194 X D220	1.00000	1.00000	1.00000	1.00000	0.61716	1.00000	1.00000	1.00000	0.08919	1.00000	1.00000	0.04534	0.32490	1.00000	**0.12857**
D194 X D231	1.00000	1.00000	1.00000	1.00000	0.61389	1.00000	0.02708	1.00000	0.84306	1.00000	1.00000	0.46319	0.18532	1.00000	**0.18730**
D194 X D238	1.00000	NA	1.00000	1.00000	0.13065	1.00000	1.00000	1.00000	0.62798	NA	NA	NA	NA	NA	**0.42927**
D194 X D246	1.00000	1.00000	1.00000	0.57510	0.13383	1.00000	1.00000	1.00000	0.79335	1.00000	1.00000	0.00417	1.00000	1.00000	**0.03720**
D194 X D251	1.00000	1.00000	1.00000	1.00000	0.34762	1.00000	1.00000	1.00000	0.35337	1.00000	1.00000	1.00000	0.57788	1.00000	**0.43988**
D220 X D231	1.00000	1.00000	1.00000	1.00000	0.02093	1.00000	1.00000	0.55020	0.07500	1.00000	0.09960	0.17510	1.00000	1.00000	**0.01181**
D220 X D238	1.00000	NA	0.39841	0.16825	0.47698	1.00000	1.00000	1.00000	0.58730	NA	NA	NA	NA	NA	**0.40764**
D220 X D246	1.00000	0.29365	1.00000	0.17440	0.71726	1.00000	1.00000	0.53661	0.71587	0.13810	0.09534	0.00268	1.00000	0.03581	**0.00486**
D220 X D251	0.17550	0.29960	0.19286	1.00000	0.35992	1.00000	1.00000	0.31131	0.24633	1.00000	0.09335	1.00000	1.00000	0.50496	**0.03313**
D231 X D238	0.00774	NA	1.00000	0.33631	0.59583	1.00000	1.00000	1.00000	0.58244	NA	NA	NA	NA	NA	**0.16518**
D231 X D246	0.13125	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	0.79315	1.00000	0.04524	0.02242	1.00000	0.38115	**0.20615**
D231 X D251	1.00000	1.00000	1.00000	1.00000	0.29653	1.00000	1.00000	0.42153	0.13581	1.00000	0.04633	0.11558	1.00000	1.00000	**0.06171**
D238 X D246	0.13214	NA	1.00000	0.05565	1.00000	1.00000	1.00000	1.00000	0.42004	NA	NA	NA	NA	NA	**0.27093**
D238 X D251	1.00000	NA	0.13651	1.00000	0.66349	1.00000	1.00000	0.42054	0.68482	NA	NA	NA	NA	NA	**0.62381**
D246 X D251	1.00000	0.10010	1.00000	1.00000	0.04633	1.00000	1.00000	1.00000	0.10774	1.00000	0.04534	1.00000	1.00000	1.00000	**0.04901**

NA no data were available due to insufficient allele data

8.6 Isolation by distance input data

Table 8.9: IBD input data

A				B				C				D			
Pop		$F_{ST}/1\text{-}F_{ST}$	km	Pop		$F_{ST}/1\text{-}F_{ST}$	km	Pop		$F_{ST}/1\text{-}F_{ST}$	km	Pop		$F_{ST}/1\text{-}F_{ST}$	km
1	2	0.13710	146	1	2	0.13710	146	1	2	0.15773	254	1	2	0.02786	27
1	3	0.21713	400	1	3	0.21713	400	1	3	0.19406	528	1	3	0.12612	181
1	4	0.15034	820	2	3	0.15773	254	2	3	0.17026	274	1	4	0.20133	170
2	3	0.15773	254									1	5	0.22922	316
2	4	0.19406	528									2	3	0.18063	182
3	4	0.17026	274									2	4	0.23604	171
												2	5	0.18453	305
												3	4	0.01237	14
												3	5	0.17280	247
												4	5	0.21676	234

Pop: Population; A, B, C, and D correspond to the tested population combinations.

A: 1 BAT, 2 OGA, 3 LAB, 4 DUA;

B: 1 BAT, 2 OGA, 3 LAB;

C: 1 OGA, 2 LAB, 3 DUA;

D: 1 LAO, 2 KAM, 3 KOR2, 4 KOR1, 5 LUW.

8.7 Beast log files

Table 8.10: Traces of six combined BEAST log files obtained from mitochondrial species tree analyses

	Statistic	mean	ESS		Statistic	mean	ESS
1	posterior	-5356.47800	6197.83	51	speciesTree.splitPopSize44	0.00112	9293.48
2	prior	2007.23500	6915.55	52	speciesTree.splitPopSize45	0.00114	4554.08
3	likelihood	-7363.71300	5446.30	53	speciesTree.splitPopSize46	0.00119	3342.71
4	species.coalescent	1732.52300	5937.22	54	species.yule.birthRate	17.03500	626.20
5	species.popSizesLikelihood	261.38100	18721.92	55	speciesTree.rootHeight	0.27400	179.17
6	speciation.likelihood	26.79200	222.86	56	CYTB_Tarsius_121009.treeModel.rootHeight	0.27600	180.06
7	species.popMean	0.00052	17340.16	57	Dloop_Tarsius_121009.treeModel.rootHeight	0.27600	179.99
8	speciesTree.splitPopSize1	0.00206	106500.00	58	CYTB_Tarsius_121009.ac	0.07762	5079.94
9	speciesTree.splitPopSize2	0.00207	90549.22	59	CYTB_Tarsius_121009.ag	1.05200	13087.68
10	speciesTree.splitPopSize3	0.00125	17679.92	60	CYTB_Tarsius_121009.at	0.02357	12611.88
11	speciesTree.splitPopSize4	0.00142	44265.11	61	CYTB_Tarsius_121009.frequencies1	0.32700	11100.13
12	speciesTree.splitPopSize5	0.00087	14846.97	62	CYTB_Tarsius_121009.frequencies2	0.29600	11484.57
13	speciesTree.splitPopSize6	0.00173	26767.87	63	CYTB_Tarsius_121009.frequencies3	0.10200	9905.02
14	speciesTree.splitPopSize7	0.00336	40756.14	64	CYTB_Tarsius_121009.frequencies4	0.27500	11360.10
15	speciesTree.splitPopSize8	0.00144	16433.04	65	CYTB_Tarsius_121009.alpha	0.15100	3399.40
16	speciesTree.splitPopSize9	0.00209	26743.32	66	Dloop_Tarsius_121009.kappa	32.40200	1245.80
17	speciesTree.splitPopSize10	0.00126	5118.04	67	Dloop_Tarsius_121009.frequencies1	0.35100	11071.44
18	speciesTree.splitPopSize11	0.00139	35859.87	68	Dloop_Tarsius_121009.frequencies2	0.24300	12985.09
19	speciesTree.splitPopSize12	0.00110	5570.30	69	Dloop_Tarsius_121009.frequencies3	0.10000	13521.71
20	speciesTree.splitPopSize13	0.00180	65851.80	70	Dloop_Tarsius_121009.frequencies4	0.30500	12874.39
21	speciesTree.splitPopSize14	0.00165	25845.06	71	Dloop_Tarsius_121009.alpha	0.23800	5699.79
22	speciesTree.splitPopSize15	0.00219	56655.40	72	CYTB_Tarsius_121009.ucld.mean	1.00000	-
23	speciesTree.splitPopSize16	0.00090	18267.96	73	CYTB_Tarsius_121009.ucld.stdev	0.43100	498.08
24	speciesTree.splitPopSize17	0.00113	2418.33	74	Dloop_Tarsius_121009.ucld.mean	4.00200	13106.52
25	speciesTree.splitPopSize18	0.00112	3412.63	75	Dloop_Tarsius_121009.ucld.stdev	0.34700	981.36
26	speciesTree.splitPopSize19	0.00108	16812.55	76	CYTB_Tarsius_121009.meanRate	1.30000	397.70
27	speciesTree.splitPopSize20	0.00109	14014.98	77	CYTB_Tarsius_121009.coefficientOfVariation	0.44900	486.32
28	speciesTree.splitPopSize21	0.00132	9128.96	78	CYTB_Tarsius_121009.covariance	-0.00157	77634.47
29	speciesTree.splitPopSize22	0.00127	9781.39	79	Dloop_Tarsius_121009.meanRate	4.00100	970.47
30	speciesTree.splitPopSize23	0.00118	10505.58	80	Dloop_Tarsius_121009.coefficientOfVariation	0.35800	972.70
31	speciesTree.splitPopSize24	0.00122	8771.36	81	Dloop_Tarsius_121009.covariance	-0.00330	126400.00
32	speciesTree.splitPopSize25	0.00130	16345.61	82	CYTB_Tarsius_121009.treeLikelihood	-4542.58800	7532.32
33	speciesTree.splitPopSize26	0.00125	14694.08	83	Dloop_Tarsius_121009.treeLikelihood	-2821.12500	11370.98
34	speciesTree.splitPopSize27	0.00126	18243.74				
35	speciesTree.splitPopSize28	0.00129	17426.63				
36	speciesTree.splitPopSize29	0.00129	20969.17				
37	speciesTree.splitPopSize30	0.00129	20247.60				
38	speciesTree.splitPopSize31	0.00126	17944.87				
39	speciesTree.splitPopSize32	0.00127	20696.15				
40	speciesTree.splitPopSize33	0.00131	18487.23				
41	speciesTree.splitPopSize34	0.00134	16675.65				
42	speciesTree.splitPopSize35	0.00128	16108.41				
43	speciesTree.splitPopSize36	0.00128	18581.01				
44	speciesTree.splitPopSize37	0.00123	16114.93				
45	speciesTree.splitPopSize38	0.00129	15982.90				
46	speciesTree.splitPopSize39	0.00129	8835.40				
47	speciesTree.splitPopSize40	0.00125	11960.57				
48	speciesTree.splitPopSize41	0.00120	14330.09				
49	speciesTree.splitPopSize42	0.00129	9591.67				
50	speciesTree.splitPopSize43	0.00114	6432.20				

ESS values < 200 are highlighted in blue.

Table 8.11: Traces of two combined BEAST log files obtained from nuclear multilocus species tree analyses

	Statistic	mean	ESS		Statistic	mean	ESS
1	posterior	-4139.04400	4252.20	51	speciesTree.splitPopSize44	0.00021	2863.59
2	prior	2513.74300	4423.20	52	speciesTree.splitPopSize45	0.00021	2605.11
3	likelihood	-6652.78700	1154.75	53	speciesTree.splitPopSize46	0.00020	947.32
4	species.coalescent	2127.42000	4172.49	54	species.yule.birthRate	824.35600	14962.03
5	species.popSizesLikelihood	337.43600	4832.58	55	speciesTree.rootHeight	0.00679	7729.49
6	speciation.likelihood	84.82700	10684.16	56	ABCA1_Tarsius.treeModel.rootHeight	0.00745	15079.74
7	species.popMean	0.00012	5240.68	57	ADORA3_Tarsius.treeModel.rootHeight	0.00753	14091.74
8	speciesTree.splitPopSize1	0.00044	9738.82	58	AXIN1_Tarsius.treeModel.rootHeight	0.00724	9918.82
9	speciesTree.splitPopSize2	0.00046	8219.69	59	RAG1_Tarsius.treeModel.rootHeight	0.00739	14791.67
10	speciesTree.splitPopSize3	0.00045	8457.88	60	TTR_Tarsius.treeModel.rootHeight	0.00735	14286.01
11	speciesTree.splitPopSize4	0.00043	9248.87	61	ABCA1_Tarsius.kappa	4.51000	2889.67
12	speciesTree.splitPopSize5	0.00036	5927.69	62	ABCA1_Tarsius.frequencies1	0.33700	1548.93
13	speciesTree.splitPopSize6	0.00034	5066.69	63	ABCA1_Tarsius.frequencies2	0.22800	1919.25
14	speciesTree.splitPopSize7	0.00041	8019.24	64	ABCA1_Tarsius.frequencies3	0.16900	1968.04
15	speciesTree.splitPopSize8	0.00045	8599.27	65	ABCA1_Tarsius.frequencies4	0.26600	1793.81
16	speciesTree.splitPopSize9	0.00047	9241.22	66	ADORA3_Tarsius.kappa	7.42700	2781.24
17	speciesTree.splitPopSize10	0.00050	9554.87	67	ADORA3_Tarsius.frequencies1	0.20600	1841.33
18	speciesTree.splitPopSize11	0.00049	7354.92	68	ADORA3_Tarsius.frequencies2	0.29700	1830.57
19	speciesTree.splitPopSize12	0.00053	7832.24	69	ADORA3_Tarsius.frequencies3	0.21200	1696.51
20	speciesTree.splitPopSize13	0.00048	8186.26	70	ADORA3_Tarsius.frequencies4	0.28600	1790.46
21	speciesTree.splitPopSize14	0.00044	8696.59	71	ADORA3_Tarsius.pInv	0.82500	3736.23
22	speciesTree.splitPopSize15	0.00059	11198.56	72	AXIN1_Tarsius.kappa	17.46300	1501.73
23	speciesTree.splitPopSize16	0.00056	12745.55	73	AXIN1_Tarsius.frequencies1	0.24200	1632.81
24	speciesTree.splitPopSize17	0.00067	10670.31	74	AXIN1_Tarsius.frequencies2	0.27800	1685.28
25	speciesTree.splitPopSize18	0.00040	9589.17	75	AXIN1_Tarsius.frequencies3	0.29300	1912.16
26	speciesTree.splitPopSize19	0.00028	7619.89	76	AXIN1_Tarsius.frequencies4	0.18700	1915.03
27	speciesTree.splitPopSize20	0.00027	6670.61	77	RAG1_Tarsius.kappa1	4.35300	1741.27
28	speciesTree.splitPopSize21	0.00017	1149.89	78	RAG1_Tarsius.kappa2	13.68100	1923.59
29	speciesTree.splitPopSize22	0.00019	3493.23	79	RAG1_Tarsius.frequencies1	0.24200	1571.76
30	speciesTree.splitPopSize23	0.00022	1824.50	80	RAG1_Tarsius.frequencies2	0.25700	1683.20
31	speciesTree.splitPopSize24	0.00021	2948.71	81	RAG1_Tarsius.frequencies3	0.29400	1769.00
32	speciesTree.splitPopSize25	0.00001	1492.58	82	RAG1_Tarsius.frequencies4	0.20800	1644.20
33	speciesTree.splitPopSize26	0.00022	4560.01	83	RAG1_Tarsius.pInv	0.74000	4166.58
34	speciesTree.splitPopSize27	0.00021	4445.53	84	TTR_Tarsius.kappa	9.02900	3072.64
35	speciesTree.splitPopSize28	0.00021	3227.40	85	TTR_Tarsius.frequencies1	0.29800	1831.61
36	speciesTree.splitPopSize29	0.00023	3783.98	86	TTR_Tarsius.frequencies2	0.19700	1799.13
37	speciesTree.splitPopSize30	0.00020	1120.81	87	TTR_Tarsius.frequencies3	0.18700	1944.44
38	speciesTree.splitPopSize31	0.00018	2750.34	88	TTR_Tarsius.frequencies4	0.31800	1395.05
39	speciesTree.splitPopSize32	0.00026	231.35	89	TTR_Tarsius.alpha	448.76500	549.74
40	speciesTree.splitPopSize33	0.00019	2987.25	90	ABCA1_Tarsius.clock.rate	3.67400	16923.07
41	speciesTree.splitPopSize34	0.00019	2615.84	91	ADORA3_Tarsius.clock.rate	5.25300	12355.34
42	speciesTree.splitPopSize35	0.00026	218.09	92	AXIN1_Tarsius.clock.rate	1.00000	-
43	speciesTree.splitPopSize36	0.00018	2902.05	93	RAG1_Tarsius.clock.rate	4.00600	16852.80
44	speciesTree.splitPopSize37	0.00017	990.84	94	TTR_Tarsius.clock.rate	3.43000	17552.17
45	speciesTree.splitPopSize38	0.00021	912.69	95	ABCA1_Tarsius.treeLikelihood	-1102.57200	2129.99
46	speciesTree.splitPopSize39	0.00021	3317.14	96	ADORA3_Tarsius.treeLikelihood	-838.89600	849.81
47	speciesTree.splitPopSize40	0.00022	4115.04	97	AXIN1_Tarsius.treeLikelihood	-1288.52900	3677.47
48	speciesTree.splitPopSize41	0.00023	5200.43	98	RAG1_Tarsius.treeLikelihood	-1575.62300	2345.54
49	speciesTree.splitPopSize42	0.00020	3557.14	99	TTR_Tarsius.treeLikelihood	-1847.16700	2310.38
50	speciesTree.splitPopSize43	0.00021	3046.22				

Table 8.12: Traces of five combined BEAST log files obtained from nuclear multilocus species tree analyses for divergence time estimation – calibration 1

ESS values < 100 are highlighted in red.

	Statistic	mean	ESS
1	posterior	-14099.41200	1099.13
2	prior	-415.97100	1075.97
3	likelihood	-13683.44100	5181.10
4	species.coalescent	-250.20400	1075.34
5	species.popSizesLikelihood	-31.92100	1719.24
6	speciation.likelihood	-94.02600	1545.16
7	species.popMean	0.27400	1871.16
8	speciesTree.splitPopSize1	1.09200	14859.14
9	speciesTree.splitPopSize2	1.09700	15024.95
10	speciesTree.splitPopSize3	1.09600	14274.87
11	speciesTree.splitPopSize4	1.06200	14337.10
12	speciesTree.splitPopSize5	1.07500	11618.46
13	speciesTree.splitPopSize6	1.06600	14428.87
14	speciesTree.splitPopSize7	1.03400	13033.15
15	speciesTree.splitPopSize8	0.88100	13210.98
16	speciesTree.splitPopSize9	0.81900	12424.95
17	speciesTree.splitPopSize10	0.96800	12191.69
18	speciesTree.splitPopSize11	1.07000	12464.81
19	speciesTree.splitPopSize12	1.11400	11390.58
20	speciesTree.splitPopSize13	1.21100	11757.40
21	speciesTree.splitPopSize14	1.22800	9769.02
22	speciesTree.splitPopSize15	1.27400	11573.90
23	speciesTree.splitPopSize16	1.16000	11817.25
24	speciesTree.splitPopSize17	1.07700	14497.06
25	speciesTree.splitPopSize18	1.47400	10645.22
26	speciesTree.splitPopSize19	1.27900	9846.36
27	speciesTree.splitPopSize20	0.96000	15542.56
28	speciesTree.splitPopSize21	0.98200	16399.82
29	speciesTree.splitPopSize22	0.63200	14366.50
30	speciesTree.splitPopSize23	0.72800	15495.07
31	speciesTree.splitPopSize24	1.09900	13715.40
32	speciesTree.splitPopSize25	1.05500	12862.01
33	speciesTree.splitPopSize26	0.46800	533.12
34	speciesTree.splitPopSize27	0.49300	4841.67
35	speciesTree.splitPopSize28	0.54200	3598.53
36	speciesTree.splitPopSize29	0.53600	4973.48
37	speciesTree.splitPopSize30	0.54000	3870.75
38	speciesTree.splitPopSize31	0.56500	6217.06
39	speciesTree.splitPopSize32	0.51900	3038.18
40	speciesTree.splitPopSize33	0.54000	3464.90
41	speciesTree.splitPopSize34	0.55200	3250.95
42	speciesTree.splitPopSize35	0.51200	2200.63
43	speciesTree.splitPopSize36	0.47300	1048.27
44	speciesTree.splitPopSize37	0.65500	299.51
45	speciesTree.splitPopSize38	0.50100	3667.16
46	speciesTree.splitPopSize39	0.50700	4026.26
47	speciesTree.splitPopSize40	0.61600	242.78
48	speciesTree.splitPopSize41	0.46400	1887.28
49	speciesTree.splitPopSize42	0.46900	1520.28
50	speciesTree.splitPopSize43	0.56600	472.39
51	speciesTree.splitPopSize44	0.52300	4408.35
52	speciesTree.splitPopSize45	0.52300	4829.53
53	speciesTree.splitPopSize46	0.59600	713.35
54	speciesTree.splitPopSize47	0.49300	3495.93
55	speciesTree.splitPopSize48	0.50600	1680.57
56	speciesTree.splitPopSize49	0.53200	3485.10
57	speciesTree.splitPopSize50	0.51900	2426.42
58	speciesTree.splitPopSize51	0.50500	1943.95
59	speciesTree.splitPopSize52	0.54800	3247.08
60	speciesTree.splitPopSize53	0.52500	2681.53
61	speciesTree.splitPopSize54	1.05400	31.46
62	speciesTree.splitPopSize55	0.82000	55.75
63	speciesTree.splitPopSize56	0.53600	3749.90
64	speciesTree.splitPopSize57	0.53600	3627.53
65	speciesTree.splitPopSize58	0.55300	1061.24
66	speciesTree.splitPopSize59	0.53200	2053.01
67	speciesTree.splitPopSize60	0.60900	282.54
68	speciesTree.splitPopSize61	0.79100	34.12
69	speciesTree.splitPopSize62	0.54700	2667.08
70	speciesTree.splitPopSize63	0.54700	5911.90
71	speciesTree.splitPopSize64	0.52900	5971.94
72	speciesTree.splitPopSize65	0.53100	7098.68
73	speciesTree.splitPopSize66	0.54100	9023.46
74	speciesTree.splitPopSize67	0.54400	6928.52
75	speciesTree.splitPopSize68	0.54600	7889.82
76	speciesTree.splitPopSize69	0.54500	2955.09
77	speciesTree.splitPopSize70	0.54600	9853.90
78	speciesTree.splitPopSize71	0.54400	9656.02
79	speciesTree.splitPopSize72	0.63700	48.83
80	speciesTree.splitPopSize73	0.56700	682.59
81	species.yule.birthRate	0.05522	39357.26
82	speciesTree.rootHeight	73.05700	1095.81
83	ABCA1.treeModel.rootHeight	74.20400	1932.94
84	ADORA3.treeModel.rootHeight	74.34800	2336.05
85	AXIN1_2.treeModel.rootHeight	74.38700	2268.77
86	RAG1.treeModel.rootHeight	74.13800	1951.09
87	TTR.treeModel.rootHeight	73.95500	1667.73
88	tmrca(ABCA1.Anthropoidea)	36.96300	418.66
89	tmrca(ABCA1.Catarrhini)	24.77000	613.06
90	tmrca(ABCA1.Haplorhini)	69.35800	562.13
91	tmrca(ABCA1.Hominidae)	14.89200	535.12
92	tmrca(ABCA1.Homininae)	5.92600	15404.09
93	tmrca(ABCA1.Hominoidea)	16.60200	482.28
94	tmrca(ABCA1.Tarsiidae)	19.39300	278.74
95	tmrca(ABCA1.Strepsirhini)	52.79400	219.01
96	ABCA1.ac	0.23000	14005.76
97	ABCA1.at	0.13300	14023.18
98	ABCA1.cg	0.38000	14128.87
99	ABCA1.gt	0.36700	14363.98
100	ABCA1.frequencies1	0.34000	5824.87

	Statistic	mean	ESS
101	ABCA1.frequencies2	0.24200	5440.08
102	ABCA1.frequencies3	0.15700	6541.27
103	ABCA1.frequencies4	0.26000	6171.51
104	ADORA3.ac	0.35900	7908.46
105	ADORA3.ag	1.64500	5565.09
106	ADORA3.at	0.08462	7773.28
107	ADORA3.frequencies1	0.21400	5555.66
108	ADORA3.frequencies2	0.27200	4997.18
109	ADORA3.frequencies3	0.20300	5464.76
110	ADORA3.frequencies4	0.31100	5109.69
111	ADORA3.alpha	0.48500	12481.52
112	AXIN1_2.kappa	9.04900	14779.32
113	AXIN1_2.frequencies1	0.23300	7352.67
114	AXIN1_2.frequencies2	0.27900	6474.93
115	AXIN1_2.frequencies3	0.29400	6362.53
116	AXIN1_2.frequencies4	0.19400	7267.62
117	AXIN1_2.alpha	0.18800	12561.63
118	RAG1.ac	0.14400	8974.10
119	RAG1.ag	0.51000	6494.13
120	RAG1.at	0.06398	9274.70
121	RAG1.frequencies1	0.25100	6212.44
122	RAG1.frequencies2	0.24200	5473.56
123	RAG1.frequencies3	0.28300	5355.73
124	RAG1.frequencies4	0.22400	5552.98
125	RAG1.alpha	0.20700	14804.01
126	TTR.ac	0.30000	14245.67
127	TTR.at	0.11700	12893.72
128	TTR.cg	0.23500	14497.04
129	TTR.gt	0.25100	13967.00
130	TTR.frequencies1	0.29200	6171.73
131	TTR.frequencies2	0.20000	6518.44
132	TTR.frequencies3	0.18800	6298.41
133	TTR.frequencies4	0.32000	5620.84
134	TTR.alpha	404.93200	1676.02
135	ABCA1.ucld.mean	0.00145	3329.06
136	ABCA1.ucld.stdev	0.29600	6852.28
137	ADORA3.ucld.mean	0.00137	4823.81
138	ADORA3.ucld.stdev	0.41600	4155.29
139	AXIN1_2.ucld.mean	0.00067	4379.92
140	AXIN1_2.ucld.stdev	0.56100	2198.20
141	RAG1.ucld.mean	0.00102	2710.14
142	RAG1.ucld.stdev	0.67200	3597.66
143	TTR.ucld.mean	0.00135	1933.94
144	TTR.ucld.stdev	0.35700	5018.80
145	ABCA1.meanRate	0.00154	3874.45
146	ABCA1.coefficientOfVariation	0.29800	5838.72
147	ABCA1.covariance	-0.00081	42548.97
148	ADORA3.meanRate	0.00132	8010.27
149	ADORA3.coefficientOfVariation	0.43000	4231.61
150	ADORA3.covariance	0.00389	42360.35
151	AXIN1_2.meanRate	0.00086	9797.41
152	AXIN1_2.coefficientOfVariation	0.58700	2096.87
153	AXIN1_2.covariance	-0.01502	36704.11
154	RAG1.meanRate	0.00109	6138.80
155	RAG1.coefficientOfVariation	0.73000	3846.68
156	RAG1.covariance	0.06551	13114.75
157	TTR.meanRate	0.00148	2465.19
158	TTR.coefficientOfVariation	0.36100	5222.92
159	TTR.covariance	-0.02172	26563.15
160	ABCA1.treeLikelihood	-2586.02400	7462.91
161	ADORA3.treeLikelihood	-1589.21500	3685.25
162	AXIN1_2.treeLikelihood	-2545.44700	15886.94
163	RAG1.treeLikelihood	-2721.86700	10432.40
164	TTR.treeLikelihood	-4240.88700	7277.10

Table 8.13: Traces of five combined BEAST log files obtained from nuclear multilocus species tree analyses for divergence time estimation – calibration 2

ESS values < 100/200 are highlighted in red/blue

	Statistic	mean	ESS
1	posterior	-14188.67100	1002.41
2	prior	-505.53100	1009.50
3	likelihood	-13683.14000	5002.87
4	species.coalescent	-318.55300	1027.94
5	species.popSizesLikelihood	-45.52600	1471.44
6	speciation.likelihood	-99.18800	1641.93
7	species.popMean	0.33000	1608.66
8	speciesTree.splitPopSize1	1.31900	14758.04
9	speciesTree.splitPopSize2	1.31700	16556.63
10	speciesTree.splitPopSize3	1.31700	14929.87
11	speciesTree.splitPopSize4	1.27500	9106.02
12	speciesTree.splitPopSize5	1.29300	13035.99
13	speciesTree.splitPopSize6	1.28600	13324.91
14	speciesTree.splitPopSize7	1.24600	12180.25
15	speciesTree.splitPopSize8	1.05800	14043.63
16	speciesTree.splitPopSize9	0.98600	10083.62
17	speciesTree.splitPopSize10	1.17300	16128.91
18	speciesTree.splitPopSize11	1.27900	12881.64
19	speciesTree.splitPopSize12	1.34800	12568.10
20	speciesTree.splitPopSize13	1.46300	12615.38
21	speciesTree.splitPopSize14	1.48800	10429.87
22	speciesTree.splitPopSize15	1.53500	10593.70
23	speciesTree.splitPopSize16	1.39400	10507.63
24	speciesTree.splitPopSize17	1.28900	11494.74
25	speciesTree.splitPopSize18	1.79600	6713.01
26	speciesTree.splitPopSize19	1.55200	5487.84
27	speciesTree.splitPopSize20	1.16100	14226.97
28	speciesTree.splitPopSize21	1.19300	15419.69
29	speciesTree.splitPopSize22	0.75700	15591.42
30	speciesTree.splitPopSize23	0.87400	17067.30
31	speciesTree.splitPopSize24	1.32000	16205.59
32	speciesTree.splitPopSize25	1.27000	14560.87
33	speciesTree.splitPopSize26	0.88400	28.46
34	speciesTree.splitPopSize27	0.75200	76.81
35	speciesTree.splitPopSize28	0.65300	8484.29
36	speciesTree.splitPopSize29	0.65200	8921.27
37	speciesTree.splitPopSize30	0.64200	2263.75
38	speciesTree.splitPopSize31	0.65600	2520.20
39	speciesTree.splitPopSize32	0.80200	35.41
40	speciesTree.splitPopSize33	0.69000	329.09
41	speciesTree.splitPopSize34	0.63900	5389.88
42	speciesTree.splitPopSize35	0.63800	1952.68
43	speciesTree.splitPopSize36	0.60200	677.43
44	speciesTree.splitPopSize37	0.64800	284.03
45	speciesTree.splitPopSize38	0.65500	856.00
46	speciesTree.splitPopSize39	0.64800	145.99
47	speciesTree.splitPopSize40	0.65500	201.01
48	speciesTree.splitPopSize41	0.61900	587.23
49	speciesTree.splitPopSize42	0.62200	1587.25
50	speciesTree.splitPopSize43	0.63600	4141.13
51	speciesTree.splitPopSize44	0.90600	83.64
52	speciesTree.splitPopSize45	1.00400	29.48
53	speciesTree.splitPopSize46	0.64100	844.25
54	speciesTree.splitPopSize47	0.66900	757.22
55	speciesTree.splitPopSize48	0.61900	893.52
56	speciesTree.splitPopSize49	0.62400	3403.28
57	speciesTree.splitPopSize50	0.67500	412.74
58	speciesTree.splitPopSize51	0.60900	1138.57
59	speciesTree.splitPopSize52	0.62600	1201.69
60	speciesTree.splitPopSize53	0.68400	2346.51
61	speciesTree.splitPopSize54	0.69500	232.34
62	speciesTree.splitPopSize55	0.85000	27.55
63	speciesTree.splitPopSize56	0.66200	3005.15
64	speciesTree.splitPopSize57	0.63000	1749.39
65	speciesTree.splitPopSize58	0.60600	2164.45
66	speciesTree.splitPopSize59	0.71700	234.12
67	speciesTree.splitPopSize60	0.61300	3783.50
68	speciesTree.splitPopSize61	0.60100	3500.56
69	speciesTree.splitPopSize62	0.71900	274.67
70	speciesTree.splitPopSize63	0.60800	999.09
71	speciesTree.splitPopSize64	0.64000	4466.96
72	speciesTree.splitPopSize65	0.66000	6689.81
73	speciesTree.splitPopSize66	0.63300	2520.56
74	speciesTree.splitPopSize67	0.61900	7291.90
75	speciesTree.splitPopSize68	0.68000	8926.64
76	speciesTree.splitPopSize69	0.67300	8809.39
77	speciesTree.splitPopSize70	0.64900	5057.74
78	speciesTree.splitPopSize71	0.64900	5440.49
79	speciesTree.splitPopSize72	0.62800	4654.04
80	speciesTree.splitPopSize73	0.60200	799.92
81	species.yule.birthRate	0.04463	32947.20
82	speciesTree.rootHeight	90.79500	628.28
83	ABCA1.treeModel.rootHeight	92.30100	813.87
84	ADORA3.treeModel.rootHeight	92.43200	858.81
85	AXIN1_2.treeModel.rootHeight	92.53400	870.70
86	RAG1.treeModel.rootHeight	92.15000	771.11
87	TTR.treeModel.rootHeight	91.91300	718.08
88	tmrca(ABCA1.Anthropoidea)	43.14100	472.93
89	tmrca(ABCA1.Catarrhini)	30.38500	759.88
90	tmrca(ABCA1.Haplorhini)	85.39600	228.74
91	tmrca(ABCA1.Hominidae)	17.73500	2087.81
92	tmrca(ABCA1.Homininae)	6.37300	14696.89
93	tmrca(ABCA1.Hominoidea)	19.96000	1572.21
94	tmrca(ABCA1.Tarsiidae)	23.40500	273.83
95	tmrca(ABCA1.Strepsirhini)	68.46900	228.62
96	ABCA1.ac	0.23000	12588.72
97	ABCA1.at	0.13200	12840.16
98	ABCA1.cg	0.38000	13338.55
99	ABCA1.gt	0.36600	14184.00
100	ABCA1.frequencies1	0.34000	5485.28

	Statistic	mean	ESS
101	ABCA1.frequencies2	0.24200	6363.28
102	ABCA1.frequencies3	0.15700	6364.27
103	ABCA1.frequencies4	0.26100	5917.46
104	ADORA3.ac	0.35900	7766.39
105	ADORA3.ag	1.64700	5303.93
106	ADORA3.at	0.08505	8822.45
107	ADORA3.frequencies1	0.21300	5791.21
108	ADORA3.frequencies2	0.27300	5417.11
109	ADORA3.frequencies3	0.20300	5872.72
110	ADORA3.frequencies4	0.31100	5143.40
111	ADORA3.alpha	0.48300	12317.33
112	AXIN1_2.kappa	9.01400	15915.06
113	AXIN1_2.frequencies1	0.23300	6399.64
114	AXIN1_2.frequencies2	0.27800	6587.45
115	AXIN1_2.frequencies3	0.29400	7016.84
116	AXIN1_2.frequencies4	0.19500	7141.53
117	AXIN1_2.alpha	0.18800	13492.42
118	RAG1.ac	0.14400	8414.13
119	RAG1.ag	0.51100	6419.15
120	RAG1.at	0.06389	10007.69
121	RAG1.frequencies1	0.25100	5783.89
122	RAG1.frequencies2	0.24200	5779.14
123	RAG1.frequencies3	0.28300	5560.64
124	RAG1.frequencies4	0.22400	5794.99
125	RAG1.alpha	0.20600	14692.16
126	TTR.ac	0.30100	14740.21
127	TTR.at	0.11600	13388.16
128	TTR.cg	0.23500	14405.20
129	TTR.gt	0.25000	15001.92
130	TTR.frequencies1	0.29200	5417.67
131	TTR.frequencies2	0.20000	6622.56
132	TTR.frequencies3	0.18800	6666.05
133	TTR.frequencies4	0.32100	5595.64
134	TTR.alpha	393.26700	1431.11
135	ABCA1.ucld.mean	0.00119	4104.38
136	ABCA1.ucld.stdev	0.26900	7240.02
137	ADORA3.ucld.mean	0.00113	4177.94
138	ADORA3.ucld.stdev	0.44700	4115.93
139	AXIN1_2.ucld.mean	0.00055	3419.04
140	AXIN1_2.ucld.stdev	0.53900	2703.36
141	RAG1.ucld.mean	0.00083	2009.53
142	RAG1.ucld.stdev	0.66700	2675.79
143	TTR.ucld.mean	0.00111	2335.10
144	TTR.ucld.stdev	0.35400	4720.12
145	ABCA1.meanRate	0.00125	3485.52
146	ABCA1.coefficientOfVariation	0.27000	7248.50
147	ABCA1.covariance	-0.00013	42259.74
148	ADORA3.meanRate	0.00107	9565.11
149	ADORA3.coefficientOfVariation	0.46400	4112.14
150	ADORA3.covariance	0.00416	38539.52
151	AXIN1_2.meanRate	0.00070	7830.53
152	AXIN1_2.coefficientOfVariation	0.56200	2663.33
153	AXIN1_2.covariance	-0.01392	38485.05
154	RAG1.meanRate	0.00088	4284.79
155	RAG1.coefficientOfVariation	0.72300	2726.33
156	RAG1.covariance	0.06628	14445.59
157	TTR.meanRate	0.00120	2505.54
158	TTR.coefficientOfVariation	0.35800	4514.92
159	TTR.covariance	-0.01907	28700.22
160	ABCA1.treeLikelihood	-2585.98400	9804.30
161	ADORA3.treeLikelihood	-1588.83800	3692.00
162	AXIN1_2.treeLikelihood	-2545.69900	13225.13
163	RAG1.treeLikelihood	-2721.80800	13796.65
164	TTR.treeLikelihood	-4240.81000	6850.26