

Molecular Phylogenetics and Chronometrics of Tarsiidae Based on 12S mtDNA Haplotypes: Evidence for Miocene Origins of Crown Tarsiers and Numerous Species within the Sulawesi Clade

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Abstract We report new mitochondrial DNA sequence data from tarsiers sampled from several populations, including the extreme northeast and southwest of the range of the *Tarsius tarsier* species complex, the most extensive sampling ever reported for this taxon. Our results provide the opportunity to produce the first ever molecular chronometric analysis of Tarsiidae. These results date the age of crown tarsiers, minimally, to the middle Miocene, and each of the 3 tarsier species groups, *Tarsius bancanus*, *T. syrichta*, and the *T. tarsier* complex, to the early or middle Miocene. Thus, each of these 3 species has evolved in isolation for a period of time that is consistent with that which would be expected for multiple speciation events. Our analysis of the *Tarsius tarsier* complex reveals 5 subclades, each of which is interpreted to represent a haplogroup at, or above, the species level, a result that is consistent with current hypotheses about numerous cryptic species within this species complex. The implications for conservation within the Sulawesi biogeographic region are that Sulawesi is subdivided into numerous subregions of

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endemism and that, by extrapolating the example of cryptic tarsier species to other taxa, biodiversity may be underestimated by an order of magnitude. The practical realities of conservation in Sulawesi are such that it is most reasonable to assume that anthropogenic extinctions are occurring, and that some species will go extinct before they have even been identified.

Keywords Biodiversity · Biogeography · Conservation · Cryptic species · Dispersal · Endemism · Phylogeography · Sulawesi · Tarsius · Vicariance

Introduction

For more than a century, the position of tarsiers within Primates has captured the attention of primate systematists, but few examined diversity within extant tarsiers. This has been particularly true for molecular systematics. In spite of the fact that published studies that included multiple tarsier species have been available for *ca.* 20 yr (Dijan and Green 1991), only comparatively recently have researchers commented on the molecular genetic differences among tarsiers. When this has happened, the results have challenged previous assumptions about extant tarsiers being closely related species (Meireles *et al.* 2003; Merker *et al.* 2009; Shekelle 2003; Shekelle *et al.* 2008a). We report the first study of DNA sequence data from wild-caught tarsier populations to include samples from locations as varied as the extreme northern and southern limits of the distribution of the *Tarsius tarsier* species complex, and use these data to examine hypotheses about the age of crown tarsiers and the validity of numerous species within that complex.

The Age of Crown Tarsiers

Hill (1955) classified tarsiers into 3 species: *Tarsius syrichta*, *T. bancanus*, and *T. spectrum* (later synonymized with *T. tarsier*), each being endemic to a distinct biogeographic area: 1) the southern Philippines; 2) parts of Sundaland (including Borneo, southwestern Sumatra, and several smaller islands); and 3) Sulawesi, respectively. A widely held belief was that these 3 species were close relatives, such that Musser and Dagosto (1987), in their landmark monograph on tarsier taxonomy, could state that the group of extant tarsiers, “comprises closely related taxa,” without reference.

Recent molecular genetic studies indicate that tarsiers are not as closely related as previously assumed. First, Shekelle (2003; Shekelle *et al.* 2008a) hypothesized the age of crown tarsiers to be *ca.* 10 Ma, beginning with the divergence of the ancestors of the *Tarsius tarsier* complex from those of a *T. bancanus*-*T. syrichta* clade. That estimate was based on 1) comparisons of genetic distances derived from DNA sequences in the 12S mitochondrial DNA (mtDNA) gene region among tarsiers with those from hominoids; 2) geological reconstructions of southeast Asia (Hall 2001); 3) palynological evidence of biotic exchange between Borneo and Sulawesi across the Makassar Straits (Morley 1998); and 4) molecular clock evidence that other taxa from Sulawesi, notably grasshoppers of *Chitaura* (Walton *et al.* 1997) and squirrels (Mercer and Roth 2003), were also compatible with a date of origin in the region of

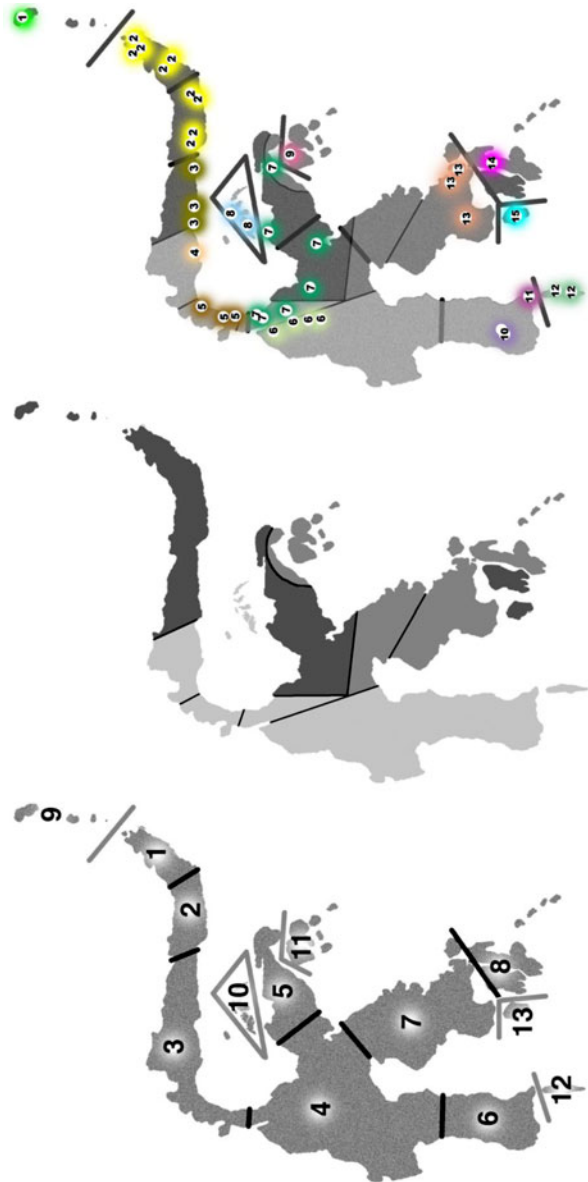
10 Ma. A second study, by Meireles *et al.* (2003), used a molecular clock approach to estimate the divergence of *Tarsius bancanus* and *T. syrichta* at 5.6 Ma, based on DNA sequences from the γ -globin gene from nuclear DNA (nDNA) (Meireles *et al.* 2003). More recently, Matsui *et al.* (2009) reported an even older molecular clock estimate for diversification of Philippine and Western Tarsiers, dating as far back as the Oligocene or possibly even the Eocene: 30.8 ± 3.9 (23.4–38.6) Ma based on protein coding mtDNA, and 20.2 ± 3.3 (14.5–27.4) Ma based on ribosomal RNA (rRNA) coding mtDNA. Thus, molecular evidence indicates that each of the 3 extant tarsier species recognized by Hill has its origins in the Miocene. The relatively ancient origin of Hill's 3 tarsier species indicates that they have been isolated for a length of time that is consistent with a hypothesis of multiple cladogenic branching events. This observation is highly relevant to the number of taxa hypothesized within the *Tarsius tarsier* complex.

Taxonomic Diversity Within the *Tarsius tarsier* Complex

Hill recognized subspecies within each of the 3 tarsier species in his classification, including 5 subspecies of *Tarsius spectrum*: *T. spectrum spectrum* (from the region around Makasar in the extreme southwestern tip of Sulawesi), *T. s. sangirensis* (from the island of Greater Sangihe, ca. 200 km north of the extreme northeastern tip of Sulawesi), *T. s. pumilus* (a uniquely small-bodied tarsier from Sulawesi's central core), *T. s. dentatus* (a tarsier of typical body proportions from Sulawesi's central core), and *T. s. pelengensis* (from the island of Peleng off the tip of Sulawesi's eastern peninsula). Subsequent taxonomic work has been unevenly distributed, with extensive fieldwork conducted on the *Tarsius tarsier* complex, whereas comparatively little work has been done on *T. bancanus* and *T. syrichta*. Studies of the *Tarsius tarsier* complex were spurred on by a report of a field study by MacKinnon and MacKinnon (1980). They noted that Sulawesi tarsiers have a conspicuous territorial duet call, that this call shows obvious geographic variation, and that the pattern of variation seemed to mirror the pattern of allopatric and parapatric species seen among the Sulawesi macaques. They concluded, therefore, that "(t)here is clearly much more taxonomic work to be done to sort out the Sulawesi tarsiers, but we would predict that there are more forms to be found in southern Sulawesi and on the offshore island groups of Selayar, Peleng, and Sangihe-Talaut" (p. 378). Thus, MacKinnon and MacKinnon implicitly recognized that each of Hill's 5 subspecies, plus an undetermined number of additional populations, were likely to be distinct species. Subsequently, all of the subspecies of *Tarsius tarsier* that Hill recognized came to be recognized as full species on the basis of morphology and, in some cases, bioacoustics: *T. pumilus* (Musser and Dagosto 1987), *T. sangirensis* (Feiler 1990; Groves 1998; Shekelle *et al.* 1997), *T. dentatus* (= *dianae*) (Brandon-Jones *et al.* 2004; Niemitz *et al.* 1991), and *T. pelengensis* (Groves 1998).

Shekelle and Leksono (2004) expanded on the MacKinnons' observations and proposed the hybrid biogeographic hypothesis for Sulawesi, which synthesized seemingly incongruent biological (Evans *et al.* 2003) and geological (Hall 2001) evidence with the distribution of the 15 known tarsier acoustic forms at that time (Fig. 1). Under their model, each of these acoustic forms was hypothetically a distinct species that arose through a process of dispersal throughout a proto-Sulawesi

Fig. 1 The hybrid biogeographic hypothesis (adapted from Shekelle and Leksono 2004). **(Left)** Biogeographic hypothesis for Sulawesi based on empirical biological evidence showing 13 areas of endemism. **(Center)** Biogeographic hypothesis for Sulawesi based on empirical geological evidence. **(Right)** Comprehensive map of tarsier acoustic forms (numbered sites), showing concordance between tarsier acoustic forms and the combined biological and geological maps.



archipelago in the Miocene and Pliocene and subsequent range fragmentation principally during the Pleistocene. Their map predicted the location of still more undiscovered species, after the proto-Sulawesi archipelago had congealed into more or less its current form and guided the fieldwork that led to the recognition of the Siau Island tarsier as a distinct species (Shekelle *et al.* 2008b). Their hypothesis has been subsequently supported by 2 independent genetic studies. Based on small regions of the 12S mtDNA gene from tarsier populations along a megatranssect (>1000 km) that sampled 7 acoustic forms, Shekelle *et al.* (2008a) found results consistent with the hypotheses of Shekelle and Leksono, i.e., that tarsier acoustic

groups diagnose species, and that these species are consistent with the hybrid biogeographic hypothesis for Sulawesi. Merker *et al.* (2009) examined the contact zone for 2 parapatric species in Shekelle and Leksono's model, *Tarsius dentatus* and *T. lariang*, in detail; their study included 12 microsatellite loci, the cytochrome *b* gene, the hypervariable region of the mitochondrial control region, and the sex-determining region on the Y chromosome from 144 tarsiers captured along a 65-km transect, and they found strong evidence of sharp concordant genetic, acoustic, and morphologic breaks at a point within an expanse of unbroken primary forest near a suture between 2 microplates, as predicted by the hybrid biogeographic hypothesis. Two more species predicted by the hybrid biogeographic hypothesis have been named: *Tarsius lariang* (Merker and Groves 2006) and *T. tumpara* (Shekelle *et al.* 2008b). Further, 1 additional species is described in this volume, and the Tinombo form, first identified by Shekelle *et al.* (1997), bringing the total number of Eastern Tarsier species to 9 (Table I).

One issue that has hampered a stable taxonomy for Eastern Tarsiers has been the lack of a well localized type specimen of *Tarsius tarsier*. Further, as all known tarsier taxa are distributed allopatrically or parapatrically with congeners—there are no known cases of sympatry among extant tarsiers—acquiring data from a representative population of this species is a high priority. As the senior taxon of the species group, all populations of Eastern Tarsiers that are not specifically classified as something else are, officially, classified as *Tarsius tarsier*. With taxonomic work progressing in the central core (Merker *et al.* 2010), this leaves *Tarsius tarsier*, in the broad sense, with an improbable disjunct distribution (Brandon-Jones *et al.* 2004). Groves *et al.* (2008) argued that Buffon's tarsier most likely came from Makassar, although the specimen itself was missing. Callou *et al.* (2010) report on the amazing rediscovery of Buffon's specimen in the Paris museum. Curiously, the specimen does not appear to have come from Makassar, but more likely from Selayar Island (Groves and Shekelle 2010). Thus, surveying populations from both Makassar and Selayar are vitally important for a stable taxonomy of tarsiers, as one or the others of these is virtually assured to be the type locality of the senior taxon.

Sulawesi Biogeography, Mode of Speciation, and Conservation

Several authors have explained the presence of numerous parapatric and allopatric taxa on Sulawesi as being the product of dispersal (range expansion), followed by isolation or fragmentation, with the correspondence of species boundaries indicating a shared set of evolutionary forces. Evans *et al.* (2003) argued that Pleistocene range fragmentation, chiefly caused by rising ocean levels, is the best explanation for the pattern of genetic diversity occurring among macaques and toads, which share regions of endemism. Shekelle and Leksono (2004) examined the distribution of 15 tarsier acoustic forms and argued that tarsiers showed even greater diversity than macaques because they arrived on Sulawesi long before the macaques, and thus experienced a history of Miocene and Pliocene dispersal throughout the proto-Sulawesi archipelago (leading to isolation on separate islands), followed by a history of the same Pleistocene vicariance events that resulted in the taxonomic structuring of the Sulawesi macaques. Key to their argument are the geological reconstructions

Table 1 A list of Eastern Tarsier taxa, showing the correspondence among acoustic forms and taxonomy, beginning with the senior taxon and moving more or less south to north

	Acoustic form	Classification
1.	Selayar form (Nietsch and Burton 2010)	<i>Tarsius tarsier tarsier</i> (=spectrum spectrum) Erxleben 1777 see Groves and Shekelle 2010
2.	Desa Ara form (Nietsch and Babo 2001)	a
3.	Bantimurung form (Nietsch and Burton 2010)	<i>T. fuscus</i> Fischer 1804; Groves and Shekelle 2010
4.	Kendari form (Nietsch and Burton 2010)	a
5.	Buton form (Nietsch and Burton 2010)	a
6.	Kabaena form (Nietsch and Burton 2010)	a
7.	Palu form (MacKinnon and MacKinnon 1980)	<i>T. lariang</i> Merker and Groves 2006
8.	Kamarora form (Niemitz et al. 1991)	<i>T. dentatus</i> Miller and Hollister 1921
9.	Peleng form (Nietsch and Burton 2010)	<i>T. pelengensis</i> Sody 1949
10.	Togian form (Nietsch and Niemitz 1993)	a
11.	Tinombo form (Shekelle et al. 1997)	<i>T. wallacei</i> Merker et al. 2010
12.	Sejoli form (Shekelle et al. 1997)	a
13.	Gorontalo form (MacKinnon and MacKinnon 1980)	a
14.	Manado form (MacKinnon and MacKinnon 1980)	
15.	Siau form (Shekelle et al. 2008b)	<i>T. tumpara</i> Shekelle et al. 2008b
16.	Sangihe form (Shekelle et al. 1997)	<i>T. sangirensis</i> Meyer 1897

^a These acoustics forms are all classified as *T. tarsier*, but that would leave this taxon with an implausibly disjunct distribution, and we consider it far more likely that each of these will be found to be taxonomically distinct

of Hall (2001), which show Sulawesi to have been an archipelago that coalesced into the modern island of Sulawesi only within the past 1–2 Ma. Macaques, it is assumed, arrived around the time of that coalescence, or even after it, and thus never experienced isolation on the many separate islands that ultimately became modern Sulawesi. Shekelle and Leksono predicted, therefore, that tarsier faunal boundaries

would be associated not only with regions of Pleistocene range contractions, but also at some microplate sutures. Their observation was, therefore, that:

- 1) On a more recent temporal scale, Sulawesi was, indeed, subdivided into the 8 regions of endemism generated through vicariance, as predicted by Evans *et al.* (2003);
- 2) On an older temporal scale, Sulawesi was also subdivided into ≥ 11 microplates, most likely generated by dispersal throughout the proto-Sulawesi archipelago, as identified by Hall (2001); and therefore,
- 3) When assessed with a relatively old endemic taxon, such as tarsiers, Sulawesi and its offshore islands were revealed to have ≥ 14 regions, which were assumed to have been generated by ancient dispersal throughout a proto-Sulawesi archipelago followed by more recent vicariance events. It is possible, of course, that the pattern of dispersal and vicariance is more complex than this simple model of dispersal followed by vicariance.

Merker *et al.* (2009) tested this hypothesis at one such suture, at the Palu-Koro fault, where populations of *Tarsius dentatus* and *T. lariang* meet in unbroken primary forest, unassociated with any current geological barrier. They associated their 1.4-Ma molecular clock estimate of the split between these 2 species with a Pleistocene glacial maximum that would have lowered sea levels greatly, possibly bringing the northern and southern halves of Sulawesi briefly into contact, allowing dispersal between the 2 land masses, before rising ocean levels once again separated the 2 populations, which went on to speciate.

Bridle *et al.* (2004) argued that there is an alternative explanation for the distribution of biodiversity on Sulawesi, that the case for vicariance had been overstated in 1 instance, and the case for adaptive evolution had been overlooked in all cases but their own. They reanalyzed the data of Evans *et al.* (2003), controlling for isolation by distance, and found that the data set for *Bufo*, the toads in the analysis of Evans *et al.*, could not refute the null hypothesis, although the macaque data could. They also revisited their own work on the grasshopper genus *Chitaura* (Bridle *et al.* 2001; Walton *et al.* 1997), and pointed out that their analysis of mtDNA, morphology, and color patterns did not fit the biogeographic model predicted by Evans *et al.* for 2 grasshopper boundaries in northern Sulawesi. Further, they argued that their morphological evidence was consistent with an adaptive response to clinal changes in ecological or climatic variables in 1 case. They cautioned against oversimplified models of evolution and speciation that rely on isolation, to the exclusion of all other mechanisms and explanations.

Evans and his colleagues contested the conclusions of Bridle and his colleagues. Evans *et al.* (2004) illustrated analytical flaws with the reanalysis of Bridle *et al.* (2004) and demonstrated that the data from Evans *et al.* (2003) are in fact consistent with fragmentation even when the same analysis of Bridle *et al.* is used. Further, Evans *et al.* (2008) collected more data from the Celebes toad and showed with coalescent simulations that a model with fragmentation is significantly preferred over a model with isolation by distance alone for this species.

Evans *et al.* (2003) argued that their results had profound implications for conservation. They stated that other taxa, even taxonomically unidentified ones, might fit this same biogeographic pattern as the macaques and toads, and therefore

conservation policy should take into account the 8 regions of endemism they identified. Shekelle and Leksono (2004) went further, elaborating on the biogeographic model to include more regions of endemism, and suggested that tarsiers could be used as flagship species to promote awareness and conservation of primary habitat in each biogeographic subregion; thus, a species-based conservation plan could be a proxy for a biogeographic/habitat-based conservation plan. They also argued that such a plan was all the more necessary because the example of the *Tarsius tarsier* complex, wherein 1 subspecies of tarsier was shown to be composed of as many as 16 or more taxonomically cryptic species, indicated, by inference, that biodiversity on Sulawesi might be underestimated by an order of magnitude. However, Bridle *et al.* (2004) cautioned that the suggestion of Evans *et al.* might be a “costly oversimplification,” because the assumption that other taxa fit the biogeography was speculative, and the underlying evolutionary mechanisms relied only on isolation and did not address adaptive evolution.

We propose to examine the hypotheses that crown tarsiers diversified in the Miocene, and that the Sulawesi clade represents numerous distinct species. We ask if our genetic data are consistent with the taxonomic hypothesis based on bioacoustics and morphology. Do the distributions of these, in turn, fit the biogeographic models being developed for Sulawesi based on empirical biological and geological data? Though our genetic data are not state-of-the-art, by any means, they could falsify these hypotheses by not showing evidence of deep branches, on the order of what would be sufficient for numerous speciations; likewise, a lack of phylogeographic structure would clearly run counter to the expectations of the hypotheses we are examining. To test these hypotheses, we add to the 12S rRNA mitochondrial data set of Shekelle *et al.* (2008a) new samples collected from the furthest southwestern portions of the range of the *Tarsius tarsier* complex, including areas thought to represent the senior taxon of the species group, i.e., Bantimurung, near Makassar, and Selayar Island. We analyze these using improved methods designed to provide a more accurate alignment of orthologous sequence and that are capable of detecting nuclear integrants of mtDNA (numts). We use maximum likelihood and Bayesian inference to identify robust genetic subclades within the Eastern Tarsiers, and use molecular clock techniques to identify the timing of key events in tarsier evolution. Finally, we place our results into the context of the literature on tarsier taxonomy and evolution, as well as Sulawesi’s biogeographic history and conservation needs.

Materials and Methods

Samples

We sampled representatives from the 3 major clades of extant tarsiers, including multiple individuals from the Eastern Tarsier group (Table II). We downloaded data from *Tarsius syrichta* (AF069976), *T. bancanus* (AF348159), and various outgroup taxa from GenBank and included them in the analyses. We designed our sampling to address the phylogenetic relationships and depth of major branching events within Tarsiidae, with a focus on Eastern Tarsiers. Figure 2 shows most of the important

Table II Individuals and taxa sampled in this study

Individual ID	Taxon	Locality	GenBank no.
Tsyr01	<i>Tarsius syrichta</i>	unknown	AF069976
Tban01	<i>T. bancanus</i>	unknown	AF348159
Tsan049	<i>T. sangirensis</i>	Sangihe Island	HM470205
Tsan050	<i>T. sangirensis</i>	Sangihe Island	HM470206
Tspec082	<i>T. tarsier sensu lato</i>	Tangkoko	HM470207
Tspec083	<i>T. tarsier sensu lato</i>	Tangkoko	HM470208
Tspec084	<i>T. tarsier sensu lato</i>	Basaan	HM470209
Tspec085	<i>T. tarsier sensu lato</i>	Basaan	HM470210
Tspec18	<i>T. tarsier sensu lato</i>	Molibagu	HM470211
Tspec19	<i>T. tarsier sensu lato</i>	Molibagu	HM470212
Tspec090	<i>T. tarsier sensu lato</i>	Suwawa	HM470213
Tspec038	<i>T. tarsier sensu lato</i>	Libuo	HM470214
Tspec074	<i>T. wallacei</i>	Tinombo	HM470215
Tspec075	<i>T. wallacei</i>	Tinombo	HM470216
Tspec096	<i>T. tarsier sensu lato</i>	Sejoli	HM470217
Tspec100	<i>T. tarsier sensu lato</i>	Sejoli	HM470218
Tspec052	<i>T. tarsier sensu lato</i>	Togian	HM470219
Tspec057	<i>T. tarsier sensu lato</i>	Togian	HM470220
Tspec058	<i>T. tarsier sensu lato</i>	Togian	HM470221
Tspec0108	<i>T. tarsier sensu lato</i>	Benteng	HM470222
Tspec0109	<i>T. tarsier sensu lato</i>	Benteng	HM470223
Tspec1	<i>T. tarsier</i>	Pattanuang	HM470224
Ttar0104	<i>T. tarsier</i>	Pattanuang	HM470225
Ttar0102	<i>T. tarsier</i>	Pattanuang	HM470226
Tlar1	<i>T. lariang</i>	Gimpu	HM470227
Tlar2	<i>T. lariang</i>	Gimpu	HM470228
Tden062	<i>T. dentatus</i>	Kamaora	HM470229
Lemur	<i>Lemur catta</i>	unknown	NC_004025
Otolemur	<i>Otolemur crassicaudatus</i>	unknown	AF179289
Nycticebus	<i>Nycticebus coucang</i>	unknown	NC_002765
Cebus	<i>Cebus albifrons</i>	unknown	NC_002763
Saimiri	<i>Saimiri sciureus</i>	unknown	FJ785425
Homo	<i>Homo sapiens</i>	unknown	NC_001807
Pan	<i>Pan troglodytes</i>	unknown	NC_001643
Pongo	<i>Pongo pygmaeus</i>	unknown	NC_001646
Papio	<i>Papio hamadryas</i>	unknown	NC_001992
Theropithecus	<i>Theropithecus gelada</i>	unknown	EU580083
Presbytis	<i>Presbytis melalophos</i>	unknown	DQ355299
Cynocephalus	<i>Cynocephalus variegatus</i>	unknown	AF460846

Classification follows Brandon-Jones *et al.* (2004) plus the subsequent work to describe the putative new species mentioned therein: Merker and Groves (2006), Groves and Shekelle (2010), and Merker *et al.* (2010).

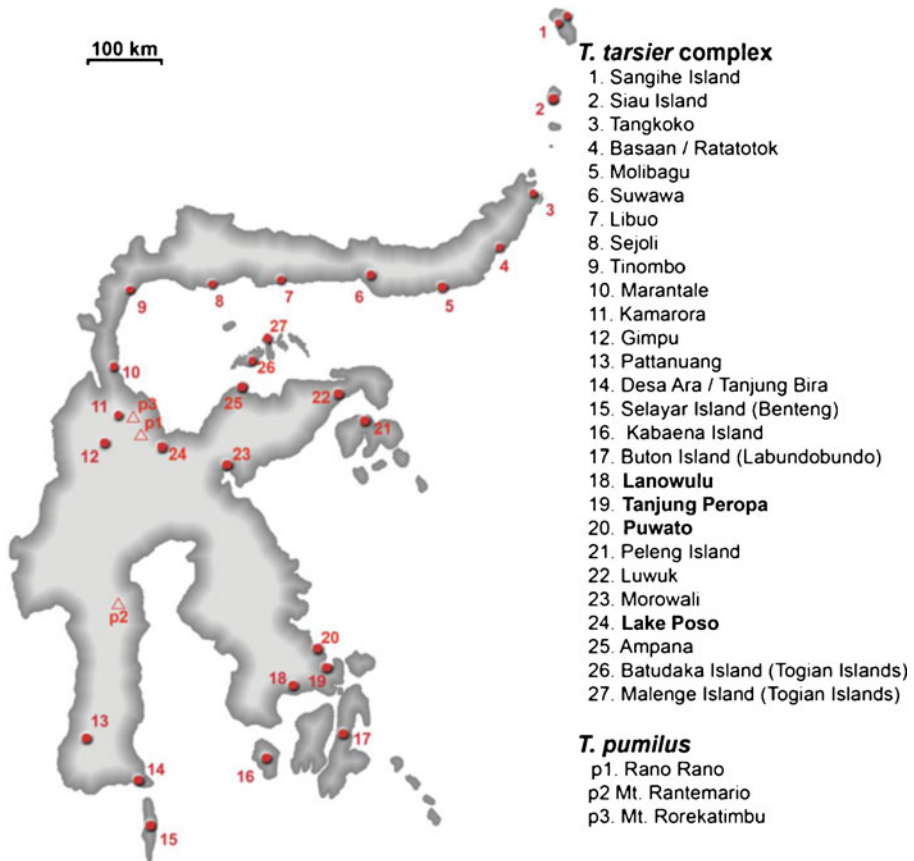


Fig. 2 Tarsier sampling localities, including all sampling points in this study and many other sampling localities from other studies.

tarsier sampling localities, including all of the sample points included in this study. Shekelle (2003) provides an in-depth discussion of the methods for collecting tarsier tissue. Our study is based on hair samples, because at the time the field work was conducted collection of ear biopsies had not yet been approved for use in studies of wild tarsiers. Prompt issuance of CITES export permits was not common when these samples were collected. Thus, we experienced significant deterioration in the amount and quality of the DNA in our samples, relative to subsequent studies that used ear biopsies (Merker *et al.* 2009). However, we kept detailed records of our genetic results, and used our experience to encourage the Indonesian Department of Forestry to issue CITES export visas for genetic samples promptly, benefiting subsequent studies.

Molecular Marker

The marker surveyed here is a partial sequence of the mitochondrial 12S ribosomal RNA gene. We chose mtDNA because of its fast mutation rate, small effective population size, and quick time to lineage fixation. These characteristics make it more likely to track the species phylogeny compared to nuclear genes, which are

generally poor at resolving short internodes (Moore 1995). We chose the 12S rRNA gene because it has been used extensively in resolving relationships within numerous mammalian groups dating back to the 1990s (McNiff and Allard 1998; Springer and Douzery 1996). Shekelle *et al.* (2008a) provide details on the methods for extraction, amplification, and DNA sequencing of the tissue samples.

Alignments

We generated 2 separate alignments for analysis. One consisted of 726 base pairs (Alignment 1), whereas the other consisted of 230 base pairs (Alignment 2) because some Eastern Tarsier samples were highly degraded and yielded shorter sequences than others. Alignment 1 is thus longer and designed to address questions regarding mitochondrial divergence dates among tarsier species. It contains numerous taxa across the primate tree and includes *Cynocephalus* as an additional outgroup. Alignment 2 is shorter but contains better sampling among the Eastern Tarsiers to address specific questions within that group. Only members of the genus *Tarsius* are included in Alignment 2, which allowed for analysis of rapidly evolving loop regions that were otherwise difficult to align with confidence.

We generated both alignments using the program R-Coffee (Moretti *et al.* 2008), which is specifically designed for multiple sequence alignment of noncoding RNA sequences. We then adjusted alignments by eye according to the structural model of mammalian 12S rRNA proposed by Springer and Douzery (1996). We used this model to check for positional covariation between sites engaged in base pairing to check for the presence of nuclear pseudogenes, although we acknowledge that this methodology is not guaranteed to detect nuclear copies of mitochondrial DNA (Olson and Yoder 2002). Finally, we excluded regions that contained missing data or that were difficult to align from all analyses, e.g., hypervariable loop regions in Alignment 1. See the [supplementary material](#) for annotated versions of the 2 alignments.

Model Choice We conducted maximum likelihood (ML) analyses in PAUP* (v4.0; Swofford 2003) and Bayesian phylogenetic analyses in BEAST (v1.5.4; Drummond and Rambaut 2007). We used the Akaike Information Criterion in jModelTest (v0.1.1) to determine that the TIM2 + I + G and TIM3 + G evolutionary models were the best fits for Alignment 1 and Alignment 2, respectively (Posada 2008). We used these in the ML analysis and the GTR + G + I evolutionary model for the Bayesian analysis because it is the closest available model in BEAST. Although the stem and loop regions of RNA genes evolve in different ways and are most appropriately modeled separately, several studies have demonstrated that the application of a single model vs. a dual model to rRNA sequence has no significant impact on phylogenetic results (Dornberg *et al.* 2008; Li *et al.* 2008; Olson *et al.* 2005). Further, BEAST currently cannot employ an appropriate model for RNA stem regions.

Phylogenetic Analyses For Alignment 1, we inferred a mitochondrial gene tree using ML methods with *Cynocephalus* set as an outgroup. We performed 100 bootstrap replicates under a heuristic search with random taxa added to the current node of the

search tree and all other parameters left as default values. For the Bayesian analysis, we designated various taxonomic groups as calibration points to infer mitochondrial divergence dates. These include Catarrhini, Hominoidea, Lorisoidea, *Pan-Homo*, *Papio-Theropithecus*, and *Cebus-Saimiri*. Table III lists the various calibration points, which were set at a normal distribution. We placed a monophyletic constraint on all Primates to the exclusion of *Cynocephalus*. We employed an uncorrelated lognormal relaxed molecular clock model with a birth-death speciation process for our analysis. We ran 100,000,000 generations, with parameters logged every 1000 generations. After discarding the first 10,000 trees from each run, we analyzed the MCMC output in TreeAnnotator (v1.5.4; Drummond and Rambaut 2007). We analyzed Alignment 2 in the same manner as Alignment 1, except the Western and Philippine Tarsiers were set as outgroups (monophyletic in the Bayesian analysis) and we employed a strict molecular clock (in BEAST). We provide the BEAST .XML files analyzed for each alignment in the [supplementary materials](#).

Results

Alignment 1

The 12S rRNA gene trees inferred from the ML and Bayesian analyses of Alignment 1 (Fig. 3) are congruent with one another, although the ML tree had more unresolved nodes. The divergence date analysis reveals an initial primate diversification between the anthropoids, strepsirhines, and tarsiers sometime between 50.4 and 85.2 Ma (see Table IV for inferred divergence dates). Crown tarsiers form a monophyletic group whose extant mitochondrial lineages began to split between 10.6 and 32.1 Ma. Within *Tarsiidae*, the Western and Philippine Tarsiers group together to the exclusion of the Eastern Tarsiers and diverged from one another between 4.8 and 18.7 Ma, and the Eastern Tarsiers form a monophyletic group

Table III Calibration points (with 95% confidence intervals) used in the divergence date analysis based on present knowledge of primate evolutionary history

Divergence	Mean (95% CI)	Fossil	Age
<i>Homo</i> – <i>Pan</i>	7.0 (6.1–8.0)	<i>Orrorin</i> ^a	6.0
<i>Cebus</i> – <i>Saimiri</i>	14.0 (12.1–15.9)	<i>Neosaimiri</i> ^b	12.5
<i>Nycticebus</i> – <i>Otolemur</i>	40.0 (33.5–43.2)	<i>Saharagalago</i> ^c	>36.9
<i>Papio</i> – <i>Theropithecus</i>	5.0 (4.2–6.0)	<i>Theropithecus</i> ^d	ca. 4.0
African ape – <i>Pongo</i>	14.0 (11.8–15.6)	<i>Sivapithecus</i> ^e	ca.12.5
Hominoid – Cercopithecoidea	26.0 (23.4–32.1)	<i>Morotopithecus</i> ^f	20.6

Dates were chosen in part so that the youngest limits of the confidence intervals approached the age of the referenced fossil taxon.

^a Senut *et al.* 2001; ^b Hartwig and Meldrum 2002; ^c Seiffert *et al.* 2003; ^d Leakey 1993; ^e Kelley 2002;

^f Young and MacLachy 2004.

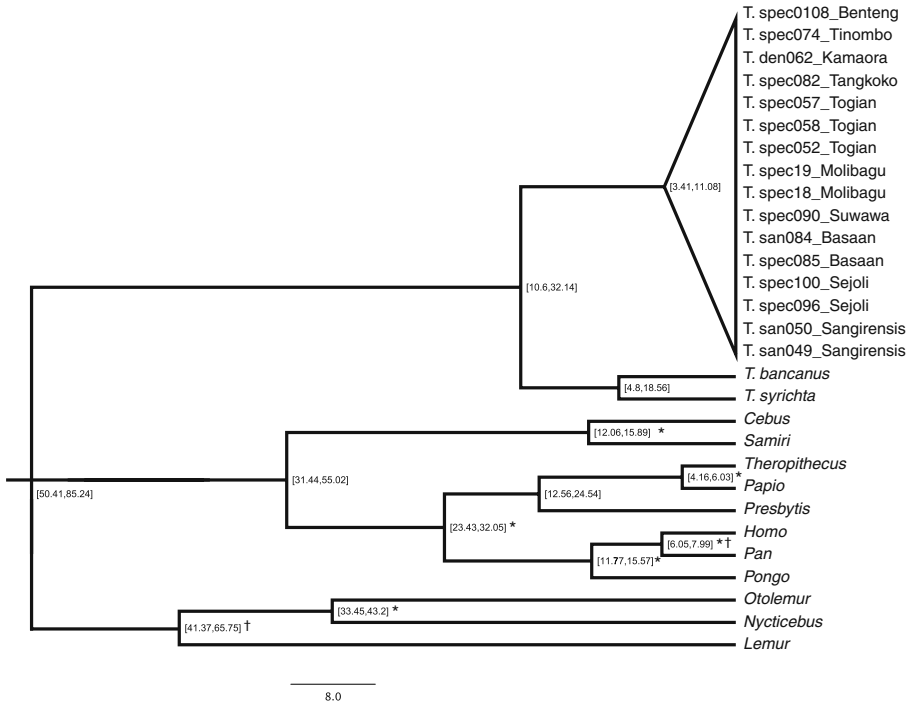


Fig. 3 Results from Alignment 1. ML and Bayesian mitochondrial gene tree and divergence dates (95% CIs shown) inferred from 726 base pairs of 12S rRNA. Asterisks (*) represent nodes that were constrained as calibration points in the analysis. All shown clades supported by bootstrap values >80 and posterior probabilities >0.90, except for clades with a cross (†), which had bootstrap values <80.

whose initial diversification of mitochondrial lineages occurred between 3.4 and 11.1 Ma. Relationships among the Eastern Tarsiers were unresolved.

Alignment 2

The Bayesian analysis of Alignment 2 recovers a monophyletic Eastern Tarsier clade, which in turn contains 5 well-supported subclades (Fig. 4). The ML analysis recovers these same nodes within the Eastern Tarsiers, but not all of them receive strong bootstrap support. These 5 clades within the *Tarsius tarsier* complex, from north to south, are as follows. Clade 1 is composed of samples from Sangihe Island, and are recognized as *Tarsius sangirensis*. Clade 2 connects the 2 northernmost sample sites

Table IV Mitochondrial divergence dates (in Ma) of *Tarsius* inferred from 726 base pairs of 12S rRNA sequence in BEAST

Divergence	Mean	Range (95% CI)
Crown tarsiers	20.4	10.6–32.1
Western Tarsier – Philippine tarsier	11.1	4.8–18.6
Crown Eastern Tarsiers	6.8	3.4–11.1

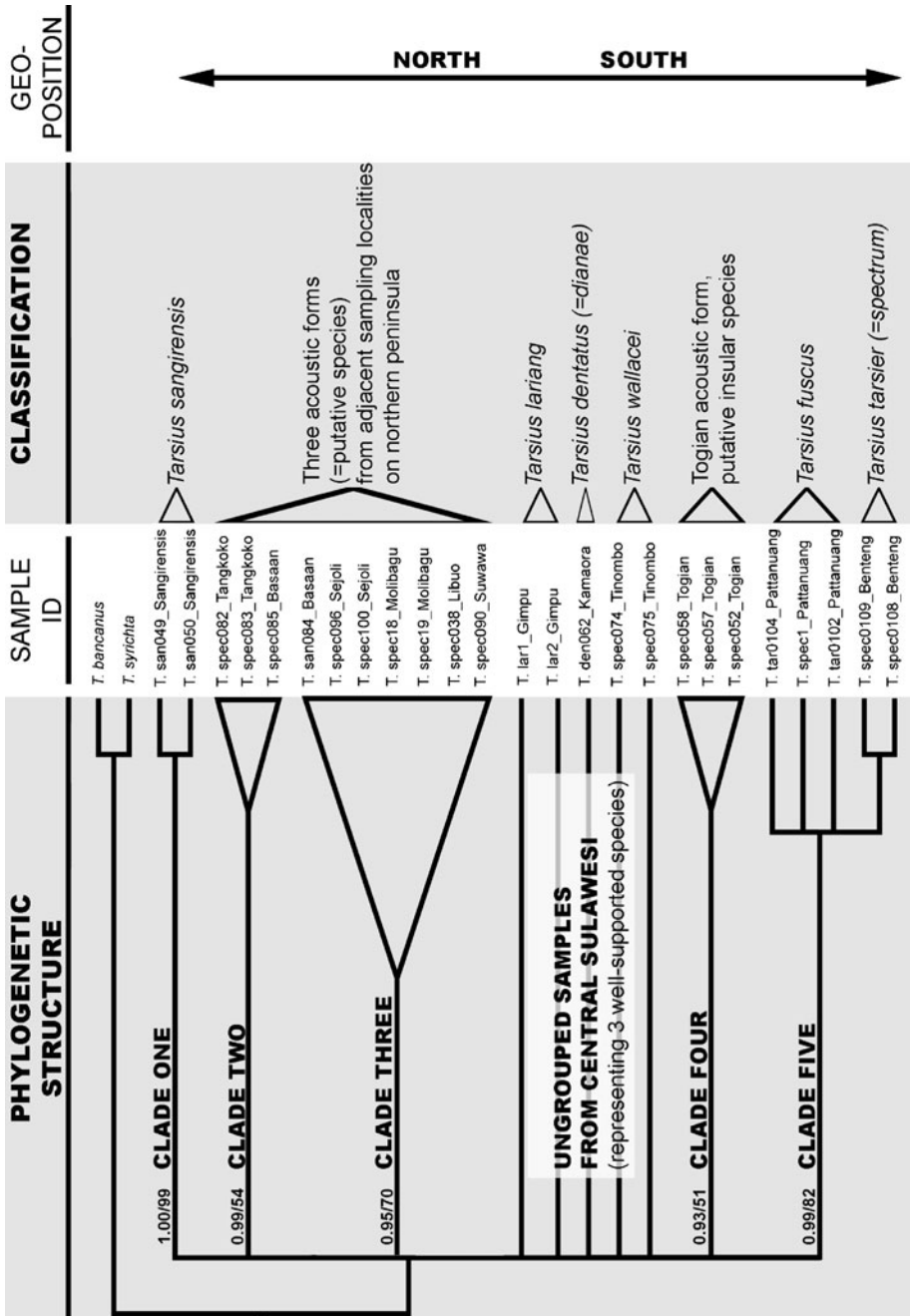


Fig. 4 Results from Alignment 2 compared with current classification. Phylogenetic structure illustrates the Bayesian mitochondrial gene tree of the tarsier clade inferred from 230 base pairs of 12S rRNA. Numbers on branches preceding clade numbering indicate posterior probability (PP) and bootstrap (BS) support (PP/BS). Geoposition refers to the spatial position of the sampling localities in this study, including islands of the extreme north, e.g., Sangihe, the Sulawesi mainland, and islands of the extreme south, e.g. Selayar.

—Tangkoko and Ratatotok— along the megatranssect sampled by Shekelle (Shekelle 2003; Shekelle *et al.* 1997, 2008a). Clade 3 connects 5 adjacent sampling localities along the northern peninsula (Ratatotok, Molibagu, Suwawa, Libuo, and Sejoli). Clade 4 contains the samples from Malenge and Batudaka Islands, both of which are located in the Togian Islands archipelago. Clade 5 includes the 2 sampling localities from the extreme southwest, Pattanuang (on Sulawesi, near Makassar) and Benteng (on Selayar Island). Five samples from the central area of Sulawesi do not fall into robust clades; these are 2 specimens from Tinombo, north of the Isthmus of Palu (recently described as *Tarsius wallacei*, Merker *et al.* 2010), 2 specimens from Gimpu (*Tarsius lariang*), and 1 specimen from Kamarora (*Tarsius dentatus*).

Discussion

By current standards, our analysis of *ca.* 700 bp of DNA sequence from the 12S gene region of the mitochondrial genome is far from state-of-the-art, and it would be unwise to draw too much inference from these results alone. Nevertheless, we identify 4 robust conclusions for further discussion. The first of these is that our data and analyses represent qualitative improvements over previous broad scale genetic sampling of tarsiers from Sulawesi. The second is that we limit our interpretations to robustly supported clades (those with a posterior probability value >0.9). Third, with these new data and analyses, we are able to make molecular clock estimates of several key events in tarsier evolution, including the relatively ancient diversification of crown tarsiers, and for each of Hill's 3 species. Finally, we focus our attention on areas where our results on Eastern Tarsiers, from admittedly limited data, intersect with similar results from complementary data sets —bioacoustics, morphology, and biogeography— thereby producing a mutually compatible and well rounded model for evolution and conservation on Sulawesi.

Our study is based on largely similar data to that by Shekelle *et al.* (2008a), but benefits from improved sampling, more objective alignment that incorporates the secondary structure of the 12S molecule, and more modern likelihood phylogenetic analyses. Our data matrix includes samples from the extreme southern limits of the range of the *Tarsius tarsier* complex, whereas the previous study focused on northern and central sites only. Thus we analyzed samples from Selayar Island, South Sulawesi, the type locality of *Tarsius tarsier* and its subjective junior synonym, *T. spectrum*, at the extreme southwestern distribution of the *T. tarsier* complex. This serves 2 purposes. 1) It provides a reasonable approximation of the haplotype of the senior taxon of this clade, which is crucial for a stable taxonomy of tarsiers. 2) Together with the previously sampled population from Sangihe Island, our data set now provides representation for the extremes of the northeast-southwest limits of the range of Eastern Tarsiers.

Chronometric Analysis: The Age of Crown Tarsiers and Tarsier Species Groups

We offer the first molecular clock estimate for crown tarsiers, 20.6 Ma (10.6–32.1). The expression “closely related” is relative, but crown tarsiers are less closely related

than are, say, orangutans, gorillas, and chimpanzees. In all likelihood, crown tarsiers diversified by the middle Miocene, and possibly as early as the early Oligocene. The inferred diversification of crown tarsiers is doubly interesting because it also dates the origins of stem Eastern Tarsiers. The 95% confidence interval from our estimated timing of this event, i.e., 10.6–32.1 Ma, is consistent with several other lines of related evidence. Based on geological reconstructions of the movement of tectonic plates during the Cenozoic, Hall (2001) found the earliest evidence of emergent land in Sulawesi to be at *ca.* 20 Ma, which was most likely a small volcanic island that later became part of the northern arm. Hall further predicted that the most likely time for faunal exchange between Asia and Sulawesi occurred *ca.* 10 Ma, hopping over relatively narrow ocean straits along landmasses connecting Sulawesi with Thailand via Java. Morley (1998) found palynological evidence of biotic exchange across the Makassar Straits at several time periods, 2 of which fall within the 95% confidence interval, i.e., 17 and 14 Ma, and a third, 9.5 Ma, nearly does. Interestingly, our mean date for the origins of Eastern Tarsiers, 20.3 Ma, is curiously similar to the 20 Ma date reported by Hall (2001) for the earliest emergent land within the proto-Sulawesi archipelago. Thus, our results indicate that 1) stem Eastern Tarsiers are a far older radiation than are Sulawesi's macaques, as predicted by Shekelle and Leksono (2004); 2) they are most likely to be ≥ 10 Ma, as predicted by Shekelle *et al.* (2008a); and 3) they could be as old as 32.3 Ma, a date near the Eocene–Oligocene boundary, and thus, far older than anyone has ever speculated for this group.

We were also able to date the split of Western Tarsiers and Philippine tarsiers, 11.1 (4.8–18.6) Ma, as well as the age of crown Eastern Tarsiers, 6.8 (3.4–11.1) Ma. The former estimate concords with the molecular clock estimate of 5.6 Ma arrived at by Meireles *et al.* (2003) based upon nDNA, but is younger than the dates of Matsui *et al.* (2009). The latter estimate indicates that Eastern Tarsiers began speciating at least 3.4 Ma and possibly as long ago as 11.1 Ma. These relatively ancient dates add crucial support for current hypotheses of numerous cryptic taxa among the Eastern Tarsiers by verifying that the clade itself is quite old and began speciating as far back as the Pliocene, or as early as the middle Miocene (Brandon-Jones *et al.* 2004). Thus, our results show that each of the 3 tarsier species groups—*Tarsius tarsier*, *T. bancanus*, and *T. syrichta* (Eastern, Western, and Philippine Tarsiers, respectively)—originated, in all likelihood, as far back as the Miocene, with the Eastern Tarsiers being the oldest of these 3 clades. Given our tremendous ignorance of the alpha taxonomy of tarsiers, any or all of Hill's 3 tarsier species could conceivably be a complex of cryptic taxa (Brandon-Jones *et al.* 2004).

Relationships and mitochondrial divergence dates within the anthropoids and strepsirhines are consistent with most previous phylogenetic studies and the fossil record.

Phylogenetic Analysis and Taxonomic Diversity Within the *Tarsius tarsier* complex

This is the first molecular study to verify a sister-taxon relationship between the Western and Philippine Tarsiers to the exclusion of the Eastern Tarsiers, a topology supported by previous morphometric studies of museum specimens (Groves 1998; Musser and Dagosto 1987). The monophyly of Eastern Tarsiers is also supported (Groves 1998; Shekelle 2008a), although the rarity of *Tarsius pumilus*, the pygmy or

mountain tarsier, precluded its inclusion in our analyses, and thus we could not test the hypothesis that it is basal to all other extant tarsiers (Shekelle 2008b). Meanwhile, the 5 subclades of Eastern Tarsiers fit an intuitively satisfying biogeographic pattern, wherein our genetic data appear to separate populations that are at, or above, the species level. We do not argue that our genetic data diagnose species, but note the concordance between our data and current classification (Fig. 4).

The robust support for clade 1 (Sangihe Island) is not surprising. Separation of the Sangihe Island tarsiers at the species level has been supported by numerous studies, including morphometric studies of museum specimens (Feiler 1990; Groves 1998), field studies of morphology and bioacoustics (Shekelle *et al.* 1997), and genetics (Shekelle *et al.* 2008a). Biogeographically these results are to be expected, as Sangihe Island is part of a volcanic arc, where the islands of the chain formed from the ocean floor. Further, the ocean floor in this area is very deep, greatly exceeding 1000 m between islands in most places, and the islands themselves are well spaced, with distances of ≥ 20 –40 km, typically, between island clusters (Shekelle and Salim 2009). Thus, it is expected that all native species on each of the islands in this chain arrived as immigrants that managed to cross the ocean, and endemism among animals that do not fly or swim is expected to be virtually 100%. Human-mitigated dispersals would be obvious exceptions.

Clades 2 and 3 link the 6 northernmost sample points on Sulawesi in what is hypothetically a cluster of 3 tarsier taxa spread across an area with 4 hypothesized regions of endemism (Shekelle and Leksono 2004). Included in this clade are 3 tarsier acoustic forms: Manado, Libuo, and Sejoli (Shekelle 2008c). Shekelle (2008c) remarked that the latter 2 of these are remarkably similar in spectrographic analysis, and are separable chiefly by field playback tests, while Alexandra Nietsch (*pers. comm.*) noted broad similarities between the Manado and the Libuo forms. Thus all 3 acoustic forms in these 2 subclades are noted for their shared similarities. Within this region are 3 known faunal boundaries. The northernmost of these is the boundary between *Macaca nigra* and *M. nigrescens*. Evans *et al.* (2003) found strong support for a recent split at this boundary using fine-scale genetic mapping of *Macaca* and the sympatric toad, *Bufo celebensis*. There is no known break in tarsier genetics, bioacoustics, or morphology that is associated with this macaque boundary, although, interestingly, our study finds paraphyly at the site Basaan/Ratatotok, which lies just north of the break found by Evans *et al.* The second faunal boundary is the well identified break at the Isthmus of Gorontalo. This is the barrier between *Macaca nigrescens* and *M. hecki*. As with the barrier between *Macaca nigra* and *M. nigrescens*, Evans *et al.* (2003) found a strongly supported genetic break in *Macaca* and *Bufo* at this point. The third faunal boundary is between the Libuo and Sejoli acoustic forms (Shekelle 2008c), a boundary that is hypothesized to correspond with a suture between 2 ophiolitic microplates identified by Hall (2001). Thus, our evidence indicates that this northern clade of tarsiers colonized the microplate on which the Sejoli form occupies (sometime before Sulawesi's colonization by macaques), was then split by the same vicariance event that split the macaques at Gorontalo, but remained less affected by the vicariance event that further split the northernmost macaques into *Macaca nigra* and *M. nigrescens*.

Clade 4 is composed of samples collected from 2 islands within the Togian Island archipelago, in Tomini Bay. Tomini Bay itself is not exceedingly deep, unlike the waters that surround Sulawesi, but it is deep enough that the Togian Islands appear to have remained geographically isolated from Sulawesi during the latter part of the Pleistocene, as evidenced by the lack of endemic macaques, which themselves are hypothesized to be a Pleistocene immigrant to Sulawesi (Evans *et al.* 2003). There is, in fact, a macaque population on Malenge Island, and for a while during the 1990s it was thought that this might be endemic, but it was ultimately shown to be a well documented human-mitigated dispersal (Evans *et al.* 2003; Lowe 2004).

Clade 5 unites 2 sample points from the extreme southwest, Pattanuang, on Sulawesi, and Benteng, on Selayar. Evidence of the geological history of Sulawesi indicates that the northern and southern parts of the island remained separate well into the Pleistocene (Hall 2001). Groves (2001) found morphological evidence in multiple mammalian taxa for separation between northern and southern populations. Thus it is not surprising that these 2 tarsier populations are genetically quite distinct from other Eastern Tarsiers. What is surprising, however, is that our analysis is unable to separate these 2 populations into robustly supported clades, Sulawesi mainland and Selayar Island, unlike the other 2 island populations in our analysis: Sangihe and Togian. Groves (1998) identified the Selayar population as a putative new taxon based on its distinctiveness in his survey of morphometric variation among museum specimens. Similarly, the bioacoustic distinctiveness of the Selayar tarsiers' duet call is addressed by Nietsch and Burton (2010). Selayar Island does not have an endemic population of *Macaca*, thus indicating the unlikelihood of any recent land connections between Sulawesi and Selayar. By inference, the population of tarsiers on Selayar is expected to be comparatively ancient. Therefore, it seems that the most likely explanation for the distinctive morphology and bioacoustics, along with the self-evident geographical isolation, is that our genetic data are not fine enough to identify some species-level clades, and this is one such example of that trend.

Finally, the central localities of Tinombo, Kamarora, and Gimpu, remained ungrouped in our study. These three populations, however, comprise three species that are well separated by genetics, bioacoustics, and morphology, and their distributions conform to the hypothesis that tarsier speciation arose as a result of dispersal throughout a proto-Sulawesi archipelago, followed by subsequent range fragmentation (Merker *et al.* 2009; Merker *et al.* 2010). Indeed, the parapatric boundary between *Tarsius dentatus* and *T. lariang* is the best studied boundary between tarsier species, with an estimated divergence of 1.4 Ma between the 2 populations. Thus, we again conclude that the most likely explanation for the well defined species boundaries between these forms, and the lack of our genetic data to resolve them into robustly supported clades, is that our data are not fine enough for that purpose and, rather, are identifying clades at, or above, the species level. Again, we do not argue that our limited genetic data are strong enough to diagnose species by themselves.

Implications for Sulawesi Biogeography, Mode of Speciation, and Conservation

Our data are consistent with the hypotheses of Evans *et al.* (2003) and Shekelle and Leksono (2004) that Sulawesi is subdivided into numerous regions of endemism,

that multiple taxa share these regions because of a shared history of isolation on separate islands of the proto-Sulawesi archipelago followed by Pleistocene range fragmentation, and these facts greatly complicate conservation on Sulawesi. The caveats of Bridle *et al.* (2004) are well taken, notably that our study does not assess adaptive evolution as a method of diversification. However, no adaptive hypothesis has yet been postulated for the diversification of Sulawesi's lowland tarsiers, although such hypotheses are possible. Lowland Eastern Tarsiers vary most notably in their duet call (MacKinnon and MacKinnon 1980; Nietsch 1999; Nietsch and Kopp 1998; Nietsch and Niemitz 1993; Nietsch and Burton 2010) and are otherwise taxonomically cryptic, with morphological variation typically noted only after bioacoustics and genetics have assisted with their classification. Thus, some minor morphological differences have been demonstrated in multivariate morphometrics (Groves 1998), ear width (Merker and Groves 2006), tail tuft (Shekelle *et al.* 2008c), and pelage coloration (Shekelle *et al.* 2008b), but researchers have not yet hypothesized any of these to offer an adaptive advantage. In contrast, the biogeographic hypothesis, based on tarsier acoustics, has been tested and supported with multiple nDNA and mtDNA markers along a limited transect between 2 putative species (Merker *et al.* 2009). We strongly suspect future analyses will find similar results for most or all of the other acoustic forms within the *Tarsius tarsier* complex. Nevertheless, our interpretations for the larger population of Eastern Tarsiers should be considered as speculative until nDNA markers have been analyzed. Similarly, we remain cautious about the interpretations of Bridle *et al.* of *Chitaura* biogeography and evolution, until those, too, have been further examined with nDNA markers.

Regarding conservation, again, we accept the caveats of Bridle *et al.* (2004), but note further that conservation policy is based on best available evidence and is unlike science, which is based on refutation of null, or alternative, hypotheses. Thus, though the model of Evans *et al.*, or any other model, might be an oversimplification, models are often shown to be oversimplifications by subsequent works, for example, Shekelle and Leksono (2004) indicate that the conservation model of Evans *et al.* (2003) omits numerous regions of endemism, and though oversimplification might be costly, we nevertheless argue that a far more costly oversimplification would be to assume that Sulawesi is composed of a single biogeographic region. We see science as a self-correcting discipline wherein, as conservation practitioners, we are required to make suggestions based on the best available evidence. We argue that the biogeographic model by Shekelle and Leksono currently offers the best available model for how to conserve the biodiversity on Sulawesi under ideal situations, though no doubt it will be improved upon in the future. In a perfect world, we would argue for blocks of habitat within each region of endemism, of each primary habitat type, to be conserved, possibly as protected areas. However, when theory meets reality, conservation strategies do not always work as planned, as noted by Bickford *et al.* (2007) in their analysis of Indonesian protected areas. Some conservationists privately voice concerns that, given the current sorry state of affairs within Sulawesi's protected areas, salvaging even 1 protected area might be a great deal to ask. We do not want to succumb to this level of pessimism, but acknowledge that the plan of Shekelle and Leksono is based on expectations for Indonesian protected areas that are at present unrealistic. The key for Sulawesi conservation,

therefore, will be to balance the increasingly taxing policy demands that result from scientific evidence, which is itself increasingly accurate and increasingly complex in its estimation of the distribution of biodiversity, with the humble realities of conservation capabilities in the region. However that balance is weighed, we argue for the increased urgency of surveying biological inventories, with the knowledge that the opportunity to do so is rapidly being lost for some taxa, and that in all likelihood some species will go extinct before they have even been identified by science.

Conclusion

Our phylogeny of tarsiers finds a robust clade uniting *Tarsius bancanus* and *T. syrichta* to the exclusion of *T. tarsier*, as predicted by morphometric studies. Further, we found 5 well supported subclades within *Tarsius tarsier*, supporting the hypothesis that this population is a species complex. By comparison with other studies, we interpret those subclades to identify haplogroups at, or above, the species level. Thus our findings are concordant with current taxonomy, which recognizes 7–9 species within the *Tarsius tarsier* complex, with the expectation that several more taxa remain undescribed.

The diversification of crown tarsiers is dated to 10.6–32.1 Ma, with a mean of 20.4 Ma. Thus, extant tarsiers are not closely related species, as was long assumed. We estimate that crown Eastern Tarsiers began to speciate sometime between 3.4 and 11.1 Ma, with a mean of 6.8 Ma, a length of time that could in theory produce a relatively speciose complex of cryptic taxa. The origins of stem Western Tarsiers and stem Philippine tarsiers are estimated to be 4.8–18.6 Ma, with a mean of 11.1 Ma. We have no evidence of genetic variability within either of these taxa, but we note that either is easily old enough to be a complex of cryptic species (Brandon-Jones *et al.* 2004). Phylogeographic studies of these taxa, along with additional studies to further understand the alpha taxonomy of Eastern Tarsiers, should be a priority. In the meantime, tarsier classifications should highlight our ignorance of diversity among tarsiers, and the rubric of using Eastern, Western, and Philippine Tarsier as a convenient shorthand for Hill's three species can be continued in light of our inadequate representation of tarsier alpha taxonomy. Alternatively, Groves and Shekelle (2010) propose elevating each of Hill's species to generic rank, with Eastern, Western, and Philippine Tarsiers classified as *Tarsius*, *Cephalopachus*, and *Carlito*, respectively; if this classification were generally accepted, the need for the former common names would decline.

There is increasing evidence that Sulawesi is subdivided into numerous regions of endemism. These regions are not merely products of Pleistocene vicariance, such as postulated in the model by Evans *et al.* (2003), but also the result of dispersal throughout the proto-Sulawesi archipelago, events that date as far back as the Miocene, before the formation of the modern island of Sulawesi. The interaction of these events has the potential to have produced ≥ 14 regions of endemism on Sulawesi, itself, and more on offshore islands. Future studies should include investigations of the effects of adaptive evolution on diversification, and not merely focus on isolation.

The implications of our results are that a conservation crisis is in process on Sulawesi and surrounding islands. If the example of tarsiers can be reasonably extrapolated to other taxa, then biodiversity may be underestimated by an order of magnitude. Effective measures to conserve each parapatric and allopatric taxon might require conservation areas in ≥ 14 regions of endemism on Sulawesi, alone, not to mention numerous offshore islands. The practicalities of conservation in the region are such that some conservationists despair in our ability to manage even one conservation area effectively, let alone 14. The extirpation of Sulawesi's amazing and unique biota is in progress, and it seems likely that some species, even charismatic ones like tarsiers, will go extinct before they are ever identified by science. Even if we are unable to slow this loss, we should make biological surveys of this area a great priority, lest we lose the chance to know what once was there.

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