



## Molecular phylogeny of treeshrews (Mammalia: Scandentia) and the timescale of diversification in Southeast Asia

Trina E. Roberts<sup>a,b,\*</sup>, Hayley C. Lanier<sup>a,c</sup>, Eric J. Sargis<sup>d,e</sup>, Link E. Olson<sup>a,f</sup>

<sup>a</sup> University of Alaska Museum, University of Alaska Fairbanks, 907 Yukon Dr., Fairbanks, AK 99775, USA

<sup>b</sup> National Evolutionary Synthesis Center, 2024 W. Main St., Suite A200, Durham, NC 27705, USA

<sup>c</sup> Department of Ecology and Evolutionary Biology, Museum of Zoology, University of Michigan, 1109 Geddes Ave., Ann Arbor, MI 48109, USA

<sup>d</sup> Department of Anthropology, Yale University, P.O. Box 208277, New Haven, CT 06520, USA

<sup>e</sup> Divisions of Vertebrate Zoology and Vertebrate Paleontology, Yale Peabody Museum of Natural History, P.O. Box 208118, New Haven, CT 06520, USA

<sup>f</sup> Institute of Arctic Biology, University of Alaska Fairbanks, 902 N. Koyukuk Dr., Fairbanks, AK 99775, USA

### ARTICLE INFO

#### Article history:

Received 8 December 2010

Revised 11 April 2011

Accepted 26 April 2011

Available online 1 May 2011

#### Keywords:

Scandentia  
Tupaiaidae  
Southeast Asia  
Borneo  
Miocene  
Treeshrews

### ABSTRACT

Resolving the phylogeny of treeshrews (Order Scandentia) has historically proven difficult, in large part because of access to specimens and samples from critical taxa. We used “antique” DNA methods with non-destructive sampling of museum specimens to complete taxon sampling for the 20 currently recognized treeshrew species and to estimate their phylogeny and divergence times. Most divergence among extant species is estimated to have taken place within the past 20 million years, with deeper divergences between the two families (Ptilocercidae and Tupaiaidae) and between *Dendrogale* and all other genera within Tupaiaidae. All but one of the divergences between currently recognized species had occurred by 4 Mya, suggesting that Miocene tectonics, volcanism, and geographic instability drove treeshrew diversification. These geologic processes may be associated with an increase in net diversification rate in the early Miocene. Most evolutionary relationships appear consistent with island-hopping or landbridge colonization between contiguous geographic areas, although there are exceptions in which extinction may play an important part. The single recent divergence is between *Tupaia palawanensis* and *Tupaia moellendorffi*, both endemic to the Philippines, and may be due to Pleistocene sea level fluctuations and post-landbridge isolation in allopatry. We provide a time-calibrated phylogenetic framework for answering evolutionary questions about treeshrews and about evolutionary patterns and processes in Euarchonta. We also propose subsuming the monotypic genus *Urogale*, a Philippine endemic, into *Tupaia*, thereby reducing the number of extant treeshrew genera from five to four.

© 2011 Elsevier Inc. All rights reserved.

### 1. Introduction

With modern methods of phylogenetic inference and divergence date estimation, biologists can form a better picture of the past than ever before. Major advances in technology have allowed the use of historical museum specimens, often collected before the advent of DNA sequencing, in genetic studies. For taxonomic groups or geographic regions for which representative samples are particularly hard to obtain, such “antique” (as opposed to truly ancient) DNA from historical collections can provide an incomparable source of evolutionary information.

An understanding of evolutionary relationships within the order Scandentia (treeshrews) has lagged behind many other

groups, despite the relatively small number of currently recognized species. No systematic study has ever included all recognized treeshrew species, both because of the group’s unstable taxonomy and because of the inaccessibility of specimens and/or samples suitable for DNA extraction. This has made it impossible to get even a moderately complete picture of morphological or biogeographic evolution in the group. In 2005, Olson et al. published a molecular phylogeny including 16 of the 20 recognized species in the order, based on the mitochondrial 12S rRNA gene. Though some relationships were well supported in their phylogeny, others were unclear, and some crucial questions depended on taxa for which no fresh samples were available. Roberts et al. (2009) analyzed six nuclear genes for a subset of taxa, but pointed out that missing taxa could affect hypothesized relationships. We build on these previous results by adding both additional mitochondrial DNA and additional taxa, in some cases using “antique” DNA from museum specimens, to present the first molecular phylogeny including all currently recognized treeshrew species. A single molecular phylogeny is no substitute for a thorough revision of the order using multiple data

\* Corresponding author address: National Evolutionary Synthesis Center, Duke Box 1044003, 2024 W. Main St., Suite A200, Durham, NC 27705, USA. Fax: +1 919 668 9198.

E-mail addresses: [trina.roberts@nescent.org](mailto:trina.roberts@nescent.org) (T.E. Roberts), [hclanier@umich.edu](mailto:hclanier@umich.edu) (H.C. Lanier), [eric.sargis@yale.edu](mailto:eric.sargis@yale.edu) (E.J. Sargis), [link.olson@alaska.edu](mailto:link.olson@alaska.edu) (L.E. Olson).

sources, but it adds substantial resolution to the consistent picture of treeshrew systematics that has begun to emerge and provides a strong framework for additional evolutionary inference.

### 1.1. Treeshrew biology and taxonomy

Treeshrews are small-bodied insectivores varying from truly terrestrial to arboreal and distributed throughout much of south-central and Southeast Asia. They comprise a single order, Scandentia, in which two families are currently recognized (Helgen, 2005): Ptilocercidae, containing one species, *Ptilocercus lowii*, and Tupaiidae, containing 19 species in four genera (*Dendrogale*, *Anathana*, *Urogale*, and *Tupaia*). The last of these, *Tupaia*, contains most of the extant diversity, with 15 of the 20 currently recognized species. *Dendrogale*, with two, has long been acknowledged as the sister group to the rest of the Tupaiidae, and recent phylogenetic studies have confirmed this (Olson et al., 2005; Roberts et al., 2009). Relationships among *Urogale*, *Anathana*, and *Tupaia* have proven much more difficult to resolve with either morphological or molecular data, as have some relationships within *Tupaia* (Butler, 1980; Han et al., 2000; Luckett, 1980; Olson et al., 2004, 2005; Roberts et al., 2009; Steele, 1973). Several authors, using molecular or morphological data, have suggested that *Tupaia* is not monophyletic with respect to *Urogale* (Han et al., 2000; Olson et al., 2005; Roberts et al., 2009; Steele, 1973). All studies have been hampered by incomplete taxon sampling, a problem further complicated by historical shifts in specific and subspecific taxonomy and the fact that nearly a century has passed since the last comprehensive taxonomic revision of Scandentia (Lyon, 1913).

Several recent studies have advanced knowledge of treeshrew ecology, behavior, and natural history (e.g., Clarke et al., 2009; Emmons, 2000; Kwartalnov, 2009; Munshi-South, 2008; Nakagawa et al., 2007; Oommen and Shanker, 2010; Schehka and Zimmermann, 2009; Timmins et al., 2003; Wells et al., 2004; Wiens et al., 2008), though detailed data are still lacking for most species. In addition, treeshrews have become an important comparative biomedical model system, and a great deal is known about some aspects of the biology, anatomy, and development of the species most often held in captivity (Bahr et al., 2003; Kobayashi and Wanichanon, 1992; Schmidt and Schilling, 2007; Vinyard et al., 2008; Vinyard et al., 2005; von Weizsacker et al., 2004). Unfortunately, a complete phylogenetic framework for understanding or combining any of this detailed knowledge has never been available. Understanding behavioral and morphological evolution within treeshrews has broader implications as well (Sargis, 2001a, 2001b, 2002a, 2002b, 2004); higher-level studies of other euarchontans, including primates, have tended to include single treeshrew species—typically *Tupaia belangeri*, *Tupaia glis*, or *Tupaia tana*—as representative of the entire order, an assumption that may not be justified and that may bias results (see Olson et al., 2005; Sargis, 2002a,b).

### 1.2. Southeast Asian biogeography

The evolution of treeshrews is inextricably tied to the geography of Southeast Asia, a region whose fauna is still remarkably poorly known despite the attention it has drawn since Wallace's study of the Malay Archipelago. The order's geographic distribution extends from India and China to central Indonesia and the Philippines, but the ranges of individual species vary greatly from widespread to extreme local endemism (Table 1 and Fig. 1). The monotypic genus *Anathana* is endemic to India, while *Urogale*, the only treeshrew east of Huxley's Line (Huxley, 1868), is found on three islands in the southern part of the oceanic Philippines. The remainder of the order's extant diversity is in

mainland Southeast Asia, from China to Malaysia, and through the Malaysian and Indonesian islands of the Sunda region, including Borneo, Sumatra, and Java. Borneo has the highest species-level diversity of any single island or region, and contains both endemic species (*Tupaia picta*, *Tupaia montana*, *Tupaia dorsalis*, *Tupaia longipes*, *Tupaia melanura*), species found on Borneo and close offshore islands (*Tupaia gracilis*, *Tupaia splendida*), and species with a broader distribution including Borneo (*Tupaia minor*, *T. tana*, *P. lowii*). Among non-Bornean species, *Tupaia chrysogaster* (Mentawai Islands), *Tupaia nicobarica* (Great and Little Nicobar Islands), *Tupaia palawanensis* (Palawan and Balabac Islands), *Tupaia moellendorffi* (Busuanga, Cuyo, and Culion Islands), and *Urogale everetti* (Mindanao, Siargao, and Dinagat Islands) are all restricted to one or a few islands in a limited geographic area. In contrast to these restricted distributions, *Tupaia javanica* is relatively widespread on the islands of the Sunda Shelf, *T. glis* is found from the Isthmus of Kra southward through Sumatra and Java, and *T. belangeri* is found from the Isthmus of Kra northward through mainland Southeast Asia, from Malaysia to Vietnam, China, and India. The distribution of extant species does not include the entire historical extent of the group; although the fossil record for treeshrews is extremely limited (reviewed in Sargis, 2004), fossil forms from India and Pakistan show that the range formerly extended farther west.

In the Holocene and Pleistocene, species diversity patterns in Southeast Asia have been largely attributed to episodic changes in sea level, which exposed landbridges and allowed contact between previously isolated populations or species. Some or all of the Sunda Shelf islands, including Sumatra, Java, Bali, Borneo, and many smaller islands, were probably connected to each other and to mainland Southeast Asia by intermittent landbridges for more than half of the last 250,000 years (Voris, 2000), and during other episodes of low sea level, which have occurred intermittently since the Oligocene (Miller et al., 2005). Such landbridge connections may have partially determined the distribution of many treeshrews. While most species are found on islands, most of those islands have at times been connected to other islands or continents. The exceptions are the islands inhabited by *T. nicobarica*, *U. everetti*, and possibly *T. palawanensis* and *T. moellendorffi* (the Palawan island group may have had a previous landbridge connection to Borneo and the Sunda Shelf). Even in these species, past landbridges have connected the islands within each island group (for example, a landbridge connection between Great Nicobar and Little Nicobar Islands connected them to each other even though they have never been connected to a larger land mass). Repeated landbridge connections can provide dispersal routes for taxa that otherwise rarely disperse over water, and can result in permanent changes in distribution. Thus, most treeshrew species distributions can be explained without invoking definite overwater colonization, which is presumably rare in this group (see Olson et al., 2005). Southeast Asia has been shaped by tectonic and volcanic activity, with many islands emerging and reaching their current position, height, and complexity in the Miocene or Pliocene (Hall, 1996, 1998, 2001; Moss and Wilson, 1998). All of these changes may have been associated with environmental alterations, including both temperature and precipitation, and the distribution of habitat has varied greatly over any reasonable evolutionary timescale (Meijaard, 2003, 2004). In addition, periods of high sea level, which have also occurred intermittently (Miller et al., 2005), may have caused inundations in low areas and isolated populations on temporary islands. Historical biogeographic research into several other Southeast Asian taxa has suggested that current species distributions and evolutionary patterns are tied to both Quaternary sea level change and Tertiary geology (e.g., Gorog et al., 2004; Inger and Voris, 2001; Roberts, 2006; Steppan et al., 2003).

**Table 1**  
Species, specimen, and sequence information.

	Species range (Fig. 1)	Voucher <sup>A,B</sup>	General locality	12S: 1002 (840) bp <sup>C</sup>	tRNA-Val: 73 (57) bp <sup>C</sup>	16S: 1644 (1243) bp <sup>C</sup>	% Complete
<i>Ptilocercus lowii</i>	S Thailand, Malay Peninsula, Singapore, Sumatra, Borneo, offshore islands (1A)	FMNH 76855 <sup>a</sup> (1950)	Malaysia, Sabah (Borneo)	867 (771) <sup>D</sup>	0 (0)	943 (780)	73
		USNM 488072 <sup>b</sup> (1971)	Malaysia, Selangor	1002 (840)	34 (25)	0 (0)	40
<i>Dendrogale melanura</i>	Northern Borneo at elevations >900 m (1E)	USNM 292552 (1951)	Malaysia, Sabah (Borneo)	1002 (840) <sup>D</sup>	34 (25)	1378 (1103)	92
<i>Dendrogale murina</i>	Mainland SE Asia (Indochina) (1C)	UAM 103000 <sup>a</sup>	Cambodia, Mondulokiri	1002 (840)	73 (57)	1644 (1243)	100
		UAM 102608 <sup>b</sup>	Cambodia, Koh Kong (Cardamom Mts.)	1002 (840)	73 (57)	1644 (1243)	100
<i>Anathana ellioti</i>	India, S of Ganges R (1A)	FMNH 91265 (1958)	India, Madhya Pradesh	1002 (840) <sup>D</sup>	73 (57)	1112 (824)	80
<i>Tupaia belangeri</i>	Southeast Asia N of Kra, and offshore islands (1C)	FMNH 165412 <sup>a</sup>	None (zoo animal)	1002 (840) <sup>D</sup>	73 (57)	1644 (1243)	100
		MVZ 186408 <sup>b</sup>	Vietnam, Vinh Phu	1002 (840) <sup>D</sup>	73 (57)	1644 (1243)	100
		USNM 583857 <sup>c</sup>	Myanmar, Mon	1002 (840) <sup>D</sup>	73 (57)	1644 (1243)	100
		UAM 102606 <sup>d</sup>	Cambodia	1002 (840)	73 (57)	1644 (1243)	100
		UAM 102607 <sup>e</sup>	Cambodia	884 (776)	73 (57)	1644 (1243)	97
		MVZ 186407 <sup>f</sup>	Vietnam, Vinh Phu	1002 (840)	73 (57)	1644 (1243)	100
		USNM 583793 <sup>g</sup> GB: NC_002521 <sup>h</sup>	Myanmar, Bago	1002 (840)	73 (57)	1644 (1243)	100
<i>Tupaia chrysogaster</i>	Mentawai Islands (1G)	USNM 121577 (1902)	Indonesia, Sumatera Barat (Pagai Selatan)	1002 (840)	73 (57)	1644 (1243)	100
		UMMZ 174427 <sup>a</sup>	Indonesia, Kalimantan Barat (Borneo)	1002 (840) <sup>D</sup>	73 (57)	1644 (1243)	100
<i>Tupaia dorsalis</i>	Borneo at <1000 m (1E)	UMMZ 174651 <sup>b</sup>	Indonesia, Kalimantan Barat (Borneo)	1002 (840)	73 (57)	1644 (1243)	100
		MVZ 192180 <sup>a</sup>	Indonesia, Sumatera Utara (Sumatra)	1002 (840) <sup>D</sup>	73 (57)	1644 (1243)	100
<i>Tupaia glis</i>	Malay Peninsula S of Kra; Sumatra, Java; many offshore islands (1B)	MVZ 192184 <sup>b</sup>	Indonesia, Aceh (Sumatra)	1002 (840) <sup>D</sup>	73 (57)	1644 (1243)	100
		USNZ 109023	Malaysia, Sabah (Borneo)	1002 (840) <sup>D</sup>	73 (57)	1644 (1243)	100
<i>Tupaia gracilis</i>	Borneo and its offshore islands at <1200 m (1F)	FMNH 47118 (1928)	Indonesia, Jawa Tengah (Java)	1002 (840) <sup>D</sup>	73 (57)	1526 (1179)	97
<i>Tupaia javanica</i>	Bali, Java, W Sumatra, Nias (1B)	JS M02b	Malaysia, Sabah (Borneo)	1002 (840) <sup>D</sup>	73 (57)	1644 (1243)	100
<i>Tupaia longipes</i>	Borneo (1F)	USNZ 109751 <sup>a</sup>		1002 (840)	73 (57)	1644 (1243)	100
<i>Tupaia minor</i>	S peninsular Thailand, Malay Peninsula, Sumatra, offshore islands, Borneo (1B)	USNZ 109988 <sup>b</sup>		1002 (840)	73 (57)	1644 (1243)	100
		USNM 477838 (1962)	Philippines, Palawan (Culion)	1002 (840)	73 (57)	1644 (1243)	100
<i>Tupaia montana</i>	Borneo; montane (1F)	USNM 449964 (1989)	Malaysia, Sabah (Borneo)	1002 (840)	73 (57)	1644 (1243)	100
<i>Tupaia nicobarica</i>	Nicobar Islands (1H)	USNM 111753 (1901)	India, Little Nicobar Island	1002 (840) <sup>D</sup>	73 (57)	1644 (1243)	100
<i>Tupaia palawanensis</i>	Palawan, Balabac (1D)	FMNH 168969	Philippines, Palawan (Palawan)	1002 (840) <sup>D</sup>	73 (57)	1644 (1243)	100
<i>Tupaia picta</i>	Borneo (1E)	FMNH 88587 (1955)	Malaysia, Sarawak (Borneo)	447 (398)	11 (11)	1476 (1086)	70
<i>Tupaia splendidula</i>	Borneo and some offshore islands (1E)	UMMZ 174429 <sup>a</sup>	Indonesia, Kalimantan Barat (Borneo)	1002 (840) <sup>D</sup>	73 (57)	1644 (1243)	100
		UMMZ 174428 <sup>b</sup>	Indonesia, Kalimantan Barat (Borneo)	1002 (840)	73 (57)	1644 (1243)	100
<i>Tupaia tana</i>	Borneo, Sumatra, offshore islands (1B)	MVZ 192193 <sup>a</sup>	Indonesia, Aceh (Sumatra)	1002 (840) <sup>D</sup>	73 (57)	1644 (1243)	100
		JS M11 <sup>b</sup>	Malaysia, Sabah (Borneo)	1002 (840) <sup>D</sup>	73 (57)	1644 (1243)	100
<i>Urogale everetti</i>	Southern Philippines: Mindanao, Siargao, and Dinagat islands (1A)	FMNH 147781	Philippines, Bukidnon (Mindanao)	1002 (840) <sup>D</sup>	73 (57)	1644 (1243)	100
<i>Galeopterus variegatus</i>		GB: NC_004031 <sup>a</sup>		1002 (840)	73 (57)	1644 (1243)	100
		GB: AF038018 <sup>b</sup>		1002 (840)	19 (13)	0 (0)	40
<i>Cebus albifrons</i>		GB: NC_002763		1002 (840)	73 (57)	1644 (1243)	100
<i>Eulemur mongoz</i>		GB: NC_010300		1002 (840)	73 (57)	1644 (1243)	100
<i>Hylobates lar</i>		GB: NC_002082		1002 (840)	73 (57)	1644 (1243)	100

Table 1 (continued)

Species range (Fig. 1)	Voucher <sup>A,B</sup>	General locality	12S: 1002 (840) bp <sup>C</sup>	tRNA-Val: 73 (57) bp <sup>C</sup>	16S: 1644 (1243) bp <sup>C</sup>	% Complete
<i>Pygathrix nemaeus</i>	GB: NC_008220		1002 (840)	73 (57)	1644 (1243)	100
<i>Tarsius bancanus</i>	GB: NC_002811		1002 (840)	73 (57)	1644 (1243)	100
<i>Nycticebus coucang</i>	GB: NC_002765		1002 (840)	73 (57)	1644 (1243)	100
<i>Daubentonia madagascariensis</i>	GB: NC_010299		1002 (840)	73 (57)	1644 (1243)	100

<sup>A</sup> GB = GenBank sequence; institutional abbreviations for vouchered specimens: FMNH = Field Museum of Natural History; USNM = United States National Museum of Natural History (Smithsonian Institution); MVZ = Museum of Vertebrate Zoology, University of California, Berkeley; UMMZ = University of Michigan Museum of Zoology; USNZ = United States National Zoo; UAM = University of Alaska Museum; JS = specimens collected by Jason Munshi-South and deposited at the Universiti Malaysia Sabah.

<sup>B</sup> Superscript letters in this column identify individuals in Figs. 2 and 3.

<sup>C</sup> Total number of non-missing alignment columns, with the number included in the analyses in parentheses.

<sup>D</sup> 12S sequence originally published by Olson et al. (2005).

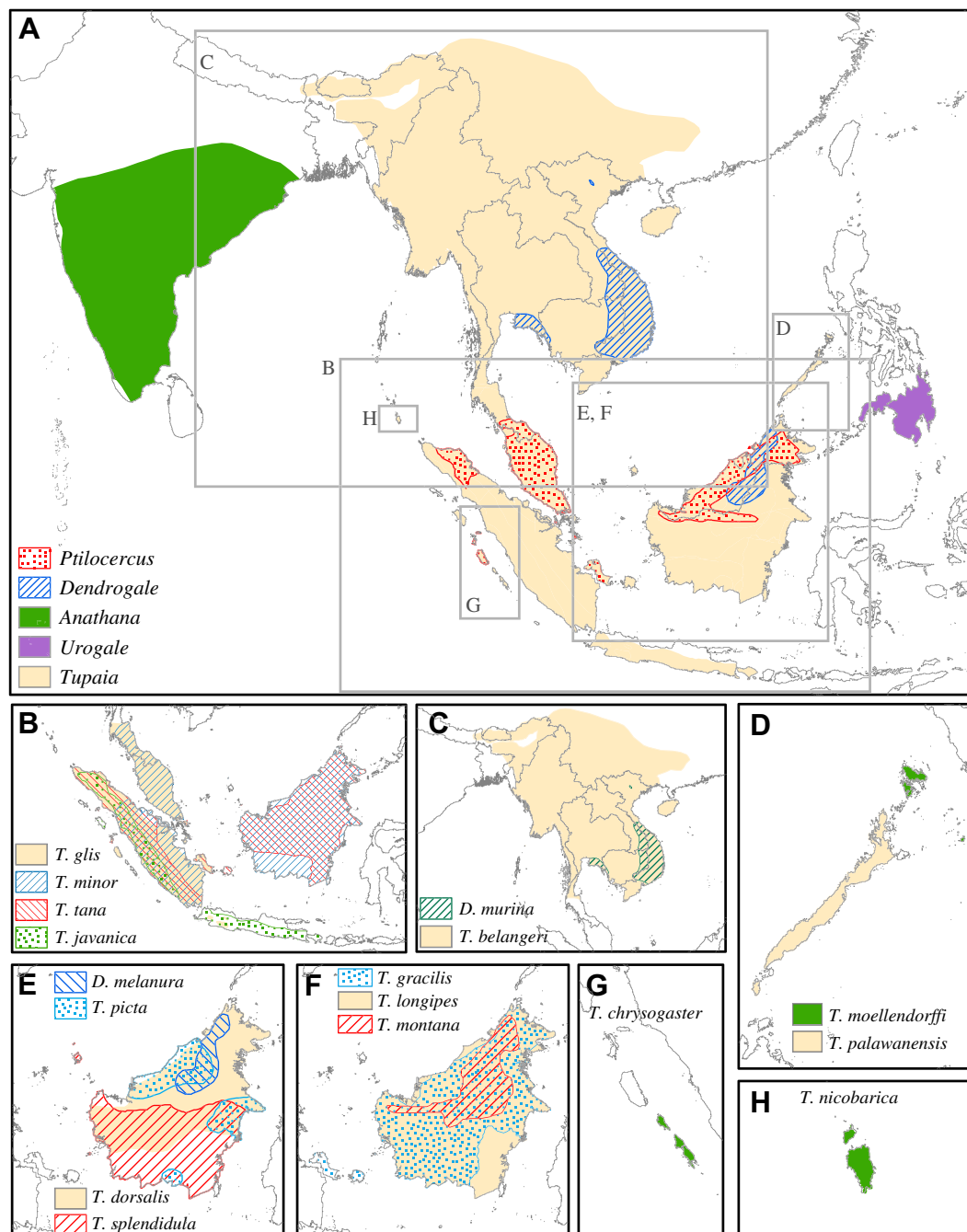


Fig. 1. Approximate distributions (extents of occurrence; IUCN, 2009) of (A) the five treeshrew genera (including the previously recognized *Urogale* (= *Tupaia everetti*), and (B)–(H) species in the genera *Dendrogale* and *Tupaia* (the other three genera are monotypic).

This raises several questions about the biogeography of treeshrew diversity that relate to evolutionary relationships within the order. We use our molecular phylogeny and modern phylogenetic divergence dating techniques to address some of the prominent biogeographic questions about treeshrews, including the relationship between Indochinese and Sundaic lineages, the affinities of the island endemics, and the origin of the high Bornean treeshrew diversity, as well as the probable timescale of colonization in some of these areas. We also use our calibrated phylogeny to analyze diversification rates in treeshrews. Diversification, including both speciation and extinction, can change as a result of extrinsic factors (including geography) as well as intrinsic or biological ones. Rising sea levels, for example, might increase allopatric speciation by fragmenting formerly continuous land, although the imprecision inherent in most dated phylogenies makes it difficult to tie rate changes to any single historical event. Previous phylogenetic studies of treeshrews have suggested that persistent lack of resolution is related to the presence of short internal branches (Olson et al., 2005; Roberts et al., 2009), a pattern that can result from rapid diversification. We test whether treeshrew diversity is consistent with a constant rate of diversification through time and when increases or decreases in net diversification rate appear to have occurred.

## 2. Materials and methods

### 2.1. Gene and taxon sampling

We sequenced the contiguous mitochondrial ribosomal genes 12S, tRNA-Val, and 16S using primers designed for amplification of short overlapping fragments (Supplementary information). The secondary structure of transcribed ribosomal genes, which contain relatively conserved pairing “stem” regions interspersed with variable nonpairing “loop” or bulge regions, lends itself to this type of primer design, which is important in using degraded sources of DNA. We included data for each of the 20 currently recognized treeshrew species (Helgen, 2005). Of these, 11 were sequenced from tissues collected specifically for genetic analysis and preserved in alcohol or buffer or by freezing (“fresh tissues” hereafter). The remaining nine were sequenced from fragments of tissue adhering to skulls or skeletons in museum collections (“crusties”). Collection years for these specimens ranged from 1901–1989, and we refer to sequences from them as “antique DNA.” Extraction, PCR amplification, and sequencing followed standard and well-established methods, although additional precautions (Section 2.2) were used for “crusty” samples. We used a combination of new and previously published primer sequences, listed in Supplement 1. Sequences for some specimens are incomplete; Table 1 details specimen and sequence information. In some cases, we included multiple individuals from the same species. This can help break up long branches and make parameter estimation more accurate, as well as providing some insight into the distribution of variation within and among recognized species. Some 12S and partial tRNA-Val sequences were originally published by Olson et al. (2005); see Table 1 for details. Four of our sequences add a single base pair to the 5' end of those published sequences. In addition, we believe the sequence of *Ptilocercus* published by Olson et al. to be a chimera, in which the final 154 bp fragment originated from *T. belangeri*. That fragment has been eliminated from this analysis. We also replaced antique specimens from the Olson et al. data set with fresh DNA sequences for *Dendrogale murina*. We used seven primate (*Cebus*, *Pygathrix*, *Hylobates*, *Tarsius*, *Eulemur*, *Nycticebus*, and *Daubentonia*) and two dermopteran (*Galeopterus*) mitochondrial sequences from GenBank as outgroups; we also included one sequence of *T. belangeri* from GenBank. All new sequences have been deposited in GenBank under accession numbers JF795291–JF795323.

### 2.2. Lab methods for antique DNA

Amplifying DNA from historic museum specimens presents challenges not typical for sequencing from fresh tissues, including particular risks for contamination. Most antique DNA must be amplified in very short fragments (100–400 bp) and target DNA often seems to be at relatively low density in samples, making the chance of accidentally amplifying contaminant DNA (treeshrew, human, dermestid, or environmental) higher than is generally true for high-quality fresh tissues (Willerslev and Cooper, 2004). Concatenating short amplicons into longer sequences also creates a high risk of generating chimeric sequences, and this can have severe effects on phylogenetic results that are difficult to predict or even to catch without careful attention (Olson and Hassanin, 2003). Our strategy for sequence verification is based on the recommendations outlined by Olson et al. (2005), including careful control of lab conditions and checking every sequenced amplicon both against GenBank and against our own extensive library of published and unpublished treeshrew sequences and sequence fragments. When necessary, some fragments were verified by re-amplification and re-sequencing, or by sequencing other individuals of the same species for comparison. In addition, pre-PCR work for some sequences generated for this study was done in the University of Alaska Museum's dedicated ancient DNA lab, a separate facility with entry control and a positive-pressure airflow system, housed in a PCR-free building. This minimizes the risk of contamination at the critical early steps of the process. Overall, our approach to antique DNA involves amplification in short fragments, spatial and temporal separation of work on fresh and antique samples, separate pipets and reagents for fresh and antique samples, and extensive, multi-step sequence verification before and after concatenation. Supplement 2 shows the complete set of shorter fragments composing each final sequence and the overlap between adjacent fragments.

### 2.3. DNA alignment and secondary structure models

Because of the complex secondary structure of ribosomal DNA, assumptions of traditional models of molecular evolution may be violated. In particular, the assumption that all sites change independently is certainly untrue, as pairing sites in stem regions often co-evolve. This is evident in any comparative ribosomal DNA alignment, in which compensatory substitutions, often maintaining canonical A–T or G–C pairs, are common and cause strong covariation between linked sites. Explicit attention to the secondary structure of these DNA regions can increase alignment accuracy and be directly used in molecular phylogenetic analysis (Kjer et al., 2009). We used secondary structure models for 12S (Springer and Douzery, 1996), tRNA-Val (Pütz et al., 2007), and 16S (Burk et al., 2002), modified by hand and using additional updated comparative sequence and structure information (e.g., Cannone et al., 2002), both for alignment and for analysis. We aligned sequences to secondary structure models manually. Some variable regions could not be confidently aligned, and we excluded 579 alignment-ambiguous positions from our phylogenetic analyses, leaving 2140 bp. The annotated alignment, including structure model and excluded characters, is available in the Supplementary information.

### 2.4. Phylogenetic analyses

We used MrBayes 3.1.2 (Altekar et al., 2004; Ronquist and Huelsenbeck, 2003) for phylogenetic analysis. MrBayes implements a “doublet” model (Schoniger and Von Haeseler, 1994) for pairing sites such as ribosomal stem regions that explicitly models the covariation in base frequency at pairing sites and is a more accurate reflection of ribosomal evolution than standard indepen-

dent models. We assigned a GTR + I + G model to both the pairing and nonpairing partitions. We chose this model based on a comparison of AIC scores for GTR and HKY models with and without rate variation, scored on a neighbor-joining tree in PAUP\* 4.0b10 (Swofford, 2002) for the pairing and nonpairing partitions. This approach provides a rough estimate of the types of model parameters that are appropriate for the data, although the specific models and implementations available in PAUP and MrBayes differ. We modeled among-partition rate variation, which is important to accurate branch-length estimation with partitioned models (Marshall et al., 2006) by assigning the rate prior a Dirichlet(1,1) distribution.

In addition, we used RAxML 7.2.7 (Stamatakis, 2006; Stamatakis et al., 2008) on the CIPRES web portal (Miller et al., 2009) for maximum-likelihood analysis. We performed a simultaneous search for the best tree and 1000 bootstrap replicates under a partitioned model with the GTR + G model assigned to unpairing sites, as recommended in the RAxML documentation, and the RNA7A model (Higgs, 2000), which is the most general seven-category model, for pairing sites.

With Markov chain Monte Carlo methods, successful analysis requires careful attention to “convergence”—usually gauged by determining whether any single run has reached a stationary distribution, whether multiple runs are sampling the same distribution, and whether all parameters are mixing well enough that samples are representative of the posterior distribution. We used R 2.8.1 (R Development Core Team, 2004) to assess burnin and Markov chain convergence in MrBayes, using both our own scripts and the R package coda (Plummer et al., 2006). We found sufficient sampling and convergence to be particularly difficult with doublet models in MrBayes. The paired-sites partition base frequency parameters mixed very poorly, were highly autocorrelated, and had very low effective sample sizes with the default search settings (4 chains, temperature 0.2). Poor mixing of individual parameters hampers their accurate estimation, and can also detrimentally (and less obviously) affect other parameters and the overall behavior of MCMC chains. In order to improve parameter mixing and increase effective sample sizes, we increased the number of chains per run to 8, with a “temperature” of 0.1, producing several “cool” chains. This substantially improved the rate of swapping by the cold chain, yielding better mixing, lower autocorrelation, and higher effective sample sizes. We ran six runs of eight chains each, four for 20 million generations and two for 19 million generations, sampling every 1000, and eliminated the first million generations (1001 samples) of each run as burnin. We checked the sufficiency of this burnin period both by visual inspection of likelihoods along the course of the run and by comparing parameter estimates for the first and second halves of the post-burnin sample.

### 2.5. Divergence time estimation

In addition to the MrBayes analysis, we used relaxed molecular dating implemented in BEAST 1.5.2 (Drummond and Rambaut, 2007; Drummond et al., 2006) to examine the timing of divergences within Scandentia. For this analysis, we included all currently recognized treeshrew species, as well as four additional lineages of *T. belangeri* that may represent synonymized species (Roberts et al., 2009). We used a birth–death prior on tree topology, with a GTR + I + G model of nucleotide substitution. We ran analyses for 10 million generations, sampling every 1000 generations, and removed the first 10% as burn-in. To improve search efficiency, we constrained monophyly in Scandentia, Primates, and the Primates + Dermoptera clade. BEAST analyses were re-run while jackknifing calibration points to look for evidence of undue influence. Analyses were checked to ensure adequate burnin, good MCMC parameter sampling, and sufficient effective sample sizes for all parameters (>200) using Tracer (Rambaut and Drummond, 2007).

We used a combination of five fossil-based calibrations on nodes within our analyses. We used the first known appearance of *Tupaia* (*T. miocenica* at 18 Mya; Mein and Ginsburg, 1997) to provide a minimum age for the base of the *Tupaia*–*Urogale*–*Anathana* clade (lognormal prior; mean 1.0, SD 1.0, offset 18.0) and used the fossil *Eodendrogale* (from the Middle Eocene; Tong, 1988) to provide a minimum age for the most recent common ancestor (MRCA) of all Scandentia (lognormal prior; mean 1.0, SD, 1.0, offset 38.2). We used lognormal priors for these calibration points because this may be the distribution best suited for modeling fossil constraints (Ho and Phillips, 2009). For calibrations outside of Scandentia, we used a lognormal prior (mean 1.0, SD 1.0, offset 23.0) with a minimum age of 23 My (Eizirik et al., 2004) for the split between *Pygathrix* (Cercopithecoidea) and *Hylobates* (Hominoidea), a normal prior with a mean of 77.5 and standard deviation of 7.0 for the MRCA of all seven primate outgroups, and a normal prior with a mean of 90 and a standard deviation of 10 for the MRCA of Euarchonta (Scandentia + Dermoptera + Primates), which is the total tree height. Both the Euarchonta and Primates calibration priors were set to broadly encompass dates associated with those splits in the Timetree of Life database (Hedges et al., 2006).

### 2.6. Diversification rate

We analyzed the pattern of diversification in treeshrews by estimating the maximum likelihood rates and breakpoints for pure-birth models with 1–5 rates using the R packages ape (Paradis et al., 2004) and laser (Rabosky, 2009), and compared these models with AIC scores. We repeated these calculations for each of the 9000 trees in the post-burnin posterior sample from BEAST, as well as for the BEAST consensus tree. We then summarized characteristics of these models across the posterior tree set, to identify diversification patterns not conditioned on a single topology.

## 3. Results

### 3.1. Sequencing historic samples

As expected, targeted amplification fragment lengths had to be much shorter (generally 100–400 bp; see Supplementary information) to successfully sequence antique DNA samples, especially when not much material was available. In general, more recent samples had higher PCR success rates than older ones, but individual samples were highly idiosyncratic.

### 3.2. Phylogeny

Well-supported clades are identical between the Bayesian and maximum-likelihood trees, and we focus on the Bayesian tree here (the full ML tree is Fig. S1). Both with and without calibration, our phylogeny reveals several well-supported clades and some unclear relationships. The expected relationships among families and some genera within treeshrews were reconstructed with long internode branch lengths and strong support—*Ptilocercus* sister to the all other treeshrews, and *Dendrogale* sister to the remainder of the Tupaiidae (Fig. 2 and Fig. 3). We found moderate support (92% posterior probability) for *Anathana* as the sister lineage to *Tupaia* and *Urogale* in the MrBayes analysis. In BEAST, however, *Anathana* falls within *Tupaia* in a majority of trees (69%).

We found strong support for a clade containing *T. glis* and *T. belangeri*, although the individuals of the latter we included were paraphyletic with respect to *T. glis* (Fig. 2 and Fig. 3). These two species are acknowledged to form a species complex with many subspecies and substantial morphological and genetic variation (Helgen, 2005; Roberts et al., 2009). There is also a strong sister relationship between *T. longipes* and *T. chrysogaster*, the Mentawai

Archipelago endemic. This clade is sister to *T. belangeri* + *T. glis*. These four species compose a well-supported clade (labeled “A” in Fig. 2) that, with the exception of *T. chrysogaster*, for which few data have been available, has been consistently recovered by phylogenetic studies with multiple data sources.

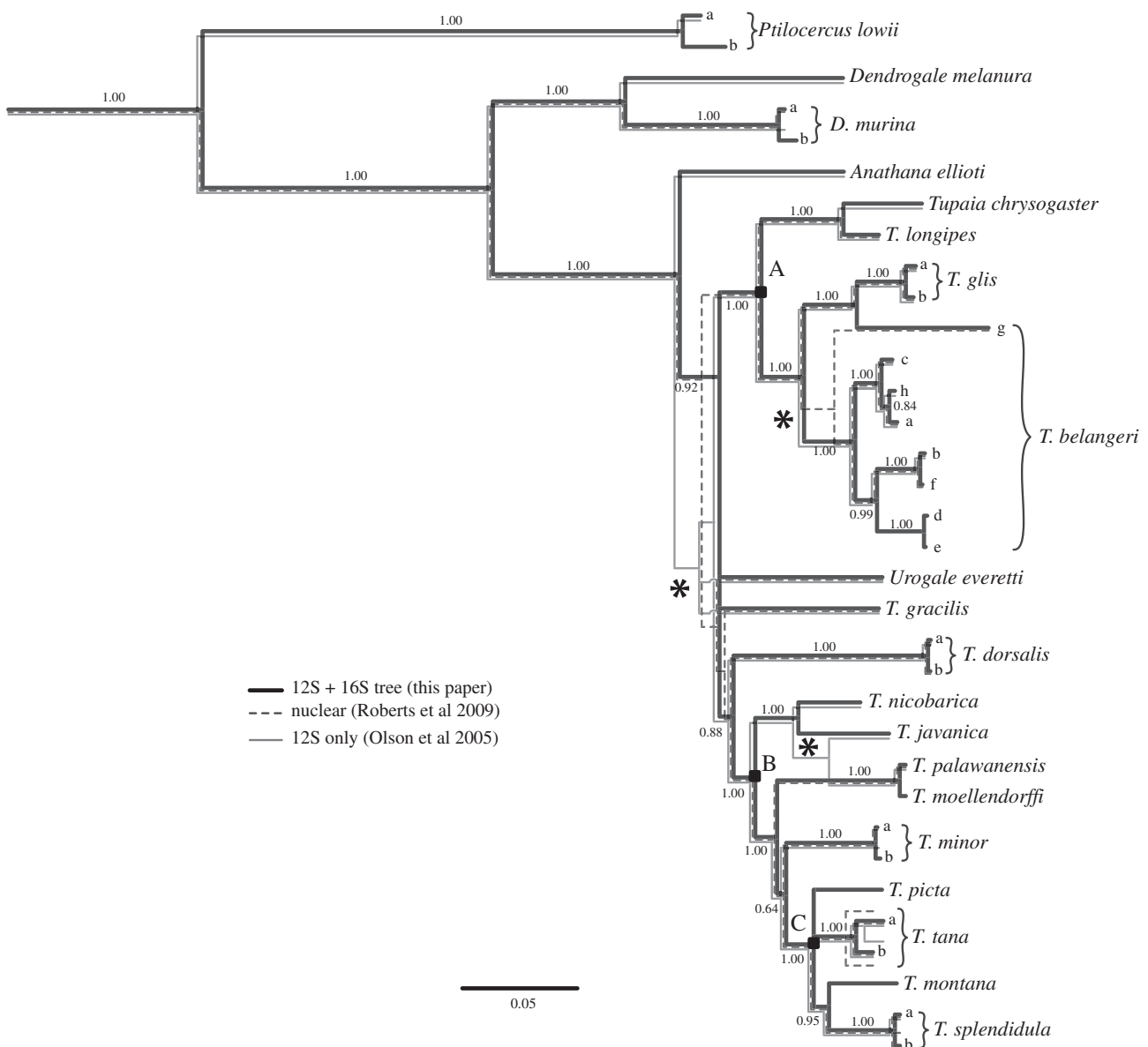
We also found strong support for a clade containing *T. montana* and *T. splendidula*, and for one containing these taxa plus *T. tana* and *T. picta* (“C” in Fig. 2). We found relatively weak support for a clade containing these four taxa and *T. minor*, but strong support for these five plus *T. palawanensis* and *T. moellendorffi* and for these seven plus *T. nicobarica* and *T. javanica*. The sister relationships between these two pairs were supported with 100% posterior probability. These nine taxa together form another well-supported clade (“B” in Fig. 2).

Relationships along the backbone of the tree within Tupaiidae were not well supported. As previously shown with nuclear data (Roberts et al., 2009) and a more limited mitochondrial data set

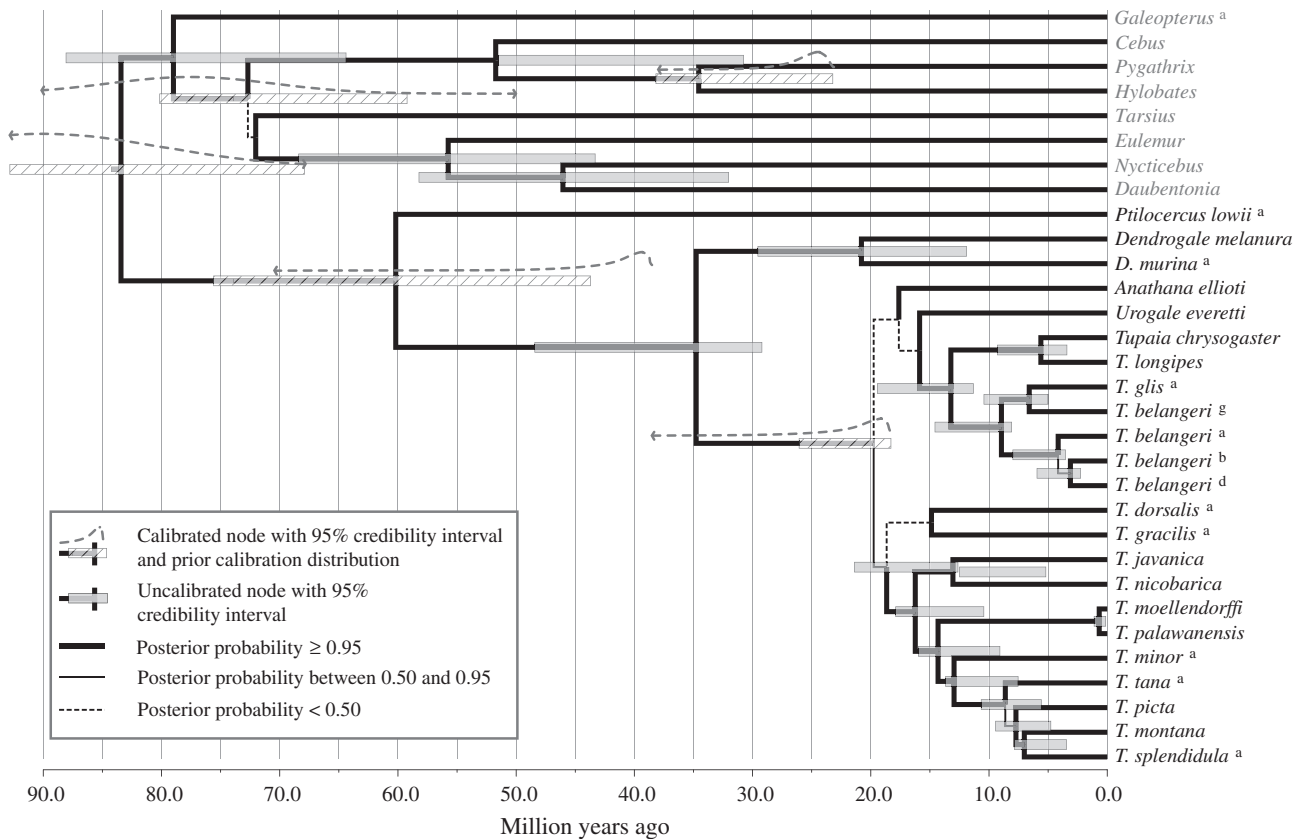
(Olson et al., 2005), the relationships among *Urogale*, *Anathana*, *T. dorsalis*, *T. gracilis*, and clades A and B (Fig. 2) are not clear. However, concordant with the nuclear data, there is very little support for the monophyly of the genus *Tupaia* (1.57% total posterior probability in the MrBayes analysis).

### 3.3. Divergence time

Our analysis puts the divergence between Scandentia and Primates/Dermoptera (the common ancestor of Euarchonta) at 83.43 Mya and the divergence between Ptilocercidae and Tupaiidae (the common ancestor of all extant Scandentia) at 60.19 Mya. Within Tupaiidae, we estimated *Dendrogale* to have diverged from the rest of the family 34.77 Mya. Divergences among *Tupaia* species, *Anathana*, and *Urogale* begin at 19.76 Mya, with several divergences (some at poorly supported nodes) between 15 and 20 Mya. The most recent divergence estimated between



**Fig. 2.** 50% consensus tree from MrBayes (in black), overlaid on previously published treeshrew topologies from Olson et al. (2005) and Roberts et al. (2009). Branch lengths and posterior probabilities apply only to the new tree; the other trees are topologies only. Asterisks indicate areas of disagreement among these trees. Diverging terminals indicate groups for which different individual specimens were sequenced in different studies. Letters at tips of some branches identify individuals as in Table 1. For clarity, outgroups are not shown. Clades A, B, and C are discussed in the text.



**Fig. 3.** Phylogeny and estimated divergence times from BEAST analysis. Hatched bars show the median and 95% credibility intervals for calibrated nodes, with the calibration distributions, while gray bars show the same values for uncalibrated nodes. Line type indicates posterior probability for bipartitions. Letters at tips of some branches identify individuals as in Table 1.

currently recognized species is 0.68 My, between *T. moellendorffi* and *T. palawanensis*. 95% credibility intervals for nodes with >50% posterior probability are shown in Fig. 3.

### 3.4. Diversification rate

The plot of treeshrew lineages through time from the consensus tree (Fig. 4) shows an apparent inflection point around 20 Mya, when the number of extant lineages rises sharply after a long static period. The best-fitting rate model for this tree has 3 rates, with breakpoints at 20.79 Mya (transition from 3 to 4 lineages) and 12.96 Mya (13–14). Some of the 9000 trees in the posterior sample support each of the 1–5 rate models, but the majority support a 3-rate model (1 rate: 547 trees out of 9000; 2 rates: 587; 3 rates: 5285; 4 rates: 1938; 5 rates: 643). Across the posterior tree sample, the inferred rate breakpoints are very consistent (Fig. 4), both in absolute time and in the node they correspond to. Multi-rate models consistently have a breakpoint and an increase in rate around 20 Mya, almost always corresponding to the transition from 3 to 4 lineages. They also consistently have a rate breakpoint with a decrease in rate in the last few branching points in the tree.

## 4. Discussion

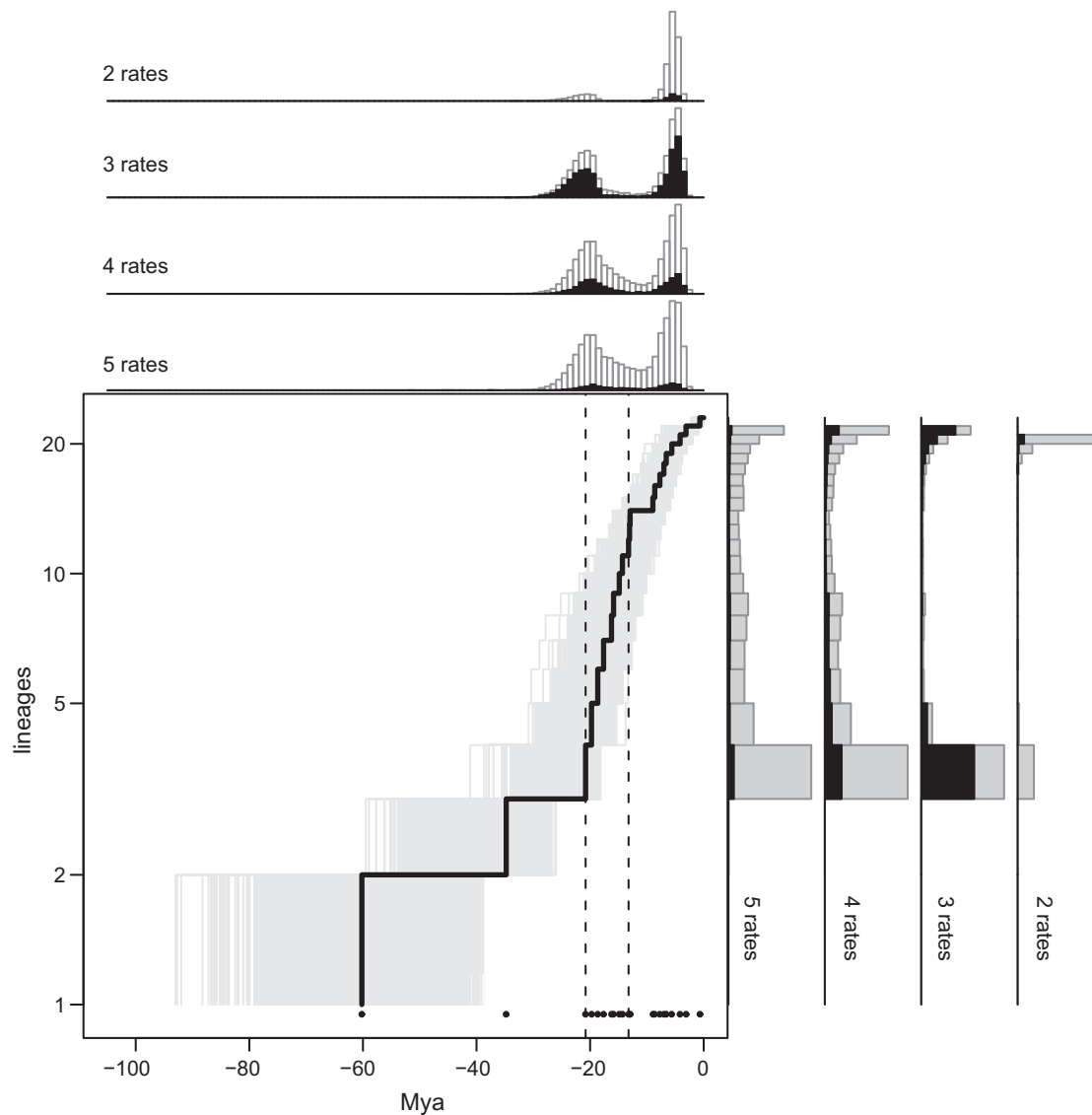
### 4.1. Overall timing and diversification

Our divergence time estimates for deep nodes in the phylogeny of Scandentia are similar to those suggested by other authors (Janecka et al., 2007); our estimates for the common

ancestor of Euarchonta and the common ancestor of Scandentia are 2–3 My younger, but the credibility intervals overlap broadly. These differences may be due to choice of calibration and branch length estimation, which can be sensitive to specific evolutionary models. Because of the limited treeshrew fossil record, some of our calibrations are derived from primate fossils and previous studies calibrating primate and Euarchontan divergences (Eizirik et al., 2004; Hedges et al., 2006; Janecka et al., 2007; Murphy and Eizirik, 2009; Steiper and Young, 2009). This parallel phylogenetic calibration is not ideal, and could introduce bias if there have been shifts in overall evolutionary rates within Euarchonta. However, the relative paucity of treeshrew fossils and the difficulty of phylogenetically placing those that have been described make this the best currently available approach to estimating treeshrew divergence times.

Credibility intervals for many divergence dates within the treeshrew radiation are quite large, but most of these dates are on the order of magnitude of millions of years, older than Pliocene or Pleistocene glaciation (with the possible exception of *T. palawanensis* and *T. moellendorffi*). We found some evidence from the accumulation of extant lineages through time (Fig. 4) that diversification rates have not been constant. In both the consensus tree and the majority of sampled trees, the lineage-through-time plot has an inflection point and a rate increase associated with the transition from 3 to 4 lineages. The subsequent period of more rapid lineage accumulation corresponds to the deep splits within the *Tupaia*/*Anathana*/*Urogale* clade. Although the absolute time of the radiation of this clade varies, the fact that it involves an inferred increase in overall diversification rate is notably consistent across the posterior tree sample. This shift in overall diversification





**Fig. 4.** Diversification model tests and lineage-through-time (LTT) plots. The thick black line is the LTT plot for the consensus tree (Fig. 3). Light gray lines show LTT plots for the 9000 trees in the posterior sample. Marginal histograms show the distribution of inferred rate breakpoints across all 9000 trees (hollow or gray histograms) and across only the trees for which a given model had the best AIC score (black histograms). Dotted lines indicate the position of inferred rate breakpoints for the best (3-rate) model for the consensus tree. Black dots indicate the positions of all branching points in the consensus tree.

rate may involve changes in extinction, speciation, or both; these cannot be distinguished without direct evidence about extinction (Quental and Marshall, 2010), which we lack.

A more recent decrease in diversification rate is also consistently inferred, although its position is less clear. In the consensus tree, the decrease is at 12.96 Mya, after which comes a 4 My period of time with no additional new lineages (in contrast, the preceding 4 My interval contained 6 branching points). However, in many of the sampled trees the rate decrease is more recent, associated with one of the last few branching points. This flattening of the lineage-through-time plot is presumably due in large part to missing taxa—actual species that are currently synonymized probably represent relatively recent divergences. Given the number of named forms that currently do not have full species status, it would certainly not be surprising if some true species were uncoun-tered in our analysis. However, it is also possible that diversification rate—again, a combination of speciation and extinction—did decrease again at some point after the earlier increase, perhaps because of renewed geologic stability.

## 4.2. Historical biogeography

### 4.2.1. Mainland–Sunda–Island dynamics

Combined with the estimation of divergence times, our phylogenetic results indicate that species flow between what is today mainland Southeast Asia and the islands of Sundaland probably happened in the Miocene. Divergences between *Dendrogale melanura* and *D. murina* and between *T. glis*/*T. belangeri* and *T. longipes*/*T. chrysogaster* in clade A support this. During the Miocene, when these divergences seem to have occurred, much of the Sunda Shelf was connected by landbridges as a single mainland, even expanding as new islands emerged (Hall, 1998, 2001). It seems likely that treeshrews spread throughout this single landmass then, and have become increasingly isolated through subsequent episodes of high sea level (Miller et al., 2005) and insularity, culminating in the current era of geographic fragmentation. Shared patterns in mammals and birds suggest that similar geographic factors may have been responsible for broad-scale divergence dynamics between Indochinese and Sundanic lineages,

even without permanent physiographic barriers to gene flow (e.g., Woodruff and Turner, 2009). However, understanding smaller-scale mainland–island patterns, including those within widespread species such as *T. glis*, *T. minor*, and *P. lowii*, will require much finer-scale data collection. It is possible that Pliocene and Pleistocene dynamics, including sea level changes, simply explain intraspecific patterns rather than interspecific ones.

The deeply divergent position of *Anathana* compared to *Tupaia* and *Urogale* is particularly interesting given that it has the farthest west distribution of any treeshrew species and that the treeshrew fossil record is also in mainland South Asia, with some Miocene fossil material found in Pakistan farther west than the current distribution. It may be that other western lineages have become extinct, leaving *Anathana* as a unique relic.

#### 4.2.2. Into Borneo, or out of Borneo?

Our results continue to clarify the role that Borneo has played as a center of treeshrew diversification, especially in clades B and C (Fig. 2). *T. tana*, *T. splendidula*, and *T. minor* have part of their range on Borneo, while *T. montana* and *T. picta* are endemic. These five taxa form a well-supported clade with divergences in the late Miocene, between 10 and 5 Mya. Unlike some of the younger volcanic islands of Southeast Asia, Borneo has old highland regions (Hall, 1998, 2002; Meijaard, 2004; Moss and Wilson, 1998). The island comprises regions with both older and younger rock, some earlier than the Cenozoic (Moss and Wilson, 1998). Highland areas have been present for at least 30 My, and the sister relationship between *T. montana* and *T. splendidula* may reflect elevational partitioning of available habitat. However, the complexity of Borneo's history creates several alternative possibilities, including secondary contact between lineages that diverged allopatrically in different parts of the island. The current island is formed of multiple geologic units that have been separate islands at times, including during the early Miocene (Meijaard, 2004).

In addition to clade C, *T. dorsalis*, *T. longipes*, and *D. melanura* are endemic to Borneo, and *T. gracilis* and *P. lowii* are present there but not endemic. The distribution of these Bornean taxa throughout the tree presents two biogeographic possibilities: treeshrews might have colonized Borneo several times, or they might have originated in Borneo and spread outward from there, with multiple dispersals to the rest of their current range (Han et al., 2000; Olson et al., 2005). Rigorously testing and distinguishing between these two scenarios may prove intractable, especially given the very limited fossil record for treeshrews and the instability of their individual distributions over time.

On this Cenozoic timescale, Borneo, like other regions of Southeast Asia, has been subject to tectonic shifts and may have been rotating and drifting (Hall, 1996, 1998, 2001; Hutchison, 2005; Moss and Wilson, 1998), so specific biogeographic reconstructions are difficult or impossible. The island is known for its high biodiversity and endemism in mammals and other taxonomic groups. Our data suggest that, for treeshrews, this high diversity may result from a combination of Borneo's age (some very old treeshrew lineages inhabit Borneo) and complexity (restricted and elevational endemics offer evidence of niche partitioning within the island). The character and diversity of habitats on the island has changed substantially with tectonic, sea level, and climatic changes through the Cenozoic, and local assemblages may well represent a combination of primary diversification and secondary contact.

#### 4.2.3. Wallacean island-hopping

Our results are consistent with stepping-stone colonization between islands in several cases. The clade composed of Philippine endemics *T. palawanensis* (Palawan and Balabac) and *T. moellendorffi* (nearby Cuyo and Calamian Islands) is sister to the Bornean radiation within clade B. The Palawan island group, including all

of these islands, may have been connected to Borneo by dry land during the Pleistocene, and this possible recent landbridge connection has frequently been invoked to explain faunal similarity between Palawan and the Sunda Shelf, which is especially high for mammals (Esselstyn et al., 2004). However, this relationship is becoming less clear as more detailed data become available (Esselstyn et al., 2010). In this case, the topology of the tree is certainly consistent with colonization—with or without a landbridge—from Borneo to Palawan and from Palawan to the Calamian Islands. However, our data suggest that the divergence between Bornean and Palawan treeshrews is between 10 and 15 Mya, well into the Miocene. Genetic divergence can certainly predate speciation and colonization, so it may be that actual colonization is somewhat later than the date on the tree, but it is unlikely to be as recent as the Pleistocene unless substantial lineage extinction means that we simply cannot see the relevant lineages. It is particularly interesting to note that the best reconstructions of landform evolution in Southeast Asia (Hall, 1998, 2001) suggest that very little of what is today Palawan was even present as dry land 10 million years ago; colonization of the island by treeshrews may have been around the time of its emergence.

Conversely, the divergence between our representatives of *T. palawanensis* and *T. moellendorffi* themselves does appear to be recent, quite possibly in the Pleistocene. The entire Palawan island group, including the ranges of both, has been repeatedly connected as sea levels have fluctuated during glacial episodes. Our estimation of divergence time strongly suggests that divergence between *T. palawanensis* and *T. moellendorffi* is consistent with recent allopatry, and that this divergence, unlike that between Palawan and Borneo, may be related to Pleistocene sea level fluctuations. However, range expansions and contractions, including colonization between islands that are now separate, may have happened numerous times, and the genetic patterns we see may reflect only the most recent events. The history of these two taxa probably involves repeated isolation and contact, possibly including limited introgression following speciation; these alternatives will require additional data sources to distinguish.

*T. nicobarica*, endemic to the Nicobar Islands northwest of Sumatra, presents another possible example of colonization by island-hopping. Our analysis places it firmly as the sister taxon to *T. javanica*, a more widespread species found on the Indonesian islands of Sumatra, Nias, Java, and Bali. All of these islands, including the Nicobars, are part of a single arc along the boundary of the Asian continental shelf. While some parts of the Sunda arc have been connected by landbridges in the past, the deep water separating the Nicobar Islands from both Sumatra and mainland Southeast Asia indicates that they are true oceanic islands and have always been isolated. Much of the tectonic and volcanic activity in the area dates from the Miocene, and many of the islands are themselves younger than 15 My (Chakraborty and Khan, 2009; Curray, 2005; Hall, 1998; Rodolfo, 1969). Our divergence time estimation suggests that the split between these two taxa is somewhat younger than this, probably 5–10 Mya, suggesting that *T. nicobarica* probably colonized the Nicobars from Indonesia during the late Miocene. However, the current large islands of Java and Sumatra did not yet exist in their current form for much of the Miocene, and may themselves have been a series of disconnected small islands (Hall, 1998, 2001; Meijaard, 2004), even when sea levels were low. Interestingly, *T. javanica* is not known to occur on the island of Lombok, a mere 30 km east of Bali but across Wallace's Line (Wallace, 1860). The distribution of *T. javanica* and other Indonesian species may have been extremely unstable on this time scale, so inferring long-ago colonization routes from current distributions and genetic relationships should be undertaken with caution.

The relationship between *T. chrysogaster*, endemic to the Mentawai Islands off the west coast of Sumatra, and *T. longipes*, endemic

to Borneo, is particularly interesting from the perspective of stepping-stone colonization. At least one frog (*Kalophrynus punctatus*; Inger, 1966) has a Borneo + Mentawais distribution, suggesting that this may be a more general pattern. Very little specific stratigraphic information is available for Sipora and Pagai (Samuel et al., 1997), the islands inhabited by *T. chrysogaster*, making it difficult to infer how long they have existed. Deep water currently separates the Mentawais and Sumatra, suggesting that they have not been directly connected even by low sea levels. Bathymetry does suggest that the Batu Islands to the northwest may have been connected both to Sumatra and to the Mentawais, forming a long, narrow peninsula parallel to Sumatra (Voris, 2000). This past connection has been used to explain the close relationship between Mentawai and Sumatran macaques (Abegg and Thierry, 2002; Roos et al., 2003). However, because neither *T. chrysogaster* nor *T. longipes* is found on Sumatra, landbridges are not a wholly satisfying explanation for this particular sister relationship. It seems likely that extinction of other closely related species, or extirpation of some populations of these two species, has obscured the true biogeographic relationships. Some authors have suggested that the Mentawais provided a rainforest refugium during past episodes of xerification (e.g., Gathorne-Hardy et al., 2002) and that Mentawai endemics represent relics of previously more widespread taxa, rather than long-distance dispersers (e.g., Brandon-Jones, 1996; Das, 2005; Ziegler et al., 2007), which may be the case here as well.

#### 4.2.4. Range stability and secondary contact

The geographic discontinuity between some closely related species in this study illustrates a broader phenomenon concerning the distributions of treeshrews (and other Southeast Asian taxa). The evolution of this group spans the entire Cenozoic, a timescale over which tectonic and volcanic activity throughout Southeast Asia, combined with recurrent changes in global sea level, have repeatedly and massively changed the presence, shape, orientation, and connectivity of islands and landmasses. Geographic instability makes it highly unlikely that current distributions reflect single dispersal or vicariance events; they are probably the result of repeated changes in range limits as a result of regional changes in land area, climate, and habitat. The limited amount of fossil data for treeshrews suggests that even on the mainland, which is stable compared to islands, distributions have not been constant and today's assemblages do not reflect the past. This may be even more true on islands, for which volcanic, tectonic, and sea level changes have been both common and intense. It is likely that treeshrew species or populations have frequently become extinct or locally extirpated, perhaps repeatedly. Even if species have persisted, their distributions may have changed drastically with the landscapes they inhabit, especially in association with changes in island area and connectivity. Competitive exclusion may also have resulted in extinction or extirpation if distinct but ecologically similar lineages or species have come into secondary contact.

Secondary contact and major distributional shifts may be particularly likely on Sumatra, an island that serves as the bridge between mainland Southeast Asia and the islands of Wallacea, and that has been repeatedly connected to both during Cenozoic episodes of climatic and sea level change. Meijaard (2004), summarizing patterns across a variety of mammalian taxa, suggested that Sumatra shares elements of its fauna with both the Bornean/Javan and Malayan regions as a result of secondary contact. This pattern appears to hold in treeshrews, in which *Tupaia glis*, which is partly a mainland species and is most closely related to another mainland species (*T. belangeri*), coexists with *T. javanica*, *T. minor*, and *T. tana* on Sumatra. The restricted distributions of *T. nicobarica* and *T. chrysogaster* could even have resulted from extirpation of Sumatran relatives. Over evolutionary timescales, the distributions of individual species and lineages may be highly unstable. Instability is even

predicted by the bulk of island biogeographic theory (MacArthur and Wilson, 1963, 1967). Overall, any attempt to reconstruct biogeographic history in this region, without fossils or evidence independent from current distributions, depends on woefully incomplete information. Future work on the geographic distribution of lineages within species, as well as increasing understanding of the geological and climatological history of the region, will help clarify these patterns.

#### 4.3. Taxonomic implications

Our results suggest several modifications to the currently recognized taxonomy of treeshrews. First, we suggest that the genetic difference between *T. moellendorffi* and *T. palawanensis* appears insufficient to merit species status. *Tupaia moellendorffi* was re-elevated to species status only recently, and this change was made “provisionally” (Helgen, 2005, p. 107). Whether either species is in fact monophyletic with respect to the other cannot be determined from the samples we included. However, the genetic distance between the two individuals in our analysis is quite small for these variable mitochondrial genes, much smaller than the distance between individuals within some other treeshrew species (e.g., *D. murina*, *T. tana*, and *T. glis*; Fig. 2). The insular, allopatric distribution of *T. palawanensis* and *T. moellendorffi*, combined with the presumed rarity of overwater colonization in treeshrews, strongly suggests a lack of substantial current gene flow between them, and their shallow genetic divergence may indicate recent, incipient, or incomplete speciation. However, a definitive answer to the status of *T. moellendorffi* will require much more extensive sampling and attention to geographic and subspecific variation, as well as a combination of genetic, morphological, and possibly other data. *Tupaia moellendorffi* and *T. palawanensis* may be an example of taxa that would be classified differently under different species criteria, most of which cannot be assessed with existing data.

Second, the monophyly of the genus *Tupaia* with respect to *Urogale* is not supported by our analysis, although the placement of *Urogale* itself is not clear. No genetic study to date has found strong support for the monophyly of *Tupaia* with respect to *Urogale* (see Roberts et al. (2009) for an in-depth examination of this issue). The taxonomic history of *Urogale* is complicated; in short, *U. everetti* was originally described as *Tupaia everetti* by Thomas (1892), who described a total of 23 treeshrew taxa (species or subspecies) over the course of his career. Seven of these are currently recognized as distinct species (Helgen, 2005). Mearns's (1905) subsequent description of *Urogale cylindrura* from southern Mindanao in the Philippines included the description of a new genus and the placement of *T. everetti* therein (*U. cylindrura* is now considered a junior synonym of *U. everetti*; Helgen, 2005). Lyon (1913) agreed that Mearns's (1905) proposed diagnostic characters were sufficient to warrant generic status for *Urogale* but went on to hypothesize that it probably shared a more recent “common ancestor” (p. 156) with *T. dorsalis* and *T. tana* (which he in turn united in a new genus *Tana*) than any of these taxa did with *Tupaia*. *Urogale* and *Tana* (later renamed *Lyonogale*), he observed, shared many unique craniodental and external features, which *Urogale* “carried to an extreme” (p. 156). Most subsequent taxonomists have not recognized *Tana/Lyonogale* (e.g. Corbet and Hill, 1992; Ellerman and Morrison-Scott, 1955; Helgen, 2005; Wilson, 1993). Previous molecular studies based on immunological (Dene et al., 1978, 1980) and DNA hybridization (Han et al., 2000) data failed to recover *T. tana* as either phylogenetically distinct from other *Tupaia* species or sister to *Urogale* (neither study included *T. dorsalis*). Every published DNA sequence-based phylogeny of treeshrews, all of which have included *T. tana*, *T. dorsalis*, and *Urogale*, has failed to recover any two of these taxa, let alone all three, in a monophyletic assemblage (Olson et al., 2005; Roberts

et al., 2009; this study). These have included both taxon- (this study) and character- (Roberts et al., 2009) rich sampling regimes, as well as explicit tests of *Urogale's* phylogenetic position with respect to *Tupaia* (Roberts et al., 2009). Collectively, this growing body of molecular evidence, as well as evidence from the postcranium (Sargis, 2004), strongly suggests that the gross craniodental and external features proposed by Lyon (1913) to be indicative of common ancestry between *Urogale*, *T. tana*, and *T. dorsalis* are instead the result of homoplasy. Since available data consistently fail to support either a split between *Tupaia* and *Urogale* or between *Tupaia* and *Tana/Lyonogale*, we hereby formally propose the subsumption of *Urogale* into *Tupaia*. *T. everetti* is unquestionably morphologically (and probably behaviorally and ecologically; Wharton, 1950) distinct from all other extant treeshrews (see Sargis, 2002a), but its distinctiveness is almost certainly, on the whole, autapomorphic.

The status of *Anathana* is much less clear; different analyses and models recover different answers to whether *Tupaia* (including “*Urogale*” *everetti*) is monophyletic with respect to *Anathana*, as shown by our BEAST, MrBayes, and RAXML analyses (Figs. 2 and 3, and S1). This may reflect differences in models and prior probability distributions, combined with the relatively short branch length connecting this monotypic genus to the rest of the tree. In BEAST, which (like any analysis of divergence times with extant taxa) infers an ultrametric tree, large variations in terminal branch length may cause the relative support for alternate topologies to change. Topological relationships that are sensitive to methods, models, and prior distributions are extremely difficult to determine with a high degree of confidence. However, the very limited availability of data for *Anathana* has also hampered attempts to determine its position in this phylogeny, and it is possible that more samples and perhaps additional sequence data will have the power to resolve the existing uncertainty.

Although we included all currently recognized species in this analysis, our taxon sampling is almost certainly not truly complete. Several treeshrew species contain multiple distinctive subspecies (currently or formerly recognized) or populations, often geographically separated (Helgen, 2005; Lyon, 1913); some of these were originally described as full species, and none have been thoroughly investigated from a phylogenetic perspective. Notable among these are *T. belangeri* and *T. glis*, which together form a species complex comprising 54 published synonyms; *T. splendidula* (6 synonyms); and *T. tana* (17 synonyms). We included multiple individuals from across the range of *T. belangeri*; the deep divergence among some of them, combined with the consistency of phylogenetic patterns across multiple loci (Roberts et al., 2009) suggests that some, at least, are probably distinct species. The paraphyly of *T. belangeri* haplotypes with respect to *T. glis* could be the result of several evolutionary processes, including both incomplete lineage sorting (deep coalescence) and biological mechanisms such as hybridization and introgression. This species complex needs much more attention before we can know which of the many possible factors have been important in its evolutionary history. However, incomplete or inaccurate taxonomy is very likely to explain some of the phylogenetic results; the decline in apparent recent diversification rate in all treeshrews (Fig. 4) suggests that (unless rates have actually changed) we are missing extant taxa, or that recent “speciation” events have not yet resulted in completely separate taxa. Close investigation into lower-level variation in treeshrews (Roberts et al., in prep.) will certainly be necessary to complete the picture we present here of their evolutionary history.

#### 4.4. Comparison to previous phylogenetic results

Molecular systematics has become more dependent on multi-locus data, acknowledging that species trees do not equate to gene

trees and often cannot be reconstructed accurately from a single molecular marker. We have used a single genetic locus here because of the difficulty of amplifying nuclear genes from degraded material. However, we note the strong concordance between nuclear data (Roberts et al., 2009) with more limited taxon sampling and the tree we present here (Fig. 2). Most well-supported clades and sister relationships are consistent with either of two mitochondrial data sets (see Olson et al., 2005) and with our previously published nuclear data, suggesting that they are robust to which genes are sampled and are likely to represent species tree relationships. This is expected for bipartitions with internal branches that are relatively long compared to the standing genetic diversity of populations, reducing the discordance among gene trees caused by incomplete lineage sorting and coalescent stochasticity.

Topological differences between the tree(s) we report here and those reported in previous molecular phylogenetic studies fall into two general categories. First, different methods and molecular markers often support slightly different resolutions of relationships among individuals within species. The sister relationship between *T. glis* and a single lineage of *T. belangeri*, which is strongly supported here, had a low total posterior probability (5.0%) in our previous analysis of nuclear DNA (Roberts et al., 2009), although it had a nonzero probability for all genes and a probability of 52.1% for one of them. Intraspecific relationships with short branch lengths are the most likely to be affected by coalescent stochasticity and incomplete lineage sorting, which may explain these differences among molecular markers. Reconstructing short internode lengths can also be affected by choice of model and method, especially when terminal branches violate assumptions of rate homogeneity.

Second, we did find several instances of conflict in interspecific relationships. Some involve relationships along the backbone of the tupauid phylogeny, where several clades and species appear to have diverged within a short period of time; taxa involved include *Urogale* (= *Tupaia*) *everetti*, *T. gracilis*, *T. dorsalis*, and *Anathana ellioti*. Internal branches around the divergences of these taxa are short and their reconstruction may be influenced by true differences between gene trees as well as by actual phylogenetic error. Individual relationships are often poorly supported by any given gene as well as discordant among genes, suggesting that there is insufficient phylogenetic information to reconstruct them unambiguously. We also found some conflict with respect to the positions of *T. javanica* and *T. palawanensis*. These two species were sister taxa in the previous maximum likelihood analysis of the 12S rRNA gene (Olson et al., 2005), although this relationship was not strongly supported. The additional data in this expanded analysis seem to have strengthened and stabilized the sister relationship between *T. nicobarica* and *T. javanica*.

We previously reported substantial areas of conflict surrounding the “backbone” taxa *Urogale* (= *Tupaia*) *everetti*, *T. dorsalis*, and *T. gracilis* with multiple nuclear genes (Roberts et al., 2009), and we find it interesting that the single locus we employ here reconstructs these areas with low confidence. This may be evidence that in this case phylogenetic ambiguity is the result of evolutionary patterns and processes that are difficult to reconstruct confidently—such as short internode distances or rapid successive divergences—rather than just differences among gene trees causing conflict in a concatenated analysis.

## 5. Conclusions

We have shown that the timescale of treeshrew evolution extends throughout the Cenozoic. Like other Southeast Asian taxa, treeshrews have evolved in a complex and changing landscape, and many lineages are Miocene and Pliocene in age. Additional

work on taxonomy in this group, especially focusing on the many named subspecies and synonymized forms (Roberts et al., in preparation), will help to develop this picture of biogeographic evolution in Southeast Asia. It is unlikely that these questions can be satisfactorily answered without additional collection of specimens for both genetic and morphological work. The severe anthropogenic pressures on Southeast Asian biodiversity (Myers et al., 2000; Schipper et al., 2008; Sodhi et al., 2004) make it even more important to determine the status, relationships, and ages of these lineages so conservation priorities can be assessed and revised.

One growing use of time-calibrated phylogenies is to tie the evolution of a focal group to the history of a broader biotic community. Recent research has highlighted potential coevolutionary relationships between *Ptilocercus* and the bertam palm *Eugeissona* (Wiens et al., 2008), in which treeshrews routinely drink fermented palm nectar without apparent intoxication, and between *T. montana* and pitcher plants in the genus *Nepenthes* (Chin et al., 2010; Clarke et al., 2009), in which pitcher size and geometry facilitate nitrogen capture from treeshrew feces. Although the timing of the *Nepenthes* radiation is unclear (Meimberg et al., 2001), it appears that *T. montana* is of relatively recent origin, suggesting that coevolution between pitcher plants and this particular treeshrew species has happened since the late Miocene or Pliocene. In contrast, *P. lowii* could be much older and might have developed its tolerance for fermented palm nectar over the course of up to 55 My, a very different coevolutionary history. Combining phylogenetic information across taxa and communities provides temporal information critical for understanding the dynamics of relationships like these.

Finally, single treeshrew species, especially *T. glis*, *T. belangeri*, *T. tana*, and *T. minor*, appear frequently as exemplars of the order in higher-level studies, sometimes with flawed assumptions about biology, taxonomy, and geography. While we realize that most authors have used the taxa most readily available to them, we also point out that incomplete, inaccurate, or inappropriate data can be a real problem in higher-level phylogenies (see Sargis, 2002a,b,c, 2004, 2007), meta-analyses, and other synthetic studies. Using single species to represent groups or characters of which they are not truly representative should be done with substantial caution, and wherever possible with attention to phylogenetic relationships and the significance of shared evolutionary history. We hope that phylogenetic information such as the results we present here can continue to inform taxon selection and strengthen tree-based inference across the breadth of modern biology.

## Acknowledgements

This research was supported by National Science Foundation Grant DEB-0542725/0542532 and an Alaska EPSCoR grant to L.E.O. and E.J.S., and startup funds from the University of Alaska Museum to L.E.O. T.E.R. was supported in part by a NESCent postdoctoral fellowship (NSF EF-0423641/DBI-0905606). DNA sequencing was conducted in the University of Alaska Fairbanks Institute of Arctic Biology Core Facility for Nucleic Acid Analysis with support from NSF EPSCoR Grant EPS-0346770. We are grateful to the following people and institutions for making tissues available for this study: W.T. Stanley and L.R. Heaney (Field Museum of Natural History); L.K. Gordon, J.F. Jacobs, and R.W. Thorington, Jr. (US National Museum of Natural History, Smithsonian Institution); J.L. Patton, C.J. Conroy, and J.P. Boubli (Museum of Vertebrate Zoology, University of California, Berkeley); A.J. Gorog and P. Myers (University of Michigan Museum of Zoology); and J. South (University of Maryland). Fresh tissues provided by J.F. Jacobs resulted from fieldwork supported by the Smithsonian's National Museum of Natural History's Biodiversity Surveys and Inventory Program and facilitated by the Myanmar Nature and Wildlife Conservation Division. We

thank Annette Olsson of Conservation International for providing the *Dendrogale murina* specimen from the Cardamom Mts. in Cambodia. Fresh tissues of *D. murina* and *Tupaia belangeri* were the result of an expedition to Cambodia funded by a grant from the National Geographic Society's Committee on Research and Exploration to E.J.S. and L.E.O., and we thank J. Walston, E. Pollard, and M. Soryun of the Wildlife Conservation Society–Cambodia and Khiev Rithy Phion of the Cambodian Forestry Administration for their invaluable assistance.

## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympmv.2011.04.021.

## References

- Aegg, C., Thierry, B., 2002. Macaque evolution and dispersal in insular South-East Asia. *Biol. J. Linn. Soc. Lond.* 75, 555–576.
- Altekar, G., Dwarkadas, S., Huelsenbeck, J.P., Ronquist, F., 2004. Parallel Metropolis-coupled Markov chain Monte Carlo for Bayesian phylogenetic inference. *Bioinformatics* 20, 407–415.
- Bahr, U., Schondorf, E., Handermann, M., Darai, G., 2003. Molecular anatomy of *Tupaia* (tree shrew) adenovirus genome; evolution of viral genes and viral phylogeny. *Virus Genes* 27, 29–48.
- Brandon-Jones, D., 1996. The Asian Colobinae (Mammalia, Cercopithecidae) as indicators of Quaternary climatic change. *Biol. J. Linn. Soc. Lond.* 59, 327–350.
- Burk, A., Douzery, E.J.P., Springer, M.S., 2002. The secondary structure of mammalian mitochondrial 16S rRNA molecules: refinements based on a comparative phylogenetic approach. *J. Mamm. Evol.* 9, 225–252.
- Butler, P.M., 1980. The tupaiid dentition. In: Lockett, W.P. (Ed.), *Comparative Biology and Evolutionary Relationships of Treeshrews*. Plenum Press, New York, pp. 171–204.
- Cannone, J.J., Subramanian, S., Schnare, M.N., Collett, J.R., D'Souza, L.M., Du, Y.S., Feng, B., Lin, N., Madabusi, L.V., Muller, K.M., Pande, N., Shang, Z.D., Yu, N., Gutell, R.R., 2002. The Comparative RNA Web (CRW) Site: An Online Database of Comparative Sequence and Structure Information for Ribosomal, Intron, and other RNAs. *BMC Bioinformatics* 3.
- Chakraborty, P.P., Khan, P.K., 2009. Cenozoic geodynamic evolution of the Andaman-Sumatra subduction margin: Current understanding. *Island Arc* 18, 184–200.
- Chin, L.J., Moran, J.A., Clarke, C., 2010. Trap geometry in three giant montane pitcher plant species from Borneo is a function of tree shrew body size. *New Phytol.* 186, 461–470.
- Clarke, C.M., Bauer, U., Lee, C.C., Tuen, A.A., Rembold, K., Moran, J.A., 2009. Tree shrew latratories: a novel nitrogen sequestration strategy in a tropical pitcher plant. *Biol. Lett.* 5, 632–635.
- Corbet, G.B., Hill, J.E., 1992. *The mammals of the Indomalayan region: A systematic review*. Oxford University Press, Oxford.
- Curry, J.R., 2005. Tectonics and history of the Andaman Sea region. *J. Asian Earth Sci.* 25, 187–228.
- Das, I., 2005. Revision of the genus *Cnemaspis* Strauch, 1887 (Sauria: Gekkonidae), from the Mentawai and adjacent archipelagos off western Sumatra, Indonesia, with the description of four new species. *Journal of Herpetology* 39, 233–247.
- Dene, H., Goodman, M., Prychodko, W., 1978. An immunological examination of the systematics of Tupaioidea. *J. Mammal.* 59, 697–796.
- Dene, H., Goodman, M., Prychodko, W., Matsuda, G., 1980. Molecular evidence for the affinities of Tupaiidae. In: Lockett, W.P. (Ed.), *Comparative Biology and Evolutionary Relationships of Tree Shrews*. Plenum Press, New York, pp. 269–291.
- Drummond, A., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* 7, 214.
- Drummond, A.J., Ho, S.W., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biol.* 4, e88.
- Eizirik, E., Murphy, W.J., Springer, M.S., O'Brien, S.J., 2004. Molecular phylogeny and dating of early primate divergences. In: Ross, C.F., Kay, R.F. (Eds.), *Anthropoid origins: New visions*. Kluwer Academic/Plenum Publishers, New York.
- Ellerman, J.R., Morrison-Scott, T.C.S., 1955. Supplement to Chasen (1940) "A handlist of Malaysian mammals" containing a generic synonymy and a complete index. *British Museum (Natural History)*, London.
- Emmons, L.H., 2000. *Tupaia: A field study of Bornean treeshrews*. University of California Press, Berkeley.
- Esselstyn, J.A., Oliveros, C.H., Moyle, R.G., Peterson, A.T., McGuire, J.A., Brown, R.M., 2010. Integrating phylogenetic and taxonomic evidence illuminates complex biogeographic patterns along Huxley's modification of Wallace's Line. *J. Biogeogr.* 37, 2054–2066.
- Esselstyn, J.A., Widmann, P., Heaney, L.R., 2004. The mammals of Palawan Island, Philippines. *Proc. Biol. Soc. Wash.* 117, 271–302.
- Gathorne-Hardy, F.J., Syaokani, Davies, R.G., Eggleton, P., Jones, D.T., 2002. Quaternary rainforest refugia in south-east Asia: using termites (Isoptera) as indicators. *Biol. J. Linn. Soc. Lond.* 75, 453–466.

- Gorog, A.J., Sinaga, M.H., Engstrom, M.D., 2004. Vicariance or dispersal? Historical biogeography of three Sunda shelf murine rodents (*Maxomys surifer*, *Leopoldamys sabanus* and *Maxomys whiteheadi*). *Biol. J. Linn. Soc. Lond.* 81, 91–109.
- Hall, R., 1996. Reconstructing Cenozoic Southeast Asia. In: Hall, R., Blundell, D. (Eds.), *Tectonic evolution in Southeast Asia*. Geological Society of London, London, pp. 153–184.
- Hall, R., 1998. The plate tectonics of Cenozoic SE Asia and the distribution of land and sea. In: Hall, R., Holloway, J.D. (Eds.), *Biogeography and geological evolution of SE Asia*. Backhuys Publishers, Leiden, pp. 99–131.
- Hall, R., 2001. Cenozoic reconstructions of Southeast Asia and the southwest Pacific: Changing patterns of land and sea. In: Metcalfe, I., Smith, J., Morwood, M., Davidson, I. (Eds.), *Faunal and floral migrations and evolution in SE Asia-Australia*. Swets and Zeitlinger, Lisse, The Netherlands, pp. 35–56.
- Hall, R., 2002. Cenozoic geological and plate tectonic evolution of Southeast Asia and the southwest Pacific: computer-based reconstructions, model, and animations. *J. Asian Earth Sci.* 20, 353–431.
- Han, K.-H., Sheldon, F.H., Stuebing, R.B., 2000. Interspecific relationships and biogeography of some Bornean tree shrews (Tupaiaidae: *Tupaia*), based on DNA hybridization and morphometric comparisons. *Biol. J. Linn. Soc. Lond.* 70, 1–14.
- Hedges, S.B., Dudley, J., Kumar, S., 2006. TimeTree: A public knowledge-base of divergence times among organisms. *Bioinformatics* 22, 2971–2972.
- Helgen, K., 2005. Order Scandentia. In: Wilson, D.E., Reeder, D.M. (Eds.), *Mammal Species of the World: A Taxonomic and Geographic Reference*, third ed. The Johns Hopkins University Press, Baltimore, pp. 104–109.
- Higgs, P.G., 2000. RNA secondary structure: Physical and computational aspects. *Quarterly Reviews of Biophysics* 33, 199–253.
- Ho, S.W., Phillips, M.J., 2009. Accounting for calibration uncertainty in phylogenetic estimation of evolutionary divergence times. *Syst. Biol.* 58, 367–380.
- Hutchinson, C.S., 2005. *Geology of North-West Borneo: Sarawak, Brunei and Sabah*. Elsevier, Amsterdam.
- Huxley, T.H., 1868. On the classification and distribution of the Alectoromorphae and Heteromorphae. *Proc. Zool. Soc. Lond.* 1868, 294–319.
- Inger, R.F., 1966. The systematics and zoogeography of the Amphibia of Borneo. *Fieldiana (Zool.)* 1–402.
- Inger, R.F., Voris, H.K., 2001. The biogeographical relations of the frogs and snakes of Sundaland. *J. Biogeogr.* 28, 863–891.
- IUCN, 2009. IUCN Red List of threatened species. Version 2009.1. <<http://www.iucnredlist.org/>>.
- Janecka, J.E., Miller, W., Pringle, T.H., Wiens, F., Zitzmann, A., Helgen, K.M., Springer, M.S., Murphy, W.J., 2007. Molecular and genomic data identify the closest living relative of primates. *Science* 318, 792–794.
- Kjer, K.M., Roshan, U., Gillespie, J.G., 2009. Structural and evolutionary considerations for multiple sequence alignment of RNA, and the challenges for algorithms that ignore them. In: Rosenberg, M.S. (Ed.), *Sequence alignment: Methods, models, concepts, and strategies*. University of California Press, Berkeley, pp. 105–150.
- Kobayashi, K., Wanichanon, C., 1992. Stereo architecture of the connective-tissue cores of the lingual papillae in the treeshrew (*Tupaia glis*). *Anat. Embryol.* 186, 511–518.
- Kvartalnov, P.V., 2009. Ecology and behavior of the slender-tailed tree-shrew (*Dendrogale murina*, Scandentia). *Zool. Zhurnal* 88, 1387–1395.
- Luckett, W.P., 1980. The suggested evolutionary relationships and classification of tree shrews. In: Luckett, W.P. (Ed.), *Comparative Biology and Evolutionary Relationships of Tree Shrews*. Plenum Press, New York, pp. 3–31.
- Lyon Jr., M.W., 1913. Treeshrews: an account of the mammalian family Tupaiidae. *Proc. US Natl. Mus.* 45, 1–188.
- MacArthur, R.H., Wilson, E.O., 1963. An equilibrium theory of insular zoogeography. *Evolution* 17, 373–387.
- MacArthur, R.H., Wilson, E.O., 1967. *The theory of island biogeography*. Princeton University Press, Princeton, NJ.
- Marshall, D.C., Simon, C., Buckley, T.R., 2006. Accurate branch length estimation in partitioned Bayesian analyses requires accommodation of among-partition rate variation and attention to branch length priors. *Syst. Biol.* 55, 993–1003.
- Mearns, E.A., 1905. Descriptions of new genera and species of mammals from the Philippine Islands. *Proc. US Natl. Mus.* 28, 425–460.
- Meijaard, E., 2003. Mammals of south-east Asian islands and their Late Pleistocene environments. *J. Biogeogr.* 30, 1245–1257.
- Meijaard, E., 2004. Solving mammalian riddles: A reconstruction of the Tertiary and Quaternary distribution of mammals and their palaeoenvironments in island South-East Asia. School of Archaeology and Anthropology, The Australian National University, Canberra, Australia, p. 349.
- Meimberg, H., Wistuba, A., Dittrich, P., Heubl, G., 2001. Molecular phylogeny of Nepenthaceae based on cladistic analysis of plastid trnK intron sequence data. *Plant Biology* 3, 164–175.
- Mein, P., Ginsburg, L., 1997. Les mammifères du gisement miocène inférieur de Li Mae Long, Thaïlande: systématique, biostratigraphie et paléoenvironnement. *Geodiversitas* 19, 783–844.
- Miller, K.G., Komazin, M.A., Browning, J.V., Wright, J.D., Mountain, G.S., Katz, M.E., Sugarman, P.J., Cramer, B.S., Christie-Blick, N., Pekar, S.F., 2005. The Phanerozoic record of global sea-level change. *Science* 310, 1293–1298.
- Miller, M.A., Holder, M.T., Vos, R., Midford, P.E., Liebowitz, T., Chan, L., Hoover, P., Warnow, T., 2009. The CIPRES portals. <[http://www.phylo.org/sub\\_sections/portal/](http://www.phylo.org/sub_sections/portal/)>. accessed March 2011.
- Moss, S.J., Wilson, M.E.J., 1998. Biogeographic implications of the Tertiary palaeogeographic evolution of Sulawesi and Borneo. In: Hall, R., Holloway, J.D. (Eds.), *Biogeography and Geological Evolution of SE Asia*. Backhuys Publishers, Leiden, The Netherlands, pp. 133–163.
- Munshi-South, J., 2008. Female-biased dispersal and gene flow in a behaviorally monogamous mammal, the large treeshrew (*Tupaia tana*). *PLoS One* 3.
- Murphy, W.J., Eizirik, E., 2009. Placental mammals (Eutheria). In: Hedges, S.B., Kumar, S. (Eds.), *The timetree of life*. Oxford University Press, Oxford, pp. 471–474.
- Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. *Nature* 403, 853–858.
- Nakagawa, M., Hyodo, F., Nakashizuka, T., 2007. Effect of forest use on trophic levels of small mammals: an analysis using stable isotopes. *Can. J. Zool.-Rev. Can. Zool.* 85, 472–478.
- Olson, L.E., Hassanin, A., 2003. Contamination and chimerism are perpetuating the legend of the snake-eating cow with twisted horns (*Pseudonovibos spiralis*). A case study of the pitfalls of ancient DNA. *Mol. Phylogenet. Evol.* 27, 545–548.
- Olson, L.E., Sargis, E.J., Martin, R.D., 2004. Phylogenetic relationships among treeshrews (Scandentia): A review and critique of the morphological evidence. *J. Mamm. Evol.* 11, 49–71.
- Olson, L.E., Sargis, E.J., Martin, R.D., 2005. Intraordinal phylogenetics of treeshrews (Mammalia: Scandentia) based on evidence from the mitochondrial 12S rRNA gene. *Mol. Phylogenet. Evol.* 35, 656–673.
- Oommen, M.A., Shanker, K., 2010. Shrewd alliances: mixed foraging associations between treeshrews, greater racket-tailed drongos and sparrowhawks on Great Nicobar Island, India. *Biol. Lett.* 6, 304–307.
- Paradis, E., Claude, J., Strimmer, K., 2004. APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics* 20, 289–290.
- Plummer, M., Best, N., Cowles, K., Vines, K., 2006. Coda: Output analysis and diagnostics for MCMC. R package version, 10–17.
- Pütz, J., Dupuis, B., Sissler, M., Florentz, C., 2007. Mamit-tRNA, a database of mammalian mitochondrial tRNA primary and secondary structures. *RNA* 13, 1184–1190.
- Quental, T.B., Marshall, C.R., 2010. Diversity dynamics: Molecular phylogenies need the fossil record. *Trends Ecol. Evol.* 25, 434–441.
- R Development Core Team, 2004. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rabosky, D., 2009. laser: Likelihood analysis of speciation/extinction rates from phylogenies. R package version 2.3.
- Rambaut, A., Drummond, A., 2007. Tracer v1.4. <<http://beast.bio.ed.ac.uk/Tracer>>.
- Roberts, T.E., 2006. Multiple levels of allopatric divergence in the endemic Philippine fruit bat *Haplopycterisischeri* (Pteropodidae). *Biol. J. Linn. Soc. Lond.* 88, 329–349.
- Roberts, T.E., Sargis, E.J., Olson, L.E., 2009. Networks, trees, and treeshrews: Assessing support and identifying conflict with multiple loci and a problematic root. *Syst. Biol.* 58, 257–270.
- Rodolfo, K.S., 1969. Bathymetry and marine geology of the Andaman Basin, and tectonic implications for Southeast Asia. *Geological Society of America Bulletin* 80, 1203–1230.
- Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572–1574.
- Roos, C., Ziegler, T., Hodges, J.K., Zischler, H., Abegg, C., 2003. Molecular phylogeny of Mentawai macaques: taxonomic and biogeographic implications. *Mol. Phylogenet. Evol.* 29, 139–150.
- Samuel, M.A., Harbury, N.A., Bakri, A., Banner, F.T., Hartono, L., 1997. A new stratigraphy for the islands of the Sumatran Forearc, Indonesia. *J. Asian Earth Sci.* 15, 339–380.
- Sargis, E.J., 2001a. The grasping behaviour, locomotion, and substrate use of the treeshrews *Tupaia minor* and *T. Tana* (Mammalia, Scandentia). *J. Zool. Lond.* 253, 485–490.
- Sargis, E.J., 2001b. A preliminary qualitative analysis of the axial skeleton of tupaiaids (Mammalia, Scandentia): functional morphology and phylogenetic implications. *J. Zool. Lond.* 253, 473–483.
- Sargis, E.J., 2002a. Function morphology of the forelimb of tupaiaids (Mammalia, Scandentia) and its phylogenetic implications. *J. Morphol.* 254, 149–185.
- Sargis, E.J., 2002b. Functional morphology of the hindlimb of Tupaiaids (Mammalia, Scandentia) and its phylogenetic implications. *J. Morphol.* 254, 149–185.
- Sargis, E.J., 2002c. The postcranial morphology of *Ptilocercus lowii* (Scandentia, Tupaiidae): an analysis of primate-morph and volitant characters. *J. Mamm. Evol.* 9, 137–160.
- Sargis, E.J., 2004. New views on treeshrews: The role of tupaiaids in primate supraordinal relationships. *Evol. Anthropol.* 13, 56–66.
- Sargis, E.J., 2007. The postcranial morphology of *Ptilocercus lowii* (Scandentia, Tupaiidae) and its implications for primate supraordinal relationships. In: Ravosa, M.J., Dagosto, M. (Eds.), *Primate Origins: Adaptations and Evolution*. Springer, New York, pp. 51–82.
- Schehka, S., Zimmermann, E., 2009. Acoustic features to arousal and identity in disturbance calls of tree shrews (*Tupaia belangeri*). *Behav. Brain Res.* 203, 223–231.
- Schipper, J., Chanson, J.S., Chiozza, F., Cox, N.A., Hoffmann, M., Katariya, V., Lamoreux, J., Rodrigues, A.S.L., Stuart, S.N., Temple, H.J., Baillie, J., Boitani, L., Lacher Jr., T.E., Mittermeier, R.A., Smith, A.T., Absolon, D., Aguiar, J.M., Amori, G., Bakkour, N., Baldi, R., Berridge, R.J., Bielby, J., Black, P.A., Blanc, J.J., Brooks, T.M., Burton, J.A., Butynski, T.M., Catullo, G., Chapman, R., Cokeliss, Z., Collen, B., Conroy, J., Cooke, J.G., da Fonseca, G.A.B., Derocher, A.E., Dublin, H.T., Duckworth, J.W., Emmons, L., Emslie, R.H., Festa-Bianchet, M., Foster, M., Foster, S., Garshelis, D.L., Gates, C., Gimenez-Dixon, M., Gonzalez, S., Gonzalez-Maya, J.F., Good, T.C., Hammerson, G., Hammond, P.S., Happold, D., Happold, M.,

- Hare, Harris, R.B., Hawkins, C.E., Haywood, M., Heaney, L.R., Hedges, S., Helgen, K.M., Hilton-Taylor, C., Hussain, S.A., Ishii, N., Jefferson, T.A., Jenkins, R.K.B., Johnston, C.H., Keith, M., Kingdon, J., Knox, D.H., Kovacs, K.M., Langhammer, P., Leus, K., Lewison, R., Lichtenstein, G., Lowry, L.F., Macavoy, Z., Mace, G.M., Mallon, D.P., Masi, M., McKnight, M.W., Medellín, R.A., Medici, P., Mills, G., Moehlman, P.D., Molur, S., Mora, A., Nowell, K., Oates, J.F., Olech, W., Oliver, W.R.L., Oprea, M., Patterson, B.D., Perrin, W.F., Polidoro, B.A., Pollock, C., Powel, A., Protas, Y., Racey, P., Ragle, J., Ramani, P., Rathbun, G., Reeves, R.R., Reilly, S.B., Reynolds III, J.E., Rondinini, C., Rosell-Ambal, R.G., Rulli, M., Rylands, A.B., Savini, S., Schank, C.J., Sechrest, W., Self-Sullivan, C., Shoemaker, A., Sillero-Zubiri, C., De Silva, N., Smith, D.E., Srinivasulu, C., Stephenson, P.J., van Strien, N., Talukdar, B.K., Taylor, B.L., Timmins, R., Tirira, D.G., Tognelli, M.F., Tsytsulina, K., Veiga, L.M., Vie, J.-C., Williamson, E.A., Wyatt, S.A., Xie, Y., Young, B.E., . The status of the world's land and marine mammals: diversity, threat, and knowledge. *Science* 322, 225–230.
- Schmidt, M., Schilling, N., 2007. Fiber type distribution in the shoulder muscles of the tree shrew, the cotton-top tamarin, and the squirrel monkey related to shoulder movements and forelimb loading. *J. Hum. Evol.* 52, 401–419.
- Schoniger, M., Von Haeseler, A., 1994. A stochastic model and the evolution of autocorrelated DNA sequences. *Mol. Phylogenet. Evol.* 3, 240–247.
- Sodhi, N.S., Koh, L.P., Brook, B.W., Ng, P.K.L., 2004. Southeast Asian biodiversity: an impending disaster. *Trends Ecol. Evol.* 19, 654–660.
- Springer, M.S., Douzery, E., 1996. Secondary structure and patterns of evolution among mammalian mitochondrial 12S rRNA molecules. *J. Mol. Evol.* 43, 357–373.
- Stamatakis, A., 2006. RaxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22, 2688–2690.
- Stamatakis, A., Hoover, P., Rougemont, J., 2008. A fast bootstrapping algorithm for the RaxML web-servers. *Syst. Biol.* 57, 758–771.
- Steele, D.G., 1973. Dental variability in the treeshrews (Tupaiaidae). In: Zingesser, M.R. (Ed.), *Craniofacial Biology of Primates: Symposium of the IVth International Congress of Primatology*. Karger, Basel, pp. 154–179.
- Steiper, M.E., Young, N.M., 2009. Primates (Primates). In: Hedges, S.B., Kumar, S. (Eds.), *The Timetree of Life*. Oxford University Press, Oxford, pp. 482–486.
- Steppan, S.J., Zawadski, C., Heaney, L.R., 2003. Molecular phylogeny of the endemic Philippine rodent *Apomys* (Muridae) and the dynamics of diversification in an oceanic archipelago. *Biol. J. Linn. Soc. Lond.* 80, 699–715.
- Swofford, D.L., 2002. PAUP\*: Phylogenetic Analysis Using Parsimony (\*and Other Methods). Sinauer and Associates, Sunderland, Massachusetts.
- Thomas, O., 1892. On some new Mammalia from the East-Indian Archipelago. *Ann. Mag. Nat. Hist.* 9, 250–254.
- Timmins, R.J., Duckworth, J.W., Robson, C.R., Walston, J.L., 2003. Distribution, status and ecology of the mainland slender-tailed treeshrew *Dendrogale murina*. *Mammal. Rev.* 33, 272–283.
- Tong, Y., 1988. Fossil tree shrews from the Eocene Hetaoyuan formation of Xichuan, Henan. *Vert. Palasiatica* 26, 214–220.
- Vinyard, C.J., Wall, C.E., Williams, S.H., Hylander, W.L., 2008. Patterns of variation across primates in jaw-muscle electromyography during mastication. *Integr. Comput. Biol.* 48, 294–311.
- Vinyard, C.J., Williams, S.H., Wall, C.E., Johnson, K.R., Hylander, W.L., 2005. Jaw-muscle electromyography during chewing in Belanger's treeshrews (*Tupaia belangeri*). *Am. J. Phys. Anthro.* 127, 26–45.
- von Weizsacker, F., Kock, J., MacNelly, S., Ren, S., Blum, H.E., Nassal, M., 2004. The tupaia model for the study of hepatitis B virus: Direct infection and HBV genome transduction of primary tupaia hepatocytes. *Methods Mol. Med.* 96, 153–161.
- Voris, H.K., 2000. Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. *J. Biogeo.* 27, 1153–1167.
- Wallace, A.R., 1860. On the zoological geography of the Malay Archipelago. *Zoological Journal of the Linnean Society of London* 4, 172–184.
- Wells, K., Pfeiffer, M., Lakim, M.B., Linsenmair, K.E., 2004. Use of arboreal and terrestrial space by a small mammal community in a tropical rain forest in Borneo, Malaysia. *J. Biogeo.* 31, 641–652.
- Wharton, C.H., 1950. Notes on the Philippine treeshrew, *Urogale everetti* Thomas. *J. Mammal.* 31, 352–354.
- Wiens, F., Zitzmann, A., Lachance, M.A., Yegles, M., Pragst, F., Wurst, F.M., von Holst, D., Guan, S.L., Spanagel, R., 2008. Chronic intake of fermented floral nectar by wild treeshrews. *Proc. Natl. Acad. Sci. USA* 105, 10426–10431.
- Willerslev, E., Cooper, A., 2004. Ancient DNA. *Proc. Roy. Soc. Lond., Ser. B: Biol. Sci.* 272, 3–16.
- Wilson, D.E., 1993. Order Scandentia. In: Wilson, D.E., Reeder, D.M. (Eds.), *Mammal Species of the World: A Taxonomic and Geographic Reference*, second ed. Smithsonian Institution Press, Washington, DC, pp. 131–133.
- Woodruff, D.S., Turner, L.M., 2009. The Indochinese–Sundaic zoogeographic transition: a description and analysis of terrestrial mammal species distributions. *J. Biogeo.* 36, 803–821.
- Ziegler, T., Abegg, C., Meijaard, E., Perwitasari-Farajallah, D., Walter, L., Hodges, J.K., Roos, C., 2007. Molecular phylogeny and evolutionary history of Southeast Asian macaques forming the *M. Silenus* group. *Mol. Phylogenet. Evol.* 42, 807–816.