

Short communication

Contrasting patterns of genetic differentiation between endemic and widespread species of fruit bats (Chiroptera: Pteropodidae) in Sulawesi, Indonesia

Polly Campbell^{a,b,*}, Andrea S. Putnam^c, Caitlin Bonney^{a,d}, Rasit Bilgin^e,
Juan Carlos Morales^f, Thomas H. Kunz^a, Luis A. Ruedas^g

^a Department of Biology, Boston University, Boston, MA 02215, USA

^b Department of Zoology, University of Florida, P.O. Box 118525, Gainesville, FL 32611, USA

^c Section of Ecology, Behavior, and Evolution, Division of Biological Sciences, University of California, San Diego, La Jolla, CA 92093, USA

^d Harvard School of Public Health, Boston, MA 02115, USA

^e Boğaziçi University, Institute of Environmental Sciences, Bebek 34342, Istanbul, Turkey

^f Division of Environmental Biology, National Science Foundation, Arlington, VA 22230, USA

^g Museum of Vertebrate Biology and Department of Biology, Portland State University, Portland, OR 97207, USA

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1. Introduction

The Indonesian island of Sulawesi is remarkable for its complex geological history and high biodiversity. Lying directly East of Wallace's original zoogeographic boundary between the Oriental and Australian Regions (Wallace, 1876), Sulawesi (Fig. 1) constitutes the largest terrestrial habitat in Wallacea, a biogeographical region prioritized as a global conservation hotspot (Myers et al., 2000). Oceanic isolation has played a significant role in the evolution of Wallacea's highly endemic fauna (Whitten et al., 1997); Sulawesi's considerable size (186,145 km²), complex topography and diversity of microclimates and habitats have provided ample opportunity for diversification and *in situ* speciation (Bridle et al., 2001). Molecular studies of Sulawesi's biota have found evidence for both historic vicariance and ecological selection as key factors driving diversification (Evans et al., 2004; Bridle et al., 2004). Geographically concordant patterns of genetic differentiation across disparate taxa (amphibians and monkeys, Evans et al., 2003a,b) support vicariance hypotheses, while low genetic divergence among ecologically differentiated congeners (shrews, Ruedi, 1995, 1998; grasshoppers, Walton et al., 1997;

Bridle et al., 2001) implicates evolutionarily recent adaptive radiation.

Although, the diversity of Old World fruit bats (Pteropodidae) is higher on Sulawesi than on the islands of the Sunda shelf and the Malay peninsula combined (Corbet and Hill, 1992), phylogenetic relationships among pteropodid bats in Wallacea are not well-defined at the species level, and neither broad-scale phylogeographic nor population-level genetic structure have been examined in any species of bat from Sulawesi. Here, we evaluate the phylogenetic status of *Cynopterus brachyotis* on Sulawesi, and compare the within-island genetic structure of this widespread fruit bat to that of a forest-restricted endemic, *Thoopterus nigrescens*.

Thoopterus nigrescens (67–99 g) is the sole member of a genus that is endemic to Sulawesi and parts of the Moluccan Island chain (Fig. 1), and is strongly associated with primary forest (Bergmans and Rozendaal, 1988). *Cynopterus brachyotis* (36–41 g) is common in disturbed habitats on Sulawesi (Bergmans and Rozendaal, 1988) and is the only Wallacean representative of a genus whose diversity in peninsular Malaysia and the Greater Sunda Islands suggests a Sunda shelf center of origin (Campbell et al., 2006). Although the range of *C. brachyotis* extends from Southwest India to Sulawesi (Corbet and Hill, 1992), recent phylogenetic analysis demonstrates that it comprises a complex of evolutionarily distinct lineages, including allopatric lineages from Sulawesi and the Philippines and two sympatric lineages from the Sunda shelf (*C. brachyotis* Forest and Sunda; Campbell et al., 2004).

* Corresponding author. Address: Department of Zoology, University of Florida, P.O. Box 118525, Gainesville, FL 32611, USA. Fax: +1 352 392 3704.

E-mail address: pcampbel@zoo.ufl.edu (P. Campbell).

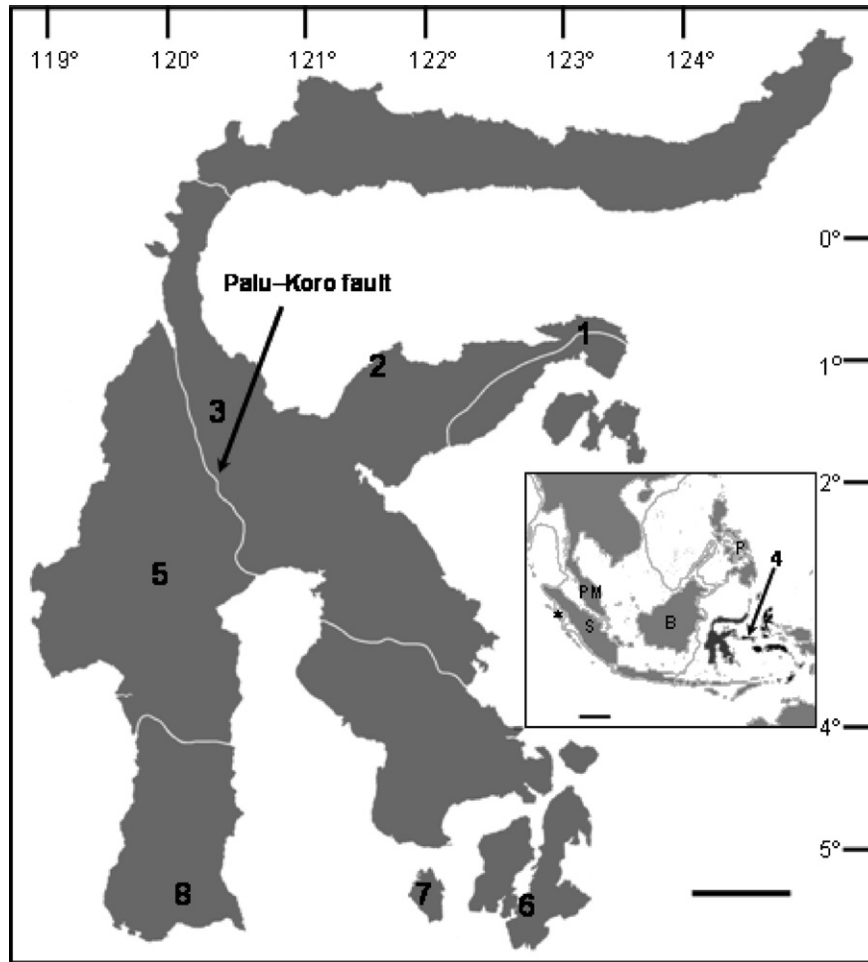


Fig. 1. Map of Sulawesi. Although currently a single island, Sulawesi was formed by accretion of a number of geologically distinct fragments. Major suture zones between fragments are shown in white. Scale bar on main map ≈ 100 km. Numbers refer to collecting localities in Table 1: 1, Siuna; 2, Poso; 3, Lore Lindu; 4, Mangole Isl. (indicated on inset); 5, Tana Toraja; 6, Buton Isl.; 7, Kabaena Isl.; 8, G. Lompobatang. Inset map localizes Sulawesi (dark gray) and the Moluccas (black) in Southeast Asia. The Sunda shelf (light grey outline) defines the approximate area of additional terrestrial habitat exposed when sea levels dropped to ≤ 120 m below present levels during Pleistocene glacial cycles. PM, peninsular Malaysia; S, Sumatra; B, Borneo; P, Philippines. Nias Island indicated by star. Scale bar on inset ≈ 1000 km.

Within Sulawesi, the taxonomic status of *C. brachyotis* remains uncertain; variation in body size has been interpreted as evidence that two allopatric populations (Bergmans and Rozendaal, 1988) or two sympatric species (Kitchener and Maharadatunkamsi, 1991) occur on the island. In a morphometric evaluation of Indonesian *Cynocephalus*, Kitchener and Maharadatunkamsi (1991) proposed that *C. brachyotis* is replaced on Sulawesi by *C. luzoniensis* and *C. minutus*, both considered synonyms of *C. brachyotis* (Corbet and Hill, 1992; Simmons, 2005), taxa respectively described from the Philippines and Nias Island, West Sumatra (see Fig. 1). This assessment has not been universally accepted (Corbet and Hill, 1992; Koopman, 1993, 1994, but see Simmons, 2005). While phylogenetic analysis of *Cynocephalus* recovered a single mitochondrial lineage from Sulawesi (Campbell et al., 2004) sampling was insufficient to evaluate the Kitchener and Maharadatunkamsi hypothesis.

In the present study, we use mitochondrial DNA (mtDNA) sequence data and nuclear microsatellites to address three main questions. (1) Was Sulawesi colonized by *C. brachyotis* from different geographic regions, as suggested by Kitchener and Maharadatunkamsi's taxonomic revision? Support for this scenario would include two Sulawesi lineages, with sister relationships to either Philippine, or Sunda shelf *C. brachyotis* clades. (2) Is there evidence that two evolutionarily distinct lineages or species occur currently on the island? Geographic overlap between reciprocally monophyletic lineages would provide strong support for this scenario. (3) Do *T. nigrescens* and *C. brachyotis* exhibit comparable or different levels of genetic structure within Sulawesi? Based on the endemism and higher habitat-specificity of *T. nigrescens*, we expected to find stronger population structure in this species relative to the geographically widespread habitat generalist, *C. brachyotis*.

2. Materials and methods

2.1. Sampling, PCR, sequencing and fragment analysis

Cynopterus brachyotis was sampled from three mainland sites on Sulawesi and two offshore islands; *Thoopterus nigrescens* was sampled from four mainland sites and one island (Fig. 1 and Table 1). Total genomic DNA was isolated from tissue samples (wing or liver) using Qiagen (Valencia, CA) DNeasy extraction kits. Approximately 640 base pairs (bp) of the 5' end of the mitochondrial (mtDNA) control region were sequenced for *C. brachyotis* ($n = 21$; see Table 1 for Genbank Accession Nos.). Primers, PCR conditions and sequencing reaction protocols are reported in Campbell et al. (2004); eight haplotypes from this earlier study were included in the present analysis.

For *T. nigrescens* ($n = 37$), complete control region was initially amplified using universal primers (Kocher et al., 1989). The sequences obtained were used to design internal primers, ThoopIntF: 5'-CCTGAAGTAAGAACCAGATG-3' and ThoopIntR: 5'-ACGGCATCTGGTTCTTTC-3', which amplified approximately 657 bp at the 5' end of the control region. Amplifications were carried out in a volume of 50 μ l containing 25 μ l of FailSafe PCR 2 \times PreMix E (Epicentre, Madison, WI), 0.15 mM of each primer and 0.5 μ l of FailSafe PCR enzyme mix. The thermal profile (94 °C for 30 s; 54 °C for 30 s; 72 °C for 1 min 30 s) was repeated for 35 cycles with an initial denaturation step at 94 °C for 2 min and a 2 min final extension at 72 °C. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen) and sequencing reactions were carried out using Big Dye terminator (Version 3.1, Applied Biosystems). Sequencing reaction products were run on an ABI Prism 3730 automated capillary sequencer (Applied Biosystems). Sequences were aligned and edited in SEQUENCHER (Version 4.0, GeneCodes Corp.).

Six microsatellite loci, originally isolated in *C. sphinx* (CSP-1, -3, -4, -5, -6, and -9; Storz, 2000), amplified in *C. brachyotis* ($n = 53$). CSP-1, -3, -5, -6, and -9 were retained for analysis as CSP-4 was monomorphic. PCR conditions were the same as those described in Campbell et al. (2006). Amplified products were run on an ABI Prism 3100 automated capillary sequencer; allele size was quantified and edited in GeneMapper (Version 3.7). A subset of the same loci (CSP-4–6), along with CSP-7, were polymorphic in *T. nigrescens* ($n = 38$). PCR reactions for *T. nigrescens* were carried out in a volume of 10 μ l with 0.5 mM of each primer, 2.5 mM MgCl₂, 2.0 mM dNTP's and 1 U of *Taq* DNA polymerase (Promega). The following thermal profile was repeated for 30 cycles with an initial denaturation step at 94 °C (2 min) and a final extension at 72 °C (2 min): 94 °C (30 s), 55 °C (30 s), and 72 °C (1 min 30 s). PCR products were run on an ABI 377 automated sequencer and fragment data were extracted and analyzed with GeneScan software (Version 3.1.2, Applied Biosystems).

2.2. Analyses—mitochondrial DNA

Intraspecific phylogenies were reconstructed using maximum parsimony (MP) and Bayesian criteria, implemented in PAUP* (Version 4.0b10; Swofford, 2002) and MRBAYES (Version 3.1.2; Huelsenbeck and Ronquist, 2001). MP analyses were run using a full heuristic search with tree-bisection-reconnection (TBR) branch-swapping and random stepwise addition (100 replicates). A *ca.* Seventy-seven base pairs deletion common to all *C. brachyotis* Sunda haplotypes (see below) was coded as a single synapomorphic character. All other gaps were treated as a fifth character state; all characters were weighted equally. Nodal support was evaluated with 1000 bootstrap replicates, each with five replicates of random taxon addition, a full heuristic search and TBR branch-swapping.

The *C. brachyotis* tree was rooted with *Megaerops ecaudatus*, a putative sister genus to *Cynopterus* (Jones et al., 2002; Giannini and Simmons, 2003). Previously published haplotypes from the Philippine-restricted *C. brachyotis* lineage, the Malaysian *C. brachyotis* Forest lineage, and the widespread *C. brachyotis* Sunda lineage (*sensu* Campbell et al., 2004) were included to evaluate the geographic origin of Sulawesi *C. brachyotis* (see Fig. 2 caption for Genbank Accession Nos.). For *T. nigrescens*, two putative sister taxa (Giannini and Simmons, 2003, 2005) were used as outgroups: *Aethalops alecto* and *Chironax melanocephalus*.

A likelihood ratio test was implemented in Modeltest (Version 3.06; Posada and Crandall, 1998) to find the best-fitting substitution model in each data set. Bayesian analyses were run under the selected GTR + Γ + I model of sequence evolution with default priors and model parameters estimated during the course of the run. Four MCMC chains were run for 2,000,000 generations. Average log-likelihood values at stationarity were compared for convergence among chains. Likelihood scores for the *C. brachyotis* and *T. nigrescens* data sets reached stationarity after approximately 200,000 generations; the consensus tree saved from the last 1,800,000 generations (post-burn-in) was used to estimate clade position probabilities.

Network representations of intraspecific genealogical relationships can provide an informative alternative to traditional phylogenetic methods, because the assumption of a strictly bifurcating gene tree is relaxed, allowing for persistence of ancestral haplotypes and reticulations resulting from recombination or homoplasy (Posada and Crandall, 2001). This approach is particularly relevant for a locus such as the control region with a high potential for homoplasy due to the rapid accumulation of mutations. We constructed a network for Sulawesi *C. brachyotis* using the statistical parsimony method (Templeton et al., 1992), implemented in TCS (Version 1.21; Clement et al., 2000) with the 10 step criterion and 95% confidence intervals. Divergence within *T. nigrescens* was too high to construct a network for this species with adequate confidence.

Genetic distances among populations and lineages were calculated in MEGA (Version 3.1, Kumar et al., 2004) under

Table 1
Localities, GenBank Accession Nos. and voucher catalogue numbers for *C. brachyotis* and *T. nigrescens* from Sulawesi

#	Locality, elevation, <i>n</i>	Lat. S	Long. E	GenBank Accession Nos.	Museum cat. #/ Collector ID
1	Bangai Dist., Siuna, 130 m <i>C.b.</i> , <i>n</i> = 7 <i>T.n.</i> , <i>n</i> = 1	0°44'25"	123°0'1"	<i>C.b.</i> , DQ777814–19 <i>T.n.</i> , DQ830488	<i>C.b.</i> , NK80083, NK80135, NK80137, NK80151, NK80165, NK80173, NK80177; <i>T.n.</i> , MSB93208
2	Poso Dist., Marowo, 10 m <i>C.b.</i> , <i>n</i> = 3	0°57'	121°27'	<i>C.b.</i> , DQ77820–22	NK80044, NK80048, NK80065, NK80073
3	Palu Dist., Lore Lindu National Park, 1050 m <i>C.b.</i> , <i>n</i> = 8 <i>T.n.</i> , <i>n</i> = 15	01°19'47"	120°2'4"	<i>C.b.</i> , DQ77823–24, AY629008, ^a AY629096, ^a AY629097, ^a AY629101, ^a AY629102, ^a AY629103 ^a ; <i>T.n.</i> , DQ829420, DQ829422, DQ829424, DQ822075, DQ822079, DQ829710, DQ822067, DQ822099, DQ822473, DQ822525, DQ830060, DQ822529, DQ822531, DQ822803	<i>C.b.</i> , PSUT24, PSUT25, PSUT43, PSUT56, PSUT195, PSUT196, PDX45, PDX49; <i>T.n.</i> , 48LIPI, 51LIPI, 52LIPI, 53LIPI, 85LIPI, 123LIPI, 127PSU, 129PSU, 131PSU, 132LIPI, 133PSU, 134LIPI, 148PSU, 160PSU, 161LIPI
4	Sula Arch., Mangole Isl., <100 m <i>T.n.</i> , <i>n</i> = 1	01°45'0"	125°50'0"	<i>T.n.</i> , DQ822911	BZM 15232-33
5	Central Sulawesi Prov., Tana Toraja, 2,150 m <i>T.n.</i> , <i>n</i> = 19	02°54'8"	119°41'50"	<i>T.n.</i> , DQ822897, DQ822903, DQ822805, DQ830388, DQ830394, DQ822809, DQ830396, DQ830386, DQ830390, DQ830472, DQ830476, DQ822905, DQ830480, DQ822907, DQ822901, DQ830482, DQ830484, DQ830486, DQ 822909	NK103513–14, NK103541, NK104009, NK104016, NK104028–29, NK104041–42, NK104049, NK104051–52, NK104054, NK104059, NK104061–62, NK104070, NK104074–75
6	Bau Bau, Buton Isl., <100 m <i>C.b.</i> , <i>n</i> = 6	05°10'12"	122°50'14"	<i>C.b.</i> , DQ77826–30, AY629094 ^a	None
7	S.E. Sulawesi Prov., Kabaena Isl., <100 m <i>C.b.</i> , <i>n</i> = 5	5°25'	122°0'	<i>C.b.</i> , DQ77831–32, AY629095 ^a	None
8	S. Sulawesi Prov., G. Lompobatang 1700 m <i>T.n.</i> , <i>n</i> = 1	05°23'24"	119°55'12"	<i>T.n.</i> , DQ822913	NK80014

C.b., *Cynopterus brachyotis*; *T.n.*, *Thoopterus nigrescens*; *n*, sample sizes for control region sequences; NK, Museum of Southwestern Biology, Albuquerque, NM; PSUT and PDX, Portland State University Museum of Vertebrate Biology, OR; LIPI and PSU specimens deposited at the National Museum of Indonesia, Cibinong; BZM, Bogor Zoological Museum, Indonesia.

Locality numbers correspond to locations on maps in Fig. 1.

^a Campbell et al., 2004.

the Tamura–Nei model of nucleotide substitution (Tamura and Nei, 1993) using the gamma distributions estimated in MrBayes (*C. brachyotis*, $\alpha = 0.44$; *T. nigrescens*, $\alpha = 0.30$). Pairwise estimates of population differentiation based on uncorrected genetic distances among haplotypes (Φ_{ST}) were calculated in ARLEQUIN (Version 3.01; Excoffier et al., 2005) for populations with sample sizes ≥ 3 . Based on the observed lack of mitochondrial or nuclear differentiation among *Cynopterus* from Buton and Kabaena Islands, samples from these sites were treated as a single population. Correlation between geographic and genetic distance (isolation by distance) was tested in *C. brachyotis* using the ISOLDE program in GENEPOP (Version 3.4, Raymond and Rousset, 1995). Linearized Φ_{ST} was regressed on straight-line distances between populations (Rousset, 1997). Insufficient sample sizes precluded this analysis for *T. nigrescens*.

Diversity was estimated by θ , which is based on the number of nucleotide polymorphisms (Watterson, 1975), and π , the average pairwise divergence per site (Tajima, 1983).

2.3. Analyses—microsatellites

Tests for linkage disequilibrium and departures from Hardy–Weinberg equilibrium were implemented in FSTAT (Version 2.9.3.2; Goudet, 1995). F_{ST} was calculated in ARLEQUIN and a test for isolation by distance in *C. brachyotis* was carried out in GENEPOP as for the mtDNA data set, with linearized F_{ST} regressed on geographic distance. We used the genotypic clustering program, STRUCTURAMA (Huelsenbeck et al., in press) to further evaluate population differentiation. STRUCTURAMA uses a Bayesian clustering approach to assign individuals to *K* populations, where the

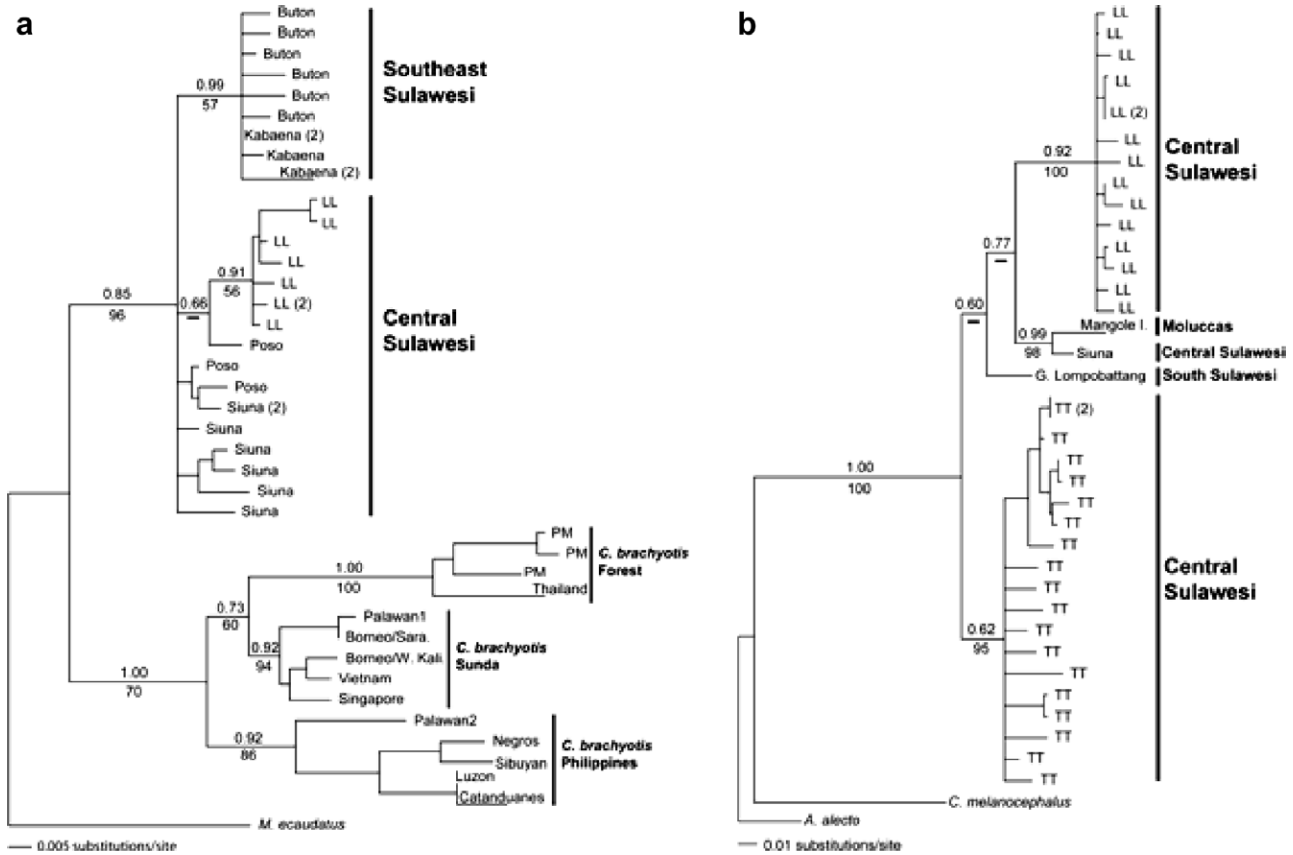


Fig. 2. Phylograms representing the consensus trees found using 2,000,000 generations of MCMC sampling in MRBAYES (Huelsenbeck and Ronquist, 2001) for (a) *Cynopterus brachyotis* and (b) *Thoopterus nigrescens*, under a GTR + Γ + I model of nucleotide substitution with parameters estimated during the course of the run. Note that branch lengths for the two phylograms are not on the same scale. Numbers above branches supporting main clades are posterior probabilities; numbers below branches are parsimony bootstrap values based on 100 bootstrap replicates, each with five replicates of random taxon addition, a full heuristic search and TBR branch-swapping. Tip labels correspond to sampling localities, LL, Lore Lindu; TT, Tana Toraja; PM, peninsular Malaysia; Sara, Sarawak; W. Kali, West Kalimantan; numbers in parentheses denote number of haplotypes. *Thoopterus nigrescens* tree rooted with *Aethalops alecto* and *Chironax melanocephalus* (Genbank Accession Nos. AY629149 and AY629150), *C. brachyotis* tree rooted with *Megaerops ecaudatus* (AY629151). Genbank Accession Nos. for Philippine, Sunda and Forest *C. brachyotis* lineages are: AY629024, AY6290047, AY629049, AY629051, AY629066, AY629090, AY629093, AY629099, AY629100, AY629104, AY629105 (Campbell et al., 2004), and AY974394, AY974429, AY974450 (Campbell et al., 2006).

posterior probabilities of observing the data given alternative values of K can be estimated with or without prior assumptions of population structure. Individuals are assigned to populations such that the squared distance among sampled partitions is minimized, where partition distance is the minimum number of individuals that must be deleted from the assignment vector to make the two partitions the same (Huelsenbeck et al., in press). Runs of 10^6 iterations were performed for *C. brachyotis* and *T. nigrescens*. Analyses for both species were run using a random variable with a Dirichlet process prior for K and prior means of the number of populations evaluated at 1, 2, 5, and 10.

3. Results

3.1. *Cynopterus brachyotis*

Mean percent nucleotide diversity in *C. brachyotis* was 1.6 (Supplementary Table 1) with 113 parsimony-informative sites. We found no evidence for multiple geographic

origins for *Cynopterus* on Sulawesi, or for strongly differentiated lineages within the island. Genetic distance among populations was low (mean $2.7\% \pm SD 0.4\%$) and both Bayesian and MP analyses recovered a single, well-supported Sulawesi clade (posterior probability = 0.85, bootstrap = 96). The Bayesian consensus tree is shown in Fig. 2a. Differences between Bayesian and MP topologies were limited to the positions of haplotypes within the four *C. brachyotis* clades included in the analysis (e.g., Sulawesi, Philippines, Sunda and Forest). Within the Sulawesi clade, the only notable difference between the two analyses was the basal placement of the Lore Lindu subclade under MP, but not Bayesian criteria. Monophyly of both the Southeast (Buton/Kabaena) and Lore Lindu subclades was well-supported by Bayesian posterior probability (0.99 and 0.91) but received low parsimony bootstrap support (57 and 56). A sister relationship between Sulawesi, and Sunda shelf plus Philippine *C. brachyotis* was recovered in both analyses but was poorly supported. The haplotype network constructed under statistical parsimony recovered

the similar relationships within Sulawesi, with Buton/Kabaena haplotypes grouped in one basal cluster, Lore Lindu haplotypes in a second cluster and Siuna plus two Poso haplotypes in a third (Supplementary Fig. 1). The Poso haplotype placed basal to the Lore Lindu clade on the Bayesian tree (Fig. 2a) could not be placed in the network with 95% confidence. The single reticulation in the network occurred within the Lore Lindu population, suggesting that homoplasmy is not a significant factor in this data set.

We identified 2–9 alleles per microsatellite locus with a small number of private alleles found at each sampling site: two each at Buton/Kabaena and Lore Lindu, one each at Poso and Siuna. None of the loci exhibited significant linkage disequilibrium or departures from Hardy–Weinberg equilibrium.

There was no correlation between genetic and geographic distance for either mtDNA ($P = 0.3$, $R^2 = 0.305$) or nuclear microsatellites ($P = 0.4$, $R^2 = 0.308$). Φ_{ST} values for mtDNA ranged from 0.061 to 0.578; all population pairs except Poso–Siuna were significantly differentiated (Table 2). Pairwise F_{ST} values for microsatellites ranged from 0.077 to 0.109, with the Siuna population significantly differentiated from all other populations (Table 2). However, genotypic clustering analysis consistently grouped all Sulawesi genotypes in a single cluster with maximum posterior probability estimates obtained for $K = 1$.

3.2. *Thoopterus nigrescens*

Mean percent nucleotide diversity in *T. nigrescens* was 1.75 (Supplementary Table 1) with 142 parsimony-informative sites. In contrast to *C. brachyotis*, *T. nigrescens* exhibited considerable genetic structure: mean genetic distance among sample sites was 7.9% (\pm SD 1.9%), genetic distance between the Tana Toraja and Lore Lindu populations was 8.7%. Under both Bayesian and MP criteria, haplotypes from Tana Toraja and Lore Lindu were split into distinct clades. The Bayesian consensus tree is shown in Fig. 2b. Posterior probability and bootstrap support for the Lore Lindu clade was high (0.92 and 100, respectively). The Tana Toraja clade was well-supported in the MP (bootstrap = 100), but not the Bayesian analysis (pos-

terior probability = 0.62). The placement of single haplotypes from other Sulawesi localities was poorly supported in both analyses: Gunung Lompobattang and Mangole Island plus Siuna were basal to the Lore Lindu clade in the Bayesian analysis and to the Tana Toraja clade under parsimony. Genetic distance between Mangole and Siuna haplotypes was comparatively low (3.7%) with a well-supported sister relationship in both analyses.

We identified 2–9 alleles per microsatellite locus; two alleles were private to Lore Lindu and six were private to Tana Toraja. No linkage disequilibrium was detected between loci but CSP-7 exhibited a significant heterozygote deficit in the Tana Toraja population ($P < 0.001$). Both mitochondrial and nuclear differentiation between the Lore Lindu ($n = 15$) and Tana Toraja ($n = 20$) populations was highly significant, with a Φ_{ST} value of 0.73 for the control region and F_{ST} of 0.48 for microsatellites (both, $P < 0.0001$).

The clustering analysis of microsatellite genotypes obtained maximum posterior probability scores for $K = 2$, with all genotypes from the Lore Lindu population assigned to cluster 1 and all Tana Toraja genotypes assigned to cluster 2. Mangole Island and Siuna genotypes were assigned to cluster 1; the G. Lompobattang genotype was assigned to cluster 2.

4. Discussion

4.1. Taxonomy and phylogeography of Sulawesi *C. brachyotis*

Concordance between mitochondrial and nuclear markers strongly suggests that the *C. brachyotis* complex is represented in Sulawesi by a single lineage. Although we were unable to resolve the biogeographic origin of the Sulawesi mitochondrial lineage, recovery of a single well-supported Sulawesi clade suggests that multiple geographic origins for Sulawesi *C. brachyotis* are unlikely.

While a Sulawesi–Philippines dispersal route has been inferred for older taxonomic groups such as birds, butterflies (Holloway and Jardine, 1968; Holloway, 1987), frogs (Emerson et al., 2000; Evans et al., 2003b) and flowering plants (Balgooy, 1987), Borneo–Philippines and Borneo–Sulawesi dispersals are implicated in the more recent diversification of mammals (Musser and Heaney, 1992; Ruedi et al., 1998; Evans et al., 1999; Lucchini et al., 2005). If *C. brachyotis* colonized Sulawesi via Borneo, transient dispersal opportunities existed from the early Pliocene (ca. 5.2 million years ago [MYA]) until the end of the Pleistocene (ca. 18,000 years ago), when maximal drops in sea level associated with glacial cycles likely reduced the shortest distance across the Makassar Strait to <50 km (Voris, 2000). Support for this hypothesis awaits more rigorous sampling of Indonesian *C. brachyotis*, particularly in Eastern Borneo, and South and North Sulawesi.

Capture records suggest that the Sulawesi lineage is ecologically similar to the *C. brachyotis* Sunda and Philippine lineages, both of which are most common in disturbed

Table 2
Pairwise population differentiation values for *Cynopterus brachyotis* sampled from four localities in Sulawesi

Population (<i>n</i>)	Buton/Kabaena (11)	Lore Lindu (8)	Poso (3)	Siuna (7)
Buton/Kabaena (12)		0.578***	0.409**	0.456***
Lore Lindu (12)	0.072		0.472**	0.507***
Poso (6)	0.060	0.037		0.061
Siuna (23)	0.109***	0.086**	0.063*	

Mitochondrial Φ_{ST} values and sample sizes (*n*) are above the diagonal, microsatellite F_{ST} values are below with sample sizes in the first column.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

habitats (Bergmans and Rozendaal, 1988; Heideman and Heaney, 1989; Heaney et al., 1989; Campbell et al., 2006). However, given that the Sulawesi lineage is restricted to a faunistically unique and geographically isolated region, it clearly deserves recognition as an evolutionarily significant unit (e.g., Moritz, 1994). Determining whether the Sulawesi *Cynopterus* lineage warrants species status awaits data from more slowly evolving mitochondrial and nuclear markers and re-evaluation of relevant type specimens. Should taxonomic revision be warranted, we suggest that *Cynopterus minor* Revilliod, 1911 (type locality: Lambuja, SE Sulawesi) is the available name for the Sulawesi *Cynopterus*. The findings of this study support the view that *C. luzoniensis* (*sensu* Kitchener and Maharadatunkamsi, 1991) is not appropriate, since the type locality associated with the name is in the Philippines, and Philippine and Sulawesi *Cynopterus* lineages are reciprocally monophyletic (Campbell et al., 2004). We suggest that *C. minutus* is also inappropriate because it implies lack of differentiation from Sunda shelf *C. brachyotis*, a relationship refuted by the monophyly of the Sulawesi mitochondrial lineage relative to both *C. brachyotis* Forest and Sunda (Fig. 2a).

4.2. Comparative population structure of Sulawesi *Cynopterus brachyotis* and *Thoopterus nigrescens*

Cynopterus brachyotis is tolerant of human disturbance on Sulawesi and belongs to a genus which has successfully colonized most of Southeast Asia and India, while *T. nigrescens* is a primary forest-associated endemic whose presence on Sulawesi likely predates the arrival of *C. brachyotis*. Our results support the prediction that these differences in ecology and evolutionary history should produce contrastingly lower genetic structure in *C. brachyotis* relative to *T. nigrescens*. However, the depth of mitochondrial divergence and concordant differentiation at nuclear microsatellites in *T. nigrescens* was unexpected. Notably, the Tana Toraja and Lore Lindu populations in Central Sulawesi are only 180 km apart but exhibit 8.7% control region divergence, are reciprocally monophyletic (Fig. 2b) and strongly differentiated at nuclear microsatellite loci. The spatial proximity of the two sites suggests that population differentiation is not an artifact of incomplete geographic sampling.

While no present-day barrier to gene flow exists, the dynamic geological history of Sulawesi provides several potential scenarios for vicariance among *T. nigrescens* populations. For example, Lore Lindu and Tana Toraja are on opposite sides of the NNW-SSE running Palu-Koro fault bisecting Central Sulawesi (Bellier et al., 2006; Fig. 1). The fault marks a suture zone between the Eastern and Western Sulawesi blocks and was a region of high tectonic activity from the early Miocene through the early Pleistocene (ca. 23–1.5 MYA), including a period of major montane uplift in the early Pliocene (ca. 5 MYA; Hall, 2002). We note, however, that no such barrier has been inferred

from the phylogeographic structure of Sulawesi macaques or the Sulawesi toad, sampled from Central Sulawesi sites on either side of the Palu-Koro fault (Evans et al., 2003a). Likewise, although the single samples from the three other sites preclude strong phylogeographic inference, control region divergence of $\geq 7\%$ among all four mainland Sulawesi sites, relative to 3.7% between sites separated by a long-standing oceanic barrier (Mangole Island and Siuna), suggests a pattern of low gene flow among Sulawesi *T. nigrescens* populations, for which vicariance is one of several potential explanations. Strong population structure can also result from aspects of species ecology or social behavior (Worthington Wilmer et al., 1994; Irwin, 2002; Lacey and Wieczorek, 2004). Concordance between nuclear and mitochondrial markers rules out structure due to sex-biased dispersal or strong male reproductive skew; however, other factors such as low natal dispersal in both sexes, small home range size or dependence on rare roost types might also reduce gene flow.

It is also possible that *T. nigrescens* comprises two or more evolutionarily distinct lineages. Numerous other mammal genera have given rise to morphologically and, in some cases, ecologically diagnosable species within Sulawesi (macaques, *Macaca*, Evans et al., 1999; shrews, *Crociodura*, Ruedi et al., 1998; rodent genera *Taeromys*, *Bunomys*, *Paruromys*, *Maxomys*, and *Rattus*, Corbet and Hill, 1992; L. Ruedas pers. obs.). While cursory analysis of general measures of body size found no significant differences between Tana Toraja and Lore Lindu *T. nigrescens* populations (Putnam, 2004), between-site altitudinal differences of >1000 m (see Table 1) provide ancillary evidence that the two populations may be ecologically differentiated. Determining whether genetic divergence within *T. nigrescens* on Sulawesi is indicative of low gene flow among populations isolated by historic or behavioral barriers, or of deeper differentiation among demographically and, possibly, ecologically distinct lineages, will require additional sampling along both latitudinal and altitudinal gradients, and analysis of slower evolving molecular markers in conjunction with morphometric and ecological data.

In *C. brachyotis*, the low level of population differentiation for microsatellites compared to mtDNA (Table 2) and lack of genotypic clustering compared to mitochondrial monophyly of the geographically isolated Buton/Kabaena clade (Fig. 2a), are consistent with the smaller effective population size and consequently shorter time to coalescence for mtDNA (Avice, 2000). Likewise, the lack of correlation between genetic and geographic distance for either mitochondrial or nuclear markers suggests that *C. brachyotis* populations have not reached demographic equilibrium, as is reasonable if colonization is an evolutionarily recent event (Slatkin, 1993). It is possible, however, that an effect of isolation by distance would be detected with more thorough geographic sampling (e.g., Bridle et al., 2004).

Taken together, the results of this study contribute to knowledge of the diversity of Old World fruit bats in a unique biogeographic region where prior studies of this

group have been limited to inclusion of single samples in deep phylogenies (Colgan and Flannery, 1995; Colgan and da Costa, 2002; Giannini and Simmons, 2003, 2005). Our finding of a monophyletic *Cynopterus* lineage in Sulawesi highlights the importance of comparing multiple lines of evidence when redefining species distributions or renaming species. High differentiation within *T. nigrescens* suggests that further molecular studies of this species, and of other endemic bats in Sulawesi, are likely to reveal additional unrecognized diversity.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.ymp.2007.02.013](https://doi.org/10.1016/j.ymp.2007.02.013).

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