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Systematics and biogeography of the arcuate horseshoe bat species complex (Chiroptera, Rhinolophidae)

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The present study sheds light on species delimitation in what has been previously described as *Rhinolophus arcuatus*, a morphologically conservative bat species complex nominally distributed throughout archipelagic South-East Asia from New Guinea to Sumatra. Given that rhinolophids tend to be relatively weak fliers, hence have low vagility, we hypothesized that some specimens attributed to *R. arcuatus*, but originating from geographically disjunct populations, may in fact represent distinct species. To test this hypothesis, we examined specimens attributed to *R. arcuatus* as well as to other species in the *Rhinolophus euryotis* species group using both morphological techniques and mitochondrial cytochrome *b* and control region sequences. Careful morphological analysis reveals heretofore cryptic but nevertheless distinct, species-level morphological differences among specimens derived from geographically isolated locations. Furthermore, molecular data illuminate the existence of several species-level sequence divergences among specimens heretofore attributed to *R. arcuatus*. These analyses similarly suggest the existence of additional species in other South-East Asian *Rhinolophus* taxa previously considered monotypic. We suggest at least one description to be undertaken of a previously unrecognized species as well as the elevation of several others from sub-specific to specific status.

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Introduction

Horseshoe bats of the genus *Rhinolophus* Lacépède, 1799 (Chiroptera: Rhinolophidae) are mostly insectivorous and distributed throughout the Old World – primarily in the tropical regions – from Africa, through Europe, to Australasia. Most of the ca. 80 species (Koopman 1994; Csorba *et al.* 2003; Simmons 2005) are specialized for seeking prey from a perch, although a few are known to glean (Norberg & Rayner 1987). The wing morphology of rhinolophids is therefore constrained by their foraging strategies: most species have short, rounded wing tips and are capable of highly manoeuvrable flight at slow speeds (Norberg & Rayner 1987). The common name for this genus comes from a specialized horseshoe-shaped structure on the rostrum, thought to direct or focus echolocation calls produced in the nasal cavities. This horseshoe structure and associated lancet, connecting process and sella structures, as well as overall size, have been used to good effect in order to distinguish among species (Peters 1871; Hill 1959, 1988, 1992; Hill & Schlitter 1982; Heaney *et al.* 1998; Csorba *et al.* 2003; Guillén-Servent *et al.* 2003). However, recent research has shown that *Rhinolophus* contains many morphologically cryptic, previously unrecognized species and that changes in echolocation call frequency are often the mechanism for speciation (Cooper *et al.* 1998; Csorba *et al.* 2003; Kingston & Rossiter 2004; Sun *et al.* 2008; Sedlock & Weyandt 2009; Chattopadhyay *et al.* 2010). The morphological conservatism pervading this genus has complicated taxonomic and phylogenetic reconstruction, resulting in conflicting phylogenetic trees over the years; indeed, even within the same book, authors of different sections have chosen to follow different species taxonomic arrangements (Csorba *et al.* 2003; Guillén-Servent *et al.* 2003)!

The focus of the present work is the putative species *Rhinolophus arcuatus*, a member of the *Rhinolophus euryotis* group, with particular focus on *R. arcuatus* from Sulawesi. Originally described from Luzon Island in the Philippines

(Peters 1871), *R. arcuatus* as currently recognized has a range extending throughout numerous islands in South-East Asia (Fig. 1): the species is distributed from New Guinea in the east to Sumatra in the west, and as far north as Luzon (Hill 1992; Csorba *et al.* 2003). However, as the range has expanded, so have taxonomic uncertainties concerning this species. Ingle & Heaney (1992), for example, noted the presence of two discrete morphotypes in the Philippines, namely *R. arcuatus* small and *R. arcuatus* large, but did not formally describe them. Sedlock & Weyandt (2009) further demonstrated the presence of two genetically (3–4% sequence divergence) and morphologically distinct morphotypes ('wide' and 'narrow' noseleaf), attributed to *R. arcuatus* from Mt. Banahaw, Luzon. One of the present authors (LAR) also noted a third discrete morphotype occurring sympatrically with *R. arcuatus* small and *R. arcuatus* large at one site on Luzon Island, suggesting the likely presence of at least three to five species on Luzon alone.

Prior to field work undertaken by LAR in 2000 and 2002, there was a single record of *R. arcuatus* from Sulawesi (Hill 1988): a specimen captured near Poso, Central Sulawesi, and attributed to *R. a. proconsulis* (Hill 1988). *Rhinolophus a. proconsulis* was originally described from specimens captured in Gua Bungoh, Bau, 1st Division, Sarawak, Borneo (Hill 1959). Subsequently, Hill (1992) modified his taxonomic opinion of the Sulawesi *R. arcuatus*, and Csorba *et al.* (2003) followed this opinion, both concluding that the Sulawesi specimen likely constituted at least a new subspecies of *R. arcuatus*.

Given the complex geological history of South-East Asia and that many species in *Rhinolophus* are relatively weak fliers, it is possible – even likely – that populations on different islands and island groups throughout the range of *R. arcuatus*, and attributed to that species, could in fact represent species distinct from, but morphologically cryptic with, *R. arcuatus*. As such, the putative species *R. arcuatus* may therefore represent a morphologically cryptic species complex remarkably rich in potential for biogeographic studies. Here, we test the hypothesis that *R. arcuatus* are conspecific throughout their range and, rejecting this hypothesis based on morphological and molecular analyses, propose the elevation of a subspecies to species level, further suggesting that there are several previously unrecognized species in the complex.

Materials and methods

Fieldwork

Whole specimens of *Rhinolophus* collected in Sulawesi by LAR and coworkers in 2000 and 2002 were preserved in 95% ethanol; specimens were deposited in the Museum of Vertebrate Biology at Portland State University, the Museum of Southwestern Biology at the University of New Mexico, and the National Museum of Indonesia (NMI, formerly Museum Zoologicum Bogoriense). The NMI specimens were not examined as part of this work. Information on collection localities is provided in Appendix I for specimens used in genetic analyses, and Appendix S1 for specimens examined in morphological analyses. *Rhinolophus* collected in the Philippines by LAR and co-workers in 1992–1993 and deposited at

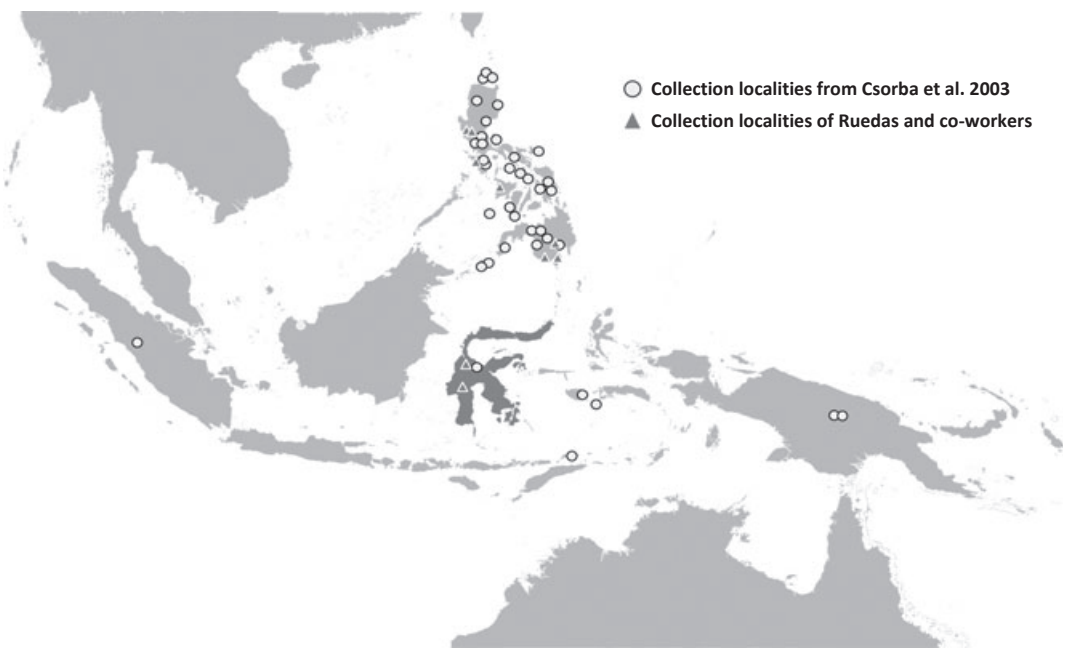


Fig. 1 Distribution map of *Rhinolophus arcuatus* showing published collection locations with circles and collection locations of LAR and coworkers in triangles. Sulawesi is indicated in dark grey.

the Cincinnati Museum of Natural History and Science were also examined in this study (Appendices I and S1).

Morphometrics

A total of 52 skull and wing measurements and indices were used in this study, following Freeman (1981) and Csorba *et al.* (2003). These are illustrated in Fig. 2 and described in the Supporting Information (Data S1).

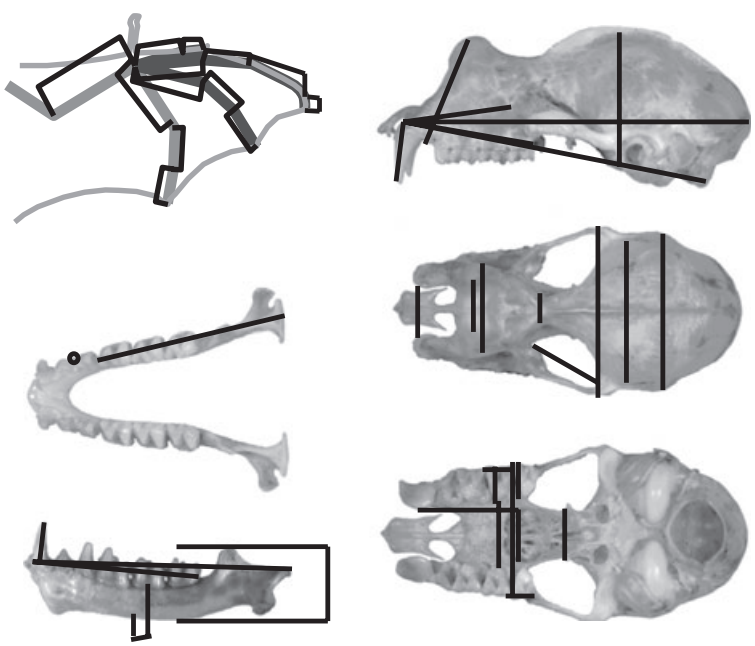
A total of 128 specimens were measured (Appendix S1), including the holotype and paratypes of *R. a. proconsulis* from Borneo, the original specimen from Sulawesi attributed by Hill to *R. a. proconsulis*, the Sulawesi *R. arcuatus* specimens collected by LAR, and numerous *R. arcuatus* specimens from the Philippines.

Measurements were taken either with digital calipers (Fowler) or from high-quality digital images using ImagePro Plus (version 4.5.0.19, Media Cybernetics, Inc, Rockville, MD USA). Log-transformed skull and wing data were analysed both separately and in combination using principal components analysis (PCA) in the SAS statistical package (Release 9.1, SAS Institute Inc., Cary, NC USA). Three-dimensional graphs of the first three principal components were produced using SigmaPlot (version 9.01; Systat Software Inc., San Jose, CA, USA).

Molecular markers and sequence data

DNA was extracted from a ca. 2 mm^3 sample of skeletal muscle or liver, or from a ca. 3 mm^2 section of wing membrane. Total genomic DNA (gDNA) was extracted using a DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol. The mitochondrial cytochrome *b* (Cyt*b*) gene and control region (CR) were sequenced as follows: the polymerase chain reaction (PCR) was carried out in an Illustra PuRe Taq Ready-To-Go PCR bead tube (GE Healthcare, Piscataway, NJ, USA) containing 3 μL gDNA (ca. 200 ng) and 2.5 μM of each primer, to a final volume of 25 μL. Primer sequences and thermal cycling profiles are shown in Table S1. PCR product was run on a 2% Agarose E-gel (Invitrogen, Grand Islans, NY, USA) and visualized under ultraviolet light. Selected amplicons were then purified using a Qiaquick PCR Purification Kit (Qiagen) or by excising the band from the gel and purifying it using a Qiaquick Gel Extraction kit (Qiagen) following the manufacturer's instructions. Cycle sequencing reactions were performed in a 10 μL total volume containing 2.5 μL of purified PCR product and 0.25 μM sequencing primer in a BigDye version 3.1 reaction mixture (Applied Biosystems, Foster City, CA, USA). Sequencing primers were the same as PCR primers. The thermal protocol for the sequencing reaction was 96 °C for 5 min and 25 cycles of 96 °C for 10 s, 50 °C for 5 s and 60 °C for 4 min. Sequencing was performed on an ABI 3100 Automated DNA Sequencer (Applied Biosystems).

A total of 111 individuals were sequenced for these analyses. A list of specimens and sequences used is provided in Appendix I and includes samples from the following locations: Sulaw-



Also: Tail, hind foot, tibia, aspect ratio, tip index, Digit3:Digit5

Fig. 2 Morphological measurements taken from each specimen. Details and names of measurements can be found in Data S1.

esi, Indonesia; Papua New Guinea (PNG); Cambodia; Luzon, Panay, Mindoro and Mindanao Islands of the Philippine Islands. In particular, we included sequences from *R.* '*arcuatus*', *R. euryotis*, and *Rhinolophus celebensis* from Sulawesi, *R. arcuatus* from near the type locality in Luzon, and *Rhinolophus* species in the *euryotis* group from the Philippines. Hereafter, we will use quotation marks in the body of the text to refer to potentially incorrect species identifications made by previous collectors.

Phylogenetic analyses

Sequences were aligned initially using MEGALIGN (version 6.1, DNA* Lasergene 6, Madison, WI, USA). Monophyly of Rhinolophidae was assumed and *Hipposideros armiger*, *H. diadema*, *H. pomona*, and *H. pratti* (Hipposideridae) were chosen as out-groups. The Cyt*b* gene and CR were analysed separately with a Bayesian inference approach using MRBAYES (version 3.1.2; Huelsenbeck & Ronquist 2001). Both genes were analysed individually for 2×10^7 generations, with trees sampled every 2000 generations. Burn-in was assessed visually and trees preceding the asymptote of the log likelihood scores' curve were discarded. In addition, GARLI (ver. 2.0; Zwickl 2006) was used to infer separate maximum-likelihood trees for each gene. GARLI was run until several searches found the same best tree. One thousand bootstrap replicates were performed for each tree.

For the CR, we used the HKY+I+Γ model of evolution. This model was chosen using MRMODELTEST (version 2.3; Nylander 2004) based on the Akaike information criterion (AIC). Individual parameters were – lnL = 6199.4316; AIC = 12410.8633; base frequencies: A = 0.3707; C = 0.2385; G = 0.1092; T = 0.2817; ti/tv ratio = 4.0971; proportion of invariable sites = 0.3104; gamma distribution shape parameter = 0.7655. For the Cyt*b* gene, MrModeltest selected GTR+I+Γ model of evolution based on the AIC. Model parameters were – lnL = 9336.3975; AIC = 18692.7949; base frequencies: A = 0.3241; C = 0.3849; G = 0.0890; T = 0.2020; rate matrix: A–C = 0.4927; A–G = 14.2629; A–T = 0.6273; C–G = 0.3700; C–T = 8.7184, G–T = 1.0000; proportion of invariable sites = 0.5011; gamma distribution shape parameter = 0.9840. A genetic distance matrix based on the GTR+I+Γ model of evolution was calculated from the Cyt*b* sequence data using PAUP (v. 4.0b10; Swofford 2000) for the clades of interest to show both inter- and intra-specific genetic distances (Table 1). Trees were assessed visually using TREEVIEW (v. 1.6.6; Page 1996) and FIGTREE (v.1.3.1, Andrew Rambaut, Institute of Evolutionary Biology, University of Edinburgh, UK). Specimens that grouped together in distinct clades were given clade designations (Tables 1 and 2) and were used particularly in population genetic analyses.

Population genetic structure and diversity

For all population genetic analyses, 'populations' were defined by clade designation, as determined in the phylogenetic analyses described above. Some clades represent separate species (i.e. *R. euryotis*), while many clades belong to the *R. arcuatus* complex (Table 2). This use of clades will capture genetic structure among populations regardless of whether *R. arcuatus* from different islands are in fact separate populations of a single species. While clade status was determined in phylogenetic analyses that used as input the same sequence data set as was used for population genetic analyses, we nevertheless gain additional insight into relationships among clades by determining within and among clade diversity and structure. To fully exploit all available sequence data, we used three separate sequence data sets as input: (i) Cyt*b* sequences only (ca. 925 bp), (ii) CR sequences only (ca. 420 bp), and (iii) Cyt*b* and CR sequences concatenated (ca. 1300 bp). We did this because while the majority of Cyt*b* and CR sequences were from the same tissue samples (specimens), in some cases, we had sequences either for Cyt*b* or CR, but not both, for a given specimen (Appendix I and Table 2). We determined genetic structure and diversity, and tested for selective neutrality using settings described below (Excoffier *et al.* 2006). All analyses of genetic structure and diversity were run in ARLEQUIN 3.11 (Excoffier *et al.* 2005).

Genetic diversity was calculated using multiple diversity indicators: (i) gene diversity 'G', calculated from the probability that two randomly chosen haplotypes are different, (ii) nucleotide diversity 'N', calculated as the probability that two randomly selected homologous sites are different, assuming no recombination and selective neutrality (Tajima 1983, 1993; Nei 1987), (iii) number of polymorphic sites '*S*' (Nei 1987), (iv) mean number of pairwise differences between every pair of haplotypes, 'π' (distance method: Tamura & Nei). Finally, we calculated two measures of theta ($\theta = 2$ Mu, where $M = 2N$ for diploid populations of size N and u is the overall mutation rate at the haplotype level): Theta *S* and Theta Pi. Theta *S* (θ_S) is calculated assuming an infinite-site equilibrium between number of segregating sites (S), the sample size (n) and θ, as well as no recombination (Tajima 1989). Theta Pi (θ_π) is estimated from the infinite-sites equilibrium relationship between the mean number of pairwise differences (π) and theta (θ).

We tested for selective neutrality under the infinite allele model using Tajima's *D* (Tajima 1989, 1996) and Fu's F_S (Fu 1997) – these analyses both assume no recombination, which is probably appropriate for short mtDNA sequences. Significant Tajima's *D* values can be the result of recent population expansion or bottlenecks, as well as selective effects. Fu's *F* statistic is also very sensitive to demographic expansion, which usually results in large negative *F* values. For all of these analyses, the observed data were tested against a null distribution of the test statistic generated by 16,000 simulations (significance level: 5%). However, the

Table 1 Partial genetic distance matrix calculated under GTR+I+Γ model of evolution showing mean percent sequence divergence and range in parentheses for cytochrome *b*. Clades are shown in Appendix I and Table 2. Bold font indicates intraclade genetic distance, missing for *D* due to this being a single distance between two individuals

Clade	A	B	C	D	E	F	G	H	I	J
A	**0.405 (0–1.4)**									
B	0.925 (0.48–1.69)	**0.283 (0.08–0.68)**								
C	2.003 (1.24–3.06)	1.961 (1.41–2.74)	**0.361 (0.16–0.53)**							
D	1.472 (1.07–2.18)	1.530 (1.17–2.32)	2.106 (1.68–2.89)	**0.488**						
E	3.190 (2.34–7.27)	3.131 (2.61–5.28)	3.227 (2.51–5.09)	2.538 (2.26–2.96)	**0.409 (0.08–0.81)**					
F	3.665 (2.94–4.81)	4.096 (3.8–4.47)	3.992 (3.53–4.82)	2.842 (2.6–3.08)	4.176 (3.78–4.65)	**0.329 (0.2–0.4)**				
G	4.995 (3.73–6.12)	5.114 (4.29–5.99)	5.163 (4.55–5.48)	3.555 (3.04–3.91)	5.895 (4.92–8.68)	4.970 (4.14–5.58)	**1.258 (0.27–2.22)**			
H	4.533 (3.62–6.5)	4.488 (3.82–6.19)	4.710 (3.91–6.07)	3.205 (2.85–3.66)	4.708 (4.32–5.34)	4.267 (3.89–4.68)	5.178 (4.33–6.00)	**0.291 (0.08–0.57)**		
I	3.729 (2.77–5.96)	3.603 (2.76–4.58)	4.143 (3.27–5.9)	2.332 (1.67–2.78)	4.082 (3.51–4.73)	3.434 (2.75–4.04)	6.206 (4.9–7.75)	2.219 (1.78–2.68)	**0.487 (0.32–0.62)**	
J	5.380 (4.83–6.79)	5.478 (5.07–6.53)	5.697 (5.27–6.52)	4.149 (3.99–4.31)	5.231 (4.92–5.92)	5.437 (5.34–5.63)	6.759 (6.25–7.31)	5.034 (4.89–5.39)	5.340 (5.13–5.71)	.

Table 2 List of islands found within each island group and the corresponding number of sequences and haplotype identifications (IDs) from that island

Island Group	Island	Sequence ID	CR Clade	Cyt*b* Clade
Papua New Guinea	Papua New Guinea	AF065090	K	Not used
		AF065091	K	Not used
		AF065089	J	Not used
Sulawesi	Sulawesi	MSB93099	H	H
		MSB93100	H	H (PDX50)
		PDX50	H	H (MSB93100)
		PSUT67	H	H
		PSUT68	H	Not used
		PSUT72	H	H
		PSUT73	H (RCD3811)	H
		PSUT74	H	H
		RCD3811	H (PSUT73)	H
		0182	I	Not used
		0183_CR	I (B10_CR)	I
		417	I	I
		421	I (B11_CR)	I
		B10_CR	I (0183_CR)	Not used
		B11_CR	I (421)	Not used
		C03_CR	I	Not used
		C09_CR	I	Not used
		418	Not used	I
Philippine Islands	Luzon	M49	B (M376/M1068)	A (M376/M1068)
		M376	B (M49/M1068)	A (M49/M1068)
		M377	C	D
		M50	E	A (M357/M159)
		M3	E	Not used
		M43	E	Not used
		M12	F	A
		M159	F (M357)	A (M50/M357)
		M357	F (M159)	A (M50/M159)
		M31	F (M348)	A
		M348	F (M31)	A
		M57	F	A
		M52	F	A (M53)
		M1	Not used	A (M353/GQ..69)
		M53	Not used	A (M52)
		M352	F	A
		M353	F	A (M1/GQ..69)
		M369	F	A
		M386	F	A
		184_CR	F	A
		M127	D	B
		M137	D	B
		M16	D	Not used
		M17	D (M195)	B
		M64	D	B (M195)
		M195	D (M17)	B (M64)
		GQ368669	Not used	A (M1/M353)
		GQ368675	Not used	A
		GQ368676	Not used	A
		GQ368683	Not used	F
		GQ368688	Not used	F
		GQ368686	Not used	F
	Panay	M461	C	A

Table 2 Continued

Island Group	Island	Sequence ID	CR Clade	Cyt*b* Clade
		M616	Not used	A (M514/M462)
		M514	C	A (M462/616)
		M525	C (M462)	Not used
		M545	C	A
		M462	C (M525)	A (M514/M616)
		M540	F	A
	Mindoro	M1068	B (M49/M376)	A (M49/M376)
	Mindanao	M1576	G	C
		M1494	G	C
		M1726	Not used	C
		M1461	A (M1634)	Not used
		M1462	A (M1651)	E
		M1463	A	E
		M1341	A	Not used
		M1651	A (M1462)	E
		M1634	A (M1461)	E
		M1457	A	Not used
		M1797	M	Not used
Cambodia	Cambodia	2005–81–7	L	J

Clade designations were determined using Bayesian analyses of control region (CR) and cytochrome *b* (Cyt*b*) sequences, respectively. Each ID occupying a unique row has a unique haplotype definition unless another ID is included within parentheses in either the CR Clade or Cyt*b* Clade columns, in which case the haplotype for that sequence is shared with the sequence indicated. For example, the only sequence from Mindoro Island shares a haplotype with Luzon Island. Samples were 'Not used' in cases where sequences were obtained for only CR or only Cytb, but not both.

Fu's F statistic should be considered significant at the 5% level if the P-value is below 0.02, and not 0.05 (Fu 1997; Excoffier *et al.* 2006). In order to determine genetic structure among clades, we ran a standard analysis of molecular variance (AMOVA, Weir & Cockerham 1984; Excoffier *et al.* 1992) and a locus by locus AMOVA. With no missing data, these two analyses should produce the same results. AMOVA takes the number of mutations between haplotypes into account when testing genetic structure of clades. For all AMOVAs, clades were clustered based on geography to form the following groups (where sequence availability permitted): Philippine Islands (clades located on Luzon, Panay, Mindoro or Mindanao), Sulawesi, PNG, and Cambodia. A Euclidean square distance matrix was used for AMOVA computations (distance method: Tamura & Nei) and to create a minimum spanning tree (MST, Rohlf 1973). The AMOVA is a non-parametric analysis in which haplotypes are permuted among populations (clades), or among population groups (geographic location, described above), to obtain a null distribution against which the empirical/observed data are tested for significance. We also computed pairwise F_{ST} among clades using Tamura & Nei distances (Tamura & Nei 1993). Significance for the AMOVA and pairwise F_{ST} was determined using null distributions calculated from 16,000 permutations with a significance level of 0.05.

Molecular dating and calibration

To simultaneously co-estimate phylogeny and divergence times within our Rhinolophoidea data set, we used a Bayesian Markov chain Monte Carlo (MCMC; Metropolis *et al.* 1953; Hastings 1970) method for performing relaxed molecular phylogenetic analysis, as implemented in the program BEAST (v. 1.5.1; Drummond *et al.* 2006; Drummond & Rambaut 2007). Molecular clocks were calibrated by assuming that the cladogenesis between lineages leading to *Hipposideros* and *Rhinolophus* occurred 45 million years ago (MYA). This assumption was based on a number of factors; we assumed (following Sigé 1990) that †*Vaylatsia* Sigé, 1990, Late Eocene and Oligocene of Europe, is either a descendant or early sister group to *Rhinolophus*. *Rhinolophus* sensu stricto is present in the Middle Eocene and another early rhinolophid, †*Palaeonycteris* Pomel 1854 (*nec* †*Palaeonycteris* Weber & Abel 1928, †Picrodontidae *incertae sedis*) in the Upper Oligocene. Like *Rhinolophus*, *Hipposideros* sensu stricto is present in the Middle Eocene. Other acknowledged hipposiderids are likewise present from the Eocene, including †*Palaeophyllopora* Revilliod, 1917 (Middle Eocene to Late Miocene) and †*Paraphyllophora* Revilliod, 1922 (Upper Eocene to Late Oligocene). Thus by the Middle Eocene, hipposiderid and rhinolophid lineages were well established. Using a midpoint of 40 MYA for the Middle Eocene, we extrapolated that cladogenesis between the two lineages should have preceded the established presence in the fossil record of quantiose genera or species in the two families by at least 5 MYA, hence our estimate of 45 MYA.

BEAST runs were conducted using substitution rates, proportion of invariable positions and gamma shape parameters obtained using MrModeltest under the GTR+I+Γ model of evolution based on the AIC, as described above (see Phylogenetic analyses). We used a relaxed lognormal molecular clock model, which has been shown to generate accurate estimates of rates with narrow highest posterior density (HPD) intervals (Drummond *et al.* 2006; Ho 2007) and a Yule prior to simulate the process of speciation, with reciprocal monophyly of the in-group (Rhinolophidae) and out-group (Hipposideridae) assumed *a priori*. Operators were tuned in successive runs of the program, with slight changes to the scaling factors until the effective sample size for each parameter exceeded 200 (Drummond & Rambaut 2007). Once optimum operator scaling was achieved, we ran the analysis twice for 2×10^7 generations (saving every 1000th generation) discarding approximately the first 10% of generations as burn-in (Shapiro *et al.* 2011), assuring that stationarity had been reached by visual inspection of the graphed output; 2×10^7 generations resulted in acceptable mixing as diagnosed using TRACER (v. 1.5; Rambaut & Drummond 2004). Convergence of the two BEAST runs likewise was assessed in TRACER.

Results

Morphology

When skull and wing data were analysed together, the first three principal components (PC) accounted for 84.5% of variation in the data (PC1: 62%, PC2: 17.5%, PC3: 5%). Eigenvectors for all measurements are given in Table S2. *Rhinolophus* '*arcuatus*' from Sulawesi show no overlap in multivariate morphological space with *R. arcuatus* from the Philippines (Fig. 3). It is also noteworthy that Bornean *R. 'a.' proconsulis* (including the holotype) are morphologically distinct from Philippine *R. arcuatus*, from the original Sulawesi *R. 'a.' proconsulis* specimen [listed by Hill (1959) as *R. proconsulis*], and from more recent specimens of *R. 'arcuatus*' collected from the highlands of Sulawesi's Central Core region. However, the original Sulawesi *R. 'a.' proconsulis* specimen is morphologically similar to more recent specimens of putative *R. 'a'. proconsulis* from Sulawesi.

Molecular markers and sequence data

A Cyt*b* genetic distance matrix for the primary clades of interest is shown in Table 1. Genetic divergence among clades of the *R. euryotis* group is ca. 4% (range: 0.48–7.75%) for the Cyt*b* gene.

Bayesian trees derived from analysis of the molecular data are shown in Fig. 4. For both genes, all recent specimens of *R. 'arcuatus*' from Sulawesi are closely related to each other, forming a monophyletic clade (clade H for both data sets) with high support values [Cyt*b*: 1.00 posterior probability (pp) and 0.99 bootstrap support (bs); CR: 1.00 pp and 0.86 bs]. Surprisingly, the sister taxon to *R. 'arcuatus*' from Sulawesi is *R. 'euryotis*' also from Sulawesi, rather than nominal *R. arcuatus* from the Philippines, with high nodal support (Cyt*b*: 1.00 pp and 0.96 bs; CR: 0.97 pp and 0.64 bs). Specimens from the Philippines do form a well-supported clade (clades A–G for both Cyt*b* and CR; Cyt*b*: 0.95 pp and 0.34 bs; CR: 1.00 pp and bs); however, taxa from each Philippine island are not monophyletic. The *R. euryotis* group proposed by Csorba *et al.* (2003) and Guillén-Servent *et al.* (2003) is upheld (Cyt*b*: 1.00 pp and 0.9 bs; CR: 1.00 pp and bs), with *R. arcuatus*, *R. euryotis*, and *Rhinolophus shameli* forming a monophyletic group.

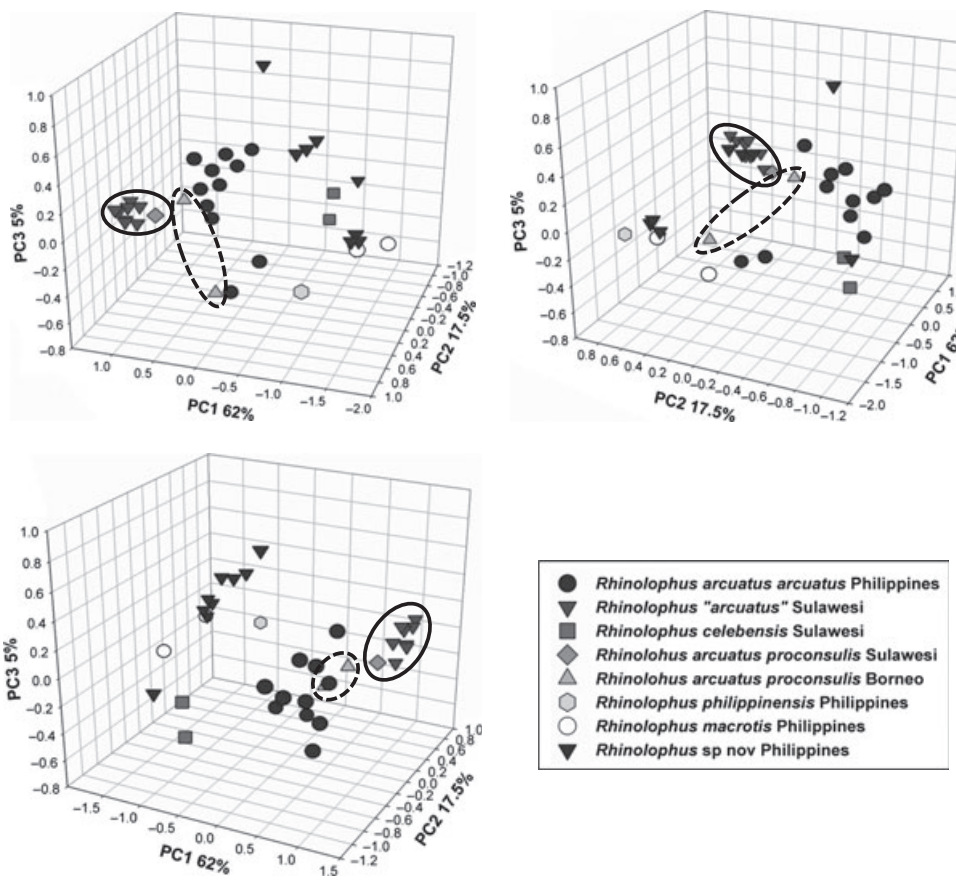


Fig. 3 Principal components analysis graphs for skull and wing data combined. All three graphs show the same data from different angles. The solid circle encloses the *Rhinolophus "arcuatus"* and *R. 'a.' proconsulis* specimens from Sulawesi. The dashed circle surrounds the specimens attributed to *R. 'a.' proconsulis* from Borneo.

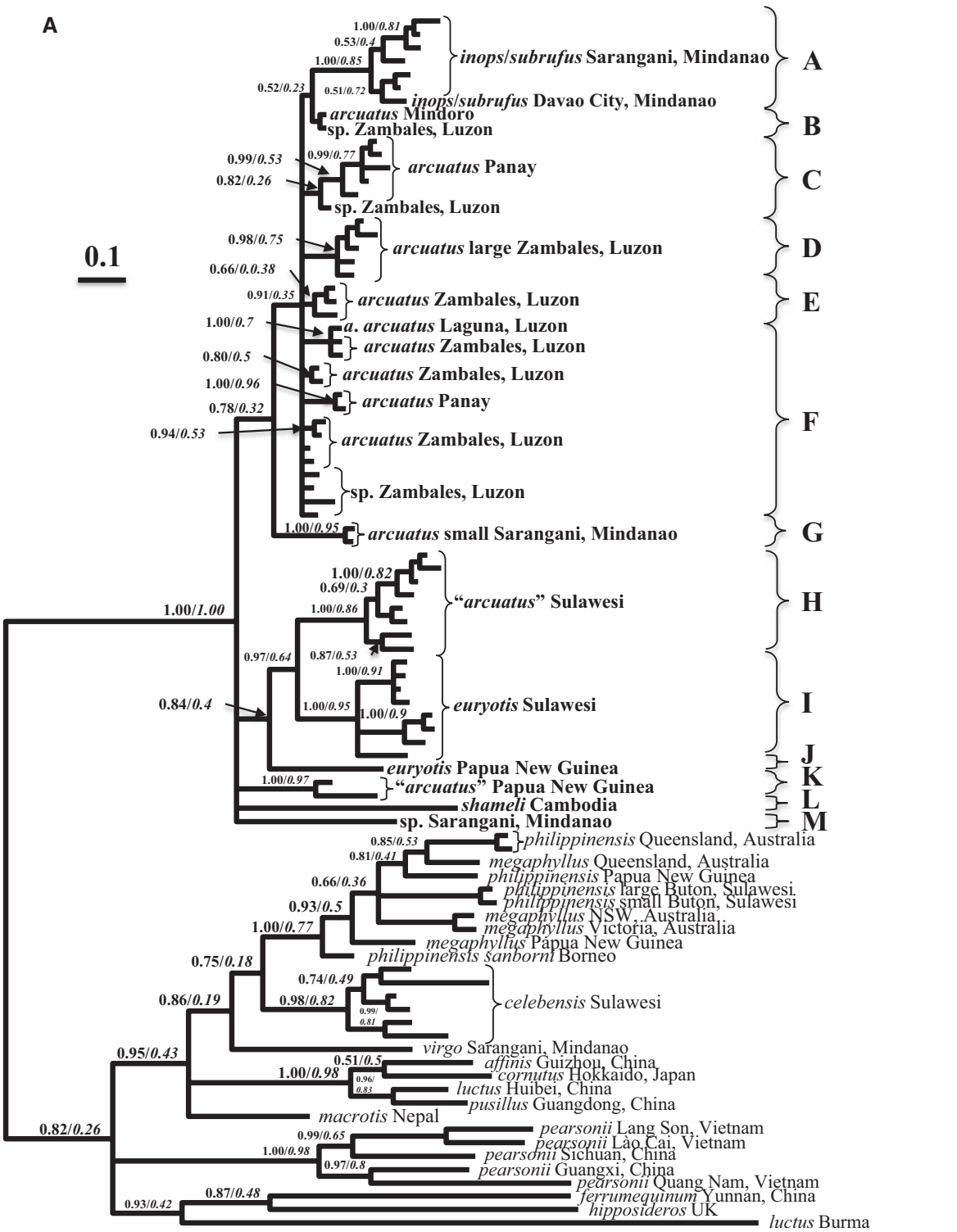


Fig. 4 Rhinolophus phylogenies inferred using Bayesian inference. Non-italicized numbers at nodes indicate Bayesian posterior probabilities while italicized numbers are maximum-likelihood bootstrap values. A. Control region tree. Lettered clades correspond to the letters in Appendix I. B. Cytochrome *b* tree. Lettered clades correspond to the letters in Table 1.

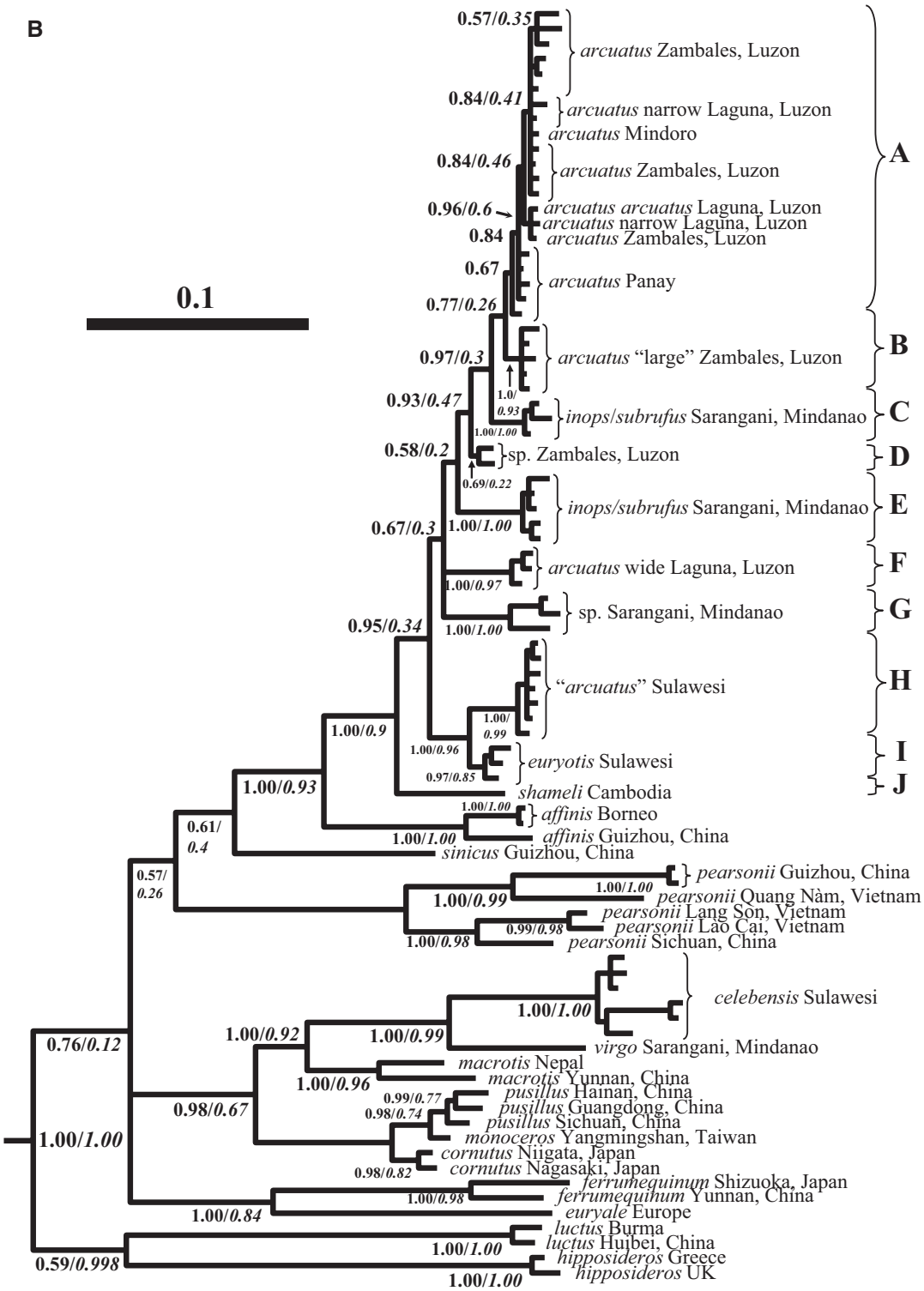


Fig. 4 (Continued).

Finally, *R. celebensis* is clearly distinct from remaining Sulawesi *Rhinolophus* and most closely associated with *R. borneensis*.

In the phylogenetic tree resulting from analysis of the CR (Fig. 4A), *R. euryotis* and *R.* '*arcuatus*' from PNG are not as closely related to each other as are *R.* '*arcuatus*' and *R.* '*euryo-*

tis' from Sulawesi: the latter two are sister taxa, with PNG *R. euryotis* basal in the same clade; PNG *R.* '*arcuatus*' have in contrast an unresolved relationship, using this marker, with a number of *arcuatus* group species from the Indo-Pacific. *Rhinolophus philippinensis* and *Rhinolophus megaphyllus* are likewise paraphyletic and share a clade with *R. celebensis* from Sulawesi. Interestingly, in the tree produced using Cyt*b* sequences (Fig. 4B), two samples tentatively identified as *Rhinolophus affinis* from Borneo and another *R. affinis* sequence from GenBank groups with the *R. euryotis* group, although *R. affinis* is a member of the *R. megaphyllus* group.

Population genetic diversity and neutrality tests

Not all sequences used in the population genetic analyses represented unique haplotypes (Table 2). Among the Philippine Islands clades, there were fewer haplotypes than sequences for clades from Luzon and Mindoro Islands. The single specimen from Mindoro (M1068) shared a common haplotype with two specimens from Luzon (M49, M376) at both CR and Cyt*b*. Also, clade designations varied between Cyt*b* and CR, which is not unexpected given the varying rates of evolution for these mitochondrial markers.

Genetic diversity measures are shown in Table 3 for all sequence data sets (Cyt*b* only, CR only, Cyt*b* + CR). For concatenated Cyt*b* + CR sequences, the two Sulawesi clades (Clade H/*R.* '*arcuatus*', and particularly Clade I/*R.* '*euryotis*') showed the highest levels of molecular diversity as indicated by nucleotide diversity and other measurements. For the Cyt*b* only data set, Clade I (Sulawesi *R.* '*euryotis*') had particularly high diversity, while Clade H (Sulawesi *R.* '*arcuatus*'), although showing high levels of genetic diversity, did not stand out from other clades. For the CR only data set, Clade K (PNG *R.* '*arcuatus*'), Clade I (Sulawesi *R.* '*euryotis*') and Clade H (Sulawesi *R.* '*arcuatus*') stand out with particularly high diversity measurements. The CR sequence data set was the only one that included samples from PNG. In general, measurements of genetic diversity were much lower with Cyt*b* only data (average molecular diversity across clades: 3.23, average nucleotide diversity across clades: 0.003) than when using only CR sequences (average molecular diversity: 6.34, average nucleotide diversity: 0.015). This is congruent with the hypothesis that the CR evolves more rapidly than Cyt*b*.

Results for tests of neutrality are summarized in Table 3. Because both tests are based on polymorphism within clades, we could not determine selective neutrality for clades represented by single samples. For the Cyt*b* + CR data set, the Clade F (CR)/Clade A (Cyt*b*) complex (Luzon/Panay: *R. arcuatus*) was negative and significantly different from neutrality (P-value < 0.05) for both Tajima's D (–1.37) and Fu's F_S (–2.72). Three other clades were not significantly different from neutral expectations using Fu's F_S, but were marginally non-neutral using Tajima's D: (i) the Clade D (CR)/Clade B (Cyt*b*) complex (*R. arcuatus* large) from Luzon (Tajima's D: 0.44), (ii) the Clade A (CR)/Clade E (Cyt*b*) complex (*Rhinolophus inops/subrufus*) from Mindanao (0.74), and (iii) the Clade H (CR)/Clade H (Cyt*b*) complex (*R.* '*arcuatus*') from Sulawesi (–0.33). Using the CR sequence data set, again Clade F (Luzon/Panay: *R. arcuatus*) exhibited significant departure from neutrality for both Tajima's D (–1.12) and Fu's F_S (–4.56). Four other CR clades were significantly non-neutral for Tajima's D, but none had large Tajima's D values: Clade C (*R. arcuatus*) from Panay (–0.44), Clade D (*R. arcuatus*) from Luzon (–0.11), Clade H (*R.* '*arcuatus*') from Sulawesi (0.03), and Clade I (*R.* '*euryotis*) from Sulawesi (0.33). For the Cyt*b* sequence data set, two clades showed significantly non-neutral and large Tajima's D and Fu's F_S values: (i) Clade A (Tajima's D: –1.54, Fu's F_S: –6.23), found on Luzon, Panay and Mindoro (*R. arcuatus*), and (ii) Clade H (*R.* '*arcuatus*') from Sulawesi (Tajima's D: –1.03, Fu's F_S: –2.68).

Population genetic structure

Global AMOVA results, as a weighted average over loci, were very similar to those of the standard AMOVA, so are not included here. Although AMOVA results for the three sequence data sets (Cyt*b* + CR, CR, Cyt*b*) all showed highly significant levels of genetic structure (P-values $\ll 0.01$), the proportion of genetic variation found among groups (geographic location), among populations (clades) within groups, and within populations varied between data sets (Tables S3a–c). For the Cyt*b* + CR data set, a clear majority of the genetic variation, 56.14%, was found among groups (Philippines, Sulawesi, Cambodia); 33.24% of the variation was contained among clades within groups; and 10.62% of the genetic variation was found within clades (Table S3a). The CR data set showed a similar trend, but much more genetic variation was found within clades, as would be expected under the model that the CR evolves faster than Cyt*b*: 47.61% of the variation was among groups (Philippines, Sulawesi, Cambodia, PNG), 35.71% among clades within groups, and 16.68% within clades (Table S3b). On the other hand, in the Cyt*b* sequence data set, most of the genetic variation was found among clades within groups (47.59%), rather than among groups (Philippines, Sulawesi, Cambodia: 44.74%), with little variation found within clades (7.67%; Table S3c). The Cyt*b* data set shows higher global F_{ST} based on the AMOVA (F_{ST}: 0.923) than does the CR data set (F_{ST}: 0.833). The large and significant proportion of variation found among populations is not surprising given that some clades actually represent distinct species, while others presently designated as populations of a single species (*R.* '*arcuatus*') may represent heretofore cryptic species.

Table 3 Measures of genetic diversity and neutrality for three sets of sequence data (control region only, cytochrome *b* only, or both concatenated)

Sequence data set	Population	Species designator	No. genes	No. haplo.	No. loci	*S*	G	π	*N*	θ_S	θ_π	Taj.'s *D*	Fu's F_S
Cyt*b* + CR	Clade A (CR), Clade E (Cyt*b*), Mnd.	*Rhinolophus inops/subrufus*	5	5	1343	14	1	7.5	0.006	6.7	7.4	0.74*	−0.75
Cyt*b* + CR	Clade C (CR), Clade D (Cyt*b*), Luz.	*Rhinolophus 'arcuatus'*	1	1	1344	0	1	NA	NA	NA	NA	NA	NA
Cyt*b* + CR	Clade L (CR), Clade J (Cyt*b*), Cam.	*Rhinolophus shameli shameli*	1	1	1341	0	1	NA	NA	NA	NA	NA	NA
Cyt*b* + CR	Clade B (CR), Clade A(Cyt*b*), Mdr.,Luz	*Rhinolophus 'arcuatus'*	1	1	1344	0	1	NA	NA	NA	NA	NA	NA
Cyt*b* + CR	Clade C (CR), Clade A (Cyt*b*), Pan.	*R. 'arcuatus'*	3	3	1344	9	1	6.0	0.004	6.0	6.0	0	0.59
Cyt*b* + CR	Clade D (CR), Clade B (Cyt*b*), Luz.	*R. 'arcuatus'* Large	5	5	1343	11	1	5.2	0.004	5.3	5.6	0.44*	0.67
Cyt*b* + CR	Clade E (CR), Clade A (Cyt*b*), Luz.	*R. 'arcuatus'*	2	2	1344	4	1	4.0	0.003	4.0	4.0	0	1.39
Cyt*b* + CR	Clade F (CR), Clade A(Cyt*b*), Luz., Pan.	*R. 'arcuatus'*	11	11	1343	35	1	8.0	0.006	11.9	8.4	−1.37*	−2.72*
Cyt*b* + CR	Clade G (CR), Clade C (Cyt*b*), Mnd.	*R. 'arcuatus'*	2	2	1344	2	1	2.0	0.001	2.0	2.0	0	0.69
Cyt*b* + CR	Clade H (CR), Clade H (Cyt*b*), Sul.	*R. 'arcuatus'*	8	8	1342	36	1	12.3	0.009	13.9	13.0	−0.33*	−1.65
Cyt*b* + CR	Clade I (CR), Clade I (Cyt*b*), Sul.	*Rhinolophus euryotis*	2	2	1343	21	1	21.4	0.016	21.0	21.0	0	3.04
CR	Clade A, Mnd.	*R. inops/subrufus*	7	5	418	11	0.90	5.3	0.013	4.5	5.2	0.90	0.38
CR	Clade B, Luz./Mdr.	*R. 'arcuatus'*	3	1	419	0	0	NA	NA	NA	NA	NA	NA
CR	Clade C, Pan./Luz.	*R. 'arcuatus'*	6	5	419	11	0.93	4.5	0.011	4.8	4.5	−0.44*	−0.55
CR	Clade D, Luz.	*R. 'arcuatus'* Large	6	5	418	9	0.93	3.9	0.009	3.9	3.9	−0.11*	−0.81
CR	Clade E, Luz.	*R. 'arcuatus'*	3	3	419	5	1	3.4	0.008	3.3	3.3	0	−0.08
CR	Clade F, Luz./Pan.	*R. 'arcuatus'*	13	11	419	21	0.97	4.9	0.012	6.8	5.0	−1.12*	−4.56*
CR	Clade G, Mnd.	*R. 'arcuatus'* Small	2	2	419	1	1	1.0	0.002	1.0	1.0	0	0
CR	Clade H, Sul.	*R. 'arcuatus'*	9	8	417	25	0.97	8.9	0.021	9.2	9.3	0.03*	−1.21
CR	Clade I, Sul.	*R. euryotis*	8	6	418	27	0.93	11.6	0.028	10.4	11.1	0.33*	1.13
CR	Clade J, PNG	*R. euryotis*	1	1	419	0	1	NA	NA	NA	NA	NA	NA
CR	Clade K, PNG	*R. 'arcuatus'*	2	2	418	13	1	13.5	0.032	13.0	15.0	0	2.71
CR	Clade L, Cam.	*R. shameli*	1	1	416	0	1	NA	NA	NA	NA	NA	NA
CR	Clade M, Mnd.	*Rhinolophus* sp.	1	1	420	0	1	NA	NA	NA	NA	NA	NA
Cyt*b*	Clade A, Luz./Pan./Mdr.	*R. 'arcuatus'*	22	15	925	22	0.96	3.2	0.003	6.0	3.5	−1.54*	−6.23*
Cyt*b*	Clade B, Luz.	*R. 'arcuatus'* Large	5	4	925	4	0.90	1.6	0.002	1.9	2.0	0.27	0.64
Cyt*b*	Clade C, Mnd.	*R. inops/subrufus*	2	2	925	1	1	1.0	0.001	1.0	1.0	0	0
Cyt*b*	Clade D, Luz.	*Rhinolophus* sp.	1	1	925	0	1	NA	NA	NA	NA	NA	NA
Cyt*b*	Clade E, Mnd.	*R. inops/subrufus*	4	4	925	6	1	3.5	0.004	3.3	3.5	0.67	−1.01
Cyt*b*	Clade F, Luz.	*R. 'arcuatus'* Wide	3	3	925	5	1	3.3	0.004	3.3	3.3	0	−0.08
Cyt*b*	Clade H, Sul.	*R. 'arcuatus'*	8	7	925	12	0.96	3.2	0.003	4.6	3.7	−1.03*	−2.68*
Cyt*b*	Clade I, Sul.	*R. euryotis*	3	3	925	10	1	6.7	0.007	6.7	9.3	1.4E + 8	1.07
Cyt*b*	Clade J, Cam.	*R. shameli*	1	1	925	0	1	NA	NA	NA	NA	NA	NA

'CR', control region; 'Cyt*b*', cytochrome *b*; '*', *P*-value < 0.05 for Tajima's *D* and <0.02 for Fu's F_S; 'No. genes', number of gene copies used in the analysis; 'No. haplo.', number of unique haplotypes; 'No. loci', number of useable loci; '*S*', number of polymorphic sites; 'G', gene diversity; 'π', molecular diversity (mean no. pairwise differences between every pair of haplotypes); '*N*', nucleotide diversity (avg. over loci); 'θ_S', Theta (*S*); 'θ_π', Theta (Pi). Location abbreviations: Sulawesi 'Sul.', Luzon 'Luz.', Panay 'Pan.', Mindanao 'Mnd.', Mindoro 'Mdr.', Cambodia 'Cam.', and Papua New Guinea 'PNG'.

We found significant and very strong genetic structure for most pairwise F_{ST} values among clades regardless of the sequence data set used in the analysis (Tables S4–S6). However, perhaps more informative were rare cases of relatively small pairwise F_{ST} values. For all sequence data sets, Clade H (Sulawesi *R.* '*arcuatus*') showed particularly large and significant pairwise F_{ST} values with all clades other than Clade I (Sulawesi *R.* '*euryotis*'), including all other clades representing *R.* '*arcuatus*' (Tables S4–S6). Regardless of the sequence data set used, Clade H had the smallest significant pairwise F_{ST} value with Clade I (Sulawesi *R.* '*euryotis*'): Cyt*b* + CR (F_{ST}: 0.75), Cyt*b* only (F_{ST}: 0.81) and CR only (F_{ST}: 0.70). In addition, several pairs of Philippine Islands clades showed small pairwise F_{ST} values relative to the average. In particular, the Clade F (CR)/Clade A (Cyt*b*) complex (*R. arcuatus*) showed relatively small pairwise F_{ST} with the Clade D (CR)/Clade B (Cyt*b*) complex (*R. arcuatus* large, F_{ST}: 0.67) and with the Clade C (CR)/Clade A (Cyt*b*) complex (*R. arcuatus*, F_{ST}: 0.48) (Table S4). These values are small only relative to the average significant F_{ST} value (Cyt*b* + CR data set: 0.82, Cyt*b* data set: 0.89, CR data set: 0.74); even the smallest of the statistically significant pairwise F_{ST} values were relatively large (Cyt*b* + CR: 0.48, Cyt*b*: 0.70, CR: 0.19; Tables S4–S6), with the possible exception of the smallest pairwise F_{ST} value from the CR data set (Table S6).

Minimum spanning trees (MST) produced from each of the sequence data sets reflect previously indicated structure (Figs S1–S3). All MSTs show that Clade H (Sulawesi *R.* '*arcuatus*') is genetically more distant from remaining *R.* '*arcuatus*' clades than Clade I (Sulawesi: *R.* '*euryotis*') is from those clades, and that Clades I and H are more genetically similar to each other than to any other clade (Figs S1–S3).

Molecular dating

Our analysis assumed an initial diversification date of 45 MYA for Rhinolophoidea (cladogenesis between Hipposideridae and Rhinolophidae) in the relaxed lognormal molecular clock model implemented by BEAST. Estimated divergence dates for the major lineages were mapped onto the tree resulting from the MCMC analysis by BEAST (Fig. S4), as were the upper and lower bounds of the 95% HPD for each node. The hypothesized estimates for major cladogenic events are listed in Table 4. The results are generally congruent with those of the Cyt*b* tree (Fig. 4B) and shed additional light on major biogeographic events in the region. Clade A identified in Fig. 4B is constituted by the 'small' and 'narrow' *R. arcuatus*. This clade separated from its sister taxon *R. arcuatus* 'large' ca. 1.2 MYA and diversified into a Mindoro and Luzon Isl. group and a Panay Isl. group ca. 775 KYA. The Luzon group includes individuals previously identified by Sedlock & Weyandt (2009) as the narrow sella morph individuals and appears distributed at least on Mt. Makiling (Luzon), Zambales Mts. (Luzon) and Mindoro Isl. These individuals appear to form a primarily montane forest species although they were sampled as low as 620 m on Mt. Makiling. Individuals in clade A from Panay – which is separated from Greater Luzon by persistent deep water channels – are distinct from the Greater Luzon individuals and appear to have diversified in Panay ca. 380 KYA. Clade B, *R. arcuatus* 'large' of Ingle & Heaney (1992) separated from the 'narrow sella' group, Clade A, 1.2 MYA, with the specimens we examined from the Zambales Mts. diversifying ca. 442 KYA. Clade C includes specimens previously referred to as *R. arcuatus* from Mindanao, including a putative 'small' form (*sensu* Ingle & Heaney 1992); this clade originated 1.62 MYA, and the specimens we sampled diversified ca. 406 KYA. Clade D is constituted by members of an undescribed species from the Zambales Mts. of Luzon Isl. and originated 2.03 MYA, diversifying ca. 1.24 MYA. Clade E, including specimens referred to as either *R. inops* or *R. subrufus*, from Mindanao originated 3.3 MYA, but had a relatively recent diversification on Mindanao based on the specimens that we examined; the most ancient event was 690 KYA, but two branching events in this clade were congruent with the Last Glacial Maximum (97 and 77 KYA). Clade F includes individuals previously identified by Sedlock & Weyandt (2009) as belonging to the 'wide sella' morph of *R. arcuatus*, from lowland forest in Luzon's Mt. Makiling. This clade originated about 4 MYA and diversified about 1 MYA. Clade G includes an undescribed species of *Rhinolophus* from Mindanao Isl., Philippines. This clade originated 4.23 MYA and diversified 2.67 MYA. Clades H and I include the undescribed *R.* '*arcuatus*' group species from Sulawesi (Clade H) as well as presumptive *R.* '*euryotis*', also from Sulawesi. The origin of the joint clades is estimated at 5.20 MYA. Diversification between Clades I and J occurred 2.14 MYA, with *R.* '*euryotis*' diversifying in Sulawesi ca. 1.28 MYA, and the undescribed *R.* '*arcuatus*', more recently at 400 KYA. The branch leading to *R. affinis* originated 10.34 MYA and includes relatively deep internal divergences, suggesting the possibility of a morphologically cryptic species complex; the two specimens from Borneo are hypothesized to have separated from the Chinese lineage 3.13 MYA. The clade including *R. celebensis*, *Rhinolophus virgo*, *Rhinolophus macrotis*, *Rhinolophus pusillus*, *Rhinolophus monoceros*, and *Rhinolophus cornutus* originated 16.88 MYA, with an internal divergence estimate of 12.50 MYA. *Rhinolophus celebensis* appear relatively homogeneous and are hypothesized to have diversified 1.81 MYA, following a cladogenic event from the lineage leading to *R. virgo* at 6.29 MYA. That lineage in turn diverged from

Table 4 Results of the temporal estimates of diversification based on the relaxed lognormal molecular clock model implemented by BEAST

Species or Species group	Clade	Origin	Diversification	Notes
Rhinolophus euryotis group	A	0.78	0.75	Luzon & Mindoro
	A	0.78	0.38	Panay
	B	1.23	0.42	*arcuatus* 'large'
	C	1.62	0.41	'*arcuatus*' Mindanao
	D	2.03	1.24	*R.* sp A, Philippines
	E	3.31	0.69	*inops* or *subrufus*
	F	3.99	0.91	*arcuatus* 'wide sella'
	G	4.23	2.67	*R.* sp B, Philippines
	H, I	5.20		Origin of H & I clades
	H	2.14	0.40	*R.* cf. *arcuatus*, Sulawesi
	I	2.14	1.28	*R. euryotis*, Sulawesi
	J	5.53	–	*Rhinolophus shameli*, Cambodia
Rhinolophus affinis		10.34	3.13	3.13 MYA distinguishes specimens between China and Borneo (62 KYA)
Rhinolophus sinicus		14.53	–	
cornutus–monoceros–pusillus–macrotis–virgo–celebensis		16.88	12.50	
Rhinolophus macrotis vs. *virgo–celebensis*		11.05	–	
R. macrotis			4.13	Separating specimens between Nepal and China
R. virgo v. *Rhinolophus celebensis*		6.29	1.81	Diversification estimate for *R. celebensis* only
cornutus–monoceros–pusillus		12.50	3.38	Origin relative to *macrotis–virgo–celebensis*
Rhinolophus cornutus		3.38	0.50	
Rhinolophus monoceros		1.70	–	
Rhinolophus pusillus		1.70	1.27	
Rhinolophus euryale– Rhinolophus ferrumequinum		18.05	11.08	
R ferrumequinum		11.08	3.58	Separates specimens between China and Japan
Rhinolophus pearsoni		19.69	9.94	Deep divergence estimates suggest a morphologically cryptic polytypic species complex
Rhinolophus luctus–R. hipposideros		21.11	19.38	
R. luctus		19.38	1.15	
R. hipposideros		19.38	0.95	
Rhinolophus, initial diversification		21.11		
Hipposideros–Rhinolophus (root)		45.0		
Hipposideros, initial diversification		24.24		
Hipposideros sp A		26.24	–	Sulawesi
Hipposideros armiger armiger–diadema–pratti		24.24	15.84	
Hipposideros diadema– Hipposideros pratti		15.48	–	

Estimated divergence dates for the major lineages were mapped onto the tree resulting from the MCMC analysis by BEAST (Fig. S4), as were the upper and lower bounds of the 95% highest posterior density (HPD) for each node. Our analysis assumed an initial diversification date of 45 MYA for Rhinolophoidea (cladogenesis between Hipposideridae and Rhinolophidae; see Materials and methods). Clade refers to the results of the Bayesian analysis of the Cytochrome *b* gene (Fig. 4B), where applicable, that is, in the *euryotis* group that includes *Rhinolophus arcuatus*. Origin refers to hypothesized estimate for the cladogenesis of the branch including that group, in millions of years. Diversification refers to the deepest internal diversification, also in millions of years (refer to Fig. S4).

the *R. macrotis* lineage at 11.05 MYA. Specimens of *R. macrotis* from Nepal and China show deep divergence suggesting that they may represent independent species; the *R. macrotis* specimen from Nepal is from south of the Himalayas, whereas the Chinese specimen is from Yunnan Province, well east of the Naga Hills that form a southward highland extension of the Himalayas terminating in the Indian Ocean. The *R. macrotis–virgo–celebensis* lineage is hypothesized to have differentiated from the *R. cornutus–monoceros–pusillus* lineage ca. 12.50 MYA, with internal diversification among species in the clade occurring ca. 3.38 MYA between *R. cornutus* and *R. monoceros*/*R. pusillus*. Cladogenesis between *R. pusillus* and *R. monoceros* is hypothesized to have occurred 1.70 MYA, with internal diversification of *R. pusillus* from the Hainan region beginning ca. 1.27 MYA. The lineage including the European *Rhinolophus euryale* and putatively widespread Eurasian *Rhinolophus ferrumequinum* diverged from the aforementioned clades ca. 18.05 MYA, with divergence between *R. euryale* and *R. ferrumequinum* hypothesized to have occurred 11.08 MYA. *Rhinolophus ferrumequinum* lineages from Yunnan and Japan diverged ca. 3.58 MYA.

The *Rhinolophus pearsoni* clade presents some interesting features: the initial lineage divergence from remaining *Rhinolophus* is hypothesized to have occurred ca. 19.69 MYA, with a relatively deep internal diversification among

specimens identified as *R. pearsoni* taking place ca. 9.94 MYA. Furthermore, that estimate separates two clades of *R. pearsoni*, each with representatives in China and Vietnam. In one clade, divergence between Chinese and Vietnamese lineages is estimated at 7.14 MYA, in the other, at 4.68 MYA. Internal (non-geographic) divergence estimates are 162 KYA (Guanxi, China) and 1.02 MYA (Lang Son, Vietnam). These data strongly suggest that there exist at least four distinct species-level taxa within what currently is recognized as *R. pearsoni*.

The final *Rhinolophus* lineage resulting from our analyses comprises *R. luctus* and *R. hipposideros*. This lineage represents an early divergence event within *Rhinolophus* (21.11 MYA), with divergence between the two species hypothesized to have taken place ca. 19.38 MYA. Internal divergence within the species are more recent: 1.15 MYA in *R. luctus* (between Burma and Huibei, China) and 950 KYA in *R. hipposideros* between Greece and United Kingdom.

Divergence estimates within *Hipposideros* were not strictly a part of this study as we used the several hipposiderid taxa as out-groups to root the *Rhinolophus* tree. Divergences among species in *Hipposideros* that we examined (15.48–26.24 MYA) were generally deeper than those in *Rhinolophus* (see Fig. S4, Supporting Information).

Discussion

Both morphological and genetic data ineluctably lead to the conclusion that specimens of *R.* '*arcuatus*' collected in Sulawesi represent an undescribed species. The morphological data show a clear distinction between Sulawesi and Philippine *R.* '*arcuatus*' notwithstanding the tendency for morphology to be highly conserved in the *R. arcuatus* complex. Likewise, Bornean *R. 'a.' proconsulis* is morphologically distinct from *R. arcuatus* from the Philippines. Based on these results, we recommend that the subspecies *proconsulis* (restricted to Borneo) be elevated to species level, as it is not morphologically congruent with *R. arcuatus*. This diagnosis is hampered by the lack of genetic samples of Bornean *R. 'a.' proconsulis* to compare to Philippine *R. arcuatus*, but the morphological data are unequivocal. Available genetic data show that the Sulawesi *R.* '*arcuatus*' are more closely related to *R.* '*euryotis*' from Sulawesi than they are to the Philippine *R. arcuatus*. Indeed, based on Bayesian analyses, the genetic distance between the Sulawesi *R.* '*arcuatus*' and the Philippine *R. arcuatus* is ca. 4%, which is the same as or greater than that between other well-established species within the *R. euryotis* group. This is greater than the 2.5% distance proposed by Bradley & Baker (2001), as suggestive of species separation, and is particularly informative in light of the 5% difference between the clearly and definitively recognized species *R. shameli* from Cambodia and *R.* '*arcuatus*' from Sulawesi. In contrast, *R.* '*arcuatus*' and *R.* '*euryotis*' from Sulawesi are only 2.2% divergent yet are considered distinct species based on nasal sella morphology.

Analyses of the cranial data alone (data not shown) are similar to those of cranial and wing data combined, supporting the contention that morphological characters of the skull are important in differentiating among species. However, when wing morphology alone is considered (data not shown), there is no distinction among taxa or geographic areas. This morphological conservatism suggests that these bats are foraging in a similar manner, and may be part of the reason these specimens have so long been considered as a single species.

As is the case in the Philippines, there is within Sulawesi the potential for recognition of more than one form of *R.* '*arcuatus*'. The most recent specimens were collected in the montane and submontane transitional forest of Tengah Province (2120 m), whereas the single other specimen previously collected was found in the lowland rainforests of Poso (Hill 1988). Elevational preferences have also been reported for *R. arcuatus* in the Philippines (Ingle & Heaney 1992; Sedlock & Weyandt 2009). Morphological data for the Sulawesi animals suggest that these specimens are poorly differentiated; however, without genetic data from lowland *R.* 'arcuatus' to corroborate the morphological results, we must remain open to the possibility of more than one species within Sulawesi *R.* '*arcuatus*'. Morphological data demonstrate a large difference between the lowland Sulawesi specimen attributed to *R.* '*a.*' *proconsulis* by Hill (1988) and Bornean *R. proconsulis* (previously *R. a. proconsulis*); it is therefore likely that they are distinct species. Indeed, it has recently been pointed out that some specimens of *R. affinis* have been erroneously identified as *R. arcuatus* (Suyanto & Struebig 2007), further confusing the identification of both new and old specimens. Perhaps the only way to sort out these issues is by using molecular techniques; unfortunately, there are no tissues associated with Hill's nominal *R.* '*a.*' *proconsulis* from Borneo or his Sulawesi specimen.

Based on the CR data, *R.* '*a.*' *mcintyrei* (Hill & Schlitter 1982) on PNG is also distinct from near topotypical *R. arcuatus* and should be elevated to specific status as *R. mcintyrei*. The type locality of *R. euryotis* is Ambon Island, in the Molucca Archipelago, on the Sahul Shelf (Australia, New Guinea and adjacent islands). Our results clearly demonstrate that island populations of *Rhinolophus* species display a propensity for speciation, a phenomenon potentially explained by Kingston & Rossiter (2004). However, in the absence of data to the contrary, it is perhaps expedient to hypothesize conspecificity to Sulawesi and Sahul Shelf *R. euryotis* (CR clade J), which would *pro tempore* retain the name. The specifically distinct species from Sulawesi (Clade I: *R.* '*euryotis*') now adopts the name *Rhinolo-*

phus tatar Bergmans & Rozendaal, 1982. We note, however, that the type locality of *R. tatar* is the Dumoga Nature Reserve, in Sulawesi's Northern Peninsula. Because there are strong differences among other taxa from the different regions of Sulawesi (Evans *et al.* 2003), which became a single island only recently (Hall 1996, 2002), and because the Sulawesi material examined herein as putative *R. tatar* is from the Central Core region of Sulawesi, it remains possible that our material represents something distinct from *R. tatar*; this hypothesis requires further testing.

Population genetic analyses indicate very strong genetic structure among clades, particularly between *R.* '*arcuatus*' from Sulawesi and the other *R. arcuatus* clades from the Philippine Islands and PNG. This is not surprising given that these clades are separated by considerable expanses of ocean. The presence of stronger genetic structure between 'populations' (clades) of the 'same' species on different islands than between different species on the same island additionally supports the hypothesis that *in situ* morphologically cryptic speciation has occurred. While a few clades from the Philippine Islands, such as Clade A (Cyt*b*) may represent very diverged populations of *R. arcuatus*, it is unlikely that even all Philippine clades presently identified as *R. arcuatus* constitute a single species. The strong structure found among the Philippine clades suggests that gene flow among islands is limited, which would be expected if bats in populations currently ascribed to the single species *R. arcuatus* are not very vagile. During the last Pleistocene glaciations, the four major island groups of the Philippines were never in contact with each other (Inger 1954; Voris 2000). Nevertheless, specimens from Luzon and Mindoro share a haplotype. These two islands are very close to each other (16 km presently, marginally closer during the last ice age), suggesting a possible minimum distance between islands insufficient to prevent dispersal, notwithstanding the deep (permanent) channel separating the two islands (Smith 1912; Voris 2000).

Levels of genetic diversity were highest for clades in Sulawesi and PNG. This is not particularly surprising in the case of PNG, as it has a larger land area (thus in theory is able to support larger effective population sizes) and was in contact with mainland Australia at various points in the Pleistocene as part of a Greater Sahul Shelf landmass; the depth of the Torres Strait is under 10 m, suggesting that land connection would have been available during much of the geological past. It was unexpected that Clades H (*R.* '*arcuatus*') and I (*R.* '*euryotis*') from Sulawesi show such high levels of genetic diversity in comparison with nominal *R. arcuatus* clades on Luzon, Panay, Mindoro, and Mindanao. These are all oceanic islands that have never had contact with the mainland. The Philippine islands are further north in the Indo-Malayan region and therefore potentially more isolated from sources of colonization than is Sulawesi. Other studies have indicated that colonization of islands seems to have occurred in a stepwise fashion with some species of bats, from west to east, with genetic diversity showing a corresponding decrease with distance from the mainland (Schmitt *et al.* 1995; Maharadatunkamsi *et al.* 2000). Unfortunately, in order to determine if this is the case with *R. euryotis* group species, we first would need to determine true biological species limits on all these islands, and further, would need samples from other islands, particularly Borneo: the putative stepping stone to both the Philippine Archipelago and Sulawesi.

Our data suggest that nominal *R. arcuatus* group species show limited dispersal even within the Philippines. This further supports the idea that *R.* '*arcuatus*' clades from Sulawesi and PNG are not merely disjunct populations of a single species, but rather that those clades are sufficiently diverged to represent different species.

One potential caveat to the genetic data presented here is that only mitochondrial genes were used to infer the phylogenies. It is possible that mitochondrial introgression could impact our conclusions based on the phylogenies; only by including nuclear data can we be sure that our mitochondrial gene trees reflect the true species trees (Funk & Omland 2003). However, our taxonomic suggestions are based on both morphological and genetic data and so should not be greatly skewed or affected by mitochondrial introgression.

The evidence presented here suggests that Rhinolophidae in South-East Asia have undergone replicated adaptive radiations, with the result that similar morphotypes appearing on different islands or archipelagoes are less closely related to each other than to different morphotypes on the same island or archipelago (R. M. Brown, pers. comm.). This phenomenon has been demonstrated by Losos *et al.* (1998) in the *Anolis* lizards of the Greater Antilles. At least two other research groups have shown this to be the case with rhinolophid bats in South-East Asia. Cooper *et al.* (1998) demonstrated that *R. philippinensis*, *R. megaphyllus*, and an intermediate form from the same geographic area, were more closely related to each other than either were to the same morphotypes from different areas in eastern Australia and PNG. Kingston & Rossiter (2004) showed similar results in the same species group from Sulawesi, and suggested that changes in primary echolocation frequency ('harmonic hopping') is the mechanism driving evolutionary change and consequent rapid speciation. Recently, Murray *et al.* (2012) found similar patterns of cryptic diversity in the genus *Hipposideros* throughout South-East Asia and hypothesized that this diversity was likely produced by mechanisms comparable to those acting on *Rhinolophus*. In

the case of the data presented here, the *R. arcuatus sensu lato* and *R. 'euryotis'* from Sulawesi are more closely related to each other than they are to their putative conspecific counterparts on different islands.

The biogeography of South-East Asia has fascinated scientists since the time of Wallace. Geological features and repeated effects of glaciations on connectivity of islands in this region have given rise to complex faunas made up of both continental and island-derived species (Stelbrink *et al.* 2012). While interesting, these phenomena have served to confound a clear picture of the systematics of many organisms. We have not provided a fully resolved phylogeny for all members of the *R. euryotis* group due to a lack of data; however, we demonstrate that it is a complex of several morphologically cryptic species-level taxa. Our revelation of replicated adaptive radiations in this biologically complex group of bats sows the seed for future biogeographic studies of this equally complex region.

Taxonomic summary

The present analysis was explicitly focused on *R. arcuatus* and the *R. euryotis* species group. Accordingly, the taxonomic summary is primarily concerned with these species. The astute reader no doubt will have noted that the results of the molecular dating analysis suggest the presence of additional morphologically cryptic species in specimens identified as belonging to *R. macrotis*, *R. ferrumequinum* and *R. pearsoni*. More detailed analyses of those taxa should be undertaken to reveal whether the hypotheses suggested in the present work indeed are borne out.

***Rhinolophus arcuatus*, Philippines**

The Philippine Archipelago populations were not the focus of this work. Hence, we will limit ourselves to summarize potential taxonomic conundrums. We strongly suspect the presence of at least five species in the *R. arcuatus* complex. Ingle & Heaney (1992) split *R. arcuatus* from Luzon into 'large' and 'small' morphs. In addition, an intermediate morph is present in Luzon's Zambales Mountains, sympatrically with the former two morphs (L. A. Ruedas, unpublished observations of specimens in National Museum of the Philippines and Cincinnati Museum of Natural History). An additional distinct morph is present in Panay (specimens in CMNH). The picture was further complicated by Sedlock & Weyandt (2009), who documented the existence of sympatric 'wide sella' and 'narrow sella' forms on Mt. Banahaw, Luzon. It is unclear whether these belong to either of the previously documented *R. arcuatus* large or *R. arcuatus* small species: Ingle & Heaney (1992) list forearm measurements as 43–45.5 mm (small morph) and 47–50 mm (large morph); the specimens of Sedlock & Weyandt (2009) range from 45.35 ± 1.83 (mean ± SD, narrow morph) to 46.58 ± 1.73 (wide morph). Thus, they overlap both previously documented morphs (measurements not available for intermediate morph of Zambales). These data suggest that there may be as many as five species present in Luzon alone, with additional species on the various remaining islands of the archipelago. We recommend that a formal description be undertaken so that at least a comparative framework may be established to assess species limits of Philippine *R. arcuatus*.

***Rhinolophus arcuatus proconsulis*, Borneo**

Our morphological data show that this taxon is morphologically distinct and unlikely to be conspecific with any *R. arcuatus* from the Philippines. Furthermore, from an ecological perspective, while Philippine Archipelago *R. arcuatus* appear to be forest species, this taxon was collected in a cave. Accordingly, this taxon should henceforth be known as *Rhinolophus proconsulis* Hill, 1959.

***Rhinolophus arcuatus proconsulis*, Sulawesi**

This specimen, another cave dwelling taxon, was assigned by Hill (1988) to *R. arcuatus proconsulis*. Our morphological data indicate that this taxon is neither conspecific with putatively topotypical Luzon *R. arcuatus* nor with *R. proconsulis* from Borneo. No name is available, but an extensive description was provided by Hill (1988), under the assumption that this taxon belonged in *R. a. proconsulis*. We suggest the name *Rhinolophus belligerator*. The name (Latin: belligerent combatant, 3rd declension nominative masculine; rather than *bellator*, a more legitimate soldier or warrior) is suggested because the single known specimen is from Permana Cave, near Poso, Sulawesi Tengah Province, Sulawesi, Indonesia. The name is suggested because the specimen originates in an area where a long-running and senseless Muslim–Christian civil war has been going on for many years. Hill (1988) provided a description and identified the sole known specimen (Natural History Museum, London, 1987.45), which becomes the holotype.

***Rhinolophus arcuatus*, Sulawesi Central Core area (this paper)**

The specimens analysed in this manuscript resulting from the collections of LAR and collaborators in the Central Core of Sulawesi (Toraja Highlands and Danau Lindu regions), all forest bats, are herein definitively shown not to be conspecific with Philippine *R. arcuatus* and are additionally distinct from the lowland troglodytic *R. belligerator*. Accordingly, they constitute a novel species. We do not wish to assign a name at this time: a full description will be forthcoming.

***Rhinolophus arcuatus mcintyrei*, New Guinea**

Based on our analysis of the mitochondrial CR sequence data, *R. a. mcintyrei* (Hill & Schlitter 1982), from PNG, is distinct from putatively topotypical *R. arcuatus*. This taxon should henceforth be known as *R. mcintyrei* Hill and Schlitter, 1982.

***Rhinolophus euryotis*, New Guinea and outlying islands (Sahul Shelf)**

As indicated in the body of the text above, this taxon is as problematic as *R. arcuatus*. Our data demonstrate the presence of at least two species: one on the Sahul Shelf (Australia, New Guinea and adjacent islands) and another on Sulawesi. The type locality of *R. euryotis* is Ambon Island, in the Molucca Archipelago, on the Sahul Shelf. Accordingly, and for now, Sahul Shelf populations (to the exclusion of Sulawesi populations, below) should retain the name *R. euryotis* Temminck, 1834.

***Rhinolophus euryotis*, Sulawesi**

Our data demonstrate that the Sulawesi populations that we examined from the Central Core region of Sulawesi and heretofore assigned to *R. euryotis* are not conspecific with *R. euryotis* as restricted above to the Sahul Shelf. Accordingly, the specifically distinct species from Sulawesi is likely *R. tatar* Bergmans and Rozendaal, 1982 which those authors note was confounded with *R. euryotis* by Tate & Archbold (1939). Readers are cautioned that – as is the case with *R. arcuatus* – there may exist additional, morphologically cryptic species in the *R. tatar* species complex present on Sulawesi (see Discussion in the text).

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References

Bergmans, W. & Rozendaal, F. G. (1982). Notes on *Rhinolophus* Lacépède, 1799 from Sulawesi, Indonesia, with the description of a new species (Mammalia, Microchiroptera). *Bijdragen tot de Dierkunde*, *52*, 169–174.

Bradley, R. D. & Baker, R. J. (2001). A test of the genetic species concept: cytochrome-b sequences and mammals. *Journal of Mammalogy*, *82*, 960–973.

Chattopadhyay, B., Schuller, G., Garg, K. M. & Kandula, S. (2010). A new phonic type of the rufous horseshoe bat *Rhinolophus rouxii* from southern India. *Current Science*, *99*, 114–118.

Cooper, S. J. B., Reardon, T. B. & Skilins, J. (1998). Molecular systematics of Australian rhinolophid bats (Chiroptera: Rhinolophidae). *Australian Journal of Zoology*, *46*, 203–220.

Csorba, G., Ujhelyi, P. & Thomas, N. (2003). *Horseshoe Bats of the World (Chiroptera: Rhinolophidae)*. Bishop's Castle, UK: Alana Books.

Drummond, A. J. & Rambaut, A. (2007). Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, 7, 1–8.

Drummond, A. J., Ho, S. Y. W., Phillips, M. J. & Rambaut, A. (2006). Relaxed phylogenetics and dating with confidence. *PLoS Biology*, *4*, e88.

Evans, B. J., Supriatna, J., Andayani, N., Setiadi, M. I., Cannatella, D. C. & Melnick, D. J. (2003). Monkeys and toads define areas of endemism on Sulawesi. *Evolution*, *57*, 1436–1443.

Excoffier, L., Smouse, P. & Quattro, J. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, *131*, 479–491.

Excoffier, L., Laval, G. & Schneider, S. (2005). Arlequin ver. 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, *1*, 47–50.

Excoffier, L., Laval, G. & Schneider, S. (2006). *Arlequin ver 3.1 User Manual Arlequin ver 3.1: An Integrated Software Package for Population Genetics Data Analysis*. Computational and Molecular Population Genetics Lab (CMPG), Institute of Zoology, University of Berne.

Freeman, P. W. (1981). A multivariate study of the Family Molossidae (Mammalia: Chiroptera): morphology, ecology, and evolution. *Fieldiana Zoology*, 7, 1–173.

Fu, Y.-X. (1997). Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, *147*, 915–925.

Funk, D. J. & Omland, K. E. (2003). Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology, Evolution, and Systematics*, *34*, 397–423.

Guillén-Servent, A., Francis, C. M. & Ricklefs, R. E. (2003). Phylogeny and biogeography of the Horseshoe bats. In G. Csorba, P. Ujhelyi & N. Thomas (Eds) *Horseshoe Bats of the World (Chiroptera: Rinolophidae)* (pp. xii–xxiv). Bishop's Castle, UK: Alana Books.

Hall, R. (1996). Reconstructing Cenozoic SE Asia. In R. Hall & D. J. Blundell (Eds) *Tectonic Evolution of Southeast Asia* (pp. 153–184). London: Geological Society.

Hall, R. (2002). Cenozoic geological and plate tectonic evolution of SE Asia and the SW Pacific: computer-based reconstructions, model and animations. *Journal of Asian Earth Sciences*, *20*, 353–431.

Hastings, W. K. (1970). Monte Carlo sampling methods using Markov chains and their applications. *Biometrika*, *57*, 97–109.

Heaney, L. R., Balete, D. S., Dolar, L., Alcala, A. C., Dans, A., Gonzales, P. C., Ingle, N. R., Lepiten, M. V., Oliver, W. L. R., Ong, P. S., Rickart, E. A., Tabaranza, B. R. Jr., & Utzurrum, R. C. B. (1998). A synopsis of the mammalian fauna of the Philippine Islands. *Fieldiana Zoology*, *88*, 1–61.

Hill, J. E. (1959). Bats in limestone of Sarawak. *The Annals and Magazine of Natural History*, *13*, 85–91.

Hill, J. E. (1988). A record of *Rhinolophus arcuatus* (Peters, 1871) (Chiroptera: Rhinolophidae) from Sulawesi. *Mammalia*, *52*, 588–589.

Hill, J. E. (1992). Order Chiroptera. In G. B. Corbet & J. E. Hill (Eds) *The Mammals of the Indomalayan Region: A Systematic Review*. New York, NY: Oxford University Press.

Hill, J. E. & Schlitter, D. A. (1982). A record of *Rhinolophus arcuatus* (Chiroptera: Rhinolophidae) from New Guinea, with the

description of a new subspecies. *Annals of Carnegie Museum of Natural History*, *51*, 455–464.

Ho, S. Y. W. (2007). Calibrating molecular estimates of substitution rates and divergence time in birds. *Journal of Avian Biology*, *38*, 409–414.

Huelsenbeck, J. P. & Ronquist, F. (2001). MRBAYES: bayesian inference of phylogenetic trees. *Bioinformatics*, *17*, 754–755.

Inger, R. F. (1954). Systematics and zoogeography of Philippine Amphibia. *Fieldiana Zoology*, *33*, 181–531.

Ingle, N. R. & Heaney, L. R. (1992). A key to the bats of the Philippine Islands. *Fieldiana Zoology*, *69*, iii–44.

Kingston, T. & Rossiter, S. J. (2004). Harmonic-hopping in Wallacea's bats. *Nature*, *429*, 654–657.

Koopman, K. F. (1994). *Chiroptera: systematics*. Berlin, Germany: W. de Gruyter.

Lacépède, B. G. E. de. (1799). Mémoire sur une nouvelle table méthodique des animaux à mamelles *Mémoires de l'Institut National des Sciences et des Arts. Sciences Mathématiques et Physiques*, *3*, 469–502.

Losos, J. B., Jackman, T. R., Larson, A., de Queiroz, K. & Rodriguez-Schettino, L. (1998). Contingency and determinism in replicated adaptive radiations of island lizards. *Science*, *279*, 2115–2118.

Maharadatunkamsi, Hisheh, S., Kitchener, D. J. & Schmitt, L. H. (2000). Genetic and morphometric diversity in Wallacea: geographical patterning in the horse shoe bat, *Rhinolophus affinis*. *Journal of Biogeography*, *27*, 193–201.

Metropolis, N., Rosenbluth, A., Rosenbluth, M., Teller, A. & Teller, E. (1953). Equations of state calculations by fast computing machines. *Journal of Chemistry and Physics*, *21*, 1087–1091.

Murray, S. W., Campbell, P., Kingston, T., Zubaid, A., Francis, C. M. & Kunz, T. H. (2012). Molecular phylogeny of hipposiderid bats from Southeast Asia and evidence of cryptic diversity. *Molecular Phylogenetics and Evolution*, *62*, 597–611.

Nei, M. (1987). *Molecular Evolutionary Genetics*. New York, NY, USA: Columbia University Press.

Norberg, U. M. & Rayner, J. M. (1987). Ecological morphology and flight in bats (Mammalia; Chiroptera): wing adaptations, flight performance, foraging strategy, and echolocation. *Philosophical Transactions of the Royal Society of London B*, *316*, 335–427.

Nylander, J. A. A. (2004). *MrModeltest*. Evolutionary Biology Centre, Uppsala University: Program distributed by author.

Page, R. D. M. (1996). TREEVIEW: an application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences*, *12*, 357–358.

Peters, W. C. H. (1871). Über die Gattungen und Arten der Huffaisennasen, Rhinolophi. *Monatsberichte der Königlichen-Preussischen Akademie der Wissenschaften zu Berlin*, *1871*, 301–322.

Rambaut, A. & Drummond, A. J. (2004). Tracer v1.5. [Computer software]. Available via http://tree.bio.ed.ac.uk/software/tracer/

Rohlf, F. J. (1973). Algorithm 76: hierarchical clustering using the minimum spanning tree. *Computer Journal*, *16*, 93–95.

Schmitt, L. H., Kitchener, D. J. & How, R. A. (1995). A genetic perspective of mammalian variation and evolution in the Indonesian Archipelago: biogeographic Correlates in the Fruit Bat Genus *Cynopterus*. *Evolution*, *49*, 399–412.

Sedlock, J. L. & Weyandt, S. E. (2009). Genetic divergence between morphologically and acoustically cryptic bats: novel niche partitioning or recent contact? *Journal of Zoology*, *279*, 388–395.

Shapiro, B., Ho, S. Y. W., Drummond, A. J., Suchard, M. A., Pybus, O. G. & Rambaut, A. (2011). A Bayesian phylogenetic method to estimate unknown sequence ages. *Molecular Biology and Evolution*, *28*, 879–887.

Sigé, B. (1990). Nouveaux Chiroptères de l'Oligocène Moyen des phosphorites du Quercy, France. *Comptes Rendus de l'Académie de Sciences, Série II*, *310*, 1131–1137.

Simmons, N. B. (2005). Order Chiroptera. In D. E. Wilson & D. M. Reeder (Eds) *Mammal Species of the World: A Taxonomic and Geographic Reference* (pp. 312–529). Baltimore, MD, USA: Johns Hopkins University Press.

Smith, H. M. (1912). The chimeroid fishes of the Philippine Islands, with description of a new species. *Proceedings of the National Museum of Natural History*, *42*, 231–232.

Stelbrink, B., Albrecht, C., Hall, R. & von Rintelen, T. (2012). The biogeography of Sulawesi revisited: is there evidence for a vicariant origin of taxa on Wallace's "anomalous island"? *Evolution*, *66*, 2252–2271.

Sun, K.-P., Feng, J., Jiang, T.-L., Ma, J., Zhang, Z.-Z. & Jin, L.-R. (2008). A new cryptic species of *Rhinolophus macrotis* (Chiroptera: Rhinolophidae) from Jiangxi Province, China. *Acta Chiropterologica*, *10*, 1–10.

Suyanto, A. & Struebig, M. J. (2007). Bats of the Sangkulirang limestone karst formations, East Kalimantan – a priority region for Bornean bat conservation. *Acta Chiropterologica*, *9*, 67–95.

Swofford, D. L. (2000). *PAUP*. Phylogenetic Analysis Using Parsimony (* and other Methods)*. Sunderland, MA: Sinauer Associates, Inc.

Tajima, F. (1983). Evolutionary relationship of DNA sequences in finite populations. *Genetics*, *105*, 437–460.

Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, *123*, 585–595.

Tajima, F. (1993). Measurement of DNA polymorphism. In N. Takahata & A. G. Clark (Eds) *Mechanisms of Molecular Evolution. Introduction to Molecular Paleopopulation Biology* (pp. 37–59). Tokyo: Japan Scientific Societies Press and Sunderland, MA: Sinauer Associates, Inc.

Tajima, F. (1996). The amount of DNA polymorphism maintained in a finite population when the neutral mutation rate varies among sites. *Genetics*, *143*, 1457–1465.

Tamura, K. & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, *10*, 512–526.

Tate, G. H. H. & Archbold, R. (1939). Results of the Archbold Expeditions. No. 24. Oriental *Rhinolophus* with special reference to material from the Archbold Collections. *American Museum Novitates*, *1036*, 1–12.

Voris, H. K. (2000). Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. *Journal of Biogeography*, *27*, 1153–1167.

Weir, B. S. & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution*, *38*, 1358–1370.

Zwickl, D. J. (2006). *Genetic Algorithm Approaches for the Phylogenetic Analysis of Large Biological Sequence Datasets Under the Maximum Likelihood Criterion*. Austin: The University of Texas at Austin.

Appendix 1 Samples used in the phylogenetic analyses Sequences generated by this study are bolded. 'Species' indicates original collector or museum identification. Field Museum of Natural History (FMNH), National Museum of Natural History (USNM), Hungarian Museum of Natural History (HMNH), Cincinnati Museum of Natural History and Science (CMNH), Portland State University Museum of Vertebrate Biology (PSUMVB), Royal Ontario Museum (ROM)

Museum number	Collector number	Species	Source (Museum or collector)	Location	CR sequence	CR clade	Used CR: Y /N (Popn genetic analyses)	Cyt*b* sequence	Cyt*b* clade	Used Cyt*b*: Y/N (Popn genetic analyses)
		Hipposideros armiger			DQ297609		No	DQ297585		No
		Hipposideros diadema			HDU95338		No	DQ219421		No
	RCD3809	*Hipposideros pomona*	PSUMVB	Indonesia, Sulawesi Island, Sulawesi Tengah Province, Kabupaten: Tana Toraja, Kecamatan: Rindingallo, Desa: Awan; Dusun: Rantekarua; 2°54.130′S, 119°41.839′E, elevation 2120 m	**JN106254**		No	**JN106319**		No
		Hipposideros pratti			DQ297608		No	DQ297584		No
		Rhinolophus affinis			DQ297606		No	DQ297582		No
	RHSP	*R. 'affinis'*	Matthew Struebig	Borneo	No		No	**JN106280**		No
	SAO4158	*R. 'affinis'*	Matthew Struebig	Borneo	No		No	**JN106274**		No
	MSB93099	*R. 'arcuatus'*	PSUMVB	Indonesia, Sulawesi Selatan Province, Kabupaten: Tana Toraja; Kecamatan: Bittuang; Desa: Tiroan; Dusun: Bolokan, 2° 56.145′ S, 119° 41.872′ E, 1571 m	**JN106184**	H	Yes	**JN106256**	H	Yes
	MSB93100	*R. 'arcuatus'*	PSUMVB	Indonesia, Sulawesi Selatan Province, Kabupaten: Tana	**JN106185**	H	Yes	**JN106257**	H	Yes

Appendix 1. Continued.

Museum number	Collector number	Species	Source (Museum or collector)	Location	CR sequence	CR clade	Used CR: Y /N (Popn genetic analyses)	Cyt*b* sequence	Cyt*b* clade	Used Cyt*b*: Y/N (Popn genetic analyses)
				Toraja; Kecamatan: Bittuang; Desa: Tiroan; Dusun: Bolokan, 2° 56.145′ S, 119° 41.872′ E, 1571 m						
	PDX50	*R. 'arcuatus'*	PSUMVB	Indonesia, Sulawesi Island, Sulawesi Tengah Province, Kabupaten: Tana Toraja, Kecamatan: Rindingallo, Desa: Awan; Dusun: Rantekarua; 2°54.130′S, 119°41.839′E, elevation 2120 m	**JN106188**	H	Yes	Yes		Yes
	PSUT67	*R. 'arcuatus'*	PSUMVB	Indonesia, Sulawesi Island, Sulawesi Tengah Province, Kabupaten: Tana Toraja, Kecamatan: Rindingallo, Desa: Awan; Dusun: Rantekarua; 2°54.130′S, 119°41.839′E, elevation 2120 m	**JN106189**	H	Yes	**JN106266**	H	Yes
	PSUT68	*R. 'arcuatus'*	PSUMVB	Indonesia, Sulawesi Island, Sulawesi Tengah Province, Kabupaten: Tana Toraja, Kecamatan: Rindingallo, Desa: Awan; Dusun: Rantekarua; 2°54.130′S,	**JN106190**	H	Yes	No		No

Appendix 1. Continued.

Museum number	Collector number	Species	Source (Museum or collector)	Location	CR sequence	CR clade	Used CR: Y /N (Popn genetic analyses)	Cyt*b* sequence	Cyt*b* clade	Used Cyt*b*: Y/N (Popn genetic analyses)
				119°41.839′E, elevation 2120 m						
	PSUT72	*R. 'arcuatus'*	PSUMVB	Indonesia, Sulawesi Island, Sulawesi Tengah Province, Kabupaten: Tana Toraja, Kecamatan: Rindingallo, Desa: Awan; Dusun: Rantekarua; 2°54.130′S, 119°41.839′E, elevation 2120 m	**JN106191**	H	Yes	**JN106267**	H	Yes
	PSUT73	*R. 'arcuatus'*	PSUMVB	Indonesia, Sulawesi Island, Sulawesi Tengah Province, Kabupaten: Tana Toraja, Kecamatan: Rindingallo, Desa: Awan; Dusun: Rantekarua; 2°54.130′S, 119°41.839′E, elevation 2120 m	Same haplotype as RCD3811		Yes	**JN106259**	H	Yes
	PSUT74	*R. 'arcuatus'*	PSUMVB	Indonesia, Sulawesi Island, Sulawesi Tengah Province, Kabupaten: Tana Toraja, Kecamatan: Rindingallo, Desa: Awan; Dusun: Rantekarua; 2°54.130′S, 119°41.839′E, elevation 2120 m	**JN106192**	H	Yes	**JN106260**	H	Yes
	RCD3811	*R. 'arcuatus'*	PSUMVB	Indonesia, Sulawesi Island, Sulawesi Tengah Province,	**JN106193**	H	Yes	**JN106261**	H	Yes

Appendix 1. Continued.

Museum number	Collector number	Species	Source (Museum or collector)	Location	CR sequence	CR clade	Used CR: Y /N (Popn genetic analyses)	Cyt*b* sequence	Cyt*b* clade	Used Cyt*b*: Y/N (Popn genetic analyses)
				Kabupaten: Tana Toraja, Kecamatan: Rindingallo, Desa: Awan; Dusun: Rantekarua; 2°54.130′S, 119°41.839′E, elevation 2120 m						
	M1	*R. (arcuatus?)*	CMNH	Philippine Is., Luzon Is., Zambales Prov., Municipaity of Masinloc, Barangay Cotó, South Slope, Mount Apoy (High Peak Range) 15.35N, 120.09E, 1160 m	**JN106213**	F	No	**JN106298**	A	Yes
	M12	*R. (arcuatus?)*	CMNH	Philippine Is., Luzon Is., Zambales Prov., Municipaity of Masinloc, Barangay Cotó, South Slope, Mount Apoy (High Peak Range) 15.35N, 120.09E, 1160 m	**JN106232**	F	Yes	**JN106297**	A	Yes
	M159	*R. (arcuatus?)*	CMNH	Philippine Is., Luzon Is., Zambales Prov., Municipaity of Masinloc, Barangay Cotó, South Slope, Mount Apoy (High Peak Range) 15.35N, 120.09E, 1160 m	**JN106215**	F	Yes	Same haplotype as M50/M357	A	Yes
	M3	*R. (arcuatus?)*	CMNH	Philippine Is., Luzon Is., Zambales Prov., Municipaity of	**JN106237**	E	Yes	No		No

Appendix 1. Continued.

Museum number	Collector number	Species	Source (Museum or collector)	Location	CR sequence	CR clade	Used CR: Y /N (Popn genetic analyses)	Cyt*b* sequence	Cyt*b* clade	Used Cyt*b*: Y/N (Popn genetic analyses)
				Masinloc, Barangay Cotó, South Slope, Mount Apoy (High Peak Range) 15.35N, 120.09E, 1160 m						
	M31	*R. (arcuatus?)*	CMNH	Philippine Is., Luzon Is., Zambales Prov., Municipaity of Masinloc, Barangay Cotó, South Slope, Mount Apoy (High Peak Range) 15.35N, 120.09E, 1160 m	**JN106233**	F	Yes	**JN106296**	A	Yes
	M348	*R. (arcuatus?)*	CMNH	Philippine Is., Luzon Is., Zambales Prov., Municipaity of Masinloc, Barangay Cotó, South Slope, Mount Apoy (High Peak Range) 15.35N, 120.09E, 1160 m	**JN106236**	F	Yes	**JN106290**	A	Yes
	M43	*R. (arcuatus?)*	CMNH	Philippine Is., Luzon Is., Zambales Prov., Municipaity of Masinloc, Barangay Cotó, South Slope, Mount Apoy (High Peak Range) 15.35N, 120.09E, 1160 m	**JN106239**	E	Yes	No		No
	M49	*R. (arcuatus?)*	CMNH	Philippine Is., Luzon Is., Zambales Prov., Municipaity of Masinloc, Barangay Cotó, South Slope,	Same haplotype as M376/M1068	B	Yes	**JN106285**	A	Yes

Appendix 1. Continued.

Museum number	Collector number	Species	Source (Museum or collector)	Location	CR sequence	CR clade	Used CR: Y /N (Popn genetic analyses)	Cyt*b* sequence	Cyt*b* clade	Used Cyt*b*: Y/N (Popn genetic analyses)
				Mount Apoy (High Peak Range) 15.35N, 120.09E, 1160 m						
	M50	*R. (arcuatus?)*	CMNH	Philippine Is., Luzon Is., Zambales Prov., Municipaity of Masinloc, Barangay Cotó, South Slope, Mount Apoy (High Peak Range) 15.35N, 120.09E, 1160 m	**JN106235**	E	Yes	**JN106299**	A	Yes
	M52	*R. (arcuatus?)*	CMNH	Philippine Is., Luzon Is., Zambales Prov., Municipaity of Masinloc, Barangay Cotó, South Slope, Mount Apoy (High Peak Range) 15.35N, 120.09E, 1160 m	**JN106242**	F	Yes	Same haplotype as M53	A	Yes
	M53	*R. (arcuatus?)*	CMNH	Philippine Is., Luzon Is., Zambales Prov., Municipaity of Masinloc, Barangay Cotó, South Slope, Mount Apoy (High Peak Range) 15.35N, 120.09E, 1160 m	**JN106230**	F	No	**JN106300**	A	Yes
	M57	*R. (arcuatus?)*	CMNH	Philippine Is., Luzon Is., Zambales Prov., Municipaity of Masinloc, Barangay Cotó, South Slope, Mount Apoy (High Peak Range)	**JN106240**	F	Yes	**JN106301**	A	Yes

Appendix 1. Continued.

Museum number	Collector number	Species	Source (Museum or collector)	Location	CR sequence	CR clade	Used CR: Y /N (Popn genetic analyses)	Cyt*b* sequence	Cyt*b* clade	Used Cyt*b*: Y/N (Popn genetic analyses)
				15.35N, 120.09E, 1160 m						
	M357	*R. (arcuatus?)*	CMNH	Philippine Is., Luzon Is., Zambales Prov., Municipaity of Masinloc, Barangay Cotó, 4.3 km N, 0.5 km E peak of Mt. High Peak 15.31N, 120.07E, 2037 m	Same haplotype as M159	F	Yes	Same haplotype as M50/M357	A	Yes
177459	JLS184	*R. arcuatus*	FMNH	Philippine Is., Luzon, Mt. Makiling	**JN106195**	F	No	**JN106268**	A	No
3279	M1494	*R. arcuatus*	CMNH	Philippine Is., Mindanao Is., Sarangani Prov., Municipality of Kiamba, Sitio Binati, S. Slope of Mount Busa, 6.05167N, 125.40537E, 900–1200 m	**JN106252**	G	Yes	**JN106311**	C	Yes
	M461	*R. arcuatus*	CMNH	Philippine Is., Panay Is., Antique Province, Municipality of Culasi, Barangay Alojipan, Hanggud Tubig (W. face of Mt. Madja-ás), 11.2331N, 122.0911E	Yes	C	Yes	**JN106294**	A	Yes
3041	M462	*R. arcuatus*	CMNH	Philippine Is., Panay Is., Antique Province, Municipality of Culasi, Barangay Alojipan, Hanggud	**JN106222**	C	Yes	Same haplotype as M514/M616	A	Yes

Appendix 1. Continued.

Museum number	Collector number	Species	Source (Museum or collector)	Location	CR sequence	CR clade	Used CR: Y /N (Popn genetic analyses)	Cyt*b* sequence	Cyt*b* clade	Used Cyt*b*: Y/N (Popn genetic analyses)
				Tubig (W. face of Mt. Madja-ás), 11.2331N, 122. 0911E						
3044	M514	*R. arcuatus*	CMNH	Philippine Is., Panay Is., Antique Province, Municipality of Culasi, Barangay Alojipan, Hanggud Tubig (W. face of Mt. Madja-ás), 11.2331N, 122. 0911E	**JN106243**	C	Yes	**JN106293**	A	Yes
	M525	*R. arcuatus*	CMNH	Philippine Is., Panay Is., Antique Province, Municipality of Culasi, Barangay Alojipan, Hanggud Tubig (W. face of Mt. Madja-ás), 11.2331N, 122. 0911E	**JN106223**	C	Yes	No		No
3045	M540	*R. arcuatus*	CMNH	Philippine Is., Panay Is., Antique Province, Municipality of Culasi, Barangay Alojipan, Hanggud Tubig (W. face of Mt. Madja-ás), 11.2331N, 122. 0911E	**JN106231**	F	Yes	**JN106292**	A	Yes
3046	M542	*R. arcuatus*	CMNH	Philippine Is., Panay Is., Antique Province, Municipality of	**JN106238**		No	No		No

Appendix 1. Continued.

Museum number	Collector number	Species	Source (Museum or collector)	Location	CR sequence	CR clade	Used CR: Y /N (Popn genetic analyses)	Cyt*b* sequence	Cyt*b* clade	Used Cyt*b*: Y/N (Popn genetic analyses)
				Culasi, Barangay Alojipan, Hanggud Tubig (W. face of Mt. Madja-ás), 11.2331N, 122. 0911E						
3047	M545	*R. arcuatus*	CMNH	Philippine Is., Panay Is., Antique Province, Municipality of Culasi, Barangay Alojipan, Hanggud Tubig (W. face of Mt. Madja-ás), 11.2331N, 122. 0911E	**JN106249**	C	Yes	**JN106291**	A	Yes
3616	M616	*R. arcuatus*	CMNH	Philippine Is., Panay Is., Antique Province, Municipality of Culasi, Barangay Alojipan, Hanggud Tubig (W. face of Mt. Madja-ás), 11.2331N, 122. 0911E	**JN106241**	C	No	**JN106295**	A	Yes
		R. arcuatus			AF065090, AF065091	K	Yes			No
180138		*R. arcuatus 'narrow'*	FMNH	Philippine Is., Luzon Is., Quezon Prov., Municipality of Tayabas, Mt. Banahaw-San Cristobal Park			No	GQ368669	A	Yes
180146		*R. arcuatus 'narrow'*	FMNH	Philippine Is., Luzon Is., Quezon Prov., Municipality of Tayabas, Mt.			No	GQ368675	A	Yes

Appendix 1. Continued.

Museum number	Collector number	Species	Source (Museum or collector)	Location	CR sequence	CR clade	Used CR: Y /N (Popn genetic analyses)	Cyt*b* sequence	Cyt*b* clade	Used Cyt*b*: Y/N (Popn genetic analyses)
				Banahaw-San Cristobal Park						
180147		*R. arcuatus 'narrow'*	FMNH	Philippine Is., Luzon Is., Quezon Prov., Municipality of Tayabas, Mt. Banahaw-San Cristobal Park			No	GQ368676	A	Yes
180154		*R. arcuatus 'wide'*	FMNH	Philippine Is., Luzon Is., Quezon Prov., Municipality of Tayabas, Mt. Banahaw-San Cristobal Park			No	GQ368683	F	Yes
180159		*R. arcuatus 'wide'*	FMNH	Philippine Is., Luzon Is., Quezon Prov., Municipality of Tayabas, Mt. Banahaw-San Cristobal Park			No	GQ368688	F	Yes
180157		*R. arcuatus 'wide'*	FMNH	Philippine Is., Luzon Is., Quezon Prov., Municipality of Tayabas, Mt. Banahaw-San Cristobal Park			No	GQ368686	F	Yes
	M127	*R. arcuatus 'big'*	CMNH	Philippine Is., Luzon Is., Zambales Prov., Municipaity of Masinloc, Barangay Cotó, South Slope, Mount Apoy (High Peak Range) 15.35N, 120.09E, 1160 m	**JN106214**	D	Yes	**JN106304**	B	Yes
	M137	*R. arcuatus 'big'*	CMNH	Philippine Is., Luzon Is., Zambales Prov., Municipaity of	**JN106250**	D	Yes	**JN106303**	B	Yes

Appendix 1. Continued.

Museum number	Collector number	Species	Source (Museum or collector)	Location	CR sequence	CR clade	Used CR: Y /N (Popn genetic analyses)	Cyt*b* sequence	Cyt*b* clade	Used Cyt*b*: Y/N (Popn genetic analyses)
				Masinloc, Barangay Cotó, South Slope, Mount Apoy (High Peak Range) 15.35N, 120.09E, 1160 m						
	M16	*R. arcuatus 'big'*	CMNH	Philippine Is., Luzon Is., Zambales Prov., Municipaity of Masinloc, Barangay Cotó, South Slope, Mount Apoy (High Peak Range) 15.35N, 120.09E, 1160 m	**JN106244**	D	Yes	**JN106283**	B	No
	M17	*R. arcuatus 'big'*	CMNH	Philippine Is., Luzon Is., Zambales Prov., Municipaity of Masinloc, Barangay Cotó, South Slope, Mount Apoy (High Peak Range) 15.35N, 120.09E, 1160 m	Same haplotype as M195	D	Yes	**JN106284**	B	Yes
	M64	*R. arcuatus 'big'*	CMNH	Philippine Is., Luzon Is., Zambales Prov., Municipaity of Masinloc, Barangay Cotó, South Slope, Mount Apoy (High Peak Range) 15.35N, 120.09E, 1160 m	**JN106251**	D	Yes	**JN106302**	B	Yes
3280	M1576	*R. arcuatus 'small'*	CMNH	Philippine Is., Mindanao Is., Sarangani Prov., Municipality of Kiamba, Sitio Binati,	**JN106227**	G	Yes	**JN106312**	C	Yes

Appendix 1. Continued.

Museum number	Collector number	Species	Source (Museum or collector)	Location	CR sequence	CR clade	Used CR: Y /N (Popn genetic analyses)	Cyt*b* sequence	Cyt*b* clade	Used Cyt*b*: Y/N (Popn genetic analyses)
				S. Slope, Mount Busa, 6.05167N, 125.40537E, 900–1200 m						
	M1068	*Rhinolophus 'inops or arcuatus L'*	CMNH	Philippine Is., Mindoro Is., Mindoro Oriental Prov., Municipality of Baco, North Ridge approach to Mount Halcon, Dulangan River Valley, 13.17.27N, 120.5932E	**JN106224**	B	No	**JN106305**	A	Yes
3299	M1461	*R. 'inops or subrufus'*	CMNH	Philippine Is., Mindanao Is., Sarangani Prov., Municipality of Kiamba, Sitio Binati, S. Slope, Mount Busa, 6.05167N, 125.40537E, 900–1200 m	**JN106247**	A	No	No		No
	M1462	*R. 'inops or subrufus'*	CMNH	Philippine Is., Mindanao Is., Sarangani Prov., Municipality of Kiamba, Sitio Binati, S. Slope, Mount Busa, 6.05167N, 125.40537E, 900–1200 m	**JN106248**	A	No	**JN106307**	E	Yes
3300	M1463	*R. 'inops or subrufus'*	CMNH	Philippine Is., Mindanao Is., Sarangani Prov., Municipality of Kiamba, Sitio Binati, S. Slope, Mount	**JN106225**	A	No	**JN106308**	E	Yes

Appendix 1. Continued.

Museum number	Collector number	Species	Source (Museum or collector)	Location	CR sequence	CR clade	Used CR: Y /N (Popn genetic analyses)	Cyt*b* sequence	Cyt*b* clade	Used Cyt*b*: Y/N (Popn genetic analyses)
				Busa, 6.05167N, 125.40537E, 900–1200 m						
3301	M1634	*R. 'inops or subrufus'*	CMNH	Philippine Is., Mindanao Is., Sarangani Prov., Municipality of Kiamba, Sitio Binati, S. Slope, Mount Busa, 6.05357N, 125.41216E, 900–1200 m	Same haplotype as M1461	A	No	**JN106313**	E	Yes
3281	M1726	*R. 'subrufus or arcuatus'*	CMNH	Pilippine Is., Mindanao Is., Sarangani Prov., Municipality of Kiamba, Sitio Binati, Mount Cabaay (Mount Busa Range), 6.03N, 124.45E, 1450–1710 m	No		No	**JN106315**	C	Yes
	446	*Rhinolophus celebensis*	Stephen Rossiter	Sulawesi	**JN106206**		No	**JN106277**		No
	469	*R. celebensis*	Stephen Rossiter	Sulawesi	**JN106207**		No	**JN106272**		No
	MSB93101	*R. celebensis*	PSUMVB	Indonesia, Sulawesi Selatan, Kabupaten County, Kecamatan: Bittuang; Desa: Tiroan; Dusun: Bolokan; Tana Toraja; 2° 56.145′ S, 119° 41.872′ E, 1571 m	**JN106186**		No	**JN106258**		No
	PDX41	*R. celebensis*	PSUMVB	Indonesia, Sulawesi Island, Sulawesi Tengah Province, Kabupaten: Tana	**JN106187**		No	**JN106265**		No

Appendix 1. Continued.

Museum number	Collector number	Species	Source (Museum or collector)	Location	CR sequence	CR clade	Used CR: Y /N (Popn genetic analyses)	Cyt*b* sequence	Cyt*b* clade	Used Cyt*b*: Y/N (Popn genetic analyses)
				Toraja, Kecamatan: Rindingallo, Desa: Awan; Dusun: Rantekarua; 2°54.130′S, 119°41.839′E, elevation 2120 m						
	RCD3802	*R. celebensis*	PSUMVB	Indonesia, Sulawesi Island, Sulawesi Tengah Province, Kabupaten: Tana Toraja, Kecamatan: Rindingallo, Desa: Awan; Dusun: Rantekarua; 2°54.130′S, 119°41.839′E, elevation 2120 m	**JN106200**		No	**JN106263**		No
	RCD3818	*R. celebensis*	PSUMVB	Indonesia, Sulawesi Island, Sulawesi Tengah Province, Kabupaten: Tana Toraja, Kecamatan: Rindingallo, Desa: Awan; Dusun: Rantekarua; 2°54.130′S, 119°41.839′E, elevation 2120 m	**JN106194**		No	**JN106262**		No
		Rhinolophus cornutus			DQ297615		No	AB085705, DQ297594		No
		Rhinolophus euryale					No	DQ120916		No
	182	*R. euryotis*	Stephen Rossiter	Sulawesi	**JN106202**	I	Yes	No		No
	183	*R. euryotis*	Stephen Rossiter	Sulawesi	**JN106203**	I	Yes	**JN106275**	I	Yes
	417	*R. euryotis*	Stephen Rossiter	Sulawesi	**JN106204**	I	Yes	Yes	I	Yes
	418	*R. euryotis*	Stephen Rossiter	Sulawesi	No		No	**JN106271**	I	Yes
	421	*R. euryotis*	Stephen Rossiter	Sulawesi	**JN106205**	I	Yes	**JN106276**	I	Yes

Appendix 1. Continued.

Museum number	Collector number	Species	Source (Museum or collector)	Location	CR sequence	CR clade	Used CR: Y /N (Popn genetic analyses)	Cyt*b* sequence	Cyt*b* clade	Used Cyt*b*: Y/N (Popn genetic analyses)
	B10 (WA0408)	*R. euryotis*	Stephen Rossiter	Sulawesi	**JN106196**	I	Yes	No		No
	B11 (WA0409)	*R. euryotis*	Stephen Rossiter	Sulawesi	**JN106197**	I	Yes	No		No
	C03 (WA0418)	*R. euryotis*	Stephen Rossiter	Sulawesi	**JN106198**	I	Yes	No		No
	C09 (SRJ0604)	*R. euryotis*	Stephen Rossiter	Sulawesi	**JN106199**	I	Yes	No		No
		R. euryotis			AF065089	J	Yes			No
		Rhinolophus ferrumequinum			DQ297599		No	AB085726, DQ297575		No
		Rhinolophus hipposideros			DQ297610		No	DQ120924, DQ297586		No
3298	M1457	*Rhinolophus inops*	CMNH	Philippine Is., Mindanao Is., Sarangani Prov., Municipality of Kiamba, Sitio Binati, S. Slope, Mount Busa, 6.05167N, 125.40537E, 900–1200 m	**JN106246**	A	Yes	No		No
	MY7	*Rhinolophus luctus*	PSUMVB	Myanmar	**JN106255**		No	**JN106264**		No
		R. luctus			DQ297619		No	DQ297596		No
	N-1	*Rhinolophus macrotis*	PSUMVB	Nepal; Pokhara	**JN106211**		No	**JN106278**		No
		R. macrotis					No	EF517311		No
		Rhinolophus megaphyllus			AF065081, AF065083, AF065088, AF065078		No			No
		Rhinolophus mehelyi					No	DQ120917		No
		Rhinolophus monoceros			DQ297600		No	DQ297581		No
111322	F44552	*Rhinolophus pearsonii*	ROM	Vietnam:Quang Nam, NgocLihn base camp, 10 km SW Nuocxa	**JN106208**		No	**JN106273**		No
116440	F47527	*R. pearsonii*	ROM	China, Guangxi, Shiwandashan Nat'l Reserve	**JN106201**		No	**JN106279**		No

Appendix 1. Continued.

Museum number	Collector number	Species	Source (Museum or collector)	Location	CR sequence	CR clade	Used CR: Y /N (Popn genetic analyses)	Cyt*b* sequence	Cyt*b* clade	Used Cyt*b*: Y/N (Popn genetic analyses)
116441	F47528	*R. pearsonii*	ROM	China, Guangxi, Shiwandashan Nat'l Reserve	No		No	**JN106270**		No
112363	F48219	*R. pearsonii*	ROM	Vietnam, Lang Son, Lan Dat, 4 km W of Huu Lien	**JN106209**		No	**JN106281**		No
112448	F48304	*R. pearsonii*	ROM	Vietnam, Lao Cai, Ta Pihn, 10 km N of Sa Pa	**JN106210**		No	**JN106282**		No
		R. pearsonii			DQ297611		No	DQ297587		No
		Rhinolophus philippinensis			AF065076, AY568642, AF065070, AF065074, AF065072, AF065078, AY568637, AY568643		No			No
		Rhinolophus pusillus			DQ297618, DQ297620		No	DQ297590, DQ297595, DQ297597		No
2005.81.7	2005.81.7	*Rhinolophus shameli*	HMNH	Cambodia, Preah Vihear Prov., 13.3400N, 104.5945E, 78 m	**JN106212**	L	Yes	**JN106269**	J	Yes
		Rhinolophus sinicus					No	EF517303		No
3307	M1341	*Rhinolophus* sp.	CMNH	Philippine Is., Mindanao Is., Davao City Province, Municipality of Toril, Barangay Baracatan, Barrio San Roque, Mount Apo Nat'l Park (Parks and Wildlife nature Center), E. face of Mount Talomo	**JN106245**	A	Yes	No		No

Appendix 1. Continued.

Museum number	Collector number	Species	Source (Museum or collector)	Location	CR sequence	CR clade	Used CR: Y /N (Popn genetic analyses)	Cyt*b* sequence	Cyt*b* clade	Used Cyt*b*: Y/N (Popn genetic analyses)
				(Mount Apo Range), 7.00642N, 125.21304E						
3304	M1651	*Rhinolophus* sp.	CMNH	Philippine Is., Mindanao Is., Sarangani Prov., Municipality of Kiamba, Sitio Binati, S. Slope, Mount Busa, 6.05357N, 125.41216E, 900–1200 m	**JN106228**	A	Yes	**JN106314**	E	Yes
3282	M1782	*Rhinolophus* sp.	CMNH	Kiamba	No		No	**JN106316**	G	No
3283	M1784	*Rhinolophus* sp.	CMNH	Kiamba	No		No	**JN106317**	G	No
3288	M1797	*Rhinolophus* sp.	CMNH	Kiamba	**JN106229**	M	Yes	**JN106318**	G	No
3289	M1798	*Rhinolophus* sp.	CMNH	Kiamba	No		No	Yes		No
	M195	*Rhinolophus* sp.	CMNH	Philippine Is., Luzon Is., Zambales Prov., Municipaity of Masinloc, Barangay Cotó, 0.5 km E, 0.5 kmS of junction Mabinial and South Lowis rivers, 15.33N, 120.06E, 125–600 m	**JN106216**	D	Yes	Same haplotype as M64	B	Yes
	M352	*Rhinolophus* sp.	CMNH	Philippine Is., Luzon Is., Zambales Prov., Municipaity of Masinloc, Barangay Cotó, 4.3 km N, 0.5 km E peak of Mt. High Peak	**JN106217**	F	Yes	**JN106289**	A	Yes

Appendix 1. Continued.

Museum number	Collector number	Species	Source (Museum or collector)	Location	CR sequence	CR clade	Used CR: Y /N (Popn genetic analyses)	Cyt*b* sequence	Cyt*b* clade	Used Cyt*b*: Y/N (Popn genetic analyses)
				15.31N, 120.07E, 2037 m						
	M353	*Rhinolophus* sp.	CMNH	Philippine Is., Luzon Is., Zambales Prov., Municipaity of Masinloc, Barangay Coto, 4.3 km N, 0.5 km E peak of Mt. High Peak 15.31N, 120.07E, 2037 m	**JN106218**	F	Yes	**JN106288**	A	Yes
	M369	*Rhinolophus* sp.	CMNH	Philippine Is., Luzon Is., Zambales Prov., Municipaity of Masinloc, Barangay Coto, 4.3 km N, 0.5 km E peak of Mt. High Peak 15.31N, 120.07E, 2037 m	**JN106219**	F	Yes	Yes	A	Yes
	M376	*Rhinolophus* sp.	CMNH	Philippine Is., Luzon Is., Zambales Prov., Municipaity of Masinloc, Barangay Coto, 4.3 km N, 0.5 km E peak of Mt. High Peak 15.31N, 120.07E, 2037 m	**JN106234**	B	Yes	**JN106306**	A	Yes
	M377	*Rhinolophus* sp.	CMNH	Philippine Is., Luzon Is., Zambales Prov., Municipaity of Masinloc, Barangay Coto, 4.3 km N, 0.5 km E peak of Mt. High Peak 15.31N, 120.07E, 2037 m	**JN106220**	C	Yes	**JN106287**	D	Yes
	M386	*Rhinolophus* sp.	CMNH		**JN106221**	F	Yes	**JN106286**	A	Yes

Appendix 1. Continued.

Museum number	Collector number	Species	Source (Museum or collector)	Location	CR sequence	CR clade	Used CR: Y /N (Popn genetic analyses)	Cyt*b* sequence	Cyt*b* clade	Used Cyt*b*: Y/N (Popn genetic analyses)
				Philippine Is., Luzon Is., Zambales Prov., Municipaity of Masinloc, Barangay Coto, 4.3 km N, 0.5 km E peak of Mt. High Peak 15.31N, 120.07E, 2037 m						
3306	M1493	*Rhinolophus subrufus?*	CMNH	Philippine Is., Mindanao Is., Sarangani Prov., Municipality of Kiamba, Sitio Binati, S. Slope Mount Busa, 6.05167N, 125.40537E, 900–1200 m	**JN106226**	A	No	**JN106310**	E	Yes
3302	M1474	*Rhinolophus virgo*	CMNH	Philippine Is., Mindanao Is., Sarangani Prov., Municipality of Kiamba, Sitio Binati, S. Slope Mount Busa, 6.05167N, 125.40537E, 900–1200 m	**JN106253**		No	**JN106309**		Yes

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Minimum Spanning Tree (MST) created using Euclidean square distance matrix (distance method: Tamura & Nei) and the CR + Cyt*b* sequence dataset as input.

Fig. S2. Minimum Spanning Tree (MST) created using Euclidean square distance matrix (distance method: Tamura & Nei) and the CR sequence dataset as input.

Fig. S3. Minimum Spanning Tree (MST) created using Euclidean square distance matrix (distance method: Tamura & Nei) and the Cyt*b* sequence dataset as input.

Fig. S4. Co–estimated phylogeny and temporal divergence times within the Rhinolophoidea dataset.

Data S1. Description of morphological measurements.

Table S1. Primers and thermal profiles used for amplification of cytochrome *b* and control region DNA.

Table S2. Eigenvalues for first three PC's for all variables analyzed together. Abbreviations as in Data S1.

Table S3. Results for analyses of molecular variance (AMOVA) run in Arlequin v 3.5.

Table S4. Pairwise F_{ST} values between clades produced with the Cyt*b* + CR sequence dataset (distance method: Tamura & Nei).

Table S5. Pairwise F_{ST} values between clades produced with the Cyt*b* sequence dataset (distance method: Tamura & Nei).

Table S6. Pairwise F_{ST} values between clades produced with the CR sequence dataset (distance method: Tamura & Nei).

Appendix S1. Specimens examined in the morphological analyses.