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Evolutionary Relationships of Flying Foxes (Genus *Pteropus*) in the Philippines Inferred From DNA Sequences of *Cytochrome b* Gene

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Six flying fox species, genus Pteropus *(four from the Philippines) were investigated using complete* cytochrome b *gene sequences (1140 bp) to infer their evolutionary relationships. The DNA sequences generated via polymerase chain reaction were analyzed using the neighbor-joining, parsimony, and maximum likelihood methods. We estimated that the first evolutionary event among these* Pteropus *species occurred approximately 13.90 ± 1.49 MYA. Within this short period of evolutionary time we further hypothesized that the ancestors of the flying foxes found in the Philippines experienced a subsequent diversification forming two clusters in the topology. The first cluster is composed of* P. pumilus *(Philippine endemic),* P. speciosus *(restricted in western Mindanao) with* P. scapulatus, *while the second one comprised* P. vampyrus *and* P. dasymallus *species based on the analysis from first and second codon positions. Consistently, all phylogenetic analyses divulged close association of* P. dasymallus *with* P. vampyrus *contradicting the previous report categorizing* P. dasymallus *under* subniger *species group with* P. pumilus, P. speciosus, *and* P. hypomelanus. *The Philippine endemic species (*P. pumilus*) is closely linked with* P. speciosus. *The representative samples of* P. vampyrus

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showed a large genetic distance of 1.87%. The large genetic distance between P. dasymallus *and* P. hypomelanus, P. pumilus *and* P. speciosus *denotes a distinct species group.*

KEY WORDS: flying foxes; Philippine endemic; *cytochrome b*; species diversification.

INTRODUCTION

The genus *Pteropus* is regarded as the most diverse group among the 42 living genera of fruitbats under the single family Pteropodidae of the suborder Megachiroptera. These flying foxes were categorized by Andersen (1912) into 17 species groups and have over 50 recognized species worldwide (see Nowak, 1991). In the Philippines, there are seven *Pteropus* species reported recently, after trimming down some synonymous species names, and they were classified into *subniger*, *pselaphon*, and *vampyrus* species groups. The seven species are distributed in different parts of the Philippine archipelago classified mostly under the *subniger* group. These are the common island flying fox (*P. hypomelanus*, a widespread species in Asia), the little golden-mantled flying fox (*P. pumilus*) endemic to the Philippines, the Philippine gray flying fox (*P. speciosus*), and the large flying fox (*P. vampyrus*) in the *vampyrus* group. It also includes the Mindoro pallid flying fox (*Pteropus sp. A*) known only from Mindoro Island (Heaney *et al.*, 1998) and the white-winged flying fox (*P. leucopterus*, a member of the *pselaphon* group), poorly known and endemic to Luzon and Dinagat Island (Ingle and Heaney, 1992). *P. speciosus* is found only in the western part of Mindanao, the Sulu faunal region, and two islands in the Java Sea (Heaney *et al.*, 1998). *P. vampyrus* is known to be distributed in Southeast Asia. Moreover, the Ryukyu flying fox (*P. dasymallus*) is another member of the *subniger* species group (Nowak, 1991) of the genus *Pteropus* found in the Ryukyu Islands of Japan to Taiwan. Its distribution also reaches into the Batanes/Babuyan region situated in the northernmost islands of the Philippines (Heaney *et al.*, 1998).

The evolutionary relationships of these flying foxes in the Philippines have received limited attention, particularly from the molecular perspective of sequence data. This led us to investigate the relationships of these flying foxes, using the complete DNA sequences of the *cytochrome b* (*cyt b*) gene in order to clarify some taxonomic issues. Purposely, we assessed the clustering of the four species (*P. pumilus*, *P. speciosus*, *P. hypomelanus*, and *P. dasymallus*) classified under the *subniger* species group to find out which one is closely linked with *P. pumilus*, the flying fox endemic to the Philippines. The genetic distances among the species as well as between the two samples of *P. vampyrus* collected from different sites were ascertained. We also calculated the date of divergence to determine the approximate event of diversification among the *Pteropus* species.

MATERIALS AND METHODS

Specimens Examined and DNA Extraction

Representative samples of flying foxes (Genus *Pteropus*) in the Philippines examined came from different islands of the country. The geographic locations of the samples are presented in Table I and Fig. 1. Two samples of *P. vampyrus* from the northeastern part of Mindanao and the western part of the Sulu archipelago (see Table I) were also included.

Total cellular DNA was extracted from the skin of specimens preserved in ethanol (95%). The sample was thoroughly cleansed with 100-mM EDTA pH 8.0 solution prior to DNA extraction. Following the standard procedure described by Sambrook *et al.* (1989), the DNA was extracted by proteinase K digestion, phenol/chloroform extraction, and ethanol precipitation.

The sequences of *P. dasymallus* (Nikaido *et al.*, 2000) under the species group of *subniger* and *P. scapulatus* (Lin and Penny, 2001) of the *scapulatus* group formed part of our analysis. These species of megabats and microbats were used as outgroups to infer the evolutionary relationships of different species of *Pteropus*. The megabats outgroup included *Eonycteris spelaea* under the subfamily

Table I. Species of Flying Foxes Examined and the Megabat/Microbat Outgroup

Specimen	Common name	Collection site (location number)[b]
Pteropus hypomelanus	Common island flying fox	Batbatan Island, Culasi, Antique Province (2)
Pteropus pumilus	Little golden mantled flying fox	Libjo, Infanta, Quezon Province (1)
Pteropus speciosus	Philippine gray flying fox	Batu-Batu, Panglima Sugala, Tawi-tawi Province (4)
Pteropus vampyrus 1[a]	Large flying fox	Tagbayagan, Rosario, Agusan del Sur Province (3)
Pteropus vampyrus 2	Large flying fox	Batu-batu, Panglima Sugala, Tawi-tawi Province (4)
Pteropus dasymallus	Ryukyu flying fox	AB042770 (Nikaido *et al.*, 2000)
Pteropus scapulatus	Little red flying fox	AF321050 (Lin and Penny, 2001)
Eonycteris spelaea	Dawn bat	Lunas, Borbon, Cebu Province (outgroup)
Cynopterus brachyotis	Dog-faced fruit bat	AB046320 (Bastian *et al.*, 2001)
Ptenochirus jagori	Musky fruit bat	AB046325 (Bastian *et al.*, 2001)
Rousettus amplexicaudatus	Rousette fruit bat	AB046329 (Bastian *et al.*, 2001)
Chalinolobus tuberculatus	New Zealand long-tailed bat	AF321051 (Lin and Penny, 2001)
Uroderma bilobatum	Tent-building bat	L28943 (Baker *et al.*, 1994)

[a] Bastian *et al.*, 2001.
[b] Numbers in parentheses refer to Fig. 1.

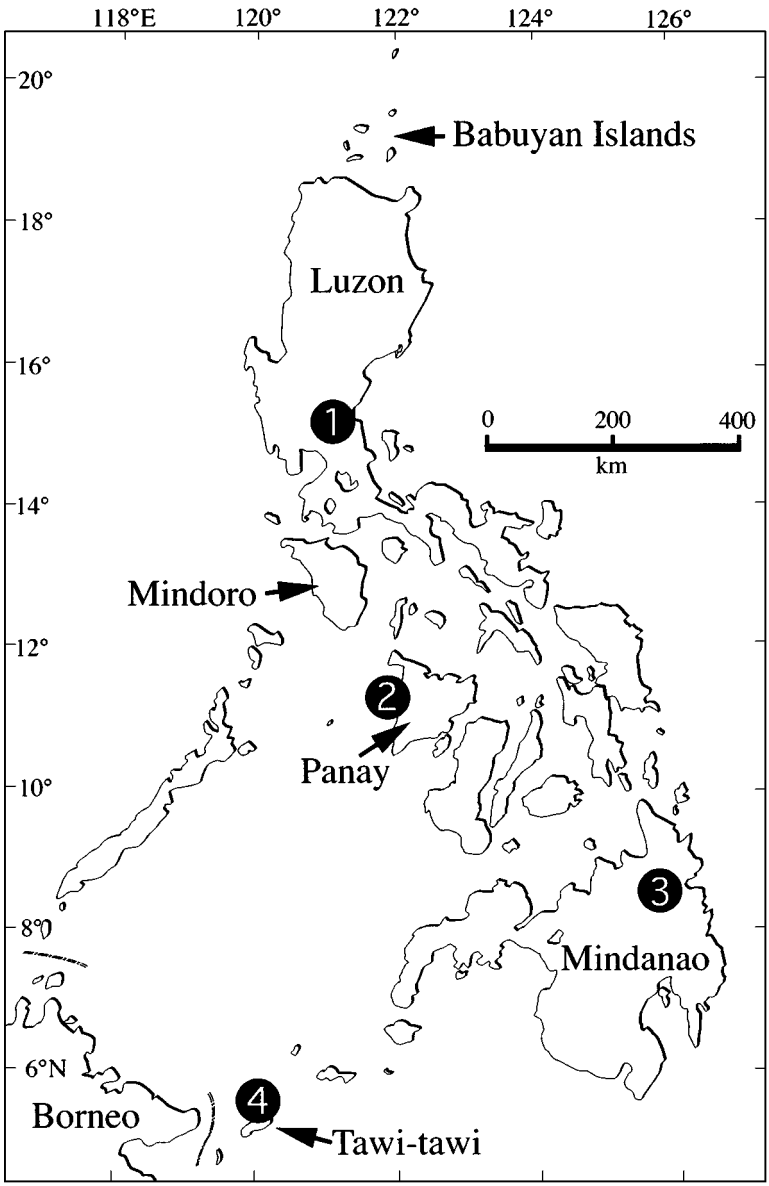

Fig. 1. The Philippines and collection sites of the different species of flying foxes examined. Numbered sites are identified in Table I.

Macroglossinae, and *Cynopterus brachyotis* (AB046320), *Ptenochirus jagori* (AB046325), and *Rousettus amplexicaudatus* (AB046329) under the subfamily Pteropodinae as listed by Bastian *et al.* (2001). The microbats species were *Chalinolobus tuberculatus* (AF321051; Lin and Penny, 2001) and *Uroderma bilobatum* (L28943; Baker *et al*., 1994).

Amplification and Sequencing of *Cyt b* Gene

Amplification of the complete *cyt b* gene (1140 bp) was done through polymerase chain reaction (PCR). The quantity of reagents used in the PCR amplification strictly followed the manufacturer's instruction (TaKaRa Taq, Takara Biomedicals, Japan). Double stranded amplification was performed using the primers L14724, 5′-CGAAGCTTGATATGAAAAACCATCGTTG-3′ (Paabo, 1989); H15915R, 5′-GGAATTCATCTCTCCGGTTTACAAGAC-3′; and L15408, 5′-ATAGACAA AATCCCATTCCA-3′ (Irwin *et al.*, 1991) and the sequence corresponding to H15576 is that of M102, 5′-TAGGCGAATAGGAAATATCATTC-3′ (Chikuni *et al.*, 1994). Primer names indicate the DNA strand (H, heavy, and L, light) and the position of the 3′ end of the oligonucleotide sequence corresponds to the human sequence (Anderson *et al.*, 1981). PCR amplification was carried out using the Takara PCR Thermal Cycler (Takara Biomedicals, Japan). The PCR conditions applied were denaturation at 94°C for 1 min, then for 40 cycles at 94°C for 1 min, 50°C for 1 min, 72°C for 2 min, and extension at 72°C for 5 min. A negative control was included in the amplification, and all mixtures were covered with light white mineral oil (Sigma). The band of interest of the PCR products was checked through 1.5% agarose electrophoresis (Seakem GTG, FMC Bioproducts, USA), stained with ethidium bromide, and viewed via ultraviolet illumination.

Purification of double-stranded PCR products was done twice using the Microspin S400 HR Column (Amersham Pharmacia Biotech, USA). Sequencing of the purified PCR product was carried out using the Dye Terminator Cycle DNA Sequencing Kit (PE Applied Biosystem, USA) as detailed in the manufacturer's manual using the same primer set utilized in the PCR amplification. Products were dissolved in 25 μL of Template Suppression Reagent (PE Applied Biosystem, USA), denatured for 2 min at 95°C and sequenced using an automated sequencer, Model 310 Genetic Analyzer (PE Applied Biosystem, USA). The whole amplified PCR fragment was sequenced with both light and heavy stranded primers. The sequences of *Pteropus* species reported in this paper and *E. spelaea* are available at the DDBJ/EMBL/GenBank with accession numbers, AB062472–AB062476.

Sequence Alignment, Saturation Analysis, and Estimate of Divergence

The DNASIS software (Hitachi Software Engineering Co., Japan) was used for alignment, connection, and homology comparison of the DNA sequences of the

cyt b gene. The published sequences of mammals (Alvarez *et al.*, 1999; Irwin *et al.*, 1991) were used to check the homology of our sequence data.

Saturation analysis was applied in our complete *cyt b* sequence data at the different codon positions of transition and transversion. The percentage of sequence divergence (uncorrected) following the methods of Griffiths (1997) was plotted against the sum of substitutions in all codon positions, both in transition and transversion. The data were considered saturated when there was an indication of plateauing or the majority of the study group exceeded the outgroup. Megabat relatives and the microbat species as mentioned above were included in the analysis to facilitate the detection of saturation.

To estimate the event of species diversification among the *Pteropus* species examined, as well as the time when these pteropodid bats (*Pteropus*) had separated from the megabat relatives and the microbats, we used the sequence differences based on transversion in all codon positions. Such sequence differences by transversion alone showed a linear relationship with time (Irwin *et al.*, 1991; Miyamoto and Boyle, 1989). Thus, we calculated the divergence time using a constant rate of change of 0.2% per million years for transversion in mammals (Miyamoto and Boyle, 1989).

Phylogenetic Analysis

We generated a separate phylogenetic analysis to infer the relationships among the six *Pteropus* species by constructing a phylogenetic tree on the basis of two approaches. First, we generated a topology from the complete nucleotide sequences of *cyt b*. Second, another topology was drawn using sequences from the first and second codon positions because of the saturated third codon position by transition. Employed in the analyses were the neighbor-joining (Saitou and Nei, 1987), maximum likelihood (Felsentein, 1981), and parsimony methods (Felsenstein, 1985) all found in the PHYLIP version 3.572c (Felsenstein, 1993). The neighbor-joining tree was produced based on the genetic distance calculated from the two-parameter model of Kimura (1980), using DNADIST software of PHYLIP. The most parsimonious tree was constructed after employing the SEQBOOT with 1000 bootstrap replicates (Felsenstein, 1985), then DNAPARS with 1000 multiple data sets and CONSENSE were used to generate a consensus tree by majority-rule and strict consensus in the PHYLIP package program. In the maximum likelihood tree the support values for internal nodes were estimated in 100 data sets.

RESULTS

Description of *Cyt b* Sequences

The complete sequence of *cyt b* gene of all *Pteropus* species and the outgroup contained 1140 bp with the initial code of ATG equivalent to methionine and ending

up with arginine (AGA or AGG). Identical sites when microbat species were used as an outgroup were accounted at 57.72% (658 positions), while 42.28% (482 positions) were found to be variable. However, between the megabat relatives and the *Pteropus* group there were identical sites at 64.91% (740 positions) with 35.09% (400 positions) remaining variable. Among the *Pteropus* species, identical sites reached 77.54% (884 positions), and the remaining 22.46% (256 positions) were variable. Most of the variable sites occurred in the third position; the second codon positions had the least variable part of the *cyt b* gene, both in transition and transversion. The substitution rate among the *Pteropus* specimen examined was 71.16% for transition and 28.84% for transversion. The percentage distribution at the respective codon positions by transition was 15.80 (first), 4.36 (second), and 79.84 (third). The transversion distributions were 17.14% (first), 7.74% (second), and 75.12% (third).

Saturation Analysis and Genetic Distance

We graphically assessed the saturation in the first, second, third, and all combined codon positions of *cyt b* gene by plotting the rates of transition and transversion separately. As shown in Fig. 2, the first and second codon positions for transition and all the codon positions for transversion were not saturated.

The genetic distance using the two-parameter model of Kimura (1980) in detecting the genetic divergence among the taxa is presented in Table II. There was a relatively large variation of 1.87% between samples of the *P. vampyrus* species collected from two different sites (see Fig. 1). The Philippine endemic species *P. pumilus* and *P. speciosus* restricted to the Philippines and nearby islands of the continental Sunda shelf had a very close genetic distance of 2.50% among the congeneric species examined. The Ryukyu flying fox (*P. dasymallus*) also showed a closer genetic affinity (4.22–4.88%) with the large flying fox (*P. vampyrus*) than its *subniger* relatives (*P. hypomelanus*, *P. pumilus*, and *P. speciosus*) having 12.41–12.78% genetic distances. Overall genetic divergence among the different species of *Pteropus* ranged from 2.50 to 14.66%.

Phylogenetic Relationships and Divergence Time

The phylogenetic analysis using different methods of phylogenetic reconstruction (neighbor-joining, parsimony, and maximum likelihood) has generated two different kinds of topologies involving the relationships of six *Pteropus* species (see Fig. 3) with the megabat relatives and microbats used as an outgroup. The phylogenetic tree produced from the complete nucleotide sequences of *cyt b* gene (Fig. 3(A)) varies from that derived from the sequences of the first and second codon positions (Fig. 3(B)) particularly in the placement of *P. scapulatus* and *P. hypomelanus*. However, both parsimony (1000 replications) and maximum likelihood (100 replications) analyses derived an identical topology (Fig. 3) using

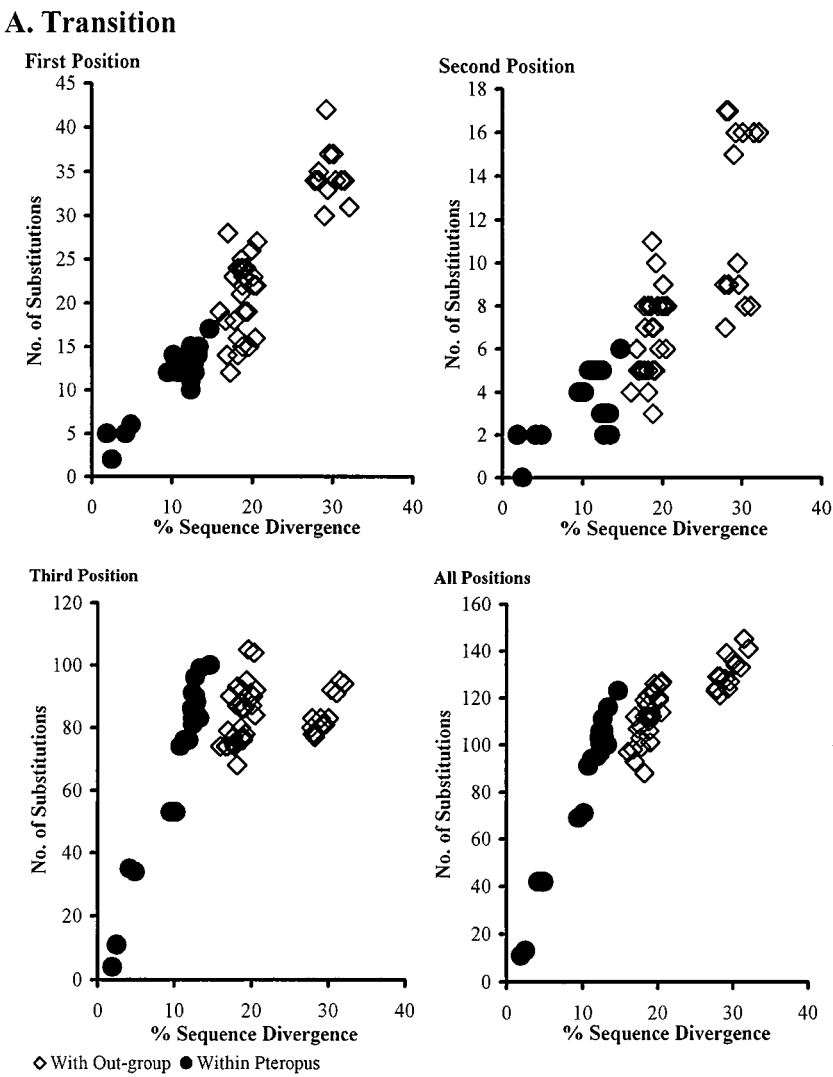

Fig. 2. Saturation assessments in the first, second, third, and all codon positions of transition (A) and transversion (B) in the complete DNA sequences of *cyt b* gene. Circle, comparisons within species of *Pteropus*. Square, comparisons with the outgroup.

two separate approaches of phylogenetic tree reconstruction. The position of the *P. dasymallus* species consistently clustered with *P. vampyrus* in all topologies. The same trend was also confirmed in the phylogenetic clustering of *P. pumilus* and *P. speciosus*.

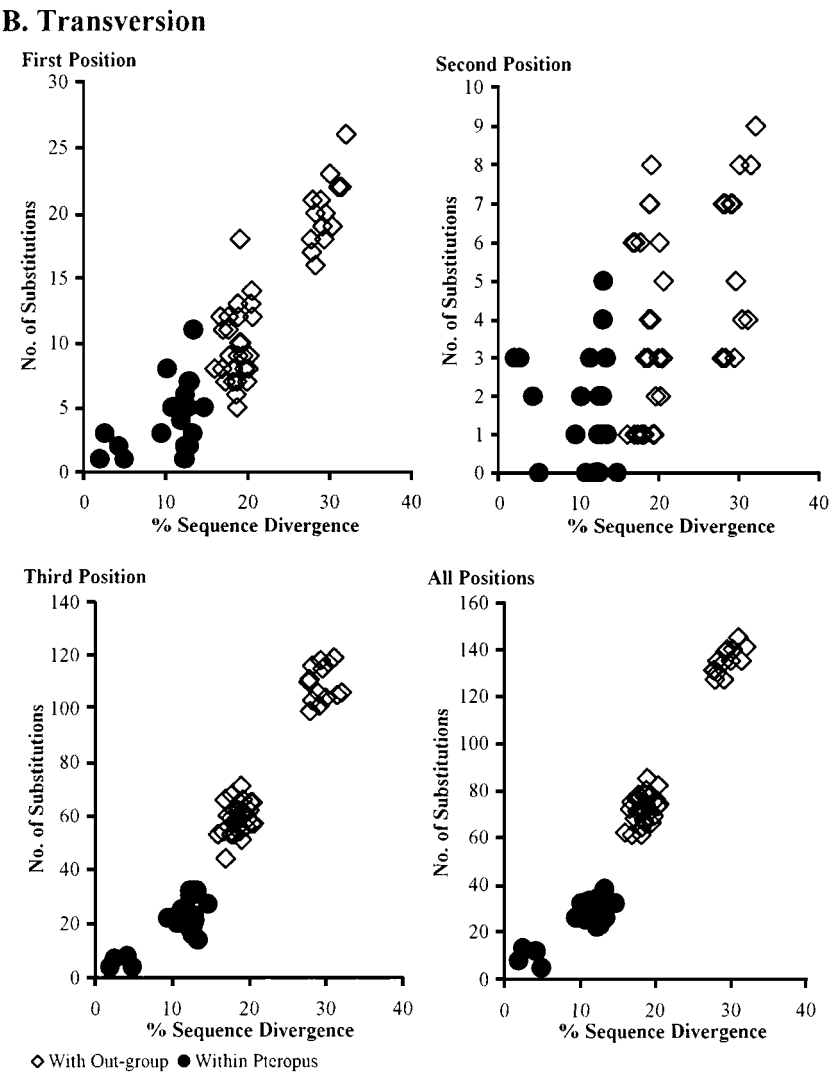

Fig. 2. (*Continued*)

Using the sum of substitutions by transversion in all codon positions, our estimate for the separation of *C. tuberculatus* and *U. bilobatum* from the megabats was approximately 58.15 ± 2.85 MYA (million years ago), whereas the split of *Pteropus* species from their megabats relatives was approximately 31.50 ± 3.13 MYA as proven by examining the complete sequences of *cyt b* and 31.74 ± 3.90

Table II. Percentage Matrix of Genetic Distances[a] and Total Substitutions of Transversion[b] in the Complete Sequences of *Cyt b* Gene Among *Pteropus* and Outgroup Species

Species	1	2	3	4	5	6	7	8	9	10	11	12	13
1. *Uroderma bilobatum*[c]		138	118	124	122	130	130	134	135	127	141	126	135
2. *Chalinolobus tuberculatus*[c]	31.59		135	132	135	131	131	139	140	135	145	131	140
3. *Rousettus amplexicaudatus*[d]	27.16	29.80		74	82	61	66	71	69	64	74	66	76
4. *Ptenochirus jagori*[d]	27.54	29.02	19.57		26	90	71	61	66	71	73	69	78
5. *Cynopterus brachyotis*[d]	26.49	30.71	18.89	12.55		93	78	62	76	77	82	73	85
6. *Eonycteris spelaea*[d]	28.04	30.92	18.22	20.24	18.25		71	76	74	61	77	72	80
7. *Pteropus dasymallus*	28.29	27.91	18.69	18.20	17.83	17.86		34	23	25	28	5	12
8. *Pteropus hypomelanus*	29.02	29.37	18.65	18.07	16.04	17.79	12.41		27	32	32	31	38
9. *Pteropus pumilus*	31.54	30.45	19.86	19.60	20.19	18.75	12.45	9.51		23	13	23	30
10. *Pteropus scapulatus*	29.25	28.27	18.21	19.42	19.16	17.13	10.77	14.66	12.66		26	26	33
11. *Pteropus speciosus*	32.12	31.10	20.60	20.37	20.51	19.23	12.78	10.23	2.50	13.40		26	33
12. *Pteropus vampyrus* 1	27.98	27.80	18.59	17.29	16.92	16.88	4.22	12.42	12.26	11.27	12.39		8
13. *Pteropus vampyrus* 2	30.08	29.65	20.06	18.85	19.04	18.82	4.88	13.28	12.91	11.91	13.04	1.87	

[a] Below the diagonal, using two-parameter model of Kimura (1980).
[b] Above the diagonal.
[c] Microbats.
[d] Megabats.

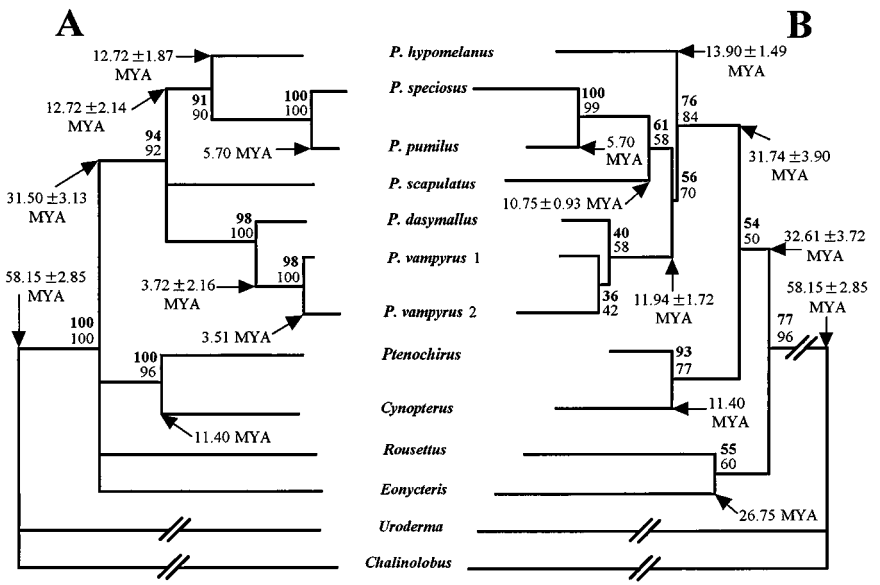

Fig. 3. Phylogenetic tree depicting the relationships of six species of flying foxes (genus *Pteropus*) based on the complete DNA sequences of *cyt b* (1140 bp) gene (A) and from the first and second codon positions (B) ascertained using the parsimony (1000 replications) and maximum likelihood (100 replications) methods. Numbers at nodes indicate the bootstrap values for parsimony (bottom number) and for the maximum likelihood method (bold). Branch lengths are proportional to the topology generated by the neighbor-joining method. Divergence time (in million years ago) on the node separating the taxa was estimated based on the total substitutions by transversion only.

MYA based on the first and second codon positions. Cynopterine and the Pteropus branched out 31.74 ± 3.90 MYA after they split with *R.* amplexicaudatus and *E. spelaea* at 32.61 ± 3.72 MYA.

We estimated that among the *Pteropus* species, the first evolutionary event arose probably 13.90 ± 1.49 MYA when *P. hypomelanus* separated from its congeneric species as depicted in the phylogenetic tree generated from the sequences of first and second codon positions. In 11.94 ± 1.72 MYA two clusters were formed in the phylogenetic tree. The first cluster was composed of *P. scapulatus* and the Philippine endemic species: *P. pumilus* and *P. speciosus*, a species restricted in western Mindanao and nearby islands in the Java Sea. Thereafter, at approximately 10.75 ± 0.93 MYA the three species split. At about 5.70 MYA speciation took place between *P. pumilus* and *P. speciosus*. The second cluster comprised *P. dasymallus* and the two samples of *P. vampyrus* collected from different sites. Divergence between *P. dasymallus* and *P. vampyrus* happened 3.72 ± 2.16 MYA. In our calculation the divergence between *P. vampyrus* samples might have occurred right after the *P. vampyrus* split with *P. dasymallus* at about 3.51 MYA.

DISCUSSION

Phylogenetic Analysis and Relationships Among Species

To establish the phylogenetic relationships of the extant *Pteropus* species inferred from the DNA sequences of *cyt b* gene, the two approaches, using the complete sequences and the sequences of the first and second codon positions, provided resolution concerning their relationships. For instance, the analysis using the first and second codon positions has clarified that *P. hypomelanus* was the first among the *Pteropus* species that diverged ahead with its congeners (13.90 ± 1.49 MYA) as shown in the phylogenetic tree (Fig. 3(B)). Such a finding was not made clear (i.e., having the same divergence time) utilizing the complete sequences in the phylogenetic analyses (see Fig. 3(A)). Perhaps it was due to the potential noise of the molecular character contributed by the saturated third codon position by transition (Griffiths, 1997).

In the phylogenetic tree (Fig. 3), it was shown that *P. pumilus* (Philippine endemic species) and *P. speciosus* (species found on western Mindanao) that were categorized under the *subniger* species group by Andersen (1912) consistently belong to the same cluster in all the phylogenetic analyses. However, the relationship of *P. dasymallus* with those species within the *subniger* species group is not sustained. Instead, this Ryukyu flying fox (*P. dasymallus*) shows a closer relationship with *P. vampyrus*. The close genetic distance between these two species and their consistent clustering in the topology using all phylogenetic methods support this finding. The large genetic distance of 1.87% detected between samples of the *P. vampyrus* species suggests that it had different populations in the Philippines that may have divided into subspecies. This finding coincided with the report of Lawrence (1939) and Rabor (1986) that *P. vampyrus* found in the Philippines, particularly Cebu, Dinagat, Leyte, Negros, Samar, Panay, Zamboanga, Basilan, and the island of Luzon, belongs to the subspecies *P. v. lanensis* (Rabor, 1977). However, three other subspecies were found in Indonesia (Tate, 1942): *P. v. malaccensis* distributed in Sumatra, *P. v. pluton* in Bali, and *P. v. natunae* in Borneo. Seemingly, the population of the subspecies of *P. vampyrus* that colonized Borneo spread into nearby islands like Tawi-tawi (Fig. 1) because of its very near geographic location and the flight ability of this mammal.

Species Diversification

The phylogenetic tree generated from the sequences of *cyt b* gene using the different phylogenetic reconstruction methods (neighbor-joining, parsimony, and maximum likelihood) provides a clear picture regarding the phylogenetic placement of the different *Pteropus* species rooted from the megabat relatives and microbat outgroup. The evolutionary relationships among *Pteropus* species are well explained

by the clustering of taxa with their corresponding dates of diversification. In our calculation, the separation of megabat species from the microbats (*C. tuberculatus* and *U. bilobatum*) took place probably during the Paleocene (58.18 ± 2.85 MYA) when the modern Philippine archipelago was still attached to the mainland of Asia (Savage and Russell, 1983). Subsequently, megabats have further diversified 32.61 ± 3.72 MYA and 31.74 ± 3.90 MYA between cynopterine and pteropodid bats. We hypothesize that the ancestors of extant megabats entered the Philippines from any area to the south (Heaney, 1991) during the late Eocene (about 35 MYA) wherein at that time the northwest-facing islands during the Paleocene to Eocene were extended from northwest Borneo to northern Luzon (Taylor and Hayes, 1980).

Our phylogenetic analysis from the first and second codon positions in six flying fox species reveals that the first evolutionary history can be dated back at about 13.90 ± 1.49 MYA (middle Miocene). The separation of *P. hypomelanus* with its congeners (see Fig. 3(B)) was the first event. Subsequent diversification also occurred 11.94 ± 1.72 MYA dividing the remaining *Pteropus* species into two clusters. The first cluster is composed of *P. speciosus, P. pumilus*, and *P. scapulatus* and diverged at about 10.75 ± 0.93 MYA, whereby the ancestors of *P. speciosus* and *P. pumilus* colonized the Philippines and *P. scapulatus* spread in Australia, New Guinea, and New Zealand (Nowak, 1991). These islands surrounded by a constellation of lesser islands share much of their fauna (Heaney, 1986). The split of *P. scapulatus* with *P. pumilus* and *P. speciosus* might be explained by the eastward subduction in the late or middle Miocene (5–12 MYA) of the Southeast Sulu Sea and its northward continuation through Panay (Mitchell *et al.*, 1986), thus separating the Philippines from mainland Asia. The subduction of western Mindanao extending through Samar and southeastern Luzon before the late Miocene followed by a subsequent westward subduction of the Philippine Sea in the Philippine Trench in the late Miocene (8 MYA) or Pliocene (5 MYA) (Mitchell *et al.*, 1986) contributed most likely to such speciation. Correspondingly, the formation of the isolated oceanic islands in the Philippines in the beginning of the Miocene towards the Pliocene might have caused the ancestors of *P. pumilus* and *P. speciosus* to undergo a substantial diversification within the Philippines. Geographically isolated populations can diverge freely, which leads to reproductive isolation (Via, 2001). Consequently, *P. pumilus* spread in the islands of Luzon, Negros, Mindanao, Leyte, and Maripipi (Heaney, 1991) while *P. speciosus* specialized in the western part of Mindanao, Sulu archipelago, and two islands in the Java Sea (Heaney *et al.*, 1998). The occurrence of geological splitting of the former continguous landmass and the vegetation that support bats in spite of apparently greater vagility contributed to species richness (Heaney, 1991).

The presence of the closest relative of the Philippine *Pteropus* species in the Sunda Shelf (i.e., *P. griseus*; Heaney, 1986) discloses that there was no evidence that megabats found in the Philippines entered from the north, rather they came from

both the Sunda Shelf of Southeast Asia and from Sulawesi/New Guinea (Heaney, 1991). Therefore, if this hypothesis is correct, the progenitor of *P. dasymallus*, noted to be phylogenetically related to *P. vampyrus* that split 3.72 ± 2.16 MYA in our calculation, has undergone speciation in the northernmost islands of the Philippines (i.e., Batanes/Babuyan region; Heaney *et al.*, 1998) towards Taiwan and Japan. Also, the subduction that happened in the trench east and west of Mindoro that resulted in the Pliocene collision (1.8–5 MYA) in Taiwan (Mitchell *et al.*, 1986) created geographical and ecological factors affecting speciation (Barraclough and Nee, 2001).

The mammalian fauna found in the Philippine islands is remarkably diverse. The number of endemic species and genera, volant or nonvolant animals, suggests that at least some species originated as a result of speciation by colonizers. The degree of divergence of some groups like murids and insectivores indicated that colonization may have begun long ago with clear evidence of natural dispersal (Heaney, 1986). The number of endemic species in the Philippines has accumulated as a result of the collision of the major Pacific lithospheric plate in the South China Sea. Reported numbers of Philippine nonvolant species from the oceanic islands (i.e., Mindoro, existed 8–10 MYA, Miocene-Pliocene, has 14 native species and Negros, with eight native species, originated during Pliocene-Pleistocene, 1–4 MYA) imply that speciation in *Pteropus* (*P. pumilus* and *P. speciosus*) began long ago by colonizers. An old landbridge island such as Palawan has a high level of endemism (Heaney, 1986). Moreover, fruit bats often have a history of successful colonization greater than seen among nonvolant mammals (Heaney, 1991).

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