# Chapter 6
# Phylogenetic Relationships of Harpyionycterine Megabats (Chiroptera: Pteropodidae)

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## ABSTRACT

After almost 70 years of stability following publication of Andersen's (1912) monograph on the group, the systematics of megachiropteran bats (Chiroptera: Pteropodidae) was thrown into flux with the advent of molecular phylogenetics in the 1980s—a state where it has remained ever since. One particularly problematic group has been the Austromalayan Harpyionycterinae, currently thought to include *Dobsonia* and *Harpyionycteris,* and probably also *Aproteles*. In this contribution we revisit the systematics of harpyionycterines. We examine historical hypotheses of relationships including the suggestion by O. Thomas (1896) that the rousettine *Boneia bidens* may be related to *Harpyionycteris*, and report the results of a series of phylogenetic analyses based on new as well as previously published sequence data from the genes *RAG1*, *RAG2*, *vWF*, *c-mos*, *cytb*, *12S*, *tVal*, *16S*, and *ND2*. Despite a striking lack of morphological synapomorphies, results of our combined analyses indicate that *Boneia* groups with *Aproteles*, *Dobsonia,* and *Harpyionycteris* in a well-supported, expanded Harpyionycterinae. While monophyly of this group is well supported, topological changes within this clade across analyses of different data partitions indicate conflicting phylogenetic signals in the mitochondrial partition. The position of the harpyionycterine clade within the megachiropteran tree remains somewhat uncertain. Nevertheless, biogeographic patterns (vicariance-dispersal events) within Harpyionycterinae appear clear and can be directly linked to major biogeographic boundaries of the Austromalayan region. The new phylogeny of Harpionycterinae also provides a new framework for interpreting aspects of dental evolution in pteropodids (e.g., reduction in the incisor dentition) and allows prediction of roosting habits for *Harpyionycteris*, whose habits are unknown.

## INTRODUCTION

During the last decade, the traditional classification of Megachiroptera (Mammalia: Chiroptera) has been challenged by a number of molecular phylogenetic studies that have called into question many of the systematic groupings established by Andersen (1912) and revised recently by Bergmans (1997). One particularly controversial megachiropteran taxon has been Harpyionycterinae Miller, 1907, an Austromalayan group currently thought to include *Harpyionycteris* Thomas, 1896, *Dobsonia* Palmer, 1898, and almost certainly *Aproteles* Menzies, 1977 (Giannini et al., 2006). There has been disagreement about the affinities of the nominate genus since its description, largely due to a suite of unique craniodental character states seen in *Harpyionycteris* (see Andersen, 1912). Thomas (1896) rather vaguely suggested placement of *Harpyionyc-*

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TABLE 1
**Internal Primers for *RAG1* and *vWF* Genes Designed for This Study**

| Gene | Primer | 5′–3′ sequence |
|---|---|---|
| *RAG1* | RAG1f2 | GCCAAGCCCTTCATTGAGACA |
| | RAG1r2 | CTATGGAAGGGACTGTCTCAATG |
| *vWF* | vWFf2 | CTTYGTGSTCAGTGGTGTGGA |
| | vWFr2 | TCCACACCACTGASCACRAAG |
| | vWFf3 | CTATGYCCAGGGCCTGAAGAA |
| | vWFr3 | GCTCCTGTTGAAATTGGCCT |

*teris* with *Rousettus* Gray, 1921, and *Boneia* Jentink, 1879. In contrast, Andersen (1912) linked *Harpyionycteris* with *Dobsonia* (see below), and *Boneia* with *Rousettus*. *Boneia* was synonymized with *Rousettus* by Bergmans and Rozendaal (1988) and this arrangement has been followed by many authors (Bergmans, 1994, 1997; Corbet and Hill, 1992; Simmons, 2005).

Andersen (1912) formally followed Miller (1907) in recognizing Harpyionycterinae as a distinct group, but wrote in extenso about a close relationship between *Harpyionycteris* and *Dobsonia*. He concluded that ''So evident is the phylogenetic connection between these two genera that *Harpyionycteris* may be said, almost with certainty, to be the peculiarly modified Philippine representative of the Austro-Malayan *Dobsonia*'' (Andersen, 1912: 803). Miller and Hollister (1921) subsequently described *Harpyionycteris celebensis* from Sulawesi, providing a further biogeographic link between *Harpyionycteris* and *Dobsonia*.

In spite of Andersen's (1912) arguments, all subsequent authors (e.g., Corbet and Hill, 1992; Koopman, 1993, 1994; Tate, 1951; Slaughter, 1970) followed Miller (1907) in considering *Harpyionycteris* as a peculiar pteropodid best placed in a subfamily of its own. However, two recent studies provided evidence supporting Andersen's (1912) hypothesis of a close relationship between *Harpyionycteris* and *Dobsonia*. Support for this grouping was found in Romagnoli and Springer's (2000) analysis of morphological data, although caution must be exercised in interpreting these results because of minimal clade support and the small number of characters used in that study. More compellingly, separate and combined parsimony analyses of two coding genes, one nuclear (exon 28 of the von Willebrand factor gene) and one mitochondrial (cytochrome *b*), recovered a highly supported clade grouping *Harpyionycteris whiteheadi* and four species of *Dobsonia* from three distinct species groups (Giannini et al., 2006). As a consequence of these results, Giannini et al. (2006) expanded Harpyionycterinae Miller, 1907, to include *Dobsonia.* They also concurred with previous authors in concluding that *Aproteles* probably also belongs in this subfamily based both on general morphological evidence (Flannery, 1995; Menzies, 1977; Giannini and Simmons, 2005; Springer et al., 1995) and results of all previous phylogenetic analyses in which *Aproteles* was included (Colgan and da Costa, 2002; Giannini and Simmons, 2003, 2005; Jones et al., 2002; Kirsch et al., 1995; Romagnoli and Springer, 2000; Springer et al., 1995).

The harpyionycterines as currently understood occupy three major Austromalayan subregions, the Philippine, Wallacean, and Papuan. The distributions of a few species marginally exceed the boundaries of those territories and enter the Australian and Sundaic regions (see Byrnes, 2005; Corbet and Hill, 1992; Koopman, 1993, 1994; Simmons, 2005). The Austromalayan region is crossed by major biogeographic boundaries, including the Wallace, Lydekker, and Weber lines. Organisms distributed across these various boundaries, such as harpyionycterine bats, may contribute to the understanding of the complex connections among the Philippine, Wallacean, and Papuan biogeographic regions and, more generally, between Asia and Australia.

In the present study, we explore further the membership and affinities of the expanded

Harpyionycterinae (sensu Giannini et al., 2006) using new molecular data as well as published sequences of relevant taxa. We test the relationship of *Boneia* and *Rousettus* to *Harpyionycteris* as well as the relationship of *Aproteles* to *Dobsonia*, and examine the problem of placing Harpyionycterinae in the megachiropteran tree. Finally, we explore the biogeographic patterns suggested by phylogenetic relationships recovered in this study.

## METHODS

### Taxa

We selected megachiropteran taxa in order to test monophyly of Harpyionycterinae as currently defined (*Harpyionycteris*, *Dobsonia*, and *Aproteles*) and investigate relationships of these taxa to other genera of megabats as previously proposed in the literature. Specifically, we sought to test (1) the association of *Boneia*, *Rousettus,* and *Harpyionycteris* proposed in the description of the latter (Thomas, 1896) versus the association of *Boneia* with *Rousettus* (Andersen, 1912; Bergmans and Rozendaal, 1988) and *Harpyionycteris* with *Dobsonia* (Andersen, 1912; Giannini et al., 2006); (2) the sister relationship of *Aproteles* and *Dobsonia* (Colgan and da Costa, 2002; Giannini and Simmons, 2003, 2005; Menzies, 1977; Romagnoli and Springer, 2000); and (3) the inclusion of *Dobsonia* and their relatives in a pteropodine clade (Romagnoli and Springer, 2000) versus a rousettine clade (Andersen, 1912; Bergmans, 1997).

Megachiropteran terminals were chosen from each main pteropodid clade following Giannini et al. (2006): *Nyctimene albiventer* and *N. vizcaccia* (Nyctimeninae), *Cynopterus sphinx* and *Ptenochirus jagori* (Cynopterinae), *Boneia bidens*, *Eonycteris spelaea*, *Rousettus amplexicaudatus,* and *R. aegyptiacus* (Rousettinae), *Myonycteris torquata* and *Megaloglossus woermanni* (Epomophorinae, Myonycterini), *Epomops franqueti* and *Epomophorus wahlbergi* (Epomophorinae, Epomophorini), *Macroglossus minimus* and *Syconycteris australis* (Macroglossinae), *Melonycteris fardoulisi* and *Melonycteris melanops* (Pteropodinae, Notopterini), and *Pteropus hypomelanus* and *P. tonganus* (Pteropodinae, Pteropodini). Harpyionycterinae megabats (sensu Giannini et al., 2006) included are: *Aproteles bulmerae*, *Harpyionycteris whiteheadi*, *H. celebensis*, and a member of each of the four currently recognized *Dobsonia* species groups (Simmons, 2005), namely *Dobsonia minor* (*minor* species group), *inermis* (*viridis* group), *D. moluccensis* (*moluccensis* group), and *D. peronii* (*peronii* group). Here *D. magna* is considered a junior synonym of *D. moluccensis* following Helgen (2007; see also Byrnes, 2005; Koopman, 1994).

Trees were rooted using *Rhinopoma hardwickii* (Rhinopomatidae) and *Artibeus jamaicensis* (Phyllostomidae). Rhinopomatids are currently thought to belong to Rhinolophoidea, which is the sister clade of megabats in multigene phylogenetic analyses, while phyllostomids represent the other major clade of echolocating bats, Yangochiroptera (Eick et al., 2005; Teeling et al., 2005; Miller-Butterworth et al., 2007).

### Sequences

We used sequences of exon 28 of the von Willebrand factor gene (*vWF*, 1231 bp) and cytochrome *b* (*cytb*, 1140 bp) from Giannini et al. (2006), and generated new sequences for harpyionycterine species for this study. We also incorporated sequences of two other nuclear coding genes, the recombination activating gene 1 (*RAG1*, 1084 bp), and the recombination activating gene 2 (*RAG2*, 760 bp) from Giannini et al. (2008) for the same taxa, and similarly generated new sequences for harpyioncterines. In order to include relevant species whose samples were not available to us, we used published sequences of the mitochondrial 12S rDNA (*12S*, ca. 970 bp) and Valine tDNA (*tVal*, ca. 70 bp) and contributed new sequences for these genes. For additional analyses (see below), we incorporated published sequences of the mitochondrial 16S rDNA (*16S*, ca. 1560 bp for complete taxa) and NADH dehydrogenase subunit 2 (*ND2*, 1044 bp) genes, and the oocyte maturation factor Mos proto-oncogene (*c-mos*, ca. 480 bp). Composition of the data set, accession numbers of all sequences used, and voucher information for new sequences are given in appendix 1.

Total DNA was obtained from preserved tissue samples with the DNeasy tissue kit

(QIAGEN). PCR amplification was carried out using previously published primers (*12S-tVal*: Springer et al., 1995; *cytb*: Bastian et al., 2002; *RAG1* and *RAG2*: Teeling et al., 2000; *vWF*: Porter et al., 1996). To obtain both forward and reverse sequences for each gene region, additional internal primers were developed for the *RAG1* and *vWF* genes (table 1). All sequences were obtained with an automated ABI 3730XL sequencer. Sequence editing and prealignment were done with the Sequencher 4.2 software (Gene Codes). Accession numbers for the new sequences are FJ218461-84. Although for some species more than one sample was available, only one was included in each analysis. The aligned dataset is provided at ftp://ftp.amnh.org/pub/group/mammalogy/downloads/.

## Phylogenetic Analyses

Sequences of our coding and ribosomal genes were submitted to parsimony analysis. In the present study, we used data for the genes for which we contributed new sequences (i.e., *12S-tVal*, *cytb*, *RAG1*, *RAG2*, and *vWF*) to conduct three principal sets of phylogenetic analyses: (1) individual gene partitions; (2) nuclear and mitochondrial partitions; and (3) all genes combined. Here we report results of the main partitions and the combined set, and comment on results from the individual genes where relevant. In additional analyses, we used genes not sequenced in our study (*16S*, *ND2*, and *c-mos* in individual and combined sets; see details below).

Analyses involving only coding genes (individual analyses for *c-mos*, *cytb*, *RAG1*, *RAG2*, *vWF*, and the nuclear partition) were performed under conventional parsimony (static alignment). The tree search strategy consisted of 500 replicates of random addition sequences of taxa (RAS) each followed by tree bisection reconnection branch swapping (TBR). An additional round of TBR was done on optimal trees so obtained. Clade support was assessed using Bremer or decay values (Bremer, 1994) and character resampling (Goloboff et al., 2003). We calculated Bremer values via incremental sampling of suboptimal trees (see Giannini and Bertelli, 2004). Briefly, we saved up to 2000 suboptimal trees one step longer than the previous optimum in successive stages. That is, we first searched for suboptimal trees 1 step longer than the optimal tree length, next saving suboptimals at every step from 2 through 10 steps longer than the optimal trees. Second, group frequency (based on unbiased symmetric resampling; Goloboff et al., 2003) was calculated on the basis of 5000 replications. These analyses were executed in TNT 1.0 (Goloboff et al., 2004, 2008).

For all analyses involving ribosomal and transfer RNA genes (i.e., individual *12S-tVal* partition, the mitochondrial partition, the combined set, and additional analyses using *16S*), we used direct optimization (DO; Wheeler, 1996). In this approach, alignment is viewed as dynamic (i.e., varying across topologies in tree space) and is therefore linked to tree search. The transformation cost of whole unaligned sequences on a topology is calculated via optimization. Costs of each transformation type (indel or substitution) were established a priori—as in every alignment procedure. According to these costs, the length of the tree is calculated as the sum of transformations on each internal node in the downpass optimization, which minimizes indels and substitutions (steps) of whole sequences on the candidate tree. Minimum-length trees are chosen among those visited. In practice, this is done by way of tree building and branch swapping. We set equal costs of indels and substitutions, and our search strategy consisted of replicated swapping + refinements (e.g., Giannini and Simmons, 2003, 2005). Briefly, 100 RAS + TBR branch swapping were run, collecting all optimal trees from each replication. Those trees were submitted to tree fusing (Goloboff, 1999). In analyses combining ribosomal and coding genes, the latter were treated as prealigned; i.e., no indels were allowed in coding genes (static alignment). All DO analyses, including searches to calculate Bremer support values, were performed using POY 4 (Varon et al., 2007).

Additional phylogenetic analyses were conducted to take advantage of additional published data. First, we added published *16S* and *c-mos* sequences to our combined set to provide a "total-evidence" analysis based on sequences available for the majority of our taxonomic samples. Second, we ran a specific

analysis of *ND2* with the few sequences of megabats available in GenBank (which unfortunately had low overlap with our taxonomic sampling) to provide a separate test of the affinities of *Boneia*. Data available included *ND2* sequences of *Dobsonia minor* among the harpyionycterines and also *Boneia bidens*. This analysis was important because our data on *Boneia* originated from a single individual (ZMA 23100) from which we extracted DNA and generated all sequences, whereas the *ND2* sequences originated from DNA that was amplified from a different individual (GenBank accession number AY504581).

In addition to the parsimony analyses, we performed a maximum-likelihood (ML) tree search for the combined nuclear data set using a GTR + Γ + I partitioned model (i.e., parameters were estimated from the data for each gene separately) using the program RAxML (Stamatakis, 2006; Stamatakis et al, 2008). Our search strategy consisted of executing 100 rapid bootstrap inferences and thereafter a thorough ML search. Statistical support was obtained with 100 bootstrap replications.

### Biogeographic Analysis

Our biogeographic analysis consisted of mapping onto the optimal tree obtained from our total-evidence phylogenetic analysis a single biogeographic character composed of six states, each of which consisted of known distribution areas of terminals (see below). We performed a partial (downpass-only) Fitch (1971) optimization in order to recover the pattern of minimal biogeographic events required by the tree. In this case, we did not intend to provide wide coverage of Megachiroptera; our aim was instead to identify the main dispersal-vicariance events of our ingroup, the Harpyionycterinae—those events that led to the origin of currently recognized genera. This attempt is necessarily incomplete as we did not include all *Dobsonia* species. Therefore we restricted our analysis to the sampled harpyionycterines and their areas of occurrence, which were coded in a single multistate, unordered character whose states consisted of broad biogeographic subregions of the eastern Austromalayan region as recognized by Corbet and Hill (1992): Sulawesi (SUL), the Philippines (PHI), Lesser Sunda Islands (LSI), the Moluccas (MOL), New Guinea (NGU), Australia (AUS), and the Solomon Islands (SOL). Taxa occurring in more than one area were scored as polymorphic (for instance, *Dobsonia minor* was scored NGU/SUL).

## RESULTS

### Nuclear Partition

Parsimony analysis of nuclear data (*RAG1*, *RAG2*, and *vWF* sequences) resulted in 10 most-parsimonious trees of 1922 steps (strict consensus tree in fig. 1). In this tree, Megachiroptera is highly supported (Bremer support value, BS = 38). Mutually monophyletic Nyctimeninae and Cynopterinae form a clade that corresponds with Andersen's (1912) *Cynopterus* section, and is sister to a poorly supported multichotomy of four clades containing all other megachiropterans. The four groups are: a reduced macroglossine clade (*Macroglossus* + *Syconycteris*); a reduced pteropodine clade including *Melonycteris* and *Pteropus* (see Bergmans, 1997; Giannini et al., 2008); a clade composed of rousettine (*Rousettus* and *Eonycteris*) and epomophorine megabats, including Myonycterini (*Megaloglossus* and *Myonycteris*) and Epomophorini (*Epomophorus* and *Epomops*); and a harpyionycterine clade (sensu Giannini et al., 2006) that includes *Boneia* as sister to *Harpyionycteris*, and both sister to *Dobsonia*. Our ML analysis (fig. 2) recovered the same major groupings as the parsimony analysis (fig. 1), resolving the backbone polytomy of the parsimony result (cf. fig. 1) with varying degrees of support (bootstrap values 32–82). This topology is identical to one of the optimal topologies obtained under parsimony (i.e., in this analysis, parsimony is conservative in reflecting conflicts in the data). Regarding harpyionycterine megabats, the branching pattern is the same in both ML and MP analyses (cf. figs. 1 and 2).

### Mitochondrial Partition

The analysis of the *12S-tVal* + *cytb* sequences under direct optimization produced a single tree at 3220 steps (fig. 3). Megachiroptera is highly supported (BS =

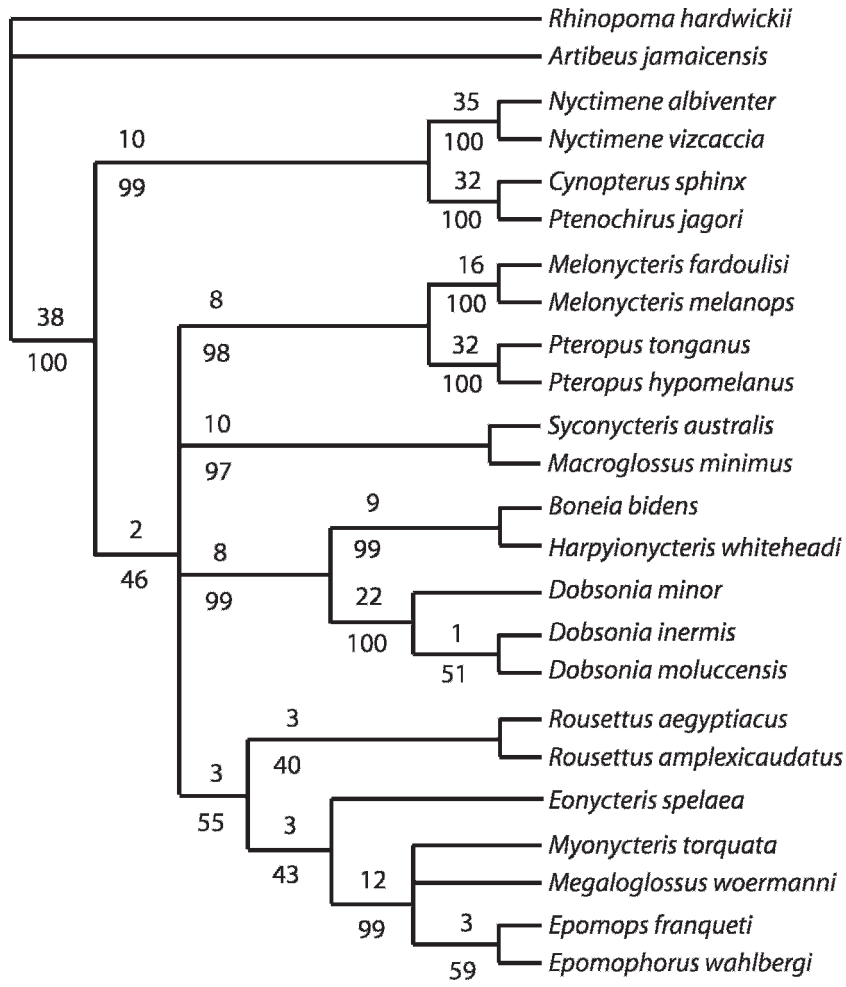


Fig. 1. Strict consensus tree resulting from a parsimony analysis of three nuclear coding genes combined (*RAG1*, *RAG2*, and *vWF*). Bremer support values are given above branches and resampling (jackknife) frequencies are given below branches.

36), but much of the backbone of the megachiropteran subtree is not very well supported ($2 \leq BS \leq 6$). *Melonycteris* appears as sister taxon to *Nyctimene*, *Syconycteris*, and a large group composed of the remainder of megachiropterans. The latter is further subdivided into a clade containing (Pteropodini + Cynopterinae), the previously reported rousettine-epomophorine clade, and another version of the harpyionycterine clade including *Aproteles*, *Boneia*, *Dobsonia*, *Harpyionycteris*, and, surprisingly, *Macroglossus*. In this clade, all intergeneric relationships are weakly supported ($1 \leq BS \leq 2$).

## Combined and Additional Analyses

A combined parsimony analysis using both the nuclear genes (*RAG1*, *RAG2*, and *vWF*) and our mitochondrial data (*cytb* and *12S-tVal*) resulted in three trees of 4451 steps (strict consensus in fig. 4). In this analysis, Megachiroptera is highly supported (BS = 97). *Melonycteris* is sister to a trichotomy including *Macroglossus*, *Syconycteris,* and a poorly supported (BS = 3) group containing the remainder of terminals, which is further subdivided into a harpyionycterine clade ((*Harpyionycteris* + *Boneia*) + (*Aproteles* +

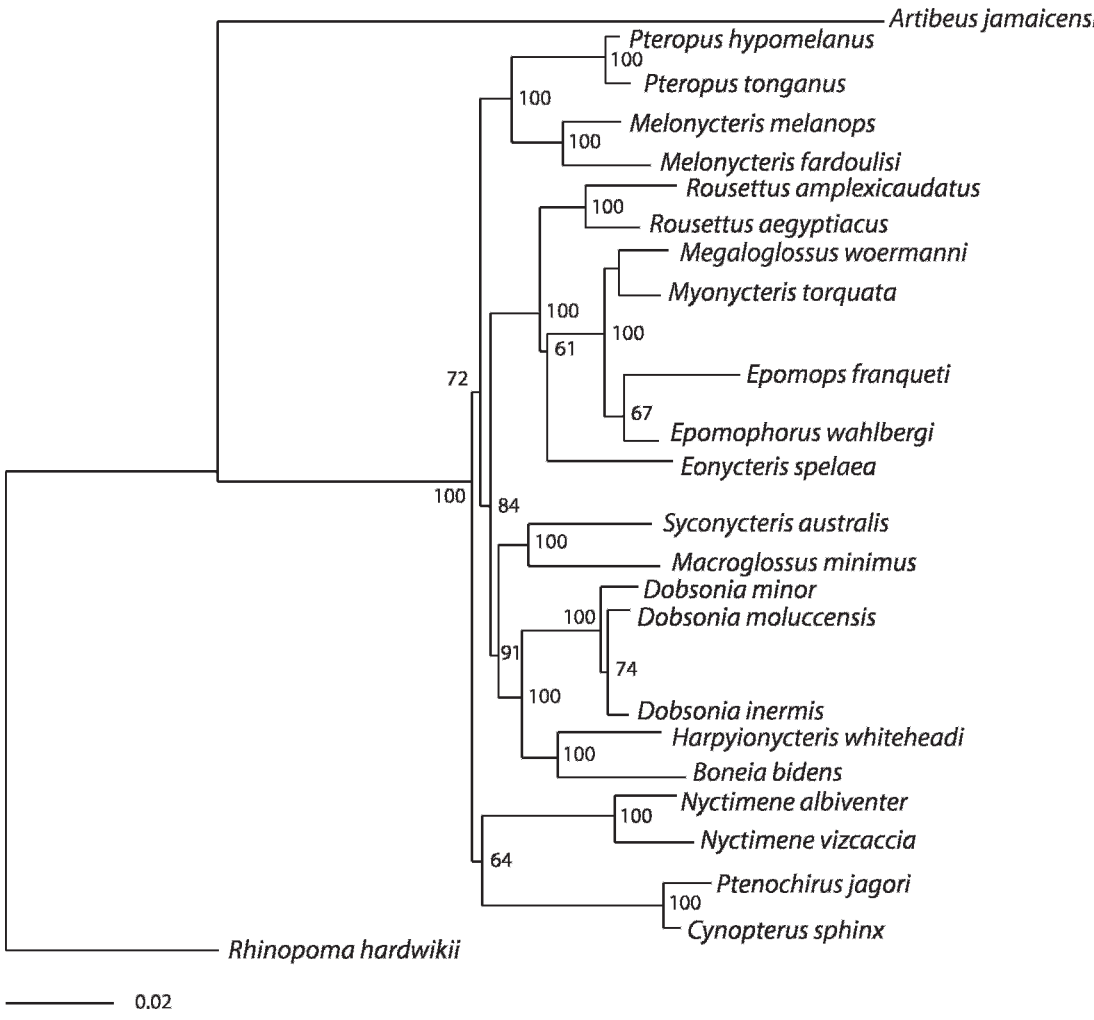


Fig. 2. Maximum likelihood tree resulting from the analysis of the nuclear dataset (*RAG1*, *RAG2*, and *vWF*; gene partitions modeled using GTR + Γ + I; ML score -11,136.227759). Values near nodes represent bootstrap estimates of clade support based on 100 replications.

*Dobsonia*)), and another group containing the rousettine-epomophorine clade and a composite clade (Pteropodini (Nyctimeninae + Cynopterinae)).

Adding to this data set published sequences of the mitochondrial 16S and the nuclear oncogene *c-mos* genes produces a single tree of 6119 steps (fig. 5). In this tree, *Nyctimene* is sister to all other megachiropterans, and this tree partition appeared highly supported (BS = 19). Successive clades recovered were (Pteropodini + Cynopterinae), the rousettine-epomophorine clade, and a highly supported harpyionycterine clade (BS = 23) in which *Dobsonia* was sister to *Aproteles*, *Boneia,* and *Harpyionycteris*. Several important dispersal-vicariance events are suggested by mapping distribution of terminals onto the optimal tree of our combined phylogenetic analysis. These patterns are discussed below.

Finally, we obtained four trees of 1717 steps in a separate analysis based on *ND2* sequences from pteropodid species available on GenBank. The strict consensus tree (fig. 6) was generally poorly supported but placed *Boneia bidens* and *Dobsonia minor* as sister taxa. This result is compatible with those of previous analyses that separately

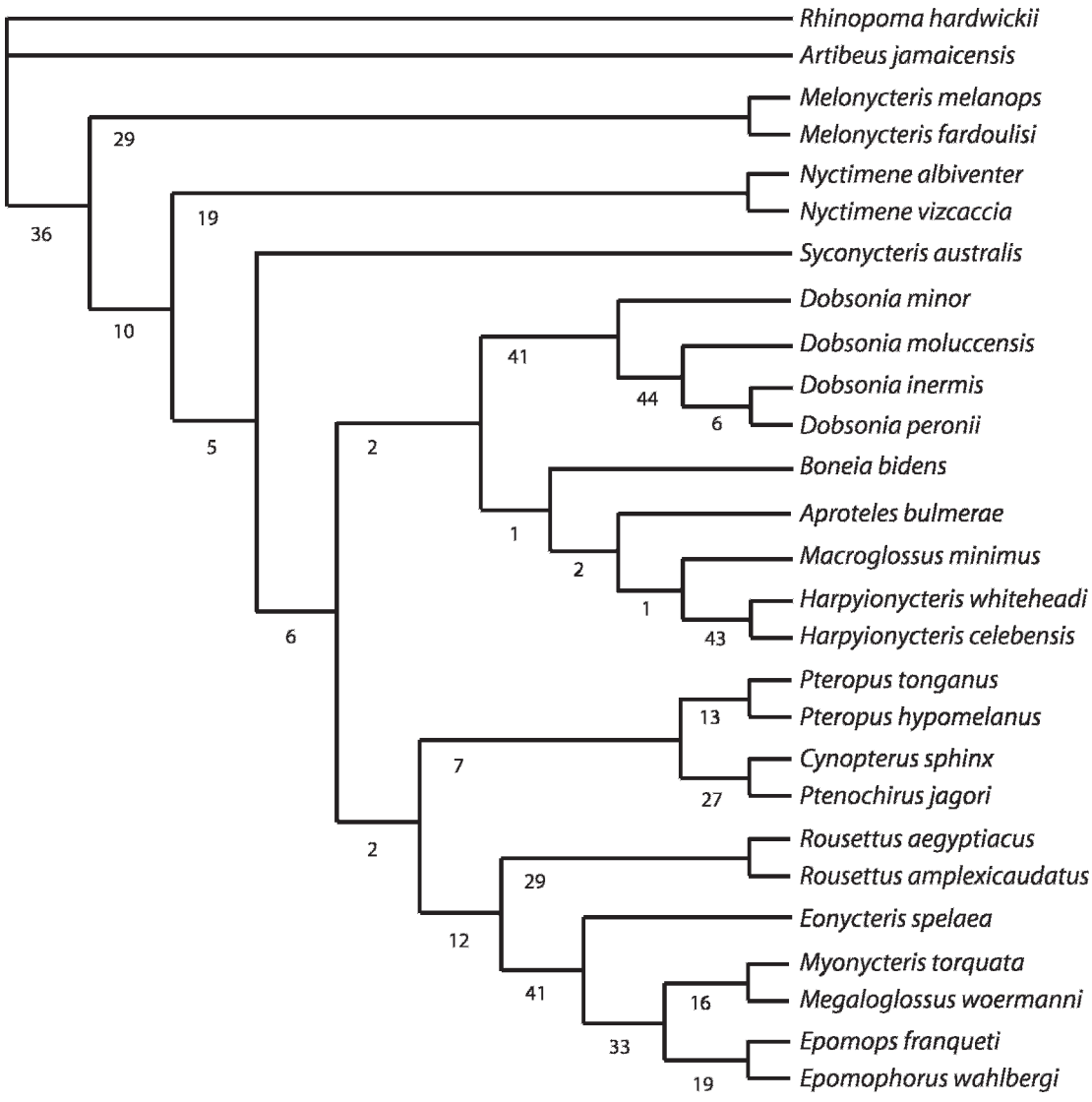


Fig. 3. Single optimal tree resulting from a direct optimization, parsimony analysis of three mitochondrial genes combined (*12S-tVal + cytb*). Bremer support values are given below branches.

support an association of *Boneia* with harpyionycterine megabats—*D. minor* among the latter.

## DISCUSSION

### Phylogenetic Relationships and Classification

The phylogenetic analyses of nuclear data recovered a harpyionycterine clade composed of *Harpyionycteris*, *Aproteles*, *Dobsonia*, and, rather unexpectedly, *Boneia*. Although *Macroglossus* was added to this group in the analysis of the mitochondrial partition (with negligible support, $1 \leq BS \leq 2$; fig. 3), the group membership of the nuclear analysis was recovered in the total-evidence analysis with high support (BS = 23; fig. 5). This represents strong evidence that *Boneia* is not a synonym of *Rousettus* but a distinct genus that belongs in Harypionycterinae. We also confirmed the

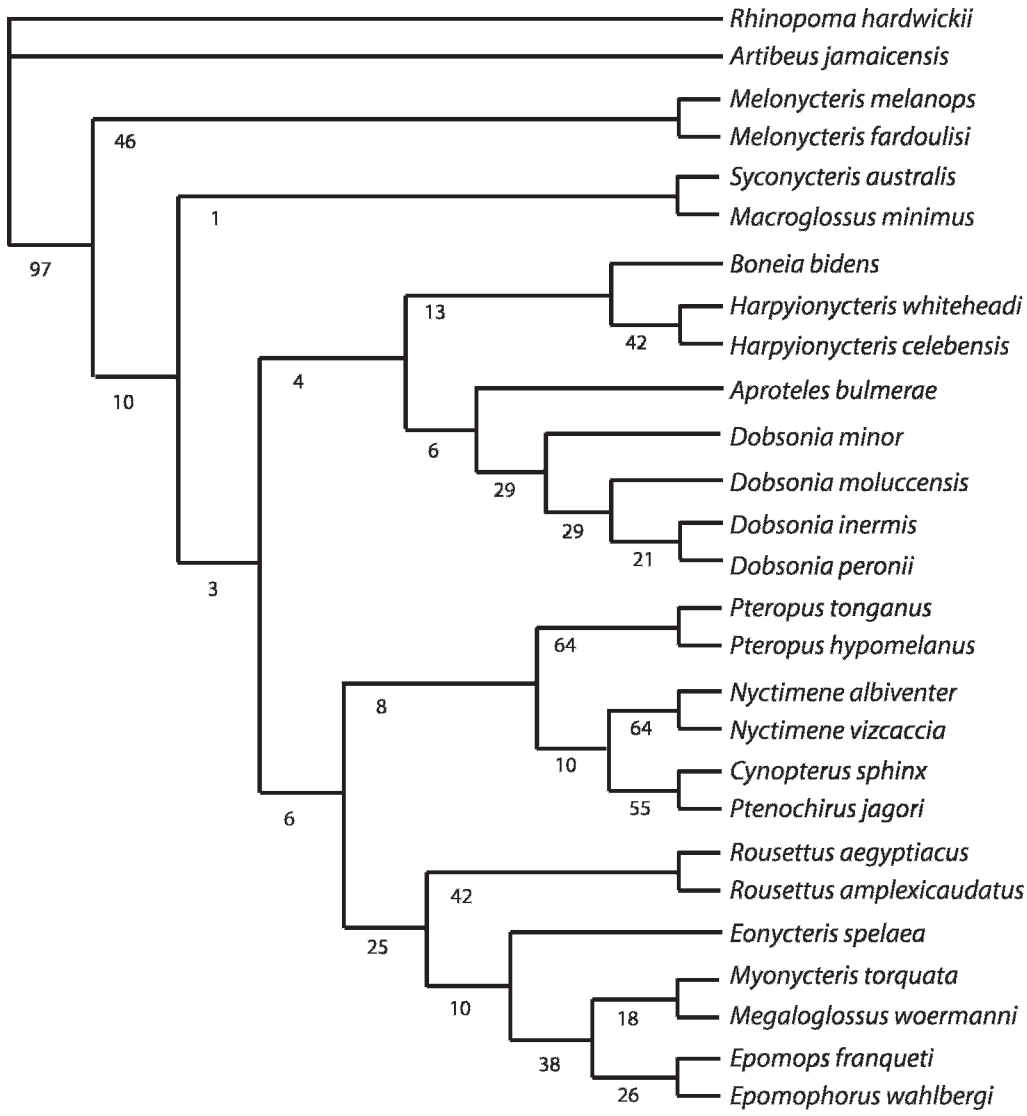


Fig. 4. Strict consensus tree of a parsimony analysis of *RAG1*, *RAG2*, *vWF*, *cytb* and *12S-tVal* genes combined. Bremer support values are given below branches.

inclusion of *Aproteles* in this group (see discussion in Giannini et al., 2006). Thus, we formally expand Harpyionycterinae Miller, 1907, to include *Boneia* Jentink, 1879, *Harpyionycteris* Thomas, 1896, *Dobsonia* Palmer, 1898, and *Aproteles* Menzies, 1977.

Resolving the composition of Harpyionycterinae has proven difficult throughout the history of megachiropteran systematics. Thomas (1896) recognized the difficulties of finding allies of *Harpyionycteris* among the megachiropterans known at the time of its description. Thomas (1896: 243) attributed the similarities in canine morphology with *Harpyia* (= *Nyctimene*) to either homoplasy (''accident'' in his words) or plesiomorphy (i.e., ''common descent from the (presumably) cuspidate-toothed ancestor of the Pteropodidae''). Thomas (1896: 243) suggested that ''On the whole … [*Harpyionycteris*] may be most conveniently placed near *Xantharpyia* [= *Rousettus*] and *Boneia*, with which it

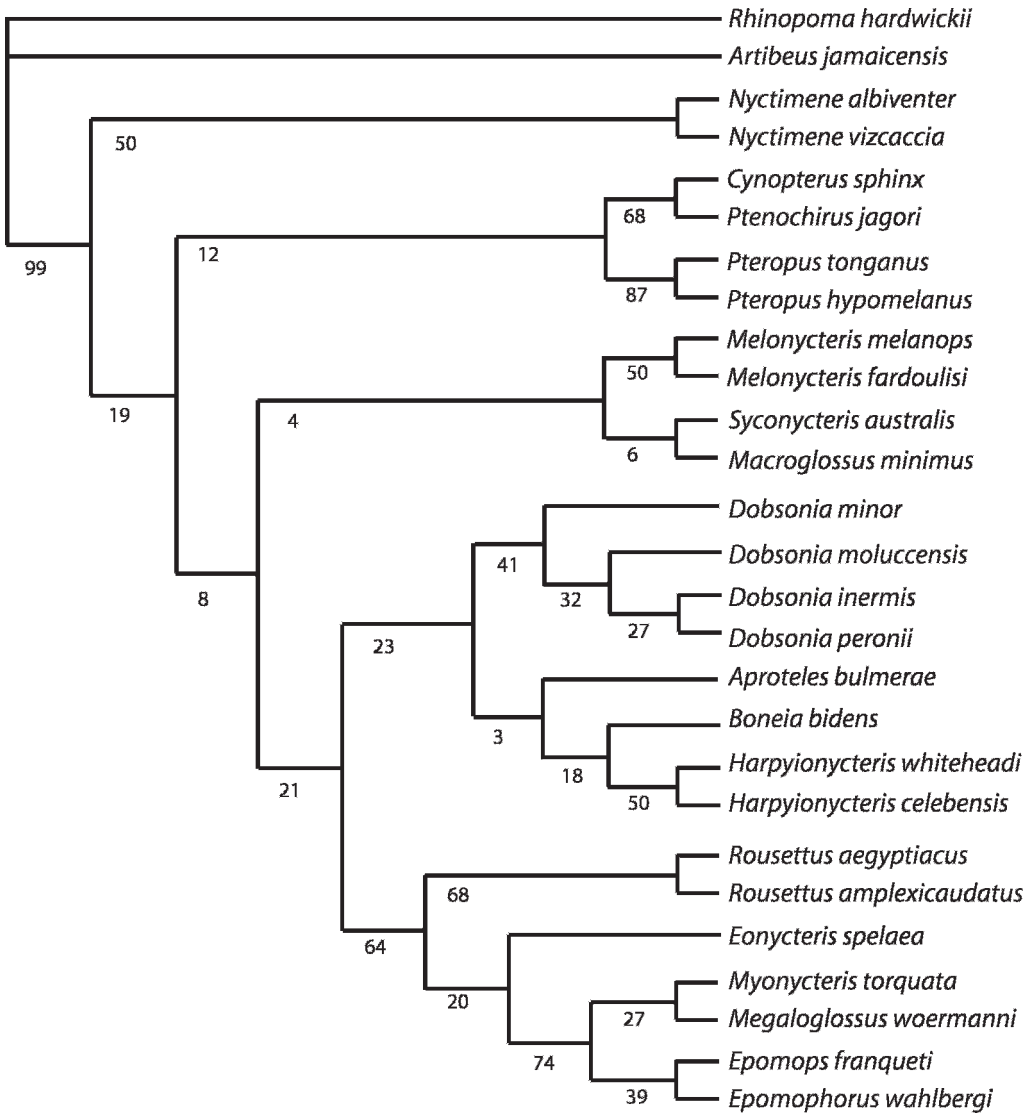


Fig. 5. Single optimal tree of a parsimony analysis of *RAG1*, *RAG2*, *vWF*, *cytb*, *12S-tVal*, *16S*, and *c-mos* genes combined. Bremer support values are given below branches.

shares certain external characters, an indical claw, and the cheek-tooth formula of P. 3/3, M. 2/3.'' The index finger claw and the cheek-tooth formula are likely plesiomorphic for Megachiroptera (see Giannini and Simmons, 2005, 2007), and the external characters were not specified (but see the external resemblance of *Harpyionycteris celebensis* and *Boneia bidens* in fig. 7). Nevertheless, it seems clear that Thomas (1896) did correctly perceive a link between these forms.

Despite Thomas's (1896) intuition and the molecular support discovered in the current study, to our knowledge, no author subsequent to Thomas considered a subfamily or tribal group inclusive of both *Harpyionycteris* and *Boneia*. This is reflected in a complete lack of potential morphological synapomorphies for such grouping in the literature. In recent systematic studies involving *Boneia*, discussion has centered on the nature of its relationship with *Rousettus* (e.g., Bergmans,

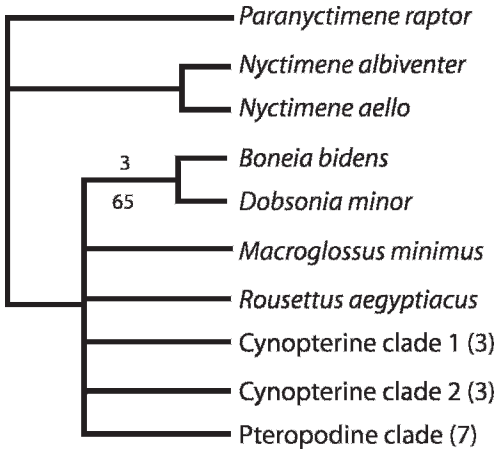


Fig. 6. Strict consensus tree of a parsimony analysis of the *ND2* gene. Bremer and jackknife values (above and below branches, respectively) are reported for the harpyionycterine clade recovered in this analysis. Numbers in parenthesis indicate number of terminals included in the cynopterine and pteropodine clades recovered.

1994; Bergmans and Rozendaal, 1988). This is not surprising given the prominent role that *Rousettus* has played in megachiropteran systematics as a result of long being considered close to the ancestral form of megabats following Andersen (1912). Although this assessment has been refuted by all cladistic studies since the early 1990s (e.g., Colgan and da Costa, 2002; Giannini and Simmons, 2003, 2005; Kirsch et al., 1995; Romagnoli and Springer, 2000; Springer et al., 1995), most megachiropteran genera have been compared with *Rousettus* on these grounds, and *Boneia* was no exception. Given the lack of unusual external features, unremarkable skull shape, dental formula close to the generalized condition of megabats, and the habit of roosting gregariously in caves, *Boneia* was considered so close an ally of *Rousettus* as to be included in *Rousettus* as a subgenus (see Bergmans and Rozendaal, 1988). The morphological evidence discussed by the authors in favor of this association includes both morphometric and qualitative data and is rather robust, which makes our finding of a *Boneia-Harpyionycteris* clade more surprising. As shown in fig. 8, the skulls of *Boneia* and *Harpyionycteris* bear little mutual resemblance. In *Boneia*, the rostrum is thin and strongly deflected relative to the basicranial axis, the dentition is simplified but otherwise typically pteropodid, the zygomatic arch is weak, and in overall appearance it resembles *Rousettus*. By contrast, *Harpyionycteris* shows a strong "dobsonine" skull with the most peculiar rostrum and dentition seen in megabats (see comments in Giannini et al., 2006).

However difficult it may seem to reconcile the current composition of the harpyionycterine clade with the morphological variation traditionally considered in megabat systematics, our phylogeny does suggest some interesting scenarios of morphological and ecological evolution. One particularly significant example involves the dentition. Harpyionycterines exhibit dental formulae that vary across taxa, but are similar in the sense that one formula can be parsimoniously transformed into any other in one-step losses or gains. A most relevant case is the first upper incisor, which is the sole tooth missing from the generalized pteropodid formula in *Boneia* (a variably deciduous tooth in this taxon; Bergmans and Rozendaal, 1988). This is also the incisor demonstrably lacking in *Dobsonia* and hypothesized to be absent in *Harpyionycteris* (see Giannini and Simmons, 2007: table 2). *Aproteles* lacks both upper incisors. Our phylogeny, once it is accepted as a supported hypothesis, allows us to suggest that the loss or reduction of I1 is effectively homologous across harpyionycterine taxa, and that the loss of incisors in *Aproteles* likely happened sequentially (I1 first lost first, followed by I2).

## Biogeographic and Ecological Patterns

Harpyionycterinae sensu Giannini et al. (2006; i.e., composed of *Harpyionycteris*, *Dobsonia*, and *Aproteles*), expanded here to include *Boneia*, comprises 18 species whose joint distribution encompasses the Philippine, Wallacean, and Papuan subregions of the Austromalayan area, plus two species that narrowly escape the biogeographic boundaries of those subregions (fig. 9; table 2). The latter two taxa are *D. peronii*, which also occurs in Bali (Sundaic subregion) and *D. moluccensis*, which also occurs in Queensland, Australia (Corbet and Hill, 1992;

Fig. 7. External appearance of live specimens of *Harpyionycteris celebensis* (top) and *Boneia bidens* (bottom). Photos: courtesy Jan Haft (with permission).

Simmons, 2005). Keeping in mind that our biogeographic interpretation rests upon (a) our limited taxonomic sampling (cf. table 2), and (b) the optimal tree from our total-evidence analysis, our biogeographic analysis reconstructed the Papuan subregion as the unambiguous area of origin of harpyionycterines (fig. 9). From this region, the ancestor of *Harpyionycteris* and *Boneia* crossed the Lyddekker's and Weber's lines westward to establish populations and speciate in Sulawesi, where differentiation of the two genera occurred. Later, an ancestral *Harpyionycteris* species split into a lineage that colonized the Philippines (crossing the Wallace line northward) giving rise to *H. whiteheadi*, and into another lineage that remained in Sulawesi to become *H. celebensis*.

The dispersal-vicariance events (sensu Ronquist, 1997) that led to the present-day


Fig. 8. Lateral (upper row) and ventral (lower row) views of the skulls of *Rousettus amplexicaudatus* (**A**, AMNH 226391), *Boneia bidens* (**B**, AMNH 254542), and *Harpyionycteris whiteheadi* (**C**, AMNH 142766). Scale = 5 mm.

diversity within *Dobsonia* can only be matter of speculation given our limited sample (but see Byrnes, 2005, for a detailed account). In our analysis, the genus also originated in New Guinea. In a more conventional scenario in which *Aproteles* is sister to *Dobsonia* as in the tree in fig. 4, the area of the ancestral harpyionycterine bat is reconstructed as Sulawesi + New Guinea. Accepting a Papuan (or Papuan-Wallacean) origin as probable (fig. 9), and taking together the distribution of all species, several *Dobsonia* lineages apparently diverged in various directions: northeastward to colonize the Bismarck Archipelago and the Solomon Islands; southward to reach Cape York Peninsula (Australia), and westward to reach the Moluccas, Lesser Sunda Islands, Sulawesi, and beyond the Wallace line in Bali (populations of *D. peronii*) and the Philippines (*D. chapmani*, restricted to Negros and Cebu Is.; Alcala et al. 2004; Heaney et al., 1998; Paguntalan et al., 2004). Other speciation events took place in land-bridge islands of New Guinea (Byrnes, 2005).

The optimal tree from our total-evidence analysis also suggests some interesting ecological patterns including insights into the

TABLE 2
**Distribution of Each of the 18 Species of Harpyionycterinae Miller, 1907**
Biogeographic regions follow Corbet and Hill (1992).

| Species | Distribution | Biogeographic regions |
|---|---|---|
| *Aproteles bulmerae* | Papua New Guinea (highlands) | Papuan |
| *Boneia bidens* | Northern Sulawesi | Wallacean (Sulawesi Division) |
| *Dobsonia anderseni* | Bismarck Arch., Admiralty Isls. | Papuan |
| *Dobsonia beauforti* | Waigeo, Batanta, Salawati, Gebe, Gag, and Biak Isls. | Papuan, Wallacean (Moluccan Division) |
| *Dobsonia chapmani* | Cebu and Negros Isls. | Philippines |
| *Dobsonia crenulata* | N Moluccas, Togian, Sangihe, Talaud, and Peleng Isls., Sulawesi | Wallacean (Sulawesi and Moluccan Divisions) |
| *Dobsonia emersa* | Biak, Numfoor, and Owii Isls. | Papuan |
| *Dobsonia exoleta* | Sulawesi, Muna, Togian, Sula Isls. | Wallacean (Sulawesi Division) |
| *Dobsonia inermis* | Solomon Islands | Solomons |
| *Dobsonia minor* | Lowlands of New Guinea and adjacent Isls., Sulawesi | Papuan, Wallacean (Sulawesi Division) |
| *Dobsonia moluccensis* | Moluccas, New Guinea and land-bridge islands, to Queensland | Papuan, Wallacean (Moluccan Division), Australian |
| *Dobsonia pannietensis* | Louisiade Arch., D'Entrecasteaux Isls., Trobriand Isls. | Papuan |
| *Dobsonia peronii* | Lesser Sunda Islands (Bali East to Babar Isls.) | Sundaic (Javan Division) and Wallacean (Lesser Sunda and Moluccan Divisions) |
| *Dobsonia praedatrix* | Bismarck Arch. | Papuan |
| *Dobsonia viridis* | C and S Moluccas, Banda, and Kai Isls. | Wallacean (Moluccan Division) |
| *Harpyionycteris celebensis* | Sulawesi | Wallacean (Sulawesi Division) |
| *Harpyionycteris whiteheadi* | Philippines | Philippine |

evolution of roosting habits (see also discussion in Byrnes, 2005). Roost availability is one of the limiting factors of bat populations (Kunz and Pierson, 1994). Megachiropterans are either cavity dwellers (occupying structures from tree holes to large caves) or foliage dwellers (a habit that varies from taking solitary refuge on leaves or vines to living in larger groups in actively defoliated trees; Kunz and Pierson, 1994). The roosting habits of *Harpyionycteris* are unknown, but this genus is nested within successive sister clades that include many typical cave-dwelling bats: *Boneia* (Bergmans and Rozendaal, 1988), *Aproteles* (Flannery, 1995; Menzies, 1977), and several *Dobsonia* species including *D. moluccensis* (Bonaccorso, 1998; Flannery, 1995; Helgen, 2007), and probably also *D. peronii* (Hutson et al., 2008) and *D. inermis*. By contrast, *D. minor* has been reported to roost both in foliage (Flannery, 1995) and in caves (Boeadi and Bergmans, 1987). The prediction we draw from reconstructing roosting habits (foliage vs. cavities) in the optimal tree of our most complete analysis is that *Harpyionycteris* could be also a cavity dweller, as are many members of the harpyionycterine clade (fig. 10). It is interesting that the harpyionycterine clade appeared in our analysis as sister to the rousettine-epomophorine clade (with high support in our analysis, BS = 21). Taken together, these two major megabat clades share a common ancestor that is also reconstructed as a cavity dweller (fig. 10). Once again, limited taxonomic sampling prevents further speculation, but we risk the hypothesis that cavity dwelling may have had a major impact in the diversification of megabats.

## CONCLUSIONS

Our results strongly confirm monophyly of a harpyionycterine clade inclusive of *Harpyionycteris*, *Dobsonia*, *Aproteles*, and *Boneia* (the latter previously included in *Rousettus* as a subgenus). Accordingly, we recognize *Boneia* as a distinct genus and recommend

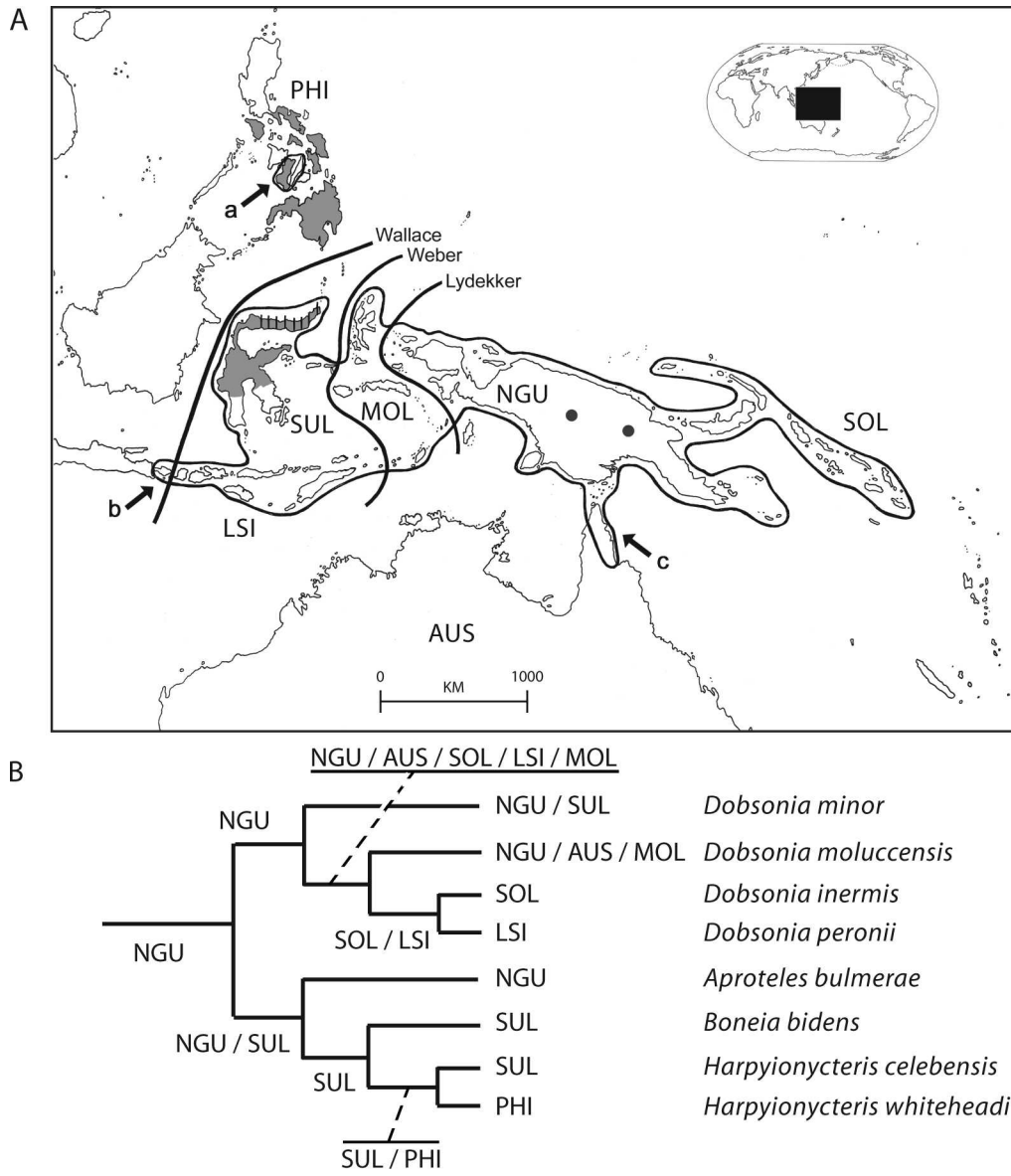


Fig. 9. Joint distribution of species in harpyionycterine megabat genera in the Austromalayan Region (**A**), and biogeographic patterns in harpyionycterine megabats (**B**). References for distributions: *Aproteles* (dots), *Boneia* (hatched area), *Dobsonia* (perimeter lines), and *Harpyionycteris* (shaded areas). Indicated are the Wallace, Weber, and Lydekker lines. Arrows indicate the distribution of *Dobsonia* species that escape the boundaries of the Wallacean and Papuan subregions: (**A**) *Dobsonia chapmani* in Negros and Cebu Islands (Philippine subregion); (**B**) *Dobsonia peronii* in Bali (Sundaic subregion, Javan Division; *D. peronii* is widespread in the Lesser Sunda Islands); (**C**) *Dobsonia moluccensis* in Queensland, Australia (*D. moluccensis*, inclusive of *D. magna*, is widespread in the Moluccas and New Guinea). Biogeographic patterns: Each species is assigned one or more of the following areas, each of which is a state in a single unordered biogeographic character: AUS Australia, LSI Lesser Sunda Islands, NGU New Guinea, PHI Philippines, SOL Solomon Islands, SUL Sulawesi. Biogeographic events are inferred by partial (downpass only) optimization of distribution areas of species in the harpyionycterine clade in the optimal tree from the most complete, combined analysis (**B**; see fig. 4).

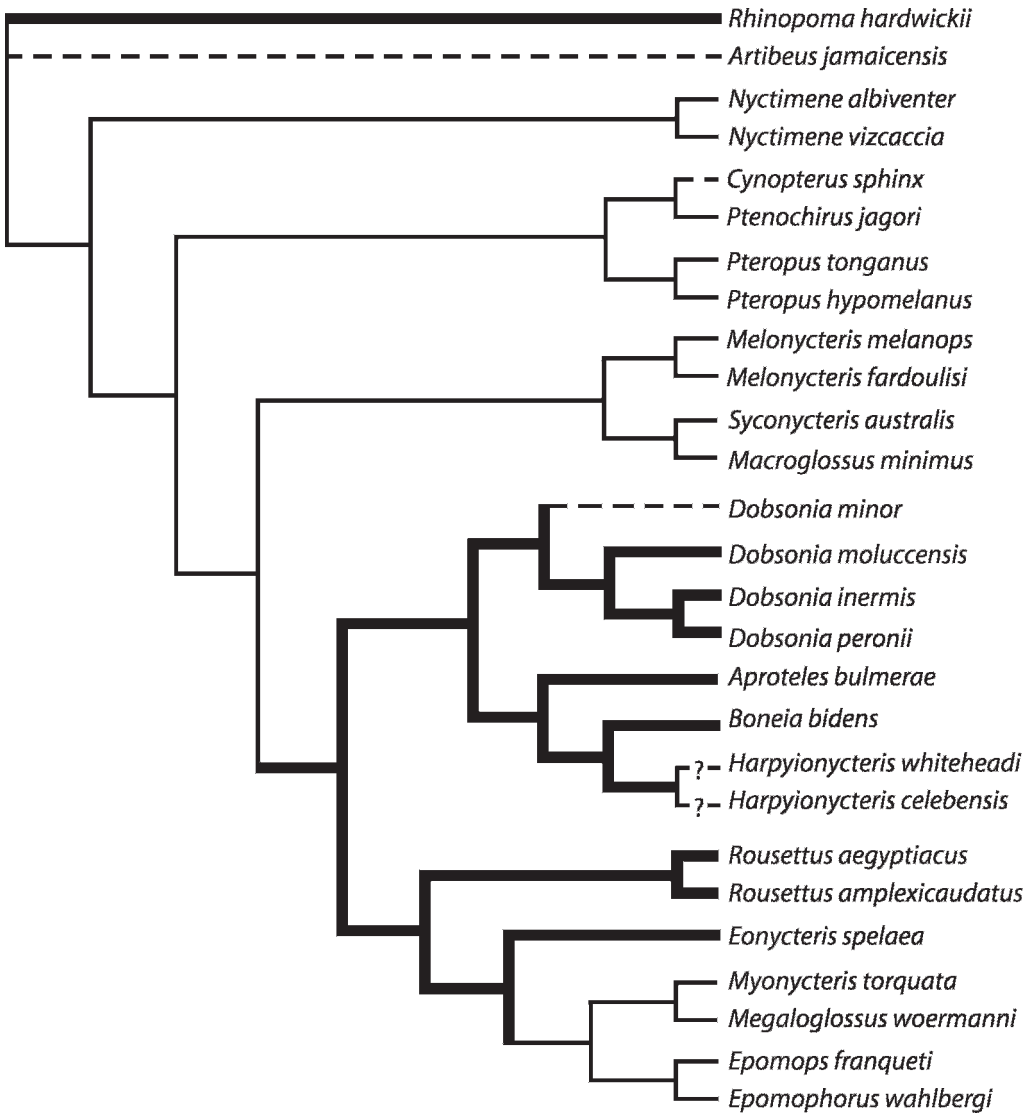


Fig. 10. Reconstruction of roosting habits (cavity versus foliage) in the most parsimonious tree from the total dataset of this study (fig. 4). Hatched lines indicate ambiguity (in this case, terminals for which both roosting habits were documented). Cavity dwelling is indicated in thick branches. Question marks indicate lack of data for *Harpyionycteris*. Note that this genus is nested in a clade of cavity dwellers (see text).

expanding Harpyionycterinae Miller, 1907, to include these four genera. Although no morphological synapomorphies are apparent for this grouping, some morphological and ecological patterns are interesting, for instance, dental-formula patterns and the prediction that *Harpyionycteris* may be a cavity-dwelling bat. Also inferred from our phylogenetic hypothesis, we showed that major biogeographic boundaries within the Austromalayan region play a key role in explaining patterns of dispersal-vicariance events in harpyionycterine bats. These results and preliminary interpretations encourage the undertaking of a full-scale phylogeny of Megachiroptera.

## ACKNOWLEDGMENTS

We are indebted with Rob DeSalle (Sackler Institute for Comparative Genomics, American Museum of Natural History) for providing laboratory space and reagents for the molecular part of this work. We thank Lawrence Heaney (Field Museum of Natural History, Chicago), Jim Patton and Carla Cicero (Museum of Vertebrate Zoology, Berkeley), John Wible and Suzanne McLaren (Carnegie Museum, Pittsburgh), Denis O'Meally (Australian Museum), Jeremy Jacobs, Louise Emmons, and James Mead (Smithsonian Institution, National Museum of Natural History, Washington DC), and Wim Bergmans (ZMA Zoölogisch Museum, Amsterdam) for access to tissue samples that made possible this contribution. We also thank Jan Haft for photographs an additional information. Finally, our appreciation to Deanna P. Byrnes for her generosity in providing two unpublished sequences for use in this study. Funding for this report was provided by the National Science Foundation (research grant DEB-9873663 to N.B.S.), Coleman and Vernay postdoctoral fellowships at the AMNH to N.P.G., a Henry MacCracken doctoral fellowship at New York University to F.C.A., and a Vernay postdoctoral fellowship at the AMNH to F.C.A. M.P.G. thanks the CONICET, Argentina.

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## APPENDIX 1

Voucher information and GenBank accession numbers of megabat specimens used in the present study, listed as appearing in the tree of figure 4. Imprecise localities are quoted. Abbreviations of Institutions: AMCC, Ambrose Monell Cryo Collection (AMNH); AMNH, American Museum of Natural History, New York; CMNH Carnegie Museum of Natural History, Pittsburgh; EBU Evolutionary Biology Unit, Australian Museum, Sydney; FMNH, Field Museum of Natural History, Chicago; MVZ, Museum of Vertebrate Zoology, University of California, Berkeley; USNM Smithsonian Institution, National Museum of Natural History, Washington DC; WAM Western Australian Museum, Perth, Australia; ZMA Zoölogisch Museum, Amsterdam. Other abbreviations refer to collector's catalog.

| Species | Origin / voucher | Tissue ID | Locality | Accession numbers | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | RAG-1 | RAG-2 | vWF | cyt *b* | 12S / tVal | 16S | *c*-mos |
| *Artibeus jamaicensis* | GenBank | - | - | AY834655 | AY834663 | AY834737 | AF061340 | AF061340 | AF061340 | - |
| *Rhinopoma hardwickii* | GenBank | - | - | AF447518 | AF447535 | AF447551 | AY629005 | AF263232 | AF263232 | - |
| *Nyctimene albiventer* | GenBank | - | - | AY249870 | AF447531 | AF447549 | - | U61077 | AF293640 | AY044669 |
| *Nyctimene vizcaccia* | AMNH PRS 2636 | AMCC 124208 | Solomon Islands, Western Prov., New Georgia Group, Vella Lavella Island | EU617959 | EU617904 | DQ445698 | DQ445711 | - | - | - |
| *Cynopterus sphinx* | AMNH 274354 | AMCC 101688 | Vietnam, Ha Giang Province, Vi Xuyen District, Cao Bo Commune, Mt Tay Con Linh II | EU617947 | EU617897 | DQ445697 | DQ445703 | - | - | - |
| *Cynopterus sphinx* | GenBank | - | - | - | - | - | - | EF139881 | EF139881 | - |
| *Ptenochirus jagori* | FMNH 175395 | LRH 6700 | Philippines, Luzon, Kalinga Prov., Balbalan Munic., Balbalasang | EU617960 | EU617910 | DQ445696 | FJ218480 | - | - | - |
| *Pteropus tonganus* | AMNH 272873 | AMCC 124962 | Tonga | EU617976 | EU617924 | DQ445695 | - | - | - | - |
| *Pteropus tonganus* | GenBank | - | - | - | - | - | AF044656 | AY044798 | AF044625 | AY044720 |
| *Pteropus hypomelanus* | Uncataloged | P 4447 | Captivity Lubee Foundation | EU617965 | EU617914 | DQ445687 | - | - | - | - |
| *Pteropus hypomelanus* | GenBank | - | - | - | - | - | AB062472 | U93073 | AF069537 | AY044710 |
| *Melonycteris melanops* | USNM 580029 | USNM 580029 | "Papua New Guinea" | FJ218465 | FJ218461 | FJ218469 | - | - | - | - |
| *Melonycteris melanops* | GenBank | - | - | - | - | - | AF044645 | AY044739 | AF044614 | AY044681 |
| *Melonycteris fardoulisi* | AMNH 275744 | AMCC 124279 | Solomon Islands, Western Prov., New Georgia Group, Vella Lavella Island | EU617957 | EU617907 | DQ445699 | FJ218478 | - | - | - |

APPENDIX 1
*(Continued)*

| Species | Origin / voucher | Tissue ID | Locality | Accession numbers | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | RAG-1 | RAG-2 | vWF | cyt *b* | 12S / tVal | 16S | *c*-mos |
| *Melonycteris fardoulisi* | GenBank | - | - | - | - | - | - | U93056 | AF293644 | AY044682 |
| *Syconycteris australis* | MVZ 140265 | MVZ 140265 | Papua New Guinea, Western Highlands Prov., Trauna Valley, Baiyer River | FJ218466 | FJ218462 | FJ218470 | FJ218479 | - | - | - |
| *Syconycteris australis* | GenBank | - | - | - | - | - | - | U93060 | AF293650 | AY044677 |
| *Macroglossus minimus* | AMNH 275761 | AMCC 124283 | Solomon Islands, Western Province, New Georgia Group, Vella Lavella Island | EU617955 | EU617905 | DQ445693 | - | - | - | - |
| *Macroglossus minimus* | GenBank | - | - | - | - | - | AF144067 | U93062 | AF293649 | AY044679 |
| *Dobsonia minor* | MVZ 140208 | MVZ 140208 | Papua New Guinea, Madang Prov., Sempi | FJ218467 | FJ218463 | DQ445701 | DQ445705 | FJ218477 | FJ218477 | - |
| *Dobsonia moluccensis* | AM M 20735 | EBU 25757 | Papua New Guinea, Sideia Mission, Milne Bay Province | EU617949 | EU617899 | EU617930 | FJ218484 | FJ218472 | - | - |
| *Dobsonia moluccensis* | GenBank | - | - | - | - | - | - | - | AF179290 | - |
| *Dobsonia inermis* | AMNH 275730 | AMCC 124428 | Solomon Islands, Western Prov., New Georgia Group, Vonavona Lagoon | EU617948 | EU617898 | DQ445686 | DQ445704 | FJ218476 | FJ218476 | - |
| *Dobsonia peronii* | WAM M30341 | M30341 | Indonesia, Sumba | - | - | - | Unsubmitted [a] | - | - | - |
| *Aproteles bulmerae* | AM 26660 | AM 26660 | New Guinea | - | - | - | Unsubmitted [a] | U93066 | AF293645 | AY044702 |
| *Boneia bidens* | ZMA 23100 | Uncataloged | Indonesia, "Sulawesi" | FJ218468 | FJ218464 | FJ218471 | FJ218481 | FJ218475 | FJ218475 | - |
| *Harpyionycteris whiteheadi* | FMNH 146646 | LRH 4811 | Philippines, Mindanao, Bukidnon Prov., Mount Kitanglad Range | EU617954 | EU617904 | DQ445690 | DQ445708 | FJ218474 | FJ218474 | - |
| *Harpyionycteris celebensis* | Uncataloged | Uncataloged | Indonesia, "Sulawesi" | - | - | - | - | FJ218473 | FJ218473 | - |
| *Rousettus aegyptiacus* | AMNH 117386 | Uncataloged | Mozambique, Zambezia, Mt. Namuli | EU617979 | EU617927 | DQ445688 | DQ445713 | - | - | - |
| *Rousettus aegyptiacus* | GenBank | - | - | - | - | - | - | AB205183 | AB205183 | - |

APPENDIX 1
*(Continued)*

| Species | Origin / voucher | Tissue ID | Locality | Accession numbers | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | RAG-1 | RAG-2 | vWF | cyt *b* | 12S / tVal | 16S | *c*-mos |
| *Rousettus amplexicaudatus* | GenBank | - | - | AF447512 | AF447529 | AY057836 | AB046329 | U93070 | AF203742 | AY044690 |
| *Eonycteris spelaea* | MVZ 176487 | MVZ 176487 | China, "Yunnan Province" | EU617951 | EU617901 | DQ445684 | FJ218482 | - | - | - |
| *Eonycteris spelaea* | GenBank | - | - | - | - | - | - | U93059 | AF203743 | AY044685 |
| *Myonycteris torquata* | AMNH 268362 | AMCC 109058 | Central African Republic, Sangha, Dzanga-Sangha | EU617958 | EU617908 | DQ445700 | FJ218483 | - | - | - |
| *Myonycteris torquata* | GenBank | - | - | - | - | - | - | AY044744 | AF044619 | AY044686 |
| *Megaloglossus woermanni* | AMNH 268358 | AMCC 109064 | Central African Republic, Sangha, Dzanga-Sangha | EU617956 | EU617906 | DQ445702 | DQ445710 | - | - | - |
| *Megaloglossus woermanni* | GenBank | - | - | - | - | - | - | U93055 | AF203741 | AY044689 |
| *Epomops franqueti* | AMNH 238356 | AMCC 109070 | Central African Republic, Sangha, Dzanga-Sangha | EU617952 | EU617902 | DQ445692 | DQ445707 | - | - | - |
| *Epomops franqueti* | GenBank | - | - | - | - | - | - | - | AF044608 | AY044687 |
| *Epomophorus wahlbergi* | AMNH 117336 | FMNH 177209 | Mozambique, Zambezia, Mt. Namuli | UE617953 | EU617903 | DQ445691 | DQ445706 | - | - | - |
| *Epomophorus wahlbergi* | GenBank | - | - | - | - | - | - | U93064 | AF203744 | |

[a] Sequences provided by Deanne Byrnes.