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Phylogeny of the owlet-nightjars (Aves: Aegothelidae) based on mitochondrial DNA sequence

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Abstract

The avian family Aegothelidae (Owlet-nightjars) comprises nine extant species and one extinct species, all of which are currently classified in a single genus, *Aegotheles*. Owlet-nightjars are secretive nocturnal birds of the South Pacific. They are relatively poorly studied and some species are known from only a few specimens. Furthermore, their confusing morphological variation has made it difficult to cluster existing specimens unambiguously into hierarchical taxonomic units. Here we sample all extant owlet-nightjar species and all but three currently recognized subspecies. We use DNA extracted primarily from museum specimens to obtain mitochondrial gene sequences and construct a molecular phylogeny. Our phylogeny suggests that most species are reciprocally monophyletic, however *A. albertisi* appears paraphyletic. Our data also suggest splitting *A. bennettii* into two species and splitting *A. insignis* and *A. tatei* as suggested in another recent paper.

Keywords: Aegothelidae; Owlet-nightjar; Phylogeny; Mitochondrial DNA; Biogeography; Maximum likelihood; Molecular systematics; Systematics

1. Introduction

The avian family Aegothelidae (commonly known as owlet-nightjars) comprises only nine extant species, all in a single genus, *Aegotheles*. Owlet-nightjars are endemic to and distributed throughout Australasia from the Maluku Islands, south throughout Australia to Tasmania, and east to New Caledonia and New Zealand. The center of diversity is in New Guinea, which has seven of the nine extant species. An additional extinct taxon was found in New Zealand Holocene caves and fissures (Rich and Scarlett, 1977; Scarlett, 1968; Worthy and Mildenhall, 1989). This unique taxon was placed in its own genus, *Megaegotheles* (Scarlett, 1968), although Olson et al. (1987) considered it to belong in *Aegotheles*.

Owlet-nightjars are small to medium-sized nocturnal birds with wide weak bills, short legs, and upright posture. Like owls, *Aegotheles* have a facial disk with eyes oriented forward. Most are cavity or hole nesters, and all (except the Australian owlet-nightjar, *Aegotheles cristatus*) are forest species. *Aegotheles* have secretive, nocturnal habits making them difficult to study. They have been sparsely collected and are consequently among the most poorly known families of birds. With increased study, the number of recognized owlet-nightjar species could rise substantially, as it has recently for owls (Marks, 2001).

Although the family has long been placed in the order Caprimulgiformes (Holyoak, 2001; Sibley and Monroe, 1990), Sibley and Ahlquist's DNA hybridization trees showed a deep branching between owlet-nightjars and the rest of the caprimulgiforms. Researchers are now recognizing several aegothelid molecular and morphological synapomorphies with the order Apodiformes (Mayr, 2002) suggesting that the Aegothelidae may be more closely allied with swifts and hummingbirds than with Caprimulgiformes. Mariaux and Braun's (1996) molecular phylogeny of Caprimulgiforms includes Aegothelidae, but their taxon sampling was not designed

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to test specifically whether Aegotheles belonged within this order.

Understanding the systematic relationships of *Aegotheles* is challenging, as each species exhibits confusing intraspecific variation in plumage color and pattern (Cleere, 1998; Holyoak, 2001; Pratt, 2000). Researchers have therefore found it difficult to identify phylogenetically informative morphological characters. Additionally, there are relatively few specimens in collections, and these are not broadly collected, so geographical ranges are unclear and the ranges of variation for morphological character-states are poorly understood. Thus it has been difficult to cluster existing specimens unambiguously into biologically relevant taxonomic units. The Starry Owlet-nightjar (*Aegotheles tatei*), recognized as a species only recently (Pratt, 2000), provides a clear example of these problems. Perhaps for these reasons, the Aegothelidae has never undergone major phylogenetic analysis or systematic revision. Aegothelidae are therefore an obvious candidate for a molecular systematic study.

At present, aegothelid taxa are underrepresented in modern tissue collections. In addition, the extinct New Zealand *Megaegotheles* is only available from subfossil bone material. We therefore sampled museum skins or subfossil bone and employed "ancient DNA" methods to obtain DNA sequences for most aegothelid taxa (Cooper et al., 1996; Sorenson et al., 1999). We examined DNA sequences from 38 individuals of 17 aegothelid taxa and amplified three regions of mitochondrial DNA. Here we analyze those combined sequences and propose a molecular phylogenetic hypothesis for the family Aegothelidae. Because of outgroup ambiguity and relative DNA distance, we included both caprimulgiform and apodiform taxa as outgroups.

2. Methods

2.1. Specimens

Our taxonomic sampling includes all of the known *Aegotheles* species (Table 1), including the possibly extinct *Aegotheles savesi*, and *Aegotheles* (*Megaegotheles*) *novazealandiae*. Our sampling also includes the majority of named subspecies (we are missing only *A. albertisi wondiwoi*, *A. cristatus tasmanicus*, and *A. wallacii manni*). Table 1 also lists the museum specimen numbers, subspecies names, localities, and type of tissue (fresh, toe pad from museum skin, and subfossil bone) for all ingroup individuals used.

2.2. DNA protocols

DNA was isolated from fresh tissues using a DNAeasy kit (Qiagen), following the manufacturer's recommended protocol. DNA from museum specimens and the sub-fossil bone was isolated in dedicated ancient DNA laboratories located in separate buildings using a phenol–chloroform and centrifugal dialysis method (Dumbacher and Fleischer, 2001). No modern DNA or amplification products are handled in these laboratories, and a number of controls are included in analyses to allow detection of contamination (see Cooper et al., 1996; Dumbacher and Fleischer, 2001; Sorenson et al., 1999; for details on ancient DNA analysis in this facility). Extractions and amplifications for key or difficult taxa were duplicated by RCF at University of Durham.

Polymerase chain reactions (PCRs) involved a number of different primer pairs (Table 2) to amplify two regions of the Cytochrome *b* gene and the ATPase subunit 8 gene. Because of the degraded nature of the DNA isolated from museum skins and the subfossil bone we could often only amplify relatively small segments of DNA (range of 98–347 bp). PCRs involved standard components and cycling profiles (Dumbacher and Fleischer, 2001), an initial 10 min denaturation at 94 °C before thermocycling up to 45 cycles (profile 92 °C denaturing/45 s, 50 °C annealing/45 s, and 72 °C extension/1 min). Success of PCRs was assessed by gel electrophoresis. PCR products were purified and both heavy and light strand DNA was sequenced using dideoxy chain termination with recommended ABI protocols and run on an ABI 373-stretch or ABI 377 automated sequencer. Genbank numbers for our sequences are AY090664–AY090698 (for cytochrome *b*) and AY090699–AY090736 (for ATPase 8), and alignments are available through GenBank. The following taxa were included as outgroups: *Chaetura pelagica* (chimney swift), *Aerodramus salanganus* (mossy-nest swiftlet), *Amazilia tzacatl* (rufous-tailed hummingbird), *Chlorostilbon aureoventris* (glittering-bellied emerald [hummingbird]), *Batrachostomus cornutus* (Sunda frogmouth), *Podargus papuensis* (Papuan frogmouth), *Caprimulgus longirostris* (band-winged nightjar), *Steatornis caripensis* (oilbird), *Nyctibius aethereus* (long-tailed potoo), and *Eurostopodus papuensis* (Papuan nightjar). Cytochrome *b* sequences for outgroups were obtained from GenBank.

2.3. Phylogenetic reconstruction

Sequences were aligned, edited, and sequences from non-contiguous gene regions were concatenated using Sequencher 4.1 software (GeneCodes) and exported in Nexus format. Sequences were checked to ensure that there were no insertions, deletions, or unexpected stop codons in protein coding regions, as such anomalies would be evidence that the sequences might be of nuclear rather than mitochondrial origin. Furthermore, patterns of DNA substitution at codon positions matched those expected for mtDNA coding genes, and thus further supported our belief that our sequences were of

Table 1
A complete list of named taxa in the family Aegothelidae, and samples used in this study

Taxon	Museum Nos.[a]	Locality
A. albertisi albertisi	AMNH 632054	Arfak Mts., Vogelkop Pen., Papua, Indonesia
A. albertisi albertisi	AMNH 632057	Arfak Mts., Vogelkop Pen., Papua, Indonesia
A. albertisi salvadorii	MV E044[b]	Tetebedi, Oro Prov., PNG
A. albertisi salvadorii	LACM 2097[c]	Mt. Kaindi, Wau, Morobe Prov., PNG
A. albertisi wondiwoi	(Not sampled)	
A. archboldi	Yale 74915	Ilaga, Nassau Range, Papua, Indonesia
A. archboldi	Yale 74917	Ilaga, Nassau Range, Papua, Indonesia
A. archboldi	Yale 74918	Ilaga, Nassau Range, Papua, Indonesia
A. archboldi	Yale 74919	Ilaga, Nassau Range, Papua, Indonesia
A. bennettii affinis	AMNH 632021	Arfak Mts., Papua, Indonesia
A. bennettii affinis	AMNH 632022	Arfak Mts., Papua, Indonesia
A. bennettii bennettii type 1	MV E636[b]	Kuriva River, Central Prov., PNG
A. bennettii bennettii type 1	PNGNM 25738	Brown River, Central Prov., PNG
A. bennettii bennettii type 1	PNGNM 23235	Mt. Lawes, Central Prov., PNG
A. bennettii bennettii type 2	AMNH 632048	Milne Bay, Milne Bay Prov., PNG
A. bennettii bennettii type 2	AMNH 632049	Boboli, China Straits, Milne Bay Prov., PNG
A. bennettii plumiferus	AMNH 632028	Goodenough Is., Milne Bay Prov., PNG
A. bennettii plumiferus	AMNH 632030	Fergusson Is., Milne Bay Prov., PNG
A. bennettii terborghi	MCZ 286269	Karimui, Simbu Prov., PNG
A. bennettii wiedenfeldi	BPBM 22678	Green River, Sandaun Prov., PNG
A. bennettii wiedenfeldi	AMNH 339724	Idenburg R., Papua, Indonesia
A. crinifrons	Yale 74911	Bacan Is., N. Maluku, Indonesia
A. crinifrons	AMNH 467339	Bacan Is., N. Maluku, Indonesia
A. cristatus cristatus	MV W0191[b]	Mabel Creek, SA, Australia
A. cristatus cristatus	AMNH 425997	Wassi Kussa River, Western Prov, PNG
A. cristatus cristatus	AMNH 425996	Wassi Kussa River, Western Prov, PNG
A. cristatus cristatus	PNG NM 25736	Brown River, Central Prov., PNG
A. cristatus cristatus	PNG NM 22128	Brown River, Central Prov., PNG
A. cristatus tasmanicus	(Not sampled)	
A. insignis	ANSP AM849[c]	Crater Mt., Eastern Highlands Prov., PNG
A. insignis	ANSP AM857[c]	Crater Mt., Eastern Highlands Prov., PNG
A. insignis	LACM 2036[c]	Bewani Mts., Sandaun, PNG
A. insignis	AMNH 632113	Upper Aroa River, PNG
A. novaezealandiae	A. Cooper	New Zealand
A. savesi	LIV T16101	Tongué, near Nouméa, New Caledonia
A. tatei	ANWC 8394	Amazon Bay, Central Prov., PNG
A. tatei	AMNH 426000	Upper Fly River, Western Prov., PNG
A. wallacii gigas	AMNH 302847	Weyland Mts., Papua, Indonesia
A. wallacii gigas	AMNH 302848	Weyland Mts., Papua, Indonesia
A. wallacii manni	(Not sampled)	
A. wallacii wallacii	AMNH 632053	Eilanden R., (SW) Papua, Indonesia
A. wallacii wallacii	AMNH 632052	Kobror, Aru Is., Papua, Indonesia

[a] Institutions that provided tissues include the Academy of Natural Sciences, Philadelphia (ANSP), American Museum of Natural History (AMNH), Museum of Victoria (MV), Papua New Guinea National Museum (PNGNM), B.P. Bishop Museum (BPBM), Los Angeles County Museum (LACM), Yale University (Yale), Liverpool Museum (LIV), Harvard Museum of Comparative Zoology (MCZ), Australian National Wildlife Collection (ANWC), and Smithsonian National Museum of Natural History (NMNH).

[b] Reported in Mariaux and Braun (1996).

[c] Denotes fresh tissue samples (frozen liver—LACM, muscle in alcohol—ANSP). All other tissues originate from museum material (toe pad, skin, or bone fragment).

mitochondrial and not nuclear origin (Sorenson and Fleischer, 1996).

Maximum likelihood (ML) analyses were performed using the complete DNA data matrix and the program PAUP*4b8 for the Linux operating system on a UNIX machine (Swofford, 2000). We used likelihood heuristic searches with a 2-rate class (transitions and transversions) model of sequence evolution with gamma correction, which is identical to the HKY85 model of sequence evolution (Hasegawa et al., 1985) with the addition of a gamma rate parameter (Yang, 1994). Our likelihood searches returned two most likely trees. We used PAUP* and the program MODELTEST 3.1 (Posada and Crandall, 1998) to determine the most appropriate model of sequence evolution for this ML tree and these genetic data. We then further tested the fit of two site-specific models of DNA evolution, and we used the best-fit model for subsequent tree searches.

Using the recommended model of DNA evolution, we performed maximum likelihood tree searches using

Table 2
Primer pairs used to amplify DNA

Primer name	Primer sequence	Region	Direction	Citation
GF t-lys (L9051)	CACCAGCACTAGCCTTTTAAG	ATPase8	Forward	(Greenberg et al., 1998; Sorenson et al., 1999)
BRUS (H9241)	TGGTCGAAGAAGCTTAGGTTC	ATPase8	Reverse	(Greenberg et al., 1998; Sorenson et al., 1999)
Cytb1-anc	CCAACATCTCTGCTTGATGAA	Cytb I	Forward	(Kocher et al., 1989)
Cytb2.k	TCAGAATGATATTTGTCCTCA	Cytb I	Reverse	(Kocher et al., 1989)
Cytb2.SH	GAATCTACTACGGCTCATAC	Cytb I	Forward	Developed by R.C.F.
Cytb.X	AGGTTTCGGATTAGTCAGCC	Cytb I	Reverse	Developed by R.C.F.
Cytb2.RC	TGAGGACAAATATCCTTCTGAGG	Cytb II	Forward	Developed by R.C.F.
Cytb.ack	CCTCCTCAGGCTCATTCTAC	Cytb II	Reverse	Developed by R.C.F.
Cytb2.wow	ATGGGTGGAATGGAATTTTGTC	Cytb II	Reverse	Developed by R.C.F.

the successive approximations method (Huelsenbeck, 1998) in PAUP* to obtain best-fit tree(s) and parameter estimates. Support for particular nodes was assessed using non-parametric bootstrap (Felsenstein, 1985) as implemented in PAUP* with 1000 fast-addition bootstrap replicates.

We also performed Bayesian phylogenetic analyses using Markov Chain Monte Carlo (MCMC) tree searches using the program MrBayes 2.01 (Huelsenbeck and Ronquist, 2001). Using the recommended model of sequence evolution, we ran four simultaneous MCMC chains for 300,000 generations, and discarded results of 15,000 early generations as "burnin" (see Section 3). Remaining trees were used by MrBayes to estimate parameters, parameter variance, and posterior probabilities of particular nodes in our phylogenetic trees.

3. Results

Our matrix includes a total of 720 bases consisting of three sequenced amplicons. The first amplicon is a 193 bp region comprising 33 bp of $tRNA^{lys}$ and 160 bases of ATPase 8. The second two amplicons comprise 307 and 220 bases of non-overlapping cytochrome *b* sequence. ATPase 8 sequences were obtained for all ingroup taxa, but the 307 base cytochrome *b* failed to sequence for five ingroup taxa [*A. albertisi salvadorii* (LACM 2097), *A. bennettii plumiferus* (AMNH 632030), *A. bennettii terborghi*, and *A. bennettii weidenfeldi* (BBM 22678), *A. novaezealandiae*, and *A. savesi*] and the 220 bp region failed to sequence for four ingroup taxa (both *A. crinifrons*, *A. savesi*, and *A. insignis* LACM 2036). Thus, our sequencing efforts produced a matrix that was 91.8% complete, and this is comparable to successes of other "ancient" DNA studies. It is notable that *A. savesi* data are only available for ATPase 8.

3.1. Model choice

We used ModelTest (Posada and Crandall, 1998; Posada and Crandall, 2001) that compares 56 models of sequence evolution for the best fit to the data. Explanations of the 56 models, their parameters, and their abbreviations can be found in ModelTest manuals (Posada and Crandall, 1998; Posada and Crandall, 2001). ModelTest recommended general-time-reversable model with gamma rate and invariant sites ($GTR + \Gamma + I$) using likelihood ratio test and recommended $TVM + \Gamma + I$ using Akaike Information Criterion (AIC). [TVM model is similar to GTR, however TVM estimates only five rate matrix parameters as opposed to six for GTR (Posada and Crandall, 1998)]. We additionally tested two site-specific models, that we call SSR_3 and SSR_7. For SSR_3 site specific models, all bases were coded as first, second, or third codon (given their generally higher rate of evolution, $tRNA^{lys}$ bases were coded as third positions). For SSR_7 models, the seven partitions were $tRNA^{lys}$, first, second, and third, positions of ATPase 8, and first, second, and third positions of cytochrome *b*. Whelan and Goldman (1999) showed that these models are not strictly nested and that likelihood ratio statistics may not strictly follow the χ^2 approximation for model testing, so we therefore used both AIC and Bayesian Information Criteria (BIC) (Schwarz, 1978) to compare these models. Both AIC and BIC suggested that site specific models improved upon both $GTR + \Gamma + I$ and $TVM + \Gamma + I$ models, and that $GTR + SSR_7$ provided the best model fit of all models considered. Using this model, PAUP* converged on a single ML tree (Fig. 1a) and parameter estimates (Table 3) after two successive approximations.

Using MrBayes and the $GTR + SSR_7$ model, four MCMC chains were run for 300,000 generations, and trees were sampled every 10 generations. Tree likelihoods began to plateau, and the frequency histogram became unimodal and stable after 15,000 generations. Thus, 1500 trees were discarded, and chains ran for another 285,000 generations, yielding 28,500 trees and parameter estimates that were used for estimating credible intervals for parameters and posterior support for nodes of the phylogenetic tree. Table 3 reports parameter estimates from PAUP* and MrBayes as well as Bayesian 95% credible intervals for parameters.

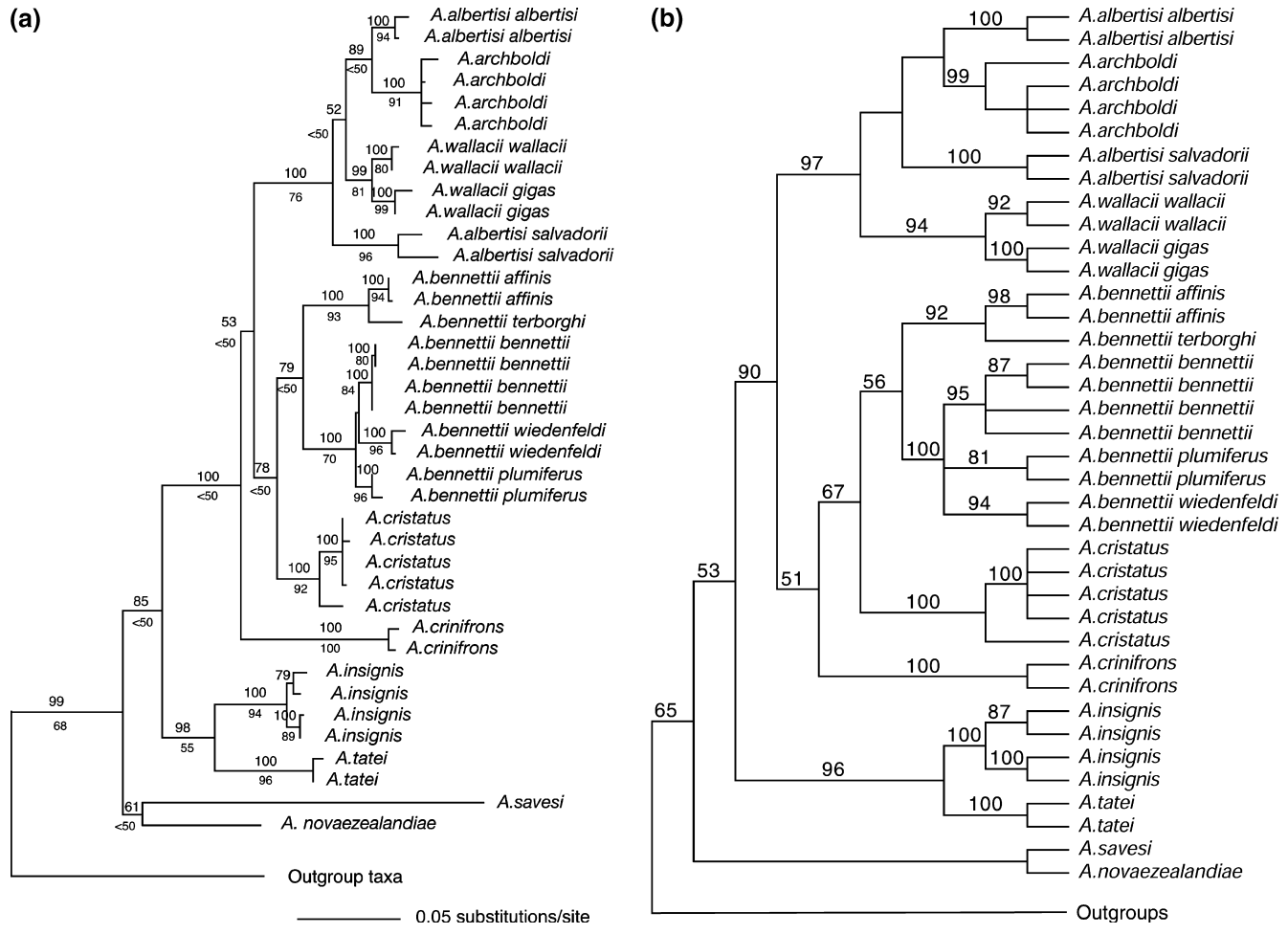

Fig. 1. Phylogenetic hypotheses. 1a is the maximum likelihood tree using GTR + SSR7 model of sequence evolution. Numbers above the branches are Bayesian posterior probabilities, and numbers below the branches are bootstrap values from 1000 fast-addition ML bootstrap replicates. 1b is a strict consensus of eight most parsimonious trees when the transition:transversion ratio equals 1:3.2. Bootstrap support is provided above each node. In both trees, outgroup relationships are poorly resolved, and form a basal polytomy. Because the node representing a single ancestor for Aegothelidae is well supported, we rooted these trees here, and depict the outgroup cluster as a single branch.

Bayesian posterior probabilities are presented for each node over 0.5, and support is given in percentages (so probabilities of 0.99 are presented as 99%).

An unweighted parsimony analysis of all sequences yielded 72 most parsimonious trees. Each tree had a length of 1003 steps, consistency index of 0.488, retention index of 0.708, rescaled consistency index of 0.345, and homoplasy index of 0.512. The parsimony consensus tree was very similar to the ML tree, however, there were a few differences. First, the position and monophyly of *A. savesi* and *A. novaezealandiae* were not supported by all 72 trees, and these two taxa were more often placed as basal to *A. crinifrons* in the ML tree. This is consistent with the low ML and Bayesian support for the position of *A. savesi* and *A. novaezealandiae* (see Fig. 1). Second, the position of *A. crinifrons* was not supported unanimously by all most parsimonious trees, and *A. crinifrons* more often appeared sister to the *A. bennettii affinis*/*A.b. terborghi* clade. Again, this is also suggested by low posterior probabilities for nodes between *A. crinifrons* and *A. bennettii affinis*/*A.b. terborghi* in the ML tree. Furthermore, all deeper nodes with Bayesian posterior probabilities of 80% or less collapsed in the consensus of the 72 most parsimonious trees. When we performed a weighted parsimony analysis with transition:transversion ratio equal to 3.2:1 (the weighting suggested by an HKY85-model likelihood parameter estimation), we obtained eight most parsimonious trees, and the consensus of these eight trees matched our ML tree for most *Aegotheles* nodes (Fig. 1b).

4. Discussion

4.1. Phylogenetics

Several issues regarding rooting and outgroup topology require mention. First, regardless of what

Table 3
Evolutionary model parameters

Parameter	Max Lik Tree[a]	Mean[b]	Variance[b]	(95% CI)[b]
Tree lnl	−5318.01625	−5384.0279	73.340301	(−5401.860000 to −5368.520000)
tl		3.007249	0.024904	(2.741944–3.366677)
r_{gt}	1	1	0	(1.000000–1.000000)
r_{ct}	6.8388547	10.498393	13.499045	(4.919051–18.064353)
r_{cg}	0.41562556	0.706404	0.091203	(0.276685–1.490061)
r_{at}	0.7811016	1.15279	0.204109	(0.468672–2.095466)
r_{ag}	5.6121085	8.840319	8.94274	(4.530709–15.443999)
r_{ac}	0.77422431	1.143567	0.188651	(0.442074–2.064183)
Π_a	0.30355	0.303048	0.000198	(0.275990–0.331056)
Π_c	0.387536	0.38974	0.000181	(0.363932–0.415852)
Π_g	0.104201	0.100705	0.000093	(0.082083–0.119523)
Π_t	0.204712	0.206508	0.000123	(0.186642–0.229194)
Tlys	0.915495	0.957351	0.063289	(0.571341–1.770753)
a8pos1	0.894888	0.965987	0.025055	(0.718220–1.337486)
a8pos2	0.526312	0.525074	0.027435	(0.399532–1.069825)
a8pos3	1.975781	2.344276	0.056818	(1.661414–2.788870)
Cbpos1	0.286234	0.268585	0.000951	(0.219836–0.300466)
Cbpos2	0.056923	0.061699	0.000071	(0.044354–0.078999)
Cbpos3	2.535601	2.408864	0.005279	(2.233259–2.468484)

Note. Likelihoods and parameters estimated by PAUP* in maximum likelihood searches and by MrBayes in MCMC searches of tree space. Tree lnl denotes tree log likelihoods, r_{xy} denotes instantaneous rate parameters in a GTR (nst = 6) rate model, Π_x denote ML estimated base frequencies, and the final seven parameters are partition rates in an SSR_7 site-specific model.

[a] Parameter estimates from PAUP* made on the most likely tree.

[b] Parameter estimates, variances, and 95% credible interval from MrBayes.

method was used to reconstruct the phylogeny, the ingroup topology is the same when outgroups were included and when outgroups were excluded. In addition to topology, the support indices for ingroup nodes were similar. Second, because biologists debate in which avian order Aegothelidae belongs (Apodiformes or Caprimulgiformes), it is unclear and confusing where to root the tree within the outgroup taxa. Third, outgroup topology is not well resolved. Outgroup topology of the best trees differs according to the reconstruction method used, and it garners little or no bootstrap or Bayesian support except for the most obvious nodes. Fourth, the relationship between Aegothelidae and the outgroups is extremely distant. Because the genetic characters used for this analysis are evolving rapidly, we do not expect that these characters could reliably resolve the outgroup topology. Thus, in our figures and discussion, we do not depict or discuss outgroup topology. The outgroups prove useful only for placing a root in the ingroup tree.

The three phylogenetic methods generated trees that contained the same better-supported nodes. Support for the monophyly of Aegothelidae was high, with roughly 99% Bayesian posterior probability (ML bootstrap support 68%). Support for most currently named species was also high; for example, *A. insignis*, *A. tatei*, *A. crinifrons*, *A. cristatus*, *A. wallacei*, and *A. archboldi* all had 99–100% Bayesian support. However, this may not be a critical test for (1) *A. archboldi*, because these were all collected from a single population or (2) *A. crinifrons*, because the population on Halmahera Is. was not represented. Molecular data confirmed the recently proposed split between species *A. insignis* and *A. tatei* (Pratt, 2000) and their position as sister taxa, with average pairwise distances of 10% (HKY85 distance). Especially striking was the close genetic similarity of the two *A. tatei* specimens (HKY85 distance = 0.004), collected nearly 1000 km apart, yet broadly allotopic with more distantly related *insignis*.

Our data provided little support for the monophyly of *A. bennettii* and contradicted the monophyly of *A. albertisi*. (Discussion for *A. bennettii* appears in the section on phylogeography.) Our data suggest that the nominate *albertisi* from the Arfak Mts. is more closely related to species *A. archboldi*, which largely replaces *A. albertisi salvadorii* in the west, however our support is not strong. Average pairwise sequence divergence between *albertisi* and eastern *salvadorii* was 7.7% (cytb only, HKY85 distance) while within group divergence was small (less than 2.3%, but sample sizes small). We remain cautious about the phylogenic and taxonomic implications of these results.

The tree supported the monophyly of *A. wallacii wallacii* and *A. wallacii gigas*, and the two forms appear closely related and conspecific, with average pairwise sequence divergence of 2.7% between these subspecies. Some authors have questioned whether they might be different species (Holyoak, 2001; Olson et al., 1987). However, these taxa appear to differ significantly only in size (T. Pratt, unpublished data).

Owlet-nightjars are currently classified as a single genus (Cleere, 1998; Holyoak, 1999), following the cautious recommendation of Olson et al. (1987). Attempts to subdivide the genus have always involved grouping the three larger aegothelid species (*A. crinifrons*, *A. insignis*, and *A. tatei*), into a single genus, *Euaegotheles* (Mathews, 1918; Schodde and Mason, 1997). Olson et al. (1987, p. 350) criticized the taxon *Euaegotheles* because *A. crinifrons* did not appear to be especially closely related to *A. insignis*, because Mathew's proposed generic characters did not hold, and because Olson et al. found no osteological synapomorphies that could justify the taxon *Euaegotheles*. Schodde and Mason (1997) identified synapomorphies for *Aegotheles* excluding *A. crinifrons* and *A. insignis* (grayer toned plumage, reduced supra-orbital tufts, round-tipped retrices, longer legs; *A. tatei* not yet recognized), but these characters do not hold (Pratt, unpubl. data). A character shared by, and unique to, *A. crinifrons*, *A. insignis*, and *A. tatei* is a distinctive, rufous juvenile plumage (Pratt, 2000). (Juvenile plumage is unknown for the fourth large owlet-nightjar, *A. savesi*). However, this rufous juvenile plumage could just as likely be a plesiomorphic character lost in the small owlet-nightjars, *A. bennettii*, *A. cristatus*, *A. wallacii*, *A. archboldi*, and *A. albertisi*. Given the topology of our molecular tree and the lack of morphological synapomorphies for *Euagotheles*, our data agree with Olson et al. (1987) that aegothelid species should continue to be classified as a single genus, *Aegotheles*.

The extinct owlet-nightjar of New Zealand, *A. novaezealandiae* was initially assigned to its own genus *Megaegotheles* (Scarlett, 1968). Olson et al. (1987) synonymized this genus because the principal characters of *Megaegotheles* were associated with large size and reduced flight apparatus and were conditions that have evolved in many island bird species. Our molecular data suggest a basal position within the Aegothelidae for a *Megaegotheles* clade. In agreement with Olson et al. (1987), *A. savesi* could not possibly be derived from a colonization by *A. cristatus*, as suggested by Mayr (1945). Instead, our data weakly associate *A. savesi* with *A. novaezealandiae*. As both these species are larger, longer-legged, hypothesized to be more terrestrial, and are island endemics, our data do not resolve the question of whether these characters are synapomorphies for a clade (*Megaegotheles*) or if they are the results of convergent evolution and adaptations for island living.

The so-called "feline" owlet-nightjars, *A. insignis* and *A. tatei*, appear to be sister taxa in our trees. This comes as no surprise, as these two species have previously been classified as a single species despite significant morphological differences in *A. tatei* (Pratt, 2000). The high average pairwise sequence divergence of ~14% from the large clade containing *A. crinifrons* and the small owlet-nightjars may justify elevating *A. insignis* and *A. tatei* to its own genus, although no historical name is available. While we are aware of morphological synapomorphies in the plumage pattern, until additional work is done, we are unwilling to offer a new generic name.

The tree grouped the small owlet-nightjars (*A. cristatus*, *A. bennettii*, *A. albertisi*, *A. archboldi*, and *A. wallacii*) together, as sister to *A. crinifrons*. Apparent morphological synapomorphies include small size and pale neck collars. *A. bennettii* and *cristatus* have always been classified together and initially were considered a single species, *A. cristatus*. The poor Bayesian and bootstrap support within this clade is due largely to the ambiguous placement of *A. crinifrons* that has weak affinities for the *A. bennettii affinis* group. When *A. crinifrons* is removed, Bayesian posterior support for *A. bennettii*/*A. cristatus* clade is 99%, and support for the monophyly of *A. bennettii* is 99%.

The other well-supported clade of owlet-nightjar species, *albertisi*, *archboldi*, and *wallacii*, retains plesiomorphic whitish spotting on the scapulars and back. Perhaps more interesting is that this group of three closely related species represents a shallower and more poorly resolved radiation than either *A. bennettii* or *A. insignis*/*A. tatei*, yet *albertisi*, *archboldi*, and *wallacii* have been more consistently split.

4.2. Phylogeography

Our data have interesting implications for the lowland biogeography of New Guinea. New Guinea's complex and isolated mountain ranges have long been credited for their ability to isolate montane taxa and lead to allopatric speciation. By contrast, because of their large, continuous tracts of lowland forest, the major lowland regions have not been fully appreciated as centers of genetic diversification and speciation. In contrast, our data suggest that the lowlands are also capable of isolating taxa, and our work suggest that lowlands are equally capable of supporting much genetic diversity. *A. bennettii*, a lowland owlet nightjar, has five named subspecies, including: (1) two small, lowland subspecies differing primarily in size, *bennettii* and *wiedenfeldi*, along the southern and northern lowlands, respectively, and (2) two larger, low-elevation montane subspecies, *affinis* of Arfak mountains in the Vogelkop Peninsula to the far west, and *terborghi* known only from the type specimen collected at Karimui in the eastern highlands. Lastly, (3) the distinctively colored, dwarf *plumiferus* inhabits the southeastern D'Entrecasteaux archipelago (Fig. 2). While support for the *A. bennettii* clade itself is not strong, *A. bennettii* included two well-supported clades that are sister in the ML tree. The first clade includes *bennettii*, *wiedenfeldi*, and *plumiferus* with average pairwise sequence divergences of 2.7% (HKY85 distance) among subspecies. Insular *plumiferus* is embedded within

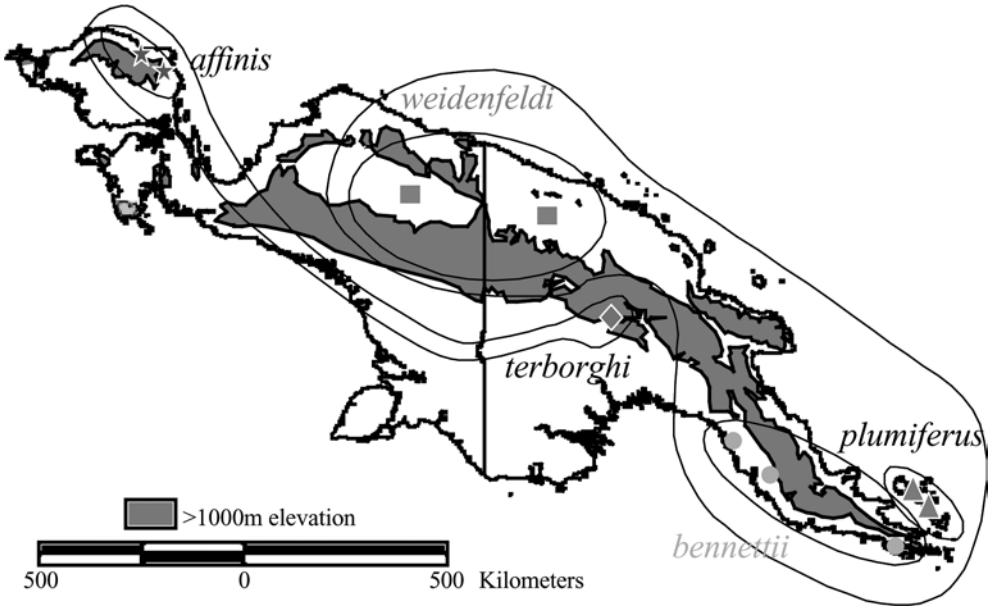

Fig. 2. Biogeographical distribution of *Aegotheles bennettii* subspecies. Symbols represent collecting localities for sampled individuals and not necessarily the entire range. Circles represent hierarchical clustering matching that of our ML phylogeny.

this polytomy despite its morphological divergence in size and plumage characters.

The remaining two subspecies of *A. bennettii*, *affinis*, and *terborghi*, appear sister to each other, with average pairwise sequence divergence of 2.5% (HKY85) between them. It is significant that these differ from the other *A. bennettii* clade by a large average pairwise sequence divergence of 8.4%. In many other studies (Johns and Avise, 1998) this divergence would be considered enough to warrant separate species status. Historically, the placement of *affinis* has shifted back and forth between *A. cristatus* (Rand and Gilliard, 1967) and *A. bennettii* (Holyoak, 2001; Mayr, 1941). Instead, it may be advantageous to split off *affinis* and *terborghi* into a new species (*A. affinis*) for three reasons. First, these groups appear to be reciprocally monophyletic. Second, between-group variation (8.4%) is much larger than within group variation (2.5% for *affinis*/*terborghi* and 2.7% for *bennettii*/*weidenfeldi*/*plumiferus*), suggesting there has been adequate time for independent evolution. Third, montane *terborghi* is allotopic with lowland taxon *bennettii*, yet separated from *affinis* far to the west by gap of approximately 1300 km, along the south slope of the Central Ranges. Few specimens of any owlet-nightjar have been collected in between, and future surveys may fill this gap. Unfortunately, little natural history is known of either *affinis* or *terborghi* that could shed light on their relationship with each other or to *bennettii*/*wiedenfeldi*.

There are several lines of evidence suggesting an Australopapuan origin for the family Aegothelidae. First, the earliest known owlet-nightjar is an early to mid-Miocene fossil, *Quipollornis koniberi*, from New South Wales, Australia. Second, no representatives of the family are known from outside the Australasian region (which for our purposes includes Maluku Islands, and Pacific islands of New Caledonia and New Zealand). Thus the geographical center of distribution would be located in Australia or New Guinea. Third, seven of the nine extant species are found in New Guinea.

Interestingly, owlet-nightjar lineages have radiated into a range of habitats as well as into geographic areas. For example, the feline *Aegotheles* clade has a lowland and a montane species (*A. tatei* and *A. insignis*, respectively), the spotted small *Aegotheles* are distributed as much by elevation as geographical region (from lowest to highest elevation: *A. wallacii*, *A. albertisi*, and *A. archboldi*), and the barred small *Aegotheles*, although mainly a lowland group (*A. cristatus* and most races of *A. bennettii*) also has a lower montane group (*A. bennettii affinis* and *A. b. terborghi*). This pattern suggests that ecological gradients (Moritz and Faith, 2000; Moritz et al., 2000) may also be important for diversification in *Aegotheles*.

Aegotheles demonstrate many of the same geographic patterns of differentiation previously described from other New Guinean taxa. These include east–west splits for montane taxa (Joseph et al., 2001) and north–south splits for the lowland taxa (Dumbacher and Fleischer, 2001). Sequence divergence between *A. albertisi albertisi* and *A. a. salvadorii* is 7.7%, which is similar to the 7.4% found in the montane logrunner *Orthonix novaeguineae*—the only other published comparison of birds from the far-western Vogelkop with those of eastern New Guinea (Joseph et al., 2001).

Aegotheles novaezealandiae and *A. savesi* could have arrived at anytime on their respective land masses, New Zealand and New Caledonia. Both archipelagos are broken-off fragments of Gondwana that have remained above sea level since the late Cretaceous and likely predate the genus *Aegotheles*. The basal position of both *A. novaezealandiae* and *A. savesi* in our phylogeny raises the question of whether owlet-nightjars dispersed to these islands at a time when they lay closer to Australia. Further supporting this hypothesis is the observation that recent Australian/New Guinean owlet-nightjar species have not reached any other island off the Sahul continental shelf. In agreement with Olson et al. (1987), our data also suggest that *A. savesi* is probably not derived from *A. cristatus* by colonization, as suggested by Mayr (1945).

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