The Wilson Journal of Ornithology

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Published by the Wilson Ornithological Society

The Wilson Journal of Ornithology 123(3):441–453, 2011

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ABSTRACT.—We constructed a phylogenetic hypothesis of the pattern of colonization of Philippine scops owls (*Otus* and *Mimizuku*). Two mitochondrial genes, ND2 and cytochrome *b*, were sequenced for 12 samples representing six Philippine endemic taxa: three endemic species, one of which has three endemic subspecies; and one endemic genus. Topology, branch length information, and sequence divergence were used to present the hypothesis for the pattern, direction, and sequence of island colonization events. Philippine scops owls are in two well-supported clades, consistent with at least two independent colonization routes. One route is represented by the montane clade of *Otus sunia*, *O. longicornis*, and *O. mirus*. The other clade is represented by three subspecies of the lowland *O. megalotis*. The basal position of *Mimizuku gurneyi* relative to the *megalotis* clade suggests early colonization of Mindanao. Branch lengths and sequence divergence data are congruent with the morphological differences among the *megalotis* races. The three races of *megalotis* differed in 15 of 16 morphological characters. Based on molecular and morphological evidence, we recognize the following *Otus megalotis* subspecies as full species: Luzon Lowland Scops Owl (*O. megalotis*), Mindanao Lowland Scops Owl (*O. everetti*), and Visayan Lowland Scops Owl (*O. nigrorum*). We also propose reassigning the Giant Scops Owl (*Mimizuku gurneyi*) to the genus *Otus* for phyletic consistency. *Received 29 November 2010. Accepted 15 April 2011.*

Archipelagos are interesting places to study phylogeography and evolution because open seas represent potential barriers to gene flow. The construction of robust species phylogenies in island archipelagos provides important insights to rates, patterns, and modes of species diversification (Lovette and Bermingham 1999, Ricklefs and Bermingham 1999, Coyne and Price 2000, Barraclough and Nee 2001, Turelli et al. 2001, Jones and Kennedy 2008a). The Philippine Archipelago, comprising >7,000 islands, has high endemism and some of the most unique avifaunal lineages in the world (Mittermeier et al. 1999, Kennedy et al. 2000, Myers et al. 2000, Peterson 2006). The archipelago is relatively recent in geological time (most islands are <35 million years ago, hereafter mya), and the chronology of oceanic island formations from sea floor tectonic events and patterns of coalescence and fragmentation are well documented (Hall 1996, 1998, 2002). These conditions provide investigators unique opportunities to test hypotheses to explain present patterns of species diversification. Recent phylogeographic studies of some vertebrate groups, such as rodents (Steppan et al. 2003, Jansa et al. 2006), thrushes (Jones and Kennedy 2008a, b), and bulbuls (Oliveros and Moyle 2010) have illuminated complex patterns of diversification within the Philippine Islands. We document the altitudinal pattern of colonization and examine the taxonomy of Philippine scops owls (*Otus* and *Mimizuku*) using two mitochondrial genes with additional evidence from morphology.

GEOLOGIC HISTORY OF THE PHILIPPINE ARCHIPELAGO

The Tertiary and Quaternary geological history of the Philippine Archipelago has been well documented (Hall 1996, 1998, 2002). Extensive studies have also investigated the role of past episodic sea level fluctuations and climate changes that shaped the configuration of the islands and formation of distinct biogeographic regions (Heaney 1986, 1991, 2000; Heaney and Rickart 1990; Musser and Heaney 1992; Steppan et al. 2003; but see Jones and Kennedy 2008a).

The Philippine Archipelago is considered geologically young with most land emerging from the sea floor <35 mya. The three distinct geological units that emerged and coalesced over the recent past are the Palawan-Mindoro, Greater Luzon, and Greater Mindanao blocks (Fig. 1). The Palawan-Mindoro block was a part of the mainland Southeast Asia continental shelf and broke away 30–35 mya. Palawan emerged about 5 mya and was connected to northern Borneo, but not to other major islands of the Philippine Archipelago. Luzon Island emerged about 30–

[1] Department of Biology, Texas Southern University, 3100 Cleburne Drive, Houston, TX 77004, USA.
[2] Houston Museum of Natural Science, 5555 Hermann Park Drive, Houston, TX 77030, USA.
[3] Maria Mitchell Association, 4 Vestal Street, Nantucket, MA 02554, USA.
[4] Corresponding author; e-mail: mirandahc@tsu.edu

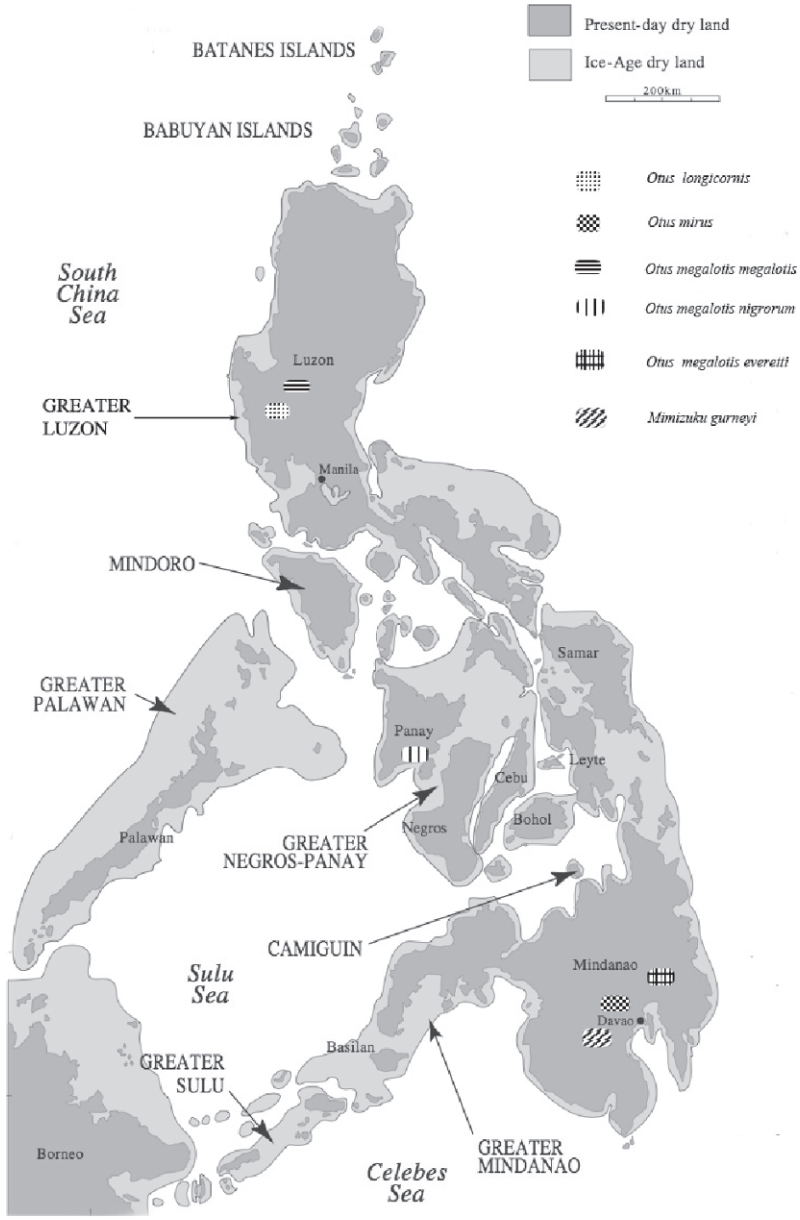


FIG. 1. Philippine Islands, showing the names of major islands, and the distribution of scops owl species and subspecies used in this study. The dark gray area represent contemporary configuration of the islands. Light gray areas represent the approximate distribution of land that was exposed above sea level during the Pleistocene glaciation cycles. (modified from Heaney 1986).

35 mya as a group of small islands, southeast of the current location. Extensive volcanism during the last 15 million years shaped the coalescence of islands to form Greater Luzon. Greater Mindanao, including present-day Samar and Leyte, arose about the same time as Luzon. The Greater Negros-Panay block is believed to have emerged as one oceanic island ~2 mya and recently subdivided into the smaller islands including Panay and Negros (Heaney 1986, 1991; Hall 1996, 1998).

One hypothesis to explain the complexity of community structure in tropical archipelagos is the elevational shifts of biomes during glacial

cycles. The contraction and expansion of biomes during these cycles led to isolation, or aided in colonization as adjacent islands merged. The montane region expanded down along mountain slopes, while lowland dipterocarp forest retracted as average temperature decreased over time. This model predicts the lowland dipterocarp forest, while retracting at the upper elevational boundary of the mountains, would have expanded as more lowland areas were exposed with descending sea level. Concomitantly, adjacent islands would be closer together, if not coalesced. This process was hypothesized to have acted as 'species pumps' in topographically diverse islands such as the Philippines, that accelerated speciation compared to less topographically diverse regions (Steppan et al. 2003). The biome elevational shift hypothesis predicts that species in montane regions of adjacent islands are sister taxa, and lowland species between adjacent islands are sister taxa.

There are two recognized strigid genera within the Philippines, *Otus* and *Mimizuku*. There are seven Philippine species of *Otus*, five of which are small island endemics: Luzon Scops Owl (*O. longicornis*), Mindoro Scops Owl (*O. mindorensis*), Mindanao Scops Owl (*O. mirus*), Philippine Scops Owl (*O. megalotis*), and Palawan Scops Owl (*O. fuliginosus*). The remaining two *Otus* are not restricted to the Philippines: *O. mantananensis* on small islands off Borneo and the Sulu Archipelago, and *O. elegans* in Batanes Islands north of Luzon (Kennedy et al. 2000).

Early classification based on morphology placed *Mimizuku gurneyi* as a relict of an ancient lineage, and clustered *nigrorum* and *longicornis* as races of the widespread *O. spilocephalus* (Burton 1973). Marshall's (1978) classification led to species status for *O. longicornis*, *O. mindorensis,* and *O. mirus*. Research based on vocalizations led to more major taxonomic rearrangements with an increase in the number of Asian scops owl species (Roberts and King 1986, Marshall and King 1988, Becking 1994, Lambert and Rasmussen 1998). Earlier systematics work on Philippine scops owls using different mtDNA genes suggested divergence of the lowland/highland clades and noted the relatively high genetic distances among endemic Philippine scops owls (Miranda et al. 1998). Merger of *Mimizuku* with *Otus* was also suggested in that paper and elsewhere (Mindell et al. 1997; Miranda et al. 1997, 1998). König and Weick (2008) cited this previous phylogenetic evidence and mentioned the differences in vocalizations among the *O. megalotis* subspecies, but did not designate the three *O. megalotis* subspecies as distinct species. Wink et al. (2008, 2009) also supported the invalidity of the genus *Mimizuku*.

An assessment of genetic structure among the three *megalotis* subspecies based on larger sampling should clarify their biogeography and taxonomy. For example, the three island blocks of Greater Luzon, Greater Mindanao, and Greater Panay-Negros were separated from each other by narrow straits of ~25 km in width (Steppan et al. 2003). There should be little or no genetic differentiation among subspecies, assuming these straits did not pose a barrier to dispersal. However, if the distinct phenotypic variation observed among the subspecies is congruent with the mtDNA substructure, one can invoke the notion of reproductive isolation due to a geographic barrier (i.e., the open sea).

We used mitochondrial DNA sequences of two genes, NADH dehydrogenase 2 (ND2) and cytochrome *b* (cyt-*b*), to address two questions. (1) Does the presence of multiple scops owl species on islands reflect speciation events or do species assemblages result from multiple colonization events? (2) Do the patterns of mtDNA divergences as shown by gene trees and morphological divergences reflect the current taxonomy for the Philippine Scops Owl (*O. megalotis*)? Resolution of taxonomy to reflect evolutionary divergences has far-reaching implications for studies of conservation and biodiversity (Zink 2004), especially in a biodiversity and conservation hotspot such as the Philippines (Mittermeier et al. 1999, Myers et al. 2000, Sodhi et al. 2004, Peterson 2006).

METHODS

Taxonomic Sampling.—Tissue samples were obtained during the 1991–1993 Philippine Biodiversity Inventory conducted by the Cincinnati Museum Center (CMC) and the Philippine National Museum. We used 12 individuals representing four species of Philippine scops owls: *M. gurneyi*, *O. longicornis*, *O. mirus*, and *O. megalotis*, which is represented by three subspecies: *O. m. megalotis* (lowland Luzon), *O. m. everetti* (lowland Mindanao), and *O. m. nigrorum* (Panay).

We used sequences of several Old World *Otus* taxa from neighboring islands to increase taxon sampling and orient colonization patterns of the

Philippine Islands, (Proudfoot et al. 2007, Fuchs et al. 2008) (Table 1). We included published sequences from two representatives of New World '*Otus*' (*Megascops*): *M. asio* and *M. hoyi* for proximal outgroups. We rooted our trees using the more distantly related *Bubo virginianus.*

Laboratory Procedures.—Samples used for DNA extraction were from muscle tissue. DNeasy Tissue Kit (Qiagen, Valencia, CA, USA) was used to isolate genomic DNA. We sequenced cytochrome *b* (cyt-*b*) and ND2 using primer pairs L14990/H15646, L15517/H16404 for cyt-*b*, and L5216/H5766, L5758/H6313 for ND2 (Sorenson et al. 1999). A new primer was designed to sequence ND2 for several taxa: L5217 (5′-CCCCATAATCTCAAAATCACATC-3′) for *O. m. nigrorum*, *O. m. megalotis,* and *O. longicornis*. Reaction tubes contained 5.0 µl of 40 mM magnesium chloride, 2.0 µl of dNTP mix (2 mM for each nucleotide), 5.0 µl of 12 pM of primers, and 5.0 µl of Platinum *Pfx* DNA polymerase enzyme in a 1:30 dilution (Invitrogen, Carlsbad, CA, USA) and 5.0 µl of template DNA. The 550–600 bp fragments were PCR-amplified in 50 µl reaction capillary tubes using Rapidcycler© (Idaho Technologies, Salt Lake City, UT, USA) with the first cycle at 94° C for 15 sec, followed by 35 cycles at 0 sec at 94° C denaturation temperature, 55–58° C annealing temperature at 0 sec, and 50 sec at 70° C extension temperature. Amplified PCR-products were purified using Wizard PCR Preps Purification System (Promega Corporation, Madison, WI, USA). Sequencing was done using an Applied Biosystems 3730 sequencer (Applied Biosystems, Foster City, CA, USA). Sequences were aligned and fragments assembled using GeneiousPro4.0.3 (Biomatters Ltd., Auckland, New Zealand). New sequences were submitted to GenBank with accession numbers JN131475 to JN131498.

Phylogenetic Reconstruction.—Maximum Parsimony (MP) and Maximum Likelihood (ML) analyses used Program PAUP* 4.0b10 (Swofford 2003). A concatenated data set was generated and analyzed. All MP searches used equal weighting, heuristic search options with 1,000 replicates, tree-bisection–reconnection branch-swapping, and random addition of taxa. Clade support for MP and ML trees was obtained with bootstrap values derived from 1,000 replications (Felsenstein 1985). Program MODELTEST (Posada and Crandall 1998), using Akaike Information Criteria, suggested General Time Reversible (GTR) plus Gamma model as the most appropriate site-specific model of evolution. We performed ML tree searches using the successive approximations method (Huelsenbeck 1998) in PAUP*4.0b10 to obtain the best-fit trees and parameter estimates. Support for particular nodes was obtained using non-parametric bootstrap (Felsenstein 1985) as implemented in PAUP*4b10 with 1,000 fast-addition bootstrap replicates in a likelihood framework.

Bayesian analysis was performed in MRBAYES Version 3.1 using Markov Chain Monte Carlo (MCMC) tree searches (Huelsenbeck and Ronquist 2001). Four independent searches were performed, each with a cold chain and three heated chains run for 400,000 generations with trees sampled every 100 generations.

Morphometric Analysis.—Specimens (Table 2) from the Delaware Museum of Natural History (DMNH) and CMC were examined at the Houston Museum of Natural Science. Only adult specimens of known gender were included, as specimens of unknown age or gender could bias the results by over- or under-representing mean measurements. Twenty-one specimens (11 males and 10 females) of *megalotis*, 18 of *everetti* (10 males, 8 females), and seven *nigrorum* (4 males, 3 females) were included (Table 2). Culmen (cere to tip) and tarsus measurements were taken with Vernier calipers, and a ruler was used to measure natural wing chord and tail length (all measurements in mm) (Table 2).

Significance of morphometric variation was assessed using ANOVA for differences between *megalotis* and *everetti*, whereas non-parametric Mann-Whitney *U*-tests were used for comparisons of *everetti* and *nigrorum*, as only small samples were available for the latter form. Differences were examined separately for males and females in light of variation between male and female owls (Amadon 1959, Brooks and Arnold 2005).

RESULTS

DNA Sequence Analysis.—All samples were sequenced from frozen tissue. We obtained 1,033 and 1,054 bp of ND2 and cyt-*b*, respectively. We truncated the first 15 bp at the 5′ end of the downloaded sequences because of ambiguous alignment in that short segment. No other insertion or deletion was observed from that point upstream. Alignments were relatively straightforward and were performed by the GeneiousAlign option within Geneious Pro 4.0.3 (Biomatters

TABLE 1. Samples, GenBank accession numbers, and reference sources used.

Species	Voucher/(Field number)	Geographic location	Cyt-*b*	Source	ND2	Source
Bubo virginianus	MVZ 18014	USA	AJ003973	Wink and Heidrich 1999	EU601050	Fuchs et al. 2008
Mimizuku gurneyi	B3294	Mindanao, Philippines	JN131488	This study	JN131476	This study
Megascops asio	MVZ 179828	USA	DQ190845	Proudfoot et al. 2007	EU601054	Fuchs et al. 2008
Otus hoyi	ZMUC 114834	Bolivia	EU601103	Fuchs et al. 2008	EU601024	Fuchs et al. 2008
O. lempiji	UWBM 73860	Captive	EU601112	Fuchs et al. 2008	EU601036	Fuchs et al. 2008
O. lettia lettia	MNHN33-4C (JF142,B)	Laos	EU601109	Fuchs et al. 2008	EU601033	Fuchs et al. 2008
O. lettia ussuriensis	UWBM 75379	Russia	EU601111	Fuchs et al. 2008	EU601035	Fuchs et al. 2008
O. longicornis	(B335)	Zambales Mts, Luzon, Philippines	JN131489	This study	JN131477	This study
O. longicornis	(B365)	Zambales Mts, Luzon, Philippines	JN131490	This study	JN131478	This study
O. longicornis	CMC B36504/(B62)	Zambales Mts, Luzon, Philippines	JN131495	This study	JN131483	This study
O. longicornis	(B398)	Zambales Mts, Luzon, Philippines	JN131496	This study	JN131484	This study
O. longicornis	FMNH 433020	Luzon, Philippines	EU601119	Fuchs et al. 2008	EU601043	Fuchs et al. 2008
O. megalotis megalotis	CMC B36509/(B9)	Luzon, Philippines	JN131487	This study	JN131475	This study
O. megalotis everetti	(B2211)	Mindanao, Philippines	JN131492	This study	JN131483	This study
O. megalotis everetti	CMC B6490/(B1392)	Mindanao, Philippines	JN131498	This study	JN131486	This study
O. megalotis everetti	B1391	Mindanao, Philippines	JN131491	This study	JN131479	This study
O. megalotis everetti	(B1746)	Mindanao, Philippines	JN131493	This study	JN131481	This study
O. megalotis	FMNH 433019	Luzon, Philippines	EU601118	Fuchs et al. 2008	EU601042	Fuchs et al. 2008
O. bakkamoena	UWBM 67567511	Captive	EU601110	Fuchs et al. 2008	EU601034	Fuchs et al. 2008
O. megalotis nigrorum	CMC B40325/(B629)	Panay, Philippines	JN131497	This study	JN131485	This study
O. mirus	CMC B38100/(B1480)	Mindanao, Philippines	JN131494	This study	JN131482	This study
O. mirus	FMNH 357429	Mindanao, Philippines	EU601126	Fuchs et al. 2008	EU601057	Fuchs et al. 2008
O. spilocephalus	MNHN 15-58	China	EU601116	Fuchs et al. 2008	EU601040	Fuchs et al. 2008
O. sunia	MNHN 6-98	Thailand	EU61117	Fuchs et al. 2008	EU601041	Fuchs et al. 2008

TABLE 2. Descriptive statistics ($\bar{x}$ = mean, r = range, n = sample size) of four measurements (mm) from male and female *O. megalotis*, *O. everetti*, *O. nigrorum*, and *Mimizuku**.

	Males				Females			
	Wing	Tail	Culmen	Tarsus	Wing	Tail	Culmen	Tarsus
	megalotis				*megalotis*			
$\bar{x}$	175.09	94.81	23.11	28.92	190.9	102.6	25.7	29.88
r	165–187	85–112	19.3–26.3	25.5–32.2	171–210	92–117	24.2–26.9	26.3–36
n	11	11	10	10	10	10	10	10
	everetti				*everetti*			
$\bar{x}$	158.5	82.1	21.29	22.08	162.87	86.5	22.02	23.74
r	152–166	70–103	20.2–22.6	20–26.4	150–176	74–99	20.2–23.4	22.3–25.2
n	10	10	10	10	8	8	7	7
	nigrorum				*nigrorum*			
$\bar{x}$	134.75	73.75	19.6	23.2	148.33	80.66	19.93	21.73
r	132–139	69–78	18–21.6	22.5–23.6	141–159	77–85	19.3–20.5	19.5–23.5
n	4	4	4	4	3	3	3	3
	Mimizuku				*Mimizuku*			
$\bar{x}$	223.6	121	30.125	34.25	263.5	145.5	35.95	38.8
r	213–230	115–126	26.5–32.1	30.5–37.2	242–285	140–151	35.4–36.5	35.5–42.1
n	5	3	4	4	2	2	2	2

* Specimens Examined. *Otus megalotis*: DMNH 2816, 3058, 3534, 3951, 14477, 52949, 52951, 55817, 66386, 70044–47, 70574–75, 70795, 74159; CMNH 36509–10, 36999, 38249. *O. everetti*: DMNH 13654–58, 19819–20, 40554, 67269; CMNH 33937, 36489–90, 38101, 38962–64, 39163, 39208. *O. nigrorum*: DMNH 13652–53; CMNH 34172, 36764–65, 36909, 40325. *Mimizuku gurneyi*: DMNH 9751–52, 19554–56; CMNH 35735, 36488.

Ltd., Auckland, New Zealand). Base composition was 30% adenine, 36% cytosine, 11% guanine, and 23% thymine. No stop codon was observed. The cyt-*b* sequences downloaded from Genbank were 890 bp in length, 164 bp shorter than the cyt-b sequences generated in our laboratory. We analyzed 24 taxa for cyt-*b* and 23 ND2 sequences. We used the 50% majority rule for the Bayesian analysis for the concatenated data set: a majority of the nodes were strongly supported, and were congruent with the ML tree (ln = 10523.52; Fig. 2). The MP analyses (9 equally parsimonious trees of 1,412 steps, CI = 0.6671, RI = 0.8135) were similar in terms of topology and values of supported nodes with both the Bayesian and ML tree. The sister relationship of *M. gurneyi* and *O. lettia ussuriensis*, however, was weakly supported in all analyses (MP and ML bootstrap value = 52 and 51, respectively; Bayes posterior probability value = 79). Topologies within species/subspecies of *O. longicornis, O. mirus, O. m. everetti,* and *O. m. megalotis* had moderate statistical support and were characterized by short internodes.

The Philippine lowland scops owls appear to be monophyletic with *O. m. megalotis* and *O. m. everetti* from Luzon forming a sister clade, and *O. m. nigrorum* of Panay/Negros positioned basal to the two other races. A subclade within the lowland *Otus* assemblage included *O. bakkamoena*, *O. lempiji*, and *O. lettia*. The second major clade comprised montane forms of *O. longicornis*, *O. mirus*, and the non-Philippine endemic *O. sunia*. Within the Philippine montane clade, *O. longicornis* of Luzon and *O. mirus* of Mindanao formed a strongly-supported clade with *O. sunia*. The Indo-Malayan *O. sunia* formed a clade together with *O. insularis*, *O. capnodes*, *O. mayottensis*, *O. rutilus*, *O. pauliani*, and *O. moheliensis* of the Comoro Islands and Madagascar (Fuchs et al. 2008). The Mindoro Scops Owl (*O. mindorensis*) was not represented in our study but was previously found to be embedded within this clade (Mindell et al. 1997, Miranda et al. 1997). Except for the *O. longicornis* clade, and the *Otus lettia*/*Mimizuku gurneyi* branch, most basal nodes were strongly supported in MP and ML with 100% bootstrap, and by 1.0 posterior probability in Bayesian analysis (Fig. 2).

Pairwise Sequence Divergence.—The uncorrected-p sequence divergence distances showed significant differences among the three clades: (1) the Philippine lowland endemic *Otus* (including *Mimizuku*), (2) the non-Philippine lowland *Otus*, and (3) the montane clade (Table 3). We selected the nearest-neighbor values as a conservative measure of distance in view of the small sample size used in this study. Pairwise estimates of

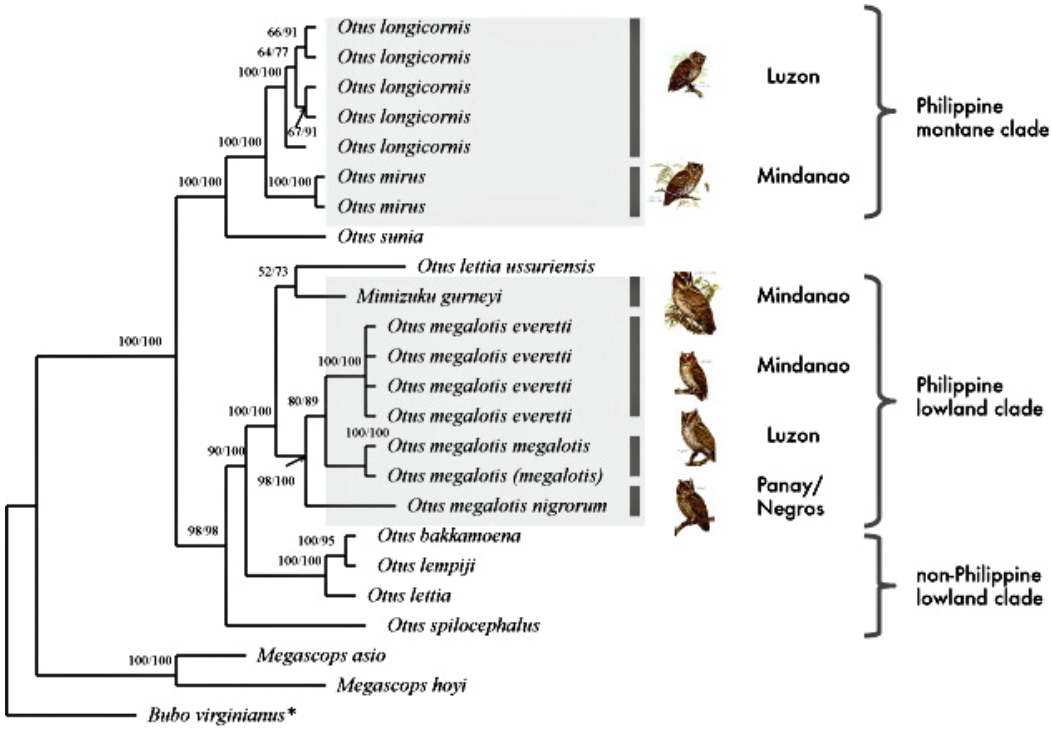
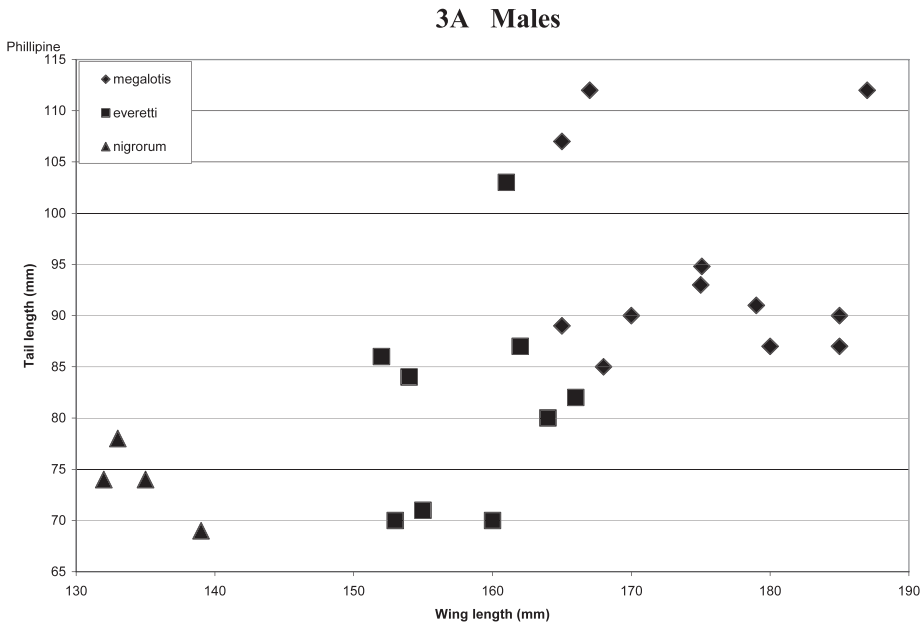


FIG. 2. Phylogenetic hypothesis of relationships of Philippine scops owls using Bayesian and Maximum Likelihood (−ln = 10523.52) methods for mitochondrial cyt-*b* and ND2 genes. The Maximum Parsimony tree is similar except for the position of *Otus lettia ussuriensis*, which is basal to *Mimizuku* and *Otus megalotis*. Numbers above the diagonal represent bootstrap support and numbers below are Bayesian posterior probability support values.

TABLE 3. Pair-wise distances of nearest-neighbor values among terminal *Otus* taxa in each of the three clades based on uncorrected p-distance and Kimura 2-parameter distance (K2P).

	Uncorrected p	K2P	Divergence dates (mya)*
Philippine lowland clade			
O. m. nigrorum/M. gurneyi	0.053	0.056	2.65–2.8
O. m. nigrorum/O. m. everetti	0.036	0.038	1.8–1.9
O. m. nigrorum/O. m. megalotis	0.042	0.044	2.1–2.2
O. m. everetti/M. gurneyi	0.058	0.060	2.9–3.0
O. m. everetti/O. m. megalotis	0.038	0.040	1.9–2.0
O. m. megalotis/M. gurneyi	0.059	0.063	2.95–3.15
Non-Philippine lowland clade			
O. bakkamoena/O. lempiji	0.003	0.003	0.15
O. bakkamoena/O. lettia	0.010	0.011	0.5–0.55
O. lempiji/O. lettia	0.012	0.012	0.6
Montane clade			
O. longicornis/O. mirus	0.038	0.038	1.9
O. longicornis/O. sunia	0.058	0.062	2.9–3.1
O. mirus/O.sunia	0.065	0.068	3.25–3.4

* Estimated divergence dates based on the putative 2% sequence divergence per million years calibration (Lovette 2004). The ranges represent the differences between the two models of molecular change (uncorrected p-distance vs. K2P).



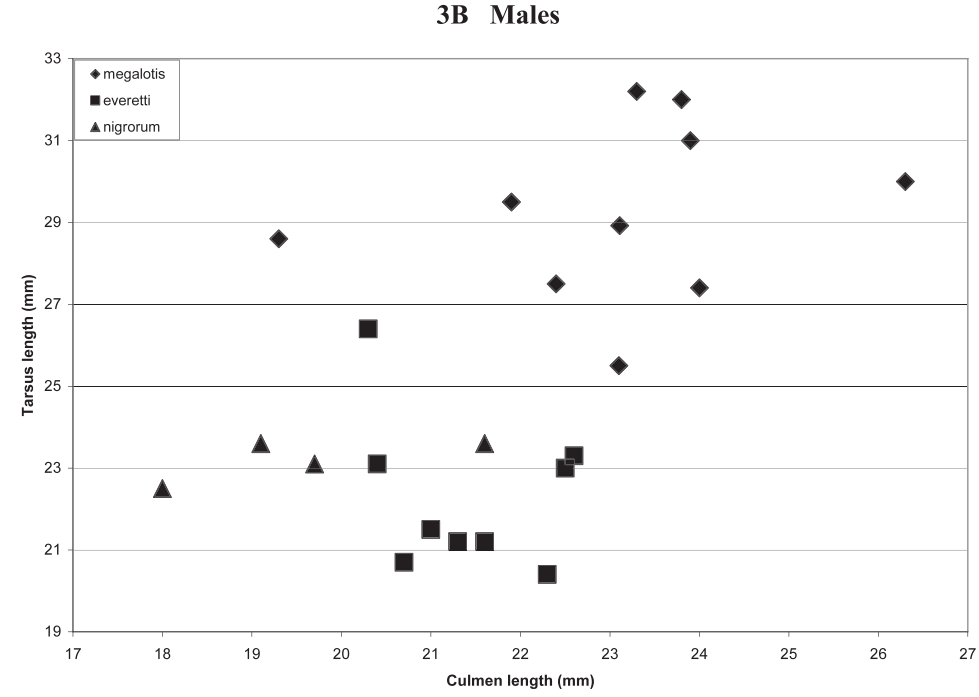
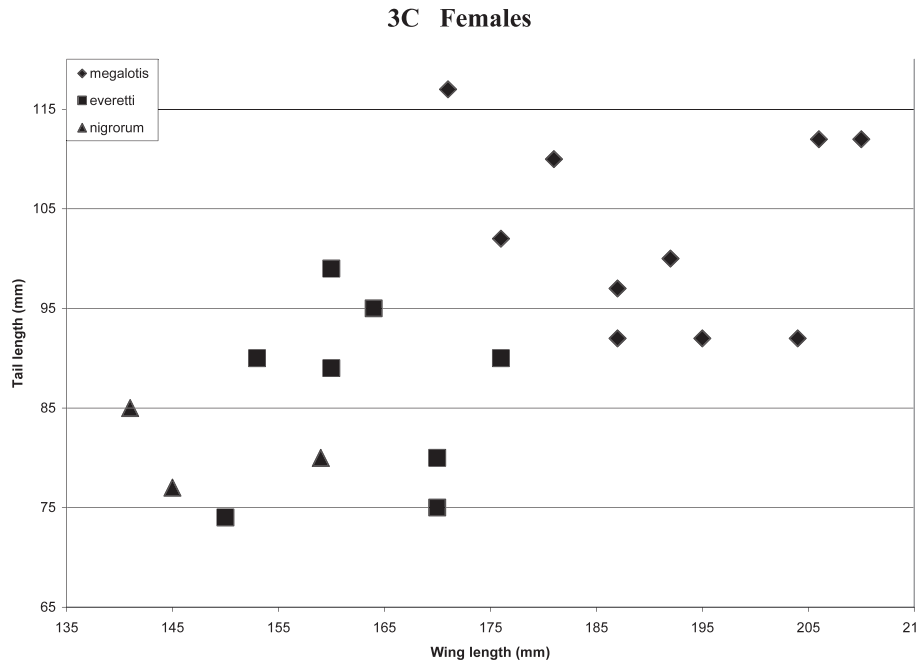


FIG. 3. Plots of comparison of morphological characters for *O. megalotis*, *O. everetti*, and *O. nigrorum*: (A) wing vs. tail length for males, (B) culmen vs. tarsus length for males, (C) wing vs. tail length for females, and (D) culmen vs. tarsus length for females.



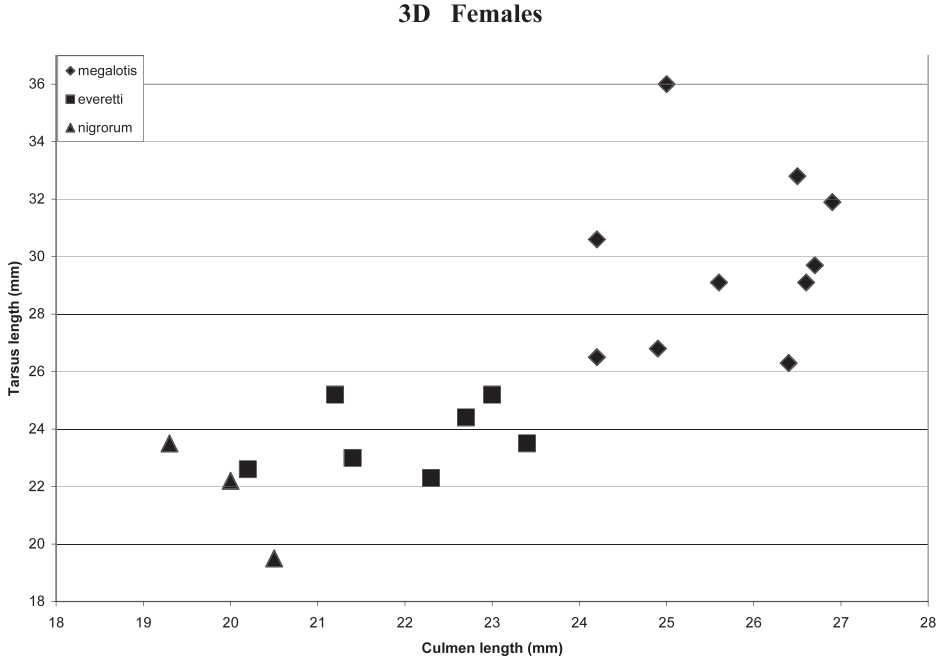


FIG. 3. Continued.

percent divergence were based on uncorrected-p and Kimura 2-parameter. The average distance within the endemic lowland clade between subspecies of *O. megalotis* including *Mimizuku gurneyi* ranged from 3.6 to 5.8% (Table 3). The pairwise sequence divergences within the lowland non-Philippine endemics clade (*O. bakkamoena*, *O. lempiji*, and *O. lettia*) were much lower at 0.03–1.2%, and were comparable to the divergence distance values among the montane clade taxa, which ranged from 3.8 to 6.5%.

Our preliminary divergence dating attempts using Beast Version 1.4.8 (Drummond and Rambaut 2007) suggested the first invasion of the archipelago started in Mindanao by *M. gurneyi* which branched off from the Indian Scops Owl group from the mainland (4.6 mya, 95% HPD = 2.6–5.8). Dispersal to the Greater Panay Island block may have occurred after emergence of the landmass (2.4 mya, 95% HPD = 1.7–3.3), followed soon after by dispersal back to Mindanao and to Luzon (2.2 mya, 95% HPD = 1.4–3.1). The montane invasion of the islands started at about the same time (2.5 mya, 95% HPD = 1.2–3.7).

Morphometric Variation.—Specimens of *megalotis* were significantly larger than *everetti*, which were significantly larger than *nigrorum* (Table 2). All 16 tests were significant except tail length was not significantly different between *everetti* and *nigrorum* (Fig. 3). Measurements of male *megalotis* were significantly greater than *everetti* for wing ($F = 29.28$, $P < 0.0001$), tail ($F = 8.08$, $P < 0.01$), culmen ($F = 8.21$, $P < 0.01$), and tarsus ($F = 48.47$, $P < 0.0001$). Female *megalotis* were also significantly larger than *everetti* for wing ($F = 26.80$, $P < 0.0001$), tail ($F = 13.12$, $P < 0.002$), culmen ($F = 46.78$, $P < 0.0001$), and tarsus ($F = 24.68$, $P < 0.0001$). Measurements of male *everetti* were significantly larger than *nigrorum* for wing ($Z = 2.82$, $P < 0.001$), culmen ($Z = 1.91$, $P < 0.02$), and tarsus ($Z = 1.62$, $P < 0.05$), but not tail ($Z = 1.55$, $P < 0.07$). The same pattern held for female *everetti*, which were significantly greater than *nigrorum* for wing ($Z = 2.04$, $P < 0.02$), culmen ($Z = 2.16$, $P < 0.01$), and tarsus ($Z = 1.59$, $P < 0.05$), but not tail ($Z = 0.91$, $P < 0.18$).

Contrary to the typical reversed sexual size dimorphism (RSD) found in owls, only one of the 16 measurements showed a larger character for males than for females. Male *nigrorum* have longer tarsus length than females (Table 2), but this should be interpreted with caution due to the limited sample size.

Plumage Variation.—We focused on diagnostic differences among the three taxa. The taxon *megalotis* has two characters the other two lacked; scapular stripes and tarsal feathers extending onto the upper part of the foot. A reddish-colored head characterizes *nigrorum* in contrast to *megalotis* and *everetti*.

The darkest taxon is *everetti*, which has dark brown underparts and is nearly black on the nape. In contrast, *nigrorum* is the lightest taxon with white-striated underparts and reddish nape. Intermediate is *megalotis* with ashy-brown underparts and brownish nape.

A red morph is also found in *megalotis*. Two of 21 (10%) specimens examined were red morph (one each from Luzon and neighboring Polilio, both females [DMNH 2816, 14477]), and three (14%) appeared to be intermediates (from Luzon, 2 females [DMNH 52949, 52951] and 1 male [CMNH 38249]).

DISCUSSION

We found that scops owls colonized the Philippines in at least two independent events with subsequent diversification occurring independently in both montane and lowland clades. This pattern is similar to that observed among endemic mammals, although time scales were different (Heaney 1986, 1991; Steppan et al. 2003).

There are three prediction models that explain patterns of colonization in islands with montane and lowland biomes, including: (1) a montane clade nested within a lowland clade on the same island is predicted when a single colonization event of the lowland biome is followed by a cladogenetic colonization by a new population of the montane biome, (2) a lowland clade nested within a montane clade within an island is predicted when a single highland colonization event is followed by lowland colonization by a new population, and (3) a separate montane and lowland clade is predicted during multiple parallel colonization events. During the latter, the lowland taxa between adjacent islands should form a monophyletic group while the montane taxa form a parallel clade. The first and second models suggest a cladogenetic speciation event within an island. The third model suggests independent parallel altitudinal colonization events. Cases supporting all three hypotheses have been reported in New World avian taxa (Rice et al. 1999).

Our study supported the third model. All montane *Otus* taxa between islands comprised one clade and the lowland *Otus* in other islands formed another parallel clade. This split within the *Otus* clade may extend deeper within the strigiform phylogeny; the extent of which can be revealed by more extensive taxon sampling and phylogenetic analysis.

The *O. megalotis* intraspecific genetic distances was relatively large with p-distance between *O. m. everetti* and *O. m. megalotis* at 3.8%, *O. m. nigrorum* and *O. m. everetti* at 3.6%, and *O. m. nigrorum* and *O. m. megalotis* at 4.2% (Table 3). These values were much higher than the p-distances between the three non-Philippine species with *O. bakkamoena* and *O. lempiji* at 0.03%, between *O. bakkamoena* and *O. lettia* at 1.0%, and between *O. lempiji* and *O. lettia* at 1.2%. The p-distance values observed among the Philippine *O. megalotis* subspecies were also comparatively higher than those observed between subspecies of other *Otus* species elsewhere (Proudfoot et al. 2007). Genetic distances are approximations of differentiation (Meier et al. 2006) and may not necessarily be diagnostic of species limits (Winker 2009, 2010). However, the distances we observed are consistent and comparable with species-level differentiation among birds (Kerr et al. 2007). These distances are congruent with the discrete morphological differences among the *Otus* taxa that we documented in this study.

Estimating divergence dates within a clade based on either island emergence chronology (Cooper and Penny 1997, Steppan et al. 2003, Weir and Schluter 2004) or sequence divergence values (Lovette 2005) are promising but with caveats (Garcia-Moreno 2004). A recent analysis supported the 2% per million years constant of the mitochondrial molecular clock for most avian lineages (Weir and Schluter 2008), and we applied this generality with caution. Our well-supported phylogeny showed the lowland invasion of the Philippine Archipelago by the lineage that led to *M. gurneyi* started in Greater Mindanao. Sequence divergence between *M. gurneyi* and non-Philippine lowland scops owls (*O. spilocephalus/O. lettia/O. lempiji/O. bakkamoena*) was calculated at 11 ± 0.06%. Assuming a 2% sequence divergence per million years (at least for the cyt-*b* gene), a divergence date of 5.5 mya coincided with the most recent date of emergence of Mindanao Island (estimated at 8 to 6 mya). Extensive volcanism followed island emergence from the sea floor, and it is likely that colonization by ancestral *M. gurneyi* occured much later, rather than earlier. The second calibration was the divergence of *O. m. nigrorum* from the ancestral *M. gurneyi*. Sequence divergence of 5.6% suggests a colonization date of the Greater Panay-Negros Island block at 2.8 mya. The Panay-Negros block emerged from the sea floor *de novo* about 2 mya (Steppan et al. 2003); the discrepancy of 0.8 mya can be explained either by (1) later estimates for the emergence of the Panay Negros block, or (2) overestimation of rates due to genetic drift, bottlenecks, and founder effects (Carson and Templeton 1984, Thorpe et al. 1994) as the Greater Panay-Negros block experienced fragmentation during the last Pleistocene ice age, 100,000 to 10,000 years ago (Heaney 1986).

We present evidence that parallel multiple colonization in two elevational zones shaped the pattern of the genus *Otus* community within the Philippine Archipelago. It is possible that Mindanao Island was colonized three times; once along the montane route (*O. mirus*), and possibly twice via the lowland route. *O. megalotis nigrorum* from the Visayas is basal to the much larger Luzon and Mindanao islands. The question for *Mimizuku* remains whether it represents a lineage from a third colonization event, or speciated *de novo* within the ancestral *megalotis*.

Taxonomic Changes.—We reviewed the literature to find the rationale for keeping the three *O. megalotis* island populations within one species (Marshall 1978, Amadon and Bull 1988). However, previous phenotypic analysis was lacking and no defined character analysis, based on either morphology or vocalizations, was conducted. Our analyses based on genetic (molecular) and morphological approaches, suggest the three *megalotis* subspecies represent evolutionarily distinct taxa under the phylogenetic species concept (PSC). We strongly suggest recognition of species status for the three *megalotis* subspecies; Luzon Lowland Scops Owl (*O. megalotis*), Mindanao Scops Owl (*O. everetti*), and Visayan Scops Owl (*O. nigrorum*). The relatively large size of the Giant Scops Owl represents an autapomorphy but its phylogenetic position as a terminal lineage does not warrant genus status. We suggest the Giant Scops Owl be designated as *Otus gurneyi*.

ACKNOWLEDGMENTS

We thank Glenn Storrs, Herm Mays (CMC), and Ron DeBry (University of Cincinnati) for tissue loans, and Jean

Woods (DMNH), and Herman Mays and Lauren Hancock (CMC) for loaning specimens from their respective institutions for examination. We thank Dries Van Nieuwenhuyse and D. H. Johnson (Global Owl Project) for help in creating Fig. 3. We thank Tim McSweeny (HMNS) for help in working with specimens and D. H. Johnson and A. E. Sieradzki (Global Owl Project), and Travis Rosenberry (The Peregrine Fund) for helping obtain obscure published descriptions of the type specimens. DMB dedicates his contribution of this manuscript to Keith Arnold, who mentored him in owl taxonomy during the early years of his graduate work. HCM dedicates his contribution to the late Pedro Alviola III, a colleague and a mentor at the University of the Philippines at Los Baños (UPLB). This work was funded by the Texas Southern University (TSU) Seed Grant Program, TSU Graduate School, and the TSU University Research Center (URC)-NASA Center for Bionanotechnology and Environmental Research (CBER)-NASA Cooperative Agreement NNX0BA47A. This manuscript was significantly improved through the comments made by G. A. Proudfoot and C. E. Braun.

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