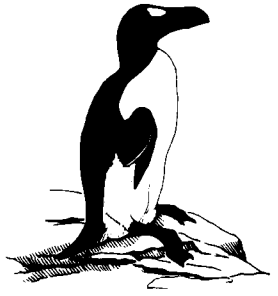

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# PHYLOGENETIC PLACEMENT OF *MIMIZUKU GURNEYI* (AVES: STRIGIDAE) INFERRED FROM MITOCHONDRIAL DNA

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ABSTRACT.—The nucleotide sequences of mitochondrial 12S rRNA (12S) and protein-coding cytochrome oxidase subunit I (COI) spanning 1,010 and 1,130 base pairs, respectively, are reported for seven owl species representing six genera: *Otus megalotis* (Philippine Scops-Owl), *O. longicornis* (Luzon Scops-Owl), *Bubo virginianus* (Great Horned Owl), *Asio flammeus* (Short-eared Owl), *Ninox philippensis* (Philippine Hawk-Owl), *Tyto alba* (Barn Owl; used as an outgroup), and the little known *Mimizuku gurneyi* (Giant Scops-Owl). Separate phylogenetic analyses of 12S, COI, and the combined 12S and COI data yielded congruent phylogenies, strongly supporting the phylogenetic affinity of *Mimizuku* with *Otus*, as opposed to *Bubo*. The conservative nature of mitochondrial DNA evolution in *Mimizuku*, in sharp contrast to its unique derived morphological attributes, suggests that *Mimizuku* is a recently evolved insular form of *Otus* that has undergone rapid morphological evolution towards gigantism, rather than being a small derivative of the much larger eagle-owls (*Bubo*) as has been suggested previously. *Received 19 August 1996, accepted 5 March 1997.*

THE GIANT SCOPS-OWL (*Mimizuku gurneyi*) is a monotypic species restricted to the Philippine islands of Mindanao, Dinagat, and Siargao. The evolutionary origin and phylogenetic placement of this species are unclear. *Mimizuku gurneyi* was first described as *Pseudoptynx gurneyi* by Tweeddale (1878), congeneric with *P. philippensis* (now *Bubo philippensis*). Hachisuka (1934) removed *gurneyi* from *Pseudoptynx* and placed it in the monotypic genus *Mimizuku*. Many authors agree and recognize *M. gurneyi* as the sole member of this distinct genus (Peters 1940, duPont 1971, Clark et al. 1978, Eck and Busse 1973, Marshall 1978, Dickinson et al. 1991), whereas others treat *Mimizuku* as a synonym of *Otus* (Delacour and Mayr 1946, Grossman and Hamlet 1964, Burton 1973, Clements 1974, Gruson 1976). Amadon and Bull (1988: 304) state that *Mimizuku* "... is sometimes placed in *Otus*, but may be closer to *Bubo* ..."

Its English name is no less controversial. *Mimizuku gurneyi* is widely known as the Giant Scops-Owl (Delacour and Mayr 1946, Burton 1973, Clark et al. 1978, Amadon and Bull 1988, Sibley and Monroe 1990), but more recently it has been referred to as the Lesser Eagle-Owl (Dickinson et al. 1991, Sibley and Monroe 1993) and the Mindanao Eagle-Owl (Inskipp et al. 1996). The uncertainty regarding the phyloge-

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FRONTISPIECE. Adult Giant Scops-Owl (*Mimizuku gurneyi*), an uncommon resident of low and middle elevation forests in the Philippines. Painting by Hector C. Miranda, Jr.

netic placement of *Mimizuku* reflects insufficient materials for comparative study and the lack of knowledge about its biology. During recent surveys of Mindanao conducted by the National Museum of the Philippines (NMP) and the Cincinnati Museum of Natural History and Science (CMNH), we obtained frozen tissue and a complete skeleton that have clarified whether this unique insular species represents a small eagle-owl or a large scops-owl. Resolution of this issue provides clues to the history and processes underlying avian species diversification in the Philippines and other archipelagos.

## METHODS

We obtained DNA sequence characters for the entire mitochondrial (mt) 12S rRNA gene (977 base pairs [bp] long in *M. gurneyi*; 1,010 bp long in the alignment, including gaps) from two individuals of *M. gurneyi* and single representatives of *Otus megalotis, O. longicornis, Bubo virginianus, Asio flammeus, Ninox philippensis,* and *Tyto alba,* the last being used for outgroup comparison. We also sequenced 1,130 bp of mitochondrial cytochrome oxidase subunit I (COI) from the same set of taxa except *O. longicornis.* All *Otus, Ninox,* and *Mimizuku* tissue samples were obtained during the 1992 to 1994 NMP/CMNH Philippine Biodiversity Inventory surveys. Voucher specimens for individuals analyzed are: *M. gurneyi* (CMNH Nos. 36488 and 35735), *O. longicornis* (CMNH No. 36491), *O. megalotis* (CMNH No. 36490), and *N. philippensis* (CMNH No. 36492). GenBank accession numbers for the sequences are: *O. m. everetti* (12S, U83754; COI, U83779), *O. longicornis* (12S, U84751), *M. gurneyi* (12S, U83756; COI, U83780), *B. virginianus* (12S, U83757; COI, U83777), *A. flammeus* (12S, U83758; COI, U83781), *N. philippensis* (12S, U83760; COI, U83783), and *T. alba* (12S, U83749; COI U91604).

DNA was extracted from muscle, liver, or heart tissue using standard techniques (Knight and Mindell 1993). Mitochondrial DNAs were amplified as a series of overlapping segments using the following primer pairs. For 12S rRNA, we used L613/H887 (Mindell et al. 1991), L821 (5-GCCACACCCCCAC GGGTAC TCAGCAGT-3')/H1194 (5'-TCGATTATA GAACAGGCTCCTCTAG-3'), L1091 (Kocher et al. 1989) /H1557 (Knight and Mindell 1994), and L1373 (5'-GAAATGGGCTACATTTTCT-3')/H1621 (5'-CTT [T/C][T/C]AGGTGTAAGCT[G/A][G/A]ATGCTT-3'). For COI, we used L5844 (5'-CCTCTGTAAAAA GGACTACAGCC-3')/H6290 (5'-TTGCCAGCTAGT GGGGGTA-3'), L6195 (5'-AATAACATAAGCTTC TGACT-3')/H6762 (5'-GATGTAAAGTAGGCTC GGGTGTCTAC-3'), and either L6424 (5'-ACCG CCATCAACATAAAACCCCC-3') or L6704 with H7216 (Mindell et al. 1991). Letters L and H refer to light and heavy strands, and numbers correspond to approximate starting positions in the human mtDNA sequence (Anderson et al. 1981). Bases for degenerate primer sites are shown in brackets. Double-stranded PCR amplifications were performed using *Taq* DNA polymerase and the reaction buffer (500mM KCl, 100 mM Tris-HCl, 1.0% Triton X-100) from Promega Corporation. Reaction mixtures were subjected to 35 cycles of denaturation at 94°C for 30 s, annealing from 40 to 50°C for 30 s, and extension at 72°C for 1 min. After amplification, 15 mL of the double-stranded PCR product were separated in low melting-point agarose, cut from the gel, and purified using Magic PCR Preps (Promega). Both the heavy and light strands of all amplified products were sequenced using the CircumVent Sequencing System (New England Biolabs) with direct incorporation of $^{35}$S-dATP.

Manual alignment of COI sequences is non-controversial, with no gaps being invoked. 12S rRNA alignments, however, require numerous insertions and deletions. Although phylogenetic analyses can be sensitive to order of sequence input for alignments with numerous insertions and deletions (Mindell 1991), we focused on well-aligned regions only, and these did not change significantly in alignment bouts with alternative input orders. CLUSTAL W (Thompson et al. 1994) was used to generate the alignment using the default parameters. Conserved motifs and other information regarding secondary structure (Hickson et al. 1996, Mindell et al. 1997) also were used to improve and adjust the alignment by eye. Percent difference between sequences was initially calculated using $p = z_d / z_t$ where $z_d$ is the number of nucleotide differences between two sequences and $z_t$ is the total number of nucleotides compared. Sequence differences corrected for substitution-rate differences were also calculated using Kimura's (1980) two-parameter method in the DNAdist program of PHYLIP (Felsenstein 1995). Sites with insertions and deletions were excluded from sequence-divergence estimates.

We conducted exhaustive parsimony analyses using PAUP 3.1.1 (Swofford 1993) for the COI and 12S rRNA data sets individually and combined. To explore the effect of differential weighting of transitions and transversions, we used the following character weighting schemes in alternative analyses: (1) all character changes equally weighted, (2) transversions (TVs) only, and (3) TVs:TIs (transitions) weighted 2:1. For the 12S rRNA alignments, we conducted separate analyses on all sites (1,010 aligned positions) and on well-aligned regions only (944 aligned positions), in which sequence regions with gaps were excluded to reduce homoplasy. We calculated support indices for each node within the most-parsimonious topology by finding the shortest

TABLE 1. Percent differences that are presumed transitions (above diagonal) and percent sequence divergences (below diagonal) for pairwise comparisons of mitochondrial 12SrRNA sequences based on observed sequence differences (left of slash) and Kimura's (1980) two-parameter model (right of slash).

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| 1 *Mimizuku gurneyi* | — | 71.0 | 71.2 | 65.2 | 61.7 | 58.5 | 61.4 |
| 2 *Otus megalotis* | 6.3/6.8 | — | 72.4 | 64.8 | 64.1 | 63.3 | 63.0 |
| 3 *Otus longicornis* | 11.2/11.1 | 8.0/8.7 | — | 68.6 | 66.5 | 62.3 | 59.5 |
| 4 *Bubo virginianus* | 16.4/16.1 | 14.1/17.1 | 16.1/20.0 | — | 62.5 | 66.7 | 58.8 |
| 5 *Asio flammeus* | 17.7/21.8 | 16.1/19.6 | 16.2/19.6 | 15.1/18.1 | — | 61.8 | 59.9 |
| 6 *Ninox philippensis* | 16.6/20.0 | 15.5/18.3 | 16.0/19.2 | 16.8/20.1 | 16.8/20.2 | — | 58.3 |
| 7 *Tyto alba* | 17.3/20.7 | 16.6/19.8 | 15.5/18.5 | 16.9/19.9 | 16.9/21.7 | 16.5/19.5 | — |

tree in which the particular node is not found, and denoting on branches the number of additional steps required (Bremer 1988, Kallersjö et al. 1992). In addition, bootstrap resampling (Felsenstein 1985) was done with 2,000 replications using the branch-and-bound search option.

## RESULTS

*12S RNA.*—Of the 1,010 bp examined, 55 gaps were invoked. Within the regions without gaps, 325 positions were variable and 233 of those positions were phylogenetically informative (23%). Presumed transitions for pairs of species ranged from 58.3 to 72.4% of all changes with an average of 63.9% across all comparisons (Table 1). Percent transitional differences were based on observed values. These relatively high percentages suggested that the 12S rRNA transition substitutions retained phylogenetic information, and that they were not yet saturated with change (Mindell and Honeycutt 1990, Mindell et al. 1991). Excluding sites with gaps (where alignments may be unreliable), the mean observed pairwise percent divergence across all comparisons was 15.0%, ranging from 6.3 to 17.7%. Sequence divergence between *O. megalotis* and *M. gurneyi* was 6.3%, lower than between the two *Otus* species at 8.0%. Percent sequence divergences between *M. gurneyi* and the two *Otus* species were less than that found between *Mimizuku* and *B. virginianus* at 16.4%. Transformed divergence measures using the Kimura 2-parameter model increased the average by 3.2%, but distances between *M. gurneyi* and *O. megalotis, O. longicornis,* and *B. virginianus* at 6.8%, 11.1%, and 16.1%, respectively, did not change drastically (see Table 1). As a precaution against mislabeling or contamination of samples, 500 bp were sequenced from a second *Mimizuku* individual, and no difference was found between the two individuals.

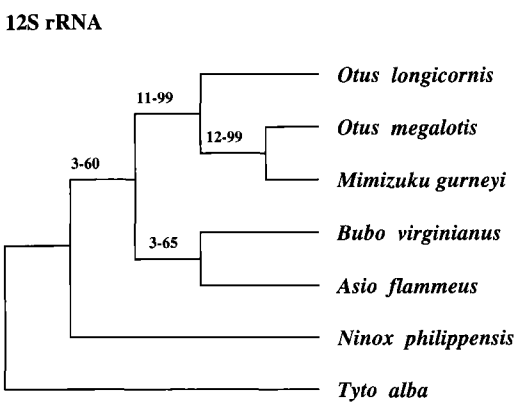


FIG. 1. The most-parsimonious phylogenetic tree demonstrating placement of *Mimizuku gurneyi,* based on all alternative analyses of the mitochondrial 12S rRNA. An analysis using transversions only yielded the same topology as above, except *Asio flammeus* was basal, rather than sister, to *Bubo virginianus. Tyto alba* was designated as the outgroup. The first number above the branch is the support index (Bremmer 1988, Kallersjö et al. 1992) denoting the number of additional steps needed to break the node, and the second number denotes bootstrap support based on 2,000 replications. These numerical calculations are based on 12S rRNA well-aligned regions only.

Using *Tyto alba* as an outgroup, the parsimony analyses were unequivocal in supporting an *Otus/Mimizuku* sister relationship (Fig. 1). Parsimony analyses of the 12S rRNA data set based on alternative character weighting approaches consistently yielded the same sister relationship. All of the most-parsimonious trees were identical, except for the transversion-only analysis for well-aligned regions in which *A. flammeus* is basal rather than sister to

TABLE 2. Mitochondrial COI percent transitional differences (above diagonal) based on first, second, and third codon positions (left of slash) and first and second codon positions only (right of slash). Values below diagonal are percent divergences for pairwise comparisons based on observed differences (left of slash) and Kimura's (1980) two-parameter model (right of slash).

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| 1 *Mimizuku gurneyi* | — | 87.1/63.9 | 93.6/77.8 | 91.1/72.2 | 86.1/59.1 | 91.6/72.3 |
| 2 *Otus megalotis* | 10.5/11.4 | — | 91.8/67.9 | 90.0/60.7 | 83.8/47.2 | 91.0/62.5 |
| 3 *Bubo virginianus* | 15.9/18.2 | 14.7/16.7 | — | 96.0/73.4 | 89.9/58.6 | 96.4/80.0 |
| 4 *Asio flammeus* | 15.4/17.6 | 16.1/18.7 | 13.3/14.8 | — | 89.2/62.5 | 95.8/76.5 |
| 5 *Ninox philippensis* | 17.2/20.0 | 17.1/20.1 | 16.3/18.4 | 15.6/17.9 | — | 89.5/57.7 |
| 6 *Tyto alba* | 18.3/21.6 | 18.3/21.8 | 16.4/19.2 | 16.6/19.2 | 19.1/23.0 | — |

*B. virginianus*. All trees that were generated, based on all regions or well-aligned regions only, showed *O. longicornis* basal to the *O. megalotis/M. gurneyi* clade. The support index showed an additional 12 steps required to break the *O. megalotis/M. gurneyi* clade and 11 additional steps to break the entire *O. longicornis/O. megalotis/Mimizuku* clade. Bootstrap values for all branches were greater than 50%, with the *O. megalotis* and *M. gurneyi* clade showing a 99% bootstrap value.

*Cytochrome oxidase I*.—COI showed 325 variable sites among five ingroup taxa. There were no indels (insertions/deletions) observed. Forty-five changes (13.9%) were at the first codon positions, 28 (8.6%) were at the second codon positions, and 252 (77.4%) were at third codon positions, a ratio of roughly 1.5:1:9. Although the substitution rate difference between the first two codons and the third codon positions is relatively high, the observed patterns are consistent with studies of other protein-coding mitochondrial genes in birds (Kocher et al. 1989, Edwards and Wilson 1990, Lanyon and Hall 1994). High percent transition values for pairwise estimates were observed, ranging from 83.8% between *O. megalotis* and *N. philippensis* to 96.4% between *T. alba* and *B. virginianus* (Table 2). This was due mainly to the rapid nucleotide change at the third codon position. Substitution bias at the third codon position has been shown to contribute to phylogenetic misinformation due to saturation (Mindell and Thacker 1996). Percent transitional changes for both first and second codon positions were lower, ranging from 47.2% (*Ninox* and *Otus*) to 80.0% (*Ninox* and *Bubo*). Overall percent sequence divergence based on the Kimura two-parameter model (Table 2) between strigiform species ranged from 11.4% (*Otus* and *Mimizuku*) to 23.0% (*Tyto* and *Ninox*). Sequence divergence between *Mimizuku* and *Bubo* was 18.2%, a value higher than that between *Otus* and *Mimizuku* or *Bubo* and *Asio*. The COI gene also showed bias in base composition, with guanine deficiency in the second and third codon positions, and relatively low thymine frequency at the third codon position, as observed in other mt protein-coding genes (Lanyon and Hall 1994). The mean COI percent base composition for the seven owl species was A: 25.4, C: 30.8, G: 16.6, and T: 24.7.

Analyses with equal weighting for all character changes, and with TVs:TIs weighted 2:1 (for first, second, and third codon positions combined) yielded a tree incongruent with the 12S topology in which *Bubo* is basal to a *Ninox/Asio* clade. Transversion parsimony for all codon positions differed, with *Asio* basal to a *Bubo/Ninox* clade. However, all topologies generated under various weighting approaches based on the first, second, and third codon positions supported the *Otus/Mimizuku* sister relationship. To reduce the amount of homoplasy due to multiple substitutions, third codon positions were given an *a priori* weighting of zero. Exhaustive parsimony analysis of all characters in the first and second codon positions resulted in a single most-parsimonious tree that united *Mimizuku* with *Otus* (Fig. 2A). Parsimony analyses also were conducted using: (1) only the first codon position, (2) only the second codon position, and (3) transversions only at the first and second codon positions. Each of these supported the sister relationship of *Otus* and *Mimizuku* relative to *Bubo*. Transversion parsimony of codon position 1 and 2 only resulted in two shortest trees, both supporting the *Otus/Mimizuku* clade, with *Bubo* basal to this branch. A consensus tree (Fig. 2A) yielded an unresolved *Bubo/Asio/Ninox* branch. Bremer and bootstrap support indices for the two resolved

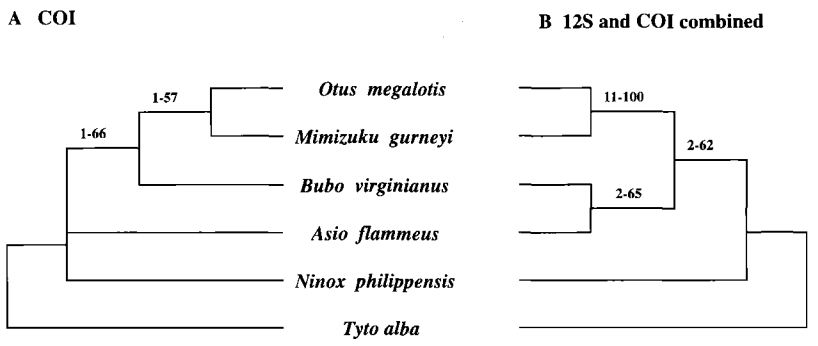


FIG. 2. (A) COI consensus of two shortest trees resulting from transversion parsimony analysis of all first and second codon position characters. (B) Single most-parsimonious tree found under all weighting schemes for 12S and COI combined, with and without exclusion of ambiguous alignment regions in 12S and the saturated third codon position in COI. The first numbers above branches represents the support index (Bremer 1988, Kallersjö et al. 1992), and the second number denotes bootstrap support with 2,000 replications.

branches were low (Fig. 2A), due in part to the small number of changes at the first and second codon positions.

*12S rRNA and COI combined.*—The combined data set comprised 2,140 nucleotide bases. Parsimony and transversion analyses of all data and all data excluding ambiguous 12S alignment regions yield a single most-parsimonious tree (Fig. 2B) that was entirely congruent with the 12S gene tree (Fig. 1). This topology is robust as indicated by the lack of sensitivity to alternative weighting approaches, and inclusion or exclusion of ambiguous alignment regions in 12S and the third codon position in COI.

*Skull morphology.*—The recent availability of a *M. gurneyi* skeleton enabled gross comparison of its skull with those of *O. megalotis* and *B. virginianus*. We evaluated the same characters used by Ford (1967): (1) medio-lateral slope of cranial side, (2) forehead size and shape, (3) cranial width relative to height, and (4) supraorbital process shape. The *O. megalotis* skull exhibits a high and steep forehead, the supraorbital process is long and pointed, and the cranium is high relative to width (Fig. 3). The *Mimizuku* skull also exhibits these features (Fig. 3). In contrast to *Otus* and *Mimizuku*, the *Bubo* skull has a low, thicker forehead and a small, knob-like supraorbital process, and the cranial height is low relative to the width (Fig. 3). The structural similarities of the major skull features in *Otus* and *Mimizuku* support the molecular evidence presented here, although a detailed phylogenetic analysis of skeletal characters is warranted.

## DISCUSSION

We present evidence that *Mimizuku* is more closely related to *Otus* than to *Bubo*, assuming the monophyly of *Bubo*. All analytical approaches for the 12S rRNA (i.e. all gene regions and the well-aligned regions only, all character changes weighted equally, transversions only, or transversions:transitions weighted 2:1) support the single shortest tree presented in Figure 1 with *Mimizuku* sister to *Otus*. This also is concordant with the COI analyses, the combined data set analyses, and the gross comparisons of skull morphology (Fig. 3). Combining data from different regions of the mitochondrial genome maximizes the amount of character evidence in parsimony analyses (Cracraft and Mindell 1989, Kluge 1989, deQueiroz et al. 1995). The apparent consistency of morphological and molecular evidence increases our confidence in the mitochondrial DNA analyses.

Our evidence indicates that *Mimizuku gurneyi* is a large scops-owl that is intermediate in size between scops-owls and eagle-owls. Accordingly, this species should be known by the English name "Giant Scops-Owl." The plumage pattern of the breast and abdomen differs from that of most scops-owls in being less cryptic, with plain black streaks along the shafts of the ventral feathers (see Frontispiece). Field observations by Miranda and Kennedy suggest that *Mimizuku*'s call (a loud, single-note "whaaack") is not typical of scops-owl vocalizations. Despite these derived characters, the mitochondrial 12S sequence divergence of

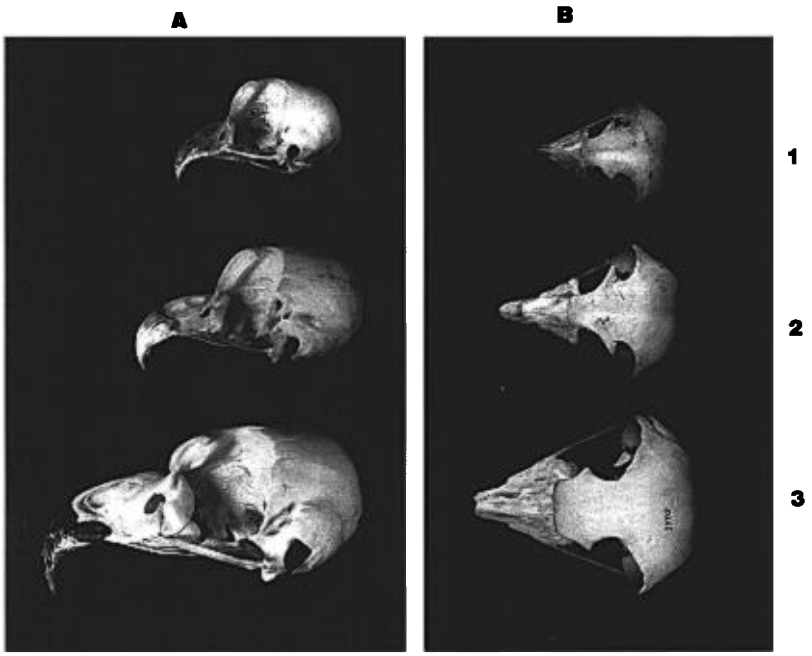


FIG. 3. Lateral (A) and dorsal (B) view of skulls of (1) *Otus megalotis* (CMNH No. 36489), (2) *Mimizuku gurneyi* (CMNH No. 35735), and (3) *Bubo virginianus* (CMNH No. 34913). Dorsal view is shown 20% smaller than lateral view.

*M. gurneyi* from *O. megalotis* is relatively low (6.8%) and is lower than the observed sequence-divergence estimates between *O. megalotis* and *O. longicornis* (8.7%). The derived phenotypic features of *Mimizuku*, in contrast to its conservative mitochondrial DNA evolution, suggest the occurrence of rapid differentiation. Discordant rates of morphological and molecular evolution also have been documented in other avian species (Shields and Wilson 1987; Avise and Zink 1988; Zink et al. 1991, 1995; Zink and Dittmann 1993) and other vertebrate lineages (King and Wilson 1975, Baverstock and Adams 1987, Schwaner and Sarre 1988, Radtkey 1996). This notion is supported by field investigations showing that speciation and evolutionary changes in morphology in birds can be rapid (e.g. Grant 1992).

*Mimizuku gurneyi* is an uncommon resident of low and middle elevation forests. On Mindanao, its habitat overlaps with that of the more widespread *Otus megalotis*, which occurs in forested areas and open woodlands. It is possible to devise a scenario wherein *Mimizuku* represent a lineage of colonizers that underwent rapid morphological changes towards gigantism. The alternative scenario, that *Mimizuku* is more closely related to the larger eagle-owls, is that *Mimizuku* evolved smaller body size, perhaps in response to competition with the Philippine Eagle-Owl (*B. philippensis*). Our data and phylogenetic analyses support the first scenario (toward gigantism) over the latter (toward size reduction). There is an active debate as to the ecological forces that drive shifts in body size among insular reptiles (Miles and Dunham 1996, Schoener 1970, Williams 1972, Roughgarden 1992). In insular forms, random genetic drift that accompanies a founder event can play a subtle but major role in rapid speciation.

Our study raises the issue of which criteria are appropriate and reasonable for delimiting genera. In the past, it was common to place a species in a separate genus if it exhibited a greater degree of morphological divergence than that observed among other related species. But, should a separate genus be recognized solely on the basis of apparent phenotypic uniqueness, regardless of how recently the taxon has evolved in evolutionary time? Or, should genera reflect phylogeny and relative age regardless of degree of morphological differentiation? Taxonomists will encounter these

questions with increasing frequency as greater emphasis is placed on phylogenetic perspectives and on historical contexts of biodiversity.

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LITERATURE CITED

AMADON, D., AND J. BULL. 1988. Hawks and owls of the world: A distributional and taxonomic list (with the genus *Otus* by J. T. Marshall and B. F. King). Proceedings of the Western Foundation of Vertebrate Zoology 3:295–357.

ANDERSON, S., A. T. BANKIER, B. G. BARRELL, M. H. L. BRUIJN, A. R. COULSON, J. DROUIN, I. C. EPERON, D. P. NIERLICH, B. A. ROE, F. SANGER, P. H. SCHREIRER, A. J. H. SMITH, R. STADEN, AND I. G. YOUNG. 1981. Sequence and organization of the human mitochondrial genome. Nature 290:457–465.

AVISE, J. C., AND R. M. ZINK. 1988. Molecular genetic divergence between avian sibling species: King and Clapper rails, Long-billed and Short-billed dowitchers, Boat-tailed and Great-tailed grackles, and Tufted and Black-crested titmice. Auk 105:516–528.

BAVERSTOCK, P. R., AND M. ADAMS. 1987. Comparative rates of molecular, chromosomal and morphological evolution in some Australian vertebrates. Pages 175–188 *in* Rates of evolution (K. S. W. Campbell and M. F. Day, Eds.). Allen and Unwin, London.

BREMER, K. 1988. The limits of amino-acid sequence data in angiosperm phylogenetic reconstruction. Evolution 42:795–803.

BURTON, J. A. (Ed.). 1973. Owls of the world. E. P. Dutton and Company, New York.

CLARK, R. J., D. G. SMITH, AND L. H. KELSO. 1978. Working bibliography of owls of the world. National Wildlife Federation Scientific and Technical Series No. 1, Washington, D.C.

CLEMENTS, J. F. 1974. Birds of the world: A checklist. Two Continents, New York.

CRACRAFT, J., AND D. P. MINDELL. 1989. The early history of modern birds: A comparison of molecular and morphological evidence. Pages 389–403 *in* The hierarchy of life (B. Fernholm, K. Bremer, and H. Jörnvall, Eds.). Elsevier Science Publishers, Amsterdam.

DELACOUR, J., AND E. MAYR. 1946. Birds of the Philippines. Macmillan, New York.

DEQUEIROZ, A., M. J. DONOGHUE, AND J. KIM. 1995. Separate versus combined analysis of phylogenetic evidence. Annual Review of Ecology and Systematics 26:657–681.

DICKINSON, E. C., R. S. KENNEDY, AND K. C. PARKES. 1991. The birds of the Philippines: An annotated check-list. British Ornithologists' Union, Checklist No. 12.

DUPONT, J. E. 1971. Philippine birds. Delaware Museum of Natural History, Greenville.

ECK, S., AND H. BUSSE. 1973. Eulen, die resenten und fossilen Formen Aves, Strigidae. Die Neue Brehm-Bucherei. A. Ziemsen, Wittenberg Lutherstadts, Germany.

EDWARDS, S. V., AND A. C. WILSON. 1990. Phylogenetically informative length polymorphism and sequence variability in mitochondrial DNA of Australian songbirds (*Pomatostomus*). Genetics 126:695–711.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783–791.

FELSENSTEIN, J. 1995. PHYLIP (Phylogeny Inference Package), version 3.57c. Department of Genetics, University of Washington, Seattle.

FORD, N. 1967. A systematic study of the owls based on comparative osteology. Ph.D. dissertation, University of Michigan, Ann Arbor.

GRANT, P. R. 1992. Hybridization of bird species. Science 256:193–197.

GROSSMAN, M. L., AND J. HAMLET. 1964. Birds of prey of the world. Bonanza Books, New York.

GRUSON, G. S. 1976. Checklist of the world's birds. Quadrangle, New York.

HACHISUKA, M. 1934. Birds of the Philippine Islands, vol.2. H. F. & G. Witherby, London.

HICKSON, R. E., C. SIMON, A. COOPER, G. S. SPICER, J. SULLIMAN, AND D. PENNY. 1996. Conserved sequence motifs, alignment and secondary struc-

ture for the third domain of animal 12S rRNA. Molecular Biology and Evolution 13:150–169.

INSKIPP, T., N. LINDSEY, AND W. DUCKWORTH. 1996. An annotated checklist of the birds of the Oriental region. Chandlers Printer Limited, East Sussex, United Kingdom.

KALLERSJÖ, M., J. S. FARRIS, A. G. KLUGE, AND C. BULT. 1992. Skewness and permutations. Cladistics 8:275–287.

KIMURA, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16:111–120.

KING, M. C., AND A. C. WILSON. 1975. Evolution at two levels in humans and chimpanzees. Science 188:107–116.

KLUGE, A.G. 1989. A concern for evidence and a phylogenetic hypothesis for relationships among *Epicrates* (Boidae, Serpentes). Systematic Zoology 38: 7–25.

KNIGHT, A., AND D. P. MINDELL. 1993. Substitution bias, weighting of DNA sequence evolution, and the phylogenetic position of Fea's viper. Systematic Biology 42:18–31.

KNIGHT, A., AND D. P. MINDELL. 1994. On the phylogenetic relationship of Colubrinae, Elapidae, and Viperidae and the evolution of front-fanged venom systems in snakes. Copeia 1994:1–9.

KOCHER, T. D., W. K. THOMAS, A. MEYER, S. V. EDWARDS, S. PÄÄBO, F. X. VILLABLANCA, AND A. C. WILSON. 1989. Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. Proceedings of the National Academy of Sciences USA 86:6196–6200.

LANYON, S. C., AND J. G. HALL. 1994. Reexamination of barbet monophyly using mitochondrial-DNA sequence data. Auk 111:389–397.

MARSHALL, J. T., JR. 1978. Systematics of smaller Asian night birds based on voice. Ornithological Monographs No. 25.

MILES, D. B., AND A. E. DUNHAM. 1996. The paradox of the phylogeny: character displacement of analyses of body size in island *Anolis*. Evolution 50:594–603.

MINDELL, D. P. 1991. Aligning DNA sequences: Homology and phylogenetic weighting. Pages 73–89 *in* Phylogenetic analysis of DNA sequence (M. M. Miyamoto and J. Cracraft, Eds.). Oxford University Press, Oxford.

MINDELL, D. P., C. W. DICK, AND R. J. BAKER. 1991. Phylogenetic relationships among megabats, microbats, and primates. Proceedings of the National Academy of Sciences USA 88:10322–10326.

MINDELL, D. P., AND R. L. HONEYCUTT. 1990. Ribosomal RNA in vertebrates: Evolution and phylogenetic applications. Annual Review of Ecology and Systematics 21:541–566.

MINDELL, D. P., M. D. SORENSON, C. J. HUDDLESTON, H. C. MIRANDA, JR., A. KNIGHT, S. J. SAWCHUK, AND T. YURI. 1997. Phylogenetic relationships among and within select avian orders based on mitochondrial DNA. Pages 211–245 *in* Avian molecular evolution and systematics (D. P. Mindell, Ed.). Academic Press, New York.

MINDELL, D. P., AND C. E. THACKER. 1996. Rates of molecular evolution: Phylogenetic issues and applications. Annual Review of Ecology and Systematics 27:279–303.

PETERS, J. L. 1940. Checklist of birds of the world, vol. 4. Harvard University Press, Cambridge, Massachusetts.

RADTKEY, R. R. 1996. Adaptive radiation of day-geckos (*Phelsuma*) in the Seychelles archipelago: A phylogenetic analysis. Evolution 50:604–623.

ROUGHGARDEN, J. 1992. Comments on the paper by Losos: Character displacement versus taxon loop. Copeia 1992:288–295.

SCHOENER, T. W. 1970. Size patterns in West Indian Anolis lizard. II. Correlations with the sizes of particular sympatric species-displacement and convergence. American Naturalist 104:155–174.

SCHWANER, T. D., AND S. D. SARRE. 1988. Body size of tiger snakes in Southern Australia, with particular reference to *Notechisater serventyi*. Journal of Herpetology 22:24–33.

SHIELDS, G. F., AND A. C. WILSON. 1987. Subspecies of the Canada Goose (*Branta canadensis*) have distinct mitochondrial DNA's. Evolution 4:662–666.

SIBLEY, C. G., AND B. L. MONROE, JR. 1990. Distribution and taxonomy of birds of the world. Yale University Press, New Haven, Connecticut.

SIBLEY, C. G., AND B. L. MONROE, JR. 1993. A supplement to distribution and taxonomy of birds of the world. Yale University Press, New Haven, Connecticut.

SWOFFORD, D. L. 1993. PAUP. Phylogenetic analysis using parsimony, version 3.1.1. Illinois Natural History Survey, Champaign.

THOMPSON, J. D., D. G. HIGGINS, AND T. J. GIBSON. 1994. Clustal W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22:4673–4680.

TWEEDDALE, A. H. 1878. Contributions to the ornithology of the Philippines, no. XI. On the collections made by Mr. A. H. Everett at Zamboanga, in the island of Mindanao. Proceedings of the Zoological Society of London 1878:936–954.

WILLIAMS, E. E. 1972. The origin of faunas. Evolution of lizard congeners in a complex island fauna: A trial analysis. Evolutionary Biology 6:47–88.

ZINK, R. M., AND D. L. DITTMANN. 1993. Gene flow, refugia, and evolution of geographic variation in

the Song Sparrow (*Melospiza melodia*). Evolution 47:717–729.

ZINK, R. M., D. L. DITTMANN, AND W. L. ROOTES. 1991. Mitochondrial DNA variation and the phylogeny of *Zonotrichia*. Auk 108:578–584.

ZINK, R. M., S. ROHWER, A. V. ANDREEV, AND D. L. DITTMANN. 1995. Trans-Beringia comparisons of mitochondrial DNA differentiation in birds. Condor 97:639–649.

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