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Source: The Auk, 130(1):118-131. 2013.
Published By: The American Ornithologists' Union
URL: http://www.bioone.org/doi/full/10.1525/auk.2012.12102

The Auk 130(1):118–131, 2013

Printed in USA.

PHYLOGEOGRAPHY OF THE VARIABLE DWARF-KINGFISHER *CEYX LEPIDUS* (AVES: ALCEDINIDAE) INFERRED FROM MITOCHONDRIAL AND NUCLEAR DNA SEQUENCES

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Abstract.—We reconstructed the phylogeographic relationships of the Variable Dwarf-Kingfisher (*Ceyx lepidus*) using DNA sequence data. Maximum likelihood and Bayesian analysis methods were used to reconstruct trees from a multilocus data set of all 15 named subspecies of the *Ceyx lepidus* species complex. The concatenated data-set length was 2,471 base pairs and included two mitochondrial genes and two noncoding nuclear introns. Support for the monophyly of *C. lepidus* was equivocal. We instead found support for a clade including all *C. lepidus* subspecies plus two endemic Philippine taxa: *C. argentatus* and *C. cyanopectus*. Relationships among subspecific taxa were not well resolved, and many nodes were collapsed into polytomies suggesting a rapid and widespread colonization. *In situ* diversification likely played a role in generating current diversity within four archipelagos: the Philippines, Malukus, Bismarcks, and Solomons. Some biogeographic patterns recovered for the Solomon Islands taxa match those seen in other bird species, such as the close relationship of taxa on Bougainville, Choiseul, and Isabel. By contrast, the sister relationship between populations on Guadalcanal and the New Georgia Group is novel. We discuss species limits and make taxonomic recommendations to treat all 15 subspecies of *C. lepidus* as species. *Received 31 May 2012, accepted 5 November 2012.*

Key words: biogeography, kingfisher, Philippines, Solomon Islands, species limits, western Pacific islands.

Phylogéographie de *Ceyx lepidus* (Aves : Alcédinidés) inférée à partir de séquences d'adn mitochondrial et nucléaire

Résumé.—Nous avons reconstruit les relations phylogéographiques de *Ceyx lepidus* à l'aide de données de séquences d'ADN. Les méthodes du maximum de vraisemblance et d'inférence bayésienne ont été utilisées pour reconstruire les arbres à partir d'un ensemble de données multiloculaires des 15 sous-espèces du complexe d'espèces de *Ceyx lepidus*. La longueur concaténée de l'ensemble de données était de 2 471 paires de bases et incluait deux gènes mitochondriaux et deux introns nucléaires non codants. Les résultats appuyant la monophylie de *C. lepidus* étaient équivoques. Nous avons plutôt trouvé que les résultats suggéraient un clade comprenant toutes les sous-espèces de *C. lepidus* ainsi que deux taxons philippins endémiques, soit *C. argentatus* et *C. cyanopectus*. Les relations entre les taxons des sous-espèces n'ont pas bien été résolues et plusieurs nœuds ont été regroupés en polytomies, suggérant une colonisation rapide et étendue. La diversification *in situ* a vraisemblablement joué un rôle dans la génération de la diversité actuelle dans les quatre archipels : les Philippines, les Moluques, l'archipel Bismarck et les îles Salomon. Certains modèles biogéographiques pour les taxons des îles Salomon concordent avec ceux observés chez d'autres espèces d'oiseaux, tels que la relation étroite entre les taxons de Bougainville, Choiseul, et Isabel. En revanche, la relation fraternelle entre les populations de Guadalcanal et de Nouvelle-Géorgie est nouvelle. Nous discutons des limites spécifiques et faisons des recommandations taxonomiques pour traiter les 15 sous-espèces de *C. lepidus* comme des espèces.

The islands of Southeast Asia and the western Pacific are home to some of the most phenotypically diverse avian species complexes in the world. Birds such as the Island Thrush (*Turdus poliocephalus*), Golden Whistler (*Pachycephala pectoralis*), and Collared Kingfisher (*Todiramphus chloris*) are well known for their widespread geographic distributions and diverse phenotypes, each having >50 subspecies (Galbraith 1956, Woodall 2001, Peterson 2007). These hyperdiverse species have served as exemplars for ornithologists and biogeographers studying evolutionary processes that lead to geographic partitioning of biological diversity on islands (Mayr and Diamond 2001).

The Variable Dwarf-Kingfisher (*Ceyx lepidus*) is another widespread, phenotypically diverse species that has long puzzled ornithologists. *Ceyx lepidus* is a highly variable species with 15

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The Auk, Vol. 130, Number 1, pages 118–131. ISSN 0004-8038, electronic ISSN 1938-4254. DOI: 10.1525/auk.2012.12102

recognized subspecies (Fry et al. 1992, Woodall 2001, Clements et al. 2011). Indeed, within its more limited distribution, *C. lepidus* is nearly as diverse as the more widespread species complexes cited above (Clements et al. 2011). Each subspecies is defined by a distinctive phenotype based on variation in breast, mantle, and rump coloration and bill color and shape. Subspecies are distributed allopatrically on islands from the Philippines to the Solomon Islands, including the Maluku Archipelago, New Guinea, and the Bismarck Archipelago (Fig. 1). *Ceyx lepidus* is a biogeographic enigma; no other bird species shares its distribution. Indeed, no biogeographic term exists to circumscribe this region (Lomolino et al. 2010). Interestingly, among terrestrial vertebrates, this distribution is mirrored closely by frogs in the genus *Platymantis* (Allison 1996, Duellman 1999); however, *Platymantis* extends east to Fiji, whereas the Solomon Islands mark the eastern boundary of *C. lepidus*.

The general plumage pattern of *C. lepidus* is blue or black above and rufous below. The breast and belly generally are rufous with a paler throat; the crown, back, wings, rump, and tail are blue or black; and a rufous loral spot and pale post-auricular stripe are present. Mayr and Diamond (2001) considered close relationships among some subspecies based on these generalized plumage patterns; however, they considered *Ceyx lepidus dispar, C. l. meeki*, and *C. l. gentianus* to be phenotypically disparate enough to warrant status as so-called "megasubspecies." *Ceyx l. gentianus*, for example, is the only taxon with a fully white breast and *C. l. dispar* is the only one with sexually dichromatic plumage (Fry et al. 1992). Notable plumage patterns also occur in the polymorphic Philippine endemic subspecies, *C. l. margarethae*, which has sympatric pale- and dark-backed morphs similar to the polymorphism described in *C. erithacus* of mainland Southeast Asia and the Sunda Shelf (Lim et al. 2010). In addition to plumage variation, bill structure and coloration vary dramatically within *C. lepidus*. Bills are either red or black; red bills tend to be dorsoventrally compressed, and black bills tend to be laterally compressed. Two taxa, *C. l. nigromaxilla* and *C. l. sacerdotis*, have intermediate bill colors with black or dusky maxillae and orange mandibles (Fry et al. 1992, Woodall 2001). Indeed, the amount of variation expressed in *C. lepidus* bill morphology matches that seen across the entire clade to which it belongs: the pygmy-kingfishers (subfamily Alcedininae).

Few attempts have been made to elucidate the phylogenetic relationships of *C. lepidus* with other pygmy-kingfishers. Fry (1980) hypothesized a closer relationship with the Philippine endemic *C. argentatus* than with the sympatric *C. melanurus* based on plumage characters. More recently, studies using molecular

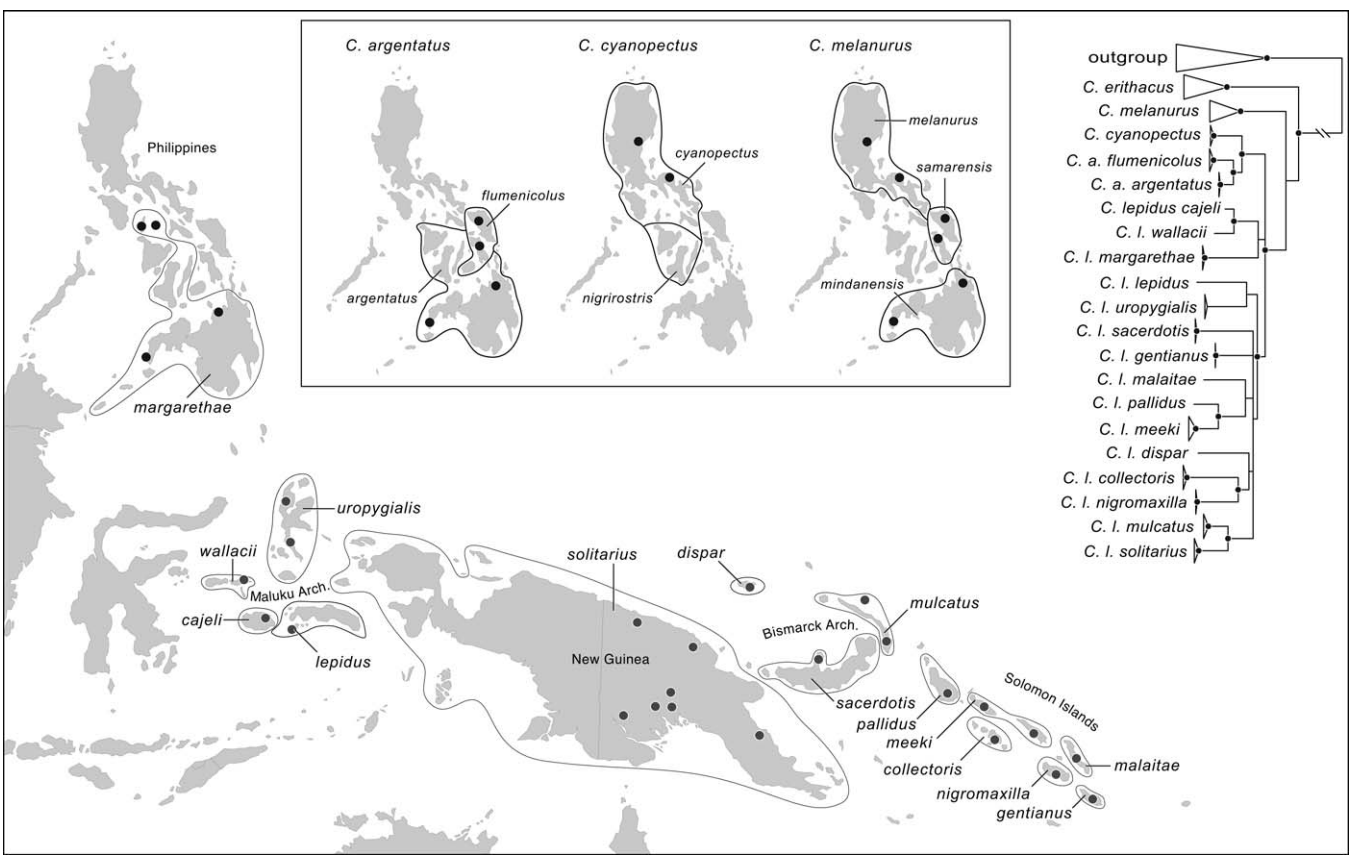

FIG. 1. Map showing the distribution of the 15 subspecies of *Ceyx lepidus* in the Philippines, Wallacea, New Guinea, and Melanesia. Inset panel depicts the distribution of closely related Philippine endemic species *C. argentatus*, *C. cyanopectus*, and *C. melanurus*. Black dots represent sampling localities used in this study; some dots represent more than one individual per locality. The reader is referred to Table 1 for the complete sampling list. The phylogeny is a cartoon of Figure 2 for topological reference. Black circles on nodes denote Bayesian posterior probability = 0.95 and maximum likelihood bootstrap support ≥70.

data recovered *C. lepidus* in a well-supported clade of three-toed pygmy-kingfishers in the genus *Ceyx* (Moyle 2006, Moyle et al. 2007) that included *C. cyanopectus*, *C. argentatus*, *C. melanurus*, and *C. erithacus*. Furthermore, Moyle et al. (2007) found evidence of a paraphyletic *C. lepidus*, but the extent of paraphyly was not known, owing to sampling deficiencies. Traditional taxonomy, based largely on plumage characters and operating within the confines of the biological species concept, has for a long time treated *C. lepidus* as one species with 15 diagnosable subspecies (Peters 1945, Clements et al. 2011). However, historically, 9 of the 15 taxa were described as species (all except *C. l. uropygialis*, *C. l. mulcatus*, *C. l. pallidus*, *C. l. collectoris*, *C. l. malaitae*, and *C. l. nigromaxilla*). Here, we reconstruct a molecular phylogeny of the *C. lepidus* species group and its closely related taxa in order to elucidate the evolutionary history and assess species limits of this group.

Methods

Taxon sampling.—Ingroup sampling included all 15 named taxa of *C. lepidus* (Clements et al. 2011) as well as representative subspecies of *C. erithacus*, *C. melanurus*, *C. cyanopectus*, and *C. argentatus* (2 of 5, 3 of 3, 1 of 2, and 2 of 2, respectively; Table 1). Outgroup sampling included all remaining taxa in the three-toed pygmy-kingfisher clade as circumscribed by Moyle et al. (2007) and *Alcedo websteri*, which was used to root trees. We sequenced 1–11 individuals per taxon, but, whenever possible, more than one sample per taxon was used to guard against errors of misidentification, mislabeling, or sample contamination.

DNA sequencing.—Total genomic DNA was extracted from frozen or alcohol-preserved muscle tissue using a Qiagen tissue extraction protocol (Qiagen, Valencia, California). All tissue samples have associated museum study-skin vouchers. For taxa with no available tissue samples, DNA was extracted from toepads of museum study skins (Table 1) in lab space separate from other *Ceyx* tissue extractions to minimize contamination risk (Mundy et al. 1997).

We sequenced the entire second and third subunits of mitochondrial nicotinamide adenine dinucleotide dehydrogenase (hereafter ND2 and ND3, respectively), the second intron of the nuclear myoglobin gene (hereafter Myo2), and the 11th intron of the nuclear glyceraldehyde-3-phosphate dehydrogenase gene (hereafter GAPDH). Target DNA fragments were amplified using polymerase chain reaction (PCR) with external and internal primers. ND2 and Myo2 primers are described by Moyle (2006) and Moyle et al. (2007). Additionally, we used internal primers 503L (Oliveros and Moyle 2010) and 562H1 (designed for this project; 5′-GATRATAATRGCYATTCAKCC-3′) to amplify ND2 and the internal primer KingMyo620R (5′-AGGTTGCAGAGCC TGGAAATATCTC-3′) to amplify Myo2 on some samples. The primer combinations L10755 and H11151 (Chesser 1999) and G3P13b and G3P14b (Fjeldså et al. 2003) were used to amplify ND3 and GAPDH, respectively.

The PCR amplifications were performed in 25-µL reactions using 5-PRIME HotMaster *Taq* DNA polymerase with a touchdown protocol for mtDNA and GAPDH (annealing temperature: 58, 54, and 50°C). We used an annealing temperature of 52°C for Myo2 following Kimball et al. (2009). Amplified PCR products were screened on high-melt, 2% agarose gels stained with ethidium bromide, and purified with 10% Exo-SAP-IT (GE Healthcare Bio-Sciences, Piscataway Township, New Jersey). We cycle-sequenced purified PCR products in both directions with the same primers used in PCR for 25 cycles, using the ABI Big Dye Terminator Cycle-Sequencing Kit, version 3.1 (Applied Biosystems, Foster City, California). Sequencing was performed on an ABI Prism 3730 high-throughput capillary electrophoresis DNA analyzer and aligned sequences by hand using SEQUENCHER, version 4.9 (GeneCodes, Ann Arbor, Michigan). Nuclear intron alignments were done by hand and checked against an automated alignment in MUSCLE (Edgar 2004).

Phylogenetic analysis and topology tests.—Phylogenetic reconstruction was performed both on the concatenated data and on each individual locus. Maximum likelihood (ML) tree searches were performed using GARLI, version 1.0 (Zwickl 2006), following the recommended default settings. We conducted 1,000 nonparametric bootstrap replicates (Felsenstein 1985) to assess clade credibility. We used SUMTREES, version 1.1.1—part of the DENDROPY, version 2.3.0, package (Sukumaran and Holder 2010)—to create bootstrap consensus trees and calculate bootstrap values. Models of DNA sequence evolution for all phylogenetic analyses were tested using Akaike's information criterion (AIC) employed in JMODELTEST, version 2.1.1 (Guindon and Gascuel 2003, Darriba et al. 2012).

Bayesian analysis (BA) was conducted using MRBAYES, version 3.2.1 (Ronquist and Huelsenbeck 2003, Altekar et al. 2004, Ronquist et al. 2012) implemented with BEAGLE (Ayres et al. 2012). The data were partitioned by codon position for mtDNA and by gene for the nuclear introns. Two independent MCMC runs of 20 million generations were conducted using default number of chains ($n = 4$) and heating conditions, sampling every 1,000 generations. TRACER, version 1.5 (Rambaut and Drummond 2007), and AWTY (Wilgenbusch et al. 2004, Nylander et al. 2008) were used to assess convergence of parameter estimates and tree splits, respectively. The average standard deviation of split frequencies (ASDSF) was used to determine topology convergence between runs. The appropriate burn-in generations (25% for all analyses) were discarded based on convergence assessments of the ASDSF passing below 0.01. The remaining trees were summarized in a 50% majority-rule consensus tree.

Finally, the monophyly of *C. lepidus* was evaluated using the approximately unbiased (AU) test (Shimodaira 2002). Using the same settings as the GARLI analyses described above, 200 ML searches were performed, 100 unconstrained and 100 with a topological constraint of *C. lepidus* monophyly. Per-site likelihoods were estimated for each tree under a partitioned model, and an AU test was performed on these values using CONSEL, version 0.1i (Shimodaira and Hasegawa 2001). The *P* value reported is the largest *P* value of all trees inferred under the constraint.

Results

Sequence attributes.—The aligned data set was 2,471 base pairs (bp) and included 75 samples from 27 named taxa. All sequences are deposited in GenBank (accession nos. KC112595–KC112848). We obtained DNA sequences for all genes for all samples, with the exception of those taken from museum skins, for which we were only able to sequence mitochondrial genes (Table 1). Alignment lengths were 1,041 bp (ND2), 352 bp (ND3), 709 bp (Myo2),

TABLE 1. Samples used to reconstruct the phylogeny of *Ceyx lepidus*, including voucher institution and locality.

Taxon	Voucher [a]	Sample number	Locality
Alcedo hercules [d]	KUNHM	10160	China: Guangxi Province
Ceyx azureus	KUNHM	96095	Papua New Guinea: Gulf Province
C. fallax [b,d,e]	AMNH	299259	Indonesia: Sulawesi
C. pusillus	UWBM	Bu67896	Solomon Islands: Western Province, New Georgia Island
C. websteri	USNM	608680[f]	Papua New Guinea: Bismarck Archipelago
C. argentatus argentatus	KUNHM	18103	Philippines: Mindanao Island
C. a. argentatus	KUNHM	19071	Philippines: Mindanao Island
C. a. argentatus	KUNHM	19252	Philippines: Mindanao Island
C. a. argentatus	KUNHM	19268	Philippines: Mindanao Island
C. a. argentatus	KUNHM	19269	Philippines: Mindanao Island
C. a. flumenicolus	KUNHM	14284	Philippines: Leyte Island
C. a. flumenicolus	KUNHM	14289	Philippines: Leyte Island
C. a. flumenicolus	KUNHM	14241	Philippines: Samar Island
C. cyanopectus cyanopectus	KUNHM	17990	Philippines: Luzon Island
C. c. cyanopectus	KUNHM	18068	Philippines: Luzon Island
C. c. cyanopectus	KUNHM	20334	Philippines: Luzon Island
C. erithacus erithacus	KUNHM	10417	China: Guangxi Province
C. e. motleyi	LSUMNS	B38586	Malaysia: Borneo, Sabah
C. e. motleyi	KUNHM	12359	Malaysia: Borneo, Sarawak
C. e. motleyi [c,d]	KUNHM	12650	Philippines: Palawan Island
C. e. motleyi [c,d]	KUNHM	12808	Philippines: Palawan Island
C. lepidus cajeli [b,c,d,e]	AMNH	637134	Indonesia: Maluku Province, Buru Island
C. l. collectoris [d]	UWBM	Bu66054	Solomon Islands: Western Province, New Georgia Island
C. l. collectoris [d]	UWBM	Bu68064	Solomon Islands: Western Province, New Georgia Island
C. l. collectoris	UWBM	Bu68077	Solomon Islands: Western Province, New Georgia Island
C. l. dispar	KUNHM	5611	Papua New Guinea: Manus Province, Manus Island
C. l. gentianus	KUNHM	12801	Solomon Islands: Makira-Ulawa Province: Makira Island
C. l. gentianus	KUNHM	13530	Solomon Islands: Makira-Ulawa Province: Makira Island
C. l. gentianus	KUNHM	13540	Solomon Islands: Makira-Ulawa Province: Makira Island
C. l. lepidus [b,c,d,e]	AMNH	637099	Indonesia: Maluku Province, Ambon Island
C. l. malaitae	UWBM	Bu66025	Solomon Islands: Malaita Province: Malaita Island
C. l. margarethae	KUNHM	14022	Philippines: Camiguin Sur Island
C. l. margarethae	KUNHM	14031	Philippines: Camiguin Sur Island
C. l. margarethae	KUNHM	14355	Philippines: Camiguin Sur Island
C. l. margarethae	KUNHM	14384	Philippines: Camiguin Sur Island
C. l. margarethae	KUNHM	14397	Philippines: Camiguin Sur Island
C. l. margarethae	KUNHM	19259	Philippines: Mindanao Island
C. l. margarethae	FMNH	344953	Philippines: Sibuyan Island
C. l. margarethae	FMNH	358316	Philippines: Sibuyan Island
C. l. margarethae	FMNH	358317	Philippines: Sibuyan Island
C. l. margarethae	KUNHM	14484	Philippines: Tablas Island
C. l. margarethae	KUNHM	14485	Philippines: Tablas Island
C. l. meeki	UWBM	Bu63203	Solomon Islands: Choiseul Province, Choiseul Island
C. l. meeki [c,d]	UWBM	Bu60194	Solomon Islands: Isabel Province, Isabel Island
C. l. meeki	AMNH	DOT6641	Solomon Islands: Isabel Province, Isabel Island
C. l. mulcatus [e]	LACM	91032	Papua New Guinea: New Ireland Province, New Ireland Island
C. l. mulcatus [d,e]	LACM	91033	Papua New Guinea: New Ireland Province, New Ireland Island
C. l. mulcatus [b,c,d,e]	AMNH	335499	Papua New Guinea: New Ireland Province, Tabar Island
C. l. nigromaxilla	KUNHM	15880	Solomon Islands: Guadalcanal Province, Guadalcanal Island
C. l. nigromaxilla	KUNHM	15892	Solomon Islands: Guadalcanal Province, Guadalcanal Island
C. l. nigromaxilla	UWBM	Bu60341	Solomon Islands: Guadalcanal Province, Guadalcanal Island
C. l. pallidus	KUNHM	5633	Papua New Guinea: Bougainville Province, Bougainville Island
C. l. sacerdotis	UWBM	Bu67945	Papua New Guinea: West New Britain Province, ~12 km SE Talasea
C. l. sacerdotis	UWBM	Bu68050	Papua New Guinea: West New Britain Province, ~12 km SE Talasea
C. l. solitarius	KUNHM	5157	Papua New Guinea: Chimbu Province
C. l. solitarius	UWBM	Bu68037	Papua New Guinea: Chimbu Province
C. l. solitarius	KUNHM	9539	Papua New Guinea: East Sepik Province
C. l. solitarius	KUNHM	5192	Papua New Guinea: Gulf Province, Ivimka Camp
C. l. solitarius	UWBM	Bu67992	Papua New Guinea: Gulf Province, Ivimka Camp
C. l. solitarius	UWBM	Bu68021	Papua New Guinea: Gulf Province, Ivimka Camp
C. l. solitarius [d]	KUNHM	7229	Papua New Guinea: Madang Province

(*continued*)

Table 1. Continued

Taxon	Voucher [a]	Sample number	Locality
C. l. solitarius	KUNHM	7295	Papua New Guinea: Madang Province
C. l. solitarius	KUNHM	6977	Papua New Guinea: Oro Province
C. l. solitarius	KUNHM	6982	Papua New Guinea: Oro Province
C. l. solitarius	KUNHM	7526	Papua New Guinea: Western Province
C. l. uropygialis [e]	YPM	74993	Indonesia: North Maluku Province, Bacan Island
C. l. uropygialis [d,e]	YPM	74989	Indonesia: North Maluku Province, Halmahera Island
C. l. uropygialis [b,c,d,e]	AMNH	637110	Indonesia: North Maluku Province, Halmahera Island
C. l. wallacii [b,c,d,e]	AMNH	637152	Indonesia: North Maluku Province, Mangole Island
C. melanurus melanurus	KUNHM	18046	Philippines: Luzon Island
C. m. melanurus	KUNHM	20203	Philippines: Luzon Island
C. m. mindanensis	KUNHM	18184	Philippines: Mindanao Island
C. m. mindanensis	KUNHM	19006	Philippines: Mindanao Island
C. m. samarensis	KUNHM	14304	Philippines: Leyte Island
C. m. samarensis	KUNHM	14226	Philippines: Samar Island

[a] Institutional abbreviations for voucher sources are as follows: AMNH = American Museum of Natural History; FMNH = Field Museum of Natural History; KUNHM = University of Kansas Natural History Museum; LACM = Los Angeles County Museum; LSUMNS = Louisiana State University Museum of Natural Science; UWBM = Burke Museum, University of Washington; USNM = National Museum of Natural History, Smithsonian Institution; and YPM = Yale Peabody Museum.
[b,c,d] Denotes samples for which data are lacking from ND3, Myo2, and/or GAPDH, respectively.
[e] Denotes samples for which DNA was extracted from museum study skins.
[f] This sample is the same as "B04021" from Moyle et al. (2007).

and 370 bp (GAPDH). The aligned data set contained 629 variable characters (25.5%) and 481 (19.5%) parsimony-informative characters. Pairwise distances in ND2 (uncorrected *p*; Table 2) ranged 8.0–11.6% between outgroup taxa and *C. lepidus* and 2.6–6.8% (mean = 4.7%) among *C. lepidus* taxa.

The ND3 gene sequence contained a single cytosine insertion at position 174 in all samples, an insertion reported in several other bird groups and turtles (Mindell et al. 1998). This insertion does not disrupt the reading frame because it is not translated. Apart from this insertion in ND3, the mitochondrial data showed no other insertions, deletions, or anomalous stop-codons; thus, there was no evidence that mtDNA sequences were of nuclear origin (i.e., pseudogenes; Sorenson and Quinn 1998). The relative divergence among codon positions was typical for mtDNA (3 > 1 > 2). Four deletions were noted in Myo2, but all were autapomorphic in the following samples: *C. pusillus* (2 bp), *C. erithacus* (1 bp in each: B38586 and 12359), and *C. l. margarethae* (2 bp; 14384). A synapomorphic 1-bp deletion was shared by all *C. erithacus* and *C. melanurus* samples in GAPDH. On the basis of model testing results, we used the GTR+I+G model of sequence evolution for all three mtDNA codon positions, HKY+I for Myo2, and HKY+G for GAPDH. All ML analyses with GARLI were done with the GTR+I+G model across the entire data set.

Phylogenetic relationships.—Individual gene trees were highly concordant (Fig. 2). The topologies recovered from analyses of mtDNA showed greater resolution than those derived from nuclear introns, which was expected given the higher rates of sequence evolution in animal mtDNA compared with nuclear DNA (Brown et al. 1979). No well-supported clades recovered from the analysis of individual genes conflicted with those from other gene trees or those from the concatenated data set, so we focused our discussion on phylogenetic relationships inferred from the concatenated data set (Fig. 3). The inferred topologies from multiple independent ML and BA runs were highly concordant. The best ML topology had a –ln likelihood score of 9605.1289, as reported in GARLI.

We recovered a well-supported clade (i.e., Bayesian posterior probability >95% and ML bootstrap >70%) that included two outgroup taxa (*C. erithacus* and *C. melanurus*) and the ingroup clade (Fig. 3, clade A), which comprised *C. lepidus*, *C. cyanopectus*, and *C. argentatus*. However, relationships among *C. erithacus*, *C. melanurus*, and clade A were unresolved, a result similar to those obtained by Moyle et al. (2007) and Lim et al. (2010). The synapomorphic indel observed in the GAPDH intron supports a sister relationship between *C. melanurus* and *C. erithacus*, but this hypothesis requires further investigation.

Support for clade A was unequivocal, but monophyly of *C. lepidus* received no support. Instead, basal relationships within clade A consisted of a polytomy among four well-supported clades: (1) the Philippine endemics *C. cyanopectus* and *C. argentatus* (Fig. 3, clade B); (2) Wallacean *C. l. cajeli* and *C. l. wallacii* (Fig. 3, clade C); (3) Philippine endemic *C. l. margarethae* (Fig. 3, clade D); and (4) a clade including the remaining 12 subspecies of *C. lepidus* (Fig. 3, clade E). Although the polytomy raised the possibility that *C. lepidus* is paraphyletic, this relationship is best considered unresolved. Indeed, an AU test failed to reject *C. lepidus* monophyly ($P = 0.230$). Despite the lack of resolution at the bases of Clades A and E, each of the 15 *C. lepidus* subspecies was monophyletic, and several sister pairs were well supported: *C. l. mulcatus* + *C. l. solitarius*, *C. l. collectoris* + *C. l. nigromaxilla*, and *C. l. pallidus* + *C. l. meeki*.

Within *C. argentatus* phylogeographic structure was concordant with named subspecies (discussed below). Conversely, we found no discernible genetic structure within two widespread *C. lepidus* subspecies: *C. l. margarethae* and *C. l. solitarius*, despite broad sampling within their ranges. This result was somewhat expected for *C. l. solitarius* and likely suggests a high amount of gene flow across the island of New Guinea. However, the lack of genetic differentiation in *C. l. margarethae* across multiple Philippine oceanic islands—and representing two color morphs—is noteworthy.

Finally, removal of the five taxa represented by only one gene sequence in our data set (*C. l. dispar*, *C. l. malaitae*, *C. l. cajeli*, *C. l. wallacii*, and *C. l. lepidus*) had little effect on results of

TABLE 2. Uncorrected ND2 pairwise *p*-distances. Mean pairwise distances are reported for taxa with more than one sample. Column headers are abbreviated with the first three letters of the subspecific epithet.

	eri.	*mot.*	*mel.*	*min.*	*sam.*	*arg.*	*flu.*	*cya.*	*caj.*	*col.*	*dis.*	*gen.*
C. erithacus erithacus	—											
C. e. motleyi	0.048	—										
C. melanurus melanurus	0.088	0.085	—									
C. m. mindanensis	0.085	0.076	0.026	—								
C. m. samarensis	0.084	0.084	0.019	0.025	—							
C. argentatus argentatus	0.085	0.080	0.070	0.065	0.070	—						
C. a. flumenicolus	0.092	0.083	0.074	0.070	0.077	0.025	—					
C. cyanopectus cyanopectus	0.092	0.080	0.072	0.068	0.073	0.034	0.041	—				
C. lepidus cajeli	0.080	0.075	0.059	0.056	0.060	0.045	0.046	0.054	—			
C. l. collectoris	0.098	0.088	0.084	0.079	0.084	0.062	0.064	0.069	0.057	—		
C. l. dispar	0.089	0.083	0.073	0.068	0.077	0.057	0.058	0.059	0.045	0.065	—	
C. l. gentianus	0.092	0.088	0.073	0.066	0.080	0.054	0.057	0.063	0.041	0.067	0.053	—
C. l. lepidus	0.086	0.082	0.069	0.064	0.065	0.045	0.050	0.057	0.047	0.063	0.055	0.057
C. l. malaitae	0.093	0.088	0.075	0.070	0.081	0.059	0.061	0.059	0.053	0.061	0.057	0.054
C. l. margarethae	0.086	0.080	0.073	0.066	0.073	0.055	0.064	0.063	0.046	0.061	0.062	0.062
C. l. meeki	0.085	0.085	0.074	0.067	0.077	0.056	0.060	0.061	0.047	0.068	0.056	0.063
C. l. mulcatus	0.099	0.086	0.077	0.067	0.078	0.062	0.062	0.063	0.052	0.067	0.062	0.052
C. l. nigromaxilla	0.094	0.089	0.078	0.071	0.081	0.061	0.064	0.065	0.048	0.053	0.059	0.059
C. l. pallidus	0.083	0.083	0.076	0.068	0.080	0.052	0.062	0.062	0.049	0.070	0.060	0.061
C. l. sacerdotis	0.100	0.092	0.076	0.073	0.082	0.060	0.061	0.069	0.047	0.073	0.061	0.051
C. l. solitarius	0.093	0.085	0.078	0.073	0.082	0.066	0.067	0.070	0.044	0.066	0.065	0.054
C. l. uropygialis	0.089	0.082	0.071	0.067	0.077	0.051	0.052	0.061	0.051	0.065	0.058	0.054
C. l. wallacii	0.083	0.076	0.062	0.058	0.061	0.050	0.048	0.058	0.016	0.061	0.054	0.048

	lep.	*mal.*	*mar.*	*mee.*	*mul.*	*nig.*	*pal.*	*sac.*	*sol.*	*uro.*	*wal.*
C. l. lepidus	—										
C. l. malaitae	0.056	—									
C. l. margarethae	0.060	0.064	—								
C. l. meeki	0.045	0.053	0.062	—							
C. l. mulcatus	0.060	0.058	0.060	0.060	—						
C. l. nigromaxilla	0.058	0.056	0.064	0.057	0.058	—					
C. l. pallidus	0.051	0.050	0.062	0.032	0.060	0.068	—				
C. l. sacerdotis	0.061	0.060	0.067	0.063	0.060	0.063	0.066	—			
C. l. solitarius	0.060	0.061	0.066	0.064	0.033	0.065	0.068	0.063	—		
C. l. uropygialis	0.048	0.056	0.060	0.057	0.055	0.063	0.053	0.062	0.061	—	
C. l. wallacii	0.052	0.056	0.050	0.052	0.056	0.060	0.053	0.048	0.054	0.055	—

Bayesian analysis of the concatenated data set. Bayesian posterior probabilities and the backbone topology were extremely similar between analyses with and without the five taxa (results not shown).

DISCUSSION

Biogeography.—Here, we present the first fully sampled molecular phylogeny of *Ceyx lepidus*. Although the abundance of unresolved relationships precluded quantitative biogeographic analysis, some biogeographic insights are evident from our results.

Overall, the most striking aspect of the phylogeny is that each taxon in the *C. lepidus* complex is monophyletic and substantially diverged from all other taxa (2.6–6.8% divergent in uncorrected ND2 *p*-distance). We interpret this pattern of shallow internodes at the base, long stem lineages, and shallow divergences within each taxon as support for a scenario in which *C. lepidus* achieved its full geographic distribution rapidly, followed by little or no subsequent gene flow among most island populations. This biogeographic pattern of rapid and widespread colonization across Southeast Asia and the Pacific islands is thought to have occurred in other widespread polytypic species complexes such as *Todiramphus chloris*, *Pachycephala pectoralis*, and *Turdus poliocephalus* (Mayr and Diamond 2001). However, densely sampled phylogenetic hypotheses are not available to test this hypothesis in these groups (but see Jones and Kennedy 2008b, Jønsson et al 2008).

The highest diversity in this group of dwarf-kingfishers (under present taxonomy) occurs in the Philippine archipelago, where one to three species are present on each major island. It appears that *in situ* diversification of *C. argentatus* and *C. cyanopectus* (clade B) in the Philippines played a role in generating this diversity. Our results also indicate that multiple colonization events contributed to the diversity of dwarf-kingfishers in the Philippines; the ancestors of *C. erithacus* appear to have invaded the western Philippines from the Sunda Shelf, and at least two other colonization events were responsible for the presence of *C. melanurus*, *C. cyanopectus*, *C. argentatus*, and *C. l. margarethae* in the archipelago. These four taxa occur in sympatry on some islands,

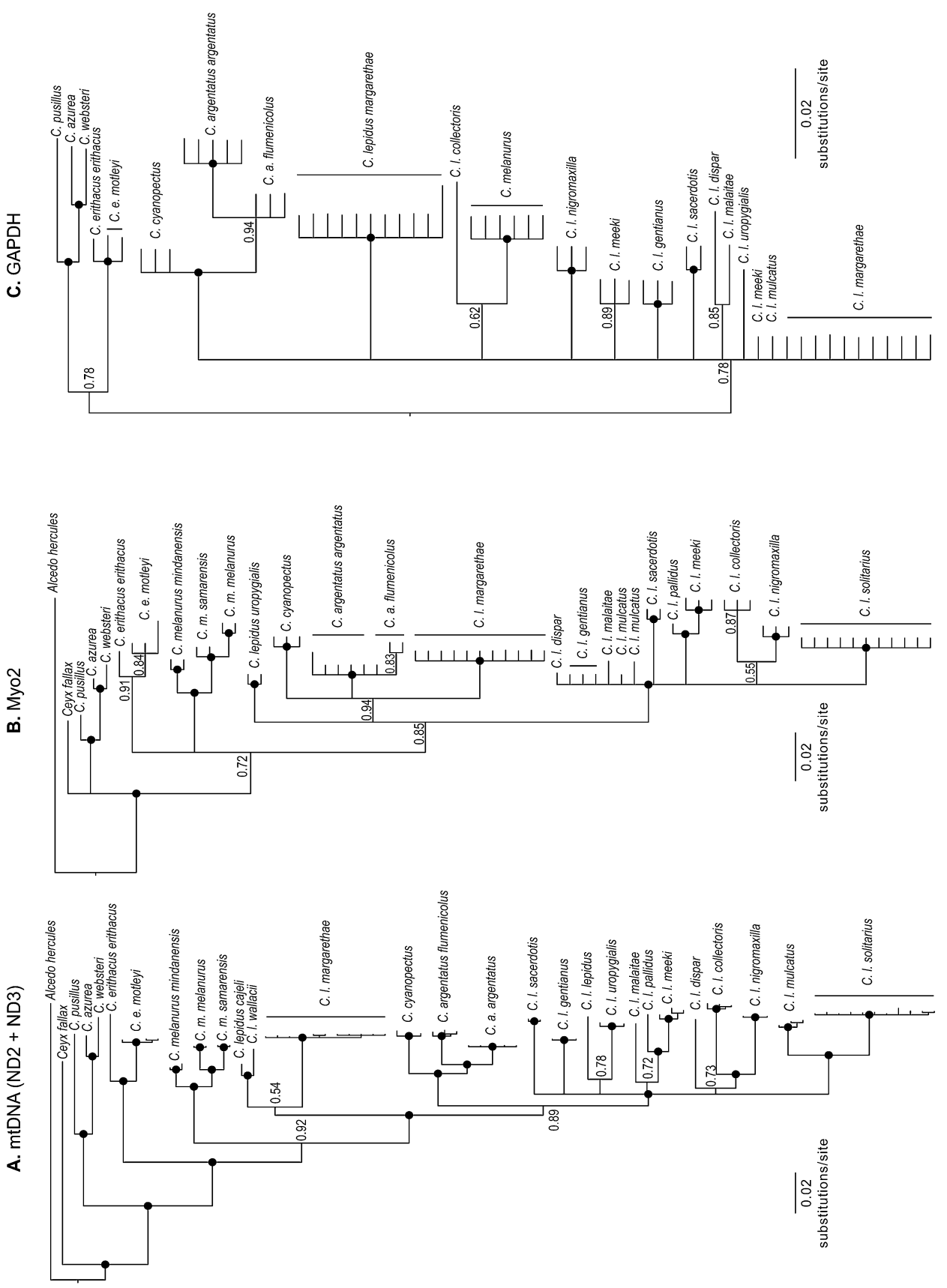

FIG. 2. Bayesian phylogenies of the *Ceyx lepidus* complex for each of the three independently segregating loci: (A) mtDNA: ND2+ND3, (B) Myo2, and (C) GAPDH. Black circles on nodes denote Bayesian posterior probability = 0.95 and maximum likelihood bootstrap support ≥70. Numbers by nodes indicate Bayesian PP. Some taxa are missing from trees B and C because data are lacking for Myo2 and GAPDH, respectively.

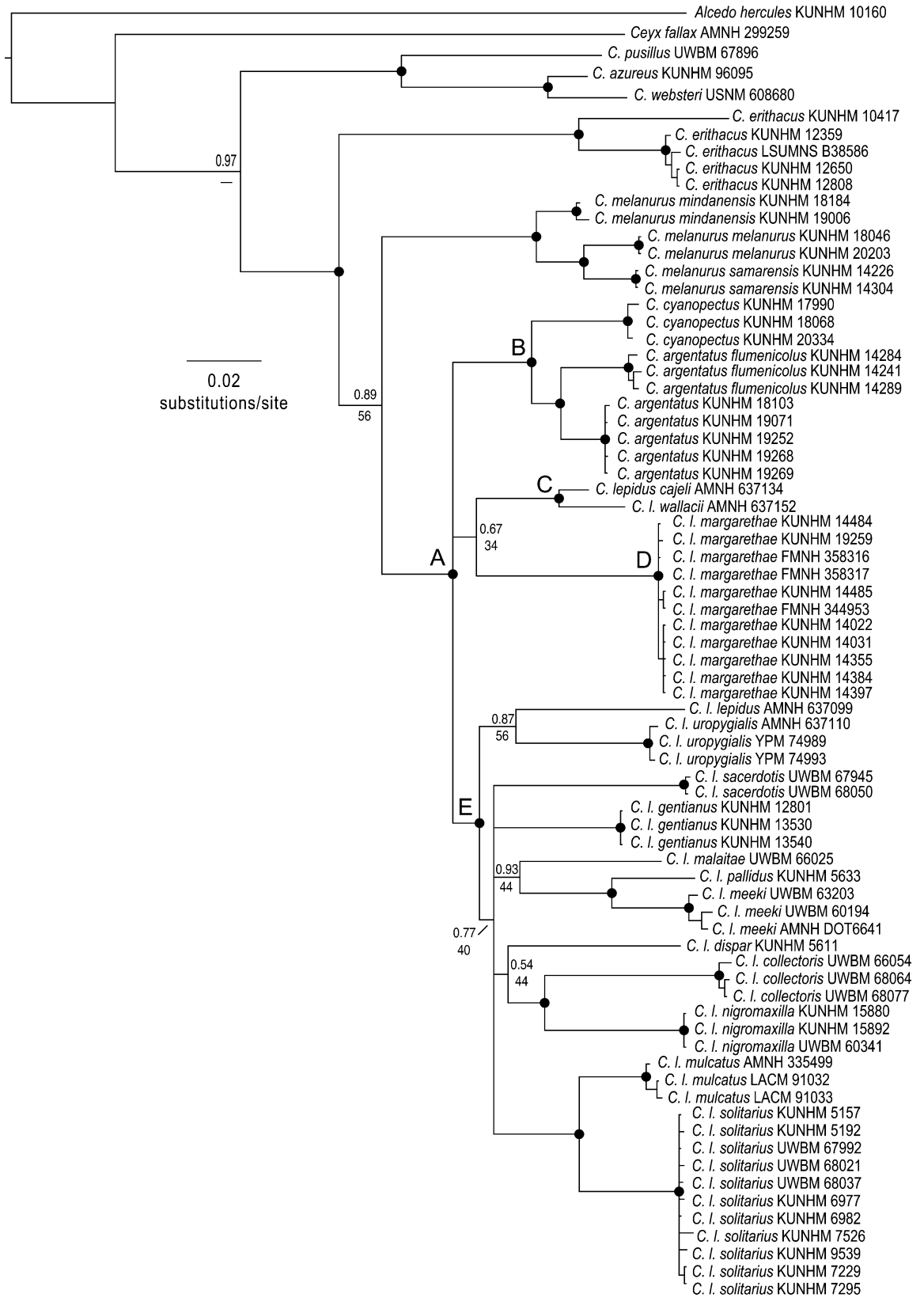

FIG. 3. Bayesian phylogeny of the *Ceyx lepidus* complex based on a concatenated data set of two mitochondrial coding genes and two nuclear introns. Black circles on nodes denote Bayesian posterior probability (PP) = 0.95 and maximum likelihood (ML) bootstrap support ≥70. Numbers by nodes detail unresolved nodes, with numbers above branches indicating Bayesian PP and those below branches ML bootstrap.

but it is uncertain whether they occur syntopically. For instance, *C. argentatus*, *C. melanurus*, and *C. l. margarathae* all occur on Mindanao, and although natural history data are sparse for these taxa, preliminary observations suggest that *C. argentatus* is a stream-associated species, whereas *C. melanurus* and *C. l. margarethae* are forest species with no affinity to water (P. Hosner pers. comm.). This pattern suggests that at least some level of ecological partitioning helps separate these otherwise broadly sympatric taxa.

Some geographic insight can be gleaned from sister relationships in the *C. lepidus* species group. For instance, dwarf kingfishers of the Malukus are derived from two well-supported but unrelated pairs of sister taxa (*C. l. cajeli* and *C. l. wallacii*; *C. l. lepidus* and *C. l. uropygialis*), which indicates the combined role of colonization and local diversification in generating diversity. *In situ* diversification is also evident in the Solomon Islands with the recovery of two pairs of sister taxa within the island group. The first pair, *C. l. pallidus* and *C. l. meeki*, reflects the close affinities of Bougainville, Choiseul, and Isabel, which form part of the Pleistocene island of Greater Bukida (Mayr and Diamond 2001). The close affinity of fauna within Greater Bukida, especially between Choiseul and Isabel, is documented in multiple avian lineages (Smith and Filardi 2007, Uy et al. 2009a) and also has been observed in bats (Pulvers and Colgan 2007). Bougainville tends to have taxa more divergent from the rest of the Greater Bukida islands (Mayr and Diamond 2001), and this pattern is also seen in *Ceyx*. The second sister pair within the Solomon Islands, *C. l. collectoris* and *C. l. nigromaxilla*, reveals a close relationship between the New Georgia Group and Guadalcanal, a biogeographic pattern not recovered in other avian studies (Filardi and Smith 2005, Smith and Filardi 2007, Uy et al. 2009a). The mostly unresolved relationships among the lineages in clade E obscure the number of colonization events on the Solomon Islands. Lastly, the sister relationship of *C. l. solitarius* and *C. l. mulcatus* unites New Guinea with the northern Bismarck Archipelago islands of New Ireland, New Hanover, Tabar, and Lihir. This result suggests that at least two independent colonization events were involved in assembling the dwarf-kingfishers of the Bismarcks. This sister pairing is cohesive with respect to plumage because they are nearly identical—both are rufous below, with whitish throats, pale rufous loral spots, dark blue backs, and black bills. This pattern of similarly plumaged sister taxa was not upheld throughout the rest of the tree, which highlights the need for revisionary taxonomy not based solely on plumage patterns in polytypic, insular species complexes (Peterson 2007).

Plumage polymorphism.—Examples of plumage polymorphism in birds are numerous and have received much attention (Roulin 2004). *Ceyx l. margarethae*, an endemic of central and southern Philippines, is the only *C. lepidus* subspecies for which polymorphism within single-island populations occurs. *Ceyx erithacus* is the only other *Ceyx* species to exhibit within population plumage polymorphism (Fry et al. 1992). Lim et al. (2010) found evidence for polymorphism in *C. erithacus* as a result of admixture of historically separate and genetically well-differentiated populations across Southeast Asia and the Sunda Shelf. We sampled widely throughout the range of *C. l. margarethae*, including pale- and dark-backed individuals from the Philippine islands of Camiguin Sur, Tablas, Mindanao, and Sibuyan, but we did not recover genetic structure in *C. l. margarethae* with respect to geography or plumage polymorphism. Recent studies have found that single point mutations in the melanocortin-1-receptor gene are associated with plumage polymorphisms in Bananaquits (*Coereba flaveola*) and monarchs (Monarchidae; Theron et al. 2001, Uy et al. 2009b). It is possible that a single point mutation is driving plumage polymorphism in *C. l. margarethae*, and investigations on the role of this gene in polymorphism in *Ceyx* species are recommended.

The subspecies *C. l. dispar*, from the Admiralty Islands of Papua New Guinea, is sexually dichromatic—the male has the typical blue head, whereas the female is orange-headed. This pattern is reminiscent of the *Ispidina* pygmy-kingfishers of Africa (Fry et al. 1992). Only one other ingroup taxon, *C. cyanopectus*, is sexually dichromatic; males have a double breast band, whereas females have only one breast band (Kennedy et al. 2000). Sexual dichromatism thus appears to have evolved twice in the ingroup. Indeed, other instances of differential patterns of sexual dichromatism in polytypic insular bird species are known. For example, *Turdus poliocephalus niveiceps* and *T. p. carbonarius* are sexually dichromatic on Taiwan and New Guinea, respectively, but not elsewhere (Peterson 2007). Interestingly, *Pachycephala pectoralis feminina* on Rennell Island in the Solomon Islands is sexually monochromatic; in this instance, the male reverts to female plumage (Galbraith 1956).

Taxonomy.—Our discussion of taxonomy is based largely on an evolutionary species concept (Simpson 1961, Wiley 1978) and its extension, the general lineage-based species concept (de Queiroz 1999). We draw upon details of genetic divergence, biogeography, and plumage pattern as the most prescient evidence. Application of lineage-based species concepts to island systems is preferable to the biological species concept (Mayr 1963) because reproductive isolation between allopatric insular taxa cannot be assessed. Instead, we employ a lineage-based species concept to recognize ancestor–descendant populations with unique evolutionary histories.

Two Philippine species warrant discussion on species limits: *C. argentatus* and *C. melanurus*. *Ceyx argentatus* is distributed throughout the central and southern Philippines (Fig. 1, inset). Two subspecies of *C. argentatus* are described: *C. a. argentatus* and *C. a. flumenicolus*, though both were originally described as species (Peters 1945). We recovered these two subspecies as sister clades diverged by 2.3% ND2 uncorrected *p*-distances. Our results support the suggestion of Collar (2011), which was based on morphological data, to treat *C. argentatus* and *C. flumenicolus* as full species, but we acknowledge that this pair of taxa requires further investigation to determine whether there is gene flow between them. On the other hand, *C. melanurus* consists of three subspecies, which are distributed along the eastern arc of the Philippines (Fig. 1, inset): *C. m. melanurus*, *C. m. samarensis*, and *C. m. mindanensis*. We sampled all three subspecies and found strong support for the sister relationship of *C. m. mindanensis* and a clade comprising *C. m. melanurus* and *C. m. samarensis*. Morphologically, these forms differ in the extent of black on the wings and the presence or absence of a blue streak on the side of the head (Fry et al. 1992). A comprehensive study of the genetic structure and morphological variation in this species is ongoing (P. Hosner unpubl. data); thus, we refrain from recommending taxonomic changes in this group. In both *C. argentatus* and *C. melanurus*, our data demonstrate the genetic

distinctiveness of species on the eastern Philippine islands of Samar, Leyte, and Bohol, despite their land connection to Mindanao during the last glacial maximum. This result provides another example showing the distinctiveness of avian populations in this group of islands (Sánchez-González and Moyle 2011, Sheldon et al. 2012) and supports a nascent, but growing, body of studies in recognizing that the paradigm of late Pleistocene aggregate islands explaining the distribution of diversity in the Philippines, as proposed by Heaney (1986), is overly simplistic for mammals, reptiles, amphibians, and birds (Evans et al. 2003, Jones and Kennedy 2008a, Esselstyn and Brown 2009, Linkem et al. 2010, Oliveros and Moyle 2010, Siler et al. 2010).

Plumage differences among the 15 *C. lepidus* subspecies were described by Fry et al. (1992) and Woodall (2001) and are summarized in the Appendix. Below, we discuss in detail an example of highly divergent plumage and an example in which the plumage differentiation is minimal, and the reader is referred to the Appendix for details of plumage differences that are not discussed in the text. *Ceyx l. gentianus* from Makira Island in the Solomon Islands is one of the most morphologically disparate taxa. It is entirely white below, lacking the rufous tones found in most other forms of *C. lepidus* (Fry et al. 1992, Dutson 2012). Other described taxa have more subtle plumage differences, and the two most similar taxa occur in the Solomon Islands. The Bougainville taxon, *C. l. pallidus*, one of the few taxa originally described as a subspecies of *C. lepidus*, is slightly paler than *C. l. meeki* from Choiseul and Isabel islands. In his description, Mayr (1935) noted that *C. l. pallidus* is "similar to *Ceyx lepidus meeki*, but [its] under parts [are] pale yellowish buff, instead of golden-yellowish ochre." *Ceyx l. pallidus* appears to be only weakly differentiated from *C. l. meeki* in plumage, but our data support a well-differentiated genetic split (3.3% ND2 uncorrected *p*-distance) between these sister taxa. This divergence is substantially higher than the 2.3% divergence between *C. a. argentatus* and *C. a. flumenicolus*, two morphologically divergent sister taxa. It appears that in the case of *C. l. pallidus* and *C. l. meeki*, morphology was conserved while their populations diverged.

Although our phylogeny does not resolve the apparent rapid and widespread geographic diversification of *C. lepidus* in a bifurcating fashion, it provides a basis for a reevaluation of species limits in this group. We propose recognizing all 15 *C. lepidus* subspecies as species for the following reasons: (1) each subspecies is morphologically distinct; (2) these taxa exhibit a relatively uniform and high degree of genetic differentiation among lineages (2.6–6.8% in ND2 uncorrected *p*-distance; Table 2), which is higher than in two sister taxa (*C. argentatus* and *C. flumenicolus*) that are closely related to *C. lepidus*; and (3) the 15 subspecies have allopatric distributions and, therefore, are experiencing their own evolutionary fate.

Our results support an improved understanding of the high degree of morphologic and cryptic genetic diversity found not only in Philippine birds (Lohman et al. 2010) but more broadly in the archipelagos of Southeast Asia and the Pacific. Recognizing subspecies of *C. lepidus* as full species will have important conservation implications, especially because most taxa are endemic to small islands or island groups.

Acknowledgments

We thank the following museum curators and collection managers for assistance with specimen loans: P. Sweet, American Museum of Natural History; D. Willard, Field Museum of Natural History; M. Robbins, University of Kansas Biodiversity Institute; K. Garrett, Los Angeles County Museum; D. Dittman, Louisiana State University Museum of Natural Science; C. Milensky, National Museum of Natural History, Smithsonian Institution; S. Birks, Burke Museum, University of Washington; and K. Zyskowski, Yale Peabody Museum. We are also grateful to the specimen collectors whose efforts in the field helped make this project possible. H. Shult assisted with molecular lab work at the University of Kansas, and P. Hosner, R. Jones, J. Manthey, and two anonymous reviewers offered insightful comments on the manuscript. This work was supported by the National Science Foundation (DEB-0743491 to R.G.M.). For permission to undertake research in the Philippines, we thank the Department of Environment and Natural Resources of the Philippines and the Protected Areas and Wildlife Bureau.

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Associate Editor: L. Joseph

Appendix. Phenotypic characters of the 15 subspecies of *Ceyx lepidus*, based on examination of specimens at the University of Kansas Biodiversity Institute and from Fry et al. (1992).

Subspecies	Bill	Head	Wings	Frontal spot	Malar stripe	Neck blaze	Back	Rump	Uppertail coverts	Chin and throat	Breast and belly	Legs and feet
C. l. cajeli	Red but stouter	Blackish, almost without dark blue	Blackish, almost without dark blue	Yellow-orange			Brilliant silvery-blue	Brilliant silvery-blue	Brilliant silvery-blue	White	Yellow-orange	Orange
C. l. collectoris	Red but stouter	Black, washed and spotted with very dark blue	Black, washed and spotted with very dark blue	Orange			Brilliant purple-blue	Brilliant purple-blue	Brilliant purple-blue	Yellowish-white	Rich orange	Orange
C. l. dispar	Red	Male: black, washed and spotted with dark blue; Female: orange, with blue-black restricted to a stripe on the hindcrown and an area in front of neck blaze	Black, spotted and washed with ultramarine	Male: orange; female: lore and small area in front of eye black		White	Brilliant pale silvery-blue	Brilliant pale silvery-blue	Brilliant dark ultramarine–blue	Male: yellowish-white; female: white	Breast dark rufous, belly rich orange	Red
C. l. gentianus	Black	Black, washed and spotted with dark blue	Black, washed and spotted with dark blue	White		White	Brilliant ultra-marine-blue	Silvery-blue	Brilliant ultramarine-blue	White	White	Orange
C. l. lepidus	Red	Black, washed and spotted with dark blue	Black, washed and spotted with dark blue	Orange	Blue-black		Brilliant ultra-marine-blue	Silvery-blue	Brilliant ultramarine-blue	Yellowish-white	Rich orange	Orange
C. l. malaitae	Upper mandible mainly black, lower mandible yellow	Black, washed and spotted with very dark blue	Black, washed and spotted with very dark blue	Orange			Brilliant purple-blue	Brilliant purple-blue	Brilliant purple-blue	Yellowish-white	Rich orange	Orange
C. l. margarethae	Red	Pale morph: pale cobalt blue; dark morph: ultramarine blue	Pale morph: pale cobalt blue; dark morph: ultramarine blue	Dark chestnut	Orange		Pale morph: pale cobalt blue; dark morph: ultra-marine blue	Pale morph: pale silvery cobalt blue; dark morph: ultramarine blue	Pale morph: pale cobalt blue; dark morph: ultramarine blue	Yellowish-white	Breast rich orange, belly pale yellow	Orange

(*continued*)

APPENDIX. Continued.

Subspecies	Bill	Head	Wings	Frontal spot	Malar stripe	Neck blaze	Back	Rump	Uppertail coverts	Chin and throat	Breast and belly	Legs and feet
C. l. meeki	Black	Blue-black, spangled with pale blue	Blue-black, spangled with pale blue	Pale yellowish-buff on male, more orange-yellow on female		Male: pale yellowish-buff; Female: pale orange-yellow	Lower mantle pale blue	Silvery	Pale blue	Pale yellowish-buff on male, more orange-yellow on female	Pale yellowish-buff on male, more orange-yellow on female	Flesh-pink
C. l. mulcatus	Black	Black, washed and spotted with dark blue	Black, washed and spotted with dark blue	Orange			Brilliant ultramarine-blue	Silvery-blue	Brilliant ultramarine-blue	Yellowish-white	Rich orange	Orange
C. l. nigromaxilla	Upper mandible mainly black, lower mandible mainly orange-red	Black, washed and spotted with very dark blue	Black, washed and spotted with very dark blue	Orange			Brilliant purple-blue	Brilliant purple-blue	Brilliant purple-blue	Yellowish-white	Rich orange	Orange
C. l. pallidus	Black	Blue-black, spangled with pale ultramarine blue	Blue-black, spangled with pale ultramarine blue	Pale yellowish-buff		Pale yellowish buff	Brilliant ultramarine-blue	Brilliant ultramarine-blue	Cobalt blue	Pale yellow	Pale orange-yellow	Yellow
C. l. sacerdotis	Upper mandible dusky red, lower mandible red	Black, washed and spotted with dark blue	Black, washed and spotted with dark blue	Orange			Brilliant ultramarine-blue	Brilliant ultramarine-blue	Brilliant ultramarine-blue	Yellowish-white	Breast rich orange, belly and undertail coverts very pale orange-yellow	Orange
C. l. solitarius	Black	Black, washed and spotted with dark blue	Black, washed and spotted with dark blue	Yellowish-white			Brilliant ultramarine-blue	Brilliant ultramarine-blue	Brilliant ultramarine-blue	Yellowish-white	Rich orange	Orange
C. l. uropygialis	Red, more slender	Black, softly washed and spotted with dark blue	Black, softly washed and spotted with dark blue	Orange			Brilliant ultramarine-blue	Silvery-blue	Brilliant ultramarine-blue	Rich yellowish-white	Dark rich orange	Orange
C. l. wallacii	Red	Black, washed and spotted with blue (paler)	Black, washed and spotted with blue (paler)	Orange			Brilliant pale cobalt-blue	Brilliant pale cobalt-blue	Ultramarine-blue	Yellowish-white	Rich orange	Orange