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RESEARCH ARTICLE

Extensive paraphyly in the typical owl family (Strigidae)

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ABSTRACT

The typical owl family (Strigidae) comprises 194 species in 28 genera, 14 of which are monotypic. Relationships within and among genera in the typical owls have been challenging to discern because mitochondrial data have produced equivocal results and because many monotypic genera have been omitted from previous molecular analyses. Here, we collected and analyzed DNA sequences of ultraconserved elements (UCEs) from 43 species of typical owls to produce concatenated and multispecies coalescent-based phylogenetic hypotheses for all but one genus in the typical owl family. Our results reveal extensive paraphyly of taxonomic groups across phylogenies inferred using different analytical approaches and suggest the genera *Athene*, *Otus*, *Asio*, *Megascops*, *Bubo*, and *Strix* are paraphyletic, whereas *Ninox* and *Glaucidium* are polyphyletic. Secondary analyses of protein-coding mitochondrial genes harvested from off-target sequencing reads and mitochondrial genomes downloaded from GenBank generally support the extent of paraphyly we observe, although some disagreements exist at higher taxonomic levels between our nuclear and mitochondrial phylogenetic hypotheses. Overall, our results demonstrate the importance of taxon sampling for understanding and describing evolutionary relationships in this group, as well as the need for additional sampling, study, and taxonomic revision of typical owl species. Additionally, our findings highlight how both divergence and convergence in morphological characters have obscured our understanding of the evolutionary history of typical owls, particularly those with insular distributions.

Keywords: insular distributions, morphological convergence, owls, phylogenomics, taxonomy, UCEs

Parafilia extendida en la familia típica de los búhos (Strigidae)

RESUMEN

La familia típica de los búhos (Strigidae) abarca 194 especies en 28 géneros, 14 de los cuales son monotípicos. Las relaciones adentro y entre géneros en los búhos típicos han sido difíciles de discernir debido a que los datos mitocondriales han producido resultados contradictorios y a que muchos géneros monotípicos han sido omitidos de los análisis moleculares previos. En este trabajo, colectamos y analizamos secuencias de ADN de elementos ultra-conservados (EUCs) provenientes de 43 especies de búhos típicos para generar hipótesis filogenéticas concatenadas y multi-especies basadas en coalescencia para todos los géneros excepto uno en la familia típica de los búhos. Nuestros resultados revelan la presencia de una parafilia extendida en los grupos taxonómicos a través de las filogenias inferidas usando diferentes enfoques analíticos, y sugiere que los géneros *Athene*, *Otus*, *Asio*, *Megascops*, *Bubo*, y *Strix* son parafiléticos, mientras que *Ninox* y *Glaucidium* son polifelíticos. Los análisis secundarios de genes mitocondriales que codifican proteínas obtenidos de lecturas de secuenciación por fuera del objetivo y genomas mitocondriales descargados de GenBank generalmente apoyan el grado de parafilia que observamos, aunque existen algunos desacuerdos a niveles taxonómicos más altos entre nuestras hipótesis filogenéticas nuclear y mitocondrial. En general, nuestros resultados demuestran la importancia del muestro de los taxones para entender y describir las relaciones evolutivas en este grupo, así como la necesidad de muestreos, estudios y revisiones taxonómicas adicionales de las especies típicas de búhos. Adicionalmente, nuestros hallazgos subrayan cómo la divergencia y convergencia en los caracteres morfológicos han dificultado nuestro entendimiento de la historia evolutiva de los típicos búhos, particularmente aquellos con distribuciones insulares.

Palabras clave: búhos, convergencia morfológica, distribuciones insulares, EUCs, filogenómica, taxonomía

INTRODUCTION

Owls (Strigiformes) are among the most iconic birds, easily recognizable because of their large eyes, distinctive facial discs, and "fluffy" plumage. The derived features of owls enable the unique lifestyle of a nocturnal predator and are involved in soundless flight (Sarradj et al. 2011, Geyer et al. 2013), complex inherited vocalizations (König et al. 1999), and a sophisticated auditory system (Norberg 1968, Payne 1971, Konishi 1973). These and other adaptations have enabled more than 200 owl species (Dickinson and Remsen 2013) to occupy a diversity of habitats around the globe from tundra to dense forests (Marks et al. 2018).

The taxonomic and systematic history of owls has been complex and confusing (reviewed in Sibley and Ahlquist 1972). Early studies of owls separated them into 2 taxonomic groups: the barn owls (Tytonidae, Wetmore 1960; 19 species, Dickinson and Remsen 2013) and the typical owls (Strigidae, Wetmore 1960; 194 species, Dickinson and Remsen 2013). This division was proposed during the mid-1800s (Nitzsch 1840) and supported by numerous morphological characters (Ridgway 1914, Bock and McEvey 1969). Subsequent classifications of taxa within Strigidae relied on characters related to the external ear and facial discs: Bonaparte (1850) proposed 3 subfamilies within the group (Figure 1A) while Kaup (1862) suggested 2 (Figure 1B). In 1940, Peters reclassified members of the group into 2 subfamilies: Buboninae (21 genera) and Striginae (6 genera; Figure 1C). Subsequently, several authors (Kelso 1940, Voous 1964; see also Norberg 1977) found that the external ear was not diagnostic for all taxa and may be subject to convergence because of its role in prey location. To avoid potential bias introduced by these convergent characters, Ford (1967) used comparative osteological analyses and divided the typical owls into 3 subfamilies: Striginae (scops and screech owls, 13 genera), Surniinae (hawk owls, 8 genera), and Asioninae (eared owls and relatives, 2 genera; Figure 1D). These taxonomic designations were adopted until 2008 (König and Weick 2008), when molecular data began to inform classifications.

Early molecular systematics of a small sample of owl species (Heidrich and Wink 1998, Wink and Heidrich 2000) used partial cytochrome b sequences to confirm the monophyly of barn owls and typical owls and resolve 2 clades that were broadly consistent with previous taxonomy: Striginae (*Otus+Asio+Bubo+Strix*; Kaup 1862) and Surniinae (*Glaucidium+Athene+Aegolius+Surnia*; Kaup 1862, Ford 1967). Subsequent studies analyzed mitochondrial and nuclear data that included additional taxa to generate phylogenetic hypotheses, all of which conflicted with Ford's subfamily taxonomy based on osteology (Wink et al. 2004, 2008, 2009; Wink 2016). Instead,

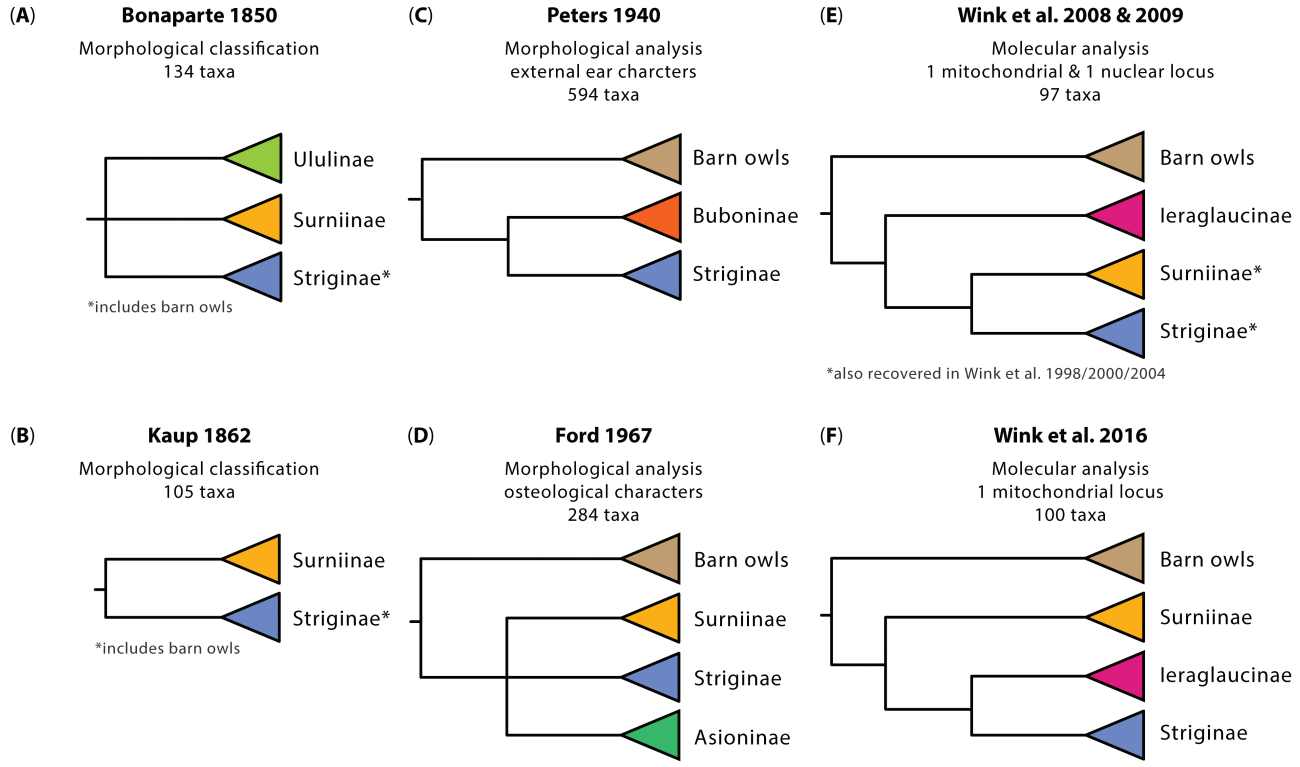

FIGURE 1. Previous hypotheses of Strigidae relationships. Note that although some subfamily names have remained consistent, the genera comprising these subfamilies have changed throughout time.

Wink et al. (2008) suggested typical owls comprised 3 subfamilies: Ieraglaucinae, Surniinae, and Striginae (which included Asioninae; Figure 1E). However, in their most recent study, Wink et al. (2016) disagreed with their previous findings (Wink et al. 2008, 2009) with respect to the placement of typical owl subfamilies, reversing the position of Ieraglaucinae and Surniinae (Figure 1F) and the relationships among genera within subfamilies (Wink et al. 2008, 2009; Wink 2016). As summarized by Kelso (1940), "the subdivision of the family Strigidae has long been a source of disagreement."

One of the challenges affecting molecular systematics of typical owls is the difficulty of obtaining genetic samples from the many monotypic genera in the family—of 14 monotypic genera (including *Mimizuku*), 8 at most were included in previous molecular studies (Wink et al. 2009, Miranda et al. 2011, Dantas et al. 2016, Wood et al. 2016, Koparde et al. 2018). The 6 unsampled genera (*Uroglaux*, *Margarobyas*, *Xenoglaux*, *Pyrroglaux*, *Nesasio*, and *Jubula*) include species poorly known to science and poorly represented in biological collections. A more complete understanding of owl systematics requires inclusion of these species and the use of techniques that allow us to collect and analyze genetic data from historical specimens, like the enrichment of nuclear loci from museum specimen toepads (McCormack et al. 2015).

Here, we use targeted enrichment and analysis of ultraconserved elements (UCEs) collected from modern tissues and historical museum specimens to infer relationships among species of typical owls (Strigidae), and we include 6 monotypic genera that have not been used in previous molecular studies. We analyzed the collected data using concatenated maximum-likelihood, concatenated Bayesian, and multispecies coalescent approaches, and we also analyzed protein-coding regions of whole mitochondrial genomes to understand how different molecular markers may have affected the inference of the evolutionary relationships among members of this family.

METHODS

Taxonomy

Throughout this manuscript, we follow the taxonomy of Dickinson and Remsen (2013), with the exception of how we treat *Mimizuku/Otus gurneyi*. Although Dickinson and Remsen (2013) used the genus *Mimizuku* in their taxonomy, Miranda et al. (2011) showed that this species is nested within *Otus*. As a result, and to make clear that our findings are similar to those of Miranda et al. (2011), we have referred to this taxon as *Otus (Mimizuku) gurneyi*. We have also followed Dickinson and Remsen (2013) with respect to the subfamily name Ieraglaucinae, which has precedence over Ninoxinae (Wink et al. 2008) per ICZN (1999).

Sampling and DNA Extraction

Our study included 39 tissue and 7 toepad samples (between 51 and 115 yr old) from 45 specimens comprising all but one of 28 Strigidae genera and 1 specimen representing each of the 2 Tytonidae genera (Table 1). Generally, we selected samples based on availability from museum collections and assumed tissue quality, and we preferred to sample taxa that had not been included in previous molecular phylogenetic studies, when possible. For genera with more than 4 species, we included samples of 2 or more taxa. Finally, because *Xenoglaux loweryi* had not been included in previous molecular phylogenies and because multiple tissue samples were readily available to us, we included 2 individuals of this taxon so that we could be more confident in our phylogenetic placement of this monotypic genus. Although we received a toepad from *Heteroglaux blewitti* (the remaining monotypic genus), we were unable to include these data because they appeared to be contaminated with DNA from another bird. From all other tissue and toepad samples, we extracted DNA using either a Qiagen DNeasy Blood & Tissue Kit (Qiagen, Valencia, California, USA) following the manufacturer's instructions (tissues) or a phenol-chloroform protocol (toepads; Tsai et al. 2019). Because sequence capture of a 1931 specimen of *Margarobyas lawrencii* failed to recover any UCEs, we sampled a 1960 specimen of the same species from which we were able to recover UCEs. We were able to reconstruct a partial mitochondrial genome from the off-target sequencing reads of the 1931 specimen but the same procedure did not work well with the 1960 specimen. As a result, we included the 1960 *Margarobyas lawrencii* specimen in our nuclear UCE results and the 1931 specimen in our mitochondrial results. We also included data for the remaining barn owl genus and 3 outgroup taxa (Table 1) that we harvested from existing genome assemblies using PHYLUCE (Faircloth 2016) following the PHYLUCE Tutorial III guidelines (Faircloth 2015a).

Sequence Capture and Next-Generation Sequencing

Following DNA extraction, we collected sequence data using targeted enrichment of UCEs (Faircloth et al. 2012). To prepare tissue samples for targeted enrichment, we used an ultrasonicator (Qsonica, Newtown, Connecticut, USA) to shear 65 µL of DNA at 10 ng μL^{-1} to a peak size distribution of 400–600 base pairs (bp). Due to DNA degradation in the toepad samples, as noted by other researchers (McCormack et al. 2015, 2017), peak size distribution was already between 100 and 300 bp, and we did not sonicate these samples. From both sample types, we prepared dual-indexed genomic libraries using the KAPA Hyper Prep library preparation kit at one-half volume (F. Hoffmann-La Roche AG, Basel, Switzerland) and custom indexes (Glenn et al. 2019). Following library preparation, we combined 7 to 8 libraries into 6 pools for targeted enrichment, and we

TABLE 1. Sample information and genomic summary statistics.

Species	Museum / Source	Catalog no. / Tissue no. / Accession no.	Locality	Read pairs	UCEs	Average locus length	mtDNA reads	Average mtDNA coverage
Aegolius funereus	KU	28868	Mongolia	3,272,796	4,688	896	112,124	413
Apaloderma vittatum	Genbank	JMFV00000000	Tanzania	–	4,753	1,107	–	–
Asio clamator	UWBM	93240	Honduras	2,587,895	4,788	962	2,861	16
Asio flammeus	KU	21727	Kansas, USA	8,166,478	4,671	795	11,125	55
Asio otus	KU	15937	Kansas, USA	7,949,210	4,749	851	2,521	10
Athene cunicularia	KU	31327	Missouri, USA	3,891,484	4,689	912	34,383	112
Athene noctua	KU	21790	Spain	8,139,398	4,653	848	7,504	30
Athene superciliaris	FMNH	384685	Madagascar	2,537,748	4,821	964	2,703	11
Bubo cinerascens	KU	15360	Ghana	4,713,695	4,709	800	10,795	64
*Bubo nipalensis** (1949)	FMNH	189733	India	2,384,623	4,760	449	1,262	5
Bubo scandiacus	KU	27634	Kansas, USA	4,259,627	4,727	913	5,910	36
Ciccaba virgata	KU	4964	El Salvador	3,084,402	4,680	836	99,358	612
Falco peregrinus	Genbank	AKMT00000000	UAE[a]	–	4,819	1,113	–	–
Glaucidium brodiei	AMNH	10781	Vietnam	8,888,820	4,722	859	–	–
Glaucidium capense	UWBM	104493	Malawi	590,271	4,700	1,086	2,592	11
Glaucidium gnoma	KU	29507	Oregon, USA	3,306,411	4,715	926	17,790	64
Glaucidium tephronotum	AMNH	12422	Liberia	9,337,613	4,697	867	20,037	92
Haliaeetus leucocephalus	Genbank	JPRR00000000	USA	–	4,502	407	–	–
*Jubula lettii** (1933)	FMNH	270520	Cameroon	1,228,395	314	252	551	5
Ketupa ketupu	AMNH	9666	Unknown	6,915,649	4,725	895	–	–
Lophostrix cristata	LSU	40834	Peru	5,971,341	4,690	894	18,858	103
*Margarobyas lawrencii** (1931)	ANSP	111914	Cuba	–	–	–	874	13
*Margarobyas lawrencii** (1960)	LSU	142308	Cuba	576,351	982	240	–	–
Megascops albogularis	KU	29421	Peru	3,835,053	4,722	876	100,730	535
Megascops asio	KU	29844	Missouri, USA	4,436,977	4,719	890	24,476	126
Megascops nudipes	LSU	11317	Puerto Rico	4,625,746	4,722	952	3,255	6
Micrathene whitneyi	LSU	38772	Texas, USA	1,210,952	4,791	1,034	3,279	15
Nesasio solomonensis	AMNH	6621	Solomon Islands	873,912	4,743	1,062	1,378	8
Ninox boobook	KU	10706	Australia	5,637,018	4,692	885	48,692	245
Ninox jacquinoti	AMNH	6636	Solomon Islands	3,843,012	4,608	836	8,317	27
Otus (Mimizuku) gurneyi	KU	19248	Philippines	7,180,958	4,688	889	6,591	43
Otus elegans	KU	10975	Philippines	2,589,384	4,669	910	43,626	324
Otus rufescens	KU	17816	Malaysia	2,007,177	4,712	906	29,993	185
Phodilus badius	AMNH	10244	Singapore	11,279,405	4,676	865	–	–
*Pseudoscops grammicus** (1961)	DMNH	2203	Jamaica	1,398,677	4,512	329	1,921	8
Psiloscops flammeolus	LSU	20019	California, USA	859,340	4,757	1,021	1,280	8
Ptilopsis granti	LACM	115604	Aviary	4,902,354	4,852	682	1,185	7
Pulsatrix melanota	KU	18423	Peru	9,769,518	4,683	788	23,846	133
Pyrroglaux podargina	KU	23683	Palau	8,508,853	4,694	837	195,768	1419
*Sceloglaux albifacies** (1903)	AMNH	230260	New Zealand	559,371	2,273	247	–	–
*Scotopelia peli** (1962)	FMNH	262870	Botswana	854,858	4,534	376	6,778	27
Strix aluco	KU	6764	England	5,445,075	4,662	881	8,373	49
Strix rufipes	KU	11745	Argentina	1,996,628	4,726	906	20,356	124
Strix varia	KU	22621	Florida, USA	6,966,076	4,733	807	55,177	327
Strix woodfordii	KU	29079	DRC[b]	2,092,763	4,701	886	42,293	236
Surnia ulula	KU	9490	Minnesota, USA	6,854,350	4,660	855	18,961	76
Tyto alba	Genbank	JJRD00000000	USA	–	4,690	1,094	–	–
Uroglaux dimorpha	KU	31387	Papua New Guinea	269,946	3,594	416	–	–
Xenoglaux loweryi	LSU	44203	Peru	2,573,555	4,692	904	42,556	200
Xenoglaux loweryi	LSU	44364	Peru	893,654	4,416	912	8,688	36
*Average (toepad samples)				1,167,046	2,896	315	2,277	12
Average (tissue samples)				4,673,450	4,675	880	28,816	160
Average all samples				4,205,929	4,459	815	25,711	143

* Denotes toepad sample (year collected).
[a] United Arab Emirates.
[b] Democratic Republic of Congo.

made sure to create separate pools of tissue libraries and toepad libraries. We enriched each library pool for 5,060 UCE loci using a MYbaits_Tetrapods-UCE-5K kit (Arbor Biosciences, Ann Arbor, Michigan, USA) following a protocol (Faircloth et al. 2018) modified from Faircloth et al. (2012). Following enrichment, we used 16 cycles of PCR to increase the amount of enriched DNA, and we ran the amplified, post-enrichment pools on a Bioanalyzer (Agilent Technologies, Santa Clara, California, USA) to verify peak size distributions and check for the presence of adapter-dimer. When necessary, we used additional SPRI bead cleanups (Rohland and Reich 2012) to remove remaining adapter dimer. Finally, we quantified pools using a KAPA qPCR quantification kit (F. Hoffmann-La Roche AG), and we combined pools together at equimolar ratios prior to 150-bp paired-end (PE150) sequencing using 2 lanes of an Illumina HiSeq 3000 run (Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma, USA).

Bioinformatic Processing, Assembly, and Alignment of UCEs

The sequencing center returned FASTQ sequence data to us, and we used illumiprocessor (Faircloth 2013), a wrapper around Trimmomatic (Bolger et al. 2014), to remove adapter sequences from the data and trim raw reads for quality. Because some libraries received a larger number of FASTQ reads than others, we used *seqtk* (Li 2012) to randomly downsample libraries having more than 2 million cleaned read pairs (i.e. 4 million reads, in total). We then assembled reads into contigs using PHYLUCE (Faircloth 2016) and SPAdes (Bankevich et al. 2012). To check assembled libraries for the correct species identification and potential contamination, we ran the PHYLUCE program *match-contigs-to-barcodes* (Faircloth 2016) using a *Bubo virginianus* COI sequence (NCBI GenBank EU525335.1) as a reference. We then input extracted contigs that matched the reference COI to NCBI BLAST (Johnson et al. 2008) to compare the extracted sequences to sequences in NCBI GenBank, confirm the identity of each sample, and check for any contaminating (different species identity) COI sequences. For the remainder of the data processing steps, we followed the PHYLUCE Tutorial I guidelines (Faircloth 2015b): we identified contigs containing UCE loci by matching contigs to the UCE probe sequences (Table 1), we aligned UCE loci with MAFFT 7.13 (Katoh and Standley 2013), and we performed internal trimming of alignments with default parameters in GBLOCKS 0.91b (Castresana 2000). We removed loci that were missing data from more than 13 taxa to produce a 75% complete data matrix containing 4,253 UCE loci.

Mitochondrial Genome Assembly and Alignment

We wanted to compare our phylogenetic results from nuclear UCE loci to prior studies that have primarily analyzed data from mitochondrial genes. Because data from targeted enrichment can include reads from genomic regions other than those targeted, some of these reads can be assembled into entire mitochondrial genome sequences (Picardi and Pesole 2012, Raposo do Amaral et al. 2015). We used MITObim 1.9 (Hahn et al. 2013), a PERL wrapper around MIRA 4.0.2 (Chevreux et al. 1999), to reconstruct mitochondrial genomes from the FASTQ data for 40 of our samples, using a mitochondrial genome of *Otus scops* from NCBI Genbank as a reference (Supplemental Material Table S1). We also downloaded 15 mitochondrial genome assemblies representing additional ingroup and outgroup taxa (Supplemental Material Table S1) from NCBI GenBank. Then, we used Geneious 6.0.5 (https://www.geneious.com) to propagate the annotation of the *Otus scops* mitochondrial genome to the other assemblies assuming 54% sequence similarity (the threshold at which the Geneious live annotation tool identified the protein-coding genes across all other assemblies), and we extracted 13 mitochondrial protein-coding genes from each mitochondrial genome assembly (Supplemental Material Table S1). We aligned each extracted gene independently using the Geneious Aligner with default parameters in Geneious 6.0.5, trimming poorly aligned edges where necessary, and we concatenated all 13 protein-coding gene alignments using Sequence Matrix 1.7.8 (Vaidya et al. 2011).

Concatenated UCE Phylogenies

For the UCE dataset, we analyzed the unpartitioned, concatenated data matrix using both maximum likelihood (ML) and Bayesian inference (BI) approaches. For the ML analysis, we used ExaML 3 (Kozlov et al. 2015) with the GTRGAMMA model to perform 20 searches for the optimal ML tree and 100 bootstrap replicate analyses, and we reconciled the best ML tree with the bootstrap replicates using Sumtrees 3.3.1 (Sukumaran and Holder 2010) with default parameters and the option to output support values as percentages. For the BI analysis, we used ExaBayes 1.5 (Aberer et al. 2014) with the GTRGAMMA model to sample 4 independent runs of 2 Markov chains each for 2 million generations. After discarding the first 10% of generations as burn-in, we assessed convergence between the runs by ensuring that the average standard deviation of split frequencies between the sampled trees was <0.5%, the effective sample size of all parameters across the combined log files was >200, and the potential scale reduction factor of all parameters across all runs was <1.1. We used the *consense* program in ExaBayes to produce a 50% majority-rule consensus tree with 10% burn-in.

Coalescent-Based UCE Phylogenies

Because UCE data are collected from putatively independent, nuclear loci, we used 2 coalescent species tree estimation methods to account for heterogeneous gene/

locus histories. First, we used PHYLUCE (Faircloth 2016) to perform site- and locus-wise resampling (Seo 2008) of the 75% data matrix and generate 100 bootstrapped subsets of this data matrix. Then, we used RAxML 8.2.8 (Stamatakis 2014) with the GTRGAMMA model and GNU Parallel 3+ (Tange 2011) to infer gene trees for each UCE locus in the bootstrapped subsets, and we used PHYLUCE to sort the resulting gene trees among resulting subsets. Finally, we input each bootstrapped subset of loci into ASTRAL-III 5.6.1 (Zhang et al. 2017) using GNU Parallel 3+ (Tange 2011) to infer species trees from all 100 subsets of multilocus bootstraps, and we generated a majority rule consensus tree from the 100 bootstrapped trees using Sumtrees 3.3.1 (Sukumaran and Holder 2010) with default parameters other than enabling the options to output support values as percentages and collapse nodes having less than 70% support.

We also analyzed the UCE data using SVDquartets (Chifman and Kubatko 2014), which is a coalescent approach (Wascher and Kubatko 2019) that does not rely on gene tree estimation, which can be error prone (Roch and Warnow 2015). Specifically, we used RAxML 8.2.8 (Stamatakis 2014) to generate 100 bootstrap replicates from our concatenated UCE alignment and PAUP* 4.0a161 (Swofford 2002) to generate quartet trees by singular value decomposition. We then used the *max-cut-trees* method in Quartet MaxCut 3.0 (Snir and Rao 2012) to infer species trees from quartet trees, which we summarized using Sumtrees 3.3.1 (Sukumaran and Holder 2010).

Mitochondrial Genome Phylogeny

Because the mitochondrial dataset was smaller and because partitioning mitochondrial data is straightforward, we used the *rcluster* search algorithm with unlinked branch lengths in PartitionFinder2 2.1.1 (Lanfear et al. 2017) to select an appropriate partitioning scheme and nucleotide substitution models for the data (Lanfear et al. 2014). Specifically, we tried partitioning the data by gene, as well as by codon position within each gene, and we ultimately selected a 15-partition scheme that accounted for codon position (AIC_c score 392503.159647, log-likelihood −194180.089966). We then used RAxML 8.0.19 (Stamatakis 2014) with the GTRGAMMA model to perform 100 searches for the optimal ML tree, perform bootstrap replicate searches using the autoMRE option, and reconcile the best tree with the bootstrap replicates.

RESULTS

Recovery of UCEs and Mitochondrial Genomes

We obtained an average of 4.6 million read pairs from tissue samples (range: 269,946–11,279,405 reads) and 1.1 million read pairs from toepad samples (range 358,877–2,384,623) (Table 1). After downsampling libraries, we used these reads to assemble an average of 142,107 contigs per tissue sample (mean length = 880 bp) and 29,071 contigs per toepad sample (mean length = 315 bp). Among the assembled contigs, we identified an average of 4,675 UCE loci from tissue samples and 2,896 UCE loci from toepad samples (Table 1). We enriched a total of 4,325 UCEs shared by at least 36 ingroup and outgroup taxa, producing a GBLOCKS-trimmed, concatenated, 75% complete data matrix containing 2,034,468 characters and 99,241 parsimony informative sites. Two toepad samples, *Jubula lettii* and *Margarobyas lawrencii*, underperformed during enrichment, and we recovered fewer than 1,000 UCE loci from each (Table 1). Although it was sequenced from a recently collected tissue sample, *Uroglaux dimorpha* yielded the lowest number of read pairs and a short average contig length consistent with the toepad samples (Table 1). To examine the effects of including/excluding suboptimal samples on our results, we also produced a filtered, concatenated, GBLOCKS-trimmed 75% complete data matrix excluding all toepad samples and *Uroglaux dimorpha*, which included 4,642 UCE loci; 3,181,795 characters; and 245,724 parsimony informative sites.

We were able to use off-target reads and MitoBim to reconstruct mitochondrial genomes for 40 of the 45 taxa, including 5 toepad samples—one from which we were unable to recover UCEs (Table 1). The mitochondrial genomes we assembled from tissue samples had an average coverage of 156X while mitochondrial genomes we assembled from toepad samples had an average coverage of 12X (Table 1). Because samples with low coverage are prone to assembly problems (Hubisz et al. 2011), we excluded mitochondrial assemblies where coverage was lower than 5X. After extracting and aligning genes from the 40 mitochondrial genomes we assembled, as well as 15 mitochondrial genome assemblies that we downloaded from NCBI GenBank, we created a 97% complete data matrix from 13 protein-coding mitochondrial gene alignments across 55 taxa to produce a concatenated alignment of 11,366 characters and 5,999 parsimony informative sites (Supplemental Material Table S1).

Concatenated UCE Phylogenies

ML and BI analyses of our concatenated UCE data produced identical, highly supported phylogenetic hypotheses of relationships among the sampled lineages (Figure 2A; see Supplemental Material Figure S1 for branch lengths). These concatenated analyses resolve barn owls and typical owls as reciprocally monophyletic and suggest that typical owls comprise 2 sister clades, which we have labeled Clades A and B (Figure 2A). Clade A comprises (1) a small Australasian group, tribe Ninoxini (Weick 2006), containing *Uroglaux dimorpha* of New

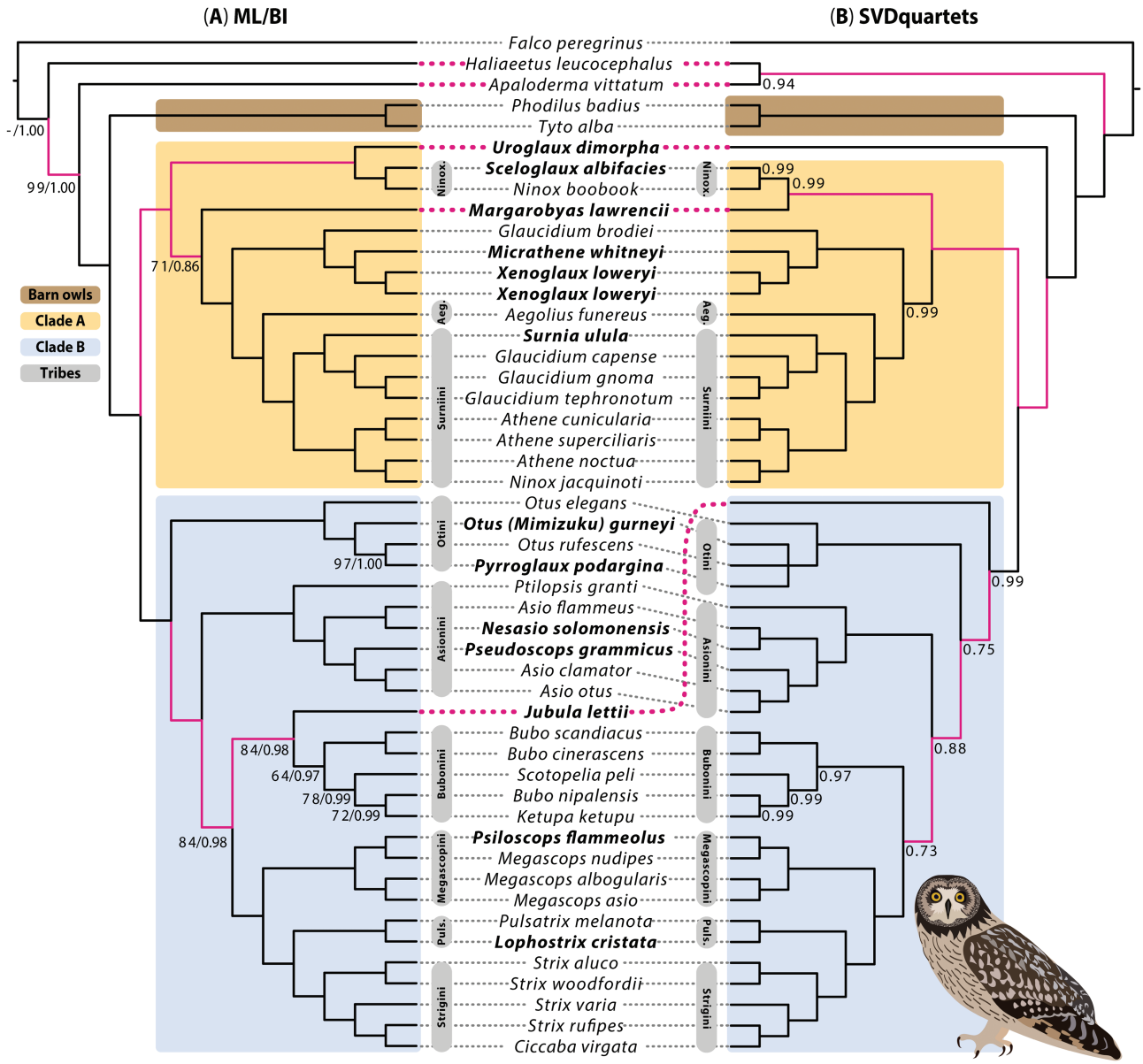

FIGURE 2. Cladogram of 46 owls (44 typical owls, 2 barn owls) inferred with (**A**) maximum likelihood (ML) and Bayesian inference (BI) analyses and (**B**) SVDquartets analysis of 4,235 nuclear ultraconserved element loci. ML and BI analyses produced identical topologies. Taxa in bold represent monotypic genera. Rounded gray boxes bracketing species names denote typical owl tribes: Ninox. = Ninoxini, Aeg. = Aegolini, Puls. = Pulsatrigini. All nodes received 100% bootstrap support / Bayesian posterior probability (BPP) unless otherwise labeled (ML bootstrap support/BPP). Nodes with <70% support have been collapsed, and pink branches and dotted lines indicate conflicting relationships. See Supplemental Material Figure S1 for a phylogram of the same relationships.

Guinea (monotypic) as sister to a clade of *Sceloglaux albifacies* of New Zealand (monotypic) and *Ninox boobook* of Australasia, and (2) a larger clade that includes the previously unsampled, monotypic *Margarobyas lawrencii* of Cuba as sister to a group of taxa that are known as subfamily Surniinae (Wink et al. 2008). Interestingly, our results confirm previous supposition that *Xenoglaux loweryi* belongs within Surniinae (as presented in Wink et al. 2008) and strongly suggest *Glaucidium brodiei* is sister to a clade comprising *Micrathene whitneyi* and *Xenoglaux loweryi* rather than forming a clade with other species of *Glaucidium*.

Clade B corresponds largely to a group of taxa known as Striginae (Wink et al. 2008). Within this group we resolve a clade of *Otus* relatives (tribe Otini, including *Pyrroglaux podargina*; Wink et al. 2008) sister to the remainder of taxa within the subfamily. This remaining group includes a clade of *Ptilopsis granti* + *Asio* relatives (tribe Asionini;

Wink et al. 2008) sister to a large clade containing species in tribes Bubonini, Megascopini, Pulsatrigini, and Strigini—the membership of which our results corroborate Wink et al. (2008). Novel relationships that we recover are resolution of *Jubula lettii* as sister to tribe Bubonini (cf. coalescent results, below) rather than tribe Strigini (Weick 2006, Wink et al. 2008), and the placement of *Margarobyas lawrencii* within Surniinae rather than tribe Otini (Weick 2006, Wink et al. 2008).

To examine the effects of suboptimal samples in our analyses, we also inferred an ML tree without the toepad samples or *Uroglaux dimorpha*, which resolves the same backbone topology as Figure 2 with improved support, particularly within tribe Bubonini (Supplemental Material Figure S2).

Coalescent-Based UCE Phylogenies

Our results from SVDQuartets generally agree with our concatenated analyses except that this analysis resolves *Uroglaux dimorpha* as sister to all other Strigidae, *Margarobyas lawrencii* as sister to Australasian tribe Ninoxini, and *Jubula lettii* as sister to all remaining lineages within Striginae (Figure 2B). Our results from the coalescent-based program ASTRAL-III diverge much more from our concatenated and SVDQuartets analyses (Supplemental Material Figure S3). However, support for many of these different relationships was low, and many of the taxa from which we collected toepad sequence data were pulled toward the root of the tree—a pattern that can result as an artefact of including short UCE contigs assembled from toepad data in some coalescent-based analyses (Hosner et al. 2016, Moyle et al. 2016). Because of the problems associated with including toepads in ASTRAL analyses, we focus our discussion of coalescent-based results on the SVDQuartets tree.

Mitochondrial Genome Phylogeny

Our ML phylogeny of 13 protein-coding regions extracted from 55 mitochondrial genome assemblies (Supplemental Material Figure S4) differs substantially from our concatenated and coalescent (SVDQuartets) analyses of nuclear DNA, while largely agreeing with topologies previously resolved using a combined 2-gene mito-nuclear dataset (Wink et al. 2008, 2009). Specifically, we recover a clade "C" sister to Clades A and B that roughly corresponds to Ieraglaucinae (Wink et al. 2008, 2009). Clade A comprises the Surniinae, and the relationships among the taxa in this group do not differ from our concatenated and coalescent analyses of UCE data. Similarly, within Clade B, the mitochondrial analyses resolve a clade of *Otus* relatives (tribe Otini, including *Pyrroglaux podargina*; Wink et al. 2008) sister to remaining taxa within Striginae, and the mitochondrial data mirror the UCE results by resolving *Ptilopsis granti* + *Asio* relatives (tribe Asionini; Wink et al. 2008) sister to the remaining species in tribes Bubonini, Megascopini, Pulsatrigini, and Strigini (note, however, the reversed positions of *Asio clamator* and *Pseudoscops grammicus*). The major differences between the mitochondrial and UCE data occur among the relationships we resolve for these remaining tribes: rather than recovering *Jubula lettii* + tribe Bubonini as sister to a clade comprising Megascopini, Pulsatrigini, and Strigini, the mitochondrial data suggest that tribe Megascopini is sister to an unresolved group that includes tribes Pulsatrigini, *Jubula lettii* + Bubonini, and Strigini.

DISCUSSION

Previous phylogenetic studies of typical owls have differed demonstrably in resolution of relationships among clades (Figure 1) and placement of lineages. Some of these contrasting results reflect differences in the types of morphological characters or genetic data analyzed, whereas others can be attributed to incomplete overlap in taxon sampling. Here, we tried to overcome several of these difficulties by collecting and analyzing thousands of ultraconserved nuclear loci from 43 Strigidae species that represent all but one of the 28 genera within the group.

The topologies we recover from concatenated and SVDQuartets analyses of thousands of nuclear UCE loci are well resolved and stable between analytical paradigms, although they differ, at several levels, from existing hypotheses of Strigidae relationships inferred from fewer loci. At the subfamily level, we recover 2 main clades of typical owls that we have designated Clade A and Clade B, and which generally correspond to hypotheses of relationships within Strigidae proposed by Wink et al. (2008, 2009; see below). It is important to note that the placement of *Uroglaux dimorpha* within Clade A in our concatenated results conflicts strongly with the placement of *Uroglaux dimorpha* as sister to Clades A and B in our coalescent-based analyses. We regard the position of *Uroglaux dimorpha* as uncertain, and this discrepancy could be caused by the amount of data missing between *Uroglaux dimorpha* and its putative sister lineage(s)—a problem that has been observed elsewhere (Oliveros et al. 2019). To better understand this problem, future empirical studies of this group should use higher-quality samples from this taxon, and future simulation studies should investigate the impact of missing data on SVDQuartets analyses.

Within Clades A and B, the concatenated and SVDQuartets results recover topologies that generally support higher-level taxonomic designations of typical owl tribes described in previous studies such as Weick (2006) and Wink et al. (2008, 2009). However, at the genus level, our results suggest that a number of generic names are problematic with respect to current taxonomy (Dickinson

and Remsen 2013), as demonstrated by the paraphyly of *Athene*, *Otus*, *Asio*, *Megascops*, *Bubo*, and *Strix* and the polyphyly of *Ninox* and *Glaucidium*. As we observed with *Uroglaux dimorpha*, it is important to note that *Jubula lettii* appears in 2 very different positions between our concatenated and coalescent-based results, making it hard to determine whether this lineage is sister to all remaining taxa within Clade B or whether *Jubula lettii* should be included in tribe Bubonini.

Although our concatenated and coalescent-based SVDQuartets analyses largely agree, comparison of these topologies with the species tree inferred using ASTRAL was difficult. This was due to poor resolution of the ASTRAL tree and the fact that most of the taxa having data generated from toepads were placed in uncertain phylogenetic positions. The poor resolution of the ASTRAL species tree is not entirely surprising for the following reasons: (1) we recovered fewer loci from low-quality DNA extracts, and ASTRAL species tree reconstruction can be negatively affected by having too few loci (Shekhar et al. 2018); (2) DNA extracts from low-quality sources like toepads or degraded tissues produce relatively short contigs that can lead to gene tree estimation error and poor resolution of the species tree (Roch and Warnow 2015, Hosner et al. 2016, Moyle et al. 2016); and (3) the inclusion in alignments of short DNA sequences for some taxa with longer DNA sequences from others (i.e. "type-II" missing data) can produce inaccurate species trees (Hosner et al. 2016, Sayyari et al. 2017), sometimes pulling tips having missing data erroneously toward the root (Moyle et al. 2016). For example, the sequence data we collected from a degraded tissue sample of *Uroglaux dimorpha* and 6 toepad samples of other taxa assembled to mean contig lengths ~562 bp shorter (95 CI: 330 ± 64 bp) than the mean contig length we observed for UCE contigs assembled from tissues (95 CI: 892 ± 24 bp; Supplemental Material Figure S3, Table 1). When analyzing these data using ASTRAL, samples having fewer loci and shorter contig assemblies were pulled toward the root of the ASTRAL species tree (Supplemental Material Figure S3), although support for these relationships was low. Because SVDQuartets analyzes quartets of concatenated alignments as input, SVDQuartets should be affected to a smaller degree by the inclusion of degraded or historical samples having a large number of short contigs relative to gene tree reconciliation approaches (Hosner et al. 2016), and we have focused on the SVDQuartets results. As noted above, future simulation studies would help quantify the circumstances under which SVDQuartets performs optimally.

When we compare results from thousands of nuclear loci (Figure 2) to those we inferred from 13 protein-coding mitochondrial genes (Supplemental Material Figure S4), we corroborate some of the findings reported by earlier studies (Figure 1; Wink et al. 2008, 2009; Wink 2016). For example, our mitochondrial analysis recovers "Clade C," (*Margarobyas*, *Sceloglaux*, and *Ninox*) that is sister to Clades A+B and largely corresponds to the typical owl subfamily Ieraglaucinae (Figure 1E; Wink et al. 2008, 2009; Wink 2016). Within Clades A and B, the mitochondrial analysis recovers relationships among and within most of the tribes that are similar to the results from thousands of nuclear DNA loci, although notable differences exist in the positions of tribes Bubonini and Megascopini and the branching order of lineages within tribes Otini and Asionini. The mitochondrial analyses also generally support the results from our nuclear DNA analyses, which suggest that *Athene*, *Otus*, *Asio*, *Megascops*, *Bubo*, and *Strix* are paraphyletic, while *Ninox* and *Glaucidium* are polyphyletic, although some of the taxa that we used and some of the placements of these taxa are different in the mitochondrial topology. Although the potentially conflicting evolutionary histories we observe between mitochondrial and nuclear DNA topologies are interesting and reflect patterns observed in other studies (Zarza et al. 2016, Platt et al. 2018), we place more weight on the concatenated and coalescent topologies we inferred from thousands of putatively independent nuclear loci (Figure 2) vs. results generated from the single evolutionary history encoded in the mitochondrial genes we used (Supplemental Material Figure S4).

Subfamily Relationships

Throughout this paper, we have generally ignored subfamily taxonomy and referred to Clades A, B, and C in our phylogenetic results to reduce confusion. However, these clade designations sometimes correspond with traditional typical owl subfamily taxonomy. For example, in our concatenated UCE analysis, we recover Clade A, which includes *Uroglaux dimorpha* and generally corresponds to subfamilies Surniinae + Ieraglaucinae. Ford (1967) thought these 2 subfamilies should be lumped ("Surniinae") based on osteological evidence. Our coalescent-based, SVDQuartets analysis makes interpreting exact membership of Clade A more complicated, however, because we resolved *Uroglaux dimorpha* as a representative of a group that is sister to Clades A+B. Moving beyond the exact membership of Clade A, both concatenated and coalescent analyses resolve the membership of Clade B, which generally corresponds to subfamily Striginae as recognized by Wink et al. (2008) and Dickinson and Remsen (2013). Importantly, neither concatenated nor coalescent-based analyses of thousands of nuclear loci recover Clade C (comprising *Margarobyas*, *Ninox*, and *Sceloglaux*) as sister to Clades A+B (Wink et al. 2008, 2009) or Clade A as sister to Clades B+C (Wink 2016). The conflicting evidence from mitochondrial data (Wink et al. 2008, 2009; Wink 2016) and a dearth of clear osteological evidence (Ford 1967) suggest that further genetic and morphological investigation is needed to verify the subfamily status of Ieraglaucinae.

Novel Relationships among Typical Owls

Above, we have focused on a number of higher-level results suggested by our concatenated and coalescent phylogenetic hypotheses, although many of the difficulties inferring high-level relationships among typical owls are recapitulated within and among typical owl genera and species. Below, we have provided additional detail relative to a number of interesting results at the genus and species levels.

Sceloglaux. Our concatenated and coalescent-based analyses (as well as our concatenated mitochondrial data) confirm previous mitochondrial analyses that placed the extinct New Zealand endemic *Sceloglaux albifacies* within a clade containing *Ninox* species (Wood et al. 2016). These results are consistent with the biogeography of these species, given the primarily Australasian distribution of the genus *Ninox* and the fact that *Sceloglaux albifacies* was a New Zealand endemic.

Uroglaux. Our concatenated nuclear DNA results suggest that *Uroglaux dimorpha* of New Guinea is sister to *Ninox boobook* + *Sceloglaux albifacies* (*Ninox* + *Sceloglaux*, hereafter) consistent with the biogeography of these species and previous speculation regarding the phylogenetic affinities of *Uroglaux* (Weick 2006, Wink et al. 2008). However, the differences we observed in the placement of *Uroglaux dimorpha* between our concatenated and coalescent-based topologies suggest that increased taxon sampling and/or the inclusion of higher-quality DNA extracts are needed to definitively establish whether *Uroglaux dimorpha* (1) is sister to all remaining typical owl lineages, (2) is sister to the clade of *Ninox* + *Sceloglaux,* or (3) may be nested within the *Ninox* + *Sceloglaux* clade.

Margarobyas. Sequence capture from 2 *Margarobyas lawrencii* specimens was suboptimal, possibly due to the age of the specimens and/or means of preservation. Even after collecting additional sequencing reads for each sample, we enriched zero UCE loci from the 1931 specimen and 982 UCEs from the 1960 specimen. Additionally, the 982 UCE loci from the second sample were short, having a mean locus length of 240 bp (Table 1). Although the exact placement of *Margarobyas lawrencii* differs between our concatenated and coalescent analyses, both analyses strongly suggest that *Margarobyas lawrencii* is a member of Clade A, contradicting previous supposition that *Margarobyas lawrencii* was closely related to *Megascops nudipes* (Sclater and Salvin 1868, Olson and Suarez 2008), a taxon we recover deeply nested within Clade B.

Micrathene* and *Xenoglaux. One of the most surprising results of these analyses is our confirmation of a close relationship between *Glaucidium brodiei*, *Micrathene whitneyi*, and *Xenoglaux loweryi.* These results support an idea initially proposed by O'Neill and Graves (1977) in their description of *Xenoglaux* based on shared similarity of skeletal, morphological, and plumage characters, and they better resolve earlier mito-nuclear analyses that sampled fewer taxa and genetic loci and placed *Micrathene* sister to *Aegolius*, *Athene*, *Surnia*, and *Glaucidium* (Wink et al. 2008). Although our resolution of the relationships among *Glaucidium*, *Micrathene*, *and Xenoglaux* differs from earlier findings, the relationships we resolve are supported across the concatenated, SVDQuartets, and ASTRAL analyses of UCE data (Figure 2, Supplemental Material Figure S3). This result is also supported in our mitochondrial analysis (Supplemental Material Figure S4), using a *Glaucidium brodiei* mitochondrial genome sequence from NCBI GenBank (Supplemental Material Table S1).

The close relationship between *Glaucidium brodiei*, *Micrathene*, and *Xenoglaux* suggests that these species may be relicts of a once-widespread lineage of owls. Similar biogeographic patterns have been observed in passerine birds, such as superfamily Bombycilloidea, which arose during the early Miocene (~20 MYA; Oliveros et al. 2019) and includes monotypic families endemic to Hispaniola, Sulawesi, and the Hawaiian archipelago, as well as species-poor Mesoamerican and panarctic families (Fleischer et al. 2008, Spellman et al. 2008, Oliveros et al. 2019). Time-calibrated analyses and expanded taxonomic sampling of owls will help elucidate biogeographic hypotheses of how this clade formed and may uncover additional members of this lineage.

Ninox jacquinoti. All analyses of nuclear and mitochondrial data recover *Ninox jacquinoti* of the Solomon Islands in a clade of *Athene* species within tribe Surniini. There is some precedent for this result: a recent analysis of 24 species of *Ninox* from Wallacea using 2 mitochondrial and 5 nuclear genes included *Ninox jacquinoti* and *Athene superciliaris* as outgroups, and the authors found *Ninox jacquinoti* was more closely related to *Athene superciliaris* than to the ingroup *Ninox* species (Gwee et al. 2017). *Athene superciliaris*, a Madagascar endemic, was once placed in *Ninox*, until analysis of cytochrome b and *RAG-1* genes suggested that it was nested within the *Athene* clade (Wink et al. 2008). Our analyses confirm these findings. Interestingly, *Ninox jacquinoti* was originally described as a member of *Athene* (Bonaparte 1850), and it is unclear when or why it was assigned to *Ninox* (Peters 1940). Because our analyses suggest *Ninox jacquinoti* is a member of the genus *Athene* and because we resolve *Ninox* as polyphyletic, future studies should thoroughly sample all proposed taxa in *Ninox* and *Athene.*

Pyrroglaux* and *Otus (Mimizuku). All analyses of nuclear and mitochondrial data suggest that *Pyrroglaux podargina* is a member of tribe Otini. In the original species description of *Pyrroglaux podargina*, Hartlaub and Finsch (1872) placed the species in *Noctua* (*Ninox*), describing it as "one very indifferent specimen" most similar to *Noctua (Ninox) ochreacea* of Sulawesi. *Pyrroglaux podargina* was elevated to the genus *Pyrroglaux* by

Yamashina (1938), who noted its close affinity with *Otus*, although Yamashina concluded that it deserved monotypic status because of several morphological traits, including lack of ear tufts, reduced facial disks, reduced number of rectrices, and bare tarsi and toes. Our analyses suggest that *Pyrroglaux podargina* is an *Otus* species, and its unusual morphological features may have evolved under intense drift and selective pressure(s) after its ancestors reached the Palau Archipelago. This scenario is similar to that of *Otus* (*Mimizuku*) *gurneyi*. Formerly placed in its own genus, *Mimizuku* (Hachisuka 1934), due to its large size, mitochondrial data subsequently showed that *Mimizuku gurneyi* was nested within the *Otus* clade and that gigantism evolved once this species reached the Philippines (Miranda et al. 1997, 2011). Our analyses confirm this placement of *Otus* (*Mimizuku*) *gurneyi*.

***Nesasio* and *Pseudoscops*.** Our concatenated and SVDQuartets results strongly suggest that the monotypic *Nesasio* and *Pseudoscops* are nested within *Asio*. In his description of *Nesasio*, Peters (1937) discussed the parallels between *Nesasio solomonensis* of the Solomon Islands and *Pseudoscops grammicus* of Jamaica, noting that both species show enlarged bills and feet, shorter wings, and emarginated primaries when compared with mainland species of *Asio*. Peters used this comparison to argue that *Nesasio solomonensis* and *Pseudoscops grammicus* were insular forms derived from widespread *Asio* species. However, in a detailed analysis of skeletal anatomy, Olson (1995) noted similarities between *Pseudoscops grammicus* and *Rhinoptynx clamator*, a widespread species from southern Mexico to Argentina, and he used these data to place *Rhinoptynx clamator* in the previously monotypic genus *Pseudoscops*. Olson (1995) used cranial morphology to suggest that these species and *Nesasio solomonensis* were relictual forms of a once widespread Asioninae lineage. Subsequent mitochondrial analysis suggested *Pseudoscops clamator* was nested within Asionini (Wink et al. 2004), and *Pseudoscops clamator* was renamed *Asio clamator*, leaving the monotypic status of *Pseudoscops grammicus* in question, once again. Supporting Peter's (1937) hypothesis, our analyses demonstrate that all 3 species are nested within Asionini, although *Asio clamator* and *Pseudoscops grammicus* are not sister taxa, as once thought (Olson 1995).

***Jubula*.** Likely due to the challenges associated with capturing a sufficient number of nuclear loci from a single, relatively old (1933; Table 1) toepad, we are unable to resolve relationships of *Jubula lettii*. Although both concatenated UCE and concatenated mitochondrial data suggest that *Jubula lettii* is a member of tribe Bubonini, our SVDQuartets result strongly conflicts with this placement (Figure 2) and our ASTRAL topology does not provide additional clarification (Supplemental Material Figure S3). The SVDQuartets placement for *Jubula* should be interpreted cautiously, as noted above, because the sensitivity of quartet-based analyses to a small number of low-quality DNA sequences has not been well tested. There is some precedent for the placement of *Jubula lettii* within Bubonini, although the taxonomic history of *Jubula* is muddy. In his description, Büttikofer (1889) noted plumage similarities between *Jubula lettii* and *Bubo (Lophostrix) cristata* and assigned *Jubula lettii* to *Bubo.* Reichenow (1900) later moved it to *Lophostrix*, until it was placed in its own genus, *Jubula*, on the basis of "peculiarly long and shaggy plumage" (Bates 1929). Although both our concatenated and coalescent-based results contradict the hypothesis of affinity between *Jubula lettii* and *Lophostrix cristata*, further sampling of additional *Jubula* specimens and members of Bubonini are needed to resolve relationships of this taxon.

Taxonomic Recommendations

Based on our results, we suggest several changes to current Strigidae taxonomy. Within Clade A, we propose transferring *Ninox jacquinoti* to *Athene* and subsuming *Sceloglaux* within *Ninox*, as suggested by Wood et al. (2016). Within Clade B, we propose *Pyrroglaux* and *Mimizuku* (Miranda et al. 1997, Miranda et al. 2011) be subsumed within *Otus*, and *Nesasio* and *Pseudoscops* be subsumed within *Asio*. Our findings support those of previous authors (Wink et al. 2008, 2009; Wink 2016) who have suggested subsuming both *Scotopelia* and *Ketupa* within *Bubo*. Similarly, we agree that *Ciccaba* should be subsumed within *Strix* (Marks et al. 2018). Consistent with previous findings (Dantas et al. 2016), our results support a sister relationship between *Psiloscops flammeolus* and the Puerto Rican endemic *Megascops nudipes* outside the remaining *Megascops* species. Using mitochondrial data, Dantas et al. (2016) found the divergence between *Psiloscops* + *Megascops nudipes* and the rest of *Megascops* to be ~20 million yr old but refrained from proposing taxonomic changes for *Megascops nudipes* until sequence data from *Margarobyas lawrencii* could be included. *Megascops nudipes* and *Margarobyas lawrencii* were once considered conspecific (Olson and Suarez 2008), but in light of our results showing that *Margarobyas* is not closely related to this group, we suggest that *Megascops nudipes* be transferred to *Psiloscops*. Given the genetic, morphological and behavioral distinction of *Glaucidium brodiei* from its closest living relatives, *Micrathene* and *Xenoglaux*, we propose that *Glaucidium brodiei* be transferred from *Glaucidium* to a different genus. The generic name *Taenioptynx* was applied to *Glaucidium brodiei* by Kaup (1848) and appears to have priority. Recently, *Glaucidium brodiei* has been split into multiple species (Gwee et al. 2019), thus *Glaucidium brodiei* and those presumed closely related taxa (no genetic data were included in the proposed split) should be included in *Taenioptynx*.

Morphology Obscures Owl Evolutionary History

One of the broad patterns evident in our results is the strong effect of island colonization in owls—of the 12 monotypic genera of typical owls currently recognized, 7 of these species have insular distributions (*Sceloglaux*, *Pyrroglaux*, *Mimizuku*, *Nesasio*, *Pseudoscops*, *Margarobyas*, and *Uroglaux*). These island species were placed in monotypic genera due to pronounced morphological differentiation from mainland species of owls, although our results and other molecular analyses (Miranda et al. 1997, 2011; Wood et al. 2016) suggest that at least 5 of these species (*Sceloglaux*, *Pyrroglaux*, *Mimizuku*, *Nesasio*, and *Pseudoscops*) are actually nested within larger genera and do not warrant monotypic status. The contrast between molecular vs. morphological results highlights the strong effects of colonization in promoting morphological divergence in owls, and they suggest that owls are another example of convergence toward the "island syndrome" (Adler and Levins 1994) observed in other insular bird species (Wright and Steadman 2012, Wright et al. 2016), which is characterized by larger body size, longer legs, and reduced flight morphology.

In contrast to this pattern, our results also highlight the power of morphological convergence to obscure relationships among owls and the potential for genetic data to uncover hidden diversity in this group. Our study includes just 22% of the described species of typical owls, yet our results identify 2 species (*Glaucidium brodiei* and *Ninox jacquinoti*) that are miscategorized at the genus level; strikingly, both of these taxa were previously thought to be part of larger species complexes based on morphology and biogeography (Marks et al. 2018). These findings underscore the importance of comprehensive taxonomic sampling (Zwickl and Hillis 2002) in future molecular studies of owls, which may identify new species within established clades and profoundly affect our understanding of the evolutionary history of this group.

Conclusions

Here, we inferred a phylogeny for typical owls by sampling thousands of nuclear loci from all but one of the 28 described genera that comprise the Strigidae. By combining enrichment of UCE loci from tissues with recent techniques for collecting DNA sequence data from avian toepads, we were able to include 6 rare, monotypic genera that have not been included in previous molecular analyses. Our results suggest typical owls comprise 2 major sister clades that generally correspond to subfamilies Surniiniae as described by Weick (2006) and Striginae as described by Wink et al. (2008), and verify membership of typical owl tribes that have been previously described. At a finer scale, results across our expanded taxon sample suggest that as many as 8 typical owl genera are not monophyletic with respect to current taxonomy (Dickinson and Remsen 2013), including *Ninox*, *Glaucidium*, *Athene*, *Otus*, *Asio*, *Megascops*, *Bubo*, and *Strix*, affecting the taxonomy of as many as 177 of the 194 described species of typical owls. Furthermore, with limited sampling at the species level, our study likely underestimates the prevalence of paraphyly and polyphyly within typical owls; increased sampling within genera will likely reveal further examples. These findings also demonstrate how morphology can sometimes obfuscate evolutionary relationships (Hedges and Sibley 1994), the importance of taxon sampling (Zwickl and Hillis 2002), and the need for taxonomic revision within Strigidae.

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Data deposits: Raw sequencing reads are available from the National Center for Biotechnology Information (NCBI)

Sequence Read Archive (BioProject PRJNA528101). The PHYLUCE computer code used in this study is available from https://github.com/faircloth-lab/phyluce. Other custom computer code, DNA alignments, analysis inputs, and analysis outputs are available from Salter et al. (2019).

SUPPLEMENTARY MATERIAL

Supplementary material is available at *The Auk: Ornithological Advances* online.

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