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Integrative taxonomy of eared nightjars (Aves: *Lyncornis*) underscores the complementarity of morphology, vocalizations and DNA evidence

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The eared nightjars (*Lyncornis*, formerly *Eurostopodus*) comprise six taxa distributed from southern India and Southeast Asia to Sulawesi. Species limits in this group have not been evaluated since 1940. In this study, we use three datasets (morphology, acoustics and mitochondrial DNA) to assess the taxonomic status of taxa in this genus. Multivariate analyses of vocalizations and phylogenetic analysis of mitochondrial DNA both revealed the presence of four major groups. Morphological analyses also revealed four major groups, but these agreed only in part with those identified by vocalizations and DNA. *Lyncornis macrotis cerviniceps* from mainland Southeast Asia and the isolated *Lyncornis macrotis jacobsoni* on Simeulue Island, off north-west Sumatra, differed by six diagnostic plumage characters, but could not be distinguished by their vocalizations or mitochondrial DNA. Conversely, *Lyncornis macrotis macrotis* from the Philippines and *Lyncornis macrotis macropterus* from Sulawesi differed diagnosably in song and by 5% sequence divergence but could not be diagnosed by plumage. We adopt an integrative approach and propose to recognize five monotypic species: *Lyncornis temminckii*, *Lyncornis cerviniceps* (synonym: *Lyncornis bourdilloni*), *Lyncornis jacobsoni*, *Lyncornis macrotis* and *Lyncornis macropterus*. Our study illustrates that taxonomic revisions based on single lines of evidence can underestimate diversity and underscores the importance of using multiple datasets in species-level taxonomy.

ADDITIONAL KEYWORDS: alpha taxonomy – cryptic species – species delineation – taxonomic revision – taxonomy.

INTRODUCTION

Taxonomy provides indispensable data for nearly all disciplines in biology and is vital for the success of biology in general, but funding for taxonomic research remains inadequate (Wheeler *et al.*, 2004; Engel *et al.*, 2021). As a result, the choice of methods in taxonomy often boils down to a trade-off between accuracy and efficiency. Debates over the relative merits of various methods (e.g. 'DNA barcoding', single-locus vs. multilocus phylogeography) and taxonomic criteria (e.g. reciprocal monophyly) often focus on the efficiency and accuracy of these methods. Typically, proponents of a given method emphasize the success in recovering a large percentage of previously identified species or in finding previously unrecognized taxa (Avise & Ball, 1990; Hebert *et al.*, 2003), whereas others can consider these methods to be unsatisfactory if they overlook valid species or produce inaccurate species limits (Kizirian & Donnelly, 2004; Moritz & Cicero, 2004; Trewick, 2008).

Several authors have argued that taxonomy should be integrative and use as many different datasets as possible (Lee, 2004; Dayrat, 2005; Page *et al.*, 2005; Will *et al.*, 2005; Padial *et al.*, 2009). The term 'integrative

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taxonomy' was introduced simultaneously by Will *et al.* (2005) and Dayrat (2005) for similar approaches. The main tenets of integrative taxonomy are that: (1) any type of data is potentially relevant for documenting differences among species; (2) different types of data are complementary and highlight different aspects of the speciation process; (3) as many types of data as possible should be obtained; (4) absence of a particular species property does not falsify species status (i.e. no property is defining for species status); (5) taxonomic data should be integrated; and (6) taxonomic evaluations should be revisited when novel data become available (de Queiroz, 2007; Padial *et al.*, 2010; Yeates *et al.*, 2011; Sangster, 2018).

Taxonomists have long included as many data as possible to infer species limits and have readily added new types of data when these became available (Wheeler, 2008; Sangster, 2014). An unresolved issue in the debate over integrative taxonomy is whether multiple lines of evidence are required for an accurate picture of species-level diversity; for instance, if all datasets (e.g. DNA, morphology and behaviour) suggest the same taxonomic groups and it would be efficient to stick to the cheapest, easiest or fastest method. Likewise, if one type of data is always the most sensitive (i.e. best able to identify species lineages), other datasets are unnecessary for species-level taxonomy. Alternatively, if different types of data uncover different species, only a combination of multiple types of data will be able to uncover all species (e.g. McKay *et al.* 2014).

Nightjars are suitable subjects for a comparison of taxonomic datasets because, unlike many other birds, their plumage and vocalizations serve different functions (i.e. camouflage and communication, respectively) and are therefore most likely to be subject to different selection pressures. The plumage of most nightjars is cryptic and, at least in some species, there is much individual variation in plumage characters (Forero & Tella, 1997; Cleere, 1998; Holyoak, 2001). It has been suggested that in owls and nightjars, plumage data are unreliable for estimating species limits and that patterns of acoustic variation should be used instead (Marshall, 1978). According to this view, populations with different vocalizations belong to different species, whereas those with the same vocalizations should be included in the same species (Marshall, 1978). In recent years, several new species of owls and nightjars have been described or elevated to species rank based on either morphological (Louette, 1990; Rasmussen, 1998, 1999) or acoustic differences (Robbins & Ridgely, 1992; Robbins *et al.*, 1994; Garrido & Reynard, 1998; Sangster & Rozendaal, 2004; Sangster *et al.*, 2021). In cases where more than one dataset has been used, one dataset is typically used as a framework for the interpretation of variation in the other sets, which are often not analysed formally. In owls and nightjars, remarkably few studies have formally analysed and compared multiple lines of evidence, with similar taxon sampling. Consequently, it is often not clear whether taxa that are supported by one line of evidence are also distinct in others.

In this study, the taxonomic status of the eared nightjars of the genus *Lyncornis* Gould, 1838 is examined using morphological, acoustic and molecular data. *Lyncornis* are poorly known, nocturnal birds, and their species limits have not been revised since the study by Peters (1940). Until recently, *Lyncornis* was included with five Australasian species in the genus *Eurostopodus* Gould, 1838 (Cleere, 1998, 1999; Holyoak, 2001; Dickinson, 2003). However, recent studies have shown that the two genera are not closely related (Braun & Huddleston, 2009; Han *et al.*, 2010; Sigurdsson & Cracraft, 2014; White *et al.*, 2016).

Lyncornis includes six taxa, which are currently grouped into two species: the monotypic *Lyncornis temminckii* (Gould, 1838) (Malaysian eared nightjar) and the polytypic *Lyncornis macrotis* (Vigors, 1831) (great eared nightjar), consisting of five subspecies, *Lyncornis macrotis bourdilloni* Hume, 1875, *Lyncornis macrotis cerviniceps* Gould, 1838, *Lyncornis macrotis jacobsoni* Junge, 1936, *Lyncornis macrotis macrotis* (Vigors, 1831) and *Lyncornis macrotis macropterus* Bonaparte, 1850. All taxa, except for *L. m. jacobsoni*, were originally described as species. Most of these were maintained as species until the early 20th century (e.g. Hartert, 1897; Sharpe, 1900). After the introduction of the polytypic species concept, all taxa in the complex except *L. temminckii* were downgraded to subspecies rank and included in *L. macrotis* (Peters, 1940). All these taxa have allopatric breeding ranges, except for *L. temminckii* and *L. m. cerviniceps*, which overlap in north-western Peninsular Malaysia (Wells, 1999; Fig. 1). No explicit rationale has been offered to explain why only *L. temminckii* should be treated as a full (monotypic) species, whereas all other taxa [including *L. cerviniceps* (Gould, 1838)] are treated as subspecies of *L. macrotis*. Despite poor documentation of species limits, this taxonomic arrangement has been maintained in recent reference works (Cleere, 1998, 1999; Holyoak, 2001; Dickinson & Remsen, 2013; Gill *et al.*, 2021). The taxonomy and relationships of this species complex have received little attention from systematists. The lack of a modern evaluation of species limits in the *L. macrotis* complex makes it difficult to assess the peculiar biogeographical pattern observed in this complex, in which *L. temminckii* is surrounded by four subspecies of *L. macrotis* (Fig. 1). Wells (1999) noted that the taxonomic position of the isolated Simeulue Island taxon *L. m. jacobsoni* might be clarified by comparing its vocalizations with those of other taxa.

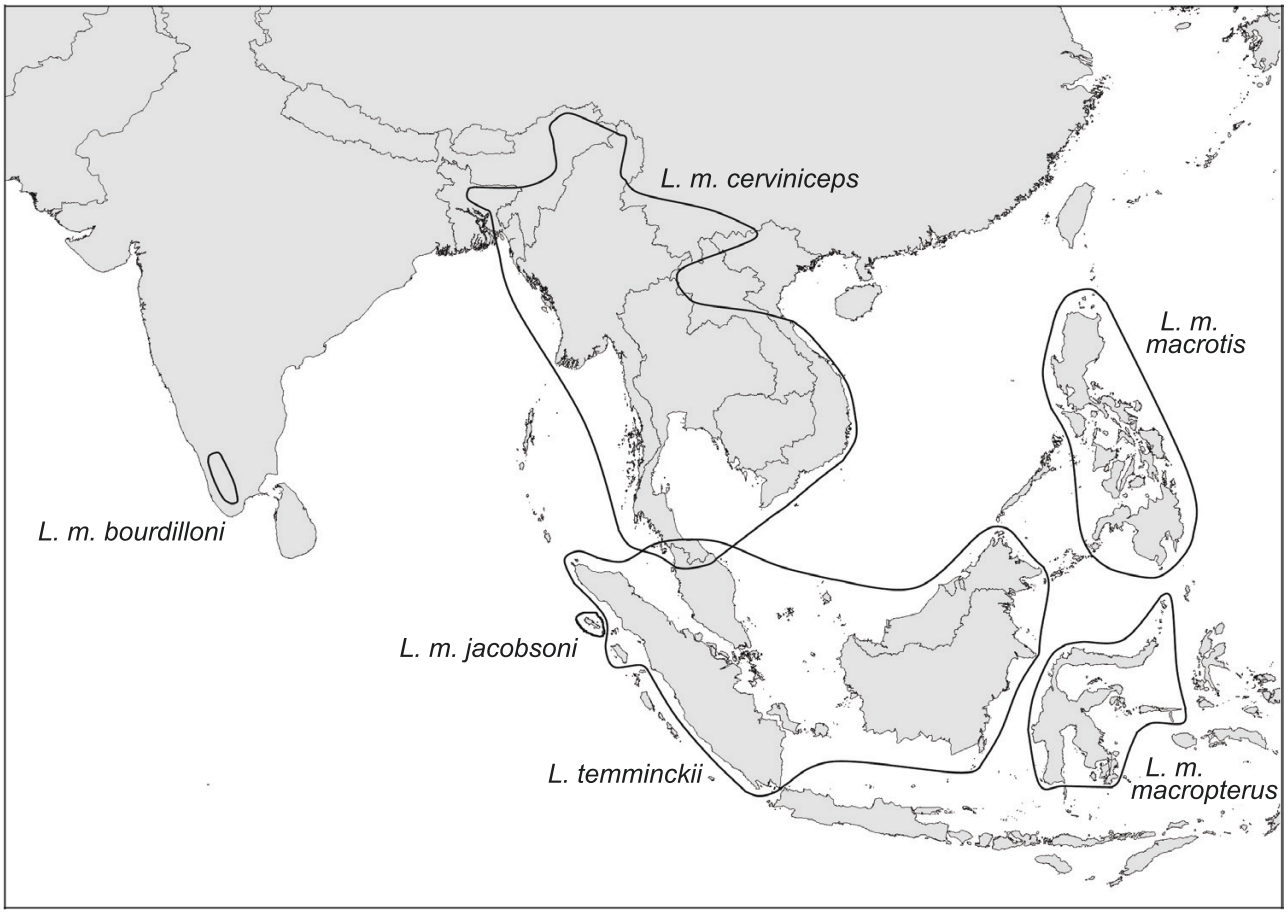

Figure 1. Map showing range of currently recognized taxa in the *Lyncornis macrotis* complex. Taxonomy follows Cleere (1998).

MATERIAL AND METHODS

MITOCHONDRIAL DNA

Sampling

We included 27 samples of all six taxa of *Lyncornis*. As outgroups, two species each of *Eurostopodus* and *Caprimulgus* Linnaeus, 1758 and one species each of *Antrostomus* Bonaparte, 1838, *Chordeiles* Swainson, 1832, *Gactornis* Han, Robbins & Braun, 2010 and *Nyctipolus* Ridgway, 1912 were included (Supporting Information, Table S1). All samples were from vouchered museum specimens.

DNA extraction, amplification and sequencing

DNA was extracted using the QIA Quick DNEasy Kit (Qiagen) according to the manufacturer's instructions. Primers used for PCR amplification are listed in the Supporting Information (Table S2). We sequenced the mitochondrial cytochrome *b* gene (*Cytb*). Although mitochondrial DNA (mtDNA) provides only a single locus and might not reflect the true species phylogeny accurately, this marker has proved extremely useful in detecting (additional) species, which have been corroborated by other evidence (e.g. Rising & Avise, 1993; Burbidge *et al.*, 2003; Baker *et al.*, 2007; Barrowclough *et al.*, 2011). Amplification and sequencing followed the protocols described by Sangster *et al.* (2016). We used the following five lines of evidence to evaluate the authenticity of the sequence fragments: (1) electropherograms were inspected for two clear peaks at one or more nucleotides, which is indicative of a mixture of mitochondrial and nuclear sequences (Den Tex *et al.*, 2010); (2) we checked for differences in overlapping sequence fragments, which represents another indication of a mixture of mitochondrial and nuclear sequences; (3) we checked the translated consensus sequence for the presence of stop codons or frameshift mutations (inferred insertions and deletions), which are clear indications that a sequence does not represent that of a protein-coding gene; (4) we checked whether nucleotide substitutions were primarily found at the third codon, which is expected when a sequence is of a

protein-coding gene [in old nuclear mitochondrial DNA segments (NUMTs), the distribution of substitutions is expected to be equal across all three codon positions (Zink & Barrowclough, 2008)]; and (5) we assessed the phylogenetic position of the six fragments separately in a maximum likelihood (ML) analysis to verify that all fragments were derived from the same locus. The sequences have been deposited at GenBank (accession numbers OM830960–OM830985).

Phylogenetic analyses

Sequences were aligned using CLUSTALW in MEGA7 (Kumar *et al.*, 2016). Molecular phylogenies were estimated by Bayesian inference using MRBAYES v.3.1.2 (Ronquist & Huelsenbeck, 2003). Posterior probabilities were calculated under a general time-reversible (GTR) model (Lanave *et al.*, 1984; Tavaré, 1986; Rodríguez *et al.*, 1990), assuming rate variation across sites according to a discrete gamma (Γ) distribution with four rate categories (Yang, 1994) with an estimated proportion of invariant (I) sites (Gu *et al.*, 1995). The choice of model was determined based on the Akaike information criterion (Akaike, 1973) calculated in MEGA7. Four Metropolis-coupled Markov chain Monte Carlo chains with an incremental heating temperature of 0.2 were run for two million generations and sampled every 1000 generations. Two runs were made simultaneously, starting from random trees, and the results were compared to ascertain that the chains had reached the same target distributions. The first 25% of generations, long after the chain reached apparent stationarity, were discarded and the posterior probability was estimated for the remaining generations. The samples from the stationary phases of the independent runs were pooled to obtain the final results. Maximum likelihood bootstrapping (1000 replicates) was performed in RAXML v.7.7.1 (Stamatakis *et al.*, 2008) using the same model as in the Bayesian inferences, with the dataset partitioned by codon and support calculated from 1000 bootstrap pseudoreplicates. Uncorrected pairwise sequence divergence was calculated in MEGA7.

VOCALIZATIONS

Recordings

Recordings of all six named taxa in the complex were available for analysis. Locations and recordists for the recordings are listed in the Supporting Information (Table S3). To augment sample size, recordings published by Marshall (1978), Bruckert (1993), Thomas & Thomas (1994), Hérelle (1997), Supari (2003), Skeoch & Koschak (2004), Scharringa (2005) and Teeuwen (n.d.) were included.

Vocalizations

Recordings of the territorial song were used to delimit species because, in nocturnal birds, this vocalization is most likely to carry information concerning the identity of the bird (Oba, 1996) and to function as sexual and territorial advertisement (Cramp, 1985; König, 1998, 2000). This vocalization has also been described as a 'call' (e.g. Holyoak, 2001), 'song' (e.g. Cleere, 1998) or 'advertising call' (Cramp, 1985). In the eared nightjars, the territorial song is a commonly heard vocalization. Songs are often given at dusk and dawn but can continue through the night and the into first hour of daylight (Holyoak, 2001; G. Sangster, pers. obs.). Singing takes place mostly in flight (Wells, 1999; Holyoak, 2001; G. Sangster, pers. obs.).

An 'element' was defined as a part of a note. A 'note' was defined as an unbroken trace on a spectrogram. A 'song' was defined as the smallest stereotypic repetition of similar note sequences.

Measurements of acoustic characters

Sonograms used in illustrations were made with SYRINX (Burt, 2001). All measurements were made using RAVEN PRO v.1.5 (Bioacoustics Research Program, 2014). The durations of elements, notes and songs were measured in seconds, expressed to the nearest thousandth. Characters were defined on the basis of sonograms (Fig. 2). The following measurements were recorded: (1) F1 = frequency at the start of the main note (in herz); (2) F2 = frequency at the start of the concave part of the main note (in herz); (3) F3 = frequency at the lowest point of the concave part of the main note (in herz); (4) F4 = frequency at the end of the concave part of the main note (in herz); (5) F5 = frequency at the end of the song (in herz);

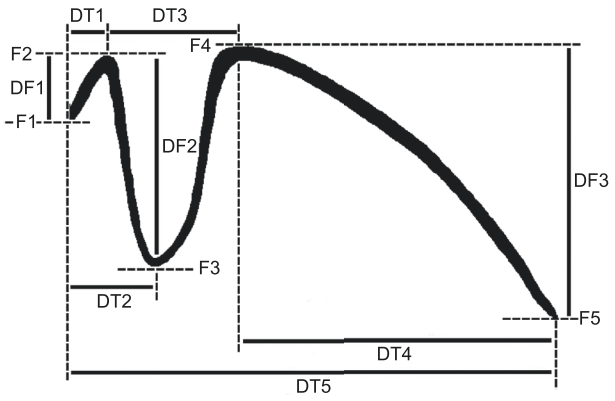

Figure 2. Measurement of acoustic variables. For definitions of acoustic variables, see '*Measurements of acoustic characters*'.

(6) DT1 = interval between start of the main note to the concave part of the main note (in seconds); (7) DT2 = interval between start of the main note to the lowest point of the concave part of the main note (in seconds); (8) DT3 = duration of the concave part of the main note (in seconds); (9) DT4 = duration of the last leg (i.e. from the end of the concave part of the main note and the end of the song; in seconds); (10) DT5 = total duration of main note (in seconds); (11) DF1 = frequency range of the start of the main note to the concave part of the main note (in herz); (12) DF2 = frequency range of the start of the concave part of the main note to the lowest point of the concave part (in herz); (13) DF3 = frequency range of the end of the concave part of the main note and the end of the song (in herz); and (14) DF4 = change in frequency between the end of the concave part of the main note and the end of the song (in herz per second). In order to give equal weight to individuals, means of up to five songs were computed for each recording. These means were used as sample points from which ranges, means and standard deviations were computed.

Statistical analysis

Principal components analysis (PCA) was used to explore the dataset. Principal components analysis reduces multiple variables to a limited number of uncorrelated variables. Analysis of variance (ANOVA) and Bonferroni post hoc comparisons were used to test whether the groups defined by PCA differed from each other.

Canonical discriminant function analysis (DFA) was applied to the acoustic variables of individuals to test whether the recordings could be assigned correctly to their taxon. Discriminant function analysis generates a set of criteria to assign individuals to groups that are defined before the analysis. Before DFA, a tolerance test was conducted to assess the independence of each variable. Variables that failed the tolerance test (i.e. which were an almost linear combination of other variables) were excluded from the analyses. Two DFAs were performed: (1) a 'descriptive' DFA, in which the observations used to develop the criteria were then subjected to these criteria; and (2) a 'predictive' DFA, which used a jackknife procedure to obtain a more accurate test of the predictive performance of the DFA. In the jackknife procedure, the DFA was recalculated using the combination of variables of the initial DFA, with one individual removed from the dataset. The criteria were then used to classify the removed individual. This process was repeated for all individuals of the dataset.

The effect size, expressed as Cohen's d, was calculated to show the strength of the acoustic differences between taxa. For interpretation of effect size data, we used the classification of Cohen (1988), which was updated and expanded by Sawilowsky (2009). Thus, we regarded an effect size of $d \geq 0.1$ as 'very small', $d \geq 0.2$ as 'small', $d \geq 0.5$ as 'medium', $d \geq 0.8$ as 'large', $d \geq 1.2$ as 'very large' and $d \geq 2.0$ as 'huge'.

SPSS v.27.0 (IBM) was used to calculate all descriptive statistics and perform ANOVAs, Mann–Whitney U-tests, PCAs and DFAs.

MORPHOLOGY

To identify morphological differences among taxa, we examined 91 adult-plumaged specimens of all taxa in the complex (Supporting Information, Table S4). Specimens in juvenile or downy plumage or with damaged, extensively worn or moulting wing and tail feathers were excluded. Museum specimens are housed in Naturalis Biodiversity Center, Leiden, The Netherlands (NBC) (formerly Rijksmuseum voor Natuurlijke Historie; RMNH), The Natural History Museum, Tring, UK (NHMUK) and the Swedish Museum of Natural History, Stockholm, Sweden (NRM). Characters and character states are described in the Appendix.

RESULTS

MITOCHONDRIAL DNA

Sequence characteristics

We obtained a 1047 bp portion of the coding *Cytb* for 26 individuals, of which 341 positions (32.6%) were parsimony informative. The *Cytb* sequence was amplified in six partly overlapping fragments of 201–229 bp. Five aspects indicated that these were genuine mitochondrial sequences rather than nuclear pseudogenes: (1) electropherograms exhibited no double peaks; (2) overlapping sequence fragments were identical; (3) the alignment showed no stop codons, insertions or deletions; (4) nucleotide substitutions were mostly found at the third codon position (150 of 182) and resulted in only 18 amino acid substitutions; and (5) all fragments analysed separately yielded the same topology.

Phylogeny and genetic divergence

The phylogenetic tree (Fig. 3) showed strong support for the monophyly of *Lyncornis*. *Lyncornis temminckii* was sister to a monophyletic *L. macrotis*, again with strong support. Three major clades in *L. macrotis* were well supported: (1) *L. m. cerviniceps*, *L. m. bourdilloni* and *L. m. jacobsoni*; (2) *L. m. macrotis*; and (3) *L. m. macropterus*. Genetic divergence among these three clades was substantial: mean divergence between

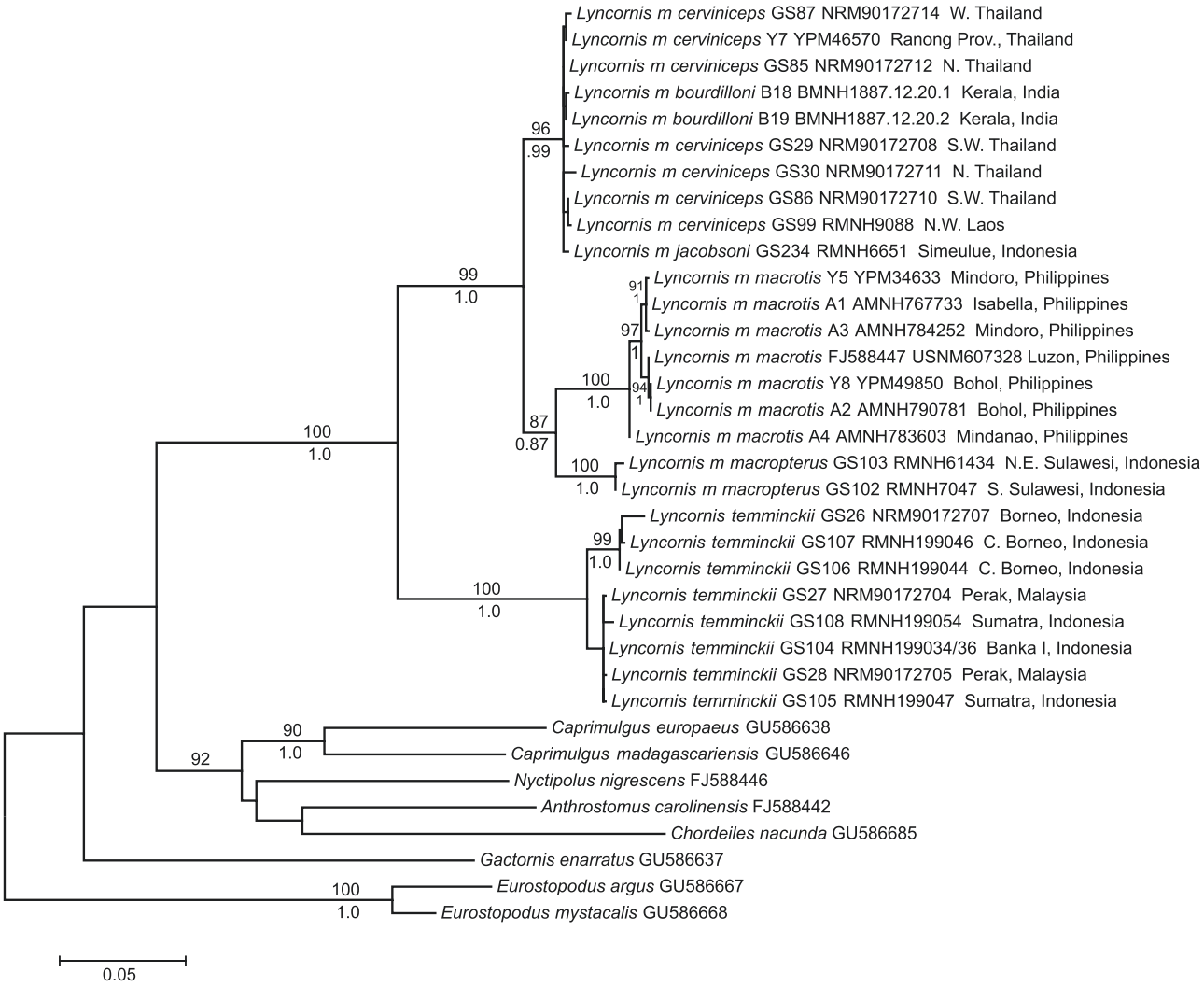

Figure 3. Maximum likelihood tree of cytochrome *b* sequences of the genus *Lyncornis* and various outgroups. Bootstrap proportions (> 70%) and posterior probabilities (> 0.8) are indicated above and below branches, respectively.

the sister taxa *L. m. macrotis* and *L. m. macropterus* was 5.6%, whereas the mean divergence between these two and members of the clade formed by *L. m. cerviniceps*, *L. m. bourdilloni* and *L. m. jacobsoni* was 5.0%. Divergence between *L. m. cerviniceps* and *L. m. bourdilloni* was small (0.3%), as was that between *L. m. cerviniceps* and *L. m. jacobsoni* (0.4%). *Lyncornis temminckii* differed from all other taxa by 11.1% (mean divergence).

VOCALIZATIONS

Sonograms of the territorial songs of the six examined taxa of the *L. macrotis* complex are given in Figure 4. All territorial song types are robust-sounding pure tones, and songs have almost no energy in their overtones. The fundamental frequency is therefore the only visible part in the spectrogram. All song types consist of a brief introductory note and a second note with strong frequency modulation and changes in amplitude.

Principal components analysis

The songs of 122 individuals representing all six taxa were used in the PCA. The results of the PCA on the 14 measurements are summarized in Table 1. Four components with eigenvalues > 1 were extracted from the dataset. Principal component (PC)1 accounted for 42.5% of the variance. Principal components 2, 3 and 4 accounted for an additional 18.6, 14.1 and 11.3% of the variance, respectively. Principal component 1 was represented by both frequency (F1–F4 and DF5) and time (DT4 and DT6) variables; PC2 was also determined

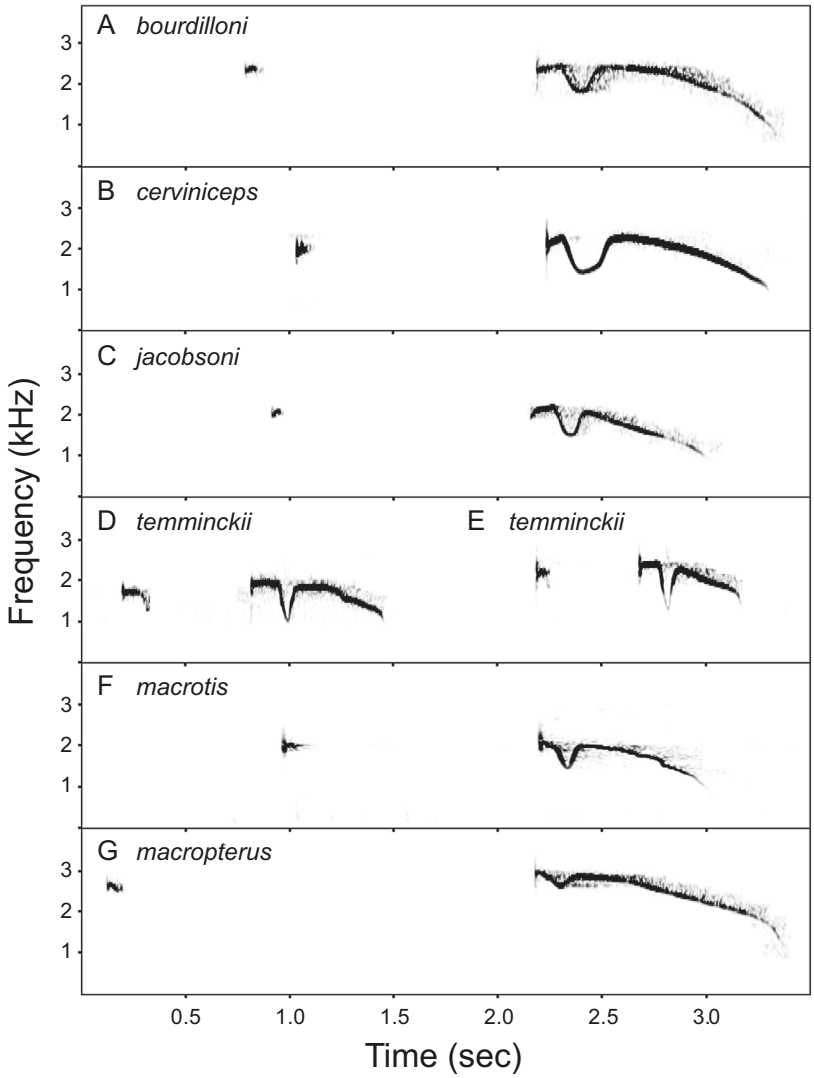

Figure 4. Sonograms of territorial songs of *Lyncornis*. A, *Lyncornis macrotis bourdilloni*, Kerala, India, B. King. B, *Lyncornis macrotis cerviniceps*, Thailand, J. C. Roché (BLSA 42510). C, *Lyncornis macrotis jacobsoni*, Simeulue Island, F. Verbelen. D, *Lyncornis temminckii*, Johore, Malaysia, T. C. White (BLSA 6414). E, *L. temminckii*, Way Kambas, Sumatra, A. B. van den Berg (ML 70527). F, *Lyncornis macrotis macrotis*, Mindanao, A. Greensmith (BLSA 34287). G, *Lyncornis macrotis macropterus*, Tangkoko Batuangus, Sulawesi, G. Sangster (GS 1841).

by frequency (DF1) and time (DT2) variables; PC3 was determined mostly by a frequency variable (F5); and PC4 mostly by time variables (DT1 and DT3).

Plotting individuals of the six taxa on PC1 vs. PC2 resulted in four distinct clusters (Fig. 5): (1) *L. m. bourdilloni*, *L. m. cerviniceps* and *L. m. jacobsoni*; (2) *L. m. macropterus*; (3) *L. m. macrotis*; and (4) *L. temminckii*. One-way ANOVA was used to test whether the four groups identified by PCA differed in any of the four PCs. The groups differed in the first three PCs ($P < 0.001$). Each of the four groups differed from all other groups by at least one PC (Tukey's post hoc comparisons).

Discriminant function analysis

The songs of the six subspecies were used as operational taxonomic units in the DFA. All characters passed the tolerance test, except for DF1, DF2 and DF3, which were excluded from the test. The descriptive DFA was highly significant (Wilks' $\lambda = 0.001$; $\chi^2_{55} = 698.7$; $P < 0.001$). The variables most important in the discrimination were DT4, DT5, F1 and F2 (Table 2). A scatterplot of the first two discriminant functions is shown in Figure 5 and showed the same clusters as the PCA plot.

The initial DFA led to a 90.2% correct classification of the individuals into the six taxa. All but one of the

Table 1. Factor loadings of the 14 acoustic variables on the four principal components; eigenvalues and thepercentage of variance explained by the respective components are given at the bottom of the table (N = 62)

Variable	PC1	PC2	PC3	PC4
F1	0.876	0.094	0.421	0.137
F2	0.859	0.287	0.377	0.151
F3	0.954	−0.084	0.059	0.073
F5	0.898	0.211	0.329	0.161
F5	0.171	−0.363	0.799	0.139
DT1	−0.295	0.159	−0.248	0.891
DT2	−0.362	0.817	−0.015	0.096
DT3	0.106	0.520	0.182	−0.806
DT4	0.860	−0.047	−0.443	−0.040
DT5	0.805	0.203	−0.470	−0.031
DF1	−0.132	0.731	−0.201	0.042
DF2	−0.616	0.477	0.339	0.051
DF3	0.759	0.482	−0.276	0.054
DF4	−0.326	0.578	0.393	0.152
Eigenvalue	5.944	2.608	1.968	1.578
Variance explained (%)	42.5	18.6	14.1	11.3
F (ANOVA)	202.9	84.8	26.9	0.8
Significance (ANOVA)	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.54$
d.f. (ANOVA)	121	121	121	121

Abbreviation: PC, principal component. For definitions of acoustic variables, see '*Measurements of acoustic characters*'.

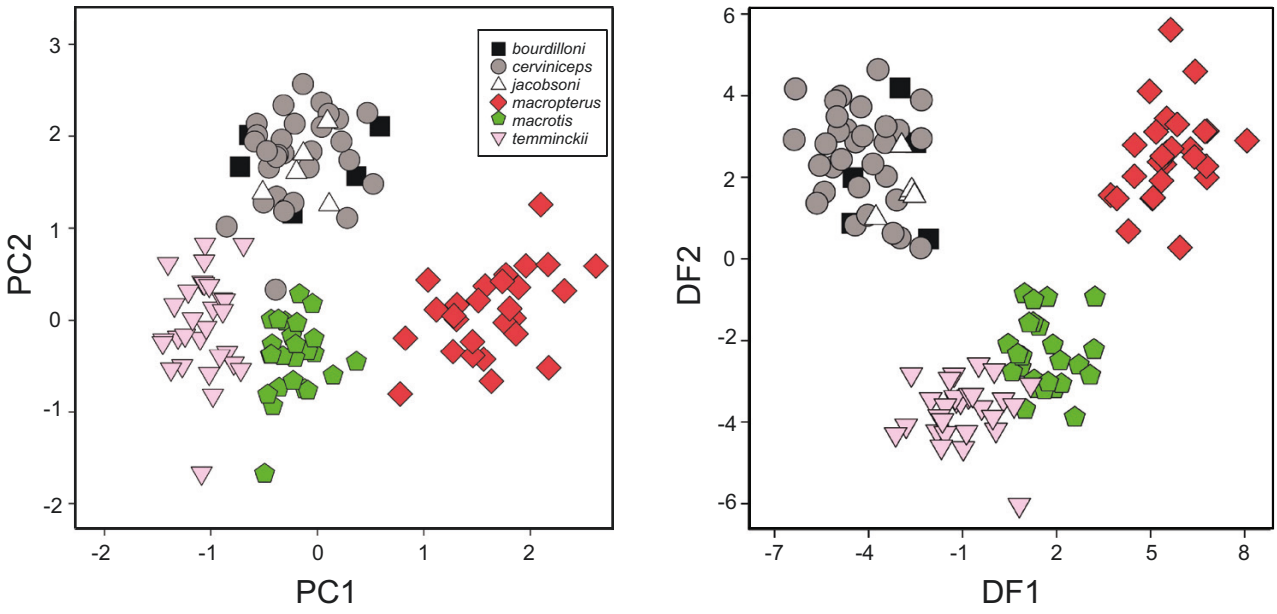

Figure 5. Multivariate analysis of acoustic variables measured for territorial songs of the *Lyncornis macrotis* complex (N = 122). Left panel, principal components analysis; right panel, discriminant function analysis. Abbreviations: DF, discriminant function; PC, principal component.

mismatches were among the taxa *L. m. bourdilloni*, *L. m. cerviniceps* and *L. m. jacobsoni*. The first of the three discriminant functions accounted for 56.9% of the variation, and the second and third accounted for 33.7% and 8.7% of the variation, respectively (Table 2). The jackknife procedure correctly assigned 85.2% of individuals to their subspecies; again, all but one of the mismatches were among the taxa *L. m. bourdilloni*, *L. m. cerviniceps* and *L. m. jacobsoni*.

Table 2. Standardized canonical discrimination function coefficients examining trends in variance of 11 acoustic variables measured for territorial songs of the *Lyncornis macrotis* complex; eigenvalues and the percentage of variance accounted for by each discriminant function (DF1-3) are given at the bottom of the table (N = 122)

Variable*	DF1	DF2	DF3
F1	0.929	−0.941	−0.640
F2	−0.449	1.170	−0.388
F3	0.421	0.421	0.460
F4	−0.355	0.129	0.531
F5	0.230	−0.040	−0.838
DT1	−0.216	1.660	3.182
DT2	−0.141	−0.324	−0.386
DT3	−0.122	1.796	3.256
DT4	3.927	−1.447	3.529
DT5	−3.584	1.775	−3.822
DF4	−0.008	−0.128	−0.371
Eigenvalue	13.821	8.188	2.108
Variance explained (%)	56.9	33.7	8.7
Wilks' λ	0.002	0.030	0.273
χ^2	698.7	395.4	145.9
d.f.	55	40	27
Significance	$P < 0.001$	$P < 0.001$	$P < 0.001$

*DF1, DF2 and DF3 failed to pass the tolerance test and were excluded from the analysis. For definitions of acoustic variables, see '*Measurements of acoustic characters*'.

Univariate analyses

Territorial song characteristics of all taxa of the *L. macrotis* complex are given in Table 3, and the statistical significance and effect size of pairwise comparisons are given in Table 4. Pairwise comparisons of *L. temminckii* and the five other taxa revealed between nine and 13 significant differences, one to five characters with non-overlapping ranges and between nine and 11 character differences with 'very large' (Cohen's $d > 1.2$) or 'huge' (Cohen's $d > 2.0$) effect size. Eight variables differed significantly in comparisons of the sister taxa *L. m. macropterus* with *L. m. macrotis*, and four of these also showed non-overlapping ranges (F2, F3, F4 and F5); there were nine character differences with a 'very large' or 'huge' effect size. A total of 14 variables differed significantly in comparisons of *L. m. macropterus* with *L. m. cerviniceps*, of which three (F1, F3 and DT2) showed no overlap; there were 12 character differences with 'very large' or 'huge' effect size. Comparisons of *L. m. macrotis* with *L. m. cerviniceps* revealed 13 significant differences and one variable (DT2) that showed no overlap between the two groups. There were eight character differences between *L. m. macrotis* and *L. m. cerviniceps* with a 'very large' or 'huge' effect size.

In contrast, songs of the taxa *L. m. bourdilloni*, *L. m. cerviniceps* and *L. m. jacobsoni* were not distinct and showed hardly any differences. One variable (F6) differed significantly between *L. m. bourdilloni* and *L. m. cerviniceps*, and there were no non-overlapping differences or differences with a 'very large' or 'huge' effect size. The taxa *L. m. cerviniceps* and *L. m. jacobsoni* showed neither any significant or non-overlapping differences nor any differences with a 'very large' or 'huge' effect size.

Morphology

All 17 characters were polymorphic in at least one taxon (Table 5). There were no diagnostic differences among the sexes in *L. m. cerviniceps*, *L. m. jacobsoni*, *L. m. macropterus*, *L. m. macrotis* and *L. temminckii*. For *L. m. bourdilloni*, only two males could be examined. A summary of diagnostic morphological character states is presented in Table 6. Comparison of the 17 morphological variables revealed 52 diagnosable differences between pairs of the six groups (Table 6), representing 20.4% of 255 comparisons. *Lyncornis temminckii* was diagnosable from all other taxa by three to six character states. Within *L. temminckii*, no diagnostic differences were found among specimens from Peninsular Malaysia (N = 4), Sumatra (N = 10), Banka Island (N = 4) and Borneo (N = 5). The sister taxa *L. m. macrotis* and *L. m. macropterus* could not be diagnosed from each other in morphology but differed from the other taxa by two to four character states. *Lyncornis m. cerviniceps* did not differ in any morphological character from *L. m. bourdilloni*, but it was diagnosable from all other taxa by two to six character states. *Lyncornis m. jacobsoni* was diagnosable by two to six character states, except from *L. bourdilloni* (seven diagnostic differences). *Lyncornis m. jacobsoni* and *L. m. cerviniceps* differed in six character states (Table 6; Figs 6, 7).

DISCUSSION

Sensitivity of morphological data

Our study documents divergence in three classes of data that play a major role in the taxonomy of birds: morphology, vocalizations and DNA sequences. However, to our knowledge, this is the first time that these three datasets have been analysed in detail in a revision of species limits of nightjars.

In the taxonomy of owls and nightjars, morphology has been considered less useful for inference of species limits than vocalizations owing to its involvement in camouflage and lack of involvement in mate recognition (Marshall, 1978). However, our results show that morphology can be as informative

Table 3. Descriptive statistics of 14 acoustic variables measured for territorial songs of *Lyncornis* nightjars

Variable	*Lyncornis macrotis bourdilloni* ($N = 5$)	*Lyncornis macrotis cerviniceps* ($N = 31$)	*Lyncornis macrotis jacobsoni* ($N = 5$)	*Lyncornis macrotis macropterus* ($N = 27$)	*Lyncornis macrotis macrotis* ($N = 25$)	*Lyncornis temminckii* ($N = 29$)
F1	2132 ± 147 (1939–2270)	2158 ± 167 (1697–2451)	2154 ± 180 (1874–2311)	2754 ± 174 (2523–3207)	1921 ± 86 (1675–2036)	2017 ± 152 (1708–2295.4)
F2	2317 ± 121 (2149–2479)	2319 ± 152 (1917–2557)	2323 ± 129 (2105–2433)	2783 ± 157 (2545–3207)	1958 ± 92 (1730–2116)	2046 ± 153 (1686–2344.4)
F3	1389 ± 240 (1157–1694)	1448 ± 164 (1113–1763)	1533 ± 193 (1334–1785)	2353 ± 194 (2040–2771)	1501 ± 190 (1036–1857)	1075 ± 181 (672–1483)
F4	2139 ± 232 (1862–2413)	2197 ± 167 (1829–2480)	2208 ± 135 (1995–2325)	2685 ± 159 (2408–3058)	1929 ± 75 (1729–2061)	1932 ± 135 (1708–2234)
F5	957 ± 73 (871–1068)	1071 ± 125 (856–1287)	989 ± 144 (749–1100)	1395 ± 233 (1036–1800)	968 ± 121 (714–1179)	1355 ± 188 (924–1667)
DT1	0.092 ± 0.033 (0.051–0.120)	0.124 ± 0.028 (0.066–0.192)	0.119 ± 0.020 (0.102–0.152)	0.019 ± 0.021 (0.000–0.084)	0.064 ± 0.198 (0.000–1.013)	0.095 ± 0.016 (0.073–0.139)
DT2	0.201 ± 0.026 (0.157–0.222)	0.222 ± 0.030 (0.163–0.297)	0.201 ± 0.018 (0.188–0.230)	0.090 ± 0.017 (0.051–0.127)	0.096 ± 0.016 (0.062–0.130)	0.140 ± 0.025 (0.046–0.192)
DT3	0.209 ± 0.028 (0.188–0.258)	0.210 ± 0.025 (0.164–0.283)	0.174 ± 0.025 (0.148–0.213)	0.138 ± 0.025 (0.094–0.186)	0.129 ± 0.020 (0.075–0.170)	0.099 ± 0.012 (0.079–0.124)
DT4	0.708 ± 0.173 (0.531–0.970)	0.616 ± 0.106 (0.342–0.802)	0.611 ± 0.031 (0.577–0.645)	1.001 ± 0.251 (0.479–1.511)	0.729 ± 0.132 (0.509–1.070)	0.321 ± 0.067 (0.202–0.466)
DT5	1.009 ± 0.184 (0.840–1.290)	0.950 ± 0.108 (0.724–1.123)	0.894 ± 0.040 (0.846–0.935)	1.158 ± 0.258 (0.612–1.650)	0.882 ± 0.135 (0.623–1.229)	0.515 ± 0.074 (0.367–0.665)
DF1	185 ± 71 (111–279)	161 ± 69 (60–364)	169 ± 67 (70–231)	29 ± 44 (–32–143)	38 ± 53 (–83 to 143)	29 ± 68 (–239 to 126)
DF2	928 ± 156 (676–1051)	870 ± 139 (610–1202)	790 ± 186 (596–1099)	430 ± 119 (198–691)	457 ± 177 (142–870)	971 ± 203 (485–1444)
DF3	1182 ± 230 (969–1477)	1126 ± 183 (705–1487)	1219 ± 214 (1014–1576)	1291 ± 283 (742–1858)	961 ± 118 (771–1162)	577 ± 169 (234–903)
DF4	1686 ± 119 (1523–1825)	1912 ± 477 (1029–2960)	1996 ± 321 (1572–2455)	1355 ± 395 (822–2462)	1354 ± 261 (929–1862)	1819 ± 471 (795–2760)

For definitions and units for acoustic variables, see '*Measurements of acoustic characters*'.

Table 4. Significance levels of ANOVA or Mann Whitney *U*-tests, the effect of size (expressed as Cohen's *d*) and interpretation of the effect of size by Cohen (1988) and Sawilowsky (2009) for vocal character differences among *Lyncornis* nightjars

Taxa	Variable						
	F1	F2	F3	F4	F5	DT1	DT2
Lyncornis m. cerviniceps/	n.s.†	n.s.*	n.s.*	n.s.†	$P < 0.05$†	n.s.*	n.s.†
L. m. bourdilloni	0.16 (very small)	0.01 (negligible)	0.35 (small)	0.34 (small)	0.97 (large)	1.15 (large)	0.71 (medium)
Lyncornis m. cerviniceps/	n.s.†	n.s.*	n.s.*	n.s.†	n.s.†	n.s.*	n.s.†
L. m. jacobsoni	0.02 (negligible)	0.03 (negligible)	0.52 (medium)	0.07 (negligible)	0.66 (medium)	0.20 (small)	0.74 (medium)
Lyncornis m. cerviniceps/	< 0.001†	< 0.001†	< 0.001†	< 0.001†	< 0.001†	< 0.001†	< 0.001†
L. m. macropterus	3.56 (huge)	3.06 (huge)	5.16 (huge)	3.04 (huge)	1.80 (very large)	4.27 (huge)	5.37 (huge)
Lyncornis m. cerviniceps/	< 0.001†	< 0.001†	n.s.*	< 0.001†	< 0.01†	< 0.001†	< 0.001†
L. m. macrotis	1.76 (very large)	2.85 (huge)	0.30 (small)	2.03 (huge)	0.85 (large)	0.46 (small)	5.15 (huge)
Lyncornis m. cerviniceps/	< 0.005†	< 0.001†	< 0.001†	< 0.001†	< 0.001†	< 0.001†	< 0.001†
L. temminckii	0.90 (large)	1.82 (very large)	2.21 (huge)	1.77 (very large)	1.82 (very large)	1.30 (very large)	2.97 (huge)
Lyncornis m. jacobsoni/	< 0.001†	< 0.001†	< 0.001†	< 0.001†	< 0.001†	< 0.001†	< 0.001†
L. m. macropterus	3.54 (huge)	3.10 (huge)	4.37 (huge)	3.16 (huge)	1.88 (very large)	4.90 (huge)	6.87 (huge)
Lyncornis m. jacobsoni/	< 0.05†	< 0.001†	n.s.*	< 0.001†	n.s.†	< 0.001†	< 0.001†
L. m. macrotis	2.31 (huge)	3.85 (huge)	0.17 (very small)	3.36 (huge)	0.18 (very small)	0.31 (small)	6.84 (huge)
Lyncornis m. jacobsoni/	n.s.†	< 0.001†	< 0.001†	< 0.001†	< 0.001†	< 0.001†	< 0.001†
L. temminckii	0.91 (large)	1.90 (very large)	2.58 (huge)	2.10 (huge)	2.07 (huge)	1.50 (very large)	2.58 (huge)
Lyncornis m. jacobsoni/	n.s.†	n.s.*	n.s.*	n.s.†	n.s.†	n.s.*	n.s.†
L. m. bourdilloni	0.15 (very small)	0.05 (negligible)	0.74 (medium)	0.40 (small)	0.31 (small)	1.10 (large)	0.02 (negligible)
Lyncornis m. macropterus/	< 0.001†	< 0.001†	< 0.001†	< 0.001†	< 0.001†	n.s.*	n.s.†
L. m. macrotis	6.11 (huge)	6.48 (huge)	4.53 (huge)	6.14 (huge)	2.32 (huge)	0.33 (small)	0.35 (small)
Lyncornis m. macropterus/	< 0.001†	< 0.001†	< 0.001†	< 0.001†	n.s.†	< 0.001†	< 0.001†
L. temminckii	4.60 (huge)	4.85 (huge)	6.94 (huge)	5.22 (huge)	0.19 (very small)	4.13 (huge)	2.41 (huge)
Lyncornis m. macropterus/	< 0.001†	< 0.001†	< 0.001†	< 0.001†	< 0.001†	< 0.001†	< 0.001†
L. m. bourdilloni	3.76 (huge)	3.16 (huge)	4.96 (huge)	3.31 (huge)	2.07 (huge)	3.28 (huge)	6.39 (huge)
Lyncornis m. macrotis/	< 0.05†	< 0.05†	< 0.001†	n.s.†	< 0.001†	< 0.001†	< 0.001†
L. temminckii	0.78 (medium)	0.70 (medium)	2.35 (huge)	0.03 (negligible)	2.46 (huge)	0.24 (small)	2.15 (huge)
Lyncornis m. macrotis/	< 0.01†	< 0.001†	n.s.*	n.s.†	n.s.†	< 0.001†	< 0.001†
L. m. bourdilloni	2.25 (huge)	3.84 (huge)	0.59 (medium)	1.95 (very large)	0.10 (very small)	0.16 (very small)	6.29 (huge)
Lyncornis temminckii/	n.s.†	< 0.001†	< 0.01†	n.s.†	< 0.001†	n.s.*	< 0.001†
L. m. bourdilloni	0.78 (medium)	1.87 (very large)	1.71 (very large)	1.42 (very large)	2.31 (huge)	0.14 (very small)	2.51 (huge)

Table 4. Continued

Taxa	Variable						
	DT3	DT4	DT5	DF1	DF2	DF3	DF4
Lyncornis m. cerviniceps/	n.s.*	n.s.†	n.s.†	n.s.*	n.s.*	n.s.†	n.s.*
L. m. bourdilloni	0.06 (negligible)	0.81 (large)	0.50 (medium)	0.36 (small)	0.42 (small)	0.31 (small)	0.52 (medium)
Lyncornis m. cerviniceps/	n.s.*	n.s.†	n.s.†	n.s.*	n.s.*	n.s.†	n.s.*
L. m. jacobsoni	1.47(very large)	0.05 (negligible)	0.56 (medium)	0.12 (very small)	0.57 (medium)	0.51 (medium)	0.19 (very small)
Lyncornis m. cerviniceps/	< 0.001†	< 0.001†	< 0.001†	< 0.001†	< 0.001†	< 0.05†	< 0.001†
L. m. macropterus	2.94 (huge)	2.08 (huge)	1.10 (large)	2.27 (huge)	3.44 (huge)	0.71 (medium)	1.29 (very large)
Lyncornis m. cerviniceps/	< 0.001†	< 0.005†	< 0.05†	<0.001†	< 0.001†	< 0.001†	< 0.001†
L. m. macrotis	3.59 (huge)	0.97 (large)	0.57 (medium)	2.01 (huge)	2.68 (huge)	1.07 (large)	1.44 (very large)
Lyncornis m. cerviniceps/	< 0.001†	< 0.001†	< 0.001†	< 0.001†	< 0.05†	< 0.001†	n.s.*
L. temminckii	5.71 (huge)	3.35 (huge)	4.78 (huge)	1.96 (very large)	0.59 (medium)	3.16 (huge)	0.20 (small)
Lyncornis m. jacobsoni/	< 0.01†	< 0.005†	< 0.01†	< 0.001†	< 0.001†	n.s.†	< 0.005†
L. m. macropterus	1.50 (very large)	1.72 (very large)	1.13 (large)	3.00 (huge)	2.86 (huge)	0.27 (small)	1.72 (very large)
Lyncornis m. jacobsoni/	< 0.001†	< 0.05†	n.s.†	< 0.001†	< 0.005†	< 0.005†	< 0.001†
L. m. macrotis	2.24 (huge)	1.00 (large)	0.10 (very small)	2.46 (huge)	1.93 (very large)	1.96 (very large)	2.46 (huge)
Lyncornis m. jacobsoni/	< 0.001†	< 0.001†	< 0.001†	< 0.001†	n.s.*	< 0.001†	n.s.*
L. temminckii	5.46 (huge)	4.69 (huge)	5.58 (huge)	2.13 (huge)	0.93 (large)	3.77 (huge)	0.40 (small)
Lyncornis m. jacobsoni	n.s.*	n.s.†	n.s.†	n.s.*	n.s.*	n.s.†	n.s.*
/*L. m. bourdilloni*	1.44 (very large)	0.87 (large)	0.96 (large)	0.26 (small)	0.90 (large)	0.18 (very small)	1.43 (very large)
Lyncornis m. macropterus/	n.s.*	< 0.001†	< 0.001†	n.s.*	n.s.*	< 0.001†	n.s.*
L. m. macrotis	0.39 (small)	1.37 (very large)	1.35 (very large)	0.17 (very small)	0.19 (very small)	1.53 (very large)	0.00 (none)
Lyncornis m. macropterus/	< 0.001†	< 0.001†	< 0.001†	n.s.*	< 0.001†	< 0.001†	< 0.001†
L. temminckii	2.05 (huge)	3.83 (huge)	3.51 (huge)	0.01 (negligible)	3.28 (huge)	3.14 (huge)	1.09 (large)
Lyncornis macropterus/	< 0.001†	< 0.05†	n.s.†	< 0.001†	< 0.001†	n.s.†	< 0.05†
L. bourdilloni	2.87 (huge)	1.25 (very large)	0.62 (medium)	3.29 (huge)	4.12 (huge)	0.40 (small)	0.92 (large)
Lyncornis m. macrotis/	< 0.001†	< 0.001†	< 0.001†	n.s.*	< 0.001†	< 0.001†	< 0.001†
L. temminckii	1.92 (very large)	4.06 (huge)	3.52 (huge)	0.15 (very small)	2.74 (huge)	2.64 (huge)	1.22 (very large)
Lyncornis m. macrotis/	< 0.001†	n.s.†	n.s.†	< 0.001†	< 0.001†	< 0.05†	< 0.05†
L. m. bourdilloni	3.86 (huge)	0.16 (very small)	0.91 (large)	2.72 (huge)	2.79 (huge)	1.64 (very large)	1.40 (very large)
Lyncornis temminckii/	< 0.001†	< 0.001†	< 0.001†	< 0.001†	n.s.*	< 0.001†	n.s.*
L. m. bourdilloni	7.60 (huge)	4.54 (huge)	5.38 (huge)	2.36 (huge)	0.23 (small)	3.50 (huge)	0.31 (small)

For definitions of acoustic variables, see '*Measurements of acoustic characters*'.
*ANOVA.
†Mann–Whitney *U*-test.

Table 5. Morphological character states of nightjars in the *Lyncornis macrotis* complex. Characters (M1-17) and character states (a, b, c, d) are described in the Appendix

Variable	*L. m. bourdilloni*	*L. m. cerviniceps*	*L. m. jacobsoni*	*L. m. macropterus*	*L. m. macrotis*	*L. temminckii*
M1	a (2)	a (22) b (4)	a (8)	a (21)	a (11)	a (23)
M2	a (2)	a (19) b (7)	a (8)	a (21)	a (11)	a (23)
M3	a (1) b (1)	a (14) b (12)	a (8)	a (21)	a (11)	a (22) b (1)
M4	b (2)	b (26)	a (8)	a (1) b (1) c (19)	a (2) c (9)	a (23)
M5	b (2)	a (2) b (23)	b (8)	b (20)	a (2) b (9)	a (22)
M6	b (2)	b (26)	b (8)	a (1) b (20)	b (9)	a (18) b (4)
M7	a (2)	a (25)	b (8)	a (20)	a (10)	a (17) b (4)
M8	b (2)	a (12) b (11)	b (8)	a (2) b (18)	a (1) b (10)	a (8) b (14)
M9	b (2)	a (13) b (13)	a (8)	a (1) b (20)	a (10) b (1)	a (18) b (4)
M10	c (2)	c (21) d (1)	c (8)	c (21)	c (7) d (4)	a (22)
M11	b (1)	a (5) b (14)	a (8)	a (21)	a (8) b (3)	a (17) b (5)
M12	b (2)	b (18)	a (8)	a (20)	a (4) b (6)	a (23)
M13	b (2)	b (18)	c (8)	a (21)	a (1) b (1) c (9)	a (22) d (1)
M14	a (2)	a (5) c (13)	b (8)	a (2) b (19)	b (11)	a (22) b (1)
M15	a (1) b (2)	b (18) c (1)	a (8)	a (2) b (2) c (14)	c (9)	a (22)
M16	b (2)	b (18) c (1)	b (8)	a (3) c (18)	c (11)	a (22)
M17	a (1) b (1)	a (7) b (12)	a (8)	a (21)	a (11)	a (23)

Numbers in parentheses denote the sample size for each character state.

for taxonomic purposes as vocalizations. First, morphological and acoustic data showed similar numbers of diagnostic differences; 52 (20.4%) of the 255 pairwise comparisons of morphological characters in our dataset showed a diagnostic difference, a level similar to that of the acoustic dataset, in which 39 (18.6%) of the 210 pairwise comparisons showed no overlapping of ranges.

Second, morphology, vocalizations and mtDNA recovered the same number of groups. Principal components analysis of vocalizations (and phylogenetic analysis of mtDNA) recovered four groups: (1) *L. m. bourdilloni*, *L. m. cerviniceps* and *L. m. jacobsoni*; (2) *L. m. macrotis*; (3) *L. m. macropterus*; and (4) *L. temminckii*. Analysis of our morphological dataset also indicated that four groups can be distinguished (Table 6): (1) *L. m. macrotis* and *L. m. macropterus*; (2) *L. m. jacobsoni*, (3) *L. m. cerviniceps* and *L. m. bourdilloni*; and (4) *L. temminckii*. The fact that both morphology and vocalizations failed to document a group that the other dataset supported shows that neither dataset is sufficient to recover all major groups in the *Lyncornis* complex.

The finding that *L. m. jacobsoni* differed in six mophological characters from *L. m. cerviniceps* was surprising, because these taxa did not differ in vocalizations or mtDNA. Morphological differences

Table 6. Summary of diagnostic morphological character states of nightjars in the *Lyncornis macrotis* complex. Characters (M1-17) are described in the Appendix

Taxon	Variable																	
	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15	M16	M17	Total
L. m. cerviniceps/*L. m. bourdilloni*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0
L. m. cerviniceps/*L. m. jacobsoni*	–	–	–	X	–	–	X	–	–	–	–	X	X	X	X	–	–	6
L. m. cerviniceps/*L. m. macropterus*	–	–	–	–	–	–	–	–	–	–	–	X	X	–	–	–	–	2
L. m. cerviniceps/*L. m. macrotis*	–	–	–	X	–	–	–	–	–	–	–	–	–	–	X	–	–	2
L. m. cerviniceps/*L. temminckii*	–	–	–	X	–	–	–	–	–	X	–	X	X	–	X	X	–	6
L. m. jacobsoni/*L. m. macropterus*	–	–	–	–	–	–	X	–	–	–	–	–	X	–	–	X	–	3
L. m. jacobsoni/*L. m. macrotis*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	X	X	–	2
L. m. jacobsoni/*L. temminckii*	–	–	–	–	X	–	–	–	–	X	–	–	X	–	–	X	–	4
L. m. jacobsoni/*L. m. bourdilloni*	–	–	–	X	–	–	X	–	X	–	X	X	X	X	–	–	–	7
L. m. macropterus/*L. m. macrotis*	–	–	–	–	–	–	–	–	–	X	–	–	–	–	–	–	–	0
L. m. macropterus/*L. temminckii*	–	–	–	X	X	–	–	–	–	X	–	–	–	–	–	–	–	3
L. m. macropterus/*L. m. bourdilloni*	–	–	–	–	–	–	–	–	–	–	X	X	X	–	–	X	–	4
L. m. macrotis/*L. temminckii*	–	–	–	–	–	–	–	–	–	X	–	–	–	–	X	X	–	3
L. m. macrotis/*L. m. bourdilloni*	–	–	–	X	–	–	–	–	–	–	–	–	–	X	X	X	–	4
L. temminckii/*L. m. bourdilloni*	–	–	–	X	X	–	–	–	–	X	–	X	X	–	–	X	–	6
Total diagnosable	0	0	0	7	3	0	3	0	1	5	2	6	8	3	6	8	0	52

included the pattern of the lower throat, undertail covers, tail, primaries and tertials. The nature of these differences suggests that it is unlikely that these were caused by environmental factors, such as differences in temperature or humidity, which affect the size and intensity of coloration (James, 1970; Zink & Remsen, 1986). Furthermore, our findings cannot be attributed to inadequate sampling, because we used multiple specimens of both taxa (Supporting Information, Table S4). We suggest that the morphological differences evolved rapidly and recently in *L. m. jacobsoni*, leaving insufficient time for vocal differences to evolve and for the mtDNA gene tree to coalesce.

Two pairs of taxa did not differ in morphology (*L. m. cerviniceps* and *L. m. bourdilloni*; *L. m. macrotis* and *L. m. macropterus*). The absence of differences in vocalizations and mtDNA suggests that the lack of morphological differences between *L. m. cerviniceps* and *L. m. bourdilloni* is likely to be a result of incorrect taxonomy (i.e. there is no evidence that *L. m. bourdilloni* represents a separate species). The substantial divergence in mtDNA of *L. m. macrotis* and *L. m. macropterus* indicates prolonged isolation and suggests that their lack of morphological divergence represents stasis.

Integrative taxonomy of *Lyncornis*

If species are viewed as population lineages, no particular dataset should be considered as essential or 'defining' for species rank (Mayden, 1997; de Queiroz 1999, 2007; Padial & de la Riva, 2010). In integrative taxonomy, traditional species properties (monophyly, levels of genetic divergence, reproductive isolation, diagnosability of morphological and acoustic characters) represent complementary ways to 'find' species (de Queiroz, 2007; Padial *et al.*, 2009). Given that lineages can differentiate in many different ways, species properties do not necessarily evolve in the same order in all lineages or possess all species properties. In our dataset, this is clearly shown by comparing the species pair *L. m. macropterus* and *L. m. macrotis*, which show differences in vocalizations but not morphology, with *L. m. cerviniceps* and *L. m. jacobsoni*, which show differences in morphology but not vocalizations. Clearly, evidence for evolutionarily unique lineages can come from different data sources, and each of these might recover species not recovered by the other sources.

Phylogenetic analysis of mtDNA (Fig. 3) and multivariate (Fig. 5) and univariate analyses of vocalizations (Table 6) support the distinctiveness of four groups: (1) *L. m. cerviniceps*, *L. m. bourdilloni* and *L. m. jacobsoni*; (2) *L. m. macropterus*; (3) *L. m. macrotis*; and (4) *L. temminckii*. The sequence divergence of these four groups was 5–11%. This is

Figure 6. Ventral aspect of four taxa in the *Lyncornis macrotis* complex, illustrating differences in size, pattern and coloration. Note the marked difference in size between *L. m. cerviniceps* and *L. m. jacobsoni*/*L. m. macropterus*. G. Sangster/©Naturalis Biodiversity Center, Leiden.

Figure 7. Upper tail of four taxa in the *Lyncornis macrotis* complex, illustrating differences in pattern and coloration. Note the marked differences in pattern and coloration between *L. m. cerviniceps* and *L. m. jacobsoni*. G. Sangster/©Naturalis Biodiversity Center, Leiden.

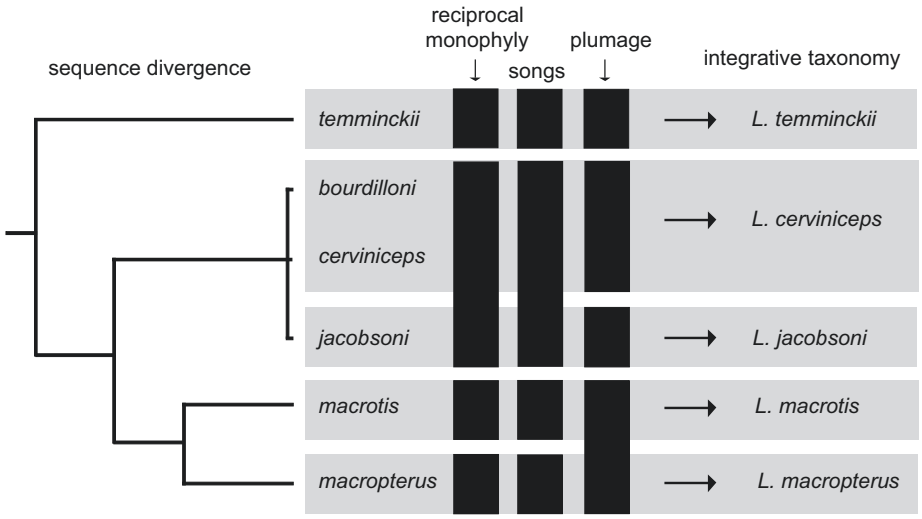

Figure 8. Integrative taxonomy of *Lyncornis* nightjars, illustrating contrasting sensitivities of datasets and the failure of each dataset to recover all five species.

much higher than levels usually found in subspecies but is typical of levels found in closely related species (Aliabadian *et al.*, 2009). The combination of reciprocal monophyly, high levels of sequence divergence and distinctive vocalizations indicates that at least these four groups should be treated as species. Treatment of these four groups as species is consistent with the treatment of other nightjars with distinct vocalizations (Robbins & Ridgely, 1992; Robbins *et al.*, 1994; Sangster & Rozendaal, 2004).

The taxonomic status of *L. m. jacobsoni* is less straightforward owing to the discordance among datasets. Multivariate and univariate analyses of vocalizations could not separate *L. m. jacobsoni* from *L. m. cerviniceps* owing to complete overlap in both PCA and DFA and lack of diagnostic differences in all 14 variables. In our phylogeographical analysis, *L. m. jacobsoni* is nested in *L. m. cerviniceps*, yet these two taxa differ in six diagnostic morphological character states. As noted above, the multiple morphological differences that characterize *L. m. jacobsoni* cannot be dismissed as a result of morphological plasticity (e.g. attributable to environmental differences). Morphological differences have always been regarded as evidence for evolutionary distinctiveness. Although *L. m. jacobsoni* and *L. m. cerviniceps* are not reciprocally monophyletic and do not differ acoustically, these properties cannot be regarded as essential for species rank (de Queiroz, 2007). In view of its phylogenetic position within *L. m. cerviniceps*, the multiple diagnostic morphological character states of *L. m. jacobsoni* are most likely to have evolved recently, perhaps facilitated by its isolation on a small island. We argue that *L. m. jacobsoni* is a morphologically distinct lineage that merits species rank. Treatment of *L. m. jacobsoni* as a species is consistent with the taxonomic treatment of the owls *Otus beccarii* (Salvadori, 1876), *Otus insularis* (Tristram, 1880) and *Otus magicus* (Müller, 1841). These taxa have identical songs (Marshall, 1978; G.S., pers. obs.) but are widely treated as species based on differences in morphology (Cleere, 1999; Clements, 2007; König *et al.*, 2008; Dickinson & Remsen, 2013; Gill *et al.*, 2021).

Lyncornis m. bourdilloni has long been considered a dubious taxon. It was synonymized with *L. cerviniceps* by Hartert (1892: 604). Baker (1927) treated it as a subspecies of *L. cerviniceps* but noted that it was 'perhaps a little darker with less buff and more grey on the wings and upper plumage'. Ali & Ripley (1983) noted that it was doubtfully distinguishable from *L. m. cerviniceps* except by smaller size, and they reiterated Baker's statement. Nevertheless, it was retained as a subspecies of *L. macrotis* by most authorities (Cleere, 1998; Dickinson & Remsen, 2013; Gill *et al.*, 2021). The validity of *L. m. bourdilloni* was once again questioned by Holyoak (2001: 319), who noted that it 'may well be better treated as a synonym of *cerviniceps*'. Our analyses yielded no diagnostic differences between *L. m. bourdilloni* and *L. m. cerviniceps* in vocalizations or morphology, and their mtDNA divergence was negligible. Thus, there is no evidence that *L. m. bourdilloni* represents a separate species (*sensu* de Queiroz, 1999). This taxon also does not meet modern criteria for subspecies, including reciprocal monophyly (Avise & Ball, 1990) and diagnosability (Remsen, 2010). We think that *L. m. bourdilloni* is best treated as a junior synonym of *L. cerviniceps*. We conclude that the *Lyncornis* complex is best treated as five monotypic species (Fig. 8), as follows:

1. *Lyncornis cerviniceps* (Gould, 1838), great eared nightjar

Found in two disjunct areas: in Kerala, south-west India (Ali & Ripley, 1983) and Southeast Asia south to northern Malaysia (Wells, 1999). This includes *L. bourdilloni*, which was placed into synonymy by Hartert (1892) and is followed here based on our evidence.

2. *Lyncornis jacobsoni* (Junge, 1936), Simeulue eared nightjar

Endemic to Simeulue Island off western Sumatra, Indonesia. Given that the type series was collected in 1913 (Junge, 1936), there were no published records of this taxon (Ripley, 1944; Van Marle & Voous, 1988; Holmes, 1994) until it was sound recorded by Martjan Lammertink in 2001 (Scharringa, 2005) and subsequently by Philippe Verbelen and Bram Demeulemeester in 2009 (F. Verbelen, in litt.).

3. *Lyncornis macropterus* (Bonaparte, 1850), Sulawesi eared nightjar

Sulawesi, Sangihe, Peleng (Banggai Island) Taliabu and Mangole (Sula Islands) (Holyoak, 2001).

4. *Lyncornis macrotis* (Vigors, 1831), Philippine eared nightjar

Endemic to the Philippines, where it is found on most islands (Dickinson *et al.*, 1991). Synonym: *Lyncornis mindanensis* Tweeddale, 1878.

5. *Lyncornis temminckii* (Gould, 1938), Malaysian eared nightjar

Restricted to southern mainland Malaysia, Sumatra, Banka and Borneo.

Conclusions

Our study updates the influential approach of Marshall (1978) to the taxonomy of owls and nightjars. According to Marshall (1978), populations of nocturnal birds with different vocalizations belong to different species, but those with the same vocalizations are the same species. We found that populations with different vocalizations are also distinct in morphology and highly divergent in DNA but that the reverse is not true; populations with the same vocalizations might still possess multiple diagnostic differences in morphology and represent distinct species taxa. Morphology and vocalizations were equally informative for species limits of *Lyncornis* nightjars and should be viewed as complementary data sources.

In our study, the three datasets did not differ in sensitivity, but none found all five species because each dataset 'overlooked' one species. This study underscores the importance of using multiple datasets. No particular dataset can be considered to be best (i.e. most sensitive) or sufficient to recover all taxa.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

DATA AVAILABILITY

All DNA sequence data are available at GenBank under accession numbers OM830960–OM830985.

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APPENDIX

Morphological character states

M1. Crown, extent of spots:
- **a.** on fore-, mid- and hindcrown;
- **b.** only on midcrown, a few dark spots.

M2. Crown, size of spots:
- **a.** medium to large;
- **b.** small.

M3. Crown, shape of spots:
- **a.** round (in some or all spots);
- **b.** elongated (no round spots).

M4. Crown, background coloration:
- **a.** greyish with rufous tinge;
- **b.** pale greyish with rufous tinge;
- **c.** greyish with slight rufous tinge.

M5. Hindneck collar, colour of pale terminal feather edges:
- **a.** buff (without rufous);
- **b.** rufous-buff.

M6. Uppermost throat, feather edges, breadth and coloration:
- **a.** black with medium/broad chestnut edges;
- **b.** black with narrow chestnut edges.

M7. Throat, pattern and extent of white:
- **a.** all feathers on sides of lower throat entirely white (no dark bases);
- **b.** feathers on sides of lower throat with dark basis and broad white edges.

M8. Upper breast, breadth of chestnut feather edges:
- **a.** broad (≥ 2 mm);
- **b.** narrow (< 2 mm).

M9. Central breast feathers (first two rows of feathers below dark breastband), subterminal bars (or spots):
- **a.** absent;
- **b.** present.

M10. Lower breast feathers, pattern:
- **a.** with broad terminal spots at sides of feathers. Some feathers have a second pale band at one side;
- **b.** with dark basis and a single narrow pale band;
- **c.** as state a but with subterminal bar or subterminal spots;
- **d.** with broad, pale buff terminal band.

M11. Belly feathers, breadth of pale terminal feather edges:
- **a.** narrow (< 6 mm);
- **b.** broad (≥ 6 mm).

M12. Undertail coverts, pattern and coloration:
- **a.** pale (rufous-buff) and dark coloration about equal (no large pale patches);
- **b.** pale rufous-cream coloration predominates, with narrow dark bars and small spots (large pale patches).

M13. Uppertail, pattern and coloration:
- **a.** feathers with three types of bands with different coloration: (1) a buff band with dark spots; (2) a dark brown band with buff spots; and (3) a narrow dark brown band;
- **b.** feathers with three types of bands with different coloration: (1) a broad warm cinnamon band with a few dark brown spots; (2) a grey/buff-brown band, densely spotted dark brown; and (3) a narrow solid dark brown band, broadest at feather shaft;
- **c.** feathers with three types of bands with different coloration: (1) a buff band with brown or black spots; (2) a dark brown/black band with small buff spots; and (3) a broad solid black/brown band, with even breadth;
- **d.** feathers with two types of bands with different coloration: (1) irregular cinnamon spots; and (2) dark brown.

M14. Primaries, pattern and coloration:
- **a.** outer primary with cinnamon-coloured bands; these are 4× as narrow as the dark brown parts of the primaries;
- **b.** outer primary predominantly dark brown with narrow, indistinct cinnamon-coloured bands or spots;
- **c.** outer primary with cinnamon-coloured and dark brown bands, which are equally wide (i.e. with broad cinnamon coloured bands).

M15. Tertials, pattern and coloration:
- **a.** cream to pale rufous-buff, densely dark spotted, with a few dark patches; or dark brown with buff spots on side;
- **b.** buff with small dark spots; some feathers have warm brown outer or inner edges; dark subterminal spot at feather tips;
- **c.** buff/silver-coloured with dark subterminal spot near feather tip.

M16. Lower scapulars, pattern and coloration:
- **a.** pale buff, densely spotted dark brown, with large dark subterminal patch on outer vane;
- **b.** predominantly cream with small dark spots, with large dark subterminal patch;
- **c.** buff/silver-coloured, with dark subterminal patch near tip.

M17. Wing coverts, pattern and coloration:
- **a.** predominantly dark brown, with rufous-brown bars;
- **b.** rufous chestnut, with fine and sharp dark brown bars.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Tissue samples used for DNA analysis.
Table S2. Primers used for polymerase chain reaction amplification and sequencing.
Table S3. Sound recordings examined.
Table S4. Museum specimens examined.