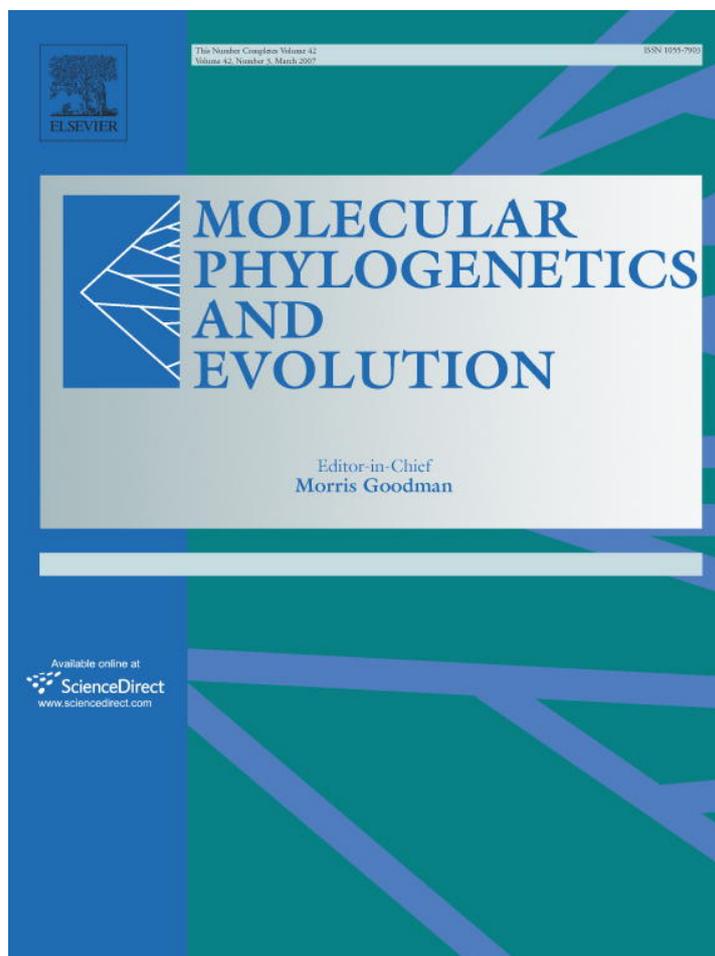


Provided for non-commercial research and educational use only.
Not for reproduction or distribution or commercial use.



This article was originally published in a journal published by Elsevier, and the attached copy is provided by Elsevier for the author's benefit and for the benefit of the author's institution, for non-commercial research and educational use including without limitation use in instruction at your institution, sending it to specific colleagues that you know, and providing a copy to your institution's administrator.

All other uses, reproduction and distribution, including without limitation commercial reprints, selling or licensing copies or access, or posting on open internet sites, your personal or institution's website or repository, are prohibited. For exceptions, permission may be sought for such use through Elsevier's permissions site at:

<http://www.elsevier.com/locate/permissionusematerial>

A molecular phylogeny of the nightjars (Aves: Caprimulgidae) suggests extensive conservation of primitive morphological traits across multiple lineages

Carl Larsen^a, Michael Speed^b, Nicholas Harvey^c, Harry A. Noyes^{b,*}

^a *Deanery of Sciences and Social Sciences, Liverpool Hope University, Hope Park, Liverpool L16 9JD, UK*

^b *School of Biological Sciences, University of Liverpool, Crown Street, Liverpool L69 7ZD, UK*

^c *East Malling Research, East Malling, Kent ME19 6BJ, UK*

Received 19 January 2006; revised 13 September 2006; accepted 4 October 2006

Available online 14 October 2006

Abstract

We report a molecular re-assessment of the classification of the nightjars which draws conclusions that are strongly at odds with the traditional, morphology-based classifications. We used maximum likelihood and Bayesian methods to compare the cytochrome *b* gene for 14 species from seven of the 15 genera of the Caprimulgidae and partial cytochrome *b* sequence data was available for a further seven species including three further genera. We found that within the Caprimulgidae there were four geographically isolated clades with bootstrap support greater than 70%. One of these clades contained just *Chordeiles* species, the remaining three clades each contained a mixture of genera including *Caprimulgus* sp. A clade of exclusively South American nightjars included the genera *Caprimulgus*, *Uropsalis*, *Eleopthreptus* and *Hydropsalis*. A clade of African and Eurasian birds included *Caprimulgus* and *Macrodipteryx*. *Phalaenoptilus nuttallii* and *Caprimulgus vociferous* formed a clade of North American birds. Two ecological factors appear to make morphological classification potentially misleading: first, the apparent retention of primitive anti-predator and foraging-related traits across genetically divergent groups; second, rapid divergence in other traits, especially those related to mating, which generate high levels of morphological divergence between species that are genetically very similar. The cytochrome *b* data suggests that the genus *Caprimulgus* is not monophyletic and is restricted to Africa and Eurasia and that *Caprimulgus* species from outside this area have been misclassified as a consequence of retention of primitive adaptations for crepuscular/nocturnal living. Some other genera also appear to have little support from the cytochrome *b* data.

© 2006 Elsevier Inc. All rights reserved.

Keywords: *Eurostopodus*; *Caprimulgus*; Cytochrome *b*; Evolution; Conserved morphology; Primitive traits; MrBayes; Paup; Caprimulgidae; Caprimulgiformes

1. Introduction

The avian nightjar family Caprimulgidae is globally distributed, comprising of 89 species of largely nocturnal insectivorous birds. Molecular classifications by Sibley and Ahlquist (1990) and Mariaux and Braun (1996), confirmed Peters (1940) morphological subdivision of the Caprimulgidae into the subfamily Chordeilinae (Nighthawks) that are restricted to the Americas and consist of 10 species, within 4 genera, and the subfamily Caprimulginae (nightjars,

pauraque, poorwills and whip-poor-wills) that occur worldwide. Sizes and distribution of genera within the family vary widely, so that whilst the largest and most cosmopolitan genus, *Caprimulgus*, contains 57 species distributed worldwide, all others are geographically restricted, including the smallest such as *Nyctidromus* and *Phalaenoptilus* that are each represented by only a single species.

Nightjar classification and identification are currently phenetic, based predominantly on morphology, song and to a lesser degree behaviour, rather than on molecular characteristics. Typical morphology of Caprimulgidae consists of cryptic and vermiculated plumage, long pointed wings, long tails that vary in form, and a small weak bill with a large

* Corresponding author.

E-mail address: harry@liv.ac.uk (H.A. Noyes).

gape (Huxley, 1867; Beddard, 1886; Hartert, 1892; Glenny, 1953; Hoff, 1966; Bühler, 1970; Cramp, 1985; Sibley and Ahlquist, 1990; Holyoak, 2001). Separation of the Caprimulgidae into the Caprimulginae and Chordeilinae is based on palate structure that is schizognathous in Caprimulginae and desmognathous in Chordeilinae (Oberholser, 1914); Caprimulginae also possess conspicuous rictal bristles although semi-bristles are present in many *Chordeiles* (Bent, 1989). Typical behaviour consists of maintaining crypsis and includes remaining motionless in a prone position when incubating or perched during the daytime; nesting directly on the ground in the majority of species, maternal incubation but with some male involvement especially if double-brooded, (but paternal in *Phalaenoptilus*), and most species perform some kind of nest defence and are territorial (Cramp, 1985; Bent, 1989; Cleere and Nurney, 1998; Holyoak, 2001). At the generic level assignment to genus is based almost exclusively on plumage modifications, song and behaviour (Peters, 1940; Holyoak, 2001), but such differences are increasingly recognised as of specific rather than generic significance (see recent studies on Pici, Gabulae, and Coracii, Johansson and Ericson, 2003). Most recent studies have been concerned with relationships of families within the order Caprimulgiformes and the relative position of the order to the Strigiformes, Trochiliformes and Apodiformes (Fidler et al., 2004; Iwaniuk et al., 2006; Mayr, 2002), and not the reliability of each genus within the family Caprimulgidae. Characteristics that have been used to classify Caprimulgidae genera include relative measurements of body size and shape, wing and tarsi length and extent of feathering, plumage characteristics including moult, and are often the records of fieldworkers. Rigorous Hennigan methods have not been used to classify species or genera within the Caprimulgidae and it may be the case that certain plumage characters that have been used to establish new genera have been given excessive weight. Indeed, the most contemporary review of nightjar systematics (Holyoak, 2001) refers to the current classification of the genus *Caprimulgus* as a ‘rag bag’ containing all Caprimulginae lacking any character striking enough to demand treatment as separate genera (Holyoak, 2001, p. 374). Although *Caprimulgus* may require further subdivision it is also possible that some genera such as *Phalaenoptilus* should be ‘merged with *Caprimulgus*’ (Holyoak, 2001, p. 343). The generic status of two other genera not sampled in this study, *Podager* and *Nyctidromus*, was questioned by Hartert (1892) and by Holyoak (2001) who suggested that they should be suppressed and assigned to *Caprimulgus*. Morphological characteristics associated with vocalisation are also employed to distinguish members of the Caprimulgidae e.g. the anatomy of the voice organ and palate (Beddard, 1886); skull bones, (Huxley, 1867); skull and jaw bones (Bühler, 1970); mouth size, (Jackson, 1985); as well as the songs themselves (Bent, 1989; Cleere and Nurney, 1998; Holyoak, 2001). Indeed, vocalisation has prevailed as one of the primary agents in the ‘lumping’ and ‘splitting’ of genera and races (Holyoak, 2001; Cleere and Nurney, 1998;

Turner et al., 1991; Fry, 1988; Davis, 1962, 1979). In all these cases there is a risk that patterns of variation in morphological or behavioural traits (De Queiroz and Wimberger, 1993) that are mediated by sexual selection and adaptation to habitat gain greater weight in the classifications than characters that are measures of common descent. Furthermore, there is such paucity of behavioural information for many genera within the Caprimulgidae, that a thorough inter-generic comparison of behaviour is not feasible. Hence, it is presently unclear whether the relatively widespread distribution of nightjars assigned to the genus *Caprimulgus* is a consequence of worldwide dispersal of a monophyletic genus, a consequence of misclassification due to retained primitive traits across genera or the result of multiple convergence events of distinct genera.

We have constructed phylogeny from the partial sequence of the cytochrome *b* gene of a representative range of caprimulgid species to test the hypothesis that the genus *Caprimulgus* is monophyletic.

2. Methods

2.1. Samples

Blood or tissue samples were collected from live and road kill specimens by licensed bird handlers. Samples were collected from 14 species spread across seven genera. Partial cytochrome *b* sequence data was available for a further seven species including three further genera from Mariaux and Braun (1996) and Espinosa de los Monteros (2000). Sequence data available at GenBank (see Table 1) for representatives of the Strigidae, Batrachostomidae, Podargidae, Nyctibiidae and Steatornidae, also from Mariaux and Braun (1996) were used as outgroups for phylogenetic analysis.

2.2. Whole-genomic DNA extraction

2.2.1. Blood, and liver tissues

Samples were stored in 20% DMSO at -20°C , with the exception of tissue from *Nyctidromus albicollis* and blood from *Caprimulgus fossii*, and *C. plumipes*, which were resuspended in Cell Lysis Solution (GenomicPrep Cells and Tissue DNA Isolation Kit, Amersham) and 10 mg/ml of proteinase K solution and then homogenised and incubated overnight at 55°C . DNA was extracted from samples resuspended in Cell Lysis solutionDNA using ‘GenomicPrep Cells and Tissue DNA Isolation Kit’ following the manufacturers’ guidelines. DNA was prepared from samples stored in DMSO by phenol–chloroform extraction followed by ethanol precipitation. *Feathers*: It was necessary to extract DNA from feathers for only one species, *Macrodipteryx longipennis*, using the silica-gel membrane in QIAquick spin columns (QIAquick™ (QIAGEN®) PCR Purification Kit). Unfortunately, samples available for a further four species: *Caprimulgus donaldsoni*, *C. ruficollis*, and *C. parvulus*; and *Nyctidromus albicollis* did not yield mtDNA of suitable quality for PCR amplification.

Table 1
Summary of samples, origins of data and Genbank location codes

Family and scientific name	Sample and source code	Location	GenBank Accession No.	Bases used
Steatornithidae				
<i>Steatornis caripensis</i>	Tissue (a)	Genbank	X95773	101–751
Podargidae				
<i>Podargus papuensis</i>	Tissue (a)	Genbank	X95772	101–751
Batrachostomidae				
<i>Batrachostomus cornutus</i>	Tissue (a)	Genbank	X95776	101–751
Nyctibiidae				
<i>Nyctibiu aethereus</i>	Tissue (a)	Genbank	X95781	101–751
Caprimulgidae				
<i>Chordeiles rupestris</i>	Tissue (a)	Genbank	X95778	101–751
<i>Chordeiles acutipennis</i>	Tissue (CW)	N. America	DQ062148	101–751
<i>Chordeiles minor</i>	Tissue (CW)	N. America	DQ062142	101–751
<i>Eurostopodus mystacalis</i>	Tissue (a)	Genbank	X95779	101–751
<i>Eurostopodus papuensis</i>	Tissue (a)	Genbank	X95780	101–751
<i>Phalaenoptilus nuttallii</i>	Blood/tissue (CW)	N. America	DQ062141	101–751
<i>Nyctiphrynus mcleodii</i>	(c)	Genbank	U89195	101–751
<i>Caprimulgus vociferus</i>	(c)	N. America	U89194	101–751
<i>Caprimulgus longirostris</i>	Tissue (b)	S. America	X95777	101–751
<i>Caprimulgus maculicaudus</i>	Blood (RC)	Paraguay	DQ062137	101–751
<i>Caprimulgus europaeus europaeus</i>	Blood, tissue	UK	DQ062139	101–751
<i>Caprimulgus europaeus unwini</i>	Blood (DP)	Kenya	DQ062135	101–751
<i>Caprimulgus europaeus plumipes</i>	Blood (DP)	Kenya	DQ062147	101–751
<i>Caprimulgus fraenetus</i>	Blood, tissue (DP)	Kenya	DQ062136	101–751
<i>Caprimulgus inornatus</i>	Blood (DP)	Kenya	DQ062138	101–751
<i>Caprimulgus climacurus</i>	Blood, tissue (CB)	Gambia	DQ062144	101–751
<i>Caprimulgus candicans</i> ^a	Blood (RC)	Paraguay	DQ062140	101–751
<i>Macrodipteryx longipennis</i>	Tissue, feather (CB)	Gambia	DQ062143	101–751
<i>Hydropsalis brasiliensis</i>	Blood (RC)	Paraguay	DQ062146	101–751
<i>Uropsalis segmentata</i>	(c)	Genbank	AF115898	128–424
<i>Eleothreptus anomalus</i>	Blood (RC)	Paraguay	DQ062145	101–751
Strigidae				
<i>Bubo bubo africanus</i>	Muscle, blood		AJ003952	101–751
<i>Bubo bubo</i>	Muscle, blood		AJ003965	101–751

Source of sequence data. Taxa with accession numbers in the range DQ062135–DQ062148 were determined in the present study. The bases used column indicates the positions in the complete cytochrome *b* sequence of *C. vociferus* to which the sequences were aligned. Abbreviations: CB, Clive Barlow; CW, Chris Woods; DP, David Pearson; RC, Rob Clay; PNG, Papua New Guinea. (a) Sequence determined by Mariaux and Braun. (b) Sequence determined by Peter Arctander. (c) Sequence determined by Espinosa de los Monteros.

^a Also termed *Eleothreptus candicans* (Cleere, 2002).

The primer used for PCR of light-strand sequence was L14841 (Kocher et al., 1989). The heavy-strand primer was H15498 (5'-AAACTGCAGGGAATAAAGTTATCTGGG TCTC-3'; Mariaux and Braun, 1996). PCR products were purified using a QIAquick™ Gel Extraction Kit (QIAGEN®) and sequenced on an ABI373A automated sequencer using the PRISM Dye-Deoxy sequencing system (ABI).

DNA sequences were aligned with Clustal. The aligned matrix consisted of 27 taxa Table 1. and 652 nucleotides between bases 101 and 750 in the cytochrome *b* gene of *C. vociferus* GenBank accession number U89194. The bases used relative to *C. vociferus* are shown in Table 1. Bayesian posterior probabilities were computed with MrBayes 3.2.1 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) by running 1,000,000 MCMC generations using the program default priors on the GTR model. Model parameters were inferred by MrBayes from the data. 1000 trees were sampled from the posterior probability distribution (one every 1000 generations) and 25% were discarded as burn-in to ensure that chains had become stationary.

The most appropriate model for maximum likelihood analysis was identified using hierarchical likelihood ratio tests in Modeltest 3.06 (Posada and Crandall, 1998). Trees were identified using a heuristic search with maximum likelihood in PAUP 4.10b (Swofford, 1998) with the model and parameters identified in Modeltest. One hundred bootstrap replicates were also evaluated using a heuristic search in PAUP. The constancy of the rate of substitution in different branches of the tree was tested using the likelihood ratio test (Felsenstein, 1981). Alternative tree topologies were evaluated by the method of Shimodaira and Hasegawa (1999) implemented in PAUP.

3. Results

3.1. Monophyly of the caprimulgidae

Trees of 21 caprimulgid taxa were compiled using Bayesian and maximum likelihood (ML) using a single member of each of the following Caprimulgiformes genera *Steator-*

nis, *Podargus*, *Nyctibius*, and *Batrachostomus* to root the Caprimulgidae, the complete tree was rooted on two species of *Bubo* (Fig. 1).

Seven of the 14 genera recognised by Cleere and Nurney (1998) in the Caprimulgidae were represented in these classifications. These genera clustered in a single clade although it had no statistical support in either Bayesian or ML trees. The genus *Eurostopodus* is included in the family Caprimulgidae by Holyoak (2001) but not by Cleere and Nurney (1998), *Eurostopodus* was external to the Caprimulgidae in the best Bayesian and ML trees although since the Bayesian and ML support for the relevant nodes was low the classification of this genus cannot be inferred from this data. The inclusion of *Eurostopodus* in a clade containing the other Caprimulgidae genera was not rejected by the Shimodaira and Hasegawa test. Therefore, although the definition of Caprimulgidae of Holyoak (2001) that included *Eurostopodus* cannot be excluded, the definition used by Cleere and Nurney (1998) that did not include *Eurostopodus* will be used here.

3.2. Monophyly of genera within the caprimulgidae and geographical clustering

Within the Caprimulgidae there were three clades with posterior probabilities greater than 0.99 (Fig. 1). The same clades were seen in the ML tree with bootstrap support greater than 92%. Additionally, a Nearctic clade containing *Phaelaenoptilus nuttallii* and *Caprimulgus vociferus* had weak bootstrap support (72%) and a Bayesian posterior probability of (0.94). *Nyctiphrynus mcleodii* did not cluster with any other Caprimulgidae included here. One clade contained just *Chordeiles* species and appeared monophyletic. The well supported Neotropical and African clades each contained a mixture of genera including *Caprimulgus*. Although the clades of Caprimulgidae contained a mixture of taxa there was strong geographic clustering. One weakly supported North American clade contained *Caprimulgus vociferus* and *Phaelaenoptilus nuttalli* and another just *Chordeiles* spp.; a South American clade contained three *Caprimulgus* species, and *Eleothreptus anomalus*, *Hydropsalis*

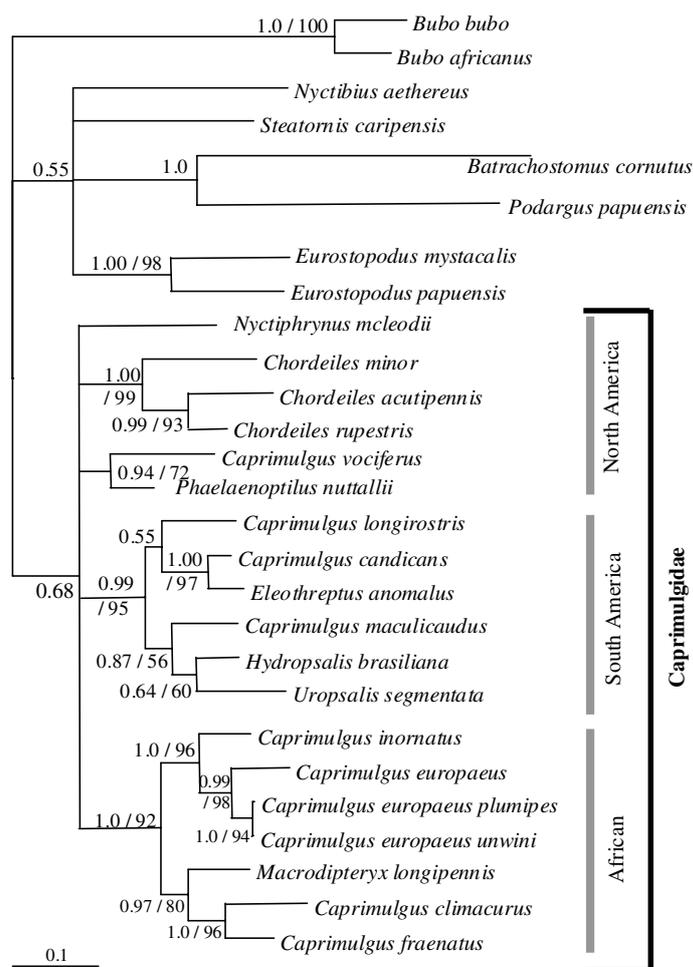


Fig. 1. Bayesian tree of Caprimulgiformes, rooted on the Strigidae, compiled with Mr. Bayes. A ML tree had identical topology for supported nodes. Bootstrap values from the ML tree are shown after the Bayesian posterior probabilities where they were greater than 50. The ML tree was compiled with PaupV4.10b using the General Time Reversible Model with gamma distribution of variability of sites. GC composition was 50% but other parameters were highly skewed, relative transition and transversion rates were: A ↔ C 0.85; A ↔ G 9.54; A ↔ T 2.17; C ↔ G 0.41; C ↔ T 11.90; G ↔ T 1.00; proportion of invariable sites 0; gamma distribution rate parameter 0.257.

brasiliensis and *Uropsalis segmentata*; and an African clade contained *Macrodipteryx longipennis*, *Caprimulgus europaeus*, (which migrates to the western Palaearctic to breed), and three other *Caprimulgus* species which are permanent residents of Africa. The hypothesis that species within the genus *Caprimulgus* as currently described are a monophyletic clade was compared with the alternative hypothesis represented by the tree presented in Fig. 1 using the Shimodaira–Hasegawa test. This test rejected the hypothesis that the genus *Caprimulgus* is monophyletic ($p < 0.05$).

A parsimony tree was also compiled which contained the same four major clades of Caprimulgidae as the ML tree (not shown). Bootstrap values were generally lower and there were two differences in topology within the major clades between the parsimony and ML trees that are described in the appropriate sections below.

3.3. Evolutionary rates and relationships among clades

None of the methods could determine the relationship between any of the four Caprimulgidae clades with confidence. A series of likelihood ratio test was applied by systematically removing clades or species within the Caprimulgidae to identify the largest set of Caprimulgidae taxa for which the likelihood ratio test did not reject the molecular clock. The molecular clock hypothesis could not be rejected for the Caprimulgidae if just *P. nuttallii* was excluded ($p = 0.52$) or if just *C. europaeus* was excluded ($p = 0.16$). Since both *P. nuttallii* and *C. europaeus* could individually cause the data to appear non-clocklike the effect of excluding both these taxa on bootstrap values was tested. However, despite the exclusion of these taxa, bootstrap values for relationships between the major geographical groups were still less than 50% and therefore, it seems unlikely that the difficulty in resolving relationships between major clades of Caprimulgidae is due to rate variation. It is possible that the difficulty in resolving relationships within the Caprimulgidae is due to a combination of a rapid radiation event followed by saturation of the relatively rapidly evolving cytochrome *b* gene over the long periods of time that this group appears to have existed (Cibois et al., 1999; DeFilippis and Moore, 2000). The mean transition/transversion ratio between taxa within each of the four clades within the Caprimulgidae was 5.0 whilst the mean ratio between taxa in different Caprimulgidae clades was 2.3, this being indicative of some degree of saturation. A phylogeny of a less rapidly evolving gene nuclear may help to resolve these relationships (Hughes, 1999; Sheldon et al., 2000), but a radiation event may be impossible to resolve. The Caprimulgidae appear to be an exception to the observations of Moore and DeFilippis (1997) who concluded that the utility of cytochrome *b* is most effective at resolving avian diversification at the level of families through to sub-species.

4. Discussion

The most striking feature of the cytochrome *b* classification is the clustering based on geography rather than exist-

ing nomenclature. Incongruencies between geographical clades based on molecular phylogenetic hypotheses and those derived from morphology have been demonstrated in a diverse range of organisms including Asian wood-feeding cockroaches (Maekawa et al., 1999), fire salamanders (García-París et al., 2003) and tragopans (Randi et al., 2000). Such geographical clustering suggests that many *Caprimulgus* species have been misclassified as a consequence of excessive weight being attached to some morphological characters; for example the exaggerated secondary sexual features of *Macrodipteryx* and *Uropsalis* and the consequent creation of distinct genera for birds that have very similar cytochrome *b* genes. Convergent evolution has confounded classifications of birds in some cases, for example, the Gymnogene (*Polyboroides typus*) and the Crane Hawk (*Geranoospiza caerulescens*), from South America and Southern Africa were believed to be closely related based on shared morphology and a remarkably mobile intertarsal joint, but mitochondrial and nuclear DNA sequences have shown these features to be convergent adaptations for extracting prey from rock crevices (Lerner and Mindell, 2005). The Caprimulgidae form the most striking example of conserved primitive traits within birds, of which we are aware, that has confounded the morphology based classification. This is particularly the case in South America where there are four caprimulgid genera (*Caprimulgus*, *Hydropsalis*, *Uropsalis* and *Eleothreptus*) that have less genetic diversity between them (maximum Jukes Cantor distance 0.112) than found within the *Caprimulgus* species of the Old World (maximum distance 0.131). Indeed, *Hydropsalis brasiliensis* and *Uropsalis segmentata* are no more genetically divergent (Jukes Cantor distance 0.079) than the subspecies *Caprimulgus e. europaeus* and *C. e. plumipes* (0.081). Hence, Caprimulgidae present a remarkable case study in which key components of morphology that have been used for classification turn out to be unreliable phylogenetic markers. *Hydropsalis* spp. differ from *Caprimulgus* in having trident shaped tails that are especially evident in the males, and *Uropsalis* spp. males have extremely elongated outer tail feathers (Cleere and Nurney, 1998). Otherwise, these genera are similar in habit and appearance to other Neotropical *Caprimulgus* spp. It would appear that species from Africa and South and North America have been included in the genus *Caprimulgus* because primitive traits (morphology, cryptic plumage and behaviour) have been retained because the lifestyle of crepuscular/nocturnal hunting on the wing with diurnal ground nesting has been retained. In contrast, local adaptation and sexual selection may have led to morphological/behavioural changes that have appeared to be sufficiently large in the context of the general uniformity of *Caprimulgus* to warrant genus status for *Hydropsalis* and *Uropsalis* when compared with *Caprimulgus*. The minor differences in the cytochrome *b* gene associated with these morphological changes suggest that the underlying genetic differences between *Hydropsalis* and *Uropsalis*, on the one hand, and *Caprimulgus*, on the other, are small. The *Eleothreptus anomalus* and *Caprimulgus can-*

dicans clade had high (99%) bootstrap support and a high posterior probability (1.00). This is consistent with observations of similarity made in the field (Cleere and Nurney, 1998; Cleere, 2002; Clay, personal communication). Indeed, Cleere (2002) has assigned *C. candicans* to the genus *Eleothreptus* on the basis of morphological, vocal and behavioural similarities. Similarities include plumage detail, outer primaries that are slightly bent inwards, a short tail, broad bill with particularly long rictal bristles and partially feathered tarsi. Both species use mechanical wing sounds, which are thought to emanate from the modified outer primaries, during courtship display. Some of these features occur widely in Neotropical *Caprimulgus* and could simply be convergent adaptations or indeed primitive traits. *Eleothreptus anomalus* and *Caprimulgus candicans* are considered allopatric, possibly parapatric in places. There is paucity in distribution data but both breed in grasslands with scattered trees (Cleere and Nurney, 1998). *Eleothreptus anomalus* shows a preference for grassland near to wet areas (Lowen, personal communication). *C. longirostris* is reported as being more similar to *C. candicans* than other *Caprimulgus* sp. in possessing a square tail, stout rictal bristles, extensive white in outer primaries and retrices, rounded wings, and also size, (Holyoak, 2001) and this was supported by the ML and Bayesian trees but not the parsimony one.

Within the African clade *Macrodipteryx longipennis* clustered with *C. fraenatus* and *C. climacurus* in the ML and Bayesian trees although with weak support. In a parsimony tree *Macrodipteryx longipennis* was external to all other African *Caprimulgus*, although with only weak bootstrap support (65%). There are two species of *Macrodipteryx*; *M. vexillarius* (not included here) and *M. longipennis*. They are distinguished from *Caprimulgus* by extremely elongated second primaries P2, which are displayed during courtship, a short cranium and very short, triangular bill (Holyoak, 2001). *Macrodipteryx* spp. are two of the few nightjars that are known to be polygynous and this may account for the exaggerated plumage of these two species. *Macrodipteryx* is also a lek breeder and, as with other lek species, such as *Uropsalis*, there may have been rapid evolution of display plumes due to extreme sexual selection leading to such sexual dimorphism and dichromatism (Hoglund, 1989; Oakes, 1992). Therefore, it is possible that *Macrodipteryx* is a relatively recent descendant of a *Caprimulgus* ancestor. The only other member of the genus, *Macrodipteryx vexillarius*, is considered a close relative of *M. longipennis* but is considerably heavier, possessing a flatter skull, and differing in the form of feather P2, development of PP3-7, and amount of white in plumage (Holyoak, 2001; Fry, 1988).

The Nearctic clade containing *Phalaenoptilus nuttallii* and *Caprimulgus vociferous* has 72% ML bootstrap support (Fig. 1). *Phalaenoptilus nuttallii* is a monotypic genus and is morphologically similar to *Caprimulgus*, the genus to which *P. nuttallii* was originally ascribed when first described by Audubon (1844). *Phalaenoptilus nuttallii* may have diverged

from *Caprimulgus* relatively recently which is consistent with the sequence of divergence proposed by Sibley and Ahlquist (1990). Morphological similarities of *Caprimulgus* to *Phalaenoptilus* include a proportionately large head, soft plumage, slightly rounded tail and the length of the uppertail coverts (Bent, 1989). A tenuous morphological dissimilarity is that the tarsi in *Phalaenoptilus* are unfeathered, although tarsal feathering varies considerably in the Old World true nightjars (Holyoak, 2001).

There was clear evidence of saturation in the cytochrome *b* sequence data, suggesting that the nightjar family might be relatively ancient compared to other bird families since the cytochrome *b* is generally useful for resolving relationships within avian families (Moore and DeFilippis, 1997). If this is the case, then a more slowly evolving marker may help resolve the relationships of major clades within this family. However, it is also possible that the geographical clades radiated over a relatively short period of time making it difficult to resolve the relationships between them by any method.

The polyphyly of the genera of the family Caprimulgidae provides evidence that this is a particularly difficult family to classify on morphological grounds. This may be attributed to the constraints imposed by night-time aerial feeding. Primarily, the requirement for camouflage to avoid detection by diurnal predators results in the cryptic plumage characteristic of all Caprimulgiformes, and also of the related crepuscular order the Strigiformes. The crepuscular/nocturnal feeding niche is divided between aerial feeders, such as the Caprimulgidae and those other Caprimulgiformes that feed in the air and on the ground, namely the Potoos (Nyctibiidae), Frogmouths (Podargidae) and the Owllet-nightjars (Aegothelidae) that are largely terrestrial feeders. Morphological characteristics of the nightjars are their cryptic plumage, large eyes and gape, weak bills, rictal bristles (reduced in Chordeilinae), long tail and wings, and low wing loads. The polyphyly of the family Caprimulgidae suggests that these characteristics are primitive traits that have been retained in the African, North American and South American clades. The alternative hypothesis, that these common characteristics are a consequence of convergent evolution on a morphology adapted for aerial feeding and cryptic plumage for diurnal ground nesting, cannot be excluded, but is less parsimonious since it would require at least two separate convergences on the same morphology.

5. Evolution

Although insufficient fossil data is available to calibrate the phylogeny of the Caprimulgidae for the time scales which are resolved in the trees presented here, the trees do have some implications for the evolution of the family Caprimulgidae. The DNA–DNA hybridisation data for the divergence of Strigiformes and Caprimulgiformes of 44.9 MYA and the Caprimulgidae at 39.7 MYA renders an early date for the radiation of the family associated with Continental break-up improbable (Sibley and Ahlquist, 1990). *Ventivorus ragei* is the earliest fossil Caprimulgidae

and has been dated to the Eocene about 40 MYA in France ((2)), which provides little support for a very early origin of the family and supports the DNA–DNA hybridisation hypothesis. However, the earliest Caprimulgiform fossil, *Prefica nivea* dates from the Lower Eocene (50.5–54.9 MYA), suggesting that the DNA–DNA hybridisation dates for divergence are too low. It is possible, therefore, that the Caprimulgidae radiated in the Eocene and that descendants of that radiation evolved independently on the separate continents but retained many features of the primitive morphology.

Caprimulgus europaeus, *Caprimulgus ruficollis*, *Caprimulgus aegypticus* and *Caprimulgus nubicus* are the only species of *Caprimulgus* that breed in the Western Palaearctic and all migrate from Africa each spring. There are 20 Afrotropical species of *Caprimulgus* (Cleere and Nurney, 1998). The current migrations of *Caprimulgus europaeus* subspecies can only have developed since the end of the last ice age, presumably from birds resident in Africa or southern European refugia. This is supported by the ‘Southern ancestral home theories’, demonstrated for many other avian species including Chaffinch *Fringilla coelebs* (Griswold and Baker, 2002), Blackcap *Sylvia atricapilla* (Pérez-Tris et al., 2004) and Great Tit, *Parus major*, (Kvist et al., 1999), that colonised seasonally suitable habitat from southern refugia during glacial retreat. The maximum likelihood and parsimony trees show that the closest relatives of *Caprimulgus europaeus* are African suggesting that *Caprimulgus europaeus* evolved from an African species. Indeed, Fry (1988) concluded the *C. europaeus* is closely related to *C. rufigena* (not included in this study) that is a resident breeder in South Africa. The alternative hypothesis, that *Caprimulgus europaeus* was indigenous to Europe and Asia and acquired a migratory habit to Africa to adapt to the cooling climate, cannot be excluded until a calibrated tree is available. The addition of Asian species of *Caprimulgus* to the tree may help resolve the origin of *C. europaeus*.

6. Conclusion

Fossil Caprimulgidae are known from the Eocene (Holyoak, 2001; Cleere and Nurney, 1998; Mourer-Chauvire, 1988). They have radiated into a diverse range of habitats and colonised all the continents except Antarctica. If the modern birds are descendants of that original radiation, their highly conserved morphology is even more remarkable, and demonstrates the extreme physiological constraints imposed by adaptation to crepuscular/nocturnal living combined with ground nesting. It would seem that such constraints are so severe that morphological similarities are genuinely homologous; the alternative explanation of strongly convergent evolution is in our view less parsimonious, requiring the independent evolution of extensive morphological similarity in phylogeographically distinct groups.

Of equal importance, analysis of cytochrome *b* sequence data provides evidence for rapid morphological evolution

of certain behaviours and secondary sexual characteristics. For example, unlike *Caprimulgus*, *Macrodipteryx* and *Uropsalis* are polygynous. Members of these latter genera are distinguished by exaggerated tail and wing feathers used in courtship displays. We suggest that such changes in mating system may have driven rapid morphological change that appears significant in relation to the similarity between the 57 species of *Caprimulgus* but may not be good markers for significant genetic divergence. Consequently, the nightjars appear to contain examples of morphologies and behaviours that remain stable over tens of millions of years, as well as examples of rapid morphological changes in response to changing behaviours.

Acknowledgments

We would like to thank the field workers who sent us tissue samples including C. Barlow, R. Clay D. Pearson, and C. Woods. We thank N. Cleere for providing critical comments on an earlier draft of this paper. Funding, for C. Larsen was provided by the Deanery of Science and Social Science, Liverpool Hope University. We also wish to thank two anonymous reviewers for their valuable comments on the manuscript.

References

- Audubon, J.J., 1844. The Birds of America, from drawings made in the United States and their territories (Vol. 7). Dorsey, New York.
- Beddard, F.E., 1886. On the syrinx and other points on the anatomy of the Caprimulgidae. Proc. Zool. Soc. Lond., 147–153.
- Bent, A., 1989. Life histories of North American cuckoos, goatsuckers, hummingbirds, and their allies. Dover Publications, Inc., New York.
- Bühler, P., 1970. Schädelmorphologie und Kiefermechanik der Caprimulgidae (Aves). The morphology of the skull and the mechanics of the jaw in Caprimulgidae. Z. Morph. Tiere 66, 337–399.
- Cibois, A., Pasquet, E., Schulenberg, T.S., 1999. Molecular systematics of the Malagasy babblers Passeriformes: Timaliidae and warblers Passeriformes: Sylviidae., based on cytochrome *b* and 16S rRNA sequences. Mol. Phylogenet. Evol. 13, 581–595.
- Cleere, N., 2002. A review of the taxonomy and systematics of the Sicklet-winged and White-winged nightjars Caprimulgidae. Bull. B.O.C. 122, 168–179.
- Cleere, N., Nurney, D., 1998. Nightjars: A Guide to Nightjars and Related Nightbirds. Pica Press, East Sussex.
- Cramp, S., 1985. The Birds of the Western Palearctic (vol. IV). Oxford University Press, Oxford. pp. 620–637.
- Davis, L.I., 1962. Acoustic evidence of relationships in *Caprimulgus*. Texas J. Sci. 14, 72–106.
- Davis, L.I., 1979. Acoustic evidence of relationships in Caprimulginae. Pan Am. Studies 1, 22–57.
- DeFilippis, V.R., Moore, W.S., 2000. Resolution of phylogenetic relationships among recently evolved species as a function of amount of DNA sequence: An empirical study based on woodpeckers (Aves: Picidae). Mol. Phylogenet. Evol. 16, 143–160.
- De Queiroz, A., Wimberger, P.H., 1993. The usefulness of behavior for phylogeny estimation: Levels of homoplasy in behavioral and morphological characters. Evolution 47, 46–60.
- Espinosa de los Monteros, A., 2000. Higher-level phylogeny of Trogoniformes. Mol. Phylogenet. Evol. 14, 20–34.
- Felsenstein, J., 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. J. Mol. Evol. 17, 368–378.

- Fidler, A.E., Kuhn, S., Gwinner, E., 2004. Convergent evolution of strigiform and caprimulgiform dark-activity is supported by phylogenetic analysis using the arylalkylamine *N*-acetyltransferase *Aanat* gene. *Mol. Phylogenet. Evol.* 33, 908–921.
- Fry, C.H., 1988. Skulls, songs and systematics of African nightjars. *Proc. Pan-African Ornithol. Congr.* 6, 105–131.
- García-Paris, M., Alcobendas, M., Buckley, D., Wake, D., 2003. Dispersal of viviparity across contact zones in Iberian populations of fire salamanders (salamandra) inferred from discordance of genetic and morphological traits. *Evolution* 57, 129–143.
- Glenny, F.H., 1953. A systematic study of the main arteries in the region of the heart Aves XX. Caprimulgiformes. Pt. 1. *Ohio J. Sci.* 53, 356–357.
- Griswold, C., Baker, A., 2002. Time to the most recent common ancestor and divergence times of populations of common chaffinches (*Fringilla coelebs*) in Europe and North Africa: Insights into Pleistocene refugia and current levels of migration. *Evolution* 56, 143–153.
- Hartert, E., 1892. Catalogue of Birds in the British Museum (vol. 16). British Museum, London.
- Hoff, K.M., 1966. A comparative study of the Appendicular Muscles of the Strigiformes and Caprimulgoformes. Ph.D., Washington State University, Arbor.
- Hoglund, J., 1989. Size and plumage dimorphism in lek-breeding birds. A comparative analysis. *Am. Nat.* 134, 72–87.
- Holyoak, D.T., 2001. Nightjars and their Allies. Oxford University Press, Oxford.
- Hughes, A.L., 1999. Adaptive Evolution of Genes and Genomes. Oxford University Press, New York.
- Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17, 754–755.
- Huxley, T.H., 1867. On the classification of birds; and on the taxonomic value of the modifications of certain of the cranial bones observable in that class. *Proc. Zool. Soc. Lond.*, 415–472.
- Iwaniuk, A.N., Hurd, P.L., Wylie, D.R.W., 2006. The comparative morphology of the cerebellum in caprimulgiform birds: Evolutionary and functional implications. *Brain Behavior Evol.* 67, 53–68.
- Jackson, H.D., 1985. Mouth size in *Macrodipteryx* and other African nightjars. *Bull. B.O.C.* 105, 51–54.
- Johansson, U., Ericson, P., 2003. Molecular support for a sister group relationship between Pici and Galbulae Piciformes sensu Wetmore 1960. *J. Avian Biol.* 34, 185–197.
- Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Pääbo, S., Villablanca, F.X., Wilson, A.C., 1989. Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci. USA* 86, 6196–6200.
- Kvist, L., Ruokonen, M., Lumme, J., Orell, M., 1999. The colonization history and present-day population structure of the European great tit (*Parus major major*). *Heredity* 82, 495–502.
- Maekawa, K., Lo, N., Kitade, O., Miura, T., Matsumoto, T., 1999. Molecular phylogeny and geographic distribution of wood-feeding cockroaches in East Asian islands. *Mol. Phylogenet. Evol.* 13, 360–376.
- Lerner, H.R.L., Mindell, D.P., 2005. Phylogeny of eagles, Old World vultures, and other Accipitridae based on nuclear and mitochondrial DNA. *Mol. Phylogenet. Evol.* 37, 327–346.
- Mariaux, J., Braun, M., 1996. A molecular phylogenetic survey of the nightjars and allies Caprimulgiformes with special emphasis on the potoos (Nyctibiidae). *Mol. Phylogenet. Evol.* 6, 228–244.
- Mayr, G.H., 2002. Osteological evidence for paraphyly of the avian order Caprimulgiformes (nightjars and allies). *J. Ornithologie* 143, 82–97.
- Moore, W.S., DeFilippis, V.R., 1997. The window of taxonomic resolution for phylogenies based on mitochondrial cytochrome *b*. In: Mindell, D.P. (Ed.), *Avian Molecular Evolution and Systematics*. Academic Press, Ann Arbor, pp. 83–113.
- Mourer-Chauvire, C., 1988. Le gisement du Bretou phosphorites du Quercy, Tarn-et-Garonne, France. et sa faune de vertebres de l'Eocene superieur. *II Oiseaux. Palaeontographica A* 205, 34–36.
- Oakes, E.J., 1992. Lekking and the evolution of sexual dimorphism in birds: Comparative approaches. *Am. Nat.* 140, 665–684.
- Oberholser, H.C., 1914. A monograph of the genus *Chordeiles* Swainson, type of a new family of goatsuckers. *Bull. US Nat. Museum* 86: pp. vii +123.
- Pérez-Tris, J., Bensch, S., Carbonell, R., Helbig, A.J., 2004. Historical diversification of migration patterns in a passerine bird. *Evolution* 58, 1819–1832.
- Peters, J.L., 1940. Check-list of Birds of the World (vol. 4). Harvard University Press, Cambridge.
- Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14, 817–818.
- Randi, E., Lucchini, V., Armijo-Prewitt, T., Kimball, R., Braun, E., Ligon, J., 2000. Mitochondrial DNA phylogeny and speciation in the Tragopans. *Auk* 117, 1003–1015.
- Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572–1574.
- Sheldon, F.H., Jones, C.E., McCracken, K.G., 2000. Relative patterns and rates of evolution in heron nuclear and mitochondrial DNA. *Mol. Biol. Evol.* 17, 437–450.
- Shimodaira, H., Hasegawa, M., 1999. Multiple comparison of log likelihoods with applications to phylogenetic inference. *Mol. Biol. Evol.* 16, 1114–1116.
- Sibley, C.G., Ahlquist, J.E., 1990. *Phylogeny and Classification of Birds: A Study in Molecular Evolution*. Yale Univ. Press, New Haven, CT.
- Swofford, D.L., 1998. PAUP Phylogenetic Analysis Using Parsimony* and Other Methods. Version 4.
- Turner, D.A., Pearson, D.J., Zimmerman, D.A., 1991. Taxonomic notes on some East African birds, Part 1—Non-passerines. *Scopus* 14, 84–91.