



## Owl melanin-based plumage redness is more frequent near than away from the equator: implications on the effect of climate change on biodiversity

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Climate change acts as a major new selective agent on many organisms, particularly at high latitudes where climate change is more pronounced than at lower latitudes. Studies are required to predict which species are at a high risk of extinction and whether certain phenotypes may be more affected by climate change than others. The identification of susceptible phenotypes is important for evaluating the potential negative effect of climate change on biodiversity at the inter- and intraspecific levels. Melanin-based coloration is an interesting and easily accessible candidate trait because, within certain species, reddish pheomelanin-based coloration is associated with adaptations to warm climates. However, it is unclear whether the same holds among species. We tested one prediction of this hypothesis in four owl genera (wood, scops, screech, and pygmy owls), namely that darker reddish species are more prevalent near the equator than polewards. Our comparative analysis is consistent with this prediction for the northern hemisphere, suggesting that pale reddish species may be adapted to cold climates and dark reddish species to warmer climates. Thus, climate change may have a larger negative impact on pale pheomelanin owls and favour dark pheomelanin species. © 2011 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2011, **102**, 573–582.

**ADDITIONAL KEYWORDS:** colour plumage – colour polymorphism – local adaptation.

### INTRODUCTION

Human activities have led to global environmental changes at the scale of the planet. As a consequence, many species are critically endangered, have disappeared or will disappear as a direct result of climate change (Thomas *et al.*, 2004). The degree of warming is more important at high than low latitudes (IPPC, 2007) and leads to massive extinction in polar species and a shift in phenology (Parmesan, 2006). It is therefore of central importance to investigate which phenotypic traits might be involved in genetic adaptations to climate change, particularly at higher latitudes. Melanin-based coloration is a candidate trait because it often covaries with other physiological attributes (Ducrest, Keller & Roulin, 2008) that may be important to adapt to climate change. Accordingly, Galeotti *et al.* (2009) found that, in Italy, the propor-

tion of reddish pheomelanin scops owls (*Otus scops*) increased from 1870 to 2007 at the expense of the grey non-pheomelanin morph, probably as a response to the concomitant increase in ambient temperatures.

We further appraised the hypothesis that pheomelanin-based coloration also could be involved in adaptations to climate between species and be a trait to predict the species response to climate change. Accordingly, we carried out comparative analyses in four owl genera (scops owls *Otus*, screech owls *Megascops*, wood owls *Strix*, and pygmy owls *Glaucidium*) to test whether, at the interspecific level, the extent of pheomelanin-based coloration is correlated with latitude on both hemispheres. In many owl species, individuals display one of several colour morphs that vary in the degree of reddishness. In Italy, the Eurasian scops owl's reddish morph increased in proportion during the last century (Galeotti *et al.*, 2009); in Italy, rufous tawny owls (*Strix aluco*) were found dead more often in cool-dry years

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and grey individuals in warm–wet years (Galeotti & Cesaris, 1996); and, in North America, rufous coloured screech owls (*Otus asio*) appear to be unfit during cold, dry and snowy weather compared to grey individuals (Van Camp & Henny, 1975; Mosher & Henny, 1976; Gehlbach, 1986). Thus, from these intraspecific patterns, we predict that owl species are more often reddish near the equator than near the poles, which may indicate that pheomelanism is not selectively neutral with respect to climate change. This would be of interest in terms of suggesting that climate change may select for a higher degree of pheomelanin-based coloration in poleward regions.

## MATERIAL AND METHODS

### DATASET

We collected data on owl plumage coloration from König, Wieck & Becking (1999). This book provides detailed information on plumage coloration of each owl species by indicating whether the plumage is rufous (i.e. reddish, orange, rufous, ochraceous, fulvous, tawny, yellowish, golden, buff, rusty, ferruginous, chestnut, maroon), brown (i.e. brown, wood, chestnut, cinnamon, chocolate, sepia) or grey. We thus counted the number of times the words rufous, brown, and grey (and their synonymous as well) were used to describe plumage colour of each species in relation to the total number of words used to describe the plumage in general. These proportions were then used to test whether owls are redder, browner or greyer near or far from the equator. An alternative method to score plumage coloration would have been to visit museum collections; however, this would have been time consuming in terms of visiting many museums and having access to a large number of owl species (which would not have helped resolve the problem of climate change). Reddish coloration is very likely to reflect pheomelanin-based coloration, as shown in both the barn owl (*Tyto alba*) and tawny owl (*Strix aluco*) (Roulin *et al.*, 2008; Gasparini *et al.*, 2009). The concentration in both pheomelanin and eumelanin pigments was lower in grey than reddish tawny owls, indicating that greyish individuals are not 'blacker' than reddish conspecifics but less pheomelanin (Gasparini *et al.*, 2009). We restricted our analysis to the four genera that comprised an important number of species: *Otus* (41 species with information on plumage coloration), *Megascops* (26 species), *Glaucidium* (29 species), and *Strix* (21 species) (Appendix 1). On average,  $26.6 \pm 1.4$  words (mean  $\pm$  SE; range 2–89) were used per species to describe plumage. The number of words was not correlated with the proportion of words used to describe plumage as reddish, brown or grey (Pear-

son's correlations:  $P > 0.08$ ). For each species, we considered the mean latitude of their distributional range. For 63 owl species, the mean latitude was located in the northern hemisphere and, for 54 species, in the southern hemisphere.

To examine whether our method of counting words to describe plumage coloration is reliable, one of the authors (S.A.), being naïve about the research question, ranked *Strix* species from the most to least reddish based on the drawings of each species that can be found in the colour plates of the same book (König *et al.*, 1999). The same procedure was then carried out for the *Otus*, *Megascops*, and *Glaucidium* genera. The proportion of words that described plumage as reddish was strongly correlated with ranks (mixed model analysis of variance with genus as random variable and proportion of words as dependent variable, rank:  $F_{1,107} = 25.68$ ,  $P < 0.0001$ ), showing that our method of assessing plumage coloration gives an appropriate proxy of the degree of pheomelanin-based coloration; rank was also associated with the proportion of words that described plumage as grey (similar model,  $F_{1,106.5} = 12.99$ ,  $P = 0.0005$ ) but not as brown (similar model,  $F_{1,101.4} = 2.77$ ,  $P = 0.099$ ). Colour morphs as categorized by the human eye are strongly associated with reflectance spectra obtained with the spectrometry of feathers (Gasparini *et al.*, 2009; Pannkuk, Siefferman & Butts, 2010).

### PHYLOGENY

Species considered in the present study are given in the Appendix 1. To reconstruct the phylogeny of owls to be used for phylogenetic correction, we compiled a data set containing cytochrome *b* (*cytb*) sequences (999 bp; data available for 57 species) and sequences of the recombination activating gene 1 (*RAG1*) (948 bp; 31 species) for all species contained in our dataset for which these were available in the GenBank database (Appendix 1). Sequences from the barn owl (*Tyto alba*) (accession numbers: *cytb*, FJ588458; *RAG1*, EU348946) were added as outgroup, and sequences of the Northern saw-whet owl (*Aegolius acadicus*) (accession numbers: *cytb*, U89172; *RAG1*, EU348862), little owl (*Athene noctua*) (*cytb*, AJ003949; *RAG1*, EU348872), and Northern hawk owl (*Surnia ulula*) (*cytb*, AJ004069; *RAG1*, EU348942) as additional ingroups in the phylogenetic reconstructions. Sequence assembly and translation into amino acid sequences before alignment were performed in MEGA, version 4.0 (Tamura *et al.*, 2007). Amino acid sequences were aligned using the CLUSTALW algorithm (Thompson *et al.*, 1994) and translated back to nucleotide sequences.

Partitioned phylogenetic reconstructions using all 57 *cytb* sequences or the concatenated 31 *cytb* and

*RAG1* sequences were performed with MrBayes, version 3.1 (Ronquist & Huelsenbeck, 2003), using a full partition strategy (i.e. each codon position from each gene was entered in a separate partition). The best fitting nucleotide substitution model for each codon position per gene was evaluated using MRMODELTEST, version 2.2 (Nylander, 2004) according to the Akaike information criterion (Akaike, 1974). Bayesian analyses included two independent runs each with four chains of  $10^7$  generations. Convergence was evaluated using the average standard deviation of split frequencies between runs and the potential scale reduction factor. Posterior distributions were examined in TRACER, version 1.4 (Rambaut & Drummond, 2007). The first half of the topologies was discarded as burn-in. The 50% majority consensus tree was rooted using the barn owl. Ultrametric branch lengths for this tree were obtained using the nonparametric rate smoothing algorithm (Sanderson, 1997) implemented in the APE package (Paradis, Claude & Strimmer, 2004) in R software. Before further analyses, barn owl and the additional ingroup species not contained in the colour data set were pruned from the tree using Mesquite, version 2.71 (Maddison & Maddison, 2009), leaving 57 (*cytb* tree) and 31 (*cytb* + *RAG1* tree) species, respectively.

#### STATISTICAL ANALYSIS

We performed two sets of analyses. First, we considered the whole sample of owls to investigate whether there is a link between the percentage of words used to describe plumage as reddish, brown or grey and absolute latitude on both hemispheres. We therefore ran three separate mixed model analyses of covariance (one for each colour type) with the proportion of words describing a grey (versus brown and then reddish) plumage as dependent variable, genus (*Otus*, *Megascops*, *Glaucidium*, and *Strix*) as a random variable, hemisphere (north versus south) as a fixed factor, and absolute latitude as a covariate. We introduced the interaction between hemisphere and absolute latitude to examine whether the covariation between coloration and latitude differs between the two hemispheres.

Second, comparative analyses correcting the above described models for phylogenetic relationships among species were performed using generalized estimating equations *sensu* Paradis & Claude (2002) and implemented in the APE package (Paradis *et al.*, 2004) in R software. Instead of considering the genus as random variable, such as if performed without controlling for phylogeny, these models use the phylogeny as a covariate and remove its effect before analyses of the desired variables. Models were run

using the 57 species contained in the *cytb* tree and using the 31 species contained in the tree based on combined *cytb* and *RAG1* sequences (Appendix 1).

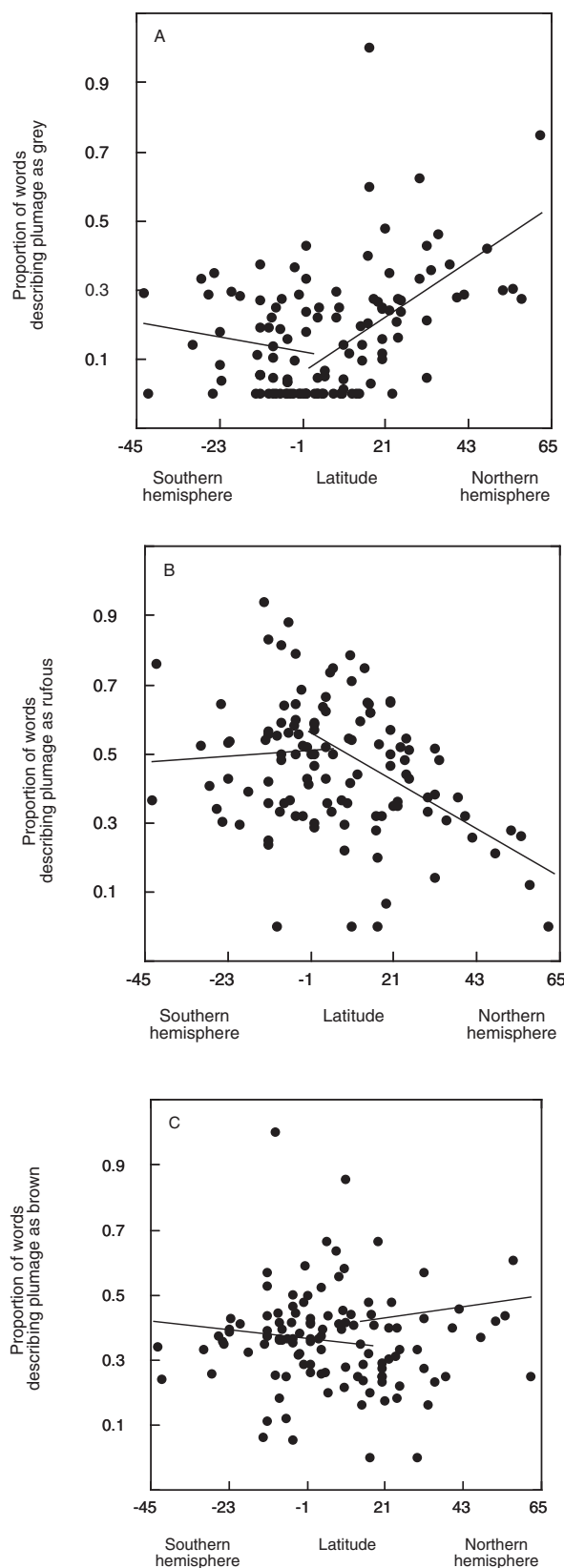
#### RESULTS

Without controlling for phylogeny, the proportion of words used to describe owl plumage as grey increased with latitude but only on the northern hemisphere, whereas the proportion of words describing a rufous plumage decreased with latitude, also only on the northern hemisphere; the proportion of words used to describe plumage as brown tended to decrease with latitude only on the northern hemisphere (Fig. 1, Table 1). When restricting our analyses to monomorphic owl species, we obtained similar results (i.e. owls were less greyer but redder close than away from the equator on the northern hemisphere) ( $F_{1,33.85} = 30.09$ ,  $P < 0.0001$  versus  $F_{1,35.96} = 9.00$ ,  $P = 0.0049$ ).

For the comparative analyses that controlled for phylogenetic relationships among species, the topologies reconstructed from *cytb* only and from *cytb* + *RAG1* differed by the position of the genus *Megascops*. In the *cytb* tree, *Megascops* clustered with *Otus*, which is consistent with the fact that *Megascops* species were previously considered as belonging to the *Otus* genus. In the *cytb* + *RAG1* tree, *Megascops* clusters with *Strix*, such as in other recent studies (Fuchs *et al.*, 2008). Nevertheless, the comparative analyses appear to be only marginally influenced by this difference in topology, and yielded results similar to the analysis that did not correct for phylogenetic relationships, with some exceptions (Table 1). Analyses with both tree topologies revealed significant interactions between latitude and hemisphere for the proportions of words describing the plumage as rufous and as brown but not as grey. On the northern hemisphere, the percentage of words used to describe plumage as grey (versus rufous) increased (versus decreased) with latitude; interestingly, the same pattern was found on the southern hemisphere with respect to grey coloration, with species located closer to the pole being more greyish than those located closer to the equator. Similar to the analyses not controlling for phylogeny, owls were more brownish closer to the equator. Owls located on the southern hemisphere were more greyish, less brownish, and less rufous than species on the northern hemisphere.

#### DISCUSSION

In four owl genera (*Otus*, *Megascops*, *Glaucidium*, and *Strix*), the extent to which the plumage was reddish pheomelanic decreased from the equator to the North Pole. In the southern hemisphere, we found a significant relationship between latitude and the



**Figure 1.** Relationship between the degree of pheomelanin-based coloration and latitude in worldwide-distributed wood owls (*Strix* spp.), scops owls (*Otus* spp.), screech owls (*Megascops* spp.), and pygmy owls (*Glaucidium* spp.). Each data point represents a species. The proportion of words used to describe plumage as grey (i.e. pale pheomelanin) (A), reddish (i.e. dark pheomelanin) (B) and brown (i.e. intermediately pheomelanin) (C) is shown. Regression lines for the two hemispheres are shown for illustrative purposes.

degree of greyness but only with the *cytb* typology. Thus, our comparative analysis suggests that this colour trait may be associated with adaptations to ecological factors that vary clinally at least in the northern part of the globe.

Previous studies in the tawny owl, screech owl, and scops owl suggested that reddish owls have a higher survival in warm–wet years than in cold–dry years (Van Camp & Henny, 1975; Mosher & Henny, 1976; Gehlbach, 1986; Galeotti & Cesaris, 1996; Galeotti *et al.*, 2009). Thus, the degree of pheomelanin-based coloration may be associated with the ability to cope with warm climates, whereas a non-pheomelanin coloration may be adapted to colder climates. In accordance with Gloger's rule, plumages are darkly coloured in habitats where relative humidity is high and pale where it is low; this rule is consistent with the results obtained in the present study, and also received empirical support in other bird species (Zink & Remsen, 1986). This finding could be explained by predator–prey relationships if a reddish coloration enhances camouflage near the equator rather than at higher latitudes, and vice versa for a greyish coloration. Unfortunately, no data are yet available to address this possibility. Alternatively, a dark reddish coloration may be selected near the equator as a result of indirect selection. Pheomelanin-based coloration can be genetically correlated with several physiological traits, including immune defence and energy homeostasis as found in the tawny owl (Gasparini *et al.*, 2009), which are traits that may be differentially selected at high and low latitudes. For example, if investment in immunity is strongly selected around the equator (Guernier, Hochberg & Guégan, 2004), this may indirectly drive the evolution of plumage coloration towards darker pheomelanin coloration.

Assuming that, in wood, scops, screech and pygmy owls, pheomelanin-based coloration is associated with adaptations to climatic variables, climate change, which is more pronounced near the poles, may have dramatic consequence in non-pheomelanin owl species located at high latitudes. Colour polymorphic species may be at an advantage because reddish individuals, which would currently have a selective advantage

**Table 1.** Mixed models and generalized estimation equations (GEE) analyses testing whether, in wood owls (*Strix* spp.), scops owls (*Otus* spp.), screech owls (*Megascops* spp.), and pygmy owls (*Glaucidium* spp.), the degree of pheomelanin-based coloration is associated with absolute latitude on both hemispheres

	Analyses including both hemispheres			Northern hemisphere	Southern hemisphere
	Latitude	Hemisphere	Interaction	Latitude	Latitude
Analyses without controlling for phylogeny (mixed models)					
Grey					
<i>F</i>	<b>19.03</b>	1.95	<b>8.37</b>	<b>38.06</b>	0.70
d.f.	<b>1, 100.5</b>	1, 98.21	<b>1, 100.5</b>	<b>1, 52.35</b>	1, 37.17
<i>P</i>	<b>&lt; 0.001</b>	0.17	<b>0.0047</b>	<b>&lt; 0.001</b>	0.41
Brown					
<i>F</i>	3.78	0.18	0.01	3.89	0.36
d.f.	1, 100.9	1, 98.31	1, 99.82	1, 53.58	1, 23.32
<i>P</i>	0.055	0.68	0.92	0.054	0.56
Rufous					
<i>F</i>	3.88	1.97	<b>4.36</b>	<b>12.87</b>	0.06
d.f.	1, 73.24	1, 100.14	<b>1, 100.2</b>	<b>1, 53.94</b>	1, 6.14
<i>P</i>	0.053	0.16	<b>0.04</b>	<b>&lt; 0.001</b>	0.81
Analyses controlling for phylogeny (GEE <i>cytb</i> )					
Grey					
<i>t</i>	<b>4.46</b>	<b>-3.50</b>	-0.23	<b>2.89</b>	1.83
d.f. <i>P</i>	<b>15.04</b>	<b>15.04</b>	15.04	<b>9.81</b>	8.76
<i>P</i>	<b>&lt; 0.001</b>	<b>0.004</b>	0.82	<b>0.02</b>	0.11
Brown					
<i>t</i>	<b>5.11</b>	<b>3.96</b>	<b>-4.19</b>	<b>5.72</b>	-0.95
d.f. <i>P</i>	<b>15.04</b>	<b>15.04</b>	<b>15.04</b>	<b>9.81</b>	8.76
<i>P</i>	<b>&lt; 0.001</b>	<b>0.002</b>	<b>0.002</b>	<b>&lt; 0.001</b>	0.38
Rufous					
<i>t</i>	<b>-8.03</b>	-1.43	<b>3.91</b>	<b>8.15</b>	-0.98
d.f. <i>P</i>	<b>15.04</b>	15.04	<b>15.04</b>	<b>9.81</b>	8.76
<i>P</i>	<b>&lt; 0.001</b>	0.18	<b>0.002</b>	<b>&lt; 0.001</b>	0.36
Analyses controlling for phylogeny (GEE <i>cytb</i> + <i>RAG1</i> )					
Grey					
<i>t</i>	<b>5.65</b>	0.46	-1.42	2.56	<b>3.33</b>
d.f. <i>P</i>	<b>8.70</b>	8.70	8.70	6.39	<b>5.27</b>
<i>P</i>	<b>0.002</b>	0.65	0.22	0.058	<b>0.04</b>
Brown					
<i>t</i>	<b>4.46</b>	2.43	<b>-4.01</b>	<b>6.19</b>	-1.40
d.f. <i>P</i>	<b>8.70</b>	8.70	<b>8.70</b>	<b>6.39</b>	5.27
<i>P</i>	<b>0.007</b>	0.06	<b>0.01</b>	<b>0.003</b>	0.25
Rufous					
<i>t</i>	<b>-11.06</b>	<b>-4.45</b>	<b>6.35</b>	<b>-14.03</b>	-1.88
d.f. <i>P</i>	<b>8.70</b>	<b>8.70</b>	<b>8.70</b>	<b>6.39</b>	5.27
<i>P</i>	<b>&lt; 0.001</b>	<b>0.002</b>	<b>0.008</b>	<b>&lt; 0.001</b>	0.15

Different models were carried out for the extent to which species were grey (i.e. pale pheomelanic), brown (i.e. intermediately pheomelanic), and reddish (i.e. dark pheomelanic). Genus was introduced as random variable in mixed models that did not control for phylogeny. GEE models controlled for phylogenetic relationships between species. A first set of analyses were performed on species located on both hemispheres and two other analyses by considering only species located on the northern hemisphere versus southern hemisphere. Significant results are shown in bold.

Mixed models included 117 species (63 for the northern hemisphere and 54 for the southern hemisphere), GEE (*cytb*) models included 57 species (29 for the northern hemisphere and 28 species for the southern hemisphere), and GEE (*cytb* + *RAG1*) models included 31 species (18 for the northern hemisphere and 13 species for the southern hemisphere).

over non-reddish conspecifics, would decrease the risk of species extinction. If all individuals are non-reddish, the risk of extinction may be higher than in polymorphic species, unless mutations allowing the expression of a pheomelanic plumage are sufficiently frequent. We thus predict that monomorphic owl species displaying a non-reddish plumage are at a risk of extinction and, in polymorphic owl species, the frequency of the reddish morph may increase particularly at higher latitudes.

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## APPENDIX 1

List of the wood owls (*Strix* spp.), scops owls (*Otus* spp.), screech owls (*Megascops* spp.), and pygmy owls (*Glaucidium* spp.) considered in the present study. Species presenting more than one colour morph in most populations are referred as 'polymorphic' and otherwise 'monomorphic'; monomorphic species showing a rare morph in some part of the distributional range are not considered as polymorphic. We indicate whether the mean latitude of their distributional range is located on the northern or southern hemisphere, absolute latitude, and the number of times the words (and their synonymous, see Material and methods) 'grey', 'brown', and 'rufous' are used to describe plumage coloration in '*Owls, a guide to the owls of the world*' (König *et al.*, 1999). For species used to carry out analyses where we controlled for phylogeny, we indicate the accession number of the *cytb* and *RAG1* sequences used for the reconstruction of the phylogenetic relationships.

Species	Polymorphism	Hemisphere	Absolute latitude	Grey	Brown	Rufous	Accession number <i>cytb</i>	Accession number <i>RAG1</i>
<i>Otus alfredi</i>	Monomorphic	South	9	1	8	13		
<i>Otus alius</i>	Monomorphic	North	7.05	0	7	4		
<i>Otus angelinae</i>	Monomorphic	South	5	0	11	20		
<i>Otus bakkamoena</i>	Monomorphic	North	20	8	8	16	AJ004020	EU348919
<i>Otus balli</i>	Polymorphic	North	10	1	10	13		
<i>Otus beccarii</i>	Polymorphic	South	1	0	6	6		
<i>Otus brookii</i>	Monomorphic	South	0	0	12	17		
<i>Otus brucei</i>	Monomorphic	North	30	5	0	3	EU348985	EU348920
<i>Otus capnodes</i>	Polymorphic	South	12.2	1	10	8	EU601114	
<i>Otus collari</i>	Monomorphic	North	3	0	3	5		
<i>Otus elegans</i>	Polymorphic	North	15	3	5	13	EU123911	
<i>Otus enganensis</i>	Monomorphic	South	5.2	0	10	14		
<i>Otus flammeolus</i>	Polymorphic	North	32	7	9	17	AJ004022	
<i>Otus fuliginosus</i>	Monomorphic	North	9.3	0	7	5		
<i>Otus hartlaubi</i>	Polymorphic	South	0	5	10	13		
<i>Otus icterorhynchus</i>	Monomorphic	North	4.1	0	5	14		
<i>Otus insularis</i>	Monomorphic	South	4.4	0	4	5	EU601102	
<i>Otus ireneae</i>	Polymorphic	South	3.2	8	7	7	EU601113	
<i>Otus lempiji</i>	Polymorphic	South	0	10	11	21	EU348987	EU348922
<i>Otus lettia</i>	Polymorphic	North	25	5	7	9	EU348988	EU348923
<i>Otus longicornis</i>	Monomorphic	North	14	0	7	13	AJ004031	
<i>Otus magicus</i>	Monomorphic	South	9	5	13	18		
<i>Otus manadensis</i>	Monomorphic	South	2.1	8	8	12		
<i>Otus mantananensis</i>	Polymorphic	North	11.5	4	15	15		
<i>Otus megalotis</i>	Polymorphic	North	14.4	6	5	20	AJ004033	EU348924
<i>Otus mentawi</i>	Polymorphic	South	2.1	0	12	13		
<i>Otus mindorensis</i>	Monomorphic	North	13.1	0	2	6		
<i>Otus mirus</i>	Monomorphic	North	8	2	5	2	EU601126	
<i>Otus moheliensis</i>	Monomorphic	South	12.2	0	10	13	EU601121	
<i>Otus pauliani</i>	Polymorphic	South	12.1	3	3	2	EU601125	
<i>Otus pambaensis</i>	Polymorphic	South	5.1	3	1	15	EU601124	
<i>Otus rufescens</i>	Polymorphic	South	0	0	21	30		
<i>Otus rutilus</i>	Polymorphic	South	20	10	14	10	EF198289	
<i>Otus sagittatus</i>	Monomorphic	North	5	1	4	15		
<i>Otus scops</i>	Monomorphic	North	48	8	7	4	AJ004038	EU348925
<i>Otus semitorques</i>	Monomorphic	North	38	9	6	9	AY422980	
<i>Otus senegalensis</i>	Polymorphic	South	0	6	4	4	EU601127	
<i>Otus silvicola</i>	Monomorphic	South	9	3	12	14		
<i>Otus spilocephalus</i>	Monomorphic	North	15	6	18	39	EU601116	EU348926
<i>Otus sunia</i>	Polymorphic	North	25	11	9	21	EU348990	EU348927
<i>Otus umbra</i>	Monomorphic	North	2.4	0	4	7		
<i>Megascops albogularis</i>	Monomorphic	South	12.2	1	2	15	AJ004012	
<i>Megascops asio</i>	Polymorphic	North	33	20	9	27	AJ004015	EU348906

APPENDIX 1 *Continued*

Species	Polymorphism	Hemisphere	Absolute latitude	Grey	Brown	Rufous	Accession number <i>cytb</i>	Accession number <i>RAG1</i>
<i>Megascops atricapillus</i>	Polymorphic	South	23	5	11	12	AJ004013	EU348907
<i>Megascops barbarus</i>	Polymorphic	North	16.2	10	8	7		
<i>Megascops choliba</i>	Polymorphic	South	10	9	12	26	AJ004021	EU348908
<i>Megascops clarkii</i>	Monomorphic	North	9.6	0	3	11		
<i>Megascops colombianus</i>	Polymorphic	South	0.2	10	11	9		
<i>Megascops cooperi</i>	Monomorphic	North	16.5	3	1	1		
<i>Megascops guatemalae</i>	Polymorphic	North	22	8	7	8	AJ004023	
<i>Megascops hoyi</i>	Polymorphic	South	24.5	8	8	7	AJ004025	EU348911
<i>Megascops ingens</i>	Monomorphic	South	7.1	3	4	9		
<i>Megascops kennicottii</i>	Polymorphic	North	35	12	6	8	EU348980	EU348912
<i>Megascops koepkeae</i>	Monomorphic	South	9.1	4	8	6		
<i>Megascops lambi</i>	Monomorphic	North	16.5	2	0	0		
<i>Megascops marshalli</i>	Monomorphic	South	13.3	0	1	15		
<i>Megascops napensis</i>	Polymorphic	South	12.2	1	7	10		
<i>Megascops nudipes</i>	Polymorphic	North	18	6	9	7		
<i>Megascops petersoni</i>	Monomorphic	South	3.4	0	6	13	AJ004034	
<i>Megascops roboratus</i>	Polymorphic	South	6.4	6	8	8	AJ004035	
<i>Megascops roraimae</i>	Monomorphic	North	2.8	0	3	6		
<i>Megascops sanctaecatarinae</i>	Polymorphic	South	30	3	7	11	AJ004036	
<i>Megascops seductus</i>	Monomorphic	North	19.1	4	10	1		
<i>Megascops trichopsis</i>	Polymorphic	North	21	11	4	8	DQ190856	
<i>Megascops usta</i>	Polymorphic	South	3.1	2	8	11	AJ004039	
<i>Megascops watsonii</i>	Monomorphic	North	8.1	5	7	5	AF295003	
<i>Megascops vermiculatus</i>	Polymorphic	North	8.6	7	11	10		
<i>Strix albitarsus</i>	Monomorphic	South	7.1	0	3	22		
<i>Strix aluco</i>	Polymorphic	North	52	15	21	14	AJ004051	EU348933
<i>Strix bartelsi</i>	Monomorphic	South	9	0	7	31		
<i>Strix butleri</i>	Monomorphic	North	24.4	3	2	6	EU348994	EU348935
<i>Strix chacoensis</i>	Monomorphic	South	28	9	7	11		
<i>Strix davidi</i>	Monomorphic	North	32	3	3	1		
<i>Strix fulvescens</i>	Monomorphic	North	17	1	15	18		
<i>Strix huhula</i>	Monomorphic	South	10	0	7	0		
<i>Strix hylophila</i>	Monomorphic	South	25	0	10	18		
<i>Strix leptogrammica</i>	Monomorphic	North	10	1	22	56	AJ003914	
<i>Strix nebulosa</i>	Monomorphic	North	62	15	5	0	AJ004059	EU348936
<i>Strix newarensis</i>	Monomorphic	North	3	1	11	9		
<i>Strix nigrolineata</i>	Monomorphic	North	10	1	6	0		
<i>Strix occidentalis</i>	Monomorphic	North	32	1	12	8		
<i>Strix ocellata</i>	Monomorphic	North	20	3	6	17		
<i>Strix rufipes</i>	Monomorphic	South	42	0	6	19	AJ004061	EU348937
<i>Strix seloputo</i>	Monomorphic	North	12	0	13	19		
<i>Strix uralensis</i>	Polymorphic	North	55	14	20	12	AJ004062	EU348938
<i>Strix varia</i>	Monomorphic	North	42	10	16	9	AF115874	
<i>Strix virgata</i>	Polymorphic	South	5	1	11	18		
<i>Strix woodfordii</i>	Monomorphic	South	5	1	14	15	EU348995	EU348940
<i>Glaucidium albertinum</i>	Monomorphic	South	1.4	0	10	7		



APPENDIX 1 *Continued*

Species	Polymorphism	Hemisphere	Absolute latitude	Grey	Brown	Rufous	Accession number <i>cytb</i>	Accession number <i>RAG1</i>
<i>Glaucidium bolivianum</i>	Polymorphic	South	17.5	13	15	18	AJ003975	EU348894
<i>Glaucidium brasilianum</i>	Polymorphic	South	13	10	31	48	AY859390	EU348895
<i>Glaucidium brodiei</i>	Monomorphic	North	20	7	12	25		
<i>Glaucidium californicum</i>	Polymorphic	North	40	7	10	8	AJ003993	EU348896
<i>Glaucidium capense</i>	Monomorphic	South	23	4	19	26	AJ003864	
<i>Glaucidium castanonotum</i>	Monomorphic	North	9	0	5	6		
<i>Glaucidium castanopterum</i>	Monomorphic	South	8	0	9	16		
<i>Glaucidium cuculoides</i>	Monomorphic	North	23	0	12	13		
<i>Glaucidium gnoma</i>	Monomorphic	North	20	4	10	26	AJ003994	EU348897
<i>Glaucidium griseiceps</i>	Monomorphic	North	16.2	9	21	14	AJ003995	EU348898
<i>Glaucidium hardyi</i>	Monomorphic	South	8	7	11	10	AJ003996	EU348899
<i>Glaucidium hoskinsii</i>	Monomorphic	North	30	5	5	5		
<i>Glaucidium jardinii</i>	Polymorphic	North	5	3	20	23	AJ003998	EU348900
<i>Glaucidium minutissimum</i>	Monomorphic	South	22.5	1	12	15	AJ003867	
<i>Glaucidium nanum</i>	Polymorphic	South	43	12	14	15	AJ003999	EU348901
<i>Glaucidium nubicola</i>	Monomorphic	North	4.4	0	4	2		
<i>Glaucidium palmarum</i>	Monomorphic	North	24	6	9	14		
<i>Glaucidium parkeri</i>	Monomorphic	South	12.2	4	12	5		
<i>Glaucidium passerinum</i>	Monomorphic	North	57	9	20	4	AJ004003	EU348902
<i>Glaucidium perlatum</i>	Monomorphic	South	5	1	16	8	EU348979	EU348903
<i>Glaucidium peruanum</i>	Polymorphic	South	12.2	16	22	21	AJ004005	EU348904
<i>Glaucidium radiatum</i>	Monomorphic	North	3	6	7	14		
<i>Glaucidium ridgwayi</i>	Polymorphic	North	20	11	13	21		
<i>Glaucidium sanchezi</i>	Monomorphic	North	24.2	4	10	11		
<i>Glaucidium siju</i>	Polymorphic	North	22	6	10	9		
<i>Glaucidium sjostedti</i>	Monomorphic	South	0	0	12	16		
<i>Glaucidium tephronotum</i>	Monomorphic	North	3.5	7	11	10	AJ004006	
<i>Glaucidium tucumanum</i>	Polymorphic	South	26	17	22	20	AJ003992	EU348905

APPENDIX 2

Tree topologies and ultrametric branch-lengths used in comparative analyses correcting for phylogeny. A, tree based on *cytb*. B, tree based on *cytb* + *RAG1*.

