

Molecular taxonomy and systematics of owls (Strigiformes) - An update

Atualização da taxonomia e sistemática moleculares em rapinas noturnas (Strigiformes)

Michael Wink^{1*}, Hedi Sauer-Gürth¹

¹ Heidelberg University, Institute of Pharmacy and Molecular Biotechnology, INF 364, D-69120 Heidelberg, Germany

* Corresponding author: wink@uni-heidelberg.de



ABSTRACT

According to the latest genomic avian tree of life hypothesis, owls are closely related to diurnal raptors, but different from falcons and nightjars. We reconstructed a molecular phylogeny based on nucleotide sequences of a mitochondrial gene (cytochrome b) and a nuclear gene (RAG-1), and used it as a taxonomic framework. In accordance to the rules of cladistics, several changes (splitting or lumping of taxa) have been proposed. Strigiformes are divided into 2 families Tytonidae and Strigidae. The Tytonidae are subdivided into the subfamilies Tytoninae (with 25 species of *Tyto*) and Phodilinae (with *Phodilus*). The Strigidae cluster in 3 subfamilies: Striginae, Surniinae and Ninoxinae (with the genera *Ninox* and possibly the monotypic *Uroglaux* and *Sceloglaux*). The Surniinae are subdivided into 2 tribes Surniini (with *Surnia*, *Athene*, *Glaucidium*) and Aegolini (with *Aegolius*). The Striginae are subdivided into 6 tribes: Bubonini (with *Bubo*, including the former *Nyctea*, *Ketupa*, *Scotopelia*), Strigini (with *Strix*, *Jubula*), Pulsatrigini (with *Pulsatrix*, *Lophostrix*), Megascopini (with *Megascops*, *Psilosops*), Otini (with *Otus*, *Mimizuku*), and Asionini (with *Asio*, *Ptilopsis*, and possibly the monotypic *Nesasio*). New sequence data show that *Ninox superciliaris* from Madagascar does not group with *Ninox* but with *Athene* and thus should become *Athene superciliaris*. Sequences from *Scotopelia peli* and *S. ussheri* cluster within the genus *Bubo* and should better be classified as *Bubo peli* and *Bubo ussheri*.

Keywords: cytochrome b, owl systematics, phylogeny, phylogeography, RAG-1

RESUMO

De acordo com a mais recente árvore genômica aviária da hipótese da vida, as rapinas noturnas apresentam uma relação estreita com as rapinas diurnas, mas diferem dos falcões e dos noitibós. Através da construção de uma filogenia molecular baseada em sequências de nucleótidos de genes mitocondriais (principalmente o citocromo b) e de genes nucleares (por exemplo, RAG-1), foi estabelecida uma estrutura taxonômica. Em conformidade com as regras da cladística, foram propostas várias alterações (divisão ou agregação de táxones). Os Strigiformes estão divididos em 2 famílias Tytonidae e Strigidae. O Tytonidae estão subdivididos nas subfamílias Tytoninae (com 25 espécies de *Tyto*) e Phodilinae (com *Phodilus*). Os Strigidae dividem-se em três subfamílias: Striginae, Surniinae e Ninoxinae (com o gênero *Ninox* e possivelmente os monotípicos *Uroglaux* e *Sceloglaux*). Os Surniinae estão subdivididos em duas tribos Surniini (com *Surnia*, *Athene* e *Glaucidinum*) e Aegolini (com *Aegolius*). Os Striginae estão subdivididos em 6 tribos: Bubonini (com *Bubo*, que inclui as formas anteriores *Nyctea*, *Ketupa*, *Scotopelia*), Strigini (com *Strix*, *Jubula*), Pulsatrigini (com *Pulsatrix*, *Lophostrix*), Megascopini (com *Megascops*, *Psilosops*), Otini (com *Otus*, *Mimizuku*) e Asionini (com *Asio*, *Ptilopsis* e, possivelmente, o monotípico *Nesasio*). Novos dados de sequenciação mostram que *Ninox superciliaris* de Madagascar não agrupa com *Ninox* mas sim com *Athene*, devendo portanto passar a *Athene superciliaris*. Sequências de *Scotopelia peli* e *S. ussheri* agrupam-se dentro do gênero *Bubo* e deveriam ser classificadas como *Bubo peli* e *B. ussheri*.

Palavras-chave: : citocromo b, filogenia, filogeografia, RAG-1, sistemática

Introduction

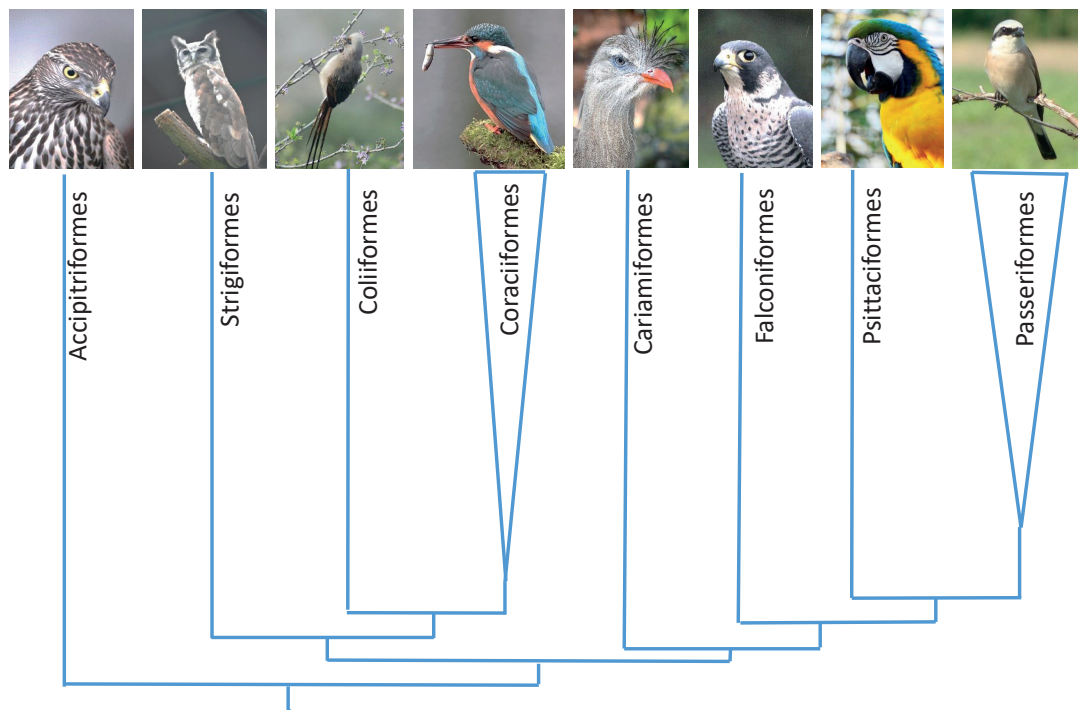
For a long time, ornithologists have discussed the phylogenetic position of owls among birds. Since owls exhibit a raptorial lifestyle, Linné had included them in the Accipitres. Already in 1927 L'Herminier separated the owls from diurnal raptors as an independent order. Nocturnal birds, such as owls, nightjars and stone-curlews share a number of common characters, such as large eyes and camouflaged plumage. Consequently, owls and nightjars had been regarded as closely related bird orders (Fürbringer 1888, Gadow 1892, Mayr & Amdon 1951, Sibley & Ahlquist 1990). However, Cracraft (1981) assumed a closer relationship between owls and falcons. The recent genome analyses (Zhang et al. 2014, Jarvis et al. 2015, Prum et al. 2015) have largely settled this open question (Fig. 1). Thus, falcons

and nightjars are not related to owls. Diurnal raptors, the Accipitriformes (including New and Old World vultures, ospreys, secretary bird, eagles, kites, harriers, buzzards and hawks) take a position at the base of a clade of land birds, which includes two other major lineages. One lineage comprises owls, mouse birds (Coliiformes) and Coraciimorphae (trogons, hoopoes, bee-eaters, hornbills, kingfishers, woodpeckers and toucans), the other lineage Seriemas, falcons (which no longer belong to diurnal raptors), parrots and the large order of Passeriformes (also see Kraus & Wink, 2015, Wink 2011, 2013, 2015a, Zhang et al. 2014).

Almost 30 years ago, we started to analyse systematics and phylogeny of raptors and owls by sequencing their DNA, mostly the mitochondrial cytochrome b and the nuclear

Figure 1 - Position of owls in the avian tree of life (simplified after Prum et al. 2015). The original phylogeny comprises genome sequences of 198 species (259 nuclear genes with 390 000 nucleotides for each taxon).

Figura 1 - Posição das rapinas noturnas na árvore da vida aviária (simplificada de acordo com Prum et al. 2015). A filogenia original compreende sequências dos genomas de 198 espécies (259 genes nucleares com 390 000 nucleótidos por cada táxon).



RAG-1 (Seibold et al. 1993; Wink 1995, 2000, 2014; Wink & Sauer-Gürth 2000, 2004; Wink & Heidrich 1999, 2000; Wink et al. 1996, 2000, 2004, 2008, 2009). In 2009, and we had presented a global phylogeny of owls, which was based on nucleotide sequences of the cytochrome b and RAG-1 genes (Wink et al. 2009).

Owls are monophyletic: the Strigiformes are divided into two monophyletic families Tytonidae and Strigidae (Sibley & Monroe 1990, Del Hoyo et al. 1999, Weick 2006, 2013, König & Weick 2008, Mikkola 2013). The Tytonidae are a small owl family with two subfamilies with a single genus each (*Tyto*, and *Phodilus* with two species). The Strigidae can be grouped into three subfamilies Striginae, Ninoxinae and Surniinae with more than 230 species (Table. 1; Fig. 2).

In this work, we summarize the existing data and add sequences of *Ninox supercili-*

aris, *Scotopelia peli*, *Scotopelia ussheri*, new subspecies of some owls and DNA sequences which had been submitted to GenBank (from other studies, Appendix S1). Our analysis thus provides a rather comprehensive update on the phylogeny of owls, although some taxa are still missing.

Methods

This publication is a follow-up of Wink et al. (2009) and includes DNA sequences, which were submitted to Genbank and previously published by us. They are included in Appendix S1, in that they show both an Institute of Pharmacy and Molecular Biotechnology (IPMB) and Genbank accession number. In case of DNA sequences from other laboratories, an IPMB number is absent. In this

Table 1 - Systematics of owls according to nucleotide sequences of mtDNA and the nuclear RAG-1 gene (after Wink et al. 2009).

Tabela 1 - Sistemática das rapinas noturnas com base em seqüências nucleotídicas de mtDNA e do gene nuclear RAG-1 (Wink et al. 2009).

FAMILY	SUBFAMILY	TRIBE	GENERA
Tytonidae	Tytoninae		<i>Tyto</i>
	Phodilinae		<i>Phodilus</i>
Strigidae	Striginae	Bubonini	<i>Bubo</i> (including the former genera <i>Nyctea</i> , <i>Ketupa</i> , <i>Scotopelia</i>)
		Strigini	<i>Strix</i> , <i>Jubula</i>
		Pulsatrigini	<i>Pulsatrix</i> , <i>Lophostrix</i>
		Megascopini	<i>Megascops</i> , <i>Psilosops</i>
		Asionini	<i>Asio</i> , <i>Ptilopsis</i>
		Otini	<i>Otus</i> , <i>Mimizuku</i>
		Surniinae	Surniini
	Aegolini	<i>Aegolius</i>	
	Ninoxinae		<i>Ninox</i> , <i>Uroglaux</i> , <i>Sceloglaux</i>

study, we also included nucleotide sequences of taxa, which were not studied before.

For the new samples, we isolated DNA from blood, tissues, feathers or buccal swabs of owls. Using polymerase chain reaction (PCR), the cytochrome b gene was amplified (primer sequences and PCR conditions were identical as in Wink et al. 2009) and sequenced (>1000 base pairs). The Sanger sequencing was executed on an ABI 3730 automated capillary sequencer (Applied Biosystems, Carlsbad, CA, USA) with the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit 3.1 (carried out by STARSEQ GmbH, Mainz, Sequencing Germany).

Sequences from our own collection and from GenBank (Appendix S1) were aligned and evaluated with standard software programmes, such as MEGA7. In most cases, one or two sequences per taxon were selected for the illustration, although over 800 sequences exist in our database from samples stored

at the Institute of Pharmacy and Molecular Biotechnology at Heidelberg University (Germany). Results of such analyses are phylograms, which reflect the phylogenetic relationships between species, genera, tribes, families and subfamilies (Fig. 2). The methodology has been described in more detail in Wink & Sauer-Gürth (2000, 2004), Wink et al. (2009), Storch et al. (2013) and Wink (2013).

The Molecular Phylogenetic analysis was done by the Maximum Likelihood method using MEGA7 (Kumar et al. 2016): The evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible model (Nei & Kumar, 2000). The tree with the highest log likelihood (-32973,50) is shown in Figure 2. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ

algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.8414)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 24.87% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 200 nucleotide sequences (mostly, two per taxon). Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 1,143 positions in the final dataset. A green dot indicates the nodes, which are supported by bootstrap values (500 replications) above 80% (for both NJ and ML analyses). GenBank accession numbers are documented in Appendix S1.

Results and Discussion

Tytonidae

Barn Owls look morphologically very similar over their entire range and Linné therefore classified them as a single species, *Tyto alba*, with cosmopolitan distribution. It has become evident however during the last decades, especially through DNA studies, that *Tyto alba* represents a taxon-rich species complex. Several new species and altogether 25 species have been defined already (Sibley & Monroe 1990, König et al. 1999; Weick 2006, 2013; König & Weick 2008, Mikkola 2013). As Barn Owls are typically resident birds, even more distinct taxa might exist on islands or in other isolated places.

Two major lineages are apparent from DNA studies, which are probably separated by more than 15 million years (Fig. 2): Lineage 1 comprises several Barn Owls from the Australasian region (except *T. capensis*) including *T. novaehollandiae*, *T. sororcula*,

T. almae, *T. manusi*, *T. castanops*, *T. multipunctata*, *T. longimembris*, *T. capensis* and *T. tenebricosa*. It is surprising that *T. capensis* occurs in southern Africa far away from its closely related Asian sisters. Lineage 2 leads to three sublineages: one Australasian sublineage comprises the widely distributed *T. delicatula* (including *T. sumbaensis*, and a few other species from oceanic islands) and *T. javanica* (Fig. 2). The next two lineages are sister groups: One comprises Barn Owls from Europe and Africa (*Tyto alba* with the subspecies *T. a. alba*, *T. a. guttata*, *T. a. ernesti*, *T. a. erlangeri*, *T. a. gracilirostris* and the African *T. a. affinis*), the other Barn Owls of the New World (the widely distributed *T. furcata* in the North, Central and South America with further local species/subspecies, and *T. glaucops* from Hispaniola). *Tyto soumagnei* from Madagascar clusters at the base of the European/African/New World clade.

Strigidae

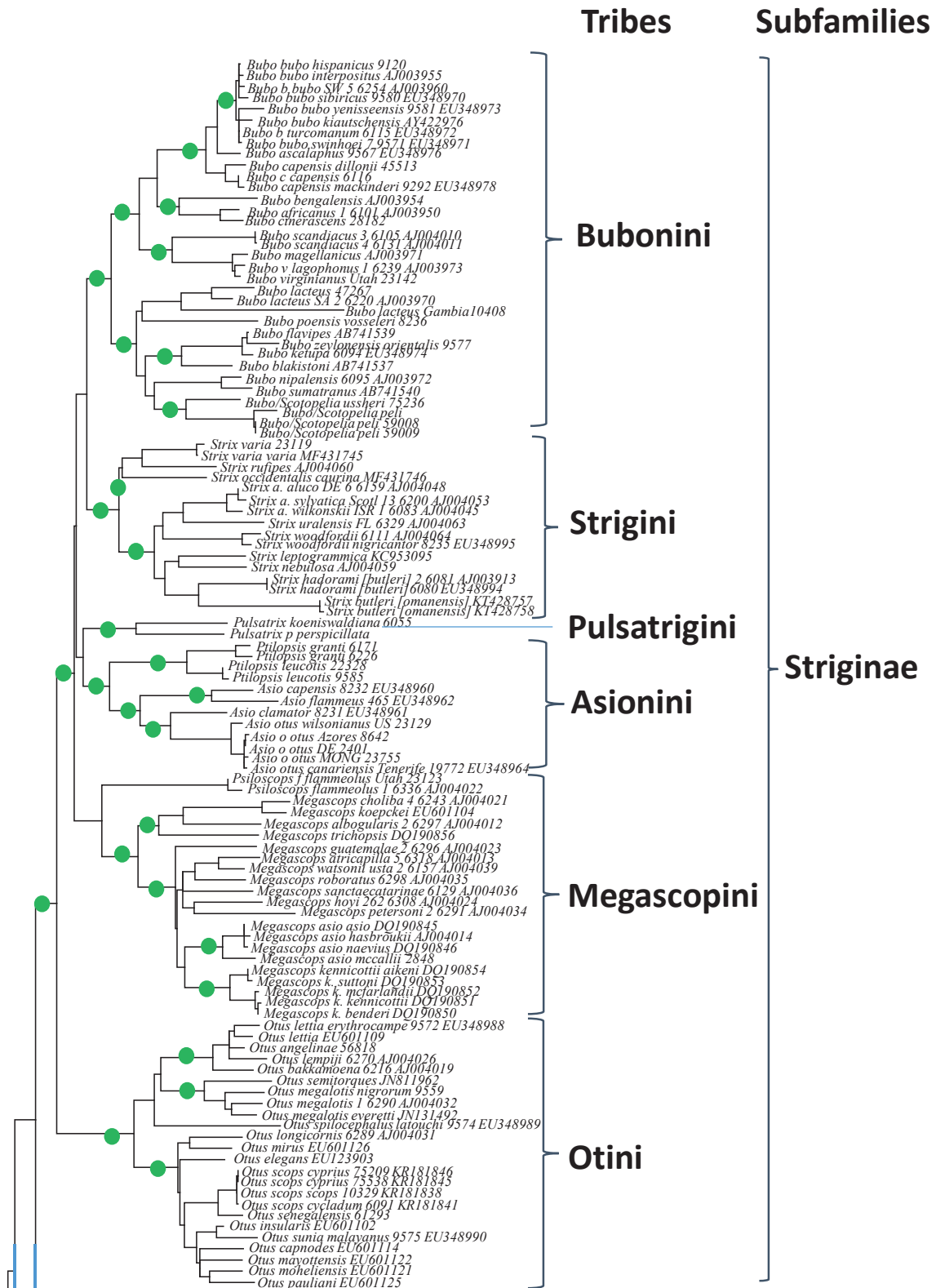
The Strigidae comprises over 230 species and is thus the biggest family in the order Strigiformes. The Strigidae are subdivided into three subfamilies according to DNA data: Striginae, Ninoxinae and Surniinae. The Striginae has been further divided into six tribes: Strigini, Bubonini, Pulsatrigini, Asionini, Megascopini and Otini (Table. 1).

In the tribe Strigini we find the genera *Strix*, and possibly *Jubula lettii* (West and Central Africa). *Strix* is a monophyletic genus with 23 species (Mikkola 2013), which clusters as a sister group to the *Bubo* complex (Bubonini) (Fig. 2). *Strix woodfordii* occurs in East Africa, and Africa and in the Near East. Two distinct taxa have been discovered which are not closely related to *Strix woodfordii*: *Strix butleri* (= *S. omanensis*) from Oman to Iran and *S. hadorami* for all other areas (formerly described as *S. butleri*) (Robb et al. 2016).

The genus *Pulsatrix* occurs in the New World and comprises three species (Mikkola 2013). A cytochrome b sequence in Gen-

Figure 2 - Molecular phylogeny of owls based on the analysis of the cytochrome b gene (see Methods). Nodes supported by bootstrap above 80% are marked with a green dot.

Figura 2 - Filogenia molecular das rapinas noturnas com base na análise do gene citocromo b (ver Métodos).



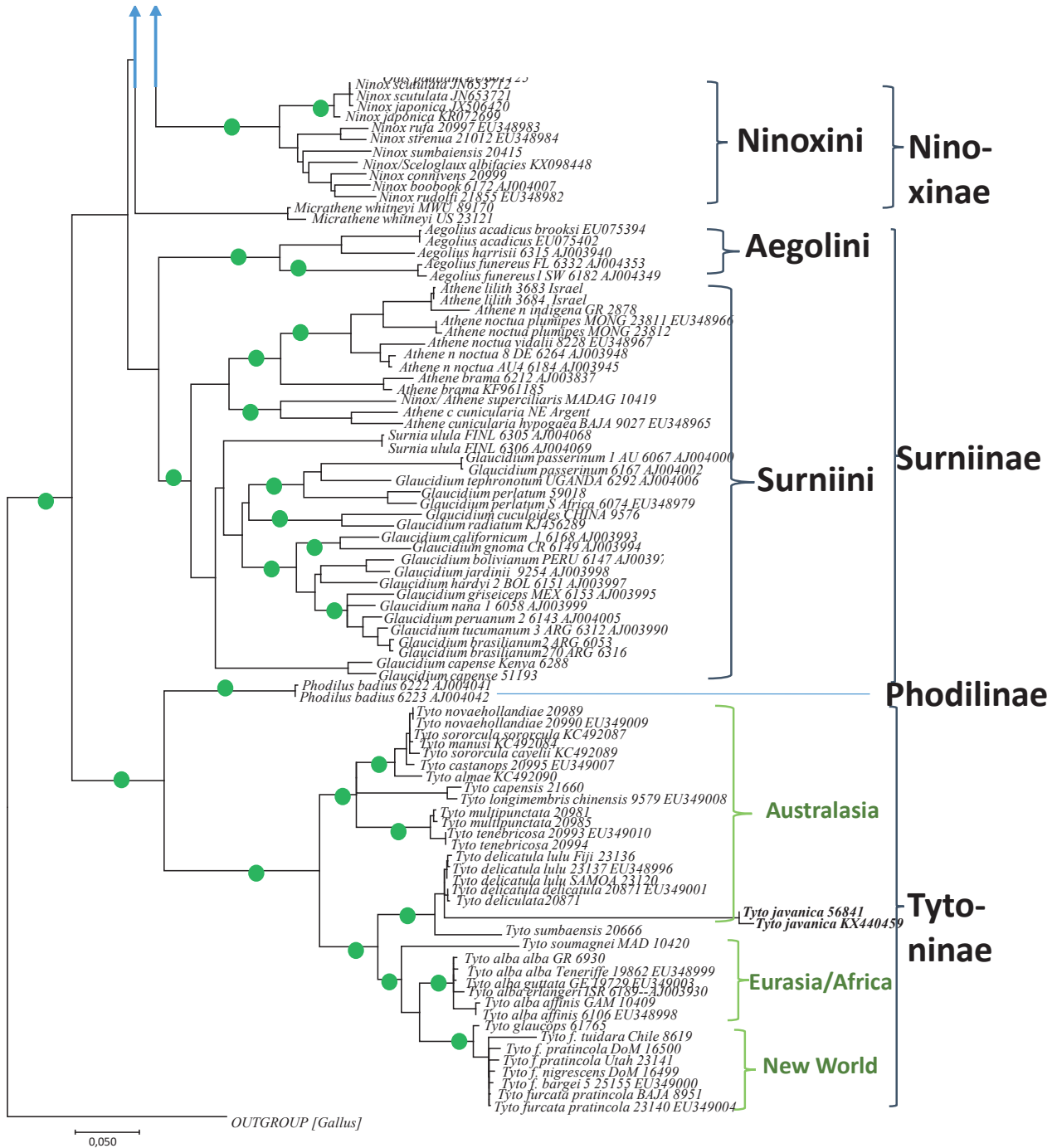
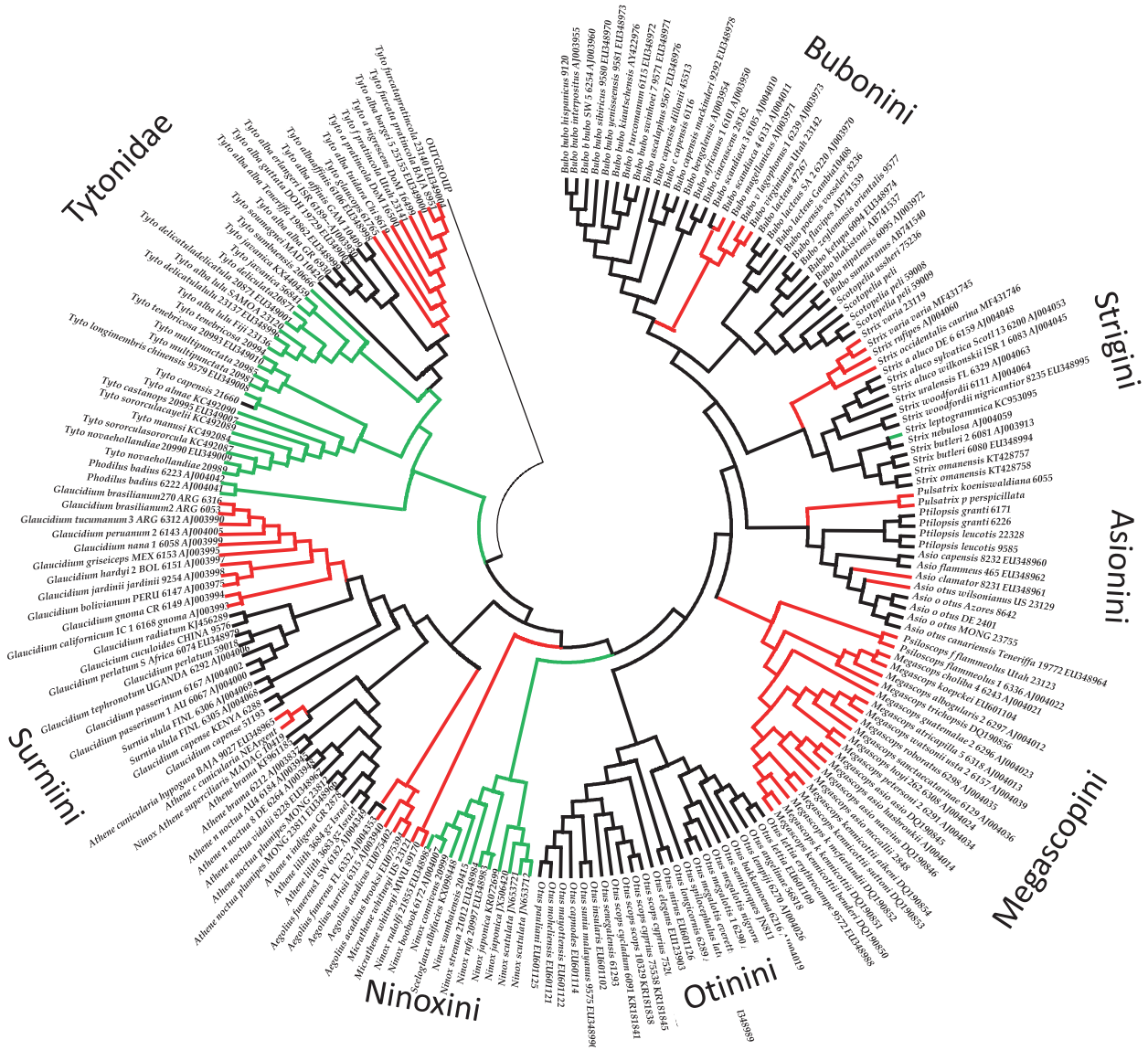


Figure 3 - Phylogeography of owls. The phylogeny is illustrated as a radiary cladogram. The colour of branches indicate their main distribution areas: Black = Europe, Africa, Central and East Asia; green = Australasia; red = New World.

Figura 3 - Filogenia das rapinas noturnas representada por um cladograma radial. A cor das ramificações indica as suas principais áreas de distribuição: preto = Europa, África, e Centro e Este da Ásia; verde = Australásia; vermelho = Novo Mundo



Bank (which was not included in our analysis because it was too short) suggests, that *Lophotrix cristata* (Central and South America) is a sister to *Pulsatrix* (Wink et al. 2008); it has been suggested to create a new tribe Pulsatrigini for the genera *Lophotrix* and *Pulsatrix*. Alternatively, these taxa could be included in the tribe Asionini as they appear to have a common ancestor (Fig. 2 & 3).

The tribe Bubonini is monophyletic and traditionally included the genera *Bubo*, *Nyctea*, *Ketupa* and *Scotopelia*. DNA analyses have shown, that the taxa of *Nyctea*, *Ketupa* and *Scotopelia* all cluster within the genus *Bubo* which would become paraphyletic (Fig. 2) (Wink & Heidrich 1999, König & Weick, 2008, Wink et al. 2008, Mikkola 2013, Omote et al. 2013, Wink 2013). The former members of the Fish-owls *Ketupa* cluster as sister to *Bubo nipalensis* and *B. sumatranus*, which share morphological similarities. In order to avoid paraphyletic groupings we had suggested to lump these genera and include them in a common genus *Bubo* (Fig. 2) (Wink & Heidrich 1999, König & Weick, 2008, Wink et al. 2008; Wink 2013, Mikkola 2013). This suggestion has been widely accepted for *Nyctea* and *Ketupa*. In this publication, we have included sequences from two species of the African Fishing-owl *Scotopelia*, which cluster together with *Bubo nipalensis* and *B. sumatranus*, as do the Asian Fish-owls. Therefore, we suggest lumping *Scotopelia* in *Bubo*.

We had detected three lineages in the former tribe Otini (with the genera *Otus*, *Megascops*, *Macabro*, *Pyrroglaux*, *Gymnoglaux*, *Psilosops* and *Mimizuku*), which revealed several para- and polyphyletic groups. Therefore, we had suggested to change genus names and genus delimitations and to create three new tribes: Asionini, Megascopini and Otini (Wink & Heidrich 1999, König & Weick 2008, Wink et al. 2009, Mikkola 2013). Screech-owls of the New World with 28 species no longer belong to *Otus* but to the genus *Megascops* (Wink et al. 2008, 2009). Only *Otus flammeolus* was maintained in a monotypic *Psilosops flammeolus*

because of its isolated position at the base of the lineage leading to *Megascops* (Penhallurick 2002, Weick 2006, König & Weick 2008, Wink et al. 2009, Mikkola 2013, Dantas et al. 2015). The species-rich Old World Scops-owls (at least 47 species) remain in the genus *Otus* (Wink et al. 2009, Mikkola 2013). Pons et al. (2013) have analysed the systematics of Scops-owls of Socotra, East Africa and the Arabian peninsula. For Cyprus, a new species *Otus cyprius* was described recently (Flint et al. 2015). Within *Otus*, two lineages are apparent: A lineage with mostly European/African taxa (*Scops* clade), the other with mostly East Asian taxa (*Letitia* clade). It is likely that more *Otus* taxa have been overlooked and exist.

The African White-faced Owls (formerly *Otus leucotis*) cluster differently from *Otus* as a sister to *Asio* (Fig. 2) and were placed in a new genus *Ptilopsis*. White-faced Owls from Central and southern Africa differ in plumage and DNA sequences: They have been split into two taxa: *P. leucotis* (West-, Central- and East Africa) and *P. granti* (southern Africa) (König & Weick 2008, Wink et al. 2009, Mikkola 2013).

The genus *Asio* with Long-eared and Short-eared Owls (Mikkola 2013) and the monotypic genus *Nesasio* were traditionally placed in Asioninae (Weick 2006). Since *Asio* clusters as a sister to *Ptilopsis* (Fig. 2) it could be treated as a tribe Asionini (Table 1).

The Surniinae has been traditionally divided into three tribes (Weick 2006): Surniini, Aegolini and Ninoxini. The tribe Surniini comprises the species-rich genus of Pygmy-owls (*Glaucidium*) with 33 species and the monotypic Hawk-owl *Surnia*, whose populations are quite similar around the Arctic circle. Pygmy-owls share similar morphology and a wide distribution in the Old and New World. However, they strongly differ in vocalisations and DNA sequences (König 1994b, Heidrich et al. 1995b). Pygmy owls of the Old World are monophyletic, as are the Pygmy owls of the New World; both groups might have been separated since more than 6

million years (Wink & Heidrich 1999). The African *G. capensis* clusters away from the other Pygmy-owls (Fig. 2) and apparently represents a different genetic lineage, which has been placed in the subgenus *Taenioglaux* Kaup 1848 with nine other species in the Old World (König & Weick 2008, Mikkola 2013, Wink et al. 2008). König & Weick (2008) suggest the use of the genus *Taenioglaux* for these species.

Little Owls (*Athene*) form another genus within the Surniinae with traditionally three species: *Athene noctua* (in Eurasia), *A. brama* (SE Asia) and *A. blewitti* (India); the latter taxon has been separated as *Heteroglaux blewittii* (Mikkola 2013). DNA data suggest that *Athene noctua* is a species complex, which might be subdivided into new species, like the situation in the *Tyto* complex (Fig. 2) (see van Nieuwenhuyse et al. 2008). *A. n. plumipes* from Mongolia and China apparently is a distinct taxon (Fig. 2; Mikkola 2013), which is also true for *A. lilith* in the Middle East (Fig. 2). The Burrowing Owls of the New World (formerly *Speotyto*) cluster as a sister to the Old World Little owls, separated by roughly 6 million years (König et al. 1999, König & Weick 2008, Wink et al. 2009). Burrowing Owls represent a species complex, with several geographically defined subspecies. As discussed later in the *Ninox* section, *Ninox superciliaris* from Madagascar clearly clusters as a sister to *A. cucularia*. Its taxonomic position thus needs to be changed.

Boreal Owls (genus *Aegolius* with 4 species) are monophyletic and cluster as a sister to the formerly described tribe Surniini and are better separated in its own tribe Aegolini (Fig. 2). Within populations of *A. funereus* genetic differentiation is low (Wink et al. 2009), but a little bit higher than within the two subspecies of the New World *A. acadicus* (Withrow et al. 2014).

The Australasian genus *Ninox* is species-rich with at least 26 taxa. Superficially, they resemble Pygmy and Little Owls and replace them in the Australasian region (Mik-

kola 2013). They are better placed in a subfamily Ninoxinae: the former tribes Aegolini and Surniinae differ strongly from the tribe Ninoxini (Table. 1; Fig. 2). Gwee et al. (2017) reported a phylogeny based on a multilocus analysis of 24 taxa of the *Ninox* complex exploring the radiation of this genus in Wallacea. The White-browed Owl from Madagascar (*Ninox superciliaris*) is not a member of the genus *Ninox* but much closer to the genus *Athene* (Fig. 2). Wink (2014) had suggested changing its name to *Athene superciliaris*. The extinct *Sceloglaux albifacies* is a member of the genus *Ninox* and should be lumped within it.

Global Phylogeography of Owls

The ancestors of owls evolved with the beginning of the Tertiary, about 66 million years ago; the split between Tytonidae and Strigidae occurred in the middle of the Eocene (Prum et al. 2015). Owls occur on all continents, but several lineages are geographically restricted. The basal taxa of Barn Owls occur in the Australasian region (Fig. 3), suggesting that owls developed in Australia. A second lineage of Barn Owls leads to a clade of African/European taxa on one hand and a clade with American Barn Owls on the other hand; both lineages are sister clades. This suggests that the American Barn owls came from the Old World (Fig. 3).

The Surniinae has its main distribution in Europe, Asia and Africa. A few taxa, however, colonized the New World, such as the Burrowing Owl (*Athene cucularia*) where it developed into a species complex. The genus *Aegolius* has one species, the Boreal Owl *A. funereus*, with a circumpolar distribution, whereas all the other three taxa of this genus are restricted to America (Fig. 3). In the genus *Glaucidium*, a sister group with two main lineages is apparent, one with Pygmy-owls

of America, the other with Pygmy-owls of Europe, Asia and Africa. A similar split can be seen in the genus *Strix*, in which Old and New World taxa diverge as separate lineages. *Strix nebulosa* clusters in the Old World lineage but developed a subspecies *S. n. nebulosa* in North America (Fig. 3). The genus *Ninox* has its centre of distribution in the Australasian region, where it partly replaces Pygmy and Little Owls (Fig. 3).

Whereas Screech-owls of the genera *Megascops* and *Psiloscops* are exclusively New World taxa, *Scops* owls replace them in Europe, Africa and Asia (Fig. 3). According to Fig. 3, the ancestors of *Megascops* probably came from the Old World.

The members of the tribe Asioninae are mostly Old World taxa, except for the Latin American *Asio clamator*. Among Short-eared and Long-eared Owls, a radiation occurred from the Old World to the Americas with the evolution of several subspecies.

Eagle-owls (genus *Bubo*) has a centre of radiation in Europe, Africa and Asia. However, it also comprises a New World clade, with Great Horned Owl and Snowy Owl (Fig. 3). The latter species probably evolved in America but presently shows a circum-polar distribution.

Conclusions

Although research on the biology, vocalisations and molecular phylogeny of owls has intensified during the last decades (König & Weick 2008, Weick 2013, Mikkola 2013), many questions remain as most owl taxa have not been studied in detail.

Phylogenetic analyses have discovered that several owl species represent species complexes which can be divided in several new taxa (e.g., within *Tyto*, *Athene*, *Glaucidium*, *Scops*, *Megascops*, *Bubo*, *Strix*). Since many owls are mainly resident (not migratory), we can expect isolated and genetically divergent populations on islands, in mountains or in the

tropical rain forests, which differ from recognized species. Consequently, the number of owl species will go up. Because many isolated owl populations are small and threatened by habitat loss some will likely become extinct in the future.

Phylogenetic analyses have discovered several para- and polyphyletic groups. As a result paraphyletic species are either lumped together with the majority of related species in a common genus (such as some genera of the *Bubo* complex, including *Ketupa*, *Nyctea* and *Scotopelia*) or existing genera are split into new genera (as shown for members of the former genus *Otus*).

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