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Phylogenetic Relationships in Owls based on nucleotide sequences of mitochondrial and nuclear marker genes

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ABSTRACT

Together with morphological and vocal characters, nucleotide sequences help to define phylogenetic relationships in owls. Since owls are nocturnal their morphological traits are often very similar a systematic differentiation or speciation event can easily be overlooked. A recent example is *Ninox sumbaensis* from the island of Sumba. An unknown owl that had been observed there had been assumed to be a member of *Otus*. DNA data unequivocally showed (Olsen *et al.* 2002) that it belongs to the genus *Ninox*. It was named *Ninox sumbaensis*. About 80 species of the Strigidae and 16 species of Tytonidae have been studied so far in our laboratory and a phylogeny based on cytochrome b data has been published (Wink & Heidrich 1999). We have enlarged our cytochrome b data base and additionally have sequenced a nuclear marker (LDHb intron DNA). Basically, the ncDNA data support the results obtained from mtDNA.

INTRODUCTION

Since owls are nocturnal their morphological traits are often very similar (König *et al.*, 1999) a systematic differentiation or speciation event can easily be overlooked. Together with morphological and vocal characters nucleotide sequences of marker genes help to define phylogenetic relationships in owls. Nucleotide sequences of the mitochondrial cytochrome b gene have already been employed to study the systematics and evolution of diurnal raptors and owls (Griffiths 1997; Mindell 1997; Groombridge *et al.* 2002, Krucken-hauser *et al.*, 2003, Riesing *et al.*, 2003; from our laboratory: Seibold *et al.*, 1993, 1996; Heidrich & Wink 1994, 1998; Heidrich *et al.*, 1995a,b, 1996; Wink 1995, 1998, 2000; Wink & Seibold 1996; Wink & Sauer-Gürth 2000; Wink *et al.* 1996, 1998; Wink & Heidrich 1999, 2000; Seibold & Helbig 1995, 1996; Olsen *et al.* 2002).

In our studies we regularly amplify and sequence the mitochondrial cytochrome b gene. Since this gene shows a good resolution at the genus level and since we already have quite a large data base for this gene, we use this gene as a platform for our owl project which aims to cover all species of owls. Nuclear genes, such as intron sequences, need to be analysed to corroborate findings of mtDNA, especially relationships between genera.

About 80 taxa of the Strigidae and 16 of Tytonidae have been studied so far in our laboratory and a phylogeny based on cytochrome b data has been published (Wink & Heidrich 1999, 2000).

We have enlarged our cytochrome b data base and have additionally sequenced a nuclear marker (intron of LDHb-DNA) for all groups that were critical. Basically, the ncDNA data support the results obtained from mtDNA.

MATERIAL AND METHODS

We have isolated total DNA from feather, blood or tissue samples (see Wink 2000) which had been kindly supplied by several colleagues (W. Bednarek, G. Ehlers, O. Hatzofe, R. Krahe, D. Reynolds, A. Kemp, C. Fentzloff, W. Grummt, C. König, J. Yom-Tov, D. Ristow, H.H. Witt, E. Thaler, B. Etheridge, D. Engelbrecht and U. Schneppat, J. Penhallurick, J. Olsen). The cytochrome b gene was amplified by PCR (primer sequences in Wink & Sauer-Gürth 2000) and sequenced by using AlfExpress (Amersham Pharmacia Biotech) or ABI 3100 (Applied Biosystems) instruments. Sequences of 1000 and more base pairs were aligned manually and analysed with the software packages PAUP* (Swofford 2002) and MEGA2 (Kumar *et al.* 2001) (see Wink 2000; Wink & Sauer-Gürth 2000; Wink *et al.* 2002; Broders *et al.* 2003 for further details).

RESULTS AND DISCUSSION

The original data set (Heidrich & Wink 1998, Wink & Heidrich 1999, 2000) contained cytochrome b sequences from at least two specimens of each species; in several instances 10 and more individuals were sequenced to assess the intraspecific sequence variation. The present analysis to which a large number of new sequences were added, covers approximately 45% of all owl species and approximately 70% of all owl genera. We have performed Maximum Parsimony (MP), Neighbour Joining (NJ) and Maximum Likelihood (ML) analyses.

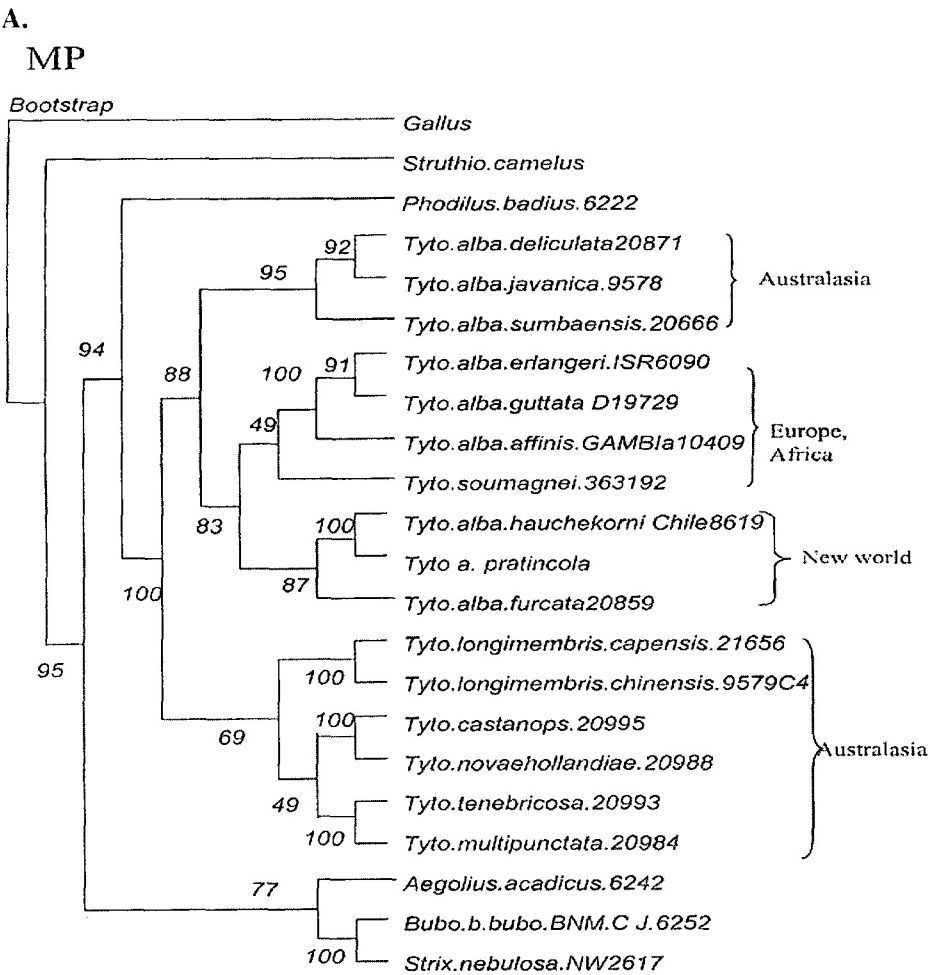
A strict consensus MP cladogram is shown in Figure 1 to illustrate the overall phylogenetic relationships of owls based on nucleotide sequences of the cytochrome b gene. The following clades were recognised unequivocally (bootstrap support usually higher than 90%) by all three methods of tree reconstruction:

- monophyletic subfamily Tytonidae
- monophyletic subfamily Strigidae
- monophyletic genus *Tyto*
- monophyletic genus *Strix*
- monophyletic genus *Aegolius*

- monophyletic genus *Athene*
- monophyletic genus *Glaucidium*
- monophyletic genus *Ninox*
- paraphyletic *Bubo* complex which comprises the genera *Bubo, Scotopelia, Ketupa* and *Nyctea*
- polyphyletic *Otus* complex, in which Old and New World members of the genus *Otus* form independent monophyletic groups.

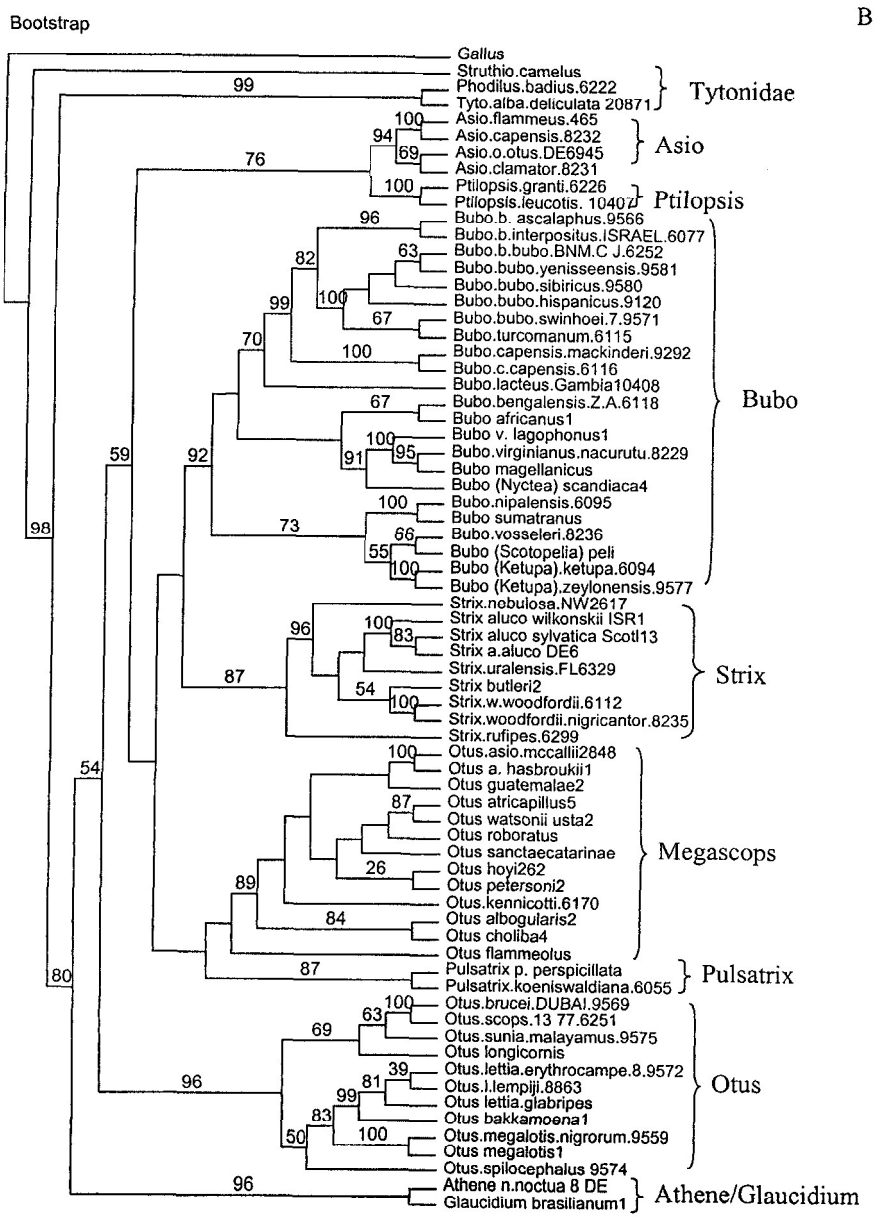
Figure 1. Molecular phylogeny of owls inferred from nucleotide sequences of the cytochrome b gene

Reconstruction with maximum parsimony; representation as a strict consensus cladogram.



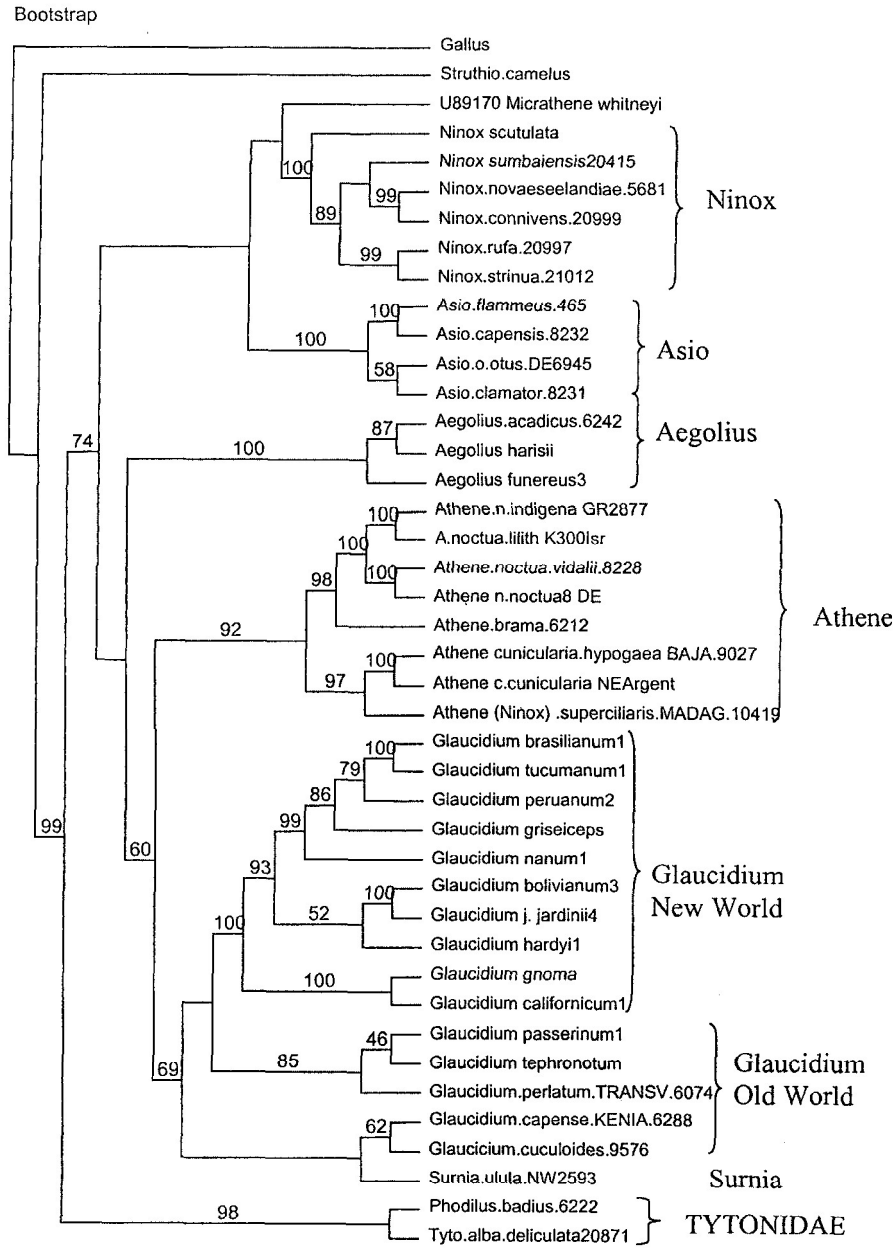
B.

MP


Bootstrap
B
Gallus
Struthio.camelus
Phodilus.badius.6222
Tyto.alba.deliculata 20871
Tytonidae
Asio.flammeus.465
Asio.capensis.8232
Asio.o.otus.DE6945
Asio.clamator.8231
Asio
Ptilopsis.granti.6226
Ptilopsis.leucotis. 10407
Ptilopsis
Bubo.b. ascalaphus.9566
Bubo.b.interpositus.ISRAEL.6077
Bubo.b.bubo.BNM.C J.6252
Bubo.bubo.yenisseensis.9581
Bubo.bubo.sibiricus.9580
Bubo.bubo.hispanicus.9120
Bubo.bubo.swinhoei.7.9571
Bubo.turcomanum.6115
Bubo.capensis.mackinderi.9292
Bubo.c.capensis.6116
Bubo.lacteus.Gambia10408
Bubo.bengalensis.Z.A.6118
Bubo africanus1
Bubo v. lagophonus1
Bubo.virginianus.nacurutu.8229
Bubo magellanicus
Bubo (Nyctea) scandiaca4
Bubo.nipalensis.6095
Bubo sumatranus
Bubo.vosseleri.8236
Bubo (Scotopelia) peli
Bubo (Ketupa).ketupa.6094
Bubo (Ketupa).zeylonensis.9577
Bubo
Strix.nebulosa.NW2617
Strix aluco wilkonskii ISR1
Strix aluco sylvatica Scotl13
Strix a.aluco DE6
Strix.uralensis.FL6329
Strix butleri2
Strix.w.woodfordii.6112
Strix.woodfordii.nigricantor.8235
Strix.rufipes.6299
Strix
Otus.asio.mccallii2848
Otus a. hasbroukii1
Otus guatemalae2
Otus atricapillus5
Otus watsonii usta2
Otus roboratus
Otus sanctaecatarinae
Otus hoyi262
Otus petersoni2
Otus.kennicotti.6170
Otus albogularis2
Otus choliba4
Otus flammeolus
Megascops
Pulsatrix p. perspicillata
Pulsatrix.koeniswaldiana.6055
Pulsatrix
Otus.brucei.DUBAI.9569
Otus.scops.13 77.6251
Otus.sunia.malayamus.9575
Otus longicornis
Otus.lettia.erythrocampe.8.9572
Otus.l.lempiji.8863
Otus lettia.glabripes
Otus bakkamoena1
Otus.megalotis.nigrorum.9559
Otus megalotis1
Otus.spilocephalus 9574
Otus
Athene n.noctua 8 DE
Glaucidium brasilianum1
Athene/Glaucidium

C.

MP


Bootstrap
Gallus
Struthio.camelus
U89170 Micrathene whitneyi
Ninox scutulata
Ninox sumbaiensis20415
Ninox.novaeseelandiae.5681
Ninox.connivens.20999
Ninox.rufa.20997
Ninox.strinua.21012
Ninox
Asio.flammeus.465
Asio.capensis.8232
Asio.o.otus.DE6945
Asio.clamator.8231
Asio
Aegolius.acadicus.6242
Aegolius harisii
Aegolius funereus3
Aegolius
Athene.n.indigena GR2877
A.noctua.lilith K300lsr
Athene.noctua.vidalii.8228
Athene n.noctua8 DE
Athene.brama.6212
Athene cunicularia.hypogaea BAJA.9027
Athene c.cunicularia NEArgent
Athene (Ninox) .superciliaris.MADAG.10419
Athene
Glaucidium brasilianum1
Glaucidium tucumanum1
Glaucidium peruanum2
Glaucidium griseiceps
Glaucidium nanum1
Glaucidium bolivianum3
Glaucidium j. jardinii4
Glaucidium hardyi1
Glaucidium gnoma
Glaucidium californicum1
Glaucidium New World
Glaucidium passerinum1
Glaucidium tephronotum
Glaucidium.perlatum.TRANSV.6074
Glaucidium.capense.KENIA.6288
Glaucicium.cuculoides.9576
Glaucidium Old World
Surnia.ulula.NW2593
Surnia
Phodilus.badius.6222
Tyto.alba.deliculata20871
TYTONIDAE

Whereas these clades were identified by all reconstructions, differences were found in some instances regarding the phylogenetic relationships between clades, although an *Athene, Surnia, Glaucidium* cluster, usually including *Aegolius* or a *Strix/Bubo* cluster was recovered by all programmes.

A few new results have been obtained (as compared to data shown in Wink & Heidrich (1999, 2000):

Recently, a new owl was discovered on Sumba island which was assumed to be a member of the genus *Otus*. DNA analysis revealed unequivocally that it is a member of the genus *Ninox*. It was described as *Ninox sumbaiensis* (Olsen *et al.* 2002).

Ninox superciliaris which occurs on Madagascar clusters clearly in the clade of *Athene*, which would also agree with its plumage characters.

Members of the genus Tyto show a high degree of genetical divergence. A number of taxa from the Australasian region have been added, indicating the existence of several *Tyto* species. Some of them have been attributed species rank already (König *et al.* 1999); others, such as *T.a. deliculata, T.a. sumbaiensis* could easily raised to species rank according to the genetic divergences found.

A major surprise of the cytochrome b analysis was the finding that the genus *Bubo* was paraphyletic (it included the genera *Ketupa, Nyctea* and *Scotopelia*) and the polyphyly of *Otus*. *Otus* shows three clades, the screech owls of the New World, the *Scops* owls of the Old World and the white-faced *Scops* owls of Africa. Critics could argue that cytochrome b represents a maternal lineage only and that hybridisations might have distorted the phylogeny. In order to assess this argument we have amplified and sequenced the nuclear LDHb intron gene (Fig. 2). The results obtained corroborate the cyt b findings, in that *Bubo* comprises *Ketupa* and *Nyctea* (*Scotopelia* was not available for the LDH analysis). The three *Otus* clades are polyphyletic also in the LDH data set (Fig. 2). White faced *Scops* owls again cluster as a sister group to the genus *Asio*. Figure 2 gives a LDHb phylogram of the *Bubo/Otus/Strix* assemblage.

In a next step we have combined our cytochrome b and LDHb datasets. A short version of a phylogram is shown in Figure 3. Also this reconstruction confirms previous findings, i.e. the paraphyly of the *Bubo* group and the polyphyly of the *Otus* assemblage.

Figure 2. Molecular phylogeny of owls inferred from nucleotide sequences (516 base pairs) of the LDHb intron DNA

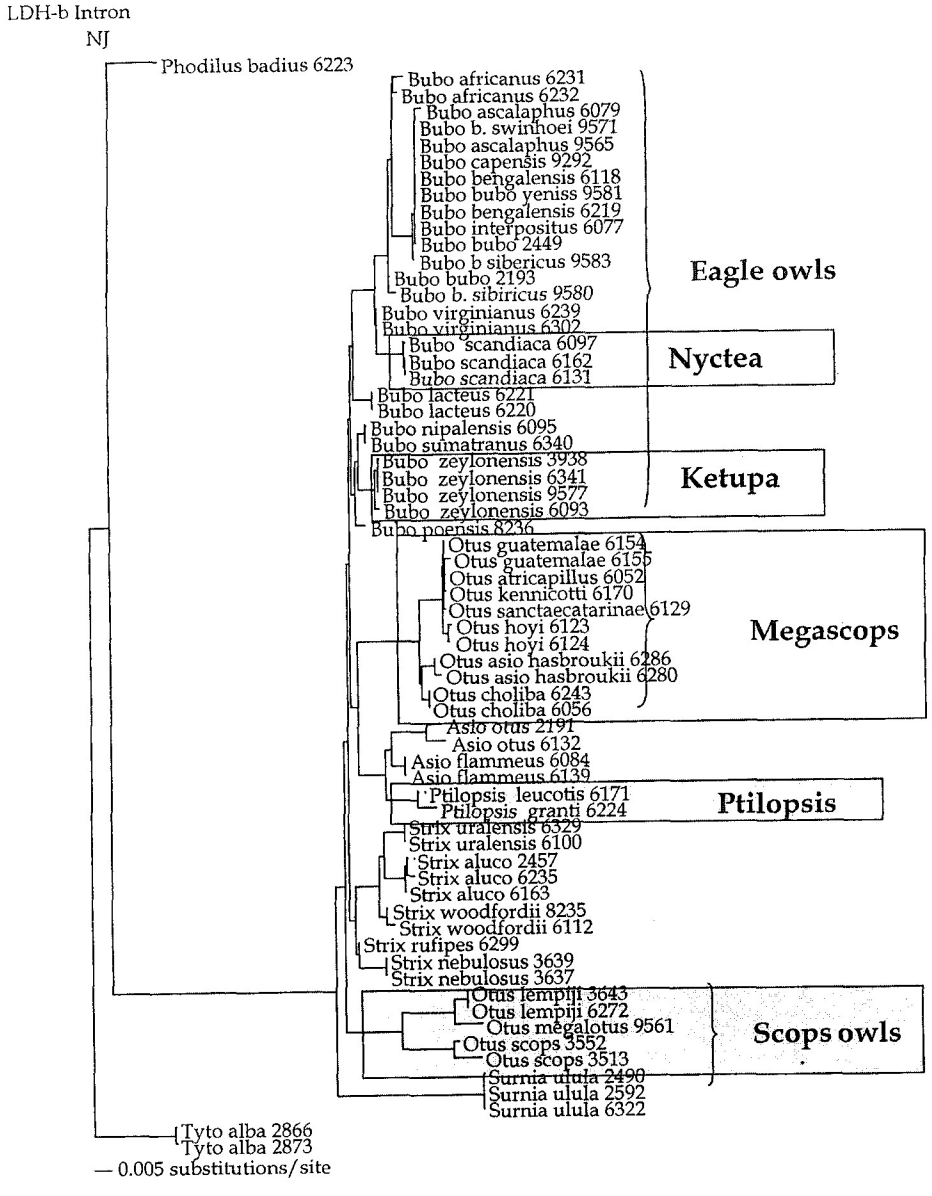
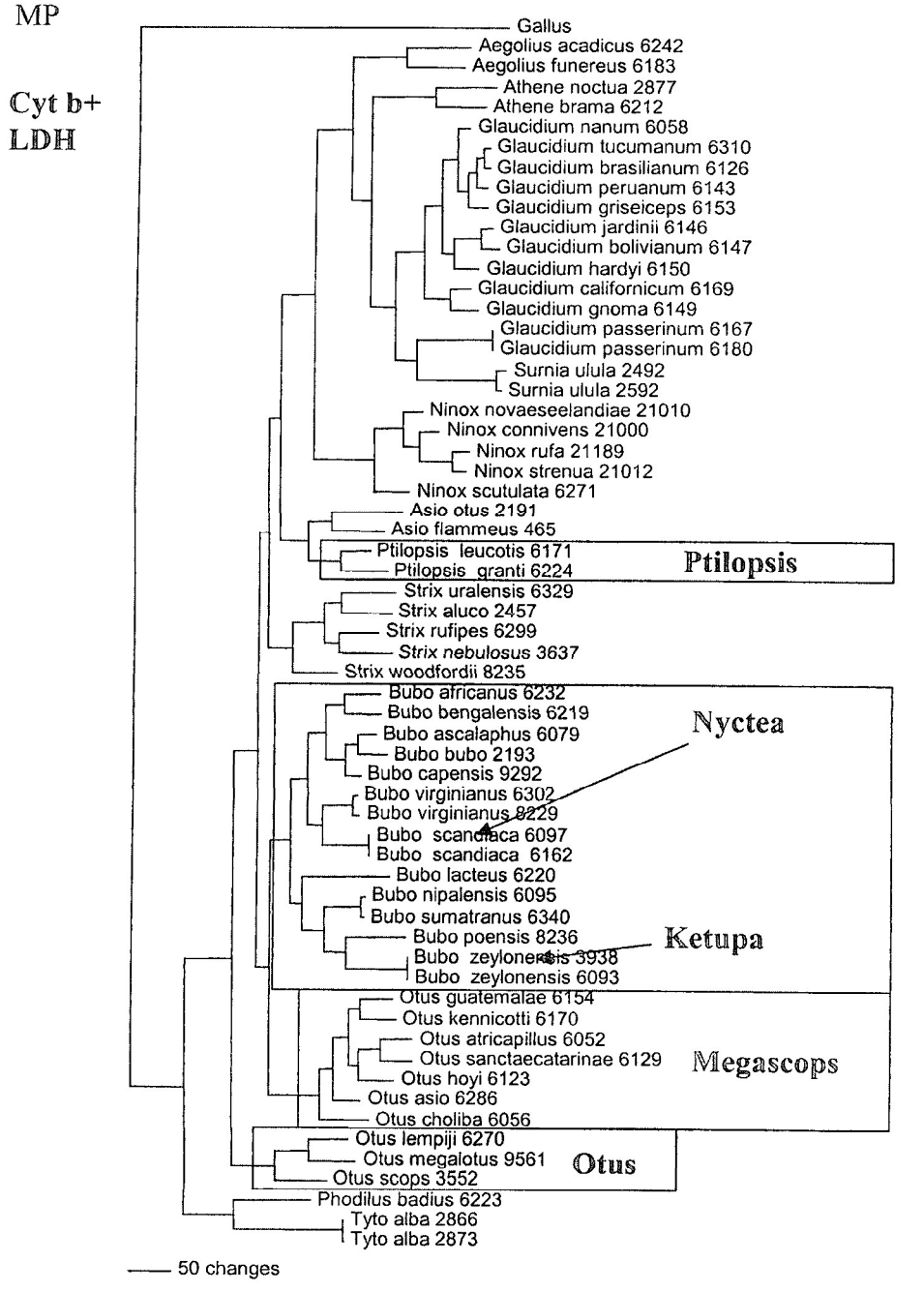


Figure 3. Molecular phylogeny of owls inferred from a combined data set of nucleotide sequences of cytochrome and LDHb



CONCLUSIONS

A few taxonomic conclusions can be drawn (Penhallurick & Wink 2004) which imply to change the following names:

New World *Otus* □ *Megascops*

New World *Glaucidium* □ *Phalaenopsis*

Otus leucotis □ *Ptilopsis leucotis*

Scotopelia □ *Bubo*

Ketupa □ *Bubo*

Nyctea □ *Bubo*

The results presented here are still preliminary as several species are not yet represented.

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