

# Tawny Owl (*Strix aluco*) and Hume's Tawny Owl (*Strix butleri*) Are Distinct Species: Evidence from Nucleotide Sequences of the Cytochrome *b* Gene

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The cytochrome *b* gene of the Tawny Owl (*Strix aluco*), Hume's Tawny Owl (*Strix butleri*) and the African wood owl (*Strix woodfordii*) was amplified by polymerase chain reaction (PCR) and partially sequenced (300 base pairs). Sequences differ substantially (9 to 12% nucleotide substitutions) between these taxa indicating that they represent distinct species, which is also implicated from morphological and biogeographic differences. Using cytochrome *b* sequences of *S. aluco*, *S. butleri*, *S. woodfordii*, *Athene noctua* and *Tyto alba* phylogenetic relationship were reconstructed using the "maximum parsimony" principle (PAUP 3.1.1) and the neighbour-joining method (MEGA).

## Introduction

According to Sibley and Monroe (1990) the genus *Strix* (family Strigidae, order Strigiformes) consists of 18 species occurring in North and South America, Africa, Europe and Asia. Whereas most taxa are unequivocally recognized as distinct species according to morphological, acoustic and other biological characters, the status of others is still a matter of debate: *Strix butleri* is sometimes treated as a subspecies of *S. aluco*; *S. varia* and *S. fulvescens* as *S. occidentalis varia* and *S. occidentalis fulvescens*; *S. davidi* as *S. nebulosa davidi*, and *S. nigrolineata* as *S. huhula nigrolineata* (Sibley and Monroe, 1990). Using morphological and biological characters alone, the decision whether a taxon has the status of a species or subspecies will remain difficult. Methods of molecular systematics might help to decide these issues (Erich, 1989; Hillis and Moritz, 1990; Hoelzel, 1992; Innis *et al.*, 1990). Most resolution can be obtained by comparing the nucleotide sequences of phylogenetically informative marker genes (Hoelzel, 1992; Kocher *et al.*, 1989; Innis *et al.*, 1990; Cooper *et al.*, 1992; Edwards *et al.*, 1991).

The mitochondrial cytochrome *b* gene has been selected as a marker gene in recent years, since its sequence has been found informative for many

phylogenetic and taxonomic problems of animals, especially of birds (Kocher *et al.*, 1989; Richman and Price, 1992; Helbig *et al.*, 1993, 1994; Seibold *et al.*, 1993, 1994a, b; Wink *et al.*, 1993a, b, 1994).

*Strix aluco* represents a polytypic species with a broad distribution range over Europe, parts of North Africa and Asia: Nominate *S. a. aluco* L., 1758, breeds in Europe from Belgium, Netherlands, German Rhineland, Vosges, Jura and Alps east to c. 35° E in central European Russia, Ukraine, and Crimea, south to northern Italy and Balkans; *S. a. siberiae* Dementiev, 1933, in Ural mountains and western Siberia; *S. a. sylvatica* Shaw, 1809, in Britain, France, Iberia, Turkey, Levant, southern Italy and Greece; *S. a. mauritanica* (Witherby, 1905) in north-west Africa; *S. a. willkenskii* (Menzbier, 1896) in Caucasus, Transcaucasia, north-east Turkey, Iran, Turkmeniya; *S. a. sanctinicolai* Zarudny, 1905, in Zagros mountains in western Iran. In addition, 5 subspecies have been described in central and eastern Asia (Cramp 1985; Glutz and Bauer 1980). *S. butleri* has a restricted distribution in Israel and the Sinai peninsula (Cramp, 1985; Glutz and Bauer, 1980). The African wood owl (*S. woodfordii*) occurs in West, Central and South Africa, reaching Somalia and Ethiopia in the East. *S. aluco* and *S. butleri* show some similarities in morphology and are mostly parapatric and but locally sympatric in the Near East. It has been questioned by some authors, *e.g.*, Mikkola in (Sibley and Monroe, 1990) that both taxa are distinct species.

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In this study the cytochrome *b* nucleotide sequences of the Tawny Owl (*Strix aluco*), the African Wood Owl (*Strix woodfordii*) and Hume's Tawny Owl (*Strix butleri*) were determined and used to evaluate their degree of speciation and to reconstruct a phylogenetic relationship between them and other members of the order Strigiformes.

## Materials and Methods

### Origin of birds

Blood was collected from *Strix aluco* (Israel, Germany), *Strix butleri* (Israel) and *Strix woodfordii* (South Africa).

### DNA-methods

Blood was stored in a modified EDTA-buffer (Arctander, 1988) at ambient temperatures in the field. DNA was extracted after digestion with proteinase K (Boehringer) according to (Swatschek

*et al.*, 1993, 1994). Employing the polymerase chain reaction PCR (Erich, 1989; Innis *et al.*, 1990) the cytochrome *b* gene was partially amplified and purified by agarose gelelectrophoresis according to Helbig *et al.* (1993), Seibold *et al.* (1993, 1994a,b), and Wink *et al.* (1993a,b, 1994). Double-stranded PCR products were directly sequenced by the chain termination method (Sambrook *et al.*, 1989) using [ $\alpha$ -<sup>35</sup>S]dATP (NEN Dupont) as a tracer. Sequences were determined manually from autoradiograms and aligned with the cytochrome *b* sequence of *Gallus gallus domesticus* (Desjardins and Morais, 1990). Sequence data were evaluated according to the maximum parsimony principle with the phylogeny program PAUP 3.1.1 (Swofford, 1993), which has been considered to be very reliable and useful for this purpose (Steward, 1993). In addition, a neighbour-joining analysis was employed as a distance method using the program package MEGA (Kumar *et al.*, 1993).

|                      |             |             |            |            |            |            |
|----------------------|-------------|-------------|------------|------------|------------|------------|
| <i>Gallus</i>        | CATAGCCCAC  | AAAGGCGGTG  | GCTATGAGTG | TGAGGAGGAG | GATTACTCCT | GTGTTTCAGG |
| <i>S. butleri</i>    | ??????????  | ??????????  | .....A..A. | .C..T..... | A..G..A... | .....      |
| <i>S. aluco</i>      | ??????????  | .....T      | .....A..G. | .C..T.AA.. | A..G..A... | .....      |
| <i>S. woodfordii</i> | ???.....T.. | G..A.....T  | .....A..A. | .C..C..... | A..G..A... | .....      |
| <i>Gallus</i>        | TTTCCTTGTA  | GAGGTAGGAG  | CCGTAGTATA | GGCCTCGTCC | GATGTGAGGG | AAGATACAGA |
| <i>S. butleri</i>    | ....T.....  | T.....      | .....      | ....A..... | ...A..T... | T....G..A. |
| <i>S. aluco</i>      | ....T.....  | C.....      | ..A.....   | .....      | .....T...  | T...C..... |
| <i>S. woodfordii</i> | ....T.....  | C.....      | .....      | .....      | .....T...  | T....G.... |
| <i>Gallus</i>        | TGAAGAGAGAA | TGAGGCGCCG  | TTTGCCTGGA | GATTCCGGAT | TAGTCAGCCG | TATTGTACGT |
| <i>S. butleri</i>    | .....G      | .....T...   | .....A.... | .G..G....G | .....A     | .....      |
| <i>S. aluco</i>      | .....G      | .....T...   | .....A.... | .G..A....  | G.....A... | .A.....A.  |
| <i>S. woodfordii</i> | .....A.G    | .....T..A   | .....A.... | .G..A..A.. | .....A     | .....A.    |
| <i>Gallus</i>        | TCCGGCAGGT  | GTGGGCTACG  | GAGGAGAGGG | CTAGGGATGT | GTCTGCTGTG | TAGTGCATGG |
| <i>S. butleri</i>    | .T.....G..  | .....AC..T  | ...TA...?  | .....TA.A  | ...G.....A | .....GGCA. |
| <i>S. aluco</i>      | .T.....G..  | A..T.A....  | ..T.T..... | .....TG.A  | A..G.....A | .....GG... |
| <i>S. woodfordii</i> | .T.....G..  | A.....AG.T. | ...TA..A.  | .....TA.A  | ...G.....A | .....GGC.. |
| <i>Gallus</i>        | CTAGTAGTAG  | GCCGGTGAGG  | ATTTGGGTCA | TGAGGCAGAC | TGCTAATAGG | AAGCCGAAT  |
| <i>S. butleri</i>    | .G..G..G..  | ...A....T.  | .....CT.   | .T.....T   | .C.C.TG... | ??.....??  |
| <i>S. aluco</i>      | .A..G..G..  | .....T.     | .....TG    | CT.A.....T | .C...G.... | G.....G.   |
| <i>S. woodfordii</i> | .G..G..G..  | .....T.     | .....G     | CT.....T   | .C...GC... | G.T.....G. |

Fig. 1. Partial nucleotide sequences of the cytochrome *b* gene of *Strix aluco*, *S. butleri* and *S. woodfordii*. The data correspond to positions 14846–15145 of the mitochondrial genome (Desjardins and Morais, 1990).

## Results and Discussion

DNA from *Strix aluco*, *S. butleri* and *S. woodfordii* was amplified by PCR using primers specific for the mitochondrial cytochrome *b* gene (Kocher *et al.*, 1989; Helbig *et al.*, 1993; Seibold *et al.*, 1993, 1994a,b; Wink *et al.*, 1993a,b, 1994; Wink and Wehrle, 1994). This gene was only partially sequenced since enough information could be obtained from a 300 bp fragment to answer the given taxonomic problems (Fig. 1): Within the genus *Strix* we found 49 variable sites, of which 40 were parsimony informative. When *Athene* and *Tyto* were included we obtained 103 variable and 53 informative sites. Most nucleotide substitutions occur in the third position of a codon, which do not lead to amino acid substitutions.

At least 2–3 specimens were analyzed for each taxon. Sequences of *S. woodfordii* showed no intraspecific variation, whereas those of *S. aluco* and *S. butleri* showed some geographic variation (Fig. 2). However, the degree of intraspecific variation which consisted of 4–8 base substitutions, was small as compared to the differences encountered between the sequences of *S. aluco*, *S. woodfordii* and *S. butleri* (substitution rate 9.3–13%) (Table I). Thus the DNA sequence data confirm that the differences in morphological and geographical characters between *S. aluco* and *S. butleri* (Table II) are indeed indicative for treating both taxa as distinct species. Both *S. butleri* and *S. woodfordii* replace *S. aluco* where they occur. They are allopatric and sequence differences suggest that these taxa were separated from a common ancestor probably several (approx. 5 to 6) million years ago (Table I, Fig. 1) assuming a constant molecular clock for mitochondrial genes (Quinn *et al.*, 1991, Wilson *et al.*, 1987).

As a next step we have analyzed the phylogenetic relationship between the *Strix* owls and two other members of the order Strigiformes; taxa selected (Heidrich *et al.*, 1994) are: Family Strigidae: *Athene noctua*; family Tytonidae: *Tyto alba*. Sequence data were evaluated by the maximum parsimony method employing exact algorithms ("Branch & Bound") and the neighbour-joining method using Kimura-2 and Jukes-Cantor distance algorithms. Both approaches resulted in trees with identical topology; the shortest tree was 145 steps long (minimal length possible, 123 steps).

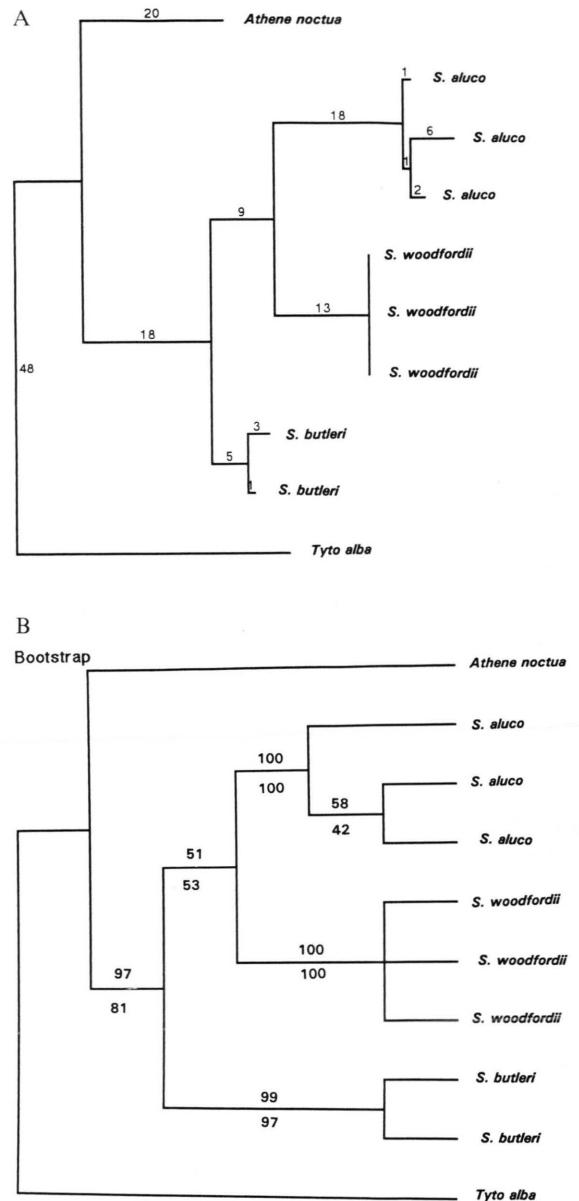


Fig. 2. Reconstruction of phylogenetic relationships of the genus *Strix* as compared to *Tyto alba* (family Tytonidae) and *Athene noctua* (family Strigidae). Tree topology was identical using parsimony and neighbour-joining methods. Percent bootstrap frequencies (200 replicates) are indicated for parsimony (branch & bound, equal character weights) below and for neighbour-joining search (Kimura 2-parameter distance) above each furcation.

A. Phylogram; numbers refer to nucleotide substitutions between taxa; branch length is proportional to substitution rates.

B. Cladogram; bootstrap values (in %) give confidence estimates for each furcation.

Table I. Pairwise genetic distances between sequences of the cytochrome *b* gene of *S. aluco* (3 individuals), *S. butleri* (2 individuals), *S. woodfordii* (3 individuals) and representative other species (Heidrich *et al.*, 1994). Above diagonal are mean values (1.0 = 100%), below diagonal absolute number of nucleotide exchanges.

|                            | 1  | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   |
|----------------------------|----|------|------|------|------|------|------|------|------|------|
| 1. <i>Athene noctua</i>    | —  | 0.20 | 0.20 | 0.20 | 0.16 | 0.15 | 0.20 | 0.20 | 0.20 | 0.23 |
| 2. <i>Strix aluco</i>      | 56 | —    | 0.03 | 0.01 | 0.12 | 0.11 | 0.11 | 0.11 | 0.11 | 0.23 |
| 3. <i>Strix aluco</i>      | 53 | 8    | —    | 0.03 | 0.12 | 0.11 | 0.11 | 0.11 | 0.11 | 0.24 |
| 4. <i>Strix aluco</i>      | 52 | 4    | 8    | —    | 0.12 | 0.11 | 0.10 | 0.10 | 0.10 | 0.25 |
| 5. <i>Strix butleri</i>    | 44 | 34   | 32   | 31   | —    | 0.01 | 0.09 | 0.09 | 0.09 | 0.23 |
| 6. <i>Strix butleri</i>    | 42 | 31   | 29   | 28   | 4    | —    | 0.09 | 0.09 | 0.09 | 0.22 |
| 7. <i>Strix woodfordii</i> | 54 | 31   | 28   | 25   | 26   | 26   | —    | 0.00 | 0.00 | 0.23 |
| 8. <i>Strix woodfordii</i> | 54 | 31   | 28   | 25   | 26   | 26   | 0    | —    | 0.00 | 0.23 |
| 9. <i>Strix woodfordii</i> | 54 | 31   | 28   | 25   | 26   | 26   | 0    | 0    | —    | 0.23 |
| 10. <i>Tyto alba</i>       | 63 | 66   | 63   | 64   | 65   | 63   | 66   | 66   | 66   | —    |

Table II. Comparison of morphological and other biological characters of *Strix aluco* and *Strix butleri* (Cramp, 1985; Glutz and Bauer, 1980).

| Character     | <i>Strix aluco</i>                   | <i>Strix butleri</i>         |
|---------------|--------------------------------------|------------------------------|
| Distribution  | resident Europe, Asia, North Africa  | resident Israel, Sinai       |
| Size          | 37–39 cm                             | 37–38 cm                     |
| Wing-span     | 94–104 cm                            | 95–98 cm                     |
| Head contours | broad, round head                    | broad, round head            |
| Plumage       | highly variable, 2 morphs            |                              |
| head          | grey-brown, pale cream-white stripes | buff-white, faintly marked   |
| wing-coverts  | dark brown, dull grey                | buff, mottled brown          |
| Eyes          | dark                                 | orange-yellow                |
| Voice         | differs from <i>S. butleri</i>       |                              |
| Habitat       | woodlands, parks                     | deserts, gorges with springs |

Fig. 2A illustrates a phylogram of the *Strix* complex, in which figures correspond to the number of nucleotide substitutions between species, whereas Fig. 2B represents a cladogram calculated by the “bootstrap” procedure (confidence values for the parsimony analysis are given below, those for the distance analysis above each furcation) (Swofford, 1993). Most furcations are well supported according to this bootstrap evaluation.

The analysis shows that the genus *Strix* represents a distinct clade. *S. aluco* could be closer related to *S. woodfordii* than to *S. butleri*, but the bootstrap values are not significant. *Athene noctua* is another “ear-less” owl, which is not related to the *Strix* group but to the *Glaucidium* complex (Heidrich *et al.*, 1994). *Tyto* (family Tytonidae) figures as an outgroup and differs by 22–25% nucleotide substitutions from the other taxa of the family Strigidae.

This first analysis is in good agreement with the current view of owl systematics as outlined in Sibley and Monroe (1990). Thus methods of molecular biology can be helpful to elucidate and interpret taxonomic and ecological problems which were a matter of dispute using traditional methods. In consequence, the molecular approach does not oppose traditional methods but rather complements them.

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