

THE BUZZARDS OF CAPE VERDE *Buteo (buteo) bannermani* AND SOCOTRA *Buteo (buteo) ssp.* : FIRST RESULTS OF A GENETIC ANALYSIS BASED ON NUCLEOTIDE SEQUENCES OF THE CYTOCHROME b GENE

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Les Buses du Cap Vert *Buteo (buteo) bannermani* et de Socotra *Buteo (buteo) ssp.* Premiers résultats d'une analyse des nucléotides du gène du cytochrome b.

L'origine des populations de buses qui sont isolées et sédentaires en situation symétrique par rapport au continent africain dans l'archipel du Cap Vert *Buteo (buteo) bannermani* et sur l'île de Socotra *Buteo (buteo) ssp.* a suscité deux hypothèses : 1) expansion en zone tropicale de la répartition des *Buteo* eurasiatiques à l'occasion d'un épisode glaciaire pléistocène qui aurait également conduit à l'installation de *Buteo oreophilus* dans les montagnes africaines. A Socotra cette colonisation aurait été facilitée par les migrations vers l'Afrique de *Buteo b. vulpinus* (MOREAU, 1966). 2) les peuplements des Buses du Cap Vert et de Socotra auraient une origine commune, portée avec la Buse féroce *Buteo rufinus*, à partir d'un stock ancestral de "Protobuteo" qui aurait occupé l'Afrique continentale avant la désertification du Sahara, au cours du Pléistocène, (de NAUROIS 1973, 1994). Dans cette hypothèse reprise par HAZEVOET (1995) et MARTINS & PORTER (1996) les populations insulaires auraient une histoire évolutive beaucoup plus ancienne et leur isolement serait antérieur à la différenciation des taxons polarctiques et afro-tropicaux tels *vulpinus* ou *oreophilus*. Certains caractères écomorphologiques des Buses du Cap Vert et de Socotra les distinguent de *Buteo buteo* : absence ou faible variabilité du plumage, éléments morphométriques plus proches de *Buteo rufinus*, situation exclusivement rupestre des aires, période de reproduction de novembre à janvier-février.

Nous avons analysé les séquences de nucléotides du gène du cytochrome b de 6 espèces de *Buteo* afin de contribuer à préciser l'origine et la position taxinomique de deux buses insulaires.

L'arbre phylogénétique (FIG. 1) met en évidence la communauté d'origine de *Buteo rufinus* et *B. hemilasius*. Les Buses du Cap Vert et de Socotra appartiennent au même groupe que *B. rufinus* ce qui suggère que les deux formes insulaires ont une affinité plus ancienne avec *B. rufinus* qu'avec *B. buteo*. Les distances génétiques sont relativement faibles au sein du complexe *buteo/rufinus*. *Buteo bannermani* et *B. socotrae* ont des séquences de nucléotides identiques, et diffèrent de 0,18 % de *B. rufinus* et de 0,5-0,7 % de *B. b. vulpinus* ou *B. b. buteo*. En se référant à une horloge moléculaire de 2 % de divergence de nucléotides par millions d'années, le complexe des buses européennes et africaines apparaît relativement jeune et aurait évolué au cours des derniers 300 000 ans. La divergence entre *rufinus* et *bannermani/socotrae* aurait pu ainsi débiter il y a 90 000 ans.

Avec les réserves qu'impose un échantillonnage aussi limité, ces premiers résultats confirment donc l'affinité des Buses du Cap Vert et de Socotra entre elles et avec *Buteo rufinus*. Ils montrent la distinction de ces trois éléments de l'ensemble des taxons paléarctiques (y compris la Buse des Açores *Buteo buteo rothschildi* et *Buteo b. vulpinus*) et africain : *Buteo (b.) oreophilus* dont la proximité avec *Buteo buteo* avait déjà été mise en évidence par WINK. Cette analyse apporte cependant une première confirmation à l'hypothèse de NAUROIS et constitue un argument à la décision d'HAZEVOET d'élever *Buteo bannermani* au rang d'espèce. Ces résultats incitent, suivant le concept d'espèce phylogénétique, à proposer de conférer un statut identique à la Buse de Socotra qui pourrait être également nommée *bannermani* (en raison de l'identité des séquences de nucléotides) ou *Buteo socotrae* du fait de l'éloignement et de l'isolement des deux populations insulaires.

Mots clés : *Buteo*, Cap-Vert, Gène du cytochrome b, Phylogéographie intraspécifique, Socotra

Key words : *Buteo*, Cap Verde, Cytochrome b gene, Intraspecific phylogeography, Socotra

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INTRODUCTION

The origins of isolated sedentary buzzard populations on either side of the African continent: the Cape Verde archipelago *Buteo (buteo) bannermani* and the island of Socotra *Buteo (buteo)* spp., off the southern edge of their continuous palearctic distribution have led to the formulation of two hypotheses:

- MOREAU (1966) considered that there had been an expansion into the tropical regions by Eurasian *Buteo* populations during a Pleistocene ice age which would have also led to *Buteo oreophilus* becoming established in the African mountains. On Socotra, this colonisation would have been facilitated by migrations, towards Africa, of *Buteo b. vulpinus*. A study of the plumage of individuals from Socotra (FROST & SEIGFREID, 1970) reported characteristics intermediate between those of *Buteo oreophilus* and *Buteo b. vulpinus* while retaining a closer resemblance to the latter.

- According to de NAUROIS (1973, 1994), the buzzard populations of Cape Verde and Socotra have a common origin, shared with *Buteo rufinus*, which he suggests arose from ancestral stock of "Proto**buteo**" occupying Continental Africa before the formation of the Sahara desert, during the Pleistocene. In this hypothesis, also reported by HAZEVOET (1995) and MARTINS & PORTER (1996) the insular populations could represent a separate evolutionary lineage which became isolated prior to the separation of the Palearctic and Afro-tropical taxa such as *oreophilus* and *vulpinus*.

Some ecomorphological characteristics differentiate the insular buzzards from the Eurasian *Buteo buteo*. Little plumage variation typifies the two populations (de NAUROIS 1973; HAZEVOET, 1995; MARTINS & PORTER, 1996; CLOUET *et al.*, 1998). Certain plumage characteristics and morphological criteria in the Cape Verde birds are very similar to those of *Buteo rufinus cirtensis* (de NAUROIS, 1973). All the eyries found, both on Socotra (OGILVIE-GRANT & FORBES, 1903; CLOUET *et al.*, 1994, 1998; MARTINS & PORTER, 1996) and on Cape Verde (except one case: de NAUROIS, 1973), were on cliff faces. The laying period takes place in November-December on Socotra and December-February on

Cape Verde *i. e.* a very different period from that of Eurasian birds. Finally, from a behavioural point of view, the Socotra birds were found to be very aggressive (CLOUET *et al.*, 1998).

With the aim of proposing a new element in the discussion on the origin and taxonomy of the two insular buzzards the present genetic study was performed on an individual from Cape Verde and one from Socotra and contributes to the richly documented phylogenetic study of WINK (1998).

We have analyzed the mitochondrial cytochrome b gene which is a good marker for the reconstruction of bird phylogenies especially at the genus and family level (AVISE, 1994; HEIDRICH *et al.*, 1998, MINDELL, 1997, SEIBOLD *et al.*, 1996, WINK, 1995, 1998; WINK *et al.*, 1996, 1998). We have analyzed sequences of 6 *Buteo* taxa; from *Buteo buteo* we have included samples from Germany, France and Portugal (*B. b. buteo*), and from the Azores (*B. b. rothschildi*) in order to assess the systematic status of the two island buzzards (*B. bannermani* and *B. socotrae* in the text).

MATERIALS AND METHODS

Origin of DNA, PCR and DNA-Sequencing

Blood and tissues were either preserved in an EDTA buffer or in ethanol (WINK, 1998) and stored at -20°C until processing. DNA was extracted using the proteinase K protocol. The mitochondrial cytochrome b gene was amplified by PCR using primers MT-A3 ctcccagcccatccacatctcagcatgatgaactctgc and MT-F2 ctaagaagggtgagctctca gtttttggttacaagacaaatg. PCR products were sequenced directly using the dideoxy chain termination method with the cycle sequencing Kit (Amersham Life Science, RPN 2438/RPN 2538) in combination with internal CY5 labelled primers. For cycle sequencing a two stage program containing an initial denaturing step at 94°C for 4 min and 25 cycles at 60°C (40 sec), and 94° (30 sec) was used. The primers employed were MT-C2-CY xgaggacaatatcattctgagg, MT-U2-CY xggggtgagagtttctgggtc, MT-C4-CY xagtgtgggtgtctactga, and MT-U1-CY xtccmggctcaacaacccttagg. Radioactive fragments were separated on a PAGE gel apparatus (Stratagene, Base Ace Sequencer) while CY5 labelled fragments were analysed on an

automated Sequencer (Pharmacia, ALF- Express II). Sequences of > 900 nt were obtained directly from ALF-Express and aligned.

Sequences of ca. 700 nt were used for this analysis, employing PAUP* with Maximum Parsimony (MP), Neighbour Joining (NJ), and Maximum Likelihood (MLE) to reconstruct phylogenetic trees. More details on the methodology can be found in WINK (1998, 1999) and HEIDRICH *et al.* (1998).

RESULTS AND DISCUSSION

Phylogenetic reconstructions based on MP, NJ, and MLE produced trees of almost identical topology. A Maximum Likelihood tree is shown in Figure 1 which reflects the phylogenetic relationships in Eurasian and African buzzards. *Buteo rufinus* and *B. hemilasius* share common ancestry, as assumed by DEL HOYO *et al.* (1994) or SIBLEY & MONROE (1990).

B. bannermani and *B. socotrae* cluster in the same group with *B. rufinus* suggesting that both island species share a closer ancestry with *B. rufinus* than with *Buteo buteo*, as has been suggested by most handbooks (DEL HOYO *et al.*, 1994; SIBLEY & MONROE, 1990). This finding supports the view of de NAUROIS (1973, 1994) and HAZEVOET (1995) who had favoured a closer relationship with *B. rufinus*.

Genetic distances are quite small within the *buteo/rufinus* complex. *B. bannermani* and *B. socotrae* have identical nucleotide sequences and differ by 0.18% from *B. rufinus* and 0.5-0.7% from *B. b. vulpinus* or *B. b. buteo*. Assuming a molecular clock with 2% nucleotide divergence in 1 million years (TARR & FLEISCHER, 1993; WILSON *et al.*, 1987), we can speculate that the European/African buzzard complex is relatively young and evolved during the last 300,000 years. The divergence between *rufinus* and *bannermani/socotrae* could have started approximately 90,000 years ago.

With all the reservations imposed by such a limited sample size, the first results tend to confirm the close similarity between buzzards from Cape Verde and Socotra and also between them and *B. rufinus*, which breeds on the African mainland, on the Arabian peninsula, and in the near East. These three buzzard taxa appear distinct from the

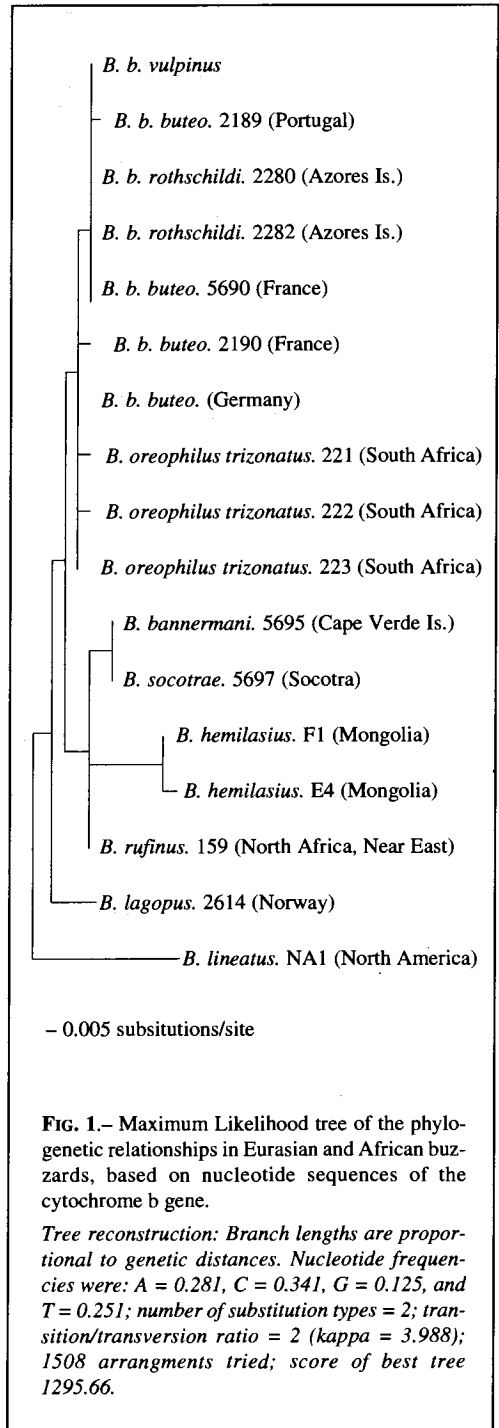


FIG. 1.— Maximum Likelihood tree of the phylogenetic relationships in Eurasian and African buzzards, based on nucleotide sequences of the cytochrome b gene.

Tree reconstruction: Branch lengths are proportional to genetic distances. Nucleotide frequencies were: A = 0.281, C = 0.341, G = 0.125, and T = 0.251; number of substitution types = 2; transition/transversion ratio = 2 ($\kappa = 3.988$); 1508 arrangements tried; score of best tree 1295.66.

Eurasian Buzzard and its subspecies (including the Azores Buzzard *B. b. rothschildi* and *B. b. vulpinus*) and the African taxa *B. oreophilus trizonatus*. The close relatedness between *B. oreophilus* and *B. buteo* has already been reported by WINK (1998) and WINK & SAUER-GÜRTH (1999).

A larger sample would be necessary to confirm this preliminary phylogeographic outline. However, this result provides a first confirmation of the hypothesis of de NAUROS (1973, 1994) and a support for HAZEVOET's proposal to consider *B. bannermani* as a distinct species according to the phylogenetic species concept (HAZEVOET 1995). If the buzzard of Cape Verde is treated as a distinct species, our findings imply that the buzzard of Socotra should obtain the same status and could be named *bannermani* (because of the identical nucleotide sequence) or alternatively *Buteo socotrae* owing to its isolated and remote situation.

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REFERENCES

- AVISE (J. C.) 1994.— *Molecular markers, natural history and evolution*. Champan and Hall, London.
- CLOUET (M.), CANU (J. G.) & LIPINSKI (F.) 1994.— Sur la nidification de la Buse de Socotra *Buteo buteo* ssp. *Alauda*, 62: 144-145. • CLOUET (M.), GOAR (J. L.) & BARRAU (C.) 1998.— Contribution à l'étude ornithologique de l'île de Socotra. *Alauda*, 66: 235-246.
- DEL HOYO (J.), ELLIOTT (A.), SARGATAL (J.) 1994 - *Handbook of the birds of the world*. Vol. 2, Lynx Edition, Barcelona.
- FROST (P. G. H.) & SEIGRIED (W. R.) 1970.— Notes on the plumage of Buzzards from Socotra. *Bull. Brit. Orn. Club*, 90: 136-142.
- HAZEVOET (C.) 1995.— *The birds of the Cape Verde Islands*. B. O. U. Check list 13. B. O. U. Herts. • HEIDRICH (P.), AMENGUAL (J.) & WINK (M.) 1998: Phylogenetic relationships in Mediterranean and North Atlantic *Puffinus* Shearwaters (Aves: Procellariidae) based on nucleotide sequences of mtDNA. *Biochemical Systematics and Ecology*, 26: 145-170.
- MARTINS (R. P.) & PORTER (R. F.) 1996.— The *Buteo* population in Socotra. *Sandgrouse*, 17: 134-137.
- MINDELL (D. P.) 1997.— *Avian molecular evolution and systematics*. Academic Press, San Diego.
- MOREAU (R. E.) 1966.— *The birds faunas of Africa and its islands*. Academic Press, New-York.
- NAUROS (R. de) 1973.— Recherches sur la Buse (*Buteo buteo* L.) de l'Archipel du Cap Vert. *Livro de Homenagen oa Professor Fernando Frade Viegas da Costa*. Lisbonne. • NAUROS (R. de) 1994.— *Les Oiseaux de l'Archipel du Cap Vert*. Instituto de Investigação Científica Tropical. Lisbonne.
- OGILVIE-GRANT (W. R.) & FORBES (H. O.) 1903.— Birds of Socotra. In: Forbes (H. O.) *The Natural History of Socotra and Abd-el-Kuri*. Porter. London.
- RIPLEY (S. D.) & BOND (G. M.) 1966.— The Birds of Socotra and Abd-el-Kuri. *Smithsonian Misc. Coll.*, 151: 1-37.
- SEIBOLD (I.), HELBIG (A. J.), MEYBURG (B. U.), NEGRO (J.) & WINK (M.) 1996.— Genetic differentiation and molecular phylogeny of European Aquila eagles according to cytochrome b nucleotide sequences. In: B. U. Meyburg & R. Chancellor (eds). *Eagle studies*. WWGBP. Berlin, London & Paris. • SEIBOLD (I.), HELBIG (A.) & WINK (M.) 1993.— Molecular systematics of falcons (family Falconidae). *Naturwissenschaften*, 80: 87-90. • SIBLEY (C. G.) & MONROE (B. L.) 1990.— *Distribution and taxonomy of birds of the world*. Yale University Press, New Haven.
- TARR (C. L.) & FLEISCHER (R. C.) 1993.— Mitochondrial DNA variation and evolutionary relationships in the amakihi complex. *Auk*, 110: 825-831.
- WILSON (A. C.), OCHMAN (H.) & PRAGER (E. M.) 1987.— Molecular time scale for evolution. *Trends Genetics*, 3: 241-247. • WINK (M.) 1995.— Phylogeny of Old and New World vultures (Aves: Accipitridae and Cathartidae) inferred from nucleotide sequences of the mitochondrial cytochrome b gene. *Z. Naturforsch.*, 50c: 868-882. • WINK (M.) 1998.— Application of DNA-markers to study the ecology and evolution of raptors. In: R. D. CHANCELLOR, B. U. MEYBURG & J. J. FERRERO. (eds). *Holarctic Birds of Prey*. Adenex & WWGBP. Berlin & Mérida. • WINK (M.) & SAUER-GÜRTH (H.) 1999.— Advances in the molecular systematics of African Raptors. *Proc. WWGPB*. South Africa. • WINK (M.), HEIDRICH (P.) & FENTZLOFF (C.) 1996.— A mtDNA phylogeny of sea eagles (genus *Haliaeetus*) based on nucleotide sequences of the cytochrome b gene. *Biochemical Systematics and Ecology* 24: 783-791. • WINK (M.), SEIBOLD (I.), LOTFIKHAH (F.) & BEDNAREK (W.) 1998.— Molecular systematics of Holarctic Raptors (Order Falconiformes). In: R. D. CHANCELLOR, B. U. MEYBURG & J. J. FERRERO (eds). *Holarctic Birds of Prey*. Adenex-WWGBP. Berlin & Mérida.