Towards a molecular systematics of the genus *Criniger*, and a preliminary phylogeny of the bulbuls (Aves, Passeriformes, Pycnonotidae)

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Pasquet É., Han L.-X., Khobkhet O. & Cibois A. 2001. — Towards a molecular systematics of the genus *Criniger*, and a preliminary phylogeny of the bulbuls (Aves, Passeriformes, Pycnonotidae). *Zoosystema* 23 (4): 857-863.

ABSTRACT
The analysis of DNA fragments of two mitochondrial genes (12S and 16S) from 27 species of bulbuls (Pycnonotidae), belonging to eight African and Asiatic genera, demonstrates the polyphyly of the genus *Criniger* Temminck, 1820, with two clades: one with the African species, the other with the Asiatic species. We propose to maintain *Criniger* for the African species and use *Alophoixus* Oates, 1889 for the Asiatic ones. This study also brings the first elements on the monophyly of African bulbuls, with the exception of *Pycnonotus* species which are of probable recent diversification in Africa.

KEY WORDS
Aves,
Passeriformes,
Pycnonotidae,
Criniger,
phylogeny,
mitochondrial genes,
molecular systematics.

RÉSUMÉ

Vers une systématique moléculaire du genre Criniger *et une phylogénie préliminaire des bulbuls (Aves, Passeriformes, Pycnonotidae).*

L'analyse de fragments d'ADN de deux gènes mitochondriaux (12S et 16S) de 27 espèces de bulbuls (Pycnonotidae), appartenant à huit genres africains et asiatiques, a permis de démontrer la polyphylie du genre *Criniger* Temminck, 1820, formé de deux clades, l'un comprenant les espèces asiatiques, l'autre les espèces africaines. Il est proposé de conserver *Criniger* pour les espèces africaines et d'utiliser *Alophoixus* Oates, 1889 pour les asiatiques. Cette étude apporte aussi des éléments permettant de formuler l'hypothèse de la monophylie des bulbuls africains, si l'on exclut les espèces du genre *Pycnonotus*, probablement diversifiées récemment en Afrique.

MOTS CLÉS
Aves,
Passeriformes,
Pycnonotidae,
Criniger,
phylogénie,
gènes mitochondriaux,
systématique moléculaire.

INTRODUCTION

Bulbuls (Pycnonotidae Gray, 1840) are tropical forest birds distributed in Africa and Asia. Among some 15 genera, only two, *Pycnonotus* Boie, 1826 and *Criniger* Temminck, 1820, are common to both continents. All African *Pycnonotus* (superspecies *P. barbatus* Hall & Moreau, 1970) seem clearly related to the Arabian species (*P. xanthopygos* [Ehrenberg, 1833], *P. leucogenys* [Gray, 1835]) and to some other Asiatic species (e.g., *P. cafer* [Linné, 1766], *P. aurigaster* [Vieillot, 1818], *P. jocosus* [Linné, 1758], *P. xanthorrhous* Anderson, 1869) (Keith 1992). Conversely, the five African and six Asiatic bearded bulbuls of the genus *Criniger* (*sensu* Rand & Deignan 1960) are now more and more seen as making an incongruous genus (Hall & Moreau 1970; Sibley & Monroe 1990; Inskipp *et al.* 1996). As here used, in the broad sense, the generic name *Criniger* is better characterised by its conspicuous throat patch with long feathers and by their numerous and long hair-like feathers on the nape, than by the general shape of the bill – strong, high and hooked – or the plumage – very long and soft. These African and Asiatic species were considered congeneric for some 30 years (Delacour 1943; Rand & Deignan 1960), before Hall & Moreau (1970) proposed to "[…] dissociate the Asiatic species [of *Criniger*] from any African genus […]", arguing that it is "[…] misleading to consider the [African *Criniger* species] congeneric with a handful of Asiatic bulbuls […]". To test this hypothesis and help elucidate the relationships between *Criniger* species, we used molecular data (part of the 12S and 16S RNA mitochondrial genes) from 27 species of bulbuls (Table 1). Three other species were used as outgroups: *Tyrannus melancholicus* Vieillot 1819, *Corvus corone* Linné, 1758 and *Coracina melaschitos* (Hodgson, 1836). Outgroup species were limited intentionally, as the aim of this study was not to test the monophyly of the Pycnonotidae family; another study is in preparation on that topic, which will integrate more Sylvioid species (*sensu* Sibley & Alhquist 1990). In addition to certain conclusions regarding the genus *Criniger*, the present study proposes a first, although preliminary, phylogenetic view of the relationships between African and Asiatic bulbul genera.

MATERIAL AND METHOD

Table 1 gives the list of the bird samples used in this study and their origin.

Total genomic DNA was extracted from preserved tissues using the Dneasy Tissue Kit (Qiagen). Polymerase Chain Reaction (PCR) were carried on for 35 cycles at annealing temperature of 50-53 °C. We used the primers L1319 (5'- AAA GAC TTA GTC CTA ACC TTA C -3'), and H2250 (5'- CTA AGT GCA CCT TCC

Table 1. — List of samples used in this study, and their origin. Abbreviations: **AMNH**, American Museum of Natural History; **ANSP**, Academy of Natural Sciences in Philadelphia; **FMNH**, Field Museum of Natural History; **MNHN**, Muséum national d'Histoire naturelle; **1**, cf Chappuis & Érard 1993.

Species	Origin	Collection and Number	Genbank numbers	
			12S	16S
Tyrannus melancholicus	South America	MNHN, n°12-33	AF386462	AF135058
Coracina melaschitos	Laos	MNHN, n°6-69	AF386464	AF391229
Corvus corone	France	MNHN, n°13-16	AF386463	AF094643
Bleda notata[1]	Nditam, Cameroon	MNHN, n°2-13	AF386474	AF391203
Bleda syndactyla	“	MNHN, n°1-02	AF386466	AF391204
Ixonotus guttatus	Ebogo, Cameroon	MNHN, n°3-03	AF386470	AF391208
Baeopogon indicator	Ngambé, Cameroon	MNHN, n°2-49	AF386465	AF391205
Andropadus latirostris	“	MNHN, n°2-52	AF386467	AF096477
Andropadus virens	Nditam, Cameroon	MNHN, n°2-01	AF386477	AF391214
Phyllastrephus icterinus	Somalomo, Cameroon	MNHN, n°E-22	AF386469	AF391207
Phyllastrephus albigularis	Nditam, Cameroon	MNHN, n°1-42	AF386476	AF391213
Criniger chloronotus	Ituri, Zaire	FMNH, n°3774	AF386487	AF391224
Criniger ndussumensis	Mbouma, Cameroon	MNHN, n°3-10	AF386472	AF391210
Criniger calurus	Republic of Central Africa	AMNH, n°PB222	AF386483	AF391220
Criniger olivaceus	Liberia	AMNH, n°8275	AF386488	AF391225
Criniger phaeocephalus	Sabah, Borneo	ANSP, n°1058	AF386491	AF391228
Criniger bres	“	ANSP, n°1056	AF386490	AF391227
Criniger flaveolus	Umphang, Thailand	MNHN, n°4-5M	AF386478	AF391215
Criniger ochraceus	Khao Chong, Thailand	MNHN, n°1989-90	AF386482	AF391219
Criniger pallidus	Korat, Thailand	MNHN, n°4-4I	AF386471	AF391209
Hypsipetes criniger	Sabah, Borneo	ANSP, n°1179	AF386489	AF391226
Hypsipetes propinquus	Umphang, Thailand	MNHN, n°4-4D	AF386475	AF391212
Hypsipetes mcclellandii	“	MNHN, n°4-4H	AF386468	AF391206
Hypsipetes leucocephalus	Gaoligong Shan, Yunnan	MNHN, n°15-60	AF386481	AF391218
Hypsipetes phillipinus	Mindanao, Philippines	FMNH, n°6590	AF386486	AF391223
Pycnonotus finlaysoni	Umphang, Thailand	MNHN, n°4-3I	AF386473	AF391211
Pycnonotus atriceps	Korat, Thailand	MNHN, n°4-3C	AF386484	AF391221
Pycnonotus jocosus	Umphang, Thailand	MNHN, n°4-4B	AF386480	AF391217
Pycnonotus barbatus	Yaoundé, Cameroon	MNHN, n°2-21	AF386479	AF391216
Pycnonotus xanthorrhous	Gaoligong Shan, Yunnan	MNHN, n°14-19	AF386485	AF391222

GGT -3') for the 12S, and the primers L3214 (5'- CGC CTG TTT ATC AAA AAC AT -3', Hedges 1994) and H3783 (5'- CCG GTC TGA ACT CAG ATC ACG T -3', Hedges & Sibley 1994) for the 16S, which delimit portions of respectively 930 and 513 DNA bases in the chicken complete mitochondrial genome (Desjardin & Morais 1990). PCR were purified using the Qiaquick Gel Extraction Kit (Qiagen). Sequencing reactions were performed in both directions, with both external and, for 12S only, internal primers designed from derived sequences, using the CEQ Dye Terminator Cycle Sequencing kit (Beckman). Sequences were obtained by capillary electrophoresis using the Beckman CEQ 2000 automatic sequencer. Sequences were managed with MUST package (Philippe 1993). The alignment of the dataset of the 12S and 16S sequences, taken as a whole, was made using POY (Gladstein & Wheeler 1996), which is more efficient than the previously used MALIGN (Gladstein & Wheeler 1994), as, allowing for heuristic search and branch swapping, it optimises directly the length of the sequences and gives a shortest tree (Wheeler 1996); the options used were gap 3, random 25, multibuild 25, slop 5, checkslop 10. The resulting aligned dataset was 1404 bases long. A manual alignment, keeping to the minimum the number of introduced gaps, gave a length of 1379 bases (respectively 864 bases for 12S and 515 bases for the 16S). The analysis of

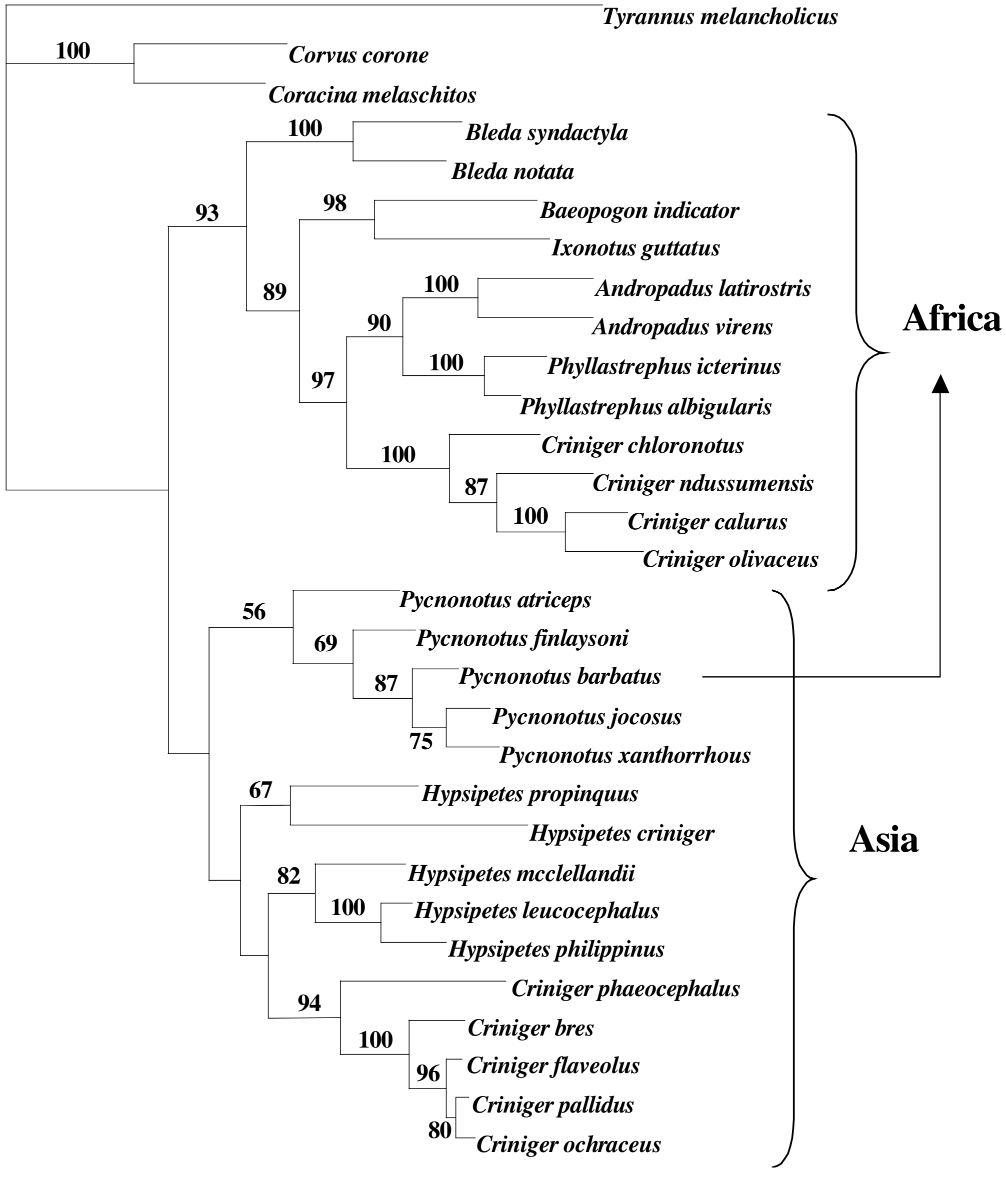

FIG. 1. — Most-parsimonious phylogenetic tree (length= 1595, CI= 0.495, RI= 0.574), obtained with PAUP* on combined partial 12S and 16S sequences, aligned with POY. Values indicate percentages of bootstrap replicates, when > 50%.

transition/transversion ratio reveals no saturation phenomena (not shown).

Neighbour-Joining (NJ) and Maximum-Parsimony (MP) topologies were obtained with PAUP* (Swofford 1999), respectively with uncorrected distances (NJ) and using the heuristic algorithm, TBR swapping algorithm, with 100 random addition-sequence replicates (MP). 1000 bootstraps were performed in both analyses. The polyphyly of the genus *Criniger* was evaluated by the test of Kishino-Hasegawa (1989).

RESULTS

The POY aligned dataset of 1404 bases long gives 578 variable sites, 398 of them being informative from the phylogenetic point of view. Both analyses, NJ and MP, lead to exactly the same unique most parsimonious phylogenetic topology (Fig. 1). Similar topology also is obtained from the manually aligned dataset.

A large part of the tree topology (Fig. 1) appears well-structured, with nodes supported with high bootstrap values. A large clade unites all the African bulbuls studied, except the African *Pycnonotus barbatus* (Desfontaines, 1789) which is placed with the other species of the genus *Pycnonotus*. All internal nodes inside this African clade are also well-supported. The genera *Phyllastrephus* Swainson, 1831, and *Andropadus* Swainson, 1832, are the first united and the following other genera appear in more basal successive paraphyletic positions: the African *Criniger* first, then a clade formed by *Baeopogon* Heine, 1860, and *Ixonotus* Verreaux, 1851, and finally *Bleda* Bonaparte, 1857, at the base of the clade. Inside this African *Criniger* clade, *C. calurus* (Cassin, 1857), and *C. olivaceus* (Swainson, 1837), are the closest species, *C. chloronotus* (Cassin, 1860), being the basal species.

The group of the five Asiatic *Criniger* species is also well-supported, but is very distantly placed from the African *Criniger*. The Kishino-Hazegawa test gives a high confidence (< 0.0001) to the splitting of the genus *Criniger* into the two continental entities. Inside this Asiatic *Criniger* clade, three species (*C. pallidus* Swinhoe, 1870, *C. ochraceus* Moore, 1854, *C. flaveolus* [Gould, 1836]) are very closely related, *C. bres* (Lesson, 1832) being more distantly related and *C. phaeocephalus* (Harlaub, 1844), the basal most species.

The five *Pycnonotus* form a clade, which though not well-supported at base, has its two most internal nodes with bootstrap proportions higher than 75%. The last genus studied, *Hypsipetes* Vigors, 1831, does not form a single clade: its species are distributed in two groups, one relatively well-supported comprises the *Hypsipetes mcclellandii* Horsfield, 1840, *H. leucocephalus* (Gmelin, 1789) and *H. philippinus* (Forster, 1795). Although useful in a preliminary way, no basal structured relationships between Asiatic groups can yet be proposed.

DISCUSSION

The main result of this study is the strong evidence for the polyphyletic grouping of the classical genus *Criniger*. African and Asiatic species of this genus are very well-separated. A taxonomic distinction is here fully justified and indeed Hall & Moreau (1970) wrote : "The Asiatic species usually placed in *Criniger* show as wide a variation in colour, pattern, size and shape of tail as that between many of the African genera, and are united chiefly by having a conspicuously coloured throat. Among African bulbuls this character is not confined to *Criniger* and does not necessarily indicate close relationships. We believe it would be desirable to dissociate the Asiatic species from any African genus: the name *Alophoixus* Oates, 1889 is available. The alternative would be to consider most of the African bulbuls congeneric which, while avoiding difficult and possibly erroneous decisions, does not seem very desirable".

The generic name *Criniger* Temminck, 1820 must remain linked to its type species, the African *C. barbatus*, because of the Principle of Typification (ICZN 1999: Art. 61) (*Trichophorus barbatus* Temminck, 1821, having been properly designated as the type species of the genus *Criniger* Temminck, 1820 and was, until recently, considered as conspecific with *C. chloronotus*); it is therefore necessary to use another name for the Asiatic species. Comments by Hall & Moreau (1970) are twofold and suggest: 1) to limit the genus *Criniger* to the African bearded bulbuls, which is clear and immediately feasible; and 2) to use *Alophoixus* for the Asiatic *Criniger*, a proposition which would have required more taxonomic assessment. These taxonomic suggestions were both used without any comments by Sibley & Monroe (1990), followed by Inskipp *et al.*

(1996). *Alophoixus* Oates, 1889 was originally used to separate the Grey-headed bearded bulbul (named Crestless White-throated Bulbul in Oates' work) *Criniger phaeocephalus* (Hartlaub, 1844) from the other species (Asiatic and/or African?). Indeed Oates (1889) did not state in his text to what other species he referred to: "It differs from that genus in entirely wanting a crest, a character of sufficient importance, in my estimation, to warrant its separation from *Criniger*, in which genus the crest is remarkably long and conspicuous". Wolters (1979) was well aware of this distinction, restricting *Alophoixus* to the species *C. phaeocephalus*, and he placed the other Asiatic *Criniger* in two other unnamed subgenera, the first one comprising all the *Criniger* species that we sampled (*C. bres*, *C. flaveolus*, *C. ochraceus* and *C pallidus*), and the second comprising only *C. finschii* Salvadori, 1871, which is morphologically very different. Without explanation, Sibley & Monroe (1970) also included *Criniger affinis* Hombron & Jacquinot, 1841 (= *Hypsipetes affinis* sensu Rand & Deignan 1960) into the genus *Alophoixus*. Unfortunately we could not sample *C. finschii* and *C. affinis* (Table 1). The present molecular study shows that our five Asiatic *Criniger* form a clade, and that it would be possible to use *Alophoixus* for these species. Alternatively, because of the basal position of *C. phaeocephalus* within this clade, it would be also possible to keep *Alophoixus* for *C. phaeocephalus* alone (as did Wolters) and introduce a new genus name for the four other species which are actually morphologically and genetically closely related. In our opinion, the morphological difference (absence of crest) that led Oates (1889) to distinguish *C. phaoeocephalus* from the other *Criniger* is not so important; a rapid study of skin measurements of these species (unpublished data) reveals that *C. phaeocephalus* differs mostly by its isometric smaller size. The definition of a genus being mainly a matter of opinion, we recommend the use of *Alophoixus* for all the Asiatic *Criniger* studied here, unless elaborated morphological study proposes alternative results. Recognition of a new genus would lead to an unjustified over-splitting of the classification. Tentatively, we propose to include also *C. finshii* in *Alophoixus* , but to keep *C. affinis* in genus *Hypsipetes,* until molecular work gives results concerning these species.

The monophyly of the African species (excepting *Pycnonotus*) is well-supported, with the most basal species of the clade being the very distinctive species of the genera *Bleda*, *Baeopogon* and *Ixonotus,* but the monophyly of the Asiatic genera is not; this phylogeny certainly needs to be studied with more Pycnonotid genera and species including outgroups from various related families. Nevertheless, the hypothesis of separate radiations in both continents is now conceivable; it has never been proposed before, neither in the pattern of relationships suggested by Delacour (1943) nor in the phylogenetic work of Sibley & Ahlquist (1990). The relationship of *Pycnonotus barbatus,* falling well inside the clade of the other *Pycnonotus* studied, is different. In contrast of the other African species studied which are forest birds, *Pycnonotus* comprises mostly species of open areas (Kalyakin 1999) and is represented in Asia by 33 species (Inskipp *et al.* 1996). It is probably a recent genus in Africa, linked to the Asiatic species by the Middle East and the Arabian peninsula.

Acknowledgements

We are grateful to colleagues who provided tissues: P. Beresford (American Museum of Natural History), D. Willard and J. Bates (Field Museum of Natural History), N. Rice and L. Joseph (Academy of Natural Sciences in Philadelphia), B. Slikas (National Zoological Park, Smithsonian Institution) and F. Sheldon (Louisiana State University). The various field works were supported by Kasetsart University, Bangkok, Thailand by the "Programme pluri-formations Asie du Sud-Est" and by the "Réseau national de biosystématique" (ACC-SV7). The molecular work was conducted at, and supported by, the Service de Systématique moléculaire, CNRS-FR1541, MNHN. This is a publication of the "EA 2586: Systématique et évolution des vertébrés tétrapodes".

We are also grateful to E. Dickinson, who suggested this work, offered some useful advice and reviewed the text, and to Ch. Érard for comments on a previous draft.

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Submitted on 7 March 2001;
accepted on 6 June 2001.