

A phylogeny of cockatoos (Aves: Psittaciformes)  
inferred from DNA sequences of the seventh intron of nuclear  $\beta$ -fibrinogen gene

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According to Forshaw (1989), cockatoos belong to subfamily Cacatuinae; family Psittacidae; order Psittaciformes. The subfamily Cacatuinae consists of 21 species of six genera (*Calyptorhynchus*, *Probosciger*, *Nymphicus*, *Callocephalon*, *Eolophus* and *Cacatua*) of three tribes (Calyptorhynchini, Calopsittacini and Cacatuini). They are distributed in the Pacific region including Australia and eastern part of Indonesia. Some previous authors have made grouping and evolutionary relationships of cockatoos based on morphological characters (e.g. Smith, 1975), isozyme (Adam *et al.*, 1984), and mitochondrial 12S ribosomal small subunit (Brown and Toft, 1999). However, their relationships are still controversial, especially concerning the position of *Nymphicus*. Since the nuclear  $\beta$ -fibrinogen gene has been recognized useful for phylogenetic studies of some birds (e.g. Pritchko and Mooers, 1997; 2000; Johnson and Clayton, 2000; 2000a; Pritchko and William, 2003;), this study employed the DNA sequence of seventh intron of this gene (Fibint7) to construct phylogenetic relationships of cockatoos.

DNA was extracted from blood of 15 cockatoo species (6 genera, 3 tribes). A single fragment of Fibint7 was amplified using nucleotide primers FIB-B17U and FIB-B17L (Pritchko and Moore, 1997), in PCR condition of 94 °C at 5 minute, 35 cycles of [94 °C - 30 sec., 46 °C - 30 sec., 72 °C - 60 sec.]. Amplified fragments were purified

using PEG (Poly ethylene glycol) method. Direct sequencing was performed to obtain the sequence data from each individual of cockatoos. DNA sequence data were aligned using DNASIS software. Sequence characteristics, including base composition, base deletions and insertions (indels) and saturation of substitutions were analyzed from the sequence data. Phylogenetic trees were constructed by neighbor-joining (NJ) and maximum-parsimony (MP) methods.

Several indels were found in the sequence data of Fibint7 in cockatoos. Due to the presence of indels, the sequence length varied among taxa. Thymine was most abundant, followed by adenine, cytosine and guanine. The variable and informative sites were 310 and 164, respectively. Transitions seemed to be unsaturated over transversions and transversions did not appear to be saturated over genetic distances.

The tribe Cacatuini composed of three genera *Cacatua*, *Eolophus* and *Callocephalon* was monophyletic. Although the relationships between species of the genus *Cacatua* were not resolved well, *C. alba*, *C. galerita*, *C. goffini*, *C. sanguinea*, *C. moluccensis* and *C. sulphurea* were grouped together and *C. leadbeateri* was relatively distant from other congeneric species. The tribe Calyptorhyncini consisting of *Probosciger* and *Calyptorhynchus* was paraphyletic and genus *Calyptorhynchus* was likely diverged earlier than *Probosciger*. *Calyptorhynchus* seemed monophyletic. *Nymphicus* (tribe *Calopsittacini*) seemed to be a basal clade of cockatoos. The phylogenetic trees suggest that cockatoos originated from Australia and then diverged in the Pacific region including the Indonesian Archipelago.

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