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KATA PENGANTAR

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Phylogenetic relationships within Cockatoos (Aves: Psittaciformes) Based on DNA Sequences of The Seventh intron of Nuclear β-fibrinogen gene

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ABSTRAK

Hubungan Kekerabatan Kakatua (Aves: Psittaciformes) Berdasarkan Sekuen DNA dari Intron ke Tujuh dari Gen β--fibrinogen. Hubungan kekerabatan diantara burung kakatua masih menjadi perdebatan, khususnya menyangkut posisi dari *Nymphycus hollandicus*. Intron pada gen *β*-fibrinogen telah diketahui berguna untuk mempelajari filogeni dari beberapa kelompok burung, oleh karena itu penelitian ini menggunakan sekuen DNA dari intron ketujuh pada gen ini (β-fibint7) untuk mengkonstruksi filogeni dari enam genus (*Cacatua, Callocephalon, Eolophus, Calyptorhynchus, Probosciger,* dan *Nymphicus*) yang terdapat di dunia berdasarkan analisis neighbor-joining (NJ) and maximum-parsimony (MP). Ditemukan beberapa indel (insersi-delesi) pada sekuen DNA dari â-fibint7 kakatua, sehingga panjang sekuen DNA bervariasi diantara taxa-taxa yang diteliti. Tidak terjadi saturasi antara substitusi transisi dengan transversi, dan juga antara transversi dengan jarak genetik. Tribe Cacatuini yang terdiri dari tiga genus *Cacatua, Callocephalon, dan Eolophu*s bersifat *monophyletic*. Meskipun hubungan diantara spesies dari genus *Cacatua* tidak terungkap dengan jelas, tetapi *C. alba, C. galerita, C. goffini, C. sanguinea, C. moluccensis* dan *C. sulphurea* berada di dalam satu group, dan *C. leadbeateri* relatif terpisah dari species-species *congeneric* lainnya. *Callocephalon* secara filogenetik terpisah jauh dari genus-genus lainnya pada tribe Cacatuini. Tribe Calyptorhyncini yang terdiri dari *Probosciger* dan *Calyptorhynchus* adalah *paraphyletic*. *Calyptorhynchus* tampak monophyletic. *Nymphicus* (tribe *Calopsittacini*) menjadi *clade* basal dari kakatua.

Key words: Kekerabatan, Kakatua, Sekuen DNA, β--fibrinogen

INTRODUCTION

Approximately 340 living species of Psittaciformes have a pan-tropical and southern distribution. Of these birds, the cockatoos, with 18 –21 species are member of six genera (*Cacatua, Eolophus, Callocephalon, Probosciger, Calypthorhynchus,* and *Nymphicus*) which form one of the most characteristic groups (Forshaw 1989). Some authors classified cockatoos belonged to the family Cacatuidae (e.g. del Hoyo *et al.* 1997; Dickinson 2003), but (Forshaw 1989) placed cockatoos into subfamily Cacatuinae and into family Psittacidae (Dickinson 2003). The cockatoos have always been thought to

form a natural, monophyletic group within the order Psittaciformes.

Cockatoos are characterized by movable crest. They are lack in dyck-texture which produces green in the plumage of other parrots (Smith 1975; Forshaw 1989), and have long been recognized as a unique group of Psittaciformes. They have several characteristics that separate them from other parrots (Adam 1984). Most authors have aligned them into major lineages: the predominantly black Calyptorhynchini (*Calyptorhynchus* and *Probosciger*) and the predominantly white Cacatuini (*Eolophus*, *Callocephalon*, and *Cacatua*). Various studies have been conducted to make grouping of cockatoos, however, the branching and the evolutionary relationships of cockatoos remain unclear and have not been tested and there were several unsolved taxonomic problem (Smith 1975; Adam 1984). One of the controversial problems is the phylogenetic position of cocktail (*Nymphycus*). For example, Smith (1975) and Homberger (1980) amassed compelling morphological and behavioral evidence to assume it as a diminutive cockatoos. However, Walters and Condon (1975) grouped *Nymphycus* with the polytelitine or platycercine parrots. More recently taxonomists (Adam 1984; Forshaw 1989; del Hoyo 1997; Par 1998) agree that *Nymphicus* is belong to cockatoos, but its position within cockatoos is still controversial, and belong to monotypic tribe Calopsittacini (Forshaw 1989). The phylogenetic position of *Callocephalon* within cockatoos has also been in doubt, it is more closely related to black Calyptorhynchini or to white Cacatuini. At species level, controversy has arisen over the position of *Cacatua leadbeateri*.

Several phylogenetic studies had used sequence data of mitochondrial DNA, but this study employs nuclear DNA sequence data. Contrary to mitochon-drial DNA, nuclear DNA sequences were rarely analyzed for the phylogenetic studies of birds including cockatoos, partly because the substitution rates of most nuclear genes in animals are too low to examine the phylogeny of closely-related birds (Allen & Omland 2003; DeBry & Seshadri, 2001; Johnson & Clayton 2000; Palumbi *et. al.* 2001).

In animals, nuclear introns were initially considered to be too slowly evolving, susceptible to incomplete lineage sorting, and overly shued due to recombination and gene conversion for resolving interspecific phylogenies when compared to mtDNA (Allen & Omland 2003; DeBry & Seshadri 2001; Johnson & Clayton 2000; Palumbi *et. al.* 2001). Yet, several studies have successfully used nuclear introns for resolving species level phylogenies in a diversity of animal groups (Beltran *et. al.* 2002; Driskell & Christidis 2004; Lavoue *et. al*. 2003; Peters *et. al.* 2005). Although there are many benefits for employing nuclear intron sequence data for phylogeny reconstruction, they remain a relatively unexploited resource because of the difficulties in isolating orthologous loci (Doyle *et. al*. 2003).

However, since Prychitko & Moore (1997) pointed out that introns are attractive candidates for phylogenetic

analysis because of their abundance in the nuclear genome, their convenient length, and potentially easy amplification by the PCR, the utility of introns for phylogenetics studies of bird has been increasing (Johson &Clayton 2000ab; Prychitko & Moore 1997, 2000, 2003).

Nuclear-gene introns sequence data have several properties that would seemingly make them ideal for phylogenetic studies, because they evolve more rapidly than exons (Prychitko & Moore 2003). Introns are also attractive candidates for phylogenetic analysis because of their abundance in the nuclear genome, their convenient lengths, and potentially easy amplification by the PCR (Prychitko & Moore 1997).

β-fibrinogen is one of the nuclear genes consisting of exons and introns. Single seventh intron of β-fibrinogen gene is known as a non protein coding gene (Prychitco & Moore 1997) and has been described with regard to phylogenetic & Clayton 2000) and family levels (Moyle & Marks 2006; Dor *et. al*. 2010; Gonzalez *et. al.* 2009). Analysis of nuclear intron demonstrated the ability of the seventh intron of β-fibrinogen for the phylogenetic studies (Prychitco & Moore 1997).

The present study was addressed to reveal the relationships among species, genera, and tribes of cockatoos inferred from DNA sequence of the seventh intron of nuclear β-fibrinogen gene (β-fibint7). Objectives of this study were to 1) resolve the phylogenetic relationships within cockatoos (Cacatuinae phylogeny), 2) determine the position of *Cacatua leadbeateri, Callocephalon*, and *Nymphicus*, and 3) determine the monophyly or paraphyly of each tribe

MATERIALS AND METHODS

Blood samples were collected from each individual bird at zoos and captive breeding, and preserved in the 99 % of ethanol and used as DNA resources for polymerase chain reaction (PCR) and DNA sequencing. In total 18 species belonging to six extant genera (*Cacatua, Callocephalon, Eolophus, Probosciger, Calyptorhynchus*, and *Nymphicus*) of three tribes (Cacatuini, Calyptorhynchini, and Calopsittasini) of cockatoos (Tabel 1) were used in this study. The nomenclature follows Forshaw (1989).

Genomic DNA was extracted from approximately 5-20 mg of each dry blood or tissue sample using Qiamp Mini Kit DNA (QIAGEN), according to manufacture's protocol. One or more individuals from each species were sequenced in order to minimize the possible effects of intraspecific variation on phylogenetic hypotheses (Smouse *et. al.* 1991).

A DNA region of β-fibint7 was analyzed in this study. The DNA fragments were amplified by the polymerase chain reaction (PCR). A single fragment of β-fibint7 was amplified using a nucleotide primer pair FIB-B17U and FIB-B17L (Prychitko & Moore1997) in the following PCR conditions: one cycle of 94 °C at 5 minute, 35 cycles of [94 °C- 30 sec., 46 °C-30 sec., 72 °C-60 sec], and one cycle of 72 °C for 7 min.

PCR products were electrophoresed in 1.5 % agarose gels, stained with Ethydium Bromide, and visualized under UV light. A single fragment of amplification products was cleaned by PEG (Polyethelene glycol) and used for DNA sequencing. Sequences of both strands for each sample were obtained using ABI 3100 automated sequencer with a BigDye Terminator Kit version 1.1 or version 3.1 (Biosystems).

In the nucleotide DNA sequence alignment, the presence of indels (insertion and deletion) was analyzed by using Proseq software. Base composition was assessed using PAUP* Version 4.0b (Swofford 2000). PAUP* was also used to determine the number of variable and informative sites.

The number of nucleotide substitutions and genetic distances were calculated using DNAsa and MEGA2 software (Kumar *et. al*. 2001). Nucleotide saturations were tested by plotting the numbers of transitions (ts) and transversions (tv) against genetic distances for all pairwise species.

Phylogenetic trees were constructed using maximum-parsimony (MP) and neighbor- joining (NJ) methods adopting *Accipiter* and *Columba livia* as outgroups. Kimura's 2-parameter distances were calculated for NJ tree. All phylogenetic analyses were assessed using PAUP* Version 4.0b (Swofford 2000). In the parsimony analysis, heuristic search option in PAUP* was selected with a random taxon addition sequence (100 replications) and three bisection-reconstruction (TBR) branch swapping. The random addition of sequences increases the effectiveness of heuristic searches (Maddison, 1991). The bootstrap values were computed using 1000 replicates for NJ tree and 100 full heuristic replicates for MP tree.

Tabel 1: Cockatoo species used in this study.

No.	Species/subspecies	Tribe
1.	*Cacatua alba*	Cacatuini
2.	*C. galerita galerita*	
3.	*C. g. triton*	
4.	*C. g. eleonora*	
5.	*C. goffini*	
6.	*C. moluccensis*	
7.	*C. sanguinea*	
8.	*C. s. sulphurea*	
9.	*C.s. citrinocristata*	
10.	*C. leadbeateri*	
11.	*Eolophus roseicapillus*	
12.	*Callocephalon fimbriatum*	
13	*Probosciger aterrimus*	Calyphtorhynchini
14.	*Calyptorhynchus banksii*	
15.	*C. lathami*	
16.	*C. baudinii*	
17.	*C. latirostris*	
18.	*Nymphicus hollandicus*	Calopsittacini

RESULTS

Based on aligned sequences, several indels occurred in β-fibint7 of cockatoos, ranging from 1 to 9 bp. Indels were more common and concentrated toward the middle of the intron. Due to the presence of several indels, the fragment length of the β-fibint7 varied from 808 to 817 bp in cockatoos (Cacatuinae). In comparison with outgroups (*Columba* and *Accipiter*), total aligned fragments consisted of 817 characters.

The mean of base frequencies of β-fibint7 was highes in thymine 31.45 %, followed by adenine (28.67 %), cytosine (21.63 %), and guanine (18.25 %). Nucleotide substitutions ranged from 1-474 sites. When all characters including indels were analyzed, there were 507 monomorphic sites, 310 polymorphic sites, and 164 parsimony- informative sites. Based on the sequence data obtained, there were variations in the sequences of the β-fibint7 gene in cockatoos.

The numbers of transition were plotted against transversion substitutions, the graph show a linear that transition appeared to be not saturated to trasnversion (Figure 1), and also when all nucleotide substitutions, including transition and transversion were plotted against genetic distance, there was no saturation signal (Figure 2).

Grouping and relationships among cockatoos

Phylogenetic relationships among cockatoos examined were presented in Figure 1 and Figure 2. Both phylogenetic trees; neighbor-joining (NJ) and maximum-parsimony (MP) show that tree genera (*Cacatua*, *Eolophus*, and *Callocephalon*) in which belong to tribe Cacatuini group together and seem to be monophyletic, with the bootstrap values 71 % in NJ and 60 % in MP trees.

Six species of white cockatoos (genus *Cacatua*); *C. sulphurea*, *C. galerita*, *C. alba*, *C. moluccensis*, *C. goffini*, and *C. sanguinea* group together and form a monophyletic group,

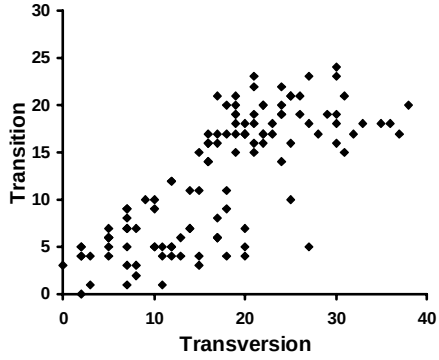

Figure 1. Plot of transition across transversion substitutions of seventh intron of *â*-fibint7) gene in cockatoos.

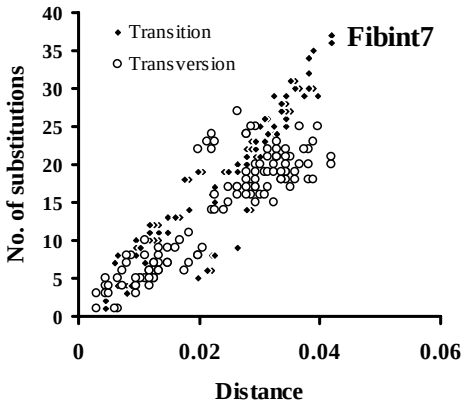

Figure 2. Plot of nucleotides substitutions (transition and transversion) across Kimura 2-parameter distances of of â-fibint7 gene in cockatoos.

supported by bootstrap values 84 % and 57 % in NJ and MP tree, respectively, however *C. leadbeateri* has a relatively distant relationship to other congeneric species, while posistion of others two genera; *Eolophus* and *Callocephalon* were unclear.

Relationships between species of genus *Cacatua* were not well resolved by Fibint7 gene sequence. Both trees show that *C. goffini* is close to *C. sanguinea* supported by 84 % bootstrap value, *C. alba* and *C. moluccensis* group together, and *C. sulphurea* closer to *C. galerita* than to others congeneric species.

β-fibint7 supported paraphyly of Calypthorhynchus and distant relationship of Nymphicus and other cockatoos. Two genera belong to tribe *Calyptorhynchini* (*Calyptorhynchus* and *Probosciger*) formed paraphyletic group, in which *Probosciger* closer to genera of Cacatuini supported by bootstrap values 67 % in NJ and 57 % in MP, than to *Calyptorhynchus.* Four species of *Calyptorhynchus; C. banksii, C. lathami, C. baudinii, and C. latirostris* group together and formed a monophyletic group supported by bootstrap values 91 % in NJ and 69 % in MP , with *C. banskii* is close to *C. lathami,* and C. *baudinii* is close to *C. latirostris*. *Nymphycus* as a monotypic genus of Calypsittacini appear to be a basal clade of cockatoos.

DISCUSSION

As reported by previous authors (Prychitko & Moore 2000; Johnson & Clayson 2000a), the â-fibint7 sequences were rich in thymine and adenine. Overall, the results of their studies were similar to those of the present study. As reported by Lewin (1997), the number and positions of introns in a gene are usually highly conserved through evolution, but the length of intron is somewhat variable as the result of indels, and the nucleotide sequence is highly variable. Previous studies reported that several indels occur in Dove and Pigeon fibint7 sequences, ranging from 1 to 125 bp (Johnson &

Clayton 2000a) and from 1 to 695 bp (Johnson & Clayton 2000b), respectively, which are almost consistent with the range of 1 to 169 indels in parrot â-fibint7. Aligned â-fibint7 sequences contained several inferred insertions or deletions (indels) (Moyle & Marks 2006). The â-fibint7 gene alignment contained several indels. The Canarian pigeons possess autapomorphic indels (Gonzalez *et. al.* 2009). Unlike woodpeckers (Prychitco & Moore 2000) and doves (Johson & Clayton 2000) in which b-fibint7 is AT rich.

No saturation effects on â-fibint7 analyzed in this study were also supported by Prychitco and Moore (1997), in that nuclear DNA usually exhibits relatively low substitution rates and no saturation on substitutions.

Phylogenetic relationships of present study was not congruent to previous studies. Biochemical analysis conducted by Adams *et. al.* (1994) has placed *Nymphicus* as one group with *Calypstorhynchus* in which both of them were basal clade of Cockatos. Whilts characters analysis conducted by del Hoyo *et. al.* (1997) proposed *Nymphicus* was independent to others, and mitochondrial 12S placed *Nymphicus* into one group of *Calyptorhynchus* and *Probosiger* (Figure 5). My parsimony and neighbor-joining analyses generated

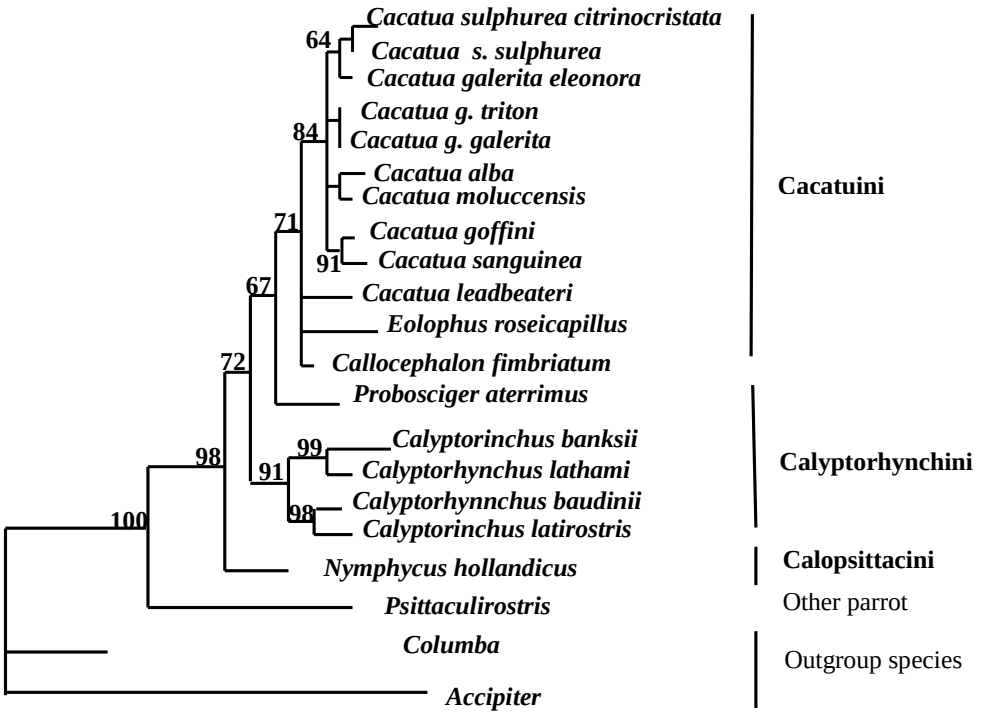

Figure 3: A neighbor-joining (NJ) tree of six genera of cockatoos based on DNA sequences of â-fibin7 gene. Numbers above indicate bootstrap values > 50 %

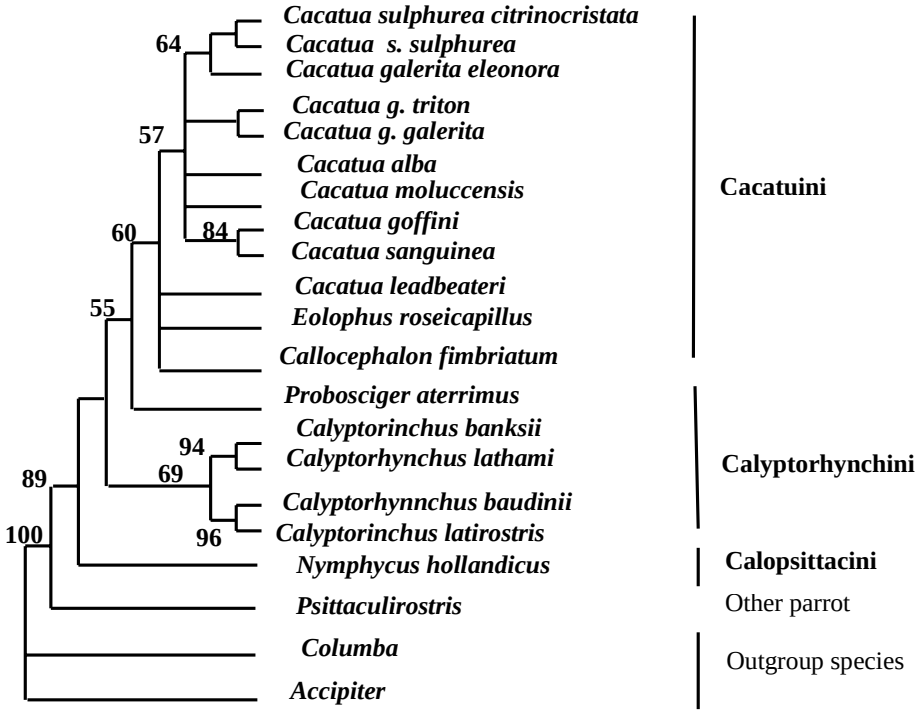

Figure 4: A maximum-parsimony (MP) tree of six genera of cockatoos based on DNA sequences of â-fibint7 gene. Numbers above indicate bootstrap values > 50 %

different results with both morphological and mitochondrial 12S analysis, however it was likely to biochemical analysis which presumable *Nymphycus* was a basal clade of cockatoos.

Relationships within genus *Cacatua* in this study could not be compared to previous studies, because the species used were different. However, within the tribe Cacatuini, the recent study agrees that *Eolophus* was closer *Cacatua* than to others genera, and *Callocephalon* was phylogeneticaly distance from other congeneric genera. Whilst the position of *Probosciger* in the present study; in which *Probosciger* is close to Cacatuini (*Cacatua*, *Eolophus*, and *Callocephalon*) was not supported by mitochondrial 12S and morphological analyses (Figur 5).

CONCLUSIONS

Relationships between species of genus *Cacatua* were not well resolved by fibint7 gene sequence. *Cacatua leadbeateri* was relatively distant from congeneric species. Tribe Cacatuini (*Cacatua*, *Eolophus* and *Callocephalon*) was monophyletic. *Callocephalon* was phylogeneticaly distant from other congeneric genera of Cacatuini. Tribe Calyptorhynchini (*Calyptorhynchus* and *Probosciger*) was paraphyletic.

Nymphicus was presumably a basal clade of cockatoos.

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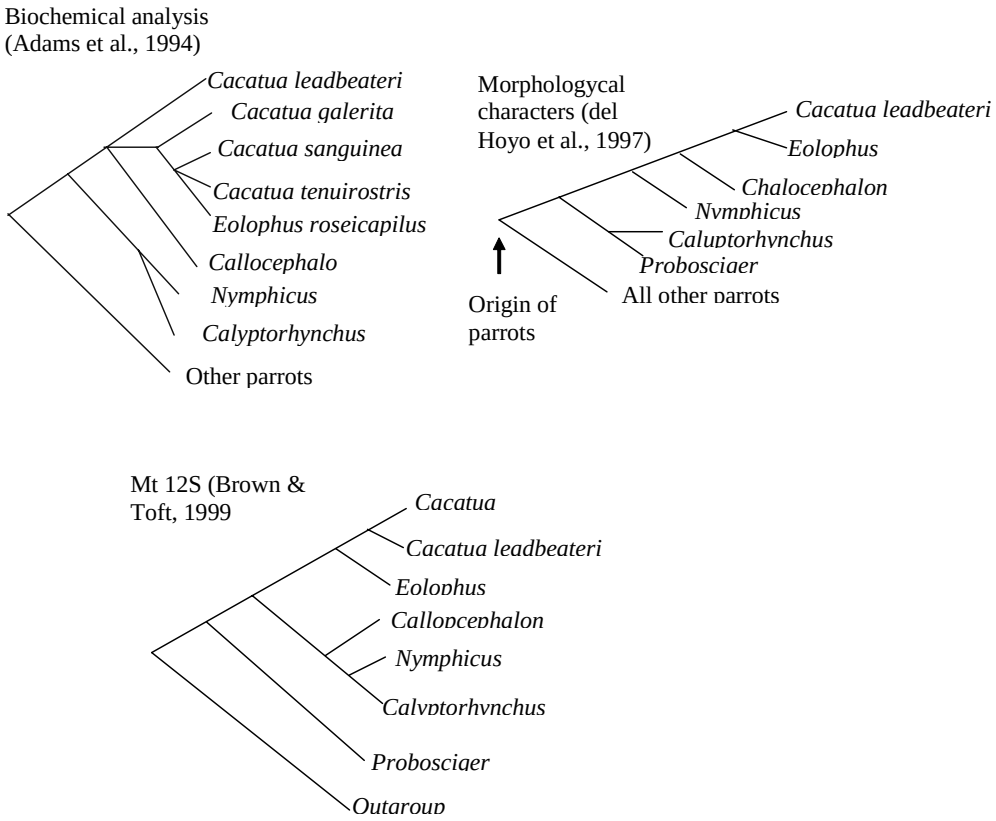

Figure 5: Tree topologies of cockatoos relationships based on other characters

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PANDUAN PENULIS

Naskah dapat ditulis dalam bahasa Indonesia atau bahasa Inggris. Naskah disusun dengan urutan: JUDUL (bahasa Indonesia dan Inggris), NAMA PENULIS (yang disertai dengan alamat Lembaga/ Instansi), ABSTRAK (bahasa Inggris, maksimal 250 kata), KATA KUNCI (maksimal 6 kata), PENDAHULUAN, BAHAN DAN CARA KERJA, HASIL, PEMBAHASAN, UCAPAN TERIMA KASIH (jika diperlukan) dan DAFTAR PUSTAKA.

Naskah diketik dengan spasi ganda pada kertas HVS A4 maksimum 15 halaman termasuk gambar, foto, dan tabel disertai CD. Batas dari tepi kiri 3 cm, kanan, atas, dan bawah masing-masing 2,5 cm dengan program pengolah kata *Microsoft Word* dan tipe huruf *Times New Roman* berukuran 12 point. Setiap halaman diberi nomor halaman secara berurutan. Gambar dalam bentuk grafik/diagram harus asli (bukan fotokopi) dan foto (dicetak di kertas licin atau di scan). Gambar dan Tabel di tulis dan ditempatkan di halam terpisah di akhir naskah. Penulisan simbol α, β, χ, dan lain-lain dimasukkan melalui fasilitas insert, tanpa mengubah jenis huruf. Kata dalam bahasa asing dicetak miring. Naskah dikirimkan ke alamat Redaksi sebanyak 3 eksemplar (2 eksemplar tanpa nama dan lembaga penulis).

Penggunaan nama suatu tumbuhan atau hewan dalam bahasa Indonesia/Daerah harus diikuti nama ilmiahnya (cetak miring) beserta Authornya pada pengungkapan pertama kali.

Daftar pustaka ditulis secara abjad menggunakan sistem nama-tahun. Contoh penulisan pustaka acuan sebagai berikut :

Jurnal :

Hara, T., JR. Zhang, & S. Ueda. 1983. Identification of plasmids linked with polyglutamate production in B. subtilis. *J. Gen. Apll. Microbiol*. 29: 345-354.

Buku :

Chaplin, MF. & C. Bucke. 1990. *Enzyme Technology*. Cambridge University Press. Cambridge.

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Gerhart, P. & SW. Drew. 1994. Liquid culture. Dalam : Gerhart, P., R.G.E. Murray, W.A. Wood, & N.R. Krieg (eds.). *Methods for General and Molecular Bacteriology*. ASM., Washington. 248-277.

Abstrak :

Suryajaya, D. 1982. Perkembangan tanaman polong-polongan utama di Indonesia. Abstrak Pertemuan Ilmiah Mikrobiologi. Jakarta . 15 –18 Oktober 1982. 42.

Prosiding :

Mubarik, NR., A. Suwanto, & MT. Suhartono. 2000. Isolasi dan karakterisasi protease ekstrasellular dari bakteri isolat termofilik ekstrim. Prosiding Seminar nasional Industri Enzim dan Bioteknologi II. Jakarta, 15-16 Februari 2000. 151-158.

Skripsi, Tesis, Disertasi :

Kemala, S. 1987. Pola Pertanian, Industri Perdagangan Kelapa dan Kelapa Sawit di Indonesia.[Disertasi]. Bogor : Institut Pertanian Bogor.

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Schulze, H. 1999. Detection and Identification of Lories and Pottos in The Wild; Information for surveys/Estimated of population density. http//www.species.net/primates/loris/ lorCp.1.html.