Sequence diversity and phylogenetic utility of nuclear RAG-1 gene in parrots

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Background

Indonesia is one of mega biodiversity countries and inhabited by 300.000 animal species including 1,531 bird species which constitutes 15 % of all bird in the world (Mittermeier *et al*. 1997, Andrew 1992). IUCN recorded 104 bird species to be endangered.

In the world, about 350 parrot species are distributed primarily in hotspots of biodiversity such as Brazil, tropical Africa and Indonesia. This bird group retains worldwide popularity, because they have several unique characteristics and economic value. The over-hunting has drastically depopulated the parrots including some endangered species, therefore, all parrot species are listed in CITES appendix.

Indonesia is inhabited by 76 parrot species of 25 genera, constituting 12 % of world's parrot species (Forshaw 1989). Of these, 32 species are endemic Indonesia (Andrew 1992), and this country is the second largest after Argentina in the export of parrots (WCMC 1992). In addition, the over-exploitation and fragmentation of their habitats have endangered some species to extinction. Therefore, conservation planning is urgently required, and this study was planned to provide some basic information about phylogeny of Indonesian parrots. In our previous study, we used a mitochondrial gene cytochrome-*b*. In this study, therefore, we used a nuclear exon gene RAG-1.

Materials and Methods

DNA sequences of 639 bp of nuclear RAG-1 gene were obtained from 50 individuals of 33 species representing 17 genera, three subfamilies (Cacatuinae, Loriinae, and Psittacinae). The RAG-1 gene was amplified using primers F1 (5'GAAGCAACTTTGCCGCA TCTGTGG-3') and C1 (TACCAGATCAGTAGGGAAGCAAGG-3') under the following conditions: 1 cycle of 94 °C for 5 min; 40 cycles of 95 °C for 30 sec, 57 °C for 30 sec, and 72 °C for 1 min; and 1 cycle of 72 °C for 10 min. We analyzed nucleotide composition, sequence variation and Tamura-Nei distance and conducted saturation analyses by plotting transitional substitutions against tranversional substitutions and by plotting transitional substtutions at each codon position against genetic distances. Thereafter, phylogenetic trees were constructed by maximum parsimony (MP) and neighbor joining (NJ) methods, adopting *Accipiter* and *Columba livia* as outgroups. The bootstrap values were computed using 1000 replicates for NJ tree and 10 full heuristic replicates for MP tree.

Results and Discussion

Make-up of RAG-1 sequences

The RAG-1 exon gene of parrot was relatively rich in A-T (57.6 %). Average nucleotide composition was 30.8% of adenine, 26.3% of thymine, 21.3% of cytocine, and 21.7% of guanine. First and second codon positions were rich in adenine (36.0% and 33.8%, respectively), but third codon position was rich in thymine (34.1 %). Compared with outgroup species, the examined parrots showed three bases deletion (CAA) events and no stop codons in the sequenced region of

any taxa. Transitional substitutions (ts) were plotted as a function of transversional substitutions (tv) as shown in Fig. 1A. The ts : tv ratio did not significantly differ from 1:1. Moreover, transitional substitutions at each codon position linearly increased with genetic distances (Fig. 1B), suggesting that the RAG-1 sequence of parrots is not saturated with substitutions yet even at third codon position as reported by Growth and Barrowclough (1999). Therefore, we used all substitutions and unweighting parsimony in constructing phylogenetic trees.

Sequence diversity

The intraspecific variation of the DNA sequence was so little that, even if there is, only one substitution was detected between individuals. The low sequence variation was also detected between species of genus *Cacatua*, and between some genera of subfamily Loriinae. Of 639 sites, 140 (21.8%) were variable and 98 (15.3%) were parsimoniously informative. Most of the variable sites and the informative sites were located at third codon position followed by first and second codon positions.

Phylogenetic relationships

Topology was similar between maximum-parsimony and neighbor-joining trees, both suggesting that parrots are divided into two large groups: Cacatuinae and Psittacinae-Loriinae. This result is consistent with previous studies which have demonstrated that Cockatoos are distant from other groups (Smith, 1975, Christidist *et al*., 1991, Astuti *et al*., 2003). On the other hand, the phylogeny inferred from RAG-1 gene could not well resolve the relationships within genus *Cacatua (C. goffini, C. sanguinea, C. sulphurea, C. galerita, C. alba* and *C. moluccensis*), and among five genera of subfamily Loriinae (*Chalcopsitta, Eos, Pseudeos, Lorrius* and *Trichoglossus*), suggesting that those five genera of Loriinae are very closely related but distant from genus *Charmosyna*. In contrast, the species or genera belonging to Psittacinae appeared clearly diverse. The phylogenetic trees suggest that the genera of Lorrinae have evolved relatively more recently than these of subfamily Cacatuinae (*Probosciger* and *Cacatua*) or Psittacinae (*Aprosmictus, Alisterus, Cyclopsitta, Eclectus, Loriculus, Psittacula, Psittaculirostris, Psittrichas* and *Tanygnathus*).

In conclusion, this gene seems appropriate for resolving the phylogeny between subfamilies. Monophyly of Cacatuinae and paraphyly of Psittacinae were recognized in MP and NJ trees with high bootstrap values (Fig. 2A and Fig. 2B), though monophyly of Loriinae was not supported.

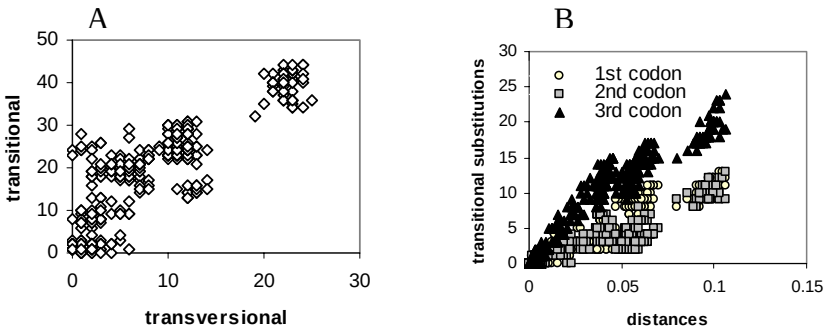

Fig. 1. Plots of transitional substitutions against transversional substitutions (A) and of transitional substitutions at each codon position against Tamura-Nei distances (B).

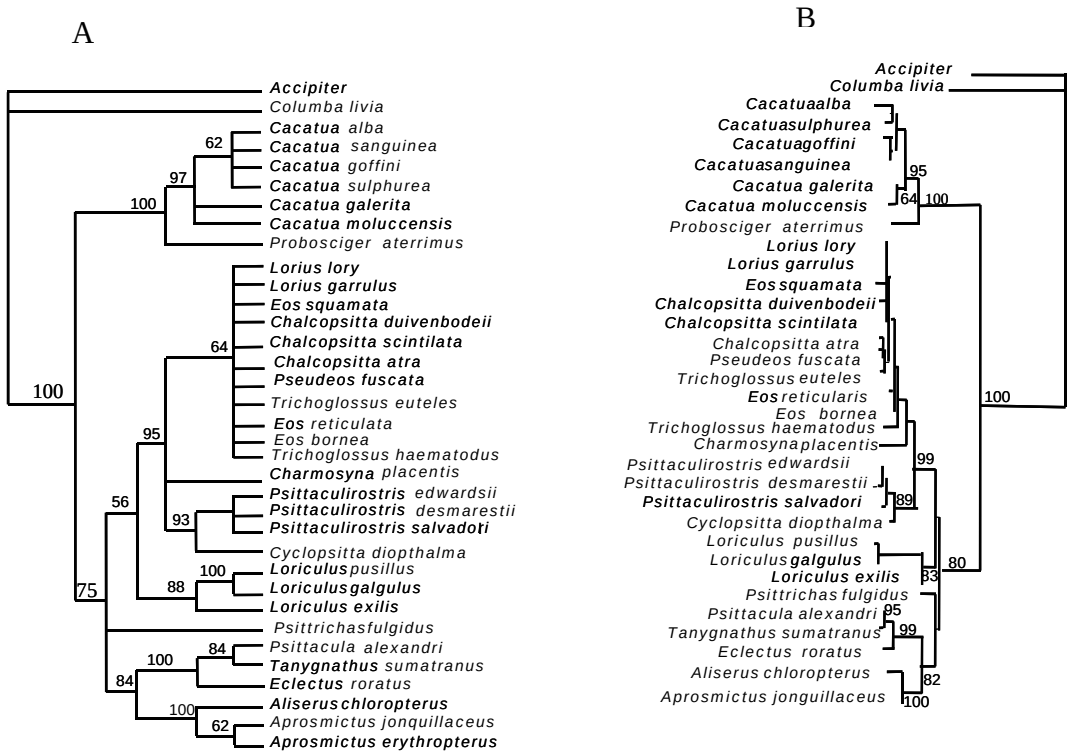

Fig. 2. Maximum -parsimony tree (A) and neighbor-joining tree (B). Numbers above branches indicate bootstrap values.

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