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Editor's note

It is great that Treubia volume 37 can be published in year 2010. Recently, it was difficult to get appropriate papers since animal taxonomy has not been an attractive subject in the field of biology. There was a lack of submitted manuscripts in 2009 that made Treubia could not be published in year 2009.

This volume of TREUBIA contains five papers of vertebrates and invertebrates. Three papers (nematode, rats and land snail) were from the results of field works in eastern part of Indonesia i.e. West Papua which was rarely explored.

Also, this year Indonesian zoologist' community lost the pioneer and expert in parasite taxonomy, Dr. Sampurno Kadarsan. His name has been used to name new species of leeches, tick, rat, lizard and frog by his successors to acknowledge his impact and contribution. He served as an editor of Treubia from 1992 to 1997 and was a proof reader for some years until his permanent retarded eye sight. So, his death was a great lost for all of us especially for the Museum Zoologicum Bogoriense.

Finally, I would like to thank all of the co-editors, referees, computing assistant, secretary and administrative assistant for their collaborative work. I acknowledge financial support from the Director of Research Centre for Biology LIPI to publish this precious journal.

Cibinong, 15 December 2010

Dewi M. Prawiradilaga
Chief Editor

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THE DNA SEQUENCE PERFORMANCE OF COI GENE IN WHITE COCKATOOS (*CACATUA*, PSITTACIFORMES)

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ABSTRACT

Performance of nucleotide sequencing of 807-bp segments of mitochondrial *c* cytochrome oxidase I (COI) was analyzed to study the relationship and grouping of six species of white cockatoos: *Cacatua galerita, C. sulphurea, C. alba, C. moluccensis, C. sanguinea,* and *C.goffini*. Two species (*Aprosmictus erythropterus* and *Prioniturus platurnus*) were used as outgroups in this study. The sequences contained a mean composition of 25.9 % tymine, 30.8 % cytosine, 26.0 % adenine, and 17.4 % guanine. Based on Kimura 2-parameter analyses, the genetic distance between individuals within a species (intraspecific) ranged from 0.0000 (*C. alba*) to 0.0026 ± 0.0012 (*C. galerita*) and the genetic distance between individuals of different species ranged from 0.0299 ± 0.0057 (*C. sulphurea* vs *C. galerita*) to 0.0991 ± 0.0120 (*C. moluccensis vs C. sanguinea*). Sequence variations and haplotypes were found in *Cacatua*. In total, 196 (%) variable sites were identified with 189 sites being parsimoniously informative. Neighbor-joining (NJ) and maximum parsimony (MP) analyses showed two main groups in *Cacatua*: (*C. sanguinea* + *C. goffini*), and (*C. alba* + *C. moluccensis*) + (*C. galerita* + *C. sulphurea*).

Key words: Performances, DNA sequences, COI, white cockatoos, *Cacatua*,

INTRODUCTION

Cytochrome *c* Oxidase I (COI) genes is one of the 13-protein coding genes located in the mitochondria area of animal cells (Lewin 1997, Brown 1999). The length of this gene is about 2000-bp. Recently COI genes have become popular and are believed to have the characteristics to be “DNA

barcode" standards/guidelines. Approximately 650-bp DNA sequence of particular regions of COI genes could be used to identify animal species (Hebert *et al*. 2003, 2004) and could also be a complement that provides information to assist in taxon selection (Hajibabaei *et al*. 2007). This short DNA sequence could confirm individuals of an animal species, because of the genetic variation in individuals within a species greatly differs from the genetic variation in individuals from different species (Hebert *et al*. 2004). Research to test the accuracy of species identification using COI DNA barcodes has been successfully performed (Hebert *et al*. 2004, Hajibabaei *et al*. 2006). However, DNA barcode could only be used for species identification if the COI DNA sequence is not shared among other species, but the fact is DNA sequence sharing a common occurrence (Meier *et al*. 2006). In addition, it is not easy to amplify COI genes in DNA target fragments using DNA primers. In this study, COI gene fragment was not precisely located on the DNA barcode position. By using other primers, fragments of COI genes along 860-bp were analyzed to study its nucleotide performance and construct a phylogenetic tree for species of white cockatoos in the *Cacatua* genus.

White cockatoos (*Cacatua*) is a group of parrots in the Psittaciformes order. According to taxonomy classification, *Cacatua* belongs to Psittacidae (Forshaw 1989, Juniper & Parr 1998, Sibley & Ahlquist 1990, Dickinson 2003) and Psittacinae subfamily (Forshaw 1989). Other researchers (del Hoyo *et al*. 1996) grouped *Cacatua* into the Cacatuidae. Regardless of grouping differences, this study was only focused on the genus level, *Cacatúa*, without accounting for the controversy of subfamilies and families. Basically, all researchers agree that white cockatoos are in the genus *Cacatua*.

There are 11 species of white cockatoos in the world, six species occur in Indonesia: *Cacatua sanguinea, C. goffini, C. galerita, C. sulphurea, C. alba,* and *C. moluccensis* (Forshaw 1989, Dickinson 2003). Each *Cacatua* species is endemic in a certain island in Indonesia. All species in the *Cacatua* genus are listed in CITES Appendices I and II.

In relation to the biodiversity of *Cacatua,* correct species identification is fundamental. Therefore, COI genes were analyzed in this study to learn its DNA sequence performance and to demonstrate the grouping and relationship of white cockatoo species within the *Cacatua*.

MATERIALS AND METHODS

Sample collection, DNA extraction and DNA visualization

Twenty blood samples of white cockatoos (*Cacatua*) consisted of 6 samples of *Cacatua galerita triton*, 1 sample of *C. sulphurea*, 2 samples of *C. alba*, 2 samples of *C. moluccensis*, 5 samples of *C. sanguinea*, and 4 samples of *C. goffini* were collected from zoos, captive breeding, and traders in Indonesia from 1995 until 2001. As outgroups, 7 blood samples were also collected from two other parrot species (4 samples of *Aprosmictus erythropterus* and 3 samples of *Prioniturus platurus*). Total DNA was extracted from each blood sample using Qiagen DNA Kit. DNA was then separated through electrophoresis, and visualized under UV rays.

PCR amplification and sequencing

COI gene fragments were amplified through PCR (Polymerase Chain Reaction) using a pair of primers, L6858 (5'-AATAACATAAGCTTCTGACT-3')

and H7827 (5'-CCAGAGATTAGAGGGAATCAGTG-3') (Mindell *et al*. 1999). The PCR condition used in this study was 93 ºC for 5 minutes, 35 X (93ºC for 1 minute, 55ºC for 1 minute, 72ºC for 1.5 minutes), and 72ºC for 5 minutes.

To clarify amplified COI fragments, the PCR products were visualized through MUPID electrophoresis. The presence of target fragments was confirmed by analyzing results of the UV ray photograph. COI fragments were then sequenced to reveal the base sequence. DNA primers used in sequencing were the same with those for PCR. The base sequence data of COI gene were taken through two-ways (Forward and Reverse) which complements each other.

Analysis of sequence data and grouping of *Cacatua*

DNA sequence data were analyzed using DNAsys and Mega-3 software to calculate the amount of transition (si) and transversion (sv) DNA substitution, and the transition:transversion ratio (si/sv). Base composition and genetic distances between individuals in one species and between species were analyzed using Mega-3 software.

The process of grouping individuals and species of *Cacatua* were done by constructing phylogenetic trees using neighbor-joining (NJ) and maximum-parsimony (MP) analyses on Mega-3 software. To measure the accuracy, bootstraps values were also calculated. NJ and MP trees were constructed based on all base substitutions (transversion and transitions) that occur in all codon positions. NJ bootstrap values were calculated with 1000 repetitions. MP tree was constructed using branch swapping addition and the bootstrap value was calculated with 100 replications.

RESULTS

1. DNA Sequence performances of COI gene in *Cacatua*

PCR produced fragments which were approximately 860-bp. Fragments were amplified from all *Cacatua* examined. From 860-bp, only 807-bp was used for further data analysis. This was because base sequences of primers used and doubting sequence data were not included in data analysis.

1.1. Base composition of COI gene

Data analysis of base sequence along 807-bp resulted in an average base composition as shown in Table 1. This composition shows that GC and AT contents were 48.2% and 51.9%. This is common in protein-coding genes which has relatively balanced compositions of bases; not AT -rich although the percentage of AT > GC. The order from the highest percentage of nucleotide was C, A, T, and G. The first codon position was dominated by guanine (G), the second position by thymine (T), and the third position by cytosine (T).

Table 1. The average percentage of base composition (%) in COI gene of *Cacatua*

Codon Position	Thymine (T)	Cytosine (C)	Adenine (A)	Guanine (G)	Total bases
All position	25,9	30,8	26,0	17,4	807
First	20,4	23,8	25,3	30,5	269
Second	39,4	26,4	18,2	16,0	269
Third	17,4	42,8	33,1	7,1	269

1.2. Genetic distance

The genetic distances between individuals and between species of *Cacatua* are shown in Table 2 and Table 3. The genetic distance reflects sequence divergences of the *Cacatua* analyzed. It showed that individuals within a cockatoo species had an average genetic distance from 0.0000 (*C. alba*) to 0.0026 ± 0.0012 (*C. galerita*) (Table 2). Two *C. alba* analyzed had identical DNA sequences; no base substitution was found among the DNA sequence of both birds. The average genetic distance between species in the *Cacatua* genus was 0.0299 ± 0.0057 (*C. sulphurea* vs *C. galerita)* to 0.1021 ± 0.01170 (*C. galerita* vs *C. sanguinea*) (Table 2) or 2.99% - 9.91% of sequence divergence.

Table 2. Average genetic distance and number of haplotypes between individuals in the species/subspecies of *Cacatua* and two outgroup species based on all base substitution in 807-bp COI gene

Taxon group	Total individuals	Genetic distance (± SE)	haplotype
Prioniturus platurus	3	0.0017 ± 0.0012	
Aprosmictus erythropterus	4	0.0031 ± 0.0014	
Cacatua galerita triton	6	0.0026 ± 0.0012	4
C. sulphurea	1	-	-
C. moluccensis	2	0.0025 ± 0.0016	2
C. alba	2	0.0000 ± 0.0000	0
C. sanguinea	5	0.0010 ± 0.0007	2
C. goffini	4	0.0021 ± 0.0012	3

Table 3. Average genetic distance between species in the *Cacatua* and two outgroup species based on all base substitution in 807-bp COI gene

Taxon group	Genetic distance (d ± SE)						
	1	**2**	**3**	**4**	**5**	**6**	**7**
1. *Prioniturus platurus*							
2. *Aprosmictus erythropterus*	0.1168 ± 0.0118						
3. *Cacatua galerita triton*	0.1391 ± 0.0135	0.1456 ± 0.0132					
4. *C.sulphurea sulphurea*	0.1303 ± 0.0129	0.1361 ± 0.0127	0.0299 ± 0.0057				
5. *C. moluccensis*	0.1397 ± 0.0141	0.1520 ± 0.0138	0.0580 ± 0.0087	0.0464 ± 0.0076			
6. *C. alba*	0.1397 ± 0.0137	0.1468 ± 0.0138	0.0567 ± 0.0086	0.0492 ± 0.0080	0.0417 ± 0.0077		
7. *C. sanguinea*	0.1300 ± 0.0139	0.1408 ± 0.0136	0.1021 ± 0.01170	0.0866 ± 0.0104	0.0991 ± 0.0120	0.0986 ± 0.0117	
8. *C.goffini*	0.1320 ± 0.0139	0.1370 ± 0.0136	0.1010 ± 0.0112	0.0860 ± 0.0104	0.0970 ± 0.0119	0.0950 ± 0.0111	0.0338 ± 0.0062

1.3. DNA sequence variation and haplotype

From the 807-bp DNA sequence analyzed in this study, there were 196 variation sites and of which 189 were parsimoniously informative. The number of haplotypes in cockatoo COI DNA sequences studied is shown in Table 4. It indicates that haplotypes occur among individuals of *Cacatua*. The number of haplotype in each species is *C. galerita* (4 haplotypes), *C. alba* (0

haplotype), *C. moluccensis* (2 haplotypes), *C. sanguinea* (2 haplotypes), and *C. goffini* (3 haplotypes).

1.4. Transition and transversion substitutions

Result of the analysis of transistion and transversion substitutions in 807-bp COI gene in *Cacatua* show that the number of transition substitution (43 bases) was greater than the number of transverse substitution (6 bases), with a transition / transverse ratio (si / sv) of 6.7. The occurrence of transition base substitution was more frequent than transverse substitution. Base substitution was highest in the third codon position, followed by the first codon position and was lowest in the second codon position. The data shows that there was no base substitution in the second codon position.

2. Grouping and genetic relationship of *Cacatua*

The grouping of *Cacatúa* was constructed with neighbor-joining (NJ) and maximum-parsimony (MP) analyses based on Kimura-2 parameters in Mega-3 software. Construction of the NJ tree was established by entering all base substitutions in all codon positions (the first, second and third position) of all *Cacatua* individuals studied. The phylogenetic tree based on NJ analysis is shown in Figure 1.

The NJ phylogenetic tree shows that each individual in one species groups together in one cluster. In this case, to speed up the process of maximum parsimony analysis, both for the construction of the MP tree and calculation of bootstrap values, only 1-2 individuals per species were used in the analysis. The MP phylogenetic tree is shown in Figure 2.

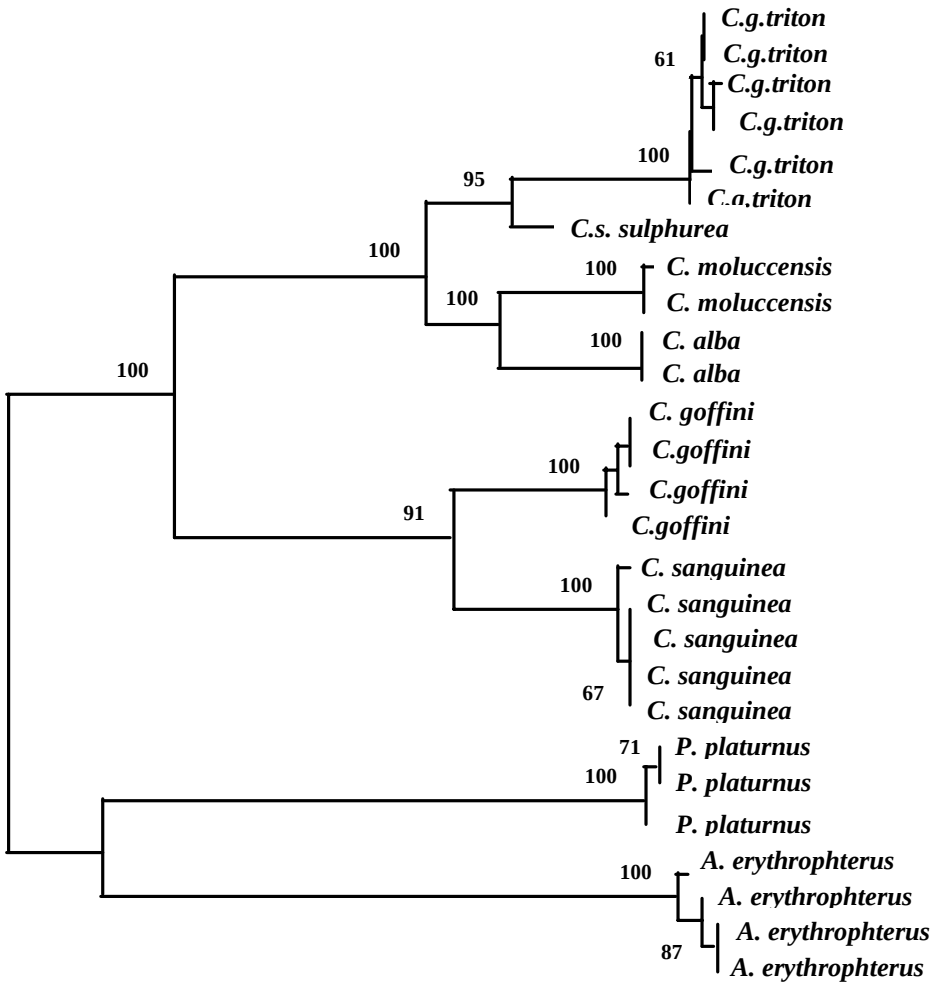

Figure 1. NJ tree based on analysis of all base substitutions (si and sv) at all codon positions in 807-bp COI gene of the *Cacatua*. *Prioniturus platurus* and *Aprosmictus erythropterus* were used as outgroups. The numbers shown above are bootstrap values

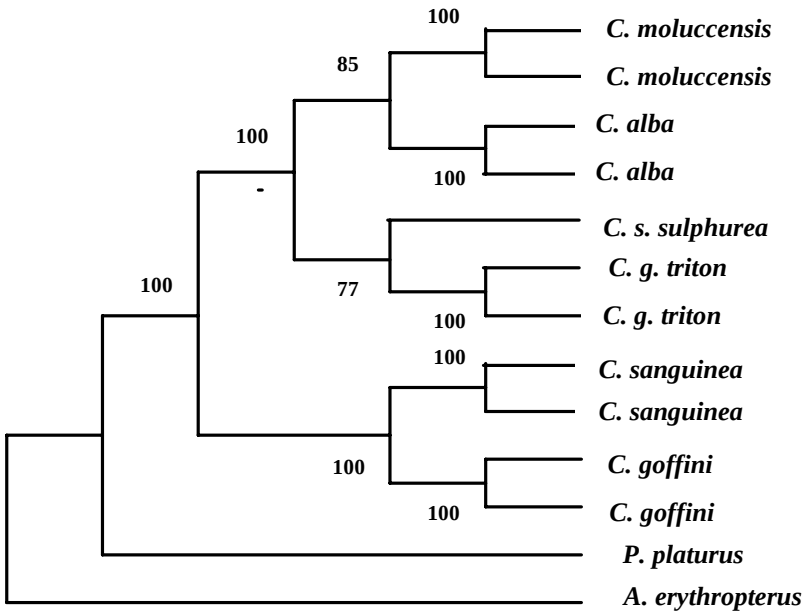

Figure 2. MP tree based on analysis of all base substitutions (si and sv) at all codon positions in 807-bp COI gene of the *Cacatua*. *Prioniturus platurus* and *prosmictus erythropterus* were used as outgroups. The numbers shown above are bootstrap values

Result of NJ (Figure 1) and MP (Figure 2) analyses illustrate that of the six studied species there were two major groups in the *Cacatúa* genus. The first group consisted of *Cacatúa sanguinea* and *C. goffini*, supported with 100% (NJ) and 100% (MP) bootstrap values. The second group consisted of *C. alba* and *C. moluccensis*, supported by 91% (NJ) and 85% (MP) bootstrap values, and *C. galerita* and *C. sulphurea* with 95% (NJ) and 77% (MP) bootstrap values. Meanwhile, the (*C.alba C. moluccensis*) + (*C.sulphurea C. galerita*) relationship has a very convincing 100% bootstrap value.

DISCUSSION

The base composition of AT and GC in COI genes in the *Cacatua* was relatively balanced. This is common in protein-coding genes which are generally not dominated by AT (AT –rich) even though the percentage of AT > GC. The order from the highest percentage of nucleotide was C, A, T, and G. Lowest guanine compositions are commonly found in the DNA, particularly in protein-coding genes in the mitochondrial DNA such as ND2 (Zuccon *et al*. 2006), cyt-b (Astuti *et al*. 2006), and also on COI in other bird groups (Weibel & Moore 2002).

The genetic distance reflects sequence divergence in the *Cacatua*. From the genetic distance, it is known that the divergence sequence value was between 0% - 0.26% for individuals in one species and 2.99 % - 10.2% for different species in the *Cacatua*. It showed clearly that the genetic distances between species were higher than between individuals.

Transition base substitution is more frequent than transverse substitution. This is very reasonable because it is easier for bases to change from purine to purine or pyrimidine to pyrimidine than from purine to pyrimidine or pyrimidine to purine (Li & Graur 1991). This base substitution pattern is also similar to the substitution pattern of other protein-coding genes in the mitochondrial DNA, such as ND2 (Ericson *et al*. 2003), cyt-b (Astuti *et al*. 2006), and COI in other birds; the *Poicoides* genus (Weibel & Moore 2002).

Relatively high bootstrap values indicate the grouping has a high accuracy. The grouping and genetic relationship among species in the *Cacatua* based on COI gene with NJ and MP analyses suggest that COI gene sequences seemingly reflect to the morphological characteristics of *Cacatúa*.

Morphologically, C. *sanguinea* and *C. goffini* have flat wings, small beaks and short crests, while other groups (*C. galerita*, *C. sulphurea*, *C. alba* and *C. moluccensis*) have rounded wings, thick beaks, and large crests (Forshaw 1989)

CONCLUSION

The research concluded that cockatoo birds in the same species were grouped together in the same cluster, while birds from different species were separated. The sequence divergence value between individuals in the same species differs greatly from the divergence value between individuals from different species. COI genes can reveal the grouping and genetic relationship of many species of white cockatoos: (*C. sanguinea* + *C. goffini*) and (*C. galerita* + *C. sulphurea*) + (*C. alba* + *C. moluccensis*).

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REFERENCES

Astuti, D., N. Azuma, H. Suzuki & S. Higashi, 2006. Phylogenetic relationships within Parrots (Psittacidae) inferred from mitochondrial cytochorome-b gene sequences. *Zoological Sciences* **23**: 191-198

Brown, T.A., 1999. *Genome*. Bios Scientific Publishers Ltd., 472 pp.

Del Hoyo J., A. Elliot & M. Sargatar, 1996. *Handbook of the Bird of the World*. Vol. 4. Lynk Editions. Barcelona.

Dickinson, E.C, 2003. *The Howard and Moore Complete Checklist of the Birds of the World*. Third Ed. Christopher Helm, London, 1039 pp.

Ericson, P.G.P., L. Christidis, M. Irestedt & J.A. Norman, 2003. Systematic affinities of the lyrebirds (Passeriformes: Menura), with a novel classification of the major groups of passerine birds. *Molecular Phylogenetic and Evolution* **25**: 53-62.

Forshaw, J.M. & W.T. Cooper, 1989. *Parrots of the World*. Third (revised) Ed. Landowns, 672 pp.

Hajibabaei, M., D.H. Janzen, J.M. Burns, W. Hallwachs & P.D.N. Hebert, 2007. DNA barcodes distinguish species of tropical Lepidoptera. *Proceedings of the National Academy of Sciences, USA* **103**: 968–971.

Hebert, P.D.N., A. Cywinska, S.L. Ball & J. R. deWaad, 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences* **270**: 313–321.

Hebert, P.D.N., M.Y. Stoeckle, T.S. Zemlak, & C.M. Francis. 2004. Identification of birds through DNA barcodes. *Public Library of Science, Biology* **2**: 1657-1663.

Juniper, T.M. & M. Parr, 1998. *Parrots. A Guide to the Parrots of the World*. Yale University Press, New Haven and London. 584 pp

Kerr, K.C.R, M.Y. Stoeckle, C.J. Dove, L.A. Weigt, C.M. Francis, & P.D.N. Hebbert. 2007. Comprehensive DNA barcode coverage of North American birds. *Molecular Ecology Notes* **7**: 535–543.

Lewin, B., 1997. *Gene VI*. Third Ed. John Wiley & Son. New York, 761 pp.

Li, W. & D. Graur, 1991. *Fundamental of Molecular Evolution.* Sinauer Ass. Inc. Publisher, Sunderland, Massachusetts, 284 pp.

Meier, R., K. Shiyang, G. Vaidya, & P.K.L. Ng, 2006. DNA barcoding and taxonomy in Diptera: a tale of high intraspecific variability and low identification success. *Systematic Biology* **55**: 715–728.

Mindell, D.P., M.D. Sorenson, D.E. Dimcheff, M. Hasegawa, J.C. Ast & T. Yuri, 1999. Interordinal relationships of birds and other reptiles based on whole mitochondrial genome. *Systematic Biology* **48**(1): 138-152.

Sibley, C.G. & J.E. Ahlquist, 1990. *Phylogeny and Classification of Birds*. Yale University Press, New Haven, 976 pp.

Weibel A.C. & S. Moore, 2002. Molecular phylogeny of cosmopolitan group of woodpeckers (Genus *Picoides*) based on COI and cyt b mitochondrial gene sequences. *Molecular Phylogenetic and Evolution* **22** (1): 65-75.

Zuccon D., A. Cibois, E. Pasquet & P.G.P. Ericson, 2006. Nuclear and mitochondrial sequence data reveal the major lineages of starling, mynas and related taxa. *Molecular Phylogenetic and Evolution* **41**: 333-344.

OBITUARY

Dr. Sampurno Kadarsan

Late Dr. Sampurno Kadarsan passed away in Bandung on 17 September 2010 at the age of 81 years old. He was survived by his wife and four married daughters. He was born in Surabaya on 11 August 1929. However, he lived in Bogor for much of his time.

Education

He entered Diploma in biology in 1955 under the Ministry of Agriculture. Then, he joined the University of California, Berkeley – USA and achieved BSc. degree in Entomology & Parasitology in 1959. Upon returning to Indonesia, he undertook further study at Bandung Institute of Technology (ITB) and achieved his first degree in biology in 1964. Then, he got an opportunity to enter the University of Maryland, College Park, USA for postgraduate study and achieved his PhD degree in 1971.

Working Career

He started working in the division of Marine Fishery (*Djawatan Perikanan Laut*) in Jakarta. Then, he moved to the division of Nature Research (*Djawatan Penyelidikan Alam*) in Bogor as an assistant in biology. In 1960 he became the director of Museum Zoologicum Bogoriense, under the Centre for Nature Research Institute (*Lembaga Pusat Penyelidikan Alam*). In 1977 he became a senior professor at the Fakulti Perubatan, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia. He became a senior scientist at the National Biological Institute-Indonesian Institute of Sciences (LIPI) in 1981. In 1986 he was the Director of the Research & Development Centre for Biology and Head of the Indonesian Botanic Gardens, LIPI. He achieved Principle Scientist in 1990. Since 1993 he obtained professorship in parasitology at the Faculty of Veteriner – Bogor Agriculture University. He was the editor of journal of Treubia from 1992 to 1997, and remained as a proof reader until 2007.

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   MacKinnon, J. & K. Phillips, 1993. *Field Guide to the Birds of Borneo, Sumatra, Java and Bali*. Oxford University Press, Oxford, 491 pp.

   Stork, N.E., 1994. Inventories of biodiversity: more than a question of numbers. *In*: Forey,P.L., C.J. Humphries & R.I. Vane-Wright (eds.), *Systematics and Conservation Evaluation.* Clarendon Press (for the Systematics Association), Oxford, pp. 81-100.

   Maddison, D.R., 1995. Hemiptera. True bugs, cicadas, leafhoppers, aphids, etc.. Version 01 January 1995 (temporary). *http://tolweb.org/Hemiptera /8239/1995.01.01*. *In*: The Tree of Life Web Project, *http://tolweb.org/* (accessed on 27 November 2007).

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