Phylogenetic position of *Psittacula* parakeet bird from Enggano Island, Indonesia based on analyses of cytochrome b gene sequences.

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5th International Conference on Biodiversity. Madrid, Spain.
10th – 12th March 2016

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CORRECTION in ABSTRACT

1. 2rd sentence : ND2 **(delete)** -----→ cyt b

2. 5th sentence : ND2 and COI **(delete)**
genes (delete) ----→ gene

3. 9th sentence : 3.70 % for (*P.l. modesta* vs *P.l. defontainei*), 2.50 % for (*P.l. defontainei* vs *P.l. longicauda*) , and 1.60 % for (*P.l. modesta vs P l. longicauda*) ---- **delete**

↓

2.16 % for (*P.l. modesta* vs *P.l. defontainei*), 1.51 % for (*P.l. defontainei* vs *P.l. longicauda*), and 2.37 % for (*P.l. modesta vs P l. longicauda*)

About Enggano Island of Indonesia

MAP of Indonesia

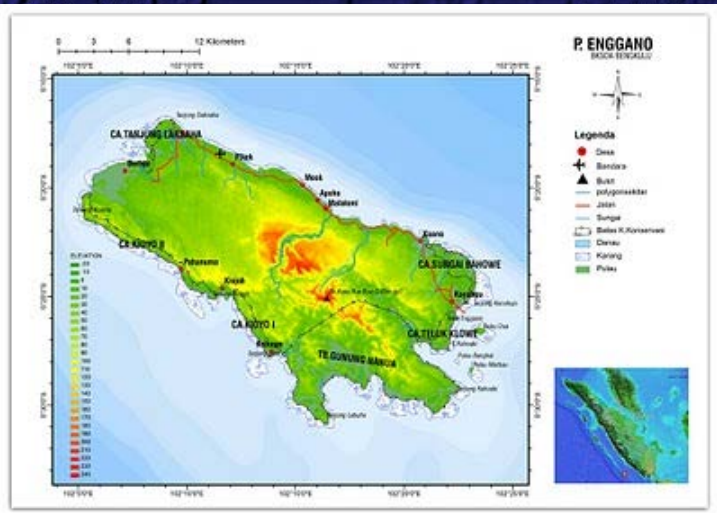
Indonesia hast housands islands; 5 are big : Sumatra is., Java is., Borneo/Kalimantan is., Celebes/Sulawesi is., and Papua is.



Enggano island

- Located in Indian Ocean, approx. 100 km SW offshore of mainland Sumatra.
- 5° 17’ - 5° 31’ S & 102° 05’ - 102° 25’ E .
- One of Indonesia’s outermost island.
- Geologically unique = Oceanic Island.
- It had never been a part of Sundaland.
- Separated from mainland Sumatra by marine basin of 2000 m deep = A perfect oceanic island.
- Bird speciation proses?
- Biologically unique = high endemicity





ABOUT *Psittacula* Parakeets BIRD

Psittaciformes

Psittacidae

Loriinae

Cacatuinae

Psittacinae



Calopsittacini

Cacatuini







Nestorini



Micropsittini



Psittaculini



Strigopini



Psittrichasini



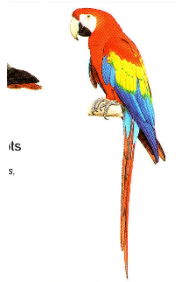
Cyclopsittacini



Psittacini



Arini



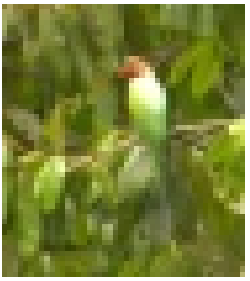
Platycercini

Lophopsittacini

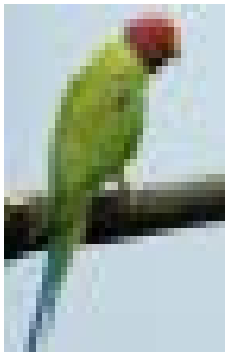
extinct



Psittacula parakeets



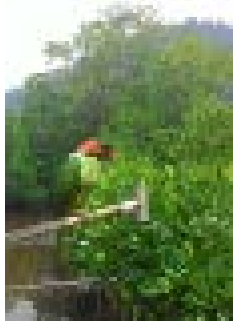
P. longicauda modesta
(Enggano Is.)



Psittacula cyanocephala
(Linné 1766), India,Pakistan



P. longicauda longicauda
(Sumatra,Borneo, Nias,
Bangka, Singapore)



P. longicauda defontainei
(Natuna is.)



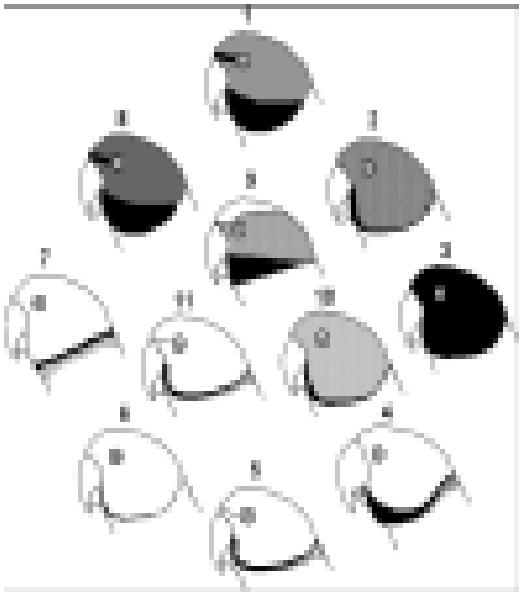
P.alexandri (Java is.)

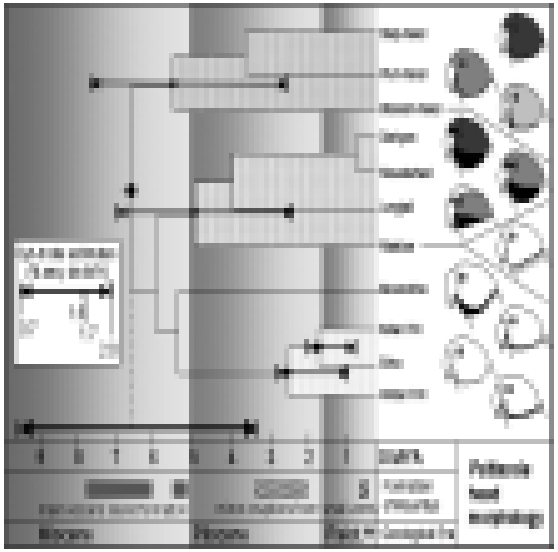
In the world there are **15-16 species** of *Psittacula* : *P. eupatria* , *P. wardi*, *P. krameri, P. echo, P. exsul, P. (himalayana) himalayana, P. (himalayana) finschii, P. intermedia, P. cyanocephala, P. roseata, P. columboides, P. calthropae, P. derbiana, P. caniceps*, *P. alexandri,* and ***P. longicauda***

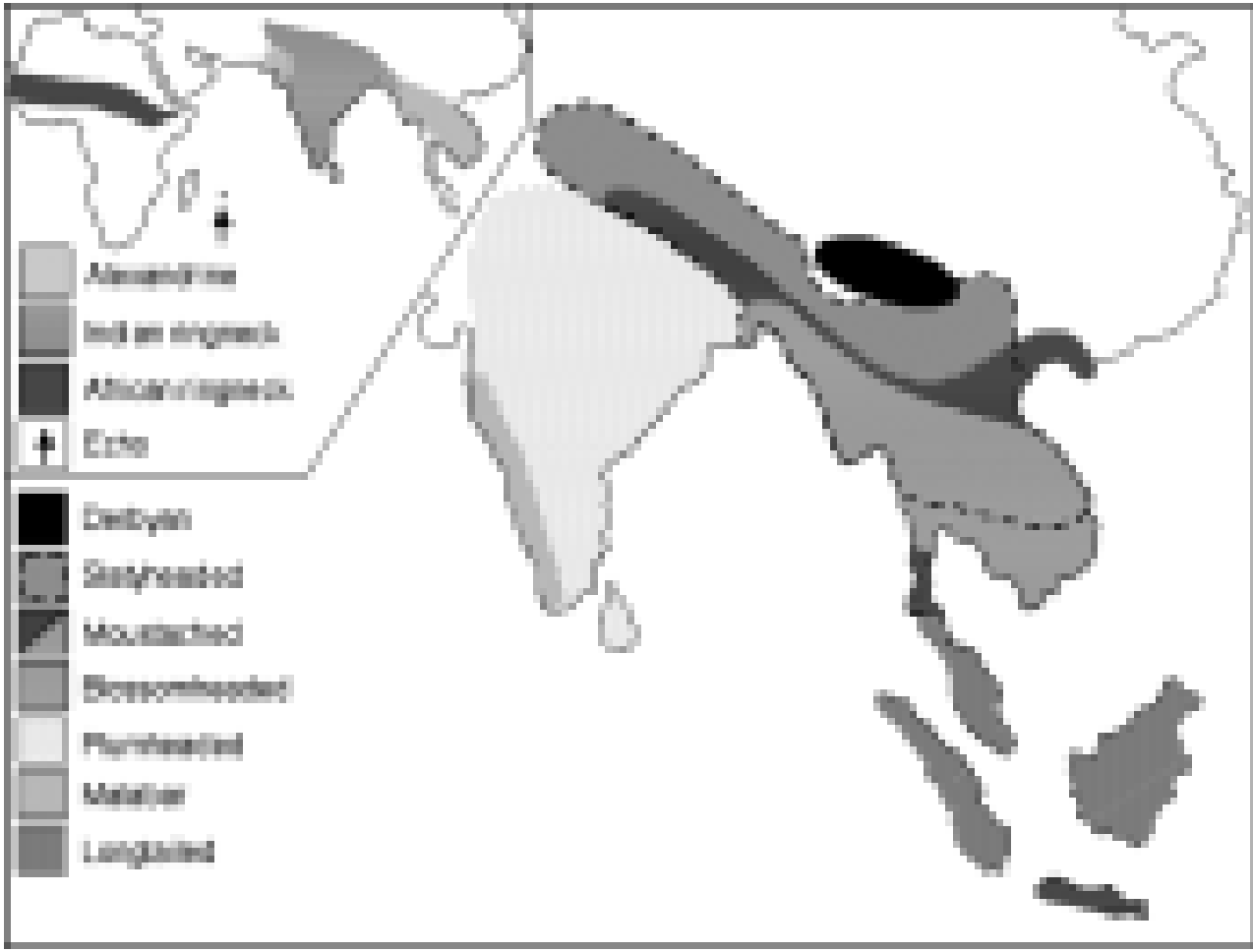
In the world: 5 subspecies of *P. longicauda* :
P. l. tytlery, P. l. nicobarica, and *P. l. longicauda, P. l. defontainei,* and *P. l. modesta*.

in Indonesia:
P. l. longicauda (Sumatra, Borneo, Riau Is.),
P. l. defontainei (Natuna Is.), and
P. l. modesta (Enggano Is.)

Distribution of *Psittacula Parakeet* forms (Groombridge J.J *et al.*, 2004)



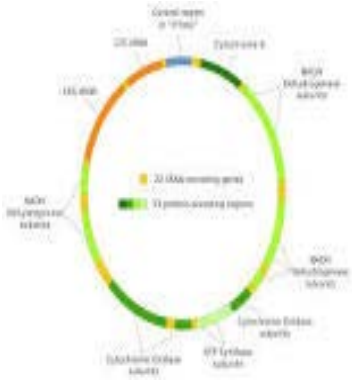




Distribution of Long-tailed Parakeet *Psittacula longicauda* (Boddaert 1783)



(Source: http://home.online.nl/psittaculaworld/Species/P-longicauda.htm)



MITOCHONDRIAL DNA



The mitochondrial genome of vertebrates is a small, circular molecule of 15-20 kb, with a compact and conserved organization in most, but not all, of the studied species (Wolstenholme, 1992).

In animals, it contains 37 genes: 13 protein-coding genes (3 units of cytochrome ocsidase (CO I-III), 7 subunit NADH-dehydrogenase (ND1-6 and ND 4L, 2 subunits ATPase (6 and 6L), cyt b), 22 transfer RNA genes (tRNAs) and 2 ribosomal RNA genes (12S and 16S rRNAs), and the control region (D-loop).

Because their structures were relatively simple and mutation rates were higher than nuclear DNA, the mtDNA sequences were used frequently to estimate phylogenetic relationships among animal taxa, genetic diversity, population genetics and molecular evolution

About cytochromre b gene

The cytochrome b gene (cyt b) from the mitochondrial genome contains speciesspecific information and has been used in phylogeny as well as in forensic investigations in previous studies. Thus, it has been confirmed the usefulness of cyt b analysis in identifying the biological origin of casework specimens (Parson, et al., 2000)

Mitochondrial cyt b sequences have been used to understand the genetic diversity for better conservation management of threatened species animals

Cytochrome-b gene (~1,143 bp) is reported as the most useful marker in recovering phylogenetic relationships among closely related taxa, at a variety of taxonomic levels

AIMS

1. How far the difergencies (sequence divergences) between *Psittacula* from Enggano island to others subspecies of *Psittacula longicauda* are

2. How far the difergencies (sequence divergences) between species of *Psittacula* are

3. Where phylogenetically the position of *Psittacula longicauda modesta* from Enggano island is

4. Genetically, whether *Psittacula* from Enggano is separated from others subspecies of Psittacula longicauda

5. Genetically, whether *Psittacula* from Enggano is as subspecies of *P. longicauda* or become a new species

METHODS

Blood samples taken from each live bird

preserved in 99% ethanol

DNA , extracted from 5 – 20 mg of dry blood

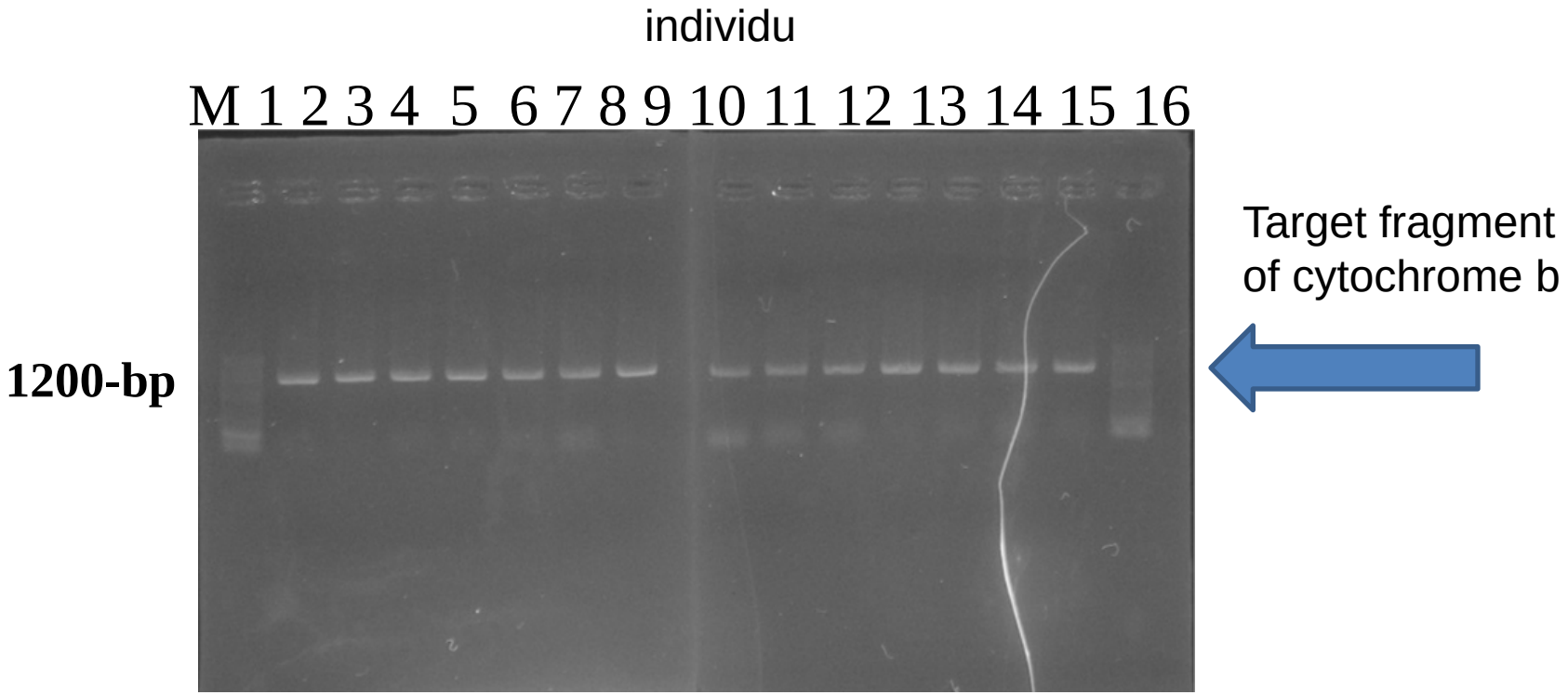
Cytochrome b gene Amplification by (PCR)

DNA sequencing

DNA sequences alignment, using MEGA5 softwarw

Phylogenetic trees analyses (MEGA5 software)

Target fragment of cyochrome b gene in this studi amplified by PCR



Bases composition (%) in the 869-bp of cytochrome b gene of Psittacula

Cytochrome b is protein coding ---→ amino acid ---→ codons

No.	Codon position	Tumine (T) %	Citosine (C) %	Adenin (A) %	Guanine (G) %	Total base
1	1st codon	10.32	48.66	37.65	3.37	290
2	2nd codon	23.55	29.67	24.66	22.11	290
3	3rd codon	37.23	28.50	20.24	14.03	289
4	Total	23.68	35.62	27.52	13.17	869

RESULTS

Genetic distances (bellow diagonal) and st. error (above diagonal) between species/subspecies of Psittacula

No.	Species name	1	2	3	4	5	6	7	8	9	10
1	*P. columboides*		0,0122	0,0121	0,0095	0,0096	0,0108	0,0096	0,0096	0,0096	0,0095
2	*P. finschii*	0,0926		0,0057	0,0078	0,0119	0,0106	0,0106	0,0099	0,0105	0,0103
3	*P. himalayana*	0,0937	0,0380		0,0092	0,0111	0,0109	0,0123	0,0107	0,0116	0,0116
4	*P. cyanochepala*	0,0749	0,0437	0,0651		0,0098	0,0105	0,0109	0,0107	0,0103	0,0104
5	*P. krameri*	0,0595	0,0855	0,0764	0,0767		0,0121	0,0112	0,0105	0,0102	0,0111
6	*P. roseata*	0,0808	0,0780	0,0851	0,0665	0,0825		0,0112	0,0106	0,0122	0,0114
7	*P. alexandri alexandri*	0,0661	0,0900	0,1003	0,0888	0,0712	0,0825		0,0081	0,0047	0,0033
8	*P. longicaoda defontainei*	0,0640	0,0866	0,0911	0,0878	0,0665	0,0712	0,0615		0,0080	0,0085
9	*P. longicaoda modesta*	0,0652	0,0844	0,0896	0,0832	0,0667	0,0665	0,0547	0,0216		0,0058
10	*P. longicauda longicauda*	0,0665	0,0886	0,0978	0,0874	0,0738	0,0852	0,0664	0,0151	0,0237	

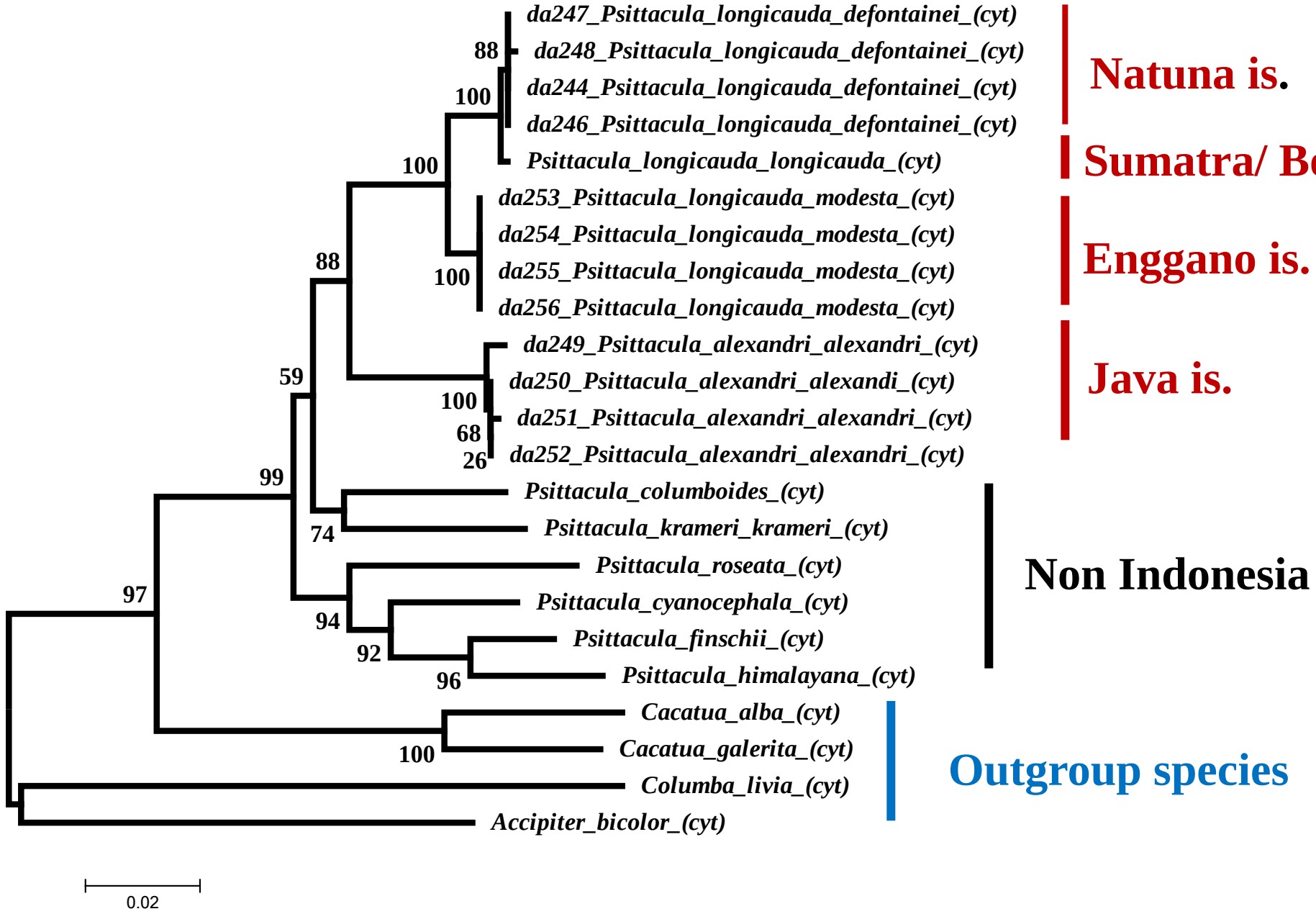
VARIABLE SITES in the 869-bp of cytochrome b gene sequences

Site

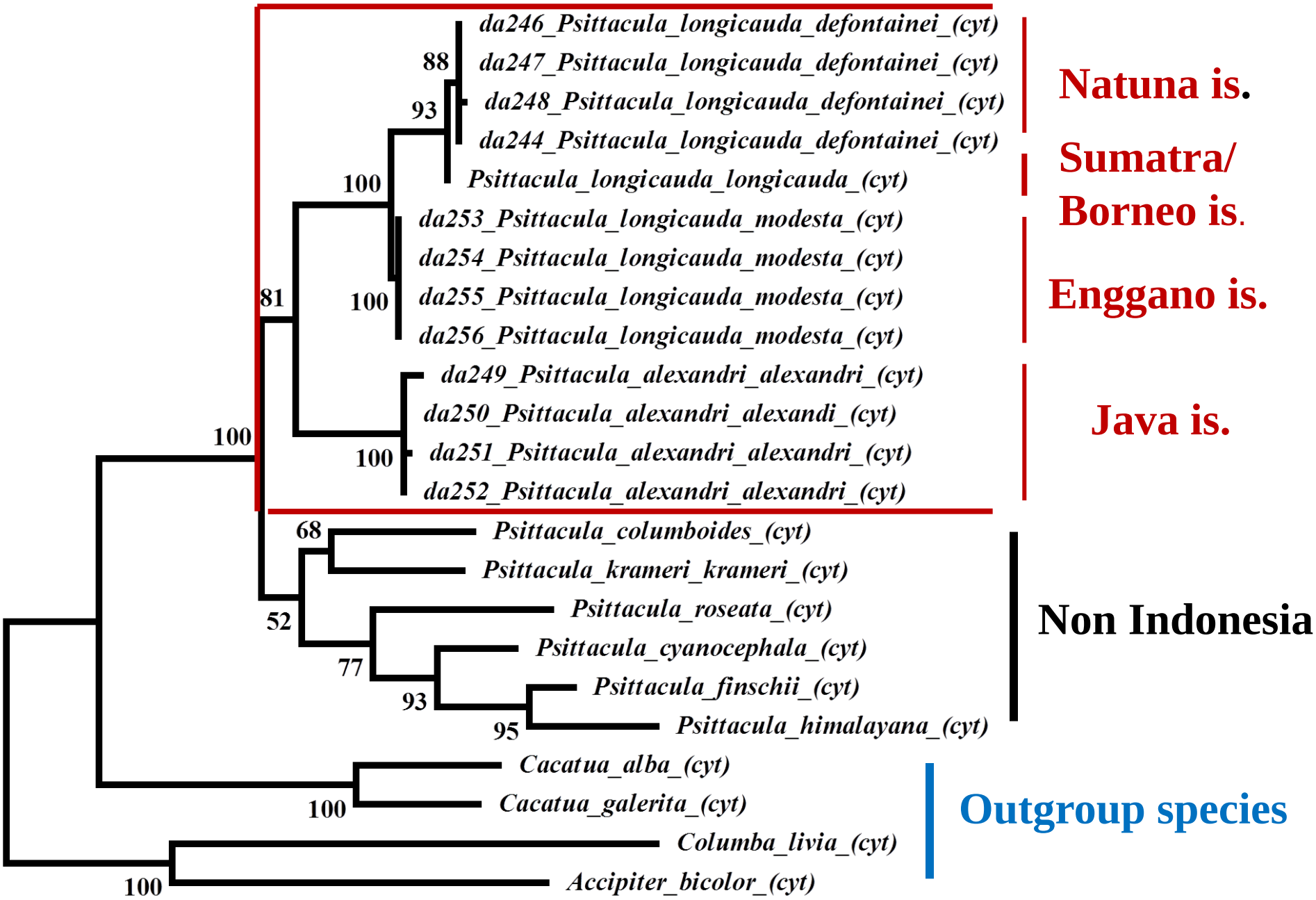
Species name	7	10	16	23	33	45	48	58	70	79	82	97	100	103	107	121	133	139	145	148	154	160	164	171	172	181	187	193	195	202	205	206	211	226	229	235	268	274	277	287	295	298	299	307	310	325	331	340	355
P. longicauda_longicauda_(cyt)	C	C	C	C	C	G	C	C	A	T	T	C	A	C	T	C	T	C	C	C	C	G	C	A	G	A	A	C	T	A	T	A	C	T	T	C	G	A	T	T	C	C	A	C	C	C	G	T	T
P.finschii	.	.	T	T	T	A	T	.	G	C	.	.	C	A	C	.	.	.	.	.	.	A	T	.	A	G	.	T	.	.	C	.	.	C	C	T	A	G	C	.	.	T	G	T	T	T	A	.	.
P. himalayana	.	.	T	T	T	A	T	.	G	C	.	.	C	A	C	.	.	.	.	T	T	A	T	T	.	.	.	T	.	.	C	.	.	C	.	T	A	.	C	.	.	T	G	T	T	T	A	.	C
P. cyanocephala	.	.	T	T	T	A	.	T	.	C	.	.	.	.	C	.	C	.	.	.	T	A	.	.	A	.	.	T	.	.	C	G	T	C	C	.	A	.	C	.	.	T	.	.	.	T	.	C	C
P. roseata	T	.	.	.	.	A	.	.	.	C	C	.	.	.	C	.	C	.	.	.	T	A	.	.	.	.	.	T	.	C	C	.	T	.	.	T	.	.	.	C	.	.	.	.	.	.	A	.	C
P. longicauda_defontainei_1	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
P.longicauda_defontainei_2	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
P.longicauda_defontainei_3	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
P. longicauda_defontainei_4	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
P. alexandri_1	.	T	.	.	T	A	.	T	.	C	C	T	.	.	C	T	.	A	.	.	T	A	.	.	A	.	G	T	C	.	.	.	.	.	.	.	.	.	C	C	T	.	.	.	.	T	.	.	.
P. alexandri_2	.	T	.	.	T	A	.	T	.	C	C	T	.	.	C	T	.	A	.	.	T	A	.	.	A	.	G	T	C	.	.	.	.	.	.	.	.	.	C	C	T	.	.	.	.	T	.	.	.
P. alexandri_3	.	T	.	.	T	A	.	T	.	C	C	T	.	.	C	T	.	A	.	.	T	A	.	.	A	.	G	T	C	.	.	.	.	.	.	.	.	.	C	C	T	.	.	.	.	T	.	.	.
P. alexandri_4	.	T	.	.	T	A	.	T	.	C	C	T	.	.	C	T	.	A	.	.	T	A	.	.	A	.	G	T	C	.	.	.	.	.	.	.	.	.	C	C	T	.	.	.	.	T	.	.	.
P. longicauda_modesta_1	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
P. longicauda_modesta_2	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
P. longicauda_modesta_3	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
P. longicauda_modesta_4	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.

	365	379	381	400	403	413	424	442	445	448	469	472	473	475	477	490	496	508	517	527	530	535	539	544	553	554	555	562	568	577	583	586	589	592	595	601	610	622	625	629	634	643	646	652	658
gicauda_longicauda_(cyt)	**C**	**T**	**C**	**A**	**T**	**A**	**C**	**C**	**T**	**A**	**A**	**C**	**A**	**T**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**G**	**G**	**T**	**C**	**T**	**C**	**T**	**C**	**C**	**C**	**C**	**G**	**A**	**A**	**C**	**C**	**C**	**C**	**A**	**C**	**T**	**T**	**C**	**A**
chii	.	**C**	.	.	**C**	**G**	.	.	**C**	.	**G**	.	**C**	**C**	**A**	.	**T**	**A**	**T**	**T**	.	**A**	**A**	**C**	.	**C**	**T**	.	.	.	.	.	**A**	.	.	.	.	.	.	.	.	**C**	**C**	.	.
nalayana	.	**C**	.	.	**C**	**G**	.	.	**C**	.	**G**	.	**C**	**C**	**A**	.	**T**	**A**	**T**	.	**T**	.	**A**	**C**	.	**C**	**T**	**C**	.	.	.	.	**A**	**G**	**G**	.	.	.	.	.	**T**	.	**C**	**T**	**G**
nocephala	.	.	.	.	**C**	**G**	.	**T**	**C**	.	.	.	**C**	**C**	**A**	.	**T**	**A**	.	.	.	**A**	**A**	**C**	.	**C**	**T**	.	.	**T**	.	**T**	**A**	.	.	.	.	.	.	.	.	**C**	**C**	.	.
eata	**T**	**C**	.	.	**C**	**G**	**T**	.	**C**	**G**	.	**T**	**C**	**C**	.	**T**	**T**	**G**	.	.	.	**A**	**A**	**C**	**T**	**C**	**T**	**C**	.	.	**T**	.	**A**	.	**G**	**A**	**T**	.	**T**	**G**	.	**C**	**C**	.	.
gicauda_defontainei_1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**A**	.	.	.	.	.	.	.	.	.	.	.	.
gicauda_defontainei_2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**A**	.	.	.	.	.	.	.	.	.	.	.	.
gicauda_defontainei_3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**A**	.	.	.	.	.	.	.	.	.	.	.	.
gicauda_defontainei_4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**A**	.	.	.	.	.	.	.	.	.	.	.	.
xandri_1	.	**C**	.	**G**	**C**	.	.	.	**C**	**G**	.	.	.	**C**	.	.	.	.	.	.	.	**A**	.	.	.	.	**T**	**C**	**T**	.	.	.	**A**	.	**G**	.	.	**T**	.	.	.	.	.	.	.
xandri_2	.	**C**	.	**G**	**C**	.	.	.	**C**	**G**	.	.	.	**C**	.	.	.	.	.	.	.	**A**	.	.	.	.	**T**	**C**	**T**	.	.	.	**A**	.	**G**	.	.	**T**	.	.	.	.	.	.	.
xandri_3	.	**C**	**T**	**G**	**C**	.	.	.	**C**	**G**	.	.	.	**C**	.	.	.	.	.	.	.	**A**	.	.	.	.	**T**	**C**	**T**	.	.	.	**A**	.	**G**	.	.	**T**	.	.	.	.	.	.	.
xandri_4	.	**C**	.	**G**	**C**	.	.	.	**C**	**G**	.	.	.	**C**	.	.	.	.	.	.	.	**A**	.	.	.	.	**T**	**C**	**T**	.	.	.	**A**	.	**G**	.	.	**T**	.	.	.	.	.	.	.
gicauda_modesta_1	.	.	.	.	.	.	.	.	**C**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**C**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
gicauda_modesta_2	.	.	.	.	.	.	.	.	**C**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**C**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
gicauda_modesta_3	.	.	.	.	.	.	.	.	**C**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**C**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
gicauda_modesta_4	.	.	.	.	.	.	.	.	**C**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**C**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

NJ tree of Psittacula based on 868 bp of cytochrome b gene sequences



ML tree of *Psittacula* based on 868- bp of cytochrome b gene sequences



CONCLUSIONS

1. Sequence divergences within *Psittacula longicauda :*

1. - *P. l. modesta* vs *P. l. longicauca* was 2.37 %
   - *P.l. modesta vs P.l. defontainei* was 2.16 %
   - *P. l. longicauda* vs *defontainei* was 1. 51 %

2. Mean sequence divergences between species of *Psittacula* was 5.12 %, ranged from 3.80 % (*P. finschii vs P. himalayana)* to 10.03 % (*P. himalayana vs P. alexandri*

3. Genetically, *Psittacula* from Enggano is (*P.l.modesta*) was separated from others subspecies of *Psittacula longicauda* (*P. l. longicauda + P. l. defontainei)*

4. Phylogenetically , the position of *Psittacula* from Enggano island (*Psittacula longicauda modesta*) was sister group of others subspecies of *Psittacula longicauda* (*P. l. longicauda + P. l. defontainei*)

5. My opinion, genetically, *Psittacula* from Enggano (*P. l. modesta*) is still a subspecies of *P. longicauda* or was not a new species of *Psittacula*

Acknowledgements

The recent study was supported by Enggano Expedition Project of Indonesian Institute of Sciences, Indonesia.

THANK YOU