Proceedings of the 1st International Conference on Natural Resources Engineering & Technology 2006
24-25th July 2006; Putrajaya, Malaysia, 77-97

Phylogenetic Relationships of Fruit Bats (Family: Pteropodidae) in Malaysia Inferred from partial mtDNA Cytochrome *b* gene

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Abstract

The taxonomic relationships of the Old World fruit bat family, Pteropodinae, by Anderson (1912) using morphological characters have been challenged by several authors. Previous studies using mitochondrial DNA (mtDNA) found major disagreement between morphology and molecular data in inferring the phylogeny of the fruit bats. Fifty-eight samples from 14 species of the Malaysian fruit bats (Family: Pteropodidae) was used in this study to examine on the phylogenogenetic relationship between species of fruit bats using 395 base pairs of partial mtDNA cytochrome *b* (cyt *b*) gene. Our phylogenetic analysis using neighbour-joining and maximum parsimony methods failed to support the monophyly of both the rousettine and cynopterine groups of the Subfamily Pteropodinae.

Keywords: Pteropodinae, mitochondrial DNA, phylogeny, Malaysia

1.0 Introduction

Bats are the members of the Order Chiroptera is widely distributed and the second most numerous groups after rodents in the world [1]. Chiroptera is divided into two distinct suborders, Megachiroptera and Microchiroptera, consist of 188 modern genera and about 977 modern species [2]

Megachiroptera includes all frugivorous bats, feeding on fruits, flower, nectar and pollen, while Microchiroptera consists of insects eating and carnivorous species bats [1, 3, 4]. According to Nowak (1994), the suborder of Megachiroptera consists of only one family, the Pteropodinae, with 42 genera and 166 species recorded worldwide. Pteropodids consist of all flying foxes and Old World fruit bats which are further divided into four subfamilies, namely, the diverse subfamily Pteropodinae, subfamily Macroglossinae (which consist of six genera of blossom bats, dawn bats, long-tongued fruit bats and relatives), the aberrant subfamily Harpyionycterinae, and the subfamily Nyctimeninae [2]. The Malaysia pteropodids consist the subfamily Pteropodinae, which are specialized fruit and flower eating genera and the subfamily Macroglossinae, which contains the genera that are specialised on diet of pollen and nectar [2].

The classical taxonomy by Andersen (1912) had categorised the subfamily Pteropodinae into three sections, namely, rousettine, epomophorine and cynopterine; and in the subfamily

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Macroglossinae are the eonycterine and notopterine bats. Macroglossinae is monophyletic sister-group to the *Rousettus, Cynopterus* and *Epomophorus* [5].

Species of megachiropterans can be found throughout the Old World tropics and subtropics from Africa through southern Asia to Australia and on the islands in Indian and also western Pacific Oceans [3]. Pteropodids are relatively small to very large bats with the forearm length from 40 to 220mm. They have large eyes and are dependent on sight and smell to navigate and find food [3]. They become active during dusk and dawn and some species fly relatively long distances to search for food. The species of fruit bats are very important pollinators and seed disperse for 433 species of plants, which are economically important to human including natural products useful to man from about 163 species of plants [3]. Javier *et al*. (1999), stated that the Malaysian-Indonesian rainforest region along with the African rainforest belt across the Congo basin as the two areas with the highest diversity of fruit bats [6]. Malaysia alone has 18 species of pteropodids making it the fourth highest in terms of world diversity [7].

Many may aspects of the equilibrium island biogeography [8], systematic, diversity pattern, ecology and conservation have been explored within selected Malaysian mammalian fauna [9, 10]. In the biogeographical context, speciation and timing of diversification within set of closely related species have not been well documented among mammals [8]. Origin and evolutionary of bats are poorly understood with recent identification of morphologically cryptic species showed that the diversity within the order Chiroptera maybe under estimated by current taxonomy [3, 10].

There are various studies of the ecology and morphological of Chiroptera in Southeast Asia and are well documented. There are some molecular studies were carried out on in megachiropteran to review the general evolutionary framework suggested by Andersen (1912) and the molecular studies focused more on the Australasian species rather than Indo Malayan species [4, 5]. Kitchener *et al.* (1995), study on the morphological variation in *Pteropus lombocensis* in Indonesia [11]. The study that there are two broad morphological forms occurred among the species in Nusa Tenggara from the analysis. Tuen *et al.* (2002) have conducted an inventory of the bat species found in the Crocker Range area and added eight species of bats to the record of 41 species in the area [12].

Bastian *et al.* (2001) has analysed five species of the existed pteropodids in Philippines using the complete sequences of the cyt *b* gene and found the genetic divergence between *Rousettus amplexicaudatus*, *Eonycteris spelaea and Cynopterus brachyotis* was small [13]. Recent study by Abdullah *et al.* (2000), Abdullah (2003), Jayaraj *et al.* (2004), Campbell *et al*. (2004) and Ahmad (2005) discovered at least two cryptic species within the *C. brachyotis* complex [10, 14,15,.16,17]. Kirsch *et al.* (1995) have conducted hybridization study of 19 genera using single-copy DNA differs with the monophyly of the cynopterine section and suggested the rousettine section are not monophlyletic taxa [18]. The finding from Hollar and Springer (1997) used 12S ribosomal RNA gene and Ahmad (2005) used cyt *b* mtDNA gene agree with the single-copy DNA hybridization work by Kirsch *et al.* (1995) in opposing rousettine monophyly [17 -19] .

The cyt *b* has been considered one of most useful genes for the phylogenetic work and probably best known mitochondrial gene with respect to structure and function of its protein product [20]. Cyt *b* is chosen as a phylogenetic probe because it may easier to align a protein coding sequences that has evolved over the period spanning the origin of mammalian order

and has been used for the diversity of systematic questions because it sufficiently variable for population analysis and conservative enough for phylogenetic analysis [10, 20]. Cyt *b* is one of the best known of the 9-10 protein which makes up complex III of mitochondrial oxidative phosphorylation system and it is the only one of them encoded by mitochondrial genome. This gene has proved to be informative and appropriate for studies aimed at intraspecific and interspecific variation across a variety of mammals. The cyt *b* gene of the mtDNA is perhaps the most well studies gene segment in fishes and several study have pointed out the usefulness of this particular gene segment I elucidating evolutionary patterns in fishes and mammals [10, 21. It has the ability to retain a history of past isolation, which has been well demonstrated [22, 23]. Furthermore, the cytochrome *b* gene has an advantage over those studies before by cloning and sequencing from taxonomically graded series of vertebrate mitochondria.

Nevertheless, the classifications on fruit bats by Andersen (1912) remain the most comprehensive evolutionary framework on the relationships among approximately 200 species of pteropodids [4]. Due to much contradiction between the morphological and genetic data, the current taxonomic status and phylogenetic relationship of the Malaysian pteropodids remains unclear. Therefore, in this study, we attempt to infer the phylogenetic relationship and determine the taxonomic status among species of pteropodids using partial cyt *b* gene.

2.0 Materials and methods

2.1 Study area and DNA extraction

Bats were collected from various locations throughout Sarawak, Sabah and Malay Peninsular (Table 1, Appendix A). Bats were collected using standard mist nets and then euthanized using chloroform, dissected exposing the stomach and intestine for better preservation and further preserved in 95% ethanol prior to genetic analysis. A total of 58 pteropodids were collected and were identified following keys provided by Payne *et al.* (1985) [24]. Some samples were obtained from UNIMAS museum collections, museum of the Department of Wildlife and National Parks and Sabah Parks museum. Three additional specimens of microchiroptera (a *Megaderma spasma* and two *Nycteris tragata*) were used in this study as outgroup.

Total DNA was extracted from muscle tissue using modified cetyltrimethylammonium bromide CTAB method with the presence of Proteinase K. The quality and approximate yield of DNA were determined by electrophoresis run in a 1% agarose gel containing ethidium bromide at 90V for 30 minutes and visualized under UV light [25].

2.2 Amplification, purification and Sequencing

Amplifications were carried out in a thermocycler machine (BIO-RAD My Cycler). Optimization of PCR was done using 25µL reaction mixtures containing 2µL DNA (~50-100ng), 2.5µL 10X PCR Buffer, 0.5µL dNTPs, 1.5µL $MgCl_2$ (Promega), 1.25µL of each primer, and 0.5µL *Taq* polymerase (Promega). Two cyt *b* primers were used, namely 5'-TGACT TGAAR AACCA YCGTT G- 3', known as GluDG-L (Palumbi *et al*., 1991) and 5'-CCCTC AGAAT GATAT TTGTC CTCA- 3', known as CB2-H [26]. Amplification was done using the following PCR profile; a initial denaturation at 94^{o}C for 2 min followed by 30 cycles of 94^{o}C for 1 min, annealing at 56 ^{o}C for 1 min and extension at 72 ^{o}C for 2 min. This

was followed by a final extension period of 72 ^{o}C for 5 min. Each PCR product was run on 1% agarose gel for confirmation of equal length against appropriate size marker. The PCR products obtained were later purified using DNA purification kits (Fermentas) according to the manufacturer's instructions. The purified PCR products were directly sequenced using the ABI PRISM BigDye terminator cycle sequencing kit (ACGT) only for the forward strand. Sequencing was carried out on an ABI 377 automated sequencer (PE Applied Biosystem).

2.3 Sequence Alignment, Estimate of Divergence and Phylogenetic Analyses

Multiple alignments of the nucleotide sequences were done using the program ClustalX program (version 1.81; Thompson *et al*., 1997) and subsequently aligned by eye [27]. The analysis involving pair-wise distance using the Kimura two-parameter model (Kimura, 1980) were done to estimate genetic distances among the species of pteropodids using *MEGA* version 2.1 [28, 29](Kumar *et al*., 2001). The nucleotide compositions were also estimated for each species using *MEGA.*

Phylogenetic trees were constructed using the neighbour-joining (NJ) and unweighted maximum parsimony (MP) methods implemented in PAUP (version 4.0b4; Swofford, 1998) [30]. The NJ clustering was performed using the Kimura two-parameter model while MP analysis corresponds to the HKY85 evolutionary model [31](Hasegawa *et al*., 1985). All trees were rooted with three Microchiroptera sequences from *Megaderma spasma* and *Nycteris tragata* as outgroups. Phylogenetic confidence was estimated by bootstrapping (Felsenstien, 1985) with 1000 replicate data sets for NJ and 100 replicate data sets for MP methods[32].

3.0 Results and Discussion

3.1 Sequence Analysis

Partial sequences for length of 395-bp comprising of the cyt *b* gene from fourteen species of the Malaysian pteropodids (58 specimens) were successfully sequenced and aligned (Appendix B). Comparing with the 1140-bp mitochondrion genome (using *C. brachyotis* taken from GenBank, AB046320), our sequences begins at the 8-bp until the 402-bp of the 1140-bp mitochondrial sequence. The base composition showed an anti-G bias, as shown in Table 2, (Appendix A) which is characteristic for the mitochondrial gene [33, 34] (Cantatore *et al*., 1994; Briolay *et al*., 1998). From the 395-bp sequence, 212 (53.7%) were polymorphic (segregating) sites were observed. Among the 212 variable sites, 198 (93.4%) were parsimoniously informative sites. Part of the sequences were registered with GenBank and given accession number DQ087395 to DQ087396 and DQ097804 to DQ097835 while the rest were still in process.

The pairwise genetic distances (number of nucleotide substitutions per site) calculated using the Kimura two-parameter model is shown in Table 3, (Appendix A). Pairwise comparisons among all the sequences range from of 5.3% to 30.0% in differences. Within the Subfamily Pteropodinae, distances range from 5.3% to 29.2% in differences, with the least differences observed between *C. brachyotis* (FA < 59.0 cm) and *C. horsfieldi*. All the pteropodids sequences were distantly related to both the outgroup sequences with an average distance value of 25.0% (data not shown).

3.2 Phylogenetic Tree Analyses

Phylogenetic trees constructed using NJ and MP methods are summarised in figure 1 and 2, respectively. Both topologies of the phylogenetic trees construction obtained a similar manner where differing slightly in grouping the genera. High confidence values were observed within the genus relationship (*Pteropus, Cynopterus, Megaerops* and *Rousettus,* with bootstrap values of 90% and above). Weak bootstrap values were observed for some inter genera relationships.

NJ method produced a tree, which formed five major clades (Figure 1, Appendix A). The first clade was formed by the genera *Cynopterus* and *Megaerops* with 53% bootstrap value. *Pteropus* (with 94% bootstrap value), *Dyacopterus* (with 100% bootstrap value) and *Rousettus* (with 90% bootstrap value) form the second, third and fourth clades respectively while *Aethalops, Chironax, Balionytcteris* and *Penthetor* formed the fifth group.

Using MP methods, where all characters were weighted equally, the tree length was 762 with consistency index (CI) of 0.4291 and retention index (RI) of 0.8312. Tree topology separates the pteropodids into six major clades with a 99% bootstrap value (Figure 2, Appendix A). The first group consist of the *Cynopterus* (*C. brachyotis*, *C. horsfieldi* and *C. sphinx*) with a 100% bootstrap value. *Megaerops* with 99% bootstrap value, *Dyacopterus* with 100% bootstrap value, *Rousettus* with 91% bootstrap value and *Pteropus* with 88% bootstrap value form the second, third, fourth and fifth group respectively. *Aethalops, Chironax, Balionytcteris* and *Penthetor* form the sixth group.

Currently, our study using molecular approaches is not consistent with Andersen (1912) classification and proposed for a re-organisation on their taxonomic status. Our phylogenetic analyses were able to clarify some aspects of the positioning of the pteropodids at suprageneric level (i.e. grouping of the cynopterine group) and successful elucidate the intra-species categories (relationships between cynopterine group).

According to Andersen (1912), the genus *Rousettus* and *Pteropus* are assembled within the group rousettines. From our phylogenetic trees, both genera did not from a cluster together which is in concordance with Ahmad (2005), Bastian *et al.* (2001), Hollar and Springer (1997) and Kirsch *et al.* (1995). Instead, *Rousettus* clustered with *Eonycteris* (Bastian *et al*., 2001 where genus *Eonycteris* is from the Subfamily Macroglossinae). The clustering of *Rousettus* and *Pteropus* are in agreement with the findings by Bastian *et al*. (2001), where they construct phylogenetic tree that *Pteropus* formed separately from *Rousettus*. We reject on the observation by Bastian *et al*. (2001) on the sister taxon of *Pteropus* with *Cynopterus* and *Ptenochirus* as our study find that the clear reflect that *Megaerops* as the sister taxon of *Cynopterus.*

Our study clearly rejects the monophyletic status of some groups; cynopterine and rousettines as established by the other molecular studies [13, 17, 18, 19]. This first attempt using the molecular approach is focus on the existed fruit bats species in Malaysian region.

The analysis using partial cyt *b* gene sequences among the Malaysian fruit bats species provided evidence of their phylogenetic relationship. Positioning of some species in phylogenetic tree that were grouped referring to their suprageneric (rousettine and cynopterine) grouping by Andersen (1912) was clarified. The approach study method on the nucleotide sequences data employing construct phylogeny trees (neighbor-joining and

maximun-parsimony) with bootstrap analysis helped us to clarify the relationship. At suprageneric level, all the methods of phylogenetic construction revealed that *Pteropus* formed separately from *Rousettus* and was excluded from the rousettine section. Overall, the phylogenetic analysis in this study was able to elucidate some confusion on the relationships among pteropodids. In conclusion, our results reconfirm some of the findings by several authors (using molecular approaches) particularly about the obscure monophyletic status of *Pteropus* and *Rousettus*, and also the polyphyletic status of the cynopterine group. Nevertheless, an increased in the number of species analysed will be able to discriminate and explain the most possible relationships between the species of pteropodids analysed.

Acknowledgement

This study was supported by the UNIMAS Fundamental Grant 232(23)/2000 and the Ministry of Science, Technology and Innovation IRPA grant number 09-02-09-1022-EA001. We wish to thank the Department of Wildlife and National Parks, Sarawak Forest Department, Sabah Wildlife Department, Sabah Parks and Director General, Department of Wildlife and National Parks, Kuala Lumpur (PERHILITAN Museum); for their permission to examine and collect samples during this study, Sarawak Forest Corporation for facilities and help. Also thanks Besar Ketol, Wahap Marni, Isa Sait, Faculty of Resource Science and Technology administrative staff for various supports and their assistance throughout the study; Universiti Malaysia Sarawak librarians for providing loans of references materials and Universiti Malaysia Sarawak for various administrative and logistic assistance.

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APPENDIX A

Table 1 Coding, localities, deposition and GenBank accession number for each individuals used in study.

Coding	Species	Localities	Sex	FA (cm)	Deposit	GenBank Acc. No.
RV005	*A. alecto* 1	Murud, S'wak	F	43.27	Zoological Museum, UNIMAS	-
RV006	*A. alecto* 2	Murud, S'wak	M	44.39	Zoological Museum, UNIMAS	-
TK004	*A. alecto* 3	Sabah Park, Sabah	M	-	Zoological Museum, UNIMAS	-
Pb001	*A. alecto* 4	Borneo Height,S'wak	M	43.72	Zoological Museum, UNIMAS	DQ097819
C468	*B. maculata* 1	Taman Negara, PMalaya	M	43.60	Perhilitan Museum, PMalaya	-
C0882	*B. maculata* 2	Taman Negara, PMalaya	F	40.60	Perhilitan Museum, PMalaya	-
B0217	*B. maculata* 3	Mt. Gading, S'wak	F	43.80	Zoological Museum, UNIMAS	-
BH75	*B. maculata* 4	Borneo Height,S'wak	F	43.26	Zoological Museum, UNIMAS	-
KNP021	*B. maculata* 5	Kubah, S'wak	F	39.52	Zoological Museum, UNIMAS	-
KNP039	*B. maculata* 6	Kubah, S'wak	F	39.56	Zoological Museum, UNIMAS	-
C025	*C. melanocephalus* 1	Tanah Rata, PMalaya	F	48.20	Perhilitan Museum, PMalaya	-
C0473	*C. melanocephalus* 2	Raub, PMalaya	F	47.60	Perhilitan Museum, PMalaya	-
A96340	*C. melanocephalus* 3	Kubah, S'wak	M	45.15	Zoological Museum, UNIMAS	-
A6322	*C. melanocephalus* 4	Lambir, S'wak	F	44.41	Zoological Museum, UNIMAS	-
M286	*C. brachyotis* 1	Taiping, PMalaya	F	65.10	Perhilitan Museum, PMalaya	-
M504	*C. brachyotis* 2	UM, PMalaya	F	60.30	Perhilitan Museum, PMalaya	-
PL003	*C. brachyotis* 3	Lakei Island, S'wak	M	64.53	Zoological Museum, UNIMAS	-
BNP229	*C. brachyotis* 4	Bako NP, S'wak	F	67.04	Zoological Museum, UNIMAS	-
JC75	*C. brachyotis* 5	Jambusan Cave, S'wak	-	-	Zoological Museum, UNIMAS	DQ097805
Ba004	*C. brachyotis* 6	Batang Ai, S'wak	M	51.67	Zoological Museum, UNIMAS	-
Ba014	*C. brachyotis* 7	Batang Ai, S'wak	M	52.99	Zoological Museum, UNIMAS	-
SD40	*C. brachyotis* 8	Sg. Dusun, PMalaya	-	-	Zoological Museum, UNIMAS	DQ097826
M215	*C. brachyotis* 9	Perlis SP, PMalaya	M	57.10	Perhilitan Museum, PMalaya	DQ097806
M310	*C. brachyotis* 10	Taiping, PMalaya	M	58.10	Perhilitan Museum, PMalaya	DQ097809
A96318	*C. horsfieldi* 1	Kubah, S'wak	M	72.91	Zoological Museum, UNIMAS	DQ097813
A96323	*C. horsfieldi* 2	Kubah, S'wak	F	77.20	Zoological Museum, UNIMAS	DQ097814
Pb002	*C. horsfieldi* 3	Borneo Height,S'wak	F	77.29	Zoological Museum, UNIMAS	DQ097820
JC70	*C. horsfieldi* 4	Jambusan Cave, S'wak	-	-	Zoological Museum, UNIMAS	DQ097804
MH033	*C. horsfieldi* 5	Perlis SP, PMalaya	M	71.30	Perhilitan Museum, PMalaya	DQ097810
SD24	*C. horsfieldi* 6	Sg. Dusun, PMalaya	-	-	Zoological Museum, UNIMAS	DQ097825

continued Table 1

Coding	Species	Localities	Sex	FA	Deposit	GenBank Acc. No.
P29	*C. sphinx* 1	Taiping, PMalaya	-	-	Perhilitan Museum, PMalaya	DQ097818
P10	*C. sphinx* 2	Selama, PMalaya	-	-	Perhilitan Museum, PMalaya	DQ097816
MS095	*C. sphinx* 3	Perlis SP, PMalaya	-	-	Perhilitan Museum, PMalaya	DQ097812
WK72	*C. sphinx* 4	Wang Kelian, PMalaya	-	-	Perhilitan Museum, PMalaya	DQ097830
MS070	*C. sphinx* 5	Perlis SP, PMalaya	-	-	Perhilitan Museum, PMalaya	DQ097811
10	*D. spadiceus* 1	ITM site, S'wak	F	-	Zoological Museum, UNIMAS	DQ097831
P024	*D. spadiceus* 2	Mt. Pueh	F	-	Zoological Museum, UNIMAS	DQ097817
BH103	*D. spadiceus* 3	Borneo Height,S'wak	F	76.63	Zoological Museum, UNIMAS	-
MWC005	*D. spadiceus* 4	Matang WC, S'wak	F	78.34	Zoological Museum, UNIMAS	-
	M. spasma 1				Zoological Museum, UNIMAS	-
Pb010	*M. ecaudatus* 1	Borneo Height,S'wak	M	53.43	Zoological Museum, UNIMAS	DQ087396
Pb025	*M. ecaudatus* 2	Borneo Height,S'wak	M	52.93	Zoological Museum, UNIMAS	-
RV036	*M. ecaudatus* 3	Murud, S'wak	F	59.98	Zoological Museum, UNIMAS	-
B035	*M. ecaudatus* 4	Sg. Karang, PMalaya	F	56.00	Zoological Museum, UNIMAS	-
F920824	*M. wetmorei* 1	Kuala Lompat, PMalaya	F	50.70	Perhilitan Museum, PMalaya	-
F920825	*M. wetmorei* 2	Kuala Lompat, PMalaya	F	52.80	Perhilitan Museum, PMalaya	-
	N. tragata 1				Zoological Museum, UNIMAS	-
	N. tragata 2				Zoological Museum, UNIMAS	-
D.005	*P. lucasi* 1	Musang Cave, PMalaya	F	64.00	Zoological Museum, UNIMAS	-
RA011	*P. lucasi* 2	Beruas, PMalaya	M	64.33	Zoological Museum, UNIMAS	-
KNP048	*P. lucasi* 3	Kubah, S'wak	M	62.18	Zoological Museum, UNIMAS	-
Pb031	*P. lucasi* 4	Borneo Height,S'wak	F	63.83	Zoological Museum, UNIMAS	-
R96790	*P. hypomelanus* 1	Langkawi, PMalaya	-	-	Perhilitan Museum, PMalaya	-
PH03	*P. hypomelanus* 2	Talang Kecil ,S'wak	-	-	Sarawak Museum, Sarawak	-
PH04	*P. hypomelanus* 3	Talang Besar, S'wak	-	-	Sarawak Museum, Sarawak	DQ097823
PP1	*P. vampyrus* 1	Patok-Patok, Sarawak	M	-	Zoological Museum, UNIMAS	DQ097824
SR1	*P. vampyrus* 2	Serian, Sarawak	F	-	Zoological Museum, UNIMAS	DQ097828
SR2	*P. vampyrus* 3	Serian, Sarawak	-	-	Zoological Museum, UNIMAS	DQ097829
1019	*R. amplexicaudatus* 1	P. Balambangan, Sabah	F	-	Zoological Museum, UNIMAS	DQ097832
1021	*R. amplexicaudatus* 2	P. Balambangan, Sabah	F	-	Zoological Museum, UNIMAS	DQ097833
SNP41	*R. spinalatus* 1	Similajau, S'wak	-	-	Zoological Museum, UNIMAS	-

Table 2 Nucleotide Composition For Each Species Used In This Study

No	Species	Nucleotide Composition			
		A	C	T	G
1	*C. brachyotis* 1	21.7	29.1	29.6	14.2
2	*C. brachyotis* 2	21.7	29.1	29.6	14.2
3	*C. brachyotis* 3	26.8	29.6	29.1	14.4
4	*C. brachyotis* 4	27.1	29.4	28.9	14.7
5	*C. brachyotis* 5	27.1	29.4	29.1	14.4
6	*C. horsfieldi* 1	26.1	30.4	28.6	14.9
7	*C. horsfieldi* 2	26.3	30.1	28.6	14.9
8	*C. horsfieldi* 3	26.3	30.1	28.4	15.2
9	*C. horsfieldi* 4	26.3	30.1	28.6	14.9
10	*C. horsfieldi* 5	26.1	30.4	28.4	15.2
11	*C. horsfieldi* 6	26.1	30.4	28.6	14.9
12	*C. brachyotis* 6	25.6	30.9	27.3	16.2
13	*C. brachyotis* 7	25.3	31.1	27.3	16.2
14	*C. brachyotis* 8	26.1	30.4	27.3	16.2
15	*C. brachyotis* 9	26.8	29.6	27.8	15.7
16	*C. brachyotis* 10	25.6	30.9	27.6	15.9
17	*C. sphinx* 1	27.1	29.6	28.9	14.4
18	*C. sphinx* 2	27.1	29.6	28.9	14.4
19	*C. sphinx* 3	27.6	29.4	29.1	13.9
20	*C. sphinx* 4	26.6	30.1	28.6	14.7
21	*C. sphinx* 5	26.8	29.9	28.6	14.7
22	*M. ecaudatus* 1	28.6	27.6	28.1	15.7
23	*M. ecaudatus* 2	28.1	28.1	28.1	15.7
24	*M. ecaudatus* 3	26.8	29.4	28.4	15.4
25	*M. ecaudatus* 4	27.8	28.4	28.6	15.2
26	*M. wetmorei* 1	27.3	29.1	28.6	14.9
27	*M. wetmorei* 2	27.3	29.1	28.6	14.9
28	*D. spadiceus* 1	27.1	27.6	28.9	16.5
29	*D. spadiceus* 2	27.3	27.3	29.4	15.5
30	*D. spadiceus* 3	26.6	28.2	28.7	16.5
31	*D. spadiceus* 4	25.6	29.1	29.4	15.9
32	*R. amplexicaudatus* 1	27.1	30.9	27.3	14.7
33	*R. amplexicaudatus* 2	26.8	31.1	27.6	14.4
34	*R. spinalatus* 1	24.8	32.4	27.3	15.4
35	*P. hypomelanus* 1	25.1	30.9	28.9	15.2
36	*P. hypomelanus* 2	25.1	30.9	28.9	15.2
37	*P. hypomelanus* 3	25.1	30.9	28.9	15.2
38	*P. vampyrus* 1	27.1	29.1	28.4	15.4
39	*P. vampyrus* 2	27.1	29.1	28.4	15.4
40	*P. vampyrus* 3	27.1	29.1	29.1	14.7
41	*P. lucasi* 1	29.1	27.1	30.6	13.2
42	*P. lucasi* 2	29.1	27.1	30.6	13.2
43	*P. lucasi* 3	29.9	26.6	30.6	12.9
44	*P. lucasi* 4	29.6	26.8	30.9	12.7
45	*B. maculata* 1	25.8	31.1	28.4	14.7
46	*B. maculata* 2	25.1	31.4	28.4	14.7

continued Table 2

No	Species	Nucleotide Composition			
		A	C	T	G
47	*B. maculata* 3	25.1	32.2	28.1	14.7
48	*B. maculata* 4	25.1	31.9	27.8	15.2
49	*B. maculata* 5	25.3	31.6	27.8	15.2
50	*B. maculata* 6	25.3	31.6	28.1	14.9
51	*C. melanocephalus* 1	28.1	31.1	26.3	14.4
52	*C. melanocephalus* 2	28.1	31.1	26.3	14.4
53	*C. melanocephalus* 3	27.6	31.6	26.8	13.9
54	*C. melanocephalus* 4	27.8	31.4	26.3	14.4
55	*A. alecto* 1	27.8	29.9	27.3	14.9
56	*A. alecto* 2	27.6	30.1	27.3	14.9
57	*A. alecto* 3	27.3	30.4	27.3	14.9
58	*A. alecto* 4	27.1	31.1	26.8	14.9
59	*M. spasma* 1	27.9	29.9	27.4	14.7
60	*N. tragata* 1	28.4	29.4	27.2	15.0
61	*N. tragata* 2	24.9	33.8	25.6	15.7
	Average (outgroups excluded)	26.8	29.9	28.4	14.9
	Average (outgroups included)	26.8	29.9	28.3	14.9

Table 3 Pairwise distances in percentage among fourteen species of malaysian pteropodids analysed based on the partial cyt *b* gene. The distances were calculated using kimura's two-parameter model of nucleotide substitution

No	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	*C. brachyotis* (FA > 59.0 cm)	-																
2	*C. horsfieldi*	10.7	-															
3	*C. brachyotis* (FA <59.0 cm)	10.6	5.3	-														
4	*C. sphinx*	11.0	7.1	8.5	-													
5	*M. ecaudatus*	19.2	17.9	19.0	17.8	-												
6	*M. wetmorei*	22.9	20.8	22.0	22.0	13.3	-											
7	*D. spadiceus*	20.6	16.6	19.1	18.1	20.1	22.7	-										
8	*R. amplexicaudatus*	18.0	17.0	19.1	19.5	23.3	21.5	18.0	-									
9	*R. spinalatus*	20.7	19.1	19.8	21.1	21.4	22.3	19.4	13.1	-								
10	*P. hypomelanus*	19.9	18.4	19.5	17.5	23.1	23.6	18.9	16.7	16.7	-							
11	*P. vampyrus*	22.4	17.5	19.0	19.2	21.0	24.5	20.8	20.3	18.3	10.9	-						
12	*A. alecto*	22.8	21.9	22.5	22.2	20.7	20.8	23.1	21.1	18.9	18.6	18.8	-					
13	*C. melanocephalus*	21.8	24.5	22.9	21.1	26.7	25.8	22.4	23.9	23.5	22.1	22.0	20.7	-				
14	*P. lucasi*	23.1	24.4	27.9	24.6	25.4	27.3	23.9	24.5	23.4	22.1	20.4	21.6	18.6	-			
15	*B. maculata*	26.5	26.0	27.6	28.7	28.2	29.2	25.6	26.9	23.5	23.9	21.4	19.9	22.6	19.5	-		
16	*M. spasma*	19.5	18.9	20.4	19.3	19.3	19.7	20.2	19.8	18.7	17.1	20.4	21.4	21.6	25.9	24.8	-	
17	*N. tragata*	25.9	22.9	25.5	21.8	26.7	26.8	24.7	23.4	23.6	22.0	24.6	24.4	27.4	30.0	29.7	17.1	-

FA=forearm length

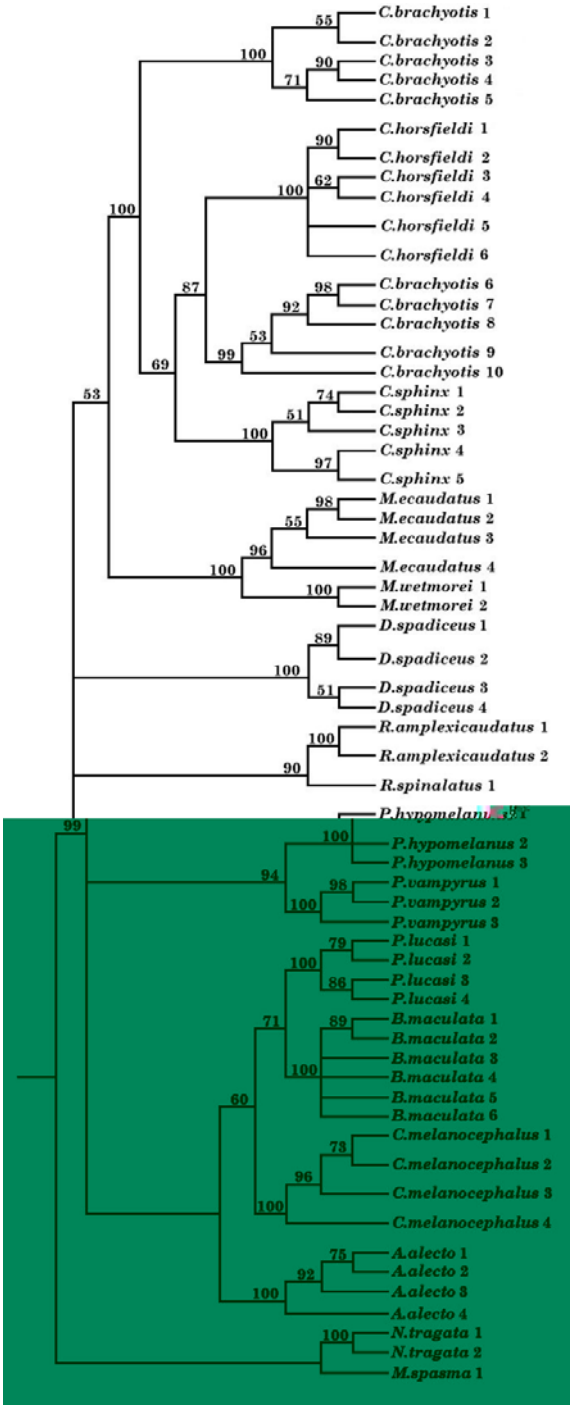

Figure 1 Rooted NJ tree generated using the cyt b gene segment of the pteropodids species used in this study (only bootstrap values >50% are shown). Values on the branches represent NJ bootstrap estimates, based on 1000 replicates. Only bootstrap values >50% are shown.

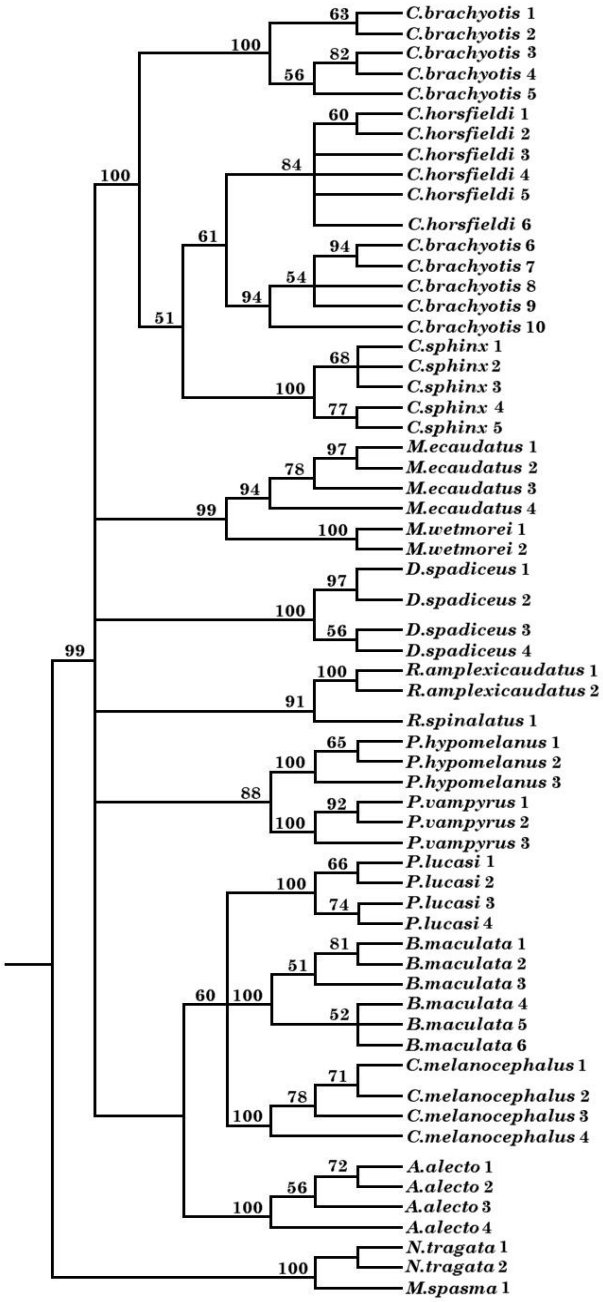

Figure 2 Unweighted and rooted MP tree generated using the cyt b of the megachiropteran species used in this study (only bootstrap values >50% are shown). Upper values on the branches represent MP bootstrap estimates, based on 100 replicate (tree length=762; CI=0.4291; RI=0.8312). Only bootstrap values >50% are shown.

APPENDIX B

The aligned sequences of the cytochrome *b* sequences for each species use in this study. Positions in the sequences are given above the sequences.

```
                    111 111 111 122 222 222 223 333 333 333 444 444 444 455 555 555 556 666 666 666 777 777 777 788 888 888 889
                123 456 789 012 345 678 901 234 567 890 123 456 789 012 345 678 901 234 567 890 123 456 789 012 345 678 901 234 567 890
C. brachyotis 1  ACA TCC GAA AAT CCC ACC CAC TAT TTA AAC TAA TTA ACG ACG CAT TAA TTG ACC TCC CAG CCC CCT CAA ACA TCT CCT CAT GAT GAA ATT
C. brachyotis 2   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
C. brachyotis 3   ... ... .C. ... ... ... ... ... ... ... ... ... ... ... ... ..G ... ... ... ... ... ... ... ... ... ... ... ... ...
C. brachyotis 4   ... ... .C. ... ... ... ... ... .C. ... ... ... ... ... ... ..G ... ... ... ... ... ... ... ... ... ... ... ... ...
C. brachyotis 5   ... ... .C. ... ... ... ... ... .C. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
C. horsfieldi 1   ... ... .C. ... ... ... ... ... .C. ... ... .C. ... ... ... ..G .C. ... ... ... ... ... ... ... ... ... ... ... .C.
C. horsfieldi 2   ... ... .C. ... ... ... ... ... .C. ... ... .C. ... ... ... ..G .C. ... ... ... ... ... ... ... ... ... ... ... .C.
C. horsfieldi 3   ... ... .C. ... ... ... ... ... .C. ... ... .C. ... ... ... ..G .C. ... ... ... ... ... ... ... ... ... ... ... .C.
C. horsfieldi 4   ... ... .C. ... ... ... ... ... .C. ... ... .C. ... ... ... ..G .C. ... ... ... ... ... ... ... ... ... ... ... .C.
C. horsfieldi 5   ... ... .C. ... ... ... ... ... .C. ... ... .C. ... ... ... ..G .C. ... ... ... ... ... ... ... ... ... ... ... .C.
C. horsfieldi 6   ... ... .C. ... ... ... ... ... .C. ... ... .C. ... ... ... ..G .C. ... ... ... ... ... ... ... ... ... ... ... .C.
C. brachyotis 6   ... ... .C. ... ... ... ... C.. .C. ... ... ... .T. ... ... ..G .C. ... ... ... ... ... ... ... ... ... ... ... .C.
C. brachyotis 7   ... ... .C. ... ... ... ... C.. .C. ... C.. ... .T. ... ... ..G .C. ... ... ... ... ... ... ... ... ... ... ... .C.
C. brachyotis 8   ... ... .C. ... ... ... ... ... .C. ... ... ... .T. ... ... ..G .C. ... ... ... ... ... ... ... ... ... ... ... .C.
C. brachyotis 9   ... ... .C. ... ... ... ... ... .C. ..T ... ... .T. ... ..C ..G .C. .T. ... ... ... ... ... ... ... ... ... ... .C.
C. brachyotis 10  ... ... .C. ... ... ... ... ... .C. ... ... ... .T. ... ... ..G .C. ... ... ... ... ... ... ... ... ... ... ... .C.
C. sphinx 1       ... ... .C. ... ... ... ... ... .C. ... ... ... ... ... ... ... .C. ... ... ... ... .T. ... ... ... ... ... ... .C.
C. sphinx 2       ... ... .C. ... ... ... ... ... .C. ... ... ... ... ... ... ... .C. ... ... ... ... .T. ... ... ... ... ... ... .C.
C. sphinx 3       ... ... .C. ... ... ... ... ... .C. ... ... ... ... ... ... ... .C. ... ... ... ... .T. ... ... ... ... ... ... .C.
C. sphinx 4       ... ... .C. ... ... ... ... ... .C. ... ... ... ... ... ... ... .C. ... ... ... ... .T. ... ... ... ... ... .G. ... .C.
C. sphinx 5       ... ... .C. ... ... ... ... ... .C. ... ... ... ... ... ... ... .C. ... ... ... ... .T. ... ... ... ... ... .G. ... .C.
M. ecaudatus 1    ... ... ..G ... ... ... ... ... .C. ... ... .C. .T. ... ... ... ... .T. .T. ... .T. ... ... .T. ... ... ... ... .C.
M. ecaudatus 2    ... ... ..G ... ... ... ... ... .C. ... ... .C. .T. ... ... ... ... .T. .T. ... .T. ... ... ... ... .T. ... ... .C.
M. ecaudatus 3    ... ... ..G ... ... ... ... ... .C. ... ... .C. .T. ... ... ... ... .T. .T. ... .T. ... ... .T. ... .T. ... ... .C.
M. ecaudatus 4    ... ... ..G ... ... ... ... ... .C. ... ... .C. .T. ... ... ... ... .T. .T. ... ... ... ... .T. ... .T. ... ... .C.
M. wetmorei 1     ... ... .GG ... ... ... ... ... .C. ... ..G .C. .T. ... ... ... ... GT. .T. ..A .T. ... ... .T. ... .T. ... ... .C.
M. wetmorei 2     ... ... .GG ... ... ... ... ... .C. ... ..G .C. .T. ... ... ... ... GT. .T. ..A .T. ... ... .T. ... .T. ... ... .C.
D. spadiceus 1    ... .T. ..G ... ... ... ... ... .C. ..A .T. .C. .T. ... ..C .TG .A. ... .A. ... ... .A. ... G.. ... .G. ... ... .C.
D. spadiceus 2    ... .T. ..G ... ... ... ... ... .C. ..A .T. .C. .T. ... ..C .TG .A. ... .A. ... ... .A. ... G.. ... .A. ... ... .C.
D. spadiceus 3    ... .T. ..G ... ... ... ... ... .C. ..- .T. .C. .T. ..A .GC .C. .A. ... .A. ... ... .A. ... G.. ... .A. ... ... .C.
D. spadiceus 4    ... .T. ..G ... ... ... ... ... .C. ..A .T. .C. ... ..A .GC .T. .A. ... .A. ... ... .A. ... G.. ... .A. ... ... .C.
R. amplexicaudatus 1 ... ... ... ..G .A. ... ... ... .C. ..A ... .C. ... ..T ..C ..G .A. ... .T. ..A ... ... ... GT. ... ... ... ... ... ...
R. amplexicaudatus 2 ... ... ... ..A .A. ... ... ... .C. ..A ... .C. ... ..T ..C ..G .A. ... .T. ..A ... ... ... GT. ... ... ... ... ... ...
R. spinalatus 1   ... ... ..G ..A .A. ... ... ... ... ..A .G. .C. ... ..T ..C ..G .A. .T. .T. ..A ... ... ... G.. .T. ... .G. ... ... .C.
P. hypomelanus 1  ... C.. ... ... .A. ... ... ... ... ..G .C. .C. ... ..T ..C .G. .C. ... .A. .C. ... TA. ... GT. ... ... ... ... ... .C.
P. hypomelanus 2  ... ... ... ... .A. ... ... ... .C. ..G .C. .C. ... ..T ..C .G. .C. ... .A. .C. ... TA. ... GT. ... ... ... ... ... .C.
P. hypomelanus 3  ... ... ... ..C .A. ... ... ... ... ..G .C. .C. ... ..T ..C .G. .C. ... .A. .C. ... TA. ... GT. ... ... ... ... ... .C.
P. vampyrus 1     ... ... ... ... .A. ... ..T ... .C. ..A .T. .C. ... ..T ..C ... .C. ... .G. .C. ... .A. ... .T. ... .T. .G. ... ... .C.
P. vampyrus 2     ... ... ... ... .A. ... ..T ... .C. ..A .T. .C. ... ..T ..C ... .C. ... .G. .C. ... .A. ... .T. ... .T. .G. ... ... .C.
P. vampyrus 3     ... ... ... ... .A. ... ..T ... .C. ..A .T. .C. ... ..T ..C ... .C. ... .A. .C. ... .A. ... .T. ... .T. ... ... ... .C.
P. lucasi 1       ... .T. ... ... ... ... .CT ..C ... ..A .C. .C. ... .TT .GC ... ... .T. ... ... ... .A. ... G.. ... ... .C. ..C ... ...
P. lucasi 2       ... .T. ... ... ... ... .CT ..C ... ..A .C. .C. ... .TT .GC ... ... .T. ... ... ... .A. ... G.. ... ... .C. ... ... ...
```

```
P. lucasi 3          ... .T. ... ... ... ... .CT ..C ... ..A .T. ... ..T .TT .GC ... ... .T. ... ... ... .A. ... G.. ... ... .C. ... ... ...
P. lucasi 4          ... .T. ... ... ... ... .CT ..C .C. ..A .T. ... ..T .TT .GC ... ... .T. ... ... ... .A. ... G.. ... ... .C. ... ... ...
B. maculata 1        ... .T. ... ... ... ... .TT ..C ... ..A .C. .C. ... .TC AC. ... .C. .T. ... ... .T. .A. .C. G.. ... ... .C. ... ... .C.
B. maculata 2        ... .T. ... ... ... ... .TT ..C ... ..A .C. .C. ... .TC AC. ... .C. .T. ... ... .T. .A. .C. G.. ... ... .C. ... ... .C.
B. maculata 3        ... .T. ... ... ... ... .CT ..C ... ..A .C. .C. ... .TC ..C ... .C. .T. ... ... .T. .A. .C. G.. ... ... .C. ... ... .C.
B. maculata 4        ... .T. ... ... ... ... .T. ..C .C. ..A .C. .C. ... .TC .GC ... .C. .T. ... ... .T. .A. .C. G.. ... ... .C. ... ... .C.
B. maculata 5        ... .T. ... ... ... ... .TT ..C .C. ..A CC. .C. ... .TC GC. ... .C. .T. ... ... .T. .A. .C. G.. ... ... .C. ... ... .C.
B. maculata 6        ... .T. ... ... ... ... .TT ..C .C. ..A .C. .C. ... .TC ..C ... .C. .T. ... ... .T. .A. .C. G.. ... ... .C. ... ... .C.
C. melanocephalus 1  ... .T. ... ... ... ... .CT ..C .C. ..A .T. ... ..T .TT ..C ... ... .T. ... ... ..T .A. ... G.. ..C ... .C. ... ... ...
C. melanocephalus 2  ... .T. ... ... ... ... .CT ..C .C. ..A .T. ... ..T .TT ..C ... ... .T. ... ... ..T .A. ... G.. ..C ... .C. ... ... ...
C. melanocephalus 3  ... .T. ... ... ... ... .CT ..C ... ..A .T. ... ..T .TT ..C ... ... .T. ... ... ... .A. ... G.. ..C ... .C. ... ... ...
C. melanocephalus 4  ... .T. ... ... ... ... .CT ..C .C. ..A .T. ... ..T .TT ..C ... ... .T. ... ... ... .A. ... G.. ... ... .C. ... ... ...
A. alecto 1          ... ... ... .G. .A. ... ..T ..C .C. ..A .CG .C. ... .TC ..C ... .C. .T. .T. ... ... .A. .C. G.. ... ... .C. ... .G. .C.
A. alecto 2          ... ... ... ... .A. ... ..T ..C .C. ..A .CG .C. ... .TC .GC ... .C. .T. ... ... ... .A. .C. G.. ... ... .C. ... .G. .C.
A. alecto 3          ... ... ... .G. .A. ... ..T ..C .C. ..A .CG .C. ... .TC ..C ... .C. .T. ... ... ... .A. .C. G.. ... ... .C. ... .G. .C.
A. alecto 4          ... ... ... ... ... ... .CT ..C .C. ..A .CG .C. ... ..C ..C ... .C. .T. ... ... ... .A. .C. G.. ... ... .C. ... .G. .C.
N. tragata 1         ... ... .-. ..A .A. ... .GT ..A ... ..A .C. ... ..A G.T ... .C. .C. ... .A. .T. .T. ... .G. G.. ... .T. .C. ... ... .C.
N. tragata 2         ... ... .-. ..A .A. ... .GT ..G ... ..A .C. ... ..A G.T ... .C. .C. ... .A. .T. .T. ... .G. G.. ... .T. .C. ... ... .C.
M. spasma 1          ... ... .-G ... ... ... ... ... .C. ..A .C. .C. .T. ..T ... .C. ... ... .A. .C. .T. ... ... GT. ... ... .C. ... ... .C.

                                 111 111 111 111 111 111 111 111 111 111 111 111 111 111 111 111 111 111 111 111 111 111 111 111 111 111 111
                     999 999 999 000 000 000 011 111 111 112 222 222 222 333 333 333 344 444 444 445 555 555 555 666 666 666 677 777 777 778
                     123 456 789 012 345 678 901 234 567 890 123 456 789 012 345 678 901 234 567 890 123 456 789 012 345 678 901 234 567 890
C. brachyotis 1      TTG GCT CCC TAC TCG GAA TTT GTT TAC TTA TTC AAA TTC TAA CAG GAC TAT TTC TAG CCA TAC ATT ACA CAT CAG ATA CAG CAA CAG CAT
C. brachyotis 2      ... ... ... ... .T. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
C. brachyotis 3      .C. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... T.. ... ... ... ... ...
C. brachyotis 4      .C. ... ... ... ... ... ... ... ... ... ... ... ... ... ... .G. ... ... ... ... ... ... ... ... T.. ... ... ... ... ...
C. brachyotis 5      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .G. ... ... ... ... ... ... ... ... ... ... ... ... ... ...
C. horsfieldi 1      ... ... ... ... .T. ... .C. .C. ... .C. ... ... .C. ... ... ... .C. .C. ... ... ... .C. .T. ... ... .C. ... .G. ... ...
C. horsfieldi 2      ... ... ... ... .T. ... .C. .C. ... .C. ... ... .C. ... ... ... .C. .C. ... ... ... .C. .T. ... ... .C. ... .G. ... ...
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C. horsfieldi 4      ... ... ... ... .T. ... .C. .C. ... .C. ... ... .C. ... ... ... .C. .C. ... ... ... .C. .T. ... ... .C. ... .G. ... ...
C. horsfieldi 5      ... ... ... ... .T. ... .C. .C. ... .C. ... ... .C. ... ... ... .C. .C. ... ... ... .C. .T. ... ... .C. ... .G. ... ...
C. horsfieldi 6      ... ... ... ... .T. ... .C. .C. ... .C. ... ... .C. ... ... ... .C. .C. ... ... ... .C. .T. ... ... .C. ... ... ... ...
C. brachyotis 6      ... ... ... ... .T. ... .C. .C. ... .C. ... ... .C. .G. ... ... .C. .C. .G. ... .G. .C. .T. ... .G. ... .G. ... ... ...
C. brachyotis 7      ... ... ... ... .T. ... .C. .C. ... .C. ... ... .C. .G. ... ... .C. .C. .G. ... .G. .C. .T. ... .G. ... .G. ... ... ...
C. brachyotis 8      ... ... ... ... .T. ... .C. .C. ... .C. ... ... .C. .G. ... ... .C. .C. .G. ... .G. .C. .T. ... .G. ... .G. ... ... ...
C. brachyotis 9      ... ... ... ... .T. ... .C. .C. ... .C. ... ... .C. ... ... ... .C. .C. .G. ... .G. .C. .T. ... .G. ... ... ... ... ...
C. brachyotis 10     ... ... ... ... .T. ... .C. .C. ... .C. ... ... .C. .G. ... ... .C. .C. .G. ... .G. .C. .T. ... ... .C. .G. ... ... ...
C. sphinx 1          ... ... .T. ... .T. ... .C. .C. ... .C. ... ... .C. ... ... ... .C. .C. .G. ... .G. ... .T. ... ... .C. ... ... ... ...
C. sphinx 2          ... ... .T. ... .T. ... .C. .C. ... .C. ... ... .C. ... ... ... .C. .C. .G. ... .G. ... .T. ... ... .C. ... ... ... ...
C. sphinx 3          ... ... .T. ... .T. ... .C. .C. ... .C. ... ... .C. ... ... ... .C. .C. .G. ... .G. ... .T. ... ... .C. ... ... ... ...
C. sphinx 4          ... ... .T. ... .T. ... .C. .C. ... .C. ... ... .C. ... ... ... .C. .C. .G. ... .G. ... .T. ... ... .C. ... ... ... ...
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M. ecaudatus 1       ... .G. ... ... .A. TG. .G. .C. .GG ... .C. ... .C. .C. ... ... ... .CT ... ... ... ... .T. ... ... .C. .C. .T. ... .C.
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M. ecaudatus 4       ... .A. ... ... .A. CG. .A. .C. .GG ... .C. ... .C. .T. ... ... ... .CT ... ... ... ... .T. ... ... ... .T. .C. ... .C.
M. wetmorei 1        ... .G. ..T ... .A. C.. .A. .C. ..G .C. ... ... .C. .T. ... A.. ... .C. ... .T. ... .C. ... .C. ... .C. .CA .C. ..A .C.
M. wetmorei 2        ... .G. ..T ... .A. C.. .A. .C. ..G .C. ... ... .C. .T. ... A.. C.. .C. ... .T. ... .C. ... .C. ... .C. .CA .CG ..A .C.
D. spadiceus 1       ... .G. ... ... .A. ..G .C. .CC .TG GC. ... ... .C. .T. ... ... ... .C. ... .A. ... ... ... .C. ... .C. ... ... ... ...
D. spadiceus 2       ... .G. ... ... .A. ..G .C. .CC .TG GC. ... ... .C. .T. ... ... ... .C. ... .A. ... ... ... .C. ... .C. ... ... ... ...
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R. spinalatus 1       .C. .T. .A. ..T .A. .G. .C. .CC ..G GC. .C. .G. .CT .G. .C. ... ... .C. ... .A. ... .C. ... .C. ... .C. ... ... .C. .C.
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P. hypomelanus 3      ... ... .A. ... .A. .C. .C. .GC ..G CC. .C. ... .C. ... ... ... ... .G. .C. ... .T. ... .C. ... .C. ... .C. ..A ... .C. .C.
P. vampyrus 1         .C. ... .A. .T. .A. .C. .C. .CC ..G CC. .C. ... .CT ... .G. ... ... .C. .G. ... ... .C. ... .T. .G. .C. ..A .G. .C. .C.
P. vampyrus 2         .C. ... .A. .T. .A. .C. .C. .CC ..G CC. .C. ... .CT ... .G. ... ... .C. .G. ... ... .C. ... .T. .G. .C. ..A .G. .C. .C.
P. vampyrus 3         .C. ... .A. .T. .A. .C. .C. .CC ..G CC. .C. ... .CT ... .G. ... ... .C. .G. ... ... .C. ... .T. .G. .C. ..A .G. .C. .C.
P. lucasi 1           .C. ... ..T ..T .A. ... ... .CC ..A CA. ... ... .CT ... ... ... ... .CT ... ... ... ... ... ... .C. .C. .C. .CA .T. .C. .C.
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P. lucasi 3           .C. ... ..T ..T .A. ... ... .CC ..A CA. ... ... .CT ... ... .G. ... .CT ..A ... ... ... ... ... .C. .C. .C. .CA .T. .C. .C.
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B. maculata 1         .C. .T. .AT .T. .A. ... .C. .CC .GG CCT .C. ... .C. ... ... ... ... ... ... ... ... ... .C. ... .C. ... .C. .CA TT. .C. .C.
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B. maculata 4        .C. .T. .AT .T. .A. ... .C. .CC .GG CCT .C. ... .C. ... ... .G. ... ... ... ... ... .C. ... .C. ... .C. .CA TT. .C. .C.
B. maculata 5         .C. .T. .AT .T. .A. ... .C. .CC .GG CCT .C. ... .C. ... ... .G. ... ... ... ... ... .C. ... .C. ... .C. .CA TT. .C. .C.
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C. melanocephalus 1   .C. A.. ..T ... .A. ... .C. .CC ..A CC. ... ... .C. .T. .T. .G. .G. .C. .C. ... .GT ... ... .C. .C. .C. ... ... .C. .C.
C. melanocephalus 2   .C. ... ..T ... .A. ... .C. .CC ..A CC. ... ... .C. .T. .T. .G. .G. .C. .C. ... .GT ... ... .C. .C. .C. ... ... .C. .C.
C. melanocephalus 3   .C. A.. ..T ... .A. ... .C. .CC ..A CC. ... ... .C. .T. .T. .G. .G. .C. .C. ... .G. ... ... .C. .C. .C. ... ... .C. .C.
C. melanocephalus 4   .C. ... ..T ... .A. ... .C. .CC ..A CC. ... ... .C. .T. .T. .G. .G. .C. .C. ... .G. ... ... .C. .C. .C. ... ... .C. .C.
A. alecto 1           ... .T. .A. .T. .A. .G. ... .C. ..A .A. .C. ... ..T .G. ... ..T ... ... ... .T. .G. .C. ... .C. ... .C. .CT .T. .C. .C.
A. alecto 2          ... .T. .A. .T. .A. .G. ... .C. ..A .A. .C. ... ..T .G. ... ..T ... ... ... .T. .G. .C. ... .C. ... .C. .CT .T. .C. .C.
A. alecto 3           ... .T. .A. .T. .A. .G. ... .C. ..A .A. .C. ... ..T .G. ... ..T ... .C. ... .T. .G. .C. ... .C. ... .C. .CT .C. .C. .C.
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N. tragata 1          ... .A. ... .T. .A. ... .C. .CC ..G G.C .A. ... .C. ... ... .T. ... .C. ... .A. ... .C. .T. .C. .C. .C. ... .C. ... .C.
N. tragata 2          ... .G. .T. .C. .A. ... .C. .CC ..G G.C .A. ... .C. ... ... .T. ... .C. ... .A. ... .C. .T. .C. .C. .C. ... .C. ... .T.
M. spasma 1           ... ... ... .T. .A. .GG .C. .C. ..G CC. .C. ... .C. ... ... .G. ... .C. ... .A. ... .C. ... .C. ... .C. ... .T. .G. .C.

                      111 111 111 111 111 111 122 222 222 222 222 222 222 222 222 222 222 222 222 222 222 222 222 222 222 222 222 222 222 222 222
                      888 888 888 999 999 999 900 000 000 001 111 111 111 222 222 222 233 333 333 334 444 444 444 555 555 555 556 666 666 667
                      123 456 789 012 345 678 901 234 567 890 123 456 789 012 345 678 901 234 567 890 123 456 789 012 345 678 901 234 567 890
C. brachyotis 1       TCC AAT CCG TCA CTC ATA TCT GCC GAG ACG TAA ACT ACG GGT GAA TCC TAC GGT ACT TAC ACG CCA ACG GAG CAT CCA TGT TTT TCA TCT
C. brachyotis 2       ... ..C ... ... ... ... ... ... ... .T. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
C. brachyotis 3       ... .G. ... ... ... ... ... ... ... .T. ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
C. brachyotis 4       ... G.. ... ... ... ... ... ... ... .T. ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
C. brachyotis 5       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
C. horsfieldi 1       ... ... ... .T. ... .C. ... .T. ... ... ... ... ... .A. ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ... ...
C. horsfieldi 2       ... ... ... .T. ... .C. ... .T. ... ... ... ... ... .A. ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ... ...
C. horsfieldi 3       ... ... ... .T. ... .C. ... .T. ... ... ... ... ... .A. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
C. horsfieldi 4       ... ... ... .T. ... .C. ... .T. ... ... ... ... ... .A. ... ... ... .A. ... ... ... ... ... ... ... ... ... ... ... ...
C. horsfieldi 5       ... ... ... ... ... .C. ... .T. ... ... ... ... ... .A. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
C. horsfieldi 6       ... ... ... ... ... .C. ... .T. ... ... ... ... ... .A. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
C. brachyotis 6       ... .G. ... .T. ... .C. ... ... ... ... ... ... ... ..A ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..C
C. brachyotis 7       ... .G. ... .T. ... .C. ... ... ... ... ... ... ... ..A ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... C..
C. brachyotis 8       ... ... ... .T. ... .C. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
C. brachyotis 9       .T. ... ... .T. ... .C. ... ... ... .T. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
C. brachyotis 10      ... ... ... ... ... .C. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
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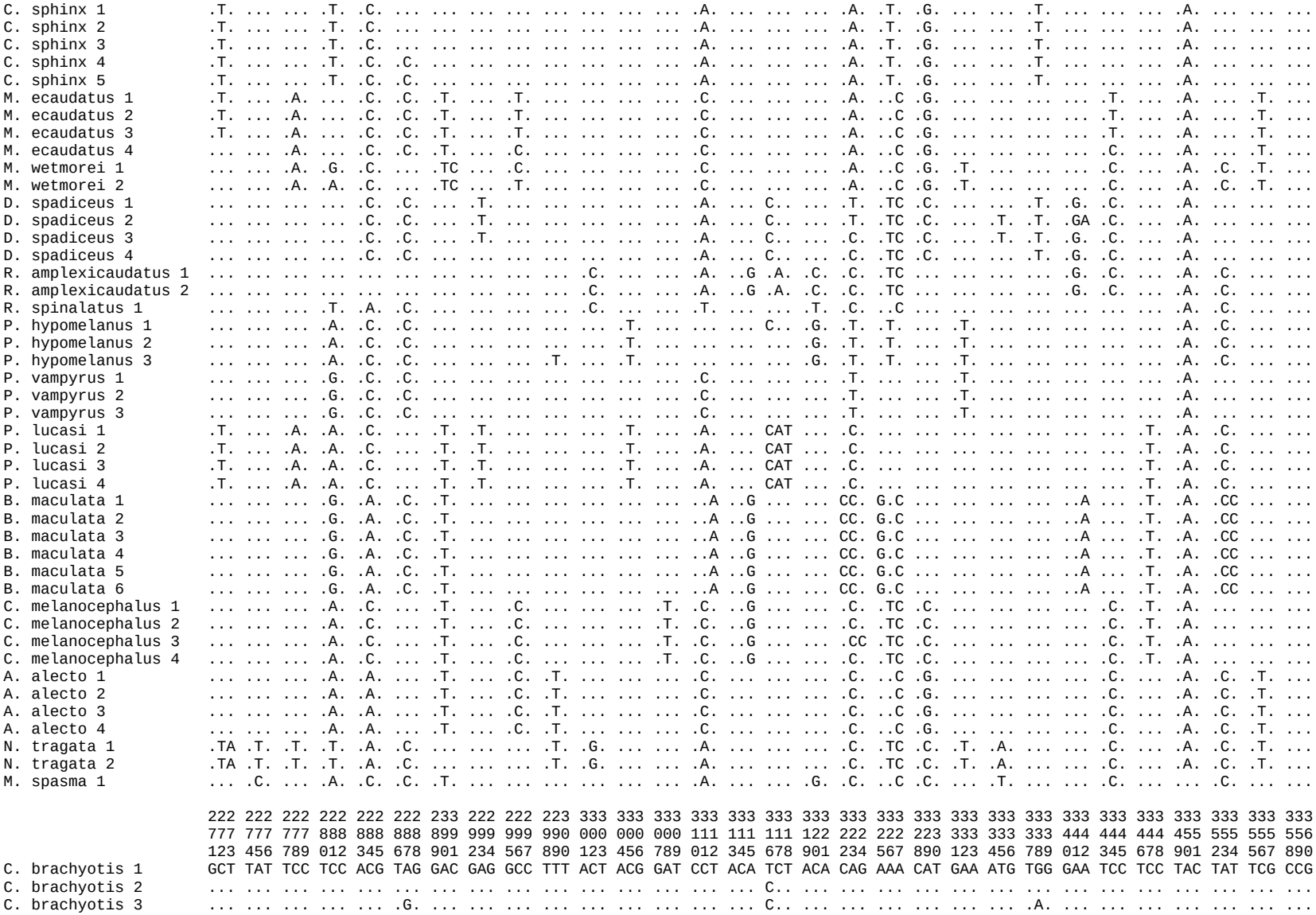
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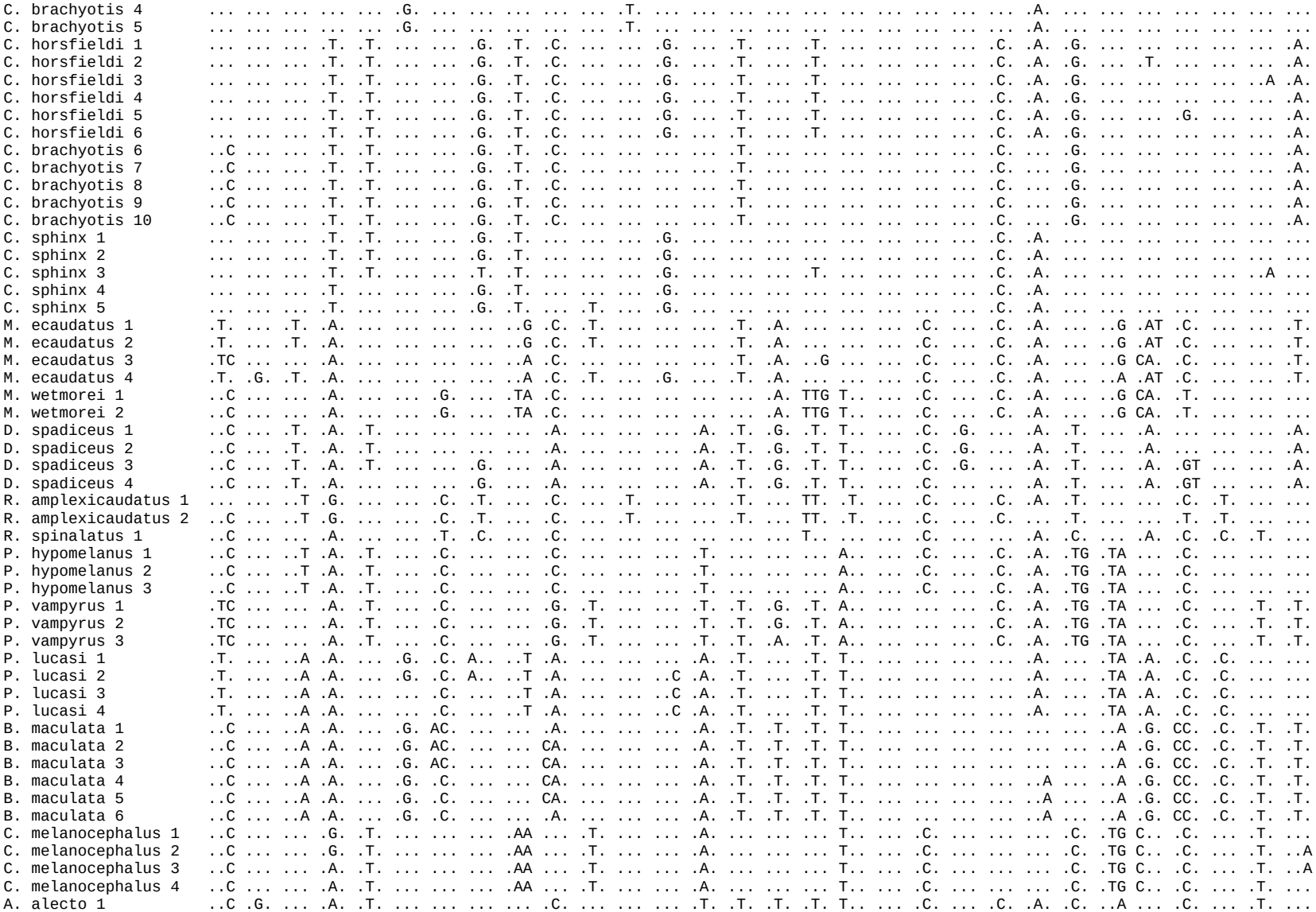
C. sphinx 2
C. sphinx 3
C. sphinx 4
C. sphinx 5
M. ecaudatus 1
M. ecaudatus 2
M. ecaudatus 3
M. ecaudatus 4
M. wetmorei 1
M. wetmorei 2
D. spadiceus 1
D. spadiceus 2
D. spadiceus 3
D. spadiceus 4
R. amplexicaudatus 1
R. amplexicaudatus 2
R. spinalatus 1
P. hypomelanus 1
P. hypomelanus 2
P. hypomelanus 3
P. vampyrus 1
P. vampyrus 2
P. vampyrus 3
P. lucasi 1
P. lucasi 2
P. lucasi 3
P. lucasi 4
B. maculata 1
B. maculata 2
B. maculata 3
B. maculata 4
B. maculata 5
B. maculata 6
C. melanocephalus 1
C. melanocephalus 2
C. melanocephalus 3
C. melanocephalus 4
A. alecto 1
A. alecto 2
A. alecto 3
A. alecto 4
N. tragata 1
N. tragata 2
M. spasma 1
222 222 222 222 222 222 233 222 222 223 333 333 333 333 333 333 333 333 333 333 333 333 333 333 333 333 333 333 333 333
777 777 777 888 888 888 899 999 999 990 000 000 000 111 111 111 122 222 222 223 333 333 333 444 444 444 455 555 555 556
123 456 789 012 345 678 901 234 567 890 123 456 789 012 345 678 901 234 567 890 123 456 789 012 345 678 901 234 567 890
C. brachyotis 1 GCT TAT TCC TCC ACG TAG GAC GAG GCC TTT ACT ACG GAT CCT ACA TCT ACA CAG AAA CAT GAA ATG TGG GAA TCC TCC TAC TAT TCG CCG
C. brachyotis 2
C. brachyotis 3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C. brachyotis 5
C. horsfieldi 1
C. horsfieldi 2
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C. horsfieldi 5
C. horsfieldi 6
C. brachyotis 6
C. brachyotis 7
C. brachyotis 8
C. brachyotis 9
C. brachyotis 10
C. sphinx 1
C. sphinx 2
C. sphinx 3
C. sphinx 4
C. sphinx 5
M. ecaudatus 1
M. ecaudatus 2
M. ecaudatus 3
M. ecaudatus 4
M. wetmorei 1
M. wetmorei 2
D. spadiceus 1
D. spadiceus 2
D. spadiceus 3
D. spadiceus 4
R. amplexicaudatus 1
R. amplexicaudatus 2
R. spinalatus 1
P. hypomelanus 1
P. hypomelanus 2
P. hypomelanus 3
P. vampyrus 1
P. vampyrus 2
P. vampyrus 3
P. lucasi 1
P. lucasi 2
P. lucasi 3
P. lucasi 4
B. maculata 1
B. maculata 2
B. maculata 3
B. maculata 4
B. maculata 5
B. maculata 6
C. melanocephalus 1
C. melanocephalus 2
C. melanocephalus 3
C. melanocephalus 4
A. alecto 1

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A. alecto 2          ..C .G. ... .A. .T. ... ... ... ... .C. ... ... ... .T. .T. .T. .T. T.. ... .C. ... .C. .A. .C. ..A ... .C. ... .T. ...
A. alecto 3          ..C .G. ... .A. .T. ... ... ... ... .C. ... ... ... .T. .T. .T. .T. T.. ... .C. ... .C. .A. .T. ..A ... .C. ... .T. ...
A. alecto 4          ..C .G. ... .A. .T. ... ... ... ... .C. ... ... ... .T. .T. .T. .T. T.. ... .C. ... .C. .A. .T. ..A ... .C. ... .T. ...
N. tragata 1         ..C .G. AT. ... .T. ... .C. ... .A. .C. ... ... .T. ... ... CA. TT. T.. ... .T. ... .C. .C. ... .T. ... ... .T. ... ...
N. tragata 2         ..C ... AT. .T. .T. ... .C. ... .A. .C. ... ... .T. ... ... CA. TT. T.. ... .T. ... .C. .C. ... .T. ... ... .T. ... ...
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                    666 666 666 777 777 777 888 888 888 899 999 999 99
                    123 456 789 012 345 678 901 234 567 890 123 456 45
C. brachyotis 1     TAA TAG CAA CAG CCT TTA TAG GCT ACG TAC TCC CA
C. brachyotis 2     ... ... ... ... ... ... ... ... ... ... ... ..
C. brachyotis 3     ... ... ... ... ... ... ... ..A ... ... ... ..
C. brachyotis 4     ... ... ... ... ... ... ... ..A ... ... ... ..
C. brachyotis 5     ... ... ... ... ... ... ... ... ... ... ... ..
C. horsfieldi 1     ... ... ... ... ... ... ... .T. .T. ... ... ..
C. horsfieldi 2     ... ... ... ... ... ... ... .T. .T. ... ... ..
C. horsfieldi 3     ... ... ... .G. ... ... ... .T. .T. .G. ... ..
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C. horsfieldi 6     ... ... ... .G. ... ... ... .T. .T. ... ... ..
C. brachyotis 6     ... ... ... ... ... ... ... ... .T. ... ... ..
C. brachyotis 7     ... ... ... ... ... ... ... ... .T. ... ... ..
C. brachyotis 8     ... ... ... ... ... ... ... ... .T. ... ... ..
C. brachyotis 9     ... ... ... ... ... ... ... ... .T. ... ... ..
C. brachyotis 10    ... ... ... ... ... ... ... ... .T. ... ... ..
C. sphinx 1         ... ... ... ... ... .C. ... .T. ... ... ... ..
C. sphinx 2         ... ... ... ... ... .C. ... .T. ... ... ... ..
C. sphinx 3         ... ... ... ... ... .C. ... .T. ... ... ... ..
C. sphinx 4         ... ... ... ... ... .C. ... .T. ... ... ... ..
C. sphinx 5         ... ... ... ... ... .C. ... .T. ... ... ... ..
M. ecaudatus 1      ... ... .C. ... ... .C. .G. ... ... ... .G. ..
M. ecaudatus 2      ... ... .C. ... ... .C. .G. ... ... ... .G. ..
M. ecaudatus 3      ... ... .C. ... ... .C. .G. ... .T. ... .G. ..
M. ecaudatus 4      ... ... .C. ... ... .C. .G. ... ... ... .G. ..
M. wetmorei 1       ... ... .C. ... ... ... ... ... .T. ..T .G. ..
M. wetmorei 2       ... ... .C. ... ... ... ... ... .T. ..T .G. ..
D. spadiceus 1      .T. .G. ... ... ... ... ... .G. .T. ... .T. ..
D. spadiceus 2      .T. .G. ... ... .T. ... ... .G. ... ... .T. ..
D. spadiceus 3      .T. ... ... ... ... ... ... .G. ... ... ... ..
D. spadiceus 4      .T. ... ... ... ... .C. ... .A. .T. ... ... ..
R. amplexicaudatus 1 ... ... ... ... ... .C. ... ... .T. ... .T. ..
R. amplexicaudatus 2 ... ... ... ..A ... .C. ... ... .T. ... .T. ..
R. spinalatus 1     ... ... ... ... ... .C. ... ... .T. ... ... .G
P. hypomelanus 1    ... ... ... ... ... .C. ... .G. ... ... ... ..
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P. hypomelanus 3    ... ... ... ... ... .C. ... .G. ... ... ... ..
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P. vampyrus 2       ... ... ... ... ... .C. ... .A. .T. .T. ... ..
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P. lucasi 2         ... ... ... ... ... .C. ... .A. .T. .T. .T. ..
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B. maculata 1       ... .G. ... ... ..C ... ... .AC ... .C. ... ..
B. maculata 2       ... .G. ... ... ..C ... ... .AC ... .C. ... ..
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B. maculata 4       ... .G. ... ... ..C ... ... .A. ... .C. ... ..
B. maculata 5       ... .G. ... ... ..C ... ... .A. ... .C. ... ..
B. maculata 6       ... .G. ... ... ..C ... ... .AC ... .C. ... ..
C. melanocephalus 1 ... ... ... .C. ... ... ... ... ... .C. ... ..
C. melanocephalus 2 ... ... ... .C. ... ... ... ... ... .C. ... ..
C. melanocephalus 3 ... ... ... .C. ... ... ... ... ... .C. ... ..
C. melanocephalus 4 ... ... ... .C. ... ... ... ... ... .C. ... ..
A. alecto 1         ... ... ... ... ... .C. ... ... ... ... .A. ..
A. alecto 2         ... ... ... ... ... .C. ... ... ... ... .A. ..
A. alecto 3         ... ... ... ... ... .C. ... ... ... ... .A. ..
A. alecto 4         ... ... ... ... ... .C. ... ... ... ... .A. ..
N. tragata 1        ... ... ... .C. ... .C. ... .T. ... ... ... ..
N. tragata 2        ... ... ... .C. ... .C. ... .T. ... ... ... ..
M. spasma 1         ... ... ... .C. ... .C. ... ... ... .C. ... ..
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