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MORPHOLOGICAL AND GENETIC STUDIES OF THE MASKED FLYING FOX, *PTEROPUS PERSONATUS*; WITH A NEW SUBSPECIES DESCRIPTION FROM GAG ISLAND, INDONESIA

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ABSTRACT

The study on the specimens of Masked Flying Fox, *Pteropus personatus* from Gag and Moluccas Islands, Indonesia was conducted by using morphological and genetic analyses. Morphologically, the specimens from Gag are different from the other populations in Moluccas Islands by the smaller size of skull, dental and other external measurements. Based on the measurements of the specimens, the population from Gag Island is identified as *P. personatus acityae* n. subsp. The phylogenetic reconstruction based on partial cytochrome *b* sequences also support the differences between *P. personatus acityae* n. subsp and *Pteropus personatus personatus*. Thus, recently two subspecies of *P. personatus* are recognised from its distribution areas.

Key words: flying fox, Gag Island, new subspecies, *Pteropus personatus*

INTRODUCTION

The direct and indirect of long term histories of geology epoch affected the species number and endemicity of mammals. For instance, South West Pacific and Moluccas Islands which have more than 230 indigenous species of mammals are higher compared to 196 species in Sumatra, 183 species in Java, 126 species in Sulawesi and 180 species in New Guinea (Flannery 1995, Helgen 2005). Among them, bats are the best represented mammals which have approximately 64 % of the total fauna.

A small island of Gag is located between Gebe-Halmahera Islands and Waigeo-Raja Ampat Islands. Up to the present, only few mammal studies have been carried out in this island. In July 1997, Maryanto and Kitchener conducteda short survey and recorded 13 species of mammals from this very small island which is only 56 km^2 (Maryanto & Kitchener 1999). It is an interesting finding in comparison with the other geographically close islands, such as Gebe (145 km^2) with 17 species, Halmahera (17780 km^2) with 35 species and Waigeo (3155 km^2) with 14 species (Flannery 1995). Furthermore, the most notable difference is that all Gebe, Halmahera and Waigeo have representatives of intermediate size and small marsupial species, whereas no marsupials were recorded in Gag Island (Maryanto & Kitchener 1999).

A small masked flying fox, *Pteropus personatus,* was another interesting finding. All individuals from Gag Island show differences in term of some measuring dimensions compared to

the specimens from the type locality (Ternate) and other nearby distribution areas, such as Tidore, Bisa, Bacan, and Obi Islands. Thus, this study compared the populations from those islands based on morphological and genetic analyses by using partial mitochondrial DNA cytochrome *b* (mtDNA cyto *b*) gene sequences.

MATERIALS AND METHODS

Morphological study

Specimens of the species which were suspected to be taxonomically putative subspecies were identified and compared to the specimens of the same species from its other distribution islands such as Ternate, Tidore, Bacan and Obi Islands. Those specimens are deposited in Museum Zoologicum Bogoriense (MZB). In total, 25 skulls, dental and external characters were measured (in mm) by using 0.01 digital calliper following Kitchener *et al.* (1993), including GSL (greatest skull length), ZB (zygomatic breadth), LIW (least inter orbital width), POW (post orbital width), BW (braincase width), CDL (condyle dentary length), ONL (orbit nasal length), PL (palatal length), MFW (mesopterygoid fossa width), CC (inner canine breadth), P3P3 and P4P4 (inner upper premolar 3 and 4 breadth), M1M1 and M2M2 (inner upper molar 1 and 2 breadth), P3L, P4L, M1L, M2L (upper premolar 3 and 4 and molar 1 and 2 length), C1M2 (lower canine to molar 2 length), RAP (ramus angular process height), CPL(condyle dentary length), FA (forearm length), Tibia (tibia length), HB (head and body length), Ear (ear length) and also weight (in gram).

Principle component analysis (PCA) and Discriminant Function Analysis (DFA) were initially run for all characters. The DFA was run for a subset of 3-4 characters based on the criteria of minimized Wilks's Lambda to select the best discriminating variables. Results obtained for the reduced set were very similar to those based on all characters.

Genetic study

Genetic materials (liver and tissue) of *Pteropus personatus* were taken from the specimens deposited in MZB. Total DNA was extracted using the CTAB (cetyl-tri-methyl ammonium bromide) protocol (Ducroz *et al.* 1998, Lecompte *et al.* 2005). 700 µl 2X CTAB buffer and 8 mg of proteinase K were added to the 1 mm^3 minced tissue sample to denature the proteins to eliminate proteins which can affect the DNA product (Di Mito and Betschart 1998). Then, 700 µl of chloroform-isoamyl alcohol was added to inhibit the lysis process by proteinase K while incubating in a water bath at 60°C for 1-3 hours. After centrifugation at 13000 rpm for 10 minutes, 500-550 µl of the upper aqueous layer that contained DNA was transferred into a 1.5 ml fresh microcentrifuge tube. Then absolute ethanol of the same amount was added. Absolute ethanol was used to precipitate the DNA product and avoid interference by other products (Gari *et al.* 2006). After the second centrifugation, the supernatant was removed into a new tube with 600 µl cold 70% ethanol

and 25 µl 3M NaCl. After the third centrifugation, the excess ethanol was discarded and the pellet was air-dried. Finally, the DNA was resuspended in 30 µl of double distilled water (ddH_2O). The extracted DNA samples were kept at -80ºC in the freezer for later use.

Twenty-five µl of reaction volume was used for the PCR amplification. This volume included 1 µl of DNA extract, 1.2 µl of each primer pair (10 mM Glud-GL and 10 mM CB2H), 1.5 µl of 25 mM $MgCl_2$, 0.5 µl of 10 mM dNTPs, 0.5 µl of *Taq* DNA polymerase with appropriate buffer and ddH_2O to volume. Thermal profiles of amplifications started with three minutes of denaturation at 93^{o}C, followed by 30 cycles at 93^{o}C (1 min), 47.2^{o}C (1 min 15 s) and 72^{o}C (2 min), with a final extension at 72^{o}C (5 min). To amplify the partial cytochrome *b* gene, the primers used were GludG-L, (5'-TGACCTGAARAACCAYCGTTG-3') and CB2H (5'-CCCTCAGAATGATAT TTGTCCTCA-3') (Palumbi *et al.* 1991). Purification of the PCR product was carried out using the Promega Purification Kit following the protocol provided by the manufacturer (Wizard® Genomic DNA Purification Kit-Instruction for use of product). The purified PCR products were sequenced by a private laboratory (1st Base, Selangor, Malaysia) using the ABI 3730 Genetic Analyzer.

CHROMAS version 1.45 (MacCarthy 1996) was used to display fluorescence nucleotide bases of the DNA sequence for analysis. Multiple sequence alignments were done by using CLUSTAL X version 1.81 program (Thompson *et al.* 1997) and subsequently aligned by eye. Molecular Evolutionary Genetics Analysis (MEGA version 6) (Kumar *et al.* 2004) was used to perform the analysis of base frequencies and distance matrix. Genetic divergence (Saitou & Nei 1987) analysis was done using Kimura two-parameter model (Kimura 1980). Phylogenetic relationship was constructed using Neighbour-Joining (NJ) and Maximum Likelihood (ML) implemented in MEGA version 6.

RESULTS

Morphological and genetic analyses reveal that there are two subspecies of *Pteropus personatus* in the northern Moluccas. One is the nominate subspecies, *Pteropus personatus personatus*, which is restricted to Halmahera group of islands; whereas the other population from Gag Island forms a distinct subspecies, i.e. *Pteropus personatus acityae* n. subspec. The descriptions of the subspecies are presented as follows:

Systematic Account

Family Pteropodidae Gray, 1821
Genus *Pteropus* Brisson, 1762
Pteropus personatus personatus Temminck, 1825 (Fig. 1B)

Pteropus personatus Temminck, 1825: 189 (type locality: Indonesia, Moluccas Islands, Ternate).

Type Specimens.–2 Syntypes RMNH 37846,9

Material examined.–3 females (MZB 15041, 15042, 15043), Bisa Island; 7 males (MZB

15038, 15039, 32269, 32267, 32264, 32266, 32265), Ternate Island; 3 females (MZB 15040, 32259, 32260), Ternate Island; 1 male (MZB 25241), Tidore Island; 1 female (MZB 15241), Tidore Island.

Description (based on Anderson 1912).–Fur shorter and length of back about 9 mm, mantle 11 mm, belly 8-9 mm, fur on upperside of tibia extending backward to ankle and in a thin line along upper side of metatarsus to base of phalanges, short scattered hairs on phalanges. Colour, back and rump silvery white with a slight creamy tinge, this colour an confined to shorter tips of the hairs, long base of fur constrasting seal brown or vandyck brown. Dark base of fur showing slightly through on back much more so on rump, thighs and tibia, breast belly and flanks buffy or pale woodbrown, base of fur contrasting seal brown. Mantle golden buffy or ochraceous buffy, shading to cream buff or silvery whitish posteriorly, sides of neck and fore neck buffy or ochraceous buffy like front of mantle, colour of mantle extending forward on occiput and crown lightening to cream buffy on face. A dark vandyck brown stripe on each side of muzzle from rhinarium to front of eye continued backward as a superciliary stripe to temporal region, there bending vertically down ward, midway between eye and ear to throat, where it meets and unites with corresponding stripe from opposite side, a short narrow horizontal line of similar colour from hinder corner of eyes to vertical temporal stripe, upper and lower eyelid dark vandyck-brown. Interoccular space between supraorbital stripes, an elongated patch above and behind each eye, region below eyes including margins of upper and lower lips and chin silvery-white or creamy-white.

Distribution.–Ternate,Tidore, Bacan, Halmahera, Bisa (Obi Islands)

Pteropus personatus acityae n. subsp. (Fig. 1A)

Pteropus personatus: Maryanto & Kitchener, 1999: 193.

Material examined.–Type series. Holotype: male (MZB31379)**,** collected by I Maryanto & MH Sinaga from Valley Forest (00°15′27″S 129°18′53″E), Gag Island in 2008. Paratypes: 2 females (MZB 31286, 31378), same locality, collectors and date as holotype; 1 male (MZB 18205), collected by I Maryanto & DJ Kitchener from coconut plantation (00°26′18″ S 129°54′ 06″ E), Gag Island in 1997. Skulls were extracted, carcass fixed in formalin 5% in the field and preserved in ethanol 70% in the laboratory.

Description.–*Pteropus personatus acityae* n. subsp. from Gag Island is different from *P. personatus* from Ternate, Tidore, Bisa and Obi Islands by average smaller size in some characters (Table 1). For example (all measurements in mm), greatest skull length (42.96 mm±1.51) for Gag versus 45.01±0.97 for Ternate and Tidore, and 55.97±0.53 for Bisa; zygomatic breadth 24.91±1.71 versus 26.14±0.54 for Ternate and 25.63±0.22 for Tidore; orbit to nasal length 14.10±0.4 versus 14.77±0.63 for Ternate and Tidore and 15.22±0.04 for Bisa; but larger post

orbital width 9.03±0.51 versus 8.68±0.59 for Ternate and Tidore and 8.44±0,07 for Bisa; inner P3P3 7.29±0.5 versus 6.92±0.32 for Ternate and Tidore and 6.77±0.25 for Bisa; inner P4P4 7,60±0,39 versus 7.59±0.26 for Ternate and Tidore and 7.43±0.06 for Bisa; upper premolar 4 length 1.78±0.25 versus 1.75±0.25 for Ternate and Tidore and 1.72±0.22 for Bisa; upper molar 1 length 1.81±1.10 versus 1.75±0.10 for Ternate and Tidore and 1.75±0.23 for Bisa. All external characters of Gag specimens smaller than those of Ternate, Tidore and Bisa Islands, i.e. forearm 90.87±3.04 ersus. 92.27±3.35 for Ternate and Tidore and 103.2±0.88 for Bisa; tibia length 41.16±3.02 versus 43,54±2.08 for Ternate and Tidore.

The morphology of skull and externals characters of the Gag population is smaller than those of other populations in the diagnosis. The external performances including stripe in the face and surounding eyes, length of fur in the back, rump and belly, and also mantle are similar. Fur colour in the back silvery white with creamy tinge hair and brown in the base of fur. Brown fur on the rump through tibia. Pale brown fur on belly and breast with dark brown base. Specimen from Gag has golden buffy mantle, shading to silvery whitish posteriorly.

Etymology.–The new subspecies is named after the lovely daughter of the second author, Acitya, for her patience when her father does his fieldworks.

Distribution.–Indonesia, Gag Island.

Figure 1. (A) Photograph of the skulls of *Pteropus personatus acityae* n. subsp. from Gag Island, and (B) *P. personatus personatus* from Ternate Island, showing cranium and lower mandible in ventral and lateral views.

Morphological analyses

Based on the 25 measurements of skull, dental and external characters indicate that all characters are not significantly influenced by sex. Measurements of skull, dental and external characters in *Pteropus personatus* from Ternate, Tidore, Bisa, Obi, and Gag Island are shown in Table 1.

The Gag samples were compared with the specimens from Tidore, Ternate, Bisa and Obi Islands. PCA which was run on 25 skull and dental characters indicated that there were extracted three coefficients factors with the total variance 86.71%. Factor 1, 2 and 3 were 74.14%, 7.10% and 5.47%, respectively (Table 2, Fig. 2). In order to avoid over fitting the data, a problem inherent in analyzing large sets of characters in DFA, the data sets for skull characters were reduced to subsets of four characters. These skull characters: Ramus angular process height (RAP), inner upper premolar 3 and 4 breadth (P3P3) and greatest skull length (GSL) were selected to minimize the value of Wilks's lambda (Table 3, Fig. 3). These skull characters provided similar clusters for all of *P. personatus* in discriminant function space (as the full set of characters did too). All three skull characters are important in the discriminant function and their coefficient values loaded heavily (> 0.5) on Function 1. A total 78% of individual were classified to their correct group, the characters that separated between *P. Personatus acityae* n. subsp. from Gag Island and others, which have loadings more than 0.5 in Function 1. Function 1 explained 88.6% (df=6, Wilks's lambda=0.201, x^2=22.44, P=0.001), and Function 2 explained 11.4% (df=2, wilks's lambda=0.74, x^2=4.16, P=0.125).

Regarding the multivariate analysis, PCA and DFA shown that *P. personatus* on Factor 1 (PCA analysis) and Function 1 (DFA), *P. Personatus acityae* n. subsp. from Gag Island is separated from others population in Ternate, Tidore and Obi, Bisa Islands. Furthermore, populations from Bisa and Obi also tend to be part of Ternate and Tidore.

Measurement of some external characters indicated that the Gag population has smaller size, for example the forearm lenght is 90.87 mm in average (Table 1) whereas Ternate and Tidore is 103.2 mm and 94.33 mm, respectively. The tibia length of specimens from Gag also smaller than other groups of Ternate, Tidore and Bisa-Obi. The statistic test of PCA was run on 12 external characters and the results show that the island population grouping form three coefficients factor with the total variance 93.548%. Factor1, 2 and 3 explained 72.39%, 15.92% and 5.24%, respectively (Table 4, Fig. 4). The analysis of external characters by DFA indicated that all four external characters are important in the discriminant function. Three of them (tibia length, metacarpal 4 phalanx 1 and metacarpal 5 phalanx 2) wereloaded heavily (> 0.5) (Table 5, Fig. 5). Based on the external characters, *P. personatus acityae* n. subsp. from Gag is smaller than *P. personatus personatus* from Ternate, Tidore and Bisa Islands.

Table 1. Measurements in mm for skull, dentary, dental and external characters

Location	**Ternate, Tidore (n=12)**				**Gag (n=4)**				**Bisa, Obi (n=3)**	
Sex	**Male**		**Female**		**Male**		**Female**		**Female**	
Characters	**mean**	**std**	**Mean**	**stdv**	**Mean**	**Std**	**Mean**	**Std**	**Mean**	**std**
GSL	44,68	1,02	45,67	0,37	43,30	0,38	42,64	2,51	45,97	0,54
ZB	25,97	0,50	26,51	0,46	25,37	0,49	24,45	2,79	25,63	0,23
LIW	6,81	0,47	7,15	0,24	6,82	0,10	6,54	0,35	6,78	0,23
POW	8,96	0,50	8,12	0,30	8,72	0,60	9,35	0,11	8,44	0,07
BW	15,89	0,40	15,89	0,67	15,49	0,43	14,82	0,06	15,72	0,54
CDL	42,87	1,17	43,67	0,38	41,44	0,70	40,95	2,84	44,00	0,71
ONLL	14,47	0,49	15,39	0,37	14,29	0,35	13,91	0,47	15,22	0,04
PL	22,08	0,76	22,77	0,28	21,66	0,08	20,81	0,75	22,68	0,21
MFW	6,01	0,41	6,27	0,46	5,62	0,24	5,84	0,38	5,93	0,23
CC	5,82	0,21	6,05	0,10	5,84	0,03	5,82	0,80	6,03	0,06
P3P3	6,85	0,25	7,07	0,46	7,17	0,47	7,42	0,69	6,78	0,25
P4P4	7,52	0,19	7,75	0,36	7,43	0,33	7,78	0,50	7,43	0,06
M1M1	8,33	0,22	8,50	0,44	8,02	0,17	8,44	0,62	8,12	0,08
M2M2	8,81	0,28	8,97	0,35	8,60	0,20	8,57	0,45	8,56	0,06
CL	1,97	0,13	2,08	0,04	1,94	0,01	1,90	0,16	2,16	0,06
P3L	1,52	0,11	1,67	0,07	1,55	0,01	1,59	0,04	1,52	0,22
P4L	1,79	0,06	1,69	0,46	1,78	0,05	1,79	0,06	1,73	0,22
M1L	1,72	0,07	1,81	0,15	1,85	0,13	1,79	0,11	1,76	0,23
M2L	1,01	0,04	0,99	0,03	0,85	0,23	0,93	0,04	0,91	0,09
C1M2	16,72	0,86	17,20	0,26	16,36	0,26	16,04	0,01	17,33	0,49
CPL	32,97	1,27	33,89	1,47	31,88	0,63	30,57	0,83	33,49	0,48
RAP	15,21	0,46	16,05	0,62	14,06	0,91	13,07	1,54	15,05	0,76
HB	134,55	10,45	145,92	10,94	123,98	4,21	129,87	11,12	150,00	8,66
FA	91,30	3,20	94,22	3,13	91,50	2,12	90,26	4,66	103,20	0,89
TB	42,78	1,84	45,07	1,85	42,26	0,98	40,05	4,64	50,90	1,61
E	21,25	1,37	24,41	7,51	21,17	0,17	19,94	2,92	22,77	1,00
W	114,88	16,75	141,00	23,43	93,50	0,71	92,00	26,87	142,00	14,42
M2_1	45,64	2,94	48,75	1,20	46,13	1,32	45,12	1,92	52,28	2,43
M2_2	13,24	1,10	13,43	0,71	14,20	0,03	13,53	0,47	14,65	0,33
M3_1	63,45	3,07	65,89	1,52	63,72	1,10	62,23	1,46	69,45	2,64
M3_2	47,00	1,87	48,45	3,93	47,69	0,03	46,41	2,43	55,24	1,55
M4_1	62,14	3,10	66,86	1,14	63,28	0,23	61,97	4,60	67,99	3,50
M4_2	37,42	1,95	37,35	5,14	37,25	0,83	35,40	3,26	41,96	2,04
M5_1	65,85	2,97	69,46	2,06	67,24	2,63	65,12	4,43	72,09	3,16
M5_2	29,02	0,78	30,58	0,66	28,04	0,24	28,96	1,72	33.24	0,31

Table 2. Skull and dental coefficient Factor of PCA of *P. personatus* from Bisa and Obi Islands, the type locality of Ternate, Tidore and Gag Island

	Raw			Rescaled		
	Component			Component		
	1	2	3	1	2	3
GSL	1,380	-0,010	-0,222	0,978	-0,007	-0,157
ZB	0,816	0,457	0,044	0,821	0,460	0,044
LIW	0,123	0,143	-0,026	0,322	0,375	-0,068
POW	-0,322	0,030	0,042	-0,582	0,055	0,076
BW	0,395	-0,138	0,257	0,726	-0,253	0,472
CDL	1,354	0,032	-0,418	0,935	0,022	-0,289
ONLL	0,537	-0,114	-0,041	0,846	-0,180	-0,065
PL	0,697	-0,082	-0,124	0,864	-0,101	-0,154
MFW	0,157	0,139	0,145	0,394	0,350	0,365
CC	0,168	0,041	-0,075	0,638	0,154	-0,286
P3P3	0,019	0,039	-0,072	0,049	0,102	-0,186
P4P4	0,085	0,065	-0,022	0,306	0,235	-0,079
M1M1	0,149	0,088	0,004	0,465	0,273	0,014
M2M2	0,164	0,141	0,066	0,539	0,464	0,216
CL	0,052	-0,048	0,012	0,420	-0,384	0,093
P3L	0,010	0,012	-0,001	0,089	0,105	-0,008
P4L	0,011	-0,005	0,119	0,051	-0,024	0,564
M1L	0,004	-0,032	0,033	0,036	-0,284	0,290
M2L	0,025	0,021	0,028	0,280	0,239	0,318
C1M2	0,551	-0,387	0,015	0,781	-0,549	0,022
CPL	1,359	-0,364	0,401	0,925	-0,248	0,273
RAP	0,890	0,452	0,321	0,812	0,412	0,293

Table 3. Skull and dental characters standardised and unstandardised (in bracket) of Canonical Discriminant Function Coefficients

	Function	
Characters	1	2
RAP	0.574 (0.731)	1,118 (1.424)
P3P3	-0.843 (-2.303)	0.250 (0.684)
GSL	0.649 (0.600)	-1,108 (-1.025)
Constant	-21.678	19.608

Table 4. External characters Coefficient Factor of PCA of *Pteropus personatus* from Bisa, Obi Islands, the type locality of Ternate, Tidore-Halmahera Islands and Gag Island.

Characters	Component		
	1	2	3
HB	0,95752	-0,28607	0,009922
FA	0,83319	0,34187	-0,33317
TB	0,85549	0,313827	-0,26676
E	0,390787	-0,02431	0,832626
M2_1	0,661485	0,6624	-0,02078
M2_2	0,211281	0,77257	-0,18811
M3_1	0,712411	0,606503	0,125516
M3_2	0,620249	0,662983	0,179753
M4_1	0,713892	0,47153	0,062627
M4_2	0,536621	0,614839	0,309891
M5_1	0,728325	0,607645	0,061077
M5_2	0,755112	0,519714	-0,06598

Table 5. External characters standardised and unstandardised (in bracket) of Canonical Discriminant Function Coefficients

Characters	Function	
	1	2
FA	-0,495 (-0,16)	2,254 (0,728)
TB	1,323(0,59)	-2,177 (-0,971)
M4_1	-1,646 (-0,493)	0,542 (0,162)
M5_2	1,506(1,502)	-0,179 (-0,178)
Variation explained	95.5%	4.5%
(Constant)	-24,357	-30,411

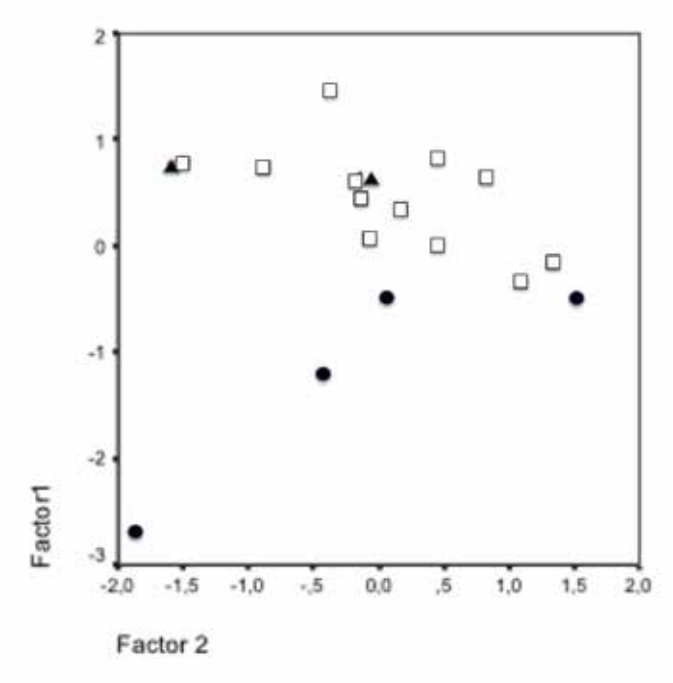

Figure 2. PCA based on the skull and dentary measurements of *P. personatus acityae* n. subsp. (circle), *P. personatus personatus* from Tidore, Ternate (square) and from Bisa and Obi (triangle).

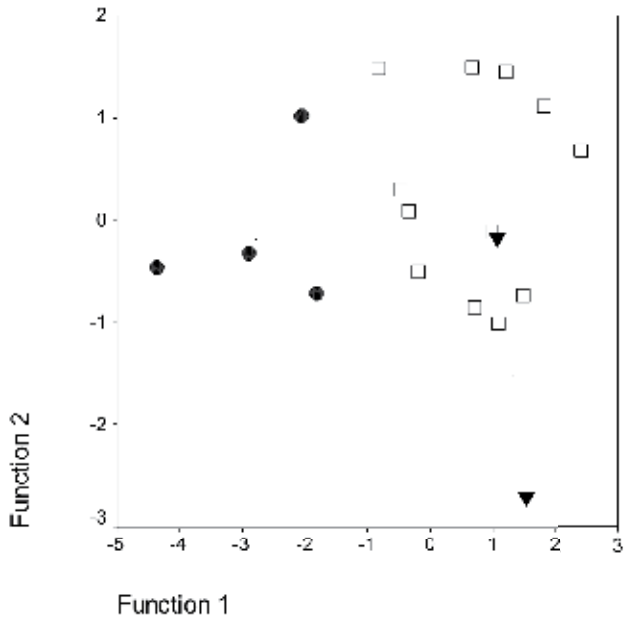

Figure 3. DFA based on the skull and dentary measurements of *P. personatus acityae* n. subsp. (circle), *P. personatus personatus* from Tidore, Ternate (square) and from Bisa and Obi (triangle).

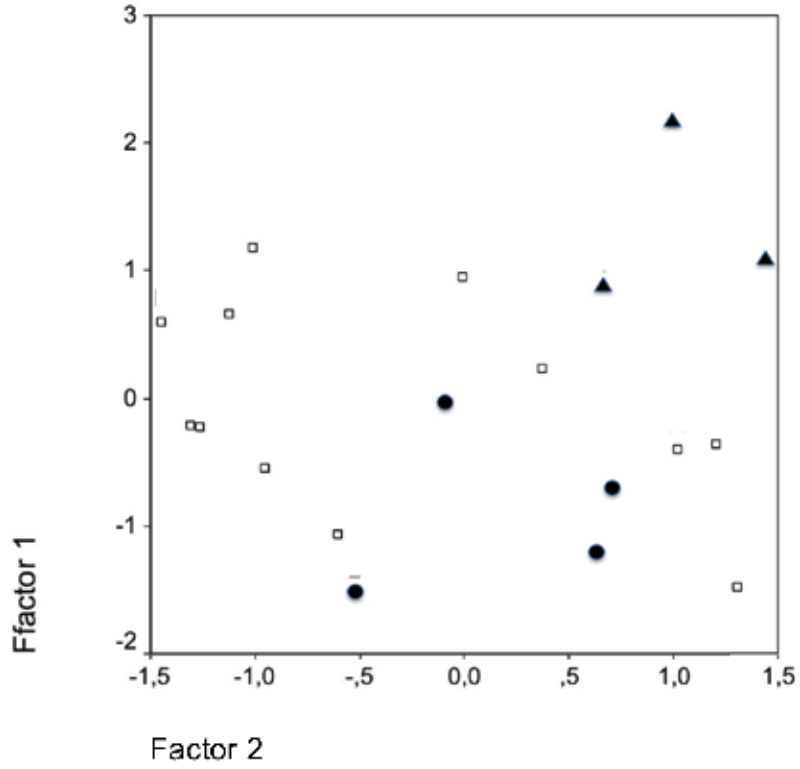

Figure 4. PCA based on the external measurements of *P. personatus acityae* n. subsp. (circle), *P. personatus personatus* from Tidore, Ternate (square) and from Bisa and Obi (triangle).

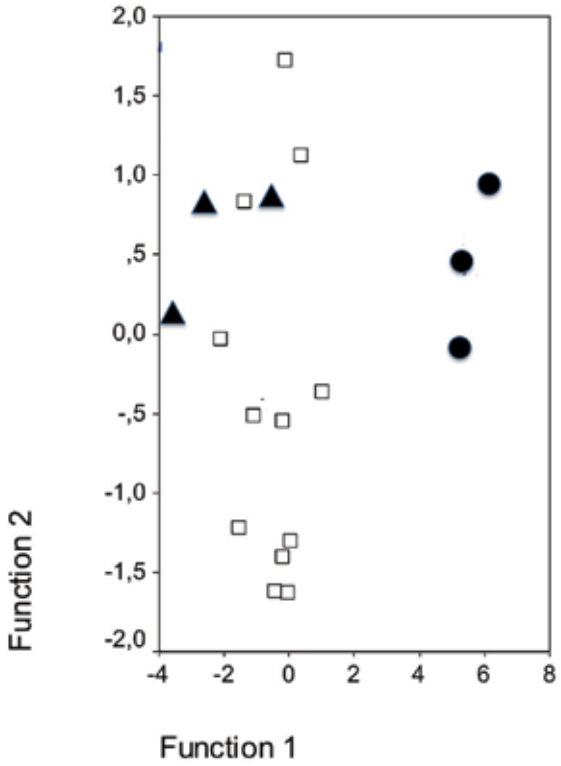

Figure 5. DFA based on the external measurements of *P. personatus acityae* n. subsp. (circle), *P. personatus personatus* from Tidore, Ternate (square) and from Bisa and Obi (triangle).

Phylogenetic reconstruction

A total of 10 partial sequences of 404 bp each of mtDNA cyto *b* were obtained and used in the phylogenetic analysis. These sequences consisted of six sequences of *P. personatus personatus* from Ternate, two sequences of *P. personatus acityae* n.subsp. from Gag and two sequences of *P. conspicillatus* and *P. hypomelanus* as outgroups.

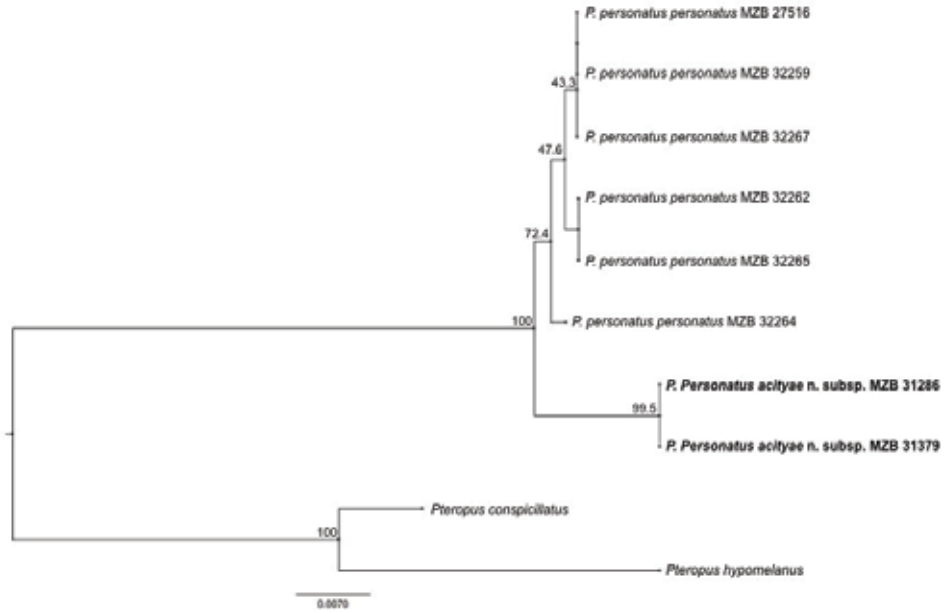

Figure 6. NJ tree showing the relationships between *P. personatus acityae* n.subsp. from Gag Island and. *P. personatus personatus* from Ternate Island based on 404 bp mtDNA cyto *b* sequences.

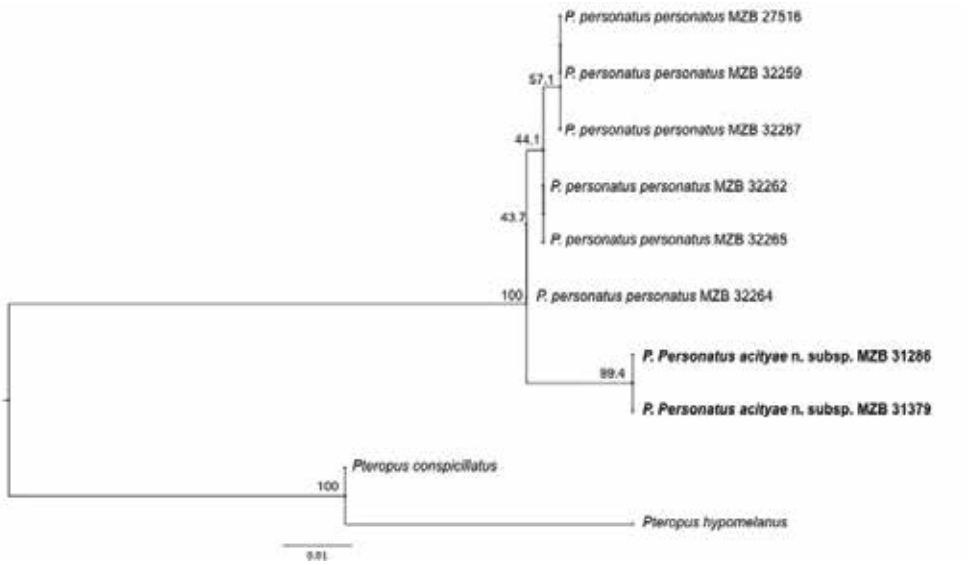

Figure 7. ML tree showing the relationships between *P. personatus acityae* n.subsp. from Gag Island and. *P. personatus personatus* from Ternate Island based on 404 bp mtDNA cyto *b* sequences.

The phylogenetic trees which were constructed using NJ and ML showed similar topology (Figs. 6 and 7). *Pteropus personatus* grouped into a monophyletic clade and *P. personatus acityae* n. subsp. is separated from the *P. personatus personatus* supporting by high bootsrap values, 100% in both NJ and ML analyses.

The genetic distance was calculated by using the Kimura two-parameter model (Table 6). Genetic distance within *P. personatus personatus* is between 0-0.5 %, while between two sequences of *P. personatus acityae* n. subsp. is 0%. Furthermore, genetic distance between *P. personatus acityae* n. subsp. and *P. personatus personatus* is between 1.5-1.8 % which clearly supported that *P. personatus acityae* n. subsp. is a separate subspecies. The results were correspond with the genetic species concept of mammals by using Cytochrome *b* gene of Bradley & Baker (2001). They considered that species contained more than two recognised subspecies shows genetic distance values between 0.09-2.34 %, with average 1.04%. Hence this genetic reconstruction is in concordant with the morphological analyses and affirms that *P. personatus acityae* n. subsp. is a distinct subspecies.

Table 6. Genetic distances (%) among *P. personatus* specimens and other two outgroups analysed from 404 bp mtDNA cyto *b* gene.

		a	b	c	d	e	f	g	h	i
a.	*P. p. personatus* MZB 32262	-								
b.	*P. p. personatus* MZB 32265	0.0	-							
c.	*P. p. personatus* MZB 32264	0.2	0.2	-						
d.	*P. p. personatus* MZB 32267	0.2	0.2	0.5	-					
e.	*P. p. personatus* MZB 27516	0.2	0.2	0.5	0.0	-				
f.	*P. p. personatus* MZB 32259	0.2	0.2	0.5	0.0	0.0	-			
g.	*P. p. acityae* n.subsp. MZB 31286	1.8	1.8	1.5	1.5	1.5	1.5	-		
h.	*P. p. acityae* n.subsp. MZB 31379	1.8	1.8	1.5	1.5	1.5	1.5	0.0	-	
i.	*P. conspicillatus*	9.4	9.4	9.1	9.1	9.1	9.1	10.3	10.3	-
j.	*P. hypomelanus*	11.8	11.8	11.5	11.5	11.5	11.5	12.1	12.1	3.9

DISCUSSION

The morphological and genetic study of the Masked Flying fox, *Pteropus personatus* species group revealed that this species group has high variation. Previous study by Flannery (1995) stated that research in progress indicated there were two subspecies, but he did not describe and name the subspecies. The nominate subspecies is restricted to Halmahera group of islands. While other populations outside this region was predicted as distinct subspecies (Fig. 8).

Figure 8. Distribution map of *Pteropus personatus personatus* in the northern Moluccas Islands with the exception for *P. personatus acityae* n. subsp in Gag Island (in red circle).

Based on the morphological analyses, *P. personatus acityae* n. subsp. from Gag Island shows differences in term of morphological characters. The size of skull and external characters of the Gag population is smaller than the other populations from Ternate, Tidore, Bisa and Obi Islands. However the external performances are similar. The phylogenetic reconstructions are also congruent with the morphological analyses. Samples of *P. personatus acityae* n. subsp. from Gag Island is separated from the others supported by high bootstrap values and also the higher genetic distance, with an average 1.04% indicating as distinct subspecies level (Bradley and Baker, 2001).

This study confirmed that *P. personatus acityae* n. subsp. From Gag Island as a subspecies distinct from *P. personatus personatus* from Ternate, Tidore, Obi, Bisa, Bacan and Halmahera Islands. This result corroborate with Maryanto & Kitchener (1999) who stated that the single specimen from Gag Island almost certainly is a representative of the nominate subspecies. Furthermore, our finding also shows the high variation of island population of species in Indonesia, especially in Moluccas Archipelago.

Record of *P. personatus acityae* n. subsp. from Gag Island is the first record of this subspecies from Papua Barat Province (Maryanto & Kitchener, 1999). Up to the present, Gag Island known as the furthest east of the distribution area of this species, and it has never been found in Papua region. Flannery (1995) found this species particularly common in garden areas. In Gag Island, single specimen was caught in coconut plantation, whereas the other three individuals were found in Valley Forest (Maryanto & Kitchener 1999).

Based on the previous survey and also the collection of MZB, there are 9 species and subspecies of bats recorded from Gag Islands, i.e. *Pteropus neohibernicus, P. conspicillatus, P. personatus acityae* n. subsp., *Nyctimene* sp*., Dobsonia beauforti, Syconycteris australis, Macroglossus minimus, Emballonura alecto,* and *Mosia nigrescens*. It shows that this small island has relatively high bat diversity.

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