CHAPTER 4
RESULTS

Climate at Chiang Dao Wildlife Research Station in 2009

The overall annual rainfall in 2009 was 3495.0 mm with an average monthly rainfall of 291.2 mm (SE = 80.4). The area has two distinctive rainfall periods. Between May and October is the rainy season; May (732.0 mm), June (657.0 mm), July (435.0 mm), August (613.0 mm), September (409.0 mm), and October (308.0 mm). January, February, November, and December have no rain and there is a low amount of rain in March (76.0 mm) and April (265.0 mm).

There were distinct temperature differences between months of the year in which both minimum and maximum temperatures fluctuated with seasonal rainfalls throughout the year. Generally, maximum temperatures were lowest in January (22.6 ± 0.4 ^{o}C), then continuously increased in February (28.0 ± 0.5 ^{o}C) and March (32.4 ± 0.5 ^{o}C), and were highest in April (33.8 ± 0.6 ^{o}C). The maximum temperature lowered in May (31.7 ± 0.5 ^{o}C) and June (28.9 ± 0.5 ^{o}C), then fluctuated in a narrow range from June and October (June, 28.9 ± 0.5 ^{o}C; July, 28.9 ± 0.5 ^{o}C; August, 29.8 ± 0.4 ^{o}C; September, 30.3 ± 0.4 ^{o}C; October, 29.6 ± 0.3 ^{o}C). The maximum temperature distinctively decreased to the lowest point at the end of the year in November (25.8 ± 0.4 ^{o}C) and December (23.4 ± 0.3 ^{o}C). Minimum temperatures showed the same patterns of maximum temperatures. Minimum temperatures were lowest in January (11.4 ± 0.3 ^{o}C), then continuously increased in February (12.9 ± 0.2 ^{o}C), March (16.3 ± 0.4 ^{o}C), and April (20.1 ± 0.3 ^{o}C). Minimum temperatures were steady between April and October (April, 20.1 ± 0.3 ^{o}C; May, 21.0 ± 0.1 ^{o}C; June, 21.0 ± 0.2 ^{o}C; July, 21.7 ± 0.1 ^{o}C; August, 21.5 ± 0.2 ^{o}C; September, 21.4 ± 0.2 ^{o}C; October, 20.2 ± 0.3 ^{o}C). The minimum temperatures distinctively decreased to the lowest point at the end of the year (November, 16.2 ± 0.5 ^{o}C; December, 12.6 ± 0.4 ^{o}C).

The weather conditions at the Chiang Dao Wildlife Research Station in 2009 are shown in Figure 2. The data shows the typical climate condition of northern Thailand, and is consistent with the previous years at Doi Tung, Chiang Rai province (Maxwell, 2007) and northern Thailand (Singhrattna *et al*., 2009).

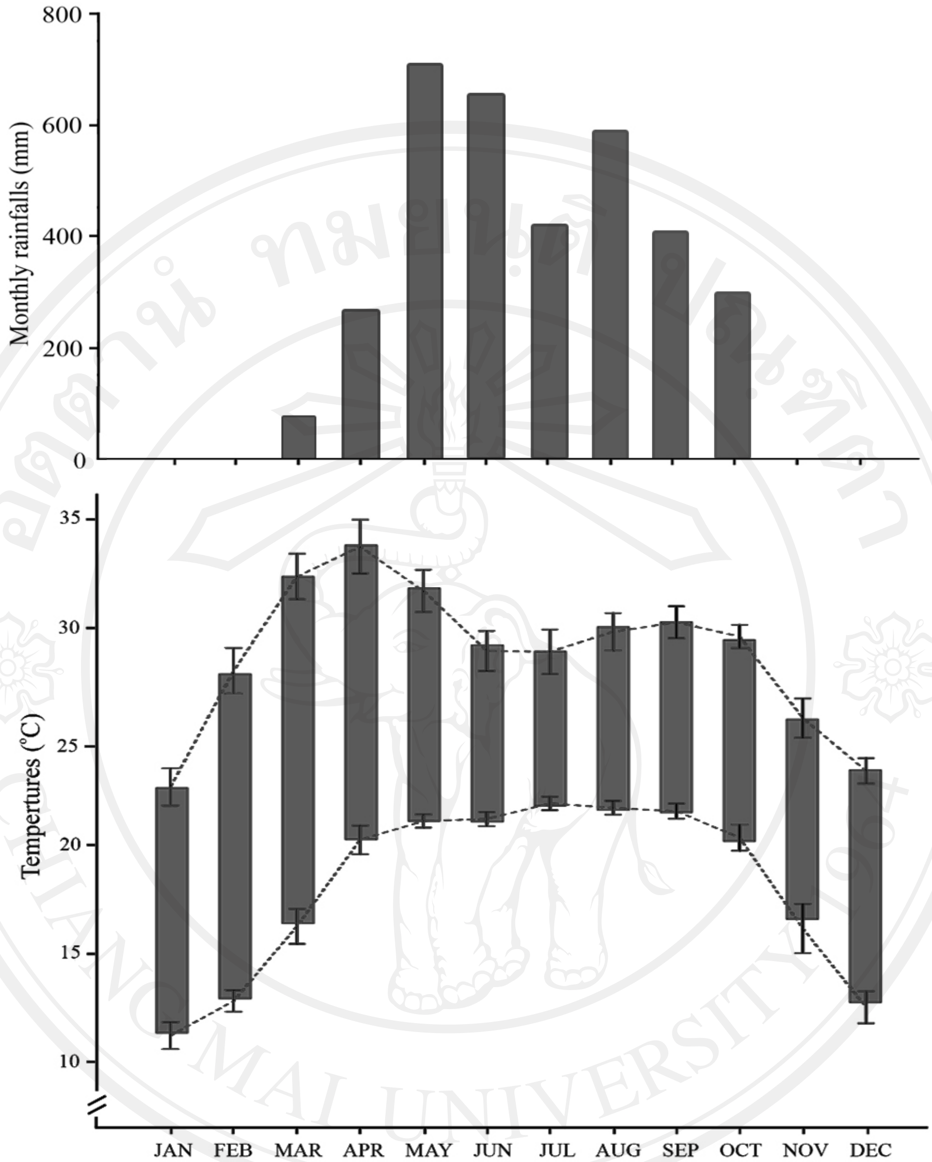

Figure 2. Climatic variations at Chiang Dao Wildlife Research Station in 2009 showing monthly rainfall (mm) and average daily minimum and maximum temperature (^oC).

Species-distinctiveness by vocal communication

Songs of six species of *Pycnonotus* bulbuls were recorded and measured in northern Thailand. All songs included in this study are homogenous in the context of communication, which is particularly important for defending territory and food resources. Statistics describing structural features of songs of the six species are presented in Table 1.

Description of vocal behaviour

Black-headed Bulbul (*Pycnonotus atriceps*)

Vocal behaviour. Black-headed Bulbuls sing a song that consists of repeated downslurred tonal elements (Figure 3a). This species has the highest number of elements per song, but just a single element type, with a narrow frequency bandwidth of ~2.3 kHz. Their songs contain the highest number of syllables, with 15.74 ± 4.69 syllables in one song. The songs have a narrow frequency range (the average minimum frequency = 2.27 ± 0.15 kHz, average maximum frequency = 4.62 ± 0.24 kHz, and average frequency bandwidth = 2.3± 0.35 kHz). This gives their songs a distinctive high pitch. This repeated-note song is very distinctive compared to other species of this group, and is the longest of the six species' songs with an average length of 3.79 ± 1.19 sec. This species was most often recorded singing in dense vegetation (e.g. within bamboo thickets and inside the middle storey of the forest).

Black-crested Bulbul (*Pycnonotus flaviventris*)

Vocal behaviour. Black-crested Bulbuls have a complex songs composed of frequency-modulated tones (Figure 3b). Their short songs contain various element types which usually start at a low frequency, then increase to a high terminal frequency, with a frequency bandwidth of ~2.0 kHz (minimum frequency = 1.72 ± 0.20 kHz, maximum frequency = 3.72 ± 0.35 kHz). Songs are short (0.69 ± 0.13 sec) and contain an average of 3.59 ± 0.78 syllables per song, including 3.58 ± 0.76 different syllable types per song. Songs start at a low frequency, then increase to a high terminal frequency, with a frequency range of 2.00 ± 0.35 kHz. This species is among the most common birds that inhabit the low elevation forest habitat in

Thailand, and was the most frequently encountered among the six bulbul species (28% of recorded songs). Birds were most often recorded singing from the topmost part of the canopy of the forest.

Streak-eared Bulbul (*Pycnonotus blanfordi*)

Vocal behaviour. Streak-eared Bulbuls sing trilled songs, consisting of repeated notes with short element duration (Figure 3c; element duration less than 0.1 s). The repeated element is a harsh, rising note, with a frequency bandwidth of ~4.0 kHz and very short syllable lengths (0.04 ± 0.05 sec). The minimum frequency was 1.25 ± 0.30 kHz, and the maximum frequency was 5.22 ± 0.58 kHz. Songs have an average length of 1.54 ± 0.55 sec with 4 to 21 syllables per song (average number of syllable per song = 8.55 ± 2.76). In contrast with other bulbuls, this shy and plain-coloured bulbul was often found in bushy habitat where it was difficult to observe due to dense vegetation (e.g. in dense bamboo thicket, thicket habitat along the forest edge, and forest understorey).

Stripe-throated Bulbul (*Pycnonotus finlaysoni*)

Vocal behaviour. Stripe-throated Bulbuls sing complex song composed of various frequency-modulated elements (Figure 3d). Song is short in length (0.73 ± 0.25 sec). This species' song has the lowest maximum frequency of 3.04 ± 0.47 kHz and also the lowest minimum frequency of 1.20 ± 0.15 kHz, when compared to the other bulbul species, with an average frequency bandwidth of ~1.8 kHz.. Among the six species, Stripe-throated Bulbuls show the highest element diversity per song (syllable diversity per song: 3.90 ± 1.04) with various element types comprise the 3 to 19 syllables per song (5.35 ± 1.97 syllables per song, on average). Morphologically, this plain-coloured bulbul is very similar to Streak-eared Bulbul; in the absence of vocalizations, distinguishing between these two species is difficult, particularly since both live in dense vegetation.

Red-whiskered Bulbul (*Pycnonotus jocosus*)

Vocal behaviour. Red-whiskered Bulbuls sing a complex song consisting of various frequency-modulated element types (Figure 3e). The songs are short and have an average song length of 0.64 ± 0.12 sec. The songs consist of varying syllable types, with an average of 3.72 ± 0.94 syllables per song and an average number of syllable types per song of 3.47 ± 0.88. The song has a moderate frequency, with an average frequency bandwidth of 2.25 ± 0.31 kHz, an average minimum frequency of 1.69 ± 0.22 kHz, and an average maximum frequency of 3.94 ± 0.37 kHz. Birds typically sang from high, exposed perches (e.g. tree tops, electric wires, roof tops), particularly in the early mornings. This species was found often in anthropogenically modified habitats, at forest edges, in parks, and near human habitation.

Sooty-headed Bulbul (*Pycnonotus aurigaster*)

Vocal behaviour. Sooty-headed Bulbuls have a simple song structure (Figure 3f). Their songs have the shortest length of the six described species (0.40 ± 0.10 sec) and songs contain the lowest number of syllables (average number of syllables per song = 3.30 ± 0.68). Their songs span a narrow frequency range (frequency bandwidth = 1.56 ± 0.63 kHz), with an average minimum frequency of 1.88 ± 0.26 kHz and a maximum frequency of 4.5 ± 0.47 kHz. Birds usually sing from tree tops, and the birds were observed feeding in small groups of 5 - 10 individuals at all times of the year.

Table 1. Measurements of the fine structural features of the songs of six species of *Pycnonotus* bulbuls recorded in northern Thailand. Values for each species are given as means ± SE.

	Black-headed Bulbul (N = 16)	Black-crested Bulbul (N = 48)	Streak-eared Bulbul (N = 20)	Stripe-throated Bulbul (N = 29)	Red-whiskered Bulbul (N = 41)	Sooty-headed Bulbul (N = 32)
Song duration (sec)	3.8 ± 1.2	0.7 ± 0.1	1.5 ± 0.6	0.7 ± 0.3	0.6 ± 0.1	0.4 ± 0.1
Maximum frequency of the song (kHz)	4.6 ± 0.2	3.7 ± 0.4	5.2 ± 0.6	3.0 ± 0.5	3.9 ± 0.4	3.5 ± 0.5
Minimum frequency of the song (kHz)	2.3 ± 0.2	1.7 ± 0.2	1.3 ± 0.3	1.2 ± 0.2	1.7 ± 0.2	1.9 ± 0.3
Average element duration (sec)	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1
Average max. frequency of elements (kHz)	4.1 ± 0.2	3.1 ± 0.3	4.5 ± 0.5	2.6 ± 0.4	3.6 ± 0.4	3.1 ± 0.3
Average min. frequency of elements (kHz)	2.6 ± 0.1	2.0 ± 0.2	1.7 ± 0.4	1.6 ± 0.3	2.0 ± 0.2	2.2 ± 0.2
Longest element (sec)	0.1 ± 0.0	0.2 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.1
Average inter-element interval (sec)	0.3 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	0.1 ± 0.0
Number of types of element	1.0 ± 0.0	3.6 ± 0.8	1.0 ± 0.0	3.9 ± 1.0	3.5 ± 0.9	2.5 ± 0.7

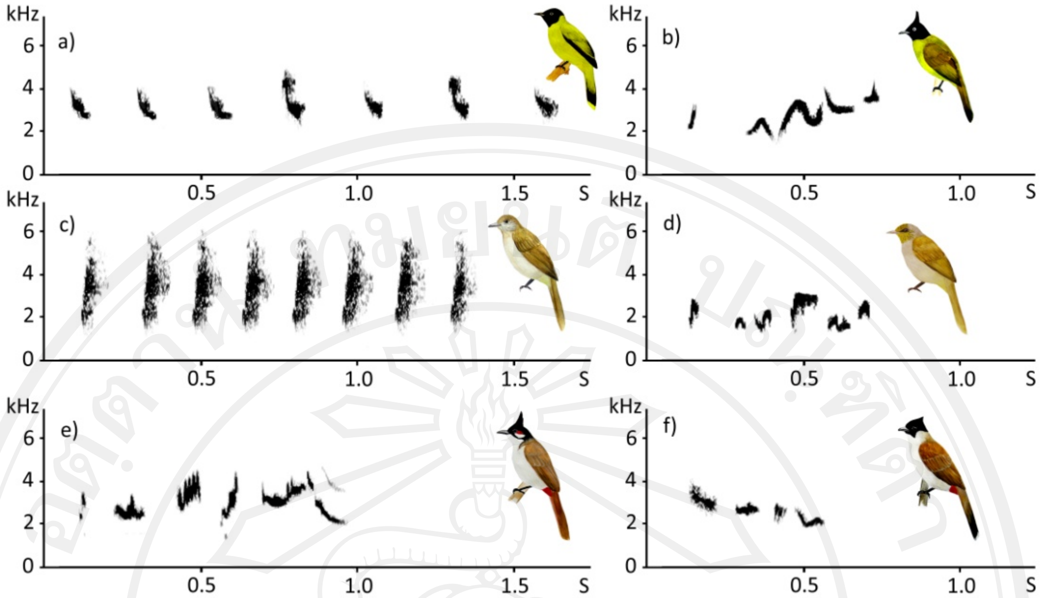

Figure 3. Sound spectrograms showing the typical song recorded from each of the six species of *Pycnonotus* bulbul in northern Thailand: a) Black-headed Bulbul, b) Black-crested Bulbul, c) Streak-eared Bulbul, d) Stripe-throated Bulbul, e) Red-whiskered Bulbul, and f) Sooty-headed Bulbul.

Multi-species comparison

Discriminant function analysis distinguished between the six species of *Pycnonotus* bulbuls based on nine fine structural variables (Wilks' lamda = 0.003; *df* = 45, $p < 0.0001$; Figure 4). This analysis, with cross-validation, correctly predicted the species identity with an accuracy of 88.7%, which is significantly higher than the 16.7% level expected by chance (Binomial test: $p < 0.0001$). Discriminant function analysis generated three canonical axes that explained 94.0% of the total variance in structural characteristics of the songs of the six species (Table 2). The first canonical axis explained 63.6% of the variance, and showed a strong positive relationship with the number of elements in the song, and strong negative relationship with song duration; high values for this canonical axis reflect heterogeneous element and short songs. The second canonical axis explained 17.3% of the variance, and showed a strong positive relationship with minimum frequency of the entire song; high values for this canonical axis reflect songs with a high minimum frequency. The third canonical axis explained 13.1% of the variance, and showed a strong positive relationship with inter-element interval, and strong negative relationship with number of types of element within a song; high values for this canonical axis reflect songs with well separated element interval and homogeneous element songs.

Multivariate analyses of song structure reveals that each species produces distinctive vocalizations, which provide acoustic cues of species identity. The results from discriminant analysis showed that the song structures of all six species can be subdivided into two discrete groups (Figure 4). First group is Black-headed Bulbul and Streak-eared Bulbul. The former sings narrow-bandwidth, down-slurred elements at a slow pace, whereas the latter sings broad-bandwidth, up-slurred elements at a fast pace. These two species sing trilled songs, which can be readily distinguished from each other based on these differences. Second group is the remaining four species that produce songs composed of various frequency-modulated tones: Black-crested Bulbul, Stripe-throated Bulbul, Red-whiskered Bulbul, and Sooty-headed Bulbul. Red-whiskered Bulbuls have the most complex songs, with many elements featuring rapid frequency modulations. Sooty-headed Bulbuls have the simplest songs of those described here, with few elements that descend in pitch over the course of the song. The remaining two species can be distinguished on the basis of their frequency range (Stripe-throated Bulbuls have the lowest-pitched frequency-modulated songs in the group) and the organization of elements (Black-crested Bulbul songs tend to rise in pitch more dramatically than the other species).

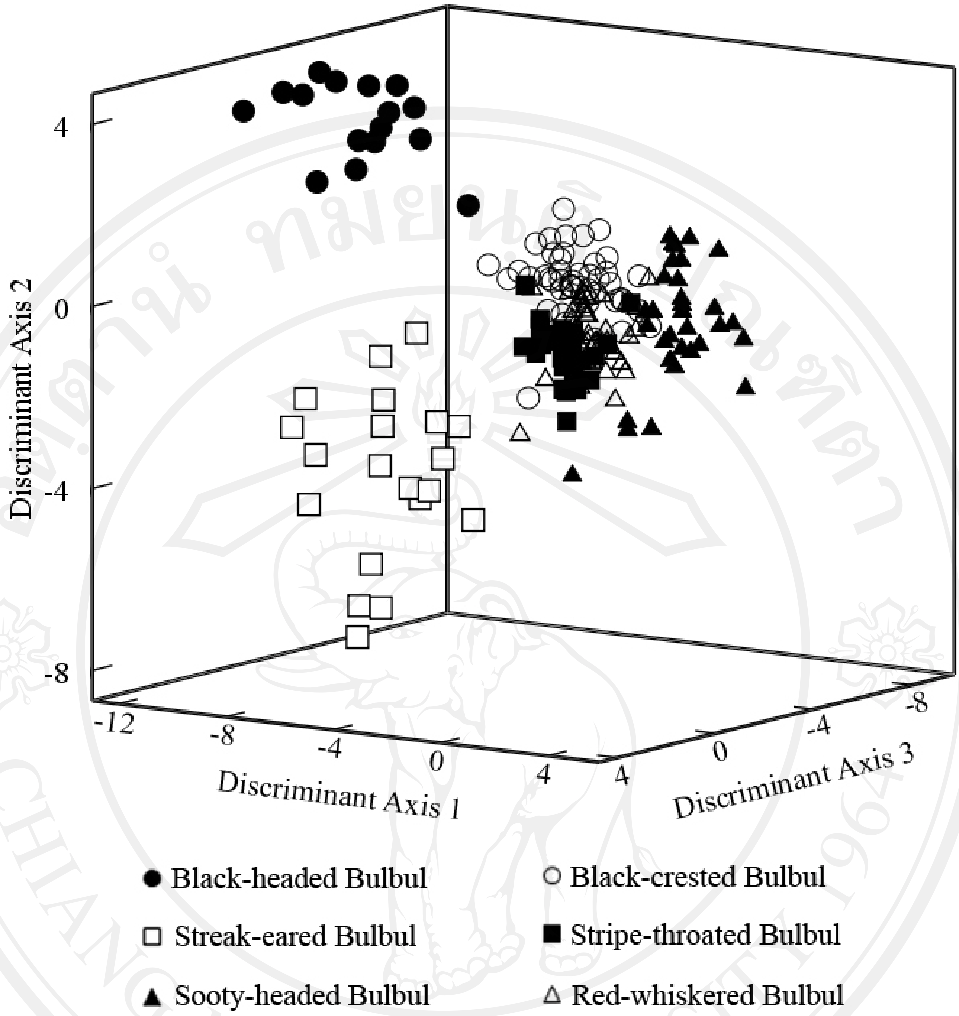

Figure 4. A scatterplot of the first three canonical discriminant functions of acoustic features resulting from the discriminant analysis explained correctly 94.0% of variances.

Table 2. Discriminant function analysis of the songs of six species of *Pycnonotus* bulbuls produced three factors summarizing nine fine structural features of songs, represented here as the correlation coefficients between the original data and the three factors; variables with the strongest relationships (correlations > 0.60) are indicated in bold font.

Variable	Factor 1	Factor 2	Factor 3
Song duration	**-0.75**	0.40	0.04
Maximum frequency of the entire song	-0.38	-0.23	-0.03
Minimum frequency of the entire song	0.03	**0.65**	-0.38
Element duration	0.49	0.05	0.34
Maximum frequency of each element	0.05	-0.52	0.14
Minimum frequency of each element	0.09	0.32	-0.21
Duration of longest element	0.25	-0.01	-0.56
Inter-element interval	-0.21	0.49	**0.87**
Number of types of element within a song	**0.73**	0.37	**-0.66**
Eigenvalue	11.55	3.15	2.38
% of variance explained	63.55	17.31	13.11

Three additional pair-wise discriminant function analysis were performed to evaluate whether song structure could differentiate between pairs of species with similar plumage characters (as assessed by human observers; see Figure 5). Acoustic differences were compared between pairs of species with the highest plumage similarities, based on the assumption that similar plumage indicates closely-related species with heightened risks of hybridization. Field data demonstrate that these pairs of visually-similar species routinely encounter one another, based on the proportion of locations where the pairs of species were both encountered: Black-headed Bulbuls (20/23 locations) versus Black-crested Bulbuls (20/48 locations included both species); Streak-eared Bulbuls (17/20 locations) versus Stripe-throated Bulbuls (17/29 locations included both species); and Red-whiskered Bulbuls (23/41 locations) versus Sooty-headed Bulbuls (23/32 locations included both species). All six species in the same foraging flock were detected on at least one occasion, and on multiple occasions encountering flocks containing four or five of the species in different combination. Pair-wise encountering rates of all six species were summarized in Table 3.

In pair-wise comparisons of species with high plumage similarity, it was substantial divergence in acoustic features of song (Figure 5 and Table 4). In a comparison of Black-headed versus Black-crested Bulbuls (Wilks' lambda = 0.027; *df* = 9, $p < 0.0001$), cross-validated DFA separated the species based on song measurements (100% assigned to the correct species, significantly higher than the chance rate of 50%; binomial test: $p < 0.0005$); the canonical axis showed a strong negative relationship with song length, maximum frequency of songs measures, and a strong positive relationship with average element length, maximum frequency of each element and number of types of element. Similarly, in a comparison of Streak-eared versus Stripe-throated Bulbuls (Wilks' lambda = 0.052; *df* = 9, $p < 0.0001$), cross-validated DFA separated the species based on song measurements (100% assigned to the correct species, significantly higher than the chance rate of 50%; binomial test: $p < 0.002$); the canonical axis showed a strong negative relationship with minimum frequency of each element, and a strong positive relationship with the length of the element and maximum frequency of each element. Similarly, in a comparison of Red-whiskered versus Sooty-headed Bulbuls (Wilks' lambda = 0.20; *df* = 9, $p < 0.0001$), cross-validated DFA separated the species based on song measurements (100% assigned to the correct species, significantly higher than the chance rate of 50%; binomial test: $p < 0.0001$); the canonical axis showed a strong negative relationship with the longest element, and a strong positive relationship with inter-element interval, and number of types of elements.

Table 3. Encountering rates between species showing that visually-similar species were routinely encountered based on the proportion of locations where species were encountered; highest proportions of encounter rate between paired visual similar speceis are indicated in bold font. Species abbreviations; BHB = Black-headed Bulbuls, BCB = Black-crested Bulbuls, SEB = Streak-eared Bulbuls, STB = Stripe-throated Bulbuls, SHB = Sooty-headed Bulbuls, and RWB = Red-whiskered Bulbuls.

	BHB	BCB	SEB	STB	SHB	RWB
BHB		**20/23**	4/23	3/23	1/23	2/23
BCB	**20/48**		7/48	12/48	2/48	6/48
SEB	4/20	7/20		**17/20**	12/20	15/20
STB	3/29	12/29	**17/29**		6/29	7/29
SHB	1/41	2/41	12/41	6/41		**23/41**
RWB	2/32	6/32	15/32	7/32	**23/32**	

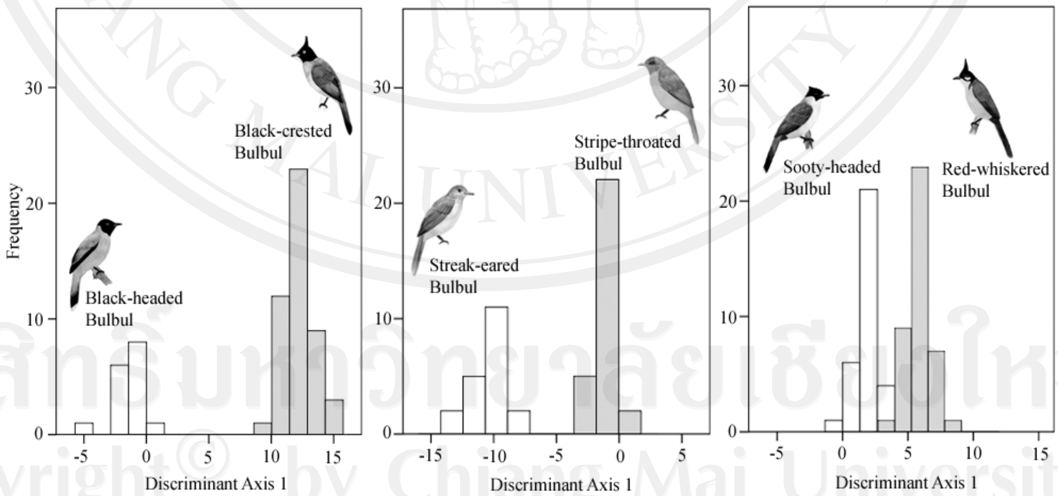

Figure 5. Histograms showing the discriminant scores for pair-wise comparisons of *Pycnonotus* bulbuls that share similar plumage features, based on three separate discriminant analyses of nine structural features of songs.

Table 4. Discriminant function analysis of the songs of pair-wise comparisons of *Pycnonotus* bulbuls that share similar plumage features, based on three separate discriminant analyses of nine structural features of songs; variables with the strongest relationships (correlations > 0.60) are indicated in bold font. Species abbreviations: BHB = Black-headed Bulbuls, BCB = Black-crested Bulbuls, SEB = Streak-eared Bulbuls, STB = Stripe-throated Bulbuls, SHB = Sooty-headed Bulbuls, and RWB = Red-whiskered Bulbuls.

Variable	BHB-BCB	SEB-STB	SHB-RWB
Song duration	**-0.72**	-0.28	0.27
Maximum frequency of the entire song	**-0.64**	-0.44	0.24
Minimum frequency of the entire song	0.04	-0.31	-0.06
Element duration	**1.55**	**0.70**	0.54
Maximum frequency of each element	**0.80**	**-0.60**	0.47
Minimum frequency of each element	-0.11	**0.70**	-0.44
Duration of longest element	-0.22	0.29	**-1.05**
Inter-element interval	-0.23	-0.59	**0.99**
Number of types of element within a song	**1.38**	0.58	**0.66**
Eigenvalue	31.61	19.64	4.35
% of variance explained	100.00	100.00	100.00

Morphometric analyses

Sexes of all six *Pycnonotus* species were determined using P2/P8 primers (Griffiths and Korn, 1997). Amplified fragments are readily discernible in 2% agarose gel which based on the differences sizes of PCR products of CHD-W gene (~415bp) are longer than CHD-Z gene (~350bp). Size of PCR products of this study matched with DNA fragments for sexual identification provided in previous studies i.e. ~345bp for CHD-W and ~293bp for CHD-Z (Chang *et al*., 2010). In this study, a total of 134 adult birds were PCR, and it was found that the 70 individuals have only single band of CHD-ZZ but 64 individuals showed two differences bands of CHD-ZW; these results indicated that there were 70 males and 64 females, respectively. Therefore, this method is effectively to identify sex of in all the six *Pycnonotus* studied here. Gel electrophoresis demonstrating sexual identification by assessing two differences PCR fragments was shown in Figure 6.

Sexual difference of all six *Pycnonotus* species is difficult to distinguish because of similarity in both plumage and body size. Morphometric comparisons indicate that males are slightly bigger than females in all six species (Table 5). There was only a small amount of evidence (20%) that males are significantly bigger than females but in most cases sizes were not diffifferent (80%). There were no sexual differences in Black-headed Bulbuls and Sooty-headed Bulbuls for all morphometric traits. Males were bigger than females for several species (i.e. Black-crested Bulbuls' bill length, *t-test* = -2.915, *df* = 38, *p* = 0.006; Stripe-throated Bulbuls' wing lengths, *t-test* = -2.132, *df* = 29, *p* = 0.049; Red-whiskered Bulbuls' tail lengths, *t-test* = -2.252, *df* = 13, *p* = 0.042). The most differences between sexes was found in the Streak-eared Bulbuls in which males had significantly bigger wing lengths (*t-test* = -3.197, *df* = 25, *p* = 0.004), tarsus lengths (*t-test* = -2.288, *df* = 25, *p* = 0.032), and body weight (*t-test* = -2.500, *df* = 25, *p* = 0.020), however, significant difference in bill length (*t-test* = -1.958, *df* = 25, *p* = 0.061) and tail length (*t-test* = -1.185, *df* = 25, *p* = 0.247).

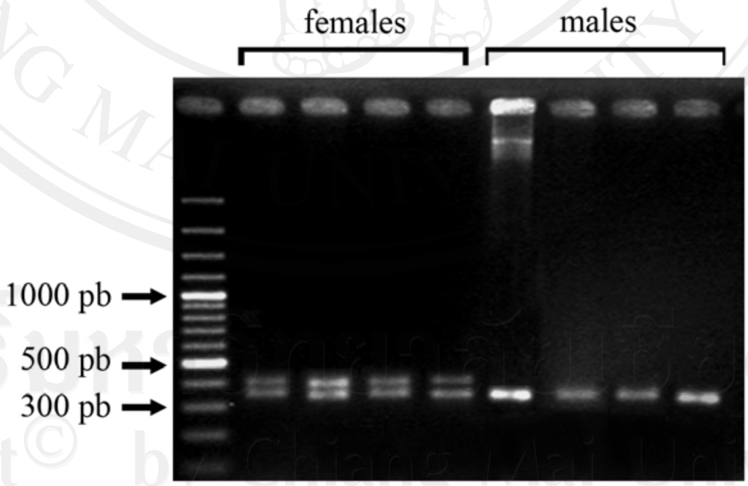

Figure 6. Gel electrophoresis showing sex identification using the primers P2/P8. Two different PCR fragments were amplified from females (double bands) and males (single band).

Table 5. Sexual comparisons using morphometric data of the six *Pycnonotus* bulbuls in Chiang Dao. Data shows the average ± SE.

Species	Bill length (mm)	Wing length (mm)	Tarsus length (mm)	Tail length (mm)	Weight (g)
Black-headed Bulbul					
female (n=5)	16.0 ± 0.3	79.8 ± 1.0	14.2 ± 0.5	70.8 ± 1.1	24.4 ± 0.8
male (n=5)	16.7 ± 0.3	82.0 ± 1.1	15.1 ± 0.2	70.8 ± 1.2	26.4 ± 0.9
p-value	0.067	0.171	0.113	1.000	0.117
Black-crested Bulbul					
female (n=19)	15.9 ± 0.3	84.3 ± 0.5	15.6 ± 0.2	84.0 ± 0.6	27.2 ± 0.5
male (n=21)	16.8 ± 0.1	85.6 ± 0.5	15.9 ± 0.2	84.6 ± 0.6	27.6 ± 0.4
p-value	0.006**	0.101	0.174	0.512	0.559
Streak-eared Bulbul					
female (n=12)	19.2 ± 0.2	84.8 ± 0.8	21.1 ± 0.3	88.6 ± 1.0	32.5 ± 0.6
male (n=15)	19.7 ± 0.2	88.3 ± 0.8	21.8 ± 0.2	90.8 ± 1.5	34.4 ± 0.5
p-value	0.061	0.004**	0.032*	0.247	0.020*
Stripe-throated Bulbul					
female (n=18)	18.3 ± 0.2	79.7 ± 0.4	19.6 ± 0.3	81.6 ± 0.7	28.5 ± 0.7
male (n=13)	17.7 ± 0.5	81.9 ± 1.0	19.2 ± 0.3	83.8 ± 0.9	30.4 ± 0.8
p-value	0.213	0.049*	0.317	0.054	0.086
Sooty-headed Bulbul					
female (n=5)	19.8 ± 0.4	88.0 ± 1.1	22.4 ± 0.4	85.3 ± 1.5	33.2 ± 1.1
male (n=5)	19.9 ± 0.5	89.0 ± 1.2	22.0 ± 0.8	84.6 ± 2.3	35.8 ± 0.8
p-value	0.890	0.571	0.686	0.827	0.081
Red-whiskered Bulbul					
female (n=5)	18.9 ± 0.4	83.8 ± 1.4	20.5 ± 0.7	83.4 ± 1.4	27.9 ± 1.5
male (n=11)	19.4 ± 0.2	84.7 ± 0.6	20.2 ± 0.3	86.8 ± 0.8	29.4 ± 0.5
p-value	0.203	0.476	0.703	0.042*	0.272

Discriminant function analysis distinguished the females of all six species based on five morphometric features (Wilks' lamda = 0.012; *df* = 25, $p < 0.0001$; Figure 7a). This analysis with cross-validation, correctly predicted the species identity with an accuracy of 79.4%, which is significantly higher than the 16.7% level expected by chance (Binomial test: $p < 0.0001$). Discriminant function analysis generated three canonical axes that explained 98.7% of the total variance in the mophometric of the six species (Table 6). The first canonical axis explained 76.6% of the variance, and showed a strong positive relationship with tail length and tarsus length, and a strong negative relationship with wing length; high values for this canonical axis reflect morphometric traits of species with long tail and long tarsus but short wings. The second canonical axis explained 13.0% of the variance, and showed a strong positive relationship with wing length; high values for this canonical axis reflect morphometric traits for species with long wings. The third canonical axis explained 9.1% of the variance, and showed a strong positive relationship with wing length, and a strong negative relationship with tail length; high values for this canonical axis reflect morphometric traits for species with long wings but short tails.

Discriminant function analysis distinguished the males of all six species based on five morphometric features (Wilks' lamda = 0.016; *df* = 25, $p < 0.0001$; Figure 7b). This analysis, with cross-validation, correctly predicted the species identity with an accuracy of 81.4%, which is significantly higher than the 16.7% level expected by chance (Binomial test: $p<0.0001$). Discriminant function analysis generated three canonical axes that explained 96.4% of the total variance in structural characteristics of the mophometric of the six species (Table 6). The first canonical axis explained 80.6% of the variance, and showed a strong positive relationship with tarsus length; high values for this canonical axis reflect morphometric traits with long tarsus species. The second canonical axis explained 10.4% of the variance, and showed a strong positive relationship with wing length, and a strong negative relationship with tail length; high values for this canonical axis reflect morphometric traits of species with long wings. The third canonical axis explained 5.5% of the variance, and showed a strong positive relationship with wing length; high values for this canonical axis reflect with long wings.

The DFA using morphometric data revealed morphological distinctiveness among the six *Pycnonotus* species with a consistent prediction in both female and male morphometrics (Figure 7). Black-headed Bulbuls is the most separated species and has the smallest body size compared to the other *Pycnonotus* bulbuls studied here regarding wing length, tarsus length, tail length, and weight. Black-crested Bulbuls seem to have unique morphometric features, but not highly distinctive. This bird has a comparatively short bill and tarsus, but long wings and tail with a moderate body mass. The remaining four species have a relatively close relationship with an overlap mostly in morphometric features, which might indicate that these four species are ecologically similar.

Table 6. Discriminant function analysis of the morphometric data of six species of *Pycnonotus* bulbuls produced three factors summarizing five morphometric features, represented here as the correlation coefficients between the original data and the three factors; variables with the strongest relationships (correlations > 0.60) are indicated in bold font.

Variables	Female			Male		
	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3
Bill length	0.30	-0.40	-0.14	0.05	0.23	-0.01
Tail length	**0.70**	0.46	**-1.12**	0.44	-1.18	0.15
Wing length	**-0.61**	**0.60**	**1.15**	-0.46	**0.65**	**0.86**
Tarsus length	**0.81**	-0.04	0.34	**0.88**	-0.01	-0.25
Weight	-0.01	0.13	0.21	0.23	0.39	0.15
Eigenvalue	10.38	1.76	1.24	10.17	1.31	0.69
% of variance explained	76.60	13.00	9.10	80.60	10.40	5.50

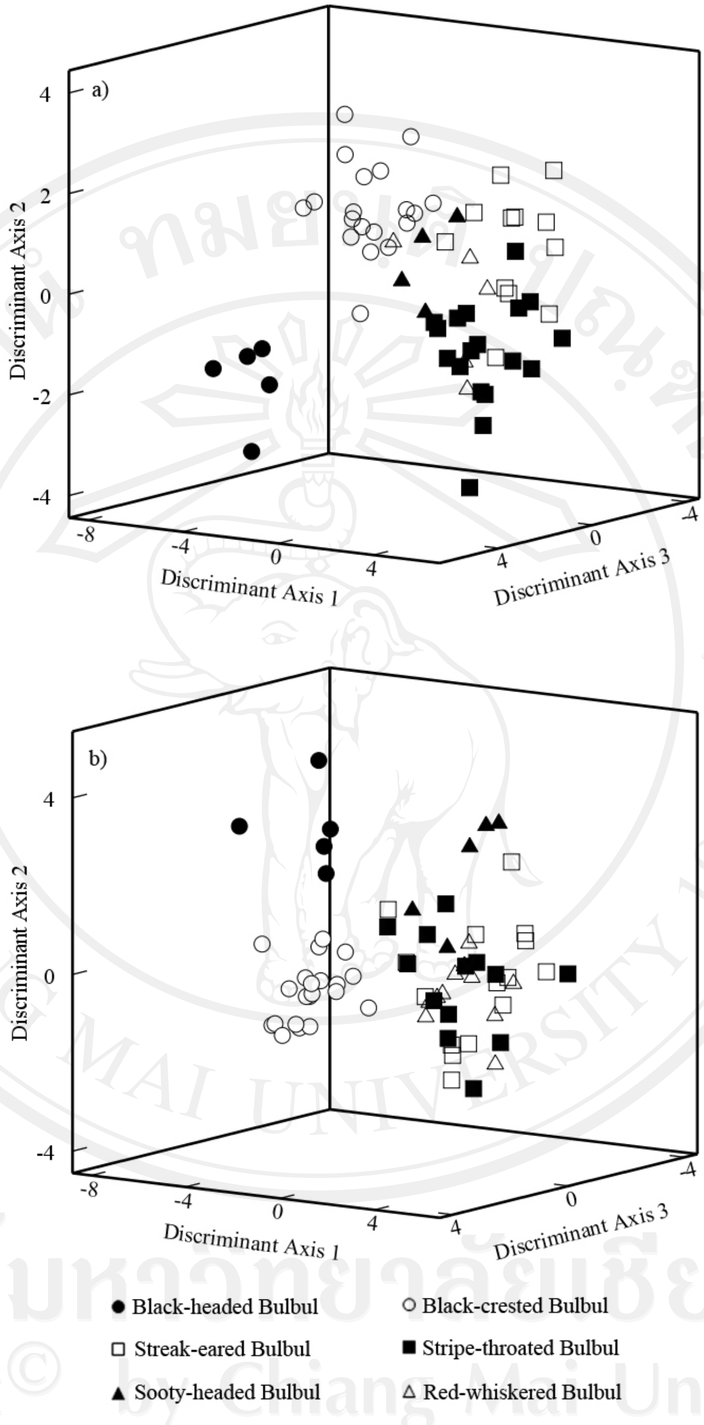

Figure 7. Scatterplot of the first three canonical discriminant functions shows the results from the discriminant analysis; a) cross-validated DFA correctly explained 98.7% of variances from female morphometric features, and b) cross-validated DFA correctly explained 96.4% of variances from male morphometric features.

Sequence analyses of 16s mitochondrial DNA

The partial sequences of the 16s mtDNA (487bp) were obtained from 23 birds. The overall nucleotide diversity ($\pi_{overall}$) across six species was 0.044. The numbers of haplotypes were six polymorphic sequences, and 49 single nucleotide polymorphisms (SNPs) were detected with no genetic variations within species sequences (Figure 8). Nucleotide composition analyses of all species showed base frequencies more heavily represented by adenine and cytosine (A = 0.304, C = 0.272) and with less Thymine and Guanine (T = 0.209, and G = 0.214). This base frequency bias is consistent with the base composition of mtDNA studied in other groups of birds (i.e. Forktails; Moyle *et al*., 2005; Fulvettas; Zou *et al*., 2007). Maximum composite likelihood estimated the overall transition/transversion bias is *R* = 3.818, and the transition/transversion rate ratios of 4.457 (purines) and 11.857 (pyrimidines). Sequence nucleotide compositions of each species were summarized in Table 7.

Table 7. Sequence analyses show nucleotides composition bias of excess adenine and cytosine bases, which is consistent for all the six *Pycnonotus* species. Codon positions included in this analysis were $1^{st}+2^{nd}+3^{rd}$ included gaps and missing positions.

Species	A	T	C	G
Black-headed Bulbuls	0.306	0.217	0.260	0.217
Black-crested Bulbuls	0.306	0.217	0.260	0.217
Streak-eared Bulbuls	0.307	0.202	0.276	0.214
Stripe-throated Bulbuls	0.303	0.202	0.285	0.210
Sooty-headed Bulbuls	0.305	0.202	0.276	0.216
Red-whiskered Bulbuls	0.305	0.214	0.267	0.214

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#01BHB  TGT TAA ACG GCC GCG GTA TCC TAA CCG TGC AAA GGT AGC GCA ATC AAT  [48]
#02BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#03BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#04BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#05BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#06BCB  ... .T. ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#07BCB  ... .T. ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#08BCB  ... .T. ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#09BCB  ... .T. ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#10BCB  ... .T. ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#11SEB  ... .C. ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#12SEB  ... .C. ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#13SEB  ... .C. ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#14STB  C.. .C. ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#15STB  C.. .C. ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#16STB  C.. .C. ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#17STB  C.. .C. ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#18STB  C.. .C. ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#19SHB  G.. .C. ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#20SHB  G.. .C. ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#21SHB  G.. .C. ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#22RWB  C.. .T. ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#23RWB  C.. .T. ... ... ... ... ... ... ... ... ... ... ... ... ... ...


#01BHB  TGT CCC ATA AAT CGA GAC TAG TAT GAA TGG CTA AAC GAG GTC TTA ACT  [96]
#02BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#03BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#04BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#05BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#06BCB  ... ... ... ... ..G ... C.. ... ... ... ... ... ... ..T C.. ...
#07BCB  ... ... ... ... ..G ... C.. ... ... ... ... ... ... ..T C.. ...
#08BCB  ... ... ... ... ..G ... C.. ... ... ... ... ... ... ..T C.. ...
#09BCB  ... ... ... ... ..G ... C.. ... ... ... ... ... ... ..T C.. ...
#10BCB  ... ... ... ... ..G ... C.. ... ... ... ... ... ... ..T C.. ...
#11SEB  ... ... G.. ... ..G ... C.. ... ... ... ... ... ... ... ... ...
#12SEB  ... ... G.. ... ..G ... C.. ... ... ... ... ... ... ... ... ...
#13SEB  ... ... G.. ... ..G ... C.. ... ... ... ... ... ... ... ... ...
#14STB  ... ... G.. ... ..G ... C.. ... ... ... ... ... ... ... ... ...
#15STB  ... ... G.. ... ..G ... C.. ... ... ... ... ... ... ... ... ...
#16STB  ... ... G.. ... ..G ... C.. ... ... ... ... ... ... ... ... ...
#17STB  ... ... G.. ... ..G ... C.. ... ... ... ... ... ... ... ... ...
#18STB  ... ... G.. ... ..G ... C.. ... ... ... ... ... ... ... ... ...
#19SHB  ... ... G.. ... ..G ... C.. ... ... ... ... ... ... ... ... ...
#20SHB  ... ... G.. ... ..G ... C.. ... ... ... ... ... ... ... ... ...
#21SHB  ... ... G.. ... ..G ... C.. ... ... ... ... ... ... ... ... ...
#22RWB  ... ... G.. ... ..G ... C.. ... ... ... ... ... ... ... ... ...
#23RWB  ... ... G.. ... ..G ... C.. ... ... ... ... ... ... ... ... ...
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#01BHB  GTC TCT TAC AGG CAA TCA GTG AAA TTG ATC TCC CTG TGC AAA AGC AGG  [144]
#02BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#03BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#04BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#05BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#06BCB  ... ... ... ... T.. ... ... ... ... ... ... ... ... ... ... ...
#07BCB  ... ... ... ... T.. ... ... ... ... ... ... ... ... ... ... ...
#08BCB  ... ... ... ... T.. ... ... ... ... ... ... ... ... ... ... ...
#09BCB  ... ... ... ... T.. ... ... ... ... ... ... ... ... ... ... ...
#10BCB  ... ... ... ... T.. ... ... ... ... ... ... ... ... ... ... ...
#11SEB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#12SEB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#13SEB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#14STB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#15STB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#16STB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#17STB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#18STB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#19SHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#20SHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#21SHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#22RWB  ... ... ... ... ... ... ... ... ... ... .T. ... ... ... ... ...
#23RWB  ... ... ... ... ... ... ... ... ... ... .T. ... ... ... ... ...
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#01BHB  GAT AAC TAC ATA AGA CGA GAA GAC CCT GTG GAA CTT CAA AAA CAG CAA  [192]
#02BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#03BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#04BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#05BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#06BCB  ... ... C.. ... ... ... ... ... ... ... ... ... ... ... ... ...
#07BCB  ... ... C.. ... ... ... ... ... ... ... ... ... ... ... ... ...
#08BCB  ... ... C.. ... ... ... ... ... ... ... ... ... ... ... ... ...
#09BCB  ... ... C.. ... ... ... ... ... ... ... ... ... ... ... ... ...
#10BCB  ... ... C.. ... ... ... ... ... ... ... ... ... ... ... ... ...
#11SEB  ... ... C.. ... ... ... ... ... ... ... ... ... ... ... ... ...
#12SEB  ... ... C.. ... ... ... ... ... ... ... ... ... ... ... ... ...
#13SEB  ... ... C.. ... ... ... ... ... ... ... ... ... ... ... ... ...
#14STB  ... .C. C.. ... ... ... ... ... ... ... ... ... ... ... ... ...
#15STB  ... .C. C.. ... ... ... ... ... ... ... ... ... ... ... ... ...
#16STB  ... .C. C.. ... ... ... ... ... ... ... ... ... ... ... ... ...
#17STB  ... .C. C.. ... ... ... ... ... ... ... ... ... ... ... ... ...
#18STB  ... .C. C.. ... ... ... ... ... ... ... ... ... ... ... ... ...
#19SHB  ... ... C.. ... ... ... ... ... ... ... ... ... ... ... ... ...
#20SHB  ... ... C.. ... ... ... ... ... ... ... ... ... ... ... ... ...
#21SHB  ... ... C.. ... ... ... ... ... ... ... ... ... ... ... ... ...
#22RWB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#23RWB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
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#01BHB  CCA CCC CGA AAT ACA TAC ACC CCC ACT ACG GGC TCA CTT ACC T-A ACG  [240]
#02BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... .-. ...
#03BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... .-. ...
#04BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... .-. ...
#05BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... .-. ...
#06BCB  ... ... .A. ... ... ... C.. ... ..C C.. ... ... ... .AT .AT ...
#07BCB  ... ... .A. ... ... ... C.. ... ..C C.. ... ... ... .AT .AT ...
#08BCB  ... ... .A. ... ... ... C.. ... ..C C.. ... ... ... .AT .AT ...
#09BCB  ... ... .A. ... ... ... C.. ... ..C C.. ... ... ... .AT .AT ...
#10BCB  ... ... .A. ... ... ... C.. ... ..C C.. ... ... ... .AT .AT ...
#11SEB  ... ... .A. ... ... ..T ..A ... ..C C.. ... ... ... .A. C-C ...
#12SEB  ... ... .A. ... ... ..T ..A ... ..C C.. ... ... ... .A. C-C ...
#13SEB  ... ... .A. ... ... ..T ..A ... ..C C.. ... ... ... .A. C-C ...
#14STB  ... ... .A. ... ... CT. T.. ... ..C C.. ... ... ... .AT .-C ...
#15STB  ... ... .A. ... ... CT. T.. ... ..C C.. ... ... ... .AT .-C ...
#16STB  ... ... .A. ... ... CT. T.. ... ..C C.. ... ... ... .AT .-C ...
#17STB  ... ... .A. ... ... CT. T.. ... ..C C.. ... ... ... .AT .-C ...
#18STB  ... ... .A. ... ... CT. T.. ... ..C C.. ... ... ... .AT .-C ...
#19SHB  ... ... .A. C.. ... ... ..A ... ..C T.. ... ... ... .A. C-C ...
#20SHB  ... ... .A. C.. ... ... ..A ... ..C T.. ... ... ... .A. C-C ...
#21SHB  ... ... .A. C.. ... ... ..A ... ..C T.. ... ... ... .A. C-C ...
#22RWB  ... ... .A. C.. ... ... ..A ... ... C.. ..T ... ... .A. C-C ...
#23RWB  ... ... .A. C.. ... ... ..A ... ... C.. ..T ... ... .A. C-C ...
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#01BHB  GGC GAT TGG TCT GCA TTT TTT CGG TTG GGG CGA CCT TGG AGC AAA GCA  [288]
#02BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#03BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#04BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#05BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#06BCB  ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... A..
#07BCB  ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... A..
#08BCB  ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... A..
#09BCB  ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... A..
#10BCB  ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... A..
#11SEB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... A..
#12SEB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... A..
#13SEB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... A..
#14STB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... A..
#15STB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... A..
#16STB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... A..
#17STB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... A..
#18STB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... A..
#19SHB  ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... A..
#20SHB  ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... A..
#21SHB  ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... A..
#22RWB  ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... A..
#23RWB  ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... A..
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#01BHB  AAA CCT CCA AAT ATT GGA CCA TTC CTC TAG ACT AAG AGC AAC TTC TCG  [336]
#02BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#03BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#04BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#05BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#06BCB  ... ... ... ..C ... A.. ... ..A ... ... ... ... ... ... C.. ...
#07BCB  ... ... ... ..C ... A.. ... ..A ... ... ... ... ... ... C.. ...
#08BCB  ... ... ... ..C ... A.. ... ..A ... ... ... ... ... ... C.. ...
#09BCB  ... ... ... ..C ... A.. ... ..A ... ... ... ... ... ... C.. ...
#10BCB  ... ... ... ..C ... A.. ... ..A ... ... ... ... ... ... C.. ...
#11SEB  ... ... ... ... ... ... ... .C. ... ... ... G.. ... ... CC. ..A
#12SEB  ... ... ... ... ... ... ... .C. ... ... ... G.. ... ... CC. ..A
#13SEB  ... ... ... ... ... ... ... .C. ... ... ... G.. ... ... CC. ..A
#14STB  ... ... ... ..C .C. A.. ... .C. ... C.. ... ... ... ... CC. ..A
#15STB  ... ... ... ..C .C. A.. ... .C. ... C.. ... ... ... ... CC. ..A
#16STB  ... ... ... ..C .C. A.. ... .C. ... C.. ... ... ... ... CC. ..A
#17STB  ... ... ... ..C .C. A.. ... .C. ... C.. ... ... ... ... CC. ..A
#18STB  ... ... ... ..C .C. A.. ... .C. ... C.. ... ... ... ... CC. ..A
#19SHB  ... ... ... ..C ... A.. ... .C. ... ... ... G.. ... ... CC. ..A
#20SHB  ... ... ... ..C ... A.. ... .C. ... ... ... G.. ... ... CC. ..A
#21SHB  ... ... ... ..C ... A.. ... .C. ... ... ... G.. ... ... CC. ..A
#22RWB  ... ... ... ..C ... A.. ... .C. ... ... ... ... ... ... .C. ..A
#23RWB  ... ... ... ..C ... A.. ... .C. ... ... ... ... ... ... .C. ..A
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#01BHB  ACG TGC TAA TAG CAA CCA GAC CCA ATA T-A TTG ATC AAT GGA CCA AGC  [384]
#02BHB  ... ... ... ... ... ... ... ... ... .-. ... ... ... ... ... ...
#03BHB  ... ... ... ... ... ... ... ... ... .-. ... ... ... ... ... ...
#04BHB  ... ... ... ... ... ... ... ... ... .-. ... ... ... ... ... ...
#05BHB  ... ... ... ... ... ... ... ... ... .-. ... ... ... ... ... ...
#06BCB  ... ... ... ... T.. ... ... ... ... .T. ... ... G.. ... ... ...
#07BCB  ... ... ... ... T.. ... ... ... ... .T. ... ... G.. ... ... ...
#08BCB  ... ... ... ... T.. ... ... ... ... .T. ... ... G.. ... ... ...
#09BCB  ... ... ... ... T.. ... ... ... ... .T. ... ... G.. ... ... ...
#10BCB  ... ... ... ... T.. ... ... ... ... .T. ... ... G.. ... ... ...
#11SEB  ... ... ... ... ... ... ... ... ... CA. ... ... ... ... ... ...
#12SEB  ... ... ... ... ... ... ... ... ... CA. ... ... ... ... ... ...
#13SEB  ... ... ... ... ... ... ... ... ... CA. ... ... ... ... ... ...
#14STB  ... ... ... ... ... ... ... ... ... .A. ... ... ... ... ... ...
#15STB  ... ... ... ... ... ... ... ... ... .A. ... ... ... ... ... ...
#16STB  ... ... ... ... ... ... ... ... ... .A. ... ... ... ... ... ...
#17STB  ... ... ... ... ... ... ... ... ... .A. ... ... ... ... ... ...
#18STB  ... ... ... ... ... ... ... ... ... .A. ... ... ... ... ... ...
#19SHB  ... ... ... ... T.. ... ... ... ... .A. ... ... ... ... ... ...
#20SHB  ... ... ... ... T.. ... ... ... ... .A. ... ... ... ... ... ...
#21SHB  ... ... ... ... T.. ... ... ... ... .A. ... ... ... ... ... ...
#22RWB  .T. ... ... ... T.. ... ... ... ... .A. ... ... G.. ... ... ...
#23RWB  .T. ... ... ... T.. ... ... ... ... .A. ... ... G.. ... ... ...
```

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#01BHB  TAC CCC AGG GAT AAC AGC GCA ATC TCC TCC AAG AGT CCA TAT CGA CGG  [432]
#02BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#03BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#04BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#05BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#06BCB  ... ... ... ... ... ... ... ... ... .T. G.. ... ... ... ... .AA
#07BCB  ... ... ... ... ... ... ... ... ... .T. G.. ... ... ... ... .AA
#08BCB  ... ... ... ... ... ... ... ... ... .T. G.. ... ... ... ... .AA
#09BCB  ... ... ... ... ... ... ... ... ... .T. G.. ... ... ... ... .AA
#10BCB  ... ... ... ... ... ... ... ... ... .T. G.. ... ... ... ... .AA
#11SEB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..A
#12SEB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..A
#13SEB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..A
#14STB  ... ... ... ... ... ... ... ... ... .T. ... ... ... ... ... ..A
#15STB  ... ... ... ... ... ... ... ... ... .T. ... ... ... ... ... ..A
#16STB  ... ... ... ... ... ... ... ... ... .T. ... ... ... ... ... ..A
#17STB  ... ... ... ... ... ... ... ... ... .T. ... ... ... ... ... ..A
#18STB  ... ... ... ... ... ... ... ... ... .T. ... ... ... ... ... ..A
#19SHB  ... ... ... ... ... ... ... ... ... .T. ... ... ... ... ... ...
#20SHB  ... ... ... ... ... ... ... ... ... .T. ... ... ... ... ... ...
#21SHB  ... ... ... ... ... ... ... ... ... .T. ... ... ... ... ... ...
#22RWB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#23RWB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

#01BHB  GGA GGT TTA CGA CCT CGA TGT TGG ATC AGG ACA TCC TAG TGG TGC AGC AG- CTA C [487]
#02BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..- ... .
#03BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..- ... .
#04BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..- ... .
#05BHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..- ... .
#06BCB  ... ... ... ... ... ... ... ... ... ... ... ... ... C.. ... ... ..- ... .
#07BCB  ... ... ... ... ... ... ... ... ... ... ... ... ... C.. ... ... ..- ... .
#08BCB  ... ... ... ... ... ... ... ... ... ... ... ... ... C.. ... ... ..- ... .
#09BCB  ... ... ... ... ... ... ... ... ... ... ... ... ... C.. ... ... ..- ... .
#10BCB  ... ... ... ... ... ... ... ... ... ... ... ... ... C.. ... ... ..- ... .
#11SEB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..- ... .
#12SEB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..- ... .
#13SEB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..- ... .
#14STB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..- ... .
#15STB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..- ... .
#16STB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..- ... .
#17STB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..- ... .
#18STB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..- ... .
#19SHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..- ... .
#20SHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..- ... .
#21SHB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..- ... .
#22RWB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... .
#23RWB  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... .
```

Figure 8. Alignment of 16s mtDNA gene sequences of all six species of the *Pycnonotus* Bulbuls. The consensus sequence is shown above the sequence alignment with nucleotide identities depicted as dots (.) and sequence deletions as dashes (-). The numbers refer to the nucleotide positions of each amplified fragment. Species abbreviations; BHB = Black-headed Bulbuls, BCB = Black-crested Bulbuls, SEB = Streak-eared Bulbuls, STB = Stripe-throated Bulbuls, SHB = Sooty-headed Bulbuls, and RWB = Red-whiskered Bulbuls.

Acoustic, morphometric, and genetic data predicted species relationship

Hierarchical cluster analysis constructing based on acoustic features created well-resolved species relationship which reflects species' song structure and song complexities. The prominent songs structure of the *Pycnonotus* bulbul are the present of trill structure which could be subtitle as two distinctive groups including; 1) Black-headed and Streak-eared Bulbuls both sing trilled songs composed of one type of repetitive element, 2) the remaining four species (i.e. Black-crested, Stripe-throated, Sooty-headed, and Red-whiskered Bulbuls) produce complex songs composed of various frequency-modulated tone, or non-trilled song. Hierarchical cluster analyses of the six *Pycnonotus* based on Euclidean distance of acoustic features is shown in Figure 9.

Hierarchical cluster analyses based on morphometric features indicate monophyletic relationship of all six species. The results show unresolved group of all the *Pycnonotus*. These may be because of all the *Pycnonotus* have similar foraging tactics, and which based on field observation most of the bulbuls studied here is capable to perform mixed-species foraging flock. In addition, based on this analysis of morphometric features, there were no morphological trends within this sympatric group. Hierarchical cluster analyses of the six *Pycnonotus* based on Euclidean distance of female and male morphometric features are shown in Figure 10.

Phylogenetic tree was constructed from 16s mitochondrial gene sequences obtained from 23 individuals of the genus *Pycnonotus*. Genetic analysed of maximum composition likelihood are presented in Figure 11. According to birds' plumage and habitat preferences, species' sequences produced well-resolved genetic relationship that all the six *Pycnonotus* were divided into two group; 1) Black-headed Bulbuls, and Black-crested Bulbul, and 2) Sooty-headed Bulbuls, Red-whiskered Bulbuls, Streak-eared Bulbuls, and Stripe-throated Bulbuls. The former is a group of yellow species and were regularly found in the mature forest. The latter is a group of species that foraging relative with urban areas and secondary forest habitat, though the plumage character of this group is a mixture.

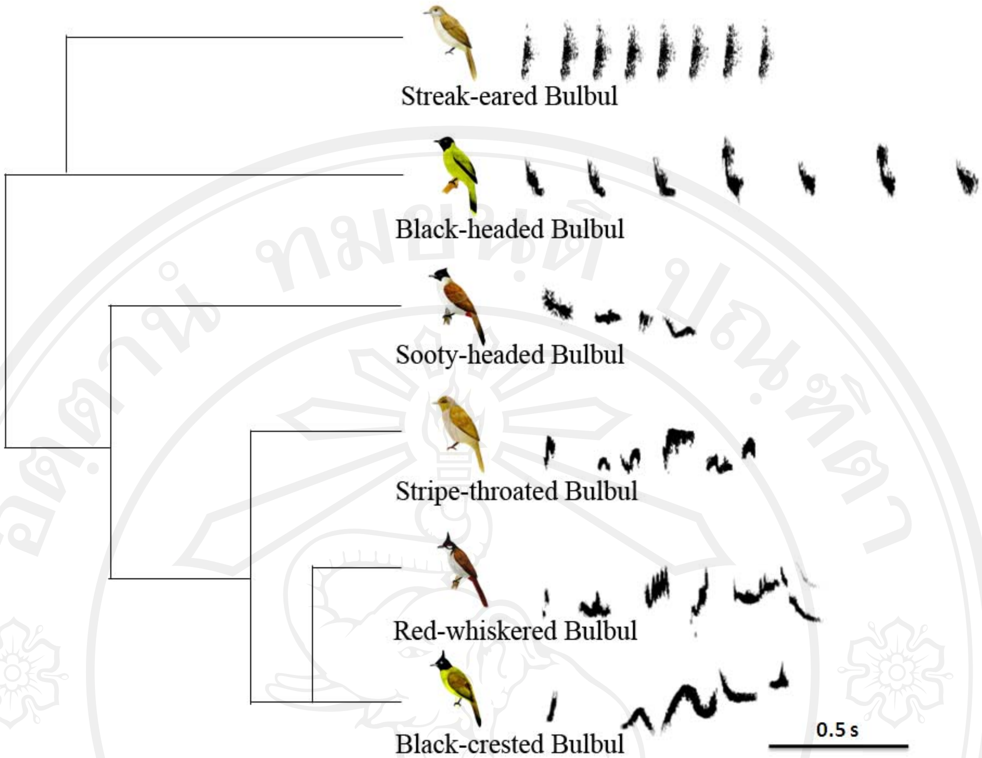

Figure 9. Hierarchical cluster analyses of the six *Pycnonotus* based on Euclidean distance of acoustic features. Spectrograms show typical song structure of each species. Dendrogram shows two discrete species' song; *trilled songs* i.e. Black-headed Bulbul and Streak-eared Bulbul, and *frequency-modulated tones* i.e. Sooty-headed Bulbul, Stripe-throated Bulbul, Red-whiskered Bulbul, and Black-crested Bulbul.

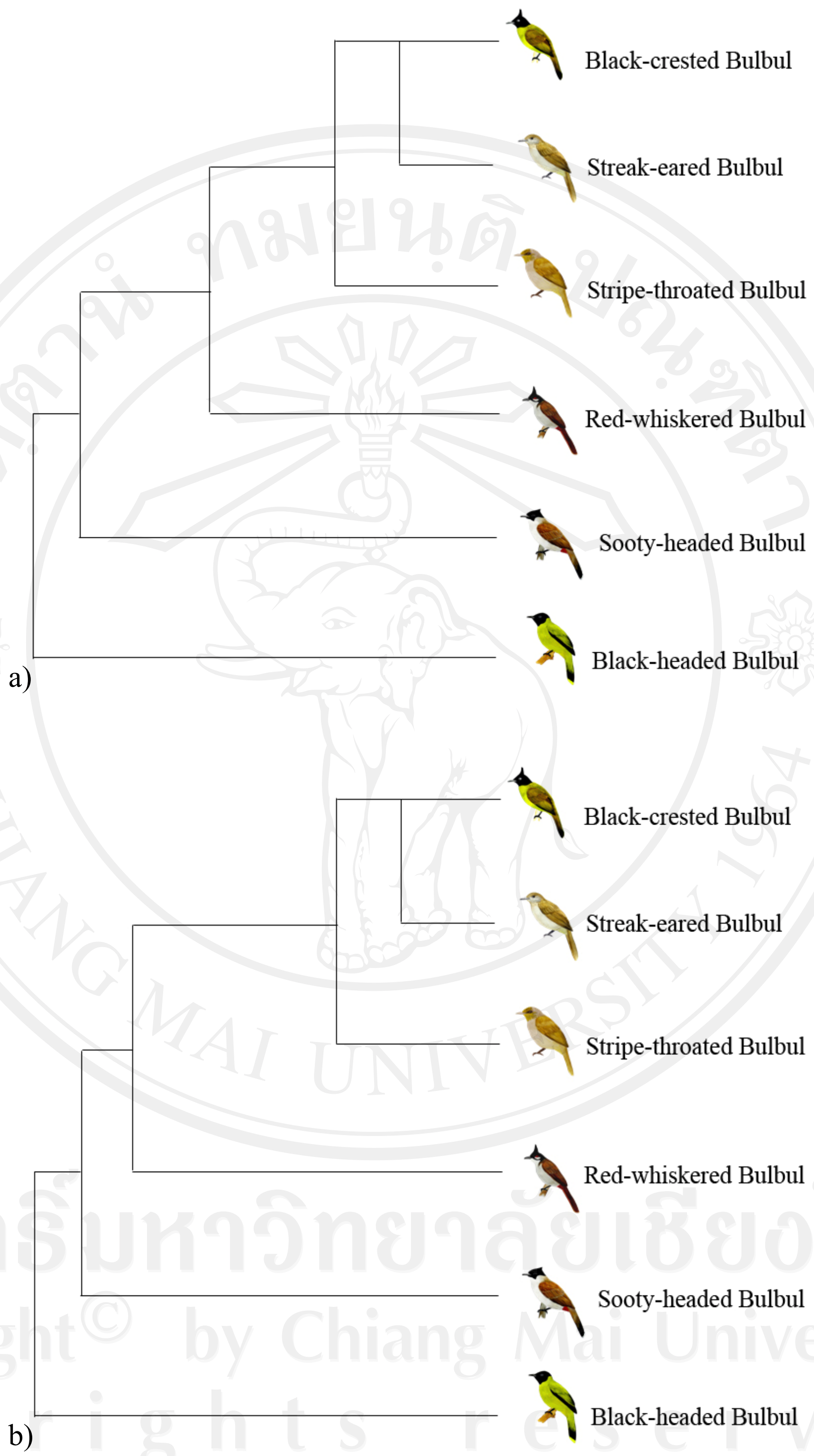

Figure 10. Hierarchical cluster analyses of the six *Pycnonotus* based on Euclidean distance of female morphometric features (a) and male morphometric features (b). Species clustering indicate monophyly of all six *Pycnonotus* Bulbuls with similar dendrograms of both sexes.

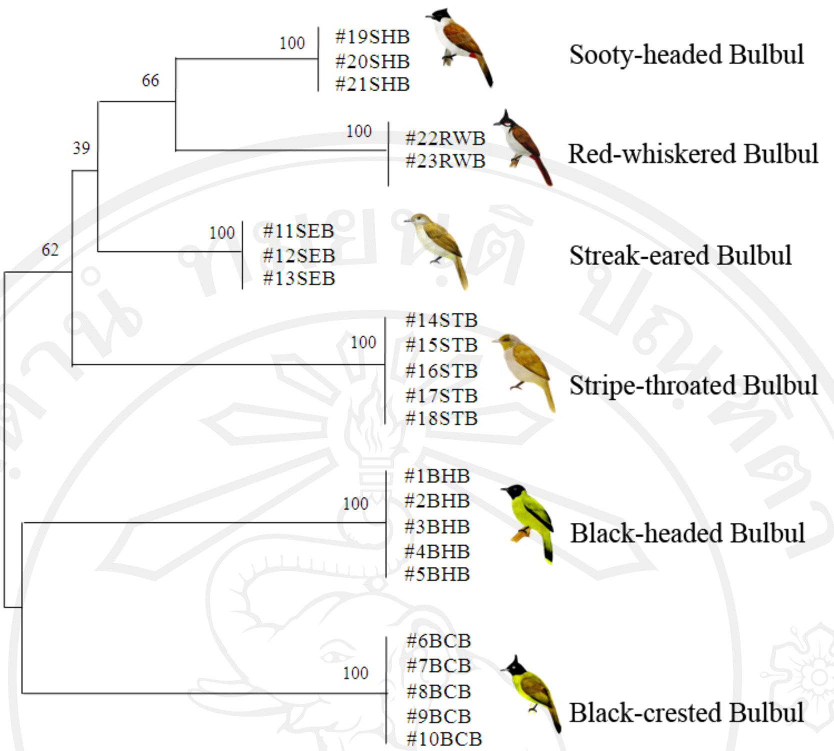

Figure 11. Molecular phylogeny based on 16s mtDNA (487bp) indicated two separated groups of the *Pycnonotus* Bulbuls. Numbers above nodes indicate bootstrap (replications = 1000) of the maximum composition likelihood.

Test for acoustic distance correlated with morphometric and genetic distance

Species' pair-wise comparisons are presented in three matrices of the Euclidean distances; Table 8 for acoustic distance, Table 9 for morphometric distance, and Table 10 for genetic distance (species abbreviations; BHB = Black-headed Bulbuls, BCB = Black-crested Bulbuls, SEB = Streak-eared Bulbuls, STB = Stripe-throated Bulbuls, SHB = Sooty-headed Bulbuls, and RWB = Red-whiskered Bulbuls).

Based on a calculation of the Euclidean distance of 15 paired cases using the functions at group centroids derived from DFA, the pair-wise acoustic distance of all six species has a range between 1.039 and 1.330 (Table 8). The results show that acoustic distance is the shortest between Black-crested Bulbul and Red-whiskered Bulbul, which reflects they have the most similar song structure, as both sing frequency-modulated songs with high song complexities. In contrast, acoustic distances are the longest between the following two paired species: Black-headed

Bulbul versus Black-crested Bulbul, and Black-headed Bulbul versus Stripe-throated, which indicated they have the least similar song structure.

The pair-wise distance of female morphometric features range between 0.890 and 1.740 (Table 9 upper diagonal). The results show that the morphometric distance of females is the shortest between Black-crested Bulbul and Streak-eared Bulbul reflecting they have the highest morphometric similarity. These two species are most similar in wing length and tail length. In contrast, female morphometric distance is the longest between Black-headed Bulbul and Sooty-headed Bulbul. These two species are most different in body size: the former having a relatively small body size, whereas the latter has a relatively large body size. The pair-wise distance of male morphometric features range between 0.894 and 1.621 (Table 9 lower diagonal), which are strongly consistent with female morphometric features (*Paired-samples t-test* = -1.724, df = 14, p = 0.107). The results show that the morphometric distance of males is the shortest between Black-crested Bulbul and Streak-eared Bulbul, which reflects they have the highest morphometric similarity. These two species are most similar in wing length and tail length. Male morphometric distance is also the longest between Black-headed Bulbul and Sooty-headed Bulbul. Similarly, these two species are most different in body size: the former with a relatively small body size and the latter with a relatively large body size.

Pair-wise composition distances of 16s mtDNA were calculated using the maximum composite likelihood (MCL distance) method. Analyses of the 16s mtDNA reveal that all six *Pycnonotus* species are closely related and share a common evolutionary history. Pair-wise genetic distances of all six species ranges between 0.023 and 0.063 (Table10). Genetic distance is the shortest between Streak-eared Bulbul and Sooty-headed Bulbul. Genetic distances are the longest between two paired-species: Black-headed Bulbul versus Black-crested Bulbul and Black-headed Bulbul versus Stripe-throated Bulbul. As mentioned above, these two paired-species also have the longest distance of acoustic features.

Table 8. Pair-wise Euclidean distances of acoustic variables for all six species.

Species	BHB	BCB	SEB	STB	SHB	RWB
BHB	0.000					
BCB	1.330	0.000				
SEB	1.152	1.055	0.000			
STB	1.330	1.064	1.262	0.000		
SHB	1.324	1.042	1.248	1.179	0.000	
RWB	1.271	1.039	1.210	1.086	1.110	0.000

Table 9. Pair-wise Euclidean distances of morphometric variables between all six species. Euclidean distances within sex were calculated independently; males (lower diagonal) and females (upper diagonal).

Species	BHB	BCB	SEB	STB	SHB	RWB
BHB	0.000	1.299	1.407	1.203	1.704	1.423
BCB	1.407	0.000	0.890	0.921	1.243	1.143
SEB	1.525	0.894	0.000	0.954	1.300	1.097
STB	1.388	1.154	1.166	0.000	1.282	1.092
SHB	1.621	1.301	1.185	1.502	0.000	1.485
RWB	1.425	1.083	1.077	1.057	1.448	0.000

Table 10. Pair-wise nucleotide composition distance using 16s mtDNA of all six species. The distance scores indicate a relatively close genetic relationship between all six species.

Species	BHB	BCB	SEB	STB	SHB	RWB
BHB	0.000					
BCB	0.059	0.000				
SEB	0.046	0.056	0.000			
STB	0.059	0.050	0.034	0.000		
SHB	0.055	0.050	0.023	0.034	0.000	
RWB	0.052	0.056	0.038	0.047	0.025	0.000

Based on the expectation that increasing acoustic distance between species also increases the distance in morphometric and genetic features, the Mantel test was applied for matrix comparisons of acoustic versus morphometric distance and acoustic versus genetic distance. The Mantel test revealed substantial song differences among the six species of *Pycnonotus* Bulbuls. The results show that acoustic distances have a non-significant positive correlation with morphometric distances (females, Mantel test: Pearson's r = 0.433, p = 0.107, Figure 12a; males, Mantel test: Pearson's r = 0.512, p = 0.051, Figure 12b). Similarly, acoustic distances have a non-significant positive correlation with genetics distances (Mantel test: Pearson's r = 0.041, p = 0.884, Figure 12c.)

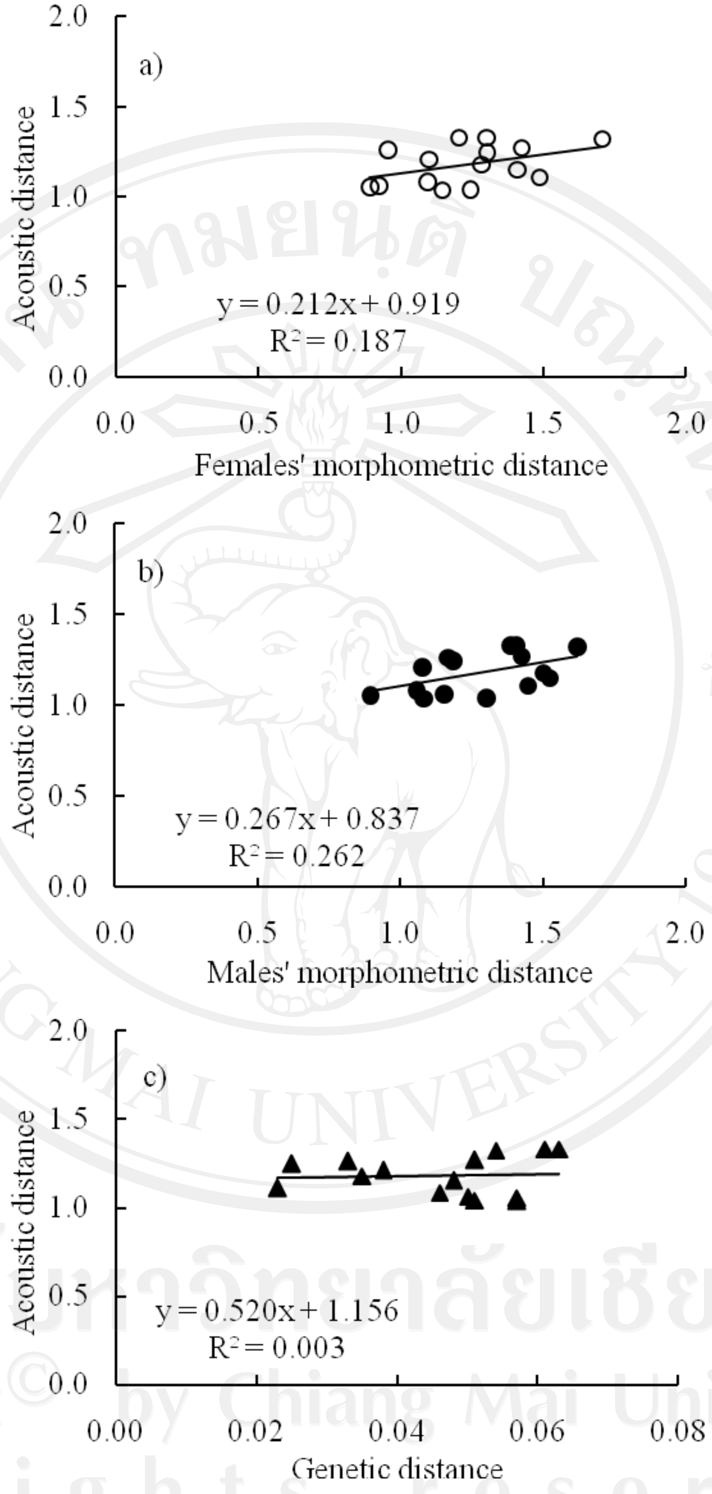

Figure 12. Scatter plots showing the positive correlation of acoustic distance compared with female morphometric distance (a), male morphometric distance (b), and genetic distance (c).