ADVERTISEMENT CALL CHARACTERISTICS OF MALE SARAWAK FROGS (FAMILY RANIDAE)

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Abstract: The family Ranidae of Sarawak frogs comprises the genera *Hylarana*, *Pulchrana*, *Abavorana*, *Chalcorana*, *Staurois*, *Meristogenys*, *Odorrana*, and *Huia.* Studies revealed that the advertisement call characteristics emitted by male frogs vary in different species. This study aims to describe the characteristics of the male frogs in the advertisement call and analyse the call characters that are useful for species discrimination. Field samplings were conducted to record the frog calls for the sound call characteristic analysis. The findings indicate that advertisement call characteristics could discriminate the frog species. Pulse duration, call energy, and call frequencies are among the useful characteristics that revealed a high correlation between dominant frequency and pulse duration and body size. The call properties are also useful in distinguishing individuals of the frog species among populations. In conclusion, this database will serve as a resource for future studies in understanding frog distribution and adaptation in their habitat.

Keywords: Sarawak, Ranidae frogs, bioacoustics, body size, species discrimination.

Introduction

There are several type of frog calls: Advertisement calls (consisting of courtship, territorial, and encounter calls), reciprocation calls, release calls, and distress calls (Xie *et al*., 2018). Among all call types, advertisement calls are the most powerful, useful, and diverse pattern of calls produced by the male frog (Vèlez & Guajardo, 2021). However, some frog species do not emit advertisement calls for mating purposes. Frogs emit calls to effectively promote their existence in their natural habitat and maturity and to protect their territory (Gerhardt & Bee, 2007; Mattison, 2011). Female frogs rarely produce calls because some species lack vocal cords, and some only have rudimentary or immature vocal cords. The male advertisement call is one of the frog behaviours' and is also well understood as male-male physical aggression (Tolosa, 2020). This behaviour is a very high-cost activity as it consumes a large amount of energy for a successful vocalisation.

Frogs have a fascinating display of vocalisations, which are mostly used for mating. They have complicated acoustic reception systems that permit them to differentiate among species and between conspecifics. Acoustic signals of frogs are also useful for discriminating between two species that are morphologically similar but of different species (Köhler *et al*., 2017). Scientists and local people sometimes easily recognise different species of frogs in the wild through their vocalisation. However, some species of frog do not produce audible calls but at a much higher frequency than our human threshold hearing level (above 20 kHz) such as *Odorrana tormota* in China (Zhang *et al.*, 2015) and *Huia cavitympanum* of Borneo (Cobo-Cuan *et al*., 2020). *H. cavitympanum* is among the well-known frog species in Southeast Asia that produce ultrasonic sound in its fast-flowing water habitat.

Many studies have been conducted to describe call characteristics of Bornean frogs in the past decade (Marly *et al*., 2017; Vallejos *et al.*, 2017; Amram *et al*., 2018; Amram *et al*., 2020; Yi *et al*., 2021). Male advertisement calls were used to identify new frog species in this region (Matsui & Nishikawa, 2014;

Shimada *et al*., 2015; Eto *et al*., 2016). Based on Bernardy *et al.* (2024), female frogs much prefer temporal characteristics of the male advertisement calls. Advanced technologies have enabled more studies to be conducted by scientists, and it has been discovered that there are variations in frog calls. The earliest studies on the Bornean frog call revealed that advertisement call characteristics of *Leptolalax heteropus* differed from the others, suggesting it is phylogenetically diverged (Matsui, 1997). Following that, frog call characteristics were also used as one of the characters to revalidate *Pulchrana laterimaculata* from the synonymy of *Pulchrana baramica* (Leong *et al*., 2003). In addition, Lardner and Lakim (2004) investigated call preferences in female tree-hole frogs and since then no other intensive study on frog advertisement calls of Bornean frogs has been carried out until Arch *et al*. (2009) on the ultrasonic sound of *Huia cavitympanum* and call characteristics of Bornean ranids (Zainudin *et al*., 2010; Zainudin *et al*., 2011) and Bornean Bufonidae (Amram *et al*., 2018; Amram *et al*., 2020).

The call characteristics vary between species and are normally divided into two main characters: Static call characteristics (dominant frequency and call amplitude) and dynamic call characteristics (call rates, mean pulse per call, and call durations). Previous studies showed that call characters that can discriminate frogs' species were the temporal and spectral properties of the call emitted (Arini *et al*., 2016; Xie *et al*., 2016; Indraswari *et al*., 2018; Xie *et al*., 2018; Gan *et al*., 2020). The earliest studies proved that the arytenoid cartilages also play a role in vibrating to produce sounds (Ryan & Tuttle, 1983; Sullivan & Malmos, 1994) and explained that the variations of call characteristics are due to differences in the vocal apparatus structure (Fears, 2010). According to Deka *et al.* (2015), Bornean ranid's vocal apparatus structure varied in size and shape, which reflected the differences in their call characteristics. Based on the findings reported by Deka *et al.* (2015), this current study hypothesised that each Bornean ranid frog might emit different call properties due to variations in vocal apparatus structure. Therefore, there are two objectives for this study: Firstly, to describe the advertisement call characteristics of the male Ranidae in Sarawak and secondly, to determine if the call characteristics could be useful to discriminate between species and among populations of one species from different localities.

Methodology

Documentation of Sound

Data collection was conducted between January 2011 and December 2012. During field sampling at Bako NP, Kubah NP, Gading NP, Batang Ai NP, Mulu NP, and Matang Wildlife Centre, the calling male frog was identified. Vocalisation of the calling male was recorded using a portable Olympus linear PCM digital recorder (LS-11 US). The recorder was attached with a SONY unidirectional microphone and a TASCAM Dynamic Stereo Headphones HP-VT1. The recording ended after three to 15 minutes, depending on the call gap of each species. During the sound recording, the movement of the frog's vocal sac was also filmed using a camcorder SONY DCR-SR68E. All recorded sound and video files were kept in electronic versions for further analysis.

Temperature and Humidity

Ambient temperature and humidity data were recorded using a HOBO ProV2 device. A HOBO data logger (HOBO Optic USB Base Station) was connected to a HOBO wireless data node (Coupler U23 Pro V2) to record the data. Upon completion of the sound recording, the calling individual was captured. A pair of gloves was put on before capturing the frog to avoid heat from the hands affecting the frog's body temperature. While capturing the frog, only the frog legs were grabbed to minimise surfaces of body contact. This is important when handling the frog while taking its anal temperature. The frog's anal temperature was detected using a thermo coupler (KE-EN TM02 Type K Thermometer). Once the probe was inserted inside the frog's

anal opening, the thermometer was switched on, and the first reading was taken. All recorded data were then transferred into an electronic device or a notebook for further analysis.

Sound Analysis

Audio files were transferred into a portable personal computer. Videos were trimmed using Picture Motion Browser (PMB version 5.2.00.03250). All the video and audio files were saved in external drives for future reference. The sound was analysed by using SoundRuler 0.9.6.0 2002 to 2007 Marcos Gridi-Papp software. Sample size (n) was indicated as the number of calls or note calls analysed in 60 seconds, and means are given for the number of calls recorded. The call variables were measured following Zainudin *et al.* (2010) (Table 1).

Table 1: Call characters measured in this study as follows Zainudin *et al.* (2010)

No.	Group	Variables	Measured Characteristics
1	Calls	Note	A group of pulse
2		Note duration	Time calculated from the beginning of the first pulse to the end of the last pulse in a note
3		Note gap	Time calculated from the beginning of one note to the beginning of the next note
4		Note repetition rate	Number of notes per second
5	Pulses	RelPulsePeak	Pulse maximum amplitude relative to call maximum amplitude
6		PulDur_0	Pulse duration between 0% amplitude marks
7		PulDur_10	Pulse duration between 10% amplitude marks
8		PulDur_50	Pulse duration between 50% amplitude marks
9		PulDur_90	Pulse duration between 90% amplitude marks (sustain)
10		PulOn_90	Pulse time for onset to 90% (attack)
11		PulOn_peak	Pulse time for onset to peak (rise)
12		PulOff_peak	Pulse time for peak to offset (fall)
13		PulOff_90	Pulse time for 90% to offset (decay)
14		PulShapeOn	Pulse shape (10:50% onset/10:90% onset)
15		PulShapeOff	Pulse shape (50:10% offset/90:10% offset)
16		PulInter	Pulse interval
17		PulPeriod	Pulse period (time peak to peak)
18		PulDuty	Pulse duration/period (Duty cycle)
19		Crest Factor	Pulse peak/rms
20	Energy	Ener-0-10-Beg	The energy between the initial 0:10% peak amplitude
21		Ener-10-50-Beg	The energy between the initial 10:50% peak amplitude
22		Ener-50-90-Beg	The energy between the initial 50:90% peak amplitude
23		Ener-90-Peak-Beg	The energy between the initial 90% peak amplitude
24		Ener-Peak-90-End	The energy between the final peak: 90% amplitude
25		Ener-90-50-End	The energy between the final 90:50% peak amplitude
26		Ener-50-10-End	The energy between the final 50:10% peak amplitude
27		Ener-10-0-End	The energy between the final 10:0% peak amplitude

28		PulseDomFreq	Dominant frequency of the pulse
29		PulseFundFreq	Fundamental frequency of the pulse
30	Frequency	PulseMinFreq	Minimum of dominant frequency in the pulse
31		PulseMaxFreq	Maximum of dominant frequency in the pulse
32		PulseOnFreq	Onset pulse dominant frequency
33		PulseOffFreq	Offset pulse dominant frequency
34		PulseHalfFM	Prop of duration to reach half frequency modulation
35		Tuning-6dBSPL	Tuning: Peak freq/bandwidth at 50% Peak amplitude (Q-20dBSPL)
36	Intensity	Tuning-6dBSPL	Tuning: Peak freq/bandwidth at 10% Peak amplitude (Q-20dBSPL)
37		relAmpl-H1	Relative amplitude of harmonic 1
38		relAmpl-H3	Relative amplitude of harmonic 3

Statistical Analysis

Seven species of the family Ranidae with a total number of 28 individuals were recorded. To ensure a reliable statistical outcome could be obtained from the call analysis, replicates are required with at least two or more individuals per species successfully recorded. The recorded calls of Ranidae belong to *Pulchrana baramica* (N = 10), *Chalcorana raniceps* (N = 3), *Pulchrana picturata* (N = 3), *Pulchrana signata* (N = 4), *Pulchrana glandulosa* (N = 3), *Abavorana luctuosa* (N = 2), and *Meristogenys phaeomerus* (N = 3). Calls of the outgroups (*Pedostibes hosii*, N = 2) used in this study were also analysed.

Multivariate analysis was employed in this study to examine all characters and to identify patterns of variation based on similarities of characters among and within samples. All samples were subjected to a hierarchical Cluster Analysis (CA) using UPGMA in the Multivariate Statistical Package, MVSP ver 3.13d programme. This analysis used the Euclidean algorithm to group samples based on the pairwise measures of similarity of the variables. This unweighted method will give equal weight to each point in each cluster. Then, the distances between each pair of points in the two clusters can be measured. Hence, the mean of these distances can be used as the distance between the clusters. This is one of the most commonly used agglomerative hierarchical methods, by means the dendrogram is produced first by clustering all samples separately. Then, the samples will be combined into a single, hierarchical group based on the most similar characters. The clustering was based on the similarities of the advertisement call characters shared between each species. The result is in the form of a dendrogram, which shows the most similar characters linked most closely together. The level of the vertical lines joining two cases or clusters indicates the level of similarity between them.

The sounds were further analysed using Principal Component Analysis (PCA) to determine which variables or call characters best describe the calls of each species. All highest loadings of call characters observed in PCA were further analysed to test the significance level of correlations between the frog snout-vent-length and call characters. Correlation analysis was performed in SPSS version 21.0 using Pearson's Correlation. Discriminant Function Analysis (DFA) was also conducted in the program software Statistical Package for Social Science (SPSS) version 21.0 to analyse which call character shows the most significant function in this study. A probability of $p < 0.05$ was considered significant in all analyses, where a total of 38 variables of call were analysed

for all statistical analyses in this study. The call variables were grouped into five groups consisting of calls, pulses, energy, frequency, and call intensity.

Character Coding for Phylogeny Reconstruction of Call Characteristics

All highest call character loadings based on PCA were further analysed for character coding to infer the phylogeny relationship of the frog species in this study. Each variable was first tested for dissimilarity among species using One-way analysis of variance (ANOVA). The test was done using Minitab 2002 version 13.2 software and the result was illustrated in a dot plot. Each dot or circle represents each character (Figure 1). Therefore, frog species that share similar characters were coded with the same number, represented as one dot or circle as they overlapped on the dot plot graph. The character coding was then simplified in a table and further analysed using MacClade version 4.08 and saved into a Nexus file. The nexus file will be used with PAUP 4b10 software to construct a phylogeny tree.

Results and Discussion

Call Variables of Sarawak Ranids

Figure 2 showed that out of 38 call characters analysed, a total of 29 variables were meaningful to discriminate each species. The principal component analysis successfully extracted useful characters (in bold) that were meaningful in identifying each species from the others. The graph showed that the highest loadings were dominated by three out of five variable groups such as pulse duration, call energy, and call frequency. This result was consistent with Zainudin *et al.* (2009) in discriminating call characteristics of Sarawak ranids.

(1) Note, (2) note duration, (3) note gap, (4) **repetition rate of note per 60 seconds**, (5) **pulse maximum amplitude relative to call maximum amplitude**, (6) **period pulse between 0%**, (7) **pulse duration of 10%**, (8) **pulse duration of 50%**, (9) **pulse duration between 90% (resistant)**, (10) **pulse to induced to 90% (attack)**, (11) **time pulse for the spark to the top (up)**, (12) **time pulse for the spark to the balancing (fall)**, (13) **time**
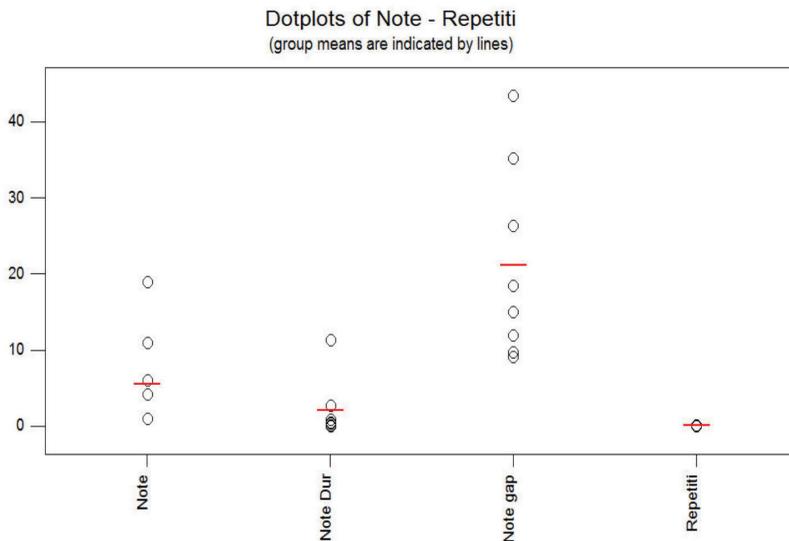

Figure 1: Dot plot obtained from ANOVA. Each circle was coded with a number, which represents a character
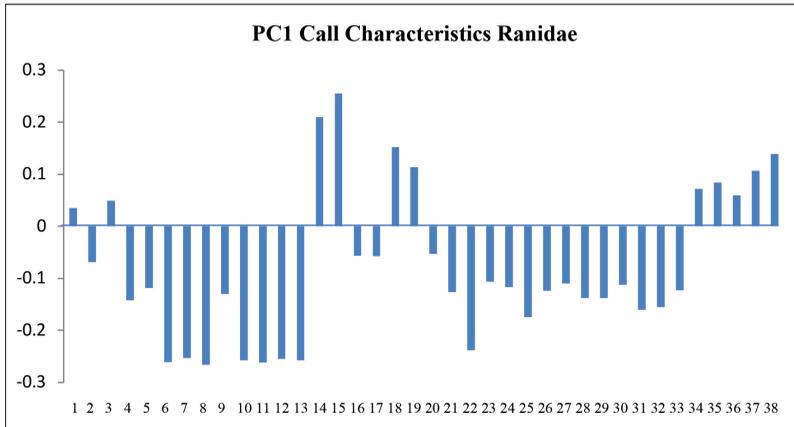
Figure 2: Variables loadings of call characteristics of family Ranidae in Principal Component Analysis 1 (PCA 1) with meaningful call variables (in bold)

pulse for 90% to counterbalance (decay), (14) **form the heart (10:50% spark/10:90% flash)**, (15) **heart shape**, (16) time between pulse, (17) time pulse (peak to peak), (18) **pulse duration (duty cycle)**, (19) **crest factor**, (20) Energy between initial peak amplitude of 0:10%, (21) **energy between initial amplitude of 10:50% peak**, (22) **energy between initial peak amplitude of 50:90%**, (23) **energy between initial peak: peak amplitude of 90%**, (24) **energy between flexion peak: peak amplitude of 90%**, (25) **energy between suffix of 90:50% peak amplitude**, (26) **energy between suffix of 50:10% peak amplitude**, (27) **energy 10:0% between peak amplitude of flexion**, (28) **dominant frequency**, (29) **fundamental frequency**, (30) **dominant frequency minimum**, (31) **dominant frequency maximum**, (32) **induced dominant frequency**, (33) **balancer dominant frequency**, (34) ratio of the period to reach half a modulation frequency, (35) tuning: peak frequency or band width at 50% peak amplitude, (36) tuning: peak frequency or band width at 10% peak amplitude, (37) **relative amplitude harmonic 1**, and (38) **relative amplitude harmonic 2**.

These variables were then illustrated in a scatter plot in which principal component one (axis x) against principal component two (axis y), as shown in Figure 3. The scatter plot revealed that *Pulchrana baramica*, *P. picturata,*,

P. signata, and *Abavorana luctuosa* were grouped on the right side of the graph. While *P. glandulosa* was on the top left side of the graph, *Meristogenys phaeomerus* was at the bottom left. This graph supports Zainudin *et al.* (2010), which indicates that the Sarawak ranids were grouped based on call characteristics incongruent with the frog's body size (snout-vent-length). *P. glandulosa* possessed larger body sizes (70 mm to 80 mm) while the other ranid species had much smaller sizes (in the range of 30 mm to 55 mm). However, *M. phaeomerus* is also small (35 mm to 40 mm), but this species does not group with the other ranid species of the same size. Therefore, a question arose: Does the body size of frogs in this study reflect the call characteristics of Sarawak frogs? As suggested in Zainudin *et al*. (2009).

Pearson's correlation test was used to clarify that the frog's body size does influence the frog's call characteristics. The correlations of 29 meaningful variables from PCA with body size are shown in Table 2. Some of the call characters showed significant results but with negative values of the correlation coefficient such as Pulse maximum amplitude relative to call maximum amplitude (RelPulsePeak) with r = -0.650 and p = 0.006, crest factor with r = -0.630 and p = 0.008, energy of 10% to end (r = -0.505; p = 0.033), pulse dominant frequency (r = -0.505; p = 0.033), pulse fundamental frequency (r =
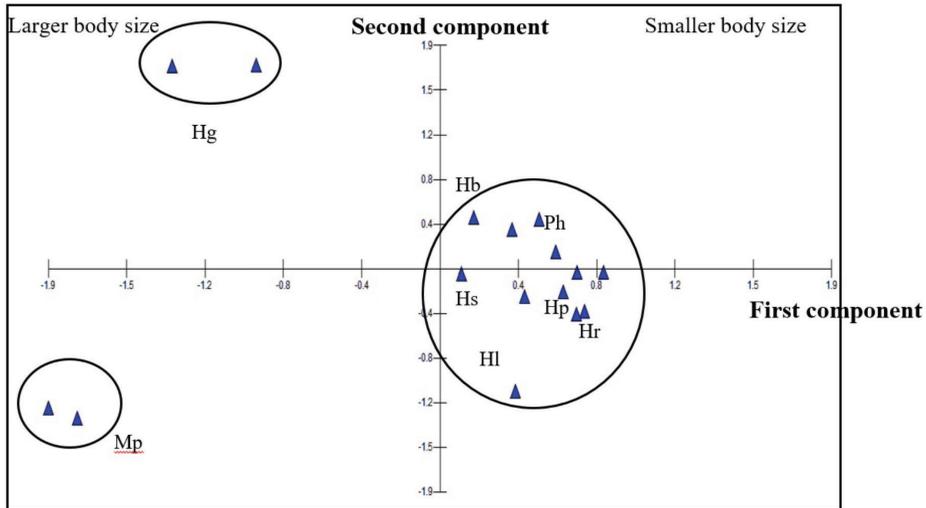
Figure 3: Scatter plot of PCA1 (axis-X) against PCA2 (axis-Y) of call characteristics of Sarawak Ranids. Hg = *Pulchrana glandulosa*; Mp = *Meristogenys phaeomerus*; Hb = *Pulchrana baramica*; Hs = *Pulchrana signata*; Hp = *Pulchrana picturata*; Hl = *Abavorana luctuosa*; Hr = *Chalcorana raniceps*

Table 2: Pearson's correlation test of call characteristics with SVL of frogs (N = 14)

	Group	Variables	Correlation between Character and SVL	
			The Correlation Coefficient (r)	Significance Test (p)
1	Calls	Note repetition rate	-0.154	**0.300
2	Pulses	RelPulsePeak	**-0.650**	**0.006; *0.01**
3		PulDur_0	**0.496**	**0.036; *0.05**
4		PulDur_10	**0.538**	**0.024; *0.05**
5		PulDur_50	0.036	**0.452
6		PulDur_90	**0.786**	**0.000; *0.01**
7		PulOn_90	0.374	**0.094
8		PulOn_peak	**0.461**	**0.048; *0.05**
9		PulOff_peak	**0.516**	**0.029; *0.05**
10		PulOff_90	**0.487**	**0.039; *0.05**
11		PulShapeOn	0.018	**0.475
12	Energy	PulShapeOff	-0.123	**0.338
13		PulDuty	**0.576**	**0.016; *0.05**
14		Crest Factor	**-0.630**	**0.008; *0.01**
15		Ener-0-10-Beg	**0.685**	**0.003; *0.01**
16		Ener-10-50-Beg	0.023	**0.469
17		Ener-50-90-Beg	**0.518**	**0.029; *0.05**
18		Ener-90-Peak-Beg	**0.740**	**0.001; *0.01**
19		Ener-Peak-90-End	0.413	**0.071

20		Ener-90-50-End	**0.692**	**0.003; *0.01**
21		Ener-50-10-End	**0.551**	**0.021; *0.05**
22		Ener-10-0-End	**-0.505**	**0.033; *0.05**
23	Frequency	PulseDomFreq	**-0.505**	**0.033; *0.05**
24		PulseFundFreq	**-0.522**	**0.028; *0.05**
25		PulseMinFreq	**-0.481**	**0.041; *0.05**
26		PulseMaxFreq	**-0.484**	**0.040; *0.05**
27		PulseOnFreq	**-0.511**	**0.031; *0.05**
28	Intensity	relAmpl-H1	0.126	**0.333
29		relAmpl-H3	-0.035	**0.453

*Arterisk indicates significant level; **arterisk indicates insignificant characters with SVL; r = Pearson correlation coefficient.

-0.522; p = 0.028), pulse minimum frequency (r = -0.481; p = 0.041), pulse maximum frequency (r = -0.484; p = 0.040), and onset pulse dominant frequency (r = -0.511; p = 0.031), indicating the call character was not linearly related with the frog body size. The negative relationship between dominant frequency and SVL obtained in this study supports Augusto-Alves *et al.* (2021), though Ziegler *et al.* (2016) revealed that dominant frequency and frog body size were consistently correlated, as shown in Figure 4.

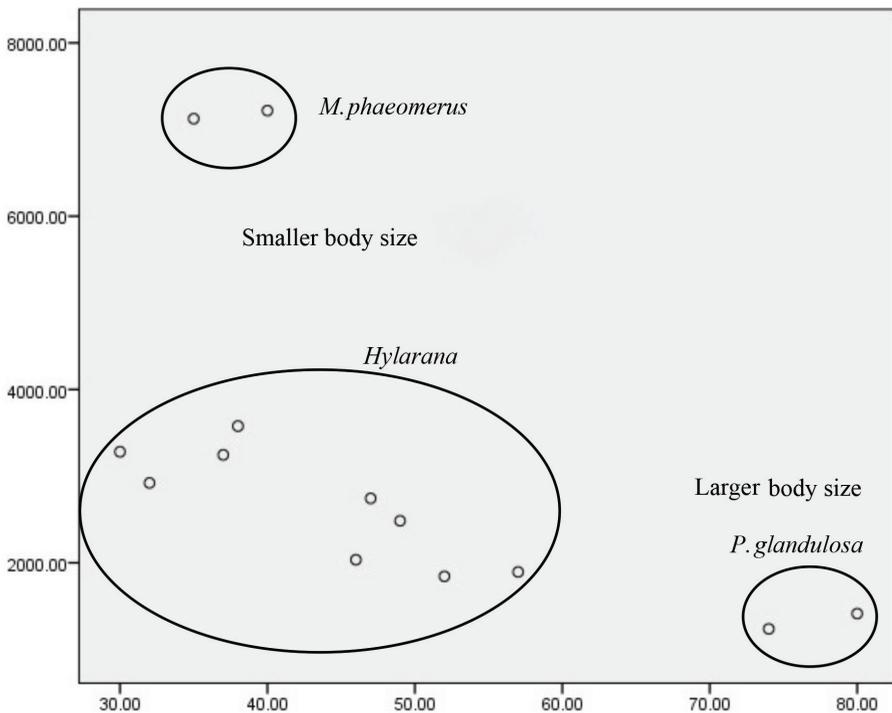**Dominant Frequency (kHz)**

Figure 4: Correlation graph showing the relationship between dominant frequencies of frog calls with body size of the frogs (snout-vent-length)

The high correlation in the relationship between pulse duration of 90% (resistant) of call and with body size of the frogs (r = 0.786, p = 0.000) indicates a strong relationship between these two characters, as illustrated in Figure 5. The graph supports the suggestion that *Pulchrana glandulosa*, with larger body sizes than other frogs, refuses to give in to the pulse between 90% and therefore, consumes a much longer time at this stage. This result was consistent with Köhler *et al.* (2017) and Röhr *et al.* (2020), who reported that call duration and pulse rate showed positive relationships with the frog's body size. Strong correlations between energy consumed by the frogs to produce calls and the frog body size were also revealed in this study. Thus, this result supports Zainudin *et al.* (2010), who also revealed a strong relationship between call energy and the body size of the frogs.

Out of 38 total call characters analysed in this study, the discriminant function analysis extracted three significant functions. Each function explained 60.6%, 27.0%, and 11.5% of the variance with eigenvalues 481.94, 215.09, and 91.14, respectively (Table 3). Wilk's Lambda test supports the result showing significant value for the three functions in which Wilk's Lambda value for Function 1, Function 2, and Function 3 is 0.000, respectively while the *p*-value for Function 1 and Function 2 is 0.000 and *p*-value for Function 3 is 0.006 (Table 4).

The canonical discriminant function coefficient values in both Function 1 and Function 2 show call characteristics of frogs in which three groups of characters were apparent: The pulse duration, crest factor, and call energy (Table 5). Figure 6 illustrates that these characters in the ranid species, *Pulchrana glandulosa* and *Meristogenys phaeomerus* differ
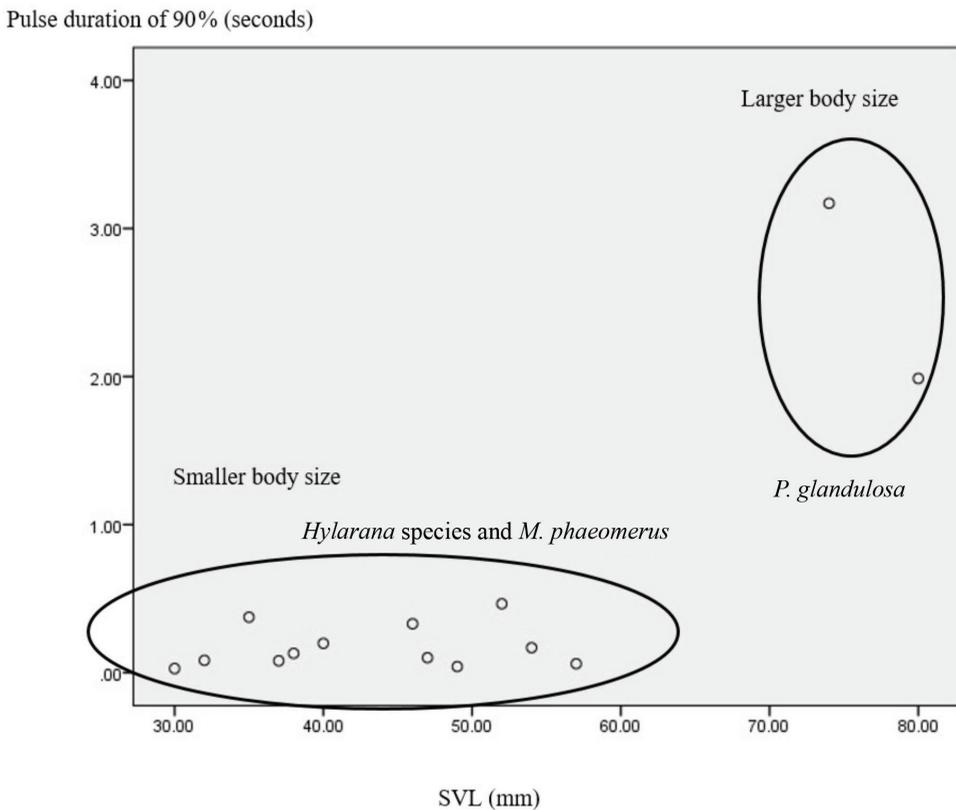

Figure 5: Correlation graph showing the relationship between pulse duration between 90% (resistant) of frog calls with body size of the frogs (snout-vent-length)

by their body size (SVL). However, how strongly do the call variables discriminate conspecifics from different localities? Thus, for this study, individuals of *P. baramica* were collected from several localities (Matang, Mulu, and Bako) and analysed to determine if there are any variations in the advertisement calls of the same species.

Table 3: Eigenvalues for DFA of call characteristics

Functions	Eigenvalue	Percentage of Variance (%)	Cumulative (%)	Canonical Correlation
1	481.938[a]	60.6	60.6	0.999
2	215.088[a]	27.0	87.7	0.998
3	91.136[a]	11.5	99.1	0.995

a = First six canonical discriminant functions were used in the analysis.

Table 4: Wilk's Lambda test for DFA

Test of Functions	Wilk's Lambda	Chi-square	df.	Sig.
1 through 6	0.000	261.282	120	0.000
2 through 6	0.000	177.853	95	0.000
3 through 6	0.000	105.282	72	0.006
4 through 6	0.038	44.218	51	0.738
5 through 6	0.201	21.664	32	0.916
6	0.628	6.287	15	0.975

Table 5: Standardised canonical discriminant function coefficients

	Functions	
	1	2
Note	-1.548	-2.203
Duration of note	7.597	16.535
Note gap	-1.464	-2.653
Repetition of note	-3.396	.249
Max. pulse amplitude relative to max. sound amplitude	-5.977	-2.125
Period pulse between 0%	-7.895	4.489
Pulse duration of 50%	**11.351**	**3.020**
Pulse duration between 90% (resistant)	**-15.741**	**-11.987**
Pulse to induced to 90% (attack)	-1.475	-17.509
Form the heart (10:50% spark/10:90% flash)	2.720	5.087
Heart shape	1.427	-4.125
Duration between pulse	1.229	-19.395
Pulse duration (duty cycle)	2.128	-1.763
Crest factor	8.206	8.550
The energy between the initial peak amplitude of 0:10%	**14.109**	**20.074**
The energy between the initial amplitude of 10:50% peak	1.964	1.874

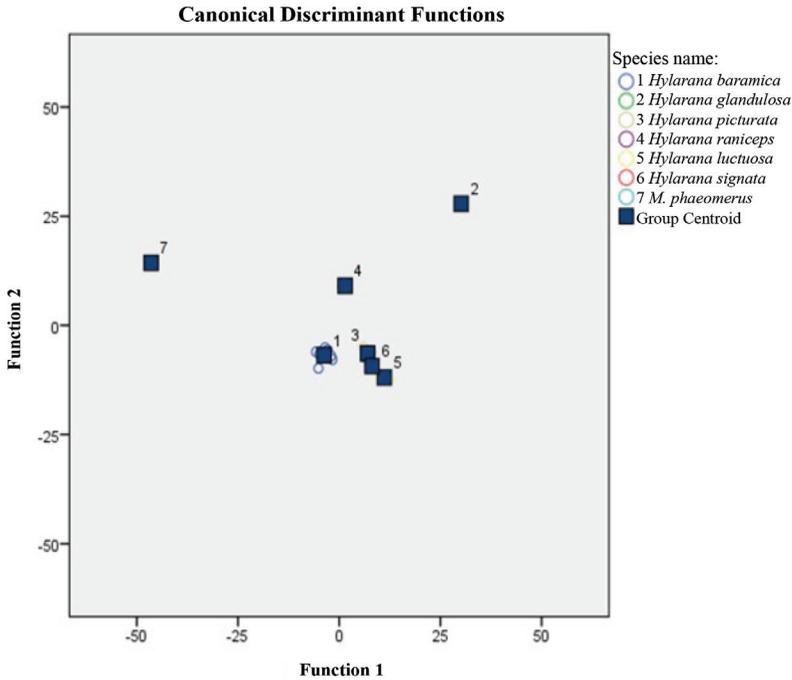|		
The energy between the initial peak amplitude of 50:90%	-2.255	8.338
The energy between the initial peak: peak amplitude of 90%	**8.089**	**10.446**
The energy between suffix of 50:10% peak amplitude	-2.367	-7.617
The ratio of the period to reach half a modulation frequency	-1.826	-2.221

*Diagnostic characters in each function.

Figure 6: Canonical discriminant functions of Function 1 and Function 2 for call characteristics Sarawak ranids were plotted. This figure reflects the highest character loadings observed in both Function 1 and Function 2, which included pulse duration between 90% (resistant) (-15.741 and -11.987, respectively) and energy between initial peak amplitude of 0:10% (14.109 and 20.074, respectively)

Variation of Call Characteristics among Individuals of Pulchrana Baramica from Different Localities

The discriminant function analysis of advertisement call characteristics belonging *to Pulchrana baramica* showed one significant function in the analysis. The eigenvalue for Function 1 is 346.538, with the highest percentage of variance in this function being 99.6% (Table 6). The Wilk's Lambda test of Function 1 through Function 2 value is 0.001 with a *p*-value is 0.021 (Table 7). Among all seven meaningful call characters (Table 8), the noted gap was the most useful character

that may elucidate the discrimination of this species based on advertisement call characters by populations. Table 8 showed that in both Function 1 and Function 2, the note gap canonical coefficient value was the highest value with 21.276 and 1.393, respectively in both functions. This result was supported by the canonical discriminant function graph (Figure 7) in which the individuals of *P. baramica* were successfully allocated based on each locality.

Table 6: Eigenvalues for DFA of *P. baramica* call characteristics

Functions	Eigenvalue	Percentage of Variance (%)	Cumulative (%)	Canonical Correlation
1	346.538[a]	99.6	99.6	0.999
2	1.272[a]	0.4	100.0	0.748

a = First two canonical discriminant functions were used in the analysis.

Table 7: Wilk's Lambda for DFA of *P. baramica* call characteristics

Test of Functions	Wilk's Lambda	Chi-square	df.	Sig.
1 through 2	0.001	26.685	14	0.021
2	0.440	3.282	6	0.773

Table 8: Standardised canonical discriminant function coefficients

	Functions	
	1	2
Note	16.588	0.720
Note duration	-7.106	0.454
Note gap	**21.276**	**1.393**
Max. pulse amplitude relative to the max. sound amplitude	-3.202	0.338
Period pulse between 0%	**17.754**	**1.155**
Pulse duration of 50%	2.537	0.913
Pulse to induced to 90% (attack)	-17.605	-0.896

*Diagnostic characters in each function.

The result also showed that call character of note gap was the most significant and informative in discriminating the populations of *P. baramica* based on the topography of the three localities; Matang was located in between Bako National Park in southern Sarawak and Gunung Mulu National Park of the northern part of Sarawak (Figure 7). The oscillograms of all individuals from each locality showed similar temporal call patterns, with rapid calling notes in 60 seconds. The repetitive notes per 60 seconds varied among populations, with three repetitive notes, five repetitive notes, and eight repetitive notes produced by individuals from each locality (Matang, Mulu, and Bako). On the other hand, there was no difference in the call pitch as they all produced pitches of call frequency ranging from 1 to 3 kHz. This may explain why the call repetition rates may influence the note gap period, thus, making it a meaningful variable for discriminating between conspecifics from different populations.

Discrimination of Frog Species Based on Similarities of the Advertisement Call Characters

A dendrogram showed that closely related frog species successfully clustered together with each other (Figure 8) with respect to the outgroup, *Pedostibes hosii* (Bufonidae). Two major groups were apparent in the dendrogram. The first major group clustered *Pulchrana baramica* and *P. glandulosa* together, which was congruent with their taxonomic status, indicating that these two species were sister taxa. The second major group consists of two subgroups in which the first subgroup clustered *P. picturata* and *P. signata* into one group. This result also

successfully emphasised that these two species are closely related sibling species. The second subgroup, on the other hand, consists of three other species, namely, *Chalcorana raniceps*, *Abavorana luctuosa*, and *Meristogenys* *phaeomerus*, respectively. Overall, the result indicates that this cluster analysis method successfully clustered the frog species based on their advertisement call characteristics.
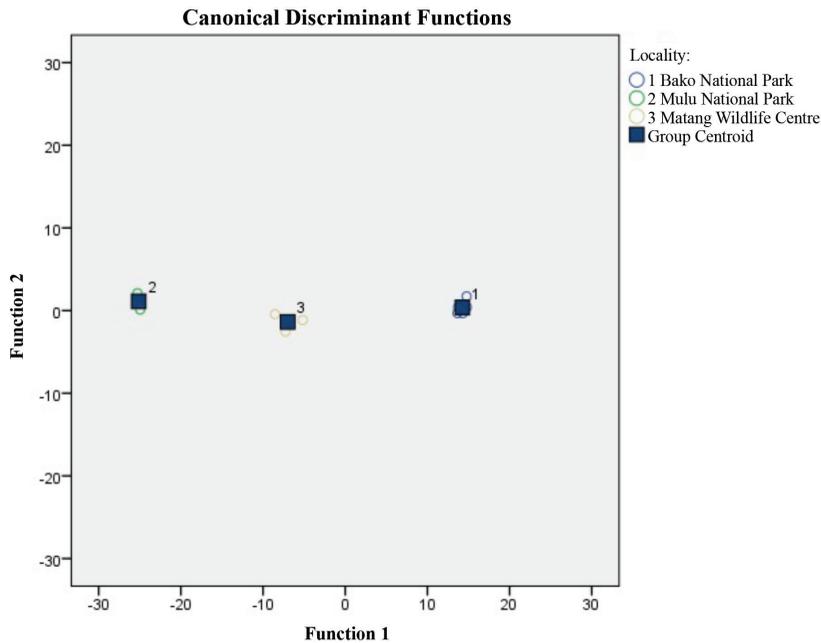

Figure 7: Canonical discriminant functions of Function 1 against Function 2 for call characteristics *Pulchrana baramica* based on note gap and pulse duration between notes of the call
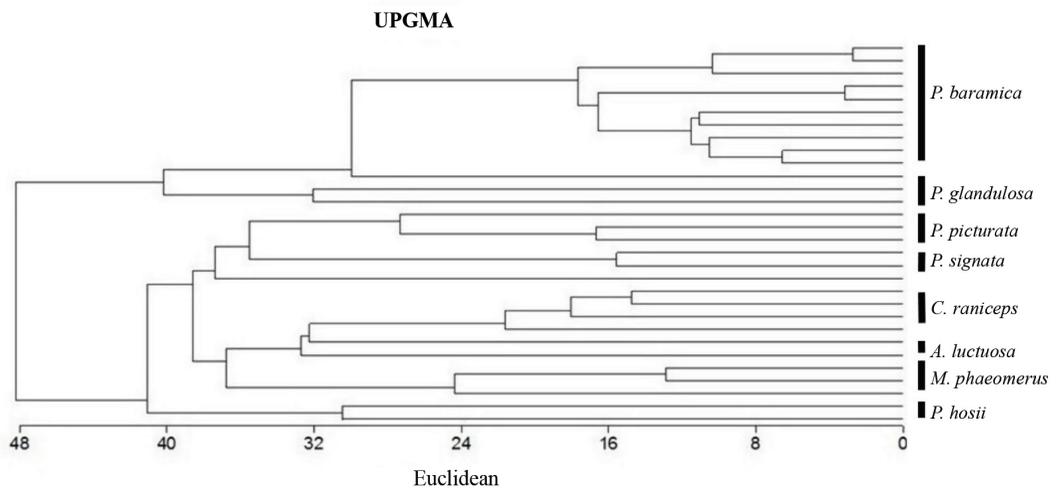

Figure 8: Dendrogram of UPGMA based on similarities of call characteristics of family Ranidae by using cluster analysis in MVSP ver3.13d (Kovach, 1996)

Advertisement Call Characteristics of the Calling Species of Family Ranidae

a. *Pulchrana baramica*

The oscillogram for the advertisement call of *P. baramica* analysed in 60 seconds showed that this species produced monophasic calls, which consist of 8 similar repetitive notes [Figure 9 (a)]. Note 8 consists of 18 pulses [Figure 9 (b)]. The spectrogram showed that this species emit low-pitched calls ranging from 1 to 3 kiloHertz (kHz) [Figure 9 (c)]. Only one peak of the highest amplitude spectrum is observed in Figure 9 (d). The result was consistent with Zainudin *et al.* (2011), who studied the call of *P. baramica* from the Matang ranges.

b. *Pulchrana glandulosa*

The oscillogram and spectrogram for the call of *P. glandulosa* are shown in Figure 10. *P. glandulosa* also produced monophasic calls similar to *P. baramica* and possesses one pulse in one note, as recorded in Zainudin *et al.* (2011). There are 29 repetitive notes with frequencies ranging from 0 to 5 kHz. Though *P. baramica* and *P. glandulosa* inhabited almost similar habitats, their advertisement calls were very different.

c. *Pulchrana picturata*

P. picturata produced two to three pulsed notes [Figure 11 (a)] and was conclusive with a total of seven repetitive notes per 60 seconds for this study. The spectrogram showed that this species emits low pitch with frequency ranging from 1 to 4 kHz [Figure 11 (c)]. The amplitude spectrum appeared to have only one highest peak, as shown in Figure 11 (d).
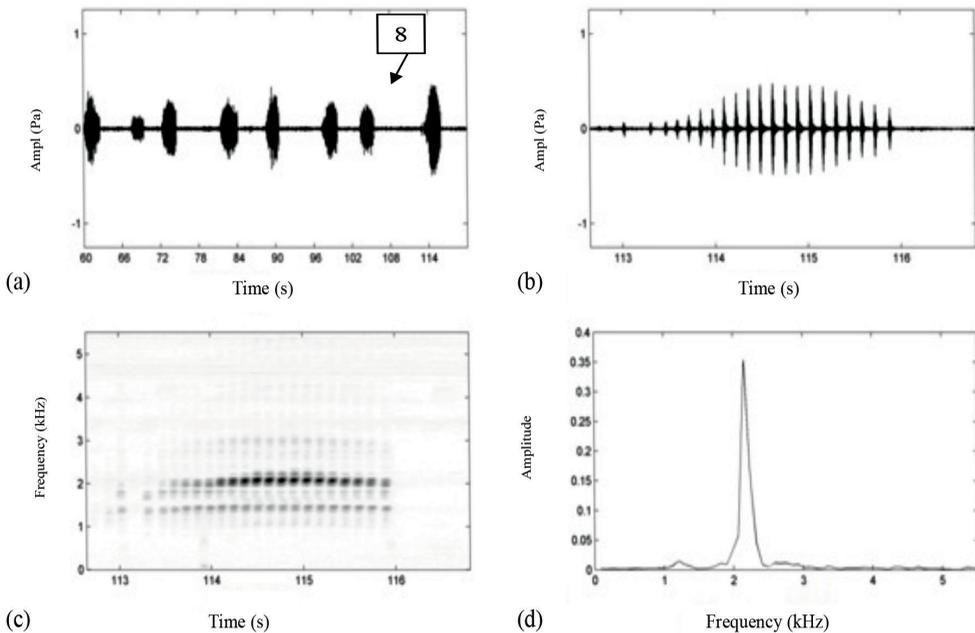

Figure 9: (a) The oscillogram of the call produced by *P. baramica* recorded in 60 seconds at Matang Wildlife Centre; (b) the oscillogram of one note; (c) the spectrogram of one note; and (d) the amplitude spectrum of one note
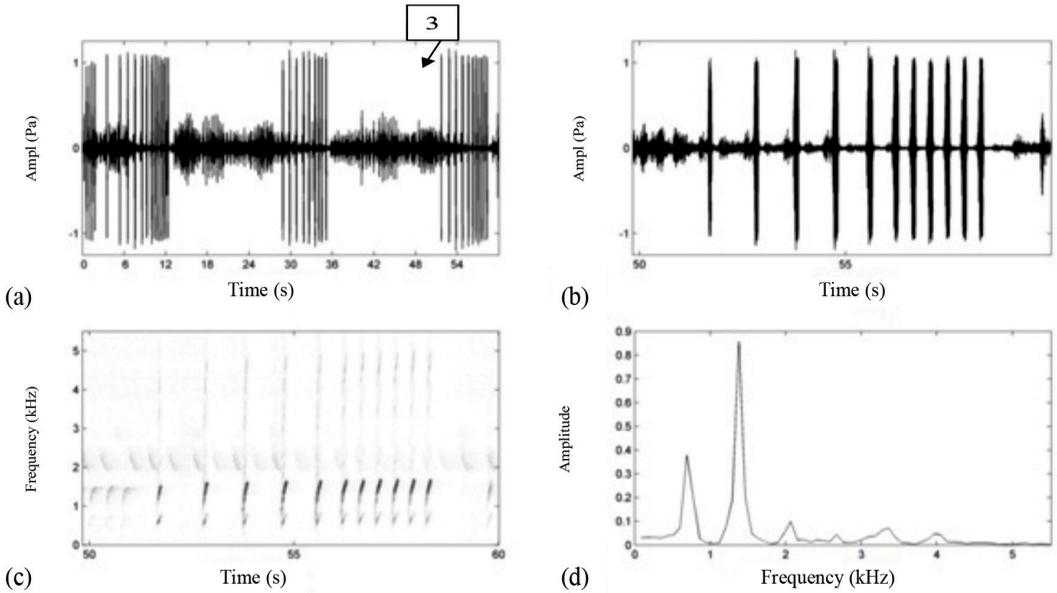
Figure 10: (a) The oscillogram of the call produced by *P. glandulosa* recorded in 60 seconds at Bako National Park; (b) the oscillogram of one note; (c) the spectrogram of one note; and (d) the amplitude spectrum of one note
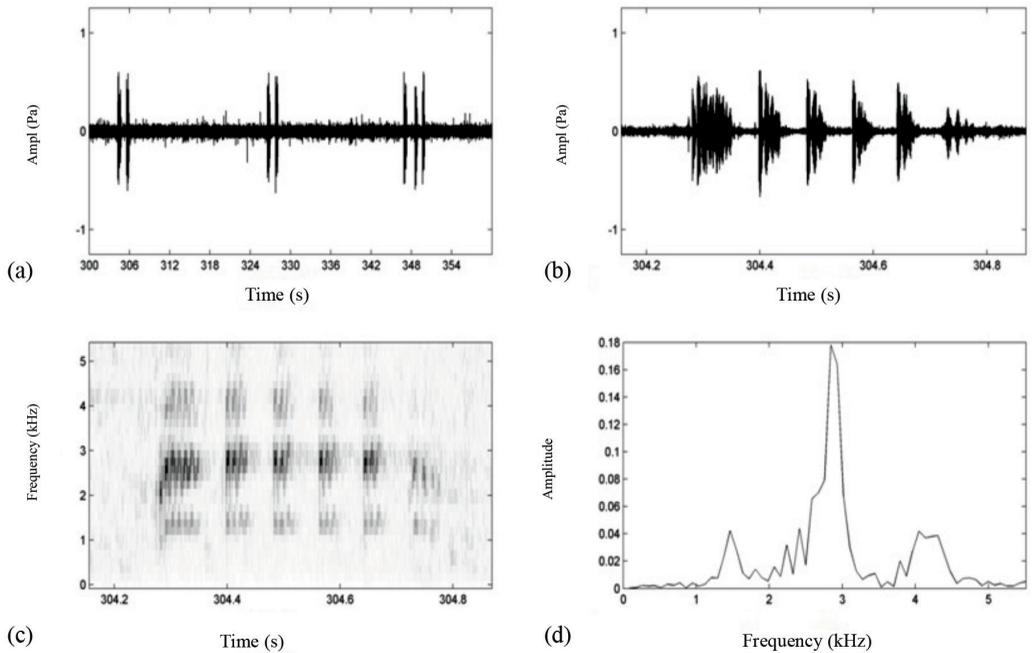

Figure 11: (a) The oscillogram of the call produced by *P. picturata* recorded in 60 seconds at Kubah National Park; (b) the oscillogram of one note; (c) the spectrogram of one note; and (d) the amplitude spectrum of one note

d. Pulchrana signata

The other closely related species to *P. picturata*, *P. signata* also produced almost similar calls to *P. picturata* but with a lower calling rate. *P. signata* possesses only one note in 60 seconds in this study. The call note consists of four repetitive pulses [Figure 12 (b)] with low-pitched (1 to 4 kHz) [Figure 12 (c)].

e. Chalcorana raniceps

The advertisement call of *C. raniceps* consists of four repetition notes in 60 seconds with two repetitive pulses in one note [Figure 13 (b)]. *C. raniceps* produced a short but loud "chuck-chuck" call with a range of frequency from 1 to 5 kHz [Figure 13 (c)].

Figure 12: (a) The oscillogram of the call produced by *P. signata* recorded in 60 seconds at Bako National Park; (b) the oscillogram of one note; (c) the spectrogram of one note; and (d) the amplitude spectrum of one note
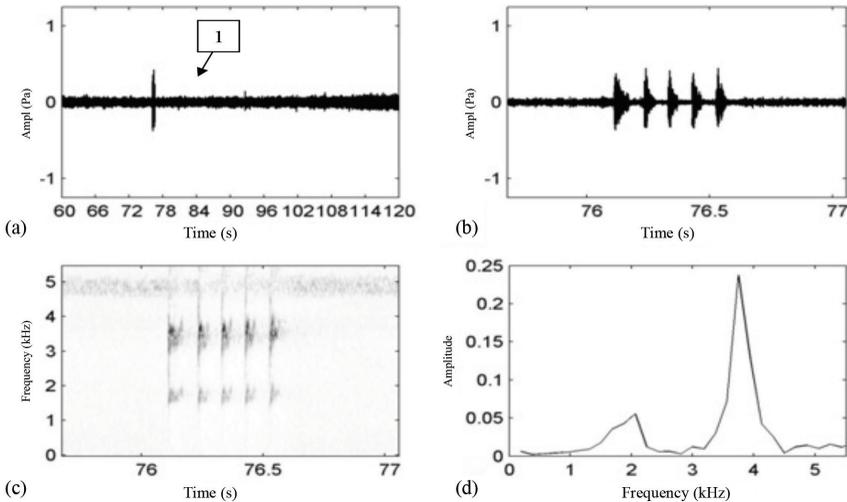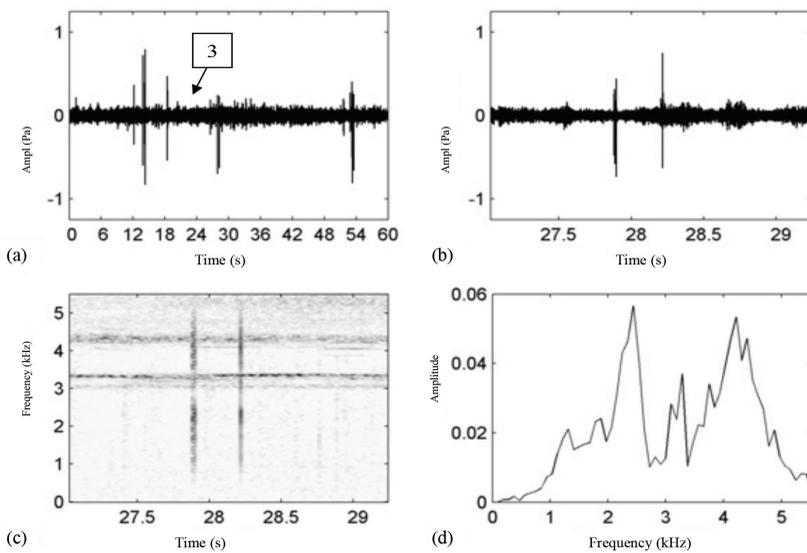

Figure 13: (a) The oscillogram of the call produced by *C. raniceps* recorded in 60 seconds at Bako National Park; (b) the oscillogram of one note; (c) the spectrogram of one note; and (d) the amplitude spectrum of one note

f. *Abavorana luctuosa*

A. luctuosa consists of a long unpulsed single note in 60 seconds. The call has a low pitch ranging from 1 to 2 kHz with only one peak shown in the amplitude spectrum [Figure 14 (d)]. During sound recording, this species was heard producing a "meew" call.

g. *Meristogenys phaeomerus*

M. phaeomerus is another calling species of the genus *Meristogenys* in this study that produced calls during field sampling. This species produced two notes in 60 seconds, each of which consisted of one pulse [Figure 15 (b)].

Figure 14: (a) The oscillogram of the call produced by *A. luctuosa* recorded in 60 seconds at Bako National Park; (b) the oscillogram of one note; (c) the spectrogram of one note; and (d) the amplitude spectrum of one note
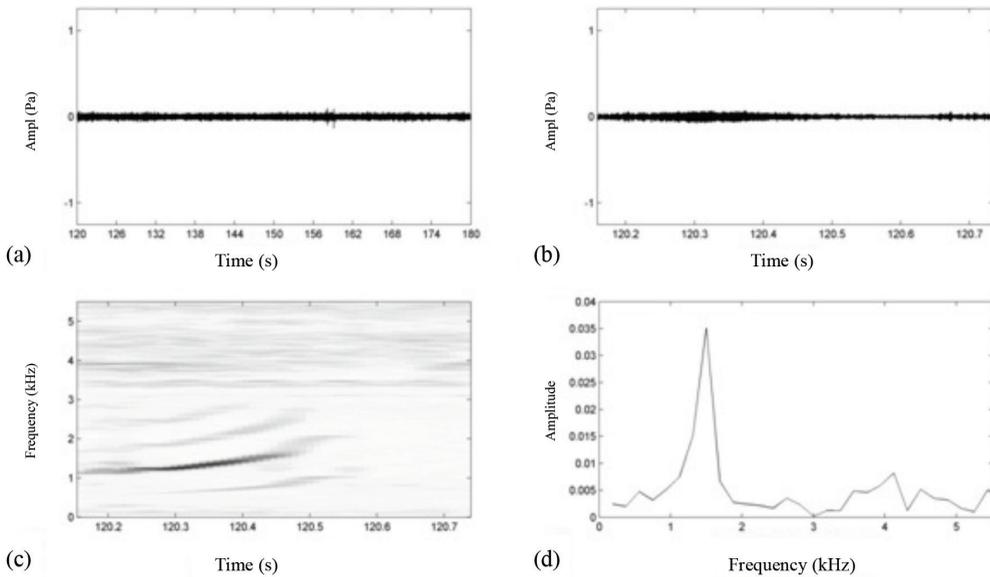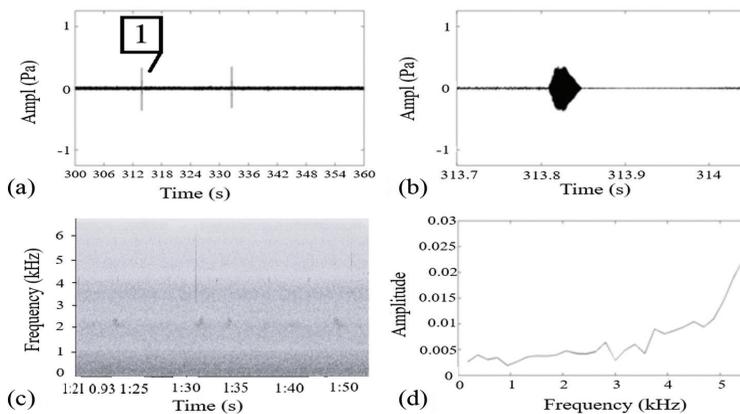

Figure 15: (a) The oscillogram of the call produced by *Meristogenys phaeomerus* recorded in 60 seconds at Bako National Park; (b) the oscillogram of one note; (c) the spectrogram of one note; and (d) the amplitude spectrum of one note

Phylogeny Reconstruction of Sarawak Ranids Based on Call Character Coding

Based on the PCA result, there were 29 meaningful characters of the male advertisement calls. The mean for each species for each of the 29 meaningful call characters was recorded in Table 9. The character coding was derived from the dot plots of ANOVA and then simplified in a table. Characters coding derived from this test were numbered from range 1 to 8 (Table 10). A maximum parsimony tree was constructed, as shown in Figure 16.

Advertisement call of male frogs is the most energetically expensive behaviour among all living vertebrates. Call energy is one of the call characters that is highly correlated with the call variations among frog species. Frogs use energy to fill their lungs with air and to rebound their lungs back to resting size after expelling the air through the larynx. When air passes through the vocal cords at the vocal apparatus, thus, the sound is emitted due to the vibration of the cords. The call energy was measured by the strength of the cords vibrating dependent on the volume of air forced from the lungs passed through the vocal cords. However, in this condition, the metabolic rate is relatively high as a large amount of oxygen was consumed when a high level of calling (call length increase and complex call pattern) was produced. Voituron *et al.* (2012) also suggested that the longer a frog emits a call, the more rapid the call rate and the more energy the call consumes.

PCA showed all call groups were meaningful to discriminate conspecific of *Pulchrana baramica*. Pulse rate and note gap were the most significant characters based on the PCA. Röhr *et al.* (2020) stated that pulse rate is sufficient as the key factor to distinguish the advertisement calls among different populations. They also suggested that the variable is the basis for discrimination of the mating partner. This reflects the finding in Figure 16, where the most meaningful call characters had been computed to reconstruct the maximum parsimony tree and had shown that the call variables could successfully discriminate between frog species,

respectively, supported by high consistency and retention indices. However, the tree reconstructed also demonstrated that the placement of species may be influenced by environmental factors that correspond to the call emission in the natural habitat such as ambient temperature and humidity. Besides, a previous study reported that call variables may also be influenced by environmental conditions, mainly by geographical factors or geographic variations (Willacy *et al.*, 2015). The result may explain the influence of their habitat descriptions on their adaptation to different types of geographical structures. Both Gunung Mulu National Park and the Matang ranges are in mountainous areas where this species has quiet environments to break the silence with their calls. Samples that were collected at Bako National Park must compete with the surrounding noises, particularly the sound of crashing waves because the habitat is located near the beach area. This suggestion was supported by Zhao *et al.* (2018) and Forti *et al.* (2022), who stated that masking interference against background noise and most geographic variation influences the call variable of frogs to gain a successive signal in such conditions.

A similar condition occurs in *Huia cavitympanum*, a species that is also well-known as the only frog that produces pure ultrasound for vocalisation (Cobo-Cuan *et al.*, 2020). *H. cavitympanum* was discovered to emit ultrasonic signals to overcome the difficulties of signal detection during communication among conspecifics. Arch *et al.* (2009) stated that *H. cavitympanum* increased its signal-to-noise ratio by producing a high frequency of call against the low frequency of fast fast-flowing medium-sized stream it inhabited. A similar case was observed on *Amolops tormotus* also known as *Odorrana tormota* (Feng *et al.*, 2006) and showed a high correlation between the fundamental frequency of its call with low background noise frequency (Narins *et al.*, 2004; Feng *et al.*, 2009). This is further explained by the fact that producing a high frequency of calls may decrease the variation

Table 9: Mean of each call character for each species of frog were recorded

Species	1 NR	2 CF	3 CG	4 CH	5 CI	6 CJ	7 CK	8 CL	9 CM	10 CN	11 CO	12 CP	13 CS	14 CT	15 CV
P. baramica	0.08	0.70	9.85	9.70	1.11	0.40	3.65	3.76	5.95	5.65	0.76	0.77	0.90	1.77	0.18
P. glandulosa	0.04	0.65	24.06	23.96	3.17	1.24	9.68	10.03	13.93	13.04	0.60	0.57	1.39	1.46	2.04
C. raniceps	0.03	0.83	1.51	1.26	0.33	0.06	0.42	0.42	0.84	0.78	0.65	0.81	0.03	2.65	0.01
M. phaeomerus	0.05	1.00	23.15	20.77	15.13	0.29	10.58	10.58	12.48	12.19	0.37	0.32	-1.00	1.96	0.06
P. picturata	0.09	0.77	7.88	7.66	1.06	0.07	2.81	2.81	4.85	4.78	0.77	0.86	0.46	2.21	0.08
P. signata	0.03	0.71	4.91	4.82	0.30	0.10	2.09	2.14	2.68	2.62	0.82	0.78	0.67	2.06	0.04
A. luctuosa	0.02	0.70	2.70	2.62	0.39	0.11	1.16	1.77	1.44	1.34	0.70	0.78	0.64	2.26	0.00
P. hosii	0.03	0.69	6.17	6.06	1.52	0.96	2.40	2.54	3.52	2.69	0.77	0.85	0.85	1.85	0.06

Species	16 CW	17 CX	18 CY	19 CZ	20 DA	21 DB	22 DC	23 DD	24 DE	25 DF	26 DG	27 DH	28 DK	29 DN
P. baramica	0.05	0.01	0.02	0.03	0.34	0.00	1,939.24	969.62	1,829.46	2,021.80	1,873.95	1,979.36	1.81	-12.40
P. glandulosa	0.23	0.09	0.13	0.28	4.01	0.00	1,367.69	683.84	1,354.61	1,390.23	1,354.62	1,390.22	2.99	-21.52
C. raniceps	0.01	0.00	0.01	0.00	0.01	0.00	3,101.02	1,550.51	3,101.02	3,101.75	3,101.02	3,101.75	4.95	-10.73
M. phaeomerus	0.34	0.00	0.02	0.35	0.06	0.00	7,171.88	3,585.94	6,000.00	8,671.88	8,343.75	6,468.75	5.06	-40.73
P. picturata	0.05	0.00	0.01	0.04	0.16	0.00	2,613.95	1,306.98	2,537.21	2,686.44	2,575.38	2,627.54	3.91	-15.43
P. signata	0.00	0.00	0.00	0.00	0.05	0.00	3,410.98	1,705.49	3,408.87	3,811.37	3,409.81	3,410.73	12.57	-24.58
A. luctuosa	0.00	0.00	0.00	0.00	0.00	0.00	3,813.65	1,906.82	3,813.37	3,814.89	3,813.79	3,814.48	44.61	-29.88
P. hosii	0.02	0.00	0.04	0.00	0.09	0.00	1,477.99	739.00	1,468.45	1,488.31	1,476.10	1,481.14	9.17	-34.31

*Note: NR = note repetition rate; CF = relative pulse peak; CG = pulse duration at 0%; CH = pulse duration at 10%; CI = pulse duration at 50%; CJ = pulse duration at 90%; CK = pulse onset at 90%; CL = pulse onset at peak; CM = pulse offset at peak; CN = pulse offset at 90%; CO = pulse shape on; CP = pulse shape off; CS = pulse duty; CT = crest factor; CV = energy 0% to 10% begin; CW = energy 10% to 50% begin; CX = energy 50% to 90% begin; CY = energy 90% to peak begin; CZ = energy peak to 90% end; DA = energy 90% to 50% end; DB = energy 50% to 10% end; DC = energy 10% to 0% end; DD = pulse dominant frequency; DE = pulse fundamental frequency; DF = pulse minimum frequency; DG = pulse maximum frequency; DH = pulse on frequency; DK = relative amplitude-Harmonic 1; DN = relative amplitude-Harmonic 3

Table 10: Character coding for call characteristics of male Bornean frogs, family Ranidae

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
P. baramica	1	1	2	2	1	1	1	1	2	2	1	1	1	1	2	2	1	1	2	4	1	3	3	3	3	3	3	5	7
P. glandulosa	1	1	3	4	1	1	2	2	3	3	1	1	1	1	3	3	2	2	3	5	1	1	1	1	1	1	1	6	5
C. raniceps	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	5	5	5	5	5	5	7	8
M. phaeomerus	1	1	3	3	2	1	2	2	3	3	1	1	1	1	1	4	1	1	4	2	8	8	8	8	8	8	8	1	1
P. picturata	1	1	2	2	1	1	1	1	2	2	1	1	1	1	1	2	1	1	2	3	4	4	4	4	4	4	4	5	6
P. signata	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	6	6	6	6	6	7	6	4	4
A. luctuosa	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	7	7	7	7	7	7	7	3	3
P. hosii	1	1	2	2	1	1	1	1	2	2	1	1	1	1	1	1	1	1	1	2	1	2	2	2	2	2	2	2	2

*Note: 1 = NR (note repetition rate); 2 = CF (relative pulse peak) ; 3 = CG (pulse duration at 0%); 4 = CH (pulse duration at 10%); 5 = CI (pulse duration at 50%); 6 = CJ (pulse duration at 90%); 7 = CK (pulse onset at 90%); 8 = CL (pulse onset at peak); 9 = CM (pulse offset at peak); 10 = CN (pulse offset at 90%); 11 = CO (pulse shape on); 12 = CP (pulse shape off); 13 = CS (pulse duty); 14 = CT (crest factor); 15 = CV (energy 0% to 10% begin); 16 = CW (Energy 10% to 50% begin); 17 = CX (energy 50% to 90% begin); 18 = CY (energy 90% to peak begin); 19 = CZ (Energy peak to 90% end); 20 = DA (energy 90% to 50% end); 21 = DB (energy 50% to 10% end); 22 = DC (energy 10% to 0% end); 23 = DD (pulse dominant frequency); 24 = DE (pulse fundamental frequency); 25 = DF (pulse minimum frequency); 26 = DG (pulse maximum frequency); 27 = DH (pulse on frequency); 28 = DK (relative amplitude-Harmonic 1); 29 = DN (relative amplitude-Harmonic 3)

between call wavelength and the size of its vocal sac. Rossing (2007) stated that high frequencies of sound with short wavelengths have a high effect on surface tension (here is the tension of vocal cords). Thus, this may require high energy with a relatively high and efficient metabolic rate to ensure continuity of vocalisation. However, the call energy of the ultrasonic signal produced by *H. cavitympanum* was incomparable in this study because no recorded data was obtained. Therefore, this study lacks evidence to support the call characteristics of this species.

After all, the call variables of frogs also have a strong correlation with ambient temperature and the frog's body size as well. These hypotheses have been widely argued and the vocalisation of animals is dependent on the metabolism rate of the organism, which varies based on the body size and temperature (Verberk *et al*., 2020). Köhler *et al.* (2017) stated that when ambient temperature increases, the frog's call frequency will increase while the call repetition rate will decrease with increasing body size (the snout-vent-length). Body size also affects the temporal call characteristics of frogs (Röhr *et al*., 2020). In this study, the body size has a positive correlation with the dominant frequency and pulse duration of call characters. The larger the body size, the lower the dominant frequency produced by the frog. This result was consistent with Zainudin *et al.* (2009) and Forti *et al.* (2022).

Conclusions

Findings in this study of call characteristics of male Bornean frogs were consistent with findings in other studies. Many studies have revealed that the most varied call characters of frog calls are the pulse repetition rate, call intensity, and call energy. This study supports the result of Zainudin *et al.* (2011), as most of the samples for the analysis conducted were mostly from the same species from Sarawak. On the other hand, it was recommended that Huia cavitympanum record ultrasonic signals to enable a complete comparison of call variables (especially call
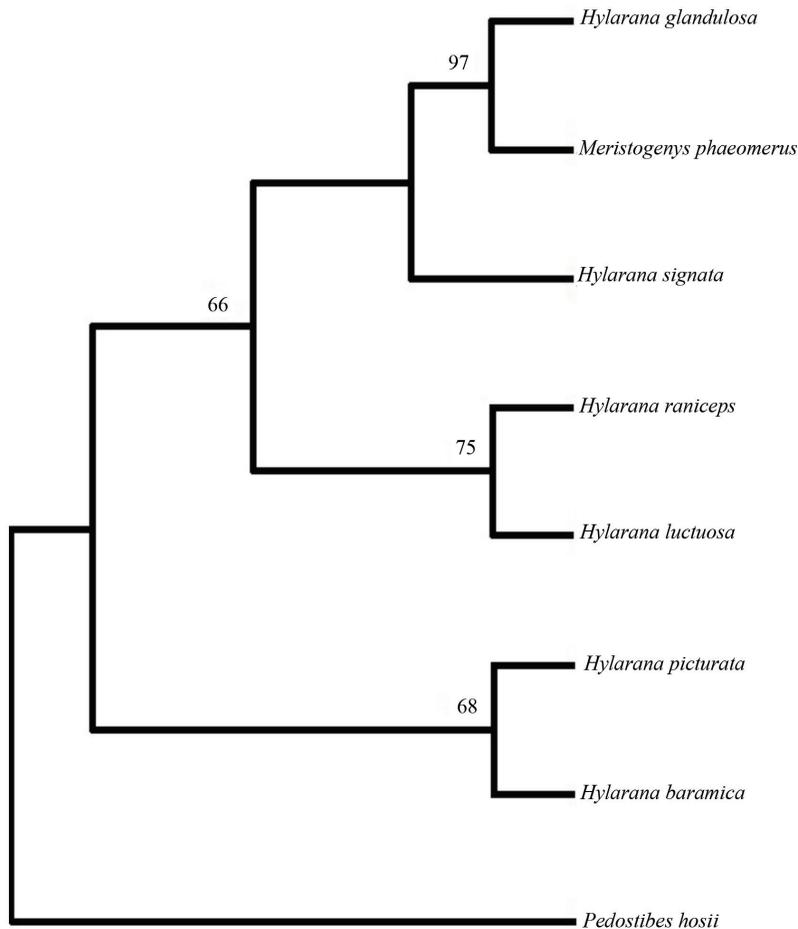
Figure 16: A maximum parsimony tree constructed using PAUP 4biOb software with a bootstrap value above the branch. Tree length is 81 with consistency index, CI = 1.000, and retention index, RI = 1.000

energy and call frequency) between ultrasound signals and the harmonic call variables produced by other audible frog species. The changes in vocalisation from harmonic calls to ultrasonic signals are likely an evolutionary adaptation to noisy environments.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

References

Amram, M. F., Zainudin, R., & Wahid, H. A. (2018). Mating calls of selected Sarawak toads (Amphibia: Anura: Bufonidae). *Sains Malaysiana*, *47*(1), 1-7.

Amram, M. F., Zainudin, R., & Wahid, H. A. (2020). Notes on advertisement calls

playback by three species of Sarawakian frogs. *Borneo Journal of Resource Science and Technology*, *10*(1), 51-60.

Arch, V. S., Grafe, T. U., Gridi-Papp, M., & Narins, P. M. (2009). Pure ultrasonic communication in an endemic Bornean frog. *PLOS ONE*, *4*(4).

Arini, K., Noer, M. I., Wulandari, A., Amalia, R., & Auliandina, T. (2016). Temporal and spectral variation in advertisement call of males *Microhyla achatina* (Tschudi, 1838) are sufficient for individual discrimination. Towards the sustainable use of biodiversity in a changing environment: From basic to applied research. In *AIP Conference Proceedings*, *1744*, 020032. AIP Publishing.

Augusto-Alves, G., Dena, S., & Toledo, L. F. (2021). Acoustic allometry, background stream noise and its relationship with large-bodied and voiceless rheophilic frogs. *Zoologischer Anzeiger*, *295*, 156-162.

Bernardy, J. V., Melo, I., Llusia, D., Diniz-Filho, J. A. F., & Bastos, R. P. (2024). Female preferences for dominant frequency in frogs: Constraints and impact on sexual size dimorphism. *Behavioral Ecology and Sociobiology*, *78*(4).

Cobo-Cuan, A., Grafe, T. U., & Narins, P. M. (2020). Beyond the limits: Identifying the high-frequency detectors in the anuran ear. *Biology Letters*, *16*, 20200343.

Deka, E. Q., Su'ut, L., Wahid, H. A., & Zainudin, R. (2015). Apparatus structure of the Sarawak frogs (Amphibia: Anura: Ranidae). *Sains Malaysiana*, *44*(9), 1289-1299.

Eto, K., Matsui, M., & Nishikawa, K. (2016). A new highland species of dwarf litter frog genus *Leptobrachella* (Amphibia: Anura: Megophryidae) from Sarawak. *Raffles Bulletin of Zoology*, *64*, 194-203.

Fears, B. A. C. (2010). *Laryngeal apparatus and call structure in North American Hylids* [Master's thesis, Missouri University of Science and Technology].

Feng, A. S., Narins, P. M., Xu, C. H., Lin, W. Y., Yu, Z. L., Qiu, Q., Xu, Z. M., & Shen, J. X. (2006). Ultrasonic communication in frogs. *Nature*, *440*(7082), 333-336.

Feng, A. S., Riede, T., Arch, V. S., Yu, Z., Xu, Z., Yu, X., & Shen, J. (2009). Diversity of the vocal signals of concave-eared torrent frogs (*Odorrana tormota*): Evidence for individual signatures. *Ethology*, *115*, 1015-1028.

Forti, L. R., de Melo Sampaio, M. R., Pires, C. R., Szabo, J. K., & Toledo, L. F. (2022). Torrent frogs emit acoustic signals of a narrower spectral range in habitats with longer-lasting biotic background noise. *Behavioural Processes*, *200*, 104700.

Gan, H., Zhang, J., Towsey, M., Truskinger, A., Stark, D., van Rensburg, B. J., Li, Y., & Roe, P. (2020). Data selection in frog chorusing recognition with acoustic indices. *Ecological Informatics*, *60*, 101160.

Gerhardt, H. C., & Bee, M. A. (2007). Recognition and localization of acoustic signals. In Narins, P. M., Feng, A. S., Fay, R. R., & Popper, A. N. (Eds.), *Hearing and sound communication in amphibians*. Springer Handbook of Auditory Research (Vol. 28). New York, NY: Springer.

Indraswari, K., Bower, D. S., Tucker, D., Schwarzkopf, L., Towsey, M., & Roe, P. (2018). Assessing the value of acoustic indices to distinguish species and quantify activity: A case study using frogs. *Freshwater Biology*, *65*(1), 142-152.

Köhler, J., Jansen, M., Rodriguez, A., Kok, P. J. R., Toledo, L. F. Emmrich, M., Glaw, F., Haddad, C. F. B., Rödel, M-O., & Vences, M. (2017). The use of bioacoustics in anuran taxonomy: Theory, terminology, methods and recommendations for best practice. *Zootaxa*, *4251*(1), 001-124.

Lardner, B., & Lakim, M. (2004). Female call preferences in tree-hole frogs: Why are there so many unattractive males? *Animal Behaviour*, *68*(2), 265-272.

Leong, T. M., Matsui, M., Yong, H. S., & Hamid, A. A. (2003). Revalidation of *Rana laterimaculata* Barbour et Noble, 1916 from the synonymy of *Rana baramica* Boettger, 1901. *Current Herpetology*, *22*, 17-27.

Marly, M. A. A., Zainudin, R., & Amram, M. F. (2017). Identification keys on advertisement call characters for the genus *Pulchrana* (Anura: Ranidae) in Sarawak. *Malaysian Applied Biology*, *46*(4), 15-21.

Matsui, M. (1997). Call characteristics of Malaysian *Leptolalax* with a description of two new species (Anura: Pelobatidae). *Copeia*, *1997*(1), 158-165.

Matsui, M., & Nishikawa, K. (2014). Description of a new species of *Limnonectes* from Sarawak, Malaysian Borneo (Dicroglossidae, Anura). *Current Herpetology*, *33*(2), 135-147.

Mattison, C. (2011). *Frogs and toads of the world*. Princeton and Oxford: Princeton University Press.

Narins, P. M., Feng, A. S., Lin, W., Schnitzler, H., Denzinger, A., Suther, R. A., & Xu, C. (2004). Old world frog and bird vocalizations contain prominent ultrasonic harmonics. *Journal Acoustic Social Amphibia*, *115*(2), 910-913.

Röhr, D. L., Camurugi, F., Paterno, G. B., Gehara, M., Junca, F. A., Alvares, G. F. R., Brandao, R. A., & Garda, A. A. (2020). Variability in anuran advertisement call: A multi-level study with 15 species of monkey tree frogs (Anura, Phyllomedusidae). *Canadian Journal of Zoology*, *98*(8), 495-504.

Rossing, T. D. (2007). *Handbook of acoustics*. Springer. pp: 473-490.

Ryan, M. J., & Tuttle, M. D. (1983). The ability of the frog-eating bat to discriminate among novel and potentially poisonous frog species using acoustic cues. *Animal Behaviour*, *31*, 827-833.

Shimada, T., Matsui, M., Nishikawa, K., & Eto, K. (2015). A new species of *Meristogenys* (Anura: Ranidae) from Sarawak, Borneo. *Zoological Science*, *32*(5), 474-484.

Sullivan, B. K., & Malmos, K. B. (1994). Call variation in the Colorado river toad (*Bufo alvarius*): Behavioural and phylogenetic implications. *Herpetologica*, *50*(2), 146-156.

Tolosa, Y. (2020). First record of male-male aggressive behavior in the yellow-striped poison frog, *Dendrobates truncates* Cope, 1861 (Anura: Dendrobatidae), with description of the aggressive call. *Herpetology Notes*, *13*, 925-929.

Vallejos, J. G., Grafe, T. U., Ahmad Sah, H. H., & Wells, K. D. (2017). Calling behavior of males and females of a Bornean frog with male parental care and possible sex-role reversal. *Behavioral Ecology and Sociobiology*, *71*(95).

Vèlez, A., & Guajardo, A. S. (2021). Individual variation in two types of advertisement calls of Pacific tree frogs, *Hyliola (=Pseudacris) regilla*, and the implications for sexual selection and species recognition. *The International Journal of Animal Sound and Its Recording*, *30*(4), 437-457.

Verberk, W. C. E. P., Atkinson, D., Hoefnagel, K. N., Hirst, A. G., Horne, C. R., & Siepel, H. (2020). Shrinking body sizes in response to warming: Explanations for the temperature-size rule with special emphasis on the role of oxygen. *Biological Reviews*, *96*(1), 247-268.

Voituron, Y., Brepson, L., Richardson, C., Joly, P., & Lengagne, T. (2012). Energetics of calling in the male treefrog *Hyla arborea*: when being large means being sexy at low cost. *Behaviour*, *149*, 775-793.

Willacy, R., Mahony, M., & Newell, D. (2015). If a frog calls in the forest: Bioacoustic monitoring reveals the breeding phenology of the endangered Richmond Range mountain frog (*Philoria richmondensis*): Breeding phenology of *P. richmondensis*. *Austral Ecology*, *40*(6), 10.1111.

Xie, J., Indraswari, K., Schwarzkopf, L., Towsey, M., Zhang, J., & Roe, P. (2018). Acoustic classification of frog within-species and

species-specific calls. *Applied Acoustics*, *131*, 79-86.

Xie, J., Towsey, M., Zhang, J., & Roe, P. (2016). Acoustic classification of Australian frogs based on enhanced features and machine learning algorithms. *Applied Acoustics*, *113*, 193-201.

Yi, J. D., Cobo-Cuan, A., Marquez, R., Sheridan, J. A., Grafe, T. U., Farina, A., & Narins, P. M. (2021). Novel acoustic snapshot of a Sarawak forest. *Journal of Ecoacoustics*, *5*(1), 1. DOI: 10.35995/jea5010001.

Zainudin, R., Rahman, M. A., Zain, B. M., Nor, S., Ahmad, N., & Inger, R. (2009). Ciri-ciri panggilan katak Borneo (Genus: Hylarana) daripada populasi Sarawak, Malaysia. *Sains Malaysiana*, *38*(5), 619-624.

Zainudin, R., Rahman, M. A., Zain, B. M. M., Nor, S. M., Inger, R., & Ahmad, N. (2010). Mating calls description of five species of frogs from the genus *Hylarana* Tschudi 1838 (Amphibia, Anura, Ranidae) from Sarawak, Malaysia. *Sains Malaysiana*, *39*(3), 363-369.

Zainudin, R., Wahid, H. A., & Rahman, M. A. (2011). *Croaks of the Bornean frog: The Hylarana of Sarawak*. Universiti Malaysia Sarawak.

Zhang, F., Chen, P., Chen, Z., & Zhao, J. (2015). Ultrasonic frogs call at a higher pitch in noisier ambience. *Current Zoology*, *61*(6), 996-1003.

Zhao, L., Sun, X., Chen, Q., Yang, Y., Wang, J., Ran, J., Brauth, S. E., Tang, Y., & Cui, J. (2018). Males increase call frequency, not intensity, in response to noise, revealing no Lombard effect in the little torrent frog. *Ecology and Evolution*, *8*(23), 11733-11741.

Ziegler, L., Arim, M., & Bozinovic, F. (2016). Intraspecific scaling in frog calls: The interplay of temperature, body size and metabolic condition. *Oecologia*, *181*, 673-681.

Abbreviations:

PulDur_0	Pulse duration between 0% amplitude marks
PulDur_10	Pulse duration between 10% amplitude marks
PulDur_50	Pulse duration between 50% amplitude marks
PulDur_90	Pulse duration between 90% amplitude marks (sustain)
PulOn_90	Pulse time for onset to 90% (attack)
PulOn_peak	Pulse time for onset to peak (rise)
PulOff_peak	Pulse time for peak to offset (fall)
PulOff_90	Pulse time for 90% to offset (decay)
PulShapeOn	Pulse shape (10:50% onset/10:90% onset)
PulShapeOff	Pulse shape (50:10% offset/90:10% offset)
RelPulsePeak	Pulse maximum amplitude relative to call maximum amplitude
PulDuty	Pulse duration/period (Duty cycle)
Crest Factor	Pulse peak/rms
Ener-0-10-Beg	Energy between initial 0:10% peak amplitude
Ener-10-50-Beg	Energy between initial 10:50% peak amplitude
Ener-50-90-Beg	Energy between initial 50:90% peak amplitude
Ener-90-Peak-Beg	Energy between initial 90%: Peak amplitude
Ener-Peak-90-End	Energy between final peak: 90% amplitude
Ener-90-50-End	Energy between final 90:50% peak amplitude

Ener-50-10-End	Energy between final 50:10% peak amplitude
Ener-10-0-End	Energy between final 10:0% peak amplitude
PulseDomFreq	Dominant frequency of the pulse
PulseFundFreq	Fundamental frequency of the pulse
PulseMinFreq	Minimum of dominant frequency in the pulse
PulseMaxFreq	Maximum of dominant frequency in the pulse
PulseOnFreq	Onset pulse dominant frequency
PulseOffFreq	Offset pulse dominant frequency
PulseHalfFM	Prop of duration to reach half frequency modulation
Tuning-6dBSPL	Tuning: peak freq/bandwidth at 50% peak amplitude (Q-20dBSPL)
Tuning-6dBSPL	Tuning: peak freq/bandwidth at 10% peak amplitude (Q-20dBSPL)
relAmpl-H1	Relative amplitude of harmonic 1
relAmpl-H3	Relative amplitude of harmonic 3
SVL	Snout Vent Length
DFA	Discriminant Function Analysis
UPGMA	Unweighted Pair Group Method with Arithmetic mean
P. hosii	*Philautus hosii*
C. raniceps	*Chalcorana raniceps*
MVSP	MultiVariate Statistical Package
Pa	Amplitude
s	Second
NR	Note Repetition rate
CF	Relative pulse peak
CG	Pulse duration at 0%
CH	Pulse duration at 10%
CI	Pulse duration at 50%
CJ	Pulse duration at 90%
CK	Pulse onset at 90%
CL	Pulse onset at peak
CM	Pulse offset at peak
CN	Pulse offset at 90%
CO	Pulse shape on
CP	Pulse shape off
CS	Pulse duty
CT	Crest factor
CV	Energy 0-10% begin
CW	Energy 10-50% begin
CX	Energy 50-90% begin
CY	Energy 90% to peak begin
CZ	Energy peak to 90% end

DA	Energy 90-50% end
DB	Energy 50-10% end
DC	Energy 10-0% end
DD	Pulse dominant frequency
DE	Pulse fundamental frequency
DF	Pulse minimum frequency
DG	Pulse maximum frequency
DH	Pulse on frequency
DK	Relative amplitude-Harmonic 1
DN	Relative amplitude-Harmonic 3
CI	Consistency Index
RI	Retention Index
kHz	kilohertz
H. cavitympanum	*Huia cavitympanum*
NP	National Park
PMB	Picture Motion Browser
PCA	Principal Component Analysis
SPSS	Statistical Package for Social Science
ANOVA	Analysis of Variance
P. baramica	*Pulcharana baramica*
A. luctuosa	*Abavorana luctuosa*
P. picturata	*Pulcharana picturata*
P. signata	*Pulcharan signata*
P. glandulosa	*Pulcharana glandulosa*
M. phaeomerus	*Meristogenys phaeomerus*
mm	millimetre