Vocalizations of *Microhyla achatina* Tschudi, 1838 (Anura: Microhylidae) from the foot hills of Mount Salak, West Java
[Vokalisasi Kodok *Microhyla achatina* Tschudi, 1838 (Anura: Microhylidae) Asal Kaki Gunung Salak, Jawa Barat]

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ABSTRACT

Vocalizations of *Microhyla achatina* have never been described before. The advertisement calls of six individual males of *M. achatina* which originated from the foot hills of Mount Salak, West Java were recorded in September 2011 at air temperatures of 21.0°C-23.4°C. Call components were obtained from 95 calls, consisting of 855 pulses, which were then analyzed to obtain the characteristics of sound waves by using software of Adobe Audition 3.0 and SAP2011. Sound waves of *M. achatina* mainly consists of impulses whose sound spectrum ranges from 1327.5-2789.1 Hz, while the band width of the spectrum is 1461.6 Hz. Results of the analysis showed that the frequency of the three pulse-forming elements (dominant frequency, maximum frequency and minimum frequency) was markedly modulated; frequency modulation was clearly visible in the minimum frequency, which was modulared by 1500 to 2700 Hz modulation. The modulation of the dominant frequency and the maximum frequency was not too broad, i.e. between 3000-3500 Hz. Results of linear regression analysis of the dominant frequency versus minimum frequency and dominant frequency versus maximum frequency showed a strong correlation between the dominant frequency versus minimum frequency, but a weak correlation between the dominant frequency versus the maximum frequency.

Keywords: vocalization, *Microhyla achatina*, West Java.

ABSTRAK

Vokalisasi jenis kodok *M. achatina* belum pernah dideskripsikan sebelumnya. Suara dari enam individu jantan *M. achatina* asal kaki Gunung Salak, Jawa Barat direkam pada bulan September 2011 pada suhu udara 21,0°C-23,4°C. Komponen suara yang didapat terdiri dari 95 suara panggilan dan 855 *pulse*, yang kemudian dianalisa untuk mendapatkan ciri gelombang suaranya dengan menggunakan perangkat lunak *Adobe Audition* 3.0 dan SAP2011. Gelombang suara jantan *M. achatina* bertipe *impulse* dengan spektrum suara panggilan berkisar dari 1327,5-2789,1 Hz, sedangkan lebar spektrum adalah 1461,6 Hz. Hasil analisis gelombang suara memperlihatkan frekuensi dari tiga unsur pembentuk *pulse* (frekuensi dominan, frekuensi maksimum dan frekuensi minimum) terdapat modulasi; modulasi ini terlihat jelas pada frekuensi minimum, yang mana modulasi ini pada kisaran 1500-2700 Hz. Modulasi pada frekuensi dominan dan frekuensi maksimum tidak terlalu besar, yaitu antara 3000-3500 Hz. Hasil analisis regresi linier dari parameter frekuensi dominan dengan frekuensi minimum dan frekuensi dominan dengan frekuensi maksimum memperlihatkan korelasi yang kuat antara frekuensi dominan dengan frekuensi minimum; sedangkan korelasi lemah antara frekuensi dominan dengan frekuensi maksimum.

Kata kunci: vokalisasi, *Microhyla achatina*, Jawa Barat

INTRODUCTION

The Javan Chorus Frog, *Microhyla achatina* Tschudi, 1838 (Figure 1) is not a forest frog; it is generally found in habitats that have been disturbed or in open habitats that are dominated by herbaceous plants, such as rice fields, fish ponds or open grassy marshland (Kurniati & Sumadijaya 2012). Vertical distribution of the frog is quite broad, ranging from 0 meter

(Kurniati *et al.* 2000) up to 1500 meters above sea level (asl) (Kurniati 2006; Liem 1973). Distribution of *M. achatina* is quite wide, includes Java (Iskandar 1998), Bali (McKay 2006), and also found in the southern tip of Sumatra (Iskandar & Mumpuni 2004).

The Javan Chorus Frog is a small frog species with the body length (SVL) of the adult male ranging between 20-22 mm (Kurniati 2006) in which the SVL is not much different from the adult male of *M. malang* (former name *M. borneensis*), which is between 18-22 mm (Matsui 2011; Dehling 2010). Generally, males of *M. achatina* call in a dense chorus; usually they release their advertisement calls in a rice field, along a permanent or impermanent pool side or at the edge of the ditch overgrown with grassy vegetation.

Vocalizations of some species of Family Microhylidae which occur in Asia have been described by some authors, included *M. heymonsi* by Heyer (1971), *M. nepenticola* by Das & Haas (2010) who later Matsui (2011) revised this species as a synonym of *M. borneensis*, *M. ornata* by Kuramoto & Joshy (2006), *M. orientalis* by Matsui *et al.* (2013), *M. malang* and *M. petrigena* by Dehling (2010) and *Micryleta innornata* by Wang *et al.* (1989). Although individuals of *M. achatina* are very easily to find in Java, but the vocalizations of this species has never been described.

METHODS

Advertisement calls of six individual males of *M. achatina* were recorded in Curug Nangka area (S 6°40'22.8"; E 106°43'53.5"; 730 m asl), at the foot hills of Mount Salak, West Java. Time of recording was on 22-24 September 2011 (air temperature 21.0° C-23.4°C) by using a recorder Sony PCM-M10 at frequency 94 kHz at a rate of 24 bits.. Adobe Audition 3.0 software was used to describe the oscillogram and audiospectrogram of the calls; while SAP2011 software was used to calculate parameters of sound waves, included the maximum and minimum frequency, dominant frequency, average frequency, amplitude, amplitude modulation (AM) and frequency modulation (FM). All data was tested by SPSS version 16.0. Correlation and linear regression analysis was used to test every parameter that produced wave characters. Linear regression analysis was used to explain the weak correlation when the R^2 value was low (e.g. $R^2 \leq 0.10$) even if $P \leq 0.01$. Standard deviation (SD) was used (except for the coefficient of Variance=CV) to show deviation around the average value. Data with a highly skewed distribution, median and range were used instead of the average value and standard deviation (Howard & Young 1998). Ratio of the Coefficient of Variance (CV) were calculated to determine the "static" and "dynamic" of vocalizations; ratio CV ≥ 12% indicates a call discrimination among individuals within a species (Gerhardt 1991).

Methodology to measure time or duration of the calls, such as call duration, call interval, pulse duration, pulse interval, pulse period

Figure 1. *Microhyla achatina* that was found in the foot hill of Mount Salak, West Java (Photograph by A. Sumadijaya).

and frequency dominant followed Pettitt *et al*. (2012) as shown at Figure 2.

RESULTS

Males of *M. achatina* only have one type of advertisement call, in which the type of wave consists of impulses (see Figure 3). In these impulses, the number of pulse periods ranges from 7 -10 periods, and the impulse duration reaching 4-8 ms (mean: 5.5 ms ± 1.2). Pulse repetition rate/ second was 16.9 to 30.3 (mean: 21.6 ± 2.6). One advertisement call (Figure 4) consisted of 6-11 pulses (mean: 8.5 ± 0.8), which for the duration of the call was 259-508 ms (mean: 375ms ± 42.4). Call repetition rate/second was 0.5 to 2.1 (mean: 1.1 ± 0.4).

The sound spectrum of *M. achatina* ranges from 1327.5 to 2789.1 Hz, while the bandwidth of the sound spectrum is 1461.6 Hz. The bandwidth of the spectrum of *M. achatina*'s advertisement call is not much different from the bandwith of the spectrum of *M. malang*'s advertisement call, occurring in Sarawak, North Borneo, which is about 1500 Hz (Dehling 2010). Dominant frequency or the maximum energy of *M. achatina*'s advertisement call ranges between 2718-3375 Hz (mean: 2976 Hz ± 143.1).

Based on the analysis of sound waves by using the software SAP2011, the frequency of the three elements of pulse (dominant frequency, maximum frequency and minimum frequency) of *M. achatina* was modulated (see Figure 6); modulation was clearly visible in the minimum frequency, which is the frequency at the beginning and end of the pulse is lower than the frequencies of pulses in the middle range. Frequency spectrum of modulation of minimum frequency were between 1500 to 2700 Hz. Frequency spectrum of modulation of the dominant frequency and the maximum frequency was narrow, i.e. between 3000-3500 Hz. Sound waves of *M. achatina* are similar to the sound waves of *M. malang* that was described by Dehling (2010), in which amplitude on the first and last pulse of *M. malang* was lower than the amplitude at the middle range, but

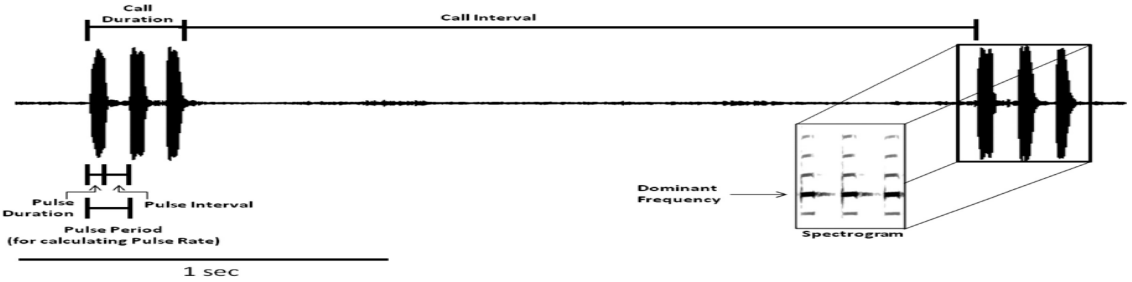

Figure 2. Duration of the sound wave measurements (an example is the call on frogs *Anomaloglossus bebeei*) by Pettitt *et al.* (2012).

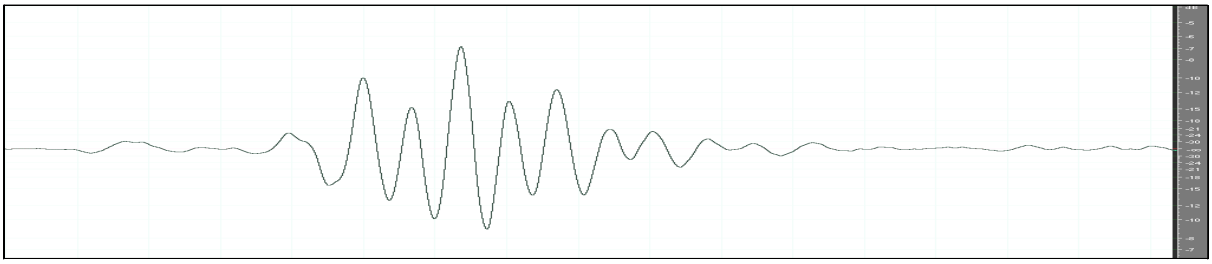

Figure 3. Waves of one pulse which has seven periods of advertisement call of *M. achatina* from West Java.

Dehling (2010) did not give detailed explanation on the modulation, because it was only based on the dominant frequency that was found by FFT method. Dynamics of dominant frequency, maximum frequency and minimum frequency of 95 calls (855 pulses) on the six individual males of *M. achatina* is shown at Figure 7.

Results of linear regression analysis (Figure 8) of the dominant frequency parameter versus minimum frequency and the dominant frequency versus maximum frequency of 855 pulses on the six individual males of *M. achatina* showed a strong correlation between the dominant frequency versus minimum frequency (Y=0.8178X-387,4; R^2=0.557; p=0.000), while a weak correlation exists between the dominant frequency versus maximum frequency (Y=0.4637X-2234,3; R^2=0.3914; p=0.89). These results indicate that the dynamics of the dominant frequency of *M. achatina* is strongly related to the dynamics of the minimum frequency, which it was also supported by the results of CV ratio value of the dominant frequency of 18% and the minimum frequency of 28%, but it was not supported by the maximum frequency of 11% (Table 1). According to Gerhardt (1991), vocalizations are "dynamic" when CV ratio ≥ 12%. and "static" when CV ratio <12%. Static parameters are call variables with low values of are more likely to be used for species recognition, because they remain

Table 1. Mean value, SD and CV ratio of the parameter dominant frequency, minimum frequency and maximum frequency of 95 calls (855 pulse) on six individual males of *M. achatina*.

Parameters	Dominant Frequency	Minimum Frequency	Maximum Frequency
Mean value	3069.99 Hz	2123.29 Hz	3657.81 Hz
Standard Deviation	541.38	593.24	401.24
Ratio of Coefisien Variant (CV)	0.18	0.28	0.11

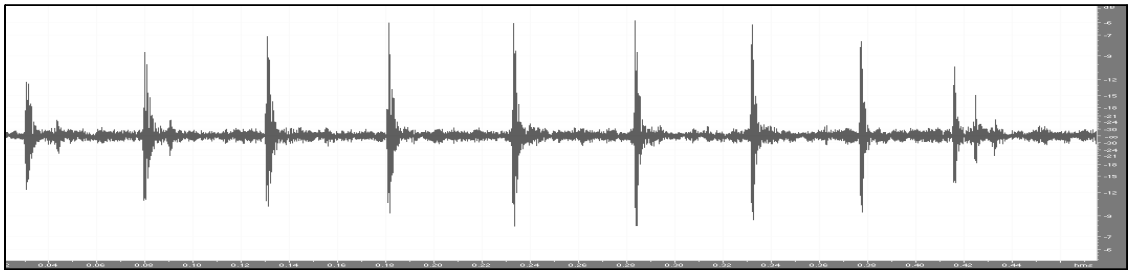

Figure 4. Oscillogram of an advertisement call that has nine pulses of *M. achatina* from West Java.

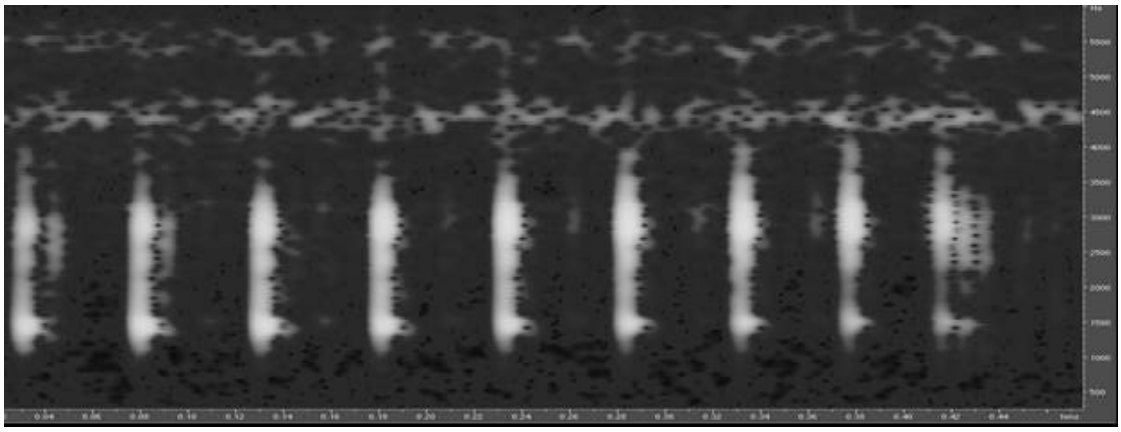

Figure 5. Audiospectrogram or sound spectrum of advertisement call of *M. achatina* from West Java.

fairly constant among individuals of a population, at least within a calling session; however dynamic parameters are more likely to be used in sexual selection within a single species (Marquez & Eekhout 2006).

DISCUSSIONS

Audiospectrograms of advertisement calls of *M. achatina* is similar to the audiospectrogram of some species of *Microhyla* that occur in Asia

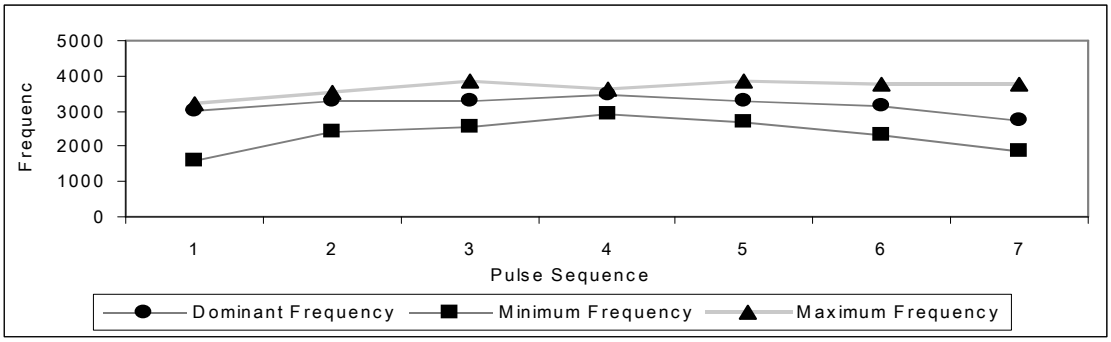

Figure 6. Result of analysis software SAP2011: dominant frequency, minimum frequency and the maximum frequency of one advertisement call of *M. achatina* that consisted of seven pulses.

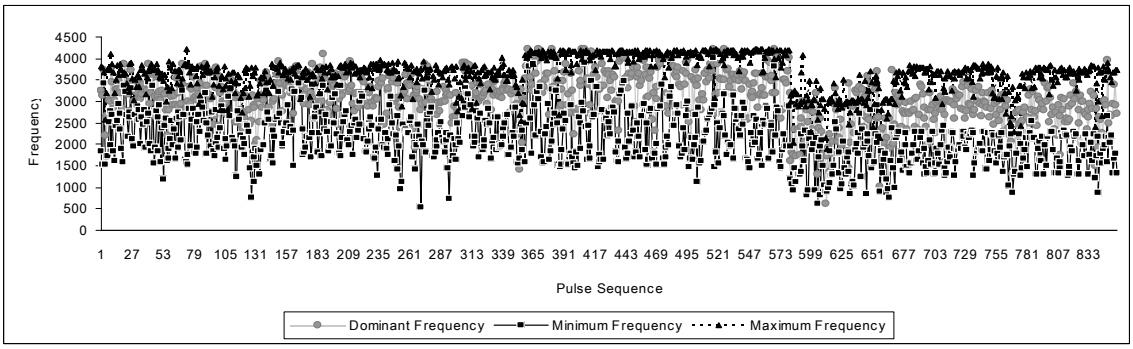

Figure 7. Dynamics of the dominant frequency, minimum frequency and maximum frequency of 855 pulses of 95 advertisement calls on six individual males of *M. achatina*.

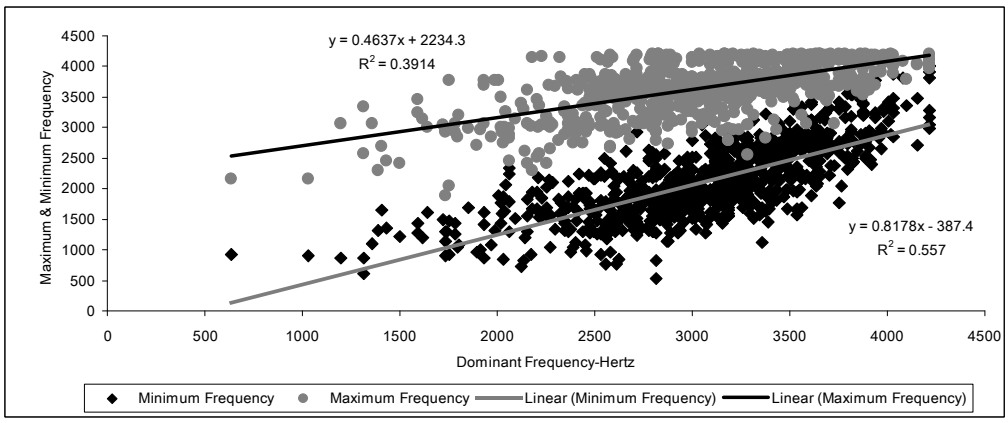

Figure 8. Results of linear regression of the dominant frequency versus the minimum frequency and the maximum frequency of 95 advertisement calls on six individual male of *M. achatina* consisting 855 pulses.

including Southeast Asia, such as *M. borneensis* (Sukumaran *et al*. 2010; Dehling 2010) which was later revised to a new species, namely *M. malang* (Matsui 2011), *M. petrigena* by Dehling (2010), *M. heymonsi* by Heyer (1971) and *M. orientalis* by Matsui *et al*. (2013). Calling behavior of the males *M. achatina* and its relatives is almost the same; they like to call in a dense chorus (see Table 3), except for *M. petrigena* (Dehling 2010) and *Micryleta innornata* (Wang *et al*. 1989); although Heyer (1971) did not describe the calling behavior of *M. heymonsi*, but individual males of this frog that were found in Sumatra also call in a dense chorus (personal observation). Advertisement call of *M. achatina* consists of a series of pulses also belongs to relatives that are listed in Table 3, except for *M. innornata* (Wang *et al*. 1989) and *M. borneensis* (synonym of *M. nepenticola*) (Das & Haas 2010). At the first time of analysis by using FFT method of Adobe Audition software, advertisement calls of *M. achatina* did not have any signs of on frequency modulation; but after using the software SAP2011, the frequency modulation can be detected in the calls (Figure 6 and 7). It is most likely that frequency modulation will be visible on the other species that are listed at Table 3 when the software SAP2011 can be implemented on analyzing of their calls.

Based on comparison of genetic distances by Matsui *et al*. (2013), the genetic distance between *M. achatina* and *M. innornata* is 17.2%, it is the farthest distance among the seven species with *M. achatina* (see Table 2). Differences in genetic distance can be seen from the pattern of the sound waves of the two species; sound wave pattern of *M. achatina* are impulses with a wide spectrum, however the sound wave pattern of *M. innornata* is a pure tone with three harmonics (see Table 3). Genetic distance between *M. achatina* and *M. petrigena* is quite far, i.e. 15.8% (see Table 2). The two species have similar call pattern, which is a series of pulses, but the maximum energy and the dominant spectral frequencies on *M. petrigena* are higher than those belonging to *M. achatina*; the duration of the call and the call repetition rate are more faster on *M. petrigena* than those belonging to *M. achatina*; contradictory between *M. achatina* and *M. ornata* (genetic distance 12.1%), *M. ornata* has a higher call repetition rate and pulse rate than *M. achatina*, on the other side the dominant frequency spectrum of *M. ornata* is much more wide, i.e. 1000-4000 Hz (see Table 3).

According to Matsui *et al.* (2013), *M. achatina* can be included into one genetic group with *M. orientalis*, *M. borneensis*, *M. malang* and *M. heymonsi*. In terms of the type of sound wave (Figure 9), advertisement calls of *M. achatina* are more similar to advertisement calls of *M. malang* which has a genetic distance between the two species of 9.7%; however genetic distance between *M. achatina* and *M. orientalis* and between *M. achatina* and *M. borneensis* is 7.3% and 8.2% re-

Table 2. Comparison of genetic distance (in %) of *M. achatina* with its relatives that are found in Asia by using 16S rRNA fragments (892 bp), based on Matsui *et al*. (2013).

Species	*Microhyla borneensis*	*Microhyla heymonsi*	*Microhyla malang*	*Microhyla ornata*	*Microhyla orientalis*	*Microhyla petrigena*	*Micryleta innornata*
Microhyla achatina	8.2	10.7	9.7	12.1	7.3	15.8	17.2

Table. 3. Comparison of vocalization on *M. achatina* from West Java with its relatives in the Family Microhylidae that are found in Asia.

Species	SVL of males	Air temperature during recorded	Call repetition rate/second	call duration (ms)	Number of pulse/call	Pulse length (ms)	Pulse repetition rate/second	Dominant frequency spectrum (Hz)	Energy maximum (Hz)	Vocalization type	Remark	Source
Microhyla achatina (West Java)	20-22 mm	21.0°C-23.4°C	1.1 ± 0.4 (0.5–2.1)	375 ± 42.4 (259–508)	8.5 ± 0.8 (6–11)	5.5 ± 1.2 (4-8)	21.6 ± 2.6 (16.9–30.3)	1327.5-2789.1	2976 ± 143.1 (2718–3375)	1. single pulsed note and is emitted in series 2. No marked frequency modulation within and between the single pulses 3. The pulses in the middle of the note had a greater amplitude than the ones at the beginning and the end	-Males called in a dense chorus -A total number of 95 calls that consis-ted of 855 pulses were analyzed.	This study
Microhyla heymonsi (Thailand)	20.0-21.5 mm (Berry 1975)	28.0°C	-	480	11	-	23	1700-3000	-	1. single pulsed note and is emitted in series 2. No marked frequency modulation within and between the single pulses	-	Heyer (1971)
Microhyla nepenticola (synonim= *M. borneensis*) (Sarawak, Borneo)	10.6–12.8 mm	-	-	696–736	1-2 and 5-9	-	-	3000–5500	-	-	-Males called in a dense chorus	Das & Haas (2010)
Microhyla ornata (Bajipe, India)	22.1-24.9	25.5°C	1-1.5	290 ± 21	12.1 ± 0.64	26 ± 1	38.1 ± 1.09	1000-4000	-	1. A series of pulse group (notes) each consisting of short and fast repeating pulse 2. No marked frequency modulation; the call did not show a clear harmonic structure, but had a wide range (ca. 1-4 kHz) of dominant frequencies, which become narrower (ca. 2-3 kHz) toward later pulses.	-Males called from banks adjacent to water or from a floating posture in water. -A total number of 13 calls were analyzed.	Kuramoto & Joshy (2006)

Table. 3. Continue

Microhyla orientalis (Bali)	16–17 mm	26.0°C	0.07 ± 0.01 (0.01–0.08)	540 ± 130 (370–970)	4.1 ± 0.7 (3–5)	-	-	1000–5300	3400 ± 100 (3200–3600).	1. single pulsed note and is emitted in series 2. No marked frequency modulation within and between the single pulses 3. The pulses in the middle of the note had a greater amplitude than the ones at the beginning and the end	-Males called in a dense chorus -A total number of 33 calls were analyzed.	Matsui *et al*. (2013)
Microhyla petrigena (Sarawak, Borneo)	14-16 mm	24.1°C	0.66 ± 0.08 (0.52–0.83)	133 ± 28 (69–174)	12.1 ± 3.0 (6–17)	-	89 ± 5 (80–100)	3800-4600 to 4500–5100	4430 ± 322 (3850–5050)	1. single pulsed note and is emitted in series 2. No marked frequency modulation within and between the single pulses	-	Dehling, (2010)
Microhyla malang (Sarawak, Borneo)	17–21 mm	23.5–24.7°C	1.2 ± 0.1 (1.1–1.4)	169 ± 37 (104–242)	5.7 ± 1.1 (4–8)	6.7 ± 0.5 (6–7)	30.3 ± 1.4 (27.7–32.2)	1400-2900	2404 ± 94 (2250–2530)	1. Single pulsed note and is emitted in series 2. No marked frequency modulation within and between the single pulses 3. The pulses in the middle of the note had a greater amplitude than the ones at the beginning and the end	-Males called in a dense chorus -A total number of 76 calls were analyzed.	Dehling, (2010)
Microhyla borneensis (synonim= *M. malang)* (Sarawak, Borneo)	17-18 mm (Inger & Stuebing 2005)	-	-	-	04-Okt	06-Agust	-	-	2430	-	-	Sukumaran *et al.* (2010)
Microhyla innornata (synonim= *Micryleta innornata*) (Southern tip of Taiwan)	23.0 ± 1.14 mm	25.0°C	-	1390 ± 270 (1110-1920)	82.6 ± 1.5 (67-113)	-	59.4 ± 2.5 (56-65)	4400-5700	-	1. Two harmonics: the second and third harmonic was apparent at 8.8-10.0 and 14.0-15.0 kHz. 2. Modulation of frequencies is lacking. The dominant and the second harmonic were slightly lower at the very beginning. The third harmo-nic was weak in the proximal two thirds and strengthened in the terminal third, but was too weak to be detected in so	A total number of 10 calls were analyzed.	Wang et al. (1989)

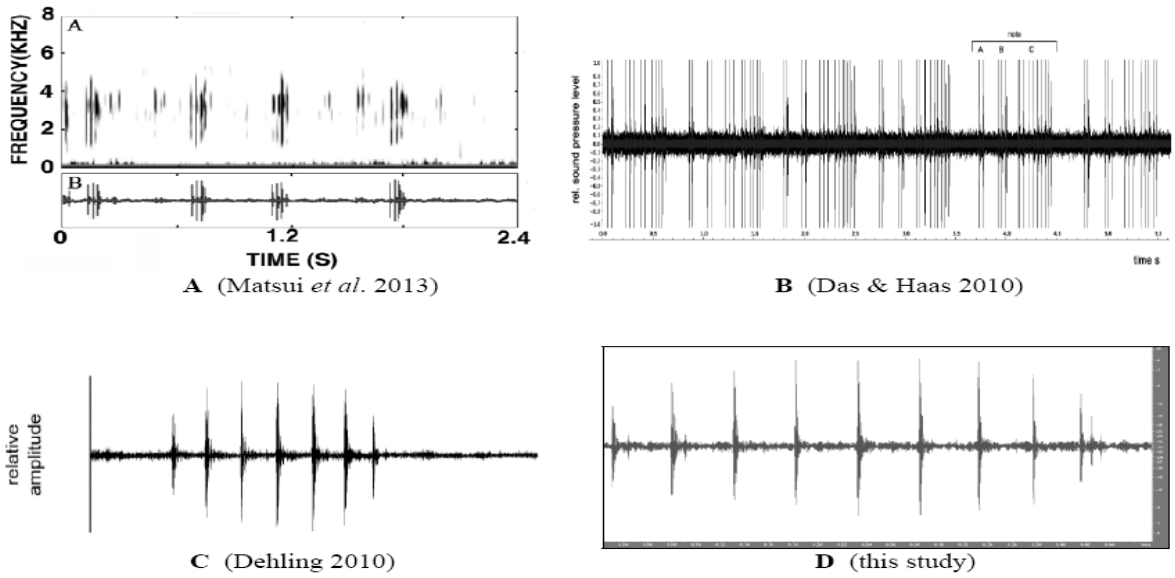

Figure 9. Oscillogram of advertisement call: (A) *Microhyla orientalis*; (B) *Microhyla borneensis*; (C) *Microhyla malang*; (D) *Microhyla achatina*.

spectively (see Table 2), but the types of sound wave are quite different between *M. achatina* and *M. orientalis* and between *M. achatina* and *M. borneensis* (see Figure 9). There is also overlap between the dominant frequency spectrum, pulse duration, call duration as well as call rate between *M. achatina* and *M. malang*; however overlap value of the parameters between *M. achatina* and *M. orientalis* and between *M. achatina* and *M. borneensis* is very low (see Table 3). The main reason of vocalization differences between *M. achatina* and *M. orientalis* and between *M. achatina* and *M. borneensis* is on the body size; body size of *M. orientalis* and *M. borneensis* is smaller than body size of *M. achatina* (Table 3). Generally, frog species that has large body size will have calling frequencies lower than the frequency of the call of frog species which has small body size (Kime *et al.* 2000).

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