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Bioacoustic analysis of advertisement calls of two ground-frogs of the genus *Platymantis* in Mount Magdiwata, San Francisco, Agusan del Sur, Philippines

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This study described the advertisement calls of two ground-dwelling species of frogs of the genus *Platymantis* from Mount Magdiwata, San Francisco, Agusan del Sur, Philippines. The two species shared a common micro-habitat preference being found on rocks or edges of slope ground, leaf litter, and on steep slopes of stream banks. The researchers analyzed the calls for their temporal and spectral characteristics. Audiospectrograms, oscillograms and power spectra presented the numerical parameters and graphical representations of the advertisement calls. The vocalization of both species revealed significant differences in terms of the dominant frequency, call duration, number of notes, note duration, and intercall at $p < 0.01$. Statistical analysis of *Platymantis magdiwata* species A showed a positive correlation between the body size length and note duration ($R^2 = 0.377$, $p < 0.01$). On the other hand, the body size length of *Platymantis magdiwata* species B showed a negative correlation with the dominant frequency ($R^2 = -0.248$; $p < 0.01$), and a positive correlation with the call duration and the number of notes ($R^2 = 0.698$ and $R^2 = 0.699$; $p < 0.01$). Both species possess unique advertisement calls that distinguish them from each other.

Key words: Bioacoustics, advertisement call, *Platymantis*, Philippines.

INTRODUCTION

Frogs of the genus *Platymantis* are noted for their unusual geographic distribution (Brown, 1997a), micro-habitat diversity, and their advertisement calls (Brown et al., 1997a, 1999) that represent a wide range of ecological, morphological and behavioral variations (Brown et al., 2002).

In the Philippine archipelago, current status in the

number of species of this genus increases to 27 species of *Platymantis* (Brown and Gonzales, 2007) from 26 species and dozens of newly discovered species that are still awaiting for description (Diesmos et al., 2002). The recognition of these species groups of *Platymantis* is based on conspicuous characteristics of external morphology and ecology (Brown et al., 1997a, 1997b, 1999). To some extent, advertisement call is also used in the identification of the *Platymantis* species (Alcala et al., 1998; Alcala and Brown, 1999; Brown et al., 1997a). Because bioacoustic study is not well established in the country, a group of herpetologists began to work on the study with the use of bioacoustic characters to solve the

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problem of species boundaries of this genus. The description of new individuals of *Platymantis* frogs will soon increase the number of Philippine amphibians that will eventually increase its species richness.

One interesting behavior of anuran species is the acoustic signals or calls they emit which exhibit acoustical differences based on its temporal and spectral characteristics between and among species (Gerhardt, 1982; Kelly, 2004; Marquez et al., 2005). These differences are useful for call recognition of the species in a population (Hall, 1994; Wells, 1977). An emerging tool currently used by many herpetologists to describe amphibian species is bioacoustic analysis of advertisement calls (Roy and Elepfandt, 1993; Parris, 1999; Hsu et al., 2005). This type of analysis could provide substantial information, more robust than traditional morphological analysis (Schneider and Sinsch, 1992; Schneider et al., 1993) bringing forth an important character; a specific call for each species (males and females) that could act as an isolating mechanism that could distinguish one species from the other (Roy, 1994; Asquith et al., 1988; Marquez and Eekhout, 2006; Taylor et al., 2007). Aside from this, a collection of anuran vocalizations is helpful in studying population ecology and distribution of many frog species (Bosch and De la Riva, 2004), and of great contribution on conservation (Laiolo, 2010).

The description of the two *Platymantis* magdiwata species is underway and is currently being finalized through a monograph of Philippine *Platymantis* by Dr. Rafe Brown and Dr. Arvin C. Diesmos (Diesmos and Brown, pers. comm.). Only the advertisement calls of the two species investigating their spectral and temporal structures are described in this study. The advertisement calls of *Platymantis* magdiwata species A and *Platymantis* magdiwata species B are markedly different. Comparison of these two acoustically described *Platymantis* magdiwata species will be made to all presumably related species of *Platymantis* in the Philippines. Current understanding of *Platymantis* species diversity throughout this topographically and geographically complex archipelago (Brown and Gonzales, 2007) is underestimated and in need of comprehensive review.

This study provides description and analysis of the advertisement calls of *Platymantis* magdiwata species A and *Platymantis* magdiwata species B in Mount (Mt.) Magdiwata, San Francisco, Agusan del Sur, Philippines. Temporal and spectral characters of the advertisement calls are described with the following parameters: call duration, intercall, and note per call, note duration, internote, call period, and the dominant frequency. Comparisons of these characters are made between the two species. Both are occupying the same microhabitat as ground-dweller individuals, which influences the specific call recognition of each species and their coexistence in the same environment. Likewise, the body size length (Snout-Vent Length) is correlated with the dominant

frequency, call duration, number of notes, and note duration. This body size of anurans constrains the dominant frequency and intensity of their vocalizations.

MATERIALS AND METHODS

Study area and site description

The province of Agusan del Sur is located in the Caraga region in Mindanao, Philippines and its capital is the municipality of Prosperidad. Mt. Magdiwata is located in the municipality of San Francisco, province of Agusan del Sur (Figure 1). This mountain stands 633 m high and has an area of 1,658 ha which contains forest covered areas rich in rare and unique species of flora and fauna (Department of Tourism, 2011). The sampling area was moderately diverse with emergent and canopy trees, canopy epiphytes and vines, understory and ground cover plants, and grasses and sedges (Figure 2). Tree taxa observed in the area were yemane (*Gmelina arborea*), binunga (*Macaranga tanarius*), hagimit (*Ficus minahassae*), balete (*Ficus balete*), coconut (*Cocos nucifera*) and other fruit density. Ground cover plants were composed of ferns, and mosses were moderately abundant on rocks and logs. Fallen logs, exposed rocks, leaf litter depth, and body of water such as stream were also observed.

Weather pattern particularly the amount of precipitation was noted during the sampling period. During October 28, 2008, precipitation was 0.19 inches which indicated a light rain shower between 5 to 9 pm. Sky during that day was 25% clear, cold and wet atmosphere in the morning. During October 29, 2008, precipitation was 0.63 inches which indicated light rain shower between 1 to 5 pm, and a moderate rain shower the rest of the night. On October 30, 2008, the precipitation was 0.20 inches. The sky was 25% clear throughout the day with small scattered rain shower between 2 to 3 pm. Moderate to light rain showers were observed at 10 pm to 5 am. On October 31, 2008, the precipitation was only 0.02 inches. The sky was 50% clear throughout the day. On November 1, 2008, the precipitation was 0.38 inches. The sky was 50% clear throughout the day, and has a moderate rain shower between 12 to 2 am. The relative humidity was 98%.

Data gathering and analysis

Call recordings were done every 1800 to 2300 h every night during the sampling period. Recording periods were conducted for duration of 20 to 25 min per sample for a total of eight individuals per species. Calls were recorded at a distance of 1.0 to 1.5 m and ambient, body, and substrate temperatures were taken immediately after acoustic recordings. Frog calls were recorded and the frog samples were captured by hand, measured (Snout-to-vent length (SVL) to the nearest 0.1 mm), weighed (to the nearest tenth of a gram (0.1 g), photographed, and processed to serve as voucher specimen.

Advertisement calls were recorded under field conditions with a solid state recorder PMD670 Marantz, with a unidirectional microphone. All calls were recorded within a temperature range of 0.5°C, so no temperature corrections of the data were undertaken during call analysis due to the narrow range of ambient temperatures recorded.

Audiospectrograms, oscillograms, and power spectra were generated to quantify the temporal and spectral features of each species (Marquez et al., 1993). The audiospectrogram was used to measure the frequency of the call over time. The oscillograms (waveform) represented the sound wave pressure over time which described the elements of the call. They were presented for the entire calls and for a 5.0 s section of the calls of each species to



Figure 1. Map of the Philippines showing the sampling area in San Francisco, Agusan del Sur, Philippines.



Figure 2. Sampling sites in Mt. Magdiwata, San Francisco, Agusan del Sur.



Figure 3. *Platymantis magdiwata* species A.

show the pulse structure. The bioacoustic analysis were performed using the program Adobe Audition 1.5 and the software Raven lite with a sampling rate of 44.100 kHz and 16 bits per sample in the mono pattern, in format "Windows PCM". Call duration was measured to the nearest 0.01 s on the waveform. Spectrogram views were produced with a Hann-filter with a sample size of 512. Frequency information was obtained through Fast Fourier Transform (FFT, frame width 1024 points). For analysis and interpretations the following terms were used:

Call duration: duration from the beginning of a call to its end measured in seconds (Roy and Elepfandt, 1993).

Intercall: refers to the time from end of a call to the beginning of the next call measured in seconds.

Notes per call group: the actual number of notes in a given call (Duellman and Pyles, 1983).

Note duration: duration from the beginning of a note to its end measured in seconds.

Internote: refers to the time from the end of a note to the beginning of the next note.

Call period: duration from the beginning of a call to the beginning of the next call (Roy and Elepfandt, 1993).

Fundamental frequency: the lowest frequency in the harmonic spectrum, measured in Hz on amplitude display section (Duellman and Pyles, 1983).

Dominant frequency: the emphasized harmonic in the frequency spectrum, measured in Hz on amplitude display section (Duellman and Pyles, 1983).

The mean, standard deviation, and range were taken to get the distribution of the spectral and temporal characteristics of the two *Platymantis* species. To test for the statistical differences between the sound parameters, a t-test for independent samples was conducted. The correlation between body size (SVL) with the dominant frequency, call duration, number of notes, and note duration were calculated using Pearson Product Moment Correlation (Pearson-r). For all these statistical tests, SPSS Version

17.0 was used.

RESULTS

Platymantis magdiwata species A

Platymantis magdiwata species A (Figure 3) was found on rocks or edges in slope ground and on steep stream bank. This species is moderate in size (SVL ranges 35.9 to 53.7 mm; n=23), grey brown body coloration with numerous tubules and ridges. The call of *Platymantis magdiwata* species A sounds to human ear in a brief, loud -tik-tik.....tik-tik.....tik-tik-, and primarily describes the species as "cliff-loud" because of its loud calls in a slope ground.

The characteristics of the advertisement call of *Platymantis magdiwata* species A are summarized in Table 1. Call duration ranged from 0.09 to 5.74 (mean= 2.2 ± 1.64 ; n=71) s. The call period and intercall vary from 26.86 to 313.16 (mean= 91.0 ± 48.01 ; n=71) s and from 26.76 to 310.60 (mean= 88.8 ± 47.37 ; n=71) s, respectively. The note duration was from 0.08 to 0.11 s (mean= 0.094 ± 0.006 ; n=71) s. The interval between notes ranged from 0.40 to 1.31 (mean= 0.5 ± 0.35 ; n=71) s.

The dominant frequency was from 2656.17 to 3000 (mean= 2833.9 ± 56.83 ; n=71) Hz. Calling activity began after sunset at 1800 h and continued until 2400 h. The advertisement call consisted of 1 to 7 notes per call group (Figure 4A). Advertisement calls of *Platymantis magdiwata* species A lasted up to 5 s. Males of this

Table 1. Characteristics of seven variables of the advertisement calls of the two species of *Platymantis* in Mt. Magdiwata, San Francisco, Agusan del Sur, Philippines. Data are expressed as mean \pm standard deviation, minimum-maximum

Variable	Number of notes	Call duration (s)	Call period (s)	Intercall (s)	Note duration (s)	Internote (s)	Dominant frequency (Hz)
<i>Platymantis magdiwata</i> species A (n=71)	4 \pm 1.71	2.2 \pm 1.64	91.0 \pm 48.01	88.8 \pm 47.37	0.094 \pm 0.006	0.5 \pm 0.35	2833.9 \pm 56.83
	1-7	0.09-5.74	26.86-313.16	26.76-310.60	0.08-0.11	0.40-1.31	2656.17-3000.0
<i>Platymantis magdiwata</i> species B (n=60)	25 \pm 15.9	15.5 \pm 9.62	68.5 \pm 59.95	51.4 \pm 52.08	0.08 \pm 0.006	0.62 \pm 0.1325	2728.8 \pm 333.01
	3-65	1.49-40.87	15.70-342.50	5.88-313.26	0.07-0.09	0.40-1.00	1593.44-3031.19

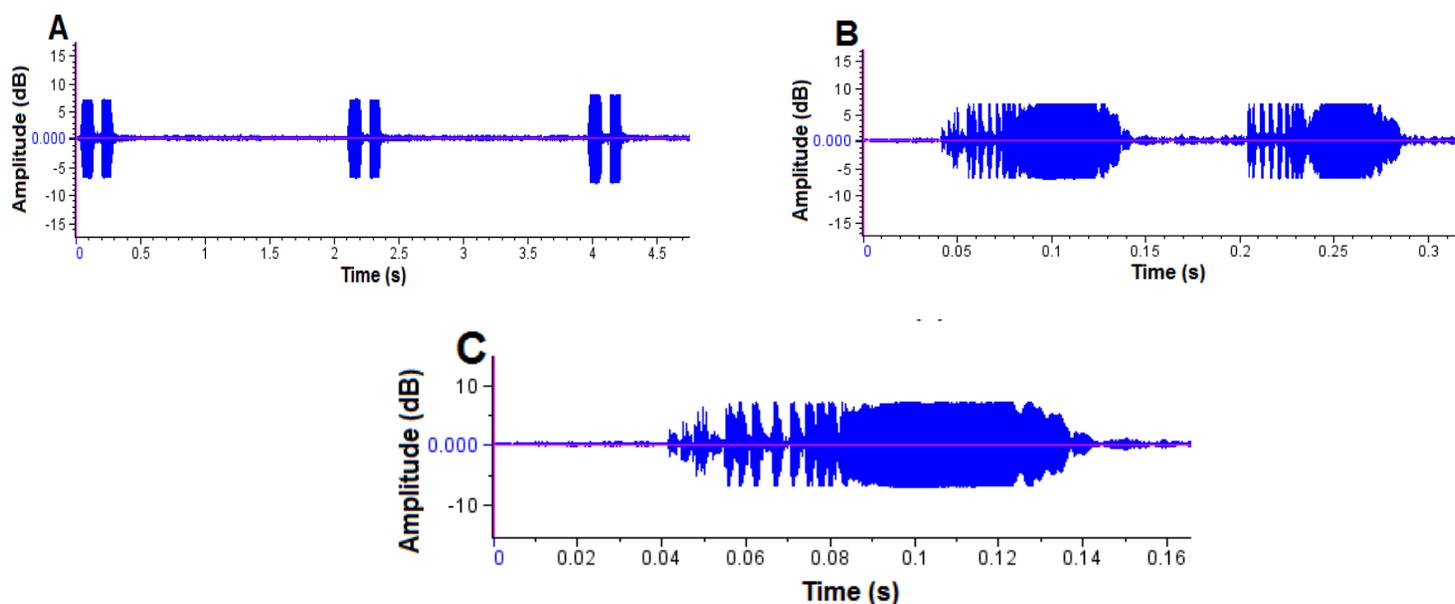


Figure 4. Call structure of *Platymantis magdiwata* species A. (A) Oscillogram (relative amplitude vs. time in seconds) with large time scale; (B) Selected piece of oscillogram shown at larger temporal resolution; (C) Single note of the call. (Temperatures: cloacal: 22.5-24°C, ambient: 20.0-21.5°C, substrate: 20.0-24.0°C).

species produced their vocalizations in pairs except for the single note call (Figure 4B). The call amplitude of the first pulse was very low (Figure

4C). The first pair consisted of two notes with shorter time interval followed by the next pair of notes with a delay of almost 2 s from the previous

notes depicted on audiospectrogram (Figure 5A). The fundamental frequency is at 2656 to 3000 Hz of the frequency spectrum bands (Figure 5B).

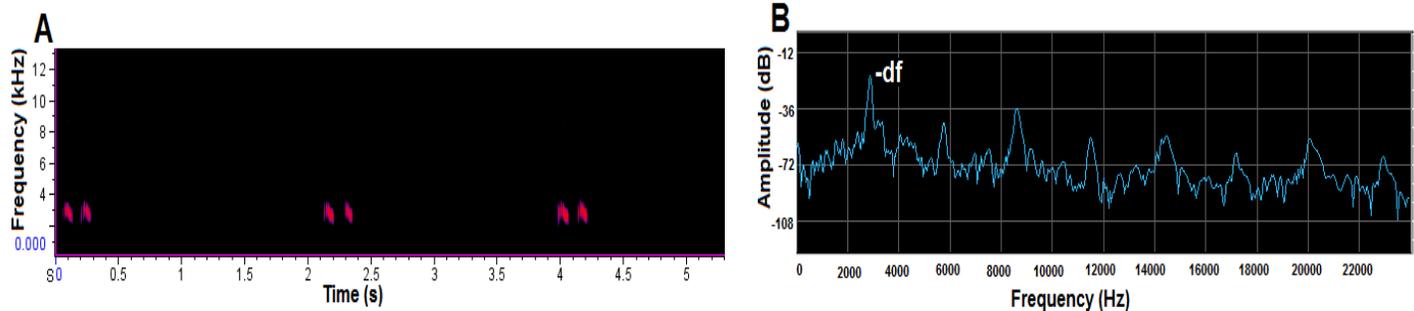


Figure 5. Call structure of *Platymantis magdiwata* species A. (A) The sonogram (audiospectrogram: relative frequency in Hz vs. time in s); (B) Power spectrum (Fast Fourier Transform, FFT), relative amplitude vs. frequency in Hz. (Temperatures: cloacal: 22.5-24°C, ambient: 20.0-21.5 °C, substrate: 20.0-24.0°C).



Figure 6. *Platymantis magdiwata* species B.

***Platymantis magdiwata* species B**

Platymantis magdiwata species B (Figure 6) was found on edges in sloping ground. Individuals of this species were found hiding under leaf litter. Snout-vent length ranges from 29.9 to 35.4 mm (n=13). Its body coloration is brown with irregular transverse bands on the limbs. The call of *Platymantis magdiwata* species B sounds to human ear in a long, successive “click..click..click..click..click..click.....” and primarily describes the species as “clicker”.

Call duration ranged from 1.49 to 40.87 (mean= 15.5 ± 9.62; n=60) s (Table 1). The call period and intercall vary from 15.70 to 342.50 (mean= 68.47 ± 59.95; n=60) s and 5.88 to 313.26 (mean= 51.4 ± 52.08; n=60) s, respectively. The note duration was from 0.07 to 0.09 s

(mean= 0.08 ± 0.006; n=60) s. The interval between notes ranged from 0.40 to 1.00 (mean= 0.62 ± 0.1325; n=60) seconds. The dominant frequency was from 1593.44 to 3031.19 (mean= 2728.8 ± 333.01; n=60) Hz (Table 1).

Oscillogram of the advertisement call of *Platymantis magdiwata* species B consisted of complex periodic trains of notes (Figure 7A). The waveform showed that the notes of *Platymantis magdiwata* species B consisted of two different groups of pulses (Figure 7B), depicted in the power spectra composed of two different frequency components. The pulses of a single note showed varying amplitude (Figure 7C). The amplitude of the first and the second note makes up the fundamental frequency. The note increased gradually until it reached its peak. This amplitude continued until the end of the call.

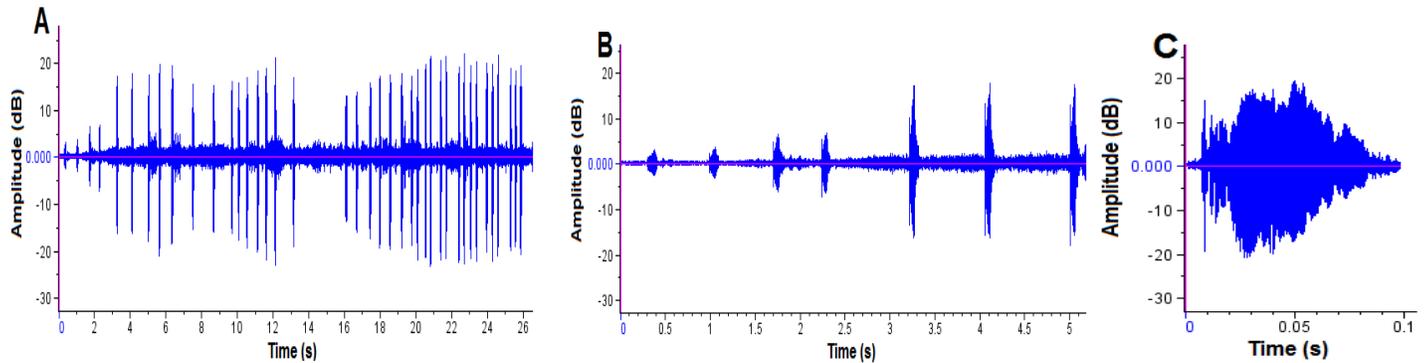


Figure 7. Call structure of *Platymantis magdiwata* species B. (A) Oscillogram (relative amplitude vs. time in seconds) with large time scale; (B) Selected piece of oscillogram shown at larger temporal resolution; (C) Single note of the call. (Temperatures: cloacal= 23.5-25°C, ambient: 21.0-21.5°C, substrate: 21.0-21.0°C).

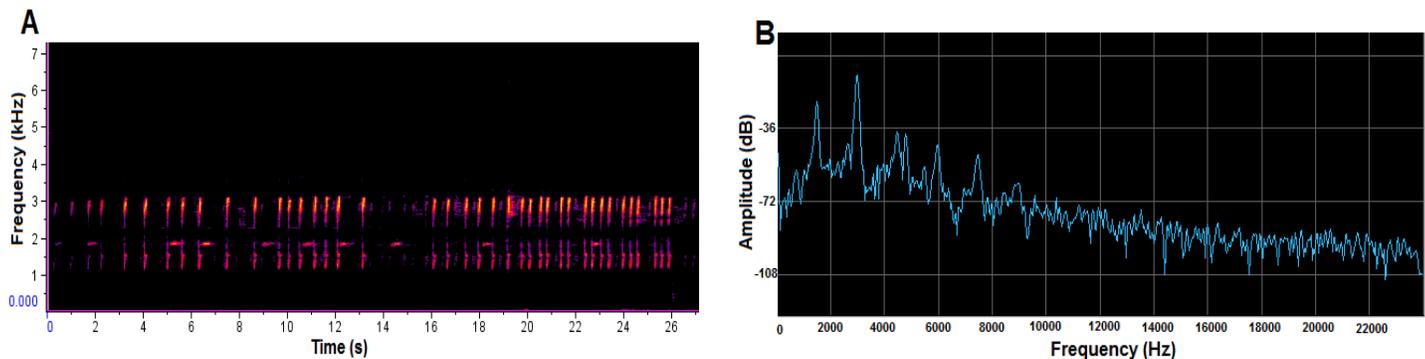


Figure 8. Call structure of *Platymantis magdiwata* species B. (A) The sonogram (audiospectrogram: relative frequency in Hz vs. time in s); (B) Power spectrum (Fast Fourier Transform, FFT), relative amplitude vs. frequency in Hz. (Temperatures: cloacal= 23.5-25°C, ambient: 21.0-21.5°C, substrate: 21.0-21.0°C).

The calls of *Platymantis magdiwata* species B have two distinct spectral components (Figure 8A). The audio-spectrogram demonstrated that the majority of energy in each note is divided between the fundamental frequency of 1300 to 1460 Hz and the dominant frequency of 2800 to 3100 Hz. The sound energy was concentrated in the dominant frequency. The emphasized dominant frequency shifted from the first to the second frequency component between 5 to 15% of the call duration.

The power spectra (Figure 8B) revealed that majority of the call is packed between the two frequency intervals, the first is the fundamental frequency and the second is the dominant frequency. The sound parameters were significantly different in the two species (Table 2) at $p < 0.01$.

Correlation of body size length (SVL) with dominant frequency, call duration, number of notes and note duration

Dominant frequency of the *Platymantis magdiwata*

species B is negatively correlated with the SVL, $R^2 = -0.248$ (Table 3) at $p < 0.01$. As the dominant frequency decreases, the SVL increases. Call duration and number of notes of the *Platymantis magdiwata* species B is positively correlated with the SVL ($R^2 = 0.698$ and $R^2 = 0.699$), at $p < 0.01$. Note duration of the *Platymantis magdiwata* species B has a low positive correlation with the SVL ($R^2 = 0.377$) at $p < 0.01$. The remaining ones are correlated but were found to be insignificant at $p < 0.05$. Data could not provide sufficient evidence to support such relationships.

DISCUSSION

The Philippine *Platymantis* species are grouped into three based on their morphological characters and habitat preferences; the *Platymantis dorsalis* (the litter frogs), *Platymantis guentheri* (the rain frogs) and *Platymantis hazelae* (the cloud frogs). The two species of *Platymantis* described herein combine the characteristics of the *P. dorsalis* group by having a terrestrial

Table 2. Statistical differences between *Platymantis magdiwata* species A and *Platymantis magdiwata* species B based on their seven acoustic parameters.

Parameters	<i>Platymantis magdiwata</i> species	Mean \pm standard deviation	t-value	Sig.
Call duration	Species A	2.2 \pm 1.64	11.341	p<0.001
	Species B	15.5 \pm 9.62		
Call period	Species A	91.0 \pm 48.01	2.271	0.025
	Species B	68.5 \pm 59.95		
Number of notes	Species A	4 \pm 1.71	10.875	p<0.001
	Species B	25 \pm 15.9		
Intercall	Species A	88.8 \pm 47.37	4.103	p<0.001
	Species B	51.4 \pm 52.08		
Note duration	Species A	0.094 \pm 0.006	12.453	p<0.001
	Species B	0.08 \pm 0.006		
Internote	Species A	0.5 \pm 0.35	1.591	0.014
	Species B	0.62 \pm 0.13		
Dominant frequency	Species A	2833.9 \pm 56.83	2.597	0.010
	Species B	2728.8 \pm 333.01		

Table 3. Correlation between body size length (SVL) and dominant frequency of *Platymantis magdiwata* species A and *Platymantis magdiwata* species B.

Variables	Type	SVL	
		R ²	P-value
Dominant frequency	<i>P. magdiwata</i> species A	- 0.100	0.408
	<i>P. magdiwata</i> species B	- 0.248	0.044
Call duration	<i>P. magdiwata</i> species A	- 0.168	0.168
	<i>P. magdiwata</i> species B	0.698	p<0.001
Number of notes	<i>P. magdiwata</i> species A	- 0.136	0.261
	<i>P. magdiwata</i> species B	0.699	p<0.001
Note duration	<i>P. magdiwata</i> species A	0.377	0.001
	<i>P. magdiwata</i> species B	0.139	0.248

microhabitat preference typically the ground-dweller frogs, and an amplitude-modulated advertisement call of the *P. guentheri* group (Brown et al., 1997a). Calls of *Platymantis magdiwata* species A may be compared to the relatively brief, complex calls of *P. dorsalis* group which has common characteristics of tonal frequency-modulated notes and vibrational amplitude-modulated calls. An example is the call of *P. spelaeus* which sounds to the human ear like the complex di-syllabic call of a small bird: "pee-coh, pee-coh....pee-coh, pee-coh". Other members of the *P. dorsalis* group (Table 4) that possess

two-note calls include *P. indeprensus*, *P. pseudodorsalis*, *P. cagayanensis*, *P. mimulus*, *P. naomiae* and several currently unnamed species awaiting formal taxonomic description (Brown et al., 1997b, 1997c, 1999).

Calls of *Platymantis magdiwata* species B may be compared to the longer, highly pulsed, amplitude modulated calls of *P. guentheri* species group (Table 4). But there are only three terrestrial frog species of the *P. guentheri* group in the Philippine islands; the first is *Platymantis insulatus* which is endemic to the islands of the Gigante group (Brown et al., 2002), the second is the newly

Table 4. Advertisement calls of the two groups of Philippine *Platymantis* species.

<i>Platymantis dorsalis</i> group		<i>Platymantis guentheri</i> group	
Species	Calls	Species	Calls
<i>Platymantis cagayanensis</i>	"Cree-eek...cree-eek."	<i>Platymantis cornuta</i>	"tutututututut..."
<i>Platymantis pseudodorsalis</i>	"Tseeo-lek...Tseeo-lek."	<i>Platymantis insulatus</i>	"Tik...tik...tik..tik-tik-tik,"
<i>Platymantis naomiae</i>	"psik...psik."	<i>Platymantis negrosensis</i>	"Kwek-kwek-kwek"
<i>Platymantis mimulus</i>	"osek...sek...sek."	<i>Platymantis luzonensis</i>	"Kwenk...kwenk...kwenk."
<i>Platymantis spelaea</i>	"Pee-coh, pee-coh... pee-coh, peecoh"	<i>Platymantis banahao</i>	"Tut-tut-tut-tut-tut-tut tut..."
<i>Platymantis indepressus</i>	"eeyak...eeyakeeyak....eeyak...eeyakeeyak."	<i>Platymantis rabori</i>	"Churenk...churenk"

The data above were taken from Brown (2004).

described species *Platymantis diesmosi* of the Bicol peninsula, Luzon Island (Brown and Gonzales, 2007), and *Platymantis bayani*, the third terrestrial member of the *P. guentheri* group from Eastern Samar Island (Siler et al., 2009). The three species groups of *Platymantis* that are recognized on their external morphology show morphological variation, as well as their advertisement calls and preferred microhabitats of the species (Brown et al., 2002; Brown and Gonzales, 2007).

One body of evidence that has been brought to bear on specific problems in platymantine frog taxonomy is acoustical analyses of male advertisement calls. Several recent studies (Brown et al., 1997b, 1999) have demonstrated the value of studying species-specific acoustic signals for elucidation of species boundaries and identification of cryptic species (Brown et al., 2002). Analysis of patterns of mate-recognition signals has been crucial in identifying numerous unrecognized, new species, especially in the *P. dorsalis* species group (Brown et al., 1997a, 1999). In fact, dozens of new species especially frogs of the genus *Platymantis* are currently in the process of being described (Diesmos et al., 2002).

On the other hand, in terms of the correlation of the body size (Snout-Vent Length) with the dominant frequency, call duration, number of notes, and note duration, the body size of the species determines the dominant frequency emitted by their calls. In a study made by Preininger et al. (2007), the sampling size was probably too low to produce the dependency of SVL on the dominant frequency. This may be the reason why the correlation between dominant frequency and call duration with the SVL of *Platymantis magdiwata* species A does not show significantly as well as the correlation between the note duration and SVL of *Platymantis magdiwata* species B. Another study by Hodl (1977) on call differences of anurans stated that dominant frequency appears to be somewhat related to body size. Small species generally have a higher frequency than larger ones. Also, the study of Bee et al. (1999) in the assessment of the size in simulated territorial encounter between male green frogs revealed similarly. The study of Hoskins et al. (2009) on frog call-body size relationship reveals a highly significant negative relationship between

body size and dominant frequency, however relationship-between body size and call duration does not show a significant relationship which further explained that call duration is likely to be the direct indicator of selection for all differentiation.

Conclusion

The advertisement calls of the two *Platymantis* species show significant differences on their temporal content and spectral structure although they share microhabitat preference as ground-dwelling species and they coexist in the same environment. Their advertisement calls keep them unique from all other *Platymantis* species. *Platymantis magdiwata* species A and *Platymantis magdiwata* species B still need to be described and identified. They are presumably new species of the most diverse genus of anuran assemblage in the Philippines. It is recommended to have a comparative study of all presumably related species of Philippine *Platymantis* based on their morphology and ecology supported by acoustic techniques in taxonomic studies to solve the knowledge gaps on amphibian systematics. Intense survey efforts are required to assess the conservation status of the two species in Mt. Magdiwata, San Francisco, Agusan del Sur.

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