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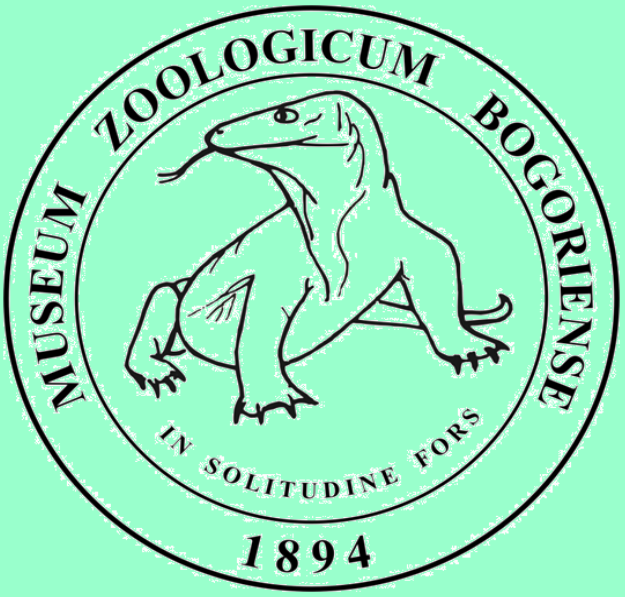

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Editor's Note

Another yearly volume of Treubia is published. I have only recently become involved in the publication of this journal and I can say that the research in this issue is increasingly interesting. I hope to remain actively involved in the publication of this journal and that we can continue to reach a larger audience as time goes on.

This volume of TREUBIA contains 5 papers of vertebrates and invertebrates. The contents of these papers vary widely from vocalizations of frogs to tropical forest spider communities. I can only hope in the future that we continue to receive interesting submissions from all areas of zoology of the Indo-Australian Archipelago.

Also this year two esteemed colleagues from LIPI retired from the service of science, Dr. Mas Noerdjito who studied the ecology of birds and Dr. Agustinus Suyanto who dedicated his life to the study of mammals.

Finally I would like to thank all of the co-editors, referees, computing assistants, secretaries and administrative assistants for their collaborative work without which this journal could not be published. I also acknowledge financial support from the Director of Research Center for Biology, LIPI to publish this essential journal.

Cibinong, December 2011

Chief Editor

Treubia 2011, 38 : 1-13

VOCALIZATION OF ASIAN STRIPED TREE FROGS, *Polypedates leucomystax* (GRAVENHORST, 1829) AND *P. iskandari* RIYANTO, MUMPUNI & McGUIRE, 2011

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ABSTRACT

Morphometric study has showed that Asian Striped Tree Frog populations from Sulawesi can be separated from populations discovered in other islands (Sumatra, Java and Kalimantan) and become accepted as a new species, namely *Polypedates iskandari* (Riyanto *et.al*., 2011). However, the results on analysis of vocalizations sequences have indicated that, *P. iskandari* and *P. leucomystax* from Java population use similar acoustic bandwidth frequencies in the major call. The different between the two species can only be found in minor calls; *P. iskandari* has a higher dominant frequency range than *P. leucomystax*. This study shows that minor calls are not important in communication among males. Therefore, based on acoustic analysis, there is no sufficient evidence to classify the Sulawesi population of *P. leucomystax* as a new species.

Keywords: vocalization, *Polypedates leucomystax, P. iskandari*, Sulawesi, Java.

INTRODUCTION

The Asian Striped Frog, *Polypedates leucomystax* has a wide distribution in Indonesian archipelago, including Sumatra (Inger & Iskandar 2005; Kamsi 2003; Kurniati 2009; Teyniei *et al*. 2010), Kalimantan (Inger 2005), Java (Iskandar 1998; Kurniati 2003; Liem 1973), Bali, Lombok (McKay 2006) and Sulawesi (Brown *et al*. 2010; Gillespie *et al*. 2005; Inger & Voris 2001; Wanger *et al*. 2011). Riyanto *et al.* (2011) argue that the wide distribution of *P. leucomystax* is likely to be the cause of the evolution of cryptic species, especially individuals that inhabit Sulawesi island which is bordered by Wallace's line. Based on morphological measurements, populations of *P. leucomystax* from Sulawesi are considered as a new species, namely *P. iskandari* which is separated from those occurring in Sumatra, Kalimantan and Java (Riyanto *et.al.* 2011). Based on DNA analysis using 16S Mt DNA comparing frogs of the Southeast Asian island archipelago with *P. leucomystax* and its close

relatives, it was concluded that the percentage of clade genetic diversity of *P. leucomystax* populations between Sulawesi and the Southern Sundas (including Java) is between 1.4%-2.0%, which is still categorized as a low divergence level (Brown *et al*. (2010). To prove the existence of *P. iskandari* as a separate species of *P. leucomystax*, it is necessary to examine their advertisement calls. Advertisement calls are species specific in almost all anuran species (Duellman & Trueb 1986) and can be used to determine a new species (Gunther 2009).

Asian Striped Frogs found in Thailand have four types of calls including normal, staccato, cackle and bark (Christensen-Dalsgaard *et al.* 2002; Sheridan 2008), however Narins *et al*. (1998) found only two types of calls including notes and staccato. In this study, normal calls are grouped as major call, whereas staccato, cackle and bark are grouped as minor calls because the wave form of staccato, cackle and bark are similar, it is different only in tempo between the two pulses (see Figure 1).

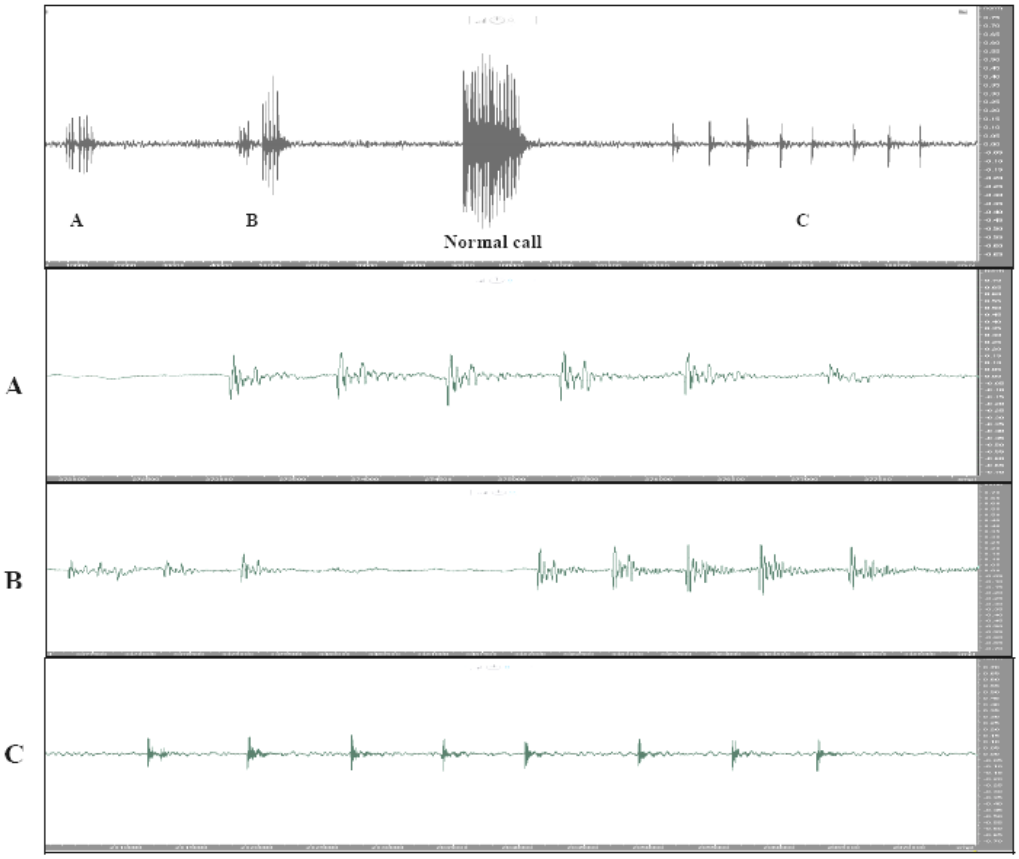

Figure 1. Oscillogram of (A) bark; (B) cackle and (C) staccato calls of *P. leucomystax* from Curug Nangka, West Java.

MATERIALS AND METHODS

P. iskandari vocalizations were recorded from 2 individuals on 21 November and 19 December 2010 in a swamp area of Mount Mekongga (S 03^0 6' 43.44"; E 121^0 09' 7.60"), SE Sulawesi, at 391 m above sea level (asl). However, *P. leucomystax* vocalizations were recorded on 18 August and 25 September 2010 in Sentul, West Java (S 06^0 34' 54.92"; E 106^0 53' 11.62") at 281 m asl (1 individual), 22-24 September 2011 in Curug Nangka, West Java (S 6^0 40' 22.8"; E 106^0 43' 53.5") at 730 m asl (6 individuals).

The frequencies of major and minor calls of both species were analyzed. Environmental temperature only influences calling rate and not call frequency (Wells 2007). Calls of *P. leucomystax* from Sentul and Curug Nangka (West Java) and *P. iskandari* from Mekongga (SE Sulawesi) were recorded with an Audio Technica AT875R microphone which has a linear frequency response between 90 Hz and 23 kHz. The sound was recorded on a Fostex FR 2LE in wav format with a sampling frequency of 88.2 kHz and a bit rate of 24 bits. Call sample size that was recorded: 21 major calls and 29 minor calls of *P. leucomystax* from one individual from Sentul; 20 major calls and 21 minor calls of *P. leucomystax* from six individuals from Curug Nangka; 7 major calls and 8 minor calls of *P. iskandari* individual 1 from Mekongga; 6 major calls and 5 minor calls of *P. iskandari* invidual 2 from Mekongga.

Since *P. leucomystax* uses very short impulses, the conventional method of measuring frequency by means of Fourier transformation is unlikely to yield sufficient resolution to detect subtle differences between populations. Therefore manual zero-crossing analysis was applied to measure the exact frequencies of the impulses in major and minor calls. Of each impulse, the loudest 5-10 cycles (major call) and 2-4 cycles (minor call) were selected and normalized by using Adobe Audition and the number of samples between start and end was measured. The average duration of 1 cycle was then calculated and converted into duration by dividing the average with the sampling frequency. Cycle duration was subsequently converted into instantaneous frequency by taking its inverse (1/duration).

To check independency of each individual's frequency of the two species, all raw data of frequencies (major and minor calls) of each individual were analysed by using one-way ANOVA statistic of SPSS version 16.0. Coefficient Variant (CV) ratio was calculated to determine "static" and "dynamic" of vocalization (Gerhardt 1991). Calculation of CV of frequency within and between individuals of the two species followed Krebs (1989).

RESULTS AND DISCUSSION

Based on coloration characters, individual frogs inhabiting swamp or forest edge in Mekongga-SE Sulawesi are different from frogs found in West Java. Individuals found in Mekongga are lighter and the dorsal stripes are nearly invisible. Moreover, black lines and blotches on the dorsal and lateral side of individuals from West Java are much more pronounced but coloration of individuals from Sulawesi (holotype and paratype) becomes dark in preservation (Riyanto *et al*. 2011). In general, color patterns of the populations in Mekongga and West Java are rather distinct.

Figure 2. Male of *P. iskandari* from Mekongga, SE Sulawesi (left) and male of *P. leuco mystax* from Curug Nangka, West Java (right).

A. Major Calls Analysis

The typical major call of *P. iskandari* and *P. leucomystax* consists of impulses having sequences spanning over a large frequency range (broad band). *P. iskandari* calls consists of 10-12 pulses/call and one pulse contains of 7-13 periods/pulse (Figure 3); however in *P. leucomys-tax,* one major call consists of 9-19 pulses/call and one pulse contains 6-9 periods/pulse (Figure 4). The number of pulses per call ranges from 12-14 individuals from West Java; similar to those "advertisement call 1" (12-14 pulses/call) from Thailand (Narins *et. al*. 1998). This number is different from those of individuals from Mekongga that has 10-12 pulses/ call. However Marquez & Eekhout (2006) found 12-23 pulses/call of in-dividuals from Bali. The major call was higher than *P. iskandari*'s but similar with that of *P. leucomystax* from West Java. The difference of the pulses number/call could be influenced by temperature as described by Wells (2009). The ambient temperature during this study ranged from 20°C-23°C, 20.5°C-21.0°C, about 26°C, 24.5°C–29°C (Marquez & Eek-hout 2006), and 25°C (Narins *et. al*. 1998) in Mekongga, Curug Nangka, Sentul; Bali and in Thailand respectively.

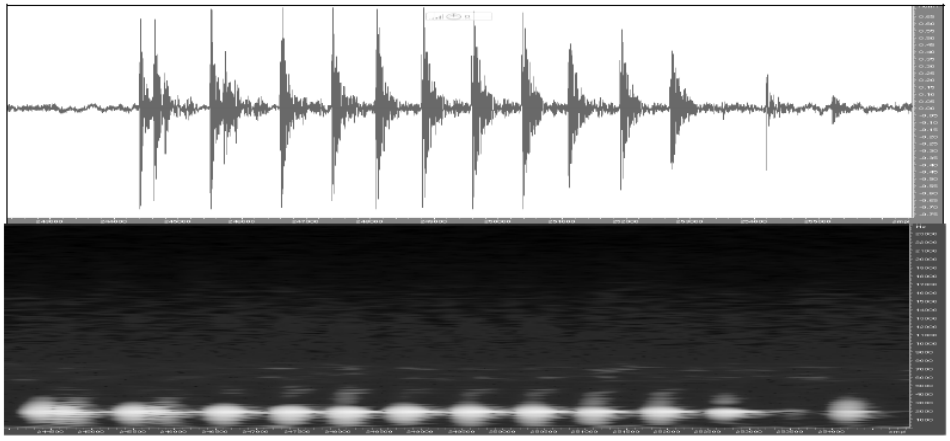

Figure 3. Oscillogram and audiospectrogram of major call of *P. iskandari* individual 1st from Mekongga, SE Sulawesi.

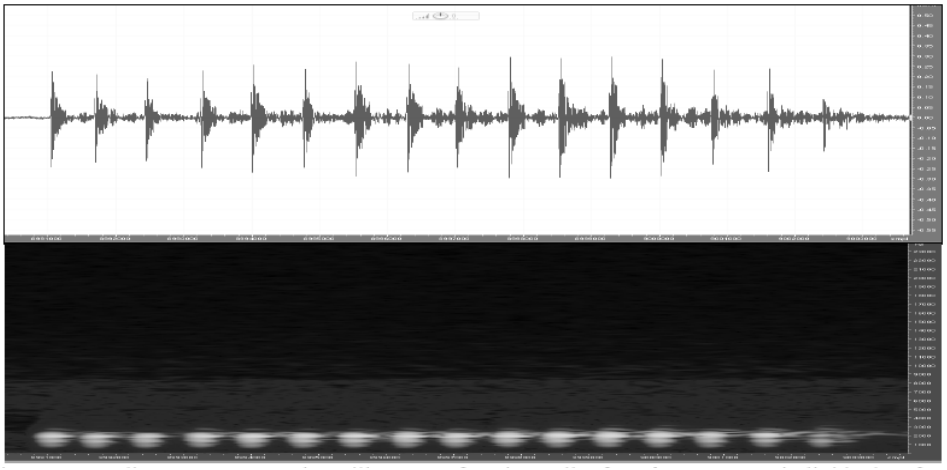

Figure 4. Oscillogram and audiospectogram of major call of *P. leucomystax* individual 5th from Curug Nangka, West Java.

Since *P. leucomystax* uses a complex vocal communication system (Christensen-Dalsgaard *et al.* 2002; Narins *et. al*. 1998; Sheridan 2008), some vocalization characters might be influenced by environmental factors, including call duration, call intensity, intercall duration and pulse rate. The only character that relatively stable is frequency (Wells 2007). The results of the zero-crossing analysis for major call of *P. iskandari* and *P. leucomystax* are shown on Table 1. Based on one-way ANOVA, the bandwidth of major calls between the two species is not significantly different ($p>0.05$; see Figure 5). The lowest frequency of major call of both species is approximately 2000 Hertz which is lower

compared to *P. leucomystax* in Bali counted of 2320.6 Hertz. The highest frequency of to *P. leucomystax* in Bali was 2677.7 Hertz (Marquez & Eekhout 2006). This different in frequency may be the result of method used in this study, the calls of *P. iskandari* and *P. leucomystax* were examined using zero crossing analysis, while the calls of *P. leucomystax* in Bali were examined using Fast Furier Transform (FFT) (Marquez & Eekhout 2006). However, the frequency range of the major calls of *P. leucomystax* in Bali is still with in the range of *P. iskandari* in Mekongga and *P. leucomystax* in West Java.

The calculation of the CV ratio of major calls between two species showed that the values were not different (see Table 1). The CV ratio for *P. iskandari* was 1.12 (112%), while the ratio for *P. leucomystax* was 1.17 (117%). The different in ratios is only 5%. Gerhardt (1991) mentioned that, the CV ratio of *P. iskandari* and *P. leucomystax* can be categorized as dynamic advertisement calls (≥ 12%); however, the ratio might indicate inter-individual discrimination of both species. The values of the CV ratio in *P. iskandari* and in *P. leucomystax* from West Java were not different from the CV ratio found in *P. leucomystax* from Bali: 1.18 (Marquez & Eekhout 2006). Based on major calls, there was no significant different between the *P. iskandari* from Mekongga, SE Sulawesi and *P. leucomystax* from West Java.

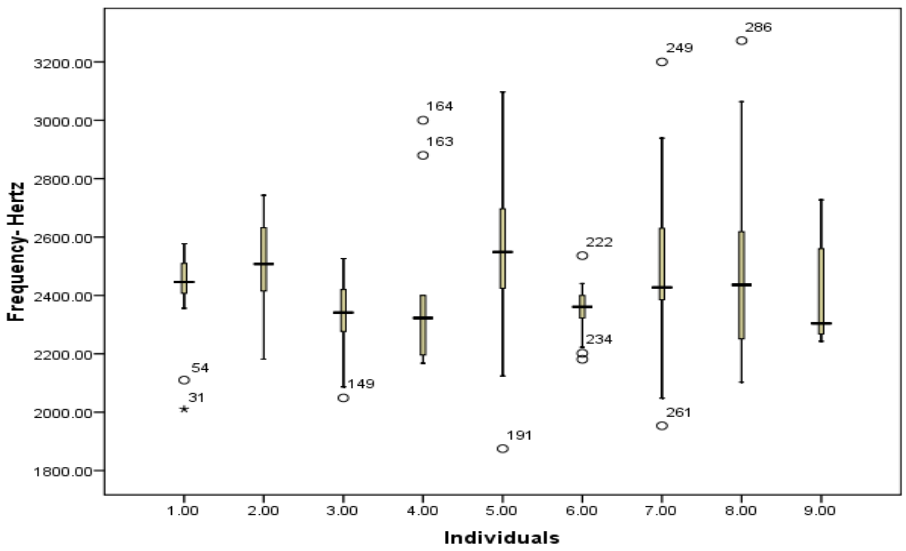

Figure 5. Box plot frequency with 95% confident of major calls of *P. iskandari* (1,2), *P. leucomystax* from Sentul (3) and *P. leucomystax* from Curug Nangka (4,5,6,7,8,9).

Table 1. Mean, Standard Deviation (SD), range frequency, bandwidth and Coefficient Variation (CV) of major call broad band frequencies between two species (n=number of calls).

	P. iskandari (individual 1-Mekongga) n=7	*P. iskandari* (individual 2-Mekongga) n=6	*P. leucomystax* (individual 1-Sentul) n=21	*P. leucomystax* (individual 1-Curug Nangka) n=1	*P. leucomystax* (individual 2-Curug Nangka) n=5	*P. leucomystax* (individual 3-Curug Nangka) n=1	*P. leucomystax* (individual 4-Curug Nangka) n=3	*P. leucomystax* (individual 5-Curug Nangka) n=8	*P. leucomystax* (individual 6-Curug Nangka) n=2
Mean-Hertz	2440.58	2505.40	2333.79	2426.97	2552.91	2353.58	2491.21	2454.78	2400.41
Standard Deviation (SD)-Hertz	90.10	126.59	112.23	303.47	202.06	98.89	244.04	225.03	161.08
Frequency range-Hertz	2010.47-2577.18	2181.82-2742.86	2048.78-2526.32	2167.74-3000.00	1875.00-3096.77	2181.18-2536.31	1953.49-3200.00	2102.9-3272.73	2242.99-2727.27
Bandwidth-Hertz	566.71	561.04	477.54	832.26	1221.77	355.13	1246.51	1168.83	484.28
CVwithin	0.04	0.05	0.04	0.12	0.08	0.04	0.10	0.09	0.07
CVbetween	0.04					0.09			
CVratio	1.12					1.17			

B. Minor Calls Analysis

The typical minor call of *P. iskandari* and *P. leucomystax* consists of impulses in which each having a broad bandwidth. *P. iskandari* minor calls consist of 5-8 pulses/call each with 2-3 periods/pulse (Figure 6). *P. leucomystax* also has a similar typical minor call with *P. iskandari* (Figure 7). Both species use a similar wave pattern of pulse in the minor call. Based on one-way ANOVA, bandwidth in the minor calls between the two species was significantly different ($p<0.05$). The bandwidth frequency of *P. iskandari* was higher than the bandwidth frequency of *P. leucomystax* (see Table 2 and Figure 8).

The calculation of CV ratio between two species showed that the ratios were different (Table 2). The CV ratio of minor calls of *P. iskandari* and *P. leucomystax* were 0.86 (86%) and 0.73 (73%) respectively; minor calls CV ratio difference between the two species is 13%. Minor calls of the two species also had dynamic advertisement calls ($\geq$ 12%) (Gerhardt 1991) indicating that minor calls of both species had high potential for inter-individual discrimination within the *Polypedates* population in Mekongga and in West Java. Minor calls of *P. iskandari* seemed to be similar to staccato calls (dominant frequency 1935 Hertz) of non-striped *P. leucomystax* from Ulu Gombak, Peninsular Malaysia (Narins *et. al*. 1998).

Based on field observations, many minor calls were produced by solitary males (shown by *P. leucomystax* individual 1 from Sentul and Curug Nangka, see Table 2), whereas major calls were often released by males that aggregated around the pool. Minor calls seem to share less important role in communication between males. It seems that females of *P. leucomystax* typically choose signals with more pulses/call (personal

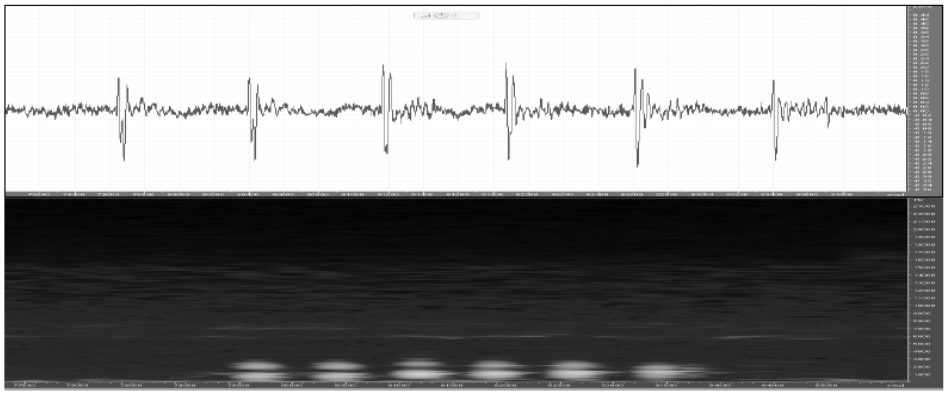

Figure 6. Oscillogram and audiospectrogram of minor call of *P. iskandari* individual 1st from Mekongga, SE Sulawesi.

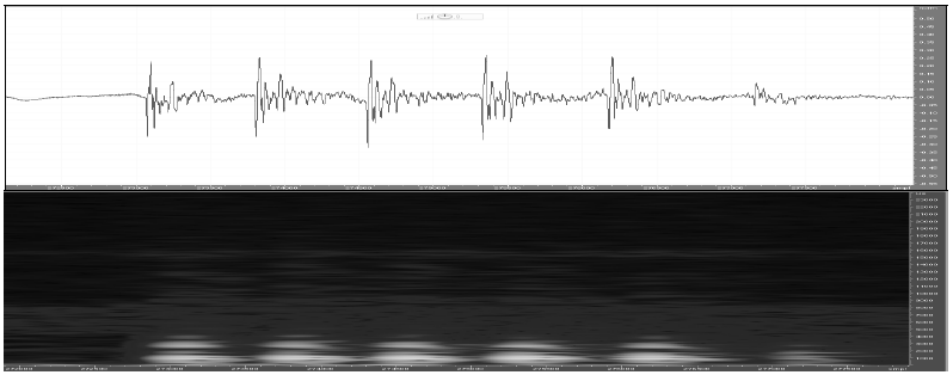

Figure 7. Oscillogram and audiospectrogram of minor call of *P.leucomystax* individual 1st from Curug Nangka, West Java.

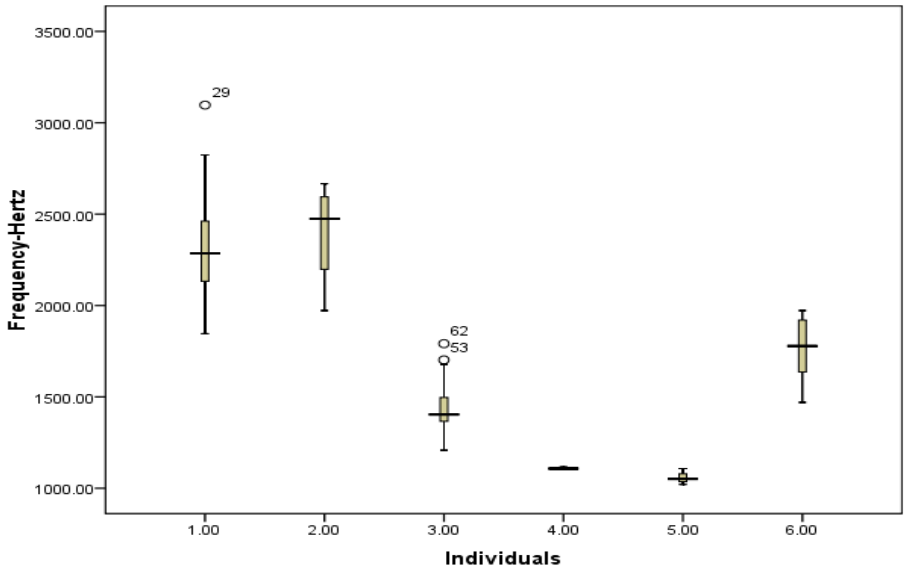

Figure 8. Box plot frequency with 95% confident of minor calls of *P. iskandari* (1,2), *P. leucomystax* from Sentul (3) and *P. leucomystax* from Curug Nangka (4,5,6).

observation); this is a characteristic of major calls of male *P. leucomystax* (this study, Marquez & Eekhout 2006; Sheridan 2008; Narins *et al*. 1998). These phenomena have also been shown in *Crinia georgiana* (Gerhardt *et al*. 2000; Smith & Robert 2003).

Based on a genetic study by Brown *et al*. (2010), there is low level divergence on 16S Mt DNA (1.4%-2.0%) between populations of *P. leucomystax* from Sulawesi and Java. However, the results of vocalization analyses on both populations, they are only different significantly in the

Table 2. Mean, Standard Deviation (SD), range frequency, bandwidth and Coefficient Variation (CV) of minor calls broad band frequencies between two species (n=number of calls).

	P. iskandari **(individual 1 -Mekongga) n=8**	***P. iskandari*** **(individual 2- Mekongga) n=5**	***P. leucomystax*** **(individual 1- Sentul) n=29**	***P. leucomystax*** **(individual 1- Curug Nangka) n=1**	***P. leucomystax*** **(individual 4- Curug Nangka) n=2**	***P. leucomystax*** **(individual 6- Curug Nangka) n=18**
Mean-Hertz	2294.39	2413.48	1440.01	1110.55	1058.49	1779.04
Standard Deviation (SD)-Hertz	264.08	232.12	115.42	4.94	31.16	153.32
Frequency range-Hertz	1846.15-3096.77	1972.60-2666.67	1207.55-1791.04	1107.69-1116.28	1021.28-1107.69	1469.39-1972.60
Bandwidth-Hertz	1250.62	694.07	583.49	8.50	86.41	503.21
CVwithin	0.11	0.10	0.08	0.004	0.03	0.09
CVbetween	0.09			0.15		
CVratio	0.86			0.73		

frequency of minor calls. *P. iskandari* uses a higher broadband frequency than the broadband frequency produced by *P. leucomystax* from Java (see Figure 8). High level divergence in genetic traits usually can distinguish a species, including: *P. leucomystax* from southern Sundas (include Java) and *P. colletti* (10.0%–12.8%), *P. leucomystax* from southern Sundas and *P*. cf. *megacephalus* (3.2%–7.9%) (Brown *et al*. 2010). Gunther & Knop (2006) showed that the genetic distances within a range of 4.5 % to 13 % was sufficient to separate *Xenorhina varia* and *Xr. oxycephala* as different species, because the two species uses different vocalizations. However, based on the results of acoustic analyses of *P. leucomystax* and of *P. iskandari* and also the results of genetics study of the species by Brown *et al*. (2010), there is no sufficient evidence to classify the population of *P. leucomystax* from Sulawesi as a new species

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INSTRUCTIONS FOR AUTHORS

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MacKinnon, J. & K. Phillips, 1993. *Field Guide to the Birds of Borneo, Sumatra, Java and Bali*. Oxford University Press, Oxford, 491 pp.

Stork, N.E., 1994. Inventories of biodiversity: more than a question of numbers. *In*: Forey,P.L., C.J. Humphries & R.I. Vane-Wright (eds.), *Systematics and Conservation Evaluation*. Clarendon Press (for the Systematics Association), Oxford, pp. 81-100.

Maddison, D.R., 1995. Hemiptera. True bugs, cicadas, leafhoppers, aphids, etc.. Version 01 January 1995 (temporary). *http://tolweb.org/ Hemiptera/8239/1995.01.01*. *In*: The Tree of Life Web Project, *http:// tolweb.org/* (accessed on 27 November 2007).

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