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CALL CHARACTERISTICS AND SYSTEMATIC RELATIONSHIPS OF A MALAYAN TREEFROG *NYCTIXALUS PICTUS* (ANURA, RHACOPHORIDAE)

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Nyctixalus Boulenger, 1882 is a small genus among the Old World tree frog family Rhacophoridae, and includes only three or four species that are confined to Southeast Asia (Frost, 1985). The genus has been variously treated taxonomically as *Hazelia* (Taylor, 1920; Liem, 1970), *Philautus* (Inger, 1966), or *Edwardtayloria* (Marx, 1975). The genus is unique both in morphology and in breeding habit (Inger, 1966; Liem, 1970), and its systematic position among rhacophorids was once assessed chiefly on the basis of morphological evidence (Liem, 1970). Acoustic characteristics of species of the family Rhacophoridae have been rather well-studied among Asian anurans (e.g., Kuramoto, 1986; Matsui & Wu, 1994), but species of *Nyctixalus* have never been acoustically studied until now (Inger & Stuebing, 1989). In this short note, I will report the call characteristics of *N. pictus* from Sarawak, Borneo, which is the type locality of the species, and discuss the systematic relationships of this genus to other Asian rhacophorid genera.

Calls of three males of *N. pictus* were recorded at the Park Headquarters, Mulu National Park, Miri Division, Sarawak, Malaysia on 28 and 29 July 1993. Possible advertisement calls (see below) were recorded in the field with a cassette tape recorder (Sony TC-D5) with an external microphone (Sony ECM-23F) and analyzed using computer programs, SoundEdit Vers. 2 or SoundEdit Pro (MacroMind-Paracomp, Inc.) by a Macintosh computer. Air temperatures recorded near the calling males were 23.6-23.7°C.

In the following description, note interval means the time from the end of one note to the beginning of the next note. For statistic analyses, Dunn's multiple comparison test (Zar, 1984) was utilized. The significance level was set at P=0.05.

Males were found calling from low vegetation (< 1 m) at night in the forest. The places where they were calling were not near any obvious breeding sites. Calls were soft and weak, being very similar to those of insects (Fig. 1) and included six to 11 notes (Table 1), each of which was clearly divided into three types (A-C).

The type A note is a long "*fiii*". The note (Fig. 1) was unpulsed and the length varied from 224 to 368 ms with the mean (±SD) of 284.7±40.9 ms (n=15). The note was very clearly modulated in frequency (Fig. 1), beginning from 2650 to 2800 hz (mean ±SD=2716.0±47.6, n=15) and ended at 3020 to 3700 hz (mean±SD=3281.3±180.9, n=15) (Fig. 1). The dominant frequency measured at the middle of the note varied from 2845 to 3240 hz (mean±SD=2998.7±93.7, n=15). Due to heavy background noise of mainly insect calls, it was difficult to analyze frog calls at frequency ranges of 4000 to 8000 hz (Fig. 1), but harmonics were detected at about 8000-10000 hz, although they were very weak and only partial. The type A note was usually emitted singularly, but when the type B notes followed it, the interval between them ranged from 161 to 931 ms (mean±SD=577.0±338.8, n=5) .

The type B note was also unpulsed, but a shorter "*pi*". The note length varied from 46 to 161 ms (mean±SD=114.7±28.8, n=54). The note lacked harmonics and a frequency modulation. The dominant frequency varied from 3025 to 3100 hz (mean±SD=3069.3±20.7, n=54). The type B note was seldom emitted singularly; it was sometimes uttered following the type A note, and was often followed by the type C note (Fig. 1). The interval between successive notes varied from 115 to 426 ms (mean±SD=209.2±29.7, n=52). The note repetition rate varied from 3.17 to 3.82 per second (mean ±SD=3.54±0.21, n=10).

The type C note was again unpulsed, and even shorter than the type B. The note length varied from 23 to 104 ms with the mean of 49.4±25.1 ms (n=19). The note lacked harmonics but usually had a marked frequency modulation (Fig. 1). The frequency began from 2900 to 3150 hz (mean±SD=2967.3±66.7, n=19) but increased rapidly and ended at 3150 to 3400 hz (mean±SD=3276.3±67.4, n=19) (Fig. 1). The range of the dominant frequency at the middle of the note was from 3025 to 3275 hz (mean±SD=3121.8±52.9, n=19).

The type C note always followed the type B. Usually several type C notes were emitted successively, and the interval between successive notes varied from 92 to 249 ms (mean±SD=147.9±50.3, n=10). Thus the note repetition rate varied from 4.82 to 9.49 per second (mean±SD=7.01±2.04, n=4). The type C note was similar for the human ear to the end notes of the syntopic *Rhacophorus appendiculatus*, but was rather softer.

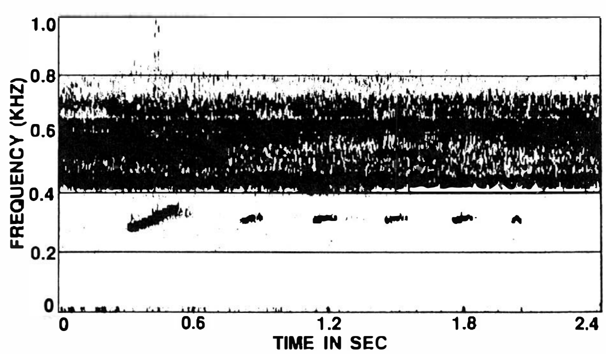

FIG. 1. A sonagram of *Nyctixalus pictus* call including successive notes of one type A, four type B, and one type C recorded at 23.6°C.

Thus the complete call was composed, in order, of the note types A, B, and C, but the combination of only B and C types was more often heard. The type B notes predominated in number within a call in most cases. The total duration of a call varied from 862 ms in the call with three notes to 3571 ms in the call with eight notes (Table 1).

When pairs of notes were compared, lengths of type A notes were always longer than those of type B, which in turn were longer in duration than type C (Dunn's multiple comparison test: $P<0.05$). The interval between the type A and the subsequent type B notes, however, was not different from the note interval in the type B, though both intervals were significantly longer than in the type C. The frequency measured at the initial point was not different between types B and C, both of which were significantly higher than type A note. By contrast, the frequency measured at the final point did not differ between the types A and C, both of which were significantly higher than type B notes. Finally, the dominant frequency measured at the middle portion of the note differed significantly among the three types, type C being the highest and type A the lowest.

During observations in the field, no mating, courtship, or male-male fighting was seen in *Nyctixalus pictus*. However, calls of one male were found to elicit another's calls. Therefore, the calling activity surely involves at least the male-male relationship and the recorded calls are assigned to the advertisement call category (Duellman & Trueb, 1986).

In a study of Thai species of Rhacophoridae, Heyer (1971) assumed that the call prototype for this family would consist of a component with a wide frequency range, lacking harmonics. He did not actually mention, however, the degree of primitiveness for the six species of four genera he studied.

TABLE 1. Variation in the number of note elements and total duration of calls in *Nyctixalus pictus*.

No. note types				Call length (ms)		
A	B	C	Total	Mean	Range	*n*
0	3	0	3	862		1
0	4	0	4	1276	(1196-1357)	2
0	4	2	6	1564	(1495-1691)	3
0	4	4	8	1875		1
0	5	1	6	1748	(1564-1932)	2
0	5	2	7	1936	(1587-2116)	6
0	5	3	8	2421	(2243-2599)	2
0	7	2	9	2312		1
0	7	3	10	2455	(2300-2611)	2
0	8	3	11	2864		1
1	4	1	6	1657		1
1	5	1	7	2536	(2381-2691)	2
1	5	3	9	2576		1
1	6	2	9	2645		1
1	7	0	8	3571		1

Later, Kuramoto (1986) suggested a possible course of acoustic divergence in the rhacophorids from Taiwan and Japan. He indicated that the single-pulsed note as seen in *Polypedates megacephalus* (as *leucomystax*) was the most primitive and the multipulsed note with harmonic bands as seen in *Rhacophorus moltrechti* or *R. owstoni* the most advanced. In order to examine the validity of these hypotheses, however, further accumulation of acoustic data for many more rhacophorid species is required.

Kuramoto (1986) at the same time noted that long pulses had not been reported in Rhacophoridae. The notes of *Nyctixalus pictus* are here expressed as "unpulsed", but they, especially in the type A, are indeed regarded as long pulses as defined by Kuramoto (1986). In this respect, the call of this species does not resemble calls of any other rhacophorid species hitherto studied (Heyer, 1971; Dring, 1987; Matsui *et al.* in preparation).

From the phenetic analyses of morphology, Liem (1970) proposed that *Nyctixalus* (as *Hazelia*) is related to *Chirixalus* and *Theloderma*. Channing (1989) reanalyzed Liem's (1970) data cladistically and concluded that *Nyctixalus* has a sister relationship with *Theloderma* but not with *Chirixalus*. Calls of *Chirixalus* species (Heyer, 1971; Matsui, unpub. data) are judged to be quite different from those of *Nyctixalus*. Acoustic traits of *Theloderma*, on the contrary, have never been reported, and are required for comparisons with *Nyctixalus*.

On the other hand, the unique calls of *N. pictus*, with unpulsed notes may be related to the habit of breeding in tree holes. Two microhylid species of the genus *Metaphrynella* also breed in tree holes and have unique calls with an unpulsed note (Matsui *et al.*, in preparation).

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