Rhacophorus leucomystax in Vietnam with Acoustic Analyses of Courtship and Territorial Calls

TANYA L. TRÉPANIER[1, 2], AMY LATHROP[1,2] AND ROBERT W. MURPHY[1,2,3]

[1]*Department of Zoology, University of Toronto, 25 Harbord Street, Toronto, Ontario, Canada M5S 3G5, e-mail: tanyat@rom.on.ca;* [2]*Centre for Biodiversity and Conservation Biology, Royal Ontario Museum, 100 Queen's Park, Toronto, Ontario, Canada M5S 2C6;* [3]*Corresponding author: e-mail: drbob@rom.on.ca*

Abstract.- Acoustic parameters of both courtship and territorial calls were analysed for two populations of Vietnamese treefrogs, genus *Rhacophorus*. These treefrogs from Ba Be National Park and nearby Na Hang Nature Reserve were identified as *R. leucomystax* as opposed to *R. megacephalus*, based on an acoustic analysis of courtship calls. They represent the first confirmed record of this species outside of Borneo and suggest that the species may also occur in China. The courtship call, composed of a long note of similar pulses and pitch, has a mean dominant frequency of 1940 ± 154 Hz at Ba Be National Park and 1950 ± 70 Hz at Na Hang Nature Reserve. The territorial call, a two to three note call, has a mean dominant frequency of 1940 ± 124 Hz at Ba Be National Park and 2190 ± 256 at Na Hang Nature Reserve. No significant differences were found between the call characters of these two populations of Vietnamese *R. leucomystax*. There was no significant association between snout-vent length and dominant frequencies.

Key words.- Amphibia, Anura, call analysis, Rhacophoridae, *Rhacophorus leucomystax*, Vietnam, vocalizations.

Introduction

Until recently, the amphibian fauna of Vietnam has been based on the works of Bourret (1942), and accounts from neighbouring China (e.g., Yang, 1991; Zhao and Adler, 1993; Zhao, 1995). The Chinese occurrence of one species of treefrog, *Rhacophorus leucomystax*, was recently placed into doubt by Matsui et al. (1986) based on chromosomal, morphological and acoustic evaluations. These authors reported that *R. leucomystax* was restricted in distribution to Borneo whereas a similar species, *R. megacephalus*, occurred in Taiwan. They stated that mainland Chinese populations of these frogs required further study, but in the interim, mainland Chinese populations should be referred to as *R. megacephalus*. Zhao and Adler (1993) reported that *R. leucomystax* did not occur in China, but rather that *R. megacephalus* occurred throughout the country. Our recent field work in northern Vietnam revealed the presence of two apparent forms of treefrogs similar in appearance to *R. megacephalus* and/or *R. leucomystax*. We successfully recorded vocalizations of one form from two allopatric populations. Herein, we report our analysis of the acoustic parameters of both courtship and territorial calls, including the dominant frequency, which is species specific.

Anurans are capable of different types of vocalizations which serve different functions. The courtship call, also known as the mating or breeding call, is emitted by males and has two functions: the attraction of conspecific females and the announcement of an occupied territory to other males of the same or different species. There are three types of courtship calls: the courtship call produced by males in attempt to attract a conspecific female, a territorial call produced by a resident male in response to an courtship call received above a critical threshold of intensity, and an encounter call evoked during close range agnostic interactions between males (Duellman and Trueb, 1994). The courtship call acts as a courtship isolating mechanism (Duellman and Pyles, 1983). A second type of call, the territorial call, is comprised of acoustic signals and is accompanied by corporal vibrations produced by a male or an unreceptive female in response to amplexus. Other types of calls include the reciprocation call which is given by a receptive female, and the distress call which is delivered in response to an enemy or predator for defence.

Signals are found to vary between and within individuals and between geographically separated populations of a single species (Cocroft and Ryan, 1995). Anurans use these differences in call characteristics to identify individuals of the same or different species and to signal intent. Frost and Platz (1983) proposed that these interspecific and intraspecific differences in fundamental call characteristics such as pulse rate, duration of call, dominant frequency, or a combina-

tion of these parameters, permits females to reduce the likelihood of error in mate choice. Anurans recognize individuals of the same and different species by their dominant frequencies.

Duellman and Pyles (1983) discovered that smaller frogs tend to call at higher frequencies and have a reduced auditory sensitivity compared with larger frogs. They concluded that an upper limit of approximately 5000 Hz is most common. Furthermore, the reception of acoustic signals is affected by habitat and interference from synchronously calling species. In rainforest habitats, which are complex habitats, anurans were found to produce sounds at lower frequencies because the dense vegetation attenuates sound waves, notably those at higher frequencies. Therefore, transmission frequencies of less than 4000 Hz would be most effective for anuran communication in such complex habitats. Partitioning of the acoustic community can be affected by several factors such as type of call produced, oviposition site, environment and the onset of the breeding season (Duellman and Pyles, 1983). But within any given community, the available acoustic environment is partitioned distinctly based on its particular ecological and geographic assemblage of anurans. Although selection may operate to maximize acoustic properties for species recognition, acoustic interference from factors of the physical habitat and from community members also operates to minimize acoustic variability. This implies that members of the same breeding fauna have voices with common characteristics (Duellman and Pyles, 1983).

Material and Methods

Frog calls were recorded and calling specimens collected from two different localities in northern Vietnam. Approximately 24 frogs of the *R. leucomystax* complex were recorded and collected in Ba Be National Park (22°24'N 105°37'E) from 15 to 29 May 1995. Five *R. leucomystax* were recorded and collected in the village of Pac Ban (22°21'N 105°23'E) located in Na Hang Nature Reserve, from the 23 May to 3 June, 1996. In both locations, frogs were selected at random and a sufficient call sample was recorded using a Realistic™ unidirectional microphone and a Marantz™ PMD 201 portable cassette recorder. Air temperatures were recorded in degrees Celsius at Ba Be National Park at the time of capture, using a thermometer; each specimen was collected during evening hours from a concrete pool adjacent to the Park's research centre. In Pac Ban, frogs were collected from various locations, including in rice patties, on trees, and in ponds. All specimens were euthanised, preserved and deposited in the herpetology collections of the Royal Ontario Museum, Toronto, Canada. Calls were transferred from the cassette recorder to a Macintosh computer using a Macrorecorder™ digitizer. Sound Edit Pro™ version 2.0.5 for Macintosh computer systems was used for call analyses. Note duration, dominant frequency, fundamental frequency, notes per call, pulses per note and time between segments were measured. Descriptive analyses were performed for all courtship and territorial calls, using Excel™ 5.0 (Microsoft). Snout-vent length (SVL) was measured for all specimens to the nearest 0.1 mm using digital calipers.

Results

Of the 24 specimens recorded and digitized at Ba Be National Park, 42 courtship calls were analysed from 22 individuals, and 33 territorial calls from 18 individuals. Table 1 summarizes measurements of call parameters for all mating calls.

In most cases, the fundamental frequencies overlapped the dominant frequencies, rendering it impossible to obtain exact measurements. However, in those that were observed, the fundamental frequency appeared to be approximately one half the value of the dominant frequency (900-1000 Hz). Air temperatures did not vary significantly, with a mean of 26.2°C for territorial and courtship calls, thereby having little effect on call characteristics. All courtship calls had one note, and pulses ranged from 2 to 17 per note. In contrast, all territorial calls were either 2 or 3 notes per call, the most common being the former, with variability in numbers of pulses in all three notes (Figure 1).

Of the 5 specimens recorded and digitized in Pac Ban, 2 courtship calls were analysed from 2 individuals, and 21 territorial calls from 5 individuals. Call parameters are summarized in Table 1. Fundamental frequencies were obtained from the sonograms, where values were noted as approximately one half the value of the dominant frequency (800-900 Hz). All courtship calls had one note, and pulses ranged from 4 to 16 notes per call. In contrast, all territorial calls were either 2 or 3 notes per call, the most common being the former, with variability in numbers of pulses in all three notes.

The courtship call resembled the sound of a trill, or long sequence of pulses of similar pitch, whereas the territorial calls were more similar to the sound of clicks. Dominant frequencies were consistent within all calls; mean dominant frequency in courtship calls were 1940 Hz for the Ba Be population and 1950 Hz

Table 1. Call measurement summary of note duration, notes/call, pulses/note and dominant frequencies for *Rhacophorus leucomystax* in Ba Be National Park and Pac Ban in Na Hang Nature Reserve.

	Ba Be National Park		Na Hang Nature Reserve	
	Courtship	Territorial	Courtship	Territorial
Note duration (secs)	0.33±0.24	0.08±0.02	0.64±0.54	0.08±0.02
Notes/call (range)	1	2-3	1	2-3
Pulses/note (range)	2-17	1-3, 1-5, 2	4-16	1-3, 2-4, 1-4
Dominant frequency (Hz)	1940±154	1940±125	1950±70	2190±256

for the Pac Ban population. For territorial calls, the mean dominant frequency was also 1940 Hz in Ba Be and 2190 Hz in Pac Ban. Therefore, *R. leucomystax* has a species-specific dominant frequency of approximately 2000 Hz. A consistency between segments or pulses was observed for the territorial calls. The mean time between segments was 0.02 seconds (standard deviation ± 0.003).

Snout-vent length was measured for all specimens and correlated with dominant frequency. Our regression analyses failed to confirm a significant association ($F=1.948$, $p=0.176$, $r^2=0.08$) between SVL and dominant frequency.

Discussion

Call duration and pulse rate are temperature dependent (Platz, 1989). Regression analysis of call characteristics and temperatures achieve the highest correlation, where a correction function is generated. The correlation function is then used to correct data for temperature differences revealing little variation. For example, a few obvious differences were discovered in the values of the call characteristics as shown in the sonograms for the eastern and western samples of *Rana pipiens* when differences in temperature between the localities and variation within the localities were taken into account (Dunlap and Platz, 1981). However, because temperatures were not significantly variable, temperature values were not used to account for variation in our data. Furthermore, our regression analyses revealed that snout-vent length did not account for variation in our acoustic data, as it does in some other anuran species (Duellman and Pyles, 1983).

Because dominant frequency is characteristic of species, we compared our data with those of Matsui et al. (1986). The dominant frequency of *Rhacophorus leucomystax* ranged from 2250-2550 Hz whereas it ranged from 1040-1070 Hz in *R. megacephalus*. Further, Matsui et al. (1986) found that *R. leucomystax* had one note/call whereas in *R. megacephalus* it ranged from 2-4 notes/call. Given that the mating calls of our frogs from northern Vietnam had a dominant frequency of 1.94-2.49 kHz (Table 1) and consisted of a single note/call, we conclude that our specimens are best identified as *R. leucomystax* pending additional morphological evaluations (in preparation). Our study sites at Ba Be National Park and Na Hang Nature Reserve are located only 125 km south of Yunnan Province, China. Consequently, given that *R. leucomystax* ranges from at least Borneo to northern Vietnam, the species likely also occurs in China and elsewhere in Asia. Further, it seems likely that it occurs sympatrically with *R. megacephalus*, at least in Vietnam.

Disturbance to the environment may have negative impact on anuran populations. As a result of increased accessibility to Vietnam, tourists are beginning to exploit the relatively primitive country. Such exploitation will likely contribute to increased deforestation and wildlife loss. Human population growth continues to rise in Vietnam at an alarming rate (ca. 2.3%/yr) and deforestation is increasing in quest for construction materials and agriculture for both in-country needs and cash-crop export, especially coffee. Species mining is occurring for export of animals to foreign countries, regardless of species type, number or Vietnamese efforts to protect the species. Littlejohn and Roberts (1975) remarked that the environment of north central Victoria, Australia, had been greatly modified over the last 130 years by increased land clearing for agriculture, timber cutting for building and mining, and by the establishment of extensive irrigation and drainage systems. They suggested that these alterations have influenced the position, extent, and nature of the main zones of intergradation, which may further increase dispersal due to global warming

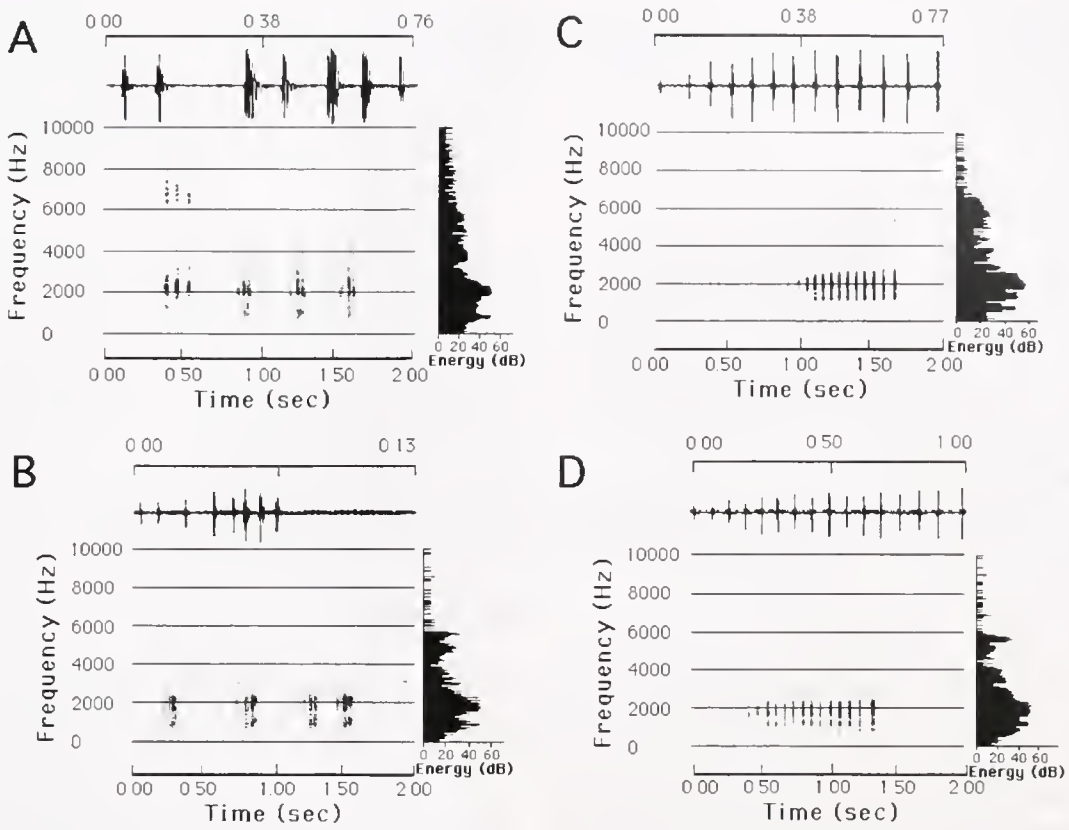

Figure 1. Call characteristics for *Rhacophorus leucomystax* as illustrated through audiospectrograms (frequency vs. energy) and sonograms (frequency vs. time). An expanded oscillogram (above) shows finer details of the call. Territorial calls from A) Babe (ROM 19334); B) Pac Ban (ROM 6514), and courtship calls from C) Babe (ROM 19486); and D) Pac Ban (ROM 6514).

and the concomitant northward movement of anuran populations. The possibility for divergence in mating call structure could then result increasing the frequency of mismatching and nonviable offspring. Like Victoria, it is not known what the future beholds for the relatively fragile, undiscovered and endemic species of Vietnam. Therefore, identification of species for many areas of study, such as behavioural, ecological and evolutionary fields, will be critical in the next few years so that as many species as possible can be studied, identified and conserved.

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Literature Cited

Bourret, R. 1942. Les batraciens de l'Indochine. Mémoires de l'Institut Océanographique de l'Indochine 6:x+517.

Cocroft, R. B., and M. J. Ryan. 1995. Patterns of advertisement call evolution in toads and chorus frogs. Animal Behaviour 49: 283-303.

Duellman, W. E., and R. A. Pyles. 1983. Acoustic resource partitioning in anuran communities. Copeia 1983: 639-649.

Duellman, W. E. and L. Trueb. 1994. Biology of Amphibians. The John Hopkins University Press, Baltimore, Maryland

Dunlap, D. G., and J. E. Platz. 1981. Geographic variation of proteins and call in *Rana pipiens* from the north central United States. Copeia 1981: 876-879.

Frost, J. S., and J. E. Platz. 1983. Comparative assessment of modes of reproductive isolation among four species of leopard frogs (*Rana pipiens* complex). Evolution 37: 66-78.

Littlejohn, M. J., and J. D. Roberts. 1975. Acoustic analysis of an intergrade zone between two call races of the *Limnodynastes tasmaniensis* complex (Anura: Leptodactylidae) in south-eastern Australia. Australian Journal of Zoology 23: 113-122.

Matsui, M., T. Seto, and T. Utsunomiya. 1986. Acoustic and karyotypic evidence for specific separation of *Polypedates megacephalus* from *P. leucomystax*. Journal of Herpetology 20: 483-489.

Platz, J. E. 1989. Speciation within the chorus frog *Pseudacris triseriata*: Morphometric and mating call analyses of the boreal and western subspecies. Copeia 1989: 704-714.

Yang, D. 1991. The Amphibia-Fauna of Yunnan. China Forestry Publishing House [In Chinese].

Zhao, E.-M. (ed.) 1995. Amphibian Zoogeographic Division of China. Sichuan Journal of Zoology, 1995 Supplement. [In Chinese].

Zhao, E.-M. and K. K. Adler. 1993. Herpetology of China. SSAR in cooperation with CSSAR, Contributions to Herpetology 10, 522 pp.