

# Mating Behavior of Hansen's Bush Frog (*Chiromantis hansenae*) at Sakaerat Environmental Research Station, Northeastern Thailand

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## ABSTRACT

The influence of meteorological and ecological factors on the breeding of Hansen's bush frog, *Chiromantis hansenae* was investigated during October 2008 to September 2009 at the Sakaerat Environmental Research Station, Northeastern Thailand. Meteorological factors (air temperature, relative humidity and rainfall) were analyzed using an ordination method. The principal component analysis output indicated a correlation between mating behavior (amplexus, laying egg behavior and advertisement calls) and termination of rainfall; however, reproduction was prolonged and lasted through the entire rainy season. While the temperature showed a significant relationship with mating behavior (a higher temperature resulted in an increase in mating), humidity did not appear to influence the mating. In Northeastern Thailand the breeding habitat of these frogs is in ponds. Male frogs advertise from elevated branches of trees and shrubs hanging over the water body including stems of grasses around the pond. Females normally attach eggs on leaves and take care of them until the eggs hatch.

**Keywords:** *Chiromantis hansenae*, mating behavior, meteorological factors, ecological factors

## INTRODUCTION

Since 1980, there has been a continuing reduction in amphibians and more than 400 species that exist now are being threatened due to habitat depletion as a result of logging. A reduction in amphibian species is associated with intensive human activities (Stuart *et al.*, 2004). Many wetlands which are important habitats for amphibians, have been destroyed by human intervention and continued infrastructure development (Cui and Zhang, 2002). Modification of the aquatic natural habitat for agricultural use and anthropogenic disturbance are also important causes of direct and indirect effects on resident animals (Schoonover *et al.*, 2006).

Wetlands are very important aquatic ecosystems (Yin and Ni, 1998). The riparian zone in a wetland is the interface between the land and the river or stream and has a high abundance of plant communities that act to filter the water with the associated chemical processes able to reduce nitrogen, sulfate and carbon (Alewell *et al.*, 2008). Therefore, wetland ecosystem preservation is necessary (Yin and Ni, 1998), specifically of the amphibians that live in such areas, which suggests the importance of preservation of both terrestrial and aquatic ecosystems (Berger *et al.*, 1998; Li and Zhang, 2002). Populations of amphibians are dependent on breeding success; therefore, study on the breeding activity of amphibians and their conservation is necessary. For example,

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*Rhacophorus malabaricus* (Rhacophoridae) starts its breeding call activity after 3 to 4 heavy monsoon showers, which wash the binding material between the leaves to open the foam nest and act as a vehicle for the developing embryos to drop into water (Kadadevaru and Kanamadi, 2000).

Hansen's bush frog (*Chiromantis hansenae*) is an amphibian species that lives in wetlands. The adult frogs forage around the water and lays egg masses on leaves over hanging ponds. Sheridan and Ocock (2008) had noted the adult of *C. hansenae* displays extensive egg attendance (day and night) over the short time to hatching. Tadpoles of *C. hansenae* live in the water of wetlands (Noikotr, 2002), and are scavengers; thus they are important for wetland ecosystems. Ecological studies are important for the conservation of this species and the other frogs. Little is known about the breeding behavior of Hansen's bush frog throughout its range. The purpose of this study was to document the breeding biology of Hansen's bush frog in Northeastern Thailand.

## MATERIALS AND METHODS

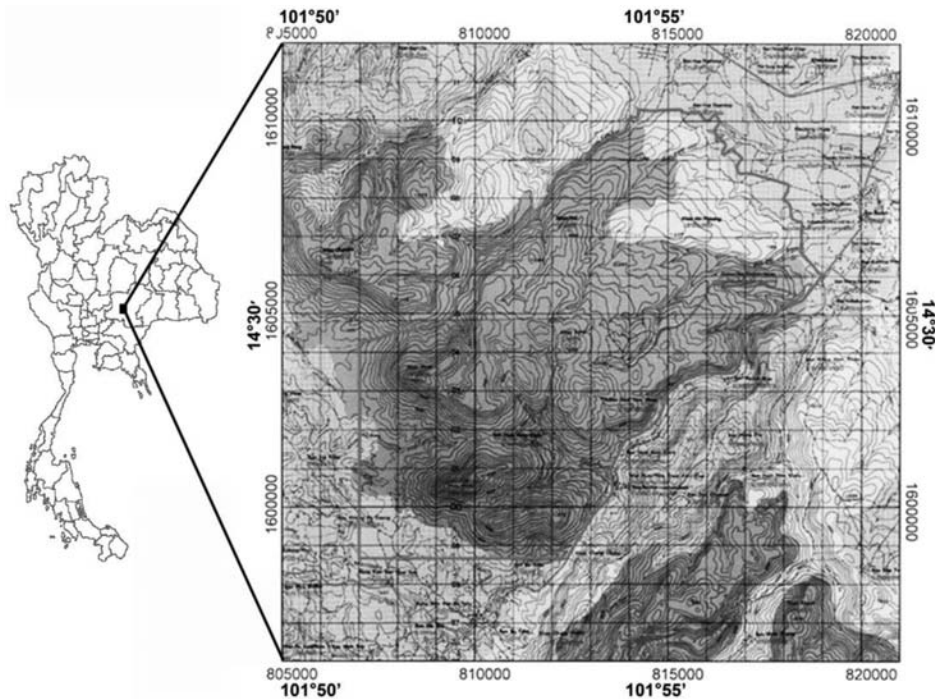
### Study species

Hansen's bush frog was first described as *Philautus hansenae* by Cochran in 1927, based on specimens from Nong Khor, Southeastern Thailand. The holotype (USNM 70109) was deposited in the Smithsonian National Museum of Natural History (Frost *et al.*, 2006). According to Frost *et al.* (2006), *Chiromantis hansenae* is known to occur in East-Central and Southeastern Thailand, and also presumably in adjacent Cambodia. Stuart and Emmett (2006) suggested that *C. hansenae* may be a junior synonym of *C. vittatus*. However, the present study refers to a population of tree frogs from Northeastern Thailand, that is *C. hansenae* (Frost *et al.*, 2006; Sheridan and Ocock, 2008).

This species is one of the anuran amphibians that exhibits parental care on mainland, Southeast Asia. The sex of an adult Hansen's bush frog can be determined visually by size—females are slightly larger (25 mm) than males (20–22 mm). Moreover, males lack a nuptial pad and vocal sacs and the sex can be confirmed only by the mating calls. In addition, only females attend the egg clutches during the breeding season. The clutch of *C. hansenae* eggs is attached to the near-vertical sides of a boulder beside a pond or on a leaf of vegetation hanging over a water body. The female sits on the clutch of eggs until the eggs hatch. During egg attendance, the ventral surface of female on the posterior half or two-thirds of the body covers the eggs, but occasionally all eggs are covered by entire ventral side of female (Sheridan and Ocock, 2008).

### Study site

The Sakaerat Environmental Research Station (SERS) is located on the southwestern edge of the Khorat Plateau, in Nakhon Ratchasima province, Northeastern Thailand (14°30' N, 101°55' E) as shown in Figure 1. The following climatic data is taken from Sakaerat Environmental Research Station (2012). The climate of this area is tropical; the summer is hot and humid, while the winter is cool and dry. The mean annual temperature ranges from 16 to 35 °C. The rainy season occurs from May to mid-October, with rainfall peaking in May and September. The mean annual precipitation is 1,200 mm. The entire area of SERS is about 80 km<sup>2</sup>. The elevation in this area ranges from 250 to 762 m above sea-level; Khao Phiat is the highest mountain. Bedrock is exposed only along streams; elsewhere there is heavy soil and vegetation cover. The entire area appears to be underlain with sandstone. The SERS was declared as a United Nations Educational, Scientific and Cultural Organization (UNESCO) biosphere reserve in 1977 (Thailand Institute of Scientific and Technological Research, 2001).



**Figure 1** Location of Sakaerat Environmental Research Station.

SERS has two major natural forest types, both of which are primary forests (more than 400 y old). Most of the area is dense, dry evergreen forest, except for the North and Northeast sections where open, dry dipterocarp forest occurs. Several small areas of bamboo clumps are found in the dry evergreen forest at higher elevations. The dry evergreen forest is dense with a four-storey canopy and covers 60% of the station area. Covering 18% of the station area, the dry dipterocarp forest occupies the rolling hills where sandstone boulders and laterite are common. The dry dipterocarp forest is open, generally consisting of uniformly spaced trees with sparse foliage allowing the sun's rays to reach the ground easily (Sakaerat Environmental Research Station, 2012).

The Tam Jong Ang (TJA) area and the Dam Pond (DAM) area were the sites for field observation on the mating behavior of *C. hansenae* in SERS. The TJA area is a water body in the dry evergreen forest that has a community of plants including: *Streblus ilicifolius* (Vidal)

Corner., *Albizia myriophylla* Benth., *Caryota mitis* Lour, *Microcos tomentosa* Sm., *Azelia xylocarpa* (Kurz) Craib, *Mitrephora vandaeflora* Kurz, *Pakia sumatrana* Miq. subsp. *streptocarpa* (Hance) H.C.F. Hopkins, *Millettia leucantha* Kurz, *Ziziphus oenoplia* (Linn.) Mill., *Zingiber zerumber*(Linn.) Smith, *Crinum asiaticum* Linn., *Dorypteris ludens* (Wall.) J. Smith, *Cissus* sp., *Lygodium flexuosum* (Linn.) Sw. and *Costus speciosus* (Koenig) Sm.

The TJA water body is a pool in a big stone lying in the stream. In the dry season, this stream lacks running water but the pool in the big stone still retains some water. In the late rainy season, the stream has abundant running water. The vegetation in the pond in the TJA area consists of *Ottelia alismoides* (Linn.) Pers. and *Leersia hexandra* Sw. among other species.

The DAM area consists of an artificial water body in the dry evergreen forest that was constructed to block off a stream to create a water supply for SERS and is located upstream of the

TJA area. In the dry season, the DAM area lacks water but has abundant water in the late rainy and early winter seasons. The plant community at the DAM water body boundary is dry evergreen forest containing species such as *Dipterocarpus turbinatus* Gaertn., *Streblus ilicifolius* (Vidal) Corner.), *Caryota mitis* Lour, *Ziziphus oenoplia* (Linn.) Mill., *Gonacaryum lobbianum* (Miers) Kurz, *Syzygium siamense*(Craib) Chantar. & J. Parn., *Aquilaria crassna* Pierre ex Lecomte and *Garcinia speciosa* Wall.

The water level of the DAM changes during the season with no water in the dry season, making the area barren. In the early to mid rainy season, rainfall stimulates plants to grow resulting in vegetative cover over the entire area. However, water is still absent in the reservoir. It is only in the latter part of the rainy season that heavy rainfall fills the reservoir. The vegetation in the pond of the DAM grows in response to the rainfall and includes: *Lygodium flexuosum*(Linn.) Sw., *Costus speciosus* (Koenig) Sm., *Chromolaena odorata*(Linn.) R. M. King, *Ludwigia hyssopifolia* (G. Don) Exell, *Melochia corchorifolia* Linn., *Mikania cordata* (Burm. F.) B. L. Robinson, *Ceratopteris thalictroides* (Linn.) Brongn and *Leersia hexandra* Sw. These plant species play an important role as a breeding site for *C. hansenae*.

### Study methods

Field observations were made in two ponds: the Tam Jong Ang area (TJA) and the Dam Pond (DAM) area. Observations were made on the mating behavior (amplexus, laying egg behavior and advertisement calls) of *C. hansenae* from October 2008 to September 2009. The study sites were surveyed 2–3 d every month. In the breeding season, the calls of males and eggs attached to vegetation were considered as evidence of mating behavior. The mating behavior of females was detected by amplexus and egg laying behavior. During daylight hours, egg clutches of *Chiromantis hansenae* were detected on leaves

hanging over the ponds (about 0.3–2 m above the water level) and also on rocks beside the ponds. Mating behavior of males was determined from advertisement calls of the frogs and amplexus. The advertisement calls of males are single note calls with a short peak “tik”. Males call from elevated leaves or branches or grass around the pond (about 0.3–8 m above the ground) to attract females. Advertisement calls were investigated by call surveys around the ponds during daylight and night time. Meteorological factors (rainfall, air temperature and relative humidity) of both study sites were based on meteorological data recorded at SERS. For data analysis, the meteorological factor data were transformed using general relativization. The mating behavior data (laying egg and advertisement calls) were transformed to a presence/absence format. The association of meteorological factors and mating behavior were analyzed using an ordination method (principal components analysis; PCA) as part of the multivariate analysis in the software package PC-ORD (version 4; MjM Software Design; Gleneden Beach, OR, USA).

## RESULTS

### Mating behavior of *C. hansenae*

The observations on the mating behavior of *Chiromantis hansenae* were made from 20 pairs, though males were seen to outnumber females in the areas studied. These frogs mated only at night but advertisement calls from males did start during the day. Adult males called from elevated perches such as tree branches or the stems of grasses around the pond. After entering amplexus (Figure 2) the female carried the male from his perch to the egg-laying site on a leaf or stem of a plant or sometimes a rock. The eggs were deposited almost immediately at the egg-laying site or the amplexus could continue for several minutes depending upon the condition of the female. While the female was being clasped, the male alternately squeezed her to stimulate oviposition. Once the male had mated

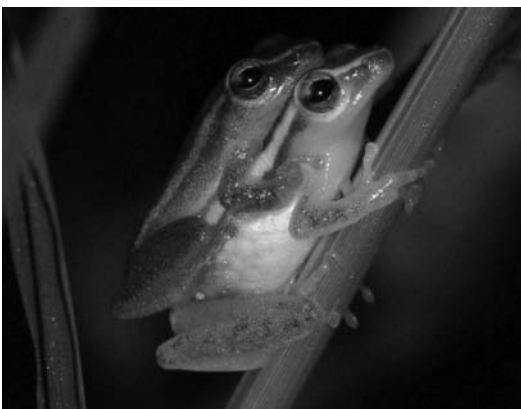
with the female, he returned to the calling site and commenced advertising for another female. After laying eggs, the female collected the eggs into a set by using her hind limbs and exhibited parental care in the form of egg attendance. The female attended to the eggs that were attached to the leaf until the eggs had hatched and the tadpoles then dropped into the water or were washed off the leaf by rain.

**Factors affecting mating behavior in *Chiromantis hansenae***

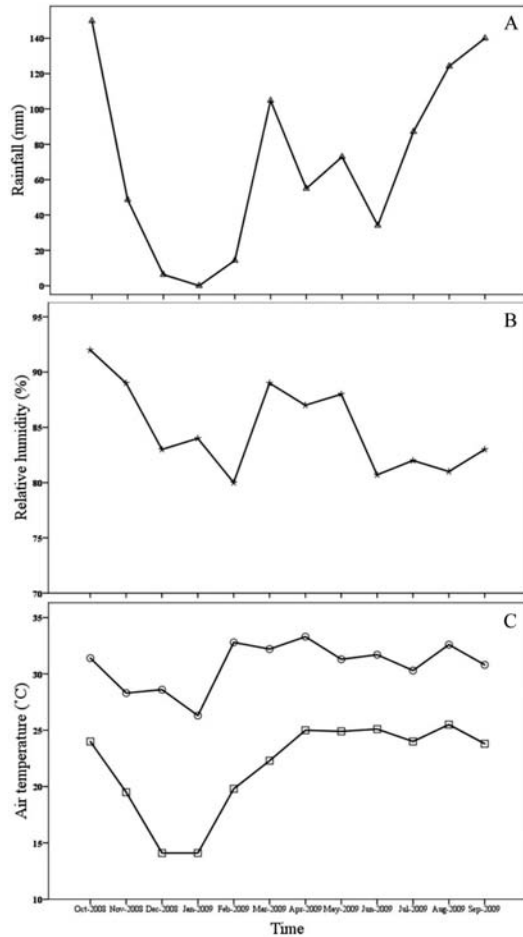
Generally, the meteorological data did not differ much from October 2008 through to September 2009 between the TJA and DAM areas. The TJA area had a mean monthly and mean annual minimum air temperature of 14.1 and 21.8 °C, respectively, a mean monthly and mean annual maximum air temperature of 33.3 and 30.8 °C, respectively, a mean annual relative humidity of 84.9% and accumulated rainfall over the period of 837.2 mm (Figure 3). The DAM area on the other hand had a mean monthly and mean annual minimum air temperature of 14.2 and 22.4 °C, respectively, a mean monthly and mean annual maximum air temperature of 34 °C and 29.7 °C, respectively, a mean annual relative humidity of 87.4% and accumulated rainfall over the period

of 931.8 mm (Figure 4). The meteorological data used in this study were based on data obtained corresponding to the period of the visits to the respective sites.

The meteorological and ecological factors that affect mating behavior of *C. hansenae* were



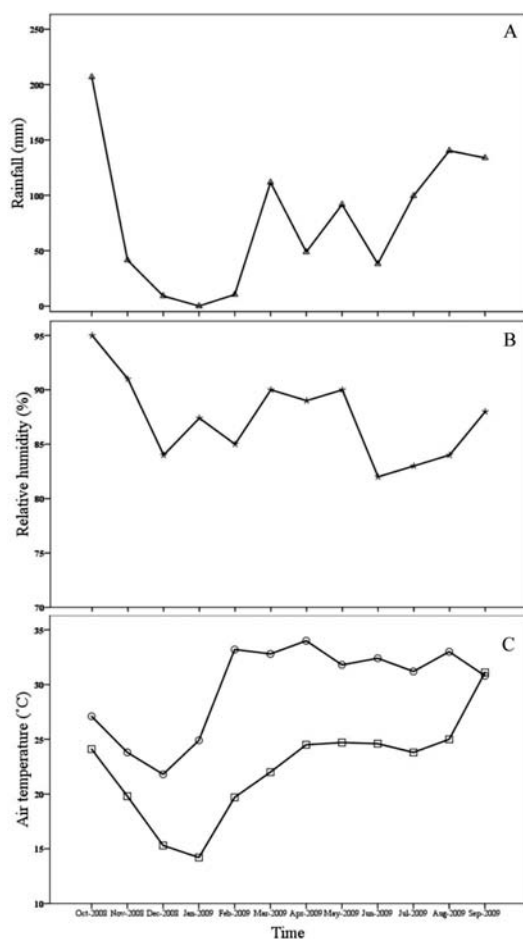
**Figure 2** *Chiromantis hansenae* male (left) catching hold of the shoulder of the female (right).



**Figure 3** Meteorological data of Tam Jong Ang area in Sakaerat Environmental Research Station during the study period: (A) Rainfall; (B) Relative humidity; and (C) Maximum and minimum air temperature. ( $\Delta$  = Monthly rainfall; \* = Mean monthly relative humidity;  $\circ$  = Mean monthly maximum air temperature; and  $\square$  = Mean monthly minimum air temperature)

analyzed by using an ordination method (PCA). PCA was utilized to investigate the correlation between the meteorological factors and mating behavior. The PCA biplot analysis is shown in Figure 5. The first axis shows that precipitation was correlated with mating behavior. The second

axis shows that temperature was correlated with mating behavior. Thus, precipitation had a higher correlation with mating behavior than temperature, while relative humidity showed the least correlation with mating behavior (Figure 5).



**Figure 4** Meteorological data of Dam Pond area in Sakaerat Environmental Research Station during the study period: (A) Rainfall; (B) Relative humidity; and (C) Maximum and minimum air temperature. ( $\Delta$  = Monthly rainfall;  $*$  = Mean monthly relative humidity;  $\circ$  = Mean monthly maximum air temperature; and  $\square$  = Mean monthly minimum air temperature)

From October 2008 to September 2009 at each study site, the mating behavior (amplexus, laying egg behavior and advertisement calls) of *C. hansenae* was also analyzed in relation to ecological factors (or ecological niches). The ecological factors of *C. hansenae* observed were a rock adjacent to the water, leaves hanging over the water or sometimes the stems of grass as mentioned before. The study found that the TJA area has suitable ecological niches for *C. hansenae* for about 9 mth. Flooding occurred during October to November 2008, but by early August 2009, drought conditions were evident. The other area, (DAM), had suitable ecological niches for *C. hansenae* for approximately 5 mth during October to December 2008 and August to September 2009.

In the TJA area, advertisement calls of the male tree frogs started approximately from March and lasted till September 2009. The female tree frogs in the TJA area mated from March to September (except during June and July 2009 when there was less precipitation), though males made advertisement calls throughout the period. In the DAM area, the males called from October to November 2008 and again in May, August and September 2009. However, the females deposited eggs only in August and September 2009. Thus, the mating behavior of males appeared to be a response to the meteorological factors to a lesser extent than for the females.

## DISCUSSION

*Chiromantis hansenae* exhibits maternal care in the form of egg attendance. Sheridan and Ocock (2008) reported that *C. hansenae* is the only species that shows parental care in Thailand.



and similarly in *Rhacophorus malabaricus*, the calling activity increases after 3–4 heavy monsoon showers (Kadadevaru and Kanamadi, 2000). Therefore, reproduction of Hansen's bush frog is prolonged and lasts through the entire rainy season. On the other hand, *Chiromantis nongkhorensis* was observed to have a short breeding season which occurred in the middle of the rainy season (August) in the SERS area.

A minor factor affecting breeding activity was the air temperature. Higher air temperatures increased mating behavior whereas lower air temperatures decreased mating behavior. The relative humidity had the least correlation with the mating behavior of *C. hansenae*.

The egg laying sites of *C. hansenae* were composed of leaves and branches of trees or shrubs hanging over a water body including grass around the pond as do other tree frog species such as *Rhacophorus malabaricus*. In contrast, *Chirixalus eiffengeri* uses the stem of bamboo for egg deposition (Kam *et al.*, 1996). Overall, heavy rain creates the suitable ecological and environmental conditions necessary for mating of *C. hansenae* and also other rhacophorids. Interference with any of these factors interferes with mating, which was quite evident in the TJA area, as this area has suitable ecological conditions for mating of *C. hansenae* all year round, but mating activity occurred after heavy rainfall (March 2009), when the environmental factors were also conducive.

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