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# Sex-based variation in the Central American frog *Rana juliani* Hillis and deSa, with notes on reproductive ecology

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THE Central American frog Rana juliani is I endemic to the Maya Mountains of Belize. Originally discovered by Julian Lee (Lee, 1976; as R. maculatum), who collected the first known specimens, it was formally described in his honour by Hillis & deSa (1988). The latter study included the species as part of the Rana sierramadrensis species group (highland species), sister to R. maculata and R. sierramadrensis, based on adult and larval morphological characters. A more recent molecular treatise (Hillis & Wilcox, 2005) placed juliani in the Rana palmipes clade of lowland species, as sister to R. vaillanti. Interestingly, this species also occurs in Belize, and juliani may be a highland form derived from it, as its morphological characters appear to be generally convergent with highland species. In spite of previous phylogenetic work, R. juliani remains a little known species, with mostly male specimens available for analysis. Lee (1996) stated that 'there are too few [female] specimens to assess sexual size dimorphism'. During postgraduate study in Belize in May and June 2004, I had the opportunity to study a population of R. juliani in an area of which it is fairly common.

to the nearby Monkey Tail River. During my visits at the beginning of the rainy season (25th, 28th and 29th May 2004), standing water was present in the creek for about 1 km of its length. Water seems to be present year-round at the creek, with some pools up to 5 feet in depth. With the onset of the rains, pools fill up and presumably flow towards the river. In addition to R. juliani, other anuran species seen at the creek were Rana berlandieri (= Rana brownorum), Eleutherodactylus sabrinus, Smilisca baudinii and Smilisca cvanosticta. All three Rana species recorded from Belize occur in sympatry at the creek, R. vaillanti having previously also been recorded in the vicinity by Gardner et. al. (2001) and Hawthorne et. al., 2003). A visual encounter survey (VES) technique was used to search for frogs along a preestablished transect that ran along the bank on the west side of the creek for 500 m (see Gardner et. al., 2001). Searches were conducted on days with rainfall, once during the day (commencing at 13:00 hrs) and once at night (commencing at 21:00 hrs) (see dates above). To standardise effort, each search was limited to one hour, giving an average

Figure 1. Adult female Rana juliani.

#### **STUDY AREA AND METHODS**

The study area consisted of a creek approximately 4 km east of the Las Cuevas Research Station (500 m elevation, 16°44'N, 88°59'W) in the Chiquibil Forest Reserve, Maya Mountains, Belize (Cayo District). The surrounding forest is of lowland tropical broadleaved rainforest type situated on underlying limestone geology. The creek itself was formed from limestone base rock and extends for approximately 5 km





**Figure 2.** Diagram of frog in dorsal view illustrating morphometric measurements taken from each specimen: 1 = internarial distance, 2 = orbit to nare distance, 3 = snout-vent length (SVL), 4 = interorbital distance, 5 = orbit to tympanum distance, 6 = head width (measured from posterior edge of tympanum), 7 = foot length (ankle to tip of outstretched 4<sup>th</sup> toe digit), 8 = tibial length, 9 = tarsal length (ankle to tip of tarsal bone when phalanges bent at 90° angle to tarsal bone), 10=thigh length (vent to tip of knee).



**Figure 3.** Diagram of lateral view of head region of frog, illustrating morphometric variables measured: 11 = orbit diameter, 12 = tympanum diameter (at widest point), 13 = head length (posterior of jaw gape to tip of snout).

Date	Day/night	berlandieri	juliani	vaillanti	Rana spp.
25/05/04	Day	0	0	0	3
	Night	2	2	0	1
28/05/04	Day	0	0	0	2
	Night	1	3	0	0
29/05/04	Day	0	0	0	1
	Night	0	8	0	0

Table 1. VES counts for Rana spp. at the creek.

search time rate of 0.5 km/h. Animals visible and/or active on the ground surface were counted, as conducting surveys after rain gave maximum chance of encountering animals in this way. All animals encountered were caught to confirm identification. Animals not caught were labelled as '*Rana* spp'. Visual encounter results are shown in Table 1. Morphometric variables recorded for each specimen were as follows: snout-vent length (SVL), interorbital distance, internarial distance, orbit-nare distance, tibial length, tarsal length, thigh length, foot length, head length, head width, orbit diameter, tympanum diameter, and orbittympanum distance (see Figs. 2 and 3). All measurements were made with digital calipers.

#### **RESULTS AND DISCUSSION**

All but one specimen of R. juliani was caught at night. Some were encountered during the day, but were well-camouflaged and too quick for capture. These may have been R. vaillanti or R. juliani, but could not be identified. Large tadpoles were also seen but were in deep pools and could not be caught. Since the tadpoles of all species of Belizean Rana attain a similar size, the tadpoles could have belonged to any of these species. No vaillanti were seen at night. Rana juliani were fairly easy to spot after dark by means of their orange 'eye shine' reflected back from torchlight. In most cases the frogs were easy to approach, not moving until a net was placed over them. One large female preserved as a voucher specimen (SSLC011, 92.6 mm SVL) contained a large 28.6 mm SVL undigested coleopteran in the stomach.

Sex	SVL	interorbital	internarial	eye - nares	head length	head width	eye diameter
Female <i>n</i> =12	83.50 ± 12.2	7.83 ± 1.16	6.91 ± 1.13	8.28 ± 1.26	28.42 ± 4.16	29.43 ± 4.46	10.73 ± 1.58
Male n=10	59.92 ± 5.78	5.11 ± 0.18	$5.12 \pm 0.62$	5.75 ± 0.51	19.41 +/- 1.50	19.79 ± 1.52	$7.68 \pm 0.74$
Sex	tympanum diam.	eye – tympanum	tibial length	tarsal length	thigh length	foot length	
Female n=12	7.19 ± 1.35	2.82 ± 0.63	55.01 ± 7.46	28.23 ± 3.88	33.93 ± 7.04	73.90 ± 10.16	-
Male <i>n</i> =10	5.21 ± 0.88	1.89 ± 0.23	36.14 ± 3.22	18.48 ± 1.61	29.86 ± 1.88	48.76 ± 4.24	-

# **Sex-based variation**

Morphometric measurements (means and standard deviations in millimetres) were taken from specimens from the creek supplemented with material from other collections (see appendix for list of specimens). These data are shown in Table 2. Maxillary teeth are present in both males and females, and vomerine tooth counts range from 5-7 (males) to 5–9 (females). In both males (n = 4) and females (n = 7) the vomerine dentary process is elliptical in shape. This characteristic does not appear to be a sexually dimorphic in R. juliani, although further specimens are needed to corroborate this. Cochran & Goin (1970) describe the vomerine teeth in Colombian Rana vaillanti and Rana palmipes (then both known as R. palmipes) as distributed 'in two small, slanting, widely separated series between the choanae' but there are no records of sexual dimorphism for this characteristic.



Figure 3. Adult female *R. juliani* in shallow water of creek having recovered after 'playing dead'.

**Table 2.** Morphometric measurements (means andstandard deviations in mm.) for *R. juliani* specimens.

## **Reproductive ecology**

There are a few details available on reproduction in *Rana juliani*. Males lack vocal slits and sacs, and most published accounts treat the species as mute and/or voiceless. However, *R. juliani* appears capable of making a distress call. One female that I handled produced loud cries and then 'played dead', assuming a completely limp posture in my hand. This behaviour had great effect in that, fearing the worst, I placed the frog in shallow water at the edge of the creek, whereupon it made an immediate recovery and escaped (see Fig. 3). I did not handle enough males to observe this behaviour. Male *R. juliani* are not known to produce an advertisement call, and it is not known how they attract females (elicit female choice),



**Figure 4**. Adult female *R. juliani* photographed at night part-buried in leaf litter.



Figure 5. Variation among individuals of *R. juliani* in character of dorsal markings.

intimidate other males, and/or hold territories. In species of Asian voiceless Rana that also lack vocal sacs, males possess fangs (odontoid processes on the lower jaw), hypertrophied jaw muscles, have longer, wider heads than females, and are also larger (Emerson & Berrigan, 1993). Male R. juliani show none of these characteristics, and have similar sexually dimorphic traits that other voiced species possess, i.e. nuptial pads on the pollex in the breeding season and smaller body size than in females. Three general causes have been cited for voicelessness in frogs: the lack of closely related species in the same habitat, the presence of a high level of background noise in the habitat, or the use of permanent breeding sites (Duellman & Trueb, 1994). Rana vaillanti, the most closely related species to R. juliani (Hillis & Wilcox, 2005) is also present at the creek, but it is interesting to note that no vocalisations have been recorded from this species in this location. Campbell (1998) describes R. juliani as a typical stream-breeding species lacking vocal slits and sacs, alluding to the aforementioned hypothesis of background noise (cited above). During my observations at the beginning of the rainy season, females were gravid but flow rate at the creek was nil. The rains had not begun in full, but I do not think that background noise could have been a significant factor in this case. An Asian voiceless species, Rana blythi (= Rana leporina) displays a female-biased sex ratio and non-clumped distribution, and males may also hold territories (Emerson & Inger, 1992).

The reproductive ecology of R. juliani has not previously been investigated. Of the total number of specimens observed at the creek, 77% were females, and almost all of these were found at or near the water's edge (one was partially buried in leaf litter; see Fig. 4). Individuals were not found in close proximity to each other, but with no quantitative data available on spatial distribution, further analysis is clearly needed to determine whether the females keep a minimum distance away from each other. Due to the timing of the onset of the rains, and the reproductive condition of the individuals concerned, the distribution pattern observed may represent females waiting for breeding males. This behaviour, however, has not been observed in R. juliani or other Rana species. All dissected females (n = 5) contained eggs, and one particularly large specimen (SSLC018, 97.7 mm SVL) collected on 29th June had a minimum clutch size of 1210 eggs. This information is concordant with the suggestion by Lee (1996) that breeding occurs during the summer rainy season. Tadpoles take around six weeks to metamorphose (Lee, 2000); the larger tadpoles I observed in the creek are possibly those of another, unidentified Rana sp. that either breeds outside the rainy season, or are from the previous year's reproductive effort and in which metamorphic development has been extended. Gravid R. juliani have also been found in February (Lee, 2000). Reproduction in R. berlandieri (= brownorum) is associated with summer rains. Rana vaillanti seems similar in this respect, but egg clutches have been found in February in Belize. It is possible that the large number of females encountered is due to their congregation at a suitable breeding site at the onset of the rains. Further study of these frogs outside the rainy season is needed to investigate sex ratios, home range sizes, and dispersion. As R. juliani is essentially restricted to riparian habitats, it may breed year round where the presence of water is permanent, with a concentrated period of reproduction during the rainy season. Some of the smaller streams along which this species occurs, however, are know to dry out in the dry season (Meyer & Farneti Foster, 1996). The position of

females along the creek banks may also indicate that they hold territories in breeding seasons. If so, sexual selection in this species may involve male choice, although the selection factors for this are unclear. Sympatric *R. juliani* and *R. vaillanti* may also hybridise.

There is an obvious requirement for the further study on the ecology and natural history of *R. juliani*. These frogs seem to be more active and easier to locate after dark, and are easily approachable once located by torchlight. One can get close enough to photograph or sketch individuals, an advantage in that each frog seems to have unique dorsal markings (see Fig. 5). Dark flecks and spots were present on the dorsum of all individuals and these marks from a unique 'fingerprint' with which to identify individual frogs. This allows for the possibility of a long-term behavioural study in the future that could shed light on the reproductive ecology and selection for voicelessness in these frogs.

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#### **Appendix: Specimens Examined**

Sam Shonleben collection (donated to the Natural History Museum, London [BMNH], awaiting accession): SSLC004–005, 008–018; creek, approx. 4 km E of Las Cuevas Research Station, Chiquibil Forest Reserve, Cayo District, Belize. BMNH 1973.2361 – 2365; Double Falls, British Honduras. UMRC 97–1 (series of 4 specimens; JCL field numbers 7029–7032); Little Quartz Ridge, Toledo District, Belize.