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# Notes on behaviour and reproduction in captive *Allobates kingsburyi* (Anura: Dendrobatidae), with comments on evolution of reproductive amplexus

P. CASTILLO-TRENN & L. A. COLOMA
*Museo de Zoología, Escuela de Biología, Pontificia Universidad Católica del Ecuador, Aptdo. 17-01-2184, Quito, Ecuador*
*E-mail: pataluca@hotmail.com*

Behaviour, reproduction and vocalizations of captive dendrobatid frogs *Allobates kingsburyi* are reported for the first time. *Allobates kingsburyi* is diurnal. Males defended their territory aggressively using acoustic and visual signals, and physical combats. Females showed aggressive behaviours only as a sign of rejection of the ♂ during courtship or during competition for food. Calls recorded in the field were a long trill of 25 · 4 seconds and 96 notes per call. The courtship was complex and it could last for several days. Males emitted acoustic signals as a long-distance attractant to the ♀, and in short-distance they performed visual and tactile signals. The most conspicuous behaviour in ♂♂ was showing the vocal sac inflated in front of possible rivals or mate. Amplexus was absent, but an axillary position with no grasping was recorded. Females lay 11–39 eggs per clutch; time of development from fertilization to hatching was 19–24 days. Males guarded the clutch and transported, for up to about 3 hours, between seven and 22 tadpoles on their dorsum to the water. Tadpoles took 88–157 days from hatchling to end of metamorphosis. Novel hypotheses are proposed about trends of evolution of reproductive amplexus in dendrobatids.

*Key-words:* *Allobates kingsburyi*; amplexus; behaviour; courtship; Dendrobatidae; parental care; reproduction; vocalization.

## INTRODUCTION

Generic and subfamily nomenclature follows Grant *et al.* (2006), although we do not follow their taxonomic split of the Dendrobatidae into two families (i.e. Aromobatidae and Dendrobatidae), but consider Dendrobatidae as a single family. *Allobates kingsburyi* (Boulenger, 1918) (Plate 1) is endemic to Ecuador (Coloma, 2005–2007); it is known from the eastern slopes of the Andes (1140–1300 m) in the vicinity of the Volcán Reventador, from the Río Pastaza trench and from several locations in the Provincia Zamora Chinchipe (Coloma, 1995; Castillo-Trenn, 2004). This species is listed as Endangered by The World Conservation Union (IUCN, 2004, 2006). Until now, nothing was known about the social and reproductive behaviour of *Al. kingsburyi*. Castillo-Trenn (2004) described the tadpole and supported the phylogenetic hypotheses of Santos *et al.* (2003) that placed *Al. kingsburyi* as the sister taxon of *Allobates femoralis* and *Allobates zaparo*. Phenetic resemblance among tadpoles of *Al. kingsburyi* and *Al. femoralis* was remarkable (Castillo-Trenn, 2004). Thus, some behavioural similarities between *Al. kingsburyi* and members of the *Allobates* clade (Grant *et al.*, 2006) were expected.

Herein, we describe the behaviours, vocalizations and reproduction of *Al. kingsburyi* and discuss aspects of their evolution among dendrobatids.

## MATERIALS AND METHODS

### Collection sites

Nine adult *Al. kingsburyi* were collected on 12 and 13 March 2000. Two ♂♂ and one ♀ were collected in Chicaña, 840 m above sea level (a.s.l.) (03°43′27 · 35″S, 78°45′20 · 8″W), and three ♂♂ and three ♀♀ in Panguitza, 800 m a.s.l. (03°53′48 · 61″S, 78°48′38 · 7″W), Provincia Zamora Chinchipe. All individuals were collected in primary and secondary forest near stream banks on

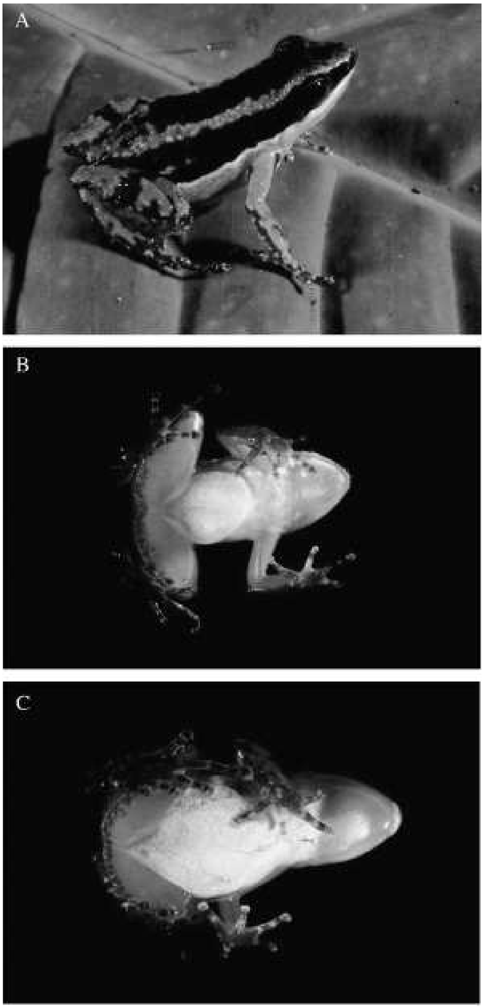

**Plate 1. (A) Adult ♀** ***Allobates kingsburyi*** **(QCAZ 27 303) from north of Panguitza (11 km south of Yantzaza), Provincia Morona Santiago, Ecuador. (B) Ventral view of adult ♂** ***Allobates kingsburyi*****. C. Ventral view of adult ♀** ***Allobates kingsburyi. Luis A. Coloma, QCAZ*****.**

vegetation and leaf litter during the morning after rain.

## Laboratory maintenance

Terrariums were designed in the manner described by Zimmermann & Zimmermann (1981) and Quiguango-Ubillús & Coloma (in press). Three terrariums were used; one with two ♀♀ and one ♂, and two terrariums with one ♀ and two ♂♂ (see individual localities in Table 1). All terrariums were 60 cm × 35 cm × 35 cm deep and were slightly inclined to sustain water. The dry portion of the terrariums was filled with leaf litter and half a coconut shell to provide shelter for the frogs. Temperature and relative humidity inside the terrariums were measured using a Hydro-Thermostat Extech. Temperature ranged between 16 and 26 °C. Terrariums were sprayed with water once a week, relative humidity averaged 86%. Adults were fed two or three times a week with adults and larvae of fruit flies *Drosophila* spp, snout beetles (Curculionidae) and young crickets *Gryllus* sp.

## Behaviour

Descriptions of most behaviours follow Zimmermann & Zimmermann (1988) and Quiguango-Ubillús & Coloma (in press). Behaviours were recorded from June 2000 to February 2002, between 0500 and 2400 hours. A total of 4388 observations (4209 behaviours and 179 inactivity events) was registered for ♂♂; 1390 observations (870 behaviours and 520 inactivity events) for ♀♀ (Fig. 1). For reproductive amplexus analyses, we used the Grant *et al.* (2006) phylogeny and characters were mapped using MacClade 4.0 software.

## Vocalizations

Captive ♂♂ were recorded between 0500 and 2400 hours. Measurements of temperature and relative humidity were taken with each vocalization. Calls were recorded with a Marantz PMD 222 tape recorder and an Optimus Condenser Microphone PA. Two 60 minute-long Maxell XL II tapes were deposited in the tape archive at the Museum of Zoology of the Pontificia Universidad Católica del Ecuador (QCAZ) (series QCAZ 103 and 104). Recordings in the field were made between 22 and 25 November 2003 at the Río Chicaña trench, 875 m a.s.l. (03°43′21·6″S, 78°45′2·6″W), Provincia Zamora Chinchipe, using a Sony Walkman Professional WM-D3 cassette recorder and a

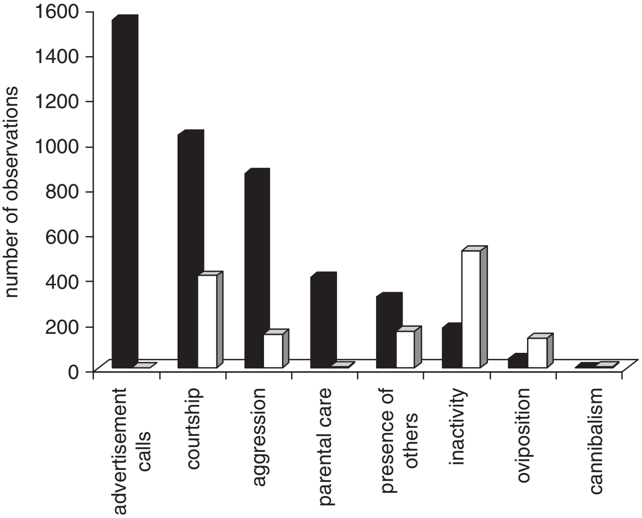

**Fig. 1. Number of behavioural features observed in the ♂♂ (black) and ♀♀ (white)** ***Allobates kingsburyi*** **in eight different contexts.**

| | GENDER | COLLECTION DATA |
|---|---|---|
| Terrarium 1 | two ♂♂ and one ♀ | collected 12 and 13 March 2000 at the Río Chicaña trench, 840 m a.s.l. (03°43′27 · 35″S, 78°45′20 · 8″W), Provincia Zamora Chinchipe |
| Terrarium 2 | two ♂♂ and one ♀ | collected 12 and 13 March 2000 1 km west of Panguitza, 800 m a.s.l. (03°53′48 · 61″S, 78°48′38 · 7″W), in the Zamora-Zumbi road, Provincia Zamora Chinchipe |
| Terrarium 3 | one ♂ and two ♀♀ | collected 12 and 13 March 2000 1 km west of Panguitza, 800 m a.s.l. (03°53′48 · 61″S, 78°48′38 · 7″W), in the Zamora-Zumbi road, Provincia Zamora Chinchipe |

**Table 1. Individuals of** ***Allobates kingsburyi*** **studied under laboratory conditions: a.s.l. above sea level.**

Sennheiser K6-ME66 microphone. One 60 minute-long Maxell XL II tape was deposited in the tape archive at QCAZ (QCAZ 152). The microphone was placed 0 · 5–1 · 5 m from the calling ♂♂, following Heyer (1994). All calls were analysed using a Macintosh computer equipped with Canary 1.2 software.

## RESULTS

### Agonistic behaviour

Males differ from ♀♀ by having a grey gular region (Plate 1). Males of *Al. kingsburyi* are territorial and constantly vocalized to establish their territories. Females are not as aggressive as ♂♂ and they moved freely in the terrariums. Female-initiated aggressive encounters between ♀♀ or between ♀♀ and ♂♂ occurred only during competition for food or when ♀♀ rejected ♂♂ during courtship. Males had preferred calling sites within their territories, either on the terrarium floor or perching on slightly elevated positions, such as small branches, rocks or on top of the coconut shell. Territories occupied by ♂♂ were used exclusively for courtship and reproduction, oviposition and parental care. Females did not establish territories and on several occasions two ♀♀ were found sharing the same refuge. Only calling ♂♂ aggressively defended their territories against other ♂♂ (non-calling and calling ♂♂) (Fig. 2). Only

**Fig. 2. Sequence of ♂–♂ aggression in** *Allobates kingsburyi* **(from top to bottom).**

when ♂♂ were guarding the clutch did they also defend their territories against all intruders, including ♀♀. The initial response of a territorial ♂ when another ♂ entered his territory was to emit loud aggressive calls and adopt an elevated body position; ♂♂ pulsated their inflated vocal sac when other ♂♂ were close to their territory, or they kept it inflated but stationary when they were not close. In most cases, visual and acoustic displays were successful for resident ♂♂ (66%) and intruders retreated without any physical combat. In 37 instances, physical encounters (fights) between ♂♂ occurred (29%). Generally, the territorial ♂ jumped in front of the intruder ($n = 35$ occurrences in 37 fights), hitting body against body ($n = 28$ occurrences in 37 fights) and repeatedly chasing the intruder away ($n = 67$ occurrences in 37 fights). Male–male wrestlings (5%), only occurred when the intruders failed to retreat ($n = 7$). The resident ♂ would jump on an intruder's head and press his body against their back ($n = 10$); he also would climb on the intruder's back and clasp the opponent's waist dorsally ($n = 26$). On 28 occasions within each fight ($n = 7$), the intruders tried to repel the attack by pushing the resident ♂ with their feet. Finally, the aggressive encounters ended with the resident ♂ chasing the intruder out of his territory. In all observations, resident ♂♂ always were successful in defending their territories.

## Courtship

Twenty-nine courtship encounters were observed. However, courtship was successful and ended in oviposition only on seven occasions. In the remaining 22 courtship encounters (75%) the ♀♀ (1) did not respond to the calling ♂♂; (2) left courtship and hid under their refuges; (3) displayed aggressive behaviours, chasing or attacking the approaching ♂♂ ($n = 130$, total aggressive ♀ behaviours observed in 22 courtship encounters). Courtship in *Al. kingsburyi* was complex and could last 2–8 days until oviposition was complete. The ♂ usually displayed several visual signals, while vocalizing from his territory. The most conspicuous behaviour in ♂ *Al. kingsburyi* was showing the inflated vocal sac in front of possible mates or rivals (see ♂ agonostic behaviour). Male *Al. kingsburyi* did not discriminate between ♀♀ and rarely left courtship, except when defending his mate or territory. However, ♀♀ discriminated between ♂♂; in terrariums with one ♀ and two ♂♂ (see Table 1) there was a significant tendency for ♀♀ (73%) to mate with the ♂ who kept his vocal sac inflated during courtship. Successful courtships occurred when the ♀ entered the territory of a calling ♂ and remained stationary. The ♂ would then approach her, move his elevated body up and down in front of the ♀ (*n* = 17 occurrences in 29 courtship encounters), and keep his vocal sac inflated between calls (*n* = 62 occurrences in 29 courtship encounters). As a sign of acceptance, the ♀ would circle the ♂ (*n* = 16 occurrences in 29 courtship encounters), stop and flatten her body in front of the ♂ (*n* = 46 occurrences in 29 courtship encounters). In 34 instances the ♂ lifted his forelimbs, one at the time, in front of the ♀, in 13 instances he touched her head and body flanks with his pads, and in 14 instances the ♂ put his inflated vocal sac onto the ♀'s head. The ♀ signalled courtship acceptance by putting her head or limbs onto the ♂'s body or head (*n* = 22 occurrences in 29 courtship encounters) or touching the ♂'s body with her snout (*n* = 18 occurrences in 29 courtship encounters). These tactile interactions between the pair lasted from several minutes to days and they were repeated between three and seven times during the courtship. On eight occasions the pair adopted a cloaca–cloaca position. The ♂ then would lead the ♀ to an oviposition site, climb on her back and amplex her in an axillary, not cephalic, position until oviposition was complete. We did not observe the time of fertilization.

## Oviposition

In captivity *Al. kingsburyi* ♀♀ laid their eggs in hidden places under the leaf litter or the coconut shell, or on slightly elevated positions, such as the leaves of live plants inside the terrariums. Oviposition can be described as follows (see also Fig. 3). During amplexus the ♀ bent her body onto the oviposition site (*n* = 11 occurrences in seven oviposition events), then she presented abdominal peristaltic movements (*n* = 7). The ♀ rotated her body several times (*n* = 77 times during seven oviposition events) before and after she laid the eggs. The pair remained in axillary position over the eggs (*n* = 14) for 7–16 minutes (*n* = 2). Finally, the ♂ released the ♀ and left the oviposition site (*n* = 12). In most cases, the ♀ left the oviposition site after the ♂ and on five occasions she remained over the eggs for 5–13 minutes. After the ♀ left, the ♂ immediately returned to the oviposition site and sat over the eggs.

## Clutches and egg hatching

Variations in clutch size and egg number are presented in Table 2. Clutches were rounded with small, pigmented eggs, covered by a gelatinous capsule. Egg number per clutch averaged 17 · 12 ± 5 · 09 (11–39; *n* = 34). Within the first day of oviposition (stages 1–2, Gosner, 1960) clutches measured between 14 · 5 mm × 11 · 3 mm and 20 · 2 mm × 14 · 2 mm (length × width) (*n* = 5); egg diameter averaged 1 · 74 ± 0 · 07 mm (1 · 58–1 · 88 mm; *n* = 78). At the blastula stage (Gosner, 1960; Del Pino *et al.*, 2004) diameter of 11 clutches ranged between 16 · 5 mm × 12 · 8 mm and 26 · 2 mm × 24 · 8 mm (length × width); egg diameter averaged 1 · 88 ± 0 · 08 mm (1 · 67–2 · 09 mm; *n* = 213). Egg hatching ranged 19–24 days (21 · 10 ±1 · 40; *n* = 10) (Table 3). Embryos had a synchronized development; however, unfertilized eggs were also found in the clutches (*n* = 23 unfertilized eggs found in ten clutches). Clutch viability (i.e. the number of eggs that hatch *vs* the number laid) averaged 83 · 73% (63 · 6–95%; *n* = 10 clutches) (Table 3). At stage 25 (Gosner, 1960) egg hatching occurred. The tadpoles took between 88 and 157 days to reach metamorphosis under laboratory conditions.

## Parental care

Parental care in *Al. kingsburyi* was carried out exclusively by the ♂♂ (*n* = 219 observations). However, on five occasions the ♀ was close to the eggs when the ♂ was vocalizing outside his refuge. Females were not aggressive when sitting near the clutch. Males

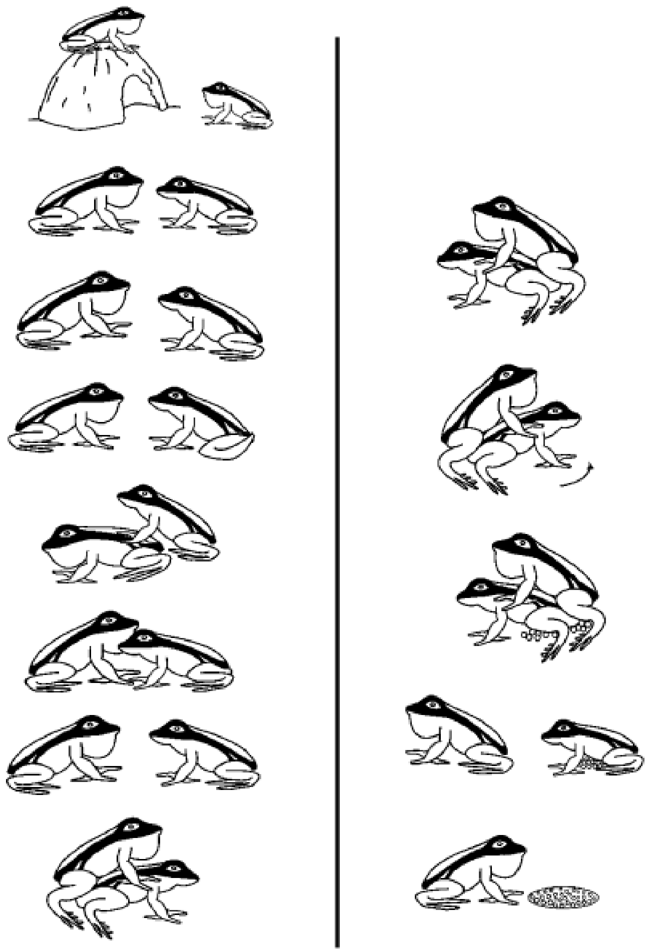

**Fig. 3. Sequence of courtship on left-hand side and sequence of oviposition on right-hand side (♂ has an inflated vocal sac) in *Allobates kingsburyi*, from top to bottom.**

displayed aggressive behaviours just after oviposition was completed. Parental care can be described as follows. Generally, the ♂ sat close to the clutch but when another frog entered his territory the ♂ vocalized (the vocal sac remained inflated between calls), raised his body and sat over the clutch (*n* = 47 observations); usually, the ♂ covered the eggs with his venter and encircled the clutch with his limbs. Males sat on top of the clutch for 15–80 minutes and, while in this position, the ♂ rotated his body over the clutch (*n* = 47 observations) and released fluids from his cloaca to moisten the eggs (*n* = 44 occasions). On 14 occasions the ♂ touched the eggs with his feet after he moistened the clutch and on 16 occasions he moved the eggs closer to his venter, which usually occurred when the clutch was large. The ♂ only left the clutch when another frog entered or passed by his territory; in these cases, the ♂ vocalized and chased away the intruder. The ♂ also left the clutch for feeding and for further courtship, which was observed on two occasions in one of the terrariums (one ♂ and two ♀♀; see localities in Table 1). The ♂ guarding the clutch began courtship with a second ♀, leading her under the

| CLUTCH SIZE (MM) | STAGE OF DEVELOPMENT | NO. EGGS PER CLUTCH | EGG DIAMETER (MM) |
|---|---|---|---|
| 14·50 × 11·30 | 1 | 11 | 1·70 ± 0·06 (1·59–1·76) |
| 16·2 × 15·30 | 2 | 14 | 1·74 ± 0·03 (1·69–1·80) |
| 16·96 × 14·90 | 2 | 21 | 1·75 ± 0·05 (1·64–1·82) |
| 15·20 × 11·80 | 2 | 15 | 1·67 ± 0·05 (1·58–1·72) |
| 17·40 × 14·80 | 3 | 17 | 1·83 ± 0·03 (1·78–1·88) |
| 17·40 × 15·60 | 7–8 | 17 | 1·88 ± 0·05 (1·88–1·96) |
| 18·90 × 16·40 | 7–8 | 15 | 1·86 ± 0·06 (1·67–1·89) |
| 18·64 × 15·5 | 7–8 | 13 | 1·82 ± 0·03 (1·76–1·88) |
| 18·50 × 17·80 | 7–8 | 15 | 1·89 ± 0·03 (1·84–1·94) |
| 26·20 × 24·80 | 7–8 | 39 | 1·94 ± 0·02 (1·90–1·96) |
| 16·52 × 12·80 | 7–8 | 20 | 1·77 ± 0·05 (1·72–1·88) |
| 16·96 × 14·60 | 7–8 | 15 | 1·95 ± 0·09 (1·74–2·09) |
| 18·24 × 16·5 | 8–9 | 16 | 1·86 ± 0·06 (1·74–1·92) |
| 20·18 × 14·96 | 8–9 | 19 | 1·81 ± 0·07 (1·68–1·89) |
| 24·18 × 21·63 | 8–9 | 23 | 1·94 ± 0·03 (1·88–1·98) |
| 19·60 × 18·87 | 8–9 | 21 | 1·90 ± 0·05 (1·82–2·01) |

**Table 2. Clutch size and number of eggs of *Allobates kingsburyi* at different stages of development (Gosner, 1960) Egg-diameter values are means ± SD followed by the range in parentheses.**

| NO. QCAZ TADPOLES | NO. EGGS PER CLUTCH | NO. TADPOLES TADPOLES/ CLUTCH | TIME TO HATCH (DAYS) | NO. TADPOLES CARRIED BY ♂ | CLUTCH (%) |
|---|---|---|---|---|---|
| | 11 | 7 | 19 | 7 | 63·64 |
| 24 643 | 11 | 9 | 20 | 8 | 81·82 |
| 24 642 | 12 | 10 | 21 | 10 | 83·33 |
| 24 646* | 13 | 10 | 21 | — | 76·92 |
| 24 641 | 15 | 12 | 20 | 11 | 80·00 |
| 24 639 | 17 | 14 | 21 | — | 82·35 |
| | 17 | 15 | 22 | 15 | 88·24 |
| | 19 | 17 | 21 | — | 89·47 |
| 24 640 | 23 | 22 | 24 | 20 | 95·65 |
| 24 644 | 24 | 23 | 22 | — | 95·83 |
| Mean | 16·2 ± 4·7 | 13·9 ± 5·4 | 21·1 ± 1·4 | 11·8 ± 4·9 | 83·73 |
| Sample size | 10 | 10 | 10 | 6 | 10 |

**Table 3. Egg developmental time, number of eggs per clutch, number of tadpoles per clutch and clutch viability of *Allobates kingsburyi*. Some data (—) represent tadpoles not carried by the ♂. Values are means ± SD over the sample size. *Male abandoned the clutch; tadpoles were released into the water.**

coconut shell where he was guarding the first clutch, and the second ♀ laid her eggs a few centimetres away from the first clutch. The ♂ then guarded both clutches. On 20 occasions ♂♂ abandoned their clutches and all these were found rotten and apparently with fungal infections. On one occasion the ♂ abandoned one clutch with ten tadpoles (see Table 3). Tadpole data are provided and discussed by Castillo-Trenn (2004).

Only the ♂ transported the tadpoles on his back. When the ♂ sat over the clutch and curved his dorsum the movements of the tadpoles' heads and tails were evident. Tadpoles climbed onto the ♂'s dorsum via his legs, flanks or inguinal region, and adopted a vertical-line position close to each other. This process was only observed on two occasions; the ♂ took 70 and 50 minutes to pick up the entire clutch of 15 and ten tadpoles, respectively. The ♂ transported an average of 12·5 ± 5·4 tadpoles on his back (7–22; *n* = 6) for 1 hour 23 minutes up to about 3 hours, until he released them all into the water. On four occasions the ♂ abandoned tadpoles in the clutch, abandoning two tadpoles in the same clutch and three other tadpoles in three different clutches. Only two tadpoles were preserved in 10% formalin and deposited at QCAZ (QCAZ 24 643a and 24 641a); the remaining tadpoles were infected with fungi and did not survive. On five occasions a ♀ ate the tadpoles that were deposited in the water by the ♂. On four of these occasions, it was uncertain if the ♀ who ate the tadpoles was the mother as this behaviour was observed in one of the terrariums with one ♂ and two ♀♀ (see Table 1). Only on one occasion in the terrarium with one ♀ and two ♂♂, was the ♀ (i.e. the mother) observed to eat the tadpoles that were in the water; however, we were unable to determine if the cannibalism occurred immediately after deposition because the tadpoles were found in the water.

### Vocalizations

The advertisement call of captive *Al. kingsburyi* was a long trill with an average length of 16 · 87 ± 0 · 6 seconds (16 · 30–17 · 52; *n* = 14). Average notes per call was 37 · 22 ± 4 · 51 (34 · 25–42 · 40; *n* = 14). Note duration ranged 74 · 22–91 · 58 ms (80 · 29 ± 9 · 79); intervals between notes ranged 317 · 45–435 · 22 ms (379 · 67 ± 59 · 17). The fundamental frequency averaged 1788 · 14 ± 88 · 43 Hz (1699 · 37–1876 · 24); the dominant frequency averaged 3407 · 23 ± 338 · 29 Hz (3017 · 53–3625 · 41).

The first author recorded a series of 29 calls of *Al. kingsburyi* at the Río Chicaña locality, 875 m a.s.l. (03°43′21 · 6″S, 78°45′2 · 6″W), Zamora Chinchipe. Males vocalized on the substrate, under the leaf litter or on slightly elevated position above the ground. Calls were recorded between 0800 and 1900 hours. However, the highest calling activity was recorded between 1500 and 1900 hours, when the temperature ranged 27–34 °C and the relative humidity was 71–86%. Calls were long trills with an average length of 21 · 01 ± 3 · 3 seconds (18 · 18–25 · 43; *n* = 29) and ranged 62 · 80–96 · 20 notes per call (81 · 18 ± 14 · 01; *n* = 29). The duration of the note ranged 29 · 07–41 · 43 ms (35.07 ± 6.23); intervals between notes ranged 200 · 38–269 · 70 ms (235 · 68 ± 34 · 37). The fundamental frequency averaged 2247 · 76 ± 75 · 65 Hz (2163 · 55–2347 · 47) and the dominant frequency averaged 4460 · 68 ± 179 · 82 Hz (4270 · 33–4704 · 30) (Fig. 4).

## DISCUSSION

Behaviours reported herein for *Al. kingsburyi* are remarkably similar to those observed in other members of the *Allobates* genus. Extensive territorial defense, as described for ♂♂ of *Al. kingsburyi*, has also been observed in *Al. femoralis* (Roithmair, 1992; Narins *et al.*, 2003) and *Allobates marchesianus* (Juncá, 1998). Territories seem to serve different functions for ♂♂ and ♀♀ in *Al. kingsburyi*. Females had hidden places under the leaf litter that were used exclusively for shelter, and they only became aggressive when feeding and when rejecting courting ♂♂. This behaviour differed from *Hyloxalus* and *Epipedobates* spp for which the ♀♀ are very aggressive and territorial (e.g. *Hyloxalus toachi*, Quiguango-Ubillús & Coloma, in press). Fights between *Al. kingsburyi* ♂♂ served to maintain their territorial status and provide them with the space required for mate attraction and reproduction.

Diurnal species of anurans use visual displays for communication (e.g. Wells, 1980; Haddad & Giaretta, 1999) and to minimize the risk of physical damage from combat. Males of *Al. kingsburyi* were extremely aggressive when defending their territories; however, the first response to an intruder was the display of both acoustic and visual signals. The most conspicuous behaviour in ♂♂ of *Al. kingsburyi* was showing the inflated vocal sac in front of possible rivals or a mate. The evolution of visual communication in some species of frogs has apparently been favoured by the availability of ambient light (see references in Haddad & Giaretta, 1999). During field observations at the Rio Chicaña trench, ♂♂ of *Al. kingsburyi* were particularly inconspicuous when calling, resembling the dark colour of the substrate; however, the venter and throat collar were weakly pigmented. This contrasting colour of the gular

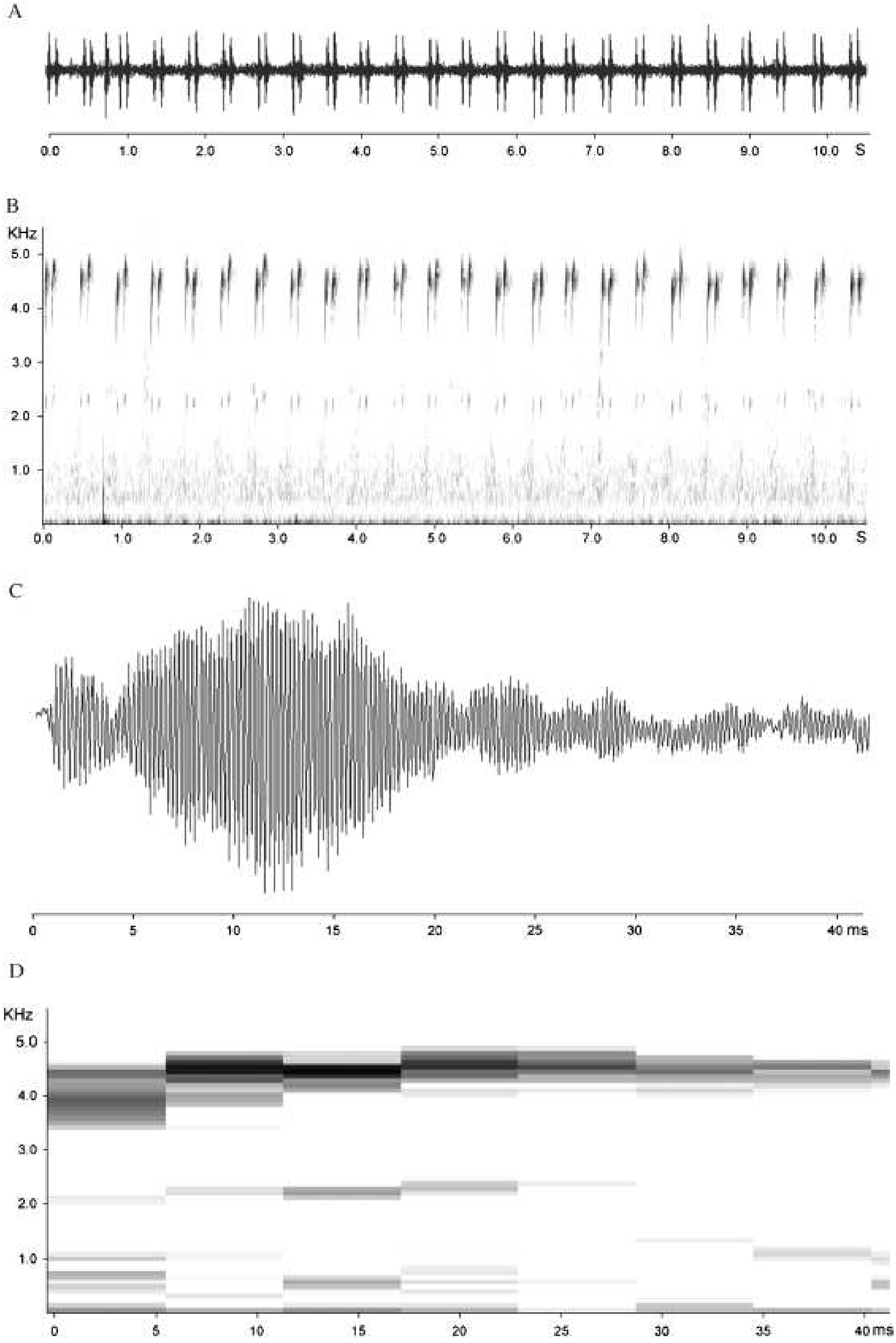

**Fig. 4. Advertisement call of *Allobates kingsburyi*: (A) section of an oscillogram of 15 seconds, (B), section of a spectrogram (C), note and (D) spectrogram of a note.**

region was evident and it might explain why ♂♂ of *Al. kingsburyi* display the vocal sac constantly in front of conspecifics, and probably it serves as a visual signal for territorial advertisement. The latter has also been experimentally tested in *Al. femoralis* (Narins *et al.*, 2003); physical attacks by a territorial ♂ of *Al. femoralis* are provoked only in response to dynamic bimodal stimuli (vocalizations and vocal-sac pulsations). Several species with non-evident sexual dimorphism (e.g. *Mantella laevigata*: Heying, 2001; *Al. kingsburyi*, pers. obs) display visual behaviours to distinguish between sexes. The display of the vocal sac in *Al. kingsburyi* was not only effective as a sign of territoriality but also it seems to increase the ♂ reproductive success. Females were discriminatory and usually left courtship before oviposition; however, ♀♀ most frequently responded to ♂♂ that appear to display their inflated vocal sac longer when calling. Usually, courtship was interrupted when the ♀ was not receptive or when the ♂ ceased courtship to chase the other ♂ away from his territory. Males were polygenic and did not discriminate between ♀♀; this behaviour has also been observed in *Ameerega pictus* (Weygoldt, 1987).

Cannibalism seems to be a significant source of mortality for tadpoles of a variety of tropical frogs that breed in extremely small pools (Crump, 1992). However, it is uncertain why the ♀♀ of *Al. kingsburyi* in our study showed cannibalism and it is possibly that this behaviour was induced by conditions in captivity and needs to be investigated further.

The call of captive *Al. kingsburyi* differs from that of ♂♂ from Chicaña, Zamora Chinchipe, in: length (17 *vs* 21 seconds); notes per call (37 *vs* 81 notes); note length (80·29 *vs* 35·07 ms); intervals between notes (379·67 *vs* 235·68 ms). The call also has a lower dominant frequency (3407·23 *vs* 4460·68 Hz) (Table 4). The big differences between frequencies and other call parameters of captive and field calls of *Al. kingsburyi* are perplexing and require further research. Thus, researchers should use extreme caution with these kind of data (with different recording conditions) to discrimi-

| | *n* | CALL DURATION (SECONDS) | INTERVAL BETWEEN CALLS (SECONDS) | NOTES PER CALL | NOTE DURATION (MS) | INTERVAL BETWEEN NOTES (MS) | FREQUENCIES | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | FUNDAMENTAL (HZ) | DOMINANT (HZ) |
| A | 4 (29) | 21·0102 | 17·6544 | 81·1786 | 35·0735 | 235·6832 | 2247·764 | 4460·6801 |
| Range | | 18·1837–25·4253 | 16·8117–19·3373 | 62·8000–96·2000 | 29·0649–41·4340 | 200·3827–269·7030 | 2163·5477–2347·4670 | 4270·3331–4704·2996 |
| SD | | 3·3390 | 1·1495 | 14·0096 | 6·2302 | 34·3680 | 75·6526 | 179·8161 |
| B | 3 (14) | 16·8707 | 19·8232 | 37·2167 | 80·2852 | 379·6659 | 1788·1374 | 3407·2270 |
| Range | | 16·2955–17·5216 | 13·2104–27·5545 | 34·2500–42·400 | 74·2176–891·5757 | 317·4473–435·2168 | 1699·3693–81 876·2352 | 3017·5320–3625·4064 |
| SD | | 0·6166 | 7·2372 | 4·5045 | 9·7869 | 59·1672 | 88·4349 | 338·2913 |

**Table 4. Comparison of seven characters between field (A) and laboratory (B) calls of ♂ *Allobates kingsburyi*: *n*, sample size, number of ♂♂ and (in parenthesis) the number of calls analysed. Values are means, ranges and standard deviations (SD).**

nate between species or in comparative studies. It might play a role factors such as temperature differences, interference present in the two different habitats where the calls were recorded, atmospheric pressure differences (lowland field site *vs* high-altitude laboratory, T. Grant, pers. comm.), size and health condition of animals in the field *vs* terrariums.

## Comments on reproductive amplexus

Current progress on the knowledge of dendrobatid phylogeny (Santos *et al.*, 2003; Vences *et al.*, 2003; Grant *et al.*, 2006) has provided the framework for either the reinterpretation of previous hypotheses of dendrobatid behaviour or the proposal of novel ones. However, missing behavioural data for most species, small sampling of some observed behaviours, doubts regarding observation in captive animals, and problems with accurate definitions of characters and transformation series, should not be dismissed. We recognize the aforementioned factors as a source of problematic or erroneous interpretations (following Grant *et al.*, 2006: 97). Nonetheless, here we focus, on reproductive amplexus and its variation in light of new information gathered in our laboratory studies (Quiguango-Ubillús & Coloma, in press; this study).

Variation in reproductive amplexus of dendrobatids and outgroup taxa has been coded by Grant *et al.* (2006) in three states: absent, axillary and cephalic. Herein, the two latter are defined as having a grasping component. Grant *et al.* (2006) proposed that axillary amplexus was lost in the most recent common ancestor of dendrobatids and that cephalic amplexus evolved independently in *Anomaloglossus* and (by implication) in other dendrobatids. Also, Grant *et al.* (2006) suggested that cephalic grasping (used for wrestling and courtship) is a first intermediate step towards cephalic reproductive amplexus. Both hypotheses of Grant *et al.* (2006) are contrary to some previous interpretations (e.g. Duellman & Trueb, 1986; Myers *et al.*, 1991), but their first hypothesis supports Weygoldt (1987) assertion that cephalic amplexus is secondarily derived.

Our observations suggest new and more complex scenarios of trends on the evolution of reproductive amplexus. Anomaloglossinae dendrobatids [as represented by *Rheobates palmatus* (see Lüddecke, 1999)], Allobatinae (as represented by *Al. kingsburyi)* and a derived clade within Hyloxalinae [as represented by *H. toachi* (see Quiguango-Ubillús & Coloma, in press)] do not have the grasping component of the axillary amplexus, but they have an axillary position during oviposition, although the axillary position of *Al. kingsburyi* differs slightly from that of *H. toachi* (compare Fig. 3 *vs* Fig. 4F of Quiguango-Ubillús & Coloma, in press). In contrast, complete absence of axillary amplexus apparently is a plesiomorphic feature within Dendrobatidae. It is absent in *Anomaloglossus beebei*, Dendrobatinae and *Hyloxalus azureiventris* (sensu Grant *et al.*, 2006). A possibility exists that samples of *An. beebei* (from Guyana: Mount Ayanganna 1490–1550 m a.s.l.) for which molecular data were analysed by Grant *et al.* (2006), and frogs (from Guyana: Kaieteur Falls, 450 m a.s.l.) for which behavioural observations were made by Bourne *et al.* (2001), are different taxa. The altitudinal difference between both samples is striking. Bourne *et al.* (2001) suggested that the frogs they studied did not belong to dendrobatinae, the latter suggestion is also supported by T. Grant (pers. comm.), based on the occurrence of a median lingual process in both samples. In any case, further verification of species identification of samples from Mount Ayanganna is required, as well as a molecular phylogenetic analysis including topotypic samples. Also, it is noteworthy that axillary amplexus is also lost in a Hylodidae frog *Crossodactylus gaudichaudii* (see Weygoldt & Potsch de Carvalho e Silva, 1992), a family that is hypothesized to be the sister group of Dendrobatidae (Grant *et al.*, 2006). Thus, axillary amplexus with a grasping component (sensu stricto) was lost in a recent common ancestor of dendrobatids and possibly hylodids, and modified to an axillary position with no grasping in some basal taxa, such as Allobatinae and *R. palmatus*.

Cephalic amplexus is ovipositional (OCA) or preovipositional (POCA, pseudo-amplectant cephalic grasping for courtship of Grant *et al*., 2006: 100). Available data indicate that OCA is only present in Colostethinae (although there are no observations in *Silverstonia*), and *Al. marchesianus*, whereas POCA may have evolved one to three times independently in dendrobatids (in *An. beebei*, *H. toachi* and/or *Minyobates steyermarki*). Observations of POCA in *An. beebei* and *M. steyermarki* are scarce and require further verification. Because *H. toachi* is not basal taxa in the Hyloxalinae clade it remains to be confirmed how widespread POCA is in that clade. In this current scenario, there is neither support that POCA is a first intermediate step towards cephalic reproductive amplexus as suggested by Grant *et al*. (2006), nor support for POCA being a vestigial remnant of an OCA as suggested by Myers *et al*. (1991). An alternative hypothesis is that the cephalic grasping used for wrestling provided the ancestral condition from which OCA and POCA evolved independently several times. Nonetheless, further data are clearly required.

The cloacal apposition recorded as present in *Al. kingsburyi* is intriguing because it is currently considered an unambiguous synapomorphy of *Oophaga* (Grant *et al*., 2006). Closer attention to this feature needs to be placed in future behavioural studies to determine if either is a convergence in *Oophaga* and *Al. kingsburyi*, or it is more widespread among dendrobatids.

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## PRODUCTS MENTIONED IN THE TEXT

**Canary 1.2:** analysis software for Mac computers, developed by Bioacoustics Research Program of the Cornell Lab of Ornithology, Ithaca, NY 14850, USA.

**Hygro-Thermometer:** humidity and temperature meter, manufactured by Extech Instruments Corporation, Waltham, MA 02451, USA. http://www.extech.com

**MacClade 4.0:** an analytical computer program for phylogenetic analysis, written by David Maddison and Wayne Maddison, USA. http://macclade.org

**Macintosh:** computer, manufactured by Apple, Cupertino, CA 95014, USA.

**Marantz PMD 222:** portable cassette recorder, manufactured by Marantz America, Inc., Mahwah, NJ 07430-2041, USA.

**Maxell XL II:** audio-cassette tape, manufactured by Maxell Latin America, Plaza Btesh, Calle 50, Panama City, Panama.

**Optimus:** condenser microphone PA, manufactured by RadioShack Corporation, Fort Worth, TX 76102-1964, USA.

**Sennheiser K6-ME66:** microphone, manufactured by Sennheiser electronic GmbH & Co. KG, 30900 Wedemark, Germany. http://www.sennheiser.com

**Sony Walkman Professional WM-D3:** recorder, manufactured by Sony Corporation of America, Sony Drive, Park Ridge, NJ 07656, USA.

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**Supplementary material**

The following supplementary material is available for this article online:

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