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Reproductive Behavior of the Amazonian Nurse-frog *Allobates paleovarzensis* (Dendrobatoidea, Aromobatidae)

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Abstract. *Allobates paleovarzensis* is a diurnal leaf-litter anuran of the Dendrobatoidea superfamily inhabiting Amazonian forests. Based on behavioral field observations, conducted between 2008–2017 at three localities, we describe the courtship, mating, parental care behavior and territoriality of the species. Vocal activity is more intense in the morning and during the rainy season. Resident males respond aggressively to other males that invade their territories, whereas females are not territorial. Females are attracted by the advertisement call of males and enter their territories to breed. The male courtship call has a lower frequency than the advertisement call, indicating that males can modulate the frequency characteristics of their vocalizations. Amplexus stimulates the release of oocytes by females. Males attend multiple egg clutches within their territories and transport tadpoles on their backs to aquatic habitats. Males carry up to 60 tadpoles on a single transport event—the largest number ever recorded for *Allobates.* We did not record male nor female cannibalism of tadpoles. In summary, we found that *Allobates paleovarzensis* is a polygamous species with uniparental care performed by the male, complex courtship behavior, high fecundity, and territoriality related to male competition for space and females.

Keywords. Courtship; Mating; Parental care; Territoriality.

INTRODUCTION

The neotropical Dendrobatoidea superfamily, composed of 307 species in the families Dendrobatidae and Aromobatidae (Grant et al., 2006; Frost, 2017), includes species with complex reproductive behavior, territoriality, multimodal signaling, prolonged reproductive periods, costly courtship behaviors and a variety of forms of parental care (e.g., Weygoldt, 1987; Lötters et al., 2007; Wells, 2007). Several reproductive modes are present in the group, with different forest strata being used for growth and development of the young, with or without associated development within water bodies (Haddad and Prado, 2005; Wells, 2007). Parental care may involve one or both parents and offspring at different stages of development (Lötters et al., 2007).

Among the genera of the Dendrobatoidea superfamily, *Allobates* Zimmermann and Zimmermann, 1988 is the second most species-rich, with 51 described taxa (Grant et al., 2006; Frost, 2017). However, the reproductive biology of most species has never been described. Reproductive traits are useful and often used in determining species limits within the genus, since the morphological similarity between many of them contrasts with the high interspecific variability in advertisement and courtship calls, as well as spawning and parental care (Grant et al., 2017; Tsuji-Nishikido et al., 2012). An exception is *A. femoralis* (Boulenger, 1884), for which several aspects of natural history have been investigated, including courtship behavior (e.g., Montanarin et al., 2011), vocalization activity (e.g., Kaefer et al., 2012), territoriality (e.g., Roithmair, 1992; Ringler et al., 2011) and communication (e.g., Narins et al., 2003; Hödl et al., 2004). This accumulation of knowledge about the species has allowed hypotheses-testing about broader issues such as cognition (e.g., Pašukonis et al., 2013, 2014), sexual selection and the evolution of parental care (e.g., McKeon and Summers, 2013; Ringler et al., 2013, 2015, 2016, 2017).

The dendrobatoid frog *Allobates paleovarzensis* Lima et al., 2010 is a recently described species. While it has already been studied for its genetic, morphological and acoustic traits (Kaefer and Lima, 2012; Kaefer et al., 2013), the reproductive behavior is poorly known (Rocha et al., 2018). Thus, this study aimed to describe vocalization, courtship and mating behavior, parental care and territoriality of *A. paleovarzensis.* This knowledge might aid future studies on the systematics and evolution of reproductive behavioral traits in *Allobates,* as well as in Dendrobatoidea generally.

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MATERIALS AND METHODS

Field work was conducted at three localities (WGS84; EPSG: 4623), all in the state of Amazonas, Brazil: municipality of Careiro da Várzea (03°22′26.3″S, 59°52′06.4″W), type-locality of *Allobates paleovarzensis* (Lima et al., 2010); Hiléia (03°11′52.80″S, 60°26′33.00″W) in the municipality of Manacapuru; municipality of Barcelos (00°54′57.70″S, 62°58′14.30″W). According to genetic and phenotypic evidences, populations from these localities are *A. paleovarzensis* (Kaefer et al., 2013). The mean annual precipitation varies from 2000–2400 mm (Sombroek, 2001). The rainy season occurs from November–April, and the average annual temperature is about 26°C. *Allobates paleovarzesis* inhabits the litter of Amazonian paleo-várzea forest, which grows on alluvial deposits of Andean origin (Assis et al., 2014). Paleo-várzeas are considered transitions between *terra firme* forests (which are never flooded), and *várzea* forests (which are flooded seasonally). The region's floristics contains both *várzea* and *igapó* species (Assis et al., 2014).

Data collection was carried out on two sample track grids subdivided into 12 quadrants, each of 10 m². These were visited 6 d per week between 04:30–18:30 (GMT-04:00) from November 2008–April 2011, December 2015–April 2016, and December 2016–February 2017 in Careiro da Várzea (402 d), June 2009–April 2011 in Hiléia (131 d), and January–February 2009 in Barcelos (10 d). Captured individuals were measured for snout–vent length (SVL in mm) and adults were tagged via toe clipping (Phillott et al., 2007) to allow individual monitoring over time. Toe clipping was performed on up to two digits on each individual, a procedure found not to interfere with *Allobates femoralis* recapture rates (Ursprung et al., 2011a) and, hence, considered unharmful. Individuals were sexed by color: yellow throat in females, and violet throat in males (Lima et al., 2010).

Territories were defined on the basis of the locations of actively vocalizing males, which were monitored from 05:00–18:00 on 12 sampling days at each study locality. Females were located and georeferenced at the time of courting or in movement, and these points were used to measure the areas they inhabited. Ambient air temperature was measured with a digital thermometer each time the number of males vocalizing within the grid was estimated. Such censuses occurred for 10 min every hour, from 05:00–18:00, once per week. During these surveys a researcher walked along a standardized transect, counting actively vocalizing individuals. Records of daily patterns of vocalization activity were based on 226 hourly censuses conducted from November–January, the period of most intense vocal activity. Data for monthly total precipitation were obtained using measurements from Pluviometric Station No. 360,000 (Hydrological Information System, HidroWeb), located in Manaus, ca. 30 km from Careiro da Várzea and 110 km from Hiléia.

The number of spawns within each territory was estimated by daily censuses, during which surface leaves of the leaf-litter were examined for evidence of spawning. Each spawn was marked with a numbered plastic flag and the developmental stage, following Gosner (1960), was visually estimated every 2 d. All male individuals carrying tadpoles to bodies of water were tracked and the number of tadpoles attached to their backs was counted.

Behavioral data were obtained by the *ad libitum* and focal animal methods (Altmann, 1974). The number of observations for each behavior (*n*) refers to distinct individuals, thus constituting independent observations. Some behavioral units were observed in all individuals and in these cases the number of samples was omitted. Visual signals were described according to the classification proposed by Hödl and Amézquita (2001), Hartmann et al. (2005) and Montanarin et al. (2011).

We also recorded courtship calls of two males. Each male was recorded for approximately 3 min using a Marantz PMD660 digital recorder (44.1 kHz sampling rate; 16-bit resolution) and a Sennheiser K6/ME66 directional microphone positioned approximately 1 m from the calling site. After each recording, we measured air temperature with a digital thermometer. Data on advertisement calls derive from Kaefer and Lima (2012) and were collected and analysed according to the same methods described here.

Call measurements were obtained from recordings in Raven Pro v.1.4 (Bioacoustics Research Program, 2011). The call of *Allobates paleovarzensis* consists of groups of single notes separated by silent intervals (Lima et al., 2010). For each recording, we chose ten calls with less background noise for detailed analyses. Note measurements, including the spectral traits, were taken from the middle or next note of each call. Spectral measurements were taken after a fast Fourier transform with frequency resolution of 82 Hz and 2,048 points. We considered 10 call traits, following Kaefer and Lima (2012): Note rate (NR, in notes/s); Note duration (ND, in s); Internote interval, as the silent interval between two consecutive notes of a call (InI, in s); Call rate (CR, in calls/s); Call duration (CD, in s); Intercall interval, as the silent interval between two consecutive calls (IcI, in s); Maximum frequency (MF, in Hz); Lowest frequency (LF, in Hz); Highest frequency (HF, in Hz); Note modulation, as the difference between the highest and the lowest frequencies of the call (NM, in Hz).

Data analysis

Student's *t*-test was used to determine the difference between male and female body size and between the size of their territories and home ranges. To test the relationship between total monthly precipitation and reproductive activity (presence of spawn), a binary linear logistic regression model was used. The relationship between the number of vocalizing males each hour and the air temperature was tested using linear regression. All analyses were conducted in the R computer environment (R Core Team, 2016). The size of male and female home ranges was estimated using the Minimum Convex Polygon (MCP) method (Kenward and Hodder, 1995), with all individual location points sampled via Ranges v.9 (Kenward and Hodder, 1996).

RESULTS

Snout–vent length and individual spatial distributions

Snout–vent length varied between 19.6–22.4 mm in males ($\overline{X}$ = 20.95; *SD* = 0.67; *n* = 60), and 18.4–22.0 mm in females ($\overline{X}$ = 20.62; *SD* = 0.87; *n* = 33) and showed no significant difference between the sexes ($t = 0.34$; $P = 0.73$). The male:female sex ratio at Careiro da Várzea was 1:1 (*n* = 14:14) and at Hiléia was 1.2:1 (*n* = 17:14). Male territory size varied between 0.31–44.62 m^2 ($\overline{X}$ = 12.19; *SD* = 16.40; *n* = 9) at Careiro da Várzea and from 0.57–13.88 m^2 ($\overline{X}$ = 8.17; *SD* = 5.66; *n* = 6) at Hiléia. Female home range size varied from 5.14–15.31 m^2 ($\overline{X}$ = 10.59; *SD* = 5.12; *n* = 3) at Careiro da Várzea and from 2.99–22.10 m^2 ($\overline{X}$ = 12.55; *SD* = 13.51; *n* = 2) at Hiléia. There was no difference in male territory size between Careiro da Várzea and Hiléia ($t = 0.67$; $P = 0.51$), nor for the female home ranges at these localities ($t = -0.19$; $P = 0.87$). There was no significant difference in the size of male territories and female home ranges (Careiro da Várzea: $t = 0.25$; $P = 0.80$; Hiléia: $t = -0.44$; $P = 0.72$).

Annual reproductive activity and precipitation

The reproductive activity of *Allobates paleovarzensis,* as indicated by presence/absence of egg clutches, was related to total monthly precipitation ($z = 0.59$, $P = 0.03$, $n = 31$ months; Fig. 1), with oviposition occurring during the rainy season. Reproductive activity ceased in the driest months, when total rainfall fell below 100 mm (June–November). All observed oviposition and tadpole transportation occurred on rainy days.

Daily patterns of vocalization

Males were recorded vocalizing exclusively during the day. Call activity was most intense during the morning, with a peak around 06:00 (GMT-04:00; (Fig. 2), and was negatively related to the ambient temperature ($r^2 = 0.24$; $P < 0.01$; $n = 226$ hours).

Male *Allobates paleovarzensis* emitted four types of vocalizations: advertisement (described in Lima et al. [2010] and Kaefer and Lima [2012]), territorial, agonistic, and courtship calls (described below). Territorial calls (Video S1) were emitted by a male when an intruder entered its territory and had a lower frequency than advertisement calls. Agonistic calls (not recorded) comprised a single note in the form of an agonistic scream emitted during physical combats between males.

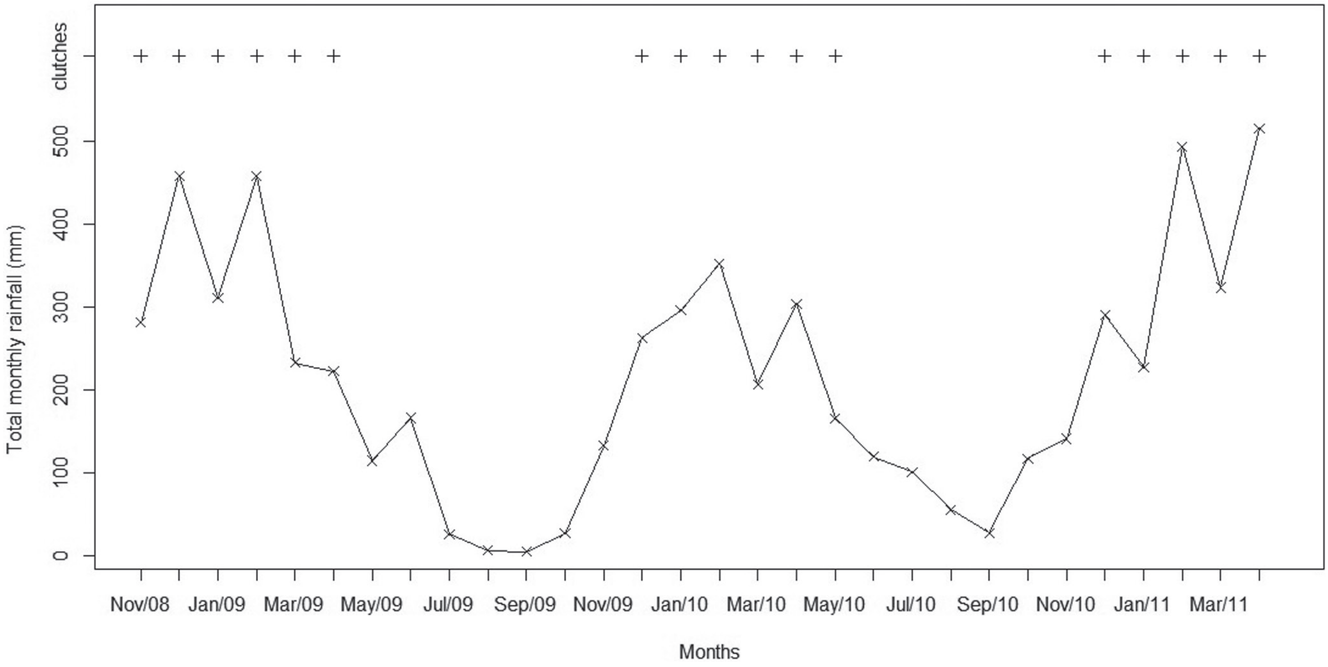

Figure 1. Reproductive activity (presence of egg clutches, +) of *Allobates paleovarzensis* in relation to total monthly rainfall (X) in the study region.

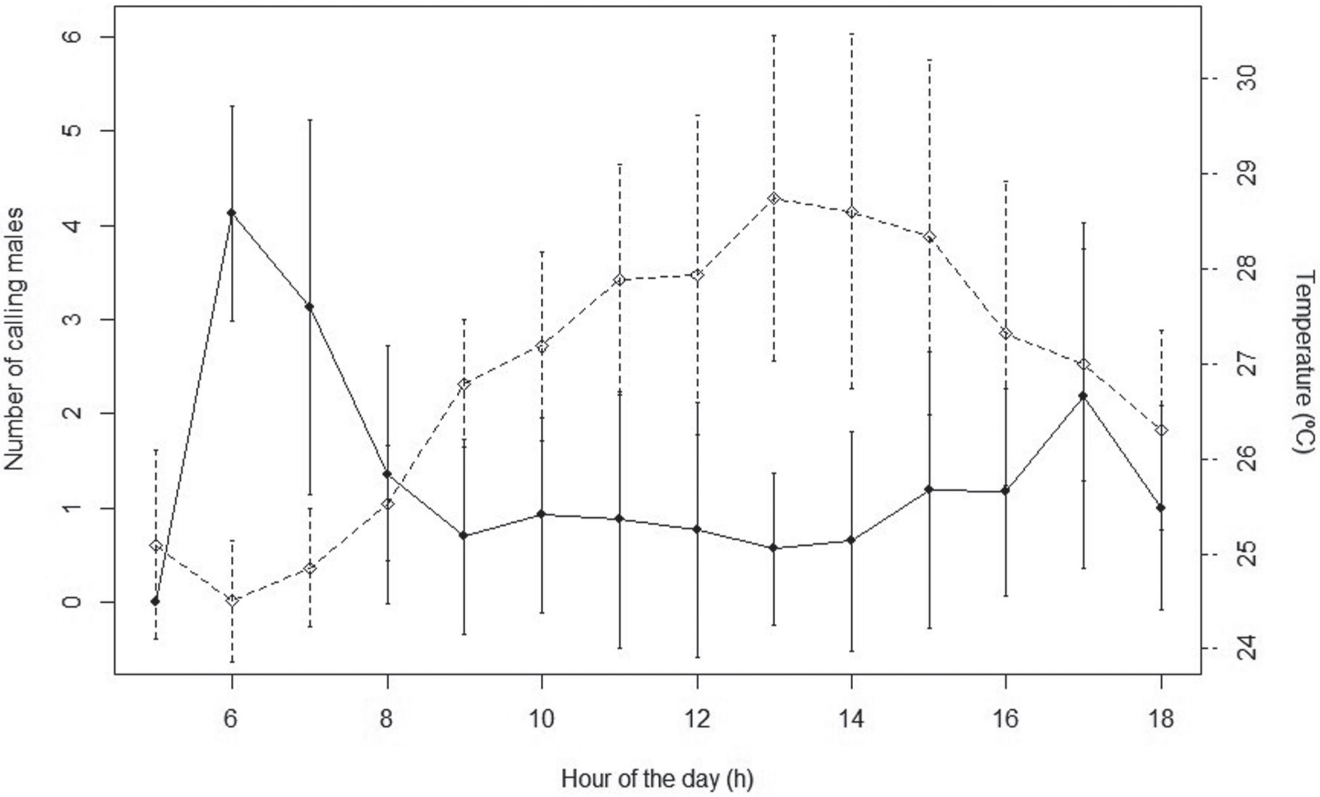

Figure 2. Daily cycle of *Allobates paleovarzensis* calling activity at Careiro da Várzea and Hiléia, Amazonas, Brazil. Black dots represent the mean number of calling males recorded in each interval at the study site. Empty diamonds represent the average air temperature in each interval at the study site. Vertical bars correspond to standard deviations.

Courtship call

Courtship calls of two males at Hiléia and Barcelos were recorded. These calls were emitted while mating pairs were close together, early in the morning (06:35, 24.2°C and 06:52, 25.3°C, respectively), after a courtship march (Rocha et al., 2018) and before cephalic amplexus. The temporal structure of the courtship call of *Allobates paleovarzensis* was that of the advertisement call (Lima et al., 2010; Kaefer and Lima, 2012), consisting of the continuous emission of groups of notes separated by silent intervals (Fig. 3A–B). However, spectral properties clearly differed from those of advertisement calls (Fig. 3C–D): courtship calls had a lower lowest frequency (3,260.3 ± 131.43, n = 10 calls and 3,446.0 ± 107.51 Hz, n = 10 calls, respectively) and higher note modulation (1,356.2 ± 214.6, n = 10 calls, and 1,639.4 ± 196.4 Hz, n = 10 calls, respectively).

Courtship, mating, and oviposition

Reproductive behavior in *Allobates paleovarzensis* included acoustic, tactile, and visual signals. We observed 39 successful courting events, all followed by amplexus and oviposition (Video S2). In almost all cases, the leaves used as nests were used for only one spawning. We recorded a single case of two spawns deposited on a single leaf. As these were of the same age, they were probably laid by different females. We also observed 23 courting attempts that were unsuccessful due to female rejection. In these cases, the female entered the male's territory but left prior to amplexus.

Pair-formation began at dawn the day before amplexus (n = 6), when the male emitted an advertising call from a raised area of leaves, branches, trunks or roots to attract the female to his territory (Fig. 4A). Early the following morning, after having detected a female in his territory, the male started emitting the courtship vocalization (n = 18). The male then moved through the territory (running-jumping display) while vocalizing, and the female followed (Fig. 4B). Males and females performed rapid up-and-down movements that involved lifting one limb and often standing on wet leaves (n = 22). The male moved onto a leaf while continuing to emit courtship calls. The female followed the male and moved onto the leaf. Some females remained on the first leaf (n = 12), while others followed the male onto subsequent leaves. After the female chose a nest leaf, the male mounted the female and grasped her head with his arms, with the dorsal surfaces of his hands contacting the female's throat and face (Fig. 4C). The pair remained in cephalic amplexus for 80 seconds on average (33–165; *SD* = 32; *n* = 25). The male moved one of his hands dorsally, sliding it in front of the female's mouth (*n* = 11). The male then moved to the side of the female and returned to his calling platform to emit advertisement calls. The female rotated around on her own axis (circling) while contracting her abdomen and releasing oocytes onto the leaf, taking 89 s on average to release the oocytes (50–140; *SD* = 33; *n* = 12). The cluster of oocytes deposited by a female on a single leaf was considered as a single spawn (nest). The female remained on the spawn for 169 seconds on average (70–285; *SD* = 101; *n* = 9) and then left the nest. Some females remained in the male territory from

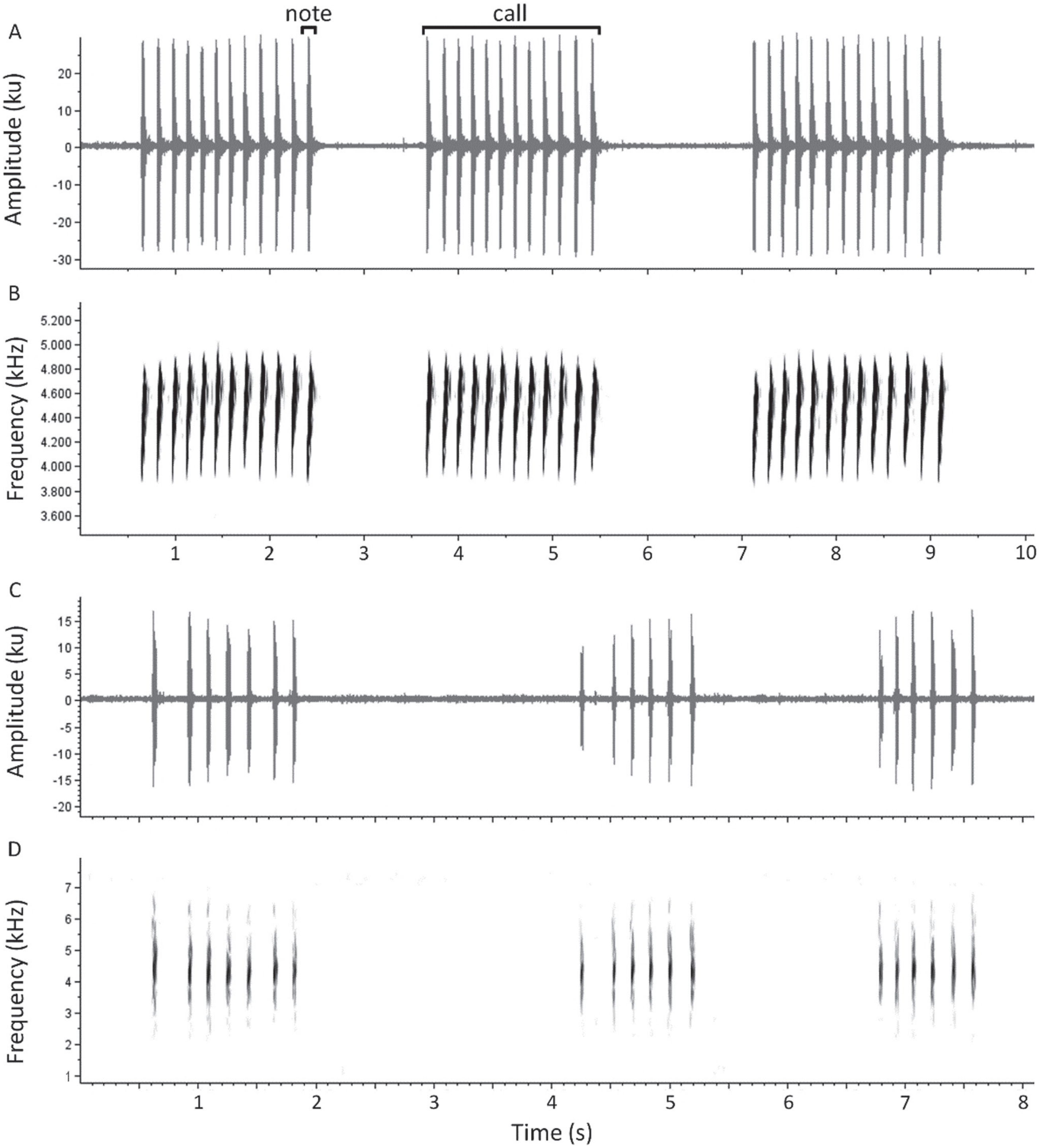

Figure 3. Acoustic signals of *Allobates paleovarzensis*. **(A)** Oscillogram and **(B)** spectrogram of three multinote advertisement calls of male *A. paleovarzensis* at its type-locality in Careiro da Várzea, Brazil. **(C)** Oscillogram and **(D)** spectrogram of three multinote courtship calls of male *A. paleovarzensis* at Barcelos, Brazil.

Figure 4. Reproductive behavior stages of *Allobates paleovarzensis*. **(A)** Male of *A. paleovarzensis* vocalizing on a leaf. **(B)** Male vocalizing and moving across its territory, followed by a female. **(C)** Cephalic amplexus. **(D)** Spawn after oviposition, in a leaf nest. **(E)** Spawning at the free tadpole stage. **(F)** Male leaving the leaf, transporting the tadpoles to a water body. Photos: SMCR.

oviposition until the next day (*n* = 8). Following oviposition, all males sat on moist substrates, such as wet leaves, before returning to the oviposition site.

Following oviposition, the male sat on the oocyte mass, pressing the eggs against the leaf with his belly and moving over the spawn, spinning around on his own axis (circling). In all observations, the gelatinous mass covering of the spawn increased in size immediately after contact with the male body. Four spawns were completely unviable from oviposition onwards, and in these cases the males did not return to the oviposition site after amplexus.

Clutch size and parental care

Mean clutch size was 29 eggs (15–50; *SD* = 7.81; *n* = 79). Recently deposited eggs were usually found in the central part of the leaf, surrounded by an opaque, gray, gelatinous substance (Fig. 4D). The mean development time from egg to tadpole (approximately stage 20–25) was 21 d (17–29; *SD* = 3.59; *n* = 17). Initially all fertile eggs had a uniform milky-gray pigmentation. After 48 h, it was possible to distinguish two distinct poles within the eggs. After fertilization, the eggs developed to embryos within gelatinous capsules (stages 1–25). During this period, the gelatin absorbs a large amount of water (Gosner, 1960). Embryos developed until the encapsulated tadpole stage, with presence of tail, operculum, as well as developed oral and digestive structures. Encapsulated tadpoles actively wriggled until their bodies were outside the capsule (Fig. 4E) and could be transported to a body of water. Successful males continued to vocalize and mate after an oviposition event and simultaneously cared for multiple egg clutches in their territory. Each clutch was usually composed of young at the same stage of development, except for any infertile eggs or embryos that died during development. Summing all the observations, an average of 44.12% of laid eggs did not develop through to hatching (0–100%; *SD* = 44.01). We recorded only one case of two clutches deposited on the same leaf (composed of 30 eggs and 27 eggs, respectively). All the tadpoles of the double spawning survived and were transported by the male in two transport events.

Nine tadpole transport events were monitored, all performed by parental males. While transporting the tadpoles to a water body, the male moved over the clutch, in the center of the jelly, and moved its feet vertically while the tadpoles actively ascended onto his dorsum. The male remained on the spawn for 480 seconds on average (300–7,200; *SD* = 216; *n* = 3). After the tadpoles were attached, the male exited the nest (Fig. 4F). During transport, the mean number of tadpoles adhering to a male's back was 25 (*SD* = 17.67; *n* = 7), ranging from 3–60 tadpoles in a single transport event. Males moved through the study area, crossing the territory of other males and even puddles, but only released tadpoles from their dorsum when they reached the edge of a body of flowing water. Here, they adopted a partially submerged position, with their heads above the water. Then the male moved his legs vertically and the tadpoles swam away from him. The male then exited the water and returned to its territory. In some cases, males submerged several times in different bodies of water until all tadpoles were released. All observed males returned to their territories following tadpole release.

Mating system and confrontation between males

Males courted different females simultaneously and mated with up to three females consecutively within a single territory, with a minimum interval of less than 20 min between two amplexi. Polygyny was common, with males having spawns from up to five different females in simultaneous development. Females were also observed in amplexus with different males, with average interval of 23 d between each amplexus (19–27; *SD* = 4.04; *n* = 3), characterizing polyandry.

We recorded fifteen events of aggressive interactions between males (e.g., Video S1). These occurred when two males attempted to attract the same female (*n* = 5) or when one male entered another male's territory (*n* = 10). Conflict began with one or both males displaying their vocal sac, either in silence or accompanied by vocalizations. The territorial call had a lower frequency than the advertisement call and was emitted simultaneously by competitors during a confrontation. Each male held his body erect and moved in small jumps next to his opponent. After such displays, one of the opponents walked away and was followed by the winner. Disputes lasted 1,260 seconds on average (240–2,700; *SD* = 889; *n* = 10). The losing male then left the territory, closely followed by the winning male. In one of the observed conflicts, which resulted in territory loss by the owner, this pursuit continued until the two individuals were approximately 12 m from the territory boundary. In this case, the nest of the loser male remained in the territory and the embryos died by desiccation.

We recorded only one confrontational event with physical contact. In this case, the intruder did not exit and was caught by the resident male. The resident male jumped on the intruder and seized its head, leaning over him and pressing him toward the ground. The resident male retained this position for 205 seconds until the intruder emitted an agonistic call. The victorious resident male then hopped to the side of the losing intruder, who remained motionless for 4 min, at which time it left the territory and concealed itself in the leaf-litter. The winning male emitted territorial calls from the start of the contest until 7 min after releasing the losing by male. In all interactions between observed males, the winning male differed from the losing male by vocalizing throughout the bout without interruption. We did not observe aggression among females.

DISCUSSION

Allobates paleovarzensis is a polygamous species with uniparental care carried out by the male and territoriality related to male–male competition for space and females. There is high investment in egg number, with the largest number of simultaneously transported tadpoles yet-known in *Allobates*. The behavioral patterns of *A. paleovarzensis* are consistent with the range of behaviors known for other species of Dendrobatoidea. Despite the sparse knowledge of the reproductive biology of species among dendrobatoids, studies show that the conquest of the terrestrial environment result in behavioral and physiological strategies that increase species reproductive success (Wells, 2007), such as polygamy and strong intra-sexual competition (Ursprung et al., 2011b), reproduction-associated territoriality (Pröhl, 2005), high investment in offspring (e.g., Lüddecke, 1999; Downie et al., 2005; Quiguango-Ubillús and Coloma, 2008), and parental care during the most vulnerable phases of offspring development (e.g., Caldwell and Oliveira, 1999; Killius and Dugas, 2014).

The lack of sexual dimorphism in body size observed here for *Allobates paleovarzensis* has also been reported for *A. marchesianus* (Melin, 1941) (Lima and Keller, 2003) and *A. bacurau* (Simões, 2016). In addition, body size is greater in *A. paleovarzensis* than many other species of the genus, although *A. alessandroi* (Grant and Rodríguez, 2001) and *A. melanolaemus* (Grant and Rodríguez, 2001), *A. femoralis* (Montanarin et al., 2011), *A. hodli* Simões et al., 2010, and *A. undulatus* (Myers and Donnelly, 2001) are as large or larger. Body size is highly correlated with reproductive investment and, for females, a large body size influences clutch size, egg size and the amount of stored energy associated with each oocyte (Summers et al., 2006; Wells, 2007). Thus, females with large body sizes can develop and carry a larger number of eggs, with more space available for egg development and possibly more yolk allocated to each egg. For males, large body size facilitates performance during confrontations with other males and the better vocal performance during territorial, courtship and territorial vocalization bouts, influencing mating (Wells, 2007). Males of some frogs may use vocalization characteristics of other males to evaluate the body size of potential opponents in agonistic encounters (e.g., Davies and Halliday, 1978; Arak, 1983; Robertson, 1984; Wagner, 1989). Ramer et al. (1983) showed that male frog *Lithobates clamitans* (Latreille in Sonnini de Manoncourt and Latreille, 1801) showed different aggressive response levels depending on the size of the potential competitor male. For *A. femoralis,* Luna et al. (2010) showed experimentally that males attacked smaller models more frequently than large ones.

During the reproductive period, male *Allobates paleovarzensis* establish territories and defend them against invading males. The territory is that part of the home range that is delimited and defended during the reproductive period and includes vocalization platforms and courtship and oviposition sites where the embryos develop to the tadpole stage (Pröhl, 2005). Exclusive courting areas might allow females to be courted without interference from other males, which is particularly important in species like *A. paleovarzensis* with prolonged, complex courtship.

Female *A. paleovarzensis* move within their home ranges, assessing males that vocalize to attract them. The home range of a female includes the territories of several different males, enabling females to evaluate different partners during the reproductive period. In *A. femoralis,* the number of effective partners per female is significantly related to the number of male candidates within an area of 20 m (Ringler et al., 2012). Polygynandry in *A. femoralis* can therefore act as a mechanism that counteracts the risk of negative effects associated with single mating decisions, especially with regard to paternal care, since females do not perform parental care and so cannot influence reproductive success after oviposition (Ursprung et al., 2011b). Studies involving the reproductive biology of several species of dendrobatoids show that polygamous mating is common and an evolutionarily stable strategy for the group (Summers, 1989, 1990; Pröhl, 2002).

In *Allobates paleovarzensis* reproductive activity is restricted to the regional rainy season. During the mating season, males vocalize throughout the day with a drop-in activity around mid-day, when the air temperature is highest. Vocalization, courtship, egg deposition, and tadpole transport all took place only on the mornings of cloudy, rainy days when the local temperature was lowest. Similar patterns of seasonal and daily cycles in reproductive activity have been reported for *A. femoralis* (Kaefer et al., 2012) and *A. subfolionidificans* (Lima et al., 2007) (Souza et al., 2017). Thus, water availability seems to be the determining factor in *Allobates* reproductive seasonality, whereas ambient temperature, which affects evapotranspiration and neuromuscular activity in ectotherms (Prestwich, 1994), appears to be the main modulator of daily vocalization patterns.

Courtship in *Allobates paleovarzensis* involves both visual and acoustic signals. Females appeared to signal visually in response to courtship calls, following males and engaging in limb lifting displays. Males appeared to display visual cues as they guided the females to the leaves and also into the nest before amplexus. Further, an acoustic signal for reproduction was emitted males in the form of a courtship call, which in many species of anurans is a modified advertisement call (Wells, 2007; Kollarits et al., 2017). However, the lack of detailed observations of the courting behavior for many species of *Allobates* means patterns of variability in this behavior cannot currently be evaluated for the group. The overall structure of the acoustic signal of *A. paleovazensis* did not differ between courtship and advertisement calls. Nonetheless, courtship calls could be clearly distinguished by static (low frequency) and dynamic (frequency modulation) properties. Future studies involving playback experiments, as well as the description of the mating call for additional species, should clarify the evolutionary implications of spectral changes in frog calls during mating interactions.

The cephalic amplexus exhibited by *Allobates paleovarzensis* is also found in other species of Dendrobatoidea (Grant et al., 2006), while the absence of amplexus in some dendrobatoids is considered a derived loss (e.g., Duellman and Trueb, 1986; Haas, 2003). The absence of amplexus can also be explained by the variety of pseudoamplectic positions employed in aggressive behavior and during courtship (Grant et al., 2006). Females of *A. paleovarzensis* showed partner choice on the day preceding amplexus, as also recorded for *A. femoralis,* where the female remains within the male territory for up to 2 d before oviposition and is courted daily by the resident male (Roithmair, 1994). Some *A. paleovarzensis* females left the territory before the amplexus, indicating that female choice is also influenced by courtship signals after attraction to a territory. In addition, females showed acceptance or rejection of the nest site prior to oviposition, actively selecting an appropriate leaf to host the egg mass. This behavior has been discussed, by many authors and for different species, as a form of prezygotic parental care (Kirkpatrick, 1985; Shuster and Wade, 2003; Royle et al., 2012).

Allobates paleovarzensis has the largest known number of eggs per reproductive event of any species in the genus, as *A. caeruleodactylus* (Lima and Caldwell, 2001) (10–30 eggs; Lima and Caldwell, 2001; Lima et al., 2002), *A. magnussoni* Lima et al., 2014 (13–18 eggs; Lima et al., 2014), *A. tapajos* (23–27 eggs; Lima et al., 2015), and *A. subfolionidificans* (5–15 eggs; Souza et al., 2017) lay fewer eggs per clutch. *Allobates paleovarzensis* also transports the largest number of tadpoles in a given transport event, although knowledge of this aspect of reproductive biology is sparse for *Allobates*. The number of tadpoles transported varies among *Allobates* spp., with some species carrying small numbers of tadpoles, including *A. magnussoni* (Lima et al., 2014) and *A. flaviventris* (Melo-Sampaio et al., 2013) with 7 tadpoles per transport, while others are known to carry large numbers, like *A. subfolionidificans* (24 tadpoles; Souza et al., 2017), *A. marchesianus* (37 tadpoles; Lima and Keller, 2003), *A. caeruleodactylus* (48 tadpoles; Lima et al., 2002). The transport of tadpoles to aquatic environments is a synapomorphy of the Dendrobatoidea (Grant et al., 2006). As in most other species of this taxon (e.g., Lima and Keller, 2003, Castillo-Trenn and Coloma, 2008; Souza et al., 2017), tadpole transport in *A. paleovarzensis* is performed by the male. Male *A. paleovarzensis* gather tadpoles from different nests, enabling them to maximize the number of tadpoles transported in a single event and possibly reduce the total transport cost per reproductive season. During transport, the male is exposed increasd predation risk and loss of territory, which can be occupied by another male, resulting in the loss of any remaining spawn (e.g., Downie et al., 2005; Ringler et al., 2013; Rojas, 2014; Pašukonis et al., 2016).

The disputes between *Allobates paleovarzensis* males can be considered as progressive stages in a combat ritual, with displays, chasing and physical confrontation. This is also known for *A. femoralis* (Luna et al., 2010). Ritualized fighting might be an evolutionarily stable strategy to avoid injury (Maynard-Smith and Price, 1973; Maynard-Smith, 1974). To minimize the risk of physical damage resulting from combat, territorial frogs exhibit a graded series of behaviors prior to physical combat (Narins et al., 2003). Results from playback experiments conducted with robotic frogs suggest that vocal sac pulsation combined with the emission of territorial calls and possession of a male body size are multimodal requirements for triggering resident male *Allobates femoralis* to attack simulated intruders (Narins et al., 2003; Luna et al., 2010). Thus, if the individuals involved in a confrontation adopt the ritualized display strategy without initial injury, as observed in most *A. paleovarzensis* bouts, disputes can be resolved without bodily harm to the participants.

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ONLINE SUPPORTING INFORMATION

The following Supporting Information is available for this article online:

Video S1. Confrontation between two males of *Allobates paleovarzensis,* with progressive stages in a ritual combat including visual displays, territorial call, agonistic call, and chasing. Video recorded with a hand-held camera by SMCR during fieldwork at Careiro da Várzea, Amazonas, Brazil.

Video S2. Male and female of *Allobates paleovarzensis* in a courtship sequence: courtship march, courtship call, amplexus, and oviposition. Video recorded with a hand-held camera by SMCR during fieldwork at Careiro da Várzea, Amazonas, Brazil.