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Reproductive biology and sexual dimorphism of the poorly known frog *Chiasmocleis alagoana* (Microhylidae, Gastrophryninae), with an updated diagnosis for the species

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ABSTRACT

Chiasmocleis is the most species-rich New World microhylid genus. Among Atlantic Forest species in the genus, *C. alagoana* has the northernmost distribution. The species was described two decades ago; however, little is known about its natural history. Herein, we provide information on its reproductive biology, advertisement call, and external morphology based on specimens from the type locality and the type series. Reproduction occurs in the rainy season but only on days with torrential rains. The advertisement call consists of a series of pulsed notes common for most species of the genus. Structurally, each note consists of two groups of distinct pulses with amplitude modulations. This call structure is a candidate of synapomorphy for a subclade of the genus consisting of *C. alagoana, C. cordeiroi, C. crucis*, and *C. schubarti*. Morphologically, the species exhibit sexual dimorphism with females being larger and twice heavier than males. Both sexes have fringes on fingers and toes. Moreover, we report the presence of dermal spines over the body and webbing on feet in males and include these characters in an update diagnosis of the species.

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Introduction

Microhylidae is one of the most diverse anuran family in the world with almost 700 described species and a pantropical distribution (de Sá et al. 2012; Frost 2020). Most species have terrestrial or semifossorial habits, but some are arboreal (e.g., some cophylines from Madagascar, Duellman & Trueb 1994; Zug et al. 2001; Andreone et al. 2005). The phylogenetic relationships of this family have been the subject of several studies that recognized 12 subfamilies (e.g., de Sá et al. 2012; Peloso et al. 2015), most of them recovered with strong support (Tu et al. 2018). Neotropical microhylids consist of three subfamilies: Adelastinae, Otophryninae, and Gastrophryninae. The last one being the most diverse, currently consisting of 11 genera and 79 species (Frost 2020).

Among gastrophrynines, *Chiasmocleis* Méhely,1904 is the most species-rich genus with 36 currently recognized species (Frost 2020), with some of them consisting of clusters of cryptic lineages (de Sá et al. 2019a, 2019b). The genus is distributed in the Atlantic Forest, Amazon, Cerrado, and Chaco biomes (Caramaschi & Cruz 1997; Cruz et al. 1997; Peloso et al. 2014). Its complex phylogenetic history is marked by multiple connections between Atlantic Forest and Amazon regions promoting independent speciations processes (de Sá et al. 2019a, 2019b). A revision of the genus synonymized the genus *Syncope* Walter, 1973 with *Chiasmocleis* (Peloso et al. 2014); however, a recent phylogenetic analysis recovered three subclades and recognized them as subgenera within *Chiasmocleis*: subgenus *Unicus* de Sá et al. 2019 to accommodate *C. gnoma* Canedo et al., 2004, and two larger subgenera: *Syncope* (10 species) and *Chiasmocleis* (25 species) (de Sá et al. 2019a, 2019c)

Chiasmocleis exhibits sexual dimorphism in several characters: snout-vent length (i.e., larger females), dark gular region in males, presence and distribution of dermal spines, and presence and degree of development of foot webbing (Cruz et al. 2007a; Tonini et al. 2014; Forlani et al. 2017). Development of foot webbing was used in the diagnosing of species (Cruz et al.

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2007b) and served as the basis for Cruz et al. (1997) to split Atlantic Forest *Chiasmocleis* species into two groups: one with developed webbing and the other with no or only basal foot webbing. However, these two groups were not recovered as monophyletic (de Sá et al. 2012; de Sá et al. 2019a, 2019b).

Advertisement call characteristics of *Chiasmocleis* are useful to identify species and to assess species relationships based on note structure (Santana et al. 2012; Forlani et al. 2013) or dominant frequency (Santana et al. 2009). *Chiasmocleis* calls consist of a series of notes formed by several pulses, a pattern suggested as a synapomorphy for the genus (Hartmann et al. 2002). The fossorial habit of *Chiasmocleis*, and its explosive breeding lasting only a few days, have hindered our knowledge of the acoustic repertoire and other aspects of the genus reproductive biology. Nonetheless, interesting aspects have been reported as bromeliad dwelling species with endotrophic tadpoles (Krügel & Richter 1995) and species with putative direct development (Moravec & Kohler 2007).

Among Atlantic Forest *Chiasmocleis, C. alagoana* Cruz et al., 1999 is the species with the northernmost distribution within the domain and is the only species to occur north of the São Francisco river (Cruz et al. 1999; Forlani et al. 2017). The species is known from the type locality (Municipality of Maceió, Alagoas State) and in a few localities in the States of Paraíba and Pernambuco in the northeastern Brazil (Santana et al. 2008; Santos & Amorim 2010; Moura et al. 2011; Almeida et al. 2016). The species was included in the group of species without foot webbing (Cruz et al. 1999); however, a recent phylogeny placed *C. alagoana*, with other northern Atlantic Forest species, in the subgenus *Chiasmocleis* (de Sá et al. 2019a, 2019b). *Chiasmocleis alagoana* was listed as Data Deficient (DD) in the IUCN Red List of Threatened Species (Freire & Silvano 2004). Nevertheless, it is listed as Endangered in the Brazilian Threatened Fauna Red List (ICMBio 2018) due to its restricted distribution within forest fragments undergoing severe degradation and probably genetic isolation among populations. The tadpole was previously described (Nascimento & Skuk 2006); however, overall the species remains poorly known.

The reproductive biology of *C. alagoana* was observed during field trips to the type locality. Herein, we report on the breeding behavior, including its advertisement call, and assess its sexual dimorphism to complement and update the diagnosis of the species.

Material and methods

Study area

The study was conducted at the type locality of *Chiasmocleis alagoana*, a remnant of Atlantic Forest north to the São Francisco river known as Mata do Catolé (9°32’42 “S, 35°47’49 “W, 113 m a.s.l.; datum WGS84) within the Environmental Protection Area of Catolé and Fernão Velho. The area comprises about 37 km2 in the municipalities of Maceió, Rio Largo, Satuba, Santa Luzia do Norte, and Coqueiro Seco (Oliveira et al. 2014), Alagoas State, Brazil. The area vegetation is classified as an ombrophilous forest (Assis 2000) and its climate consists of a dry season (October to March) with high temperatures (~21–29ºC) and little rainfall (~400 mm) and a wet season (April to September) with lower temperatures (~19–28°C) and higher rainfall (~1300 mm; Vilela et al. 2015).

We sampled at two temporary ponds within the forest, about 300 m from the edge of the fragment, separated about 1.4 km from each other. The smallest pond (i.e., pond 1) has approximately 470 m2 and 0.5 m maximum deep whereas pond 2 is approximately 950 m2 with 0.6 m maximum deep. Both ponds had clear water, bottom surface with extensive sediments, and dense semi-aquatic vegetation.

Data collection and analysis

We carried biweekly field surveys from April to November 2011 (total 15 days of field work) from the beginning of water accumulation and ponds’ formation to their complete drying. Sampling usually began at 5:00 p.m. (sunset) and lasted until 11:00 p.m. when the species calling activity decreased. At each pond, we recorded air temperature and relative air humidity with a digital thermo-hygrometer (0.1°C precision) and recorded pond size (i.e., measured area covered by water and maximum depth). The precipitation data for fieldwork days were downloaded from a nearby station (UFAL-INMET station, Secretaria de Estado de Meio Ambiente e Recursos Hídricos de Alagoas; http://www.semarh.al.gov.br, accessed in October 2015). During fieldwork, we observed the presence/absence of adults, juveniles, and tadpoles of the species and adults breeding activity by means of *ad libitum* and animal-focal sampling recording all behaviors of interest (Altmann 1974). The perimeter of each pond was covered three times to quantify all males in calling activity, as well as their microhabitat, we kept only the route that had the maximum number of individuals (adapted from Gottsberger & Gruber 2004).

We recorded calls from seven males (two vouchers collected, MUFAL 10962 and 10981) on July 12 and 23 August 2011 (air temperature = 20.8–20.9°C; relative air humidity = 100%). Recordings were made with a Sony PCM-D50 digital recorder coupled with a Sennheiser ME66/K6 unidirectional external microphone, with a sample frequency rate of 44.1 kHz and 16-bit resolution (WAVE file format), and positioned about 0.5 m from the calling males. Sound samples were analyzed with Raven Pro 1.4 software for Windows (Cornell Lab of Ornithology, Bioacoustic Research Program, 2011). For temporal parameters, we used the waveform window. The spectrogram parameters were Hann window, fast Fourier transformation 256 points, 3 dB filter bandwidth 248 Hz, 75% overlap window, hope size 64, DFT size 512 samples, grid spacing 86.1 Hz, and brightness 60%. We used the note-centered approach (identifying continuous units of sound as notes and their entirety as a call) and terminology follows Köhler et al. (2017). Acoustic parameters analyzed are call duration, numbers of notes, note duration, note rate, number of pulses per note, and fundamental and dominant frequencies. The dominant frequency was obtained through the peak frequency function of the software. Recordings were deposited in the Fonoteca Neotropical Jacques Vielliard (FNJV) at Universidade Estadual de Campinas, São Paulo, Brazil (catalog numbers: FNJV 45033–45039). For all parameters, we report mean (χ), standard deviation (±), and minimum and maximum values (range).

Amplectant couples were collected and placed in plastic bags with water from the pond for eventual spawning. Subsequently, they were taken to the laboratory where they were euthanized with 5% lidocaine solution. Body weight was measured in recently euthanized individuals with a digital scale to 0.01 g; females were measured after spawning. Specimens were fixed in 10% formalin and preserved in 70% alcohol. Egg clutches (n = 3) were preserved in 10% formalin, about six hours after laying. One egg clutch was kept in an aquarium to assess the time until hatching and the stage at which the larvae hatch (*sensu* Gosner 1960). The total number of eggs and/or larvae per spawning was recorded and the maximum diameter of the eggs measured (without considering the gelatinous layers) from a randomly selected sample (n = 100), using an ocular micrometer installed on a Coleman NSZ 405 stereomicroscope. Tadpoles were also collected to confirm species identification, which was done by direct comparison with the same lot used in the original description of the tadpole of *C. alagoana* (Nascimento & Skuk 2006). All of them were euthanized with 5% lidocaine and preserved in 10% formalin.

Adult specimens were measured for snout-vent length (SVL) with digital callipers (0.1 mm accuracy) and examine morphological characters using a Leica® MZ6 stereomicroscope to assess variations within the population and/or sexual dimorphism. Morphological characters follow standards for the genus (Cruz et al. 1997, 1999, 2007a; Tonini et al. 2014; Peloso et al. 2014; e.g., presence of fringes on fingers and toes, presence and development of webbing between toes, gular coloration, presence and distribution of dermal spines, and presence of dorsal medial line and of femoral line).

Additional topotypic specimens and almost the entire type series of the species (including holotype and eight paratypes) were included in the morphological analyses. We considered the minimum adult body size (mabs) the smallest male and female collected in amplexus; individuals smaller than mabs were not included in analyses. Difference between body mass and SVL of male and female were assessed using Student's t-test for two independent samples; furthermore, to verify that difference in morphological characters are sex related, we performed a chi-square test for each character separately.

We also optimized characters from advertisement call and morphology to assess patterns of evolution within *Chiasmocleis* using the parsimony algorithm in Mesquite 3.20 (Maddison & Maddison 2006). The analysis was implemented on a recent *Chiasmocleis* phylogeny (de Sá et al. 2019a, 2019b) and characters coding for other species of genus derives from the available literature.

All collected and examined specimens are housed at the Museu de História Natural, Universidade Federal de Alagoas (MUFAL) and Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ, see Appendix).

Results

Reproductive biology

The reproductive season of *Chiasmocleis alagoana* largely coincides with the wet season (April–September). The first tadpoles appeared in June (specimens found during July 29th fieldwork) when the ponds are almost at their maximum volume and are found until mid-November (November 17th fieldwork) when there was still some water only in pond 2 (area ~ 28 m2). Visual encounter of calling males occurred on July 12 and August 23, coinciding with the days with greatest rainfall accumulated within the last 24 hours (>50 mm) and air temperature near 20ºC

(Figure 1); no adults or juveniles were seen during other fieldwork days. Furthermore, the species was most abundant in both ponds on those days; with more than 50 individuals calling in pond 1 (July 12 and 23 August 2011, Figure 2A) and about 20 individuals in pond 2 (August 23). Other species calling simultaneously with *C. alagoana*, but at lower density (four to 20 individuals per species), were *Boana albomarginata* (Spix, 1824), *Dendropsophus haddadi* (Bastos and Pombal, 1996), *D. minutus* (Peters, 1872), *Leptodactylus natalensis* Lutz, 1930, *Macrogenioglottus alipioi* Carvalho, 1946, *Ololygon skuki* (Lima, Cruz, and Azevedo, 2011), and *Scinax x-signatus* (Spix, 1824).

Chiasmocleis alagoana began calling at sunset (about 5:30 pm) with the peak occurring about an hour later and a considerable decrease in the calling activity three hours later. Males seemed to actively search for females (calling-moving-calling) during calling periods. Males called on leaf litter around the ponds and at the edge of the water with their body partially submerged (Figure 2B–C); amplexus is axillary and male legs are stretched. Highers density of amplectant couples was observed around 6:30 pm and on three different substrates: (1) leaf litter (Figure 2D), (2) fallen tree trunks (Figure 2E), and (3) partially submerged at edge of pond (Figure 2F). At the peak of breeding activity (~7:00 p.m.), we observed an interspecific amplexus between a *C. alagoana* male with an amplectic pair of *Ololygon skuki* (Figure 2G).

Advertisement call

All recordings of *C. alagoana* occurred during strong rainfalls; recorded specimens (n = 7 males) called on leaf litter or partially submerged in water. The advertisement call (n = 13 calls, 420 notes) is emitted almost continuously at intervals that varied from 9 to 57 seconds (s). The call consists of a long series of pulsed notes with a duration of 10.5 to 49.2 s (χ = 32.3 ± 10.9 s; Figure 3A). The number of notes per call ranging from 83 to 379 (χ = 249.2 ± 80.4 notes), with intervals between notes ranging from 0.02 to 0.06 s (χ = 0.05 ± 0.04 s). Each note ranged from 0.07 to 0.14 s (χ = 0.09 ± 0.0032 s) with a note rate ranging from 6.9 to 9.0 notes/second (χ = 7.8 ± 0.6 notes/second). The fundamental frequency was about 2250 Hz. The notes showed a structure with up to six distinct harmonics. The dominant frequency corresponding to the second harmonic, ranging from 3962.1 to 4823.4 Hz (χ = 4466.7 ± 199.46 Hz; Figure 3B, D). Structurally, each note consists of 10 to 17 distinct pulses (χ = 13.4 ± 0.7 pulses) divided into two groups, more evident in some notes than in others, based on the modulation pattern (Figure 3C). The first group consists of calls with three to six pulses, the first and last ones have lower intensity while the second pulse is more stronger

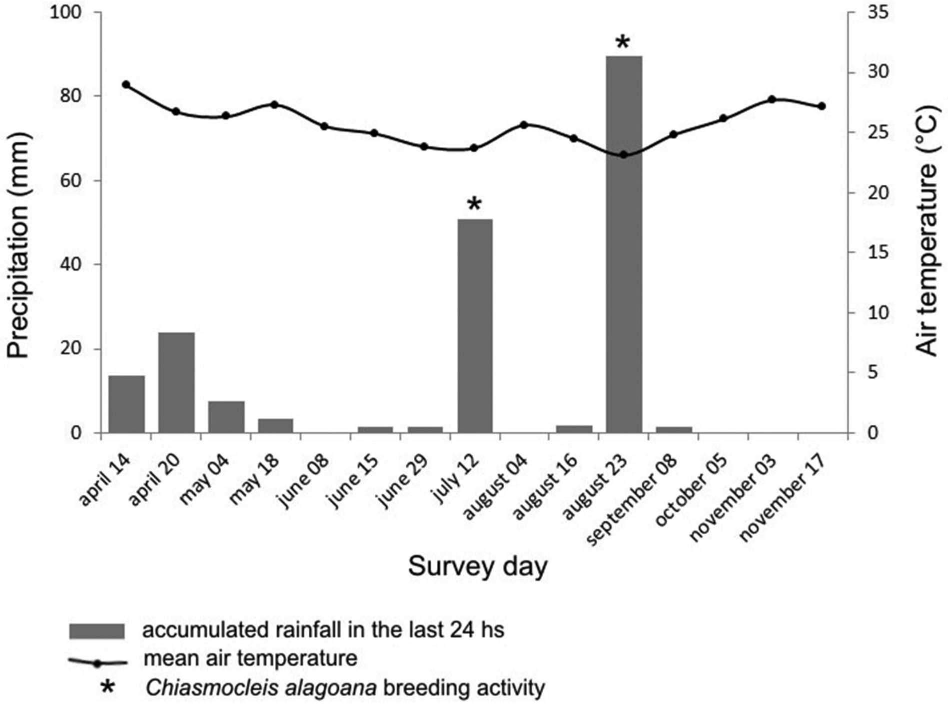

Figure 1. Breeding activity of *Chiasmocleis alagoana* in relation to daily accumulated rainfall and air temperature.

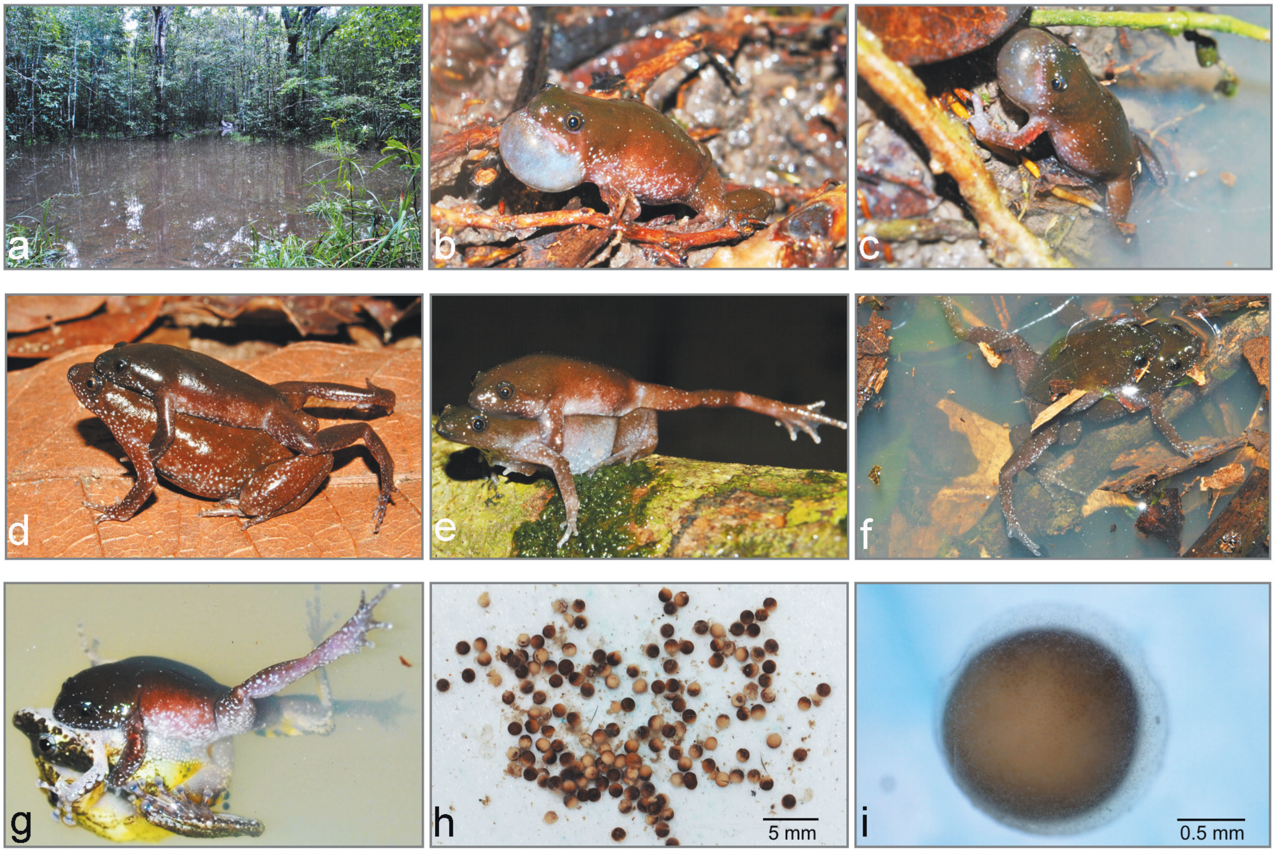

Figure 2. Breeding activity of *Chiasmocleis alagoana* from Mata do Catolé, Maceió, Alagoas, Brazil. (A) One of the temporary ponds (pond 2) where breeding activity occurred; (B) male calling on the leaf litter; (C) male calling with the body partially submerged in the water; (D) couple in amplexus on the leaf litter; (E) couple in amplexus on fallen tree trunks, (F) couple in amplexus partially submerged in the water; (G) interspecific amplexus between a male of *C. alagoana* and a couple already in amplexus of *Ololygon skuki*; (H) egg clutches laid in the plastic bag; and (I) detail of one of the eggs.

forming an ascending-descending pattern. The second group consists of seven to 11 pulses with an ascending amplitude modulation. Overall, the higher maximum amplitude usually occurred in the second group (range 6868–18,804 u, n = 120 notes) than in the first one (range 5225–13,649 u, n = 120 notes).

Some calls began with an introductory note with slightly different parameters from the subsequent notes. These notes are shorter in duration, ranging from 0.03 to 0.07 s (χ = 0.05 ± 0.01 s, n = 12), and consist of fewer pulses (5–10). Structurally, they did not have a static form, showing a clear ascending amplitude modulation usually with the first two pulses slightly separated from other pulses; however, sometimes this introductory notes have almost evenly spaced pulses without a clear modulation (Figure 3E). The dominant frequency ranged from 3789.8 to 4651.2 Hz (χ = 4282.7 ± 170.7 Hz).

One male (not collected) produced a slightly different call right after emitting two typical advertisement calls (Figure 3F–G). This call (n = 30 analyzed notes) consisted of a series of notes with duration ranging from 0.12 to 0.14 s (χ = 0.13 ± 0.005 s), more pulses per note (ranging from 16 to 29, χ = 18 ± 0.9 pulses), and lower note rate (5.8 notes/second). The dominant frequency oscillated between 4823.4 and 4995.7 Hz (χ = 4834.9 ± 43.7 Hz). Structurally, the two groups of pulses are separated by a small but distinct space (~0.007–0.009 s). In addition, the maximum amplitude of the second group was much higher than the first one (8423–9899 u vs. 3209–4398 u, respectively).

Egg clutches

The two clutches analyzed had 201 and 249 eggs (MUFAL 10983–10984, respectively; Figure 2H). The eggs were free or slightly adhered to one another by a gelatinous mass. Eggs are small (χ = 1.45 mm ± 0.07, range 1.33–1.86 mm, n = 100), pigmented, with the color ranging from light to dark brown on the animal pole. It was possible to visualize at least two gelatinous

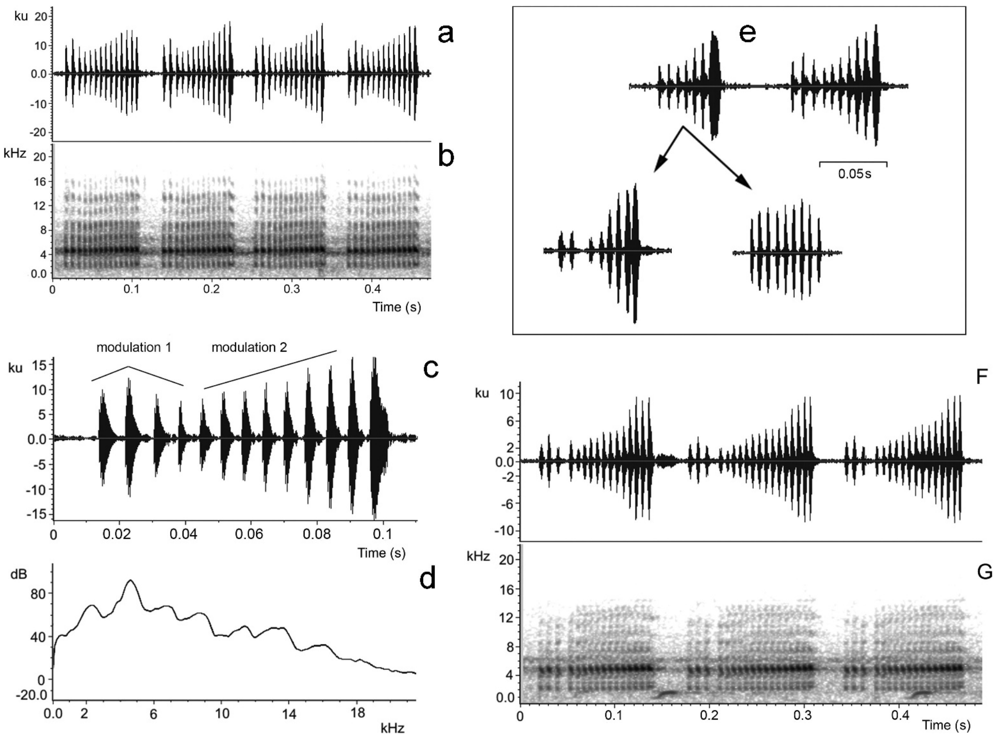

Figure 3. Advertisement call of *Chiasmocleis alagoana* from Mata do Catolé, Maceió, Alagoas, Brazil. (A) Oscillogram and (B) spectrogram of a call segment (voucher MUFAL 10981); (C) oscillogram of one note of the call, showing the pulses structure regarding the modulation pattern; (D) frequency spectrum of the same note; (E) three different types of introductory notes of the call; (F) oscillogram and (G) spectrogram of a segment of a slightly different call emitted right after a typical advertisement call (specimen not collected).

layers (Figure 2I). The embryos were between stages 1 and 6 (Gosner 1960). The third clutch, which was kept in the laboratory (unaccounted eggs), gave rise to 33 larvae that hatched two days after laying (MUFAL 11021); they were between stages 21 and 22 (total length χ = 4.8 mm ± 0.5, range 3.8–5.6 mm) and were translucent.

Morphological analysis

Chiasmocleis alagoana has pronounced sexual dimorphism, with females about 5 mm larger (χ = 25.6 ± 1.8, range 23.3–29.2 mm, n = 12) than males (χ = 20.6 ± 1.1, range 19.0–22.8 mm, n = 23, $t_{1,33} = 9.986$, $p < 0.001$) and about twice heavier (χ = females 1.5 ± 0.3, range 1.3–2.2 g, n = 6; χ = males 0.7 ± 0.1, range 0.6–0.9 g, n = 7, $t_{1,12} = 6.134$, $p < 0.001$). Morphologically, both sexes showed fringes on fingers and toes; however, females' fringes are less developed than those of males. All males examined differed from females by (1) gular region markedly darker than other ventral surfaces and (2) having webbed feet. There is variation on foot webbing among examined males (n = 23), with 12 individuals having webbing with its margins cropped. Dermal spines on dorsum, venter, chin, dorsal and lateral surface of limbs, and laterally on fingers and toes occurred in 87% of the analyzed males and were absent in all females (n = 13; Figure 4). Females have few dermal spines limited to the area around the cloaca. A light mid-dorsal line, usually extending from the internarial region to the cloaca, was present in 13% of the males and 23% of the females. However, a chi-square test did not reveal a significant correlation of the light mid-dorsal line with sex ($p = 0.756$) nor did the presence of femoral line (present in 30% of males and 46% of females, $p = 0.561$).

Discussion

In tropical environments, rainfall seems to be the main extrinsic factor that determines the time of breeding activity in anurans, different from temperate regions, whose temperature plays a relevant role (Duellman & Trueb 1994; Bevier 1997). The reproductive period of *Chiasmocleis alagoana* occurred during the rainy season in the region (April to September) and breeding activity occurred only on days of heavy rains (>50 mm), characterizing the temporal reproductive pattern of the species as explosive (*sensu* Wells 1977). Explosive

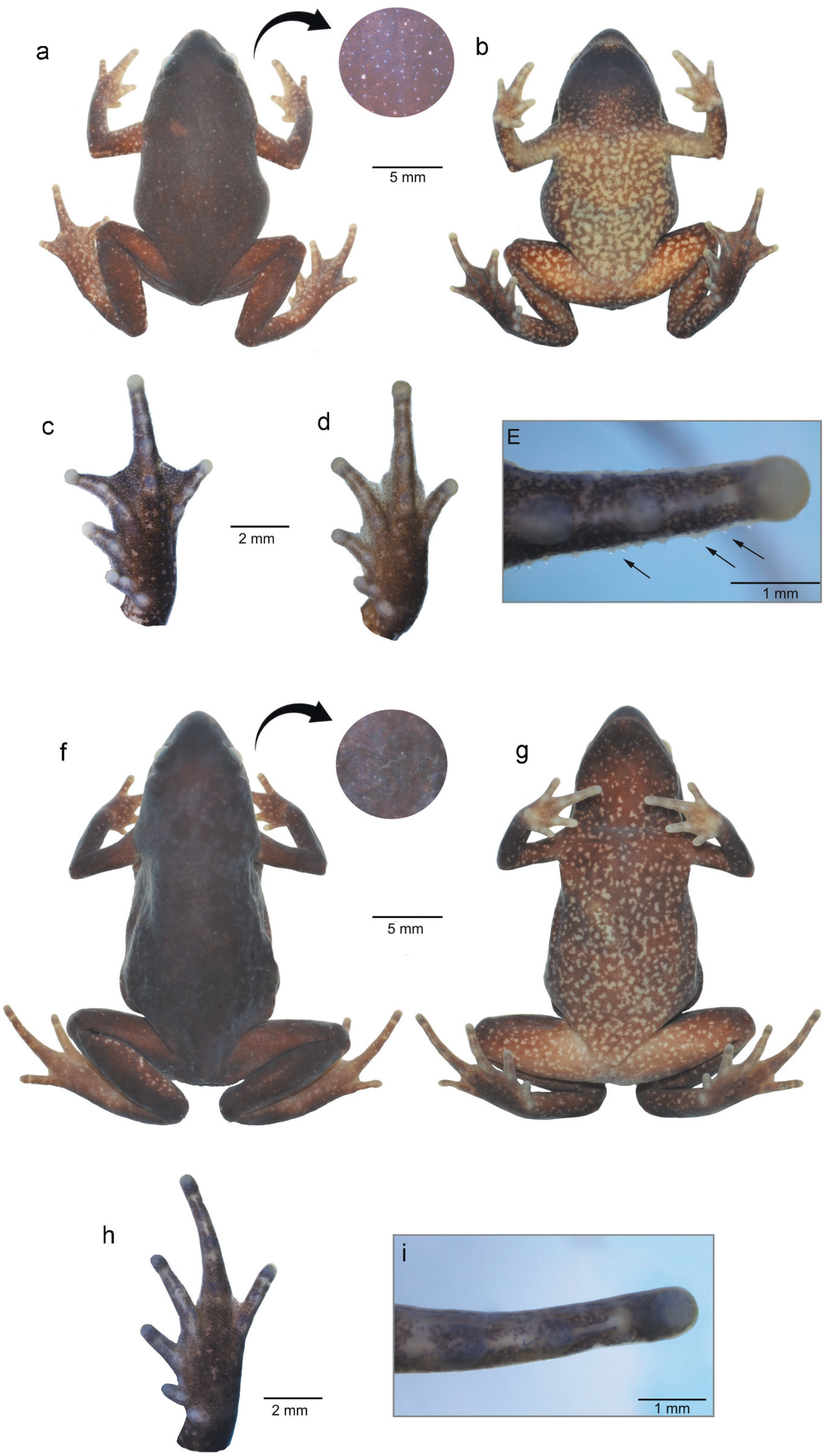

Figure 4. Specimens of *Chiasmocleis alagoana* from Mata do Catolé, Maceió, Alagoas, Brazil. (A) Male in dorsal and (B) ventral views; in detail a section of the dorsal region is shown, highlighting the dermal spines (MUFAL 10967); (C) male toes with webbing (MUFAL 10967); (D) male toes with cropped webbing (MUFAL 10981); (E) detail of the male toe IV highlighting the dermal spines over the fringes (MUFAL 10967); (F) Female in dorsal and (G) ventral views; in detail a section of the dorsal region is shown, highlighting the absence of dermal spines; (H) female toes with webbing absent; and (I) detail of the female toe IV highlighting the absence of dermal spines (MUFAL 10977).

reproduction have been reported for other species in the genus, i.e., *C. atlantica* Cruz et al., 1997 (Wogel et al. 2004), *C. albopunctata* (Boettger, 1885) (Oliveira-Filho & Giaretta 2006), *C. capixaba* Cruz et al., 1997 (Wogel et al. 2004), *C. hudsoni* Parker, 1940 (Rodrigues et al. 2008; Miranda et al. 2015), *C. lacrimae* Peloso et al. 2014 (Wogel et al. 2004; Cordioli et al. 2019), *C. mehelyi* Caramaschi and Cruz 1997 (Prado et al. 2005), *C. shudikarensis* Dunn, 1949 (Gottsberger & Gruber 2004), and *C. royi* Peloso et al. 2014 (Schlüter & Salas 1991) as well as for other microhylids (e.g., Rodrigues & Duellman 1994; Kwet & Di-Bernardo 1998; Rodrigues et al. 2003; Hirschfeld & Rödel 2011). Heavy rains (>50 mm) also occurred at the beginning of the season (April and May); however, larvae appeared only in June, probably as a result of both torrential rains and substantial accumulation of water in the ponds. The available reports on *Chiasmocleis* reproduction have no information on the amount of water in ponds during the reproductive season but a pattern similar to that of *C. alagoana* seems to occur in *C. albopunctata* ['… males call from artificial ponds in open (grassland) areas, just after heavy rain showers filled them'; Oliveira-Filho and Giaretta 2006]. Ecological factors, e.g., larval competition, predator abundance, offspring size, and breeding frequency (Wells 1977) may be linked to the minimization of the risk of offspring mortality by desiccation of the ponds (Hirschfeld & Rödel 2011).

Chiasmocleis alagoana reproductive activity was restricted to days of heavy rains; however, it was the most abundant species in those days, forming large agglomerations around the ponds. Previous work suggested that agglomerations of explosive breeding species may decrease the risk of predation, result in stronger emission of male calls, and likely increasing the chance of attracting more and distant females (Wells 1977; Ryan 1983; Olson 1989).

Density of individuals at a breeding site may interfere with males' courtship behavior (Wells 1977). For instance, at low densities, *Lithobates sylvaticus* (LeConte, 1825) males tended to remain stationary, while at higher densities, they actively search for females (Woolbright et al. 1990). During breeding days, *C. alagoana* males were observed actively moving around the pond, apparently in search for females; periods of active searching alternated with stationary periods when males actively call. This agrees with previous reports that male's reproductive success is determined by the frequency of encountering females and direct competition with other males for females (Wells 1977; Pombal & Haddad 2007). Females seem to have low or no selectivity for males (Pombal & Haddad 2005). A direct consequence of this high density of individuals and active search for females is that the acquisition of partners becomes a process of trial and error, which may foment interspecific amplexus (Wells 2007; Mollov et al. 2010; Rocha et al. 2015), as we observed between a *C. alagoana* male and an amplectant couple of *Ololygon skuki*.

Males of *C. alagoana* have their legs fully stretched out during amplexus. A similar behavior was reported in *C. royi* (Schlüter & Salas 1991; also see plate 12 H in Peloso et al. 2014). Neotropical anurans under stress or to avoid predation show a behavior known as 'stiff-legged' (i.e., animal remains immobile with the body flattened against the substrate and the legs stretched back; e.g., Garcia 1999; Toledo & Zina 2004; Bertoluci et al. 2007; Toledo et al. 2011; Costa et al. 2017). However, it is an unlikely cause of stretched legs in amplectant males of *C. alagoana* since we observed it in all amplectant males but not in females, indicating a posture associated directly to the amplexus of the species.

The advertisement calls have been reported for 20 of the 36 currently species of *Chiasmocleis* (Table 1). Structurally, the call of *C. alagoana* consists of a series of multipulsed notes, a pattern known to all species of *Chiasmocleis*, except *C. mantiqueira* Cruz, Feio, and Cassini, 2007 and *C. parkeri* Almendáriz C., Brito-M., Batallas-R., Vaca-Guerrero, and Ron, 2017 in which the notes of the calls have no distinct pulses (Santana et al. 2012; Almendáriz et al. 2017). The temporal and spectral parameters of the call agree with previous reports for the genus.

Based on the advertisement calls, the species in the genus with pulsed notes are grouped into three distinct groups: (1) most *Chiasmocleis* have advertisement calls composed of pulses with relatively uniform amplitudes and evenly spaced from each other (Wogel et al. 2004; Santana et al. 2009; Barros et al. 2010), (2) *Chiasmocleis albopunctata* and *C. mehely* have notes consisting of two groups of pulses separated by a short interval between them (De-la-Riva et al. 1996; Hartmann et al. 2002; Oliveira-Filho & Giaretta 2006; Carvalho et al. 2013); also, *C. supercilialba* was reported to have a call with two groups of pulses; however, the separation of the notes is not clear in the original report (Morales & McDiarmid 2009); and (3) a group of species with notes formed by pulses structured in amplitude modulations; these pulses may be divided into two clusters with different modulation patterns (Forlani et al. 2013; this study). This group consists of *C. cordeiroi* Caramaschi and Pimenta (2003), *C. crucis* Caramaschi and Pimenta 2003, and *C. alagoana*. The modulation of *C. alagoana* is most similar to that of *C. cordeiroi*, with

Table 1. Advertisement calls described for *Chiasmocleis* species. Values are presented as mean standard±deviation (range). If the species has introductory notes, these were not considered. ^{1}The authors description was based on the combined calls of three specimens, including a reanalysis of Santana et al.'s (2009) recording. ^{2}The authors description was based on the combined calls of three specimens, including one specimen also analyzed by Rodrigues et al. (2008).

Species	Subgenus	Source	Specimens (calls)	Call duration (s)	Number of notes	Note duration (s)	note rate (notes/s)	number of pulses per note	dominant frequency (Hz)
Chiasmocleis bassleri	*Syncope*	Santana et al. (2009)	1	–	–	0.059 ± 0.007 (0.039–0.074)	–	4.83 ± 0.64 (3–6)	3268.66 ± 3.01 (3266.67–3273.17)
Chiasmocleis bassleri	*Syncope*	Peloso et al. (2014)^1	3	–	–	0.0533 ± 0.0111 (0.015–0.163)	9.47	5.2 ± 1.2 (2–16)	2747.0 ± 145.8 (2584–3125)
Chiasmocleis haddadi	*Syncope*	Peloso et al. (2014)	–	–	–	0.33 ± 0.04 (0.15–0.445)	2.73	10.6 ± 4.07 (5–22)	4146.5 ± 60.1 (4125.0–4312.5)
Chiasmocleis hudsoni	*Syncope*	Rodrigues et al. (2008)	8 (5)	49.8 ± 13.5 (32.4–67.3)	–	0.986 ± 0.0117 (0.071–0.126)	–	–	4624.2 ± 153.5 (4263–4866)
Chiasmocleis hudsoni	*Syncope*	Peloso et al. (2014)^2	3	–	–	0.954 ± 0.0022 (0.007–0.198)	8.3	5.6 ± 0.6 (5–10)	4845.2 ± 379.0 (3962.1–6373.8)
Chiasmocleis parkeri	*Syncope*	Almendáriz et al. 2017	1(1)	14.78	72	0.00736 ± 0.00107 (0.055–0.01)	5.24 ± 0.53 (3.59–6.25)	nonpulsed	4040 ± 30 (3960–4090)
Chiasmocleis supercilialba	*Syncope*	Morales and McDiarmid (2009)	–	12.4	–	–	–	–	3100
Chiasmocleis alagoana	*Chiasmocleis*	here	7(13)	32.3 ± 10.9 (10.5–49.2)	249.2 ± 80.4 (83–379)	0.09 ± 0.003 (0.07–0.14)	7.8 ± 0.6 (6.9–9.0)	13.4 ± 0.7 (10–20)	4466.7 ± 199.46 (3962.1–4823.4)
Chiasmocleis albopunctata	*Chiasmocleis*	De-la-Riva et al. (1996)	1(15)	–	–	0.052 ± 0.008 (0.023–0.06)	10.7*	7.1 ± 0.8 (5–8)	4431.5 ± 101.7 (4311.0–4664.4)
Chiasmocleis albopunctata	*Chiasmocleis*	Oliveira-Filho and Giaretta (2006)	2(10)	–	–	0.06 ± 0.004 (0.05–0.06)	(7.05–8.35)	9	4306
Chiasmocleis albopunctata	*Chiasmocleis*	Carvalho et al. (2013)	7	17.2 ± 4.6 (5.8–34.2)	203 ± 56.3 (69–400)	0.053 ± 0.021 (0.033–0.061)	11.8 ± 0.4 (11–13)	8.7 ± 0.6 (6–10)	4520 ± 170 (3940–4880)
Chiasmocleis albopunctata	*Chiasmocleis*	Carvalho et al. (2013)	1	25	298	0.056 ± 0.013 (0.054–0.059)	12	8.4 ± 0.5 (8–9)	4260 ± 150 (4130–4480)
Chiasmocleis atlantica	*Chiasmocleis*	Wogel et al. (2004)	–	–	–	0.137 ± 0.022 (0.064–0.186	–	16.7 ± 2.1 (7–22)	3540 ± 110 (3320–3750)
Chiasmocleis avilapiresae	*Chiasmocleis*	Barros et al. (2010)	1(128)	–	–	0.098 ± 0.0098 (0.043–0.117)	4.9	15.9 ± 1.5 (7–18)	3368.2 ± 73.2 (2937.5–3500)
Chiasmocleis capixaba	*Chiasmocleis*	Wogel et al. (2004)	1	–	–	0.052 ± 0.005 (0.047–0.061)	–	8.7 ± 0.8 (8–10)	4750 ± 60 (4610–4870)
Chiasmocleis cordeiroi	*Chiasmocleis*	Forlani et al. (2013)	2(9)	7.22 ± 7.80 (1.31–26.86)	48.56 ± 52.93 (9–182)	0.12 ± 0.01 (0.07–0.14)	6.76 ± 0.37 (6.20–7.46)	20.13 ± 1.59 (13–24)	4761.48 ± 62.34 (4500–4898)
Chiasmocleis crucis	*Chiasmocleis*	Forlani et al. (2013)	1 (7)	3.54 ± 19.55 (5.97–13.89)	59.50 ± 19.55 (33–77)	0.159 ± 0.01 (0.105–0.179)	5.39 ± 0.21 (5.17–5.59)	20.78 ± 1.70 (14–23)	4324 ± 61 (4069–4435)
Chiasmocleis lacrimae	*Chiasmocleis*	Nelson (1973)	(5)	3.4 ± 1.4 (1.8–5.0)	–	–	–	–	4529 ± 71 (4465–4650)
Chiasmocleis lacrimae	*Chiasmocleis*	Hartmann et al. 2002	3(11)	3.02 ± 1.6 (0.07–4.83)	29.8 ± 12.3 (9–48)	–	–	9.08 ± 1.06 (6–12)	4000–4900
Chiasmocleis lacrimae	*Chiasmocleis*	Wogel et al. (2004)	–	–	–	0.048 ± 0.005 (0.042–0.054)	–	9.3 ± 0.9 (8–10)	4840 ± 70 (4690–4960)
Chiasmocleis leucosticta	*Chiasmocleis*	Nelson (1973)	(6)	12.4 ± 4.2 (7.5–16.8)	–	–	–	–	3647 ± 177 (3400–4000)
Chiasmocleis mantiqueira	*Chiasmocleis*	Santana et al. (2012)	1	0.04 ± 0.02 (0.012–0.072)	one	same as call duration	–	nonpulsed	3146.23 ± 829 (1884.06–4944.23)
Chiasmocleis mehelyi	*Chiasmocleis*	Hartmann et al. (2002)	1(5)	16.34 ± 6.79 (8.56–26.74)	147.75 ± 44.85 (91–196)	–	–	9.1 ± 0.93 (7–11)	4700–5400

(*Continued*)

Table 1. (Continued).

Species	Subgenus	Source	Specimens (calls)	Call duration (s)	Number of notes	Note duration (s)	note rate (notes/s)	number of pulses per note	dominant frequency (Hz)
Chiasmocleis royi	*Chiasmocleis*	Schlüter (2005)	–	–	–	–	–	–	5000–7000
Chiasmocleis royi	*Chiasmocleis*	Peloso et al. 2014	1	–	–	0.0895 ± 0.0052 (0.077–0.104)	8.38	14.6 ± 0.7 (12–16)	7629.1 ± 1204.0 (5512.5–13,953.5)
Chiasmocleis schubarti	*Chiasmocleis*	Nelson (1973)	(2)	5.1–6.0	–	–	–	–	3886 ± 136 (3700–4100)
Chiasmocleis shudikarensis	*Chiasmocleis*	Zimmerman and Bogart (1988)	1(20)	–	–	0.04 ± 0.01 (0.03–0.06)	–	–	5890–7510
Chiasmocleis shudikarensis	*Chiasmocleis*	Zimmerman and Bogart (1988)	1(20)	–	–	0.03 ± 0.01 (0.01–0.05)	–	–	5530–7460
Chiasmocleis shudikarensis	*Chiasmocleis*	Lescure and Marty (2000)	–	–	–	0.098	–	–	3380–3750
Chiasmocleis ventrimaculata	*Chiasmocleis*	Nelson (1973)	–	–	–	0.13 ± 0.01 (0.10–0.18)	–	–	3562 ± 110 (3350–3700)

the first cluster of pulses formed by an ascending-descending amplitude pattern, while the second cluster has only an ascending amplitude modulation. In *C. crucis*, both cluster of pulses have an ascending-descending pattern.

We transform these three types of notes into character-states and optimize them on the phylogeny of the *Chiasmocleis* (Figure 5). We found that the notes have a strong phylogenetic signal. Calls structured in pulses with amplitude modulations may be a synapomorphy for the subclade consisting of *C. alagoana, C. cordeiroi, C. crucis*, and C. *schubarti* Bokermann 1952. Unfortunately, the description of the call of *C. schubarti* did not report or figured the note structure (Nelson 1973). Also, a note formed by two groups of pulses separated by a short interval could also represent a synapomorphy for the subclade consisting of *C. albopunctata, C. mehely, C. bicegoi* Miranda-Ribeiro, 1920, and *C. centralis* Bokermann 1952. This supported synapomorphy awaits the description of the unknown calls of *C. bicegoi* and *C. centralis.*

Chiasmocleis alagoana sometimes produce an introductory note, i.e., first note of the call, that is shorter in duration, consists of fewer pulses, and it is not always structured as the other notes in the call. We cannot determine the intrinsic or extrinsic factors that caused the emission of these notes. Introductory notes were also reported for *Chiasmocleis hudnosi* and *C. albopunctata*. Introductory notes for *C. hudsoni*

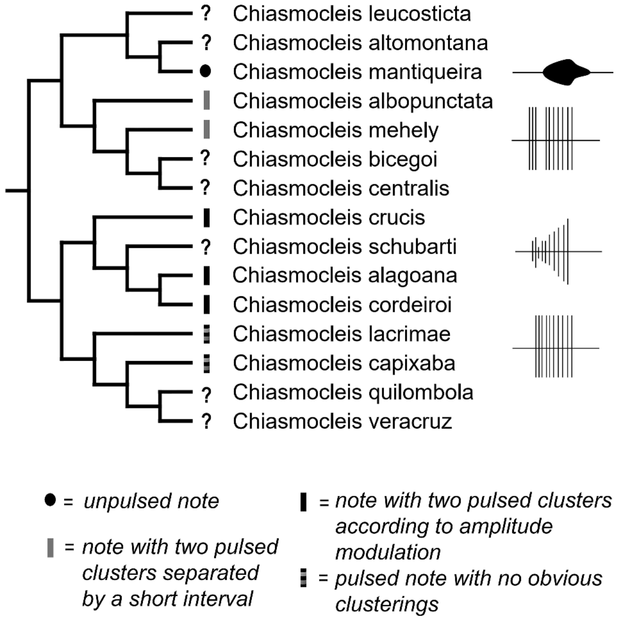

Figure 5. Optimization of the type of note structure in the advertisement calls of *Chiasmocleis* from Atlantic Forest and open habitats. Tree topology from de Sá et al. (de Sá et al. 2019a, 2019b). Data from the species, except *C. alagoana*, were acquired from literature (see Table 1).

were present in three out of eight analyzed specimens (Rodrigues et al. 2008); these notes were longer and had a lower dominant frequency than other notes in the call. Introductory notes were also found in six of seven males analyzed of *C. albopunctata* but they lack the pulse structure of other notes and had a lower dominant frequency (Carvalho et al. 2013).

A recorded individual of *C. alagoana* emitted a different call shortly after the usual advertisement call. Variation in anuran calls could be due to: (i) motivation or energetic constraints, (ii) social context, (iii) change of position at calling site, (iv) changing temperature, and (iv) response to changing acoustic environment (Köhler et al. 2017). We can only reject a 'change of position at calling' in *C. alagoana* since the recorded individual remained in the same position while producing this call. A 'sporadic call,' i.e., different from the usual advertisement call, was reported for *C. hudsoni* at the beginning of the breeding activity (Rodrigues et al. 2008).

During our fieldwork we did not observe egg-laying of *Chiasmocleis alagoana*; three egg clutches laid in the plastic bag by collected specimens consist of a gelatinous mass containing a large number of brown pigmented eggs (>200 eggs per clutch), with each egg measured about 1.5 mm in diameter, similar to most species of the genus (Crump 1974; Schlüter & Salas 1991; Haddad & Hödl 1997; Lima et al. 2005; Cordioli et al. 2019); these eggs were reported to float on the water surface (Nascimento & Skuk 2006; Cordioli et al. 2019). Exceptions to this pattern are found in the subgenus *Syncope*, e.g., in *Chiasmocleis antenori* (Walker, 1973), the clutch has a maximum of six pigmented and slightly larger eggs (about 2 mm each), the tadpole is endotrophic and develops in bromeliads (Krügel & Richter 1995). Also, *C. carvalhoi* (Nelson 1975) and *C. magnova* Moravec and Köhler, 2007 have unpigmented and few (about 5–10) and larger eggs (about 2.2 mm in *C. carvalhoi*); potentially suggesting that these species may have direct development or a similar mode to that of *C. antenori* (Nelson 1975; Rodriguez & Duellman 1994; Moravec & Kohler 2007; Peloso et al. 2014). In addition, a clutch of four pigmented eggs was reported for *C. parkeri* (Almendáriz et al. 2017).

Sexual dimorphism in size is quite common in *Chiasmocleis*, especially among species of the subgenus *Chiasmocleis* (Caramaschi & Cruz 1997; Cruz et al. 1997, 2007a, b; Peloso & Sturaro 2008; Cordioli et al. 2019) where females are about twice the size of males. Other markedly dimorphic characters in *Chiasmocleis* are the presence of vocal sac, darkened gular region in males, and the presence of dermal spines and webbing between toes; the last two characters only present, or at least more developed, in males. Toe webbing can be used as a diagnostic character for several species of the genus (Cruz et al. 1997, 2007b; Peloso & Sturaro 2008; Peloso et al. 2014) but have also caused taxonomic confusions, e.g., the descriptions of *Engystoma leucostictum* Boulenger, 1888 and *Nectodactlylus spinulosus* Miranda-Ribeiro, 1924, were based on a male and a female of the sema species, respectively (Carvalho 1954). The diagnosis of *C. alagoana* described hand and foot not webbed, fingers and toes without lateral dermal spines, and dorsal and ventral surface of body and limbs without dermal spines (Cruz et al. 1999). However, our examination of specimens from the type locality showed that all males have webbing between the toes and the majority of them (87%) also have dermal spines on dorsal, ventral, and lateral surfaces of the body and limbs, and laterally on fingers. Moreover, females of the species only possessed them around the cloaca, a pattern similar to that found in other *Chiasmocleis* (Peloso et al. 2014; Forlani et al. 2017; Cordioli et al. 2019).

The type series of *Chiasmocleis alagoana* consists of a female holotype (MNRJ 21856) and nine paratypes, four of them males (MNRJ 21859–60, 21862, MUFAL 1637). We examined three of these four males. Our examination of MUFAL 1637 showed it is actually a female based on (1) the absence of a darkened gular region, (2) dermal spines found only around the cloaca, and (3) SVL = 25.9 mm (female range 23.3–29.2 mm). The specimen MNRJ 21,860 has SVL = 21.3 mm, within the range for males (19.0–22.8 mm) but it was dehydrated, preventing further analyses. The specimen MNRJ 21859 has a darkened gular region and SVL = 22.4 mm but lack webbing on feet; we consider the specimen to be an anomalous male (Figure 6).

Dimorphic characters such as toe fringes and dermal spines are more distinct during the breeding season (Peloso et al. 2014; Forlani et al. 2017). The type series of *C. alagoana* was collected during the day and outside the reproductive season (September to December, Cruz et al. 1999) which may explain the absence of dermal spines reported in the species description. Herein, we provide additional characters to the diagnosis of *Chiasmocleis alagoana*: (1) presence of webbing between toes in males, (2) dermal spines over the dorsum, venter, chin, on limbs, and laterally on fingers and toes in males, and (3) dermal spines restricted to area surrounding the cloaca in females.

Dermal spines were considered plesiomorphic for *Chiasmocleis* with three independent losses (Peloso et al. 2014). When we re-analyzed the optimization of

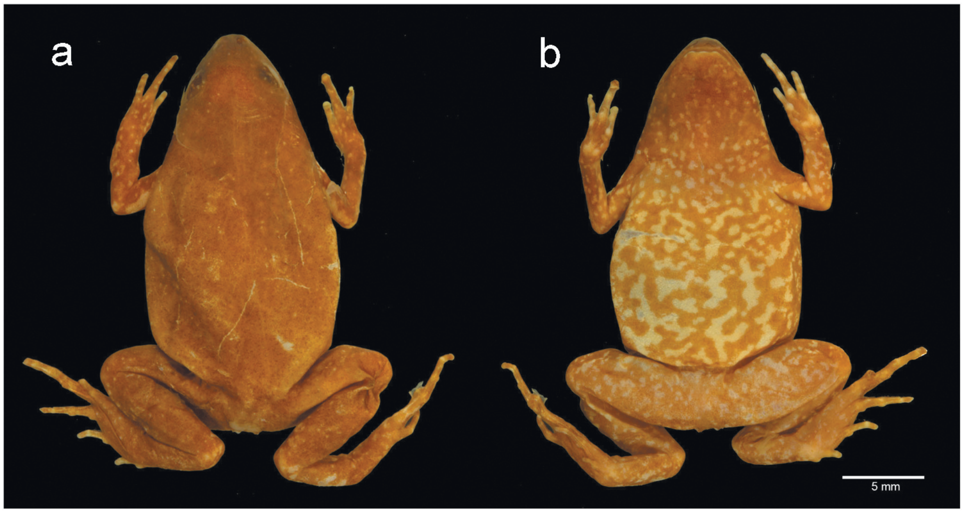

Figure 6. Paratype MNRJ 21859 of *Chiasmocleis alagoana* in (A) dorsal and (B) ventral views.

these two characters on the recent phylogeny for the genus (de Sá et al. 2019a, 2019b; Figure 7), the evolution of dermal spines agrees with the previous report on the number of losses [the clade *C. carvalhoi* + *C.* tridactyla (Duellman and Mendelson, 1995); in *C. antenori*; and in *C. papachibe* (Peloso et al. 2014)], but herein we corrected the information for *C. alagoana* as having dermal spines. No information is available in the literature for this trait in *C. bicegoi*, *C. centralis*, and *C. devriesi* Funk and Cannatella 2009.

The occurrence of feet webbing is limited to the subgenus *Chiasmocleis*. It was previously reported to have evolved independently four times (Peloso et al. 2014): (1) in the clade *C. avilapiresae* Peloso and Sturaro 2008 + *C. shudikarensis*, (2) in *C. anatipes* Walker and Duellman, 1974, (3) *C. leucosticta*

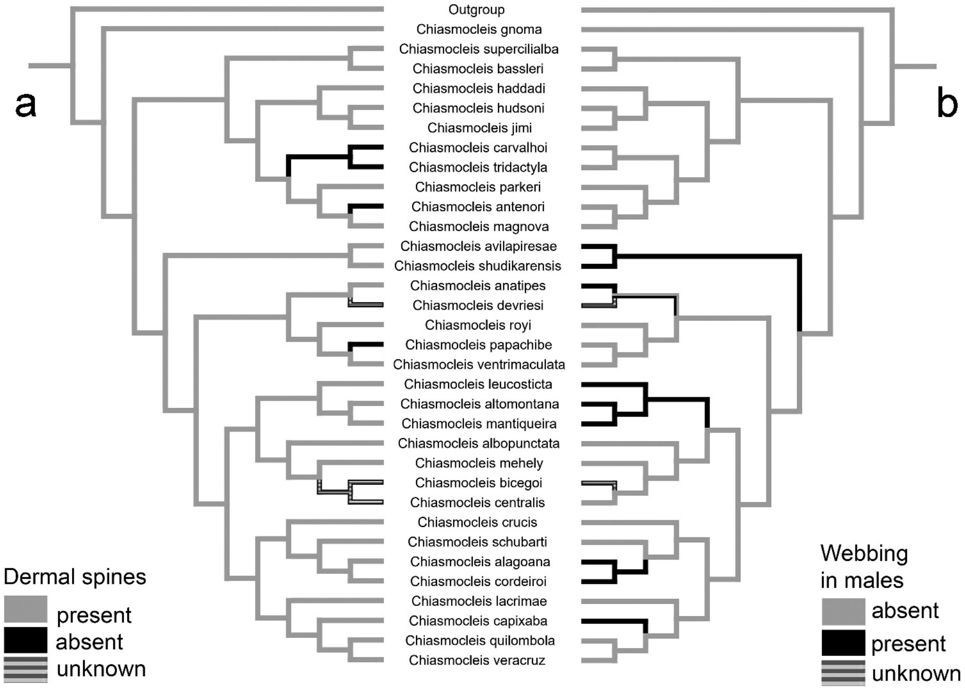

Figure 7. Optimization of dermal spines and webbing on feet in males of *Chiasmocleis* species. Tree topology from De Sá et al. (2019a, 2019b). Data from the species, except *C. alagoana*, were acquired from literature (Miranda-Ribeiro 1920; Bokermann 1952; Caramaschi & Cruz 1997; 2001; Caramaschi & Pimenta 2003; Canedo et al. 2004; Morales & McDiarmind 2009; Cruz et al. 2007a, 2007b; Funk & Cannatella 2009; Peloso et al. 2014; Tonini et al. 2014; Almendáriz et al. 2017; Forlani et al. 2017).

(Boulenger, 1888) and (4) in *C. capixaba*. Our analysis of this trait on the phylogeny adopted here suggests that feet webbing evolve independently five times: (1) in the clade *C. avilapiresae* + *C. shudikarensis*, (2) in *C. anatipes*, (3) in the clade *C. leucosticta* + (*C. altomontana* Forlani, Tonini, Cruz, Zaher, and de Sá, 2017 + *C. mantiqueira*), (4) in the clade *C. alagoana* + *C. cordeiroi* and (5) in *C. capixaba*. Furthermore, *C. alagoana* is the only species currently known in which this character is polymorphic, i.e., webbing with continuous or cropped margins. Presence of foot webbing was previously correlated to the male ability to swim or float on water (Peloso et al. 2014). Males of *C. alagoana* were seen actively swimming in the ponds in search of partners.

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Disclosure statement

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Appendix

Specimens examined

Chiasmocleis alagoana: Brazil: Alagoas: Rio Largo, Mata do Cedro (MUFAL 1637–1639, MNRJ 21857–61, paratypes); Maceió, Mata do Catolé (MNRJ 21856, holotype; MUFAL 4268, 4270, 4275–4276, 5499–5505, 9537, 10962–10981, 11274–11275; tadpoles: MUFAL 9785, 11021; eggs: MUFAL 10983–10984).