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ORIGINAL ARTICLE

Geographic call variation and further notes on habitat of *Ameerega flavopicta* (Anura, Dendrobatidae)

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We describe habitat and inter-populational call variation of the dendrobatid frog *Ameerega flavopicta.* Data were collected in the Brazilian states of Minas Gerais and Goiás. Principal component analysis separated the Goiás population from others because of its higher call rates and shorter calls. The Paranaíba River may represent the major geographic barrier. We recognize the cephalic amplexus as the main type for the species. Although habitat disturbances increased since 1990, we did not notice differences in the density of calling males at Serra do Cipó. *Ameerega flavopicta* appears to be quite resistant to alterations in its natural habitats caused by human activities.

Keywords: dendrobatid frog; vocalization; cephalic amplexus; abundance; Brazil

Introduction

Ameerega flavopicta (= *Epipedobates flavopictus*) (Lutz, 1925) is a distinctive dendrobatid species which reproduces in open and rocky environments subject to broad variations in humidity and temperature (Costa et al. 2006). As presently recognized, this species presents a wide distribution in the Brazilian states of Minas Gerais, Goiás, Tocantins, Pará, and Maranhão (Haddad & Martins 1994; Colli et al. 2002; Costa et al. 2006; Giaretta et al. 2008). The breeding activity of *A. flavopicta* is diurnal and its egg-clutches are deposited on the ground or under rocky piles beside seasonal or permanent streams. Males can transport tadpoles on their backs to adjacent water bodies, where they complete larval development (Toledo et al. 2004; Costa et al. 2006). Inter-populational differences have been reported in color pattern (Haddad & Martins 1994), and in call features (Haddad & Martins 1994; Costa et al. 2006). In the present study we report new data on inter-populational call differences and the existence of cephalic amplexus in the species. We also comment on habitat and abundance.

Materials and methods

Field works were carried out sporadically between November 2004 and November 2007 in three municipalities in Minas Gerais state: Araguari (MG) (two sites: 18°27′S, 45°85′W; 18°27′S, 48°31′W; 600 m a.s.l.), Perdizes (MG) (19°11′S, 47°07′W; 890 m a.s.l.), and Santana do Riacho (MG) (19°17′S, 43°35′W; Serra do Cipó, 900 m a.s.l.) and one municipality in Goiás state: Caldas Novas (GO) (17°46′S, 48°39′W; 906 m a.s.l.). The two most distant localities (Santana do Riacho and Caldas Novas) were about 550 km apart in an east–west direction. All these localities were originally covered by Cerrado (Brazilian savanna) vegetation (Oliveira & Marquis 2002; Eterovick & Sazima 2004; Giaretta et al. 2008). We also took records of the habitats used in these localities.

Calls were recorded with a Boss 864 digital recorder (44.100 Hz, 16 bit resolution) and a Sennheiser ME67 microphone. Sound analyses were performed in the Sound Ruler software (Gridi-Papp 2004) using a FFT (Fast Fourier Transformation) length of 512. Air temperature range of call records from Araguari was 29–31°C, Perdizes 20–20.9°C, from Serra do Cipó 16.5–18.2°C and from Caldas Novas 23.5–25.8°C. All recordings were done during the morning in Araguari ($n = 3$), Perdizes ($n = 2$), and Serra do Cipó ($n = 3$); in Caldas Novas, two males were recorded during the morning and one in the afternoon (see Costa et al. 2006 for the bimodal calling period of the species).

Within a call, the minimum frequency corresponded to the median frequency of the first pulse; the maximal frequency was that of the last pulse. Population differences in advertisement call parameters (minimum and maximum frequencies, call duration,

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call rate, pulse number, and inter-call intervals) were evaluated through a principal components analysis (PCA) applied on a correlation matrix (Manly 1986) considering mean values (n = 6 calls/individual) for each recorded male. Measured call features were standardized prior to analysis. The PCA was performed in the Fitopac 1.5 software (Shepherd 2005). Call parameters, as summarized by the first principal component (PC I) scores, were correlated with call temperature through the Spearman rank coefficient (Zar 1999). To eliminate temperature effects from the ordination, we carried out a partial PCA entering temperature as covariate using the CANOCO 4.5 statistical software (Ter Braak & Šmilauer 2002). Again, call values of parameters were standardized prior to analysis (Zar 1999).

One amplectant pair was observed while courting and egg-laying; the most important behaviors are reported. At Serra do Cipó (2007) we recorded and counted calling males along a 500 m transect and took records of the habitat where individuals were heard or sought in our last visit. A voucher specimen is in the anuran collection (AAG-UFU 2526, Caldas Novas, GO) of the Museu de Biodiversidade do Cerrado, Universidade Federal de Uberlândia, MG, Brazil. Photos of live specimens and recordings of advertisement calls (Caldas Novas, GO) can be accessed at AmphibiaWeb (http://amphibiaweb.org/search/search_photos.shtml). Data from Serra do Cipó is of particular interest because it is close (60 km, NE) to the type locality (Belo Horizonte) and from a site where a populational decline has been suggested (Eterovick et al. 2005).

Results

Calling habitat

In Araguari (MG), males were found calling within a forest (n = 5) and along erosive trenches in pastures or along road cut banks of the BR 050 highway; all sites in steep terrain draining to the Paranaíba River. In Perdizes (MG), males were found calling along the border of deep (≤2.5 m deep; 0.5–1.0 m wide) erosive trenches in two sites: (1) at the Estação Ambiental Galheiro [a private (CEMIG) reserve with 2800 ha] and (2) along the MG 452 highway. At the Estação Ambiental Galheiro, males called from an area covered by scattered low (<1.5 m) shrubs and grass-like vegetation, in a steep (45°) terrain at the border (150 m away) of the lake of the Nova Ponte hydroelectric dam. There was water in scattered pools at the bottom of these trenches. Close to the highways, males were heard calling along banks of the road cut (≥10 m from the asphalt-paved line) in areas covered by sparse tall (≤2 m) grass tufts.

At Serra do Cipó (MG) we found ca. 20 males calling along a 500 m transect parallel to a second-order creek in disturbed (cattle and horse farming) rocky fields and two other males beside human settlements. All males called from distances larger than 50 m from the water. Another individual was found hidden under rocks beside a creek in a recently (<3 months) burnt area.

In Caldas Novas (GO) we found males calling at two sites: (1) at the Parque Estadual da Serra de Caldas Novas (PESCAN) and (2) along the GO 139 highway. At the PESCAN, males called mainly from rocky areas covered by scattered low shrubs and grass-like vegetation. Close to the highway, ca. 50 males were heard calling along banks of the road cut (≥10 m from the asphalt-paved line) in areas covered by sparse tall (≤2 m) grass tufts.

Advertisement calls and variation

The advertisement call parameters of different populations are summarized in Table 1 and Figure 1. In all populations the call was a single pulsed note with ascending modulation from the beginning to the end. The first two axes of the PCA accounted for 66.8 and 19.7% of total variance in advertisement calls, respectively (Figure 2). This plot showed a separation between the populations from Perdizes (MG) and Serra do Cipó (MG) from those of Caldas Novas (GO) and Araguari (MG) along the first component (Figure 2). The main call parameters that contributed to the first component were call duration (auto vector = 0.45) on the positive side and call rate (number of calls per

Table 1. Acoustic parameters of the advertisement call of *Ameerega flavopicta* from four localities in the states of Minas Gerais (MG), and Goiás (GO), Brazil.

Localities	n (males)	Call duration (s)	Inter-call interval (s)	Pulse/call	Call rate (call/min)	Max. frequency (Hz)	Min. frequency (Hz)
Araguari (MG)	3	0.10–0.13 (0.11 ± 0.01)	0.25–0.42 (0.31 ± 0.04)	7–9	123–146	3788–4285 (4045)	3457–3981 (3708)
Perdizes (MG)	2	0.16–0.18 (0.17 ± 0.01)	0.36–0.50 (0.43 ± 0.05)	6–9	94–109	3374–4340 (3889)	3181–3954 (3561)
Serra do Cipó (MG)	3	0.11–0.18 (0.15 ± 0.03)	0.33–0.69 (0.46 ± 0.12)	6–10	78–129	3292–3705 (3503)	2878–3457 (3203)
Caldas Novas (GO)	3	0.09–0.15 (0.11 ± 0.02)	0.23–0.29 (0.26 ± 0.02)	6–8	140–175	3650–4367 (4016)	3319–4119 (3723)

Values are ranges; those in parentheses are means or mean ± SD.

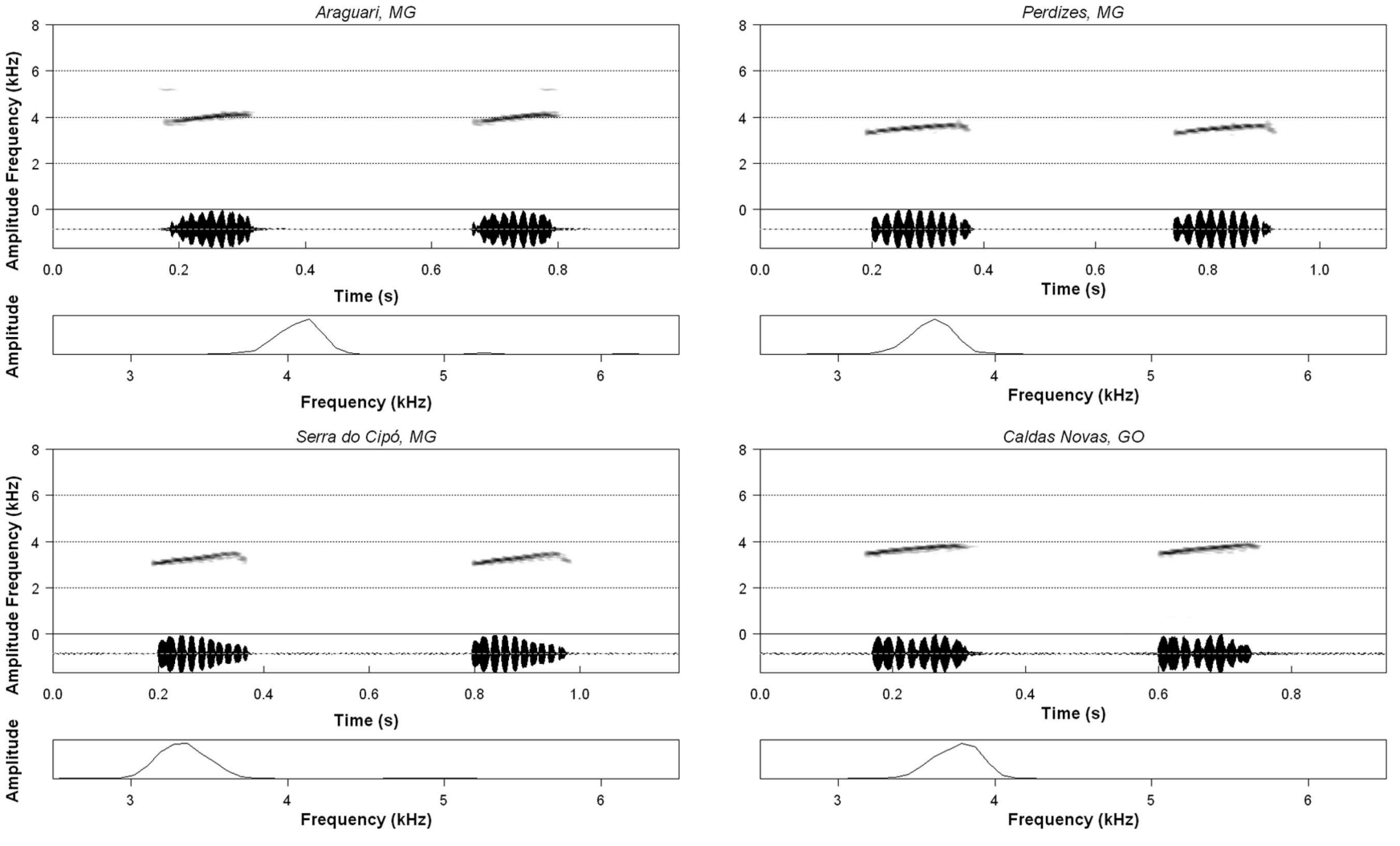

Figure 1. Advertisement calls of *Ameerega flavopicta* from four localities in the states of Minas Gerais (MG) and Goiás (GO), Brazil. Audiospectrogram (upper), oscillogram (middle) and power spectrum (lower) of two calls of one individual of each population are presented. Araguari (MG), air temperature (T-air) = 29.0°C, 07:45 h, November 2007. Perdizes (MG), T-air = 20.9°C, 05:45 h, November 2005. Serra do Cipó (MG), T-air = 18.2°C, 07:05 h, December 2005. Caldas Novas (GO), T-air = 23.5°C and water temperature = 22.7°C, 18:15 h, November 2004. AAG record files: AmerflavopMG9LMb; AmerflavopMG5AAGb, AmerflavopMG8AAGb, AmerflavopGO1AAGb, respectively.

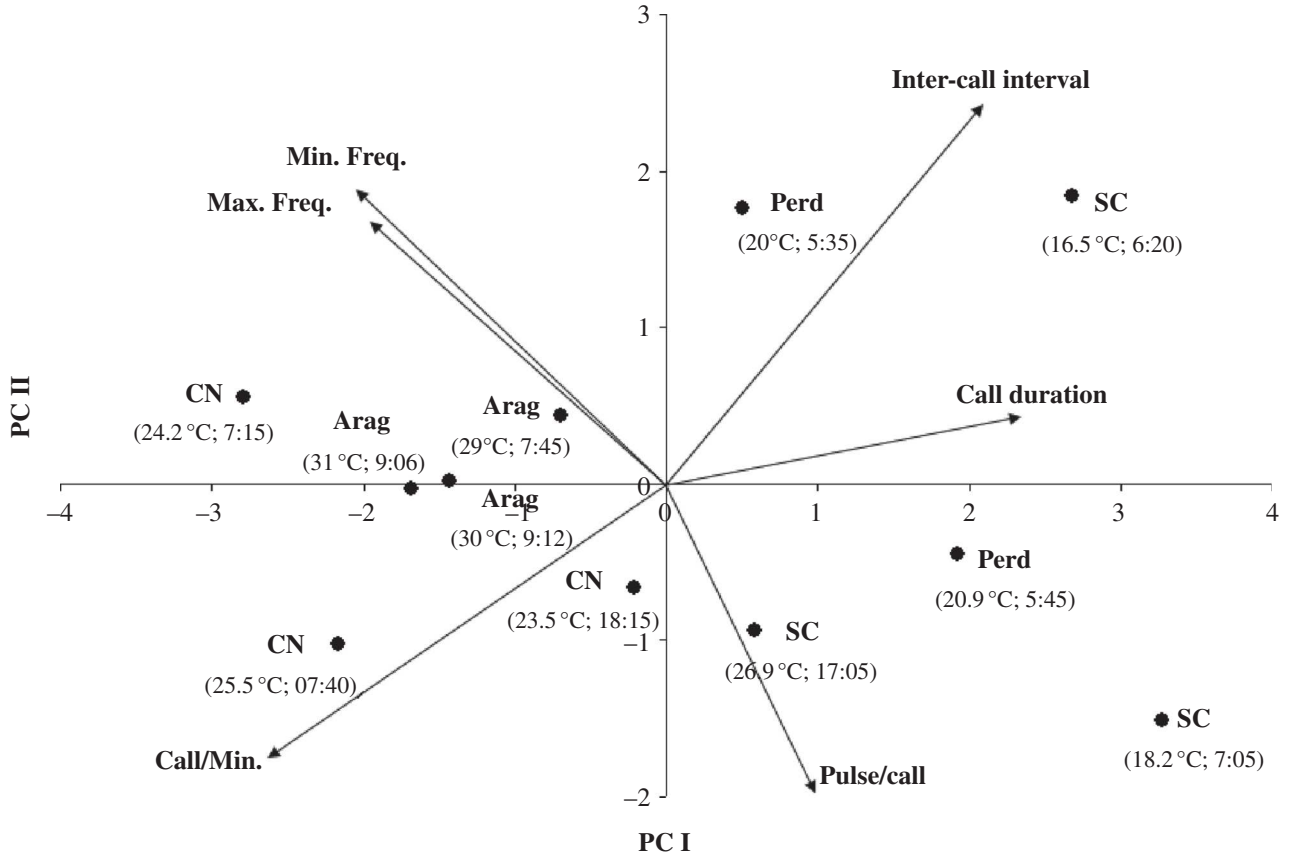

Figure 2. Principal components analysis on parameters of *Ameerega flavopicta* advertisement calls taken from records from four localities in the states of Minas Gerais (MG) and Goiás (GO), Brazil. Arag, Araguari (MG); Perd, Perdizes (MG); SC, Serra do Cipó (MG); CN, Caldas Novas (GO); Max. Freq., maximum frequency; Min. Freq., minimum frequency.

minute) (–0.44) and minimum (–0.43) and maximum (–0.41) frequencies on the negative side. The second component represented basically pulse number (auto vector = –0.54) on the negative side and inter-call intervals (0.54) on the positive side. Summarizing, the advertisement calls of Caldas Novas and Araguari populations differed from those of Perdizes and Serra do Cipó in both temporal and spectral (frequency) features. Individuals from Araguari and Caldas Novas had shorter calls with higher minimum and maximum frequencies and were emitted in a higher rate. Intra-populational variation in pulse number was greater than that among populations. Temperature was negative and significantly correlated with PCI scores (r_s = –0.682; n = 11; $P < 0.05$) and the studied localities also tended not to shuffle along the axis (Figure 3). The first two axes of the partial PCA with temperature as covariable explained 48.5% of the total variance and separated the Caldas Novas population from the others. Eliminating (partitioning out) the effect of temperature, which explained 38.8% of the total variance, males from Caldas Novas still had more calls per minute and shorter calls and inter-call intervals.

Reproductive behaviour

In December 2005 (07:00 h), we followed a courting pair at Serra do Cipó. Five minutes after detection of the couple in a narrow rocky crevice, it entered amplexus where the rock contacted the earth, 20 cm deep into the crevice. Amplectic position varied along the observation time (45 min) from axillary to mid-body to cephalic during which with the dorsal surface of the male's first fingers contacted the corner of the female's mouth. This last position was the most frequent.

Discussion

Among frogs, intraspecific variation in advertisement call parameters have been reported for widely distributed species, sometimes taking the form of gradients or clines, and for highly isolated populations. Geographical call variation can be found in parameters

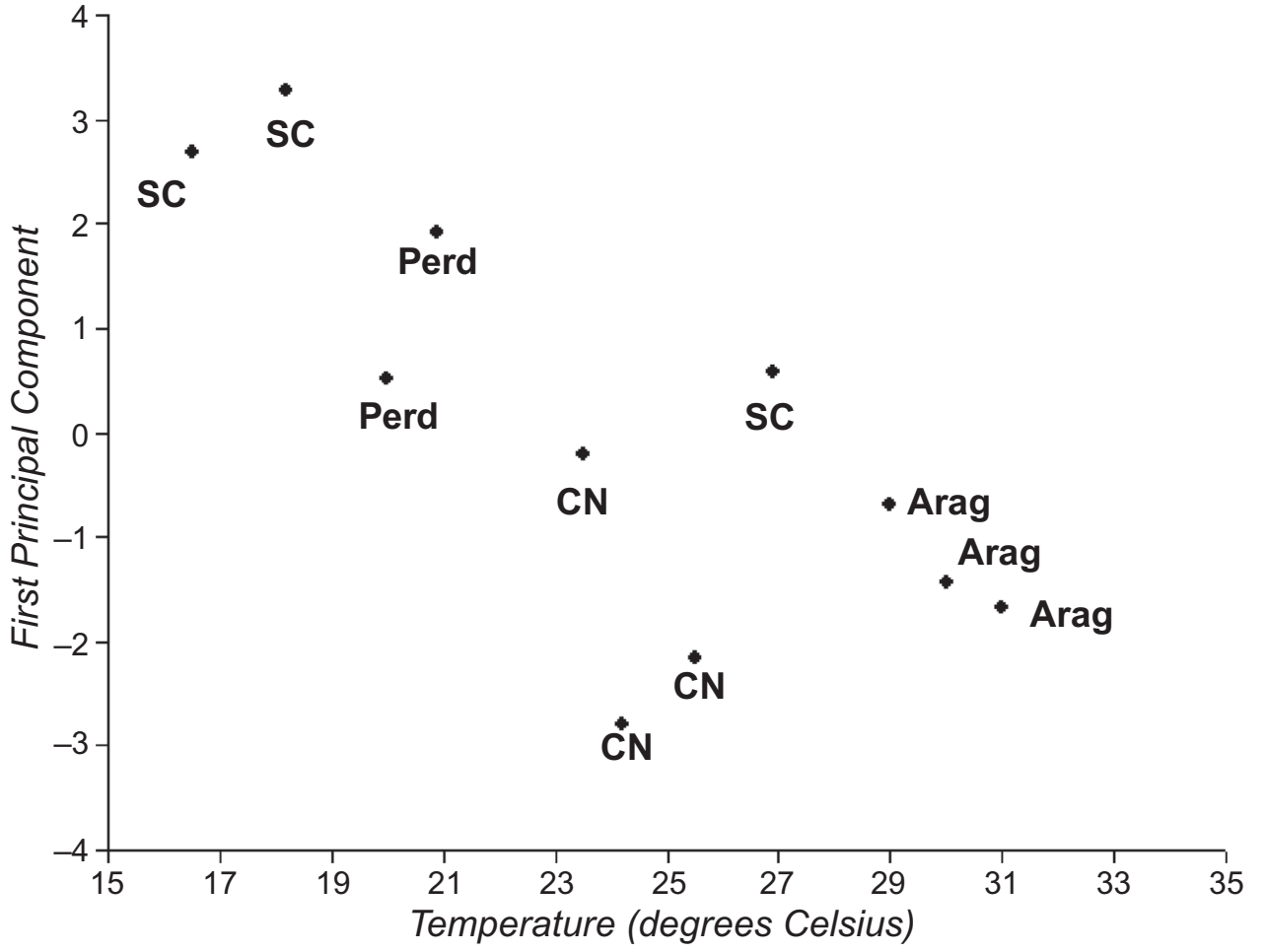

Figure 3. A plot of the first principal component (PC I) scores of the *Ameerega flavopicta* advertisement calls against air temperature of records from four localities in the states of Minas Gerais (MG) and Goiás (GO), Brazil. Main features summarized by the PC I were call rate (positive) and call duration (negative). Arag, Araguari (MG); Perd, Perdizes (MG); SC, Serra do Cipó (MG); CN, Caldas Novas (GO).

such as call duration, pulse number, frequency range, and emission rate (Smith et al. 2003; Bernal et al. 2005), such as we report here for *A. flavopicta*.

Based on a larger sample, here we reinforce our previous suggestions (Costa et al. 2006) that the advertisement calls of *A. flavopicta* of western populations (Caldas Novas, GO) present more calls per minute, shorter calls and smaller call intervals than those from eastern populations (e.g., Serra do Cipó, MG), which represent localities at the extremes of our sample data. The negative correlation we found between temperature and PCI scores indicates that, in general, in hotter localities the calls are shorter and emitted at higher rates, an expected relationship (Narins et al. 2007). We also found that localities do not mix up in the temperature × PCI plot, indicating that the geographical component of the call differences variation was not just an artifact generated by the differences in temperature. At present, the Paranaíba River (about 200 m wide) is a major geographic barrier and historically can be responsible for the isolation of the populations of *A. flavopicta* of Caldas Novas.

According to Grant et al. (2006), cephalic amplexus is a synapomorphy of the clade Colostethinae (Dendrobatidae), which is composed of the genera *Ameerega*, *Colostethus*, *Epipedobates* and *Silverstoneia*. Previously, Costa et al. (2006) reported on axillary amplexus in *A. flavopicta.* Herein we consider the amplectic position as variable (axillary, mid-body, and cephalic) in this species but recognize the cephalic amplexus as the main type, as expected for a typical member of this group (Juncá 1998; Grant et al. 2006).

One of us (A.A.G.) visited the population of Santana do Riacho at the margins of the MG 10 highway in the southern part of the Serra do Cipó three

times (1990, 1997, and 2005). This area had already been altered by human settlements (locals and tourists) and pastures (for cattle and horse farming) in 1990, and since then the situation has become worse by visitation, large amounts of improperly disposed domestic garbage, and asphalt pavement of the highway. In spite of these increased disturbances, a high number of calling males was recorded in all our visits. Together with other experts A.A.G. reviewed the status of *A. flavopicta* in Minas Gerais and considered it as a species of "Least Concern" (for IUCN definitions see Azevedo-Ramos et al. 2004). *Ameerega flavopicta* appears to be quite resistant to stressing climatic conditions and to habitats modified by cattle farming, removal of natural vegetation (by fire clearings or otherwise), and vehicle traffic (Costa et al. 2006; present study). If some local populations are really declining at Serra do Cipó as claimed by Eterovick et al. (2005), causes other than direct local anthropogenic effects, for example natural populational fluctuation or metapopulational dynamics, may be involved (Wissinger & Whiteman 1992; Blaustein et al. 1994; Sarkar 1996; Davidson et al. 2001).

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