Arquivos do Museu Nacional, Rio de Janeiro, v.63, n.2, p.275-282, abr./jun.2005
ISSN 0365-4508

ADVERTISEMENT CALLS OF SPECIES OF THE *HYLA ALBOSIGNATA* GROUP (AMPHIBIA, ANURA, HYLIDAE) [1]

(With 4 figures)

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ABSTRACT: We describe and compare the spectral and temporal characteristics of the advertisement calls of six species of the *Hyla albosignata* group (*H. albosignata*, *H. callipygia*, *H. cavicola*, *H. ibirapitanga*, *H. leucopygia*, and *H. sibilata*), and relate the ecological and morphological relationships among these species based on the phenetic analysis resulted from the acoustic parameters. Advertisement calls of species of the *H. albosignata* species group are quite similar, each consisting of one repeated note containing harmonic structure. Duration of calls range from about 0.1s (*H. sibilata*) to 0.5s (*H. cavicola*) and are repeated at different rates, from three calls per second (*H. sibilata*) to one call for each three seconds (*H. callipygia*). The main difference among the advertisement calls is related to their spectral properties. The arrangement of the phenetic tree does not corroborate the division proposed in the literature based on morphological differences of the cloacal region, nor is related to species body size. Sympatric species with similar calls are not syntopic, and syntopic species show more differentiated calls.

Key-words: Advertisement call; Cluster analysis; *Hyla albosignata*; *Hyla callipygia*; *Hyla cavicola*; *Hyla ibirapitanga*; *Hyla leucopygia*; *Hyla sibilata*.

RESUMO: Cantos de anúncio de espécies do grupo de *Hyla albosignata* (Amphibia, Anura, Hylidae).

Descrevemos e comparamos as características espectrais e temporais dos cantos de anúncio de seis espécies do grupo de *Hyla albosignata* (*H. albosignata*, *H. callipygia*, *H. cavicola*, *H. ibirapitanga*, *H. leucopygia* e *H. sibilata*) e confrontamos as relações ecológicas e morfológicas entre essas espécies baseadas em análise fenética resultante dos parâmetros acústicos. Os cantos de anúncio das espécies do grupo de *Hyla albosignata* são bastante semelhantes, cada um consistindo de uma nota com estrutura harmônica, emitida repetidamente. A duração dos cantos variou entre 0,1s (*H. sibilata*) e 0,5s (*H. cavicola*) e são repetidos em taxas diferentes, desde três cantos por segundo (*H. sibilata*) até um canto a cada três segundos (*H. callipygia*). A principal diferença entre os cantos de anúncio é relacionada às suas propriedades espectrais. O arranjo da árvore fenética não corrobora a divisão proposta em literatura baseada em diferenças morfológicas na região cloacal, nem está relacionada ao tamanho corporal das espécies. Espécies simpátricas de cantos similares não são sintópicas, enquanto espécies sintópicas apresentam cantos mais diferenciados.

Palavras-chave: Canto de anúncio; Análise de agrupamento; *Hyla albosignata*; *Hyla callipygia*; *Hyla cavicola*; *Hyla ibirapitanga*; *Hyla leucopygia*; *Hyla sibilata*.

INTRODUCTION

The number of ecological, taxonomic, and systematic studies using acoustic parameters as specific behavioral characters to clarify relationships among species groups has increased in the last ten years (*e.g.*, MÁRQUEZ, DE LA RIVA & BOSCH, 1993; COCROFT, 1994; HEYER, GARCÍA-LOPEZ & CARDOSO, 1996; DE LA RIVA, MÁRQUEZ & BOSCH, 1997; HADDAD & POMBAL, 1998; HARTMANN, HARTMANN & HADDAD, 2002). In the Neotropical region,

[1] Submitted on November 18, 2004. Accepted on March 11, 2005.
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relationships among taxa are particularly complex and the advertisement call (*sensu* WELLS, 1977), which plays a crucial role in anuran species recognition and female mate choice (*e.g.*, LITTLEJOHN & LOFTUS-HILLS, 1968; WELLS, 1977), is an important alternative character since it determines differences and similarities among phenetic groups (DE LA RIVA, MÁRQUEZ & BOSCH, 1997).

The *Hyla albosignata* species group is characterized by green color on dorsal surfaces, iris with two distinct color zones, and presence of anal milium and calcar appendage (CRUZ & PEIXOTO, 1984). Seven species are currently placed in the group: *Hyla albosignata* Lutz & Lutz, 1938; *H. callipygia* Cruz & Peixoto, 1984; *H. cavicola* Cruz & Peixoto, 1984; *H. fluminea* Cruz & Peixoto, 1984; *H. ibirapitanga* Cruz, Pimenta & Silvano, 2003; *H. leucopygia* Cruz & Peixoto, 1984; and *H. sibilata* Cruz, Pimenta & Silvano, 2003. The *H. albosignata* species group is restricted to the Atlantic Rain Forest from the State of Santa Catarina, southern Brazil, to the State of Bahia, northeastern Brazil (CRUZ & PEIXOTO, 1984; CRUZ, PIMENTA & SILVANO, 2003). Data about vocalizations are available only for *H. leucopygia* (BOKERMANN, 1967; HEYER *et al.*, 1990; HADDAD & SAWAYA, 2000), and *H. callipygia* (BOKERMANN, 1967), both referred to as *H. albosignata* in BOKERMANN (1967) (see HADDAD & SAWAYA, 2000, for discussion). Herein we describe and compare features of the advertisement calls of six species of the *H. albosignata* group (*H. albosignata*, *H. callipygia*, *H. cavicola*, *H. ibirapitanga*, *H. leucopygia*, and *H. sibilata*), and discuss our findings with respect to data obtained by other studies (BOKERMANN, 1967; HEYER *et al.*, 1990; HADDAD & SAWAYA, 2000). We also relate and comment on the ecological and morphological relationships among these species based on the phenetic analysis derived from the acoustic parameters of the advertisement calls.

MATERIAL AND METHODS

From 1993 to 2002, field activities in different Atlantic Rain Forest localities of eastern Brazil generated records of the advertisement calls of *H. albosignata*, *H. callipygia*, *H. cavicola*, *H. ibirapitanga*, *H. leucopygia*, and *H. sibilata* (Tab.1). *Hyla fluminea*, a species restricted to its type-locality at Serra dos Órgãos, State of Rio de Janeiro, Brazil, was the only species from the *H. albosignata* group in which our attempts to find calling males were unsuccessful.

Table 1. Record site locality, recorder model, microphone model, and number of recorded males of species of the *Hyla albosignata* group.

SPECIES	RECORD SITE LOCATION (COORDINATES; ALTITUDE; MUNICIPALITY, STATE)	RECORDER MODEL	MICROPHONE MODEL	NUMBER OF RECORDED MALES
H. albosignata	Fazenda São Luiz (24°13'S, 48°45'W; 875m Ribeirão Branco, São Paulo)	UHER-4000 Report	UHER	1
H. callipygia	Monte Verde (22°52'S, 46°02'W; 1600m; Camanducaia, Minas Gerais)	Sony TCM 5000-EV	Sony FV5	5
H. cavicola	(19°54'S, 40°35'W; 800m; Santa Teresa, Espírito Santo)	TASCAM DA-P1	Seinnheiser ME66	1
H. ibirapitanga	Fazenda Pedra Formosa (13°57'S, 39°27'W; 270m; Ibirapitanga, Bahia); RPPN Estação Veracruz (16°23'S, 39°10'W; 80m; Porto Seguro, Bahia); Fazenda Taquara (15°58'S, 39°22'W; 160 m; Belmonte, Bahia)	Sony DAT TCD-D8	Seinnheiser ME66	3
H. leucopygia	Serra do Japi (23°11'S, 46°52'W; 800m; Jundiaí, São Paulo)	UHER-4000 Report	UHER	4
H. sibilata	Fazenda Alto São Roque (13°51'S, 39°40'W; 670m; Itamari, Bahia); Fazenda Pedra Formosa (13°57'S, 39°27'W; 270m; Ibirapitanga, Bahia)	Sony DAT TCD-D8	Seinnheiser ME66	2

We digitized vocalizations in a PC Pentium at a sampling frequency of 22.05kHz, 16 bit resolution, FFT with 256 points, 100% Frame, Hamming window, 75% Overlap, and analysed them using the softwares Avisoft-Sonagraph Light 1, version 2.7, and Cool Edit 2000. Advertisement call terminology follows DUELLMAN & TRUEB (1986).

Nine acoustic parameters were measured: call duration (s), call interval (s), note number, fundamental frequency (kHz), dominant frequency (kHz), peak time (s) (time from the beginning of the call to the point of maximum amplitude), proportional rise time (ratio between peak time and call duration, which provide envelope form: linear, exponential or inverse exponential; see GERHARDT, 1998), frequency modulation (present or not present; corresponds to the difference between the frequencies at the end and the beginning of the call), and call structure (harmonic and/or pulsed). Waveforms and sonograms were produced using the Avisoft-Sonagraph Light 1 software, and spectrograms with the Cool Edit 2000 software. We used four acoustic parameters for clustering analysis: call duration, fundamental frequency, dominant frequency, and proportional rise time. The phenetic tree resulted from an UPGMA analysis (using Euclidean distance) of these four numerical parameters.

Voucher specimens are deposited at Museu Nacional, Rio de Janeiro, Brazil (MNRJ), and Célio F.B. Haddad collection (CFBH), housed at Departamento de Zoologia, Universidade Estadual Paulista, Rio Claro, Brazil.

RESULTS

The advertisement calls of the species in the *H. albosignata* group are quite similar, sharing as common characteristic a harmonic single note, frequently repeated. Waveforms, sonograms, and spectrograms of the advertisement calls of these species are in figures 1, 2, and 3, respectively. A summary of temporal and spectral acoustic parameters is presented in table 2. Accounts of descriptions and comparisons of calls are presented in accordance to the order of the phenetic tree (Fig.4), which arranged the species in two clusters, each consisting of three species: *H. albosignata*, *H. leucopygia*, and *H. sibilata* in the first cluster, and *H. callipygia*, *H. ibirapitanga*, and *H. cavicola* in the second cluster.

Hyla albosignata is a medium-sized treefrog (males snout-vent length [SVL] 39.0–46.5mm; CRUZ & PEIXOTO, 1984), associated with mountain rivulets at altitudes <850m, from the State of São Paulo to the State of Santa Catarina (CRUZ & PEIXOTO, 1984). The mean duration of its advertisement call is 0.2s (Fig.1A), emitted at regular intervals of about 0.7s. The mean dominant frequency is 2.63±0.01kHz, the highest among the group, corresponding to the third harmonic (Figs.2A and 3A). The peak time occurs right after the middle of the call and envelope form has approximately a linear rising form (Fig.1A).

Hyla leucopygia is a medium-sized member of the group (males SVL 36.8-45.1mm; CRUZ & PEIXOTO, 1984) and occurs in mountain rivulets and temporary ponds in the states of Rio de Janeiro and São Paulo, at elevations between 800 and 1200m (CRUZ & PEIXOTO, 1984; HADDAD & SAZIMA, 1992). Herein we extend the range of this species to the State of Espírito Santo (Municipality of Castelo, 20°36'S, 41°11'W). Males usually call from the vegetation above water (HADDAD & SAWAYA, 2000) and emit a series of short calls (0.1s; Fig.1B), half the duration of the call of *H. albosignata*, at intervals of about 0.9s. The mean dominant frequency is 2.41±0.04kHz, corresponding to the third harmonic (Figs.2B and 3B), as in *H. albosignata*. The peak time occurs at the beginning of the call and envelope form has an inverse exponential rising form (Fig.1B). The advertisement calls of *H. leucopygia* described by BOKERMANN (1967) (referred as *H. albosignata*; see HADDAD & SAWAYA, 2000) from Paranapiacaba, and HEYER *et al.* (1990) from Boracéia, are very similar to the call presented in our study. HADDAD & SAWAYA (2000) have already reported the acoustic similarities among different populations of *H. leucopygia*. Small differences among acoustic parameters of different populations could be attributed to air temperature and precipitation, number of calling males, and proximity of neighbouring males or females.

Hyla sibilata is the smallest treefrog of the group (males SVL 30.0–33.6mm; CRUZ, PIMENTA & SILVANO, 2003). It is restricted to the State of Bahia, occurring in lowlands as much as in mountain rivulets, from 20 to 720m in altitude (CRUZ, PIMENTA & SILVANO, 2003). Males call from different microhabitats, occupying several strata of the forest, from leaf litter to shrubs and

trees, but always above water and along rivulets. The advertisement call (Fig.1C) is the shortest (0.08s) among the species of the *H. albosignata* group and is repeated at the shortest time interval (0.3s). The mean dominant frequency is 2.44±0.05kHz (Figs.2C and 3C), the same as *H. leucopygia*. However, in *H. sibilata* the dominant frequency corresponds to the second harmonic. The peak time occurs at the end of the first third of the call and envelope form has an inverse exponential rising form (Fig.1C).

Hyla callipygia is the largest treefrog of the group (males SVL 40.0–50.7mm; CRUZ & PEIXOTO, 1984). It is distributed in mountain rivulets in the states of São Paulo, Rio de Janeiro, and Minas Gerais (GOMES & PEIXOTO, 1997), at altitudes of about 1500m. Males call from the muddy margins of the rivulets, above water (CRUZ & PEIXOTO, 1984). The advertisement call of *H. callipygia* (Fig.1D) has a mean duration of 0.3s (the second longest call), and is emitted with the greatest intercall time interval (2.8s) for the group. The mean dominant frequency is 1.04±0.59kHz, a value that does not correspond to any particular harmonic. The dominant frequency corresponds either to the fundamental frequency (about 0.8kHz in 84% of analysed calls) or to the third harmonic (about 2.4kHz in 16% of analysed calls). In fig.2D, we present the sonogram of the most common type of call. It is possible to observe on the spectrogram (Fig.3D) that there is only a small difference (at about 3.8±2.3dB; range=0.9-9.8dB; n=15 calls) between the intensity of the first and third harmonics. The peak time occurs in the middle of the call and the envelope form has approximately a linear rising envelope (Fig.1D). The advertisement call of *H. albosignata* from Bocaina (BOKERMANN, 1967; recognized as *H. callipygia* by HADDAD & SAWAYA 2000) has a dominant frequency concentrated at the third harmonic and longer duration, according to the sonogram in BOKERMANN (1967).

Hyla ibirapitanga is a medium-sized species (males SVL 37.0-41.0mm; CRUZ, PIMENTA & SILVANO, 2003). It is distributed in lowlands and mountain rivulets in the states of Bahia and Minas Gerais, at altitudes ranging from 60 to 635m (CRUZ, PIMENTA & SILVANO, 2003). Males call under the leaf litter at the muddy margins of rivulets or perched on vegetation above water (CRUZ, PIMENTA & SILVANO, 2003). Its advertisement call (Fig.1E) has half of the duration (0.2s) of the call of *H. cavicola* and is also given at a greater rate (number of calls per minute), being repeated at intervals of about 1.0s. The dominant frequency (0.82±0.05kHz) corresponds to the fundamental frequency (Figs.2E and 3E) and is the same as that of the most common dominant frequency of *H. callipygia*. The peak time and envelope form (Fig.1E) are quite similar to those of *H. callipygia* (Fig.1D).

Hyla cavicola is the second smallest species of the group (males SVL 33.8–37.3mm (CRUZ & PEIXOTO, 1984). Its range includes the mountain

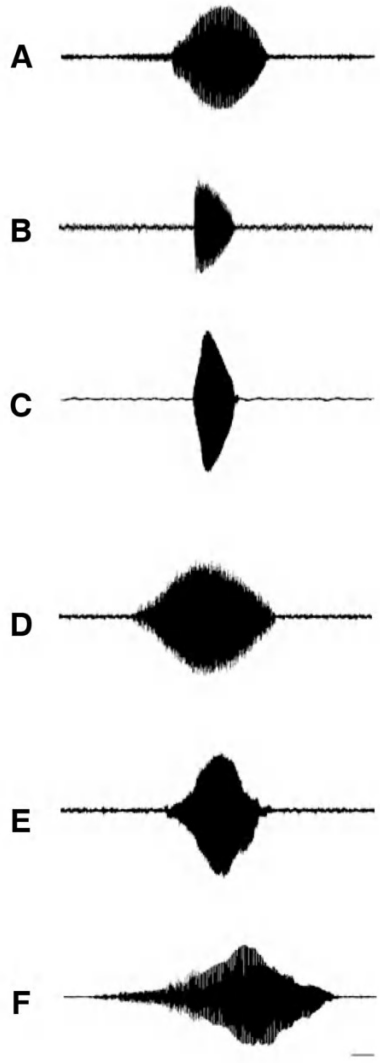

Fig.1- Waveforms of the advertisement calls of species of the *H. albosignata* group: (A) *H. albosignata* (t_{air}=20.0°C), (B) *H. leucopygia* (t_{air}=18.0°C), (C) *H. sibilata* (t_{air}=26.2°C), (D) *H. callipygia* (t_{air}=17.5°C), (E) *H. ibirapitanga* (t_{air}=28.6°C), and (F) *H. cavicola* (t_{air}=20.5°C). scale bar = 0.05s.

rivulets in the states of Espírito Santo and Minas Gerais, and it is found at altitudes between 200 and 850m (CRUZ & PEIXOTO, 1984). Males call inside burrows in the muddy margins of rivulets, in open areas or inside forests. The advertisement call is the longest (0.5s) among species of the group (Fig.1F) and is emitted at intervals of 1.8s. The dominant frequency is the lowest of the group (0.70±0.02kHz), corresponding to the fundamental frequency (Figs.2F and 3F). The peak time occurs just after the middle of the call and the envelope form has an exponentially rising envelope (Fig.1F). It is the only species in the *H. albosignata* group whose advertisement call has slight frequency modulation (Fig.2F).

DISCUSSION

In the call-derived phenetic tree, we can observe two distinct groups. The most important parameter for the result produced was the dominant frequency: the first cluster grouped species with a high dominant frequency (ranging from 2.4 to 2.6kHz) concentrated at the second (*H. sibilata*) or third harmonics (*H. albosignata* and *H. leucopygia*), whereas the second cluster grouped species with a low dominant frequency (ranging from 0.7 to 1.0kHz) concentrated at the fundamental harmonic (*H. callipygia*, *H. cavicola*, and *H. ibirapitanga*). The species arrangement inside each cluster is related to the envelope form of the call. *Hyla leucopygia* and *H. sibilata* have an exponentially rising call envelope, whereas *H. albosignata* has a nearly linear rising envelope. *Hyla callipygia* and *H. ibirapitanga* have an approximately linear rising call envelope, whereas *H. cavicola* has an exponentially inverse envelope.

According to DUELLMAN & PYLES (1983), calls of closely related allopatric species tend to be more similar than those of closely related sympatric species, and closely related sympatric species having similar calls are not syntopic and/or synchronous breeders. The study of MÁRQUEZ, DE LA RIVA & BOSCH (1993) differs from the findings of DUELLMAN & PYLES (1983) regarding syntopic species having more similar calls, but suggests that in this condition species should exhibit some level of resource partitioning, as they found. Although our study does not deal with communities as taxonomically diverse as those studied by DUELLMAN & PYLES (1983), but rather with a group of closely related species, we expected to find a similar relationship between

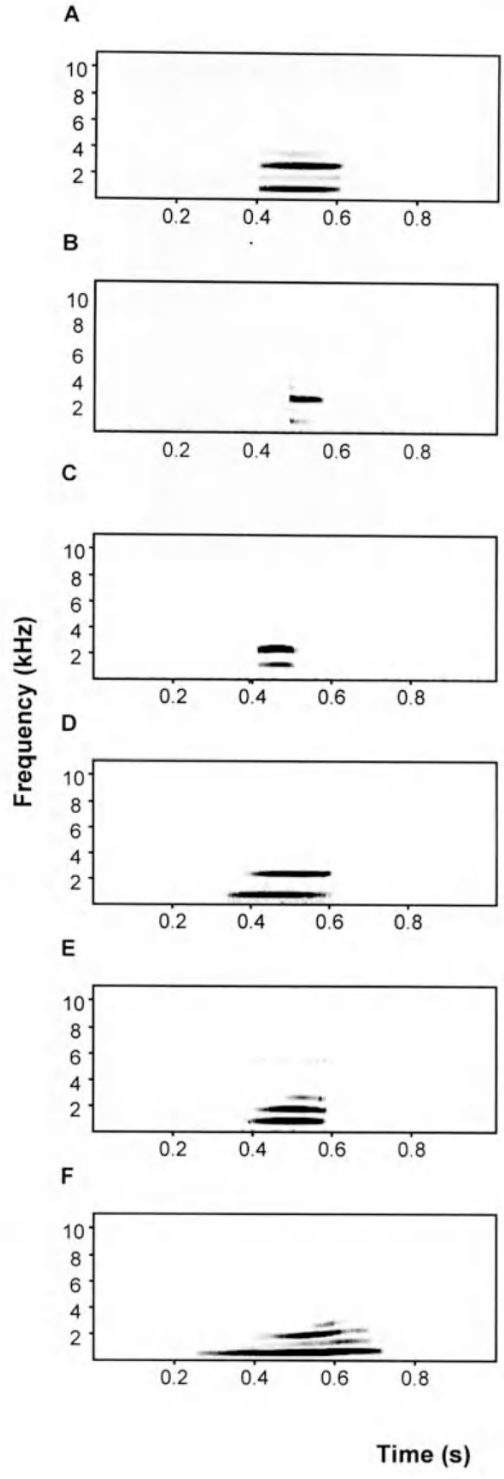

Fig.2- Sonograms of the advertisement calls of species of the *H. albosignata* group: (A) *H. albosignata* (t_{air}=20.0°C), (B) *H. leucopygia* (t_{air}=18.0°C), (C) *H. sibilata* (t_{air}=26.2°C), (D) *H. callipygia* (t_{air}=17.5°C), (E) *H. ibirapitanga* (t_{air}=28.6°C), and (F) *H. cavicola* (t_{air}=20.5°C).

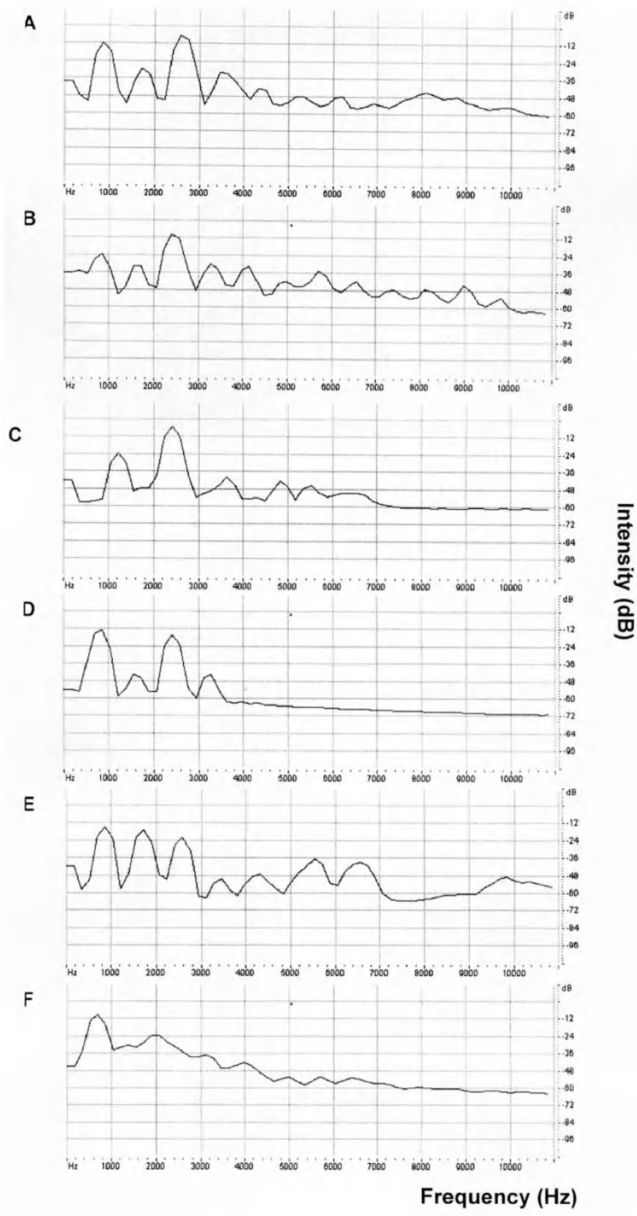

Fig.3- Spectrograms of the advertisement calls of species of the *H. albosignata* group: (A) *H. albosignata* (t_{air}=20.0°C), (B) *H. leucopygia* (t_{air}=18.0°C), (C) *H. sibilata* (t_{air}=26.2°C), (D) *H. callipygia* (t_{air}=17.5°C), (E) *H. ibirapitanga* (t_{air}=28.6°C), and (F) *H. cavicola* (t_{air}=20.5°C).

call structure and distribution. From two pairs of species having sympatric occurrence (*H. ibirapitanga* and *H. sibilata* in the State of Bahia; *H. albosignata* and *H. leucopygia* in the State of São Paulo), *H. ibirapitanga* and *H. sibilata*, which are syntopic, present more differentiated acoustic parameters, and were placed in different clusters. In the other hand, *H. albosignata* and *H. leucopygia* present more similar calls and were arranged at the same cluster, but not paired. These species were never found in syntopy, corroborating the findings of DUELLMAN & PYLES (1983). The remaining species (*H. cavicola* and *H. callipygia*), which are allopatric, are placed in the same cluster.

The division of two subgroups proposed by CRUZ & PEIXOTO (1984) and followed by CRUZ, PIMENTA & SILVANO (2003) based on the morphological differences of the cloacal region (1-presence of anal flap and a continuous milium around the cloacal region: *H. cavicola*, *H. ibirapitanga*, and *H. leucopygia*; 2-absence of anal flap, and anal milium not continuous: *H. albosignata*, *H. callipygia*, *H. fluminea*, and *H. sibilata*), is not corroborated by our call-based phenetic tree.

ZIMMERMAN (1983) found a negative correlation between SVL and dominant frequency of the advertisement call of different species. The *H. albosignata* species group does not seem to follow this pattern. In our phenetic tree, the range of male SVL overlapped in both clusters (30.0 to 46.5mm in the first cluster of *H. albosignata*, *H. leucopygia*, and *H. sibilata*; 33.8 to 50.7mm in the second cluster of *H. callipygia*, *H. ibirapitanga*, and *H. cavicola*). Moreover, the smallest species (*H. sibilata*) does not exhibit the highest dominant frequency and the largest species of the group (*H. callipygia*) does not exhibit the lowest dominant frequency. Thus, size also does not explain our phenetic tree.

The analysis of advertisement calls allows the inclusion of one more diagnostic character for members of the *H. albosignata* group: calls with a single harmonic note. Distances observed among the advertisement calls of syntopic and allopatric species on the phenetic tree does not reflect, necessarily, hypothesis of phylogenetic relationships. The differences found in syntopic species, for example, could be a result of putative selection avoiding the overlap on acoustical space. Future studies concerning the relationships among the members of this group using morphological, molecular, and additional acoustic data should be obtained to corroborate or refute these discrepancies between our conclusions and those of CRUZ & PEIXOTO (1984) and CRUZ, PIMENTA & SILVANO (2003).

Table 2. Acoustic parameters of the advertisement calls of species of the *Hyla albosignata* group. Values are presented as mean ± SD (range) (number of calls analyzed).

	Hyla albosignata	*Hyla leucopygia*	*Hyla sibilata*	*Hyla callipygia*	*Hyla ibirapitanga*	*Hyla cavicola*
CALL DURATION (s)	0.22±0.006 (0.21-0.23) (10)	0.11±0.01 (0.10-0.13) (19)	0.08±0.02 (0.05-0.11) (41)	0.32±0.04 (0.27-0.41) (63)	0.19±0.01 (0.16-0.22) (31)	0.47±0.04 (0.36-0.58) (29)
CALL INTERVAL (s)	0.68±0.06 (0.60-0.78) (10)	0.92±0.40 (0.45-1.63) (17)	0.32±0.09 (0.19-0.49) (36)	2.80±1.21 (1.08-5.90) (54)	1.00±0.77 (0.46-3.51) (26)	1.80±0.85 (0.56–3.26) (25)
FUNDAMENTAL FREQUENCY (kHz)	0.87±0.01 (0.85-0.88) (10)	0.82±0.02 (0.79-0.85) (19)	1.23±0.02 (1.22-1.28) (41)	0.79±0.03 (0.75-0.88) (53)	0.82±0.05 (0.74-0.90) (31)	0.70±0.02 (0.68-0.79) (29)
DOMINANT FREQUENCY (kHz)	2.63±0.01 (2.61-2.66) (10)	2.41±0.04 (2.39-2.56) (19)	2.44±0.05 (2.37-2.52) (41)	1.04±0.59 (0.75-2.48) (63)	0.82±0.05 (0.74-0.90) (31)	0.70±0.02 (0.68-0.79) (29)
PEAK TIME	0.12±0.01 (0.11-0.15) (10)	0.01±0.008 (0.005-0.03) (10)	0.03±0.004 (0.03-0.04) (10)	0.13±0.02 (0.11-0.16) (10)	0.09±0.01 (0.07-0.11) (10)	0.30±0.01 (0.28-0.31) (10)
PROPORTIONAL RISE TIME	0.56±0.06 (0.48-0.66) (10)	0.15±0.08 (0.04-0.29) (10)	0.34±0.03 (0.29-0.40) (10)	0.47±0.05 (0.40-0.58) (10)	0.53±0.07 (0.39-0.63) (10)	0.64±0.03 (0.60-0.68) (10)

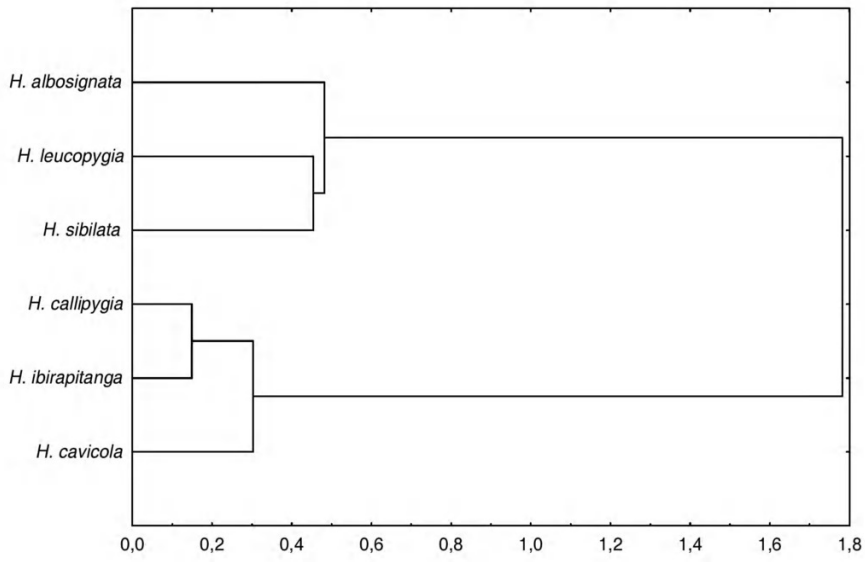

Fig.4- Phenetic tree resulting from the cluster analysis among acoustic parameters (call duration, fundamental frequency, dominant frequency, and proportional rise time) of the advertisement calls of species of the *H. albosignata* group. Vertical axis: species; horizontal axis: linkage distance.

ACKNOWLEDGEMENTS

To R.P.Bastos (Universidade Federal de Goiás), J.P.Pombal Jr. (Museu Nacional - Rio de Janeiro - MNRJ), and H.Wogel (MNRJ), for helpful comments on the manuscript; R.T.Moura and R.V.Lopes, for support in the fieldwork; owners of Fazenda Pedra Formosa and Fazenda Alto São Roque, for permission to work at their areas; VERACEL Celulose, for the permission and support to work at RPPN Estação Veracruz; J.P. Pombal Jr., for kindly providing his recordings of *Hyla cavicola* calls. To Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), for financial support. This work is partially a result of the sub-project "Ecological Approaches and Economic Instruments for the Establishment of the Discovery Corridor: a Strategy to Revert Forest Fragmentation of the Atlantic Forest at Southern Bahia", supported by the Project on the Conservation and Sustainable Use of the Brazilian Biological Diversity/Brazilian Ministry of the Environment (PROBIO/MMA), Center for Applied Biodiversity Science/Conservation International (CABS/CI), The World Bank, and coordinated by the Institute for Social and Environmental Studies of Southern Bahia (IESB).

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