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THE ADVERTISEMENT CALL OF *HYLA ATLANTICA* CARAMASCHI & VELOSA, 1996, WITH CONSIDERATIONS ON ITS TAXONOMIC STATUS (AMPHIBIA, ANURA, HYLIDAE) [1]

(With 6 figures)

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ABSTRACT: The advertisement call of *Hyla atlantica* is described upon specimens from the Municipality of Ituberá, Bahia, Brazil. The call comprises four to eight notes. Call duration 0.19-0.44s, and note duration 12-22.37ms. Two spectral bandwidths (harmonics) are distinct. The lowest-pitched harmonic ranges from 0.68-1.90kHz, and the highest from 2-3.68kHz. Overall dominant frequencies 0.84-1.28kHz (fundamental frequency), with exception of four calls (3.25%) in which values reached 2.18-2.53 kHz. Comparisons of the advertisement calls of *H. atlantica* and *H. punctata* resulted in two different suppositions in respect to the taxonomic status of the former. The overall analysis of the literature indicated that the calls of *H. atlantica* and *H. punctata* from Manaus (Amazonas, Brazil), Santa Cecilia (Ecuador), and Puerto Almacén (Bolivia) are similar, which does not support *H. atlantica* as a valid species. In a complementary analysis, using recordings of the call of *H. punctata* available on commercial digital media from Bolivia and French Guiana, and another from Maranhão (Brazil), significant differences between the calls of *H. atlantica* and *H. punctata* were obtained, which lead to the conclusion that may exist at least three distinct species involved: *H. atlantica* from Bahia (Brazil); *H. punctata* from northern South America (Manaus/Amazonas and Maranhão, Brazil; Ecuador; French Guiana; Suriname); and a third species from Bolivia (type locality of *H. p. rubrolineata*), Acre (Brazil), and Chacoan Argentina. The latter hypothesis is more congruent with the geographic distribution of the samples analyzed, and also with the current taxonomic positions of the taxa involved.

Key-words: Hylidae. *Hyla atlantica*. *Hyla punctata*. Taxonomy. Advertisement call.

RESUMO: O canto de anúncio de *Hyla atlantica* Caramaschi & Velosa, 1996, com considerações sobre sua posição taxonômica (Amphibia, Anura, Hylidae).

O canto de anúncio de *Hyla atlantica* é descrito a partir de espécimes do Município de Ituberá, Bahia, Brasil. O canto compreende quatro a oito notas. Amplitude de duração do canto 0,19-0,44s; tempo de duração da nota 12-22,37ms. Duas faixas espectrais (harmônicos) são distintas. Harmônico de freqüência mais baixa 0,68-1,90kHz; harmônico de freqüência mais alta 2-3,68kHz. Freqüência dominante 0,84-1,28kHz (freqüência fundamental), com exceção de quatro cantos (3,25%), nos quais há valores que alcançam 2,18-2,53kHz. Comparações dos cantos de *H. atlantica* e *H. punctata* resultaram em duas suposições diferentes quanto à posição taxonômica de *H. atlantica*. A análise da literatura indicou que os cantos de *H. atlantica* e *H. punctata* de Manaus (Amazonas, Brasil), Santa Cecília (Equador) e Puerto Almacén (Bolívia) são similares, o que não suporta *H. atlantica* como espécie válida. Em análises complementares, utilizando gravações do canto de *H. punctata* da Bolívia e Guiana Francesa, disponíveis em mídia digital comercial, e outra do Maranhão (Brasil), observaram-se diferenças significativas entre os cantos de *H. atlantica* e *H. punctata*, o que levou à conclusão que podem existir ao menos três espécies distintas envolvidas: *H. atlantica* da Bahia (Brasil); *H. punctata* do norte da América do Sul (Manaus/Amazonas e Maranhão, Brasil; Equador; Guiana Francesa; Suriname); e uma terceira espécie da Bolívia (localidade-tipo de *H. p. rubrolineata*), Acre (Brasil) e Chaco Argentino. A última hipótese é mais congruente com a distribuição geográfica das amostras analisadas e igualmente com as posições taxonômicas correntes dos táxons envolvidos.

Palavras-chave: Hylidae. *Hyla atlantica*. *Hyla punctata*. Taxonomia. Vocalização.

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INTRODUCTION

Hyla atlantica is a green tree frog that inhabits open flooded habitats in Atlantic Tropical Forest areas of southern State of Bahia, Brazil. CARAMASCHI & VELOSA (1996) described this taxon based on specimens from the Municipality of Itabuna, Bahia. SILVANO & PIMENTA (2002) extended its range ca. 102km northwards to Nilo Peçanha and Camamu, Bahia. This taxon is closely related to *Hyla punctata* (Schneider, 1799), and is distinguished from the latter by proportions of the head and shape of vomerine teeth and choanae (CARAMASCHI & VELOSA, 1996). *Hyla punctata* comprises disjunct populations widespread in South America, occurring in seasonally flooded forests of the Amazon Basin of Ecuador, Peru, Bolivia, and Brazil, Orinoco Basin, Central Brazil, Chaco of Paraguay and Argentina, Guyana, Suriname, French Guiana, northern Colombia, Venezuela, and Trinidad (FROST, 2004). This extensive distribution is followed by minor variations in external morphology and color patterns (DUELLMAN, 1974; HEYER, 1977), which lead some authors in recent past to consider some of these morphotypes as distinct species or subspecies (see CARAMASCHI & VELOSA, 1996 for a brief summary), but all synonymized to *H. punctata* by DUELLMAN (1974). The similarity shared in external morphology by *H. punctata* and *H. atlantica* makes necessary clarifications of the taxonomic status of the latter, mainly by non-morphologic methods.

Fieldworks at Ituberá, southern Bahia, lead us to record the advertisement call of *H. atlantica*. As this species is morphologically very similar to *H. punctata*, the description of its vocalization and comparisons with advertisement calls of *H. punctata* are desirable and presented herein.

MATERIAL AND METHODS

Recordings of two specimens of *H. atlantica* were obtained in 19 June 2004 during a survey of anurans in Ituberá, Bahia, Brazil. These specimens were found at night, calling horizontally positioned on grassy vegetation in river edges, in open habitat. Two other specimens were captured in a second flooded area. Frog vocalizations were recorded in the field with a Marantz Portable Cassette Recorder PMD222 with a Sennheiser ME66 directional microphone. Tapes were analyzed with Avisoft-SASLab Light for Windows, version 3.74, and Sound Ruler Acoustic Analysis, version 0.941. The vocalizations were digitized with a sampling frequency of 8 and 11kHz; the sonograms were made with Fast Fourier Transform length (FFT) of 256, overlap 87.5%, frame 100%, and window Flat Top. Call parameters were compared using Kruskal-Wallis test and Mann-Whitney *U*-test (SOKAL & ROHLF, 1981). All tests were two-tailed. When multiple post hoc comparisons were carried out on the same set of subjects, the α-level of significance ($P \leq 0.05$) was Bonferroni corrected (BLAND, 2004). Nonparametric tests were used because most variables were not normally distributed. Specimens were deposited in the amphibians' collection of the Museu de Zoologia of the Universidade Federal da Bahia (UFBA), along with a cassette tape with the advertisement calls of *H. atlantica* from Ituberá, Bahia (UFBA 001-K7). The terminology used for the description of the advertisement calls follows DUELLMAN & TRUEB (1986). Snout-vent length (SVL) was measured for each specimen captured.

RESULTS

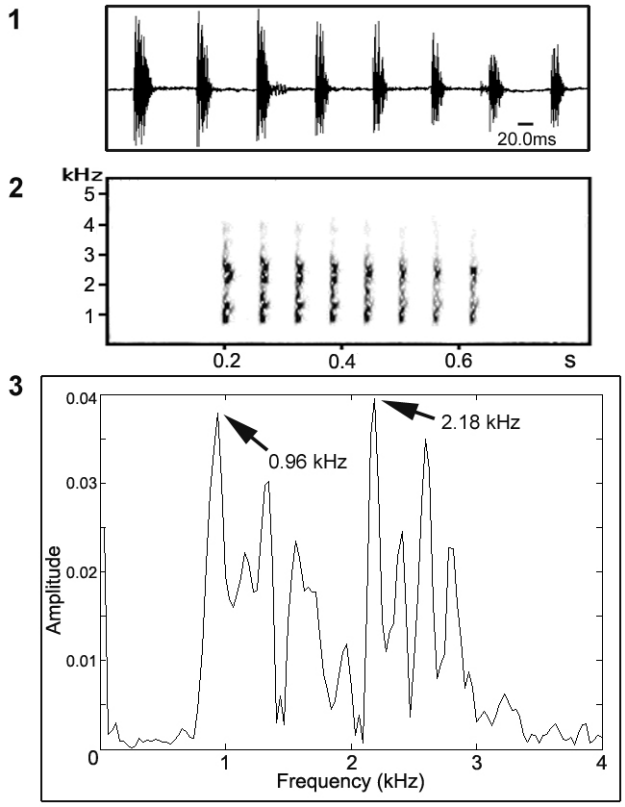
The following description is based on advertisement calls of two specimens of *H. atlantica*, approximately two meters distant from each other, both captured, but only one with its vocalization correctly assigned to him (UFBA 3090; SVL 35.9mm), given that the second specimen was accidentally deposited with other individuals of *H. atlantica* during the fieldwork (UFBA 3091-3093; SVL 34.9-36.7mm). For the purposes of this paper, specimen UFBA 3090 will be named NU1, and the second specimen NU2 (specimens numbers one and two, respectively). The specimens NU1 and NU2 were approximately one and two meters distant from the microphone, respectively. The calls were emitted sporadically, and therefore we decided to not measure some temporal parameters (intercall intervals; calls per minute; notes per minute).

Calls (Fig.1-3) comprised four to eight notes (note=pulse). NU1 had seven calls (41.17%) with six notes, six calls (35.29%) with five notes, two calls (11.76%) with seven notes, one call (5.88%) with four notes, and one call (5.88%) with eight notes. NU2 had six calls (75%) with four notes, and two calls (25%) with five notes.

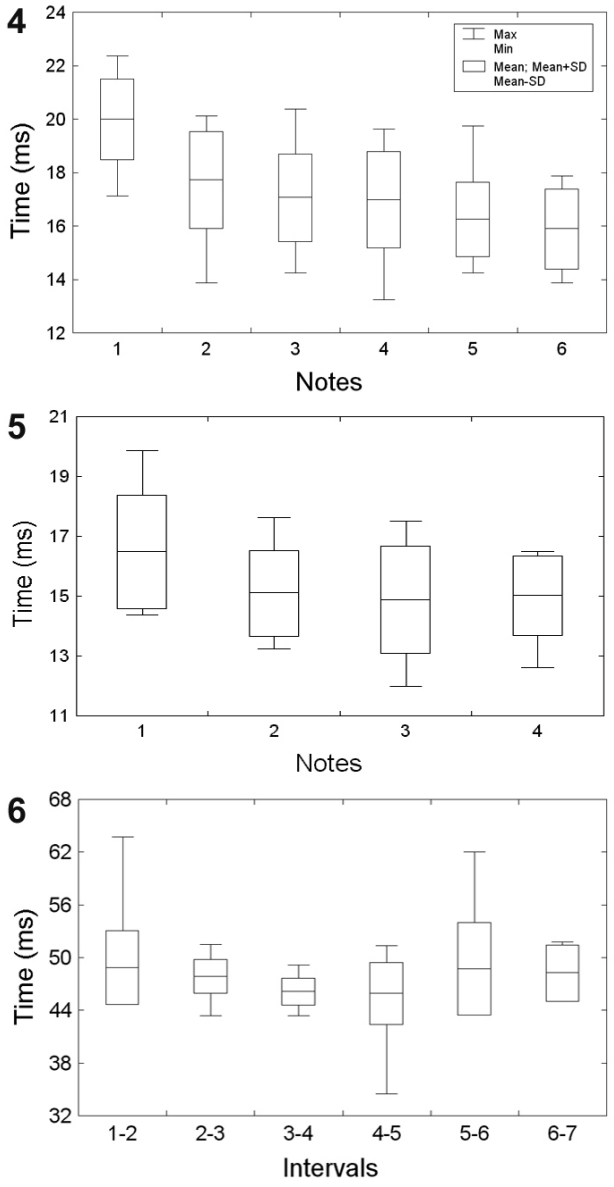
Temporal Structure – Calls with four notes had duration of 0.19-0.26s ($\bar{x}$=0.21; *N*=7; SD=0.02); with five notes 0.26-0.29s ($\bar{x}$=0.27; *N*=8; SD=0.01); with six notes 0.32-0.34s ($\bar{x}$=0.33; *N*=7; SD=0.01); with seven notes 0.38-0.39s ($\bar{x}$=0.39; *N*=2; SD=0.00); with eight notes 0.44s (*N*=1) (Fig.1). Overall duration of a note varied from 12-

22.37ms ($\bar{x}$=16.88; *N*=127; SD=2.16). The duration of notes in specimen NU1 were not equal (Kruskal-Wallis test; $H_{5, 89}$=33.25; *P*<0.0001), and decreased from the first to the last note (Fig.4); note 1 differed from notes 2-6 (Mann-Whitney *U*-test; Bonferroni corrected *P*<0.0033; U_{1-2}=47; N_1=16; N_2=17; *P*<0.0014; U_{1-3}=28; N_1=16; N_2=17; *P*<0.0001; U_{1-4}=20.5; N_1=16; N_2=16; *P*<0.0001; U_{1-5}=8.5; N_1=16; N_2=15; *P*<0.0001; U_{1-6}=3.5; N_1=16; N_2=8; *P*<0.0002); notes 2-6 did not differ from each other. The duration of notes in specimen NU2 were not significantly different ($H_{3, 31}$=3.83; *P*<0.29), but note 1 tended to be longer than notes 2-5 (Fig.5). Intervals between successive notes varied from 34.50-68.87ms ($\bar{x}$ =49.11; *N*=102; SD=5.42). Specimen NU1 had intervals significantly different ($H_{5, 79}$=13.51; *P*<0.0190), which decreased from notes 1-3, and increased from notes 4-6 (Fig.6).

Harmonic structure – Two dominant bandwidths (harmonics) are clearly distinguishable from the audiospectrogram and power spectrum (Fig.2-3). The lowest-pitched harmonic (fundamental frequency) ranged from 0.68-1.53kHz. The highest-pitched harmonic ranged from 1.90-3.68kHz. Some calls (12%) had peaks of dominant frequencies in both bandwidths (one per note, never co-dominants), but often only in the lowest-pitched harmonic (88%), which resulted in specimens in an overall dominant frequency range of 0.84-1.28kHz ($\bar{x}$ =0.93; *N*=119; SD=0.07), with exception of four calls (3.25%) in which overall dominant frequencies reached values from 2.34-2.53kHz (Fig.3). The peaks of energy in the second bandwidth (highest-pitched harmonic) ranged from 2-2.99kHz ($\bar{x}$ =2.37; *N*=137; SD=0.17). The frequency amplitude ranged from 0.51-4.73kHz.



Advertisement call of *Hyla atlantica*, UFBA 3090, SVL 35.9mm, Municipality of Ituberá, Bahia, Brazil, 19/VI/2004, air temperature 22°C – fig.1- oscillogram; fig.2- sonogram; fig.3- power spectrum. Two distinct bandwidths (harmonics) can be viewed in figure 2 and figure 3. The overall dominant frequencies most often occur (96.75%) in the low-pitched harmonic (fundamental frequency: 0.84-1.28 kHz), but in four calls analyzed (3.25%) the dominant frequencies occurred in the highest-pitched harmonic (2.18-2.53 kHz), as observed in figure 3.



Box and whisker plots of temporal parameters of the advertisement call of *Hyla atlantica* from Ituberá, Bahia, Brazil – fig.4 and fig.5- duration of notes 1-6 of specimens NU1 (UFBA 3090) and NU2, respectively; fig.6- intervals between successive notes (1-7) of specimen NU1.

DISCUSSION

The advertisement call of *H. punctata* was described for distinct localities in South America by different authors. HÖDL (1977), DUELLMAN (1978), DUELLMAN & PYLES (1983), CARDOSO & VIELLIARD (1990), and MÁRQUEZ, DE LA RIVA & BOSCH (1993) furnished temporal and spectral parameters for calls of *H. punctata* from Manaus (Amazonas, Brazil), Santa Cecilia (Ecuador), Acre (Brazil), and Puerto Almacén (Bolivia). The dominant frequencies reported by them ranged from 0.78-1.40kHz ($\bar{x}$=0.92-1.07kHz), and are similar to the values obtained for *H. atlantica* ($\bar{x}$ =0.93kHz; 0.84-1.28kHz), with exception of that from Acre, in which the dominant frequencies were around 2.4kHz. Sonograms of the calls of *H. punctata* figured by HÖDL (1977) and CARDOSO & VIELLIARD (1990) clearly denoted two bandwidths of dominant frequencies, which is similar to the pattern observed for *H. atlantica* (Fig.2). HOOGMOED (1979) provided two sonograms of calls of *H. punctata* from Suriname, a short call (with about four pulses), and a long call (with about 15 pulses), without comments on temporal or spectral parameters. The general structure of the call resembles that obtained for *H. punctata* from other localities, but the former clearly seems to have shorter intervals between notes. The range for number of notes per call reported in the literature for *H. punctata* is variable. MÁRQUEZ, DE LA RIVA & BOSCH (1993) cited 4-8 notes/call, which is equal to the values observed for *H. atlantica*. HÖDL (1977), DUELLMAN (1978), and DUELLMAN & PYLES (1983) reported 5-15 ($\bar{x}$=9.6), 3-17 ($\bar{x}$ =8.4), and 5-17 ($\bar{x}$ =5.7) notes/call, respectively. These variations may be explained by the observations of CARDOSO & VIELLIARD (1990) for *H. punctata* from Acre (Brazil). The latter authors reported that the vocalization of *H. punctata* typically have 6-8 notes/call. Under undisturbed conditions, three specimens of *H. punctata* emitted 5-8, 7-10, and 4-6 notes/call, but one of them, when excited or perturbed, emitted one call with three notes, and two calls with 20 and 34 notes. Other three excited specimens emitted 5-9 and 4-10 notes/call, but with variations ranging from 12-23 notes/call. These data indicate that the variation in number of notes per call observed by other authors for geographically different population samples of *H. punctata* is possibly related to excitatory and disturbance conditions, and should not be considered as inter-specific variation. This conclusion is supported by HÖDL (1977), who obtained 5-15 notes/call from choruses (not from isolated individuals; ZIMMERMAN, 1983), and MÁRQUEZ, DE LA RIVA & BOSCH (1993), which stated that single calls were sometimes followed by long sequences of notes, like a rapid drumming, when large numbers of individuals were calling

together. The call duration of *H. atlantica* ($\bar{x}$ =0.28s; *N*=25; SD=0.06; 0.19-0.44s) is similar to the values from HÖDL (1977) for *H. punctata* ($\bar{x}$ =0.32s; *N*=39; 0.16-0.47s). The duration of notes of *H. atlantica* ($\bar{x}$=16.8ms; *N*=127; SD=2.1; 12.0-22.3ms) is similar to that obtained by MÁRQUEZ, DE LA RIVA & BOSCH (1993) for *H. punctata* ($\bar{x}$=17.4ms; *N*=49; SD=1.2; 14.9-22.0ms). BARRIO (1965) described the advertisement call of *H. punctata rubrolineata* Lutz, 1951 from the Chacoan Argentina, but not assigned the call to a voucher specimen. The sonogram figured has three notes, and does not have a good resolution (duration of call 0.25s; dominant frequency 2.70kHz; frequency range up ca. 8.0kHz). With exception of the frequency amplitude, these parameters are comprised in the ranges cited for *H. punctata* and *H. atlantica*. However, the author described the voice of *H. p. rubrolineata* as being a "slash on a crystal glass" very similar to that of *Hyla albofrenata*. This latter statement is at least curious, because the voice of *H. albofrenata* Lutz, 1924 is absolutely different from that of *H. punctata* and *H. atlantica* (personal observation).

The overall analysis of the literature lead us to conclude that the advertisement calls of *H. punctata* from Manaus (Amazonas, Brazil), Santa Cecilia (Ecuador), and Puerto Almacén (Bolivia) are similar in spectral and temporal parameters, and that the call of *H. atlantica* is similar to them, mainly in the ranges of dominant frequencies. Therefore, the differences observed did not support the taxon *H. atlantica* as a valid species, which possibly only represents an Atlantic Forest population of *H. punctata*. The calls of *H. punctata* from Acre (Brazil) and Chacoan Argentina have higher dominant frequencies (in the second harmonic) than *H. atlantica* and the northernmost sample calls of *H. punctata* (in the first harmonic), which lead us to suppose that the former samples could represent a distinct species. The sonogram of the call of *H. punctata* from Suriname apparently differs from all other calls, mainly by shorter intervals between successive notes.

The literature data, however, do not agree entirely with recordings of the call of *H. punctata* available on commercial digital media. A sample of the voice of *H. punctata* available in "Sounds of Frogs and Toads of Bolivia" (MÁRQUEZ *et al.*, 2002) revealed different acoustical parameters from that published by MÁRQUEZ, DE LA RIVA & BOSCH (1993) for Bolivian specimens. In this sample, the dominant frequencies range from 1.37-3.27kHz ($\bar{x}$ =3.02; *N*=14; SD=0.48), denoting the highest-tuned call observed; two calls have three notes and two, four notes; intervals between successive notes range from 63.8-77.9ms ($\bar{x}$=71.2; *N*=8; SD=4.8). These parameters are similar to that published by BARRIO (1965) for *H. p. rubrolineata* from Chacoan Argentina, and by CARDOSO & VIELLIARD (1990) for *H. punctata* from Acre (Brazil), in which the dominant frequencies are located in the second harmonic. Another sample of the call of *H. punctata* is available in "Sound Guide to The Tailless Amphibians of French Guiana" (MARTY & GAUCHER, 1999). The dominant frequencies range from 1.20-1.37kHz ($\bar{x}$ =1.21; *N*=36; SD=0.04); six calls have six notes; intervals between successive notes range from 31.7-38.6ms ($\bar{x}$ =34.3; *N*=30; SD=2.2). These parameters agree with the sonograms published by HOOGMOED (1979) for *H. punctata* from Suriname, and are clearly distinguishable from that available for Bolivian specimens (MÁRQUEZ *et al.*, 2002) by lower dominant frequencies and shorter intervals between successive notes. The advertisement call of *H. punctata* from Maranhão, Brazil (recorded by G.V. Andrade), have dominant frequencies ranging from 1.03-2.06 kHz ($\bar{x}$=1.52; *N*=40; SD=0.51); calls have 5-15 notes; intervals between successive notes range from 17.2-34.3ms ($\bar{x}$ =25.5; *N*=32; SD=4.0). The spectral parameters are similar to that of *H. punctata* from French Guiana, Manaus (Amazonas, Brazil), and Santa Cecilia (Ecuador), and the temporal parameters and number of notes resemble that from French Guiana, Manaus (Amazonas, Brazil), Santa Cecilia (Ecuador), and Suriname. Statistical comparisons (Kruskal-Wallis test; Mann Whitney *U*-test; $P \leq 0.05$) among the call samples analyzed (*H. atlantica*: Bahia; *H. punctata*: Bolivia, French Guiana, and Maranhão) for the parameter "intervals between successive notes" revealed that all are significantly different. Moreover, it is clearly noticeable that the northernmost population samples (French Guiana and Maranhão) have shorter intervals, and Bolivia the longest. The dominant frequencies are not statistically different between calls from French Guiana and Maranhão, but are significantly different for all other pair-wise comparisons, where *H. punctata* from Bolivia have the highest dominant frequencies, and *H. atlantica* from Bahia the lowest. Given these results, we conclude that at least three distinct species could be involved: *H. atlantica* from Bahia (Brazil); *H. punctata* from northern South America (Manaus/ Amazonas and Maranhão, Brazil; Santa Cecilia,

Ecuador; French Guiana; Suriname); and a third from Bolivia (type locality of *H. p. rubrolineata*), Acre (Brazil), and Chacoan Argentina. This alternative hypothesis is more congruent with the geographic distribution of the samples involved, and with the current taxonomic positions of the taxa involved.

It is important to note that we did not make an extensive morphological analysis of *H. punctata*, nor have additional sample calls or other kinds of data, like molecular studies, which could subsidies one of these two suppositions. The current analysis was essentially based on literature data, somewhat biased by differences in the methods employed by each author in the acoustical analyses, and also limited by the degree of detail of each description. Moreover, the differences observed in Bolivian data (MÁRQUEZ, DE LA RIVA & BOSCH, 1993; MÁRQUEZ *et al.*, 2002) allow two different conclusions in respect to the Bolivian specimens of *H. punctata*. The contradictions and limitations of the analyzed data claim for caution in the decision to consider *H. atlantica* as a junior synonym of *H. punctata*, or instead, to consider *H. p. rubrolineata* as a full species. Therefore, we prefer to maintain the taxonomic status of *H. atlantica* and *H. p. rubrolineata* until more detailed morphologic and acoustical data from all known geographic populations of *H. punctata* and *H. atlantica* are available.

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