

Bioacoustics supports genus identification in piranhas^{a)}

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ABSTRACT:

In different teleost species, sound production can utilize specific coding schemes to avoid confusion between species during communication. Piranhas are vocal Neotropical fishes, and both *Pygocentrus* and *Serrasalmus* produce similar pulsed sounds using the same sound-producing mechanism. In this study, we analysed the sounds of three *Pygocentrus* and nine *Serrasalmus* species to determine whether sounds can be used to discriminate piranha species at both the species and genus levels. Our analysis of temporal and frequency data supports the idea that the sounds of *Serrasalmus* and *Pygocentrus* species are species specific, and that different acoustic features can be used to differentiate taxa at the genus level. Specifically, the sounds of *Serrasalmus* species are shorter, louder, and have a shorter pulse period (as determined after correction for standard length). This suggests that sounds can be used to support taxonomy at the genus level as well as the species level. © 2023 Acoustical Society of America.

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I. INTRODUCTION

In fish, sound production plays a critical role in various social communication contexts, including reproduction,^{1,2} territory defense,³ and conspecific/heterospecific fighting.^{1,4,5} During the reproduction, the use of specific coding schemes is essential to prevent confusion among species.⁶ Therefore, many fish species have evolved calls that are presumed to be species specific.^{7–9} The production of sound in fish has independently emerged multiple times.^{10,11} In some fish taxa, all species can produce similar acoustic signals because they share a common sound-producing mechanism.¹² In this case, differences in specific calls can be attributed to small modifications at the level of the sound-producing mechanism^{8,13–16} or the neurophysiology associated with the mechanism.¹⁷ Modifications in neural firing can correspond to differences in call duration or pulse period.^{17,18} Although sounds are expected to be species specific, the use of common mechanisms supports the hypothesis that closely related species should exhibit shared acoustic features that allow the identification of a supraspecific level. Testing this hypothesis requires working on a fish taxon exhibiting at least some modifications at the level of the sound-producing apparatus.

Piranhas (Serrasalminae, Characiformes) are endemic to the Neotropics and are widely distributed in all major river basins of South America.¹⁹ At least 14 species from four carnivorous genera (*Pygopristis*, *Catoprion*, *Serrasalmus*, and *Pygocentrus*) (Table I) produce sounds by using sonic muscles that act on the swim bladder,⁸ implying common

characteristics in the calling features. However, significant differences can be found in the sound production mechanisms (e.g., muscles extending until the third or the fourth rib),^{20,21} and the histology of the sonic muscles,²² which support differences in acoustic abilities. Recently, it has been shown that sounds can be used to discriminate different species from the genus *Pygocentrus*.⁸ Although determining the specific nature of the sounds was not the subject of the study, some differences were also reported between several species of *Serrasalmus*,²¹ where single variables were not sufficient to distinguish between piranha species, multivariate differences were highlighted.²³

This study aims to use different species-specific sounds to test whether they can be used to discriminate the *Serrasalmus* genus from the *Pygocentrus* genus. This study is divided into two parts: the first aims to determine whether *Serrasalmus* species possess species-specific sounds, while the second aims to determine whether *Serrasalmus* and *Pygocentrus* species possess acoustic features, allowing discrimination between their respective genera.

II. METHODS

A. Ethical issue

The experiment received approval from the ethical commission of the University of Liège (case 1532). The capture of piranha specimens was carried out under a licence issued by the Brazilian Ministry of the Environment (number: 9722–1, solicitation number: 10 306). In the Paraopeba River, fish were captured downstream near the Retiro Baixo Dam under authorisation number PT-04/07/2018 granted by Retiro Baixo Energética. In the Parque Estadual do Rio Doce, fish were sampled with authorisation from the Instituto

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TABLE I. True piranha species reported in the literature and their sound recordings status. The term “true piranhas” is defined following Ref. 24. Species validity and distribution information are updated from Ref. 25. Countries: A, Argentina; Bo, Bolivia; Br, Brazil; C, Colombia; E, Ecuador; FG, French Guiana; G, Guyana; Pa, Paraguay; Pe, Peru; S, Suriname; U, Uruguay; V, Venezuela. Rivers and watersheds: A, Amazon; At, Atabapo; B, Branco; BC, Northeastern Brazilian coastal rivers; C, in a tributary of Casiquiare and one of Atabapo rivers; E, Essequibo; G, Guaporé; GR, Guyana rivers; GS, North and East of the Guiana shield; M, Madeira; N, Negro; O, Orinoco; Pa, Padauri; PP, Paraguay–Paraná; SF, São Francisco; T, Tocantins; Uc, Ucayali; Ua, Uatumã; V, Coastal rivers of Venezuela.

Species	Countries	Distribution	Sound production
<i>Pristobrycon aureus</i> (Spix and Agassiz, 1829)	Br, G	Lower A, GR	NR ^a
<i>Pristobrycon calmoni</i> (Steindachner, 1908)	Br, G, V	Lower-middle A and O, GR	NR ^a
<i>Pristobrycon careospinus</i> Fink and Machado-Allison, 1992	V	At, O	NR ^a
<i>Pristobrycon maculipinnis</i> Fink and Machado-Allison, 1992	V	C	NR ^a
<i>Pristobrycon striolatus</i> (Steindachner, 1908)	Br, FG, G, S, V	A, O, GS	r ^b (Ref. 26)
<i>Pygocentrus cariba</i> (Humboldt and Valenciennes, 1821)	C, V	O	Ref. 8
<i>Pygocentrus nattereri</i> Kner, 1858	A, Bo, Br, C, E, G, Pa, Pe, U	A, PP, E, BC	This study (Refs. 8, 23, 21, 26–29)
<i>Pygocentrus palometa</i> ^c Valenciennes, 1850	V	O	NR ^a
<i>Pygocentrus piraya</i> (Cuvier, 1819)	Br	SF	This study (Ref. 9)
<i>Pygopristis denticulata</i> (Cuvier, 1819)	Br, FG, G, S, V	O, GS, lower A	Ref. 20
<i>Serrasalmo emarginatus</i> ^d Jardine in Schomburgk, 1841	G	E	NR ^a
<i>Serrasalmo scotopterus</i> ^d Jardine in Schomburgk, 1841	Br	B	NR ^a
<i>Serrasalmo stagnatilis</i> ^d Jardine and Schomburgk in Schomburgk, 1841	G	Upper E	NR ^a
<i>Serrasalmo undulatus</i> ^d Jardine and Schomburgk in Schomburgk, 1841	Br	Pa	NR ^a
<i>Serrasalmus altispinis</i> Merckx <i>et al.</i> , 2000	Br	A and Ua	NR ^a
<i>Serrasalmus altuvei</i> Ramírez, 1965	V	O	NR ^a
<i>Serrasalmus brandtii</i> (Lütken, 1875)	B	SF	This study
<i>Serrasalmus compressus</i> Jégu <i>et al.</i> , 1991	Bo, Br, Pe	Middle A	^c (Refs. 21, 23)
<i>Serrasalmus eigenmanni</i> Norman, 1929	Br, FG, G, S, V	A, GS	^c (Refs. 21, 23)
<i>Serrasalmus elongatus</i> Kner, 1858	Bo, Br, E, Pe, V	A, O	Refs. 21, 26, 28
<i>Serrasalmus geryi</i> Jégu and Santos, 1988	Br	T	NR ^a
<i>Serrasalmus gibbus</i> Castelnau, 1855	Br	T	NR ^a
<i>Serrasalmus gouldingi</i> Fink and Machado-Allison, 1992	Br, V	A, O	NR ^a
<i>Serrasalmus hastatus</i> Fink and Machado-Allison, 2001	Br	N	NR ^a
<i>Serrasalmus hollandi</i> Eigenmann, 1915	Bo, G?	M, GR	r ^b (Ref. 26)
<i>Serrasalmus humeralis</i> Valenciennes, 1850	Bo, Br, Pe	A	NR ^a
<i>Serrasalmus irritans</i> Peters, 1877	V	O	NR ^a
<i>Serrasalmus maculatus</i> Kner, 1858	A, Bo, Br, C, Pa, Pe, U	A, PP	This study (Refs. 23, 30)
<i>Serrasalmus manueli</i> (Fernández-Yépez and Ramírez, 1967)	Br, V	A, O	Ref. 21
<i>Serrasalmus marginatus</i> Valenciennes, 1837	A, Br, Pa, U	PP	This study (Refs. 21, 31)
<i>Serrasalmus medinai</i> Ramírez, 1965	V	O	NR ^a
<i>Serrasalmus nalseni</i> Fernández-Yépez, 1969	V	O	NR ^a
<i>Serrasalmus neveriensis</i> Machado-Allison <i>et al.</i> , 1993	V	V	NR ^a
<i>Serrasalmus nigricans</i> (Agassiz, 1829)	Br	A	NR ^a
<i>Serrasalmus rhombeus</i> (Linnaeus, 1766)	Bo, Br, C, E, FG, G, Pe, S, V	A, O, GS, BC	Refs. 21, 26, 28
<i>Serrasalmus sanchezei</i> Géry, 1964	Pe	Uc	^c (Ref. 23)
<i>Serrasalmus serrulatus</i> (Valenciennes, 1850)	Br, G?, Pe	A, E?	r ^b (Ref. 28)
<i>Serrasalmus spilopleura</i> Kner, 1858	Br	G	Refs. 21, 28

^aNR, no recording.

^br, sound production reported.

^c*Nomen dubium* species *Pygocentrus palometa* Valenciennes, 1850 (in bold).

^dSpecies *inquirendae* from genus *Serrasalmo* (in bold).

^eSpecies considered uncertain by the authors of the sound description.

Estadual de Florestas (Governo do Estado de Minas Gerais) number 049/2018. In the Araguari River, fish were captured downstream near both the Amador Aguiar I and II dams under authorisation from Consórcio Capim Branco.

B. Sampling

Five piranha species from three different river basins were sampled in July 2018 (Table II). Thirty-four specimens

of *Serrasalmus brandtii* [standard length (SL): 185 ± 43 mm] and three specimens of *Pygocentrus piraya* (SL: 185 ± 86 mm) were captured using gillnets and hook-and-lines in the Paraopeba River (São Francisco River basin, Brazil; 18°52'35.34" S, 44°46'49.08" W). A total of 150 specimens of *P. nattereri* (SL: 178 ± 24 mm) were captured using gillnets in Dom Helvécio Lake (Parque Estadual do Rio Doce, Brazil; 19°46'29.42" S, 42°35'57.44" W).

TABLE II. Numbers of specimens and sounds of piranhas recorded in this study.

Species	River and watershed	N (specimens)	N (sounds)
<i>Serrasalmus brandtii</i>	Paraopeba River (São Francisco Basin)	34	332
<i>Serrasalmus marginatus</i>	Araguari River (Paraná Basin)	55	550
<i>Serrasalmus maculatus</i>	Araguari and Grande River (Paraná Basin)	40	400
<i>Pygocentrus piraya</i>	Paraopeba River (São Francisco Basin)	3	30
<i>Pygocentrus nattereri</i>	Parque Estadual do Rio Doce (Doce Basin)	150	1500
<i>Pygocentrus cf. piraya</i>	Araguari River (Paraná Basin)	11	107

Fifty-five specimens of *S. marginatus* (SL: 169 ± 17 mm) and 11 specimens of *P. cf. piraya* (SL: 328 ± 18 mm) were captured using gillnets and hook-and-lines in the Araguari River (Paraná River basin, Brazil; $18^{\circ}39'37.41''$ S, $48^{\circ}26'19.94''$ W). Due to the uncertain status of the population of *P. cf. piraya* from the Araguari River,⁸ it was analysed separately. Finally, 40 specimens of *S. maculatus* (SL: 209 ± 44 mm) were captured using gillnets—five at a second location on the Araguari River ($18^{\circ}46'37.87''$ S, $48^{\circ}10'9.66''$ W) and 35 in the Grande River (Paraná River basin, Brazil; $20^{\circ}10'19.06''$ S, $48^{\circ}6'34.63''$ W). Between the fish capture and fish sound recording, the captured fish were first placed in a tank on the boat with a bubbler for a period of time (between 10 and 40 min). Subsequently, they were transferred to a mesocosm located in the river/lake, where they were given a minimum of 20 min to recover before the sound recording sessions. SL, body mass (BM), and body height (BH) were measured for all the individuals.

To increase the number of species in our comparison, the acoustic recordings from previous studies on the communication in Serrasalminae^{8,21} were reanalysed specifically for this study, including the following species: *S. compressus*, *S. eigenmanni*, *S. elongatus*, *S. manueli*, *S. rhombeus*, *S. spilopleura*, and *P. cariba*. Furthermore, the six *Serrasalmus* species were utilized to enhance the acoustic discrimination within the *Serrasalmus* genus.

C. Recordings and sound analysis

The sounds collected for this study were recorded using the same method as in previous works^{8,21} to enable data integration. In Brazil, the recordings were made in a glass tank using an HTI-96-min hydrophone (High Tech Inc., Long Beach, USA) (sensitivity: -164.4 dB re 1 V μPa^{-1}) positioned in the center of the aquarium and connected to a calibrated TASCAM DR5 recorder (TEAC Corporation, Tokyo, Japan). The water temperature in the aquarium was maintained at $26 \pm 2^{\circ}\text{C}$. The fish were gently hand held between the index and the thumb at a distance of approximately 5 cm from the hydrophone to homogenize sounds as much as possible. For each specimen, ten sounds were analysed except for *S. brandtii* and *P. nattereri* from the Araguari River, in which some individuals produced fewer than ten sounds (Table II). The sounds were digitalized in mono-channel at 44.1 kHz with a 16 bit resolution. Subsequently, they were sub-sampled at 4 kHz, and a high-pass filter at 50 Hz was applied. The analysis was conducted

using Avisoft sas-Lab Pro 5.2 (Avisoft Bioacoustics, Glienicke, Germany).

Differences in acoustic features that could have been attributed to the method of capture, fish size (SL, BM, and BH), or sex, were preliminarily checked (See supplementary material for details regarding the effects of fish size, BH, and BM effects).³² The following features were measured on the oscillograms of the sounds: duration (d) in ms, number of peaks (N) unitless, the period between consecutive peaks (p) in ms, maximal amplitude (Amp_{Max}) in dB re $1 \mu\text{Pa}$ at 5 cm, duration of the energetic zone, i.e., the continuous zone with an intensity of maximum 3 dB less than the maximum intensity peak⁸ (d_{ez}) in ms (Fig. 1), number of peaks in the energetic zone (N_{ez}) unitless, percentage of peaks present in the energetic zone ($\%N_{\text{ez}}$) unitless, and period in the energetic zone (p_{ez}) in Hz. The following features were measured on the power spectra of the sounds: fundamental frequency (F_0) in Hz (Fig. 1) and corresponding amplitude ($\text{Amp}F_0$) in dB re $1 \mu\text{Pa}$ at 5 cm,^{8,31} dominant frequency (F_{AmpMax}) in Hz (Fig. 1), and corresponding amplitude ($\text{Amp}F_{\text{AmpMax}}$) in dB re $1 \mu\text{Pa}$ at 5 cm,^{8,31} frequency ratio (H) unitless, defined as $H = F_0 \times F_{\text{AmpMax}}^{-1}$, quartile frequencies (F_{Q1} , F_{Q2} , and F_{Q3} , in Hz) obtained by integration and dividing the instantaneous spectra into four equal parts, minimum and maximum frequencies (F_{min} and F_{max}) in Hz³³ (Fig. 1), and bandwidth (BW) in Hz (Fig. 1) defined as $BW = F_{\text{max}} - F_{\text{min}}$.

The analysis of sounds of the seven piranha species from the literature was conducted in the same manner as for sounds recorded directly in the field. For them, ten sounds per individual from three individuals of each species were used. However, the amplitude was not included due to the lack of calibration in the recorders used in previous studies.

D. Statistics

Statistical analyses were conducted in two stages. Initially, the analyses were performed exclusively on the *Serrasalmus* species, and then on all species from both *Pygocentrus* and *Serrasalmus* genera. For each specimen, values from the ten sounds were averaged to avoid pseudoreplication. To investigate the correlation between acoustic features and SL, correlation matrices were calculated using Spearman correlation coefficients (r_s) associated with p-values adjusted using the Holm method. Correlations were deemed statistically significant when $p \leq 0.05$ and were considered strong when $p \leq 0.05$ and $r_s > |0.60|$. To reduce the

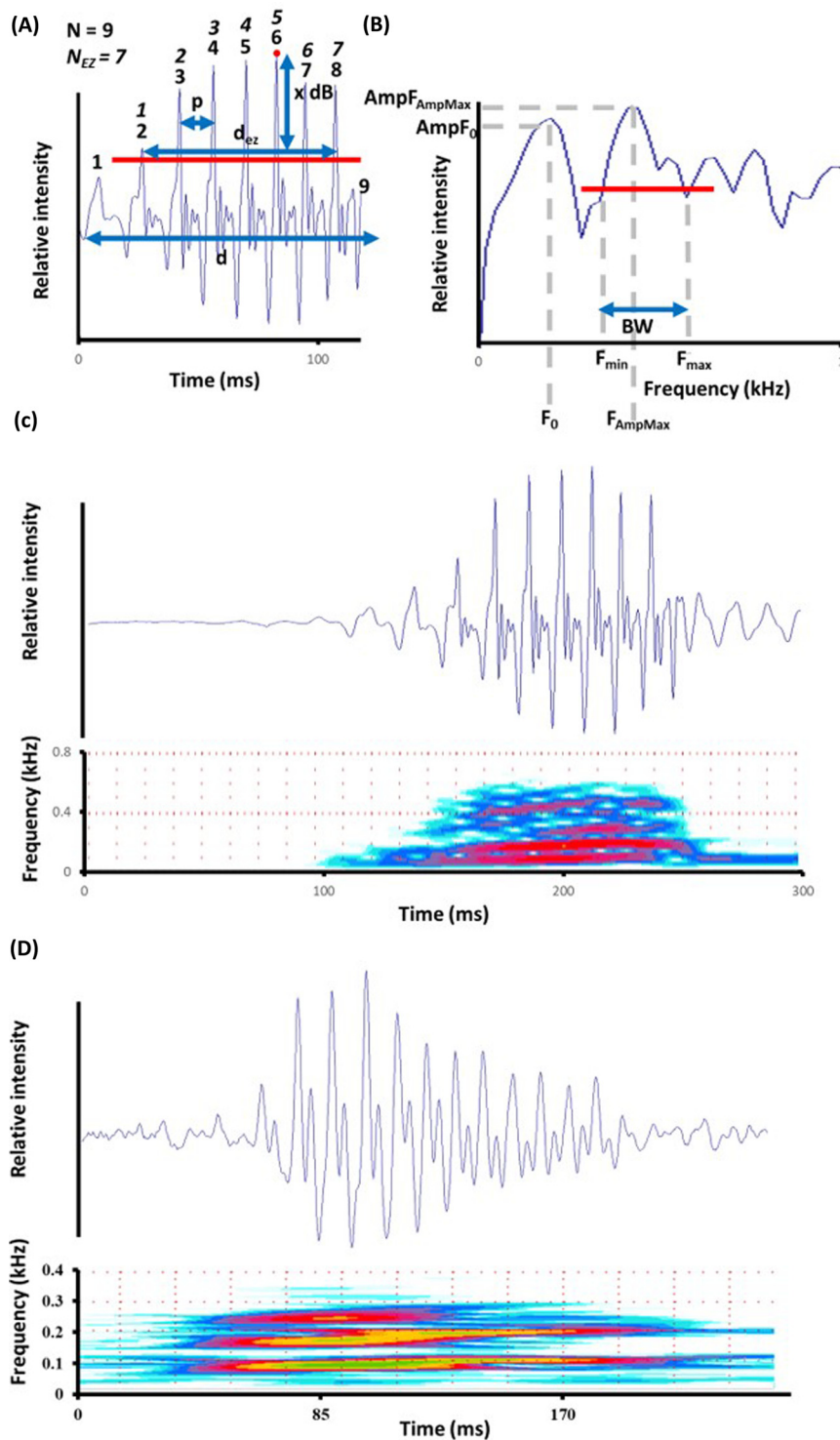


FIG. 1. (Color online) (A) Illustration of several measurements on an oscillogram of a sound produced by *Serrasalmus brandtii* ($x = 3$ dB), (B) a power spectrum of a sound produced by *Pygocentrus nattereri*, (C) and (D) waveform (above) and spectrogram (below) of a sound produced by (C) *Serrasalmus brandtii* and (D) *Pygocentrus nattereri*. N , number of peaks; N_{EZ} , number of peaks in the energetic zone; d , duration of the sound; d_{EZ} , duration of the energetic zone; BW , bandwidth; F_0 , fundamental frequency; $AmpF_0$, amplitude at the fundamental frequency; F_{AmpMax} , dominant frequency; $AmpF_{AmpMax}$, amplitude at the dominant frequency; F_{min} , minimum frequency; F_{max} , maximum frequency. Roman numerals indicate the position of the peaks in the sound, while italic numerals indicate the position of the peaks in the energetic zone. Blue lines illustrate the measurements while red lines illustrate thresholds used for the energetic zone and the bandwidth related measurements. Fast Fourier transform (FFT) length: 512; window: Hamming; frame: 100%.

impact of SL effect, linear regressions were performed on the features correlated with the SL.⁸ Differences were assessed using Mann–Whitney tests or Kruskal–Wallis tests followed by Dunn *post hoc* tests with Benjamini–Hochberg corrections. To address the multivariate nature of the data, principal components analyses (PCA) were conducted on all the features except those correlated with SL, wherein the residuals (res) from the linear regressions were employed instead. The principal components (PCs) are presented alongside their

corresponding percentage of variance. Linear discriminant analyses (LDA) were utilized to discriminate between groups. Prior to conducting LDA, all features (including residuals, when applicable) were standardised by subtracting the mean and dividing by the standard deviation. The presence of a phylogenetic signal was evaluated using Blomberg’s K based on species-level means.³⁴ A Blomberg’s K value greater than one suggests a positive association between the trait and phylogeny, while a value less than one suggests a negative

association. A Blomberg’s K value equal to one indicates no association, with statistical significance determined by the p-value. This test was conducted both with and without considering the conflicting *P. cf. piraya*, yielding similar results. All statistical analyses were conducted using R 3.6.1. (GNU General Public License) with a significance level set at $\alpha = 0.05$.

III. RESULTS

A total of 2919 sounds were recorded from 293 piranha specimens sampled in three river basins in Southeast Brazil (Table II). Since this is the first report of sounds from *S. brandtii*, a species endemic to the São Francisco River basin,²⁵ we provide a brief description of their sounds. Sounds produced by *S. brandtii* ($n_{\text{specimens}} = 34$, $n_{\text{sounds}} = 332$) consisted of 8 ± 2 (mean \pm standard deviation) continuous peaks, each lasting approximately 13 ± 1 ms (Fig. 1). The total sound duration was 107 ± 19 ms, with a fundamental frequency of 76 ± 7 Hz, a dominant frequency of 107 ± 43 Hz, and a frequency ratio (H) of 0.82 ± 0.22 . The frequency distribution was as follows: $F_{Q1} = 128 \pm 32$ Hz, $F_{Q2} = 198 \pm 52$ Hz, and $F_{Q3} = 322 \pm 76$ Hz. The energetic zone of the sound lasted for 31 ± 14 ms and comprised 3.5 ± 1 pulses, accounting for $45 \pm 11\%$ of the total number of pulses. Within the energetic zone, the individual peaks had a period of 12 ± 2 ms (Fig. 1).

A. Acoustic discrimination of *Serrasalmus* species

Statistically significant differences in SL were observed among the nine species ($p < 0.0001$, Kruskal–Wallis test), and SL showed correlations with all features ($0.26 \leq |r_S| \leq 0.94$; $p < 0.0001$ except for d_{EZ} , F_{max} , and H : $p = 0.0007$, 0.015 , and 0.039) except BW and d ($r_S = -0.13$ and -0.004 , $p \approx 1$). The separation of the different species was achieved by the first principal component (PC1, 40.1% variance), PC2 (29.7%), and PC3 (9.5%) (all $p < 0.0001$, Kruskal–Wallis tests). PC1 was primarily influenced by BW and F_{max} . The LDA demonstrated overall correct classification rates of 90.47% (without size correction, i.e., with all the raw variables) and 86.72% (with size correction). The lower correct classification rate with size correction might be attributed to the variation in the number on individuals among the different species; see Raick *et al.*⁸ for an example on piranha sounds. By using the residuals for the LDA analysis, we focus on the variation that was not accounted for by the regression. Notably, a high number of observations for a given species significantly contributes to the unexplained variation in the data, potentially leading to suboptimal, but still highly significant, species classification in the LDA.

B. Acoustic discrimination of genera *Pygocentrus* and *Serrasalmus*

When analysing the data from all species collectively, all features except for four (H , d , BW , and F_{max} ; $p = 0.18$, ≈ 1 , ≈ 1 , and ≈ 1 , respectively) showed correlations with SL ($0.22 \leq |r_S| \leq 0.67$; $p < 0.0001$ except for d_{EZ} and $\%N_{EZ}$:

0.0028 and 0.0031 , respectively). PCA was conducted on these four features and on the residuals obtained from linear regressions of all other features. PC1 (36.5%) exhibited a predominantly positive correlation with F_{Q1} and F_{max} ($r_S = 0.61$ and 0.69 ; both $p < 0.0001$) while displaying a negative correlation with H ($r_S = -0.69$, $p < 0.0001$). PC2 (23.7%) demonstrated a mainly positive correlation with BW ($r_S = 0.68$, $p < 0.0001$) and a negative correlation with d_{EZ} and N_{EZ} ($r_S = -0.77$ and -0.68 , both $p < 0.0001$). Both PC1 and PC2 effectively distinguished between the two genera ($W = 4993$ and $10\ 315$, $p < 0.0001$ and 0.029 ; Mann–Whitney U test). In pairwise comparisons of different species using Dunn *post hoc* tests, 92.86% of the statistically significant comparisons pertained to species from different genera (separating a species from the genus *Pygocentrus* from one of the genus *Serrasalmus*) for PC1, whereas it was only 50% for PC2. The LDA demonstrated that sounds were associated with the correct genus (Table III). The overall correct classification rate was 84.9% (87.8% on the residuals). For *Pygocentrus*, the correct classification rate was 83.6% (87.9% on the residuals) and for *Serrasalmus*, it was 86.3% (87.7% on the residuals). Sounds were also associated with the correct species, with an overall correct classification rate of 76.5% (80.1% on the residuals). The correct classification rate for species within the genus *Pygocentrus* was 83.6% (87.9% on the residuals), while for species within the genus *Serrasalmus*, it was 68.5% (71.2% on the residuals). There was no phylogenetic signal for the acoustic features ($0.34 < K < 0.76$, all $p > 0.05$) except for the duration ($K = 1.03$, $p = 0.024$) when considering all the species altogether.

To improve the classification rate, we conducted a more complete analysis on individuals for which we were able to

TABLE III. Coefficients of linear discriminants (LD1) for predicting the genus and mean values for each genus (*Pygocentrus* and *Serrasalmus*). Residuals were not computed for the last four features (BW , d , H , and F_{max}) as they did not show any correlation with the standard length. Z, the standardised feature; Zres, the standardised residuals of the feature.

Acoustic feature	LD1		<i>Pygocentrus</i>		<i>Serrasalmus</i>	
	Z	Zres	Z	Zres	Z	Zres
d_{EZ}	-1.03	-0.45	0.17	0.20	-0.18	-0.22
F_0	0.72	0.77	-0.33	-0.32	0.37	0.37
F_{AmpMax}	-1.20	-1.21	-0.27	-0.24	0.30	0.27
F_{min}	-1.46	-0.21	-0.23	-0.20	0.27	0.23
F_{Q1}	1.24	1.01	-0.37	-0.37	0.43	0.42
F_{Q2}	-1.26	-0.81	-0.34	-0.34	0.39	0.39
F_{Q3}	0.42	0.48	-0.33	-0.32	0.37	0.37
N	-1.97	-1.28	0.13	0.23	-0.14	-0.24
N_{EZ}	2.02	1.05	-0.071	-0.012	0.085	0.027
p	-1.36	-0.83	0.38	0.43	-0.42	-0.49
p_{EZ}	0.52	-0.039	0.35	0.39	-0.39	-0.44
$\%N_{EZ}$	-0.15	-0.080	-0.24	-0.21	0.26	0.24
BW	-5.37	-1.20	-0.27		0.31	
d	0.43	0.33	0.52		-0.57	
H	-0.33	-0.38	0.20		-0.22	
F_{max}	5.75	1.48	-0.33		0.37	

TABLE IV. Z values and corresponding adjusted p-value for Dunn tests conducted following Kruskal–Wallis tests on the first scores of the PCA (PC1). Two species from the same genus exhibit equivalent scores, while scores differ for all comparisons involving species from different genera. Significant values are highlighted in bold.

	Species1	Species2	Z	P adj
Same genus	<i>Serrasalmus maculatus</i>	<i>Serrasalmus marginatus</i>	1.99	0.069
Different genera	<i>Serrasalmus maculatus</i>	<i>Pygocentrus nattereri</i>	-5.52	0.0001
Different genera	<i>Serrasalmus marginatus</i>	<i>Pygocentrus nattereri</i>	-8.81	0.0001
Different genera	<i>Serrasalmus maculatus</i>	<i>Pygocentrus piraya</i>	-2.73	0.011
Different genera	<i>Serrasalmus marginatus</i>	<i>Pygocentrus piraya</i>	-3.45	0.0012
Same genus	<i>Pygocentrus nattereri</i>	<i>Pygocentrus piraya</i>	-1.11	0.33
Different genera	<i>Serrasalmus maculatus</i>	<i>Pygocentrus cf. piraya</i>	-3.75	0.00045
Different genera	<i>Serrasalmus marginatus</i>	<i>Pygocentrus cf. piraya</i>	-5.11	<0.0001
Same genus	<i>Pygocentrus nattereri</i>	<i>Pygocentrus cf. piraya</i>	-0.94	0.40
Same genus	<i>Pygocentrus piraya</i>	<i>Pygocentrus cf. piraya</i>	0.55	0.62
Same genus	<i>Serrasalmus maculatus</i>	<i>Serrasalmus brandtii</i>	0.13	0.90
Same genus	<i>Serrasalmus marginatus</i>	<i>Serrasalmus brandtii</i>	-1.76	0.10
Different genera	<i>Pygocentrus nattereri</i>	<i>Serrasalmus brandtii</i>	5.33	<0.0001
Different genera	<i>Pygocentrus piraya</i>	<i>Serrasalmus brandtii</i>	2.76	0.011
Different genera	<i>Serrasalmus brandtii</i>	<i>Pygocentrus cf. piraya</i>	3.76	0.00050

measure amplitude values: *S. brandtii*, *S. maculatus*, *S. marginatus*, *P. piraya*, and *P. nattereri*. Eleven features (d_{ez} , F_0 , F_{AmpMax} , F_{min} , F_{Q1} , F_{Q2} , F_{Q3} , N , N_{ez} , p , and p_{ez}) were found to be correlated with SL. Consequently, linear regressions were performed on these features. The multiple comparison of PC1 (30.1%, correlated with all amplitude features, F_{AmpMax} , F_{min} , and F_{Q1} with $r_S > |0.60|$) revealed that PC1 values were consistently similar for two species from the same genus while PC1 values differed for two species from different genera (Table IV) (See supplementary material for Table I).³² PC2 (26.2%, correlated with BW , d , F_{max} , and N with $r_S < |0.60|$) and PC3 (13.1% variance, correlated with d_{ez} with $r_S < |0.60|$) separated certain species but could not distinguish between the two genera. Sounds were associated with the correct genus, yielding an overall correct classification rate of 90.1% (91.4% on the residuals). The correct classification rate for *Pygocentrus* was 82.5% (89.6% on the residuals), and for *Serrasalmus*, it was 93.0% (93.8% on the residuals). The LDA highlighted the significance of BW and F_{max} . However, once the size effect was accounted for, the amplitude features played a crucial role in discrimination. There was a phylogenetic signal for Amp_{Max} ($K = 1.72$, $p = 0.04$), d ($K = 1.31$, $p = 0.028$), and the residuals of F_{min} ($K = 1.12$, $p = 0.032$). There was no phylogenetic signal for the other acoustic features ($0.76 < K < 1.76$, all $p > 0.05$), possibly because of the small number of species in the phylogeny.

IV. DISCUSSION

Many studies have documented species-specific sounds in various biological groups, but only a few have investigated whether sounds produced by sister species can be representative of the genus level. In Felidae (Pantherinae and Felinae), distinct types of so-called *friendly close-range sounds* (gurgle, prusten, and puffing) have been identified. These sounds are congruent with the phylogeny and appear

to correspond to the evolution of acoustic communication signals.³⁵ The gurgle represents the primitive sound type among felids, while the prusten is a derived vocalization that emerged exclusively within the lineage comprising seven felid species that do not produce gurgle (Pantherinae).³⁵ The most derived sound, known as puffing, is only exhibited by the sister-species lion, *Panthera leo*, and leopard, *P. pardus*.^{35–37} Acoustic features also differentiate genera within Lissodelphininae (Cetacea),³⁸ where *Lissodelphis borealis* produces broadband clicks while *Cephalorhynchus* species generate narrow-band clicks.³⁹ In Hylidae (Anura), acoustic distinctions are observed between *Dryophytes*, *Hyla*, and *Pseudacris* genera, both in terms of frequency (higher amplitude at the fundamental or dominant frequency) and temporal characteristics (gradual onset or decay).^{40,41}

Different morphological features (Table V)^{42–45} and molecular data⁴⁶ are currently used to distinguish between *Pygocentrus* and *Serrasalmus*. However, some of these features are highly challenging to utilize in the field for non-trained people. Based on nine out of 24 *Serrasalmus* species and all three *Pygocentrus* species, we propose that sounds could serve as a potential additional useful tool for identifying genera since the species have been correctly assigned to their respective genera. Within the different acoustic features used, $H (F_0 \times F_{AmpMax}^{-1})$ proves to be valuable as it differs between sounds produced by the two genera. *Serrasalmus* species produced shorter louder sounds with shorter pulse periods (after being adjusted by the SL), lower H values, and higher BW and F_{max} values. In the genus *Pygocentrus*, intraspecific variation (including variation between small and large individuals) was found to be substantial, concealing the interspecific variation that only became apparent once the size effect was eliminated.^{8,23} However, further studies in the field are required to understand how sounds with a lower signal-to-noise ratio, emitted under varying environmental conditions such as depth or temperature, can be effectively utilized for accurate species

TABLE V. Distinction between *Pygocentrus* and *Serrasalmus* genera. Some morphological characters present a high overlap between the two genera and can be variable, depending on the size of the fish, and need comparable body sizes.^{43,44}

	<i>Pygocentrus</i>	<i>Serrasalmus</i>
	Morphology	
Number of prepelvic serrae	13–21 (average: 17)	17–29 (average: >20)
Posterior chamber of the gas bladder	Much truncated	Elongate or somewhat truncate
Frontal, parietal, and pterotic bones	Crests surrounding the sensory canals of the head	Not the case
Hypobranchial bones	Covered by well-developed dermal tooth plates	Not the case
Head	Wide (width: 10.7%–19.5% of SL)	Narrow (width: 7.8%–14.1% of SL)
	Bioacoustics	
Amplitude of the sound ^a (dB re 1 μPa at 5 cm)	Amp _{Max} : 140 (min 98 to max 158) Amp _{F0} : 124 (min 82 to max 146)	Amp _{Max} : 151 (min 132 to max 160) Amp _{F0} : 135 (min 147 to max 135)
Standardised duration of the sound ^b (ms mm ⁻¹)	0.86 (min 0.33 to max 2.25)	0.69 (min 0.33 to max 2.66)

^aIn *Serrasalmus* species, data available only for *S. brandtii*, *S. maculatus*, and *S. marginatus*.

^bData refer to duration × size.⁻¹

identification without knowledge of their size. Future research should also investigate the efficacy of (relative) amplitude features at increased distances from the hydrophones and explore the significance of sound emission rhythms. These aspects need to be considered to advance our understanding of sound-based species identification in Neotropical freshwater acoustic environments.

V. CONCLUSION

In conclusion, this study on piranhas demonstrates that acoustic features can support taxonomy at a higher taxonomic level than just the species level.

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