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Research Article

Keywords: Bioacoustics, Conservation, Geographic isolation, Suboscines, Vocal taxonomy

Posted Date: March 13th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8611017/v1

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Additional Declarations: No competing interests reported.

Vocal conservatism in disjunct populations of the Marsh Tapaculo, *Scytalopus iraiensis* (Aves: Rhinocryptidae), in the Brazilian Atlantic Forest

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Abstract

Population fragmentation has the potential to cause divergences between individuals, which can result in allopatric speciation, with vocal manifestations standing out as a characteristic susceptible to differentiation. This evolutionary dynamic differs in birds of the suborder Tyranni, which are historically characterized by innate and genetically determined vocalizations; however, recent evidence points to exceptions, such as the plasticity of certain lineages. In this context, we investigated the song of *Scytalopus iraiensis* (Rhinocryptidae), an endangered species restricted to dense swamp vegetation and with a disjunct distribution between the South and Southeast macroregions of Brazil, to test the hypothesis of acoustic divergence driven by geographic isolation. For this, we used 80 recordings encompassing both subpopulations, from which spectral and temporal parameters were extracted and analyzed using Principal Component Analysis (PCA), K-means clustering, and Permutational Multivariate Analysis of Variance (PERMANOVA). The results revealed remarkable vocal conservatism, with K-means cluster analysis segregating vocalizations into two groups with a mixture of individuals from both macroregions. PERMANOVA confirmed the absence of significant differentiation between subpopulations, with geography explaining only 1.5% of the total acoustic variation. It is concluded, therefore, that the vocal conservatism found results from the synergy between the biomechanical constraints of the suborder Tyranni, strong habitat specialization, ancestral polymorphism, and probable recent historical connectivity through wetland corridors. This corroborates the recognition of the species as a single Evolutionarily Significant Unit (ESU) and the need for future phylogeographic, molecular, and in situ experimental investigations.

Keywords Bioacoustics · Conservation · Geographic isolation · Suboscines · Vocal taxonomy

Introduction

The population fragmentation has the potential to cause divergences in genotypes, phenotypes, or behavior among individuals, processes that can result in allopatric speciation (Haffer and Prance 2002; Repenning 2017; Lima and Mariano 2019). Thus, vocal manifestations stand out as a characteristic susceptible to differentiation, increased by cultural changes and regional adaptations (Podos and Warren 2007; Cardoso 2016; Lima and Mariano 2019). This evolutionary dynamic presents a unique scenario in birds of the suborder Tyranni (Passeriformes), which are historically characterized by innate and genetically determined vocalizations, dispensing with social learning (Dias 2009; Da Silva and Vielliard 2011; Oliveira 2014). This invariability has been corroborated by decades of studies (*e.g.*, Nottebohm 1972; Lovell and Lein 2004; Foote et al. 2012; Lima and Mariano 2019). However, recent evidence points to exceptions, such as the occurrence of plasticity in certain lineages (Saranathan et al. 2007; Kroodsma et al. 2013), as well as the emergence of acoustic divergences due to geographic isolation and environmental filters in disjunct populations, even in the absence of vocal learning. Furthermore, bioacoustics has proven to be a primary tool for revealing cryptic diversity of this taxon with conserved morphology, where divergence in the acoustic space anticipates the recognition of new evolutionary lineages (Areta and Monteleone 2023; Burnham 2023; Lima and Vaz 2025).

The family Rhinocryptidae (Tyranni) comprises 12 genera with a Neotropical distribution, among which *Scytalopus* stands out for its high diversity, resulting from intense speciation processes in mountainous areas, notably in the Andes and the Brazilian Atlantic Forest (Sick 1997; Bornschein et al. 1998; Maurício et al. 2008). In this taxon, morphological evolution is conservative, resulting in cryptic species where bioacoustics may play a central role in species recognition and reproductive segregation (Cadena et al. 2020; García-Navas et al. 2025). Recent studies indicate that, although syntopy strongly drives acoustic divergence to avoid hybridization, allopatry processes also shape vocal evolution in this radiation, allowing the diagnosis of cryptic taxa through quantitative analysis of song (Areta and Monteleone 2023; García-Navas et al. 2025). Its diversification dates back to the Middle Miocene (~10 Ma), a period that marks the divergence between the lineages of *Scytalopus* and its sister group, *Eleoscytalopus* (Cadena et al. 2020). A second pulse of diversification in Rhinocryptidae occurred at the end of the Pliocene (1.8-2.4 Ma), giving rise to most extant species (Mata et al. 2009).

Paleoclimatically, the beginning of the Pliocene in southeastern Brazil was marked by arid and semi-arid conditions, with increases in humidity levels occurring only in the second half of the period (Grazziotin et al. 2006; Mata et al. 2009). These fluctuations resulted in severe forest contractions, fragmenting Rhinocryptidae populations. Given the strict association of these species with humid and mountainous environments, their persistence was restricted to refuges where orographic rainfall ensured environmental stability over geological time (Mata et al. 2009). Consequently, the current disjunct distribution of *Scytalopus* in eastern Brazil is interpreted as a direct product of these climatic oscillations and the refuge theory (Haffer and Prance 2002).

The Marsh Tapaculo, *Scytalopus iraiensis* (Bornschein, Reinert & Pichorim, 1998), is a species restricted to dense vegetation in alluvial forests and floodplains (Mata 2005; Klemann Jr. and Vieira 2013; del Hoyo *et al*. 2016). Its geographic distribution is disjunct, occurring in the states of the South macroregion (Paraná, Santa Catarina, and Rio Grande do Sul) and Southeast macroregion (Minas Gerais). Currently, the species is classified globally as "Endangered" (EN) due to the continuous degradation of its habitat (BirdLife International 2016; Pacheco 2021). Vocally, the song of the Marsh Tapaculo is described as a series of "tchek"-type notes, preceded by longer introductory notes, while the call consists of a characteristic "pic-pic" (BirdLife International 2016).

Despite the conservative morphology of the genus, bioacoustics remains an important tool for delimiting taxonomic units, i.e., species (Gwee et al. 2019). However, to date, no study has compared the vocalizations across the entire distribution of Marsh Tapaculo. Considering this geographic isolation, coupled with the potential for cryptic diversity in Rhinocryptidae, the present study aimed to analyze and characterize the species' song and test the hypothesis of acoustic divergence between its disjunct populations in southern and southeastern Brazil.

Methods

Data acquisition and selection

Recordings of "song" type vocalizations belonging to the Marsh Tapaculo were compiled from three online databases that store audio recordings, encompassing the files available on August 12, 2025. The survey included data from Xeno-canto, the Macaulay Library, and Wiki Aves, totaling 29, 52, and 176 recordings, respectively. Considering that the Wiki Aves records do not have exact geographic coordinates associated with the audio files, spatial standardization was performed by assigning the coordinates of the centroids of the corresponding municipalities, georeferenced via Google Maps.

For the constitution of the final sample, the files were subjected to rigorous curation based on inclusion and exclusion criteria. Vocalizations of the "call" type, incomplete recordings, inaudible recordings, or recordings with acoustic overlap of other species in the notes of the Marsh Tapaculo were discarded. The evaluation of sound quality followed the guidelines of the Xeno-canto platform, restricting itself to recordings classified as A (loud and clear), B (clear, with slight interference or distance), and C (moderately clear). For the Macaulay Library and Wiki Aves databases, the same classification was applied manually, since they do not have this native metric. Consequently, recordings of inferior quality (D and E) were excluded (Xeno-canto, 2025). After applying the filters, the sample resulted in 95 valid songs for analysis, 39 from the Southeast subpopulation and 56 from the South subpopulation. These selected files were converted from their original format (MP3) to WAVE and subsequently subjected to manual inspection to isolate and extract a single song per recording, prioritizing the example with the highest quality and acoustic integrity. Both steps were performed using Audacity software (v. 3.7.5).

All analyses were performed using R software (v. 4.5.2). Initially, the spectrotemporal characteristics of the songs were manually selected, including the initial and final times, and the minimum and maximum frequencies, by spectrogram inspection (Hanning FFT window, wl = 512). The number of notes for each song, in turn, was determined manually by listening to and counting them one by one, in order to avoid processing errors. This data was tabulated in the same dataset already extracted, to be used as "guide" data in the automated extraction.

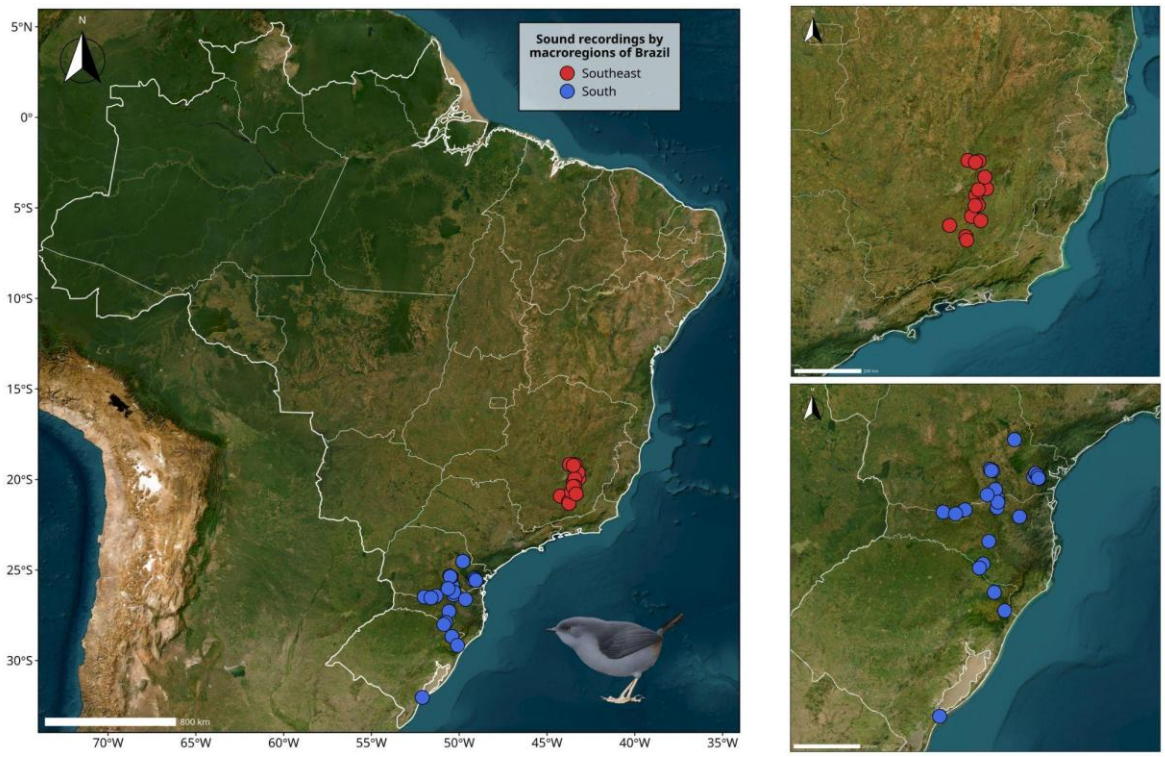
Acoustic parameter extraction

To standardize sampling effort and mitigate selection biases during automated extraction of acoustic parameters, a time-slicing protocol was applied to recordings longer than 27 seconds. From this time interval, the first 2 seconds of the recordings were discarded to exclude possible initial artifacts, such as initial silence or

"clicks" from the recorder activation, followed by extraction of the next 25-second continuous segment for use in statistical analyses. Acoustic parameters were extracted using the *spectro_analysis* function from the *warbleR* package, along with the *seewave* package, and employing parallel processing to optimize machine efficiency (Sueur et al. 2008; Araya-Salas and Smith-Vidaurre 2017; script available in Online Resource 1). By using this time interval to extract acoustic parameters, it was possible to perform robust sampling with a computationally feasible approach.

The algorithm was configured in a 512 window, with a bandpass filter calibrated within the range of manually selected frequency coordinates (maximum and minimum frequencies) and a 15% amplitude threshold for detecting acoustic characteristics. A comprehensive set of spectral and temporal features was measured, including the dominant frequency range, modulation index, spectral entropy, peak time, among others (Online Resource 2). Furthermore, files that consisted of absolute silence or insufficient signal-to-noise ratio for extraction were automatically discarded during the process. After this, the final dataset for validation and analysis comprised 80 songs: 31 belonging to the Southeast subpopulation and 49 to the South subpopulation (Fig. 1; Online Resources 3 and 4), totaling a sample of 2,000 seconds (= 33 minutes).

Fig. 1 Geographic distribution of the Marsh Tapaculo *Scytalopus iraiensis* sound recordings analyzed in this study. The map highlights two distinct sampling clusters within Brazil: red circles represent the population in the Southeast macroregion, while blue circles represent the population in the South macroregion. Right panels display zoomed-in views of the sampling localities overlaid on satellite imagery. The inset provides an illustration of the species (from Krabbe & Schulenberg, 2020).



Statistical processing and analysis

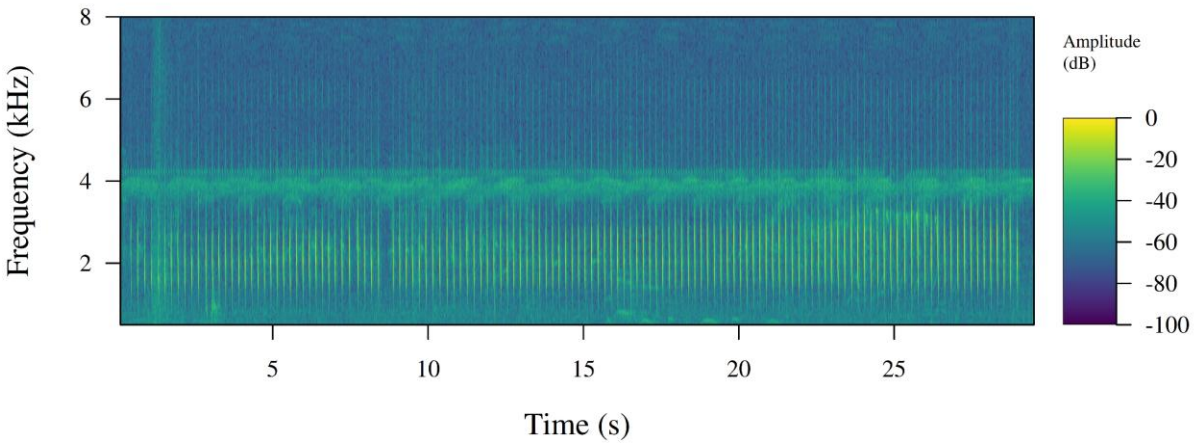
To mitigate multicollinearity, a correlation filter was applied to the raw dataset, eliminating redundant variables with a Pearson correlation coefficient greater than or equal to $|r| \geq 0.6$. The normality of the remaining variables was assessed using the Shapiro–Wilk test, and a logarithmic transformation ($\log_{10}$) was applied to those that did not meet the assumptions ($p < 0.001$). Subsequently, a Principal Component Analysis (PCA) was performed on the correlation matrix (standardized data) to reduce dimensionality. Only the Principal Components (PCs) that cumulatively explained at least 95% of the total variance were retained.

The internal acoustic structure of the species was evaluated without prior assumptions (data-driven) through an unsupervised cluster analysis (K-means clustering) on the retained PC scores. The ideal number of clusters (K) was determined by the silhouette width method (Malik and Tuckfield 2019; Almeida 2022). Finally, to test the hypothesis of vocal conservatism among disjunct populations, the acoustic dissimilarity between the South and Southeast macroregions was evaluated using a Permutational Multivariate Analysis of Variance (PERMANOVA) (Anderson and Braak 2003; Da Silva et al. 2022). This analysis was based on Euclidean distances calculated from the PC scores, using 9,999 permutations. The homogeneity of multivariate dispersions was verified using the BETADISPER test to ensure that significant differences in PERMANOVA were due to location effects and not dispersion artifacts (Kavazos et al. 2022). These analyses were performed using the *vegan*, *cluster*, *factoextra*, and *tuneR* packages.

Results

The analyzed Marsh Tapaculo songs consist of a series of 27 to 3,237 monosyllabic notes (mean: 417.6 ± 450.9 notes), occupying a bandwidth between 0.23 kHz and 5.96 kHz, with a mean dominant frequency of 2.06 ± 0.56 kHz, and exhibiting a remarkably long mean duration of 90.8 ± 91.6 s (amplitude: 6.1-641.5 s) (Fig. 2). The PCA reduced the dimensionality of these data to seven components that cumulatively explained 95% of the acoustic variation. The first component (PC1) was strongly associated with variables related to the shape of the spectrum (Spectral skewness and Spectral entropy) and dominant frequencies (Minimum dominant frequency and Dominant frequency at the end of the signal), while PC2 mainly represented frequency modulation (Slope of the dominant frequency) and spectral variability (Standard deviation of the mean frequency).

Fig. 2 Representative spectrogram of the Marsh Tapaculo *Scytalopus iraiensis* song. The visualization displays frequency (kHz) over time (s), where the color gradient indicates relative amplitude (dB). Yellow areas represent peak energy frequencies, while dark blue represents lower amplitude background noise.



Determining the ideal number of clusters using the Silhouette method indicated that partitioning into two groups (k = 2) represented the best structure for the data. Consequently, unsupervised cluster analysis (K-means) segregated the vocalizations into two distinct groups that captured the intrinsic acoustic variation of the sample: Cluster 1 (Vocal type 1), composed of 34 vocalizations, and Cluster 2 (Vocal type 2), grouping the remaining 46 (Figs. 3 and 4). This composition of both clusters revealed a mixture of individuals from the South and Southeast populations, independent of geographic origin.

Fig. 3 Variation in acoustic parameters between the two Marsh Tapaculo *Scytalopus iraiensis* vocal types. The panels compare Dominant Frequency Range, Modulation Index, Spectral Skewness, and Spectral Entropy for Vocal type 1 (green) and Vocal type 2 (orange). In the box plots, the central line represents the median, the box limits indicate the interquartile range (25th and 75th percentiles), and the whiskers extend to 1.5 times the interquartile range. Points outside the whiskers represent outliers.

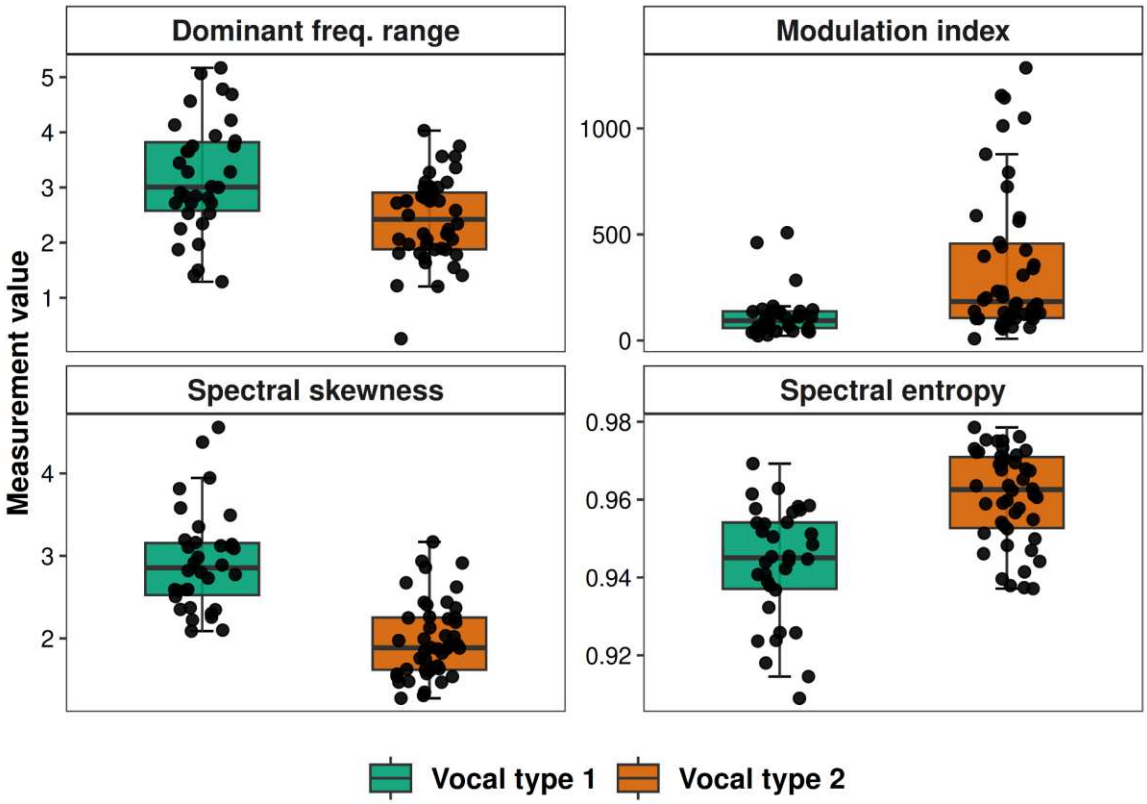
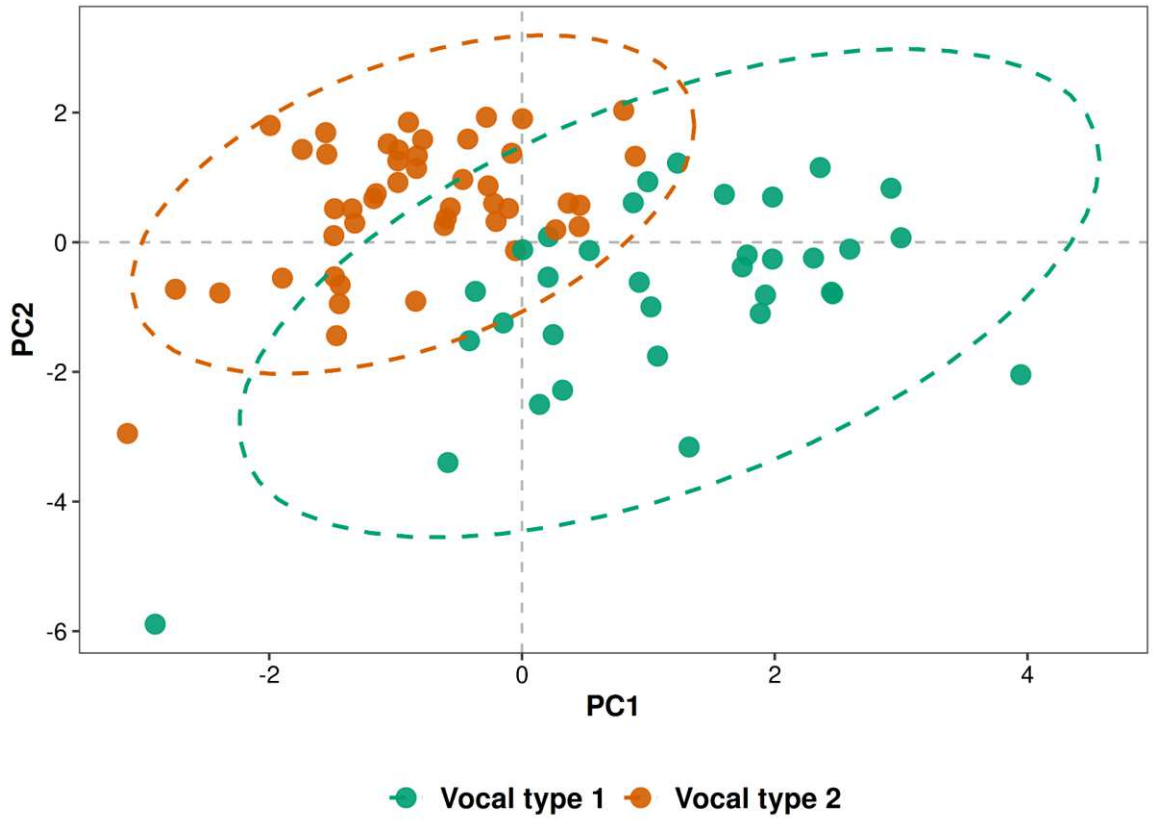
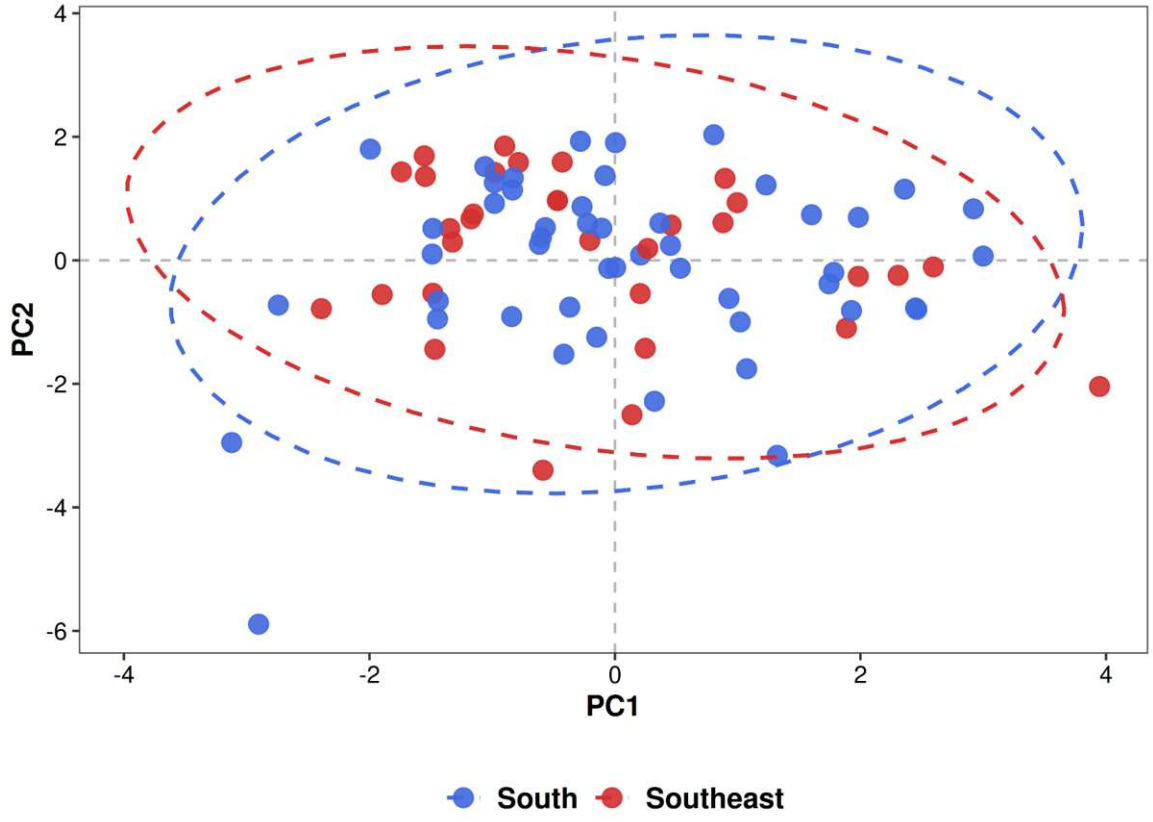


Fig. 4 Acoustic space segregation in Marsh Tapaculo *Scytalopus iraiensis*. The scatterplot displays the first two principal components (PC1 and PC2) derived from the acoustic parameters. Colors represent the distinct vocal clusters identified by K-means analysis: Vocal type 1 (green) and Vocal type 2 (orange). Dashed ellipses denote the 95% confidence intervals for each group.



Finally, when explicitly grouping the vocalizations by location to test the effect of geography, PERMANOVA analysis did not reveal significant acoustic differences between the disjunct populations of Marsh Tapaculo ($F_{1,79} = 1.25$, $R^2 = 0.016$, $P = 0.279$). The geographic region explained only 1.5% of the total acoustic variation. Furthermore, the multivariate dispersion of acoustic parameters did not differ significantly between regions (Betadisper: $F_{1,79} = 0.64$, $P = 0.428$) (Fig. 5), indicating homogeneity in vocal variability throughout the species' distribution area. These results corroborate the existence of vocal conservatism.

Fig. 5 Projection of Marsh Tapaculo *Scytalopus iraiensis* acoustic samples onto the first two principal components (PC1 and PC2), grouped by Brazilian macroregion. Samples from the South are shown in blue, while those from the Southeast are in red. Dashed lines indicate the 95% confidence ellipses for each geographic group.



Discussion

Geographic disjunction is widely recognized as a factor generating divergent selective pressures, including variations in vegetation cover, social interactions (*e.g.*, social/sexual selection), temperature, humidity, and wind speed, which frequently lead to differentiation in functional characteristics, such as vocalizations (Räsänen and Hendry 2008; Hermes et al. 2016; Sementili-Cardoso and Donatelli, 2018). However, this effect was not observed in the subpopulations of the Marsh Tapaculo, whose current results revealed a remarkable vocal conservatism, reflecting the morphometric and color uniformity of the species, already reported by Vasconcelos et al. (2008a). The acoustic space of the Southeast subpopulation is completely nested within the variation observed in the South, as evidenced by unsupervised cluster analysis (K-means): although two discrete vocal groups ("Vocal types 1 and 2") were identified, the presence of individuals from both macroregions in both clusters demonstrates that this variability constitutes a maintained ancestral polymorphism, and not an incipient divergence between the subpopulations due to geographic influence (Maldonado-Coelho et al. 2023).

This stability contrasts with evolutionary patterns observed in other lineages that possess innate vocalizations, for example, in the *Glaucidium brodiei* (Burton, 1836) owl complex, belonging to the order Strigiformes, where geographic isolation led to a clear acoustic divergence between insular and continental populations, acting as the primary characteristic for species delimitation (Gwee et al. 2019). Even in insular systems with land bridges, analogous to the historical connectivity proposed here, isolation can promote rapid acoustic differentiation in Tyranni, as evidenced for *Thamnophilus atrinucha* Salvin & Godman, 1892 on Gorgona Island (Sedano-Cruz et al. 2024). Similar patterns occur in other Neotropical birds with genetically determined

vocalizations, such as *Hemitriccus diops* (Temminck, 1822) in the Brazilian Atlantic Forest and *Sakesphoroides cristatus* (Wied-Neuwied, 1831) in the Caatinga of northeastern Brazil, where geographic and fluvial barriers, respectively, drove significant acoustic differentiation (Capelli et al. 2020; Acero-Murcia et al. 2021). These cases demonstrate that species with innate song are not immune to geographic variation.

Such vocal stability can be attributed to the intrinsic physiological constraints of the suborder Tyranni, because unlike Passeri, which rely on complex neural circuits for vocal learning and refined muscle control, sound production in this group is primarily regulated by syrinx biomechanics and subsyringeal pressure modulations (Goller et al. 2021; Amador and Mindlin 2023). This anatomical architecture acts as an evolutionary filter: as demonstrated by Goller et al. (2021), the fundamental frequency in these birds exhibits a strong phylogenetic signal and a negative correlation with body mass, a physical limitation absent in vocally learned lineages. This indicates that significant alterations in the acoustic space would require mandatory structural changes in the syrinx or body size. Thus, even under the influence of genetic drift, the morphological conservatism of the genus *Scytalopus* imposes a "physical" restriction, limiting the exploration of new spaces and slowing down phenotypic evolution (Goller et al. 2021).

The maintenance of this vocal uniformity is strongly supported by the Acoustic Adaptation Hypothesis (AAH) in a scenario of strong environmental pressure and selection. As is known, the structure of the physical environment acts as the main predictor of spectral parameters, along with stochastic divergence, as demonstrated by Seddon (2005) with birds of the Thamnophilidae family. The Marsh Tapaculo is a specialist in dense marsh vegetation and alluvial plains, dominated by species of rushes and tall grasses (Mata 2005; Klemann Jr. and Vieira 2013). And it is this type of habitat that imposes a severe degradation of high-frequency signals, unlike open environments, where selective pressure favors high frequencies and rapid temporal modulation to overcome wind turbulence (Morton 1975).

Recent macroevolutionary analyses corroborate that vocal complexity in Tyranni represents an evolutionary trade-off with these environmental constraints (Yang et al. 2025), and that the efficiency of vocal signals depends on the precise coupling between the fundamental frequency of the sound source and tracheal resonance (Riede et al. 2019). Therefore, the marshy environment exerts strong stabilizing selection, favoring a specific transmission window characterized by low frequencies and filtering out variants that deviate from this adaptive optimum, since they would be energetically costly and functionally ineffective in overcoming the dense vegetation structure (Barreira and García 2019).

Furthermore, the absence of the capacity for rapid vocal variations in this taxon reinforces this conservatism, because while Passeri benefit from the learning advantage to adjust their songs and overcome noise or acoustic barriers, such as the aforementioned wind turbulence and vegetation (Ríos-Chelén et al. 2012), Tyranni depend on innate and genetic patterns. Since Marsh Tapaculo does not exhibit the behavioral flexibility to explore new acoustic spaces, it remains "anchored" to a vocal character that, evolutionarily, maximizes propagation efficiency in its habitat. Given that the biotic and abiotic structure of these areas is analogous between the South and Southeast, the selective pressure remains uniform, which guarantees the retention of the ancestral acoustic signal (Maldonado-Coelho et al. 2023).

As an alternative explanation, there is the hypothesis of recent historical connectivity, which offers a parsimonious explanation for the absence of divergences. Geographic isolation can lead to adaptations and, consequently, to vocal differentiations in certain lineages of innate vocalizers, as described in populations of

Sciaphylax hemimelaena (Sclater, 1857) isolated for less than 3,000 years (Seddon and Tobias 2007). However, the time of separation does not act as the sole determinant of these events, as in *Formicarius colma* Boddaert, 1783, which has disjunct populations distributed north of the Amazon River and along the Atlantic Forest for about 6.5 million years, and still maintains high acoustic similarity despite this profound geographic separation (Lima and Mariano 2019). Other factors influence this, including climatic pressure, as described by Weir and Wheatcroft (2011), showing that tropical species exhibit lower rates of physical and vocal differentiation when compared to species from temperate climates, having an intrinsic stability in these regions.

It is plausible that gene flow in the Marsh Tapaculo persisted until recently via stepping-stone dispersal, facilitated by wetland corridors formed during Pleistocene marine regressions (Mata et al. 2009). This dependence on physical corridors is consistent with the biology of the genus *Scytalopus*, historically characterized by low dispersal capacity and sedentary lifestyle, which restricts gene flow through inadequate habitat barriers (Cadena et al. 2020). The viability of this past connection is corroborated by the existence of suitable marshy phytophysiognomies in the intermediate region of the state of São Paulo, such as in the Serra do Mar State Park (Vasconcelos et al. 2008a, b; Klemann Jr. and Vieira 2013). Although there are no contemporary records of the species in these areas, such zones may likely act as historical ecological corridors, preventing effective isolation for a sufficient time for the accumulation of mutations. This scenario illustrates a case of niche conservatism (Wiens and Graham 2005), where strict fidelity to ancestral habitat limits geographic expansion to ecologically specific routes.

From a taxonomic and conservationist perspective, the uniformity of the acoustic signal reinforces the status of the Marsh Tapaculo as a single Evolutionarily Significant Unit (ESU), since, in cryptic genera marked by plumage stasis, vocalization often constitutes the main pre-zygotic barrier to gene flow (Casacci et al. 2014; Cadena et al. 2020). In this context, the bioacoustics of the species emerges as an evolutionary product of the interaction between strict habitat specialization, biomechanical constraints intrinsic to Tyranni, and morphological conservatism. It is concluded, therefore, that the absence of vocal divergence reflects the synergism between recent historical connectivity and niche conservatism (Wiens and Graham 2005; Mata et al. 2009), a hypothesis that defines the roadmap for future phylogeographic and molecular dating investigations, as well as for in situ song playback experiments aimed at testing intraspecific recognition.

Acknowledgments

We would like to thank Mônica da Costa Lima for providing the initial script for extracting acoustic parameters and for her valuable help in clarifying doubts, which were fundamental to the completion of this study.

Author contributions

Conceptualization: José Alan Sales Souza; Methodology: José Alan Sales Souza, Bruno Pinho de Lucena, Erich de Freitas Mariano; Formal analysis and investigation: José Alan Sales Souza, Ricardo Henrique Pereira da Silva, Bruno Pinho de Lucena, Gabriel Rian Alexandre Ferreira, Alan Roberto Ferreira Silva, Iasmin Duarte da Silva; Writing - original draft preparation: José Alan Sales Souza, Ricardo Henrique Pereira da Silva, Bruno

Pinho de Lucena, Gabriel Rian Alexandre Ferreira, Alan Roberto Ferreira Silva, Iasmin Duarte da Silva; Writing - review and editing: Bruno Pinho de Lucena, Gabriel Rian Alexandre Ferreira; Supervision: Erich de Freitas Mariano.

Declarations

Ethics approval: None.
Conflict of interest: The authors declare no competing interests.
Funding: No funding, grants, or other support was received.
Financial interests: The authors declare they have no financial interests.
Consent: All authors consent to participate in and publish this study.
Data availability: The authors declare that the data used can be accessed (Online Resources 1, 2, 3 and 4 as supplementary material).

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Figure Captions:

Fig. 1 Geographic distribution of the Marsh Tapaculo *Scytalopus iraiensis* sound recordings analyzed in this study. The map highlights two distinct sampling clusters within Brazil: red circles represent the population in the Southeast macroregion, while blue circles represent the population in the South macroregion. Right panels display zoomed-in views of the sampling localities overlaid on satellite imagery. The inset provides an illustration of the species (from Krabbe & Schulenberg, 2020).

Fig. 2 Representative spectrogram of the Marsh Tapaculo *Scytalopus iraiensis* song. The visualization displays frequency (kHz) over time (s), where the color gradient indicates relative amplitude (dB). Yellow areas represent peak energy frequencies, while dark blue represents lower amplitude background noise.

Fig. 3 Variation in acoustic parameters between the two Marsh Tapaculo *Scytalopus iraiensis* vocal types. The panels compare Dominant Frequency Range, Modulation Index, Spectral Skewness, and Spectral Entropy for Vocal type 1 (green) and Vocal type 2 (orange). In the box plots, the central line represents the median, the box limits indicate the interquartile range (25th and 75th percentiles), and the whiskers extend to 1.5 times the interquartile range. Points outside the whiskers represent outliers.

Fig. 4 Acoustic space segregation in Marsh Tapaculo *Scytalopus iraiensis*. The scatterplot displays the first two principal components (PC1 and PC2) derived from the acoustic parameters. Colors represent the distinct vocal clusters identified by K-means analysis: Vocal type 1 (green) and Vocal type 2 (orange). Dashed ellipses denote the 95% confidence intervals for each group.

Fig. 5 Projection of Marsh Tapaculo *Scytalopus iraiensis* acoustic samples onto the first two principal components (PC1 and PC2), grouped by Brazilian macroregion. Samples from the South are shown in blue,

while those from the Southeast are in red. Dashed lines indicate the 95% confidence ellipses for each geographic group.

Supplementary Information (SI) Captions:

Online Resource 1. R script providing the complete workflow for bioacoustic parameter extraction, data pre-processing, and multivariate statistical analyses (PCA, K-means clustering, and PERMANOVA) of *Scytalopus iraiensis* vocalizations.

Online Resource 2. Description of spectral and temporal bioacoustic parameters extracted from *Scytalopus iraiensis* vocalizations using the R package *warbleR* (Araya-Salas & Smith-Vidaurre, 2017).

Online Resource 3. Metadata of *Scytalopus iraiensis* sound recordings analyzed in this study, including source databases, quality ratings, and geographic coordinates.

Online Resource 4. Compressed audio dataset (ZIP archive) containing the sound recordings used in the final analysis. The file comprises the 80 valid songs of *Scytalopus iraiensis* included in the study: 31 belonging to the Southeast subpopulation and 49 to the South subpopulation.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ESM1.docx
- ESM2.xlsx
- ESM3.xlsx
- ESM4.zip