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Acoustic clue: bringing echolocation call data into the distribution dilemma of *Pteronotus* (Chiroptera: Mormoopidae) complexes in Central America

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In Central America, the distributional limits and the contact zones of some *Pteronotus* species, such as the naked-backed bats and the lesser mustached bats, are unclear. To elucidate the distributional dilemma of the species groups *Pteronotus fulvus* + *Pteronotus davyi* and *Pteronotus psilotis* + *Pteronotus personatus* in Central America, we studied the acoustic variation of their echolocation calls along the range of possible contact zones and the existence of distinct phonic groups. We performed hierarchical *k*-means clustering on principal components (HCPC) using acoustic samples from Mexico, Honduras, El Salvador, Nicaragua and Costa Rica to describe the global acoustic diversity, possibly overlooking differences between species groups. We assessed whether those acoustic differences were related to the geographical distance and geographical location. We found evidence of sympatry for three phonic groups within each species complex, without a clear correspondence to the known distribution of species. The changes in frequency of their echolocation calls seem to follow a similar pattern to the geographical variation in body size of the species. Future studies in Central America should include an integrative sampling of individually captured, tagged and recorded bats to help in resolution of the distribution dilemma raised here.

ADDITIONAL KEYWORDS: acoustic identification – HCPC – phonic groups – *Pteronotus davyi* – *Pteronotus fulvus* – *Pteronotus personatus* – *Pteronotus psilotis*.

INTRODUCTION

The Mormoopidae are a Neotropical bat family consisting of two genera of living species, *Mormoops* (ghost-faced bats) and *Pteronotus* (mustached bats and naked-backed bats). Mormoopids have a wide geographical distribution, ranging from the southern USA into Central America, from the Caribbean to central Brazil and from west Andes to Peru (Koopman, 1993). Species of the family inhabit tropical rainforest, semi-arid and arid environments < 3000 m a.s.l. (Smith, 1972; Emmons, 1997; Patton & Gardner, 2007). All species are insectivorous, gregarious and obligatory cave dwellers (Koopman, 1993; Simmons & Conway, 2001).

Multiple lines of evidence (morphological, morphometric, ecological, acoustic and molecular; e.g. Smith, 1972; Simmons & Conway, 2001; Dávalos, 2006; Mancina *et al*., 2012; Clare *et al*., 2013; Pavan & Marroig, 2016, 2017) have allowed a better understanding of species delimitation and evolutionary history within this family, particularly for the genus *Pteronotus*. The most recent phylogenetic hypothesis recognizes the high diversity of this genus and its subdivision into three subgenera and four clades (Pavan & Marroig, 2016).

In general, *Pteronotus* species from distinct species complexes overlap in terms of their geographical distribution, whereas species within the same clade (species group) show separate distributions (Pavan & Marroig, 2016). However, in Central America, for some species groups, such as the naked-backed bats and the lesser mustached bats, with supposedly allopatric distribution, the distributional limits and contact zones are unclear (Smith, 1972; Pavan & Marroig, 2016, 2017).

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The recognized distribution of Thomas's naked-backed bat, *Pteronotus fulvus* (Thomas, 1892), and Davy's naked-backed bat, *Pteronotus davyi* Gray, 1838, primarily follows the subspecies geographical range proposed by Smith (1972), with *P. fulvus* ranging from southern Mexico to eastern Honduras and El Salvador and *P. davyi* from Nicaragua to northern South America (Pavan & Marroig, 2016; Pavan, 2019). Based on morphological geographical variation, allopatric populations may be distinguished easily on the base of cranial and external size, with *P. fulvus* being smaller than *P. davyi*, gradually increasing in size southward (Smith, 1972). Smith (1972) proposed a narrow intergradation zone in northern Nicaragua and eastern Honduras and El Salvador. However, the lack of molecular data for populations of *P. davyi* at its northern distribution limit inhibits the determination of its exact geographical limits (Pavan & Marroig, 2016, 2017). Unpublished molecular data (A. C. Pavan, personal communication) suggest the occurrence of *P. fulvus* and *P. davyi* in sympatry in Costa Rica, which might correspond to a secondary contact zone.

Likewise, the recognized distribution of Wagner's lesser mustached bat, *Pteronotus psilotis* Wagner, 1843, and Dobson's lesser mustached bat, *Pteronotus personatus* Dobson, 1878, is based on the geographical range of the subspecies proposed by Smith (1972), with *P. psilotis* occurring from southern Mexico to eastern Honduras and El Salvador and *P. personatus* from western Costa Rica to South America. Considering cranial and external size, *P. psilotis* is smaller than *P. personatus*, gradually increasing in size southward, with a probable intergradation zone in southeastern Honduras and eastern El Salvador (Smith, 1972).

The high levels of genetic differentiation and the significant morphometric variation of the lesser mustached bats throughout their distribution indicate that this clade is a species complex with at least five lineages, corresponding to a new undescribed subgenus (Pavan & Marroig, 2016; Zárate-Martínez *et al*., 2018). Nevertheless, the phylogenetic status of Central American *P. personatus* populations has not been assessed, and the northern limits of its distribution are unclear, probably extending to Nicaragua or Costa Rica (Pavan, 2019). Pavan & Marroig (2017) summarized the findings of Smith (1972), indicating a possible contact zone located in Nicaragua and Costa Rica.

In general, the echolocation calls of mormoopids show low levels of interspecific variation (Fenton, 1994; Ibáñez *et al*., 1999; Macías & Mora, 2003). In particular, the echolocation calls of the aforementioned *Pteronotus* species groups share a similar design, with multiple harmonics and with initial and terminal components of constant frequency (CF) linked by a frequency-modulated (FM) component (Griffin & Novick, 1955; Novick & Vaisnys, 1964; O'Farrell & Miller, 1997; Ibáñez *et al*., 1999; Macías & Mora, 2003). Acoustic identification relies on species-specific frequencies of the CF segment of the second harmonic (Macías *et al*., 2006), which is less susceptible to changes in frequency during distinct behavioural circumstances (Ibáñez *et al*., 1999). Acoustic information on those species is scarce and available mainly from a few localities in Mexico (e.g. Morelos, Oaxaca, Yucatán and Veracruz), Central America (e.g. Belize, Costa Rica and Panama) and the Caribbean (Puerto Rico, French Guiana and Guadeloupe) (for a review on this topic, see Arias-Aguilar *et al*., 2018).

Given that the areas of sympatry are uncertain, and in the absence of an ideal acoustic integrative sampling (including individual acoustic, morphological and molecular information), species identification from common passive acoustic monitoring in those areas might be ambiguous. There is a need to identify and describe acoustic groups before assigning them to a particular species. The study of acoustic variation along the range of possible contact zones might contribute to elucidation of the distributional dilemma of those species.

Here, we aim to study the acoustic variation of the species groups *P. fulvus* + *P. davyi* and *P. psilotis* + *P. personatus* in Central America within their possible contact zones. Specifically, we aim to investigate the existence of distinct phonic groups and their correspondence to the known or estimated distribution of the different species. We hypothesize that different phonic groups will occur within the species intergradation zone, and we expect that the acoustic variation will follow a similar pattern to the morphological geographical variation proposed by Smith (1972).

MATERIAL AND METHODS

Acoustic data

Through our own recordings and donations, we compiled echolocation calls from species of the genus *Pteronotus* from Mexico, Honduras, El Salvador, Nicaragua and Costa Rica, corresponding to populations within the recognized distribution of *P. fulvus*, *P. davyi* (Fig. 1), *P. psilotis* and *P. personatus* (Fig. 2; Supporting Information, Table S1). Recordings were made on free-flying bats. Given that the recordings were made with different bat detectors and different sampling frequencies, in order to maintain the same frequency resolution for all files (188 Hz) we resampled those recordings with a different sampling rate (192 kHz) before the acoustic analysis.

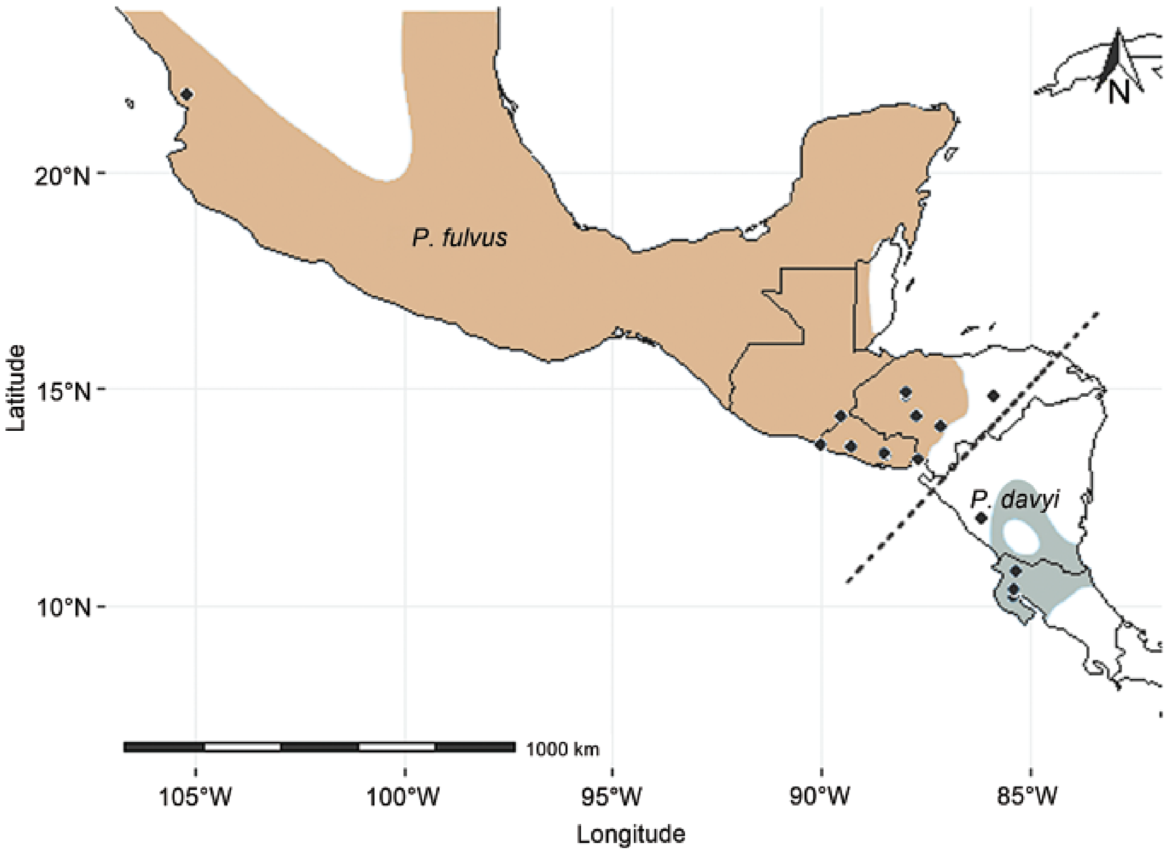

Figure 1. Map showing the localities of acoustic monitoring (black dots), with shaded areas representing part of the distribution of *Pteronotus fulvus* (in brown) and *Pteronotus davyi* (in grey) (modified from Pavan, 2019). The dotted line indicates the area of species intergradation proposed by Smith (1972).

ACOUSTIC ANALYSIS

Spectrograms were generated with 1024-point fast Fourier transformation (FFT), FlatTop window, 100% frame size and 98.43% overlap. The echolocation call parameters were measured from the second harmonic of each pulse using AVISOFT SAS Lab Pro software, v.5.2.13, R. Specht, Berlin, Germany. Frequencies under and above the second harmonic were filtered. When possible, ten pulses (minimum of five) were measured for each file, including consecutive and non-consecutive calls. Only search phase calls with no overlap or overloading and clearly distinguished from the background (signal-to-noise ratio > 20 dB) were measured.

We used the automatic parameter-measurement tool to compute the following parameters: the peak frequency at the start (f.start) and end (f.end) of the element (call); the lowest peak frequency (f.min); the highest peak frequency (f.max); the mean peak frequency (f.mean); and the maximum amplitude of each element (f.me). We obtained the derived parameters bandwidth (difference between f.max and f.min), sumentire (frequency change from the start to the end) and meanentire (average frequency slope of the element, expressed in kiloherz per millisecond). The units for all temporal measurements were seconds and for all frequency measurements herz.

We measured the duration of each element from start to end. Additionally, we measured the duration of the initial and terminal CF components, with a frequency-change threshold of 2000 Hz.

STATISTICAL ANALYSIS

We used two different datasets, one including *P. fulvus* and *P. davyi*, hereinafter referred to as complex *P. fulvus*/*P. davyi* (containing 250 files and a total of 2330 echolocation calls) and one for *P. psilotis* and *P. personatus*, hereinafter referred to as complex *P. psilotis*/*P. personatus* (containing 123 files and a total of 1040 echolocation calls). Each file summarized the mean values of the acoustic parameters for all the calls of the individual within that file. We selected only recordings with calls undoubtedly belonging to the same individual, although the same individual might have been recorded in different files. Nonetheless,

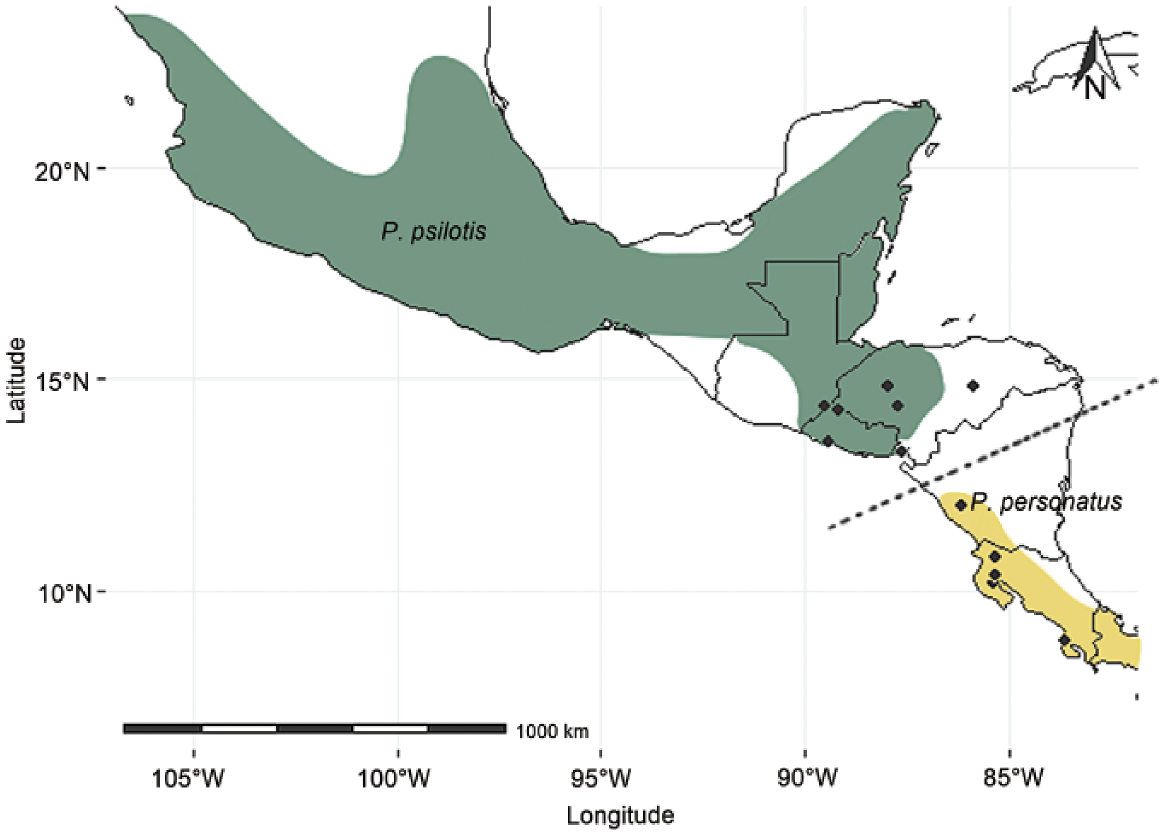

Figure 2. Map showing the localities of acoustic monitoring (black dots), with shaded areas representing part of the distribution of *Pteronotus psilotis* (in green) and *Pteronotus personatus* (in yellow) (modified from Pavan, 2019). The dotted line indicates the area of species intergradation proposed by Smith (1972).

for the sake of simplicity we will refer to the data regarding the mean values of the acoustic parameters obtained in each file as an individual acoustic sample.

The following analyses were performed for each dataset. To reduce multicollinear variables in the analysis, the maximal information coefficient was determined with the R package 'minerva' (Albanese *et al.*, 2013). All variables with correlations > 0.7 were discarded. The following parameters were kept for the analyses: call duration, duration of CF terminal component, f.min, f.max, f.me, f.mean, slope, sumentire (frequency change from the start to the end) and bandwidth. Bandwith was used only for *P. fulvus*/*P. davyi*, because bandwidth maximal information coefficient for the other pair was > 0.7.

HIERARCHICAL *K*-MEANS CLUSTERING ON PRINCIPAL COMPONENTS

To describe the global acoustic diversity, possibly overlooking differences between species groups, we initially carried out a mixed principal components analysis (PCA) as a preprocessing step to the hierarchical clustering analysis (HCA). We included the following as active quantitative variables in the PCA: call duration, f.min, f.max, f.me and f.mean; as quantitative supplementary variables: duration of terminal CF component, slope, fchange and bandwidth; and as qualitative supplementary variables: locality and site. We then performed a hierarchical *k*-means clustering on principal components (HCPC), considering the two main dimensions retained from the PCA. The HCPC delineates clusters of individuals (acoustic samples) with similar characteristics. For the hierarchical tree, we used Ward's criterion and Euclidean distance and built it without any prespecified number of clusters. Tree partitioning was consolidated by the centroid-based algorithm k-means partitioning. We successively combined the samples into clusters, minimizing the within-cluster variation and maximizing the between-cluster variation. The HCPC was performed using the package FACTOMINER (Lê, Josse & Husson, 2008).

GEOGRAPHICAL VARIATION

To assess whether differences in the frequency parameters with a major contribution to cluster separation (f.mean, f.min and f.max) were related to the geographical distance, we calculated a dissimilarity matrix of acoustic distances using frequency differences (in hertz) between localities and a geographical distance matrix using the physical distance (Haversine distances) between localities. Then, we compared the acoustic and geographical distance matrices using a Mantel test based on Pearson correlation with 9999 permutations, using the R packages vegan (Oksanen *et al.*, 2016) and geosphere (Hijmans, 2019). To test the association between frequency parameters and the geographical location, we conducted linear regressions of f.mean, f.min and f.max means against the longitude and latitude of each locality. All analyses were conducted using R software, v.3.6.3 (R Foundation for Statistical Computing, 2016).

RESULTS

Complex *P. fulvus*/*P. davyi*

The PCA was applied to a matrix of 250 acoustic samples characterized by 11 variables (Supporting Information, Fig. S1). In the decomposition of the total inertia, the first two principal components (PC1 and PC2) accounted for 87.57% of the total data variance. Therefore, the variability of the data was well reflected in the first projection plane and was used to interpret the data for the next classification step. The main characteristics of this first dimension are summarized in Table 1. Note that the f.mean, the site Mexico, and the localities Metapan (El Salvador), Montecito (El Salvador) and PNLT (Honduras) were highly correlated with dimension 1 (respective correlations of 0.94, 0.98, 0.9, 0.95 and 0.98). Within this dimension, only samples from Mexico were clearly separated from those of other sites (Supporting Information, Fig. S1A). Besides, there was no clear separation when the acoustic samples were classified according to the real species distribution (Supporting Information, Fig. S1B).

The HCPC returned a set of three clusters, grouping samples broadly similar to each other. Each could be positioned, within the cluster to which it belonged on a factor map to visualize individual positions in relationship to dimension 1 (Dim1) and dimension 2 (Dim2) of the PCA (Fig. 3A). The cluster analysis was performed initially according to variables, then according to individuals (acoustic samples). The main variables that best described the partitioning of the clusters were f.mean, f.min and f.max ($\eta^2 = 0.73$, 0.71 and 0.69, respectively; $P < 0.0001$; Fig. 3B; Supporting Information, Table S2).

Cluster 1 was composed of individuals sharing low values for the variables f.mean, f.min and f.max, meaning that calls within this cluster had significantly lower frequencies than the overall files. These samples had significant lower values of coordinates in dimension 1 than samples overall (Supporting Information, Table S3). All samples from the localities PV_M3 (Costa Rica) and Catacamas (Honduras) belonged to this cluster, as did ~47.1% of samples from Costa Rica and 8.9% from El Salvador (Supporting Information, Table S4).

Cluster 2 was composed of individuals sharing high values for the variable f.mean and low values for the variables call duration and duration of CF end section, meaning that acoustic samples within this cluster had significantly higher frequencies and shorter duration than the samples overall. Also, they showed significantly lower values of coordinates in dimension 1 and higher values of coordinates in dimension 2 than the samples overall (Supporting Information, Table S3). All samples from El Tigre (El Salvador), 90.9% of those from La Naturaleza (Honduras) and 89.7% of those from PV_M2 (Costa Rica) belonged to this cluster (Supporting Information, Table S4).

Cluster 3 was characterized by high values for the variables f.max, f.min and f.mean, meaning that calls within this cluster had significantly higher frequencies. Acoustic samples here also had significant higher values of coordinates in dimension 1 (Supporting Information, Table S3). All samples from Mexico and 26.7% of those from El Salvador belonged to this cluster, as did 80.0% of samples from Cueva Viejo (Honduras) and 70.0% from Montecito (El Salvador) (Supporting Information, Table S4).

Clustering also involved the identification of paragons, which were the individuals whose coordinates were closest to the barycentre of each group. Accordingly, the profile of this sample best characterized the cluster to which it belonged. The paragons were the acoustic samples 96 from Palo Verde, Costa Rica (cluster 1), 217 from El Tigre, El Salvador (cluster 2) and 144 from Cueva Viejo, Honduras

Table 1. Results of principal components analysis for the acoustic datasets of *Pteronotus fulvus*/*Pteronotus davyi* and *Pteronotus psilotis*/*Pteronotus personatus*

Dataset	Principal component	Eigenvalue	Variance (%)	Cumulative variance (%)
P. fulvus/*P. davyi*	PC1	3.482	69.649	69.649
	PC2	0.896	17.925	87.574
P. psilotis/*P. personatus*	PC1	3.370	67.397	67.397
	PC2	0.922	18.441	85.838

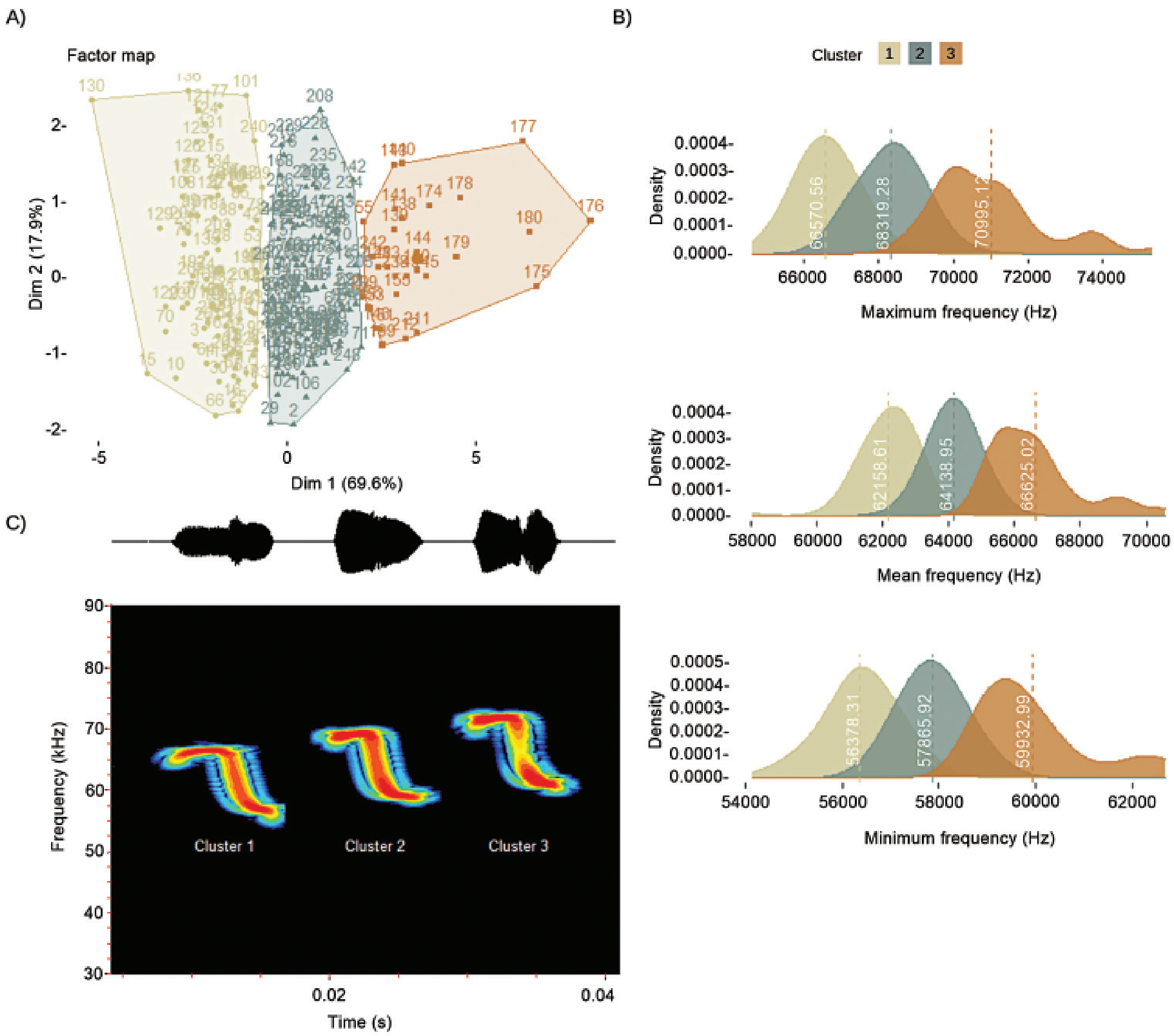

Figure 3. Characterization of the echolocation calls of the complex *Pteronotus fulvus*/*Pteronotus davyi* according to the results of the hierarchical clustering on principal components (A), density plots of the main frequency variables characterizing the three phonic groups (clusters 1–3; B) and spectrograms of representative echolocation calls (paragons) for each cluster of the complex *P. fulvus*/*P. davyi* (C).

(cluster 3). Their profiles defined the typical acoustic variables of each cluster (Fig. 3C). Samples most distant from other clusters were 130 from Catacamas, Honduras (cluster 1), 208 from Alegría, El Salvador (cluster 2) and 176 from Santiago de Ixcuintla, Mexico (cluster 3), representing the more specific calls in each cluster.

Geographical variation

The mean, minimum and maximum frequency variation was significant and positively associated with geographical distances between localities (Mantel test for f.mean, $r = 0.68$, $P < 0.05$; f.min, $r = 0.72$, $P < 0.01$; f.max, $r = 0.70$, $P < 0.01$; Fig. 4), meaning that the more distant the populations, the more distinct the frequencies. Regression analyses showed significant correlations between all the frequency parameters and longitude (f.mean, $r^2 = 0.53$; f.min, $r^2 = 0.57$; f.max, $r^2 = 0.57$; $P < 0.001$) and latitude (f.mean, $r^2 = 0.34$; f.min, $r^2 = 0.43$; f.max, $r^2 = 0.45$; $P < 0.01$), exhibiting a tendency toward frequency increase westward and northward (Fig. 5; Supporting Information, Fig. S2). Nevertheless, when we excluded Mexico (considered statistically as an outlier) from the analysis, the significance of the test result was lost.

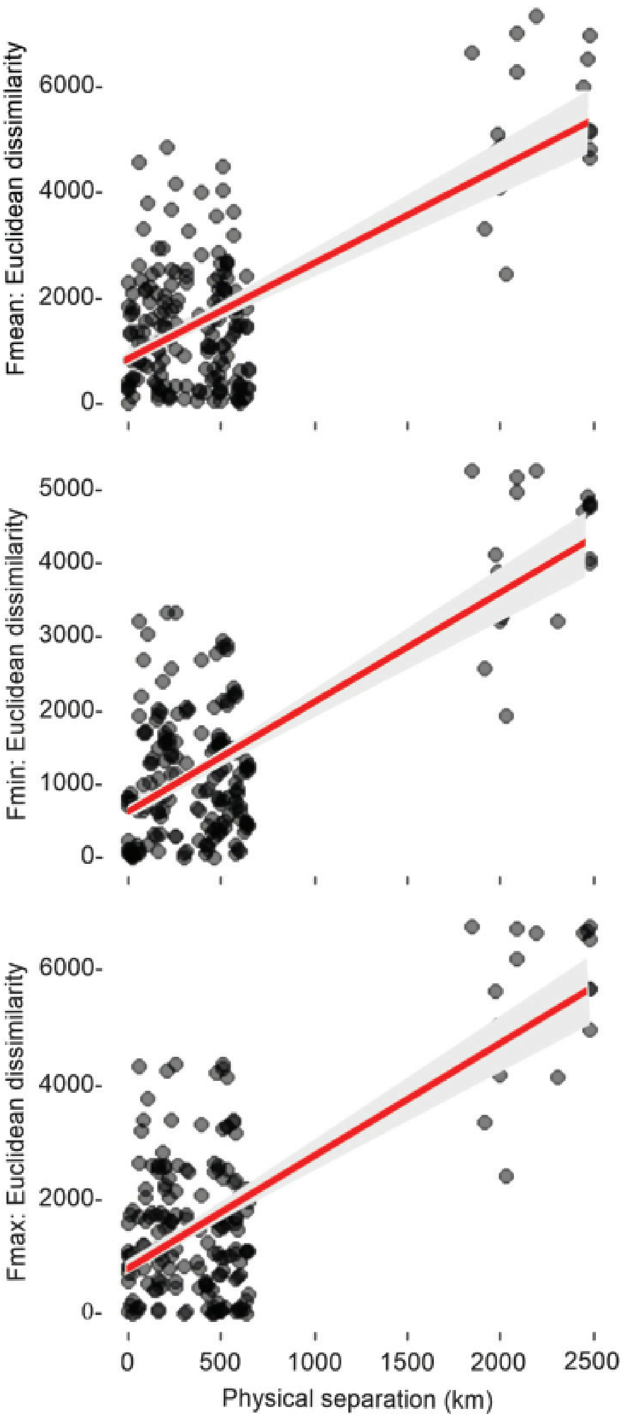

Figure 4. Relationships between frequency parameters for the echolocation calls of the complex *Pteronotus fulvus*/*Pteronotus davyi* and geographical distance.

COMPLEX *P. PSILOTIS*/*P. PERSONATUS*

The PCA was conducted on a matrix of 123 acoustic samples characterized by ten variables (Supporting Information, Fig. S3). In the decomposition of the total inertia, PC1 and PC2 accounted for 85.84% of the total data variance. Therefore, the variability of the data was well reflected in the first projection plane and was used to interpret the data for the next classification step. The main characteristics of this first plane are summarized in Table 1. Note that the f.mean, the sites El Salvador and Honduras and the locality El Flor (El Salvador) are highly correlated with dimension 1 (> 0.90). Within this dimension, there was no clear separation of individuals (acoustic samples) according to site or species (Supporting Information, Fig. S3A, B).

The HCPC returned a set of three clusters, grouping acoustic samples that were broadly similar to each other. Each sample could be positioned within the cluster to which it belonged on a factor map to visualize individual positions in relationship to Dim1 and Dim2 of the PCA (Fig. 6A). Initially, the cluster analysis was performed according to variables and then according to individuals (acoustic samples). The main variables that best described the partitioning of the clusters were f.mean, f.max and f.min ($\eta^2 = 0.80$, 0.73 and 0.71, respectively; $P < 0.0001$; Fig. 6B; Supporting Information, Table S5).

Cluster 1 was composed by individuals sharing low values for the variables f.min, f.max and f.mean, meaning that calls within this cluster had significantly lower frequencies (Supporting Information, Table S6). Approximately 85.7% of samples from El Salvador, including all those from El Flor and ~66.7% of those from Metapan, belonged to this cluster (Supporting Information, Table S7).

Cluster 2 was composed by individuals sharing high values for the variable duration of the terminal CF component and lower values for slope and f.change, meaning that calls within this cluster had a significantly longer CF component at the end of the call and more modulation (Supporting Information, Table S6). Approximately 78.1% of samples from Costa Rica and 40.0% of those from Honduras were included in this cluster (Supporting Information, Table S7).

Cluster 3 was characterized by high values for the variables f.mean, f.max and f.me, meaning that calls within this cluster had significantly higher frequencies (Supporting Information, Table S6). Approximately 52.5% of samples from Honduras, including 70.0% of those from Catacamas and 64.3% from Golfo de Fonseca, belonged to this cluster. Also, 83.3% of samples from Palo Verde M2 were within this cluster (Supporting Information, Table S7).

The paragons identified were the samples 64 from Amapala, Honduras (cluster 1), 28 from Palo Verde M3, Costa Rica (cluster 2) and 110 from Catacamas, Honduras (cluster 3). Ther profiles defined typical acoustic variables for each cluster (Fig. 6C). Samples most distant from other clusters were the files 110 from El Flor, El Salvador (cluster 1), 115 from Catacamas, Honduras (cluster 2) and 114 from Catacamas, Honduras (cluster 3), representing the more specific calls in each cluster.

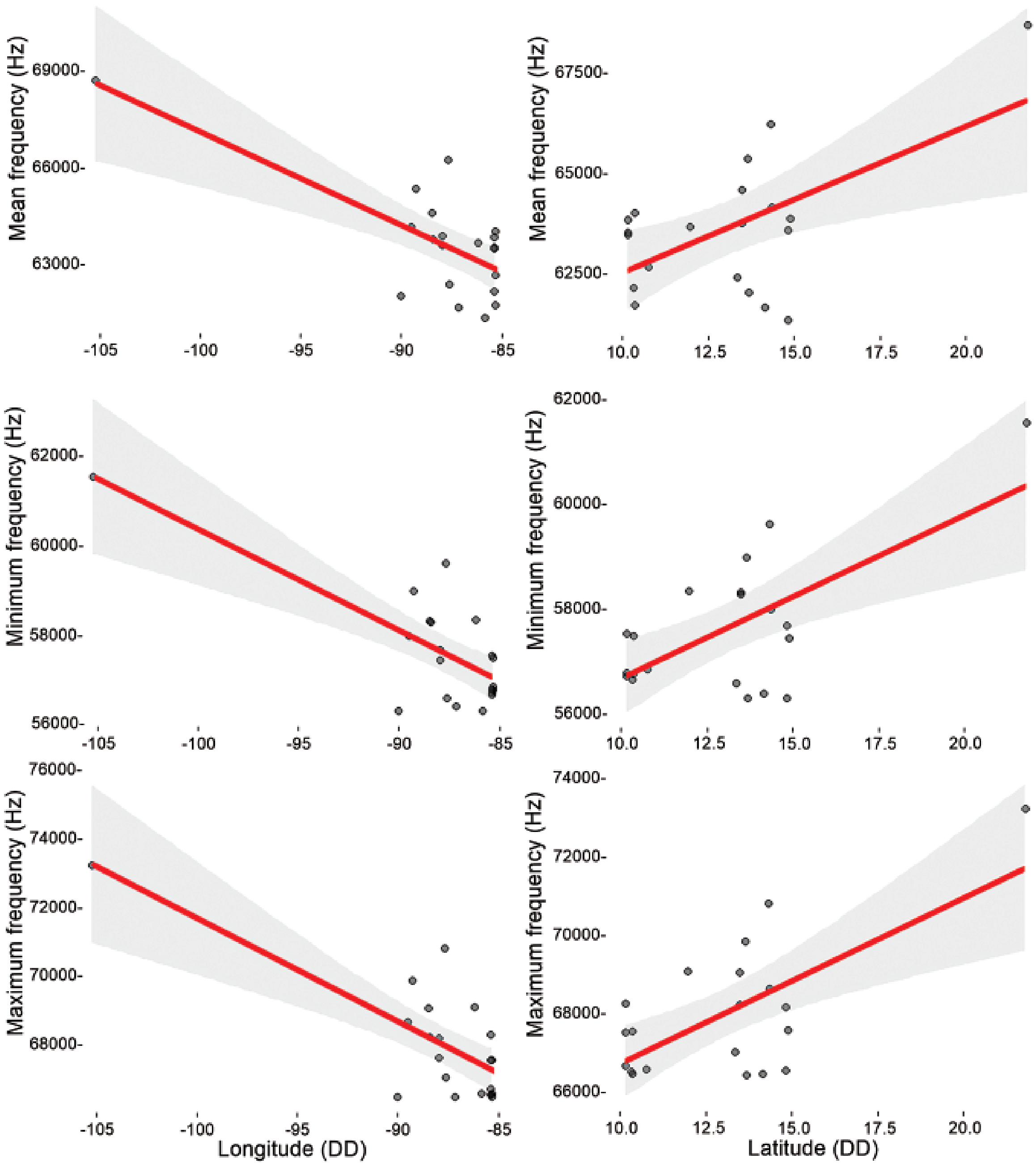

Figure 5. Relationships between frequency parameters for the echolocation calls of the complex *Pteronotus fulvus*/*Pteronotus davyi* and longitude or latitude.

Geographical variation

The Mantel test did not show any significant association between the main frequency parameters with major contributions for the description of the clusters and the geographical distances. Regression analyses showed only a significant correlation between minimum frequency and longitude ($r^2 = 0.27$; $P < 0.05$), exhibiting a tendency toward an eastward increase in frequency (Fig. 7; Supporting Information, Fig. S4).

DISCUSSION

We provide the first insights into the acoustic variation of sister species *Pteronotus fulvus* + *P. davyi* and *P. psilotis* + *P. personatus* within their possible contact zones in Central America. Furthermore, for the two species complexes we found evidence of sympatry for the three phonic groups within each complex, mainly separated by frequency parameters and without a clear correspondence to the known or estimated distribution

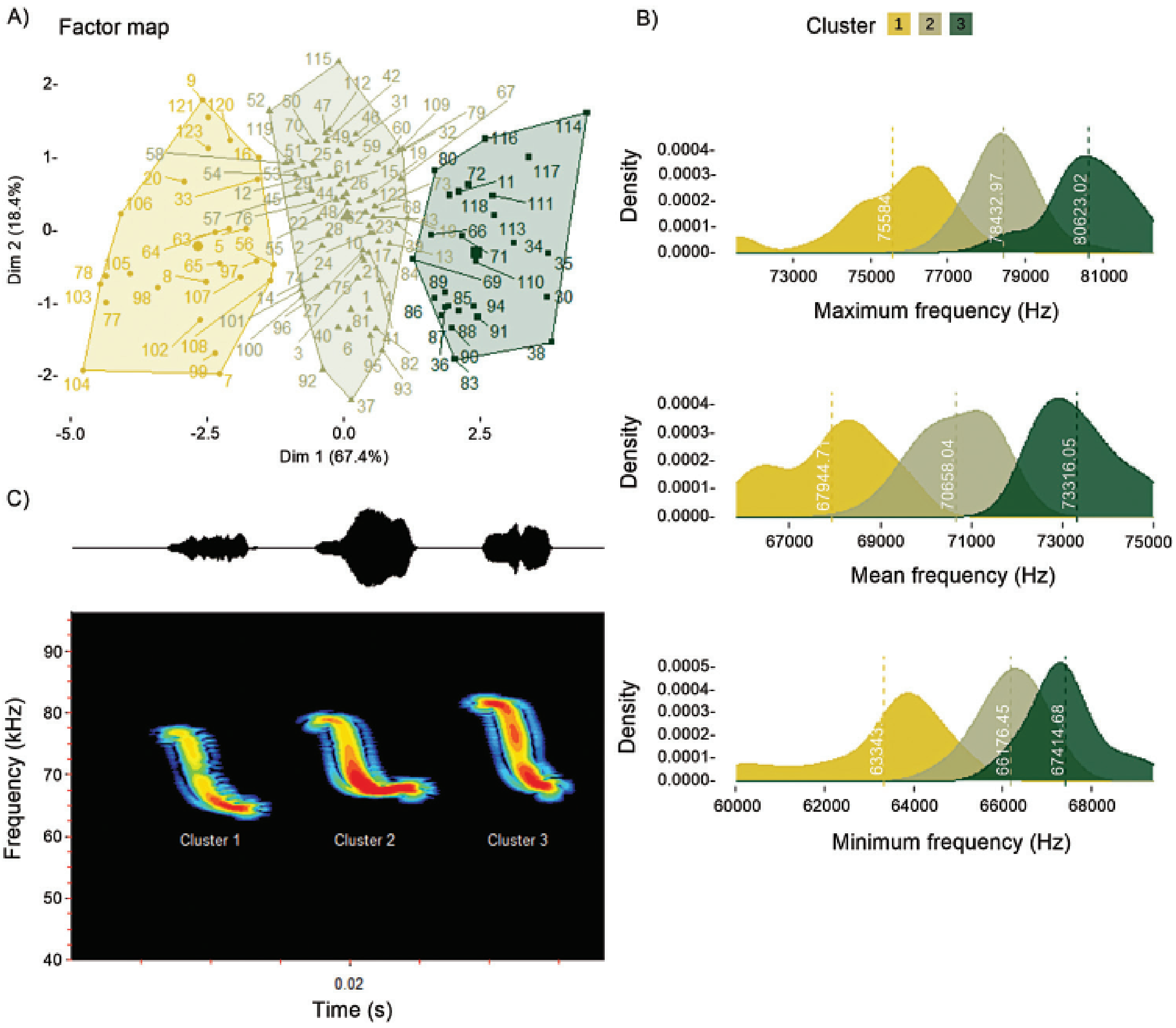

Figure 6. Characterization of the echolocation calls of the complex *Pteronotus psilotis*/*Pteronotus personatus* according to the results of the hierarchical clustering on principal components (A), density plots of the main frequency variables characterizing the three phonic groups (clusters 1–3; B) and spectrograms of representative echolocation calls (paragons) for each cluster of the complex *P. psilotis*/*P. personatus* (C).

for each of the species (Supporting Information, Figs S5, S6). However, the changes in frequency of the echolocation calls for both species complexes seem to follow a similar pattern to the geographical variation in body size of the species.

For the complex *P. fulvus*/*P. davyi*, the three phonic groups were found to coexist in El Salvador, Honduras, Nicaragua and Costa Rica, probably suggesting that the narrow intergradation zone proposed by Smith (1972) in the northern border of Nicaragua is wider and, possibly, that those species, supposedly allopatric (Pavan, 2019), occur in sympatry at least in some parts of this region. Besides, the lack of a clear separation in frequency between the species of the complex might reflect similarities in body size in the Central American localities, as was noted by Smith (1972) for populations of *P. fulvus* (larger individuals from the southern part of their distribution) and *P. davyi* (smaller individuals in the northern part of their distribution) in northern Nicaragua and eastern Honduras and El Salvador.

Although the data from Mexico might be viewed as statistical outliers, they seem to provide relevant biological information. There is an acoustic information gap between the Central American localities and Mexico that needs to be filled, but there is morphological evidence suggesting geographical variation along the

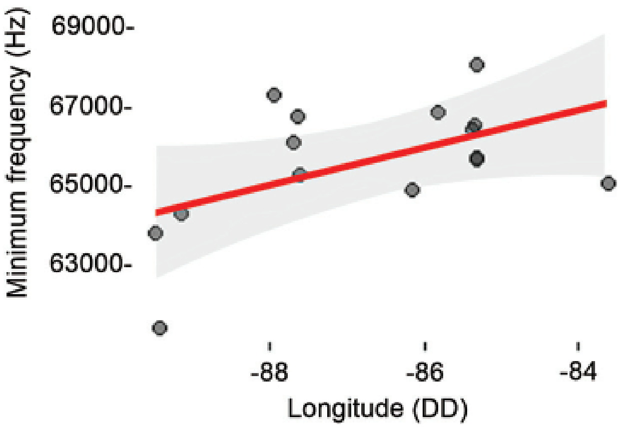

Figure 7. Relationship between minimum frequency of the echolocation calls of the complex *Pteronotus psilotis*/*Pteronotus personatus* and longitude.

sampled sites; hence, a similar acoustic trend is to be expected. Indeed, the geographical variation in the echolocation call parameters and the trend towards an increase in frequency at higher longitudes and latitudes mirror the geographical variation in body size found by Smith (1972) and the frequency scaling with body size known to occur in bat echolocation calls (Jones, 1999). Owing to the physical properties of sound production, even small differences in body size can result in different frequencies (Lin *et al.*, 2014). Recently, Méndez-Rodríguez *et al.* (2021) examined size variation and the hybridization process between *P. fulvus* and *Pteronotus gymnonotus* from Mexico to Costa Rica and found individuals with forearm size intermediate between both species, corroborating the correlation with the latitudinal gradient. Although Méndez-Rodríguez *et al.* (2021) identified the individuals from Nicaragua and Costa Rica as *P. fulvus*, including the intermediate forms based on nuclear genes and microsatellites, the geographical delimitation of the lineages *P. fulvus* and *P. davyi* identified by Clare *et al.* (2011) and Pavan & Marroig (2016) in Central America remains unclear. Further comprehensive sampling is required (individual capture and recording) to corroborate body size–frequency trends and to verify species correspondence to the phonic groups.

Likewise, the three phonic groups found for the complex *P. psilotis*/*P. personatus* did not follow the species distribution and occurred in sympatry in most of the sampling localities. The acoustic differences between the phonic groups and their occurrence in part of the intergradation zone proposed by Smith (1972) could also mirror the overlap in size found by the author between the species in this area.

Central America is recognized as the origin of *P. personatus s.l.*, with a basal clade from Guatemala and two diversification routes, one towards Mexico and another towards South America (Pavan & Marroig, 2017; Zárate-Martínez *et al.*, 2018). Can the acoustic variation of the phonic groups and the trend of increasing frequency to the east reflect the distribution patterns of the species? A comprehensive sampling that couples bioacoustics and genetic evidence is crucial to check the correspondence between the phonic groups and the distinct lineages occurring in Central America.

For the two species complexes, we found significant but slight acoustic variation (< 6 kHz) between the sympatric phonic groups. Likewise, López-Baucells *et al.* (2018) found small differences in the frequency of maximum energy (f.me) between sympatric populations of *Pteronotus rubiginosus* and *Pteronotus alitonus*, not likely to be related to prey size detection or resource partitioning. Kingston *et al.* (2001) pointed out that acoustic divergence below 10 kHz in sympatric populations is not enough for significant resource partitioning and more likely to be a result of local adaptation and restrictive social interactions leading to selection for non-interference in acoustic signals between populations. Indeed, they suggest that ecological segregation might be achieved by differences in the use of microhabitat. Concerning *P. fulvus* vs. *P. davyi* and *P. psilotis* vs. *P. personatus* in Central America, morphological, molecular and dietary analyses and analyses of fine-scale habitat use of individually captured, tagged and recorded bats will undoubtedly be a valuable aim for future studies focusing on resolution of the distribution dilemma raised here.

Future sampling locations in Central America should include the remnants of the Central American dry forest, an ecoregion listed as globally threatened (Janzen, 1988; Gillespie *et al.*, 2000), and areas of aerobic caves. Special attention should be paid to sampling in Nicaragua and Costa Rica, because they represent the major discrepancy zone of the distribution dilemma. We suggest as priority localities to be sampled the areas of importance for the conservation of bats (AICOMs; https://www.relcomlatinoamerica.net/) Masaya Volcano National Park and Barra Honda National Park, because they house large colonies of syntopic bats of the genus *Pteronotus* in their caves (Leiva, 2012; Girón-Galván, 2020; Medina *et al.*, 2020). Finally, we would like to highlight the importance and the necessity of local bat acoustic libraries to support the acoustic identification of these and other species widely distributed across the Neotropical region.

CONCLUSION

This study examines the acoustic variation of the species groups *P. fulvus* + *P. davyi* and *P. psilotis* + *P. personatus* within their possible contact zones in Central America. For both species groups, our acoustic analysis revealed the existence of three phonic groups without a clear correspondence to the known distribution of the species but with a frequency variation that mirrors geographical variation in body size. The paucity of integrative information, including morphological, molecular and ecological analyses, requires further study, especially in Nicaragua and Costa Rica, the apparent key point of the distribution dilemma. Until then, we recommend reporting the f.mean, f.max and f.min of echolocation calls obtained from free-flying species recordings and keeping them as species complexes owing to the lack of other integrative information.

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DATA AVAILABILITY

The data underlying this article will be shared on reasonable request to the corresponding author.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Acoustic sampling localities of *Pteronotus davyi*, *P. fulvus*, *P. psilotis* and *P. personatus*, with the number of sequences and echolocation calls from each locality.
Table S2. Correlation ratio between the cluster variable and the statistically significant ($P < 0.0001$) quantitative variables. Acoustic dataset for *P. fulvus*/*P. davyi*.
Table S3. Cluster description by the quantitative variables. Acoustic dataset for *P. fulvus*/*P. davyi*.
Table S4. Cluster description by the qualitative variables. Acoustic dataset for *P. fulvus*/*P. davyi*.
Table S5. Correlation ratio between the cluster variable and the statistically significant ($P < 0.0001$) quantitative variables. Acoustic dataset for *P. psilotis*/*P. personatus*.
Table S6. Cluster description by the quantitative variables. Acoustic dataset for *P. psilotis*/*P. personatus*. *SD, standard deviation.
Table S7. Cluster description by the qualitative variables. Acoustic dataset for *P. psilotis*/*P. personatus*.
Figure S1. Principal components analysis biplot of the complex *P. fulvus*/*P. davyi* acoustic datasets, showing the individuals (acoustic samples) coloured by group site (A) and species (B), with a map of species distribution and sampling localities (black dots). Active variables are in light blue: call duration (call_dur), minimum frequency (f.min), maximum frequency (f.max), mean frequency (f.mean) and frequency of maximum energy (f.me). Supplementary variables are in navy blue: duration of the constant frequency (CF) component, slope, frequency change and bandwidth.
Figure S2. Histograms and density plots of main frequency parameters recorded for naked-backed bats (complex *P. fulvus*/*P. davyi*) from Mexico (MX), Honduras (HN), El Salvador (ES), Nicaragua (NIC) and Costa Rica (CR). Dotted lines indicate average frequency values per site.
Figure S3. Principal components analysis biplot of the complex *P. psilotis*/*P. personatus* acoustic datasets, showing the individuals (acoustic samples) coloured by group site (A) and species (B), with a map of species distribution and sampling localities (black dots). Active variables are in light blue: call duration (call_dur),

minimum frequency (f.min), maximum frequency (f.max), mean frequency (f.mean) and frequency of maximum energy (f.me). Supplementary variables are in navy blue: duration of the constant frequency (CF) component, slope and frequency change.

Figure S4. Histograms and density plots of main frequency parameters recorded for lesser mustached bats (complex *P. psilotis*/*P. personatus*) from Honduras (HN), El Salvador (ES), Nicaragua (NIC) and Costa Rica (CR). Dotted lines indicate average frequency values per site.

Figure S5. Map showing the localities of acoustic monitoring of the complex *P. fulvus*/*P. davyi*, with per-locality pie-charts of the proportional number of acoustic samples of the three phonic groups (clusters C1, C2 and C3).

Figure S6. Map showing the localities of acoustic monitoring of the complex *P. psilotis*/*P. personatus*, with per-locality pie-charts of the proportional number of acoustic samples of the three phonic groups (clusters C1, C2 and C3).