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ORIGINAL ARTICLE

Social calls characterisation of the island-restricted Madeiran Pipistrelle (*Pipistrellus maderensis*)

Rym Nouioua[1,*], Nia Toshkova[2], Ricardo Rocha[3], Marlotte Jonker[4]

[1] Department of Botany and Biodiversity Research, Faculty of Life Sciences, University of Vienna; Austria.

[2] Vertebrates Department, National Museum of Natural History at the Bulgarian Academy of Science - Sofia; Bulgaria.

[3] Department of Biology, University of Oxford, OX1 3SZ, Oxford; United Kingdom.

[4] Department of Wildlife ecology and management, University of Freiburg; Germany.

*Corresponding author:
rym.nouioua@univie.ac.at

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ABSTRACT

The natural history of island-restricted bats remains poorly understood, particularly with regard to their social behaviours. Here, we present the first description of the social calls of aggression, distress and isolation in the IUCN endangered Madeira pipistrelle (*Pipistrellus maderensis*, Dobson, 1878). We recorded *P. maderensis* during two nights of mist netting at two different sites on Madeira Island, Portugal. We classified species-specific calls into four main categories of social calls, with variations (A; B; C.i; C.c; D) indicating specific types of behaviour. This information on the social call structures and variability of *P. maderensis* not only enhances our understanding of social communication but also provides valuable insights into its behavioural ecology, helping researchers to assess its habitat use and social dynamics. These findings, together with our recordings, which are made publicly available in an open repository, provide an important basis for further research and conservation efforts aimed at protecting this endemic island species and its unique ecological niche.

RESUMO

A história natural dos morcegos endémicos das ilhas é ainda pouco conhecida, particularmente no que diz respeito aos seus comportamentos sociais. Apresentamos a primeira descrição dos chamamentos sociais de agressão, angústia e isolamento do morcego-da-Madeira (*Pipistrellus maderensis*, Dobson, 1878), espécie ameaçada de extinção segundo a IUCN. Registámos *P. maderensis* durante duas noites de captura com redes de neblina em dois locais diferentes na Ilha da Madeira, Portugal. Classificámos os chamamentos específicos da espécie em quatro categorias principais de chamamentos sociais, com variações (A; B; C.i; C.c; D), que indicam tipos específicos de comportamento. Esta informação sobre as estruturas e variabilidade dos chamamentos sociais de *P. maderensis* não só melhora a nossa compreensão da sua comunicação social, como também fornece informações valiosas sobre a sua ecologia comportamental, ajudando os investigadores a avaliar a utilização do seu habitat e a dinâmica social. Estas descobertas fornecem uma base importante para mais investigação e esforços de conservação destinados a proteger esta espécie endémica insular e o seu nicho ecológico único.

INTRODUCTION

The Madeira Pipistrelle (IUCN Vulnerable) is an insectivorous bat endemic to Macaronesia. Its range extends across the oceanic archipelagos of Madeira, the Canary Islands (Spain) and the Azores (Portugal) (Rocha 2021, Nóbrega et al. 2023), where it preys on a wide range of arthropods, including multiple agriculture and forestry pest species (Gonçalves et al. 2024). It is a small bat with a forearm length of 29.5-34.0 mm (Teixeira & Jesus 2009), and it is an edge-space forager, emitting QCF (quasi constant frequency) or FM-QCF (frequency modulation - quasi constant frequency) echolocation calls in a frequency range of 41-52 kHz with a duration of 2.1-9.5 ms (Ferreira et al. 2022). Island-restricted bats are poorly studied (Conenna et al. 2017), and despite recent advances in the development of computer programs for the analysis and description of European bat calls, the calls of island-restricted bats in the region remain inadequately studied and documented. Proper descriptions of these calls can serve as reliable mechanisms for precise species discrimination in different environmental conditions and habitats, as well as better understanding of the inter/intraspecific variations between island and mainland populations, as this trait is primarily based on the distinct species-specific features embedded in their social vocalisations (Obrist et al. 2004, Jones & Siemers 2011). Although *Pipistrellus maderensis* has a similar echolocation call structure to its close relative *Pipistrellus kuhlii* (Kuhl, 1817), some types of their social calls have not been described yet (Russo et al. 2009).

While echolocation calls are primarily utilised for orientation and inadvertently convey information to others (Knörnschild et al. 2012), social calls have specifically evolved to communicate with other individuals (Jones & Siemers 2011). Despite their significance, research on social calls remains limited compared to studies focused on echolocation (Russo & Jones 2002, Fenton 2003, Jones & Siemers 2011). Presently, social calls are broadly categorized into four main categories, including aggression, distress, isolation, and advertisement calls. The four types of social calls are generally different in their behavioural context (Fenton 2003) and spectro-temporal features (Pfalzer & Kusch 2003). For example, bat songs, which are a subset of advertisement calls, are characterised by complex vocalisations with longer, repetitive intervals and serve multiple functions such as courtship, advertising, and territorial defence (Schöner et al. 2010). However, the study of social calls in bats remains an understudied area, necessitating further research to expand our existing knowledge.

This study set out to describe the spectro-temporal features and the frequencies of social calls of *P. maderensis*, proceeding from the assumption that the parameters of spectro-temporal features and frequency of *P. maderensis* social calls will be similar to social calls emitted by its close relative and better studied *P. kuhlii*. By distinguishing the calls into the four main categories known for social calls, we fill a gap in current knowledge of European bat species.

MATERIAL AND METHODS

Study area and bat mist-netting surveys

Bats were recorded at two sites across Madeira Island. Each site was surveyed with a total of six mist nets (3 with a length of 6m, 2 with a length of 9 m and 2 with a length of 12 m; 3.45 m height and a mesh size of 16x16 mm) for 1 night per site. Bat sampling took place in the summer on 05/06.08.2021 and 22/23.08.2021 (see SM Table 1) using the acoustic bat-detector Echo Meter Touch 2 (Wildlife Acoustics Inc., USA, Android App Version 2.8.22). Species-specific social calls were recorded during handling and manually analysed using Kaleidoscope Pro Software (v. 5.4.2, Wildlife Acoustics Inc., USA). We set mist-nets to 3.45 m height over the stream of Curral das Freiras at 32°44’34.6”N 16°57’58.1’’W (date: 05/06.08.2021) and in the Ecological Park of Funchal at 32°42’18.4”N 16°54’08.8”W (date: 22/23.08.2021).

The nets were set in the evenings just before sunset, from 8:30 pm until 2 am (WET time zone). Captured bats were collected from the nets and placed into a small cotton bag. The procedures were undertaken in accordance with the ethical guidelines set out by UNEP/Eurobats (Hutson et al. 2019) and at the recommendation of the IUCN (Germán et al. 2020). In accordance with established methods (Dietz & Kiefer 2016), we identified the bats’ species and recorded relevant information, including their sex, reproductive

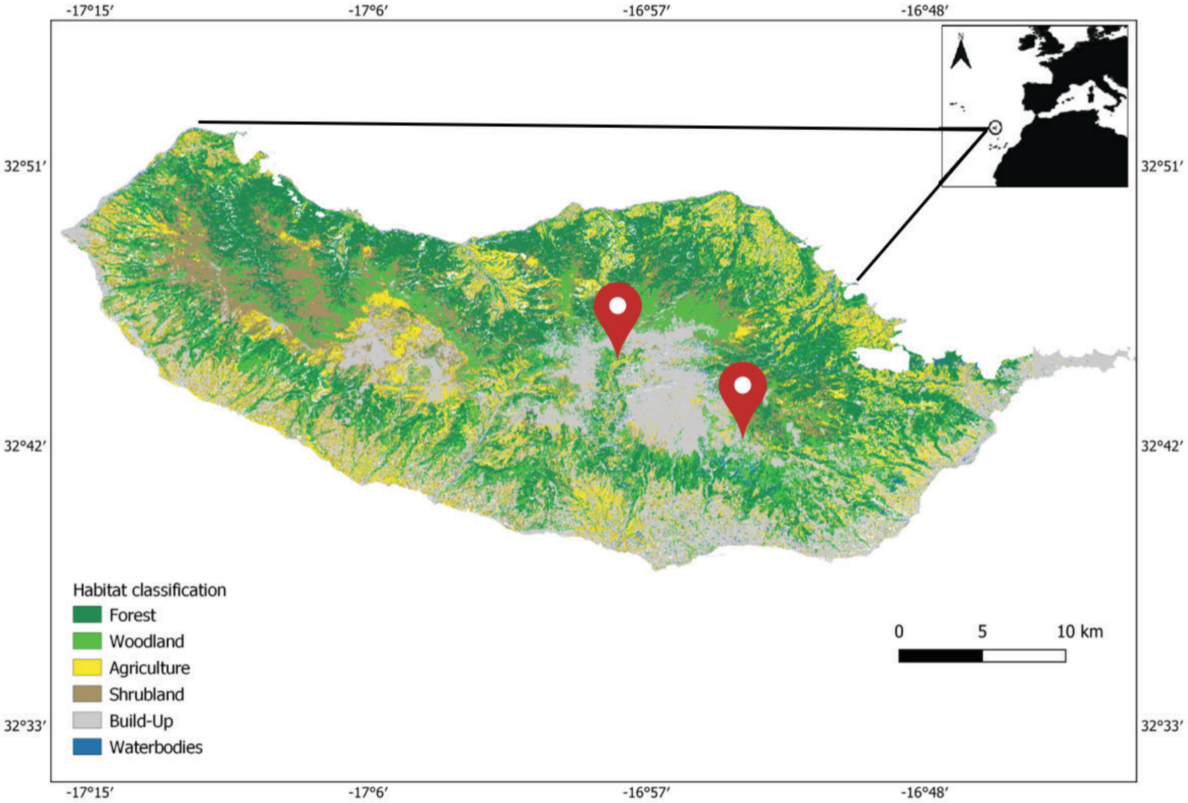


Fig. 1 - Map of Madeira Island classified according to the main habitat types and displaying the two sampling sites for mist netting (marks): Mist-nets over the stream of Curral das Freiras, in Câmara de Lobos (left marking) and in the Ecological Park of Funchal close to a water pond (right marking).

Fig. 2 - Photos showing the field settings: **(A)** Curral das Freiras River, Concelho Câmara de Lobos at 32°44’34.6”N 16°57’58.1’’W (date: 05/06.08.2021); **(B)** Funchal, Ecological Park at 32°42’18.4”N 16°54’08.8”W (date: 22/23.08.2021).

condition, and age (Anthony 1988) (see SM Table 1). During the first survey on the Curral das Freiras river, we managed to capture two juvenile individuals of *P. maderensis*. During our second survey at the Funchal Ecological Park, we captured one adult of *P. maderensis* (see SM Table 1).

The research was conducted under a permit (№ 10/IFCN/2019) granted by the Institute of Forests and Nature Conservation of the Madeiran Autonomous Region.

Acoustic analyses

For the bioacoustic analyses, we considered and measured calls from the two juveniles on the first night and one adult of *P. maderensis* captured on the second night. Recordings were made after capture, during handling, and during the resting period between capture and handling, starting at 9 pm until 2 am (WET time zone), using hand-held detectors (i.e. the EchoMeter Pro detector, Wildlife Acousrics, USA). The Echo Meter Touch 2 recorded the data with a sampling rate of 256 kHz, capturing frequencies up to 128 kHz with a 12-dB gain. The minimum trigger length was set to 5 s, and the maximum trigger length was set to 15 s to ensure that recording sequences fall within the range of 5 to 15 s, totalling in 423 WAV files within the two sampling nights.

The mating calls analysed in this study were recorded during flight observations of *P. maderensis*. These recordings were obtained from free-ranging individuals within the study areas at the same time as the bat nettings took place. The identification of these calls as advertisement calls was based on their similarity to advertisement calls from other *Pipistrellus* species, as described in Pfalzer & Kusch (2003) and Middleton et al. (2014). Species identification was confirmed through a comprehensive assessment of echolocation characteristics. *P. maderensis* is the sole *Pipistellus* species documented on Madeira Island, distinguished by noticeable differences from the other two species present: *Nyctalus leisleri verrucosus* and *Plecotus austriacus*. These differences are supported by echolocation call analyses, which show distinct acoustic signatures for each species. Specifically, *P. maderensis* exhibits unique call characteristics compared to other regional species (Teixeira & Jesus 2009), while its social calls differ significantly from those of its close relative, *P. kuhlii* (Russo et al. 2009).

All bioacoustic analyses were conducted using Kaleidoscope Pro software (v. 5.4.2, Wildlife Acoustics Inc., USA), with manual validation. The recordings were visualized and measured using Kaleidoscope Pro software, presenting them as amplitude oscillograms and colour spectrograms with an FFT size of 256 and a window size of 128.

Our process involved identifying distinct sequences within the recorded WAV files, delineated by time intervals (Tstart and Tend), and defining frequency analysis windows (Fstart and Fend). Social calls were classified by call types based on their signal structure, spectro-temporal features and measurements of various call parameters. The nomenclature of the call structure followed that proposed by Middleton et al. (2014) for social calls of the family of *Vespertilionidae*. The initial classification was based on Pfalzer & Kusch (2003), focusing on call features, while behavioural contexts were assigned according to Middleton et al. (2014). We classified the social calls into five types (A, B, C.i, C.c, and D) based on their spectro-temporal characteristics (Table 1). We used the following eight parameters to describe and analyse social calls namely: Call dur (ms) (Length or duration of individual component or pulse within the sequence), Fmin (kHz) (minimum frequency of the fundamental component or pulse), Fmax (kHz) (maximum frequency of the fundamental component or pulse), Fpeak (kHz) (frequency at which the component or pulse has the highest intensity of energy), Fmean (mean frequency = (2 × Fpeak (kHz) + Fmin (kHz) + Fmax (kHz)) / 4), Inter-pulse interval (ms) (time interval between successive components or pulses), call shape (Frequency modulation change over time of components), call structure (organisation and arrangement of various elements (e.g. repetition of components or number of syllables)). While some terminology was inspired by Middleton et al. (2014), the detailed definitions of these measurements were adapted from standard acoustic analysis practices (e.g. Obrist et al. 2004) and refined based on our own methodological framework. Parameters such as call shape (change in frequency modulation over time) and call structure (organisation and arrangement of elements, such as repetition of components or number of syllables) were adapted using general guidelines from the literature (e.g., Fenton 2003) and tailored to the specific context of our study (see SM Table 2 for definitions of terms and parameters).

Table 1 - Classification of social call types (A, B, C.i, C.c, and D) with considered purpose/behaviour and spectro-temporal features. The categorisation was adapted and further interpreted using the frameworks of Pfalzer & Kusch (2003) and Middleton et al. (2014).

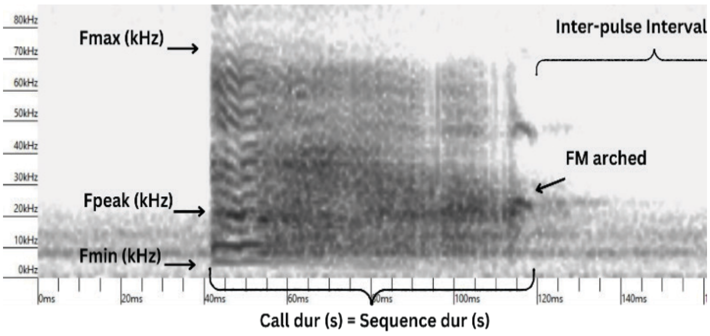
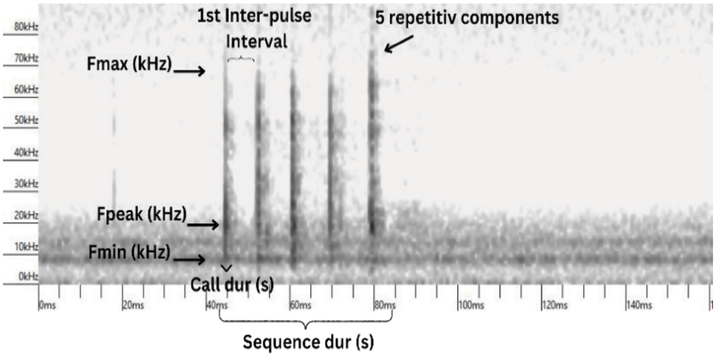
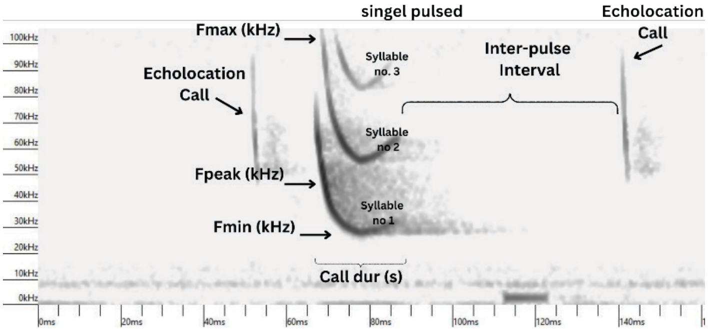
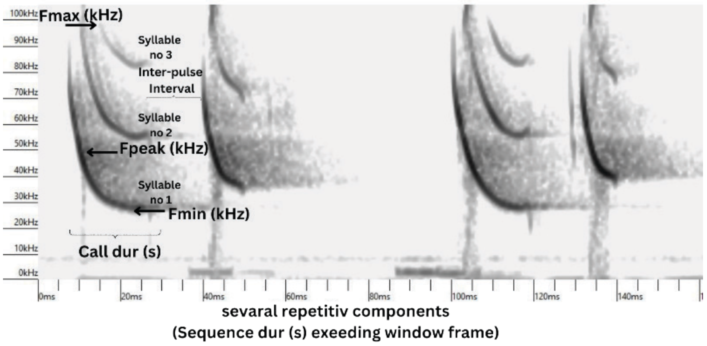
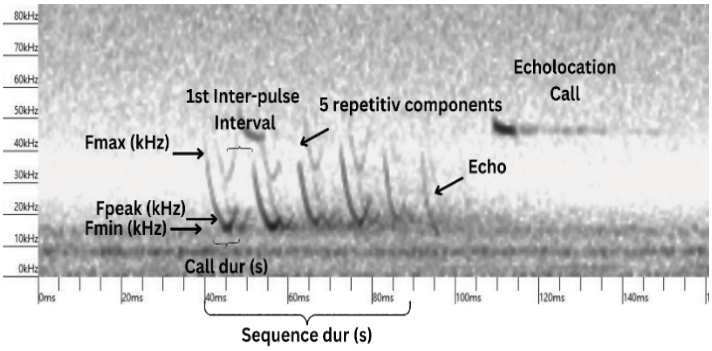
Call Type	Considered Purpose/Behaviour and detection context	Spectro-temporal features and characteristics
Type A	**Aggression / Thread** Is considered to be used aggressively (Pfalzer & Kusch 2003, Middleton et al. 2014) **Detection context (this study)** Only detected from captured juveniles while handled	 • long trill QCF, FM arched at end • multi harmonic / modulated oscillated • long duration
Type B	**Distress** Is considered to be used when caught or restricted, may be used to alert others about danger (Middleton et al. 2014) **Detection context (this study)** Predominantly detected by captured adult while handled, in net and in bag (resting)	 • steep broadband FM • 3-6 repetitive components
Type C.i	**Isolation / location** Is considered to be used in mother/ infant communication (Middleton et al. 2014) **Detection context (this study)** Detected from both captured juveniles and adult in bag (resting)	 • descending FM-CF (2-3 syllables) • single pulsed or occasionally two pulses • wide bandwidth

Table 1 - Continuation: Classification of social call types (A, B, C.i, C.c, and D) with considered purpose/behaviour and spectro-temporal features. The categorisation was adapted and further interpreted using the frameworks of Pfalzer & Kusch (2003) and Middleton et al. (2014).

Call Type	Considered Purpose/Behaviour and detection context	Spectro-temporal features and characteristics
Type C.c	**Co-ordination / cohesion / chase** Is considered to be used for group co-ordination, maintaining cohesion, and during chases (Middleton et al. 2014) **Detection context (this study)** Detected from both captured juveniles and adult while handled, in net and in bag (resting)	 - descending FM-CF (2-3 syllables) - repeated number of pulses (up to 37 pulses measured in one sequence) - wide bandwidth
Type D	**Advertisement** Is considered to be emitted by males as mating behaviour or advertising to females / territorial boundaries and in flight or stationary (Pfalzer & Kusch 2003, Middleton et al. 2014) **Detection context (this study)** Detected from fee ranging bats	 - Shallow narrowband FM (V shaped) - 4-5 components (repetitive) - repeated constantly between echolocation calls

We conducted manual measurements of all call parameters, supplemented where possible by Kaleidoscope Viewer analysis, to ensure accuracy. For all call type parameters were measured per component. For social call types B and D, typically occurring in repetitive components with short inter-pulse intervals, measurements were taken as averages per component and inter-pulse interval per sequence, comprising a group of closely associated components separated by time (Table 1). Initially, social calls were classified based on call features following the framework of Pfalzer & Kusch (2003). Behavioural contexts were then assigned according to the descriptions provided by Middleton et al. (2014). This combined approach allowed us to capture nuanced acoustic variations across different call types and their structural contexts efficiently. The classification of type C calls into subtypes C.i (isolation/location) and C.c (coordination/cohesion/chase) follows the definitions of Middleton et al. (2014). C.i calls are structurally simple, typically consisting of a single broadband descending FM sweep, and are typically used in the context of isolation or mother-infant communication. For this reason, we distinguished C.i calls based on their occurrence as single calls interspersed with echolocation pulses. In contrast, C.c calls are characterised by longer, repetitive sequences without intervening echolocation pulses and are associated with group coordination, cohesion or chase behaviour. Further, for each social call type, we also measured both average values and their corresponding standard deviations to capture variability across the measured parameters. To explore the multivariate structure and patterns within social call types, we performed principal component analysis (PCA) on the measured social call parameters for all social call types represented, using a correlation matrix to account for scale differences between variables. The analysis was performed using the FactoMineR package (Husson et al. 2019). We additionally performed a group-level PCA to analyse patterns specific to each social call type by calculating their PCA scores and summarising their contributions to the total variance along each principal component. This approach allowed us to quantify the variability explained for specific groups (Jolliffe 2002).

RESULTS

From the 423 recorded files, with a maximal length of 15 s, 197 were identified as originating from *P. maderensis*, primarily comprising echolocation call sequences, alongside 17 sequences containing feeding buzzes, which were excluded from this study. Among the remaining files including social calls of *P. maderensis*, 18 were specifically chosen for detailed analysis due to their representation of recording quality and exclusive origin from *P. maderensis* (SM Table 5). We classified the social call patterns into four main call types with one subcategory following the call categories established by Middleton et al. (2014): Types A (aggression and threat), B (distress), and C (isolation, cohesion, and coordination), D (advertisement). Social calls of *P. maderensis* (i.e. call types D) have been described in previous studies and compared with their sister species *P. kuhlii* (Russo et al. 2009). However, to our knowledge, call types A, B, and C have not yet been described. Specifically, we delineate five sequences featuring type A social calls, 12 with type B social calls, 83 with type C social calls, and 5 with type D social calls (Table 2). All four call types showed a variable frequency modulated signal structure, as it is typical for the genus *Pipistrellus*. For each classification and measurement, we provide selected images of example sequences to illustrate them.

The results of the average measurement parameters per social call type reveal distinct acoustic patterns across social call types A, B, C.i, C.c, and D, highlighting their unique characteristics (Table 2). Type A calls, sampled five times, stand out as the longest and most complex among the calls, featuring an average call duration of 71.90 ms and a wide frequency range from 6.9 kHz to 71.3 kHz. They exhibit a distinct long trill quasi constant frequency (QCF) with frequency modulation (FM) arching at the end, incorporating multi-harmonic and modulated oscillated components. In contrast, Type B calls, sampled twelve times, show a much shorter average duration per component of 4.20 ms and a narrower frequency range from 14.0 kHz to 48.0 kHz, characterised by a steep broadband FM pattern and 3-6 repetitive components per selected sequence. Type C calls, with very similar component durations on average (13.86 and 14.13 ms for C.i and C.c, respectively), demonstrate descending FM-Constant Frequency (CF) patterns with 2-3 syllables, differing mainly in pulse repetition and inter-pulse intervals. Lastly, Type D calls, recorded five times, feature a shallow narrowband FM (V-shaped) pattern reaching from Fmin 13.4 kHz up to an average of 18.7 kHz with 4-5 repetitive short components (7.25 ms), showcasing a unique spectral signature distinct from the other call types described.

All Type A social calls were emitted by one of the two captured juveniles, whereas most Type B calls were emitted by adult *P. maderensis* individuals. The longest sequences identified as C.c social calls were emitted by one of the juveniles, lasting over 3.5 s with 37 repetitive pulses. In addition, C.i type calls were emitted by both adult and juvenile individuals, indicating a wider distribution across age groups.

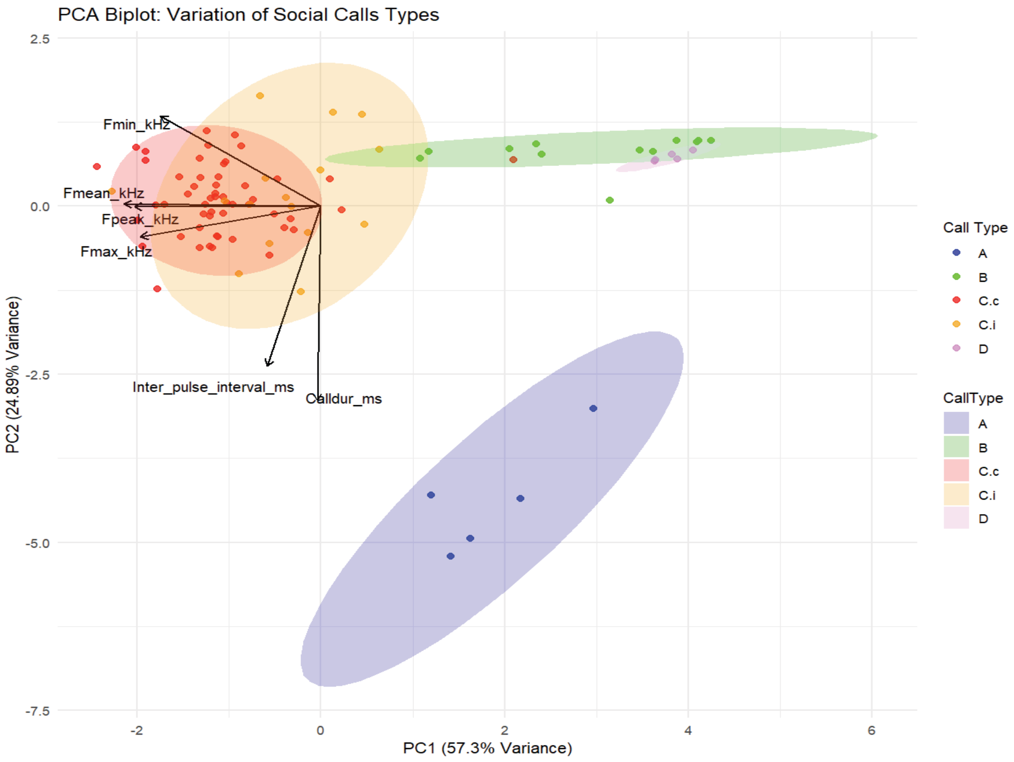


Fig. 3 - Principal component analysis (PCA) of social call characteristics measurements. The plot shows the distribution of social call types along PC1 and PC2 (82.20% of the total variance). The plot displays the distribution of social call types, color-coded by category (A: blue, B: green, C.i: red, C.c: orange, D: violet), in relation to the first two principal components (PC1 and PC2), which together explain the majority of the variance in the dataset (see Table A.3 for details). The arrows indicate the direction and magnitude of influence of the measured parameters (Call duration [ms], Fmin [kHz], Fmax [kHz], Fmean [kHz], Fpeak [kHz], and Inter-pulse interval [ms]) on the PCA axes.

Table 2 - Average and standard deviation of measurement parameters for all types of social call described

SC Call Type	Sample Size	Calc.	Sequence dur (ms)	Call dur (ms)	Fmin (kHz)	Fmax (kHz)	Fmean (kHz)	Fpeak (kHz)	Inter-pulse interval (ms)	Call shape	Components
A	5	Average	78.46	71.90	6.9	71.3	31.8	24.5	161.58	long trill QCF, FM arched at end	multi component / multiple harmonics and modulated oscillations
		SD	24.76	21.86	0.6	9.6	5.1	5.6	108.25		
B	12	Average	204.68	4.20	14.0	48.0	25.4	19.8	8.77	steep broadband FM	3-6 components (repetitive)
		SD	158.99	2.61	3.0	22.6	8.8	7.5	3.67		
C.i.	30	Average	136.88	14.13	27.4	82.9	49.4	43.6	54.90	descending FM-CF (2-3 syllables)	single pulsed or occasionally two pulses
		SD	252.31	9.00	4.0	13.9	6.0	8.9	36.37		
C.c.	53	Average	780.74	16.35	29.8	93.2	54.1	46.7	54.46	descending FM-CF (2-3 syllables)	sequence (lasting up to 3,5 s) comprising up to 37 individual repeated number of pulses
		SD	1361.24	7.11	6.1	9.2	4.8	6.5	34.70		
D	5	Average	43.40	7.25	13.4	29.5	18.7	15.9	9.40	shallow narrowband FM (V shape)	4-5 components (repetitive)
		SD	10.32	1.02	0.7	4.6	1.4	0.7	2.88		

The PCA results show associations between the measured acoustic parameters and the social call types of *Pipistrellus maderensis*. PC1 and PC2 together explained 82.20% of the total variance in the dataset (PC1: 57.31%, PC2: 24.90%). In the group level PCA scores, the variances explained by PC1 and PC2 for each social call type indicate how these components capture the variability specific to each group (SM Table 3). For Type A calls, PC2 explained 64.76% of the variance and was primarily associated with temporal features, including call duration and inter-pulse interval (SM Table 4). These features contribute significantly to the differentiation of Type A calls, which have longer durations and distinct temporal patterns. In contrast, for Type B calls, PC1 accounted for 30.87% of the variance and was mainly driven by spectral features such as mean frequency (Fmean), peak frequency (Fpeak) and maximum frequency (Fmax) (SM Table 4).

The PCA plot (Fig. 3) illustrates the distribution of social call types along PC1 and PC2. While some separation between call types is evident, there is considerable overlap between C.c and C.i calls. This overlap is consistent with their shared structural and functional characteristics, including very similar frequency ranges (Table 2). C.i calls are typically associated with isolation contexts (e.g., single or double pulses), whereas C.c calls are characterised by longer, repetitive sequences associated with group coordination and cohesion. Although the measured parameters were not strongly associated with Type D calls, the PCA results suggest distinct acoustic characteristics for these call types.

DISCUSSION

Our results provide the first detailed description of the social calls of *P. maderensis*, categorised into Types A (aggression and threat), B (distress), and C (isolation, cohesion, and coordination), following the call categories established by Middleton et al. (2014) and aligning with the approach proposed by Pfalzer & Kusch (2003). The analysis presented in this study is based on a focused dataset of 18 recording files of *P. maderensis* social calls. The sequences presented in Table 1 have been carefully selected to provide the most representative samples with high-quality resolution achievable within the field conditions, with the aim of highlighting the characteristics under investigation while acknowledging the inherent challenges of data collection in this area. We opted to make all parameter measurements manually, as only this can reliably measure the complex structure of social calls. Additionally, they are generally not recognised by commonly used analysis programs. The automated classifiers based on reference databases from echolocation recordings falsely identify social calls as distinct species, noise or without Identification (NoID). For example, Springall et al. (2019), in reference to the in-flight social calls recorded in their study, considered automated classifiers with a match ratio of less than 0.6 not to be identified. Despite this identification problem, many researchers nevertheless depend on these classifiers and advanced algorithms to streamline the workflow of bat sound analysis. Indiscriminately deleting files which are flagged as noise by classifiers can thereby lead to the loss of important scientific information.

According to Pfalzer & Kusch (2003), social calls of a resembling type vary not only among species but also within species. In our analysis, we observed that the arched hook at the end found in Type A calls appears to be unique to *P. maderensis* (juveniles) and has not been described as part of Type A calls in other *Pipistrellus* species based on current knowledge. The comparison of type D social calls of *P. maderensis*, in contrast to its morphologically similar relative *P. kuhlii,* has already shown that call structure displays considerable similarity between species even though echolocation calls differ significantly (Russo et al. 2009, Teixeira & Jesus 2009). In addition, Russo et al. (2009) showed that, in Madeira, *P. maderensis* significantly reduced its flight activity when advertisement calls of *P. kuhlii* were played. This phenomenon has also been noted in other members of the same genus (Starik & Göttert 2022) and confirms the assumption that one function of certain social calls is to defend feeding territory.

Furthermore, our observations revealed distinct patterns of call types between adult and juvenile *P. maderensis* individuals. Specifically, all Type A social calls were emitted by one of the two captured juveniles, whereas most Type B calls were predominantly emitted by the captured adult. These differences may indicate differences in social communication strategies within the species and between age groups, as well as nuanced behavioural repertoires. This observation is partially consistent with research on learning vocal production in bats by Knörnschild (2014), who discusses how bats modify their vocalisations through learning processes. In particular, Knörnschild highlights the role of mother-infant interactions in the development of vocal communication skills, emphasising the importance of vocal learning. While our results suggest different patterns of call use between juvenile and adult *P. maderensis*, the small sample size in this study limits the generalisability of these observations, and they may reflect chance or context-specific behaviours. It is also important to note that the distinction between Type A and B calls as age-specific vocalisations in this study should be interpreted with caution. For example, previous studies, including Middleton et al. (2014) and Pfalzer & Kusch (2003), have not reported age-related effects for these call types. Furthermore, Type A calls have been documented in adult bats of several species, including *P. kuhlii* and other *Pipistrellus* species (Huang et al. 2018). This suggests that Type A calls are not exclusive to juveniles, as noted in Table 2. Future studies with larger sample sizes and more diverse capture contexts will be essential to validate these patterns, and to further explore the potential influence of vocal learning processes on social call usage in *P. maderensis*.

Particularly intriguing was the identification of successive pulses of astonishingly long sequences, categorised as repetitive C.c social calls, emitted by one of the juveniles, which lasted over 3.5 s and consisted of 37 repetitive components. This observation hints at the complex social dynamics within distinct age groups of *P. maderensis*, showcasing the variability inherent in their social interactions. This variability in social interactions is in a similar trend to the flexibility observed in the echolocation calls of *P. kuhlii* in different flight situations (Berger-Tal et al. 2007), highlighting the adaptive nature of bat vocalisations to specific behavioural contexts.

The observed directional patterns in the PCA analysis provide further insight into the acoustic characteristics and usage patterns of the social call types of *P. maderensis*. The variation in call intensity and structure that we observed in the C.i and C.c calls of *P. maderensis* may reflect adaptations to optimise energy expenditure during vocalisation. Although we did not measure the energetic cost of these calls, the stronger association of parameters such as Fmean, Fpeak, Fmin and Fmax with Type C.i/C.c calls suggests structural differences that could influence energy requirements. Previous studies (e.g. Jones & Holderied 2007, Currie et al. 2020) have examined energy costs, but in the context of bat echolocation call characteristics and have shown that higher frequency and more intense calls may have greater energy costs, providing a potential framework for interpreting our results. Future studies directly measuring call energy are needed to test this hypothesis. In particular, the study by Currie et al. (2020) showed that there is a limit to the intensity at which bats can call due to exorbitant energy costs above a certain sound pressure level. Although these two call types are very similar in terms of call component and call shape, type C.i calls are often single-pulsed, occasionally double-pulsed, and more often have 2 instead of 3 syllables compared to type C.c, while type C.c calls with long repetitions may also require more energy to produce.

The PCA plot highlights distinct groupings based on measured acoustic characteristics, particularly at the level of the main social call categories (A, B, C (generalised) and D), underlining the importance of these parameters in distinguishing these groups. The overlap between Type C.c and Type C.i calls, as subcategories within the broader understanding of the Type C group, is consistent with their similarities described in Table 2. This overlap highlights their shared structural characteristics, which may limit a clear separation based on measured parameters alone. In particular, the primary distinction between these subcategories lies in the repetitiveness of calls, with Type C.c characterised by long sequences of repetitive components, whereas Type C.i calls are typically shorter and less repetitive.

Further investigation into why Type B and Type D calls do not match the measured acoustic parameters is warranted. A possible explanation for Type D calls is their emission context during bat flight, which may involve lower energy demands compared to other social calls recorded in a netting situation and, therefore, a stressful context for individuals of *P. maderensis*.

From these observations, we can infer the potential role of eco-evolutionary changes in the call structure of insular bat species. This inference raises multiple questions: issues meriting further consideration include whether factors such as competition between species, niche dynamics, or urban development contribute to alterations in call structures, as suggested by recent research conducted by Starik & Göttert (2022).

More samples would be necessary to do the statistical analysis required to confirm this hypothesis, but based on the calls measured, we agree with Russo et al. 2009 that, given the similarity of social calls, the respective sister species *P. kuhlii* can be expected to respond to the social calls of *P. maderensis* and vice versa. There is potential to test this hypothesis further in reference to the social calls A, B and C which have been described above. In addition, it is known that certain social calls are also used to attract bats to new roosts (Schöner et al. 2010). This can affect the research and conservation efforts, especially in contexts where no data on permanent roosts is available (Schöner et al. 2010), which is the case for Madeira Island. As evidenced by studies on other species, such as *Nyctalus* sp. and *Myotis* sp. (Barros et al. 2014, Schmidbauer & Denzinger 2019), these recent findings have the potential to enhance and revise our understanding of various aspects of bat ecology across Europe. This includes identifying potential new locations for mating, swarming, and hibernation, as well as gaining insights into the duration of the mating season, migratory patterns, and overall behaviour of bat populations (Barros et al. 2014, Schmidbauer & Denzinger 2019).

The development of a larger database of social calls *for P. maderensis* and other island-restricted species is necessary. The aim would be to include *P. maderensis* in the comparative databases of European bats and thus obtain statistical evaluations. While Middleton et al. (2014) have released limited examples of bat social calls online, a well-established, comprehensive and publicly accessible library of European bat social calls remains uncharted.

Therefore, the acoustic recordings used in this study have been archived on ChiroVox (https://www.chirovox.org) under the UIDs A006112 to A006129. Furthermore, they are accessible on Phaidra (https://phaidra.univie.ac.at), linked to the DOI associated with this publication. However, this gap in available resources underscores the importance of initiatives aimed at building comprehensive databases of bat vocalizations, including social calls, to support research and conservation efforts. Such databases would facilitate comparative studies across species and regions and contribute to a deeper understanding of bat communication and behaviour. For example, recent advances in sensory and cognitive ecology, as highlighted by Page & Ter Hofstede (2021), emphasise the need for these databases to encompass not only vocalisations but also the cognitive and sensory aspects of bat communication. We advocate for collaborative efforts within the scientific community to establish and maintain open-access repositories of bat social calls, which would be invaluable for advancing our knowledge of bat ecology and evolution.

CONSERVATION CONSIDERATIONS

Recording and analysis of bat echolocation data to assess their activity is already a fundamental component of conservation research. The ability to interpret social calls accurately is beneficial to several research areas, but especially for conservation science (Russo et al. 2009). A proper understanding of social calls enables better interpretation of bioacoustic data, which can assist in the assessment of bat behaviour and the development of appropriate solutions to mitigate anthropogenic threats.

Additionally, our findings hold potential significance for future studies involving mist-netting assessments. Understanding social calls, including those related to aggression, distress, and isolation, could inform the development and refinement of lures used to attract bats during mist-netting. This information might be particularly crucial for researchers refining mist-netting protocols and effective lure designs, as insights into social calls enable the development of more targeted and less intrusive capture methods, minimizing harm to bat populations and promoting ethical research practices.

A 1992 study by Kalko & Schnitzler (1993) examining the variation of echolocation signals with habitat structure provides valuable insights into how bats adapt their echolocation behaviour in different environments. Their research has already shown significant variation in signal structure between European *Pipistrellus* species, suggesting adaptive responses to environmental conditions. By further understanding how bats adapt not only their echolocation signals in different habitats, we can improve our interpretations of bat behaviours and movement patterns, even in situations where direct observation may be limited.

The study of social calls should be integrated into conservation research because, in addition to the activity measurements (via echolocation data) that they provide researchers, they allow us to assess movement, foraging and social behaviour, for example, by identifying hotspots and swarming areas, or recording mother-infant interactions. Because echolocation allows us to track the social activity of insectivorous bats, we can infer specific movements, such as those towards foraging areas or roosts, from social calls, even when flight orientation within the habitat is not directly observable. By studying how calls adapt to environmental pressures such as food scarcity and competition, we have been able to trace how the species has evolved over time. Consequently, observations of the social call structure of *P. maderensis* in island ecosystems with limited food resources may also help researchers trace the evolutionary development of this species (Russo et al. 2009). In addition, differences in social call characteristics between bat populations may indicate genetic divergence, revealing patterns of population differentiation and contributing to our understanding of a species' evolutionary history.

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