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Decoding the overlap: diagnostic acoustics to distinguish the endangered Magnificent Broodfrog, *Pseudophryne covacevichae* Ingram & Corben, 1994, from the common Montane Toadlet, *Uperoleia altissima* Davies et al., 1993 in northern Queensland, Australia

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Abstract. Accurate species identification from passive acoustic monitoring data is critical for threatened species, particularly when vocal overlap with sympatric species risks misidentification. The Magnificent Broodfrog (*Pseudophryne covacevichae*), a narrowly endemic species of the Australian Wet Tropics listed as Endangered (IUCN) and Vulnerable (Australia EPBC), co-occurs and reproduces alongside the common Montane Toadlet (*Uperoleia altissima*), and the two species produce advertisement calls that overlap in structure and frequency. This study provides a diagnostic reference to reduce the potential risk of misidentification during acoustic analysis, particularly by those working in poorly documented areas between the Magnificent Broodfrog's two main populations (Atherton Tablelands and Paluma Range). The advertisement and threat calls of the Magnificent Broodfrog are described in detail and compared with the advertisement call of the Montane Toadlet. Principal Component Analysis showed distinct clustering by species, and an analysis using the Multi-Response Permutation Procedure confirmed call duration contributes most strongly to species discrimination. The Magnificent Broodfrog's advertisement call was longer, lower in frequency, and had more variation in pulse number than the Montane Toadlet's short, repetitive call. These findings provide an applied framework to support accurate identification of the Magnificent Broodfrog from recordings and enhance the reliability of acoustic assessments for this threatened species.

Keywords. Advertisement call, desktop identification, myobatrachid, passive acoustic monitoring, spectrogram, threat call, vocalisation profile.

Introduction

Anuran vocalisations are crucial for a variety of ecological and behavioural functions and can assist in assessing taxonomic and phylogenetic information (Hoskin et al., 2011; Donnellan et al., 2023; Parkin et al., 2024). Most typically performed by males, vocalisations serve as the primary means of conveying information of an individual's reproductive state, maintaining and defending territories, and assisting in species recognition (Wells, 2019). For conservation, vocalisations provide a non-invasive and efficient method for monitoring frog populations, particularly for elusive or range-restricted species that are otherwise difficult to detect (Cutajar et al., 2022). Passive acoustic monitoring is a method that involves the use of in-situ recording devices (passive acoustic monitors, PAMs) to continuously capture sounds over extended periods This approach enables researchers to collect large datasets on species presence, activity patterns, and population dynamics without the need for direct human observation (Rayment et al., 2011; Xie et al., 2015; Hagens et al., 2018; Brodie et al., 2021; Pérez-Granados and Schuchmann, 2021; Schwarzkopf et al., 2023). PAMs have transformed wildlife conservation by providing a non-invasive, cost-effective method for monitoring auditory species (Blumstein et al., 2011).

In the context of frog conservation, PAMs are particularly valuable for detecting the reproductive calls produced by breeding males, termed 'advertisement calls'. The use and benefit of PAMs in anuran research is extensive and has allowed for species diversity and richness to be measured (Dorcas et al., 2009; Anunciação et al., 2022), species distributions estimated (Campos-Cerqueira and Aide, 2016), and patterns in breeding phenology established (Bolitho et al., 2023; Measey et al., 2017; Willacy et al., 2015).

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This can provide important knowledge for tracking changes in anuran biodiversity, allowing for the implementation of targeted conservation actions. While PAMs have enhanced our ability to monitor wildlife populations, their effectiveness hinges on accurate identification of the target species. Misidentification of threatened species can lead to conservation management errors, including the misallocation of resources, ineffective management strategies, and the overlooking of occupied habitat (Solow et al., 2012; Webster et al., 2023). This can result in inaccurate conclusions about species distribution, population dynamics and habitat requirements, compromising the effectiveness of conservation plans (e.g., Hunt, 2015; Webster et al., 2022).

Many Australian frogs do not have vocalisation profiles available to assist in their identification in desktop analyses of acoustic recordings. This knowledge gap is concerning given the country's high frog diversity, and it can hinder efforts to identify and monitor species, particularly those that are rare or have localised distributions (Guerra et al., 2018; Gibb et al., 2019). Expanding our understanding of the vocal profiles of Australian frogs is essential for accurate species identification (Donnellan et al., 2023; Elliott-Tate and Rowley, 2024; Parkin et al., 2024) and will facilitate the effective use of data derived from continent-wide PAM initiatives.

Here, the vocalisation profile of the threatened Magnificent Broodfrog, *Pseudophryne covacevichae* from northern Queensland is established by providing a description of the advertisement and threat calls, which are the most frequently emitted vocalisations. In addition, the advertisement call of the common Montane Toadlet, *Uperoleia altissima*, is also described. Although these two species are easily distinguished visually, their vocalisations share acoustic similarities when viewed on spectrograms, including having two prominent peaks and an overlapping bandwidth (i.e., the range of frequencies within a call). There is a risk of misidentification for those unfamiliar with the species, particularly when partial calls are recorded, vocalisations are obscured by background noise, or there is poor signal attenuation due to vegetation or recording distance (Ross et al., 2023; Winiarska et al., 2024). To establish the call characteristics that are best used to differentiate the two species on recordings, a comparison is performed on select vocalisation parameters. The evaluation aims to enhance the accuracy of distinguishing the two species during desktop analysis, thereby improving monitoring and conservation outcomes for the Magnificent Broodfrog.

Materials and Methods

Study species. The Magnificent Broodfrog is undergoing a rapid rise in population monitoring due to proposed habitat alteration across areas of its known and potential distribution (Global Energy Monitor, 2024). The species is listed as Endangered on the *IUCN Red List* (IUCN SSC Amphibian Specialist Group, 2022) and as Vulnerable under both Australia's federal legislation (*Environment Protection and Biodiversity Conservation Act 1999*) and under Queensland state legislation (*Nature Conservation Act 1992*). Magnificent Broodfrog distribution is fragmented and restricted to wet sclerophyll forests in high-elevation mountain ranges on the western edge of the Australian Wet Tropics. A larger population exists in the southern Atherton Tablelands and a smaller population occurs approximately 160 km to the southeast in the Paluma Range (McDonald, 2002; Zozaya and Hoskin, 2015). However, new populations are still being discovered between these two major populations (e.g., Windlab, 2023; E. Rush, unpublished data).

These frogs reside within grassy tussocks or underneath leaf litter along narrow ephemeral drainage lines on first and second order streams, where they occur in low densities and can be difficult to detect if surveys are not timed correctly (Rush et al., 2025). Consequently, acoustic recorders present an appropriate and reliable sampling technique for the species. Vocalisations of the Magnificent Broodfrog exhibit similarities to those of better-studied members of the genus *Pseudophryne* (Pengilley, 1971; Byrne, 2008) but its vocalisation profile has not been described beyond the less-frequently heard courtship call (Groffen et al., 2024).

The Montane Toadlet is a small, non-threatened myobatrachid, similar in size to the Magnificent Broodfrog at 3 cm body length (Anstis, 2017). They have a larger, more continuous distribution in northern Queensland occurring from Laura in Cape York and south to the Paluma Range (Zozaya and Hoskin, 2015; Cutajar et al., 2022). The species shares habitat with the Magnificent Broodfrog and uses temporary ponds and creeks for breeding. The two species call in proximity to each other, but they often remain physically separated at a breeding site, with the Magnificent Broodfrog calling inconspicuously, directly at the edge of breeding habitat (e.g., drainage line, seep), and the Montane Toadlet typically vocalising in more exposed surrounding habitat (pers. obs.).

Study area. Magnificent Broodfrog vocalisations were recorded between 2013 and 2024 at six locations in the Wet Tropics bioregion of northern Queensland (Fig. 1).

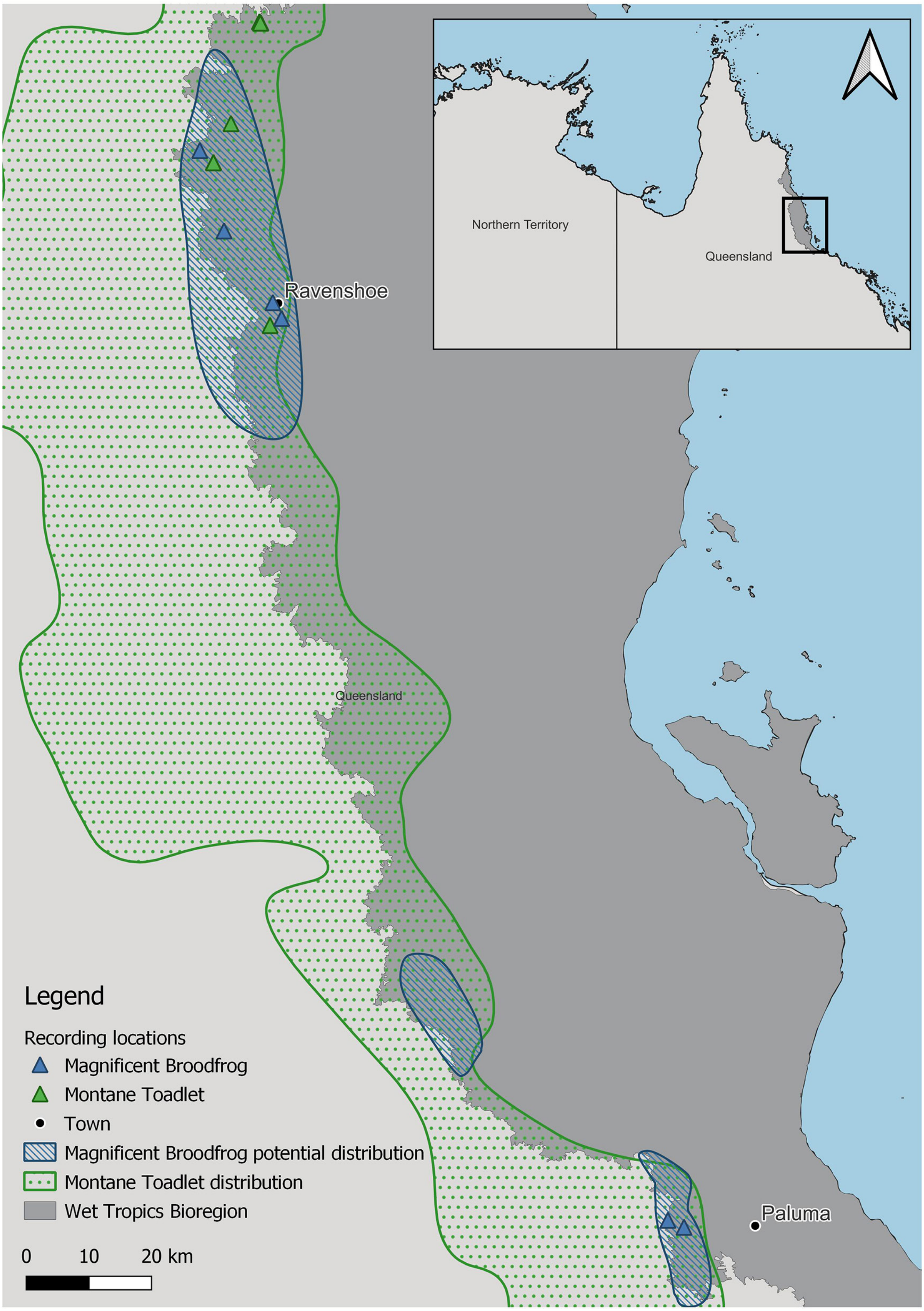

Figure 1. Recording locations of the Magnificent Broodfrog (*Pseudophryne covacevichae*) and Montane Toadlet (*Uperoleia altissima*), overlaid with current distributions of both species in the study area. Due to the scale, two sites overlap for the Montane Toadlet. The Magnificent Broodfrog distribution is estimated based on the latest records. The Montane Toadlet distribution shapefile was sourced from Cutajar et al (2022).

Recordings were made using either a handheld Sennheiser ME66 shotgun microphone (Sennheiser, Wedemark, Germany) coupled with a Zoom H4n Pro (Zoom, Tokyo, Japan) or an Edirol R09 recorder (Roland, Hamamatsu, Japan), or using an in-situ PAM with either BAR-LT (Frontier Labs, Brisbane, Australia) or song meter SM4 recorders (Wildlife Acoustics, Maynard, USA). PAMs were positioned 1.8 m high on a tree at the edge of a breeding area. The recordings obtained from PAMs were filtered manually and those with a strong signal were utilised. In total, 11 recordings were used from the six locations. All audio recordings were saved in WAV format.

All but one of the Montane Toadlet vocalisation recordings were provided by FrogID™, a citizen science project led by the Australian Museum, where participants use smartphones to record and upload frog calls for validation by experts (Rowley et al., 2019). The other recorded vocalisation was collected using the Sennheiser microphone with the Edirol recorder. The recordings were made between 2013 and 2023, and 11 recordings were used from five sites (Fig. 1). Multiple recordings were made at two of the sites (2–3 records per site), however, the calls were recorded 25–60 m apart. Recordings made with the FrogID app are saved as MPEG AAC audio files. These were converted to WAV format using FreeConvert software (FreeConvert, 2024) on Google Chrome.

In instances where multiple recordings were obtained from a single site, recordings were chosen from different nights to reduce the likelihood that it was the same frog calling. For each recording, 3–10 consecutive calls from a single individual were selected (Aland and Hoskin, 2013; Weaver et al., 2020). Typically, the first sequence of calls in the recording were utilised unless the quality was poor or the signal was obscured (e.g., invertebrate noise or multiple frogs calling), in which case the sequence of calls was selected from a section of the recording with less disturbance. Where a recording had multiple individuals calling, the individual frog closest to the microphone was selected for measurements (Weaver et al., 2020).

Terminology. Definitions of vocalisations and analyses by Köhler et al. (2017) were adopted, and a note-centred approach was used for the descriptive terminology. This approach defines a "coherent unit of sound" as the 'call', which may have subunits ('notes') separated by periods of silence ('internote intervals'). For both the Magnificent Broodfrog and Montane Toadlet, each call was therefore defined as a single note. Characteristics of both the advertisement and threat call of the Magnificent Broodfrog are described and analysed. For the Montane Toadlet only the advertisement call is described and analysed, which is then used to compare to the characteristics of the Magnificent Broodfrog advertisement call.

Data analysis. All audio recordings were imported into Raven Pro sound software v1.6.5 (Cornell Lab of Ornithology, ravensoundsoftware.com) for processing and analysis. Each recording had the spectrogram window size set to 512 with 50% overlap and were band-filtered to reduce background noise. To visually represent the frequency and temporal structure of the calls, representative audio files were converted into spectrograms using R statistics software v4.2.2 (R Core Team, 2023) using the *seewave* package (Sueur et al., 2008). The call structure was described qualitatively, noting descriptive pattern (shape and structure), note arrangement (sequence and organisation) and tonal features (inflections) as well as quantitatively through parameter measurements.

The following call traits were measured from spectrograms: duration from the beginning of a note to the end of the note (s); dominant frequency, at which the note is of greatest intensity (Hz); internote interval (s); and number of pulses per note. These call characteristics are commonly used in anuran vocalisation analyses (Hoskin et al., 2011; Köhler et al., 2017; Weaver et al., 2020). Descriptive statistics (mean, standard deviation, range) were calculated based on the individual note measurements in R. For comparison purposes, box plots were produced for call duration, dominant frequency, and internote interval for advertisement calls using the *ggplot2* package (Wickham, 2011).

Principal Component Analysis (PCA) was employed to explore patterns of variation and potential clustering of species based on their advertisement vocalisation parameters. The PCA used call duration, peak frequency, and internote interval, with data scaled to ensure comparability. The principal components were examined and a biplot of the highest contributing components was produced to illustrate the distribution of the parameters by species, with ellipses representing the 95% confidence interval for each group.

We then tested for differences in these vocalisation parameters using a Multi-Response Permutation Procedure (MRPP). MRPP evaluates whether there is a significant difference between groups by comparing within-group similarity against what would be expected by chance (Cai, 2006). For each parameter, data were scaled, and a Euclidean distance matrix was computed. 10,000 permutations were used to determine the significance of the MRPP test statistic (δ), which measures within-group homogeneity. A lower observed δ compared to the expected δ suggests that observations within the same group (i.e., species) are more similar to each other than would be expected by chance. Additionally, the chance-corrected within-group agreement statistic (A) was used to assess effect size. An A-value near 1 indicates the groups are highly distinct, and an A-value close to 0 suggest minimal difference beyond random expectation.

Results

In total 87 advertisement calls from 11 individual Magnificent Broodfrogs and 21 threat calls from four individuals were analysed. For the Montane Toadlet 110 advertisement calls from 11 individuals were analysed.

The Magnificent Broodfrog emits a series of distinct notes, including a powerful but brief 'creek-k', recognised as the advertisement call (Fig. 2A). The 'creek-k' is made of two syllables, the first syllable contains 2–5 pulses separated by a silent interval, which is followed by a second syllable that is longer and pulsatile (i.e., fast alternating amplitude modulation without intermittent silence and no clearly countable peaks; Köhler et al., 2017). The advertisement call had a mean call duration of 0.13 ± 0.02 s, with a mean peak frequency of 2372 ± 269 Hz and an internote interval averaging 6.38 ± 3.80 s, with variation in the duration of the interval (0.40–27.72) (Table 1). The number of pulses per note were not able to be measured for every call due to differences in distances from the recording device, which made clear visualisation on a spectrogram difficult. From the 47 observations able to be visualised, the mean pulse number of the first syllable was 3.19 ± 0.88.

In addition to the advertisement call, the Magnificent Broodfrog emitted a harsher vocalisation within a call series that was distinguished from the advertisement call as a single syllable, tonal note, devoid of pulsatile elements (Fig. 2A). This was termed a threat call, and it had a longer mean duration (0.18 ± 0.04 s) and higher mean peak frequency (2579 ± 178 Hz) than the advertisement call (Table 1). This threat call was singular or paired and occurred on average 0.39 ± 0.22 s after an advertisement call, or if paired 0.32 ± 0.07 s after the initial threat call.

The advertisement vocalisation of the Montane Toadlet is characterised by brief, rapidly repeated 'crick' notes. This note always had two distinct pulses separated by a brief silent interval (Fig. 2B). Compared to the Magnificent Broodfrog, the advertisement call of the Montane Toadlet exhibited a shorter call duration, with a mean of 0.04 ± 0.01 s, higher mean peak frequency of 2775 ± 206 Hz and shorter mean internote interval (0.77 ± 0.27 s), which showed more regularity in interval duration (0.43–1.58 s; Table 1).

Table 1. Summary of call parameters of Magnificent Broodfrog (*Pseudophryne covacevichae*) and Montane Toadlet (*Uperoleia altissima*). Values are displayed as mean ± standard deviation with the range in parentheses and n denoting the number of vocalisations analysed. Interval A represents the interval between the advertisement call and first threat call, Interval T represents the interval between the first threat call and the second threat call, if present.

Species	Call Type	Call Duration (s)	Peak Frequency (Hz)	Pulses	Internote Interval (s)
Magnificent Broodfrog	Advertisement	0.13 ± 0.02 (0.09–0.21) *n* = 86	2372 ± 269 (1723–2719) *n* = 86	3.19 ± 0.88 (2–5) *n* = 47	6.38 ± 3.80 (0.40–27.72) *n* = 83
Magnificent Broodfrog	Threat	0.18 ± 0.04 (0.11–0.25) *n* = 21	2579 ± 178 (2063–2813) *n* = 21	None	Interval A 0.39 ± 0.23 (0.09–0.80) *n* = 15 Interval T 0.32 ± 0.07 (0.16–0.37) *n* = 6
Montane Toadlet	Advertisement	0.04 ± 0.01 (0.03–0.06) *n* = 110	2775 ± 206 (2153–3101) *n* = 110	2.00 (2–2) *n* = 110	0.77 ± 0.27 (0.43–1.58) *n* = 99

Box plots suggested there was minimal overlap in vocal parameters between the two species, particularly for call duration which showed complete separation (Fig. 3). This was confirmed by the PCA biplot, which demonstrated distinct clustering for each species (Fig. 4). The first principal component (PC1) explained 74% of the variance, while the second principal component (PC2) accounted for 19%, together capturing 93% of the total variance.

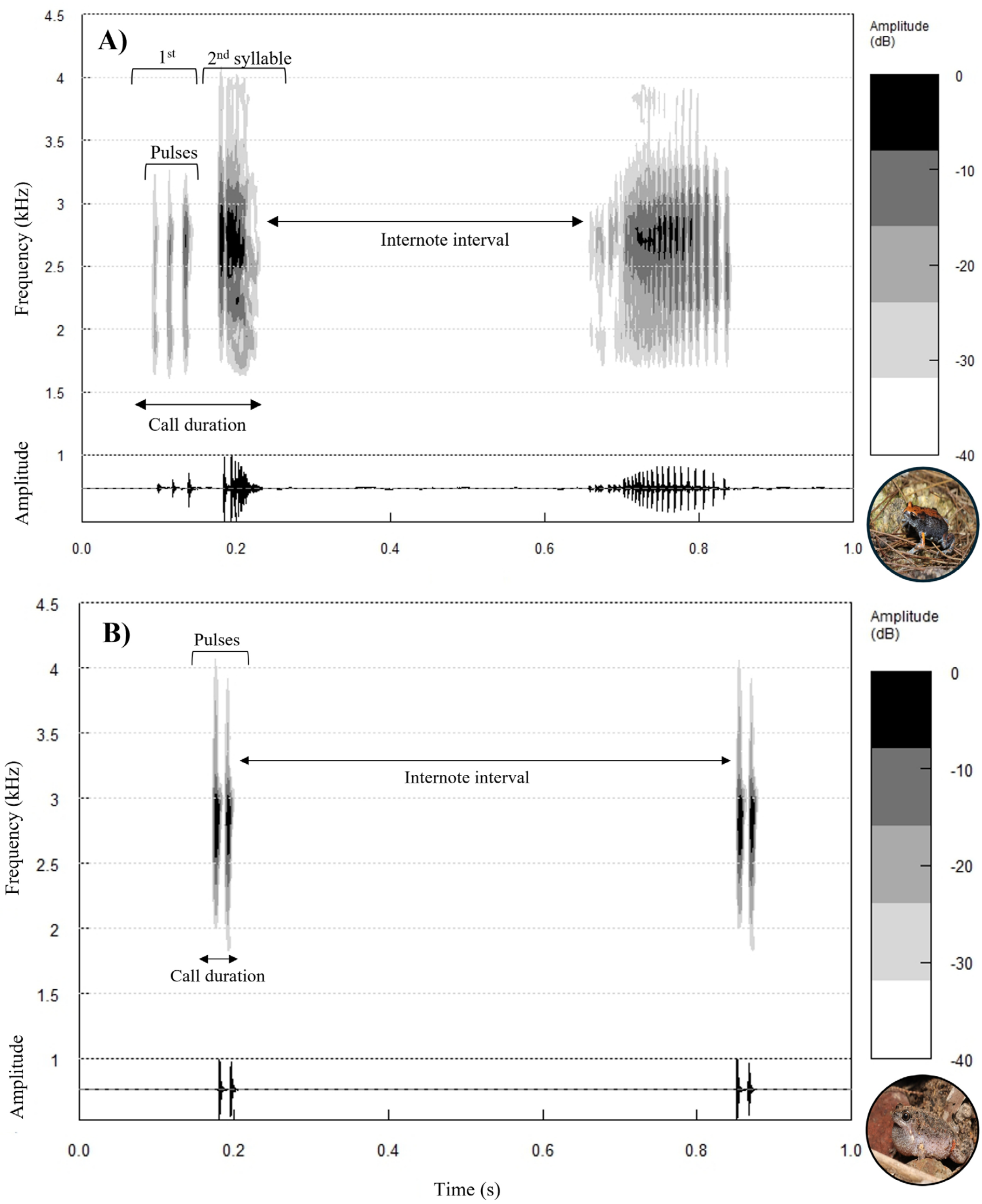

Figure 2. Spectrogram and oscillation of a single representative male Magnificent Broodfrog (*Pseudophryne covacevichae*) and Montane Toadlet (*Uperoleia altissima*) vocalisation, including the measured call parameters. Peak frequency is not shown but was determined based on highest amplitude within a call. (A) shows two Magnificent Broodfrog vocalisations, the first depicts the two-syllable advertisement call and the second is a threat call. (B) shows two advertisement calls of the Montane Toadlet.

Call duration and internote interval were the main contributors to PC1, while peak frequency contributed most to PC2.

The MRPP demonstrated differences between the two species across all vocal parameters. The strongest separation was evident in call duration, which showed a high *A*-value (0.701, $p < 0.0001$) and a much lower observed than expected δ (0.326 vs. 1.089, respectively). This indicates strong separation between species in call duration. Similarly, internote interval also exhibited a strong difference ($A = 0.503$, $p < 0.0001$), with observed $\delta = 0.471$ (expected $\delta = 0.949$) indicating that this parameter can also distinguish the species' calls, though not as markedly as call duration. Peak frequency showed moderate differentiation between species ($A = 0.267$, $p < 0.0001$) with an observed $\delta = 0.807$ (expected $\delta = 1.102$).

Discussion

Establishing reliable acoustic resources is essential for accurate species identification, particularly in monitoring threatened Anura where vocalisations serve as a key diagnostic trait. Here, a detailed description of the advertisement and threat call of the Magnificent Broodfrog are presented and its vocalisation profile is established when considered alongside the previously described courtship call (Groffen et al., 2024). Additionally, the advertisement call of the Montane Toadlet is described, and the most reliable acoustic markers to assist with desktop analysis of PAM recordings are identified. While the two species are taxonomically distinct, confusion may arise during acoustic analysis, particularly in regions between the two main populations of the Magnificent Broodfrog, where survey efforts are increasing in response to development pressure (Global Energy Monitor, 2024). This work provides reference material to support consultants and practitioners who may be less familiar with the species, helping to reduce the risk of misidentification and improve accuracy in distribution assessments.

The vocalisations of the Magnificent Broodfrog are characterised as a 'complex call' (see Köhler et al., 2017) encompassing distinct call types within a sequence, which aligns with the description of vocalisations in other *Pseudophryne* species (Littlejohn and Martin, 1969; Pengilley, 1971; Byrne, 2008).

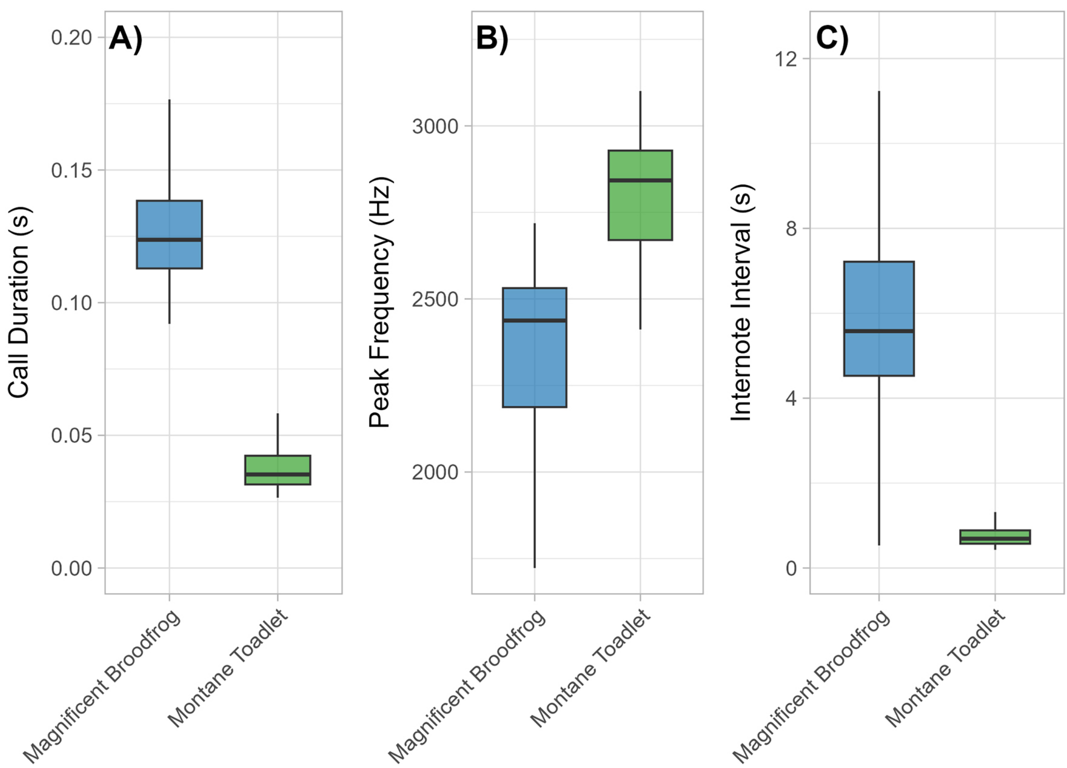

Figure 3. Boxplots comparing key advertisement call characteristics of the Magnificent Broodfrog (*Pseudophryne covacevichae*) and Montane Toadlet (*Uperoleia altissima*). (A) shows call duration, (B) peak frequency, and (C) the internote interval.

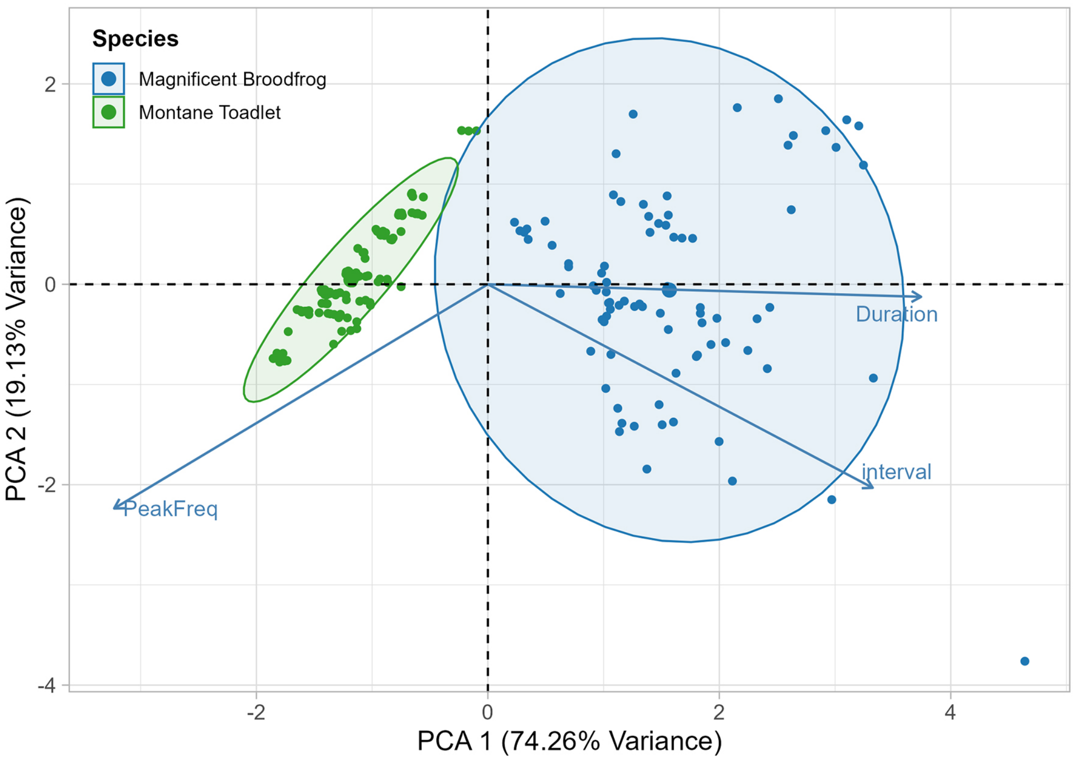

Figure 4. Principal Component Analysis (PCA) of the three compared call characteristics of the Magnificent Broodfrog (*Pseudophryne covacevichae*) and Montane Toadlet *(Uperoleia altissima)* advertisement calls. Ellipses show 95% confidence intervals.

The most encountered call was the short, two-syllable advertisement call. This melodic 'creek-k' was emitted with irregularity, which may have been a factor of environmental conditions or male excitement at the time of recording (Byrne, 2008; Terry, 2022). The first syllable of the advertisement call varied between 2–5 pulses, followed by a brief silent interlude before the pulsatile second syllable. The variation in pulse number is consistent with the advertisement call of the southern congeneric Bibron's Toadlet, *Pseudophryne bibronii* Günther, 1858. Byrne (2008) suggested this was a response to the presence of conspecifics, where more investment is put into an individual call when there is an increased opportunity for mating or competition.

In contrast, the Montane Toadlet had a 'simple call', which was characterised by a regularly repeated, brief 'crick' note, defined by two pulses. Confusion between the two species advertisement calls may arise due to them both having two distinct 'peaks', as well as their shared bandwidth. These peaks were defined as being two syllables for the Magnificent Broodfrog and two pulses for the Montane Toadlet. This was based on syllables typically being of longer duration, compared to pulses which are a short burst of sound energy (Pengilley, 1971; Köhler et al., 2017). The pulses in the Magnificent Broodfrog recordings obtained on PAM were obscured due to recording distance, making them an unreliable feature in identification. In these instances, the advertisement call appeared as two peaks, similar to the Montane Toadlet. Though the species' calls fall within a similar bandwidth, the Magnificent Broodfrog had a lower average peak frequency when compared to the Montane Toadlet. The complex structure and variable internote interval of the Magnificent Broodfrog create a noticeably different acoustic profile to the consistent advertisement calls of the Montane Toadlet, providing some visual markers for desktop identification.

Threat or aggressive calls are used to by anurans to establish dominance, maintain and defend territories, or deter rivals during competitive interactions (Fellers, 1979; Wells, 2019; Silva et al., 2025). The Magnificent Broodfrog could rapidly interchange its advertisement call with the longer tonal, threat call.

Whereas the advertisement call could be issued singularly or in a sequence, the threat call was never issued on its own. In the Southern Corroboree Frog (*Pseudophryne corroborree* Moore, 1953), Dendy's Toadlet (*P. dendyi* Lucas, 1892), and Bibron's Toadlet, both a 'short' and 'long' threat call have been documented (Pengilley, 1971). No distinct differences were observed in the recordings of this study, but it is possible that Magnificent Broodfrogs also have variations in their threat calls. No other call types were identified for the Montane Toadlet, though presumably the species have additional vocalisations not documented here (e.g., Robertson, 1984, 1986).

The Montane Toadlet displayed a restricted range in the PCA, indicating higher consistency in its vocalisations. In contrast, the Magnificent Broodfrog exhibited variability within its vocalisation parameters. The strongest separation was evident in call duration and internote interval, where the Montane Toadlet had a markedly lower δ value compared to the Magnificent Broodfrog, suggesting reduced within-group dissimilarity. In contrast, a moderate degree of separation for peak frequency highlights that it is a less distinctive trait for distinguishing between the two species. This result demonstrated that distinctions in vocal traits, particularly call duration and internote interval, can provide a reliable method of delineating between the two species, reducing the risk of misidentification in areas of sympatry.

The intraspecific variation identified in the Magnificent Broodfrog advertisement calls, evident in the PCA, is common in anurans and can be attributed to a combination of physiological, environmental and social factors (Röhr et al., 2020; Weaver et al., 2020; Itakura et al., 2023; Elliott-Tate and Rowley, 2024). For example, body size and the presence of competing males can influence call frequencies and amplitude (Byrne, 2008; Wang et al., 2012), and temperature and precipitation can influence call duration and repetition rates (Mitchell, 2001; Byrne, 2008). The variation observed in the Magnificent Broodfrog may be natural among the individuals sampled, or alternatively it may reflect the quality of recordings and methodology.

Six of the 11 Magnificent Broodfrog calls were collected from in-situ PAMs, which were positioned approximately 1.8 m high, to protect the equipment from fire. In contrast, the Montane Toadlet calls were all recorded on mobile phone devices, likely held in close proximity to the calling frog. Though Magnificent Broodfrog calls were carefully selected, the distance of the calling individual to the recorder was unknown. Greater distances can reduce clarity and the signal-to-noise ratio, as acoustic energy attenuates over distance and high-frequency components are disproportionately absorbed (Yip et al., 2017; Zhang et al., 2024). This attenuation, combined with environmental noise, can diminish the resolution of vocal parameters, potentially affecting analysis of fine-scale features (Brumm and Slabbekoorn, 2005; Metcalf et al., 2023). Though recording limitations may influence the resolution of vocal parameters they do not hinder the detection of meaningful variation observed in the results. Those applying these findings may observe reduced variability, particularly in peak frequency, when using handheld recording equipment or close-range recordings, as these methods minimise environmental interference and improve signal quality.

This study is the first detailed description of the Magnificent Broodfrog's vocalisations, establishing call parameters that will inform future acoustic and ecological studies of this threatened, little-known species. In addition, key acoustic differences from the sympatric Montane Toadlet are defined, with call duration and internote interval emerging as the most reliable markers for species differentiation in overlapping regions. By establishing these differences and providing qualitative descriptions of the two species calls, this study provides an accessible method for desktop identification. This is particularly vital in areas where conservation interventions may hinge on the accurate identification of Magnificent Broodfrog populations.

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