

Evidence-based practice

Bioacoustics reveals the uniqueness of an ex-situ tree hyrax population

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Abstract

Tree hyraxes *Dendrohyrax* spp. are nocturnal, morphologically cryptic and consequently highly understudied mammals. Their taxonomy has always been puzzling, thus their species-level diversity is probably underestimated. Prominent and species-specific songs and loud calls emitted by tree hyraxes are one of the key cues in recognising their diversity. In 2020, a population of tree hyraxes with a specific vocal repertoire not fully matching the vocal repertoire of any known tree hyrax species was discovered in Taita Hills in Kenya. This population might represent a taxon unknown to science. In 2015, calls of a captive group of tree hyraxes that originated from Tanzania and had previously been identified as the southern tree hyrax *D. arboreus* were recorded in Ostrava Zoo, Czech Republic. Here, calls recorded from this captive group are compared to those previously recorded from other known tree hyrax taxa. The vocal repertoire of this captive population mostly matches the vocal repertoire of the population occurring in Taita Hills. No loud calls nor even the components typically emitted by the southern tree hyrax were recorded from this captive population. Therefore, these captive ambassadors of rarely kept tree hyraxes may have been misidentified as the southern tree hyrax, and may actually be conspecific with the population currently known from Taita Hills in Kenya. Species identification of this tree hyrax population should be a subject of further examination and the captive animals may bring important insights. These findings highlight the importance of mutual cooperation among zoos, academic institutions and museum collections.

Introduction

Hyraxes Hyracoidea that naturally occur in Africa and the Middle East are traditionally classified in three genera, which are believed to be strikingly distinct in their ecology and behaviour (Shoshani et al. 2013a). Rock and bush hyraxes *Procavia* and *Heterohyrax* are diurnal or crepuscular social animals that occupy rocky outcrops, while arboreal tree hyraxes *Dendrohyrax* are believed to be rather nocturnal and solitary (Djossa et al. 2012; Kundaali 1976; Shoshani et al. 2013b; but see Milner and Harris 1999; Rosti et al. 2023a). Within the order, the species to which a hyrax belongs is difficult to discern through morphology, therefore there is some difference of

opinion across various authors as to the total number of species recognised. In particular, the nocturnal tree hyraxes represent a highly understudied mammalian group whose species-level diversity might be underestimated (Bloomer 2009; Hoeck 2017; Roberts et al. 2013; Rosti et al. 2020). Three species have been generally accepted until recently. The western tree hyrax *Dendrohyrax dorsalis* is distributed in the rainforests stretching from Sierra Leone to Uganda. The southern tree hyrax *D. arboreus* occurs in forested areas of eastern and south-central Africa. The eastern tree hyrax *D. validus* is patchily distributed in forests of eastern Tanzania, some adjacent islands and southern Kenya (Shoshani et al. 2013a). A fourth species, the Benin tree hyrax *D. interfluvialis* that occurs in forests stretching between the Niger and Volta rivers in western Africa, was

formally described only in 2021, although it has been known for a relatively long time that populations from this area emit quite distinctive loud calls (Oates et al. 2021).

Unlike their morphological appearance, calls of tree hyraxes that can be heard over long distances are highly species-specific as the acoustic structure of these calls is relatively steady within species but remarkably different between species. As such calls, they represent a promising tool for taxonomic delineation and species identification (Oates et al. 2021; Roberts 1999, 2001; Rosti et al. 2020). The loud call of the western tree hyrax is termed a 'klaxon' and consists of a series of relatively uniform, regularly repeated units resembling screams or cries. The series typically starts quietly and gradually becomes louder as the call progresses (Oates et al. 2021). The loud call of the southern tree hyrax includes more unit types and typically starts with a series of rattles which gradually turn into a series of screams (Milner and Gaylard 2013; Roberts 1999). The call of the Benin tree hyrax starts with rattles that are interspersed with short squawks and grades into a series of rapid barking sequences (Oates et al. 2021). The vocal repertoire of the eastern tree hyrax seems to be more complex (but see Milner and Harris 1999 describing the vocal repertoire of the southern tree hyrax) as it includes several different call types previously termed 'knock', 'snort', 'hac', 'strangled thwack' and 'wheeze', however, some of these probably represent synonyms used by different authors (Roberts 2001; Rosti et al. 2023a). Some of these calls can be combined into prolonged series of calls or more complex vocalisations (Rosti et al. 2023b).

Recent research conducted in Taita Hills in South Kenya, focusing on nocturnal arboreal mammals, has shown that local tree hyraxes *Dendrohyrax* sp. (Figure 1A) also possess a rich vocal repertoire including three call types corresponding to 'strangled thwack', 'hac' and 'wheeze' which can form more complex songs (Rosti et al. 2020, 2023b). Therefore, the vocal repertoire of tree hyraxes occurring in Taita Hills fits that found in the eastern tree hyrax to some extent. However, tree hyraxes from Taita Hills differ in how frequently they use certain call types as well as in the detailed acoustic structure of these calls. Moreover, tree hyraxes from Taita Hills combine 'wheezes' into more complex songs than

have been found in eastern tree hyraxes to date (Rosti et al. 2020, 2023b). This indicates that the population of tree hyraxes from Taita Hills may represent a taxon previously unknown to science (Rosti et al. 2020).

Diurnal rock and bush hyraxes are frequently exhibited in zoos as unique representatives of African fauna or as unexpected relatives of elephants (Veselovský 1977). On the other hand, tree hyraxes are extremely rare in zoos as they are probably less attractive to visitors and are challenging to keep and breed, partly due to their browser diet and shyness. According to the Species360 Zoological Information Management System (Species360 2022), there were no tree hyraxes in zoos worldwide at the beginning of this century. From 1960 to 1992, five zoos (Chicago, San Diego, Seattle and Washington, US; and Twycross, UK) kept them, however, without pronounced breeding success. Five individuals (two males and three females) from Togo were imported in 2016 to Ostrava Zoo, Czech Republic. Based on their morphology and origin they were identified as the western tree hyrax. Another nine individuals (four males and five females) were imported in 2009 and 2010 from Tanzania to three Czech zoos (Ostrava, Plzeň and Prague). Based on their morphology and origin they were determined as the southern tree hyrax. Nevertheless, due to taxonomic issues accompanying tree hyraxes they are referred to as a captive Tanzanian population in this study (Figure 1B).

To increase the breeding success and expand the knowledge on both species kept, the Ostrava Zoo has continuously collected data on their body mass, diet composition and veterinary reports. Calls of five individuals from the captive Tanzanian population were recorded in 2015. However, these calls had not been further analysed because the calling individuals and contexts were unknown; this discouraged any comparisons with already known tree hyrax calls. Therefore, the aim of the present study was: (1) to examine these calls in light of the most recent findings on vocal repertoires of tree hyraxes (Rosti et al. 2020, 2023a) and (2) based on the vocal repertoire, to verify whether the captive Tanzanian population indeed belongs to the southern tree hyrax or could be conspecific with the population of tree hyraxes from Taita Hills in South Kenya.

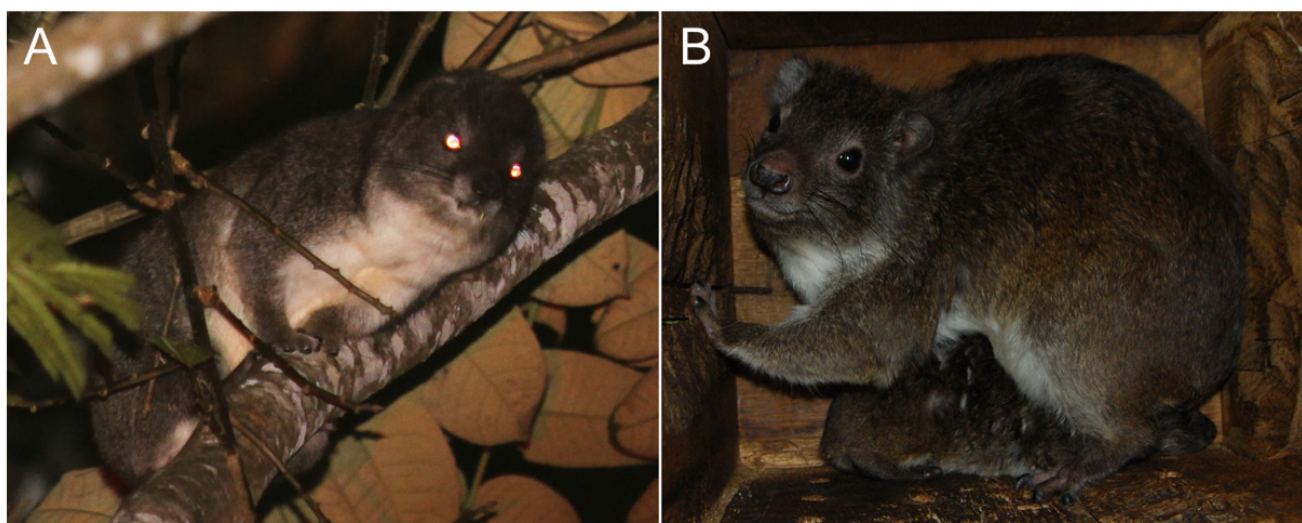


Figure 1. A) A tree hyrax *Dendrohyrax* sp. from a population occurring in Taita Hills, Kenya. Photo: Hanna Rosti; B) An adult tree hyrax and juveniles from a captive population that originated in Tanzania and is currently kept by several Czech zoos. Photo: Jan Pluháček

Materials and methods

Recording of captive Tanzanian tree hyrax population

The tree hyraxes that provided calls for this study were five individuals from the captive Tanzanian population which were housed in an off-show area located at Ostrava Zoo, Czech Republic. The tree hyraxes were housed in two separate enclosures (2 m × 4 m × 2.5 m) within one room. One enclosure was occupied by a wild-caught male (local ID 207806) and female (ID 207808) of non-determined age. The other enclosure was occupied by a wild-caught male of a non-determined age (ID 207810) and his two almost two-year-old offspring, a male (ID 209173) and female (ID 209172). The mean temperature in the room was kept at approximately 24°C as recorded by internal sensors built into the recorder used in this study (see below). There was natural lighting in the room; at the time of the recording, sunrise in Ostrava was at approximately 0720 and sunset at approximately 1640 (Thorsen 2015). Each enclosure was equipped with several branches and wooden shelters and the animals were fed once a day with a mixture of herb leaves, vegetables, pellets for leaf-eating monkeys and a sufficient amount of variable browse (e.g. willow, beech, oak, birch and raspberry).

The tree hyraxes shared the room with two rodent species, northern and southern Luzon giant cloud rats *Phloeomys pallidus* and *P. cumingi*, housed in separate enclosures. According to local keepers, these species do not vocalise extensively. Thus, it was considered unlikely that their calls would be confused with those recorded from tree hyraxes. The tree hyraxes were recorded from 30 January to 7 February 2015 with an automated Song Meter SM2+ recorder (Wildlife Acoustics, Concord, Massachusetts, USA). The recorder was placed in the room outside the animals' enclosures and set to record at a sampling rate of 44.1 kHz and 16-bit resolution with a time schedule from 1930 to 2130 and from 0200 to 0500. This time schedule was chosen based on the authors' previous experience of when the highest calling rate might be expected and in order to save the memory card and battery capacity at the same time (but see Rosti et al. 2023a).

Acoustic analysis of recordings of captive Tanzanian tree hyrax population

In total, 49.5 hours of recordings were collected from the captive Tanzanian population at the Ostrava Zoo, Czech Republic. All single calls, defined as a single, uninterrupted trace on the spectrogram, and all multiple syllable calls, defined as several uniform or variable

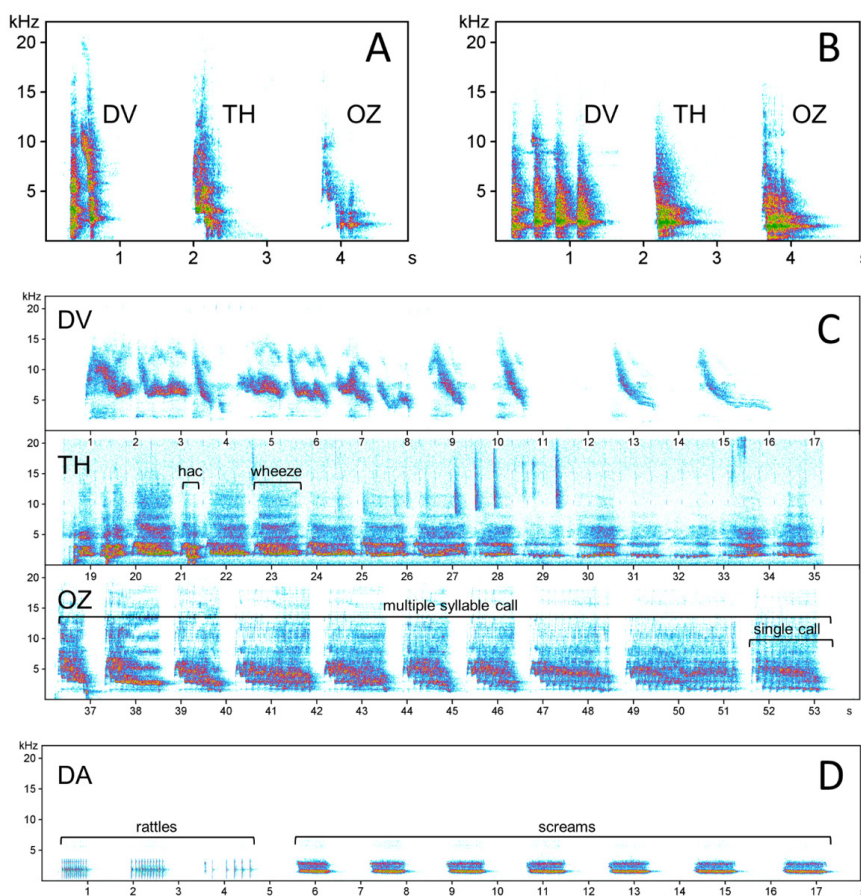


Figure 2. Spectrograms of single calls and multiple syllable calls recorded from four different tree hyrax populations, the eastern tree hyrax *Dendrohyrax validus* (DV), a population of *Dendrohyrax* sp. occurring in Taita Hills, Kenya (TH), a captive Tanzanian population from Ostrava Zoo (OZ) and the southern tree hyrax *D. arboreus* (DA). A) 'Strangled thwack', B) 'hac', C) song illustrating the definition of single call examples, 'hace' and 'wheeze', and a multiple syllable call and D) loud call with its indicated components, rattles and screams. All recordings were standardised in Avisoft SASLab Pro 5.2 software to sampling frequency 44.1 and spectrograms were created by using the following settings: FlatTop window, FFT length 1024 points, frame size 100% and overlap 75%.

calls produced in series with intervals between the calls shorter than 10 seconds (Figure 2C), were manually labelled in the main window of Avisoft SASLab Pro 5.2 software (Avisoft Bioacoustics, Berlin, Germany). Labelled calls were further classified based on their acoustic similarity with calls of tree hyraxes described previously (Roberts 2001; Rosti et al. 2020).

A total of 75 single calls (25 per call type) were randomly selected from obtained recordings to measure four acoustic parameters in Raven Pro 1.5 software (Cornell University, Ithaca, New York, USA). These acoustic parameters were identical to four acoustic parameters measured for calls emitted by tree hyraxes from Taita Hills (Rosti et al. 2020) and included the fundamental frequency (FF), highest frequency (HF), maximum frequency (MF), i.e. the frequency with maximum amplitude, and duration (DC) of the calls. These measurements were conducted with the use of spectrograms with the following settings: FFT size 512, 50% overlap, Hann 86.1 Hz, sample rate 44.1 kHz, 16-bit signed.

Comparison with other tree hyrax taxa

Calls produced by the captive Tanzanian population were further compared to calls of all known tree hyrax taxa. For the purpose of this comparison, songs and loud calls were considered to be the most comparable vocalisations within the vocal repertoires of all tree hyraxes. These vocalisations share the common trait that they can be heard over long distances and show the highest acoustic complexity in all the taxa. Therefore, they were used for comparison in the present study but it should be noted that the vocal repertoires of tree hyraxes are still unexplored, thus, homology of certain calls remains questionable (Odom et al. 2021). Likewise, although it is obvious that ‘wheeze’ is an important component of songs, the exact composition of these complex vocalisations in tree hyraxes should be a subject of further study. Three songs recorded from the captive Tanzanian population, four songs from the population from Taita Hills recorded for the purpose of another study (Rosti et al. 2020) and one song from the eastern tree hyrax also recorded for the purpose of another study (Rosti et al. 2023b) were selected. Additionally, three loud calls from the southern tree hyrax, one loud call from the western

tree hyrax and one loud call from the Benin tree hyrax collected and deposited by the Nocturnal Primate Research Group at Oxford Brookes University, United Kingdom (Table 1) were used. These loud calls were recorded either in the wild (southern and Benin tree hyraxes) or in Ostrava Zoo (western tree hyrax). They had relatively long durations, therefore, a shorter fragment of each call was used, which fully represented the typical acoustic structure (e.g. included all the typical components—rattles, screams and squawks).

Avisoft SASLab Pro 5.2 software was used to convert the sampling frequency of all recordings to 32 kHz and to edit them to achieve a suitable quality of spectrograms that could be further used for cross-correlation. Their volume was standardised using the ‘Change volume’ editing tool and the background noise was removed using the ‘Noise reduction’ filter tool provided by the software. If there were any apparent remnants of loud noise in the recordings not emitted by hyraxes, e.g. noise generated by insects or bats, spectrograms were created and the noise was manually removed using the ‘Remove erased spectrogram sections from waveform’ tool. These spectrograms were created using the following settings: Hamming window, FFT length 1024 points, frame size 75% and overlap 50%.

The R 4.2.2 software (R Core Team 2021) and the packages warbleR and ape were used to calculate the pairwise similarity of all spectrograms by means of time-frequency cross-correlation. Additionally, a phylogenetic tree based on hierarchical cluster analysis using a set of dissimilarities for these spectrograms and complete-linkage metrics was generated (Araya-Salas and Smith-Vidaurre 2017).

Results

Acoustic analysis of recordings of captive Tanzanian tree hyrax population

Tree hyraxes from Ostrava Zoo produced on average 11.7 single calls or multiple syllable calls per hour and 274 single calls and 304 multiple syllable calls were found and labelled in total. Based on their acoustic structure and auditory quality, single calls could

Table 1. The list of songs and loud calls recorded from all known tree hyrax taxa and used in this study for comparison with songs recorded from a captive Tanzanian tree hyrax population living in Ostrava Zoo, Czechia. Songs of the eastern tree hyrax *Dendrohyrax validus* and a population from Taita Hills, Kenya *Dendrohyrax* sp., were recorded for the purpose of another studies, while loud calls of the western *D. dorsalis*, southern *D. arboreus* and Benin *D. interfluvialis* tree hyraxes were collected and deposited by the Nocturnal Primate Research Group at Oxford Brookes University, UK. These loud calls were recorded either in the wild or in captivity.

Taxon		Locality	Recordist or study	Record label
Southern tree hyrax	<i>D. arboreus</i>	Loita Hills, Kenya	Y. de Jong and T. Butynski	D. arboreus Loita Hills
		Mt. Kenya, Kenya	Y. de Jong and T. Butynski	D. arboreus Mt. Kenya
			Y. de Jong and T. Butynski	D. arboreus Nairobi
Western tree hyrax	<i>D. dorsalis</i>	Ostrava Zoo, Czech Republic	H. Rosti	D. dorsalis Ostrava zoo
Benin tree hyrax	<i>D. interfluvialis</i>	Okomu, Nigeria	S. Bearder	D. interfluvialis Okomu
Eastern tree hyrax	<i>D. validus</i>	Vipingo, Kenya	Rosti et al. (2023)	D. validus Vipingo
Taita Hills population	<i>Dendrohyrax</i> sp.	Mbololo forest, Taita Hills, Kenya	Rosti et al. (2020)	Dendrohyrax sp. Mbololo 1
				Dendrohyrax sp. Mbololo 2
		Ngangao forest, Taita Hills, Kenya	Rosti et al. (2020)	Dendrohyrax sp. Ngangao 1
				Dendrohyrax sp. Ngangao 2
captive Tanzanian population		Ostrava Zoo, Czech Republic	present study	Ostrava zoo 1
				Ostrava zoo 2
				Ostrava zoo 3

be classified into three distinct types strongly corresponding to those previously reported by Rosti et al. (2020, 2023a) for the population of tree hyraxes from Taita Hills in South Kenya and for the eastern tree hyrax from coastal Kenya (Figure 2). The most frequently uttered call type was the ‘strangled thwack’ (Figure 2A) produced either singly or in multiple syllable calls as the only call type or combined with the ‘hac’ (Figure 3). Calls corresponding with or being highly similar to ‘hac’ (Figure 2B) were also produced as single calls or multiple syllable calls as the only call type or combined with the ‘strangled thwack’ or ‘wheeze’. The third call type was the ‘wheeze’ (Figure 2C) which was produced as a single call. However, it was mostly combined with ‘hac’ and as such formed an essential part of more complex vocal expressions highly similar to the song previously recorded from tree hyraxes from Taita Hills (Figure 2C, Rosti et al. 2020). None of the calls or multiple syllable calls corresponded to a typical loud call or units forming this call, rattle or scream, typically produced by the southern tree hyrax (Figure 2D). Table 2 provides means and standard deviations of four acoustic parameters measured from the three call types uttered by the captive Tanzanian population.

Comparison with other tree hyrax taxa

The phylogenetic tree based on dissimilarities of spectrograms of 13 songs and loud calls produced by all known tree hyrax taxa as well as the captive Tanzanian tree hyrax population comprises two main clusters. Figure 4 shows in detail which loud calls and songs form the particular clusters.

Discussion

The results demonstrate that the tree hyraxes living in Ostrava Zoo that have been previously determined as the southern tree hyrax do not emit complex loud calls, nor any of the components, rattles and screams that are typical for this species (Milner and Gaylard 2013). Moreover, they emit several call types whose acoustic structure and auditory quality rather correspond to calls previously reported for the eastern tree hyrax (Rosti et al. 2023b), or even more likely, a population of tree hyraxes occurring in moist montane forests in Taita Hills in South Kenya (Rosti et al. 2020). Although there are some differences in values of measured acoustic parameters between the captive and wild population (see Rosti et al. 2020 and the present study), these differences are probably within the intra-species variability found in calls of many mammalian taxa. For example, geography (Lameira et al. 2010), individuality (Koren and Geffen 2011), sex-related variability (Matrosova et al. 2011) and disparity in in-situ and ex-situ recording conditions are potential sources of intra-species variation in vocalisations (Matrosova et al. 2010). The tree hyraxes from the captive Tanzanian population as well as those

occurring in Taita Hills most frequently utter a call type termed ‘strangled thwack’. Striking similarities in vocal repertoires of the captive Tanzanian population and the population occurring in Taita Hills indicate that these populations might be conspecific and they might represent a taxon unknown to science. Species identification of these tree hyraxes should be a subject of further examination. Additionally, more recordings and data are needed to investigate vocal repertoires and variability of calls and songs with more robust statistical analyses.

Zoos have established cooperative breeding programmes to maintain viable ex-situ populations in order to fulfil their conservation mission (Tribe and Booth 2003). Identification of a group of individuals that represents a meaningful taxonomic unit (i.e. species or subspecies) is an essential base to manage these programmes. The present study demonstrates that phenotypically cryptic nocturnal mammals still pose a challenge when it comes to taxonomic delineation and species recognition. Moreover, many of these taxa are still undergoing significant taxonomic changes (e.g. slow lorises (Munds et al. 2013), galagos (Svensson et al. 2017), mouse lemurs (Schüßler et al. 2020), sugar gliders (Cremona et al. 2021) and tree hyraxes (Oates et al. 2021)). Molecular genetics is an effective tool to elucidate taxonomic identification, however, utilising molecular data is still uncommon in the management of ex-situ populations (Jensen et al. 2020; Norman et al. 2019; Russello and Amato 2007). Bioacoustics represents another powerful tool for species recognition when morphology provides only limited conclusions (Pozzi et al. 2019). Its application and perspectives in zoos have been reviewed by Volodina and Volodin (1999). However, this approach is even more disregarded than molecular genetics within zoo and aquaria communities. Although bioacoustics requires some equipment and expertise, recording and analysing animals’ calls can be relatively cheap, quick and overall a relatively available method for collection management and improving husbandry of ex-situ populations (e.g. Schneiderová and Vodička 2021). Therefore, the authors encourage zoo managers and the community to consider this approach in appropriate situations, and to collect recordings of specimens and populations that may represent problematic taxa.

The recent description of the vocal repertoire of an apparently unique population of tree hyraxes occurring in South Kenya (Rosti et al. 2020) has quickly led to a discovery that several individuals probably conspecific with this population are currently being kept by several Czech zoos. This was due to the fact that one of the zoos has been more intensely studying these animals and is involved in various related research projects, including those focused on bioacoustics and nocturnal mammals (e.g. Schneiderová et al. 2016). This has opened the doors for mutually beneficial cooperation; the captive tree hyraxes allow close observations as

Table 2. Descriptive statistics of four acoustic parameters, fundamental frequency (FF), highest frequency (HF), maximum frequency (MF) and duration (DC), measured from three call types ‘strangled thwack’, ‘hac’ and ‘wheeze’, recorded from a captive tree hyrax group from Ostrava Zoo, Czechia that represents a population that originated in Tanzania.

Acoustic parameter	Strangled thwack (mean ± SD)	Hac (mean ± SD)	Wheeze (mean ± SD)
FF (Hz)	229.8±82.7	190.8±43.6	1438.7±727.2
HF (Hz)	18295.8±1676.6	17184.0 ±1304.1	5508.2±1777.7
MF (Hz)	2167.1±1453.6	1378.1±279.1	4303.2±1413.4
DC (s)	1.1±0.1	0.9±0.2	1.5±0.4

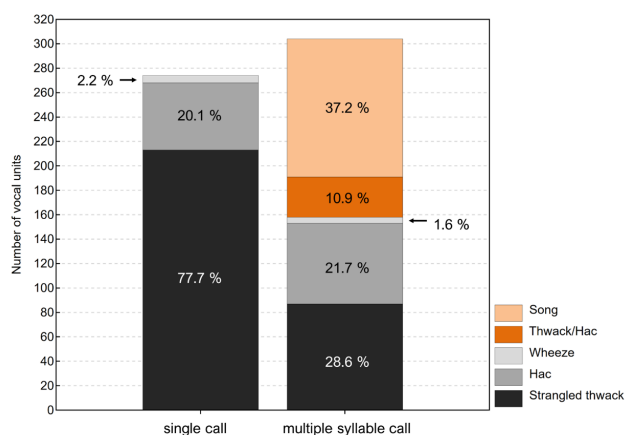


Figure 3. Bar plot showing the number of single calls or multiple syllable calls found in recordings of a captive Tanzanian population living in Ostrava Zoo, Czechia. Vocal repertoire basically consisted of three call types, ‘strangled thwack’, ‘hac’ and ‘wheeze’ that could be combined into multiple syllable calls containing uniform (‘strangled thwack’, ‘hac’ or ‘wheeze’) or variable call types (‘thwack/hac’ combination or song). Songs in captive animals were predominantly formed by ‘wheezees’ combined with ‘hac’-like call types.

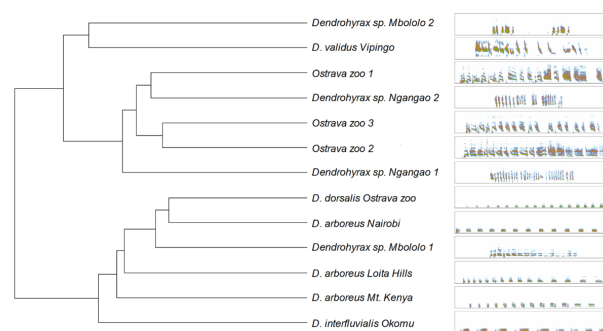


Figure 4. A phylogenetic tree based on dissimilarities of spectrograms of 13 songs and loud calls produced by all known tree hyrax taxa as well as captive Tanzanian population living in Ostrava Zoo, Czechia. One cluster is exclusively formed by spectrograms of songs produced by the eastern tree hyrax, population of tree hyraxes from Taita Hills, Kenya and the captive Tanzanian population from Ostrava Zoo, Czechia. The second cluster is predominantly formed by spectrograms of loud calls recorded from the western, southern and Benin tree hyraxes, but also one song recorded from a population of tree hyraxes from Taita Hills, Kenya. More details on the recordings used to create the spectrograms and the tree can be found in Table 1.

well as data and sample collections that are hard to conduct in the wild. On the other hand, knowledge on ecology and behaviour acquired from wild populations (e.g. Rosti et al. 2022) can help to improve husbandry protocols and increase ex-situ breeding success. Therefore, the present study also adds to the growing body of evidence highlighting and underlining the importance of zoo research and mutual benefits of collaboration among zoos and aquaria, academic institutions and museum collections (Fernandez and Timberlake 2008; Lawson et al. 2008; Poo et al. 2022; Ryder and Feistner 1995).

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