



# Evidence of individual discrimination in the maned wolf long-distance extended-bark



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## ABSTRACT

The acoustic channel is an efficient long-distance signalling system that may be especially effective for animals moving in the dark in a vast home range. The maned wolf's extended-bark is a long-range vocalization that functions as a mechanism to increase spatial distance among conspecifics as well as to enable pair-mate reunion. Individual variations in this vocalization have been reported, but the possibility that they can be perceived and used by the species has never been tested. In our study, we used ABAB playback experiments to test if captive maned wolves could perceive individual variations. We ran 19 playback sessions with ten different subjects from six different zoos. After discarding nine sessions that did not fulfil minimal experimental conditions, in all except two of the ten valid trials, the subjects responded with displaying Oriented Attention. To our knowledge, this is the first demonstration that the maned wolf is capable of discriminating among extended-barks of different individuals.

## 1. Introduction

The acoustic channel is an efficient long-distance signalling system that may provide instant information about senders - such as location and identity - and may be especially effective for animals with crepuscular/nocturnal habits. The so-called long-range calls (Darden et al., 2003) or loud calls (due to its high level of sound pressure; de Cunha et al., 2015) are defined as acoustic signals broadcasted to individuals that are often out of view (Owings and Morton, 1998). Many mammals adopt loud calls to signal territory occupancy (e.g., lion *Panthera leo*: McComb et al., 1994; Grinnell and McComb, 2001; other great cats: Darden et al., 2003; gray wolf *Canis lupus*: Harrington and Mech, 1983; chimpanzees *Pan troglodytes*: Mitani and Nishida, 1993), to attract mates and even to stimulate premature ovulation, as it indicates male quality (i.e. physical attributes or health condition; e.g., “roar” in *Cervus elaphus*: Clutton-Brock and Albon, 1979; Reby and McComb, 2003; in *Panthera tigris*: Ji et al., 2013; “songs” of the fin-whale, *Balaenoptera physalus*: Croll et al., 2002). It has been demonstrated that individuals of some species are capable of distinguishing familiar vocalizations from unfamiliar ones, even when they are far away from the sender

(e.g., gray wolf *Canis lupus*: Tooze et al., 1990; rhesus monkey *Macaca mulatta*: Rendall et al., 1996; lion *Panthera leo*: Grinnell and McComb, 2001; African elephant *Loxodonta africana*: McComb et al., 2000).

The maned wolf's extended-bark is a loud vocalization whose abrupt start and broadband frequency favour its perception and its location source (McGregor, 2005). If this vocalization involves the provision of clues about the sender's identity, either to permit reproductive partner recognition or to signal territory property, such vocalization might have recognisable individual variations.

The maned wolf (*Chrysocyon brachyurus*, Illiger, 1815) is a large canid inhabiting open vegetation areas, mainly savanna's phytosociology (Batalha, 2011) of Cerrado (Dietz, 1985). The species has been described as having crepuscular/nocturnal habits and being solitary since the members of a reproductive pair are rarely found together during activity periods (Dietz, 1984). It would be more precise, however, to define the maned wolf as a level two or three of sociality species (i.e. aggregation for one or two activities, solitary to others: Lee, 1994), since the pair mates may share a vast territory (Kleiman, 1972; Dietz, 1984) from 20 to 115 km<sup>2</sup> (Dietz, 1984, 1985; de Carvalho and Vasconcellos, 1995; de Melo et al., 2007), may even rest and hunt

**Abbreviations:** ABAB, the habituation-discrimination paradigm of Friedman (1972), which we adopted as playback protocol, modified from Rendall et al. (1996), where A represents habituation phases that are interspersed by experimental phases B (playback discrimination); WO, Wolverine; LO, Logan; ME, Mel; OR, Orfeu; FR, Frutti; TU, Tutti; HOR, Hortelã; MO, Mostarda; FA, Fantasma; NY, Nymeria; PE, Perônio; TÍ, Tíbio; CO, Colombo; PL, Pluto; LU, Lupin; MOG, Mogli

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together (Bandeira de Melo et al., 2007). The species tends to monogamy and both male and female invest in parental care (Bestelmeyer, 2000; Veado, 2005).

To the best of our knowledge, the only published studies that address the acoustic behaviour of the maned wolf were made in the 70's and 80's by Encke (1970, 1971 *apud* Kleiman, 1972), Kleiman (1972); Tembrock (1976); Brady (1981) and Dietz (1984). Since then, there is a gap on studies focusing on the maned wolf's acoustic repertoire. Brady (1981) has described ten different signals, including the loud "single-bark" and the "roar-bark" (called "single-bark" by Kleiman, 1972; and "extended-bark" by Dietz, 1984, which is the term we adopted in our work). The extended-bark resembles an extended variation of the bark of a large domestic dog (Dietz, 1984), and is emitted in sequences either by males and females or in duetting (Dietz, 1984; personal observations). Kleiman (1972) suggested that the extended-barks function as a spacing mechanism among individuals, such as the roars of the great cats. After observing several exchanges of vocalizations between pairs and between pair-mates, Dietz also suggested that it occurs at all times of the year (most commonly during June through August, in that year of 1979), and may have a role in aiding the localization between pair-mates.

The maned wolf's body is large and requires significant energy supply. As its food is scarce and dispersed (mostly fruits and small vertebrates such as rodents and birds – De Arruda Bueno et al., 2003; occasionally, larger animals, Bestelmeyer and Westbrook, 1998) the best strategy for this species is to hunt alone and use mutually exclusive foraging areas. If a strict territoriality has increased the survival of individuals, then natural selection must have favoured characteristics that emphasize the property of an area (Dietz, 1984). In fact, the maned wolf exhibits scent-marking behaviours with urine and faeces and produces a long distance vocalization (the extended-bark), which are presumably related to territorial defence in close proximity and at longer range, respectively (Dietz, 1984; Kleiman, 1972).

Brady (1981) reported individual variations in maned wolf extended-barks and stated that a human could distinguish individual differences in extended-barks at a distance of 1 km. Nevertheless, the possibility that this variability can be used by maned wolves for individual recognition has never been tested. One should expect these individual variations to be perceived by the species, for only in this scenario would it be plausible for the extended-bark to function as a mechanism to increase spatial distance among conspecifics and to enable pair-mate reunion. If this individual variability is not perceived by conspecifics, the efficiency of this vocalization at long distances would be compromised, as the receiver would not be able to identify the sender as its reproductive partner or a potential rival. We used playback experiments to test if individual variations in the extended-bark of maned wolves can be perceived by the species. To the best of our knowledge, the ability to discriminate individuals through the voice itself has never been demonstrated in this great carnivore of Latin-America.

## 2. Methods

### 2.1. Study sites and animals

We carried out all acoustic and behavioural data collection in captivity, in seven different zoos of the State of São Paulo, Brazil. The total number of maned wolves participating in this study was 16 (Table 1). From this total, only six (four males and two females) produced extended-barks that could be used to create the soundtracks to be used in playback trials. Playback trials were conducted with 10 subjects (three pairs and seven solitary males). In all zoos, the enclosures permitted visitors to see the animals; hence, the maned wolves were accustomed to human presence.

Besides the captive maned wolves, we also had the chance to observe two wild individuals. The first (HOR) was a free-living animal

from the ecological reserve bordering the zoo of São Carlos and that often entered the zoo through a hole in the outer fence. We assumed it was a male from observation and unofficial information from the zoo's employees. The second wolf (MOG) inhabited the Ecological Station of Itirapina and its surroundings.

### 2.2. Recordings

Vocalizations were recorded manually in a single channel by a solid-state digital recorder (Marantz PMD661-MKII) attached to a professional unidirectional microphone (Sennheiser ME67 head with K6 powering module - frequency response: 50–20,000 Hz) suited with a windshield (Rycote classic-softie windshield). Since the maned wolf's extended-barks can reach 20 kHz (as noticed with MOG recorded barks and known by unpublished dissertations), we chose a sample rate of 44.1 kHz. All files were saved in uncompressed 16 bits PCM-WAVE format. We also monitored the vocal patterns maned wolves using remote sound recordings with an automated digital recorder (Wildlife Acoustics, SongMeter 2+), that permitted continuously recording from 17:00 to 07:00, without the experimenter presence. Recorders were installed in the open area of the enclosures. Recording sessions (manual and automatic) occurred in each zoo continuously from 17:00 to 07:00 (UTC-3), from 2013 to 2015, in thirteen 10-day field campaigns. The distance between the emitter and the manual microphone varied from 7 to no more than 15 m, except for the FR wolf to whom it was impossible to be closer than 40 m. To get recordings with similar conditions in all of the zoos, we measured the sound pressure level (of vocalizations and environmental noise in dB) using a sound level meter (Instrutemp, ITDEC-4080; range 30 dB to 130 dB). The amount of data collected was then scanned manually in Song Scope software (Wildlife Acoustics). We also used some of these vocalizations automatically acquired as stimuli for some of the playback tests (see topic 2.4.1 for details).

### 2.3. Playback soundtracks preparation

Playback tracks were edited in Raven Pro (1.4 version) composed of individual notes (playback stimulus) set in a temporal series similar to a natural extended-bark (Fig. 1, top). To avoid sex and oestrous stage influences on vocalizations, we used only notes from males (FA, TU, and OR) as playback stimuli. The notes were randomly selected in R program among those with a good signal-to-noise ratio. The intervals between two stimuli (notes) respected the natural sequence patterns of the maned-wolf extended-bark, of about 5 s. We avoided repeating notes in the soundtracks (*i.e.* using different notes to be the stimulus A: A<sub>7</sub>, A<sub>8</sub>, A<sub>9</sub>, B, A<sub>10</sub>) and we used new test soundtracks for each playback session. That approach increases the consistency of our results by preventing simple pseudo replication (Kroodsmma et al., 2001).

The quality of the sound played by our equipment was verified. We recorded a playback soundtrack when it was being played during the first playback session, and compared that to the original recording, using Raven Pro. The spectrogram analysis of the recorded playback showed no distortion or anomaly in the notes.

### 2.4. Experimental procedure

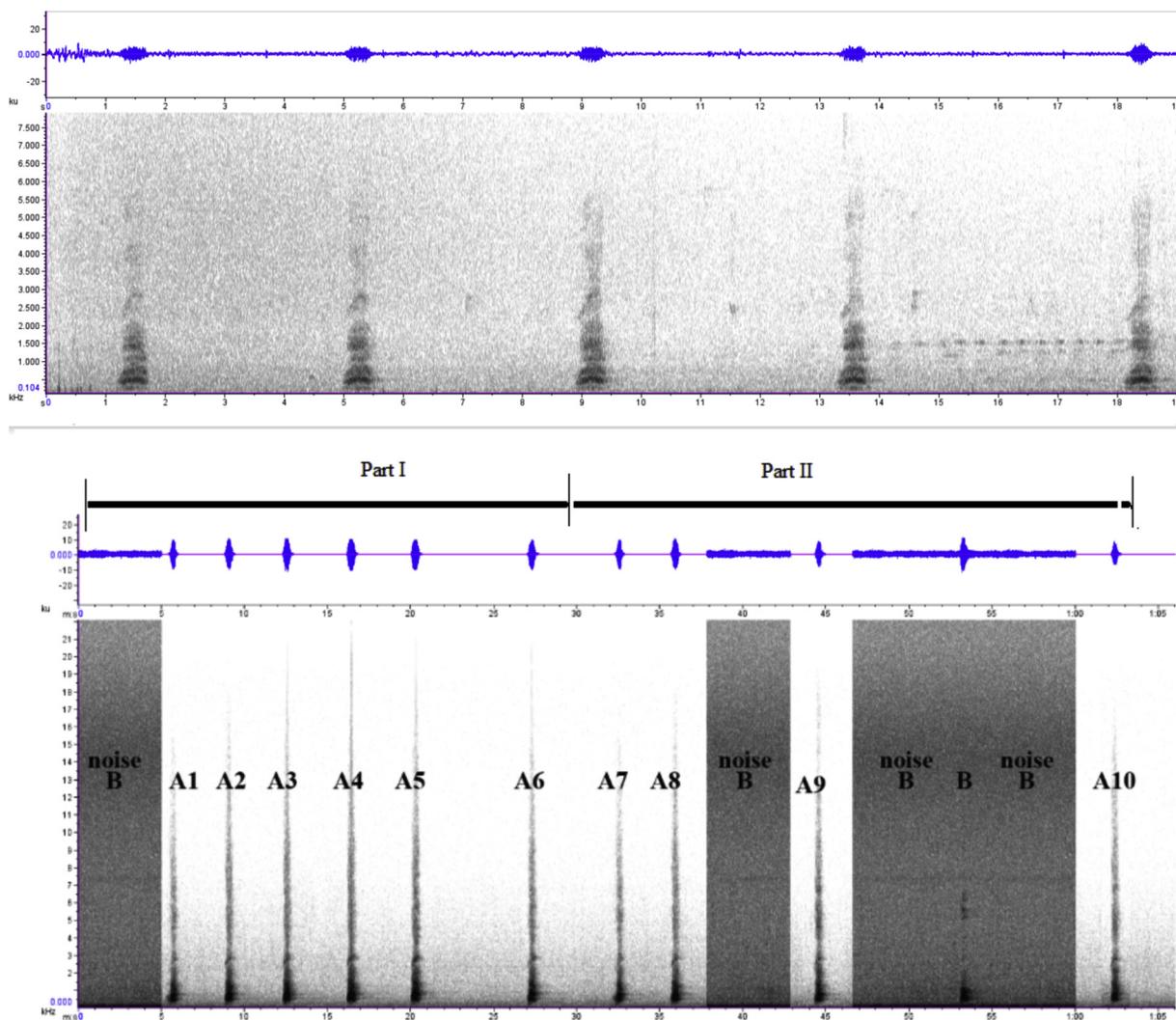
If the maned wolf is capable of distinguishing its reproductive partner from a possible competitor using only the extended-bark, then we expected this vocalization to have individual variations that provide clues about the sender's identity. In case our hypothesis was valid, the maned wolves would be able to distinguish among different conspecifics' extended-barks. In order to test this prediction, we used the habituation-discrimination paradigm (Friedman, 1972; Cheney and Seyfarth, 1988; Rendall et al., 1996; Hauser, 1998; Reby and McComb, 2003; Mumm et al., 2014; Palacios et al., 2015). Habituation is defined as a gradual decrease in the magnitude and/or frequency of the response due to repeated presentations of a stimulus (Catania, 1999;

**Table 1**

Maned wolves that participated in this study. They were held in captivity in different zoos of the State of São Paulo, Brazil, either isolated or in pairs. Sexes are presented as M = male, F = female. The contribution of each animal in the different phases of the research is presented in the last column.

CITY/STATE	SUBJECT	SEX	PARTICIPATION
Botucatu/ SP	Wolverine (WO)	M	Playback tests
Botucatu/ SP	Logan (LO)	M	Playback tests
Catanduva/ SP	Mel (ME)	F	Recording
Ribeirão Preto/ SP	Orfeu (OR)	M	Recording
São Carlos/ SP	Frutti (FR)	F	Recording
São Carlos/ SP	Tutti (TU)	M	Recording and Playback tests
São Carlos/SP	Hortelã (HOR)**	M	Recording
São José do Rio Preto/ SP	Mostarda (MO)	M	Recording
Sorocaba/ SP	Fantasma (FA)***	M	Recording and Playback tests
Sorocaba/ SP	Nymeria (NY)***	F	Recording and Playback tests
Sorocaba/ SP	Perônio (PE) <sup>†</sup> , ****	M	Playback tests
Sorocaba/ SP	Tíbio (TÍ) <sup>†</sup> , ****	M	Playback tests
Americana/SP	Colombo (CO)	M	Recording and Playback tests
Paulínia/SP	Pluto (PL)	M	Playback tests
Piracicaba/SP	Lupin (LU)	M	Recording and Playback tests
Itirapina/SP	Mogli (MOG)	Unknown	Recording (pilot procedures)

<sup>†</sup>All subjects out of PE and TI, were adults (more than 2 years old according to Rodden et al., 2004). \*\*HOR was a free-living male (unofficial information from Zoo's employees and personal verification) at reserve bordering the Zoo of São Carlos, that occasionally visited Tutti and Frutti's enclosure. The only wolves that were kept in pairs were: (1) FA and NY; (2) TÍ and PE. TU and FR shared the same enclosure during part of the day, but were kept apart most of the time.



**Fig. 1.** Spectrograms of a natural sequence of extended-arks (top) and a soundtrack used in standard protocol playback test (bottom). In the natural sequence, five notes are present and showed in detail at the 0.2–3 kHz band. In the edited sequence below, Part I corresponds to the habituation phase: it was played continuously (i.e. in loop mode) until the subject was habituated. Part II corresponds to the test phase (discrimination soundtrack). The dark regions are background noise extracted from track B. The spectrogram was generated in Raven Pro 1.4.

**Table 2**

List of the valid standard playback protocol trials. Discrimination between stimulus B and A occurred when “Oriented Attention” in the beginning of the habituation phase was recorded, and during/immediately after B.

Subject	Session	DID IT DISPLAY ORIENTED ATTENTION?					Discrimination
		A <sub>1</sub> - A <sub>6</sub> (BEGINNING)	A <sub>1</sub> - A <sub>6</sub> (END)	A <sub>7</sub> - A <sub>9</sub>	B	A <sub>10</sub>	
CO	2 <sup>nd</sup>	Yes	No	No	Yes	No	Yes
FA	1 <sup>st</sup>	Yes	No	No	Yes	No	Yes
FA*	2 <sup>nd</sup>	Yes	No	No	No	No	No
LO	1 <sup>st</sup>	Yes	No	No	Yes	No	Yes
LU	1 <sup>st</sup>	Yes	No	No	Yes	No	Yes
NY	1 <sup>st</sup>	Yes	No	No	Yes	No	Yes
NY*	2 <sup>nd</sup>	Yes	No	No	No	No	No
TU	1 <sup>st</sup>	Yes	No	No	Yes	No	Yes
TU	2 <sup>nd</sup>	Yes	No	No	Yes	No	Yes
TU	3 <sup>rd</sup>	Yes	No	No	Yes	No	Yes

\* “Inconclusive”, since the subjects failed discrimination in the second trial, but responded as expected in the first trial.

Rankin et al., 2009). The typical habituation-discrimination paradigm involves presenting repeatedly a given stimulus (*i.e.* habituation stimulus; *e.g.* extended-bark from individual ‘A’) at fixed time intervals until the subject habituates to it, followed by the presentation of a different stimulus (*i.e.* test stimulus; *e.g.* extended-bark from individual ‘B’). If this subsequent exposure to the test stimulus immediately increases the magnitude and/or frequency of the response, discrimination between stimuli A and B occurred.

Sessions started within the period of activity of the species (Dietz, 1984), in the early morning (6:00), to take advantage of photoperiod for video documentation. After preparing all playback and video recording apparatus, we waited approximately 10 min in silence and motionless before starting each test, to familiarize the subjects to our presence and the apparatus. Acoustic stimuli were played back using a powered speaker (Boxer M-10; output power 100 W; impedance 4–8 Ω; frequency range 0.05 kHz–20 kHz). The speaker was positioned 30 m away from the subjects and out of their sight. Volume level was adjusted to ensure the stimuli were audible by the subjects and simulated a natural barking bout that we witnessed during the recording phase. To prevent changes in the wave sound caused by ground attenuation (Wiley and Richards, 1978), we placed the powered speakers at a distance of approximately 0.5 m from the ground. During the tests, we documented the maned wolves’ behaviours with a Sony Handy cam, except for the couple FA and NY that requested individual cameras.

#### 2.4.1. Standard playback protocol

To test whether the maned wolf was able to discriminate among extended-barks of different individuals (A and B), we adopted the following playback protocol (modified from Rendall et al., 1996):

**2.4.1.1. Habituation phase.** we used a sequence of 6 extended-barks (A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub>, A<sub>4</sub>, A<sub>5</sub>, A<sub>6</sub>) from wolf A as the habituation stimulus. The sequence was presented to the subject repeatedly until it was habituated (for details see topic 2.5). We chose to use a sequence as stimulus, rather than a single extended-bark, to expose the subject to six different samples of class A stimulus. Thus, the subject was habituated to the individual content of wolf A’s extended-barks, not to the particular acoustic characteristics of a single note, a more reliable approach to the natural situation of emission.

**2.4.1.2. Habituation control phase.** subsequent to the subject’s habituation, we broadcast new extended-barks from wolf A. According to our prediction, these new stimuli (A<sub>7</sub>, A<sub>8</sub>, A<sub>9</sub>) would retain the acoustic characteristics to which the subject had been habituated. Thus, if the subject had not recovered from habituation, we would assume that it had habituated to the acoustic features virtually present in all stimuli from class A. Otherwise, our postulate

would be discarded and we would conclude that the subject had only habituated to the initial habituation series (*i.e.* A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub>, A<sub>4</sub>, A<sub>5</sub>, A<sub>6</sub>). Again, we choose to use three new stimuli rather than a single one.

**2.4.1.3. Discrimination phase.** after the habituation control phase, we broadcast a single stimulus from wolf B (the interval between this discrimination stimulus B and the precedent class A stimulus was of about 10 s). Stimulus B was also new. Therefore, if stimulus B elicited a response similar to that obtained at the beginning of the habituation phase (*i.e.* before the wolf was habituated), we would assume that the subject had discriminated from maned wolves A and B extended-barks.

**2.4.1.4. Re-habituation phase.** at last we presented a new stimulus from class A (*i.e.* A<sub>10</sub>). If we had observed a decrease in the magnitude of response, or even no response at all, we would endorse the statement that the subject was able to discriminate between A and B. Albeit we could re-exposure the subject to a stimulus of type A that had already been used, we preferred to broadcast a new one (A<sub>10</sub>) as a way to increase experimental control.

Sessions on a single zoo were separated by at least 1 week from one another, except in Sorocaba’s zoo, for logistical reasons (1-day interval between sessions). We ran the test more than once in several sessions to test habituation to B. However, we only considered the first trial of each session as valid, since the habituation control phase stimuli (A<sub>7</sub>, A<sub>8</sub>, A<sub>9</sub>) and the re-habituation stimuli (A<sub>7</sub>) were no longer new after the first trial (Table 2).

#### 2.4.2. Complementary playback protocol for CO wolf

The first playback session of the subject CO was harmed by confounding variables, particular to that zoo: loud background noise, frequent human interference, dogs barking and others. Under such adverse conditions, the subject did not show a clear response to the stimuli change in the standard protocol. One of the possible reasons for the absence of response was that the subject did not perceive the B stimulus along the track since there were too many distractions during the test. Another possibility was that the subject did not have enough time to react to B stimulus. Thus, even if it had started to react to B, the following stimulus (*i.e.* A<sub>8</sub>) could have interrupted it. We could not elongate the interval between notes since the silence itself could be a confounding factor. So, we ran a new test: (1) we played a series of stimulus from wolf A (A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub>, A<sub>4</sub>, A<sub>5</sub>, A<sub>6</sub>), and then (2) a series of stimulus from wolf B (B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>4</sub>, B<sub>5</sub>, B<sub>6</sub>). The criteria of response (see topic 2.5) and the procedure were the same of topic 2.4.1.

#### 2.4.3. Control playback procedure evaluations

**2.4.3.1. Background noise effect evaluation.** Since recordings were made in uncontrolled environments, the background noise differed from one

recording to another. That difference was especially pronounced between automatic and manual recordings. The recordings obtained by Song Meter are much noisier (*i.e.* more background noise is captured with the signal of interest), probably due to the nature of its microphones, and this difference could not be eliminated without inserting other confounding variables (*e.g.*, filtering natural acoustic characteristics of that environment). To guarantee that the subjects' responses occurred due to the change in the stimuli and not to the difference among the types of background noise of each track, we used two forms of control:

- (1) We inserted background noise extracted from track B (background noise B) in the habituation soundtrack and just before stimulus B (Fig. 1). This strategy allowed us to know if alteration in the subjects' behaviour was due to the change of stimulus (*i.e.* from A to B) or due to the background noise change. If the subject did not react to the inserted fragments of background noise, we assumed that discrimination between A and B took place. The duration of each background noise fragment was of approximately 5 s (the same adopted in extended-bark series).
- (2) We also held a playback trial with subjects NY and FA using only noise as stimuli to test their responses to the difference in background noise alone. The protocol we used was simpler than the standard one: we played a track which contained an excerpt of noise extracted from the stimulus A recording (background noise A), followed by an excerpt of noise extracted from the stimulus B recording (background noise B). Our expectation was that the subject would react to none of them or, if there was a reaction, the behaviours presented would differ from the ones that occurred in the presence of the extended-barks. For this test, we used only background excerpts that evidently differed from each other, such as background from Song Meter recordings against the background from manual recordings.

**2.4.3.2. Stimulus control test.** With one of the subjects (male TU), we had the opportunity to play an audio track comprising 15 min of surround sound which had been previously recorded before initiating the extended-barks playback tests. This control-track allowed us to evaluate the animal's reaction to artificially played sounds. If the subject responded in some way to the playback of surround sound it would have been harder to guarantee that its reaction to the other playback tests (all artificially played) was due to the stimuli.

## 2.5. Behavioural analysis

During the habituation phase of each playback trial, we studied the responses shown by the subjects in order to judge which could be elected as good estimators of the maned wolves' reaction to the stimuli. We adopted the following criteria: (1) the response would have to follow a habituation pattern (*i.e.* gradually decreased in magnitude and/or frequency after repeated presentations of a stimulus); (2) the occurrence of the response would have to be easily identified by an untrained observer; (3) the duration of the response would have to be brief (less than 10 s), since the interval between the discrimination stimulus and the re-habituation stimulus was of about 10 s. The response that best matched all requirements was the "Oriented Attention" (Fig. 2): the maned wolf turns its head towards the speaker and remains with ears raised, eyes wide open and outer ears facing the speaker during at least 1 s. The reaction can occur just after the stimulus or have a latency of most 4 s. (Adapted from Palacios *et al.*, 2015).

In order to comply with the habituation-discrimination paradigm, we only analysed tests in which the subjects displayed the Oriented Attention response at the beginning of the habituation phase. However, any response to the test stimulus (*i.e.* B) might have been influenced by the fact that the subject had already heard extended-barks. Thus, to consider the subject was habituated during playback procedures, both

the frequency of the Oriented Attention response and the magnitude of the subject's overall "disturbance state" (examined by the experimenter under Konrad Lorenz, 1962's "Gestalten" concept 1959 *apud* Hutt and Hutt, 1974; the experimenter conclusion was checked later by five untrained observers, as explained below) should decrease. Since we used Oriented Attention as a measure of the response, we only played the test soundtrack (*i.e.* A<sub>7</sub>, A<sub>8</sub>, A<sub>9</sub>, B, A<sub>10</sub>) when the subject was not facing the speaker (we waited until it happened, that never took more than one minute) and when no other noticeable acoustic stimulus was present.

To score the subjects responses, we watched the playback videos in the Camtasia Studio software (version 8.6.0; TechSmith Corporation). This software displays the video and the audio's waveforms on the same screen, which allowed us to accurately visualize the timing between stimulus (sound) and response (image). To measure the subjects' responses to the stimuli we used a single measure: Oriented Attention presence. Thus, discrimination between A and B happened if the subject displayed Oriented Attention only in the beginning of the habituation phase and during/immediately after B. To prevent "experimenter bias" we elected five untrained observers to judge the validity of our "gestalt" Disturbance. Each judge watched video excerpts from CO's playback tests. Four of the excerpts corresponded to moments in which we considered the subject was "very perturbed" (*i.e.* high magnitude level of Disturbance). The other four excerpts were from moments we considered it "less perturbed" (*i.e.* lower magnitude level of Disturbance). We randomly mixed the video excerpts and, as a result, we obtained four pairs of video containing excerpts we judged as different "gestalten", one of them corresponding to the transition from stimuli A to stimuli B (Table 3). Judges were asked to watch each pair of video excerpts and score if the animal's activity had decreased or increased from the first excerpt to the second. The judges were familiar with our definition of the "gestalt" Disturbance and evaluated the videos on mute mode. To determine inter-observer agreement, we used Fleiss' Kappa statistic calculated with and without author's scores. To determine the strength of agreement, we adopted Landis and Koch (1977) benchmarks.

## 3. Results

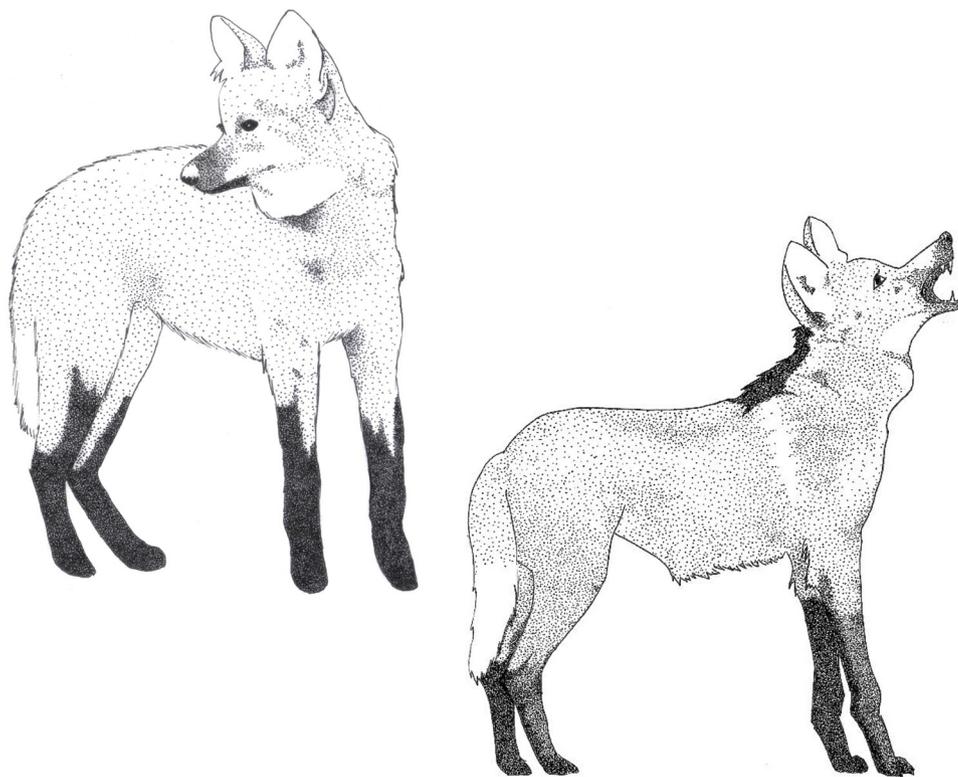
### 3.1. Recordings

We sampled nine captive maned wolves (6 males and 3 females) and one wild wolf (unknown sex) and gathered 1911 notes distributed in 107 sequences (Table 4). We recorded five duet events between the couples FA-NY ( $n = 3$ ) and FR-TU ( $n = 2$ ) (Fig. 3). Male and female were out of each other's sight before and during the events.

During fieldwork in the São Carlos zoo, we recorded two very long sequences with the automatic recorder. The first sequence contained 712 extended-barks emitted in a row and the second one contained 208. Both sequences were emitted on the same day, 20 min apart from each other. Unfortunately, all these sequences, those of FA, NY and CO ( $n = 34$ ) and most of LUs notes ( $n = 42$ ), could not be used due to recording or digitalization errors.

### 3.2. Standard playback protocol

We ran 19 playback sessions with 10 different subjects from six different zoos. We discarded nine of the sessions, which did not fulfil minimal experimental conditions. We considered as invalid every trial in which the subject did not display Oriented Attention in the beginning of the habituation phase (*i.e.*, did not habituate: 1st sessions of CO, PE, PL, TI, WO, and 2nd or 3rd sessions of FA, LU, NY, PL). All of them, except in WO sessions, were harmed by confounding variables such as loud background noise, human interference; subject was not used to unfamiliar humans. In two of the 10 valid trials, the subjects (FA and NY) did not respond as expected.



**Fig. 2.** The “Oriented Attention” posture (left) of the maned wolf that characterized stimulus discrimination: the animal turns its head towards the speaker and remains with ears raised, eyes wide open and outer ears facing the speaker during at least 1 s. Barking (right): maned wolf emits a sequence of extended-barks. The wolf is usually standing up with its tail relaxed. Between one note and another, its ears rotate in a back and forth movement as if searching for sounds (Drawings made by Juliana Takata based on video excerpts of our playback tests and author’s verbal description, respectively).

**3.3. Complementary playback protocol**

The Complementary Playback Protocol to which CO was submitted generated a clear reaction: the magnitude of the “gestalt” Disturbance suddenly increased in the presence of the B stimuli (excerpts from Video-B). The judgment on validity of our “gestalt” Disturbance indicated moderate agreement without the author as judge (Fleiss’ kappa statistic:  $k = 0.56$ ;  $n$  judges = 5; P-value 0.001) and substantial agreement with the author as judge (Fleiss’ kappa statistic:  $k = 0.625$ ;  $n$  judges = 6; P-value 0.001). The agreement in the increase of Disturbance magnitude from stimuli A to stimuli B was unanimous in both cases (Table 3).

**3.4. Control playback procedures evaluations**

**3.4.1. Background noise effect**

The difference of background noise did not produce any response in the subjects. We used the first type of control in four playback soundtracks (two with TU; two with FA and NY) and the subjects did not show any response to the fragment of background noise preceding the test stimulus. In addition, the subjects FA and NY did not react to the second type of control.

**3.4.2. Stimulus control test**

The subject TU did not show any response to the background noise playback. It remained resting before, during and after the test, which

**Table 3**

Evaluation of the “gestalt” Disturbance based on video excerpts of the subject CO by 5 naive adult judges of both sexes.

	Author	Judge 1	Judge 2	Judge 3	Judge 4	Judge 5
Video-A	Increases	Increases	Increases	Increases	Decreases	Increases
Video-B <sup>†</sup>	Increases	Increases	Increases	Increases	Increases	Increases
Video-C	Increases	Increases	Increases	Increases	Decreases	Increases
Video-D	Decreases	Decreases	Decreases	Decreases	Decreases	Decreases

<sup>†</sup>Video-B corresponds to the transition from stimuli A to stimuli B and the agreement among judgments was unanimous.

**Table 4**

Recording samples acquired during 13 field campaigns in 7 Zoos. The total number of notes is indicated, followed by number of sequences (parenthesis) and the individual average number of notes per sequence. Recording procedure is indicated: automatic (recorded with SongMeter 2+) or manual (recorded with Marantz).

Subject	Notes (sequences)	Average Notes/Sequence	Recording Procedure	Recording Effort	Zoo’s Location
OR	337 (8)	42,1	Automatic	37:52 h	R. Preto
ME	0	0	Automatic	111:40 h	Catanduva
MO	0	0	Automatic	181:58 h	S. J. R. Preto
NY	111 (12)	9,2	Manual	221:00 h	Sorocaba
FA	145 (33)	4,4	Manual	221:00 h	Sorocaba
FR	54 (7)	7,7	Manual	120:00 h	São Carlos
TU	32 (3)	10,7	Manual	120:00 h	São Carlos
CO**	34 (5)	6,8	Manual	1:00 h	Americana
LU**	52 (5)	10,4	Manual	1:00 h	Piracicaba
LU	323 (30)	10,8	Automatic	98:00 h	Piracicaba
FA or NY*	215 (31)	6,9	Automatic	50:00 h	Sorocaba
HOR or FR*	931 (3)	310,3	Automatic	5:00 h	São Carlos

\* Since there was more than one wolf near the autonomous recorder, it is not possible to be certain of the emitter (FA or NY in Sorocaba’s Zoo, and FR or HOR in São Carlos’ Zoo). \*\* All the vocalizations of the CO and most vocalizations of the LU were anomalous and exceeded the typical highest frequency threshold of extended-barks. Thus, the sample rate previously adjusted for the recordings was insufficient leading to aliasing artefacts in all notes emitted by CO ( $n = 34$ ) and most of the ones emitted by LU ( $n = 42$ ).

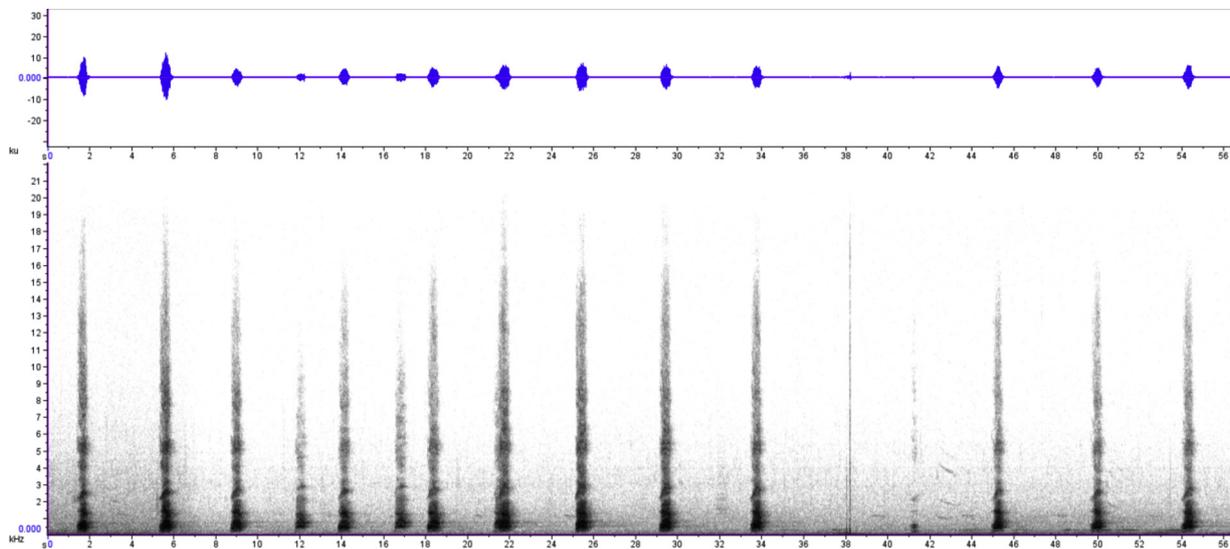


Fig. 3. Duetting extended-bark of FR and TU from São Carlos' Zoo personally recorded by Flora Balieiro. The sequence is initiated by the female FR. The TU males' first note occurred after female's third. The female emitted 12 notes and the male 2.

increases the credibility of the other playback tests' results.

#### 4. Discussion

Our study showed that the maned wolves are able to distinguish among the different extended-barks of conspecifics. Individual variations in this vocalization have been reported, but the possibility that they could be perceived by the species was not known until now.

The extended-bark functioning as a mechanism to increase the spatial distance among conspecifics is in accordance with the “distance communication hypothesis” (Bouchet et al., 2012), which states that individual acoustic distinctiveness is related to the typical distance between the sender and the target receivers (Bouchet et al., 2012; Marler, 1967). The key idea is that the acoustic channel may be the only efficient modality for individual recognition of the animals that are out of each other's visual range (Bouchet et al., 2012). As the vocalizations used for long-distance communication cannot benefit from visual or olfactory clues, they should be easier to recognise than the ones used for short-range communication (Bouchet et al., 2012). In addition, this individual distinctiveness should be especially pronounced in species whose individuals are often separated from each other (Bouchet et al., 2012; e.g. penguins: Aubin and Jouventin, 2002) and have nocturnal habits (coyote, *Canis latrans*, Mitchell et al., 2006). Both characteristics are found in the maned wolf, a species with nocturnal habits in which the spatial distance between male and female varies according to the female's reproductive period (Dietz, 1984). The emission rate of the maned wolf's extended-barks increases during the breeding season (Dietz, 1984) and couples' duets were observed only when male and female were out of each other's visual range (Dietz, 1984; personal observations). Thus, the extended-bark may also function in aiding the localization between pair-mates (Dietz, 1984) and it is expected to contain individual acoustic variation (Brady, 1981).

Vocal individuality only has biological relevance if it can be perceived by receivers. There are two ways in which receivers can perceive vocal individuality: (1) *discrimination* when the receiver uses the similarity or dissimilarity of acoustic stimuli to discriminate between senders (Mumm et al., 2014) [note that the term “discrimination” does not follow the behaviourist concept, defined by differences in responding in the presence of different stimuli; Catania, 1999]; and (2) *recognition* when the receiver matches the individual distinctive characteristics of the emitter's signal with specific information about this emitter (‘true recognition’ for Tibbetts and Dale, 2007). Although these definitions are not always distinct among authors (Tibbetts and

Dale, 2007; Mumm et al., 2014), they may have different implications. Recognition implies that the emitter is somehow familiar to the receiver and, in this sense, one may expect that the latter's ability to learn must be quite sophisticated and requires memory capacity. On the other hand, one could state that if the receiver is not able to perceive similarities or dissimilarities in the acoustic stimuli, it would not be able to recognise it either. Thus, discrimination is a previous condition to recognition.

The maned wolves should be able to recognise at least their reproductive partner's extended-barks and discriminate them from unfamiliar ones. If they are not able to do that, the efficiency of this vocalization at long distances would be compromised, as the hearer would not be able to discriminate its reproductive partner from a rival. We have focused our efforts on testing discrimination of senders through extended-barks vocal individuality and our results present clear evidence of it. In the future, it might be interesting to further investigate the recognition capacity of conspecifics through extended-barks, given the potential relevance in social dynamics of the maned wolf. Since discrimination is a prerequisite to recognition, our results are the first step to further research in that direction.

The “single measure approach” (McGregor et al., 1992) assumes that the response elicited by playback is adequately described by a single measure. The use of this approach can be inadequate if the measure chosen is not sufficient to describe the subject response (McGregor et al., 1992). The criteria of response chosen for the standard playback tests (*i.e.* Oriented Attention) leaves no doubt regarding the subjects' responses: they clearly discriminate between stimuli. Many mammals adopt loud vocalizations to signal territory occupancy (*e.g.* Lion *Panthera leo*: McComb et al., 1994; Grinnell and McComb, 2001; other great cats: Darden et al., 2003; gray wolf *Canis lupus*: Harrington and Mech, 1983; chimpanzees *Pan troglodytes*: Mitani and Nishida, 1993). A loud vocalization would be expected to occur in a species with wide territories such as the maned wolf (ranging from 20 to 115 km<sup>2</sup>: Dietz, 1984, 1985; de Carvalho and Vasconcellos, 1995; Bandeira de Melo et al., 2007). The loud extended-bark presents several acoustic characteristics that may favour its perception and source location (*e.g.* high amplitude, abrupt start and broadband frequency: McGregor, 2005), making it the proper vocalization for territorial defence. Other evidences of the extended-bark territorial function were recorded during the recording phase in the present study. The vocalization was usually emitted at dawn and dusk, optimal times for sound transmission in open areas such as the Cerrado's phytogeography in which the species occurs (*i.e.* Wiley and Richards, 1978; Brady, 1981). In addition,

we noticed that the female NY always climbed the mound of dirt placed on the centre region of the enclosure (the highest place there) before vocalizing and often turned its head up during the emission of the extended-barks (Fig. 2), which may have facilitated the sound spreading.

The extended-bark functioning for territorial boundaries disputes may find support in the very long sequences (712 notes and 208 notes) recorded in São Carlos's Zoo, where the captive wolves, male TU and female FR, lived in interaction with the free-living male HOR. The longest extended-bark sequences recorded in the wild by Dietz (1984), and in captivity by Kleiman (1972), were of only 23 and 30 notes, respectively. We noted many events of aggressive interactions between the captive wolves and HOR; when HOR was around the enclosures, the younger male TU was always submissive and we observed agonistic interactions between HOR and FR. Thus, the caretakers had reported that another male wolf (which shared the enclosure with FR before TU) had been injured in a fight with HOR, through the fences. The long extended-bark sequences may be an honest signal of the wolf's physical conditions and could be used to avoid physical confrontation (if TU had the chance to get away from HOR, maybe he would). A wolf in better physical condition may be able to broadcast a longer sequence of extended-barks, thus communicating its disposition to fight for territory, and identifying itself to familiar competitors.

In addition to the difficulty of gathering data in so many different places, there were numerous technical and logistical problems to overcome. These problems may have limited the validity of some results. As previously reported, our soundtrack stimuli were restricted to the maned wolves FA, TU and OR. The maned wolves FA and TU were manually recorded with Marantz, and OR was automatically recorded with SongMeter 2+. Since we could not play the stimuli produced by one wolf to itself, all playback soundtracks played to the subjects TU and FA (including NY, that shared the enclosure with FA), contained stimuli recorded with different types of equipment. The difference between background noises from different recording equipment was controlled in every trial by a simple procedure (see "Background noise effect evaluation"). By exploiting every relevant variable, we eliminated as many confounding factors as possible, given the quasi-experimental character of our work. Thus, the number of subjects is not crucial to the validity of our experiment. In fact, even if only one subject had corroborated our hypothesis, it would be more plausible to generalize the validity of our results to the whole species than to assume that the one subject tested was the only wolf capable of discriminating between stimuli. Furthermore, the robust and consistent responses of the subjects make it unlikely that the results are odd or deviant.

## 5. Conclusions

Individual variations in the extended-bark can be discriminated by maned wolves. If they perceive differences, they can probably declare territorial limits by voice, and at a distance. That makes the acoustic channel an efficient long-distance signalling system for a species that moves in the dark in a vast home range and whose hunting territory is a valuable resource to be defended.

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