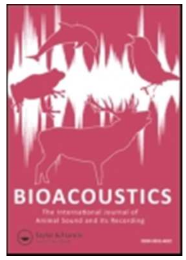

Acoustic monitoring of the golden jackals in Europe: setting the frame for future analyses

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Keywords:	Howling, Carnivores, Sound Analysis, Vocalizations

To: The Editors,
Bioacoustics

Subject: Submission of the revised manuscript titled "Acoustic monitoring of the golden jackals in Europe: setting the frame for future analyses".

Dear Editor-in-Chief and Editorial Board,

Enclosed is a revised version of the manuscript titled "Acoustic monitoring of the golden jackals in Europe: setting the frame for future analyses" by Comazzi, Mattiello, Friard, Filacorda and Gamba to be considered for publication as a research article in Bioacoustics.

As already mentioned during our previous submission, this multi-annual study demonstrates that golden jackals are regularly ranging in Italy after a long absence from the records. Groups and single individuals move throughout a portion of the Friuli-Venezia Giulia region and in some cases possibly maintain persistent spatial position over years. The team of Prof. Stefano Filacorda initiated a jackal howling activity years ago. In the present study, we collected the recordings obtained by the emission of the howling stimuli, which lead to the collection of the first acoustic recordings of the golden jackals in Italy. All the basic information about the sounds recorded is described in this Manuscript. We have evaluated the minimum number of vocalizing individuals and quantitatively analyzed vocalizations using Dynamic Time Warping to understand structural variation and set the frame for future investigations in Italy and Eastern Europe.

We are still convinced that the results are important for future studies and provide readers with insights into the understanding of species behavioral ecology. These results are of great practical interest in conservation planning, also because few previous quantitative investigations studies that have deepened howling structure and inter-group dynamics in the golden jackals.

We are encouraged by the referees' comments we obtained on the previous version and we are convinced that the current revised version is largely improved, also thanks to the constructive work of the referees. We accessed the files suggested by Referee #1, which were useful for the validation of our estimation of the number of vocalizing individuals but failed to enter the automatic DTW and the cluster analysis because of their low quality.

We did neither publish nor submit the paper anywhere else. All the authors of the paper approved the revised version of the manuscript and agreed in submitting it to your journal.

We hope that the current version is acceptable for publication.

Thank you.

Best regards.

for the Authors

Carlo Comazzi

Acoustic monitoring of the golden jackals in Europe: setting the frame for future analyses

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Abstract

The golden jackal (*Canis aureus)* utters complex howls that can be used to monitor the population density and distribution in a specific area. However, little is known of the vocal behaviour of this species. In the present paper we show the first results of the acoustic analysis that followed the acoustic monitoring of the golden jackal in Friuli–Venezia Giulia during 2011–2013. We estimated the number of callers by screening the fundamental frequency of the emissions within a howl. We analysed 42 vocalizations given by a single jackal or multiple individuals. The howling duration significantly increased with the number of emitters, which ranged between one and three in our estimates. Twenty-nine howls were then submitted to a quantitative semi–automatic analysis procedure based on dynamic time warping. On the basis of the resulting dissimilarity indices, vocal emissions were clustered in six different acoustically uniform groups, which showed a potential for these procedures to be developed into future monitoring tools. The results suggest the need for integration between jackal howling, bioacoustics and camera trapping.

Introduction

Acoustic monitoring has raised more attention in the recent years, and can represent a primary source to derive measures of animal abundance (Marques et al. 2013). Passive acoustic monitoring (PAM) is now commonly used to detect marine mammal acoustic signals (McDonald and Fox 1999; Van Parijs et al. 2009), and it has been increasingly used to study other taxa (Dawson and Efford 2009; Nagy and Rockwell 2012), including terrestrial mammals (Blumstein et al. 2011). Moreover, passive acoustics is also highly amenable to automated data collection and processing while this information can be gathered in environments where it is not easy for a human observer to work (Marques et al. 2013).

The golden jackal is an opportunistic omnivore with a widespread distribution in several countries of the African continent, Middle East, Asia and Europe (Kryštufek et al. 1997; Lapini 2003; Jhala and Moehlman 2004; Humer et al. 2007; Lapini et al. 2009); data on its density are reported by several authors (Spassov and Markov 2004; Giannatos et al. 2005; Humer et al. 2007; Spassov 2007; Tóth et al. 2009; Arnold et al. 2011). As for Italy, the current distribution is fragmented and probably underestimated, but recent information from the regions Veneto and Trentino Alto Adige, together with documented breeding events in Friuli–Venezia Giulia (Lapini et al. 2009), suggests a stable distribution across the north–west of the country (Lapini 2010). The presence of a new predator may create potential conflicts with other wild species living in the same area and also with farming activities. In fact, occasional occurrence of predation events on livestock has already been observed (Benfatto et al. 2014). An accurate monitoring of the population is important to estimate population trend (distribution and consistency) and pack size (Filibeck 1982), which may be useful in predicting the impact of predators on other wild and domestic species (Marucco and McIntire 2010).

Information about jackals' vocal behaviour is still scanty. As for other Canid species, the golden jackal exhibits a complex vocalization repertoire (Jhala and Moehlman 2004), including single and group howls. These calls mainly serve to maintain group cohesion and play a role in finding a reproductive partner and in territorial defence. They are usually more frequent in the reproductive period (Jaeger et al. 1996) and in areas at high population density (Giannatos 2004; Jaeger et al. 2007). Giannatos et al. (2005) reports that solitary individuals vocalize less frequently than those in a pack, possibly due to their young age or to their attempt to avoid fights with resident packs. Other than howls, the vocal repertoire includes hisses, huffs and roars (Lapini 2010) and a species–specific alarm call elicited by the presence of other large carnivores as wolves, hyenas and tigers (Jerdon 1874 in Jhala and Moehlman 2004).

The aim of this study was to acquire a deeper knowledge on jackals vocal behavior, in order to set the basis for the refinement of the existing monitoring tools and possibly for the development of new non–invasive monitoring methods, which can also lead to individual censuses. First, we examined the acoustic structure of the howl to estimate the minimum number of vocalizers. This first step allowed gathering information about the minimum number of jackals in a pack, which is crucial to infer about the size of the population (Barrientos 2000). We then performed a quantitative semi–automatic analysis based on dynamic time warping that can serve developing further acoustic monitoring techniques and may provide researchers with an important basis for management tools

(Azzolin et al. 2014). Although still not comparable with the vast evidence of voice studies (Rabiner and Schafer 1978; Salvador and Chan 2007; Muda et al. 2010), the application of dynamic time warping has been useful for the classification of animal sounds in various species (Trawicki et al. 2005; Clemins and Johnson 2006; Ranjard and Ross 2008; Tao et al. 2008; Brown and Smaragdis 2009; Meliza et al. 2013; Gamba et al. 2015). Dynamic time warping is a spectrogram alignment procedure that allows comparing sounds belonging to large datasets. The procedure is based on a method commonly used in speech science, that relies on the calculation of cepstrum coefficients (Davis and Mermelstein 1980). These coefficients provide a representation of the energy distributed at the various frequencies in the sound spectrum and, even if the computation of cepstral coefficients is usually performed to match the sensitivity of human ear, they have been shown to be useful in the study of animal calls (Ranjard et al. 2010; Riondato et al. 2013).

Material and methods

Data collection

We recorded jackal vocalizations in Friuli–Venezia Giulia (North–Eastern Italy) from summer 2011 to spring 2013 during a jackal–howling monitoring activity carried out by the University of Udine (Confalonieri et al. 2012). The study area consisted of 149 GIS-based grid cells of 3x3 km each. Because of the rough morphology of the study area, grid cells were reduced in respect to those used by Giannatos et al. (2005) and Krofel (2008) in order to obtain an approximate listening radium of 1.5 km. For the present study, the area was divided into five macroareas. In each macroarea, six stations were semi-opportunistically selected for howling emissions to increase the probability of detecting jackals' presence. For the howling emissions, we took into account different factors. A station (i) was located near the centre of the cell, possibly in an elevated position thus to allow a better broadcast of the stimulus. The station (ii) was at a minimum distance of approximately 2.0 km from villages to avoid masking due to excessive environmental noise. The station (iii) was accessible by car or after a short walk to optimise the logistics. We selected a total of 30 stations (Fig. 1). Each station was visited approximately once every 30 to 45 days to avoid overstimulation of the jackals.

In a single night, we emitted the playback stimuli, starting from one hour after sunset until maximum one hour before sunrise, in random order from each of the six stations of a macroarea, trying to minimise acoustic disturbance mainly related to anthropogenic activities. Each playback session consisted on average of about five emissions (min 1, max 8 emissions) of 30 seconds each.

In between each emission, there was a 3–minute silence. At the end of each session, we waited for 10 minutes in case of possible delayed answers by the animals. Sound intensity was increased at each emission and played towards a different direction to cover 360° degrees. In case of rain or strong wind, the activity was suspended, therefore in some cases we could not complete all the sessions. A total of 145 playback sessions and 679 emissions was carried out.

For playback activities, we used a custom–made portable audio speaker (Audio Source s.r.l., Udine, Italy) and pre–recorded howls. The unit contains an exponential horn sized 270 x 170 x 215 mm driven by a 20 W power amplifier and an on–board equalizer, which guarantee a flat frequency response of 550 Hz–3 kHz. The howls were previously available in the laboratory of the Department of Agriculture, Food, Environmental and Animal Sciences and they originated from Greece. During the reproductive period we played back a chorus track, while a pair track was played back during the rest of the year. Recordings were made using digital solid–state recorders (Sound Devices 702 and Sony PCM–M10) equipped with different microphone systems (Sennheiser MKH60, Telinga Pro 7 + Stereo Dat Mic + parabolic dish). Recordings were digitized at 48 kHz sampling rate (24 bit depth) and WAV file format.

Data processing

We recorded a total of 42 vocalizations, which were then processed using four different programs. The recordings obtained were referred to as group howls or choruses, in the case we could recognize multiple vocalizers, or as howls, in the case we could indicate the utterance of a single jackal during the spectrographic inspection. Pro Tools 9.0 (Avid Technology Inc.) was used to edit each recording session and to select those parts including jackal calls. The sounds were then exported to Raven Pro 1.4 (Cornell Lab of Ornithology), where they were precisely edited and spectrographically inspected (by aureal and visual inspection) to detect the minimum number of vocalizing individuals and to measure the duration of playback responses (for details, see Electronic Supplemental Online Material). We estimated the minimum number of vocalizers by considering whether more than one fundamental frequency present at a particular time occurred during the chorus (Fig. 2). We measured the duration and estimated the minimum number of emitters of all howlings (n = 42). Sound files were then pre–processed using Praat 5.3.52 (Boersma and Weenink, University of Amsterdam), before dynamic time warping analysis. In Praat, each soundfile was normalized using a *scale to peak* function. Sample rate and bit depth were set at 44.1 kHz and 16 bit respectively.

A sample of 29 recordings, in which the quality of the recording (signal to noise ratio) allowed further analysis, were then submitted to an acoustic distance calculation using a dynamic time warping analysis. Thirteen recordings failed to enter the analysis because of their low quality (e.g.; insufficient signal to noise ratio). Because the duration of the recordings may change dramatically, we standardized the duration of each sample by selecting the initial 10 seconds of the recorded signal, of either a howl or a chorus. To limit anthropogenic noise, we used a frequency range of 350 to 1850 Hz.

We used a method currently implemented in the package called DTWave (University of Auckland). A sequence of cepstrum coefficients was computed for each signal by means of a Mel filterbank (Ranjard et al. 2010) using the Hidden Markov Model Toolkit (Young 1994). When acoustic signals were submitted to the Hidden Markov Model Toolkit we used a target rate of 50,000 ns and a window size of 100,000 ns. Once all cepstral coefficients were aligned and rescaled, the software constructed an average vector sequence. Then, dynamic time warping calculated the pairwise distances between all the signals in the dataset until only the sequence representing an average of all howl sequences remained (see Ranjard and Ross 2008). Previous studies showed that duration may have a critical impact on the dissimilarity calculation (Gamba et al. 2015).

Data analysis and validation

Because the distribution was not normal, we used the Mann–Whitney U test (MWW) to understand whether the howls emitted by a different number of jackals differed in duration.

To identify independent groupings and to visualize emerging groups of signals (Nowicki and Nelson 1990), we clustered the howls on the basis of their degree of dissimilarity, as measured by the pairwise comparison. We used the Affinity Propagation (AP) tool (Frey and Dueck 2007) using the *apcluster* package in R (Bodenhofer et al. 2011; Hornik 2013). The AP clustering requires a limited number of assumptions and simultaneously considers all the data points as potential cluster centres ('exemplars'). It then chooses the final centres through an iterative process, after which the clusters also emerge. Although the user does not define the number of clusters or the number of exemplars (Bodenhofer et al. 2011), the preference (p) is a critical parameter. The preference with which a data point is chosen as a cluster centre determines the number of clusters in the final solution. Moreover, because AP clusterization does not automatically converge to an optimal solution, we used an external validation procedure. This validation was based on a q–scanning process (where q corresponds to the sample quantile of p, Gamba et al. 2015). We evaluated the clusters obtained using different preferences by the Adjusted Rand Index (Hubert and Arabie 1985) to assess the stability of successive cluster solutions (Hennig 2007). We used the exemplars in the final clustering solution to label the respective clusters. We obtained the most stable cluster solutions (Adjusted Rand Index = 1.000) for $q > 0.5$. Thus, we used $q = 0.5$ for the AP clustering presented in the Results.

To test our estimation of the number of vocalizing individuals, we have accessed additional jackal recordings of captive groups with known size. We used sound files available from an online library (http://www.tierstimmenarchiv.de) identified with "TSA: Canis_aureus_S_" plus the following codes: 136, 137, 141, 146, 147, 153, 162, 232, 239. All the files were recorded in German zoos (Tierpark Berlin, Zoo Halle, Zoo Berlin) before 1960. They were analysed using Raven Pro 1.4 (Cornell Lab of Ornithology), and the estimated number of vocalizing individuals was then compared with the information available in the online description of each file.

Results

We obtained responses from surveys in two of the five macro areas, MA2 (Carnia) and MA5 (Goritian Karst). Eighteen out of 42 responses (43%) were given by single individuals. In choruses, usually a single animal started the emission with one or two notes at relatively low frequency (Fig. 2).

Number of emitters

The minimum number of emitters for each howl ranged from one jackal (N = 18), to two (N = 13) or three animals (N = 11). Howling duration ranged between 0.76 s to 62.78 s (average duration 29.9 ± standard deviation 3.7; Fig. 3). The duration of the howls emitted by a single jackal (20.23±14.40 s) significantly differed from that measured in howls emitted by two (31.27±12.23 s; MWW, $U = 52.00$, $z = –2.52$, $p = 0.011$) or three animals (40.36±12.03 s; MWW, $U = 20.00$, $z = –3.55$, $p < 0.001$). The differences between the duration of howls emitted by two versus three animals approached statistical significance (MWW, $U = 38.00$, $z = –1.94$, $p = 0.055$) (Fig. 3). The analysis of the sound files recorded in captivity revealed that the estimation of the number of vocalizers correctly matched with group size in eight sounds out of nine. In the case of "TSA: Canis_aureus_S_146_2_1" we indicated two vocalizing jackals, whereas the available notes reported a single individual.

Cluster analysis

The clustering procedure based on the dissimilarity indices indicated six clusters including four to six howls per cluster (Fig. 4). The analysis included 171 iterations (input preference = –1.24; sum of similarities = –17.40; sum of preferences = –7.46; net similarity = –24.86). The *affinity propagation* process identified an *exemplar* for each cluster. The spectrogram of each *exemplar* is shown in Fig. 4. The cluster analysis grouped howls according to their acoustic structure as follows:

– *Cluster* 1 (N = 4). We found here strongly frequency–modulated signals with multiple emitters overlapping each other. The first and second harmonics were clearly visible in the spectrogram. The howls grouped in this cluster were recorded across different seasons in 2011 (N=3) and 2012 (N=1).

– *Cluster* 2 (N = 6). The howls that clustered here had strong frequency modulation and showed multiple emitters overlapping each other. All signals grouped in this cluster have a weaker second harmonic. We found in this cluster three howls recorded, in different seasons, in 2011 and three recorded in 2012.

– *Cluster* 3 (N = 4). The howls showed moderate frequency modulation and higher harmonics. A howl was recorded in August 2011 and three in 2012 (March, July, and October).

– *Cluster* 4 (N = 4). The howls clustered here have notes with strong frequency modulation, with or without overlapping between individuals, often separated by short gaps. The howls that were grouped in cluster 4 were recorded in 2012 (N = 3, in March and July) and in 2013 (in February).

– *Cluster* 5 (N = 6). The signals featured long single notes with moderate frequency modulation, without overlapping between individuals, separated by silent gaps. We found in this cluster three howls recorded in 2011, in August, and three recorded in 2012 (in March and April).

– *Cluster* 6 (N = 5). The howls in this cluster have long notes showing high frequency modulation. We found two howls recorded in August 2011, two recorded in 2012 (in April and July), and a howl recorded in February 2013.

Discussion

The analyses presented in this paper are the first attempt to investigate the golden jackal howls quantitatively. We hope they will serve as a pilot study for future research.

Estimates of the number of callers

The minimum number of emitters within a chorus estimated by acoustic analysis was lower compared to the numbers reported during on–field monitoring sessions (Comazzi et al. 2015), where authors indicated numbers of synchronous singers of up to five individuals in one session. The overestimation/underestimation of the number of emitters can be due to different factors. The first is related to the pattern in which animals participate to the howl. In many species, mainly in those in which animals vocalize to advertise occupation of a territory, emitters turn their heads in different directions to maximize the broadcasting range of their calls (wolves – *Canis lupus*, Harrington and Mech 1979; Harrington 1989; indris – *Indri indri*, Torti et al. 2013). The perception of intensity variation during the playback response could provide listeners with the impression of a larger number of emitters. The same effect can then also be produced by frequency overlapping and from the simultaneous emission of different signals. It is also possible that the minimum number of vocalizers we estimated did not correctly match with the number of individuals within a pack. In fact, some members might be silent, or they can intervene in the howling at different times as it happens in wolves (Harrington and Mech 1979) and chorusing primates (Giacoma et al. 2010). The spectrogram inspection still appears a useful method to detect a minimum number of individuals within a pack or an area, assumed their responsiveness to jackal howling. The analysis of captive jackal choruses and howls provided the first validation to our estimation of the number of vocalizers. In all but one case we estimated the correct number of animals in the group. For the single case that revealed a difference in the estimated number, we think that there might be a mistake in the description of the data set. Of course, direct observation of wild packs or larger captive groups are needed for further consideration. Data coming from camera traps and scat analysis may then complement this information.

In agreement with previous studies, we recorded both single and group howls (Giannatos et al. 2005; Krofel 2008). Most of the responses (57%) were emitted by groups of animals, in agreement with the results obtained by Krofel (2008), who recorded 62% of group responses. According to Giannatos et al. (2005), this may be explained by the fact that lone and free–ranging young jackals usually respond less frequently than those belonging to a family group. However, individual responses do not necessarily indicate the presence of an isolated jackal. In fact, other animals belonging to the same group may temporarily be in different areas of their territory and, therefore, did not answer to the stimulation. Also, Giannatos et al. (2005) noticed that not all animals in a group always respond: for example, sub–adults do not always vocalize (confirmed by CC personal observations). In a restricted area, where the presence of at least two animals had been previously confirmed using spectrogram inspection and camera traps, we occasionally recorded individual responses (Comazzi, pers. obs.).

Duration and howling structure

The duration of the howls increased with the number of emitters and significantly differed between one and two or three animals. We can hypothesise that this longer duration may be because more animals join the chorus and reciprocally stimulate each other, inducing a prolonged duration of the howling. This effect of the number of vocalizers appears in agreement with what observed by Nowak et al. (2007) in wolves.

Our observations confirm that the structure of jackals' howling follows a fixed pattern, similar to that reported for wolves (Harrington and Mech 1979). A single animal usually starts with one or two notes, emitted at relatively low frequency. In most cases, a second individual intervenes on the second note with a howl at a higher frequency, and the howls of the two animals continue to overlap to form a chorus of frequency–modulated howls. The chorus then gradually evolves into short and distinct howls, yelps, barks and woofs, which become more accentuated at the end. In Carnia, in a single macroarea, we listened to isolated, scarcely frequency-modulated howls. We referred these calls to the observations of Giannatos et al. (2005), which reports that solitary individuals vocalize less frequently than those in a pack, possibly due to their young age or to their attempt to avoid fights with resident packs. Indeed, they probably indicated the presence of dispersed jackals or satellite individuals.

Cluster analysis

The clustering analysis conducted in this study is the first attempt to quantitatively evaluate variability between the jackals' howls. We also aimed to understand whether semi–automatic analyses could be applied to the emissions of this species, in a case where other techniques (e.g. Root–Gutteridge et al. 2013; Torti et al. 2013) could not be implemented because of the lack of information about vocalizers' identity. In fact, the structure of the howl is not related to seasonal effects and can therefore possibly be attributed to individual or group differences, to a particular social context, or to a different acoustic structure. As we recorded responses only from two of the five macro areas, we can hypothesize that we have recorded a pack repeatedly (see Zaccaroni et al. 2012). Unfortunately, these hypotheses could not be further investigated at the moment, because of the lack of additional information on the emitters.

In general, we obtained a small sample compared to our sampling effort, but we are confident that the present study will be important in a scenario in which the density of carnivores is increasing in Italy (Chapron et al. 2014; Galaverni et al. 2015).

Further studies on semi–automatic analyses, implemented with the use of camera traps and scat genetic analysis, may be useful to set a frame for the development of new non–invasive monitoring methods, which can also lead to individual censuses (Terry et al. 2005; Zimmer 2011). However, the implementation of these systems requires larger data collection and an accurate evaluation of the intra–specific variability joint with individual recognition.

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FIGURE LEGENDS

Fig. 1 Schematized map of the study areas in Friuli Venezia Giulia. The surveyed macroareas are indicated by codes MA1 (Val Aupa/Glazzat), MA2 (Carnia), MA3 (Plain), MA4 (Julian pre-alps/Natisone), MA5 (Goritian Karst). For each macroarea, the six howling stations are indicated by filled dots.

Fig. 2 Spectrogram of a typical golden jackal howl showing how a single animal started the emission with one or two notes at relatively low frequency. The spectrogram is generated in Praat with the following parameters: window length: 0.035 s, time range as shown (0–10 s); frequency range: 350–2000 Hz. We overlaid a Praat textgrid showing the information related to the number of emitters (S=silent interval; 1 = one emitter; 2 = two emitters).

Fig. 3 Box plot of howling duration (s) depending on the number of emitters.

Fig. 4 Polar dendrogram (center) showing how vocalizations of the golden jackals cluster together. For each cluster, we show a spectrogram of the exemplar chosen during the affinity propagation process. All spectrograms are generated in Praat with the following parameters: window length: 0.035 s, time range: 0–10 s); frequency range: 350–2000 Hz.

Acoustic monitoring of the golden jackals in Europe: setting the frame for future analyses

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Abstract

The golden jackal (*Canis aureus)* utters complex howls that can be used to monitor the population density and distribution in a specific area. However, little is known of the vocal behaviour of this species. In the present paper we show the first results of the acoustic analysis that followed the acoustic monitoring of the golden jackal in Friuli–Venezia Giulia during 2011–2013. We estimated the number of callers by screening the fundamental frequency of the emissions within a howl. We analysed 42 vocalizations given by a single jackal or multiple individuals. The howling duration significantly increased with the number of emitters, which ranged between one and three in our estimates. Twenty-nine howls were then submitted to a quantitative semi–automatic analysis procedure based on dynamic time warping. On the basis of the resulting dissimilarity indices, vocal emissions were clustered in six different acoustically uniform groups, which showed a potential for these procedures to be developed into future monitoring tools. The results suggest the need for integration between jackal howling, bioacoustics and camera trapping.

Introduction

Acoustic monitoring has raised more attention in the recent years, and can represent a primary source to derive measures of animal abundance (Marques et al. 2013). Passive acoustic monitoring (PAM) is now commonly used to detect marine mammal acoustic signals (McDonald and Fox 1999; Van Parijs et al. 2009), and it has been increasingly used to study other taxa (Dawson and Efford 2009; Nagy and Rockwell 2012), including terrestrial mammals (Blumstein et al. 2011). Moreover, passive acoustics is also highly amenable to automated data collection and processing while this information can be gathered in environments where it is not easy for a human observer to work (Marques et al. 2013).

The golden jackal is an opportunistic omnivore with a widespread distribution in several countries of the African continent, Middle East, Asia and Europe (Kryštufek et al. 1997; Lapini 2003; Jhala and Moehlman 2004; Humer et al. 2007; Lapini et al. 2009); data on its density are reported by several authors (Spassov and Markov 2004; Giannatos et al. 2005; Humer et al. 2007; Spassov 2007; Tóth et al. 2009; Arnold et al. 2011). As for Italy, the current distribution is fragmented and probably underestimated, but recent information from the regions Veneto and Trentino Alto Adige, together with documented breeding events in Friuli–Venezia Giulia (Lapini et al. 2009), suggests a stable distribution across the north–west of the country (Lapini 2010). The presence of a new predator may create potential conflicts with other wild species living in the same area and also with farming activities. In fact, occasional occurrence of predation events on livestock has already been observed (Benfatto et al. 2014). An accurate monitoring of the population is important to estimate population trend (distribution and consistency) and pack size (Filibeck 1982), which may be useful in predicting the impact of predators on other wild and domestic species (Marucco and McIntire 2010).

Information about jackals' vocal behaviour is still scanty. As for other Canid species, the golden jackal exhibits a complex vocalization repertoire (Jhala and Moehlman 2004), including single and group howls. These calls mainly serve to maintain group cohesion and play a role in finding a reproductive partner and in territorial defence. They are usually more frequent in the reproductive period (Jaeger et al. 1996) and in areas at high population density (Giannatos 2004; Jaeger et al. 2007). Giannatos et al. (2005) reports that solitary individuals vocalize less frequently than those in a pack, possibly due to their young age or to their attempt to avoid fights with resident packs. Other than howls, the vocal repertoire includes hisses, huffs and roars (Lapini 2010) and a species–specific alarm call elicited by the presence of other large carnivores as wolves, hyenas and tigers (Jerdon 1874 in Jhala and Moehlman 2004).

The aim of this study was to acquire a deeper knowledge on jackals vocal behavior, in order to set the basis for the refinement of the existing monitoring tools and possibly for the development of new non–invasive monitoring methods, which can also lead to individual censuses. First, we examined the acoustic structure of the howl to estimate the minimum number of vocalizers. This first step allowed gathering information about the minimum number of jackals in a pack, which is crucial to infer about the size of the population (Barrientos 2000). We then performed a quantitative semi–automatic analysis based on dynamic time warping that can serve developing further acoustic monitoring techniques and may provide researchers with an important basis for management tools

(Azzolin et al. 2014). Although still not comparable with the vast evidence of voice studies (Rabiner and Schafer 1978; Salvador and Chan 2007; Muda et al. 2010), the application of dynamic time warping has been useful for the classification of animal sounds in various species (Trawicki et al. 2005; Clemins and Johnson 2006; Ranjard and Ross 2008; Tao et al. 2008; Brown and Smaragdis 2009; Meliza et al. 2013; Gamba et al. 2015). Dynamic time warping is a spectrogram alignment procedure that allows comparing sounds belonging to large datasets. The procedure is based on a method commonly used in speech science, that relies on the calculation of cepstrum coefficients (Davis and Mermelstein 1980). These coefficients provide a representation of the energy distributed at the various frequencies in the sound spectrum and, even if the computation of cepstral coefficients is usually performed to match the sensitivity of human ear, they have been shown to be useful in the study of animal calls (Ranjard et al. 2010; Riondato et al. 2013).

Material and methods

Data collection

We recorded jackal vocalizations in Friuli–Venezia Giulia (North–Eastern Italy) from summer 2011 to spring 2013 during a jackal–howling monitoring activity carried out by the University of Udine (Confalonieri et al. 2012). The study area consisted of 149 GIS-based grid cells of 3x3 km each. Because of the rough morphology of the study area, grid cells were reduced in respect to those used by Giannatos et al. (2005) and Krofel (2008) in order to obtain an approximate listening radium of 1.5 km. For the present study, the area was divided into five macroareas. In each macroarea, six stations were semi-opportunistically selected for howling emissions to increase the probability of detecting jackals' presence. For the howling emissions, we took into account different factors. A station (i) was located near the centre of the cell, possibly in an elevated position thus to allow a better broadcast of the stimulus. The station (ii) was at a minimum distance of approximately 2.0 km from villages to avoid masking due to excessive environmental noise. The station (iii) was accessible by car or after a short walk to optimise the logistics. We selected a total of 30 stations (Fig. 1). Each station was visited approximately once every 30 to 45 days to avoid overstimulation of the jackals.

In a single night, we emitted the playback stimuli, starting from one hour after sunset until maximum one hour before sunrise, in random order from each of the six stations of a macroarea, trying to minimise acoustic disturbance mainly related to anthropogenic activities. Each playback session consisted on average of about five emissions (min 1, max 8 emissions) of 30 seconds each.

In between each emission, there was a 3–minute silence. At the end of each session, we waited for 10 minutes in case of possible delayed answers by the animals. Sound intensity was increased at each emission and played towards a different direction to cover 360° degrees. In case of rain or strong wind, the activity was suspended, therefore in some cases we could not complete all the sessions. A total of 145 playback sessions and 679 emissions was carried out.

For playback activities, we used a custom–made portable audio speaker (Audio Source s.r.l., Udine, Italy) and pre–recorded howls. The unit contains an exponential horn sized 270 x 170 x 215 mm driven by a 20 W power amplifier and an on–board equalizer, which guarantee a flat frequency response of 550 Hz–3 kHz. The howls were previously available in the laboratory of the Department of Agriculture, Food, Environmental and Animal Sciences and they originated from Greece. During the reproductive period we played back a chorus track, while a pair track was played back during the rest of the year. Recordings were made using digital solid–state recorders (Sound Devices 702 and Sony PCM–M10) equipped with different microphone systems (Sennheiser MKH60, Telinga Pro 7 + Stereo Dat Mic + parabolic dish). Recordings were digitized at 48 kHz sampling rate (24 bit depth) and WAV file format.

Data processing

We recorded a total of 42 vocalizations, which were then processed using four different programs. The recordings obtained were referred to as group howls or choruses, in the case we could recognize multiple vocalizers, or as howls, in the case we could indicate the utterance of a single jackal during the spectrographic inspection. Pro Tools 9.0 (Avid Technology Inc.) was used to edit each recording session and to select those parts including jackal calls. The sounds were then exported to Raven Pro 1.4 (Cornell Lab of Ornithology), where they were precisely edited and spectrographically inspected (by aureal and visual inspection) to detect the minimum number of vocalizing individuals and to measure the duration of playback responses (for details, see Electronic Supplemental Online Material). We estimated the minimum number of vocalizers by considering whether more than one fundamental frequency present at a particular time occurred during the chorus (Fig. 2). We measured the duration and estimated the minimum number of emitters of all howlings (n = 42). Sound files were then pre–processed using Praat 5.3.52 (Boersma and Weenink, University of Amsterdam), before dynamic time warping analysis. In Praat, each soundfile was normalized using a *scale to peak* function. Sample rate and bit depth were set at 44.1 kHz and 16 bit respectively.

A sample of 29 recordings, in which the quality of the recording (signal to noise ratio) allowed further analysis, were then submitted to an acoustic distance calculation using a dynamic time warping analysis. Thirteen recordings failed to enter the analysis because of their low quality (e.g.; insufficient signal to noise ratio). Because the duration of the recordings may change dramatically, we standardized the duration of each sample by selecting the initial 10 seconds of the recorded signal, of either a howl or a chorus. To limit anthropogenic noise, we used a frequency range of 350 to 1850 Hz.

We used a method currently implemented in the package called DTWave (University of Auckland). A sequence of cepstrum coefficients was computed for each signal by means of a Mel filterbank (Ranjard et al. 2010) using the Hidden Markov Model Toolkit (Young 1994). When acoustic signals were submitted to the Hidden Markov Model Toolkit we used a target rate of 50,000 ns and a window size of 100,000 ns. Once all cepstral coefficients were aligned and rescaled, the software constructed an average vector sequence. Then, dynamic time warping calculated the pairwise distances between all the signals in the dataset until only the sequence representing an average of all howl sequences remained (see Ranjard and Ross 2008). Previous studies showed that duration may have a critical impact on the dissimilarity calculation (Gamba et al. 2015).

Data analysis and validation

Because the distribution was not normal, we used the Mann–Whitney U test (MWW) to understand whether the howls emitted by a different number of jackals differed in duration.

To identify independent groupings and to visualize emerging groups of signals (Nowicki and Nelson 1990), we clustered the howls on the basis of their degree of dissimilarity, as measured by the pairwise comparison. We used the Affinity Propagation (AP) tool (Frey and Dueck 2007) using the *apcluster* package in R (Bodenhofer et al. 2011; Hornik 2013). The AP clustering requires a limited number of assumptions and simultaneously considers all the data points as potential cluster centres ('exemplars'). It then chooses the final centres through an iterative process, after which the clusters also emerge. Although the user does not define the number of clusters or the number of exemplars (Bodenhofer et al. 2011), the preference (p) is a critical parameter. The preference with which a data point is chosen as a cluster centre determines the number of clusters in the final solution. Moreover, because AP clusterization does not automatically converge to an optimal solution, we used an external validation procedure. This validation was based on a q–scanning process (where q corresponds to the sample quantile of p, Gamba et al. 2015). We evaluated the clusters obtained using different preferences by the Adjusted Rand Index (Hubert and Arabie 1985) to assess the stability of successive cluster solutions (Hennig 2007). We used the exemplars in the final clustering solution to label the respective clusters. We obtained the most stable cluster solutions (Adjusted Rand Index = 1.000) for $q > 0.5$. Thus, we used $q = 0.5$ for the AP clustering presented in the Results.

To test our estimation of the number of vocalizing individuals, we have accessed additional jackal recordings of captive groups with known size. We used sound files available from an online library (http://www.tierstimmenarchiv.de) identified with "TSA: Canis_aureus_S_" plus the following codes: 136, 137, 141, 146, 147, 153, 162, 232, 239. All the files were recorded in German zoos (Tierpark Berlin, Zoo Halle, Zoo Berlin) before 1960. They were analysed using Raven Pro 1.4 (Cornell Lab of Ornithology), and the estimated number of vocalizing individuals was then compared with the information available in the online description of each file.

Results

We obtained responses from surveys in two of the five macro areas, MA2 (Carnia) and MA5 (Goritian Karst). Eighteen out of 42 responses (43%) were given by single individuals. In choruses, usually a single animal started the emission with one or two notes at relatively low frequency (Fig. 2).

Number of emitters

The minimum number of emitters for each howl ranged from one jackal ($N = 18$), to two ($N = 13$) or three animals ($N = 11$). Howling duration ranged between 0.76 s to 62.78 s (average duration 29.9 ± standard deviation 3.7; Fig. 3). The duration of the howls emitted by a single jackal (20.23±14.40 s) significantly differed from that measured in howls emitted by two (31.27±12.23 s; MWW, $U = 52.00$, $z = -2.52$, $p = 0.011$) or three animals (40.36±12.03 s; MWW, $U = 20.00$, $z = -3.55$, $p < 0.001$). The differences between the duration of howls emitted by two versus three animals approached statistical significance (MWW, $U = 38.00$, $z = -1.94$, $p = 0.055$) (Fig. 3). The analysis of the sound files recorded in captivity revealed that the estimation of the number of vocalizers correctly matched with group size in eight sounds out of nine. In the case of "TSA: Canis_aureus_S_146_2_1" we indicated two vocalizing jackals, whereas the available notes reported a single individual.

Cluster analysis

The clustering procedure based on the dissimilarity indices indicated six clusters including four to six howls per cluster (Fig. 4). The analysis included 171 iterations (input preference = –1.24; sum of similarities = –17.40; sum of preferences = –7.46; net similarity = –24.86). The *affinity propagation* process identified an *exemplar* for each cluster. The spectrogram of each *exemplar* is shown in Fig. 4. The cluster analysis grouped howls according to their acoustic structure as follows:

– C*luster* 1 (N = 4). We found here strongly frequency–modulated signals with multiple emitters overlapping each other. The first and second harmonics were clearly visible in the spectrogram. The howls grouped in this cluster were recorded across different seasons in 2011 (N=3) and 2012 (N=1).

– C*luster* 2 (N = 6). The howls that clustered here had strong frequency modulation and showed multiple emitters overlapping each other. All signals grouped in this cluster have a weaker second harmonic. We found in this cluster three howls recorded, in different seasons, in 2011 and three recorded in 2012.

– C*luster* 3 (N = 4). The howls showed moderate frequency modulation and higher harmonics. A howl was recorded in August 2011 and three in 2012 (March, July, and October).

– C*luster* 4 (N = 4). The howls clustered here have notes with strong frequency modulation, with or without overlapping between individuals, often separated by short gaps. The howls that were grouped in cluster 4 were recorded in 2012 (N = 3, in March and July) and in 2013 (in February).

– C*luster* 5 (N = 6). The signals featured long single notes with moderate frequency modulation, without overlapping between individuals, separated by silent gaps. We found in this cluster three howls recorded in 2011, in August, and three recorded in 2012 (in March and April).

– C*luster* 6 (N = 5). The howls in this cluster have long notes showing high frequency modulation. We found two howls recorded in August 2011, two recorded in 2012 (in April and July), and a howl recorded in February 2013.

Discussion

The analyses presented in this paper are the first attempt to investigate the golden jackal howls quantitatively. We hope they will serve as a pilot study for future research.

Estimates of the number of callers

The minimum number of emitters within a chorus estimated by acoustic analysis was lower compared to the numbers reported during on–field monitoring sessions (Comazzi et al. 2015), where authors indicated numbers of synchronous singers of up to five individuals in one session. The overestimation/underestimation of the number of emitters can be due to different factors. The first is related to the pattern in which animals participate to the howl. In many species, mainly in those in which animals vocalize to advertise occupation of a territory, emitters turn their heads in different directions to maximize the broadcasting range of their calls (wolves – *Canis lupus*, Harrington and Mech 1979; Harrington 1989; indris – *Indri indri*, Torti et al. 2013). The perception of intensity variation during the playback response could provide listeners with the impression of a larger number of emitters. The same effect can then also be produced by frequency overlapping and from the simultaneous emission of different signals. It is also possible that the minimum number of vocalizers we estimated did not correctly match with the number of individuals within a pack. In fact, some members might be silent, or they can intervene in the howling at different times as it happens in wolves (Harrington and Mech 1979) and chorusing primates (Giacoma et al. 2010). The spectrogram inspection still appears a useful method to detect a minimum number of individuals within a pack or an area, assumed their responsiveness to jackal howling. The analysis of captive jackal choruses and howls provided the first validation to our estimation of the number of vocalizers. In all but one case we estimated the correct number of animals in the group. For the single case that revealed a difference in the estimated number, we think that there might be a mistake in the description of the data set. Of course, direct observation of wild packs or larger captive groups are needed for further consideration. Data coming from camera traps and scat analysis may then complement this information.

In agreement with previous studies, we recorded both single and group howls (Giannatos et al. 2005; Krofel 2008). Most of the responses (57%) were emitted by groups of animals, in agreement with the results obtained by Krofel (2008), who recorded 62% of group responses. According to Giannatos et al. (2005), this may be explained by the fact that lone and free–ranging young jackals usually respond less frequently than those belonging to a family group. However, individual responses do not necessarily indicate the presence of an isolated jackal. In fact, other animals belonging to the same group may temporarily be in different areas of their territory and, therefore, did not answer to the stimulation. Also, Giannatos et al. (2005) noticed that not all animals in a group always respond: for example, sub–adults do not always vocalize (confirmed by CC personal observations). In a restricted area, where the presence of at least two animals had been previously confirmed using spectrogram inspection and camera traps, we occasionally recorded individual responses (Comazzi, pers. obs.).

Duration and howling structure

The duration of the howls increased with the number of emitters and significantly differed between one and two or three animals. We can hypothesise that this longer duration may be because more animals join the chorus and reciprocally stimulate each other, inducing a prolonged duration of the howling. This effect of the number of vocalizers appears in agreement with what observed by Nowak et al. (2007) in wolves.

Our observations confirm that the structure of jackals' howling follows a fixed pattern, similar to that reported for wolves (Harrington and Mech 1979). A single animal usually starts with one or two notes, emitted at relatively low frequency. In most cases, a second individual intervenes on the second note with a howl at a higher frequency, and the howls of the two animals continue to overlap to form a chorus of frequency–modulated howls. The chorus then gradually evolves into short and distinct howls, yelps, barks and woofs, which become more accentuated at the end. In Carnia, in a single macroarea, we listened to isolated, scarcely frequency-modulated howls. We referred these calls to the observations of Giannatos et al. (2005), which reports that solitary individuals vocalize less frequently than those in a pack, possibly due to their young age or to their attempt to avoid fights with resident packs. Indeed, they probably indicated the presence of dispersed jackals or satellite individuals.

Cluster analysis

The clustering analysis conducted in this study is the first attempt to quantitatively evaluate variability between the jackals' howls. We also aimed to understand whether semi–automatic analyses could be applied to the emissions of this species, in a case where other techniques (e.g. Root–Gutteridge et al. 2013; Torti et al. 2013) could not be implemented because of the lack of information about vocalizers' identity. In fact, the structure of the howl is not related to seasonal effects and can therefore possibly be attributed to individual or group differences, to a particular social context, or to a different acoustic structure. As we recorded responses only from two of the five macro areas, we can hypothesize that we have recorded a pack repeatedly (see Zaccaroni et al. 2012). Unfortunately, these hypotheses could not be further investigated at the moment, because of the lack of additional information on the emitters.

In general, we obtained a small sample compared to our sampling effort, but we are confident that the present study will be important in a scenario in which the density of carnivores is increasing in Italy (Chapron et al. 2014; Galaverni et al. 2015).

Further studies on semi–automatic analyses, implemented with the use of camera traps and scat genetic analysis, may be useful to set a frame for the development of new non–invasive monitoring methods, which can also lead to individual censuses (Terry et al. 2005; Zimmer 2011). However, the implementation of these systems requires larger data collection and an accurate evaluation of the intra–specific variability joint with individual recognition.

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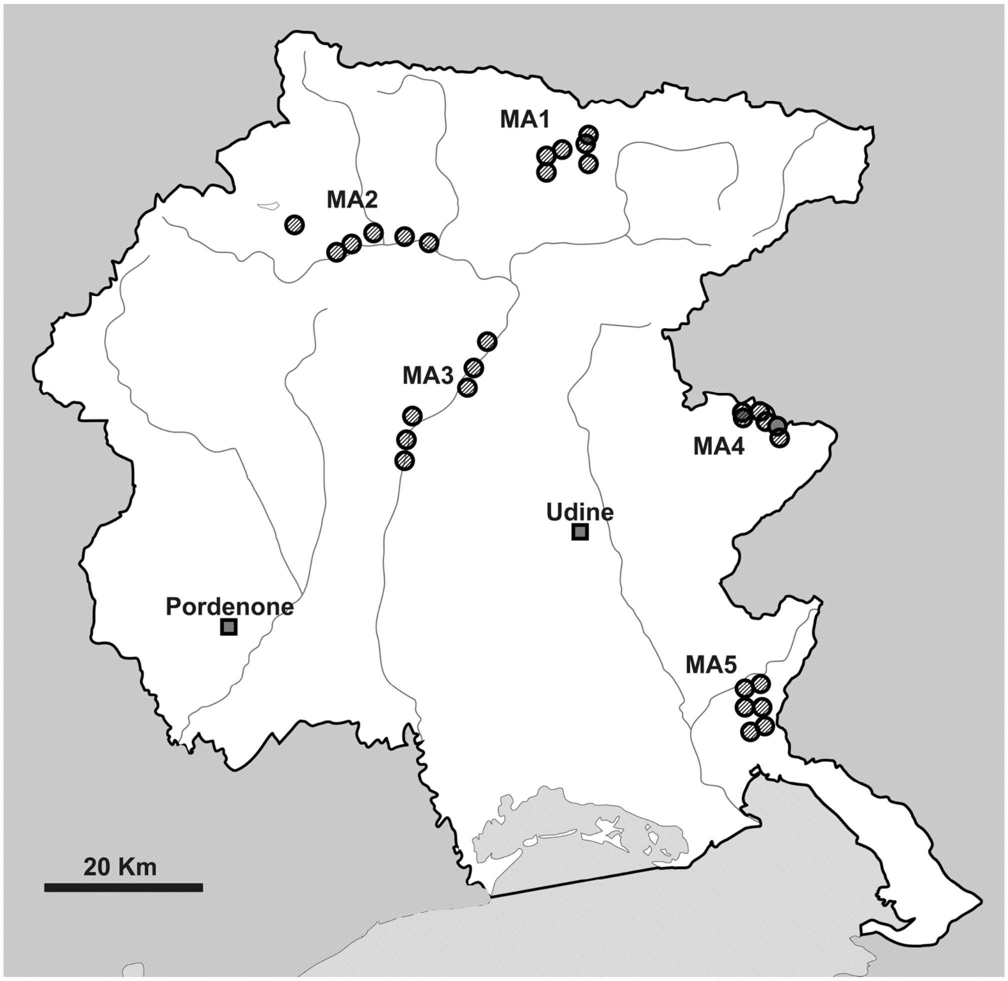

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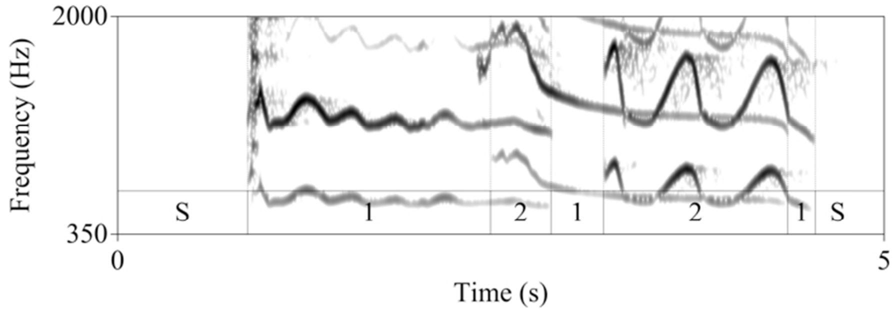

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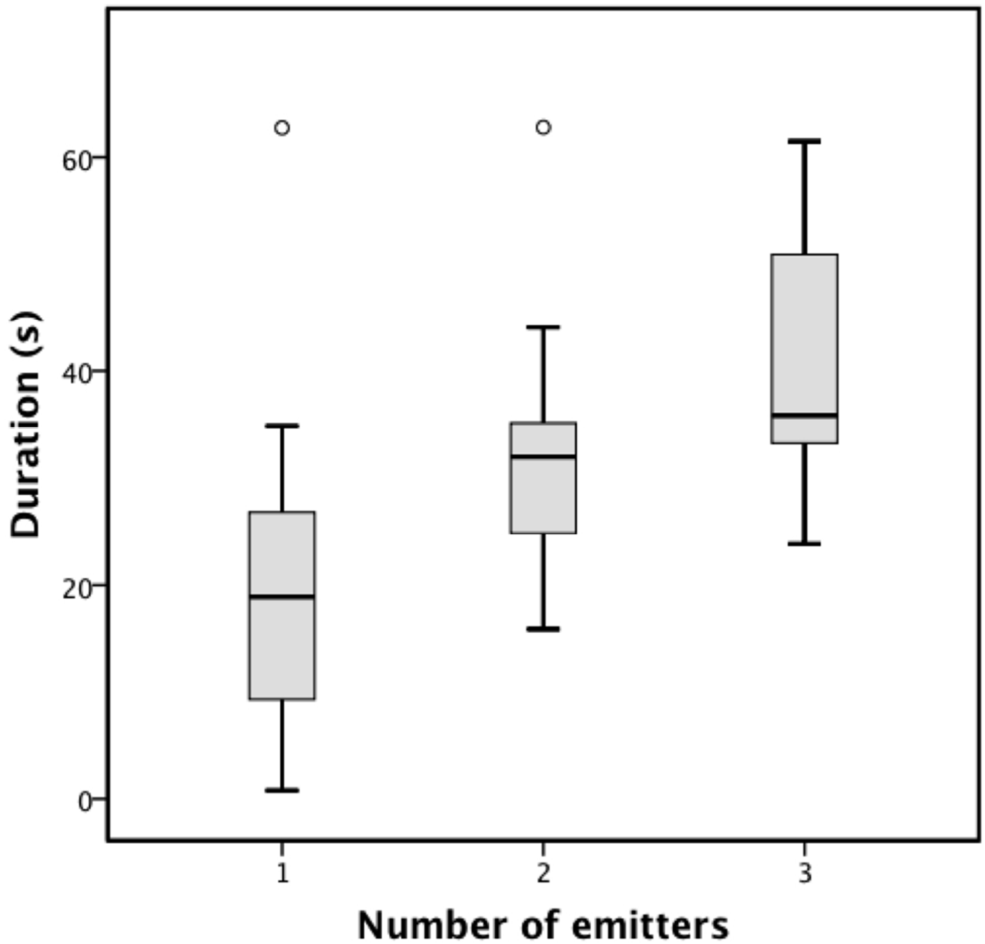

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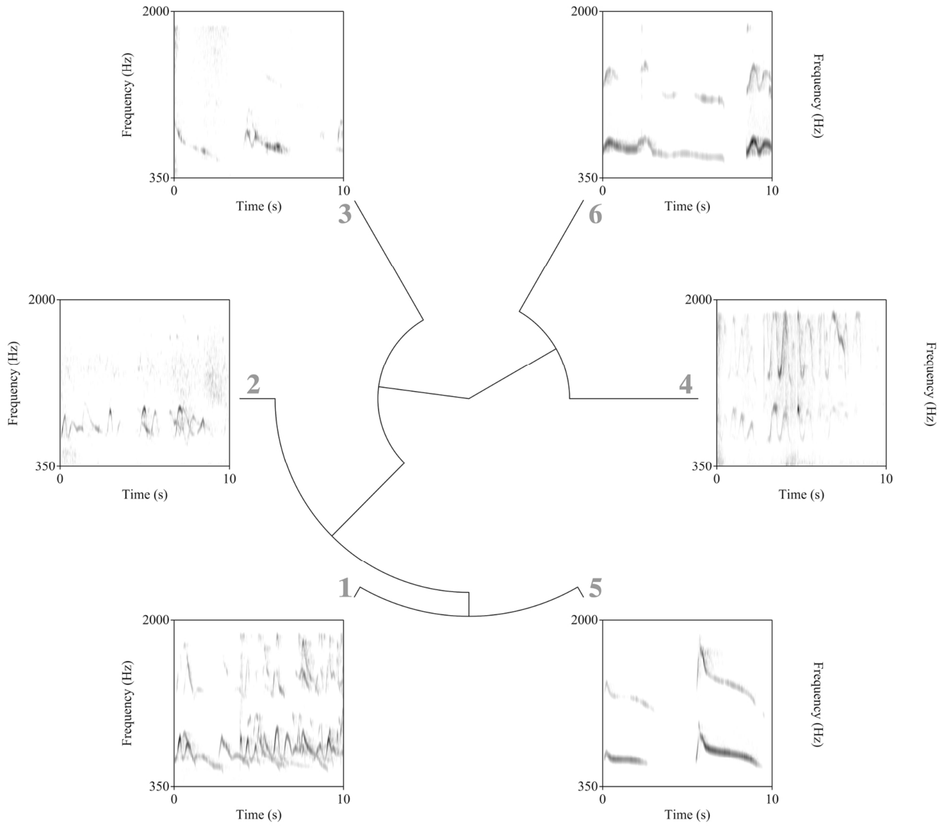

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Electronic supplementary material for Comazzi, Mattiello, Friard, Filacorda, Gamba "Acoustic monitoring of the golden jackals in Europe: setting the frame for future analyses".

Table 1

Detailed information on the recordings analysed in the current study: name of the sound file (Id. name); date of the recording (Date); duration of the howling (Duration); estimated minimum number of emitters (N. of emitters). An asterisk (*) denotes vocalizations submitted to dynamic time warping and cluster analysis.

Id. name	Date	Duration (s)	N. of emitters
2011_08_25_Carnia_001	2011.08.25	35.606	3
2011_08_26_Carnia_002	2011.08.26	34.854	1
2011_08_26_Carnia_003	2011.08.26	23.351	1
2011_08_29_Carso_004	2011.08.29	53.181	3*
2011_08_29_Carso_005	2011.08.29	48.571	3*
2011_08_29_Carso_006	2011.08.29	32.31	2*
2011_08_29_Carso_007	2011.08.29	15.914	2*
2011_08_29_Carso_008	2011.08.29	17.053	1*
2011_08_29_Carso_009	2011.08.29	20.09	1*
2011_08_30_Carnia_010	2011.08.30	12.304	1*
2011_08_30_Carnia_011	2011.08.30	30.378	1*
2011_08_30_Carnia_012	2011.08.30	23.602	2
2011_08_30_Carnia_013	2011.08.30	16.899	1
2011_12_07_Carso_014	2011.12.07	31.081	3*
2011_12_07_Carso_015	2011.12.07	54.028	3*
2011_12_07_Carso_016	2011.12.07	23.835	3*
2012_03_03_Carso_019	2012.03.03	35.163	2*
2012_03_03_Carso_020	2012.03.03	31.962	2*
2012_03_03_Carso_020_2	2012.03.03	32.899	2*
2012_03_03_Carso_021	2012.03.03	35.78	2*
2012_03_03_Carso_021_2	2012.03.03	44.066	2*
2012_03_03_Carso_022	2012.03.03	25.795	2*
2012_04_09_Carso_023	2012.04.09	26.828	1*
2012_04_09_Carso_024_2	2012.04.09	62.731	1*
2012_04_09_Carso_024	2012.04.09	33.016	1
2012_04_09_Carso_025_2	2012.04.09	62.778	2*
2012_04_09_Carso_025	2012.04.09	19.69	1
2012_07_28_Carso_026	2012.07.28	18.135	1*
2012_07_28_Carso_027	2012.07.28	9.174	1*
2012_07_28_Carso_028	2012.07.28	0,763	1
2012_07_28_Carso_029	2012.07.28	19.927	1*
2012_07_28_Carso_030	2012.07.28	9.308	1*
2012_10_03_Carso_031	2012.10.03	35.851	3
2012_10_03_Carso_032	2012.10.03	35.359	3*
2012_10_03_Carso_033	2012.10.03	6.489	1
2012_12_03_Carso_034	2012.12.03	3.225	1
2012_12_03_Carso_035	2012.12.03	28.437	3*
2013_02_05_Carso_036	2013.02.05	24,882	2
2013_02_05_Carso_037	2013.02.05	24,957	2
2013_02_07_Carso_038	2013.02.07	17,324	2
2013_02_07_Carso_039	2013.02.07	36,526	3
2013_02_07_Carso_040	2013.02.07	61,477	3*