VOCAL SIMILARITY AND DIFFERENCES IN FOUR SPECIES OF THE GENUS ANTHUS: IMPLICATION ON COMMUNICATION AND TAXONOMIC RELATEDNESS

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ABSTRACT

Similar flight call (*tsip*) of two closely related species (*Anthus pratensis*, *Anthus spinoletta*) showed species-specific characteristics, that allows to discriminate the two species only with acoustic means. Discriminant function analysis was able to correctly classify 98,4% of the recordings. Three acoustic parameters showed the highest power in the discrimination process: the frequency modulations of the call, the maximum frequency value and the minimum frequency value of the peak frequency contour. Using these three values I proposed a simple method for recognizing these two species, that allowed a correct classification of 96% of recordings. Three different call types (*tsip*, *soft* and *alarm*) of four species of the genus Anthus (*A. pratensis*, *A. spinoletta*, *A. petrosus*, *A. cervinus*) were also studied to investigate their acoustic similarity and difference. Alarm calls of the four species showed a very strong similarity, while the other two call types were more distinctive showing a much lower similarity, with soft call seeming to have a lower similarity between species and hence a higher distinctive power. These results supported the hypothesis that alarm call is similar, because it retains features of a common ancestor and may ease heterospecific communication, while the other two call types (*tsip* and *soft*) showed a decreasing similarity and more species-specific features. Integrative taxonomy is commonly used in phylogenetic studies and besides genetic and morphological information, acoustic data play a major role in taxonomic designation of birds. The song of passerines was extensively investigated in phylogenetic comparative studies, but the data reported in this paper suggest that also the comparisons of call repertoires in closely related species may be a useful tool for taxonomic studies.

KEYWORDS: call similarity - acoustic recognition - genus Anthus - vocal behaviour - taxonomy

INTRODUCTION

Two congeneric similar species, sharing the same wintering area and often the same habitat, cope with problems of intraspecific and interspecific communication. They should have finely tuned capability of call

discrimination between species, but in some cases they might also benefit from interspecific communication. Meadow pipits (*A. pratensis*) and Water pipits (*A. spinoletta*) are morphologically similar species, they have partly overlapping wintering range and often share feeding areas (Cramp 1983; Alström and Mild 2003; Brichetti and Fracasso 2007). Outside the breeding season the vocal repertoire of these two species rely mainly on two call types, a *tsip* call used more often in flight and a *soft* call typically uttered between members of a flock as a contact signal (Elfström 1992; Alström and Mild 2003). The former shows a very similar spectrographic structure for both species (Alström and Mild 2003; Bergmann et al. 2008), although it can sound slightly different for a much trained ear (Brichetti and Fracasso 2007). In this paper I try to answer two questions: i) are there objective measurable differences among vocal parameters of *tsip* call for these two species ?; ii) is there a measurable vocal threshold that allows a safe identification of the species ?

Elfström (1992) showed that calls of *A. pratensis* and *A. petrosus* contain species-specific characteristics and that these species are able to discriminate between conspecific and heterospecific calls of similar configuration at a distance. Finding comparable species-specific differences also for *tsip* call of *A. pratensis* and *A. spinoletta* would support the hypothesis that this call type is mainly used for intraspecific communication.

In addition, vocal identification can be a useful tool for field work when the two sister species share the same habitat and in the case of passive recording of migratory birds. Indeed, flight call recording and identification of nocturnal migrant birds is a method used to study this fascinating behaviour (Hamilton 1962; Gal 2003; Farnsworth 2005). Some evidences suggest that birds of the genus Anthus, although mainly diurnal migrants (Elkins 2005), can migrate also by night (Ebenhöh 1998; Gal 2003; Wang 2019; Briedis et al. 2020).

For years bird calls were considered innate (see for example Hamilton 1962), because subjects raised away from their own species developed abnormal songs but normal calls. After the discovery of developmental plasticity in the calls of several bird species (Mudinger 1970; Mudinger 1979; Marler 2004), learning appeared to play a role in the call repertoire of some passerines. Particularly flight calls of cardueline finches showed remarkable plasticity and dialects were discovered for other call types (Baker et al. 2000; Marler 2004). Although bird calls have not been studied as extensively as bird song, today it is commonly accepted that some calls are learned while others appear to be innate (Zann 1985; Williams 2008), of course we need more data and more species studies to increase our knowledge about this topic and about intra/interspecific communication. Investigating the similarities and differences among closely related species can be particularly fruitful for this task, therefore I also collected some preliminary data on three different call types of four Anthus species (*A. pratensis, A. spinoletta, A. petrosus, A. cervinus*). Some calls can share very similar characteristics in sister species (Stefanski and Falls 1972; Fallow et al. 2011; Gayk et. al 2021), while others can be more or less distinctive for intraspecific recognition and/or communication (Elfström 1992; Kroodsma 2000; Fijen 2014). Genetic relatedness should favour a stronger similarity mainly for those innate calls that convey crucial information involved with immediate issues of life and death. On the other hand there are also calls, either genetically determined or developed by learning processes, which contains

distinctive features and are important for species-specific recognition. Studies of interspecific and intraspecific variability in the calls of closely related species can be particularly helpful to shed some light on these topics.

METHODS

I recorded calls of *Anthus pratensis* and *Anthus spinoletta* in central Italy with a Fostex FR2 digital recorder in wav uncompressed format (48 kHz sampling rate, 16 bit resolution). The microphone was a Sennheiser Mkh20 in the focus of a 22" Telinga parabolic dish. The species identification was made visually (with binoculars, cameras and spotting scopes) before starting the recording session. Moreover almost all recordings of *A. spinoletta* were made in nesting areas of this species, where *A. pratensis* is absent.

34 good quality recordings of *A. pratensis* and 31 of *A. spinoletta* were made from 2004 to 2021 in different places of Central Italy, therefore it is very unlikely that I have recorded the same bird more than once. Many recordings contain vocalizations of two or three different birds that are easily separable, however to avoid any risk of pseudoreplication, each recording was always considered as one single independent data. The *tsip* call type (in flight) was selected from these recordings following the classification and spectrographic representation available in the literature (Cramp 1983; Elfström 1992; Alström and Mild 2003; Bergmann 2008; Fijen 2014; Garner et al. 2015). All spectrograms were made with Raven Pro 1.6.1 (Center for Conservation Bioacoustics 2019): window and DFT size 1024 samples, overlap 75%, window type Hann (3dB filter bandwidth 67.5 Hz).

One *tsip* call template of the two species (Fig. 1) was chosen among good quality recordings, with a signal to noise ratio above 10 calculated on the amplitude values expressed by Raven in arbitrary units. This call type has at least three visible harmonics and the second is always the dominant one, therefore a bandpass filter was used for limiting the analysis to this harmonic. As shown in Figure 1 most sound energy of the template calls is concentrated between 4 and 8.5 kHz, for this reason I used the band limited energy detector of Raven Pro to select all the *tsip* calls to be analysed. The detector parameters were: minimum frequency 4000 Hz, maximum frequency 8500 Hz, minimum duration 0.03 s, maximum duration 0.2 s, minimum separation 0.02 s, minimum occupancy 50%, SNR (signal to noise ratio) threshold 5 dB. The detections showing unwanted signals (e.g. overlapped calls of the target species or of other species, various noises) were discarded. A detailed acoustic analysis was performed on 212 *A. pratensis tsip* calls from 50 different birds and 165 *A. spinoletta tsip* calls from 42 different birds and data were averaged for each recording, yielding 34 and 31 independent data for the two species; the following parameters were calculated by means of the appropriate Raven Pro functions: Center Frequency (CF), Frequency Contour Percentile 75 (FC75), Peak Frequency Contour Max Frequency (PFCMa), Peak Frequency Contour Min Frequency (PFCMi), Duration 90% (DUR90). The difference between PFCMa and PFCMi was calculated as a measure of the frequency bandwidth (BW). I counted for each call the number of relative maximum plus minimum values

(RMM) found by the FC75 function. The time interval between the beginnings of consecutive call selections (TI) was calculated. In 2020/2021 further 5 recordings of *A. pratensis tsip* calls were made in Northern and Central Italy and other 5 recordings of *A. spinoletta tsip* calls from Central Europe and Northern Italy were provided by Xeno-Canto recordists in wav uncompressed original format. These 10 recordings (45 calls by14 different birds) were used to test the ability of DFA to discriminate the two species.

The three different call types (*tsip*, *soft* and *alarm*) of the four Anthus species (*A. pratensis*, *A. spinoletta*, *A. petrosus*, *A. cervinus*) were compared following the classification and spectrographic representation of Elfström (1992) and Alström and Mild (2003). Figure 1 shows the template calls chosen for each call type of the three species. Recordings of *A. petrosus* and *A. cervinus*, recordings of *A. pratensis alarm* call and some recordings of *A. spinoletta alarm*, *tsip* and *soft* call were audio files published on Xeno-Canto under Creative Common licence (CC BY-NC-ND). Unfortunately most of these recordings were not available in an original non-compressed (wav) format, but only as mp3 files. Since all the recordings were not homogeneous as file format, recording tools and editing processes, a deep analysis of the acoustic parameters, as made for *A. pratensis* and *A. spinoletta tsip* call, could have yielded unreliable results. To avoid this drawback I preliminarily tested the capability of Raven Pro FC75 function to assess in a sufficiently precise manner the general morphological traits and modulations of the same calls recorded in three different ways: wav original uncompressed format, mp3 compressed file of medium quality (170/210 kbps), mp3 compressed format of low quality (145/185 kbps). The results of this test (not shown in the present paper) suggested that FC75 function was able to assess correctly the broad morphological characteristics, modulations and shapes for all three different quality levels. Therefore the comparison among the three call types was made using only the FC75 parameter, calculated over 84 randomly chosen calls for each call type of the four Anthus species. The *alarm* call recordings were 10 for each Anthus species (each giving an average independent data calculated over 7 – 9 calls), the *tsip* call recordings were 13 (each giving an average of 6 – 8 calls) and the *soft* call recordings were 12 (each giving an average of 6 – 8 calls). Most recordings contained vocalisations from more than one bird, but the independent data used for the statistical analysis was always the average of randomly chosen calls for each recording. The call selection was made with the same method described above, using the band limited energy detector of Raven Pro (band limits: 4000 - 8500 Hz, 3000 - 7000 Hz, 3500 - 7000 Hz for *tsip*, *soft* and *alarm* call respectively). All recordings were filtered with a bandpass filter for limiting the analysis to the dominant harmonic where most of sound energy was found.

Statistical analysis.

Where not specified otherwise all the statistical tests were performed using PAST v. 4.09 package (Hammer et al. 2001). Multivariate normality of data was checked with omnibus test by Doornik and Hansen (2008). The comparison of *A. pratensis* versus *A. spinoletta tsip* call was performed with PERMANOVA based on Euclidean distance measure (Anderson 2001). Principal components analysis (PCA) was used to find hypothetical variables (components) accounting for as much as possible of the variance in these multivariate data (Legendre and Legendre 1998). Coefficient of variation (CV) was calculated with the following

formula: CV =100*(1+1/4n)*sd/m (Scherrer 1984; Aubin et al. 2004), where n = number of observations, SD = standard deviation, m = mean. I have also calculated a CVr as the ratio of interspecific (CVis) versus average intraspecific coefficient of variation (CVin). CVr is a measure of the identification power of each vocal parameter. The equivalence of the variance/covariance on multivariate samples was checked with Box's M test (Rencher 2002); this is a prerequisite to perform the discriminant function analysis (DFA), used to find the differences among acoustic parameters of *A. pratensis* versus *A. spinoletta tsip* calls.

The comparison among four Anthus species (*A. pratensis, A. cervinus, A. spinoletta, A. petrosus*) was performed using FC75 to find similarity and differences in the three different call types (*alarm, tsip, soft*). The randomly selected call contours were averaged for each recording and an averaged contour was then calculated for the four species in the three different call types. Each averaged contour represented a series of frequency data evenly spaced in time (5 ms apart). Mantel correlogram (Legendre and Legendre 1998), that is a multivariate extension to autocorrelation based on any similarity or distance measure, made a first comparison among all four species contours of each call type. The correlation index of Mantel test showed the similarity between the averaged call contours shown in Figure 3. A second approach was made with a more classical cross-correlation. All paired comparisons were performed between average call contours from every recording of the four species for each different call type with the following constraints: each recording must be used only once, all recordings must be used, each pair of recording comparison was randomly chosen. This scheme yielded for example 10 comparisons for *alarm* call of *A. pratensis* versus *A. spinoletta*, 10 comparisons of *A. pratensis* versus *A. cervinus* ...and so on. An average correlation index was calculated for the four species on each call type at two different lags (lag 0 and lag of maximum correlation index); the average value of the lag accounting the maximum correlation index was also calculated. Of course a higher correlation index at lag 0 or near 0 expresses a higher similarity among different species for that particular call type. The cross-correlation was calculated using the R function ccf (R core Team 2020).

RESULTS

Comparison between *tsip* call of *A. pratensis* versus *A. spinoletta*. Table 1 shows *tsip* call parameters of *A. pratensis* (average of 212 calls selected from 34 recordings of 50 different birds) and *A. spinoletta* (average of 165 calls selected from 31 recordings of 42 different birds). Doornik and Hansen (2008) omnibus test indicates a highly significant (p<0,001) departure from normality of these *tsip* call data. Therefore the two species were compared by means of non parametric PERMANOVA test, which showed a highly significant difference (F=45; P<0,001) of acoustic features.

Principal component analysis (PCA) of all multivariate data (Figure 2) was performed. The three first components accounted for 89,4% of the variance and PCA loadings of the variables were all comparable with values ranging between 0,33 to 0,38 except for TI, that showed a lower value (0,28). Since TI has

several missing data and a very high CVis (Table 1) with a quite low CVr, it was excluded from further analyses because of its unreliability.

CVr was higher than 1 for all acoustic parameters (Table 1) except BW, showing a good potential predictor power of these acoustic features for the recognition of the two sister species.

The Box's M test (Rencher 2002) for equality of variance-covariance of multivariate data (excluding TI parameter) yielded a not significant result (F=1,38). Moreover the normality test (Doornik and Hansen 2008) showed a slightly significant value (p=0,045), accounting for a slight departure from normality of the data. Hence the prerequisites to perform DFA were satisfied, since Sever et al. (2005) showed the DFA to be highly robust to non-normal data.

DFA correctly classified 98,4% of calls with jack-knifed validation. Only one *A. pratensis* recording was misclassified as *A. spinoletta*. Further 5 recordings of *A. spinoletta* and 5 of *A. pratensis*, included in the same discriminant function as unknown specimens, were all correctly classified. The acoustic parameters with higher DFA loadings, accounting for a better discriminant power, were RMM, PCFMa, PCFMi and BW (Table 1). DUR90 has a very low loading and CF a near zero value.

I have calculated threshold values that can discriminate the tsip call of the two species (Table 1), as median of range between mean + SD of lower value parameter and mean - SD of higher value parameter. I tested the acoustic data using threshold values of three parameters (RMM, PCFMa and PCFMi) with the following rule: i) at least two parameters must be in agreement; ii) one of the two must be the RMM. This method was able to classify correctly 96% of all available recordings (n=75).

Similarity and differences among four Anthus species calls. Figure 3 shows the average contours of *alarm*, *tsip* and *soft* calls for *A. spinoletta*, *A. pratensis*, *A. cervinus* and *A. petrosus.* An objective measure of similarities among species for each different call type was calculated with two approaches. A multivariate Mantel correlogram (Legendre and Legendre 1998), computed with correlation index, was applied on the averaged time series of Figure 3. This test showed a high similarity of *alarm* call of the four species (0,981) and lower values for *tsip* (0,660) and *soft* calls (0,421).

Table 2 shows the results of a classical cross-correlation applied to the six possible comparisons between the four species for each of the three different call type. Average value of *alarm* call is again by far higher (0,943) than *tsip* and *soft* calls (0,441 and 0,329). Cross-correlation indexes, either at lag(0) or lag(m), were not normally distributed and variance-covariance was significantly unequal. Therefore I performed the comparisons among the three call types with Kruskall-Wallis test. Cross-correlation indexes, both at lag 0 and at lag of maximum correlation ("avg. lag m" of Table 2), showed a significant difference among the three call types (Hc=75,9 p<0,001; Hc=78,3 p<0,001). Mann Whitney post hoc test was significant for the comparisons *alarm* vs *tsip* call (p<0,001) and *alarm* vs *soft* call (p<0,001), while the comparison *tsip* vs *soft* call was not significant. The average lag of maximum correlation was 0,05 for *alarm* call, but shifted to 1,71 for *tsip* and to 3,31 for *soft* call. Again Kruskall-Wallis test showed a significant difference among these lag

data (Hc=54,5 p<0,001). Mann Whitney post hoc test showed a significant shift of lag for *tsip* and *soft* versus *alarm* call (p<0,001), but also a significantly higher shift for *soft* versus *tsip* call (p<0,01). An outlier data was produced by the comparisons between *A. spinoletta* versus *A. petrosus* both for *tsip* and *soft* calls (Table 2), which yielded a maximum correlation coefficient of 0,889 and 0,860 at lag 0. These two data, although significantly lower than *alarm* call coefficient (p<0,05), were clearly by far higher than the other coefficient resulting from these two call types. Moreover, for *A. spinoletta* versus *A. petrosus* comparison, the average lag of maximum correlation (avg. lag m) did not shift significantly from zero also for *tsip* and *soft* calls.

DISCUSSION

This paper showed that a similar flight call (*tsip*) of two sister species, *A. pratensis* and *A. spinoletta*, contains species-specific characteristics, that allows to discriminate the two species only with acoustic means. Some results of my investigation are similar to those reported by Elfström (1992) for *A.pratensis* and *A. petrosus*: for both studies, although the parameters are measured in different ways, *A. pratensis* has a lower call duration, a reduced frequency range (BW of Table1 in this paper) and a less frequency modulated call, represented by RMM parameter in this paper and by pulse number in Elfström's work (1992). The top frequency figure of *A.pratensis* (PFCMa Table 1) is quite consistent with that reported by Elfström (1992). Higher and lower frequencies of *A. spinoletta* are close to those reported by Fijen (2014). Multivariate analysis of all acoustic features showed a highly significant difference (Table 1) between the two species. The first question is: what parameters are most effective and most reliable to discriminate the two species? The PCA analysis, the ratio of coefficient of variability (CVr in Table 1) and interspecific coefficient of variability (CVis) helps to discard time interval between calls (TI), which shows a low CVr, a very high CVis and a low PCA loading. Frequency bandwidth (BW) proved to be less reliable because of its very low CVr (1,05) and high variability (CVin = 29,7).

The second question is: what is the best method to recognize acoustically the species and to classify new unknown data? DFA is a commonly used method in bioacoustics to classify multivariate parameters extracted from bird vocalizations (Galeotti and Sacchi 2001; Lengagne 2001; Christie et al. 2004; Terry *et al.* 2005) and it allows classifying new unknown specimens that are not part of the dataset used to create the discriminant function, so that future observations can be correctly grouped. Moreover DFA helps to understand what are the most important acoustic features to distinguish the two sister species. In this paper I found that the discriminant function is able to correctly classify 98,4% of the recordings and group assignment is validated by a leave-one-out cross-validation (jack-knifing) procedure. Only one bird out of 65 was misclassified. The DFA correctly classified all further 10 recordings of the two Anthus species that were added to the discriminant function as unknown specimens. An analysis of the DFA loadings (Table 1) shows that two parameters (central frequency CF and call duration DUR90) have very low values (respectively 0,20 and 7,88) compared with the other ones, therefore their discrimination power is relatively low. Since BW has a good DFA loading, but, as stated above, it is less reliable than the other parameters, the

most important acoustic features in the discrimination process are the remaining three parameters: RMM representing the frequency modulations of the tsip call, PFCMa that is the maximum frequency value of the peak frequency contour and PFCMi that is the minimum frequency value of the peak frequency contour. Among these last three parameters RMM shows the highest loading value for the DFA and can thus be considered the most critical acoustic feature for the recognition of these two bird species. I also tested a simpler and lighter method to discriminate the *tsip* calls of *A. pratensis* and *A. spinoletta*, which needs only the three above mentioned parameters. Table 1 at the sixth row shows for each acoustic parameter the lower and higher values yielded by the mean minus the SD and the mean plus SD: these ranges can reasonably used to find a threshold that separates the two species. The threshold for the three most important parameters (RMM, PFCMa and PFCMi) was calculated as the median of the ranges (Table 1). The threshold values of these three parameters were used to classify all available recordings (n = 75) following these rules: i) at least two comparisons with thresholds must be in agreement; ii) one of the two must be the comparison with RMM threshold. This method allowed a correct classification of 96% of recordings, where only 2 *A. pratensis* and 1 *A. spinoletta* were misclassified over 75 recordings. Of course either this simpler method or the DFA may be successfully used only if the methods of acoustic analysis exposed in this paper are carefully followed.

This paper showed that two closely related species, with an overall similar vocalization, nevertheless possess fine, but significant differences of acoustic features that may be important to transmit species-specific information and to discriminate between conspecific and heterospecific vocalizations, like found by Elfström (1992) for *A. petrosus* and *A. pratensis*. This finding is confirmed by other authors for some species of the genus Anthus (Fijen 2014; Garner et al. 2015) and for bird species belonging to other taxa (Stiffler et al. 2018; Tietze et al. 2008). On the other hand, many bioacoustics researches found interspecific communication mediated by various bird call types (Johnson et al. 2003; Goodale and Kotagama 2005; Magrath et al. 2007; Templeton and Greene 2007; Magrath et al. 2009; Fallow et al. 2011; Fallow et al. 2013). The advantages of interspecific eavesdropping and communication by means of calls are documented in the literature (Nuechterlein 1981; Burger 1984; Forsmann and Mönkkönen 2001; Goodale and Kotagama 2005; Goodale et al. 2010) and this raise the question of how birds recognize heterospecific calls and their meaning. Learning has been suggested to explain avian recognition and response to heterospecific calls (Curio 1971; Hurd 1996; Magrath 2009), a second suggested hypothesis is that similar acoustic features facilitate heterospecific recognition (Marler 1957; Stefanski and Falls 1972; Johnson *et al.* 2003; Fallow *et al.* 2011) and a third hypothesis combines both previous ones: Hurd (1996) suggested that interspecific recognition results from both associative learning and common acoustic properties. Interestingly, the acoustic similarity of some call types in different species can be the result of retaining features from a common ancestor (De Kort and Ten Cate 2001) or convergence in acoustic structure on the most effective design for a particular environment (Marler 1955; Marler and Slabbekoorn 2004). Broadening the view on other species of the genus Anthus and investigating the acoustic similarity and differences of different call

types seemed me a useful approach to shed some light on these topics. Therefore three different call types of four Anthus species were studied.

The choice to analyse only the call contour (see Methods) was made for the following reasons: my aim was to represent objectively the overall call morphology, underlining the trend of dominant frequencies, which seem particularly involved in heterospecific recognition (Johnson et al. 2003); since most recordings were from Xeno-Canto mp3 files, with unknown quality level, I previously tested the reliability of contour representation (see Methods), that was enough for the aims of the present work; finally, call representation by mean of average spectrogram contour is particularly suitable to be combined with cross-correlation as an objective method of evaluating vocal similarity. Combining cross-correlation with frequency contour is considered a method that may capture more precisely how signals vary over the duration of an acoustic unit (Odom *et al.* 2021).

The results of the comparison among *A. spinoletta*, *A. pratensis*, *A. cervinus* and *A. petrosus* on three different call types (*tsip*, *soft* and *alarm*) show that similarity of *alarm* calls in the four species is significantly and by far higher than the other two call types, with a tendency of a decrease in similarity from *tsip* to *soft* call. Indeed average correlation coefficient of the four species is significantly higher for *alarm* call either at lag 0 or at lag of the maximum correlation (Table 2). Since a shift from lag 0 proves a decreased similarity on the overall time shape of the calls, it is worth to analyse the average lag values where the correlation coefficient reach its maximum. For *alarm* it is 0,05, for *tsip* is 1,71 and for *soft* call is 3,31, where the differences of these values are highly significant showing a decrease of similarity among the four species. While the similarity among *alarm* calls of the four species is very strong and by far higher than the other two call types, the mean values of the correlation coefficients of *tsip* and *soft* calls don't differ significantly, although the lag 0 values show a higher similarity for *tsip* than for *soft* call. This slight difference is confirmed by Mantel correlogram index: 0,660 for *tsip* and 0,421 for *soft* call. I can conclude that alarm calls of the four species show a very strong similarity, while the other two call types are more distinctive showing a much lower similarity, with *soft* call seeming to have a slightly lower similarity between species and hence a higher distinctive power. Since all four species are closely related from a taxonomic point of view, the results of my investigation support the hypothesis that *alarm* calls retain features of a common ancestor, while the other two call types (*tsip* and *soft*) show a decreasing similarity between the four Anthus species, resulting in a higher content of species-specific characteristics. The *alarm* call type studied in the present paper is only given in the vicinity of nest or in the breeding territory (Alström and Mild 2003; Bergmann et al. 2021; Dragonetti pers. obs.) therefore it is a very strong signal of danger, that the evolutive process may have maintained almost unaltered for these Anthus species, because it eases the heterospecific communication. On the other hand *tsip* call is the most common call uttered in many different context mainly in flight and soft *call* is typically uttered between members of a flock as a contact signal (Elfström 1992; Alström and Mild 2003), therefore for these two calls the needs of intraspecific communication increase and evolution may have increased their acoustic divergence, as shown in the present paper.

Interestingly the comparison *A. petrosus* versus *A. spinoletta* yields a somewhat outlier data (see Table 2 row 5): indeed both *tsip* and *soft* calls show values (0,889 and 0,860 respectively) very close to that of *alarm* call (0,960) and a nearly null shift from lag 0, accounting for a greater similarity of this couple of species than the other ones. The taxonomy of "Water Pipit complex" has been much debated and until recently *A. petrosu*s and *A. spinoletta* were treated as a single species (Ali and Ripley 1998). Arctander *et al.* (1996) found only 1,2 % difference in mitochondrial cytochrome b sequences between *A. spinoletta* and *A. petrosus*, for this reason they expressed their reluctance to treat them as separate species. Today they are commonly treated as separate species, but there is no doubt that they are more closely related from a genetic point of view than the other species studied in this paper (see Voelker 1999) and this may explain the higher similarity indexes reported in Table 2.

A further consideration can be made about alarm call similarity in the genus Anthus, checking the eighteen species living in Europe, Asia and North America and visually comparing specimen spectrograms of alarm call available in the literature (Cramp and Simmons 1983; Alström and Mild 2003; Pieplow 2019; Bergmann et al. 2021). The *alarm* calls of six species were unknown, the remaining twelve might be classified as follows: three show a completely different alarm call compared to those studied in this paper, seven show a highly similar call and two show a lower degree of similarity but are very reminiscent of the *alarm* described in this paper. If we look to the maximum likelihood tree of the genus Anthus based on the mitochondrial cytochrome b gene (from Voelker 1999), we can find all the former three species in the 4th group (the "large" pipits), while the remaining nine are all in the 3rd group (the "small" pipits).

The results of the present paper support the hypothesis that *alarm* call of four Anthus species is highly similar, because it retains features of a common ancestor and may ease heterospecific communication. The call similarities of closely related species can be important for phylogenetic comparative studies, as suggested by the comparisons made in the Anthus genus. The method commonly used in phylogenetic studies is integrative taxonomy (combination and integration of multiple types of evidence, see Sangster 2018): together with genetic and morphological information, acoustic data play a major role in taxonomic designation of birds. The song of passerines, considered to be one of the most important traits promoting differentiation among species (Price 2008), was extensively investigated in phylogenetic comparative studies, but the data I reported here suggest that also the comparisons of call repertoires may be a useful tool for taxonomic studies.

The results showed in the present study have to be considered preliminary and further research is needed to shed light on the role of call similarity and divergence for the taxonomic status of closely related species.

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REFERENCES

Ali S, Ripley S D (1998) Handbook of the birds of India and Pakistan. Volume 9 second edition Oxford University Press, Delhi

Alström P, Mild K (2003) Pipits and Wagtails of Europe, Asia and North America. Christopher Helm, London

Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Australian Ecology 26:32-46

Arctander P, Folmer O, Fjeldså J (1996) The phylogenetic relationships of Berthelot's Pipit *Anthus Berthelotii* illustrated by DNA sequence data, with remarks on the genetic distance between Rock and Water Pipits *A. spinoletta*. Ibis 138:263-272

Aubin T, Mathevon N, Da Silva ML, Vielliard JME, Sebe F (2004) How a simple and stereotyped acoustic signal transmits individual information: the song of White-browed warbler Basileuterus leucoblepharus. An. Acad Bras de Ciências 76:335-344

Baker MC, Howard TM, Sweet PW (2000) Microgeographic variation and sharing of the gargle vocalization and its component syllable in black-capped chickadee (Aves, Paridae, *Poecile atricapillus*) populations. Ethology 106:819-838

Bergmann H H, Helb HW, Baumann S (2008) Die stimmen der Vögel Europas. Aula Verlag GmbH, Wiebelsheim

Bergmann H H, Engländer W, Baumann S (2021) Die stimmen der Vögel Europas. Aula Verlag GmbH, Wiebelsheim

Brichetti P, Fracasso G (2007) Ornitologia Italiana. Volume 4 Oasi Alberto Perdisa Editore, Bologna

Briedis M, Beran V, Adamic P, Hanh S (2020) Integrating light-level geolocation with activity tracking reveals unexpected nocturnal migration patterns of the tawny pipit. Journal of Avian Biology. https://doi.org/10.1111/jav.02537

Burger J (1984) Grebes nesting in gull colonies: protective association and early warning. The American Naturalist 123:327-337

Center for Conservation Bioacoustics (2019) Raven Pro: interactive sound analysis software. Version 1.6.1. The Cornell Lab of Ornithology, Ithaca, NY

Christie PJ, Mennill DJ, Ratcliffe LM (2004) Chickadee song structure is individually distinctive over long broadcasting distances. Behaviour 141:101-124

Cramp S, Simmons KEL (1983) The Birds of the Western Palearctic. Volume 3 Oxford University Press, Oxford.

Curio E (1971) Die akustische Wirkung von Feindalarmen auf einige Singvogel. Journal für Ornithologie 112:365-372

De Kort SR, Ten Cate (2001) Response to interspecific vocalizations is affected by degree of phylogenetic relatedness in Streptopelia doves. Animal Behaviour 61:239-247

Doornik J, Hansen H (2008) An Omnibus Test for Univariate and Multivariate Normality. Oxford Bulletin of Economics and Statistics 70(s1):927-939

Ebenhöh H, Hoffrichter O (1998) Beobachtungen an einem winterlichen Schlafplatz des Bergpiepers1 (*Anthus spinoletta*) bei Köndringen, Landkreis Emmendingen. Naturschutz südl. Oberrhein 2:181-194.

Elfström ST (1992) Tsip calls of meadow and rock pipits: interspecific comparisons and playback experiments. Animal Behaviour 43:795-802

Elkins N (2005) Weather and bird migration. British Birds 98:238-256

Farnsworth A (2005) Flight calls and their value for future ornithological studies and conservation research. The Auk 122(3):733-746

Fallow P, Gardner JL, Magrath RD (2011) Sound familiar? Acoustic similarity provokes responses to unfamiliar heterospecific alarm calls. Behavioral Ecology 22:401-410

Fallow P, Pitcher BJ, Magrath RD (2013) Alarming features: birds use specific acoustic properties to identify heterospecific alarm calls. Proceedings of The Royal Society B. https://doi.org/10.1098/rspb.2012.2539

Fijen TPM (2014) Flight call identification of Rock Pipit and Water Pipit. Dutch Birding 36:87-95

Forsman JT, Mönkkönen M (2001) Responses by breeding birds to heterospecific song and mobbing call playbacks under varying predation risk. Animal Behaviour 62:1067-1073

Gal A, (2003) A New Technique for Studying Nocturnal Bird Migration. Semantic Scholar. https://www.semanticscholar.org/paper/A-New-Technique-for-Studying-Nocturnal-Bird-Gal/eb621c8c3cbba823ca9b9133cb4f1e75d3bd621c

Galeotti P, Sacchi R (2001) Turnover of territorial Scops Owls Otus scops as estimated by spectrographic analyses of male hoots. Journal of Avian Biology 32:256-262

Garner M, Perlman Y, Kiat Y, Collison MJ (2015.) Water Pipits: three species rather than one? British Birds 108:42-48

Gayk ZG, Simpson RK, Mennill DJ (2021) The evolution of wood warbler flight calls: Species with similar migrations produce acoustically similar calls. Evolution 75(3):719-730

Goodale E, Kotagama S (2005) Alarm calling in Sri Lankan mixed-species bird flocks. The Auk 122(1):108-120

Goodale E, Beauchamp G, Magrath RD, Nieh JC, Ruxton GD (2010) Interspecific information transfer influences animal community structure. Trends in Ecology and Evolution 25:354-361

Hamilton WJ III (1962) Evidence concerning the function of nocturnal call notes of migratory birds. Condor 64:390-401

Hammer Ø, Harper DAT, Ryan PD (2001) PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica 4(1):1-9

Hurd CR (1996) Interspecific attraction to the mobbing calls of black capped chickadees (*Parus atricapillus*). Behavioural Ecology and Sociobiology 38:287-292

Johnson FR, McNaughton EJ, Shelley CD, Blumstein DT (2003) Mechanism of heterospecific recognition in avian mobbing calls. Australian Journal of Zoology 51:577-585

Kroodsma DE (2000) Vocal behavior. In Home Study Course in Bird Biology. Cornell Laboratory of Ornithology (ed), Ithaca, New York, Chapter 7 pp 1-18

Legendre P, Legendre L (1998) Numerical Ecology. 2nd English ed. Elsevier, Amsterdam

Lengagne T (2001) Temporal stability in the individual features in the calls of Eagle Owl (*Bubo bubo*). Behaviour 138:1407-1419

Magrath RD, Pitcher BJ, Gardner JL (2007) A mutual understanding? Interspecific

responses by birds to each other's aerial alarm calls. Behavioral Ecology 18(5):944-951

Magrath RD, Pitcher BJ, Gardner JL (2009) Recognition of other species' aerial alarm calls: speaking the same language or learning another? Proceedings of The Royal Society B 276:769-774

Marler P (1955) Characteristics of some animal calls. Nature 176:6-8

Marler P (1957) Specific distinctiveness in the communication signals of birds. Behaviour 11:13-38

Marler P, Slabbekoorn H (2004) Nature's music. The science of birdsong. Elsevier Academic Press, San Diego

Mudinger PC (1970) Vocal imitation and individual recognition of finch calls. Science 168:480-482

Mudinger PC (1979) Call learning in the Carduelinae: Ethological and systematic considerations. Systematic Zoology 28:270-283

Nuechterlein GL (1981) 'Information parasitism' in mixed colonies of western grebes and Forster's terns. Animal Behaviour 29:985-989

Odom KJ, Araya-Salas M, Morano JL et al (2021) Comparative bioacoustics: a roadmap for quantifying and comparing animal sounds across diverse taxa. Biological Reviews 96(4):1135-1159

Pieplow N (2019) Peterson Field Guide to Bird Sound of Western North America. Houghton Mifflin Harcourt, New York

Price T (2008) Speciation in Birds. Roberts and Company, Greenwood Village, CO

R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing

Rencher AC (2002) Methods of multivariate analysis. 2nd ed. Wiley, New York

Sangster G (2018) Integrative taxonomy of birds: The nature and delimitation of species. In: Tietze DT (ed) Bird Species, Springer International Publishing, Cham, pp 9-37

Scherrer B (1984) Biostatistique. Gaetan Morin Press, Quebec

Sever M, Lajovic J, Rajer B (2005) Robustness of the Fisher's Discriminant Function to Skew-Curved Normal Distribution. Metodoloski Zvezki 2(2):231-242

Stefanski RA, Falls JB (1972) A study of distress calls of song, swamp, and white-throated sparrows (Aves: Fringillidae). II. Interspecific responses and properties used in recognition. Canadian Journal of Zoology 50:1513-1525.

Stiffler LL, Schroeder KM, Anderson JT, McRae SB, Katzner TE (2018) Quantitative acoustic differentiation of cryptic species illustrated with King and Clapper rails. Ecology and Evolution 8:12821–12831.

Templeton CN, Greene E (2007) Nuthatches eavesdrop on variations in heterospecific chickadee mobbing calls. Proceedings of the National Academy of Sciences USA 104:5479-5482

Terry AM, Peake TM, McGregor PK (2005) The role of vocal individuality in conservation. Frontiers in Zoology https://doi.org/10.1186/1742-9994-2-10

Tietze DT, Martensa J, Sun Y, Packert M (2008) Evolutionary history of treecreeper vocalisations (Aves: Certhia). Organism, Diversity and Evolution 8:305-324

Voelker G (1999) Molecular evolutionary relationships in the Avian genus Anthus (pipits: Motacillidae). Molecular Phylogenetics and Evolution 11:84-94

Wang Y, Shi Y et al (2019) Patterns of bird nocturnal migration at Shenyang Taoxian International Airport, Northeast China. The Journal of applied ecology 30(1):292-300

Williams H (2008) Birdsongs and singing behavior. In: Neuroscience of Birdsongs, Zeigler HP, Marler P (eds), Cambridge University Press, Cambridge, pp 5-32

Zann R (1985) Ontogeny of the zebra finch distance call. I. Effects of cross fostering to bengalese finches. Z. Tierpsychol 68:1-23

TABLES AND FIGURES

	RMM (n)	CF (Hz)	PFCMa (Hz)	PFCMi (Hz)	BW (Hz)	DUR90 (ms)	TI (ms)
A. pratensis	5,01 +/- 0,62	6643 +/- 280	7271 +/- 273	5928 +/- 448	1343 +/- 461	56 +/- 6	293 +/- 305
A. spinoletta	7,24 +/- 0,91	6078 +/- 370	6655 +/- 408	5037 +/- 386	1618 +/- 396	72 +/- 8	1255 +/- 638
CVis	22,08	6,82	6,23	11,14	30,62	16,36	92,58
CVr	1,75	1,31	1,25	1,45	1,03	1,53	1,18
DFA loadings	76,07	0,20	47,91	47,55	47,68	7,88	n.c.
T/range	5,63 – 6,33	n.c.	6998 – 7063	5423 – 5480	1222 – 1804	62 - 64	n.c.
Threshold	5,98	n.c.	7030	5451	1513	63	n.c.

Table 1. *Comparison of tsip call vocal parameters between A. pratensis and A. spinoletta.* **CV** *= coefficient of variation (see Methods);* **CVis** *= interspecific coefficient of variation;* **CVr** *= ratio between CVis and average CV intraspecific of the two species.* **DFA** *= discriminant function analysis.* **T/range** *= range between mean + SD of lower value parameter and mean - SD of higher value parameter.* **Threshold** *= median of T/range values taken as separation threshold between the two species.* **RMM** *= number of relative minimum and maximum values of call frequency contour;* **CF** *= center frequency;* **PFCMa** *= maximum of the peak frequency contour measured from spectrogram slices;* **PFCMi** *= minimum of the peak frequency contour;* **BW** *= call frequency bandwidth calculated from (PFCMa - PFCMi);* **DUR90** *= call duration containing 90% of the call energy;* **TI** *= time interval between start of subsequent calls; n.c. = not calculated. A. pratensis n=34, A. spinoletta n=31.*

	n (a)	alarm(0)	alarm(m)	avg. lag (m)	n (t)	tsip(0)	tsip(m)	avg. lag (m)	n (s)	soft(0)	soft(m)	avg. lag (m)
pr vs sp	10	0,869+/-0,151	0,905+/-0,085	0,29+/-0,49	13	0,433+/-0,173	0,502+/-0,128	1,14+/-0,90	12	0,463+/-0,132	0,650+/-0,089	1,86+/-0,38
pr vs ce	10	0,962+/-0,021	0,962+/-0,021	0,00	13	0,313+/-0,196	0,447+/-0,070	2,71+/-3,15	12	-0,06+/-0,394	0,711+/-0,140	3,71+/-1,38
pr vs pe	10	0,935+/-0,041	0,935+/-0,041	0,00	13	0,367+/-0,234	0,455+/-0,145	1,29+/-1,25	12	0,388+/-0,118	0,582+/-0,106	2,00+/-0,82
sp vs ce	10	0,963+/-0,045	0,963+/-0,045	0,00	13	0,318+/-0,194	0,462+/-0,085	2,43+/-1,40	12	0,175+/-0,450	0,481+/-0,182	4,86+/-3,76
sp vs pe	10	0,960+/-0,034	0,960+/-0,034	0,00	13	0,889+/-0,034	0,889+/-0,034	0,00	12	0,860+/-0,077	0,871+/-0,068	0,43+/-0,053
ce vs pe	10	0,968+/-0,027	0,968+/-0,027	0,00	13	0,325+/-0,131	0,383+/-0,061	2,71+/-4,03	12	0,146+/-0,371	0,437+/-0,212	7,00+/-3,37
Mean	60	0,943+/-0,074	0,949+/-0,049	0,05+/-0,22	78	0,441+/-0,263	0,523+/-0,192	1,71+/-2,34	72	0,329+/-0,407	0,622+/-0,198	3,31+/-3,00

Table 2. *Cross correlation results (average +/- SD) among contours of three different call type (alarm call, tsip call, soft call) in four Anthus species;* **pr** = *A. pratensis;* **sp** = *A. spinoletta;* **ce** = *A. cervinus;* **pe** = *A. petrosus.* **Mean** = *average of all the cross-correlations of all comparisons.* **(0)** = *cross-correlation results at lag 0;* **(m)** = *cross-correlation results at lag of maximum correlation coefficient;* **avg. lag(m)** = *average lag value corresponding to the maximum correlation coefficient;* **n(a)** = *number of cross-correlations performed for alarm calls;* **n(t)** = *number of cross-correlations for tsip calls;* **n(s)** = *number of cross-correlations for soft calls. Each contour submitted to cross-correlation is an average of a minimum 6 and maximum 9 randomly selected calls from each recording of a given species. The total number of randomly selected calls is always 84 for each recording set and for every call type of the four species. Most recordings contain calls of more than one bird.*

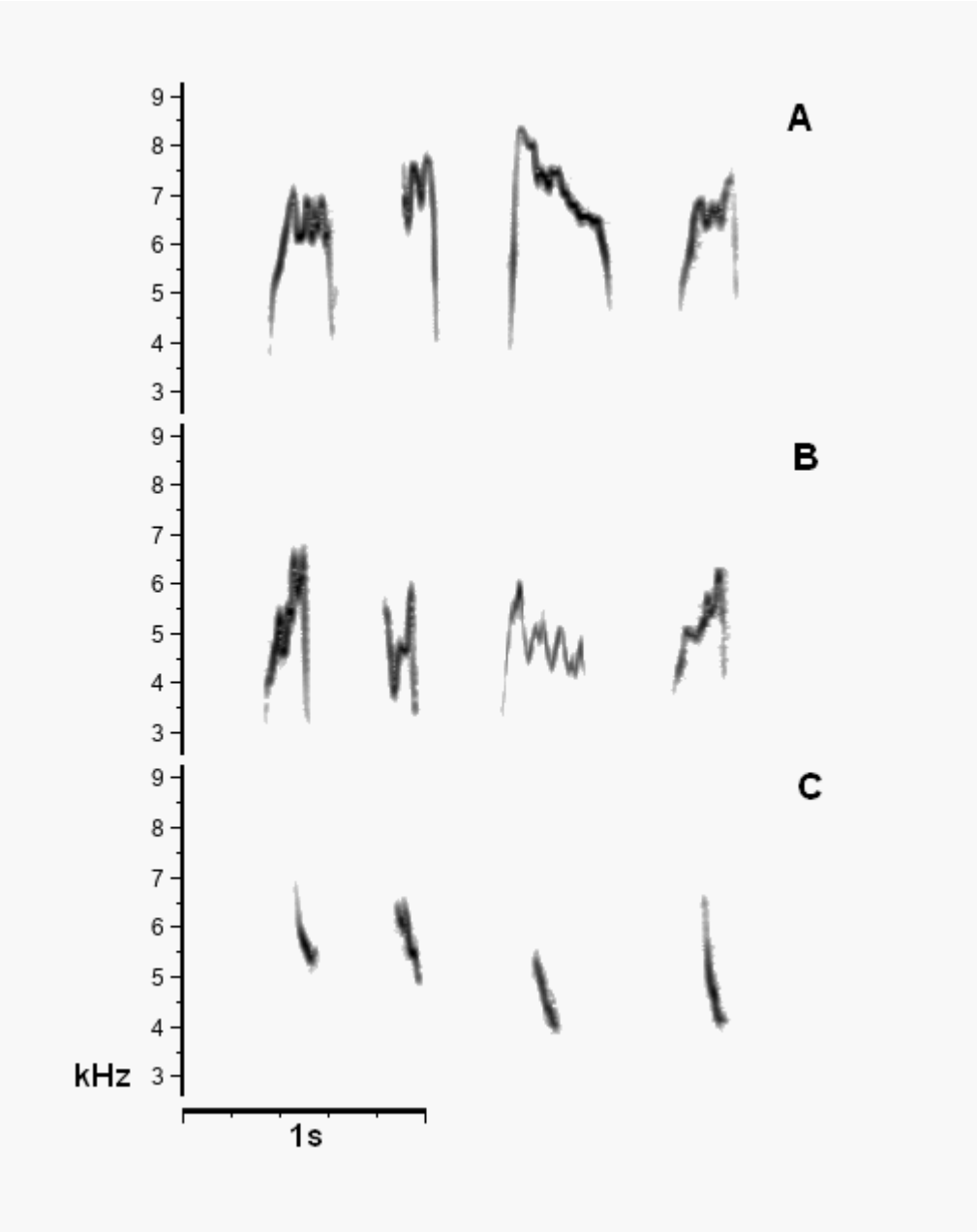

Figure 1. *From left to right call spectrograms of A. spinoletta, A. pratensis, A. cervinus, A. petrosus.* **A)** *tsip calls;* **B)** *soft calls;* **C)** *alarm calls. See Methods for call type classification.*

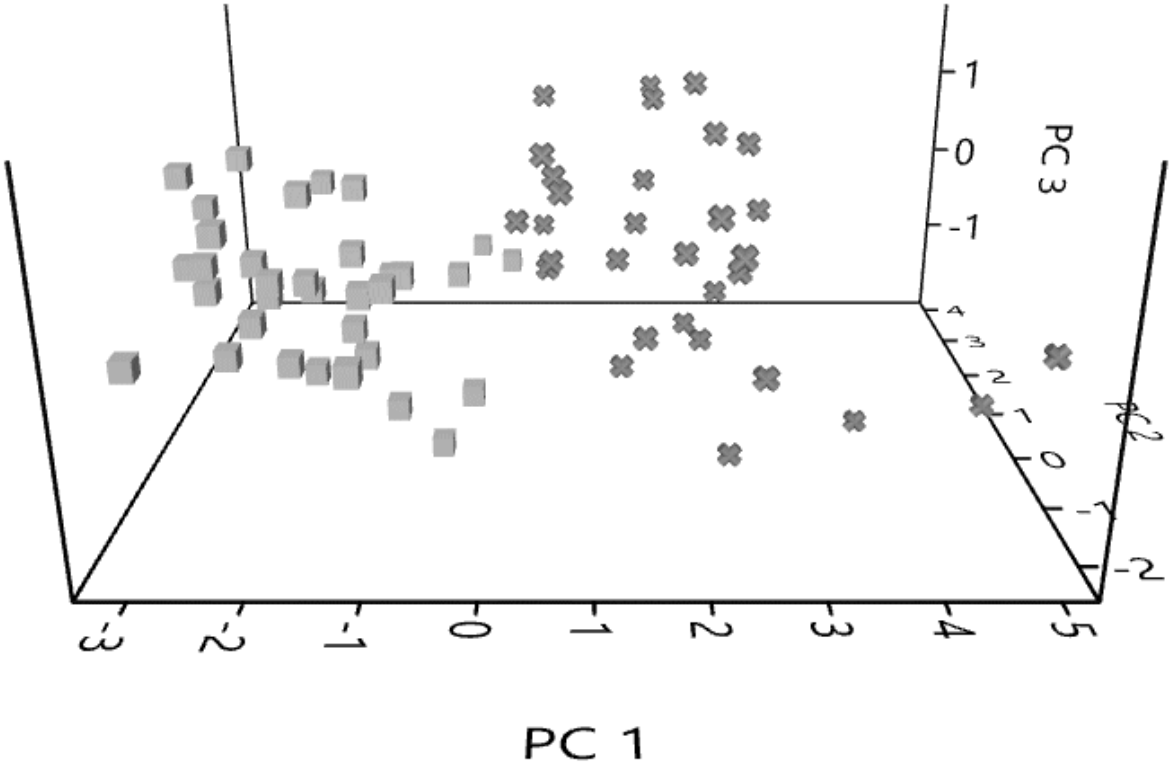

Figure 2 . *Comparison of tsip call vocal parameters between A. pratensis and A. spinoletta. Scatter plot in the coordinate system given by the first three PCA components that account for the 86,4 % of the variance. Cubes = A. pratensis tsip call; Crosses = A. spinoletta tsip call*

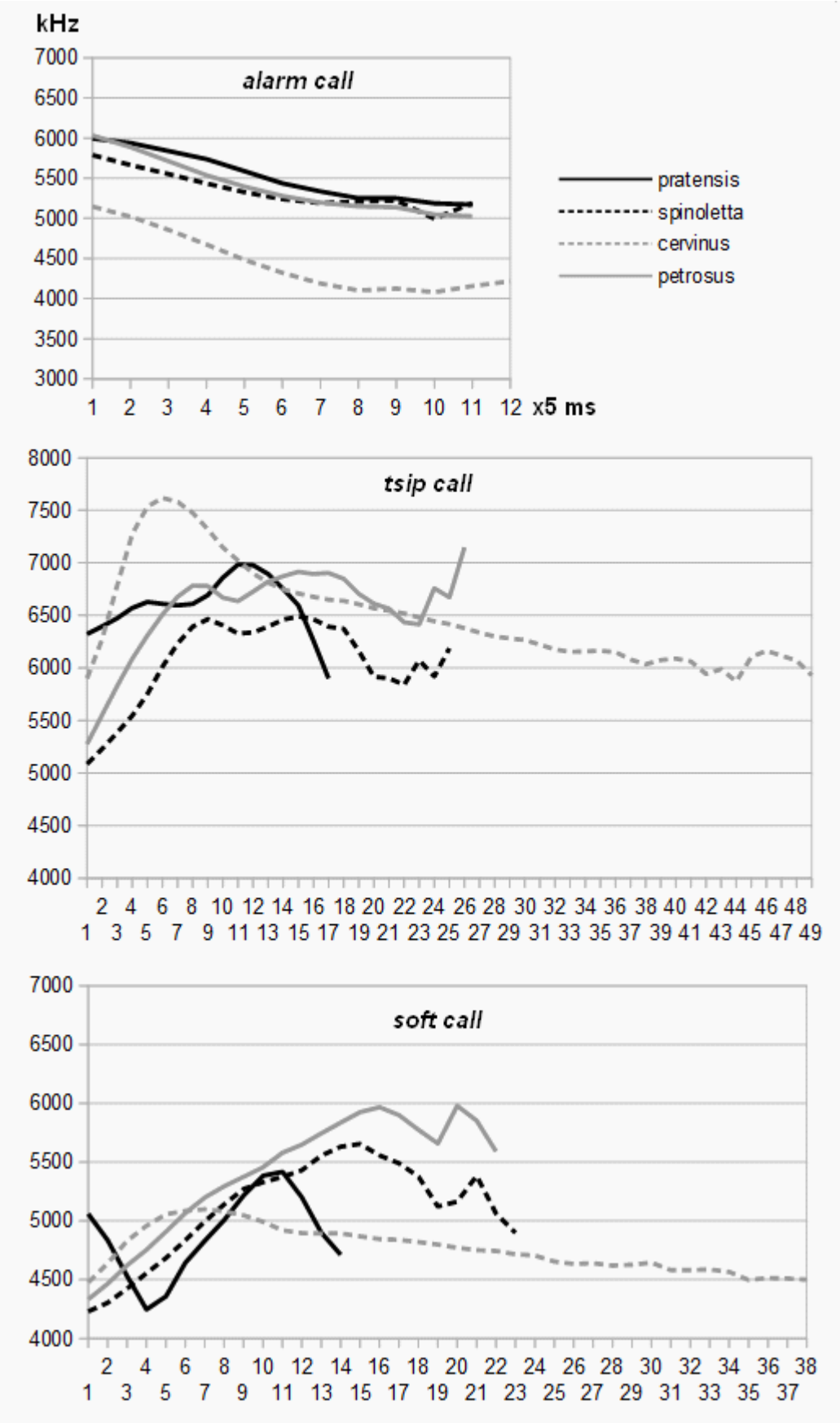

Figure 3 . *Average frequency contours (FCP75, see Methods) of three different calls in four Anthus species.* ***Alarm call****: average of 10 recordings for each species.* ***Tsip call****: average of 13 recordings for each species.* ***Soft call*** *: average of 12 recordings for each species. The total number of randomly selected calls for every call type of each species is always 84. Time series (x axis) are made by points evenly spaced 5 ms apart*