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ACOUSTIC DISCRIMINATION OF *PIPISTRELLUS KUHLII* AND *PIPISTRELLUS NATHUSII* (CHIROPTERA: VESPERTILIONIDAE) AND ITS APPLICATION TO ASSESS CHANGES IN SPECIES DISTRIBUTION

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A rapid range expansion of *Pipistrellus kuhlii* (KUHL, 1817) can be observed throughout Europe based on new records gathered in the last two decades. Data of new occurrences were obtained by different methods (mist netting, dead specimens, checking buildings for roosts, etc.), providing only a rough picture about the exact pattern of the expansion. The aim of this study was to show an effective acoustic method that can be used for quantifying the differences in the occurrence of *Pipistrellus kuhlii* in two given areas or within the same area at different times. Therefore this method can be used to investigate the process of area expansion in this species. The large overlap in the echolocation call parameters of *Pipistrellus kuhlii* and *Pipistrellus nathusii* (KEYSERLING & BLASIUS, 1839) made it necessary to create a sound library from the calls of the two species. Discriminant function analysis (DFA) was used to classify the calls with 81.5% accuracy. We visited and acoustically surveyed 71 settlements in the summer of 2008. Based on our results, *Pipistrellus kuhlii* is a widespread species in the settlements of the southern part of Hungary, while in the northern part it is quite rare.

Key words: climate change, area expansion, acoustic monitoring, species identification

INTRODUCTION

Pipistrellus kuhlii is a widespread bat species, its range extends from Western Europe eastwards to Afghanistan, Turkmenistan and Kazakhstan, and it ranges all over North Africa (DIETZ *et al.* 2009). Until the 1990s, the European *P. kuhlii* was known primarily in the Mediterranean region, but since then a rapid range expansion has been observed (SACHANOWICZ *et al.* 2006). In Hungary, the species was noted for the first time in 1993 and in the same year its breeding was also confirmed (FEHÉR 1995). The species has since become widespread in Hungary, where many breeding colonies were found, mainly in the southern part of the country (FEHÉR 2007*b*). Unfortunately, due to the lack of an organized Hungarian *P. kuhlii* monitoring program, we only have data collected with different and incomparable methods, and the results reflect the effort of bat researchers conducting surveys in different areas with divergent aims (FEHÉR 2007*b*). Therefore we are missing a detailed picture about the change of the species distribution within different parts of the country. Hence, the reasons for the expansion could not be studied. Nevertheless, based on the observed tendency thus far in Hungary and in Europe, we expect further changes in the *P. kuhlii* distribution.

No long-term studies have been published showing that the effects of global warming on European bats is a plausible explanation of the area extension of *P. kuhlii*, although one paper has discussed the hypothetical effects of different climate change scenarios on European bat species (REBELO *et al.* 2010). Other reports suggest that the extension of wintering area of *Pipistrellus nathusii* (SACHANOWICZ & CIECHANOWSKI 2006) and the range expansion of *Hypsugo savii* in many European countries (REITER *et al.* 2010) may be linked to climate change. The lack of informative data on this topic can be explained by the fact that the data have not been collected within the framework of a unified European monitoring program. *P. kuhlii* is a house-dwelling bat species and thus roosting places are difficult to count. Another commonly used method is mist-netting, but in many cases there are few suitable netting sites (for example drinking spots) in urban areas which makes it hard to get sufficiently reliable occurrence data on the species. Moreover, for quantifying bat occurrences, the netting and roost searching techniques require substantial time spent surveying larger areas, especially compared to acoustic methods. The use of acoustic methods during bat surveys is becoming more and more important for estimating habitat associations (FISCHER *et al.* 2009) and anthropogenic impacts (GEHRT & CHELSVIG 2004, JONES *et al.* 2009) without any disturbance.

Echolocation calls of *P. kuhlii* can be separated clearly from those of other bat species, with the exception of *P. nathusii*, based on frequency parameters. The echolocation calls of both *P. kuhlii and P. nathusii* start with a frequency modulated (FM) part followed by a quasi constant frequency (CF) part, a so called FM – CF call type (KALKO & SCHNITZLER 1993). The frequency and time parameters of the calls from these two species largely overlap, therefore, the possibility of confusing these two species is a considerable problem in areas where both species occur (OBRIST *et al.* 2004, RUSSO & JONES 2002). However, using multivariate techniques like discriminant function analysis (DFA) or synergetic classifiers, the accuracy of identification can reach about 80% or better (OBRIST *et al.* 2004). It must be mentioned that social calls of these two species can be used to distinguish them with high accuracy, although occurrences of these call types largely depend on the situation and period of the year (BARLOW & JONES 1996, RUSSO & JONES 1999). Therefore, the usage of social calls for identification is quite limited, while echolocation calls are always present, regardless of the context in which they are made.

Our aim in this paper is first, to present an effective acoustic method that can be used for quantifying the differences in the occurrence of *P. kuhlii* between areas or comparing the occurrence in the same area at different times. Thus, this method can be used for investigating the progress of area expansion in case of this species on a larger scale. Our method is based on recording echolocation calls with point-count monitoring technique, as well as using multivariate classification of calls with DFA. To exclude the geographical effect on echolocation call parameters, a Hungarian sound library was built based on *P. nathusii* and *P. kuhlii* echolocation calls. Second, we used our method and protocol to compare the relative activity of *P. kuhlii* in two areas in order to show the usability of the technique and to get new information about the present distribution pattern of this species in Hungary.

MATERIALS AND METHODS

Building the sound library and analysis of echolocation calls

Echolocation calls of *P. nathusii* and *P. kuhlii* were collected from free flying specimens, close to known colonies or when communication calls were present in order to ensure accurate species identity. We used only a single sequence from each individual in order to increase variances in the call parameters. Therefore, only those echolocation sequences were used, which had been recorded far away from each other, or in cases where visual confirmation was made that sequences originated from different individuals.

We collected 93 sequences from *P. kuhlii* (from 13 sites) and 67 sequences from *P. nathusii* (from 5 areas) throughout Hungary (Fig. 2). The ultrasound recordings were made with Pettersson D240× ultrasound detectors with a sampling rate of 307 kHz and a frequency range of 10–120 kHz. Time expanded sounds were recorded with Sony WM-D6C professional cassette recorder or M-Audio MicroTrack II digital audio recorder on the field and later transmitted to the computer in 48 kHz sampling rate and 16 bits quality.

The echolocation calls were segmented and measured by a self-written MATLAB 7.7 (Mathworks, Inc.) script. For creating the sonograms and taking the measurements, we used a 256 point FFT length, and Hanning window with 96% overlap settings. First, the frequency having the highest amplitude was localized and measured in each call, than the start and endpoint of the calls were determined by taking the frequencies at the points where amplitudes were 30 dB below the peak amplitude (similar to SIEMERS & KERTH 2006). The following classical parameters were measured: F_{start} – start frequency, measured at the start of the call, F_{end} – end frequency, measured at the end of the call, F_{center} – centre frequency, measured in the middle of the call, F_{max} – frequency with the maximum intensity, F_{band} – frequency bandwidth, measured as the difference between F_{start} and F_{end}, T_{dur} – duration of the call, T_{PI} – pulse interval, which is the time interval between the beginning of two consecutive calls.

Mean values of call parameters in each sequence (6.8±3.6 calls per sequence) were computed and used later in this study for descriptive statistics and in the DFA to reduce errors of measurement and recording.

The other reason for using average values was that the identification key gave slightly better results when average values were used. Descriptive statistics (mean, standard deviation and range) were computed for all the parameters. Univariate analyses were used to test the differences between species. For normally distributed variables we used ANOVA, for those that did not conform to normality we used the Mann-Whitney U-test.

Quadratic discriminant function analysis with 10-fold cross-validation was applied to the above-mentioned call parameters for discriminating the two species. Effective variables were chosen by using a backward stepwise method. All statistical analyses were performed in Statistica 8 (StatSoft, Inc.) and MATLAB.

Point-count survey

Urban areas are the primary habitats of *P. kuhlii* (BOGDANOWICZ 2004), thus our bat detector survey focused on this type of habitat in order to gather sufficient occurrence data. Seventy-one settlements in two areas of Hungary (N and S Hungary) were visited once in the second half of June 2008, under ideal weather conditions (above 20 °C, no precipitation and no wind). The number of chosen settlements located in high and low altitudes, as well as the number of smaller and larger settlements was similar in the two areas. The locations in the two areas were visited simultaneously in the same period. The survey started 30 minutes after sunset and continued for three hours each day. The "greenest" place (e.g. park or place with trees) was selected in every settlement (one location in each settlement) with the help of Google Earth prior to the survey. At each location, all passing bats were recorded for 10 minutes.

The recording, processing and measuring procedure for monitoring echolocation calls were the same as used for building the sound library and creating the discriminant functions. Communication calls were not considered, and only those echolocation calls were processed that possibly belonged to *P. kuhlii* or *P. nathusii.* The averages of echolocation call parameters in each sequence were used for identification. Using the discriminant functions resulted from the classification described above, we identified the sequences as *P. kuhlii* or *P. nathusii* with a self-written MATLAB script.

In each settlement, there were four possible options for occurrence: none of the two species observed, only *P. kuhlii*, only *P. nathusii*, or both present. The comparison of the activity rate of these species between the two areas was investigated with a Yates's corrected χ^2 test.

RESULTS

Call parameters and species discrimination

Altogether 160 sequences were collected and analyzed to build the sound library. We found significantly higher values for *P. nathusii* in the F_{end} (Mann-Whitney Z = –6.77, p < 0.001), F_{max} (Mann-Whitney Z= –2.55, p = 0.011), and F_{center} (Mann-Whitney Z = –3.15, p < 0.001) parameters. Descriptive statistics of the echolocation calls from *P. kuhlii* and *P. nathusii* are summarized in Table 1.

Table 1. Describing statistics of echolocation call parameters in *P. kuhlii* and *P. nathusii* with the mean±standard deviation and the range in brackets.

	P. kuhlii (N = 92)	*P. nathusii* (N = 67)
F_{start} (kHz)	58.6±11.8 (38.4–93.2)	59.6±13.7 (39.3–97.3)
F_{end} (kHz)	38.3±1.7 (32.1–44.4)	40.3±1.7 (36.3–44.3)
F_{center} (kHz)	41.2±2.6 (36.3–48.8)	42.2±2.2 (37.5–48.5)
F_{max} (kHz)	41.6±3.3 (36.6–52.3)	42.3±2.6 (37.4–48.9)
F_{band} (kHz)	20.5±11.3 (2.7–52.1)	19.6±13.4 (1.7–55.6)
T_{dur} (msec)	5.2±1.4 (2.1–9.9)	5.6±1.5 (2.9–9.1)
T_{PI} (msec)	108±31 (68–242)	106±38 (52–283)

In the DFA five effective variables were selected by using a forward stepwise method. A MANOVA indicated significant discrimination of the model (Wilk's λ = 0.614, $F_{5,154} = 19.38$, $p < 0.0001$). Wilk's λ values showed the following discrimination power for the 5 variables: $F_{end} > T_{dur} > F_{max} > F_{band} > F_{start}$. Only one canonical variable was created by the quadratic DFA (Fig. 1). The species discrimination was investigated by 10-fold cross-validation process and resulted in 81.5% accuracy based on the confusion matrix (Table 2), the random data classification would be 50% correct.

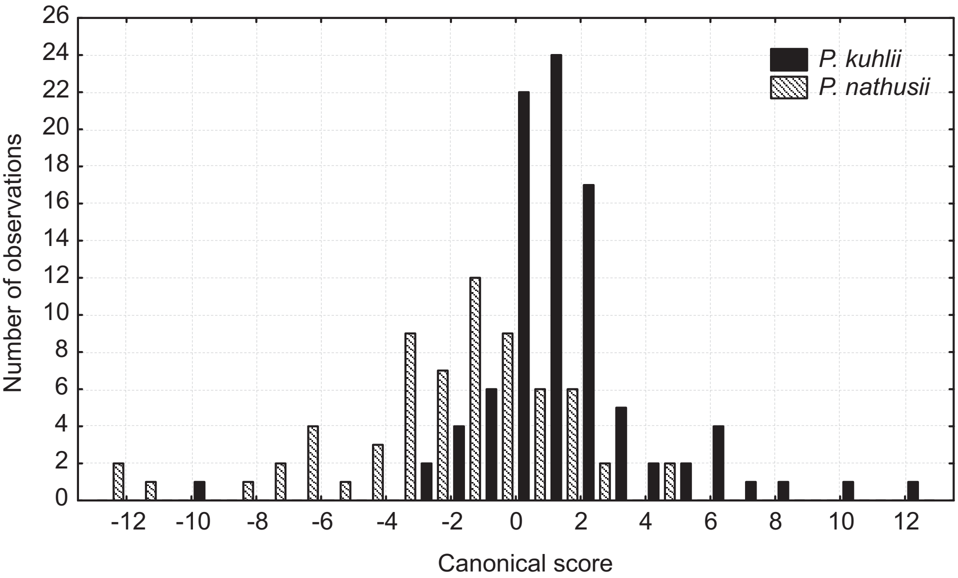

Fig. 1. The distribution of the canonical scores between *P. kuhlii* and *P. nathusii* resulted from the discriminant function analysis based on 5 call parameters

Table 2. Classification matrix for *P. kuhlii* and *P. nathusii* discrimination from the DFA with 10-fold cross-validation procedure. The average accuracy of the correct classification is 81.5%.

	P. kuhlii observed	*P. nathusii* observed
P. kuhlii predicted	85%	15%
P. nathusii predicted	22%	78%

Results of the survey

We conducted the point-count sampling by means of ultrasound recording in 71 settlements. We found at least one echolocation sequence from *P. kuhlii* or *P. nathusii* in 37 settlements, and altogether 128 sequences were collected (3.2±2.5 sequences/site). Using the discriminant functions from DFA described above, we identified the sequences recorded in the survey (Figs 2 & 3). A significant difference in the activity rate of *P. kuhlii* was found between the two areas (χ^2 test, df = 1, $\chi^2 = 31.9$, $p < 0.0001$), with activity in the northern area being higher. The activity rate of *P. nathusii* did not differ between sites (χ^2 test, df = 1, $\chi^2 = 3.60$, $p = 0.058$).

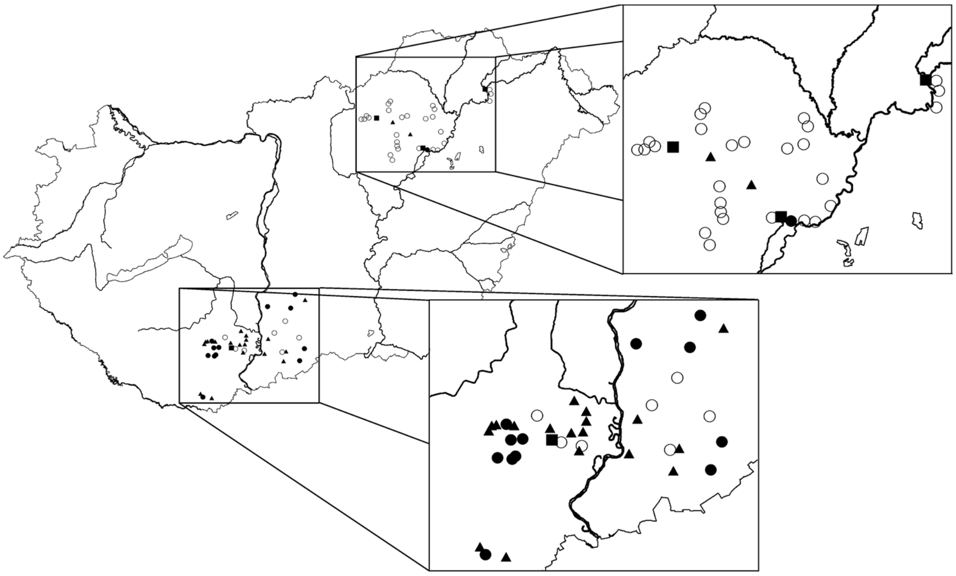

Fig. 2. Occurrences of the two species in the two studied areas from North and South Hungary. (open circle = none of the species found, black square = *P. nathusii*, black triangle = *P. kuhlii*, black circle = both species found)

DISCUSSION

Call parameters and species discrimination

Values of call parameters used for the identification of *P. kuhlii* may differ considerably from study to study depending on the geographic area of sampling or on the recording equipment and the context (animal in hand, releasing from hand or free-flying animals), or on the parameter-measuring technique (PARSONS & SZEWCZAK 2009). For example, the value of the end frequency of a *P. kuhlii* echolocation call – which is the most important parameter in our DFA – may vary considerably in different studies, e.g. 33.6±1.3 kHz (OBRIST *et al.* 2004), 39.6±1.71 kHz (RUSSO & JONES 2002), 37.5±1.3 kHz (PAPADATOU *et al.* 2008) and in this study it is 38.3±1.7 kHz. However, the relation of some parameters between *P. kuhlii* and *P. nathusii* seems stable. For example, both in the literature and in our study, the lowest frequency values are slightly, but consistently lower in *P. kuhlii* than in *P. nathusii* (OBRIST *et al.* 2004, PAPADATOU *et al.* 2008).

In most cases it is hard to separate the effects mentioned above, and to identify how the geographical distance might influence call parameters. However, to avoid this problem, we used our own call library to make discriminant functions valid for the Hungarian area, and we suggest doing the same in other countries prior to the identification of acoustic data gathered.

The classification accuracy from discriminant function analysis of this study is very similar to the DFA results of OBRIST *et al.* (2004) from Switzerland based on similar parameters (T_{dur}, F_{max}, F_{start}, F_{end}). However, OBRIST *et al.* got slightly better results when they used a synergetic classifier algorithm. Our discrimination result, which had an accuracy of around 81.5%, is not suitable for indicating new species within a given area, at a confidence of 100%. However, in case of monitor-

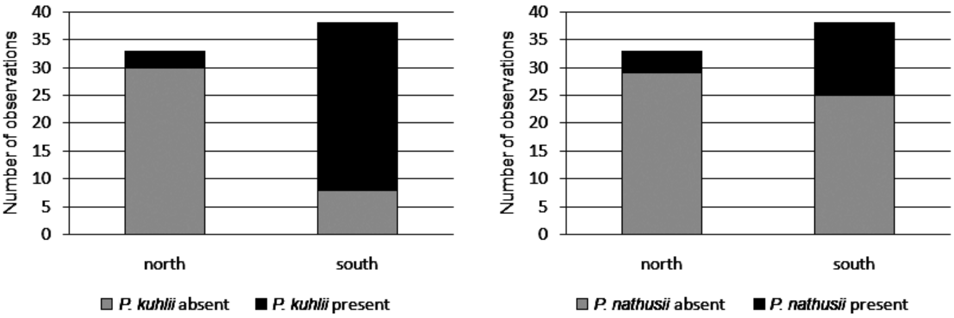

Fig. 3. Bar graph about the number of settlements where *P. kuhlii* and *P. nathusii* occurred or were absent in case of the two studied areas, from North and South Hungary

ing studies, where changes are to be monitored within an area or where two areas are to be compared in terms of activity rate, this technique might be feasible alternative, provided that the method is sensitive enough and the sample size is sufficient (VAUGHAN *et al.* 1997, RUSSO & JONES 2003, OBRIST *et al.* 2011).

Interpretation of survey results

A significant difference was found in the activity rate of *P. kuhlii* between the northern and southern areas sampled. Our results revealed that *P. kuhlii* is a widespread species in the settlements monitored in the southern part of Hungary, while in northern part, it is quite rare. These results may be beneficial to prove the usability of the method, despite of the classification accuracy being only 81.5%. Prior to this survey, we had information about *P. kuhlii* occurrence in numerous settlements throughout the country, and especially in the bigger cities (FEHÉR 2007*b*, ESTÓK, GÖRFÖL & ZSEBŐK unpubl. data), but such a large difference between the southern and northern area was not expected. Unfortunately, we cannot follow changes in the abundance of *P. kuhlii* during previous decades in Hungary, because we have no comparable dataset. We can only see that 15 years after *P. kuhlii* was first identified in Hungary in 1993 (FEHÉR 1995), it has become a common species in the southern studied area. In the future, regular use of this method within other areas should allow us to produce comparable datasets, and hence enable us to follow the area expansion of *P. kuhlii* in Hungary.

No significant difference between the two areas was found in the activity rate of *P. nathusii*. In Hungary, this species mainly inhabits lowlands, with a major abundance in forested areas near larger rivers and lakes (FEHÉR 2007*a*). Therefore, *P. nathusii* was not expected to be a common species in human settlements, although the possibility of the species occurring in urban habitat cannot be excluded, provided that preferential conditions exist. Moreover, *P. nathusii* is a widespread species throughout the country, thus these results comply with our expectations.

The method in general

The method used in this study might be useful to monitor the area expansion of other bat species, such as *Hypsugo savii*, which would be one of the "winners" of climate change (REBELO *et al.* 2010). Although this species is not as widespread in Hungary as *P. kuhlii*, more and more data are available regarding their breeding in urbanized areas in Central Europe (BARTONIČKA & KAŇUCH 2006, GÖRFÖL *et al.* 2007). Another interesting application of our method would be to monitor the change in abundance of other sympatric bat species (for example *P. pygmaeus*) in the settlements where *P. kuhlii* abundance is increasing. Our above-described technique is well suited for use in studies of potential inter-specific competition.

On a larger scale, it would be important to include all concerned European countries in the comparison of occurrence data deriving from different larger areas, and to follow changes in the abundance of species in a long term study. In order to accomplish this it would be necessary to use identical or similar techniques, where comparison of results is possible. One example of such an international program is the iBats car survey (JONES *et al.* 2011). Compared to the iBats program, our method is more concentrated on urbanized areas, where many settlements can be sampled in a short time and with relatively inexpensive equipment. Studying the rapid range expansion of *P. kuhlii* using quantifiable and comparable occurrence data produced by means of the method we present in this paper might facilitate the study of the impact of climatic change.

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