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Song Characteristics of Oriental Cuckoo *Cuculus optatus* and Himalayan Cuckoo *Cuculus saturatus* and Implications for Distribution and Taxonomy

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Canwei Xia, Wei Liang, Geoff J. Carey, and Yanyun Zhang (2016) Song features during the breeding season are important in identifying species of cuckoos. Whether Oriental Cuckoo *Cuculus optatus* and Himalayan Cuckoo *C. saturatus* inhabiting the Palearctic and Oriental realms respectively can be distinguished according to song characteristics is uncertain. In this study, we performed a thorough investigation of the song characteristics of these taxa by collecting and analyzing recordings of song in their distribution areas. We found that songs could be divided into two groups based on the number of notes per syllable, and significant differences in other frequency and temporal features were also found between these two groups. The group with a song comprising two notes per syllable was shown to breed in Russia, Japan and China including Xinjiang, Inner Mongolia, Heilongjiang and Taiwan, while the group with a song containing more than two notes per syllable was found to breed in the Himalayas and central China, extending northeast through north China as far as northeast Hebei, and south to southwest China. The distribution of these two groups was broadly related to the published distribution of populations of *optatus* and *saturatus*, respectively. Our data supported the separation of *optatus* and *saturatus* based on their song features, and also suggested refinements to the distribution of these two taxa, as follows: birds in north mainland China are *saturatus*, and those in Taiwan Island are *optatus*.

Key words: Distribution, Himalayan cuckoo, Oriental cuckoo, Song characteristics, Taxonomy.

BACKGROUND

Song is a vocal behavior of birds that serves as a means of territory defense and/or mate attraction during the breeding season (Marler and Slabbekoorn 2004; Catchpole and Slater 2008). As an important reproductive isolation mechanism, song is an essential basis for classification among Passeriformes (Slabbekoorn and Smith 2002; Alström and Ranft 2003). In non-Passeriformes, vocalizations are often simple and stereotyped, with no song behavior. However, certain non-Passeriformes, such as waders, rails, owls, pigeons and barbets, utilize a song that, although stereotyped, is important in territory defense (Kroodsma and Miller 1996). This is the case with cuckoos (*Cuculus* spp.), whose vocalizations during the breeding season serve as a means of territory defense and mate attraction, similar to the songs of Passeriformes (Payne 2005). Since cuckoo song is relatively conserved with little intraspecies variation, it can serve as an important basis for interspecies classification (Payne 2005).

Oriental Cuckoo *Cuculus optatus* (previously *C. horsfieldi*, a junior synonym) and Himalayan Cuckoo *Cuculus saturatus* were originally

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described as species before Peters (1940) treated *optatus* as a subspecies of *saturatus*. The latter approach was generally followed into the 1990s (*e.g.* Sibley and Monroe 1990; Howard and Moore 1991) until Payne (1997) accorded *optatus* specific status based on differences in song. Johnsgard (1997) classified the taxa according to their distributions during the breeding season, with *optatus* breeding in the Palearctic realm and *saturatus* in the Oriental realm. King (2005) and Payne (2005) provided further evidence of their specific status based on song features, and this is the approach of the International Ornithological Congress (Gill and Donsker 2014), which has adopted Oriental Cuckoo (*optatus*) and Himalayan Cuckoo (*saturatus*) as the English names, which we follow here. However, Lindholm and Lindén (2007) found that song of presumed *optatus* in Hebei and Shanxi, mainland China (located in the Palearctic realm) showed greater resemblance to the song of *saturatus*, whereas the song of presumed *saturatus* from Taiwan Island (located in the Oriental realm) resembled more that of *optatus*, implying song features are highly variable within species. Based on this research, Erritzøe et al. (2012) believed there was an overlap between the songs of *saturatus* and *optatus*, and treated *optatus* as a subspecies of *saturatus*. As suggested by Payne (2005), in cuckoos, "if populations have the same calls and songs, they are considered one species, and if they have different calls, they are different species." Thus, whether there are consistent differences in song between *saturatus* and *optatus* is of importance in determining whether they should be treated as one or two species.

Cuculus optatus and *C. saturatus* have significant differences neither in mitochondrial DNA (Payne 2005) nor in morphology, other than the larger size of the former (Leven 1998); as a result, song differences between the two have been the focus of taxonomic discussions (King 2005; Payne 2005; Erritzøe et al. 2012). Payne (2005) classified *optatus* and *saturatus* as two species based on the following (see Fig. 1 for song terms): (1) the first note in syllable of *saturatus* has a higher frequency than the second note, which is not observed in *optatus*; and (2) the syllable frequency of *optatus* is lower than that of *saturatus*. King (2005) also supported this distinction between the two species, and believed that song differences could be summarized as follows: (1) the first note of each syllable of *saturatus* has a higher frequency than the second, which is not observed in *optatus*, and (2) the number of notes per syllable of *optatus* is strictly two, while that of *saturatus* is greater than two. In this study, we aimed to perform a thorough analysis of the song of *saturatus* and *optatus* in order to shed further light on distribution and classification. We tested whether songs can be classified into two groups based on the three features proposed by Payne (2005) and King (2005): (1) the number of notes per syllable; (2) the frequency ratio of the first note over the second note; and (3) the syllable frequency. On the basis of above research, we also analyzed other differences in song features and discussed distribution areas and taxonomy of these two groups accordingly.

MATERIALS AND METHODS

Audio acquisition

We obtained 119 recordings from our own data as well as online databases (Macaulay Library [http://macaulaylibrary.org], Xeno-canto [http://www.xeno-canto.org], and AVoCet: Avian Vocalizations Center [http://avocet.zoology.

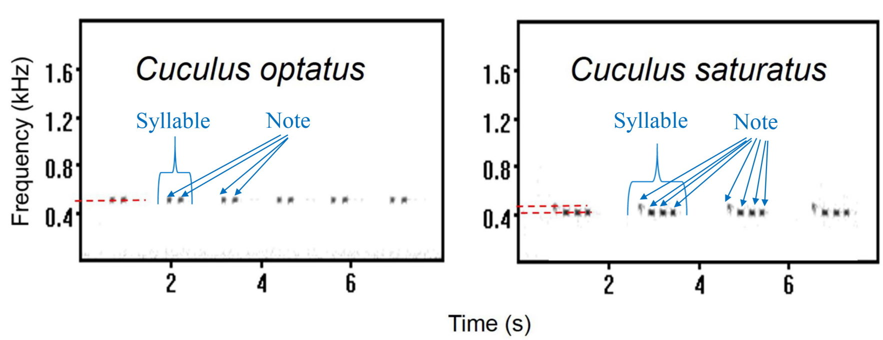

Fig. 1. Spectrograms of the songs of *Cuculus optatus* and *Cuculus saturatus* and song terms. The red dashed line indicates the peak frequency of note.

msu.edu]). Goldwave 5.25 audio processing software (Goldwave, Canada) was used for audio resampling with the following parameters: sampling precision, 16 bit; sampling frequency, 8,000 Hz; and audio format, .wav. For multiple recordings collected on the same day at the same location such that individuals could not be identified, one recording was randomly selected for analysis to avoid false repetition. Recordings with background noise that affected measuring were also removed. A total of 83 usable recordings were obtained (Additional file 1).

As differences between *optatus* and *saturatus* in molecular date (*e.g.* mitochondrial DNA, Payne 2005) is not significant, in plumage and size are minor, and song features are overlap (Lindholm and Lindén 2007; Erritzøe et al. 2012), now, the taxa are mainly based on distribution areas. One aim of this study is to refine distribution areas, so we didn't link the recordings with the corresponding species based on names in online databases, which is mainly based on distribution areas. We firstly mixed all recordings together, and then linked the recordings with the corresponding taxa based on song features, if there are any song features can successfully split the group.

Audio measurements

Avisoft-SASLab Pro 5.2 audio analysis software (Avisoft Bioacoustics, Germany) was used to generate spectrograms. Different spectrogram parameters were used to measure temporal and frequency features to achieve higher precision. The spectrogram parameters for temporal feature measurement were as follows: Hamming window; FFT length, 64; frequency bandwidth, 163 Hz; and time resolution, 4 ms. The spectrogram parameters for frequency feature measurement were as follows: Hamming window; FFT length, 1,024; frequency bandwidth, 10 Hz; and time resolution, 64 ms. Terms used in analysis are illustrated in figure 1. A note refers to a trace continuously appearing in the spectrogram, while a syllable is composed of multiple notes, with relatively longer time intervals between syllables. Based on previous studies (King 2005; Lindholm and Lindén 2007) and the requirements of this study, an automatic measurement method was applied to measure the following variables: duration and peak frequency of the first note; duration and peak frequency of the second note; and duration, peak frequency and note number of syllable. Peak frequency refers to the frequency associated with the maximum energy, and be called frequency for short below. The frequency ratio of the first note relative to the second note was also calculated.

As noted by Lindholm and Lindén (2003), the song of 'Oriental Cuckoo' *sensu lato* usually begins with the 'soft phrase' (a softer and faster sequence of 4-10 notes), followed by a series of syllables. The soft phrase is uttered once only in each song series, and, given the unpredictable nature of cuckoo song utterance, was consequently absent in many of our recordings. Our pragmatic solution to this problem was to focus on song features in the remainder of the song, an approach also adopted by King (2005) and Lindholm and Lindén (2003, 2007).

A sample of 39 individuals that had more than five consecutive syllables clearly recorded was first selected, and five consecutive syllables were measured for each individual. After demonstrating that song variables were highly conserved in the same recording (Table 1) using reliability analysis (Rankin and Stokes 1998), one syllable was randomly selected and measured from each of the recordings (sample size 83).

Data analysis

The following frequency distribution was proposed for the selected syllables from recordings: (1) the number of notes per syllable; (2) the frequency ratio of the first note relative to the second note in each syllable; and (3) the syllable

Table 1. Reliability of song features within recording

Reliability	Frequency of the first note	Duration of the first note	Frequency of the second note	Duration of the second note	Note number per syllable	Sentence frequency	Sentence duration	First note frequency/ second note frequency
Mean	0.980	0.870	0.991	0.851	0.971	0.988	0.974	0.960
95% low	0.968	0.805	0.986	0.778	0.954	0.981	0.959	0.937
95% up	0.988	0.922	0.995	0.910	0.983	0.993	0.985	0.976

frequency. Song features were plotted based on the quartiles of the above-mentioned variables.

Note number per syllable was found to have a bimodal distribution; accordingly, vocalizations were classified into two groups based on this, and differences in other song features were compared between these two groups. We applied a MANOVA to assess the overall differences between the two groups, followed by an independent sample t-test for each variable. Discriminant and principal component analyses were employed to examine whether features other than note number per syllable differed between the two groups. In discriminant analysis, results from jack-knifed classifications, in which each recording was assigned to a group on the basis of a discriminant function calculated from all recordings in the data set except the one being classified, are reported as percentages of recordings correctly assigned. In principal component analyses, varimax rotation was used, because it provided a clearer (very high loadings for some of the variables and very low loadings for the others) interpretation of the principal components (PCs) than without varimax rotation.

Statistical analysis was performed using SPSS 21.0. Data were presented as the mean ± standard deviation. Differences with P values of less than 0.05 were considered significant. Permission for this study (recording cuckoo vocalizations) was granted by Ethic and Animal Welfare Committee in Beijing Normal University (license number CLS-EAW-2013-016).

RESULTS

Note number per syllable was higher in Himalayan areas (where 4-6 notes were recorded) and central China (where four notes dominated), and lower in Russia, Japan and some region of China including Xinjiang, Inner Mongolia, Heilongjiang and Taiwan, where only two notes per syllable were recorded (Fig. 2). The frequency ratios of the first note relative to the second note were higher in Himalayan areas, central China and Beijing/Hebei, and lower in Russia, Japan and some region of China including Xinjiang, Inner Mongolia, Heilongjiang and Taiwan, respectively (Fig. 3). The highest syllable frequency was observed in Taiwan Island, while the lowest syllable frequency was observed in Russia; however, syllable frequency varied substantially among adjacent locations in Himalayan areas and central China (Fig. 4). There was a significant correlation between the note number per syllable and the frequency ratio of the first note relative to the second note (Kendall correlation coefficient = 0.612, P < 0.001). In contrast, there were no significant correlations of either of these parameters with syllable frequency (Kendall correlation coefficient = 0.079, P = 0.317; Kendall correlation coefficient = 0.081, P = 0.360, respectively).

Among the aforementioned variables (Figs. 2A, 3A, and 4A), note number per syllable showed a clear bimodal distribution with peak values of 2 and 4. Furthermore, after mapping note number per syllable (Fig. 2B), it was clear that there was broad congruence with published distributions of

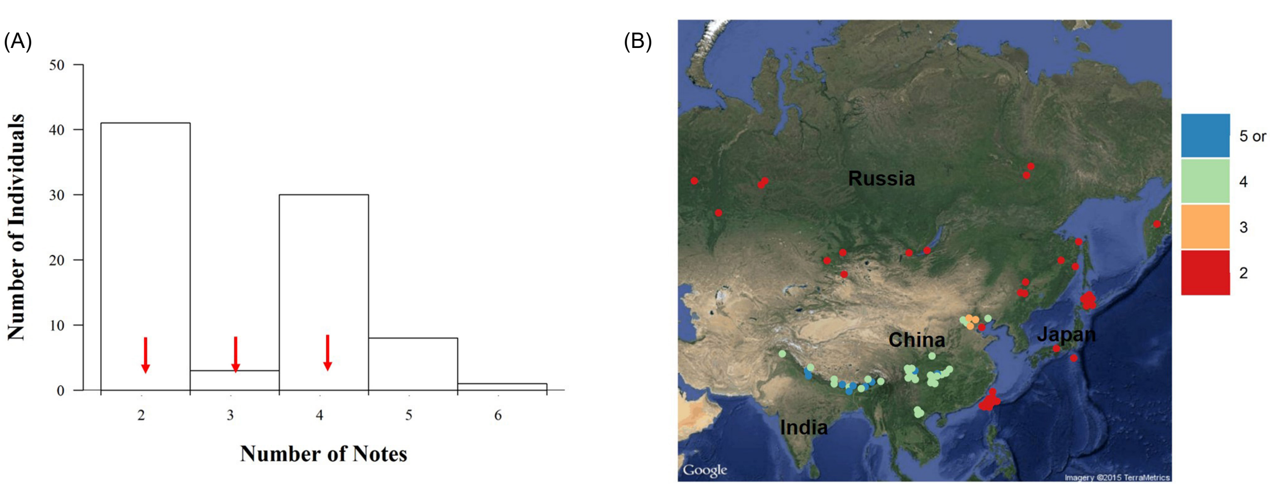

Fig. 2. Frequency distribution (A) and geographical distribution (B) of the note number per syllable in each syllable. The red arrow indicates the quartile. Geographic base map in (B) is from Google Maps (Google, USA).

optatus and *saturatus*, and with the differentiation described by King (2005), with birds having two notes per syllable thus considered referable to *optatus*, and birds with more than two per syllable referable to *saturatus*. Consequently, we set note numbers of two or more than two per syllable as criteria to divide birds into two groups (n = 42 and n = 41, respectively).

Other song features (except for the number of notes per syllable) are significantly different between two groups (MANOVA: Pillai's Trace = 0.934, $F_{7, 75}$ = 152.315, P < 0.001). For the group with two notes, the first note had a relatively lower frequency and longer duration, the second note had a relatively shorter duration, the duration of the syllable was relatively short, and the frequency ratio of the first note over the second note was relatively small (Table 2). Discriminant analysis using other song features (except for the number of notes per syllable) achieved 100% accuracy in distinguishing between the two groups. Principal components analysis generated three principal components with eigenvalues > 1.0, explaining 92.4% of the variance in the original seven variables (Table 3). The two groups could also be clearly separated by conducting principal component analysis with song features other than note number per syllable and plotting with the first two principal components (Fig. 5).

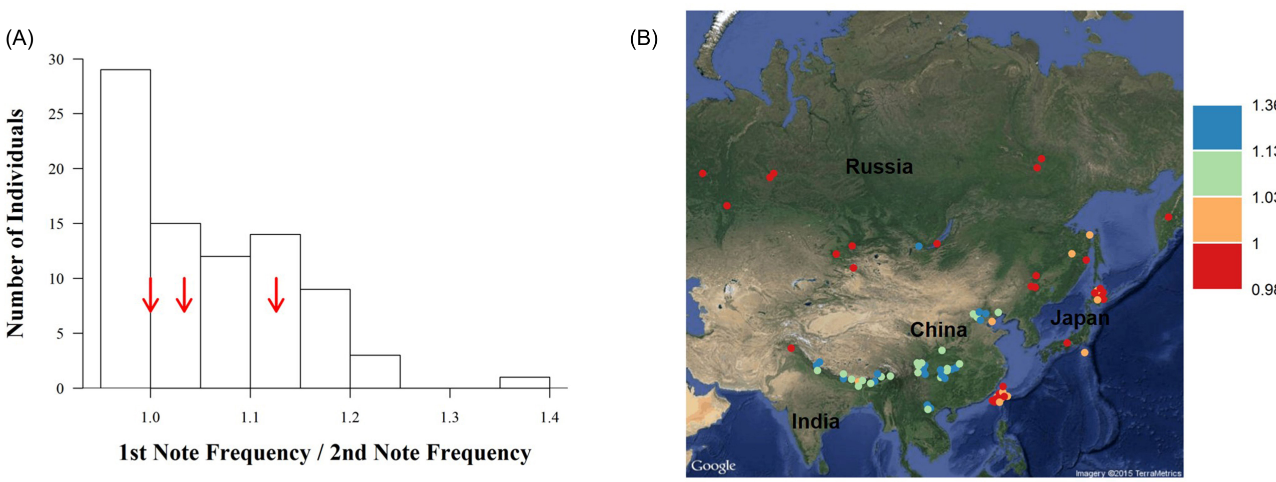

Fig. 3. Frequency distribution (A) and geographical distribution (B) of the frequency ratio of the first note over the second note. The red arrow indicates the quartile. Geographic base map in (B) is from Google Maps (Google, USA).

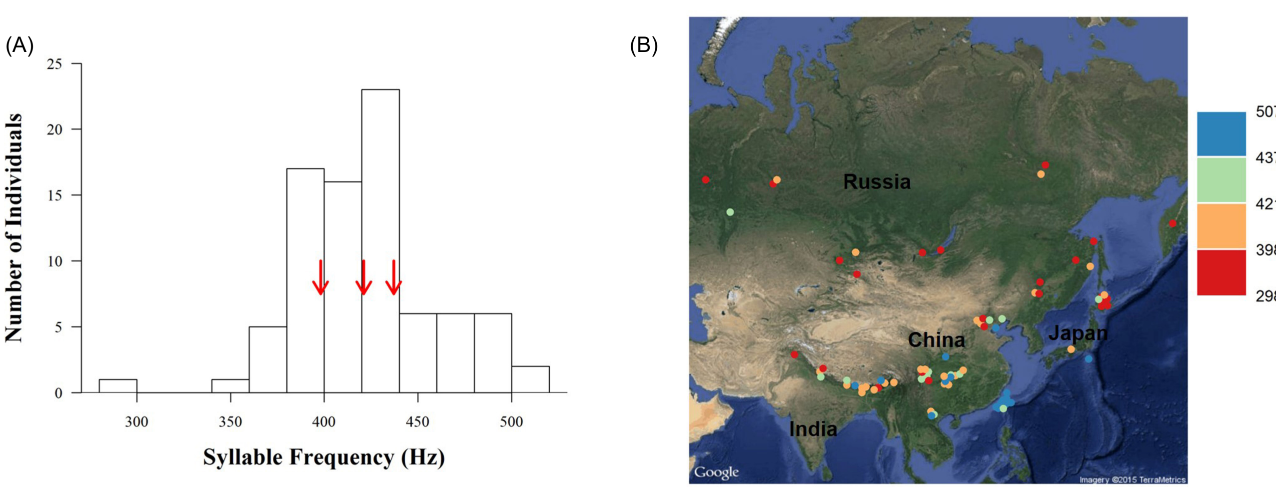

Fig. 4. Frequency distribution (A) and geographical distribution (B) of syllable frequency. The red arrow indicates the quartile. Geographic base map in (B) is from Google Maps (Google, USA).

DISCUSSION

Song difference between *optatus* and *saturatus*

In this study, we performed an in-depth investigation of song in *optatus* and *saturatus*. Our data showed that song could convincingly be used to distinguish birds into two groups, and that the distributions of these two groups was broadly related to the published distribution of populations of *optatus* and *saturatus*, respectively. Unlike the previous studies (*e.g.* King 2005; Lindholm and Lindén 2007), which have broadly accepted published distributions (Johnsgard 1997) and interpreted vocal data accordingly, we firstly tested whether song features could be used to classify

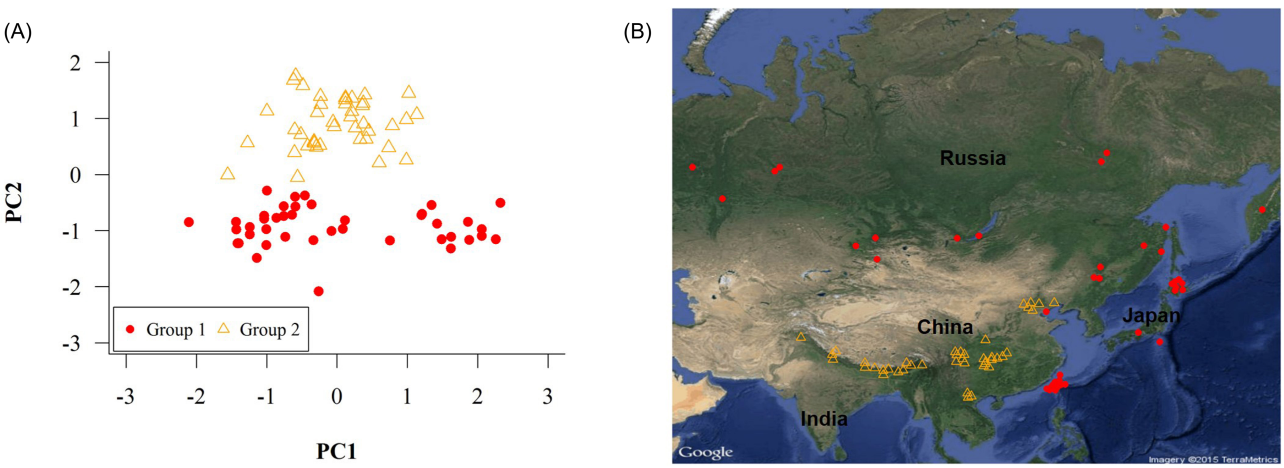

Fig. 5. Differentiation between different groups based on the first two principal components of song features (A) and geographical distribution (B). Geographic base map in (B) is from Google Maps (Google, USA). The relationship between principal components and song variables are shown in table 3.

Table 2. Comparison between song variables of different cuckoo groups (independent sample *t*-test)

Song variables	Group 1 (Mean ± SD)	Group 2 (Mean ± SD)	t_{81}	*P*
Frequency of the first note (Hz)	428.56 ± 48.09	472.02 ± 32.39	-4.840	< 0.001
Duration of the first note (s)	0.08 ± 0.02	0.04 ± 0.01	11.329	< 0.001
Frequency of the second note (Hz)	426.29 ± 45.97	421.88 ± 21.06	0.564	0.574
Duration of the second note (s)	0.09 ± 0.02	0.11 ± 0.02	-3.292	0.001
Syllable frequency (Hz)	426.29 ± 45.97	422.26 ± 21.19	0.515	0.608
Syllable duration (s)	0.30 ± 0.03	0.82 ± 0.14	-23.621	< 0.001
First note frequency/second note frequency	1.00 ± 0.01	1.12 ± 0.06	-12.791	< 0.001

Table 3. Eigenvalues and factor loadings for principal components analysis of song variables

Song variables	PC1	PC2	PC3
Frequency of the first note (Hz)	0.828	0.501	0.171
Duration of the first note (s)	-0.048	-0.919	0.189
Frequency of the second note (Hz)	0.993	-0.054	-0.055
Duration of the second note (s)	-0.013	0.128	0.971
Syllable frequency (Hz)	0.994	-0.047	-0.043
Syllable duration (s)	0.003	0.893	0.193
First note frequency/second note frequency	0.075	0.851	0.336
Eigenvalue	3.155	2.309	1.007
% Variance explained	45.077	32.983	14.384

recordings into two groups, and then refined the distribution areas of the two groups based on song features.

Cuckoo songs were quantified, and note number per syllable was found to have a clear bimodal distribution; thus, we used note number per syllable as the criterion for differentiation. Groups divided by this criterion also had significant differences in other song features, and the accuracy of discriminant analysis was as high as 100%. The method used to distinguish between *saturatus* and *optatus*, *i.e.*, using note number per syllable, was the same in this study as in a previous study by King (2005). Also consistent with the latter, we found that the song of *optatus* had two notes, while the song of *saturatus* had more than two notes. Because it is quite straightforward to identify the number of notes, this criterion can also be used to distinguish singing birds in the field easily. The frequency ratio of the first note relative to the second note was also a criterion used by Payne (2005) and King (2005). According to data from our study, this feature was positively correlated with note number, and the distributions of the two parameters were generally consistent. Hence, the frequency ratio of the first note relative to the second note can also be used as a criterion to distinguish between the two groups. Payne (2005) stated that song frequency of *optatus* was lower than that of *saturatus*. According to our data, frequency tended to be higher in southern regions and lower in northern regions; however, substantial variations were found across adjacent areas, especially in Himalayan areas. For this reason, we cannot unequivocally recommend using song frequency to distinguish between these two groups.

Distributions and taxonomy of *optatus* and *saturatus*

Johnsgard (1997) classified *optatus* and *saturatus* as two subspecies according to their distributions during the breeding season, with *optatus* breeding in the Palearctic realm and *saturatus* in the Oriental realm; this division of the distribution areas has generally been utilized by other authors (*e.g.* Payne 2005; Erritzøe et al. 2012). Both King (2005) and Lindholm and Lindén (2007) found that the song of cuckoos in Taiwan Island (located in the Oriental realm), which had syllables composed of two notes, is more similar to *optatus*; while Lindholm and Lindén (2007) also found that the song of cuckoos in Hebei and Shanxi (located in the Palearctic realm), which had syllables composed of three to four notes, is similar to *saturatus*. Results of our study supported these findings, as all 11 recordings made in Taiwan Island had syllables composed of two notes, and six of seven recordings from Beijing and Hebei had syllables composed of three or four notes. The one recording that had syllables composed of two notes, recorded in May on a small island ('Happy Island' or Shi Jiu Tuo) off the coast of Hebei, is likely to have been a migrant rather than a locally-breeding bird, as the island appears to lack breeding habitat for both Oriental cuckoo and Himalayan cuckoo. Unlike Erritzøe et al. (2012) who believed that song is not discriminant between *optatus* and *saturatus*, we believe a simpler and more straightforward explanation is that birds in north mainland China are, indeed, *saturatus*, and those in Taiwan Island are *optatus*. We thus set the distribution boundary between *optatus* and *saturatus* in Northern China (Fig. 6), which is in line with distribution boundary of some species pairs based on recent researches, for example, Blue-and-white Flycatcher (*Cyanoptila cyanomelana*) and Zappey's Flycatcher (*Cyanoptila cumatilis*) (Leader and Carey 2012), Songar Tit (*Parus songarus*) and Willow Tit (*Parus montanus*) (Kvist et al. 2001), Silver-throated Bushtit (*Aegithalos glaucogularis*) and Long-tailed Tit (*Aegithalos caudatus*) (Päckert et al. 2010). We obtained

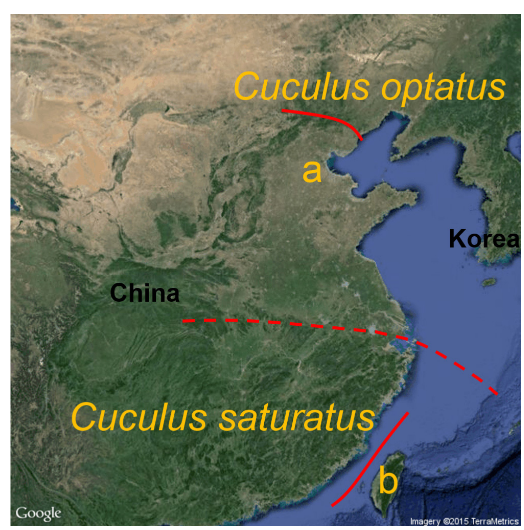

Fig. 6. Boundary in China between distribution areas of *Cuculus optatus* and *Cuculus saturatus* during breeding season. The dashed line is the boundary that Johnsgard (1997) used to separate *Cuculus optatus* and *Cuculus saturatus*. The solid line is the boundary suggested by this study. a: Northern mainland China, b: Taiwan Island. Geographic base map is from Google Maps (Google, USA).

recordings from *optatus* and *saturatus* across most range of their breeding area, the exception being central Russia. This defect was alleviated by examining songs from 58 males in Russia presented in Lindholm and Lindén (2007). All these belong to *optatus* according the criteria in this study, as there are two notes per syllable and a smaller frequency change between the first note and the second note (Lindholm and Lindén 2007).

As cuckoo song is innate and may have a strictly genetic basis (Mayr 1942), it is most useful in identifying species (Payne 2005). With regard to *optatus* and *saturatus*, Payne (1997) accorded both specific status mostly based on differences in their songs, while Erritzøe et al. (2012) treated the two as a single species after Lindholm and Lindén (2007) found apparent overlap between their songs. In this study, after a thorough investigation of the song characteristics of these taxa, we found the songs can clearly be split into two groups, and there is no song characteristic overlap between *optatus* and *saturatus* after a fine adjustment in distribution area of these two taxa. Thus, our data support the separation of *optatus* and *saturatus* into two species, as suggested by Payne (1997, 2005). Three notes per syllable are present in three recordings from Beijing and Hebei, near the northernmost part of the range of *saturatus*. The number of notes per syllable in these recordings lies between *optatus* (with two notes per syllable) and other *saturatus* (with four to six notes per syllable). This may indicate past interbreeding between *optatus* and *saturatus* at this area. Although song difference is not absolutely definitive in taxonomy without other evidence such as morphology and genetic markers, given the high degree of conformity of song with published distributions, there is strong evidence to support the conclusions. At least, our data deny there is an overlap between the songs of *saturatus* and *optatus*, which was proposed by Lindholm and Lindén (2007) and Erritzøe et al. (2012).

CONCLUSIONS

In this study, the song features of birds from across the range of *optatus* and *saturatus* were analyzed. Based on note number per syllable, birds could be split into two groups, and significant differences in other song features were also found between these two groups. The group with a song comprising two notes per syllable was shown to breed in Russia, Japan and China including Xinjiang, Inner Mongolia, Heilongjiang, Taiwan Island, while the group with a song containing more than two notes per syllable was found to breed in the Himalayas and central China, extending northeast through north China as far as northeast Hebei, and northern southeast Asia. The data largely agree with the size-related distribution described by Leven (1998), and with published distributions of *optatus* and *saturatus*, respectively (Johnsgard 1997; Payne 2005; Erritzøe et al. 2012). As the songs can be clearly split into two groups, our data support Payne (1997, 2005) in treating *optatus* and *saturatus* as two species.

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Additional file 1. Recordings used in this study and original measurement data. (download)