Song characteristics of Oriental Cuckoo *Cuculus optatus* and Himalayan Cuckoo *Cuculus saturatus* and implications for taxonomy and distribution

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Song features during the breeding season are important for determining classifications. 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 areas of distribution. We found that these cuckoos could be divided into two groups based on the number of notes in their songs. In addition, we observed significant differences in song duration and frequencies such that these two species could be clearly distinguished based on discriminant analysis of song features. Songs of Oriental cuckoos had two notes, whereas songs of Himalayan cuckoos had more than two notes. Thus, our data supported the separation of the two species based on their song features. The areas of distribution of the two species are thus refined, with Oriental Cuckoo distributed in Northeast China, Xinjiang in northwest China, Japan and Russia, and Himalayan Cuckoo distributed in the Himalayas through central and northern China to northeast Hebei, and the northern part of southeast Asia (Guangxi, China). The taxonomic status of birds in Taiwan indicates a closer relationship to *C. optatus*, which is contrary to the prevailing interpretation and requires further study.

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4 Abstract

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22	Keywords: Oriental cuckoo, Himalayan cuckoo, song variation, distribution, taxonomy
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34 Introduction

Song is a vocal behaviour of birds during the breeding season that serves as a means of territory defense and/or mate attraction (Marler & Slabbekoorn, 2004; Catchpole & Slater, 2008). In Passeriformes, as an important reproductive isolation mechanism, song is an essential basis for classification (Slabbekoorn & Smith, 2002; Alstrom & Ranft, 2003). In non-Passeriformes and sub-oscine passerines, vocalisations are often simple and stereotyped, with no song behaviour. However, certain non-passeriformes, such as waders, rails, owls, pigeons and barbets, utilize a song that, although stereotyped, is important in territory defense (Kroodsma & Miller, 1996). This is also the case with cuckoos (*Cuculus* spp.), whose vocalisations during the breeding season serve as a means of territory defence and mate attraction, similar to the songs of Passeriformes. 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 described as species before Peters (1940) treated *optatus* as a subspecies of *C. saturatus*. The latter approach was generally followed into the 1990s (e.g. Sibley & Monroe, 1990; Howard & Moore, 1991) until Payne (1997) accorded *optatus* specific status based on differences in song. King (2005) and Payne (2005) provided further evidence of their specific status, and this is the approach of the International Ornithological Congress (Gill & Donsker, 2014), which has adopted Oriental Cuckoo (*C. optatus*)

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and Himalayan Cuckoo (C. saturatus) as the English names, which we follow here.

C. optatus and C. saturatus have significant differences neither in mitochondrial DNA (Payne, 2005) nor in morphology, other than the larger size of the latter (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 C. optatus and C. saturatus as two species based on the following (see Fig. 1 for song terms): (1) the first note in a typical C. saturatus song syllable has a higher frequency than the second note, which is not observed in C. optatus; and (2) the song frequency of C. optatus is lower than that of C. saturatus. King (2005) also supported this distinction between the two, and believed that song differences could be summarized as follows: (1) the first note in a typical song syllable of C. saturatus has a higher frequency than the second, which is not observed in C. optatus, and (2) the number of notes in the main song syllable of C. optatus is strictly two, while that of C. saturatus has more than two notes. However, Lindholm and Lindén (2007) found that song of presumed C. optatus in Hebei and Shanxi, China (located in the Palearctic realm) showed greater resemblance to the song of C. saturatus, whereas the song of presumed Himalayan cuckoos from Taiwan (located in the Oriental realm) resembled more that of C. optatus. Based on Wells and Becking (1975) and Lindholm and Lindén (2007), Erritzøe et al. (2012) believed there was an overlap between the songs of C. saturatus and C. optatus, and treated them as two subspecies of the same species. Thus, whether there are differences in song between C. saturatus and C. optatus is of importance

in determining whether they should be treated as one or two species, and in determining the distribution of two taxa for which differences in plumage are very minor and differences in size minor.

In this study, we aimed to perform a thorough analysis of the song of *C. saturatus* and *C. optatus* in order to shed further light on species limits. We tested whether the taxa can be classified into two groups based on the three features proposed by Payne (2005) and King (2005): (1) the frequency ratio of the first note over the second note; (2) the syllable frequency; and (3) the number of notes. We also analyzed other differences in song features of the two groups and reviewed the distribution areas of the two groups based on this analysis.

Materials and Methods

Audio acquisition

We obtained 119 recordings of song of the two taxa acquired 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.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 testing were also removed.

A total of 83 usable recordings were obtained (Table S1, Data S1).

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Audio measurements

Avisoft-SASLab Por 5.2 audio analysis software (Avisoft Bioacoustics, Germany) was used to generate sonograms. Different sonogram parameters were used to measure temporal and frequency features to achieve higher precision. The sonogram parameters for temporal feature measurement were as follows: Hamming window; FFT length, 64; frequency bandwidth, 163 Hz; and time resolution, 4 ms. The sonogram 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 Fig. 1. A note refers to a curve continuously appearing in the sonogram, while a syllable is composed of multiple notes, with relatively longer time intervals between syllables. Based on previous studies (King, 2005; Lindholm & Linden, 2007) and the requirements of this study, an automatic measurement method was applied to measure the following variables: duration and frequency of the first note; duration and frequency of the second note; and duration, frequency and note number of syllable. Frequency refers to the frequency associated with the maximum energy. 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 a softer and faster sequence of 4-10 notes, which sometimes also occur irregularly in other parts of the song sequence; this they termed the 'soft phrase'. Both King (2005) and Lindholm and Lindén (2003; 2007) excluded this syllable from measurements, and we also saw fit to do this.

Eleven individuals were first selected (4 *C. optatus*, and 7 *C. saturatus*), 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 & Stokes, 1998), one syllable was randomly selected and measured from each of the individuals.

Data analysis

After removing the first syllable as described above, the following frequency distribution was proposed for the selected syllables from recordings of the 83 individuals: (1) the frequency ratio of the first note in each syllable relative to the second note; (2) the syllable frequency; and (3) the number of notes in each syllable. Song features were plotted based on the quartiles of the above-mentioned variables.

Note number was found to have a bimodal distribution; accordingly, vocalisations were classified into two groups based on this, and differences in other song features were compared

between the two groups. Discriminant and principal component analyses were employed to examine whether features other than note number differed between the two groups. The results of this analysis were used to inform assessment of the distribution of the two taxa; this is in contrast to previous studies, which have broadly accepted published distributions and interpreted vocal data accordingly.

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

137 Results

As shown in 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 northeastern China, Taiwan, Japan, and Russia, respectively. As shown in Fig. 3, highest syllable frequency was observed in Taiwan, while the lowest syllable frequency was observed in Russia; however, syllable frequency varied substantially among adjacent locations in Himalayan areas and central China. Note number was higher in the Himalayan areas (where 4-6 notes were recorded) and central China (where four notes dominated), and lower in northeast China, Taiwan, Japan, and

Russia, where only two notes were recorded (Fig. 4). There was a significant correlation between the frequency ratio of the first note relative to the second note and the note number (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 (Fig. 2a, 3a, and 4a), note number showed a clear bimodal distribution with peak values of 2 and 4. Following the differentiation between C. optatus and C. saturatus described by King (2005), we set note numbers of two or more than two as criteria to divide birds into two groups (n = 42 and n = 41, respectively). The two groups had significantly different song features (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 sentence 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) fully separated the two groups. The two groups could also be clearly separated by conducting principal component analysis with song features other than note number and plotting with the first two principal components (Fig. 5).

Discussion

In this study, we performed an in-depth investigation of song in *C. optatus* and *C. saturatus* in order to determine whether song variations could be used in differentiation. Our data showed that song could convincingly be used to distinguish between the two taxa and that this was related to the distribution of populations. Thus, these data supported the separation of the two species based on song features alone.

Cuckoo songs were quantified, and note number was found to have a clear bimodal distribution; thus, we used note number 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 *C. saturatus* and *C. optatus*, i.e., using note number, was the same in this study as in a previous study by King (2005). Also consistent with the study by King (2005), we found that the song of *C. optatus* had two notes, while the song of *C. saturatus* had more than two notes (see sonograms in Fig. 6). Because it is quite straightforward to identify the number of notes, this criterion can also be used to distinguish between singing *C. saturatus* and *C. optatus* in the field.

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 species. Payne (2005) stated that song

frequency of *C. optatus* was lower than that of *C. 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 *C. saturatus* and *C. optatus*.

Distributions of C. saturatus and C. optatus

Johnsgard (1997) classified the taxa 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). King (2005) noted the allopatric nature of their breeding season distributions, with *C. optatus* breeding from northeast Europe across the Palearctic to Japan and northeast China, and *C. saturatus* breeding across the Himalayas, southern China, Taiwan (though suggesting a taxonomic reassessment of birds on that island was required) and the extreme northern part of southeast Asia (apparently referring to Guangxi, China, a location from where we also acquired data).

Leven (1998) indicated that, based on a specimen from Xinjiang China, on size birds in that province were probably *C. saturatus*. The sole recording we analysed from that province was

made in the Altai Mountains in the north of the province, and was unequivocally *C. optatus*. The specimen measured by Leven (1998) was number 38300 in Academia Sinica, Beijing, and is labelled from Altai and dated 13 August. This would appear to be anomalous, and is not considered sufficient to propose sympatry of the two species.

Both King (2005) and Lindholm and Lindén (2007) found that the song of Oriental cuckoos in Taiwan is more similar to that of *C. optatus* than that of *C. saturatus*. Results of our current study supported these findings, as all 11 of the cuckoo recordings made in Taiwan had syllables composed of two notes.

Lindholm and Lindén (2007) found that the song of Oriental cuckoos in Hebei and Shanxi, north China are similar to those of *C. saturatus*, and that syllables were composed of three to four notes, but stated that further data and analysis were needed to determine the (sub)specific status of these birds. Based on this and the similarity of the song of birds from Taiwan with *C. optatus* in Russia, Erritzøe et al. (2012) suggested that the two taxa had no definitive differences in song and suggested that they should be combined into a single species.

Among the seven Oriental cuckoos from Beijing and Hebei analysed in this study, one had syllables composed of two notes, three had syllables composed of three notes, and three had syllables composed of four notes. The individual singing with two syllables was recorded in May on a small island off the coast of Hebei ('Happy Island' or Shi Jiu Tuo), and is likely to have been a migrant rather than a locally-breeding bird, especially as the island appears to lack

optimum breeding habitat for either species; in view of this and the number of syllables, it is likely to have been *C. optatus*. Based on song features, we thus suggest that cuckoos breeding in northern China from Shanxi northeast to northeast Hebei belong to *C. saturatus* (see distribution map in Fig. 7). Such a phenomenon would be in line with other forest or forest-edge species for which the main distribution lies in the area of the Himalayas and central China, but which also range through mountainous areas to Beijing or northern Hebei, e.g. Claudia's Leaf Warbler *Phylloscopus claudiae*, Chinese Leaf Warbler *P. yunnanensis*, Large-billed Leaf Warbler *P. magnirostris*, Plain-tailed Warbler *Seicercus soror*, Long-tailed Minivet *Pericrocotus ethologus*, White-bellied Redstart *Hodgsonius phoenicuroides* (see Zheng, 2005; Brazil, 2009).

230 Conclusions

In this study, the song features of birds from across the range of *C. optatus* and *C. saturatus* were analysed. Based on this, 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 was shown to breed in Russia, northern Xinjiang in the Altai Mountains, northeast China, Japan, and Taiwan, while the group with a song containing more than two notes was found to breed in the Himalayas and central China, extending northeast through north China as far as northeast Hebei, and northern southeast Asia (Guangxi, China). The data largely agree with the size-related distribution described by Leven (1998). Overall, there is strong evidence to

239 indicate that *C. optatus* and *C. saturatus* should be treated as separate species.

In addition, while Erritzøe et al. (2012), based on the presence of birds uttering song of *saturatus* in Shanxi and Hebei and on the occurrence of song similar to *optatus* in Taiwan, believed that song is not discriminant, we believe a simpler and more straightforward explanation is that birds in north China are, indeed, *C. saturatus*. This is supported by a similar distribution of certain passerine species for which the breeding range is mainly concentrated in the Himalayan foothills, including west and central China. With regard to birds in Taiwan, we suggest these are either *C. optatus*, or a taxon closely-related; in either case, further study is required.

Acknowledgements

The recordings from online databases (Macaulay Library [http://macaulaylibrary.org], Xenocanto [http://www.xeno-canto.org], and AVoCet: Avian Vocalizations Center [http://avocet.zoology.msu.edu]) used in this study were much appreciated. We thank Kuankuoshui and Dongzhai National Nature Reserves for support and permission to carry out this study, Lijun Zhang and Chentao Wei for offering their recordings. Valuable comments on a draft were provided by Michael Leven and Paul Leader, and information was by Paul Holt.

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297 TABLE CAPTIONS

298 **Table 1.** Reliability of song features within individual cuckoo.

		Duration	Frequency	Duration				
D 1: 1:1:	Frequency	of the	of the	of the	Note	Sentence	Sentence	First note frequency/
Reliability	of the first	first	second	second	number	frequency	duration	second note frequency
± St	note	note	note	note				
Mean	0.968	0.821	0.994	0.960	0.973	0.993	0.979	0.935
95% low	0.927	0.647	0.986	0.909	0.939	0.983	0.952	0.856
95% up	0.990	0.939	0.998	0.987	0.992	0.998	0.994	0.979

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

0 :11	Group 1 (Mean ±	Group 2 (Mean	t ₈₁	P
Song variables	SD)	± SD)		
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
C-11-1.1- 11 (-)	0.20 + 0.02	0.82 ± 0.14	-	< 0.001
Syllable duration (s)	0.30 ± 0.03	0.82 ± 0.14	23.621	
First note frequency/second note	1.00 + 0.01	1 12 + 0.06	-	< 0.001
frequency	1.00 ± 0.01	1.12 ± 0.06	12.791	

Figure 1. Song terms.

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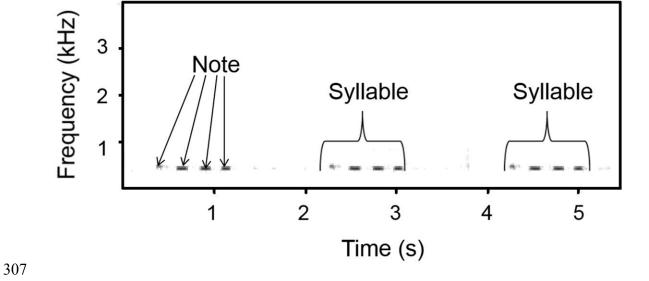
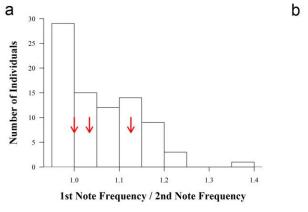


Figure 2. Frequency distribution (a) and map distribution (b) of the frequency ratio of the first note over the second note. The red arrow indicates the quartile. Geographic base map in (b) is from Google Maps (Google, USA).





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Figure 3. Frequency distribution (a) and map distribution (b) of syllable frequency. The red arrow indicates the quartile. Geographic base map in (b) is from Google Maps (Google, USA).

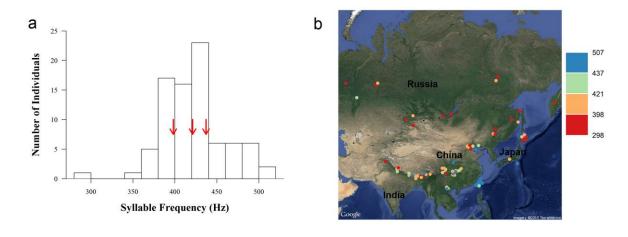
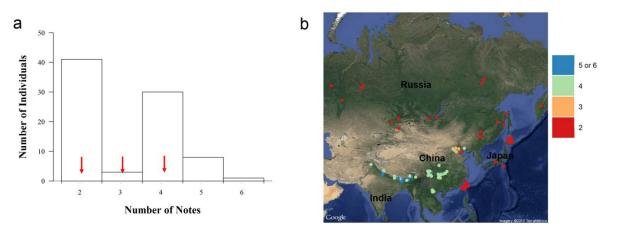


Figure 4. Frequency distribution (a) and map distribution (b) of the note number in each syllable.

The red arrow indicates the quartile. Geographic base map in (b) is from Google Maps (Google,

323 USA).



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Figure 5. Differentiation between different groups based on the first two principal components of song features (a) and map distribution (b). Geographic base map in (b) is from Google Maps (Google, USA).

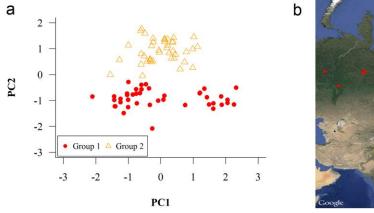




Figure 6. Sonograms of the songs of *Cuculus optatus* and *Cuculus saturatus*.

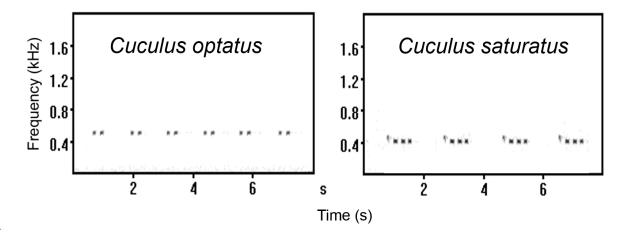


Figure 7. Boundary in China between distribution areas of *Cuculus optatus* and *Cuculus*341 *saturatus* during breeding season. The dashed line is the boundary that Johnsgard (1997) used to
342 separate Oriental Cuckoo and Himalayan Cuckoo. The solid line is the boundary suggested by
343 this study. a: Northern China, b: Taiwan. Geographic base map is from Google Maps (Google,
344 USA).

