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A phylogeographic break and bioacoustic intraspecific differentiation in the Buff-barred Warbler (*Phylloscopus pulcher*) (Aves: Passeriformes, Phylloscopidae)

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Abstract

Background: According to current taxonomy only three out of 27 Sinohimalayan leaf warbler species (Phylloscopidae) are considered genetically uniform across their entire breeding range along the Southeastern margin of the Qinghai-Tibetan Plateau, the Buff-barred Warbler (*Phylloscopus pulcher*) being one of them. Because marked differentiation among Himalayan and Chinese populations has been recently demonstrated for a number of *Phylloscopus* species (or sister species) we investigated the intraspecific variation of a mitochondrial gene, songs and morphology of *P. pulcher* in a phylogeographic approach.

Methods: We sequenced a fragment of the mitochondrial cytochrome *b*, reconstructed haplotype networks and analyzed DNA polymorphism among Himalayan and Chinese populations. We measured time and frequency parameters of two distinct song types and analyzed among population-differentiation in a principal component analysis and a discriminant analysis. We also compared measurements of body size dimensions taken from museum specimens.

Results: The mitochondrial haplotype network (*cytb*) was divided into two distinct clusters corresponding to geographic origin of samples. Pairwise genetic distances among Himalayan and Chinese mtDNA lineages account for 1.3% which coincides with Pleistocene lineage separation at roughly 650,000 years ago. Genetic diversity is slightly higher in the Chinese part of the species' range with respect to haplotype and nucleotide diversity while the less diversified Himalayan population lineage shows signs of recent range expansion. The vocal repertoire of *P. pulcher* comprises two distinct verse types that are combined with short interspersed click notes to long continuous song displays. Trill verse types showed significant differences among regions in almost all measured frequency and time parameters: Chinese males displayed more rapid and more broad-banded trills at a lower pitch. In contrast, warbling verse types showed a distinctively different structure among regions: Himalayan songs consisted of repeated syllables while Chinese songs comprised repetitions of single, long and strongly modulated elements. Subtle morphological differences among specimens from the two study regions could only be confirmed for plumage coloration but not for metric characters.

Conclusions: Based on the genetic and bioacoustic distinctiveness of Chinese Buff-barred Warbler populations, we recommend that the name *Phylloscopus pulcher vegetus* Bangs, 1913 should be re-validated for this taxon.

Keywords: Phylloscopidae, Phylogeography, East–west divide, Territorial songs, *Phylloscopus pulcher vegetus*

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Background

Phylogeographic patterns of Sino-Himalayan forest passerines are widely congruent for terminal sister taxa equally on the species level as well as on the subspecies level (Martens et al. 2011; Päckert et al. 2012). The consistency of these patterns across different passerine families implies a common onset and cause of differentiation among the extant taxa concerned: Of many Chinese bird species (particularly of those occupying subalpine breeding habitats) their closest relative inhabits the corresponding elevational forest belt of the Himalayas and/or the adjacent Indo-Burmese mountain ranges. In many cases the origin of the respective sister lineage split and extant vicariance could be dated back to Pleistocene range fragmentation (Päckert et al. 2009a, 2012). So far, information on the extent of secondary contact zones among such close, but genetically distinct relatives is scarce except for several sister taxa in the Himalayas (Martens and Eck 1995; Martens et al. 2011; Päckert et al. 2012). Recent phylogeographic studies on Chinese bird populations even located some areas of range overlap at the south-eastern margin of the Qinghai-Tibetan Plateau (QTP) in the Hengduan mountain range that in some cases well correspond with the postulated Mekong-Salween divide (Dai et al. 2011; Zhao et al. 2012). Likewise, similar phylogeographic breaks have been found across the Himalayas at the southern QTP margin, e.g. between Himalayan and Indo-Burmese sister lineages (Päckert et al. 2012) and even for Central Nepal four distinct subspecies transition areas were defined by (Martens and Eck (1995); (Figure Forty-one)).

Among a large number of phylogenetic and phylogeographic studies of Asian birds, leaf warblers (Phylloscopidae; sensu Alström et al. 2006; taxonomic review in Martens 2010) are among the best studied families. To date, nearly all currently accepted Asian species have been firmly placed in a molecular phylogeny (Johansson et al. 2007; Price 2010; Päckert et al. 2012; Alström et al. 2013) and phylogeographic relationships among close relatives of single subclades have been evaluated (Helbig et al. 1995, 1996; Irwin et al. 2001a, 2001b, 2005; Martens et al. 2004, 2008; Olsson et al. 2004, 2005; Päckert et al. 2004, 2009b; Rheindt 2006) including several recent descriptions of new species from China, Vietnam and Laos (Martens et al. 1999; Alström and Olsson 1999; Martens et al. 2008; Alström et al. 2010). Many species and subspecies pairs of *Phylloscopus* leaf warblers reflect a common phylogeographic pattern of East–West splits between Chinese and Himalayan sister species with most of them corresponding to genetic distances between 1% and 3% (late to early Pleistocene divergence according to the revisited molecular clock for avian cytochrome *b* by Weir and Schluter 2008; Table 1) and only a few dating back to earlier periods of lineage separation (e.g.

subspecies of *P. humei*, *P. affinis*/*P. occisnensis*, Table 1). These phylogeographic East–West divides at the southern/south-eastern QTP margin appear to be a quite universal pattern not only in leaf warblers and it is remarkable that among 27 Chinese and Himalayan *Phylloscopus* species (Clements et al. 2013), only three are currently considered genetically uniform without any notable internal splits across a wide trans-Sino-Himalayan range: the Ashy-throated Leaf Warbler (*Phylloscopus maculipennis*), the Large-billed Leaf Warbler (*Phylloscopus magnirostris*) and the Buff-barred Warbler (*Phylloscopus pulcher*).

The Buff-barred Warbler (*Phylloscopus pulcher*; sometimes also named Orange-barred Leaf Warbler) occupies a wide breeding distribution from the NW Himalayas along the southern margin of the Qinghai-Tibetan Plateau to the Hengduan Mountains and stretching to N and E Myanmar and to SW and C China (map with detailed records in von Vietinghoff-Scheel 1984; Cheng 1987; Clement 2006; Martens 2010). Within the wide breeding range two subspecies are currently accepted (Clement 2006; Martens 2010): *Phylloscopus pulcher kangrae* in the NW Himalayas to Kashmir and nominate *P. p. pulcher* covering the whole remaining eastward range (Hartert 1910; Vaurie 1959). Forest breeding habitats of the species cover a broad altitudinal range from median elevations up to subalpine forests occasionally above 4000 m including some downhill movements outside the breeding season (Clement 2006). Local breeding records from Nepal cover a rather narrow range from between 3250 up to 4100 m a.s.l. and wintering records suggest local movements from beginning of October down to 1400–2700 m a.s.l. (Martens and Eck 1995). For the drier western Himalayas, Price et al. (2003) give breeding range limits at slightly lower elevations between 3050 and 3500 m a.s.l. (for the western subspecies *kangrae* a breeding range between 3250 and 3960 m is given in Hartert 1932). In Sikkim, the species is highly abundant in conifer and dwarf rhododendron association up to 3960 m during breeding season with downhill movements in winter (between 1220 and 2130 m; Hartert 1910; Ali 1962). During the breeding season Buff-barred Warblers occupy the coniferous forest belt with *Abies*, *Tsuga* and *Picea* intermixed with *Quercus* and *Rhododendron* at the lower limit up to subalpine birch and juniper with some dwarf-rhododendron undergrowth as well as semi-open bush vegetation above treeline (Martens and Eck 1995; Clement 2006).

Presently, nominate *P. p. pulcher* is considered morphologically uniform with respect to plumage coloration or proportions throughout its entire Sino-Himalayan breeding range (e.g. Clements et al. 2013). However, there was first evidence of subtle differentiation of territorial songs among populations from the Himalayas and from China based on a limited number of sound recordings (Fischer et al. 2011). Accordingly, we at least expect a

Table 1 Phylogeographic breaks among Himalayan and Chinese sister taxa of Old World warblers (*Phylloscopus*) indicated by mtDNA genetic distances (cytochrome *b*, in %)

Taxon 1 Himalayas	Taxon 2 China	p-dist	Source
<i>Phylloscopus trochiloides trochiloides</i>	<i>Phylloscopus trochiloides obscuratus</i>	0.4	Irwin et al. 2001b, 2005; Päckert et al. 2012
<i>Phylloscopus trochiloides ludlowi</i> W ^a	<i>Phylloscopus t. ludlowi</i> E/P. <i>t. trochiloides</i>	4.3	Richman 1996; Irwin et al. 2005; Päckert et al. 2012
<i>Phylloscopus fuligiventer fuligiventer</i>	<i>Phylloscopus fuligiventer weigoldi</i>	1.3	Martens et al. 2008
<i>Phylloscopus cantator</i>	<i>Phylloscopus ricketti</i> /P. <i>calciatilis</i>	2.2–3.4	Olsson et al. 2005; Päckert et al. 2009a; Alström et al. 2010
<i>Phylloscopus reguloides reguloides</i> ^b	<i>Phylloscopus reguloides ticehursti</i>	2.9–3.6	Olsson et al. 2005; Päckert et al. 2009b
<i>Phylloscopus xanthoschistos</i> ^c	<i>Phylloscopus davisoni</i>	3.6	Olsson et al. 2005; Päckert et al. 2009a
<i>Phylloscopus chloronotus</i> ^d	<i>Phylloscopus forresti</i>	4.2	Martens et al. 2004
<i>Phylloscopus affinis</i>	<i>Phylloscopus occisinensis</i>	7.4	Martens et al. 2008
<i>Phylloscopus humei humei</i>	<i>Phylloscopus humei mandellii</i>	8.7–8.8	Irwin et al. 2001a; Päckert et al. 2012

^aLocal sympatry of members from the two clades proven.

^bIncluding eastern subspecies *assamensis*.

^cIncluding eastern subspecies *tephrodiras*.

^dIncluding western subspecies *simlaensis*.

subtle phylogeographic break between Himalayan and adjacent Chinese populations of *Phylloscopus pulcher* as found in many other leaf warbler species, too. We test this hypothesis using one molecular marker (mitochondrial cytochrome *b*) and sonographic analysis of an extended data set of song recordings.

Methods

Molecular genetics and voucher specimen morphology

A total of 58 blood or tissue samples of Buff-barred Warblers from Nepal and China were available for molecular analysis (for origin of samples see map in Figure 1 and Additional file 1: Appendix). DNA extraction from blood and tissue samples was performed in a chloroform-isoamyl isolation or with a peqLab tissue/blood kit according to the manufacturer's instructions. An approximately 990-bp long fragment of the mitochondrial cytochrome-*b* gene was amplified using the forward primer L14841 (5'-AAA AAG CTT CCA TCC AAC ATC TCA GCA TGA TGA AA-3'; Kocher et al. 1989) and the reverse primer H15917 (5'-TAG TTG GCC AAT GAT GAT GAA TGG GTG TTC TAC TGG TT-3'; Dietzen et al. 2003) under PCR settings according to Dietzen et al. (2003). PCR products were purified by adding 0.2 µL ExoSap-IT solution in 4 µL distilled water to each sample (cycling program: 37°C for 30 min and 94°C for 15 min). Sequencing of the PCR products was performed with BigDye™ v. 3.0 and v. 3.1 Dye Terminator Cycle Sequencing Kits (Applied Biosystems) according to the manufacturers' instructions. Cycle sequencing products were purified by salt/ethanol precipitation or by using Sephadex (GE Healthcare, München, Germany) and sequenced in both directions on an ABI 3130xl DNA sequencer. An additional internal reverse primer H15547 (5'-AAT AGG AAG TAT CAT TCG GGT TTG ATG-3'; Edwards et al. 1991) was used for sequencing of some samples. The sequences were aligned

by ClustalW using MEGA 4 (Tamura et al. Tamura et al. 2007) and slightly adjusted by eye.

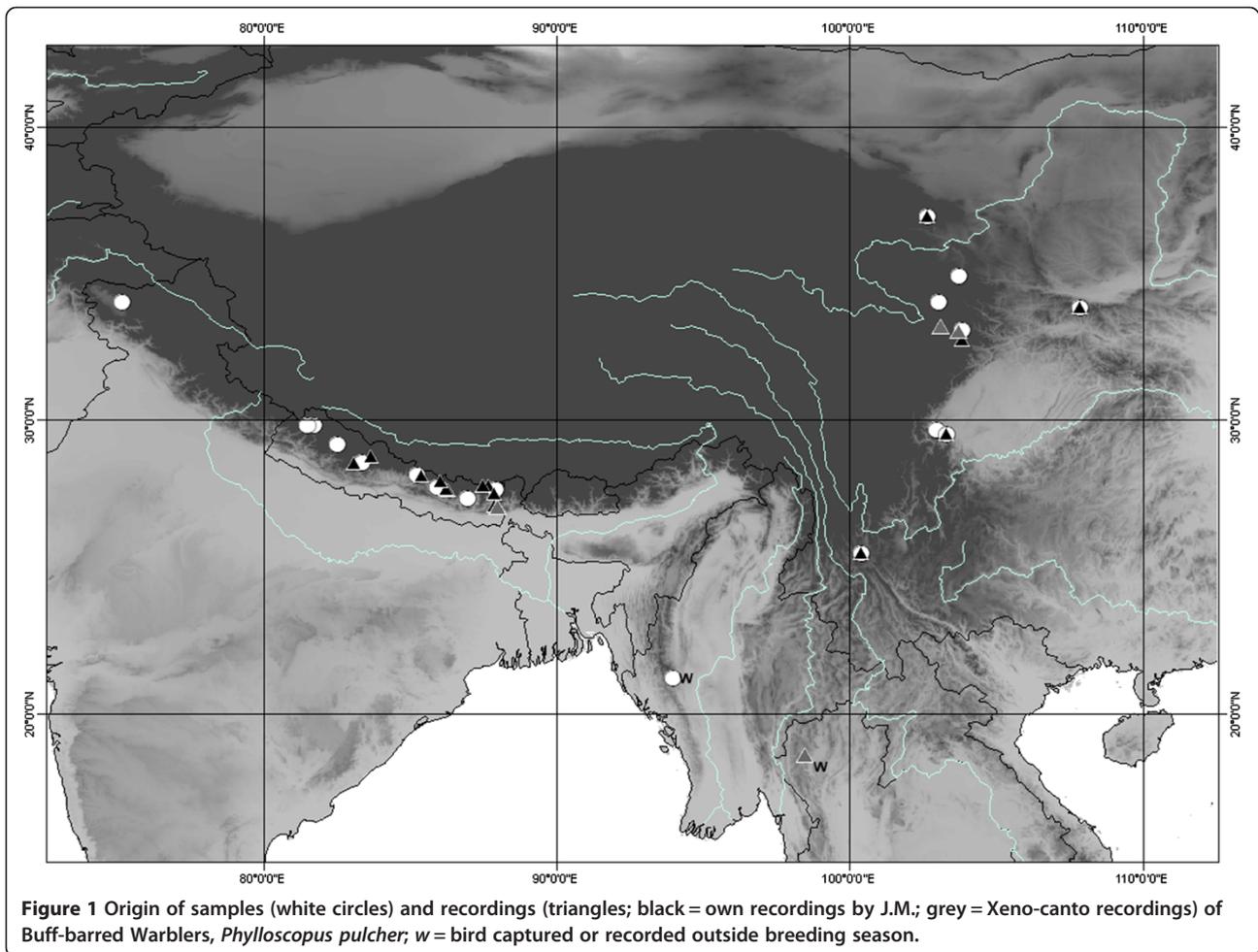
A minimum spanning haplotype network was constructed using TCS (Clement et al. 2000) and pairwise distances were computed using MEGA 4 and genetic diversity parameters and mismatch distributions were estimated using DnaSP v5.1 (Librado and Rozas 2009). Cytochrome-*b* sequences were deposited on GenBank under accession numbers KJ567540–KJ567596 (Additional file 1: Appendix).

For potential phenotypic distinction among Himalayan and Chinese birds we took morphological measurements from specimens available to us (compare Additional file 1: Appendix) of wing length, tail length, beak length and tarsus length according to the methods given in Eck et al. (2011): wing length (W_{max}) maximum chord, ruler with zero-stop; tail length (T1) taken with divider from the point where the central rectrices meet the skin to longest rectrix tip; tarsus length (Tar1) taken with calipers according to Eck et al. (2011); beak length (Bsk) with divider from tip to skull front.

Bioacoustics

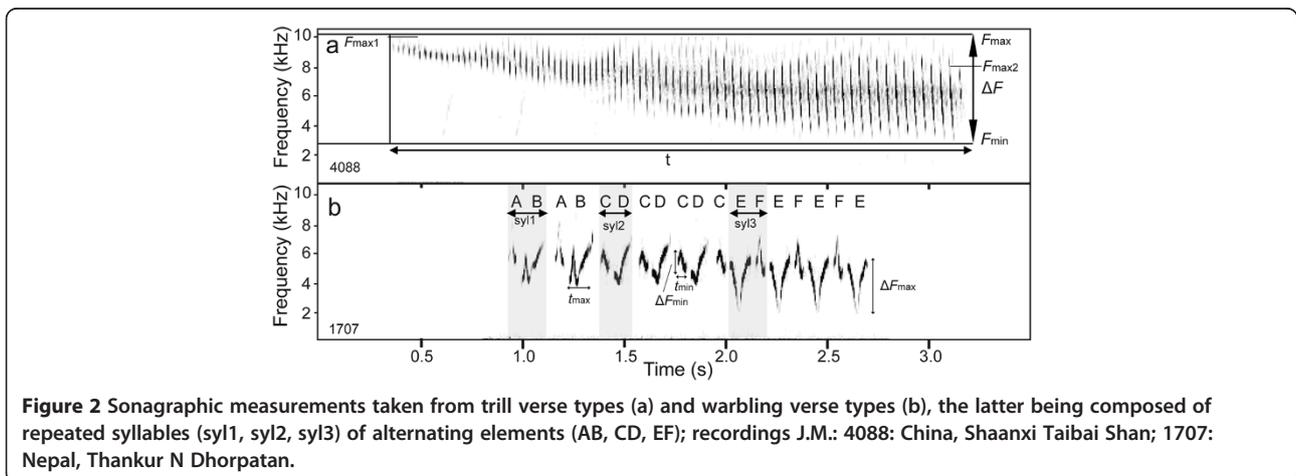
Buff-barred Warbler songs are quite stereotypic and are usually displayed in a song continuum of distinct verses which are connected by short high-pitched (call like) notes.

Two distinct verse types have been described for Nepalese populations by Martens (1980): rattling trill verse types and warbling verse types (Figure 2). In fact, the different auditory impressions of trills and warbles were already recognized as distinct song parts by Hartert (1932). Both types can be displayed separately in long series by one male, but also in quick alteration, thus both together seem to be likewise relevant in territorial context and are typically combined to long continuous song displays. The high-pitched trills consist of a series of rapidly repeated short and steeply descending elements



of the same type (Figure 2a) and have been commonly described as similar to songs of the Wood Warbler (*P. sibilatrix*), and of the Brooks's Leaf Warbler (*P. subviridis*) (Martens 1980; Clement 2006). Even though trills do not necessarily predominate in male territorial behavior and courtship, this verse type seems to be

commonly regarded as the typical vocalization of *P. pulcher* and it is only this type that has been listed for this species in Clement (2006); also in Williamson (1976). In contrast, warbling song types of *P. pulcher* have been described as long series of repeated syllables consisting of two elements of syntax ABABAB and



often songs are divided into distinct parts combining different syllables of syntax ABABCDCDCD (Figure 2b; compare Martens 1980). Because different syllables can be combined in warbling songs, they are more variable within and between males compared to trill songs (2 to 5 different warbling song types per individual male; Martens 1980). Both trill and warbling song types are introduced by a short call-like click note that is a typical structure of other leaf warbler songs, too (Päckert et al. 2009b). The human ear can hardly distinguish the songs from either areas; it is unable to resolve the distinct patterns of warbling song parts (recordings from Nepal and China documented in Martens (2013)).

Prior to sonographic analyses all original recordings were converted to wave format with a sampling rate of 22.1 kHz and in 16 bit mono and analyzed with Avisoft SASLab Light v3.74 (Specht 1996). Sonographic measurements were taken for individual verse types (trills and warbles) excluding the introductory click note. The following parameters were measured (compare Figure 2): maximum frequency of the whole verse, of the first and the last note (F_{\max} , $F_{\max 1}$, $F_{\max \text{end}}$), minimum frequency and frequency span of verse (F_{\min} , $\Delta f = F_{\max} - F_{\min}$), frequency course of verse from the first to the last element ($\Delta F_{\max} = F_{\max \text{end}} - F_{\max 1}$), total duration of verse (t) plus maximum and minimum duration of longest and shortest element (t_{\max} , t_{\min}), maximum and minimum frequency bandwidth of broadest and narrowest element (Δf_{\max} , Δf_{\min}), total number of elements (z) and of different element types (z_{el}). Tempo of verses was calculated as a function of a) total number of elements per time unit (z/t ; speed) or b) number of repeated units (elements or syllables) per time unit (repetition rate). A maximum of five verses per type (trills and warbles) were measured for the individual male and means were calculated separately for each the two verse types of each male. Among- and within-species differentiation of verse types was analyzed using principal-component analysis and discriminant analysis with SPSS 11.5.

Results

Mitochondrial DNA

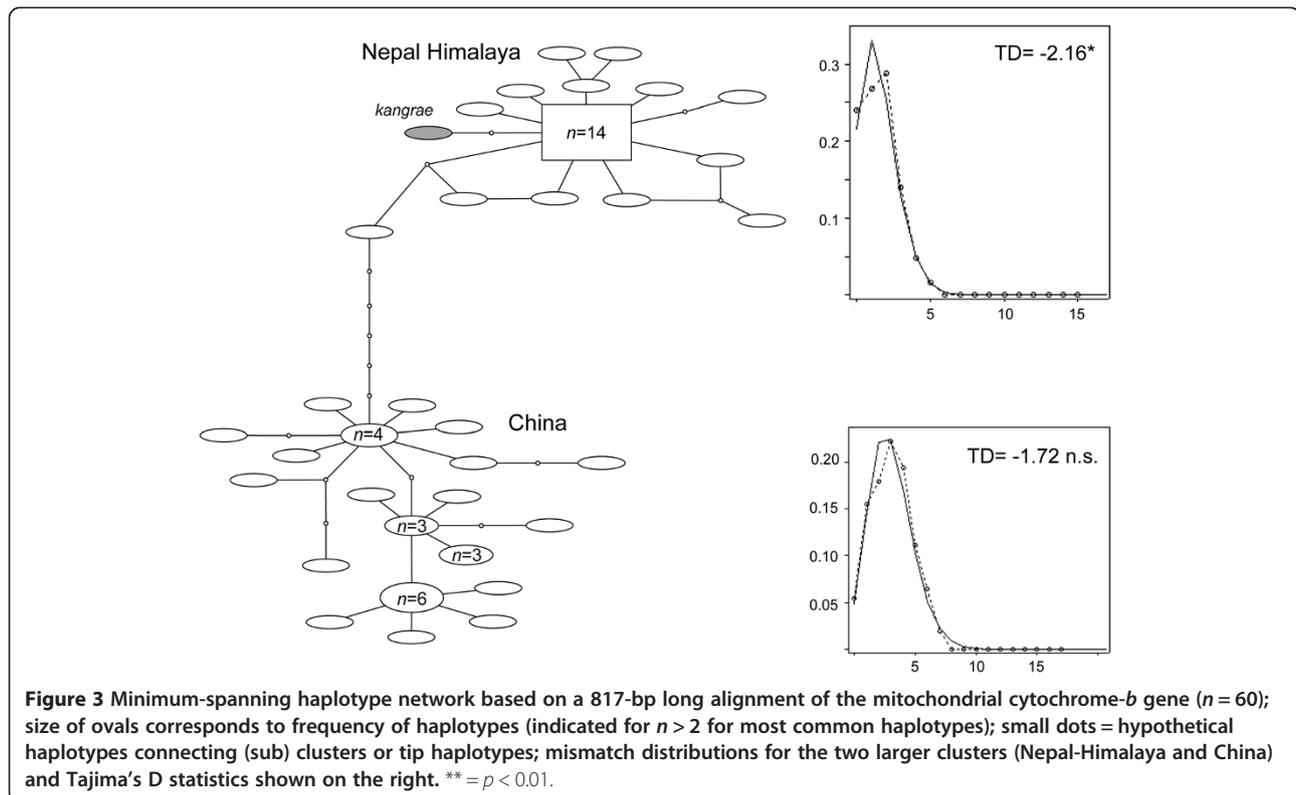
The 817-bp long alignment of 56 original sequences and four further sequences from GenBank (Additional file 1: Appendix) included 44 variable sites of which 17 were parsimony informative. The minimum-spanning network is shown in Figure 3. The network was divided into two distinct clusters which clearly corresponded to the geographic origin of samples. The Himalayan cluster comprised 15 haplotypes arranged in a star-like cluster with the central haplotype being the most common one and present in 14 individuals from all populations. All other haplotypes were private haplotypes found in a single individual only. Tip haplotypes differed at a maximum of three substitutions

from the center of the network including one haplotype of subspecies *kangrae* (GenBank sequence, sample from Kashmir) which was firmly nested in the Himalayan cluster (Figure 3).

The Himalayan cluster was separated by a minimum of six substitutions from a second cluster including all sequences from Chinese samples and two samples from Myanmar (the maximum difference among tip haplotypes was 15 substitutions; Figure 3). The latter Burmese birds were captured in March and must be considered winter visitors/spring migrants from a Chinese population (Figure 1). Genetic diversity of the Chinese cluster is somewhat higher compared to the Himalayan ones (Figure 3): Among all Chinese samples, 20 haplotypes were found and most common haplotypes were present in six, four and three individuals, respectively. Haplotype diversity (HD) and nucleotide diversity (π) were both higher in the Chinese population than in the Himalayan population: $HD_{\text{China}} = 0.947 \pm 0.023$, $HD_{\text{Nepal}} = 0.717 \pm 0.103$; $\pi_{\text{China}} = 0.0037 \pm 0.0004$, $\pi_{\text{Nepal}} = 0.0016 \pm 0.0004$. Both geographical subsamples showed strictly unimodal mismatch distributions corresponding to a growth-decline model, but only the Nepal-Himalayan population had a significantly negative Tajima's D indicating past population expansion (Figure 3). However, both Fu and Li's D and F statistics were significantly negative for the Chinese population, too ($D = -3.025$, $p < 0.05$; $F = -3.052$, $p < 0.05$). The mean genetic distance between the Himalayan and the Chinese cluster was 1.2% compared to within-group distances of 0.4% for each of both clusters. The mean genetic distance between one sample of subspecies *kangrae* and the samples of nominate *pulcher* from Nepal ranged at 0.3%.

Morphology

We compared 19 specimens from the Himalayas (W to E Nepal, 1999–2003) to 11 from China (Sichuan, Gansu; 5 from 1914, 6 from 1998–2007), all from the breeding season (May to early July). Except for one Chinese bird (from 30 July) all birds were adults and not in moulted plumage. Specimens from both regions were pretty similar in plumage coloration of underpart and identical in upperpart coloration. Slight differences are only noticeable in a series. Nepal: Throat and breast light grey noticeably interspersed with scattered yellow feathers causing patchy ornamentation, towards broad middle of belly vividly yellowish-greenish, sides of belly, flanks, in continuation of throat and belly light greyish. China: Similar to nominate *pulcher* from Nepal, throat and breast grey but distinctly more homogeneously darker stone grey with hardly any yellow feathers, no patchy distribution of greyish-greenish markings like in specimens from Nepal; middle of belly less vividly yellowish-green than in Himalayan specimens, flanks similar in specimens from both regions.



Measurements: No significant differences in body size dimensions (wing length, tail length, tarsus length and beak length) were found among populations from China and from the Nepal Himalayas (Mann–Whitney U test; > 0.05 , n.s.). It made no difference whether all measures were pooled or analysed separately for sexes. Males were significantly larger than females in wing, tail and beak dimensions but not in tarsus dimensions (Table 2; Mann–Whitney U test, $p < 0.01$, pooled measurements of all Himalayan and Chinese specimens).

Territorial songs

A total of 168 verses of 29 individual males (45 means of individual verse types, trills and warbles) were measured and included in our analysis. Trill verse types had the same structure all over the entire range of the species ($n = 28$) while warbling verse types were structurally more complex and variable throughout the trans-Sino-Himalayan range (Figure 4). Differences among regions were subtle, but significant for most frequency and time parameters (only three out of eleven parameters were not significantly different among regions: duration, maximum frequency and minimum element range; Mann–Whitney U-test, n.s.).

The first three principal components had eigenvalues greater than one and PC1 explained 46.8% of the total variation (PC2 explained a cumulative 62.6% and PC3 a cumulative 74.2% of the total variation). Scatterplots of PC1 vs. PC2 and PC3, respectively, showed individual

trills clustering according to the two regions (Figure 5). On average Chinese trills ranged at significantly lower minimum frequencies and covered a significantly broader frequency range (Mann–Whitney U-test, $p < 0.01$; Table 3). Trills of Chinese males were significantly more rapid compared to slower trills in Himalayan populations (Mann–Whitney U-test, $p < 0.01$) and they have a significantly steeper frequency decrease towards the end (Table 3). Trill notes of Chinese verses were significantly shorter and cover a significantly broader frequency range (only Δf_{\max}) than trill notes of Nepalese verses (Table 3).

Warbling verses of Himalayan males (Figure 4) were structurally complex including steady repetitions of one syllable (syntax: AB AB AB AB), two syllables (AB AB AB CD CD CD) and even three syllables (AB AB AB CD CD CD EF EF EF). Despite very similar auditory impression, Chinese warbles were distinctive against those from the Himalayas comprising only repeated single elements instead of syllables (syntax: AAAAA, AAA BBB or AAA BBB CCC; Figure 4d). The strong frequency modulation of the single element in Chinese verse types (v-shaped or w-shaped in the sonagram) is the reason for their warbling sound quality, although these song types do not contain syllables of alternating notes. No repetitions of syllables were found in Chinese warbles and no repetitions of single elements were found in Himalayan warbles, respectively. In contrast to trill verse types, warbling verse types did not show strong differences of continuous

Table 2 Body size dimensions of Buff-barred Warblers (*Phylloscopus pulcher*) from the Himalayas and China (all in mm); means and standard deviations [range of individual measures]

Population/sex	Wing	Tail	Tarsus	Beak
Nepal/males (n = 14)	59.4 ± 1.8 [56.0–62.5]	43.1 ± 1.9 [40.0–45.8]	19.1 ± 1.2 [16.4–20.0]	11.3 ± 0.7 [10.0–13.0]
Nepal/females (n = 5)	53.0 ± 2.9 [50.0–58.0]	40.2 ± 1.0 [38.5–41.0]	18.4 ± 1.3 [17.0–20.2]	10.4 ± 0.6 [9.5–10.8]
China/males (n = 7)	59.6 ± 1.8 [58.0–62.0]	44.0 ± 1.4 [42.5–46.5]	18.7 ± 1.1 [17.0–20.0]	11.0 ± 0.7 [10.0–12.0]
China/females (n = 3)	55.5 ± 1.3 [55.0–57.0]	40.7 ± 2.0 [39.0–43.0]	17.9 ± 0.2 [17.7–18.0]	10.0 ± 0.06 [10.0–10.1]

frequency and time parameters among regions. Only four out of twelve parameters differed between Chinese and Himalayan warblers and all of these corresponded to the more complex structure of the latter including syllables of alternating short and long elements. Compared to Himalayan populations, warbling songs from Chinese populations are significantly slower and less variable with respect to different element types per single verse (Table 3). Note types of Chinese verses are significantly longer (only t_{\min}) and cover a significantly

broader frequency range (only Δf_{\min}) compared to Nepalese note types (Table 3).

Rarely, mixed verse types have been found in single individuals combining trills followed by a short warbling part in both regions (Figure 4h, i).

Discussion

Phylogeography

According to our expectations we confirmed a Sino-Himalayan East–West divide in the Buff-barred Warbler,

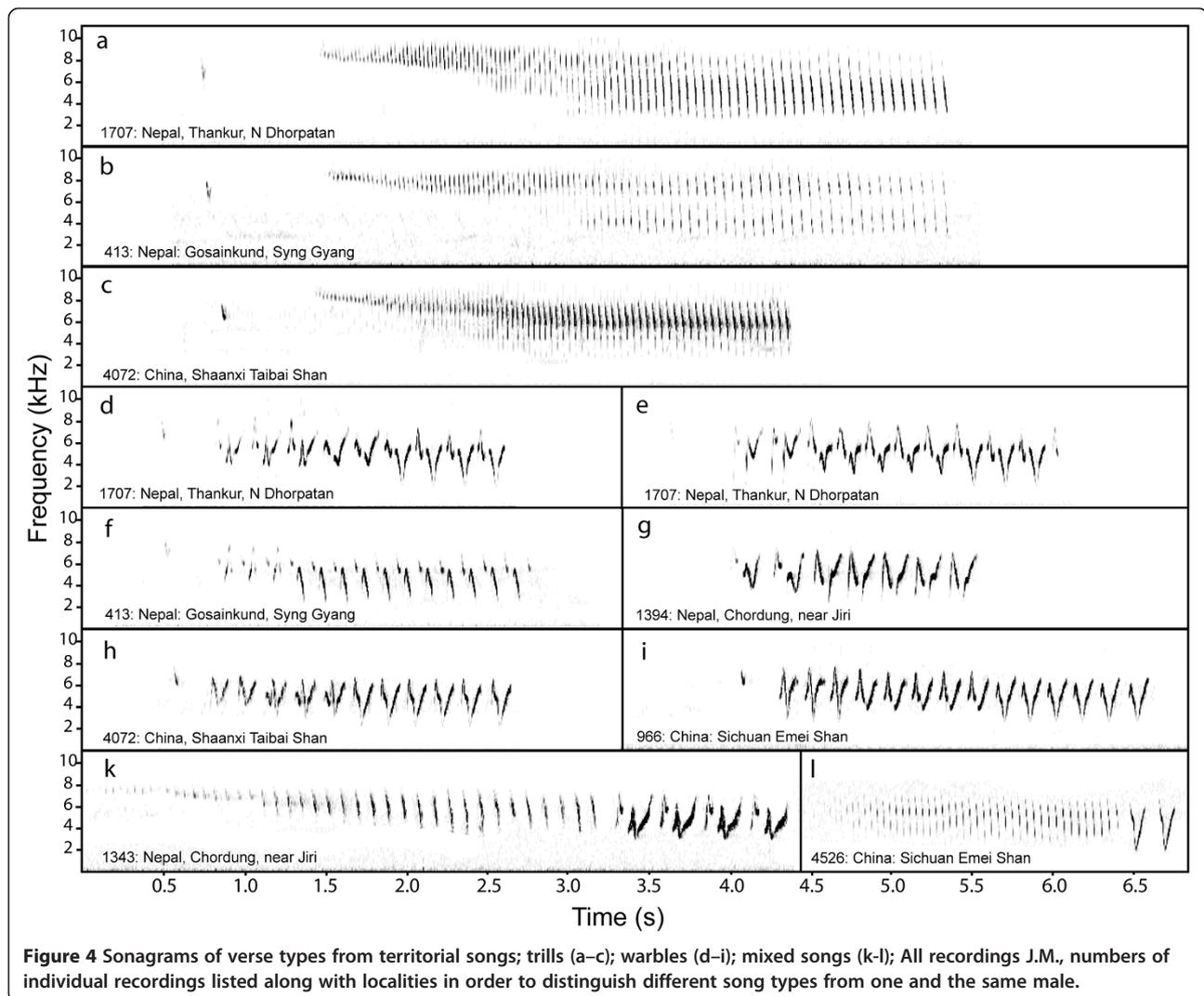
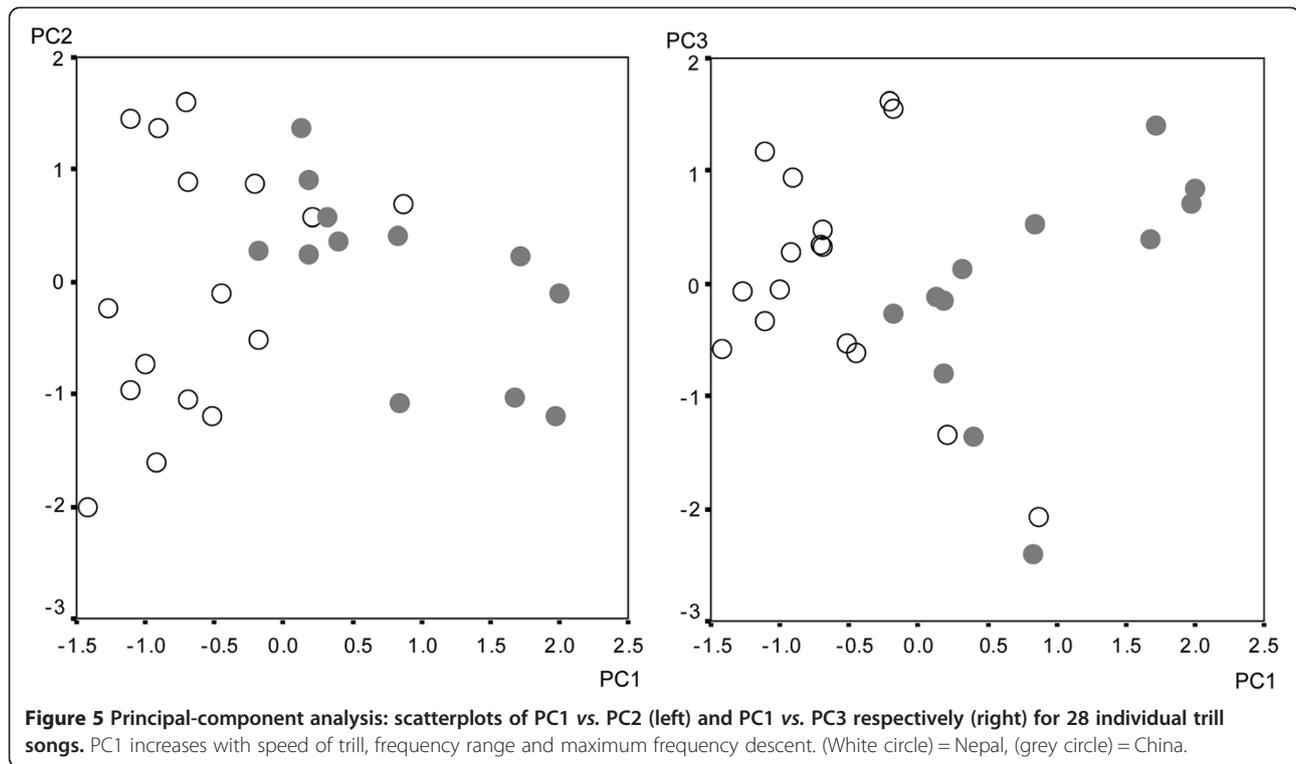


Figure 4 Sonograms of verse types from territorial songs; trills (a–c); warbles (d–i); mixed songs (k–l); All recordings J.M., numbers of individual recordings listed along with localities in order to distinguish different song types from one and the same male.



P. pulcher. Assuming an empirical substitution rate of 0.0105 substitutions per lineage, site and million years (approximately 2% among lineages for cytochrome *b*, Weir and Schluter 2008) the respective genetic distance of 1.2% corresponds to a late Pleistocene separation among Himalayan and Chinese mtDNA lineages roughly 650000 years ago. This value compares to intraspecific distances in other leaf warbler taxon pairs showing the same phylogeographic break (e.g. *P. f. fuligiventer*/*P. f. weigoldi*: Table 1; Martens et al. 2008) and even to interspecific distances among sister species of leaf warblers: *Phylloscopus ogilviegranti* and *P. hainanus*; *Seicercus grammiceps* and *S. castaniceps* (both pairs having a cytochrome-*b* distance of 1.5%; Olsson et al. 2004, 2005; Päckert et al. 2004, 2009b).

East–West divides across the southern and south-eastern QTP margin are a quite general pattern in forest-dwelling passerines and many of them date back to Pleistocene impact on differentiation processes in disjunct forest refuges (Martens et al. 2011; Päckert et al. 2012). Typically, Himalayan taxa might be genetically less diverse than their respective Chinese counterparts due to a loss of genetic variation in smaller, strongly isolated Himalayan refuge populations (Päckert et al. 2009a). This is partly reflected by slight differences of genetic diversity parameters and haplotype network structure in *P. pulcher*, but a broader sampling would be required to substantiate these findings. Likewise, the eastward extent of the Himalayan haplogroup of *P. pulcher* and possible secondary contact

with the Chinese haplogroup cannot be reliably confirmed due to a wide sampling gap in the eastern Himalayas and southern China. Thus further study of material particularly from south-western Chinese provinces (particularly SW Yunnan and SE Tibet) and adjacent areas in N Myanmar and NW India is required. In fact, secondary overlap of distinct mtDNA lineages in the latter region has been demonstrated for tits and long-tailed tits, Paridae and Aegithalidae (Dai et al. 2011; Zhao et al. 2012; Wang et al. 2013a, 2013b) and can be hypothesized for other leaf warbler taxa, too (e.g. for *P. xanthoschistos* and *P. davisoni*, *P. r. reguloides* (incl. *assamensis*) and *P. r. ticehursti*; Olsson et al. 2005; Päckert et al. 2009b, 2012).

Along the southern QTP margin in the Himalayas, East–West divides and secondary contact between mtDNA lineages were documented for different regions and at different degrees of genetic divergence. In the Central Himalayas, Martens and Eck (1995) defined four subspecies transition areas that largely correspond to intraspecific splits among mtDNA sister lineages, e.g. in *Periparus* tits (*P. ater* in the Dhaulagiri transition area and *P. rubidiventris* in the Kathmandu transition area). In contrast, secondary overlap in the Western Himalayas often corresponds to a faunal transition among Central Asian and Himalayan faunal elements that correspond to deeper and older phylogenetic splits (and do not even represent sister clades); e.g. *Periparus rufonuchalis*/*P. rubidiventris* (Martens et al. 2006); *Aegithalos leucogenys*/*Ae. concinnus*, *Ae. niveogularis*/*Ae. iouschistos* (Päckert et al. 2010).

Table 3 Mean values (plus standard deviations) of frequency and time parameters of trill song and warbling song of *Phylloscopus pulcher*

Trill song													
Population	<i>n</i>	tges	zel**	speed**	zeltype**	<i>t</i> _{max} **	<i>t</i> _{min} **	<i>F</i> _{max}	<i>F</i> _{min} **	Δf **	Δf _{max} *	Δf _{min}	<i>f</i> _{mod} **
China	12	3.25 ± 0.42	96.4 ± 13.5	29.6 ± 1.4	1.4 ± 0.5	0.03 ± 0.004	0.01 ± 0.001	9.77 ± 0.71	2.93 ± 0.23	7.00 ± 0.74	5.64 ± 0.87	0.87 ± 0.37	-1.9 ± 0.71
Nepal	16	3.32 ± 0.92	70.6 ± 20.7	21.4 ± 2.1	1.0 ± 0.1	0.05 ± 0.012	0.02 ± 0.016	9.37 ± 0.51	3.56 ± 0.58	5.81 ± 0.77	4.97 ± 0.88	0.73 ± 0.27	-0.9 ± 0.83
Warbling song													
Population	<i>n</i>	tges	zel	speed**	zeltype*	<i>t</i> _{max}	<i>t</i> _{min} **	<i>F</i> _{max}	<i>F</i> _{min}	Δf	Δf _{max}	Δf _{min} **	<i>f</i> _{mod}
China	9	1.76 ± 0.24	12.1 ± 3.0	6.9 ± 1.3	2.5 ± 0.7	0.13 ± 0.009	0.09 ± 0.028	7.46 ± 0.38	2.42 ± 0.26	5.04 ± 0.44	4.75 ± 0.44	3.36 ± 0.62	-0.4 ± 0.32
Nepal	8	1.61 ± 0.27	15.9 ± 6.2	9.7 ± 2.3	3.4 ± 0.9	0.11 ± 0.028	0.05 ± 0.009	7.72 ± 0.60	2.43 ± 0.44	5.30 ± 0.99	4.36 ± 0.91	2.05 ± 0.76	-0.3 ± 0.41

n = number of individual male recordings measured; significant differences among Chinese and Himalayan (Nepal) populations: * = Mann-Whitney-U test, *p* < 0.05;

** = Mann-Whitney-U test, *p* < 0.01.

To date only a few Asian passerine species were shown to have a homogeneous mitochondrial gene pool across the entire southern and south-eastern QTP margin, e.g. *Pyrrhula nipalensis* (Töpfer et al. 2011). Intraspecific mtDNA differentiation might also be absent in two further leaf warbler species (*P. maculipennis*, *P. magnirostris*), but this remains to be verified. Furthermore, mtDNA phylogeographies have limited explanatory power, because nuclear gene flow might widely extend beyond the limits of phylogeographic mtDNA divides as in the Green-backed Tit (*Parus monticolus*) (Wang et al. 2013a, 2013b).

Taxonomy

For the Chinese populations of the Buff-barred Warbler the name *Reguloides pulcher vegetus* Bangs 1913 is available. The original description was based on three specimens from W Sichuan collected by W. R. Zappey. The holotype was collected at Yachiakun on July 14, 1908, by W. R. Zappey on 3810 m a.s.l. Bangs later compared the material from Zappey with two specimens from Sikkim which he considered to be “very different from the Chinese bird” (Bangs 1913). According to Bangs’ description his newly described *P. p. vegetus* should be “much paler and duller in color throughout” compared to nominate *pulcher* from the Himalayas with some slight differences in yellow underpart and rump patch coloration, too. Later on, Chinese *P. p. vegetus* received a tentative treatment only by taxonomists (e.g. Hartert 1932), presumably due to a lack of significant material for comparison. Riley (1931) considered all Chinese specimens belonging to subspecies *vegetus* until further revisiting of specimens from Nepal. Greenway (1933) analysed seven Buff-barred Warbler specimens from Yunnan and Tibet and considered them even “slightly darker and greener below than the type of *vegetus*” and concluded that for the time being he had insufficient Indian material at hand for comparison. Most subsequent taxonomic authorities synonymized that taxon with the nominate form *P. p. pulcher* (Ticehurst 1938; Vaurie 1959) and, in his field guide, Williamson (1976) considered the Chinese form *vegetus* “not separable from the typical race”. Thus, in current taxonomy *P. p. vegetus* is not recognized as a valid subspecies anymore (Cheng 1987; Dickinson 2003; Clements et al. 2013). In fact, we could confirm morphological distinctiveness of Chinese *P. p. vegetus* only for subtle differences from Himalayan birds in plumage coloration but not by metric morphological traits of specimens examined. Our mean values of morphological parameters were fully congruent with body size dimensions listed for birds from Sikkim by Ali (1962) and for birds from Nepal by Martens and Eck (1995). Differences among sexes were already documented for Nepal birds by Eck and Martens (1995) but based on different material than analyzed here.

Compared to Himalayan populations of subspecies *P. p. pulcher* and *P. p. kangrae*, Chinese *P. p. vegetus* has more homogeneously dark greyish throat and breast plumage with hardly any yellow feathers and underparts less vividly yellowish-green. In addition, warbling song types provide a diagnostic character for subspecies distinction of Chinese *vegetus* populations for their unique syntax pattern of repeated single notes (AAA BBB CCC) instead of repeated syllables in the Himalayan part of the species breeding range (ABABAB CDCDCD). Apart from subspecies diagnosability solely by song pattern, pairwise distances between Himalayan and Chinese populations notably exceed the intraspecific distance value among NW Himalayan *P. pulcher kangrae* and nominate *P. p. pulcher* from the Central Himalayas.

We therefore recommend revalidation of the name *Phylloscopus pulcher vegetus* Bangs 1913 for Chinese populations of the Buff-barred Warbler. We refrain from assigning cytochrome-*b* hapotypes as diagnostic characters and restrict subspecies diagnosis to its subtle phenotypic characteristics and to song pattern of single note repetitions as outlined above. According to our data, the Chinese breeding area of *P. pulcher vegetus* extends from the provinces Gansu and Qinghai in the North across Shaanxi and Sichuan to Yunnan in the South – and possibly even to adjacent sub-Himalayan N Myanmar in the Southwest (breeding records from this region remain to be confirmed, since our own field records were made outside the breeding season). During the breeding season the birds occupy broad-leaf and mixed forests from mean elevations at 2350 m to mountain summits at 3200 m (at Lianhua Shan, Gansu).

Though slight differences between *P. p. pulcher* and *P. p. vegetus* are obvious, the original description of *vegetus* by Banks differed from the judgment of later authors as outlined above. Though we generally agree with the original morphological diagnosis by Bangs (1913) and use the name *vegetus* here, a re-examination of the type material is advisable.

Conclusions

Chinese populations of the Buff-barred Warbler (*Phylloscopus pulcher*) turned out to be distinctive against their Himalayan counterparts by the structural pattern of warbling song types and by slight differences in plumage coloration. That distinctiveness is backed by a genetic distance of 1.3% among two separate mitochondrial lineages (cytochrome *b*) from the Himalayas and China, respectively, and by significant differentiation of frequency and timely dimensions of trill song types. Based on the notable differentiation of three independent character sets outlined here (morphology, acoustics, genetics) we recommend the revalidation of the taxon name *Phylloscopus pulcher vegetus* Bangs, 1913 for Chinese populations of the Buff-barred Warbler.

Additional file

Additional file 1: Appendix. Origin of samples used for genetic analyses, all collection J.M. (MAR); museum acronyms of voucher specimens: NME = Museum für Naturkunde Erfurt, Germany; MTD = Senckenberg Natural History Collections Dresden; ZFMK = Zoologisches Forschungsmuseum Alexander Koenig Bonn, Germany.

Competing interests

The authors declare that they have no competing interests.

Authors' contribution

MP designed the study, performed all analyses and drafted the manuscript. BF carried out lab work and pilot analysis within the scope of his Diplom thesis. JM provided most samples and sound recordings that he collected over five decades. YHS coordinated all field work in China. DTT co-supervised BF's thesis and provided additional samples. All authors have read and approved the final draft of the paper.

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