A new contact zone in the Chiffchaff distribution range: Caucasian and European subspecies meet in the south of Russia

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The study of contact zones between closely related taxa of animals sheds light on many important issues of evolution biology and taxonomy. Several such zones were described earlier within the huge distribution range of the chiffchaff species complex (*Phylloscopus collybita*). We have documented for the first time the existence of a new contact zone between Caucasian (*Ph. c. caucasicus*) and European (*Ph. c. abietinus*) subspecies of the Chiffchaff in Ciscaucasia (southern Russia). We predicted the occurrence of hybridization between these subspecies belonging to the same group of "greenish" chiffchaff. In eastern Ciscaucasia, a population was found in which the *abietinus* and *caucasicus* haplotypes co-occur in a significant number. A mixed pair of Chiffchaff (male *caucasicus* and female *abietinus*) was also found here. The colour of the plumage of Chiffchaffs in Ciscaucasia varies more widely compared to allopatric populations. In many specimens bearing the *abietinus* haplotype in Ciscaucasia, the wing formula is identical to that typical for *caucasicus* and differs from that typical for *abietinus* in allopatric populations. The tret calls typical of *caucasicus* are included in the song of Chiffchaffs, which carry the *abietinus* haplotype and do not have notes characteristic of *caucasicus* in their song. Chiffchaffs from Ciscaucasia occupy an intermediate position between *abietinus* and *caucasicus* in the duration of individual song phrases and syllable time-frequency characteristics. During the captures, the males of *caucasicus* readily reacted to the broadcast of the *abietinus* song, and vice versa. Thus, all the results suggest hybridization between *abietinus* and *caucasicus* in Ciscaucasia.

1. Introduction

Zones of secondary contact and hybridization are called "windows into the evolutionary process" (Harrison 1990). Their study provides unique opportunities for investigating the processes of differentiation of populations and speciation in nature. Therefore, it is not surprising that such zones remain in the focus of researchers' attention across many decades (Mayr 1966, Dixon 1989, Aliabadian *et al.* 2005, Swenson & Howard 2005, Rheind & Edwards 2011, Carpenter *et al.* 2022). The current stage of ornithological research in this area is marked by the synthesis of morphological, genetic and bioacoustic approaches. Songbirds are particularly attractive for such studies. Many of them have complex vocalizations, which could be involved in the analysis along with other signs

Many hybrid zones arise due to anthropogenic changes in natural landscapes, leading to the introduction of one species into the range of another. For example, artificial afforestation and watering in the once-treeless steppe areas create excellent opportunities for the arboreal bird species to settle here. A well-known example is the Great Plains of North America, where many previously completely isolated western and eastern bird taxa began to disperse towards each other and form zones of contact and hybridization (Remington 1968, Rising 1983, Rhymer & Simberloff 1996). A similar situation exists in Ciscaucasia, the southern part of European Russia, the area between the foothills of the Greater Caucasus in the South, the Manych Depression in the North, and the coasts of the Azov and Caspian Sea in the West and East. The length of this area, from south to north, is about 200 km. Vast territories of the formerly treeless steppe of Ciscaucasia have seen massive forest cultivation and the development of forest belts for more than 100 years. Currently, this region holds many old, artificially planted forests. In addition, almost all of its territory is covered with a dense network of forest belts of different ages, widths, and vegetation compositions established in 1940–1950. Such large-scale transformation of Ciscaucasian landscapes induced a massive redistribution of various bird taxa. Caucasian species and subspecies moved from the South to the North, and European boreal species settled towards them from the North to

the South. As a result, modern Ciscaucasia has become an arena of mass mixing of northern and southern closely related forms, many of which enter into hybridization here (Belik 2009).

In particular, the history of the Chiffchaff, represented here by the Caucasian (*Phylloscopus collybita caucasicus*) and the Eastern European subspecies (*Ph. c. abietinus*), is interesting. According to count results conducted in the early 1970s, the Chiffchaff number in Ciscaucasia was indicated as extremely low (Belik 2009). It nested in large numbers only in the floodplain of the Kuban River, which at that time was essentially the northern boundary of the distribution of the Caucasian subspecies (Belik *et al.* 1981, Loskot 1991). To the north of the Kuban River, the Caucasian Chiffchaff nested only in the forests of the Stavropol upland, without penetrating the Ciscaucasian plain (Likhovid & Tertyshnikov 1994). The Eastern European Chiffchaff, on its move in a southerly direction, arrived at the lower reaches of the Don only by the mid-1950s. Until that time, Chiffchaff had not been found here. Thus, in the early 1970s of the past century, vast areas of the Ciscaucasian steppes between the Kuban and Don rivers were not inhabited by Chiffchaff. In particular, it did not nest in artificial forest belts, which by this time were widely represented in this region (Belik 2009).

Half a century later, the situation has changed dramatically. We found the Chiffchaff to be numerous breeding species throughout Western Ciscaucasia, from the Kuban Valley up to the central districts of the Rostov region. In Eastern Ciscaucasia, the Chiffchaff was found nesting from the northern parts of the Stavropol region in the south up to Salsk in the north. At present, the Chiffchaff nests everywhere in great numbers, not only in artificially planted forests, but also in all forest belts, being one of the most numerous birds here (up to 12 singing males per 1 km). Such a large-scale expansion raises many intriguing questions. Among them is the question of which subspecies were involved in the settlement and whether there is a zone of contact and hybridization between them.

In this article, we describe a new zone of contact and probable hybridization of two Chiffchaff subspecies in the south of Russia in addition to the previously described ones in the

Pyrenees (Salomon & Hemin 1992, Helbig *et al.* 2001), in Scandinavia (Hansson *et al.* 2000), and in the Southern Urals (Marova *et al.* 2017). We analyze the morphological, bio-acoustic, and genetic differentiation of Chiffchaff in western and eastern Ciscaucasia: from Krasnodar and North Ossetia to the north up to the central regions of the Rostov region and from the northern part of the Stavropol region to the North up to Salsk (Fig. 1). For comparison, we use our bioacoustic data from the Kursk and morphological data from the Moscow and Kursk populations of *abietinus*.

2. Materials and methods

2.1. Studied taxa

Both *abietinus* and *caucasicus* belong to the group of "greenish" Chiffchaffs. Both subspecies are coloured very similarly and have almost the same body size, so their distinction by external appearance is problematic. However, these subspecies differ well in wing formula, mitochondrial DNA, and song (Helbig *et al.* 1996, Marova *et al.* 2021). In particular, the tret calls in the songs of *abietinus* and *caucasicus* differ well and are the same throughout the vast distribution range of each of these subspecies (Ivanitskii *et al.* 2021).

2.2. Capturing method and genetic analysis

Field studies were conducted in May-June 2017, 2019 and 2021. Singing males were tape-recorded and then captured in an Ecotone mist net (6 x 2.5 m, mesh 16 mm) after being lured by song playback. Once photographed, measured and blood sampled (from *vena brachialis*), the birds were ringed and immediately released. We analysed 66 blood samples (dried on a paper
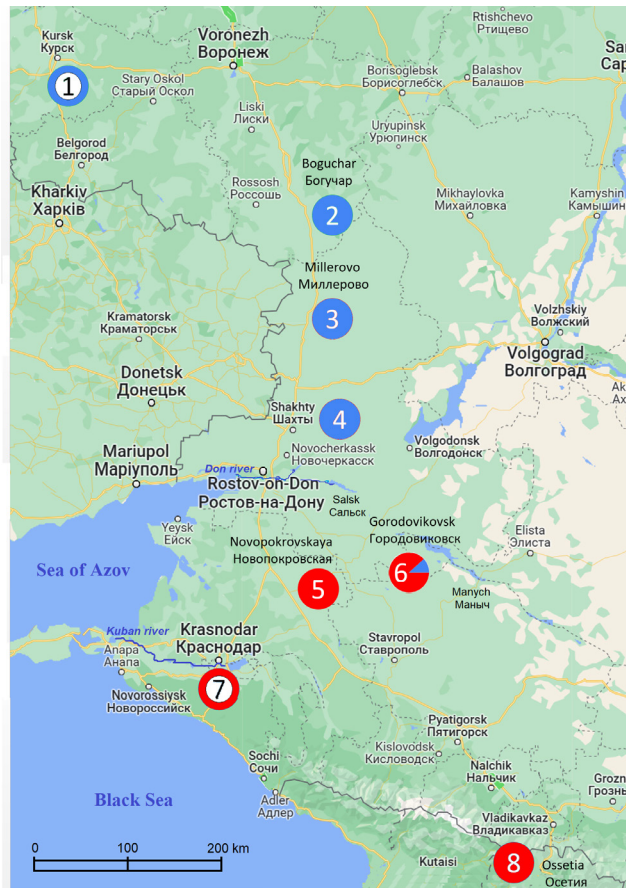

Fig. 1. Chiffchaff populations studied (the number of males tape-recorded in the population is indicated in square brackets): 1 – Kursk region [19]; 2 – Boguchar (Don river valley) [13]; 3 – Millerovo [8]; 4 – Shakhty [12]; 5 – Novopokrovskaya [20]; 6 – Gorodovikovsk (western Kalmykia) [20]; 7 – Krasnodar [20]; 8 – North Ossetia [20]. Blue circles represent pure *abietinus*; red circles represent pure *caucasicus*; white circles with blue and red represent allopatric populations not typed for mtDNA.

filter) collected in five locations: Gorodovikovsk (Kalmykia), n=34; Millerovo, n=8; Shakhty, n=4; Novopokrovskaya, n=6; Krasnodar, n=14 (Fig. 1). DNA was isolated from blood samples using the Diatom™ DNA Prep 100 kit (Isogen Lab.ltd., Russia). We examined the mtDNA cytochrome *b* gene (902 bp). The cytochrome *b* fragments were amplified with the primers L-14995 and H-16065 (Helbig *et al.* 1996). PCR was performed under the following conditions: initial denaturation of DNA (94°C for 2.5 min), 35 cycles of amplification (30 s at 93°C, 30 s at

54°C, and 180 s at 72°C), and final elongation (72°C for 3 min) with a GenPak® PCR Core kit (Isogen Lab.ltd., Russia). PCR products were purified with a Diatom™ DNA Clean-up purification kit (Isogen Lab. Ltd., Russia). Sequences of cytochrome *b* were initially aligned and then optimized manually using the BioEdit Sequence Alignment Editor 5.0.9 (Hall 1999).

The phylogenetic relationships between the haplotypes were reconstructed by Median Joining Network using PopART v. 1.7 (Leigh & Bryant 2015). To determine the subspecies of samples, we conducted the phylogenetic analysis for cytochrome *b* sequences using the Bayesian analysis with MrBayes v. 3.2.7 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003). In addition, we analysed the cytochrome *b* sequences of six different subspecies of Chiffchaff and a sequence of willow warbler (*Phylloscopus trochilus*) as an external group from the GenBank. The accession numbers of all analyzed sequences are shown in Supplementary Table 1. The phylogenetic tree was built after Markov chain Monte Carlo run for 300,000 generations with a burn-in of 10,000 generations.

2.3. Morphology

A total of 56 Chiffchaffs (55 males and 1 female) from populations localized in a possible contact zone were captured, measured (wing and tail length), and photographed. In addition, 57 specimens from allopatric populations in North Ossetia (*caucasicus*), Kursk and Moscow regions

(*abietinus*) were captured and processed. For each specimen, a verbal description of the colour of its plumage was prepared, and the wing formula (the position of the tips of the primary flight feathers relative to each other) was determined. All the measurements and wing formula descriptions were made according to Svensson (1992).

2.4. Song recording and analysis

For sound recording, we used solid-state recorders (Marantz PMD-660) with Sennheiser K6-ME66 cardioid condenser microphones (sampling rate 44.1 kHz, 16-bit resolution). Each individual male was recorded only once from a distance of 10–15 m. Since the birds were not marked, we made special efforts to avoid recording the same males. In particular, in dense settlements, we usually started recording the next male only when we heard the previous one. One recording lasted 5–10 minutes. In total, we analysed the recordings of 132 males from eight populations. The geographical position of the latter corresponds to the transition from pure *abietinus* (in the north) to pure *caucasicus* (in the south) (Fig. 1). We used recordings from two pure populations of *abietinus*, two pure populations of *caucasicus* and four populations located in the area of contact between these subspecies in Ciscaucasia and the Rostov region. Data on the number of males recorded in each population are presented in the caption of Figure 1.

We used common terminology to describe song units (Prúchová *et al.* 2017). An "element"

Table 1. Haplotypes and wing formulas of Chiffchaff in Ciscaucasia and in allopatric populations.

Wing formula	Populations in Ciscaucasia			Allopatric populations			
	Total Chiffchaffs	Genetically identified as *P. c. abietinus*	Genetically identified as *P. c. caucasicus*	Total Chiffchaffs	*P. c. abietinus*		*P. c. caucasicus*
					Moscow	Kursk	North Ossetia
7<2<6	5	3	2	25	13	12	0
8<2<7	24	9	15	6	0	6	16
9<2<8	23	4	19	0	0	0	10
10<2<9	4	0	4	0	0	0	0
Total	56	16	40	57	13	18	26

is a single continuous trace on the spectrogram. A "syllable" consists of one or more elements that are always combined in the same way. Usually, gaps between elements are very short, so they appear as a single sound to the human ear. The largest gap between elements within a syllable was about 0.05 ms. Elements, or syllables, thus represent the smallest building blocks of songs. Chiffchaff's song consists of well-differentiated syllables separated by clear pauses. The syllables are grouped into phrases separated by much longer gaps. The repertoire of syllables was determined for each male recorded. Then, for each population sample, we determined the number of males whose repertoire contained a particular syllable (as a percentage of the total number of males in this sample).

To analyse the frequency and temporal characteristics of the song, three samples were created: pure *abietinus* (Kursk and Boguchar), pure *caucasicus* (Krasnodar and North Ossetia), and Chiffchaffs from a contact zone in Ciscaucasia (Novopokrovskaya and Gorodovikovsk). Each sample included recordings of 20 males: 10 males from each of the two populations. We randomly selected three 3-second samples from the continuous singing of each male for detailed analysis. Each segment included 9–10 syllables. Thus, 180 segments from 60 males were included in the analysis. For each segment, we determined: 1) maximum frequency (top limit of a frequency range); 2) minimum frequency (bottom limit of a frequency range); 3) mean length of the song syllables; 4) mean length of the pauses between song syllables. In addition, for each male included in the analysis, we measured the duration of 10 individual song phrases randomly selected from his recording. We calculated medians for all parameters for each male for subsequent statistical analysis.

The spectrograms were created in Syrinx 2.5s (software developed by John M. Burt; University of Washington, Department of Psychology, Seattle, WA 98195, USA) with settings FFT=512 and Blackman window. For automatic sound measurement, we used AviSoft-SASLab Pro with spectrograms created with settings FFT=512 and Blackman window. The spectrogram's time resolution was 2.9 ms, and its frequency resolution was 170 Hz. To fix the upper and lower limits of the frequency range, we used a default threshold of –20 dB (referenced to the peak amplitude). Consequently, the maximum and minimum frequencies could be identified as the highest and, respectively, the lowest frequencies, at which the amplitude was –20dB or more. We applied a "hold time" parameter of 50 ms in order to fix the beginning and end of the elements.

2.5. Statistics

We used the software packages STATISTICA V. 8.0 (StatSoft, Inc.USA) and PAST V. 2.13 (Hammer *et al.* 2001) for statistical data processing. The results obtained from the individual repertoires study were subjected to cluster analysis. We used a matrix containing 8 rows (populations) and 74 columns (elements). Each cell of the matrix contained the number of males performing this element in this population (as a percentage of the total number of recorded males). We used the Bray–Curtis similarity index, the unweighted paired group average as an agglomeration method, and 999 replicates for a bootstrapping calculation. The Kruskal–Wallis nonparametric test was used to assess the statistical significance of the differences between the three song samples by two frequencies (maximum and minimum frequency) and three temporal characteristics (the length of the song syllables, the length of pauses between them and the phrase length). The PCA analysis was carried out on these data. For this, we used a matrix containing five columns (song characteristics) and 60 rows (males). Prior to analysis, the data was standardized. Then, the significance of the differences between the values of the first main component was evaluated using the Kruskal–Wallis nonparametric test.

3. Results

3.1. Genetics

According to phylogenetic analysis, 16 of the birds we studied were associated in the same clade with 7 specimens of *abietinus* from the GenBank, and 50 other birds were combined in the other well-separated clade with two specimens

of *caucasicus* from the GenBank (Fig. 2). We identified 66 sequences of cytochrome *b* of 902 bp in length. In this sample, 25 haplotypes were found. The Median-Joining network of haplotypes showed a clear separation between two subspecies groups, differing in at least five nucleotide substitutions (Fig. 3). The *caucasicus* haplotypes prevail in most of the studied areas in both western (Novopokrovskaya) and eastern (Gorodovikovsk) Ciscaucasia (Fig. 1, circles 5 and 6). Only the *abietinus* haplotype was found to the north of Rostov-on-Don.

Both haplotypes were found in Western Kalmykia's population and the adjacent parts of the Stavropol Region (Fig. 1, circle 6). Four of the 34 (11.8%) specimens carried the *abietinus* haplotype, and the rest carried the *caucasicus* one. Here, we succeeded in capturing both members of one breeding pair from which the male was determined as *caucasicus* and the female as *abietinus* according to their haplotypes.

3.2. Morphology

We found no significant differences in tail and wing length between *abietinus* and *caucasicus* males (Marova *et al.* 2021). However, the wing formula is a trait that reliably separates individuals taken from the Moscow and North Ossetia allopatric populations. The wing of *abietinus* is sharper; the tip of the second primary is between the 6 and 7 primaries. The wing of the *caucasicus* is more rounded; the tip of the second primary is between 7 and 8, 8 and 9, or even 9 and 10. The situation looks much less clear in the Kursk region, especially in Ciscaucasia. In six of the 18 Chiffchaffs caught there, the wing formula was closer to *caucasicus* than to the typical *abietinus*. In Ciscaucasia, most of the Chiffchaffs bearing the haplotype *abietinus*, had a rounded wing, and only three individuals had a wing shape typical of allopatric populations. Two Chiffchaffs carrying the *caucasicus* haplotype did not differ in wing formula from typical *abietinus*s (Table 1).
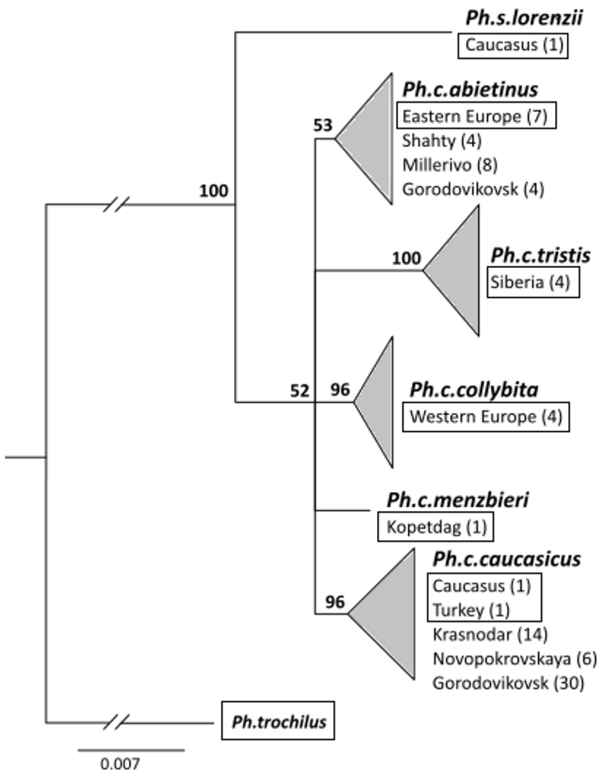

Fig. 2. The phylogenetic tree of the Chiffchaff subspecies cytochrome *b* haplotypes has been confirmed using Bayesian analysis (the figures at the bottom of the cluster show its support). The figures in parentheses right of the geographical names indicate the number of individuals studied. Rectangles indicate specimens from the GenBank.
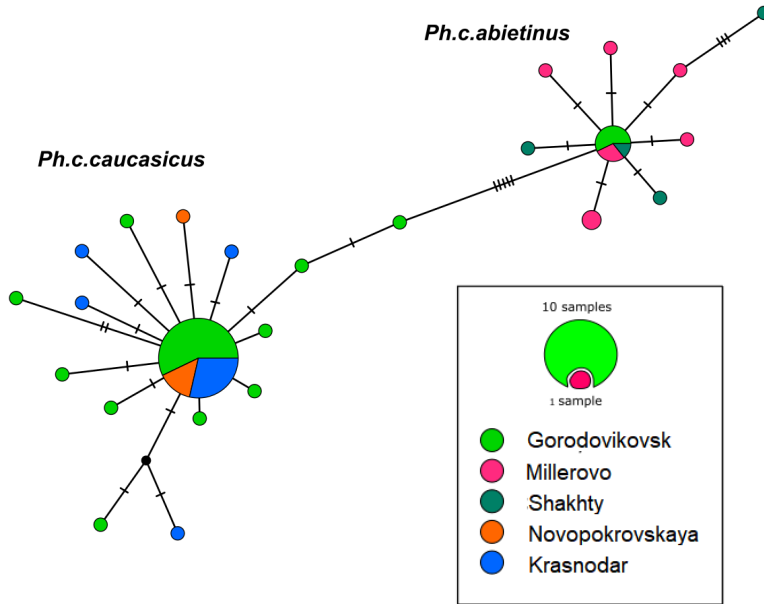
Fig. 3. Minimum-spanning network between the observed 25 mitochondrial haplotypes, representing five populations and two subspecies of Chiffchaff. Hash marks between the haplotypes indicate the number of nucleotide differences separating each haplotype. The size of the circle representing the individual haplotypes corresponds to the abundance of that haplotype. The black dot indicates an unobserved intermediate haplotype.

3.3. Song analysis

We identified 74 syllable types in our recordings. Syllables with a V-shaped frequency modulation (Fig. 4B) are characteristic of the *caucasicus* song (40% of males perform) and are completely absent in the *abietinus* song. We did not find any specific syllables in *abietinus*: *caucasicus* males presented all the syllables performed by males of the latter. The differentiation of syllables between the studied populations was rather weak (Fig. 4A). One cluster combines three populations: a typical *caucasicus* from Krasnodar and two populations from the transition zone between *caucasicus* and *abietinus*: from Western Kalmykia (Gorodovikovsk) and Novopokrovskaya. The population of the Shakhty located 200 km north of Novopokrovskaya adjoins the same cluster, but with much less bootstrap support. The second cluster combines two populations of pure *abietinus* (Boguchar and Kursk) and a population of Millerovo localised 130 km north of the Shakhty. Finally, the North Ossetia population occupies a separate position in relation to all others.

The data on the tret calls in the song shows a slightly different picture. Paradoxically, the tret calls typical of *caucasicus* are found in the song of Chiffchaffs, carrying the *abietinus* haplotype

and having no notes characteristic of *caucasicus* in their song (Shakhty and Millerovo) (Fig. 4C).

The subspecies studied differ significantly in all measured frequency and time parameters of their song. The analysis of the main components identified six factors, of which two had eigenvalues greater than 1. The first factor (the first main component) selects 43.5% of the variation, the second factor selects 18.1%. In all characteristics, including the first main component values, the song of the Chiffchaffs from Ciscaucasia falls in between the songs of *abietinus* and *caucasicus* (Fig. 5).

4. Discussion

Modern Ciscaucasia, whose natural environment has been radically transformed by human activity, has become a real contact zone hotspot. At least 10 pairs of subspecies and closely related species, spreading towards each other from the north and the south, formed contact zones in Ciscaucasia (*Dendrocopos major candidus / D. m. tenuirostris, Garrulus glandarius glandarius / G. g. krynicki, Lanius collurio collurio / L. c. kobylini, Aegithalos caudatus caudatus / Ae. c. major, Sylvia atricapilla atricapilla / S. a. dammholzi, Luscinia luscinia*
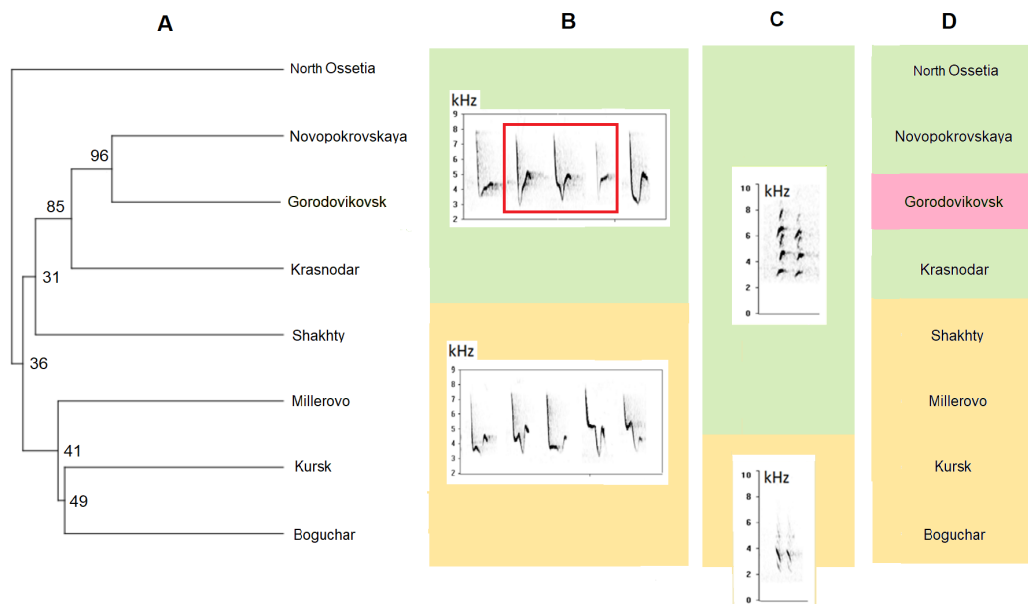
Fig. 4. Distribution of the acoustic and genetic traits among the populations studied. Column A – the song similarity (the numbers show bootstrap support for the cluster); column B – the most characteristic song syllables (the *v*-shaped syllables most typical of the *caucasicus* song are highlighted in a red frame); column C – tret calls; column D – haplotypes. Green colour is *caucasicus*; yellow colour is *abietinus*; pink colour is mixed population.

/ *L. megarhynchos, Fringilla coelebs coelebs* / *F. c. caucasica, Chloris chloris chloris* / C*h. ch. bilkevitchi, Carpodacus erythrinus erythrinus* / *C. e. kubanensis, Coccothraustes coccothraustes coccothraustes* / *C. c. nigricans*) (Belik 2009).

We have documented for the first time the existence of a new contact zone between two subspecies of the Chiffchaff. The main results obtained are as follows:

1) In many specimens bearing the *abietinus* haplotype in Ciscaucasia, the wing formula is identical to that typical of *caucasicus* and differs from that typical of *abietinus* in allopatric populations;

2) The tret calls typical of *caucasicus* are included in the song of Chiffchaffs, which carry the *abietinus* haplotype and do not have notes characteristic of *caucasicus* in their song.

3) Chiffchaffs from Ciscaucasia occupy an intermediate position between *abietinus* and *caucasicus* in the duration of individual song phrases and syllable time-frequency characteristics.

4) In the eastern Ciscaucasia, a population was found in which the *abietinus* and *caucasicus* haplotypes co-occur in a significant number. A mixed pair of Chiffchaff (male *caucasicus* and female *abietinus*) was also found here. Mixed populations have not been found in Western Ciscaucasia. It should be noted, however, that a rather small number of haplotypes were analyzed in this area, while the unexplored gap between Novopokrovskaya and Shakhty populations (Fig. 1, circles 4 and 5) is about 200 km. It is quite likely, for example, that both forms co-occur in the large urban parks of Rostov-on-Don, located just in the middle between Novopokrovskaya and Shakhty.

An additional result is the increased variability in the plumage coloration of Chiffchaffs in Ciscaucasia compared to allopatric populations. Both taxa studied have similar plumage coloration. However, there are certain differences as well. In *caucasicus*, dull greyish and brownish colours are more pronounced, whereas *abietinus* is more brightly coloured, with a greater proportion of greenish and yellowish lores. In almost all the populations studied in Ciscaucasia, specimens were caught with a bright colouration typical of *abietinus*, together with specimens with a duller

colouration characteristic of *caucasicus*, as well as specimens with a variety of intermediate colouration. The mismatch between the plumage colour and the haplotype was also noted. Eleven specimens bearing the *caucasicus* haplotype were coloured more like *abietinus* and three specimens bearing the *abietinus* haplotype were coloured more like *caucasicus*.
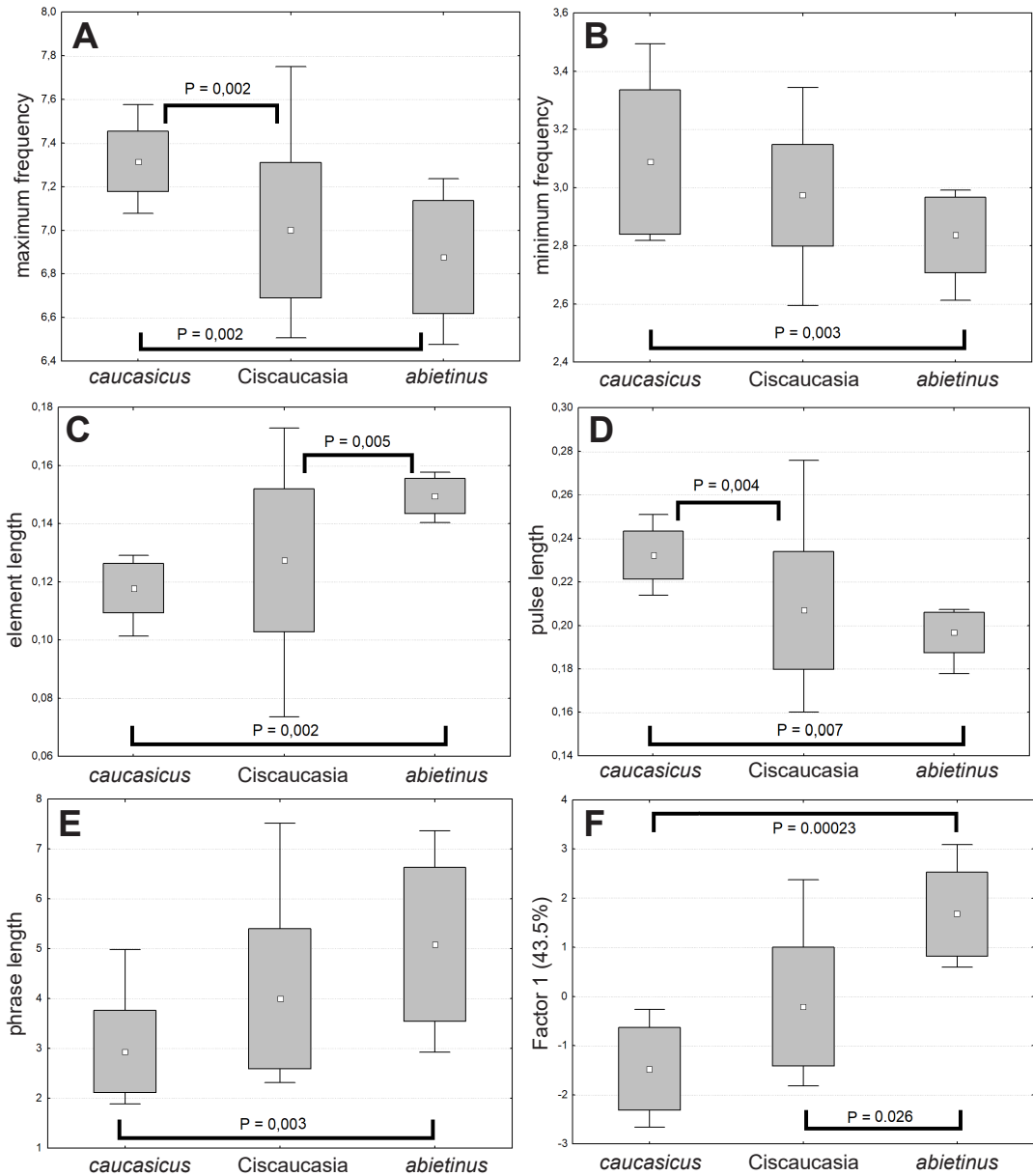

Fig. 5. Variations of song parameters in pure populations of *caucasicus* (Krasnodar and North Ossetia), in pure populations *abietinus* (Boguchar and Kursk), and in Ciscaucasia. A – maximal frequency (kHz), B – minimal frequency (kHz), C – syllable length (s), D – duration of inter-syllable pause (s), E – song phrase length (s), F – first principal component values. Significant differences in individual characteristics are illustrated (Kruskal–Wallis test; 2-tailed multiple comparison). Means, standard deviations, and limits are shown.

Although we did not conduct playback experiments, it is interesting to note that, during the captures, the males of *caucasicus* readily reacted to the broadcast of the *abietinus* song and vice versa, which we also observed earlier (Marova *et al.* 2021).

Thus, the results suggest hybridization between *abietinus* and *caucasicus* in Ciscaucasia. A significant argument in favour of this assumption is the mismatch between haplotypes and phenotypic traits (Helbig *et al.* 2001, Fedorov *et al.* 2009, Marova *et al.* 2017). For example, the Chiffchaffs from Shakhty and Millerovo carry the haplotypes of *abietinus*, and their songs do not contain syllables characteristic of *caucasicus*. However, their songs contain tret calls typical of *caucasicus*, not *abietinus*. As noted earlier, the tret calls in the songs of Chiffchaff of all subspecies, including *caucasicus*, are performed unchanged in the most remote populations of the subspecies (Ivanitskii *et al.* 2021). This suggests the probably innate nature of tret calls, with the haplotype mismatch of which may be among the evidence of hybridization.

The mismatch between the haplotype and the wing formula seems to be particularly intriguing. Only three of the 16 specimens genetically identified as *abietinus* had a wing formula identical to that in allopatric populations. The wing of the remaining 13 specimens was rounded like that of *caucasicus*. Most of these 13 specimens were caught in populations of Western Ciscaucasia (Shakhty and Millerovo) where the *caucasicus* haplotype has not been found. So the differences in morphology between *abietinus* populations in the northern (allopatric) and southern (sympatric) parts of the distribution range could arise due to differences in the length of migration routes. The distance between the populations of Ciscaucasia and the allopatric populations of the Moscow region, where the measurements were made, is about 1,000 km. This is confirmed by our data on the Kursk population, which, according to both wing formula and geographical location (480 km from Moscow), occupies an intermediate position between Moscow and Ciscaucasia. It is quite possible that the inter-population differences in the wing formula are a consequence of clonal variability. It is well known that the wing sharpness of migrating birds increases in proportion to the

increase in the length of their migration routes (Bowlin & Wikelski 2008, Corman *et al.* 2014).

Among all three contact zones of the Chiffchaff subspecies described earlier, the situation in Sweden (Hansson *et al.* 2000) has the greatest similarity with the situation in Ciscaucasia. In both cases, the very first stages of contact zone formation proceed right before our eyes. Despite the fact that the *collybita* and *abietinus* ranges in Sweden are separated by 500 km of a territory unpopulated by Chiffchaffs, 7 out of 60 individuals carried an mt-haplotype, which did not match with the population from where they were sampled. In Ciscaucasia, the gap between the ranges of phenotypically pure *abietinus* and *caucasicus* is absent (at least in the eastern area), and 4 out of 40 individuals carried a haplotype alien to the population in which they were caught. An important difference between the two situations is the degree of habitat similarity between the subspecies. In southern Sweden *collybita* inhabit rich, deciduous forests, while in the northern region *abietinus* is found in old coniferous forests. The authors believe that such differences will prevent hybridization after the formation of a geographical contact zone. In contrast, there is no difference in habitat between *abietinus* and *caucasicus*. In the south of Russia, both *abietinus* and *caucasicus* inhabit broad-leaved forests, which undoubtedly should contribute to hybridization between them.

Thus, according to our data, haplotypes of *caucasicus* currently prevail throughout the studied area of Ciscaucasia. The *abietinus* haplotype dominates only within the range of this subspecies north of Rostov-on-Don. Although the absence of *abietinus* haplotypes south of Rostov-on-Don may be due to a poorly studied gap between the extreme southern (Novopokrovskaya) and extreme northern (Shakhty) parts of the contact zone (Fig. 1), we assume that *caucasicus* moves north faster than *abietinus* moves south. Over the past half-century, the Caucasian subspecies has covered a distance of about 150 km, settling northward. We suggest that the increased mobility of *caucasicus,* at least in part, may be related to its hybrid origin (*menzbieri* x *brevirostris*), which was hypothesized earlier (Marova *et al.* 2021). There are cases when hybrids are better adapted and have greater reproductive success than

parental forms (Barton & Hewitt 1985, Moore & Koenig 1986, Arnold *et al.* 2001).

Uusi kontaktivyöhyke tiltalttien levinneisyysalueella: Kaukasian ja Euroopan alalajit kohtaavat Etelä-Venäjällä

Lähisukuisten eläinlajien kontaktivyöhykkeiden tutkimus auttaa vastaamaan moniin tärkeisiin evoluutiobiologian ja taksonomian kysymyksiin. Tiltaltilla (*Phylloscopus collybita*) on erityisen laaja levinneisyysalue ja sen alalajien kontakti-vyöhykkeitä on kuvattu paljon. Tässä tutkimuk-sessa dokumentoimme kahden tiltaltin alalajin, Kaukasian (*Ph. c. caucasicus*) ja Euroopan (*Ph. c. abietinus*) tiltaltin, uuden kontaktivyöhykkeen Etelä-Venäjän Ciskaukasiassa.

Ennustimme, että näiden kahden samaan "vihreiden tiltalttien" ryhmään kuuluvan alalajin välillä tapahtuu hybridisaatiota. Löysimme itäisestä Ciskaukasiasta populaation, jossa *abietinus*- ja *caucasius*-haplotyypit esiintyvät merkittävissä määrin yhdessä. Havaitsimme siellä myös hybridisoivan parin (*caucasicus*-koiras ja *abietinus*-naaras). Tiltalttien höyhenpeitteen väri vaihteli Ciskaukasiassa enemmän verrattuna al-lopatrisiin populaatioihin. Monilla *abietinus*-hap-lotyyppiä kantavilla yksilöillä Ciskaukasiassa siiven muoto (*wing formula*) oli identtinen *cauca-sius*-haplotyypin tyypillisen siipimuodon kanssa ja poikkesi abietinuksen tyypillisestä muodosta allopatrisissa populaatioissa. Ciskaukasian tiltal-teille ominaisia toistokutsuja (*tret calls*) esiintyi *abietinus*-haplotyyppiä kantavilla yksilöillä, mutta *caucasicukselle* tunnusomaiset sävelet puuttuivat. Monet laulun ominaisuudet edustivat *abietinuksen* ja *caucasicuksen* välimuotoa. Kiinniottojen aikana *caucasicus*-koiraat reagoivat nopeasti *abietinuksen* lauluun ja päinvastoin.

Kaikki yllä kuvatut tulokset viittaavat hybri-disaatioon *abietinuksen* ja *caucasicuksen* välillä tutkimusalueella Ciskaukasiassa.

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Online supplementary material

Supplementary material available in the online version of the article (https://doi.org/10.51812/of.131719) includes Table S1.