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Anatolia: A Hotspot of Avian Genetic Diversity in the Western Palaearctic

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Abstract: Located at the crossroads of two continents and at the southeastern edge of the Mediterranean Basin, Anatolia was one of the most important Pleistocene glacial refugia in the Western Palaearctic. As part of the Irano-Anatolian, Caucasus and Mediterranean Basin biodiversity hotspots, this region is also home to a rich avian community including nearly 400 breeding species. Nevertheless, research addressing the genetic structure and diversity of local bird populations is limited, and information on glacial refugia in this region is still scant, especially when compared to other large Mediterranean peninsulas, namely the Balkan, Italian and Iberian ones. In this study, we contribute to filling this gap by addressing the biogeographic pattern of four common resident songbirds—the Eurasian blue tit (*Cyanistes caeruleus*), the great tit (*Parus major*), the Eurasian chaffinch (*Fringilla coelebs*) and the Eurasian blackbird (*Turdus merula*)—and one endemic species—the Krüper's nuthatch (*Sitta krueperi*)—by amplifying two mitochondrial DNA genes in individuals from Anatolia (n = 329) and comparing their sequences to those of conspecifics from the rest of their distribution range across the western Palaearctic (n = 357) deposited in public databases. The overall genetic structure of these species is consistent with a scenario of isolation for multiple populations in different refugia across Anatolia and subsequent secondary contact in the wake of ice retreat, which makes this region a hotspot of genetic diversity for both widespread and endemic avian species.

Keywords: biodiversity hotspot; biogeography; Caucasus; evolution; Irano-Anatolian; Mediterranean basin; mitochondrial DNA; population genetics; songbirds; refugium

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1. Introduction

The conservation and restoration of biological diversity at any of its levels, including genetic diversity, is one of the main challenges for modern human societies [1,2]. The preservation of genetic diversity is now of unquestionable and lasting relevance in the global political agenda, as testified by well-known international mandates such as the Aichi Biodiversity Targets (Strategic Goal C, Target 13) and the Post 2020 Biodiversity Framework (OECD). This caused an increasing body of research to address this topic [3] and prompted the development of specific national and international conservation programs, with a special focus on threatened taxa and ecosystems as well as on those hosting a higher species diversity and referred to as Global Biodiversity Hotspots [4]. While a first assessment had led to the identification of 25 such areas worldwide [5], a recent reappraisal increased their number up to 36 [6], 3 of which—the Mediterranean Basin, Caucasus and Irano-Anatolian

Plateau—fall (at least partially, as in the latter case) not only in the Western Palaearctic but also in one region in particular, Anatolia, which entirely lies within present-day Turkey. In terms of biodiversity conservation, this is of the utmost relevance when considering that two out of every three amphibian species, 48% of reptiles, 25% of mammals and 3% of bird species are endemic to the Mediterranean region [7], while two of the songbird species-rich regions of the Western Palaearctic are the Caucasus along with the mountains of southern and eastern Anatolia [8].

There are multiple reasons behind the origin of this high species and habitat diversity, primarily the unique palaeogeological history of this region. During the Mesozoic era, the Pontic and the Taurus mountain ranges were occupied by the Tethys Sea. At the end of the Cretaceous, sediments—mostly carbonates—which had been slowly but constantly folding, uplifted as the result of continental collusion [9]. This originated present-day rough and complex mountainous landscapes, with only 11% of Anatolia surface area lying under 250 m above the sea level (a.s.l.) as opposed to 30% consisting of elevations higher than 1500 m a.s.l. and 3% even higher than 2500 m a.s.l. [10]. These remarkable altitudinal shifts account for highly different precipitation regimes, temperature ranges and sunlight radiation variation, which makes Anatolia a region hosting one of the richest topographic, climatic and habitat diversities in the Western Palaearctic [11]. While 1299 of 2126 total terrestrial Chordata species (of which 257 out of 568 mammals, 792 out of 954 birds, 156 out of 462 reptiles and 94 out of 142 amphibians) are found throughout Europe and the Western Palaearctic, respectively, 778 total terrestrial Chordata species (168 mammals, 454 birds, 125 reptiles and 31 amphibians)—corresponding to 59.9% and 36.6% of those occurring in Europe and the Western Palaearctic, respectively—are found in Anatolia/Turkey [12]. This species diversity is also the result of Pleistocenic climatic oscillations, with Anatolia playing—similar to the Iberian, Italian and Balkan peninsulas—a primary role as a refugium for several species living further north, especially during the Last Glacial Maximum (LGM) [13]. It has actually been proposed that, by that time, Anatolia hosted multiple isolated refugia [14,15], in compliance with the refugia within refugia model [16], which greatly affected the genetic structure of the local populations [17–19]. This is reflected by the uniqueness of native populations of fallow deer (*Dama dama*) [20,21], golden jackals (*Canis aureus*) [22], European blind snake (*Typhlops vermicularis*) [17] and common (*Bufo bufo*) as well as Caucasian toad (*B. verrucosissimus*) [23], just to list some examples among terrestrial vertebrates.

The few demographic data available for Anatolian avian populations suggested that some species expanded their ranges between the Last Interglacial (LIG) and the LGM, while others did so after the LGM [14,15]. In most cases, however, neither genetic nor demographic data are available. To contribute to filling this knowledge gap, we here characterise the genetic diversity and reconstruct the demographic history of the Anatolian populations of four common and one endemic western Palaearctic songbird. Specifically, we aim to (i) compare the genetic diversity of their populations with that of conspecifics from other regions; (ii) explore the role played by local glacial refugia in these species during Pleistocenic climatic oscillations; and (iii) confirm that Anatolia is a hotspot of avian genetic diversity in the Western Palaearctic.

2. Materials and Methods

2.1. Sample Collection and DNA Extraction

Four common western Palaearctic polytypic passerine species—the Eurasian blue tit (*Cyanistes caeruleus* Linnaeus, 1758; *n* = 49), the great tit (*Parus major* Linnaeus, 1758; *n* = 61), the Eurasian chaffinch (*Fringilla coelebs* Linnaeus, 1758; *n* = 80) and the Eurasian blackbird (*Turdus merula* Linnaeus, 1758; *n* = 59)—and one endemic species—the Krüper's nuthatch (*Sitta krueperi* Pelzeln, 1863; *n* = 80)—were captured with mist nets during the breeding season (March–June, from 2013 to 2017) in Anatolia (Figure 1). A blood sample (70 µL) was obtained from the brachial vein of each individual and stored in 500 µL of 96% ethanol.

Total genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following manufacturers' instructions.

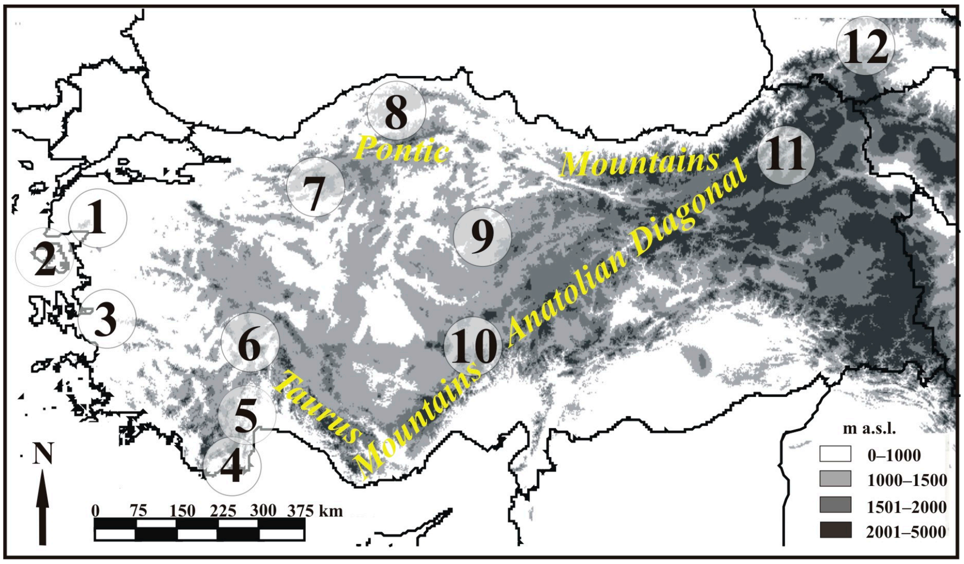

Figure 1. Sampling localities. 1. Kazdağları; 2. Lesvos Island; 3. Spil Mountain; 4. Adrasan; 5. Beydağları; 6. Burdur; 7. Kartalkaya; 8. Küredağları; 9. Yozgat; 10. Aladağlar; 11. Artvin; 12. Caucasus. See Supplementary S1 for coordinates and other details. The main mountain ranges are also indicated.

2.2. DNA Sequencing

We amplified the partial sequence of two mitochondrial genes largely used in phylogeographic studies, namely the cytochrome c oxidase subunit 1 (COI; 656 bp) with primers passerF1/passerR1 [24] and BirdF1/BirdR1 [25] along with the NADH dehydrogenase subunit 2 (ND2; 1041 bp) with primers L5215 and H1064 [26]. PCR reactions were performed in a 50 µL final volume including 1× PCR buffer (Bioron, Ludwigshafen, Germany), 100 µM dNTPs, 0.2 units of *Taq* DNA polymerase (Bioron), 200 ng of DNA and 5 pmol of primers. Thermal conditions were as follows: 5 min at 94 °C, followed by 38 cycles of 50 s at 94 °C, 40 s at 55.6 °C, 1 min at 72 °C and a final extension at 72 °C for 10 min. PCR products were sequenced on both strands at Macrogen Sequencing Facilities (Macrogen-Korea, Seoul, Korea). Newly amplified sequences were deposited in GenBank under accession numbers OR513143, OR513145–OR513157, OR513159–OR513161, OR513163–OR513413 and PP811813–PP812121.

2.3. Phylogeographic Analyses

The newly amplified sequences were aligned to those from other western Palaearctic conspecific populations retrieved from GenBank (Supplementary S1) in Geneious Prime v.2021.2.2 [27]. For the sake of clarity, there is no consensus about the intra-Palaearctic boundaries, with at least two main views—one traditionally excluding all of Iran from the western Palaearctic [28] and a new one including it in its entirety (thus featuring the Greater Western Palaearctic [29,30]). Both of these views are widely accepted, and another has been recently proposed (see [31]). However, in this paper, we will refer to the Western Palaearctic *sensu stricto*. Two separate alignments—one per gene—were produced with the MUSCLE [32] plugin, converted into amino acid sequences and visually inspected to check against internal start and stop codons that could indicate the occurrence of nuclear mitochondrial pseudogenes. Haplotype list, number of polymorphic sites (S), haplotype diversity (Hd), nucleotide diversity (π), average number of nucleotide differences (K) and number of haplotypes (h) were obtained in DnaSP v.6 [33], which was also used for the analysis of mismatch distributions. Haplotype networks were built using the median-joining methods [34] implemented in popART [34,35]. Finally, the genetic differentiation among populations was evaluated by means of ϕ_{ST} and analysis of molecular variance (AMOVA) in Arlequin v.3.5 [36], with significance assessed by performing 10,100 permutations.

2.4. Demographic History

We used MEGA v.X [37] to build Maximum Likelihood trees by selecting the best substitution model for each species and gene separately based on the lowest BIC (Bayesian Information Criterion) scores. We found that, for both loci, it was HKY+G+I for *C. caeruleus* and *S. krueperi*, HKY+G for *F. coelebs* and *T. merula* and HKY for *P. major*. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbour Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the best model and then selecting the topology with a superior log-likelihood value. We hence used both genes to infer the demographic history of the target species by means of Extended Bayesian Skyline Plot (EBSP) in BEAST2 [38]. Multiple EBSP runs relying on Markov chain Monte Carlo (MCMC) methods were performed using the following parameters: linear models, 100 million steps, parameters sampled every 10,000 steps and a 10% burn-in. We used a strict clock model with 0.027 substitutions/site/million years (Myr) mutation rate [39], and the population model factor was set to 0.5 to account for a female-only contribution to the effective population size (N_e), since all the target species exhibit male philopatry. All other operator settings were left as default. The graphics inherent to demographic reconstructions were generated using the plotEBSP.R script available online at https://www.beast2.org/tutorials/ (accessed on 1 January 2023) in R Studio v.2021.09.2 [40].

3. Results

The number of samples yielding successful amplification at each locus and species are reported in Table 1. COI and ND2 alignments consisted of 656 and 1041 bp, respectively. No internal stop codons were found. The highest and lowest COI haplotype diversity was found in *C. caeruleus* and *P. major*, respectively. As far as the ND2 is concerned, *S. krueperi* yielded the highest diversity as opposed to *C. caeruleus*, which instead yielded the lowest one (Table 1). Populations from Anatolia turned out to host a high number of haplotypes, including several that were private to this region, at both genes and across all the surveyed species, ranging from 28.6% to 82.6% for the COI and from 22.2% to 96.4% for the ND2, on average (Table 1). The highest rate of private haplotypes was found in *F. coelebs* (82.6%) followed by *T. merula* (50.0%) and *P. major* (46.2%) for the COI and in *P. major* (36.1%), *F. coelebs* (36.0%) and *T. merula* (34.3%) for the ND2 (Figure 2). The network analysis showed that Anatolian populations of the latter species diverged substantially at the ND2, while *C. caeruleus*, *P. major* and *F. coelebs* showed a mix of private and shared haplotypes. Common haplotypes featuring star-like topologies were found in *P. major* at both loci and in *F. coelebs* and *T. merula* at the COI only (Figure 3). Pairwise ϕ_{ST} values show that all Anatolian populations differ significantly, with a few exceptions, from their counterparts at one or both loci (Tables 1 and 2), with the most pronounced differentiations being found between Anatolian and non-Anatolian *C. caeruleus* and *F. coelebs* populations at the COI ($\phi_{ST} = 0.59$, $p < 0.01$) and ND2 ($\phi_{ST} = 0.65$, $p < 0.01$), respectively. When the per species partition of genetic diversity across Anatolia was considered, we found the coastal locations along western and southern Anatolia to stand out among the others (Figure 4 and Supplementary Table S1).

Table 1. Summary statistics (±SD) of genetic diversity in the studied species. COI: cytochrome c oxidase subunit 1; ND2: NADH dehydrogenase subunit 2; Hd: haplotype diversity; π: nucleotide diversity; N: sample size; S: polymorphic sites; *h*: number of haplotypes; ph: number of haplotypes private to Anatolia; WP*: Western Palaearctic except Anatolia.

	COI	**S**	**Haplotypes**		**Hd**	**π (× 10^{-3})**	**ND2**	**S**	**Haplotypes**		**Hd**	**π (× 10^{-3})**
	N		**h (%)**	**ph**			**N**		**h (%)**	**ph**		
Cyanistes caeruleus												
Anatolia	46	9	12 (86)	4	0.82 ± 0.04	3.35 ± 0.47	49	17	4 (44)	2	0.35 ± 0.08	4.57 ± 1.16
WP*	27	12	10 (71)	9	0.86 ± 0.04	5.46 ± 0.4	10	23	7 (78)	5	0.91 ± 0.08	10.27 ± 1.64
Total WP	**73**	**14**	**14**		**0.85 ± 0.02**	**5.90 ± 0.40**	**59**	**23**	**9**		**0.48 ± 0.08**	**5.72 ± 1.06**
Parus major												
Anatolia	55	9	7 (54)	6	0.27 ± 0.08	1.00 ± 0.33	61	16	16 (44)	13	0.65 ± 0.07	1.00 ± 1.00
WP*	21	24	9 (69)	6	0.76 ± 0.08	1.27 ± 2.37	89	31	23 (64)	19	0.61 ± 0.06	1.11 ± 0.18
Total WP	**76**	**26**	**13**		**0.42 ± 0.07**	**5.34 ± 1.52**	**150**	**39**	**36**		**0.63 ± 0.01**	**1.08 ± 0.13**
Fringilla coelebs												
Anatolia	80	21	22 (95)	19	0.77 ± 0.05	1.85 ± 0.20	54	20	24 (67)	18	0.91 ± 0.03	2.03 ± 0.21
WP*	10	3	4 (17)	1	0.64 ± 0.15	1.46 ± 0.47	22	28	18 (50)	12	0.97 ± 0.03	3.87 ± 0.98
Total WP	**90**	**21**	**23**		**0.75 ± 0.05**	**1.90 ± 0.20**	**76**	**41**	**36**		**0.92 ± 0.02**	**2.62 ± 0.37**
Turdus merula												
Anatolia	51	11	11 (79)	7	0.61 ± 0.07	1.69 ± 0.33	59	25	14 (40)	12	0.87 ± 0.03	6.05 ± 1.10
WP*	24	11	7 (50)	3	0.76 ± 0.06	2.28 ± 0.48	69	39	23 (66)	21	0.86 ± 0.03	4.72 ± 0.67
Total	**75**	**17**	**14**		**0.67 ± 0.05**	**1.91 ± 0.28**	**128**	**46**	**35**		**0.92 ± 0.01**	**5.90 ± 0.68**
Sitta krueperi												
Anatolia	115	16	15 (79)	13	0.78 ± 0.02	2.25 ± 0.17	48	17	27 (96)	27	0.96 ± 0.02	3.37 ± 0.20
Lesvos Island	20	0	5	0	0.00 ± 0.00	0.00 ± 0.00	0					
Caucasus	28	4	5 (26)	4	0.38 ± 0.11	0.74 ± 0.24	1	1	1 (4)	1		
Total	**163**	**18**	**19**		**0.77 ± 0.01**	**2.94 ± 0.13**	**49**	**20**	**28**		**0.96 ± 0.02**	**3.49 ± 0.22**

Table 2. Genetic differentiation ϕ_{ST} between Anatolian and all other Western Palaearctic (WP) regions of the species investigated at the COI and ND2 genes. Only sample sizes (see Table 1) larger than 5 were considered. * $p < 0.01$.

	COI		**ND2**	
Cyanistes caeruleus	0.57 *		0.49 *	
Parus major	0.27 *		0.01	
Fringilla coelebs	0.04		0.57 *	
Turdus merula	0.31 *		0.34 *	
	Lesvos	**Caucasus**	**Lesvos**	**Caucasus**
Sitta krueperi	0.29 *	0.53 *	1	1

The demographic history and mismatch distribution profiles of the five songbirds are presented in Figure 5. While *C. caeruleus* shows a profile with an invariably stable N_e, those of *T. merula* and *S. krueperi* indicate a stable size up to approximately 15–20 ka followed by a rapid increase that becomes progressively less steep towards the present. *Fringilla coelebs* and *P. major* population profiles appear rather different, with both seemingly starting their expansion before 25 ka, even though the uncertainty attached with population size estimations before 18 ka (Figure 5) calls for caution in making such statement. Mismatch distributions were multimodal in *C. caeruleus*, *P. major* and *T. merula*, with no support for demographic expansion, as opposed to *F. coelebs* and *S. krueperi*, in which these were unimodal (Figure 5). The demographic reconstructions showed that while *C. caeruleus* population size apparently experienced no change over time, in the case of *P. major* and *F. coelebs*, it increased starting before 21 ka, which corresponds to the LGM, as opposed to the case of *T. merula* and *S. krueperi*, in which the change occurred after the LGM.

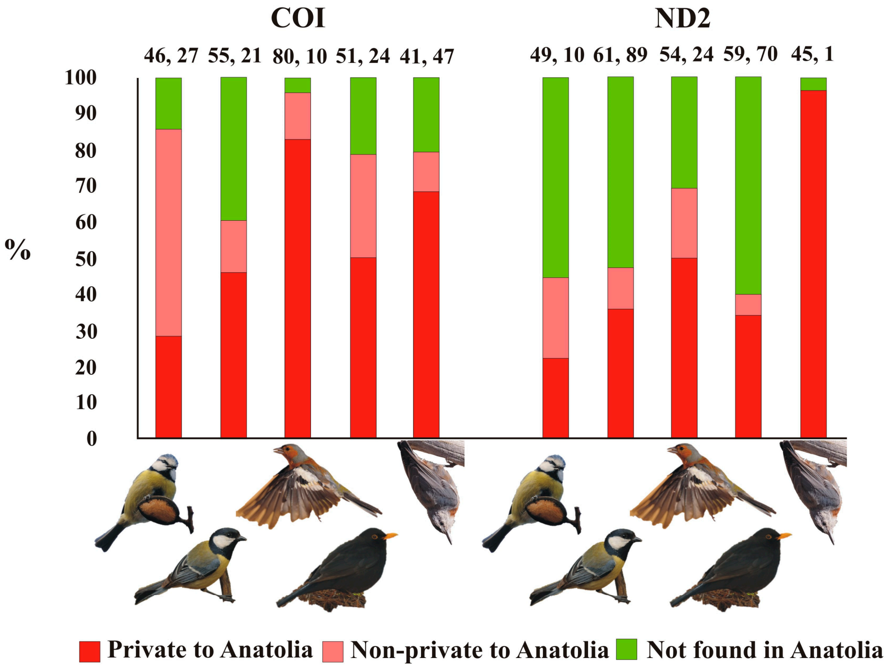

Figure 2. Percentage of COI and ND2 haplotypes private to Anatolia, found in Anatolia and found in other Western Palaearctic regions (i.e., non-private to Anatolia) and not found in Anatolia in Eurasian blue tit, great tit, Eurasian chaffinch, Eurasian blackbird and Krüper's nuthatch (**left** to **right**). Numbers on top of the columns indicate sample size within (**left**) and outside (**right**) Anatolia. Pictures not to scale.

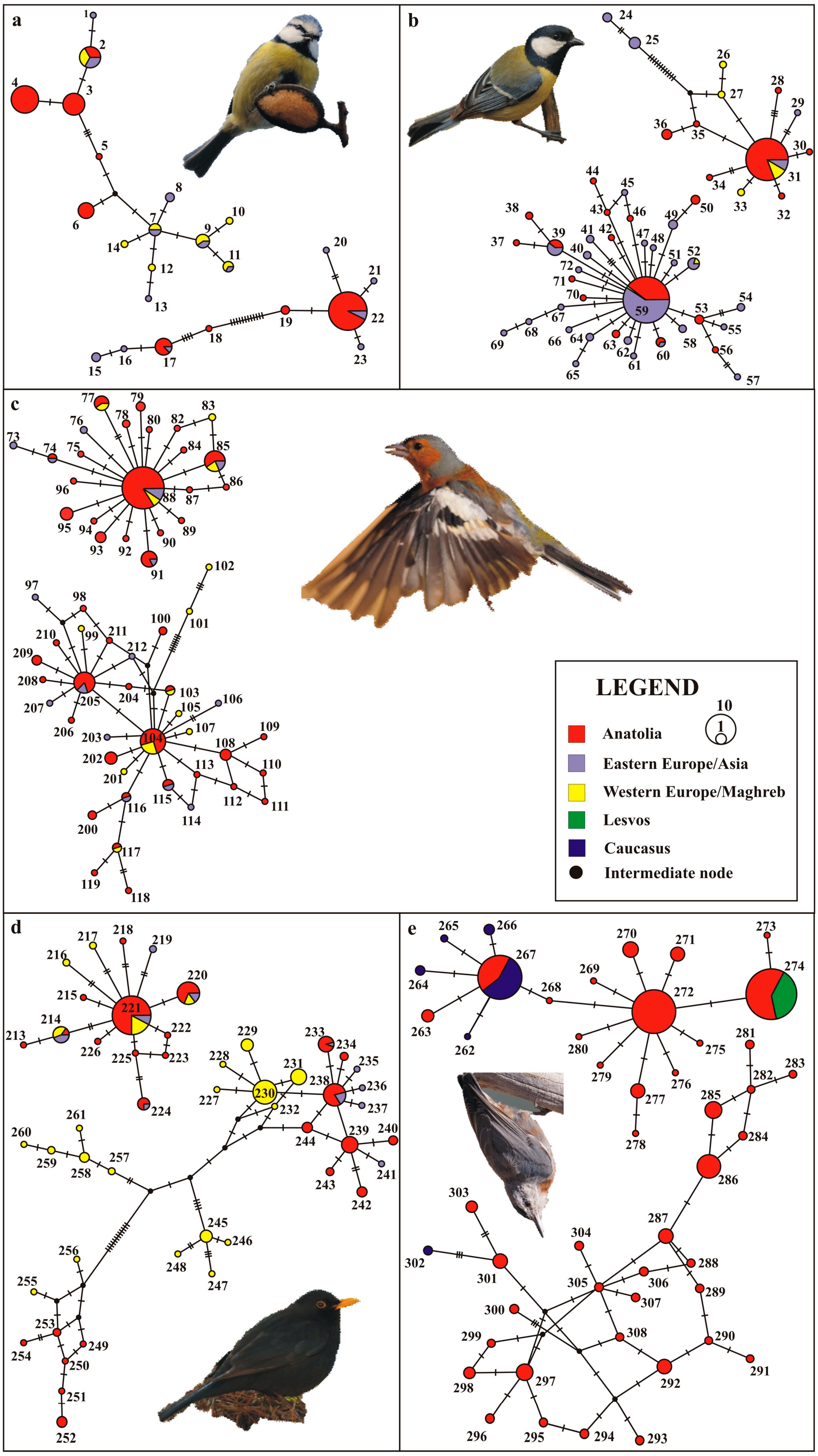

Figure 3. COI and ND2 (top and bottom left, respectively) median-joining network of target species: (**a**) Eurasian blue tit; (**b**) great tit; (**c**) Eurasian chaffinch; (**d**) Eurasian blackbird; (**e**) Krüper's nuthatch. A scale to infer the number of sequences (i.e., individuals) for each haplotype (1–308) is provided; mutational steps are indicated by hatch marks. See Supplementary S1 for coordinates and other details.

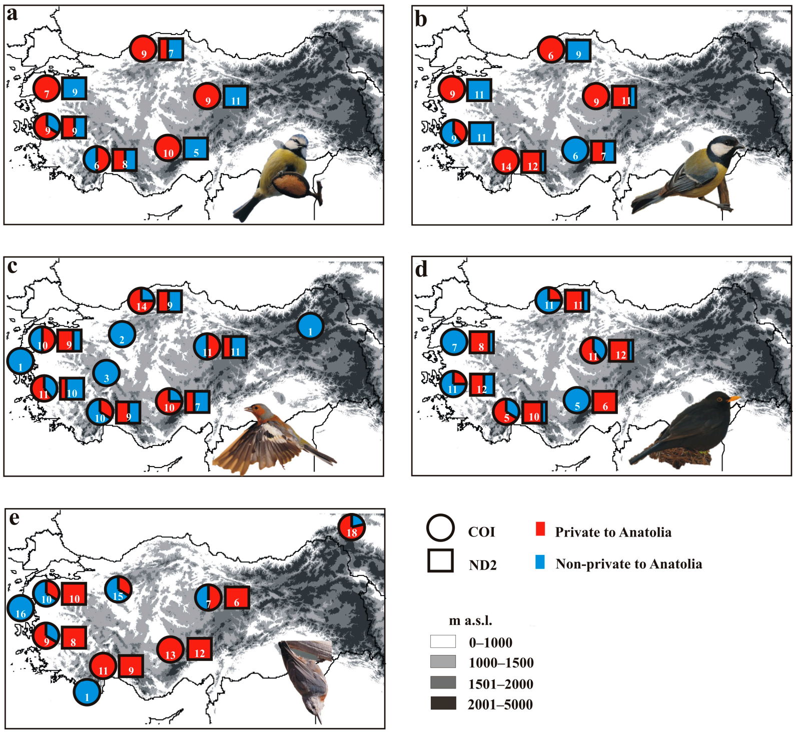

Figure 4. Proportion of private- and non-private-to-Anatolia COI and ND2 haplotypes per species and sampling locality. Sample sizes per locus and locality are also indicated (see Supplementary S1 for coordinates and other details). (**a**) Eurasian blue tit; (**b**) great tit; (**c**) Eurasian chaffinch; (**d**) Eurasian blackbird; (**e**) Krüper's nuthatch. Pictures not to scale.

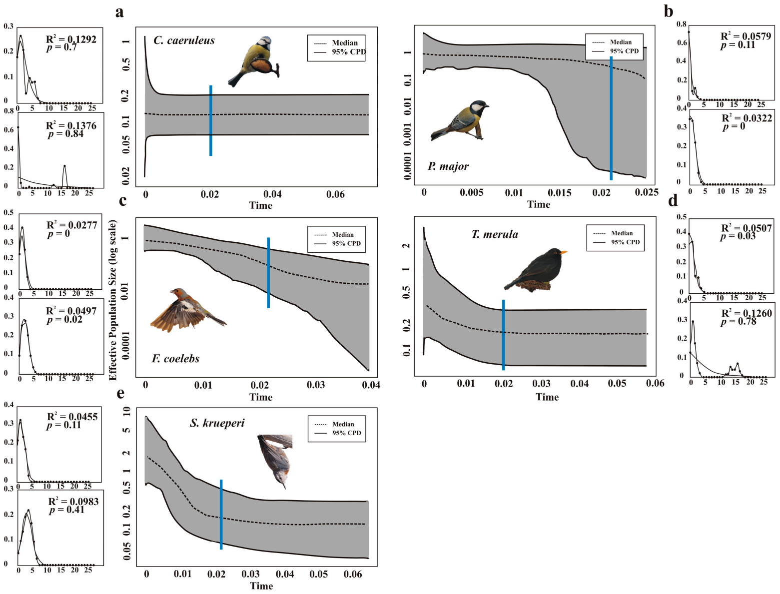

Figure 5. Extended Bayesian Skyline Plots (EBSPs) based on COI and ND2 of Anatolian populations of the five species included in this study: (**a**) Eurasian blue tit; (**b**) great tit; (**c**) Eurasian chaffinch; (**d**) Eurasian blackbird; (**e**) Krüper's nuthatch. Median estimates of effective population size (Ne) are given. The dotted line and the grey area delimit the 95% central posterior density (CPD) intervals. The x-axis represents time from the present in million years (Myr), while the blue line indicates the Last Glacial Maximum. Panels on the left evidence mismatch distributions to expected frequencies under the demographic expansion model (top: COI; bottom ND2).

4. Discussion

Located at the intra-Palaearctic boundary, Anatolia is known for its complex topography, different climates and rich habitat diversity, which translates into a high species richness and high levels of endemism, hinting at its global importance for the conservation of biodiversity [41]. In this study, five passerine species were investigated to test the hypothesis that their genetic diversity might be higher than expected due to the occurrence of multiple barriers that determined population isolation in the past prior to recolonisation and gene flow restoration among them. Provided that the sampling of western Palaearctic conspecific populations is far from being exhaustive, since it only relied on publicly accessible records for which sampling locality information was available, our results confirmed the hypothesis that the entirety of Anatolia is a hotspot of avian genetic diversity.

4.1. Genetic Diversity

When looking at the two widely distributed resident species, *C. caeruleus* and *P. major*, slightly different scenarios emerged. The haplotype and nucleotide diversity of Anatolian *C. caeruleus* was high at the COI but lower at the ND2, with haplotypes private to Anatolia in both genes (Figures 2–4) and an overall divergence of about 50% from other western Palaearctic representatives (Table 2). This finding is in line with the sharp distinctiveness between northern and southern European as well as western Asian populations [42]. Moreover, fine-scale genetic structure has emerged in other studies on Mediterranean *C. caeruleus* populations, for which habitat type rather than physical barriers has been pointed out as the main driver of divergence [43]. On the other hand, *P. major* genetic diversity was found to be lower in Anatolia than elsewhere across its range, with local populations being clearly distinct from all the others (Table 2). While the scenario we found for *C. caeruleus* points towards the separation of different populations during the LGM and the subsequent re-establishment of their connectivity (with habitat type possibly playing a role in preventing complete homogenisation), the lack of a strong genetic structure within Anatolia suggests that *P. major* local populations have never been completely isolated from each other or, at least, not for long, which is also confirmed by the occurrence of a widespread haplotype with a star-like topology (Figure 3). Concordantly, high migration and dispersal rate have been invoked to explain the weak genetic structure found in *P. major* elsewhere across its range [44].

As far as the two migratory species are concerned, *F. coelebs* and *T. merula*, both occurring in Anatolia as both a resident throughout the entire year and winter visitor, we also found slightly different scenarios. In *F. coelebs*, we expectedly found comparable genetic diversity values across all populations, a lack of differentiation among them at the COI and a widespread haplotype with a star-like topology, which is in line with the seemingly high gene flow between its continental populations reflected in only subtle phenotypic differences [45] and limited genome-wide diversity [46]. Nevertheless, ND2 genetic diversity values were higher than COI ones, with Anatolian populations being markedly different from the others (Table 2). In *T. merula*, some degree of differentiation was found at both loci, even if COI genetic diversity was lower than that of ND2, with more haplotypes private to Anatolia, which turned out in its local populations to be well differentiated from others in the western Palaearctic by at least 31% at both loci (Table 2). This pattern indicates that the effect of isolation was particularly pronounced in this species, possibly because of the long-lasting isolation suffered by its populations or the limited gene flow between their migratory and resident counterparts. Indeed, if, on one hand, *T. merula* radiation across Eurasia occurred relatively recently and rapidly [47], this points to a limited genetic distinctiveness. On the other hand, it is known that its migratory habits vary sharply with latitude [48], which may increase population isolation and divergence. Finally, *S. krueperi*, resident to Anatolia, Caucasus and Lesvos Island (Greece), where its insular population holds only one COI haplotype (Figure 4), is probably the species that was most affected by past climatic oscillations among those studied here. Overall, intraspecific genetic diversity was high (Table 2), with three COI haplogroups which are deemed to result from past population isolation in different refugia during the LGM, consistent with what was previously suggested on the basis of mitochondrial and nuclear DNA markers [14]. This is further supported by the scenario emerging on the basis of the ND2 gene, with Anatolian, Caucasian and Lesvos Island populations sharing no haplotypes at all (Figure 1).

4.2. Conclusive Remarks

The complex scenario that emerged for the five species studied, with either one or the other mitochondrial gene yielding higher diversity, is likely attributable to the fact that two of them—*T. merula* and *F. coelebs*—are also seasonal visitors other than resident, which might result in increasing genetic diversity by means of gene flow with non-resident populations. The likely reason underlying this intraspecific variability should be investigated in the high habitat and climate heterogeneity along with varied topography characterising Anatolia, which accounts for different survival strategies even within the same species. As a result, the species with the highest number of haplotypes turned out to be precisely one of those occurring also as migratory, *F. coelebs* (COI 95%; ND2 44%), followed by *C. caeruleus* (COI 86%; 44% ND2), both of which hosted some haplotypes private to Anatolia. The high number of haplotypes and the uniqueness of part of them in this region is certainly the result, in addition to migration, of isolation in different refugia, as testified by the high genetic distinctiveness characterising local populations.

Additional evidence supporting this scenario is represented by the demographic reconstructions, showing that the population size of all the studied species except for one—*C. caeruleus*—changed markedly across time. Accordingly, it has been found that there might be suitable conditions for an increase in the population size of species inhabiting Anatolia both before and after the LGM [49,50], pointing to this region as an important refugium during Pleistocenic climatic fluctuations, which greatly affected population connectivity. As an example, the decrease in sea level during the LGM caused Lesvos Island to connect to Anatolia, which made *S. krueperi* colonisation from mainland possible. The fact that the insular population hosts only one non-private haplotype is the result of the founder effect that could not be counteracted due to the rise in the sea level, preventing any further connection with the source population. The major role played by Anatolia as a refugium for species that later (re-)colonised other areas, shaping present-day European biodiversity, is well-known for non-avian taxa, this being the case of mammals such as the brown hare (*Lepus europaeus*) [49] and the beech marten (*Martes foina*) [51] but also of reptiles [52], insects [53,54] and plants [11,55].

Moreover, our data support the occurrence of multiple refugia within Anatolia, primarily along the western and southern coasts but also the northern ones, as in the case of *C. caeruleus* (and, to a certain extent, also *T. merula*), whose Anatolian populations host two highly diverging ND2 haplogroups, presumably as the result of population isolation during the LGM and secondary contact after it. The occurrence of three *T. merula* ND2 haplogroups is also in line with this scenario as well as that of private-to-Anatolia along with widespread haplotypes, which was also found in other avian species such as the common redstart (*Phoenicurus phoenicurus*) [56] (but in this case, the so-called "ghost introgression" might also play a role cf. [57]), the house sparrow (*Passer domesticus*) [58] and the chukar partridge (*Alectoris chukar*) [15]. The high haplotype diversity and, often, the occurrence of multiple haplogroups in Anatolia, which was found in a number of other taxonomic groups such as mammals [59], reptiles [60], amphibians [61] and insects [62] has been imputed to its geographic positions favouring Pleistocene bidirectional faunal exchanges between Europe and the Near East [63], as well as the occurrence of multiple refugia within Anatolia underlying cryptic diversity [17,64].

Taken together, these data point to the entirety of Anatolia as an extraordinary hotspot of avian genetic diversity in the western Palaearctic. The next step is investigating this topic further by means of genome-wide approaches. At the same time, we advocate for the urgent need to curb the relentless urbanisation and related land consumption that, as already highlighted more than a decade ago, is putting Turkey's conservation capacity at risk [65]. For this reason, boosting the development of growing conservation ethics, which is still in its infancy, along with transboundary conservation efforts in a country that ranks among the top 20 largest economies [66] is a certainly a demanding yet worthwhile task for Turkish broader society.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d16060339/s1, Supplementary Table S1. Pattern of private- (ph) and non-private-to- (non-ph) Anatolia COI and ND2 haplotypes per species and sampling locality (see Figure 1 for further details). Supplementary S1. List of sequences generated for this study and of GenBank records [67–84] downloaded for comparative purposes.

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Data Availability Statement: All data generated in this study have been reported within the main text or in the Supplementary Materials. The COI and ND2 sequences produced in this study were deposited in GenBank under accession numbers OR513143, OR513145–OR513157, OR513159–OR513161, OR513163–OR513413 and PP811813–P812121.

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