RESEARCH ARTICLE

Genome-wide data reveal paraphyly in the sand plover complex (*Charadrius mongolus/leschenaultii*)

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ABSTRACT

Correct assessment of species limits and phylogenetic relationships is a prerequisite for studies in ecology and evolution. Even in well-studied groups such as birds, species delimitation often remains controversial. Traditional avian taxonomy is usually based on morphology, which might be misleading because of the contingent nature of evolutionary diversification. The sand plover complex (genus *Charadrius*) may be such an example wherein 2 Lesser Sand Plover *C. mongolus* subspecies groups have been proposed to comprise 2 species. We use genome-wide data of 765K SNPs to show that the widely accepted taxonomic treatment of this sand plover complex appears to be a paraphyletic grouping, with two Lesser Sand Plover subspecies groups found not to be each other's closest relatives, and with the *mongolus* subspecies group being the sister taxon of Greater Sand Plover *C. leschenaultii*. Based on genomic and acoustic analyses, we propose a three-way split of the Sand Plover complex into the Siberian Sand Plover *C. mongolus*, Tibetan Sand Plover *C. atrifrons*, and Greater Sand Plover *C. leschenaultii*. The similar sizes of the Siberian and Tibetan Sand plovers may be the result of niche conservatism coupled with rapid morphological and ecological differentiation in the Greater Sand Plover. Gene flow between the non-sister Tibetan and Greater Sand plovers might have happened in phases of secondary contact as a consequence of climate-driven range expansions. We call for further studies of the Sand Plover complex, and suggest that speciation with intermittent gene flow is more common in birds than currently acknowledged.

Keywords: Asia, speciation, taxonomic revision, waders

LAY SUMMARY

- The use of an integrative approach combining molecular and phenotypic data has revolutionized modern avian taxonomy.
- Using mitochondrial and genomic-wide variants, we show that the Lesser Sand Plover (*Charadrius mongolus*) appears to be paraphyletic taxa, with its two subspecies groups ("*mongolus*" and "*atrifrons*") found not to be each other's closest relatives, and with the "*mongolus*" group being the sister taxon of Greater Sand Plover *Ch. leschenaultii*.
- The pairwise sequentially Markovian coalescent (PSMC) method indicated contrasting demographic histories between the three taxa in the sand plover complex.
- We further demonstrated that the two subspecies groups in Lesser Sand Plover differ significantly in vocalizations.
- These evidences suggest that the migratory Palearctic shorebird Lesser Sand Plover should be split into 2 species: *Ch. mongolus* Pallas, 1776, and *Ch. atrifrons* Wagler, 1829.

基因组数据揭示了蒙古/铁嘴沙鸻种组中的并系群 (*Charadrius mongolus/leschenaultii*)

摘要：经典分类学常常是基于形态学数据考虑类群间的分类学关系，然而这样的处理有时会由于进化过程中的随机性导致分类上的谬误。蒙古-铁嘴沙鸻复合种可能就是一个这样的案例，其中的蒙古沙鸻有可能实际上包含了两个独立的物种。利用线粒体基因数据、基因组数据以及声学数据，我们揭示了传统分类学处理中的沙鸻复合种中事实上包含了一个并系群。我们发现蒙古沙鸻的两个亚种群实际上并非姐妹类群，其中东部类群与铁嘴沙鸻的亲缘关系更近。我们的数据支持将蒙古-铁嘴沙鸻复合种作为三个种处理，分别为西伯利亚沙鸻、青藏沙鸻以及铁嘴沙鸻。西伯利亚沙鸻、青藏沙鸻这两种沙鸻在形态上的相似性可能是生态位保守的结果，而铁嘴沙鸻的快速体型演化则可能是由于其进入新的生境产生的适应性进化。青藏沙鸻和铁嘴沙鸻间的历史基因流可能发生在由于气候引导的分布区扩张和二次接触过程中。我们呼吁对该复合种开展更全面和深入的研究以理解该系统的分化与成种。

关键词：亚洲，水鸟，分类修订，鸻鹬

INTRODUCTION

Traditional bird taxonomy and systematics have relied on morphology, such as morphometrics and plumage traits to differentiate taxa. However, the contingent nature of evolutionary divergence and the speciation process can make such assessments misleading. While phenotypic divergence can evolve fast during adaptive radiations, evolutionary diversification does not necessarily result in morphological differentiation. Such morphologically similar or even cryptic evolutionary lineages might differ both in genetics, and in other traits such as mating behavior, ecology-related physiology, or phenology (Braune et al. 2008, Feckler et al. 2014, Taylor and Friesen 2017). Conversely, morphological similarity can also result from parallel evolution through mechanisms such as incomplete lineage sorting, introgression of genetic material from other taxa, or via different mutations (Stern 2013, Schweizer et al. 2019). Consequently, avian taxonomy has developed into an integrative process over the last decade (Sangster 2014) with taxonomic decisions being achieved by taking multiple lines of evidence into account, integrating data from genetics, morphology, vocalizations, behavior, ecology, or physiology (Sangster 2018).

Shorebirds (Charadriiformes) are not known to be a group that poses major taxonomic challenges at the species level. Different widely distributed species are characterized by weak genetic structure as a consequence of remarkable dispersal abilities, leading to frequent gene flow between geographically distant populations (Küpper et al. 2012, Conklin et al. 2016). This might be driven by nomadic polygynous males moving through a considerable part of a species' breeding range, preventing local adaptation and population divergence, as in the Pectoral Sandpiper (*Calidris melanotos*) (Kempenaers and Valcu 2017). Alternatively, some true allopatric subspecies, such as island or inland populations, might prominently differ genetically from their coastal counterparts.

An incipient species was recently revealed in the Kentish plover complex. The morphologically distinct subspecies *Charadrius alexandrinus dealbatus* of southeast Asia has differentiated genome-wide and in ecology (Wang et al. 2019a, b, Sadanandan et al. 2019) and was recently given

species status (Gill et al. 2021). It also has been argued that morphological variation in the Lesser Sand Plover (*Ch. mongolus*) might reflect species-level differentiation (Hirschfeld et al. 2000, Livezey 2010), although this has not been investigated using an integrative approach including genetic data (Küpper and dos Remedios 2019).

The Lesser Sand Plover, a long-distance migrant breeding in Central and East Asia, is usually divided into 2 subspecies groups with disjunct breeding ranges (del Hoyo et al. 2014). These are (1) the western "*atrifrons*" group containing 3 subspecies: *Ch. m. pamirensis* breeding from western Tien Shan, the Pamirs and Karakoram mountains to the western Kunlun Shan; *Ch. m. atrifrons* breeding in the Himalayas and the southern Qinghai-Tibetan Plateau; and *Ch. m. schaeferi* from the eastern part of the Qinghai–Tibetan plateau north to southern Mongolia; and (2) the eastern "*mongolus*" group comprising the nominate subspecies *Ch. m. mongolus* breeding inland in East Siberia and the Russian Far East, as well as the subspecies *Ch. m. stegmanni* breeding in north-eastern Siberia from Chukotka Autonomous Okrug south to Kamchatka, Commander Islands, and North Kuril Island (Lappo et al. 2012). These 2 groups differ notably in breeding plumage. While individuals of the *atrifrons* group generally have more black coloring on the forehead, those of the *mongolus* group show a white forehead patch and a broader and more deeply red breast-band usually with a black upper border, although a considerable proportion of individuals breeding on the Chukotskiy Peninsula in Siberia (*mongolus* group) seem to have a similar forehead pattern to birds from the *atrifrons* group (Hirschfeld et al. 2000). The issue is complicated by the morphologically similar Greater Sand Plover (*Ch. leschenaultii*), which breeds from Minor Asia and the Levant over Central Asia to Mongolia, western China, and southern Siberia, and is considered to be a sister species of the Lesser Sand Plover *Ch. mongolus* (*s.l.*) based on molecular phylogenetic data (Dos Remedios et al. 2015). The Greater Sand Plover is generally larger and bulkier than the Lesser Sand Plover; however, its smallest subspecies *Ch. l. columbinus* approaches the latter in size (Hirschfeld et al. 2000). The breeding range of the Greater Sand Plover extends between those of the *mongolus* and *atrifrons*
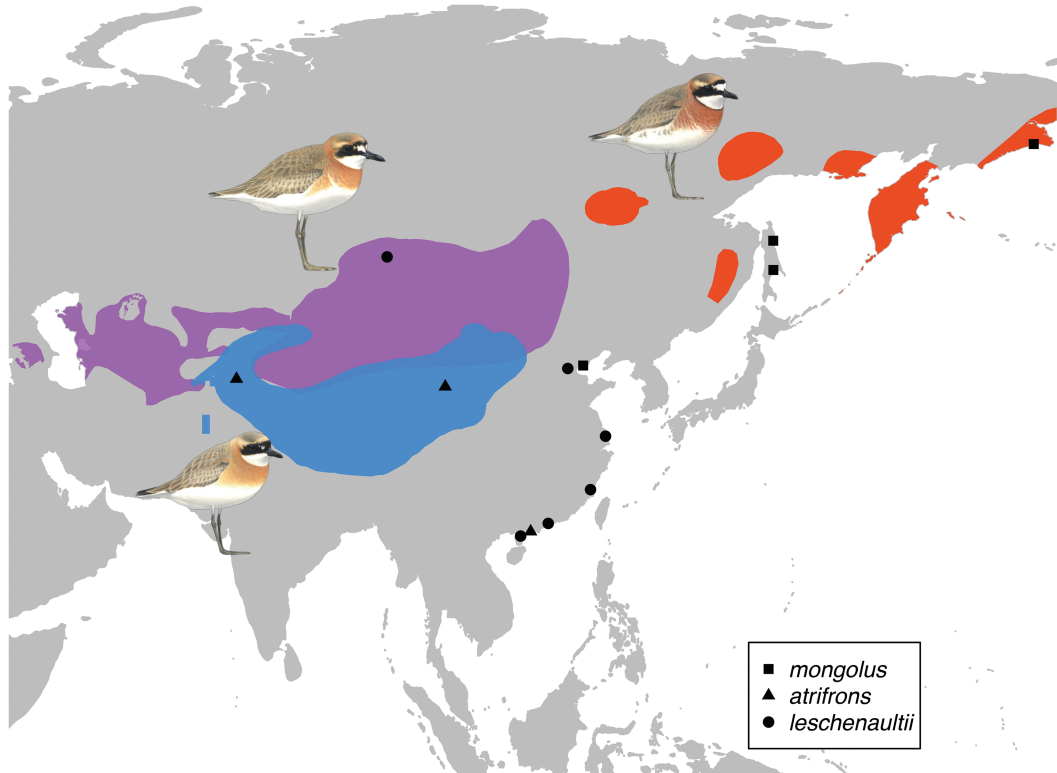
FIGURE 1. Breeding distribution and sampling sites of *Charadrius mongolus/leschenaultii* complex. The species distribution was derived from BirdLife International data zone (http://datazone.birdlife.org/species/requestdis). It is important to note that some of the sampling sites are from migratory routes. Plover drawings are courtesy of Ian Lewington.

groups of the Lesser Sand Plover in Mongolia and Russian Transbaikalia. Interestingly, based on cladistic analyses of 1,024 phenotypic characters, Livezey (2010) found the *mongolus* group to be more closely related to the Greater Sand Plover than to the *atrifrons* group.

We use genome-wide data to investigate the phylogenetic relationships and phylogeographic history of the *mongolus/leschenaultii* complex of sand plovers. Combined with additional acoustic data, we assess the taxonomic status of members of this complex, building upon previous assessments based on morphological data.

METHODS

Sample Collection, DNA Extraction, and Sequencing
From the *mongolus*' group, we sampled 14 individuals from the breeding range of *Ch. m. stegmanni* in Chukotka Autonomous Okrug, as well as 7 individuals from migratory routes in Sakhalin Island, and Northern China. From the *atrifrons* group, we included 19 individuals from the breeding range of *Ch. m. schaeferi* from Qinghai, China, and one *Ch. m. pamirensis* from the Kuhistani Badakhshan Autonomous Region, Tajikistan, as well as one migratory individual from the coastal area of Southern China (Figure 1, Supplementary Material Table S1). We also included

one migratory individual from the coastal area of Eastern China on the migration route of the *mongolus* group. We included 11 individuals of *Ch. leschenaultii*, 1 from Tyva Republic, Russia, collected during the breeding season, and others comprising migratory individuals from China (Figure 1, Supplementary Material Table S1). Genomic DNA was extracted using DNeasy Blood & Tissue kits (Qiagen, Hilden, Germany). We amplified 2 mitochondrial genes, cytochrome *b* (cyt *b*) and cytochrome *c* oxidase I (COI), for all individuals following the PCR protocols of Liu et al. 2020b (see Supplementary Material Table S2 for gene and primer details).

MtDNA Phylogenetic Analysis
Sequences were edited manually using CodonCode Aligner (CodonCode Corporation, Dedham, MA, USA), and then aligned with Clustal W under default settings (Thompson et al. 1994) in MEGA 7.0 (Kumar et al. 2016). We determined the best-fitting substitution model for the 2 genes with jModelTest 2.1.4 based on the Bayesian information criterion (Darriba et al. 2012) and implemented the models accordingly in phylogenetic analyses (see Supplementary Material Table S2 for details). We inferred a dated mitochondrial phylogeny (gene tree) using Bayesian Inference (BI) in BEAST 2.5.1 (Drummond and Bouckaert 2015) with

3 individuals of *Ch. alexandrinus* selected as an outgroup. A birth–death model was selected as the tree prior. A relaxed uncorrelated lognormal distribution was used as a clock model for both mtDNA markers; clock rates (ucld. mean parameters) were estimated implementing a prior from a published substitution rate (Weir and Schluter 2008) of 0.0135 substitutions/site/million year for cyt *b*, while a uniform prior was used for COI.

Markov chain Monte Carlo (MCMC) analysis was run 3 times independently for 50 million generations, with sampling every 5,000 generations. Tracer 1.7 (Rambaut et al. 2018) was used to compare posterior probabilities of all parameters to assess convergence among the 3 independent runs, and to confirm adequate effective sample sizes (i.e. ESS > 200) of the posterior distribution. The 3 independent runs were then combined each using LogCombiner 2.4.7 (Drummond and Bouckaert 2015) with 20% burn-in. Finally, the trees were summarized to produce a maximum clade credibility tree with TreeAnnotator 1.8.2 (Rambaut and Drummond 2007).

Whole-Genome Resequencing
Genome-wide data was generated for 11 individuals, comprising 5 of the *mongolus* and 4 of the *atrifrons* groups, and 2 of *Ch. leschenaultii*. For each of the 3 groups, 1 individual was selected and re-sequenced at 30X depth, and others were re-sequenced at 5X depth. For each individual, 1–3 μg of DNA was sheared into fragments of 200–800 base pairs (bp) with the Covaris system. DNA fragments were then end-repaired, A-tailed, ligated to paired-end adaptors, and PCR amplified with 500-bp inserts for library construction. Sequencing was performed on the Illumina HiSeq 2500 platform, and 100-bp paired-end reads were generated. We then carried out quality control and filtered out reads using FastQC (Andrews 2010), Duplication 150 (https://github.com/Holt59/cakephp3-bootstrap-helpers/pull/150), and Trimmomatic (Bolger et al. 2014) based on the following criteria: (1) any reads with adapter sequence, allowing ≤10% mismatches; (2) any reads with ≥50% bases having a Phred quality score <5; and (3) any reads with ≥10% unidentified nucleotides (N).

Reads Mapping and Single Nucleotide Polymorphisms (SNPs) Calling
After quality control, reads were mapped to the previously published Kentish Plover (*Ch. alexandrinus*) genome (Wang et al. 2019b) using BWA (Li 2013). Single nucleotide polymorphisms (SNPs) were first hard filtered using GATK 3.7 (DePristo et al. 2011) at settings: Strand Odds Ratio > 3.0, Qual by Depth < 2.0, Fisher Strand > 60.0, Mapping Quality < 40.0, Mapping Quality Rank Sum Test < −12.5, and Read Pos Rank Sum Test < −8.0. SNPs were then filtered using VCFtools (Danecek et al. 2011), GATK 3.7 (DePristo et al. 2011), and Plink (Purcell et al. 2007)

in accordance with the following criteria: (1) missing rate ≤0.05; (2) each 10 bp ≤3 SNPs; and (3) allele frequency >0.05. Because the calling was based on the Kentish Plover genome, we further filtered all homogeneous SNPs of sand plovers to remove SNPs between sand plovers and the Kentish Plover, leaving 765,092 SNPs for further analyses.

Genome-wide Population Structure and Phylogeny
To infer genetic structure within the sand plover complex, a principal component analysis (PCA) was conducted based on SNPs using PLink (Purcell et al. 2007). A maximum-likelihood tree search was done to reconstruct phylogenetic relationships using RAxML 8.2.12 (Stamatakis 2014) under the GTRGAMMA model, with 100 bootstrap pseudoreplicates. SNPs of a Kentish Plover individual called using the same reference genome as above (Wang et al. 2019b) were used as the outgroup in the RAxML analysis.

We applied the Patterson's *D*-statistic, also known as the ABBA-BABA test (Green et al. 2010, Durand et al. 2011), to investigate whether introgression happened within the complex. This method assesses introgression between pairwise comparisons of one target taxon (P3) and either of 2 other taxa (P1 or P2), where their phylogenetic relationships are (((P1, P2), P3), Outgroup). Given the phylogeny we recovered, we tested if the *atrifrons* group (P3) shared more derived alleles than expected by chance either with the *mongolus* (P1) or *Ch. leschenaultii* (P2) groups. We used bam files of the 3 individuals sequenced at 30X depth to run the test, and a Kentish Plover genome (Wang et al. 2019b) as an outgroup. Counts of "ABBA" and "BABA" sites were calculated with the program ANGSD (Korneliussen et al. 2014). *D*-statistics and the *Z* score were then calculated using the JackKnife.R script in ANGSD. We adopted the commonly used significance threshold of $|Z| > 3$ (Reich et al. 2011, Ottenburghs et al. 2017).

Demographic History
To reconstruct the demographic history of different taxa, a pairwise sequentially Markovian coalescent (PSMC) analysis was conducted to estimate change in effective population size (N_e) through time using the 3 individuals sequenced at 30X depth. SAMtools1.9 and the BCFtools1.9 consensus callers were used to call variants in individual samples, with the Kentish Plover genome used as reference (Wang et al. 2019b). A diploid consensus for each individual was then created with vcfutils.pl, using a minimum read depth of 10 and a maximum depth of 100 with the following parameter settings: −N25 −t15 −5 −b −p "4+30*2+4+6+10." Following Wang et al. (2019b), the generation time was set to 2.5 years, and a mutation rate of 0.08×10^{-8} per generation time was implemented. To check for variance in N_e, we performed 100 bootstrap replicates.

Bootstrapping was conducted by randomly sampling with replacement 5-Mb sequence segments obtained from the consensus genome sequence.

Acoustic Analysis

Field recordings of calls from several sand plover populations of 7–10 individuals of the *atrifrons* (A), *Ch. leschenaultii* (L), and *mongolus* (M) groups (Supplementary Material Table S3) were obtained by the authors (VA, recorded 14 individuals), by other bird sound recordists, or from the online sound library www.xeno-canto.org, and/or a published commercial LP recording (Veprintsev 1982–86). All digital audio recordings were transformed into mono wave files; the sampling frequency was set to 44.1 kHz with 16-bit using Adobe Audition 3.0. Sonograms were created in Syrinx 2.6h (Burt 2006). Our samples included only one call made by the same individual. Sand plover calls are series of 3–6 similar elements (units, separated by pauses); one note consists of an ascending and a descending part ("shoulders"), so that on the sonogram it has a shape of a caret sign; the ascending shoulder is usually longer than the descending one. For each call, we measured elements, speed, frequency, range, shape, rise, slope, and speedup (see Supplementary Material Table S4 for definitions). These 8 variables were used in 2 multidimensional statistical methods: PCA and Linear Discriminant Analysis (LDA), carried out in R 3.3.2 (R Core Team 2016).

RESULTS

Phylogenetic Relationships

The final alignment of the 2 mitochondrial markers consisted of 706 bp for cyt *b* and 651 bp for COI (see Supplementary Material Table S1 for GenBank accession numbers). The maximum clade-credibility gene tree of the BEAST analysis revealed 3 major robustly supported clusters, one comprising all individuals of the *atrifrons* group, one all of the *mongolus* group, and the third containing all individuals of *Ch. leschenaultii*. Surprisingly, *Ch. leschenaultii* was revealed to be a sister group to the *mongolus* group (Figure 2A). All relationships were robustly supported. The *mongolus* group and *Ch. leschenaultii* diverged at ~1.2 (95% highest posterior density (HPD): 0.8–1.7) million years ago (mya), while the split between the *atrifrons* group and the former 2 taxa occurring ~2.0 (95% HPD: 1.3–2.8) mya (Figure 2A). The close relationship between the *mongolus* group and *Ch. leschenaultii* in comparison to the *atrifrons* group was also revealed in the phylogeny inferred with 765,092 SNPs (Figure 2B).

Population Genomic Structure

The PCA analysis based on a 765K SNP set of 11 individuals showed a pattern consistent with the results from phylogenetic analyses (Figure 2C). PC1 explained 26.4% of the variance and clearly separated the 6 individuals belonging to the *atrifrons* group from those of the *mongolus* group (3 individuals) and *Ch. leschenaultii* (2 individuals). The latter 2 groups were separated in PC2 which explained 10.7% of the variance.

Demographic History and Introgression

The PSMC analysis showed contrasting demographic histories between the *atrifrons* group and the remaining 2 lineages (Figure 3). The effective population size (N_e) of the *atrifrons* group declined from ~2.5 mya onwards, while N_e of the *mongolus* group and *Ch. leschenaultii* increased from ~1 mya, mostly pronounced in the *mongolus* until ~0.1 mya. The N_e of *Ch. leschenaultii* reached a plateau between 0.7 and 0.2 mya and then decreased. In contrast, the N_e of *mongolus* group also plateaued during the similar period, but then experienced a quick expansion during ~0.2–0.1 mya before sharply declining.

The significantly positive *D* statistic resulting from the ABBA-BABA tests (Table 1, $D = 0.057$, $Z = 55.1$, $P < 0.001$) points towards introgression between *Ch. leschenaultii* and the *atrifrons* group during their evolutionary history.

Acoustic Divergence

The first PCA component explained 37% of variance and was correlated with variables describing pitch diapason of a call ("range," "rise," and "slope"; Supplementary Material Table S4), and the second PC explained 17% of variance and was correlated with time properties of a call ("speed" and "speed-up"; Supplementary Material Table S4). The 3 groups are well separated in PC space except for small overlap between *Ch. leschenaultii* and the *atrifrons* group (Figure 4). The LDA reliably classifies the 3 sand plover groups. The overall proportion of correct classification is 96%, with 100% in the *mongolus* group and *Ch. leschenaultii*, and 90% in the *atrifrons* group.

DISCUSSION

Genome-Wide Variation and Vocalization Indicate a 3-Species Hypothesis in the Sand Plover Complex

By analyzing genome-wide data, we demonstrate the 2 Lesser Sand Plover *Ch. mongolus* (*s.l.*) subspecies groups to not be each other's closest relatives, with individuals from the *mongolus* group forming the sister clade to Greater Sand Plover *Ch. leschenaultii*. Paraphyletic groupings have been revealed in other traditionally accepted species complexes in birds (Schweizer and Shirihai 2013, Alaei Kakhki et al. 2018, Schweizer et al. 2019), to which we add a new case.

The *mongolus* and *atrifrons* groups, traditionally included in Lesser Sand Plover *Ch. mongolus* (*s.l.*), are similar in morphometrics, while *Ch. leschenaultii* clearly differs in
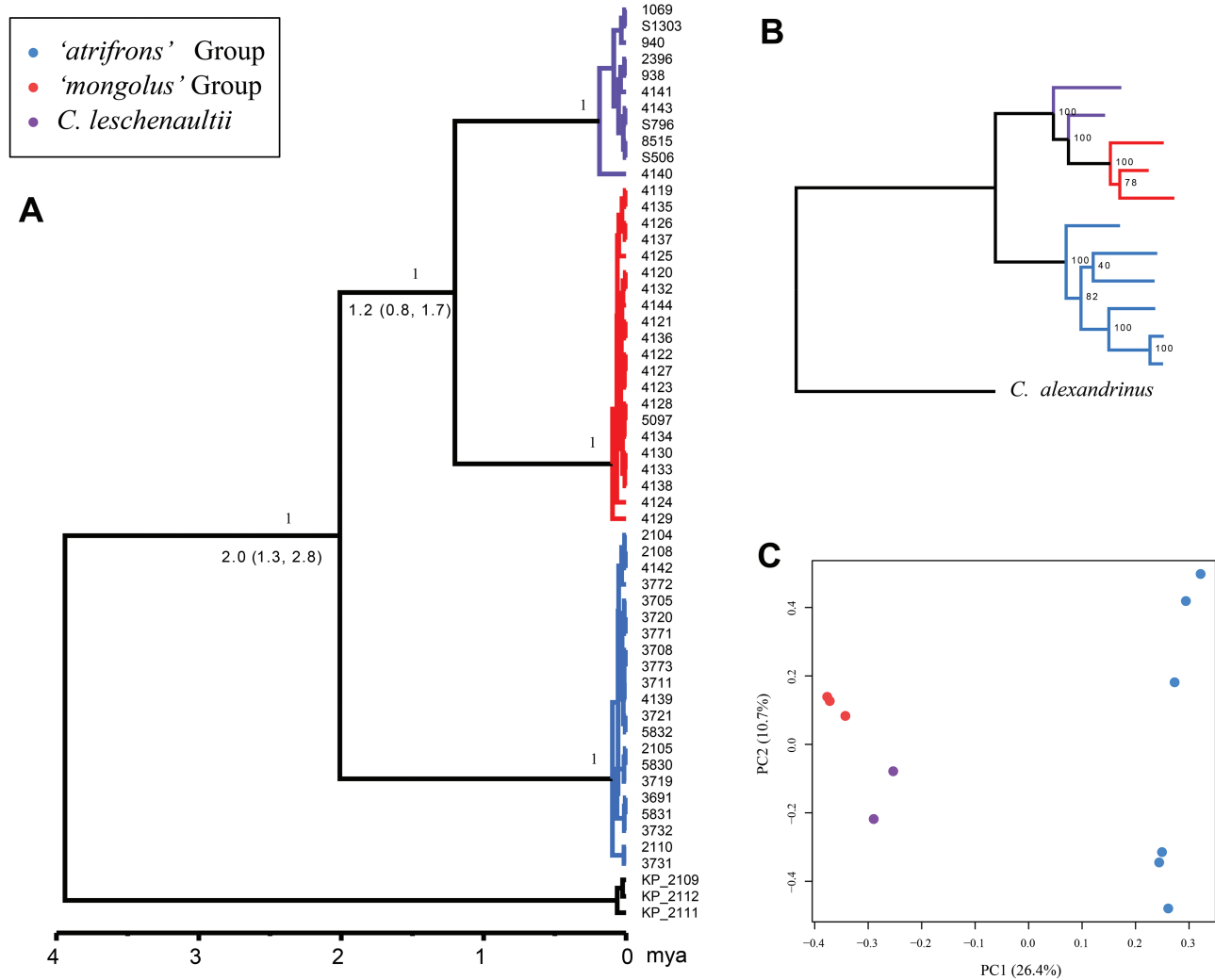
FIGURE 2. Phylogeny and population structure of the *Charadrius mongolus/leschenaultii* complex; samples from different taxa are labeled with different colors. (A) A phylogenetic tree reconstructed with 2 mitochondrial genes using BEAST; posterior probabilities are shown above nodes and divergence times are shown below nodes with a 95% HPD in parentheses (mya = million years ago); (B) a phylogenetic tree reconstructed with genome-wide SNPs data using RaxML, with bootstrap values on nodes; (C) principal component analysis of 765,092 SNPs.
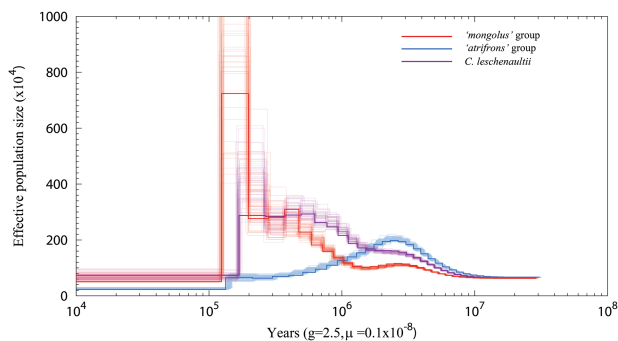
FIGURE 3. Pairwise sequentially Markovian coalescent estimates of changes in the effective population size over time for the *Charadrius mongolus/leschenaultii* complex, with bootstrap results indicated with thin lines.

being generally larger and bulkier (Hirschfeld et al. 2000). However, the close relationship between *Ch. leschenaultii* and the *mongolus* group was already indicted by a cladistic analysis of 1,024 phenotypic characters (Livezey 2010). In addition, differences in plumage and morphology between the *mongolus* and *atrifrons* groups have been described (Cramp 1983). We further demonstrate that these 2 taxa clearly differ in vocalizations and have undergone different demographic histories.

Although the breeding range of *Ch. leschenaultii* contacts that of the *atrifrons* group, interbreeding is not documented and the 2 differ in their preferred habitat. While *Ch. leschenaultii* is more of a lowland bird of deserts, semi-deserts, or steppes, both the *atrifrons* and *mongolus* groups breed above or beyond the treeline (Cramp 1983).

TABLE 1. ABBA-BABA test for the *Charadrius mongolus/leschenaultii* complex. Taxa are arranged such that the dominant pattern is ABBA

P1	P2	P3	*D* statistic	*Z* score	*P* value
mongolus group	*Ch. leschenaultii*	*atrifrons* group	0.057	55.1	<0.001
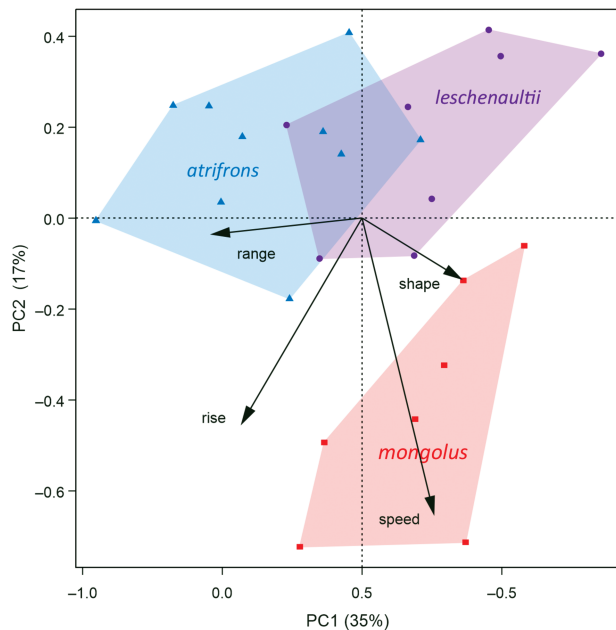
FIGURE 4. Principal component analysis of calls in the *Charadrius mongolus/leschenaultii* complex. Arrows indicate the loadings of original acoustic variables. Speed, the number of elements per second; range, the mean base frequency diapason of an element; shape, the mean relative length of the ascending shoulder; rise, the tangent of the angle of an ascending shoulder.

In winter, the distributional range of *Ch. leschenaultii* overlaps with both Lesser Sand plover groups, but these 2 groups have disjunct winter ranges—the *mongolus* group mainly winters in the region of the Chinese Sea, the Philippines, eastern Indonesia, Melanesian Islands, and Australia, while the winter range of the *atrifrons* group extends from the Gulf of Thailand, Malaysia and the Greater Sundas west to the Persian Gulf and coastal eastern Africa (Cramp 1983).

A 3-way split best explains the evolutionary diversity of the 3 independent lineages of the sand plover complex regardless of the species concept applied. Consequently, the Lesser Sand Plover should be split into 2 species: *Ch. mongolus* Pallas, 1776, with subspecies *Ch. m mongolus* and *Ch. m. stegmanni*, Portenko, 1939; and *Ch. atrifrons* Wagler, 1829, with subspecies *Ch. a. pamirensis*, (Richmond, 1896) nominate *Ch. a. atrifrons*, and *Ch. a. schaeferi*. We also propose a new set of English names which may better reflect the geographical distribution of the taxa in this revised complex. We suggest the new names "Siberian Sand Plover" for *Ch. mongolus* (*s.s.*), and "Tibetan Sand Plover"

for *Ch. atrifrons*. Because the name Lesser Sand Plover is no longer in use, we also propose to rename the Greater Sand Plover to Desert Sand Plover to better reflect breeding distributions and habitats, respectively.

A shortcoming of this study is that only the nominate subspecies of the Desert Sand Plover from the eastern part of the species' range was included, whereas Transcaspian *Ch. l. scythicus* and Middle Eastern *Ch. l. columbinus* could not be sampled. The latter is characterized by being smaller and having a weaker bill, both attributes similar to *Ch. mongolus* and *Ch. atrifrons*. However, its morphology is otherwise more similar to the 2 subspecies of *Ch. leschenaultii* than to the latter 2 species (see Hirschfeld et al. 2000). Nonetheless, further sampling is needed to characterize the phylogeographic structure of Desert Sand Plover.

Evolutionary Diversification and Speciation in the Sand Plover Complex

Diversification in the sand plover complex started in the early Pleistocene with the split from the Tibetan Sand Plover from the common ancestor of the Siberian and Desert Sand Plovers, which split in the mid Pleistocene. Their diversification falls in a period of increased aridity in Central Asia (Favre et al. 2015), followed by an intensification of climate fluctuations that increased in duration and amplitude after the mid-Pleistocene transition 1.2–0.7 mya (Sun et al. 2019). The reconstructed demographic histories indicate that Tibetan Sand Plover might have been affected by climate fluctuations in a different way to the 2 other species. Although the eastern part of the Palearctic was less glaciated compared to Europe or North America during late-Pleistocene cold periods (Svendsen et al. 2004), glaciers did repeatedly extend onto the Tibetan plateau and adjacent mountains such as the Tian Shan (Xu et al. 2010, Heyman 2014). This might have led to range retraction and a general decrease in effective population size of the Tibetan Sand Plover in these mountain areas today following the mid-Pleistocene climate transition. In contrast, an increase in Tundra-like habitats and open vegetation in Siberia and Central Asia during dry late-Pleistocene glacial periods might have promoted range expansions and an increase in effective population sizes of the other 2 species. Such range expansions of the ancestor of the Desert Sand Plover might have led to contact with the ancestor of the Tibetan Sand Plover, resulting in gene flow as indicated by the ABBA-BABA analyses. Speciation with intermittent

gene flow seems to be more common in birds than previously thought (Rheindt et al. 2011, Ottenburghs et al. 2017, Singhal et al. 2021), and might be triggered in phases of secondary contact as a consequence of climate-driven range expansions (Schweizer et al. 2018, Wang et al. 2019a).

Siberian and Tibetan Sand plovers, although not each other closest relatives, are similar in size and habitat preferences, which differ in the Desert Sand Plover (Cramp 1983). While this similarity might be the result of parallel evolution, a more parsimonious explanation would assume niche conservatism coupled with comparatively rapid morphological and ecological differentiation in the Desert Sand Plover. The divergence in acoustic signals and plumage of the Siberian and Tibetan Sand plovers demonstrates that the tempo of evolution may differ in different dimensions of phenotypic traits (Winger and Bates 2015). Niches and associated traits generally evolve slowly and are usually retained over evolutionary timescales in most animal species (Peterson et al. 1999, Wiens et al. 2010, Liu et al. 2020a). However, rapid changes might be associated with colonization of new habitats (Losos and Ricklefs 2009, Yoder et al. 2010, Cooney et al. 2017) which may have been the case in the Desert Sand Plover.

Conclusions

We show that a current, widely accepted taxonomic treatment of the sand plover complex includes a paraphyletic grouping. We propose to split the traditionally recognized Lesser Sand Plover into 2 species, namely the Siberian Sand Plover in the east and the Tibetan Sand Plover in the west. We thus highlight that species-level taxonomy based on few morphological features is potentially error-prone and call for a thorough analyses of species complexes with allopatric distributions, preferably including genetic data from many independent loci. Signs for ancestral gene flow between the nonsister Tibetan and Desert Sand plover species are consistent with growing evidence for intermittent gene flow during the speciation process being more prevalent than previously thought.

The phylogeographic history of bird lineages is poorly studied in Central and East Asia compared to Europe and North America. As is evident from the sand plover complex, however, regional drivers shaped by variable effects of past climate and associated environmental change in combination with different ecological specialization might lead to different phylogeographic histories even among closely related species, making generalizations difficult (Hung et al. 2017, Liu et al. 2020a).

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Ornithology* online.

LITERATURE CITED

Alaei Kakhki, N., M. Aliabadian, M. I. Förschler, S. M. Ghasempouri, B. H. Kiabi, L. D. Verde Arregoitia, and M. Schweizer (2018). Phylogeography of the *Oenanthe hispanica–pleschanka–cypriaca* complex (Aves, Muscicapidae: Saxicolinae): Diversification history of open-habitat specialists based on climate niche models, genetic data, and morphometric data. Journal of Zoological Systematics and Evolutionary Research 56:408–427.

Andrews, S. (2010). FastQC: A quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/fastqc

Bolger, A. M., M. Lohse, and B. Usadel (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120.

Braune, P., S. Schmidt, and E. Zimmermann (2008). Acoustic divergence in the communication of cryptic species of nocturnal primates (*Microcebus* ssp.). BMC Biology 6:1–10.

Burt, J. (2006). Syrinx, version 2.6 h. University of Washington, Seattle, WA, USA.

Conklin, J. R., J. Reneerkens, Y. I. Verkuil, P. S. Tomkovich, P. J. Palsbøll, and T. Piersma (2016). Low genetic differentiation between Greenlandic and Siberian Sanderling populations implies a different phylogeographic history than found in Red Knots. Journal of Ornithology 157:325–332.

Cooney, C. R., J. A. Bright, E. J. R. Capp, A. M. Chira, E. C. Hughes, C. J. A. Moody, L. O. Nouri, Z. K. Varley, and G. H. Thomas (2017). Mega-evolutionary dynamics of the adaptive radiation of birds. Nature 542:344–347.

Danecek, P., A. Auton, G. Abecasis, C. A. Albers, E. Banks, M. A. DePristo, R. E. Handsaker, G. Lunter, G. T. Marth, S. T. Sherry, G. McVean, R. Durbin, and 1000 Genomes Project Analysis Group (2011). The variant call format and VCFtools. Bioinformatics 27:2156–2158.

Darriba, D., G. L. Taboada, R. Doallo, and D. Posada (2012). jModelTest 2: More models, new heuristics and parallel computing. Nature Methods 9:772.

DePristo, M. A., E. Banks, R. Poplin, K. V Garimella, J. R. Maguire, C. Hartl, A. A. Philippakis, G. del Angel, M. A. Rivas, M. Hanna, A. McKenna, T. J. Fennell, A. M. Kernytsky, A. Y. Sivachenko, K. Cibulskis, S. B. Gabriel, D. Altshuler, and M. J. Daly (2011). A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nature Genetics 43:491–498.

Drummond, A. J., and R. R. Bouckaert (2015). Bayesian Evolutionary Analysis with BEAST. Cambridge University Press, Cambridge, UK.

Durand, E. Y., N. Patterson, D. Reich, and M. Slatkin (2011). Testing for ancient admixture between closely related populations. Molecular Ecology and Biology 28:2239–2252.

Favre, A., M. Päckert, S. U. Pauls, S. C. Jähnig, D. Uhl, I. Michalak, and A. N. Muellner-Riehl (2015). The role of the uplift of the Qinghai-Tibetan Plateau for the evolution of Tibetan biotas. Biological Reviews 90:236–253.

Feckler, A., J. P. Zubrod, A. Thielsch, K. Schwenk, R. Schulz, and M. Bundschuh (2014). Cryptic species diversity: An overlooked factor in environmental management? Journal of Applied Ecology 51:958–967.

Gill, F., D. Donsker, and P. Rasmussen (Editors) (2021). IOC World Bird List (v11.2). doi:10.14344/IOC.ML.11.2

Green, R. E., J. Krause, A. W. Briggs, T. Maricic, U. Stenzel, M. Kircher, N. Patterson, H. Li, W. Zhai, M. H. Y. Fritz, and N. F. Hansen (2010). A draft sequence of the Neandertal genome. Science 328:710–722.

Heyman, J. (2014). Paleoglaciation of the Tibetan plateau and surrounding mountains based on exposure ages and ELA depression estimates. Quaternary Science Reviews 91:30–41.

Hirschfeld, E., C. S. Roselaar, and H. Shirihai (2000). Identification, taxonomy and distribution of Greater and Lesser Sand Plovers. British Birds 93:162–189.

del Hoyo, J., N. J. Collar, D. A. Christie, A. Elliott, and L. D. C. Fishpool (2014). HBW and BirdLife International Illustrated Checklist of the Birds of the World: Non-passerines. Lynx Edicions, Barcelona, Spain.

Hung, C., S. V Drovetski, and R. M. Zink (2017). The roles of ecology, behaviour and effective population size in the evolution of a community. Molecular Ecology 26:3775–3784.

Kempenaers, B., and M. Valcu (2017). Breeding site sampling across the Arctic by individual males of a polygynous shorebird. Nature 541:528–531.

Korneliussen, T. S., A. Albrechtsen, and R. Nielsen (2014). ANGSD: Analysis of next generation sequencing data. BMC Bioinformatics 15:356.

Kumar, S., G. Stecher, and K. Tamura (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33:1870–1874.

Küpper, C., S. V. Edwards, A. Kosztolányi, M. Alrashidi, T. Burke, P. Herrmann, A. Argüelles-Tico, J. A. Amat, M. Amezian, A. Rocha, H. Hötker, A. Ivanov, J. Chernicko, and T. Székely (2012). High gene flow on a continental scale in the polyandrous Kentish plover Charadrius alexandrinus. Molecular Ecology 21:5864–5879.

Küpper, C., and N. dos Remedios (2019). Defining species and populations: Molecular genetic studies in plovers. In The Population Ecology and Conservation of Charadrius Plovers (M. A. Colwell and S. M. Haig, Editors). CRC Press, Boca Raton, FL, USA. pp. 16–43

Lappo, E., P. Tomkovich, E. Syroechkovskiy, and Y. V. Zharikov (2012). Atlas of Breeding Waders in the Russian Arctic. Institute of Geography, Russian Academy of Sciences, Moscow, Russia.

Li, H. (2013). Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv preprint arXiv:1303.3997.

Liu, S., Y. Liu, E. Jelen, M. Alibadian, C. Yao, X. Li, N. Kayvanfar, Y. Wang, F. S. M. Vahidi, and J. Han (2020a). Regional drivers of diversification in the late Quaternary in a widely distributed generalist species, the common pheasant Phasianus colchicus. Journal of Biogeography 47:2714–2727.

Liu, S., C. Wei, P. J. Leader, G. J. Carey, Y. Jia, Y. Fu, P. Alström, and Y. Liu (2020b). Taxonomic revision of the Long-tailed Rosefinch Carpodacus sibiricus complex. Journal of Ornithology 161:1061–1070.

Livezey, B. C. (2010). Phylogenetics of modern shorebirds (Charadriiformes) based on phenotypic evidence: Analysis and discussion. Zoological Journal of the Linnean Society 160:567–618.

Losos, J. B., and R. E. Ricklefs (2009). Adaptation and diversification on islands. Nature 457:830–836.

Ottenburghs, J., H. J. Megens, R. H. S. Kraus, P. Van Hooft, S. E. Van Wieren, R. P. M. A. Crooijmans, R. C. Ydenberg, M. A. M. Groenen, and H. H. T. Prins (2017). A history of hybrids? Genomic patterns of introgression in the True Geese. BMC Evolutionary Biology 17:1–14.

Peterson, A. T., J. Soberón, and V. Sánchez-Cordero (1999). Conservatism of ecological niches in evolutionary time. Science 285:1265–1267.

Purcell, S., B. Neale, K. Todd-Brown, L. Thomas, M. A. R. Ferreira, D. Bender, J. Maller, P. Sklar, P. I. W. De Bakker, and M. J. Daly (2007). PLINK: A tool set for whole-genome association and population-based linkage analyses. The American Journal of Human Genetics 81:559–575.

R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.r-project.org/

Rambaut, A., and A. J. Drummond (2007). TreeAnnotator. Program and documentation distributed by the author. http://beast.bio.ed.ac.uk/TreeAnnotator

Rambaut, A., A. J. Drummond, D. Xie, G. Baele, and M. A. Suchard (2018). Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Systematic Biology 67:901.

Reich, D., N. Patterson, M. Kircher, F. Delfin, M. R. Nandineni, I. Pugach, A. M. S. Ko, Y. C. Ko, T. A. Jinam, M. E. Phipps, N. Saitou, A. Wollstein, M. Kayser, S. Pääbo, and M. Stoneking (2011). Denisova admixture and the first modern human dispersals into Southeast Asia and Oceania. American Journal of Human Genetics 89:516–528.

Dos Remedios, N., P. L. M. Lee, T. Burke, T. Székely, and C. Küpper (2015). North or south? Phylogenetic and biogeographic origins of a globally distributed avian clade. Molecular Phylogenetics and Evolution 89:151–159.

Rheindt, F. E., T. Székely, S. V Edwards, P. L. M. Lee, T. Burke, P. R. Kennerley, D. N. Bakewell, M. Alrashidi, A. Kosztolányi, and M. A. Weston (2011). Conflict between genetic and phenotypic differentiation: the evolutionary history of a 'lost and rediscovered'shorebird. PLoS One 6:e26995.

Sadanandan, K. R., C. Küpper, G. W. Low, C. T. Yao, Y. Li, T. Xu, F. E. Rheindt, and S. Wu (2019). Population divergence and gene flow in two East Asian shorebirds on the verge of speciation. Scientific Reports 9:1–9.

Sangster, G. (2014). The application of species criteria in avian taxonomy and its implications for the debate over species concepts. Biological Reviews 89:199–214.

Sangster, G. (2018). Integrative taxonomy of birds: The nature and delimitation of species. In Bird Species (D. Tietze, Editor). Springer, Cham, Switzerland. pp. 9–37.

Schweizer, M., and H. Shirihai (2013). Phylogeny of the Oenanthe lugens complex (Aves, Muscicapidae: Saxicolinae): Paraphyly of a morphologically cohesive group within a recent radiation of open-habitat chats. Molecular Phylogenetics and Evolution 69:450–461.

Schweizer, M., H. Shirihai, H. Schmaljohann, and G. M. Kirwan (2018). Phylogeography of the House Bunting complex: Discordance between species limits and genetic markers. Journal of Ornithology 159:47–61.

Schweizer, M., V. Warmuth, N. Alaei Kakhki, M. Aliabadian, M. Förschler, H. Shirihai, A. Suh, and R. Burri (2019). Parallel plumage colour evolution and introgressive hybridization in Wheatears. Journal of Evolutionary Biology 32:100–110.

Singhal, S., G. E. Derryberry, G. A. Bravo, E. P. Derryberry, R. T. Brumfield, and M. G. Harvey (2021). The dynamics of introgression across an avian radiation. Evolution Letters. https://doi.org/10.1002/evl3.256

Stamatakis, A. (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312–1313.

Stern, D. L. (2013). The genetic causes of convergent evolution. Nature Reviews Genetics 14:751–764.

Sun, Y., Q. Yin, M. Crucifix, S. C. Clemens, P. Araya-Melo, W. Liu, X. Qiang, Q. Liu, H. Zhao, and L. Liang (2019). Diverse manifestations of the mid-Pleistocene climate transition. Nature Communications 10:1–11.

Svendsen, J. I., H. Alexanderson, V. I. Astakhov, I. Demidov, J. A. Dowdeswell, S. Funder, V. Gataullin, M. Henriksen, C. Hjort, and M. Houmark-Nielsen (2004). Late Quaternary ice sheet history of northern Eurasia. Quaternary Science Reviews 23:1229–1271.

Taylor, R. S., and V. L. Friesen (2017). The role of allochrony in speciation. Molecular Ecology 26:3330–3342.

Thompson, J. D., D. G. Higgins, and T. J. Gibson (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22:4673–4680.

Wang, X., P. Que, G. Heckel, J. Hu, X. Zhang, C. Y. Chiang, N. Zhang, Q. Huang, S. Liu, J. Martinez, and E. Pagani-Núñez (2019a). Genetic, phenotypic and ecological differentiation suggests incipient speciation in two Charadrius plovers along the Chinese coast. BMC Evolutionary Biology 19:1–18.

Wang, X., K. H. Maher, N. Zhang, P. Que, C. Zheng, S. Liu, B. Wang, Q. Huang, D. Chen, X. Yang, Z. Zhang, T. Székely, A. O. Urrutia, and Y. Liu (2019b). Demographic histories and genome-wide patterns of divergence in incipient species of shorebirds. Frontiers in Genetics 10:1–14.

Weir, J. T., and D. Schluter (2008). Calibrating the avian molecular clock. Molecular Ecology 17:2321–2328.

Wiens, J. J., D. D. Ackerly, A. P. Allen, B. L. Anacker, L. B. Buckley, H. V Cornell, E. I. Damschen, T. Jonathan Davies, J. Grytnes, and S. P. Harrison (2010). Niche conservatism as an emerging principle in ecology and conservation biology. Ecology Letters 13:1310–1324.

Winger, B. M., and J. M. Bates (2015). The tempo of trait divergence in geographic isolation: Avian speciation across the Mara non Valley of Peru. Evolution 69:772–787.

Xu, X., L. Wang, and J. Yang (2010). Last Glacial Maximum climate inferences from integrated reconstruction of glacier equilibrium-line altitude for the head of the Urumqi River, Tianshan Mountains. Quaternary International 218:3–12.

Yoder, J. B., E. Clancey, S. Des Roches, J. M. Eastman, L. Gentry, W. Godsoe, T. J. Hagey, D. Jochimsen, B. P. Oswald, and J. Robertson (2010). Ecological opportunity and the origin of adaptive radiations. Journal of Evolutionary Biology 23:1581–1596.