1 Supplementary Information for

2	Genomic Consequences of Long-term Population Decline in Brown Eared Pheasant
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31 **DNA sample collection**

Tissue for *de novo* whole genome sequencing was obtained from a female Brown eared 32 pheasant (Crossoptilon mantchuricum) from the central population in the Pangquangou 33 National Natural Reserve, Jiaocheng, Shanxi Province, China. For genome re-34 sequencing, tissue samples were obtained from 11, 18, and 11 individuals from Shanxi 35 36 (Central population, Brown-C), Shaanxi (Western population, Brown-W), Hebei, and Beijing (Eastern population, Brown-E), respectively. The blue eared pheasant (C. 37 auritum) was used as the control group in this study, of which 11 individuals were 38 sampled for re-sequencing (the geographic distribution of the studied populations can 39 be seen in Fig. 1a and S12. Sampling information can be seen in Dataset S9). All 40 samples were stored at -80 °C at Beijing Normal University. For genome annotation, 41 we also sequenced the transcriptome of the C. mantchuricum individual used for the de 42 novo genome assembly using blood samples, developing tail feathers, and primaries. 43 44 The tissues used to isolate the RNA were mixed with an RNA preservation solution (RNA-In-Safe; Sangon Biotech, Beijing, China). All tissues used for transcriptome 45 sequencing were stored in liquid nitrogen. Sample collection was performed by an 46 accredited veterinarian, and all sampling procedures were approved by the Forestry 47 Department of China and the Ethics and Animal Welfare Committee at the College of 48 Life Science, Beijing Normal University, China (Approval Number CLS-EAW-2015-49 50 012).

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52 DNA, RNA isolation, sequencing, and data filtering

53 DNA and RNA isolation and library construction were performed at the Novogene 54 Sequence Center (Beijing, China). DNA and RNA quality and quantity were evaluated 55 using a Nanodrop spectrophotometer, a Qubit Fluorometer, an Agilent 2100, and gel 56 electrophoresis. Fragment libraries (insert length 250 bp) and jumping libraries (insert 57 lengths 2000 bp and 5000 bp) were constructed for *de novo* genome sequencing. For *de*

novo genome sequencing, the targeted total amount of sequences from the fragment 58 libraries and jumping libraries was ~ 150 Gb ($\sim 150\times$) and ~ 100 Gb ($\sim 100\times$), 59 respectively. A total of 51 fragment libraries (insert length 350 bp) were constructed for 60 genome re-sequencing, targeting more than 15× coverage per individual. The targeted 61 total amount of transcriptome sequences was ~190 Gb from the RNA libraries (insert 62 length 250 bp). All libraries were sequenced on the Illumina HiSeq platform at the 63 paired-end 150 bp. Adapter content was trimmed and five base pairs were removed 64 65 from both the 5' and 3' ends of each read in Trim Galore v0.4.2, with other default parameters (http://www.bioinformatics.babraham.ac.uk/projects/trim galore/). FastQC 66 v0.11.5 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) was used to 67 assess the quality of the raw sequencing data and clean data. 68

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70 Genome assembly and evaluation

After removing the low-quality data, 220.6 Gb of DNA data (1.37 billion reads, 195.6-71 fold sequence coverage) were used for the *de novo* genome assembly of C. 72 73 mantchuricum in ALLPATHS-LG v52488 (Butler, et al. 2008). Basic metrics (e.g., scaffold and contig N50) were calculated from the genome assembly with a minimum 74 contig length of 100 bp. We used BUSCO v3 (Simão, et al. 2015) to evaluate the 75 completeness of the draft genome by searching for 4915 Benchmarking Universal 76 Single-Copy Orthologs from 40 avian lineages (aves odb9 dataset) in the C. 77 mantchuricum assembly. 78

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80 Genome annotation

81 The *de novo* repetitive elements annotation was performed in RepeatModeler-open v4.0.6 (http://www.repeatmasker.org/) with repeat masker libraries v20160829 (Bao, 82 al. 2015) finding programs (RECON v1.08 83 et and repeat (http://selab.janelia.org/recon.html) RepeatScout v1.0.5 84 and (http://repeatscout.bioprojects.org/)). Repetitive elements were identified with 85 RepeatMasker-open v4.0.6, in which repetitive elements were searched against de novo 86

repetitive elements and homologous sequences (Dfam 2.0 database (Hubley, et al. 2015)
and RepBase database v20160829 (Bao, et al. 2015)). Tandem repeats were identified
across the genome using Tandem Repeats Finder v404 (Benson 1999). The repetitive
elements were masked in the whole genome for annotation purposes.

91

We employed an integrative approach to identify a protein-coding gene set of the 92 repeated-masked in MAKER v2.31.8 (http://www.yandell-93 genome 94 lab.org/software/maker.html), which used homologous genes, ab initio predictions, expressed sequence tags (ESTs), and RNA sequence evidence. The homologous gene 95 sets were from human, zebra finch, chicken, and turkey protein sequences downloaded 96 from Ensembl genome browser 87 (http://useast.ensembl.org/info/data/ftp/index.html). 97 The *ab initio* prediction was performed in both SNAP (Korf 2004) and AUGUSTUS 98 v3.0.3 (Stanke and Waack 2003). The 80.0-Gb clean RNA sequences were used to 99 produce splice junction information of the repeat-masked draft genome in TopHat 100 v2.0.13 (http://ccb.jhu.edu/software/tophat/index.shtml) and to de novo assemble 101 102 mRNA-sequences in Trinity v2.3.2 (Grabherr, et al. 2011). To improve the accuracy of the annotation procedure, we ran MAKER twice. For the initial MAKER run, both the 103 AUGUSTUS chicken models and SNAP chicken models that we trained were used to 104 predict the coding genes. The assembled chromosomes and annotation file of 105 Gallus gallus-5.0 (Consortium 2004) were used to train the SNAP chicken models. 106 After the initial MAKER run, we used the gene models produced during the initial 107 MAKER run to retrain AUGUSTUS and SNAP. For the final MAKER run, we used the 108 retrained gene predictors to obtain a consensus set of protein-coding genes. In both 109 110 MAKER runs, the minimum length required for single exon ESTs was set to 250 bp. The gene set preserved genes that encoded 20 or more amino acids (Eilbeck, et al. 2009). 111 To identify protein function, Interproscan-5.27-66.0 (Zdobnov and Apweiler 2001) was 112 employed to analyze proteins from MAKER with known functional domains and GO 113 terms from the Pfam database. Then, the genes were filtered to remove those without 114 domain content support or those with a quality metric (Annotation Edit Distance, AED) 115

116 greater than or equal to 1 (Eilbeck, et al. 2009).

117

To assess annotation quality, two summary statistics were considered: the percentage of the genes with an AED value less than 0.5, and the percentage of the genes with recognizable domain content. The gene set from annotation was also assessed using BUSCO v3 (Simão, et al. 2015), which was performed on the protein sets from MAKER with 4915 Benchmarking Universal Single-Copy Orthologs from 40 avian lineages (aves_odb9 dataset).

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125 Read mapping

Cleaned reads in all the fragment libraries for each individual were mapped to the C. 126 mantchuricum reference genome produced in this project and a complete mitochondrial 127 genome that was downloaded from the GenBank database (accession number: 128 KP259807) with a Burrows-Wheeler alignment-maximal exact matches (BWA-MEM, 129 v0.7.9a) algorithm (Li and Durbin 2009). Samtools v1.3.1 was used to calculate the per-130 131 base sequence depth, and was then used to calculate the mean coverage for each scaffold (Li, et al. 2009). Sorting and marking of duplicate sequences and generation of 132 alignment metrics performed with Picard Tools v2.8.0 133 were (https://broadinstitute.github.io/picard/). 134

135

Local realignment around indels and recalibration of base quality scores was performed 136 using the Genome Analysis Toolkit v3.7 (GATK) pipeline according to the GATK best 137 practice recommendations (McKenna, et al. 2010). To obtain a database of known 138 139 polymorphic sites for realignment and recalibration, the raw variants were called in three programs: FreeBayes v0.9.20 (Garrison and Marth 2012), which required at least 140 five supporting observations to consider a variant, the GATK UnifiedGenotyper tool 141 that set the minimum phred-scaled quality score threshold to 50, and samtools mpileup 142 v1.3.1 with default parameters (Li, et al. 2009). The genotypes identified by all three 143 approaches were extracted with bcftools v1.2 (Li, et al. 2009) and used as a database of 144

145 known genotypes for realignment and recalibration. To obtain convergence between the
146 average reported quality scores and empirical quality scores, this process was repeated
147 four times.

148

149 Identification of sex chromosomes and mitochondrial genome-linked scaffolds

We employed two criteria to identify scaffolds belonging to either the sex chromosomes 150 or the mitochondrial genome. For one, we used the proportion of the chicken (Gallus 151 152 gallus) sex chromosome and mitochondrial orthologous genes on each scaffold. We employed the tblastn and blastp functions with an e-value of 1 e⁻⁵ in BLAST+ v2.2.28 153 (Camacho, et al. 2009) to search for the one-to-one orthologous genes of G. gallus and 154 C. mantchuricum. The sex chromosomes and mitochondrial protein sequences of G. 155 gallus were downloaded from Ensembl Release 87 (ftp://ftp.ensembl.org/pub/release-156 $\underline{87/}$). We used in-house scripts to calculate the proportion of orthologous genes on each 157 scaffold. For the second criterion, we used the ratio of scaffold coverage between males 158 and females. We calculated the per-scaffold ratio of sequence coverage between males 159 160 and females, which is expected to be equal to 1 on autosomes and 2 on Z-linked scaffolds. Considering the coverage bias, we assumed that scaffolds with a ratio 161 between 1.6 and 2.4 belong to the Z chromosome. Based on this coverage ratio criteria, 162 we assumed that the scaffolds that have equal to or more than 25% orthologous sex and 163 mitochondrial genes are sex chromosomes and mitochondrial genome-linked scaffolds. 164 Because we mapped the re-sequencing reads to a complete sequence of Crossoptilon 165 crossoptilon mitochondrion DNA, we excluded all mitochondrial genome-linked 166 scaffolds and SNPs in the downstream analyses. 167

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For visualization and downstream analysis purposes, all autosome-linked scaffolds were ordered based on their alignment to autosomes in the *G. gallus* genome (<u>ftp://ftp.ensembl.org/pub/release-87/fasta/gallus_gallus/dna/</u>). All autosome-linked scaffolds were aligned to each *G. gallus* autosome using the PROmer function in the MUMmer V 3.23 package (Kurtz, et al. 2004). After alignment, we merged all Delta files together and filtered the results with the following criteria: retain the longest consistent alignment, minimum alignment identity is 50, and minimum alignment length is 500. After filtering, we determined the location of each scaffold based on the position of the longest alignment.

178

179 SNP calling

After realignment and recalibration, sites with a phred-scaled confidence ≥ 30 were 180 used to perform joint genotyping with the GATK HaplotypeCaller and 181 GenotypeGVCFs tool. Raw variant sets on autosomes, the Z-chromosome, and the 182 mitochondrial genome were generated separately based on our designation scaffolds. 183 The quality metrics of each raw variant were extracted with vcftools v0.1.14 (Danecek, 184 et al. 2011) and plotted on the density curve of each parameter in RStudio. The 185 distribution of each parameter was used to set the filtering criteria based on the 186 suggestion of the GATK best practices. Based on the distribution of each parameter, 187 quality filtering of the raw variants on autosomes was performed with the following 188 189 criteria to maintain high-quality SNPs: minor allele frequency > 0.05, strand odds ratio < 3, quality by depth > 2, Fisher Strand < 60, RMS mapping quality > 50, mapping 190 quality rank sum > -4, read position rank sum > -2, minimum depth (summing all 51 191 samples) > 870, and maximum depth (summing all 51 samples) < 1304; explanation of 192 these parameters can be found in the GATK user manual (McKenna, et al. 2010). These 193 high-quality variants were used in the analyses performed in this study. Variants on the 194 Z chromosome were filtered using the same quality thresholds as above, except that the 195 minimum and maximum depths were 545 and 1207. Considering the huge amount of 196 197 mitochondrial DNA in cells, we set the minimum depth to 966 for filtering the raw variants. The percentage of missing SNPs in each individual was calculated using 198 vcftools v0.1.14 (Danecek, et al. 2011). 199

200

201 Inference of kinship

202 In population genetic analyses, it is often necessary to exclude closely related

individuals. The three populations of C. mantchuricum are all small isolated wild 203 populations where individuals may have familial relationships. We estimated the 204 relatedness of all samples in each population using KING v 2.1.3 (Manichaikul, et al. 205 2010) to estimate the kinship coefficient using our library of autosomal SNPs with no 206 missing sites. Pairs with a kinship coefficient > 0.177 (twins and first-degree 207 relationship) were not included in subsequent analyses. There were 14 samples with 208 close (duplicate/twins, first degree) relationships to other individuals in the data. These 209 210 samples were removed, leading to a total of 37 samples that were retained in downstream analyses (Fig. S13; Dataset S10). 211

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213 **Phylogenetic inference and the mitochondrial haplotype network**

214 Phylogenetic relationships were inferred separately using a maximum-likelihood (ML) approach with autosomes, the Z chromosome, and mitochondrial SNP data. Aligned 215 autosome sequences were generated from the SNP library using the SNPhylo pipeline 216 with the filtering criteria of a linkage disequilibrium coefficient ($R^2 < 0.2$) and no 217 218 missing sites (Lee, et al. 2014). After filtering with SNPhylo, 2,274,011 autosome SNPs were used to infer phylogenetic relationships. Sequences for the Z chromosome and 219 mitochondria were generated with in-house scripts and aligned in the Mafft v 7.271 220 program (Yamada, et al. 2016). The phylogenetic trees were constructed in PhyML v 221 222 3.0 (Guindon, et al. 2010) with 1000 bootstrap replicates for autosomes, the Z chromosome, and mitochondrial sequences. Nucleotide frequencies, 223 the transition/transversion ratio, and the value of the gamma shape parameter were 224 estimated by ML. Tree topology, branch length, and substitution rate parameters were 225 226 optimized in this program. The median joining haplotype network of mitochondria was constructed using Popart (Leigh and Bryant 2015). 227

228

229 **Population structure and principal component analysis**

Principal component analysis (PCA) of the genotypes from 37 individuals was
 performed using a stringently filtered set of 49,701 autosomal SNPs (no missing sites,

Hardy-Weinberg equilibrium exact test p-value > 0.00001, linkage disequilibrium 232 coefficient, $r^2 < 0.2$) in the Genome-wide Complex Trait Analysis (GCTA) v 1.91.3 233 (Yang, et al. 2011). Using the same SNP set, population structure was inferred using 234 ADMIXTURE v 1.3.0 (Alexander, et al. 2009) with cross-validation (CV) (Alexander 235 and Lange 2011) and 100 bootstraps. This approach estimates the ancestry proportion 236 of each sample based on SNP data, given the *a priori* specification of K ancestral 237 populations. To test the stability of the results, we performed the analysis using all 37 238 239 samples, and subsets of individuals belonging to the putative populations C. mantchuricum: Blue, Brown-W, Brown-C, Brown-E. We predefined the K value from 240 1 to 10 for all individuals (including C. auritum and C. mantchuricum), and from 1 to 241 6 within C. mantchuricum. For the isolated populations (Blue, Brown-W, Brown-C, and 242 Brown-E), we predefined the K value from 1 to 3. For each K value, the analysis was 243 performed ten times with different random seeds, which aimed to obtain the most stable 244 results. 245

246

247 Estimation of genetic diversity

To assess the genetic diversity within and among C. mantchuricum populations, we 248 calculated the heterozygosity of each sample, defined as the proportion of heterozygous 249 sites in all callable sites across the autosomes. For each population or species, we 250 defined the genetic diversity as the mean heterozygosity per individual within each 251 population or species. We compared the diversity estimates of the two species to 21 252 other avian species for which comparable estimates were available, some of which are 253 also highly inbred, such as White tailed eagle (Haliaeetus albicilla), Bald eagle 254 255 (Haliaeetus leucocephalus), Crested ibis (Nipponia nippon), and Dalmatian pelican (Pelecanus crispus). 256

257

We also computed population genetic statistics including pairwise nucleotide diversity (θ_{π}) and Watterson's expected nucleotide diversity (θ_{W}) on both synonymous (four-fold degenerate sites) and non-synonymous sites (zero-fold degenerate sites). These

summary statistics were calculated based on the site frequency spectrum (SFS) with in-261 house scripts. In both the synonymous and non-synonymous sites, low-quality and 262 missing genotypes were inferred separately for each population using the program 263 BEAGLE v 4.1 (Browning and Browning 2016). To test whether C. mantchuricum has 264 accumulated more missense mutations than C. auritum, we computed Θ_{π} (zero-fold 265 degenerate sites) / Θ_{π} (four-fold degenerate sites) for each population. If the populations 266 of C. mantchuricum have accumulated more missense mutations, then this value is 267 268 expected to be higher in C. mantchuricum than that in C. auritum. To compare these summary statistics between populations, these statistics were calculated using 1,000 269 bootstrap replicates (resampling with replacement). Because the variance of any 270 summary statistics between two randomly selected populations was not homoscedastic, 271 non-paired Welch two-sample t-tests were used to test whether the summary statistics 272 of the two populations were significantly different from each other. 273

274

275 Estimating inbreeding patterns

Estimation of inbreeding using whole genome resequencing data has been shown to outperform estimates using extensive pedigree data (Kardos, et al. 2018). To understand the genomic extent of inbreeding in *C. mantchuricum*, we identified genome-wide patterns of runs of homozygosity (ROH), estimated linkage disequilibrium (LD), and the inbreeding coefficient (F_{is}) in each of the three populations of *C. mantchuricum*. For comparison, we also performed the same analysis in the sampled population of *C. auritum*.

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ROH arises through the process of inbreeding that gives offspring the opportunity to inherit identical haplotypes from each parent (Ceballos, et al. 2018). The location and number of ROHs were identified in each sample using plink v1.90 with default parameters (Howrigan, et al. 2011). Because meiosis can break down the segments that are identical-by-descent, we assume that longer ROH (≥ 0.5 Mb) arose from recent inbreeding, and shorter ROH (< 0.5 Mb) arose from more ancient inbreeding (Kirin, et al. 2010). To compare the relative extents of recent and ancient inbreeding, the average
fraction of the longer and shorter ROH on the autosomes was summarized for each
population.

293

To avoid biases caused by the different sample sizes of the sampled populations in the 294 LD analysis, we randomly selected six unrelated samples from each population and 295 repeated this analysis 10 times. We employed Haploview (Barrett, et al. 2004) to 296 calculate the correlation coefficient (R^2) between any pair of SNPs in each population. 297 The parameters were set as follows: -maxdistance 500 -dprime -minGeno 0.75 -298 minMAF 0.05 -hwcutoff 0.001. To plot the LD decay curves, we merged the SNPs that 299 had a similar physical distance (1 kb) into one group and calculated the mean R² for 300 each group. We estimated the inbreeding coefficient per individual in plink v1.90 301 (Howrigan, et al. 2011) and then calculated the average inbreeding coefficient for each 302 population. 303

304

305 Evaluating the genetic load

Genetic load is a factor that can drive the extinction of small populations. Evaluating the genetic load of endangered species is an important component of conservation genetics. Thus, we sought to gauge the genetic load of *C. mantchuricum* using population genomic data. We estimated the relative excess of derived loss-of-function (LOF) and missense variants in three populations of *C. mantchuricum* compared to those of *C. auritum* to test whether these isolated populations experience inbreeding depression.

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To measure the genetic load, we employed methods previously used for gorillas (Xue, et al. 2015). We used SnpEff to identify LOF, missense, and synonymous variations in the coding region (Cingolani, et al. 2012). We inferred the ancestral and derived allele at each location based on comparison to the genome of their close relatives, the White eared pheasant (*C. crossoptilon*) (Wang, et al. 2018). In order to estimate the relative number of derived LOF and missense alleles found in each population of C. *mantchuricum* compared to those in C. *auritum*, we used the following formula to calculate the number of derived LOF or missense alleles found in population A rather than in population B:

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$$L_{A,B}(C) = \frac{\sum_{i \in C} f^{A}i(1 - f^{B}i)}{\sum_{j \in I} f^{A}j(1 - f^{B}j)}$$

We used $f^{A}i = d^{A}i / n^{A}i$ to calculate the observed derived allele frequency in population 324 A, where d^{A_i} is the number of derived alleles in population A, and n^{A_i} is the total number 325 of alleles in population A. In population B, $f^{B}i$ was similarly defined. We defined C as 326 a set of protein-coding sites, and I as a set of intergenic sites. For each comparison, the 327 relative number of derived LOF or missense variations was measured by $R_{A/B}(C) =$ 328 329 $L_{A,B}(C)/L_{B,A}(C)$. If the $R_{A/B}(C)$ is larger than one, it means that the A population has more LOF or missense mutations than the B population; otherwise, the B population 330 has excess corresponding mutations. To estimate the variance of $R_{A/B}(C)$, we performed 331 a 100-block jackknife at the sites of the C set. 332

333

To evaluate inbreeding depression, we used three strategies to analyze the outcome of 334 inbreeding. We first calculated whether the ROH regions accumulated much more 335 homozygosity missense mutations than those outside the ROH regions. If the 336 homozygous lethal variations did not come from inbreeding, we expected the ratio of 337 the number of homozygous missense genotypes to the number of homozygous 338 synonymous genotypes to be equal to one; otherwise, the ratio should be less than one. 339 Next, we tested whether there were more heterozygous missense mutations compared 340 341 to the number of homozygous mutations. If one population accumulates homozygous lethal mutations, the ratio of the number of missense alleles to the number of 342 synonymous alleles in the homozygous sites would be significantly less than the ratio 343 in the heterozygous sites. To test whether there was a significant difference between the 344 homozygous and heterozygous sites, we employed the Kolmogorov-Smirnov test to 345 compare the distribution. For the third strategy, we compared the genetic diversity of 346

the functional region (MHC, conserved non-exonic elements, conserved intron, conserved intergenic region, and coding region) and non-functional region (putative non-functional region, non-conserved intron, and non-conserved intergenic region) between each population of *C. mantchuricum* and *C. auritum*. If *C. mantchuricum* experienced inbreeding depression, both the functional and non-functional regions would have a lower genetic diversity than that of *C. auritum*.

353

354 To identify the conserved non-exonic elements (CNEE), putative non-function region, conserved and non-conserved introns, and conserved and non-conserved intergenic 355 regions, we followed the method described in a comparative genomics project on 356 paleognaths (Sackton, et al. 2019) to identify the G. gallus (galGal5) CNEE coordinates. 357 After getting the information regarding the G. gallus CNEE coordinates, we aligned the 358 scaffolds of C. mantchuricum to the G. gallus genome in LAST software (Kiełbasa, et 359 al. 2011). To obtain the CNEE coordinates of C. mantchuricum, we lifted the G. gallus 360 CNEE coordinates to C. mantchuricum. We defined the putative non-functional region 361 362 as the region far away from the genes, with CNEE regions at least 50 kb and at least 1 kb in length. The conserved intron and conserved intergenic region are defined as the 363 regions with overlap between CNEE and the intron and intergenic regions, respectively. 364 Otherwise, the regions that do not have any overlap are considered non-conserved 365 introns and non-conserved intergenic regions. 366

367

As an additional assessment of the fitness of *C. mantchuricum*, we calculated the genetic diversity in the MHC region. We identified the MHC region with the most effective reciprocal hit BLAST method. By comparing the protein sequences of *C. mantchuricum* and *G. gallus*, the ortholog genes were identified. Using the annotation information of *G. gallus*, the ortholog MHC region was identified. With the in-house scripts, we calculated the genetic diversity of the MHC.

374

375 In order to more intuitively understand whether purifying selection could remove

deleterious mutations in C. mantchuricum, we investigated whether or not derived 376 missense mutations in potential adaptative genes are deleterious. Because the genomic 377 islands identified by the statistic F_{ST} may occur due to reduced diversity (Cruickshank 378 and Hahn 2014), and C. mantchuricum typically has low genome-wide diversity, we 379 employed d_{XY} , a statistic that is independent of the genetic diversity within the 380 populations, to identify the candidate adaptive genes (Cruickshank and Hahn 2014). We 381 also used pi, a measure of genetic variation, to infer the candidate adaptive genes. We 382 first employed "popgenWindows.py" written by Simon Martin at The University of 383 Edinburgh, UK, to calculate the d_{XY} between C. auritum and each population of C. 384 mantchuricum. The measure of genetic variation, pi, was also calculated using this 385 script. We set the window size to 100 kb, and the minimum number of sites within a 386 window was 10. The size of the step for the sliding window was set to 25 kb. The 387 window size was determined by the results of the linkage disequilibrium test. Then, we 388 Z-transformed the d_{XY} and pi to Zd_{XY} and Zpi. Because there was almost no genetic 389 diversity in C. mantchuricum, we only employed the Zpi of C. auritum and Zd_{XY} to 390 391 infer the candidate adaptive genes. The windows with high (top 5%) Zd_{XY} and extremum (both top 5% and bottom 5%) of Zpi were determined as the candidate 392 regions containing the genes under selection. Genes with more than 50% of their length 393 contained within the candidate regions were selected as the genes putatively under 394 positive selection. PROVEAN v 1.1.5 (Choi, et al. 2012) was used to predict whether 395 the derived missense mutations in the genes putatively under positive selection were 396 deleterious mutations. The derived missense mutations with PROVEAN scores lower 397 than -2.5 were identified as deleterious mutations. The genetic diversity of each 398 399 potential adaptive gene and the frequency of the derived missense mutations were also calculated. KOBAS v 3.0 (Wu, et al. 2006) was used to perform KEGG and GO 400 enrichment analyses for these genes putatively under positive selection. 401

402

403 Demographic History Reconstruction

404 To explore the potential historical factors that are linked to low present-day genetic

diversity, we used the Multiple Sequential Markovian Coalescence (MSMC2) model 405 (Schiffels and Durbin 2014) to reconstruct demographic histories for each of our study 406 populations. Because MSMC2 uses phased haplotypes as input data, we used SHAPEIT 407 v2.904.3.10.0 (Delaneau, et al. 2012) to phase 33 scaffolds separately, with a minimum 408 length of 132 kb. Based on the results from MUMmer, we selected at least one scaffold 409 from each chromosome. The total length of these 33 scaffolds covered 18.48% of the 410 draft genome. To prepare the input file, we followed the procedure of Heng Li's 411 412 SNPable program (http://lh3lh3.users.sourceforge.net/snpable.shtml) to generate Mappability mask files for each scaffold. Under the general guidelines of MSMC2 413 (https://github.com/stschiff/msmc/blob/master/guide.md), we ran MSMC2 414 and calculated the effective population size (N_e) of C. mantchuricum. To test the 415 convergence of the result, we performed both analyses with 100 bootstrap replicates. 416 Based on the comparison of the four-fold degenerate sites among the lineages of 417 Galliformes, we set the mutation rate of C. mantchuricum as 4.02×10^{-9} per site per 418 generation (Zhang, et al. 2014), and the generation time as 2 years per generation 419 420 (Zheng 2015).

421

We used Migrate-n v 3.6.11 (Beerli and Felsenstein 2001) to investigate whether gene 422 flow occurs between adjacent populations of C. mantchuricum. We evaluated four 423 possible gene flow patterns among the three populations of C. mantchuricum: migration 424 between adjacent populations, migration between Brown-C and Brown-W, migration 425 between Brown-C and Brown-E, or no gene flow between adjacent populations. The 426 best-fit model was inferred by the maximum likelihood in the Migrate-n. To prepare the 427 428 input data for Migrate-n, we first selected the putative non-functional regions whose lengths were longer than 1 kb and then randomly selected 1 kb from each of these 429 putative non-functional regions. Among the selected 1 kb putative non-functional 430 regions, we randomly selected 30 regions to create the bed file. In the vcftools software, 431 we extracted the phased vcf file for each bed and then employed the bcftools software 432 to produce sequences for each region of each sample. Using the 60 sequences of each 433

434 sample, we performed Migrate-n to estimate the gene flow patterns.

435

436 In order to distinguish whether the populations experienced continuous population decline or consistent small population size in C. mantchuricum, we employed 437 fastsimcoal26 (Excoffier, et al. 2013), a simulation-based framework, to infer the 438 demographic history of the three populations of C. mantchuricum. Fastsimcoal26 uses 439 SFS information to infer the most suitable evolutionary model given a range of 440 scenarios. Using this method, we also tested whether C. mantchuricum experienced 441 inbreeding and whether there was gene flow among the adjacent populations of C. 442 mantchuricum. The three-dimensional SFS of the three populations of C. mantchuricum 443 was estimated using the -doSaf and -realSFS methods in ANGSD (Korneliussen, et al. 444 2014). To minimize misidentification of the derived allele in C. mantchuricum, we only 445 selected the sites whose alleles were fixed in the population of C. auritum, and excluded 446 the sites that were not located in the putative non-functional region and were not present 447 in all individuals within each population. We first estimated the unfolded one-448 449 dimensional SFS for each of the C. mantchuricum populations. We then estimated the three-dimensional SFS among the three populations. The three-dimensional SFS was 450 used to estimate the composite likelihoods of the different evolutionary models. 451 Because we wanted to test 1) whether C. mantchuricum experienced long-term 452 population decline (D) or consistent small population size (S), 2) whether there was 453 gene flow between the adjacent populations of C. mantchuricum (no gene flow between 454 adjacent populations: I; gene flow occurred between adjacent populations: WCE; gene 455 flow occurred between the Western and Central populations: CW; gene flow occurred 456 457 between the Central and Eastern populations: CE), and 3) whether there had been inbreeding (IN) in the populations of C. mantchuricum, we devised 16 historical models 458 for the three populations. The 16 models were as follows: a) S+I; b) S+WCE; c) S+CW; 459 d) S+CE; e) D+I; f) D+WCE; g) D+CW; h) D+CE; i) S+I+IN; j) S+WCE+IN; k) 460 S+CW+IN; 1) S+CE+IN; m) D+I+IN; n) D+WCE+IN; o) D+CW+IN; and p) D+CE+IN 461 (these models are illustrated in Fig. S11). For each model, we performed 1,000,000 462

simulations to estimate the expected derived SFS, and 100 expectation conditional 463 maximization cycles were performed. We also required the minimum console output 464 and set the minimum observed SFS entry count as 20 accounting for parameter 465 estimation. The multiSFS and infinite site options were also used for estimating the 466 model parameters. To obtain the global maximum likelihood for each model, the above 467 procedure was replicated 100 times. To infer the best-fit model, the replicate with the 468 highest estimated likelihood was used to calculate the AIC values and Akaike weights 469 (w). 470

471

472 **Results**

473 Mapping and SNP quality

The sequencing coverage of the re-sequence individuals varied from 13.4 to 43.2 (the average coverage was 19.0), and the mapping rate varied from 83.6% to 98.9% (the average mapping rate was 97.3%) (Dataset S9).

477

478 The average proportion of high-quality bases (the call quality of the bases was Q20 or higher) in the BAM files was 93.30% (varied from 90.20% to 95.00%), 93.28% (varied 479 from 89.07% to 95.27%), and 95.46% (varied from 95.24% to 95.75%) of the 480 autosomes, Z chromosome, and mitochondria, respectively (Fig. S14a, Fig. S15a, Fig. 481 S16a, Dataset S11). This proportion was defined as the number of high-quality bases 482 divided by the number of aligned bases in the BAM file. In the BAM files of the 483 autosomes, Z chromosome, and mitochondrial genome, the average percentage of sites 484 covered by at least five reads was 97.58% (varied from 83.37% to 98.56%), 88.83% 485 (varied from 45.00% to 98.44%) and 100% (no variation), respectively (Fig. S14b, Fig. 486 487 S15b, Fig. S16b, Dataset S11).

488

After variant calling, we obtained 7,556,341 and 65 single-nucleotide variants for the genome (including autosomes and sex chromosomes) and mitochondria, respectively. After hard-filtering, the final SNP library contained 2,327,376 and 134,081 variable sites on the autosome and Z chromosome, respectively. In the mtDNA dataset, we
retained 24 substitution sites. In the autosome, Z chromosome, and mitochondrial SNP
library, the mean proportion of missing SNPs was 0.047% (varied from 0.006% to
0.372%), 0.392% (varied from 0.003% to 4.144%), and 0 (no variation), respectively
(Fig. S14c, Fig. S15c, Fig. S16c, Dataset S11).

497

The average number of heterozygous sites of the autosomes and Z chromosome was 498 499 230,681 (varied from 38,525 to 902,604) and 10,764 (varied from 808 to 28,595), respectively (Fig. S17, Fig. S18). In the autosome SNP library, C. auritum had the 500 highest heterozygosity (average= 0.083%, SD = 0.0055%). Among the three 501 populations of C. mantchuricum, Brown-C had the highest heterozygosity (average= 502 0.014%, SD= 0.001%), while both Brown-W and Brown-E had similar heterozygosity 503 (Fig. S14d). On the Z chromosomes, C. auritum had the highest genetic diversity 504 (average= 0.029%, SD= 0.007%), but the three populations of C. mantchuricum had 505 similar genetic diversity (Fig. S15d). For the mitochondria, the Brown-C and Brown-E 506 507 populations had a low average number of nucleotide differences (the value was 0) (Fig. S16d). 508

509

510 Scaffolds linked to the Z chromosome and mitogenome

We identified 49 Z chromosome-linked scaffolds (Dataset S12), which had 71.05 Mb 511 bases with a scaffold N50 of 2432.56 kb. The length of the Z chromosome of C. 512 mantchuricum was similar to that of G. gallus (82.53 Mb) (Consortium 2004). The 513 distribution of single-base sequencing depths across all individuals showed two peaks 514 515 for all scaffolds, but there was one single peak for the scaffolds designated as autosomelinked, indicating that the Z-linked scaffolds had effectively been removed from this 516 set (Fig. S19). Female individuals had lower sequencing coverage on the Z 517 chromosome compared to that of the male individuals, while the female and male 518 individuals had similar sequencing coverage of the autosomes (Fig. S20). All the 519 evidence indicated that the process of identifying and removing Z-linked scaffolds was 520

Interestingly, the W-chromosome did not appear in this assembly, even though the

- 521 effective.
- 522

523

individual used to generate the *de novo* assembly was a female. This may be due to the 524 typical characteristics of the avian W chromosomes, such as they tend to be highly 525 heterochromatic and degenerated (Zhou, et al. 2014; Peichel 2017). We identified 3 526 scaffolds that are on the mitochondrial genome (Dataset S12), which have 5,875 bp. 527 528 References 529 530 Alexander DH, Lange K. 2011. Enhancements to the ADMIXTURE algorithm for individual ancestry 531 estimation. BMC Bioinformatics 12:246. 532 Alexander DH, Novembre J, Lange K. 2009. Fast model-based estimation of ancestry in unrelated 533 individuals. Genome Research 19:1655-1664. 534 Bao W, Kojima KK, Kohany O. 2015. Repbase update, a database of repetitive elements in 535 eukaryotic genomes. Mobile DNA 6:11. Barrett JC, Fry B, Maller J, Daly MJ. 2004. Haploview: analysis and visualization of LD and haplotype 536 537 maps. Bioinformatics 21:263-265. 538 Beerli P, Felsenstein J. 2001. Maximum likelihood estimation of a migration matrix and effective 539 population sizes in n subpopulations by using a coalescent approach. Proceedings of the National 540 Academy of Sciences 98:4563-4568. 541 Benson G. 1999. Tandem repeats finder: a program to analyze DNA sequences. Nucleic Acids 542 Research 27:573-580. 543 Browning Brian L, Browning Sharon R. 2016. Genotype imputation with millions of reference 544 samples. The American Journal of Human Genetics 98:116-126. 545 Butler J, MacCallum I, Kleber M, Shlyakhter IA, Belmonte MK, Lander ES, Nusbaum C, Jaffe DB. 546 2008. ALLPATHS: De novo assembly of whole-genome shotgun microreads. Genome Research 547 18:810-820. 548 Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. 2009. BLAST 549 plus: architecture and applications. BMC Bioinformatics 10:421. 550 Ceballos FC, Joshi PK, Clark DW, Ramsay M, Wilson JF. 2018. Runs of homozygosity: windows into 551 population history and trait architecture. Nature Reviews Genetics 19:220-234. 552 Choi Y, Sims GE, Murphy S, Miller JR, Chan AP. 2012. Predicting the functional effect of amino acid 553 substitutions and indels. PLOS ONE 7:e46688. 554 Cingolani P, Platts A, Wang LL, Coon M, Nguyen T, Wang L, Land SJ, Lu X, Ruden DM. 2012. A 555 program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff. Fly 556 6:80-92. 557 Consortium ICGS. 2004. Sequence and comparative analysis of the chicken genome provide 558 unique perspectives on vertebrate evolution. Nature 432:695-716. 559 Cruickshank TE, Hahn MW. 2014. Reanalysis suggests that genomic islands of speciation are due 560 to reduced diversity, not reduced gene flow. Molecular ecology 23:3133-3157. 19

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643 corresponding groups. n indicates the number of BUSCO groups in the avian dataset.



Fig. S2. Annotated gene set assessment result. AED means Annotation Edit Distance, 645 which is used to measure the suitability between the annotated gene model and the 646 supporting evidence of that model. AED is represented by a number between 0 and 1. 647 An AED value of 0 indicates the annotated gene model is perfected supported by 648 available evidence, and an AED of 1 denotes that the annotated gene model is 649 completely lacks supportive evidence. (a) The pie charts represent the annotated gene 650 models with different AED values (0~0.25, 0.25~0.5, 0.5~0.75, and 0.75~1). (b) The 651 652 number of annotated gene models with different AED values from 0 to 1. (c) The curve of the cumulative fraction of the annotated gene models. 653



Fig. S3. Cross-validation (CV) values of different ancestral number (K) values. The CV
values from the ADMIXTURE analyses performed on the different population scales
with 10 independent runs with different random seeds.



658

Fig. S4. Principal component analysis (PCA) of *Crossoptilon mantchuricum* and *Crossoptilon auritum*. The numbers in brackets are the contribution values of the corresponding eigenvectors.



662

Fig. S5. Phylogenetic relationships and haplotype networks within Crossoptilon 663 mantchuricum. The colors represent sampling populations; blue represents 664 Crossoptilon auritum (Blue) as outgroups, while orange, green, and red represent the 665 Western (Brown-W), Central (Brown-C), and Eastern (Brown-E) populations of C. 666 *mantchuricum*, respectively. (a), (b), and (c) The unrooted maximum-likelihood (ML) 667 trees reconstructed using autosomes, the Z chromosome, and mitochondria, 668 respectively. The bootstrap values larger than 80% of the bootstrap number were 669 associated with the corresponding branches. (d) Median joining mitochondrial 670 haplotype network. Lines on the linking branches indicate single base variations, and 671 circle size represents the number of samples that share a single haplotype. 672



Fig. S6. Folded site frequency spectrum. (a) *C. auritum*. (b) Brown-W, the western population of *C. mantchuricum*. (c) Brown-C, the central population of *C*.

676 *mantchuricum*. (d) Brown-E, the eastern population of *C. mantchuricum*.



677

678 Fig. S7. Census of the Runs of Homozygosity (ROHs) and inbreeding coefficients (Fis).

(a) The cumulative ROH length against the total number of observed ROH segments

680 for each sample. (b) The mean inbreeding coefficient of each population. Three

asterisks indicate that the non-adjusted P values were less than 10^{-3} .



Fig. S8. Genetic diversity (Θ_w) of each population in different regions (CDS: coding region; CNEE: conserved non-exonic elements; conserved intron; conserved intergenic; putative non-function region; non-conserved intergenic; and non-conserved intron). Error bars represent 95% confidence intervals estimated from 1,000 bootstrap resampling.



Fig. S9. Manhattan plots of the genome-wide Zd_{xy} and Zpi. The points above the dotted line in the Zdxy Manhattan plots are the windows with the top 5% Zdxy. The points above the upper dotted line and the points under the lower dotted line in the Zpi Manhattan plot are the windows with top 5% and bottom 5% Zpi. The green points are the windows with the genes putatively under positive selection.



694

Fig. S10. Demographic history of *Crossoptilon mantchuricum* and *Crossoptilon auritum* estimated from the 100 times MSMC bootstrap. The MSMC results show the
demographic history of the four populations. The blue line represents *C. auritum* (Blue);
the purple, brown, and green lines represent the Western (Brown-W), Central (BrownC), and Eastern (Brown-E) populations of *C. mantchuricum*, respectively.



Fig. S11. The demographic models inferred by fastsimcoal26. S represents consistent 701 702 small population size, D represents population decline, I indicates that there was no gene flow between adjacent populations, WCE indicates that there was gene flow 703 between adjacent populations, CW indicates that the gene flow only existed between 704 the Western and Central populations, CE indicates that the migration only occurred 705 between the Central and Eastern populations, and IN indicates that inbreeding occurred. 706 w represents the Akaike weights. The D+I+IN model obtained the ideal amount of 707 support, which was marked by the asterisk. 708



Fig. S12. Sample locations. N indicates the number of samples for each population. The

711 brown regions indicate the distribution of *Crossoptilon mantchuricum*, while the light-

712 yellow region indicates the distribution of *Crossoptilon auritum*. The star represents the

- 713 Eastern population of *C. mantchuricum* (Brown-E), the square represents the Central
- 714 population of *C. mantchuricum* (Brown-C), the circle represents the Western population
- of *C. mantchuricum* (Brown-W), and the triangle represents *C. auritum* (Blue).



716

Fig. S13. Individuals' kinship within populations. Zero IBS indicates non-Identical-ByState. The dashed lines show the kinship thresholds (duplicate/MZ twin, 1st-degree, 2nddegree, 3rd-degree). (a) and (b) show the relatedness of all samples within populations
and the individuals used in the population genetic analysis in each population,
respectively.



Fig. S14. Variant quality metrics of autosomes. The corresponding sample of each sample index can be seen in Supplementary Dataset S11. (a) Individual percentage of high-quality sites in the BAM file (the call quality of the bases was Q20 or higher). (b) Individual percentage of sites in the BAM file with coverage greater than 5 X. (c) Individual percentage of missing SNPs. (d) Heterozygosity of each sample in the four populations. In the box plots, the bold black line represents the median, and the boxes limit the 25th and 75th percentiles of the distributions.



730

Fig. S15. Variant quality metrics of the Z chromosome. The corresponding sample of
each sample index can be seen in Supplementary Dataset S11. (a) Individual percentage
of high-quality sites in the BAM file (the call quality of the bases was Q20 or higher).
(b) Individual percentage of sites in the BAM file with coverage greater than 5 X. (c)
Individual percentage of missing SNPs. (d) Heterozygosity of each sample in the four
populations. In the box plots, the bold black line represents the median, and the boxes
limit the 25th and 75th percentiles of the distributions.


738

Fig. S16. Variant quality metrics of the mitogenome. The corresponding sample of each
sample index can be seen in Supplementary Dataset S11. (a) Individual percentage of
high-quality sites in the BAM file (the call quality of the bases was Q20 or higher). (b)
Individual percentage of sites in the BAM file with coverage greater than 5 X. (c)
Individual percentage of missing sites. (d) Average number of nucleotide differences
for each population (K).



745

Fig. S17. Variant number of autosomes for the 51 individuals on a population-scale.

Sample IDs with the abbreviations Blue, Brown-W, Brown-C, and Brown-E denoted
individuals from *Crossoptilon auritum* and the Western, Central, and Eastern

749 populations of *Crossoptilon mantchuricum*, respectively.



750

Fig. S18. Variant number of the Z chromosome for the 51 individuals on a population-

scale. Sample IDs with the abbreviations Blue, Brown-W, Brown-C, and Brown-E

- 753 indicate individuals from *Crossoptilon auritum* and the Western, Central, and Eastern
- 754 populations of *Crossoptilon mantchuricum*, respectively.



755

Fig. S19. Distribution of sequencing depth across 51 individuals for (a) the wholegenome (including sex chromosomes) and (b) the autosomes.



758

Fig. S20. Sequencing coverage of males and females. Blue boxes indicate the summary
of the female individuals, orange boxes indicate the summary of the male individuals.
In the box plots, the bold black line represents the median, and the boxes limit the 25th
and 75th percentiles of the distributions, while the points are the outlier values. The label
under the plots indicates the population symbol and genomic part.

	Draft genome
Complete and single-copy BUSCOs (S)	4624 (94.08%)
Complete and duplicated BUSCOs (D)	52 (1.06%)
Fragmented BUSCOs (F)	134 (2.73%)
Missing BUSCOs (M)	105 (2.14%)
Total BUSCO groups searched	4915

Dataset S1: Completeness evaluation results.

Dataset S2: Summary of the genetic diversity (heterozygous SNP rate) of inbreeding and outbreeding birds on an autosome scale. The genetic diversity of *Crossoptilon mantchuricum* and *Crossoptilon auritum* is the average value within each isolated population. The genetic diversity of other organisms is the lowest value in the corresponding reported population.

Species	Common_Name	Class	IUCN status	Heterozygous SNP rate	Seq_Type	Source DOI
Crossoptilon mantchuricum	Brown eared pheasant (Western)	Aves	Vulnerable	7.33E-05	genome-wide	Cureent study
Crossoptilon mantchuricum	Brown eared pheasant (Eastern)	Aves	Vulnerable	8.73E-05	genome-wide	Cureent study
Crossoptilon mantchuricum	Brown eared pheasant (Center)	Aves	Vulnerable	1.37E-04	genome-wide	Cureent study
Haliaeetus albicilla	White tailed eagle	Aves	Least Concern	4.00E-04	genome-wide	10.1186/s13059-014-0557-1
Haliaeetus leucocephalus	Bald eagle	Aves	Least Concern	4.30E-04	genome-wide	10.1186/s13059-014-0557-1
Nipponia nippon	Crested ibis	Aves	Endangered	4.30E-04	genome-wide	10.1186/s13059-014-0557-1
Pelecanus crispus	Dalmatian pelican	Aves	Near Threatened	6.00E-04	genome-wide	10.1186/s13059-014-0557-1
Falco peregrinus	Peregrine falcon	Aves	Least Concern	7.00E-04	genome-wide	10.1038/ng.2588
Aptenodytes patagonicus	King penguin	Aves	Least Concern	7.20E-04	RNAseq	10.1038/nature13685
Eudyptes moseleyi	Northern rockhopper penguin	Aves	Endangered	7.97E-04	RNAseq	10.1038/nature13685
Falco cherrug	Saker falcon	Aves	Endangered	8.00E-04	genome-wide	10.1038/ng.2588
Crossoptilon auritum	Blue eared pheasant	Aves	Least Concern	8.25E-04	genome-wide	Cureent study
Nestor notabilis	Kea	Aves	Endangered	9.10E-04	genome-wide	10.1186/s13059-014-0557-1
Eudyptes chrysocome filholi	Eastern rockhopper penguin	Aves	Vulnerable	1.14E-03	RNAseq	10.1038/nature13685
Ectopistes migratorius	Passenger pigeon	Aves	Extinct	1.20E-03	genome-wide	10.1073/pnas.1401526111
Cyanistes caeruleus	Eurasian blue tit	Aves	Least Concern	1.27E-03	RNAseq	10.1038/nature13685
Phalacrocorax carbo	Great black cormorant	Aves	Least Concern	1.39E-03	genome-wide	10.1186/s13059-014-0557-1
Taeniopygia guttata	Zebra finch	Aves	Least Concern	1.40E-03	genome-wide	10.1038/nature08819

Apteryx mantelli	Kiwi	Aves	Vulnerable	1.50E-03	genome-wide	10.1186/s13059-015-0711-4
Meleagris gallopavo	Wild turkey	Aves	Least Concern	2.37E-03	genome-wide	10.1371/journal.pbio.1002112
Egretta garzetta	Little egret	Aves	Least Concern	2.51E-03	genome-wide	10.1186/s13059-014-0557-1
Anas platyrhynchos	Mallard	Aves	Least Concern	2.61E-03	genome-wide	10.1038/ng.2657
Ficedula albicollis	Collared flycatcher	Aves	Least Concern	3.80E-03	genome-wide	10.1371/journal.pbio.1002112
Melopsittacus undulatus	Budgerigar	Aves	Least Concern	4.31E-03	genome-wide	10.1186/s13059-014-0557-1
Gallus gallus	Chicken	Aves	Least Concern	5.88E-03	genome-wide	10.1371/journal.pbio.1002112

Site Class	Population	Θ_{π} (%), 95% CI	Θ_{w} (%), 95% CI	Θ_{π} (zero-fold) / Θ_{π} (fold-fold)
	Blue	0.030145% [0.030136%, 0.030154%]	0.020448% [0.020442%, 0.020453%]	0.273340 [0.273220, 0.273459]
zero-fold	Brown-W	0.002670% [0.002666%, 0.002675%]	0.002237% [0.002234%, 0.002240%]	0.308766 [0.307968, 0.309564]
	Brown-C	0.005474% [0.005467%, 0.005481%]	0.004528% [0.004523%, 0.004533%]	0.318080 [0.317500, 0.318661]
	Brown-E	0.003683% [0.003678%, 0.003689%]	0.002817% [0.002813%, 0.002821%]	0.369310 [0.368405, 0.370215]
	Blue	0.110287% [0.110255%, 0.110320%]	0.074654% [0.074636%, 0.074672%]	
four fold	Brown-W	0.008657% [0.008640%, 0.008675%]	0.006903% [0.006891%, 0.006916%]	
four-fold	Brown-C	0.017218% [0.017194%, 0.017242%]	0.014164% [0.014145%, 0.014182%]	
	Brown-E	0.009983% [0.009964%, 0.010001%]	0.007687% [0.007673%, 0.007701%]	

Dataset S3: Genetic diversity of the zero-fold degenerate sites and four-fold degenerate sites. The numbers in brackets are 95% confidence intervals, which were obtained from bootstrapping 1,000 times.

Gene ID	The	frequency function (L Brown-W	of derived OF) mutat Brown-C	loss-of- ions Brown-E	Protein ID	Protein name	The site of amino acid substitutio n	Function
EARP_000 00097	0.8	0	0	0	EARP_0000 0097-RA	helix-turn- helix domain- containing protein	Q552*	regulate gene expression
EARP_000 05078	0.1	1	1	1	EARP_0000 5078-RB	ring finger protein 146	K361*	facilitate DNA repair and protect against cell death

Dataset S4: The derived loss-of-function mutations in the potential adaptive genes.

Dataset S5: The derived missense mutations in the potential adaptive genes. A PROVEAN score less than -2.5 indicates that the corresponding derived missense mutations were deleterious. Blue represents *Crossoptilon auritum*, while Brown-W, Brown-C, and Brown-E represent the Western, Central, and Eastern populations of *Crossoptilon mantchuricum*, respectively.

	The site of		Freq	uency		Proven	Unique fixed to <i>C</i> .	Unique
Protenin ID	amino acid substitution	Blue	Brown -W	Brown -C	Brown -E	scores	mantchuri cum ?	fixed to C. auritum?
EARP_00000044-RA	D411N	0.40	0.00	0.00	0.00	0.160	no	no
EARP_00000045-RA	R101G	0.00	1.00	1.00	1.00	0.867	yes	no
EARP_00000097-RA	A979T	0.70	0.00	0.00	0.00	-4.000	no	no
EARP_00000097-RA	C1895Y	1.00	1.00	1.00	1.00	-11.000	no	no
EARP_00000097-RA	D2118G	0.00	1.00	1.00	1.00	-7.000	yes	no
EARP_00000097-RA	D2650G	0.80	1.00	1.00	0.00	-7.000	no	no
EARP_00000097-RA	D28V	0.60	0.00	0.00	0.00	-9.000	no	no
EARP_00000097-RA	E631K	0.60	0.00	0.00	0.00	-4.000	no	no
EARP_00000097-RA	E63V	0.50	1.00	1.00	1.00	-7.000	no	no
EARP_00000097-RA	F19L	0.20	1.00	1.00	1.00	-6.000	no	no
EARP_00000097-RA	F2851S	0.40	1.00	1.00	1.00	-8.000	no	no
EARP_00000097-RA	F303Y	0.70	1.00	1.00	1.00	-3.000	no	no
EARP_00000097-RA	G1018D	0.00	1.00	1.00	0.00	-7.000	yes	no
EARP_00000097-RA	G39R	0.10	1.00	1.00	1.00	-8.000	no	no
EARP_00000097-RA	L2621F	0.00	1.00	1.00	0.00	-4.000	yes	no
EARP_00000097-RA	N777S	1.00	1.00	1.00	1.00	-5.000	no	no
EARP_00000097-RA	N785S	1.00	1.00	1.00	1.00	-5.000	no	no
EARP_00000097-RA	P1235L	0.00	1.00	1.00	1.00	-10.000	yes	no
EARP_00000097-RA	Q851K	0.00	1.00	1.00	1.00	-4.000	yes	no
EARP_00000097-RA	R29G	0.50	0.00	0.00	0.00	-7.000	no	no
EARP_00000097-RA	R29S	0.50	0.00	0.00	0.00	-6.000	no	no
EARP_00000097-RA	R364H	0.80	0.00	0.00	0.00	-5.000	no	no
EARP_00000097-RA	R374C	0.80	0.00	0.00	0.00	-8.000	no	no
EARP_00000097-RA	S1875P	0.90	1.00	1.00	1.00	-5.000	no	no
EARP_00000097-RA	S1899F	0.40	0.00	0.00	0.00	-6.000	no	no
EARP_00000097-RA	T1451I	0.90	1.00	1.00	0.00	-6.000	no	no
EARP_00000097-RA	T2466A	0.00	1.00	1.00	0.00	-5.000	yes	no
EARP_00000097-RA	T388S	0.40	1.00	1.00	1.00	-4.000	no	no
EARP_00000097-RA	V295I	0.00	1.00	1.00	1.00	-1.000	yes	no
EARP_00000097-RA	W1433C	0.70	0.00	0.00	1.00	-13.000	no	no
EARP_00000193-RA	V109A	0.30	1.00	1.00	0.00	-4.000	no	no
EARP_00000985-RA	P8L	1.00	0.00	0.00	0.00	-0.662	no	yes
EARP_00000986-RA	N62S	1.00	0.00	0.00	0.00	-0.005	no	yes

EARP_00000987-RA	R94Q	0.00	1.00	1.00	1.00	2.941	yes	no
EARP_00000987-RA	S28G	1.00	0.00	0.00	0.00	3.198	no	yes
EARP_00000989-RA	H1599N	0.00	0.00	0.00	0.50	-5.304	no	no
EARP_00000989-RA	M2378I	1.00	0.00	0.00	0.00	-0.534	no	yes
EARP_00000989-RA	S2400I	1.00	0.00	0.00	0.00	-1.674	no	yes
EARP_00002145-RB	R43G	0.70	1.00	1.00	1.00	-7.000	no	no
EARP_00002148-RA	K24E	0.70	0.00	0.00	0.00	-4.000	no	no
EARP_00002148-RA	R38H	0.60	0.00	0.00	0.00	-5.000	no	no
EARP_00002233-RA	S134N	0.90	0.00	0.00	0.00	-1.428	no	no
EARP_00002234-RB	A1163T	1.00	0.00	0.00	0.00	-2.246	no	yes
EARP_00002234-RB	L1777P	0.00	1.00	1.00	1.00	0.517	yes	no
EARP_00002634-RB	G162S	0.70	0.00	0.00	0.00	0.906	no	no
EARP_00002634-RB	T32I	1.00	0.00	0.00	0.00	0.037	no	yes
EARP_00003544-RA	A1335T	0.10	1.00	1.00	1.00	3.314	no	no
EARP_00003544-RA	L1630P	0.10	1.00	1.00	1.00	0.083	no	no
EARP_00003544-RA	S932P	0.20	1.00	1.00	1.00	2.139	no	no
EARP_00003544-RA	V988I	0.70	0.00	0.00	0.00	0.255	no	no
EARP_00003545-RC	T48A	0.90	1.00	1.00	1.00	0.433	no	no
EARP_00003547-RA	H651Q	0.70	0.00	0.00	0.00	0.401	no	no
EARP_00003548-RB	K103E	0.00	1.00	1.00	1.00	0.389	yes	no
EARP_00003548-RB	Y122D	0.00	1.00	1.00	1.00	2.078	yes	no
EARP_00005078-RB	G339R	0.20	1.00	1.00	1.00	-8.000	no	no
EARP_00005078-RB	H401R	0.10	1.00	1.00	1.00	-8.000	no	no
EARP_00005078-RB	H401Y	1.00	0.00	0.00	0.00	-6.000	no	yes
EARP_00005078-RB	N337S	0.20	1.00	1.00	1.00	-5.000	no	no
EARP_00005078-RB	Q247R	0.00	1.00	1.00	1.00	-4.000	yes	no
EARP_00005078-RB	R141W	1.00	0.00	0.00	0.00	-2.000	no	yes
EARP_00005078-RB	R444G	1.00	0.00	0.00	0.00	-1.000	no	yes
EARP_00005078-RB	S142F	0.00	0.00	0.25	0.88	-6.000	no	no
EARP_00005078-RB	T362I	0.10	1.00	1.00	1.00	-6.000	no	no
EARP_00005078-RB	V132M	1.00	0.00	0.00	0.00	-3.000	no	yes
EARP_00006312-RA	A162P	0.80	0.00	0.00	0.00	-5.000	no	no
EARP_00006312-RA	W409L	0.40	0.00	0.00	0.00	-13.000	no	no
EARP_00006312-RA	Y714H	0.20	1.00	1.00	1.00	-5.000	no	no
EARP_00006313-RA	V812A	0.40	1.00	1.00	1.00	-4.000	no	no
EARP_00006344-RA	G7D	0.70	0.00	0.00	0.00	-7.000	no	no
EARP_00006349-RB	I201V	0.60	0.00	0.00	0.00	0.427	no	no
EARP_00006349-RB	V326A	0.80	0.00	0.00	0.00	-0.455	no	no
EARP_00006356-RA	A95T	0.00	1.00	1.00	0.75	-0.252	yes	no
EARP_00006357-RC	V86L	0.00	1.00	1.00	1.00	0.494	yes	no
EARP_00006614-RD	E383K	1.00	0.00	0.00	0.00	-1.865	no	yes

EARP_00007488-RA	I62V	1.00	1.00	1.00	1.00	0.054	no	no
EARP_00007490-RA	E1110K	0.00	0.00	1.00	0.00	-0.487	yes	no
EARP_00007490-RA	V5M	0.40	0.00	0.00	0.00	-0.106	no	no
EARP_00007491-RA	R77S	0.00	1.00	1.00	1.00	-6.000	yes	no
EARP_00007492-RB	G579R	0.70	0.00	0.00	0.00	-7.828	no	no
EARP_00007492-RB	H1120N	1.00	0.00	0.00	0.00	-1.144	no	yes
EARP_00007492-RB	L479P	0.00	1.00	1.00	1.00	3.457	yes	no
EARP_00007492-RB	P353H	0.00	1.00	1.00	1.00	1.750	yes	no
EARP_00007492-RB	R559G	0.00	1.00	1.00	1.00	3.443	yes	no
EARP_00007492-RB	V418E	1.00	0.00	0.00	0.00	-5.531	no	yes
EARP_00007494-RB	L12V	1.00	1.00	1.00	1.00	-0.313	no	no
EARP_00007509-RD	L543P	0.00	1.00	1.00	1.00	1.766	yes	no
EARP_00007509-RD	M56L	0.00	1.00	1.00	1.00	1.702	yes	no
EARP_00007510-RC	A6V	1.00	0.00	0.00	0.00	-0.138	no	yes
EARP_00007511-RA	E41K	1.00	0.00	0.00	0.00	-4.000	no	yes
EARP_00007511-RC	G1815D	0.00	0.36	0.13	1.00	0.107	yes	no
EARP_00008168-RA	L1075F	0.00	1.00	1.00	1.00	1.431	yes	no
EARP_00008168-RA	L1144S	0.00	1.00	1.00	1.00	0.786	yes	no
EARP_00008598-RA	D338E	0.90	0.00	0.00	0.00	0.255	no	no
EARP_00008600-RA	V4M	0.60	1.00	1.00	1.00	1.026	no	no
EARP_00008602-RC	A34V	0.70	0.00	0.00	0.00	-0.068	no	no
EARP_00008602-RA	G51R	0.50	0.00	0.00	0.00	0.355	no	no
EARP_00008602-RC	L22W	0.10	1.00	1.00	1.00	-0.259	no	no
EARP_00008602-RC	T26A	0.50	0.00	0.00	0.00	-0.357	no	no
EARP_00008602-RA	Y78C	0.10	1.00	1.00	1.00	-0.378	no	no
EARP_00008602-RA	Y78H	0.10	1.00	1.00	1.00	0.000	no	no
EARP_00008603-RB	I842V	0.70	0.00	0.00	0.00	0.452	no	no
EARP_00008603-RB	T311I	0.00	1.00	0.25	0.00	3.975	yes	no
EARP_00008603-RB	V382M	0.20	0.00	0.00	1.00	-1.846	no	no
EARP_00009935-RA	F57S	0.30	1.00	1.00	1.00	-8.000	no	no
EARP_00009935-RA	V65M	0.90	0.00	0.00	0.00	-3.000	no	no
EARP_00010173-RA	A428V	0.00	1.00	0.50	0.00	-2.152	yes	no
EARP_00010175-RB	G285E	1.00	0.00	0.00	0.00	0.133	no	yes
EARP_00010175-RB	T949I	1.00	0.00	0.00	0.00	-0.011	no	yes
EARP_00011418-RA	L1448M	0.00	0.00	0.88	0.00	1.408	no	no
EARP_00011419-RB	T208A	0.70	0.00	0.00	0.00	0.000	no	no
EARP_00011419-RB	T446A	0.70	0.00	0.00	0.00	-0.356	no	no
EARP_00011586-RC	G208A	0.00	1.00	1.00	0.00	0.214	yes	no
EARP_00011586-RC	V33I	0.10	0.00	0.00	1.00	-0.297	no	no
EARP_00012749-RA	M467T	1.00	0.00	0.00	0.00	0.574	no	yes
EARP_00012749-RA	S3Y	0.80	0.00	0.00	0.00	-1.320	no	no

EARP_00012844-RA	T1112P	0.00	1.00	1.00	1.00	-0.129	yes	no
EARP_00012844-RA	T1198A	0.00	1.00	1.00	1.00	0.431	yes	no
EARP_00012846-RA	S376N	0.60	0.00	0.00	0.00	0.303	no	no
EARP_00012846-RA	V1344E	0.00	1.00	1.00	1.00	1.988	yes	no
EARP_00012848-RA	P104S	0.20	0.00	1.00	0.00	-4.667	no	no
EARP_00012944-RA	L33F	0.00	1.00	1.00	1.00	-0.391	yes	no
EARP_00013131-RA	R511Q	0.70	0.00	0.00	0.00	0.000	no	no
EARP_00013132-RA	I120V	1.00	0.00	0.00	0.00	1.000	no	yes
EARP_00013132-RA	L474S	0.00	1.00	1.00	1.00	6.000	yes	no
EARP_00013132-RA	M332V	0.00	1.00	1.00	1.00	3.000	yes	no
EARP_00013133-RA	P1884S	0.80	0.00	0.00	0.00	0.337	no	no
EARP_00013133-RA	S1767L	0.80	0.00	0.00	0.00	-1.419	no	no
EARP_00013133-RA	T2351A	1.00	0.00	0.00	0.00	-0.568	no	yes
EARP_00013133-RA	V2114A	0.80	0.00	0.13	0.00	0.655	no	no
EARP_00013134-RA	T711I	0.00	1.00	1.00	1.00	-0.500	yes	no
EARP_00013135-RA	T488K	0.40	0.00	0.00	0.00	-6.000	no	no
EARP_00013137-RA	I205M	0.00	1.00	1.00	1.00	1.217	yes	no
EARP_00013138-RA	T664A	0.60	1.00	1.00	1.00	-2.663	no	no
EARP_00013139-RA	E1834K	0.40	0.00	0.00	0.00	-0.870	no	no
EARP_00013139-RA	H1168Q	0.40	0.00	0.00	0.00	1.333	no	no
EARP_00013139-RA	I742V	0.80	1.00	1.00	1.00	0.467	no	no
EARP_00013139-RA	Q44R	0.00	1.00	1.00	1.00	-0.391	yes	no
EARP_00013139-RA	T1428A	0.50	0.00	0.00	0.00	-0.216	no	no
EARP_00013142-RA	T43S	1.00	0.00	0.00	0.00	-4.000	no	yes
EARP_00013786-RA	F42S	0.00	1.00	1.00	1.00	1.747	yes	no
EARP_00014053-RA	R263C	0.00	0.00	0.13	0.50	-3.196	no	no
EARP_00014072-RA	Y80F	0.70	1.00	0.88	1.00	-0.948	no	no
EARP_00014073-RD	R56H	1.00	0.00	0.00	0.00	-5.000	no	yes
EARP_00014240-RA	V119M	0.10	1.00	1.00	1.00	-0.100	no	no
EARP_00014598-RB	E7K	1.00	0.00	0.00	0.00	-0.036	no	yes
EARP_00014598-RB	V1549I	0.00	1.00	1.00	1.00	-0.021	yes	no
EARP_00015100-RA	A116E	0.00	1.00	1.00	1.00	0.886	yes	no
EARP_00015100-RA	E97D	0.90	0.00	0.00	0.00	-0.202	no	no
EARP_00015100-RA	G177D	1.00	0.00	0.00	0.00	1.921	no	yes
EARP_00015100-RA	L9P	0.00	1.00	1.00	1.00	-0.022	yes	no
EARP_00015807-RA	L58V	0.00	1.00	1.00	1.00	-3.000	yes	no
EARP_00015807-RA	L78P	0.10	1.00	1.00	1.00	-7.000	no	no
EARP_00015808-RA	C10R	1.00	0.00	0.00	0.00	0.349	no	yes
EARP_00015808-RA	H23Y	0.00	1.00	1.00	1.00	-0.193	yes	no
EARP_00015808-RA	L484P	0.00	1.00	1.00	1.00	3.432	yes	no
EARP_00016036-RA	P908S	0.00	1.00	1.00	0.50	1.395	yes	no

EARP_00016471-RAQ1996H0.501.001.001.00-0.655nonoEARP_00016471-RAV4I0.001.001.001.00-0.355yesnoEARP_00017025-RFA628G0.001.001.001.00-0.100yesnoEARP_00017025-RFR140H0.700.000.000.00-0.158nonoEARP_00017027-RBS286P0.700.000.000.00-0.730nonoEARP_00017032-RAV374I1.000.000.000.000.314noyesEARP_00017036-RAE450D0.101.001.001.00-1.894nonoEARP_00017036-RAR488C0.900.000.000.244nonoEARP_00017037-RDT735A0.301.001.001.000.873nonoEARP_00017072-RAQ136R0.001.001.001.00-0.347yesnoEARP_00017072-RAQ136R0.401.001.000.00-1.846nonoEARP_00017811-RAL136I0.401.001.000.00-1.846nonoEARP_00017811-RAV1139A1.001.001.000.466nono	EARP_00016471-RA	E1545K	0.90	1.00	1.00	1.00	0.792	no	no
EARP_00016471-RAV4I0.001.001.001.00-0.355yesnoEARP_00017025-RFA628G0.001.001.001.00-0.100yesnoEARP_00017025-RFR140H0.700.000.000.00-0.158nonoEARP_00017027-RBS286P0.700.000.000.00-0.730nonoEARP_00017032-RAV374I1.000.000.000.000.314noyesEARP_00017036-RAE450D0.101.001.001.00-1.894nonoEARP_00017036-RAR488C0.900.000.000.244nonoEARP_00017036-RAV85I0.201.001.001.000.873nonoEARP_00017037-RDT735A0.301.001.001.00-0.347yesnoEARP_00017811-RAL136I0.401.001.001.000.386nonoEARP_00017811-RAV1139A1.001.001.001.000.466nono	EARP_00016471-RA	Q1996H	0.50	1.00	1.00	1.00	-0.655	no	no
EARP_00017025-RFA628G0.001.001.001.00-0.100yesnoEARP_00017025-RFR140H0.700.000.000.00-0.158nonoEARP_00017027-RBS286P0.700.000.000.00-0.730nonoEARP_00017032-RAV374I1.000.000.000.000.314noyesEARP_00017036-RAE450D0.101.001.001.00-1.894nonoEARP_00017036-RAR488C0.900.000.000.244nonoEARP_00017036-RAV85I0.201.001.001.000.964nonoEARP_00017037-RDT735A0.301.001.001.000.873nonoEARP_00017072-RAQ136R0.001.001.001.000.347yesnoEARP_00017811-RAL136I0.401.001.000.00-1.846nonoEARP_00017811-RAV1139A1.001.001.000.466nono	EARP_00016471-RA	V4I	0.00	1.00	1.00	1.00	-0.355	yes	no
EARP_00017025-RFR140H0.700.000.000.00-0.158nonoEARP_00017027-RBS286P0.700.000.000.00-0.730nonoEARP_00017032-RAV374I1.000.000.000.000.314noyesEARP_00017036-RAE450D0.101.001.001.00-1.894nonoEARP_00017036-RAR488C0.900.000.000.244nonoEARP_00017036-RAV85I0.201.001.001.000.964nonoEARP_00017037-RDT735A0.301.001.001.000.873nonoEARP_00017072-RAQ136R0.001.001.001.00-0.347yesnoEARP_00017811-RAL136I0.401.001.001.000.386nonoEARP_00017811-RAV1139A1.001.001.001.000.466nono	EARP_00017025-RF	A628G	0.00	1.00	1.00	1.00	-0.100	yes	no
EARP_00017027-RBS286P0.700.000.000.00-0.730nonoEARP_00017032-RAV374I1.000.000.000.000.314noyesEARP_00017036-RAE450D0.101.001.001.00-1.894nonoEARP_00017036-RAR488C0.900.000.000.000.244nonoEARP_00017036-RAV85I0.201.001.001.000.964nonoEARP_00017037-RDT735A0.301.001.001.000.873nonoEARP_00017072-RAQ136R0.001.001.001.000.386nonoEARP_00017811-RAL136I0.401.001.001.000.466nonoEARP_00017811-RAV1139A1.001.001.001.000.466nono	EARP_00017025-RF	R140H	0.70	0.00	0.00	0.00	-0.158	no	no
EARP_00017032-RAV374I1.000.000.000.000.314noyesEARP_00017036-RAE450D0.101.001.001.00-1.894nonoEARP_00017036-RAR488C0.900.000.000.000.244nonoEARP_00017036-RAV85I0.201.001.001.000.964nonoEARP_00017037-RDT735A0.301.001.001.000.873nonoEARP_00017072-RAQ136R0.001.001.001.00-0.347yesnoEARP_00017811-RAL136I0.401.001.001.000.386nonoEARP_00017811-RAV1139A1.001.001.000.466nono	EARP_00017027-RB	S286P	0.70	0.00	0.00	0.00	-0.730	no	no
EARP_00017036-RAE450D0.101.001.001.00-1.894nonoEARP_00017036-RAR488C0.900.000.000.000.244nonoEARP_00017036-RAV85I0.201.001.001.000.964nonoEARP_00017037-RDT735A0.301.001.001.000.873nonoEARP_00017072-RAQ136R0.001.001.001.00-0.347yesnoEARP_00017811-RAL136I0.401.001.001.000.386nonoEARP_00017811-RAV1139A1.001.001.001.000.466nono	EARP_00017032-RA	V374I	1.00	0.00	0.00	0.00	0.314	no	yes
EARP_00017036-RAR488C0.900.000.000.000.244nonoEARP_00017036-RAV85I0.201.001.001.000.964nonoEARP_00017037-RDT735A0.301.001.001.000.873nonoEARP_00017072-RAQ136R0.001.001.001.00-0.347yesnoEARP_00017811-RAL136I0.401.001.001.000.386nonoEARP_00017811-RAM336I1.001.001.000.00-1.846nonoEARP_00017811-RAV1139A1.001.001.000.466nono	EARP_00017036-RA	E450D	0.10	1.00	1.00	1.00	-1.894	no	no
EARP_00017036-RAV85I0.201.001.001.000.964nonoEARP_00017037-RDT735A0.301.001.001.000.873nonoEARP_00017072-RAQ136R0.001.001.001.00-0.347yesnoEARP_00017811-RAL136I0.401.001.001.000.386nonoEARP_00017811-RAM336I1.001.001.000.00-1.846nonoEARP_00017811-RAV1139A1.001.001.000.466nono	EARP_00017036-RA	R488C	0.90	0.00	0.00	0.00	0.244	no	no
EARP_00017037-RDT735A0.301.001.001.000.873nonoEARP_00017072-RAQ136R0.001.001.001.00-0.347yesnoEARP_00017811-RAL136I0.401.001.001.000.386nonoEARP_00017811-RAM336I1.001.001.000.00-1.846nonoEARP_00017811-RAV1139A1.001.001.000.466nono	EARP_00017036-RA	V85I	0.20	1.00	1.00	1.00	0.964	no	no
EARP_00017072-RAQ136R0.001.001.001.00-0.347yesnoEARP_00017811-RAL136I0.401.001.001.000.386nonoEARP_00017811-RAM336I1.001.001.000.00-1.846nonoEARP_00017811-RAV1139A1.001.001.000.466nono	EARP_00017037-RD	T735A	0.30	1.00	1.00	1.00	0.873	no	no
EARP_00017811-RAL136I0.401.001.001.000.386nonoEARP_00017811-RAM336I1.001.001.000.00-1.846nonoEARP_00017811-RAV1139A1.001.001.001.000.466nono	EARP_00017072-RA	Q136R	0.00	1.00	1.00	1.00	-0.347	yes	no
EARP_00017811-RAM336I1.001.001.000.00-1.846nonoEARP_00017811-RAV1139A1.001.001.001.000.466nono	EARP_00017811-RA	L136I	0.40	1.00	1.00	1.00	0.386	no	no
EARP_00017811-RA V1139A 1.00 1.00 1.00 1.00 0.466 no no	EARP_00017811-RA	M336I	1.00	1.00	1.00	0.00	-1.846	no	no
	EARP_00017811-RA	V1139A	1.00	1.00	1.00	1.00	0.466	no	no

Genetic diversity (Π) Gene ID Blue Brown-W Brown-C Brown-E EARP_00000007 0.00 0.00 0.00 0.00 EARP_00000044 0.49 0.00 0.00 0.00 EARP_00000045 0.18 0.02 0.02 0.04 EARP_00000046 0.17 0.03 0.02 0.11 EARP_00000047 0.24 0.02 0.02 0.09 EARP 00000048 0.00 0.00 0.00 0.00 EARP_00000097 0.32 0.00 0.01 0.00 0.05 EARP_00000191 0.52 0.46 0.12 EARP_00000192 0.29 0.00 0.00 0.06 EARP_00000193 0.20 0.07 0.07 0.04 EARP_00000194 0.29 0.00 0.00 0.00 EARP_00009935 0.31 0.00 0.00 0.00 EARP_00010171 0.00 0.00 0.00 0.00 0.00 EARP_00010172 0.48 0.00 0.00 EARP_00010173 0.21 0.03 0.03 0.00 EARP_00010174 0.10 0.00 0.00 0.00 0.23 EARP_00010175 0.02 0.02 0.00 0.34 EARP_00002145 0.00 0.01 0.01 EARP_00002146 0.18 0.00 0.00 0.00 EARP_00002147 0.02 0.00 0.15 0.00 EARP_00002148 0.51 0.00 0.00 0.00 EARP_00002231 0.15 0.00 0.05 0.21 EARP_00002232 0.25 0.00 0.06 0.05 EARP 00002233 0.32 0.00 0.05 0.05 EARP_00002234 0.15 0.00 0.06 0.05 0.17 EARP_00002248 0.03 0.04 0.01 EARP_00002249 0.31 0.00 0.00 0.01 EARP 00002250 0.30 0.00 0.00 0.00 EARP_00011416 0.30 0.00 0.00 0.01 EARP_00011418 0.14 0.00 0.00 0.00 EARP_00011419 0.40 0.00 0.00 0.00 EARP_00011420 0.26 0.00 0.00 0.00 0.19 EARP_00011586 0.00 0.00 0.00 0.36 EARP_00011725 0.00 0.06 0.00 EARP_00002633 0.28 0.00 0.00 0.00 EARP_00002634 0.30 0.00 0.00 0.00 EARP_00002780 0.25 0.00 0.00 0.03

Dataset S6: Genetic diversity of the potential adaptive genes. The genetic diversity is the average value of sites-pi within each gene. Blue represents *Crossoptilon auritum*. Brown-W, Brown-C, and Brown-E represent the Western, Central, and Eastern populations of *Crossoptilon mantchuricum*, respectively.

EARP_00002781	0.00	0.00	0.00	0.00
EARP_00002782	0.23	0.00	0.00	0.00
EARP_00002783	0.10	0.00	0.00	0.44
EARP_00012228	0.00	0.00	0.00	0.00
EARP_00012748	0.35	0.00	0.00	0.01
EARP_00012749	0.26	0.00	0.03	0.02
EARP_00012844	0.32	0.01	0.00	0.00
EARP_00012845	0.00	0.00	0.00	0.00
EARP_00012846	0.19	0.00	0.00	0.00
EARP_00012847	0.28	0.00	0.00	0.00
EARP_00012848	0.22	0.01	0.10	0.00
EARP_00012849	0.00	0.00	0.00	0.00
EARP_00012850	0.23	0.00	0.08	0.00
EARP_00012816	0.19	0.00	0.00	0.00
EARP_00012944	0.29	0.00	0.00	0.00
EARP_00012945	0.18	0.00	0.00	0.00
EARP_00012946	0.00	0.00	0.00	0.00
EARP_00013130	0.28	0.00	0.03	0.00
EARP_00013131	0.26	0.00	0.02	0.00
EARP_00013132	0.00	0.00	0.00	0.00
EARP_00013133	0.20	0.00	0.02	0.00
EARP_00013134	0.18	0.00	0.01	0.00
EARP_00013135	0.23	0.00	0.02	0.00
EARP_00013136	0.00	0.00	0.00	0.00
EARP_00013137	0.07	0.00	0.05	0.00
EARP_00013138	0.31	0.00	0.02	0.00
EARP_00013139	0.41	0.00	0.01	0.00
EARP_00013142	0.28	0.01	0.01	0.00
EARP_00003307	0.38	0.00	0.00	0.01
EARP_00013446	0.31	0.00	0.00	0.00
EARP_00013447	0.17	0.00	0.00	0.00
EARP_00013448	0.13	0.00	0.00	0.00
EARP_00013449	0.30	0.00	0.00	0.00
EARP_00013450	0.28	0.00	0.00	0.00
EARP_00003347	0.41	0.00	0.00	0.00
EARP_00003349	0.09	0.00	0.00	0.04
EARP_00013785	0.21	0.00	0.05	0.00
EARP_00013786	0.15	0.00	0.00	0.00
EARP_00013787	0.36	0.00	0.05	0.00
EARP_00000455	0.14	0.00	0.00	0.00
EARP_00003474	0.00	0.00	0.00	0.00
EARP_00013794	0.22	0.00	0.00	0.02
EARP_00014053	0.21	0.00	0.01	0.05
EARP_00014054	0.44	0.00	0.00	0.00

EARP_00014069	0.27	0.00	0.02	0.00
EARP_00014070	0.00	0.00	0.00	0.00
EARP_00014071	0.22	0.00	0.17	0.00
EARP_00014072	0.32	0.00	0.41	0.00
EARP_00014073	0.14	0.05	0.05	0.04
EARP_00014074	0.11	0.03	0.34	0.17
EARP_00014075	0.00	0.00	0.50	0.23
EARP_00003544	0.40	0.00	0.00	0.00
EARP_00003545	0.33	0.00	0.00	0.00
EARP_00003546	0.25	0.00	0.00	0.00
EARP_00003547	0.32	0.01	0.02	0.00
EARP_00003548	0.18	0.04	0.10	0.00
EARP_00003549	0.20	0.00	0.00	0.00
EARP_00003550	0.17	0.29	0.07	0.00
EARP_00014238	0.20	0.00	0.03	0.01
EARP_00014239	0.25	0.02	0.03	0.01
EARP_00014240	0.14	0.00	0.01	0.00
EARP_00014241	0.07	0.00	0.00	0.00
EARP_00014242	0.19	0.00	0.00	0.00
EARP_00014598	0.18	0.00	0.06	0.00
EARP_00014599	0.36	0.00	0.10	0.00
EARP_00014600	0.32	0.01	0.00	0.00
EARP_00014601	0.25	0.00	0.00	0.00
EARP_00015100	0.10	0.01	0.02	0.00
EARP_00015101	0.25	0.01	0.02	0.02
EARP_00015102	0.36	0.00	0.00	0.00
EARP_00015103	0.36	0.00	0.00	0.00
EARP_00003923	0.35	0.00	0.03	0.00
EARP_00015598	0.00	0.00	0.00	0.00
EARP_00015599	0.00	0.00	0.00	0.00
EARP_00015806	0.28	0.22	0.00	0.18
EARP_00015807	0.24	0.03	0.00	0.01
EARP_00015808	0.23	0.00	0.00	0.00
EARP_00016029	0.06	0.12	0.18	0.13
EARP_00016030	0.00	0.00	0.00	0.00
EARP_00016031	0.19	0.00	0.00	0.00
EARP_00016032	0.13	0.10	0.13	0.13
EARP_00016033	0.14	0.09	0.08	0.13
EARP_00016034	0.13	0.12	0.18	0.00
EARP_00016035	0.19	0.00	0.00	0.00
EARP_00016036	0.09	0.04	0.06	0.20
EARP_00016102	0.12	0.00	0.00	0.00
EARP_00000574	0.10	0.00	0.00	0.00
EARP_00000596	0.16	0.00	0.05	0.03

EARP_00000597	0.25	0.04	0.04	0.04
EARP_00000598	0.00	0.00	0.00	0.00
EARP_00004745	0.26	0.00	0.01	0.03
EARP_00004746	0.31	0.00	0.00	0.53
EARP_00016471	0.33	0.00	0.01	0.00
EARP_00016472	0.31	0.00	0.00	0.00
EARP_00016473	0.27	0.00	0.00	0.00
EARP_00016474	0.22	0.00	0.00	0.00
EARP_00016475	0.27	0.00	0.02	0.00
EARP_00017025	0.32	0.00	0.00	0.00
EARP_00017026	0.41	0.00	0.00	0.00
EARP_00017027	0.27	0.00	0.00	0.02
EARP_00017028	0.34	0.00	0.00	0.00
EARP_00017029	0.16	0.00	0.00	0.02
EARP_00017030	0.02	0.00	0.00	0.00
EARP_00017031	0.30	0.00	0.00	0.00
EARP_00017032	0.37	0.00	0.00	0.00
EARP_00017033	0.38	0.00	0.00	0.05
EARP_00017034	0.13	0.00	0.20	0.00
EARP_00017035	0.00	0.00	0.00	0.00
EARP_00017036	0.25	0.00	0.00	0.00
EARP_00017037	0.27	0.00	0.03	0.02
EARP_00017038	0.04	0.00	0.05	0.00
EARP_00017066	0.20	0.00	0.01	0.00
EARP_00017067	0.26	0.00	0.06	0.00
EARP_00017068	0.18	0.00	0.00	0.00
EARP_00017069	0.39	0.00	0.01	0.00
EARP_00017070	0.34	0.00	0.01	0.00
EARP_00017071	0.29	0.00	0.00	0.00
EARP_00017072	0.37	0.01	0.01	0.00
EARP_00005078	0.17	0.00	0.08	0.16
EARP_00005079	0.13	0.00	0.00	0.00
EARP_00005080	0.30	0.00	0.00	0.00
EARP_00017302	0.43	0.00	0.00	0.00
EARP_00017303	0.24	0.00	0.00	0.07
EARP_00017606	0.00	0.00	0.00	0.00
EARP_00017607	0.09	0.00	0.00	0.00
EARP_00017698	0.00	0.00	0.00	0.00
EARP_00017699	0.25	0.00	0.00	0.00
EARP_00017707	0.44	0.00	0.00	0.00
EARP_00017708	0.17	0.05	0.14	0.00
EARP_00017709	0.24	0.02	0.05	0.00
EARP_00017809	0.10	0.00	0.00	0.01
EARP_00017810	0.06	0.00	0.00	0.00

EARP_00017811	0.08	0.00	0.00	0.00
EARP_00017812	0.10	0.00	0.00	0.00
EARP_00006312	0.33	0.00	0.03	0.00
EARP_00006313	0.31	0.00	0.03	0.00
EARP_00006329	0.36	0.00	0.00	0.00
EARP_00006344	0.34	0.00	0.11	0.00
EARP_00006349	0.23	0.00	0.01	0.27
EARP_00006350	0.31	0.00	0.03	0.06
EARP_00006351	0.26	0.00	0.00	0.02
EARP_00006352	0.00	0.00	0.00	0.00
EARP_00006353	0.04	0.00	0.02	0.43
EARP_00006354	0.17	0.00	0.00	0.33
EARP_00006355	0.14	0.00	0.01	0.15
EARP_00006356	0.28	0.00	0.03	0.17
EARP_00006357	0.19	0.00	0.13	0.00
EARP_00000889	0.23	0.00	0.00	0.26
EARP_00006613	0.25	0.00	0.02	0.00
EARP_00006614	0.24	0.00	0.02	0.00
EARP_00006615	0.00	0.00	0.00	0.00
EARP_00006616	0.38	0.00	0.00	0.00
EARP_00018561	0.11	0.00	0.00	0.00
EARP_00018562	0.26	0.00	0.00	0.00
EARP_00018563	0.00	0.00	0.00	0.00
EARP_00018564	0.00	0.00	0.00	0.00
EARP_00006824	0.34	0.06	0.06	0.00
EARP_00006825	0.19	0.07	0.08	0.00
EARP_00000981	0.28	0.28	0.13	0.11
EARP_00000982	0.26	0.00	0.00	0.04
EARP_00000983	0.13	0.07	0.03	0.00
EARP_00000984	0.17	0.03	0.01	0.01
EARP_00000985	0.00	0.00	0.00	0.00
EARP_00000986	0.12	0.08	0.04	0.07
EARP_00000987	0.14	0.04	0.02	0.03
EARP_00000988	0.13	0.00	0.00	0.04
EARP_00000989	0.14	0.03	0.02	0.02
EARP_00000990	0.20	0.03	0.01	0.02
EARP_00007488	0.38	0.00	0.04	0.00
EARP_00007489	0.35	0.00	0.09	0.00
EARP_00007490	0.29	0.01	0.04	0.00
EARP_00007491	0.16	0.00	0.03	0.00
EARP_00007492	0.13	0.00	0.03	0.00
EARP_00007493	0.24	0.00	0.02	0.00
EARP_00007494	0.27	0.00	0.02	0.00
EARP_00007499	0.28	0.03	0.08	0.00

EARP_00007507	0.14	0.14	0.06	0.00
EARP_00007508	0.00	0.00	0.00	0.00
EARP_00007509	0.07	0.01	0.03	0.00
EARP_00007510	0.14	0.01	0.00	0.00
EARP_00007511	0.04	0.01	0.02	0.00
EARP_00007512	0.06	0.01	0.02	0.00
EARP_00007520	0.00	0.00	0.00	0.00
EARP_00007521	0.20	0.00	0.01	0.00
EARP_00007522	0.20	0.00	0.00	0.00
EARP_00007523	0.15	0.09	0.11	0.00
EARP_00018845	0.52	0.00	0.00	0.00
EARP_00018891	0.48	0.00	0.00	0.00
EARP_00018892	0.00	0.00	0.00	0.00
EARP_00018893	0.46	0.00	0.00	0.00
EARP_00018894	0.37	0.00	0.00	0.00
EARP_00018895	0.00	0.00	0.00	0.00
EARP_00018896	0.47	0.00	0.00	0.00
EARP_00018902	0.05	0.00	0.00	0.00
EARP_00008168	0.23	0.00	0.16	0.00
EARP_00008169	0.09	0.00	0.02	0.00
EARP_00008170	0.00	0.00	0.00	0.00
EARP_00008578	0.19	0.08	0.06	0.00
EARP_00008598	0.33	0.00	0.01	0.00
EARP_00008599	0.31	0.00	0.02	0.00
EARP_00008600	0.24	0.00	0.07	0.00
EARP_00008601	0.37	0.00	0.02	0.00
EARP_00008602	0.21	0.00	0.09	0.00
EARP_00008603	0.29	0.00	0.06	0.00
EARP_00008888	0.23	0.09	0.02	0.10
EARP_00011127	0.00	0.00	0.00	0.00
EARP_00011128	0.00	0.00	0.00	0.00
EARP_00011129	0.15	0.03	0.01	0.00
EARP_00011680	0.05	0.00	0.00	0.00
EARP_00011865	0.12	0.00	0.01	0.03
EARP_00011866	0.05	0.00	0.00	0.00
EARP_00011867	0.15	0.00	0.00	0.00
EARP_00011868	0.02	0.00	0.00	0.05
EARP_00011869	0.07	0.00	0.00	0.00
EARP_00012502	0.00	0.00	0.00	0.05
EARP_00012505	0.16	0.00	0.00	0.00
EARP_00012506	0.00	0.00	0.00	0.00
EARP_00012507	0.00	0.00	0.00	0.00
EARP_00012508	0.07	0.00	0.00	0.00
EARP_00012509	0.22	0.00	0.00	0.00

EARP_00012510	0.10	0.00	0.00	0.01
EARP_00012511	0.18	0.04	0.07	0.01
EARP_00012512	0.20	0.00	0.00	0.00
EARP_00012513	0.13	0.00	0.00	0.01
EARP_00012514	0.31	0.00	0.00	0.00
EARP_00012515	0.07	0.00	0.00	0.00
EARP_00012516	0.01	0.00	0.00	0.00
EARP_00012517	0.05	0.00	0.00	0.01
EARP_00012518	0.07	0.00	0.00	0.00
EARP_00012519	0.12	0.00	0.00	0.00
EARP_00012520	0.13	0.00	0.00	0.00
EARP_00012521	0.09	0.00	0.00	0.01
EARP_00013700	0.20	0.00	0.00	0.00
EARP_00013701	0.22	0.00	0.00	0.00
EARP_00013702	0.00	0.00	0.00	0.00
EARP_00013703	0.02	0.00	0.00	0.00
EARP_00013704	0.12	0.07	0.06	0.03
EARP_00014256	0.10	0.00	0.00	0.00
EARP_00014257	0.12	0.00	0.01	0.01
EARP_00014258	0.10	0.00	0.00	0.02
EARP_00014259	0.12	0.00	0.01	0.02
EARP_00014269	0.37	0.00	0.00	0.00
EARP_00014270	0.32	0.17	0.18	0.18
EARP_00015298	0.22	0.00	0.00	0.00
EARP_00015299	0.10	0.00	0.00	0.00
EARP_00015303	0.21	0.00	0.01	0.00
EARP_00015304	0.05	0.00	0.00	0.03
EARP_00015305	0.14	0.00	0.00	0.01
EARP_00015306	0.11	0.00	0.00	0.00
EARP_00015426	0.27	0.00	0.00	0.01
EARP_00005020	0.34	0.00	0.00	0.00
EARP_00005021	0.00	0.00	0.00	0.00
EARP_00005022	0.05	0.00	0.04	0.00
EARP_00005028	0.06	0.01	0.02	0.00
EARP_00017436	0.00	0.00	0.00	0.00
EARP_00017437	0.05	0.00	0.00	0.02
EARP_00017591	0.12	0.01	0.01	0.01
EARP_00017592	0.08	0.00	0.00	0.00
EARP_00017593	0.28	0.31	0.30	0.24
EARP_00006966	0.17	0.00	0.00	0.00
EARP_00006967	0.10	0.00	0.00	0.00
EARP_00008636	0.20	0.00	0.00	0.01
EARP_00008638	0.00	0.00	0.00	0.00
EARP_00008639	0.07	0.00	0.00	0.00

EARP_00008640	0.00	0.00	0.00	0.00
EARP_00008641	0.00	0.00	0.00	0.00
EARP_00008643	0.26	0.00	0.00	0.02
EARP_00008644	0.10	0.18	0.20	0.00
EARP_00008645	0.08	0.00	0.00	0.00
EARP_00008646	0.08	0.00	0.00	0.00
EARP_00009516	0.31	0.00	0.02	0.00
Average	0.19	0.01	0.03	0.02

Dataset S7: Likelihood analysis of different evolutionary scenarios. In the models, S represents consistent small population size, D represents population decline, I indicates that there was no gene flow between adjacent populations, WCE indicates that there was gene flow between adjacent populations, CW indicates that the gene flow only existed between the Western and Central populations, CE indicates that migration only occurred between the Central and Eastern populations, and IN indicates that inbreeding occurred. The likelihood (ln) was estimated using fastsimcoal26. d represents the number of parameters in the different models. AIC represents the Akaike information criterion, w represents Akaike weight.

Models	ln(Likelihood)	d	AIC _i	Δ_{i}	w _i
S+I	-47915.76	9.00	95849.52	6566.44	0.00
S+WCE	-45039.69	23.00	90125.37	842.30	0.00
S+CW	-45231.52	16.00	90495.04	1211.96	0.00
S+CE	-44783.52	16.00	89599.04	315.97	0.00
D+I	-44617.25	28.00	89290.49	7.42	0.02
D+WCE	-54029.39	34.00	108126.79	18843.71	0.00
D+CW	-44689.21	31.00	89440.41	157.34	0.00
D+CE	-44653.58	31.00	89369.16	86.09	0.00
S+I+IN	-47926.01	12.00	95876.01	6592.94	0.00
S+WCE+IN	-45031.20	26.00	90114.40	831.32	0.00
S+CW+IN	-45232.04	19.00	90502.07	1219.00	0.00
S+CE+IN	-44771.16	19.00	89580.32	297.25	0.00
D+I+IN	-44610.54	31.00	89283.07	0.00	0.98
D+WCE+IN	-47953.29	37.00	95980.58	6697.50	0.00
D+CW+IN	-44692.62	34.00	89453.24	170.16	0.00
D+CE+IN	-44657.87	34.00	89383.75	100.67	0.00

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Model	Log(ml)	Log(bf)	Model-probability	Custom migration model
Migration between adjacent populations	-1403.43	-7.87	0.0004	Brown-W, Brown-C, Brown- E={**0 *** 0**}
Migration between Brown-W and Brown-C	-1402.01	-6.45	0.0015	Brown-W, Brown-C, Brown- E={**0 **0 00*}
Migration between Brown-C and Brown-E	-1398.59	-3.03	0.046	Brown-W, Brown-C, Brown- E={*00 0** 0**}
Isolation	-1395.56	0	0.9521	Brown-W, Brown-C, Brown- E={*00 0*0 00*}

Dataset S8: Model probability estimated in Migrate-N. Brown-W, Brown-C, and Brown-E indicate the Western, Central, and Eastern populations of *Crossoptilon mantchuricum*, respectively. ml is the marginal likelihood. bf is the Bayes factor.

Dataset S9: Sample information, coverage, and mapping rate. The asterisks mark the individuals that were excluded from the population genetic analysis because they had a close relationship (duplicate/MZ twin, 1st-degree) with at least one individual in the corresponding population. The sequence data of all individuals were deposited in the National Genomics Data Center (https://bigd.big.ac.cn/?lang=en). Readers can use the accession number to find the data in the National Genomics Data Center Datavases.

Species	Location	Tissue type	Gender	Sample index	Sample ID	Sequencing type	Sequencing coverage (×)	Mappin g rate	Accession number
C. auritum	Gansu	Blood	Female	1	CA00	Re-sequencing	41.57	98.53%	SAMC226688
C. auritum	Gansu	Blood	Female	2	CA01	Re-sequencing	16.03	97.86%	SAMC226689
C. auritum	Gansu	Blood	Male	3	CA02	Re-sequencing	17.06	98.48%	SAMC226690
C. auritum	Gansu	Blood	Female	4	CA03*	Re-sequencing	16.73	98.24%	SAMC226691
C. auritum	Gansu	Blood	Female	5	CA04	Re-sequencing	20.68	98.49%	SAMC226692
C. auritum	Gansu	Blood	Female	6	CA05	Re-sequencing	17.67	98.05%	SAMC226693
C. auritum	Gansu	Blood	Male	7	CA06	Re-sequencing	18.96	98.58%	SAMC226694
C. auritum	Gansu	Blood	Male	8	CA07	Re-sequencing	16.48	98.74%	SAMC226695
C. auritum	Gansu	Blood	Male	9	CA08	Re-sequencing	16.33	98.68%	SAMC226696
C. auritum	Gansu	Blood	Male	10	CA09	Re-sequencing	16.93	98.54%	SAMC226697
C. auritum	Gansu	Blood	Female	11	CA10	Re-sequencing	18.20	98.09%	SAMC226698
C. mantchuricum	Shaanxi(Western population)	Blood	Female	12	CMAA00	Re-sequencing	43.17	98.59%	SAMC226699
C. mantchuricum	Shaanxi(Western population)	Blood	Female	13	CMAA01	Re-sequencing	15.60	98.54%	SAMC226700

C. mantchuricum	Shaanxi(Western population)	Blood	Male	14	CMAA02 Re-sequencing	15.89	98.65%	SAMC226701
C. mantchuricum	Shaanxi(Western population)	Muscle	Female	15	CMAA03 Re-sequencing	15.31	97.56%	SAMC226702
C. mantchuricum	Shaanxi(Western population)	Blood	Male	16	CMAA04* Re-sequencing	17.64	98.85%	SAMC226703
C. mantchuricum	Shaanxi(Western population)	Blood	Female	17	CMAA05 Re-sequencing	18.86	97.42%	SAMC226704
C. mantchuricum	Shaanxi(Western population)	Blood	Male	18	CMAA06 Re-sequencing	18.56	93.89%	SAMC226705
C. mantchuricum	Shaanxi(Western population)	Blood	Female	19	CMAA07* Re-sequencing	16.07	95.51%	SAMC226706
C. mantchuricum	Shaanxi(Western population)	Blood	Female	20	CMAA08 Re-sequencing	20.81	98.60%	SAMC226707
C. mantchuricum	Shaanxi(Western population)	Blood	Female	21	CMAA09* Re-sequencing	15.53	98.75%	SAMC226708
C. mantchuricum	Shaanxi(Western population)	Blood	Female	22	CMAA10 Re-sequencing	13.42	86.63%	SAMC226709
C. mantchuricum	Shaanxi(Western population)	Blood	Male	23	CMAA11* Re-sequencing	21.99	96.29%	SAMC226710
C. mantchuricum	Shaanxi(Western population)	Blood	Female	24	CMAA12* Re-sequencing	19.77	96.96%	SAMC226711
C. mantchuricum	Shaanxi(Western population)	Blood	Female	25	CMAA13 Re-sequencing	18.19	97.43%	SAMC226712
C. mantchuricum	Shaanxi(Western population)	Blood	Female	26	CMAA14 Re-sequencing	18.36	96.47%	SAMC226713
C. mantchuricum	Shaanxi(Western population)	Blood	Female	27	CMAA15* Re-sequencing	15.59	93.59%	SAMC226714
C. mantchuricum	Shaanxi(Western population)	Blood	Male	28	CMAA16 Re-sequencing	16.82	97.81%	SAMC226715
C. mantchuricum	Shaanxi(Western population)	Blood	Male	29	CMAA17* Re-sequencing	13.60	83.55%	SAMC226716
C. mantchuricum	Shanxi(Central population)	Muscle	Male	30	CMA04* Re-sequencing	15.94	98.39%	SAMC226717

C. mantchuricum	Shanxi(Central population)	Blood	Female	31	CMA08	Re-sequencing	15.21	98.24%	SAMC226718
C. mantchuricum	Shanxi(Central population)	Muscle	Female	32	CMA09	Re-sequencing	19.23	97.51%	SAMC226719
C. mantchuricum	Shanxi(Central population)	Liver	Female	33	CMA10*	Re-sequencing	16.77	97.81%	SAMC226720
C. mantchuricum	Shanxi(Central population)	Muscle	Female	34	CMA11	Re-sequencing	19.53	96.86%	SAMC226721
C. mantchuricum	Shanxi(Central population)	Liver	Female	35	CMA12	Re-sequencing	18.26	97.80%	SAMC226722
C. mantchuricum	Shanxi(Central population)	Liver	Male	36	CMA13	Re-sequencing	21.46	97.98%	SAMC226723
C. mantchuricum	Shanxi(Central population)	Liver	Male	37	CMA14	Re-sequencing	19.13	98.39%	SAMC226724
C. mantchuricum	Shanxi(Central population)	Liver	Female	38	CMA15*	Re-sequencing	18.19	97.54%	SAMC226725
C. mantchuricum	Shanxi(Central population)	Muscle	Female	39	CMA16	Re-sequencing	16.03	97.79%	SAMC226726
C. mantchuricum	Shanxi(Central population)	Muscle	Male	40	CMA18	Re-sequencing	15.32	97.62%	SAMC226727
C. mantchuricum	Hebei(Eastern population)	Blood	Male	41	CMH00	Re-sequencing	36.59	98.26%	SAMC226728
C. mantchuricum	Hebei(Eastern population)	Blood	Female	42	CMH01	Re-sequencing	17.40	98.53%	SAMC226729
C. mantchuricum	Hebei(Eastern population)	Blood	Male	43	CMH02	Re-sequencing	19.22	98.65%	SAMC226730
C. mantchuricum	Hebei(Eastern population)	Blood	Female	44	CMH03	Re-sequencing	19.54	98.12%	SAMC226731
C. mantchuricum	Hebei(Eastern population)	Muscle	Male	45	CMH04*	Re-sequencing	18.79	97.85%	SAMC226732
C. mantchuricum	Hebei(Eastern population)	Blood	Male	46	CMH05	Re-sequencing	18.30	98.63%	SAMC226733
C. mantchuricum	Hebei(Eastern population)	Muscle	Male	47	CMH06	Re-sequencing	19.69	97.85%	SAMC226734

C. mantchuricum	Hebei(Eastern population)	Blood	Female	48	CMH07*	Re-sequencing	17.60	98.34%	SAMC226735
C. mantchuricum	Hebei(Eastern population)	Blood	Female	49	CMH08	Re-sequencing	15.51	97.95%	SAMC226736
C. mantchuricum	Hebei(Eastern population)	Blood	Female	50	CMH09	Re-sequencing	18.92	98.19%	SAMC226737
C. mantchuricum	Hebei(Eastern population)	Muscle	Male	51	CMH12*	Re-sequencing	17.60	97.77%	SAMC226738
C. mantchuricum	Shanxi(Central population)	Blood	Female	52	CMA00*	De novo sequencing	201.75	Ν	SAMC226739

Dataset S10: The relationship of each sample in each population inferred by the King program. Blue indicates *Crossoptilon auritum*. Brown-W, Brown-C, and Brown-E indicate the Western, Central, and Eastern populations of *Crossoptilon mantchuricum*, respectively. ID1: The first individual of the pair; ID2: The second individual of the pair; N_SNP: The number of SNPS that do not have missing SNPS in either of the individuals; HetHet: Percentage of SNPs with double heterozygotes; IBS0: Proportion of SNPs with 0-IBS (identical-by-state); Kinship: Kinship coefficient estimated by the program. The asterisks mark the individuals that were excluded in the downstream analysis.

Population	ID1	ID2	N_SNP	HetHet	IBS0	Kinship
Blue	CA00	CA1	2275444	15.29%	8.02%	-0.06
Blue	CA00	CA10	2275444	12.22%	12.15%	-0.20
Blue	CA00	CA2	2275444	13.75%	7.01%	0.00
Blue	CA00	CA3*	2275444	13.94%	7.15%	-0.01
Blue	CA00	CA4	2275444	11.88%	12.58%	-0.23
Blue	CA00	CA5	2275444	12.65%	9.10%	-0.10
Blue	CA00	CA6	2275444	12.14%	12.50%	-0.22
Blue	CA00	CA7	2275444	11.95%	12.38%	-0.21
Blue	CA00	CA8	2275444	12.02%	12.52%	-0.22
Blue	CA00	CA9	2275444	12.15%	12.33%	-0.21
Blue	CA1	CA10	2275444	15.29%	7.84%	-0.06
Blue	CA1	CA2	2275444	15.24%	7.92%	-0.06
Blue	CA1	CA3*	2275444	17.07%	0.30%	0.21
Blue	CA1	CA4	2275444	14.83%	8.22%	-0.09
Blue	CA1	CA5	2275444	14.68%	8.77%	-0.11
Blue	CA1	CA6	2275444	15.06%	8.01%	-0.08
Blue	CA1	CA7	2275444	15.06%	8.17%	-0.08
Blue	CA1	CA8	2275444	14.94%	8.36%	-0.09
Blue	CA1	CA9	2275444	15.15%	7.82%	-0.07
Blue	CA10	CA2	2275444	12.19%	12.07%	-0.19
Blue	CA10	CA3*	2275444	12.59%	12.35%	-0.20
Blue	CA10	CA4	2275444	13.34%	7.26%	-0.03
Blue	CA10	CA5	2275444	12.04%	11.43%	-0.18
Blue	CA10	CA6	2275444	13.54%	7.25%	-0.02
Blue	CA10	CA7	2275444	13.39%	7.44%	-0.03
Blue	CA10	CA8	2275444	13.30%	7.30%	-0.03
Blue	CA10	CA9	2275444	13.39%	7.49%	-0.03
Blue	CA2	CA3*	2275444	13.73%	7.19%	-0.02
Blue	CA2	CA4	2275444	12.00%	12.38%	-0.22
Blue	CA2	CA5	2275444	12.55%	9.02%	-0.10
Blue	CA2	CA6	2275444	12.05%	12.22%	-0.21
Blue	CA2	CA7	2275444	12.16%	12.21%	-0.20
Blue	CA2	CA8	2275444	11.95%	12.25%	-0.21
Blue	CA2	CA9	2275444	12.20%	12.12%	-0.20
Blue	CA3*	CA4	2275444	12.33%	12.66%	-0.23
Blue	CA3*	CA5	2275444	12.95%	9.17%	-0.10
Blue	CA3*	CA6	2275444	12.36%	12.56%	-0.22
Blue	CA3*	CA7	2275444	12.51%	12.56%	-0.22
Blue	CA3*	CA8	2275444	12.39%	12.66%	-0.23
Blue	CA3*	CA9	2275444	12.57%	12.53%	-0.21
Blue	CA4	CA5	2275444	11.66%	11.95%	-0.20

Blue	CA4	CA6	2275444	13.02%	7.41%	-0.03
Blue	CA4	CA7	2275444	13.20%	7.05%	-0.02
Blue	CA4	CA8	2275444	13.06%	7.44%	-0.03
Blue	CA4	CA9	2275444	13.01%	7.46%	-0.03
Blue	CA5	CA6	2275444	11.78%	11.62%	-0.18
Blue	CA5	CA7	2275444	11.91%	11.58%	-0.18
Blue	CA5	CA8	2275444	11.73%	11.61%	-0.19
Blue	CA5	CA9	2275444	11.96%	11.63%	-0.18
Blue	CA6	CA7	2275444	13.28%	7.55%	-0.03
Blue	CA6	CA8	2275444	13.00%	7.30%	-0.03
Blue	CA6	CA9	2275444	13.17%	7.09%	-0.02
Blue	CA7	CA8	2275444	13.15%	7.40%	-0.03
Blue	CA7	CA9	2275444	13.46%	6.85%	0.00
Blue	CA8	CA9	2275444	13.21%	7.50%	-0.03
Brown-W	CMAA00	CMAA1	2275444	0.98%	0.53%	-0.03
Brown-W	CMAA00	CMAA10	2275444	0.79%	0.61%	-0.20
Brown-W	CMAA00	CMAA11*	2275444	1.24%	0.20%	0.14
Brown-W	CMAA00	CMAA12*	2275444	1.02%	0.69%	-0.09
Brown-W	CMAA00	CMAA13	2275444	1.16%	0.34%	0.05
Brown-W	CMAA00	CMAA14	2275444	1.39%	0.43%	0.07
Brown-W	CMAA00	CMAA15*	2275444	1.33%	0.40%	0.07
Brown-W	CMAA00	CMAA16	2275444	1.26%	0.27%	0.09
Brown-W	CMAA00	CMAA17*	2275444	1.10%	0.60%	-0.03
Brown-W	CMAA1	CMAA10	2275444	1.05%	0.22%	0.07
Brown-W	CMAA1	CMAA11*	2275444	1.06%	0.45%	0.02
Brown-W	CMAA1	CMAA12*	2275444	0.90%	0.93%	-0.18
Brown-W	CMAA1	CMAA13	2275444	0.96%	0.35%	0.03
Brown-W	CMAA1	CMAA14	2275444	1 19%	0.44%	0.02
Brown-W	CMAA1	CMAA15*	2275444	1 30%	0.25%	0.02
Brown-W	CMAA1	CMAA16	2275444	1.56%	0.33%	0.10
Brown-W	CMAA1	CMAA17*	2275444	1.10%	0.33%	0.01
Brown-W	CMAA10	CMAA11*	2275444	0.70%	0.42%	-0.15
Brown-W	CMAA10	CMAA11	2275444	0.65%	0.30%	-0.15
Brown-W	CMAA10	CMAA12	2275444	0.05%	0.77%	-0.20
Brown-W	CMAA10	CMAA14	2275444	0.70%	0.00%	-0.15
Brown-W	CMAA10	CMAA15*	2275444	0.89%	0.50%	-0.13
Brown W	CMAA10	CMAA15	2275444	0.96%	0.0170	-0.15
Brown W	CMAA10	CMAA17*	2275444	0.73%	0.40%	-0.13
Brown W	CMAA10	CMAA17*	2275444	0.73%	0.63%	-0.23
Brown W	CMAA11*	CMAA12	2275444	1.40%	0.05%	-0.00
Brown W	CMAA11*	CMAA13	2275444	1.40%	0.03%	0.23
Brown W	CMAA11*	CMAA14	2275444	1.1270	0.43%	0.01
$\frac{DIOWII-W}{Drown}W$	CMAA11*	CMAA15	2275444	1.43%	0.1270	0.16
Brown W	CMAA11*	CMAA17*	2275444	1.37%	0.22%	0.10
Drown-W	$CMAA11^*$	$CMAA1/^{*}$	2275444	1.18%	0.27%	0.11
Brown-W	$CMAA12^*$	CMAA13	2275444	0.84%	0.70%	-0.14
Brown-w	CMAA12*	CMAA14	2275444	1.03%	0.05%	-0.09
Brown-w	CMAA12*	CMAA15*	2275444	1.10%	0.63%	-0.08
Brown-W	CMAA12*	CMAA16	2275444	1.13%	0.82%	-0.16
Brown-W	CMAA12*	CMAAI/*	2275444	0.82%	0.98%	-0.22
Brown-W	CMAA13	CMAA14	22/5444	1.16%	0.44%	-0.01
Brown-W	CMAA13	CMAA15*	2275444	1.28%	0.15%	0.12
Brown-W	CMAA13	CMAA16	2275444	1.33%	0.26%	0.08
Brown-W	CMAA13	CMAA17*	2275444	1.10%	0.35%	0.05

Brown-W	CMAA14	CMAA15*	2275444	1.27%	0.43%	0.06
Brown-W	CMAA14	CMAA16	2275444	1.42%	0.24%	0.14
Brown-W	CMAA14	CMAA17*	2275444	1.11%	0.50%	-0.02
Brown-W	CMAA15*	CMAA16	2275444	1.73%	0.02%	0.26
Brown-W	CMAA15*	CMAA17*	2275444	1.37%	0.04%	0.20
Brown-W	CMAA16	CMAA17*	2275444	1.94%	0.21%	0.23
Brown-W	CMAA00	CMAA2	2275444	0.62%	1.05%	-0.65
Brown-W	CMAA00	CMAA3	2275444	1.48%	0.59%	0.00
Brown-W	CMAA00	CMAA4*	2275444	1.20%	0.52%	0.00
Brown-W	CMAA00	CMAA5	2275444	1.33%	0.63%	-0.01
Brown-W	CMAA00	CMAA6	2275444	1.21%	0.52%	0.00
Brown-W	CMAA00	CMAA7*	2275444	0.90%	0.69%	-0.09
Brown-W	CMAA00	CMAA8	2275444	1.52%	0.60%	0.04
Brown-W	CMAA00	CMAA9*	2275444	1.02%	0.68%	-0.07
Brown-W	CMAA1	CMAA2	2275444	0.86%	0.02%	0.09
Brown-W	CMAA1	CMAA3	2275444	1.08%	0.58%	-0.09
Brown-W	CMAA1	CMAA4*	2275444	1.19%	0.62%	-0.05
Brown-W	CMAA1	CMAA5	2275444	0.83%	0.41%	-0.04
Brown-W	CMAA1	CMAA6	2275444	1.20%	0.62%	-0.05
Brown-W	CMAA1	CMAA7*	2275444	1.07%	0.95%	-0.18
Brown-W	CMAA1	CMAA8	2275444	1.08%	0.30%	0.05
Brown-W	CMAA1	CMAA9*	2275444	1.10%	0.61%	-0.05
Brown-W	CMAA10	CMAA2	2275444	0.69%	0.73%	-0.31
Brown-W	CMAA10	CMAA3	2275444	1.03%	0.63%	-0.22
Brown-W	CMAA10	CMAA4*	2275444	0.82%	0.57%	-0.20
Brown-W	CMAA10	CMAA5	2275444	0.81%	0.42%	-0.14
Brown-W	CMAA10	CMAA6	2275444	0.83%	0.56%	-0.20
Brown-W	CMAA10	CMAA7*	2275444	0.79%	0.99%	-0.38
Brown-W	CMAA10	CMAA8	2275444	0.73%	0.48%	-0.18
Brown-W	CMAA10	CMAA9*	2275444	0.85%	0.72%	-0.25
Brown-W	CMAA11*	CMAA2	2275444	0.71%	0.75%	-0.42
Brown-W	CMAA11*	CMAA3	2275444	1.30%	0.40%	0.03
Brown-W	CMAA11*	CMAA4*	2275444	1 23%	0.36%	0.06
Brown-W	CMAA11*	CMAA5	2275444	1.20%	0.38%	0.05
Brown-W	CMAA11*	CMAA6	2275444	1.20%	0.35%	0.05
Brown-W	CMAA11*	CMAA7*	2275444	1.02%	0.35%	-0.01
Brown-W	CMAA11*	CMAA8	2275444	1.02%	0.47%	0.01
Brown-W	CMAA11*	CMAA9*	2275444	1.57%	0.52%	0.03
Brown-W	CMAA12*	CMAA2	2275444	0.40%	1.08%	-0.71
Brown-W	CMAA12*	CMAA3	2275444	1.00%	0.67%	-0.14
Brown-W	CMAA12*	CMAA4*	2275444	1.00%	0.57%	-0.14
Brown-W	CMAA12*	CMAA5	2275444	1.20%	0.52%	-0.02
Brown-W	$CM\Delta\Delta 12*$	CMAA6	2275444	1.40%	0.12%	-0.02
Brown-W	CMAA12*	CMAA7*	2275444	1.21%	0.52%	-0.02
Brown-W	CMAA12*	CMAA	2275444	1.00%	0.70%	-0.12
Brown-W	CMAA12 CMAA12*	CMAA0*	2275444	1.40%	0.72%	-0.08
Brown-W	$CM\Delta \Delta 12$	$CM\Delta\Delta2$	2275444	0.64%	0.65%	-0.03
Brown W	CMA 13	CMAA2	2275444	1 100%	0.05%	-0.55
Brown W	CMAA13	CMAA3	2213444 2275111	1.17% 1 NQ0/	0.47%	-0.00 0.06
Brown W	CMAA13	CMAA4	2213444 2275111	1.00% 1.0/10/	0.31%	-0.00
Brown W	CMAA13	CMAAS	2273444 2275111	1.0 4 70 1 ∩00⁄2	0.57%	-0.01
Brown W	CMA 13	CMAA7*	2275444	0.800%	0.50%	-0.00
Brown W	CMA 12		2213444 2275111	1 000%	0.0270	-0.14
	CIVIAAIS	CIMAAO	<i>LLIJ</i> 444	1.07%	0.2370	0.05

Brown-W	CMAA13	CMAA9*	2275444	0.98%	0.46%	-0.04
Brown-W	CMAA14	CMAA2	2275444	0.66%	0.75%	-0.49
Brown-W	CMAA14	CMAA3	2275444	1.44%	0.45%	0.06
Brown-W	CMAA14	CMAA4*	2275444	1.25%	0.60%	0.01
Brown-W	CMAA14	CMAA5	2275444	1.19%	0.37%	0.07
Brown-W	CMAA14	CMAA6	2275444	1.26%	0.59%	0.01
Brown-W	CMAA14	CMAA7*	2275444	1.26%	0.88%	-0.11
Brown-W	CMAA14	CMAA8	2275444	1.36%	0.29%	0.12
Brown-W	CMAA14	CMAA9*	2275444	1.24%	0.65%	-0.02
Brown-W	CMAA15*	CMAA2	2275444	0.81%	0.54%	-0.33
Brown-W	CMAA15*	CMAA3	2275444	1.37%	0.40%	0.07
Brown-W	CMAA15*	CMAA4*	2275444	1.34%	0.50%	0.05
Brown-W	CMAA15*	CMAA5	2275444	1.57%	0.55%	0.07
Brown-W	CMAA15*	CMAA6	2275444	1.35%	0.49%	0.06
Brown-W	CMAA15*	CMAA7*	2275444	1.16%	0.66%	-0.05
Brown-W	CMAA15*	CMAA8	2275444	1.28%	0.31%	0.10
Brown-W	CMAA15*	CMAA9*	2275444	1.43%	0.60%	0.02
Brown-W	CMAA16	CMAA2	2275444	1.01%	0.52%	-0.27
Brown-W	CMAA16	CMAA3	2275444	1.50%	0.44%	0.08
Brown-W	CMAA16	CMAA4*	2275444	1.30%	0.36%	0.08
Brown-W	CMAA16	CMAA5	2275444	1.33%	0.47%	0.05
Brown-W	CMAA16	CMAA6	2275444	1.31%	0.36%	0.09
Brown-W	CMAA16	CMAA7*	2275444	1.24%	0.56%	-0.01
Brown-W	CMAA16	CMAA8	2275444	1.35%	0.37%	0.08
Brown-W	CMAA16	CMAA9*	2275444	1.28%	0.64%	-0.03
Brown-W	CMAA17*	CMAA2	2275444	0.73%	0.50%	-0.26
Brown-W	CMAA17*	CMAA3	2275444	1.30%	0.42%	0.02
Brown-W	CMAA17*	CMAA4*	2275444	1.13%	0.70%	-0.09
Brown-W	CMAA17*	CMAA5	2275444	1.05%	0.51%	-0.03
Brown-W	CMAA17*	CMAA6	2275444	1.14%	0.69%	-0.08
Brown-W	CMAA17*	CMAA7*	2275444	0.79%	0.79%	-0.16
Brown-W	CMAA17*	CMAA8	2275444	1.05%	0.56%	-0.05
Brown-W	CMAA17*	CMAA9*	2275444	0.93%	0.77%	-0.14
Brown-W	CMAA2	CMAA3	2275444	0.78%	0.84%	-0.56
Brown-W	CMAA2	CMAA4*	2275444	0.69%	0.85%	-0.55
Brown-W	CMAA2	CMAA5	2275444	0.71%	0.66%	-0.42
Brown-W	CMAA2	CMAA6	2275444	0.70%	0.84%	-0.55
Brown-W	CMAA2	CMAA7*	2275444	0.52%	1.18%	-0.77
Brown-W	CMAA2	CMAA8	2275444	0.60%	0.72%	-0.49
Brown-W	CMAA2	CMAA9*	2275444	0.61%	0.82%	-0.53
Brown-W	CMAA3	CMAA4*	2275444	1.19%	0.33%	0.06
Brown-W	CMAA3	CMAA5	2275444	1.40%	0.45%	0.06
Brown-W	CMAA3	CMAA6	2275444	1.20%	0.32%	0.07
Brown-W	CMAA3	CMAA7*	2275444	1.19%	0.61%	-0.05
Brown-W	CMAA3	CMAA8	2275444	1.60%	0.46%	0.08
Brown-W	CMAA3	CMAA9*	2275444	1.24%	0.35%	0.05
Brown-W	CMAA4*	CMAA5	2275444	1.40%	0.47%	0.07
Brown-W	CMAA4*	CMAA6	2275444	3.07%	0.00%	0.49
Brown-W	CMAA4*	CMAA7*	2275444	1.27%	0.03%	0.19
Brown-W	CMAA4*	CMAA8	2275444	1.24%	0.39%	0.07
Brown-W	CMAA4*	CMAA9*	2275444	1.34%	0.42%	0.07
Brown-W	CMAA5	CMAA6	2275444	1.42%	0.47%	0.08
Brown-W	CMAA5	CMAA7*	2275444	1.00%	0.54%	-0.04

Brown-W	CMAA5	CMAA8	2275444	1.18%	0.52%	0.02
Brown-W	CMAA5	CMAA9*	2275444	1.29%	0.49%	0.04
Brown-W	CMAA6	CMAA7*	2275444	1.28%	0.02%	0.19
Brown-W	CMAA6	CMAA8	2275444	1.25%	0.38%	0.07
Brown-W	CMAA6	CMAA9*	2275444	1.35%	0.41%	0.07
Brown-W	CMAA7*	CMAA8	2275444	1.18%	0.65%	-0.04
Brown-W	CMAA7*	CMAA9*	2275444	1.22%	0.54%	0.01
Brown-W	CMAA8	CMAA9*	2275444	1.58%	0.20%	0.19
Brown-C	CMA10*	CMA11	2275444	2.08%	1.31%	-0.06
Brown-C	CMA10*	CMA12	2275444	5.28%	0.00%	0.49
Brown-C	CMA10*	CMA13	2275444	2.18%	1.19%	-0.04
Brown-C	CMA10*	CMA14	2275444	1.86%	1.03%	-0.02
Brown-C	CMA11	CMA12	2275444	2.10%	1.30%	-0.06
Brown-C	CMA11	CMA13	2275444	2.40%	0.92%	0.04
Brown-C	CMA11	CMA14	2275444	2.37%	1.12%	0.00
Brown-C	CMA12	CMA13	2275444	2.19%	1.19%	-0.03
Brown-C	CMA12	CMA14	2275444	1.86%	1.02%	-0.02
Brown-C	CMA13	CMA14	2275444	2.69%	0.55%	0.13
Brown-C	CMA10*	CMA15*	2275444	2.10%	1.08%	-0.04
Brown-C	CMA10*	CMA16	2275444	1.98%	1.14%	-0.03
Brown-C	CMA10*	CMA18	2275444	1.71%	1.37%	-0.12
Brown-C	CMA10*	CMA4*	2275444	1.73%	1.35%	-0.11
Brown-C	CMA10*	CMA8	2275444	1.75%	1.55%	-0.24
Brown-C	CMA10*	CMA9	2275444	1.98%	1.01%	-0.021
Brown-C	CMA11	CMA15*	2275444	2 71%	0.02%	0.02
Brown-C	CMA11	CMA16	2275444	2.11%	1 29%	-0.05
Brown-C	CMA11	CMA18	2275444	1 90%	1.53%	-0.14
Brown-C	CMA11	CMA/*	2275444	1.90%	1.55%	-0.13
Brown-C	CMA11	CMA4 CMA8	2275444	1.52%	1.50%	-0.15
Brown-C	CMA11	CMA9	2275444	1.02%	1.58%	-0.20
Brown-C	CMA12	CMA15*	2275444	2 11%	1.45%	-0.03
Brown-C	CMA12	CMA16	2275444	1 00%	1.07%	-0.03
Brown C	CMA12	CMA18	2275444	1.77%	1.15%	-0.02
Brown C	CMA12	CMA18	2275444	1.7270	1.30%	-0.12
Brown C	CMA12	CMA4 [®]	2275444	1.74%	1.55%	-0.11
Brown C	CMA12	CMAO	2275444	1.70%	1.0470	-0.24
Brown C	CMA12	CMA15*	2273444	1.99%	1.1170	-0.02
Brown C	CMA13	CMA15 ¹	2273444	2.40%	1.24%	-0.01
Brown-C	CMA13	CMA10	2275444	2.23%	1.22%	-0.04
Brown-C	CMA13	CMA18	2273444	2.17%	1.03%	-0.13
Brown-C	CMA13		2275444	2.20%	1.03%	-0.14
Brown-C	CMA13	CMA8	2275444	1.57%	1.58%	-0.27
Brown-C	CMA13	CMA9	2275444	2.26%	0.85%	0.03
Brown-C	CMA14	CMA15*	2275444	2.39%	0.88%	0.03
Brown-C	CMA14	CMA16	2275444	2.18%	1.09%	0.00
Brown-C	CMA14	CMA18	2275444	2.06%	1.13%	-0.04
Brown-C	CMA14	CMA4*	2275444	2.08%	1.12%	-0.03
Brown-C	CMA14	CMA8	22/5444	1.58%	1.59%	-0.25
Brown-C	CMA14	CMA9	2275444	2.25%	1.02%	0.02
Brown-C	CMA15*	CMA16	22/5444	2.18%	1.22%	-0.05
Brown-C	CMA15*	CMA18	2275444	2.82%	0.04%	0.22
Brown-C	CMA15*	CMA4*	2275444	2.86%	0.03%	0.22
Brown-C	CMA15*	CMA8	2275444	1.73%	1.56%	-0.27
Brown-C	CMA15*	CMA9	2275444	2.37%	1.44%	-0.08

Brown-C	CMA16	CMA18	2275444	1.82%	1.49%	-0.13
Brown-C	CMA16	CMA4*	2275444	1.84%	1.47%	-0.12
Brown-C	CMA16	CMA8	2275444	1.52%	1.51%	-0.24
Brown-C	CMA16	CMA9	2275444	2.16%	1.03%	0.01
Brown-C	CMA18	CMA4*	2275444	4.93%	0.00%	0.48
Brown-C	CMA18	CMA8	2275444	1.39%	1.90%	-0.33
Brown-C	CMA18	CMA9	2275444	2.03%	1.55%	-0.12
Brown-C	CMA4*	CMA8	2275444	1.40%	1.88%	-0.32
Brown-C	CMA4*	CMA9	2275444	2.05%	1.54%	-0.11
Brown-C	CMA8	CMA9	2275444	1.72%	1.47%	-0.21
Brown-E	CMH00	CMH1	2275444	1.69%	0.31%	0.18
Brown-E	CMH00	CMH12*	2275444	1.02%	1.29%	-0.29
Brown-E	CMH00	CMH2	2275444	1.47%	0.63%	-0.09
Brown-E	CMH00	CMH3	2275444	1.32%	0.60%	-0.09
Brown-E	CMH00	CMH4*	2275444	1.03%	1.27%	-0.26
Brown-E	CMH00	CMH5	2275444	1.28%	0.77%	-0.09
Brown-E	CMH00	CMH6	2275444	1.03%	1 28%	-0.27
Brown-E	CMH1	CMH12*	2275444	1.00%	1.20%	-0.37
Brown-E	CMH1	CMH2	2275444	1.00%	0.63%	-0.15
Brown-F	CMH1	CMH3	2275444	1.22%	0.89%	-0.18
Brown-F	CMH1	CMH4*	2275444	1.43%	1 53%	-0.37
Brown-F	CMH1	CMH5	2275444	1.02%	0.90%	-0.37
Brown-E	CMH1	CMH6	2275444	1.25%	1.54%	-0.13
Brown E	CMH12*	CMH2	2275444	1.01%	0.63%	-0.37
Brown-E	CMH12*	CMH2 CMH3	2275444	1.2370	1 00%	-0.13
Brown E	CMH12*	CMH4*	2275444	2 76%	0.00%	-0.32
Brown E	CMH12*	CMH5	2275444	2.70%	0.00%	0.40
Brown E	CMH12*		2275444	2 7704	0.00%	-0.27
Drown E	CMH2	CMH2	2273444	2.77%	0.00%	0.49
Brown E	CMH2	CMH4*	2273444	2.20%	0.30%	0.17
DIOWII-E Brown E			2273444	1.27%	0.02%	-0.12
Drown E	CMH2	CMH6	2273444	1.7270	0.59%	0.00
DIOWII-E	CMII2		2273444	1.27%	0.02%	-0.14
Drown-E	CMH3 CMH2		2273444	1.07%	1.08%	-0.29
Brown-E	CMH3 CMH2	CMH5 CMH6	2275444	1.69%	0.71%	-0.01
Drown-E			2273444	1.00%	1.09%	-0.51
Brown-E	CMH4*	CMH5	2275444	1.08%	1.12%	-0.24
Brown-E			2275444	2.80%	0.00%	0.48
Brown-E	CMH5	CMH6	2275444	1.07%	1.13%	-0.26
Brown-E	CMH00	CMH/*	2275444	1.28%	0.81%	-0.09
Brown-E	CMH00	CMH8	2275444	1.04%	1.01%	-0.19
Brown-E	CMH00	CMH9	2275444	1.25%	1.37%	-0.25
Brown-E	CMHI	CMH/*	2275444	1.03%	0.93%	-0.19
Brown-E	CMHI	CMH8	2275444	1.07%	1.08%	-0.23
Brown-E	CMHI	CMH9	2275444	1.01%	1.43%	-0.34
Brown-E	CMH12*	CMH7*	2275444	0.88%	1.21%	-0.32
Brown-E	CMH12*	CMH8	2275444	1.13%	1.46%	-0.36
Brown-E	CMH12*	CMH9	2275444	0.83%	1.45%	-0.38
Brown-E	CMH2	CMH7*	2275444	1.85%	0.68%	-0.01
Brown-E	CMH2	CMH8	2275444	1.45%	0.79%	-0.11
Brown-E	CMH2	CMH9	2275444	1.52%	0.70%	-0.11
Brown-E	CMH3	CMH7*	2275444	1.67%	0.73%	-0.04
Brown-E	CMH3	CMH8	2275444	1.23%	1.17%	-0.24
Brown-E	CMH3	CMH9	2275444	1.34%	0.90%	-0.18

Brown-E	CMH4*	CMH7*	2275444	0.89%	1.20%	-0.28
Brown-E	CMH4*	CMH8	2275444	1.16%	1.45%	-0.32
Brown-E	CMH4*	CMH9	2275444	0.85%	1.44%	-0.35
Brown-E	CMH5	CMH7*	2275444	1.41%	1.02%	-0.11
Brown-E	CMH5	CMH8	2275444	0.90%	0.92%	-0.16
Brown-E	CMH5	CMH9	2275444	1.10%	0.90%	-0.16
Brown-E	CMH6	CMH7*	2275444	0.89%	1.20%	-0.30
Brown-E	CMH6	CMH8	2275444	1.15%	1.45%	-0.34
Brown-E	CMH6	CMH9	2275444	0.84%	1.44%	-0.36
Brown-E	CMH7*	CMH8	2275444	1.20%	0.46%	0.04
Brown-E	CMH7*	CMH9	2275444	1.95%	0.13%	0.25
Brown-E	CMH8	CMH9	2275444	0.96%	0.73%	-0.11
Dataset S11: Summary metrics of the SNP of each sample. The high-quality SNPs indicate the bases with a call quality of Q20 or higher. The highcoverage sites are at least covered by five reads. Heterozygosity was defined as the number of heterozygous SNPs divided by the total callable sites. Blue indicates *Crossoptilon auritum*, and Brown indicates *Crossoptilon mantchuricum*. A represents autosomes, Z represents the Z chromosome, and M represents the mitogenome.

			The ן קו	percent of uality SN	f high Ps	The percent of high coverage sites		The percent of missing SNPs			The number of heteozygous / substitutions		Heterozygosity		
Species	Sample ID	Sample index	А	Z	М	А	Z	М	А	Z	М	А	Z	А	Z
Blue	CA00	1	94.78%	93.66%	95.25%	98.50%	98.16%	100.00%	0.04%	0.28%	0.00%	760421	17881	0.00083	0.00025
Blue	CA01	2	93.13%	92.48%	95.25%	97.72%	77.31%	100.00%	0.10%	1.34%	0.00%	902604	16246	0.00099	0.00024
Blue	CA02	3	93.78%	94.36%	95.26%	97.79%	97.68%	100.00%	0.12%	0.12%	0.00%	756677	28595	0.00083	0.00040
Blue	CA03	4	93.19%	92.59%	95.26%	97.76%	80.35%	100.00%	0.10%	0.93%	0.00%	779739	15933	0.00086	0.00023
Blue	CA04	5	93.69%	92.91%	95.26%	98.15%	90.75%	100.00%	0.09%	0.62%	0.00%	722721	15973	0.00079	0.00023
Blue	CA05	6	93.39%	92.87%	95.26%	97.99%	86.72%	100.00%	0.09%	0.63%	0.00%	728717	15723	0.00080	0.00022
Blue	CA06	7	93.77%	94.35%	95.26%	97.93%	97.88%	100.00%	0.11%	0.14%	0.00%	728200	24951	0.00080	0.00035
Blue	CA07	8	94.50%	94.99%	95.26%	96.70%	97.11%	100.00%	0.22%	0.13%	0.00%	728940	26981	0.00080	0.00037
Blue	CA08	9	95.00%	95.27%	95.26%	97.04%	96.82%	100.00%	0.16%	0.22%	0.00%	721363	24411	0.00079	0.00034
Blue	CA09	10	94.22%	94.58%	95.26%	97.72%	97.57%	100.00%	0.12%	0.08%	0.00%	732472	25416	0.00080	0.00035
Blue	CA10	11	93.63%	92.70%	95.26%	98.05%	85.95%	100.00%	0.09%	0.63%	0.00%	740524	14987	0.00081	0.00021
Brown	CMAA00	12	94.68%	93.71%	95.54%	98.56%	98.30%	100.00%	0.01%	0.12%	0.00%	68230	13903	0.00007	0.00019
Brown	CMAA01	13	94.92%	93.99%	95.54%	97.34%	77.13%	100.00%	0.02%	0.53%	0.00%	63205	10840	0.00007	0.00016
Brown	CMAA02	14	94.39%	94.87%	95.54%	97.68%	97.67%	100.00%	0.01%	0.02%	0.00%	38525	1403	0.00004	0.00002
Brown	CMAA03	15	92.27%	92.00%	95.54%	97.69%	74.81%	100.00%	0.02%	0.59%	0.00%	80829	12038	0.00009	0.00018
Brown	CMAA04	16	94.16%	94.80%	95.54%	97.73%	97.68%	100.00%	0.02%	0.02%	0.00%	73818	1840	0.00008	0.00003
Brown	CMAA05	17	92.22%	91.39%	95.54%	98.10%	84.59%	100.00%	0.01%	0.41%	0.00%	74476	12417	0.00008	0.00018
Brown	CMAA06	18	93.07%	94.25%	95.54%	98.24%	98.08%	100.00%	0.01%	0.02%	0.00%	75001	1898	0.00008	0.00003

Brown	CMAA07	19	92.24%	91.79%	95.54%	97.86%	77.04%	100.00%	0.01%	0.57%	0.00%	68547	11875	0.00008	0.00018
Brown	CMAA08	20	94.48%	93.87%	95.54%	98.09%	87.52%	100.00%	0.01%	0.34%	0.00%	73400	12521	0.00008	0.00018
Brown	CMAA09	21	94.84%	94.33%	95.54%	97.00%	77.02%	100.00%	0.03%	0.53%	0.00%	70336	11406	0.00008	0.00017
Brown	CMAA10	22	91.75%	89.07%	95.54%	83.37%	45.00%	100.00%	0.37%	4.14%	0.00%	49204	10382	0.00006	0.00021
Brown	CMAA11	23	93.03%	94.07%	95.55%	98.39%	98.21%	100.00%	0.01%	0.00%	0.00%	65444	2150	0.00007	0.00003
Brown	CMAA12	24	93.93%	93.48%	95.55%	98.21%	88.87%	100.00%	0.01%	0.36%	0.00%	63348	13922	0.00007	0.00020
Brown	CMAA13	25	92.83%	92.46%	95.55%	98.08%	83.94%	100.00%	0.01%	0.32%	0.00%	58156	12005	0.00006	0.00017
Brown	CMAA14	26	94.50%	94.15%	95.55%	98.19%	85.63%	100.00%	0.01%	0.30%	0.00%	74402	13709	0.00008	0.00020
Brown	CMAA15	27	92.62%	92.41%	95.55%	97.76%	76.50%	100.00%	0.01%	0.59%	0.00%	75143	12700	0.00008	0.00019
Brown	CMAA16	28	91.92%	92.82%	95.55%	97.98%	97.82%	100.00%	0.01%	0.01%	0.00%	77779	2145	0.00009	0.00003
Brown	CMAA17	29	92.29%	93.54%	95.55%	96.23%	95.82%	100.00%	0.02%	0.02%	0.00%	64647	2054	0.00007	0.00003
Brown	CMA04	30	93.13%	94.36%	95.69%	97.34%	97.45%	100.00%	0.03%	0.04%	0.00%	120600	1294	0.00013	0.00002
Brown	CMA08	31	93.96%	93.37%	95.71%	95.55%	72.05%	100.00%	0.05%	0.97%	0.00%	101425	13094	0.00011	0.00020
Brown	CMA09	32	92.85%	92.53%	95.75%	98.28%	88.71%	100.00%	0.01%	0.34%	0.00%	128238	12343	0.00014	0.00017
Brown	CMA10	33	92.40%	92.02%	95.73%	98.00%	81.37%	100.00%	0.01%	0.53%	0.00%	127557	12243	0.00014	0.00018
Brown	CMA11	34	92.69%	92.46%	95.30%	98.15%	90.29%	100.00%	0.01%	0.39%	0.00%	133880	12653	0.00015	0.00018
Brown	CMA12	35	92.66%	92.29%	95.32%	98.21%	86.08%	100.00%	0.01%	0.39%	0.00%	128242	12283	0.00014	0.00018
Brown	CMA13	36	92.93%	94.01%	95.36%	98.41%	98.24%	100.00%	0.01%	0.02%	0.00%	136818	2837	0.00015	0.00004
Brown	CMA14	37	93.14%	94.20%	95.36%	98.16%	98.08%	100.00%	0.02%	0.02%	0.00%	126997	2454	0.00014	0.00003
Brown	CMA15	38	93.47%	93.18%	95.48%	98.18%	84.49%	100.00%	0.01%	0.27%	0.00%	144027	12458	0.00016	0.00018
Brown	CMA16	39	92.50%	92.09%	95.49%	97.86%	79.01%	100.00%	0.02%	0.52%	0.00%	127677	12222	0.00014	0.00018
Brown	CMA18	40	92.79%	93.93%	95.53%	97.38%	97.07%	100.00%	0.02%	0.03%	0.00%	119486	1346	0.00013	0.00002
Brown	CMH00	41	92.46%	93.40%	95.55%	98.49%	98.31%	100.00%	0.02%	0.08%	0.00%	70061	808	0.00008	0.00001
Brown	CMH01	42	94.17%	93.06%	95.55%	97.84%	83.94%	100.00%	0.04%	0.37%	0.00%	66657	10978	0.00007	0.00016
Brown	CMH02	43	94.00%	94.66%	95.55%	98.18%	98.44%	100.00%	0.03%	0.03%	0.00%	105279	1392	0.00012	0.00002
Brown	CMH03	44	93.44%	92.77%	95.55%	98.19%	87.08%	100.00%	0.02%	0.35%	0.00%	99277	13337	0.00011	0.00019
Brown	CMH04	45	91.98%	93.04%	95.41%	98.23%	98.09%	100.00%	0.03%	0.02%	0.00%	70004	912	0.00008	0.00001
Brown	CMH05	46	93.03%	93.95%	95.41%	98.03%	98.01%	100.00%	0.03%	0.03%	0.00%	81476	2178	0.00009	0.00003

Brown	CMH06	47	93.08%	94.18%	95.44%	98.30%	98.16%	100.00%	0.02%	0.03%	0.00%	67604	953	0.00007	0.00001
Brown	CMH07	48	93.94%	93.23%	95.44%	97.97%	84.65%	100.00%	0.03%	0.31%	0.00%	78353	12207	0.00009	0.00018
Brown	CMH08	49	92.99%	92.63%	95.44%	97.64%	77.13%	100.00%	0.03%	0.53%	0.00%	77740	11520	0.00009	0.00017
Brown	CMH09	50	93.38%	92.78%	95.45%	98.13%	87.84%	100.00%	0.02%	0.32%	0.00%	69882	12318	0.00008	0.00018
Brown	CMH12	51	90.21%	91.22%	95.24%	98.09%	97.95%	100.00%	0.03%	0.04%	0.00%	66594	812	0.00007	0.00001

Z chromosom	ne linked scaffolds	Mitochondria linked scaffolds							
Scaffolds	The percent of homologous gene (%)	Scaffolds	The percent of homologous gene (%)						
scaffold_74	25	scaffold_1343	100						
scaffold_142	41.38	scaffold_1701	100						
scaffold_34	46.24	scaffold_1847	100						
scaffold_394	50								
scaffold_397	50								
scaffold_399	57.14								
scaffold_215	68.75								
scaffold_247	70.59								
scaffold_157	73.33								
scaffold_79	73.68								
scaffold_241	75								
scaffold_380	75								
scaffold_401	75								
scaffold_254	76.47								
scaffold_187	77.08								
scaffold_62	77.5								
scaffold_212	80								
scaffold_333	80								
scaffold_403	80								
scaffold_122	80.36								
scaffold_196	81.48								
scaffold_291	81.48								
scaffold_192	82.93								
scaffold_336	85.71								
scaffold_349	85.71								
scaffold_160	86.36								
scaffold_236	86.36								
scaffold_113	86.67								
scaffold_156	86.67								
scaffold_57	87.04								
scaffold_93	87.23								
scaffold_37	88.46								
scaffold_259	88.89								
scaffold_136	89.66								

Dataset S12: Scaffolds on the sex chromosomes and mitochondrial genome.

scaffold_252	90	
scaffold_1249	100	
scaffold_1272	100	
scaffold_194	100	
scaffold_285	100	
scaffold_351	100	
scaffold_357	100	
scaffold_367	100	
scaffold_393	100	
scaffold_427	100	
scaffold_438	100	
scaffold_453	100	
scaffold_582	100	
scaffold_777	100	
scaffold_913	100	