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The genome of the Marco Polo Sheep (Ovis ammon polii)

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Abstract:	Background: The Marco Polo Sheep (Ovis ammon polii), a subspecies of argali (Ovis ammon) which is distributed mainly in the Pamir Mountains, provides a mammalian model in which to study high-altitude adaptation mechanisms. Due to over-hunting and subsistence poaching, as well as competition with livestock and habitat loss, O. ammon has been categorized as an endangered species on several lists. It can have fertile offspring with sheep. Hence a high quality reference genome of the Marco Polo Sheep will be very helpful in conservation genetics and even in exploiting useful genes in sheep breeding. Findings: A total of 1,022.43 Gb of raw reads resulting from whole-genome sequencing of a Marco Polo Sheep were generated using an Illumina HiSeq2000 platform. The final genome assembly (2.71 Gb), which has an N50 contig size of 30.7 Kb and a scaffold N50 of 5.49 Mb. The repeat sequences identified account for 46.72% of the genome and 20,336 protein-coding genes were predicted from the masked genome. Phylogenetic analysis indicated a close relationship between Marco Polo Sheep and the domesticated sheep, and the time of their divergence was approximately 2.36 million years ago (Mya). We identified 271 expanded gene families and 168 putative positively selected genes in the Marco Polo Sheep lineage. Conclusions: We provide the first genome sequence and gene annotation for the Marco Polo Sheep. The availability of these resources will be of value in the future conservation of this endangered large mammal, for research into high-altitude adaptation mechanisms, for reconstructing the evolutionary history of the Caprinae and for the future conservation of the Marco Polo Sheep.
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Response to Reviewers:	In your next (final) version, please also remove any highlighting of changes that have been made for the purpose of peer review. Reply: We have removed all highlighting in the manuscript. Regarding the corresponding authors, we must insist that you list a maximum of two co-authors with this role, as explained in our instructions for authors. Reply: We have reduced one corresponding author from the manuscript. Kun wang and Hongjiang wei were now listed as the corresponding authors. I have only one minor comment, and that concerns the heterozygosity analysis. I think the authors are probably justified in including this section, because although it is based on just one animal, it is presumably possible for researchers studying other sheep breeds to perform similar analyses and make comparisons. I wonder whether the 14% of the genome that is highly homozygous - I think this is a better word to use than 'homogeneous' (line 152) - could be due to recent inbreeding, and that the remainder of the genome is more useful for comparing levels of diversity between Marco Polo sheep and other sheep? I'm not proposing that the authors analyse these regions in any more detail though. Reply: Thank you for the suggestion, and we agree with that recent inbreeding could lead to the highly homozygous in the genome. We have re-written related sentences in line 143-148. “The genomic regions with “low heterozygosity” state that made up 14% of the genome were highly homozygous (mean heterozygosity rate = 0.003%), which could be explained by either loss of polymorphism in endangered species [20] or recent inbreeding in some specific Macro Polo sheep individuals. More samples will be required to test whether the highly homozygous status was common in this species.”
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Draft genome of the Marco Polo Sheep (*Ovis ammon polii*)

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Abstract

Background: The Marco Polo Sheep (*Ovis ammon polii*), a subspecies of argali (*Ovis ammon*) which is distributed mainly in the Pamir Mountains, provides a mammalian model to study high-altitude adaptation mechanisms. Due to over-hunting and subsistence poaching, as well as competition with livestock and habitat loss, *O. ammon* has been categorized as an endangered species on several lists. It can have fertile offspring with sheep. Hence a high quality reference genome of the Marco Polo Sheep will be very helpful in conservation genetics and even in exploiting useful genes in sheep breeding.

Findings: A total of 1,022.43 Gb of raw reads resulting from whole-genome sequencing of a Marco Polo Sheep were generated using an Illumina HiSeq2000 platform. The final genome assembly (2.71 Gb), which has an N50 contig size of 30.7 Kb and a scaffold N50 of 5.49 Mb. The repeat sequences identified account for 46.72% of the genome and 20,336 protein-coding genes were predicted from the masked genome. Phylogenetic analysis indicated a close relationship between Marco Polo Sheep and the domesticated sheep, and the time of their divergence was approximately 2.36 million years ago (Mya). We identified 271 expanded gene families and 168 putative positively selected genes in the Marco Polo Sheep lineage.

Conclusions: We provide the first genome sequence and gene annotation for the Marco Polo Sheep. The availability of these resources will be of value in the future conservation of this endangered large mammal, for research into high-altitude adaptation mechanisms, for reconstructing the evolutionary history of the *Caprinae* and for the future conservation of the Marco Polo Sheep.

Keywords: Marco Polo Sheep, genome assembly, annotation, evolution.

Data description

Introduction to *O. ammon polii*

The Marco Polo Sheep (*Ovis ammon polii*) is a subspecies of argali (*Ovis ammon*), named after the explorer Marco Polo. It was first described scientifically in 1841 by Edward Blyth [1]. This subspecies is distributed mainly in the Pamir Mountains, which consist of rugged ranges at elevations of 3,500-5,200 m [2]. The habitat of the subspecies includes the Tajikistan Pamir Mountains [3], as well as limited regions in China, Afghanistan, Pakistan, and Kyrgyzstan [4]. The Marco Polo Sheep species represents a new model to study high-altitude adaptation mechanisms adopted by mammals. Due to the sheep's impressively long horns, foreign hunters have for many years been willing to pay large amounts of money to take part in a hunt [5] and this is still the case today [2]. Recent studies on the status of the argali population have shown a decline in numbers, caused mainly by over-hunting and subsistence poaching, as well as by competition with livestock and habitat loss [6-9]. *O. ammon* has been categorized in several protection lists, such as Appendix II of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) and the IUCN (International Union for Conservation of Nature and Natural Resources) Red List, as a vulnerable or near threatened species. Conservation and restoration measures are therefore needed in order to safeguard the species, and information about its genome will be a key element in formulating an appropriate conservation strategy.

Sequencing

High molecular weight genomic DNA was extracted from fibroblast cells cultured from the ear skin biopsy sample of a male *O. ammon polii* using a Qiagen DNA purification kit. The sheep was originally captured from the Pamir Plateau of China and reared in the KaShi Zoo, Kashgar Prefecture, Xinjiang Province, China. A whole-genome shotgun sequencing strategy was applied, and a series of libraries with insert seizes ranging from 400 base pairs (bp) to 15 kilobase pairs (kb) were constructed using the standard protocol provided by Illumina (San Diego, CA, USA). To construct small-insert libraries (400, 500, 600, 700 and 800 bp), DNA was sheared to the target size range using a Covaris S2 sonicator (Covaris, Woburn, MA, USA) and ligated to adaptors. For long-insert libraries (4, 8, 10, 12 and 15 kb), DNA was fragmented using a Hydroshear system (Digilab, Marlborough, MA, USA). Sheared fragments were end-labelled with biotin and fragments of the desired size were gel purified. A second round of fragmentation was then conducted before adapter ligation. All libraries were sequenced on an Illumina HiSeq 2000 platform (**Table S1**). A total of 1,022.43 Gb of raw data was generated, and 624.74 Gb of clean data was retrieved after removal of duplicates, contaminated reads (reads with adaptor sequence) and low quality reads using the sickle software tool (https://github.com/najoshi/sickle) with a quality threshold of 10 and a length threshold of 50. We further corrected the short-insert library reads using SOAPec [10], a k-mer-based error correction package.

Evaluation of genome size

Approximately 65 Gb clean reads were randomly selected from all short libraries to

estimate the genome size using the k-mer-based method and the formula: G = k-mer_number/k-mer_depth. In this study, a total of 52,413,427,492 k-mers were generated and the peak k-mer depth was 17. The genome size was estimated to be approximately 3 Gb (**Table S2** and **Fig. S1**) and all the clean data correspond to a coverage of ~ 208-fold.

De novo genome assembly

The assembly was performed using Platanus v1.2.4 (Platanus, RRID:SCR_015531) [11], which is well suited to high-throughput short reads and heterozygous diploid genomes. Briefly, error-corrected paired-end reads (insert size < 2 kb) were input for contig assembly with the default parameters. Next, all cleaned paired-end (insert size < 2 kb) reads and mate-paired (insert size > 2 kb) reads were mapped onto the contigs for scaffold building, using default parameters except that the minimum number of links (-l) was set to 10 in order to minimize the number of scaffolding errors. After gap filling by Platanus, the gaps that still remained in the resulting scaffolds were closed using GapCloser (GapCloser, RRID:SCR_015026) [10]. The final *de novo* assembly for the Marco Polo Sheep has a total length of 2.71 Gb, including 116.91 Mb (4.3%) unknown bases. The assembly is slightly larger than that of the domestic sheep (*Ovis aries*, Oar_v3.1, 2.61 Gb) [12] and smaller than that of the domestic goat (*Capra hircus*, ARS1, 2.92 Gb) [13]. The N50s for contigs and scaffolds of the Marco Polo Sheep genome are, respectively, 30.8 kb and 5.5 Mb (**Table S3**). The assembled scaffolds represented ~ 88% of the estimated genome size, and the GC content was 41.9%,

similar to those of sheep (41.9%) and goat (41.5%) (**Fig. S2**).

We assessed the quality of the genome assembly with respect to base-level accuracy, integrity, and continuity. More than 99.65% of the short insert paired-end reads could be mapped to the assembly and more than 98.35% of the sequence have a coverage depth greater than 20-fold (**Table S4**), thus the assembly is of high level of single-base accuracy. A core eukaryotic genes (CEG) mapping approach (CEGMA, RRID:SCR_015055, v2.5 [14]) dataset comprising 248 CEGs was used to evaluate the completeness of the draft: 93.55% (232/248) of genes were completely or partially covered in the assembled genome (**Table S5**). Alongside this, we also used the BUSCO v2.0.1 (BUSCO, RRID:SCR_015008 [15]; the representative mammal gene set mammalia_odb9, which contains 4,104 single-copy genes that are highly conserved in mammals) software package to assess the quality of the genome assembly generated. The resulting BUSCO value was 95.9%, containing C: 92.5% [S: 91.3%, D: 1.2%], F: 3.4%, M: 4.1%, n: 4104 (C: complete [D: duplicated], F: fragmented, M: missed, n: genes) (**Table S6**). Both the CEGMA and the BUSCO scores are comparable to those for sheep (Oar_v3.1) and domestic goat (ARS1 and CHIR_1.0), which are known for their high quality as the references genomes of two important livestock animals, suggesting our Marco Polo Sheep assembly is of high quality and quite complete. Finally, to evaluate the trade-off between the contiguity and correctness of our assembly, we applied the feature-response curve (FRC) method [16], which predicts the correctness of an assembly by identifying ‘features’ representing potential errors or complications on each *de novo* assembled scaffold during the assembly process. The

FRC curve was calculated for the Marco Polo Sheep, sheep, taurine cattle and two versions of goat assemblies (**Fig. S3**). We found that the curve for our assembly was similar to that for the sheep and the two goat assemblies, with taurine cattle slightly different from the others, indicating the level of contiguity and correctness of the Marco Polo Sheep genome assembly is comparable to those of sheep and goat.

We mapped the reads from short-insert length libraries to the Marco Polo Sheep reference genome with BWA (BWA, RRID:SCR_010910) [17] and performed variant calling with SAMtools v0.1.19 (SAMTOOLS, RRID:SCR_002105) [18]. Applying strict quality control and filtering, we obtained a total of 3.5 million SNVs (**Table S7**) and noted that the heterozygosity rate (0.14%) was lower than that estimated for sheep (Oar_v3.1, 0.2%) and similar with that of goat (CHIR_1.0, 0.13%) [12]. We further assessed the distribution of heterozygosity ratio of non-overlapping 50K windows (Fig. S4). We assume that the heterozygosity on the genome can be divided into three states (low/normal/high) and applied Hidden Markov model with depmixS4 package [19] in R to infer the state of each window. The genomic regions with "low heterozygosity" state that made up 14% of the genome were highly homozygous (mean heterozygosity rate = 0.003%), which could be explained by either loss of polymorphism in endangered species [20] or recent inbreeding in some specific Macro Polo sheep individuals. More samples will be required to test whether the highly homozygous status was common in this species. 156 genes were overlap of more than half length with the low heterozygosity regions and the GO enrichment analysis shows that no GO category was significant enriched (Table S8). A total of 384,018 insertions and deletions (InDels)

(**Table S9**) were obtained. Similar to the findings of previous studies on yak [21] and wisent [22], the InDels in the coding regions were enriched for sizes that are multiples of three bases (**Fig. S5**).

Annotation

The transposable elements present in Marco Polo sheep sequences were identified using a combination of *de novo* and homology-based approaches. Transposable elements were identified at both the DNA and the protein levels, based on known sequences contained within the DNA repeat database (RepBase v21.01) [23], using RepeatMasker v4.0.5 (RepeatMasker, RRID:SCR_012954) [24] and RepeatProteinMask (v4.0.5, a package within RepeatMasker). For the *de novo* prediction, firstly RepeatModeler V1.0.8 (RepeatModeler, RRID:SCR_015027) was employed to construct a *de novo* repeat library, then RepeatMasker was used to identify repeats using both the *de novo* repeat database and RepBase. We then combined the *de novo* prediction and the homolog prediction of transposable elements according to the coordination in the genome. Tandem repeats were annotated with RepeatMasker and Tandem Repeats Finder (TRF, V4.07) [25]. In summary, a total of 0.87% tandem repeats and 46.60% transposable elements were identified in the Marco Polo sheep assembly, with LINEs constituting the greatest proportion, 72.48% of all repeats, and SINEs making up 24.09% of all repeats (**Table S10** and **Table S11**).

We used homology-based and *de novo* prediction to annotate protein coding genes. For homology-based prediction, protein sequences from 5 different species (*Bos taurus*,

Equus caballus, *Homo sapiens*, *Ovis aries*, *Sus scrofa*) (**Table S12**) were mapped onto the repeat-masked Marco Polo sheep genome using TblastN with an E-value cutoff of 1e-5; the aligned sequences as well as the corresponding query proteins were then filtered and passed to GeneWise (GeneWise, RRID:SCR_015054) [26] to search for accurately spliced alignments. For *de novo* prediction, we first randomly selected 1500 full-length genes from the results of homology-based prediction to train the model parameters for Augustusv3.2.1 (Augustus: Gene Prediction, RRID:SCR_008417) [27] and geneid v1.4.4 [28]. GenScan [29], Augustus v3.2.1 [27] and geneid v1.4.4 [28] were then used to predict genes based on the training set of human and Marco Polo Sheep genes. We used EVidenceModeler software (EVM, version 1.1.1) to integrate the genes predicted by the homology and *de novo* approaches and generated a consensus gene set (Table S13). The final gene set was produced by removing low-quality genes of short length (proteins with fewer than 50 amino acids) and/or exhibiting premature termination. The final total gene set consisted of 20,336 genes, and the number of genes, gene length distribution and exon number per gene were similar to those of other mammals, while the intron length was slightly larger than goat (CHIR_1.0), sheep (Oar_v3.1) and taurine cattle (UMD3.1) (Table S14 and Fig. S6, S7). The repeat content was annotated by RepeatMasker v4.0.5 [24] with unified parameters for Macro Polo sheep, domestic sheep and goat. We found that there were more LINE sequences in the intron regions of Marco Polo sheep than the other species, suggesting that transposon insertions might have contributed to intron length increasing (Fig. S8). 92.55% of all the predicted genes could be annotated using five protein databases: InterPro

(InterPro, RRID:SCR_006695) (87.17%), GO (Gene ontology, 70.99%), Swiss-Prot (91.67%), TrEMBL (92.33%) and KEGG (KEGG, RRID:SCR_012773) (Kyoto Encyclopedia of Genes and Genomes, 57.25%) (**Table S15**). In addition, we identified 2,978 noncoding RNAs in the Marco Polo Sheep genome (**Table S16**).

Genome evolution

Firstly, large-scale variations among Marco Polo Sheep, sheep and goat were identified by the synteny analysis using the program LAST (LAST, RRID:SCR_006119) [30]. A total of 2.29/2.30/2.40 Gb 1:1 alignment sequences were generated for, respectively Marco Polo Sheep vs sheep (Oar_v3.1), Marco Polo Sheep vs goat (ASR1), sheep vs goat, covering more than 88.55% of each genome (**Table S17** and **Fig. S9**). The sequences present on sheep/goat autosomes were well covered (average values: 89.65%/89.88%) by the synteny alignment, whereas only 66.09%/63.03% were covered in the case of chromosome X. The scaffolds of the Marco Polo Sheep genome that aligned to the sex chromosomes were also more fragmented. The divergence between Marco Polo Sheep vs sheep (Oar_v3.1), Marco Polo Sheep vs goat (ASR1), sheep vs goat was 0.7%, 2.2%, 2.3%, respectively, corresponding to their relatedness (**Table S17** and **Fig. S10**). Although Marco Polo Sheep, sheep and goat showed good synteny alignments, there are large numbers of inter-chromosomal rearrangements between pairs of them (**Fig. S11** and **S12**). By comparing Marco Polo Sheep and sheep/goat genomes we identified 11,756/6,026 inter-chromosomal, intra-chromosomal, or inversion breakpoints (edges of transposition events) (**Table S18**),

which may have been caused by the real translocations events between them as they have a different karyotype, errors in the assembly of the genomes or erroneous synteny alignments (false positives and false negatives). However, at this stage it is difficult to distinguish between possible artifactual and real effects. The breakpoint distributions were significantly enriched in repeat regions (**Fig S13a**), which are susceptible to rearrangements but also to assembly or alignment errors. Longer scaffolds were found to harbor fewer breakpoints (**Fig. S13b)**. Single molecule sequencing with unbiased long reads will be the best way of identifying large-scale variation.

To analyze gene families, we downloaded the protein sequences of eight additional species (Opossum, human, dog, horse, pig, taurine cattle, goat and sheep) from Ensembl (Ensembl, RRID:SCR_002344) [31] and GigaDB (GigaDB, RRID:SCR_004002) [32] (**Table S12**). The consensus gene set for the above eight species and Marco Polo Sheep were filtered to retain the longest CDS (coding sequence) for each gene, removing CDS with premature stop codons and those protein sequences < 50 amino acids in length, resulting in a dataset of 188,359 protein sequences, which was used as the input file for OrthoMCL (OrthoMCL DB: Ortholog Groups of Protein Sequences, RRID:SCR_007839) [33]. A total of 17,578 OrthoMCL families were built utilizing an effective database size of all-to-all BLASTP strategy with an E-value of 1e-5 and a Markov Chain Clustering default inflation parameter (**Table S19** and **Fig. 1a**). We identified 155 gene families that were specific to the Marco Polo Sheep when comparing with taurine cattle, sheep, goat and horse (**Fig. 1b**), and detected 271 gene families that have expanded in the Marco Polo Sheep lineage using CAFÉ

(Computational Analysis of gene Family Evolution, v4.0.1) [34] (**Fig. 1a**). The expanded gene families were enriched in 38 GO categories and their functions were mainly associated with response to stimulus, cell adhesion, G-protein coupled receptor and enzyme activity (**Table S20**).

Next, we selected 5,788 single-copy gene families from the above-mentioned 9 mammalian species and used PRANK v3.8.31 [35] with the codon option to align the CDS from each single-copy gene family. 4D-sites (fourfold degenerate sites) were extracted from all the single-copy genes and used to construct a phylogenetic tree with the GTR+G+I model in RAxML v7.2.8 (RAxML, RRID:SCR_006086) [36] (**Fig. S14**). The divergence time of each node was estimated by the PAML (PAML, RRID:SCR_014932) MCMCtree program v4.5 [37] and calibrated against the timing of the divergence of the opossum and human (124.6-134.8 Mya), human and taurine cattle (95.3-113 Mya), taurine cattle and pig (48.3-53.5 Mya), and taurine cattle and goat (18.3-28.5 Mya) [38]. The convergence was checked by Tracer v1.5 [39] and confirmed by two independent runs. The phylogenetic analysis showed that the Marco Polo Sheep has a closer relationship with sheep than with other mammals and that the divergence time between them is about 2.36 (1.94-2.61) Mya (**Fig. 1a**).

We further used the free ratio model to calculate the average Ka/Ks values and the branch-site likelihood ratio test to identify positively selected genes in the Marco Polo Sheep lineage. A total of 10,353 high confidence single-copy genes were identified by InParanoid and MultiParanoid within the human, dog, taurine cattle, goat, sheep and Marco Polo Sheep. We found that the Marco Polo Sheep has a regular level of the

average Ka/Ks values, but containing more outliers (**Fig. 1c**). A total of 168 positively selected genes were identified in the Marco Polo Sheep lineage (Table S21), and six of them were orthologous with high altitude adaptation related genes (IDE, IGF1, P2RX3, PHF6, PROX1 and RYR1) identified in Tibet wild boar [40]. Two genes were associated with hypoxia response: the ryanodine receptor protein encoded by *RYR1* (Ryanodine Receptor 1) was located in the pulmonary artery smooth muscle cells, which could subserve coupled O_2 sensor and NO regulatory functions to response to the tissue hypoxic decrease [41]; *P2RX3* (Purinergic Receptor P2X, Ligand-Gated Ion Channel, 3), is reported as a potential new target for the control of human hypertension, which could reduce the arterial pressure and basal sympathetic activity and normalize carotid body hyperreflexia in conscious rats with hypertension during P2RX3 antagonism [42]. Four genes were related with energetic metabolism: *IGF1* (Insulin-like Growth Factor 1) encodes the growth-promoting polypeptide mainly involved in the body growth and differentiation and as well as the glucose, lipid and protein metabolism [43]; *IDE* (Insulin Degrading Enzyme) encodes a zinc metallopeptidase that degrades intracellular insulin, which could accelerates glycolysis, pentose phosphate cycle, and glycogen synthesis in liver [44]; *PHF6* (PHD Finger Protein 6) encodes a protein with two PHD-type zinc finger domains and its function was associated with Börjeson-Forssman-Lehmann syndrome, which is one of the syndromic obesities in humans [45]; the protein encoded by *PROX1* (Prospero Homeobox 1) could occupy promoters of metabolic genes on a genome-wide scale to control of energy homeostasis [46]. In addition, the other identify PSGs may also be associated to high

altitude adaptation, while there are rare literature data on the function of them. Further studies will be required to clarify the roles of these genes in high altitude tolerance.

Finally, we inferred the demographic history of the Marco Polo Sheep using the Pairwise Sequentially Markovian Coalescent (PSMC) model [47]. Consensus sequences were obtained using SAMtools v0.1.19 [18] and divided into non-overlapping 100 bp bins. The analysis was performed with the following parameters: -N25 -t15 -r5 -p '4+25×2+4+6'. PSMC modeling was done using a bootstrapping approach, with sampling performed 100 times to estimate the variance of the simulated results. The effective population size (N_e) of Marco Polo Sheep shows a peak at ~1 Mya followed by two distinct declines. The most recent decline involved at least a sevenfold decrease in N_e, and occurred ~ 60,000 years ago (Fig. S15).

Conclusion

In summary, the novel genome data generated in this work will provide a valuable resource for studying high-altitude adaptation mechanisms within mammals and for investigating the evolutionary histories of the *Caprinae*, and it will have relevance for the future conservation of the Marco Polo Sheep.

Availability of supporting data

The sequencing reads of each sequencing library have been deposited at NCBI with the Project ID: PRJNA391748, Sample ID: SAMN07274464, and the Genome Sequence Archive [48] in BIG Data Center [49], Beijing Institute Genomics (BIG), Chinese

Academy of Science, under accession number PRJCA000449 (publicly accessible at http://bigd.big.ac.cn/gsa). The assembly and annotation of the Marco Polo Sheep genome are available in the *GigaScience* database GigaDB (GigaDB, RRID:SCR_004002) [50]. Supplementary figures and tables are provided in Additional file 1.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

KW and WW conceptualized the research project. KW, WW and HW designed analytic strategy and coordinated the project. YW, HW and WW collected the samples and led the genome sequencing. YY and KW led the bioinformatics analysis. YY, YW and YZ generated the genome assembly and the genome annotation. YY, RL and LC finished the synteny analysis. YW, YZ and GZ performed the gene family construction and the phylogeny analysis. YY and QQ detected the PSGs and carried out data submission. YY, WW and KW wrote the paper. All authors read and approved the final manuscript.

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Figure 1. Phylogenetic relationships and genomic comparisons between Marco Polo Sheep and other mammals. (a) Divergence time estimates for the nine mammals generated using MCMCtree and the 4-fold degenerate sites. The red dots correspond to calibration points and the divergence times. Divergence time estimates (Mya) are indicated above the appropriate nodes; blue nodal bars indicate 95% confidence intervals. Gene orthology was determined by comparing the genomes with the OrthoMCL software. (b) A Venn diagram of the shared orthologues among Marco Polo Sheep, sheep, goat, taurine cattle and horse. Each number represents a gene family number. (c) The box plot shows the ratio of non-synonymous to synonymous mutations (Ka/Ks) for Marco Polo Sheep, sheep, goat, taurine cattle, horse and human.

Additional files

Figure S1. 21-mer-based analysis carried out to estimate the size of the Marco Polo Sheep genome.

Figure S2. GC content distribution for the genomes of Marco Polo Sheep, goat and sheep.

Figure S3. FRCurve of five genome assemblies.

Figure S4. The distribution of observed heterozgosity stats within Marco Polo Sheep genome.

Figure S5. Counts of InDels in coding regions, showing an enrichment of multiples of three bases.

Figure S6. Comparison of gene structure characteristics with those of other mammals.

Figure S7. Comparison of gene structure characteristics of the 1:1 orthologs in the five mammals.

Figure S8. Comparison of the repeat content in the intron regions among Marco Polo Sheep, Sheep (Oar_v3.1) and Goat (CHIR_1.0).

Figure S9. Summary of the number of chromosomes to which a given scaffold of the Marco Polo Sheep genome could be aligned.

Figure S10. Divergence between Marco Polo Sheep, sheep and goat.

Figure S11. Synteny relationship between Marco Polo Sheep and sheep.

Figure S12. Synteny relationship between Marco Polo Sheep and goat.

Figure S13. Density of breakpoints (number per million bases) in different regions of the genome.

Figure S14. Phylogeny relationships between Marco Polo Sheep and other mammals.

Figure S15. Demographic history of Marco Polo Sheep.

Table S1. Summary of sequenced reads.

Table S2. Estimation of genome size based on 21-mer statistics.

Table S3. Statistics for the final assemblies of the Marco Polo Sheep genome.

Table S4. Numbers of reads mapped to the assembled Marco Polo Sheep genome.

Table S5. Summary of CEGMA analysis results.

Table S6. Summary of BUSCO analysis results obtained by counting matches to 4104 single-copy orthologs (mammalia_odb9).

Table S7. The distribution of SNVs in the Marco Polo Sheep genome.

Table S8. Genes located in the low heterozygosity regions.

Table S9. The distribution of InDels in the wisent genome.

Table S10. Prediction of repetitive elements in the assembled Marco Polo Sheep genome.

Table S11. Classification of interspersed repeats in the assembled Marco Polo Sheep genome.

Table S12. Data on all species used during the genome analysis.

Table S13. Prediction of protein-coding genes in the Marco Polo Sheep.

Table S14. Comparative gene statistics.

Table S15. Functional annotation of predicted genes in the Marco Polo Sheep.

Table S16. Summary statistics of non-coding RNAs in the Marco Polo Sheep.

Table S17. Summary of synteny alignments.

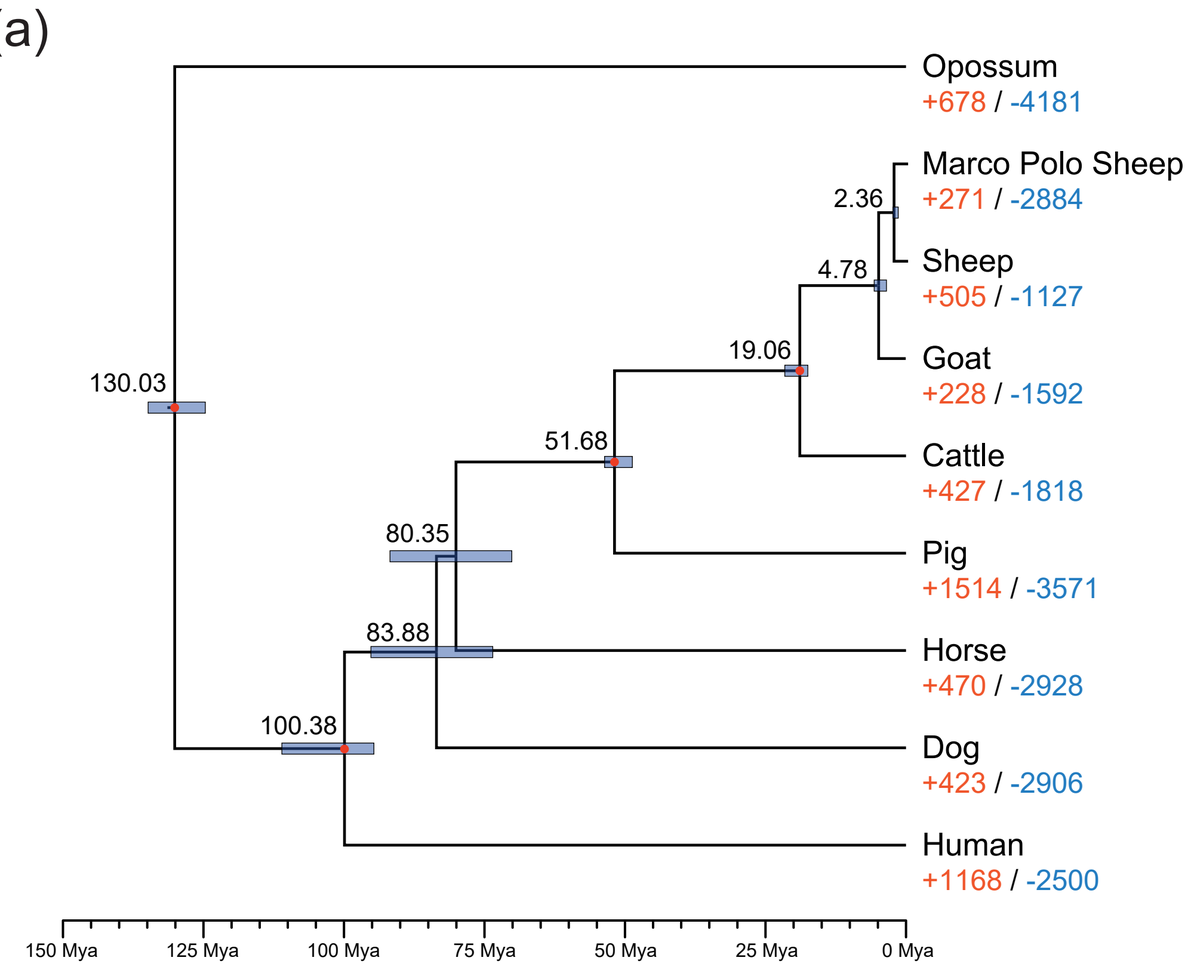
Table S18. Summary of breakpoints between Marco Polo Sheep, sheep and goat.

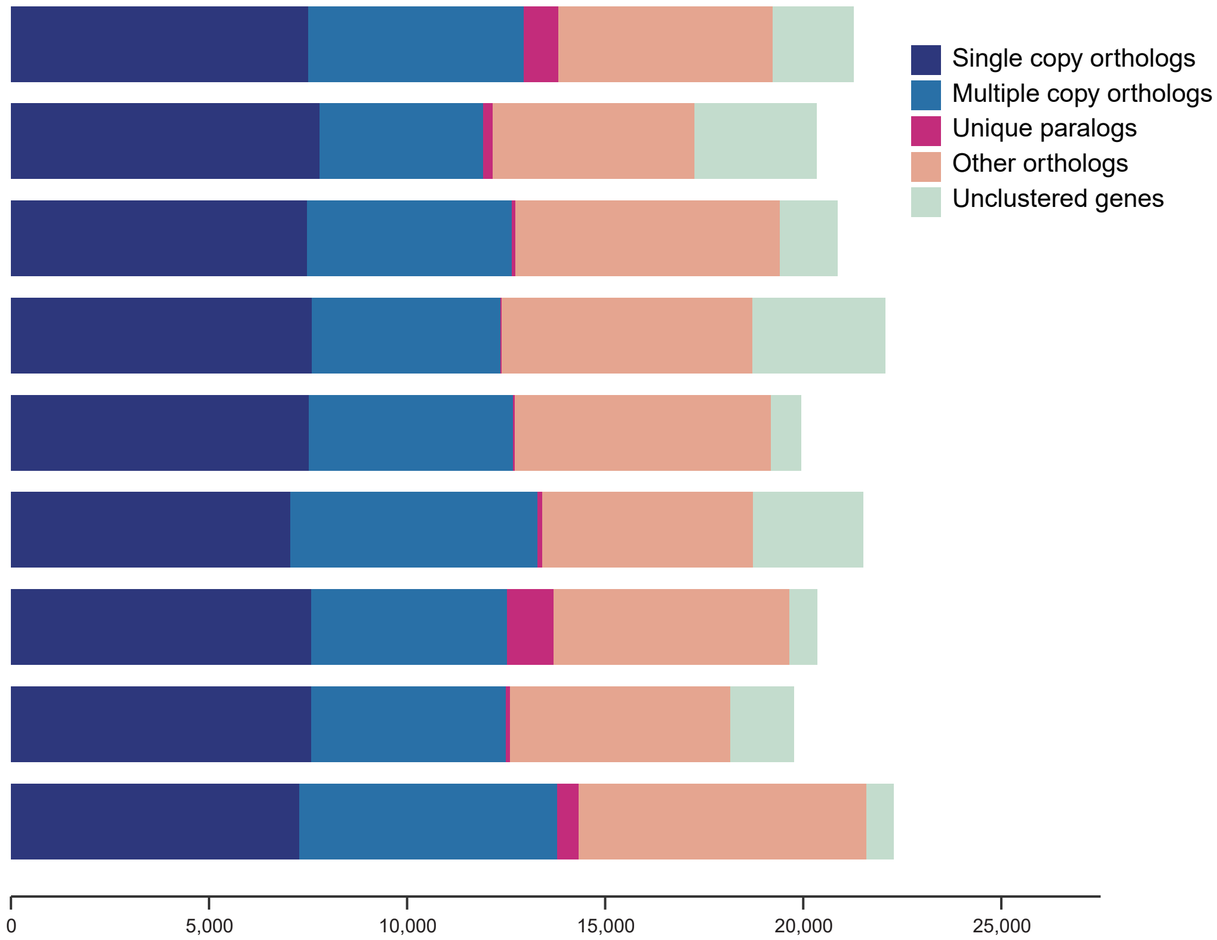
Table S19. Summary statistics of gene families in 9 species.

Table S20. GO enrichment analysis of the expanded gene families in the Marco Polo Sheep lineage.

Table S21. Candidate positively selected genes (PSGs) in the Marco Polo Sheep lineage.

(a)





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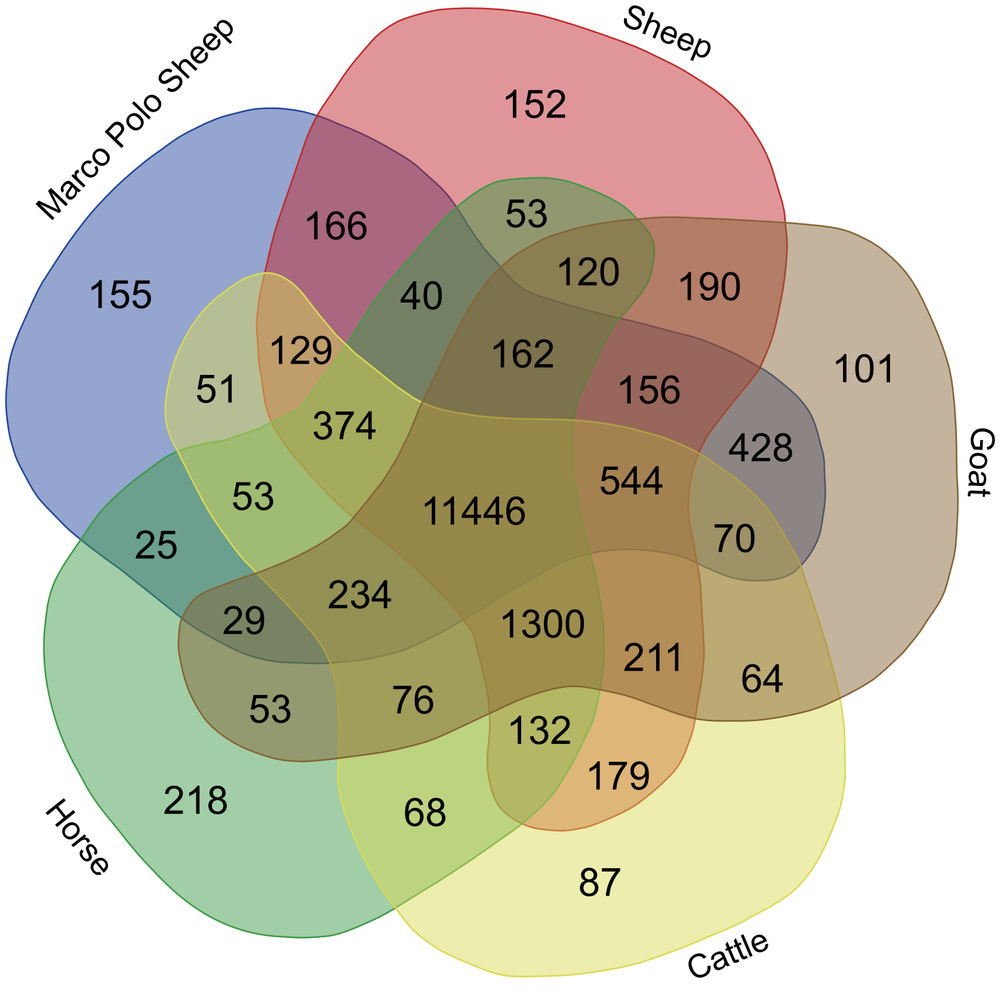

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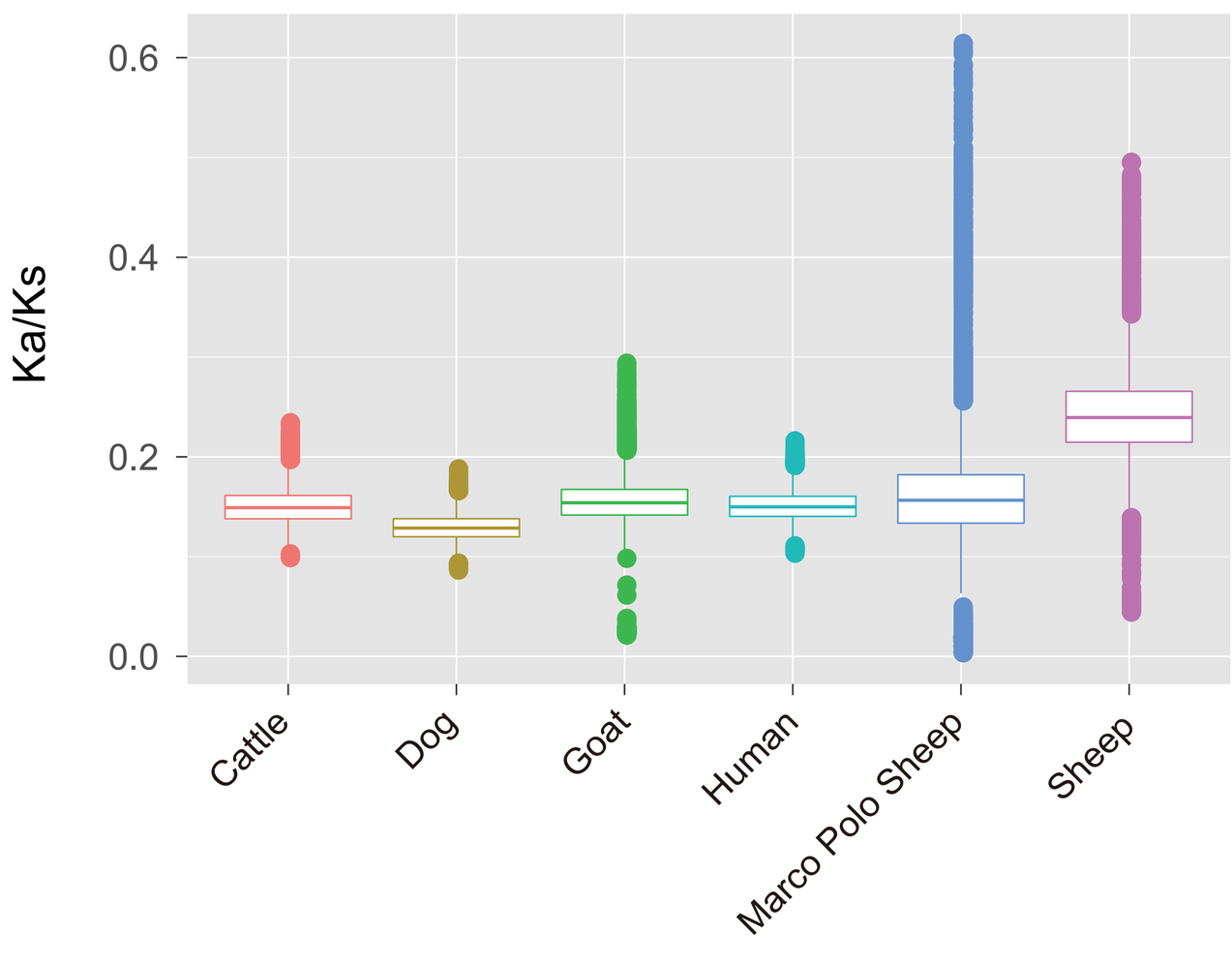

Dear Editor,

Thank you very much for returning our manuscript GIGA-D-17-00160 entitled “The genome of the Marco Polo Sheep (*Ovis ammon polii*)”.

We submit the revised manuscript here and hope the revised manuscript is more suitable for the publication in Giga Science. If you have any questions, please do not hesitate to contact the corresponding author at any time.

Thank you again for your time and efforts in handling our manuscript.

Best wishes,

Kun Wang

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