Population Genetics and Evolution Analysis Reveal Diversity and Origin of *Ammopiptanthus* in China

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Abstract

Background

Elucidating and revealing the population genetic structure, genetic diversity and recombination is essential for understanding the evolution and adaptation of species. *Ammopiptanthus*, which is an endangered survivor from the Tethys in the Tertiary Period, is the only evergreen broadleaf shrub grown in Northwest of China. However, little is known about its genetic diversity and underlying adaptation mechanisms.

Results

Here, 111 *Ammopiptanthus* individuals collected from fifteen natural populations in estern China were analyzed by means of the specific locus amplified fragment sequencing (SLAF-seq). Based on the single nucleotide polymorphisms (SNPs) and insertions and deletions (InDels) detected by SLAF-seq, genetic diversity and markers associated with climate and geographical distribution variables were identified. The results of genetic diversity and genetic differentiation revealed that all fifteen populations showed medium genetic diversity, with PIC values ranging from 0.1648 to 0.3081. AMOVA and Fst indicated that a low genetic differentiation existed among populations. Phylogenetic analysis showed that NX-BG and NMG-DQH of fifteen populations have the highest homology,while the genetic structure analysis revealed that these *Ammopiptanthus* germplasm accessions were structured primarily along the basis of their geographic collection, and that an extensive admixture occurred in each group. In addition, the genome-wide linkage disequilibrium (LD) and principal component analysis showed that *Ammopiptanthus nanus* had a more diverse genomic background, and all genetic populations were clearly distinguished, although different degrees of introgression were detected in these groups.

Conclusion

Our study could provide guidance to the future design of association studies and the systematic utilization and protection of the genetic variation characterizing the *Ammopiptanthus.*

Background

Throughout the world, many plant species are in need of conservation as a result of human activities such as overgrazing and deforestation. Endangered plants that occur in small and isolated populations often need to be managed to mitigate anthropogenic effects such as habitat fragmentation, habitat loss, or climate change[1]. Conservation over the past two decades has increasingly become reliant on genetic data and the insight it provides into the breeding behavior and overall genetic variation within endangered species[2, 3]. Endangered wild species have many characters, such as huge ornamental and medicinal value, stress resistance[4, 5]. *Ammopiptanthus* is an endangered species from the Tertiary Period, and it is

the only evergreen broadleaf shrub grown in Northwest desert of China [6, 7]. The genus *Ammopiptanthus* (*Leguminosae*) only comprises of two species, which are *A. mongolicus*(Maxim. ex Kom.) and *A. nanus*(M. Pop.). Both species are diploid with 18 chromosomes and exhibiting a wide range of adaptation [8, 9].

In China, *Ammopiptanthus* are mainly distributed in the northern and western areas, such as Inner Mongolia, Shaanxi, Gansu, Ningxia and Xinjiang, within these areas, *Ammopiptanthus* is known as a drought, cold and heat tolerant shrub[10, 11]. However, with the gradual warming of global climate, what degree the speed of future adaptation can keep pace with the climate change remains unknown. Therefore, a deep understanding of the genetic diversity and genetic regulation of adaptation in *Ammopiptanthus* is significant. Revealing polymorphisms and genes that determine adaptation would provide the basis for breeding genetically improved germplasms that can be applied in the breeding practice. Breeding resistant cultivars using resistant resources is an economic and effective approach to control this disease. Resistance breeding is also one of the important goals in the desert-plant breeding programme.

As known to all,the genetic diversity and relationships among resistant resources is importance for improving the tolerant varieties. Molecular markers reflect the actual level of genetic variation which exist among genotypes at the DNA level [12, 13]. Hence, they provide a more accurate estimate with such variation than do either phenotypic or pedigree information. In *Ammopiptanthus*, the DNA markers such as inter-simple sequence repeat (ISSR), SSR and ESTs have been used for analyzing the germplasm genetic diversity, cultivar fingerprinting and identification of abiotic stress-responsive genes[14–16]. Recently, the rapid development and application of sequence-specific markers such as SSR, EST-SSR, InDels were also reported for *sesame* [17]. Although kinds of molecular markers have been considered to be efficient tools for studying genetic structure, mating systems, gene flow, and estimating genetic variation within a species. Especially, microsatellite markers are used to analyze the effective pollen flow and seed dispersal among the populations and within a species [18, 19]. Unfortunately, a limited number of selected markers used in these studies might provide inaccurate estimates in genetic variability.

However, the limited number of molecular markers are too small to construct a high-density genetic map in most plants. Fortunately, Specific Length Amplified Fragment sequencing (SLAF-seq) was developed based on high-throughput sequencing technology [20]. This technology allows researchers to design the experimental system through bioinformatics and screen for fragments of a specific length from the constructed SLAF-seq library[21]. The efficiency of SLAF-seq was tested using rice and soybean data[22–24], and has been used to construct the highest-density genetic map of common carp[25], without a reference genome sequence. To date, SLAF-seq has been used successfully to construct highdensity genetic maps and study the genomes of many organisms, including wax ground[26], white jute[27], crape myrtle[28], cauliflower[29], *Boehmeria nivea*[30] and sesame[17]. Moreover, this method has been widely used in GWAS for important traits, as well as in the development of core germplasm[31]. However, the genetic diversity and population structure of *Ammopiptanthus* are now have not been reported.

In this study, 111 *Ammopiptanthus* samples were collected and selected from the Northwest in China and genotyped using the SLAF-seq method to evaluate their usability as an association-mapping panel. Our objectives were as follows: (1) to evaluate the genetic diversity as represented by the 111 *Ammopiptanthus* germplasm accessions; (2) to calculate the characteristics of the population structure; and (3) to estimate the linkage disequilibrium (LD) patterns occurring in the sample of *Ammopiptanthus* germplasm. Finally, Our results not only develop a valuable resource for further genome-wide association studies in Asian *Ammopiptanthus* and exploiting rich allelic variation for marker-assisted breeding, but also provides guidelines for the conservation, management, and restoration of *Ammopiptanthus.*

Results

SLAF-seq of 111 *Ammopiptanthus mongolicus* Individuals

After SLAF library construction in 111 *Ammopiptanthus mongolicus* individuals, a total of 20.92 Gb of data containing 390.06 Mb reads were obtained, the GC content and Q_{30} value are 40.35% and 91.63%, respectively. While 957,624 SLAF tags were identified in *Ammopiptanthus* individuals, with an average coverage of 13.83 per samples. SLAF tags were mapped to the reference *Ammopiptanthus* genome using the BWA software, and 624,223 SLAF tags containing polymorphic SNPs were detected among the 111 *Ammopiptanthus* individuals (Table 3 and Fig 1). Of these SLAF tags containing polymorphic SNPs, 44.02% (274,782) were located on the 18 assembled chromosomes of the *Ammopiptanthus* reference genome, similar percentage (43.30%) of the total assembled genome sequence of *Ammopiptanthus* present in the chromosome assemblies (642.5 Mb of 1.535 Gb). The number of SLAF tags per chromosome ranged from 5,673 to 17,521.

Meanwhile, A total of 3,925,962 SNPs were identified among the 111 individuals of *Ammopiptanthus*, of which 1,261,501 SNPs were identified by both GATK and SAM tools and were subsequently used as SNP markers and the integrity of SNPs in the 111 accessions ranged from 80.66 to 97.66% (Fig 2). The raw data for the 111 *Ammopiptanthus* individuals SLAF-seq have been uploaded to the Sequence Reads Archive in National Center for Biotechnology Information. The number and distribution of the polymorphic SLAF tags on each chromosome are shown in Supplementary Table 1. Insertion and deletion (INDEL) polymorphisms, are abundant in the genomes of model organisms and are expected to be abundant in *Ammopiptanthus* as well. We used our computational pipeline to mine 296,977 INDELs from two independent *Ammopiptanthus* sets, ranging from 1 bp to 831 bp in length. Frameshift INDELs (INDEL length is not multiple of three bases) are under especially strong scrutiny as they generally result in a nonsense mutation and changes in amino acid sequences (Fig 3A). In our database, we discovered different INDELs which ranging in size from −80 to 12 base pairs. The overwhelming majority (95%) of the INDELs are short, and it has the rich distribution for in-frame INDELs (Fig 3B).

Genetic diversity and Genetic differentiation of population

Genetic diversity usually refers to the sum of genetic differentiation and variation among different individuals within a population or among different populations of a species [32, 33]. Results of genetic diversity showed that the genetic diversity of *Ammopiptanthus mongolicus* (*he*=0.32522, *ho*=0.31129) was higher than that of *Ammopiptanthus nanus* (*he* = 0.26143, *ho* = 0.25834) (Table 1). The population differentiation degree of *Ammopiptanthus mongolicus* was low (F_{ST}=0.1248) (Table 2), indicating that the population differentiattion was moderate and the variation mainly existed in the population. While the differentiation degree of *Ammopiptanthus nanus* population in Xinjiang was high (F_{ST} = 0.6989), indicating that *Ammopiptanthus nanus* population was highly differentiated and the variation mainly existed between populations. The variation of *Ammopiptanthus mongolicus* and *Ammopiptanthus nanus* exists between species, which is closely related to the geographical distance between the two species.

In this study, we also assumed that cpDNA variation was in a drift-migration equilibrium [34], and considerable differentiation of the whole-genome among the *Ammopiptanthus* populations growing in different geographically remote areas and therefore a high degree of genetic disunity between them are evidenced by the high pairwise genetic distances(F_{ST}, Table 2), as well as between the *Ammopiptanthus mongolicus* and *Ammopiptanthus nanus* groups (F_{ST}= 0.5813, *P*<0.0001).

Phylogenetic Relationships Based on SNP Data

Phylogenetic trees could reflect the evolutionary relationships of different individuals and groups, and which close relatives tend to gather together. So, In order to ascertain the divergence of 111 of *Ammopiptanthus* species during evolution, we performed the phylogenetic analysis, the results showed all species are clustered into five distinct branches (Fig 4), and that the majority of individuals can gather in the same group. The GS-MQ population is transplanting species out of five population (GS-MQ, GS-HSY, NX-HW, NX-BG and NMG-DQH population). The *Ammopiptanthus nanus* from Xinjiang province gathered together, while *Ammopiptanthus nanus* from Gansu province can not gather to a family group. Meanwhile, all *Ammopiptanthus mongolicus* gather to a big family group. The *Ammopiptanthus mongolicus* GS-HSY and NX-HW are clustered on a large branch, while the remaining communities in Ningxia gather on another branch. The majority of *Ammopiptanthus mongolicus* gathered together (Fig 4). Finally, all results indicated that NX-BG and NMG-DQH have the highest homology.

Population Structure and Linkage Disequilibrium Analysis

To ascertain the divergence of the NMG-DQH, NMG-ALBLG, NMG-AZQ, NMG-QLG, NMG-DKTST, NX-BG, NX-BDG, NX-BJT, NX-HW, GS-HSY, XJ-KS, XJ-AHQ, XJ -BX, XJ-WYS and GS-MQ groups during evolution, principal component, population structure analyses were performed. Additionally, the sequence diversity of the FD, SD and ND germplasms was evaluated. All of the analyses indicated that there are strong divergence between the different *Ammopiptanthus* groups.

A population structure analysis using the Admixture program and SNP data revealed that 111 *Ammopiptanthus* germplasms were mainly divided into three groups according to the cross-validation error rate (Fig 5). Of the three groups, group 1 comprised the most germplasms with 51 followed by group

2 (41 germplasms) and group 3 (19 germplasms). The *Ammopiptanthus* germplasms distributed in three groups, suggesting these *Ammopiptanthus* species were genetically diverse.

Meanwhile, Linkage disequilibrium (LD) analysis of these fifteen groups revealed that the distance of LD decay in the NX-HW and XJ-WHS group is longer than that in the other groups (Fig 6). The results indicated that *Ammopiptanthus nanus* had a more diverse genomic background.

The principal component analysis

In order to ascertain the divergence of fifteen population of *Ammopiptanthus* species during evolution, we performed the principal component analysis. The first three components explained 30.14% of the total genetic variation, of which the first two components contributed 64.67% (PC1) and 5.55% (PC2), The results showed that the NMG-DQH, NMG-ALBLG, NMG-AZQ, NMG-QLG, NMG-DKTST, NX-BG, NX-BDG, NX-BJT, NX-HW, GS-HSY, XJ-KS, XJ-AHQ, XJ -BX, XJ-WYS and GS-MQ collections were clearly distinguished (Fig 7), although existing some degrees of introgression in these groups. All results indicated that a strong divergence between different *Ammopiptanthus* groups.

Discussion

High-density genetic maps play an essential role in promoting discovery of functional genes and comparative analysis of genome structure[35]. However, most current genetic maps only contain about hundreds of markers, due to discovery technologies and genotyping costs [36, 37]. Advances in genome sequencing technologies have set the scene for vital improvements in the rapid detection of genetic variation as well as the throughput and wealth of the information obtained. Up to now, a number of reduced representation sequencing methods have been developed, such as genotyping by sequencing (GBS), typeIIB restriction site associated DNA (2b-RAD)[38], and SLAF-seq. We chose the latter for this study because of its vast advantages, such as high throughoput, lower sequencing costs, higher genotyping accuracy, and efficient detection system.

Genetic diversity is the amount of variation observed between DNA sequences from distinct individuals of a particular species[39]. This pivotal concept of population genetics has implications for species health, domestication, management and conservation. As Known to all, population diversity and structure is of fundamental importance for crop breeding programs. Normal passport (geography and/or pedigree) and phenotype data, traditionally used for the assessment of genetic architecture of the population, has been recently paralleled by the use of molecular markers. This is because the molecular markers allowed researchers to distinguish closely related samples and give more precise variation information among genotypes. SLAF-seq methods are currently used in a wide range of applications. These applications mainly include constructing high-density linkage maps[20, 40], identifying candidate genes and mutant [41, 42], drafting the genome[43] and constructing the core set for rare species[44]. However, the application of population genetics in endangered plant *Ammopiptanthus* has not been reported. In this study, a high quality of 111,735,304 80-bp long paired-end reads, 467,735 SLAFs and 1,261,501 SNPs were generated using high-throughput SLAF-seq. Then we analyzed the genetic diversity(both

morphology and molecular)and population structure of 111 kinds of *Ammopiptanthus* that grow in different locations of China in order to begin to lay a foundation for future yield-improvement endangered plant breeding programs.

Species that are considered threatened or endangered often exhibit low genetic diversity, especially when populations are small or disconnected[45]. Higher levels of genetic diversity allow species to adapt to environmental changes and reduces their susceptibility to catastrophic extinction events[46]. In this study, the 111 *Ammopiptanthus lines* were used as materials, by comparing the genetic diversity of *Ammopiptanthus mongolicus* and *Ammopiptanthus nanus*, most groups can be divided into the independent group, may be experienced the founder effect or the result of the bottleneck effect, GS-MQ group as compared to other *Ammopiptanthus nanus* on the related closer to the *Ammopiptanthus mongolicus* group, showing the geographical position close group is affected by the same climate, tending to be more consistent in evolutionary history.

The species evolution is largely related to the genetic diversity. Generally, species with high genetic diversity are better able to adapt to changing ecological environment, while species with low genetic diversity are less adaptable to the environment and are passive in the permanent evolution. In this study, the genetic diversity and genetic structure of different geographic populations in *Ammopiptanthus mongolicus* and *Ammopiptanthus nanus* was explored by SLAF for the first time. Previous studies have shown that the heterozygosity is an important measure of overall genetic diversity. Our results showed that the genetic diversity of *Ammopiptanthus mongolicus* (*he*=0.32522, *ho*=0.31129) was higher than that of *Ammopiptanthus nanus* (*he* = 0.26143, *ho* = 0.25834). The population differentiation degree of *Ammopiptanthus mongolicus* was low (F_{ST}=0.1248), indicating that the population differentiattion was moderate and the variation mainly existed in the population. While the differentiation degree of *Ammopiptanthus nanus* population in Xinjiang was high (F_{ST} = 0.6989), indicating that *Ammopiptanthus nanus* population was highly differentiated and the variation mainly existed between populations. The variation of *Ammopiptanthus mongolicus* and *Ammopiptanthus nanus* exists between species, which is closely related to the geographical distance between the two species.

Gene flow are integral to interpretation of microevolutionary patterns and geographic structure. Through gene flow, we strive to gain insights into evelutionary independence and potential for population diversification, differentiation and ultimately speciation. Gene flow can halt or reduce genetic divergence that accumulates due to population isolation. In this study, the gene flow value (Nm) among *Ammopiptanthus mongolicus* population was 3.506, it indicates that genetic differentiation between *Ammopiptanthus mongolicus* populations was small, while the gene flow value (Nm) among *Ammopiptanthus nanus* population was 0.431, its genetic differentiation is higher than *Ammopiptanthus mongolicus.* Overall, the average of gene flow (Nm) was 0.360, indicating that the gene flow level of the whole population was low and the genetic differentiation was high. The main reason may be related to geographical distribution and interspecific isolation. Our analysis results are consistent with the previous studies.

Recently, Ding[47] found that the mountain and monsoon are mainly reasons of terrestrial biodiversity, and by the early to middle Miocene, accelerated diversification and colonization of adjacent regions were likely driven jointly by mountain building and intensification of the monsoon. Similarly, based on our research, we speculate on a possible migration model for *Ammopiptanthus* species in China. The possible migration route may be from Xinjiang to Gansu, then to Ningxia and Inner Mongolia. It is why we can find some *Ammopiptanthus nanus* species in Mingqin county of Gansu province. In addition, introduction and cultivation are also the reasons of terrestrial biodiversity, this accuracy of this model needs to be verified in future experiments.

In summary, a SLAF-seq approach for *Ammopiptanthus* was developed to reveal the genetic diversity, genetic structure and relationship between *Ammopiptanthus mongolicus* and *Ammopiptanthus nanus*. Meanwhile, SNP including hundreds of genes between *Ammopiptanthus mongolicus* and *Ammopiptanthus nanus* were identifed further. Consequently, our study not only provides a cost-effective approach for *Ammopiptanthus* genome-wide screening, but also contribute to understanding the diversity and origin of *Ammopiptanthus* in future research.

Conclusions

The aim of this study was to reveal the genetic diversity and underlying adaptation mechanisms in *Ammopiptanthus*. Overall, our results indicated that all fifteen populations showing medium genetic diversity, with PIC values ranging from 0.1648 to 0.3081. AMOVA and Fst indicated that a low genetic differentiation existed among populations. Phylogenetic analysis showed that NX-BG and NMG-DQH of fifteen populations have the highest homology, while the genetic structure analysis revealed that these *Ammopiptanthus* germplasm accessions were structured primarily along the basis of their geographic collection, and that an extensive admixture occurred in each group. In addition, the genome-wide linkage disequilibrium (LD) and principal component analysis showed that *Ammopiptanthus nanus* had a more diverse genomic background, and all genetic populations were clearly distinguished, although different degrees of introgression were detected in these groups.

Methods

Plant varieties and DNA extraction

A total of 111 *Ammopiptanthus* samples were evaluated in this study, these individuals were selected from the 1200 core germplasms collected from Northwest of China in 2019 (Fig. 1). When collecting the samples, we have to get the permission from a local wildlife conservation park. The geographical coordinates of the collection area span a wide range (37°N to 41°N and 74°E to 107°E), with an altitude of 1021–2546 meters. The samples of *Ammopiptanthus* were stored in a -80℃ refrigerator of college of life science, Yulin University, Shaanxi province, China. Genomic DNA was extracted from *Ammopiptanthus* leaves according to a modified SDS method[28, 48]. Finally, all samples that are packed in dry-ice were sent to Beijing BioMarker Corporation (Beijing, China) for library construction [25, 28].

SLAF Sequencing

The genomic DNA of *Ammopiptanthus* individuals in leaves were digested with several restriction enzymes according to the SLAF-Seq method from the Beijing BioMarker Corporation[49, 50], while the SLAF tags were used as templates for PCR amplification, target fragment selection and so on[51]. The SLAF-sequencing was carried out on the Illumina HiSeq 2500 system (Illumina, Inc.; San Diego, CA, USA), and the information of *Ammopiptanthus* samples are listed in Table 1.

Evaluation of Data Quality and Identification of SNP Markers

“Dual-index” software was used to analyze the original data. BLAT software was used to cluster all SLAF reads based on sequence alignment[51]. Meanwhile, SNP markers were identified for polymorphic SLAF tags using GATK and SAM tools [52, 53].

Population Structure and Linkage

After SNPs pretreatment, the phylogenetic tree was constructed using the MEGA7.5 software. Meanwhile, Bayesian clustering and Linkage disequilibrium were analyzed by STRUCTURE software and TASSEL 6.0 software, respectively[54–56]. While the PCA was performed using the OdmicShare tools, a free online platform for data analysis (https://www.omicshare.com/tools).

Availability of data and materials

All the data pertaining to the present study has been included in table and/or figure form in the present manuscript and authors are pleased to share analyzed/raw data and plant materials upon reasonable request. And all sequencing data have submitted to NCBI SRA(https://www.ncbi.nlm.nih.gov/bioproject/PRJNA758436)

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Declarations

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Contributions

YD designed the study. GC, YD, PJ, ZD, and FK performed experiments. GC and YD analyzed the SLAF-seq data and drafted the manuscript. GC, YD and ZD revised the manuscript. All authors contributed to the article and approved the submitted version.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare absence of competing interests.

Tables

Table 1 Results of genetic diversity of *Ammopiptanthus* populations.

No.	ID	*ho*	*he*	NI	WI	PIC
1	NMG-ALBLG	0.32475	0.32029	0.34227	0.48802	0.25897
2	NMG-AZQ	0.32593	0.31756	0.33933	0.48472	0.25705
3	NMG-DKTST	0.27844	0.30579	0.32419	0.47019	0.24873
4	NMG-DQH	0.29359	0.33438	0.36577	0.50747	0.27043
5	NMG-QLG	0.29790	0.30305	0.31972	0.46526	0.24577
6	NX-BG	0.41261	0.38689	0.46427	0.57120	0.30764
7	NX-BDG	0.26544	0.29864	0.31673	0.46091	0.24319
8	NX-BJT	0.26626	0.30448	0.32101	0.46814	0.24755
9	NX-HW	0.38489	0.34623	0.37864	0.52076	0.27797
10	GS-HSY	0.26314	0.33488	0.36654	0.50737	0.27020
11	XJ-KS	0.13009	0.19482	0.20904	0.33833	0.17178
12	XJ-AHQ	0.27917	0.27342	0.31248	0.43996	0.23156
13	XJ-BX	0.20823	0.18781	0.20275	0.32706	0.16479
14	XJ-WYS	0.44472	0.38740	0.46489	0.57185	0.30805
15	GS-MQ	0.229488	0.26369	0.27965	0.42343	0.22170

Table 2 The results of nalyses of variance for populations within the entire distributional region and two species of *Ammopiptanthus*.

Grouping of species	Source of variation	d.f.	Sum of squares	Variation components	Variation (%)	Fixation index
A.mongolicus	Among populations	10	192917.72	898.12	12.48	F_{ST}= 0.1248
	Among individuals within populations	69	437522.73	44.71	0.62	
	Within individuals	80	500118.50	6251.48	86.89	
A.nanus	Among populations	4	115986.48	2322.59	69.88	F_{ST}= 0.6989
	Among individuals within populations	26	31213.04	199.64	6.01	
	Within individuals	31	24838.00	801.23	24.11	
Total*(A.mongolicus* and *A.nanus)*	Among species	15	264033.63	1211.33	58.13	F_{ST}= 0.5813
	Among populations within species	95	86721.02	40.35	1.94	F_{SC}= 0.0463
	Within populations	111	92368.00	832.14	39.93	F_{CT}= 0.6007

Note: d.f, degrees of freedom; F_{ST}, correlation within populations relative to the total; F_{SC}, correlation of haplotypes within groups relative to the total; F_{CT}, correlation within populations relative to groups.

Table 3 Population details of Chinese elms and their climate information.

Number	Species	Population Location	Abbreviation	Sample Size	Geographical Coordinates	Altitude (m)
1	*A.mongolicus*	Dongqinghu	NMG-DQH	6	40°30′69″N 106°29′91″E	1031
2		Aolunbulage	NMG-ALBLG	8	40°29′59″N 106°16′03″E	1045
3		Jilantai	NMG-AZQ	8	40°06′98″N 105°41′99″E	1072
4		qianligou	NMG-QLG	10	39°55′07″N 106°53′28″E	1269
5		Taositu	NMG-DKTST	9	40°10′64″N 106°55′66″E	1054
6		Binggou	NX-BG	3	38°30′96″N 106°34′66″E	1125
7		Biandangou	NX-BDG	9	37°42′65″N 106°19′81″E	1261
8		Baijitan	NX-BJT	10	37°54′13″N 106°27′65″E	1303
9		Hongwei	NX-HW	6	37°26′79″N 104°49′04″E	1576
10		Hongshayan	GS-HSY	11	37°30′25″N 103°49′49″E	1915
11	*A.nanus*	Kangsu	XJ-KS	8	39°42′12″N 75°03′22″E	2171
12		Bayinkulu	XJ-AHQ	4	39°49′50″N 75°35′25″E	2157
13		Biaoertuokuoyi	XJ -BX	7	39°30′28″N 74°52′46″E	2546
14		Wuheshalu	XJ-WYS	3	39°39′30″N 75°45′18″E	2250
15		Minqin,Gansu	GS-MQ	9	38°59′01″N 102°98′74″E	1375

Figures

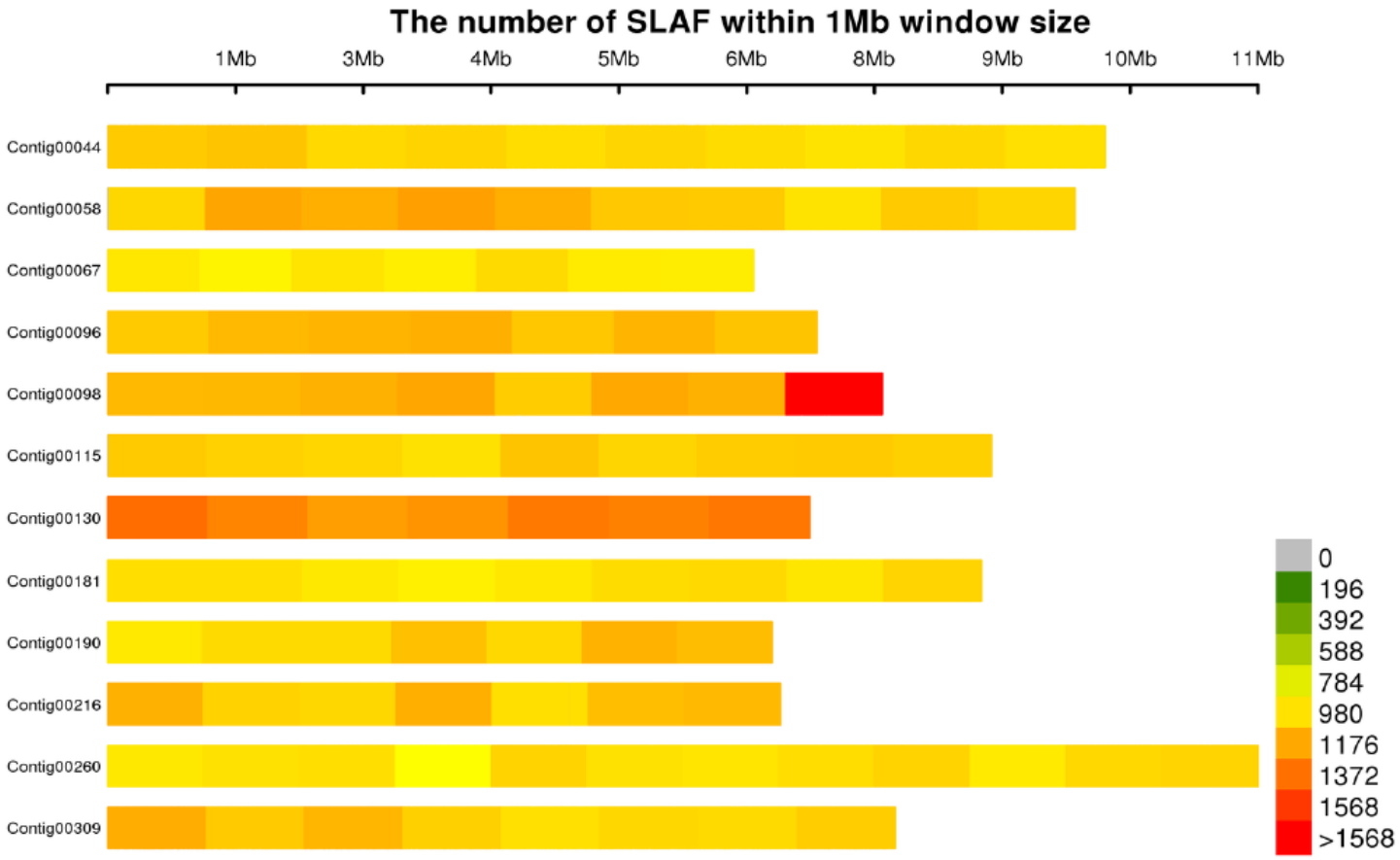

Figure 1

Distribution of SLAF tags on chromosomes of 111 Ammopiptanthus species.

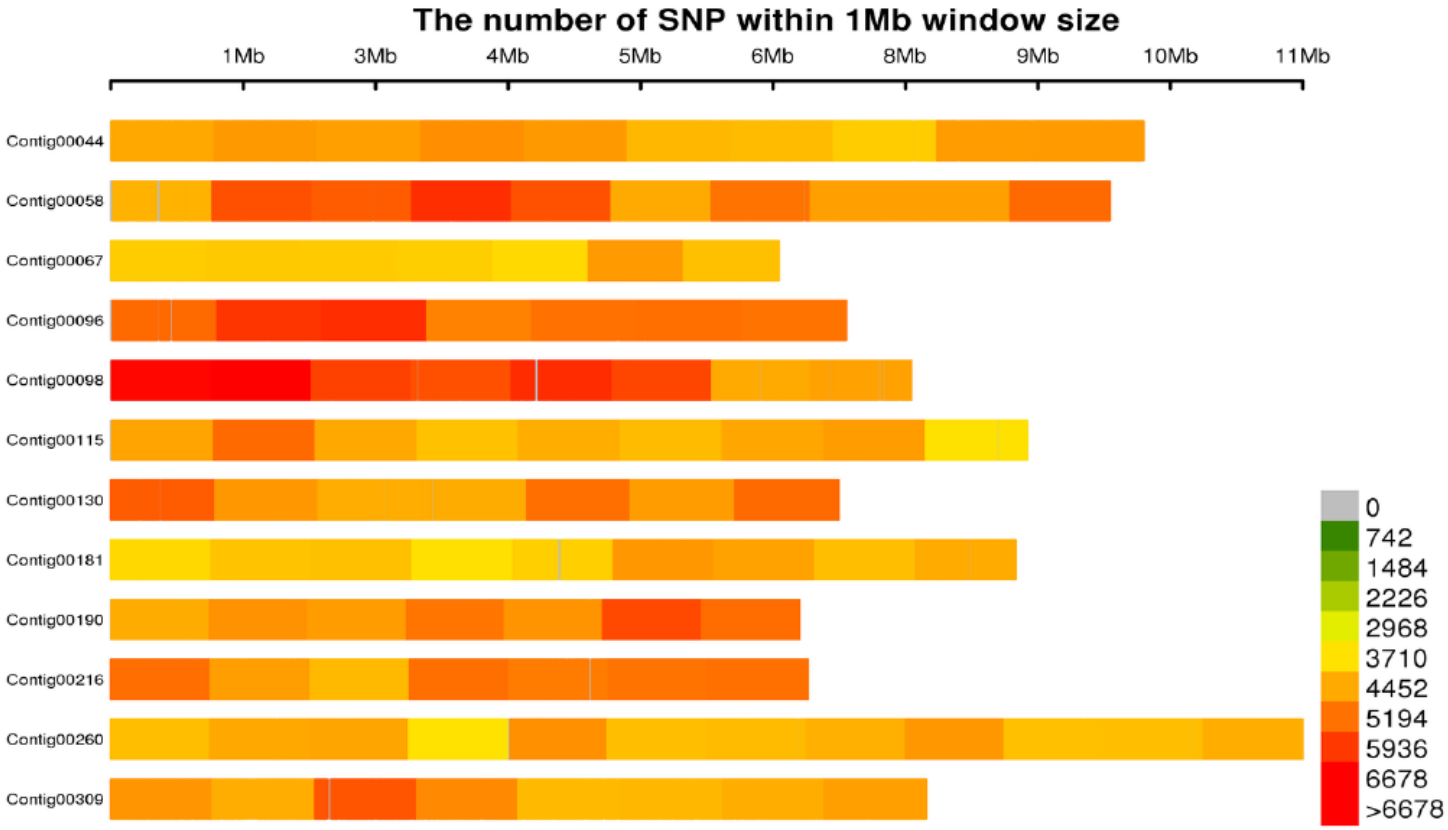

Figure 2

Distribution of SNPs on 111 Ammopiptanthus chromosomes. Thex-axis represents the physical length of the chromosome.

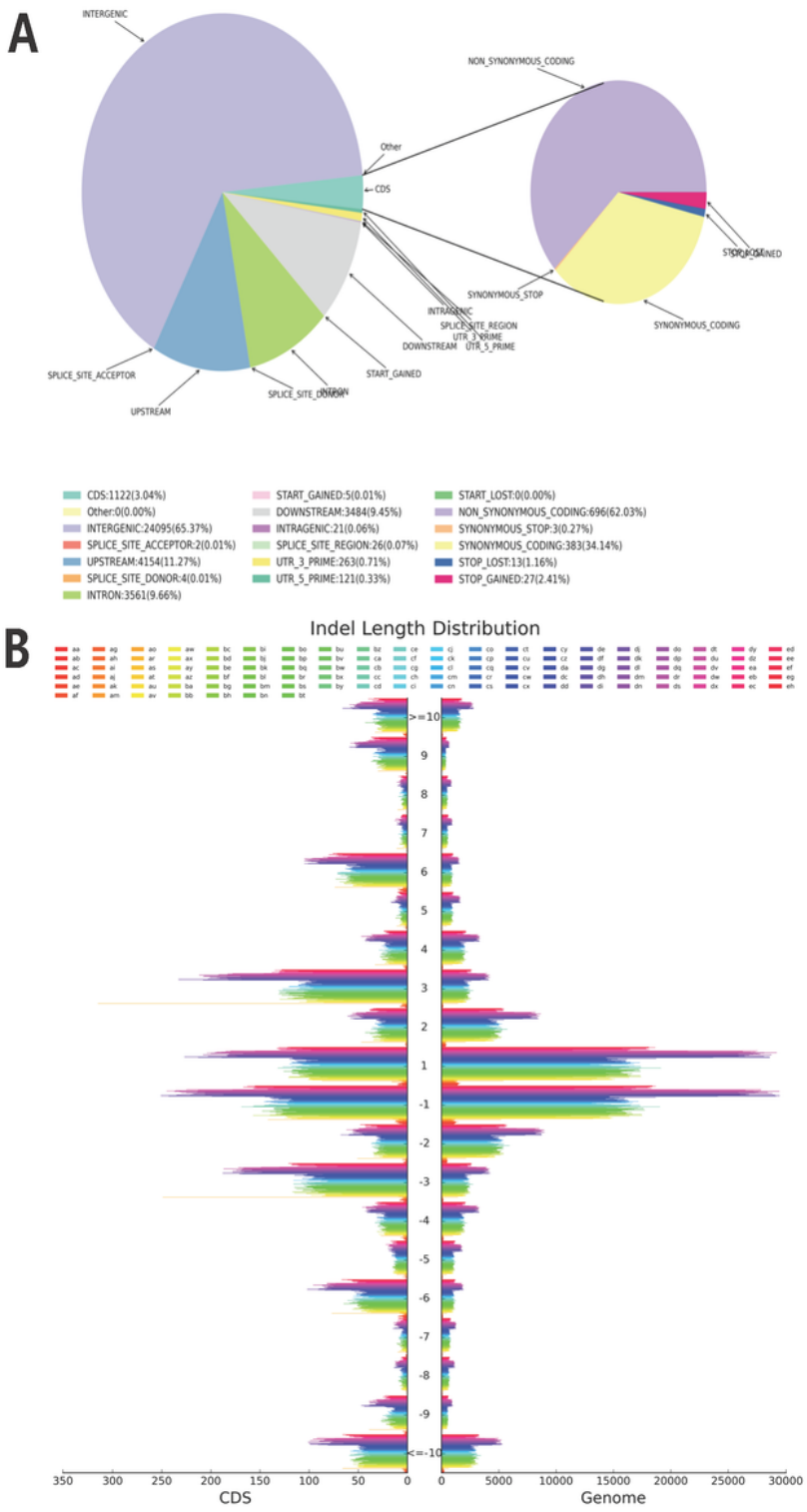

Figure 3

Annotation of InDels in 111 Ammopiptanthus species. (A) Distribution of InDels in different intergenic and genic regions among 111 Ammopiptanthus population species. In CDS region, the number of synonymous and non-synonymous InDels detected has also shown among the Ammopiptanthus species species. (B) InDels length distribution in different genomic regions for the 111 Ammopiptanthus species.

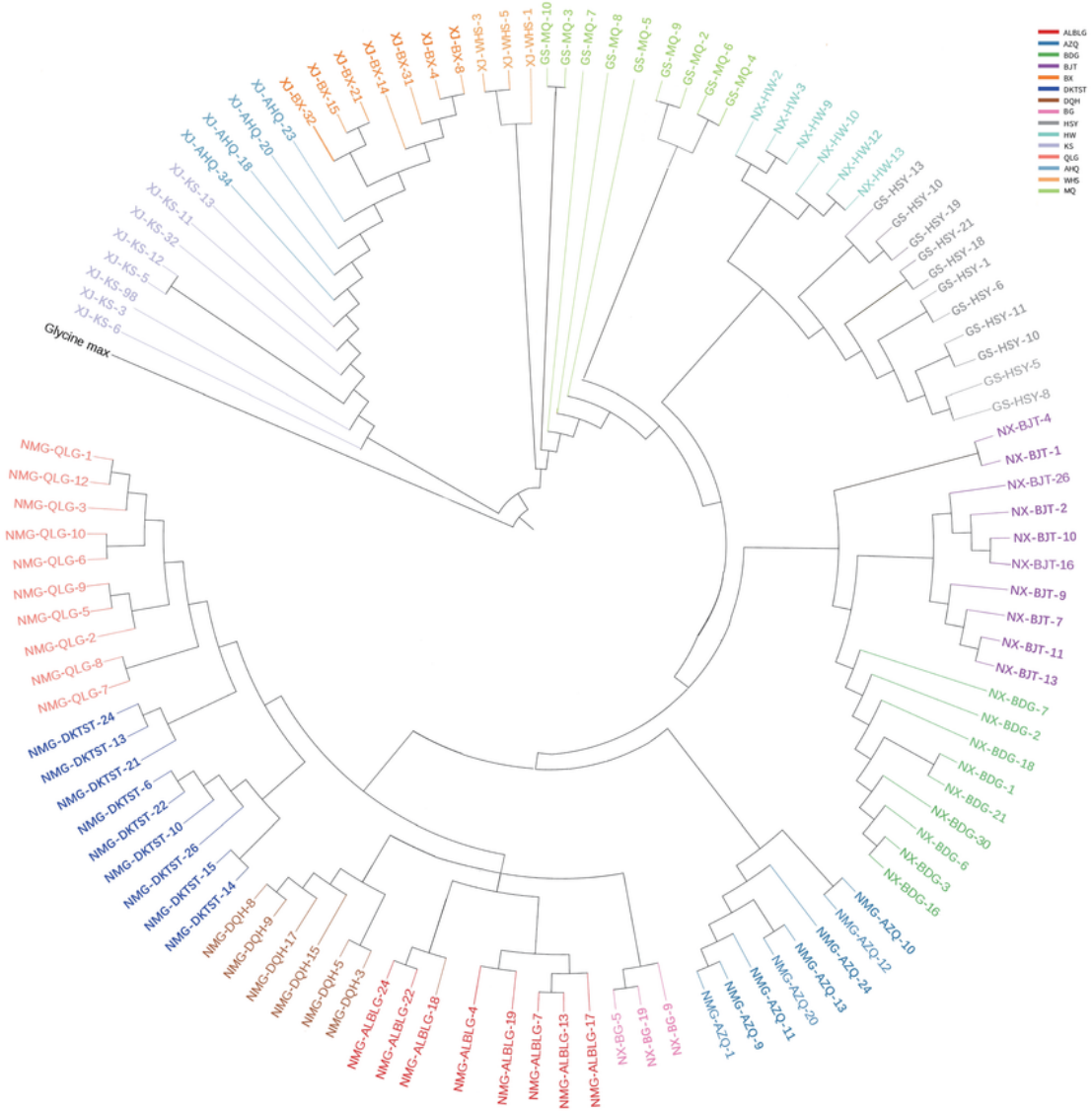

Figure 4

Phylogenetic tree of the 111 individuals based on the analysis of 457,888 single nucleotide polymorphisms (SNPs).

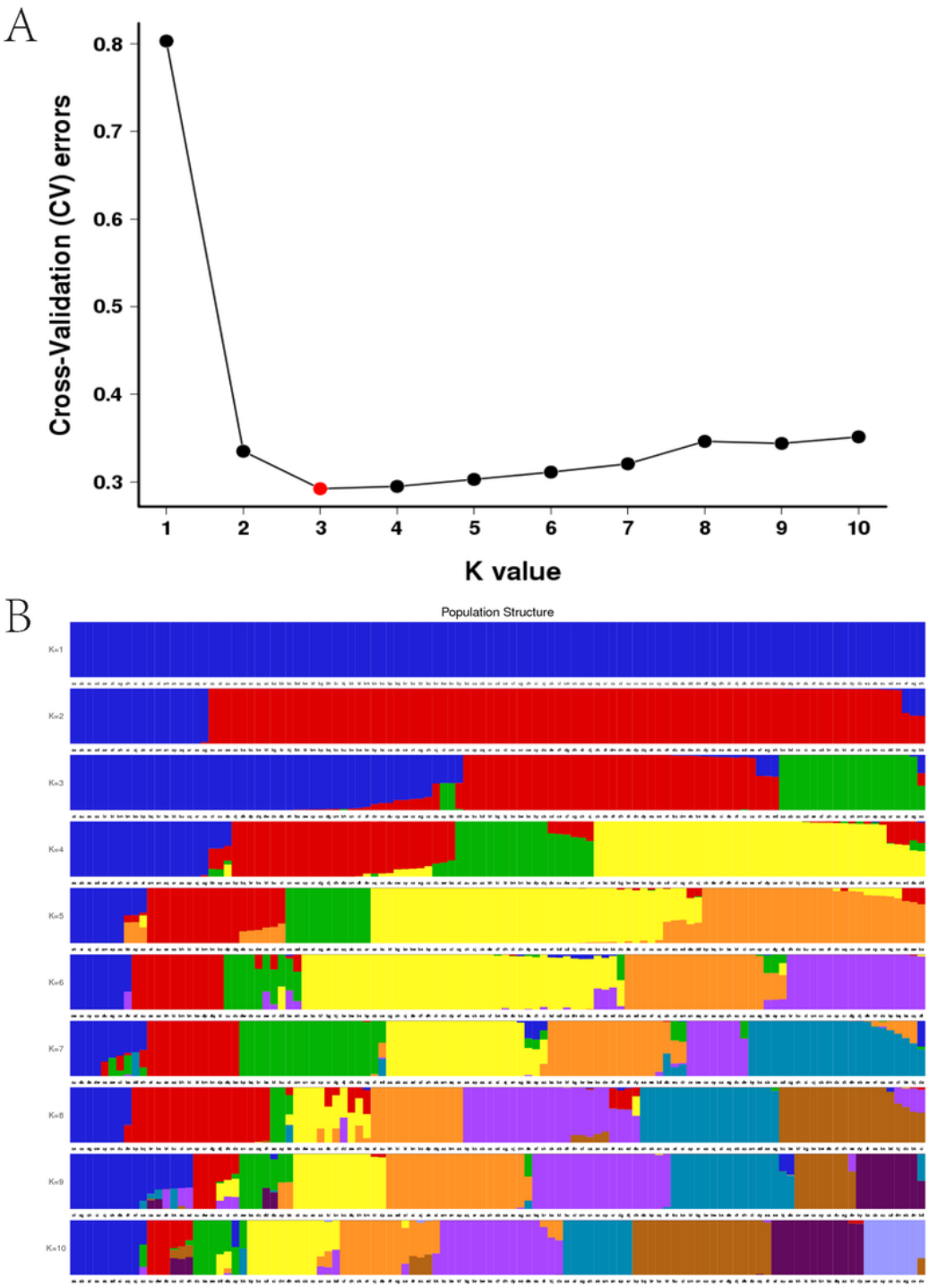

Figure 5

Population structure analysis of the Ammopiptanthus accessions (111 genotypes) by the Admixture program using genome-wide SNP markers. (A) The estimated cross-validation errors for different grouping results (K value). (B) Groups identified in the structure analysis by a predefined K (group number) from 1 to 10.

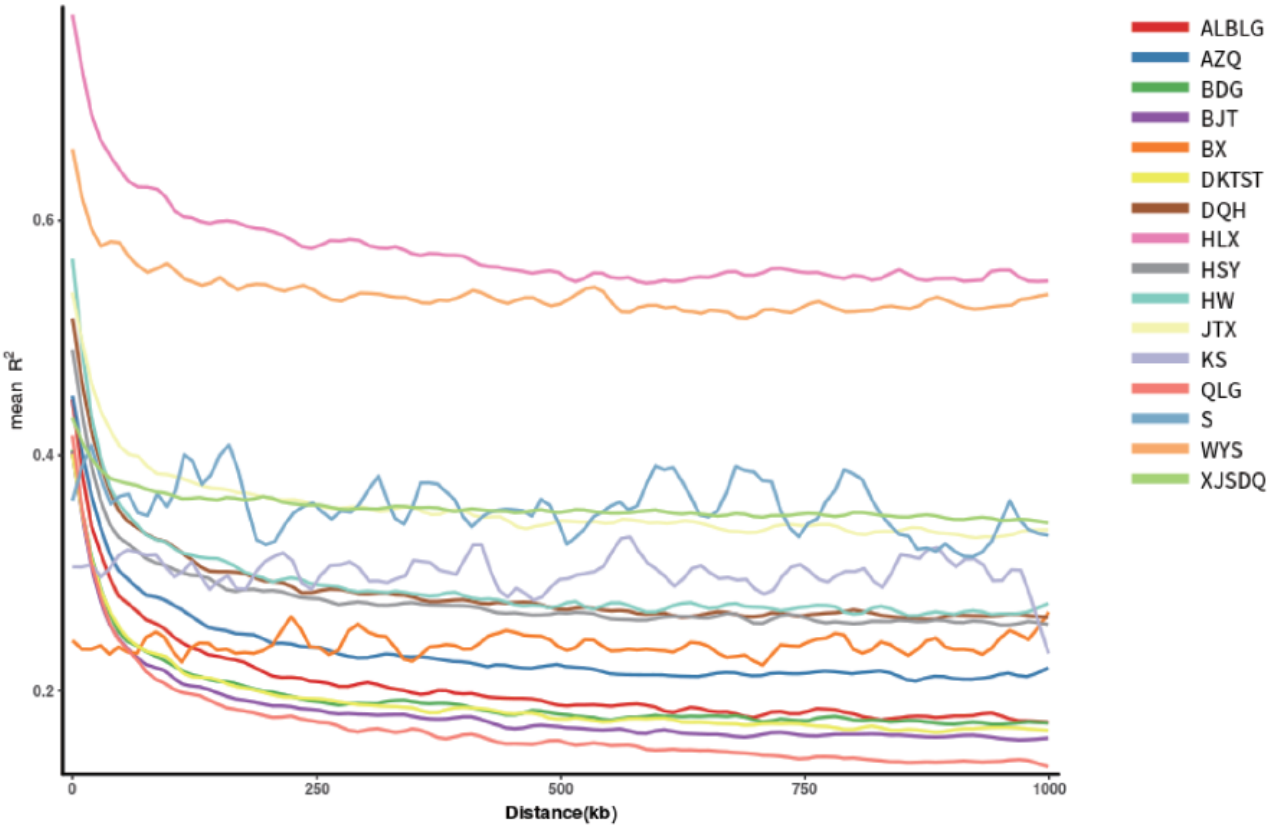

Figure 6

Linkage Disequilibrium Analysis.

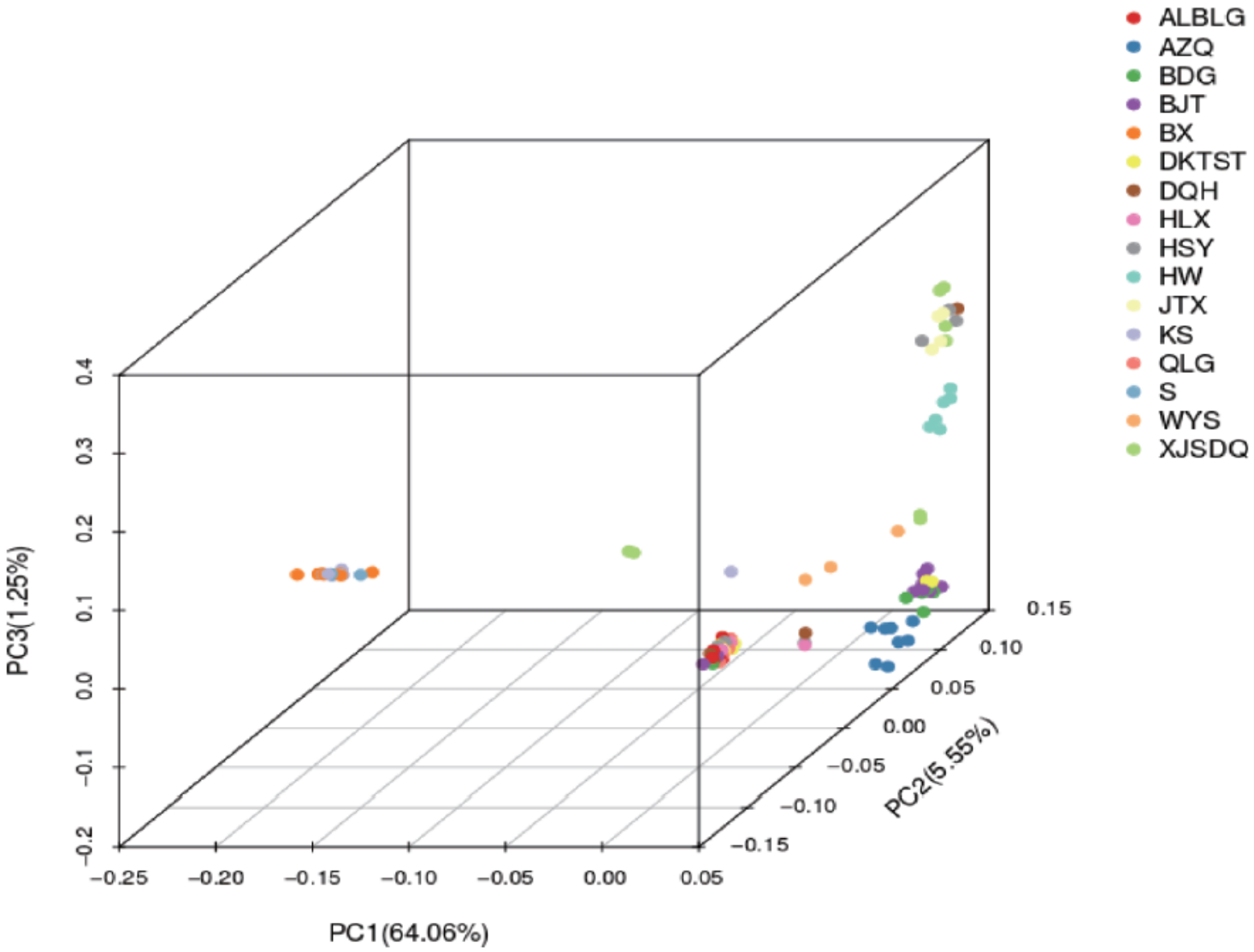

Figure 7

The principal component analysis (PCA) clustering among Ammopiptanthus samples.

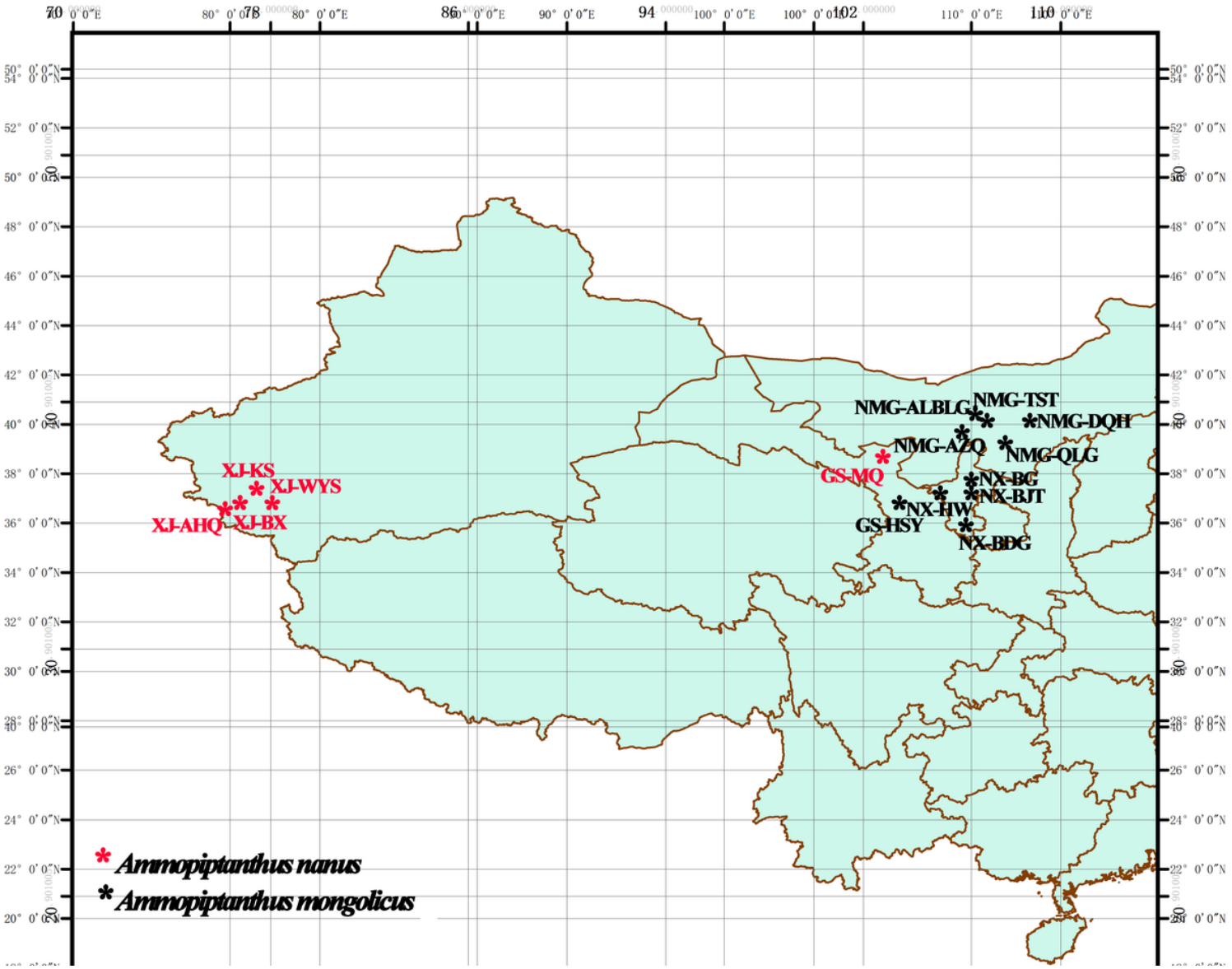

Figure 8

Map showing locations of the populations of Ammopiptanthus.

Supplementary Files

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