

Application of chloroplast genome to resolve the taxonomy and phylogenetic relationships of invasive dioecious weeds in *Amaranthus* (Amaranthaceae)

Han Xu (✉ xuhangin@gmail.com)

Research article

Keywords: Phylogenetic relationship, Chloroplast genome, Invasive alien plants, *Amaranthus palmeri* S. Watson, *Amaranthus arenicola* I. M. Johnston, *Amaranthus tuberculatus* (Moq.) J.D. Sauer, *Amaranthus* subgen. *Acnida* (L.) Aellen ex K. R. Robertson

Posted Date: December 19th, 2019

DOI: <https://doi.org/10.21203/rs.2.19304/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Background: *Amaranthus palmeri*, *A. tuberculatus* and *A. arenicola* are alien invasive dioecious amaranths originated from North America which have similar morphology and complex taxonomic relationship with their relatives. To search for effective molecular methods and accurate species boundary for detecting the alien invasive species, we sequenced whole chloroplast genome of 6 amaranths species, of which *A. palmeri*, *A. arenicola*, *A. retroflexus* and *A. dubius* are the first reports.

Results: The complete chloroplast genome of 6 species has a circular molecular structure of 150,454 to 150,939 bp in length with 36.6% of GC content and contains a total of 134 genes, including 89 protein-coding genes, 37 tRNA genes, and 8 rRNA genes. There are a total of 802 parsimony-informative (PI) sites within genes and intergenic spacers. The *rpl22-rps19*, *ndhG-I*, *rpl 32-trnLUAG*, *trnPUGG-psaJ* and *ccsA-ndhD* are the hotspots in the genus. And the 1,601 bp fragment from *rpl32* to *psaC* has contained maximum variants with 82 PI sites. *A. arenicola* differs from *A. tuberculatus* with 19 PI sites located in 14 genes and spacers separately. The regions for differentiate *A. dubius*, *A. hypochondriacus* and *A. caudatus* of the Hybrid complex only fasten on 2 coding genes and 5 intergenic spacers. The patristic distances (0.00001-0.00005) among the three species are approximate to the distance (0.00005) between individuals of *A. tuberculatus*. Conformed to dioecious and monoecious distinctions but different with previous phylogenetic studies, *A. palmeri* clustered with *A. arenicola* and *A. tuberculatus* and formed a stable clade of subgen. *Acnida*.

Conclusion: The chloroplast genome has played a role in offering enough information for discrimination and phylogenetic relationship among the *Amaranthus* subgen. *Acnida*. The most valuable regions of chloroplast genome in *Amaranthus* are intergenic spacers and could differentiate *A. arenicola* from *A. tuberculatus* better. Subsequently, much more *Amaranthus* species should be sequenced and analyzed complementally in the future.

Background

The genus *Amaranthus* comprises at least 60 species, most of them are annual weeds and distributed throughout the world's temperate and tropical regions (Mosyakin & Robertson, 2003). Several amaranths are economic crops, such as *A. hypochondriacus* L. and *A. caudatus* L. (Sauer, 1950), and a well-known vegetable and horticultural plant *A. tricolor* L. (Advisory Committee on Technology Innovation, 1984). In contrast to this, *A. palmeri* S. Watson, *A. tuberculatus* (Moq.) J.D. Sauer and *A. retroflexus* L. are famous invasive weeds (Ward et al., 2013; Costea, 2005; Steckel & Sprague, 2004). These weeds not only invade new habitats easily and cause environmental and agricultural damages, but are trouble subgroups hard to identify, especially their small similar, about 1 mm diameter seeds (Ward et al., 2013; Costea, 2005; Steckel & Sprague, 2004; Xu et al., 2014). These invasive weed seeds often been carried by grain trades through different countries and continents, and have been intercepted from imported grains and monitored on the ports in China (Xu et al., 2012, 2013). They have brought huge environmental and agricultural damage risks. But their identification always is an intractable problem.

In addition, the genus *Amaranthus* is divided into 3 subgenera presently: *Amaranthus* subgen. *Acnida* (L.) Aellen ex K. R. Robertson, *Amaranthus* subgen. *Amaranthus* and *Amaranthus* subgen. *Albersia* (Kunth) Gren. & Godr., according to dioecious or monoecious and the tepal or stamen number (Mosyakin & Robertson, 1996). *A. palmeri*, *A. tuberculatus*, and *A. arenicola* are dioecious, belong to *Amaranthus* subgen. *Acnida* (L.) Aellen ex K. R. Robertson, and often be misidentified for their similar morphology (Sauer, 1955, 1972). These three dioecious amaranths all originated from North America and have spread widely in agricultural fields and other disturbed areas (Sauer, 1955, 1972).

Palmer amaranth is one of a distinct subgroup of dioecious species within *Amaranthus* which hybridization among different species has been widely reported (Trucco et al., 2007; Steckel, 2007). Gaines et al. (2012) found the highest levels of successful hybridization (up to 0.4%) occurred between Palmer amaranth and spiny amaranth, with this cross producing viable and fertile F1 progeny (Gaines et al., 2012). All like that are often of frequent occurrence among different amaranths. Especially the confused relationships among the waterhemp complex (*A. tuberculatus* var. *rudis* (J.D. Sauer) Costea & Tardif and *A. tuberculatus* (Moq.) J.D. Sauer var. *tuberculatus*) with *A. arenicola*, that prompts us to get more details about these taxa through a new perspective.

The chloroplast genome has been studied in *Amaranthus*, but mainly referring to the species under *Amaranthus* subgen. *Amaranthus* and subgen. *Albersia*, and get better effectiveness on the phylogeny than common DNA barcodes ITS, *matK*, and *rbcl*, but poorly results were gotten at resolving the Hybrid complex (*A. hybridus*, *A. caudatus*, *A. hypochondriacus*, *A. quitensis*, *A. dubius*, *A. cruentus*) except the GBS methods (Chaney et al., 2016; Wu & Blair, 2017; Viljoen et al., 2018). ITS sequence is enough for most species of *Amaranthus*, but limited on the dioecious specie and the Hybrid complex (Xu et al., 2017a,b).

In previous studies, a high ITS sequence homology degree between Palmer amaranth and spiny amaranth has been found, and *A. palmeri* was separated from subgen. *Acnida* and then merged into subgen. *Amaranthus* (Xu et al., 2017a; Kirkpatrick, 1995). This conclusion is opposite to traditional taxonomy opinion. Whether the taxonomic status of subgen. *Acnida* is tenable needs our further studies. In our papers, we focus on seeking appropriate identified regions for amaranths based on the chloroplast. In addition, we try to find out a new viewpoint for resolve our previous doubts about the status of subgen. *Acnida*.

Results

Genomic features

The complete chloroplast genome of six *Amaranthus* spp. has a circular molecular structure of 150,454 to 150,939 bp in length with 36.6% of GC content. It has a large single copy (LSC) region of 83,747 to 84,340 bp and a small single copy (SSC) region of 17,898 to 18,044 bp, separated by a pair of identical inverted repeat regions (IRs) of 24,519 to 24,582 bp each (Table S1). The chloroplast genome contains a total of 134 genes, including 89 protein-coding genes, 37 tRNA genes, and 8 rRNA genes, 19 of which were duplicated in the inverted repeat regions (Table S2). The *rps12* not only lie in the LSC, but

duplicated in the IRs. 14 genes (*rps12*, *rps16*, *atpF*, *rpoC1*, *ndhB*, *petB*, *petD*, *ndhA*, *trnK-UUU*, *trnI-GAU*, *trnA-UGC*, *trnG-UCC*, *trnL-UAA* and *trnV-UAC*) contained a single intron, while *ycf3* and *clpP* harbored two introns separately (Table S3).

Comparative genomic analysis and hotspot regions for identification

After aligned, sequence variability was due solely to the presence of single nucleotide polymorphism (SNP) and indels. No gene rearrangements of genome or differences in gene content were observed. There are a total of 802 PI sites among 134 genes and 132 intergenic spacers (Table 1 and S3). Analysis of the distribution of genetic variability within the chloroplast genome of *Amaranthus* revealed that the most variable region is the Small Single Copy Unit (SSC) with 1.12 percent of nucleotide variation (Table 1). And the variation frequency of intergenic spacers is 1.05 percent much more than 0.32 percent of the gene regions (Table 1).

Among the 134 genes, the highest frequency of polymorphism was found in *rpl16* (1,385 bp, 2.671%) and *psbT* (102 bp, 1.961%) (Table S3). On the other side, *rpl22-rps19* (132bp, 9.091%), *ndhG-I* (146 bp, 6.849%), *rpl32-trnLUAG* (1,177 bp, 5.523%), *trnPUGG-psaJ* (422 bp, 5.213%) and *ccsA-ndhD* (201 bp, 5.473%) are the biggest concentration of polymorphic intergenic spacers (Table S4). And the 1,601 bp fragment from *rpl32* to *psaC* has contained maximum variants with 82 SNPs (Table S4).

The hotspots for discriminate *A. arenicola* with *A. tuberculatus* mainly concentrate in 6 genic regions (*ndhA*, *rpl16*, *ccsA*, *matK*, *atpF* and *ycf1*) and 8 intergenic spacers (*psbA-trnKUUU*, *trnDGUC - trnYGUA*, *ndhC - trnVUAC*, *trnEUUC - trnTGGU*, *trnTUGU - trnLUAA*, *atpB - rbcL*, *rbcL - accD*, *trnWCCA - trnPUGG*, *trnPUGG - psaJ* and *rps15 - ycf1*) each contains one or two PI sites (Table S3 and S4). The SNPs for differentiate the complex of *A. dubius*, *A. hypochondriacus* and *A. caudatus* fasten on 2 coding genes (*matK* and *rpl16*) and 5 non-coding spacers (*trnHGUG - psbA*, *rpoB - trnCGCA*, *psbC - trnSUGA*, *trnGGCC-trnfMCAU* and *ndhF - rpl32*) (Table S3 and S4).

Phylogenetic tree

The two phylograms constructed by whole chloroplast genome and 802 PI sites are consistence basically (Figure 1 and S1). Conformed to traditional taxonomy opinions, *A. palmeri* together with *A. arenicola* and *A. tuberculatus* formed the clade of subgen. *Acnida*. *A. arenicola* has apart with *A. tuberculatus* by 19 SNPs. *A. tricolor* represents subgen. *Albersia*, has closer relationship with subgen. *Acnida* rather than subgen. *Amaranthus*. *A. dubius* is closer with *A. hypochondriacus* and *A. caudatus* than *A. hybridus* ssp. *cruentus*.

Genetic distance

According to patristic distances calculated by whole plastid genome and pairwise distance based on PI sites, 2 individuals of *A. tuberculatus* have small difference (0.00005) (Table S5). *A. arenicola* differs from *A. tuberculatus* with 19 SNPs and 0.00029 patristic distance value (Table S5). The complex of *A. dubius*, *A. hypochondriacus* and *A. caudatus* have poor differences (0.00001-0.00005) and approximate to the distance (0.00005) between individuals of *A. tuberculatus*. (Table S5). And *A. hybridus* ssp. *cruentus* keeps away from the complex with above 0.0016 values (Table S5).

Discussions

Discrimination of *Amaranthus* spp. based on the chloroplast genome

Concluded from the results, the phylogram based on the chloroplast genome showed more information about relationships and species boundary among *Amaranthus* than the *ITS*, *rbcL* and *matK*. Some species with clear demarcation could identified by *ITS*, but the similar relatives or the complex cannot get a good results for their high consistence on *ITS* (Xu et al., 2017a). Different with previous studies, there are five intergenic regions *rpl22-rps19* (132bp, 9.091%), *ndhG-I* (146 bp, 6.849%), *rpl32-trnLUAG* (1,177 bp, 5.523%), *trnPUGG-psaJ* (422 bp, 5.213%) and *ccsA-ndhD* (201 bp, 5.473%) have offered more informative phylogeny for *Amaranthus*, and much better than *ndhD*, *rpoC2*, *atpE* and *rpl22* (only 1% to 1.9%) found by Viljoen et al. (2018). That's for the sequences they used to align and analyze came from the species among *Amaranthus* subgen *Amaranthus* and *Amaranthus* subgen. *Albersia*, and not involved in three subgenera, and the hotspots will be limited. Even expanded research taxa, the valuable informative sites still scatter in different genic and intergenic regions. As for the confused taxa, if we want to set apart *A. arenicola* from *A. tuberculatus*, or distinguish the species of the Hybrid complex, that needs at least 6 genic and 13 intergenic regions. Efficient molecular markers based on these SNPs should be developed for rapid identification. And more different geographical individuals of one species ought to be used in verify the availability of these variable sites.

Taxonomy of *Amaranthus* subgen. *Acnida*

From the previous classification system of *Amaranthus* to date, many scholars have put forward different revision suggestions. The primary taxonomic system for *Amaranthus* was revised by Mosyakin & Robertson (1996) and for *Amaranthus* subgen. *Acnida* was classified by Sauer (1955). But under subgenus level, the divisions of sects or species boundary are not clear and controversial and often rectified by other scholars.

In *Amaranthus* subgen. *Acnida*, *A. tuberculatus* is a complex was formed of two varieties: *A. tuberculatus* var. *rudis* (J.D. Sauer) Costea & Tardif and *A. tuberculatus* (Moq.) J.D. Sauer var. *tuberculatus*, and has more widely diversity genetic structures and viable morphology (Mosyakin & Robertson, 2003; Pratt & Clark, 2001). The species can hybridize in the wild with other relatives of subgen. *Acnida*, even with monoecious species vest in *Amaranthus* subgen. *Amaranthus* (Costea et al., 2005; Waselkov & Olsen, 2014). That's why the phylogenetic status or species boundary are always unclear, in spite of they have several distinct characteristics for discern on morphology. Chances are the stamiferous plants could be shared widely among the different dioecious species. Especially, they all originate from the same regions and distribute overlapped. That's might be *A. arenicola* is very close with *A. tuberculatus*, and both of them are hard to distinguish.

A. palmeri is very close with *A. spinosus* which belonging to *Amaranthus* subgen. *Amaranthus* nothosect. *Dubia* Mosyakin & K. R. Robertson (Mosyakin & Robertson, 1996; Xu et al., 2017a,b; Kirkpatrick, 1995). In prior studies, *A. palmeri* was divided from subgen. *Acnida* and attributed to subgen. *Amaranthus* based on the *ITS* and *ALS* (Xu et al., 2017a,b; Kirkpatrick, 1995; Xu, 2019, Unpublished data). The taxonomic status of subgen. *Acnida* seem to be broken up. But in our studies, *A. palmeri* has a longer genetic distance with subgen. *Amaranthus*, and much more close with *A. arenicola* and *A. tuberculatus*. The phylogenetic status of subgen. *Acnida* seem to be stable and form an independent clade on the plastid genome.

Phylogenetic status of *Amaranthus* subgen. *Amaranthus*

Another group is the Hybrid complex which is the most studied taxa (Sauer, 1967). Erika Viljoen et al. (2018) and Lindsay Chaney et al. (2016) all came to a poor conclusion gotten by chloroplast data for resolving recent evolution of the complex (Chaney et al., 2016; Viljoen et al., 2018). Only the SNPs acquired from whole genome (GBS) get a better result for their taxonomy (Wu & Blair, 2017). About the Hybrid complex, our research continues to be unsatisfied, and just found 2 genes and 5 intergenic spacers could be served as markers for the next studies. Besides, the genetic distance (0.00001-0.00005) among the three species are approximate to the distance (0.00005) between individuals of *A. tuberculatus*. It means that the three species might be segmented into subspecies or varieties under *A. hybridus* instead of an independent species more reasonably.

Conclusions

All above conclusions have accounted for the genus is a trouble taxa. The chloroplast genome has played a role in offering enough information for discrimination and phylogenetic relationship among the *Amaranthus*, especially the *Amaranthus* subgen. *Acnida*. That's the first reports for the subgen. *Acnida* based on the plastid. Subsequently, much more *Amaranthus* species should be sequenced and analyzed complementedly in the future.

Methods

Plant samples, DNA extraction, and sequencing

Fresh leaves of each individual of *A. palmeri*, *A. arenicola*, *A. retroflexus*, *A. dubius* and two accessions of *A. tuberculatus* var. *tuberculatus* were sampled from imported borders in China and dried using silica gel. Voucher specimens (Table S1) were deposited at the plant quarantine institute of Chinese Academy of Inspection and Quarantine (CAIQ) (Beijing, China). All the samples used in the experiment are legal evidence samples from each port submitted for species identification. The identification is part of CAIQ's laboratory functions. The basis for identification was the classification monographs of *Amaranthus* by Sauer (1972) and Mosyakin and Robertson (2003). Total genomic DNA was extracted from the silica-dried leaf tissues using Plant Genomic DNA Kit (Tiangen Biotech Co., China). Genomic DNA of each individual was indexed by a barcode and then pooled together with other samples for sequencing in one lane of HiSeq 2500 (Illumina) (Novogene, Beijing, China). The whole chloroplast genomes of *A. hypochondriacus* (GenBank accession No. MG836505), *A. caudatus* (NC040143), *A. hybridus* ssp. *cruentus* (MG836507) and *A. tricolor* (KX094399) were downloaded from NCBI (<https://www.ncbi.nlm.nih.gov/>).

Genome assembly and annotation

The paired-end sequencing data (2 × 150 bp) were used to assemble its complete chloroplast genome. Sequencing adapters and barcodes were trimmed and low quality reads with Q value ≤ 30 removed. Trimmed paired end reads were mapped to the chloroplast sequence of *A. hypochondriacus* (MG836505), with default parameters. The reads were assembled using the Geneious Prime v. 2019.1.3 (Biomatters, Auckland, New Zealand). The consensus chloroplast sequence of four *Amaranthus* spp. was retrieved separately and used as a reference for a second round of mapping of itself reads in order to validate its consensus chloroplast sequence. All trimmed and quality-filtered sequence reads have been deposited in Genbank of NCBI. Non-mapped reads, which are assumed to be of non-plastid origin, were excluded from further analysis. The complete chloroplast genome sequence was annotated using the Geneious Prime v. 2019.1.3 (Biomatters, Auckland, New Zealand) by comparing with the genome of *A. hypochondriacus* (GenBank accession No: MG836505). The assembled and annotated *Amaranthus* spp. chloroplast genome sequence was deposited at NCBI (Table S1).

Genome comparative analysis

A comparative plot of full alignment with annotations of the 10 genomes of *Amaranthus* was produced by Geneious prime v.2019.1.3 software (Biomatters, Auckland, New Zealand), using the annotation of *A. hypochondriacus* (MG836505) as a reference. To analyze nucleotide diversity and parsimony-informative (PI) sites, we extracted all the regions (including coding regions, introns, and intergenic spacers) after alignment, and put into Molecular Evolutionary Genetics Analysis (MEGA) v6.06 software (Tamura et al., 2011) for next phylogenetic analyses, patristic distance calculation and discern the hotspot regions. The extraction followed two criteria: (i) each gene and intergenic spacer; and (ii) mutation site with variant frequency above 20%. The parsimony-informative (PI) sites are much credible than single SNP for the discrimination of difficult groups and phylogenetic analyses.

Whole-plastid genome tree and parsimony-informative (PI) sites-based tree

Chloroplast genomic phylogenetic analyses were performed based on 12 sequences of 11 species in *Amaranthaceae*. These sequences were aligned using the Geneious Prime v.2019.1.3 software (Biomatters, Auckland, New Zealand) and Neighbor-Joining (NJ) analysis was conducted with *Celosia trigyna* L. (MN057637) and *Alternanthera philoxeroides* (Mart.) Griseb. (MK795965) as outgroups using Geneious Tree Builder and confidence for nodes determined using bootstrap analysis with 1000 replicates. In addition, we extracted all PI sites except indels of each gene and intergenic spacer from the whole chloroplast genome and formed a fasta forma file putting into MEGA v6.06 software (Tamura et al., 2011) for phylogenetic analyses and construct a phylogram based on the PI sites.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analysed during the current study are available in the GenBank repository, <https://www.ncbi.nlm.nih.gov/>.

Competing interests

The authors declare that they have no conflict of interest.

Funding

This work was supported by National agricultural standardization Project NBFW-14-2018, and the Basic Scientific Research Program of CAIQ (2014JK01002017JK038). The work involved in this study is part of the above projects on pest identification techniques.

Authors' contributions

XH designed the experiments and write the paper.

Acknowledgements

Thanks to Xiaoxia Zhang and Liansheng Xu of Institute of Botany, Chinese academy of sciences, for their technical guidance in the annotation of genome analysis. Thanks to the port staff who provided samples to the laboratory.

References

1. Advisory Committee on Technology Innovation. *Amaranth, modern prospect for ancient crop. Report of an Ad Hoc Panel of the Advisory Committee on Technology Innovation Board on Science and Technology for International Development Office of International Affairs, National Research Council*. Washington DC: National Academic Press; 1984.
2. Chaney L, Mangelson R, Ramaraj T, Jellen EN, Maughan PJ. The Complete Chloroplast Genome Sequences for Four *Amaranthus* Species (Amaranthaceae). *Appl Plant Sci*. 2016; 4(9): 1600063.
3. Costea M, Weaver SE, Tardif F. The biology of invasive alien plants in Canada. 3. *Amaranthus tuberculatus* (Moq.) Sauer var. *rudis* (Sauer) Costea & Tardif. *Can J Plant Sci*. 2005; 85: 507-522.
4. Gaines TZ, Ward SM, Bekun B, Preston C, Leach JE, Westra P. Interspecific hybridization transfers a previously unknown glyphosate resistance mechanism in *Amaranthus* species. *Evol Appl*. 2012; 5: 29-38.
5. Kirkpatrick BA. Interspecific relationships within the genus *Amaranthus* (Amaranthaceae). Ph.D. dissertation. College Station). TX: Texas A & M University; 1995. p. 87.
6. Mosyakin SL, Robertson KR. New infrageneric taxa and combinations in *Amaranthus* (Amaranthaceae), *Ann Bot Fennici*. 1996; 33: 275-281.
7. Mosyakin S, Robertson KR. *Amaranthus*. Magnoliophyta: Caryophyllidae, part 1. In: Flora of North America Editorial Committee editors. *Flora of North America North of Mexico, Vol. 4*. New York: Oxford University Press; 2003
8. Pratt DB, Clark LG. *Amaranthus rudis* and *A. tuberculatus*, one species or two? *J Torrey Bot Soc*. 2001; 128: 282-296.
9. Sauer JD. The grain amaranths: a survey of their history and classification. *Ann Mo Bot Gard*. 1950; 37: 561-632.
10. Sauer JD. Revision of the dioecious amaranths. *Madroño*. 1955; 13: 5-46.
11. Sauer JD. The grain amaranths and their relatives: a revised taxonomic and geographic survey. *Ann Mo Bot Gard*. 1967; 54: 103-137.
12. Sauer JD. The dioecious amaranths: a new species name and major range extensions. *Madroño*. 1972; 21: 426-434.
13. Steckel LE, Sprague CL. Common waterhemp (*Amaranthus rudis*) interference in corn. *Weed Sci*. 2004; 52(3): 359-364.
14. Steckel LE. The dioecious *Amaranthus* spp.: here to stay. *Weed Tech*. 2007; 21: 567-570.
15. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol*. 2011; 28(10): 2731-2739.
16. Trucco F, Jeschke MR, Rayburn AL, Tranel PJ. Promiscuity in weedy amaranths: high frequency of female tall waterhemp (*Amaranthus tuberculatus*) x smooth pigweed (*A. hybridus*) hybridization under field conditions. *Weed Sci*. 2005; 53: 46-54.
17. Trucco F, Zheng D, Woodyard AJ, Walter AR, Tatum TC, Rayburn AL, Tranel PJ. Nonhybrid progeny from crosses of dioecious Amaranths: implications for gene-flow research. *Weed Sci*. 2007; 55: 119-122.

18. Viljoen E, Odeny DA, Coetzee MPA, Berger DK, D.J.G. Rees DJG. Application of Chloroplast Phylogenomics to Resolve Species Relationships Within the Plant Genus *Amaranthus*. J Mol Evol. 2018; doi:10.1007/s00239-018-9837-9.
19. Ward SM, Webster TM, Steckel LE. Palmer Amaranth (*Amaranthus palmeri*): A Review. Weed Tech. 2013; 27: 12-27.
20. Waselkov KE, Olsen KM. Population genetics and origin of the native north American agricultural weed waterhemp (*Amaranthus tuberculatus*, Amaranthaceae). AJB. 2014; 101 (10): 1726-1736.
21. Wu X-B, Blair MW. Diversity in Grain Amaranths and Relatives Distinguished by Genotyping by Sequencing (GBS). Front Plant Sci. 2017; 8: 1960.
22. Xu H, Chen K, Ouyang Z-Y, Pan X-B, Zhu S-F. Threats of invasive species for china caused by expanding international trade. ES & T. 2012; 46: 7063-4.
23. Xu H, Song Y, Fan X-H, Yang S-J, Wu Y. Invasion risk and suitability analysis of three kinds of Acnida weeds (Subgen. Acnida L.) in China. Plant Quarantine. 2013; 27(4): 20-23.
24. Xu H, Li Z-Y, Liao F, Liu Y. Seed morphological studies on the genus of *Amaranthus* in China. Plant Quarantine. 2014; 28 (2): 33-38.
25. Xu H, Li Z-Y, Li J-S. Phylogenetic analysis of alien species of *Amaranthus* in China based on ITS sequences. *Guihaia*. 2017a; 37 (2): 139-144.
26. Xu H, Zhao C-Y, Liu Y-B, Chen P-C, Li J-S. Taxonomic circumscription of *Amaranthus palmeri*, *A. rudis* and *A. tuberculatus* (Amaranthaceae) based on ITS sequences and SNP analysis. *Plant protection*. 2017b; 43(5): 128-133.
27. Xu H. Detecting alien invasive *Amaranthus* weeds using *ITS* and *ALS* and *R ALS* allele biotypes were founding from China Ports. Unpublished; 2019.

Table

Table 1 Variations within genes and intergenic regions among 10 whole chloroplast genomes of 9 *Amaranthus* spp.*.

Reigon	Coding regions					Non-coding regions					Whole chloroplast genome				
	Length	SNP	Indel	Total	Variable frequency	Length	SNP	Indel	Total	Variable frequency	Length	SNP	Indel	Total	Variable frequency
LSC	56,218	176	60	236	0.42%	30,110	205	105	310	1.03%	86,328	381	165	546	0.63%
IRa	19,673	12	0	12	0.06%	4,910	6	7	13	0.26%	24,583	18	7	25	0.10%
SSC	15,509	88	4	92	0.59%	2,990	51	64	115	3.85%	18,499	139	68	207	1.12%
IRb	19,673	11	1	12	0.06%	4,910	6	6	12	0.24%	24,583	17	7	24	0.10%
Total	111,073	287	65	352	0.32%	42,920	268	182	450	1.05%	153,993	555	247	802	0.52%

*Note: The region length of genes and intergenic spacers includes gaps and originated from the consensus sequence aligned by 10 whole chloroplast genomes of 9 *Amaranthus* spp..

Figures

