

# ***Adiantum hispidulum* chloroplast genome high-throughput sequencing and comparative analysis of cpDNA inverted repeat regions of *Adiantum* species**

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*Adiantum* is a genus of family Pteridaceae that is a large family with a nearly worldwide distribution. Presently 35 genera of family Pteridaceae belongs to four subfamilies. Recent molecular studies supported five major clades within the family: the cryptogrammoids (the CR clade), the ceratopteridoids (the CE clade), the pteridoids (the PT clade), the cheilanthoids (CH clade) and the adiantoids (the AD clade). The AD clade formed by genus *Adiantum* (tribe Adiantae) together with vittarioid ferns (tribe Vittarieae) [1, 2].

*Adiantum* (commonly known as maidenhairs ferns) comprising about 200 species and widely distributed mainly in the tropical and subtropical regions. The classifications of *Adiantum* mainly based on regional studies. Authors pointed out that parallel or convergent evolution probably occurred in the frond architecture and other characters in *Adiantum*, which may have obscured the relationships among species [2, 3].

Comparative analyses of molecular markers between closely related species may provide clues to understand the intragenus phylogeny and character evolution during their different ecological niche colonization.

Analysis of level and pattern of genetic variation using the ISSR fingerprinting method previously demonstrated low level of intrageneric differentiation in four species of the fern genus *Adiantum* L., originating from South India (*A. hispidulum* Sw., *A. incisum* Forrsk., *A. raddianum* C.Presl, *A. zollingeri* Mett. ex Kuhn) [4].

Another emerging approach is to use complete chloroplast (cp) DNA sequences but molecular evolutionary and ecology analyses on ferns cpDNA are relatively few in general in comparison with those on seed plants. The complete nucleotide sequence of the chloroplast genome of the fern *Adiantum capillus-veneris* L. was determined in 2003 [5], but no more *Adiantum* full genome chloroplast sequences has been published during last decade.

This work we present sequencing and assembling the cpDNA of *A. hispidulum* using Illumina (MiSeq) sequencing technology. For sample preparation the mature alive fern was taken from the collection of the Moscow State University Botanical Garden. Total DNA (partially

enriched by cpDNA) was extracted from fresh fronds and TruSeq (NEBNext reagents) protocol was used for preparing DNA library. Two sequencing runs produced about 2,04M PE(2x300) reads. Trimming procedure resulted to about 2,01M high quality PE reads and cpDNA reached 2.5% of total data. De-novo assembly (Velvet) of cpDNA reads generated 38 contigs with sum length about 140 Kbp. Finalization of the chloroplast genome assembly is in progress.

Analysis of five plastid markers (atpA, atpB, rbcL, trnLF and rps4-trnS) was carry out to study of intraspecies variability of cpDNA and searching of relatives of MSU Botanical Garden *A. hispidulum* specimen. Comparison of our data with previously published sequences demonstrated the undoubted common origin of Russian isolate and cultivated isolate "A79" from USA: Virginia (see table 1).

We made comparative analysis of *A. hispidulum* vs *A. capillus-veneris* inverted repeats (IR) regions of cpDNA sequences using BLAST algorithm. *Adiantum capillus-veneris* chloroplast genome consists of 150568 bp [NC\_004766.1]. It contains two IR (~23Kbp) that separate large single-copy region (~82 Kbp in *A. capillus-veneris*) from small single-copy region (~21Kbp in *A. capillus-veneris*) [1] and comprise 14 genes: transfer RNA genes: trnT-TGT, trnR-ACG, trnH-GTG, trnN-GTT, trnA-TGC, trnI-GAT; ribosomal RNA genes: rrn4.5, rrn5, rrn16, rrn23; ribosomal protein genes: rps7, rps12 (exon 2); photosystem II gene: psbA; hypothetical protein gene: ycf2. Our results demonstrate the same principal organization of IR regions of cpDNA of both *Adiantum* species. The essential difference between IR regions of *A. capillus-veneris* and *A. hispidulum* is the ~180 bp region that is located between ycf2 and trnN-GTT genes. There are only two small differences between two IR regions of *A. capillus-veneris* cpDNA - at the hypothetical protein gene (ycf2) and on the boundary of IR regions and small single-copy region. Comparative analysis of two IR regions of *A. hispidulum* cpDNA reveals more differences.

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5. P.G. Wolf et al. (2003) Complete Nucleotide Sequence of the Chloroplast Genome from a Leptosporangiate Fern, *Adiantum capillus-veneris* L., *DNA Research*, **10**: 59-65.

**Table 1.** Comparison of Russian isolate *A. hispidulum* with specimens from different geographic locations using five basic plastid markers.

marker	specifications	Comparison of Russian isolate cp markers to		Nearest homolog of Russian isolate <i>A. hispidulum</i>
		isolate "A79" USA: Virginia cultivated	isolate "A69" Indonesia: Sulawesi	
<b>rbcL</b>	accession number	JF935349	JF935341	isolate "A79", USA data is presented in column with the same name
	homology	100%	99%	
	SNP numbers	none	6	
<b>atpB</b>	accession number	JF935431	JF935423	isolate "A79", USA data is presented in column with the same name
	homology	100%	99%	
	SNP numbers	none	2	
<b>atpA</b>	accession number	JF937304	JF937296	isolate "A79", USA data is presented in column with the same name
	homology	99%	99%	
	SNP numbers	1	2	
<b>rps4-trnS</b>	accession number	JF980615	JF980607	isolate "A79", USA data is presented in column with the same name
	homology	98%	97%	
	SNP numbers	17	31	
<b>trnLF</b>	accession number	JF980694	JF980686	LC004388 country="Indonesia" collector number: BA706 homology 99% SNP numbers = 1
	homology	99%	99%	
	SNP numbers	5	7	