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Sequence Analyses of ITS Regions of nrDNA from *Siphocranion nudipes* Kudo (Lamiaceae) *

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Abstract The sequence of internal transcribed spacer (ITS) regions of nuclear ribosomal DNA (nrDNA) was determined for the endemic species *Siphocranion nudipes* (Hemsl.) Kudo (Lamiaceae) from China. This is the first sequence report of the ITS regions for Lamiaceae. The entire ITS and 5.8S regions of *Siphocranion nudipes* are 609 bases long with an ITS-1 of 207 bp, an ITS-2 of 236 bp and a 5.8S of 166 bp. The overall G+C content was 59.11% with 64.25% in ITS-1, 57.63% in ITS-2, and 53.61% in 5.8S. The numerous copies of the ITS sequences within an individual in Siphocranion nudipes showed a high level of sequence homogeneity and only three nucleotide positions were ambiguous. This sequence will serve as a basis for determining the phylogenetic status of *Siphocranion nudipes* within Lamiaceae and for indepth studies on the conservation of this endemic species from subtropical areas of China.

Keywords sequence, *Siphocranion nudipes*, internal transcribed spacer regions
Classification number Q 949.777.6

Siphocranion Kudo is a genus from subtropical areas of China, northerns of India, Myanmar and Viet-Nam[1]. It consists of two species *Siphocranion nudipes* (Hemsl.) Kudo distributed in subtropical regions of China, Yunnan, Sichuan, Hubei, Guizhou, Guangdong, Jiangxi and Fujian provinces, and *S. macranthum* (Hook. f.) Wu[2] distributed in southwestern China, India, Myanmar and Viet Nam. The type-species of *Siphocranion*, *S. nudipes* Kudo was previously described as *Plectranthus nudipes* by Hemsl in 1890 and then as Hancea nudipes by Dunn in 1913. Kudo[3] put it in the genus *Siphocranion* and Wu[2] too by a perennial herb with a ligneous subterranean stem, almost without hair, verticellaeters 2-terminal, flowered, racemes, solidary or sometimes 3 together. Calyx broadly campanulate in bloom, 5-cleft subregular, and distinctly 2-lipped in fruit (3/2 type). Corolla-tube straight, narrow and elongate; stameus included, filaments glabrous.

Siphocranion nudipes is an endemic species and was previously recorded from Wushan in Sichuan Province[1]. Recently several populations were found in the Doupengshan forest region with an elevation above 1800 meters in the Dagongshan Natural Preserve, Sangzhi county, Hunan Province. The discovery of the additional populations attracted recent studies on the ecology and conservation of this species. However the phylogenetic and evolutionary status of Siphocranion nudipes within Lamiaceae has not been well investigated.

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The internal transcribed spacer (ITS) sequences of nrDNA have been commonly used for phylogenetic studies at the interspecific and intrafamilial level in plants[4~7]. At present there has been no report for the ITS sequences from Lamiaceae. In this study we examined the characteristics of the sequence of the ITS regions from *Siphocranion nudipes* and compared this sequence with that of other plant genera.

1 Materials and methods

Leaves of *Siphocranion nudipes* were collected from Doupengshan of the Dagongshan Natural Preserve, Sangzhi county, Hunan Province. Voucher specimens (Zhu 079) were deposited in South-Central Forestry University Herbarium, Hunan.

1.1 DNA isolation and purification

Total DNA was isolated from 0.2~0.3 g dried leaves using the acetone-potassium acetate method[8], and purified with glass powder.

1.2 Amplification of the ITS regions

The entire ITS regions of rDNA was amplified for *Siphocranion nudipes* via the polymerase chain reaction (PCR). Double stranded DNA and single-stranded DNA amplifications were performed following the procedure of Wen[7]. C26A and N-nc18S10 (Tab. 1) were used as amplification primers. The single-stranded PCR products were purified with ultrafree-MC filters (Millipore) to eliminate the non-incorporated primers and nucleotides.

Tab. 1 Primers used for DNA amplification and sequencing

Primer	Sequence	Use	Designer	Reference
C26A	5′-GTTTCTTTTCCTCCGCT-3′	Ampification	Suh Y modified from	[7]
N-nc18S10	5′-AGGAGAAGTCGTAACAAG-3′	Ampification	Bult C	[7]
C5.8S	5′-TGCGTTCAAAGACTCGAT-3′	Sequencing	Suh Y	[6]
ITS4	5′-TCCTCCGCTTATTGATATGC-3′	Sequencing	White et al	[5]

1.3 Sequencing the single-stranded DNA

The ITS regions were sequenced following the dideoxy chain temination method[9] using the Sequenase Version 2.0 DNA Sequencing Kit (US70770, Amersham) and alpha 35S-dATP as a radioactive tracer. C5.8S and ITS4 primers (Tab. 1) were used as sequencing primers to obtain the ITS-1 and ITS-2 regions, respectively. Products of the sequencing reactions were separated on a 6% polyacrylamide gel at 50 W. Gels were transferred to 3 mm Whatman paper, dried in an oven at 45℃ for 1.5 h, and exposed to an X-ray film (Kodak XAR) for 36~48 h at room temperature. About 325 nucleotides were obtained per sequencing run.

1.4 Sequence analysis

The sequences obtained were assembled and the boundaries between the coding and spacer regions were determined by comparing with those of Acanthopanax of Araliaceae[7]. The G+C content of ITS-1, 5.8S and ITS-2 regions was calculated for Siphocranion nudipes and 10 species of other plant families. The GC percentage of *Siphocranion nudipes* was based upon unambiguous sequence. Nucleatides of ambiguous positions were added to the respective category afterwards.

2 Results and discussion

Fig. 1 shows the sequence and the boundary of ITS-1, 5.8S, and ITS-2 regions of Siphocranion nudipes. The general organization of a nrDNA repeat is presented in Fig. 2.

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Ribosomal DNA is arranged in numerous tandem repeats in one or a few chromosomal loci. Each repeat of the nrDNA of plants consists of three major genes (18S, 26S and 5.8S) and a few spacer regions.

18S| ITS-1
TCGAAACCTG CAAAGCAGAC CGCGAACACG TGTTTAACAC CGTCGGCACG GCCTCGTCGT
GATCGTCCGC CGCTGCGTAG CCCCCGCTTC GCATCGTGCG GGCTAACGAA CCCCGGCGGA
ATGCGCCAAG GAAAACTTAA TGGAGCGTCG GCCCCCTGCC ACCCCGTTCG CGGTGCGTGC
ITS-1| 5.8S
GGGGGGAGCG GATGTCTATC GAATGTCAAA ACGACTCTCG GCAACGGATA TCTCGCGTCT
CGCATCGATG AAGAACGTAG CCAAATGCGA TACTTGGTGT GAATTGCAGA TCCCGTGAAC
TCGAGTCTTT CGAAGCAAGT TGCGCCGAAG CCATTAAGGC CGAGGCACGT CTGCCTTGGG
5.8S| ITS-2
CGTCAGCATT GCGTCGTCCC CCTATTGCCC CGTGCCCAAA CAACGCTTGG GATGGGGGAA
CGGATATTGG CCTCCGGTGC ACCCCGGTTG CGMGGCTGGC CAAAATGCGA TCCCTCGGCG
ACTCTTGTCA CGACATGTGG TGGTTGAAAA TTTCAATCTC GCTTTGTGCC GTGCTTTCGA
GTCGTTCGTA AGGGMTTCAA AAAAYGACCC AATGGCGTCG ACGCGACCGA CCTGGCCGCG
ITS-2| 26S
CGCTTTCGAC AGCGACCCC

Fig. 1 Sequence of internal transcribed spacer regions from *Siphocranion nudipes*

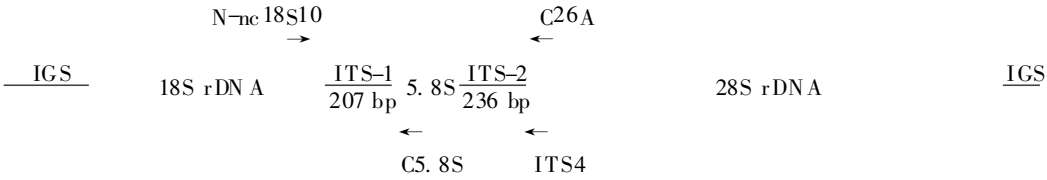

Fig. 2 Organization of nuclear ribosomal genes and spacer regions (modified from Hamby and Zimmer, 1991) with the length of ITS regions of *Siphocranion nudipes* and primer position. IGS represents intergenic spacer and ITS is the abbreviation for the internal transcribed spacer regions.

2.1 The length of ITS regions in *Siphocranion nudipes*

The length of the entire ITS and 5.8S regions in *Siphocranion nudipes* are 609 bases with an ITS-1 region of 207 bp, an ITS-2 region of 236 bp and a 5.8S region of 166 bp (Fig. 2, Tab. 2), with ITS1 region shorter than ITS2 region. Among the ITS sequences reported so far, ITS-1 region was found to be shorter than ITS-2 region in Lamiaceae and in Betulaceae[10], Scrophulariaceae[11]. ITS-1 region is longer in Asteraceae[4], Onagraceae[12], and Winteraceae[6]. The two spacers are nearly equal in length in Araliaceae[7] and Solanaceae[13]. In Fabaceae and Poaceae, the relative length of ITS-1 and ITS-2 regions varies among taxa[14,15]. ITS-1 and ITS-2 regions are each less than 300 bp in all the flowering plant (ITS-1 from 187 to 298 bp, ITS-2 from 187 to 252 bp, reviewed in Baldwin et al., 1995), in contrast to much longer spacers in some gymnosperms (e.g., pines[16]) and in some vertebrates[17]. The length of the 5.8S region is usually 163 or 164 bp and shows little variation among angiosperms. The length of ITS regions in Siphocranion nudipes falls within the narrow range of that of flowering plants.

2.2 The G+C content

Percentge G+C content in *Siphocranion nudipes* were 64.25% in ITS-1 region, 57.63% in ITS-2, and 53.61% in 5.8S region, and the G+C content for the entire ITS and 5.8S regions was 59.11%. Overall the G+C content of ITS regions varies widely across angiosperm species, from 47% in *Madinae* of Asteraceae to 75% in *Oryza sativa* of Poaceae (Tab. 2). *Siphocranion nudipes* falls in this range, but has a slightly higher ITS G+C content (only next to *Oryza sativa*). The G+C content of ITS regions are usually higher than that of 5.8S, 26S and 18S.

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Tab. 2　Sizes and G+ C content of ITS-1, 5. 8S and ITS-2 of *Siphocranion nudipes* and other angiosperms

Taxon	Family		ITS-1	5. 8S	ITS-2	Total	Reference
Siphocranion	Lamiaceae	a[1)]	207	166	236	609	This study
		b[2)]	64. 25	53. 61	57. 63	59. 11	
Aralia	Araliaceae	a	220~ 221	163	221~ 224	614~ 608	[7]
		b	60. 2~ 62	54~ 54. 6	62. 1~ 63. 8	59. 2~ 61	
Betula	Betulaceae	a	214~ 219		226~ 231		[10]
		b	62~ 63		63~ 65		
Daucus	Apiaceae	a	215		224		[15]
		b	49		52		
Epilobium	Onagraceae	a	240~ 244	164	211~ 216	616~ 622	[12]
		b	57. 4~ 58. 7	52. 0~ 53. 4	56. 3~ 56. 9	55. 9~ 56. 4	
Madinae	Asteraceae	a	255~ 261	164	216~ 223	635~ 647	[4]
		b	47. 7~ 51. 4	51. 2~ 53. 7	49. 5~ 53. 6	46. 9~ 52. 2	
Mimulus	Scrophulariaceae	a	189~ 214		203~ 225		[11]
		b	44~ 49		45~ 48		
Nicotiana	Solanaceae	a	216		217		[13]
		b	69		65		
Oryza	Poaceae	a	94		233		[14]
		b	72. 2		77. 3		
Panax	Araliaceae	a	220~ 221	163	222~ 224	606~ 608	[7]
		b	61. 1~ 61. 2	51. 5~ 54. 1	57. 8~ 63. 2	59. 2~ 61. 1	
Vicia	Fabaceae	a	235		208		[15]
		b	52		50		

1) size/bp; 2) w(G+ C)/%

2. 3　Sequence homogeneity

Overall sequence homogeneity of the ITS regions was detected in *Siphocranion nudipes*, with only three ambiguous nucleotide positions (ITS positions 453, 555, and 565, Fig. 1). This homogeneity is also found in ITS sequences of most flowering plants, although a high level of polymorphism was reported from Winteraceae[6]. This most remarkable feature of rDNA, the overall intraspecific sequence homogeneity among members of the multigene family, has been referred to as concerted evolution.

This is the first report of ITS sequence of Lamiaceae. Both the length and G+ C content percentage of ITS regions in Siphocranion nudipes fall within the range of those of flowering plants. The ITS sequence of Siphocranion nudipes is relatively homogeneous and only three nucleotide positions were ambiguous. This sequence may serve as a basis for further phylogenetic and comparative analysis of Lamiaceae. The relative effeciency of sequencing ITS regions and the lack of intraspecific sequence polymorphism makes this an attractive phylogenetic marker for this family.

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光柄筒冠花核糖体 DNA ITS区序列

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摘 要 测定了中国特有植物光柄筒冠花 (*Siphocranion nudipes*) 的 ITS区序列, 分析了其结构特征、G + C百分含质量 (w(G+ C) /%)及序列变异性, 并比较了光柄筒冠花与其他被子植物的 ITS区序列特征. 光柄筒冠花的 ITS区和 5.8S DNA的总长度为 609个核苷酸, 其中 ITS-1为 207 bp, ITS-2为 236 bp, 5.8SrDNA为 166 bp; 总的 w(G+ C)为 59.11% , 其中 ITS-1为 64.25%、ITS-2为 57.63% 和 5.8S rDNA为 53.61% . 这一序列的测定为唇形科植物的 ITS区序列分析及多态性研究打下了基础, 同时对进一步研究这一我国特有植物的生态学及物种保护学有着重要意义. 这是唇形科 ITS区序列的首次报道.

关键词 序列, 光柄筒冠花, ITS区

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