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Internal Transcribed Spacer Sequence Based Identification and Phylogenic Relationship of Herba Dendrobii

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ABSTRACT

Herba Dendrobii, commonly known as "Shi-hu", has been used as a precious traditional Chinese medicine. It is expensive and adulteration are common due to high demand. The method to distinguish the herb from adulterant species is necessary. In the present study, internal transcribed spacers (ITS) region-based analysis was employed to ascertain the phylogenetic relationship among the 11 *Dendrobium* and two adulterant species *Pholidota articulata* and *Flickingeria comate*. Results showed that the length of the ITS regions among the thirteen species ranged from 635 to 641 bp and the GC ratio in ITS (ITS1 + 5.8S + ITS2) regions ranged from 50.55% to 57.25%. *Dendrobium* species was significantly different from one another by an average of 13.20% and from *P. articulata* and *F. comate* by 42.00% and 29.00% respectively. The molecular phylogenetic trees indicated that most of *Dendrobium* species are closely related and share common clad while both the adulterants outgroup and have separated clad. Therefore, ITS regions can be used as a molecular marker to differentiate medicinal *Dendrobium* spp. from one another and also from adulterants.

Key words: Herba Dendrobii, internal transcribed spacers, molecular phylogenetic tree

INTRODUCTION

Dendrobium species is one of the most valuable Chinese medicines known as "Shi-hu" and it is used as functional health food in Taiwan. The genus *Dendrobium* (Orchidaceae) includes about 1,600 species, 15 of which are found in Taiwan[(1)]. It is widely used in both traditional Chinese and folk remedies for antipyretic, ophthalmic, and tonic purposes[(2)]. The natural resources of Herba Dendrobii are limited and great demand has led to severe shortage and high price. However, survey of market samples revealed that other cheaper and more common orchids such as *Pholidota* and *Flickingeria* genus are found as adulterants of Herba Dendrobii. Adulterants made up of these stems used clinically as Herba Dendrobii resulted in inconsistent therapeutic effects. Because of the similarity with *Dendrobium* species in appearance and tissue structure, it is difficult to distinguish the marketing products by traditional identification methods. Hence a good method for identification is urgently required.

Many attempts were made to identify *Dendrobium* species. Medicinal *Dendrobium* have been identified by pharrmacognostic and chemical analysis and is not reliable for species identification[(3)]. Taxonomy-based classification relied on the morphology and taxonomy of the fresh materials[(4)]. Various internal transcribed spacers (ITS) regions were used to authenticate *Dendrobium* species[(5-8)]. Suppression subtraction hybridization arrays were used to identify five *Dendrobium* species[(9)]. The ITS regions in nuclear ribosomal RNA genes (rDNAs), plastid genome and mitochondria genome were used as DNA barcode for the authentication of plant species[(10)]. Different species in commercial Shihu were identified by dot blot hybridization. Thus, various attempts have already been made to authenticate *Dendrobium* at the molecular level in order to differentiate it from adulterants[(11)].

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Currently, DNA diversity might be used as a valuable source not only for the evidence of biological phylogeny, but for identifying crude medicine as well. ITS region has been widely used in taxonomy and molecular phylogenetics in Angiosperms[12-15]. The ITS region in rDNAs comprises of two regions ITS1 and ITS2. The location of ITS1 is between the 18S and 5.8S rDNA, while ITS2 between the 5.8S and 28S rDNA. The 18S, 5.8S, and 28S rDNAs are highly conserved. The ITS regions are variable in different genera, species, or even subspecies, thus rendering them suitable targets for the investigation of phylogenetic relationships and can be exploited for species identification. The conserved regions of 18S and 28S rDNA have been used to design universal primers used to amplify the flanking ITS regions[14]. Since ITS1 and ITS2 regions can be amplified by using universal primers, its analysis becomes an easy, reliable, simple, cost effective, and requires less quantity of starting material.

In this study, we explored the possibility of using ITS regions of rDNA to differentiate eleven *Dendrobium* species and two adulterants. Analysis of the concerned sequences has offered more defined markers for the authentication and the assessment of the phylogeny among these species.

MATERIALS AND METHODS

I. *Materials*

The plants of *Dendrobium tosaense, D. huoshanense, D. moniliforme, D. linawianum, D. loddigesii, D. hercoglossum, D. nobile, D. chameleon, D. clavatum, D. candidum, D. fimbriatum*, *Flickingeria comate* and *Pholidota articulata*, were collected from China and Taiwan (Table 1). Selected plants were maintained in the green house of Chaoyang University of Technology. The voucher specimens were deposited in the Herbarium of China Medical University (CMU), Taichung, Taiwan. The species were identified by Professor. C. L. Kuo of CMU.

II. *DNA Extraction*

Approximately 100 mg of fresh leaf samples was pulverized under liquid nitrogen in a mortar. The powder was transferred to a 1.5 mL microcentrifuge tube and genomic DNA as extracted using a DNeasy® Plant Mini Kit (Qiagen, Germany). The harvested genomic DNA was stored at -20°C for further analysis.

III. *Polymerase Chain Reaction (PCR) Amplification*

The PCR assay was performed with 30 ng test sample in a total reaction volume of 50 μL consisting of PCR buffer (20 mM Tris-HCl, pH 8.4, 50 mM KCl, 0.2 mM (each) dATP, dGTP, dCTP, and dTTP; 1.5 mM $MgCl_2$; 0.3 mM (each) primer; and 1 U Klen*Taq* polymerase (Protech Technology Enterprise Co., Ltd., Taiwan)). The primer pairs used for amplification of the ITS region was 18S (5'-CGTAACAAGGTTTCCGTAGGTGA-3') and 28S (5'-CCTTTCATCTTTCCCTCGCGGT-3') that were modified by Lin *et al*.,[16]. PCR program consisted of a denaturation step at 94°C for 5 min followed by annealing at 47.5°C for 1 min and extension at 68°C for 1 min for the first template amplification, and then 25 cycles of 94°C for 20 sec, 54.5°C for 20 sec and 68°C for 30 sec, and a final extension step at 72°C for 10 min. Approximately 10 μL of PCR products were electrophoresed on 1% agarose gel, stained with ethidium bromide, and visualized under UV. The remaining PCR products were stored at 4°C until used.

IV. *Cycle Sequencing*

Sequencing was carried out by Tri-I Biotech, Inc., Taiwan using standard procedures. Briefly, the PCR products were purified by ethanol precipitation. Sequencing reactions were performed using BigDye® Terminator v3.1 Cycle Sequencing Kits (Applied Biosystems, Foster City, CA, USA) and run on the ABI 3730 DNA analyzer (Applied Biosystems). All the sequence analysis was done using the Sequencher 4.8 software (Gene Codes, Ann Arbor, MI, USA). All suspected variations were verified by bidirectional sequencing. The ITS region of each individual PCR product was sequenced in both 5' and 3' direction at least 3 times as to define the ITS sequences[16,17].

V. *Sequence Alignments and Phylogenetic Trees*

The DNA sequences were compared and aligned using the BioEdit (version 7.0.5.3) and MEGA 4 softwares[18] and further verified by comparing with the sequences of other species by BLAST search in the website of the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/blast/blast.cgi). Phylogenetic trees were based on the hierarchical clustering of the alignments of ITS1, 5.8S rDNA and ITS2 and produced by Neighbor-Joining

Table 1. Particulars of materials used in this study

No.	Species	Locality	Region
1	*Dendrobium tosaense*	Taichung, Taiwan	Fresh leaves
2	*D. huoshanense*	Anhui, China	Fresh leaves
3	*D. moniliforme*	Ilan, Taiwan	Fresh leaves
4	*D. linawianum*	Ilan, Taiwan	Fresh leaves
5	*D. loddigesii*	Yunnan, China	Fresh leaves
6	*D. hercoglossum*	Yunnan, China	Fresh leaves
7	*D. nobile*	Yunnan, China	Fresh leaves
8	*D. chameleon*	Ilan, Taiwan	Fresh leaves
9	*D. clavatum*	Taichung, Taiwan	Fresh leaves
10	*D. candidum*	Anhui, China	Fresh leaves
11	*D. fimbriatum*	Yunnan, China	Fresh leaves
12	*Flickingeria comata*	Taichung, Taiwan	Fresh leaves
13	*Pholidota articulata*	Yunnan, China	Fresh leaves

(NJ) and Maximum Parsimony (MP) methods using MEGA 4 software of the bootstrap values (1000 replicates).

IV. *Nucleotide Sequence Accession Numbers*

The ITS1–5.8S–ITS2 DNA sequences of referenced *Dendrobium* species were not previously available within the National Center for Biotechnology Information GenBank nor EMBL databases. The assigned sequence accession numbers are as follows: *Dendrobium tosaense* (HM590367), *D. huoshanense* (HM590368), *D. moniliforme* (HM590369), *D. linawianum* (HM590371), *D. loddigesii* (HM590374), *D. hercoglossum* (HM590381), *D. nobile* (HM590382), *D. chameleon* (HM590385), *D. clavatum* (HM590387), *D. candidum* (HM590391), *D. fimbriatum* (HM590392). Sequences from other two species *Flickingeria comate* (HM590389) and *Pholidota articulate* (HM590390) were also deposited into GenBank and listed in Table 2.

RESULTS AND DISCUSSION

Many studies indicated that the variation in nucleotide sequences among the highly conserved regions or coding sequences might be used not only as a valuable source for the evidence of biological phylogeny but also as a tool to discriminate from adulterants. *Pholidota* and *Flickingeria* genus are found as adulterants of Herba Dendrobii in market. The ITS region was amplified by PCR with the same primer pair. The sequences of ITS1, ITS2 and 5.8S region are shown in Figure 1.

I. *rDNA Sequence Analysis*

Amplification of the ITS regions between the 18S and 28S rDNA from 13 orchid species generated PCR products ranging in size from 635 to 641 bp, which were in accordance with previous report[16,17,19]. Alignment of 11 different species of *Dendrobium* and two adulterant spp. sequences demonstrated that both single-nucleotide differences and short lengths of sequence diversity are due to insertions or deletions existing in the ITS1-5.8S-ITS2 regions among different *Dendrobium* species. Both ITS1 and ITS2 regions displayed more interspecies variation, ITS1 and ITS2 contain at least one separate variable regions ranging from 3 to 5 bp in length (Figure 1). The hyper-variable nucleotide sequences of ITS regions between 18S and 28S rDNA are useful for identification of different plant species. These regions can be utilized to design species-specific primers to amplify the flanking regions. There were not as many variations in size of ITS regions in plants as in animal kingdom, where variations are very high[20].

The length of the ITS (ITS1, 5.8S and ITS2) regions among the 11 *Dendrobium* species ranged from 635 to 641 bp, and the GC content ranged from 50.55 to 55.96%. The length of ITS1 ranged from 230 to 232 bp with the GC content ranging from 44.40 to 57.58%. The length of ITS2 ranged from 242 to 247 bp, and the GC content ranged from 48.18 to 54.92% (Table 2). The length of ITS2 was longer than that of ITS1. The GC content of ITS2 was higher than that of ITS1. Interspecific variations in 5.8S rDNA region were very low among 11 *Dendrobium* species and the length ranged between 162 - 163 bp. The GC content of 5.8S rDNA region was in the range of 56.44 - 58.90% (Table 2). The two adulterants (*F. comata* and *P. articulate*) have similar ITS length. However *P. articulate* has higher GC content of 57.25% compared to 11 different *Dendrobium* species whereas lowest GC content (51.33%) was found in *F. comata* as compare to 11 different *Dendrobium* species. ITS sequences of many plants have GC content over

Table 2. ITS and 5.8S rDNA length (bp) and GC contents of *Dendrobium* spp.

Species	Length (bp)				G+C content (%)				Genbank number
	ITS	ITS1	5.8SrDNA	ITS2	ITS	ITS1	5.8SrDNA	ITS2	
Dendrobium tosaense	636	231	163	242	52.20	50.22	57.67	50.41	HM590367
D. huoshanense	635	230	163	242	53.23	50.43	58.28	52.48	HM590368
D. moniliforme	638	231	163	244	52.82	46.75	58.28	54.92	HM590369
D. linawianum	636	231	163	242	53.30	50.22	57.67	53.31	HM590371
D. loddigesii	639	232	163	244	50.86	44.40	58.28	52.05	HM590374
D. hercoglossum	636	231	163	242	52.99	50.22	58.28	52.07	HM590381
D. nobile	636	231	163	242	53.62	50.65	58.28	53.31	HM590382
D. chameleon	641	231	163	247	50.55	48.92	56.44	48.18	HM590385
D. clavatum	638	231	163	244	52.35	48.92	58.90	51.23	HM590387
D. candidum	635	230	163	242	52.44	50.87	57.67	50.41	HM590391
D. fimbriatum	638	231	163	244	55.96	57.58	57.67	53.28	HM590392
Flickingeria comata	641	231	163	247	51.33	49.78	46.63	55.87	HM590389
Pholidota articulata	641	233	162	246	57.25	54.94	57.41	59.35	HM590390

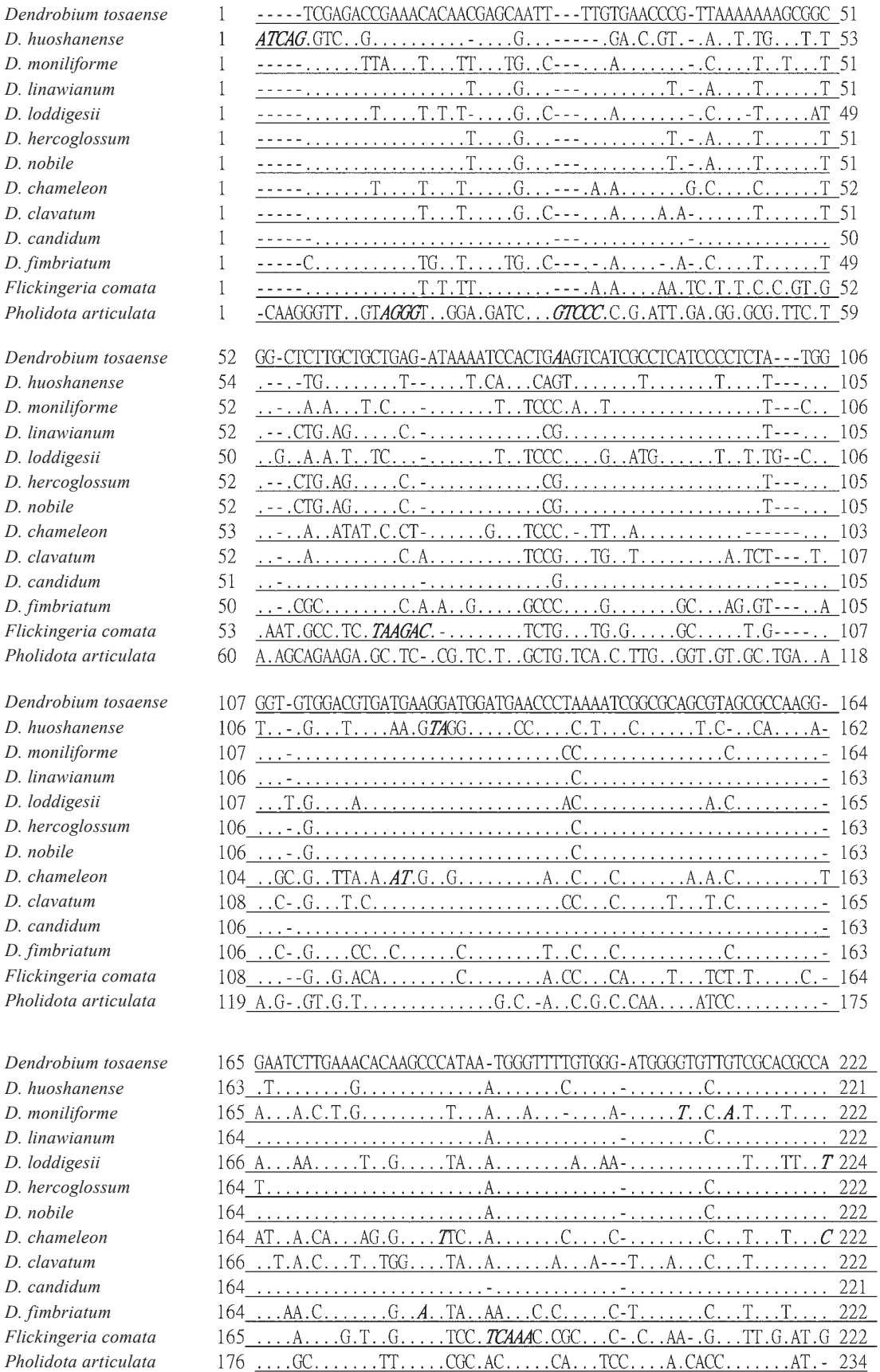

Figure 1. Alignment of ITS1 (underlined), 5.8S (highlighted in grey) and ITS2 sequences in eleven *Dendrobium* species, *Flickingeria comate* and *Pholidota articulata*. The same sequences are indicated by dot (·). Gaps (-) are introduced for the best alignment. Variable region ranged 1 - 5 bp in size, marked in bold italics.

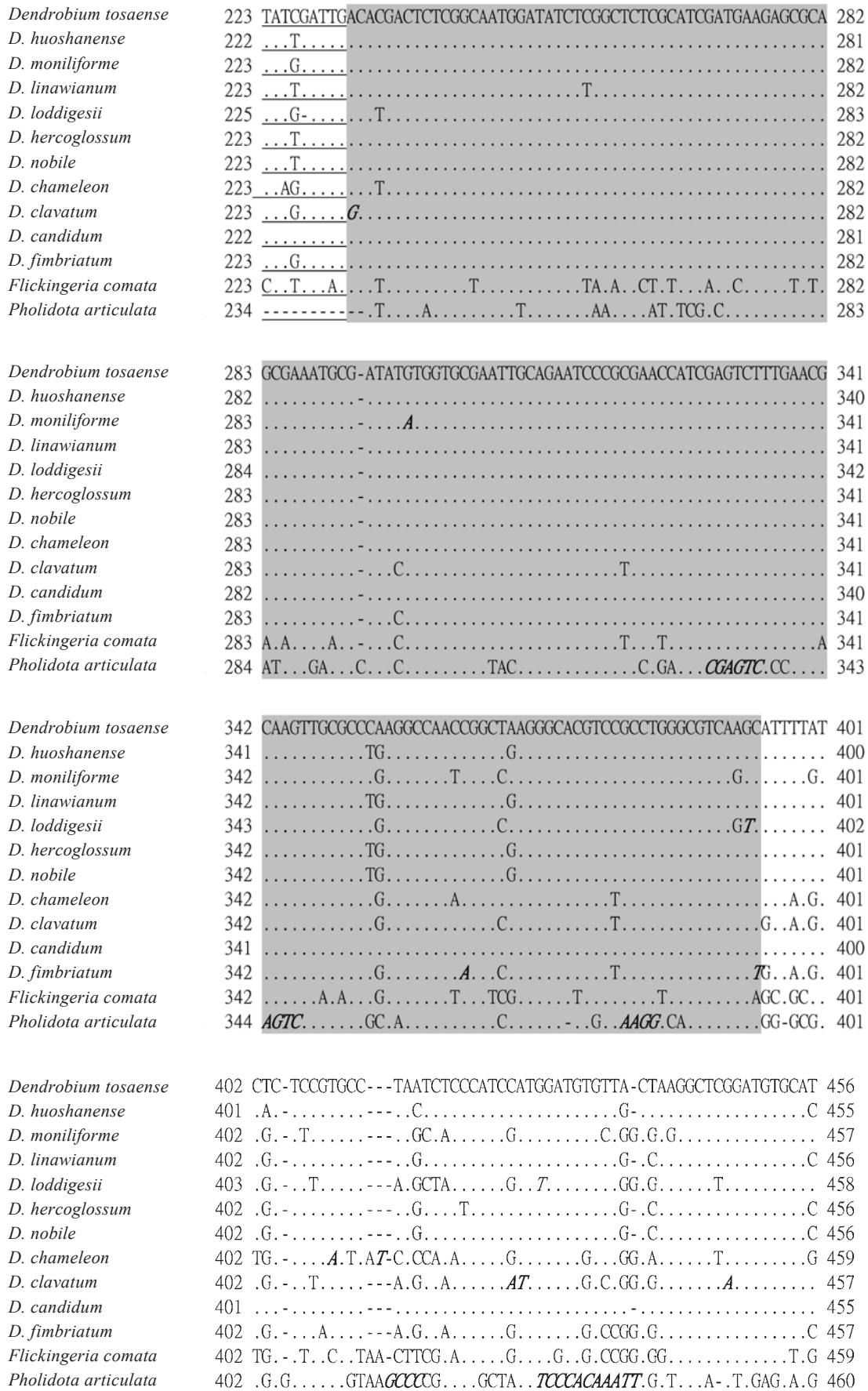

Figure 1. Continued.

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Dendrobium tosaense      457 GGTGGCTCCTCGTGCCCCTTGGTGCGGCGGGCTGAAGGGCGGGTCATCTTCTCGTTGGTT 516
D. huoshanense           456 ........G.............................................C. 515
D. moniliforme           458 ........G..........C..C.............A....A.....G...T.....C. 517
D. linawianum            457 ........G.............................................C. 516
D. loddigesii            459 TA......A..........G..C.............A..........A.........C. 518
D. hercoglossum          457 ........G.............................................C. 516
D. nobile                457 ........G.............................................C. 516
D. chameleon             460 A.......A....T.....C..A....T.........A.TA.......A.........C. 519
D. clavatum              458 ........G........T.C..CA...T.........A..........A...T...A.CC 517
D. candidum              456 ..................................................... 515
D. fimbriatum            458 A.......GC.........C.................A..........TA...T.....C. 517
Flickingeria comata      460 A.....C.A..AA...GACC...AT............A.....CTG..A.........C. 519
Pholidota articulata     461 A......TAGA.....TG.........A....G..CG.A.G...GA.GAG.GGGT.AT.CC 520

Dendrobium tosaense      517 GCCAACAATA--AGGGGTGGATT--AAAAAAGGCCTATGC-----TATTGTGATAAGCGC 567
D. huoshanense           516 ..........--..........--...T..........-----.......TC...... 566
D. moniliforme           518 ..G.......--..........T-...TG..........-----.......TCGT..AT 569
D. linawianum            517 ..........--..........--...T..........-----.......TC...... 567
D. loddigesii            519 ..G.......--.........C.G-..GGG..A..........-----.......TCGT..AT 570
D. hercoglossum          517 .T........--..........--...T..........-----.......TC...... 567
D. nobile                517 ..........--..........--...T..........-----.......TC...... 567
D. chameleon             520 ..G.T.....--..........A-...TG..T...G..T----------.....CTCGT.TAT 571
D. clavatum              518 A.G.......--..........AA-...TG..........-----.......CCGT..AT 569
D. candidum              516 ..........--..........--..............-----.............. 566
D. fimbriatum            518 ..AGG.....--.T........AA-.CGCG......T..T----------.....TCGT..AT 569
Flickingeria comata      520 ..G.......--..........GA-G.GC..A.....CAT------.T.....TCGTTG.T 571
Pholidota articulata     521 AT....GGC.CGG....G....GAG.GGTG..CA.A.AA.GCATA.G..T.TT.GT.T.G 580

Dendrobium tosaense      568 GCCCGAGAGATGATCATACTT-TTTAGGTGATCCCAATCCATGCGCTAATCCATGGATGG 626
D. huoshanense           567 ............G........-..............T......T.G............ 625
D. moniliforme           570 ...TA...........C..C.-..G...........AT.......CG............ 628
D. linawianum            568 ...T........G.........-A.............T......TCG............ 626
D. loddigesii            571 ...TA....T.........C.-..G...A........AT......TCG...T.....C.. 629
D. hercoglossum          568 ...T........G.........-A.............T......TCG...........A 626
D. nobile                568 ...T........G.........-A.............T......TCG............ 626
D. chameleon             572 ...T.....GA...T....A.A..C.T..........AT.......CG............ 631
D. clavatum              570 ....A.........T....C.-....T..........AT......TCG.....C.A.... 628
D. candidum              567 ....................-.................................... 625
D. fimbriatum            570 ...TA...T.....T.A....-.............AT......TCG.....C...... 628
Flickingeria comata      572 ...G......G..ATGC...C-C.C.A..........A.......T.G.....CA....A 630
Pholidota articulata     581 .G.GAGAGA.GA...CC.G..CA.CG..A.....GGG.........CG..G..C..G... 640

Dendrobium tosaense      627 CGTATCGAAT- 636
D. huoshanense           626 ..........- 635
D. moniliforme           629 ..CT.T....- 638
D. linawianum            627 ..........- 636
D. loddigesii            630 ..CT.T....- 639
D. hercoglossum          627 ..........- 636
D. nobile                627 ..........- 636
D. chameleon             632 ...T.T....- 641
D. clavatum              629 ...TGT....- 638
D. candidum              626 ..........- 635
D. fimbriatum            629 ...T.T....- 638
Flickingeria comata      631 T.CC.T..GAT 641
Pholidota articulata     641 A---------- 641
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Figure 1. Continued.

50.00%, while mammals have GC content of 70.00 to over 80.00%[20]. GC content of these spacer regions may be associated with structural constraints related to intramolecular RNA folding[21]. Consensus sequences were found in the ITS region of 11 *Dendrobium* species but varying sequences were present in corresponding regions in two different adulterants species. Hence, these regions may be exploited to design specific primer to detect the adulterant.

The ITS regions of 11 *Dendrobium* species have many differentiations, which are identical to the published sequences[5]. The three sequences from two species of *Dendrobium (D. fimbriatum*; LSy: AF362041, *D. nobile*; JC1 and JC2: AF372039 and AF362037) by Xu *et al*. (2001) showed variations. The length of ITS1 and 2 regions in *D. fimbriatum* by Xu *et al*. was 230 and 242 bp with GC content 50.00% and 51.30% respectively, whereas, we report 231 and 244 bp long ITS1 and 2 regions with GC content 57.58% and 53.28% respectively in *D. fimbriatum*. The nucleotide sequences of the ITS1 and 2 regions of *D. nobile* were similar to the previously reported sequences with slight variation in GC content. However, the nucleotide sequences of ITS1 region in *D. nobile* was almost identical with previous report[5]. It was clear from the sequence analysis that the inter-specific sequence divergence is much larger and each *Dendrobium* species was found to have a unique sequence (1 - 5 bp) in the ITS1 and ITS2 region (Figure 1), thus it could be used to distinguish species at the DNA level.

II. *Sequence Coefficients of Identity*

Among the 11 *Dendrobium* species, the inter-specific percentage identities range from 76.00% to 99.60% with an average of 85.50% (Table 3), the maximum percentage identities (> 99.00%) were between *D. hercoglossum* and *D. linawianum, D. nobile* and *D. linawianum, D. nobile* and *D. hercoglossum,* and *D. candidum* and *D. tosaense*, where as minimum identities (76.10%) were between *D. chameleon* and *D. huoshanense.* The percentage identities between different species of *Dendrobium* species and *F. comata* range from 63.80% to 69.90% with an average of 68.20%. The percentage identities between different species of *Dendrobium* species and *P. articulata* range from 51.10% to 53.10% with an average of 51.94% similarity. Thus these two adulterants showed lower degree of relatedness with different *Dendrobium* species. According to the data, *D. hercoglossum* has more relatedness to *D. nobile* and *D. hercoglossum*. Similarly, *D. candidum* and *D. tosaense* showed higher degree of relatedness, whereas *D. chameleon* and *D. huoshanense* showed lower degree of relatedness. These findings are in congruence with previous reports[16,19].

Pairwise distances were calculated for the 11 *Dendrobium* species and two adultrants (Table 3). The pairwise distance varied from 0-21 characters amongst 11 *Dendrobium* species. There were 21 character differences between *D. huoshanense/D. fimbriatum* and *D. huoshanense/D. chameleone,* whereas no character difference was in *D. tosaense* and *D. candidum*. These results suggested that

Table 3. The percentage identity of ITS (up diagonal) and pairwise distance (Number of differences, down diagonal) of eleven sources of Shi-hu and two out-group species

species	*Dendrobium tosaense*	*D. huoshanense*	*D. moniliforme*	*D. linawianum*	*D. loddigesii*	*D. hercoglossum*	*D. nobile*	*D. chameleon*	*D. clavatum*	*D. candidum*	*D. fimbriatum*	Flickingeria comata	Pholidota articulata
Dendrobium tosaense		87.0	85.4	93.5	82.5	92.9	93.5	79.3	82.3	99.6	80.9	67.7	51.6
D. huoshanense	11		80.2	90.3	77.5	90.0	90.6	76.1	77.5	87.0	77.0	63.8	51.4
D. moniliforme	13	18		86.6	88.6	86.2	86.6	83.2	85.9	85.2	84.6	68.6	51.9
D. linawianum	6	8	12		83.4	99.0	99.6	80.6	83.4	93.5	83.1	68.6	52.8
D. loddigesii	15	19	10	14		83.3	83.8	82.5	83.6	82.3	81.9	68.6	51.8
D. hercoglossum	7	9	13	1	15		99.3	80.4	83.1	92.9	82.8	68.3	52.8
D. nobile	6	8	12	0	14	1		80.9	83.7	93.5	83.4	68.6	53.1
D. chameleon	18	21	15	17	15	17	16		81.5	79.2	80.8	68.9	51.1
D. clavatum	16	20	13	15	15	15	15	16		82.3	87.1	69.6	51.5
D. candidum	0	11	13	6	15	6	6	18	16		80.9	67.7	51.8
D. fimbriatum	18	21	14	16	16	16	15	17	12	18		69.9	51.5
Flickingeria comata	30	33	29	29	29	29	29	28	28	30	28		48.0
Pholidota articulata	42	43	42	41	42	41	41	44	43	42	43	46	

D. fimbriatum and *D.chameleone* may be genetically more distant from *D. huoshanense* while *D. tosaense* and *D. candidum* may be genetically more similar. Pairwise distance between different *Dendrobium* species and two adulterants *F. comata* and *P. articulate* varies from 28 - 46 characters. There was 41 to 46 character difference between *P. articulate* and different *Dendrobium* species. Whereas, 28 to 33 character differences were between *F. comata* and different *Dendrobium* species. These results suggested that *F. comata* and *P. articulate* may be genetically more diverse than reported 11 *Dendrobium* species and suitable to be considered as an outgroup.

III. *Molecular Phylogenetic Tree*

Phylogenetic tree was constructed for the 11 species of *Dendrobium* and the two out group species (Figure 2). Phylogenetic trees based on ITS regions were generated by neighbor-joining (NJ) (Figure 2A) and maximum parsimony (MP) methods (Figure 2B)[16]. It was clear from the figure that the 11 *Dendrobium* species and two out grouped species were clustered separately. Within the genus *Dendrobium* single main branches differentiated the 11 *Dendrobium* species from *F. comata* and *P. articulate* into three distinct groups. A common clad shared by all studied *Dendrobium* species with a boot strap value of 100 and 99 for NJ and MP based tree respectively. *D. tosaense* and *D. candidum* had almost identical sequences (99.60%), thus, were clustered together in common clad with boot strap value of 100 for both NJ and MP based phylogenetic tree. On the other hand, *D. clavatum* and *D. fimbriatum* were clustered together with boot strap value of 94 and 82 for NJ and MP based tree respectively. *D. chameleon* could be the basal member amongst the reported *Dendrobium* species and clustered separately in both type of phylogenetic tree. Molecular sequence data supports the positioning of *D. chameleon* as a basal member as seen in Table 3.

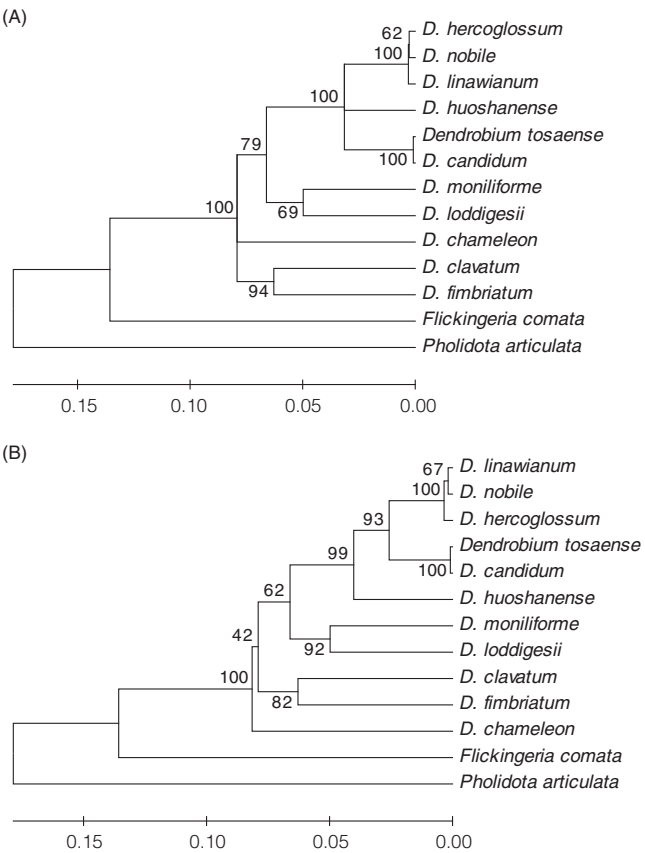

Figure 2. Phylogenetic trees based on the ITS sequence by (A) Neighbor-Joining (NJ) and (B) Maximum Parsimony (MP) methods.

CONCLUSIONS

Amplification of the ITS regions between the 18S and 28S rDNA from 11 *Dendrobium* species and 2 adulterants orchid species resulted in products ranging in size from 635 to 641 bp. The GC content was 50.55% to 57.25% in most of the species which is the general trend in angiospermic plants with GC content above 50.00%. The molecular phylogenetic analysis clearly showed relatedness among different species of *Dendrobium* and a large number of species were in similar clad while adulterants were grouped into two separate clad, which is in good agreement with the traditional classification[6,22]. Our results demonstrate that the frequency of variations is high in ITS1 and ITS2 while low in 5.8S rDNA. Hence, these ITS regions may be used as molecular marker to distinguish intraspecifically as well as from adulterants.

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