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A Phylogenetic Study of *Polygonum* sect. *Tovara* (Polygonaceae) Based on ITS Sequences of Nuclear Ribosomal DNA

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***Polygonum* sect. *Tovara* comprises three morphologically very similar species; *P. virginianum*, *P. filiforme*, and *P. neofiliforme*. Sequences of internal transcribed spacers (ITSs) of nuclear ribosomal DNA of these were determined to examine phylogenetic relationships and the levels of differentiation among them. The size of ITS 1 was 241 bp in *P. filiforme* and *P. neofiliforme*, and 242 bp in *P. virginianum*. The size of ITS 2 was 243 bp, and that of the 5.8S rRNA coding region was 163 bp. The ITS sequences clearly separate North American *P. virginianum* from the eastern Asian species. Nucleotide divergence between them ranges from 3.3% to 3.8% for ITS 1 and from 9.3% to 10.7% for ITS 2. The molecular data also revealed that two eastern Asian species are closely related but should be treated as distinct species.**

Keywords: Polygonaceae, *Polygonum* sect. *Tovara*, molecular phylogeny, ITS, disjunct distribution

Polygonum sect. *Tovara* (Adans.) Benth. & Hook. (Polygonaceae) is a highly variable taxon usually defined by large ovate to elliptic leaves, elongate spike-like inflorescences, and persistent bifid styles which become rigid and bent obliquely downward in fruit (Park *et al.*, 1992; Mun and Park, 1995). Plants of sect. *Tovara* are erect perennial herbs, which usually occupy moist habitats such as margins of swamps and lakes, rich shady forest floors, and soils of streambeds. The section shows an interesting disjunct distribution pattern: Eastern North America (with a few isolated occurrences in Mexico) and eastern Asia (Li, 1952a; Park *et al.*, 1992).

Species of sect. *Tovara* exhibit very complicated patterns of morphological variation, resulting in taxonomic confusion and difficulty in delimiting species boundaries (Nieuwland, 1912; Elmer, 1915; Nakai, 1922; Ohki, 1926; Steward, 1930; Maekawa, 1932; Li, 1952b; Hara, 1962, 1965; Graham and Wood, 1965). Recently, Park *et al.* (1992) recognized three species in the section on the basis of numerical analyses of morphological characters: *Polygonum virginianum* L. in eastern North America, and *P. filiforme* Thunb. and *P. neofiliforme* Nakai in eastern Asia. In addition, the subsequent study on flavonoid chemistry of these taxa (Mun and Park, 1995) revealed that they are closely allied but distinct species. However, phylogenetic relationships among these species have not been proposed by these authors because of the paucity of discrete qualitative morphological characters and the difficulty in estimating phylogenetic derivation for the various structural types of flavonoids (Park *et al.*, 1992; Mun and Park, 1995).

Recent molecular phylogenetic studies have demonstrated that the internal transcribed spacer (ITS) regions of nuclear ribosomal DNA (nrDNA) are very useful for assessing phylogenetic relationships at lower taxonomic levels such as among genera or species, because their rates of divergence are relatively high in comparison to protein or rRNA coding genes such as *rbc*L and 18S/26S ribosomal DNA (Baldwin, 1992; Suh *et al.*, 1993; Kim and Jansen, 1994; Sang *et al.*, 1994, 1995; Baldwin *et al.*, 1995; Downie and Katz-Downie, 1996). In this study, we analyzed the ITS sequences of nrDNA from the three species recognized in sect. *Tovara* to look into phylogenetic relationships among them and to compare the results with those from previous

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studies of morphology (Park *et al.*, 1992) and flavonoid chemistry (Mun and Park, 1995).

MATERIALS AND METHODS

Plant materials and DNA extraction

Three species recognized in sect. *Tovara* (Park *et al.*, 1992; Mun and Park, 1995) and one outgroup species were sequenced (Table 1). *Polygonum hydropiper* L. of sect. *Persicaria* (Mill.) DC. was chosen as an outgroup species, because sect. *Tovara* is considered to be most closely related to sect. *Persicaria* (Haraldson, 1978; Ronse Decraene and Akeroyd, 1988; Mun and Park, 1995). Leaves used as sources of DNA were collected in the field, and transported to the laboratory on ice. Leaves were powdered in liquid nitrogen and kept in -70°C until DNA extraction. Total DNA was extracted by the 2× CTAB method of Doyle and Doyle (1989), and further purified with Gene Clean Kit II (Bio101, CA).

PCR and sequencing

PCR was carried out in 100 μl final volume of 10 mM Tris buffer (pH 8.3) containing 0.5 ng template DNA, 2.5 units of *Taq* polymerase (Perkin-Elmer Cetus), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 200 μM of each dNTP, and 0.5 μM of each primer. PCR primers were 'ITS1' and 'ITS4' designed by White *et al.* (1990). PCR thermal cycle profile was 3 minutes at 95°C for pre-denaturation, followed by 30 cycles, each consisting of 1 minute at 95°C for denaturation, 1 minute at 55°C for anealing, and 45 seconds at 72°C for extension. Primer extension time was increased by 3 seconds with each cycle, followed by the final extension for 7 minutes at 72°C. Double stranded PCR product was directly sequenced by using the Sequenase PCR Product Sequencing Kit (USB, OH). Excess dNTPs, primers and extraneous single-stranded DNA's produced by PCR were removed by Shrimp alkaline phosphatase and Exonuclease I. In addition to PCR primers, 'ITS 2' and 'ITS3' of White *et al.* (1990) were used as internal primers for sequencing in both directions. Electrophoresis was performed with denaturing formamide gels, and glycerol tolerant buffer containing taurine was used instead of TBE buffer (protocols for Sequenase PCR Product Sequencing Kit, 2nd ed., USB, OH).

Table 1. Species used for ITS sequence analysis of *Polygonum* sect. *Tovara* and outgroup species. All vouchers are at SNU

Species	Collection number	Locality and Date
Section *Tovara*		
Polygonum filiforme	*Mun 640*	Korea, Chunam Prov., Mt. Turyun Aug. 20, 1993
P. neofiliforme	*Mun 23*	Korea, Chunam Prov., Mt. Chiri Sep. 15, 1993
P. virginianum	*Suh 9402*	U. S. A., Virginia, Alexandria Aug. 1, 1994
Section *Persicaria*		
P. hydropiper	*Mun 701*	Korea, Seoul, Mt. Kwanak Aug. 18, 1994

Sequence alignment and phylogenetic analyses

The sequence boundaries of ITS 1, the 5.8S coding region, and ITS 2 were determined by comparison with published sequences from various plant species (Yokota *et al.*, 1989; Baldwin, 1992; Suh *et al.*, 1993; Kim and Jansen, 1994). Sequences were aligned using the Clustal V program (Higgins *et al.*, 1992), and then finally adjusted by eye. Gaps were not included in the phylogenetic analysis. Phylogeny was reconstructed with Fitch parsimony as implimented in PAUP (ver. 3.1.1; Swofford, 1993). Branch and bound searches, with equal weighting of character state changes, were conducted. Sequence divergence values between species were calculated using Kimura's two-parameter method (Kimura, 1980). Kimura's distances were obtained using DNADIST program of PHYLIP (ver. 3.5; Felsenstein, 1992), with default settings (transition vs. transversion=2:1).

RESULTS

The complete sequences of ITS 1, the 5.8S coding region, and ITS 2 were determined for all three species of sect. *Tovara* and outgroup species, *P. hydropiper* of sect. *Persicaria* (Fig. 1). The size of ITS 1 was 241 bp long in *P. filiforme, P. neofiliforme,* and *P. hydropiper,* and 242 bp long in *P. virginianum.* The size of ITS 2 was 241 bp long in *P. hydropiper*, and 243 bp long in all other species. The 5.8S coding region was 163 bp in length for all species examined (Table 2). Sequence alignment required insertion of a one-base gap in ITS 1 of *P. virginianum*, and four one-base indels between the ingroup species and the outgroup in ITS 2 (Fig. 1).

GC content ranged from 61.8% to 66.5% in ITS 1, and from 65.4% to 79.8% in ITS 2 (Table 2). In the

four species of *Polygonum*, GC content of ITS 2 was higher than that of ITS 1. GC content of ITS 2 in *Polygonum virginianum* was 79.8%, which is the highest value from all *Polygonum* species in the study. Sequences of the 5.8S coding region were identical in all four species (Fig. 1), with GC content of 55.8% (Table 2).

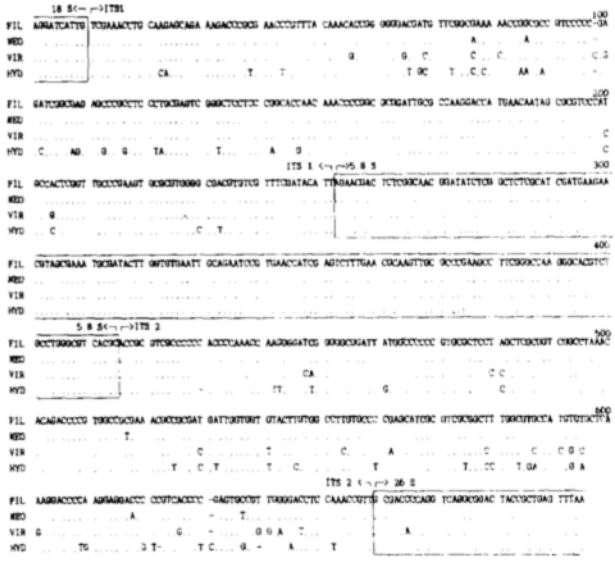

Fig. 1. Aligned sequences of ITS from *Polygonum* Sect. *Tovara* and the outgroup. Dots indicate bases identical with the first line, and dashes are gaps required for alignment. Abbrevations are: *Polygonum filiforme* (FIL); *P. neofiliforme* (NEO); *P. virginianum* (VIR); *P. hydropiper* (HYD). Sequences have been deposited to GenBank with accession numbers of U51273-51276.

Table 2. Size and GC content of ITS 1, ITS 2, and 5.8 S coding region of nuclear ribosomal DNA in *Polygonum* sect. *Tovara* and outgroup species

Species	Length in bp (GC%)		
	ITS 1	ITS 2	5.8S
P. filiforme	241 (63.5)	243 (66.7)	163 (55.8)
P. neofiliforme	241 (62.7)	243 (65.4)	163 (55.8)
P. virginianum	242 (66.5)	243 (79.8)	163 (55.8)
P. hydropiper	241 (61.8)	241 (65.6)	163 (55.8)

The range of sequence divergences (Kimura's K; Kimura, 1980) of ITS 1 was 0.8-3.8% for species of sect. *Tovara*, and 11.9-12.9% for species of sect *Tovara* and *P. hydropiper* of sect. *Persicaria* (Table 3). Sequence divergences of ITS 2 were considerably higher than those of ITS 1; they ranged from 1.3% to 10.7% between the species of sect. *Tovara*, and from 12.4% to 14.9% between the species of sect. *Tovara* and *P. hydropiper* of sect. *Persicaria*. Sequence divergences of ITS 1 and ITS 2 combined ranged from 1.0 to 7.2% for species of sect. *Tovara*, and 12.4-13.9% for species of sect. *Tovara* and *P. hydropiper* of sect. *Persicaria* (Table 3). Within sect. *Tovara*, the highest divergence value occurred between North American *P. virginianum* and eastern Asian *P. neofiliforme*.

A total of 75 variable sites including four indels was detected from the aligned sequences of 649 sites for ITS 1, the 5.8S coding region, and ITS 2 (Fig. 1). Eight of these variable sites were phylogenetically informative, with three in ITS 1 and

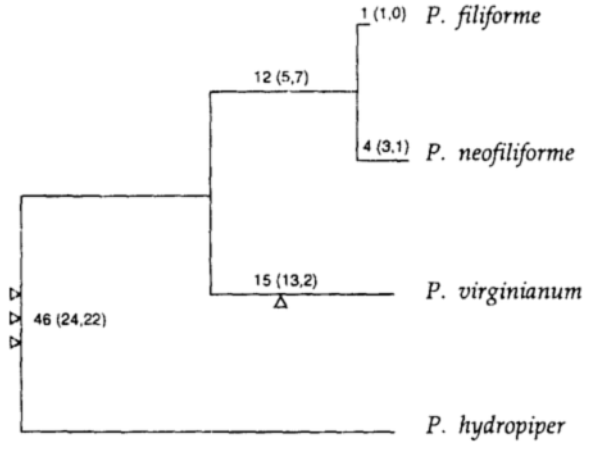

Fig. 2. The most parsimonious tree of *Polygonum* sect. *Tovara* from the phylogenetic analysis of ITS sequence variation. Numbers of nucleotide substitutions are given along the top of each branch, and followed by the number of transitions and transversions in parentheses. Δ: Indels.

Table 3. Kimura's two parameter distances (Kimura's K × 100, Kimura 1980) between ITS sequences from three species of sect. *Tovara* and one outgroup species. Divergence values above diagonal in each matrix are the proportion of divergent sites and actual numbers of divergent sites appear below diagonal. Gaps are not included in the calculation of divergence values

Species	ITS 1				ITS 2				ITS 1+ITS 2			
	1	2	3	4	1	2	3	4	1	3	3	4
1 *P. filiforme*	-	0.83	3.40	12.39	-	1.25	9.30	12.37	-	1.04	6.29	12.38
2 *P.neofilforme*	2	-	3.83	11.90	3	-	10.74	13.40	5	-	7.20	12.65
3 *P. virginianum*	8	9	-	12.88	19	22	-	14.85	27	31	-	13.86
4 *P. Hydropiper*	27	26	28	-	27	29	32	-	54	55	60	-

five in ITS 2. A single most parsimonious tree with a length of 78 steps was obtained by using unweighted Fitch parsimony (Fig. 2). The consistency index excluding uninformative sites was 0.89, and the retention index was 0.88. North American *P. virginianum* was resolved as the sister group of the two Asian species, *P. filiforme* and *P. neofiliforme* (Fig. 2).

DISCUSSION

The size of ITS 1 of *Polygonum* sect. *Tovara* as well as the outgroup species, *P. hydropiper* of *Polygonum* sect. *Persicaria*, lies intermediate in the range of that reported previously for other angiosperms (187-298 bp; reviewed in Baldwin *et al.*, 1995; Downie and Katz-Downie, 1996). However, the size of *Polygonum* ITS 2 (241-242 bp) is relatively long as compared to previous reports for other angiosperms (187-252 bp; reviewed in Baldwin *et al.*, 1995; Downie and Katz-Downie, 1996). Among ITS 2 sequences of various angiosperm groups, only that of *Cucurbita* L. (Cucurbitaceae; Torres *et al.*, 1990) is longer than ITS 2 of *Polygonum* sect. *Tovara*.

Relative sizes of ITS 1 and ITS 2 vary considerably among the groups of angiosperms (Baldwin *et al.*, 1995). In *Polygonum* sect. *Tovara*, ITS 1 and ITS 2 appear to be nearly identical in size (Table 2), as reported from *Nicotiana* L. (Venkateswarlu and Nazar, 1991) and *Lycopersicon* Mill. (Kiss *et al.*, 1988) in Solanaceae and *Hordeum* L. (Chatterton *et al.*, 1992) in Poaceae.

In most investigated groups of angiosperms, nucleotide divergence values of ITS 1 are generally similar to those of ITS 2 with a few exceptions (Baldwin *et al.*, 1995). In *Polygonum* sect. *Tovara*, however, nucleotide divergence is greater between sequences of ITS 2 than between those of ITS 1, suggesting that ITS 2 changes evolutionarily faster than ITS 1. Nucleotide divergence values of ITS 2 between eastern North American *P. virginianum* and two Asian species (*P. filiforme* and *P. neofiliforme*) are almost three times higher than those of ITS 1 (Table 3).

Especially noteworthy in ITS sequences of *Polygonum* sect. *Tovara* is the exceptionally high GC content of ITS 2 in *P. virginianum*. For studied angiosperms, GC content of ITSs ranges from approximately 30% to 77% (reviewed in Baldwin *et al.*, 1995); the value of 79.8% in *P. virginianum* ITS 2 is the highest of those reported from angiosperms to date (reviewed in Baldwin *et al.*, 1995; Downie and Katz-Downie, 1996). In the remaining species, including the outgroup, GC content of both ITSs falls within the range reported for other angiosperms (Table 3).

Parsimony analysis of ITS sequences of *Polygonum* sect. *Tovara* fully resolved phylogenetic relationships among the species (Fig. 2). The three species recognized by earlier studies of numerical analyses of morphological characters (Park *et al.*, 1992) and flavonoid chemistry (Mun and Park, 1994) are well-separated phylogenetically. In addition, interspecific relationships depicted in the ITS tree correlate with the current geographic distribution of these three species: North American *P. virginianum* is clearly separated from the two Asian species, *P. filiforme* and *P. neofiliforme*.

The ITS tree suggests that *P. virginianum* of eastern North America probably diverged from the ancestral lineage of *P. filiforme* and *P. neofiliforme* of eastern Asia prior to divergence of the latter two species (Fig. 2). The nucleotide divergence between the ITS regions of *P. virginianum* and the Asian species ranges from 6.3% to 7.2% (Table 3). This value is very high as compared to those reported from other eastern Asia-eastern North America disjunct taxa: 0.09% between *Menispermum canadense* L. and *M. dauricum* DC., 1.3% between *Caulophyllum thalictroides* (L.) Michx. and *C. robustum* Maxim., 1.6% between *Penthorum sedoides* L. and *P. chinense* Pursh, and 5.0% between *Phryma leptostachya* L. var. *leptostachya* and *P. leptostachya* var. *asiatica* Hara (Lee *et al.*, 1994).

The relatively high level of molecular divergence of *P. virginianum* from the Asian species is concordant with differences in flavonoid chemistry. The flavonoid profile of *P. virginianum* is very simple and entirely based on quercetin. In contrast, those of the Asian species are more complex, and two additional flavonol types, kaempferol and myricetin, are found in *P. filiforme* and *P. neofiliforme*, respectively (Mun and Park, 1995). In morphology, however, *P. virginianum* is distinguished from the Asian species mainly by quantitative characters related to leaf shape (Park *et al.*, 1992), suggesting that the molecular and chemical divergence did not parallel the morphological divergence in these species.

Polygonum neofiliforme, partly sympatric with *P. filiforme*, is morphologically very similar to *P. filiforme*, and some authors (Steward, 1930; Li, 1952 b; Graham and Wood, 1965) considered that the former is conspecific with the latter. Comparative analysis of ITS sequence data revealed that the two species

are phylogenetically closely related (Fig. 2) but differ in ITS sequence by five bases, with the nucleotide divergence value of 1.04% (Table 3). In general, this result is in agreement with that from flavonoid analysis (Mun and Park, 1995). In flavonoid chemistry, these two species share some identical compounds, but *P. neofiliforme* is distinguished from *P. filiforme* by the presence of myricetin derivatives and by the absence of 3-O-arabinosides of kaempferol and quercetin and quercetin 3-O-(2"-galloyl)glucoside. Morphologically, the former differs from the latter in having narrowly oblong leaves with sharp acuminate apices and acute bases as opposed to leaves with broader upper and apical portion in *P. filiforme* (Park *et al.*, 1992).

In conclusion, the ITS sequence data presented here appear to be very useful for recognizing phylogenetic relationships and levels of differentiation among the species of *Polygonum* sect. *Tovara*. The phylogenetic analysis of ITS sequence variation in the section provided fully resolved relationships among the species as well as an additional criterion for estimating their levels of differentiation. The relationships among the species depicted in the ITS tree and their nucleotide divergence values fully support the results from earlier studies of morphology (Park *et al.*, 1992) and flavonoid chemistry (Mun and Park, 1995) that *P. virginianum*, *P. filiforme* and *P. neofiliforme* are closely related but distinct species.

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LITERATURE CITED

Baldwin, B.G. 1992. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: An example from the Compositae. *Molec. Phylogenet. Evol.* **1**: 3-16.

Baldwin, B.G., M.J. Sanderson, J.M. Porter, M.F. Wojciechowski, C.S. Campbell and M.J. Donoghue. 1995. The ITS region of nuclear ribosomal DNA: A valuable source of evidence of angiosperm phylogeny. *Ann. Missouri Bot. Gard.* **82**: 247-277.

Chatterton, N.J., C. Hsiao, K.H. Asay, K.B. Jensen and R.-C. Wang. 1992. Nucleotide sequence of the internal transcribed spacer region of rDNA in the primitive oat species, *Avena longiglumis* Durieu (Gramineae). *Pl. Molec. Biol.* **20**: 163-164.

Downie, S.R. and D.S. Katz-Downie. 1996. A molecular phylogeny of Apiaceae subfamily Apioideae: Evidence from nuclear ribosomal DNA internal transcribed spacer sequences. *Amer. J. Bot.* **83**: 234-251.

Doyle, J.J. and J.L. Doyle. 1989. A rapid DNA isolation porcedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* **19**: 11-15.

Elmer, A.D.E. 1915. Two hundred twenty-six new species-II. *Leafl. Philipp. Bot.* **8**: 2719-2883.

Felsenstein, J. 1992. PHYLIP (Phylogeny Inference Package) version 3.5. Computer program distributed by the author. Seattle: The University of Washington.

Graham, S.A. and C.E. Wood, Jr. 1965. The genera of Polygonaceae in the southeastern United States. *J. Arnold Arbor.* **46**: 91-121.

Hara, H. 1962. *Sunania* Rafin. versus *Tovara* Adanson. *J. Jap. Bot.* **37**: 326-332.

Hara, H. 1965. On the genus *Antenoron* Rafin. J. Jap. Bot. **40**: 192.

Haraldson, K. 1978. Anatomy and taxonomy in Polygonaceae subfam. Polygonideae Meisn. emend. Jaretzxy. *Symb. Bot. Upsal* **22**(2): 1-95.

Higgins, D.G., A.J. Bleasby and R. Fuchs. 1992. CLUSTAL V: Improved software for multiple sequence alignment. *Computer Applic. Biosci.* **8**: 189-191.

Kim, K.-J. and R.K. Jansen. 1994. Comparisons of phylogenetic hypotheses among different data sets in dwarf dandelions (*Krigia*, Asteraceae): Additional information from internal transcribed spacer sequences of nuclear ribosomal DNA. *Pl. Syst. Evol.* **190**: 157-185.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitution through comparative studies of nucleotide sequences. *J. Molec. Evol.* **16**: 111-120.

Kiss, T.M., S.A. Kiss and F. Solymosy. 1988. Nucleotide sequence of the 17S-25S spacer region from tomato rDNA. *Nucl. Acids. Res.* **16**: 7179.

Lee, N.S., T. Sang and D. Crawford. 1994. ITS Sequence divergence between disjunct taxa in eastern Asia and eastern North America. *Amer. J. Bot.* **81**(suppl.): 167.

Li, H. 1952a. The genus *Tovara* (Polygonaceae). *Rhodora* **54**: 19-25.

Li, H. 1952b. Floristic relationships between eastern Asia and eastern North America. *Trans. Am. Phil. Soc.* 42: 371-429.

Maekawa, F. 1932. Alabastra diversa I. *Bot. Mag. (Tokyo)* **46**: 561-586.

Mun, J.-H. and C.-W. Park. 1995. Flavonoid chemistry of *Polygonum* Sect. *Tovara* (Polygonaceae): A systematic study. *Pl. Syst. Evol.* **196**: 153-159.

Nakai, T. 1922. Notulae ad plants Japoniae et Koreae XXVIII. *Bot. Mag. (Tokyo)* **36**: 117-128.

Nieuwland, J.A. 1912. New plants from various places. *Amer. Midl. Naturalist* **2**: 178-185.

Ohki, K. 1926. Polygonaceae of the Island Iki. *Bot. Mag. (Tokyo)* **40**: 48-58.

Park, C.-W., M.G. Lee and H. Shin. 1992. A systematic study on *Polygonum* sect. *Tovara* (Polygonaceae): Analysis of morphological variation. *Korean J. Bot.* **35**: 385-392.

Ronse Decraene, L.-P. and J.R. Axeroyd. 1988. Generic limits in *Polygonum* and related genera (Polygonaceae) on the basis of floral characters. *Bot. J. Linn. Soc.* **98**: 321-371.

Sang, T., D.J. Crawford, S.-C. Kim and T.F. Stuessy. 1994. Radiation of the endemic genus *Dendroseris* (Asteraceae) on the Juan Fernandez Islands: Evidence from sequences of the ITS regions of nuclear ribosomal DNA. *Amer. J. Bot.* **8**: 1494-1501.

Sang, T., D.J. Crawford, T.F. Stuessy and M. Silva O. 1995. ITS sequences and the phylogeny of the genus *Robinsonia* (Asteraceae). *Syst. Bot.* **20**: 55-64.

Steward, A.N. 1930. The Polygonaceae of eastern Asia. *Contr. Gray Herb.* **5**(88): 1-129.

Suh, Y., L.B. Thien, H.E. Reeve and E.A. Zimmer. 1993. Molecular evolution and phylogenetic implications of internal transcribed spacer sequences of ribosomal DNA in Winteraceae. *Amer. J. Bot.* **80**: 1042-1055.

Swofford, D.A. 1993. Phylogenetic Analysis Using Parsimony (PAUP). Version 3.1.1. Washington, DC: Smithsonian Institution.

Torres, R.A., M. Ganal and V. Hemleben. 1990. GC balance in the internal transcribed spacers ITS-1 and ITS-2 of nuclear ribosomal RNA genes. *J. Molec. Evol.* **30**: 170-181.

Venkateswarlu, K. and R. Nazar. 1991. A conserved core structure in the 18-25S rRNA intergenic region from tobacco, *Nicotiana rustica*. *Pl. Molec. Biol.* **17**: 189-194.

White, T.J., T. Bruns, S. Lee and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* Innis, M., D. Gelfand, J. Sninsky, and T. White (eds.), PCR protocols: A guide to methods and application. Academic Press, San Diego, pp. 315-322.

Yokota, Y., T. Kawata, Y. Iida, A. Kato and S. Tanifuji. 1989. Nucleotide sequences of the 5.8S rRNA gene and internal transcribed spacer regions in carrot and broad bean ribosomal DNA. *J. Molec. Evol.* **29**: 294-301.

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