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Molecular Phylogeny of the Genus *Hypericum* (Hypericaceae) from Korea and Japan: Evidence from Nuclear rDNA ITS Sequence Data

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As part of our ongoing phylogenetic study of genus *Hypericum*, nuclear ribosomal DNA internal transcribed spacer sequences were analyzed for 36 species of *Hypericum* as ingroup and two species of *Thornea* as outgroup. This sampling included most of the previously described species from both Korea and Japan. The ITS phylogeny suggested that the surveyed *Hypericum* species belong to a monophyletic section, *Trigynobrathys*, and a polyphyletic section, *Hypericum*. In addition, two monotypic sections, *Sampsonia* and *Roscyna*, were identified. Members of section *Hypericum* occur in four different lineages worldwide, which imply at least four independent origins. The Korean and Japanese species of section *Hypericum* form a monophyletic group, except for *H. vulcanicum*. Instead, that particular species belongs to a distinct monophyletic group with *H. scoreri* and *H. formosa* from other geographic areas, and is a sister to section *Trigynobrathys*. The Korean and Japanese species of section *Trigynobrathys* show a monophyletic origin. *H. sampsonii* is now recognized as a distinct section rather than being a member of sections *Hypericum* or *Drosocarpium*, as had been indicated previously. Our results differ somewhat from those of recent morphological and cytological studies. The phylogenetic relationships among Korean and Japanese species have now been mostly resolved via ITS phylogeny.

Keywords: cladistic analysis, Hypericaceae, *Hypericum*, maximum likelihood value, monophyletic group, nuclear ITS phylogeny

The genus *Hypericum* Linneaus (1753) includes approximately 350 to 400 species (Robson, 1977). Its name was given by the Greeks to plants hung above their religious figures to ward off evil spirits. One species is now known as Saint John's Wort (*Hypericum perporatum* L.), and pills made from crude extracts of the whole plant are sold as an antidepressant throughout the world market. This genus is usually characterized by a set of morphological characters, such as its shrubby or perennial herbaceous habits, glabrous or simple hairs with translucent and often opaque black or reddish glands, opposite or whorled leaves, four- or five-fascicled stamens, dorsifixed or basifixed anthers, and short-cylindric seeds with finely to coarsely reticulate seed coats (Robson, 1977). Because of the difficulty in handling its large numbers of species and predicting the evolutionary directions of those morphological characters, only a few systematic studies have been performed (Robson, 1977, 1990, 2001). No phylogenetic work has yet been published.

The species of *Hypericum* are distributed worldwide. Robson (1977) has hypothesized, based on distribution and evolutionary trends of their morphological characters, that they evolved in Africa and spread to Asia, America, and Australia before the disintegration of Gondwanaland. However, the lack of fossil records means those trends must be considered only as speculation, so that predictions are often impossible to make without cladistic analysis or other in-depth analytical methods. Because of the inherent difficulties in conducting thorough phylogenetic studies of this large and complex genus, we will focus here primarily on the phylogeny of northeastern Asian species, especially those from Korea and Japan.

Japanese *Hypericum* species are perennial herbs that occupy a wide range of habitats. In a monograph of Hypericaceae Juss., Kimura (1951) identified 22 native Japanese species, including 42 varieties. In that work, many intermediate forms were recognized as varieties because it was not easy to delimit the species boundary even among the most well-recognized species complexes. For example, the *Hypericum pseudopetiolatum* R. Keller complex from Japan includes three closely related species and one variety that are somewhat isolated in their reproduction, geography, and/or ecology (Kato, 1990). In contrast to Kimura's narrowly circumscribed species boundaries, Ohwi (1975) enumerated only 14 species, including 7 endemic to Japan. For the infrageneric classification,

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Kogi (1984) suggested that the native Japanese *Hypericum* species could be divided into two sections, *Roscyna* (Spach) R. Keller and *Hypericum* (= *Homotaenium* Y. Kimura), based on chromosome data. However, members of section *Drosocarpium* Spach were not considered in that chromosome study. Kimura (1951) had delineated the genera into 12 sections, including three natives (*Roscyna*, *Drosocarpium*, and *Homotaenium*), according to their morphological characters. That system was comparable to the one presented by Robson (1977), who further classified those 12 sections into 30, from all over the world.

Korean *Hypericum* species are perennial herbs or shrubs, generally lacking pubescence. Six to nine species have been reported from Korea (Park, 1974; Lee, 1979; Lee 1996a, b). Among them, six commonly occur in both Korea and Japan.

The overall number of *Hypericum* species known from Korea and Japan varies among authors because of differing circumscriptions. In addition, considerable controversy still exists regarding infraspecific and infrageneric relationships, although a few species have been the focus of morphological (Spach, 1836; Jaubert and Spach, 1842; Keller, 1925; Kimura, 1951; Robson, 1977) and karyological (Kogi, 1984) studies. A molecular phylogenetic study has never been performed with this large and problematic genus.

As part of our ongoing research, we first analyzed the Korean and Japanese species, using nuclear rDNA internal transcribed spacer (ITS) sequences. Our main objectives were to 1) examine the phylogenetic relationships among three sections [*Roscyna*, *Trigynobrathys* (Y. Kimura) N. Robson, and *Hypericum*]; 2) evaluate the monophyly of section *Hypericum*, especially in view of a previous suggestion that one member, *H. sampsonii* Hance, be excluded; and 3) investigate the geographic distribution patterns and evolutionary directions of characters for both Korean and Japanese species.

MATERIALS AND METHODS

Plant Sampling

Specimens included 36 *Hypericum* species as the ingroup taxa and two *Thorea* Breedlove & McClint species as the outgroup (Table 1). Species-level samplings of the former represented all native sections (*Roscyna*, *Trigynobrathys*, *Sampsonia* N. Robson, and *Hypericum*) from Korea and Japan, with most having been previously reported by Korean and Japanese authors (Satake et al., 1981; Kogi 1984; Ohwi, 1984; Lee, 1996a, b). To test the monophyly of *Trigynobrathys* and *Hypericum*, we included all Japanese and Korean species as well as 10 species from other regions. In addition, a single representative species from each of the five closely related sections were included. Outgroup selection was based on earlier morphological studies of Hypericaceae (Robson, 2001) plus our ongoing molecular phylogenetic study of *Hypericum* and related genera at the intergeneric level (Park and Kim, unpublished results).

DNA Isolation, PCR Amplification, and Sequencing

Total genomic DNA was isolated from both fresh or silica-dried leaves and herbarium specimens. Fresh material was obtained from the field. The CTAB method of Doyle and Doyle (1987) was used to isolate DNA from fresh and silica-dried materials, and the DNA was further purified by CsCl-EtBr gradient ultracentrifugation (Sambrook et al., 1989). Small-scale DNA isolations were also used on the herbarium samples, following the method of Loockerman and Jansen (1996).

ITS regions of nuclear DNA were amplified by polymerase chain reaction (PCR) in 50 μl reactions, using external ITS1 and ITS4 primers designed by White et al. (1990). The protocol followed that of Kim and Jansen (1994). PCR products were purified via the QIAquick PCR purification kit (Qiagen, USA). These purified products were then sequenced using a dye terminator cycle-sequencing ready reaction kit (Big Dye 2.0^{TM} Terminator Cycle Sequencing Kit; PE Applied Biosystems, USA) and an ABI 377 automatic sequencer. Sequencing primers were the same as those for PCR amplification, with occasional exceptions, i.e., primers ITS2 and ITS3 (Kim and Jansen, 1994) being used within the 5.8S coding region.

Sequence Alignment and Phylogenetic Analysis

Sequences from both directions were edited and assembled with the program Sequencer (Gene Code, USA). Clustal X (Thompson et al., 1997) was used for multiple sequence alignment, and gaps were further adjusted by hand. Parsimony analysis was performed via PAUP* version 4.0b (Swofford, 2002). Heuristic searches with 1000 random entries were conducted with the ACCTRAN, MULPRAS, and TBR options. Gaps were treated as missing data and all characters were accorded equal weight. The amount of support

Table 1. Plant materials used in this study, with sources and voucher information provided after the collector's name. The standard herbarium acronym within parentheses indicates the location of voucher specimens. All ITS sequences were generated in this study and are available under their accession numbers from the GenBank/EMBL/DDBJ database.

Taxon	Source[a)]	Accession number[b)]
Section *Hypericum*		
H. ashinae Makino	T. Yamazaki *s.n.*, Oct. 15 1988 (TI)	AY572997
H. attenuatum Choisy var. *attenuatum*	S.-J. Park 00-307, Aug. 12 2000 (YNUH)	AY572993
H. attenuatum var. *confertissimum* (Nak.) T. Lee	S.-J. Park 00-182, Aug. 25 2000 (YNUH)	AY572995
[c)]Unknown species 1	Park 00-431, Aug. 3 2001 (YNUH)	AY572996
H. erectum Thunb. ex Murray	K.-J. Kim 2000-1325, Sep. 13 2000 (YNUH)	AY572991
H. formosum Kunth	A. Huber 3382, Aug. 5 1996 (TEX)	AY573018
H. hakoense Fr. et Sav.	M. Togashi *s.n.*, Aug. 26 1961 (TI)	AY573000
H. kamtschaticum Ledeb.	S. Gage 1253, Aug. 5 1995 (MO)	AY572992
H. kinashianum Koidz.	Haga *s.n.*, Aug. 5 2000 (TI)	AY573001
H. maculatum Crantz	P. F. Filip 12, Sep. 16 1980 (TEX)	AY573007
H. oaxacum R. Keller	E. K. Balls *s. n.*, Sep. 17 1938 (TEX)	AY573003
H. oliganthum Fr. et Sav.	S.-J. Park 00-257, Aug. 13 2000 (YNUH)	AY573005
H. ovalifolium Koidz.	C. R.. Lancaster 12047, May 30 1977 (BM)	AY572998
H. pseudopetiolatum R. Keller	J. Ohwi & T. Koyama. s. n., Aug. 21 1951 (TI)	AY573002
H. sampsonii Hance	T. Yamazaki 4160, June 30 1999 (TI)	AY573011
H. scouleri Hook.	T. Makle 212, Sep. 14 1932 (K)	AY573017
H. shikokumontanum Makino	T. Yamazaki *s. n.*, Aug. 29 1989 (MAK)	AY572999
H. triquetrifolium Turra	A. Jonson *s. n.*, Aug. 21 1952 (TEX)	AY573006
H. vanoitii Lev.	S.-J. Park 00-683, Aug. 14 2000 (YNUH)	AY572994
H. vulcanicum Koidz.	M. Takahashi *s. n.*, Aug. 7 1973 (TI)	AY573016
H. *yesoense* Maxim.	M. Takahashi *s. n.*, Jun. 29 1973 (TI)	AY573004
Section *Roscyna* (Spach) R. Keller		
H. ascyron L.	S.-J. Park 00-532, Aug. 23 2000 (YNUH)	AY573014
H. ascyron var. *longystylum* Maxim.	S.-J. Park 00-487, Aug. 24 2000 (YNUH)	AY573015
Section *Spachium* (R. Keller) N. Robson (= Section *Trigynobrathys* (Y. Kimura) N. Robson)		
H. boreale (Britton) Bicknell	P. Synge 1520, Aug. ? 1935 (TEX)	AY573026
H. brevistylum Choisy	R. K. Robson *s. n.*, Dec. 12 1980 (BM)	AY573019
H. japonicum Thunb.	K.-J. Kim 1999-11465, Aug. 17 2000 (YNUH)	AY573025
[c)]Unknown species 2	K.-J. Kim 2000-1267, Aug. 19 2001 (YNUH)	AY573023
H. laxum (Bl.) Koidz.	Park 00-853, Aug. 12 2001 (YNUH)	AY573024
H. rigidum St.-Hil.	G. Hatschba 48170, Mar. 1 1984 (K)	AY573021
H. setosum L.	J. S. Miller 8310, Aug. 2 1994 (TEX)	AY573020
H. ternum St.-Hil.	V. Nicolack 63, Oct. 20 1989 (BM)	AY573022
Section *Androsaemum* (Duhamel) C. Koch		
H. androsaemum L.	P. M. Synge 1520, Jul. ? 1935 (BM)	AY573012
Section *Adenosepalum* Spach		
H. delpinicum Boiss. et Heldr.	V. E. McNeilur 99-543, Jul. 24 1999 (BM)	AY573009
Section *Olympia* (Spach) Nyman		
H. olympicum L.	S. Richard *s. n.*, Aug. 12 1972 (TEX)	AY573008
Section *Ascyreia* Choisy		
H. leschenaultii Choisy	R. Billi 271, Sep. 29 1943 (BM)	AY573010
Section *Adenotrias* (Jaub. et Spach) R. Keller		
H. aegypticum L.	H. Kanai *s. n.*, Aug. 13 1989 (BM)	AY573013
Outgroups		
Thornea calcicola (Stan. et Ste.) Breedlove et E.M. McClint	P. Adams 242, Aug. 12 1958 (TEX)	AY573028
Thornea matudue (Lundell) Breedlove et E.M. McClint	R. K. Godgre. *s.n.*, OCT. ? 1963 (TEX)	AY573027

[a]YNUH (Yeungnam University Herbarium), BM (The Natural History Museum), K (Kew, Royal Botanical Garden), MAK (Makino Herbarium, Tokyo), MO (Missouri Botanic Garden), TI (University of Tokyo), TEX (University of Texas at Austin). Collection numbers and dates are given. [b]ITS sequences are available from public data banks, e.g., NCBI, EMBL, and DDBJ homepages under the same accession number. [c]Unknown species 1 and 2 are described as *H. chejuense* Park et Kim and *H. jeonjocksanense* Park et Kim, respectively (Park and Kim, in press). Sectional grouping of species follows Robson's (1977) classification.

Table 2. Comparison of sizes, G+C contents, and sequence divergences of nuclear ITS regions among major groups of *Hypericum*.

Major Group	Number of taxa	Lengths of Sequence (bp range)	G+C contents (average %)	Sequence divergence[a] (%)
Overall	38	655-674	55.2	0.1387 ± 0.0133
Within outgroup	2	655-657	58.5	0.0125 ± 0.0058
Within ingroup	36	661-674	55.0	0.1418 ± 0.0129
Sect. *Hypericum*	20	665-673	55.4	0.0914 ± 0.0091
Sect. *Hypericum* Group I	14	669-670	55.8	0.0243 ± 0.0030
Sect. *Roscyna*	2	662	62.2	0.0000 ± 0.0000
Sect. *Trigynobrathys*	8	661-670	50.5	0.0766 ± 0.0060
Korean-Japanese species	21	662-673	56.0	0.1208 ± 0.0077

[a] Sequence divergence is corrected by the two-parameter model of Kimura's (1980). Mean values and standard deviations are given.

for monophyletic groups was evaluated by 1,000 bootstrap replicates (Felsenstein, 1985), with ACCTRAN, MULPRAS, and TBR options and random entries of taxa. Parsimony jackknifing (Farris et al., 1996) of 1,000 replications was also performed to assess support for monophyletic groups. The consistency index (CI; Kluge and Farris, 1969), retention index (RI; Farris, 1989), and g1 statistic (Hillis and Huelsenbeck, 1992) were obtained by PAUP. These g1 values were calculated from 100,000 random parsimony trees using the random TREES command. Maximum likelihood (ML) values of all equally parsimonious trees were estimated under the GTR plus gamma distribution model because the MODELTEST (Posada and Crandall, 1998) suggested that this condition provided the best fit. A separate ML analysis was performed on a raw data set under the same model conditions. In addition, sequence divergences and other statistical analyses were conducted with MEGA (Kumar et al., 2001).

RESULTS

Sequence Variation

Our 38 aligned ITS sequences were 731 b long (i.e., 276 b for ITS1, 205 b for 5.8S, and 250 b for ITS2), with 402 invariant and 329 variant positions that included 246 parsimony-informative sites. Sequence lengths ranged from 655 b (for *T. calcicola* Breedlove and McClint) to 674 b (*H. aegypticum* L.). Base-composition averages were A: 23.9%, C: 27.7%, G: 27.5%, and T: 20.9%. The transition/transversion ratio on the maximum parsimonious tree was 1.419: 1.000.

Average sequence divergence among all surveyed taxa, as determined by Kimura's (1980) two-parameter method, was 0.1387, with a standard deviation of 0.0133 (Table 2). The average divergences between outgroup species, among ingroup species, and between outgroup and ingroup species were 0.0125, 0.1418, and 0.1616, respectively. Average divergences within section *Hypericum* (21 species), within section *Hypericum* group I (14 species), within section *Roscyna* (2 species), and within section *Trigynobrathys* (8 species) were 0.0914, 0.0243, 0.0000, and 0.0766, respectively. Finally, the average sequence divergence between Korean and Japanese species (21 species) was 0.1208, with a standard deviation of 0.0077. Insertion and deletion (indel) mutations were observed at 103 locations in the aligned sequence. Most (95 of the 103) indels were 1 to 3 b long; the longest, 10 b, was recorded in the middle of ITS 1 for the two outgroup species of *Thornea*.

Phylogenetic Analysis

Phylogenetic analysis using an heuristic search, with TBR branch rearrangements and 1000 random data entries of PAUP, produced 97 equally parsimonious trees at 770 steps. We also calculated a CI of 0.567 (excluding autapomorphies), an RI of 0.826, and an RC of 0.509. Tree skewness, based on 100,000 random trees, was -0.707 (g1 value). The maximum likelihood (ML) values of 97 equally parsimonious trees ranged from -5,012.580 to -5,029.829. Fig. 1 presents one of the 97 tree topologies with the best ML (= -5,012.580). Independent ML analysis also generated the best tree topology, identical to that shown in (Fig. 1).

The monophyly of ingroup genus *Hypericum* is strongly supported by 100% bootstrap and 100% jackknife values. Several clades are recognized within that ingroup. A basal monophyletic group comprises three species of section *Hypericum* (lineage IV) and all surveyed species of section *Trigynobrathys*. The sister-group relationship between section *Trigynoblathys* and lineage IV of section *Hypericum* is supported by 75 characters as well as 100% bootstrap and jackknife values. Within this clade, the monophyly of section *Trigynobrathys* is strongly supported by 28 characters, a 94% bootstrap value, and a 95% jackknife value. Furthermore, within that same section, the three Korean and Japanese species also form a monophyletic lineage by three characters, 95% bootstrap, and 88% jackknife values. *H. boreale* (Britton) E. P. Bicknell from far-eastern Russia is a sister species to the Korean and Japanese species of section *Trigynobrathys*.

The second intersectional group in the tree (Fig. 1)

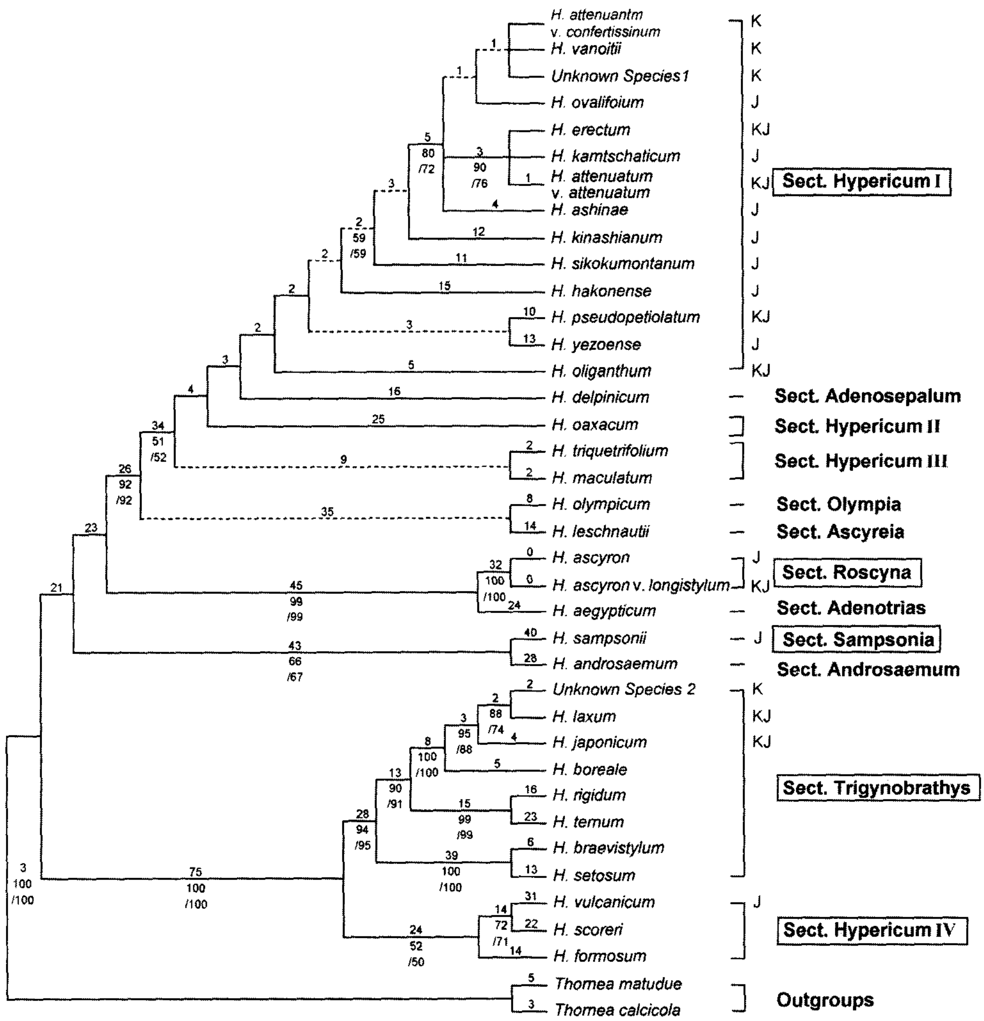

Figure 1. One of 97 equally most parsimonious trees based on 329 variable characters including 246 phylogenetically informative sites in ITS sequences for 36 *Hypericum* and two *Thornea*. This tree topology has the best maximum likelihood value. The numbers of base substitutions are indicated above each branch and the bootstrapping/jackknifing percentages (More than 50% supports are shown) from 1,000 replications are given below each internal branch, respectively. Dashed lines indicate nodes that collapsed in the strict consensus tree. The letters K and J before sectional names indicate the distribution ranges of Korea and Japan, respectively. The section *Hypericum* is a polyphyletic lineage and is grouped into four different monophyletic groups (I, II, III, and IV). Unknown species 1 and 2 are described as *H. chejuense* Park et Kim and *H. jeonjocksanense* Park et Kim, respectively (Park and Kim, in press).

contains section *Sampsonia* and section *Androsaemum* (Duhamel) Spach. A sister-group relationship between those two, however, is relatively weak, supported by 43 characters, and only 66% bootstrap and 67% jackknife values. In addition, the number of autapomorphies leading to two terminal species is relatively high (Fig. 1). A third intersectional group is represented by two taxa from section *Roscyna* and a species from section *Adenotrias* (Jaub. & Spach) R. Keller. We found *H. aegypticum* L., from the Mediterranean region, to be closely related to section *Roscyna*; the sister-group relationship could be supported strongly by 45 characters and 99% bootstrap and jackknifing values.

The remaining clade is the largest lineage, containing most members of section *Hypericum* (lineages I, II, and III) and three related sections of *Ascyreia* Choisy, *Olympia* (Spach) Nyman, and *Adenosepalum* Spach. The monophyly of this clade is supported by 26 characters and 92% bootstrap and jackknife values. Sections *Olympia* and *Ascyreia* are sister groups to lineages I to III of section *Hypericum* (including section *Adenosepalum*). Section Adenosepalum is positioned between lineages I (14 Japanese and Korean species) and lineages II to III (three American species) of section *Hypericum*. Except for *H. vulcanicum* Koid. of lineage IV (section *Hypericum*), which is closely related to section *Trigynobrathys*, all Korean and Japanese species of section *Hypericum* form a monophyletic clade as lineage I, even though the support values are low.

Therefore, within the genus *Hypericum*, three sections from Korea and Japan (*Trigynobrathys, Roscyna,* and *Sampsonia*) form independent monophyletic or monotypic groups. In contrast, section *Hypericum* is represented by four lineages (I, II, III, and IV).

DISCUSSION

Phylogenetic analysis of the ITS sequence data has provided us with several insights into the relationships within *Hypericum* from Korea and Japan. These data also offer an opportunity to examine the monophyly of several sections. Based on the morphology of 22 species and 42 varieties, Kimura (1951) recognized three native and one introduced section from Japan, including *Roscyna, Drosocarpium, Millesporum* Spach, and *Homotaenium* (*Hypericum*). Section *Millesporum* was represented by only an introduced species, *H. perforatum*. In addition, Kimura (1951) subdivided the Japanese section *Hypericum* into two series -- *Bilineata* Y. Kimura and *Elineata* Y. Kimura. A more comprehensive classification system by Robson (1977) recognized 30 sections worldwide. Among them, only three are found in Korea and Japan -- *Hypericum, Roscyna,* and *Spachium* (R. Keller) N. Robson. Therefore, compared with previous morphological classifications, our ITS tree recognizes the three monophyletic or monotypic sections represented by *Roscyna, Trigynobrathys,* and *Sampsonia,* as well as a polyphyletic section of *Hypericum* from Korea and Japan.

Section *Sampsonia*

H. sampsonii Hance historically has been treated as a member of section *Drosocarpium* (Hance, 1865; Kimura, 1951) because of its dot-shaped glandular trichomes on the capsule valves. However, Robson (1977) placed this species in section *Hypericum* but was later placed in a new section, *Sampsonia*, which consisted of two species, *H. sampsonii* and *H. assamicum* S. N. Biswas (Robson, 2001). Both species are rare plants that occur in relatively restricted areas, from southern Japan and southern China to northern Vietnam and eastern India. Our ITS tree not only suggests that section *Hypericum* is a polyphyletic lineage, but also supports the distinct phylogenetic position of *H. sampsonii*, which is probably closely related to section *Androsaemum* (Fig. 1). In addition, those two species are easily distinguished from members of section *Hypericum* by several morphological characters, e.g., their terete stems, perfoliate leaves, cupuliform flowers, 5-merous perianths, and black-glanded anthers. Therefore, both molecular and morphological data support the separation of section *Sampsonia* from section *Hypericum*.

Section *Roscyna*

A clade leading to section *Roscyna* from Korea and Japan also forms a distinct lineage in our ITS tree (Fig. 1). This section comprises two species - *H. ascyron* L. and *H. przewalskii* Maxim. (Robson, 2001), and can easily be told from other related sections because of its persistent petals and stamens. Furthermore, the karyotype data also support a distinct position for section *Roscyna* in the genus *Hypericum* (Kogi, 1984). Several varieties of *H. ascyron* are distributed in the disjunct areas of northeastern Asia and northeastern America. In contrast, *H. przewalskii* is restricted to southwestern China. Our ITS phylogeny suggest that section *Roscyna* differs from other sections by its longer branches, although here we analyzed only two varieties of *H. ascylon*. Section *Adenotrias* from the Mediterranean region forms a strong sister-group relationship with *Roscyna*, supported by 45 characters and 99% bootstrap and jackknife values. That closeness between sections, however, had never previously been hypothesized.

Section *Trigynobrathys*

H. laxum (Bl.) Koidz. and *H. japonicum* Thunb. of section *Trigynobrathys* were first considered by Kimura (1951) to be members of the genus *Sarothra*. However, Robson (1977) then included *Sarothra* in the genus *Hypericum*, and treated *H. japonicum* and *H. laxum* as members of *Hypericum* section *Spachium*. Later, Robson (1990) dismantled section *Spachium* and assigned its members to two other sections -- *Brathys* Choisy and *Trigynobrathys*. The latter consists of 52 species from America, Asia, Africa, and Oceania, and includes *H. laxum* and *H. japonicum* from Korea and Japan. These two species, together with one unknown species from Korea (now *H. jeongjocksanense* Park et Kim; Park and Kim, in press), form a clade within the monophyletic section *Trigynobrathys* in the ITS tree (Fig. 1). We have also included five additional species in widely separated geographical areas, from Asia to North America. However, our ITS data suggest that section *Trigynobrathys* is a monophyletic group, strongly supported by high statistical values (Fig. 1). In addition, the Korean and Japanese species of section *Trigynobrathys* (Robson, 2001) are of single origin.

Section *Hypericum*

The ITS phylogeny clearly shows that section *Hypericum* (Robson, 1977) is a polyphyletic assemblage. Our sampling included only 20 out of the 48 described species from that section. However, its members occur in four independent lineages in the ITS tree, even though one former member, *H. sampsonii*, has now been excluded. One unexpected example is the phylogenetic positioning of three species -- *H. vulcanicum* Koidz., *H. scoreri* Hook., and *H. formosanum* Kunth. These three species (section *Hypericum* - lineage IV in Fig. 1) form a sister clade to the monophyletic section *Trigynobrathys* rather than to the major clades of section *Hypericum* (lineages I, II, and III). This sister-group relationship between section *Trigynoblathys* and lineage IV of section *Hypericum* is strong, and is supported by 75 characters and 100% bootstrap and jackknife values. Except for *H. vulcanicum* from Japan, which is nested in lineage IV of section *Hypericum*, all 14 other species of that section (from Korea and Japan) form a monophyletic group as shown in the lineage I of section *Hypericum* (Fig. 1). This suggests a monophyletic origin in northeastern Asia. The detailed relationships among members of section *Hypericum* in our ITS tree also indicate several possibly meaningful phylogenetic relationships among species. For example, *H. erectum* Thunb. v. *attenuatum* forms a clade with *H. kamtschaticum* Ledeb. and *H. attenuatum* Choisy, with strong support values (Fig. 1). Ohwi (1984) has reported that *H. erectum* is closely related to *H. kamtschaticum*, based on morphological data. In contrast, Park (1974) has hypothesized that the former is closely related to *H. attenuatum*. Our ITS data, which are consistent with those of previous studies, suggest that *H. attenuatum* may be a polyphyletic species. Therefore, more detailed systematic research is required for this widely distributed species complex.

Sections *Olympia* (Spach) Nyman and *Ascyreia* Choisy are sister groups to lineages I to III of section *Hypericum* (including section *Adenosepalum* Spach). Monophyly of this group is relatively highly supported by 26 characters and 92% bootstrap and jackknife values. In addition, section *Adenosepalum* is nested between the three American species group of section *Hypericum* (lineages II and III) and the 14 Korean and Japanese species group of section *Hypericum* (lineage I). Therefore, recognition of section *Hypericum* invalidates the sectional-level classification in the genus *Hypericum*.

Biogeography and Phylogeny

Seven of our 36 surveyed ingroup *Hypericum* species are common to Korea and Japan. The common species scattered on several tree branches. In addition, four and 10 species are restricted to either Korea or Japan, respectively. Neither the Korean nor the Japanese endemic species form a monophyletic group in our tree. In contrast, 14 of 21 surveyed Korean and Japanese native species form a monophyletic lineage and are grouped to lineage I of section *Hypericum*. Sequence divergences among these 14 species are relatively low and suggest that the relatively recent diversification occurred in different habitats in those countries. Two previously unknown Korean species, *H. chejuense* Park et Kim and *H. cheongjocksanense* Park et Kim (Park and Kim, in press), are members of sections *Hypericum* and *Trigynobrathys*, respectively.

In general, our ITS phylogeny suggests that, section *Hypericum* is a diverse polyphyletic assemblage, whereas sections *Roscyna, Sampsonia*, and *Trigynobrathys* are monophyletic groups. In research concerning evolutionary trends of the genus *Hypericum*, Robson (1977) hypothesized that section Hypericum arose from section *Roscyna*. The ITS data, however, do not support this hypothesis because several other sections are nested between sections *Rocyna* and *Hypericum*. Therefore, to evaluate the phylogeny of the entire genus, we are increasing our sampling pool to include all its taxa. Furthermore, re-examination of the generic circumscription and interspecific relationships of *Hypericum* would benefit from further morphological studies to evaluate the taxonomic values of those characters traditionally used to delimit sections or species. Expanded sampling and additional molecular and morphological comparisons are necessary to achieve accurate placement of sectional relationships within the genus *Hypericum*.

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