



# Phylogenetics and evolution of phyllotaxy in the Solomon's seal genus *Polygonatum* (Asparagaceae: Polygonateae)

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*Polygonatum* is the largest and most complex genus in tribe Polygonateae, comprising approximately 57 species widely distributed in the warm temperate, subtropical and boreal zones of the Northern Hemisphere. However, phylogenetic relationships in the genus remain poorly understood. The objectives of this study were to reconstruct the phylogenetic relationships of the genus using four plastid markers, and to examine the evolution of leaf arrangement in *Polygonatum* in the phylogenetic context of its closely related taxa. Thirty *Polygonatum* species were sampled to infer phylogenetic relationships using maximum-likelihood and Bayesian analyses. The evolution of leaf arrangements was reconstructed using Bayesian, parsimony and likelihood methods. The phylogenetic analyses supported the current generic delimitation of *Polygonatum*, with *Heteropolygonatum* recognized as a distinct genus. Three major lineages in *Polygonatum* were well supported, largely correlated with geographical distribution and the most recent classification at the sectional level. However, our results did not support the currently recognized series, especially the two large series *Verticillata* and *Alternifolia*. Bayesian analyses support the alternate-leaf arrangement as the ancestral state for *Polygonatum*, but parsimony and maximum-likelihood analyses suggest an equivocal state for crown *Polygonatum*. Leaf arrangement was found to be evolutionarily labile. A new nomenclatural combination was made: ***Polygonatum* section *Sibirica* (L.I.Abramova) Y.Meng, comb. nov.** © 2014 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2014, 176, 435–451.

ADDITIONAL KEYWORDS: leaf arrangement – phylogeny – *Polygonatum* section *Sibirica*.

## INTRODUCTION

Polygonateae are a tribe of monocots characterized by a sympodial rhizome, an elongated aerial stem and fleshy berries (Tamura *et al.*, 1997b). The taxonomic position of the tribe has been debated; they

were originally placed in Liliaceae (Engler, 1888), then in Convallariaceae (Dahlgren, Clifford & Yeo, 1985) and Ruscaceae (Chase *et al.*, 2000; Fay *et al.*, 2000; Rudall, Conran & Chase, 2000) and more recently in Asparagaceae (APG III, 2009; Kim *et al.*, 2010). The monophyly of the tribe has been supported in most molecular studies (Rudall *et al.*, 2000; Yamashita & Tamura, 2000b, 2004; Meng *et al.*, 2008; Chen *et al.*, 2013). Two groups are

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usually recognized in Polygonatae: one comprising *Polygonatum* Mill., *Disporopsis* Hance and *Heteropolygonatum* M.N.Tamura & Ogisu (Tamura *et al.*, 1997b; Yamashita & Tamura, 2000b; Jang & Pfosser, 2002) and the other including *Maianthemum* Wigg. and *Smilacina* Desf.

*Polygonatum* (commonly known as Solomon's seal) is the largest genus in the tribe and shows great morphological diversity (Fig. 1). It contains approximately 57 species that are widely distributed in the warm temperate, subtropical and boreal zones of the Northern Hemisphere (Tang, 1978; Chen & Tamura, 2000; Judd, 2003). *Polygonatum* is also one of the most important medicinal taxa in Asia, with some species (e.g. *P. cyrtonema* Hua and *P. sibiricum* Redouté) being widely used in traditional Chinese medicine. *Disporopsis* comprises six species distributed in eastern and south-eastern Asia and is different from the other genera in that the filaments are dilated to form a corona. *Heteropolygonatum* has six species endemic to south-western and southern China, and it usually possesses terminal and axillary inflorescences, inner and outer stamens of different lengths and an epiphytic habit (Tamura, Ogisu & Xu, 1997a; Chao, Tseng & Tzeng, 2013), except that *H. xui* W.K.Bao & M.N.Tamura was reported to have similar inner and outer stamens in length (Bao *et al.*, 1999). The phylogenetic relationships of *Polygonatum*, *Disporopsis* and *Heteropolygonatum* were reconstructed by restriction site mapping of the *trnK* gene (Tamura *et al.*, 1997b); that study supported treating *Disporopsis*, *Heteropolygonatum* and *Polygonatum* as distinct genera.

The classification of *Polygonatum* has long been disputed. The most commonly used characters to differentiate species within *Polygonatum* are phyllotaxis, flower size, shape and colour, bract size and texture, filament pubescence, leaf type (cirrhose or ecirrhose) and stem angularity (Baker, 1875; Tang,

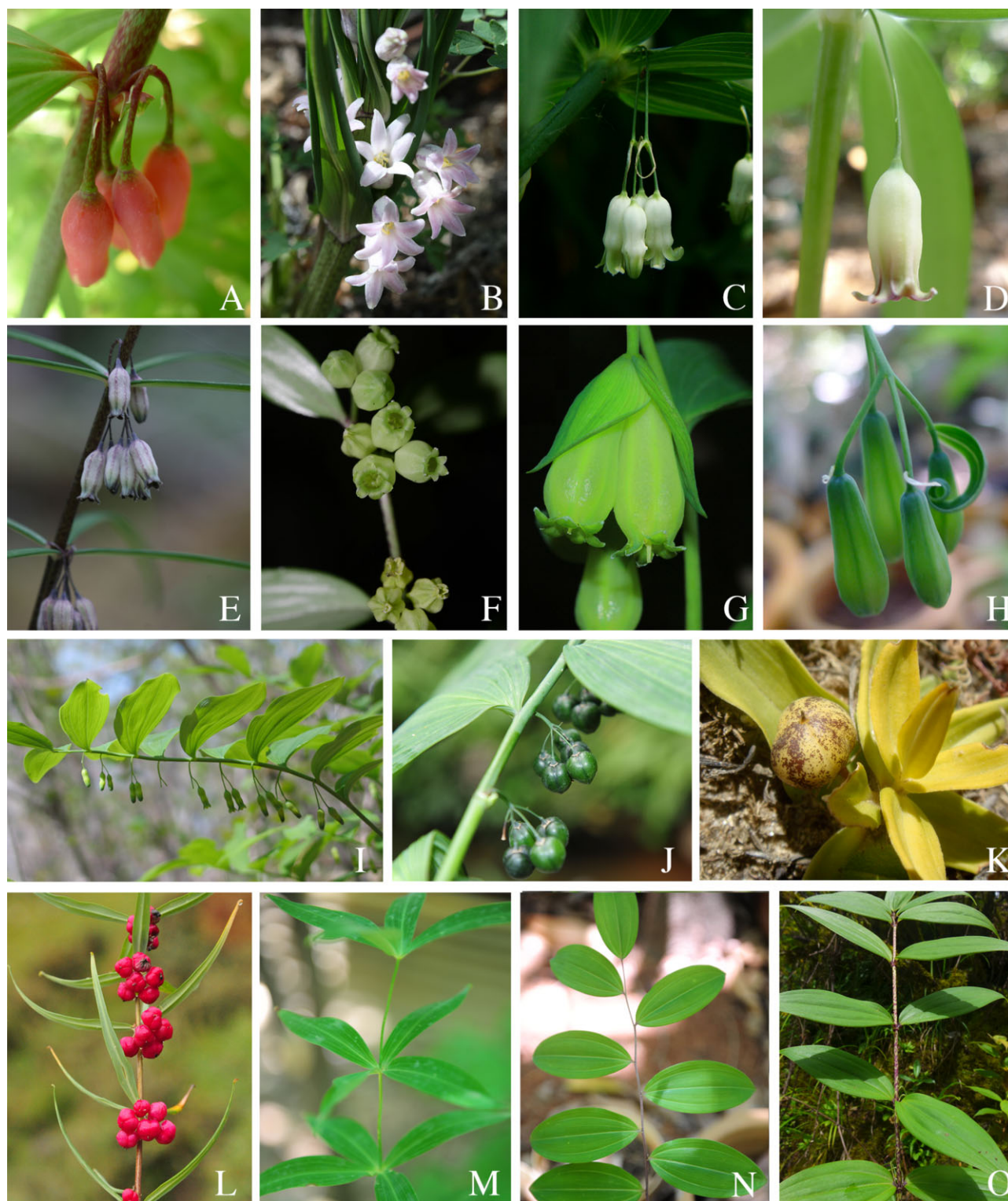
1978). Tang (1978) provided a classification, recognizing eight series for the genus (Table 1). The most recent and widely accepted classification is by Tamura (1993), who subdivided the genus into two sections, section *Polygonatum* and section *Verticillata* Baker, based on karyological and micromorphological characters of staminal filaments (Tamura, 1993; Tamura *et al.*, 1997b). Recognition of two sections was also supported by random amplified polymorphic DNA (RAPD) data (Szczecinska *et al.*, 2006). Species of section *Polygonatum* have alternate leaves, stout staminal filaments, perforated pollen exines,  $x = 9, 10$  or 11 as the base chromosome number, and karyotypes mainly consisting of metacentric and submetacentric chromosomes. Species of section *Verticillata* have mostly opposite or verticillate leaves, slender filaments, mostly reticulate pollen exines,  $x = 14$  or 15 as the base chromosome number, and karyotypes primarily consisting of long subtelocentric and short metacentric chromosomes. Molecular data based on restriction site mapping of the plastid gene *trnK* (Tamura *et al.*, 1997b) supported the monophyly of section *Polygonatum*, but there were disagreements concerning the series concepts in the genus.

Leaf arrangement has been emphasized in the infrageneric classifications of *Polygonatum* (Table 1). One factor that complicates the taxonomy and classification of *Polygonatum* is that the leaf arrangement varies greatly, which has led various botanists to place some species in different infrageneric groups (Abramova, 1975; Tang, 1978; Tamura *et al.*, 1997b). For example, *P. prattii* Baker usually has alternate leaves, but sometimes it possesses opposite leaves on the proximal part of the stem or three whorled leaves on the apical part of the stem. This species was thus placed in section *Oppositifolia* by Baker (1875), but then in series *Verticillata* by Tang (1978). Phyllotaxy is an important component of growth form in

**Table 1.** Major classifications of *Polygonatum*; number and taxonomic ranks of proposed groups are listed with the morphological characters used to define the infrageneric taxa

	Characters used	Alternate	Verticillate	Opposite
Baker (1875)	Phyllotaxis	<i>Alternifolia</i>	<i>Verticillata</i>	<i>Oppositifolia</i>
Three sections				
Tang (1978)	Phyllotaxis, size and texture of bracts, size and number of flowers, and the proportion of the connate to the free part of the tepals	<i>Altelobata*</i> <i>Alternifolia</i> <i>Bracteata</i> <i>Punctata</i>	<i>Hookeriana</i> <i>Kingiana</i> <i>Verticillata</i>	<i>Oppositifolia</i>
Eight series				
Tamura (1993)	Karyological and micro-morphological characters of staminal filaments	<i>Polygonatum</i> (series <i>Polygonatum</i> ) (series <i>Bracteata</i> ) (series <i>Inflata</i> )	<i>Verticillata</i>	
Two sections and three series				

\*Transferred to *Heteropolygonatum* by Chao *et al.* (2013).



**Figure 1.** Morphological diversity in *Polygonatum*. A–I, flowers; J–L, fruits; M–O, phyllotaxy. A, *P. kingianum*; B, *P. verticillatum*; C, *P. sibiricum*; D, *P. prattii*; E, *P. cirrhifolium*; F, *P. punctatum*; G, *P. involucratum*; H, *P. cyrtonema*; I, *P. biflorum*; J, *P. odoratum*; K, *P. hookeri*; L, *P. cirrhifolium*; M, *P. sibiricum*; N, *P. cyrtonema*; O, *P. cathcartii*.

*Polygonatum*; hence, transitions in leaf arrangement are important for understanding the evolution of the genus.

Previous phylogenetic studies (Bush, 1927; Shinwari *et al.*, 1994; Tamura *et al.*, 1997b; Jang & Pfosser, 2002; Deng, Wang & He, 2009) have primarily focused on relationships at the tribal level with only limited sampling of *Polygonatum* (Tamura *et al.*, 1997b; Jang & Pfosser, 2002). There remain many gaps in our understanding of the infrageneric classification and evolutionary relationships in the genus. A robust estimate of phylogenetic relationships for the genus is needed based on extensive sampling, especially from its diversification centre in south-western China.

We here provide the first and most comprehensive phylogenetic analysis of *Polygonatum* using data from two plastid coding regions, *rbcL* and *trnK* (*matK*), and two non-coding regions, *psbA-trnH* and *trnC-petN*. We test the monophyly of *Polygonatum*, reconstruct phylogenetic relationships and examine the character evolution of phyllotaxis in the genus.

## MATERIAL AND METHODS

### TAXON SAMPLING, DNA EXTRACTION AND SEQUENCING

In total, 129 accessions representing 30 *Polygonatum* species were sampled in our analyses (Appendix). We followed the species concept of Chen & Tamura (2000). Our sampling covered the geographical diversity of the genus (25 of the 50 recognized species in eastern Asia, three of the five species in Europe and both species in North America) and represents a wide range of morphological diversity with representative samples from both sections of Tamura *et al.* (1997b) and all eight series of Tang (1978). To test the monophyly of *Polygonatum* with all available data, a number of sequences from GenBank were combined in our dataset (Appendix). Some widespread taxa were represented by multiple accessions. To assess the monophyly of *Polygonatum*, we also sampled representative taxa from other genera in Polygonateae (*Heteropolygonatum*, *Disporopsis* and *Maianthemum*). Taxa of other tribes in Asparagaceae (e.g. *Ophiopogon* Ker Gawl., *Convallaria* L., *Reineckea* Kunth and *Calibanus* Rose) were selected as outgroups based on previous broader analyses (Rudall *et al.*, 2000; Kim *et al.*, 2010; Chen *et al.*, 2013).

Genomic DNA were extracted from approximately 15 mg silica-gel dried leaf material using the modified CTAB method (Doyle & Doyle, 1987) or the DNeasy plant mini kits (BioTeke Corporation) following the manufacturer's protocol. Polymerase chain reaction (PCR) amplifications were performed using 10 ng

genomic DNA, 4 pmol each primer, 0.5 U Taq polymerase (Promega) and 2.5 mM MgCl<sub>2</sub> in a volume of 20 µL under the following conditions: 3 min at 95 °C, followed by 30 cycles of 20 s at 94 °C, 30 s at 50 °C and 40 s at 72 °C, and then a final 5-min extension at 72 °C. Amplifications were carried out in an Eppendorf Mastercycler (Perkin-Elmer). Four plastid regions were amplified and sequenced using standard primers (Lee & Wen, 2004; Meng *et al.*, 2008), which have been applied in phylogenetic studies on related taxa in Asparagales (Fay *et al.*, 2000; Kim & Lee, 2007; Meng *et al.*, 2008; Chen *et al.*, 2013). The amplification protocol and primers for amplifying *rbcL*, *trnK* and *psbA-trnH* were described by Meng *et al.* (2008). The *trnK* region is partial, including partial *trnK* 5' intron, complete *matK* gene and partial *trnK* 3' intron. An internal primer (ATCAAGAATATCA GAATCCGA) was designed for sequencing of *trnK*. The *trnC-petN* sequences were amplified according to Lee & Wen (2004).

The PCR products were purified using the polyethylene glycol (PEG) precipitation procedure. Sequencing was carried out in 35 cycles of 97 °C for 15 s, 50 °C for 5 s and 60 °C for 4 min. The products of the cycle sequencing reactions were cleaned using Sephadex columns (Amersham Pharmacia Biotech) and dried at 60 °C in a rotary vacuum evaporator. Sequences were generated on an ABI Prism 3730 capillary sequencer (Applied Biosystems). Sequence alignment was performed by MAFFT version 6 using the default alignment parameters (Kato & Toh, 2008) followed by manual adjustment in Se-AL v2.0a11 (Rambaut, 2002).

### PHYLOGENETIC ANALYSIS

Phylogenetic analyses were performed using Bayesian and maximum-likelihood (ML) methods. Bayesian inference was conducted using MrBayes version 3.2.1 (Ronquist & Huelsenbeck, 2003). Models of nucleotide substitution were selected based on the Akaike Information Criterion (AIC) as determined by MrModelTest 2.3 (Nylander, 2004). Independent model tests were performed on each marker and model parameters (statefreq, revmat and shape) were unlinked between partitions. The Bayesian Markov chain Monte Carlo (MCMC) algorithm was run for 10 000 000 generations with four incrementally heated chains starting from random trees and sampling one out of every 1000 generations. The burn-in and convergence diagnostics were graphically assessed using AWTY (Nylander *et al.*, 2008). The trees sampled from the burn-in stage were excluded, and the remaining trees were assumed to be representative of the posterior probability (PP) distribution. Internodes with PP > 0.95 were considered

statistically significant. The ML trees were inferred with RAxML 7.6.3 (Stamatakis, 2006). Data were also partitioned into gene regions, allowing for independent parameter estimates on each partition; branch length estimates were optimized across all gene regions. We used the rapid bootstrapping algorithm to perform 1000 bootstrap replicates. The analyses employed a GTRCAT model for each partition followed by a final parameter optimization step using the GTR substitution distribution and  $\Gamma$  distribution of among-site rate variation.

#### CHARACTER EVOLUTION OF LEAF ARRANGEMENT

To understand the evolution of leaf arrangement, character state changes of phyllotaxy were reconstructed using Bayesian, parsimony and likelihood methods. A reduced dataset including 46 representative taxa from the tribe was sampled in the character evolution analyses. Species with widespread distribution were represented by multiple accessions, such as *P. cirrhifolium* (Wall.) Royle. We coded leaf arrangement based on herbarium and literature studies (Ownbey, 1944; Abramova, 1975; Tang, 1978; Jeffrey, 1980; Tamura *et al.*, 1997b; Chen & Tamura, 2000; Judd, 2003). Three states of phyllotaxy were coded: alternate (0), verticillate (1) and opposite (2). In case of some species with more than two character states, we coded the character state based on their dominant status. For example, the leaf arrangement for *P. prattii* was coded as alternate because it usually has alternate leaves but only possesses opposite or three-whorled leaves on the upper part of the stem occasionally. For *P. franchetii* Hua, its leaves were described as alternate in Tang (1978), but alternate or sometimes opposite or in whorls of three in Jeffrey (1980). However, we noted that our specimens collected from the field were mostly verticillate, sometimes with a few alternate leaves; the species was thus coded as verticillate in the analyses.

The Bayesian analysis uses a standard continuous-time Markov chain (CTMC) (Lemey *et al.*, 2009) that integrates phylogenetic uncertainty and Markov model parameter uncertainty (Sanmartín, Van der Mark & Ronquist, 2008). Each taxon can be allocated to a character state corresponding to the leaf arrangement of each species here. For each node, CTMC reconstructs the probability distribution for the different states. The Bayesian CTMC method was implemented in BEAST 1.8.0 using a 20 million MCMC chain length, saving trees every 10 000 generations (Drummond & Rambaut, 2007). We used an uncorrelated lognormal relaxed-clock model with a Yule tree prior (Drummond *et al.*, 2006) and a GTR + I +  $\Gamma$  model. We used TRACER 1.5 to check for convergence between the runs (Rambaut & Drummond, 2007) and TREEANNOTATOR 1.8.0 (Drummond & Rambaut, 2007) to build a maximum clade credibility tree from this posterior distribution of trees.

The parsimony (unordered model) and likelihood (Markov k-state one-parameter model) methods were run in Mesquite 2.75 (Maddison & Maddison, 2011). To account for topological uncertainty, we used the 'trace character over trees' option, which summarizes the ancestral states over a series of trees. All reconstructions were integrated over the last 8000 post-burn-in trees of the previous BEAST analysis and the ancestral states were summarized on the consensus tree.

#### RESULTS

The aligned combined dataset for the four loci (*rbcl*, *trnK*, *psbA-trnH* and *trnC-petN*) contained 158 accessions, including 129 *Polygonatum* taxa. The statistics related to the four plastid DNA regions are summarized in Table 2. With the exception of *psbA-trnH* (7.1% variable characters), all the DNA fragments yielded a similar percentage of variable characters

**Table 2.** Statistical summary of the plastid DNA regions used to infer phylogenetic relationships in *Polygonatum* and related taxa

	<i>rbcl</i>	<i>trnK</i>	<i>psbA-trnH</i>	<i>trnC-petN</i>	Combined dataset
No. of sequences (ingroup/outgroup)	118/29	123/27	115/20	93/16	129/29
No. of missing taxa	11	8	23	49	
Alignment length (bp)	1450	1857	729	990	5026
No. of variable characters: ingroup/all (%)	56/145 (3.8/10)	90/311 (4.8/16.7)	24/52 (3.2/7.1)	57/106 (5.7/10.7)	227/614 (4.5/12.2)
No. of potentially parsimony-informative characters: ingroup/all (%)	23/64 (1.6/4.4)	51/132 (2.7/7.1)	17/27 (2.3/3.7)	31/53 (3.1/5.4)	122/276 (2.4/5.5)
Substitution model	HKY+I + $\Gamma$	GTR+ $\Gamma$	HKY+I + $\Gamma$	GTR+I + $\Gamma$	

**Figure 2.** Bayesian 50% majority rule consensus tree of *Polygonatum* (black) and closely related taxa (grey) in Asparagaceae, with ML tree shown on the left below. The trees are based on a combined dataset of four plastid sequences (*rbcL*, *trnK*, *psbA-trnH* and *trnC-petN*). Posterior probabilities > 0.5 are shown above the branches and ML bootstrap supports > 50 below. Bold branches show the backbone leading to the three clades (I–III). Section and series are shown according to Tamura (1993) and Tang (1978), respectively. The series names of Tang (1978) are abbreviated as follows: A, *Alternifolia*; B, *Bracteata*; H, *Hookeriana*; K, *Kingiana*; O, *Oppositifolia*; P, *Punctata*; V, *Verticillata*.

(10.0% for *rbcL* to 16.7% for *matK*) and the combined matrix included 12.2% variable characters (Table 2).

Bayesian MCMC for the four combined loci reached stationarity at 5000 000 generations. The ‘compare’ plot produced by AWTY suggested that parallel MCMC runs achieved topological convergence. The ML analysis produced a similar topology to the Bayesian tree. The Bayesian consensus tree with PP and ML bootstrap (BS) values with the ML topology is shown in Figure 2.

The monophyly of *Polygonatum* was strongly supported in our analyses (PP = 1.00, BS = 88, Fig. 2). Species of *Heteropolygonatum* [*H. altelobatum* (Hayata) Y.H.Tseng, H.Y.Tzeng & C.T.Chao, *H. roseolum* M.N.Tamura & Ogisu and *H. ginfushanicum* (F.T.Wang & T.Tang) M.N.Tamura, S.C.Chen & Turland] formed a well-supported sister clade to the monophyletic *Disporopsis*. Our data supported three major clades in *Polygonatum*, which were largely correlated with regional distributions and morphological characters. As shown in Figure 2, clade I was supported with PP = 1.00 and BS = 61, including all species of section *Verticillata* (except for *P. sibiricum*) and section *Oppositifolia*. Species in this clade are mainly from south-western to southern China and adjacent regions. Clade II includes all species from section *Polygonatum* and was sister to *P. sibiricum* (clade III) of section *Verticillata* with support of PP = 1.00 and BS = 60 (Fig. 2). The New World species *P. biflorum* (Walter) Elliott and *P. pubescens* (Willd.) Pursh were nested in section *Polygonatum*, showing a close relationship to *P. filipes* Merr. ex C. Jeffrey & McEwan and *P. desoulavii* Komarov (Fig. 2).

The results of the ancestral state reconstruction of leaf arrangement are shown in Figure 3. The reconstruction yielded no conflicts with parsimony, likelihood and Bayesian analyses, except for the relationships among some deep nodes. All analyses suggested the alternate leaf arrangement as the ancestral state for the stem *Polygonatum* node or the crown tribe node (Fig. 3). The parsimony reconstruction usually produced greater uncertainty than the other two approaches (Fig. 3). For example, the parsimony analysis reconstructed higher ambiguous (equivocal) origins of the crown *Polygonatum* with both alternate and verticillate leaves, whereas the likelihood analyses (1.00 versus 0.83, Fig. 3B, C) and

Bayesian CTMC strongly supported alternate leaves as the most likely ancestral state (Fig. 3A).

## DISCUSSION

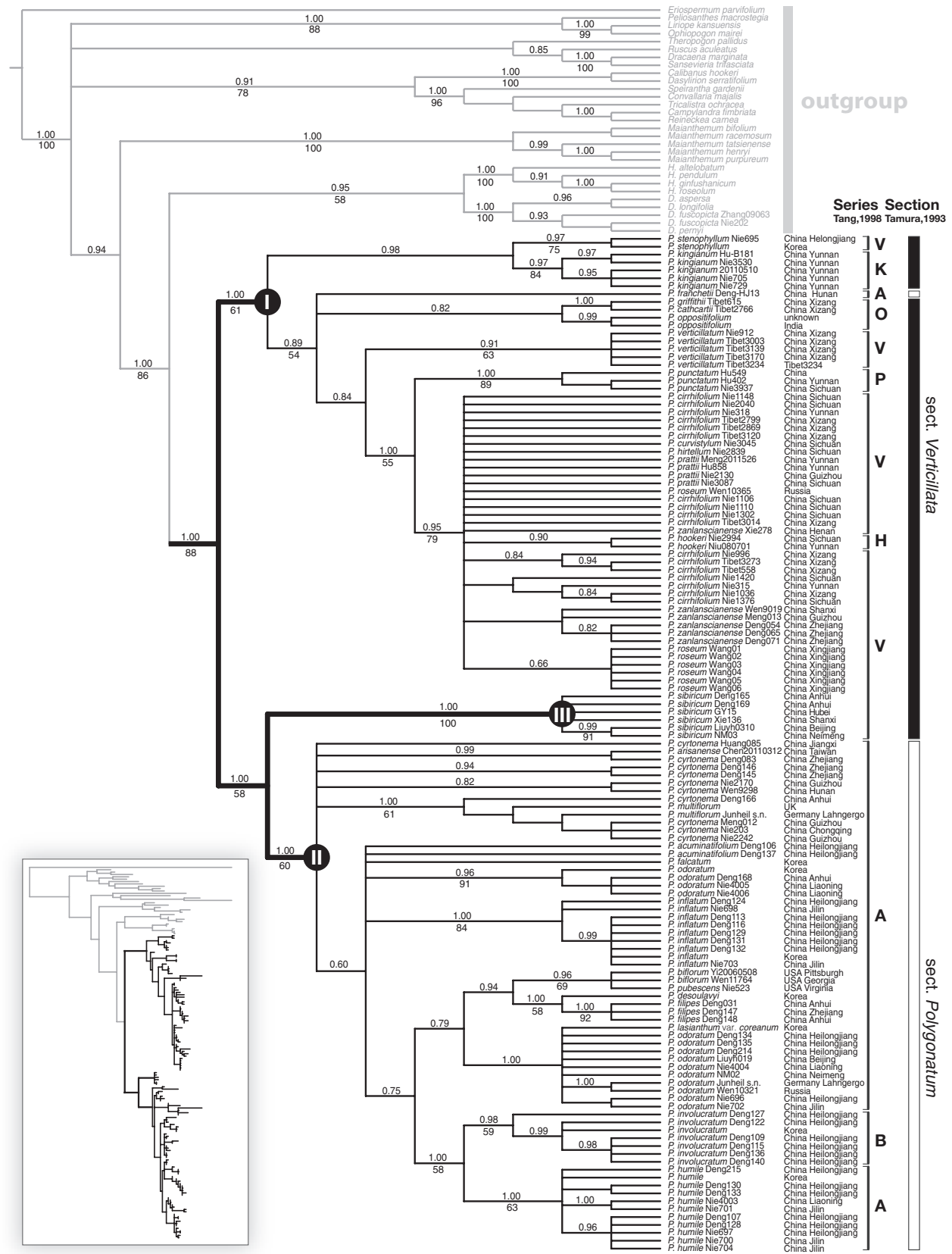
### GENERIC DELIMITATION OF *POLYGONATUM*

The monophyly of *Polygonatum* was robustly supported by the present analyses based on a comprehensive sampling scheme (PP = 1.00, BS = 88, Fig. 2), in agreement with previous studies (Tamura *et al.*, 1997b; Lü *et al.*, 2000). Synapomorphies of the genus include reduced axillary inflorescences and pendulous flowers with strongly connate tepals forming a tubular to urceolate and more or less campanulate perianth (Judd, 2003).

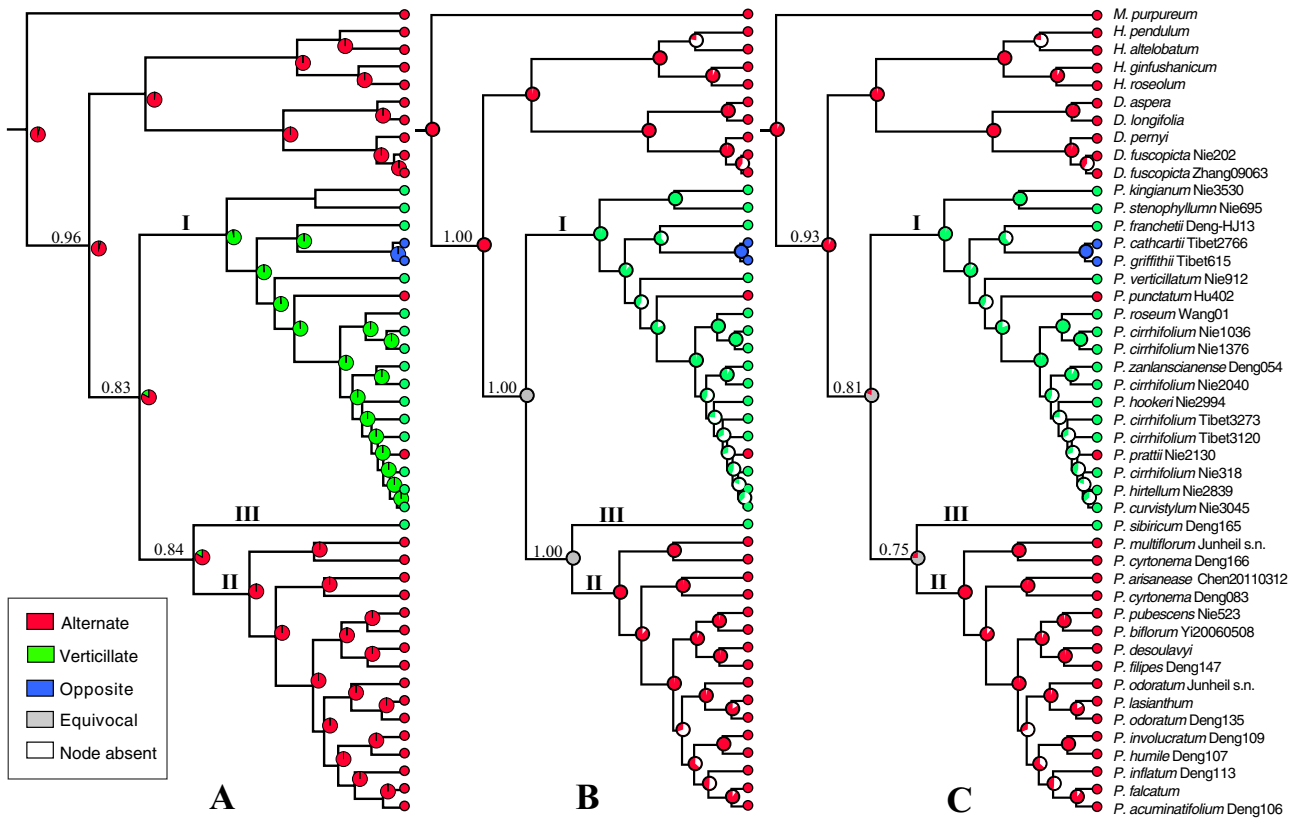
Phylogenetic relationships among *Polygonatum*, *Heteropolygonatum* and *Disporopsis* were not resolved in the previous study (Tamura *et al.*, 1997b). Our analyses placed *Polygonatum* as sister to a clade including *Heteropolygonatum* and *Disporopsis* (PP = 1.00, BS = 86, Fig. 2), supporting the recognition of *Heteropolygonatum* as a genus distinct from *Polygonatum*. All *Heteropolygonatum* species were previously placed in *Polygonatum* (Tang, 1978), but Tamura *et al.* (1997a) pointed out that *Heteropolygonatum* differs from *Polygonatum* with the former having stamens that are usually dimorphic (with the outer ones short and the inner ones long), overlapping tepals, both terminal and axillary inflorescences, epiphytic growth and a basic chromosome number  $x = 16$  (Yamashita & Tamura, 2000a). In addition, our data also supported the transfer of *Polygonatum altelobatum* to *Heteropolygonatum* (Chao *et al.*, 2013). This species is an epiphytic herb with stamens that are dimorphic in length and both terminal and axillary inflorescences (Chao *et al.*, 2013).

### INFRAGENERIC RELATIONSHIPS

Three major lineages (clades I–III) are recognizable in *Polygonatum* (Fig. 2), which are largely congruent with the sectional classification on the basis of morphology, cytology and biogeographical evidence (Baker, 1875; Jeffrey, 1980, 1982; Tamura, 1993). For instance, Jeffrey (1980) divided the eastern Asian taxa into a southern group usually with verticillate leaves and purple flowers and a northern group with alternate leaves and greenish white or yellow flowers.



**Figure 2.** See caption on previous page.



**Figure 3.** Ancestral state reconstructions of leaf arrangement for *Polygonatum* and its allied taxa based on Bayesian (A), parsimony (B) and maximum-likelihood (C) methods. Numbers above branches show the support of the most likely ancestral state of leaf arrangement.

Jeffrey's groupings largely correspond to clades I (the southern group) and II+III (the northern group) in Figure 2 in our analyses.

Clade I includes most *Polygonatum* species centred in southern to south-western China, with some species extending into Europe and Central Asia [*P. verticillatum* (L.) All. and *P. roseum* (Ledeb.) Kunth]. The only exception is *P. stenophyllum* Maxim., endemic to north-eastern Asia, probably indicating a northward dispersal in eastern Asia. This group is characterized by the combination of opposite or verticillate leaves, purple or pink flowers, red berries, slender filaments, mostly reticulate pollen exines and a base chromosome number  $x = 14$  or  $15$  (Jeffrey, 1982; Tamura, 1993). All but one of species (*P. sibiricum*) that have been assigned to sections *Verticillata* and *Oppositifolia* as proposed by Baker (1875) and Tamura (1993) belong to this clade.

According to Tang's (1978) classification, species with verticillate leaves belong to series *Kingiana*, Y.C.Tang, *Hookeriana* Y.C.Tang, *Oppositifolia* and *Verticillata*. However, our results did not support recognition of series of Tang (1978); for example, the large series *Verticillata* is clearly polyphyletic (see

Fig. 2). The karyological results also indicated that Tang's (1978) series concepts for the genus are problematic (Tamura, 1993). Except for a few species (such as *P. stenophyllum* and *P. verticillatum*), most species of series *Verticillata* and series *Hookeriana* formed a large polytomy (PP = 0.95, BS = 79, Fig. 2). Taxa from this large unresolved clade display a high degree of morphological variation and species delimitations in this lineage are particularly problematic (Tang, 1978; Chen & Tamura, 2000). Members of this group are mostly distributed from south-western China to the Himalayas and may represent a radiation of *Polygonatum* in this region. Evolutionary radiations have been commonly reported in plants of the Sino-Himalayan region studied so far (e.g. Zhou *et al.*, 2013; Wen *et al.*, 2014; Zhang *et al.*, 2014).

All taxa with typical alternate leaves formed a well-supported group corresponding to section *Polygonatum* recognized by Baker (1875) and Tamura (1993) (clade II, Fig. 2). The monophyly of this section is supported by morphological and karyological characters, such as stout staminal filaments, perforated pollen exines and a chromosome base number  $x = 9$ , 10 or 11 (Tamura, 1993). This group also shares



greenish-white or yellow flowers and blue–black berries. The group includes some widely distributed species, including *P. odoratum* (Mill.) Druce extending into south-western China and Europe, but most species are restricted to central to north-eastern China and north-eastern Asia. According to Jeffrey (1980), clade II belongs to the ‘northern’ group, mainly in the north-eastern part of the Sino-Japanese region, with some species endemic to central China (e.g. *P. cyrtoneura* and *P. filipes*) and two species (*P. biflorum* and *P. pubescens*) from North America. Phylogenetic relationships in this group are still unclear, and the group probably represents a second radiation of the genus in Asia.

Our phylogenetic results also suggested that series *Alternifolia* Baker of Tang (1978) is not monophyletic (Fig. 2). First, one of its members, *P. franchetii*, is placed in the verticillate group (clade I). Leaves of this species are usually opposite or in whorls of three (Jeffrey, 1980). Second, series *Bracteata* Kom. ex L.Abr. (represented by *P. involucreatum* (Franch. & Sav.) Maxim. in Fig. 2) is nested in the alternate group. This series is characterized by large and leaf-like bracts in *Polygonatum* (Fig. 1G), and leaves of taxa in series *Bracteata* are always alternate.

Clade III comprises only one species, *P. sibiricum*, with typical verticillate leaves (Fig. 1C), and is sister to clade II, the alternate group, with moderate support (PP = 1.00, BS = 58, Fig. 2), rather than to the verticillate group of clade I. *Polygonatum sibiricum* is widely distributed from central to northern China, Korea, Mongolia and Siberia. It frequently grows on sandy substrates and appears to be more tolerant of drier grassland or scrub conditions than the other species (Tang, 1978; Jeffrey, 1980). In addition, *P. sibiricum* has a base chromosome number  $x = 11, 12$  or  $13$  and black berries, differing from most species of section *Verticillata* (Therman, 1953; Wang, Li & Li, 1987; Ge & Li, 1989; Han *et al.*, 1998). A new chromosome base number of  $x = 15$  was reported from Sichuan province of China (Deng *et al.*, 2009) for the species, but this species is not distributed in that province; thus, the chromosome report is highly questionable. Our results indicate that *P. sibiricum* represents an independent evolutionary lineage, distinct from sections *Verticillata* and *Polygonatum*.

#### EVOLUTION OF LEAF ARRANGEMENT

Bayesian CTMC analyses suggested alternate leaf arrangement as the most likely ancestral state (0.83, Fig. 3A), although the parsimony and likelihood methods inferred an equivocal state for the crown *Polygonatum* (1.00, Fig. 3B; 0.81, Fig. 3C). Nevertheless, the alternate leaf arrangement was suggested as the ancestral state for the stem lineage of the genus in

all the analyses (Fig. 3), which is consistent with an alternate origin of *Polygonatum* phyllotaxy. Therefore, it seems that whorls of more than three leaves are derived in *Polygonatum* (Fig. 3). Jang & Kim (1998) also suggested a similar evolutionary trend from alternate leaves to verticillate leaves in *Polygonatum*.

The hypothesis that whorled or opposite leaves are usually derived from alternate ones has often been explained as a variety of asynchronous whorls (Rutishauser, 1999). In growth whorls, the whorled appearance is the result of an uneven distribution of internode elongation. The leaves are initiated along a spiral, and the  $k$ -merous growth whorls are formed when every  $k$ th internode elongates and all other internodes stay short (Kwiatkowska, 1995, 1999). This type of asynchronous leaf whorl often arises in monocots and some dicots, such as *Anagallis* L., *Euphorbia* L., *Fritillaria* L., *Lilium* L., *Peperomia* Ruiz & Pav. and *Polygala* L. (Rutishauser, 1999).

However, as shown in Figure 3, the leaf arrangement is evolutionarily labile or homoplasious in *Polygonatum*. Whorled leaves have arisen from the alternate state at least twice: once in leading to the large group of clade I and the other in *P. sibiricum* of clade III (Figs 2, 3). By contrast, verticillate leaves has reversed to alternate leaves at least twice independently, including in *P. punctatum* Royle ex Kunth and *P. prattii*, which have mainly alternate leaves but are deeply nested in the verticillate group (Fig. 3). These species with derived alternate leaves sometimes also persist with verticillate or opposite leaves. It is noteworthy that many typically verticillate species (e.g. *P. cirrhifolium*, *P. curvistylum* Hua, *P. roseum* and *P. verticillatum*) sometimes have alternate leaves, which further supports the labile state of the leaf arrangement in *Polygonatum*.

#### NOMENCLATURE TREATMENT

Evidence from our molecular analyses shows that *P. sibiricum* is distinct from sections *Polygonatum* or *Verticillata sensu* Tamura (1993). Therefore, we remove the species from section *Verticillata* as its own section, and the new combination is provided below.

*Polygonatum* section *Sibirica* (L.I.Abramova) Y.Meng, comb. nov. Basionym: *P.* series *Sibirica* L.I.Abramova, *Bot. Zhurn.* 60: 495. 1975. Type species: *P. sibiricum* Redouté.

Leaves in whorls of usually three to six, glaucous beneath and occasionally scaberulous on the veins. Flowers axillary, pendulous, white; peduncles arcuate-deflexed, usually two- to four-flowered; pedicels subtended at the base by small but conspicuous persistent scarious bracts; perianth cylindrical, more or less constricted. Berries always black, seeds four to seven.  $2n = 22, 24, 26$ .

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## APPENDIX

Species names and authorities, herbarium vouchers and geographical locality for the material included in this study. GenBank accession numbers are given for the four markers sequenced: *rbcL*, *trnK*, *psbA-trnH* and *trnC-petN*. Accessions beginning with KJ are new sequences published in this study; missing data are indicated by a dash. Herbarium acronyms follow <http://sciweb.nybg.org>.

Taxon	Voucher/Reference	Locality	<i>rbcL</i>	<i>trnK</i>	<i>psbA-trnH</i>	<i>trnC-petN</i>
<i>Calibanus hookeri</i> (Lem.) Trel.	Kim <i>et al.</i> (2010)	–	HM640472	HM640585	–	–
<i>Campylandra fimbriata</i> (Hand.-Mazz.) M.N.Tamura, S.Yun Liang & Turland	Kim <i>et al.</i> (2010)	–	HM640448	HM640562	–	–
<i>Convallaria majalis</i> L.	Z.-L.Nie 201 (KUN)	China: Heilongjiang	KJ745528	KJ745641	KJ745890	KJ745987
<i>Dasyllirion serratifolium</i> Baker	Kim <i>et al.</i> (2010); Yamashita & Tamura (2000b)	–	AB029847	HM640587	–	–
<i>Disporopsis aspersa</i> (Hua) Engl.	H.Li 22773 (KUN)	China: Yunnan	KJ745529	–	KJ745784	KJ745928
<i>Disporopsis fuscopicta</i> Hance	Z.-L.Nie 202 (KUN)	China: Chongqing	KJ745521	KJ745724	KJ745778	KJ745937
	J.-W.Zhang 09063 (KUN)	China: Chongqing	KJ745530	–	KJ745843	–
<i>Disporopsis longifolia</i> Craib	Z.-L.Nie 2362 (KUN)	China: Guangxi	KJ745582	KJ745662	KJ745836	KJ745944
<i>Disporopsis pernyi</i> (Hua) Diels	–	–	HM640452	HM640566	–	–
<i>Dracaena</i> sp.	Kim <i>et al.</i> (2010)	–	HM640469	HM640582	GQ435171	–
<i>Eriospermum parvifolium</i> Jacq.	Kim <i>et al.</i> , 2010	–	HM640477	HM640591	–	–
<i>Heteropolygonatum</i> <i>altelobatum</i> (Hayata) Y.H.Tseng, H.Y.Tzeng & C.T.Chao	J.-D.Chao 1411 (TCF)	China: Taiwan	KJ745592	KJ745742	KJ745789	KJ745975
<i>Heteropolygonatum</i> <i>ginfushanicum</i> (F.T.Wang & T.Tang) M.N.Tamura, S.C.Chen & Turland	Z.-L.Nie 225 (KUN)	China: Chongqing	KJ745621	KJ745698	KJ745782	KJ745938
<i>Heteropolygonatum pendulum</i> (Z.G.Liu & X.H.Hu) M.N.Tamura & Ogisu	Yamashita & Tamura (2000b)	–	AB029831	AB029764	–	–
<i>Heteropolygonatum roseolum</i> M.N.Tamura & Ogisu	Z.-L.Nie 4077 (KUN)	China: Yunnan	KJ745527	KJ745713	KJ745790	KJ745939
<i>Liriope kansuensis</i> (Batalin) C.H.Wright	G.-W.Hu Z890 (KUN)	China: Jiangxi	KJ745561	KJ745648	KJ745794	KJ745986
<i>Maianthemum bifolium</i> (L.) F.W.Schmidt	Y.Meng 211 (KUN)	China: Jilin	KJ745531	KJ745660	KJ745793	KJ745910
<i>Maianthemum henryi</i> (Baker) LaFrankie	Wen 9017 (US)	China: Shanxi	KJ745620	KJ745714	KJ745792	–
<i>Maianthemum purpureum</i> (Wall.) LaFrankie	Z.-L.Nie 306 (KUN)	China: Yunnan	KJ745532	KJ745767	KJ745773	KJ745909
<i>Maianthemum racemosum</i> (L.) Link	Wen 8562 (US)	USA: Virginia	KJ745533	KJ745667	KJ745772	KJ745906
<i>Maianthemum tatsienense</i> (Franch.) LaFrankie	Z.-L.Nie 2099 (KUN)	China: Sichuan	KJ745583	KJ745679	KJ745797	KJ746010

APPENDIX *Continued*

Taxon	Voucher/Reference	Locality	<i>rbcL</i>	<i>trnK</i>	<i>psbA-trnH</i>	<i>trnC-petN</i>
<i>Ophiopogon mairei</i> H.Léveillé	Z.-L.Nie 2372 (KUN)	China: Guangxi	KJ745600	KJ745642	KJ745795	KJ745988
<i>Peliosanthes macrostegia</i> Hance	Z.-L.Nie 3242 (KUN)	China: Yunnan	KJ745599	KJ745647	KJ745796	KJ745904
<i>Polygonatum acuminatifolium</i> Komarov	T. Deng 137 (KUN)	China: Heilongjiang	KJ745634	KJ745704	KJ745864	KJ745984
	T. Deng 106 (KUN)	China: Heilongjiang	KJ745576	KJ745703	KJ745863	KJ746006
<i>Polygonatum arisanense</i> Hayata	Chen20110312 (PE)	China: Taiwan	–	KJ745675	–	KJ745967
<i>Polygonatum biflorum</i> (Walter) Elliott	T.S.Yi 20060508_41 (US)	USA: Pennsylvania	KJ745562	KJ745725	KJ745897	KJ745973
	Wen 11764 (US)	USA: Georgia	KJ745628	KJ745682	KJ745855	KJ746003
<i>Polygonatum cathcartii</i> Baker	Tibet-MacArthur 2766 (US)	China: Xizang	KJ745594	KJ745753	KJ745842	–
<i>Polygonatum cirrhifolium</i> (Wall.) Royle	Z.-L.Nie 1110 (KUN)	China: Sichuan	KJ745518	KJ745686	KJ745808	KJ745914
	Z.-L.Nie 1106 (KUN)	China: Sichuan	KJ745515	KJ745764	KJ745824	KJ745917
	Tibet-MacArthur 558 (US)	China: Xizang	KJ745539	KJ745733	KJ745895	KJ745919
	Tibet-MacArthur 3234 (US)	China: Xizang	KJ745598	KJ745687	KJ745834	KJ745942
	Z.-L.Nie 2040 (KUN)	China: Sichuan	KJ745633	KJ745740	KJ745819	KJ745945
	Z.-L.Nie 315 (KUN)	China: Yunnan	–	KJ745665	KJ745780	KJ745989
	Z.-L.Nie 318 (KUN)	China: Yunnan	KJ745522	KJ745734	KJ745783	KJ745990
	Z.-L.Nie 996 (KUN)	China: Xizang	KJ745591	KJ745692	KJ745810	KJ745991
	Z.-L.Nie 1148 (KUN)	China: Sichuan	KJ745610	KJ745754	KJ745801	KJ746004
	Tibet-MacArthur 3014 (US)	China: Xizang	KJ745537	KJ745741	KJ745774	–
	Z.-L.Nie 1420 (KUN)	China: Sichuan	KJ745602	KJ745737	KJ745788	–
	Z.-L.Nie 1376 (KUN)	China: Sichuan	KJ745597	KJ745670	KJ745804	–
	Z.-L.Nie 1036 (KUN)	China: Xizang	KJ745601	KJ745729	KJ745816	–
	Z.-L.Nie 1302 (KUN)	China: Sichuan	KJ745629	–	KJ745826	–
	Tibet-MacArthur 3120 (US)	China: Xizang	KJ745563	KJ745730	KJ745838	–
	Tibet-MacArthur 2869 (US)	China: Xizang	KJ745536	KJ745756	KJ745839	–
	Tibet-MacArthur 3273 (US)	China: Xizang	KJ745538	KJ745728	KJ745893	–
Tibet-MacArthur 2799 (US)	China: Xizang	KJ745535	KJ745766	KJ745901	–	
<i>Polygonatum curvistylum</i> Hua	Z.-L.Nie 3045 (KUN)	China: Sichuan	KJ745580	KJ745666	KJ745802	KJ745912

APPENDIX *Continued*

Taxon	Voucher/Reference	Locality	<i>rbcL</i>	<i>trnK</i>	<i>psbA-trnH</i>	<i>trnC-petN</i>	
<i>Polygonatum cyrtonema</i> Hua	Z.-L.Nie 203 (KUN)	China: Chongqing	KJ745540	KJ745718	KJ745775	KJ745947	
	T. Deng 146 (KUN)	China: Zhejiang	KJ745523	KJ745644	KJ745785	KJ745949	
	Y.Meng 012 (KUN)	China: Guizhou	KJ745611	KJ745761	KJ745878	KJ745958	
	Z.-L.Nie 2242 (KUN)	China: Guizhou	KJ745574	–	KJ745879	KJ745959	
	T. Deng 145 (KUN)	China: Zhejiang	KJ745569	KJ745645	KJ745884	KJ745960	
	W.-P.Huang 085 (KUN)	China: Jiangxi	–	KJ745654	KJ745887	KJ745961	
	T. Deng 083 (KUN)	China: Zhejiang	KJ745590	KJ745643	KJ745888	KJ745962	
	T. Deng 166 (KUN)	China: Anhui	KJ745640	KJ745657	KJ745770	KJ745976	
	Z.-L.Nie 2170 (KUN)	China: Guizhou	KJ745566	KJ745650	KJ745885	KJ745980	
	Wen 9298 (US)	China: Hunan	KJ745638	KJ745651	KJ745886	KJ746001	
	<i>Polygonatum desoulavyi</i> Komarov	Chen <i>et al.</i> (2013)	Korea	JX903128	JX903537	–	–
	<i>Polygonatum falcatum</i> A.Gray	Chen <i>et al.</i> (2013)	Korea	JX903129	JX903538	–	–
<i>Polygonatum filipes</i> Merr. ex C.Jeffrey & McEwan	T. Deng 031 (KUN)	China: Anhui	KJ745609	KJ745720	KJ745769	KJ745946	
	T. Deng 147 (KUN)	China: Zhejiang	KJ745604	KJ745723	KJ745869	KJ745956	
	T. Deng 148 (KUN)	China: Anhui	KJ745605	KJ745719	KJ745867	KJ745998	
<i>Polygonatum franchetii</i> Hua	T. Deng HJ13 (KUN)	China: Hunan	KJ745558	–	KJ745833	KJ745977	
<i>Polygonatum griffithii</i> Baker	Tibet-MacArthur 615 (US)	China: Xizang	KJ745552	KJ745752	KJ745781	KJ745926	
<i>Polygonatum hirtellum</i> Hand.-Mazz.	Z.-L.Nie 2839 (KUN)	China: Sichuan	KJ745541	KJ745758	KJ745822	KJ745916	
<i>Polygonatum hookeri</i> Baker	Z.-L.Nie 2994 (KUN)	China: Sichuan	KJ745622	KJ745699	KJ745811	KJ745923	
	Y.Niu 080701 (KUN)	China: Yunnan	KJ745635	KJ745731	KJ745900	KJ745925	
	Z.-L.Nie 697 (KUN)	China: Heilongjiang	KJ745542	KJ745746	KJ745787	KJ745950	
<i>Polygonatum humile</i> Fischer ex Maxim.	Z.-L.Nie 704 (KUN)	China: Jilin	KJ745545	KJ745681	KJ745854	KJ745953	
	T. Deng 107 (KUN)	China: Heilongjiang	KJ745612	KJ745702	KJ745862	KJ745955	
	Z.-L.Nie 700 (KUN)	China: Jilin	KJ745543	KJ745673	KJ745892	KJ745963	
	Z.-L.Nie 701 (KUN)	China: Jilin	KJ745544	KJ745715	KJ745898	KJ745965	
	T. Deng 130 (KUN)	China: Heilongjiang	KJ745613	KJ745658	KJ745847	KJ745978	
	T. Deng 128 (KUN)	China: Heilongjiang	KJ745606	KJ745693	KJ745857	KJ745981	
	T. Deng 133 (KUN)	China: Heilongjiang	KJ745625	KJ745701	KJ745861	KJ745983	
	T. Deng 215 (KUN)	China: Heilongjiang	KJ745614	KJ745747	KJ745873	KJ745999	
	Z.-L.Nie 4003 (KUN)	China: Liaoning	KJ745559	KJ745745	KJ745872	–	
	Kim <i>et al.</i> (2010)	Korea	HM640455	HM640569	–	–	

APPENDIX *Continued*

Taxon	Voucher/Reference	Locality	<i>rbcL</i>	<i>trnK</i>	<i>psbA-trnH</i>	<i>trnC-petN</i>
<i>Polygonatum inflatum</i> Komarov	<i>T. Deng 124</i> (KUN)	China: Heilongjiang	KJ745639	KJ745697	KJ745859	KJ745905
	<i>Z.-L.Nie 698</i> (KUN)	China: Jilin	KJ745546	KJ745708	KJ745779	KJ745948
	<i>T. Deng 113</i> (KUN)	China: Heilongjiang	KJ745524	KJ745685	KJ745856	KJ745954
	<i>Z.-L.Nie 703</i> (KUN)	China: Jilin	KJ745547	KJ745759	KJ745899	KJ745966
	<i>T. Deng 131</i> (KUN)	China: Heilongjiang	KJ745585	KJ745700	KJ745860	KJ745979
	<i>T. Deng 129</i> (KUN)	China: Heilongjiang	KJ745615	KJ745680	KJ745853	KJ745982
	<i>T. Deng 116</i> (KUN)	China: Heilongjiang	KJ745571	KJ745646	KJ745844	–
	<i>T. Deng 132</i> (KUN)	China: Heilongjiang	KJ745616	KJ745668	KJ745850	–
<i>Polygonatum involucreatum</i> (Franch. & Sav.) Maxim.	Kim <i>et al.</i> (2010)	Korea	HM640456	HM640570	–	–
	<i>T. Deng 109</i> (KUN)	China: Heilongjiang	KJ745572	KJ745653	KJ745845	KJ745951
	<i>T. Deng 140</i> (KUN)	China: Heilongjiang	KJ745577	KJ745661	KJ745849	KJ745952
	<i>T. Deng 122</i> (KUN)	China: Heilongjiang	KJ745570	KJ745748	KJ745874	KJ746000
	<i>T. Deng 115</i> (KUN)	China: Heilongjiang	KJ745516	KJ745726	KJ745870	KJ746005
	<i>T. Deng 136</i> (KUN)	China: Heilongjiang	KJ745623	KJ745659	KJ745848	–
	<i>T. Deng 127</i> (KUN)	China: Heilongjiang	KJ745586	KJ745671	KJ745851	–
<i>Polygonatum kingianum</i> Collett & Hemsley	Kim <i>et al.</i> (2010)	Korea	HM640457	HM640571	–	–
	<i>G.-W.Hu B181</i> (KUN)	China: Yunnan	–	KJ745663	KJ745829	KJ745929
	<i>Z.-L.Nie 3530</i> (KUN)	China: Yunnan	KJ745520	KJ745684	KJ745830	KJ745930
	<i>Z.-L.Nie 729</i> (KUN)	China: Yunnan	KJ745517	KJ745691	KJ745831	KJ745941
	<i>Y.Meng 20110510</i> (KUN)	China: Yunnan	KJ745603	KJ745652	KJ745828	–
	<i>Z.-L.Nie 705</i> (KUN)	China: Yunnan	KJ745553	KJ745710	KJ745832	–
<i>Polygonatum lasianthum</i> Maxim. var. <i>coreanum</i> Nakai	Kim <i>et al.</i> (2010)	Korea	HM640458	HM640572	–	–
<i>Polygonatum multiflorum</i> (L.) All.	<i>Junheil sn</i>	Germany: Lahngergo	–	KJ745690	KJ745776	KJ746009
	de Vere <i>et al.</i> (2012)	UK: Cardiganshire	JN891841	JN895209	–	–
<i>Polygonatum odoratum</i> (Mill.) Druce	<i>Wen 10321</i> (US)	Russia: Russia	KJ745630	KJ745674	KJ745852	KJ745911
	<i>T. Deng 168</i> (KUN)	China: Anhui	KJ745607	KJ745744	KJ745871	KJ745957
	<i>Z.-L.Nie 696</i> (KUN)	China: Heilongjiang	KJ745550	KJ745696	KJ745771	KJ745968
	<i>Liuyh 019</i> (KUN)	China: Beijing	KJ745637	KJ745655	KJ745846	KJ745969
	<i>Z.-L.Nie 702</i> (KUN)	China: Jilin	KJ745551	KJ745721	KJ745868	KJ745970
<i>NM02</i> (KUN)	China: Neimeng	KJ745525	–	KJ745877	KJ745971	

APPENDIX *Continued*

Taxon	Voucher/Reference	Locality	<i>rbcL</i>	<i>trnK</i>	<i>psbA-trnH</i>	<i>trnC-petN</i>
	<i>T. Deng 135</i> (KUN)	China: Heilongjiang	KJ745564	KJ745717	KJ745866	KJ745985
	<i>T. Deng 214</i> (KUN)	China: Heilongjiang	KJ745588	KJ745709	–	KJ746002
	<i>Junheil sn</i>	Germany: Lahngergo	–	KJ745689	KJ745777	KJ746007
	<i>Z.-L.Nie 4006</i> (KUN)	China: Liaoning	KJ745549	KJ745694	KJ745858	–
	<i>T. Deng 134</i> (KUN)	China: Heilongjiang	KJ745587	KJ745716	KJ745865	–
	<i>Z.-L.Nie 4005</i> (KUN)	China: Liaoning	KJ745548	KJ745760	KJ745875	–
	<i>Z.-L.Nie 4004</i> (KUN)	China: Liaoning	KJ745593	KJ745768	KJ745876	–
<i>Polygonatum oppositifolium</i> (Wall.) Royle	Kim <i>et al.</i> (2010) Yamashita & Tamura (2000b)	Korea unknown	HM640459 AB029830	HM640573 AB029763	– –	– –
<i>Polygonatum prattii</i> Baker	Kim & Lee (2007) <i>Z.-L.Nie 3087</i> (KUN)	India China: Sichuan	– KJ745565	EF133695 KJ745669	– KJ745803	– KJ745913
	<i>Z.-L.Nie 2130</i> (KUN)	China: Guizhou	KJ745624	KJ745712	KJ745815	KJ745915
	<i>Y.Meng 2011526</i> (KUN)	China: Yunnan	–	–	KJ745827	KJ745918
	<i>G.-W.Hu 858</i> (KUN)	China: Yunnan	–	KJ745677	KJ745837	–
<i>Polygonatum pubescens</i> (Willd.) Pursh	<i>Z.-L.Nie 523</i> (KUN)	USA: Virginia	KJ745534	KJ745722	KJ745896	KJ745972
<i>Polygonatum punctatum</i> Royle ex Kunth	<i>Z.-L.Nie 3937</i> (KUN)	China: Sichuan	KJ745595	KJ745664	KJ745800	KJ745927
	<i>G.-W.Hu 549</i> (KUN)	China: Yunnan	–	–	KJ745799	KJ745940
	<i>G.-W.Hu 402</i> (KUN)	China: Yunnan	KJ745554	KJ745678	KJ745798	KJ746008
<i>Polygonatum roseum</i> (Ledebour) Kunth	<i>L.L.Wang 01</i> (KUN)	China: Xingjiang	KJ745526	KJ745757	KJ745821	KJ745903
	<i>L.L.Wang 02</i> (KUN)	China: Xingjiang	KJ745617	KJ745707	KJ745813	KJ745995
	<i>L.L.Wang 03</i> (KUN)	China: Xingjiang	KJ745618	KJ745705	KJ745812	KJ745994
	<i>L.L.Wang 04</i> (KUN)	China: Xingjiang	KJ745619	KJ745765	KJ745825	KJ745997
	<i>L.L.Wang 05</i> (KUN)	China: Xingjiang	KJ745626	KJ745688	KJ745809	KJ745993
	<i>L.L.Wang 06</i> (KUN)	China: Xingjiang	KJ745636	KJ745762	KJ745823	KJ745996
	<i>Wen 10365</i> (US)	Russia: Russia	KJ745631	KJ745676	KJ745806	KJ745922
<i>Polygonatum sibiricum</i> Redouté	<i>T. Deng 165</i> (KUN) <i>GY 15</i> (KUN)	China: Anhui China: Yunnan	KJ745581 KJ745555	KJ745706 KJ745727	KJ745880 KJ745881	KJ745931 KJ745932
	<i>Nie &amp; Meng 03</i> (KUN)	China: Neimeng	KJ745589	KJ745749	KJ745882	KJ745933
	<i>Y.-P.Xie 136</i> (KUN)	China: Shanxi	KJ745575	KJ745750	KJ745883	KJ745934



APPENDIX *Continued*

Taxon	Voucher/Reference	Locality	<i>rbcL</i>	<i>trnK</i>	<i>psbA-trnH</i>	<i>trnC-petN</i>
<i>Polygonatum stenophyllum</i> Maxim.	<i>T. Deng 169</i> (KUN)	China: Anhui	KJ745632	KJ745695	KJ745889	KJ745935
	<i>Liuyh 0310</i> (KUN)	China: Beijing	KJ745560	KJ745656	–	KJ745936
	<i>Z.-L.Nie 695</i> (KUN)	China: Helongjiang	KJ745556	KJ745763	KJ745894	KJ745964
	Kim, Kim & Kim (2012)	Korea	JF972886	JF972920	–	–
<i>Polygonatum verticillatum</i> (L.) All.	<i>Z.-L.Nie 912</i> (KUN)	China: Xizang	KJ745578	KJ745751	KJ745835	KJ745943
	<i>Tibet-MacArthur 3170</i> (US)	China: Xizang	KJ745557	KJ745739	KJ745786	–
	<i>Tibet-MacArthur 3003</i> (US)	China: Xizang	KJ745596	KJ745735	KJ745840	–
	<i>Tibet-MacArthur 3139</i> (US)	China: Xizang	KJ745573	KJ745738	KJ745841	–
<i>Polygonatum zanlanscianense</i> Pamp.	<i>T. Deng 071</i> (KUN)	China: Zhejiang	KJ745519	KJ745683	KJ745807	KJ745902
	<i>T. Deng 065</i> (KUN)	China: Zhejiang	KJ745567	KJ745732	KJ745817	KJ745920
	<i>T. Deng 054</i> (KUN)	China: Zhejiang	KJ745608	KJ745755	KJ745820	KJ745921
	<i>Wen 9019</i> (US)	China: Shanxi	KJ745627	KJ745672	KJ745805	KJ745992
	<i>Y.-P.Xie 278</i> (KUN)	China: Henan	–	KJ745711	KJ745814	KJ745924
	<i>Y.Meng 013</i> (KUN)	China: Guizhou	KJ745568	KJ745736	KJ745818	KJ745974
	<i>Z.-L.Nie 2172</i> (KUN)	China: Guizhou	KJ745579	KJ745649	KJ745791	KJ745907
<i>Reineckea carnea</i> (Andrews) Kunth						
<i>Ruscus aculeatus</i> L.	Kim <i>et al.</i> (2010)	–	HM640440	HM640554	–	–
<i>Sansevieria trifasciata</i> Prain	Kim <i>et al.</i> (2010); Bruni <i>et al.</i> (2010)	–	HM640471	HM640584	FN675812	–
<i>Speirantha gardenii</i> (Hook.) Baill.	<i>T. Deng 012</i> (KUN)	China: Anhui	KJ745584	KJ745743	KJ745891	KJ745908
<i>Theropogon pallidus</i> Maxim.	Kim <i>et al.</i> , 2010	–	HM640446	HM640560	–	–
<i>Tricalistra ochracea</i> Ridl.	Yamashita & Tamura (2000b)	–	AB029839	AB029777	–	–