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Molecular Phylogeny of *Ophiopogon* (Asparagaceae) Inferred from Nuclear and Plastid DNA Sequences

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Abstract—The East and Southeast Asian genera *Ophiopogon*, *Liriope*, and *Peliosanthes* are classified in the tribe Ophiopogoneae (Asparagaceae). Phylogenetic relationships of this group were explored using maximum parsimony, Bayesian and maximum likelihood analyses of nuclear ITS and plastid *psbA-trnH*, *matK*, *rbcL*, and *trnL-trnF* sequences. These analyses supported the monophyly of *Ophiopogon*, *Liriope*, and *Peliosanthes* within the Ophiopogoneae, although tree topologies based on nuclear and chloroplast DNA differed in the placement of many taxa. Incongruence between these two datasets may be a result of hybridization and introgression. Our results reveal that *Ophiopogon* consists of two major lineages, which we recognize at the sectional level: *O. lancangensis*, *O. multiflorus*, *O. yunnanensis*, *O. reversus*, *O. longibracteatus* (sect. *Ophiopogon*), and *O. tsaii* (sect. *Peliosanthoides*).

Keywords—*Liriope*, *Ophiopogon*, Ophiopogoneae, *Peliosanthes*, phylogenetic reconstruction.

Ophiopogon Ker Gawler (Asparagaceae) is distributed mainly in East Asia, with some species occurring in Southeast Asia (Fig. 1). Species of *Ophiopogon* are perennial, rhizomatous, or sometimes stoloniferous. *Ophiopogon* comprises about 65 species, with 38 endemic to China (Chen and Tamura 2000) and a center of diversity in southern and southeastern Yunnan and southwestern Guangxi (Yang et al. 1990). Zhang (1991) indicated that the modern focus of differentiation in the genus extends from the Himalayas to the Hengduan Mountains through southern and western Sichuan.

The correct tribal placement of *Ophiopogon* has been disputed at length, and still remains unsettled. Krause (1930) transferred *Ophiopogon*, *Liriope* Lour., and *Peliosanthes* Andrews to the tribe Ophiopogoneae within Liliaceae because the fruit wall splits irregularly at an early stage of development to expose the immature seeds. Thorne (1968, 1976), Dahlgren et al. (1985), and Takhtajan (1987) also placed the three genera in Ophiopogoneae, but within subfamily Ophiopogonoideae of Convallariaceae. Dai and Liang (1991) and Liang and Dai (1992) retained the group in Liliaceae but classified *Ophiopogon* and *Liriope* in Ophiopogoneae and *Peliosanthes* in Peliosantheae on the basis of leaf epidermis and pollen characters, respectively. Molecular phylogenetic analyses of the *matK* and *rbcL* chloroplast DNA regions by Tamura and Yamashita (2004) and Kim et al. (2010) supported the monophyly of Ophiopogoneae.

Ker-Gawler (1807) proposed the genus *Ophiopogon* based on Thunberg's species, which he recognized as misassigned to the genus *Convallaria* Linn., and designated *O. japonicus* as the type species. The first attempt at an infrageneric classification of *Ophiopogon* was carried out by Kunth (1850), who transferred the *Liriope* species into *Ophiopogon* and divided *Ophiopogon* into two groups based on the position of the peduncle joint. Rodriguez (1900) compiled 15 species of the genus *Ophiopogon* in the F*lore Gènèrale De L'Indo* and classified them into two groups based mainly on petiole and leaf shape characters. Wang and Tang (1978) published an extensive treatment of the *Ophiopogon* endemic to China using leaf shape to divide the genus into sect. *Peliosanthoides* Wang et Dai and sect. *Ophiopogon*. Furthermore, Yang and Li (1990) published a more extensive taxonomic treatment that split the genus into two sections and five series based on inflorescence position and leaf shape. Genera related closely to *Ophiopogon* include *Liriope* and *Peliosanthes*. De Loureiro (1790) proposed the genus *Liriope* for plants from Cochinchina (presently Cambodia, Laos, and Vietnam), which is the origin of the plants known presently as *L. spicata* (Thunb.) Lour. Andrew (1810) established the genus *Peliosanthes* for a plant introduced from India into England, designating *P. teta* Andr. as the type species.

Chromosome counts are reported for about 45 *Ophiopogon* species. The genus has two basic chromosome numbers: $x = 18$, which is present in most species, and $x = 17$, which represents *O. clarkei* Hook. f., *O. intermedius* D. Don, *O. japonicus* (L. f.) Ker Gawl., *O. ohwii* (L. f.) Ker Gawl., and *O. umbraticola* Hance (Wang et al. 2013). Most species are diploid, with the exception of 12 polyploid species (Zhang 1991; Wang et al. 2013). Moreover, Zhang's (1991) karyotype analyses supported Yang and Li's (1990) morphologically based classification.

Rudall et al. (2000), Tamura and Yamashita (2004), and Kim et al. (2010) included only two species of *Ophiopogon* in their broad phylogenetic analyses of Ruscaceae and other monocotyledons based on chloroplast DNA (cpDNA). Thus, the phylogenetic relationships within *Ophiopogon* have been tested only minimally using molecular data and remain unclear. In this study, we undertook a comprehensive phylogenetic analysis of *Ophiopogon*, *Liriope*, and *Peliosanthes* using DNA sequence data derived from the nuclear internal transcribed spacer region (ITS) region and several chloroplast regions (*psbA–trnH*, *matK*, *rbcL*, and *trnL–F*). Our goals were to reconstruct the phylogenetic relationships among the three genera, to re-evaluate the monophyly of *Ophiopogon*, and to elucidate the interspecific relationships within *Ophiopogon*.

Materials and Methods

Taxon Sampling—We sampled 91 specimens representing 43 species of *Ophiopogon*, *Liriope*, and *Peliosanthes* in Ophiopogoneae (Appendix 1).

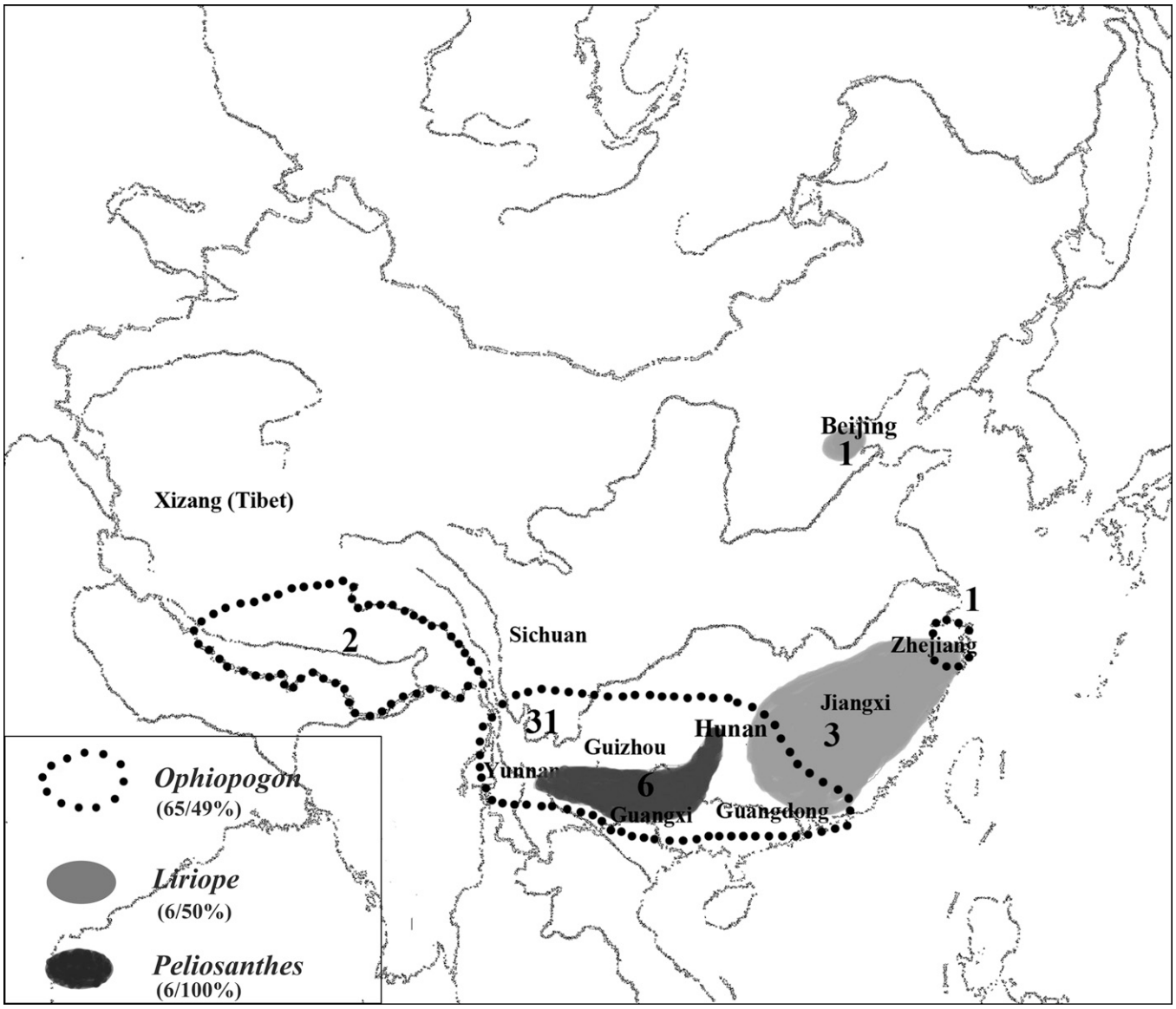

FIG. 1. Distribution of Ophiopogoneae in East and Southeast Asia. The number of species sampled and percentage of the total number of species in each geographic region are indicated.

Single accessions of *Convallaria* L., *Reineckea* Kunth, *Aspidistra* Ker Gawl., *Disporopsis* Hance, *Heteropolygonatum* M. N. Tamura et Ogisu in M. N. Tamura et al. *Maianthemum* Web., and *Polygonatum* Mill. were chosen as outgroups, in accordance with Kim et al. (2010). Living plants were cultivated in a greenhouse at the Kunming Institute of Botany, Yunnan, China. Voucher specimens were deposited in KUN.

DNA Extraction, PCR Amplification, and Sequencing—Genomic DNA was extracted from 15 mg of desiccated leaf tissue using the CTAB method of Doyle and Doyle (1987) and a plant genomic DNA extraction kit (Bioteke, Beijing, China). Each PCR amplification was performed in a volume of 20 µl using 10 ng genomic DNA (quantified with a NanoDropTM2000c, Thermo Scientific, Waltham, Massachusetts) as template, 4 pmol each primer, 0.5 U Taq polymerase (Promega, Madison, Wisconsin), and 2.5 mM $MgCl_2$ under the following conditions: 3 min at 94°C; followed by 30 cycles of 30 s at 94°C, 30 s at 50°C, and 1 min at 72°C; with a final extension of 10 min at 72°C.

To amplify the ITS region as a single fragment, the primers ITS4 and ITS5 (White et al. 1990) were used. However, if amplification of the entire ITS region was unsuccessful, the internal primers ITS2 and ITS3 (White et al. 1990) were used in the combinations ITS2/ITSmF and ITS3/ITS4 to obtain two shorter, overlapping fragments. The primers used to amplify the chloroplast DNA regions were as follows: psbA-F and trnH-R for *psbA–trnH* (Sang et al. 1997; Hamilton 1999); Z1 and 1024R for *rbcL* (Zurawski et al. 1981; Olmstead et al. 1993); 3F and 1R for *matK* (Kilian et al. 2009); and Tab-c and Tab-f for *trnL–trnF* (Taberlet et al. 1991).

The PCR products were purified using the polyethylene glycol (PEG) precipitation procedure following the manufacturer's protocols. Cycle sequencing was carried out as follows: 35 cycles of 97°C for 15 s, 50°C for 5 s, and 60°C for 4 min. The products of cycle-sequencing reactions were cleaned using Sephadex columns (Amersham Pharmacia Biotech, Piscataway, New Jersey) and dried at 60°C in a rotary vacuum evaporator. Sequence data were generated with an ABI Prism 3100 capillary sequencer (Applied Biosystems, Foster City, California). Sequences were aligned with ClustalX 1.83 (PCversion, Thompson et al. 1997), then manually edited with Bioedit (Hall 1999).

Phylogenetic Analyses—Phylogenetic trees were reconstructed using maximum parsimony (MP), Bayesian inference (BI) and maximum likelihood (ML). Parsimony analyses were performed with heuristic searches of 1,000 replicates with random stepwise addition using tree bisection–reconnection (TBR) branch swapping, MulTrees, and the Collapse option selected in PAUP* v.4.0b10 (Swofford 2003). Gaps were treated either as missing data or as new characters. All characters and character state transformations were weighted equally. The bootstrap percentages (BP) were calculated from 1,000 replicates using a heuristic search with simple addition with the TBR and MULPARS options implemented

(Felsenstein 1985). The optimal model of molecular evolution under the Akaike information criterion (AIC) was determined with Modeltest 3.7 (Posada and Crandall 1998; Posada and Buckley 2004). For each analytic method the optimal model was the general time reversible model, with rate heterogeneity modeled by assuming that a proportion of sites were invariable and that the rate of evolution at other sites could be modeled using a discrete approximation to a gamma distribution (GTR + I + C). Bayesian inference using a Markov chain Monte Carlo (MCMC) algorithm was implemented in MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001). Bayesian analyses were run for 2,000,000 generations with four incrementally heated Markov chains, starting from random trees and sampling every 100 generations. The first 2,000–5,000 trees, depending on when chains appeared to have become stationary, were discarded as 'burn-in'. The remaining trees were assumed to represent the posterior probability (PP) distribution. The 50% majority rule consensus tree and PP for each node were calculated in PAUP* 4.0 b10 (Swofford 2003). The ML was performed as implemented in GARLI 0.951 (Zwickl 2006) starting from a random tree with 10,000,000 generations per search. The ML bootstrap support (BS) values were estimated from 100 bootstrap replicates in GARLI.

To evaluate the congruence of the nuclear and plastid datasets, we employed the incongruence length difference (ILD) test (Farris et al. 1994). The ILD test was conducted using a heuristic search with 1,000 replicates and tree–bisection–reconnection branch–swapping with 10 random sequence additions in PAUP*.

Results

The aligned sequences of the ITS region comprised a data matrix of 725 base pairs (bp) with 228 parsimony-informative sites (228/725, 31.45%). The *psbA–trnH* alignment consisted of 682 bp with 11 parsimony-informative sites (11/682, 1.61%). The aligned *rbcL* sequences were 1,146 bp in length with 30 parsimony-informative sites (30/1146, 2.62%). The aligned *matK* data set comprised 869 bp, of which 44 were parsimony informative (44/869, 5.06%). The *trnL–trnF* alignment consisted of 1,029 bp, of which 38 sites were parsimony informative (38/1029, 3.69%) (Table 1). Given the absence of recombination in the chloroplast genome, we combined the plastid sequences into a single dataset. The combined cpDNA data matrix comprised 3,726 characters including 123 parsimony-informative sites (123/3726, 3.30%). The ILD test indicated that the ITS and cpDNA (*psbA–trnH*, *rbcL*, *matK*, and *trnL–trnF*) datasets were significantly incongruent ($p = 0.02$); therefore, a combined analysis of the ITS and cpDNA sequence data was not performed.

The nrDNA tree with PP and BS support is shown in Fig. 2. The cpDNA tree is shown in Fig. 3. Our analyses resolve Ophiopogoneae as monophyletic, but with low to moderate support. The genus *Ophiopogon* is monophyletic and consists of two major lineages, Clades A and B. Clade A consists of subclade A1 and A2, while Clade B is divided into subclades B1, B2, and B3. Based on the results of the phylogenetic analyses, *Ophiopogon lancangensis* Wang et Tang, *O. multiflorus* Y. Wan, *O. yunnanensis* S. C. Chen, *O. reversus* C. C. Huang, and *O. longibracteatus* D. Don were classified in sect. *Ophiopogon*, and *O. tsaii* F. T. Wang et Tang was classified in sect. *Peliosanthoides*. The monophyly of both *Liriope* and *Peliosanthes* was supported in all analyses.

Discussion

***Incongruence of Nuclear and Chloroplast Matrices*—**The ILD test (Farris et al. 1994) indicated that the nuclear and combined plastid data sets were significantly incongruent ($p = 0.02$). For example, in the nrDNA tree, *Ophiopogon chingii* Wang et Tang was sister to *O. grandis* W. W. Sm. and *O. bodinieri* Lévl., which in the cpDNA tree it was sister to *O. latifolius* Rodrig., *O. platyphyllus* Merr. et Chun and *O. dracaenoides* (Baker) Hook. f. Similarly, the clade *O. multiflorus* and *O. umbraticola* was internal to the nrDNA tree, but in the cpDNA tree, it was sister to all other clades. Theoretical and experimental studies have demonstrated that incongruence among gene trees or between gene trees and organismal phylogenies can result from a variety of factors, including sampling error, convergence, evolutionary rate heterogeneity, lineage sorting, hybridization, and introgression (Avise 1989; Rieseberg and Soltis 1991; Doyle 1992; Kadereit 1994; Rieseberg et al. 1996). One probable explanation for the differences in tree topologies is hybridization. Hybridization has been documented in *Ophiopogon* (Zhang 1991), as well as polyploidy (Zhang 1991; Wang et al. 2013). Most of the polyploid taxa within the genus occur within sect. *Ophiopogon*. Whether these are allopolyploids or autopolyploids has not yet been evaluated using a molecular approach. Another probable explanation is introgression. Inflorescences of *O. szechuanensis* Wang et Tang, *O. latifolius*, and *O. mairei* H. Lév. occur on the upper stem (unspecialized), whereas leaves are scattered (specialized); the leaf widths of *O. clarkei*, *O. intermedius*, *O. megalanthus* F. T. Wang et L. K. Dai, *O. corifolius* Wang et Dai, *O. zingiberaceus* F. T. Wang et L. K. Dai, *O. revolutus* F. T. Wang et L. K. Dai, and *O. platyphyllus* are between sect. *Ophiopogon* and sect. *Peliosanthoides*; inflorescences of *O. yunnanensis* occur on the upper stem (unspecialized), while leaves are sessile (specialized). By their combination of specialized and unspecialized inflorescence features, we infer that these species are transitional taxa.

***Relationships among Ophiopogon, Liriope, and Peliosanthes of Ophiopogoneae*—**Nuclear and chloroplast data indicated the monophyly of the tribe Ophiopogoneae with strong to moderate support (PP 1.00, BS 99%, Fig. 2; PP 0.69, BS 87%, Fig. 3), a result consistent with findings from previous studies. The monophyly of Ophiopogoneae also is supported by irregular dehiscence early during development to expose the immature seeds. The relationships of the three genera of Ophiopogoneae have been studied using morphological, cytological, and molecular data. Dai and Liang (1991), Liang and Dai (1992), and Zhang (1991) hypothesized that *Ophiopogon* and *Liriope* were closely related based on leaf epidermal and pollen characters and chromosome numbers. Those authors placed *Ophiopogon* and *Liriope* in tribe Ophiopogoneae and *Peliosanthes* in a separate tribe, Peliosantheae. Cutler (1992) also presented evidence for the close affinity of *Ophiopogon* and *Liriope* in a vegetative anatomical study of the tribe

Table 1. Summary of the DNA sequence data used in this study of Ophiopogoneae.

	ITS	*psbA–trnH*	*rbcL*	*matK*	*trnL–trnF*
Length of aligned matrix (bp)	725	682	1,146	869	1,029
Number of parsimony-informative characters	228	11	30	44	38
Percent of parsimony-informative sites	31.5%	1.6%	2.6%	5.1%	3.7%

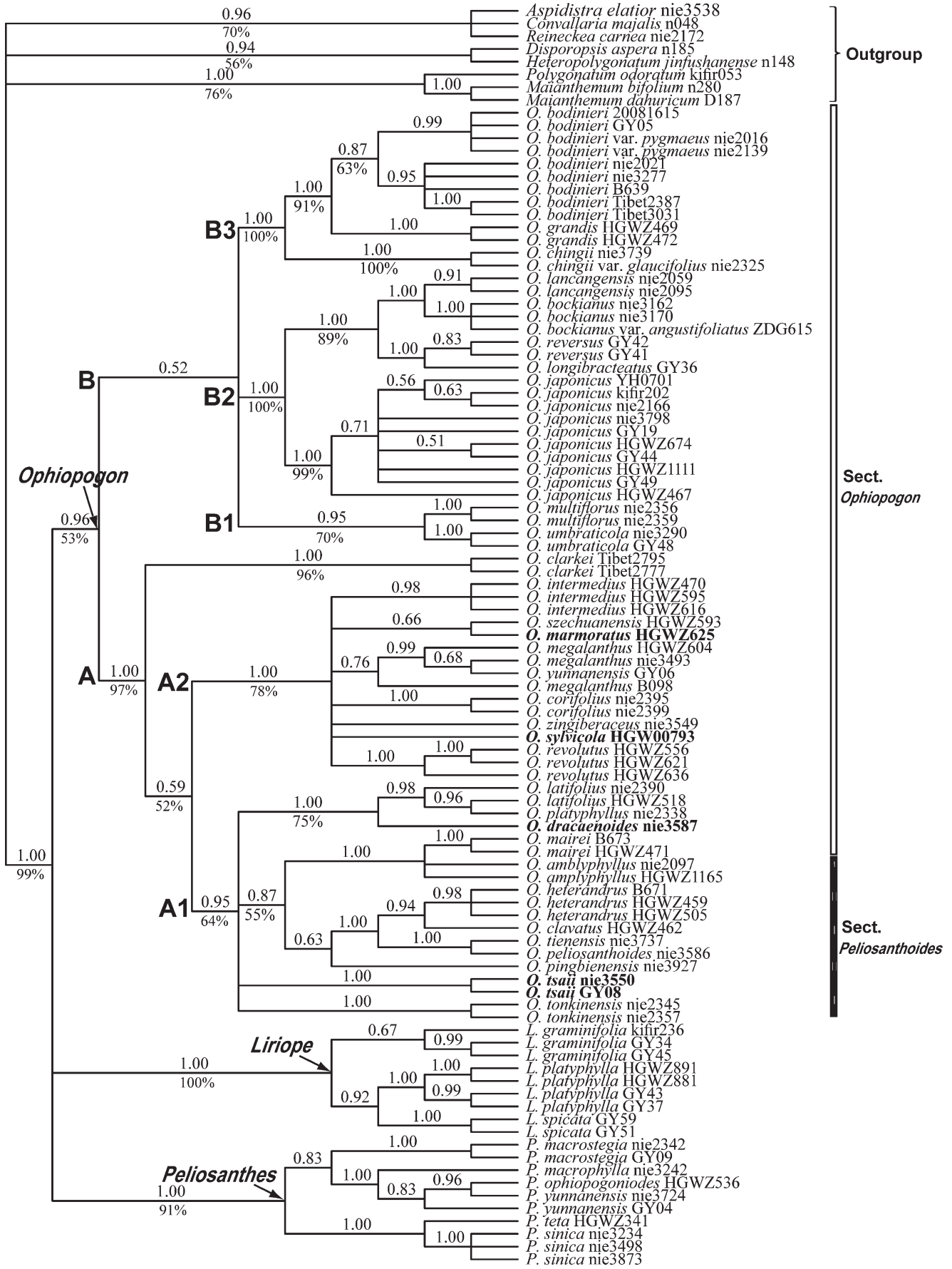

FIG. 2. Strict consensus tree of Ophiopogoneae based on nuclear ITS Sequence data (tree length = 760 steps, CI = 0.57, RI= 0.83, RC = 0.47). Values above each branch represent Bayesian posterior probabilities and those below branch (> 50%) are maximum likelihood bootstrap values from 100 replicates.

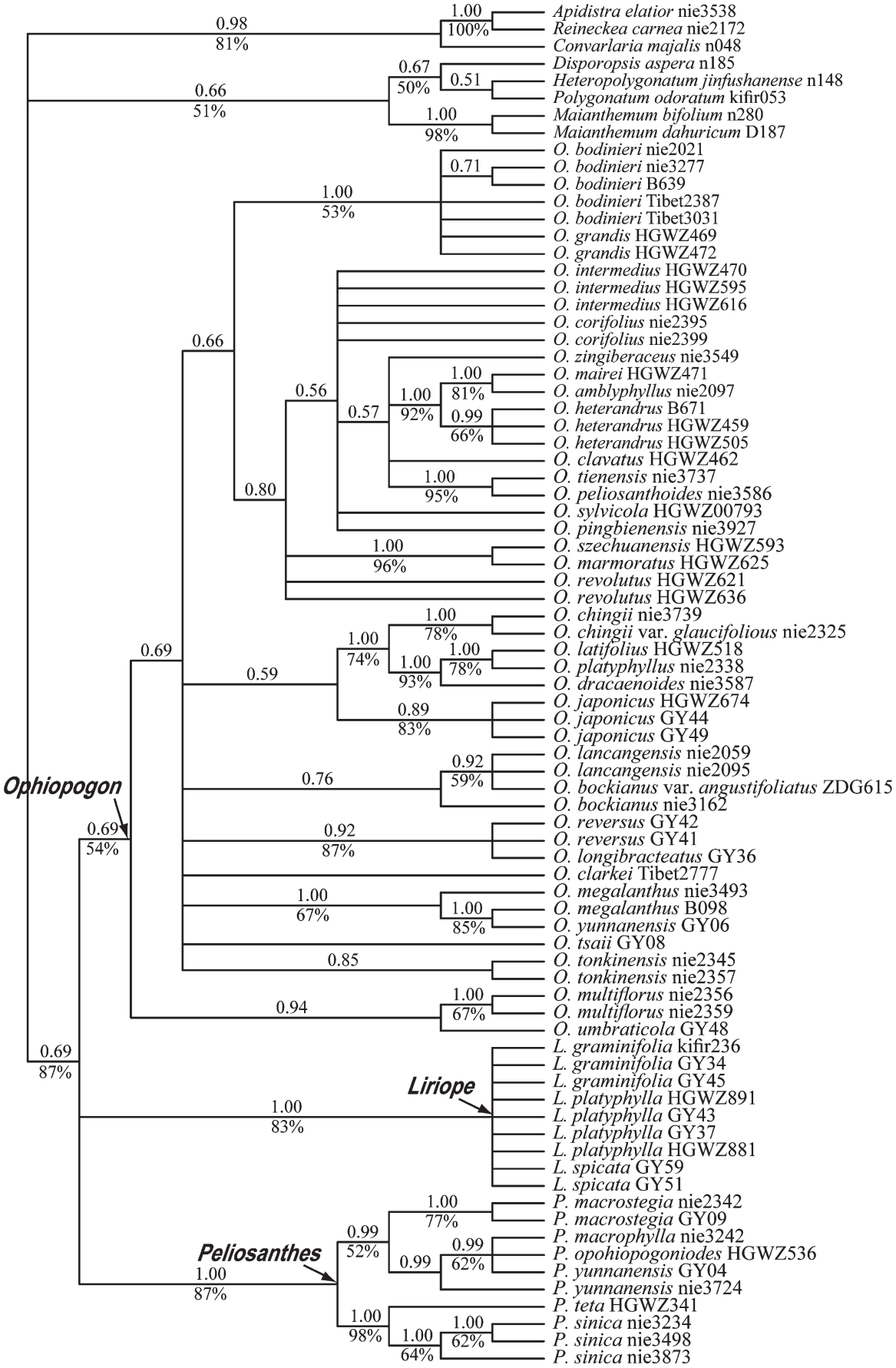

FIG. 3. Strict consensus tree of Ophiopogoneae based on chloroplast DNA sequence data (tree length = 326, CI = 0.82, RI = 0.90, RC = 0.73). Values above each branch represent Bayesian posterior probabilities and those below branch (> 50%) are maximum parsimony values.

Ophiopogoneae. Rudall et al. (2000) and Kim et al. (2010) showed that *Ophiopogon* and *Liriope* were closely related in their phylogenetic analyses of *matK* and *rbcL* sequence data. However, some authors argued that *Ophiopogon* and *Liriope* had no close relationship (Bailey 1929; Hume 1961; Skinner 1971; Mcharo et al. 2003). Furthermore, Tamura and Yamashita (2004) suggested that *Liriope* was more closely related to *Peliosanthes* based on analyses of *matK* and *rbcL* sequences. Cytological data supports the monophyly of *Ophiopogon*, *Liriope,* and *Peliosanthes* (Wang et al. 2013). In the present study, *Ophiopogon* was a distinct clade and had moderate support, while the isolated clades of both *Liriope* and *Peliosanthes* were highly supported (Figs. 2 and 3). All three genera resolve within one clade, with relatively strong support. Therefore, we suggest that *Ophiopogon*, *Liriope*, and *Peliosanthes* be maintained as distinct but closely related genera.

Monophyly of Ophiopogon—Our results show low to moderate support for the monophyly of *Ophiopogon* (ITS PP = 0.96, BS = 53%, Fig. 2; cpDNA PP = 0.69, BS = 54%, Fig. 3). The monophyly of the genus also is supported by morphological and chromosomal evidence. For example, drooping flowers, free overlapping tepals, short filaments, and a semi-inferior ovary are synapomorphic for *Ophiopogon* (Bailey 1929; Chen and Tamura 2000), whereas erect flowers, free tepals, long filaments, and a superior ovary are diagnostic of *Liriope*, and basally overlapping tepals, filaments dilated and connate in a fleshy ring, and an inferior ovary are synapomorphic in *Peliosanthes* (Chen and Tamura 2000). Most species of *Ophiopogon* share a basic chromosome number of $x = 18$, although $x = 17$ is known in a few species (e.g. *O. umbraticola*, *O. japonicus*, *O. clarkei*, *O. intermedius,* and *O. ohwii*) (Malik 1962; Sharma and Chaudhuri 1964; Nagamatsu and Noda 1971; Zhang 1991; Yamashita and Tamura 2001). Within this group, dysploid diploid, tetraploid, and hexaploid species exist only in *Ophiopogon*. *Ophiopogon* species have both 2B and 2C type chromosomes, with subtelocentric (st) chromosomes occurring rarely. *Liriope* shares the basic chromosome number of $x = 18$, but has only 2B chromosomes, with a few species possessing st chromosomes. In contrast, *Peliosanthes* species have a basic chromosome number of $x = 17$ or 18, possess 2C chromosomes, and have st chromosomes in all species (Hsu 1971; Yang et al. 1990; Zhang 1991; Wang et al. 2013).

Phylogenetic Relationships within Ophiopogon—Two major clades were revealed in *Ophiopogon* by the ITS sequence data (Fig. 2). Clade A included species from both sect. *Ophiopogon* and sect. *Peliosanthoides*. Clade B consisted of species from sect. *Ophiopogon* as classified by Wang and Tang (1978). The cpDNA data failed to provide enough variation to resolve the relationships within *Ophiopogon* (Fig. 3). Therefore, the following discussion of *Ophiopogon* is largely based on phylogenetic reconstructions from the ITS dataset.

Two well-defined, moderately to well-supported subclades, A1 and A2, were resolved within clade A (Fig. 2). All species in subclade A1 belonged to sect. *Peliosanthoides* except for *O. platyphyllus*, *O. latifolius*, *O. mairei*, and *O. tsaii*, which belonged to sect. *Ophiopogon* according to Wang and Tang (1978). In sect. *Peliosanthoides*, most species grow on karst landforms. *Ophiopogon tsaii* also grows on karst landforms but grouped within sect. *Peliosanthes*. Except for *O. tonkinensis* L. Rodr. and *O. dracaenoides*, species placed in subclade A1 have petiolate, oblong-oblanceolate leaves, solitary flowers, short filaments, and slender styles. *Ophiopogon tonkinensis* and *O. dracaenoides* have leathery leaves, clustered flowers, and obvious filaments, a discrete combination of characters suggesting that they represent a distinct lineage within the subclade. *Ophiopogon dracaenoides* is distinct from sect. *Pelioanthoides*, but its phylogenetic position within *Ophiopogon* is uncertain. Additional sampling of *O. dracaenoides* accessions could contribute to a better understanding of the species' phylogenetic relationships. *Ophiopogon dracaenoides*, *O. platyphyllus*, and *O. latifolius* formed a fairly well-supported clade (PP 1.00, BS 75%, Fig. 2). These species share woody roots and lanceolate tepals.

Subclade A2 was sister to subclade A1 but with low support (PP 0.59, BS 52%, Fig. 2). All taxa included in subclade A2 belong to sect. *Ophiopogon* except for *O. sylvicola* F. T. Wang et Tang and *O. marmoratus* Pierre ex L. Rodr., which were assigned to sect. *Peliosanthoides* by Wang and Tang (1978). Except for *O. zingiberaceus*, which has distinctive *Zingiber*-like rhizomes and deltoid-ovate tepals, all species of this subclade possess slender roots. Tanaka (1999) reduced *O. revolutus* to synonymy with *O. griffithii* D. Don on the basis of leaf shape and width. Given the broad morphological diversity exhibited by *O. intermedius*, numerous related species were synonymized by Tanaka (2001a, b). *Ophiopogon megalanthus* and *O. yunnanensis* formed a moderately supported clade, but the two species show little morphological similarity; *O. megalanthus* has rhizomes, basal leaves, and lanceolate bracts, with flowers in clusters of 2–4; *O. yunnanensis* has elongate stems, distant tufted leaves, paired flowers, and lanceolate to linear-lanceolate bracts. We classify *O. yunnanensis* in sect. *Ophiopogon* based on the possession of grass-like, sessile leaves.

Ophiopogon clarkei is distributed in Tibet and can be distinguished by its slender underground rhizome, obvious midvein leaves, white, ovate to lanceolate tepals, and long filaments. Although this species was placed in sect. *Ophiopogon* by Wang and Tang (1978), the present results indicate that *O. clarkei* occupies an isolated position in clade A as a sister species to subclades A1 and A2 with high support (PP 1.00, BS 99%, Fig. 2).

Species in the weakly supported clade B in *Ophiopogon* are characterized by grass-like leaves, nodding flowers, and short filaments (PP 0.52, Fig. 2). Three moderately to robustly supported subclades (B1, B2, and B3) also were recovered (Fig. 2).

Although the nrDNA and cpDNA phylogenies resolved similar higher-level relationships, the phylogenetic placement of several individual species differed. The most notable difference was in the position of subclade B1 (comprising *O. multiflorus* and *O. umbraticola*), which is sister to subclades B2 and B3 in the nrDNA tree but sister to all other *Ophiopogon* species in the cpDNA tree. Subclade B1 was moderately supported (PP 0.95, BS 70%, Fig. 2; PP 0.94, Fig. 3). *Ophiopogon multifolius* and *O. umbraticola* share bluish flowers and three internal tepals wider than the external ones. *Ophiopogon multifolius* has grass-like and sessile leaves and is morphologically similar to *O. bockianus* (sect. *Ophiopogon*) in flower structure (Wan 1988). After considering both morphology and our molecular data, we elected to move *O. multifolius* to sect. *Ophiopogon*.

In the nrDNA tree, subclade B2 was highly supported (PP 1.00, BS 100%, Fig. 2) and was sister to subclades B1 and B3. Two well-defined groups were resolved within subclade B2. One group comprised only accessions of the polymorphic species *O. japonicus*. Different forms of *O. japonicus* have been

distinguished as distinct species by many authors. However, the flower structure is relatively stable, especially in the slightly open tepals and basally-broadened styles. The second group comprised *O. longibracteatus*, *O. reversus*, *O. bockianus* Diels, *O. bockianus* var. *angustifoliatus* F. T. Wang et Tang, and *O. lancangensis* F. T. Wang et Tang. All of these species are well-defined morphologically and clearly distinct from each other. *Ophiopogon longibracteatus* and *O. reversus* are morphologically distinct, but co-occur in moist environments in Qingyuan County, Guangdong Province. The most striking difference between the two species is that *O. longibracteatus* has longer bracts and tepals than *O. reversus*. In light of their grass-like, sessile leaves, we placed the two species into sect. *Ophiopogon*. Species of *O. lancangensis* and *O. bockianus* formed a well-supported clade (PP 1.00, Fig. 2). Their close relationship was supported by their lanceolate bracts, which articulate below the middle. Furthermore, they occur in similar subtropical, humid climates. Combined with their possession of grass-like and sessile leaves, we classified *O. lancangensis* in sect. *Ophiopogon*.

The final group resolved within clade B was subclade B3 (PP 1.00, BS 100%, Fig. 2), where the presence of lanceolate tepals, lanceolate bracts, conspicuous filaments, and central articulate are potential synapomorphies. All species belong to sect. *Ophiopogon* according to Wang and Tang (1978). *Ophiopogon chingii* is morphologically distinct from the other members of this subclade by its possession of prostrate stems, scattered leaves, and membranous bracts. *Ophiopogon bodinieri* and *O. grandis* resolved as sister species (PP 1.00, BS 91%, Fig. 2; PP 1.00, BS 53%, Fig. 3). The main difference between these two species is that *O. bodinieri* has underground rhizomes and *O. grandis* has conspicuous filaments and articulation above the middle. In addition, *O. bodinieri* constitutes a polyploid complex of diploids, tetraploids, and hexaploids.

Previously, *O. marmoratus*, *O. sylvicola*, and *O. dracaenoides* were classified in sect. *Peliosanthoides*, and *O. tsaii* was placed into sect. *Ophiopogon* (Wang and Tang 1978). However, our analyses of nrDNA sequence data indicate that *O. marmoratus*, *O. sylvicola*, and *O. dracaenoides* belong to sect. *Ophiopogon*, and *O. tsaii* to sect. *Peliosanthoides*. Our field observations have revealed that *O. marmoratus*, *O. sylvicola*, and *O. dracaenoides* produce grass-like leaves under drought conditions but cauline leaves in humid environments. The sectional placement of *O. tsaii* (which has grass-like leaves and erect stems in the field) also has been problematic. When the grass-like leaves are emphasized, it is better classified in sect. *Ophiopogon*, but when the erect stem is emphasized, it is better classified in sect. *Peliosanthoides*. A detailed examination of morphological characters revealed that the erect stem is of primary taxonomic importance in *O. tsaii*, which is consistent with our molecular results.

Yang et al. (1990) divided *Ophiopogon* into two sections with five series based on inflorescence position and leaf shape. However, our present results show that taxa classified in these series were not resolved as clades, but were dispersed throughout the trees. This finding indicates that inflorescence position and leaf shape may not represent suitable characters for use in an infrageneric classification of *Ophiopogon*. Compared to the classification of Yang et al. (1990), molecular evidence better supports the classification proposed by Wang and Tang (1978) with respect to sects. *Ophiopogon* and *Peliosanthoides*.

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Appendix 1. Voucher information and GenBank accession numbers for Ophiopogoneae accessions used in this study. Data are in the order: taxon, voucher, locality, GenBank accession numbers for ITS, *psbA-trnH, matK, rbcL,* and *trnL-trnF*, -"indicates that this locus was not sequenced for the taxon.

Ophiopogon Ker-Gawl., Sect. Ophiopogon. ***O. bodinieri*** Levl., *20081615* (KUN), China: Yunnan, KIB, KF671232, -, -, -, -. *Gy 05* (KUN), China: Yunnan, Qiaojia, KF671233, -, -, -, -. *Nie 2021* (KUN), China: Sichuan, Leibo, KF671234, KF671391, KF671529, KF671462, KF671323. *Nie 3277* (KUN), China: Yunnan, Dali, KF671235, KF671392, KF671530, KF671463, KF671324. *B 639* (KUN), China: Yunnan, Lanping, KF671238, KF671393, KF671531, KF671464, -. *Tibet 2387* (KUN), China: Yunnan, Xianggelila, KF671239, KF671394, -, KF671465, KF671325. *Tibet 3031* (KUN), China: Tibet, Gongbujiangda, KF671240, KF671395, -, -, KF671326. ***O. bodinieri var. pygmaeus*** Wang et Dai, *Nie 2016* (KUN), China: Sichuan, Leibo, KF671236, -, -, -, -. *Nie 2139* (KUN), China: Guizhou, Weining, KF671237, -, -, -, -. ***O. grandis*** W. W. Sm., *Hgwz 469* (KUN), China: Hunan, Sangzhi, KF671241, KF671396, KF671532, KF671466, KF671327. *Hgwz 472* (KUN), China: Yunnan, KIB, KF671242, KF671397, KF671533, KF671467, KF671328. ***O. chingii*** F. T. Wang & Tang, *Nie 3739* (KUN), China: Yunnan, Malipo, KF671243, KF671398, KF671534, KF671468, KF671329. ***O. chingii var. glaucifolius*** Wang et Tang, *Nie 2325* (KUN), China: Guangxi, Fangcheng, KF671244, KF671399, KF671535, KF671469, KF671330. ***O. lancangensis*** H. Li et Y. P. Yang, *Nie 2059* (KUN), China: Sichuan, Leibo, KF671245, KF671400, KF671536, KF671470, KF671331. *Nie 2095* (KUN), China: Sichuan, Leibo, KF671246, KF671401, KF671537, KF671471, KF671332. ***O. bockianus*** Diels, *Nie 3162* (KUN), China: Sichuan, Emeishan, KF671247, KF671402, -, KF671472, KF671333. *Nie 3170* (KUN), China: Sichuan, Emeishan, KF671248, -, -, -, -. ***O. bockianus* var. *angustifoliatus*** F. T. Wang & T. Tang, *Zdg 615* (KUN), China: Hunan, Yongshun, KF671249, KF671403, -, KF671473, KF671334. ***O. reversus*** Huang, *Gy 42* (KUN), China: Guangdong, Qingyuan, KF671266, KF671404, KF671538, KF671474, KF671335. *Gy 41* (KUN), China: Guangdong, Qingyuan, KF671267, KF671405, KF671539, KF671475, KF671336. ***O. longibracteatus*** H. Li et Y. PP. Yang, *Gy 36* (KUN), China: Guangdong, Qingyuan, KF671268, KF671406, KF671540, KF671476, KF671337. ***O. japonicus*** (Thunb.) Ker Gawl, *Hgwz 467* (KUN), China: Hunan, Sangzhi, KF671259, -, -, -, -. *Yh 0701* (KUN), China: Zhejiang, YuHang, KF671250, -, -, -, -. *Kifir 202* (KUN), China: Guangxi, IBK, KF671251, -, -, -, -. *Nie 2166* (KUN), China: Guizhou, Guiding, KF671252, -, -, -, -. *Nie 3798* (KUN), China: Yunnan, Malipo, KF671253, -, -, -, -. *Hgwz 674* (KUN), China: Yunnan, KIB, KF671255, KF671407, -, KF671477, KF671338. *Hgwz 1111* (KUN), China: Sichuan, Gongxian, KF671256, -, -, -, -. *Gy 19* (KUN), China: Sichuan, Shendu, KF671254, -, -, -, -. *Gy 44* (KUN), China: Guangdong, Qingyuan, KF671257, KF671408, KF671541, KF671478, KF671339. *Gy 49* (KUN), China: Guangdong, Zhaoqing, KF671258, KF671409, KF671542, KF671479, KF671340. ***O. multiflorus*** Y. Wan, *Nie 2356* (KUN), China: Guangxi, Longzhou, KF671260, KF671410, -, KF671480, KF671341. *Nie 2359* (KUN), China: Guangxi, Longzhou, KF671261, KF671411, KF671543, KF671481, KF671342. ***O. umbraticola*** Hance, *Nie 3290* (KUN), China: Yunnan, Dali, KF671262, -, -, -, -. *Gy 48* (KUN), China: Guangdong, Zhaoqing, KF671263, KF671412, KF671544, KF671482, KF671343. ***O. clarkei*** Hook. f., *Tibet 2795* (KUN), China: Tibet, Linzhi, KF671264, -, -, -, -. *Tibet 2777* (KUN), China: Tibet, Bomi, KF671265, KF671413, -, -, KF671344. ***O. intermedius*** D. Don, *Hgwz 470* (KUN), China: Hunan, Sangzhi, KF671269, KF671414, KF671545, KF671483, KF671345. *Hgwz 595* (KUN), China: Yunnan, Jinghong, KF671270, KF671415, KF671546, KF671484, KF671346. *Hgwz 616* (KUN), China: Yunnan, Menghai, KF671271, KF671416, KF671547, KF671485, KF671347. ***O. szechuansis*** Wang et Tang, *Hgwz 593* (KUN), China: Yunnan, Jinglong, KF671272, KF671417, KF671548, KF671486, KF671348. ***O. marmortus*** Pierre ex Rodrig., *Hgwz 625* (KUN), China: Yunnan, Lancang, KF671273, KF671418, KF671549, KF671487, KF671349. ***O. megalanthus*** Wang et Dai, *Hgwz 604* (KUN), China: Yunnan, Jinghong, KF671274, -, -, -, -. *Nie 3493* (KUN), China: Yunnan, Mengla, KF671275, KF671419, -, KF671488, KF671350. *B 098* (KUN), China: Yunnan, Mengla, KF671276, KF671420, -, KF671489, KF671351. ***O. yunnanensis*** S. C. Chen, *Gy 06* (KUN), China: Yunnan, KIB, KF671277, KF671421, -, KF671490, KF671352. ***O. corifolius*** Wang et Dai, *Nie 2395* (KUN), China: Guangxi, Napo, KF671278, KF671422, KF671550, KF671491, KF671353. *Nie 2399* (KUN), China: Yunnan, Funing, KF671279, KF671423, KF671551, KF671492, KF671354. ***O. zingiberaceus*** Wang et Dai, *Nie 3549* (KUN), China: Yunnan, Wenshan, KF671280, KF671424, KF671552, KF671493, KF671355. ***O. revolutus*** Wang et Dai, *Hgwz 556* (KUN), China: Yunnan, Mengla, KF671282, -, -, -, -. *Hgwz 621* (KUN), China: Yunnan, Menglian,

KF671283, KF671426, KF671554, KF671495, KF671357. *Hgwz 636* (KUN), China: Yunnan, Puer, KF671284, KF671427, KF671555, KF671496, KF671358. ***O. latifolius*** Rodrig., *Nie 2390* (KUN), China: Guangxi, Napo, KF671285, -, -, -, -. *Hgwz 518* (KUN), China: Yunnan, Maguan, KF671286, KF671428, KF671556, KF671497, KF671359. ***O. platyphyllus*** Merr. et Chun, *Nie 2338* (KUN), China: Guangxi, Longzhou, KF671287, KF671429, -, KF671498, KF671360. ***O. mairei*** Levl., *B 673* (KUN), China: Hunan, Zhangjiajie, KF671289, -, -, -, -. *Hgwz 471* (KUN), China: Hunan, Sangzhi, KF671290, KF671431, KF671558, KF671500, KF671362. ***O. tsaii*** Wang et Tang, *Nie 3550* (KUN), China: Yunnan, Wenshan, KF671300, -, -, -, -. *Gy 08* (KUN), China: Yunnan, Heilongtan, KF671301, KF671440, KF671567, KF671509, KF671371.

Sect. Peliosanthoides. ***O. amblyphyllus*** Wang et Dai, *Nie 2097* (KUN), China: Sichuan, Leibo, KF671291, KF671432, KF671559, KF671501, KF671363. *Hgwz 1165* (KUN), China: Yunnan, Weixin, KF671292, -, -, -, -. ***O. heterandrus*** Wang et Dai, *B 671* (KUN), China: Hunan, Zhangjiajie, KF671293, KF671433, KF671560, KF671502, KF671364. *Hgwz 459* (KUN), China: Hunan, Sangzhi, KF671294, KF671434, KF671561, KF671503, KF671365. *Hgwz 505* (KUN), China: Hunan, Shimen, KF671295, KF671435, KF671562, KF671504, KF671366. ***O. clavatus*** C. H. Wright, *Hgwz 462* (KUN), China: Hunan, Sangzhi, KF671296, KF671436, KF671563, KF671505, KF671367. ***O. sylvicola*** Wang et Tang, *Hgwz 00793* (KUN), China: Yunnan, KIB, KF671281, KF671425, KF671553, KF671494, KF671356. ***O. dracaenoides*** (Baker) Hook. f., *Nie 3587* (KUN), China: Yunnan, Malipo, KF671288, KF671430, KF671557, KF671499, KF671361. ***O. tienensis*** Wang et Tang, *Nie 3737* (KUN), China: Yunnan, Malipo, KF671297, KF671437, KF671564, KF671506, KF671368. ***O. peliosanthoides*** Wang et Tang, *Nie 3586* (KUN), China: Yunnan, Malipo, KF671298, KF671438, KF671565, KF671507, KF671369. ***O. pingbienensis*** Wang et Dai, *Nie 3927* (KUN), China: Yunnan, Pingbian, KF671299, KF671439, KF671566, KF671508, KF671370. ***O. tonkinensis*** Rodrig., *Nie 2345* (KUN), China: Guangxi, Longzhou, KF671302, KF671441, KF671568, KF671510, KF671372. *Nie 2357* (KUN), China: Guangxi, Jinlong, KF671303, KF671442, -, KF671511, KF671373.

Liriope Lour. ***L. graminifolia*** (L.) Baker, *Kifir 236* (KUN), China: Zhejiang, Hangzhou, KF671304, KF671443, -, KF671512, -. *Gy 34* (KUN), China: Guangdong, Qingyuan, KF671305, KF671444, KF671569, KF671513, KF671374. *Gy 45* (KUN), China: Guangdong, Qingyuan, KF671306, KF671445, KF671570, -, KF671375. ***L. platyphylla*** Wang et Tang, *Hgwz 891* (KUN), China: Jiangxi, Lushan, KF671307, KF671446, KF671571, KF671514, KF671376. *Hgwz 881* (KUN), China: Hunan, Shimen, KF671310, KF671449, KF671574, KF671517, KF671379. *Gy 37* (KUN), China: Guangdong, Qingyuan, KF671309, KF671448, KF671573, KF671516, KF671378. *Gy 43* (KUN), China: Guangdong, Qingyuan, KF671308, KF671447, KF671572, KF671515, KF671377. ***L. spicata*** Lour., *Gy 51* (KUN), China: Guangdong, Guangzhou, KF671312, KF671451, -, KF671518, KF671380. *Gy 59* (KUN), China: Beijing, Shijingshan, KF671311, KF671450, KF671575, -, -.

Peliosanthes Andr. ***P. macrostegia*** Hance, *Nie 2342* (KUN), China: Guangxi, Longzhou, KF671313, KF671452, KF671576, KF671519, KF671381. *Gy 09* (KUN), China: Hunan, Jishou, KF671314, KF671453, KF671577, KF671520, KF671382. ***P. macrophylla*** Wall. ex Baker, *Nie 3242* (KUN), China: Yunnan, Pingbian, KF671319, KF671458, KF671582, KF671525, KF671387. ***P. ophiopogoniodes*** F. T. Wang & Tang, *Hgwz 536* (KUN), China: Yunnan, Pingbian, KF671320, KF671459, KF671583, KF671526, KF671388. ***P. yunnanensis*** Wang et Tang, *Nie 3724* (KUN), China: Yunnan, Malipo, KF671322, KF671461, KF671585, KF671528, KF671390. *Gy 04* (KUN), China: Yunnan, KIB, KF671321, KF671460, KF671584, KF671527, KF671389. ***P. teta*** Andr., *Hgwz 341* (KUN), China: Yunnan, Longchuan, KF671315, KF671454, KF671578, KF671521, KF671383. ***P. sinica*** Wang et Tang, *Nie 3234* (KUN), China: Yunnan, Honghe, KF671316, KF671455, KF671579, KF671522, KF671384. *Nie 3498* (KUN), China: Yunnan, Mengla, KF671317, KF671456, KF671580, KF671523, KF671385. *Nie 3873* (KUN), China: Yunnan, Pingbian, KF671318, KF671457, KF671581, KF671524, KF671386.