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A NOVEL SPRING-FLOWERING *LIRIOPE* (ASPARAGACEAE) FROM CENTRAL CHINA

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ABSTRACT

Liriope vernalis Avent & Floden, **sp. nov.**, is described and illustrated from specimens originating from northeastern Sichuan Province, China. This species of *Liriope* is the earliest of the genus to come into the flower. Molecular phylogenetic analyses of the nuclear ribosomal ITS region support its distinction.

Liriope Lour. is a genus of 8–10 species that occurs across the Himalaya, China, Indochina, Japan, the Philippines, and Taiwan (Chen & Tamura 2000; Conran & Tamura 1998). Considerable taxonomic work has been devoted to *Liriope* and its sister genus *Ophiopogon* Ker Gawl (Bailey 1929; Wang & Tang 1951; Hume 1961; Hsu & Li 1981; Fantz 1993, 1994, 2008a, 2008b, 2009; Nesom 2010; Lattier et al. 2014), largely within the realm of horticulture due to their prolific use as a border and groundcover. Despite the attention to the systematics of *Liriope* and study of genome size and polyploidy (Lattier et al. 2014), there is no consensus on the number of species within *Liriope*.

Nesom (2010) provided an account of *Liriope* and *Ophiopogon* species in cultivation, recognizing *L. muscari* (Dcne.) L.H. Bailey and noting the distinctiveness of *L. platyphylla* F.T. Wang & Tang, while acknowledging that further study might reveal additional justifiable species in the *L. muscari* complex. Wang et al. (2014) included multiple samples of *L. platyphylla* in their phylogenetic analyses of *Ophiopogon* and these formed two sister clades, indicating molecular differentiation among the samples. Recently Xia et al. (2012) described *L. zhejiangensis* Xia & Li, which they presumed to be placed in the *L. muscari* group. Since the late 1990's, a distinctive *Liriope* originating from Sichuan Prov., China, has been cultivated in the USA and sold as "*L. vernalis*," an invalid name. Here we validate that name with a diagnosis, description, and designation of a type specimen, and we provide preliminary molecular data that support its distinction from other species.

Methods

DNA from *Liriope vernalis* (from the type collection) was extracted using D'Neasy Plant Minikits (Qiagen, Foster City, California), following manufacturer's protocols. PCR protocols followed Schilling et al. (2007) but used primers ITS4 and ITS-Leu. Sequencing was done at the University of Tennessee Genomics Core.

Other sequences of *Liriope*, *Ophiopogon*, *Peliosanthes*, and *Tupistra* were included from Genbank. Phylogenetic analyses and sequence comparisons were performed in Geneious 7.1.7 (Biomatters Ltd.). Alignments were done in MAFTT v7.017 (Katoh et al. 2002) and RaxML 7.2.8 (Stamatakis 2006) was used to assess relationships, with 1000 iterations to provide bootstrap support values.

Results

The results of the molecular analyses of ITS show *Liriope vernalis* embedded in an unresolved clade containing multiple disparately placed samples of *L. platyphylla* and *L. muscari* (Genbank number MH558170). The phylogeny recovers a paraphyletic *L. muscari* with respect to *L. spicata* in one clade and *L. vernalis* and *L. platyphylla* in the other clade (Fig. 1). *Liriope graminifolia* is sister to a clade with *L. muscari* and *L. spicata* samples, and this clade is in turn sister to the remainder of the unresolved samples, which includes *L. platyphylla*, *L. muscari*, and *L. vernalis*. Notably, *L. kansuensis* is included with *Ophiopogon*. The placement of *L. spicata* var. *prolifera* within the two samples of *L. muscari* is also noteworthy.

Sequence comparisons of the sample of *Liriope vernalis* show between 98–99% pairwise similarities (between 5–9 bp) relative to other *Liriope* in the *L. muscari* complex and down to between 94–97% pairwise similarity to the two *L. graminifolia* samples. Some changes are shared with *L. graminifolia* and others with some samples of *L. muscari*, with one fixed difference from all other *Liriope* samples available in Genbank.

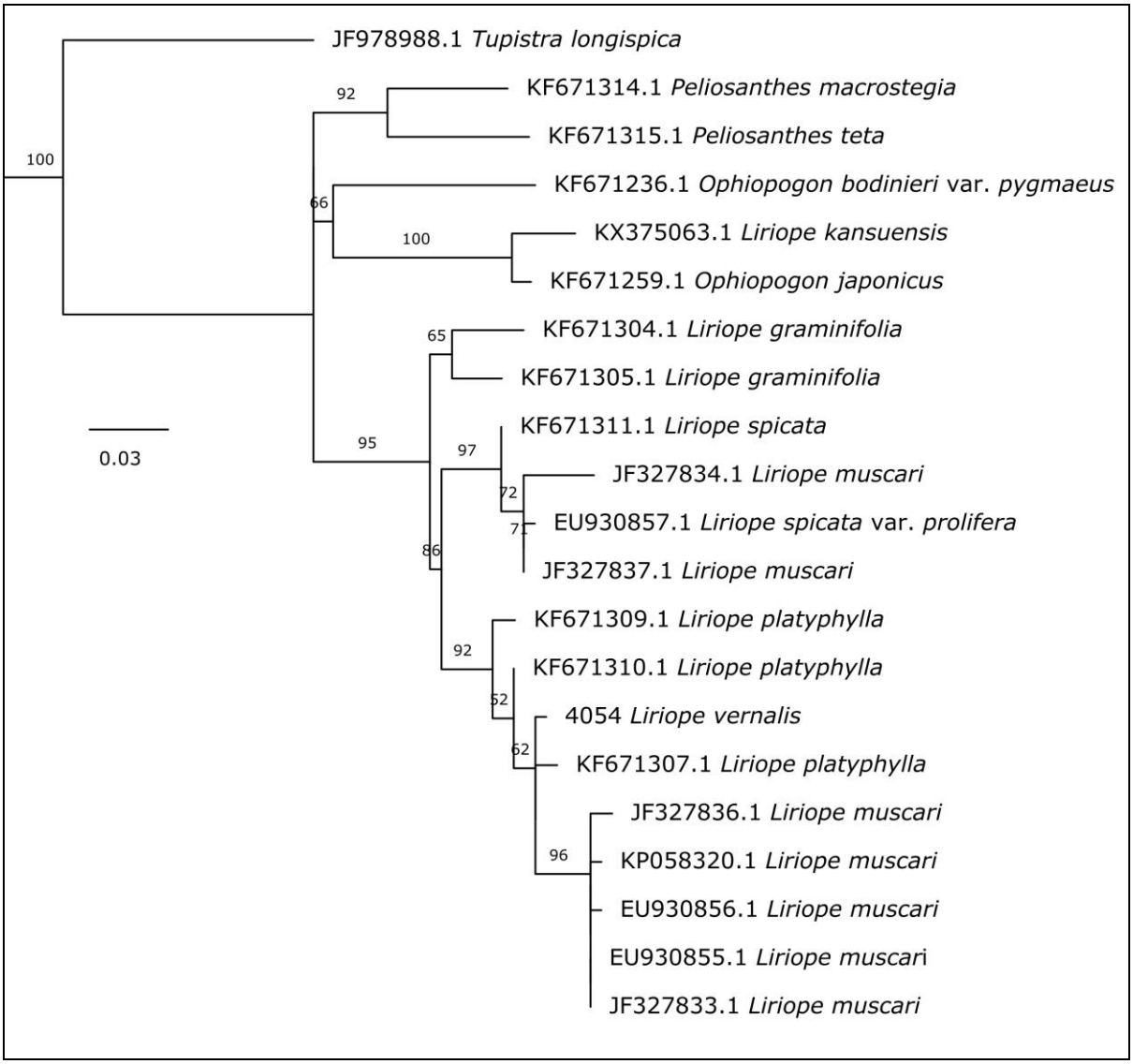

Figure 1. Phylogram of *Liriope* and outgroups showing the placement of *L. vernalis* embedded in multiple samples of *L. muscari* and *L. platyphylla*; derived from 500 maximum likelihood replications in RaxML.

Results of the molecular analyses of *Liriope* (Fig. 1) suggest that the evolutionary structure of *Liriope* is more complex and diverse than currently recognized. *Liriope vernalis* differs in ITS sequence from all other samples in Genbank. Both *Liriope muscari* and *L. platyphylla* likely comprise more than one entity, based on their polyphyly in the phylogeny and observable differences in morphology of accessions in cultivation. These results also support the treatment of Nesom (2010) in treating *L. muscari* and *L. platyphylla* as different species and shows that additional investigation is required to understand their taxonomy. Most accessions of *L. muscari* form the ultimate clade, but two others are placed with *L. spicata*. *Liriope platyphylla* samples form an unresolved grade with *L. vernalis* between them. The lack of resolution of *L. platyphylla* might be due to polyploidy that has led to genetic divergence between accessions (it has diploid and tetraploid forms—see Lattier et al. 2014) or simply due to limited sampling. Placement of *Liriope kansuensis* with *Ophiopogon* may be due to misidentification of the sample. Additionally, some of the samples of *L. muscari* in the *L. spicata* clade may be due to misidentifications. Future phylogenetic work should document the ploidy of accessions to determine monophyly of different ploidy levels of the same taxon and should be based on consistently identified vouchers.

LIRIOPE VERNALIS Avent & Floden, **sp. nov.** **TYPE**: **CHINA**. Sichuan Province. [N of Wanyuan], N 32°09.425', E 108°02.351', 2940 ft elevation, gentle slopes to almost flat area in heavy, red clay below open 30' tall pine forest (type of red pine), only *Mahonia* and tall *Liriope* here, *D. Probst CPC 5.5.01.1*, pressed from specimens cultivated by Avent and Floden from the original wild collection, collected on 7 May 2017 by AJF (holotype: MO; isotypes: CAS, NY, P, PE, US). Figures 2–4.

Differs from *Liriope longipedicellata* F.T. Wang & Tang (Fl. Reipubl. Pop. Sin. 15: 257. 1978) its roots with fusiform tubers (vs. without tubers), leaves 7–9-veined and not glaucous abaxially (vs. 5-veined and glaucous), the flower pedicels only 2–3 mm (vs. 5–8 mm), and with larger tepals 6–7 mm (vs. ca. 3 mm), and from *L. muscari* by the terminal pedicel articulation (vs. articulation at mid pedicel), leaves with fewer veins (7–9 vs 9–11), longer staminal filaments, and anthers shorter than the filaments. Differing from all *Liriope* species in its early spring flowering period (beginning late April to early June vs. July to September).

Perennial, essentially caespitose herbs, slowly increasing in clonal size through production of short rhizomes. Rhizomes 0.5–1 × 1–2 cm long, fleshy white-yellow. Roots thick, with fusiform tubers, tubers 0.5–2 cm long. Cataphylls (scale leaves) 2–14 cm long, purple-brown when fresh, apex callose, margins 2–4 mm wide, papery and membranous, center 9–12-veined. Leaves linear, 20–55 × 0.3–0.6 cm, apex obtuse, coriaceous, 7–9 veined, margins antrorsely scabrellous, ca. 10 teeth/mm. Scapes erect, dark green, 30–60 cm long including inflorescence, ridged in transverse section; inflorescence 10–20 cm with 15–30 nodes with flowers (naked for 2/3 of scape). Flowers in clusters of 3–7, typically 2–4; bracts near basal part of inflorescence up to 1 cm long, ovate-caudate acuminate, bracts near middle broadly ovate-lanceolate, and at distal portion linear lanceolate and only 2–3 mm long, bracteoles ca. 1 mm long; pedicel purple, 2–3 mm long, articulate at apex; tepals free, 6–7 × 0.2–0.3 mm, ovate-elliptic; anthers yellow, 1.7–2 mm, filaments 2–2.5 mm long; ovary ca. 1.5 mm, globose, shoulders slightly squared, style ca. 2 mm, stigma capitate; seeds black at maturity, globose, 5–8 mm in diameter. Flowering April–June, fruiting August–September.

Other specimens examined. CHINA. **Gansu Province**. Wen Xian, Motianling Shan, Baishui Jiang Nature Reserve, vicinity of town of Fanba, upstream along Heiyin Gou, remnant mixed deciduous forest with *Acer, Ulmus, Juglans, Castanea, Diospyros, Cercis*, etc., most remaining trees of economic importance, 32°42'12"N, 105°5'48"E, 825-890 m, dry slopes in forest and around ledges, 13 May 2007, *Boufford & Zhang 37608* (GH). **Hubei Province**: W Hupeh, May 2000, *Wilson 489*, as "*L. spic*. v. *longipes* var. *nov. Wang & Tang"* (K 846088, digital image). **Shaanxi Province**: Zhenping Co., Zhongbao Town, Jinping Village, 1800 m, 20 Jun 2005, *Chen et al 2829* (WUK 0495024 digital image <http://www.cvh.ac.cn/spm/WUK/0495024>).

Figure 2. A selected clone of *Liriope vernalis* in a propagation bed flowering at Juniper Level Botanic Garden from the seed collection from which the type gathering was made from. Photograph by Tony Avent, May 2017. This clone began from a seed collection in 2001.

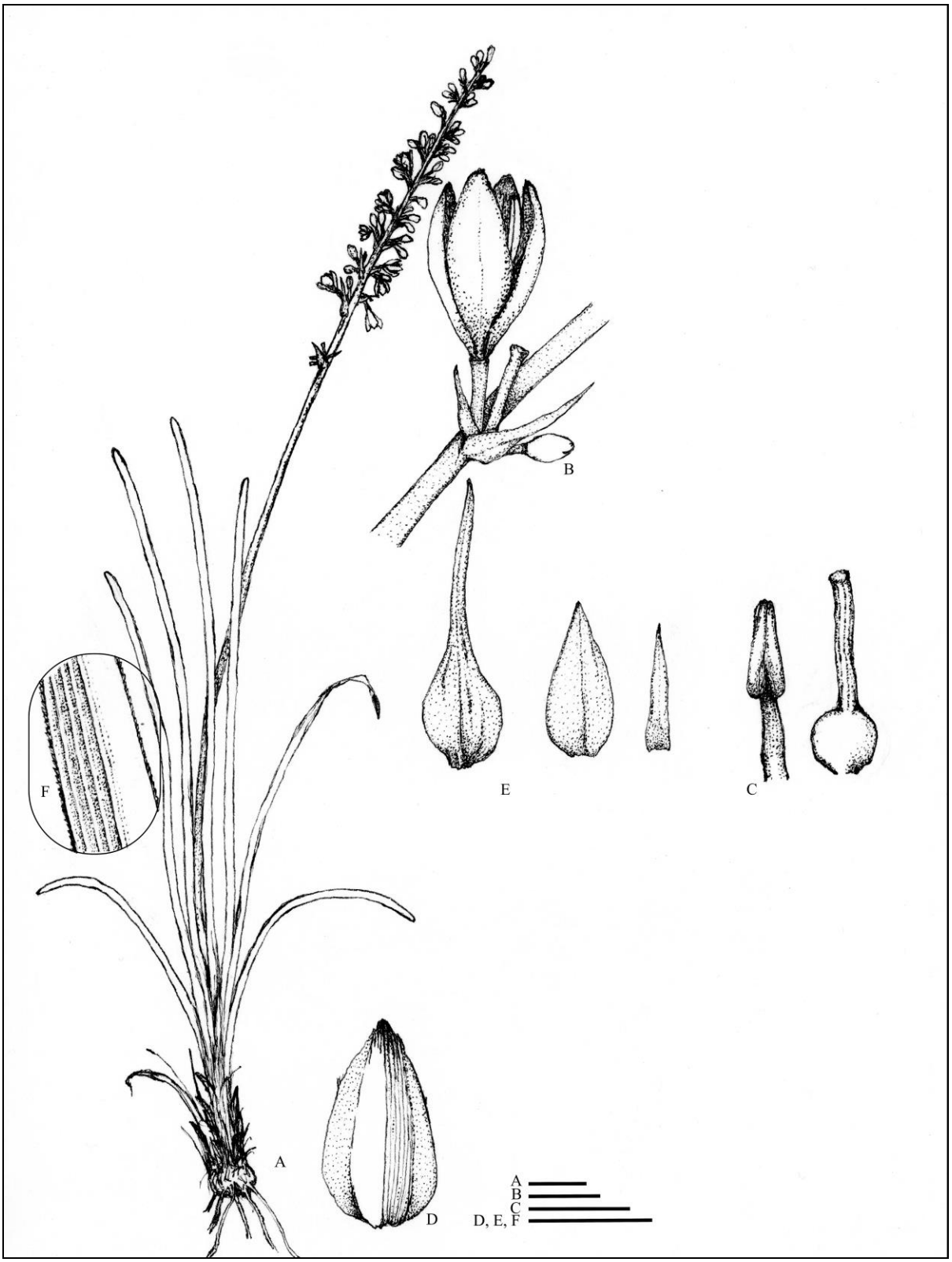

Figure 3. Line drawing from the holotype of *Liriope vernalis, D. Probst CPC 5.5.01.1* (MO). A. Whole culm. B. Single branch of the inflorescence scape. C. Anther and ovary. D. Basal scale leaves. E. Bracts of the inflorescence. F. Close up of leaf margin showing veins and scabrellous margin. Scale bars, A= 2 cm, B = 2 mm, C = 2 mm, D = 5 cm, E = 0.5 cm, F = 2 mm.

Figure 4. *Liriope vernalis* cultivated by AJF — a 7-year-old plant begun from a single seed-grown shoot from the same seed lot that the type originated from. This plant was removed from a 3-gallon pot and cleaned of growing medium. Clonal size increases slowly through short rhizomes — stolons are not produced. Root tubers are abundant. Top left: Whole clump. Top right: Root tubers. Bottom left: New shoot and center portion of plant dying out after 7 years of growth. Bottom right: Rhizome broken off from clump.

Figure 5. *Liriope vernalis* cultivated by AJF. New growth points adjacent to previous seasons shoots. From the same clump illustrated in Figure 4.

Liriope vernalis is known only from the Daba Shan in southeastern Gansu, southern Shaanxi, northeastern Sichuan, and western Hubei provinces, China. The distribution of this species is sympatric with that of the narrow endemic *L. longipedicellata* and also with *L. muscari*, though neither was observed near the type locality in May or September 2015 by AF. It occurs in grassy areas and open woodland at elevations of 800–1700 meters. Given the minimal number of collections that are documented (though over an expansive region), it should be considered data deficient (DD, IUCN 2017).

Flowering in *Liriope vernalis* begins in late April to early May and continues into early June. The epithet *vernalis* alludes to the early spring-flowering, well before all other species begin inflorescence growth. Infructescences with immature fruit are often present when sympatric species begin to flower. *Liriope longipedicellata*, which is endemic to the same region of China, begins flowering in July and can continue into September. The flowering period of *Liriope* is typically summer (Chen & Tamura 2000). In contrast, *Liriope vernalis* flowers early in the season before sympatric species and they are often in early fruit (in cultivation) before other species of *Liriope* have begun to flower. The only other species reported to flower as early as May is *L. spicata* (Chen & Tamura 2000), from which *L. vernalis* is well-differentiated in morphology by its non-stoloniferous and densely caespitose habit, more than 5-veined leaves, and its larger perigone. The type of *L. zhejiangensis* was collected on May 7, but the range of flowering dates of this species in the description is given as July–August. It and *L. vernalis* are well morphologically separated from one another in numerous characters.

Morphological delimitation of *Liriope* species is aided by characters that include whether plants are caespitose vs. mat-forming by stolons and/or rhizomes, the presence of root tubers, the number of lines on the abaxial leaf surfaces, the point of articulation on the flower pedicel, and the length of the anthers vs the stamen filaments (Hsu & Li 1981; Chen & Tamura 2000). Hsu and Li (1981) documented the significance of the presence or absence of stolons and rhizomes in species delimitation; these characters were also used by Chen and Tamura (2000). *Liriope vernalis* is distinctly clump-forming with very short rhizome internodes (essentially caespitose) and no production of stolons or longer rhizomes over a decade of cultivation by both authors (Fig. 4). It also produces root tubers in contrast to the sympatric *L. longipedicellata* and has more abaxial leaf veins, shorter pedicels, and larger tepals. *Liriope muscari* and *L. platyphylla* that were recovered in the same clade (Fig. 1) differ in their longer pedicels and subequal anthers and filaments.

Key to caespitose species of *Liriope*

(adapted from Chen & Tamura 2000 and Nesom 2010)

1. Pedicels 2–3 or 5–8 mm long; anthers slightly shorter than filaments.
    2. Roots without fleshy tubers; leaves glaucous abaxially, 5-veined; pedicels 5–8 mm long; tepals ca. 3 mm long ..... **Liriope longipedicellata**
    2. Roots with fleshy tubers; leaves not glaucous abaxially, 7–9-veined; pedicels 2–3 mm long; tepals 6–7 mm long ..... **Liriope vernalis**
1. Pedicels (2–) 4–5 mm long; anthers subequal to filaments.
    3. Scapes 20–30 cm long, rachis 6–12 cm long; fascicles 35–50; perigone tube short, <0.5 mm; anthers 1.5 mm long; anthers 1.6–1.8 mm long; bracts with hyaline margins to near apex ..... **Liriope muscari**
    3. Scapes 55–60 (–100) cm long, rachis 18–24 cm long; fascicles 60–80; perigone tube 1–1.2 mm long; anthers 1.5 mm long; bracts with hyaline margins basally ..... **Liriope platyphylla**

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