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MOLECULAR EVIDENCE SUPPORTS RECOGNITION OF *LOBELIA ROGERSII* (CAMPANULACEAE) AS A DISTINCT SPECIES

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ABSTRACT

Data from the commonly used nuclear ribosomal ITS molecular barcode marker was used to assess whether *Lobelia rogersii* represents a series of hybrids between *L. brevifolia* and *L. puberula*. Analysis of numerous samples revealed that all three species are distinct from one another at multiple positions for the ITS marker. There was no evidence in the DNA sequence from samples of *L. rogersii* of either sequence or positional polymorphism that would suggest a recent hybrid origin. These results support the recognition of *L. rogersii* as a distinct species.

The taxonomy of *Lobelia* sect. *Lobelia*, a distinctive group of species distributed primarily in the southeastern USA, was recently reviewed by Spaulding and Barger (2016). They proposed that *L. rogersii* Bowden, which was originally described as consisting of hybrids (McVaugh 1936; Bowden 1961), was actually a distinct species, though possibly of hybrid origin. The suggested parents were *L. brevifolia* Nutt. ex A. DC. and *L. puberula* Michx., based on morphological characters. Populations of plants meeting the description of *L. rogersii* are found in southern Louisiana, extending to the panhandle of Florida and slightly into Georgia, and are widespread in Alabama (Spaulding & Barger 2016). The consistent morphology and presence of *L. rogersii* in areas beyond the distribution of *L. brevifolia* suggest that these are not simply of recent hybrid origin.

Historically, few molecular phylogenetic data have been available for *Lobelia* sect. *Lobelia* that might allow testing of the hypothesis of a hybrid origin for *L. rogersii*. However, a recent molecular barcoding study showed that members of the genus found in Tennessee are all distinctive for the commonly used molecular barcode marker nuclear ribosomal ITS (Schilling et al. 2025), which would provide a data source to test for a possible hybrid origin of *L. rogersii*. Neither *L. brevifolia* nor *L. rogersii* occur in Tennessee, so they were not sampled as part of the earlier study. Here the sampling for sect. *Lobelia* is extended to include samples of both taxa, as well as other members from the southeastern USA, to test the possible hybrid origin of *L. rogersii*. Because ITS is biparentally inherited, the marker can be used to detect hybrids if the two parents differ in their sequences for this region.

Materials and Methods

Plant samples of *Lobelia* were obtained primarily from herbarium specimens (Appendix 1). Sampling included four samples each of *L. rogersii* (including one originally identified as *L. georgiana* McVaugh) and *L. brevifolia* (including one downloaded from GenBank) and two new samples of *L. puberula* in addition to one previously sampled. Samples of other members of sect. *Lobelia,* as well as sequence data on deposit at GenBank, were also included.

DNA was extracted from leaf samples, using the DNeasy Plant Mini Kit protocol (Qiagen, Valencia CA). PCR amplifications and sequencing of the ITS region followed Schilling et al. (2007). A few samples required the use of the internal primers, "5.8S 79 for" and "ITS 5.8SR," for sequencing to obtain clean sequences, either because of fungal contamination or because of length polymorphisms (Schilling et al. 2007). GenBank accession numbers are provided in Appendix 1. A maximum likelihood tree was generated using RAxML implemented in the Geneious software package. GenBank sequences for *L. jalicensis* McVaugh (sect. *Cryptostemon*) and *L. laxiflora* Kunth (sect. *Homochilus*) were utilized as the outgroup.

Results

The ITS sequences for members of *Lobelia* sect. *Lobelia* ranged in length from 602-639 bp, and alignment required insertion of numerous gaps. Sequences for *Lobelia* sect. *Lobelia* samples all exhibited a 27 bp gap in ITS-1 relative to the outgroup samples, *L. jalicensis* and *L. laxiflora*. Several samples gave sequence results that suggested the presence of sequence length polymorphisms. Some of these could be resolved by sequencing with both primers and could be inferred to involve a single insertion or deletion; in other cases, notably involving polyploid species, the result was more complex and was not resolved for the current study. Sequence data was not obtained for *L. batsonii* Pittman & Sorrie, *L. elongata* Small, *L. homophylla* F. Wimmer, and *L. kalmii* L., and multiple polymorphisms inhibited obtaining results for *L. apalachicolensis* Spaulding, Barger, & Horne and *L. feayana* A. Gray.

The sequences obtained for samples of *Lobelia rogersii* showed no evidence of either length or positional polymorphisms and were nearly identical to one another, differing by 0-3 changes. The sequences for three samples of *L. brevifolia* were identical to one another, and the fourth showed a polymorphism for presence/absence of a 3 bp duplicated segment at the very start of the ITS-1 region but was otherwise also identical to the others. The sequences for the three samples of *L. puberula* were nearly identical to one another, with one differing at two bp positions. The sequences for *L. rogersii* differed from those of *L. brevifolia* by 6 bp positions and two gaps and from those of *L. puberula* by 12 bp positions and two gaps. The sequences of *L. brevifolia* differed from those of *L. puberula* by 14 bp positions and 2 gaps. There was no evidence of polymorphism in the sequences of *L. rogersii* at any of the positions where there were differences between *L. brevifolia* and *L. puberula.*

Phylogenetic analysis of the ITS sequence data (Fig. 1) showed that samples of *Lobelia* sect. *Lobelia* formed a monophyletic group relative to the outgroup samples *L. jalicensis* (sect. *Cryptostemon*) and *L. laxiflora* (sect. *Homochilus*). The first branching (but an only weakly supported group) included the widely distributed and obligate selfer *L. inflata* L., together with the rare *L. boykinii* Torr. & Gray ex A. DC. A subsequent split separated two large groups of species. The first included the widespread *L. siphilitica* L. and *L. cardinalis* L., together with a number of other samples that included some polyploid species. Neither *L. siphilitica* nor *L. cardinalis* were monophyletic; notably, a sample of *L. cardinalis* from western North America was placed at the base of a clade that included the other two, eastern North American samples, together with *L. georgiana, L. glandulosa* Walt., and *L. amoena* Michx. In the other major group, samples of *L. canbyi* A. Gray and *L. dortmanna* L. formed a weakly supported clade, sister to a well-supported clade with samples of *L. puberula, L. brevifolia*, and *L. rogersii*. Each of these last three species formed well-supported individual clades.

Discussion

The primary result of this study is to demonstrate that *Lobelia rogersii* is a distinct species and not a series of hybrids. Its putative parents, *L. brevifolia* and *L. puberula*, differ from one another by 16 changes in ITS sequence, but there was no indication of polymorphism at any of these positions in samples of *L. rogersii*, and they differ from both suggested parents by eight and 14 changes, respectively. This result supports the recommendation of Spaulding and Barger (2016) to recognize *L. rogersii* as distinct.

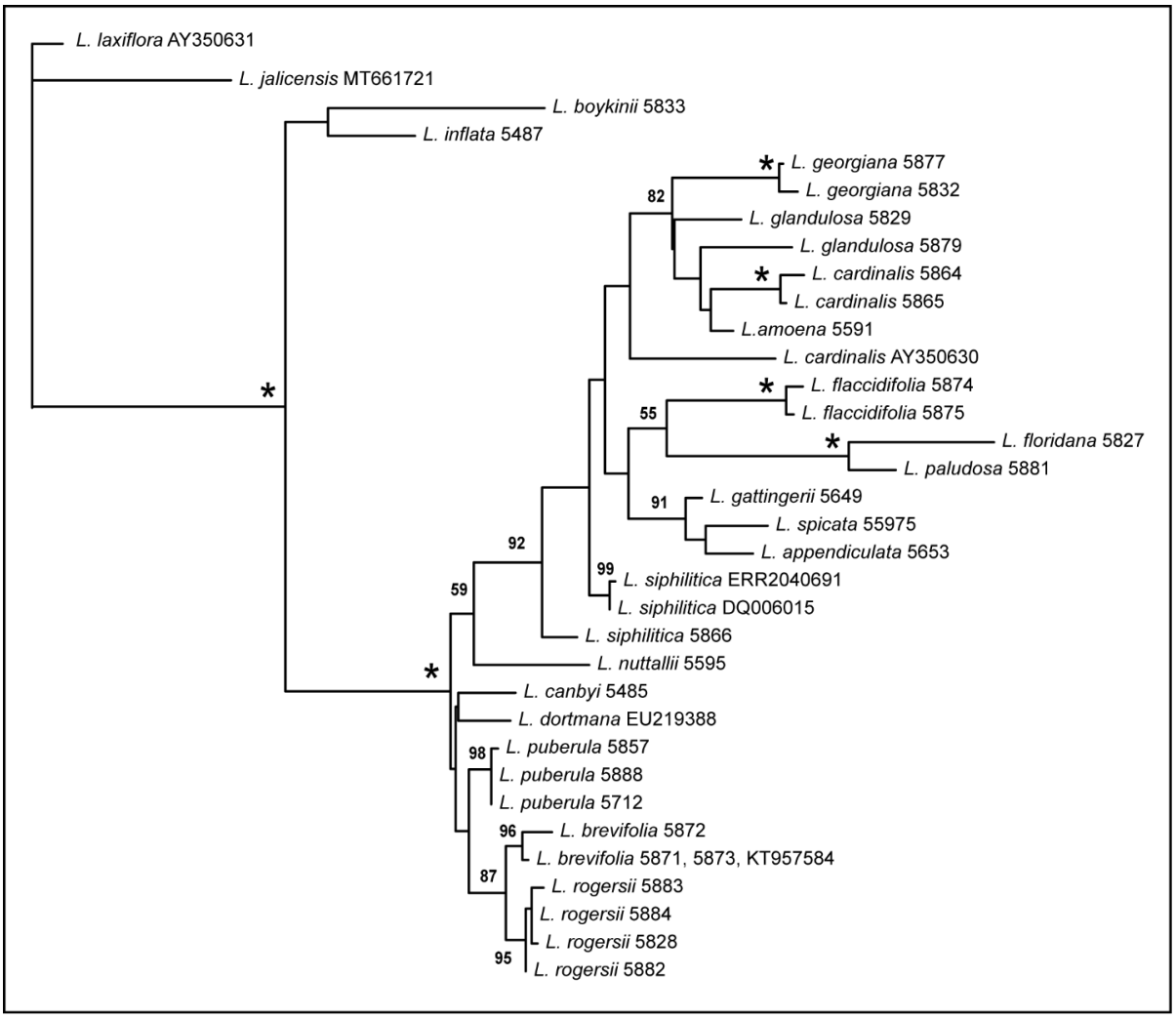

Figure 1. Phylogram of relationships among species of *Lobelia* sect. *Lobelia* showing placement of samples of *L. rogersii* (bottom) as distinct from both *L puberula* and *L. brevifolia*, based on RAxML analysis of nuclear ribosomal ITS sequence data; percent bootstrap support (500 replicates) shown above branches, * = 100%. DNA numbers provided in Appendix 1.

The phylogenetic analysis placed samples of the red-flowered *Lobelia cardinalis* in a relatively derived clade, suggesting that the red flower color evolved independently in this species compared to other red-flowered members of the genus, such as *L. laxiflora*. The separation of a sample of *L. cardinalis* from Arizona (AY350630) from our newly obtained sequences for samples of the species from eastern North America suggests that this widespread species is composed of two or more cryptic species, although further sampling is needed. There were distinctive sequence motifs from *L. cardinalis* in the ITS sequences of the tetraploids *L. amoena* and *L. glandulosa*, suggesting that *L. cardinalis* is

likely to be one of the parents of both; further work will be needed to identify the second parent in each. In contrast, the hexaploids *L. floridana* and *L. paludosa* Nutt. exhibited a completely clean ITS sequence, suggesting that they may be autoploids.

For most species of *Lobelia* sect. *Lobelia*, the ITS region will form a good barcode for species level identification. This study exemplifies this by the case of a specimen (*Kral 86973*, TENN) collected initially as *L. georgiana* but shown by the ITS sequence result to be *L. rogersii* and confirmed by re-examination of the specimen.

KEY TO RELATED SPECIES

1. Leaves mostly less than 3 cm long and very numerous (usually more than 20); calyx sinuses with well-developed, overlapping auricles that often completely cover the calyx tube; calyx lobes pectinately toothed ..... **Lobelia brevifolia** (Fig. 2)
1. Leaves 4–15 cm long and less numerous (usually less than 20); calyx sinuses lacking auricles or with conspicuous, non-overlapping auricles that rarely obscure the calyx tube; calyx lobes toothed or entire.
    2. Calyx sinuses with prominent, drooping auricles that often cover half of the calyx tube; calyx lobes distinctly toothed.....**Lobelia rogersii** (Fig. 3)
    2. Calyx sinuses lacking auricles or with very small, inconspicuous ones; calyx lobes typically entire, occasionally with a few scattered teeth ..... **Lobelia puberula** (Fig. 4)

Figure 2. *Lobelia brevifolia*. Walton Co., Florida, 20 Oct 2014. Photos: Melanie Taylor & Dan Spaulding.

Figure 3. *Lobelia rogersii*. Etowah Co., Alabama, 24 Sep 2014. Photos: Wayne Barger.

Figure 4. *Lobelia puberula*. Cleburne Co., Alabama, 2 Sep 2020. Photos: Dan Spaulding.

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Appendix 1. Samples of *Lobelia* analyzed for nrDNA ITS sequence, with GenBank accession number listed in parentheses.

Lobelia amoena Michx., Tennessee, Van Buren Co., *McNeilus 00-051*, 09 Sep 2000, TENN, DNA 5593 (OR392552). ***Lobelia appendiculata*** A.DC., Arkansas, Sebastian Co., *Thomas 165533*, 10 May 2000, TENN, DNA 5618, (OR392551). ***Lobelia boykinii*** Torr. & Gray ex A.DC., Alabama, Houston Co., *McDonald 10528*, 17 May 1997, MO, DNA 5833 (PV662412). ***Lobelia brevifolia*** Nutt. ex A.DC., Alabama, Mobile Co., *Horne 2445*, 27 Oct 2013, AMAL, DNA 5872 (PV662414); Baldwin Co., *Bryson 25429*, 30 Sep 2017, AMAL, DNA 5871 (PV662415), Florida, Walton Co., *Spaulding 14235*, 14 Oct 2014, AMAL, DNA 5873 (PV662413). ***Lobelia canbyi*** A. Gray, Tennessee, Van Buren Co., McNeilus 00-1051, 09 Sep 2000, TENN, DNA 5485 (OR392552). ***Lobelia cardinalis*** L., Tennessee, Knox Co., *Benoit* s.n., 18 Sep 2024, TENN, DNA 5864 (PV662416), *Schilling 24-4*, 21 Sep 2024, TENN, DNA 5865 (PV662417). ***Lobelia flaccidifolia*** Small, Alabama, Coffee Co., *Diamond 16460*, 14 Jul 2013, AMAL, DNA 5873 (PV662418); Crenshaw Co., *Barger & Taylor SP2051*, 08 Jun 2013, AMAL, DNA 5874 (PV662419). ***Lobelia floridana*** Chapm., Alabama, Geneva Co., *Kral 81176*, 17 Jul 1992, TENN, DNA 5827 (PV662420). ***Lobelia gattingeri*** A. Gray, Tennessee, Wilson Co., *McNeilus 00-501*, 27 May 2000, TENN, DNA 5649 (OR392553). ***Lobelia georgiana*** McVaugh, Alabama, Calhoun Co., *Spaulding 14177*, 25 Sep 2014, AMAL, DNA 5877 (PV662421), Florida, Leon Co., *Uncapher 24*, 09 Oct 1996, MO, DNA 5832 (PV662422). ***Lobelia glandulosa*** Walter, Florida, Duval Co., *Slaughter 17142*, 30 Oct 2008, TENN, DNA 5829 (PV662424), Wakulla Co., *Spaulding 14224*, 18 Oct 2014, AMAL, DNA 5879 (PV662423). ***Lobelia inflata*** L., Tennessee, Hamblen Co., *Floden & Schilling 2225*, 20 Aug 2012, TENN, DNA 5487 (OR392555). ***Lobelia nuttallii*** Roem. & Schult., Tennessee, Campbell Co., *Floden & Wofford 587*, 2 Jul 2008, TENN, DNA 5595 (OR392552). ***Lobelia paludosa*** Nutt., Florida, Wakulla Co., *Spaulding 14227*, 19 Oct 2014, AMAL, DNA 5881 (PV662425). ***Lobelia puberula*** Michx., Alabama, Randolph Co., *Kelly s.n.*, 06 Sep 2024, TENN, DNA 5857 (PV662427); Tennessee, Knox Co., *Schilling s.n.*, 04 Sep 2021, TENN, DNA 5712 (OR392557); Texas, Jasper Co., *Hill 528*, 01 Oct 2024, TENN, DNA 5888 (PV662426). ***Lobelia rogersii*** Bowden, Alabama, Autauga Co., *Keener 8659*, 05 Oct 2014, AMAL, DNA 5882 (PV662428), Cherokee Co., *Spaulding 14137*, 20 Sep 2014, AMAL, DNA 5883 (PV662429), Conecuh Co., *Kral 86973*, 16 Oct 1996, TENN, DNA 5828 (PV662431), Mobile Co., *Horne 3037*, AMAL, 31 Oct 2015, DNA 5884 (PV662430). ***Lobelia siphilitica*** L., Tennessee, Knox Co., *Benoit s.n. 9/21/2024*, 21 Sep 2024, TENN, DNA 5866 (PV662432). ***Lobelia spicata*** Lam., Tennessee, Campbell Co., *Floden & Wofford 566*, 2 Jul 2008, DNA 5597 (OR392558).