Western North American Naturalist 72(3), © 2012, pp. 407–411

TAXONOMIC CLARIFICATION OF *LUPINUS OREGANUS* AND *LUPINUS BIDDLEI* IN THE PACIFIC NORTHWEST, USA

Paul M. Severns[1], Stephen C. Meyers[2], and Tri Tran[2]

ABSTRACT.—Through a phylogenetic study using *LEGCYC1A* nucleotide sequences and a survey of historical botanical literature, we propose clarifications in the nomenclature of *Lupinus oreganus* and *Lupinus biddlei*. The former taxon has been incorrectly classified as *Lupinus sulphureus* ssp. *kincaidii* and recently as *L. oreganus* var. *kincaidii*. The latter has recently been incorrectly delimited as *L. oreganus* var. *oreganus*.

RESUMEN.—Se proponen aclaraciones en la nomenclatura de *Lupinus oreganus* y *Lupinus biddlei* a través de un estudio filogenético que utiliza secuencias nucleótidas de *LEGCYC1A* y una revisión de literatura botánica histórica. El primer taxón mencionado se ha clasificado incorrectamente como *Lupinus sulphureus* ssp. *kincaidii* y recientemente como *L. oreganus* var. *kincaidii*. El segundo taxón mencionado ha sido delimitado erróneamente como *L. oreganus* var. *oreganus.*

The taxonomic status of *Lupinus sulphureus* Douglas ex Hook. ssp. *kincaidii* (C.P. Sm.) L. Phillips (Kincaid's lupine), a threatened species endemic to western Oregon and southwestern Washington, USA (Wilson et al. 2003), has confused botanists for nearly a century. This perennial lupine with purple to pink and occasionally cream-colored flowers is found primarily in Willamette Valley grasslands and was first described as *Lupinus oreganus* from a type collected in Eugene, Lane County, Oregon (Heller 1911). Heller (1912) also described another phenotypically similar species, *Lupinus amabilis*, also from Eugene, Oregon, but failed to provide a description that adequately differentiated *L. oreganus* from *L. amabilis*. Smith (1924), while synonymizing several of Heller's recently described *Lupinus* species, also named a variety of *L. oreganus* (*L. oreganus* var. *kincaidii*) from Corvallis, Benton County, Oregon (a locality approximately 80 km north of Eugene), in honor of Trevor Kincaid. Unfortunately, Smith (1924) did not provide a trait for distinguishing *L. oreganus* var. *kincaidii* from *L. oreganus* var. *oreganus*, even though he recognized them as distinct taxa. A few years later, C.P. Smith (1927) again synonymized many more of Heller's western North American *Lupinus* species. In this revision, *L. amabilis* was synonymized with *L. oreganus* var. *oreganus,* but without justification, *L. oreganus* var. *kincaidii* was retained as a distinct taxon despite referenced collections of *L. oreganus* var. *oreganus* to the south and north of Corvallis (Smith 1927).

Until Phillips revised western North American lupines in 1955, there were hundreds of instances where Smith's eye for slight variation in *Lupinus* floral morphology created an overabundance of poorly defined lupine taxa throughout western North America (Phillips 1955). Phillips often justified his revision of western North American *Lupinus* taxa (Phillips 1955), but he did not provide a reason for recognizing *L. oreganus* as a subspecies of *L. sulphureus*. Considering that flowers of *L. sulphureus* are typically yellow with a cup-shaped banner and do not share the strongly reflexed "ruffled banner" and unbranched raceme characteristic of *L. oreganus*, Phillips' revision did nothing to clarify the taxonomic positioning of "Kincaid's lupine." Regardless of the lack of morphological support in Phillips' delimitation, *Lupinus sulphureus* ssp. *kincaidii* has remained the formally recognized name for this endemic Willamette Valley lupine for the last 55 years.

In 2011, the United States Department of Agriculture PLANTS database (USDA, NRCS 2011) listed *L. sulphureus* ssp. *kincaidii* as a synonym for *L. oreganus* var. *kincaidii* and *Lupinus biddlei* L.F. Hend. ex C.P. Sm. s.s. (a taxon with a range restricted to southeastern Oregon; Smith 1939) as the nominate subspecies,

[1]Washington State University–Vancouver, School of Biological Sciences, 14204 NE Salmon Creek Ave., Vancouver, WA 98686. E-mail: paulseverns@hotmail.com; paul.severns@wsu.vancouver.edu
[2]Department of Botany and Plant Pathology, Oregon State University, 2082 Cordley Hall, Corvallis, OR 97331.

L. oreganus var. *oreganus*. Although the authority for this change is not attributed, the circumscription is clearly in error as *L. oreganus* var. *oreganus* was originally described from the Willamette Valley of western Oregon (Heller 1911), and no taxonomic affiliation between *L. oreganus* and *L. biddlei* was ever recognized by Heller, Smith, or Phillips. In fact, Barneby (1989) considered *L. biddlei* to be synonymous with *Lupinus polyphyllus* Lindl. var. *prunophilus* (M.E. Jones) L. Phillips, which is clearly not sister to *L. oreganus*. Nonetheless, the listing by USDA PLANTS has generated more complexity surrounding "Kincaid's lupine," rendering the taxonomic situation even more ambiguous for the local, state, and federal agency botanists who rely on USDA PLANTS until the *Flora of North America* treatments are published.

The assignment of *L. biddlei* to *L. oreganus* var. *oreganus* (sensu USDA PLANTS) and *L. oreganus* to *L. sulphureus* ssp. *kincaidii* (sensu Phillips) are testable phylogenetic hypotheses. If Phillips' taxonomy is correct, *L. sulphureus* spp. *kincaidii* should be derived from, or at least sister to, *L. sulphureus* ssp. *sulphureus* in a phylogenetic analysis. Likewise, if the USDA PLANTS nomenclature is correct Kincaid's lupine (*L. oreganus* var. *kincaidii*) should be derived from *L. oreganus* var. *oreganus* (= *L. biddlei*) or sister to it. Since both "Kincaid's lupine" (U.S. Fish and Wildlife Service Threatened Species) and *L. biddlei* (globally rare but not imperiled—Oregon Biodiversity Information Center 2010) are species of conservation concern, their taxonomic status has legal ramifications. We conducted a limited phylogenetic study with *LEGCYC1A*, a gene that appears to yield greater phylogenetic resolution than other currently sequenced DNA regions within *Lupinus* (Ree et al. 2004, Hughes and Eastwood 2006), to test the derivation hypotheses proposed by Phillips (1955) and listed on USDA PLANTS.

METHODS

Leaf tissue was collected throughout the range of Kincaid's lupine (Douglas Co., Oregon to southwest Washington). The collections included a combination of fresh leaf tissue and herbarium specimens for broadly sympatric western Oregon *Lupinus* taxa, including *Lupinus onustus* S. Watson, formerly described as *Lupinus oreganus* var. *pusillulus* (Smith 1924) from southwestern Oregon (Table 1). About half of the lupine locations from which taxa were collected in this study grew in protected areas, so vouchers were not collected. However, most of the populations from which plants were sampled have voucher specimens in the Oregon State University collection (OSC). Voucher numbers for herbarium specimens used in the phylogeny are as follows: *L. albicaulis* (OSC174766, OSC105375, OSC105976), *L. biddlei* (OSC21105, OSC200872), *L. littoralis* (OSC210076, OSC200872), *L. onustus* (OSC 207526, OSC207605), *L. oreganus* (OSC165929), *L. sulphureus* ssp. *sulphureus* (OSC199950), *L. rivularis* (OSC288359, OSC177600, OSC 214252).

Genomic DNA from 50–100 mg of leaf tissue per individual was extracted with the FastDNA® kit and FastPrep® instrument according to manufacturer-recommended protocols (QBiogene, Inc., CA). *LEGCYC1A* (Citerne 2005) was amplified from 0.5–1.2 µL (10–30 ng) of genomic DNA, 1.2 µL of 10X Thermopol Buffer (New England Biolabs), 0.6 µL of 100X BSA, 1.0 µL (0.25mM) of each dNTP, 0.5 µL of each forward and reverse primer (5 pM), 0.5–1.0 units of Taq polymerase (New England Biolabs), and 7.0 µL of ddH_20 for an approximate 11-µL reaction volume. *LEGCYC1A* is a member of the *CYCLOIDEA* (circa 1100 bp) family of proteins (transcription factors) and is involved in the regulation of flower zygomorphy (Citerne et al. 2000, 2003, Ree et al. 2004, Howarth and Donoghue 2006). In papilinoid legumes, *LEGCYC1A* appears to be involved in banner petal development (Feng et al. 2006).

Sanger sequencing was performed by the Center for Genome Research and Biocomputing at Oregon State University (representative GenBank accession numbers JN628016–JN628018). Sequences were aligned with the program BioEdit for Windows 95/98 (Hall 1999). Gaps were scored as missing data, and heterozygote loci were coded according to IUPAC nucleotide combinations. Modeltest 3.7 (Posada and Crandall 1998) was used to select the model rate (F81) that best fit the data set, and the phylogenetic analysis was conducted using MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003). Bayesian searches were conducted with 1 cold and 3 heated Markov chains over 2 million generations, with sampling every 100 generations. All trees generated

TABLE 1. Approximate localities for genotyped *Lupinus* species.

Species	Location	County
L. albicaulis	Pigeon Butte, Finley National Wildlife Refuge, OR	Benton
	Coburg Ridge, Nature Conservancy Preserve, OR	Lane
	Hwy. 20, Fernview Campground, OR	Linn
	Mary's Peak (summit), OR	Benton
	Decker Rd., ~0.5 mi W of Linville Ln., OR	Benton
	Armitage County Park, OR	Lane
	Dallas, OR	Polk
L. arbustus	Coburg Ridge, Nature Conservancy Preserve, OR	Lane
	Blanton Heights Rd., Eugene, OR	Lane
	Browder Ridge Trail, OR	Linn
	Pigeon Butte, Finley National Wildlife Refuge, OR	Benton
	Basket Butte, Basket Slough National Wildlife Refuge, OR	Polk
L. biddlei	Fields-Denio Rd., 19.3 mi N of Pike Crk. Rd. junction, OR	Harney
	Rome Quad, 0.5 mi N of Crooked Crk. Ranch, OR	Malheur
L. latifolius	Mary's Peak (summit), OR	Benton
	Browder Ridge Trail, OR	Linn
L. lepidus	Mary's Peak (summit), OR	Benton
L. leucophyllus	Mt. Emily Summit Rd., 2.5 mi E of Hwy. 84, OR	Umatilla
	Lick Creek Campground, OR	Wallowa
L. littoralis	2 mi N of Waldport, Hwy. 101, OR	Lincoln
	Baker Beach Rd., 6 mi N of Florence, Hwy. 101, OR	Lane
L. onustus	Illinois River Rd., W of Selma, milepost 2, OR	Josephine
L. oreganus	Boistfort, WA	Clark
	Rockin' Easy Ranch, OR	Polk
	Basket Butte, Basket Slough National Wildlife Refuge, OR	Polk
	West Hills Rd., Corvallis, OR	Benton
	Fern Ridge Reservoir, OR	Lane
	Willow Creek Nature Preserve, Eugene, OR	Lane
	Callahan Ridge, OR	Douglas
L. polyphyllus	Near Finley Wildlife Refuge, Hwy. 99, OR	Benton
var. *polyphyllus*	Lorane, OR	Lane
L. rivularis	Armitage County Park, OR	Lane
	Richardson Butte, Fern Ridge Reservoir, OR	Lane
	McDonald State Forest, Rd. 680, OR	Benton
	3.6 mi N of Hwy. 22 at Rickreal on 99W, OR	Polk
L. sulphureus	11 mi SE of Pendleton, Hwy. 30, OR	Umatilla
var. *sulphureus*		

within the first 2000 generations of the burn-in period were discarded, and posterior probability confidence values were based only on trees found in the stationary phase.

RESULTS AND DISCUSSION

The *LEGCYC1A* phylogeny did not support Phillips' (1955) hypothesis that Kincaid's lupine is derived from or sister to *L. sulphureus* or the USDA PLANTS hypothesis that *L. biddlei* and *L. oreganus* var. *kincaidii* are sister taxa (Fig. 1). Although *L. oreganus* occurred within several different clades and not all *Lupinus* species formed monophyletic groups (Fig. 1), these patterns can be explained by either methodological or biological reasons.

We did not clone "haplotypes" of the *LEGCYC1A* gene as is typically done in other *Lupinus* phylogenetic studies (Ree et al. 2004, Hughes and Eastwood 2006), but rather, we sequenced both haplotypes simultaneously because funding limited the sequencing effort. As a result, multilocus heterozygous gene copies were found in all taxa, rendering it impossible to determine linkage among the multilocus heterozygous copies of the gene. IUPAC coding at heterozygous nucleotide positions likely diminished our ability to resolve lineages, given that most taxa had more than 10 heterozygous loci. Biologically, it is clear that *Lupinus oreganus* hybridizes and introgresses with sympatrically occurring *L. arbustus* (Liston et al. 1995, Severns in preparation) and *L. albicaulis* (Severns in preparation), and predictably, both taxa group with *L. oreganus* in the phylogeny (Fig. 1). Perennial lupines have long been recognized for their tendency to

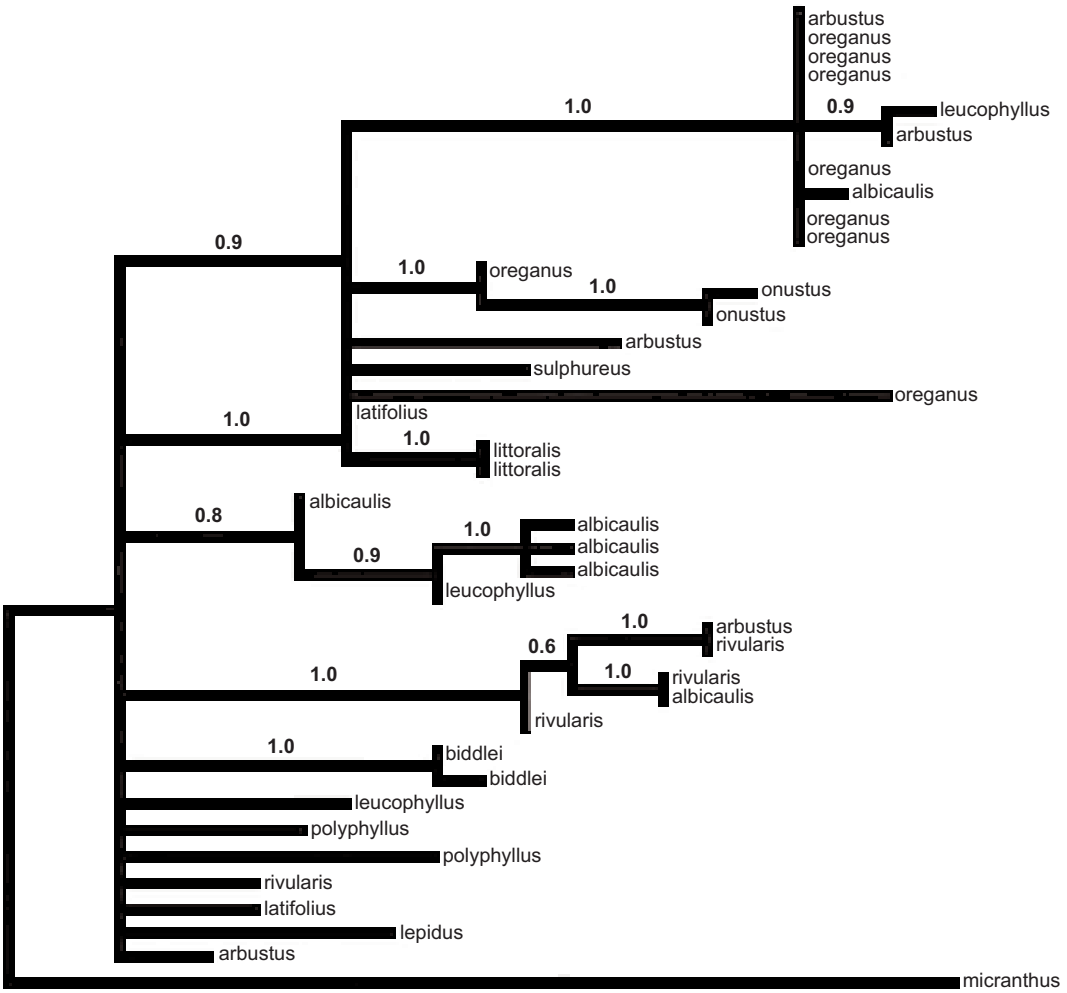

Fig. 1. Bayesian inference of *Lupinus* phylogeny. Numbers above branches are Bayesian posterior probability values.

form interspecific hybrids (Phillips 1955, Downey and Dunn 1964, Barneby 1989, Liston et al. 1995, Gupta et al. 1996). These hybridization events can generate a reticulated evolution of lineages that result in unresolved phylogenies and polytomies (Felsenstein 2004).

Regardless of biological and methodological explanations for unresolved regions of the *Lupinus* phylogeny, the systematic relationships proposed by Phillips and listed by USDA PLANTS had little support in our phylogeny. Furthermore, there were no morphological, geographical, or nomenclatural reasons to rationalize the Phillips and USDA PLANTS assignments. We therefore propose the following taxonomic assignments: "Kincaid's lupine" should be re-elevated to its original specific status as *L. oreganus* Heller, a taxon restricted to western Oregon, southwest Washington, and western British Columbia (historically). The synonymization of *L. biddlei* to *L. oreganus* var. *oreganus* listed by USDA PLANTS should not be recognized, and the taxon should remain as *L. biddlei* until a formal phylogenetic study with *L. polyphyllus* var. *prunophilus* suggests otherwise. We further recommend that the common name of "Kincaid's lupine" be used for *L. oreganus* to preserve the taxonomic history and commemorate the work of the naturalist Trevor Kincaid and his exploration of the Pacific Northwest.

While we can present a reasonable argument for the re-elevation of *L. oreganus* to species rank, resolution of *L. biddlei* is not possible

given the taxa represented in our study. Barneby (1989) considered *L. biddlei* conspecific with *L. polyphyllus* var. *prunophilus*, but we did not include *L. polyphyllus* var. *prunophilus* in our phylogeny (samples were *L. polyphyllus* var. *polyphyllus*). We address *L. biddlei* because it has been erroneously allied with *L. oreganus* in USDA PLANTS, and we provide resolution for this proposed relationship only.

LIST OF SYNONYMS

1. *Lupinus oreganus* A. Heller, Muhlenbergia 7: 89, f. 14. 1911. TYPE: U.S.A. OREGON. Lane Co.: at Eugene, 18 May 1910, A.A. Heller 10044 (HOLOTYPE: NESH).

= *Lupinus oreganus* var. *kincaidii* C.P. Sm., Bull. Torrey Bot. Club 51(7): 305. 1924. *Lupinus sulphureus* subsp. *kincaidii* (C.P. Sm.) L. Phillips, Res. Stud. State Coll. Wash. 23(3):193.

1955. *Lupinus sulphureus* var. *kincaidii* (C.P. Sm.) C.L. Hitchc., Vasc. Pl. Pacific N.W.3: 330. 1961. TYPE: U.S.A. OREGON. Benton Co.: Corvallis, [8 Jun 1898], T. Kincaid s.n. (HOLOTYPE: WU).

= *Lupinus leucopsis* J. Agardh var. *hendersonianus* C.P. Sm. Species Lupinorum 111. 1939. Hendricks Park, Eugene, Lane Co. OREGON, L.F. Henderson 14418, ISOTYPE:ORE

= *Lupinus amabilis* A. Heller, Muhlenbergia 8(10): 114–115, f. 21. 1912. TYPE: U.S.A. OREGON. Lane Co.: Eugene, 18 May 1910, A.A. Heller 10043 (HOLOTYPE:NESH).

ACKNOWLEDGMENTS

We thank land managers, private landowners, and the Oregon State University Herbarium for access to *Lupinus* specimens. Tri Tran was supported by a Portland Garden Club of America Grant for Undergraduate Research. J. Andrew Alexander, Sam Friedman, and 2 anonymous reviewers provided comments that helped us improve this manuscript.

LITERATURE CITED

BARNEBY, R.C. 1989. Intermountain flora. Vascular plants of the Intermountain West, U.S.A. Vol 3B. Fabales. New York Botanical Garden, New York, NY.

CITERNE, H.L. 2005. A primer set for specific amplification of two *CYCLOIDEA*-like genes in the genistoid clade of Leguminosae subfam. Papilionideae. Edinburgh Journal of Botany 62:119–126.

CITERNE, H.L., D. LUO, R.T. PENNINGTON, E. COEN, AND Q.C.B. CRONK. 2003. A phylogenomic investigation of *CYCLOIDEA*-like TCP genes in the Leguminosae. Plant Physiology 131:1042–1053.

CITERNE, H.L., M. MÖLLER, AND Q.C.B. CRONK. 2000. Diversity of *cycloidea*-like genes in Gesneriaceae in relation to floral symmetry. Annals of Botany 86:167–176.

DOWNEY, J.C., AND D.B. DUNN. 1964. Variation in the lycaenid butterfly *Plebejus icarioides* III. Additional data on food-plant specificity. Ecology 45:172–178.

FELSENSTEIN, J. 2004. Inferring phylogenies. Sinauer Associates, Sunderland, MA.

FENG, X., Z. ZHAO, Z. TIAN, S. XU, Y. LUO, Z. CAI, Y. WANG, ET AL. 2006. Control of petal shape and zygomorphy in *Lotus japonicus*. Proceedings of the National Academy of Sciences 103:4970–4975.

GUPTA, S., B.J. BUIRCHELL, AND W.A. COWLING. 1996. Interspecific reproductive barriers and genomic similarity among the rough-seeded *Lupinus* species. Plant Breeding 115:123–127.

HALL, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95–98.

HELLER, A.A. 1911. The North American lupines V. Muhlenbergia 7:89.

_____. 1912. The North American lupines IX. Muhlenbergia 8:114.

HOWARTH, D.G., AND M.J. DONOGHUE. 2006. Phylogenetic analysis of the "ECE" (CYC/TB1) clade reveals duplications preceding the core eudicots. Proceedings of the National Academy of Sciences 103:9101–9106.

HUGHES, C., AND R. EASTWOOD. 2006. Island radiation on a continental scale: exceptional rates of plant diversification after uplift of the Andes. Proceedings of the National Academy of Sciences USA 103:10334–10339.

LISTON, A., K. ST. HILAIRE, AND M.V. WILSON. 1995. Genetic diversity in populations of Kincaid's lupine, host plant of fender's blue butterfly. Madroño 42: 309–322.

OREGON BIODIVERSITY INFORMATION CENTER. 2010. Rare, threatened and endangered species of Oregon. Institute for Natural Resources, Portland State University, Portland, OR.

PHILLIPS, L.L. 1955. A revision of the perennial species of *Lupinus* of North America exclusive of southwestern United States and Mexico. Research Studies of the State College of Washington 23:161–201.

POSADA, D., AND K.A. CRANDALL. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14: 817–818.

REE, R.H., H.L. CITERNE, M. LAVIN, AND Q.C.B. CRONK. 2004. Heterogeneous selection on *LEGCYC* paralogs in relation to flower morphology and the phylogeny of *Lupinus* (Leguminosae). Molecular Biology and Evolution 21:321–331.

RONQUIST, F., AND J.P. HUELSENBECK. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

SMITH, C.P. 1924. Studies in the genus *Lupinus*-XI. Some new names and combinations. Bulletin of the Torrey Botanical Club 51:303–310.

_____. 1927. A taxonomic study of the Pacific States of *Lupinus*. Stanford University Press, Palo Alto, CA.

_____. 1939. Species Lupinorum 7:108–109.

USDA, NRCS. 2011. The PLANTS Database [online]. National Plant Data Team, Greensboro, NC; [cited 5 July 2011]. Available from: http://plants.usda.gov/

WILSON, M.V., T. ERHART, P.C. HAMMOND, T.N. KAYE, K. KUYKENDALL, A. LISTON, A.F. ROBINSON, C.B. SCHULTZ, AND P.M. SEVERNS. 2003. Biology of Kincaid's lupine (*Lupinus sulphureus* ssp. *kincaidii* [Smith] Phillips), a threatened species of western Oregon native prairies. Natural Areas Journal 23:72–83.

Received 1 October 2011
Accepted 15 March 2012