# The Systematics of Lithospermum L. (Boraginaceae) and the Evolution of Heterostyly

by James Isaac Cohen

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# THE SYSTEMATICS OF *LITHOSPERMUM* L. (BORAGINACEAE) AND THE EVOLUTION OF HETEROSTYLY

A Dissertation
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In Partial Fulfillment of the Requirements for the Degree of
Doctor of Philosophy

by
James Isaac Cohen
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# THE SYSTEMATICS OF *LITHOSPERMUM* L. (BORAGINACEAE) AND THE EVOLUTION OF HETEROSTYLY

James Isaac Cohen, Ph.D.

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*Lithospermum* L. (Boraginaceae) includes ca. 60 species. The genus has a nearly cosmopolitan distribution, being native to all continents except Australia and Antarctica. The center of diversity for the genus is Mexico and the southwestern United States, and approximately three-quarters of the species of *Lithospermum* are endemic to this region. *Lithospermum* and the tribe to which it is assigned, Lithospermeae, have been the subject of recent phylogenetic analyses, but these analyses have been limited in their scope. In order to examine critically the phylogenetics of *Lithospermum* and its relatives, three matrices, each consisting of 67 taxa, were constructed. The molecular matrix includes 10 chloroplast DNA regions, and the morphological matrix is composed of scores for 22 morphological characters. The combined matrix comprises the data from both of these matrices. Analyses of these matrices resolve *Lithospermum* as non-monophyletic, because members of other New World genera of Lithospermeae are found to be nested among species of *Lithospermum*. Because *Lithospermum*, as traditionally circumscribed, was resolved as non-monophyletic, the circumscription of the genus is expanded to include all species of New World Lithospermeae. Additionally, the phylogenetic distribution of various molecular and morphological characters was examined. One character, heterostyly, arose multiple times among the species of *Lithospermum*. The floral developmental patterns of three heterostylous and three homostylous species of *Lithospermum* were investigated. Although heterostyly may have originated independently in each of these three species, the floral developmental patterns of the long-style and short-style morphs of heterostylous species of *Lithospermum* are similar to those of homostylous relatives with approach and reverse herkogamous flowers, respectively. Despite similar gross floral developmental patterns, the developmental patterns of the stylar epidermal cells differ among the heterostylous species. In addition to phylogenetic and floral developmental studies, a revision of the species of *Lithospermum* of Mexico was undertaken, and species boundaries were reevaluated. Three new species are described from northern Mexico – *L. chihuahuanum*, *L. kelloggiae, and L. tenerum* – and each is known only from its type specimen.

# BIOGRAPHICAL SKETCH

James Isaac Cohen was born early in the morning on June 17, 1980. His early years were spent at Gan Hayeled Nursery School in Washington, DC. Afterwards, he attended Lafayette Elementary School, Alice Deal Junior High School, and Woodrow Wilson Senior High School, all part of the District of Columbia Public School system. During these formative years, James attended Camp Nebagamon, a boys' camp in northwestern Wisconsin. As a camper, he spent much time in the Boundary Waters Canoe Area Wilderness in northeastern Minnesota. He truly enjoyed these outdoor experiences, and because of his love for the natural world, decided to study resource ecology and management at the School of Natural Resources and Environment at the University of Michigan, Ann Arbor.

James was an excellent student in the Woody Plants course, a rite of passage at the school, and subsequently, he took other forest ecology courses. One of his graduate student instructors, John Syring, encouraged James to take a course in systematic botany. Because of his experiences in the Systematic Botany course, which was taught by William Anderson, James decided to learn more about the subject by reading books and botanizing with Dr. Anderson as well as taking courses with Robyn Burnham and Arnold Kluge. James graduated from the University of Michigan in 2002, and worked in various field botany and taxonomy positions throughout the USA. In order to continue to satisfy his interest in plant systematics, James applied, and was accepted, to Cornell University in 2004. Since his matriculation, he has learned a great deal on the subject of plant systematics, and has had wonderful experiences on the Ag Quad. Additionally, he has enjoyed his travels botanizing throughout the Americas.

# ACKNOWLEDGEMENTS

Given that a doctoral dissertation is a long journey, it could not be accomplished without the help of others. Many people have contributed to my being able to complete my dissertation, and I owe them all a great deal of thanks. My advisor, Jerry Davis, has been incredibly helpful throughout all of the stages of my dissertation. I would like to think that after six years, Jerry's attention to detail, precision, and thoughtfulness has rubbed off on me. Jerry has supported my ideas, provided guidance, and been a role model, and I hope to emulate him during my time on the other side of the lectern. The other members of my committee – Alejandra Gandolfo, Monica Geber, Melissa Luckow, Jim Miller, and Bob Turgeon – have always had their door open, and been willing to discuss various aspects of botany. In particular, the assistance and suppot that Alejandra, Melissa, and Jim have provided has been invaluable, and I will certainl not forget it.

Other faculty members in the Hortorium and in the department of Plant Biology have provided me with guidance and treated me as I believe they would treat their own students. Jeff Doyle opened his lab group to me, and I learned a great deal concerning topics I otherwise would not have explored. Jim Reveal always has been willing to discuss topics related to taxonomy and nomenclature, and I appreciate his help.

The students of the Hortorium have been wonderful companions. I believe that we have formed a tight-knit cohort that challenges and takes care of each other. Their friendship and intelligence have been a constant source of comfort.

I have taken multiple collecting trips throughout North America, and many people have helped me during these trips. In 2005, Shannon Straub was a wonderful collecting partner, even though we only found a small number of species of *Lithospermum*. In 2007, during a trip to Mexico, Hilda Flores, Helga Ochoterrena, Paty Hernandez Ledesma, Fernando Alzate, and Socorro Gonzalez were invaluable friends to have in the field, and in 2008, during another trip to Mexico, Lucia Vazquez, Paty Hernandez Ledesma, and Janelle Burke made the collecting experience memorable. Finally, during 2009, Caroline Kellogg was a beautiful and helpful collecting assistant.

In addition to trips to collect plant material, I also spent time at the New York Botanical Garden. It is through the generosity of Amy Litt and Dennis Stevenson that it was possible to visit the garden in the first place, and I am very appreciative for their help. While at the garden, Lisa Campbell, Damon Little, Larry Kelly, and Natalia Pabon Mora helped me with any questions I had regarding microscopy. These people, along with Fabian Michelangeli, Michael Sundue, and Donald McClelland, made me feel at home at NYBG, and that is always a nice feeling.

Although I collected a great deal of plant material, various gardens and organizations sent me material or allowed me to collect on their property. These include the National Botanical Garden of Belgium, the Royal Botanical Garden at Kew, the Missouri Botanical Garden, USDA – GRIN, the National Park Service, the Chicago Botanical Garden, the South African National Biodiversity Institute, and the Cornell Plantations.

Various organiziations and societies provided funding for my research: American Society of Plant Taxonomists, Molecular and Organismic Research in Plant History Cross-Disciplinary Training Grant, Cornell University Latin American Studies Program Travel Award, Cornell University Graduate School Research Travel Grant, Cornell University Sigma Xi Grant, Cornell University Mellon Funds, and the L. H. Bailey Hortorium Harold E. Moore Jr. Research Fund. Without assistance of these grants, it would have been very difficult to complete my dissertation. The funds provided are greatly appreciated.

I also have taught for eleven of my twelve semesters at Cornell. I would like to thank Karl Niklas, Tom Silva, Jerry Davis, Alejandra Gandolfo, Darlene Campbell, and Melissa Brechner for the opportunity to learn how to teach, and to work with them. Teaching at Cornell has been an interesting and fun experience, and I look forward to taking the lessons I have learned and applying them to future classrooms.

Most importantly, I would like to thank my wife, Caroline Kellogg, for her support. She has gracefully put up with me during my time in graduate school, and has been willing to read anything I write. Caroline is a wonderful person, and without her, graduate school would have been a much more trying experience.

I would be remiss if I also did not acknowledge the role that TV shows have played during my time at Cornell. As a distraction, TV shows have proven to be wonderful. In particular, the following are excellent programs for anyone in need of shutting off their brain for a short period of time: Everwood, Gilmore Girls, the OC, 30 Rock, Glee, Make It or Break It, Pretty Little Liars, House, Greek, Entourage, Veronica Mars, Robin Hood, the Simpsons, Better Off Ted, Scrubs, Law and Order, Law and Order: Special Victims Unit, Las Vegas, How I Met Your Mother, the Big Bang Theory, the Class, Bones, Pushing Daisies, and Party Down.

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# CHAPTER ONE

## INTRODUCTION

*Lithospermum* L. (Boraginaceae) includes ca. 60 species. The genus has a nearly cosmopolitan distribution, being native to all continents except Australia and Antarctica. The center of diversity for the genus is Mexico and the southwestern United States, and approximately three-quarters of the species of *Lithospermum* are endemic to this region. Although its species display diverse floral, vegetative, and palynological features, the nutlets are quite uniform in size, shape, color, and texture. Indeed, the erect, smooth, white, lustrous nutlets, which resemble small stones or pieces of porcelain, provide the genus with its common name – stone seeds. Given the structural diversity that occurs among its members, *Lithospermum* is a useful taxon for investigations on biogeography, breeding systems, morphological structures, and molecular evolution. The goals of this dissertation are to: 1) investigate phylogenetic relationships and character evolution within *Lithospermum* and among related genera of Boraginaceae; 2) examine floral developmental patterns of heterostylous and homostylous species of *Lithospermum*; and 3) revise the Mexican species of *Lithospermum*.

In Chapter 2, a phylogeny of 27 species of *Lithospermum* is presented. This analysis resolves *Lithospermum* as non-monophyletic, because members of other New World genera of Lithospermeae, including *Lasiarrhenum* I. M. Johnst., *Macromeria* D. Don, *Nomosa* I. M. Johnst., *Onosmodium* Michx., and *Psilolaemus* I. M. Johnst. (Johnston 1952, 1954a, b), are found to be nested among species of *Lithospermum*. Because *Lithospermum*, as traditionally circumscribed, was resolved as non-monophyletic, its circumscription is expanded to include all species of New World Lithospermeae. This broader *Lithospermum* is characterized by erect, smooth, white, and lustrous nutlets, and by corollas that are yellow, yellow-green, white, or orange (i.e., corollas that are not blue or purple) (Chapter 2). This small-scale study set the stage for larger, more-inclusive phylogenetic investigations.

In order to examine phylogenetic relationships within *Lithospermum* further, three matrices were constructed, each consisting of 67 taxa. These matrices include 37 species of *Lithospermum* and 30 species from related genera of three tribes of Boraginaceae, i.e., Lithospermeae, Boragineae, and Cynoglosseae. The molecular matrix consists of nucleotide sequences of 10 chloroplast DNA (cpDNA) regions, while the morphological matrix includes scores for 22 morphological characters. The combined matrix is composed of the data from both of these matrices.

Analyses of the molecular matrix reconstruct 80 most-parsimonious (MP) trees, and the strict consensus of these trees provides a detailed phylogenetic hypothesis for *Lithospermum*. Two South African species of *Lithospermum* are resolved as sisters, and the clade that includes these two species is sister to the remainder of the genus. Additionally, two Eurasian species are sisters, and the clade that includes these species is resolved as sister to the New World members of *Lithospermum*. The New World species of the genus are resolved as monophyletic.

In addition to the examination of evolutionary relationships within *Lithospermum*, issues related to taxon and character sampling were explored. Through permutations of the molecular matrix, matrices composed of various combinations of cpDNA regions and taxa were created. Analyses of these matrices suggest that a combination of regions, some that evolve more slowly and others that evolve more rapidly, allows for the greatest number of nodes to be resolved that are not contradicted by the inclusion the remainder of the data.

Analyses of the combined matrix yielded slightly different relationships than those of the molecular matrix. The combined matrix resolved seven MP trees. In the consensus tree of the combined matrix, a clade that includes both New World and

South African species is resolved as sister to the rest of the genus. This differs from the consensus tree of the molecular matrix, which resolves a clade that includes only South African species in this position. Despite this difference, many smaller clades, along with a limited number of clades that include approximately five species, are identical between the phylogenies from both matrices.

The results obtained with the combined matrix were used to investigate the evolution of various morphological characters. Some characters, such as corolla shape and color, and pollen shape, have a number of different states, and the structure of the phylogeny suggests that many character states evolved multiple times. For example, tubular corollas originated at least twice within *Lithospermum*. Other characters, including pollen pore position and the presence/absence of faucal appendages, are less variable and tend to characterize larger clades.

One character, heterostyly, appears to have originated multiple times in *Lithospermum*. Heterostyly is a complex and elegant breeding system that is defined as the occurrence in a species of two or more floral morphs that exhibit reciprocal herkogamy (herkogamy is defined as the spatial separation between anthers and stigmas [Webb and Lloyd, 1986]), and each individual plant produces flowers with only one type of herkogamy (Barrett et al., 2000; Barrett and Shore, 2009). Additionally, heterostylous species often display other physiological and structural characters, such as self- and intramorph-incompatibility and pollen and stigma polymorphisms (Barrett et al., 2000; Barrett and Shore, 2009). Given the complexity of heterostyly, it is surprising that this floral syndrome evolved multiple times in a medium-sized genus. In order to investigate the evolution of heterostyly, floral developmental patterns were examined in three heterostylous species of *Lithospermum*, *L. canescens* Lehm., *L. cobrense* Greene, and *L. multiflorum* A. Gray. These species were chosen for two reasons. First, the phylogeny suggests that heterostyly originated independently in each of these species (Chapter 3, 4). Second, these species are abundant throughout their large geographic ranges, and therefore it was possible to obtain adequate material in order to examine floral developmental patterns. In additional, floral developmental patterns were examined in three homostylous species, *L. calcicola* B. L. Rob., *L. distichum* Ortega, and *L. latifolium* Michx. The data from the homostylous relatives help to provide information concerning the ancestral developmental patterns of heterostylous species.

The floral developmental patterns of the long-style and short-style morphs of each heterostylous species of *Lithospermum* are similar to those of homostylous relatives with approach and reverse herkogamous flowers, respectively. In addition, the non-herkogamous flowers of *L. distichum* develop according to a different pattern. Therefore, three floral developmental patterns exist in *Lithospermum*: one each for approach herkogamous, reverse herkogamous, and non-herkogamous flowers (Chapter 5).

Although gross floral developmental patterns are similar between the flowers of heterostylous morphs and those of their respective homostylous, herkogamous relatives, the development of stylar epidermal cells differs. In homostylous species, these cells follow either a logarithmic or linear pattern of growth, but in heterostylous species, these cells undergo logarithmic, exponential, or linear patterns of growth. Therefore, in *Lithospermum*, different patterns of floral development exist between heterostylous and homostylous species as well as among heterostylous species.

These patterns provide evidence for the mechanisms by which different, morph-specific heights of stigmas and anthers are attained. The stigmas of each morph attain their distinct heights via different rates of elongation of stylar cells. In contrast, the position of the anthers is influenced by cell division in different areas of the corolla tube. In the long-style morph, a greater number of cell divisions occur above the point of filament attachment to the corolla tube, while in the short-style morph, the opposite pattern of cell divisions occurs (Chapter 5).

These different floral developmental patterns also have implications for the evolution of heterostyly. Two of the available models for the evolutionary development of heterostyly appear to be most applicable in this group. These two models are supported by the floral developmental data, and by the presence of variation in herkogamy that occurs within and among species of *Lithospermum*. The models differ as to whether variation in the occurrence of herkogamy is present among the individuals of an ancestral population (Sakai and Toquenaga, 2004), or appears in the form of a mutant with flowers with one type of herkogamy, which invades a population with herkogamous flowers of a different type (Lloyd and Webb, 1992).

In addition to the studies on phylogenetics and development, the species boundaries in *Lithospermum* were reevaluated. Approximately three-quarters of the species of *Lithospermum* were examined as part of a revision of the Mexican species of the genus. In total, 43 species of the genus are recognized as native to Mexico, of which 32 are endemic. Of these 43 species, three are newly described. These three species – *L. chihuahuanum*, *L. kelloggiae*, and *L. tenerum* – are from northern Mexico, and each is known only from its type specimen. With the formal description of these species, it is anticipated that more populations will be discovered, and more collections made.

The data presented in this dissertation have allowed me to answer many of the questions I set out to investigate; however, as I have collected these data, other areas of research have presented themselves. Future research questions will include:

1) How will the inclusion of nuclear and mitochondrial DNA resolve phylogenetic relationships in *Lithospermum*? The addition of DNA

sequence data from multiple genomes will allow for a more critical examination of evolutionary relationships among members of the genus.

2) What are the patterns of evolution of various micromorphological characters among species of *Lithospermum*? Although many gross morphological characters have been examined, observations of micromorphological characters, such as the structure of faucal appendages, the shape of corolla lobe epidermal cells, and the shape of stigma papillae, should be included.
3) Do all long-funnelform flowers follow the same pattern of development, and how does this floral developmental pattern compare to that of other species of *Lithospermum*? Long-funnelform corollas are unusual in Boraginaceae, and this corolla shape appears to have originated multiple times among species of *Lithospermum* (Chapter 3, 4). A study of the developmental patterns of these types of flowers will allow me to examine whether long-funnelform flowers of species of the genus develop according to the same pattern, or if those from independent origins develop in different manners.
4) What are the species boundaries in *Lithospermum*? Although a revision of the Mexican species of *Lithospermum* is presented here (Chapter 6), it would be beneficial to reevaluate the species of South America, the United States and Canada, Eurasia, and Africa. Future revisionary studies of the species of these regions will provide botanists with a stable taxonomic system for *Lithospermum*, and with keys for identification of the species of the genus.

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# CHAPTER TWO

## NOMENCLATURAL CHANGES IN *LITHOSPERMUM* (BORAGINACEAE) AND RELATED TAXA FOLLOWING A REASSESSMENT OF PHYLOGENETIC RELATIONSHIPS*

### Abstract

*Lithospermum* (Boraginaceae) comprises approximately 40 species in both the Old and New Worlds, with a center of diversity in the southwestern United States and Mexico. Using 10 cpDNA regions, a phylogeny of *Lithospermum* and related taxa was reconstructed. *Lithospermum* (including New World and Old World species) and related New World members of Lithospermeae form a monophyletic group, with *Macromeria*, *Onosmodium*, *Nomosa*, *Lasiarrhenum*, and *Psilolaemus* nested among species of *Lithospermum*. New World Lithospermeae also is a monophyletic group, with Eurasian species of *Lithospermum* sister to this group. Because *Lithospermum* is not monophyletic without the inclusion of the other New World genera, species from these genera are transferred to *Lithospermum*, and appropriate nomenclatural changes are made. New combinations are *Lithospermum album*, *Lithospermum barbigerum*, *Lithospermum dodrantale*, *Lithospermum exsertum*, *Lithospermum helleri*, *Lithospermum leonotis*, *Lithospermum notatum*, *Lithospermum oaxacanum*, *Lithospermum pinetorum*, *Lithospermum rosei*, *Lithospermum trinverium*, and *Lithospermum unicum*; new names are *Lithospermum chiapense*, *Lithospermum johnstonii*, *Lithospermum macromeria*, *Lithospermum onosmodium*, *Lithospermum rzedowskii*, and *Lithospermum turneri*.

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*Reproduced with permission from the New York Botanical Garden Press; Cohen, J. I., and J. I Davis. 2009. Nomenclatural changes in *Lithospermum* L. (Boraginaceae) and related taxa following a reassessment of phylogenetic relationships. *Brittonia* 61: 101-111.

## Introduction

The genus *Lithospermum* L. (Boraginaceae) comprises approximately 40 species (Johnston, 1954a). Although it has a cosmopolitan distribution, most species of the genus occur in the New World, from Canada southward to Peru, with a center of diversity in Mexico and the southwestern United States. *Lithospermum* and the tribe to which it belongs, Lithospermeae Dumort., were revised by Ivan Johnston in a series of papers from 1952 to 1954 (1952, 1953a, b, 1954a, b). In these papers, Johnston recognized new species within the genus, described new genera, and proposed hypotheses concerning evolutionary relationships within the tribe (1952, 1954a, b). A recent phylogeny of Boraginaceae (Langstrom and Chase, 2002) reconstructs Lithospermeae as monophyletic, but resolution is lacking within the tribe and no New World members of Lithospermeae were included in the analysis; therefore, Johnston's hypothesis that all six genera of Lithospermeae endemic to the New World (i.e., all New World genera of the tribe other than *Lithospermum*) are closely related to *Lithospermum* (Johnston, 1954a) has yet to be tested (but see Thomas et al., 2008). This group includes *Macromeria* D. Don, *Onosmodium* Michx., *Lasiarrhenum* I. M. Johnst., *Nomosa* I. M. Johnst., *Psilolaemus* I. M. Johnst., and *Perittostema* I. M. Johnst. Species of these genera occur from Canada to Guatemala, and as with *Lithospermum*, the center of diversity for the group is in Mexico. Only two of these genera, *Macromeria* and *Onosmodium*, include more than one species; *Macromeria* includes 11 species and *Onosmodium* includes seven. Johnston's generic demarcations within New World Lithospermeae are primarily based on floral characters, such as exserted vs. included anthers; flattened vs. cylindrical filaments; and campanulate, urceolate, or long funnelform vs. salverform or funnelform corolla shapes (Johnston, 1954a, b). However, phylogenetic analyses (Cohen, 2006, 2007) suggest that the genera recognized on the basis of these floral features are not monophyletic.

*Lithospermum* and other New World members of Lithospermeae share characteristic erect, smooth to slightly dimpled, white, lustrous nutlets with basal attachment, and all have three-celled glands inside the corollas, except for ca. 12 taxa in which glands, presumably, have been lost or modified. Closely related genera of Lithospermeae that share these features, such as *Buglossoides* Moench and *Lithodora* Griseb. (Thomas et al., 2008), have blue or purple corollas, but these corolla colors do not occur in *Lithospermum* or other New World members of Lithospermeae.

The taxonomic history of *Lithospermum* is complex, and the complexity arises, in part, from treatments of the genus in the 1800s. For example, in *Plantae e Familia Asperifoliarum Nuciferae* (1818), Lehmann circumscribed a broadly inclusive *Lithospermum,* which included species currently placed in *Lithospermum* (Johnston, 1954a) as well as species currently included in other genera, such as *Mertensia* Roth, *Buglossoides, Arnebia* Forssk.,and *Lithodora*. A later taxonomic treatment of *Lithospermum* in the Prodromus Systematis Naturalis Regni Vegetabilis by A. P. de Candolle (1846) was, like Lehmann's treatment, quite inclusive; however, unlike Lehmann, de Candolle divided the genus into three sections. One section, *Eulithospermum* DC. included only species currently included in *Lithospermum* (with one exception, *L. chinensis* Hook. and Arn., which is a synonym of *Heliotropium strigosum* Willd. [Zhu et al., 1995]). The other two sections in the treatment, *Rhytispermum* Link and *Margarospermum* Rchb. f., included species that are usually included in other genera, such as *Buglossoides*, *Lithodora, Neatostema* I. M. Johnst., and *Moltkia* Lehm.

Although both Lehmann and de Candolle recognized a large, inclusive *Lithospermum*, they also recognized two endemic New World genera, *Macromeria* and *Onosmodium* (as *Purshia* Lehm. [1818], non *Purshia* DC. ex Poir. [Rosacaeae]). Species from these two genera have only rarely been included in *Lithospermum* (e. g., Muhlenberg, 1813). This treatment of species in the New World differs from that of many species in the Old World in which it has been common to include species from putatively related genera of Lithospermeae, such as *Buglossoides*, *Lithodora*, and *Neatostema*, in *Lithospermum* (see Johnston, 1953a [but see Johnston, 1954a, b for taxonomic changes], Flora Iranica [Rechinger, 1967], and Flora of China [Zhu et al., 1995]).

At present, the most widely accepted taxonomic system for the genus derives from Johnston's revisionary efforts on the genus and tribe during the 1950s, but that treatment also contributed additional complexity to the group's taxonomy. Johnston placed *Arnebia* and *Echioides* Fabr. in *Lithospermum* in 1952, but two years later he removed them, noting differences in stamens and pollen. Also, he placed some newly described species in *Lithospermum* in 1953, and transferred them to *Arnebia* the following year. In his 1952 revision of *Lithospermum*, Johnston recognized the difficulty of dividing *Lithospermum* into smaller groups, stating that "The genus has great internal coherence. It is exasperatingly lacking in lines of cleavage" (1952, p. 300).

Taxonomic questions regarding generic boundaries of *Lithospermum* still arise, as seen in the treatment of Boraginaceae in the *Flora of China* (Zhu et al., 1995), in which *Lithospermum* is circumscribed as including species usually placed in *Buglossoides*. Treating *Lithospermum* in this broad sense is common in Old World literature; however, *Buglossoides* is not monophyletic (cf. Langstrom and Chase, 2002; Thomas et al., 2008; Cohen, unpublished data) and may not be sister to *Lithospermum*.

A recent taxonomic study of *Lithospermum* (Ralston, 1993) included a phylogenetic analysis of the genus utilizing a total of twenty morphological, palynological, karyological, and chemical characters. Her analysis provided support for the recognition of several species groups, but did not resolve relationships among these groups. Recent work on other taxa of New World Lithospermeae includes Turner's synopses of *Macromeria* (1994a), *Onosmodium* (1995), and *Lasiarrhenum* (1994b), and Boyd's (2003) phylogeny of *Macromeria* based on morphological characters.

The present study was undertaken to examine the phylogenetic relationships among species of *Lithospermum* and other elements of New World Lithospermeae, with two of the goals being to determine whether the non-monotypic genera *Lithospermum*, *Macromeria*, and *Onosmodium* constitute monophyletic groups, and to reevaluate the taxonomy of New World Lithospermeae.

## Materials and methods

### TAXON SAMPLING

Twenty-seven species were included in the analyses. Twenty-two belong to the ingroup, and five are outgroup taxa from related genera in Boraginaceae (Table 2.1). The ingroup sampling represents the range of variation within *Lithospermum*, and species from all but one elusive genus of New World Lithospermeae, *Perittostema*, were included. *Perittostema* was not included in the analyses because it is only known from the type, supposedly housed in Paris (P), and the type locality is unknown; however, Johnston hypothesized that it was collected in Oaxaca, Mexico (Johnston, 1935, 1954a). The majority of the taxa were collected from wild populations. For these taxa, herbarium specimens were collected and deposited at BH, and leaf tissue was dried and preserved in silica gel for subsequent DNA extraction. Taxa not collected from natural populations were obtained from gardens as leaves preserved in silica (Cornell Plantations, Missouri Botanical Garden, and National Botanic Garden of Belgium) or as DNA isolations from the DNA bank of Royal Botanic Gardens, Kew. See Table 2.1 for specimen list and information.

### DNA EXTRACTION, PCR AMPLIFICATION, AND SEQUENCING

DNA extraction was performed with dried plant tissue using a modified CTAB extraction method (Doyle and Doyle, 1990) which included 2% PVP-40 and in some cases the addition of 0.5 M glucose in the CTAB extraction buffer. PCR amplifications of the ten chloroplast DNA regions, *ndh*F - *rpl*32, *psbA* - *trn*H, *psb*J - *pet*A, the *rpl*16 intron, *trn*K - *rps*16, *trn*L - *rpl*32, *trn*Q - *rps*16, *ycf*6 - *psb*M, *trn*L - *trn*F (Shaw et al. 2005, Shaw et al. 2007), and *mat*K (390F [Cuénoud et al., 2002] and 1710R [Barfuss et al., 2005]), were performed using the published primers of the cited authors. PCR mixtures, 25 µL in volume, consisted of 67 mM Tris-HCl with 2.1% DMSO and 0.01% TritonX per reaction or 1X *Ex Taq* Buffer (Takara Bio Inc., Japan), 2 mM $MgCl_2$, 0.2 mM to 0.25 mM dNTPs , 1 µM of primers, 0.125 µL to 1 µL of *Taq* polymerase, and 0.1 µL to 2.5 µL DNA sample, depending on the DNA concentration. Amplifications were performed in an Eppendorf Mastercycler Gradient 5331 thermocycler, using the annealing temperatures listed in Table 2.2. PCR products were run on a 1% to 1.5% agarose gel and stained with ethidium bromide to determine if amplification occurred. Prior to sequencing, some PCR reactions were purified with the QIAquick PCR purification kit (Qiagen, Germany).

Sequencing reactions were performed with BigDye 3.1 (Applied Biosystems, CA U.S.A.) terminators and locus-specific amplification primers. Sequencing products were precipitated using a modification of the ethanol/EDTA/sodium acetate method (Applied Biosystems), and automated cycle sequencing was performed by the Life Sciences Core Laboratory Center at Cornell University with an Applied Biosystems (ABI) 3730 DNA Analyzer. Alternatively, sequencing reactions and subsequent steps

Table 2.1. List of taxa included in analyses along with collection or source data

| Taxon | Collection | Location |
|---|---|---|
| **OUTGROUP SPECIES** | | |
| *Anchusa leptophylla* Roem. & Schult. | 2002 1260-96 | Natl. Bot. Gard. Belgium |
| *Buglossoides arvense* (L.) I. M. Johnst. | 19792083 | Natl. Bot. Gard. Belgium |
| *Echium vulgare* L. | J. Cohen 212 | Cornell Plantations |
| *Lithodora diffusa*. (Lag.) I. M. Johnst. | M. Chase 6063 | Cantabria, Spain |
| *Onosma stellulata* Waldst. & Kit. | 1992 1317-39 | Natl. Bot. Gard. Belgium |
| **INGROUP SPECIES** | | |
| *Lasiarrhenum trinervium* (Lehm.) I. M. Johnst | J. Cohen 228 | Michoacán, Mexico |
| *Lithospermum californicum* A. Gray | J. Cohen 43 | Oregon, U.S.A. |
| *Lithospermum canescens* Lehm. | J. Cohen & S. Straub 12 | Ohio, U.S.A. |
| *Lithospermum caroliniense* MacMill. | J. Cohen 11 | Indiana, U.S.A. |
| *Lithospermum cobrense* Greene | J. Cohen 78 | Texas, U.S.A. |
| *Lithospermum erythrorhizon* Siebold and Zucc. | J. Cohen 173 | Cornell Plantations |
| *Lithospermum multiflorum* A. Gray | J. Cohen 57 | Arizona, U.S.A. |
| *Lithospermum nelsonii* Greenm. | J. Cohen 184 | Nuevo León, Mexico |
| *Lithospermum obovatum* J. F. Macbr. | J. Cohen 208 | Durango, Mexico |
| *Lithospermum officinale* L. | J. Cohen 171 | Cornell Plantations |

Table 2.1 (Continued)

| | | |
|---|---|---|
| *Lithospermum strictum* Lehm. | J. Cohen 225 | Michoacán, Mexico |
| *Lithospermum tuberosum* DC. | J. Cohen 108 | Georgia, U.S.A. |
| *Macromeria exserta* D. Don | J. Cohen 224 | Michoacán, Mexico |
| *Macromeria hispida* M. Martens & Galeotti | J. Cohen 218 | Michoacán, Mexico |
| *Macromeria leonotis* I. M. Johnst. | J. Cohen 195 | Nuevo León, Mexico |
| *Macromeria longiflora* D. Don | J. Cohen 226 | Michoacán, Mexico |
| *Macromeria notata* I. M. Johnst. | J. Cohen 188 | Nuevo León, Mexico |
| *Macromeria viridiflora* DC. | J. Cohen 141 | Arizona, U.S.A. |
| *Nomosa rosei* I. M. Johnst. | J. Cohen 207 | Durango, Mexico |
| *Onosmodium helleri* Small | J. Cohen 132 | Texas, U.S.A. |
| *Onosmodium molle* Michx | 2003-0671 | Missouri Bot. Gard. |
| *Psilolaemus revolutus* (B. L. Rob.) I. M. Johnst. | J. Cohen 199 | San Luis Potosí, Mexico |

were performed by the Life Science Core Laboratory Center at Cornell University, using Big Dye terminators and either an ABI 3700 or an ABI 3730. Sequence trace files were compiled, examined, and edited with Sequencher ver. 4.6 - 4.8 (Gene Codes Corporation, MI U.S.A.), and sequences were deposited in GenBank (GenBank accessions FJ827256 – FJ827480).

### ALIGNMENT, GAP CODING, AND PHYLOGENETIC ANALYSIS

Initial alignments were performed with MUSCLE (Edgar 2004) as implemented by the European Bioinformatics Institute’s MUSCLE server (http://www.ebi.ac.uk/Tools/muscle/index.html) using the default settings. Subsequent

Table 2.2. Annealing temperatures for the cpDNA regions amplified for the present study

| **Region** | **Annealing Temperature (°C)** |
|---|---|
| *ndh*F - *rpl*32 | 56 |
| *psb*A - *trn*H | 58 |
| *psb*J - *pet*A | 50 |
| *rpl*16 intron | 54–59 |
| *trn*K - *rps*16 | 50 |
| *trn*L - *rpl*32 | 50 |
| *trn*L - *trn*F | 50 |
| *trn*Q - *rps*16 | 50–54 |
| *ycf*6 - *psb*M | 54 |
| *mat*K | 48–50 |

adjustments were made manually in Bioedit ver. 7.0.5.3 (Hall, 1999). Gaps were coded using simple indel coding (Simmons and Ochoterena, 2000). Inversions were coded as present/absent with the inverted sequence regions excluded from the analyses (Ochoterena, 2009). All characters were weighted equally and treated as unordered. Regions that aligned ambiguously (ca. 234 bp of the *rpl*16 intron) were excluded from analyses.

Maximum parsimony phylogenetic analyses were conducted using TNT (Goloboff et al., 2000), with 10,000 trees held in memory, and 1000 parsimony ratchet iterations performed (Nixon, 1999), with 10% probability of upweighting and 10% probability of downweighting, followed by 100 cycles of tree drifting, 100 rounds of tree fusing, and random sectorial searches (Goloboff, 1999). This search strategy was repeated for ten rounds with five replications per round using the Xmult setting. In total, 50 searches, 50,000 ratchets, 5000 cycles of drift, and 5000 rounds of tree fusing were performed. Clade support was measured with TNT by conducting 10,000 jackknife replicates (36% removal probability, traditional search strategies, and groups below 1% support collapsed) (Farris et al., 1996). Consistency indices were calculated after removal of parsimony-uninformative characters.

## Results

### SEQUENCE VARIATION

A total of 8,232 aligned nucleotides from the 27 species were included in the analyses (Table 2.3). The mean number of species sequenced for each plastid DNA (cpDNA) region was 23, with all 27 species sequenced for *ndh*F - *rpl*32 and the *rpl*16 intron, and the fewest number of species, 13, sequenced for *mat*K. The sequence data yielded 207 informative characters, 176 (85%) from nucleotides, and 31 (15%) from gaps and inversions.  The sequenced cpDNA regions ranged in length from 420 bp to 1,144 bp. The most informative region was *rpl*16, with 45 informative characters (substitutions, no indels or inversions) out of 1,140 bp (although 234 aligned nucleotides were difficult to align and, therefore, not included in analyses), or 5% of the total sequence length. The least informative cpDNA region, in terms of total number of characters, was *trn*L - *trn*F, with four informative characters; however, *mat*K, with seven informative characters, was the least informative region in terms of percent informative characters (0.6% vs. 0.7% for *trn*L - *trn*F). These percentages are not strictly comparable because different numbers of taxa were sampled for each region; a more complete evaluation of variation levels among the cpDNA regions is in preparation.

## PHYLOGENETIC RESULTS

Four most-parsimonious trees of 374 steps (CI = 0.62, RI = 0.61) were discovered, the strict consensus tree is provided in Figure 2.1. Most relationships are well resolved. *Lithospermum* is not monophyletic, as sampled representatives of other genera of New World Lithospermeae are nested within it. The group including *Lithospermum* and these other genera is monophyletic with 99% jackknife support, and New World Lithospermeae is a monophyletic group with 54% jackknife support. Most of the larger clades in the group that includes *Lithospermum* and other members of New World Lithospermeae are not well supported; however, each of five species pairs, *L. officinale* L. and *L. erythrorhizon* Siebold and Zucc., *L. nelsonii* Greenm. and *M. leonotis* I. M. Johnst., *M. hispida* M. Martens & Galeotti and *M. exserta* D. Don, *O. helleri* Small and *O. molle* Michx., and *L. multiflorum* A. Gray and *M. viridiflora* DC has 98% or 99% jackknife support.

Although *Onosmodium* was found to be monophyletic, *Macromeria* is not a monophyletic group. *Psilolaemus* is sister to *Lithospermum cobrense* Greene, and *Nomosa* and *Lasiarrhenum* are sister taxa, but neither of these relationships is supported by jackknife values greater than 50%.

*Lithospermum officinale* and *L. erythrorhizon*, the two Old World species of *Lithospermum* included in the analysis, are sisters, and the clade they comprise is sister to the rest of *Lithospermum* and other members of New World Lithospermeae. *Lithodora diffusa.* (Lag.) I. M. Johnst. is sister to *Lithospermum* and other members of New World Lithospermeae with 99% jackknife support.

Table 2.3. Summary of cpDNA regions included in analyses

| **Region** | **Number of Taxa** | **Aligned Length (bp)** | **Informative Nucleotide Site Characters** | **Informative Structural Characters (gaps and inversion)** | **Total Informative Characters** | **% Informative Nucleotide Site Characters** |
|---|---|---|---|---|---|---|
| *ndh*F - *rpl*32 | 27 | 801 | 27 | 4 | 31 | 3.4% |
| *psb*A - *trn*H | 23 | 420 | 8 | 5 | 13 | 1.9% |
| *psb*J - *pet*A | 23 | 821 | 17 | 6 | 23 | 2.1% |
| *rpl*16 intron | 27 | 1140[a] | 45 | 3 | 48 | 5.0% |
| *trn*K - *rps*16 | 24 | 894 | 23 | 3 | 26 | 2.6% |
| *trn*L - *rpl*32 | 25 | 838 | 21 | 5 | 26 | 2.5% |
| *trn*L - *trn*F | 17 | 549 | 4 | 1 | 5 | 0.7% |
| *trn*Q - *rps*16 | 23 | 497 | 14 | 2 | 16 | 2.8% |
| *ycf*6 - *psb*M | 23 | 1,128 | 10 | 1 | 11 | 0.9% |
| *mat*K | 13 | 1,144 | 7 | 1 | 8 | 0.7% |
| Mean | 23 | 823 | 18 | 3 | 21 | 2.2% |
| Total | 225 | 8,232 | 176 | 31 | 207 | 2.3% |

[a](includes 234 characters deactivated, but these characters not included in number of Informative Characters or % Informative Characters)

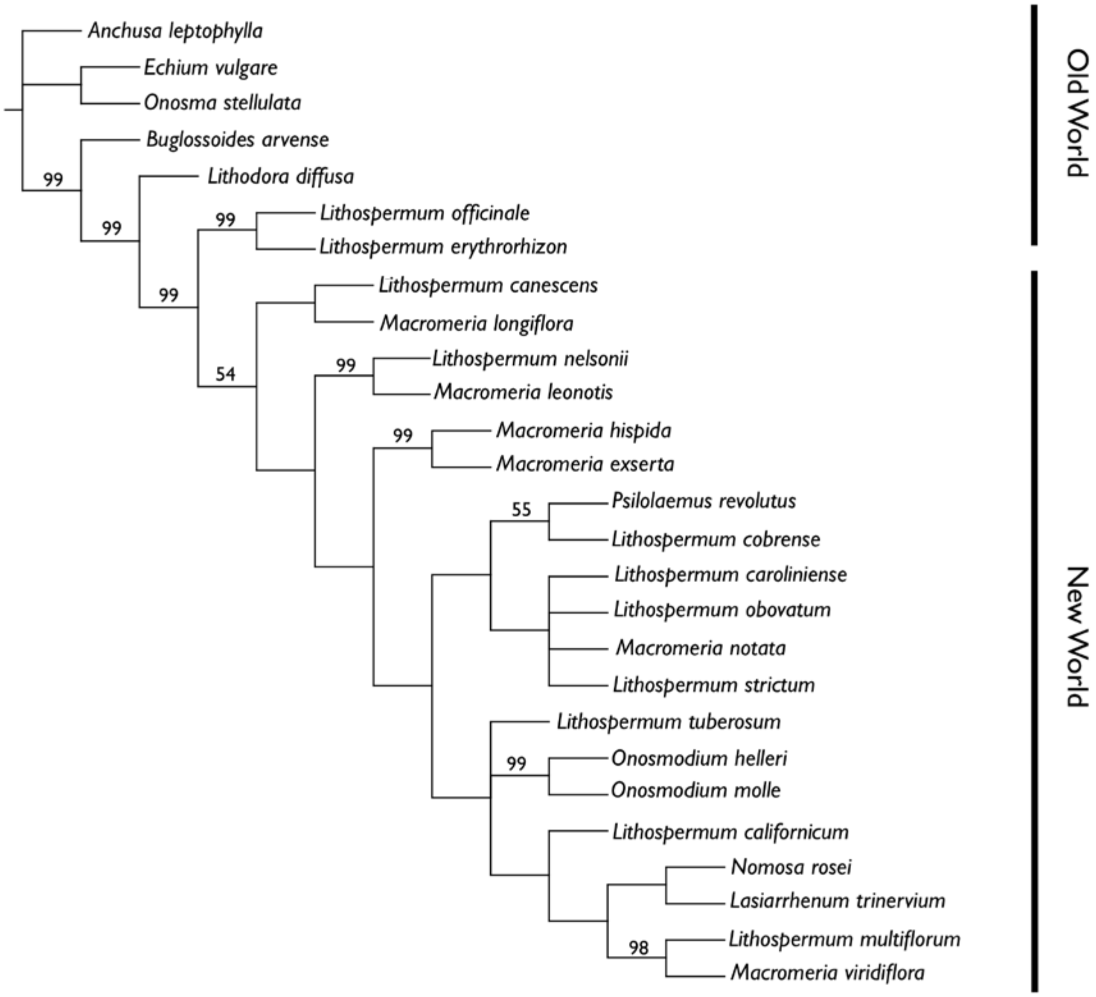

Figure 2.1. Strict consensus of 4 MP trees of 374 steps, CI = 62, RI = 61. Jackknife values >50% are shown above branches.

## Discussion

### PHYLOGENETIC ANALYSES

The analysis resolves *Lithospermum* and other New World members of Lithospermeae as a monophyletic group, and this is also the case when more taxa are added to the phylogenetic analyses (Chapter 3). However, *Lithospermum* is not monophyletic because species of other genera of New World Lithospermeae are interdigitated among species of this genus (Figure 1). Although species of *Lithospermum* differ from each other in habit, corolla shape, and corolla color, the other genera of New World Lithospermeae have been segregated from *Lithospermum* on the basis of the same sorts of features, which appear to be derived vegetative and floral characters. For example, *Nomosa* and *Lasiarrhenum* are the only two genera of New World Lithospermeae with completely white corollas and three-veined leaves. In addition, both have corolla shapes, urceolate and campanulate respectively, which are not found in any other species in the group.

*Macromeria* is a polyphyletic assemblage of species that share a similar phenotype. Species that have been placed in this genus are usually large in habit (> 0.75 m tall) with long corollas (> 4 cm) and exserted anthers and stigmas. This suite of characteristics appears to have arisen multiple times independently among species of New World Lithospermeae.

*Onosmodium*, on the other hand, is resolved as monophyletic, but only two of the seven species of the genus were included in the present study. Although the sampling is limited, the group has 99% jackknife support, and with the inclusion of other taxa in the analyses *Onosmodium* remains monophyletic (Chapter 3).

*Lithodora diffusa* is sister to the clade that includes *Lithospermum* and other genera of New World Lithospermeae. This sister relationship differs from the results obtained by Thomas et al. (2008) for *Lithodora* and related taxa, in which *Buglossoides*, rather than *Lithodora,* was placed as sister to *Lithospermum* and other genera of New World Lithospermeae. Differences in sampling of both taxa and loci could contribute to these dissimilar results.

## TAXONOMY AND NOMENCLATURE

As currently circumscribed, *Lithospermum* is not monophyletic. All of the species of New World Lithospermeae could be included in *Lithospermum*, or alternatively the clade containing *Lithospermum* and other New World Lithospermeae could be divided along different lines than have previously been drawn. I have not observed diagnostic character combinations by which a series of readily recognizable monophyletic genera can be delimited. In addition, most of the monophyletic groups that are readily delimited have five or fewer species. Splitting along these lines would result in several small genera that would be quite difficult to distinguish from each other, and this would not provide a useful taxonomy. The former option, an expansion of the genus, is more useful because *Lithospermum* and the other species of New World Lithospermeae share multiple synapomorphies, including a nutlet that is erect, smooth to slightly dimpled, white, and lustrous. Although some species of the endemic New World genera of Lithospermeae have not been included in these phylogenetic analyses, careful examination has shown that these species also share the characteristic nutlet, a synapomorphy for the clade, as well as other vegetative and floral characteristics similar to those of species that are included in the analyses. Therefore, a broader circumscription of *Lithospermum* is proposed, with the genus recognized by the characteristic nutlet in association with corollas that are yellow, yellow-green, white, or orange in color. Including all members of the New World Lithospermeae in *Lithospermum* is a useful and conservative approach to the taxonomy of the group.

Several of the species that are assigned to genera other than *Lithospermum* have previously been included in *Lithospermum*, but most others have never been included in the genus. Most species that are part of the former group retain their specific epithet when included in *Lithospermum*. Species that have not been classified as part of *Lithospermum* must be transferred to that genus. As *Lithospermum* is a Linnaean name that has proven to be a catch-all for many species with smooth, white nutlets, several common specific epithets, such as *hispida*, have been used previously. Therefore, six species require new specific epithets, and those proposed here refer to the unique characteristics and history of these species.

**Lithospermum album** (G. L. Nesom) J. Cohen, **comb. nov.** Basionym: *Macromeria alba* G. L. Nesom, Madroño 36: 28. 1989. Type: Mexico. Tamaulipas, Mpio. Gomez Farias, 5–7 km, NW of Gomez Farias, just S of Agua del Indio, 30 May 1969, *A. Richardson 1763* (holotype: TEX).

**Lithospermum barbigerum** (I. M. Johnst.) J. Cohen, **comb. nov.** Basionym: *Macromeria barbigera* I. M. Johnst. J. Arnold Arbor. 16: 189. 1935. Type: Mexico. Nuevo León, N slope of Sierra Tronconal between Canyon de los Charcos and Canyon de San Miguel, ca. 15 mi SW of Galeana, "common in dense oak woods beyond pine-fir belt," 6000-9000 ft. 4 Jun 1934, *C. H. Mueller & M. T. Mueller 741* (holotype: GH; isotypes: F-n.v., MEXU, MICH-n.v., TEX).

**Lithospermum chiapense** J. Cohen, **nom. nov.** Basionym: *Macromeria guatemalensis* I. M. Johnst., J. Arnold. Arbor. 29: 232. 1948, non *Lithospermum guatemalense* Donn.Sm., Bot. Gaz. 27: 436. 1899. Type: Guatemala. San Marcos, south facing slope of Volcán Tajumulco, between Las Canojas and top of ridge, 7 mi from San Sebastián, 3300–3900 m, 16 Feb 1940, *J. A. Steyermark 35898* (holotype: GH; isotype: F).

This species has a range restricted to Guatemala and Chiapas, Mexico; the specific epithet is based on the latter.

**Lithospermum dodrantale** (I. M. Johnst.) J. Cohen, **comb. nov.** Basionym: *Onosmodium dodrantale* I. M. Johnst., J. Arnold Arbor. 18: 22. 1937**.** Type: Mexico. Nuevo León, Mpio. de Galeana, Peak of Cerro Potosí, "Scattered in colonies in the upper pine wood," 21 July 1935, *C. H. Mueller 2259* (holotype: GH-n.v.; isotypes: F, GH n.v, MEXU, MICH-n.v.).

**Lithospermum exsertum** (D. Don) J. Cohen, **comb. nov.** Basionym: *Macromeria exserta* D. Don, Edinburgh New Philos. J. 13: 239. 1832. Type: Mexico. Without locality, 1787–1804, *Sessé & Moçiño s.n.* (holotype: not found).

**Lithospermum helleri** (Small) J. Cohen, **comb. nov.** Basionym: *Onosmodium helleri* Small, Fl. S.E. U.S. 1000, 1337. 1903. Type: U.S.A. Texas: Kerr Co., along Bear Creek, 1600 - 2000 ft, 30 Apr 1894*, A. A. Heller 1682* (holotype: NY; isotypes: UC n.v, US).

**Lithospermum johnstonii** J. Cohen, **nom. nov.** Basionym: *Macromeria hispida* M. Martens & Galeotti, Bull. Acad. Roy. Sci. Bruxelles 11: 339. 1844, non *Lithospermum hispidum* Forssk., Fl. Aegypt.-Arab. 39. 1775. Type: Mexico. Michoacán: near Morelia, Cerro de Quinzeo, 6500 - 8000 ft, 1840–1844, *Galleotti 1917* (holotype: BR n.v, isotypes: GH).

This species is named for Ivan M. Johnston, Boraginaceae systematist. His work on *Lithospermum*, Lithospermeae, and Boraginaceae has provided great insight for many botanists.

**Lithospermum leonotis** (I. M. Johnst.) J. Cohen, **comb. nov.** Basionym: *Macromeria leonotis* I. M. Johnst. [“*leontis*”], J. Arnold Arbor. 16: 188. 1935. Type: Mexico. Nuevo León: “ascent into Taray,” ca. 15 mi SW of Galeana, ca. 8000 ft, 6 June 1934, *C. H. Mueller & M. T. Mueller 754* (holotype: GH; isotype: TEX).

**Lithospermum macromeria** J. Cohen, **nom. nov.** Basionym: *Macromeria viridiflora* DC. in A. P. de Candolle & A. L. P. P. de Candolle, Prodr. 10: 68. 1846, non *Lithospermum viridiflorum* Roxb., in Carey & Wallich, Fl. Ind. 2: 4. 1824**.** Type: Mexico, without locality, 1787–1804, *Sessé & Mociño 5131* (holotype: M-n.v.; probable fragment of holotype: F-n.v., GH; photo of holotype: GH, MICH-n.v.).

This specific epithet is derived from the genus *Macromeria*, the largest genus of Lithospermeae endemic to the New World. This species was given this epithet because of its broad, international range, from western Mexico to the southwestern United States.

**Lithospermum notatum** (I. M. Johnst.) J. Cohen, **comb. nov.** Basionym: *Macromeria notata* I. M. Johnst., J. Arnold Arbor. 35: 13. 1954. Type: Mexico. Nuevo León: Ascent of Sierra Infernillo, ca. 15 mi. SW of Galeana, “Common over small areas just below the peak,” 9000–10,000 ft, 16 June

1934, *C. H. Mueller & M. T. Mueller 830* (holotype: GH; isotypes: F, MICH-n.v., TEX).

**Lithospermum oaxacanum** (B. L. Turner) J. Cohen, **comb. nov.** Basionym: *Onosmodium oaxacanum* B. L. Turner, Phytologia 78: 53. 1995**.** Type: Mexico. Oaxaca, Llano Udadi, 4 km SE of San Andreas Lagunas, *Pinus pseudostrobus* woodland, 2380 m, 10 July 1981, *A. Garcia M. 504*. (holotype: MEXU).

**Lithospermum onosmodium** J. Cohen, **nom. nov.** Basionym: *Onosmodium bejariense* DC. in A. P. de Candolle & A. L. P. P. de Candolle, Prodr. 10: 70. 1846, non *Lithospermum bejariense* DC. in A. P. de Candolle & A. L. L. P. de Candolle, Prodr. 10: 79. 1946. Type: U.S.A. Texas, "Bejar ad Rio de la Trinidad," May 1828, *J. L. Berlandier 1681*. (holotype: G-DC; isotype: GH; photo of isotype: F-n.v., GH, MICH-n.v.).

This specific epithet is derived from the genus *Onosmodium*, the second largest genus of Lithospermeae endemic to the New World.

**Lithospermum pinetorum** (I. M. Johnst.) J. Cohen, **comb. nov.** Basionym: *Lasiarrhenum pinetorum* I. M. Johnst., J. Arnold Arbor. 16: 187. 1935. *Perittostema pinetorum* (I. M. Johnst.) I. M. Johnst. J. Arnold Arbor. 35: 30. 1954. Type: Mexico. Oaxaca(?), September 1841(?), *Ghiesbreght 311* (holotype: P-n.v.).

**Lithospermum rosei** (I. M. Johnst.) J. Cohen, **comb. nov.** Basionym: *Nomosa rosei* I. M. Johnst., J. Arnold. Arbor. 35: 25. 1954. Type: Mexico, in Sierra Madre near the southern border of the state of Durango, Aug 16 1897, *J. N. Rose 2360* (holotype: GH).

**Lithospermum rzedowskii** J. Cohen**, nom. nov.** Basionym: *Macromeria pringlei* Greenm., *Proc. Amer. Acad. Arts* 34: 570. 1899, non *Lithospermum pringlei* I.M.. Johnst., *Contr. Gray Herb.* 70: 22. 1924. *Onosmodium pringlei* (Greenm.) J. F. Macbr. *Contr. Gray Herb*. n.s. 49: 20. 1917. Type: Mexico. Hidalgo, Sierra de Pachuca, 10,000 ft., 4 Aug 1898, *C. G. Pringle 6949* (holotype: GH; isotypes: F, MEXU, PH-n.v., UC-n.v., US).

This species is named for Jerzy Rzedowski. Whether by chance or by design, he has collected a great number of specimens of *Lithospermum* and other New World Lithospermeae, and these collections have been very helpful in studying the variation in these groups.

**Lithospermum trinervium** (Lehm.) J. Cohen, **comb. nov.** Basionym: *Onosma trinervium* Lehm., Pl. Asperif. Nucif. 2: 37. 1818. *Lasiarrhenum trinvervium* (Lehm.) B. L. Turner Phytologia 77: 39. 1994. Type: "Habitat in America Meridionali," *Humboldt & Bonpland s.n.*? (holotype: not located).

According to Turner (1994), the type is from Mexico, with the following information provided by Lehmann: “Habitat in America Meridionali;” however, Lehmann did not name the collector or provide a collection date for the specimen. Turner (1994) states that the type is possibly based upon a specimen from Humboldt and Bonpland’s collection from Michoacán in 1803, and that this specimen is possibly at MEL, but no specimen by that name could be found there.

**Lithospermum turneri** J. Cohen, **nom. nov.** Basionym: *Macromeria hintoniorum* B. L. Turner, Phytologia 77: 398. 1994, non *Lithospermum hintoniorum* B. L. Turner, Sida 20: 501. 2002. Type: Mexico. Guerrero, Teotepec, ca. 12˚ 27’ N, 100˚ 10’ W, pine forest, 2750 m, 16 July 1939, *G. B. Hinton et al. 14439* (holotype: NY-n.v.; isotypes: F, NY-n.v., US).

This species is named for Billie L. Turner. His work on *Lithospermum* and New World Lithospermeae has been helpful in understanding these taxa.

**Lithospermum unicum** (J. F. Macbr.) J. Cohen, **comb. nov.** Basionym: *Onosmodium unicum* J. F. Macbr., Contr. Gray Herb. n.s. 49: 21. 1917. Type: Mexico. San Luis Potosí, Alvarez, 13 - 23 July 1904, *E. Palmer 185*. (holotype: GH; isotype: F).

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# CHAPTER THREE

# MOLECULAR PHYLOGENETICS AND MOLECULAR EVOLUTION OF *LITHOSPERMUM* AND RELATED TAXA

## Abstract

*Lithospermum* and the tribe to which it is assigned, Lithospermeae, have been the subject of recent phylogenetic analyses, but these analyses have been limited in their scope. Utilizing 10 chloroplast DNA regions and thorough taxon sampling, a phylogeny of *Lithospermum* and related members of both Lithospermeae and Boraginaceae is reconstructed. *Lithospermum* is supported as monophyletic, and the genus is hypothesized to have originated in the Old World, followed by one colonization of the New World. The heterostylous breeding system is inferred to have originated at least five times within *Lithospermum*. The stability of the 10-region matrix and the number and combination of regions necessary to accurately reconstruct phylogenies are investigated. The combination of concatenated regions is important, and the following regions are recommended for future phylogenetic studies of genera of Boraginaceae: the *rpl*16 intron, *mat*K, *psb*A-*trn*H, and *trn*L-*rpl*32. Concatenation of regions that evolve slowly with those that do so quickly provides a useful combination of characters for reconstruction of the greatest number of nodes that do not conflict with those reconstructed with additional sequence data.

## Introduction

*Lithospermum* L. (Boraginaceae) comprises approximately 60 species. The genus has a cosmopolitan distribution, with a center of diversity in the southwestern United States and Mexico, and with more than half of the species endemic to this region. In the present study, relationships are examined among species of

*Lithospermum* and their close relatives. Outgroups include representatives of more distantly related members of Boraginaceae, and patterns of chloroplast DNA (cpDNA) evolution are examined within *Lithospermum*, Lithospermeae, and Boraginaceae.

Linnaeus (1753) described *Lithospermum* in *Species Plantarum*, and he included six species within it. This initial circumscription encompassed species with smooth, white, lustrous, erect nutlets, and this *Lithospermum*-type of nutlet has consistently been recognized as characterizing the genus. This is the case even as the circumscription of the genus has changed, which has happened frequently. Of the six species that Linnaeus described as members of *Lithospermum*, most were subsequently transferred to other genera, and until recently, only the type remained in the genus (Cohen and Davis, 2009). Since the mid 1700s, taxonomists have considerably broadened the circumscription of *Lithospermum* three times. The first major change occurred in the treatment of Boraginaceae for the *Prodromus* (de Candolle, 1846). De Candolle described the state of *Lithospermum* 90 years after Linneaus' original generic circumscription. De Candolle's treatment circumscribed *Lithospermum* in a very broad sense and included the addition of many new species to the genus. In this treatment, de Candolle included species currently recognized as part of *Lithospermum* as well as those now placed in other, closely related genera, such as *Buglossoides* Moench. and *Lithodora* Griseb. De Candolle recognized species currently placed in *Lithospermum* by including all of those that were known at the time in the subgenus *Eulithospermum* DC. (with the exception of *L. chinensis* Hook. & Arn., currently a member of *Heliotropium* L. [Zhu et al., 1995]).

During the early to mid 20th century, Johnston (e.g., 1935, 1952) studied and reevaluated the taxonomy of Boraginaceae. Throughout his career, Johnston studied *Lithospermum* and the tribe to which it is assigned, Lithospermeae. In doing so, he described 35 new species of *Lithospermum* and described or segregated seven new genera of Lithospermeae. His work on the genus and the tribe culminated in revisions of both taxa (Johnston 1952, 1953a, b, 1954 a, b). In these publications, Johnston noted that all of the genera endemic to the New World share a series of nutlet, pollen, and floral characters with *Lithospermum* (Johnston, 1954a, b). Though he hypothesized various relationships among the genera of the tribe, these were not explicit phylogenetic hypotheses.

Until recently, Johnston's hypotheses remained untested. In 2009, Cohen and Davis presented a phylogeny based on cpDNA sequence data that included *Lithospermum* and five of the six genera of Lithospermeae endemic to the New World. This phylogeny provided evidence that all members of these New World genera – *Lasiarrhenum* I. M. Johnst., *Macromeria* D. Don, *Nomosa* I. M. Johnst., *Onosmodium* Michx., *Perritostema* I. M. Johnst., and *Psilolaemus* I. M. Johnst. – are nested among species of *Lithospermum*. In light of these recently discovered relationships, the authors included all members of these endemic New World genera in *Lithospermum*. This inclusion increased the number of species in the genus by 18, bringing the total number to approximately 60 (Cohen and Davis, 2009). Two features diagnose this more broadly defined *Lithospermum*: the *Lithospermum*-type nutlet, and corollas that are not blue or purple; these two colors occur in closely related genera. In contrast, species of *Lithospermum* produce corollas that are yellow, yellow-green, orange, or white.

Weigend et al. (2009) also have reconstructed a phylogeny of *Lithospermum*. They included less diverse taxon sampling, and their phylogeny was not as fully resolved as that of Cohen and Davis (2009). Despite these differences, both analyses placed the New World genera among species of *Lithospermum*, but the two analyses recovered conflicting phylogenetic relationships.

Phylogenies have been reconstructed for only a small number of genera in Boraginaceae, such as *Lithodora* (Thomas et al., 2008), *Cerinthe* L. (Selvi et al., 2009), *Echium* L. (Böhle et al., 1996), *Anchusa* L. (Hilger et al., 2004), and *Nonea* Medik. (Selvi et al., 2006), but currently a comprehensive phylogeny of Boraginaceae does not exist. Difficulties, for example lack of overlap among taxa sampled in different studies, arise with attempts to cobble together a phylogeny of Boraginaceae; therefore, reevaluation of the taxonomy of Boraginaceae above the genus-level becomes problematic, especially as the family includes many small genera (i.e., with fewer than five species). Through phylogenetic investigation of larger genera, it has been found that some taxa, such as *Nonea* (Selvi et al., 2006), *Cryptantha* Lehm. ex G. Don (Hasenstab and Simpson, 2008), *Lithospermum* (Cohen and Davis, 2009), and *Cynoglossum* L. (this chapter), are not monophyletic. Although the present study focuses on the phylogenetics of *Lithospermum* and its closest relatives, the outgroup sampling allows for the examination of relationships among more distantly related species of Lithospermeae and Boraginaceae.

Most of the phylogenetic analyses of genera of Boraginaceae (e.g., Hilger et al., 2004; Selvi et al., 2006, 2009; Thomas et al., 2008; Weigend et al., 2009, 2010) employ only a few DNA regions, usually a combination of one of two cpDNA spacer regions, *trn*L-*trn*F or *trn*S-*trn*G, and the nuclear ribosomal DNA internal transcribed spacer (ITS). This sampling strategy tends to provide adequate resolution among genera or families (e.g., Thomas et al., 2008), but resolution of relationships among species can prove difficult with this strategy (e.g., Hilger et al., 2004; Weigend et al., 2009, 2010). Therefore, the present study utilizes data from 10 cpDNA regions, one of them protein-encoding and the other nine non-coding. This quantity of cpDNA sequence data serves three purposes. First, the incorporation of multiple regions provides adequate sequence data to resolve phylogenetic relationships both within the ingroup – which includes all species of *Lithospermum* sensu Cohen and Davis (2009) – and among members of the outgroup, which includes species of Lithospermeae as well as members of Cynoglosseae and Boragineae, two of the other tribes of Boraginaceae. Second, the examination of this number of cpDNA regions allows for the study of molecular evolutionary patterns within the genus, tribe, and family. Third, the utilization of 10 cpDNA regions allows me to recommend certain cpDNA region combinations for future phylogenetic studies.

## Materials and Methods

### TAXON SAMPLING

Sixty-seven species were included in the analyses (Table 3.1). Thirty-seven belong to the ingroup, and this sampling represents both the morphological and geographic range of variation within *Lithospermum* sensu Cohen and Davis (2009). The outgroup comprises 30 species from related genera of Boraginaceae: eight from Cynoglosseae, three from Boragineae, and 19 from Lithospermeae. The majority of the species were collected from wild populations. For these taxa, herbarium specimens were collected and deposited at BH, and leaf tissue was dried and preserved in silica gel for subsequent DNA extraction. Taxa not collected from natural populations were obtained from gardens (i.e., Cornell Plantations, Missouri Botanical Garden, and National Botanic Garden of Belgium) as leaf samples preserved in silica gel or as DNA isolations from either the DNA bank of Royal Botanic Gardens, Kew or the South African National Biodiversity Institute (SANBI). For some species, sequence data for the *trn*L-*trn*F spacer and *mat*K were obtained from GenBank.

Table 3.1.  List of taxa included in analyses along with collection or source data

| **Taxon** | **Collection** | **Location** |
|---|---|---|
| **Outgroup** | | |
| *Amsinckia tessellata* A. Gray | W6 27115 | USDA |
| *Anchusa leptophylla* Roem. & Schult. | 2002 1090-96 | Natl. Bot. Gard. Belgium |
| *Arnebia benthamii* (Wall ex. G. Don) I. M. Johnst. | Chase 34887 | RBG Kew |
| *Buglossoides incrassata* (Guss.) I. M. Johnst. | FJ763255 | GenBank |
| *Buglossoides purpureo-caerulea* (L.) I. M. Johnst. | 19792084, Chase 6055 | Natl. Bot. Gard. Belgium and RBG Kew |
| *Buglossoides tenuiflora* (L.f.) I. M. Johnst. | EU599939, EU599675 | GenBank |
| *Buglossoides arvensis* (L.) I. M. Johnst. | 19792083/PI 296073 | Natl. Bot. Gard. Belgium and USDA |
| *Cerinthe major* L. | Cohen 91 | Cultivated in New York |
| *Cryptantha* sp. | Cohen 227 | Michoacán, Mexico |
| *Cynoglossum amabile* Stapf & Drummond | Cohen 89 | Cultivated in New York |
| *Cynoglossum pringlei* Greenm. | Cohen 219 | Michoacán, Mexico |
| *Echium vulgare* L. | Cohen 212 | Cornell Plantations |
| *Glandora diffusa* (Lag.) D. C. Thomas | Chase 6063 | RBG Kew |
| *Glandora oleifolia* (Lapyer.) D. C. Thomas | Chase 34889, 34890 | RBG Kew |
| *Halacsya sendtneri* Dörfl. | EU919618, EU044885 | GenBank |
| *Lindelofia longiflora* Baill. | 19981513-95 | Natl. Bot. Gard. Belgium |
| *Lithodora hispidula* (Sibth. & Sm.) Griseb. | Chase 34888 | RBG Kew |
| *Lithodora zahnii* (Heldr. ex Halácsy) I. M. Johnst. | Chase 34891 | RBG Kew |
| *Maharanga emodi* DC. | FJ763269 | GenBank |
| *Mairetis microsperma* (Boiss.) I. M. Johnst. | FJ763257, EU919620 | GenBank |
| *Mertensia virginica* Link | Cohen 89 | New York, USA |
| *Moltkia petraea* (Tratt.) Griseb. | 2000 1260-54 | Natl. Bot. Gard. Belgium |
| *Neatostema apulum* (L.) I. M. Johnst. | EU919627, FJ763262 | GenBank |
| *Omphalodes cappadocica* DC | 1995-3595 | Missouri Bot. Gard. |
| *Omphalodes verna* Moench | 19830183 | Natl. Bot. Gard. Belgium |
| *Onosma stellulata* Waldst. & Kit. | 1992 1317-39 | Natl. Bot. Gard. Belgium |

Table 3.1 (Continued)

| | | |
|---|---|---|
| *Paramoltkia doerfleri* (Wettst.) Greuter & Burdet | EU919630,<br>EU044886 | GenBank |
| *Podonosma orientalis* (L.) Feinbrun | FJ763307 | GenBank |
| *Symphytum asperum* Lepech. | Cohen 221A | Chicago Bot. Gard. |
| *Trachystemon orientalis* (L.) G. Don | 1978-1795 | Missouri Bot. Gard. |
| | | |
| **Ingroup** | | |
| *Lithospermum calcicola* B. L. Rob | Cohen 191 | Nuevo León, Mexico |
| *Lithospermum californicum* A. Gray | Cohen 43, 157 | California and Oregon, USA |
| *Lithospermum calycosum* (J. F. Macbr.) I. M. Johnst. | Cohen 197 | Nuevo León, Mexico |
| *Lithospermum canescens* Lehm. | Cohen and Straub 12, Cohen 1, 176 | Ohio, Alabama, and Michigan, USA |
| *Lithospermum caroliniense* MacMill. | Cohen 6 and 11 | Indiana, USA |
| *Lithospermum cinereum* DC. | FJ763295 | GenBank |
| *Lithospermum cobrense* Greene | Cohen 78, 145, 203 | Arizona and Texas, USA and Durango, Mexico |
| *Lithospermum discolor* M. Martens & Galeotti | Cohen 216, 243 | Michoacán and Guerrero, Mexico |
| *Lithospermum distichum* Ortega | Cohen 192, 202 | Nuevo León and D. F., Mexico |
| *Lithospermum erythrorhizon* Siebold & Zucc. | Cohen 173 | Cornell Plantations |
| *Lithospermum exsertum* (D. Don) J. I. Cohen | Cohen 244 | Guerrero, Mexico |
| *Lithospermum flavum* Sessé & Moc. | Cohen 226 | Michoacán, Mexico |
| *Lithospermum gayanum* I. M. Johnst. | FJ763297 | GenBank |
| *Lithospermum helleri (Small)* J. I. Cohen | Cohen 132 | Texas, USA |
| *Lithospermum johnstonii* J. I. Cohen | Cohen 218 | Michoacán, Mexico |
| *Lithospermum latifolium* Michx. | Cohen 87 and 220A | New York, USA and Chicago Botanical Garden |
| *Lithospermum leonotis* (I. M. Johnst.) J. I. Cohen | Cohen 195 | Nuevo León, Mexico |

Table 3.1 (Continued)

| | | |
|---|---|---|
| *Lithospermum macromeria* J. I. Cohen | Cohen 141, 151 | Arizona, USA |
| *Lithospermum matamorense* DC. | Cohen and Straub 68 | Texas, USA |
| *Lithospermum molle* Michx. | 2003-0671 | Missouri Bot. Gard. |
| *Lithospermum multiflorum* Torr. ex A. Gray | Cohen 49, 57, 64, 81 | Utah, Arizona, Texas, USA |
| *Lithospermum nelsonii* Greenm. | Cohen 184 | Nuevo León, Mexico |
| *Lithospermum notatum* (I. M. Johnst.) J. I. Cohen | Cohen 188 | Nuevo León, Mexico |
| *Lithospermum oblongifolium* Greenm. | Cohen 201 | D. F., Mexico |
| *Lithospermum obovatum* J. F. Macbr. | Cohen 208 | Durango, Mexico |
| *Lithospermum officinale* L. | Cohen 171 | Cornell Plantations |
| *Lithospermum mirabile* Small | Cohen 83, Cohen and Straub 35 | Texas, USA |
| *Lithospermum mirabile* × *incisum* | Cohen and Straub 64 | Texas, USA |
| *Lithospermum revolutum* B. L. Rob. | Cohen 199 | San Luis Potosí, Mexico |
| *Lithospermum rosei* (I. M. Johnst.) J. I. Cohen | Cohen 207 | Durango, Mexico |
| *Lithospermum ruderale* Douglas ex Lehm. | Cohen 34 | Utah, USA |
| *Lithospermum scabrum* Thunb. | LHMS 2125, FJ763293 | SANBI and GenBank |
| *Lithospermum strictum* Lehm. | Cohen 225 | Michoacán, Mexico |
| *Lithospermum trinervium* (Lehm.) J. I. Cohen | Cohen 228 | Michoacán, Mexico |
| *Lithospermum tuberosum* DC. | Cohen 108 | Georgia, USA |
| *Lithospermum tubuliflorum* Greene | M. Gonzalez 4001 | Durango, Mexico |
| *Lithospermum viride* Greene | Cohen 86 | Texas, USA |

### DNA EXTRACTION, PCR AMPLIFICATION, AND SEQUENCING

DNA extraction was performed with dried plant tissue using a modified CTAB extraction method (Doyle and Doyle, 1990) that included 2% PVP-40 and in some cases the addition of 0.5 M glucose in the CTAB extraction buffer. PCR amplifications of the ten chloroplast DNA regions, *ndh*F - *rpl*32, *psb*A - *trn*H, *psb*J - *pet*A, the *rpl*16 intron, *trn*K - *rps*16, *trn*L - *rpl*32, *trn*Q - *rps*16, *ycf*6 - *psb*M, *trn*L - *trn*F (Shaw et al., 2005, Shaw et al., 2007), and *mat*K (390F [Cuénoud et al., 2002] and 1710R [Barfuss et al., 2005]), were performed using the published primers of the cited authors. The only exception was a pair of primers designed by the authors to amplify part of *mat*K for some species of *Lithospermum* (*mat*K-Lith2F 5’ CAC GAG TAT TGG AAT CCT TTT ATT 3’ and *mat*K-Lith806R 5’ TTG TGT TTC CGA GCC AAA GT 3’). PCR mixtures, 25 µL in volume, consisted of 67 mM Tris-HCl with 2.1% DMSO and 0.01% TritonX per reaction or 1X *Ex Taq* Buffer (Takara Bio Inc., Japan), 2 mM $MgCl_2$, 0.2 mM to 0.25 mM dNTPs, 1 µM of primers, 0.125 µL to 1 µL of *Taq* polymerase, and 0.1 µL to 2.5 µL DNA sample, depending on the DNA concentration. Amplifications were performed in an Eppendorf Mastercycler Gradient 5331 thermocycler with the programs and annealing temperatures listed in Cohen and Davis (2009). PCR products were separated on a 1% to 1.5% agarose gel and stained with ethidium bromide to determine whether amplification had occurred. Prior to sequencing, some PCR products were purified with the QIAquick PCR purification kit (Qiagen, Germany).

Nuclear ribosomal ITS was examined, as it provides a large number of informative characters and is used in many phylogenetic studies of genera of Boraginaceae (e.g., Thomas et al., 2008; Weigend et al., 2009). After the initial exploration of this region, it was determined that multiple copies exist in some species of *Lithospermum* and related taxa, and it proved difficult to align unambiguously the sequences of this region for the species included in the present study. Given these issues, along with time and budgetary constraints and other concerns associated with ITS (Alvarez and Wendel, 2003), the region was not included in this study. However, other cpDNA regions were employed that have not been used in other studies of Boraginaceae, such as the *rpl*16 intron, *trn*L-*rpl*32, and *ndh*F-*rpl*32.

Sequencing reactions were performed with BigDye 3.1 (Applied Biosystems, CA U.S.A.) terminators and locus-specific amplification primers. Sequencing products were precipitated using a modification of the ethanol/EDTA/sodium acetate method (Applied Biosystems), and automated cycle sequencing was performed by the Life Sciences Core Laboratory Center at Cornell University with an Applied Biosystems (ABI) 3730 DNA Analyzer. Alternatively, sequencing reactions and subsequent steps were performed by the Life Science Core Laboratory Center at Cornell University, using Big Dye terminators and either an ABI 3700 or an ABI 3730. Sequence trace files were compiled, examined, and edited with Sequencher ver. 4.6 - 4.8 (Gene Codes Corporation, MI U.S.A.). Sequences will be deposited in GenBank, and the matrix will be available at Treebase.

### ALIGNMENT, GAP CODING, AND PHYLOGENETIC ANALYSIS

***Matrix of 10 cpDNA regions*** - Initial alignments were performed with MUSCLE (Edgar, 2004) as implemented by the European Bioinformatics Institute's MUSCLE server (http://www.ebi.ac.uk/Tools/muscle/index.html) using the default settings. Subsequent adjustments were made in Bioedit ver. 7.0.5.3 (Hall, 1999) and Winclada ver. 1.7 (Nixon, 2002). Gaps were coded using simple indel coding (Simmons and Ochoterena, 2000). Inversions were coded as present/absent, and the nucleotides of inversions were excluded from analyses (Ochoterena, 2009). When informative characters were embedded in an inferred inversion, the inverted sequences were recoded with their reverse complement, and these characters were included in the analyses (Graham et al., 2000; Davis and Soreng, 2010). Additionally, unusual nucleotide motifs were coded as present/absent. An unusual nucleotide motif is defined as a short (between seven and 10 nucleotides in length) non-inverted, non-length-variable (or shorter by one nucleotide) sequence of nucleotides that occurs in two or more taxa and substantially differs in composition (more than 50% of the aligned nucleotides) from the sequences of most other species. Each unusual nucleotide motif was treated as a single character, as each is treated provisionally as having arisen from a single event. All characters were weighted equally and treated as unordered (i.e., nonadditive). Regions that aligned ambiguously were excluded from analyses.

The matrix of 10 cpDNA regions consists of 20% missing data. This percentage largely reflects the use of sequence data from GenBank for 10 species for which only one or two regions were available. When these 10 species are excluded from the matrix, the percentage of missing data drops to 5.7%. The topology of the strict consensus tree, in terms of taxa with more complete coverage, is not affected by the inclusion of these 10 species.

Individual cpDNA regions were also aligned for two subsets of taxa: species of Lithospermeae and species of *Lithospermum*. Some regions aligned ambiguously with all taxa or were excluded due to inversions, but some of these same regions aligned unambiguously with a smaller subset of taxa. Therefore, some regions that were excluded from the aligned matrix of all species of Boraginaceae or Lithospermeae were included in the aligned sequence matrix of species of Lithospermeae or *Lithospermum*, respectively.

Maximum parsimony phylogenetic analyses were conducted with the entire data matrix and with various permutations of the data matrix described below. The following search strategy was applied in all analyses except where noted: the data were analyzed using TNT (Goloboff et al., 2008), with 1,000,000 trees held in memory, and 1,000 independent iterations, with 20 trees held per iteration, of 1,000 parsimony ratchet iterations (Nixon, 1999), with 10% probability of upweighting and 10% probability of downweighting, followed by 1,000 cycles of tree drifting; afterwards, 100 rounds of tree fusion and random sectorial searches were performed (Goloboff, 1999a), followed by TBR-max, swapping among all the most-parsimonious trees until completion. Additionally, a more abbreviated, search strategy was performed: 10,000 trees held in memory, and one iteration of 1,000 parsimony ratchet iterations (Nixon, 1999), with 10% probability of upweighting and 10% probability of downweighting, followed by 1,000 cycles of tree drifting, 100 rounds of tree fusion, random sectorial searches (Goloboff, 1999a) and TBR-max. Clade support was measured with NONA (Goloboff, 1999b) by conducting 10,000 jackknife replicates (36% removal probability) (Farris et al., 1996). For each replicate 10 TBR searches were conducted, with one tree held after each replicate, and a total of 100,000 trees held in memory for the duration of the entire jackknife resampling. Consistency indices were calculated after removal of parsimony-uninformative characters.

***Exclusion analyses* –** A series of exclusion analyses was conducted in order to compare and evaluate the contribution of each cpDNA region. Each exclusion analysis involved the removal of one region, followed by analysis of a matrix consisting of the remaining nine regions. For these analyses, two initial data matrices were constructed from the 67-taxon matrix; one includes species with all 10 regions sampled (the 100% matrix), and the other consists of species with 80% (any eight of ten) of the regions sampled (the 80% matrix). The 100% matrix includes 34 species, and the 80% matrix includes 53 species. The exclusion analyses were conducted using the abbreviated search strategy described above, with ten analyses performed for each matrix, one for each cpDNA region removed. The strict consensus tree for each analysis was compared to that of the corresponding matrix with all 10 regions included. To investigate the utility of each region, the number of nodes resolved in the consensus tree and the number of contradictory nodes resolved in the consensus tree were counted and compared. A contradictory node is defined as a node in the consensus tree from an exclusion analysis matrix that conflicts with the structure of one of the most-parsimonious (MP) trees from the corresponding 10-region matrix.

***Successive-inclusion analyses*** - To determine the utility in the reconstruction of phylogenetic relationships of the cpDNA regions with the greatest number of informative characters as compared to those with the smallest number informative characters, successive-inclusion (SI) analyses were performed. These analyses involve the use of successively larger numbers of individual cpDNA regions in analyses. I conducted SI analyses by beginning with the cpDNA region with either the greatest or the smallest number of characters, and performing a phylogenetic analysis. This was followed by the addition of the region with either the next greatest or next smallest number of informative characters, respectively, performing a phylogenetic analysis with this matrix, and continuing until all regions were included. These analyses were performed on the same 100% and 80% matrices that were used in the exclusion analyses, with the abbreviated search strategy described above, with one exception. For some analyses, 100,000 trees, rather than 10,000 trees, were held in memory. A total of nine analyses were performed, one per cpDNA region added, for each type of inclusion analysis (i.e., more to fewer or fewer to more). A strict consensus tree was reconstructed for each analysis. The total number of nodes reconstructed and the total number of non-contradictory nodes reconstructed were counted, as with the exclusion analyses, and these numbers were compared to the respective consensus tree reconstructed from the matrix that includes all 10 cpDNA regions. Contradictory nodes were determined in the same manner as for the exclusion analyses.

For the 100% matrix, the order of the regions from greatest number of informative characters to fewest is *rpl*16, *trn*L-*rpl*32, *psb*J-*pet*A, *ycf*6-*psb*M, *ndh*F-*rpl*32 (tie), *mat*K (tie), *trn*K-*rps*16, *trn*Q-*rps*16, *psb*A-*trn*H, and *trn*L-*trn*F. For the 80% matrix, the order of the regions from greatest number of informative characters to fewest is *rpl*16, *psb*J-*pet*A, *mat*K, *ycf*6-*psb*M, *trn*K-*rps*16, *trn*L-*rpl*32, *ndh*F-*rpl*32, *trn*Q-*rps*16, *psb*A-*trn*H, and *trn*L-*trn*F.

***Analyses of matrices of five-cpDNA regions* –** Randomly-constructed five-cpDNA region matrices were created and analyzed in order to compare the utility of five regions, as opposed to 10, in the reconstruction of phylogenetic relationships. Using the 80% matrix of the exclusion and SI analyses, matrices for each cpDNA region were created. The matrices of these individual regions were concatenated in order to construct 100 randomly-selected five-region matrices. Each matrix was analyzed using the abbreviated search strategy described above, with one exception. Instead of holding 10,000 trees in memory, 100,000 trees were held.

The total number of resolved nodes and the number of non-contradictory nodes were counted on the consensus tree from each matrix, and the difference between these two numbers also was calculated. A non-contradictory node is defined in the same manner as above.

***Single cpDNA region analyses* –** To examine the utility of each cpDNA region at various taxonomic levels, four different matrices were constructed: the Boraginaceae matrix with 67 species of the family, the Lithospermeae matrix with 56 species of the tribe, the *Lithospermum* matrix with 37 species of the genus, and the 100% matrix of the exclusion, SI, and five-cpDNA region analyses. A total of ten analyses were performed for each matrix, one per cpDNA region, and the individual region matrices included only the taxa with that region sampled. The matrices were analyzed using the abbreviated search strategy described above, with one exception. In some analyses of single regions, more than 10,000 MP trees were discovered. Therefore, I increased the number of trees held in memory to 100,000 or 1,000,000, but 1,000,000 MP trees were found in searches for some regions.

As with the exclusion, SI, and five-cpDNA region analyses, the resulting strict consensus trees were compared to those from the respective matrix with all 10 cpDNA regions included. In the Boraginaceae, Lithospermeae, and *Lithospermum* matrices, the taxon sampling differs among single-region matrices. In order to account for bias that may result from greater resolution solely due to increased taxon sampling, the percentage of nodes resolved and the percentage of contradictory nodes resolved were calculated. The percentage of nodes resolved was calculated using the following equation: nodes resolved/total possible nodes resolved. Total possible nodes resolved is calculated by subtracting 2 from the number of taxa in the matrix. The percentage of contradictory nodes resolved was calculated using the following equation: contradictory nodes resolved/nodes resolved. Because taxon sampling is consistent among the single regions of the 100% matrix, the number of nodes resolved and the number of contradictory nodes resolved in the consensus trees were counted and compared, just as in the exclusion, inclusion, and five-region analyses. A contradictory node was determined in the same manner as described above.

## Results

### SEQUENCE VARIATION

***Boraginaceae*** - A total of 10,036 aligned nucleotides from 67 species were included in the analyses (Table 3.2). Most portions of all regions aligned unambiguously, with the exception of *trn*Q-*rps*16. The alignment of this region for species of Cynoglosseae and Lithospermeae was ambiguous; therefore, the alignments for the species of these two tribes were treated as if they were separate regions. The mean number of species sequenced for each region was 54, with the greatest number of species, 60, sequenced for the *trn*L-*trn*F spacer, and the smallest number of species, 50, sequenced for the *psb*J-*pet*A, *trn*K-*rps*16 and *trn*Q-*rps*16 spacers. The sequence data yielded 918 parsimony-informative characters, 811 (88%) from nucleotides, and 107 (12%) from gaps, inversions, and unusual nucleotide motifs. The sequenced regions ranged in length from 546 base pairs (bp) in the *trn*Q-*rps*16 spacer in species of Lithospermeae to 1,390 bp in the *ycf*6-*psb*M spacer. The regions with the greatest number of informative nucleotides were the *rpl*16 intron, with 121 informative nucleotides out of 1,310 bp (11.6% of the total sequence length), the *psb*J-*pet*A spacer, with 110 informative nucleotides out of 1,060 bp (11.1% of the total sequence length), and *mat*K, with 108 informative nucleotides out of 1,299 bp (8.4% of the total sequence length). The least informative regions in terms of total number of characters were the *trn*Q-*rps*16, *psb*A-*trn*H, and *trn*L-*trn*F spacers, which each provided 43 or 44 informative nucleotides. However, both *psb*A-*trn*H and *trn*L-*trn*F have a greater percentage of informative nucleotides, 10.6% and 7.2% respectively, than *trn*Q-*rps*16, which yields between 2% and 5% informative nucleotides. Additionally, although *ycf*6-*psb*M provides 84 informative nucleotides (almost as many as *trn*Q-*rps*16 and *trn*L-*trn*F combined), it is one of the longest regions included in the present study, and only 6.2% the nucleotides are informative. Of the ten sampled regions, the *ycf*6-*psb*M, *ndh*F-*rpl*32, and *psb*J-*pet*A spacers provide the greatest number of structural DNA characters (indels, inversions, and unusual nucleotide motifs), 18, 17, and 15 respectively. Most other regions have fewer than 10 structural DNA characters.

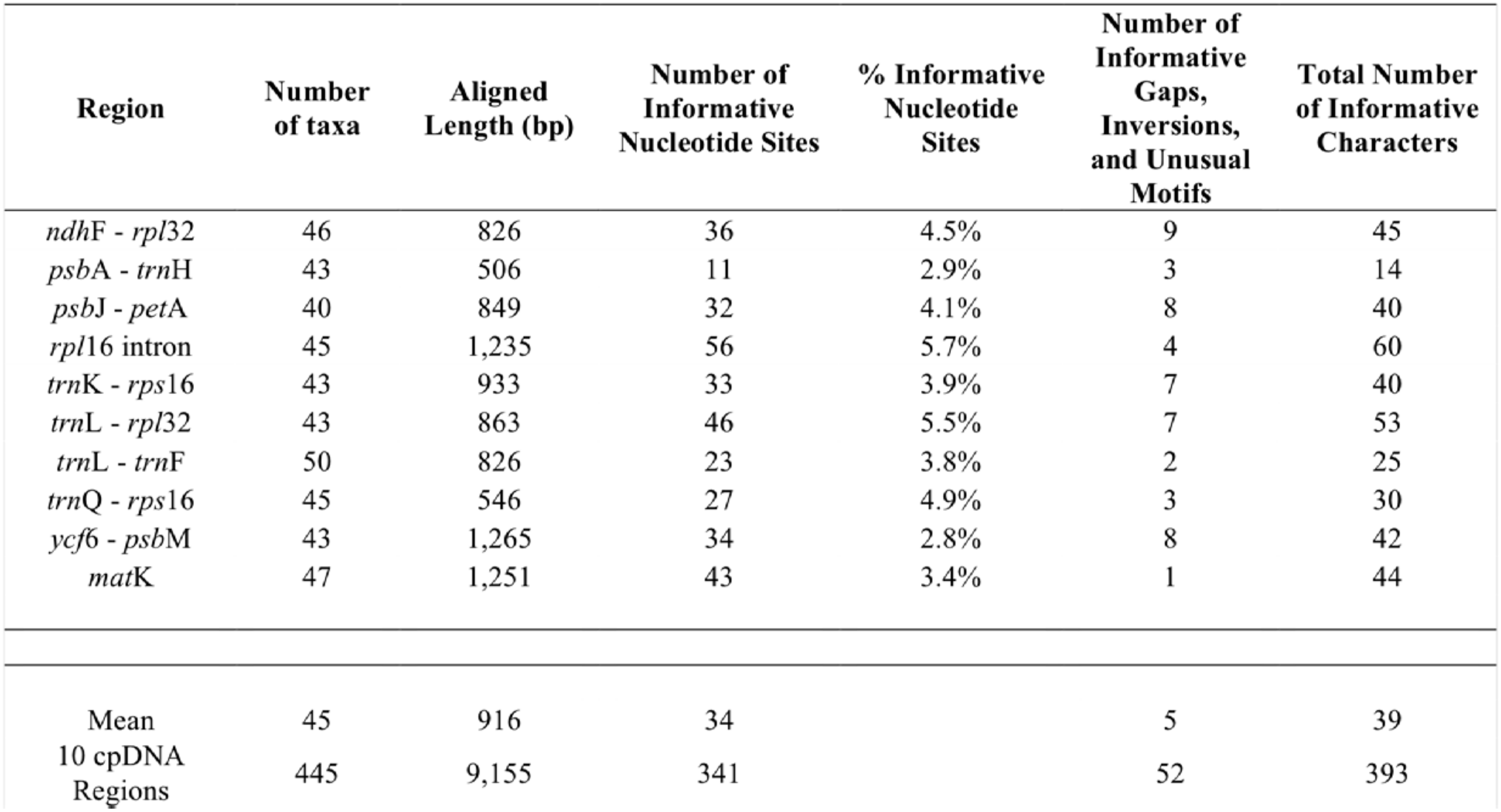
***Lithospermeae*** - The sequences from 56 species of Lithospermeae provided 9,155 aligned nucleotides (Table 3.3). The mean number of species sequenced for each region was 45, with the greatest number of species, 50, sequenced for the *trn*L-*trn*F spacer, and the smallest number of species, 40, sequenced for the *psb*J-*pet*A spacer. The sequence data yielded 393 informative characters, 341 (87%) from nucleotides, and 52 (13%) from gaps, inversions, and unusual nucleotide motifs. The sequenced regions ranged in length from 506 bp in the *psb*A-*trn*H spacer to 1,265 bp in the *ycf*6-*psb*M spacer. The region that provided the greatest number of informative nucleotides was *rpl*16, with 56 informative nucleotides out of 1,235 bp (5.7% of the total sequence length). The least informative region in terms of number of characters was the *psb*M-*trn*H spacer, with 11 informative nucleotides out of 506 bp (2.9% of the total sequence length). The *ycf*6-*psb*M spacer provides the smallest percentage of informative nucleotides (2.8%), though this region yielded 34 informative nucleotides, more than three times that of the *psb*A-*trn*H spacer.

***Lithospermum*** - The alignment of sequences from 37 species of *Lithospermum* provided 7,919 aligned nucleotides (Table 3.4). The mean number of species sequenced for each region was 33, with the greatest number of species, 35, sequenced for the *ndh*F-*rpl*32 spacer, and the smallest number of species, 30, sequenced for the *psb*J-*pet*A spacer. The sequence data yielded 163 informative characters, 141 (87%) from nucleotides, and 22 (13%) from gaps, inversions, and unusual nucleotide motifs.

Table 3.2. Summary statistics of cpDNA regions included in analyses of Boraginaceae matrix

| Region | Number of taxa | Aligned Length (bp) | Number of Informative Nucleotide Sites | % Informative Nucleotide Sites | Number of Informative Gaps, Inversions, and Unusual Motifs | Total Number of Informative Characters |
|---|---|---|---|---|---|---|
| *ndh*F - *rpl*32 | 57 | 981 | 79 | 10.2% | 17 | 96 |
| *psb*A - *trn*H | 53 | 563 | 44 | 10.6% | 4 | 48 |
| *psb*J - *pet*A | 50 | 1,060 | 110 | 11.1%% | 15 | 125 |
| *rpl*16 intron | 55 | 1,310 | 121 | 11.6%% | 6 | 127 |
| *trn*K - *rps*16 | 50 | 1,058 | 90 | 9.8%% | 9 | 99 |
| *trn*L - *rpl*32 | 51 | 973 | 88 | 9.5% | 8 | 96 |
| *trn*L - *trn*F | 60 | 856 | 44 | 7.2% | 7 | 51 |
| *trn*Q - *rps*16 | 50 (45 + 5) | 546 + 927 | 27 + 16 | 5% + 2% | 3 + 11 | 57 |
| *ycf*6 - *psb*M | 54 | 1,390 | 84 | 6.2% | 18 | 102 |
| *mat*K | 58 | 1,299 | 108 | 8.4% | 9 | 117 |
| Mean | 54 | 1,004 | 81 |  | 11 | 91 |
| 10 cpDNA Regions | 538 | 10,036 | 811 |  | 107 | 918 |

Table 3.3. Summary statistics of cpDNA regions included in analyses of Lithospermeae matrix



| Region | Number of taxa | Aligned Length (bp) | Number of Informative Nucleotide Sites | % Informative Nucleotide Sites | Number of Informative Gaps, Inversions, and Unusual Motifs | Total Number of Informative Characters |
|---|---|---|---|---|---|---|
| *ndh*F - *rpl*32 | 46 | 826 | 36 | 4.5% | 9 | 45 |
| *psb*A - *trn*H | 43 | 506 | 11 | 2.9% | 3 | 14 |
| *psb*J - *pet*A | 40 | 849 | 32 | 4.1% | 8 | 40 |
| *rpl*16 intron | 45 | 1,235 | 56 | 5.7% | 4 | 60 |
| *trn*K - *rps*16 | 43 | 933 | 33 | 3.9% | 7 | 40 |
| *trn*L - *rpl*32 | 43 | 863 | 46 | 5.5% | 7 | 53 |
| *trn*L - *trn*F | 50 | 826 | 23 | 3.8% | 2 | 25 |
| *trn*Q - *rps*16 | 45 | 546 | 27 | 4.9% | 3 | 30 |
| *ycf*6 - *psb*M | 43 | 1,265 | 34 | 2.8% | 8 | 42 |
| *mat*K | 47 | 1,251 | 43 | 3.4% | 1 | 44 |
| Mean | 45 | 916 | 34 |  | 5 | 39 |
| 10 cpDNA Regions | 445 | 9,155 | 341 |  | 52 | 393 |

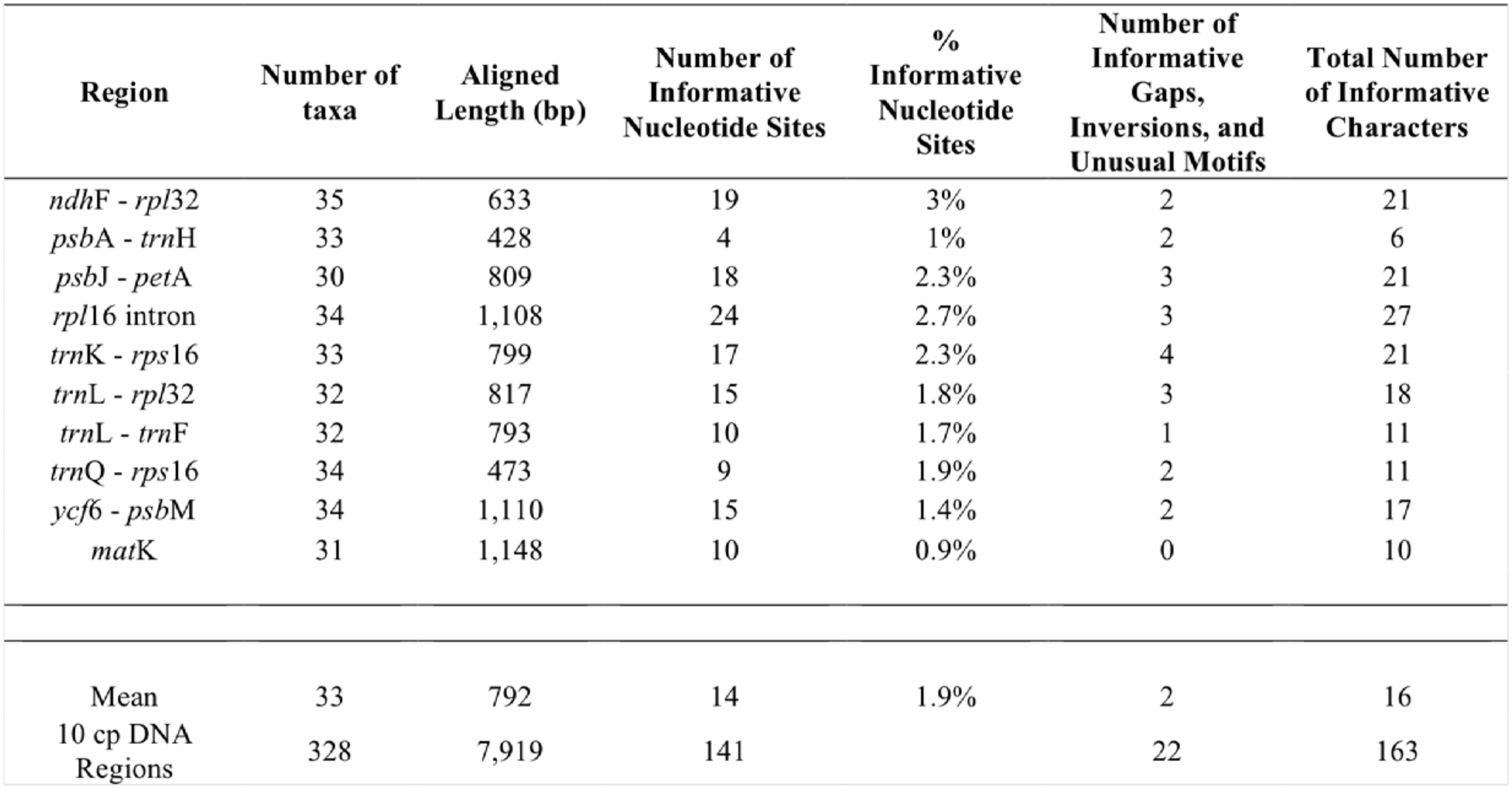
The sequenced cpDNA regions ranged in length from 428 bp in the *psb*A-*trn*H spacer to 1,148 bp in *mat*K. The *rpl*16 spacer provided the greatest number of informative nucleotides, 24 out of 1,110 bp (2.7% of the total sequence length). The least informative region in terms of number of informative nucleotides was *psb*A-*trn*H, which provided only four. However, with ten informative nucleotides, *mat*K was the least informative region in terms of percentage of informative nucleotides (0.9% vs. 1% for *psb*A - *trn*H).

### PHYLOGENETIC RESULTS

***Matrix of 10 cpDNA regions*** - Eighty most-parsimonious trees of 1,810 steps (CI - 0.61, RI - 0.80) were discovered. The strict consensus tree is provided in Figure 3.1. Relationships among species of Cynoglosseae are well-resolved and well-supported, with all branches having greater than 96% jackknife support (jk). *Omphalodes* Tourn. ex Moench is monophyletic and sister to *Mertensia* Roth. The two species of *Cynoglossum* included in the analyses, *C. officinale* L. and *C. pringlei* Greenm., do not form a monophyletic group. *Cynoglossum officinale* is sister to *Lindelofia longiflora* (Benth.) Baill., while *C. pringlei* is sister to *Amsinckia tessellata* A. Gray. Although only three species are included in the present analysis, Boragineae is supported as a monophyletic group (99% jk).

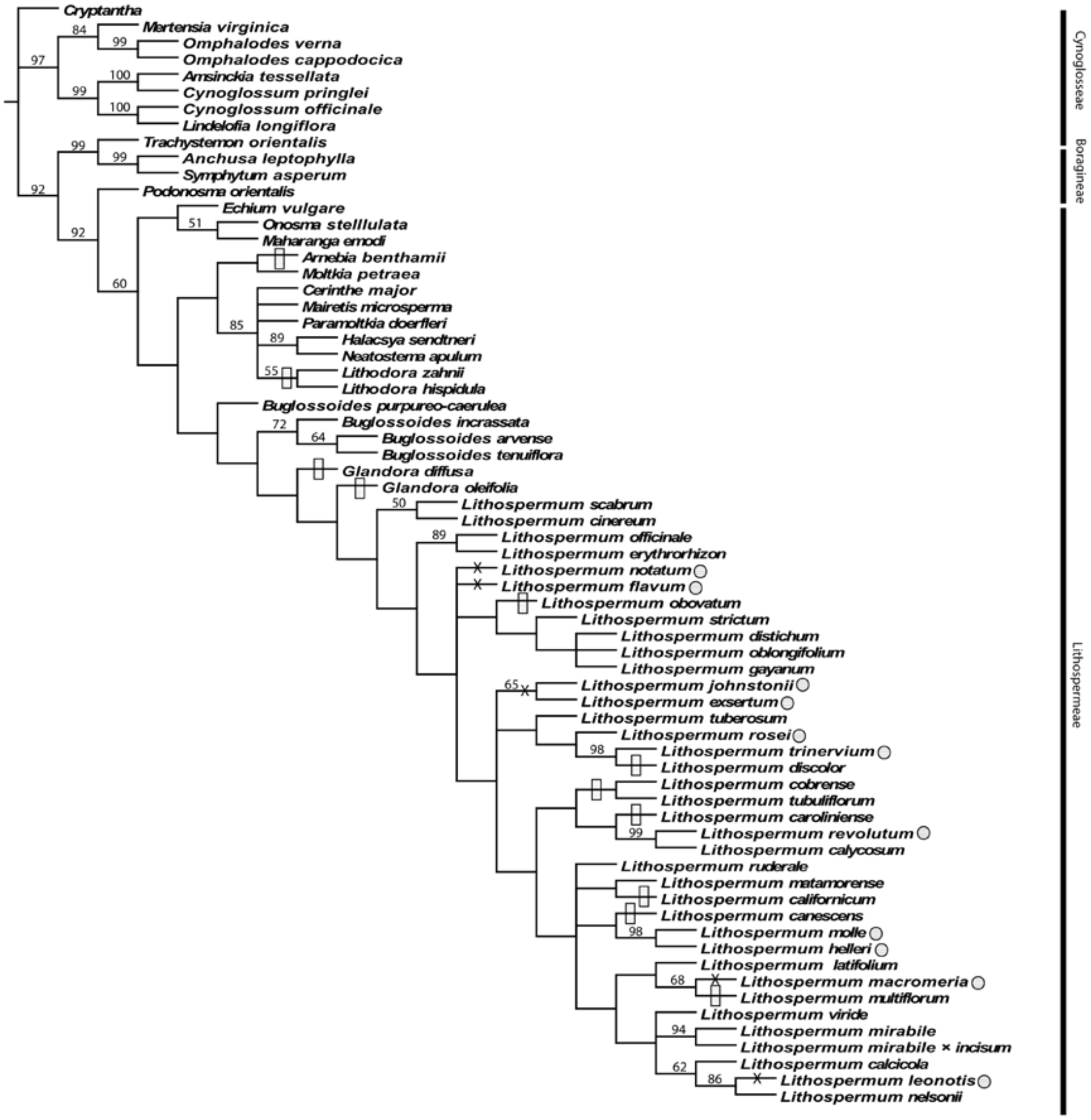
Lithospermeae is monophyletic, and the tribe forms a well-resolved and well-supported clade (92% jk). *Podonosma* Boiss. is sister to the rest of Lithospermeae (92% jk). A clade composed of *Echium* L., *Onosma* L., and *Maharanga* DC. is sister to the remainder of the genus, but this clade has weak support (< 50% jk). A clade including *Cerinthe* L., *Mairetis* I. M. Johnst., *Paramoltkia* Greuter, *Halacsya* Dörfl., *Neatostema* I. M. Johnst., and two species of *Lithodora* receives strong support (85% jk), and it is sister to a clade composed of *Arnebia* Forssk. and *Moltkia* Lehm.

Table 3.4. Summary statistics of cpDNA regions included in analyses of *Lithospermum* matrix



| Region | Number of taxa | Aligned Length (bp) | Number of Informative Nucleotide Sites | % Informative Nucleotide Sites | Number of Informative Gaps, Inversions, and Unusual Motifs | Total Number of Informative Characters |
|---|---|---|---|---|---|---|
| *ndh*F - *rpl*32 | 35 | 633 | 19 | 3% | 2 | 21 |
| *psb*A - *trn*H | 33 | 428 | 4 | 1% | 2 | 6 |
| *psb*J - *pet*A | 30 | 809 | 18 | 2.3% | 3 | 21 |
| *rpl*16 intron | 34 | 1,108 | 24 | 2.7% | 3 | 27 |
| *trn*K - *rps*16 | 33 | 799 | 17 | 2.3% | 4 | 21 |
| *trn*L - *rpl*32 | 32 | 817 | 15 | 1.8% | 3 | 18 |
| *trn*L - *trn*F | 32 | 793 | 10 | 1.7% | 1 | 11 |
| *trn*Q - *rps*16 | 34 | 473 | 9 | 1.9% | 2 | 11 |
| *ycf*6 - *psb*M | 34 | 1,110 | 15 | 1.4% | 2 | 17 |
| *mat*K | 31 | 1,148 | 10 | 0.9% | 0 | 10 |
| Mean | 33 | 792 | 14 | 1.9% | 2 | 16 |
| 10 cp DNA Regions | 328 | 7,919 | 141 |  | 22 | 163 |

Figure 3.1. Strict consensus of 80 most-parsimonious trees (L – 1,810 steps, CI/RI – 0.61/0.80). Consensus tree based on matrix that includes 67 taxa and 10 cpDNA regions. Numbers above branches are jackknife percentages greater than 50%. Open rectangles denote origins of heterostyly. Crosses represent origins of *Macromeria*-type flowers. Grey circles represent species that Johnston and other authors traditionally have not included in *Lithospermum* (see text for discussion).


Cryptantha
84
Mertensia virginica
97
99
Omphalodes verna
Omphalodes cappodocica
100
Amsinckia tessellata
99
Cynoglossum pringlei
100
Cynoglossum officinale
Lindelofia longiflora
99
Trachystemon orientalis
99
Anchusa leptophylla
Symphytum asperum
92
Podonosma orientalis
Echium vulgare
51
Onosma stelllulata
92
Maharanga emodi
Arnebia benthamii
Moltkia petraea
60
Cerinthe major
Mairetis microsperma
85
Paramoltkia doerfleri
89
Halacsya sendtneri
Neatostema apulum
55
Lithodora zahnii
Lithodora hispidula
Buglossoides purpureo-caerulea
72
Buglossoides incrassata
64
Buglossoides arvense
Buglossoides tenuiflora
Glandora diffusa
Glandora oleifolia
50
Lithospermum scabrum
Lithospermum cinereum
89
Lithospermum officinale
Lithospermum erythrorhizon
Lithospermum notatum
Lithospermum flavum
Lithospermum obovatum
Lithospermum strictum
Lithospermum distichum
Lithospermum oblongifolium
Lithospermum gayanum
65
Lithospermum johnstonii
Lithospermum exsertum
Lithospermum tuberosum
Lithospermum rosei
98
Lithospermum trinervium
Lithospermum discolor
Lithospermum cobrense
Lithospermum tubuliflorum
Lithospermum caroliniense
99
Lithospermum revolutum
Lithospermum calycosum
Lithospermum ruderale
Lithospermum matamorense
Lithospermum californicum
Lithospermum canescens
98
Lithospermum molle
Lithospermum helleri
Lithospermum latifolium
68
Lithospermum macromeria
Lithospermum multiflorum
Lithospermum viride
94
Lithospermum mirabile
Lithospermum mirabile × incisum
62
Lithospermum calcicola
86
Lithospermum leonotis
Lithospermum nelsonii
Cynoglosseae
Boragineae
Lithospermeae

*Buglossoides* does not form a monophyletic group because *B. purpureo-caerulea* L. is not a member of the moderately-supported clade (72% jk) that includes the other species of the genus sampled for the present study. *Glandora* is not monophyletic according to the structure of the present phylogeny; however, the relationships between the species of this genus have weak support. *Glandora oleifolia* (Lapeyr.) D. C. Thomas is resolved as sister to *Lithospermum*, and *Glandora diffusa* (Lag.) D. C. Thomas is sister to the clade that includes *G. diffusa* and *Lithospermum*.

Both *Lithospermum* and the New World members of *Lithospermum* are resolved as monophyletic, but these two clades, along with most others of the ingroup, receive weak support. Despite this generally weak support, each of six species pairs, *L. officinale* L. and *L. erythrorhizon* Siebold and Zucc., *L. trinervium* (Lehm.) J. I. Cohen and *L. discolor* M. Martens & Galeotti, *L. revolutum* B. L. Rob. and *L. calycosum* I. M. Johnst, *L. helleri* (Small) J. I. Cohen and *L. molle* Muhl., *L. mirabile* I. M. Johnst. and *L. mirabile* × *incisum*, and *L. nelsonii* Greenm. and *L. leonotis* (I. M. Johnst.) J. I. Cohen, is supported by greater than 85% jk, while each of three species pairs, *L. scabrum* Thunb. and *L. cinereum* DC., *L. johnstonii* J. I. Cohen and *L. exsertum* (D. Don) J. I. Cohen, *L. multiflorum* A. Gray and *L. macromeria* (DC.) J. I. Cohen, receives greater than 50% jk. In addition, *L. calcicola* B. L. Rob. is supported as sister to *L. leonotis* and *L. nelsonii* with 62% jk.

***Exclusion analyses*** **–** Analyses of the 100% matrix with all 10 regions included resulted in 156 MP trees of 630 steps. The consensus resolved 16 nodes, all of which are consistent with the consensus of the 67-taxon matrix. In general, the matrices with one region removed resolved a consensus with as much or only slightly less resolution than with all 10 regions included (Table 3.5). The analysis of the matrix with *ndh*F-*rpl*32 excluded resolved the greatest number of nodes, 28, but also resolved one contradictory node, with *L. officinale* placed as sister to *L. caroliniense* (Walter ex J. F. Gmel) MacMill. rather than to the rest of *Lithospermum*. The exclusion of other regions did not result in support for any contradictory nodes. Analysis of the matrix that excludes the *rpl*16 intron provided the most resolution, 24 nodes, without supporting any contradictory nodes. The exclusion of the *ycf*6-*psb*M spacer resulted in the fewest nodes resolved (15).

The 80% matrix with all 10 regions included resulted in the discovery of 653 MP trees of 1,686 steps, and the consensus resolved 35 nodes. As with the 100% matrix, the consensus tree was consistent with that of the 67-taxon matrix. Analyses of the matrices with one region excluded tended to resolve consensus trees with as much or slightly more resolution than that from the matrix with all 10 regions included (Table 3.5). In only two instances did the removal of a region result in the resolution of contradictory nodes.

The removal of each of two regions, *trn*Q-*rps*16 and *mat*K, resulted in consensus trees with much more resolution than that with all 10 regions included. In the analysis of the matrix with *trn*Q-*rps*16 excluded, 48 nodes were resolved, but six of the 48 nodes conflicted with those in the consensus from the 10-region matrix. Despite the increased resolution in the consensus tree, 46% of the additionally resolved nodes (six of 11) implied relationships contradictory to those of the 10-region consensus tree. This contrasts with the removal of *mat*K. The analysis of the matrix with this region excluded resolves a consensus tree with 11 more nodes than that with all 10 regions, and none of these nodes was contradictory.

***Successive-inclusion analyses*** - In general, consensus trees obtained from the initial SI analyses (i.e., with one, two, or three regions) with the successive addition of regions with the greatest number of informative characters were more fully resolved

Table 3.5. Results of exclusion analyses of 100% matrix (A) and 80% matrix (B).

A.

| Excluded Region | # of MP Trees | Length | CI/RI | Nodes Resolved | Contradictory Nodes |
|---|---|---|---|---|---|
| *ndh*F-*rpl*32 | 4 | 544 | 59/68 | 28 | 1 |
| *psb*A-*trn*H | 148 | 593 | 59/69 | 16 | 0 |
| *psb*J-*pet*A | 227 | 524 | 61/71 | 16 | 0 |
| *rpl*16 | 3 | 544 | 58/68 | 24 | 0 |
| *trn*K-*rps*16 | 425 | 569 | 59/68 | 16 | 0 |
| *trn*L-*rpl*32 | 38 | 546 | 58/68 | 19 | 0 |
| *trn*L-*trn*F | 103 | 603 | 58/68 | 17 | 0 |
| *trn*Q-*rps*16 | 28 | 582 | 59/68 | 19 | 0 |
| *ycf*6-*psb*M | 206 | 570 | 58/68 | 15 | 0 |
| *mat*K | 17 | 578 | 57/66 | 20 | 0 |
| | | | | | |
| 10 cpDNA Regions | 156 | 630 | 29/34 | 20 | n/a |

B.

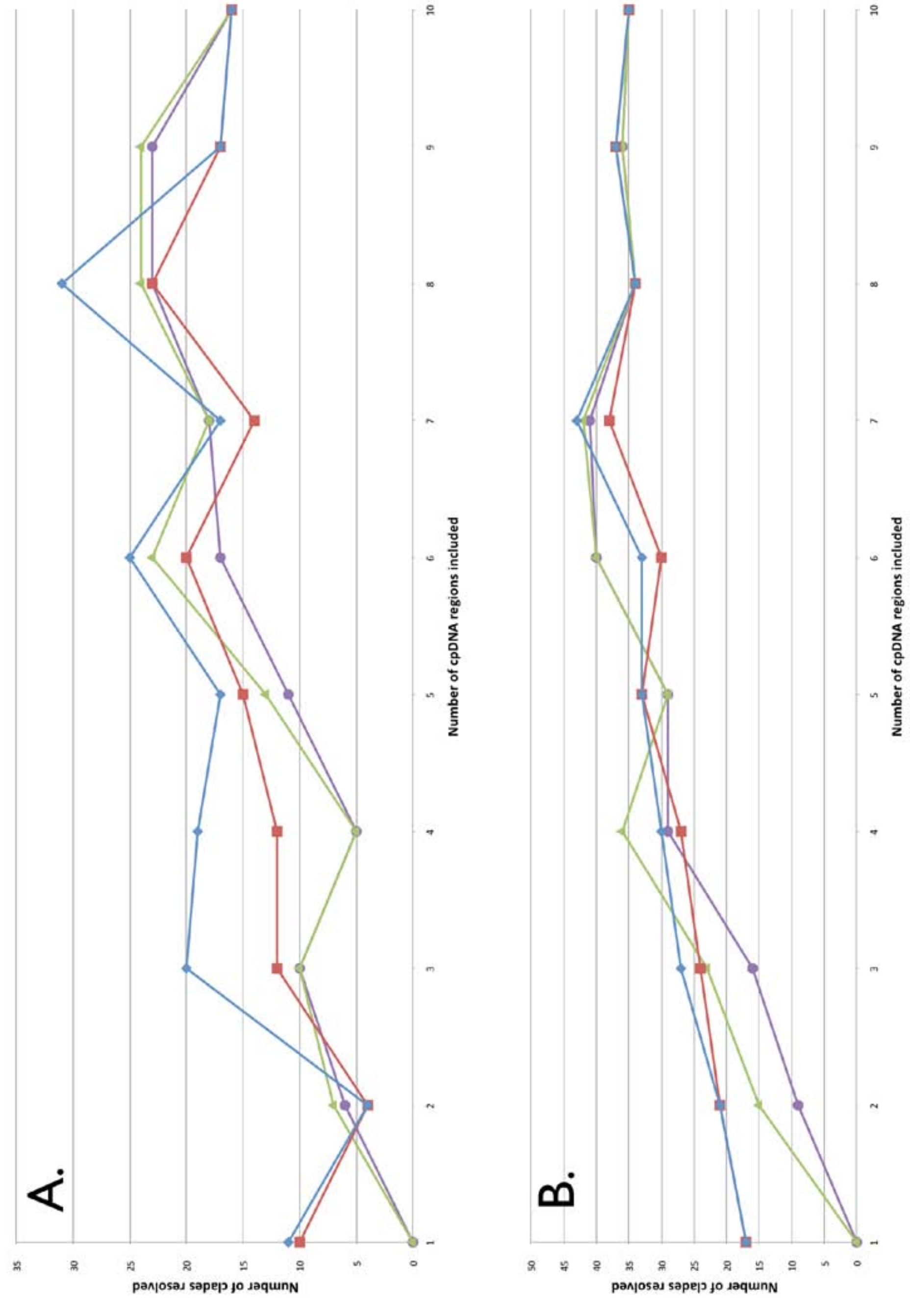
| Excluded Region | # of MP Trees | Length | CI/RI | Nodes Resolved | Contradictory Nodes |
|---|---|---|---|---|---|
| *ndh*F-*rpl*32 | 65 | 1479 | 63/80 | 37 | 0 |
| *psb*A-*trn*H | 2635 | 1572 | 63/80 | 31 | 0 |
| *psb*J-*pet*A | 954 | 1437 | 64/81 | 33 | 1 |
| *rpl*16 | 378 | 1439 | 62/79 | 36 | 0 |
| *trn*K-*rps*16 | 148 | 1505 | 62/79 | 36 | 0 |
| *trn*L-*rpl*32 | 363 | 1504 | 31/40 | 38 | 0 |
| *trn*L-*trn*F | 65 | 1602 | 63/80 | 37 | 0 |
| *trn*Q-*rps*16 | 2 | 1593 | 31/40 | 48 | 6 |
| *ycf*6-*psb*M | 890 | 1530 | 61/79 | 34 | 0 |
| *mat*K | 8 | 1493 | 31/39 | 46 | 0 |
| | | | | | |
| 10 cpDNA Regions | 653 | 1686 | 31/40 | 35 | n/a |

than those from the addition of the regions with the smallest number of informative characters (Figure 3.2). However, with the addition of more regions, this trend does not continue.  After the inclusion of four or five regions, approximately the same numbers of nodes are resolved with the addition of subsequent regions, regardless of whether the added regions have the most or the fewest informative characters.  This pattern is observed with both the 100% and 80% matrices.  The matrices that involve the addition of regions with successively larger numbers of informative characters yield consensus trees with fewer contradictory nodes than do those with the addition of regions in the opposite order.  The inclusion of additional regions to the 100% matrix does not provide as clear of a pattern as does the inclusion of regions with the 80% matrix.  In both matrices, the inclusion of 10 cpDNA regions does not result in consensus trees with the greatest number of nodes resolved; the addition of seven (80% matrix) or eight (100% matrix) regions does so.

***Analyses of matrices of five-cpDNA regions* –** In these analyses, the total number of nodes resolved in the consensus tree is usually greater than the number of non-contradictory nodes resolved.  These numbers are the same in only 17% of the cases (Figure 3.3).  Most of the randomly-constructed matrices of five-cpDNA regions resolved between 27 and 34 total nodes, with 35 nodes reconstructed from the 80% matrix with all 10 cpDNA regions included.  However, most of these matrices resulted in consensus trees with between 27 to 31 non-contradictory nodes.  The median number of nodes resolved in the consensus trees is 32 total nodes and 29 non-contradictory nodes.  The mode is 31 total nodes and 29 non-contradictory nodes.  There is a greater range in the total number of nodes reconstructed than in the number of non-contradictory nodes resolved.

Most of the randomly-constructed matrices of five-cpDNA regions resolved at least one node that is contradicted by the consensus of the 80% matrix with 10 regions included.  However, 72% of the matrices yielded three or fewer contradictory nodes,

Figure 3.2. Results of successive-inclusion analyses of 100% matrix (A) and 80% matrix (B; see text). Diamonds represent number of total nodes resolved by successive addition of regions in order of most to fewest informative characters. Squares denote number of non-contradictory nodes resolved by successive addition of regions in same order. Triangles denote number of total nodes resolved by successive addition of regions in order of fewest to most informative characters. Circles represent number of non-contradictory nodes resolved by successive addition of regions in same order.


A.
Number of clades resolved
0
5
10
15
20
25
30
35
1
2
3
4
5
6
7
8
9
10
Number of cpDNA regions included
B.
Number of clades resolved
0
5
10
15
20
25
30
35
40
45
50
1
2
3
4
5
6
7
8
9
10
Number of cpDNA regions included

and only 8% of the matrices reconstructed consensus trees with greater than seven contradictory nodes (Figure 3.3).

***Single cpDNA region analyses*** **–** The analyses of the individual cpDNA regions yielded strict consensus trees that are resolved much less fully than are those from the 10-region matrices. Despite this lack of resolution, the phylogenies reconstructed from individual regions usually did not contradict, or only slightly contradicted, phylogenies derived from matrices with all 10 regions concatenated.

The consensus trees of the individual regions from the 100% matrix resolved a mean of eight nodes (Table 3.6). The number of nodes resolved ranged from two with the *psb*A-*trn*H spacer to 19 with the *ndh*F-*rpl*32 spacer. The *ndh*F-*rpl*32 spacer and the *rpl*16 intron provided the most resolution; however, these regions yielded 32% and 18% contradictory nodes, respectively. Every region that resolved more than six nodes resulted in at least two contradictory nodes, and only those regions that resolved six or fewer nodes did not yield any contradictory nodes. Most of the analyses of the individual regions supported well-resolved outgroup relationships, and in general, these relationships were consistent with those of the 10-cpDNA region consensus tree. Relationships within *Lithospermum* were poorly resolved, with most regions only resolving four or five nodes in the ingroup.

The single-region analyses of the Boraginaceae, Lithospermeae, and *Lithospermum* matrices yielded similar results (Table 3.7, 3.8, 3.9). For the Boraginaceae matrix, the *trn*K-*rps*16 and *ndh*F-*rpl*32 spacers provided the most resolution, 47.9% and 45% respectively, of the total possible nodes; however, these regions also reconstructed 21.7% and 16% contradictory nodes, respectively. The consensus tree of the *psb*A-*trn*H spacer resolved the smallest percentage of nodes, 15.7%, and did not resolve any contradictory nodes. The only other region that did not resolve contradictory nodes

Figure 3.3. Results of randomly-constructed five-region analyses. Histograms depict numbers of occurrences of various results among a sample of analyses. A. Total number of nodes resolved (darker gray bars) and number of non-contradictory nodes resolved (lighter gray bars). B. Difference between total number of nodes resolved and number of non-contradictory nodes resolved.

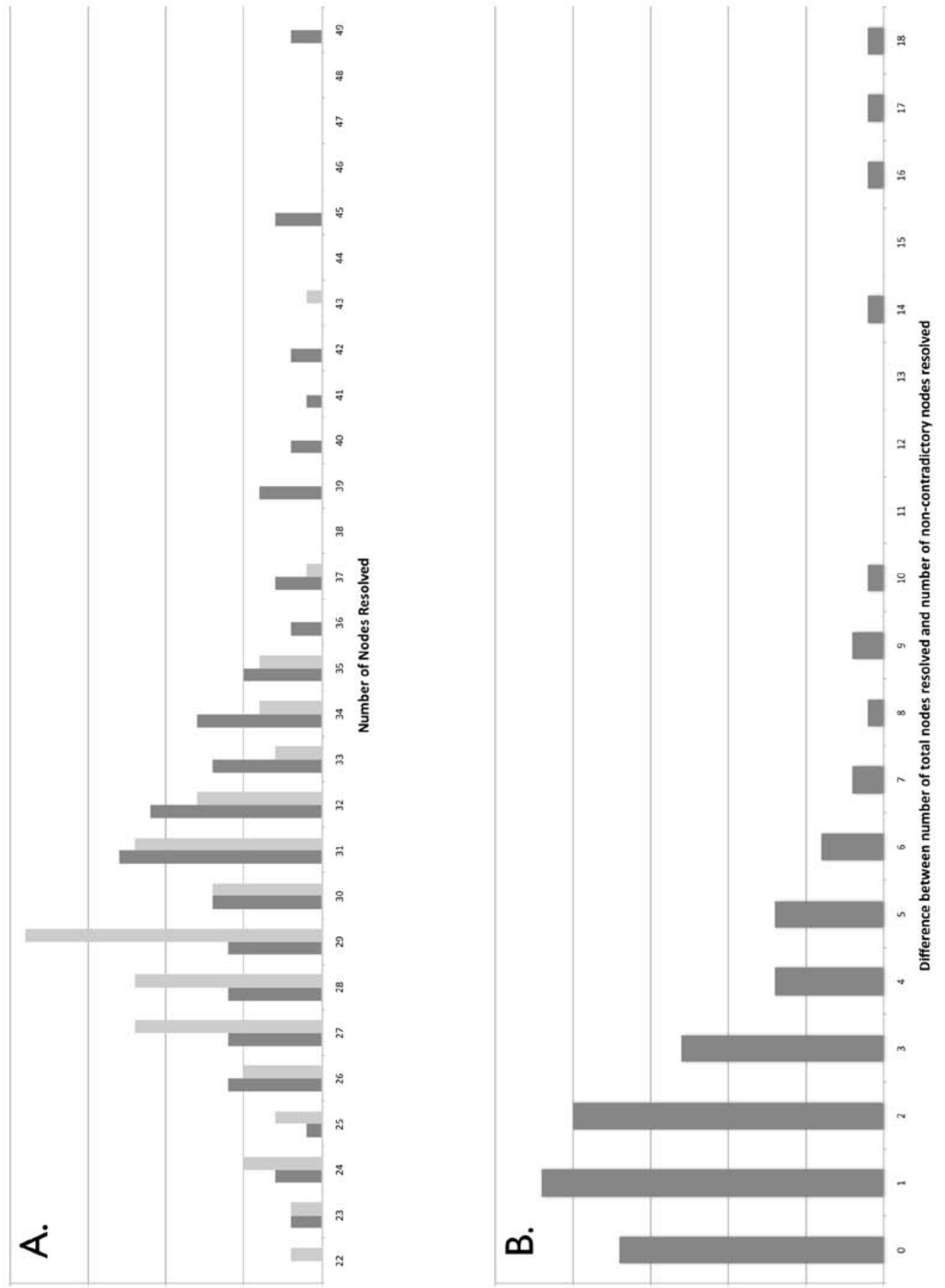

A.
Number of trees
0
5
10
15
20
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
Number of Nodes Resolved
B.
Number of trees
0
5
10
15
20
25
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
Difference between number of total nodes resolved and number of non-contradictory nodes resolved

Table 3.6. Results of single-region analyses of 100% matrix. Matrix of *trn*Q-*rps*16 spacer includes only 33 species, not 34, because of ambiguous alignment between *Amsinckia*, a member of Cynoglosseae, and species of Lithospermeae.

| Region | # of MP Trees | Aligned Length | CI/RI | Nodes Resolved | Contradictory Nodes |
|---|---|---|---|---|---|
| *ndh*F-*rpl*32 | 211 | 105 | 59/71 | 19 | 6 |
| *psb*A-*trn*H | 9168 | 33 | 57/64 | 2 | 0 |
| *psb*J-*pet*A | >1,000,000 | 89 | 55/62 | 6 | 0 |
| *rpl*16 | 11435 | 79 | 68/75 | 11 | 2 |
| *trn*K-*rps*16 | 5176 | 50 | 64/81 | 9 | 3 |
| *trn*L-*rpl*32 | 15105 | 74 | 70/77 | 4 | 0 |
| *trn*L-*trn*F | 41190 | 19 | 84/89 | 3 | 0 |
| *trn*Q-*rps*16 | 106,500 | 39 | 66/80 | 6 | 0 |
| *ycf*6-*psb*M | 609 | 60 | 71/77 | 6 | 0 |
| *mat*K | 2250 | 46 | 80/89 | 9 | 2 |

was *rpl*16; all other regions resolved between 5.8% (*trn*L-*trn*F) and 26.3% (*mat*K) contradictory nodes.

As with the Boraginaceae matrix, the *trn*K-*rps*16 and *ndh*F-*rpl*32 spacers from the Lithospermeae matrix reconstructed consensus trees with the most resolution, 41.5% and 31.8% respectively, of the total possible nodes. Despite this resolution, these regions yielded 17.6% and 28.6% contradictory nodes, respectively. The *rpl*16 intron and the *trn*L-*trn*F spacer did not resolve any contradictory nodes, and these regions reconstructed 26.7% and 14.6%, respectively, of the total possible nodes. The *psb*A-*trn*H spacer resolved the smallest percentage of nodes, 12.2% and the greatest percentage of contradictory relationships, 40%.

Table 3.7. Results of single-region analyses of Boraginaceae matrix. A phylogeny of taxa that include *trn*Q-*rps*16 spacer was not reconstructed because of ambiguous alignment between species of Lithospermeae and Cynoglosseae.

| **Region** | **MP Trees** | **Length** | **CI/RI** | **Number of Nodes Resolved** | **% Potential Nodes Resolved** | **Number of Contradictory Nodes** | **% Contradictory Nodes** |
|---|---|---|---|---|---|---|---|
| *ndh*F - *rpl*32 | 2282 | 208 | 60/80 | 25 | 45% | 4 | 16% |
| *psb*A - *trn*H | 887122 | 106 | 61/80 | 8 | 15.7% | 0 | 0% |
| *psb*J - *pet*A | >1000000 | 230 | 63/80 | 21 | 43.8% | 3 | 14.3% |
| *rpl*16 intron | >1000000 | 263 | 64/83 | 20 | 37.7% | 0 | 0% |
| *trn*K - *rps*16 | 140,004 | 171 | 69/85 | 23 | 47.9% | 5 | 21.7% |
| *trn*L - *rpl*32 | >1000000 | 174 | 66/80 | 19 | 38.8% | 4 | 21.1% |
| *trn*L - *trn*F | >1000000 | 95 | 65/81 | 17 | 29.3% | 1 | 5.8% |
| *trn*Q - *rps*16 | N/A | | | | | | |
| *ycf*6 - *psb*M | 204 | 152 | 75/88 | 20 | 38.5% | 3 | 15% |
| *mat*K | >1000000 | 202 | 71/89 | 19 | 33.9% | 5 | 26.3% |

The individual cpDNA regions from the *Lithospermum* matrix provided much less resolution than did those from either the Boraginaceae or Lithospermeae matrices. The *rpl*16 intron provided the most resolution, 31.3%, but supported 20% contradictory nodes. Three spacer regions, *psb*A-*trn*H, *psb*J-*pet*A, and *trn*Q-*rps*16, resolved fewer than 8% of the possible nodes, but the one or two nodes resolved were not contradictory. Most regions either provided little resolution or resulted in at least 20% contradictory nodes. For example, the *ndh*F-*rpl*32 spacer resolved 21.2% of the possible nodes, but 71.4% of these resolved nodes were contradictory. The only region that resolved more than 10% of its nodes without supporting any conflicting relationships was the *trn*L-*rpl*32 spacer.

Table 3.8. Results of single-region analyses of Lithospermeae matrix.

| Region | MP Trees | Length | CI/RI | Number of Nodes Resolved | % Potential Nodes Resolved | Number of Contradictory Nodes | % Contradictory Nodes |
|---|---|---|---|---|---|---|---|
| *ndh*F - *rpl*32 | 2282 | 99 | 56/74 | 14 | 31.8% | 4 | 28.6% |
| *psb*A - *trn*H | 17,397 | 31 | 54/70 | 5 | 12.2% | 2 | 40% |
| *psb*J - *pet*A | >1000000 | 93 | 53/65 | 11 | 28.9% | 4 | 36.4% |
| *rpl*16 intron | >1000000 | 112 | 65/73 | 12 | 26.7% | 0 | 0% |
| *trn*K - *rps*16 | 70,002 | 68 | 63/81 | 17 | 41.5% | 3 | 17.6% |
| *trn*L - *rpl*32 | >1000000 | 94 | 67/76 | 11 | 26.8% | 3 | 27.3% |
| *trn*L - *trn*F | >1000000 | 46 | 69/81 | 7 | 14.6% | 0 | 0% |
| *trn*Q - *rps*16 | >1,000,000 | 53 | 62/79 | 7 | 16.3% | 1 | 14.3% |
| *ycf*6 - *psb*M | 17 | 64 | 73/78 | 13 | 31.7% | 2 | 15.4% |
| *mat*K | >1000000 | 66 | 74/87 | 10 | 22.2% | 1 | 10% |

Table 3.9. Results of single-region analyses of *Lithospermum* matrix.

| Region | MP Trees | Length | CI/RI | Number of Nodes Resolved | % Potential Nodes Resolved | Number of Contradictory Nodes | % Contradictory Nodes |
|---|---|---|---|---|---|---|---|
| *ndh*F - *rpl*32 | 145,082 | 45 | 53/66 | 7 | 21.2% | 5 | 71.4% |
| *psb*A - *trn*H | 380 | 10 | 80/85 | 1 | 3.2%` | 0 | 0% |
| *psb*J - *pet*A | >1,000,000 | 38 | 60/69 | 2 | 7.1% | 0 | 0% |
| *rpl*16 intron | 22,327 | 39 | 74/76 | 10 | 31.3% | 2 | 20% |
| *trn*K - *rps*16 | 6399 | 31 | 67/81 | 8 | 25.8% | 3 | 37.5% |
| *trn*L - *rpl*32 | 831761 | 23 | 82/87 | 5 | 16.7% | 0 | 0% |
| *trn*L - *trn*F | 12,712 | 16 | 81/86 | 4 | 13.3% | 1 | 25% |
| *trn*Q - *rps*16 | 33,618 | 19 | 57/74 | 2 | 6.3% | 0 | 0% |
| *ycf*6 - *psb*M | 17 | 21 | 80/85 | 7 | 21.9% | 1 | 14.3% |
| *matK* | 827 | 112 | 90/94 | 4 | 13.8% | 1 | 25% |

## Discussion

### PHYLOGENETICS

***Phylogenetics of Lithospermum*** - *Lithospermum*, as currently circumscribed, is a monophyletic group. The results obtained in the present analysis are consistent with those of Cohen and Davis (2009) and provide additional details. The species that had been segregated into other New World genera are nested among the species of *Lithospermum*. Each of the morphological features formerly used to characterize the genus *Macromeria* – corollas greater than three centimeters in length, exserted anthers and stigmas, and leaves with evident secondary venation – originated at least four times (Figure 3.1, crosses). This contrasts with the situation in the two species formerly included in *Onosmodium*, *L. molle* Muhl. and *L. helleri* (Small) J. I. Cohen. These species are sisters, and they are characterized by adaxially glabrous, campanulate corollas, precociously exserted styles, and leaves with evident secondary venation. Although the present analysis indicates that this suite of morphological characters originated once, only 25% of the species previously included in *Onosmodium* were sampled.

Breeding systems within *Lithospermum*, along with their patterns of evolution, are diverse. Heterostyly is known to occur in eight species of *Lithospermum* (Johnston 1952), and the present phylogenetic analysis includes all of them. The structure of the phylogeny indicates that heterostyly originated within the ingroup multiple times, and origins and losses of this breeding system can be optimized in different manners. At the extremes, heterostyly either originated at least seven times or originated at least four times and was subsequently lost five times (Figure 3.1, open rectangles). An intermediate number of gains and losses also may have occurred. Cleistogamous flowers have been observed in three species of *Lithospermum*

(Johnston, 1952), and this type of flower has originated twice within the genus, once in *L. calycosum* and once in the ancestor of *L. mirabile* and *L. mirabile* × *incisum*. Levin (1972) reported cleistogamous flowers in the heterostylous species *L. caroliniense*, but I have not been able to confirm this observation.

The phylogeny suggests that *Lithospermum* originated in the Old World and colonized the New World once. The two African species included in the analyses, *L. cinereum* and *L. scabrum*, are sisters, and this result is consistent with the phylogeny of Weigend et al. (2009), who discovered that the five African species are part of a monophyletic group. In the present analysis, the clade of African species is sister to one composed of the Eurasian and New World members of *Lithospermum*. The Eurasian species, *L. officinale* and *L. erythrorhizon*, are members of a clade that is sister to the New World species (Figure 3.1). The close relationship between the African and Eurasian species of the genus is consistent with Ralston's (1993) hypothesis that the species of *Lithospermum* native to these two continents share an affinity. The phylogeny herein presented also suggests that *Lithospermum* originated in Eurasia or northern Africa. This is because *L. officinale* and *L. erythrorhizon*, along with the closest relatives of the genus – *Glandora* and *Buglossoides* – have a Eurasian and north African distribution. Subsequently, members of *Lithospermum* independently colonized both southern Africa and the New World.

The present analysis cannot test the monophyly of the five South American species of *Lithospermum* because it includes only one representative from this continent, *L. gayanum* I. M. Johnst. Weigend et al. (2009) included most of the South American species in their phylogenetic analysis, and they found the group to be monophyletic. Unfortunately, the resolution of their phylogeny does not identify the closest relative of this clade. If the South American species are monophyletic, then the structure of the present phylogeny suggests that two Mexican species, *L. distichum*

Ortega and *L. oblongifolium* Greenm., are the closest relatives of the South American members of the genus (Figure 3.1). However, no analysis has included *L. mediale* I. M. Johnst., a taxon with a form similar to that of *L. distichum* and *L. gayanum* and with a geographic distribution ranging from Guatemala to northern South America. The inclusion of this taxon in future analyses may help to determine if the distribution of the South American species originated via long distance dispersal, or if the ancestor of *L. mediale* initially colonized South America and later gave rise to a small radiation of species in the Andes.

One specimen included in the present analysis has been recognized as the putative hybrid between *L. mirabile* and *L. incisum*. This specimen has been recognized as such because the vegetative features resemble those of *L. mirabile*, while the nutlets are similar to those of *L. incisum*. This description – *L. mirabile*-like leaves and *L. incisum*-like nutlets – is similar to that of *L. confine* I. M. Johnst. Unfortunately, the only specimen of *L. confine* that I have seen is the type, and the specimens Johnston (1952) recognized as *L. confine* are unavailable. Given this limited amount of material, I have difficulty confidently assigning this specimen to this species.

Johnston (1952) hypothesized that *L. incisum*, *L. mirabile*, and *L. confine* are closely related. The putative hybrid is resolved as sister to *L. mirabile* (Figure 3.1), and this placement is consistent with the structure and form of the specimen. Future analyses that incorporate nuclear loci may help to determine more precisely the relationship of this specimen, and if it is of hybrid origin.

The phylogeny presented here differs from other recent phylogenies of the genus in two significant aspects. First, this phylogeny provides evidence that New World members of *Lithospermum* are monophyletic. Thomas et al. (2008) could not determine if the New World species are monophyletic because their phylogeny lacked the necessary resolution. Weigend et al. (2009) resolved Old World members nested among New World species. Utilizing various DNA regions and taxon combinations, Weigend et al. (2009) resolved different clades with combinations of various North American, Eurasian, and African species as sister to the remainder of the genus. In contrast, the present analysis, which includes greater DNA and taxon sampling, and thus is a more critical test of phylogenetic relationships, did not resolve any of these clades in this position. The second difference involves the amount of resolution among species of *Lithospermum* in the consensus tree. Other phylogenetic reconstructions (Thomas et al., 2008; Weigend et al., 2009) have utilized two or three DNA regions. The data from this number of DNA regions have not resolved much of a backbone for ingroup relationships, although these data have yielded some relationships towards the tips of the tree. The resolution of the consensus tree presented here provides evidence that the use of 10 regions, rather than two or three, is a superior approach to obtain resolution. Many of the ingroup relationships towards the tips of the tree, as well as those of the backbone, are well-resolved, albeit with low support in some instances (Figure 3.1).

***Phylogenetics of Lithospermeae* -** Recently, relationships within Lithospermeae have begun to take shape as Thomas et al. (2008), Cohen and Davis (2009), Weigend et al. (2009), Cecchi and Selvi (2009), and Selvi et al. (2009) have focused on the phylogenetics of members of the tribe. The relationships presented here (Figure 3.1) are consistent with those found in other studies of members of Lithospermeae, with two exceptions. In the present phylogeny, neither *Buglossoides* nor *Glandora*, the closest relatives of *Lithospermum*, are resolved as monophyletic. *Buglossoides purpureo-caerulea* is not part of the same clade as the other members of the genus included in the present analyses. The phylogenies of Langstrom and Chase (2002), Thomas et al. (2008), and Weigend et al. (2009) have suggested this relationship, but oftentimes the consensus trees of their phylogenetic analyses did not resolve this group. The phylogenies of these authors resolve a polytomy between *B. purpureo-caerulea* and other members of *Buglossoides*. Although *Buglossoides* is not found to be monophyletic, its two sections, *Margarospermum* (Rchb.) I. M. Johnst. and *Eubuglossoides* I. M. Johnst., may each be monophyletic (Weigend et al., 2009; Figure 3.1). Therefore, these sections may be closely related, but not sisters.

The present phylogeny resolves *Glandora oleifolia* as sister to *Lithospermum*, and *Glandora diffusa* as sister to *G. oleifolia* and *Lithospermum*. Even though *Glandora* is not reconstructed as monophyletic, the present analysis includes only 33% of the species of the genus. Additional taxon sampling should be conducted prior to a reconsideration of the taxonomy of *Buglossoides* and *Glandora*.

***Phylogenetics of Boraginaceae*** **-** Members of three tribes – Cynoglosseae, Boragineae, and Lithospermeae – are included in the present analyses. The tree is rooted between the *Cryptantha* Lehm. ex G. Don and the clade that includes the remainder of Cynoglosseae (Figure 3.1). With alternate rooting schemes (i.e., rooting within Boragineae or Lithospermeae), each tribe is resolved as monophyletic. These relationships are consistent with those from other analyses, such as Mansion et al. (2009) and Weigend et al. (2010).

In the present analyses, two genera of Cynoglosseae included more than one species. Of these, *Omphalodes* is monophyletic, but *Cynoglossum* is not. *Cynoglossum officinale* is reconstructed as sister to *Lindelofia longiflora*, and both are Old World species. *Cynoglossum pringlei* is resolved as sister to *Amsinckia tessellata*, and both are New World species. Given the taxon sampling in the present analyses, it is not possible to determine whether the characters that diagnose *Cynoglossum* are convergent or ancestral. Through future analyses that include greater taxon sampling within Cynoglosseae, it may be possible to better understand the evolution and evolutionary relationships of *Cynoglossum* and related genera.

### 10 cpDNA-Region Matrix

Exclusion analyses were conducted in order to determine if one region or multiple regions provide an overwhelming amount of resolving power in analyses of the 10 cpDNA-region matrix. The consensus trees from each of the exclusion analyses resolve approximately the same number of nodes as those with all 10 regions included. Therefore, it is concluded that no single region makes an overwhelming contribution to the resulting phylogeny. In some instances, the removal of one region results in a more fully-resolved phylogeny. This apparently is due to conflict between the excluded region and the other nine. For example, analyses of the 80% matrix after the exclusion of either *mat*K or the *trn*Q-*rps*16 spacer result in a phylogeny with 11 and 13 additional nodes resolved, respectively, than with all 10 regions concatenated. However, this increased resolution may not always be helpful. The analyses of the matrix without *mat*K result in a phylogeny with additional nodes resolved, and none are contradictory. In contrast, the exclusion of the *trn*Q-*rps*16 spacer yields clades that conflict with those of the 10-region consensus.

With the present data set, the number of resolved nodes also increases as taxon sampling increases. The consensus of the 80% matrix resolves a greater percentage of nodes than does the 100% matrix (68.6% vs. 62.5%). Furthermore, the consensus reconstructed with all 67 taxa and all 10 regions (Figure 3.1) resolves a greater percentage of nodes (84.6%) than either of the other two matrix subsets. Additionally, the exclusion matrices derived from the 80% matrix tend to yield consensus trees that are as much or slightly more resolved than that of the consensus from 10-region 80%

matrix. This result differs from that of the exclusion matrices derived from the 100% matrix. The latter tend to result in consensus trees with slightly less resolution than the consensus from the 10-region 100% matrix. Moreover, fewer regions need to be added to the 80% matrix than to the 100% matrix in order to reconstruct relationships that will not be contradicted (Figure 3.2). Comparisons of these analyses demonstrate that greater resolution is obtained despite an increase in the amount of missing data.

## MOLECULAR EVOLUTION OF CPDNA IN BORAGINACEAE

Of the 10 cpDNA regions, individual analyses of three spacers, *ndh*F-*rpl*32, *trn*K-*rps*16, and *psb*J-*pet*A, resolve the greatest proportion of nodes at higher taxonomic levels; however, analyses of these individual regions also result in numerous contradictory nodes. From the analyses of each individual region, a pattern has begun to emerge: as the number of resolved nodes increases, so does the number of contradictory nodes. Some of these contradictory nodes reconstruct minor differences, such as sister species not resolved as such. This result is found in analyses of *mat*K from the Boraginaceae matrix. Other conflicting phylogenetic relationships are more extreme, such as species of the ingroup placed among members of the outgroup and vice-versa. This occurs in analyses of *psb*J-*pet*A from the Lithospermeae matrix. With only one region used to reconstruct a phylogeny, it would be impossible to determine where these errors lie, and with two regions it still may prove challenging.

It should be noted that among the species of Lithospermeae and Cynoglosseae, The *trn*Q-*rps*16 spacer could not be aligned unambiguously. Consequently, a phylogeny for the family was not reconstructed from this spacer (Table 3.7). The *trn*Q-*rps*16 spacer is ca. 400 bp longer in species of Cynoglosseae than in species of Lithospermeae. The difference in the length of this spacer between species of these two tribes appears to have been caused by an indel event. However, in other regions, such as *ndh*F-*rpl*32 and *ycf*6-*psb*M, large indel events (100 - 200 bp in length) were inferred, and yet it was possible to align the remainder of the nucleotides (those not part of the indel) among all species. This was not possible for the *trn*Q-*rps*16 spacer. Therefore, an indel event did not by itself result in difficult homology assessments for the alignment of the *trn*Q-*rps*16 spacer among species of Boraginaceae, various rearrangements of the nucleotides also are hypothesized to have occurred.

Non-coding regions often provide more structural DNA characters, such as indels and inversions, than coding regions. This is because stabilizing selective pressures do not affect non-coding DNA regions to the same extent as they do coding DNA regions (Kelchner, 2000; Shaw et al., 2005). Some parts of the non-coding regions can be quite plastic, while others appear to be less prone to mutation. For example, within the present data set a 5 bp inversion in the *trn*K-*rps*16 spacer (CI - 0.08) and a 35 bp gap in the *psb*A-*trn*H spacer (CI - 0.25) each have evolved multiple times independently. Other structural characters are less plastic and provide diagnostic characters for larger clades. One of these characters is a gap in the *trn*L-*rpl*32 spacer (CI - 1), which is a synapomorphy for the clade that includes *L. viride* Greene, *L. nelsonii*, and four other species. Another diagnostic structural DNA character is a 42 bp inversion in the *psb*J-*pet*A spacer (CI - 0.5). This inversion characterizes all of the species of Cynoglosseae and some members of Boragineae. In addition, shorter structural characters, such as a 2 bp inversion in the *trn*K-*rps*16 spacer and a 4 bp inversion in the *ycf*6-*psb*M spacer, are synapomorphies of sister species.

Informative characters can provide grouping information for any number of taxa. Some of the regions examined in the present study tend to provide sequence data that allows for the resolution of sister species, while others yield grouping information for a greater number of species. For example, analyses of the *rpl*16 intron, which has the greatest number of informative characters of the 10 regions included in the present study, resulted in many species pairs in *Lithospermum*; however, analyses of this intron only resolved a few relationships above the species level. This contrasts with *mat*K and the *ndh*F-*rpl*32 spacer. Analyses of these two regions each resolved many nodes among members of the outgroup, but did not resolve many intrageneric relationships. Therefore, it seems that the most useful sampling strategy would involve a combination of these two types of regions. Unfortunately, comparative studies of various cpDNA regions are not available for most taxa.

Studies like those Shaw et al. (2005, 2007) provide excellent starting points, but as evidenced by the present study and others (e.g., Mast et al., 2004, Miller et al., 2009), the most informative regions differ from taxon to taxon. For example, Shaw et al. (2007) hypothesized that of 34 non-coding cpDNA regions, the *trn*L-*rpl*32 and *trn*Q-*rps*16 spacers should provide the greatest number of informative characters (including gaps and inversions) for angiosperms at the intrageneric level, but I obtained different results. The *trn*L-*rpl*32 spacer was often the 5th most-informative region (except in the Lithospermeae matrix, where it ranked 2nd), but the *trn*Q-*rps*16 spacer was always among the three least-informative regions. This low ranking could be due, in part, to its small length in members of Lithospermeae.

The data from the present study suggest that the *rpl*16 intron and the *psb*J-*pet*A spacer provide the greatest number of informative characters for studies of genera of Boraginaceae. Shaw et al. (2007) ranked these two regions as thirteenth and sixth most informative, respectively. Furthermore, in the present study *rpl*16, *ndh*F-*rpl*32, *psb*J-*pet*A, and *trn*K-*rps*16 each provide more than 20 informative characters at the intrageneric level. Shaw et al. (2007, their Figure 4) included these four regions among the 15 (of 34) most informative regions. In contrast, other regions that ranked quite highly for intrageneric studies do not appear to be as useful in Boraginaceae. For example, the *trn*V-*ndh*C spacer, the third most informative region of Shaw et al. (2007), provided very little information when initially screened for the present study, and was dropped.

Recently, Miller et al. (2009) conducted analyses that utilized five of the regions discussed by Shaw et al. (2007). In their study of Lycieae (Solanaceae), Miller et al. (2009) observed that two of these five regions, *ndh*F-*rpl*32 and *trn*L-*rpl*32, were most informative, and the third most informative region was *trn*D-*trn*T, a region Shaw et al. (2007) ranked as the tenth potentially most informative region. The *trn*D-*trn*T spacer was not included in the present study because it yielded too few informative characters. Shaw et al. (2007), Miller et al. (2009), and the present study all obtained different results regarding which cpDNA regions provide the greatest number of informative characters. As the availability of this type of data increases, so will our understanding of the utility of various cpDNA regions in different taxa.

One of the goals of the present study is to determine which cpDNA regions are best used in future phylogenetic investigations. The results of exclusion, SI, and randomly-constructed 5-cpDNA region analyses helped address this question. As mentioned earlier, none of the 10 regions provides an overwhelming amount of resolving power in the 10-region matrix, so the structure of the phylogeny is not primarily the result of one or two cpDNA regions. In the SI analyses, the use of five regions, either those that yield the greatest or smallest number of informative characters, results in consensus trees with approximately the same phylogenetic relationships as analyses with all 10 regions (Figure 3.2). In the present study, the three regions, *trn*L-*trn*F, *psb*A-*trn*H, and *trn*Q-*rps*16, yield the fewest informative characters. Each of these regions provides ca. 40 informative characters, which is approximately half as many as the regions with the fourth and fifth fewest informative characters. The addition of these fourth and fifth regions to the matrix that includes *trn*L-*trn*F, *psb*A-*trn*H, and *trn*Q-*rps*16 results in a sharp increase in the number of clades resolved in the consensus, and this number of clades is approximately equal to that from the analysis of the matrix that includes the five regions with the greatest number of informative characters (Figure 3.2). The results of the SI analyses provide evidence that the analysis of a matrix that includes any combination of five cpDNA regions should yield relationships that are similar to those obtained from a matrix with all 10 cpDNA regions. These results are consistent with those of other studies that have found that additional sequencing efforts appear to yield diminishing returns. Wortley et al. (2005) and Hardy et al. (2008) demonstrated this through simulations of DNA sequence data. Wortley et al. (2005) observed that additional sequence data could result in the loss of both resolution and accuracy in a phylogeny. These authors provide evidence that too much rapidly evolving sequence data can include a large number of sites that have experienced multiple substitutions, which can result in a decrease in the accuracy of phylogenetic analyses. Therefore, Wortley et al. (2005) suggest that a large amount of slowly evolving sequence data may yield the most useful sampling strategy. These results are similar to those of the SI analyses: the number of cpDNA regions included, not regions with a fast substitution rate, may yield a more well-resolved phylogeny.

Hardy et al. (2008) determined that in order to resolve ca. 70% of the nodes in a phylogeny, half as much character (nucleotide) data is needed as would be required to resolve all of the nodes. The addition of nucleotides does not result in a linear increase in resolution (Hardy et al., 2008). My results support this conclusion, and this can be visualized on the graph from SI analyses of the 80% matrix (Figure 3.2B). Analyses of matrices that include at least five regions resolve approximately the same number of nodes as do those with all 10 cpDNA regions.

Simmons and Miya (2004) reached similar conclusions to Wortley et al. (2005) and Hardy et al. (2008). In their resampling of 100 mitochondrial genomes of teleost fishes, Simmons and Miya (2004) discovered that the addition of nucleotides resulted in an increase in the number of non-contradictory clades, and in a decrease in the number of contradicotry clades, but only up to a point. After this point, the amount of resolution, both non-contradictory and contradictory, only slightly changed. With the addition of more characters and more taxa, the number of non-contradictory clades resolved steadily increased; however, the number of contradictory clades resolved remained steady. Taking into account these three studies, as well as the data presented here, it appears that after a threshold of character data is obtained, additional sequence data does not substantially increase resolution to the same extent as increased taxon sampling (Figure 3.2). However, the use of large amounts of sequence data may help resolve obstinate nodes (Wortley et al., 2005).

The results of the SI analyses provide information of only limited scope, while the results of the randomly-constructed five-cpDNA region matrices suggest a more complete understanding of character sampling strategies. Analyses of the randomly-constructed five-cpDNA-region matrices provide evidence that a range in the amount of resolution can occur with the use of different combinations of cpDNA regions (Figure 3.3). The five-region analyses indicate which regions, when excluded, cause the greatest disparity between the total number of nodes resolved and the number of non-contradictory nodes resolved. The exclusion of various combinations of the following regions result in analyses that reconstruct well-resolved strict consensus trees with multiple (often greater than five) contradictory nodes: the *rpl*16 intron, *mat*K, *psb*A-*trn*H, and *trn*L-*rpl*32. The use of these four regions, in concert with the *trn*Q-*rps*16 spacer, results in a consensus tree with 34 nodes resolved, none of which is contradictory. This is one fewer node than the consensus tree from the 80% matrix with all 10 regions included. Therefore, I recommend these five regions for future phylogenetic studies of genera of Boraginaceae, especially those that include thorough outgroup sampling. Although other combinations of cpDNA regions can provide the same amount of non-contradictory resolution, attempts to find other patterns of particular regions were unsuccessful.

My recommendation of which five cpDNA regions to sample for future phylogenetic analyses in Boraginaceae is conservative. This is because some of the randomly-constructed five-region combinations resulted in consensus trees with a greater number of nodes resolved than that of the 10-region matrix, but these consensus trees also resolved at least a few contradictory nodes (Figure 3.3). Rather than recommend five regions that resolve a large number of nodes, but at the same time have been observed to support contradictory nodes and introduce type I error, it seems more prudent to suggest five regions that will not be contradicted by the addition of more data. This error can be determined only with the addition of more sequence data, and collection of these data is not always possible.

In addition to recommending specific cpDNA regions for phylogenetic studies of genera of Boraginaceae, my results suggest that the concatenation of a few regions that evolve more slowly, such as *mat*K and *psb*A-*trn*H, along with a few regions that evolve at a more rapid rate, including the *rpl*16 intron and the *trn*L-*rpl*32 spacer, will provide the most useful combination of cpDNA regions to yield non-contradictory phylogenetic relationships. This combination of different types of regions contrasts with the idea that phylogenetic studies of intra- or intergeneric relationships should use regions which mutate at the fastest rate, and thus often provide the greatest number of informative characters among closely related species. This latter strategy may be best if only a small number of regions (i.e., one to three) can be sequenced (Figure

3.2). However, if more regions (at least five) can be obtained, then it seems beneficial to utilize this combination of more slowly and more rapidly evolving regions.

Miller et al. (2009) obtained a different result. They found that the three most informative cpDNA regions in their study supported a phylogeny that was almost identical to that from the concatenation of nine regions. However, Miller et al. (2009) did not examine phylogenies produced from other three-region combinations, which may yield phylogenies similar to that with all nine regions included. In addition, the taxon sampling of Miller et al. (2009) was more limited than that of the present study.

The results of the SI analyses provide evidence that, within *Lithospermum*, numerous regions are necessary to recover relationships that will not be contradicted with the inclusion of more sequence data. Therefore, investigators studying the phylogenetics of Boraginaceae should strive to include at least five regions in order to provide a well-resolved tree that supports relationships that will not conflict with those reconstructed with the addition of more sequence data.

The use of the cpDNA regions I recommend contrasts with recent studies of genera of Boraginaceae (e.g., Thomas et al., 2008; Weigend et al., 2009, 2010; Cecchi and Selvi, 2009; Selvi et al., 2009). These studies have relied heavily on the *trn*L-*trn*F spacer and ITS, a region that has been known to result in misleading phylogenies (Álvarez and Wendel, 2003). The *trn*L-*trn*F spacer yields fewer informative characters than most of the other regions used in the present study (Tables 3.2, 3.3, 3.4). Analyses of this region alone resolve only a few nodes, but the use of *trn*L-*trn*F rarely results in relationships that conflict with those derived from the 10-region matrix (Tables 3.7, 3.8, 3.9). Investigators initially may not want to spend time and money sequencing this region, especially as the strict consensus from analyses of this region resolves only a few nodes. However, I suggest that *trn*L-*trn*F can complement the use of other regions because analyses of it consistently resolve non-contradictory relationships.

## Conclusion

Future studies in *Lithospermum* will incorporate a greater number of cpDNA regions as well as investigations of phylogenies based on nuclear DNA.  With more cpDNA regions, it will be possible to examine whether the use of 10 cpDNA regions is sufficient to reconstruct a phylogeny of *Lithospermum*.  The inclusion of nuclear regions, such as the COSII markers of Wu et al. (2006), will allow for an investigation of congruence of the evolutionary relationships obtained via different genomes. Additionally, nuclear and cpDNA regions can be concatenated into a combined matrix in which to examine the phylogenetic relationships of species of *Lithospermum* and Boraginaceae.

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CHAPTER FOUR

# A PHYLOGENETIC ANALYSIS OF MORPHOLOGICAL AND MOLECULAR CHARACTERS OF *LITHOSPERMUM* L. AND RELATED TAXA: EVOLUTIONARY RELATIONSHIPS AND CHARACTER EVOLUTION

## Abstract

*Lithospermum* (Boraginaceae) includes approximately 60 species and exhibits a wide range of floral, palynological, and vegetative diversity. A phylogenetic analyses based on 10 chloroplast DNA regions and 22 morphological characters was conducted, to examine evolutionary relationships within *Lithospermum* and among related genera of Boraginaceae, and to investigate patterns of morphological evolution. Several morphological features, such as long-funnelform corollas, faucal appendages, reciprocal herkogamy, and evident secondary leaf venation, have evolved multiple times within the genus. In contrast, other morphological features, including the presence of glands and the position and number of pollen pores, are less plastic and tend to characterize larger clades. Some features, including the presence of glands, are interpreted as symplesiomorphic for *Lithospermum*, while others, such as evident secondary leaf venation, appear to have originated repeatedly. The range of structural diversity that occurs among the species of *Lithospermum* suggests the potential utility of this genus as a model for integrative studies of evolution, development, and molecular biology.

## Introduction

*Lithospermum* L., a genus in the family Boraginaceae, comprises approximately 60 species, with a center of diversity in Mexico and the southwestern United States. Recently, Cohen and Davis (2009) and Weigend et al. (2009) reconstructed phylogenies of *Lithospermum* and related genera of Lithospermeae, the tribe to which *Lithospermum* belongs. Cohen and Davis (2009) utilized 10 chloroplast DNA (cpDNA) regions, and Weigend et al. (2009) concatenated two cpDNA regions along with the nuclear ribosomal internal transcribed spacer. Both of these analyses resolved *Lithospermum* as non-monophyletic, with members of related genera nested among its species. In light of these relationships, Cohen and Davis (2009) expanded the circumscription of *Lithospermum*, and under this broader interpretation it appears to be monophyletic. The genus currently includes the species that traditionally have been recognized as members of *Lithospermum* (i.e., Johnston, 1952, 1954a, b) as well as all of the other New World members of Lithospermeae, previously placed in *Lasiarrhenum* I. M. Johnst., *Macromeria* D. Don, *Nomosa* I. M. Johnst., *Onosmodium* Michx., *Perittostema* I. M. Johnst., and *Psilolaemus* I. M. Johnst. With this broader generic circumscription, *Lithospermum* exhibits a wide range of floral, palynological, and vegetative diversity.

In light of the broad range of structural diversity among its species, *Lithospermum* may be a useful taxon in which to investigate character evolution. Some features, such as flowers with long-funnelform corollas (> 4 cm in length) and exserted anthers and stigmas (termed *Macromeria*-type flowers), appear to have originated multiple times (Cohen and Davis, 2009). However, the phylogenetic distributions of other characters, including type of herkogamy, pollen shape, and pattern of leaf venation, remain unexamined. A great deal of discussion has taken place during the past 20 years regarding the inclusion of morphological characters in phylogenetic analyses (e.g., Eernisse and Kluge, 1993; Scotland et al., 2003; Wiens, 2004). I believe that the most comprehensive tests of phylogenetic relationships and patterns of character evolution are conducted through the integration of multiple types of critically-examined data.

The goals of the present study are two-fold: 1) to reconstruct a phylogeny of *Lithospermum* (herein the ingroup) and other members of Lithospermeae and Boraginaceae (herein the outgroup) through a combination of cpDNA sequence data and morphological character data, and 2) to investigate patterns of morphological character evolution. The results of the present study will allow for the identification of characters that diagnose larger clades and that may prove predictive for future taxonomic classifications.

## Materials and Methods

### TAXON SAMPLING

The taxon sampling employed in the present study is the same as that used in Chapter 3. Sixty-seven species were sampled (Table 3.1). Thirty-seven belong to the ingroup, and this sampling represents both the morphological and geographic range of variation within *Lithospermum* sensu Cohen and Davis (2009). The outgroup comprises 30 species from related genera of Boraginaceae: eight from Cynoglosseae, three from Boragineae, and 20 from Lithospermeae.

### DNA SEQUENCE DATA

One protein-encoding cpDNA region – matK – and nine non-encoding cpDNA regions – *ndh*F - *rpl*32, *psb*A - *trn*H, *psb*J - *pet*A, the *rpl*16 intron, *trn*K - *rps*16, *trn*L - *rpl*32, *trn*Q - *rps*16, *ycf*6 - *psb*M, and *trn*L - *trn*F– were included in the present study. These regions and aligned sequence data are the same as those used in chapter 3.

The matrices that include the 10 cpDNA regions are missing approximately 20% of the sequence data. However, this number is largely confined to a set of 10 species for which data were obtained from GenBank. With the exclusion of these species, only 5.7% of the data is missing. The topology of the consensus tree, with respect to the remaining species, does not differ when these 10 species are excluded. Sequences generated for the present study will be deposited in GenBank, and the matrix will be available at Treebase.

### MORPHOLOGICAL CODING

The morphological matrix includes twenty-two characters (Table 4.1). Half of the characters are binary, while the other half are multi-state.

Morphological character data were gathered from living plant material, herbarium specimens from BH, CAS, F, GH, MEXU, MICH, NY, TEX/LL, US, and WISC, and digital images of species. Published descriptions also were consulted (i.e., Johnston 1952, 1953a, b, 1954a, b; Valentine and Chater, 1972; Al-Shehbaz, 1991; Zhu et al., 1995), as were other peer-reviewed publications (i.e., Díez et al., 1986; Jian-chang et al., 1995; Boyd, 2003; Selvi and Bigazzi, 2003; Aytas Akçin and Ulu, 2007; Thomas et al. 2008; Ferrero et al., 2009). I attempted to observe at least 20 specimens for each species; however, given the limited quantity of representative material for some taxa, fewer specimens sometimes were examined. This occurred more frequently with outgroup than with ingroup species. In a few cases, where it was not possible to collect data (often related to pollen) for a particular species, information for a specific character was based on observations of congeners. For the morphological matrix, 1.5% of the cells are scored as missing.

### PHYLOGENETIC ANALYSIS

Three matrices were constructed: the cpDNA matrix comprises cpDNA sequence data plus scores for structural features of DNA regions (gaps, inversions, and unusual nucleotide motifs [Chapter 3]), the morphological matrix is composed of

Table 4.1.  Morphological characters, their states, and additional information.

|  | Character | Character States | Comments |
|---|---|---|---|
| 1 | Position of leaves | (0) cauline and basal<br>(1) only cauline<br>(2) cauline and pseudobasal | A pseudobasal rosette is defined as a rosette that is sometimes present and may be ephemeral.  This type of rosette includes leaves that, although they may have short internodes between them, are not necessarily from the base of the stem. |
| 2 | Pattern of leaf venation | (0) only midvein evident<br>(1) midvein and secondary veins evident, veins sunken<br>(2) midvein and secondary veins evident, veins not sunken<br>(3) trinerviate with 1 or 2 pairs of secondary veins or midvein and 1 or 2 pairs of secondary veins | Sunken venation refers to veins that are not flush with the surface of the leaf (see Figure 4.4e) |
| 3 | Naphthoquinones | (0) present<br>(1) absent | Species that sometimes produce small amounts of naphthoquinones are coded as polymorphic. |
| 4 | Abaxial trichomes on corolla | (0) present<br>(1) absent |  |
| 5 | Cleistogamy | (0) present<br>(1) absent |  |
| 6 | Adaxial trichomes on the corolla | (0) present<br>(1) absent | Johnston (1952, 1954a) only identified a few species of *Lithospermum* with trichomes inside of the corollas.  However, some of the species that he did not mention as bearing trichomes on the inside of their corollas are polymorphic for this character, with only some individuals producing trichomes in this position. |
| 7 | Faucal appendages | (0) present<br>(1) absent |  |
| 8 | Glands inside corolla | (0) present<br>(1) absent |  |

Table 4.1 (Continued)

| | | | |
|---|---|---|---|
| 9 | Corolla color | (0) cream<br>(1) purple<br>(2) blue<br>(3) yellow<br>(4) white<br>(5) white with a yellow center<br>(6) green-yellow<br>(7) orange<br>(8) cream-green<br>(9) pink/red | Some species are coded as polymorphic for this character. In some species, the corolla is of two different colors. In other species, the flowers of one individual bear corollas that are of one color, but those of other individuals are of a different color. |
| 10 | Corolla shape | (0) salverform<br>(1) salverform-funnelform<br>(2) funnelform<br>(3) tubular<br>(4) campanulate - *Onosmodium-type*<br>(5) campanulate - *Lasiarrhenum-type*<br>(6) urceolote<br>(7) long-funnelform that gradually taper from the base to the apex<br>(8) long-funnelform flaring open<br>(9) rotate | |
| 11 | Corolla lobes | (0) reflexed (ca. 180°)<br>(1) flared (ca. 90°)<br>(2) erect/ascending (< 45°) | Some species have their corolla lobes oriented in different manners at different stages of development, or throughout their geographic range. |
| 12 | Type of herkogamy | (0) reverse herkogamy<br>(1) non-herkogamy<br>(2) approach herkogamy<br>(3) reciprocal herkogamy | Some species exhibit multiple types of herkogamy. |
| 13 | Filament shape in cross section | (0) circular<br>(1) elliptical<br>(2) triangular | |
| 14 | Anther exsertion/insertion | (0) exserted<br>(1) inserted | |
| 15 | Trichomes on the abaxial surface of anthers | (0) present<br>(1) absent | |
| 16 | Pollen shape | (0) ellipsoid<br>(1) spherical<br>(2) cylindrical<br>(3) prolate with a constricted equator (hourglass)<br>(4) ovoid | |

Table 4.1 (Continued)

| | | | |
|---|---|---|---|
| 17 | Pollen pore position | (0) equatorial<br>(1) sub-equatorial | |
| 18 | Pollen pore number | (0) three<br>(1) six to eight<br>(2) more than 10<br>(3) three to five | These character states are defined in this manner because the presence of three pores tends to be fixed in species, but variation tends to occur in species that bear pollen grains with a greater number of pores. However, most taxa are fixed within a certain range: either six to eight pores, three to five, or more than 10 pores (i.e., 12). |
| 19 | Style position | (0) exserted at anthesis<br>(1) inserted<br>(2) tardily exserted<br>(3) precociously exserted | Most species that are tardily exserted often also have inserted styles earlier in development; therefore, these species are polymorphic for this character and are coded as such. |
| 20 | Stigma | (0) terminal<br>(1) subterminal | Some species are polymorphic for this character, with some individuals producing terminal stigmas, and other individuals developing subterminal stigmas. |
| 21 | Nutlet attachment | (0) basal<br>(1) sub-medial | |
| 22 | Nutlet bases attached | (0) present<br>(1) absent | |

scores for 22 morphological characters (Table 4.1), and the combined matrix concatenates the cpDNA matrix and the morphological matrix. All characters were treated as non-additive, and all characters were weighted equally for cladistic analysis.

Maximum parsimony phylogenetic analyses were conducted with each of the three data matrices and with various permutations of the data matrix described below. The following search strategy was applied in all analyses: the data were analyzed using TNT (Goloboff et al., 2008), with 1,000,000 trees held in memory, and 1,000 independent iterations, with 20 trees held per iteration, of 1,000 parsimony ratchet iterations (Nixon, 1999), with 10% probability of upweighting and 10% probability of downweighting, followed by 1,000 cycles of tree drifting; afterwards, 100 rounds of tree fusion and random sectorial searches were performed (Goloboff, 1999a), followed by TBR-max, swapping among all the most-parsimonious trees until completion.. Clade support for the combined matrix was measured with TNT (Goloboff et al., 2008). Ten thousand 10,000 jackknife replicates (36% removal probability) (Farris et al., 1996) were conducted. For each replicate 10 TBR searches were conducted, with 10 trees held after each replicate, and a total of 100,000 trees held in memory for the duration of the entire jackknife resampling. The same search strategy was employed for the cpDNA matrix, but the data were analyzed with NONA (Goloboff, 1999b). Consistency indices were calculated after removal of parsimony-uninformative characters.

## Results

### SEQUENCE VARIATION

The cpDNA and combined matrices each include a total of 10,036 aligned nucleotides from the 67 species (Table 3.2). A total of 918 informative characters were obtained from the aligned sequenced data. This number includes 811 informative nucleotides and 107 informative gaps, inversions, and unusual nucleotide motifs. These summary statistics are the same as those from the Boraginaceae matrix of Chapter 3 (Table 3.2).

### PHYLOGENETIC ANALYSIS

Eighty most-parsimonious (MP) trees of 1,810 steps (CI - 0.61, RI - 0.80) were discovered for the cpDNA matrix, and seven MP trees of 2,054 steps (CI - 0.57, RI - 0.76) were found for the combined matrix. The strict consensus tree from the combined matrix is provided in Figure 4.1, and that from the cpDNA matrix is presented in Figure 4.2. Analysis of the 22 morphological characters alone resulted in 223 MP trees of 170 steps. The strict consensus from this matrix had little resolution (tree not shown).

Outgroup relationships for the consensus trees of the cpDNA and the combined matrices are nearly identical, with three exceptions. The first involves the clade that includes *Mairetis* I. M. Johnst., *Halacsya* Dörfl., and five additional species. This clade is more fully resolved and has greater support (67% jackknife support [JK]) in the tree from the combined matrix than in that from the cpDNA matrix. The second contrasting relationship concerns the placement of *Podonosma* Boiss. The analysis of the combined matrix results in a phylogeny in which *Podonosma* and *Echium* L. (61% JK) are sisters, with this clade sister to one that includes *Onosma* L. and *Maharanga* DC. This poorly-supported four-taxon clade is sister to the rest of Lithospermeae. In contrast, the analysis of the cpDNA matrix yields a consensus tree in which *Podonosma* is sister to the rest of Lithospermeae, and a clade comprising *Echium*, *Onosma*, and *Maharanga* is resolved as sister to the remainder of the tribe. The third relationship that differs between the two analyses involves two species of *Glandora* D. C. Thomas, Weigend, & Hilger. The consensus of the cpDNA matrix resolves *Glandora oleifolia* (Lapeyr.) D. C. Thomas as sister to *Lithospermum*, and *Glandora diffusa* (Lag.) D. C. Thomas as sister to the clade composed of *G. oleifolia* and *Lithospermum*. In contrast, the consensus of the combined matrix resolves these two species of *Glandora* as sisters (64% JK). Even though the topology of the outgroup is similar between the consensus trees of the two matrices, there is greater jackknife support for most relationships in the phylogeny of the combined matrix.

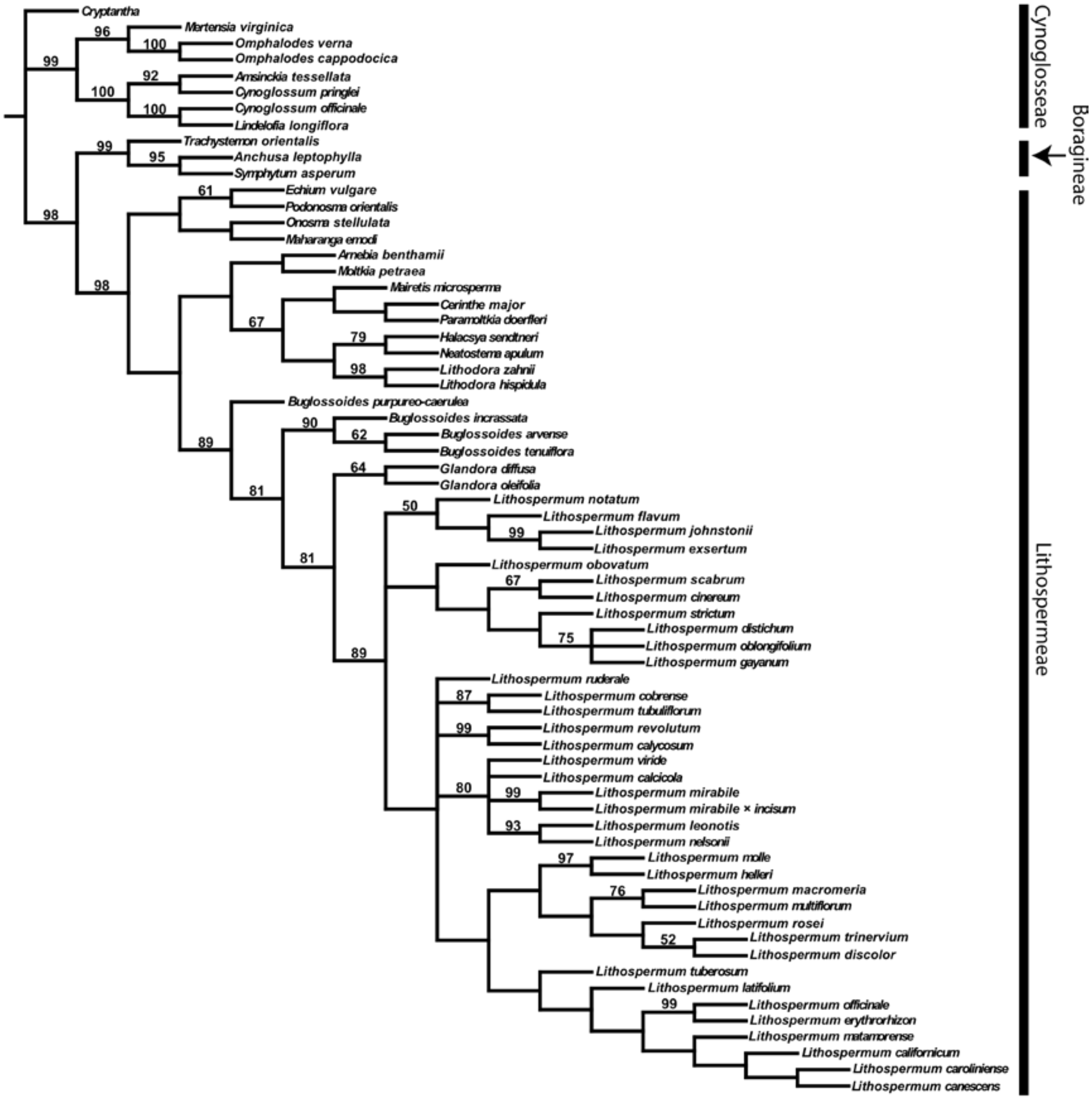


Figure 4.1. Strict consensus of combined matrix (L - 2,054; CI/RI - 0.57/0.76). Numbers above branches are jackknife values greater than 50%.

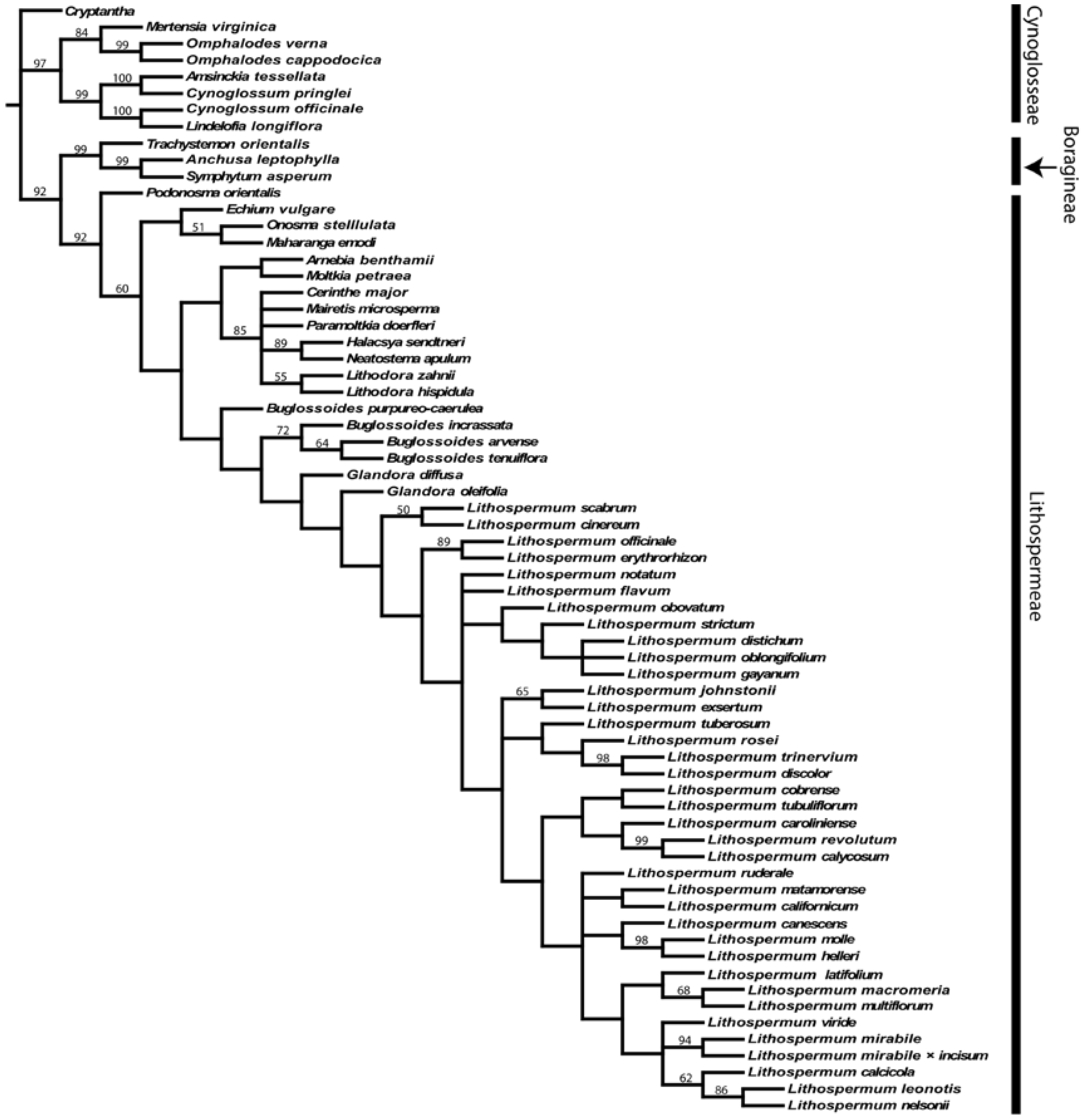


Figure 4.2. Strict consensus of 10 cpDNA-region matrix (L - 1,810; CI/RI - 0.61/0.80). Numbers above branches are jackknife values greater than 50%.

Both the cpDNA and combined matrices resolve *Lithospermum* as monophyletic, but the combined matrix provides greater support for this clade (89% JK vs. 29% JK). Apart from different jackknife support values, the consensus tree of each matrix resolves different relationships at the base of the ingroup. In the cpDNA consensus tree, two South African species, *L. scabrum* Thunb. and *L. cinereum* DC., are resolved as sisters, and two Eurasian species, *L. officinale* L. and *L. erythrorhizon* Siebold & Zucc., are sisters. The South African species are resolved as sister to the rest of the genus, and the Eurasian species are sister to the New World members of *Lithospermum* (Figure 4.2). In contrast, the combined matrix yields a different set of relationships at the base of the ingroup. The clade that is sister to the rest of the genus is composed of either *L. notatum* (I. M. Johnst.) J. I. Cohen, *L. flavum* Sessé & Moc., *L. johnstonii* J. I. Cohen, and *L. exsertum* (D. Don) J. I. Cohen, or these four species and *L. obovatum* J. F. Macbr., *L. strictum* Lehm., and five other species (Figure 4.1).

In the analyses of each matrix, nine species pairs (clades) – *L. johnstonii* and *L. exsertum*, *L. scabrum* and *L. cinereum*, *L. revolutum* B. L. Rob. and *L. calycosum* (J. F. Macbr.) I. M. Johnst., *L. leonotis* (I. M. Johnst.) J. I. Cohen and *L. nelsonii* Greenm., *L. mirabile* I. M. Johnst. and *L. mirabile* × *incisum*, *L. molle* Muhl. and *L. helleri* (Small) J. I. Cohen, *L. macromeria* J. I. Cohen and *L. multiflorum* Torr. ex A. Gray, *L. trinervium* (Lehm.) J. I. Cohen and *L. discolor* M. Martens & Galeotti, and *L. officinale* and *L. erythrorhizon* – were obtained, all of them supported by 50% JK or greater, with most supported by greater than 85% JK. The inclusion of morphological data in the analyses results in increased jackknife support for three of these clades and decreased support for one of these clades. In addition, analyses of the combined matrix yield increased support for two clades: one composed of *L. cobrense* Greene and *L. tubuliflorum* Greene (5% JK vs. 87% JK), and another that includes *L. distichum* Ortega, *L. oblongifolium* Greenm., and *L. gayanum* I. M. Johnst (48% JK vs. 75% JK). Despite low support for many clades of the ingroup, jackknife support tends to increase with the addition of morphological characters to the analyses.

Although the evolutionary relationships resolved by the two matrices are similar, only one clade composed of more than two species is resolved with the same phylogenetic relationships. This clade includes *L. viride* Greene, *L. nelsonii*, and four other species. Additionally, one clade occurs only in the results of the combined matrix. This clade, which is composed of *L. tuberosum* Rugel ex. DC., *L. canescens* Torr., and six other species, receives low jackknife support. Most of the members of this clade are scattered throughout the ingroup in the phylogeny of the cpDNA matrix (Figures 4.1, 4.2).

## MORPHOLOGICAL CHARACTERS

### ***Vegetative characters***

**Position of leaves** (Length [L] - 8, Consistency Index [CI] - 0.25) – All of the species of Cynoglosseae and Boragineae included in the present analysis develop a basal rosette, with the exception of *Amsinckia tessellata* A. Gray (Cynoglosseae), which produces a pseudobasal rosette. In contrast, the presence of only cauline leaves is resolved as the plesiomorphic condition for Lithospermeae. Among the members of the tribe, the presence of basal rosettes is a synapomorphy for the clade composed of *Halacsya* and *Neatostema*. Outside of *Lithospermum*, this is the only occurrence of this state within the tribe.

Basal rosettes originated three times among species of *Lithospermum* (e.g., in the ancestor of *L. cobrense* and *L. tubuliflorum*). Pseudobasal rosettes originated at least four times, as in *L. mirabile* and in *L. matamorense*. Three of the four origins of a pseudobasal rosette are among species that do not always produce rosettes.

**Pattern of leaf venation** (L -19, CI - 0.15) – The ancestral pattern of leaf venation in Boraginaceae cannot be reconstructed unambiguously (Figure 4.3, orange hash marks). Regardless of the ancestral condition, secondary venation, either with or without sunken veins, has evolved multiple times among members of the family (Figures 4.4e, f, and 4.3, red and blue hash marks respectively). In the ingroup, eight species are polymorphic for one of two conditions: 1) leaves with only a midvein (state 0) or with a midvein and secondary venation of either type (state 0 and either state 1 or 2), or 2) leaves with either type of secondary venation (states 1 and 2) (Table 4.1). Sunken secondary venation is a synapomorphy for a large clade composed of *L. molle*, *L. tuberosum*, and 13 other species. Within this clade, secondary venation either has been lost four times or has been lost three times and subsequently gained once. A venation pattern consisting of either a midvein and one or two pairs of secondary veins or three equal or subequal primary veins and one or two pairs of secondary veins either originated twice or arose once and was subsequently lost (Figure 4.3, green hash marks). This type of venation characterizes both *L. rosei* and *L. trinervium*.

**Naphthoquinones** (L - 8, CI - 0.12) - The production of naphthoquinones has originated at least 11 times among members of Lithospermeae, and most of these instances occur in *Lithospermum*. Five of these origins involve species, such as *L. multiflorum*, *L. officinale*, and *L. revolutum*, that frequently produce observable quantities of naphthoquinones.

Figure 4.3. Phylogenetic distribution of patterns of leaf venation optimized on one most-parsimonious tree. Orange hash marks denote acquisition of leaves in which the only evident vein is the midvein. Red hash marks represent origin of leaves that produce sunken secondary veins. Blue hash marks represent acquisition of leaves that produce secondary veins flush with the surface of the leaf. Green hash marks represent origin of leaves with venation pattern consisting of either an evident midvein and one or two pairs of secondary veins or three equal or subequal primary veins and one or two pairs of secondary veins. Hash marks in two colors represent species or clades that are polymorphic for this character.

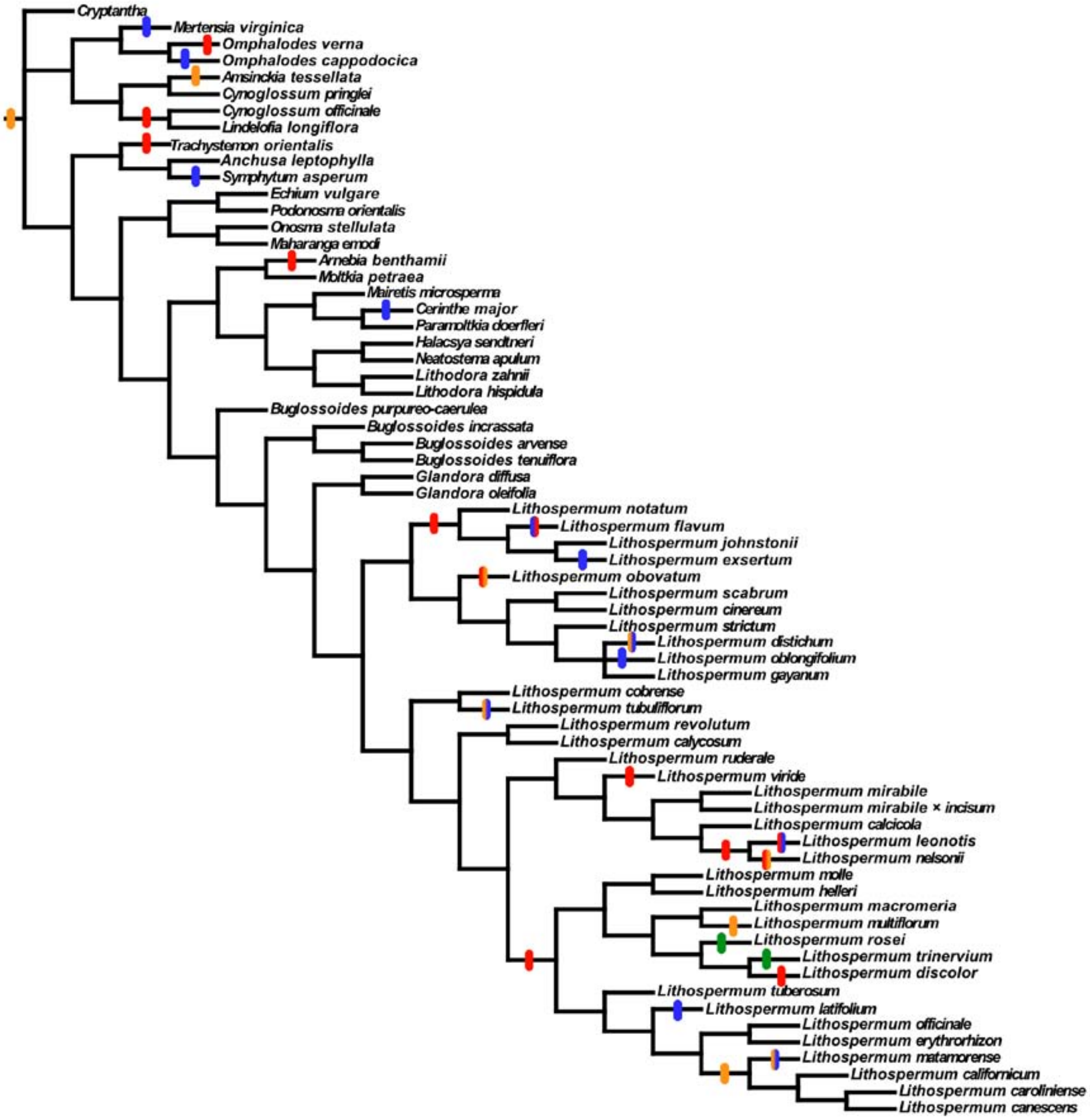
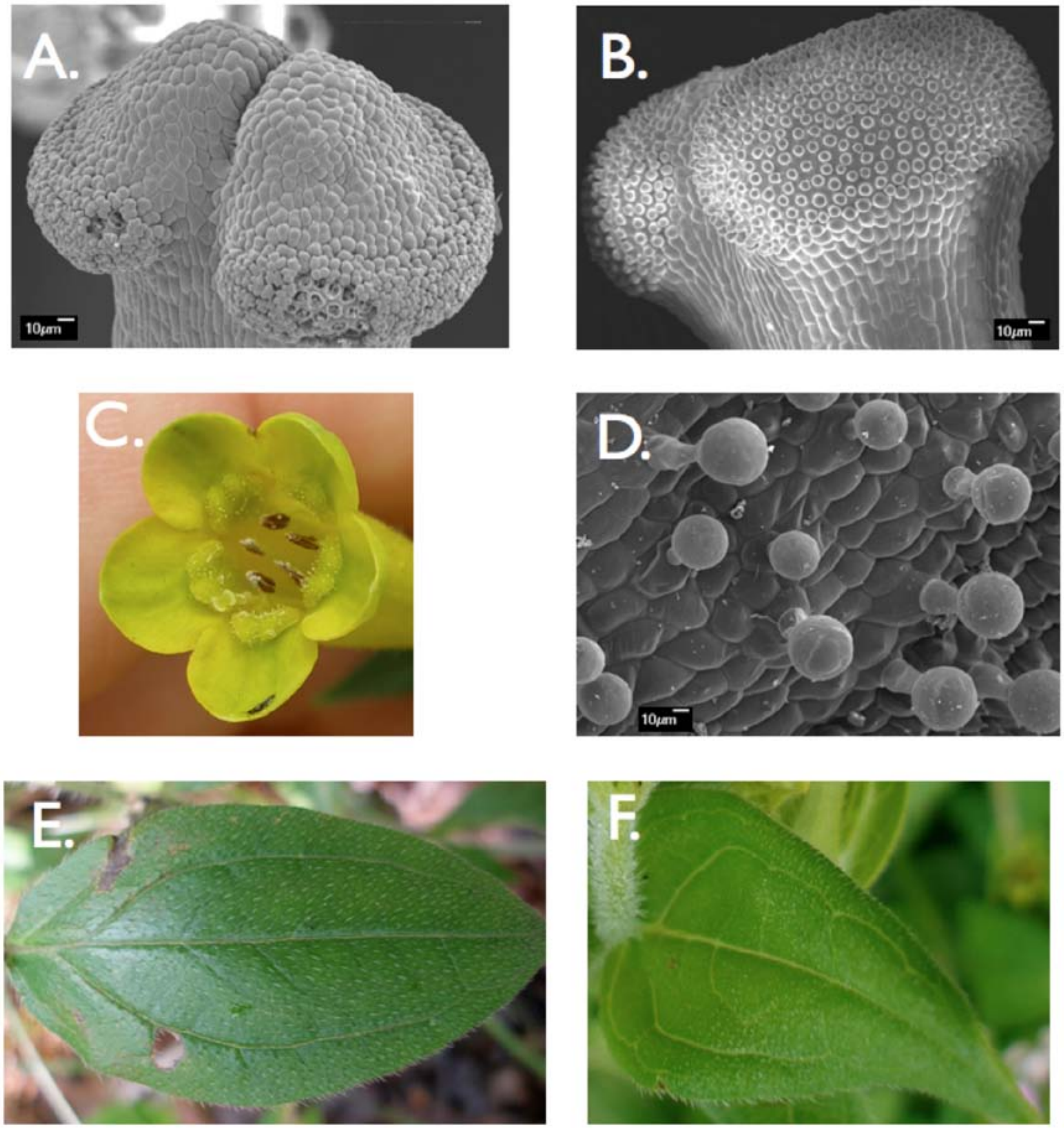

Cryptantha
Mertensia virginica
Omphalodes verna
Omphalodes cappodocica
Amsinckia tessellata
Cynoglossum pringlei
Cynoglossum officinale
Lindelofia longiflora
Trachystemon orientalis
Anchusa leptophylla
Symphytum asperum
Echium vulgare
Podonosma orientalis
Onosma stellulata
Maharanga emodi
Arnebia benthamii
Moltkia petraea
Mairetis microsperma
Cerinthe major
Paramoltkia doerfleri
Halacsya sendtneri
Neatostema apulum
Lithodora zahnii
Lithodora hispidula
Buglossoides purpureo-caerulea
Buglossoides incrassata
Buglossoides arvense
Buglossoides tenuiflora
Glandora diffusa
Glandora oleifolia
Lithospermum notatum
Lithospermum flavum
Lithospermum johnstonii
Lithospermum exsertum
Lithospermum obovatum
Lithospermum scabrum
Lithospermum cinereum
Lithospermum strictum
Lithospermum distichum
Lithospermum oblongifolium
Lithospermum gayanum
Lithospermum cobrense
Lithospermum tubuliflorum
Lithospermum revolutum
Lithospermum calycosum
Lithospermum ruderale
Lithospermum viride
Lithospermum mirabile
Lithospermum mirabile × incisum
Lithospermum calcicola
Lithospermum leonotis
Lithospermum nelsonii
Lithospermum molle
Lithospermum helleri
Lithospermum macromeria
Lithospermum multiflorum
Lithospermum rosei
Lithospermum trinervium
Lithospermum discolor
Lithospermum tuberosum
Lithospermum latifolium
Lithospermum officinale
Lithospermum erythrorhizon
Lithospermum matamorense
Lithospermum californicum
Lithospermum caroliniense
Lithospermum canescens

Figure 4.4. Morphological features of species of *Lithospermum*. A. Subterminal stigma, with apical projection. B. Terminal stigma. C. Five faucal appendages located at intersection of base of corolla lobes and apex of corolla tube. D. Three-celled glands on adaxial surface of corolla. E. and F. Leaves with evident secondary venation. Veins in *L. tuberosum* are sunken (E.), while veins in *L. exsertum* (F.) are not.


A.
10μm
B.
10μm
C.
D.
10μm
E.
F.

### *Floral characters*

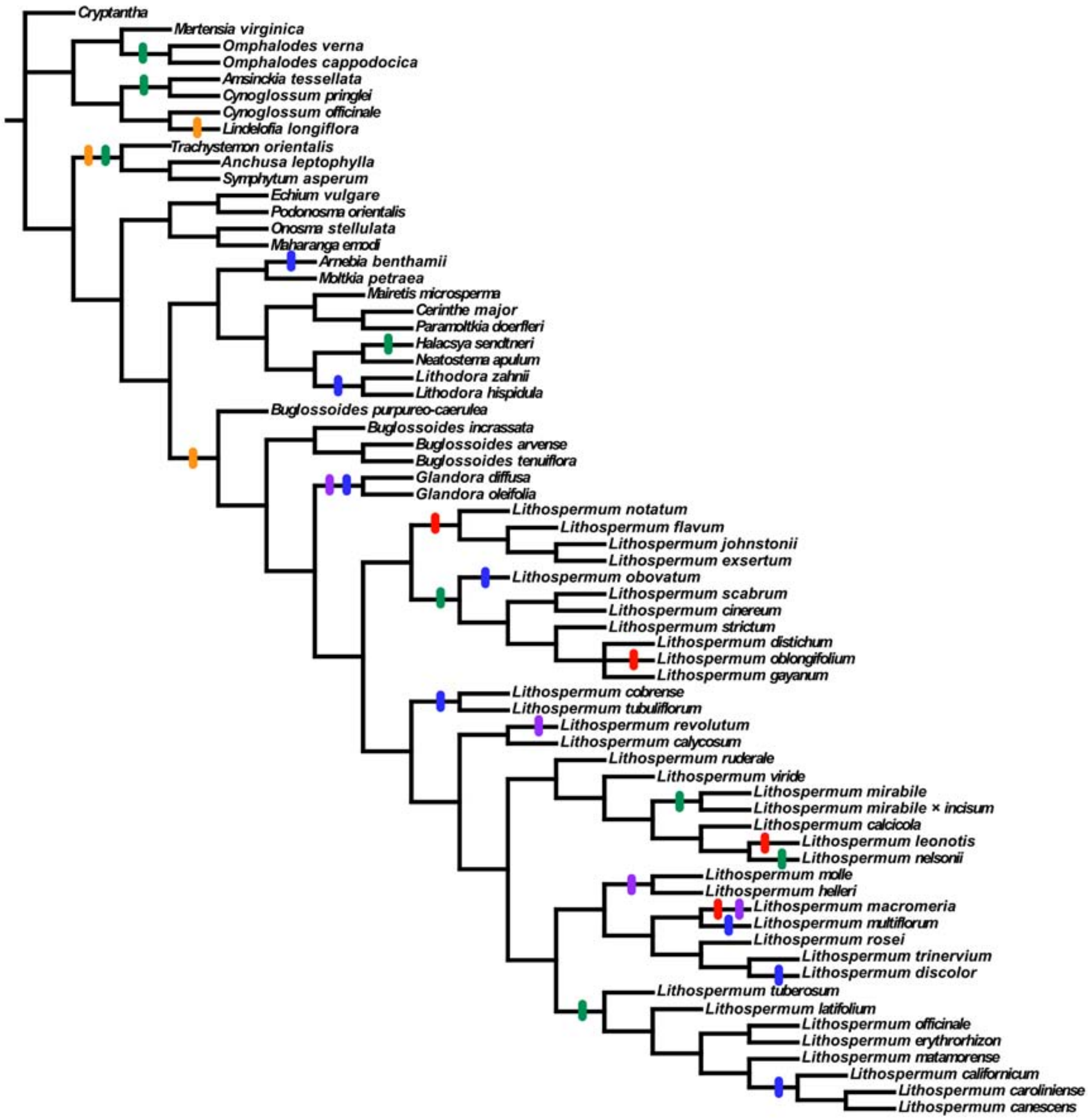
**Abaxial trichomes on the corolla** (L - 7, CI - 0.14) – Plants of all the species of Cynoglosseae included in the present analyses have corollas that are abaxially glabrous, but some members of Boragineae as well as some early-diverging members of Lithospermeae have trichomes on the abaxial surface of their corollas. The ancestral condition of this character cannot be reconstructed unambiguously from the available results. In Lithospermeae, glabrous corollas are a synapomorphy for a seven-species clade that includes *Cerinthe major* L. and *Lithodora zahnii* (Heldr. ex Halácsy) I. M. Johnst. Presence of abaxial trichomes on the corollas is a synapomorphy for the clade that includes *Buglossoides*, *Glandora*, and *Lithospermum*.

**Cleistogamy** (L - 3, CI - 0.33) – Cleistogamy arose three times within Lithospermeae: twice in *Lithospermum* and once in the monospecific genus *Neatostema* I. M. Johnst.

**Adaxial trichomes on the corolla** (L - 6, CI - 0.16) – Trichomes on the adaxial surface of corollas originated at least nine times within Lithospermeae. This character state tends to arise in a single species, and four of the origins are within species that are polymorphic for this character. The presence of corollas with adaxial trichomes is a synapomorphy for the clade composed of *L. cobrense* Greene and *L. tubuliflorum* Greene.

**Faucal appendages** (L - 9, CI - 0.11) – The presence of faucal appendages (Figure 4.4c) appears to have arisen at least eight times within Boraginaceae (Figure 4.5, green hash marks). Four of these origins are within *Lithospermum*, and only one species, *L. californicum* A. Gray, has lost faucal appendages. Additionally, the presence of faucal appendages is a synapomorphy for Boragineae.

Figure 4.5. Phylogenetic distribution of various floral features optimized on one most-parsimonious tree. Red hash marks denote acquisition of *Macromeria*-type flowers. Blue hash marks represent origin of reciprocal herkogamy. Green hash marks represent acquisition of faucal appendages. Orange hash marks denote origin of glands. Purple hash marks denote loss of glands.


Cryptantha
Mertensia virginica
Omphalodes verna
Omphalodes cappodocica
Amsinckia tessellata
Cynoglossum pringlei
Cynoglossum officinale
Lindelofia longiflora
Trachystemon orientalis
Anchusa leptophylla
Symphytum asperum
Echium vulgare
Podonosma orientalis
Onosma stellulata
Maharanga emodi
Arnebia benthamii
Moltkia petraea
Mairetis microsperma
Cerinthe major
Paramoltkia doerfleri
Halacsya sendtneri
Neatostema apulum
Lithodora zahnii
Lithodora hispidula
Buglossoides purpureo-caerulea
Buglossoides incrassata
Buglossoides arvense
Buglossoides tenuiflora
Glandora diffusa
Glandora oleifolia
Lithospermum notatum
Lithospermum flavum
Lithospermum johnstonii
Lithospermum exsertum
Lithospermum obovatum
Lithospermum scabrum
Lithospermum cinereum
Lithospermum strictum
Lithospermum distichum
Lithospermum oblongifolium
Lithospermum gayanum
Lithospermum cobrense
Lithospermum tubuliflorum
Lithospermum revolutum
Lithospermum calycosum
Lithospermum ruderale
Lithospermum viride
Lithospermum mirabile
Lithospermum mirabile × incisum
Lithospermum calcicola
Lithospermum leonotis
Lithospermum nelsonii
Lithospermum molle
Lithospermum helleri
Lithospermum macromeria
Lithospermum multiflorum
Lithospermum rosei
Lithospermum trinervium
Lithospermum discolor
Lithospermum tuberosum
Lithospermum latifolium
Lithospermum officinale
Lithospermum erythrorhizon
Lithospermum matamorense
Lithospermum californicum
Lithospermum caroliniense
Lithospermum canescens

**Glands inside corolla** (L - 8, CI - 0.12) – The presence of glands (Figure 4.4d) arose independently at least three times among species of Boraginaceae: once in *Lindelofia* Lehm., once or twice among members of Boragineae, and once in the clade containing *Buglossoides*, *Glandora*, and *Lithospermum* (Figure 4.5, orange hash marks). Most species of *Lithospermum* have retained the presence of glands, but this state has been lost at least four times (Figure 4.5, purple hash marks).

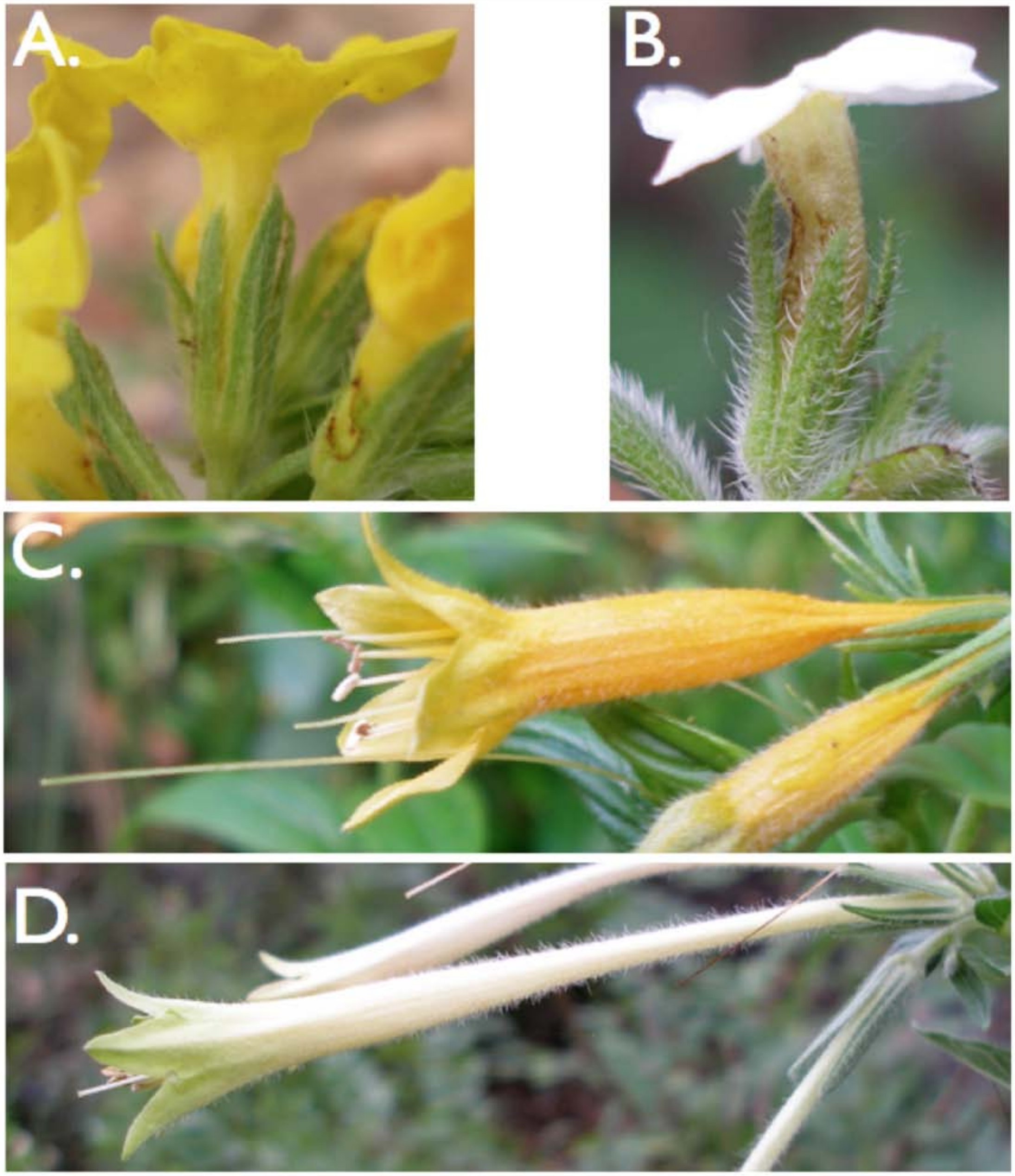
**Corolla color** (L - 23, CI - 0.30) - I recognized 10 distinct corolla colors (Figure 4.6) among the species of Boraginaceae. Each of these colors has originated multiple times throughout the family. Blue corollas are resolved as ancestral for the present phylogeny, while yellow corollas are ancestral for *Lithospermum*.

**Corolla shape** (L - 28, CI - 0.32) - I identified 10 different corolla shapes among the species of Boraginaceae. The salverform-funnelform shape (Figure 4.6a) is plesiomorphic for Lithospermeae and *Lithospermum*. Additionally, the present phylogeny provides evidence for multiple losses of this character state, particularly among early-diverging members of Lithospermeae.

The salverform corolla shape (Figure 4.6b) is the most common type in the ingroup. This corolla shape is the ancestral condition for two large clades: one includes *L. scabrum*, *L. gayanum*, and four additional species, and another is composed of *L. calcicola* B. L. Rob., *L. caroliniense* MacMill. and twenty-two other species.

In the present analyses, two different types of *Macromeria*-type flowers have been recognized. One type includes long-funnelform corollas that flare out (Figure 4.5c), and this shape originated once. The other type is composed of long-funnelform

Figure 4.6. Corollas of species of *Lithospermum* A. Yellow, salverform-funnelform corolla of *L. cobrense*. B. White with a yellow center, salverform corolla of *L. nelsonii*. C. *L. flavum* bears orange, long-funnelform corollas that flare out. D. *L. macromeria* produces green-yellow to green-cream, long-funnelform corollas that taper from the base to the apex.


A.
B.
C.
D.

corollas that gradually taper from the base to the apex (Figure 4.6d). This corolla shape arose three times (Figure 4.5, red hash marks).

**Corolla lobes** (L - 19, CI - 0.10) – The present phylogeny provides evidence that flared corolla lobes are the plesiomorphic condition for the family. Reflexed corolla lobes originated at least seven times, and ascending corolla lobes arose at least nine times. Reflexed corolla lobes do not characterize any clade with more than two species. In contrast, ascending corolla lobes characterize one clade that comprises *L. molle*, *L. trinervium*, and five other species. In the combined matrix, five species are coded as polymorphic for the presence of both flared and ascending corolla lobes.

**Type of herkogamy** (L - 17, CI - 0.17) - Approach herkogamy (stigmas positioned above the anthers) is the ancestral condition for Lithospermeae and for *Lithospermum*. This type of herkogamy has been lost multiple times in both the ingroup and the outgroup.

Although reciprocal herkogamy (Figure 4.5, blue hash marks) is present in some species of Boragineae and Cynoglosseae, the present study only included species of Lithospermeae that exhibit this floral feature. Within Lithospermeae, reciprocal herkogamy originated at least eight times, with five of these origins occurring in *Lithospermum*. The structure of the phylogeny of the combined matrix (Figures 4.1 and 4.5) does not support any losses of reciprocal herkogamy.

Reverse herkogamy (stigmas positioned below the anthers) characterizes three outgroup species, *Maharanga emodi* DC., *Mairetis microsperma* (Boiss.) I. M. Johnst., and *Buglossoides purpureo-caeurlea* (L.) I. M. Johnst., and this condition originated independently in each. In the ingroup, the presence of reverse herkogamous flowers is a synapomorphy for only one clade. This clade includes

*L. tuberosum*, *L. matamorense* DC., and six other species. Although other species of *Lithospermum* exhibit reverse herkogamy, they do not do so as a fixed state.

**Filament shape in cross section** (L - 6, CI - 0.33) - Most species of Boraginaceae produce filaments that are circular in cross section. This state is resolved as plesiomorphic for Lithospermeae. Filaments that are elliptical in cross section have arisen three to five times. In the outgroup, filaments with this shape in cross section evolved twice: once in *Cerinthe* L. and once in *Podonosma*. In the ingroup, this character state either evolved independently in each of three species, *L. macromeria*, *L. rosei* (I. M. Johnst.) J. I. Cohen, and *L. trinervium*, or was acquired once at the base of the clade that includes these three species and subsequently lost twice.

**Anther exsertion/insertion** (L - 9, CI - 0.11) – Insertion of anthers in the corolla tube is the ancestral condition for Lithospermeae and for *Lithospermum*. Anther exsertion originated at least eight times. Most of these origins are isolated instances, such as in *L. macromeria* and *L. leonotis*; however, anther exsertion is a synapomorphy for the clade composed of *L. notatum*, *L. flavum*, *L. johnstonii*, and *L. exsertum*.

**Trichomes on the abaxial surface of anthers** (L - 3, CI - 0.33) - Three species of *Lithospermum* – *L. macromeria*, *L. rosei*, and *L. trinervium* – produce trichomes on the abaxial surface of their anthers. As with the optimization of filament shape in cross section, the structure of the present phylogeny does not differentiate unambiguously between three origins of this state and one origin followed by two losses.

**Pollen shape** (L – 16 - 17, CI – 0. 23 - 0.25) - I recognized five pollen shapes among species of Boraginaceae, and all occur in *Lithospermum*. The present phylogeny provides evidence that ellipsoid pollen (Figures 4.7 and 4.8, orange hash marks) is the plesiomorphic state for Lithospermeae. This character state has been lost multiple times, including at least seven times among members of the outgroup.

Ellipsoid pollen is reconstructed as ancestral for *Lithospermum*, and this shape has been lost multiple times. Prolate pollen with a constricted equator (termed hourglass-shaped, as the pollen closely resembles this object) evolved independently at least five times within *Lithospermum* (Figure 4.8, purple hash marks). This pollen shape is a synapomorphy for the clade that includes *L. latifolium*, *L. canescens*, and five other species. Apart from this clade, this type of pollen is produced only by isolated species or one morph of a heterostylous species. Ovoid pollen (Figure 4.7c) originated once in the ingroup, and subsequently was lost at least three times (Figure 4.8, red hash marks). Spherical pollen (Figure 4.7e) characterizes the clade that includes *L. viride*, *L. mirabile* I. M. Johnst., and three other taxa. In addition, this type of pollen arose independently in both *L. exsertum* and *L. strictum* (Figure 4.8, green hash marks). Cylindrical pollen is a synapomorphy for a clade that includes two Mexican species, *L. distichum*, *L. oblongifolium*, and one South American species, *L. gayanum* (Figure 4.8, blue hash marks).

**Pollen pore position** (L - 7, CI - 0.14) - Pollen with equatorial pores is the ancestral condition for Lithospermeae. Pollen with subequatorial pores originated at least seven times. Most of these instances occur in only one or two species, but subequatorial pollen pores are a synapomorphy for a large clade of species of *Lithospermum*. This clade includes *L. molle, L. canescens*, and 13 other species. In this clade, only one reversion to equatorial pores occurs.

Figure 4.7. Five different pollen shapes of species of *Lithospermum*. A. Prolate with a constricted equator (hourglass shape). B. Cylindrical. C. Ovoid. D. Ellipsoid. E. Spherical.

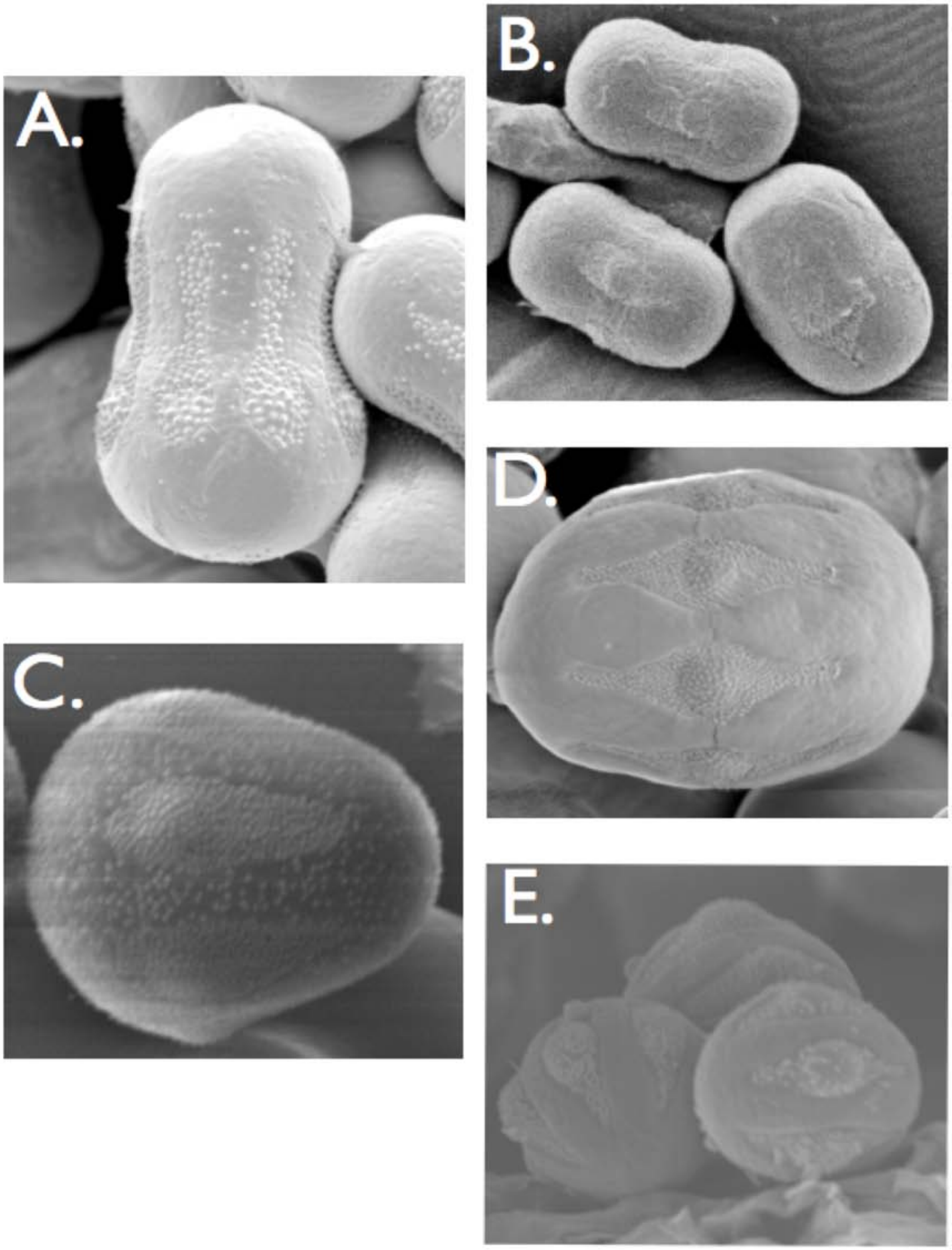

A.
B.
C.
D.
E.

Figure 4.8. Phylogenetic distribution of pollen shape optimized on one most-parsimonious tree. Purple hash marks represent origin of prolate pollen with a constricted equator (hourglass shape). Orange hash marks denote origin of ellipsoid pollen. Red hash marks represent origin of ovoid pollen. Green hash marks represent origin of spherical pollen. Blue hash marks denote origin of cylindrical pollen. Hash marks in two colors represent species or clades that are polymorphic for this character.


Cryptantha
Mertensia virginica
Omphalodes verna
Omphalodes cappodocica
Amsinckia tessellata
Cynoglossum pringlei
Cynoglossum officinale
Lindelofia longiflora
Trachystemon orientalis
Anchusa leptophylla
Symphytum asperum
Echium vulgare
Podonosma orientalis
Onosma stellulata
Maharanga emodi
Arnebia benthamii
Moltkia petraea
Mairetis microsperma
Cerinthe major
Paramoltkia doerfleri
Halacsya sendtneri
Neatostema apulum
Lithodora zahnii
Lithodora hispidula
Buglossoides purpureo-caerulea
Buglossoides incrassata
Buglossoides arvense
Buglossoides tenuiflora
Glandora diffusa
Glandora oleifolia
Lithospermum notatum
Lithospermum flavum
Lithospermum johnstonii
Lithospermum exsertum
Lithospermum obovatum
Lithospermum scabrum
Lithospermum cinereum
Lithospermum strictum
Lithospermum distichum
Lithospermum oblongifolium
Lithospermum gayanum
Lithospermum cobrense
Lithospermum tubuliflorum
Lithospermum revolutum
Lithospermum calycosum
Lithospermum ruderale
Lithospermum viride
Lithospermum mirabile
Lithospermum mirabile × incisum
Lithospermum calcicola
Lithospermum leonotis
Lithospermum nelsonii
Lithospermum molle
Lithospermum helleri
Lithospermum macromeria
Lithospermum multiflorum
Lithospermum rosei
Lithospermum trinervium
Lithospermum discolor
Lithospermum tuberosum
Lithospermum latifolium
Lithospermum officinale
Lithospermum erythrorhizon
Lithospermum matamorense
Lithospermum californicum
Lithospermum caroliniense
Lithospermum canescens

**Pollen pore number** (L - 5, CI - 0.60) - Presence of three pores originated three times, once each in *Anchusa leptophylla* Roem. & Schult., *Halacsya*, and the clade composed of *Podonosma*, *Onosma*, *Echium*, and *Maharanga*. Most other species produce pollen with six to eight pores, with the only exceptions being species of *Arnebia* Forssk. and *Symphytum* L. Species of these two genera bear pollen with more than eight (often at least ten) pores. This derived condition evolved independently in each taxon.

**Style position** (L - 20, CI - 0.15) - Style insertion has arisen or been lost numerous times throughout the evolution of the genus, tribe, and family. Multiple origins of style exsertion, whether precocious (three gains), tardy (at least two gains), or at anthesis (at least five gains), occur among members of *Lithospermum*.

**Stigma** (L - 5, CI - 0.20) - Terminal stigmas (Figure 4.4a) are plesiomorphic for Lithospermeae, with the presence of subterminal stigmas (Figure 4.4b) originating at least nine times. Most of these origins occur within species that are polymorphic for this character. Only a limited number of taxa, including a few species of *Lithospermum* and all members of *Buglossoides* Moench., exclusively develop subterminal stigmas.

***Fruit characters***

**Nutlet attachment** (L - 1, CI - 1) - Sub-medial nutlet attachment characterizes Cynoglosseae, and basal nutlet attachment characterizes Boragineae and Lithospermeae.

**Nutlet bases attached** (L - 2, CI - 0.5) - Nutlet bases that remain attached to the gynobase evolved twice: once in *Glandora* and once in *Lithodora* Griseb. This condition is restricted to the members of these genera.

## DISCUSSION

### PHYLOGENETICS

Analyses of the cpDNA and combined matrices resolve the same outgroup relationships, with two notable exceptions. The basal relationships of Lithospermeae differ between the trees of the two matrices. The cpDNA matrix reconstructs *Podonosma* as sister to the rest of Lithospermeae, but the combined matrix resolves a clade composed of *Podonosma, Echium, Onosma* and *Maharanga* as sister to the tribe. In analyses of the combined matrix, the exclusion of the character “pollen pore number” does not allow for the unambiguous resolution of which species or clade is sister to the remainder of Lithospermeae. However, with the inclusion of this character, the combined matrix resolves the aforementioned four-taxon clade in this position. Three pollen pores is a synapomorphy for this clade.

The present analysis includes representatives of only five of the nine genera of Lithospermeae that produce pollen with three pores (Johnston 1952, 1953a, b, 1954a, b). The clade composed of *Podonosma, Echium, Onosma,* and *Maharanga* contains four of the sampled taxa, while the fifth, *Halacsya*, is nested among members of the tribe that produce pollen with six to eight pores. The inclusion of additional triporate representatives of the tribe, such as *Lobostemon* Lehm. and *Vaupelia* Brand (Johnston, 1954b), will help to determine if pollen with different numbers of pores characterize two separate clades. If this is the case, then the tribe would be characterized by two clades, each diagnosed by pollen with a specific number of pores.  However, if these two clades are not resolved, then this result would provide evidence that pollen with three pores is the ancestral state for the tribe.

Another difference in the outgroup relationships obtained with and without the morphology included involves the relationship between two species of *Glandora*.  The combined matrix resolves the two species are sisters, but the cpDNA alone does not. In analyses of the combined matrix, the inclusion of two characters – “nutlet base attachment” and “type of herkogamy” – is necessary to resolve unambiguously *G. diffusa* and *G. oleifolia* as sisters.  If these characters are excluded from the analysis, the remainder of the combined matrix cannot determine whether the two species are sisters or not.  The present analysis included only two of the six species of *Glandora*, so more complete taxon sampling may help resolve the relationships among the species of this genus.

Although the cpDNA and the combined matrices resolve similar outgroup relationships, the ingroup relationships that they resolve differ to a greater extent.  One of the most striking differences involves relationships at the base of the ingroup. Analyses of the combined matrix, but not those of the cpDNA matrix, yield a clade composed of four species with *Macromeria*-type flowers at this position. Consequently, some character states that are derived in analyses of the cpDNA matrix, such as the absence of faucal appendages, and the presence of long-funnelform corollas that flare open (state 8), are resolved as ancestral in analyses of the combined matrix.

Another notable difference between the trees obtained with the two matrices is that with the inclusion of morphological data, jackknife support increases for the relationships of the close relatives of *Lithospermum*, such as *Buglossoides* and *Glandora*.  In contrast, jackknife support for larger clades of the ingroup does not tend to increase with the inclusion of morphological data.  This lack of increased support, along with the structural diversity among species of *Lithospermum*, is consistent with observed patterns for rapid radiations, or island colonizations followed by subsequent diversification (Bateman, 1999). *Lithospermum* may have undergone this type of diversification throughout the mountains and higher elevation regions of Mexico and the western United States. It is important to note that both matrices resolve many of the same sister species relationships, and some of the same larger clades composed of five or six species.

## LEAF EVOLUTION

I identified four different patterns of leaf venation among species of Boraginaceae (Table 4.1). Most of the observed variation in leaf venation occurs in the ingroup (Figures 4.3 and 4.4e, f). All outgroup members of Lithospermeae, except *Arnebia* and *Cerinthe*, produce leaves in which the only evident vein is the midvein. This is the ancestral condition for the tribe. In contrast, all species of Cynoglosseae and Boragineae included in the present study (with the exception of *Amsinckia tessellata*) produce leaves with evident secondary venation. This is the plesiomorphic condition for these two tribes. This difference between Lithospermeae, on the one hand, and Boragineae and Cynoglosseae, on the other, helps to distinguish between these groups.

In *Lithospermum,* the ancestral state is one in which only the midvein is evident. Leaves with evident secondary venation have arisen multiple times. Evident secondary venation characterizes two larger clades: one includes *L. notatum,* *L. exsertum*, *L. flavum*, and *L. johnstonii*, and the other comprises *L. molle,* *L. officinale,* and 13 other species. Seven species distributed among other clades bear leaves with evident secondary venation, but three of these species are polymorphic for this character, producing some leaves with evident secondary venation and others in which only the midvein is evident. Jones et al. (2009) studied the evolution of leaf venation in South African species of *Pelargonium* L'Hér. ex Aiton. These authors concluded that leaf venation is quite plastic in the genus. This also appears to be the case in *Lithospermum*.

Species that produce leaves with evident secondary venation tend to produce larger plants, and this may be influenced considerably by developmental patterns. Because the plants themselves are larger, this may cause the leaves to become larger and develop a greater number of evident veins. Conversely, instead of being a byproduct of development, a greater number of evident veins could allow for the growth of larger plants, due to an increased ability of the leaf to transport larger quantities of water, nutrients, and sugars to and from the rest of the plant (Roth-Nebelsick et al., 2001; McKown et al., 2010). Therefore, it is possible that evident secondary venation either allows the plant to grow larger, or that larger plants develops larger leaves with a greater number of evident secondary veins.

Some species of *Lithospermum*, such as *L. tubuliflorum* and *L. obovatum*, produce basal leaves, but not cauline leaves, with evident secondary venation. The explanations presented here could also account for this pattern. The basal leaves of these species tend to grow much larger than the cauline leaves, and therefore the basal leaves would require an increased ability to transport water, nutrients, and sugars to and from the rest of plant.

The aforementioned hypotheses concerning the presence of evident secondary venation do not explain why species with larger habits produce leaves in which the only evident vein is the midvein, or why species with smaller habits produce leaves with evident secondary venation. Phylogenetic history provides one possible explanation for why species with smaller habits, such as *L. tuberosum*, produce leaves with evident secondary venation. The ancestor of this species is resolved to have produced leaves with evident secondary venation. Consequently, even if a plant does not require the putative advantages of a greater number of evident secondary veins, the presence of this character state is retained as a symplesiomorphy.

## FLORAL EVOLUTION

***Corolla shape*** **–** I recognized nine different corolla shapes within *Lithospermum*, with all but one of these states – the salverform corolla shape (Figure 4.6b) – characteristic of fewer than four species. Salverform corollas have originated at least three times within the genus. Most of the eight other corolla shapes, such as tubular or funnelform, also have evolved multiple times. These shapes do not characterize any clade, with a few exceptions. For example, the presence of a type of campanulate corolla is a synapomorphy for *L. molle* and *L. helleri* (Small) J. I. Cohen, two species previously assigned to *Onosmodium*. The long-funnelform corollas that flare out characterize a clade composed of four species formerly included in *Macromeria* (Figure 4.6c). However, the present study sampled only two of the eight species that were part of *Onosmodium,* and only six of the eleven species included in *Macromeria*.

The diversity in corolla shapes among species of *Lithospermum* is equal to or greater than that of many larger genera. For example, Harrison et al. (1999) identified six different corolla types in their study of *Streptocarpus* Lindl. and *Saintpaulia* H. Wendl. (Gesneriaceae), genera with 130 and 20 species respectively. In their analyses of 36 species from these two genera, Harrison et al. (1999) found 10 origins of the six corolla shapes. In contrast, the present study resolved 21 transitions of corolla shape among 37 species of *Lithospermum*. In a study of *Campanula* L. (Campanulaceae), a genus composed of 350 - 500 species (6 to 8 times larger than *Lithospermum*), Roquet et al. (2008) recognized six different corolla shapes within the genus. In *Campanula,* most species tend to produce tubular-campanulate corollas, but many species are characterized by one of the other five types, each of which has originated multiple times. Roquet et al. (2008) postulate that corolla shape is quite plastic in *Campanula*, and I have reached a similar conclusion in *Lithospermum*. However, the evolution of corolla form in *Lithospermum* follows different patterns than that in *Campanula*. In *Lithospermum*, most of the modifications of corolla shape involve changes in the length of the corolla tube, the circumference of the apex of the corolla tube, and the orientation of the corolla lobes. However, in *Campanula*, substantial changes occur in the extent of petal fusion and width of the corolla tube (if a tube exists at all). In light of these differences, it appears that changes in corolla form in *Lithospermum* tend to be quantitative, rather than qualitative. Some changes involve the overall structure of the corolla, but this is infrequent. For example, in *L. rosei* and *L. trinervium*, urceolate and campanulate corolla shapes occur. These shapes are quite rare in *Lithospermum*, but are common in some Old World genera of Lithospermeae, such as *Onosma*.

*Macromeria*-type flowers arose four times (Figure 4.5, red hash marks). In three of these instances, the species that bear this type of flower are sister to species that produce short corollas (1 - 1.5 cm in length) with inserted anthers and stigmas. Flowers of a form that is intermediate between these two types have not been observed. It is possible that species of intermediate form once existed, and are now extinct.

The transition from smaller to larger flowers has been hypothesized to occur less frequently than the reverse trend (Stebbins, 1957; Johnston and Schoen, 1996; Takebayashi and Morrell, 2001; Armbruster et al., 2002; Herlihy and Eckert, 2005). Therefore, the pattern observed in *Lithospermum* and other taxa, such as *Lycium* L. (Solanaceae) (Miller and Venable, 2003), *Penstemon* Schmidel (Plantaginaceae)

(Wolfe et al., 2006), and *Ruellia* L., along with other species of Acanthaceae (Tripp and Manos, 2008; Daniel et al., 2008), is contrary to expectations.

Species with *Macromeria*-type flowers are unusual for their putative evolutionary transitions as well as for their pollination syndrome. Grant and Grant (1970) have observed hummingbirds pollinating the flowers of *L. macromeria*. Additionally, there is evidence that some other species with this floral form share the same type of pollinator (Fægri and van der Pijl, 1979; Richards, 1997; Aizen, 2003). The repeated evolution of hummingbird pollination is not uncommon (Wolfe et al., 2006; Tripp, 2007; Daniel et al., 2008; Tripp and Manos, 2008), but species of *Lithospermum* do not fit the typical hummingbird pollination syndrome, which includes long, tubular red corollas (e.g., Richards, 1997; Tripp, 2007). Even though the corollas of *Macromeria*-type flowers are long and tubular, none are red. However, hummingbirds are also known to visit flowers with green or orange tubular corollas (Richards, 1997), and many species of *Lithospermum* with *Macromeria*-type flowers produce corollas with one or both of these colors.

***Herkogamy*** - In *Lithospermum*, herkogamy can vary both inter- and intraspecifically. Most species, at least over parts of their geographic range, or during different stages of development, exhibit approach herkogamy, the most common type of herkogamy in angiosperms (Webb and Lloyd, 1986). Reverse herkogamy commonly occurs among the close relatives of *Lithospermum*, but this type of herkogamy characterizes only one clade in the ingroup.

The phylogenetic results indicate that reciprocal herkogamy, a component of heterostyly, originated at least five times within *Lithospermum* and at least three additional times elsewhere in Lithospermeae, once each in *Arnebia*, *Glandora*, and *Lithodora* (Figure 4.5, blue hash marks). Phylogenetic analyses of other taxa, such as

*Linum* (Linaceae) (McDill et al., 2009), *Lythrum* (Lythraceae) (Morris, 2007), *Narcissus* (Amaryllidaceae) (Graham and Barrett, 2004; Pérez-Barrales et al., 2006), and *Lithodora* and *Glandora* (Thomas et al., 2008; Ferrero et al., 2009), have also recovered multiple origins of reciprocal herkogamy. Despite evidence from these and other phylogenetic analyses, Kohn et al. (1996), McDill et al. (2009), Morris (2007) and others have hypothesized that multiple gains of heterostyly among close relatives are unlikely. However, Cohen (2010) suggests that under appropriate conditions, heterostyly may evolve repeatedly in some taxa, including *Lithospermum*.

It should be noted that to infer the pattern of the evolution of heterostyly, I did not examine gains/losses with either a step matrix or likelihood methods, as others (e.g., Kohn et al., 1996; McDill et al., 2009) have done. I did not employ these approaches for two reasons. First, the only component of heterostyly included in these analyses was reverse herkogamy per se. The other components, self- and intramorph incompatibility and micromorphological differences (Barrett et al., 2000), were not included because insufficient data are available for these characters in *Lithospermum* and Boraginaceae. Second, despite the complexity of heterostyly, evidence concerning its evolutionary development is lacking. Therefore, it is difficult to hypothesize an appropriate weighting scheme for the evolution of the breeding system. Current research has yet to determine if acquisition of the heterostylous syndrome is two, three, or ten times more “difficult” than its loss. Consequently, I chose to weight gains and losses of this character equally rather than to employ other analytical strategies. Although the use of equal weighting still may be considered an assumption, it minimizes the total number of assumptions.

In addition to flowers that differ in their type of herkogamy, species of *Lithospermum* exhibit variation in the position of the stigma relative to the apex of the corolla tube. The presence of stigmas that are exserted from the corolla tube originated multiple times, although the timing of stigma exsertion can occur before, during, or after anthesis (Johnston, 1952, 1954a, b). I divided the character "stigma position" into four states, and three of these states represent various types of stigma exsertion (Table 4.1). Most species of *Lithospermum* develop exserted stigmas at anthesis (pers. obs.); however, precocious stigma exsertion arose three times in the genus. It is not known if the stigma is receptive at this early stage of development, but should it be, precocious stigma exsertion would combine herkogamy and dichogamy (the temporal separation of pollen dispersal and stigmatic receptivity). This combination of herkogamy and dichogamy also may occur in species that exhibit late stigma exsertion. Future crossing studies on species of *Lithospermum* may provide evidence concerning the timing of stigma receptivity.

***Faucal appendages*** - Faucal appendages are thickenings of the corolla that are located at the intersection of the corolla tube apex and corolla lobe base (Figure 4.4c). These floral structures may play a role in constricting the apex of the corolla tube and providing a reward for pollinators, as glands are produced on the faucal appendages of some species. These appendages have originated at least three times within *Lithospermum*, and they diagnose two larger clades (Fig 4.5, green hash marks). In the present study, this character is coded in binary form (Table 4.1), but different types of appendages occur among species of the genus. An examination of the micromorphology of these appendages may determine if it is appropriate to code this character in a more complex manner.

Species in other groups of Boraginaceae also produce faucal appendages. The structure of the phylogeny suggests that these appendages have originated at least four times among members of the outgroup. Currently, it is not known if the faucal appendages that have resulted from these independent origins have a similar structure.

***Glands*** - Although not all species of *Lithospermum* produce faucal appendages, most members of the genus bear glands on the adaxial surface of their corolla tubes (Figure 4.4d). These glands are present in the upper portion of the corolla tube, on the veins that lead to the filaments, or both. I coded this character as binary (Table 4.1), but it is possible to recognize multiple states of this character based on the location of the glands. The presence of glands is a synapomorphy for two clades, one that includes both *Lithospermum* and *Buglossoides* and one composed of the members of Boragineae (Figure 4.5, orange hash marks).

In *Lithospermum*, glands have been lost at least four times (Figure 4.5, purple hash marks). The loss of glands does not appear to follow any pattern related to corolla shape, color, or size. The only correlation I can observe is that all species that lack glands also produce flowers with exserted stigmas. However, not all species with exserted stigmas lack glands. Because of the limited relationship between the absence of glands and other floral features, it is difficult to infer any benefit from the loss of glands or any possible linkage between glands and the other floral, vegetative, or pollen characters investigated in the present study.

## POLLEN EVOLUTION

An examination of the phylogenetic distribution of pollen shape can inform our understanding of the evolution of heterostylous species. Two heterostylous species of *Lithospermum*, *L. caroliniense* and *L. tubuliflorum*, produce pollen that is dimorphic in both size and shape (Johnston, 1952). In both species, the long-style morph produces hourglass-shaped pollen, and the short-style morph produces ellipsoid pollen. *Lithospermum caroliniense* is nested among species that produce hourglass-shaped pollen; therefore, it appears that ellipsoid pollen arose within the species. In contrast, the relatives of *L. tubuliflorum* produce ellipsoid pollen, so hourglass-shaped pollen originated within this species. Even though the morphs of these species exhibit the same pollen shapes, the conditions arose in the opposite order in each species (Figure 4.8).

Cylindrical pollen characterizes the clade that includes two Mexican species, *L. distichum* and *L. oblongifolium*, and one South American species, *L. gayanum*. In view of this relationship, I proposed two mechanisms for the origin of the South American species of *Lithospermum* (Chapter 3). The first is that long distance dispersal of a Mexican species from this clade may have given rise to the South American species. In contrast, it also is suggested that the ancestor of the extant *L. mediale* I. M. Johnst. – a species distributed from Guatemala to Colombia and with a floral form similar to that of both *L. distichum* and *L. gayanum* – could have colonized the Andes, and subsequently given rise to the Andean members of the genus. However, *L. mediale* produces ellipsoid, not cylindrical, pollen. Additionally, the four other South American species of *Lithospermum* produce ellipsoid, spherical, or cylindrical pollen (Johnston, 1952). Despite the small number, the South American species of the genus display a range of pollen shape variation similar to that of the species of North America. In order to resolve the ancestor and reconstruct the character evolution of the South American species of *Lithospermum*, it will be necessary to include these taxa in future phylogenetic analyses.

The present phylogenetic analysis provides evidence that pollen shape is quite evolutionarily labile in *Lithospermum*. This also appears to be the case in other genera of Boraginaceae. In a study of the pollen of Boragineae, Bigazzi and Selvi (1998) described 14 different types among members of the tribe. These authors recognized two genera, *Nonea* and *Anchusa*, that produce three and five different types of pollen, respectively.

The diversity in pollen shape among species of Boraginaceae appears to be much greater than that of many other angiosperm groups. For example, families such as Myristicaceae (Sauquet and Le Thomas, 2003), and genera, including *Gaultheria* L. (Lu et al., 2009) and *Hemerocallis* L. (Xiong et al., 1997), produce pollen that varies much more in exine sculpturing than in shape. Bigazzi and Selvi (1998) noted various types of exine ornamentation among species of Boragineae, but other pollen characters varied to a greater degree. I did not specifically evaluate exine ornamentation, but my limited observations suggest that species of *Lithospermum* do not exhibit evident differences in exine structure. Regardless of the pattern of variation in exine ornamentation, pollen shape seems more diverse and more evolutionarily labile in Boraginaceae, both at the intrafamilial and intrageneric level, than in most families of angiosperms.

Two other pollen characters – pollen pore position and pollen pore number – were investigated in a phylogenetic context. Most of the species of Boraginaceae included in the present study produce pollen with equatorial pores. In the outgroup, three taxa produce pollen with subequatorial pores, and this condition originated independently in each of them. In *Lithospermum*, the equatorial pollen pore position is ancestral. This state has been lost five times, resulting in species that produce pollen with a subequatorial pore position. The latter condition characterizes two ingroup clades (Figure 4.8). One includes *L. flavum*, *L. johnstonii*, and *L. exsertum*, and the other comprises *L. rosei*, *L. canescens*, and 13 other species. Among the members of this large clade, equatorial pollen pores have secondarily originated only in one species, *L. multiflorum*.

All species of *Lithospermum* produce pollen with six to eight pores, and this is the ancestral condition for the phylogeny. This type of pollen has been lost five times in Boraginaceae, resulting in pollen with either a larger or smaller number of pores.

One of these instances involves the evolution of pollen with three pores. Triporate pollen is a synapomorphy for the clade that includes *Echium*, *Podonosma*, *Maharanga*, and *Onosma* (described above). Pollen with more than eight pores originated twice, once each in *Symphytum* and *Arnebia*. The pollen grains produced by members of each of these genera differ. In *Symphytum*, the pores are distributed in one row around the equator of the pollen grain (Bigazzi and Selvi, 1998). In *Arnebia*, the pores are distributed in two rows, with one row above and one row below the equator of the pollen grain (Johnston, 1952). This latter condition is uncommon in Boraginaceae. Pollen shape seems to vary more than pollen pore position or pollen pore number, but this hypothesis should be examined further with increased sampling of taxa and palynological characters.

## Conclusion and Future Directions

Within *Lithospermum* and Boraginaceae, the evolution of morphological, palynological, and vegetative characters follows both simple and complex patterns. The combined analysis presented here provides only a limited amount of information concerning character evolution within Boraginaceae. The present study includes both cpDNA regions and morphological characters that can be aligned or coded for any species in the family. Additionally, with more observations of certain floral and vegetative features, it may be appropriate to recode some characters, including glands and faucal appendages, in other manners.

It is possible to integrate current molecular biology tools, classical developmental techniques, and data related to the evolution of structural features. For example, identification of the genes that affect floral organ length will enhance our understanding of the evolutionary changes necessary to alter corolla shape and type of herkogamy. Given the degree of structural diversity within *Lithospermum*, the genus may be a useful model in which to investigate the genetics, development, and evolution of morphological characters.

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# CHAPTER FIVE

# COMPARATIVE FLORAL DEVELOPMENT OF HETEROSTYLOUS AND HOMOSTYLOUS SPECIES OF *LITHOSPERMUM* AND IMPLICATIONS FOR THE EVOLUTION AND DEVELOPMENT OF HETEROSTYLY

## Abstract

In order to investigate the evolution and development of heterostyly, a comparative study of the floral developmental patterns of three heterostylous and three homostylous species of *Lithospermum* L. (Boraginaceae) was undertaken. Using light and scanning electron microscopy, flowers at various stages of maturation were observed, and developmental patterns were inferred. Although heterostyly may have originated independently in each of these heterostylous species, flowers of the long-style morph of each species follow similar developmental patterns, as do those of the short-style morph. In addition, the flowers of each heterostylous morph develop in a manner similar to those of their respective, homostylous, herkogamous relatives. Despite similarities in gross floral developmental patterns, the developmental patterns of the stylar epidermal cells differ among the heterostylous species, and between heterostylous and homostylous species. In heterostylous species of *Lithospermum*, the style of the long-style morph is composed of fewer, longer cells than that of the short-style morph; therefore, differences in the patterns of cell elongation, and possibly in cell division, contribute to the morph-specific style lengths. In contrast, the corolla tubes of each morph are composed of cells that are of approximately the same length, so differences between the heights of anthers in the flowers of each morph are attributable to differences in the number of cell divisions in various regions of the corolla tubes.

## Introduction

The structure of flowers influences the evolutionary development and pollination biology of species of plants. Endress (1994) described three different levels of floral structure: bauplan, gestalt, and mode. Of these, floral bauplan is the most genetically-ingrained pattern of development. Bauplan involves the overall organization of the entire flower. Floral mode, on the other hand, is the most evolutionarily-labile level of structure. Mode concerns superficial floral adaptations. In the microevolution of flowers, mode is the most frequently altered of these three levels of structure. Modifications to mode often involve minor changes to flowers, such as in corolla color, corolla size, and degree of herkogamy – the spatial separation between anthers and stigmas (Webb and Lloyd, 1986). Different types of herkogamy, which range from approach herkogamy, with stigmas positioned above the anthers, to reverse herkogamy, with the stigmas situated below the anthers (Webb and Lloyd, 1986), have been observed among species of a genus or those of closely related genera (Kelly, 1997; Armbruster et al., 2002; Klooster and Culley, 2009; Chapter 3, 4). Changes in the type of herkogamy a species exhibits can affect its breeding system and pollination biology (Armbruster et al., 2002; Tripp and Manos, 2008; Chapter 4).

Most species of angiosperms bear flowers that produce only one type of herkogamy, and approach herkogamy is the most common (Webb and Lloyd, 1986). A limited number of species exhibit stylar polymorphisms, syndromes that involve the occurrence of individual plants in a population that develop flowers that are fixed for one of two or three different types of herkogamy (Barrett et al., 2000). The most common stylar polymorphism is heterostyly (Barrett et al., 2000), which is defined as the occurrence in a species of two or more floral morphs that exhibit reciprocal herkogamy (Barrett, 1992; Barrett et al., 2000). In addition, heterostylous species often exhibit heteromorphic self-incompatibility, and the presence of di- or trimorphic micromorphological characters (Darwin, 1877; Barrett, 1992; Barrett et al., 2000; Barrett and Shore, 2009). This breeding system is known to occur in 28 families of angiosperms (Barrett and Shore, 2009), and in some families, such as Pontederiaceae (Kohn et al., 1996), Lythraceae (Morris, 2007), Linaceae (McDill et al., 2009), and Boraginaceae (Thomas et al., 2008; Ferrero et al., 2009; Cohen, 2010; Chapter 4), heterostyly has originated multiple times, sometimes among close relatives.

*Lithospermum* L. (Boraginaceae) includes species that variously exhibit approach herkogamy, reverse herkogamy, reciprocal herkogamy, non-herkogamy, or multiple types of herkogamy. Of the ca. 60 species in the genus, eight are heterostylous. The data presented in Chapter 4 provide evidence that, among the species of the genus, herkogamy is an evolutionarily-labile character. Phylogenetic analyses of *Lithospermum* and relatives have resolved multiple origins of heterostyly within the genus (Chapter 3, 4). Although numerous studies (e.g., Kohn et al, 1996; Morris, 2007; McDill et al., 2009; Thomas et al., 2008; Ferrero et al., 2009; Chapter 4) have investigated the evolution and phylogenetics of heterostyly, only a limited number (e.g., Riveros et al., 1987; Richards and Barrett, 1992; Richards and Koptur, 1993; Faivre, 2000) have examined the floral development of heterostylous species. The goals of the present study are 1) to investigate floral developmental patterns of heterostylous and related homostylous species of *Lithospermum*, and 2) to use these patterns of development to inform our understanding of the evolution of heterostyly.

Floral developmental studies have provided evidence for the evolution of a variety of aspects of floral architecture (Hufford, 1988; Faivre, 2000; Bissell and Diggle, 2008; Kirchoff et al., 2009). For example, Faivre (2000) has suggested changes that may have occurred during the evolution of heterostylous flowers from a homostylous ancestor. In the context of a phylogeny, floral developmental data can help provide a critical understanding of the evolutionary development of heterostyly.

## Materials and Methods

### MATERIAL

The flowers of *Lithospermum* have five sepals, a sympetalous corolla with five lobes, five stamens, with the filaments adnate to the corolla tube, and a gynobasic style that terminates in two stigmas. Some species of *Lithospermum* develop long filaments (>10 mm in length) (Cohen and Davis, 2009; Chapter 3, 4), but the species included in the present study bear filaments of only negligible length (< 1 mm). Consequently, the point of attachment of each filament to the corolla tube is the principle determinant of the height at which each anther is borne, relative to the base of the corolla. Because the style is gynobasic, the height of the stigma is determined entirely by the length of the style.

Six species of *Lithospermum* were examined for the present study. Three are heterostylous, and three are homostylous. The heterostylous species are *L. multiflorum* Torr. ex A. Gray, *L. cobrense* Greene, and *L. canescens* Lehm. The homostylous species are *L. calcicola* B. L. Rob. (approach herkogamous [AH]), *L. latifolium* Michx. (reverse herkogamous [RH]), and *L. distichum* Ortega (non-herkogamous [NH], with the stigma positioned at the same height as the anthers). Each of the heterostylous species represents a putative independent origin of the breeding system, and at least six nodes separate each of these heterostylous species from the others (Chapter 3, 4). The three heterostylous species bear corollas of approximately equal length (ca. 10 mm long). *Lithospermum multiflorum* produces corollas that are funnelform in shape, while *L. canescens* and *L. cobrense* develop corollas that are salverform-funnelform (Chapter 4). The three homostylous species produce salverform corollas (Chapter 4), and these corollas tend to be shorter (ca. < 8 mm long) than those of the heterostylous species.

Plant material was collected from wild populations in the United States and Mexico, and fixed in 70% EtOH, 70% isopropanol, or FAA. After one to seven days, the fixed material was transferred to, and preserved in, either 70% EtOH or a mixture of 70% EtOH and 10% glycerol. Herbarium vouchers of all material were deposited in the L. H. Bailey Hortorium Herbarium (BH).

### MICROSCOPY

Plant material was examined with the use of both light (LM) and scanning electron microscopy (SEM). For observations of gross floral structure, flowers at various stages of development were dissected and then observed with a Nikon SMZ 1500 light microscope. Images of dissected flowers were captured with a Nikon DMX 1200f digital camera, and viewed with Act-1 software.

Flowers at various stages of development were sectioned, and then these sections were viewed with a compound microscope. To prepare material for anatomical studies, samples were dehydrated and infiltrated. Afterwards, the material was embedded in paraffin. The blocks of paraffin were sectioned with a rotary microtome, and the sections were mounted on slides with Haupt’s adhesive. All processes, from dehydration and infiltration to mounting sections, involved the use of standard practices (e.g., Ruzin, 1999). Slides were stained using 0.5% AstraBlue and 0.2% Safranin, and the stained material was then mounted in resin. Sections were viewed with a Zeiss Axiophot compound microscope, and photographs were obtained with a Nikon DMX 1200c digital camera. The images were displayed with Act-1c software.

For SEM observations, material was dehydrated using a graded ethanol series, with multiple changes in 100% EtOH. Using a Denton Vacuum DCP-1 or a Baltec 1010 CPD, the dehydrated material was dried beyond the vapor-liquid critical point.

The dried material was mounted on stubs, and coated with either gold or palladium using a Hummer 6.2 sputter coater, a Hummer II sputter coater, or a Technics Au-Pd coater. After preparation, material was examined at 10 kV with either a JEOL model 5410-LV or an Akashi ISI DS-130C. Digital images were captured, and then viewed with Orion software (JEOL USA, Inc.).

### MEASUREMENTS AND ANALYSIS

Using the captured LM and SEM digital images, the heights and lengths of various floral organs were measured. For LM images, corolla length, stigma height, and anther height were measured (Figure 5.1). Measurements were graphed on scatter plots in Microsoft Excel. Best-fit lines were generated with Excel.

For SEM images, the lengths of stylar epidermal cells, corolla tube epidermal cells above the filaments, and corolla tube epidermal cells below the filaments were measured. At various developmental stages, the lengths of at least seven cells were measured for each of these organs or portions of an organ. For each flower, the mean and standard deviation were calculated for the lengths of the cells of each organ or each portion of an organ.

The lengths of the mature epidermal cells of each organ or each portion of an organ were investigated. Within flowers, the lengths of the corolla tube epidermal cells above the filaments were compared to those below the filaments. Cell lengths of three groups of cells – corolla tube epidermal cells above the filaments, corolla tube epidermal cells below the filaments, and stylar epidermal cells – were compared between the two morphs of each heterostylous species. The cell length data were analyzed using the Wilcoxon/Kruskal-Wallis two-sample test, as implemented in JMP version 8.0.1 (SAS Institute).

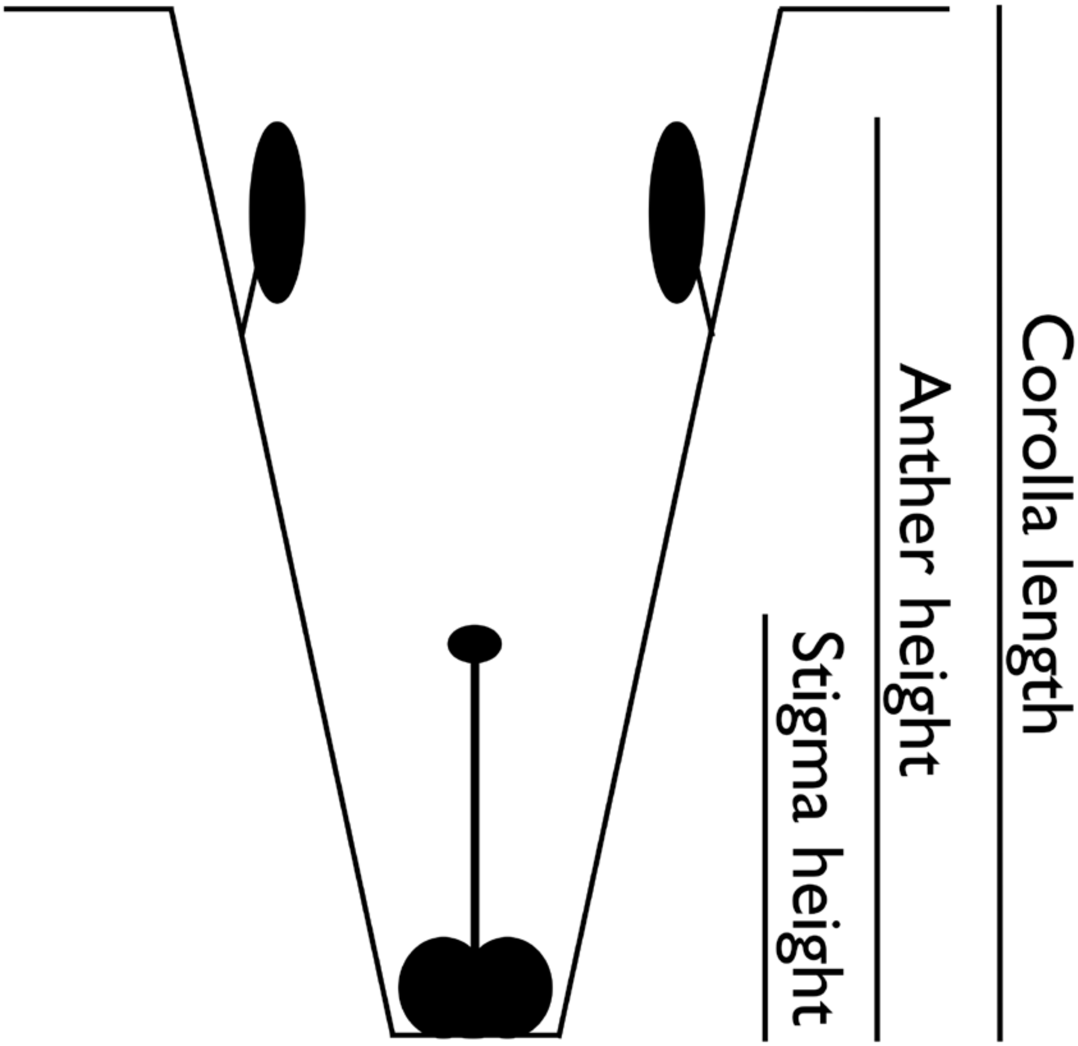


Figure 5.1. Characteristics measured in flowers of *Lithospermum.*

In order to determine the number of cells that would reach from the base to the apex of an organ when laid end-to-end, the mean length of the epidermal cells of the organ at maturity was divided by the length of the same organ at maturity.

The developmental patterns for stigma height, anther height, stylar epidermal cell length, corolla tube epidermal cell length below filaments, and corolla tube epidermal cell length above filaments were examined with JMP. A least squares line was fit to each group of data for each species or morph, and the developmental patterns were compared. The interconnection between the length of the corolla and the heights of other organs or the lengths of epidermal cells was accounted for with the use of the "cross" function in the fit model menu.

For analyses of the developmental patterns of the epidermal cells, in JMP (but not in Excel), both cell length and corolla length data were log-transformed in order to satisfy the homoscedasticty assumption of regression analysis (Eberly, 2007).

For some tests, it was necessary to account for the lack of independence of some observations (e.g., organ heights or cell lengths from the same flower); therefore, measurements from each flower were assigned a flower-specific identifier. This flower identifier was incorporated into the model, and assigned the attribute "random effect". The organs, species, and morphs that differed significantly from each other were noted, and for developmental patterns, the $R^2$, slope, and intercept were recorded. It should be noted that in JMP, all regression lines were linear, but in Excel, some regression lines were linear, while others were logarithmic.

## Results

### GROSS FLORAL DEVELOPMENTAL PATTERNS

I observed three different floral developmental patterns among species of *Lithospermum*, one each for AH, RH, and NH flowers.

***Approach herkogamous flowers* –** Developmental patterns of AH flowers are shown in Figures 5.2A, C, E and 5.3A. In the early developmental stages of these flowers the anthers are positioned above the stigmas. During development, the corolla tube and the style elongate at different rates, with the style elongating faster than the corolla tube. The position of the stigma surpasses that of the anthers when the corollas are

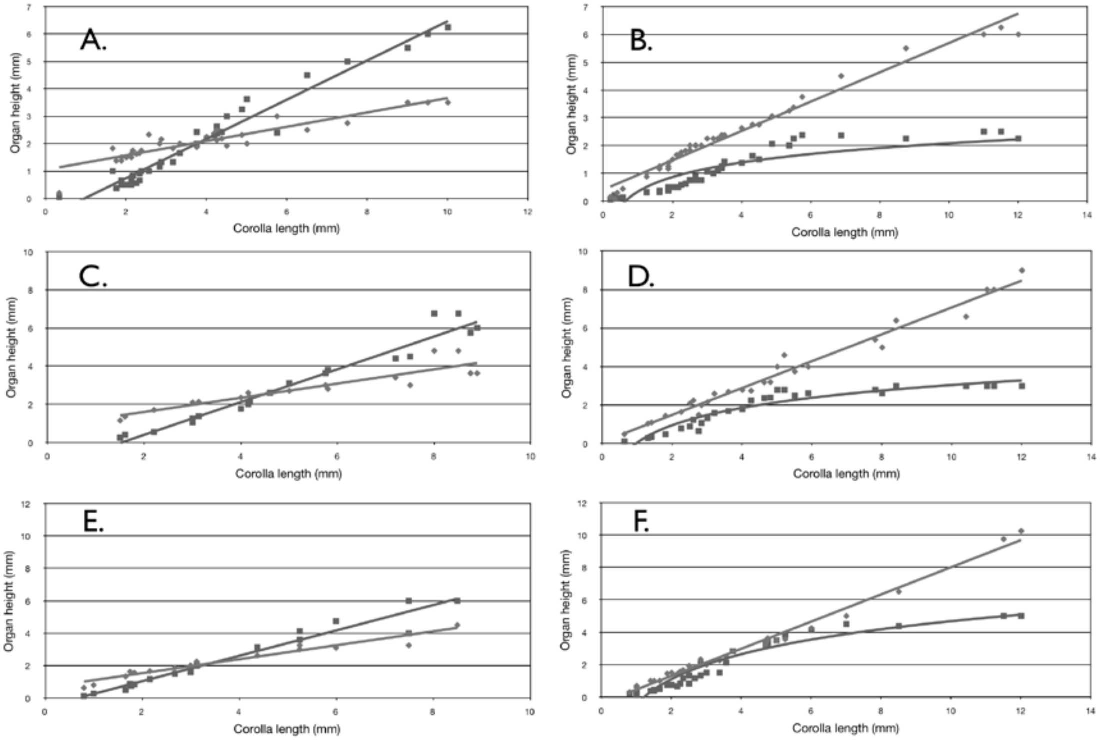


Figure 5.2. Length of corolla vs. heights of stigmas (squares) and anthers (diamonds) during development of three heterostylous species of *Lithospermum*. A. Long-style morph of *L. canescen*s. B. Short-style morph of *L. canescens*. C. Long-style morph of *L. cobrense*. D. Short-style morph of *L. cobrense.* E. Long-style morph of *L. multiflorum*. F. Short-style morph of *L. multiflorum*. Regression lines included. Scales differ among graphs.

between 2.5 and 4 mm in length, with the exact corolla length being species specific. At anthesis, the distance between the stigmas and the anthers ranges from 0.75 to 2.5 mm. In all of examined species with AH flowers, the growth rate of the style differs significantly from that of the corolla tube (Table 5.1, see Table 5.2 for the approximate rates). The rates of stylar elongation are statistically significant only between *L. cobrense* and *L. canescens,* and between *L. cobrense* and *L. calcicola* (Table 5.3).

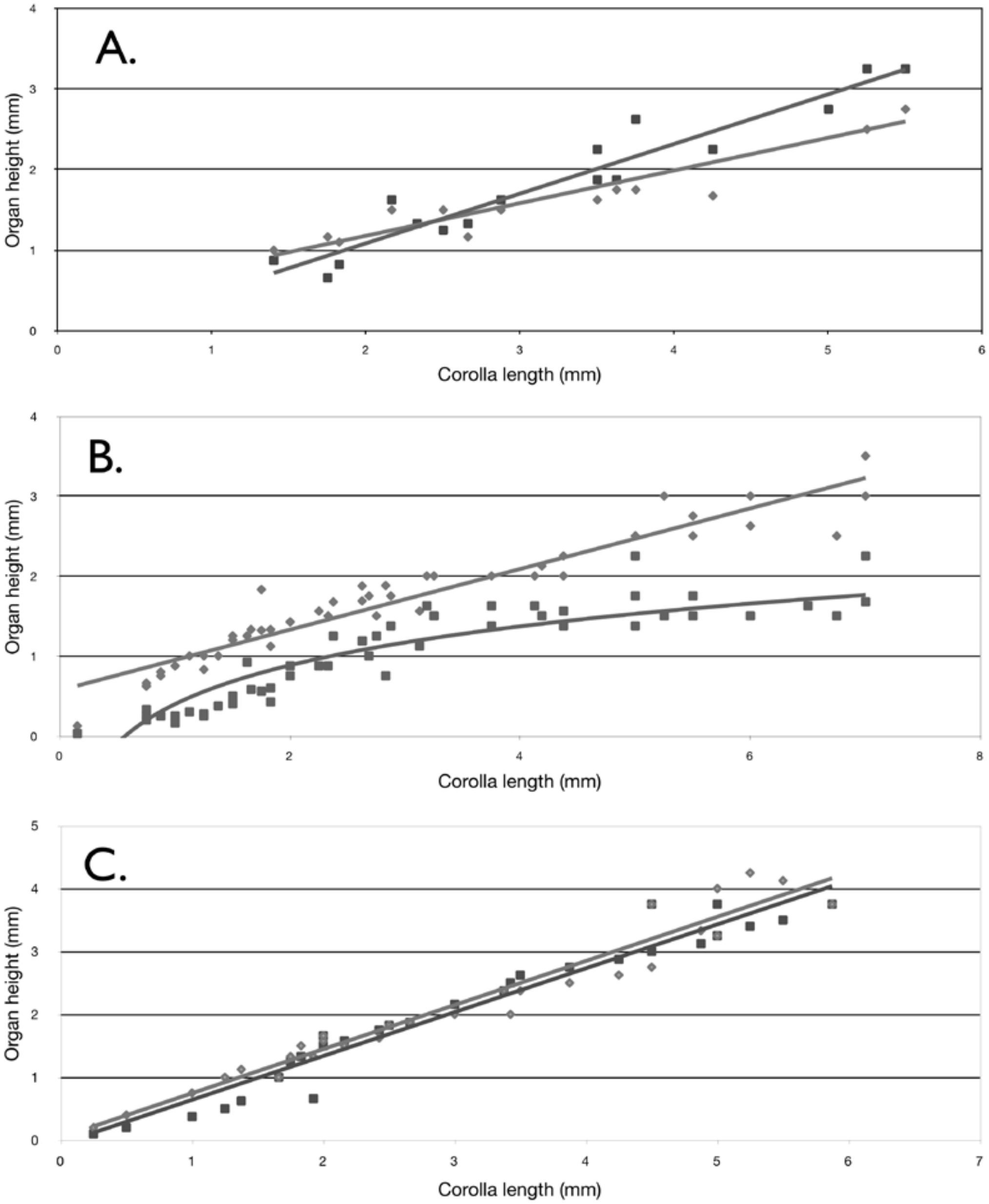


Figure 5.3. Length of corolla vs. heights of stigmas (squares) and anthers (diamonds) during development of three homostylous species of *Lithospermum*. A. Approach herkogamous *L. calcicola*. B. Reverse herkogamous *L. latifolium*. C. Non-herkogamous *L. distichum*. Regression lines included. Scales differ among graphs.

In contrast, the rate of anther height development is statistically significant between *L. canescens* and the other three species or morphs with AH flowers (Table 5.3).

***Reverse herkogamous flowers* –** Developmental patterns of RH flowers are shown in Figures 5.2B, D, F, and 5.3B. In these flowers, the corolla tube and the style initially elongate at approximately the same rate. When the corolla reaches approximately half of its final length, stylar growth ceases; however, the corolla tube continues to elongate at its initial rate. In all of examined species with RH flowers, the growth rate of the style is significantly different than that of the corolla tube (Table 5.1). In the mature flowers of the SS morph of each heterostylous species, the stigmas are separated from the anthers by 5 mm, but in the homostylous *L. latifolium*, these two organs are separated by only 2.5 mm.

The rate of stylar elongation is statistically different between *L. multiflorum* and the other three species or morphs with RH flowers. In contrast, the development of anther height among all of the studied species or morphs with RH flowers is statistically significant (Table 5.3).

***Non-herkogamous flowers* –** The present study includes only one species that develops NH flowers, *L. distichum*. Development of NH flowers in this species follows a simple pattern: the style and corolla tube elongate at the same, constant rate throughout development (Figure 5.3C). At each stage of development, the anthers and the stigmas are at the same height. In contrast to AH and RH flowers, the growth rates of the style and corolla tube are not statistically different (Table 5.1).

Table 5.1. Within-flower comparisons between stylar and corolla tube developmental patterns.

| Species or Morph | Comparative growth rates |
|---|---|
| *L. canescens* long-style morph | $p<0.05$ |
| *L. canescens* short-style morph | $p<0.0001$ |
| *L. cobrense* long-style morph | $p<0.0001$ |
| *L. cobrense* short-style morph | $p<0.0001$ |
| *L. multiflorum* long-style morph | $p<0.0001$ |
| *L. multiflorum* short-style morph | $p<0.0001$ |
| *L. calcicola* (approach herkogamous) | $p<0.005$ |
| *L. latifolium* (reverse herkogamous) | $p<0.0001$ |
| *L. distichum* (non-herkogamous) | not sig |

***Heterostylous flowers* –** In each of the three heterostylous species investigated, the rate of stylar development of the long-style (LS) morph differs statistically from that of the short-style (SS) morph. This is also the case for corolla tube development (Table 5.2).

### DEVELOPMENTAL PATTERNS OF EPIDERMAL CELLS

***Corolla tube epidermal cells* –** For both heterostylous and homostylous species, the mean length of epidermal cells of the corolla tube increases in a linear manner, both above and below the filaments (Figures 5.4 – 5.7). Additionally, the range in the length of these cells increases throughout development. The growth rates of the corolla tube epidermal cells, both above and below the filaments, are not statistically different between the morphs of each heterostylous species, with two exceptions

Table 5.2. Results of least-squares regression analyses of lengths of corollas vs stigma and anther heights. Asterisks denote significant differences between morphs. All regression lines are linear.

| Species or morph | Stigma height | | | Anther height | | |
|---|---|---|---|---|---|---|
| | $R^2$ | Slope | Intercept | $R^2$ | Slope | Intercept |
| *L. canescens* long-style morph | 0.96 | 0.71*** | -0.69*** | 0.83 | 0.26*** | 1.05*** |
| *L. canescens* short-style morph | 0.81 | 0.24*** | 0.19*** | 0.98 | 0.53*** | 0.41*** |
| *L. multiflorum* long-style morph | 0.95 | 0.78* | -0.53** | 0.95 | 0.43*** | 0.67*** |
| *L. multiflorum* short-style morph | 0.85 | 0.52* | 0.02** | 0.99 | 0.84*** | -0.37*** |
| *L. cobrense* long-style morph | 0.96 | 0.86*** | -1.32*** | 0.85 | 0.37*** | 0.85*** |
| *L. cobrense* short-style morph | 0.75 | 0.26*** | 0.54*** | 0.98 | 0.7*** | 0.11*** |
| *L. calcicola* (approach herkogamous) | 0.92 | 0.62 | -0.14 | 0.88 | 0.41 | 0.37 |
| *L. latifolium* (reverse herkogamous) | 0.80 | 0.28 | 0.15 | 0.92 | 0.38 | 0.58 |
| *L. distichum* (non-herkogamous) | 0.95 | 0.70 | -0.06 | 0.94 | 0.70 | 0.04 |

p<.0001 ***; p<.0005 **; p<.05 *

Table 5.3. Comparison of growth rates of corollas vs. stigma and anther heights for species or morphs that bear either approach or reverse herkogamous flowers. All regression lines are linear. LS – long-style morph. SS – short-style morph.

| Species or morph | Stigma height | Anther height |
|---|---|---|
| Approach herkogamous | | |
| *L. canescens* LS × *L. multiflorum* LS | not sig | p<0.0001 |
| *L. canescens* LS × *L. cobrense* LS | p<0.005 | p<0.01 |
| *L. canescens* LS × *L. calcicola* | not sig | p<0.05 |
| *L. multiflorum* LS × *L. cobrense* LS | not sig | not sig |
| *L. multiflorum* LS × *L. calcicola* | not sig | not sig |
| *L. cobrense* LS × *L. calcicola* | p<0.01 | not sig |
| Reverse herkgamous | | |
| *L. canescens* SS × *L. multiflorum* SS | p<0.0001 | p<0.0001 |
| *L. canescens* SS × *L. cobrense* SS | not sig | p<0.0001 |
| *L. canescens* SS × *L. latifolium* | not sig | p<0.0001 |
| *L. multiflorum* SS × *L. cobrense* SS | p<0.0001 | p<0.0001 |
| *L. multiflorum* SS × *L. latifolium* | p<0.0001 | p<0.0001 |
| *L. cobrense* SS × *L. latifolium* | not sig | p<0.0001 |

(Table 5.4). One exception is that between the two morphs of *L. canescens*, the growth rates of the corolla tube epidermal cells above the filaments are statistically different. The other exception is that between the two morphs of *L. cobrense*, the intercepts of the developmental patterns of the corolla tube epidermal cells below the filaments differ significantly. Although different growth rates of the corolla tube

Figure 5.4. Length of corolla vs. lengths of corolla tube epidermal cells in *Lithospermum canescens*. A. Long-style morph below filaments. B. Long-style morph above filaments. C. Short-style morph below filaments. D. Short-style morph above filaments.

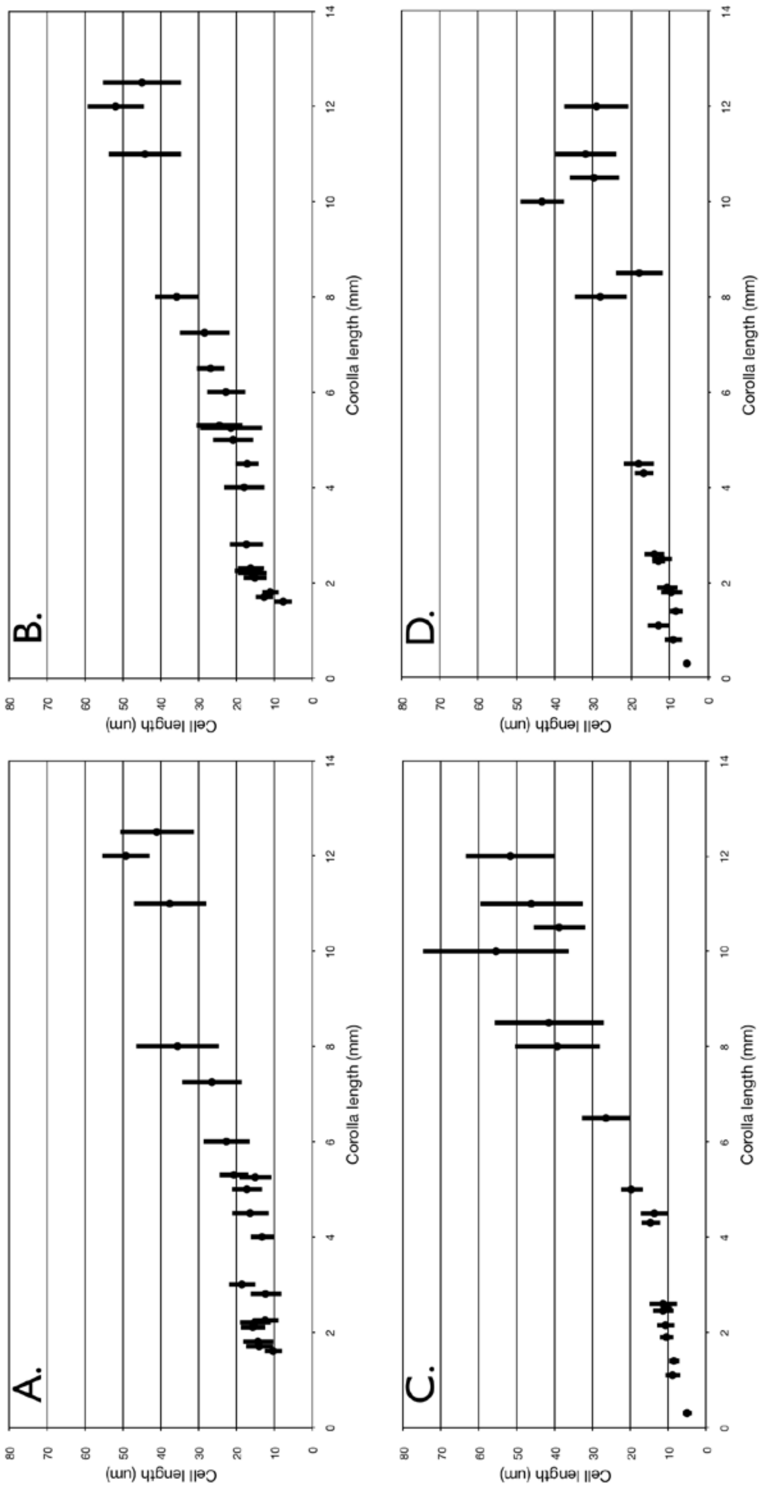

A.
B.
C.
D.
Cell length (um)
Corolla length (mm)
0
10
20
30
40
50
60
70
80
0
2
4
6
8
10
12
14

Figure 5.5. Length of corolla vs. lengths of corolla tube epidermal cells in *Lithospermum cobrense*. A. Long-style morph below filaments. B. Long-style morph above filaments. C. Short-style morph below filaments. D. Short-style morph above filaments.

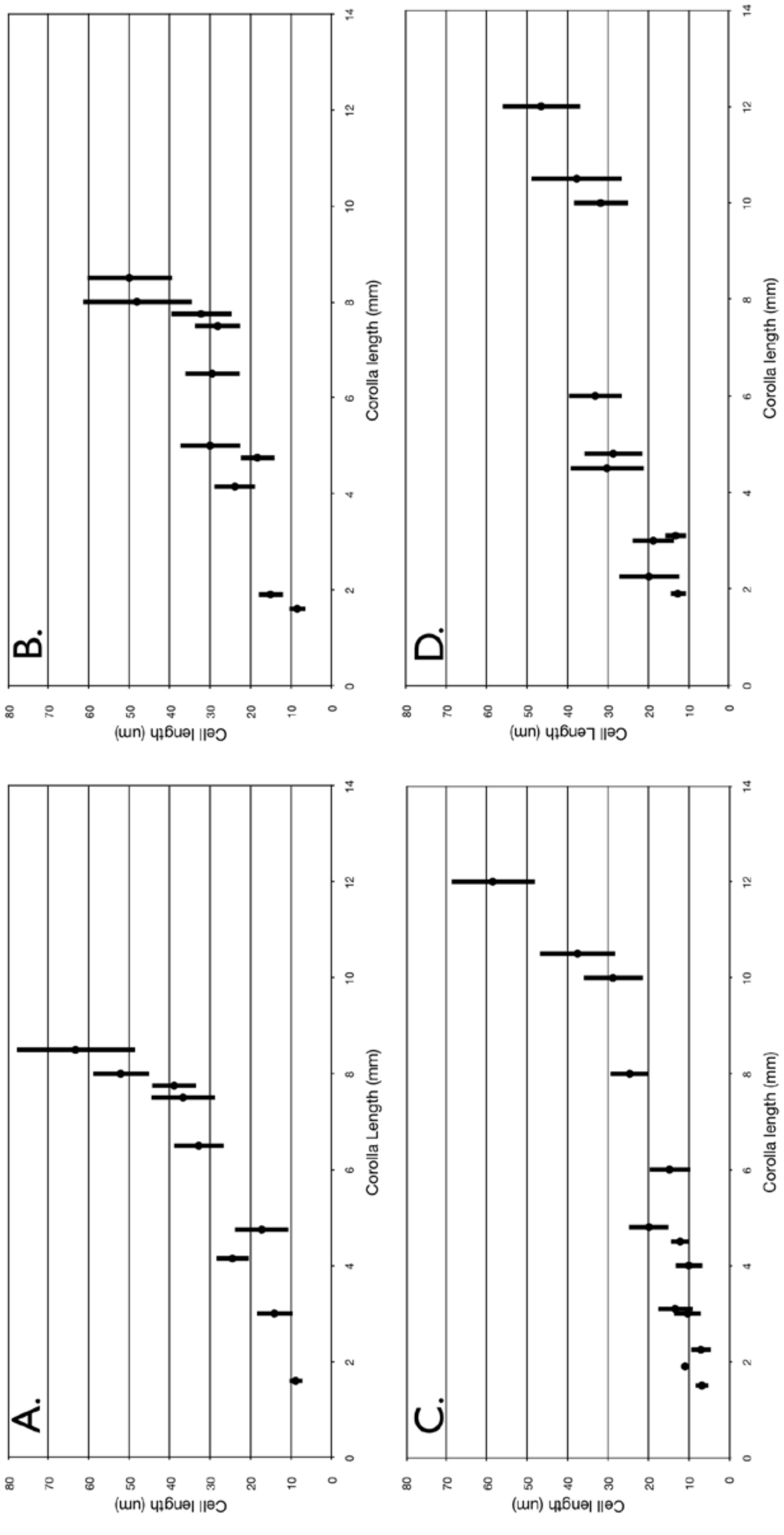
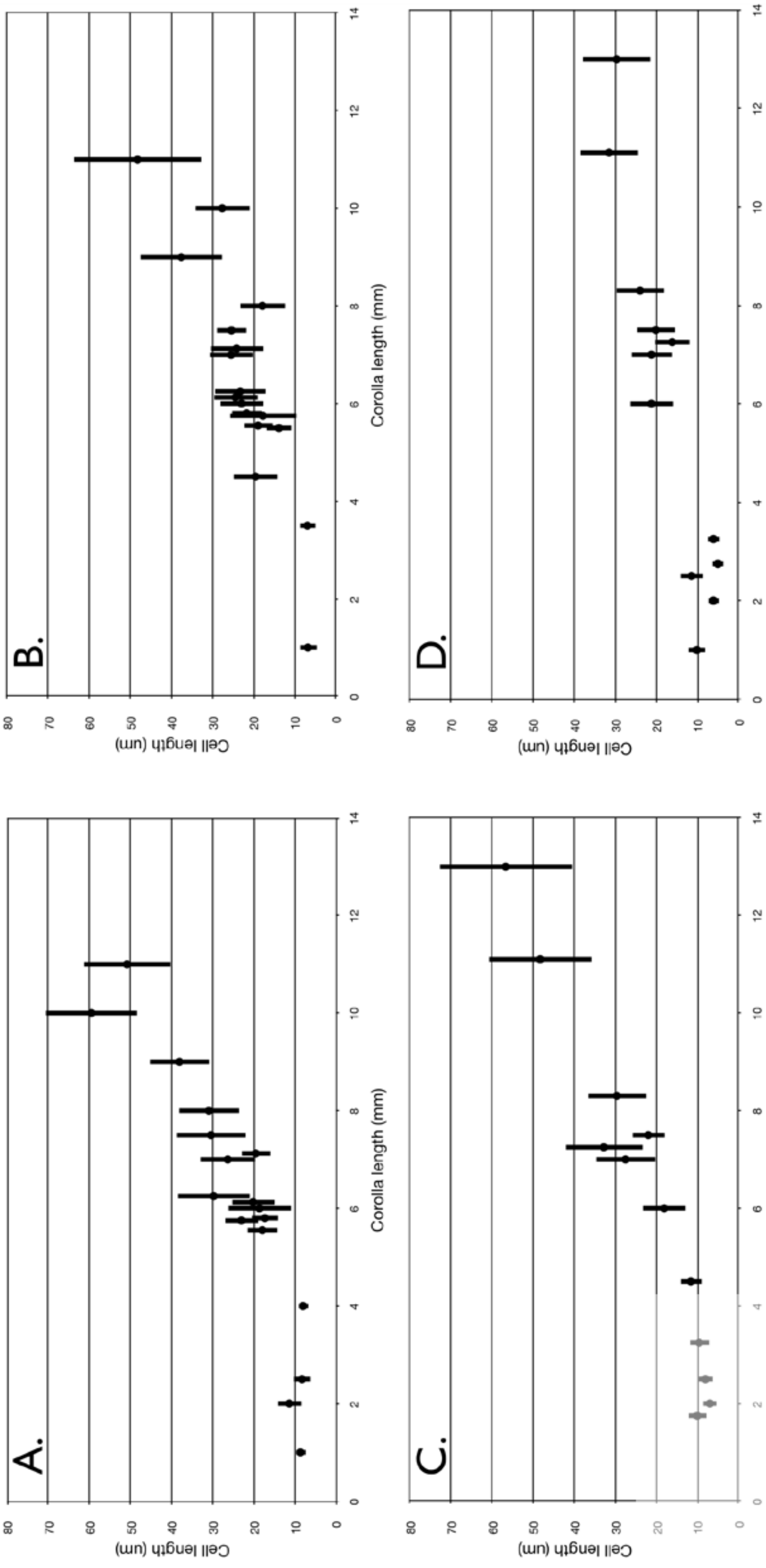

A.
B.
C.
D.
Cell length (um)
Cell length (um)
Cell length (um)
Cell Length (um)
Corolla Length (mm)
Corolla length (mm)
Corolla length (mm)
Corolla length (mm)
0
10
20
30
40
50
60
70
80
0
2
4
6
8
10
12
14

Figure 5.6. Length of corolla vs. lengths of corolla tube epidermal cells in *Lithospermum multiflorum*. A. Long-style morph below filaments. B. Long-style morph above filaments. C. Short-style morph below filaments. D. Short-style morph above filaments.


A.
B.
C.
D.
Cell length (um)
Corolla length (mm)
0
10
20
30
40
50
60
70
80
0
2
4
6
8
10
12
14

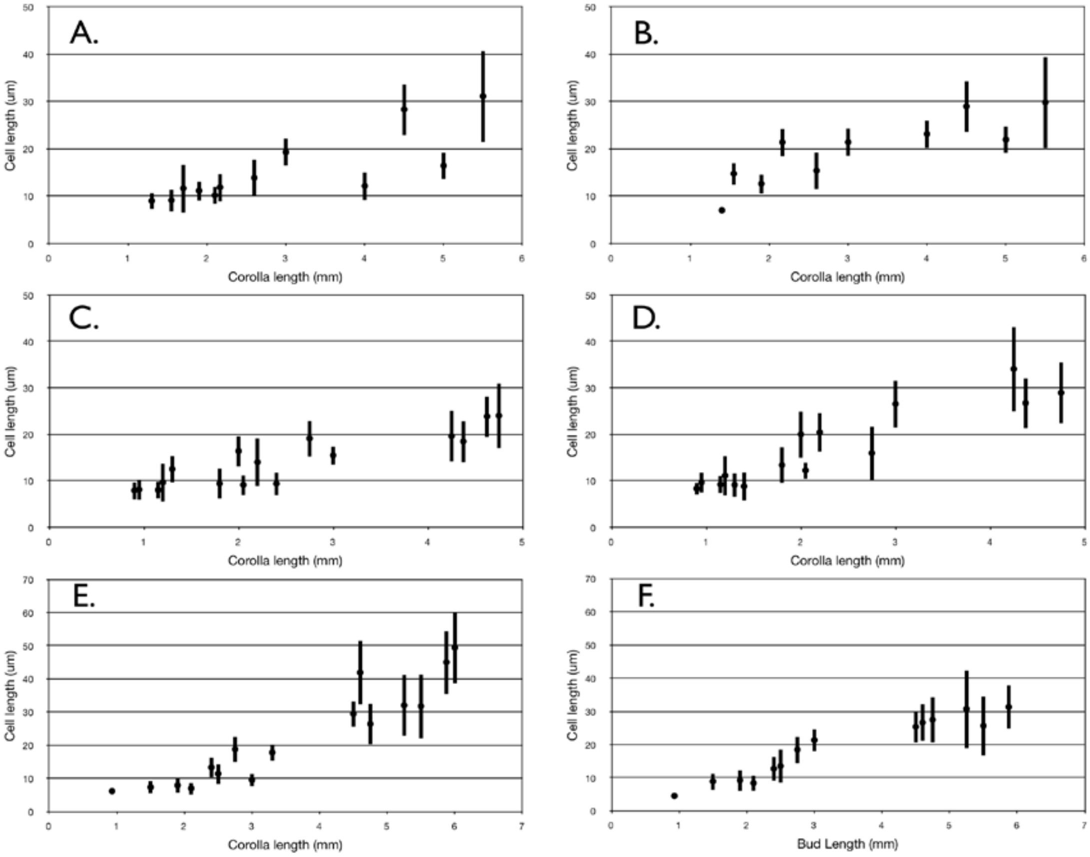


Figure 5.7. Length of corolla vs. lengths of corolla tube epidermal cells in three homostylous species of *Lithospermum*. A. Approach herkogamous *L. calcicola* below filaments. B. Approach herkogamous *L. calcicola* above filaments. C. Reverse herkogamous *L. latifolium* below filaments. D. Reverse herkogamous *L. latifolium* above filaments. E. Non-herkogamous *L. distichum* below filaments. F. Non-herkogamous *L. distichum* above filaments. Scales differ among graphs.

epidermal cells, both above and below the filaments, are uncommon between the two morphs, intrafloral differences are observed more frequently. In all species or morphs with RH flowers, the developmental patterns of the corolla tube epidermal cells above the filaments differ significantly from those of the corolla tube epidermal cells below the filaments; however, this pattern is not observed for any of the species or morphs with AH flowers (Table 5.5).

At anthesis, the lengths of the corolla tube epidermal cells can differ within or among flowers of heterostylous species. Within mature RH flowers of species of *Lithospermum*, the lengths of corolla tube epidermal cells above the filaments differ statistically from the lengths of those below the filaments (Table 5.5). These differences are not observed in species with AH flowers. The corolla tube epidermal cells below the filaments do not differ between heterostylous morphs. In contrast, the corolla tube epidermal cells above the filaments differ significantly between the morphs of *L. canescens*, and between those of *L. multiflorum* (Table. 5.6). Although these differences are significant, the difference between the lengths of corolla tube epidermal cells above and below the filaments, both within flowers and between morphs, is less than 15 μm.

In the SS morph of all heterostylous species, a greater number of corolla epidermal cells are situated below the point of filament attachment than above it, and this pattern is reversed in the LS morph. These observations on the corollas of heterostylous species differ from those of homostylous species. In homostylous species, the number of cells above and below the point of filament attachment is approximately equal (Table 5.7).

The total number of corolla tube epidermal cells (above the filaments and below the filaments) that would reach from the base of the corolla tube to its apex, if laid end-to-end, is greater in the SS morph of *L. multiflorum* and *L. cobrense* than in the LS morph of these species. In contrast, the corolla tubes of each morph of *L. canescens* are composed of approximately the same number of epidermal cells (Table 5.7).

Table 5.4. Results of least-squares regression analyses of length of corollas vs. stylar epidermal cell length, corolla tube epidermal cell length above filaments, and corolla tube epidermal cell length below filaments. All raw data were log-transformed. Asterisks denote significant differences between morphs. All regression lines are linear.

| Species or morph | Stylar epidermal cell length | | | Corolla tube epidermal cell length above filaments | | | Corolla tube epidermal cell length below filaments | | |
|---|---|---|---|---|---|---|---|---|---|
| | $R^2$ | Slope | Intercept | $R^2$ | Slope | Intercept | $R^2$ | Slope | Intercept |
| *L. canescens* long-style morph | 0.88 | 1.13*** | 1.74*** | 0.80 | 0.69* | 2.02 | 0.76 | 0.60 | 2.06 |
| *L. canescens* short-style morph | 0.86 | 0.57*** | 1.96*** | 0.84 | 0.49* | 2.16 | 0.89 | 0.71 | 1.95 |
| *L. multiflorum* long-style morph | 0.91 | 1.41*** | 1.47*** | 0.78 | 0.80 | 1.57 | 0.84 | 0.82 | 1.64 |
| *L. multiflorum* short-style morph | 0.86 | 0.87*** | 1.72*** | 0.88 | 0.67 | 1.59 | 0.88 | 1.01 | 1.25 |
| *L. cobrense* long-style morph | 0.87 | 1.32*** | 1.61 | 0.83 | 0.83 | 1.88 | 0.86 | 1.06 | 1.51** |
| *L. cobrense* short-style morph | 0.83 | 0.9*** | 1.56 | 0.73 | 0.60 | 2.25 | 0.87 | 0.89 | 1.39** |
| *L. calcicola* (approach herkogamous) | 0.74 | 0.95 | 2.18 | 0.75 | 0.77 | 2.07 | 0.74 | 0.72 | 1.92 |
| *L. latifolium* (reverse herkogamous) | 0.79 | 0.88 | 1.83 | 0.83 | 0.93 | 2.08 | 0.70 | 0.62 | 2.06 |
| *L. distichum* (non-herkogamous) | 0.79 | 0.80 | 2.49 | 0.83 | 1.05 | 1.61 | 0.91 | 1.24 | 1.43 |

*** $p<0.0001$, ** $p<0.0005$, * $p<0.05$

Table 5.5. Within-species or morph comparisons of growth rates and mature cell lengths between corolla tube epidermal cell lengths above filaments and those below filaments. All raw data were log-transformed. All regression lines are linear.

| Species or morph | Comparative growth rates of epidermal cells above and below filaments | Differences in mature epidermal cell lengths above and below filaments |
|---|---|---|
| *L. canescens* long-style morph | not sig | not sig |
| *L. canescens* short-style morph | $p<0.0001$ | $p<0.05$ |
| *L. cobrense* long-style morph | not sig | not sig |
| *L. cobrense* short-style morph | $p<0.0001$ | $p<0.05$ |
| *L. multiflorum* long-style morph | not sig | not sig |
| *L. multiflorum* short-style morph | $p<0.0001$ | $p<0.0001$ |
| *L. calcicola* (approach herkogamous) | not sig | not sig |
| *L. latifolium* (reverse-herkogamous) | $p<0.0001$ | $P<0.05$ |
| *L. distichum* (non-herkogamous) | $p<0.005$ | not sig |

***Stylar epidermal cells*** **–** The LS morph of each heterostylous species follows a different pattern of stylar epidermal cell development. In the LS morph of *L. cobrense*, the mean lengths of the stylar epidermal cells undergo exponential increase later in development (Figure 5. 8C). The mean lengths of the stylar epidermal cells in the LS morph of *L. canescens* resemble a logarithmic pattern of development, with a decreasing rate of elongation as the flowers mature (Figure 5.8A). Those of *L. multiflorum* follow a linear pattern of growth (Figure 5.8E). In contrast to the developmental patterns of the stylar epidermal cells in the LS morphs of each

Table 5.6. Lengths (mean ± standard deviation) of mature epidermal cells of various organs or portions of organs. All measurements are in µm. Asterisks denote significant differences between morphs. LS – long-style morph. SS – short-style morph. AH – approach herkogamous. RH – reverse herkogamous, NH – non-herkogamous.

| Species or morph | Corolla tube epidermal cells below filaments | Corolla tube epidermal cells above filaments | Stylar epidermal cells |
|---|---|---|---|
| *L. canescens* LS | 49.9 ± 6.3 | 51.8 ± 7.5* | 96.4 ± 23.2**** |
| *L. canescens* SS | 55.5 ± 19.3 | 43.3 ± 5.8* | 28.6 ± 7.9**** |
| *L. cobrense* LS | 63.3 ± 14.7 | 50 ± 10.6 | 147.1 ± 24.3*** |
| *L. cobrense* SS | 58.6 ± 10.5 | 46.6 ± 9.7 | 34.3 ± 10.6*** |
| *L. multiflorum* LS | 59.5 ± 11.1 | 48.2 ± 15.6** | 110 ± 36.7**** |
| *L. multiflorum* SS | 56.7 ± 16.1 | 29.6 ± 8.2** | 40.9 ± 11.7**** |
| *L. calcicola* (AH) | 31.1 ± 9.6 | 29.8 ± 6.9 | 52.2 ± 10.3 |
| *L. latifolium* (RH) | 24 ± 7 | 28.9 ± 6.6 | 26.6 ± 6.7 |
| *L. distichum* (NH) | 49.4 ± 10.9 | 31.3 ± 6.5 | 58.2 ± 15 |

* $p<0.05$, ** $p<0.01$, *** $p<0.0005$, **** $p<0.0001$

heterostylous species, those of the SS morph resemble a pattern of logarithmic growth (Figure 5.8 B, D, F). After the corolla of the SS morph has grown to approximately 40% of its mature length, the stylar epidermal cells of this morph essentially cease to elongate. The growth rates of the stylar epidermal cells between morphs of the same species are significantly different (Table 5.4). Furthermore, the development of the stylar epidermal cells of the SS morph statistically differs from that of the other three species with reverse herkogamous flowers (Table 5.8). The stylar epidermal cells of

Table 5.7. Approximate number of epidermal cells that reach from base to apex of organ or zone of organ, if laid end-to-end. See text for formula. LS – long-style morph. SS – short-style morph. AH – approach herkogamous. RH – reverse herkogamous. NH – non-herkogamous.

| Species or morph | Number of cells below filaments | Number of cells above filaments | Number of cells in style |
|---|---|---|---|
| *L. canescens* LS | 52 | 100 | 63 |
| *L. canescens* SS | 90 | 47 | 77 |
| *L. cobrense* LS | 32 | 109 | 37 |
| *L. cobrense* SS | 102 | 77 | 75 |
| *L. multiflorum* LS | 50 | 117 | 50 |
| *L. multiflorum* SS | 136 | 99 | 90 |
| *L. calcicola* (AH) | 65 | 67 | 58 |
| *L. latiflolium* (RH) | 64 | 69 | 46 |
| *L. distichum* (NH) | 58 | 41 | 59 |

*L. latifolium* and *L. distichum* resemble a linear pattern of growth, while those of *L. calcicola* follow a logarithmic pattern of development (Figure 5.9).

In all three heterostylous species, the mature lengths of the stylar epidermal cells of the LS morph differ significantly from those of the SS morph (Table 5.6). At anthesis, the stylar epidermal cells of the SS morph are 23% to 43% as long as those of the LS morph. In the LS morphs of *L. multiflorum* and of *L. cobrense*, the number of stylar epidermal cells that would reach from the base of the style to its apex, if laid end-to-end, is approximately half as much as that of the SS morph. In *L. canescens*, the LS style is composed of fewer epidermal cells than the SS style, but the difference between these numbers of cells is approximately 20%, not 50% (Table 5.7).

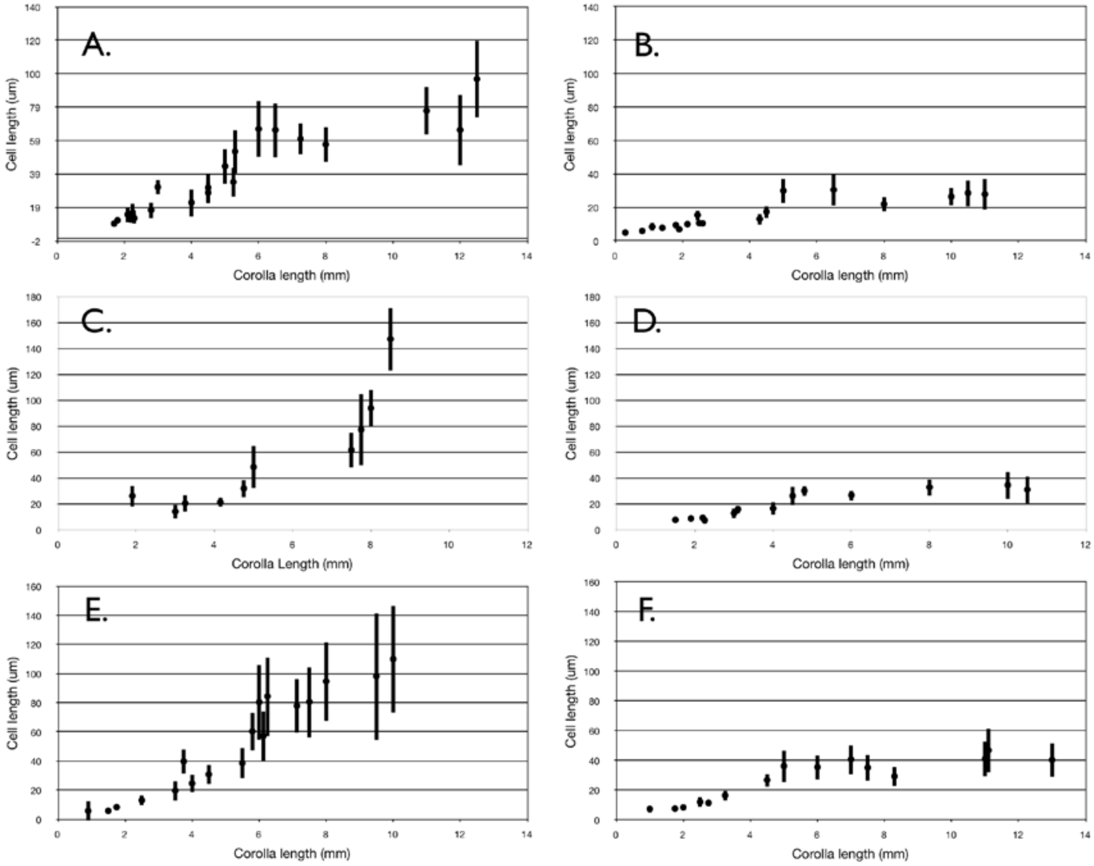


Figure 5.8. Length of corolla vs. lengths of stylar epidermal cells in three heterostylous species of *Lithospermum*. A. Long-style morph of *L. canescens*. B. Short-style morph of *L. canescens*. C. Long-style morph of *L. cobrense*. D. Short-style morph of *L. cobrense.* E. Long-style morph of *L. multiflorum*. F. Short-style morph of *L. multiflorum*. Scales differ among graphs.

The lengths of stylar epidermal cells of the homostylous species are more similar to those of the SS morph than to those of the LS morph (Table 5.7). This is especially the case for *L. latifolium*. At anthesis, the stylar epidermal cells in this species have a mean length of 26.6 ± 6.7 µm. In the other two homostylous species, *L. calcicola* and *L. distichum*, the stylar epidermal cells are approximately 30 µm longer (Table 5.7).

Table 5.8. Comparison of growth rates of stylar epidermal cells, corolla tube epidermal cells above filaments, and corolla tube epidermal cells below filaments. Growth rates are compared for species or morphs that bear either approach or reverse herkogamous flowers. All raw data were log-transformed. All regression lines are linear. LS – long-style morph. SS – short-style morph.

| Species or morph | Stylar epidermal cell length | Corolla tube epidermal cell length above filaments | Corolla tube epidermal cell length below filaments |
|---|---|---|---|
| Approach herkogamous | | | |
| *L. canescens* LS × *L. multiflorum* LS | $p<0.05$ | not sig | not sig |
| *L. canescens* LS × *L. cobrense* LS | not sig | not sig | $p<0.05$ |
| *L. multiflorum* LS × *L. cobrense* LS | not sig | not sig | not sig |
| *L. calcicola* × *L. canescens* LS | not sig | not sig | not sig |
| *L. calcicola* × *L. cobrense* LS | not sig | not sig | not sig |
| *L. calcicola* × *L. multiflorum* LS | $p<.005$ | not sig | not sig |
| Reverse herkogamous | | | |
| *L. canescens* SS × *L. multiflorum* SS | $p<.001$ | not sig | $p<.05$ |
| *L. canescens* SS × *L. cobrense* SS | $p<.01$ | not sig | not sig |
| *L. multiflorum* SS × *L. cobrense* SS | not sig | not sig | not sig |
| *L. latifolium* × *L. canescens* SS | $p<0.05$ | $p<0.0005$ | not sig |
| *L. latifolium* × *L. cobrense* SS | not sig | $p<0.05$ | not sig |
| *L. latifolium* × *L. multiflorum* SS | not sig | not sig | $p<0.05$ |

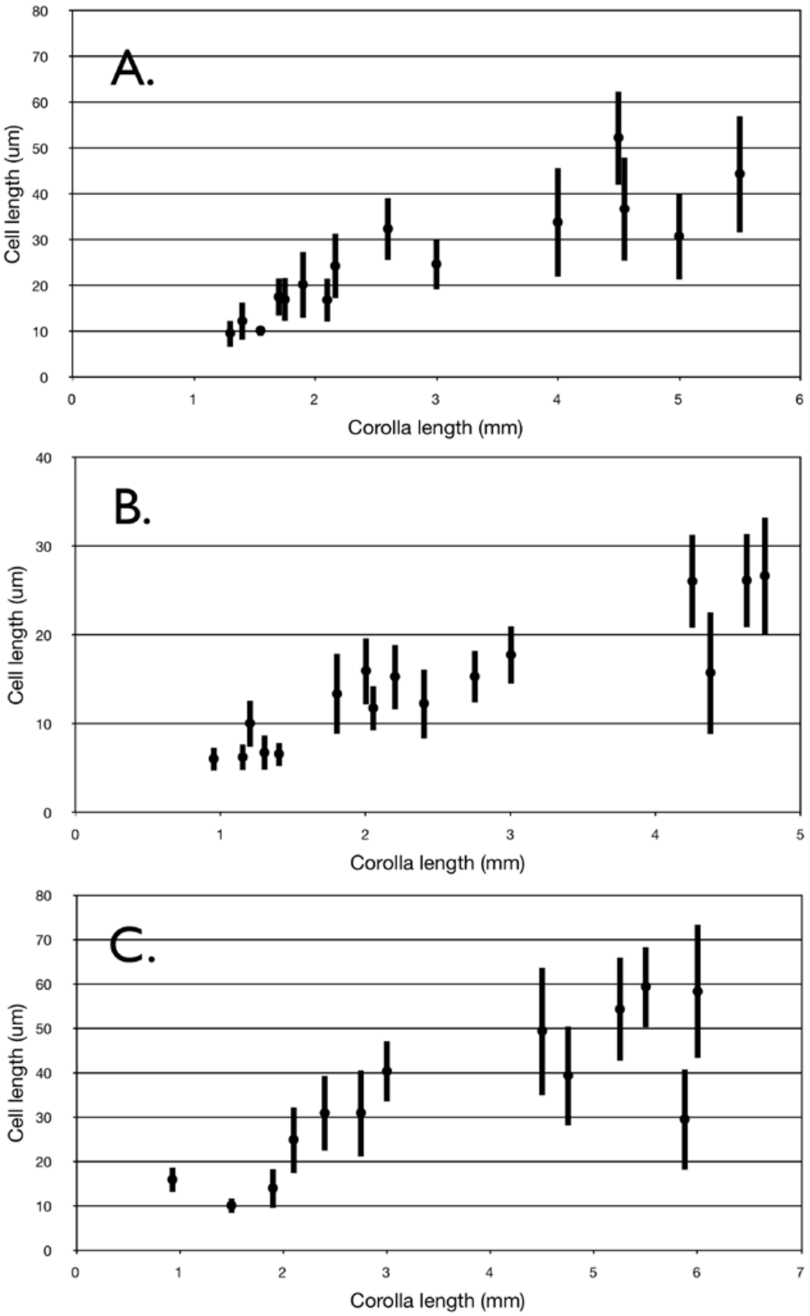


Figure 5.9. Length of corolla vs. lengths of stylar epidermal cells in three homostylous species of *Lithospermum*. A. Approach herkogamous *L. calcicola*. B. Reverse herkogamous *L. latifolium*. C. Non-herkogamous *L. distichum.* Scales differ among graphs.

## Discussion

### FLORAL DEVELOPMENTAL PATTERNS IN *LITHOSPERMUM*

The gross floral developmental patterns of each morph of the three heterostylous species are similar. However, the stylar epidermal cells, which are a proxy for the internal cells of the style, follow different developmental patterns among these species. For example, in the LS morph of each heterostylous species, elongation of the stylar epidermal cells resembles different patterns of development – logarithmic in *L. canescens*, exponential in *L. cobrense*, and linear in *L. multiflorum* (Figure 5.8A, C, E). These species-specific patterns are consistent with the independent origins of heterostyly in each of these species (Chapter 3, 4). With an investigation of only gross floral development, these differences would not have been observed.

In *Lithospermum*, the different lengths of the mature stylar epidermal cells provide additional evidence regarding independent origins of heterostyly. Phylogenetic evidence suggests that *L. multiflorum* and *L. cobrense* each arose from an AH ancestor, but for *L. canescens*, along with *L. caroliniense* MacMill. and *L. californicum* A. Gray (two heterostylous species not included in the present study), the ancestor is resolved as RH (Chapter 4). In *L. multiflorum* and *L. cobrense*, the style of the LS morph has approximately half as many epidermal cells as that of the SS morph. In *L. canescens*, however, the style of the LS morph comprises 20% fewer cells than that of the SS morph (Table 5.7). Given this difference, the origin of heterostyly in *L. canescens* may involve a different evolutionary sequence of events than in either of the two other species included in the present study. This difference may result from the origin of heterostyly by an RH ancestor, rather than one that is AH.

Changes from a homostylous ancestor that have taken place during the origin of the LS and SS morphs can be inferred from the number and lengths of the stylar epidermal cells at anthesis. Homostylous species develop styles that have elements of each heterostylous morph. The number of stylar epidermal cells is similar to that of the LS morph, while the lengths of the mature stylar epidermal cells are similar to those of the SS morph. Therefore, during the origin of heterostyly in *Lithospermum*, only one component of the stylar epidermal cells – either the number or the length – would need to be altered.

Additionally, the developmental patterns of the stylar epidermal cells provide evidence regarding the origin of heterostyly. Although the styles of AH and RH flowers of homostylous species appear to follow the same developmental patterns as those of the LS and SS morphs, respectively, differences are observed in the growth rates of the stylar epidermal cells. In homostylous species, the stylar epidermal cells develop following either a linear or a logarithmic pattern (Figure 5.9), but in heterostylous species, the developmental pattern of these cells is exponential, logarithmic, or linear in the LS morph, and logarithmic in the SS morph (Figure 8). Therefore, all AH and LS flowers do not undergo the same developmental pattern, and neither do all RH and SS flowers. Faivre (2000) came to a similar conclusion in her comparison of AH and LS flowers of species of Rubiaceae. The modification that she proposed involved changes to the overall height of the stigma, rather than specifically to the developmental pattern of the stylar epidermal cells.

Developmental changes that affect the positions of the stigmas and anthers in each morph appear to involve either temporal or spatial modifications of gene expression. In each of the two heterostylous morphs, stylar growth may entail temporal changes in gene expression, but based on the action of the gene, the pattern of expression would differ. If the gene acts to elongate the style, the gene must cease expression in the style of the SS morph, but continue in that of the LS morph. In contrast, if the gene inhibits the elongation of the style, the style of the SS morph must express the gene earlier in development than will that of the LS morph.  This hypothesis can explain the disparity in stylar epidermal cell lengths between the two morphs, but it does not address the different numbers of cells in each morph.

An alternative hypothesis for stylar development in heterostylous species of *Lithospermum* involves two genes, with each gene affecting a different aspect of cellular development.  These genes would act in concert, in order to influence the different style lengths in the LS and SS morphs.  One gene would affect cell length.  In the LS morph, one allele would cause the stylar cells to greatly elongate, but in the SS morph, a different allele would inhibit this action.  The other putative gene would influence cell division.  The action of one allele in the LS morph would result in less cell division, and the action of another allele in the SS morph would result in a greater amount of cell division.  Consequently, in the LS morph, fewer, longer cells would result, while in the SS morph, more, shorter cells would occur.  This two-gene hypothesis is supported by two observations.  The first is that the mature stylar epidermal cells of the LS morph are much longer than those of the SS morph (Table 5.6).  The second observation is that, despite having fewer cells, the style of the LS morph is longer than that of the SS morph (Table 5.7).

In contrast to stylar development, differences in anther height between the two morphs appear to involve changes in the spatial expression of hypothesized genes.  In the LS morph, the genes that control corolla tube cell division are expressed above the point of filament attachment, but in the SS morph, the genes are expressed below this point.  This expression pattern is appropriate because the epidermal cells of each morph are quite similar in length (with the exception of the SS morph of *L. multiflorum*) (Table 5.6), so changes in anther position must be controlled by active cell division in specific zones of the corolla tube, not by differences in cell elongation.

This active cell division can result from a greater rate of gene expression, or from the gene being expressed for a greater length of time.

### COMPARATIVE DEVELOPMENT OF DISTYLOUS SPECIES

Fewer than a dozen studies have focused on the intra- and interspecific developmental differences of distylous flowers. Most of these studies involve species of Rubiaceae (Richards and Barrett, 1992; Richards and Koptur, 1993; Riveros et al., 1995, Faivre, 2000; Hernández and Ornelas, 2007), the family with the greatest number of heterostylous species (Darwin, 1877). However, Riveros et al. (1987) and Webster and Gilmartin (2006) have undertaken studies of heterostylous species in Santalaceae and Primulaceae, respectively.

Among all of these taxa, researchers have identified four distinct patterns for the development of the two stigma heights in distylous species. One pattern of gynoecium development involves inhibition of growth of the style of the SS morph, and the continued elongation of that of the LS morph. This pattern has been observed in *Quinchamalium chilense* Molina (Santalaceae) (Riveros et al. 1987), *Faramea suerrensis* Donn.Sm. (Rubiaceae) (Richards and Barrett, 1992), *Primula vulgaris* Huds. (Primulaceae) (cf. Webster and Gilmartin, 2006), and now in *Lithospermum*. A variation on this pattern – a decreased rate of stylar growth, rather than complete inhibition, in the SS morph – is known to occur in *Guettarda scabra* Lam. (Rubiaceae) (Richards and Koptur, 1993) and *Palicourea padifolia* (Willd. ex. Roem. & Schult.) T. N. Taylor & Lorence (Rubiaceae) (Hernández and Ornelas, 2007). The other two patterns of gynoecium developmental do not include cessation or changes in the growth rate of the SS style. One developmental pattern entails different, morph-specific rates of gynoecial growth (Riveros et al., 1995; Faivre, 2000), and the other involves different sizes of the gynoecium early in the development of each morph (Faivre, 2000; Cohen, 2010).

In contrast to the numerous patterns of stylar development in distylous species, only one type of developmental pattern has been described for anther height (Riveros et al., 1987; Richards and Barrett, 1992; Richards and Koptur, 1993; Riveros et al., 1994, Faivre, 2000; Hernández and Ornelas, 2007; Cohen, 2010). This pattern entails different, morph-specific growth rates of the filaments and corolla tubes.

The evolution of distylous flowers from a homostylous ancestor often does not appear to involve allometric changes, which are common modifications that accompany shifts in floral form (e.g., Hufford, 1988). Although allometric changes to the flowers of heterostylous species may occur, and Faivre (2000) noted them, these types of modifications are less frequent than other types of floral changes. In order for a homostylous species to give rise to a heterostylous species, fine-scale changes to the anther and stigma heights, not large-scale changes to the shape or length of the corolla tube, seem to occur, and this is the case in the evolution of distylous flowers in *Lithospermum*.

## THE EVOLUTION OF HETEROSTYLY IN *LITHOSPERMUM*

In the past 31 years, three models have been proposed for the evolutionary origin of heterostyly. These models involve the sequence in which the components of heterostyly arise, and the manner in which they do so. Lloyd and Webb (1992) and Sakai and Toquenaga (2004) have separately proposed models in which the morphological features of heterostyly arise prior to the physiological ones. Charlesworth and Charlesworth (1979) presented a model with the opposite scenario. Two of these models, Charlesworth and Charlesworth (1979) and Lloyd and Webb (1992), explicitly discuss the conditions under which a population would acquire self-and intramorph incompatibility. The degree of incompatibility in heterostylous and homostylous species of *Lithospermum* is not well known (but see Levin [1968], Ganders [1979], and Ralston [1993]), so the floral developmental patterns in *Lithospermum* cannot be examined in this context. Although it is not possible to investigate the sequence of events that specifically led to the origin of self- and intramorph incompatibility in *Lithospermum*, it is possible to examine how applicable each of these three models is to the origin of heterostyly in the genus. Each of these models invokes an ancestral population with a different type of herkogamy. Therefore, the floral developmental data from the present study have been placed in the context of these ancestral populations.

Given the data herein presented, the most appropriate models for the evolution of heterostyly in *Lithospermum* are those of Lloyd and Webb (1992) and Sakai and Toquenaga (2004). These two models differ as to whether a mutant with herkogamous flowers of one type (i.e., RH) invades an ancestral population composed of flowers with the other type of herkogamy (i.e., AH) (Lloyd and Webb, 1992), or variation in herkogamy expression is present in the ancestral population (Sakai and Toquenaga, 2004). The model proposed by Charlesworth and Charlesworth (1979) is not applicable because none of the NH species of *Lithospermum* are resolved as close relatives to any of the heterostylous species of the genus (Chapter 4), a precondition in their model.

Lloyd and Webb (1992) proposed that heterostyly arose from an ancestral population composed of AH individuals, with a RH mutant invading the population. Because the flowers of the SS morph of each of the three heterostylous species of *Lithospermum* develop in a similar manner, each RH mutant invader would likely follow a similar floral developmental pattern. Three origins of flowers that develop according to the same pattern may seem unlikely; however, if the development of RH flowers is canalized among species of *Lithospermum*, as the data presented here suggest, then this model is applicable.

In one species, *L. canescens*, the ancestral species is resolved as RH (Chapter 4). With Lloyd and Webb's model (1992), an AH mutant would have arisen in the ancestral population of this species. Given the floral developmental data presented here, it appears that this AH invader would need to develop flowers in a manner similar to that of other AH species of *Lithospermum*. If the development of AH flowers is canalized, then an AH invader might develop flowers in a manner similar to that of other species with AH flowers. Although the flowers of the LS morph of *L. canescens* follow a gross developmental pattern similar to those of the other species or morphs with AH flowers, the development of anther height actually differs between *L. canescens* and these other three species (Table 5.3). In addition, the ratio of the number of stylar epidermal cells of the LS and SS morphs differs between *L. canescens* and both *L. cobrense* and *L. multiflorum* (Table 5.7). These differences provide evidence for independent origins of heterostyly in *Lithospermum*, and a different manner of origin of AH flowers in *L. canescens*.

The other model that appears relevant is that of Sakai and Toquenaga (2004). These authors invoked a model that involves an ancestral population that is variable in the occurrence of herkogamy. This type of variation occurs in some species of *Lithospermum* (Chapter 4). It is possible that species in the genus that exhibit multiple types of herkogamy can express the developmental programs for both AH and RH flowers. In *Lithospermum*, if the ancestor of a heterostylous species displayed variation in herkogamy, then the gross developmental patterns for both AH and RH flowers already would have been present among the members of the species. Consequently, the evolution of heterostyly might involve pollinator preference for the particular, reciprocal positions of the anthers and stigmas, and this would entail, concurrently, the fixation of the gross developmental patterns of AH and RH flowers.

The floral developmental patterns and the presence of multiple types of herkogamy within some species of *Lithospermum* are consistent with the model proposed by Sakai and Toquenaga (2004). If the evolutionary development of heterostyly followed this model, it would provide an example in which this breeding system could originate via fixation of currently-available genetic variation, rather than with the addition of an individual with a novel mutation. Furthermore, should only some members of the population evolve reciprocal herkogamy, and subsequently be involved in a speciation event, this would provide a mechanism by which sympatric speciation might occur.

## Conclusions

Due to variation in herkogamy, as well as the presence of a comprehensive phylogeny that suggests multiple, independent origins of heterostyly, *Lithospermum* presents a unique opportunity for the study of the evolution and comparative floral development of heterostyly. Although the gross floral developmental patterns suggest that heterostyly arose in the same manner in each of the three species examined, different patterns of the stylar and corolla tube development provide evidence that the development of heterostyly differed in each of these three species. Consequently, the floral developmental data are congruent with the independent origins of heterostyly within *Lithospermum*.

A study complementary to the present one might involve the examination of floral development in *L. caroliniense* and *L. californicum*, two heterostylous species that appear to have a RH ancestor (Chapter 4). If these two species have floral developmental patterns similar to those of *L. canescens*, rather than to those of either

*L. multiflorum* or *L. cobrense,* it would provide further evidence for different patterns of origins of heterostyly in *Lithospermum*.

In addition, studies of floral development of homostylous species of *Lithospermum* can facilitate investigations of the evolutionary origins of heterostyly. Through an examination of the species that exhibit different types of herkogamy throughout their range, it could be determined if one species expresses multiple gross floral developmental patterns, even if these patterns do not occur as fixed differences among individuals in a population

Finally, one of the limitations of the study is a lack of data concerning the efficiency of pollen transfer within species that exhibit different types of herkogamy. Although one can speculate regarding the conditions that may favor the evolution of heterostyly, it is difficult to determine the underlying forces that influence the development of heterostyly, as opposed to the retention of homostyly, without this type of data. Future research regarding pollen transfer can help provide evidence to account for the evolution of heterostyly among separate lineages in *Lithospermum*.

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# CHAPTER SIX

# A REVISION OF THE MEXICAN SPECIES OF *LITHOSPERMUM* L.

## Abstract

*Lithospermum* includes approximately 60 species. Of these 60 species, 43 occur in Mexico, and 32 of these 43 species are endemic to the country. A revision of the species of *Lithospermum* native to Mexico has not been undertaken in more than 50 years. Through morphological and molecular investigations, the species of the genus are reevaluated. Additionally, a broader generic circumscription is recognized, and this circumscription includes the species traditionally recognized as members of *Lithospermum* as well as the species of all other members of New World Lithospermeae, the tribe to which *Lithospermum* is assigned. A dichotomous key and descriptions of the Mexican species of *Lithospermum* are provided. Three new species are described from northern Mexico – *L. chihuahuanum*, *L. kelloggiae, and L. tenerum* – and each is known only from its type specimen. Additionally, *Lasiarrhenum confundum* is transferred to *Lithospermum*.

## Introduction

*Lithospermum* L. (Boraginaceae) includes ca. 60 species. The genus has a cosmopolitan distribution, with the center of diversity in the southwestern United States and Mexico. Approximately three quarters of the species occur in this region, with many being endemic. Forty-three species of *Lithospermum* occur in Mexico, more species than any other country, and of these 43 species, 32 are endemic. In addition to species diversity, the Mexican species of *Lithospermum* exhibit a range of morphological variation that is greater than that of species of the genus from any other region. In spite of this diversity, the group has not been examined in detail since the

1950s, when Johnston (1952, 1954a, b) revised the genus and the tribe to which it is assigned, Lithospermeae. Drawing on both morphological and molecular data, the present revision provides a critical and comprehensive reevaluation of the Mexican species of *Lithospermum*.

In 2009, Cohen and Davis expanded the circumscription of *Lithospermum* to include species traditionally recognized as members of the genus as well as all of the species of the other New World genera of Lithospermeae: *Lasiarrhenum* I. M. Johnst., *Macromeria* D. Don, *Nomosa* I. M. Johnst, *Onosmodium* Michx., *Perittostema* I. M. Johnst., and *Psilolaemus* I. M. Johnst. Under this expanded circumscription, *Lithospermum* is characterized by smooth, white, lustrous, erect nutlets, and by corollas that are yellow, yellow-green, orange, or white (Cohen and Davis, 2009). Although the diversity in both corolla color and nutlet form is consistent throughout the genus, *Lithospermum* exhibits variation in floral and vegetative features, including corolla shape, style insertion/exsertion, trichome length, leaf shape, and pattern of leaf venation (Chapter 4). Some of these characters vary intraspecifically, but many are uniform within species and differ among species. The structural diversity exhibited by species of *Lithospermum* is described below.

### HABIT

Most species of *Lithospermum* have multiple stems arising from a woody caudex, and each stem is usually less than 0.75 m tall. The base of each stem is usually sub-woody, and not greater than 4 mm in diameter. Some species, especially those previously included in other genera (i.e., *Macromeria*, *Lasiarrhenum*), can grow larger, up to 2 m tall, and develop wider stems that are sub-woody for a greater length. The stems of all species bear trichomes, with the indument of most stems being either strigose or hispid.

All species of *Lithospermum* have the ability to produce axillary branches, and most species do so. However, a few species, including *L. cobrense* and *L. obovatum*, only rarely develop axillary branches.

### INDUMENT

The stems, leaves, bracts, sepals, and petals (and rarely the anthers) bear trichomes. The most common types of indument are strigose, strigulose, or hispid. However, the type of indument is one of the most variable characters in *Lithospermum*. Some species consistently produce the same type of indument among all of their organs or throughout their geographic range. In contrast, some species can bear different types of indument on the same organ. For example, *L. discolor* produces leaves that have an indument on the adaxial surface that ranges from strigose and strigulose to strigose and hispidulose to strigose and scabrous, while on the abaxial surface, the indument is woolly. The terminology employed in the present revision to describe the types of indument is modeled after that of Harris and Woolf Harris (2001), with the exact terms and definitions listed below. It should be noted that some species of the genus produce irritating trichomes, which can break off from the plant and become embedded in the skin of the unaware botanist.

#### ***Stiff Trichomes***

**Strigose –** Appressed trichomes that are between 0.5 and 2 mm long. The trichomes are not too densely spaced, and the green of the plant can be seen.

**Long strigose –** Similar to strigose, but trichomes are longer than 2 mm.

**Dense strigose –** Similar to strigose, but the green surface is obscured.

**Sparse strigose –** Similar to strigose, but fewer trichomes are on the organ. Also, more of the green surface is exposed.

**Strigulose –** Similar to strigose, but the trichomes are less than 0.5 mm long.

**Sparse strigulose –** Similar to strigose, but the trichomes are less than 0.5 mm long. Additionally, fewer trichomes are on the organ, so more of the green surface is exposed.

**Hispid –** Trichomes are erect (ca. 90° from the organ), and they are between 0.5 and 2 mm long. The trichomes are not too densely spaced, and the green of the plant can be seen.

**Dense hispid –** Similar to hispid, but the green surface is obscured.

**Dense long hispid –** Similar to hispid, but trichomes are longer than 2 mm and the green surface is obscured.

**Sparse hispid –** Similar to hispid, but fewer trichomes are on the organ. Also, more of the green surface is exposed.

**Hispidulose –** Similar to hispid, but the trichomes are less than 0.5 mm long.

**Scabrous –** Very short trichomes, at most 0.1 mm long. One often needs to use a light microscope or hand lens to see individual trichomes clearly.

### *Soft Trichomes*

**Villous –** Wavy trichomes that are between 0.5 and 2 mm long. The trichomes usually are appressed, and the green of the plant can be seen.

**Villulous –** Similar to villous, but the trichomes are less than 0.5 mm long.

**Sparse villous –** Similar to villous, but fewer trichomes are on the organ. Also, more of the green surface is exposed.

**Woolly –** Similar to villous, but the trichomes are very close together. The surface of the organ is obscured almost entirely.

**Sericeous –** Appressed hairs that are between 0.5 and 2 mm long. These trichomes are not too densely spaced, and the green of the plant can be seen.

**Dense sericeous –** Sericeous, but the green surface is obscured.

**Sericulous –** Similar to sericeous, but the trichomes are less than 0.5 mm long.

**Pilose –** Trichomes that are erect (ca. 90° from the organ), and that are between 0.5 and 2 mm long. The green of the plant can be seen.

**Pilosulose –** Similar to pilose, but the trichomes are less than 0.5 mm long.

## NAPHTHOQUINONES

Some species of *Lithospermum* produce naphthoquinones, a class of secondary compound, in their roots. On herbarium specimens, the species that produce naphthoquinones usually develop a purple stain in the area around the roots. The ability to produce naphthoquinones originated multiple times within *Lithospermum* (Chapter 4).

Species of *Lithospermum* that produce naphthoquinones can be divided into two groups: one group consistently produces large quantities of naphthoquinones, while the other group inconsistently produces smaller quantities of these secondary compounds. Brigham et al. (1999) demonstrated that naphthoquinones can provide the plant with antimicrobial properties. These secondary compounds have been shown to have medicinal uses as an effective contraceptive (Cranston, 1945; Plunkett and Noble, 1951), and to have anti-inflammatory (Han et al., 2008) and anti-HIV properties (Yamasaki et al., 1993). Although much is known on the subject of the chemical pathway (Yamamura et al., 2003) and the medicinal properties of naphthoquinones, less is known regarding the production of these secondary compounds in wild populations of *Lithospermum*.

### LEAVES AND BRACTS

All species of *Lithospermum* produce alternate, cauline leaves arranged in a spiral pattern. In addition to cauline leaves, a small number of species produce either basal or pseudobasal rosettes. Most leaves, both cauline and basal, are sessile, but a limited number of species sometimes develop cauline leaves with short petioles. These petioles are often inconspicuous (and easily overlooked), being less than 2 mm long.

The cauline leaves of species of *Lithospermum* range in shape from elliptical to lanceolate to ovate to oblanceolate. The leaf apex is usually acute or acuminate, although some species have rounded or mucronate leaf apices. The leaf base is usually acute, rounded, or truncate**.** The basal or pseudobasal leaves usually are oblanceolate in shape, with an acute or rounded apex and a truncate base.

Venation patterns differ among species of *Lithospermum*. The majority of the species of the genus bear leaves in which the only a midvein is evident. However, a large number of species produce leaves with both an evident midvein and evident secondary veins. Three different types of evident secondary venation occur in species of the genus. These three patterns include: secondary veins that are flush with the leaf blade, secondary veins that are sunken (below the surface of the leaf blade), and leaves that either have a midvein and one or two pairs of secondary veins or have three equal or subequal primary veins and one or two pairs of secondary veins. These four types of leaf venation are not mutually exclusive in occurrence. Patterns of leaf venation can differ between cauline leaves of the same plant, and between cauline and basal leaves of the same plant (Chapter 4).

All species of *Lithospermum* produce bracts, and each bract subtends one flower. The structure of the bracts is similar to that of the leaves, with two notable exceptions: size and phyllotaxy. The bracts are smaller than the leaves, and the bracts decrease in size from the base of the inflorescence to the apex. In addition, rather than the spiral phyllotaxy exhibited by the leaves, the bracts are arranged in a distichous pattern, which reflects the arrangement of the flowers along the scorpioid cyme (Buys and Hilger, 2003).

Both the leaves and the bracts bear trichomes on the adaxial and abaxial surfaces. The most common types of leaf indument are strigose, strigulose, dense strigose, hispid, and villous. Some species produce trichomes that are at a 45° angle with the blade, so this type of indument is exactly intermediate between strigose and hispid (Harris and Woolf Harris, 2001). For the present revision, this type of indument is described as strigose; however, species with leaves or bracts that develop this type of indument often vary among individuals, with some plants producing an indument that is strigose, and others developing one that is hispid.

### INFLORESCENCE

All species of *Lithospermum* develop terminal inflorescences that are either monochasial or dichasial scorpioid cymes, and most species produce both types. Additionally, many species develop axillary monochasia, but not in all individuals.

Early in the development of the inflorescence, the internodes are short. These short internodes provide the inflorescence with a compact form. As the flowers open and mature, the lengths of these internodes increase. The maximum length of the inflorescences measured in the present study incorporates these expanded internodes, with the final lengths of the inflorescences much greater than those in their immature, compact forms.

### FLOWERS

***Calyx*** - Species of *Lithospermum* bear five sepals. The shape of each sepal is usually lanceolate, with a truncate base and an acute to acuminate apex. The sepals develop trichomes on both the abaxial and adaxial surfaces. The abaxial surface tends to be strigose, hispid, or strigulose, and the adaxial surface is usually strigulose. In many species, after the corolla withers and detaches from the receptacle, the sepals continue to increase in length. This increase in length ranges from two to seven millimeters. While a flower is developing, its calyx is actinomorphic, but during fruit development, one sepal tends to elongate more than any of the other four. Due to this developmental pattern, the calyx becomes slightly zygomorphic.

In *Lithospermum*, the increase in sepal length does not seem to have a function as it does in other genera, such as *Arnebia* Forssk. In *Arnebia*, the sepals tightly enclose the nutlets, and because the sepals bear long trichomes, the calyx and nutlets seem to form a unit for fruit dispersal (pers. obs.). The sepals in species of *Lithospermum* do not wrap around the nutlets, so this developmental syndrome may be plesiomorphic for members of the tribe, or serve another, currently unknown purpose.

***Corolla*** - The five petals of the species of *Lithospermum* are fused together for the majority of their length. The fused part of the corolla develops into a corolla tube, ranging in length from three to 60 cm. The shape of the corolla varies, the most common form being salverform (Chapter 4). However, seven other corolla shapes are found in the genus. The long-funnelform shape characterizes species formerly recognized as *Macromeria*, and one type of campanulate shape characterizes the species that were part of *Onosmodium* (Chapter 4).

In addition to corolla shape, the color of the corollas varies. Colors range from yellow to yellow-green to orange to white, but most species produce corollas that are either yellow or white with a yellow center. Corollas that are white with a yellow center are much more common in Mexico than in the United States. In the United States, only one species, *L. matamorense*, produces a corolla with this color pattern, compared to five species in Mexico. Additionally, other Mexican species bear corollas that are pure white or cream.

The corollas of most species bear glands. These glands are three-celled (Thomas et al., 2008), and are often situated toward the apex of the corolla tube. In some species, glands are present on the veins leading to the anthers. One species, *L. rosei*, produces multicellular glands on its corolla tube and filaments.

In addition to glands, many species develop five faucal appendages. These appendages are thickenings of the corolla that occur inside the corolla at the intersection of the corolla tube apex and the corolla lobe base. Each faucal appendage is located opposite one of the corolla lobes. Most species produce faucal appendages that have glands, but the faucal appendages of some species are glabrous.

Glands and faucal appendages are positioned towards the apex of the corolla tube, but many species develop an annulus at the base of the inside of the corolla. The annulus usually is composed of five or ten flanges or knobs. These flanges or knobs can be glabrous or bear trichomes. The annulus of species of *Lithospermum* can produce nectar, and sometimes the annulus can serve as a protective covering for the ovary (Johnston, 1952, 1954a, b).

All species of *Lithospermum* produce trichomes on the abaxial surface of the corolla, and oftentimes, the indument is villous or villulous. In contrast, only a few species bear trichomes on the adaxial surface of the corolla tube. In one heterostylous species, *L. obovatum*, the long-style morph bears trichomes on the adaxial surface, while the short-style morph does not (Johnston, 1952; Cohen, 2010).

The corolla lobes are free for most of their length, but they are fused at the base. Their shape ranges from elliptical to orbicular to deltoid, with a rounded, acute, or obtuse apex and a truncate base. The lobes can be erect, flared, or reflexed; most species produce corollas with flared lobes.

***Androecium*** - The five filaments of species of *Lithospermum* are adnate to the corolla tube. Most species bear short filaments (< 1 mm long); however, many of the species that were only recently assigned to *Lithospermum* (Cohen and Davis, 2009) develop longer filaments (> 10 mm long). Species with short filaments have anthers inserted in the corolla; those with longer filaments may develop either inserted or exserted anthers. The filaments of all but five species are less than 0.5 mm wide. The filaments of *L. rosei*, *L. macromeria*, *L. trinervium*, *L. confundum*, and *L. kelloggiae* are ca. 1 mm wide and flat in cross section.

Anthers are ellipsoid. Most species produce anthers that are between 0.5 and 2 mm long and 0.5 to 1 mm wide. In addition, the anthers of most species are glabrous, but those of *L. rosei*, *L. trinervium*, and *L. macromeria* bear abaxial trichomes.

***Gynoecium*** - In *Lithospermum*, the gynoecium of each flower includes a hypogynous ovary with two fused carpels, a gynobasic style, and two stigmas. Each carpel is further divided, producing an ovary with four separate locules, with a single ovule per locule.

The length of the style varies both intra- and interspecifically; however, a more noticeable, and possibly more ecologically important feature of the style is whether the stigmas are inserted or exserted from the corolla tube. In most species, stigma insertion/exsertion is a fixed character, but in a few species, such as *L. incisum* and *L. nelsonii*, the stigma can be inserted at anthesis, and exserted later in development. In contrast, a few species, such as *L. rosei* and *L. revolutum*, develop stigmas that are exserted early in development (Chapter 4).

The stigmas of most species are terminal, but a few species produce styles that terminate in an apical projection. Many of the species that develop these subterminal stigmas are polymorphic for this character (Chapter 4), but subterminal stigmas are fixed in seven species, including *L. distichum*, *L. nelsonii*, and *L. album*. In general, species that develop subterminal stigmas also produce corollas that are mostly white.

Stigmas also develop different types of papillae that cover the entire surface of the stigma. These papillae range in shape from cylindrical to umbraculate. In some species, these papillae appear to function in a type of lock-and-key pollination system (cf. Bigazzi and Selvi, 2000).

### NUTLETS

Species of *Lithospermum* produce nutlets that resemble small white pebbles or pieces of porcelain, and this similarity provides the genus with its common name: stone seeds. The nutlets are ovoid in shape, with an acute apex and a base that is round, although some species, such as *L. incisum*, produce a 0.25 mm pedestal at the base of each nutlet.

Most species bear nutlets that are white to tan or grey, but nutlets that are partially red or golden are observed as well. The nutlets of most species have smooth surfaces, although some pitting, similar to the dimples in a golf ball, may occur. Pitting is most common on either side of the adaxial scar of the nutlet, and in some species, pitting can occur over the entire surface of the nutlet. *Lithospermum mirabile* departs from this pattern in bearing nutlets that have a rugose surface.

### DISTRIBUTION AND ECOLOGY

Species of *Lithospermum* occur throughout Mexico, except in the Baja and the Yucatan peninsulas. More species of *Lithospermum* are distributed in the eastern part of Mexico than throughout the west, and are most common in the Sierra Madre Oriental, the Sierra Madre Occidental, or the Trans-Mexican Volcanic Belt at 1,500 – 3,200 meters. For example, *L. viride*, *L. oblongifolium*, and *L. nelsonii* are found in the Sierra Madre Oriental, while *L. johnstonii*, *L. obovatum*, and *L. tubuliflorum* are restricted to the Sierra Madre Occidental.

Only a few species grow in the southerm part of Mexico, or in Mesoamerica (Cohen and Miller, in press), and it is uncommon for species of *Lithospermum* to grow at lower elevations or in wet tropical forests. The formation of smooth fruits with no obvious means for dispersal, in conjunction with valleys that separate areas of suitable habitat, may help to explain the restricted distribution of many species of *Lithospermum*.

*Lithospermum* often is found in pine or oak forests. As much of the natural habitat of the species has been developed, species of *Lithospermum* can be found growing along roadsides. One species, *L. revolutum*, which is endemic to San Luis Potosí, is a halophyte, but this is unusual.

## Materials and Methods

Measurements were taken on ca. 555 herbarium specimens from a total of ca. 750 specimens that were examined. Material was obtained from BH, CAS, F, GH, MEXU, MICH, MO, NY, TEX/LL, US, and WISC. In addition, multiple collecting trips were made to Mexico and the southwestern United States in order to observe wild populations of species of *Lithospermum*. Geographic data for each specimen was recorded.

Morphological, geographical, and phenological data helped inform the species concept employed in the present revision. Species are defined as a group of populations characterized by a suite of diagnostic features, and the particular combinations of features vary among groups of populations (Nixon and Wheeler, 1990; Davis and Nixon, 1992). The aforementioned species concept generally is consistent with that of previous treatments of *Lithospermum*, such as those by Johnston (1952, 1954a, b), de Candolle (1846), and those of related genera, including Turner (1994a, b, 1995). Although the specific circumscriptions largely remain unchanged, the generic circumscription differs from that of past treatments (i.e., Candolle, 1846; Johnston, 1952, 1954a, b; Ralston, 1993; Turner, 1994a, b, 1995; Boyd, 2003).

## Key to the species of *Lithospermum* of Mexico

1. Stigma exserted from corolla tube; style usually longer than 1 cm - 2 (alternative couplet on p. 211)

    2. Anthers completely exserted from corolla tube; corolla tubes usually > 35 mm long, leaves with evident secondary veins; plants usually 50 cm to 2 m tall (formerly *Macromeria*) - 3 (alternative couplet p. 205)

        3. Lobes of corolla reflexed 180° - 4

            4. Color of corolla cream white to light yellow; style 42 - 61 mm long; corolla tube suddenly flaring - *L. johnstonii*

            4. Color of orolla yellow; style usually longer, 55 - 121 mm long; corolla tube either gradually or suddenly flaring - 5

                5. Flowers pendant, actinomorphic; corolla golden yellow, anthers exserted 8 - 15 mm from the apex of the corolla tube; style 55 - 85 mm long; stems up to 65 cm tall; plants of northeastern Mexico - *L. leonotis*

                5. Flowers horizontal, slightly zygomorphic, corolla light yellow to yellow, anthers exserted 25 - 55 mm

from the apex of the corolla tube; style (87) 100 - 121 mm long; stems up to 2 m tall; plants of western and southern Mexico - *L. exsertum*

3. Lobes of corolla ascending to flared (sometimes the apex of each corolla lobe reflexed, but the entire lobe never reflexed 180°) - 6

6. Corolla lobes with a medial line of glands on the adaxial surface, these sometimes present at the apex of the corolla throat as well - 7

7. Flowers with white corollas; naphthoquinones absent; plants of southern Tamaulipas - *L. album*

7. Flowers with yellow and yellow-green corollas; naphthoquinones usually present; plants of Coahuila and and Nuevo León - *L. notatum*

6. Corolla lobes glabrous to pubescent but lacking glands in a medial line on the adaxial surface- 8

8. Color of corollas orange to orange-yellow, the lobes usually tipped with green - *L. flavum*

8. Color of corollas yellow-green to yellow, the lobes usually the same color as the tube - 9

9. Stems hispid and long hispid to dense hispid and dense long hispid; plants of Nuevo León and Tamaulipas - *L. barbigerum*

9. Stems strigose to strigulose to hispid, but not long hispid; plants of central, southern, or northwestern Mexico and the southwestern US - 10

10. Corollas yellow; plants of Guerrero and Oaxaca - *L. turneri*

10. Corollas yellow-green; plants of Mexico and SW US - 11

11. Corollas lacking glands on the abaxial surface; the tube gradually tapering from the base of the tube to the apex; filaments wide elliptical in cross section, ca. 1 mm wide; anthers with abaxial trichomes; stigmas terminal - *L. macromeria*

11. Corollas with glands on the abaxial surface; the tube suddenly flaring; filaments round in cross section, ca. 0.25 mm wide; anthers glabrous; stigmas usually subterminal, rarely terminal - *L. rzedowskii*

2. Anthers not completely exserted from corolla tube, usually fully included, but sometimes partially exserted; corolla tubes usually < 35 mm long (> 35

mm in some species), leaves with or without evident secondary veins; plants usually less than 50 cm tall - 12

12. Corolla campanulate or urceolate - 13 (alternative couplet p. 208)

13. Leaves with evident midveins, the other veins obscure; margins revolute - 14

14. Flower with yellow corollas; plants restricted to Michoacán - *L. kelloggiae*

14. Flowers with white corollas; plants restricted to Oaxaca - *L. pinetorum*

13. Leaves with an evident midvein and evident secondary veins, margins rarely revolute - 15

15. Corollas pure white; filaments wide elliptical in cross section, ca. 1 mm wide; plants of western Mexico - 16

16. Leaves 1.4 - 3.3 mm wide; multiple pairs of secondary veins evident; glands absent from the corolla tube and filaments - *L. confundum*

16. Leaves 0.2 - 1.8 mm wide; trinerviate or 1 - 2 pairs of secondary veins evident; glands present on the corolla tube or filaments - 17

17. Corolla urceolate; stigma precociously exserted; filaments with multicellular glands; restricted to Durango and northern Jalisco - *L. rosei*

17. Corolla campanulate; stigma exserted at anthesis; filaments glabrous, but glands present in the apex of the corolla tube; plants of western Mexico - *L. trinervium*

15. Corollas yellow to yellow-green; filaments round in cross section, ca 0.1 mm wide; plants of eastern or southern Mexico (formerly *Onosmodium*) - 18

18. Stems, leaves, and calyx with conspicuous glandular trichomes - *L. unicum*

18. Stems leaves, and calyx lacking glandular trichomes - 19

19. Trichomes > 2 mm long; pseudobasal and cauline leaves present; plants of Oaxaca - *L. oaxacanum*

19. Trichomes < 2 mm long; cauline leaves present, pseudobasal leaves lacking; plants of Nuevo León and Tamaulipas - *L. dodrantale*

12. Corolla salverform, long-funnelform, or tubular - 20

20. Color of corollas white, cream, or white with a yellow center - 21

21. Flowers with corollas that are white with a yellow center; faucal appendages present; leaves with midvein and usually with one or two pairs of secondary veins; plants restricted to Nuevo León - *L. nelsonii*

21. Flowers with corollas that are white to cream (rarely light yellow); faucal appendages absent; leaves an evident midvein, but no evident secondary veins; plants of eastern Mexico - *L. calcicola*

20. Color of corollas yellow or yellow-green - 22

22. Shape of corolla tubular or long-funnelform - 23 (alternative couplet p. 210)

23. Corollas yellow, adaxially glabrous; the lobes erect; leaves coriaceous; plants of San Luis Potosí - *L. revolutum*

23. Corollas green-yellow, adaxially glanduliferous; the lobes flared or reflexed; leaves fleshy; plants of eastern Mexico - 24

24. Faucal appendages present - 25

25. Tube of corolla 16 - 37 mm long; corolla long-funnelform; corolla lobes flared; style (13 - 19) 28 - 36 mm long; leaves with a midvein and evident secondary veins, margins not revolute; trichomes on all parts of abaxial surface of leaf - *L. oblongifolium*

25. Tube of corolla 13 - 20 mm long; corolla tubular; corolla lobes erect; style 15 - 23 mm long; leaves with a midvein and lacking evident secondary veins, margins revolute; trichomes usually solely along midvein of the abaxial surface of leaf - *L. muelleri*

24. Faucal appendages absent - 26

26. Leaves with only an evident midvein; corolla tube 6 - 13 mm long; corolla lobes usually fimbriate - *L. calycosum*

26. Leaves with both an evident midvein and conspicuous secondary venation; corolla tube 20 - 33 mm long; corolla lobes entire - 27

27. Glands present at apex of corolla tube; corolla lobes erect to reflexed; corolla tubular to long-funnelform; leaves 0.3 - 1.3 cm wide; plants of Chiapas, Mexico and Guatemala - *L. guatemalense*

27. Glands present on veins leading to filaments; corolla lobes reflexed; corolla tubular; leaves 0.4 - 2.4 cm wide; plants of northeastern Mexico - *L. viride*

22. Shape of corolla salverform or funnelform - 28

28. Secondary venation on leaves conspicuous; nutlets with a red tint; all flowers chasmogamous - *L. pringlei*

28. Secondary venation on leaves obscure, only the midvein evident; nutlets white to tan, not red; flowers chasmogamous or cleistogamous - 29

29. Faucal appendages absent; corolla yellow to yellow-green - *L. calycosum*

29. Faucal appendages present; corolla yellow - 30

30. Corolla funnelform; only cauline leaves present, pseudobasal leaves lacking; corolla tube 9 mm long; nutlet base rounded; flowers chasmogamous - *L. tenerum*

30. Corolla salverform; both cauline and pseudobasal leaves sometimes present; corolla tube 11 - 15 mm long; nutlet base on a 0.25 mm pedestal; flowers chasmogamous and cleistogamous - 31

31. Margins of corolla lobes fimbriate; nutlets smooth; style of chasmogamous flowers 16 - 34 mm long - *L. incisum*

31. Margins of corolla lobes entire; nutlets rugose; style of chasmogamous flowers 11 - 14 mm long - *L. mirabile*

1. Stigma included in corolla tube; style usually less than 1 cm (but longer in a few species) - 32 (first half of couplet on p. 203)

32. Species producing only cleistogamous flowers; styles 3 mm long; plants of Coahuila and Nuevo León - *L. confine*

32. Species producing chasmogamous or cleistogamous flowers; styles often longer than 3 mm (but see *L. matamorense*); plants of various regions of Mexico - 33

33. Corolla white, cream, or white with a yellow center - 34 (alternative couplet on p. 214)

34. Cauline leaves with a midvein, and multiple pairs of evident secondary veins usually present on most, if not all, leaves- 35

35. Leaf venation trinerviate with one to two pairs of secondary veins; corolla white, narrow campanulate; leaves cauline and pseudobasal - *L. chihuahuanum*

35. Leaf venation of evident secondary veins; corolla white with a yellow center; salverform or funnelform; all leaves cauline - 36

36. Secondary veins 2 or more per leaf; faucal appendages absent; corolla funnelform; leaf indument dense villous on abaxial surface; species heterostylous; leaves 0.5 - 2.6 cm wide; plants of western and southern Mexico - *L. discolor*

36. Secondary veins 1 - 2 pairs per leaf; faucal appendages present; corolla salverform; leaf indument

strigose to hispid on abaxial surface; species homostylous; leaves 0.4 - 1.6 cm wide; plants of northeastern Mexico - 28

37. Corolla tube 3 - 5 mm long; style <3 mm long; stems to 40 cm tall; restricted to area around Ciudad Victoria, Tamaulipas - *L. berlandieri*

37. Corolla 11 - 14 (19) mm long; style >12 mm long; stems to 70 cm tall; restricted to Nuevo León - *L. nelsonii*

34. Cauline leaves usually with a midvein and no secondary veins, secondary veins only rarely present, and if present, only one or two pairs on some leaves - 38

38. Tube of corolla 1 - 2 mm long; style 1 mm long; nutlets pitted, white to gray; annual - *L. matamorense*

38. Tube of corolla tube > 3.5 mm long, style > 2 mm long; nutlets smooth to slightly pitted, white to tan; perennial - 39

39. Color of corolla white to cream; faucal appendages absent, flowers unscented; all leaves cauline - *L. calcicola*

39. Color of corolla white with a yellow center; faucal appendages present; flowers scented; leaves cauline and sometimes pseudobasal - *L. distichum*

33. Corolla yellow to yellow-green - 40

40. Species heterostylous - 41

41. Leaves cauline, basal leaves lacking; trichomes absent inside corolla; corollas funnelform; naphthoquinones present - *L. multiflorum*

41. Leaves both basal and cauline; trichomes present inside the corolla (with the exception of the short-style morph of *L. obovatum*); corollas funnelform or salverform-funnelform; naphthoquinones absent - 42

42. Basal leaves with only an evident midvein, faucal appendages absent; stigmas usually terminal - *L. cobrense*

42. Basal leaves with an evident midvein and evident secondary veins; faucal appendages present or absent; stigmas usually subterminal - 43

43. Corollas salverform-funnelform; faucal appendages present - *L. obovatum*

43. Corollas funnelform; faucal appendages absent - *L. tubuliflorum*

40. Species homostylous - 44

44. Faucal appendages absent - 50

45. Corolla light yellow, salverform; corolla tube usually < 6 mm long; corolla lobes entire; indument strigose to hispid; flowers chasmogamous - *L. calcicola*

45. Corolla yellow to yellow-green; salverform to tubular; corolla tube usually > 6 mm long; indument strigose to sericeous; flowers chasmogamous or cleistogamous - *L. calycosum*

44. Faucal appendages present - 46

46. Corolla tube > 15 mm long; flowers chasmogamous and cleistogamous; leaves cauline, pseudobasal rosette sometimes present - 47

47. Margins of corolla lobes fimbriate; nutlets smooth; corollas 16 - 34 mm long - *L. incisum*

47. Margins of corolla lobes entire; nutlets rugose; corollas 11 - 14 mm long - *L. mirabile*

46. Corolla tube < 15 mm long; flowers chasmogamous flowers, cleistogamous flowers lacking; all leaves cauline, pseudobasal rosette lacking - 48

48. Indumentum woolly; restricted to Mt. Jimulco in Coahuila - *L. jimulcense*

48. Indumentum strigose to dense strigose, but not woolly; central and southern Mexico - 49

49. Corollas yellow to yellow-green; leaves with a midvein, secondary veins lacking; nutlets white to grey, not red - *L. strictum*

49. Corollas yellow; leaves with a midvein and multiple secondary veins; nutlets usually slightly red - 50

50. Tube of corolla 5 - 9 mm long; style 6 - 8 mm long - *L. pringlei*

50. Tube of corolla 3 - 5.5 mm long; style < 3mm long - *L. indecorum*

GEOGRAPHIC REGIONS AND THE STATES THEY INCLUDE

Northern Mexico – Chihuahua, Coahuila, Durango, Nuevo León, Sonora, and Tamaulipas

Northeastern Mexico – Coahuila, Nuevo León, and Tamaulipas

Western Mexico – Chihuahua, Colima, Guerrero, Jalisco, Michoacan, Nayarit, Sinaloa, and Sonora

Central Mexico – D.F., Guanajuato, Hidalgo, Mexico, Morelos, Puebla, Querétaro, Tlaxcala, and central Veracruz

Southern Mexico – Chiapas, Guerrero, Puebla, Oaxaca, and southern Veracruz

## Taxonomic Treatment

*Lithospermum* L. *Sp. Pl.* 132. 1753. TYPE: *Lithospermum officinale*

*Batschia* J. F. Gmel. *Syst. Nat., ed. 13[bis.]* 2: 315. 1791. TYPE: *Batschia caroliniensis* [= *Lithospermum caroliniense*]

*Cyphorima* Raf. *Amer. Monthly Mag. & Crit. Rev.* 4: 191, 357. 1819. TYPE: *Cyphorima latifolium* [= *Lithospermum latifolium*]

*Lasiarrhenum* I. M. Johnst. *Contr. Gray Herb.* 70: 15. 1924. TYPE: *Lasiarrhenum strigosum* [= *Lithospermum trinervium*]

*Macromeria* D. Don *Edinburgh New Philos. J.* 13:239. 1832. TYPE: *Macromia exserta* [= *Lithospermum exsertum*]

*Nomosa* I. M. Johnst. *J. Arnold Arbor*. 35: 24. 1954. TYPE: *Nomosa rosei* [= *Lithospermum rosei*]

*Onosmodium* Michx. *Fl. Bor.-Amer*. 1: 132, t. 15. 1803. TYPE: *Onosmodium hispidum* [= *Lithospermum bejariense*]

*Pentalophus* A. DC. *Prodr*. 10: 86. 1846. TYPE: *Pentalophus longiflorus* (**designated here**) [= *Lithospermum incisum*]

*Perittostema* I. M. Johnst. *J. Arnold. Arbor.* 35: 30. 1954. TYPE: *Perittostema pinetorum* [= *Lithospermum pinetorum*]

*Psilolaemus* I. M. Johnst. *J. Arnold. Arbor.* 35: 33. 1954. TYPE: *Psilolaemus revolutus* [= *Lithospermum revolutum*]

Erect perennial, rarely annual, herbs up to 2 m tall with 1--few stems arising from a woody caudex, naphthoquinones present or absent, stems with various types of indument, trichomes eglandular, rarely glandular. Leaves cauline, sometimes basal or pseudobasal, sessile, sometimes petiolate; blades lanceolate to elliptical to ovate to obovate to oblanceolate, apex acute to rounded to acuminate, rarely mucronate, base rounded to truncate to acute, margins entire, planar or revolute, indument of various types, midvein evident, other orders of veins sometimes evident, sunken or not. Inflorescence scorpioid cymose, terminal dichasium or monochasium, sometimes axillary monochasia, each flower subtended by a bract, bracts sessile, sometimes petiolate, blades lanceolate to elliptical to ovate to obovate to oblanceolate, apex acute to rounded to acuminate, rarely mucronate, base rounded to truncate to acute, margins entire, planar or revolute, indument of various types, midvein evident, other orders of veins sometimes evident, sunken or not. Flowers bisexual, homostylous or heterostylous, chasmogamous, rarely also cleistogamous; pedicellate; sepals 5, lanceolate to linear, persistent**,** apex acute to acuminate, base truncate, indument of various types, margins entire; petals 5, corolla sympetalous, tubular to funnelform to long-funnelform to salverform, rarely campanulate or urceolate, actinomorphic, rarely slightly zygomorphic, white to cream to green-yellow to yellow, rarely orange, indument villullous to villous to woolly, tube 1--60 x 0.3--11 mm, adaxial trichomes present or absent, lobes 1--27 x 0.5--7 mm, erect to flared to reflexed, orbicular to elliptical to deltoid, connate at the base, apex acute to acuminate to obtuse, base truncate, margins entire, rarely fimbriate, glands present or absent, faucal appendages 5, present or absent, annulus present or absent, if present of various forms of knobs and flanges; stamens 5, epipetalous, filaments 0.1--60 x 0.1--1 mm, glabrous, rarely with glands, anthers ellipsoid, 0.5--3 x 0.3--1 mm, glabrous, rarely with trichomes abaxially, inserted to exserted, theca 4; style 1, gynobasic, 1--121 x 0.1--0.25 mm, inserted to exserted up to 61 mm from the apex of the corolla tube, sometimes precociously or tardily exserted, persistent; stigma 2-lobed, terminal to subterminal; ovary 2-carpellate and 4-lobed.  Nutlets 4 or fewer by abortion, attached basally, ovoid, 2--5 x 1.5--3 mm, smooth to pitted, white to slightly red to brown to grey, scar adaxial, prominent or not, base rounded or with a pedestal, apex acute.

## List of Species

1.  *Lithospermum album* (G. L. Nesom) J. I. Cohen *Brittonia* 61: 108.  2009. Basionym: *Macromeria alba* G. L. Nesom *Madroño* 36: 28.  1989.  TYPE: Mexico. Tamaulipas: Mpio. Gomez Farias, 5 – 7 km, NW of Gomez Farias, just S of Agua del Indio, May 30, 1969, *A. Richardson 1263* (holotype, TEX [digital image]!).

Erect perennial herb up to 43.5 cm tall with 1--few stems, each 2 mm wide, arising from a woody caudex, naphthoquinones not observed, stems strigose to sparsely strigose. Leaves cauline, sessile or petiolate, petioles 2 x 1 mm; blades lanceolate, 7.7-

-9.6 x 1.6--2.9 cm, apex acute to acuminate, base rounded to acute, margins entire, planar, adaxially scabrous to strigulose and abaxially strigose to strigose and strigulose, more densely strigose on veins, midvein evident, secondary veins evident, sunken. Inflorescence terminal monochasium, up to 14 cm long, bracts sessile or petiolate, petioles 2 x1 mm; blades lanceolate, 4.5--7.1 x 0.7--2.4 cm, apex acute to acuminate, base rounded to acute, margins entire, planar, adaxially scabrous and abaxially strigose, more densely strigose on veins, midvein evident, secondary veins evident, sunken. Flowers homostylous, chasmogamous; pedicels 0--13 mm long; sepals linear, 7--8(--up to 12 in fruit) x 1 mm, apex acute, abaxially strigose and adaxially strigulose; corolla long-funnelform with a bulge, white, abaxially villous, tube 39 x 5 mm, adaxial trichomes absent, lobes 7 x 2.5--3 mm, erect, deltoid, apex acute, glands present as a line in the middle of each lobe and at the apex of the tube, faucal appendages absent, annulus not observed; stamens exserted, filaments 9 x 0.1 mm, attached 34 mm above the base of the corolla, exserted up to 8 mm from the apex of the corolla tube, anthers 0.5 x 0.1 mm, ellipsoid; style 50 x 0.1 mm, exserted up to 12 mm from the corolla throat; stigma subterminal. Nutlets 3 x 3 mm, ovoid, tan, smooth, base a slight pedestal, apex acute.

Distribution: Endemic to Tamaulipas.

Phenology: Flowers from late May to late June, fruiting from late June.

Discussion: This species is known from only three collections by Alfred Richardson. Diagnostic characters for this species are the white, long-funnelform corollas. No other species of *Lithospermum* produces corollas of this type. In addition to corolla color, *L. album* shares with *L. notatum* the presence of glands in a medial on each corolla lobe; glands of this sort do not occur in any other species of *Lithospermum*. Given this unique character, Nesom hypothesized that these two species are closely-related (Nesom, 1989).

Specimens Examined: MEXICO. **Tamaulipas**: Between Indian Springs and Agua Linda turnoff, June 26, 1968, *A. Richardson 366* (TEX).

2. *Lithospermum barbigerum* (I. M. Johnst.) J. I. Cohen *Brittonia* 61: 108. 2009. Basionym: *Macromeria barbigera* I. M. Johnst. *J. Arnold Arbor.* 16: 189. 1935. TYPE: Mexico. Nuevo León: N slope of Sierra Tronconal between Canyon de los Charcos and Canyon de San Miguel, ca. 15 mi SW of Galeana, "common in dense oak woods beyond pine-fir belt," 6000–9000 ft., June 4, 1934, C. *H. Mueller & M. T. Mueller 741* (holotype, GH!; isotypes, F, MEXU!, MICH [digital image]!, TEX [digital image]!)

Erect perennial herb up to 1(--1.3) m tall with 1--few stems, each 3--5 mm wide, arising from a woody caudex, naphthoquinones not observed, stems hispid and long hispid to dense hispid and dense long hispid. Leaves cauline, sessile or petiolate, petioles 1 x 1(--3) mm; blades ovate to elliptical, rarely lanceolate, 3.5--13.5 x 1.3--5.2 cm, apex acute to acuminate, rarely cuspidate, base acute, sometimes rounded or truncate, margins entire, planar, adaxially strigose to hispid and abaxially strigose, sometimes hispid, midvein evident, secondary veins evident, sunken or not. Inflorescence terminal dichasium or monochasium and sometimes axillary monochasia, up to 30.5 cm long, bracts sessile or petiolate, petioles 1 x 1 mm, blades ovate to lanceolate to elliptical, 2.3--8.1(--9.8) x 0.4--6 cm, apex acute to acuminate to cuspidate, base acute to rounded, margins entire, planar, adaxially and abaxially strigose, sometimes dense strigose or hispid, midvein evident, secondary veins evident, sunken or not.  Flowers homostylous, chasmogamous; pedicels 0--9 mm long; sepals linear, 12--20 x 0.75--1 mm, apex acute, abaxially strigose and long strigose to hispid and long hispid, denser towards the base, and adaxially strigose and strigulose; corolla long-funnelform with a bulge, yellow, abaxially villous, tube (40--)47--55 x 4--6(--8) mm, adaxial trichomes absent, lobes 2--5 x 2--4 mm, erect, deltoid, apex obtuse, glands present at the apex of the corolla tube and the base of the corolla lobes, faucal appendages absent, annulus of 4--10 membranous flanges; stamens exserted, filaments 6--10 x 0.25--0.3 mm, attached 41--53 mm above the base of the corolla, exserted up to 3--5 mm from the apex of the corolla tube, anthers 2--2.25 x 0.75--1 mm, ellipsoid; style 53--59 x 0.1 mm, exserted up to 7 mm from the corolla tube; stigma terminal.  Nutlets 3--5 x 2--3 mm, ovoid, white to white and brown, smooth, rare pitting, scar evident, but not prominent, base rounded, apex acute.

Distribution: Northeastern Mexico in Nuevo León and Tamaulipas.

Phenology: Flowers late April to August, fruits from August.

Discussion: Unlike many species of *Lithospermum*, the indument on the stems of *L. barbigerum* is long hispid.  This type of indument, along with the long-funnelform corollas, allows this species to be distinguished from others with similar corolla shapes.

Specimens Examined: MEXICO.  **Nuevo León**: Near top of "M" ridge above Chipinque, May 7, 1960, *R. F. Smith M191* (TEX); Mpio. Zaragoza, Cerro El Viejo, 2200 m., July 7, 1992, *Hinton et al. 22117* (TEX); Mpio. Galeana, Agua Blanca → Sn.

Miguel, 2070 m., Aug. 28, 1991, *Hinton et al. 21351* (TEX); Sierra Madre Oriental, arroyos and canyon slopes about the head of Santa Barbara Canyon, 15 miles SW of Galeana, alt. 7500 ft., May 25, 1934, *C. H. and M. T. Mueller 563* (TEX); Mpio. Montemorelos, Trail from La Trinidad up Sierra de la Cebolla, Aug. 20, 1939, *C. H. Muller 2871* (LL); Sierra Madre Oriental, San Francisco Canyon, about 15 miles SW of Pueblo Galeana, alt. 75 - 8000 ft., May 10, 1934, *C. H. and M. T. Mueller 287* (TEX); Sierra Madre Mts., Monterrey, July 29, 1933, *C. H. and M. T. Mueller 174* (F, TEX); Sierra Madre Mts., Monterrey, July 4, 1933, *C. H. and M. T. Mueller 173* (F, TEX). Hacienda Pablillo, Galeana, Aug. 17, 1936, *M. Taylor 191* (F). **Tamaulipas**: Mpio. Hidalgo, road from Sta. Engracia toward Dulces Nombres, N.L., above Puerto Purificacion, ca. 1 km SSE straight line from Cerro Purificación, ca. 8 road miles NE of Los Caballos, ca. 19.5 road miles from Dulces Nombres, ca. 1300 m., Mar. 3, 1995, *G. Nesom 7862b* (TEX); Mpio. Hidalgo, Arroyo Obscuro, along road to Dulces Nombres, Nuevo León, 2.0 road miles NE of Los Caballos towards Cañada El Mimbre, 15.0 road mi from the lowermost crossing of arroyo El Mimbre, N 23° 59' 09" W 99° 28' 37", 1800 m., Sep. 23, 1994, *M. H. Mayfield 2089* (TEX); La Caballada, 2 k E of Hidalgo, 1735 m., Apr. 29, 1995, *Hinton et al. 25283* (TEX); Mpio Hidalgo, Los Caballos → Rancho Las Flores, 1690 m., Sep. 23, 1994, *Hinton et al. 24906* (TEX).

3. *Lithospermum berlandieri* I. M. Johnst. *J. Arnold. Arbor.* 23: 357. 1952. TYPE: Mexico. Tamaulipas: Oak forests on Jaumave road about 13 miles southwest of Ciudad Victoria, elev. about 1000 m., abundant, fl. white, May 13, 1949, *R. McVaugh 10517* (holotype, GH!; isotypes, MEXU!, NY!).

Erect perennial herb up to 38 cm tall with 1--few stems, each 1.5-3 mm wide, arising from a woody caudex, naphthoquinones not observed, stems hispid, rarely strigose. Leaves cauline, sessile; blades elliptical to obovate, rarely oblanceolate or spatulate, 1.4--6.7 x 0.4--1.5 cm, apex rounded to acute to mucronate, base acute, entire, planar, sometimes revolute, adaxially strigose to strigulose to hispid to hispidulose and abaxially strigose to hispid, margin entire, planar, midvein evident, usually 1--2 pairs of secondary veins evident, not sunken. Inflorescence terminal dichasium or monochasium and sometimes axillary monochasia, up to 25 cm long, bracts sessile; blades elliptical to lanceolate, 0.5--3.4(--5.5) x 0.1--1.3 cm, apex acute, base acute, margins entire, planar, adaxially strigose to hispid, sometimes strigulose and abaxially strigose to hispid, midvein evident, other orders of veins not evident. Flowers homostylous, chasmogamous; pedicels 0--6 mm long; sepals linear to lanceolate, 2--6 x 0.75--1 mm, apex acute, abaxially hispid to strigose and adaxially strigulose; corolla salverform, white with a yellow center, abaxially villous towards the apex and glabrous towards the base, tube 2.5--4 x 1--2 mm, adaxial trichomes absent, lobes 1.5--2 x 1--1.5 mm, erect to flared, elliptical to orbicular, apex rounded, glands present at the apex of the corolla tube, faucal appendages present, annulus of a membranous ring; stamens inserted, filaments 0.25--0.5 x 0.1 mm, attached 2--3 mm above the base of the corolla, anthers 1 x 0.25--0.5 mm, ellipsoid; style 2--3 x 0.1 mm, inserted; stigma subterminal. Nutlets 3--4 x 2 mm, ovoid, white, pitting around prominent scar, base with a 0.25 mm pedestal, apex acute.

Phenology: Flowering late March to early May, and fruiting from late March.

Distribution: Restricted to the area around Ciudad Victoria, Tamaulipas.

Discussion: *L. berlandieri* appears to have an affinity with either *L. nelsonii* or *L. matamorense*. Both *L. berlandieri* and *L. nelsonii* bear leaves with an evident midvein and one or two pairs of evident secondary veins, and produce pollen that is prolate with a constricted equator. However, the corollas of *L. berlandieri* are similar in size and shape to those of *L. matamorense*. Although *L. berlandieri* has not been examined in a phylogenetic context, the leaves and pollen of *L. berlandieri* suggest that it is more closely-related to *L. nelsonii* than to *L. matamorense*.

*L. berlandieri* has a very narrow distribution. Given its small geographic range, species of *Lithospermum* that bear white corollas with a yellow center, and grow in the vicinity of Ciudad Victoria can confidently be identified as this species. Although it may be easy to recognize the species, it may be difficult to find *L. berlandieri*. I have not seen any specimens collected within the past 20 years.

Specimens Examined: MEXICO. **Tamaulipas**: C. Victoria, April 1930, *H. W. Viereck* 252 (US); South of Victoria, alt. 1000 m., Mar. 25, 1925, *R. Runyon 747* (US); Mountains near Victoria, 400 m., April 4, 1926, *R. Runyon 921* (US); Mountains along route 70, ca. 9 miles south of Ciudad Victoria, April 7, 1961, *R. M. King 4539* (NY, US); Mina de Asbesto, a 25 km al Oeste de La Libertad y a 35 km de Cd. Victoria, Dec. 9, 1987, *F. González Medrano 16944* (MEXU); 17.6 km SW of CD. Victoria on Mex. 101, 1150 mnm., Apr. 16, 1985, C. P. Cowan 5203 (NY); Sierra new Jaumave, July 1932, *H. W. Von Rozynski 567* (F).

4. *Lithospermum calcicola* B. L. Rob. *Proc. Amer. Acad. Arts* 27: 182. 1892. TYPE: Mexico. San Luis Potosí: San Jose Pass, July 11, 1890, *C. G. Pringle 3529* (holotype, GH!; isotypes, F, MEXU!).

*Lithospermum conzattii* Greenm. *Bull. Field Mus. Nat. Hist.* 2: 339. 1912. *Lithospermum calcicola* var. *conzattii* (Greenm.) I. M. Johnst. *Contr. Gray Herb.* 70: 27. 1924. TYPE: Mexico. Oaxaca: de Huanclilla a Nochixtlán, Rancho Pozuelos, 2400 m., June 19, 1907, *C. Conzatti 1846* (holotype, F [digital image]!; isotypes, MEXU!, GH!).

*Lithospermum hoyasense* Brand *Repert. Spec. Nov. Regni Veg.* 28: 15. 1930. TYPE: Mexico. Oaxaca: las Hoyas Canyon, 5500 ft., November 2, 1894, *C. G. Pringle 6026* (lectotype, GH!) **lectotype designated here**, see discussion below.

*Lithospermum madrense* Brand *Repert. Spec. Nov. Regni Veg.* 28: 14. 1930. TYPE: Mexico. Nuevo León: Sierra Madre above Monterey, 2500 ft., April 4, 1906, *C. G. Pringle 10196* (lectotype, GH!; lectotype duplicate, PH) **lectotype designated here**, see discussion below.

Erect perennial herb up to 40 cm tall with 1--few stems, each 1--3 mm wide, arising from a woody caudex, naphthoquinones not observed, stems strigose to hispid. Leaves cauline, sessile; blades lanceolate to elliptical to ovate, rarely obovate, 0.9--5.5(--8.6) x 0.2--1.1(--1.4) cm, apex acute, base rounded to acute, margins entire, planar, abaxially and adaxially strigose, midvein evident, other orders of veins not evident. Inflorescence terminal dichasium and axillary monochasia, up to 15 cm long, bracts sessile, lanceolate to ovate to elliptical, sometimes linear, 0.7--3.8 x 0.1--1 cm, apex acute, rarely rounded, base rounded to acute, margins entire, planar, abaxially and adaxially stigose to densely strigose, midvein evident, other orders of veins not evident. Flowers homostylous, chasmogamous; pedicels 0--4(--6) mm long; sepals linear to lanceolate, 3--6 x 0.5--1.25 mm, apex acute, abaxially hispid, more densely hispid towards the base than at the apex, adaxially sparse strigulose; corolla salverform, yellow-white to creamy white to white with a light yellow center to light yellow, abaxially villous, denser towards the apex than towards the base, tube 3.5--5(--7) x 2--3 mm, adaxial trichomes absent, lobes orbicular to elliptical, 1--3 x 1.5--3 mm, flared, rarely erect or slightly reflexed, apex rounded to obtuse, glands present in the upper portion of the corolla tube, faucal appendages absent, annulus of a ring of 10 membranous flanges; stamens inserted, filaments 0.1 x 0.75 mm, attached 3--7 mm above the base of the corolla, anthers 1--1.5 x 0.5 mm, ellipsoid; style 2--7(--8) x 0.1 mm wide, usually inserted, sometimes exserted up to 1(--1.75) mm from the apex of the corolla tube; stigma terminal. Nutlets 3--4 x 1--2.25(--2.5) mm, ovoid, white to brown, base rounded, apex acute.

Phenology: Flowers from mid- to late April until September, fruits can be found starting in May.

Distribution: This species can be found from northeastern Mexico south to Oaxaca.

Discussion: The corolla color in this species varies from white with a yellow center to cream with a yellow center to light yellow. Individuals with light yellow corollas usually have been recognized as *L. indecorum*, but this assgnation is incorrect. Individuals of *L. indecorum* bear leaves with evident secondary venation, and this is not the pattern of leaf venation observed in individuals of *L. calcicola*.

A small number of individuals from Coahuila develop 1 mm long glands that are arranged in a ring around the apex of the corolla tube. The stigmas of these individuals are exserted up to 2 mm from the apex of the corolla tube. Although the latter feature is not unusual (other individuals of *L. calcicola* have an exserted style; although to a lesser extent), the long glands are unique to these specimens.

It also should be noted that the holotypes of the species described by Brand were housed in the Berlin herbarium, and unfortunately these specimens were destroyed during World War II (Hiepko, 1987). Therefore, many of the species of *Lithospermum* described by Brand are lectotypified in the present revision.

Specimens Examined: MEXICO. **Coahuila**: Mpio. de Muzquiz, Rincón de Maria, 28° 27’ 30” N, 102° 04’ W, August 23, 1975, *T. Wendt, E. Lott, D. H. Riskind 1275* (LL); Mpio. Arteaga, Las Vigas, Cañon de la Carbonera, Sierra de Arteaga, 25° 20’ N, 100° 39’ W, 2100 - 2600 m., June 5, 1987, *J. A. Villarreal & M. A. Carranza 3766* (LL); Rincón de Maria, on Hacienda La Babia, which is ca. 70 mi. by road NW of Muzquiz, 28° 27’ 30” N 102° 04’ 30” W, 1550 m., April 27, 1975, *T. Wendt & D. Riskind 908* (LL); Serranias del Burro, Rcho. El bonito, Mpio. de Zaragosa (approx. 29° 01’ 30” N 102° 07’ 30” W), April 12, 1976, *D. H. Riskind & T. F. Patterson 1974*. (LL); Mpio. Arteaga, Los Lirios → El Cercado, 2410 m., July 29, 1995, *Hinton et al. 25443* (LL); Mpio. Arteaga, Los Lirios → El Cercado, 2405 m., July 29, 1995, *Hinton et al. 25546* (LL); Mpio. Arteaga, Los Lirios → La Jacinta, 2390 m., September 15, 1995, *Hinton et al. 25432* (LL); ca. 28 (air) miles E. of Saltillo on the S. side of the Sierra de Viga, ca. 4 miles E. of Jamé along, woodcutters road, near 25° 21’ N, 100° 33’ W, 9,500 – 10,500 ft., *J. Henrickson with D. Riskind, R. Engard, & T. Daniel 16,089*, (TEX). **Hidalgo**: on mountain side, km. 231 of highway between Zimapan and Jacala, August 19, 1943, *C. L. Lundell & A. A. Lundell 12387* (LL, US). **Nuevo León**: San Francisco Canyon about 15 miles SW of Pueblo Galeana, alt. 7500 - 8,000 ft., May 9, 1934, *C. H. & M. T. Mueller 266* (TEX); N. of Santa Rita, 2360 m., May 14, 1981, *G. B. Hinton 18251* (TEX); El Jonuco, Santa Catarina, 1760 m., September 30, 1996, *Hinton et al. 25932* (LL); Mpio. Santiago, Los Lirios → La Jacinta, 2050 m., September 15, 1995, *Hinton et al. 25582* (LL); Near top of “M” ridge above Chipinque, Monterrey, May 7, 1960, *R. F. Smith M175* (TEX); Sierra Madre Mts., Monterrey, *C. H. & M. T. Mueller 172*, (TEX); 1 - 2 miles southwest of Pablillo, July 21, 1958, *D. S. Correll & I. M. Johnston 19934* (LL); S.E. Potosí, 2360 m., Galeana, September 27, 1981, *G. B. Hinton 18352* (TEX); “Taray”, to Rio Santa Ana, Pabillo, southeast of Galeana, alt. 2000 - 2100 m., July 2 - 3 1934, *F. W. Pennell 17216* (US); Cañon de los Capulines, above San Enrique, Hacienda San Jose de Raices, Mpio. de Derrumbadero, August 6, 1935, *C. H. Mueller 2414* (US); “Cieneguillas”, Pablillo, southeast of Galeana, alt. 2450 - 2550 m. June 28 - 30, 1934, *F. W. Pennell 17085* (US); Mpio. Galeana, on road from Galeana to Reynosa, 24° 70.752’ N, 100° 5.414’, alt. 5900 ft. June 13, 2007, *J. Cohen, 187* (BH); Mpio. Zaragoza, near road from La Encantada to San Antonio Peña Nevada, June 14, 2007, *J. Cohen 191* (BH). **Oaxaca**: 9.4 mi. SE of Nochixtlan, August 27, 1967, *O. F. Clarke, B. T. Gittins, H. Haid, & E. Lathrop s.n.* (LL). **Puebla**: The vicinity of San Luis Tultitlanapa, Cerros de Paxtle & Santa Lucia, July 1907, *C. A. Purpus 2701* (US). **San Luis Potosí**: Charcas, July - August 1934, *C. L. Lundell 5,314* (US); Minas de San Rafael, May 1911, *C. A. Purpus 5381* (US). **Tamaulipas**: San Lucas, July 1930, *H. W. Viereck 615* (US); 3 mi N of Miquihuana, July 15, 1959, *Stanford, Lauber, & Taylor 2459* (US, NY). **Veracruz**: Cerros calizos al SE del pueblo de Alchichica, mapa. 36.5 21.5, May 16, 1968, *C. H. Ramos 180* (LL).

5. *Lithospermum calycosum* (J. F. Macbr.) I. M. Johnst. *Contr. Gray Herb.* 70: 30. 1924. Basionym: *L. strictum* var. *calycosum* J. F. Macbr. *Contr. Gray Herb.* 48: 56. 1916. TYPE: Mexico. Guanajuato: montagnes, terrains pierreux, June 1907, *A. A. D. Dugès 5* (holotype, GH!).

*Lithospermum obtusifolium* I. M. Johnst. *Contr. Gray Herb.* 70: 27. 1924. TYPE: Mexico. San Luis Potosí: in arenosis prope San Miguelito, 1876, *J. G. Schaffner 728* (holotype, GH!).

*Lithospermum galeottii* Brand Repert. *Spec. Nov. Regni Veg.* 28: 17. 1930. TYPE: Mexico. Monte Tanja, July 1844, *H. G. Galeotti s.n.* (lectotype, GH!) **lectotype designated here**, see discussion under *L. calcicola*.

Erect perennial herb up to 39.5 cm tall with 1--few stems, each 1--2 mm wide, arising from a woody caudex, naphthoquinones not observed, stems dense strigose to strigose to sericeous. Leaves cauline and pseudobasal, sessile; cauline blades linear to elliptical, sometimes ovate or spatulate, 0.8--5 x 0.1--1 cm, apex rounded to acute, base truncate to rounded to acute, margins entire, usually revolute, adaxially and abaxially densely strigose to strigose to sericeous, midvein evident, other orders of veins not evident; basal blades linear to spatulate, 1--3.8 x 0.2--0.7 cm, apex rounded, base acute, margins entire, planar, sometimes revolute, adaxially and abaxially densely strigose to strigose to sericeous, midvein evident, other orders of veins not evident. Inflorescence terminal and sometimes axillary monochasia, up to 21.5 cm long, bracts sessile; blades linear to ovate, rarely elliptical, (0.4--)0.6--2.7 x 0.1--0.6(--2.5) cm, apex rounded to acute, base truncate, margins entire, planar or revolute, dense strigose to strigose to sericeous, midvein evident, other orders of veins not evident. Flowers homostylous, chasmogamous and cleistogamous; pedicels 0--7 mm long; chasmogamous flowers with sepals linear to lanceolate, 4--13(-16) x (0.25--)0.75--2 mm, apex acute, rarely round, abaxially strigose to strigulose and adaxially strigose to strigulose; corolla tubular to salverform, green-yellow to yellow, abaxially villous to woolly, denser towards the apex and sparser towards the base, tube 6--13 x 2--3 mm, adaxial trichomes absent, lobes 1--3 x 1.5--3.25 mm, flared to erect, orbicular to elliptical, apex rounded, margins fimbriate, glands present in the upper portion of the tube, faucal appendages absent, annulus of 5 membranous flanges with sparse trichomes; stamens inserted, filaments 0.1--0.25 x 0.1 mm, attached 7--12 mm above the base of the corolla, anthers 1--1.5 x 0.25--0.5 mm; style (3--)5--13 x 0.1 mm, inserted; stigma terminal, rarely subterminal; cleistogamous flowers with sepals linear to lanceolate, 5.5--10 x 0.75--1.5 mm, apex acute, abaxially strigose to dense strigose and adaxially strigose; style 3--4 x 0.1 mm, inserted, terminal.  Nutlets 2.5--4 x 1.5--2.5, ovoid, white, sometimes pitting along the scar, base round to slightly truncated, apex acute.

Distribution: Eastern Mexico, and disjunct in Guatemala.

Phenology: Flowers May to September, fruiting from May onward.

Discussion: Given the form of the chasmogamous flowers of *L. calycosum*, it is easy to imagine why Macbride (1916) described this species as a variety of *L. strictum*. However, other morphological features in *L. calycosum*, including the leaves and the presence of both chasmogamous and cleistogamous flowers, are dissimilar to these features in *L. strictum*.  Indeed, the aforementioned features are more similar to those in *L. mirabile* and in *L. incisum*.  Despite these floral and vegetative similarities, *L. calycosum* is sisters with *L. revolutum* (Cohen and Davis, 2009; Chapter 3, 4).

The corollas of *L. calycosum* do not bear faucal appendages; nevertheless, in some of the flowers, glands are aggregated into pad-like appendages.  These “pads” may perform a similar function to faucal appendages.  However, the “pads” are longer and more diffuse than are faucal appendages. These pad-like appendages do not constrict the apex of the corolla tube to the same extent as faucal appendages.

Johnston (1952) reported that this species develops both chasmogamous and cleistogamous flowers, but I have not observed any intact cleistogamous flowers. Without the presence of one, it is difficult to determine indisputably if the species produces both types of flowers. This difficutly arises because the range in the lengths of the styles of chasmogamous flowers overlaps with those of putative cleistogamous flowers. In Texas, I have found individuals of each of two species of *Lithospermum*, *L. incisum* and *L. mirabile*, that produce cleistogamous, but I have not had the opportunity to do so for *L. calycosum*. Furthermore, in *L. incisum* and in *L. mirabile*, the styles of chasmogamous flowers are much longer than those of cleistigamous flowers, and therefore, even without the corolla present, it is possible on herbarium specimens to identify flowers of each type. Because of Johnston's observations, I have recognized that *L. calycosum* produces chasmogamous and cleistogamous flowers. However, it is possible that the flowers Johnston identified as cleistogamous are actually chasmogamous flowers with a short style.

Specimens Examined: MEXICO. **Guanajuato**: San Miguel Allende, el Escalón, arriba de la cañada, cerca de la Presa del Obraje, alt. 1850 m., Aug. 9, 1979, *J. Kishler 729* (MEXU). **Hidalgo**: Ixmiquiplau, July 1905, *C. A. Purpus 1403* (F). **Michoacán** : E. de Morelia, alt. 2100 m., June 26, 2009, *G. Arsène 7235* (US); Barranca qui descend au NO. du Punguato, alt. 1900 m., October 18, 1909, *G. Arsène 3,153* (US). **Nuevo León**: San Francisco Canyon, about 15 miles SW of Pueblo Galeana, alt. 7500-8000 ft., May 10, 1934, *C. H. & M. T. Mueller 292* (F, TEX, MICH); Mpio. Zaragoza, along roadside on road from San Antonia Peña Nevada to la Encantada, 23° 48' 50.3" N 99° 53' 37.7" W, alt. 7700 ft., June 15, 2007, *J. Cohen 197* (BH); Mpio.

Derrumbadero, Cañon de los Capulines above San Enrique, Hacienda San José de Raices, August 6, 1935, *C. H. Mueller 2378* (F). **San Luis Potosí**: 4 km al S. de Aguaje de Garcías, 6 km al W. de Guadalcázar, alt. 2050 m., Sep. 1, 1955, *Rzedowski 6379* (F, TEX, MICH); Sierra de Alvarez, cerca del Puerto Huerta, alt. 2350 m., Sep. 5, 1954, *Rzedowski 4050* (TEX); alt. 6000-8000 ft., *C. C. Perry & Ed. Palmer 622* (F); 26 miles east of San Luis Potosí, along highway 86 to Rio Verde, elev. ca. 8000 ft., July 14, 1963, *R. L. McGregor, L. J. Harms, A. J. Robinson, R. del Rosario, & R. Segal 702* (US). **Veracruz**: Actopan, Cofre de Perote, 10500 ft., May 27, 1938, *E. K. Balls 4647* (US). **Zacatecas**: Sierra del Astillero (approached from southeast, from Tanque El Alto), 24° 34’ - 24° 34’ 30” N 101° 04’ 30” - 101° 06’ W, 2,500-3,195 m., July 2, 1973, *M. C. Johnston, T. L. Wendt, & F. Chiang C. 11556A* (LL).

6. *Lithospermum chihuahuanum* J. I. Cohen **sp. nov.** Type: Mexico. Chihuahua: Mpio. Ocampo, parque nacional Cascada de Casaseachic, alt. 1920 m., bosque de pino-encino, Aug. 5, 1994, *C. Yen & E. Estrada 2918* (holotype, NY!).

Erect perennial herb up to 25.5 cm tall with 1--few stems, each 2 mm wide, arising from a woody caudex, naphthoquinones not observed, stems strigose and hispid to strigose and long hispid to dense strigose and hispid to dense strigose and long hispid. Leaves cauline and basal, sessile; cauline blades ovate to elliptical to obovate to lanceolate, 1.9--2.5 x 0.6--0.9 cm, apex acute, rarely acuminate, base acute to truncate, margins entire, planar, adaxially strigose and strigulose and abaxially dense strigose and hispid, trinerviate, usually 1--2 pairs of secondary veins, sunken; basal blades obovate to oblanceolate, 2--3.2 x 0.6--1 cm, apex acute, base truncate, margins entire, planar, adaxially strigulose and strigose, sometimes also long strigose, and abaxially strigose and hispid to strigose and dense hispid, midvein evident, secondary veins evident, sunken. Inflorescence terminal monochasium, up to 8.5 cm long, bracts sessile, lanceolate, 0.7--1.6 x 0.2--0.5 cm, apex acuminate, base acute, margins entire, planar, abaxially strigulose and strigose to strigulose and dense strigose to strigose and hispid to strigulose and dense hispid and adaxially strigulose and hispid to dense strigulose and hispid, midvein evident, secondary veins sometimes evident, sunken. Flowers homostylous, chasmogamous; pedicels 0--7 mm long; sepals linear, 6(--up to 12 in fruit) x 1 mm, apex acute, abaxially dense strigose and hispid and adaxially strigulose to strigose; corolla narrow campanulate, white or light yellow, abaxially dense villous, tube 13 x 2 mm, trichomes not observed, lobes 0.5 X 1 mm, erect, deltoid, apex acute, glands not observed, faucal appendages not observed, annulus not observed; stamens not observed; style 11--12 X 0.1 mm, inserted; stigma terminal. Nutlets 3 x 2 mm, ovoid, white, base rounded, apex acute.

Distribution: Endemic to Chihuahua.

Phenology: Flowers and fruits in early July.

Discussion: *L. chihuahuanum* is known from only one specimen. Although the specimen only has one flower, the vegetative parts of the plant allow this specimen to be recognized as a new species. The plant produces abundant trichomes, many of which are longer than 2 mm. In addition, the cauline leaves develop a venation pattern that is trinerviate with one or two pairs of evident secondary veins. This pattern of leaf venation is similar to that of *L. trinervium* and *L. rosei*.

Although *L. chihuahuanum* has not been examined in a phylogenetic context, it appears to be closely-related to *L. trinervium* and *L. rosei*. In addition to the particular pattern of leaf venation, the one flower present on the type specimen appears to be similar in shape and in color to those of these two species. However, the indument of *L. chihuahuanum* resembles that of *L. obovatum*, another species of northwestern Mexico.

This species is named after the Mexican state, Chihuahua, in which it was collected.

Specimens Examined: MEXICO. **Chihuahua**: Chihuahua: Mpio. Ocampo, parque nacional Cascada de Casaseachic, alt. 1920 m., Aug. 5, 1994, *C. Yen and E. Estrada 2918* (NY!).

7. *Lithospermum cobrense* Greene *Bot. Gaz.* 6: 157. 1881. TYPE: U.S.A. New Mexico: no locality, *C. Wright 1563* (lectotype, GH!; lectotype duplicate, GH!). **lectotype designated here**.

Erect perennial herb up to 44 cm tall with 1--few stems, each 1--3 mm wide, arising from a woody caudex, naphthoquinones absent, stems strigose to dense strigose to sericeous. Leaves cauline and basal, sessile; cauline blades 0.6--4(--5) x 0.1--0.6**,** linear to narrow elliptical to lanceolate**,** apex acute to rounded, base acute to truncate, margins entire, usually revolute, adaxially and abaxially strigose to strigose and strigulose to dense strigose to sericeous, midvein evident, other orders of veins not evident; basal blades (0.4--)1.1--6 x 0.1--0.8 cm**,** apex rounded to acute, base truncate, margins entire, planar, sometimes revolute, adaxially strigose to strigose and strigulose to sericeous and abaxially strigose to sericeous, midvein evident, other orders of veins not evident. Inflorescence terminal dichasium or monochasium and infrequently axillary monochasia, up to 25.5 cm long, bracts sessile, blades linear to narrow elliptical to lanceolate, 0.5--2.7(--3.5) x 0.1--0.6 cm, apex acute, rarely rounded, base truncate to acute, margins entire, usually revolute, adaxially and abaxially strigose to strigose and strigulose to dense strigose to sericeous, midvein evident, other orders of veins not evident. Flowers heterostylous, chasmogamous; pedicels 0--6 mm long; Long style flowers with sepals lanceolate, 4--8(--14) x 1(--2) mm, apex acute, abaxially strigose to strigose and strigulose to dense strigose and adaxially strigose to strigulose, rarely dense strigose; corolla salverform-funnelform, yellow, abaxially villulous, tube 6--9 x 2--3 mm, adaxial trichomes present, villous in the lower half of the tube, lobes 3--8 x (2--)4--6 mm, flared, elliptical, apex rounded to obtuse, glands present at the apex of tube, faucal appendages absent, annulus of 5 knobby flanges with trichomes; stamens inserted, filaments 0.25 x 0.1 mm, attached 2--3 mm above the base of the corolla, anthers 1--2 x 0.5 mm, ellipsoid; style 4--7 x 0.1 mm, inserted; stigma terminal, sometimes subterminal. Short style flowes with sepals lanceolate, 3--9(--up to 12 in fruit) x 0.75--1.5 mm, apex acute, abaxially and adaxially strigose to strigulose to dense strigose; corolla salverform-funnelform, yellow, abaxially villous to villulous, tube 5--10 x 2--3 mm, adaxial trichomes present, villous in the lower half of the tube, lobes 2--9 x 2--7 mm, flared, elliptical, apex rounded, glands present at apex of tube, faucal appendages absent, annulus of 5 hairy flanges; stamens inserted, filaments 0.25--0.5 x 0.1 mm, attached 4--7 mm above the base of the corolla, anthers 1.5--2 x 0.5 mm, ellipsoid; style 2--3 x 0.1 mm, inserted; stigma terminal, rarely subterminal. Nutlets 2--3 x 1.5--2 mm, ovoid, white to tan, smooth to some pitting around the prominent scar, base rounded, apex acute.

Distribution: Northern Mexico, primarily Chihuahua and Durango, and Arizona, New Mexico, and western Texas.

Phenology: Flowers mid-June until early September, fruits late June onward

Discussion: In his description of *L. cobrense*, Greene (1881, p 158) stated "Doubtless all [specimens] that have been called *L. canescens* from New Mexico and Arizona is of this species." Both *L. cobrense* and *L. canescens* produce corollas that are similar in shape, size, and texture; therefore, it is easy to understand why specimens of *L. cobrense* initially were assigned to *L. canescens*. However, the two species are certainly distinct. Apart from different, non-overlapping geographic ranges, the corollas of *L. cobrense* are yellow, while those of *L. canescens* are orange. In addition, *L. cobrense* produces both basal and cauline leaves, while *L. canescens* only bears cauline leaves.

*L. cobrense* is sister to *L. tubuliflorum* (Chapter 3, 4), and the ranges of the two species overlap in Mexico. Both species are heterostylous and produce basal rosettes, but the two species differ in other floral and vegetative characteristics. *Lithospermum cobrense* develops corollas that are salverform-funnelform in shape, while those of *L. tubuliflorum* are funnelform. Additionally, the basal leaves of *L. cobrense* only bear an evident midvein, but those of *L. tubuliflorum* produce an evident midvein and evident secondary veins.

Specimens Examined: MEXICO. **Chihuahua**: 5 miles west of Hidalgo de Parral, 5800 ft., Oct. 3, 1965, *H. D. Ripley & R. C. Barneby 13937a* (NY); Hwy 16, approximately 30 miles W. of La Junta Jct., alt. 6950', July 19, 1975, *Ellis, Dunn, & Torke 1022* (NY); Mpio. Guachochi, Agua Azul, 12 km. al E. de Guachochi, 2100 m., Aug. 12, 1982, *R. Hernández M. 8724* (MEXU); 4 mi E northeast Gomez Farias, 7000 ft, July 2, 1985, *P. S. Martin s.n.* (MEXU); Mpio Guachochi, June 13, 1987, *R. Bye 17571* (MEXU); Mpio. Bocoyna, below the railroad bed NE of Creel, July 12, 1971, *R. A. Bye 1506* (MEXU); Mpio. Janos, Carretas, border of Chihuahua and Sonora, elev. 4800 ft., Aug. 26 – 28, 1939, *S. S. White 2575* (MEXU); District of Bocoyna, near San Juanito, alt. 7800 ft., July 26, 1937, *F. Shreve 8024* (F); District of Cusihuiriachic, 17 mi. N of Rubio, alt. 7000 ft., July 23, 1937, *F. Shreve 7964* (F); Near Colonia Garcia in the Sierra Madres, alt. 7500 ft., June 8, 1899, *C. H. Townsend and C. M. Barber 85* (F); El Cima, June 29, 1936, *H. LeSueur 867* (F); Majalca, June 24, 1936, *H. LeSueur 861* (F); Creel, alt. 2400 m., July 20, 1983, *Y. Saiki M-32* (F). **Durango**: Mpio. Súchil, on side of road from Suchil to Michilía ,23 31’ 2” N, 104 8’ 14” W, 2100 m., June 23, 2007, *J. Cohen 203* (BH); Between La Zarca and Rodeo, June 23, 1967, *C. Molsee M1365* (MEXU); Mpio. Mezquital, 2 km. de Laguna del Chivo por el camino a San Antonio, alt. 2250 m., July 30, 1986, *M. González 1960* (MEXU); Mpio. Tepehuanes, 30 km al Noroeste de Tepehuanes, alt. 2000 m., July 24, 1982, *R. Hernández M. 8245* (MEXU); 14 miles west of Durango, elev. ca. 7500 ft., July 22, 1955, *M. C. Johnston 2676b*; about 5 miles north of railroad at Coyotes (45 airline miles west of C. Durango), elev. 2400 – 2500 m., June 28, 1950, *J. H. Maysilles 7178* (MEXU); Mpio. El Salto, 46 km del Entronque de la Brecha a San Miguel de Cruces con la Carr. Durango-Mazatlán, 2200 m., July 8, 1982, *P. Tenorio L. 880* (MEXU); Mpio. Santiago Papasquiaro, 10 km, al W de Garame de Abajo, July 15, 1982, *P. Tenorio L. 1016* (MEXU); Mpio. Mezquital, 1 km. al N. de Laguna del Chivo, alt. 2250 m., July 20, 1985, *M. González et al. 1774* (MEXU); El Salto, alt. 8000 – 8700 ft., July 12, 1898, *E. W. Nelson 4540* (US); *P. Ibana García 313* (US); Along the road north of Indé toward Mexico Highway 45, about 13 miles north of Indé at Puerta de Cabreras, Aug. 10, 1971, *J. L. Reveal 2732* (US); Indé, alt. 2000 m., Aug. 1927, *B. P. Reko 5215* (US); Otinapa, July 25 to Aug. 5, 1906, *E. Palmer 555* (F); Tobar, May 28 – 31, 1906, *E. Palmer 246* (F); City of Durango and vicinity, Apr. to Nov. 1896, *E. Palmer 370* (F); 28 miles southwest of Durango, Aug. 11, 1957, *U. T. Waterfall 13573* (F). **Sonora**: Mpio. Agua Prieta, alt. 1100 m., Sept. 1972, *O. F.*

*Francke II* (MEXU). **Zacatecas**: Near Plateado, on the road from Colotlan, Aug. 31, 1897, *J. N. Rose 2707* (US); Near Plateado, Sep. 2, 1897, *J. N. Rose 3636* (US)

8. *Lithospermum confine* I. M. Johnst. *J. Arnold Arbor*. 33: 346. 1952. TYPE: Mexico. Nuevo León: Mpio. Derrumbadero, Cañon de los Capulines above San Enrique, Hacienda San José Raices, Aug. 6, 1935, *C. H. Mueller 2378* (holotype, GH; isotype, US!).

Erect perennial herb up to 29 cm tall with 1--few stems, each 2 mm wide, arising from a woody caudex, naphthoquinones not observed, stems strigose. Leaves cauline, sessile; blades narrow elliptical to oblanceolate, 1.6--4.7 x 0.2--0.5 cm, apex rounded to acute, base acute to truncate, margins entire, planar, sometimes revolute, adaxially and abaxially strigose, midvein evident, other orders of veins not evident. Inflorescence terminal and axillary monochasia, up to 10.7 cm long, bracts sessile; blades narrow elliptical to oblanceolate, 2--4.5 x 0.2--0.5 cm, apex rounded to acute, base acute to truncate, margins entire, planar or revolute, adaxially and abaxially strigose, midvein evident, other orders of veins not evident. Flowers homostylous, cleistogamous; pedicels 0--6 mm long; sepals linear, 8--14 x 1 mm, apex acute, abaxially strigose to hispid and adaxially strigulose; corolla not observed; androecium not observed; style 3 x 0.1 mm; stigma terminal. Nutlets 3--3.5 x 2 mm, ovoid, white, smooth with pitting around prominent scar, base rounded to a short 0.25 mm pedestal, apex acute.

Distribution: Coahuila and Nuevo León and Arizona and Texas, USA.

Phenology: Flowers late June onward, and starts fruiting in early July.

Discussion: In his discussion of *L. confine*, Johnston (1952, p 347) stated “At one time or another they [the specimens assigned to *L. confine*] have been questionably identified as *L. obtusifolium*, *L. calycosum*, *L. multiflorum*, *L. cobrense*, and *L. incisum*, and even their possible hybrids, as well.” *L. confine* is a unique member of the genus because it appears only to produce cleistogamous flowers. Because of this feature, along with its habit, *L. confine* seems to have an affinity with *L. incisum*, *L. calycosum*, or *L. mirabile*. However, given the lack of phylogenetic data, the evolutionary relationships are unknown. Since 1952, when Johnston described *L. confine*, no new specimens of this species have been collected. Perhaps with more intensive collecting efforts, molecular phylogenetic investigations, and breeding system studies, the relationships, and perhaps the origin, of *L. confine* will be elucidated.

Specimens Examined: MEXICO. **Nuevo León**: Mpio. Derrumbadero, Cañon de los Capulines above San Enrique, Hacienda San Jose Raices, Aug. 6, 1935, *C. H. Mueller 2378* (US).

9. *Lithospermum confundum* (B. L. Turner) J. I. Cohen **comb. nov.** Basionym: *Lasiarrhenum confundum* B. L. Turner *Phytologia* 77: 41. 1994. TYPE: Mexico. Durango: Mpio. El Salto, SE face of El Espinazo del Diablo, 20 km SE of La Ciudad, elev. 2770 m., Sep. 12, 1973, *D. E. Breedlove 43946* (holotype, CAS!).

Erect perennial herb up to 91 cm tall with 1--few stems, each 2 mm wide, arising from a woody caudex, naphthoquinones not observed, stems strigose and hispid to strigose and long hispid. Leaves cauline, sessile; blades elliptical to oblanceolate, rarely ovate, 5--8.5 x 1.4--3.3 cm, apex acute to acuminate, rarely mucronate, base acute to truncate, margins entire, planar, adaxially strigulose and strigose and abaxially dense strigulose and sparse strigose, midvein evident, secondary veins evident, sunken. Inflorescence terminal dichasia and monochasia, up to 19 cm long, bracts sessile, blades lanceolate, 0.6--2(--3.8) x 0.1--0.6(--1.8) cm, apex acuminate, base acute to truncate, margins entire, planar, adaxially strigulose and strigose and abaxially dense strigulose and strigose, midvein evident, secondary veins sometimes evident, sunken. Flowers homostylous, chasmogamous; pedicels 0--11 mm long; sepals lanceolate, 5--7(--up to 11 in fruit) x 1 mm, apex acuminate, abaxially strigose and strigulose to strigose and hispid and adaxially strigulose; corolla campanulate, white with greenish tips, abaxially villous to dense villous, tube 15--16 x 5--7 mm, adaxial trichomes absent, lobes 2 x 2 mm, erect, deltoid, rarely orbicular, apex acute, rarely rounded, margins entire, glands absent, faucal appendages absent, annulus absent; stamens inserted, filaments 3--4 x 1 mm, attached 8--10 mm above the base of the corolla, anthers 3 x 1 mm, ellipsoid; style 16--20 x 0.1 mm, exserted up to 7 mm from the corolla tube; stigma terminal.  Nutlets 3 x 2--2.5 mm, ovoid, white to dark tan, smooth, scar not prominent, base rounded, apex acute.

Distribution: Restricted to Durango.

Phenology: Flowers and fruits in mid-September.

Discussion: Turner (1994b) described this species and assigned it to *Lasiarrhenum*. *Lithospermum confundum* produces flowers that are very similar to those of *L. trinervium*, the type of *Lasiarrhenum*; however, the leaves of the two species differ. Whereas *L. trinervium* bears narrow leaves with one or two pairs of secondary veins, *L. confundum* develops broad leaves with multiple pairs of secondary veins. The broad leaves of *L. confundum* are similar to those of *L. flavum*. Furthermore, the only known specimen of *L. confundum* was found towards the northern edge of the geographic ranges of both *L. trinervium* and *L. flavum*. Given the similar flowers, this species is probably closely-related to *L. trinervium*; however, the existence of *L. confundum* raises questions concerning pollination biology and barriers to gene flow at the edge of ranges.

Specimens Examined: MEXICO. **Durango**: Mpio. El Salto, SE face of El Espinazo del Diablo, 20 km SE of La Ciudad, elev. 2770 m., Sep. 12, 1973, *D. E. Breedlove 43946* (CAS).

10. *Lithospermum discolor* M. Martens & Galeotti *Bull. Acad. Roy. Sci. Bruxelles* 11: 337. 1844. TYPE: Mexico. 1840. *H. G. Galeotti 1261* (holotype, BR [digital image]!; isotype, GH!).

*Lithospermum obtusiflorum* Sessé & Moç. *Naturaleza (Mexico City) ser. 2*, 29. 1893. TYPE: Mexico. México: Mountains, Valle de Bravo, *Sessé & Moçiño 1737* (lectotype, MA).

*Lithospermum chersinum* J. F. Macbr. *Contr. Gray Herb.* 49: 22. 1917. TYPE: Mexico. Guerrero: Cerro Verde, near the boundary between Michoacán and Guerrero, 1400 m., Nov. 6 1898, *E. Langlassé 581* (holotype, GH!).

*Lithospermum hypoleucum* I. M. Johnst. *Contr. Gray Herb.* 69: 23. 1924. TYPE: Mexico. Michoacán: Vicinity of Morelia, Lorna Santa Maria, alt. 1850 m., July 29, 1909, *Bro. G. Arsène 2511* (holotype, GH!; isotype, US!).

Erect perennial herb up to 1.5(--2.5) m tall with 1--few stems, each 2--5 mm wide, arising from a woody caudex, naphthoquinones rarely present, stems strigose and strigulose to dense strigose and dense strigulose to hispid and strigulose to dense hispid and dense strigulose, rarely also long hispid, trichomes rarely glandular. Leaves cauline, sessile or petiolate; petioles 1--2(--3) x --2 mm; blades (2.1--)2.5--10 x 0.5--2.6(--3.4) cm, lanceolate to elliptical, rarely ovate, apex acute to acuminate, rarely cuspidate, base acute to rounded, margins entire, planar, adaxially strigose and strigulose to strigose and hispidulose to strigose and scabrous and abaxially woolly, rarely villous, midvein evident, other orders of veins evident, sunken. Inflorescence terminal dichasium or monochasium and sometimes axillary monochasia, up to 33 cm long, bracts sessile or petiolate, petioles 1--2 x 0.5--1 mm; blades lanceolate, rarely ovate, 0.6--6.8 x 0.15--2.1(--2.6), apex acuminate, sometimes acute, base acute to rounded, margins entire, planar, adaxially strigose to strigose and strigulose to strigose and hispidulose to strigose and scabrous and abaxially woolly, rarely villous, midvein evident, other orders of veins evident, sunken. Flowers heterostylous, chasmogamous; pedicels 0--9 mm; Long style flowers with sepals lanceolate, 4--9(--up to 14--17 in fruit) x 0.75--1.25(--up to 2 in fruit) mm, apex acute to acuminate, abaxially strigose to dense strigose, rarely strigose and strigulose to dense strigose and dense strigulose and adaxially strigulose; corolla funnelform, white with a yellow center, abaxially villous to dense villous, tube 7--11 x 2--3 mm, adaxial trichomes present, villous in the lower two-thirds to half of the tube, lobes 2--5 x 2--4 mm, flared, elliptical, apex rounded to obtuse, rarely acute, glands present in the upper half to quarter of the tube, faucal appendages absent, annulus of 5 membranous flanges; stamens inserted, filaments 0.25--0.5 x 0.1 mm, attached 2--3 mm above the base of the corolla, anthers 1.25--2 x 0.5 mm, ellipsoid; style 6--10(--11) x 0.1 mm, inserted; stigma terminal. Short style flowers with sepals lanceolate, 5--7(--up to 14 in fruit) x 0.75--1(--up to 1.5 in fruit) mm, apex acute to acuminate, abaxially strigose to strigose and strigulose to dense strigose and adaxially strigulose; corolla funnelform, white with a yellow center, abaxially villous to dense villous, tube 9--13 x 2--4 mm, adaxial trichomes present, villous in the lower three-quarters to two-thirds of the tube, lobes 2--5 x (1.5--)2.5--5 mm, flared, elliptical, apex rounded, glands present in the upper quarter to third of the tube, faucal appendages absent, annulus of 5 membranous flanges to a membranous ring; stamens inserted, filaments 0.25--1 x 0.1 mm, attached (7--)8--9(--11) mm above the base of the corolla, anthers 1--1.75 x 0.5 mm, ellipsoid; style 2--3 x 0.1 mm, inserted; stigma terminal. Nutlets 2.5--4 x 2--3 mm, ovoid, white to tan, usually smooth, sometimes dimpling around not prominent scar, base rounded, apex acute.

Distribution: Western and southern Mexico.

Phenology: Flowers late July until late October, fruits late July.

Discussion: *L. discolor* differs from other heterostylous species of *Lithospermum* in three manners: it bears white corollas, its cauline leaves have evident secondary veins that are sunken, and individuals can grow taller than 80 cm. Most other heterostylous species of *Lithospermum* bear yellow or orange corollas, have cauline leaves with an evident midvein and no secondary veins, and produce individuals that are shorter than 50 cm.

Although this species is mainly found in southwestern Mexico, such as in Guerrero, Michoacán, Nayarit, and Oaxaca, some populations are present in Jalisco, and one specimen was collected in Chihuahua. It would not be surprising to find that *L. discolor* has an even broader geographic range, perhaps with populations in Zacatecas or Durango.

Specimens Examined: MEXICO. **Chihuahua**: Sierra Charuco, Rio Fuerte, July 23, 1935, *H. S. Gentry 1536* (F). **Colima**: Comala, 2 – 3 km al SW de Zacualpan, 500 – 1000 m., al S de Campo Cuatro, Camino Juluapan-Campo Cuatro, 19° 21' N 103° 50' W, 1000 – 1200 m.s.n.m., Sep. 6, 1990, R. Cuevas G. & L Guzman H. 3963 (MEXU). **Guerrero**: Mpio. Chichihualco, 25 km al NE de Filo de Caballo camino a Xochipala, alt. 1700 m.s.n.m., Sep. 8, 1983, *E. Martínez S. 4284* (MEXU); Mpio. Chichihualco, 12 km al SO de Xochipala, 1700 m.s.n.m., Sep. 25, 1983, *G. Lozano 294* (MEXU); On road to Filo de Caballo, N 17° 45' 51.9" W 99° 42' 17", alt. 1865 m., Aug. 10 – 17, 2008, *J. Cohen 243* (BH); On road to Filo de Caballo, N 17° 46' 33" W 99° 42' 16.1", alt. 1733 m., Aug. 10 – 17, 2008, *J. Cohen 246* (BH). **Jalisco**: Volcano of Colima, July 14, 1892, *M. E. Jones 324* (US); Mpio. Zumpango, 7 km al sur de Miraval, 1973, *F. González Medrano 6231* (MEXU); Mpio. Jocotepec, Cerro Viejo, Paraje, Barranca del Agua, subiendo por Zapotitán de Hidalgo, 2300 m.s.n.m., Oct. 1, 1991, *M. Cházaro B., J. Metzger, & R. Acevedo 6762* (MEXU, TEX); Mpio. Mascota, 20 km al NE de Mascota, alt. 1500 m., Oct. 5, 1982, *R. Hernández M. 9096* (MEXU). **México**: 5 km S of Temascaltepec, 18° 57' N 100° 5' W, alt. 1900 m., Sep. 3, 1965, *K. Roe, E. Roe, & S. Mori 1703* (MEXU); Mpio. Tejupilco, Pie de la Loma, elev. 600 m.s.n.m., Nov. 20, 1973, *G. Medrano F. 6491* (MEXU); Tejupilco, Cerro Nanchititla, Sep. 8, 1954, *E. Matuda 31529* (MEXU); 3 km al SW de Temascaltepec, sobre la carretera a Tejupilco, atl. 2000 m., Sep. 3, 1965, *Rzedowski 20754* (MEXU); 6 km S of Temascaltepec on Hwy 130, alt. 2000 m., 18° 57’ N 100° 5’ W, alt. 1900 m., Sep. 3, 1965, *K. Roe, E. Roe, & S. Mori 1679* (F); Temascaltepec, Rincón del Carmen, 1340 m., Oct. 3, 1932, *Geo. B. Hinton 1951* (F). **Michoacán :** Coalcoman, Puerto de Las Cruces, 1300 m., Sep. 12, 1938, *Hinton et al. 12179* (F, US); Vicinity of Morelia, Sep. 8, 1910, 1900 m., *Bro. G. Arsène 6993* (US); Arteaga, Fresnal, 1400 m., Sep. 27, 1939, *Hinton et al. 15233* (LL, US); Uruapan, Tancitaro, 1725 m., Oct. 18, 1940, *Hinton et al. 15552* (F, MEXU, US); Coalcoman, Sierra Torricillas, 2340 m., Oct. 17, 1938, *Hinton et al. 12405* (F, US); East of San Juan Nuevo, (ca. 8 kms. south of Uruapan), elev. ca. 6100 ft., Oct. 11-15, 1961, *R. M. King & T. R. Soderstrom 4775* (US); Vicinity of Morelia, Cerro Azul, alt. 2200 m., Sep. 15, 1909, *Bro. G. Arsène 3380* (US); Vicinity of Morelia, Lorna Santa Maria, alt. 1950 m., Aug. 15, 1909, *Bro. G. Arsène 2938* (US); Uruapan, Nov. 15, 1900, *C. G. Pringle 13671* (US); Vicinity of Morelia, Punguato, alt. 2100 m., Aug. 29, 1912, *Bro. G. Arsène s.n.* (US); Vicinity of Morelia, Lorna Santa Maria, alt. 1950 m., Aug. 28, 1910, *Bro. G. Arsène 5844* (US); Tancitaro, pedregal lava flow 2 mi. south of Tancitaro, elev. 6500 ft. Aug. 15, 1940, *Wm. C. Leavenworth 568* (F, LL); Mpio. Coalcoman, 0.3 mi (0.5 km) NE of KM 50 on road from Coalcomán to Tepalcatepec, 14.1 mi (22.6 km) NE of Coalcomán and 31.4 mi (50.7 km) SW of Tepalcatepec, 1160 m., Sep. 13, 1985, *C. P. Cowan 5683* (TEX); Thirteen miles east of Moralia, Aug. 13, 1947, *F. A. Barkley, J. B. Paxson, & G. L. Webster 2753* (TEX); On road from Morelia from Hidalgo, near a waterfall, ca. 13 miles E of Morelia, and E of the waterfall on a hillside, N 19° 39.216’ W 100° 56.972’, 7256 ft., *J. Cohen 216* (BH); Mpio. Apatzingan, Tancitaro, above La Presa, elev. 3600 ft., Aug. 25, 1941, *Wm. C. Leavenworth & H. Hoogstraal 1774* (F); Abou 18 kms. from Uruapan on road to Apatzingán, alt. 1300 m., Nov. 14, 1949, *H. E Moore Jr., E. Hernandez X., & H. Porras H. 5737* (BH). **Nayarit**: Cerro de San Juan, W. of Tepic, 1000 m., Sep. 19, 1926, *Y. Mexia 712* (US); Mpio. Tepic, 10 km al E de

La Yerba, carretera La Yerba – Tepic o al SE de la desviación La Peñita, 21.31.20 N 105.00.57 W, alt. 945 m., Sep. 15, 1994, *G. Flores-Franco 3691* (MEXU); Mpio. Xalisco, km 4 del camino de terraceria de Xalisco a la Estación de Microondas Cerro Boludo, 21.27 N 104.56 W, alt. 1250 m., Oct. 3, 1993, *O. Tellez-Valdes, G. Flores, & A. Dominguez 12997* (MEXU); Mpio. Tepic, slope of Volcán San Juan along small road at km 6 on road from Tepic to Jalcocotan, elev. 900 m., Aug. 8, 1980, *D. E. Breedlove 45196* (MEXU). **Oaxaca**: Miahuatlan, Campamento San Mateo, 14 kms. al oeste de San Jerónimo, alt. 1990 m.s.n.m., Oct. 23, 1980, *R. Hernández M. 5162* (MEXU); 19 km southwest of Sola de Vega along road to Puerto Escondido, elev. 7000 ft., Aug. 30, 1965, *D. E. Breedlove 12285* (MEXU); 15 km (by road) SE of Miahuatlan on road to Puerto Angel in high mountains of Sierra Madre del Sur, 16° 12’ N 96° 30’ W, alt. ca. 2400 m., July 6, 1969, *B. Marcks & C. Marcks 1032* (LL); Western slope of Sierra Madre del Sur, 35 km S of Juchatengo on road to Zimatlan at km 178, alt. ca. 1700 m., 16° 15’ N 97° 5’ W, July 25, 1965, *K. Roe, E. Roe, & S. Mori 579* (F).

11. *Lithospermum distichum* Ortega *Nov. Rar. Pl. Descr. Dec.* 8. 1797. *Batschia disticha* G. Don *Gen. Hist.* 4: 326. 1838. TYPE: Mexico. No State: “Cuba”, grown in the Madrid Botanical Garden, *Sessé & Moçiño s.n.* (possibly 1732) (holotype, MA).

*Lithospermum spathulatum* M. Martens & Galeotti *Bull. Acad. Roy. Sci. Bruxelles* 11: 337. 1844. TYPE: Mexico. No State: *H. G. Galeotti 1259, 1273* (syntype, BR [digital image]!).

*Lithospermum sordidum* Brand *Repert. Spec. Nov. Regni Veg.* 28: 15. 1930. TYPE: Mexico: Prope San Jose del Oro, June 1831, *C. J. W. Schiede s.n.* (lectotype, GH). **lectotype designated here**, see discussion under *L. calcicola*.

*Lithospermum approximatum* Brand *Repert. Spec. Nov. Regni Veg.* 28: 15. 1930. TYPE: Mexico. México: District Tlalmanalco, bergwald bei San Rafael, Aug. 18, 1907, *C. Seler & E. G. Seler 5322*. (lectotype, GH). **lectotype designated here**, see discussion under *L. calcicola*.

*Myosotis grandiflora* Kunth *Nov. Gen. Sp.* 3: 90, t. 199. TYPE: Mexico. No State: Real del Monte, Humboldt & Bonpland 4083 (holotype, P [IDC microfiche Humboldt and Bonpland Herbarium 66/1]!).

*Myosotis foliosa* Lehm. *Pl. Asperif. Nucif.* 1: 99. 1818. TYPE: Mexico. No State: America meridional, *Humboldt s.n. (MEL 2340047)* (holotype, MEL [digital image]!).

*Lithospermum laevigatum* Sessé & Moc. *Naturaleza (Mexico City)* ser. 2, 32. 1893. TYPE: Mexico. D.F.: Mountains, San Nicolás, *Sessé & Moçiño 5226* (according to Sutherland [1997]) (lectotype, MA).

*Anchusa mexicana* Sessé & Moc. *Pl. Nov. Hisp.* 21. 1888. TYPE: Mexico. Michoacán: Pátzcuaro, San Nicolás, *Sessé & Moçiño s.n.* (holotype, not located).

Erect perennial herb up to 44.5 cm tall with 1--few stems, each 1--4 mm wide, arising from a woody caudex, naphthoquinones rarely present, stems strigose to strigulose to dense strigose. Leaves cauline and sometimes pseudobasal, sessile or petiolate, cauline leaves petioles 1--3 x 1--2 mm; blades elliptical to lanceolate to oblanceolate, sometimes ovate, 1.1--7.8(--10.5) x 0.3--1.7(--2.5) cm, apex rounded to acute, base rounded to truncate to acute, margins entire, planar, adaxially and abaxially strigose to strigulose, midvein evident, other orders of veins sometimes evident; basal leaves epetiolate; blades oblanceolate, rarely spatulate, 0.9--11.3 x 0.3--2.1 cm, apex acute to rounded, base acute to truncate, margins entire, planar, adaxially and abaxially strigose to strigulose, midvein evident, other orders of veins often evident. Inflorescence terminal monochasium, dichasium, trichasium, up to 19.5 cm long, bracts sessile or petiolate, petioles 0.5--2 x 1--2 mm; blades lanceolate to ovate to elliptical, 0.4--3 x 0.1--1.3 cm, apex acute, rarely rounded, base rounded to truncate to acute, margins entire, planar, adaxially and abaxially strigose to strigulose, rarely dense strigose, midvein evident, other orders of veins not evident. Flowers homostylous, chasmogamous; pedicels 0--7 mm long; sepals lanceolate, 2--5(--up to 7 fruit) x 0.75--1 mm, apex acute, abaxially strigose to strigulose to dense strigose and adaxially strigulose; corolla salverform, white with a yellow center, abaxially villulous, tube 3--5(--7) x 1--2 mm, adaxial trichomes absent, lobes 2--5 x 2--4 mm, flared, elliptical to orbicular, apex rounded to obtuse, margins entire, glands present towards the apex, faucal appendages present, annulus of 5 flanges in a continuous ring; stamens inserted, filaments 0.25--1 x 0.1 mm, attached 2--3(--6) mm above the base of the corolla, anthers 1 x .5 mm; style (2--)3--5 x 0.1 mm, inserted; stigma subterminal. Nutlets 2--3 x 2 mm, white to tan, smooth with pitting around slightly prominent scar, base rounded, apex acute.

Distribution: Widely distributed throughout eastern, central, and southern Mexico, and into Guatemala.

Phenology: Flowers from late March to October, fruiting begins late April.

Discussion: *L. distichum* is the most widespread Mexican species of *Lithospermum*. Its geographic range stretches from Nuevo León to Guatemala. Throughout its distribution, the form of *L. distichum*, especially that of its leaves, varies. Most of the structural variation does not exhibit any geographic pattern, but there are two exceptions: stature and indument. In Veracruz, the individuals of *L. distichum* are much shorter than those in other states, and towards the northern part of its distribution, the indument of individuals of *L. distichum* is usually much denser than that of individuals in more southern areas.

Johnston (1952) recognized both *L. sordidum* and *L. distichum*; however, in the present revision *L. sordidum* is treated as a synonym of *L. distichum*. According to Johnston (1952), individuals of *L. sordidum* are smaller than those of *L. distichum*, and bear a spreading indument. Despite this distinction, the features that supposedly separate individuals of the two species grade into each other.

Specimens Examined: MEXICO. **Chiapas**: Ametenango del Valle, alt. 1835 m., June 12, 1945, *E. Matuda 5876* (F, LL); San Cristobal Las Casas, cerro San Cristobal, elev. 7000 ft., July 5, 1964, *D. E. Breedlove 6017* (F); Mountains along route 190, about 5 miles east of San Cristóbal de Las Casas, June 24, 1960, *R. M. King 3005* (MICH, TEX, US); Tapisca, May 9, 1904, *E. A. Goldman 957* (US); Mountains along route 190, about 5 miles east of San Cristóbal de Las Casas, June 11, 1960, *R. M. King 2808* (MICH, TEX, US). **DF**: Ajusco near Rancho Ecologia, N 19° 6’ 34” W 99° 11’ 27.5”, alt. 2885 m., June 20, 2007, *J. Cohen 202* (BH); Contreras, Aug. 9, 1910, *C. R. Orcutt 3502* (F); La Cima de Ajusco, 9800 ft., Aug. 2, 1906, *C. G. Pringle 13775* (F, MICH); N.E. of Cerro Xitle, alt. 8400 ft., Sep. 13, 1930, *P. G. Russell & M. J. Souviron 177*

(US); Fields along route 95, 42 km. south of Mexico City, June 19, 1960, *R. M. King 2901* (MICH, TEX, US); Contreras, Sep. 1937, *E. Lyonnet 1646* (US); Contreras, alt. 3000 m., July 26, 1941, *P. Lyonnet 3262* (US); Sierra de Ajusco, July 18, 1901, *J. N. Rose & R. Hay 5532* (US); La Cima De Ajusco, alt. 9800 ft., Aug. 2, 1906, *C. G. Pringle 13775* (TEX, US); Cañada de Contreras, Sep. 1927, *E. Lyonnet 123* (US); Cima, Aug. 24, 1910, *C. R. Orcutt 6619* (US); Cima de Toluca, alt. 3250 m., Apr. 1924, *E. Lyonnet 31* (US); Contadero, delegación de Cuajimalpa, alt. 2800 m., Aug. 19, 1965, *Rzedowski 20437* (TEX, US); Ladera sur del Xitle, alt. 3100 m., June 19, 1980, *M. A. Panti Madero 125* (MEXU); Delegación de Milpa Alta, Paraje de Cuauhte, alt. 2700 m., July 4, 1976, *A. Ventura A. 1731* (MEXU); Highway 190, between Puebla and Mexico City, May 27, 1965, *D. Ahshapanek 700* (TEX); At Estacion La Cima, W. of hwy 95 (libre) from Cd. Mexico to Cuernavaca, alt. 3169 m., Aug. 3, 1979, *M. A. Lane 2570* (TEX); Twenty miles south of Sierra Pelado, July 12, 1947, *Miranda, Barkley, & Webster 2257a* (TEX). **Hidalgo**: Sierra de Pachuca, alt. 9500 ft., July 28, 1898, *C. G. Pringle 7587* (F); El Chico, July 29, 1938, *E. Lyonnet 2227* (US); Between Pachuca and Real del Monte, July 19, 1905, *J. N. Rose, Jos. H. Painter, & J. S. Rose 8674* (US); Sierra de Pachuca, summit of ridge ca. 2 kms. south of Real del Monte, alt. ca. 2980 m., July 7, 1959, *J. H. Beaman 2750* (TEX, US); District Pachuca, near Zerezo and below Parque Nacional El Chico, alt. 3000 m., June 20, 1947, *H. E. Moore Jr. 3144* (BH); District Pachuca, above Pueblo Nuevo and below Parque Nacional El Chico on road from Real del Monte to El Chico, alt. ca. 3000 m., July 6, 1948, *H. E. Moore Jr. 3699* (BH); District Pachuca, near Zerezo and below Parque Nacional El Chico, alt. 3000 m., May 12, 1947, *H. E. Moore Jr. 2805* (BH). **Jalisco**: Valley of Zapotlan, alt. 5000 ft., Aug. 7, 1905, *P. Goldsmith 105* (F, US); Mpio. Real del Monte, cerca de la Mina de Morán, alt. 2550 m., May 25, 1999, *G. Ocampo & S. Zamudio 739* (MEXU, TEX). **México**: Valley of Toluca, Aug. 1982, *C. G. Pringle 4203* (F, US); South of Mexico City, elev. 9500 ft., July 12, 1941, *Mr. & Mrs. Wm. Leavenworth 951* (F); Old road Mexico-Puebla, alt. 8500 ft., June 8, 1981, *D. D. Soejarto & C. Compadre 5378* (F); Mpio. Ixtapaluca, Estación de Experimental de Investigacion y Enseñaza de Zoquiapan, 8 km. S. de Río Frio, camino 4, 4 km. S. entronque con camino 3, alt. 3300 m., June 20, 1975, *S. D. Koch 75204* (F); Amacameca, alt. 8100 ft., July 26, 1924, *Geo. L. Fisher 263* (F, US); Salto de Agua, Dec. 1905, *C. A. Purpus 1767* (F); Iztaccihuatl, south side of mtn. between Altzomoni and La Joya, 0.7 km. south of La Joya, alt. ca. 3980 m., July 7, 1960, *J. H. Beaman 3493* (US); 55 km SE of Mexico City, alt. 10500 ft., July 5, 1942, *J. N. Weaver 725* (US); Cerro Pelado, July 1937, *E. Lyonnet 1974* (US); Ojos de Agua, Nevada de Toluca, alt. 12000 ft., July 14, 1938, *E. K. Balls 5048* (US); Paraje Provincial, Popocatepetl, alt. 11800 ft., April 12, 1938, *E. K. Balls 4175* (US); Istaccihuatl, alt. 3950 to 4000 m., July 30, 1958, *J. H. Beaman 1946* (TEX, US); 33 miles east of Mexico, D.F., on road to Puebla, ca. 10000 ft., July 9, 1940, *C. L. Hitchcock & L. R. Stanford 7026* (US); Near Tultenango, July 13, 1901, *J. N. Rose & R. Hay 5444* (US); Temascaltepec, alt. 2430 m., June 6, 1932, *G. B. Hinton 846* (US); Slope of Popocatepetl, below Puerto Tlamacas, Aug. 12, 1943, *C. L. Lundell & A. Lundell 12368* (TEX, US); Mpio. Villa Victoria, Cerca de la Presa, 6 km al SO de Villa Victoria, elev. 2520 m., July 6, 1985, *J. C. Soto Nuñez 8953* (MEXU); Mpio. Otumba, Cerro Buena Vista, alt. 2700 m., July 14, 1975, *A. Ventura A. 710* (MEXU); Mpio. Ocoyoacac, Camino a Ocoyoacac desde la autopista México-Toluca, 19° 16’ 45.4” N 99° 29’ 3.5” W, alt. 2594 m., June 10, 2001, *H. Vibrans 7286* (MEXU); Mpio. Río Frio, Parque Nacional Zoquiapan, Paraje Loma de San Diego, alt. 3350 m., Sep. 27, 1979, *A. Mancera O. 12* (MEXU); 5 km al NE de Amecameca; laderas del Cerro de la Coronilla, alt. 2650 m., Sep. 22, 1968, *A. Pineda R. 563* (LL); Road beyond Capulin at 20 km. marker, from junction road to Temescaltepec and Nevada de Toluca, July 30, 1977, alt. 11000 m., *Dunn, Torke, Bennett, & Wieder 22567* (TEX); Mpio. Amecameca, slopes of Popocatepetl between 10000 and 12000 ft., July 4, 1943, *C. L. Gilly and D. Dodds 16* (TEX); About 15 km S of Toluca along the road to Tenancingo and 3.5 km E on road through village of S. M. Chapultepec, 19° 12' N, 99° 33' W, Aug. 18, 1977, *R. W. Sanders 1069* (TEX); 35 miles northwest of Toluca, Aug. 14, 1947, *F. A. Barkley, C. M. Rowell, Jr., & G. L. Webster 2803* (TEX); Highway 150 (libre) near Selva Obscura, 67 km s of Mexico City, Aug. 1, 1971, *A. C. Gibson & L. C. Gibson 2540* (TEX); Mountains 20 miles south of Mexico City, Sep. 1, 1946, *A. I. Ortenburger, J. B. Paxon, & F. A. Barkley s.n.* (TEX); Mpio. Ixtapaluca, Campo Experimental de Investigación y Enseñanza de Zoquiapan, carretera México-Puebla 8 km. al SE de Río Frío, July 8, 1978, *J. García P s.n.* (TEX); 5 miles east of Lerma, alt. 9500 ft., July 29, 1956, *O. S. Fearing & J. S. Thompson 96* (TEX); 20 m. east of Toluca, on way to Mexico D.F., alt. ± 9000ft., July 1, 1957, *E. Meyer 295* (BH). **Michoacán**: Next to dirt road that parallels the road from Nahuatzen to Pátzcuaro, Aug. 10 – 17, 2008, *J. Cohen 223* (BH); Along road from Caraupan to Cheran, around km 20, Aug. 10 – 17, 2008, *J. Cohen 229* (BH); Mpio. Zinapécuaro, Cerro San Andrés, alt. 3400 m., Aug. 29, 1987, *S. Zamudio R. 5566* (MEXU); At km. 19 (by road) S. of Carapan (on road to Uruapan), ca. 19° 45' N, 102° 3' W, alt. 2300 m., July 27, 1960, *H. H. Iltis & R. Koeppen 476* (TEX); Mpio. Pátzcuaro, 2 km al SW de Los Tanques, alt. 2400 m., H. Díaz Barriga 2431 (TEX). **Morelos**: Along route 95, 15 miles North of Cuernavaca, Aug. 16, 1961, *A. M. Powell & J. Edmondson 737* (F, TEX). **Nuevo León**: Mpio. Zaragoza, approx. 20 km from San Antonia Peña Nevada, N 23° 49' 25.2" W 99° 52' 57.4", alt. 8680 ft., June 15, 2007, *J. Cohen 194* (BH); Mpio. Zaragoza, along roadside, June 17, 2007, *J. Cohen 192* (BH); On road from Zaragosa to Aserradero la Encantada, 10.7 road miles south of Zaragosa, May 20, 1988, *B. L. Westlund 5.20.88.36* (TEX); Mpio. Zaragoza, Trail from Cañada La Tinaja to Rancho La Encantada, 23° 57’ N 99° 49’ W, alt. 2600 – 2700 m., July 4, 1988, *T. F. Patterson 5861* (TEX); Mpio. Zaragoza, Trail from Canada La Tinaja to Rancho La Encantada, 23° 57’ N 99° 49’ W, alt. 2600 – 2700 m., July 4, 1988, *T. F. Patterson 5864* (TEX); Mpio. Zaragoza, Camino de la Encantada a la Tinaja, 23° 57’ N 99° 54’ W, alt. 2500 – 2800 m., May 25, 1992, *L. Hernadez S. 2767* (TEX). **Oaxaca**: NW side of summit of Mt. Zempoaltepec, alt. 10000 – 11000 ft., July 9, 1894, *E. W. Nelson 657* (US); Near Reyes, alt. 7500 – 10400 ft., Oct. 17, 1894, *E. W. Nelson 1732* (US); Eighteen miles southwest of the City of Oaxaca, alt. 7500 – 9500 ft., Sep. 10 – 20, 1894, *E. W. Nelson 1399* (US); Mpio. Miahuatlan, S. side of Cerro Quiexobra, 1 – 3 km NE of La Cieneguilla, on road to summit of mountain, alt. 2900 m., Oct. 2, 1990, *A. McDonald 2983* (TEX); Miahuatlán, Neverías, alt. 2650 m., *Hinton et al. 26531* (LL); Distrito de Coixtlahuaca, Cerro verde, 5 km S de Coixtlahuaca, alt. 2600 – 2850 m., July 7, 1986, *A. García M., 2351* (TEX). **Puebla**: Mt. Orizaba, July 4, 1904, *Heller & Barber 31* (F); Puerto Tlamacas, Volcán Popocatépetl, alt. 12000 ft., July 4, 1943, *C. L. Lundell 12180* (LL, US). San Luis Potosí: 1879, *J. G. Schaffner 360/729* (US); Vicinity of Puebla, Mayorazgo, alt. 2120 m., July 4, 1907, *Bro G. Arsène 1349* (US); Cerro El Pinar, Aug. 28, 1945, *E. Hernandez X. 2266* (MEXU); Mpio. Cholula, Camino Cerro Teoton lado Este San Pedro Yancuictlalpan, 19° 4’ 30” - 19° 4’ 50” N 98° 29’ - 98° 27’ 30” W, alt. 2450 – 2600 m., June 6, 1987, *M. Tlapa A. & G. Ubierna 1308* (TEX); Mpio. Cholula, Santiago Xalitzintla entrada por el lado sur, 19° 4’ 45” - 19° 3’ 30” N 98° 31’ - 98° 30’ W, alt. 2580 – 2650 m., Nov. 3, 1987, *T. Tlapa A. & G. Ubierna 1246* (TEX); Mpio. Tlachichuca, 10.9 km (12.3 mi) from Tlachichuca on dirt road to summit of Orizaba, near Miguel Hidalgo, June 7, 1983, *C. P. Cowan 3885* (TEX); Pico de Orizaba, below pueblo of Hidalgo, ca. 10 km NE of Tlachichuca, elev. 10700 ft. (3240 m.) June 17, 1974, *R. Sanders 74084* (LL); Mpio. Cholula, Camino a Santiago Xalitzintla entrada lado oriente, 19° 4’ 15” - 19° 3’ 45” N 98° 29’ 30” - 98°

31’ W, alt. 2500 – 2580 m., Oct. 29, 1987, *T. Tlapa A. & G. Ubierna 1228* (TEX); Mpio. Esperanza, Esperanza, alt. 2375 m., July 29, 1971, *F. Ventura A. 3964* (TEX); Mpio. Cholula, Tapetzingo camino por el lado Norte de antiago, 19° 4’ 45” - 19° 5’ 15” N 98° 31’ - 98° 31’ 30” W, alt. 2580 – 2650 m., Oct. 8, 1987, *T. Tlapa A. & G. Ubierna 762* (TEX). **Veracruz**: Cofre de Perote, ca. 1 km below the summit, 11 km (by air) Se of town of Perote, 19° 30’ N 97° 11’ W, alt. 3650 – 3700 m., June 27, 1982, *G. Diggs, N. Nee & G. Schatz 2616* (F); Mpio. Calcahualco, Pico de Orizaba, 5 km. SW of Jacal, part of the Coscomatepec-Escola-Jacal road going over the pass to Miguel Hidalgo and Tlachichuca in Edo. Puebla, 19° 5’ N 97° 15’ W, alt. 3450 – 3700 m., July 6, 1982, *M. Nee & G. Diggs. 24812* (F); Mpio. Maltrata, along hwy. Mex. 150, 5.5 km. by road below Edo. Puebla-Veracruz state line, just below village of Guadalupe Magueyes, 6 km. by air N of Maltrata, 18° 52’ N 97° 16” W, alt. 2175 m., May 1, 1983, *M. Nee & K. Taylor 27019* (F); Cerca de las Vigas, camino a los Manantiales, Rafael Ramirez, alt. 2920 m., Mar. 21, 1976, *J. I. Calzada, C. Horvitz, & Victor T. 2285* (F); Below Lomogrande, Mt. Orizaba, 9000 ft., Apr. 5, 1938, *E. K. Balls 4425* (US); Cofre de Perote, alt. 10300 ft., May 2, 1939, *E. K. Balls 4563* (US); Mpio. Prof. Rafael Ramirez, at microwave station, 16 km along SSW of Las Vigas, 19° 31’ N 97° 9’ W, al. ca. 3400 m., Oct. 26, 1983, *B. L. Turner 15385* (TEX).

12. *Lithospermum dodrantale* (I. M. Johnst.) J. I. Cohen *Brittonia* 61: 108. 2009. Basionym: *Onosmodium dodrantale* I. M. Johnst. *J. Arnold Arbor.* 18: 22. 1937. TYPE: Mexico. Nuevo León: Mpio. Galeana, the peak of Cerro Potosi, July 21, 1935, *C. H. Mueller 2259* (holotype, GH, isotypes, F [digital image]!, GH, MEXU!, MICH [digital image]!, MO!)

Erect perennial herb up to 28 cm tall with 1--few stems, each 1--3 mm wide, arising from a woody caudex, naphthoquinones not observed, stems strigose to hispid. Leaves cauline, sessile; blades lanceolate to narrow elliptical, 1.5--5 x 0.3--1.2 cm, apex acute, sometimes rounded, base acute to rounded, margins entire, planar, rarely revolute, adaxially strigose, rarely dense strigose, and abaxially strigose, sometimes dense strigose, often dense strigose on veins, midvein evident, secondary veins evident, sunken. Inflorescence terminal dichasium or monochasium, up to 4 cm long, bracts sessile, blades lanceolate, sometimes narrow elliptical, 1--3.7 x 0.2--0.8 cm, apex acute, base rounded to acute, margins entire, planar, rarely revolute, adaxially strigose and abaxially strigose, sometimes dense strigose, often dense strigose on veins, midvein evident, secondary veins evident, sunken. Flowers homostylous, chasmogamous; pedicels 0--7 mm long; sepals lanceolate, 6--11 x 1--2 mm, apex acute, abaxially strigose to dense strigose and adaxially strigulose; corolla campanulate, yellow with green lobes, abaxially villous towards the apex and glabrous towards the base, tube (7--)9--13 x 4--5 mm, adaxial trichomes absent, lobes 2--3 x 1.5--2 mm, erect, deltoid, apex acute, glands absent, faucal appendages absent, annulus absent; stamens inserted, filaments 0.5--1 x 0.1 mm, attached 7--9 mm above the base of the corolla, anthers 2--2.5 x 0.5--1 mm, ellipsoid; style 14--27 x 0.25 mm, exserted up to 7--10(--17) mm from the apex of the corolla tube; stigma terminal. Nutlets 3.5--4 x 3 mm, ovoid to deltoid, white to tan, smooth to slight dimpling around non-prominent scar, base rounded to truncate, apex acute.

Distribution: Restricted to Nuevo León and Tamaulipas.

Phenology: Flowers early May onward, and starts fruiting in early August.

Discussion: Of the three Mexican species previously included in *Onosmodium*, *L. dodrantale* is the least distinctive. It does not bear glandular trichomes, as *L. unicum* does, and its indument is strigose to hispid, rather than hispid to long hispid, like that of *L. oaxacanum*. Although the ranges of *L. dodrantale* and *L. unicum* overlap, *L. unicum* has a broader and more southerly distribution.

Specimens Examined: MEXICO. **Nuevo León**: Sierra Madre Oriental, ascent of Infernillo, about 15 miles SW of Galeana, alt. 9-10000 ft., June 29, 1934, *C. H. and M. T. Mueller 923* (F); Mpio. Dr. Arroyo, San Antonio Peña Nevada, May 9, 1978, alt. 2740 m., *Hinton et al. 17378* (MEXU). **Tamaulipas**: On mountain top 7 kilo. southwest of Miquihuana, 23˚ 35' N 99˚ 38' W, Aug. 5, 1941, *L. R. Stanford, K. L. Retherford, and R. D. Northcraft 909* (CAS, MO).

13. *Lithospermum exsertum* (D. Don) J. I. Cohen *Brittonia* 61: 108. 2009. Basionym: *Macromeria exserta* D. Don, *Edinburgh New Philos. J.* 13: 239. 1832. TYPE: Mexico. Without locality, 1787–1804, *Sessé & Moçiño s.n.* (holotype, BM).

*Echium longiflorum* Sessé & Moç. *Pl. Nov. Hisp.* 20. 1888. TYPE: Mexico. Guererro: Mountains of Maztlán and Chilpancingo, *Sessé & Moçiño 859* (holotype, MA [negative at F]!; possible isotype, GH).

*Macromeria exserta* var. *imparta* J. F. Macbr. *Contr. Gray Herb.* 49: 22. 1917. TYPE: Mexico. Oaxaca: 1842. *M. Ghiesbreght s.n.* (holotype, GH [digital image]!; isotype, US!).

Erect perennial herb up to 2 m tall with 1--few stems, each 4--8(--12) mm wide, arising from a woody caudex, naphthoquinones not observed, stems strigose to dense strigose to hispid to dense hispid to long hispid. Leaves cauline, sessile; blades lanceolate to ovate to elliptical to obovate, rarely spatulate, 2.8--13.1(--18.5) x 1--4.2(--4.7) cm, apex acute to mucronate, base acute to rounded, margins entire, planar, adaxially and abaxially strigose, rarely hispid, strigulose, or dense strigose, midvein evident, secondary veins evident, not sunken. Inflorescence terminal dichasium or monochasium and axillary monochasia, up to 38 cm long, bracts sessile; blades ovate to lanceolate, 1.9--5.5(--8.5) x 0.5--2.7 cm, apex acute, base rounded, rarely acute, margins entire, planar, adaxially and abaxially strigose, sometimes dense strigose or strigulose, midvein evident, secondary veins evident, not sunken. Flowers homostylous, chasmogamous; pedicels 0--11 mm long; sepals linear to lanceolate, 13--34(--up to 39 in fruit) x 1--4(--up to 5 in fruit) mm, apex acute, sometimes acuminate, abaxially strigose to strigose and strigulose, usually more densely strigose towards the base, and adaxially strigulose; corolla long-funnelform with a bulge, slightly zygomorphic, yellow, abaxially villous, tube (33--40--)47--60 x 4--11 mm, adaxialy trichomes absent, lobes (8--)12--27 x 3--5(--7) mm, reflexed, deltoid, apex obtuse to acute, glands present at the apex of the corolla tube, faucal appendages absent, annulus absent; stamens exserted, filaments (32--)40--60 x 0.25--0.5 mm, attached 35--48(--58) mm above the base of the corolla, exserted 30--55 mm from the corolla throat, anthers 2--3 x 0.1--1 mm, ellipsoid; style (87--)100--121 x 0.25--0.5 mm, exserted up to 61 mm from the corolla throat; stigma terminal. Nutlets 3--5 x 3 mm, ovoid, white, sometimes tan towards the apex, smooth, scar slightly prominent, base rounded, apex acute.

Distribution: Western and southern Mexico, but most common in Guerrero, Michoacán, and Oaxaca.

Phenology: Flowers from July to September, and fruits from mid-August onwards.

Discussion: *L. exsertum* is one of the most conspicuous species of the genus, and it is the best example of a species with *Macromeria*-type flowers (Chapter 4). The corolla tubes of *L. exsertum* can be up to 6 cm long, and the style can extend up to 6 cm beyond the apex of the corolla tube. In addition, the plant can grow up to 2 m tall. *L. exsertum* grows in open forests as well as in grazed pastures and along roadsides.

*L. exsertum* is sister to *L. johnstonii*. The two species share two floral features: slightly zygomorphic corollas and reflexed corolla lobes.

Specimens Examined: MEXICO. Specimens Examined: MEXICO. **Guerrero**: Manchóm-Fundiciones, distrito Mina, September 4, 1936, *Hinton et al. 9442* (F, NY, US); Mpio. Chichihualco, "El Carrizal", 9 km al W de Iyotla, Carr. a Filo de Caballo, alt. 2000 m., August 12, 1982, *P. Tenorio L. 1277* (CAS, NY); North of Iguala, August 12, 1945, *E. J. Alexander & E. Hernandez X. 2171* (NY); Chilapa de Alvarez, km 75.5 de la carretera Chilapa-Tlapa, alt. 2100 m., September 4, 1991, *J. L. Panero 2294* (TEX); Mpio. Chichihualco, Puerto Chico, 10 km. al W de Camotla, alt. 2500 m., August 8, 1964, *Rzedowski 18512* (TEX); Mpio. Zirándaro, en La Ciénaga, 18 km al NW de Los Cimientos, August 24, 1978, *J. C. Soto Nuñez & J. R. Hernández 923* (TEX); on the road to Filo de Caballo, N 17° 45' 51.9" W 99° 42' 17", 1865 m., August 10 - 17, 2008, *Cohen 244* (BH); 20 miles northwest of Chilpancingo at Olmitemi, elev. 7000 ft., Aug. 1, 1967, *D. E. Breedlove 15823* (CAS); 1 – 2 k E of Omiltemi, vicinity of Omiltemi, ca. 60 km W of Chilpancingo, alt. ± 2000 m., July 10

– 12, 1963, *S. S. Tillett 637-156* (US); Mina, Teotepec, 2500 m., July 11, 1939, *Hinton et al. 14809* (US); Mpio. Malinaltepec, Malinaltepec, 17° 14’ N 98° 40’ W, alt. 1700 msnm, Aug. 3, 1991, *I. Wagenbreth 699* (MO). **Jalisco**: South of Zapotlan, alt. 5000, Aug. 8, 1905, *P. Goldsmith 113* (F, US); Sierra del Tigre, 3 miles south of Mazamitla, alt. 2100 - 2200 m., Sep. 16, 1952, *R. McVaugh 12945* (MICH); Mpio. Tamazula, Monte Cerrado, 19 km directamente al E de Cd. Guzmán, sobre breecha de Vista Hermosa a El Durazno, alt. 1880 m., Aug. 29, 1988, *M. Fuentes O. 601* (MO). **México**: 3 km al SE de Temascaltepec, carretera a Tejupilco, alt. 1900 m., August 27, 1984, *R. Fernández N. 2547* (NY); Volcán Temascaltepec, alt. 1490 m., August 9, 1932, *Hinton et al. 1296* (NY, US); 3 km al SW de Temascaltepec, sobre la carretera a Tejupilco, alt. 2000 m., September 3, 1965, *Rzedowski 20759* (CAS, LL); 6 km S of Temascaltepec on Hwy 130, alt. 2000 m., 18° 57’ N 100° 05’ W, Sep. 3, 1965, *K. Roe, E. Roe, S. Mori with J. Rzedowski 1682* (US); 4 km (air) SSE of Luvianos, 18° 50’ N 100° 20’ W, alt. 1500 m., Sep. 2 – 3, 1965, *K. Roe, E. Roe, S. Mori with J. Rzedowski 1643* (US). **Michoacán** : Zitacuaro-San Felipe, distrito Zitacuaro, 1950 m., August 15, 1938, *Hinton et al. 13107* (F, LL, MO, NY, US); Highway 37 17 miles S. of Uruapan, 1600 m., July 31, 1990, *A. Jack & M. Sydor 87* (TEX); ca. 4 km S of Jesus del monte, N 19° 38’ 37.3” W 101° 9’ 20.5”, 2172 m., August 10 - 17, 2008, *Cohen 217* (BH); Mpio. Apatzingan, Tancitaro, forest above Acahuato, alt. 3400 ft., Aug. 23, 1941, *WM. C. Leavenworth and H. Hoogstraal 1773* (F, MO); Mpio. Maravatío. Cerro El Calvario, San Miguel, alt. 2600 m., Aug. 21, 1991, H. Díaz Barriga y Cols. 6826 (MO); Cerro Azul, vicinity of Morelia, alt. 2000 – 2300 m., Sep. 1, 1909, *Bro. G. Arsène s.n.* (US). **Nayarit**: Trail to Santiago from Tepic, 1000 m., Sept. 15, 1926, *Y. Mexia 625* (CAS, US); Cerro de San Juan, southwest of Tepic, alt. 1100 – 1200 m., Aug. 18, 1935, Pennell 19759 (MEXU, GH). **Oaxaca**: La Herradura, carretera Nochixtlán-Oaxaca, distrito Nochixtlán, alt. 2300 m., August 1, 1988, *A. G. Mendoza*

*4014* (NY); Sierra de San Felipe, alt. 7000 ft., July - August 1894, *C. G. Pringle 4820* (NY, US); Along road from Oaxaca to Guelatao de Juárez and Tuxtepec, 6.5 miles north of intersection with Mex. Hwy 190, elev. 1850 m., July 18, 1968, *W. R. Anderson & C. Anderson 4818* (NY); Mihuatlán, ca. km 116, October 14, 1967, *T. MacDougall S.N.* (NY); 7 to 8 miles southeast of Miahuatlan along new road over Sierra Madre del Sur, elev. 6500 to 7000 ft., September 2, 1952, *H. S. Gentry 12089* (LL); 5 miles northeast of Mexican Highway 190 near Oaxaca along road to Ixtlán de Juárez, elev. 6500 ft., August 26, 1965, *D. E. Breedlove 12206* (LL); 25 km al S de Tonaltepec, carr. Teotitlán-Oaxaca, distrito de Etla, alt. 2130 m., August 2, 1985, *R. Torres C. 6940* (TEX); Hiway 175, 19.7 mi. S of Suchixtepec, August 22, 1980, *D. K. Northington, B. L. Turner, W. Elisens, & J. Johnson 1154* (TEX); 15 km (by road) SE of Miahuatlan on road to Puerto Angel in high mountains of Sierra Madre del Sur, (16° 12' N, 96° 30' W), alt. 2400 m., July 6, 1969, *B. Marcks & C. Marcks 1027* (LL); 11.4 km al SO de Ayutla, dist. Mixe., carr. Totontepec-Mitla, alt. 1900 m., August 7, 1985, *R. Torres C. 7049* (MO, TEX); Camino Monselobos, alt. 2000 m., June 23, 1907, *C. Conzatti 1843* (F); Camino Monselobos, alt. 2000 m., June 23, 1907, *C. Conzatti 1907* (F); Nacaltepec, alt. 2000 m., Aug. 23, 1976, *R. Hernández M. 2676* (CAS); Mpio. Nacaltepec, Cerro el Veinte, ca. 7 km al S de Tonaltepec, alt. 2000 - 2200 m, 17° 33' N 96° 58' O, Aug. 26, 1992, *A. Salinas T. 7038* (CAS, MO); Dto. Sola de Vega, Mpio. San Vicente Lachixio, 1 km al SE de San Vicente Lachixio camino a El Vado 16° 40' 44" N, 96° 59' 36" W, alt. 1940 m., July 13, 2001, *A. García-Mendoza 7166* (US); Cerro San Felipe, 2000 m., Aug. 18, 1921, *C. Conzatti 4164* (US); El Cumbre Mountains, 16° 55' N, 96° 20' W, July 21, 1966, *J. Schoenwetter JSOX-32* (US); Valley of Oaxaca, 7500 ft., 1894, *E. W. Nelson 1180* (US); Distrito Centro, Cerro San Felipe, 2000 m., Sep. 14, 1933, *Conzatti y hijo 4974* (MO); Distr. Juztlahuaca, Mpio. San Sebastián Tecomaxtlahuaca, a 7 km de San

Sebastián Tecomaxtlahuaca, senda para el cerro Las Cuevas, 17° 19’ 09.40” N 98° 04’ 34.50” W, alt. 1870 m.s.n.m, Sep. 17, 1996, J. I. Calzada 21442 (MO). **Sinaloa**: along Mexican Highway 40 6 - 8 km SE of El Palmito, elev. 2000 m., Sep. 11, 1979, *D. E. Breedlove 43896* (CAS).

14. *Lithospermum flavum* Sessé & Moc. *Naturaleza (Mexico City) ser 2. 32*. 1893. *Macromeria longiflora* D. Don *Edinburgh New Philos. J.* 13:329. 1832. *Onosmodium longiflorum* (D. Don) J. F. Macbr. *Contr. Gray Herb.* 49: 21. 1917. TYPE: Mexico. Mexico: Mountains between Zitácuaro, Michoacán, and Malacatepec, *Sessé & Moçiño 1738* (holotype, BM; isotype, GH [digital image of fragments and holotype]!).

*Macromeria discolor* Benth. *Pl. Hartw.* 49. 1840. *Onosmodium discolor* (Benth.) J. F. Macbr. *Contr. Gray Herb.* 49: 20. 1917. TYPE: Mexico. Michoacán: no date, *Graham 256* (holotype, K [digital image]!).

Erect perennial herb up to 1.8 m tall with 1--few stems, each 3--6 mm wide, arising from a woody caudex, naphthoquinones not observed, stems strigose to dense strigose. Leaves cauline, sessile or petiolate, petioles 1--2(--4) x 1--2 mm; blades elliptical to ovate to lanceolate, rarely obovate, 2.5--12.3 x 0.8--3.5(--5) cm, apex acute to acuminate, sometimes cuspidate, base acute to rounded, margins entire, planar, adaxially scabrous, rarely strigose, and abaxially strigose to dense strigose, midvein evident, secondary veins evident, sunken, rarely not sunken. Inflorescence terminal dichasium or monochasium and usually axillary monochasia, up to 38.5 cm long, bracts sessile or petiolate, petioles 1 x 1 mm; blades lanceolate to ovate, sometimes elliptical, 1.2--6(--7) x 0.2--2.4(--3.1) cm, apex acute to acuminate, base rounded to acute, margins entire, planar, adaxially scabrous and abaxially strigose to dense strigose, midvein evident, secondary veins evident, sunken. Flowers homostylous, chasmogamous; pedicels 0--12(--20) mm long; sepals lanceolate, 5--20 x 0.75--1.5(--2) mm, apex acute, abaxially strigose to dense strigose to hispid and adaxially strigulose; corolla long-funnelform with a bulge, orange to yellow, usually the lobes tipped with green, abaxially villous, tube 28--45 x 4--7 mm, adaxial trichomes only rarely present, lobes 7--16 x 2.5--5 mm, erect to flared to reflexed, deltoid, rarely elliptical, apex acute, glands sometimes present at the apex of the corolla tube and the base of the corolla lobes and on the veins of the filaments, faucal appendages absent, annulus absent; stamens exserted, filaments 8--16 x 0.25(--0.5) mm, attached 29--36 mm above the base of the corolla, exserted 4--10 mm from the apex of the corolla tube, anthers 2--2.5 x 0.5--1 mm, ellipsoid; style 41--59 x 0.1 mm, exserted up to 15 mm from the apex of the corolla tube; stigma terminal. Nutlets 3--4.5 x 2--3 mm, ovoid, white to grey-brown, sometimes tan towards the apex, smooth, scar not prominent, base rounded, apex acute.

Distribution: Western and southern Mexico.

Phenology: Flowers late July to mid-November, and fruits from late July.

Discussion: *L. flavum* is one of the most widespread Mexican species of *Lithospermum*, with a geographic range stretching from Durango to Oaxaca. The corollas of this species are orange, and this color is unique among the Mexican species of the genus. The only two other species of *Lithospermum* that produce orange corollas are found in the eastern United States. In addition, hummingbirds have been observed visiting the flowers of this species.

Specimens Examined: MEXICO. **Colima**: Cuchilla, NE Volcano Colima, alt. 10000 ft., July 22, 1905, *P. Goldsmith 65* (F, MO, NY, US). **Durango**: Cerro Chupadero, 45 miles south of El Salto, and just west of Pueblo Nuevo, elev. 2000 - 2500 m, July 28, 1955, *J. H. Maysilles 8250* (NY, TEX); Mpio. Mezquital, 48 km west northwest of Huejuquilla El Alto, Jalisco on road to Canoas, elev. 2530 m., October 21, 1983, *D. E. Breedlove 59048* (CAS, NY, TEX). **Guanajuato**: Mpio. Jerecuaro, El Moro, alt. 2600, Aug. 4, 1985, *A. Rubio 14* (F, MICH); **Guerrero**: Ca 15 air km W-SW of Chichihualco and 10 air km S-SE of Filo de Caballos on the side road to Balzamar S of the main rd from Chichihualco - Filo de Caballos, along 2 km down the road towards Balzamar, 17° 34’ 57” N 99° 48’ 25” W, 2400 m., July 23, 1991, *Mayfield, A. Hemple, & A. Jack 1002* (TEX); Vicinity of Omiltemi, ca. 60 km W of Chilpancingo, along road bordered by partly cutover forest, 1 - 2 k E of Omiltemi, alt. ± 2035 m., July 10 - 12, 1963, *S. S. Tillett 637-139* (CAS); Mpio. Chichihualco, Puerto Chico, 10 km al W de Camotla, Aug. 8, 1964, *Rzedowski 18513* (CAS); 1 – 2 km E of Omiltemi, ca. 60 km W of Chilpancingo, alt. ± 2035 m., July 10 – 12, 1963, *S. S. Tillett 637-139* (US). **Jalisco**: Tapalpa, elev. 7000 ft., August 7, 1969, *S. Walker 69010* (NY); Volcán Tequila, due south of Tequila, elev. 2750 m., August 11, 1968, *W. R. Anderson & C. Anderson 5124* (MO, NY); Mpio. Tlajomulco, vertiente N del Cerro Viejo, Arroyo de Aguas, alt. 2200 m., August 15, 1970, *Rzedowski 27516* (CAS, F, LL, MO, NY, TEX, US); Mpio. Tapalpa, adelante de Tapalpa rumbo a San Gabriel, alt. 2000 m., July 24, 1994, *J. A. Machuca N., M. Cházaro B., & M. Huerta N. 7122* (TEX); Mpio. Teocuitatlan, Cerro de García, July 28, 1991, *M. Cházaro B., J. A. Machuca N., & P. Hdz de Cházaro 6651* (TEX); Summit of Cerro Tequila, ca. 20 road km S of Tequila, August 26, 1980, *M. J. Warnock, S. McCormick, & J. Norris 2177* (TEX); Mpio. Autlán de Navarro, along logging road from El Chante to El Guisar in the Sierra de

Manantlan, elev. 2160 m., Aug. 17, 1980, *D. E. Breedlove 45730* (CAS); Mpio. Gómez Farías, 15 km al NE de Cd. Guzman, carr. a San Andrés Ixtlán luego 19 k al E por brecha a Concepción de Buenos Aires, alt. 1980 m., Sep. 2, 1988, *M. Fuentes O. 625* (CAS, MO); Mpio. Tequila, Cerro de Tequila, km 17 junto a la cerro de microndas, Sep. 6, 1988, *S. González Casillas 87* (CAS); Mpio. Tequila, Cerro de Tequila, alt. 2300 m., July 13, 1971, *R. González T. 238* (CAS); Volcano of Colima, July 13, 1892, *M. E. Jones 326* (US); Along secondary road into mountains, 3.8 mi n. of jct. with hwy to Tapalpa, *R. Kral 27585* (MO). **Mexico**: Mpio. Villa Nicolás Romero, 3 km al NW de Cahuacán, alt. 2600 m., July 18, 1976, *Rzedowski 34254* (NY); Temascaltepec, September 1, 1935, *Hinton et al. 8257* (F, NY, US); Sierra de Alcaparrosa, 5 km al NW de Tepozotlán, alt. 2800 m., July 29, 1971, *Rzedowski 28272* (CAS, TEX, US); Tenancingo, alt. 8200 ft., November 13, 1930, *D. Reddick 351* (BH); District of Temascaltepec, Las Cruces, alt. 3350 m., July 15, 1932, *G. B. Hinton 1045* (US); District of Temascaltepec, Rincón, alt. 1960 m., Aug. 22, 1933 *G. B. Hinton 4452* (MO, US); Zempoala, Nov.. 1954, *E. Lyonnet 541100002* (MO). **Michoacán** : Mountains around Pátzcuaro, July 22, 1892, *C. G. Pringle 4146* (F, MO, NY, US); Coalcoman, Sierra Torricillas, October 4, 1939, *Hinton et al. 15274* (F, LL, MO, NY, US); S. Torricillas, Coalcoman, alt. 2300, August 14, 1941, *Hinton et al. 15981* (NY, US); At Km. 19 (by road) S. of Carapan (on road to Uruapan) (Ca. 19° 45' N 102° 3' W), ca. 2300 m., July 27, 1960, *H. H. Iltis & R. Koeppen 471* (TEX); On side of road by mile marker 19 from Carupan to Cheran, 19° 42' 49.2" N 102° 00' 51.7" W, alt. 2250 m., August 11 - 17, 2008, *J. Cohen 226* (BH); Mpio. Maravatío, Cerro El Calvario, San Miguel, alt. 2600 m., Aug. 21, 1991, *H. Díaz Barriga y Cols. 6825* (CAS); Mpio. Tancitaro, July 5, 1941, *Wm C. Leavenworth and H. Hoogstral 4017* (F, MO); Cerro Azul, vicinity of Morelia, alt. 2200 m., 1910, *Bro. G. Arsène 6736* (US); Quincea, vicinity of Morelia, alt. 2800 m., *Bro. G. Arsène 3248* (MO, US).

**Morelos**: Cuernavaca, Aug., Sep. 1950, *E. Lyonnet 5008000045* (CAS, MO); Mpio. Tancitaro, on Cerro Tancitaro, elev. 11000 ft., July 18, 1940, *Wm. C. Leavenworth 286* (F, MO). **Nayarit**: WNW of Huejuquilla El Alto along road to Jesus Maria near side road to Santa Lucia de la Sierra, elev. 2380 m., Sep. 9 1984, *D. E. Breedlove 61496* (CAS); Mpio. Xalisco, 7 km al SW de Xalisco, camino a la estacion de microondas, Cerro Boludo, 21.27.19 N, 104.58.49 W, alt. 1500 m., Oct. 20, 1994, *G. Flores-Franco 4487* (MO). **Oaxaca**: 16.8 - 25.6 km by road from Teotitlán del Camino on road to Huautla de Jiménez, elev. 2050 - 2200 m, July 11 - 12, 1968, *W. R. Anderson & C. Anderson 4741* (MO, NY); Dto. De Ixtlán, Sierra de Juárez, camino de La Cumbre (Ruta 175) a Corral de Piedra, cerca de la Torree de TRM, alt. 3050 m., July 31, 1985, *D. H. Lorence 4654* (F); On road between Oaxaca and Puerto Angel, 35.5 km S of Miahuatlan, 16° 07' N 96° 29' W, elev. 2500 m., July 2, 1979, *G. Diggs w/ C. Corcoran 2313* (WISC); Cerro San Felipe, 2200 m., Aug. 26, 1921, *C. Conzatti 4183* (US); Hwy 175 ca. 24 miles north of Pochutla, Aug. 19, 1970, *D. H. Norris & D. J. Taranto 16115* (MO). **Sinaloa**: Río Florido, alt. 1200 m., Sep. 19, *M. P. Dehesa 1527* (US); Quebrada alt. 1550 m., Sep., *M. P. Dehesa 1517* (US). **Zacatecas**: Sierra Madre, alt. 7500 m., Aug. 16, 1897, *J. N. Rose 3520* (US).

15. *Lithospermum guatemalense* Donn. Sm. *Bot. Gaz.* 27: 436. 1899. TYPE: Guatemala. Huehuetenango: alt. 1800 m., Aug.1896, *Seler 3082* (holotype, US!).

Erect perennial herb up to 71.5 cm tall with 1--few stems, each 3--4 mm wide, arising from a woody caudex, naphthoquinones not observed, stems hispid to strigose, sometimes dense hispid. Leaves cauline, sessile; blades lanceolate to elliptical, rarely linear, (1.3--)2.4--7.2(--9) x 0.3--1.3(--1.6) cm, apex acute, base acute to rounded to truncate, margins entire, planar, adaxially and abaxially strigose, dense strigose on abaxial portion of veins, midveins evident, secondary veins evident, sunken. Inflorescence terminal dichasium or monochasium and sometimes axillary monochasia, up to 27 cm long, bracts sessile; blades lanceolate, 0.8--4 x 0.2--0.75 cm, apex acute, base rounded, rarely truncate, margins entire, planar, adaxially and abaxially strigose, dense strigose on abaxial portion of veins, midvein evident, sometimes secondary veins evident, sunken. Flowers homostylous, chasmogamous; pedicels 0--7(--12) mm; sepals lanceolate to linear, (3--)5--15 x 0.75--1 mm, apex acute, abaxially strigose to densely strigose towards the base, and adaxially strigose to sparsely strigose; corolla tubular to long funnnelform, yellow, abaxially villous towards the apex and villous to sparsely villous towards the middle and base, tube 15--33 x 2.5--4 mm wide, adaxial trichomes rarely present on the corolla lobes and at the base of the tube, lobes 1--4 x 1.5--3.75 mm, erect to reflexed, orbicular to elliptical, apex rounded to obtuse, glands at the apex of the corolla tube, faucal appendages absent, annulus of 5 knobs; stamens inserted, filaments 1 x 0.1 mm wide, attached 19--28 mm above the base of the corolla, anthers 1.75--2 x 0.5--0.75 mm wide, ellipsoid; style (16.5--)20--30 x 0.1--0.2 mm wide, exserted up to 4 mm from the apex of the corolla tube; stigma terminal to subterminal. Nutlets 3.5 x 2--2.5 mm wide, ovoid, tan, smooth, base rounded, apex acute.

Phenology: Flowers in May, fruiting from late June.

Distribution: Chiapas, Mexico and Huehuetenango, Guatemala.

Discussion: The habit, leaves, and flowers of *L. guatemalense* are similar to those of *L. viride* and *L. oblongifolium*. However, the leaves of *L. guatemalense* are much narrower than those of either of species, and *L. guatemalense* produces yellow, rather than yellow-green, corollas. *Lithospermum guatemalense* has a very restricted geographic range, with the majority of the specimens collected in Chiapas. Despite its epithet, I only know of one specimen from Guatemala.

*L. guatemalense* should not be confused with *L. chiapense*, a species with which it has an overlapping range. *Lithospermum chiapense* bears flowers with exserted stamens and exserted stigmas, while *L. guatemalense* produces flowers with inserted anthers and exserted stigmas.

Specimens Examined: MEXICO. **Chiapas**: 1864-1870, *Ghiesbreght 804* (GH); Teopisca, May 11, 1904, *E. A. Goldman 968* (US); Mpio. Venustiano Carranza, 3 miles south of Aguacatenango along road to Pinola Las Rosas, elev. 5600 ft., May 28, 1965, *D. E. Breedlove 10084* (MICH, US); Mpio. San Cristóbal de las Casas, Rancho Pellizzí en el arroyo, 2 a 3 km al E. de San Cristóbal, June 20, 1985, *Alonso Méndez G. 8321* (MEXU); Mpio. La Independencia, 6-10 km north-northeast of La Soledad along logging road from Las Margaritas to Campo Alegre, elev. 1600 m., May 5, 1973, *D. E. Breedlove 34790* (MEXU).

16. *Lithospermum incisum* Lehm. *Pl. Asperif. Nucif.* 2: 303. 1818. *Batschia longiflora* Pursh *Fl. Amer. Sept.* 1: 132. 1813. *Lithospermum longiflorum* (Pursh.) Spreng. Syst. Veg. (ed. 16) 1: 544. 1824, non *Lithospermum longiflorum* Salisb. *Prodr. Stirp. Chap. Allerton* 113. 1796. *Pentalophus longiflorus* A. DC. in A. P. de Candolle & A. L. P. P. de Candolle, *Prodr*. 10: 87. 1846. *Cyphorima longiflora* (Nutt.) Raf. *Amer. Monthly Mag. & Crit. Rev.* 4: 191. 1819. TYPE: No Location. *T. Nuttall. s.n.* (holotype, PH [digital image]!).

*Lithospermum angustifolium* Michx. *Fl. Bor.-Amer.* 1: 130. 1830, non *Lithospermum angustifolium* Forssk. *Fl. Aegypt.-Arab.* 39. 1775. *Cyphorima angustifolia* Nieuwl. *Amer. Midl. Naturalist* 3: 194. 1914. TYPE: USA. No State: Pres les Rapides de l'Ohio, *A. Michaux s.n.* (holotype, P [IDC microfiche Michaux Herbarium 31/12]!)

*Batschia decumbens* Nutt. *Gen. N. Amer. Pl.* 1: 114. 1818. *Lithospermum decumbens* (Nutt.) Torr. *Ann. Lyceum Nat. Hist. New York* 2: 225. 1828, non *Lithospermum decumbens* Vent. *Descr. Pl. Nouv*. 37. 1800. *Cyphorima decumbens* (Nutt.) Raf. *Amer. Monthly Mag. & Crit. Rev.* 4: 191. 1819. *Lithospermum mandanense* Spreng. *Syst. Veg. (ed. 16)* 1: 544. 1824. (nom. illeg.) *Pentalophus mandanesnsis* (Spreng.) A. DC. in A. P. de Candolle & A. L. P. P. de Candolle, *Prodr*. 10: 87. 1846. *Cyphorima mandanensis* (Spreng.) Lunell *Amer. Midl. Naturalist* 4: 515. 1916. TYPE: No Location. *T. Nuttall s.n.* (holotype, BM [digital image]!)

*Lithospermum breviflorum* Engelm. & A. Gray *Boston J. Nat. Hist.* 5: 252. 1845. TYPE: USA. Texas: Austin Co., industry, Apr. – May 844, *F. J. Lindheimer Fasc. II.278* (type, GH; isotype, GH)

*Lithospermum asperum* A. Nelson *Bull. Torrey Bot. Club* 26: 244. 1899. TYPE: USA. Wyoming: Point of rocks, June 15, 1898, *A. Nelson 4737* (holotype, US!; isotype, NY)

*Lithospermum albicans* Greene *Pittonia* 4: 91. 1899. TYPE: USA. Colorado: Arboles, 6000 ft., June 10, 1899, *C. F. Baker s.n. (NDG – 43207)* (syntypes, ND [digital image]!, F [digital image]!, GH!, NY!, US!)

*Lithospermum ciliolatum* Greene *Pittonia* 4: 92. 1899. TYPE: USA. Colorado: Los Pinos, May 18, 1899, *C. F. Baker s.n. (NDG – 43209)* (holotype, ND [digital image]!)

*Lithospermum oblongum* Greene *Pittonia* 4: 92. 1899. TYPE: USA. New Mexico: Aztec, Apr. 26, 1899*, C. F. Baker s.n. (NDG – 43211)* (holotype, ND [digital image]!; isotype: F [digital image]!, G, NY!)

Erect perennial herb up to 40 cm tall with 1--few stems, each 1--3 mm wide, arising from a woody caudex, naphthoquinones sometimes present, stems strigose to dense strigose. Leaves cauline and pseudobasal, sessile; cauline blades narrow elliptical to linear to lanceolate, sometimes oblanceolate, 1--6.6 x 0.1--0.5 cm, apex acute, base truncate to acute, margins entire, usually revolute, adaxially and abaxially strigose to strigulose, midvein evident, other orders of veins not evident; basal blades oblanceolate, sometimes lanceolate or narrow elliptical, 2.4--5.7 x 0.2--0.7 cm, apex acute to rounded, base acute to truncate, margins entire, planar, sometimes revolute, adaxially and abaxially strigose to strigulose, midvein evident, other orders of veins not evident. Inflorescence terminal dichasium or monochasium and axillary monochasia, up to 17 cm long, bracts sessile, blades linear to lanceolate to narrow elliptical, (0.6--)1.3--4.5 x 0.1--0.4 cm, apex acute, base truncate to acute, margins entire, usually revolute, adaxially and abaxially strigose to strigulose, midvein evident, other orders of veins not evident. Flowers homostylous, chasmogamous and cleistogamous; pedicels 0--5 mm long; chasmogamous flowers with sepals lanceolate, 4--12(--up to 14 in fruit) x 0.75--1.5 mm, apex acute, abaxially strigose to strigulose to dense strigose and adaxially strigose to strigulose; corolla salverform, yellow, abaxially villous, sometimes sparse villous towards the apex, tube 21--38 x 1--3(--5) mm, adaxial trichomes absent, lobes 3--7 x 2--6 mm, flared, elliptical, apex rounded, margins fimbriate, glands present in the upper portion of the tube, faucal appendages present, annulus of 5 or 10 knobs with trichomes; stamens inserted, filaments 0.5--1 x 0.1 mm, attached 21--32 mm above the base of the corolla, anthers 1.25--2 x 0.5 mm; style 16--34 x 0.1 mm, inserted to late exserted up to 3 mm from the apex of the corolla tube; stigma terminal; cleistogamous flowers with sepals lanceolate, 4--9 x 1--1.5(--up to 2 in fruit) mm, apex acute, style 1--2 x 0.1 mm, inserted, stigma terminal. Nutlets 3--4 x 2 mm, ovoid, white to tan, frequent pitting, scar prominent, base rounded with a 0.25 mm pedestal, apex acute.

Distribution: Uncommon throughout northern Mexico, but quite common throughout the Great Plains and intermountain region of the United States.

Phenology: Flowers early March, starts fruiting in March.

Discussion: *L. incisum* is a widespread species in the United States, but only a few populations occur in northern Mexico. *Lithospermum incisum* produces both chasmogamous and cleistogamous flowers. The chasmogamous flowers have long, yellow, salverform corollas with fimbriate lobes, and this combination of floral features is unique among species of the genus.

Specimens Examined: MEXICO. **Chihuahua**: Santa Eulalia Mountains, between Santa Eulalia and San Antonio, Mar. 7, 1985, P. F. Zika 8472 (MO). **Coahuila**: Above Chorro de Agua, route #75, May 2, 1959, *D. S. Corell & I. M. Johnston 21362* (NY). **Nuevo León**: Mpio. Galeana, Arroyo Hondo, Hacienda San Jose de Raices, Aug. 1, 1935, *C. H. Mueller 2340* (F, MO, US). **No locality**: 1847, *A. Fendler 629* (F); 1850-1851, *C. Wright 1560* (F).

17. *Lithospermum indecorum* I. M. Johnst. *J. Arnold Arbor*. 33: 350. 1952. TYPE: Mexico. Nuevo León: Mpio. Derrumbadero, Cañon de los Capulines abv. San Enrique, Hacienda San José de Raices, Aug. 6, 1935, *C. H. Mueller 2380* (holotype, GH!, isotype, MO!)

Erect perennial herb up to 37.5 cm tall with 1--few stems, each 2 mm wide, arising from a woody caudex, naphthoquinones not observed, stems strigose to hispid. Leaves cauline, sessile; blades elliptical, 1.8--3.9 x 0.3--0.8 cm, apex acute, base acute, margins entire, planar, sometimes revolute, adaxially strigose, sometimes also strigulose and abaxially villous to strigose, midvein evident, other orders of veins evident, sunken or not. Inflorescence terminal and axillary monochasia, up to 17 cm long, bracts sessile; blades elliptical to lanceolate, 2.1--3.7 x 0.5--1 cm, apex acute, base acute, margins entire, planar, sometimes revolute, adaxially strigose to strigulose and abaxially hispid to hispidulous, midvein evident, usually other orders of veins evident, sunken or not. Flowers homostylous, chasmogamous; pedicels 0--5 mm long; sepals linear, 7--14 x 0.75--1.5 mm, apex acute, abaxially strigose to hispid, more densely strigose and hispid towards the base, and adaxially strigose and strigulose; corolla yellow, strigose, tube 0.4--0.9 x 0.2 cm, trichomes not observed, lobes 0.2 x 0.1--0.2, margins entire, glands present, faucal appendages present, annulus not observed; stamens inserted, filaments not observed, anthers not observed; style 3 X 0.1 mm, inserted; stigma subterminal. Nutlets 3.5--4 X 2 mm, ovoid, white to slightly reddish or brown, pitted, usually most heavily around prominent scar, base rounded, apex acute.

Distribution: This species is restricted to Nuevo León and Tamaulipas.

Phenology: Fruits in August.

Discussion: *L. indecorum*, a species known only from three specimens, bears leaves with multiple, evident secondary veins, and produces nutlets that are usually partially red. It shares these two features with *L. pringlei*.

Since 1952, only a few specimens have been assigned to *L. indecorum*, and none of these show these particular leaf or nutlet characteristics. I have not seen any specimens, apart from those that Johnston (1952) mentioned in his description of the species, which accurately could be assigned to *L. indecorum*. Any specimen that has been recognized as *L. indecorum* probably should be assigned to *L. calcicola* (see discussion of *L. calcicola*).

Specimens examined: MEXICO. **Tamaulipas**: Between Marcella and Hermosa, July 22, 1949, *Stanford, Lauber, and Taylor 2637* (NY).

18. *Lithospermum jimulcense* I. M. Johnst. *J. Arnold Arbor.* 23: 351. 1952. TYPE: Mexico. Coahuila: Summit of Mt. Jimulco, 13 kilo. east of Jimulco, 25º N 103º W, elev. 1730 m. June 29, 1941, *L. R. Stanford, K. L. Retherford, & R. D. Northcraft 100* (holotype, GH!, isotypes, MO!, NY!).

Erect perennial herb up to 12.5 cm tall with 1--few stems, each 2 mm wide, arising from a woody caudex, naphthoquinones not observed, stems woolly. Leaves cauline, sessile; blades linear to narrow elliptical to lanceolate, 1--1.5 x 0.5 -- 0.2 cm, apex acute, base truncate to rounded to acute, margins entire, revolute, abaxially and adaxially, woolly, midvein evident, other orders of veins not evident. Inflorescence terminal dichasium or monochasium, up to 1 cm long, bracts sessile; blades linear, 0.6

x 0.1 cm, apex rounded to acute, base rounded to truncate, margins entire, revolute, woolly, midvein evident, other orders of veins not evident. Flowers homostylous, chasmogamous; pedicel 0 mm long; sepals linear, 2 x 0.5 mm, apex acute, abaxially woolly, adaxially villous to woolly; corolla salverform, yellow, abaxially woolly, the bottom quarter of tube glabrous, tube 2.5--4 x 1 mm, adaxial trichomes absent, lobes 1--1.25 x 1--1.5 mm, flared, elliptical to orbicular, apex round, glands present below the filaments, faucal appendages present, annulus of 5 or 10 membranous to hairy flanges; stamens inserted, filaments 0.1 x 0.1 mm, attached 3 mm above the base of the corolla, anthers 1 x 0.5 mm, ellipsoid; style 1.5--2 x 0.1 mm, inserted; stigma terminal. Nutlets not observed.

Distribution: Restricted to Mt. Jimulco in Coahuila, Mexico.

Phenology: Flowers in June, fruiting unknown.

Discussion: *L. jimulcense* is only known from its type specimen. This species is quite distinct because of the woolly indument that covers the entire plant,. Johnston (1952) hypothesized an affinity between *L. jimulcense* and *L. indecorum*, but this has yet to be examined in a phylogenetic context.

Specimens Examined: MEXICO. **Coahuila**: Summit of Mt. Jimulco, 13 kilo. east of Jimulco, 25º N 103º W, elev. 1,730 m. June 29, 1941, *L. R. Stanford, K. L. Retherford, & R. D. Northcraft 100* (GH, MO, NY).

19. *Lithospermum johnstonii* J. I. Cohen *Brittonia* 61: 108. 2009. Basionym: *Macromeria hispida* M. Martens & Galeotti, *Bull. Acad. Roy. Sci. Bruxelles* 11: 339.

1844, non *Lithospermum hispidum* Forssk., *Fl. Aegypt.-Arab.* 39. 1775. *Macromeria longiflorum* var. *hispida* (M. Martens & Galeotti) DC. *Prodr*. 10:68. *Onosmodium longiflorum* var. *hispidum* (M. Martens & Galeotti) J. F. Macbr. *Contr. Gray Herb.* 49: 21. 1917. TYPE: Mexico. Michoacán: near Morelia, Cerro de Quinzeo, 6500 - 8000 ft, 1840 - 1844, *Galeotti 1917* (holotype, BR; isotype, GH!).

Erect perennial herb up to 1 m tall with 1--few stems, each 4--6 mm wide, arising from a woody caudex, naphthoquinones not observed, stems strigose, more densely strigose towards the apex and more sparsely strigose towards the base. Leaves cauline, sessile; blades lanceolate to elliptical, 3.4--10.2 x 0.6--2.1 cm, apex acute to acuminate, base rounded to truncate, margins entire, planar, adaxially and abaxially strigose, usually more densely strigose on veins, midvein evident, 1--2 pairs of secondary veins evident, sunken. Inflorescence terminal dichasium and axillary monochasia, bracts sessile; blades lanceolate, 1.5--4.7 x 0.2--1 cm, apex acute to acuminate, base acute to rounded to truncate, margins entire, planar, adaxially and abaxially strigose to densely strigose, midvein evident, sometimes 1 pair of secondary veins evident, sunken or not. Flowers homostylous, chasmogamous; pedicels 0--15 mm long; sepals linear, rarely lanceolate, 10--25 x 1--3 mm, apex acuminate, abaxially and adaxially strigose to densely strigose; corolla long-funnelform with a bulge, slightly zygomorphic, cream to yellow-cream, abaxially villous, tube 35--53 x 4--7 mm, adaxial trichomes absent, lobes (3--)5--8 x 2--4.5 mm, erect to reflexed, deltoid, apex obtuse, glands present at the apex of the tube, faucal appendages absent, annulus absent; filaments 6--11 x 0.15--0.25 mm, attached 34--41 mm, exserted 3--5(--11) mm from the apex of the corolla tube, anthers 2--3 x 0.5--1 mm, ellipsoid; style 42.5--61 x 0.1 mm, exserted up to 11 mm from the apex of the corolla tube; stigma terminal. Nutlets not observed.

Distribution: Western Mexico, from Sinaloa to Michoacán , the state in which it is most abundant.

Phenology: Flowers late June to mid-August, fruiting begins in mid-August.

Discussion: Although de Candolle (1846) placed *L. johnstonii* as a variety of *L. flavum, L. johnstonii* is resolved as sister to *L. exsertum* (Cohen and Davis 2009, Chapter 3, 4). Both species produce slightly zygomorphic corollas with reflexed lobes. Additionally, the two species have similar geographic ranges.

Despite these similarities, the two species differ in vegetative and floral characteristics. *Lithospermum johnstonii* is smaller, and produces narrower leaves than does *L. exsertum*. The corollas of *L. johnstonii* are cream to yellow-cream, as opposed to those of *L. exsertum*, which are yellow.

Specimens Examined: MEXICO. **Jalisco**: Cerro Viejo, south face, above Zapotitán de Hidalgo, a village about 1 mi. N of highway Mex. 15, about 25 mi. due south, or 45 road miles from Guadalajara, alt. 1,890 m., June 27, 1956, *D. P. Gregory & G. Eiten 208* (MO, NY); Fraccionamiento Los Cazos, al sur de Mazamitla, July 9, 1995, *J. A. Machuca N. 7,350* (CAS, TEX). **Michoacán** : Along highway from Morelia to Quiroga, 19° 37’ 42.5” N 101° 25’ 50.6” W, 2,347 m., August 2008, *J. Cohen 218* (BH); 3.3 mi. E of Mazamitla, along Hwy 15, 107 mi. W of Zacapu, 6,950 ft., July 26, 1975, *K. Torke, Dunn, & Wallace 286* (LL, MEXU, NY); Vicinity of Morelia, alt. 1,900 m., August 14, 1910, *Bro. G. Arsène 5493* (MO, NY, US); Morelia, 1840, *legit Hartweg 372* (NY); Between Pátzcuaro and Quiroga, July 21, 1948, *H. E. Moore Jr. & C. E. Wood, Jr. 4,041* (BH); Morelia, Aug. 19, 1938, *L. A. Kenoyer A287* (F, MO);

Mpio. Taintzuntzan, cerro Tariaqueri, alt. 2160 m., July 21, 1986, *R. Olivo 111* (MEXU); ± 10 km al SW de Morelia, por la carretera a Pátzcuaro, alt. ± 2000 m., July 27, 1999, *S. Zamudio R. 11084* (MEXU); Mpio. Morelia, 2.5 km sureste del Cerro de Quinceo, alt. 2400 m., July 30, 1985, *V. M. Huerta 54* (MEXU); Mpio. Tiripetío, Rancho Los Patores, alt. 2100 m., July 2, 1986, *J. M. Escobedo 946* (MEXU); Colonia L. Cárdenas, cerca de Tzintzuntzan, alt. 2100 m., Aug. 6, 1985, Rzedowski 38931 (MEXU); Grassy area near Highway 15, at K 387, 3 miles southeast of Naranja, alt. 7100 ft., July 13, 1963, *E. Molseed & H. Rice 224* (MEXU); 3 km de la desviación a Cuto de la Esperanza, alt. 2150, Aug. 14, 1986, H. Díaz B. & Nery López 2578 (MEXU); Mpio. Penjamillo, carretera de cuota Guadalajara-México, entre los cruceros de La Piedad y Zacapu, 5 km antes de llegar al Puente La Medina, km 219 – 320, 20° 23’ 0” N 103° 32’ 0” W, alt. 1500 m, July 14, 1995, *A. Rodríguez & J. Villand 2666* (MEXU); Morelia, Punguato, 2300 m., July 16, 1909, *Bro. G. Arsène 2775* (MO, US); North of Lake Pátzcuaro, 19 miles southeast of Zacapu, Aug. 19, 1961, *U. T. Waterfall 16435* (US); Hills near Pátzcuaro, July 19, 1892, *C. G. Pringle 5606* (MO, US). **Nayarit**: near km. 886, ca. 10 miles southeast of Tepic (2 miles southeast of El Refugio); elev. 1020 – 1100 m., Aug. 30, 1957, *R. McVaugh 16577* (MEXU, US). **No location**: 1787 - 1795 - 1804, *Sessé, Mociño, Castillo, and Maldonado 5217* (F).

20. *Lithospermum kelloggiae* J. I. Cohen **sp. nov.** TYPE: Mexico. Michoacán : Mpio. Erongariocuaro, camino a Zinzirio, alt. 2400 m., July 22, 1991, *J. M. Escobedo 2069* (holotype, IEB!).

Erect perennial herb up to 40 cm tall with 1--few stems, each 2 mm wide, arising from a woody caudex, naphthoquinones not observed, stems strigose. Leaves cauline, sessile; blades lanceolate, 1.5--3.8 x 0.1--0.3 cm, apex acute, base rounded to truncate, margins entire, usually revolute, adaxially strigose and abaxially strigose to strigose on midvein and glabrous on remainder of blade, midvein evident, other orders of veins not evident. Inflorescence terminal monochasium, 11 mm long bracts sessile, lanceolate, 0.5--2.2 x 0.1--0.2 cm, apex acute, base rounded to truncate, margins entire, usually revolute, adaxially strigose to strigulose and abaxially strigose to strigulose to sparse strigose and sparse strigulose, veins strigose, midvein evident, other orders of veins not evident. Flowers homostylous, chasmogamous; pedicels 0--3 mm long; sepals lanceolate, 4(--up to 7 in fruit) x 1 mm, apex acute, abaxially strigose to dense strigose and adaxially strigulose; corolla campanulate, yellow, abaxially villous to villulous, tube 7 x 4 mm, adaxial trichomes absent, lobes 5 x 3 mm, erect, elliptical, apex rounded to acute, glands associated with faucal appendages, faucal appendages present, annulus absent; stamens inserted, filaments 1 x 0.75 mm, attached 5 mm above the base of the corolla, anthers 2 x 1 mm, ellipsoid; style 16--17 x 0.1 mm, exserted up to 9 mm from the apex of the corolla tube; stigma terminal. Nutlets 2 x 1.5 mm, ovoid, base rounded, apex acute.

Distribution: Restricted to Michoacán.

Phenology: Flowers and fruits in late July.

Discussion: This species is known from only one specimen that was collected in Michoacán. The individuals of *L. kelloggiae* bear a strong resemblance to those of *L. trinervium*, and the geographic ranges of the two species overlap. The two species differ in one floral and one vegetative feature. *Lithospermum kelloggiae* bears leaves in which only the midvein is evident, as opposed to *L. trinervium*, which produces leaves with an evident midvein and evident secondary veins. Additionally, the corollas of *L. kelloggiae* are yellow, while those of L. trinverium are white.

The form of *L. kelloggiae* is very similar to that of another species of *Lithospermum*, *L. pinetorum*. However, *L. pinetorum* produces shorter corollas, shorter styles, and faucal appendages that are below, rather than above, the summit of the corolla throat (Johnston, 1954a).

This species is named after my wife, who is my inspiration and my greatest champion.

Specimens Examined: MEXICO. **Michoacán** : Mpio. Erongariocuaro, camino a Zinzirio, alt. 2400 m., July 22, 1991, *J. M. Escobedo 2069* (IEB).

21. *Lithospermum leonotis* (I. M. Johnst.) J. I. Cohen *Brittonia* 61: 108. 2009. Basionym: *Macromeria leonotis* I. M. Johnst. [“*leontis*”], *J. Arnold Arbor.* 16: 188. 1935. TYPE: Mexico. Nuevo León: “ascent into Taray,” ca. 15 mi SW of Galeana, ca. 8000 ft, June 6, 1934, *C. H. Mueller & M. T. Mueller* 754 (holotype, GH!; isotype, TEX [digital image]!).

Erect perennial herb up to 65 cm tall with 1--few stems, each 3--5 mm wide, arising from a woody caudex, naphthoquinones not observed, stems hispid and hispidulose, rarely strigose or sericeous. Leaves cauline, sessile; blades elliptical to lanceolate to ovate, rarely oblanceolate, 1.5--8.1 x 0.5--3.3 cm, apex acute, base acute, rarely rounded, margins entire, planar, adaxially strigose and strigulose to hispid and hispidulose and abaxially pilose and pilosulose, sometimes strigose, midvein evident, secondary veins evident, sunken or not. Inflorescence terminal dichasium or monochasium, rarely also axillary monochasia, up to 29.5 cm long, bracts sessile;

blades ovate to lanceolate, 1.9--4.2(--5.5) x 0.8--1.8(--2.4) cm, apex acute, base rounded, rarely acute, margins entire, planar, adaxially strigose and strigulose to hispid and hispidulose and abaxially sericeous to pilose, midvein evident, secondary veins evident, sunken or not. Flowers homostylous, chasmogamous; pedicels 0--10 mm long; sepals lanceolate to linear, 12--23(--up to 29 in fruit) x 1--2 mm, apex acuminate, abaxially hispid to strigose and adaxially strigose to strigulose; corolla long-funnelform, yellow, abaxially villous, tube (42--)49--75 x 4--9 mm, adaxial trichomes rarely present on veins leading to filaments or on filaments, lobes 5--11 x 3--6(--8) mm, reflexed, elliptical, apex rounded to obtuse, sometimes acute, glands present at the apex of the tube, faucal appendages absent, annulus of 7 membranous flanges to a membranous ring with a few aggregations of trichomes; stamens exserted, filaments 9 -14(--20) x 0.25--0.75 mm, attached (27--)55--69 mm, above the base of the corolla, exserted (3--)7--14 mm from the apex of the corolla tube, anthers 2--3 x 0.5--1 mm, ellipsoid; style 55--85 x 0.1--0.25 mm, exserted up to 20 mm from the apex of the corolla tube; stigma subterminal, rarely terminal. Nutlets 4 x 3 mm, ovoid, white to partially light golden, smooth, scar prominent, base with a 0.25 mm pedestal, apex acute.

Distribution: Nuevo León and western Tamaulipas.

Phenology: Flowers April to August, fruits begin developing in late June.

Discussion: With the presence of pendant rather than horizontal flowers, *L. leonotis* differs from most other species of *Lithospermum*. This feature, in combination with yellow, long-funnelform corollas with reflexed lobes, does not occur in any other member of the genus.

*L. leonotis* is not common, although it may be locally abundant. Apart from my collection in 2007, I do not know of any collections that have been made since the early 1990s. The closest relative of *L. leontis* is *L. nelsonii*. These two species do not share many structural features, with the exception of the presence of subterminal stigmas. Although the two species may not have similar forms, they have similar geographic ranges.

Specimens Examined: MEXICO. **Nuevo León**: Aramberri, La Escondido → San Francisco, 2410 m., July 23, 1993, *G. B. Hinton 23060* (NY, TEX); Sierra Madre Oriental, descent into Alamar, about 15 miles SW of Galeana, May 29, 1934, *C. H. and M. T. Mueller 594* (TEX); Mpio. Galeana, E. Slope Potosi, 2050 m., July 22, 1983, *Hinton et al. 18523* (TEX); Mpio. Galeana, Agua Blanca, below 2270 m., July 4, 1991, *Hinton et al. 22277* (TEX); Mpio. Galeana, Cerro El Gallo, 2250 m., June 15, 1991, *Hinton et al. 21026* (TEX); Mpio. Zaragoza, 2440 m., June 19, 1979, *G. B. Hinton 17566* (TEX); Mpio. Zaragoza, 18 km al NE de San Antonio Peña Nevada 23° 51’ N 99° 54’ W, 2700 m., May 24, 1992, *L. Hernandez S. 2725* (TEX). Mpio. Galeana, Cerro El Gallo, 2160 m., Apr. 9, 1989, *G. B. Hinton 19375* (TEX); Mpio. Zaragoza, 18 km al NE de San Antonio Peña Nevada 23° 51’ N 99° 54’ W, 2700 m., May 24, 1992, *L. Hernandez S. 2724* (TEX); Mpio. Zaragoza, approx. 20 km to Encansada from San Antonio Peña Nevada, 23° 49’ 33.4” N 99° 52’ 36.6” W, 8625 ft., June 15, 2007, *J. Cohen 195* (BH, MEXU). **Tamaulipas**: 4 km west of Miquihuana, 23° 42’ N 99° 45’ W, 3110 m., Aug. 4, 1941, *L. R. Stanford, K. L. Retherford, & R. D. Northcraft 660* (CAS, MO, NY); Mpio. Tula, 10.2 mi SE of Tula (centro) on road to Ocampo, 22° 56.5’ N 99° 36’ W, 1440 m., June 16, 1987, *G. Nesom 6013* (CAS, TEX).

22. *Lithospermum macromeria* J. I. Cohen *Brittonia* 61: 108. 2009. Basionym: *Macromeria viridiflora* DC. in A. P. de Candolle & A. L. P. P. de Candolle, *Prodr*. 10: 68. 1846, non *Lithospermum viridiflorum* Roxb., in Carey & Wallich, *Fl. Ind.* 2: 4. 1824. TYPE: Mexico. No State: Without locality, 1787-1804, *Sessé & Mociño 5231* (holotype, MA [photograph]!; probable fragments of holotype, F!, GH [digital image]!; photo of holotype, GH!, MICH!).

*Onosmodium thurberi* A. Gray *Syn. Fl. N. Amer.* 2(1): 205. 1878. *Macromeria thurberi* (A. Gray) Mack. *Bull. Torrey Bot. Club.* 32: 496. 1905. *Macromeria viridiflora* var. *thurberi* (A. Gray) I. M. Johnst. *J. Arnold. Arbor.* 35: 12. 1954. TYPE: U.S.A. New Mexico: Grant Co., “Copper mines,” Aug. 1851. *G. Thurber 1106* (holotype fragment, GH [digital image]!; isotype, NY!).

Erect perennial herb up to 1.5 m tall with 1--few stems, each 3--8 mm wide, arising from a woody caudex, naphthoquinones not observed, stems strigose and strigulose to strigose and hispid. Leaves cauline, sessile; blades lanceolate to ovate to elliptical, 2.3--10.7 x 0.7--3 cm, apex acute, base rounded to acute, margins entire, planar, adaxially strigose and strigulose, sometimes dense strigose and dense strigulose, and abaxially strigose and strigulose to dense strigose and dense strigulose, midvein evident, secondary veins evident, sunken. Inflorescence terminal dichasium or monochasium, and sometimes axillary monochasia, up to 27.5 cm long, bracts sessile; blades lanceolate to ovate, 0.9--6.9(--7.9) x 0.3--2.7 cm, apex acute, base rounded to acute, margins entire, planar, adaxially strigose and strigulose to strigose and dense strigulose and abaxially strigose to strigulose to dense strigulose, midvein evident, secondary veins evident, sunken. Flowers homostylous, chasmogamous; pedicels 0--13 mm long; sepals linear, 9--22(--25 in fruit) x 1--2 mm, apex acute, abaxially strigose to dense strigose to strigulose to dense strigulose and abaxially strigose to strigulose, sometimes dense strigulose; corolla long-funnelform, yellow-green to off-white with green, abaxially villous, tube (41--)47--61(--72) x 4--5.5 mm, adaxial trichomes usually present, sparsely villous to sparsely villulous, lobes 4--10 x 2--6 mm, erect, deltoid, apex acute, glands absent, faucal appendages absent, annulus absent; stamens exserted, filaments 8--13(--18) x 0.75--1 mm, attached 42--62 mm above the base of the corolla, exserted 5--8 mm from the apex of the corolla tube, anthers (2--)3--4 x 0.75--1 mm, ellipsoid, abaxially sparsely strigose to sparsely strigulose; style 52--86 x 0.1--0.25 mm, exserted up to 16.5(--19) mm from the apex of the corolla tube; stigma terminal.  Nutlets 3--4 x 2--2.75 mm, ovoid, white to white and tan, smooth, scar slightly prominent or not, base rounded, apex acute.

Distribution: Northwestern Mexico, with some scattered populations in Arizona and New Mexico, USA.

Phenology: Flowers late June onward, and starts fruiting in early July.

Discussion: *L. macromeria* is distinguished from other species of the genus by its green to off-white, long-funnelform corolla that gradually tapers from the base of the corolla to its apex.  Of all the species formerly included in *Macromeria*, it has the northernmost range. *L. macromeria* is closely-related to *L. multiflorum*, a heterostylous species (Cohen and Davis, 2009; Chapter 3, 4).  The two species have very different floral and vegetative forms, but their geographic ranges overlap.

Grant and Grant (1970) observed hummingbirds pollinating the flowers of *L. macromeria*.  This is the only published study concerning hummingbird pollination in *Lithospermum*, but given the floral forms of some species in the genus, it appears likely that other species will share this type of pollination syndrome (Aizen, 2003; Chapter 4).

Specimens Examined: MEXICO. **Chihuahua**: Near Colonia Garcia in the Sierra Madres, alt. 7500 ft., July 1, 1899, *C. H. T. Townsend & C. M. Barber 62* (F, MO, NY, TEX, US); 10 km. E of Colonia Pacheco on the road to Colonia Juárez, just below the Indian Cave at "Arch Flat, " in canyon of "Little Strawberry Creek," 30 7' N 108° 15' W, 1900 m., July 31, 1972, *E. Wilson, L. A. & M. C. Johnston 8520* (LL); 61.8 mi from El Vergel, 8 mi before Guadalupe y Calvo along dirt-gravel road (continuation of hwy 24) that winds around Mt. Mohinora, August 9, 1978, *V. A. Funk & R. W. Sanders 2769a* (TEX, US); 61.8 mi from El Vergel, 8 mi before Guadalupe y Calvo along dirt-gravel road (continuation of hwy 24) that winds around Mt. Mohinora, August 9, 1978, *V. A. Funk & R. W. Sanders 2769b* (US); 4 mi NW of Pacheco/Willy jct. on rd. to Rancho Willy, 6800 ft., July 10, 1997, *J. Spencer & D. Atwood 562* (TEX); 18.4 mi S of jct of La Hunta-Tomochic road and road to Creel, along road to San Juanito, August 10, 1978, *V. A. Funk & R. W. Sanders 2771* (TEX); 25.6 mi from jct of La Hunta-Tomochic road and road to Creel along road to San Juanito, Aug. 10, 1978, *V. A Funk & R. W. Sanders 2772* (US); Norogachi, elev. approx. 5000 ft., July 19, 1955, *C. Pennington 691* (TEX); Mpio. Bocoyna, N of Choquita, between Creel and Bocoyna, ca. Km 21+700, August 2, 1977, *R. Bye & W. A. Weber 7839* (TEX); Tributary of Rio Loera, just southeast of Nabogame, 6000 ft. alt., October 11, 1959, *D. S. Correll & H. S. Gentry 23040* (LL); 3.3 road miles (5.3 km) WNW of Chuhuichupa on road to La Norteana about 50 yds., (15.2 m) to the N of road, elev. ca. 6400 ft. (1950.7 m.), August 9, 1977, *M. A. Lane 2253* (LL); Majalca, June 24, 1936, *H. LeSueur 895* (CAS, F, TEX, US); Soldier Canyon, Sierra Madre Mts, alt. 6600 ft., Sep. 16, 1903, *M. E. Jones s.n.* (F, MO, US); Mpio. Guerrero, 6 mi.

N. of Picachíc, alt. 7000 ft., July 25, 1937, *F. Shreve 8018* (F); Cerro Mohinora, 10 miles south of Guadalupe y Calvo, 2300 - 2400 m elev., Aug. 10, 1960, *R. M. Straw and M. Forman 1921* (CAS, MO); Sierra Madre, June 21 – July 29, 1899, *E. W. Nelson 6053* (US); at base of Mt. Mohinor, 8 miles from Guadalupe y Calvo, alt. 7000 – 7500 ft., Aug 23 – 31, 1898, *E. W. Nelson 4830* (US); Chuichupa, Sierra Madre Mts., alt. 7000 ft., Sep. 26, 1903, *M. E. Jones s.n.* (US). **Durango**: Mpio. El Salto, "El Capulin," 4 km al NE de El Pueblo de 1010, June 28, 1982, *P. Tenorio L. 703* (CAS, MO, NY); Coyoter, 7800 ft., July 22, 1978, *S. Walker 75H33* (NY); Base of cliffs in Mimbres Canyon, 26 miles west of Durango, route #40, July 24, 1958, *D. S. Correll & I. M. Johnston 20130* (LL); Mpio. Santiago Papasquiaro, Sierra Madre Occidental, about 22 air km WNW Santiago Papasquiaro, 3.3 mi. by Topia Rd. W from jct. rd. to antenna at E edge crest of sierra (approx. 25° 4' N 105° 51' W), elev. 8750 ft., July 9, 1983, *R. C. Diaz 251 & R. D. Worthington 10957* (TEX); Mpio. Santiago Papasquiaro, 35 km, del Entronque de la Brecha a Topia con la Carr. Santiago Papasquiaro-Tepehuanes, July 18, 1982, *P. Tenorio L. 1063* (CAS, MO); Mpio. El Salto, 32 kms. al W de El Salto, 2300 m.s.n.m. July 10, 1982, R. Hernández M. 7830 (US); 75 km by air W of Durango, 37 km by road N or Est. Coyotes, rd. to San Juan Causas, 24° 00' N, 105° 27' W, alt. 2000 m., Nov 25, 1978, *R. Sanders & A. McDonald 1345* (US); Near El Salto, alt. 8000 – 8700 ft., July 12, 1898, *E. W. Nelson 4551* (US); Sierra Madre Occidental, El Indio, along Hwy 40 (Mazatlan-Durango Hwy) 9 km E of Revolcaderos (23° 38.5; N 105° 49' W), alt. 2320 – 2400 m, Sep. 4, 1997, *A. C. Sanders 21488* (MO). **Sinaloa**: Ocurahui, Sierra Surotato, ± 6000 – 7000 ft., Sep. 1 - 10, 1941, *H. S. Gentry 6374* (CAS). **Sonora**: Region of the Rio de Bavispe, Puerto de los Aserraderos, Aug. 4 - 9, 1940, *S. S. White 3155* (MICH); Region of the Rio de Bavispe, between Las Tierritas and El Tigre, Aug. 22, 1940, *E. A. Phillips 673* (MICH).

23. *Lithospermum matamorense* DC. *Prodr*. 10: 76. 1846. TYPE: Mexico. Tamaulipas: Matamoros, Apr. 1831, *Berlandier 2311* (holotype, G [IDC microfiche Herbarium A. P. Candolle 1712/14]!; isotypes, F [digital image]!, GH!, MO!).

*Lithospermum prostratum* Buckley *Proc. Acad. Nat. Sci. Philadelphia* 1861: 462. 1861. TYPE: U.S.A. Texas: Llano County, *S. B. Buckley s.n.* (holotype, PH).

Erect perennial herb up to 36.5 cm tall with 1--few stems, each 1--2(--3) mm wide, arising from a woody caudex, naphthoquinones not observed, stems hispid to hispid and strigose, rarely strigose. Leaves cauline, sessile; blades usually spatulate to elliptical to obovate, 1--6 x 0.3--1.5(--1.8) cm, apex rounded, sometimes acute, base acute to rounded, margins entire, planar, adaxially and abaxially hispid to strigose, usually more densely hispid or densely strigose on the abaxial midvein, midvein evident, sometimes one or two pairs of secondary veins evident, not sunken. Inflorescence terminal dichasium or monochasium and sometimes axillary monochasia, up to 25 cm long, bracts sessile; blades elliptical to obovate, 0.3--3.2 x 0.1--1.1 cm, apex rounded, base acute to rounded, margins entire, planar, adaxially and abaxially hispid to strigose, sometimes densely hispid or densely strigose, midvein evident, other orders of veins rarely evident, not sunken. Flowers homostylous, chasmogamous; pedicels 0 -- 3 mm long; sepals linear, (1.5--)2--5(--6) x (0.3--)0.5--1 mm, apex acute, abaxially strigose to densely strigose and adaxially strigulose; corolla salverform, white with a yellow center, abaxially sparsely villous, tube 1--2 x 1 mm, adaxial trichomes absent, lobes 1--2 x 0.5--1 mm, erect to flared, elliptical to orbicular, apex rounded, glands present in the upper portion of the tube, faucal appendages present, annulus of 5--10 hairy knobs; stamens inserted, filaments 0.1 x 0.1 mm, attached 0.75 mm above the base of the corolla, anthers 0.5 x 0.3 mm, ellipsoid; style 1 x 0.1 mm, inserted; stigma terminal. Nutlets 2--3 x 1.5--2 mm, ovoid, white to brown, sometimes mottled, abundant dimpling, rarely slightly rugose, base rounded to with a 0.5 mm pedestal, apex acute.

Phenology: Flowers in March, fruiting from April onward

Distribution: Northeastern Mexico and Texas, United States

Discussion: *L. matamorense* differs from other members of the genus in three manners. First, *L. matamorense* is the only annual member of *Lithospermum*. Second, it has nutlets with abundant pitting. Third, its corollas and styles are very short, even shorter than in cleistogamous flowers of species of the *Lithospermum*. *Lithospermum matamorense* is closely-related to *L. californicum*, the forms of the two species are quite different. However, both species bear pollen that is prolate with a constricted equator (Chapter 4).

Specimens Examined: MEXICO. **Coahuila**: Rio Grande Valley near Diaz, 700 ft., April 21, 1900, *C. G. Pringle 8,302* (MEXU, US); Mpio. de Muzquiz, camino al Nacimiento (de los Negros), NW de Muzquiz, 28º 00’ N 101º 45’ W, 580 - 600 m., May 5, 1989, *J. A. Villarreal 4,860* (MEXU). **Nuevo León**: Mpio. Higueras or Carmen, on W side of old route of Mex 85, ca. 0.2 km N of road to estacion de microondas Mamalique, just S of top of pass, ca. 36 km S of Sabinas Hidalgo, Mamalique Pass, Mamalique 1:50,000 Quad., 26º 11’ 56” N 100º 06’ 27” W, elev. 640 - 660 m., April 7, 1990, *E. L. Bridges & L. Woodruff 13,126* (MEXU); San Juan, near Monterey, Mar. 1, 1906, *C. G. Pringle s.n.* (US); Monterrey, May 1891, *C. K. Dodge. 146* (US); Monterey, May 1891, *C. K. Dodge. 137* (US); Monterey, 1846, *D.*

*Edwards & Maj. Eaton 282* (NY); Guajuco, 27 miles southeast of Monterey, early March 1880, *Dr. E. Palmer 901* (NY). **Tamaulipas**: Mpio. San Carlos, Sierra de San Carlos, ca. 5 mi S of San Carlos, N side of Bufa El Diente, 770 m., 24º 31.5' N 98º 57.6' W, June 17, 1987, *G. Nesom 6,055* (MEXU).

24. *Lithospermum mirabile* Small *Fl. S.E. U.S.* 999, 1337. 1903. *Lithospermum longiflorum* var. *mirabile* (Small) Brand *Repert. Spec. Nov. Regni Veg.* 28: 14. 1930. TYPE: USA. Texas: San Antonio, Apr. 1853, *G. Thurber s.n.* (holotype, NY!; isotype, GH!).

*Lithospermum parksii* I. M. Johnst. *J. Arnold Arbor.* 33: 345. 1952. TYPE: USA. Texas: Val Verde County, rocky limestone hills above dam at foot of Devils Lake, about 20 miles northnorthwest of Del Rio, Mar. 31, 1947, *R. McVaugh 7725* (holotype, GH!).

*Lithospermum parksii* var. *rugulosum* I. M. Johnst. *J. Arnold Arbor.* 33: 345. 1952. TYPE: Mexico. Nuevo León: Sierra Madre Oriental, descent into Alamar about 15 miles southwest of Galeana, dry openly wooded and unwooded slopes, May 29, 1934, *C. H. & M. T. Mueller 598* (holotype, GH!).

Erect perennial herb up to 43 cm tall with 1--few stems, each 1--2(--3) mm wide, arising from a woody caudex, naphthoquinones sometimes present, stems strigose. Leaves cauline and pseudobasal, sessile; cauline blades linear to oblanceolate, 1.2--5(--6.4) x 0.1--0.6 cm, apex rounded, base truncate to acute, rarely acuminate, margins entire, usually revolute, adaxially and abaxially strigose to densely strigose, midvein evident, other orders of veins not evident; basal blades linear to oblanceolate, 1.2--7.5

x 0.2--1.1 cm, apex rounded, base acute to truncate, margins entire, planar, sometimes revolute, adaxially and abaxially strigose to densely strigose, midvein evident, other orders of veins not evident. Inflorescence terminal and axillary monochasia, rarely dichasia, up to 13 cm long, bracts sessile; blades linear, 0.6--2.6 x 0.1--0.3 cm, apex rounded, base truncate, margins entire, usually revolute, adaxially strigose to strigose and strigulose to densely strigose and abaxially strigose to densely strigose, midvein evident, other orders of veins not evident. Flowers homostylous, chasmogamous and cleistogamous; pedicels 0--6 mm long; chasmogamous flowers with sepals linear, 4--13 x 0.5--1 mm, apex acute, adaxially strigose to densely strigose and abaxially strigulose; corolla salverform, yellow, abaxially villous, denser towards the apex and sparser to glabrous towards the base, tube (8--)10--15 x 1.25--2.75 mm, adaxial trichomes absent, lobes 2.5--7 x 2--7 mm, flared, orbicular to elliptical, apex rounded, glands present in the upper portion of the tube, faucal appendages present, annulus of 5 hairy knobs; stamens inserted, filaments 0.25--0.75 x 0.1 mm, attached 11--12 mm above the base of the corolla, anthers 1.5--2 x 0.5--0.75 mm; style 11--14 x 0.1 mm, inserted; stigma terminal; cleistogamous flowers not observed. Nutlets 2--3 x 1.5--2 mm, ovoid, white to greenish-white, rugose, base rounded with a 0.25 mm pedestal, apex acute.

Distribution: Northeastern Mexico and adjacent Texas.

Phenology: Flower from March to June, fruiting from late March.

Discussion: *L. mirabile* is allied with *L. incisum*, and the two share many characteristics, including corollas that are long, yellow, and salverform. However, the two species differ in the margins of their corolla lobes. Those of *L. mirabile* are entire, while those of *L. incisum* are fimbriate. The nutlets of *L. mirabile* are unique among members of the genus. Rather than being smooth, its nutlets are rugose. These floral and fruit characters can be used to differentiate between *L. incisum* and *L. mirabile*. These differences are useful to note, as specimens of these two species are often confused for each other.

Johnston (1952, p 346) described *L. parksii* as a species distinct from *L. mirabile*. According to Johnston, the nutlets of *L. mirabile* are "distinctly angulate", while those of *L. parksii* are "covered with warts or are distinctly rugose." Despite these descriptions, the two species produce nutlets with similar surfaces. Therefore, *L. parksii* should not be recognized as distinct from *L. mirabile*.

Specimens Examined: MEXICO. **Chihuahua**: slopes of Santa Eulalia and San Antonio, Mar. 8, 1985, *P. F. Zika 8494* (NY). **Coahuila**: La Favorita Ranch on N slope of Cañon Bonanza, SE end of Sierra de la Purisima, 26° 25' - 26° 26' N 101° 30' - 101° 28' W, alt. 1300-1650 m., Mar. 19, 1973, *M. C. Johnston, T. L. Wendt, & F. Chiang 10295B* (MO, NY); 20 mi South of Allende, Mar. 23, 1970, *N. D. Atwood 2110* (NY); 20 km W of Ciudad Acuna, south floodplain of the Rio Grande, March 15, 1976, *S. R. Hill & D. R. Taller 4025* (NY); Road from Muzquiz to USA, near a dirt road, 28° 43' 58.8" N 102° 30' 38.2" W, June 11, 2007, *J. Cohen 180* (BH); Muzquiz, April 12, 1936, *E. G.  108* (F); Muzquiz, Spring 1935, *E. G. Marsh 143* (F). **Nuevo León**: 8 km al SE de Galeana, sobre la carratera a Linares, alt. 1700 m., Mar. 24, 1970, Rzedowski 27179 (MICH); Sierra Madre Oriental, descent into Alama about 15 miles SW of Galeana, May 29, 1934, *C. H. & M. T. Mueller 598* (F). **Tamaulipas**: SW of Victoria, 600 m., March 23, 1925, *R. Runyon 726* (US); Mikihuaua, June 1930, *H. W. Viereck 533* (US).

25. *Lithospermum muelleri* I. M. Johnst. *J. Arnold Arbor.* 16: 187. 1935. TYPE: Mexico. Nuevo León: Common in pine belt above Mesa de la Camisa on the north slope of Sierra Tronconal between Cañon de los Charcos and Cañon de San Miguel, Sierra Madre Oriental, ca. 25 km. s.w. of Galeana, alt. 1800 - 2700 m., June 4 1934, *C. H. & M. T. Mueller 739* (holotype, GH!; isotype, F [digital image]!).

Erect perennial herb up to 41.5 cm tall with 1--few stems, each 1--3 mm wide, arising from a woody caudex, naphthoquinones not observed, stems strigose. Leaves cauline, sessile; blades lanceolate to elliptical, rarely oblanceolate or spatulate, 1--4.9 x 0.1--0.8 cm, apex acute, base rounded to truncate, sometimes acute, margins entire, usually revolute, adaxially strigose to strigulose, abaxially strigose on the midvein, sometimes densely strigose or strigulose, rarely sparsely strigose on the remainder of the blade, midvein evident, other orders of veins not evident. Inflorescence terminal dichasium or monochasium, up to 10.5 cm long, bracts sessile; blades lanceolate, 0.3--3.2 x 0.1--0.65 cm, apex acute, base truncate, sometimes rounded, margins entire, usuall revolute, adaxially strigose to strigulose, abaxially strigose on the midvein and sparsely strigose on the blade, midvein evident, other orders of veins not evident. Flowers homostylous, chasmogamous; pedicels 0--5 mm long; sepals lanceolate, 3--6 x 0.5--1.25(--2) mm, apex acute, abaxially strigose, densely strigose towards the base, adaxially strigulose; corolla tubular, yellow-green, abaxially villous to woolly towards the apex, villous towards the middle, sparsely villous towards the base, tube (13--)15--21 x 1.5--4 mm, adaxial trichomes absent, lobes 1--1.5(--2) x 1--1.25 mm, erect, orbicular to elliptical, apex rounded, glands on veins leading to the filaments, faucal appendages present, annulus of 5 flanges with trichomes; stamens inserted, filaments 0.75--1 x 0.1 mm, attached 12--16 mm above the base of the corolla, anthers 1.25--1.75 x 0.5--0.75 mm, ellipsoid; style 15--23 x 0.1 mm, exserted up to 5 mm from the apex of the corolla tube; stigma terminal. Nutlets 2.5--2.75 x 1.5--1.75 mm, ovoid, mottled dusty grey and white, smooth and lustrous, scar is a very prominent ridge, base rounded, apex acute.

Phenology: Flowers and fruits from mid-May to July.

Distribution: Chiefly in the mountains of Nuevo León, but also higher elevations in Coahuila, Tamaulipas, and Querétaro.

Discussion: The vegetative and floral forms of *L. muelleri* are similar to those of *L. strictum*; however, the two species differ in a few characters. The indument on the abaxial surface of the leaves of *L. muelleri* consists of trichomes that are usually restricted to the midvein. This contrasts with that on the abaxial surface of the leaves of *L. strictum*, in which trichomes are distributed on all parts of the surface. Additionally, *L. muelleri* develops a tubular corolla with an exserted stigma, rather than a salverform corolla with an inserted style, as occurs in *L. strictum*. Although the affinity between the two species has been hypothesized (Johnston, 1952), *L. muelleri* has yet to be included in a phylogenetic analysis. *Lithospermum muelleri* is rare, being known from only 10 collections, the most recent of which is from 1992.

Specimens Examined: MEXICO. **Coahuila**: Sierra de la Marta (100° 21 - 28’ N, 25° 11 - 13’ W), elev. 8500 ft., May 17, 1981, *J. M. Poole 2343* (TEX). **Nuevo León**: N. or Santa Rita, 2360 m., May 14, 1981, *G. B. Hinton 18247* (TEX, MEXU); Area of Cerro Pene Nevada, ca. 12 km NE of San Antonio Pene Nevada, 30 km E of Doctor Arroyo, N and NW slope of mt. known locally as Picacho Onofre, and ridges and valleys ca. 5 km to the NW of this peak, July 1977, *C. Wells & G. Nesom 437* (LL);

Mpio. Zaragoza, 6 km al n de la Siberia en el camino a la Encantada, 23° 54’ N 99° 54’ W, 2700 - 3000 m., May 25, 1992, *L. Hernandez S. 2748* (TEX); “Cieneguillas”, Pablillo, southeast of Galeana, alt. 2350 - 2450 m., June 28 - 30, 1934, F. W. Pennell 17068 (US); Mt. “El Infernillo”, Pablillo, southeast of Galeana, alt. 2650 - 2750 m., June 29, 1934, F. W. Pennell 17110 (US); Cerro Potosí, road from Galeana to microwave tower, between km 11 and the tower, alt. 8300 ft., May 24, 1974, *McPherson 896* (MICH); Mpio. Galeana, above Santa Rita, alt. 2210 m., May 14, 1981, *Hinton et al. 18243* (MEXU). **Querétaro**: Cerro Boludo, 10 km al S de Pinal de Amoles, 2850 m. alt., June 8, 1986, *R. Fernández N. & J. Rzedowski 3347* (MEXU). **Tamaulipas**: en route to Pena Nevada out of Hermosa, July 16, 1949, *Stanford, Lauber, & Taylor 2507* (GH, MO, US).

26. *Lithospermum multiflorum* Torr. ex A. Gray *Proc. Amer. Acad. Sci.* 10: 51. 1874. TYPE: U.S.A. Colorado: El Paso Co., near the head of Fountain Creek, north of Colorado Springs, July 20, 1843, *J. C. Frémont 447* (syntypes, NY!, GH!).

*Lithospermum pilosum* A. Gray (nom. nud.) *Proc. Acad. Nat. Sci. Philadelphia* 15: 73. 1863, non *Lithospermum pilosum* Nutt. *J. Acad. Nat. Sci. Philadelphia* 7: 43. 1834.

*Lithospermum cognatum* Greene ex Spengle *Oesterr. Bot. Z.* 68: 118. 1919. TYPE: Colorado: Piedra, 7000 ft., July 1899, *C. F. Baker 556* (lectotype, ND [digital image]!; lectotype duplicates, F [digital image]!, NY!). **lectotype designated here**.

Erect perennial herb up to 60 cm tall with 1--few stems, each 1.5--4 mm wide, arising from a woody caudex, naphthoquinones present, stems strigose, sometimes strigulose. Leaves cauline, sessile; blades 1.4--5.8 x 0.2--0.7(--1.1) cm, lanceolate to narrow elliptical, sometimes linear, apex acute, rarely rounded, base acute to rounded to truncate, margins entire, usually revolute, adaxially strigose to strigulose and abaxially strigose, rarely hispid, midvein evident, other orders of veins not evident. Inflorescence terminal dichasium or monochasium and frequently axillary monochasia, up to 19.5 cm long, bracts sessile; blades lanceolate to narrow elliptical to linear, 0.6--3.1(--4.2) x 0.1--0.4(--1) cm, apex acute, base rounded to truncate to acute, margins entire, usually revolute, adaxially and abaxially strigose to strigulose, midvein evident, other orders of veins not evident. Flowers heterostylous, chasmogamous; pedicels 0--7 mm. Long style flowers with sepals 3--6(--10) x 0.5--1 mm, lanceolate, sometimes linear, apex acute, abaxially strigose and adaxially strigulose; corolla funnelform, yellow, abaxially villous to villulous, tube 6--13 x 2--4 mm, adaxial trichomes absent, lobes 1--3 x 1--3 mm, flared to erect, elliptical to orbicular, apex rounded, glands present in the upper portion of the tube, faucal appendages absent, annulus of 5 flanges with trichomes; stamens inserted, filaments 0.5--0.75 x 0.1 mm, attached 3--5 mm above the base of the corolla, anthers 1.5--2 x 0.5 mm, ellipsoid; style 6--10 x 0.1 mm, inserted; stigma subterminal. Short style flowers with sepals 3--9 x 0.75--1 mm**,** lanceolate to linear, apex acute, abaxially strigose to strigulose and adaxially strigulose; corolla funnelform, yellow, abaxially villous to villulous, tube (7--)9--13 x 2--3 mm, adaxial trichomes absent, lobes 1--3.5 x 1--3(--4.5), flared to erect, elliptical to orbicular, apex rounded, glands present in the upper portion of the tube and sometimes on the veins leading to the filaments, faucal appendages absent, annulus of 5 flanges with trichomes; stamens inserted, filaments 0.5--0.75 x 0.1 mm, attached (6--)7--8(--10) mm above the base of the corolla, anthers 1.25--2 x 0.5--0.75 mm, ellipsoid; style 3.5--5 x 0.1 mm, inserted; stigma subterminal. Nutlets 2--4 x 1.5--2 mm, ovoid, white to tan, some dimpling around prominent scar, base rounded, apex acute.

Distribution: The southern extent of the geographic range this species reaches Chihuahua and Coahuila, but *L. multiflorum* is more common throughout the western mountains of the United States.

Phenology: Flowers from early June to October, fruits from late June.

Discussion: *L. multiflorum* is quite common throughout the mountains of the western United States, but in Mexico, it has a much more limited distribution. I have not come across a specimen from Mexico that has been collected within the last thirty years.

The form of *L. multiflorum* is quite similar to that of *L. tubuliflorum*. Both species are heterostylous and produce yellow, funnelform flowers. However, *L. multiflorum* only produces cauline leaves, while *L. tubuliflorum* develops both cauline and basal leaves. Johnston (1952) noted that despite the large number of collections he observed, only a few were in fruit. I also found this to be the case, with only 20% of the specimens that I examined bearing fruit.

Specimens Examined: MEXICO. **Chihuahua**: East from Pearson, July 25, 1911, *B. Barlow s.n.* (F); Santa Clara Mts., Aug. 17, 1936, *H. LeSueur 865* (F); St. Clara Canyon, Oct. 10, 1935, H. *LeSueur Mex-315* (F); **Coahuila**: Madera del Carmen, at head of road above Campo Cuatro, alt. 7500 ft., June 21, 1976, *P. Fryxell 2698* (MO, NY).

27. *Lithospermum nelsonii* Greenm. *Proc. Amer. Acad Arts* 40: 31. 1904. TYPE: Mexico. Nuevo León: Cerro la Silla, near Monterrey, Mar. 20, 1902, *E. W. Nelson 6682* (holotype, GH!).

Erect perennial herb up to 71 cm tall with 1--few stems, each 1--2.5(--4) mm wide, arising from a woody caudex, naphthoquinones not observed, stems hispid. Leaves cauline, sessile; blades elliptical to obovate, sometimes ovate or spatulate, (1.9--)2.5--6.3 x 0.6--1.6 cm, apex acute, sometimes mucronate, base acute to acuminate, margins entire, planar, adaxially and abaxially strigose to hispid, midvein evident, usually 1--2 pairs of secondary veins evident, sunken or not. Inflorescence terminal dichasium or monochasium and sometimes axillary monochasia, up to 20 cm long, bracts sessile; blades ovate to elliptical to spatulate, 0.8--4.5 x 0.2--1.1(--1.3) cm, apex acute, base rounded to acute, margins entire, planar, adaxially and abaxially strigose to hispid, rarely also hispidulose, midvein evident, sometimes 1--2 pairs of evident secondary veins, sunken or not. Flowers homostylous, chasmogamous; pedicels 0--6 mm long; sepals linear to lanceolate, (2--)5--8(--10) x 0.5--1.25 mm, apex acute to acuminate, abaxially strigose to hispid, dense strigose or dense hispid towards the base, adaxially strigose to strigulose, if both types present, usually strigose towards the base and strigulose towards the apex; corolla salverform, white with a yellow center, abaxially villous towards the apex, glabrous towards the base, tube 10.5--14 x 2--3 mm, adaxial trichomes absent, lobes (2--3.5--)5--8 x 3--7 mm, flared, orbicular to elliptical, glands present just below the filaments and apex of corolla throat, faucal appendages present, annulus of 5 membranous flanges to a membranous ring; stamens inserted, 0.75--1 x 0.1 mm, attached 8--10 mm above the base of the corolla, anthers 1.25--1.5 x 0.5 mm, ellipsoid; style (5.5--)12--18(--20) x 0.1 mm, usually inserted, sometimes exserted up to 1.75 mm from the apex of the corolla tube; stigma subterminal. Nutlets 3 x 2 mm, ovoid, pitting common, more abundant towards the base than the apex, base a 0.25 mm pedestal, apex acute.

Distribution: Restricted to Nuevo León.

Phenology: Flowers March to August, fruits from mid-June.

Discussion: *L. nelsonii* has corollas that are white with a yellow center, and this color pattern is similar to that of the corollas of *L. distichum* and of *L. matamorense*. Despite this similarity, *L. nelsonii* does not appear to be closely-related to either of these species. *Lithospermum nelsonii* is resolved as sister to *L. leonotis*. These two species have quite different floral and vegetative forms (Cohen and Davis, 2009; Chapter 3, 4).

Because plants of *L. nelsonii* produce leaves with a particular type of venation pattern – a midvein and one or two pairs of sunken secondary veins, this species can be distinguished from others that bear corollas of a similar color pattern. The aforementioned pattern of leaf venation is found only in one other species of *Lithospermum*, *L. berlandieri*. *Lithospermum nelsonii* has larger corollas than *L. berlandieri,* and the former is restricted to Nuevo León, while the latter is found only around Ciudad Victoria, Tamaulipas.

Specimens Examined: MEXICO. **Nuevo León**: Sierra Madre above Monterry, 2500 ft., Apr. 4, 1906, *C. G. Pringle 10233* (F, MEXU, MICH, MO, US); Sierra Madre Oriental, Guadjuco Canyon, El Cercado, about 30 miles south of Monterrey, Aug. 10, 1934, *C. H. & M. T. Mueller 1307* (F, MICH); Chipinque, 10 miles south of Monterrey, April 5, 1964, *E. Gonzalez-Arroyo 118* (TEX); Monterrey, trails just W of Chipinque Lodge, ca. 6800 ft., Mar. 10, 1980, *B. L. & G. Turner 80-07M* (MEXU, TEX); Sierra Madre Mts., Monterrey, July 13, 1933, *C. H. & M. T. Mueller 168* (F, TEX); Monterrey, Parque Chipinque, on trail to V2 – veer off and head up the trail to the top, not to the hotel, 25° 37.012’ N 100° 21.405’ W, June 12, 2007, *J. Cohen 184* (BH); Monterrey, Chipinque, alt. 1700 ft., May 15, 1947, M. M. Lacás 328 (F); Mpio. Villa de Santiago, elev. 2500 ft., June 20, 1940, *Wm. C. Leavenworth 133* (F).

28. *Lithospermum notatum* (I. M. Johnst.) J. I. Cohen *Brittonia* 61: 108. 2009. Basionym: *Macromeria notata* I. M. Johnst. *J. Arnold. Arbor.* 35: 13. 1954. TYPE: Mexico. Nuevo León: Ascent of Sierra Infernillo, ca. 15 mi. SW2009] of Galeana, “Common over small areas just below the peak,” 9000 - 10,000 ft, 16 June 1934, *C. H. Mueller & M. T. Mueller 830* (holotype, GH!; isotypes, F!, MICH [digital image]!, TEX [digital image]!).

Erect perennial herb up to 50 cm tall with 1--few stems, each 2--4 mm wide, arising from a woody caudex, naphthoquinones present, stems hispid and strigose, usually dense strigose towards the apex. Leaves cauline, sessile; blades lanceolate to elliptical, (2.3--)3.3--7.6 x 0.5--2.4, apex acute, rarely acuminate, base rounded to acute, margins entire, revolute, adaxially sparsely strigose to strigose and abaxially sparsely strigose to strigose, more densely strigose on veins, midvein evident, secondary veins evident, sunken. Inflorescence terminal dichasium or monochasium, rarely axillary monochasia, up to 16.5 cm long, bracts sessile; blades lanceolate, 1.9--3.7(--5.2) x 0.4--1.2(--1.6) cm, apex acute, base rounded to acute, margins entire, revolute, adaxially strigose, sometimes strigulose, and abaxially sparse strigose to strigose, more densely strigose on veins, midvein evident, secondary veins evident, sunken. Flowers homostylous, chasmogamous; pedicels 0--9 mm long; sepals linear, 9--15 x 0.75--1(--2) mm, apex acute, abaxially strigose, usually more densely strigose towards the base, with margins hispid, adaxially strigulose; corolla long-funnelform, yellow towards the base and yellow-green towards the apex, abaxially villous, villulose towards the apex of the lobes, tube 35--48 x 3--4.5 mm, adaxial trichomes absent, lobes 4--6(--8.5) x 3--4(--5) mm, elliptical, apex acute, glands present as a line in the middle of each lobe and at the apex of the tube, faucal appendages absent, annulus absent; stamens exserted, filaments 7--10 x 0.25--0.3 mm, attached 34--39 mm above the base of the corolla, exserted 3--6.5 mm from the apex of the corolla tube, anthers 2--3 x 0.75--1 mm, ellipsoid; style 41--56 x 0.1--0.3 mm, exserted 7--15 mm from the apex of the corolla tube; stigma terminal to subterminal.  Nutlets 4 x 3 mm, ovoid, white, smooth to sparse pitting around the scar, base rounded, apex acute.

Distribution: Center of diversity is Cerro Potosi in Nuevo León, the species has been found in neighboring Coahuila.

Phenology: Flowers May to October, fruits from July.

Discussion: *L. notatum* has a small distribution, being known from Nuevo León and Coahuila.  *Lithospermum notatum* shares with *L. album* the presence of glands as a line in the middle of each lobe and at the apex of the throat. In the phylogenetic analysis that Boyd (2003) conducted, these species were resolved as sisters.

Approximately half of the specimens of *L. notatum* that I examined for the present study have been assigned to *L. oblongifolium*.  The gross vegetative and floral features of the two species are quite similar, and therefore it can be easy to mistake the species for each other.  However, the two species differ in two floral features.  The flowers of *L. notatum* lack faucal appendages and produce exserted anthers, while those of *L. oblongifolium* develop faucal appendages and have inserted anthers.  In addition, the two species are separated geographically, with *L. notatum* restricted to the northeastern part of Mexico, and *L. oblongifolium* growing farther south.

Specimens Examined: MEXICO. **Coahuila**: El Coahuilón, Sierra de la Marta (Arteaga), 25° 14’ 12” N 100° 18’ 40” W, 3600 m., October 17, 1989, *J. A. Villarreal 5483* (TEX). **Nuevo León**: on the road to the top of Cerro Potosí, near the top of the cerro, at the weather station, about halfway through the pine belt, 24° 52’ N 100° 14.5’ W, 3500 m., May 23, 1988, *B. L. Westlund 5.23.88.24* (TEX); Mpio. Galeana, road to summit of Cerro Potosí, 12.4 mi from Dieciocho de Marzo, May 18, 1982, *L. J. Dorr 2272* (TEX); Sierra de la Marta, El Renacer de la Sierra, 25° 12’ 28” N 100° 21’ 43” W, 2895 m., May 14, 2004, *J. A. Encina 1332* (TEX); Mpio. Galeana, 18 km on the road on the way to the top of Cerro Potosí, 24° 53.148’ N 100° 13.311’ W, 10269 ft., June 13, 2007, *J. Cohen 188* (BH); Cerro Potosí, just below northeast summit of mtn., ca. 3640 m., September 18, 1961, *J. H. Beaman 4505* (US); Cerro Potosí, near microwave tower, ca. 9000 ft., July 8, 1963, *R. L. McGregor, L. J. Harms, A. J. Robinson, R. del Rosario, & R. Segal 346* (US); Mpio. Galeana, cima del Cerro Potosí, 3650 m., August 15, 1989, *A. García 148* (MEXU); Mpio. Galeana, cañon below Las Canoas on Cerro Potosí, July 20, 1935, *C. H. Mueller 2238* (F, US); Mt. “El Infernillo”, Pablillo, southeast of Galeana, alt. 3000 – 3100 m., June 29, 1934, *F. W. Pennell 17123* (US); Cima de la Sierra de la Marta, 40 km al E de San Antonio de las Alazanas, 25° 09’ N 100° 23’ W, 3400 m. May 19, 1995, *J. A. Villareal 8134* (MO).

29. *Lithospermum oaxacanum* (B. L. Turner) J. I. Cohen *Brittonia* 61: 109. 2009. Basionym: *Onosmodium oaxacanum* B. L. Turner *Phytologia* 78: 53. 1995. TYPE: Mexico. Oaxaca: Llano Udadi, 4 km SE of San Andreas Lagunas, alt. 2380 m., July 10, 1981, *A. Garcia M. 504* (holotype, MEXU!).

Erect perennial herb up to 40 cm tall with 1--few stems, each 2--3 mm wide, arising from a woody caudex, naphthoquinones not observed, stems hispid to long hispid. Leaves cauline and pseudobasal, sessile; cauline blades lanceolate to narrow elliptical, 2.7--10 x 0.7--1.3 cm, apex acute, base rounded, rarely acute, margins entire, planar, sometimes revolute, adaxially strigose to long hispid and abaxially hispid to long hispid, midvein evident, other orders of veins sometimes evident, not sunken; basal blades oblanceolate to narrow elliptical, 5--9.7 x 0.9--1.3 cm, apex acute to rounded, base truncate, margins entire, planar, sometimes revolute, adaxially and abaxially hispid to long hispid, midvein evident, other orders of veins sometimes evident, not sunken. Inflorescence terminal dichasium, up to 8.5 cm long, bracts sessile, lanceolate, 0.8--3 x 0.2--1 mm, apex acute, base rounded, margins entire, planar, sometimes revolute, adaxially and abaxially strigose to hispid, rarely long hispid, midvein evident, other orders of veins not evident. Flowers homostylous, chasmogamous; pedicels 0--7 mm long; sepals lanceolate, 9--11(--up to 17 in fruit) x 1--2 mm, apex acute, abaxially hispid, more dense hispid towards the base, and adaxially hispid; corolla campanulate, yellow-green, abaxially dense villous towards the apex and sparse villous towards the base, tube 9--11 x 4--5 mm, adaxial trichomes present, villous throughout tube, lobes 3--4 x 1--1.5 mm, erect, deltoid, apex acute, glands absent, faucal appendages absent, annulus of 5 knobs with trichomes; stamens inserted, filaments 0.75 x 0.1 mm, attached 8 mm above the base of the corolla, anthers 2.5 x 0.75 mm, ellipsoid; style 19 x 0.1--0.25 mm, exserted up to 9 mm from the apex of the corolla tube; stigma terminal. Nutlets 4 x 3 mm, grey, smooth, scar prominent.

Distribution: Restricted to Oaxaca.

Phenology: Flowers and fruiting starting in mid-July.

Discussion: *Lithospermum oaxacanum* is the southernmost species of those formerly included in *Onosmodium*. *Lithospermum oaxacanum* is distinctive, as it is one of the few species of *Lithospermum* that produces an indument that ranges from hispid to long hispid. The long distance between *L. oaxacanum* and both *L. unicum* and *L. dodrantale*, the two other Mexican species previously included in *Onosmodium*, raises questions concerning dispersal and convergent evolution.

Specimens Examined: MEXICO. **Oaxaca**: 4 km al N de Guadalupe Tixa, sobre el camino a San Andres Lagunas, distrito de Teposcolula, alt. 2260 m, July 15, 1986, *A. G. Mendoza 2657* (MO).

30. *Lithospermum oblongifolium* Greenm. *Proc. Amer. Acad. Arts* 32: 300. 1897. TYPE: Mexico. D.F.: Serrania de Ajusco, 10000 ft., Aug. 18, 1896, *C. G. Pringle 6451* (holotype, GH!, isotypes, BR [digital image]!, CAS, MEXU!, NY [digital image]!, US!).

*Lithospermum euryphyllum* Brand *Repert. Spec. Nov. Regni Veg.* 28: 16. 1930. TYPE: Mexico. D.F.: Serrania de Ajusco, 10000 ft., Aug. 18, 1896, *C. G. Pringle 6451* (type, F [digital image]!).

Erect perennial herb up to 68 cm tall with 1--few stems, each 2--4 mm wide, arising from a woody caudex, naphthoquinones not observed, stems strigose and hispid. Leaves cauline, sessile; blades elliptical to ovate, sometimes obovate or lanceolate, 2--12.6(--14) x (0.3--)0.5--2.6(--3.3) cm, apex acute, base rounded, sometimes acute, margins entire, planar, adaxially strigose to strigose and strigulose to strigose and sparsely strigulose and abaxially strigose to strigose and strigulose, midvein evident, secondary veins evident, not sunken. Inflorescence terminal dichasium, rarely axillary monochasia, up to 28.5 cm long, bracts sessile; blades ovate to lanceolate to elliptical, 1--6.2(--7.2) x 0.2--2.5 cm, apex acute, base rounded to truncate, rarely acute, margins entire, planar, adaxially and abaxially strigose and strigulose, midvein evident, secondary veins evident, not sunken. Flowers homostylous, chasmogamous; pedicels 0--13(--19) mm long; sepals linear, 5--15(--20) x 0.75--1.5(--3) mm, apex acute to acuminate, abaxially strigose to strigose and hispid, margins hispid, adaxially strigulose; corolla long-funnelform, yellow to yellow-green, rarely lightly pink in parts, abaxially villous to villous and villulose, tube (16--)20--37 x (2--)3--4 mm, adaxial trichomes absent, lobes (1--)1.5--4 x 2-5 mm, erect, orbicular to elliptical, apex rounded, glands present on veins leading to filaments and apex of corolla throat, faucal appendages present, annulus absent; stamens inserted, filaments 0.75--1 x 0.1--0.25 mm, cylindrical, attached 28--34 mm above the base of the corolla, anthers 1.75--2 x 0.5--0.75 mm, ellipsoid; style (13--19--)28--36(--41) x 0.1--0.25 mm, exserted up to 6 mm from the apex of the corolla tube; stigma terminal to subterminal. Nutlets 3--4 x 2--3.5 mm, ovoid, white to white and tan, smooth, base rounded, apex acute.

Distribution: This species is found in the mountains of the central Mexico.

Phenology: Flowers from May to August, fruits from early July.

Discussion: *Lithospermum oblongifolilum* is unique among species of *Lithospermum*, as it is the only species to produce the following combination: long-funnelform corollas, faucal appendages, and inserted anthers. The latter two features usually are associated with salverform corollas, not ones that are long-funnelform.

*Lithospermum oblongifolium* is closely related to *L. distichum*. These species share the presence of both cylindrical pollen and faucal appendages (Chapter 4). It also should be mentioned that plants of this species produce sharp trichomes, so take precautions when collecting it.

Specimens Examined: MEXICO. **Distrito Federal**: Estación La Cima, Serranía del Ajusco, alt. 3000 m., Aug. 22, 1965, *Rzedowski 20443* (TEX); Alrededores de la Estación La Cima, Serranía del Ajusco, alt. 3000 m., Aug. 14, 1960, *Rzedowski 12608* (F, LL, MICH, US); Milpa Alta, a 300 m en LR al oeste del Volcán Ocotecatl, 19° 05' 07" N 99° 01' 25 W, alt. 3,320 msnm., *J. E. Rivera-H. 3859* (MEXU); Alrededores de la Estación La Cima, Serranía del Ajusco, alt. 3000 m., June 13, 1965, *Rzedowski 19910* (MEXU); Cima del Ajusco, July 17, 1908, *C. G. Pringle 15624* (F, US); Side of road in Ajusco near Rancho Ecologico, 19° 6' 34.0" N 99° 11' 27.5 W, alt. 2885 m., June 20, 2007, *J. Cohen 201* (BH). **Mexico**: Mpio. Ixtapaluca, Llano Grande, alt. 3250 m., July 23, 1983, *E. Ventura V. 1128* (TEX); Cerro Acacoaya, cerca del km 50, carretera México – Puebla, May 15, 1974, *L. Paray 3470* (LL, MICH); Cerros Tenayo, Sonayo, alt. 2600 m., July 27, 1952, *Leg. E. Matuda 26256* (TEX, MEXU); Telapón, July 5, 1967, *Leg. Wolfgang Boege 571* (MEXU); N-facing slopes just S. of Pan-Am Highway at Pass of Monte Rio Frio (5 km WNW of Rio Frio) at Llano Grande , alt. ca. 3100 - 3200 m., 19° 22' N 98° 43' W, Aug.18, 1960, *H. H. Iltis, R. Koeppen, & F. Iltis 1088* (MEXU); "Cerro Gordo" or "Cerro Cuatillo Grande", high hill of layers of volcanic ash, ¼ km. S of La Cima, ¼ km. S. of La Cima R.R. Station, alt. 3050 - 3150 m., just N and W of old highway 95 (19° 6' - 7' N, 99° 12' W), July 12, 1960, *H. H. Iltis, R. Koeppen, & F. Iltis 155* (MEXU, MICH); Mpio. Calpulapan, 6 km al S del pueblo “La Soledad”, elev. 3000 m., Apr. 27, 1991, *L. Aguilera 542* (MEXU); Llano Grande, Pinagua, alt. 3000 m., Aug.10, 1941, *P. Lyonnet 3293* (US); Llano Grande, July 1930, *E. Lyonnet 683* (MICH, US); 55 km SE of Mexico City, alt. 10500 ft., July 6, 1942, *J. N. Weaver 741* (US); Temascaltepec, Crucero Agua Blanca, elev. 3170 m., Aug. 29, 1933, *Hinton 4609* (F); Mpio. Ixtapaluca, Estación Experimental de Investigación y Enseñanza de Zoquiapan, 8 km. S de Río Frio, camino 4, 6 - 7 km. S of camino 3, elev. 3410 m., June 27, 1975, *S. D. Koch 75210* (F). **Puebla**: 4 km E of Rio Frio between new and old highway 190 at km 65, alt. ca. 3000 m., (19° 20’ N, 98° 35’ W), July 19, 1965, *K. Roe, E. Roe, & S. Mori 358* (US). **Tlaxcala**: Mt. Malinche, above San Francisco, alt. 9000 ft., June 22, 1938, *E. K. Balls 4885* (US).

31. *Lithospermum obovatum* J. F. Macbr. *Contr. Grey Herb.* 48: 56. 1916. TYPE: Mexico. Durango: Quebrada Honda, May 20 & 21, 1906, *E. Palmer 216* (holotype, GH!; isotype, K [digital image]!).

*Lithospermum gentianoides* Brand *Repert. Spec. Nov. Regni Veg.* 28: 15. 1930. TYPE: Mexico. Chihuahua: vicinity of Tierra Colorado, or Rio Colorado, Sierra Madre, ca. 2400 m., Apr. 19, 1906, *Endlich 1235* (lectotype, GH!). **lectotype designated here**, see discussion under *L. calcicola*.

Erect perennial herb up to 31.5 cm tall with 1--few stems, each 1--2 mm wide, arising from a woody caudex, naphthoquinones rarely present, stems hispid to dense hispid. Leaves cauline and basal, sessile; cauline blades 0.8--2.6 x 0.1--0.55 cm**,** linear to elliptical to lanceolate**,** apex rounded to acute, base truncate to obtuse, margins entire, usually revolute, adaxially strigose to dense hispid, rarely hispid and abaxially hispid to dense hispid, rarely strigose, midvein evident, other orders of veins not evident;

basal blades 1.3--9.5 x 0.3--3.7 cm, apex rounded to acute, base acute to acuminate, margins entire, planar, adaxially strigose, rarely long strigose or hispid and abaxially hispid, usually more densely hispid on veins, midvein evident, secondary veins evident, sunken. Inflorescence terminal monochasia, up to 12.5 cm long, bracts sessile; blades lanceolate to linear, 0.4--1.8 x 0.1--0.4 cm, apex rounded to acute, base truncate, margins entire, usually revolute, adaxially strigose to dense strigose, rarely hispid and abaxially hispid to dense hispid, rarely strigose; midvein evident, other orders of veins not evident. Flowers heterostylous, chasmogamous; pedicels 0--4 mm; Long style flowers with sepals linear, 3--6(--8) x 0.5--1 mm, apex rounded to acute, abaxially strigose to dense strigose, usually more densely strigose towards the base, and adaxially strigulose; corolla funnelform-salverform, yellow, abaxially villous to dense villous, tube 7--10 x 2--2.25 mm, adaxial trichomes usually present above filaments, villous, lobes 3--6 x 2--5 mm, flared, orbicular to elliptical, apex rounded, glands present in the upper portion of the tube, faucal appendages present, annulus of 5 knobs with trichomes; stamens inserted, filaments 0.5 x 0.1 mm, attached 1.75--2 mm above the base of the corolla, anthers 1.5--1.75 x 0.5 mm; style 5.5--7(--12) x 0.1 mm, inserted; stigma subterminal. Short style flowers with sepals linear, 3--5(--8) x 0.75--1 mm, apex acute to acuminate, abaxially strigose to hispid and adaxially strigulose; corolla funnelform-salverform, yellow, abaxially villous, sparsely villous towards the base and dense villous towards the apex, tube 8--13 x 2--3(--4), adaxial trichomes absent, lobes 4.5--7.5 x 4--7 mm, flared, orbicular to elliptical, apex rounded, glands present above filaments, faucal appendages present, annulus of 5 knobs with trichomes; stamens inserted, filaments 0.5 x 0.1 mm, attached 7--9 mm above the base of the corolla, anthers 1.75--2 x 0.5 mm, ellipsoid; style 1.5--2 x 0.1 mm, inserted; stigma terminal to subterminal. Nutlets 3 x 2 mm, ovoid, white to tan, smooth to sparse pitting by prominent scar, base rounded, apex acute.

Distribution: Chihuahua and Durango.

Phenology: Flowers April to June, fruits develop in June.

Discussion: *L. obovatum* is one of the four heterostylous species of *Lithospermum* that grows in northwestern Mexico. A combination of floral and vegetative characters can be used to distinguish among these four species. The three most common species, *L. obovatum*, *L. tubuliflorum*, and *L. cobrense*, each produce basal and cauline leaves. However, *L. cobrense* bears basal leaves in which only the midvein is evident, and this contrasts with the venation pattern of both *L. obovatum* and *L. tubulifloum*. These two species produce basal leaves with an evident midvein and evident secondary veins. Although the patterns of leaf venation are similar between *L. obovatum* and *L. tubuliflorum*, the shape of corollas of the two species differs. *Lithospermum obovatum* develops corollas that are funnelform-salverform, while those of *L. tubuliflorum* are funnelform. The fourth species that grows in northwestern Mexico is *L. multiflorum*, and unlike *L. obovatum*, *L. cobrense*, and *L. tubuliflorum*, it only produces cauline leaves.

Specimens Examined: MEXICO. **Chihuahua**: area of Cascada de Basaseachic at the confluence of Rio Basaseachic and Rio Durazno, ca. 2 mi S of village of Basaseachic, between campground and top of falls, 2100--2200 m., Apr. 27, 1986, *G. Nesom 5433* (TEX); 26.4 mi. W of Tomochic, 6.9 mi. E of jct to San Juanito on road to Basaseachic, 2200 m., Apr. 25, 1986, *G. Nesom 5421* (TEX); Mpio. Guachochic, Northwest of Cusarare, near boundary with San Ignacio Arareco (Mpio. de Bocoyna), Oct. 15, 1973, *R. A. Bye 5439* (MEXU); Mpio. Ocampo, Parque Nacional “Cascada de

Basaseachic," along the trail from the parking lot to the top of the Cascada, 28° 10' N, 108° 12' 30" W, elev. 1980 m., June 17, 1993, *R. Spellenberg, L. Brouillet, & E. Ulaszke 11878* (NY); Mpio. Guerrero, 40 km by rock road W of Tomochic on the way to Basaseachic, near top of pass and the limits of the municipio, ca. 28° 17' N, 108° 3' W, elev. 2290 m., Apr. 26, 1995, *R. Spellenberg, R. Soreng, & R. Corral 8005* (NY). **Durango**: District Santiago Papasquiaro, northeast of Ataes, alt. 8000 ft., Apr. 20, 1943, *C. L. Lundell 13015* (LL); Mpio. San Dimas, Mirvalles, UCODEFO 4. 24° 17' N 105° 32' W, 2,460 m., May 22, 1992, *S. González 5114* (CIIDIR); Mpio. San Dimas, on road out of Vencedores, to exit of town closer to San Miguel de Cruces, 24° 26' 11" N 105° 43' 14" W, 2,486 m., June 25, 2007, *J. Cohen 208* (BH, MEXU); Mpio. San Dimas, Mesa del Roble, San Miguel de Cruces, 24° N 105° W, 2740 m., May 26, 1990, *A. García 341* (CIIDIR).

32. *Lithospermum pinetorum* (I. M. Johnst.) J. I. Cohen *Brittonia* 61: 109. 2009. Basionym: *Lasiarrhenum pinetorum* I. M. Johnst. *J. Arnold. Arbor.* 16: 187. 1935. *Perittostema pinetorum* (I. M. Johnst.) I. M. Johnst. *J. Arnold Arbor.* 35: 30. 1954. TYPE: Mexico. Oaxaca(?): Sep. 1844, *Ghiesbreght 311* (holotype, P [digital image]!).

Erect perennial herb up to 15 cm tall with 1--few stems, each 1--2 mm wide, arising from a woody caudex, naphthoquinones not observed, stem indument not observed. Leaves cauline, sessile; blades lanceolate to linear, 1--3 x 0.15--0.2 cm, apex acute to acuminate, base truncate, margins entire, revolute, adaxially and abaxially strigulose, midvein evident, other orders of veins not evident. Inflorescence terminal monochasium, up to 2 mm long, bracts sessile, linear to lanceolate, 0.75--1 x 0.1 cm, apex acuminate, base truncate, margins entire, revolute, adaxially and abaxially strigulose, midvein evident, other orders of veins not evident. Flowers homostylous, chasmogamous; pedicel 0--1 mm long; sepals lanceolate, 4--4.5 x 0.75 mm, apex acuminate, sepal indument not observed; corolla campanulate, tube 6--8 x 2--2.5 mm, adaxial trichomes absent, lobes 1--1.5 x 1 mm, erect, deltoid, apex acute, glands present below the faucal appendages, annulus absent; stamens inserted, filaments not observed, anthers 2 x 1 mm, ellipsoid; style 17--18 x 0.1--0.25 mm, exserted up to 4 mm from the apex of the corolla tube; stigma terminal. Nutlets not observed.

Distribution: Although the exact locality of this species is unknown, given the evidence, Johnston (1954a) believed it was collected in Oaxaca.

Phenology: Flowers in September, fruits not observed.

Description: *L. pinetorum* is known from only one collection made by Ghiesbreght. Johnston (1954a) was quite taken with this species, and he described, in detail, its floral characteristics. Given that this species has not been collected since the mid-1800s, it is possibly extinct.

Specimens Examined: MEXICO. **No Locality**: Sep. 1841(?), *Ghiesbreght 311* (P).

33. *Lithospermum pringlei* I. M. Johnst. *Contr. Gray Herb.* 70: 22. 1924. TYPE: Mexico. Hidalgo: Rocky hills near Metepec Station, 8300 ft., June 25, 1904, *C. G. Pringle 13123* (holotype, GH!, isotype, F!, K [digital image]!).

*Lithospermum seleri* I. M. Johnst. *Contr. Gray Herb.* 70: 28. 1924. TYPE: Mexico. Puebla: Prope, July 1888, *C. Seler & E. G. Seler 847* (holotype, GH!; isotype, US!).

Erect perennial herb up to 82 cm tall with 1--few stems, each 1.5--3(--5) mm wide, arising from a woody caudex, naphthoquinones not observed, stems strigose and hispid. Leaves cauline, sessile; blades elliptical to lanceolate, rarely oblanceolate, or obovate, (1.5--)2.4--9.4 x 0.35--1.5(--1.8) cm, apex acute, rarely rounded, base acute, margins entire, planar, adaxially strigose and strigulose and abaxially strigose to strigose and strigulose, more densely strigose on veins, midvein evident, secondary veins evident, sunken. Inflorescence terminal dichasium and axillary monochasia, up to 26.5 cm long, bracts sessile; blades lanceolate to elliptical, rarely linear, 0.9--5 x 0.2--1(--1.5) cm, apex acute, base acute, margins entire, planar, adaxially strigose and strigulose, rarely densely strigose and densely strigulose and abaxially strigose to strigose and strigulose, rarely densely strigose or hispid, usually more densely strigose on the veins, midvein evident, other orders of veins evident or not. Flowers homostylous, chasmogamous; pedicels 0--7 mm long; sepals linear, 4--12(--16) x 0.5--1 mm, apex acute, abaxially strigose, usually more densely strigose towards the base, and adaxially strigulose to strigose; corolla tubular to funnelform-tubular, yellow, abaxially villous to woolly, tube 5--9 x 1--2 mm, adaxial trichomes absent, lobes 1--2(--2.5) x 1--2(--2.5) mm, flared, orbicular to elliptical, apex rounded to obtuse, glands absent, faucal appendages present, annulus of 5 knobs with trichomes; stamens inserted, filaments 0.5--1 x 0.1 mm, attached 6--7 mm above the base of the corolla, anthers 1--1.1 x 0.5 mm, ellipsiod; style 6--8(--11) x 0.1 mm, usually inserted, sometimes exserted up to 0.5 mm from the apex of the corolla tube; stigma subterminal. Nutlets 3--4 x 2--2.5 mm, ovoid, white to dull gold to white and red, maroon, or pink, pitting around the scar to around upper portion of nutlet, base rounded, apex acute.

Distribution: Restricted to the mountains, and usually lava flows, of central Mexico.

Phenology: Flowers late June to August, and fruits late June onwards.

Discussion: *L. pringlei* appears to share an affinity with *L. indecorum* (a species only known from its type). Both species produce leaves with evident secondary venation, and the leaves of these two species are the smallest in the genus to develop this venation pattern. Although the vegetative characteristics of these two species are similar, *L. pringlei* produces corollas that are much longer than those of *L. indecorum*. The two species also have different geographic ranges. *Lithospermum indecorum* grows in the northern part of Mexico, and *L. pringlei* is distributed throughout Hidalgo and D.F.

Specimens Examined: MEXICO. **Distrito Federal**: Pedregal de San Angel, cerca de la Ciudad Universitaria, 2300 m., July 27, 1970, *Rzedowski 27379* (MICH, US); Sierra de Ajusco, alt. 10000 ft., August. 18, 1896, *C. G. Pringle 7391* (US); Pedregal, August 15, 1938, *E. Lyonnet 2332* (US); Near Tlalpam, Valley of Mexico, 1905, *J. N. Rose, Jos. H. Painter, & J. S. Rose 8490* (US); Pedregal, Valley of Mexico, 7500 ft., August 1896, *C. G. Pringle 6416* (F, NY, US); Pedregal de San Angel, 2250--2300 m., August 25, 1941, *P. Lyonnet 3301* (US); Sierra de Guadalupe, 7000 ft., July 21, 1938, *E. K. Balls 5068* (US); Sierra de Santa Catarina, delegación de Ixtapalapa, alt. 2600 m., July 10, 1975, *Rzedowski 33870* (MEXU, MICH). **Hidalgo**: Rocky hills near Metepec Station, 8300 ft., June 25, 1904, *C. G. Pringle 13123* (F, US); Mpio. Tepeapulco, Cerro de Xihuingo, alt. 2700 m., October 4, 1974, *A. Ventura A. 369* (MEXU); Sierra de Pachuca, alt. 9000 ft., July 16, 1898, *C. G. Pringle 7573* (F, MO).

34. *Lithospermum revolutum* B. L. Rob. *Proc. Amer. Acad. Sci.* 27: 182. 1892. *Onosmodium revolutum* (B. L. Rob.) J. F. Macbr. *Contr. Gray Herb.* 49: 21. 1917. *Psilolaemus revolutus* (B. L. Rob.) I. M. Johnst. *J. Arnold. Arbor.* 35: 34. 1954. TYPE: Mexico. San Luis Potosí: Alkaline meadows, Hacienda de Angostura, July 10, 1891, *C. G. Pringle 3802* (holotype, GH!, isotypes, BR [digital image]!, F!, K [digital image]!; MEXU!, MICH!, US!).

Erect perennial herb up to 54 cm tall with 1--few stems, each 1--4 mm wide, arising from a woody caudex, naphthoquinones present, stems strigose. Leaves cauline, sessile; blades lanceolate to elliptical to oblanceolate, 1.4--6.8 x 0.3--1.6 cm, apex acute to rounded, base acute to acuminate to truncate, rarely rounded, margins entire, usually revolute, adaxially strigose to strigulose and abaxially strigose to strigulose, midvein evident, sometimes secondary veins evident, not sunken. Inflorescence terminal and axillary monochasia, up to 29.5 cm long, bracts sessile; blades ovate to lanceolate to elliptical, 0.5--2.7 x 0.2--0.9 cm, apex acute, base rounded, sometimes acute, margins entire, usually revolute, adaxially and abaxially strigose to strigulose, rarely dense strigose, midvein evident, other orders of veins not evident. Flowers homostylous, chasmogamous; pedicels 0--6 mm long; sepals lanceolate, 6--9(--up to 12 in fruit) x 0.75--1.25 mm, apex acute, abaxially strigose and strigulose to dense strigose and dense strigulose and adaxially strigulose; corolla tubular, yellow, abaxially villous, tube 7--9 x 1--2 mm, adaxial trichomes absent, lobes 1--2 x 1--1.25 mm, erect, deltoid, apex acute, glands absent, faucal appendages absent, annulus of a ring with trichomes; stamens inserted, fllaments 1 x 0.1--0.25 mm, attached 7--8 mm above the base of the corolla, anthers 1--1.5 x 0.5, ellipsoid; style 9--15 x 0.1 mm, precociously exserted, at anthesis exserted up to 5 mm from the apex of the corolla tube; stigma terminal. Nutlets 2 x 1--1.5 mm, ovoid, white to grey to brown, smooth to sparse pitting, usually around not prominent scar, base rounded, apex acute.

Distribution: Restricted to salty, wet areas in San Luis Potosí.

Phenology: Flowers from late May to August, and fruits from early June.

Discussion: *L. revolutum* is quite distinctive among the members of *Lithospermum*. Its leaves are more coriaceous than those of other species, and it grows in salty, wet areas. In addition, *L. revolutum* bears yellow, tubular, eglandular corollas. Corollas of this type are not produced by other species of the genus.

Most collections of this species were made over 30 years ago. In 2007, I collected near Las Tablas, and I found individuals of *L. revolutum* growing along the side of the road as well as in a ditch in a meadow. Given its restricted geographic range, this species may go extinct if the countryside of San Luis Potosí becomes more developed.

Specimens Examined: MEXICO. **San Luis Potosí**: Just N of Las Tablas, N 22˚ 15' 41.7' W 99˚ 51' 52.8", alt. 3382 ft., June 16, 2007, *J. Cohen 199* (BH); 0.65 km (0.4 mi) N of the Escuela Primaria in Las Tablas, on gravel road running between hwys 70 & 80, 1097 m (3600'), July 27, 1979 *M. A. Lane 2543a* (TEX); 1.3 mi S of Colonia Libertad on the high road 07 mi N of Las Tablas, May 31, 1981, *M. Lane 2912* (TEX); Mpio. Río Verde, 2 km as S de Miguel Hidalgo, alt. 950 m.s.n.m., June 23, 1979, *H. Puigh 6772* (MEXU); Sierra Madre Oriental, Las Tablas, alt. 1000-1100 m., Aug. 9-10, 1934, *F. W. Pennell 18046* (MEXU, NY); Minas de San Rafael, Angostura, June, 1911, *C. A. Purpus 5380* (F, MEXU, NY); Media luna, near Rio Verde, June 2-8, 1904, *E. Palmer 66* (F, NY).

35. *Lithospermum rosei* (I. M. Johnst.) J. I. Cohen *Brittonia* 61: 109. 2009. Basionym: *Nomosa rosei* I. M. Johnst. *J. Arnold. Arbor.* 35: 25. 1954. TYPE: Mexico. Durango: most southern tip of Durango, alt. 1700--5100 ft., Aug. 14 1897 - Aug. 16 1897, *J. N. Rose 2360* (holotype, GH!).

Erect perennial herb up to 49 cm tall with 1--few stems, each 2--3 mm wide, arising from a woody caudex, naphthoquinones not observed, stems sericeous to dense sericeous. Leaves cauline and sometimes pseudobasal, sessile; blades lanceolate to elliptical to linear, rarely ovate or obovate, 1.5--4.5(--7.3) x 0.2--1.1 cm, apex acute, base rounded to truncate, margins entire, planar, adaxially and abaxially sericeous to dense sericeous, trinerviate, usually with 1 pair of secondary veins evidents or midvein evident, 1--2 pairs of secondary veins evident, sunken, sometimes not. Inflorescence terminal dichasium or monochasium, up to 13 cm long, bracts sessile; blades lanceolate to linear, 0.5--3 x 0.1--0.6 cm, apex acute, base rounded to truncate, margins entire, planar, adaxially and abaxially soft sericeous to dense sericeous, trinerviate or midvein evident, sometimes 1--2 pairs of secondary veins evident, sunken or not. Flowers homostylous, chasmogamous; pedicels 0--10 mm long; sepals linear, 5--15 x 1--2 mm, apex acute to acuminate, abaxially and adaxially sericeous to dense sericeous; corolla urceolate to campanulate, white, abaxially sericeous to dense sericeous, tube 12--16 x 0.45--6.5(--8) mm, adaxial trichomes absent, lobes 1.5--3 x (1.5--)2--3 mm, erect, orbicular to elliptical, apex acute, glands on filaments and the tube around the base of the filaments, faucal appendages absent, annulus of 5 knobs with trichomes or flanges with trichomes; stamens inserted, filaments 7.5--9 x 0.75

mm, attached 4 mm above the base of the corolla, anthers 3.5--5 x 0.75--1 mm, ovoid, abaxially strigulose; style 20--27 x 0.1--0.5 mm, precociously exserted, at anthesis exserted up to 12 mm from the apex of the corolla tube; stigma terminal. Nutlets not observed.

Distribution: Higher elevations in Durango and Jalisco.

Phenology: Flowers June to September, fruiting unknown.

Discussion: Without the collections of Dra. Socorro González, we would know much less than we currently do on the subject of *L. rosei*. Dra. González has made at least half of the collections of this species, which, until recently, was only known from Durango. Given a recently discovered specimen at the Instituto de Ecología (IEB), the geographic range of this species now is known to include Jalisco.

*L. rosei* is unique among members of *Lithospermum*, and this species only barely resembles others in the genus. *Lithospermum rosei* originally was described in the monospecific genus *Nomosa*, an anagram of the Eurasian genus *Onosma* L. Johnston (1954a) named the genus as such because the flowers of the two genera are very similar. The corollas of *L. rosei* are urceolate or campanulate, and no other species of *Lithospermum* produces urceolate corollas. Additionally, the corolla and filaments bear multicellular filamentous glands, another unique feature among the members of the genus. Although our knowledge of the structural diversity and distribution of *L. rosei* has grown since Johnston described it over 50 years ago, the fruits still have not been observed.

Specimens Examined: MEXICO. **Durango**: Mpio. Súchil, Reserva de la Biosfera La Michilía, Cerro Blanco, Las Estañeras, 23° 27’ N 104° 18’ W, 2,550 m., Aug. 13, 1990, *S. González 4800* (CIIDIR); Mpio. Súchil, Reserva de la Biosfera La Michilía, Cerro El Purgatorio, Sierra de Urica, 23° 20’ N 104° 7’ W, 2,620 m., July 7, 1990, *S. González 4679* (CIIDIR); Mpio. Súchil, on side of road from Súchil to Charcos, a couple km E of Charcos, 23° 2’ 9” N 104° 17’ 48” W, 2790 m., June 24, 2007, *J. Cohen 207* (MEXU, CIIDIR); Mpio. Durango, Salto del Agua, 5 km al N (en línea recta), 23° 34’ 49” N 104° 57’ 32” W, 2500 m., Sep. 8, 1997, *S. González 5948* (CIIDIR); Mpio. El Mizquital, Reserva de la Biosfera La Michilía, mesa Altos de San Pedro, camino Súchil - Los Charcos, 23° 20’ 38” N 104° 17’ 19” W, 2600 m., June 15, 1996, *S. González 5686* (CIIDIR); Approx. 77 rd. km S of Durango by highway to La Flor, 4 km N of La Flor by rd. to city of Durango at mountain meadow area called Bajio De Los Ejes (approx. 23° 32’ N 104° 42’), approx. 9000 ft. elev., Aug. 18, 1982, *R. D. Worthington 8828* (TEX). **Jalisco**: Mpio. de Mezquitic, Los Amoles, km. 20 de la brecha Bolaños-Tenzompa, alt. 2400 m., Aug. 8, 1989, *R. Ramírez Delgadillo & González Tamayo 1275* (IEB).

36. *Lithospermum rzedowskii* J. I. Cohen *Brittonia* 61: 109. 2009. Basionym: *Macromeria pringlei* Greenm., *Proc. Amer. Acad. Arts* 34: 570. 1899, non *Lithospermum pringlei* I. M. Johnst., *Contr. Gray Herb.* 70: 22. 1924. *Onosmodium pringlei* (Greenm.) J. F. Macbr. *Contr. Gray Herb. n.s.* 49: 20. 1917. TYPE: Mexico. Hidalgo, Sierra de Pachuca, 10,000 ft., 4 Aug 1898, *C. G. Pringle 6949* (holotype, GH!; isotypes, F!, MEXU!, MO!, NY!, PH, UC, US!).

Erect perennial herb up to 1 m tall with 1--few stems, each 3--5 mm wide, arising from a woody caudex, naphthoquinones not observed, stems strigose to sericeous.

Leaves cauline, sessile or petiolate, petioles 2 x 1--3 mm; blades lanceolate, sometimes narrow elliptical, 2--10.4 x 0.4--2.5 cm, apex acute to acuminate, base rounded to acute, margins entire, planar, adaxially and abaxially strigose to sericeous to scabrous, sometimes more dense abaxially, midvein evident secondary veins evident, sunken, rarely not. Inflorescence terminal dichasium and axillary monochasia, up to 27.5 cm long, bracts sessile or petiolate, petioles 1--2 x 1 mm; blades lanceolate, rarely narrow ellptical, 1.8--8.5 x 0.3--2.3 cm, apex acuminate, rarely acute, base rounded to acute, margins entire, planar, adaxially strigose to sericeous to scabrous and abaxially strigose to dense strigose to sericeous to dense sericeous to scabrous, midvein evident, secondary veins evident, sunken, sometimes not. Flowers homostylous, chasmogamous; pedicels 0--14 mm long; sepals linear, 4--15 x 0.75--1(--2) mm, apex acuminate, abaxially strigose to dense strigose to sericeous to dense sericeous and adaxially strigose to strigulose to sericeous to sericulous; corolla long-funnelform with a bulge, yellow, abaxially villous, tube 28--39 x 5--8 mm, adaxial trichomes absent, lobes 4--8(--10) x 3--5 mm, erect, deltoid, apex obtuse, glands present at the apex of the tube and the base of the lobes, faucal appendages absent, annulus absent; stamens exserted, filaments 6--9 x 0.1--025 mm, attached 32--46 mm above the base of the corolla, exserted 3--6 mm from the apex of the corolla tube, anthers 2--2.25 x 0.5--0.75 mm, ellipsoid; style (35--)41--48 x 0.1 mm, exserted up to 6.5--11 mm from the apex of the corolla tube; stigma subterminal, rarely terminal. Nutlets 4--5 x 2.5--3.5 mm, ovoid, white to slightly grey to brown, smooth, scar not prominent, base rounded with a 0.25 mm pedestal, apex acute.

Distribution: Hidalgo and Veracruz.

Phenology: Flowers late April to August, fruits beginning in late June.

Discussion: As it has no unique features or combinations of features, *L. rzedowskii* is the least distinctive of all the species formerly included in *Macromeria*. In comparison to many other species of *Lithospermum* that produce long-funnelform corollas, the corollas of *L. rzedowskii* are quite short, not reaching more than 40 mm in length.

Specimens Examined: MEXICO. **Hidalgo**: Mpio. Mineral del Chico, 19 km. al N. de la carretera Pachuca-Zacualtipán, por la carretera que pasa por el parque y va a Mineral del Chico, alt. 2700 m., Apr. 20, 1979, *José García P. 975* (CAS, F, LL, MEXU, MO, NY); Cerro de Las Ventanas, 6 km al N de Pachuca, alt. 2900 m., May 11, 1969, *Rzedowski 26767* (LL); Mpio. El Chico, 3 km al S de El Chico, alt. 2850 m., June 11, 1988, *R. Hernández R. 123* (CAS, MEXU, MO, TEX); Mpio. El Chico, Los Cedros, 5.5 km al NNE de Pachuca, alt. 2800 m., May 11, 1986, *M. Medina Cota 3008* (MEXU, NY, TEX); Sierra de Pachuca, 10000 ft., Aug. 23, 1902, *C. G. Pringle 11044* (BH, F, LL, MO, NY, US); Mpio. Pachuca, near Pueblo Nuevo, about 2 miles from Real del Monte on road to El Chico, alt. 8500 ft., June 20, 1947, *H. E. Moore Jr. 3105* (BH, WISC); Cerro de Las Ventanas, Parque Nacional El Chico, alt. 2950 m., July 19, 1970, *González Reyes 21* (CAS); Parque Nacional del Chico a 2 km al sur del Mpio., alt. 3000 m., May 11, 1969, *Luz Ma. Villarreal de Puga 3000* (MEXU); El Chico, Sep. 1935, *E. Lyonnet 1095* (MEXU); Mpio. El Chico, Cerro de Las Ventanas, alt. 2900 – 3000 m., May 16, 1981, *A. Gentry, J. Rzedowski, & Ramamoorthy 32169* (MEXU, MO); El Chico, July – Sep. 1929, *E. Lyonnet 104* (MEXU), El Chico, July 19, 1938, *E. Lyonnet 2214* (US). **Querétaro**: Mpio. Pinal de Amoles, 2 km al NW del Puerto el Tejamanil, alt. 2350 m., July 10, 1990, *E. Carranza 2486* (MEXU).

**Veracruz**: Orizaba, 1855, *Legit Miller 1358* (NY). **No location**: 1853, *Legit. F. Müller S.N.* (NY)

37. *Lithospermum strictum* Lehm. *Pl. Asperif. Nucif.* 2: 303. 1818. *Anchusa tuberosa* Kunth *Nov. Gen. Sp. [H.B.K.]* 3: 92. 1818. non *Lithospermum tuberosum* Rugel ex DC. *Prodr*. 10: 76. 1846. *Heliotropium lithospermoides* Willd. ex Roem. & Schult. *Syst Veg., Ed. 15* 4: 737. 1819. TYPE: Mexico. No Locality: *A. J. A. Bonpland and F. W. H. A. von Humboldt s.n.* (holotype, MEL; isotype, B-Willd [digital image]!).

*Heliotropium mexicanum* Sessé & Moç. *Pl. Nov. Hisp.* 20. 1888. TYPE: Mexico. No Locality: 1787–1804, *Sessé & Moçiño s.n.* (holotype, MA [negative from F]!).

*Lithospermum rosmarinifolium* Sessé & Moç. *Pl. Nov. Hisp.* 20. 1888. non *Lithospermum rosmarinifolium* Ten. *Fl. Napol. dup.* 1: lxvi. 1811-1815. TYPE: Mexico. No Locality: 1787–1804, *Sessé & Moçiño 1734* (holotype:, MA).

*Lithospermum angustifolium* Sessé & Moç. *Naturaleza (Mexico City) ser. 2,* 29. 1893, non *Lithospermum angustifolium* Forssk. *Fl. Aegypt.-Arab.* 39. 1775. TYPE: Mexico. No Locality: 1787–1804, *Sessé & Moçiño 5234* (holotype, MA; isotype, GH [digital image]!).

Erect perennial herb up to 57 cm tall with 1--few stems, each 1--2 mm wide, arising from a woody caudex, naphthoquinones not observed, stems strigose. Leaves cauline, sessile; blades linear to lanceolate, sometimes elliptical to oblanceolate, 0.8--4(--4.7) x 0.1--0.5(--0.9) cm, apex acute, base truncate to rounded, rarely acute, margins entire, usually revolute, adaxially and abaxially strigose to strigulose, rarely sparsely strigose to sparsely strigulose, midvein evident, other orders of veins not evident. Inflorescence a terminal monochasium, rarely a dichasium, and axillary monochasia, up to 21.5 cm long, bracts sessile, linear to lanceolate, 0.4--2.3(--2.9) x 0.1--0.3 cm, apex acute, base truncate, margins entire, usually revolute, adaxially and abaxially strigose to strigulose, midvein evident, other orders of veins not evident. Flowers homostylous, chasmogamous; pedicels 0--4(--10) mm long; sepals linear to lanceolate, 2--5(--up to 8 in fruit) x 0.75--1.5 mm, apex acute, abaxially strigose to strigulose, usually more dense strigose towards the base and adaxially strigulose; corolla salverform, yellow to yellow and green-yellow, abaxially villous to woolly, tube 7--12(--15) x 1--2 mm, adaxial trichomes absent, lobes 1.5--3 x 1--2 mm, erect to flared, elliptical, apex rounded to obtuse, glands present, associated with faucal appendages, faucal appendages present, annulus of 4--5 flanges with trichomes; stamens inserted, filaments 0.5 x 0.1 mm, attached 7--9(--10.5) mm above the base of the corolla, anthers 1--1.25 x 0.25--0.5 mm, ellipsoid; style 5--12 x 0.1 mm; stigma terminal. Nutlets 2--3 x (1--)1.5--2 mm, ovoid, white to mottled white and grey, smooth, scar prominent, base rounded, apex acute, crest-like from apical extension of the scar.

Distribution: Generally found growing in the states along the Trans-Mexican Volcanic Belt.

Phenology: Flowers and fruits early July until September.

Discussion: *L. strictum* is a well-defined species of the Trans-Mexican Volcanic Belt, and individuals of this species grow in forests or in more-open areas. *Lithospermum strictum* has a distinctive inflorescence that resembles a Mohawk hairstyle. This species is related to *L. distichum* and *L. oblongifolium*. These three species have overlapping ranges, but despite this overlap, *L. strictum* does not usually grow at the same location as either of these species.

*L. strictum* and *L. muelleri* share similar floral and vegetative forms. There are multiple features that differ between these species (see *L. muelleri* for a discussion of these).

Specimens Examined: MEXICO. **Distrito Federal**: Lomas de Mixcoac, Aug. 13, 1938, *E. Lyonnet 2287* (US); Parres, Aug. 30, 1910, *C. R. Orcutt 6567* (TEX, US); Terrenos de Topilejo, delegación Tlalpan, alt. 2650 m., July 27, 1976, *A. Ventura A. 1681* (MEXU); San Bartolo Xicomulco, delegación Milpa Alta, alt. 2500 m., July 31, 1976, *A. Ventura A. 1927* (MEXU); One mile east of Sierra Pelado, July 12, 1947, *Barkley, Rowell, & Webster 2224* (TEX); Lomas de Chapultepec, July 30, 1947, *Barkley & Rowell 7481* (TEX); Santa Fé, valley of Mexico, Aug. 22, 1903, *J. N. Rose 6502* (US); Valle de México, San Angel, 1865 – 1866, *M. Bourgeau 129* (US); Santa Fé, valley of Mexico, 1905, *J. N. Rose 8649* (US); Lomas de Mixcoac, Sept. 1936, *E. Lyonet 1388* (US). **Hidalgo**: Collected between Somoriel and Las Lajas, Aug. 5, 1905, *J. N. Rose, Jos. H. Painter, & J. S. Rose 9216* (US); Wet meadows near Buena Vista Station, 8300 ft., Aug. 6, 1904, *C. G. Pringle 13124* (F, US); Sierra de Pachuca, 9000 ft., July 1898, *C. G. Pringle 6928* (F, US); El Chico, Sep. 1927, *E. Lyonnet 188* (US). **Mexico**: 55 km se of Mexico City, July 2, 1942, *J. N. Weaver 716* (US); Flor de Maria, Sep. 4, 1890, *C. G. Pringle 3245* (F, US); Flor Maria, July 12 + 13, 1901, *J. N. Rose & R. Hay 5452* (US); Near cima, Sep. 19, 1903, *J. N. Rose & Jos H. Painter 7186* (US); Mpio. Tezcoco, lado sur de la Cañada de Aguas, 13.5 km. al SE de Tezcoco, (11 km. al ESE de Coatlinchán), alt. 2740 m., July 29, 1977, *J. García P. 348* (MEXU); Mpio. Ecatepec, Sierra de Guadalupe, entre Cuautepec y San Andrés, July 14, 1973, *S. Moreno G. 291* (MEXU); Mpio. Huixquilucan, Fraccionamiento “La

Herradura”, alt. 2350 m., July 7, 1968, *C. Guzmán G. 44* (MEXU); Mpio. Ixtapaluca, Llano Grande, alt. 3100 m., July 18, 1983, *E. Ventura V. 1105* (MEXU); Mpio. Ixtapaluca, Estación Experimental de Investigación y Enseñanza de Zoquiapán, 8 km. al S. de Río Frio. ± a 200 m. al N. de Llano Aculco , sobre Camino 6, alt. 3220 m., June 29, 1978, *R. V. Aviña 215* (NY); mt. meadow so. of Mexico City, alt. 9500 ft., July 12, 1941, *Mr. and Mrs. Wm. C. Leavenworth 956* (F); 18.2 km (11.3 mi) W of Toluca, measure from intersection of hwy 15 with the road to Tlalcilalcalpa and Valle de Bravo, bluffs on S side of road, alt. 2590 m., Aug. 22, 1979, *M. A. Lane 2656* (TEX); *Dos Rios, Sep. 6 1913, F. Salazar s.n.* (US); Cerca del rancho La Libertad, 25 km al SO de Toluca, carretera a Zitácuaro, alt. 2500 m., July 3, 1984, *J. C. Soto Nuñez 6407* (MEXU); Along Hwy 15, km124, 7 km east of Bosencheve, elev. 7800’, Aug. 1, 1965, *S. M. Mertz 165* (MEXU); At pineland, Valle de México, alt. 2800 m., July 9, 1950, *E. Matuda 19028* (F); Mpio. Temascaltepec, Las Cruces, 3350 m., Aug. 2, 1933, *G. B. Hinton 4394* (F, US). **Michoacán** : Zitácuaro, Aug. 7, 1938, *Hinton et al. 13023* (F, LL, US); Mpio. Coeneo, 3 km. al E de Comanja, sobre la carretera a Quiroga, alt. 2100 m., June 26, 1985, *Rzedowski 38630* (MEXU); Near bend in road at Sevina on the way to Comachuen, N 19˚ 37’ 11.2” W 101˚ 54’ 7.9”, alt. 2410 m., Aug. 11 – 17, 2008, *Cohen 225* (BH); Along road from Caraupan to Cheran, around km 20, Aug. 11 – 17, 2008, *Cohen 230* (BH); Next to dirt road that parallels the road from Nahuatzen to Pátzcuaro, Aug. 11 – 17, 2008, *Cohen 222* (BH). **Morelos**: Toro, alt, 9800 ft., Aug. 5, 1924, *G. L. Fisher 261* (US); Along Hwy. Mex. 15, ca. 20 mi. W. of Morelia, alt. 7600 ft., July 27, 1975, *Watkins, Dunn, & LeDoux 621* (LL). **Puebla**: Mayorazgo, vicinity of Puebla, alt. 2120 m., July 18, 1907, *Bro. G. Arséne s.n.* (US); Rancho Posadas, July 5, 1909, *F. Nicolas s.n.* (US); Mayorazgo, vicinity of Puebla, July 5, 1909, *Bro. G. Arséne 256* (US); Barranca de l’Alseseca, hacienda Batan, près de Totimehuacan, alt. 2120 m., vicinity of Puebla, June 13, 1907, *Bro. G. Arséne 1452*

(US); au Mayoraz sur l’Ayotac, alt. 2120 m., 1907, *G. Arsén 1124* (US); Baranca de Manzanilla July 5, 1909, *F. Nicolas 255* (US); Entre las haciendas Santa Bárbara et Cristo, sur l’Alseseca, alt. 2150 m., *Bro. G. Arséne s.n.* (US); Esperanza, June 1907, *C. A. Purpus 2485* (F, US); Near Hacienda Santa Barbara, barranca de la Alseseca, alt. 2150 m., Vicinity of Puebla, May 2, 1907, *Bro. G. Arséne 10187* (US); Mayorazgo, sur l’Atoyac, alt. 2120 m., Vicinity of Puebla, May 20, 1907, *Bro. G. Arséne 7113* (US); Hacienda Santa Barbara, vicinity of Puebla, June 2, 1907, *Bro. G. Arséne 10193* (US); Mpio. Tecamachalco, El Ocotal, alt. 2340 m., July 10, 1972, *F. Ventura A. 5703* (NY); ca 16 road km NW of Perote (Veracruz), along dirt road, 19˚ 42’ X 97˚ 28’, alt. 2950 m., Sep. 24, 1983, *B. L. Turner 15286* (TEX); Mount Orizaba, Julyl 25 & 26, 1901, *J. N. Rose 5814* (US); Mount Orizaba, Esperanza, 8000 ft., Aug. 14, 1891, *H. E. Seaton 337* (F, US). **Oaxaca**: Llano de las Flores on the Oaxaca-Valle Nacional highway 20 kilometers east of Ixtlan; alt. ca. 2870 m., July 22, 1960, *J. H. Beaman 3720* (US). **San Luis Potosí**: 1876, *J. G. Schaffner 399* (US); Tamazunchale, 1937, *L. A. Kenoyer 864* (F). **Tlaxcala**: Cantadero, Aug. 3, 1901, *J. N. Rose & R. Hay 5964* (US). **No Locality**: En route from San Luis Potosí to San Antonio, Texas, Aug. 1878, *C. C. Parry 620* (US); Mexico, *A. Duges s.n.* (US).

38. *Lithospermum tenerum* J. I. Cohen **sp. nov.** Type: Mexico. Nuevo León: 14 km by winding road west of Tokio on the San Roberto-Galeana highway, 24° 41' N and 100° 07' W, alt. 2000 m, rolling hills, gypseous calcareous gravelly soil, pinyon and ponderosa pines, May 17, 1973, *M. C. Johnston, T. L. Wendt, and F. Chiang 11054C* (holotype, LL!).

Erect perennial herb up to 20 cm tall with 1--few stems, each 2 mm wide, arising from a woody caudex, naphthoquinones not observed, stems strigose to dense strigose.

Leaves cauline, sessile; blades linear to lanceolate, 1.1--1.8 x 0.1--0.2 cm, apex acute, base truncate, margins entire, revolute, adaxially and abaxially dense strigose, midvein evident, other orders of veins not evident. Inflorescence terminal dichasium, 9.5 cm in length, bracts sessile, lanceolate, 0.7--1 x 0.2 cm, apex acute, base rounded to truncate, margins entire, revolute, adaxially strigose and strigulose to dense strigose and abaxially strigose to dense strigose, midvein evident, other orders of veins not evident. Flowers homostylous, chasmogamous; pedicels 0--1 mm long; sepals lanceolate, 5--6 x 1 mm, apex acute, abaxially strigose to dense strigose and adaxially strigose to strigulose; corolla funnelform, yellow, abaxially villous, tube 9 x 2 mm, adaxial trichomes absent, lobes 2--3 x 2 mm, erect, elliptical, apex rounded, glands present around faucal appendages and veins leading to filaments, faucal appendages present, annulus of a membranous ring; stamens inserted, filaments 0.75 x 0.1 mm, attached 7 mm above the base of the corolla, anthers 1 x 0.5 mm, ellipsoid; style 11--13 x 0.1 mm, exserted up to 3 mm from the apex of the corolla tube; stigma terminal. Nutlets 3 x 2 mm, ovoid, white, abundant pitting around non-prominent scar, base rounded, apex acute.

Distribution: Restricted to Nuevo León.

Phenology: Flowers and fruits in mid-May.

Discussion: *L. tenerum* is known from only one collection made more than 35 years ago. The specimen has been assigned to two different species: *L. sordidum* and *L. strictum*; however, it does not appear to belong to any of the currently recognized species of the genus. The leaves are similar to those of *L. incisum*, *L. mirabile*, *L. calycosum*, and *L. strictum*, but the leaves of *L. tenerum* are narrower than those of these species. The flowers bear some resemblance to those of *L. multiflorum* and *L. tubuliflorum*, but the stigma is exserted from the corolla tube, a feature neither of these species posseses. Therefore, this specimen is recognized as a new, distinct species. The delicate nature of the type specimen provides the species with its epithet.

Specimens Examined: MEXICO. **Nuevo León**: 14 km by winding road west of Tokio on the San Roberto-Galeana highway, 24° 41' N and 100° 07' W, alt. 2000 m, May 17, 1973, *M. C. Johnston, T. L. Wendt, and F. Chiang 11054C* (LL).

39. *Lithospermum trinervium* (Lehm.) J. I. Cohen *Brittonia* 61: 109. 2009. Basionym: *Onosma trinervium* Lehm., *Pl. Asperif. Nucif*. 2: 37. 1818. *Lasiarrhenum trinvervium* (Lehm.) B. L. Turner *Phytologia* 77: 39. 1994. TYPE: Mexico. “Habitat in America Meridionali,” *Humboldt & Bonpland s.n.* (holotype, not located).

*Onosma strigosum* Kunth, *Nov. Gen. Sp. [H.B.K.]* 3:93. 1819. *Lithospermum longifolium* Willd. ex Roem. & Schult, *Syst. Veg., ed. 15 bis [Roemer & Schultes]* 4:742. 1819. *Onosmodium strigosum* (Kunth) G. Don, *Gen. Hist.* 4:317. 1837. *Lasiarrrhenum stngosum* (Kunth) I. M. Johnst., *Contr. Gray Herb*. 70:15. 1924. TYPE: Mexico. Helier de l’Amérique équitoriale, *Bonpland 4341* (holotype, P [digital image]!).

*Onosmodium eriocaulon* DC. *Prodr*. 10:70. 1846. TYPE: Mexico. "Cordillera de Guchilaque", *J. L. Berlandier 1020* (holotype, P; holotype fragments, GH, G).

*Lasiarrhenum lundelli* I. M. Johnst. *Wrightia* 2:15. 1959. TYPE: Mexico. Oaxaca: Mpio. Tlacolula, mountain side in pine land, above Tejocote, July 25, 1943, *C. L. Lundell 12296* (holotype, LL [digital image]!; isotype, MICH [digital image]!).

Erect perennial herb up to 1 m tall with 1--few stems, each 2--5 mm wide, arising from a woody caudex, naphthoquinones not observed, stems hispid to hispid and long hispid to hispid, long hispid and strigose to dense hispid to dense hispid and dense long hispid to dense hispid, dense long hispid, and dense strigose. Leaves cauline, sessile; blades lanceolate to elliptical, rarely oblanceolate or ovate, 2.3--10 x 0.2--1.8 cm, apex acute to acuminate, base acute to rounded to truncate, margins entire, planar, adaxially and abaxially strigose to strigose and strigulose to dense strigose to dense strigose and dense strigulose, trinerviate, usually with 1 pair of secondary veins evidents or midvein evident, 1--2 pairs of secondary veins evident, sunken, sometimes not. Inflorescence terminal dichasium or monochasium and sometimes axillary monochasia, up to 23.5 cm in length, bracts sessile; blades lanceolate, 0.5--4.5(--5.2) x 0.2--1.3(--1.6) cm, apex acute to acuminate, base acute to rounded to truncate, margins entire, planar, adaxially and abaxially strigose to strigose and strigulose to dense strigose to dense strigose and dense strigulose, trinerviate or midvein evident, sometimes 1 pair of secondary veins evident, sunken or not. Flowers homostylous, chasmogamous; pedicels 0--17 mm long; sepals lanceolate, rarely elliptical, 5--15(--up to 19 in fruit) x 1--2(--up to 3 in fruit) mm, apex acute, abaxially strigose to dense strigose, rarely also strigulose, and adaxially strigose to strigulose; corolla campanulate, white, abaxially sericeous to dense sericeous, tube 11--16 x 4--6(--7.5) mm, adaxial trichomes absent, lobes 1--2 x (1--)2--3 mm, erect, elliptical to deltoid, apex acute, glands present in the upper half of the corolla tube, faucal appendages absent, annulus of a membranous ring; stamens inserted, filaments 3--4 x 1 mm, attached 5--9 mm above the base of the corolla, anthers 3.5--4.5 x 0.75--1 mm, ellipsoid, abaxially strigose; style 15--21(--23.5) x 0.1--0.5 mm, exserted up to 7 mm from the apex of the corolla tube; stigma terminal. Nutlets 3.5--4 x 2--3 mm, ovoid, white, sometimes tan towards the apex, smooth, scar not prominent, base rounded, apex acute.

Distribution: Western, central, and southern Mexico.

Phenology: Flowers from late July onward, and fruits starting mid-August.

Discussion: *L. trinervium* is unusual among members of the genus. Johnston found this species to be sufficiently distinct from other genera of Boraginaceae, so he erected the genus *Lasiarrhenum* and placed this species as its sole member (Johnston, 1924). *Lithospermum trinervium* has a particular type of leaf venation and pure white, campanulate corollas.

This species has an affinity with *L. rosei* (Cohen and Davis, 2009; Chapter 4), and these two species share multiple features, including pure white corollas, wide filaments, and anthers with trichomes on the abaxial surface. The relationship between *L. trinervium* and *L. confundum*, a species restricted to Durango, is unknown. Given their similar flowers, these species are probably very closely-related.

The geographic range of *L. trinervium* appears to be expanding. Turner (1994b) reported that this species is distributed throughout the trans-volcanic mountain range. Currently, specimens are known from as far north as Chihuahua, and it would not be surprising to find specimens from Durango. However, despite its wide range in the western part of the country, this species is not known to occur in the Sierra Madre Oriental.

Specimens Examined: MEXICO. **Chihuahua**: Mpio. Ixtapaluca, SE de Chapingo, Canada Xaltomatla, alt. 2600 m., Aug. 13, 1984, *D. Randolph 300* (MEXU). **D.F.**: Mpio. Juchitepec, Cerro "Dos Cerros", 10 km al. SE de Milpa Alta, alt. 3100 m., Sep. 4, 1982, *F. G. Lorea 2208* (MEXU); Cañada de Contreras, Aug. 1911, *C. Reicke s.n.* (MEXU). **Hidalgo**: Mpio. Epazoyucan, Cerca de El Guajolote, alt. 2900 m., Aug. 3, 1975, *Rzedowski 33425* (CAS). **Jalisco**: Volcán Tequila, due south of Tequila, 7.4 miles from summit on road from Tequila, elev. 1900 m., Aug. 11, 1968, *W. R. Anderson and C. Anderson 5137* (NY); Concepción de Buenos Aires, Sierra del Tigre, alt. 2100 m., Aug. 13, 1972, *L. M. Villareal 3932* (MEXU). **Mexico**: Temascaltepec, Cumbre-Cruz., Aug. 9, 1935, *Hinton et al. 8274* (LL, MEXU); on mountain side in ravine near Valle de Bravo, Nov. 3, 1943, *C. L. Lundell 12647* (LL); Mpio. de Naucalpan, 2 km al SE de San Francisco Chimalpa, alt. 2600 m., Sep. 3, 1967, Rzedowski 24321 (LL); 8 mi. N of Temascaltepec, 19.08 N, 100.06 W, alt. 7200 ft., Sep. 11, 1976, *G. L. Webster 21170* (MEXU); Mpio. Amecameca, 1 km al E del poblado de San Antonio, Sep. 27, 1981, alt. 2500 m., *R. M. Tapia G. 18* (MEXU); Dos Rios, *J. Salazar s.n.* (MEXU). **Michoacán** : 18 miles E of Uruapan, alt. 6500 ft., Oct. 14, 1970, *D. Bates, O. Blanchard, and P. Fryxell 1633* (BH, NY); 2 kms. west of Zacapu, km. 403 – 406 on Guadalajara highway, alt. ca. 7000', July 21, 1948, *H. E. Moore Jr. and C. E. Wood. Jr. 4049* (BH, MEXU); Along road from Caraupan to Cheran, around km 20, Aug. 10 – 17, 2008, *J. I. Cohen 228* (BH); Across the street and up the hill from Restaurant Bella Vista, on highway from Morelia to Caraupan, Aug. 10 – 17, 2008, *J. I. Cohen 221* (BH); Across the street and up the hill from Restaurant Bella Vista, on highway from Morelia to Caraupan, N 19° 37' 27.4" W 101° 27' 46.6", alt. 2326 m., Aug. 10 – 17, 2008, *J. I. Cohen 220* (BH); Mpio. de Santa Clara del Cobre, Zirahuén, alt. 2150 m., Sep. 3, 1985, *J. M. Escobedo 51* (TEX); Route 15, 0.5 km north of K 416, ca. 19 km northwest of Zacapu, elev. ca. 2560 m., July 14, 1966, *R. W. Cruden 1064* (LL, MEXU); 4.5 miles N. of Cheran, 2400 m., July 30, 1990, *A. Jack and M. Sydor 083* (TEX); A 17 km al NE de Uruapan, carr. a Pátzcuaro, alt. 1700 m., Sep. 9, 1978, *J. C. Soto Nuñez 1016* (TEX); along Hwy. Mex. 15, ca. 20 mi. W. of Morelia, alt. 7600', July 27, 1975, *Watkins, Dunn, and LeDoux 622* (LL); About 12 miles east of Zacapu on road from Guadalajara to Morelia, July 22, 1950, *J. R. Reeder, C. G. Reeder, and L. N. Goodding 1451* (TEX); 2 miles northwest of Quiroga, Aug. 13, 1947, *F. A. Barkley, B. L. Westlund, and G. L. Webster 2732* (TEX); Km 49, off highway 37, south of Paracho, July 2, 1969, *F. Ramos M. M1444* (MEXU); Cascada de la Tzararacua, ca. 5 miles south of Uruapan, ca. 5000 ft. alt., Aug. 23, 1957, *R. M. Straw and D. P. Gregory 1220* (MEXU); Hills about Pátzcuaro, July 22, 1892, *C. G. Pringle 4131* (MEXU); Mpio. Erongarícuaro, Cerro El Huacapian, alt. 2300 m., Aug. 15, 1978, *J. Caballero y C. Mapes 419* (MEXU); Parte alta del Cerro Huashán, aprox. 2 km al S de Nahuatzen, alt. 2550 m., July 28, 1990, *E. García and E. Pérez 2775* (MEXU); 2 km N & NE of Puentacillas (ca. 7 km N. of Ario de Rosales), alt. 2200 – 2400 m., (ca. 19° 18' N, 101° 40' W), July 24, 1960, *H. H. Iltis, R. Koeppen, and F. Iltis 373* (MEXU); Mpio. Cherán, Cerro San Marcos, Aug. 16, 1990, alt. 3050 m., *E. García L. y E. Pérez C. 3055* (MEXU); A 8 km al S de Acuitzio del Canje, carr. a Villa Madero, alt. 2320 m., Oct. 1, 1982, *J. C. Soto Nuñez 4795* (MEXU); A 6 km al SW de Morelia carretera a Guadalajara, elev. 2340 m., Aug. 21, 1980, *J. C. Soto Nuñez 2383* (MEXU). **Morelos**: Cuernavaca, Sierra Morelos, alt. 2000 m., Jan. 24, 1970, *Hinton et al. 17513* (BH, TEX); Mountainside of Cuernavaca, alt. 8000 ft., Oct. 13, 1900, *C. G. Pringle 9124* (BH); 26 miles south of Mexico City along highway 95, alt. 8000 ft., Aug. 1, 1956, *O. S. Fearing and J. S. Thompson 128* (TEX); Parque Alarcón, Aug. 4, 1940, *F. Miranda 544* (MEXU); 20 km. N.E. Cuautla, alt. 6500 ft., July 26, 1951, *C. Humphreys 14* (MEXU). **Nayarit**: Mpio. Xalisco, 8 km al W de Xalisco camino de terraceria de

Xalisco al Malinal, 21.25.56 N, 104.57.26 W, alt. 1500 m., Oct. 16, 1994, *G. Flores-Franco, A. Martinez, and A. Dominguez 3965* (MEXU). **Oaxaca**: Nochixtlán, medio kilometro al norte de La Joya (comarca a 4 – 6 kilometros al sur de Nochixtlán) sobre la carretera Oaxaca-Huajuápan de León, 2175 m., Oct. 2, 1993, *J. L. Panera 3616* (NY); 7.3 km al N de Ojo de Agua, carr. Puerto Escondido-Oaxaca, alt. 1510 m., Aug. 1, 1984, *R. Torres C. 5837* (MEXU, TEX); 15 km northwest of Zaachila on road to San Miguel Peras, elev. 2290 m., Nov. 11, 1983, *D. E. Breedlove 60082* (MEXU); Dto. Juxtlahuaca, Mpio. San Juan Mixtepec, Caba yáa (Peña de Aguilas), a 7 km al S de San Juan Mixtepec, elev. 2000 m., Sep. 3, 1989, J. Reyes S. 1925 (MEXU). **Puebla**: San Miguel Tecuanipa, Sep. 14, 1966, *L. W. Boege 233* (MEXU). *Tlaxcala*: Nanacamilpa, alt. 2710 m., Nov. 1985, *E. Dominguez Sendero s.n.* (MEXU). **Veracruz**: Carretera Mexico-Orizaba, cerca del limite con Puebla, Veracruz, Mexico (18-51 N, 97-17 W), alt. 2850 m., July 26, 1971, *Nevling y Gomez-Pompa 1974* (MEXU).

40. *Lithospermum tubuliflorum* Greene *Pittonia* 1: 155. 1888. TYPE: Mexico. Durango: From the Sierra Madre, west of Durango, alt. 8100 ft., Sep. and Oct. 1881, *A. Forrer s.n.* (ND – 043164) (holotype, ND [digital image]!)

*Lithospermum lasiosiphon* I. M. Johnst. *Contr. Grey Herb.* 70: 22. 1924. TYPE: Mexico. Chihuahua: Sierra Madres near Colonia Garcia, alt. 7500 ft., Aug. 10, 1899, *C. H. T. Townsend and C. M. Barber 248* (type, GH!, isotypes, F!, K [digital image]!, MO!).

*Lithospermum worthingtonii* B. L. Turner *Phytologia* 52: 493. 1983. TYPE: Mexico. Durango: Sierra Madre Occidental, 5.1 dr. mi SW of El Salto at Arroyo de Agua (By hwy. 40), (approx. 23 45' N-105 24' W), alt. approx. 8000 ft., Aug. 19, 1982, *R. D. Worthington 8904* (holotype, TEX [digital image]!).

Erect perennial herb up to 50 cm tall with 1--few stems, each 2--3 mm wide, arising from a woody caudex, naphthoquinones rarely present, stems strigose to hispid to villous to woolly. Leaves cauline and basal, sessile; cauline blades 1.2--6.4 x 0.2--0.8 cm**,** lanceolate to linear, rarely oblanceolate**,** apex acute to rounded, base truncate to rounded, margins entire, planar, sometimes revolute, adaxially strigose to strigose and strigulose to villous to woolly and abaxially strigose to villous to woolly, usually denser on veins, midvein evident, other orders of veins not evident; basal blades 1.5--9.6 x 0.3--2 cm**,** apex rounded to acute, base truncate, rarely acute, margins entire, planar, sometimes revolute, adaxially and abaxially strigose to villous to woolly, usually denser on abaxial veins, midvein evident, other orders of veins evident, sunken or not. Inflorescence terminal dichasium or monochasium, sometimes axillary monochasia, up to 13 cm long, bracts sessile; blades lanceolate, 0.6--2(--2.9) x 0.2--0.7 cm, apex acute, base truncate to rounded, margins entire, planar, sometimes revolute, adaxially and abaxially strigose to villous to woolly; midvein evident, other orders of veins not evident. Flowers heterostylous, chasmogamous; pedicels 0--7 mm; Long style flowers with sepals lanceolate, 3--5 x 1--1.25 mm, apex acute, adaxially strigose to dense strigose and abaxially strigulose; corolla funnelform, yellow, abaxially villous to woolly, tube 8--11 x 2--3 mm, adaxial trichomes present, villous, lobes 1.25--2 x 1.25--2 mm, flared, elliptical to orbicular, apex rounded, glands present at apex of the tube, faucal appendages absent, annulus of 5 flanges with trichomes; stamens inserted, filaments 0.5--1 x 0.1 mm, cylindrical, attached 1--2 mm above the base of the corolla, anthers 1.25--1.5 x 0.3--0.5 mm; style 7.5--10 x 0.1 mm, inserted; stigma terminal to subterminal. Short style flowers with linear, sepals 3.5--

6.5 x 0.5--1.25 mm, apex acute, abaxially strigose to dense strigose and adaxially strigose to strigulose; corolla funnelform, yellow, abaxially villous to woolly, tube (5--)8--13 x 2--3 mm, adaxial trichomes present, dense villous, lobes 1--2.5 x 1.5--2.5 mm, flared, orbicular to elliptical, apex rounded, glands absent, faucal appendages absent, annulus of 5 knobs with trichomes; stamens inserted, filaments 0.25--0.5 x 0.1 mm, attached 7 mm above the base of the corolla, anthers 1 x 0.5 mm, ellipsoid; style 3 x 0.1 mm, inserted; stigma subterminal. Nutlets 3 x 2 mm, ovoid, white to white with a tint of grey, smooth, base rounded, apex acute.

Distribution: Higher elevation forests (>2000 m.) of Chihuahua, Durango, and Sonora.

Phenology: Flowers late June to September, fruits late July.

Discussion: *L. tubuliflorum* is most closely-related to another heterostylous species, *L. cobrense*. Despite this close relationship, the flowers of *L. tubuliflorum* bear more of a resemblance to the flowers of a different heterostylous species, *L. multiflorum*, than to those of *L. cobrense*. Although the flowers of *L. tubuliflorum* and *L. multiflorum* are similar, the leaves of the two species differ. *Lithospermum tubuliflorum* produces cauline leaves and basal rosette, but *L. multiflorum* only develops cauline leaves. In Chihuahua, both *L. tubuliflorum* and *L. multiflorum* are much less common than *L. cobrense*.

Of the five heterostylous species of *Lithospermum* that grow Mexico, *L. tubuliflorum* is the only one that produces pollen that is dimorphic in both size and shape. The long-styled flowers produce pollen that is prolate with a constricted equator, while the short-styled flowers produce ellipsoid pollen. Apart from *L. tubuliflorum*, the only other species in the genus to have this feature is

*L. caroliniense*, a species found in the eastern United States (Johnston, 1952; Chapter 4).

Specimens Examined: MEXICO. **Chihuahua**: Sierra Madres near Colonia Garcia, alt. 7500 ft., Aug. 10, 1899, *C. H. T. Townsend & C. M. Barber 248* (F, MO). **Durango**: San Luis, 51 road miles northwest of Coyotes (about 70 airlines miles west of C. Durango), elev. 2600 m., July 1, 1950, *J. H. Maysilles 7216 (*MICH); 11 km SW of La Ciudad near Buenos Aires, elev. 2900 m., Aug. 13, 1974, *D. E. Breedlove 36471 (*MICH); 17 km al S de El Salto, cerca de paraje La Remuda, July 25, 1981, *S. & M. González 1780 (*MEXU, TEX); Mpio. Pueblo Nuevo, Peñitas, approx. 13 km al SW de El Salto, 23° 39’ 54” N 105° 24’ 50” W, 2843 m., June 23, 2007, *M. González 4001*(CIIDIR). **Sonora**: Mesa El Campanero, Arroyo Largo, upper tributary of Barranca El Salto, 28° 21½’ N 109° 2’ W, 2080 m., Sep. 9, 1995, *M. Fishbein, 2589 (*TEX); Mesa El Campanero, Arroyo Largo, upper tributary of Barranca El Salto, 28° 21’ 18” N 109° 1’ 48” W, 2000 m., July 14, 1997, *T. R. Van Devender 97-689* (NY).

41. *Lithospermum turneri* J. I Cohen *Brittonia* 61: 109. 2009. Basionym: *Macromeria hintoniorum* B. L. Turner, *Phytologia* 77: 398. 1994, non *Lithospermum hintoniorum* B. L. Turner, *Sida* 20: 501. 2002. TYPE: Mexico. Guerrero: Teotepec, ca. 12 □ 27’ N, 100 □ 10’ W, pine forest, 2750 m, July 16, 1939, *G. B. Hinton et al. 14439* (holotype, NY!; isotypes, F, NY!, US!).

Erect perennial herb up to 1.8 m tall with 1--few stems, each 3 mm wide, arising from a woody caudex, naphthoquinones not observed, stems strigose. Leaves cauline, sessile or petiolate, petioles 1--3 x 1 mm; blades lanceolate to ovate, 4--8.5 x 1.5--2.1 cm, apex acuminate, base acute, margins entire, planar, adaxially strigose to strigulose to scabrous and abaxially strigose, usually more densely strigose on veins, midvein evident, secondary veins evident, sunken. Inflorescence terminal dichasium and sometimes axillary monochasia, up to 36 cm long, bracts sessile or petiolate, petioles 1--2 x 1 mm; blades lanceolate, 3.2--6.1 x 0.9 -1.6 cm, apex acute to acuminate, base rounded to acute, margins entire, planar, adaxially strigose to strigulose to scabrous and abaxially strigose, more densely strigose on veins, midvein evident, secondary veins evident, sunken. Flowers homostylous, chasmogamous; pedicels 0--8 mm long; sepals lanceolate, 10--16(--up to 18 in fruit) x 1--1.5 mm, apex acuminate, abaxially strigose to densely strigose and adaxially strigulose; corolla long-funnelform with a bulge, yellow, abaxially villous, tube 32--40 x 4--6 mm, adaxial trichomes absent, lobes 10--12 x 4--5 mm, erect to flared, deltoid to elliptical, apex rounded to acute, glands absent, faucal appendages absent, annulus not observed; stamens exserted, filaments 8--10 x 0.5--0.75 mm, attached 22--33 mm above the base of the corolla, anthers 3 x 0.75 mm, ellipsoid; style (43--)53--60 x 0.25 mm, exserted up to 13 mm from the apex of the corolla tube; stigma terminal. Nutlets 3.5 x 2.5 mm, beige.

Distribution: Restricted to Guerrero and Oaxaca.

Phenology: Flowers and fruits in mid-July.

Discussion: Only three specimens of *L. turneri*, including the type specimen, are known. This species has leaves that are similar to those of *L. pringlei*, flowers similar to those of *L. flavum,* and a habit similar to that of *L. chiapense*. Given the particular combination of floral and vegetative characters, *L. turneri* appears to be a distinct species; however, because of the small number of specimens, it is difficult to determine whether this species should be segregated from or included in any of three aforementioned species.

Turner (1994a) postulated that *L. turneri* shares an affinity with *L. chiapense*, and Boyd (2003) resolved these two species as sisters in her phylogeny of *Macromeria*. Neither of these species has been included in a phylogenetic analysis that utilizes molecular data and broad taxon sampling.

Specimens examined: MEXICO. **Guerrero**: Teotepec, ca. 12 ° 27’ N, 100 ° 10’ W, pine forest, 2750 m, July 16, 1939, *G. B. Hinton et al. 14439* (US); W. of Puerto El Gallo along road to Toro Muerto, elev. 2530 m., October 9, 1986, *D. E. Breedlove 65042* (TEX).

42. *Lithospermum unicum* (J. F. Macbr.) J. I. Cohen *Brittonia* 61: 109. 2009. Basionym: *Onosmodium unicum* J. F. Macbr. *Contr. Gray Herb. n.s.* 49: 21. 1917. TYPE: Mexico. San Luis Potosí: Alvarez, 13-23, July 1904, *E. Palmer 185* (holotype, GH; isotype, F).

Erect perennial herb up to 80(--100) cm tall with 1--few stems, each 3--5 mm wide, arising from a woody caudex, naphthoquinones not observed, stems strigose and hispid to strigulose and hispid, trichomes usually glandular. Leaves cauline, sessile; blades lanceolate to narrow elliptical, 2.8--9 x 0.5--2.7 cm, apex acute, rarely acuminate, base acute, margins entire, planar, adaxially strigose and strigulose to strigose and hispidulose and abaxially villous and strigose to dense villous and strigose, midvein evident, secondary veins evident, sunken. Inflorescence terminal dichasium or monochasium, up to 32.5 cm long, bracts sessile, lanceolate, sometimes narrow elliptical or ovate, 1--5.7 x 0.2--1.3(--2.2) cm, apex acute to acuminate, base acute, margins entire, planar, adaxially strigose and hispidulose and abaxially dense villous and strigose, midvein evident, secondary veins evident, sunken, rarely not. Flowers homostylous, chasmogamous; pedicels 0--12 mm long; sepals lanceolate, 6--12(--up to 16 in fruit) x 1--1.5(--up to 2 in fruit) mm, apex acute, abaxially strigose to strigose and strigulose and adaxially strigulose, trichomes usually glandular; corolla campanulate, yellow with green lobes, abaxially villous towards the apex and sparse villous or glabrous towards the base, tube 7--12 x 4--6 mm, adaxial trichomes absent, lobes 1--3 x 1--2 mm, erect, deltoid, apex acute, glands absent, faucal appendages absent, annulus of a membranous ring; stamens inserted, filaments 0.5--1 x 0.1 mm, attached 6--8 mm above the base of the corolla, anthers 1--3 x .0.25--0.5 mm, ellipsoid; style 13--23 x 0.1--0.25 mm, exserted up to 5--10 mm from the apex of the corolla tube; stigma terminal.  Nutlets 3--4 x 2--3 mm, spherical to ovoid, white, sometimes tan, smooth, scar an sunken line, base a short 0.1--0.25 mm pedestal, apex acute.

Distribution: Central eastern to northeastern Mexico.

Phenology: Flowers early June onward, and starts fruiting in early July.

Discussion: *L. unicum* differs from all other species of *Lithospermum* due to the presence of abundant glandular trichomes on the stems, leaves, and sepals of plants. Additionally, *L. unicum* has the broadest geograhic range of the three Mexican species formerly included in *Onosmodium*.

Specimens Examined: MEXICO.  **Hidalgo**: 13.5 mi by road SW of Jacala, alt. 6300 ft., June 11, 1962, *G. L. Webster, W. P. Adams, K. Miller, and L. Miller 11319* (F, MEXU); Barranca de San Vicente, near km. 238 on highway between Zimapán and Jacala, alt 1800-2000 m., Aug. 9, 1948, *H. E. Moore Jr. 4454* (BH, MEXU); Barranca de las Verduras, north side of El Monte on trail from Zimapán to mines of El Monte, alt. 7500 – 8400 ft., Aug. 11, 1948, *H. E. Moore Jr. 4503* (BH); Mpio. Zimapán, upper end of Barranca de San Vicente, alt. 2000 m., Sep. 25, 1946, *H. E. Moore Jr. 1303* (BH); Slopes and ravines by Rio Panotlán, between Zacualtipán and Olotla on road to Metztitlán, 1600 – 2000 m., July 3, 1947, *H. E. Moore Jr. 3271* (BH). **Nuevo León**: Zaragoza, Cerro El Viejo, 1850 m, July 29, 1993, *Hinton et al. 23253* (NY). **Querétaro**: Mpio. San Joaquin, Ruinas Las Ranas, 2 km al N de San Joaquin, alt. 2300 m., Sept. 26, 1991, *R. Fernandez N. 4800* (CAS, MEXU). **San Luis Potosí**: Alvarez, Sierra de Alvarez, Sierra Madre Oriental, alt. 2200-2400 m., July 30-31, 1934, *F. W. Pennell 17861* (MEXU). **Tamaulipas**: Mpio. Hidalgo, 7 km al Sureste de Puerto Purificación, June 6, 1990, *F. González, Medrano 17482* (MEXU); Mpio. Gómez Farías, Reserva de la Biosfera "El Cielo", 23° 10' N, 99° 15' W, alt. 1986 msnm., Aug. 31, 1994, *L. Hernandez 3383* (MEXU); Dulces Nombres, Nuevo León, and just east of border into Tamaulipas, 25˚ N 99.5˚ - 100.5˚ W, alt. 1800 m., July 14, 1948, *F. G. Meyer and D. J. Rogers 2775* (MO).

43. *Lithospermum viride* Greene *Bot. Gaz.* 6: 158. 1881. TYPE: U.S.A. New Mexico: Near Santa Rite del Cobre, 1877, *E. L. Greene s.n. (ND – 043203)* (neotype, ND [digital image]!) **neotype designated here**.

*Lithospermum palmeri* S. Watson *Proc. Amer. Acad. Arts* 18: 122. 1883. TYPE: Mexico. Coahuila or Nuevo León: Sierra Madre, south of Saltillo, Feb. to Oct. 1880, *E. Palmer 903* (holotype, US!; isotype, K [digital image]!).

Erect perennial herb up to 75 cm tall with 1--few stems, each 2--4 mm wide, arising from a woody caudex, naphthoquinones not observed, stems strigose to strigulose to hispid. Leaves cauline, sessile or petiolate, petioles 1--2 x 1--2 mm; blades elliptical to lanceolate to ovate, rarely oblanceolate, 1.3--8.6(--10.5) x 0.4--2.4(--3) cm, apex acute, base acute to rounded, margins entire, planar, adaxially strigose to hispid to strigose and strigulose to hispid and hispidulose and abaxially strigose to hispid to woolly, midvein evident, secondary veins evident, sunken, sometimes not. Inflorescence terminal dichasium or monochasium and sometimes axillary monochasia, up to 25 cm long, bracts sessile, ovate to lanceolate, 1.1--4 x 0.2--2.2 cm, apex acute, sometimes acuminate, base rounded to acute, margins entire, planar, adaxially strigose to strigulose to hispid to hispidulose and abaxially strigose, sometimes strigulose, hispid and hispidulose, villous, and woolly, midvein evident, secondary veins usually evident, sunken, sometimes not. Flowers homostylous, chasmogamous; pedicels 0--10 mm long; sepals lanceolate, 7--17(--up to 19 in fruit) x 0.75--2 mm, apex acute, abaxially strigose to dense strigose to hispid and adaxially strigose to strigulose, rarely hispid; corolla tubular, yellow-green, sometimes yellow to light yellow, abaxially villous to pilose, tube (13--17--)23--32(--35) x 3--6 mm, adaxial trichomes absent, lobes 2--4(--5) x 1.5--3 mm, reflexed, elliptical, apex rounded to obtuse, glands present on veins leading to filaments, faucal appendages absent, annulus of 5--10 membranous flanges; stamens inserted, filaments 0.75--1.5 x 0.1 mm, attached 23--30 mm above the base of the corolla, anthers 2--2.5 x 0.5--1 mm, ellipsoid; style (18--)27--39 x 0.1 mm, exserted up to 6 mm from the apex of the corolla tube; stigma terminal. Nutlets 4 x 2--3 mm, ovoid, white to tan to grey, sometimes pitting, primarily around the not prominent scar, base with a 0.25 mm pedestal, apex acute.

Distribution: Northern Mexico, from Chihuahua to Tamaulipas, and adjacent United States.

Phenology: Flowers from late March to late July, and fruits from late June onward.

Discussion: *L. viride* has some of the features of the species formerly included in *Macromeria*, such as leaves with an evident midvein and evident secondary veins, long corollas, and exserted stigmas. Despite these features, *L. viride* always has been included in *Lithospermum*. This is because the species produces tubular corollas and short filaments. Consequently, *L. viride* probably does not appear to share the same type of pollinator as many of the species with long-funnelform corollas. The corollas vary in length throughout its range, but the variation does not seem to follow any geographical pattern.

Specimens Examined: MEXICO. **Chihuahua**: Cañon del Rayo, a large canyon on the northeastern side towards the northern end of the Sierra del Diablo, about Lat. 27˚ 20' N, July 25 – 29, 1941, *R. M. Stewart 914* (F); Sierra de la Parra across Rio Grande from Sierra Vieja, 30˚ 00' 00" - 30˚ 02' 30" N., 104˚ 52' 30" - 104˚ 53' W., alt. 1450 - 2158 m., June 13, 1973, *M. C. Johnston, T. L. Wendt, & F. Chiang 11314*. **Coahuila**: Sierra de Parras, July 1910, *C. A. Purpus 4596* (F, US); Canyon in eastern face of Sierra de Almagre, 27˚ 36' - 27˚ 38' 30" N., 103˚ 51' 30" - 103˚ 54' W., alt. 1400 – 1900 m, May 5, 1973, *M. C. Johnston, T. L. Wendt, & F. Chiang 10827* (F, NY); 24 km northwest of Fraile on mountain, 25˚ 3' N., 101˚ 18' W., elev., 2500 m., July 15, 1941, *L. R. Stanford, K. L. Retherford, & R. D. Northcraft 377* (NY); Mpio. Sierra Mojada, Cañon de San Salvador, Sierra Mojada, Sep. 14, 1939, *C. H. Muller 3303* (MICH); La Escondida – Lirios Arteaga, alt. 2600 m., July 3 – 23, 1943, *P. Lyonnet*

*4010* (MEXU); La Escondida – Lirios Arteaga, alt. 2600 m., July 3 – 23, 1943, *P. Lyonnet 4024* (MEXU); Sierra Zapalinamé, alt. 2100 m., *P. Lyonnet 3542* (MEXU); Sierra de San Marcos, May 4 – 5, 1969, *Keil, Meyer, Lewis, & Pinkava p 6085* (US). **Nuevo León**: Sierra Madre above Monterrey, 3000 ft., March 31, 1906, *C. G. Pringle 10195* (F, MEXU, NY, US); Mpio. Derrumbadero, Cañon de los Capulines above San Enrique, Hacienda San Jose de Raices, Aug. 6, 1935, *C. H. Mueller 2390* (F); Mountains near Monterrey, July 1933, *C. H. & M. T. Mueller 170* (F); Mountains near Monterrey, beyond Puerta, July 1933, *C. H. & M. T. Mueller 171* (F); Monterrey, June 25, 1938, *L. A. Kenoyer A285* (F); Mpio. Derrumbadera, Cañon de los Capulines, above San Enrique, Hacienda San Jose de Raices, Aug. 6, 1935, *C. H. Mueller 2390* (MICH); Sierra Madre Oriental, ascent of Sierra Infernillo, about 15 miles S.W. of Galeana, alt. 9 – 10000 ft., June 16, 1934, *C. H. & M. T. Mueller 817* (MICH). San Luis Potosí: Mpio. Armadillo, cañon cercano a Palomas, alt. ± 2000 m., *Rzedowski 7900* (MEXU); Mpio. Santiago, Rancho San Benito, alt. 2230 m., June 22, 1994, *Hinton et al. 24393* (MEXU); Mt. "El Infernillo," Pablillo, southeast of Galeana, Sierra Madre Oriental, alt. 2500 – 2650 m., *F. W. Pennell 17122* (US); Mpio. Galeana, 8 km. E of Potosi, alt. 2320 m., July 3, 1984, *Hinton et al. 18718* (US). **Tamaulipas**: between Hermosa and Miquihuana, July, 23, 1949, *Stanford, Lauber, & Taylor 2676* (NY); Mpio. Bustamante, cañon situado 1.5 km al O de Bustamante rumbo a Joya de Herrera, 33˚ 25 ' N y 99˚ 48' O, alt. 1900 m., June 2, 1994, *A. Mora-Olivo 05296* (MEXU).

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