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Phylogenetic Relationships and the Evolution of Gender Dimorphism in *Lycium* (Solanaceae)

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ABSTRACT. *Lycium* (Solanaceae) is a genus of ~ 75 species found worldwide inhabiting arid to semi-arid environments. Phylogenetic relationships were inferred for 25 species of *Lycium* and three closely-related *Grabowskia* species using sequences of the internal transcribed spacer (nr-ITS) regions of nuclear ribosomal DNA and 27 morphological characters. The nr-ITS and morphological data sets were congruent and a combined analysis showed strong support for a clade containing several North American species that have distinctive floral and fruit morphologies. In addition, there was strong support for a single origin of gender dimorphism among North American *Lycium* species. Inclusion of a dimorphic species from South Africa suggests that gender dimorphism has evolved independently among African *Lycium*. Results strongly suggest that *Lycium* is not monophyletic, but includes the genus *Grabowskia*. Further, North American *Lycium* are paraphyletic and current sectional circumscriptions for the American species are inadequate.

Lycium L. (Solanaceae) is a genus of ~ 75 species distributed worldwide, but the genus is particularly species-rich in South America (30 species), southwestern North America (21 species), and southern Africa (17 species) (Hitchcock 1932; Chiang-Cabrera 1981; Joubert 1981; Bernardello 1986a, 1987). *Lycium* belongs to the tribe Lycieae A.T. Hunziker in the subfamily Solanoideae, which also contains the Chilean endemic *Phrodus microphyllus* Miers (Bernardello and Hunziker 1987) and six species of *Grabowskia* Schltdl. (Hunziker 1979, 1997). *Lycium* species are long-lived perennial shrubs, and the majority inhabit arid to semi-arid environments though some are found in coastal saline habitats (D'Arcy 1991). Plants are usually hermaphroditic, having perfect flowers, and most produce red, fleshy, multi-seeded berries (Hitchcock 1932). Functional dioecy has been described for three species of *Lycium* in North America (Chiang-Cabrera 1981; Gilmartin 1983; Miller 2000) and six species in South Africa (Minne et al. 1994; Venter et al. 1999), though in North America the functionally male plants are morphologically perfect and capable of low levels of fruit set (Miller 2000).

Since Hitchcock's (1932) revision, infrageneric classifications in *Lycium* have been based on characters of the ovary and fruit. In his treatment of North and South American *Lycium*, Hitchcock (1932) questioned previous sectional groupings that were based on characters of the calyx and corolla (e.g., Dunal 1852 and Miers 1854, cited in Hitchcock 1932). In Dunal's sectional classification, the relative length of the calyx tube to the calyx lobes was an important character, whereas in Miers classification the ratio of corolla tube to lobe length was central. While Hitchcock (1932) agreed that these characters were useful taxonomically, he argued that intraspecific variation complicated their utility as sectional characters (see also Bernardello 1987). Instead, he proposed that the American species be divided into three sections based primarily on ovary characters, which Hitchcock noted had little within species variation. The first section, *Eulycium*, included all of the North American species with one exception (*L. californicum*) and the majority of the South American species. *Eulycium* was characterized by the presence of two- to many-ovuled carpels and two- to many-seeded fruits. By contrast, relatively few species were included in each of the two remaining sections, *Sclerocarpellum* and *Selidophora*. The sexually dimorphic North American species *L. californicum* and the South American species *L. ameghinoi* were placed in section *Sclerocarpellum*. These species have one-ovuled carpels and a hardened (i.e., indurate), two-seeded fruit. Section *Selidophora*, which included the South American species *L. chilense* and *L. ciliatum*, was not characterized by ovary or seed characters that resemble those in section *Eulycium* (i.e., ovaries are many-ovuled and fruits many-seeded), but by enlarged glands present at the base of the filaments, these being bordered by a row of cilia.

Chiang-Cabrera (1981; see also Chiang 1983) studied the North American species and restructured Hitchcock's classification in two ways. First, he clarified the correct names of two of the three sections, resulting in the renaming of section *Eulycium* as section *Lycium* and section *Selidophora* as section *Schistocalyx*. Second, he transferred four species (*L. cooperi*, *L. macrodon*, *L. puberulum*, and *L. schaffneri*) from Hitchcock's *Eulycium* into section *Sclerocarpellum*. These four species were transferred into *Sclerocarpellum* based on the presence of an indurated endocarp in the fruit. Ber-

nardello (1986b) recognized the three sections above (*Lycium*, *Schistocalyx*, and *Sclerocarpellum*) for the South American species and added another section *Mesocope*. Species in section *Mesocope* have a prominent and protruding red nectary at the base of the ovary (Bernardello 1986b). The sectional classification for the American species in Table 1 follows the most recent circumscription by Bernardello and Chiang-Cabrera (1998).

Grabowskia, also in the tribe Lycieae is morphologically similar to *Lycium* in terms of both floral morphology (Hunziker 1977, 1979; Bernardello 1987) and pollen characteristics (Bernardello and Luján 1997). In their recent analysis of Solanaceae, Olmstead et al. (2000) found that *Lycium* may in fact include members of *Grabowskia*, though this larger scale analysis of Solanaceae included only five species of *Lycium* and a single species of *Grabowskia*.

In order to understand the direction of character change in *Lycium*, particularly as it relates to the evolution of gender dimorphism, a hypothesis of phylogenetic relationships is needed. This study represents a first step towards developing a phylogenetic hypothesis for the genus *Lycium* using both molecular sequence data and morphological characters. Specifically, I was interested in determining the number of times gender dimorphism evolved in North America and identifying the closest relatives of the sexually dimorphic species. Inclusion of *Grabowskia* allowed tests of the monophyly of the genus *Lycium*, and an analysis using morphological data in combination with the molecular data was conducted to test the sectional circumscription of American *Lycium*.

Materials and Methods

Species Sampling and Outgroup Selection. As I was particularly interested in the evolution of gender dimorphism in North American *Lycium*, 16 of the 21 (76%) North American species were included here, including all three North American sexually dimorphic species (*L. californicum*, *L. exsertum*, and *L. fremontii*). To test the monophyly of North American *Lycium*, three species of *Lycium* from South America (*L. cestroides*, *L. ciliatum*, *L. chilense*), four from Africa (*L. ferocissimum*, *L. shawii*, *L. tenue*, *L. tetrandum*), and one each from Asia (*L. barbarum*) and Australia (*L. australe*) also were included (Table 1). One of the African species included (*L. tetrandum*) is sexually dimorphic (Minne et al. 1994). Intraspecific variation was investigated using multiple accessions from different individuals for three species (*L. cestroides*, *L. exsertum*, and *L. fremontii*).

For the majority of *Lycium* species, young leaf tissue was collected from plants in the field during 1997 and 1998 and either dried in silica gel or kept on ice until stored at −80°C. Leaf tissue or genomic DNA also was provided by G. Bernardello (Instituto Multidisciplinario de Biología Vegetal, Córdoba, Argentina), R. G. Olmstead (Department of Botany, University of Washington, U. S. A.), B. Tan (Strybing Arboretum and Botanical Gardens, San Francisco, U. S. A.), and A. L. Hempel (Texas A & M University, Kingsville, TX, U. S. A.) (Table 1). To test the monophyly of *Lycium*, three species of the closely related (Olmstead and Palmer 1986; Olmstead et al. 2000), predominantly South American (Hunziker 1979, 1997) genus *Grabowskia* (*G. boerhaaviaefolia*, *G. duplicata*, and *G. glauca*) were included. *Atropa belladonna*, *Jaborosa integrifolia*, and four species in the related genus *Nolana* were included as outgroups according to previous data of Olmstead et al. (2000) (Table 1).

DNA Extraction, Amplification, and Sequence Alignment. Total genomic DNA was extracted from leaf tissue using a modified CTAB procedure from Doyle and Doyle (1987). The internal transcribed spacers (ITS1 and ITS2) and the 5.8S coding region were amplified from total genomic DNA by the polymerase chain reaction (PCR). Total volume of the reactions was 25 μl (rarely 50 μl) and included 6 μl template DNA (diluted to 10 ng/μl), 9.42 μl sterile H_2O, 2.5 μl 10X PCR buffer, 0.5 μl dNTPs, 1.5 μl $MgCl_2$, 1.0 μl primers: *C26A*, 5′-GTTTCTTTTCCTCCGCT-3′ and *Nnc18s10*, 5′-AGGAGAAGTCGTAACAAG-3′ (Wen and Zimmer 1996), 1.5 μl 50% glycerol, 1.5 μl DMSO, and 0.08 μl Taq polymerase. All PCR reactions included both positive and negative controls. Optimal annealing temperatures varied from 46–58°C and were initially determined for each species separately. Later in the project, a touchdown procedure was employed rather than optimizing each taxon separately (McDade et al. 2000). The touchdown profile began with two cycles at an annealing temperature of 58°C. The annealing temperature was subsequently lowered 1°C every two cycles until a temperature of 48°C was reached. Finally, thirty additional cycles at 48°C were repeated. This touchdown profile was used for 17 *Lycium* accessions and *Jaborosa integrifolia*. Genomic DNAs for *Lycium berlandieri* and *L. torreyi* did not amplify initially and were gel purified prior to PCR. PCR products were visualized on 2.0% agarose gels, purified with the Qiagen™ qiaquick purification kit and sequenced in both directions using the same primers as in amplification on an ABI-377 automated sequencer at the University of Arizona sequencing facility.

To generate consensus sequences, the two sequences (one in each direction) for each sample were aligned and edited using Autoassembler™ v1.4.0 (Applied Biosystems 1995). Consensus sequences for all species were aligned manually using SeqApp (Gilbert 1992). Alignment of the outgroup taxa (*Atropa*, *Jaborosa*, and *Nolana*) to *Lycium* and *Grabowskia* was achieved readily. Gaps within the ingroup were rare, typically of only a single base, and were coded as missing data. The percentage of missing data was 0.5% (88 of 18924 bp) and nearly all of the missing data was due to incomplete sequences obtained from *L. ciliatum* (missing 31 bp) and *L. torreyi* (missing 48 bp).

Morphological Characters for Phylogenetic Analysis. Data for 27 morphological characters were compiled for the American *Lycium* species included in the molecular analysis and also for the three *Grabowskia* species (Appendix 1). For the North American species, the majority of characters were assessed from field observations, preserved material, and voucher specimens collected from source populations. Herbarium specimens were also used to confirm character scoring for the North American species (Appendix 2). For the three North American gynodioecious species, those characters that were sexually dimorphic between females and hermaphrodites were scored using hermaphrodites. South American *Grabowskia* and *Lycium* species were scored using herbarium material (Appendix 2) and by consulting the literature (Hitchcock 1932; Bernardello 1986a; Bernardello and Chiang-Cabrera 1998 for *Lycium*; and Hunziker 1997 for *Grabowskia*). Twenty-one of the 27 morphological characters were coded as binary, five characters had three states and a single character had six states (Appendix 1). All characters were treated as unordered and weighted equally in analyses.

Molecular Phylogenetic Analyses. Data matrices were prepared in MacClade (Maddison and Maddison 1992) and are available upon request from the author. Molecular data (ITS1 and ITS2) were analyzed using PAUP* 4.0b5a (Swofford 2000). Phylogenies were inferred under parsimony using heuristic searches with 1000 random addition sequence replicates and Tree Bisection Reconnection (TBR) branch swapping. Phylogenetic signal in the data set was estimated using standard measures (CI, consistency index; RI, retention index) excluding uninformative characters. Two hundred bootstrap searches (BS; Felsenstein 1985), each with 10 random addition sequence replicates and TBR branch swapping, were done to assess the consistency of these data in reconstructing branching patterns. Decay indices (DI; Bremer 1988; Donoghue et

TABLE 1. Taxon, provenance and source, voucher information, and GenBank accession numbers for nuclear ITS sequences included in this study. Sectional classification for the American *Lycium* species follows Bernardello and Chiang-Cabrera (1998). Abbreviations for herbaria follow Holmgren et al. (1990). STRY refers to Strybing Arboretum, San Francisco, U.S.A.; WAIM refers to Waimea Botanical Garden, Hawaii; BIRM refers to Birmingham University Botanical gardens. * DNA provided by R. G. Olmstead, Department of Botany, University of Washington, U.S.A. † Leaf tissue provided by G. Bernardello, Instituto Multidisciplinario de Biología Vegetal, Córdoba, Argentina. ‡ Leaf tissue provided by B. Tan, Strybing Arboretum, San Francisco, CA, U.S.A. § Leaf tissue provided by A. Hempel, Texas A & M University, Kingsville, TX, U.S.A. ¶ Sequence obtained from GenBank.

Taxon	Provenance and source	Voucher	GenBank accession
Lycium section *Lycium*			
Lycium andersonii A. Gray	Baja California, Mexico	*J. S. Miller 97-12* ARIZ	AF238988
Lycium berlandieri Dunal	Pima County, Arizona, U.S.A.	*J. S. Miller 01-1* ARIZ	AF238989
Lycium brevipes Benth.	Baja California Sur, Mexico	*J. S. Miller 97-19* ARIZ	AF238991
Lycium cestroides Schltdl.*	South America	*Olmstead S-34* WTU; S.0368 BIRM	AY028134, AY028152
Lycium cestroides Schltdl.†	Córdoba, Argentina	*Bernardello 878* CORD	AY028135, AY028153
Lycium pallidum Miers	Pinal County, Arizona, U.S.A.	*J. S. Miller 97-20* ARIZ	AF238986
Lycium parishii A. Gray	Pima County, Arizona, U.S.A.	*J. S. Miller 97-22* ARIZ	AF238990
Lycium torreyi A. Gray	Mohave County, Arizona, U.S.A.	*J. S. Miller 01-5* ARIZ	AF238992
Lycium section *Mesocope*			
Lycium carolinianum Walter§	Kleberg County, Texas, U.S.A.	*A. L. Hempel 843* TAIC	AY028133, AY028151
Lycium exsertum A. Gray	Pinal County, Arizona, U.S.A.	*J. S. Miller 95-1* ARIZ	AF238994
Lycium exsertum A. Gray	Pima County, Arizona, U.S.A.	*J. S. Miller 01-3* ARIZ	AY028138, AY028156
Lycium fremontii A. Gray	Pinal County, Arizona, U.S.A.	*J. S. Miller 95-2* ARIZ	AF238995
Lycium fremontii A. Gray	Baja California, Mexico	*J. S. Miller 97-9* ARIZ	AY028140, AY028158
Lycium fremontii A. Gray	Pima County, Arizona, U.S.A.	*J. S. Miller 01-4* ARIZ	AY028141, AY028159
Lycium sandwicense A. Gray*	Hawaii (cultivated at WAIM)	*74P2091* WAIM	AY028142, AY028160
Lycium shockleyii A. Gray	Mineral County, Nevada, U.S.A.	*J. S. Miller 98-1* ARIZ	AF238987
Lycium section *Schistocalyx*			
Lycium chilense Bertero†	San Luis, Argentina	*Bernardello 877* CORD	AY028137, AY028155
Lycium ciliatum Schltdl.†	Córdoba, Argentina	*Bernardello 876* CORD	AY028136, AY028154
Lycium section *Sclerocarpellum*			
Lycium californicum Nutt. ex Gray	Pima County, Arizona, U.S.A.	*J. S. Miller 01-2* ARIZ	AF238993
Lycium cooperi A. Gray	Mohave County, Arizona, U.S.A.	*J. S. Miller 97-1* ARIZ	AF238984
Lycium macrodon A. Gray	Pinal County, Arizona, U.S.A.	*J. S. Miller 97-21* ARIZ	AF238983
Lycium puberulum A. Gray	Brewster County, Texas, U.S.A.	*R. Levin 97-6* ARIZ	AF238985
Other *Lycium* species			
Lycium sp.	Clark County, Nevada, U.S.A.	*J. S. Miller 97-23* ARIZ	AY028146, AY028164
Lycium australe F. Muell.*	Australia	*Symon 14834* AD	AY028131, AY028149
Lycium barbarum L.*	Asia (cultivated in Michigan, U.S.A.)	*Olmstead S-35* WTU	AY028132, AY028150
Lycium ferocissimum Miers‡	Africa (cultivated at STRY)	*98-0143* STRY	AY028139, AY028157
Lycium shawii Roem. & Schult.*	Africa	*Olmstead S-36* WTU; S.1194 BIRM	AY028143, AY028161
Lycium tenue Willd.*	Cape Province, South Africa	*R. Olmstead 99-13* WTU	AY028144, AY028162
Lycium tetrandum Thunb.*	West Coast National Park, South Africa	*R. Olmstead 99-24* WTU	AYO28145, AY028163
Grabowskia			
Grabowskia boerhaaviaefolia Schltdl.*	El Alto, Cabo Blanco, Peru	*T. Plowman 5401* US	AF238981
Grabowskia duplicata Arnott*	Buenos Aires, Argentina	S.0258 BIRM	AF238982
Grabowskia glauca Johnston¶	Chile	N / A	AB019289, AB019949

TABLE 1. Continued.

Taxon	Provenance and source	Voucher	GenBank accession
Outgroups			
Atropa belladonna var. *lutea* L.*	Worldwide (cultivated at BIRM)	S.0078 BIRM	AY028129, AY028147
Jaborosa integrifolia Lam.*	South America (cultivated at Botanic Garden, Genoa University, Italy)	S.0290 BIRM	AY028130, AY028148
Nolana arenicola I. M. Johnst.¶	Peru	N/A	AB019294, AB019954
Nolana galapagensis (Christoph.) Johnst.¶	Galapagos Islands	N/A	AB019306, AB019966
Nolana inflata Ruiz & Pav.¶	Peru	N/A	AB019311, AB019971
Nolana mollis (Philippi) I. M. Johnst.¶	Chile	N/A	AB019314, AB019974

al. 1992) also were calculated with PAUP* after using the ''PAUP Decay Commands'' option in MacClade.

Combined Analysis. For the American species in the analysis above, molecular and morphological characters were combined to test sectional relationships for American *Lycium*. Phylogenetic inference and calculation of bootstrap values and decay indices were as described above. Before combining the molecular and morphological data, a partition homogeneity test (Farris et al. 1995, as implemented in PAUP*) was conducted to determine if the nr-ITS and morphological data sets were congruent. One thousand replicates were performed, each with 10 random addition sequence replicates and TBR branch-swapping. Due to memory constraints, the MULTREES option was disabled such that only one tree was saved per replicate.

In addition, using the combined data set (with one exception, see below) and MacClade, constraint trees were constructed requiring (a) monophyly of the genus *Lycium* (excluding *Grabowskia*), (b) the North American *Lycium* as a monophyletic group distinct from an exclusively South American monophyletic group of *Lycium*, (c) the Old World *Lycium* as a monophyletic group separate from a monophyletic New World *Lycium* (using the molecular data set only), (d) monophyly of section *Sclerocarpellum* within *Lycium*, (e) monophyly of section *Mesocope* within *Lycium*, and (f) monophyly of section *Lycium* within *Lycium*. These trees were loaded into PAUP* and heuristic searches performed to determine the shortest trees consistent with each of the constraints. These alternative topologies were compared statistically to the maximally parsimonious trees of the unconstrained analyses using Templeton (1983) tests as implemented in PAUP*.

RESULTS

Analysis of Molecular Data. The aligned data matrix of ITS1 and ITS2 for all species was 498 bp in length. ITS1 and ITS2 included 267 and 231 nucleotide bases, respectively (Table 2). Of these 498 aligned bases, 104 (20.9%) were phylogenetically informative; they were equally distributed between ITS1 (55 bp, 20.6%) and ITS2 (49 bp, 21.2%). The 5.8S region was 155 nucleotide bases in length and phylogenetically uninformative. Percent GC content of ITS1 and ITS2 was 67% and 69%, respectively. Pairwise sequence-divergence ranged from 0–9.8% within the ingroup to 0–21.2% among all species included in the study.

The heuristic search using data from both ITS1 and ITS2 yielded six most-parsimonious trees of 298 steps (CI excluding uninformative characters = 0.49; RI = 0.69). The strict consensus of these six trees is well resolved, with the only ambiguity being resolution of the basal position in the clade containing *L. cestroides* plus *L. shawii*, which switches position with *L. tetrandum*, and the terminal relationships in the clade containing *L. parishii*, *L. torreyi*, and an unidentified *Lycium* species (note the dashed arrows in Fig. 1). There is strong support (>75% BS) for the monophyly of *Lycium* including *Grabowskia*, and for the monophyly of *Grabowskia* nested within *Lycium*. Further, the data strongly support a clade composed of the North American dimorphic species, *L. californicum*, *L. exsertum*, and *L. fremontii*. The close relationship between the South American species *L. ciliatum* and *L. chilense*, the sister taxon relationship between *L. carolinianum* and Hawaiian *L. sandwicense*, and the two accessions of *L. cestroides* also were strongly supported (Fig. 1).

Analysis of Combined Data. The partition homogeneity test did not detect significant incongruence be-

TABLE 2. Characteristics of the nuclear ribosomal ITS region in 32 accessions of *Lycium* and *Grabowskia*. *ITS1 for *L. ciliatum* is missing a 31 bp region and was not included. † ITS2 for *L. torreyi* is missing a 48 bp region and was not included.

	ITS1	ITS2
Raw length	246–254*	218–225†
Aligned length	267	231
Variable sites	116 (43.4%)	74 (32.0%)
Parsimony informative sites	55 (20.6%)	49 (21.2%)
Pairwise distances, mean (range)	5.2% (0–12.5%)	4.1% (0–8.7%)
GC content, mean (range)	0.67 (0.62–0.70)	0.69 (0.65–0.71)

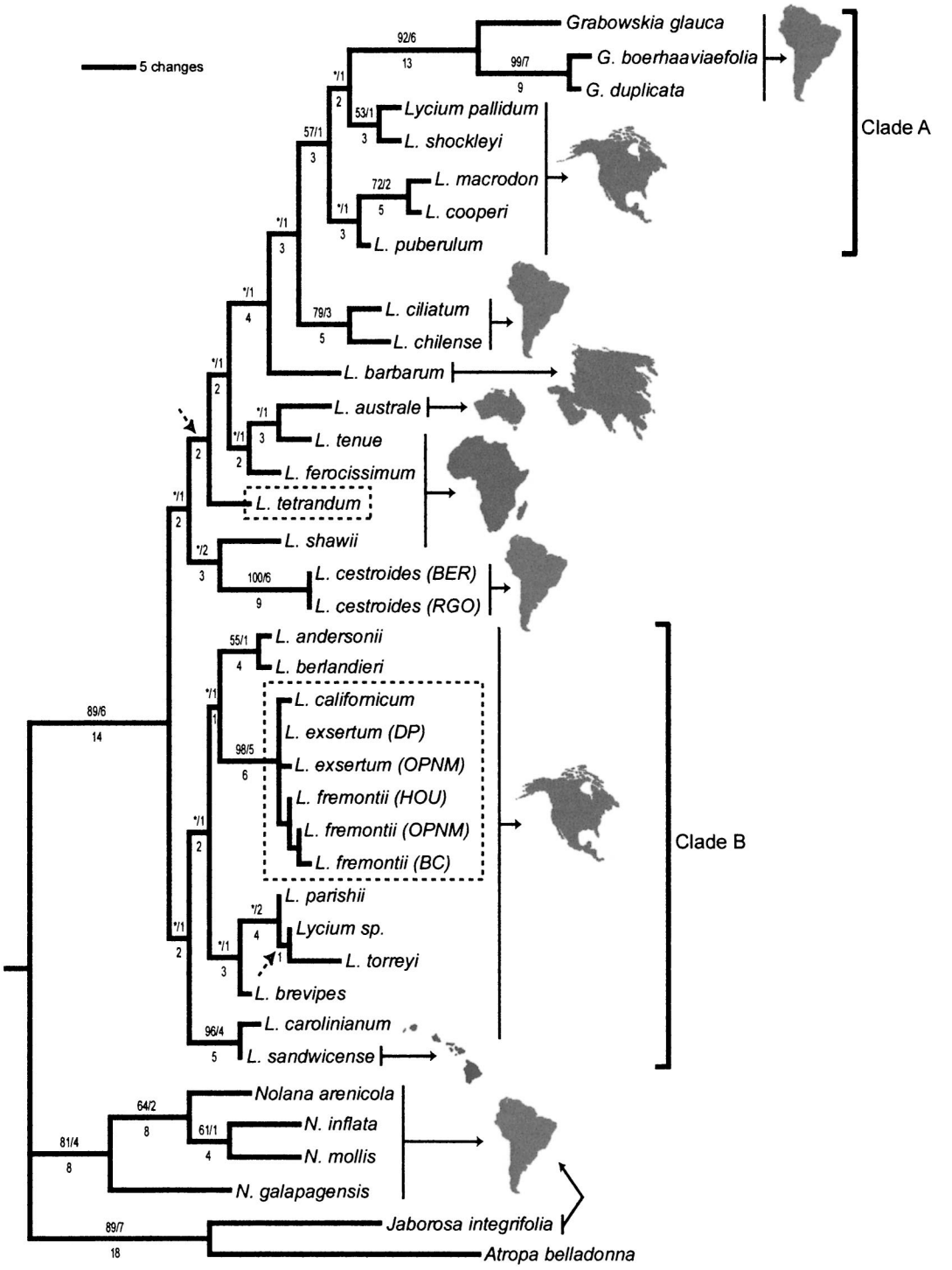

FIG. 1. One of six most-parsimonious trees (length = 298) from heuristic parsimony analysis of ITS1 and ITS2 sequence data for *Lycium* and *Grabowskia. Atropa belladonna, Jaborosa integrifolia* and *Nolana* spp. were used as outgroups. Numbers above the branches are bootstrap percentages / decay indices; below are branch lengths. The sexually dimorphic taxa are in dashed boxes. The abbreviations RGO, BER, DP, OPNM, HOU, and BC represent different accessions within species. Asterisks indicate nodes supported by < 50 percent of bootstrap replicates; dashed arrows indicate nodes that collapse in a strict consensus of the six most-parsimonious trees. Biogeographic regions are indicated at right.

FIG. 2. The single most-parsimonious tree (length = 340) from heuristic parsimony analysis for molecular (ITS1 and ITS2) and morphological data combined for American species of *Lycium* and *Grabowskia*. *Atropa belladonna*, *Jaborosa integrifolia* and *Nolana* spp. were used as outgroups. Numbers above the branches are bootstrap percentages; below are decay indices. Asterisks indicate nodes supported by < 50 percent of bootstrap replicates. At right are the sections for *Lycium* as circumscribed by Bernardello and Chiang-Cabrera (1998).

tween the morphological and molecular data sets (P = 0.133); thus, they were combined in a subsequent parsimony analysis. The combined analysis of American *Lycium* species yielded a single most-parsimonious tree of 340 steps (CI excluding uninformative characters = 0.51, RI = 0.72). The combined data are in agreement with the molecular-only data set in placing *L. ciliatum*, *L. chilense*, *L. pallidum*, *L. shockleyi*, *L. puberulum*, *L. macrodon*, *L. cooperi*, and the three *Grabowskia* species together (Fig. 2). The addition of morphology strengthens this relationship, resulting in a well supported clade in the combined analysis (molecular data set, BS

< 50%, DI = 1; combined data set, BS = 77%, DI = 4; Fig. 2). In addition, within this group relationships among species are clarified and have increased support with the addition of morphological characters (compare relationships and support for this clade in Figs. 1, 2). The combined data also provide increased evidence for relationships within the dimorphic clade, supporting a sister taxon relationship between *L. exsertum* and *L. fremontii* (BS = 83%, DI = 2; Fig. 2).

Several differences between the molecular data set and the most parsimonious tree from the combined analysis are evident. Most notably, placement of the basal members of clade B (i.e., clades containing *L. carolinianum* and *L. parishii*; Fig. 1) switch to the base of the large clade containing *L. chilense* plus the *Grabowskia* species in the combined analysis (Fig. 2). In addition, *L. torreyi* and *L. cestroides* also shift positions in the combined analysis. However, support for the placement of these groups is not supported at the 50% bootstrap level in either analysis.

Discussion

***Phylogenetic Relationships in* Lycium.** *Lycium* is not monophyletic as currently circumscribed, but includes the genus *Grabowskia* (Figs. 1, 2). This result concurs with a family-level study of Solanaceae, in which *Grabowskia duplicata* is nested within *Lycium* (represented by five species) in an analysis of chloroplast sequence and restriction site data (Olmstead et al. 2000). Although in the present study constraint trees requiring the monophyly of *Lycium* excluding *Grabowskia* are only 2.4% longer than the original tree, Templeton's (1983) test indicates that the constrained topology is significantly longer than the most-parsimonious topology (constrained topology is eight steps longer, $P < 0.0001$). The molecular data are also consistent with a morphological analysis of tribe Lycieae in which *Grabowskia* is nested within *Lycium* (Bernardello and Chiang-Cabrera 1998). The *Grabowskia* species studied here share many morphological characters with *Lycium* (Bernardello 1987; Bernardello and Luján 1997), particularly with those *Lycium* species in the clade containing *Grabowskia*. Thus, the molecular data appear to be congruent with the morphological information that is currently available, and suggest that *Grabowskia* species may comprise a clade of very divergent *Lycium*.

North American *Lycium* are not supported as monophyletic, having members in two distinct clades (Figs. 1, 2). Constraining the North American *Lycium* to be a monophyletic lineage separate from a monophyletic group of South American species results in trees that are 4.7% longer than the most-parsimonious tree, and the most-parsimonious topology is significantly shorter than the constrained topologies as assessed by Templeton's (1983) test (constrained topologies are 16 steps longer, $P < 0.0001$). The North American members in clade A (see Fig. 1) typically have pendulous, whitish flowers that are relatively large compared to a second clade containing North American species (see clade B in Fig. 1). In addition, the North American members in clade B (Fig. 1; *L. californicum* excepted), all have the typical multi-seeded, orange or red, fleshy berry. By contrast, species in clade A (Fig. 1) have fruits that are modified in various ways from the typical fleshy berry (Chiang-Cabrera 1981; see discussion below on the sectional circumscription of *Lycium*).

Further, Old and New World *Lycium* appear polyphyletic (Fig. 1), contrary to the analyses of Olmstead et al. (2000), though sampling of *Lycium* in Olmstead et al. was limited to three Old World and two New World species. Despite low bootstrap support for this result in my analysis (Fig. 1), a constraint tree requiring the monophyly of the Old and New World species as two distinct lineages is significantly longer than the most-parsimonious trees (constrained topology is 13 steps longer, $P < 0.0001$). The fleshy, red berries present in most *Lycium* are certainly attractive to bird species, which could possibly disperse the seeds long distances, potentially complicating the relationships among Old and New World *Lycium*. Providing further support for long range dispersal is the Hawaiian taxon *L. sandwicense*, which is thought to be a variety of *L. carolinianum* (Hitchcock 1932; Chiang-Cabrera 1981); the nr-ITS results strongly support this relationship. *Lycium carolinianum* is a wide ranging species found from the islands in the West Indies to the coastal regions of Florida, East Texas, and Mexico (Chiang-Cabrera 1981). *Lycium sandwicense* has an eastern Pacific Island distribution, and Symon (1991) has suggested that birds are responsible for the dispersal of this species to islands in the Pacific.

In a recent paper, Fukuda et al. (2001) used chloroplast sequence data to investigate relationships among *Lycium* and found similar results. Neither the New World *Lycium* (represented by ten species) nor the North American subset of these (represented by six species) were supported as monophyletic in their analyses. In addition, the seven species of southern African species included were also not monophyletic (Fukuda et al. 2001). The analysis of Fukuda et al. (2001) used data from four regions of the chloroplast genome including the *matK* coding region, two intergenic spacers (*trnT-trnL* and *trnL-trnF*), and the *trnL* intron. Furthermore, only ten species were shared between Fukuda et al. (2001) and this study. Despite these differences, it is interesting that results from the two studies are largely congruent. For example, the strongly supported sister taxon relationships presented here between *Lycium andersonii* and *L. berlandieri*, and *L. carolinianum* and *L. sandwicense* are consistent with Fukuda et al. (2001). In addition, their study provides additional ev-

idence for the sister relationship (weakly supported here, see Figs. 1, 2) between the North American dimorphic species and the clade containing *L. andersonii* plus *L. berlandieri*.

Evolution of Gender Dimorphism. There is strong support for a single origin of gender dimorphism in North America (BS = 98%, DI = 5 in Fig. 1 and BS = 87%, DI = 4 in Fig. 2). The functionally dioecious species from South Africa included in this study (*L. tetrandum*) is not related to the North American dimorphic species. This argues for a minimum of two evolutionary origins of gender dimorphism within *Lycium*, depending on the patterns of relationships among African *Lycium* (only one dimorphic and three cosexual African *Lycium* species were included here). More such transitions may have occurred, as there are six dimorphic species in Africa (Minne et al. 1994; Venter et al. 1999). Dimorphic *Lycium* in Africa appear to be morphologically more advanced towards full dioecy compared to their North American relatives, as flowers on staminate plants in Africa have gynoecia that are either underdeveloped and possess only a rudimentary style and stigma, or the gynoecia are entirely absent (Minne et al. 1994). In North America, flowers on staminate (i.e., hermaphroditic) plants always produce female structures, though for hermaphrodites of *L. californicum* and *L. fremontii* these are somewhat reduced in size (Miller 2000).

The association of polyploidy and gender dimorphism is also notable. The North American dimorphic species are either tetraploid or octoploid, while the dimorphic South African species is hexaploid. Interestingly, all of the cosexual species with known chromosome counts are diploid (2n = 24 for 37 *Lycium* species), and polyploidy and gender dimorphism would seem to have evolved in concert (Miller and Venable 2000).

***Sectional Circumscription of American* Lycium.** Current sectional divisions of American *Lycium* are inadequate (Fig. 2). As currently circumscribed (Chiang 1983; Bernardello and Chiang-Cabrera 1998), section *Sclerocarpellum* is not monophyletic (Fig. 2). Constraining the monophyly of *Sclerocarpellum* requires trees to be 7.9% longer compared to the unrestricted analysis, and the constraint tree is significantly different than the unconstrained trees (constrained topology is 27 steps longer, $P < 0.0001$). Hitchcock (1932) originally designated section *Sclerocarpellum* as including only *L. californicum* and *L. ameghinoi* based on their unique one-ovuled carpels and two-seeded fruit. Though *L. ameghinoi* was not included in this analysis, Fukuda et al. (2001) found no support for a close relationship between *L. californicum* and *L. ameghinoi*, making it unlikely that inclusion of *L. ameghinoi* here would result in unifying *Sclerocarpellum*. Further, strong support for the close relationship of *L. californicum* (section *Sclerocarpellum*) with *L. exsertum* and *L. fremontii* (both section *Mesocope*) (BS = 98%, DI = 5; Fig. 1) in the analyses here makes it very unlikely that section *Sclerocarpellum* is monophyletic as currently circumscribed.

However, when *L. californicum* is excluded, other members of section *Sclerocarpellum* (included here, *L. cooperi*, *L. macrodon*, and *L. puberulum*) form a monophyletic group (Figs. 1, 2; not included here, *L. ameghinoi* and *L. schaffneri*). These species also are related closely to *L. shockleyi* (section *Mesocope*) and *L. pallidum* (section *Lycium*) (Fig. 2). All species in this group of five North American species have relatively large (compared to other North American species), typically white, pendulous flowers and long calyx lobes. They also have distinctive fruits. Fruits of *L. macrodon*, *L. puberulum*, and *L. cooperi* all possess a transverse split that separates the fruit into upper and lower compartments and an indurated endocarp that partially encloses the seeds. In *L. macrodon* and *L. puberulum* the separation between the compartments is complete, but in *L. cooperi* the separation is incomplete. In addition, these species have a reduced number of seeds (typically < 8) and fruits that are yellowish-green at maturity and presumably mammal dispersed (Chiang-Cabrera 1981; J. S. Miller, unpub. data). Though *L. shockleyi* does not have a transverse separation that divides the fruit into upper and lower compartments, it does possess a suture or fold in this position across the outside of the fruit (Muller 1940, 1961). Chiang-Cabrera (1981, p. 141) notes the suture described by Muller, but attaches no significance to this finding, as he finds no indurated portion in fruits of *L. shockleyi*. Nevertheless, in further support of a close relationship with *L. macrodon*, *L. cooperi*, and *L. puberulum*, *L. shockleyi* shares a reduction in the number of ovules and seeds, typically having four seeds (Muller 1940; Chiang-Cabrera 1981; J. S. Miller, unpub. data). *Lycium pallidum*, also closely related to these species (Figs. 1, 2), has a multi-seeded berry with no indurated portion, but even here the otherwise fleshy berries have a small sclerified beak present in the distal region of the berry (J. S. Miller, unpub. data). One variety of *L. pallidum* (var. *oligospermum*; Hitchcock 1932; Chiang-Cabrera 1981) has only four to eight seeds, similar to the reduction found in the other species, though it is not known whether this variety is basal within *L. pallidum*. Also included in this group are the *Grabowskia* species, which have distinctive four-loculed fruits, each locule having one or two seeds. It is interesting that much of the variation in fruit morphology occurs in this one clade (see clade A in Fig. 1). The final species classified in section *Sclerocarpellum*, but not included in the analyses here, is *L. schaffneri*. *Lycium schaffneri* shares many morphological characters with species in clade A (see Fig. 1), including vegetative characteristics resembling *L. pallidum* and fruits similar to those in *L. macrodon*,

L. cooperi, and *L. puberulum* (Chiang-Cabrera 1981). Thus, inclusion of *L. schaffneri* would likely result in its placement within clade A.

The exclusively South American section *Schistocalyx* includes only the species *L. ciliatum* and *L. chilense* (Bernardello 1986a, 1987) and is supported as monophyletic by both the nr-ITS and the combined analyses (Figs. 1, 2). Fruits of these two species are fleshy berries and many-ovuled and -seeded, as in the majority of *Lycium* species (Bernardello 1986a). Members of section *Schistocalyx* share the presence of an enlarged, ciliated gland at the base of the filaments as noted by Hitchcock (1932) and Bernardello (1986a). Given the results of the nr-ITS data, the presence of this morphological character appears to be a synapomorphy for this section (see also Bernardello and Chiang-Cabrera 1998), though increased sampling of South American *Lycium* is necessary to confirm this result.

Two additional sections of *Lycium*, sections *Lycium* and *Mesocope*, have been proposed for the American taxa (Chiang 1983; Bernardello 1987; Bernardello and Chiang-Cabrera 1998). Section *Lycium* is not supported by any synapomorphic characters and appears to include all *Lycium* species not placed in other sections. Section *Mesocope* includes those species having an ovary with a protruding, conspicuous red basal nectary (Bernardello 1986a,b, 1987). However, this character is either symplesiomorphic or has evolved in parallel, as several taxa placed in other sections also possess this trait (Appendix 3). Constraint trees requiring the monophyly of sections *Lycium* and *Mesocope* are 4.1% and 7.9% longer, respectively, than the most parsimonious topology (both $P < 0.0001$).

Future Directions. Despite many regional treatments of the genus *Lycium* (Hitchcock 1932; Feinbrun 1968; Haegi 1976; Chiang-Cabrera 1981; Joubert 1981; Bernardello 1986a; Bernardello and Chiang-Cabrera 1998), little attention has been directed at a worldwide classification of the genus. The results presented here would suggest that a geographically unrestricted treatment is necessary, given that the Old and New World *Lycium* species do not appear to be monophyletic and that North American *Lycium* are not monophyletic (Fig. 1; see also Fukuda et al. 2001).

In terms of generating variable sites, comparison of nucleotide statistics reveals that ITS1 and ITS2 are more appropriate choices compared to many chloroplast regions. For example, the total number of variable sites in the analysis of Fukuda et al. (2001) across four chloroplast regions was 57 bp compared to 55 bp for ITS1 and 49 bp for ITS2 (compare Table 4 in Fukuda et al. 2001 with Table 2, this paper). Furthermore, the ratio of informative sites to total sequence length was far greater for nr-ITS sequence data (20.6% and 21.2% for ITS1 and ITS2, respectively; see Table 2) compared to chloroplast sequence data (0.8%, 1.1%, 0.5%, and 0.6% for *matK*, *trnT-trnL*, *trnL-trnF*, and the *trnL* intron, respectively). Thus, continued use of ITS and other regions with similarly high levels of variation, as well as combined analyses, will be crucial to resolving relationships within *Lycium*.

This study has provided direction for addressing a variety of questions of evolutionary interest in *Lycium*. For example, increased sampling from Africa could resolve the number of times gender dimorphism and polyploidy have evolved in concert in both the African species and the genus as a whole (see Miller and Venable 2000). In addition, questions concerning the biogeography of *Lycium* could be addressed with the inclusion of additional South American, African, Asian, and island species (see Fukuda et al. 2001). It would also be interesting to investigate fruit evolution among those species in clade A (see Fig. 1), including the ecological importance (e.g., dispersal mechanisms) of the different fruit types present in this group. Lastly, further work on the infrageneric classification and sectional circumscription of *Lycium* is needed. Increased sampling of *Lycium*, particularly in South America where the genus is most species-rich, and the inclusion of genes that evolve fast enough to capture infrageneric variation will strengthen hypotheses of relationships and allow for future investigations of ecologically and evolutionarily interesting traits in *Lycium*.

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APPENDIX 1.

Morphological characters used in the phylogenetic analysis.

1. *Pedicel length* (0: sessile or subsessile, pedicel much shorter than the calyx tube, 1: pedicel present and as long or longer than the calyx tube).

2. *Flower orientation* (0: upright, 1: pendulous).

3. *Corolla tube shape* (0: campanulate, 1: tubular, 2: funnelform).

4. *Corolla lobe length* (0: corolla lobes always shorter than the corolla tube, 1: corolla lobes as long as or longer than the corolla tube).

5. *Corolla lobe position* (0: spreading, 1: reflexed).

6. *Corolla lobe margins* [0: margins glabrous or sparsely (with only a few trichomes along the margin) ciliate, 1: margins densely (trichomes present along the entire margin) ciliate, 2: margins densely ciliate-lanate, with a ''wooly'' appearance].

7. *Corolla color* (0: greenish-white, never purple, 1: typically white, sometimes pale purple, 2: purple, often deep violet, but never white). Corolla color can be quite variable, however, field observations of multiple populations for many of the North American species made scoring of this character possible. In addition,

corolla color was often indicated on herbarium specimens for the South American species.

8. *Merosity* (0: five or mostly five, 1: four or mostly four). This character refers only to the calyx, corolla, and stamens as all *Lycium* species have bicarpellate gynoecia.

9. *Filament adnation* (0: adnate at or just below the mid-point of the corolla tube, 1: adnation extending to the distal half of the corolla tube). Several species have very short filaments that are adnate high in the corolla tube, contrasting with those species having short adnation distances and longer, free filaments.

10. *Filament base* (0: glandless, 1: with a large gland, fringed by a row of cilia).

11. *Filament pubescence* (0: free portion of filament glabrous, 1: free portion of filament pubescent).

12. *Anther height* [0: anthers equal or nearly so (subequal), 1: anthers unequal]. Hitchcock (1932) reported that the relative length of the stamens, and thus the height of the anthers was a character of taxonomic importance.

13. *Stamen position* [0: stamens always included (or nearly so) in the corolla tube, 1: stamens exerted from the corolla tube].

14. *Nectary* (0: green and not protruding from the ovary wall, 1: orange or red and protruding from the ovary wall). Nectaries are located at the base of the ovary in *Lycium* and vary with regard to their morphology and color.

15. *Fruit type* (0: fleshy berry, 1: berry with a distal sclerified ''beak,'' 2: incompletely indurated endocarp with an incomplete transverse split, 3: incompletely indurated endocarp with a transverse split, 4: completely indurated endocarp with a longitudinal split, 5: completely indurated, four-loculed fruit).

16. *Fruit color at maturity* (0: orange or red, 1: yellow, green, or brown).

17. *Number of seeds* (0: greater than ten, 1: two to four, rarely eight).

18. *Calyx lobe length* (0: always shorter than the calyx tube, 1: as long as or longer than the calyx tube).

19. *Calyx lobe shape* [0: at least as broad as long (e.g., triangular, deltoid), 1: longer than broad (e.g., lanceolate, linear)].

20. *Calyx tube shape* (0: campanulate, 1: tubular, 2: cup-shaped).

21. *Calyx pubescence* (0: glabrous to sparsely pubescent, 1: densely pubescent). Pubescence is always multicellular and typically glandular for the American species studied here. Calyces were coded as either densely (most of the calyx surface covered with trichomes) or glabrous to sparsely (with only a few scattered trichomes) pubescent.

22. *Calyx post anthesis growth* (0: calyx not accrescent, 1: calyx accrescent, enlarging with the fruit).

23. *Leaf type* (0: thin, membranaceous, 1: fleshy, succulent).

24. *Leaf trichomes* (0: glabrous or only sparsely pubescent, 1: densely pubescent). Leaves were coded as either densely (most of the leaf surface covered with trichomes) or glabrous to sparsely (with only a few scattered trichomes) pubescent.

25. *Leaf color* (0: green, 1: glaucous-green).

26. *Plant habit* (0: upright shrub, 1: creeping, prostrate shrub). Most *Lycium* are erect multi-branched shrubs, however, a few species grow low to the ground and do not typically exceed 1 m in height.

27. *Chromosome number* (0: n=12, 1: n=24, 2: n=48). Several literature sources were consulted for this character including Chiang-Cabrera (1981), Bernardello (1986a), Bernardello et al. (1990), and Hunziker (1997).

APPENDIX 2.

Herbarium specimens studied in the morphological character analysis. Sectional circumscription follows Bernardello and Chiang-Cabrera (1998) and abbreviations for herbaria follow Holmgren et al. (1990).

Lycium* section *Lycium. ***Lycium andersonii*** (*J. S. Miller 97–12* ARIZ, *L. Benson 10093* ARIZ, *F. W. Reichenbacher et al. 468A* ARIZ, *A. Harlan & F. W. Telewski 136* ARIZ, *R. S. Felger 92–756* ARIZ, *R. S. Felger 92–21* ARIZ, *R. Perrill 5861* ARIZ, *Butterwick & Hillyard 5716* ARIZ, *M. Ames et al. 66* ARIZ, *P. C. Fischer 5978* ARIZ); ***Lycium berlandieri*** (*J. S. Miller 01–1* ARIZ, *R. Perrill 5153* ARIZ, *T. L. Burgess 5947* ARIZ, *R. S. Felger 9419* ARIZ, *G. J. Harrison et al. 7295* ARIZ, *G. J. Harrison et al. 7996* ARIZ, *R. H. Whittaker & W. A. Niering 7 Aug 1963* ARIZ, *T. R. Van Devender 87–231* ARIZ, *J. E. Bowers 897* ARIZ, *J. E. Bowers 1522* ARIZ); ***Lycium brevipes*** (*J. S. Miller 97–19* ARIZ, *T. L. Burgess 6197* ARIZ, *J. R. Hastings & R. M. Turner 71–127* ARIZ, *R. M. Turner 61–97* ARIZ, *J. R. Hastings & R. M. Turner 64–263* ARIZ, *T. R. Van Devender & M. C. Kearns 18 Feb 1977* ARIZ, *R. S. Felger 86–2* ARIZ); ***Lycium cestroides*** (*R. H. Fortunato 5170* ARIZ, *P. Cantino 378* ARIZ, *A. Krapovickas & C. L. Cristóbal 17358* MO, *W. G. D'Arcy & A. T. Hunizker 13955* MO, *I. G. Vargas 3042* MO, *J. C. Solomon 10900* MO, *A. Krapovickas et al. 18817* MO, *A. Krapovickas et al. 27944* MO, *A. Krapovickas & C. L. Cristóbal 27156* MO, *A. L. Cabrera 34863* MO, *K. Fiebrig 2213* US, *N. Rosengurtt B-3647* US, *S. A. Renvoiae 3383* US, *E. P. Killip 39564* US, *S. A. Pierotti 81561* US, *H. H. Bartlett 19245* US, *A. L. Cabrera 2065* US, *S. Venturi 5431* US, *S. Venturi 2459* US); ***Lycium pallidum*** (*J. S. Miller 97–20* ARIZ, *A. F. Whiting 1072* ARIZ, *S. Mclaughlin & R. McManus 197* ARIZ, *G. J. Harrison & T. H. Kearney 6665* ARIZ, *R. R. Halse 89* ARIZ, *R. R. Halse 504* ARIZ); ***Lycium parishii*** (*J. S. Miller 97–22* ARIZ, *J. S. Miller 01–7* ARIZ, *J. E. Bowers 1052* ARIZ, *J. E. Bowers & B. K. Mortenson 1112* ARIZ, *T. R. Van Devender et al. March 5 1983* ARIZ, *J. E. Bowers et al. 1584* ARIZ, *R. S. Felger 92–662* ARIZ, *R.*

S. Felger 93–69 ARIZ, *T. H. Kearney & R. H. Peebles 10828* ARIZ, *T. R. Van Devender 87–233* ARIZ); ***Lycium torreyi*** (*J. S. Miller 01–5* ARIZ, *J. S. Miller 01–11* ARIZ, *R. H. Peebles 6446* ARIZ, *D. D. Porter et al. 1165* ARIZ, *J. J. Thornber 8837* ARIZ, *E. U. Clover 6321* ARIZ, *C. F. Deaver 2453* ARIZ, *R. K. Grevisch 4628* ARIZ).

Lycium* section *Mesocope*. *Lycium carolinianum (*C. S. Wallis 8293* ARIZ, *E. U. Clover 61331* ARIZ, *C. L. Lundell & D. S. Correll 15214* LL, *D. S. Correll & H. B. Correll 28501* LL, *R. D. Thomas et al. 80245* MO, *J. C. Solomon 2728* MO, *D. F. Austin 4360* MO, *A. W. Lievens 2934* MO, *S. R. Hill 13446* MO, *R. Runyon 5773* TEX, *F. Chiang 701* TEX, *S. M. Tracy 48* US, *R. Runyon 273* US, *R. Runyon 692* US, *R. L. Crockett 7110* US, *L. F. Ward 9–16–1877* US, *J. N. Rose 24204* US, *J. N. Rose 24257* US, *V. L. Cory 50952* US, *W. C. Brumbach 7756* US, *E. P. Killip 31523* US, *E. P. Killip 44492* US, *F. Duckett 215* US); ***Lycium exsertum*** (*J. S. Miller 95–1* ARIZ, *J. S. Miller 01–3* ARIZ, *J. S. Miller 01–8* ARIZ, *R. H. Peebles 7476* ARIZ, *R. H. Peebles & G. J. Harrison 7495* ARIZ, *S. Mclaughlin 1006* ARIZ, *C. D. Bertelsen 91–001* ARIZ, *D. Ducote 705* ARIZ, *Butterwick & Hillyard 5702* ARIZ, *Fishbein 890* ARIZ, *H. J. Fulton 6435* ARIZ); ***Lycium fremontii*** (*J. S. Miller 95–2* ARIZ, *J. S. Miller 97–9* ARIZ, *J. S. Miller 01–4* ARIZ, *J. S. Miller 01–6* ARIZ, *R. H. Peebles 7471* ARIZ, *R. H. Peebles 7480* ARIZ, *R. S. Felger & M. A. Dimmitt 87–292* ARIZ, *R. S. Felger 90–468* ARIZ); ***Lycium sandwicense*** (*D. Herbst 2346* MO, *D. Herbst 6025* US, *D. Herbst 6035* US, *D. Herbst 6122* US, *A. A. Heller 2093* US, *F. R. Fosberg 43546* US, *C. R. Long 1654* US, *C. R. Long 1677* US); ***Lycium shockleyii*** (*J. S. Miller 98–1* ARIZ, *J. L. Reveal 4435* COLO).

Lycium* section *Schistocalyx*. *Lycium chilense (*R. H. Fortunato 4288* ARIZ, *P. Cantino 400* ARIZ, *O. Zöllner 8163* MO, *A. Krapovickas et al. 22427* MO, *P. C. Hutchison 33* US, *P. C. Hutchison 362* US, *T. G. Lammers et al. 7745* US, *I. M. Johnston 4989* US, *A. Burkart 20.458* US, *H. H. Bartlett 19453* US, *H. H. Bartlett 19954* US, *H. H. Bartlett 20587* US, *W. Fischer 17* US, *H. A. Fabris 833* US, *H. Sleumer 1470* US); ***Lycium ciliatum*** (*R. H. Fortunato 5163* ARIZ, *Accession 192212* ARIZ, *A. Krapovickas 27644* MO, *A. Krapovickas & C. L. Cristóbal 17552* MO, *L. Bernardello 15199* MO, *S. M. Bottz & D. C. Miconi 581* MO, *A. Jimenez 88* US, *H. Sleumer & F. Vervoorst 2337* US, *H. Sleumer & F. Vervoorst 2389* US, *H. H. Bartlett 20236* US, *A. Burkart 10428* US, *S. Venturi 903* US, *S. Venturi 1681* US, *S. Venturi 5689* US, *M. Cárdenas 727* US).

Lycium* section *Sclerocarpellum*. *Lycium californicum (*J. S. Miller 01–2* ARIZ, *J. S. Miller 01–9* ARIZ, *J. S. Miller 01–10* ARIZ, *R. H. Peebles & G. J. Harrison 3546* ARIZ, *R. H. Peebles 13241* ARIZ, *W. B. McDougall 87* ARIZ, *G. Nabhan 367* ARIZ, *K. F. Parker 82612* ARIZ); ***Lycium cooperi*** (*J. S. Miller 97–1* ARIZ, *S. P. Mclaughlin 4442* ARIZ, *Butterwick & Hillyard 5841* ARIZ, *R. S. Pauty 6/15/38* ARIZ, *R. E. Coombs & C. F. Bundy 2479* ARIZ, *G. J. Harrison et al. 7614* ARIZ, *T. H. Kearny & R. H. Peebles 11137* ARIZ); ***Lycium macrodon*** (*J. S. Miller 97–21* ARIZ, *R. H. Peebles 11407* ARIZ, *D. Ducote 746* ARIZ, *E. Lehto 17621* ARIZ, *R. A. Darrow Mar 16 1941* ARIZ, *T. F. Daniel & M. Butterwick 2568* ARIZ, *L. Benson 10617A* ARIZ, *R. H. Peebles & H. F. Loomis 6432* ARIZ, *C. T. Mason 3378* ARIZ); ***Lycium puberulum*** (*R. Levin 97–6* ARIZ, *C. S. Lieb 1261* COLO).

Grabowskia*. *Grabowskia boerhaaviaefolia (*P. Cantino 640* ARIZ, *A. Richardson 2007* LL, *T. Plowman 5401* MO, *T. Plowman 5510* MO, *A. Sagástegui 8532* MO, *R. Ferreyra 19160* MO, *M. O. Dillon & M. Whalen 4008* TEX, *J. T. Howell 9911* US, *M. O. Dillon & A. Sagástegui 6097* US, *P. C. Hutchison 538* US, *F. R. Fosberg 27975* US, *O. V. Nuñez 1870* US, *F. R. Fosberg 27670* US, *T. H. Goodspeed 33085* US, *E. Asplund 18348* US, *O. Haught 15* US, *I. L. Wiggins & D. M. Porter 510* US); ***Grabowskia duplicata*** (*A. Krapovickas 15786* LL, *J. F. Casas 4475* MO, *A. L. Cabrera 28071* MO, *R. Degen 1022* TEX, *Ragonese 2290* US, *Del Puerto 5374* US); ***Grabowskia glauca*** (*A. Gentry et al. 19143* MO, *I. M. Johnston 5127* US, *I. M. Johnston 5608* US).

APPENDIX 3. Taxon by character matrix. Question marks denote missing data.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Lycium andersonii	1	0	1	0	0	1	1	0	0	0	1	1	1	0	0	0	0	0	0	2	0	0	1	0	0	0	0
Lycium berlandieri	1	0	1	0	0	1	1	0	0	0	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Lycium brevipes	1	0	2	1	0	1	2	0	0	0	1	0	1	0	0	0	0	1	1	0	1	0	1	1	0	0	0
Lycium californicum	1	0	0	1	0	0	1	1	0	0	1	0	1	1	4	0	1	0	0	0	0	0	1	0	0	1	1
Lycium carolinianum	1	0	2	1	0	0	1	1	0	0	1	0	1	1	0	0	0	1	0	2	0	0	1	0	0	0	0
Lycium cestroides	1	0	1	0	0	2	2	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Lycium ciliatum	1	0	2	1	0	1	1	0	1	1	1	0	1	0	0	0	0	1	1	2	1	0	0	0	0	0	0
Lycium chilense	1	0	2	1	0	1	1	0	1	1	1	0	1	0	0	0	0	0	0	2	1	0	0	1	0	0	0
Lycium cooperi	1	1	1	0	1	1	0	0	1	0	0	0	0	?	2	1	1	1	1	0	1	1	0	1	0	0	0
Lycium exsertum	1	1	1	0	1	0	1	0	0	0	1	1	1	1	0	0	0	0	0	1	1	0	1	1	0	0	1
Lycium fremontii	1	0	1	0	0	0	2	0	0	0	1	1	0	1	0	0	0	0	0	1	1	0	1	1	0	0	2
Lycium macrodon	0	1	1	0	1	0	1	0	1	0	1	0	0	1	3	1	1	1	1	0	1	1	0	1	1	0	0
Lycium pallidum	1	1	0	0	0	1	0	0	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	1	0	0
Lycium parishii	1	0	2	1	0	1	2	0	0	0	1	0	1	0	0	0	0	1	1	0	1	0	1	1	0	0	0
Lycium puberulum	0	1	1	0	1	0	0	0	1	0	0	0	0	1	3	1	1	1	1	0	1	1	0	1	1	0	0
Lycium sandwicense	1	0	2	1	0	0	1	1	0	0	1	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0
Lycium shockleyi	0	1	1	0	1	1	0	1	1	0	0	0	0	1	0	1	1	1	0	1	0	1	1	0	0	1	?
Lycium torreyi	1	0	1	0	0	2	1	0	0	0	1	1	1	?	0	0	0	0	0	2	0	0	1	0	0	0	0
Grabowskia duplicata	1	0	0	0	0	0	0	0	1	0	1	0	1	0	5	?	1	0	0	2	0	0	0	0	1	0	0
Grabowskia glauca	1	0	0	0	0	0	0	0	?	0	1	0	1	?	5	?	1	1	1	2	0	1	0	0	1	0	?
Grabowskia boerhaaviaefolia	1	0	0	0	0	0	0	0	?	0	1	0	1	?	5	0	1	0	1	2	0	1	0	0	1	0	0