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RELATIONSHIPS WITHIN TRIBE LYCIEAE (SOLANACEAE): PARAPHYLY OF *LYCIUM* AND MULTIPLE ORIGINS OF GENDER DIMORPHISM[1]

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We infer phylogenetic relationships among *Lycium*, *Grabowskia*, and the monotypic *Phrodus microphyllus*, using DNA sequence data from the nuclear granule-bound starch synthase gene (GBSSI, *waxy*) and the chloroplast region *trnT-trnF*. This is the first comprehensive molecular phylogenetic study of tribe Lycieae (Solanaceae). In addition to providing an understanding of evolutionary relationships, we use the phylogenetic hypotheses to frame our studies of breeding system transitions, floral and fruit evolution, and biogeographical patterns within Lycieae. Whereas *Lycium* is distributed worldwide, *Phrodus* and the majority of *Grabowskia* species are restricted to South America. Tribe Lycieae is strongly supported as monophyletic, but *Lycium* likely includes both *Grabowskia* and *Phrodus*. Results also suggest a single dispersal event from the Americas to the Old World, and frequent dispersal between North and South America. The diversity of fruit types in Lycieae is discussed in light of dispersal patterns and recent work on fruit evolution across Solanaceae. Dimorphic gender expression has been studied previously within *Lycium*, and results indicate that transitions in sexual expression are convergent, occurring multiple times in North America (a revised estimate from previous studies) and southern Africa.

Key words: GBSSI; gender dimorphism; *Grabowskia*; *Lycium*; *Phrodus*; Solanaceae; *trnT-trnF*; *waxy*.

Tribe Lycieae A.T. Hunziker (Solanaceae) includes *Lycium* (ca. 80 spp.), *Grabowskia* (four spp.) and *Phrodus* (one sp.) (Hunziker, 2001). In recent years, the genus *Lycium* has received considerable attention; there have been studies of breeding system evolution (Miller and Venable, 2000, 2002), sexual dimorphism (Miller and Venable, 2003), species interactions (Nogales et al., 1998), phylogenetics (Bernardello and Chiang-Cabrera, 1998; Fukuda et al., 2001; Miller, 2002), and self-incompatibility systems (Richman, 2000; Richman and Kohn, 2000). Lycieae is an ideal group within which to study evolutionary relationships, and a phylogenetic hypothesis will provide the necessary framework for future evolutionary, ecological, and developmental studies. In the present study, such a hypothesis will be immediately applicable to questions regarding the evolution of gender dimorphism, fruit evolution, and the biogeography of the tribe.

Within Lycieae, both *Grabowskia* and *Phrodus* are predominantly South American, with *Phrodus* endemic to Chile, and most *Grabowskia* species limited to Argentina and adjacent countries. However, *G. boerhaviaefolia* is fairly widespread, occurring in a small area of southern Mexico, the Galapagos Islands, Peru, Bolivia, Chile, and Argentina (Hunziker, 1997, 2001). In contrast to the more restricted distribution of *Grabowskia* and *Phrodus*, the large genus *Lycium* is distributed in temperate and subtropical regions worldwide. The genus is disjunct between the northern and southern hemispheres, since *Lycium* is absent from both the Old and New World tropics. Areas of greatest species richness are in South America (Hitchcock, 1932; Bernardello, 1986), southwestern North America (Hitchcock, 1932; Chiang-Cabrera, 1981), and southern Africa (Venter, 2000), with fewer species in Eurasia (Feinbrun, 1968; Zhang et al., 1994) and at least two taxa found primarily on islands (Hitchcock, 1932; Yamazaki, 1991; Nogales et al., 1998). Accordingly, previous taxonomic treatments of *Lycium* have been regional in focus (Hitchcock, 1932; Feinbrun, 1968; Chiang-Cabrera, 1981; Bernardello, 1986; Venter, 2000). As in *Grabowskia* and *Phrodus*, *Lycium* species are long-lived perennial shrubs or small trees, and the majority inhabit arid to semiarid environments, though some are halophytic and found in coastal saline habitats. Plants are usually hermaphroditic with perfect flowers; however, there are a few species in North America and southern Africa that are dimorphic in gender expression (Miller and Venable, 2000, 2002; Minne et al., 1994; Venter, 2000, 2003a, b).

Fruit type has been the main character used to distinguish *Grabowskia* and *Phrodus* from *Lycium*, with fleshy fruits containing two pyrenes of 1–2 seeds each in *Grabowskia* and mucilaginous, multi-seeded berries with an accrescent calyx and two hard sclerified regions at the berry apex in *Phrodus* (Bernardello and Hunziker, 1987; Hunziker and Bernardello, 1995; Bernardello and Chiang-Cabrera, 1998; Hunziker, 2001). Red, fleshy, multi-seeded (>10) berries are the most common fruit type in *Lycium*, but a few species in the Americas produce drupaceous fruits with a hardened endocarp and two seeds (Miller, 2002). Additionally, several other taxa possess modified berries that are partially sclerified and have a reduced number of seeds (Miller, 2002).

Recently there has been much interest in phylogenetic relationships within Lycieae (Bernardello and Chiang-Cabrera, 1998; Fukuda et al., 2001; Miller, 2002). A morphological study including American *Lycium* species shows very little resolution of relationships and implies that North and South

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American *Lycium* do not comprise reciprocally monophyletic lineages (Bernardello and Chiang-Cabrera, 1998). Further, Bernardello and Chiang-Cabrera (1998) suggest that *Grabowskia* and *Phrodus* may not be distinct from *Lycium*. The monophyly of *Lycium* has also been questioned by Olmstead et al. (1999) in a Solanaceae-wide study using chloroplast DNA data and sampling one *Grabowskia* species as well as five *Lycium* species.

The first phylogenetic study of worldwide *Lycium* species was conducted by Fukuda et al. (2001), who examined evolutionary relationships among 23 *Lycium* species using chloroplast DNA sequence data from *matK* and *trnT-trnF*. A somewhat larger sampling of *Lycium* (25 species) and three *Grabowskia* species was included in Miller's (2002) study of the genus based on nuclear ribosomal ITS data. In accordance with Bernardello and Chiang-Cabrera (1998) and Olmstead et al. (1999), results of Miller (2002) suggest that *Grabowskia* is likely nested within *Lycium*. In addition, all three previous phylogenetic studies (Bernardello and Chiang-Cabrera, 1998; Fukuda et al., 2001; Miller, 2002) found little support for the existing infrageneric classification (Chiang-Cabrera, 1981; Chiang, 1983; Bernardello, 1986, 1987). Further, Fukuda et al. (2001) and Miller (2002) conflict as to whether Old World *Lycium* comprise a monophyletic group; increased taxon sampling is needed to resolve this conflict.

Because the close relatives of Lycieae are restricted to South America (Di Fulvio, 1961; Tago-Nakazawa and Dillon, 1999; Hunziker, 2001), and species richness within Solanaceae, Lycieae, and *Lycium* is very high in that area of the world, the origin of all these taxonomic groups is thought to be in South America (Hitchcock, 1932; Bernardello, 1987; D'Arcy, 1991; Hunziker, 2001). South America has the greatest diversity of *Lycium*, with ca. 30 species (Bernardello, 1986). Both Fukuda et al. (2001) and Miller (2002) sampled *Lycium* from throughout its biogeographic range, but both suffered from an underrepresentation of South American species. Of the 23 species of Lycieae included in Fukuda et al. (2001), only four were South American, and only six South American species (including three *Grabowskia* species) of 28 total species of Lycieae were included in Miller (2002). Because both Fukuda et al. (2001) and Miller (2002) did not find the North and South American taxa to comprise reciprocally monophyletic groups, it is necessary in this study to include more South American taxa to better understand evolutionary relationships within the genus as a whole.

Across the genus *Lycium*, dimorphic gender expression is known in three species in North America and six species in southern Africa. Inclusion by Miller (2002) of a wide sample of North American taxa, including all three of the American dimorphic species, allowed inference of a single evolutionary transition to gender dimorphism in North America. However, recently it has been shown that one of these species, *L. californicum*, is not uniformly dimorphic; rather there are both monomorphic and dimorphic populations (Yeung et al., 2005; Miller and Levin, unpublished data). Thus, these results demand the reconsideration of a single origin of dimorphism in North America. Here we include three accessions of *L. californicum* from both dimorphic and monomorphic populations to further investigate gender dimorphism in North American *Lycium*. The number of times that dimorphism has evolved among the southern African species is as yet unknown; both Fukuda et al. (2001) and Miller (2002) only included one dimorphic African taxon in their analyses, suggesting the need for increased taxon sampling.

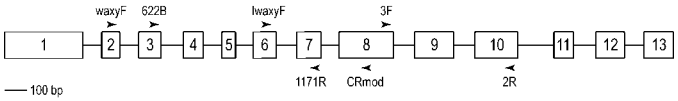

Fig. 1. Diagram of the granule-bound starch synthase (GBSSI, *waxy*) gene, with the locations of the various primers used for amplification and sequencing in this study. Boxes indicate exons. Exon 1 is preceded by an untranslated exon that is not shown.

These previous DNA sequence-based studies (Fukuda et al., 2001; Miller, 2002) provide a good start for understanding relationships within Lycieae and the genus *Lycium*; however, less than a third of the total *Lycium* species were included in these analyses, and neither included *Phrodus microphyllus*. Even collectively, both studies contained only 37 of ca. 80 *Lycium* species and only five South American species. Further, weak resolution limited many conclusions regarding relationships within the genus. Thus, in the present study we examine relationships among a larger set of Lycieae, with broad geographic sampling including the addition of many South American and African taxa, as well as the inclusion of multiple *Grabowskia* species and *Phrodus microphyllus*. Evolutionary relationships are inferred using DNA sequence data from both the nuclear (GBSSI or *waxy*) and chloroplast (*trnT-trnF*) genomes. The specific goals of this study are to (1) test the monophyly of tribe Lycieae and the genus *Lycium*, (2) examine phylogenetic relationships within *Lycium*, (3) reexamine the number of times that dimorphism has evolved in *Lycium*, and (4) better understand biogeographical patterns within the genus and, specifically, determine whether species from the same geographic region are monophyletic. We also discuss fruit evolution in light of phylogenetic relationships.

MATERIALS AND METHODS

Taxon sampling—Included in this study are 48 species of *Lycium* (60%) from across its geographic range, including 16 North American species (one species ranges into the Pacific islands), 13 South American species, 14 African species, and five Eurasian and Australian species. In addition to multiple accessions of the three American dimorphic taxa, we also include five (one is an undescribed species) dimorphic taxa from Africa to help determine the number of times that dimorphism has evolved within the genus. We have included sampling of the other taxa in tribe Lycieae, including three *Grabowskia* species and the monotypic *Phrodus microphyllus*. Also included are representatives of those genera thought to be close relatives of Lycieae, including *Nolana*, *Sclerophylax*, and *Jaborosa* (Olmstead et al., 1999; R. Olmstead, University of Washington, personal communication). All 65 taxa with voucher information and GenBank accession numbers are listed in the Appendix.

DNA extraction, amplification, and sequencing—Total genomic DNA was extracted from fresh or silica gel dried leaf material using the protocols described in Miller (2002) and Levin et al. (2004).

waxy—Amplification of the 3′ end of exon 3 through the 5′ end of exon 8 of the nuclear GBSSI gene (Fig. 1) was done using primers 622 B (GBSSI B: 5′-CAC TGC TAT AAA CGT GGG GTT GA-3′; Peralta and Spooner, 2001) and CRmod [5′-GGC ATA GTA TGG GCT CAC AGT AA-3′; modified from primer GBSSI CR of Peralta and Spooner (2001)]. Twenty-five microliter reactions contained 1× buffer, 2.5 mM $MgCl_2$, 0.20 mM dNTPs, 0.40 μM of each primer, 1× Qiagen Q-solution (Qiagen, Valencia, California, USA), 0.625 units of *Taq* polymerase, and 1 μL DNA. The thermal cycler program used was a touchdown procedure with an initial denaturing at 94°C

TABLE 1. Comparison of the 58 taxa data sets for the *waxy* and *trnT-trnF* regions.

Statistic	*waxy*	*trnT-trnF*
Range of raw length	851–1866 bp[a]	1563–1610 bp
Aligned length	1963 bp	1691 bp
Variable sites (proportion)	300 (0.15)	81 (0.048)
PI sites (proportion)	140 (0.071); Intron only 94 (0.114)	30 (0.018)
Range of pairwise distances	0–0.113	0–0.028
CI (RC); RI	0.93 (0.89); 0.95	0.98 (0.97); 0.99

Note: PI = parsimony-informative, CI (RC = rescaled CI) = consistency index, RI = retention index.
[a] The large range for raw length is due to the use of different primers (Fig. 1), rather than true differences in length.

for 4 min; 14 cycles at 94°C for 30 s, 57°C–51°C (decreasing one degree every two cycles) for 1 min, 72°C for 1 min 30 s; 26 cycles at 94°C for 30 s, 50°C for 1 min, 72°C for 1 min 30 s; ending with an extension at 72°C for 10 min. Occasionally amplifications were done with forward primers 181F (Walsh and Hoot, 2001) or waxyF (Levin et al., 2006) and 2R (Miller et al., 1999; but note that one base is missing in the primer sequence given in this reference, see Levin et al., 2006) using the protocols of Levin et al. (2006). PCR products were cleaned using either polyethylene glycol precipitation and ethanol cleanup (Morgan and Soltis, 1993) or the QIAquick PCR purification kit (Qiagen, Valencia, California, USA). Sequencing was completed on an ABI automated sequencer (Applied Biosystems, Foster, California, USA) by the DNA Sequencing Facility of the Biotechnology Resource Center at Cornell University, Ithaca, New York, USA. Cycle sequencing was done with both amplification primers 622B and CRmod; when amplification was done with 181F or waxyF and 2R, sequencing was done with the primers used for amplification as well as internal primers 1171R (5′-TCA TAC CCA TCA ATG AAA TC-3′; Walsh and Hoot, 2001) and IwaxyF (5′-ATT CCC TGC TAC CTG AAG TC-3′; a *Lycium*-specific version of primer 1058F, Levin et al., 2006); occasionally 3F (5′-GAT ACC CAA GAG TGG AAC CC-3′; Miller et al., 1999) was also used (Fig. 1).

trnT-trnF—We amplified the chloroplast region between the *trnT* and *trnF* genes, including the intergenic spacer between *trnT* and *trnL*, the *trnL* intron, the *trnL* 3′ exon (we sequenced only a few bases of the *trnL* 5′ exon), and the intergenic spacer between the *trnL* 3′ exon and *trnF*. For ease of amplification, this piece was amplified with two separate PCRs; one reaction used primers a and b, and the other reaction used primers c and f (Taberlet et al., 1991). This set of primers resulted in a sequence gap of 24 bp in the *trnL* 5′ exon, and 26 bp at the 3′ end of the *trnL* intron. For both sets of primers, 50 μL reactions were done using 1× buffer, 2.0 mM $MgCl_2$, 0.20 mM dNTPs, 0.36 μM of each primer, 8.8 ng BSA, 1.25 units of *Taq* polymerase, and 1–2 μL DNA. The thermal cycler program used with primers a and b was a touchdown procedure with an initial denaturing at 94°C for 4 min; 12 cycles at 94°C for 1 min, 54°C–49°C (decreasing one degree every two cycles) for 1 min, 72°C for 1 min 30 s; 28 cycles at 94°C for 1 min, 48°C for 1 min, 72°C for 1 min 30 s; ending with an extension at 72°C for 7 min. For PCR amplifications using primers c and f, two different thermal cycler programs were used: 94°C for 4 min; 30 cycles at 94°C for 1 min, 54°C for 1 min, 72°C for 1 min 30 s; 72°C for 7 min; or 94°C for 4 min; 40 cycles at 94°C for 45 s, 52°C for 1 min, 72°C for 1 min; 72°C for 7 min. PCR products were cleaned and sequenced as before using the same primers as for amplification.

Sequence alignment—Sequences of all primers were edited and aligned using Autoassembler DNA Sequence Assembly Software, version 1.4.0 (Applied Biosystems, 1989–1995) to construct a consensus sequence for each taxon. Taxon sequences were aligned manually in SeAl (Rambaut, 2002) and MacClade 4.0 (Maddison and Maddison, 2000).

Parsimony analyses—The two data sets were analyzed separately (Table 1), combined, and both with and without indels coded as additional binary characters. Parsimony analyses were conducted in PAUP* version 4.0b10 (Swofford, 2002) using heuristic searches with 100 random addition sequence replicates and tree-bisection-reconnection (TBR) branch swapping. Constant characters were excluded, and gaps were treated as missing data. For the *waxy* only and the combined data sets, due to large numbers of equal length trees, each addition replicate was limited to 200 trees following the analysis protocol in Levin et al. (2004). The strength of support for individual tree branches was estimated using bootstrap values (BS) (Felsenstein, 1985) and decay indices (DI) (Bremer, 1988; Donoghue et al., 1992). Bootstrap values were from 500 full heuristic bootstrap replicates, each with 10 random addition sequence replicates. The MulTrees option was not in effect. Decay values for each branch were determined using the PAUP decay index command file in MacClade to prepare a set of trees each with a single branch resolved. To find the shortest trees consistent with each constraint, this file was executed in PAUP* using the heuristic search option with 100 random addition sequence replicates and the MulTrees option disabled. The decay index for each branch is the difference in length between the shortest trees consistent with each constraint and the globally shortest trees.

In the *trnT-trnF* only analysis, all 65 taxa in the Appendix were included. For the *waxy* only and the *waxy* + *trnT-trnF* combined analyses, 58 taxa were included, because *waxy* data were either missing or considerably incomplete for seven species. The *trnT-trnF* only analysis was rooted with *Jaborosa integrifolia* and *J. squarrosa*; the other analyses were rooted with *J. squarrosa*, because *waxy* data were incomplete for *J. integrifolia*.

Congruence of the 58 taxa data sets was tested using the incongruence length difference test (ILD; Farris et al., 1994, 1995) as implemented by the partition homogeneity test in PAUP*. One thousand heuristic partition homogeneity replicates were completed, each with 10 random addition sequence replicates, TBR branch swapping, MulTrees off, gaps treated as missing data, and constant characters excluded.

Maximum likelihood analysis—An analysis using a maximum likelihood (ML) model was conducted with the 58 taxa combined data set. ML model parameters were determined using Modeltest version 3.5 (Posada and Crandall, 1998). This program tests the fit of 56 substitution models to the data; based on a hierarchical likelihood ratio test, a model that best fits the data is identified. The best model was used in an ML analysis in PAUP* using the heuristic search option, all 94 most parsimonious trees from a parsimony analysis of the combined data set (100 random addition sequence replicates, Multrees disabled) as the starting trees, TBR branch swapping, and the MulTrees option in effect. As with the parsimony analysis of the combined data set, *Jaborosa squarrosa* was defined as the outgroup. An ML bootstrap analysis was also conducted using 100 full heuristic bootstrap replicates, each with three nearest neighbor interchange (NNI) swapping replicates; the MulTrees option was not in effect.

RESULTS

waxy—Sequences across all 58 taxa had an aligned length of 1963 bp (because of primer choice, 16 taxa had sequences that started in the 3′ end of exon 2 and extended through most of exon 10; the rest began in the 3′ end of exon 3 or the 5′ end of intron 3 and extended through the 5′ end of exon 8) (Fig. 1, Table 1). Of these 1963 characters, 140 were parsimony informative (PI), and phylogenetic analysis yielded the maximum number (20 000) of most parsimonious trees (MPTs)

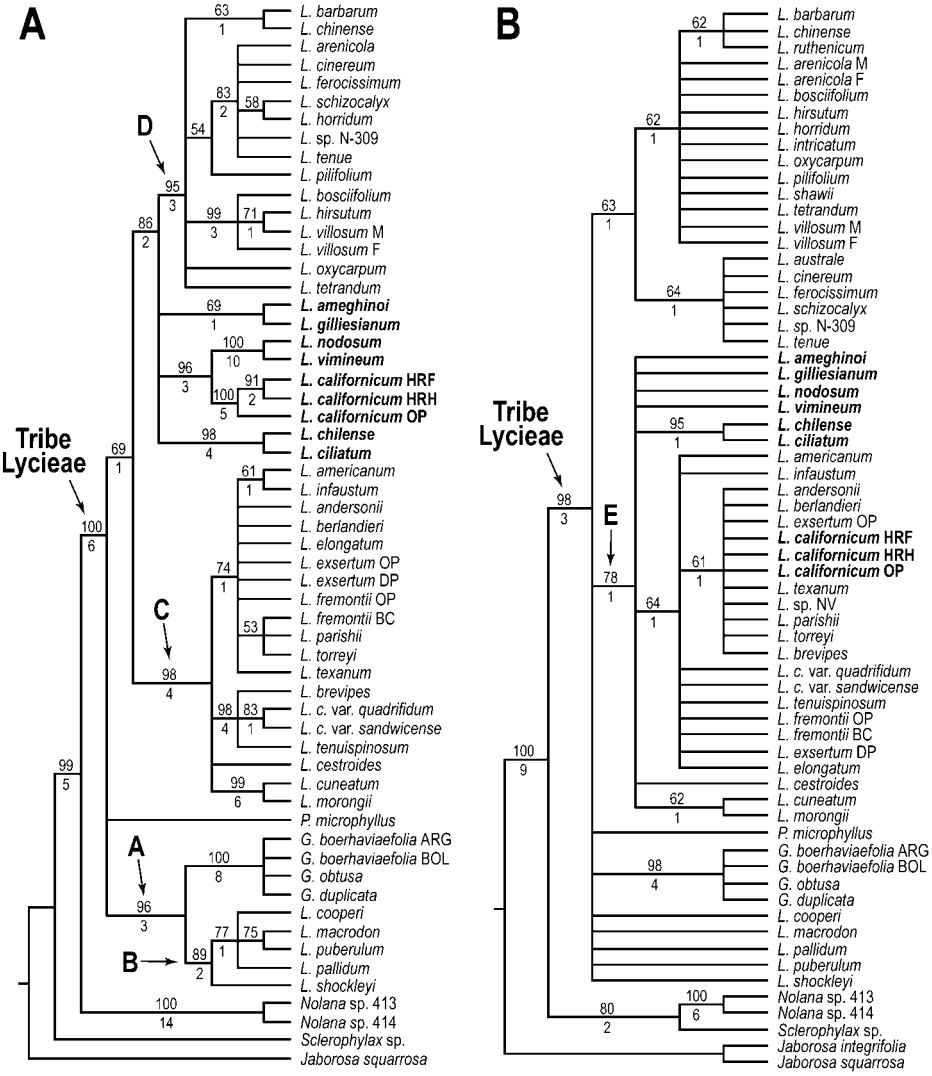

Fig. 2. Bootstrap consensus trees inferred from parsimony analyses of the 58 taxa nuclear *waxy* data set (A) and the 65 taxa cp *trnT-trnF* data set (B). Bootstrap values ≥50% are shown above the branches, decay indices below. Branches with bootstrap values <50% or with decay indices <1 have been collapsed. Major clades are labeled with letters and discussed in the text. Taxa in bold differ in placement between the topologies inferred from the two data sets. *L. c.* = *L. carolinianum*.

allowed under the search method, with a tree length of 360 steps.

The *waxy* data show strong support for many relationships within tribe Lycieae (Fig. 2A). A clade comprising *Nolana* spp. sister to a monophyletic Lycieae (*Lycium* + *Grabowskia* + *Phrodus*; BS = 100; DI = 6) is well-supported (BS = 99; DI = 5). Within this clade, *Grabowskia* is monophyletic (BS = 100; DI = 8), and is part of a well-supported group (clade A; BS = 96; DI = 3) in which it is sister to a group of *Lycium* comprised of *L. cooperi*, *L. macrodon*, *L. pallidum*, *L. puberulum*, and *L. shockleyi* (clade B; BS = 89; DI = 2). There is a basal trichotomy within tribe Lycieae that includes *Phrodus microphyllus*, clade A (i.e., *Grabowskia* + clade B, Fig. 2A), and a weakly supported clade comprised of the majority of *Lycium* species (BS = 69; DI = 1). Within this latter clade of *Lycium*, there are two well-supported lineages: clade C (BS = 98; DI = 4) and a clade (BS = 86; DI = 2) that includes the strongly supported clade D (BS = 95; DI = 3), *L. nodosum* + *L. vimineum* + *L. californicum* (BS = 96; DI = 3), *L. chilense* + *L. ciliatum* (BS = 98; DI = 4), and *L. ameghinoi* + *L. gilliesianum* (BS = 69; DI = 1) (Fig. 2A). Within clade C there is not much resolution, although *L. brevipes* + *L. carolinianum* var. *quadrifidum* + *L. carolinianum* var. *sandwicense* + *L. tenuispinosum* are strongly supported (BS = 98; DI = 4), as is the sister relationship of *L. cuneatum* + *L. morongii* (BS = 99; DI = 6) (Fig. 2A). The clade comprised of *L. brevipes* + *L. carolinianum* var. *quadrifidum* + *L. carolinianum* var. *sandwicense* + *L. tenuispinosum* is further supported by a large (59–62 bp) insertion. There is also an 11-bp deletion supporting the sister relationship of *L. cuneatum* + *L. morongii*. Inclusion of indels as additional binary characters in a phylogenetic analysis (topology not shown) did not affect either the topology or support values for the *waxy* only analysis.

trnT-trnF—Sequences across 65 taxa had an aligned length of 1691 bp. Of these 1691 characters, 42 were PI, and phylogenetic analysis yielded five MPTs of 98 steps. Although

there was strong signal in the data [consistency index (CI) = 0.97], there were too few characters to yield a well-supported topology (Fig. 2B). Unlike the *waxy* only analysis, *Nolana* + *Sclerophylax* (BS = 80; DI = 2) are sister to a monophyletic tribe Lycieae (BS = 100; DI = 9). However, within the well-supported clade containing *Lycium* + *Grabowskia* + *Phrodus* (BS = 98; DI = 3), there is generally limited resolution of relationships. *Grabowskia* is well supported as monophyletic (BS = 98; DI = 4), as is the sister species relationship between *L. chilense* and *L. ciliatum* (BS = 95; DI = 1). Notably, seven taxa including *L. ameghinoi*, *L. californicum*, *L. chilense*, *L. ciliatum*, *L. gilliesianum*, *L. nodosum*, and *L. vimineum* are included within the moderately supported clade E (BS = 78; DI = 1; Fig. 2B) in the chloroplast only analysis, whereas these taxa are all placed as sister to clade D (BS = 86; DI = 2; Fig. 2A) in the *waxy* only analysis.

In Lycieae there are four indels >1 bp within *trnT-trnF*. A 6-bp deletion is shared by all taxa in a clade (BS = 64; DI = 1) that is nested within clade E (Fig. 2B), and a separate 6-bp deletion is shared by *L. barbarum* + *L. chinense* + *L. ruthenicum* (BS = 62; DI = 1; Fig. 2B). There is also a 12-bp deletion shared by six taxa (*L. americanum*, *L. carolinianum* var. *quadrifidum*, *L. carolinianum* var. *sandwicense*, *L. elongatum*, *L. infaustum*, and *L. tenuispinosum*). In addition, there is a 6-bp deletion shared by *L. cooperi*, *L. pallidum*, and *L. puberulum*. Not surprisingly, the strict consensus topology inferred from a *trnT-trnF* analysis including indels as additional characters (not shown) is the same as Fig. 2B, except that the six taxa with the 12-bp deletion have weak support (BS = 64) as a monophyletic group. Similarly, a clade of *L. cooperi* + *L. pallidum* + *L. puberulum* also has weak support (BS = 62) in this analysis.

Data sets combined—Results of an ILD test comparing the *waxy* and *trnT-trnF* data sets suggest that they are not congruent ($P = 0.001$). Visual examination of the topologies suggests that the source of the conflict between the topologies inferred from chloroplast *trnT-trnF* and the nuclear *waxy* data is primarily the placement of seven taxa (bolded in Fig. 2), although there is limited support for the placement of these taxa in the cp only analysis. To investigate the effects of these seven taxa, an ILD test was conducted with these taxa excluded; however, the data sets remained incongruent ($P = 0.001$). Because *Sclerophylax* sp. was placed somewhat differently by the cp and *waxy* data, this taxon was excluded from an additional ILD analysis, with no effect on data set incongruence. Visual examination of the topologies inferred from these two regions suggests few differences in relationships (except those previously outlined), with the topologies differing mainly at the level of resolution (Fig. 2). Contributing to the significant incongruence may be the large disparity in the number of PI characters (Table 1), as well as a difference in the substitution rates between the relatively slowly evolving *trnT-trnF* region and the faster evolving *waxy* region (see also Dolphin et al., 2000; Barker and Lutzoni, 2002; Dowton and Austin, 2002).

Thus, despite the apparent conflict between data sets, 58 taxa were included in a combined analysis of *waxy* and *trnT-trnF* data. This analysis included 170 PI characters, resulting in 20 000 MPTs (the maximum saved under the search method, see Materials and Methods) of 459 steps, with CI = 0.91, retention index (RI) = 0.94. Despite the large number of MPTs, the bootstrap consensus tree shows considerable resolution among taxa (Fig. 3). Tribe Lycieae is strongly supported as a monophyletic group (BS = 100; DI = 10) and contains two supported clades (clades A and F in Fig. 3), as well as *Phrodus microphyllus*, which cannot be placed within either clade A or clade F. Clade A (BS = 92; DI = 3) is comprised of the monophyletic *Grabowskia* (BS = 100; DI = 13) that is sister to a group of North American *Lycium* species (clade B; BS = 89; DI = 2). The large clade F (BS = 86; DI = 2) includes a single Old World *Lycium* clade with high support (clade D, BS = 100; DI = 5) that is sister to a weakly supported group of American *Lycium* species (BS = 60; DI = 1). However, within this weakly supported group of American species, there are a number of well-supported clades, including a large clade comprised of North and South American species plus one Pacific island taxon *L. carolinianum* var. *sandwicense* (clade C; BS = 99; DI = 5), *L. californicum* + *L. nodosum* + *L. vimineum* (BS = 94; DI = 3), and the strongly supported *L. chilense* + *L. ciliatum* (BS = 100; DI = 7). As in the separate analyses, inclusion of indels as binary characters had little effect on either the topology or the support values for the combined analysis of the *trnT-trnF* and *waxy* data sets (topology not shown). However, bootstrap support for the 16 taxa clade nested within clade C in Fig. 3 (BS = 73; DI = 1) did increase to 92%, likely due to the 6-bp deletion in *trnT-trnF*

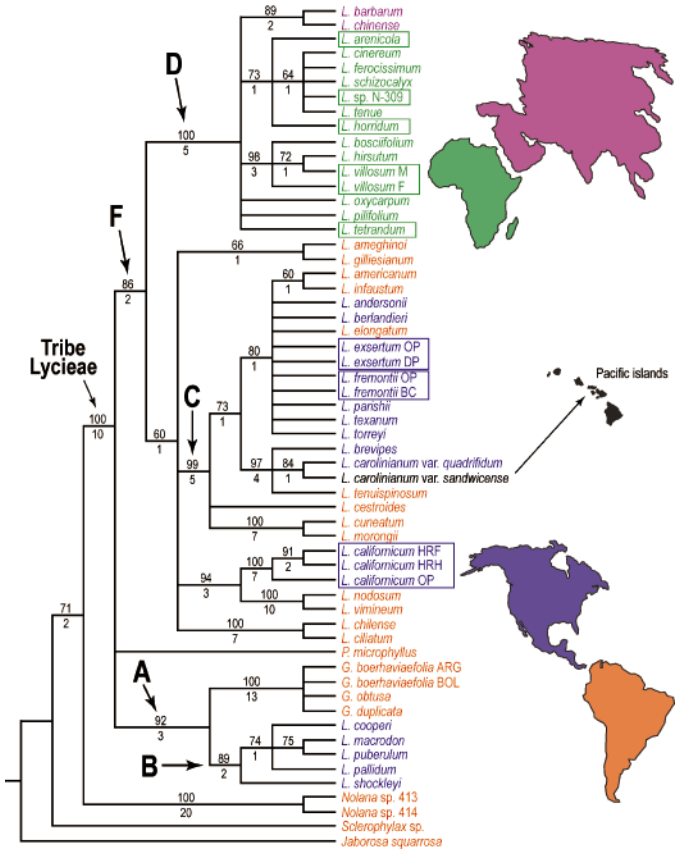

Fig. 3. The bootstrap consensus tree inferred from a parsimony analysis of the *waxy* and *trnT-trnF* data sets combined. Bootstrap values ≥50% are shown above the branches, decay indices below. Branches with bootstrap values <50% or with decay indices <1 have been collapsed. Major clades are labeled with letters and discussed in the text. Taxon names are colored according to geographic region, and boxes denote dimorphic species.

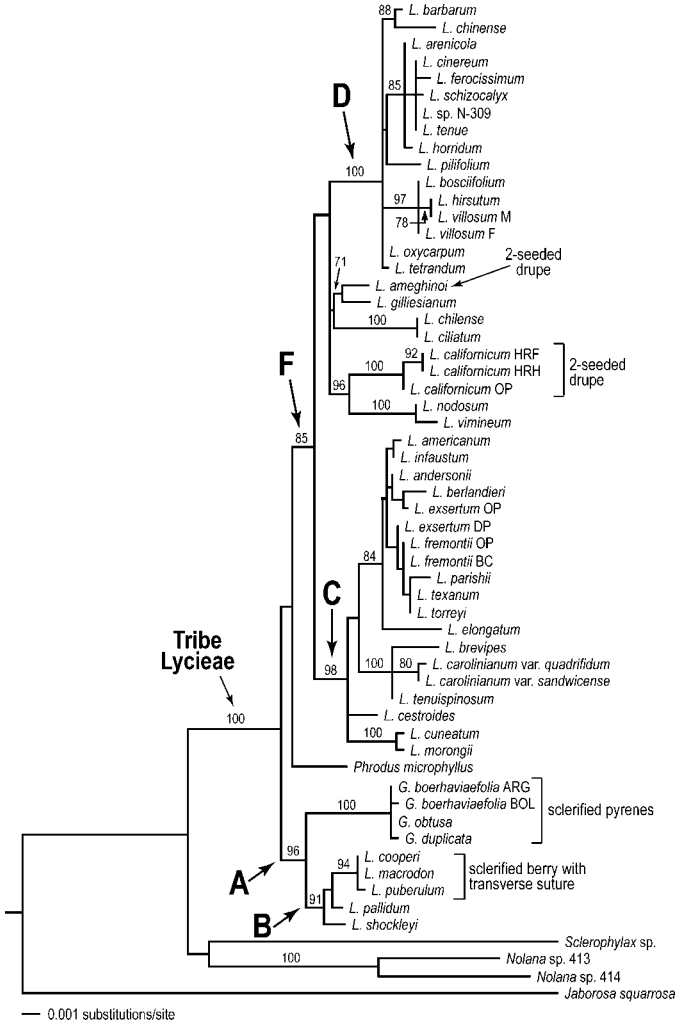

Fig. 4. One of four most likely trees inferred from the maximum likelihood analysis. This tree differs from the strict consensus only in the level of resolution among *Lycium exsertum*, *L. fremontii*, and closely related taxa. Major clades are labeled with letters and discussed in the text. Branches with bootstrap values ≥70% are shown above the branches. Fruit types of special interest within Lycieae (see text) are indicated to the right of taxon labels.

shared by all taxa in the clade (BS = 64; Fig. 2B) nested within clade E (Fig. 2B).

Maximum likelihood—Maximum likelihood (ML) analysis of the 58 taxa combined data set was conducted with parameters estimated using Modeltest. The AIC procedure indicated that the GTR + G model best fit the data. The ML model parameters included nucleotide frequencies of A = 0.3156, C = 0.1787, G = 0.1828, and T = 0.3229; a substitution rate matrix of A to C: 1.0034, A to G: 1.9793, A to T: 0.5, C to G: 1.3265, C to T: 2.4417, and G to T: 1; assumed proportion of invariant sites = none; and a gamma rate distribution at variable sites with shape (alpha) = 0.2477. Using this model, the analysis yielded four trees with −lnL = 8160.18425. In general, results of the ML analysis (Fig. 4) are very similar to those from the combined parsimony analysis (Fig. 3). However, as in the *waxy* only parsimony analysis (Fig. 2A), the seven American taxa are placed in a clade with the Old World taxa (see taxa in bold in Fig. 2A), although this relationship has low ML bootstrap support (Fig. 4; BS = 53). In addition, as in the chloroplast only parsimony analysis (Fig. 2B), *Sclerophylax* is sister to *Nolana*. Maximum likelihood phylograms (Fig. 4) show very short branch lengths among *Lycium* species; in particular, the Old World taxa (clade D) have short branch lengths, as do the species that comprise clade C. Further, the taxa outside of Lycieae are all separated from Lycieae by very long branches.

DISCUSSION

Comparison of waxy vs. trnT-trnF—The *waxy* data provide greater resolution of relationships compared to the *trnT-trnF* data (Fig. 2). Although both the cp *trnT-trnF* and nuclear *waxy* regions have strong phylogenetic signal, with similarly high consistency and retention indices, *waxy* is more phylogenetically useful, with a considerably higher percentage of PI sites (Table 1). This high level of information is likely due to the mix of noncoding introns and coding exons (Fig. 1). In fact, introns accounted for 67% of the total PI characters observed for *waxy* (Table 1). The distribution of PI characters across the *waxy* region is similar to that observed by Levin et al. (2005) for *Solanum*. For those introns with complete sampling among Lycieae (Fig. 1, introns 3–7), both Lycieae and *Solanum* have the highest number of PI characters in intron 3 and the lowest number in intron 5.

There is an apparent conflict (Fig. 2) in the phylogenetic placement of the seven previously mentioned American taxa (bold, Fig. 2) that group with the Old World species (clade D; Fig. 2A) in the *waxy* only topology (BS = 86; DI = 2; Fig. 2A), whereas these same taxa are part of a larger American set of species in the *trnT-trnF* topology (clade E; BS = 78; DI = 1; Fig. 2B). To test whether there are true topological differences between the data sets, each data set was constrained to find the MPTs consistent with the placement of these seven taxa in the position suggested by the other data set. When a parsimony analysis of the *waxy* data is constrained to place the seven taxa in clade E (Fig. 2B), there is a cost of two steps; such constrained topologies are not significantly less likely than unconstrained topologies (one-tailed nonparametric Shimodaira-Hasegawa [S-H] test; $P > 0.05$). However, when a parsimony analysis of the *trnT-trnF* data is constrained to place the seven taxa in a clade with all taxa in clade D (Fig. 2A), there is a cost of five steps; these constrained topologies are significantly less likely than unconstrained topologies (one-tailed S-H test; $P < 0.05$). Thus, there is stronger evidence that these seven taxa belong within the American clade E (Fig. 2B), a finding not surprising given that these taxa are also placed in this position in the combined analysis (Fig. 3), albeit with weak support. It is likely that increased taxon sampling within American *Lycium* will help determine the true affinities of these seven species.

Monophyly of Lycieae and Lycium—Tribe Lycieae, including 48 *Lycium* species, three *Grabowskia* species, and the monotypic *Phrodus*, is strongly supported as monophyletic in all analyses (Figs. 2–4). This result concurs with Olmstead and Bohs (University of Washington and University of Utah, personal communication), in which a monophyletic Lycieae (4 *Lycium*, 2 *Grabowskia*, *Phrodus microphyllus*) was recovered using cp *ndhF* and *trnL-trnF* data. Regarding the monophyly of *Lycium*, results from the present study corroborate previous work (Olmstead et al., 1999; Miller, 2002) and confirm that

Grabowskia is nested within *Lycium* (clade A) and sister to a small North American group of *Lycium* (clade B), most of which possess berries with sclerifications and a reduced seed number (see Discussion). *Grabowskia* species share several morphological characters with this group of North American *Lycium* in addition to their sclerified fruits, including relatively large (compared to other *Lycium* species), typically white, pendulous flowers, calyx lobes that are longer than the calyx tube, and flattened, often glaucous leaves. The ML topology (Fig. 4) suggests that this clade of *Grabowskia* and *Lycium* species diverged first within Lycieae, with *Phrodus microphyllus* sister to all *Lycium* species except those species in clade A. However, as these relationships are not well supported, more data are needed to clarify basal relationships within Lycieae.

Relationships within Lycium—*Lycium sandwicense* occurs on islands across the Pacific (Easter Island, Hawaiian Islands, and Ogasawara Islands and Daitou Island in Japan), and is the only *Lycium* species found in both the northern and southern hemispheres (Hitchcock, 1932; Chiang-Cabrera, 1981; Yamazaki, 1991). It has been thought to be closely related or conspecific with *L. carolinianum*, a species found from Florida to Texas in the United States and in Mexico (Hitchcock, 1932; Chiang-Cabrera, 1981). Fukuda et al. (2001) and Miller (2002) confirmed this close relationship, supporting the nomenclatural combination of this taxon as *L. carolinianum* var. *sandwicense* (Gray) C.L. Hitchcock (Hitchcock, 1932). However, their sampling did not allow determination of the affinities of these taxa beyond being related to various American species. In agreement with previous studies, *L. carolinianum* var. *sandwicense* is sister to *L. carolinianum* var. *quadrifidum*, and results of the present study strongly support the close relationship of *L. carolinianum* var. *quadrifidum* and *L. carolinianum* var. *sandwicense* with *L. brevipes* and *L. tenuispinosum* (BS = 97; DI = 4; Fig. 3). These four species are also supported by a large indel in *waxy* intron 7. This clade is one of two well-supported lineages within the strongly supported American clade C (Fig. 3).

Sister to the aforementioned *L. carolinianum* lineage is a clade of 10 species (BS = 80; DI = 1; Fig. 3), among which there is no resolution. Understanding phylogenetic relationships within this clade is especially important, because it contains North and South American species and both hermaphroditic and gender dimorphic species. Gender expression has been studied by Miller and Venable (2002) for *L. fremontii*, *L. exsertum*, *L. berlandieri*, and *L. parishii*, and much is known regarding the evolution of the *S* gene controlling self-incompatibility for *L. andersonii* (Richman, 2000; Richman and Kohn, 2000) and *L. parishii* (A. E. Savage and J. S. Miller, Amherst College, unpublished manuscript). Detailed knowledge of evolutionary relationships within this well-supported clade will contribute to microevolutionary studies of transitions between sexual systems. Branch lengths for these taxa in the ML topology (Fig. 4) are short, suggesting the rapid radiation of taxa in this group. We are currently exploring the utility of other gene regions to infer relationships among a larger set of *Lycium*, including these 10 taxa (R. A. Levin and J. S. Miller, unpublished data).

As in previous studies (Bernardello and Chiang-Cabrera, 1998; Fukuda et al., 2001; Miller, 2002), there is little support for infrageneric sections as earlier circumscribed (Hitchcock, 1932; Chiang-Cabrera, 1981; Bernardello, 1986). The exception, however, is the exclusively South American section *Schistocalyx*, including two species: *L. chilense* and *L. ciliatum*. This section is defined by filaments with an enlarged ciliate base (Bernardello, 1986, 1987), and both the previous analyses (Fukuda et al., 2001; Miller, 2002) plus the present study concur in supporting a sister relationship between *L. chilense* and *L. ciliatum*. However, despite a great increase in sampling among South American *Lycium*, the closest relatives of these species remain elusive. *Lycium chilense* is a geographically widespread species occurring throughout Argentina and central Chile, from near sea level to at least 3470 m elevation in the Andes. Likely associated with its widespread geographic distribution, this species is also morphologically quite diverse, and it has been divided into eight varieties (Bernardello, 1986). Plants can be large, upright shrubs (up to 2 m tall) or nearly prostrate (<40 cm tall), and leaves are densely pubescent to glabrous and either membranous or fleshy. Inclusion of additional accessions that encompass this variation will enable us to determine if this variable species is monophyletic, and sampling of more species from South America may help determine the closest relatives of *L. chilense* and *L. ciliatum*.

Evolution of gender dimorphism—Previous analyses by Miller (2002) based on nrITS sequence data suggested that *L. californicum* was sister to *L. exsertum* + *L. fremontii*, resulting in the inference of a single evolutionary transition to dimorphism among North American *Lycium*. However, the present study suggests that the situation is more complicated and that dimorphism in *L. californicum* arose separately from that in *L. exsertum* and *L. fremontii*. Not only do our results place *L. californicum* as distinct from the other North American dimorphic species (Fig. 3), but the presence of both monomorphic (i.e., hermaphroditic) and dimorphic populations of *L. californicum* suggests a separate origin of gender dimorphism in this species (Yeung et al., 2005; J. S. Miller and R. A. Levin, unpublished data). Population sexual expression (monomorphism vs. dimorphism) and the ploidy level (diploidy vs. tetraploidy) of individuals in this species are clearly associated (Yeung et al., 2005). Specifically, diploid populations are always hermaphroditic, whereas tetraploid populations have separate hermaphroditic and female plants; a result consistent with the ploidy-driven hypothesis of gender dimorphism proposed by Miller and Venable (2000). Despite variation in sexual systems among populations, accessions of *L. californicum* from a dimorphic, tetraploid population (HRF and HRH) and an accession from a monomorphic, diploid population (OP) are clearly supported as monophyletic in our analyses (Fig. 3). In addition, a more extensive study including 15 accessions from 10 populations of *L. californicum* also confirms the monophyly of this species (Yeung et al., 2005).

Results from the present study cannot definitively place *L. exsertum* as sister to *L. fremontii*, such that dimorphism could have evolved separately in each of these three taxa. However, *L. exsertum* and *L. fremontii* share a number of morphological characters and are likely each other's closest relative. Thus, it seems that dimorphism has evolved at least twice within North America.

Previous analyses have not included sufficient taxon sampling among African *Lycium* to determine the number of times that dimorphism evolved on this continent. Here we have included five African dimorphic species (*L. arenicola*, *L. horridum*, *L. tetrandum*, *L. villosum*, and an undescribed *Lycium* species), but lack of resolution among the African species limits inference of the number of times dimorphism has arisen.

Given current topologies and levels of support (Fig. 3), it is likely that dimorphism evolved a minimum of twice in southern Africa, but perhaps more, because there are two additional dimorphic species in southern Africa (Venter, 2003a, b) that have not been included.

Venter (2000, 2003a, b) suggested that hybridization has been important in the evolution of African *Lycium*. The presence of dioecy and tetraploidy in *L. villosum* distinguishes this taxon from the closely related *L. hirsutum* (Venter, 2000); thus, Venter (2000) proposed that *L. villosum* is of hybrid origin, with *L. hirsutum* as one of the parents. Our data are consistent with this hypothesis and support a close relationship between these two species (Figs. 2A, 3). Additionally, she hypothesizes that hexaploid *L. arenicola* is a hybrid, with either *L. horridum* or *L. tetrandum* as a likely parent. Our data place *L. arenicola* closer to *L. horridum* (than to *L. tetrandum*) with moderate support (Figs. 2A, 3), which is consistent with the cytological observations of Venter (2000). However, at present the chloroplast marker is uninformative with regard to the African *Lycium*. Venter (2000, 2003a, b) also suggests hybrid origins for two additional polyploid African *Lycium* species, *L. strandveldense* and *L. gariepense*, which are not included in this study. Inclusion of these taxa in future studies will allow evaluation of Venter's ideas, as well as exploration of the more general phenomenon of introgression following hybridization (e.g., Okuyama et al., 2005).

Finally, Bernardello (1986) has suggested that gender dimorphism may be present in *L. minimum*, a Galapagos endemic not included in the present study. However, field observations of *L. minimum* are needed to confirm gender dimorphism in this species.

Biogeography—The present study finds a clearly monophyletic group of Old World taxa (Fig. 3, BS = 100; DI = 5) in agreement with Fukuda et al. (2001), including all Old World taxa except for the Pacific island taxon *L. carolinianum* var. *sandwicense*, which is derived from a dispersal event of an American ancestor onto islands in the Pacific. The Old World taxa appear as a well-supported clade nested within the American species, which comprise the rest of Lycieae. Among the New World species, neither the North or South American species are monophyletic, a finding consistent with Fukuda et al. (2001) and Miller (2002). This is not surprising, since most *Lycium* have red, fleshy, bird-dispersed fruits that could easily be moved between North and South America. Current data support one dispersal event from America to the Old World, but our data are silent on dispersal patterns within the Old World. All of the *Lycium* species in the Old World have fleshy berries, a pattern consistent with past bird dispersal of the seeds from a berry-fruited American *Lycium*. No subsequent shift to sclerified fruits has occurred in the African and Eurasian lineage.

Fruit evolution within Lycium—A red, fleshy, bird-dispersed berry is the dominant fruit type in *Lycium* (Bernardello, 1983; Bernardello and Chiang-Cabrera, 1998). Bernardello (1983) suggests that fruit evolution in *Lycium* has been from a berry (ancestral condition) to a berry with sclerifications (initially at the berry apex) to drupaceous with a reduced seed number. Knapp (2002) examined fruit evolution across the entire family and found that berries likely evolved three times within Solanaceae, including along the branch leading to subfamily Solanoideae, which includes tribe Lycieae. Berries containing stone cells (i.e., sclerified areas) are found across the subfamily (Knapp, 2002), suggesting either that berries with stone cells are the symplesiomorphic condition for Solanoideae or that such modification to the berry fruit in Solanaceae is easily accommodated evolutionarily.

Berries with stone cells have been reported from across tribe Lycieae (Bernardello and Chiang-Cabrera, 1998), and in some species the amount of sclerification is considerable (Fig. 4). For example, fruits of *L. cooperi*, *L. macrodon*, and *L. puberulum* have a hardened endocarp that partially encloses the seeds, a transverse split separating the upper and lower halves of the fruit, and a reduced number of seeds (Miller, 2002). Although *L. shockleyi* and *L. pallidum* lack the hardened endocarp, *L. shockleyi* and *L. pallidum* var. *oligospermum* share a reduced seed number with the other species (Miller, 2002). Further, fruits of *L. pallidum* have a sclerified apex, and fruits of *L. shockleyi* have a transverse suture. Several other taxa (*L. ameghinoi*, *L. californicum*, Fig. 4; *L. athium* and *L. minimum*, not included here) have distinctive, two-seeded drupaceous fruits, which may be inferred to have evolved multiple times. Additionally, the genus *Grabowskia* has been defined by its unique fruits that are fleshy with two sclerified pyrenes. In all of the ML topologies (Fig. 4), the earliest diverging lineages within Lycieae are clade A and *Phrodus microphyllus*, both of which have fruits with some type of sclerification, suggesting that fruits with stone cells may be ancestral in the tribe.

The closest relatives of Lycieae have fairly distinctive fruits within Solanaceae, offering little insight as to the fruit type of the common ancestor of all Lycieae. Tago-Nakazawa and Dillon (1999) suggested that the unique mericarp fruits of *Nolana* are derived from a berry (Tago-Nakazawa and Dillon, 1999, as cited in Knapp, 2002), likely with structures similar to stone cells playing a role in development (Knapp, 2002). Among the other relatives, *Sclerophylax* have dry, two-seeded indehiscent fruits (Di Fulvio, 1961), and *Jaborosa* have berries (Hunziker, 2001). Understanding the development of these fruits is necessary to more confidently infer the likely ancestral fruit type for Lycieae.

Conclusions—Tribe Lycieae is strongly supported as monophyletic, and it is likely that both *Grabowskia* and *Phrodus microphyllus* are nested within the largest genus *Lycium*. *Lycium* is not monophyletic; a small group of North American *Lycium* species are more closely related to *Grabowskia* than they are to other *Lycium* species. Nomenclatural revision of the tribe will be forthcoming, particularly with regard to this group of *Lycium* plus *Grabowskia*. Further analyses (in progress) including greater taxon sampling and additional sequence data will strengthen understanding of relationships among the rest of Lycieae.

Contrary to Miller (2002), gender dimorphism appears to have evolved at least twice in North America. In southern Africa, dimorphism has evolved at least twice, but perhaps three or more times. Old World *Lycium* comprise a monophyletic group nested within a group of North and South American lineages. The diversity of fruit morphologies within Lycieae offers the opportunity to better understand fruit evolution. Berries with stone cells may be ancestral within Lycieae; however, developmental studies are necessary to assess homologies among fruit types.

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APPENDIX. Taxa, localities, vouchers, and GenBank accession numbers for all sequences included in this study. are listed in the following order: *waxy, trnT-trnL, trnL-trnF*. A dash indicates that the region was not sampled for that accession. BIRM samples have the seed accession number of the Solanaceae collection at the University of Birmingham, UK; Nijmegen accession numbers refer to the Solanaceae collection at the University of Nijmegen, Netherlands. Notation in parentheses is used in Figs. 2–4 for species with multiple accessions. Voucher specimens are deposited in the following herbaria: AD = Plant Biodiversity Centre, Adelaide, Australia; ARIZ = University of Arizona; BLFU = University of the Free State; CORD = Universidad Nacional de Córdoba; MASS = University of Massachusetts; NY = New York Botanical Garden; TAIC = Texas A&M University, Kingsville; US = Smithsonian Institution; UT = University of Utah; WTU = University of Washington.

Taxon, Locality, *Voucher information*; GenBank accession numbers: *waxy, trnT-trnL, trnL-trnF*.

Tribe Lycieae Hunz.

Grabowskia Schltdl.

G. boerhaviaefolia Schltdl.—Argentina, *Bernardello 894* (CORD); DQ124496, DQ124431, DQ124554 (ARG). ***G. boerhaviaefolia*** Schltdl.—Bolivia, *Nee 51864* (NY); DQ124499, DQ124434, DQ124557 (BOL). ***G. duplicata*** Arnott—Argentina, *Bernardello & Vesprini 898* (CORD); DQ124497, DQ124432, DQ124555. ***G. obtusa*** Arnott—Argentina, *Bernardello 891* (CORD); DQ124498, DQ124433, DQ124556.

Lycium L.

L. ameghinoi Speg.—Argentina, *Forcone 790* (CORD); DQ124501, DQ124437, DQ124560. ***L. americanum*** Jacq.—Argentina, *Barboza 525* (CORD); DQ124502, DQ124438, DQ124561. ***L. andersonii*** A. Gray—Mexico, *Miller 97–12* (ARIZ); DQ124503, DQ124439, DQ124562. ***L. arenicola*** Miers—South Africa, *Venter 647* (BLFU); DQ124504, DQ124440, DQ124563 (M). ***L. arenicola*** Miers—South Africa, *Venter 648* (BLFU); —, DQ124441, DQ124564 (F). ***L. australe*** F. Muell.—Australia, *Symon 14834* (AD); —, DQ124442, DQ124565. ***L. barbarum*** L.—Cult. Michigan, USA, *Olmstead S-35* (WTU); DQ124505, DQ124443, DQ124566. ***L. berlandieri*** Dunal—Arizona, USA, *Miller 01–1* (ARIZ); DQ124506, DQ124444, DQ124567. ***L. boscciifolium*** Schinz—South Africa, *Venter 654* (BLFU); DQ124507, DQ124445, DQ124568. ***L. brevipes*** Benth.—Mexico, *Miller 97–19* (ARIZ); DQ124508, DQ124446, DQ124569. ***L. californicum*** Nutt. ex A. Gray—Pima Co., Arizona, USA, *Miller 01–2* (ARIZ); DQ124511, DQ124449, DQ124572 (OP). ***L. californicum*** Nutt. ex A. Gray—Pinal Co., Arizona, USA, *Miller & Levin 04–12* (MASS); DQ124510, DQ124448, DQ124571 (HRH). ***L. californicum*** Nutt. ex A. Gray—Pinal Co., Arizona, USA, *Miller & Levin 04–15* (MASS); DQ124509, DQ124447, DQ124570 (HRF). ***L. carolinianum*** Walt. var. *quadrifidum* (Moc. & Sessé ex Dun.) C. L. Hitchcock—Texas, USA, *Hempel 843* (TAIC); DQ124512, DQ124450, DQ124573. ***L. carolinianum*** Walt. var. *sandwicense* (Gray) C. L. Hitchcock—Hawaii, USA, *Lorence 9367* (US); DQ124538, —, —. Hawaii, USA, cultivated Waimea Bot. Garden 74P2091; —, DQ124478, DQ124601. ***L. cestroides*** Schltdl.—Argentina, *Bernardello 878* (CORD); DQ124513, DQ124451, DQ124574. ***L. chilense*** Bertero—Argentina, *Bernardello 877* (CORD); DQ124514, DQ124452, DQ124575. ***L. chinense*** Mill.—China, cult. Waimea Bot. Garden, Hawaii, USA, *Olmstead 92–212* (WTU); DQ124515, DQ124453, DQ124576. ***L. ciliatum*** Schltdl.—Argentina, *Bernardello 876* (CORD); DQ124516, DQ124454, DQ124577. ***L. cinereum*** Thunb.—South Africa, *Venter 649* (BLFU); DQ124517, DQ124455, DQ124578. ***L. cooperi*** A. Gray—Arizona, USA, *Miller 97–1* (ARIZ); DQ124518, DQ124456, DQ124579. ***L. cuneatum*** Dammer—Argentina, *Bernardello & Vesprini 897* (CORD); DQ124519, DQ124457, DQ124580. ***L. elongatum*** Miers—Argentina, *Bohs 2940* (UT); DQ124520, DQ124458, DQ124581. ***L. exsertum*** A. Gray—Pima Co., Arizona, USA, *Miller 01–3* (ARIZ); DQ124521, DQ124459, DQ124582 (OP). ***L. exsertum*** A. Gray—Pinal Co., Arizona, USA, *Miller 95–1* (ARIZ); DQ124522, DQ124460, DQ124583 (DP). ***L. ferocissimum*** Miers—South Africa, cult. Strybing Arboretum and Botanical Gardens 98–0143; DQ124523, DQ124461, DQ124584. ***L. fremontii*** A. Gray—Mexico, *Miller 97–9* (ARIZ); DQ124525, DQ124463, DQ124586 (BC). ***L. fremontii*** A. Gray—Pima Co., Arizona, USA, *Miller 01–4* (ARIZ); DQ124524, DQ124462, DQ124585 (OP). ***L. gilliesianum*** Miers—Argentina, *Forcone 789* (CORD); DQ124526, DQ124464, DQ124587. ***L. hirsutum*** Dunal—South Africa, *Venter 646* (BLFU); DQ124527, DQ124465, DQ124588. ***L. horridum*** Thunb.—South Africa, *Venter 655* (BLFU); DQ124528, DQ124466, DQ124589. ***L. infaustum*** Miers—Argentina, *Bernardello 893* (CORD); DQ124529, DQ124467, DQ124590. ***L. intricatum*** Boiss.—Canary Islands, cult. Nijmegen #814750031; —, DQ124468, DQ124591. ***L. macrodon*** A. Gray—Arizona, USA, *Miller 97–21* (ARIZ); DQ124530, DQ124469, DQ124592. ***L. morongii*** Britton—Bolivia, *Nee 46749* (NY); DQ124531, DQ124470, DQ124593. ***L. nodosum*** Miers—Argentina, *Barboza 515* (CORD); DQ124532, DQ124471, DQ124594. ***L. oxycarpum*** Dunal—South Africa, no voucher; DQ124533, DQ124472, DQ124595. ***L. pallidum*** Miers—Arizona, USA, *Miller 97–20* (ARIZ); DQ124534, DQ124473, DQ124596. ***L. parishii*** A. Gray—Arizona, USA, *Miller 97–22* (ARIZ); DQ124535, DQ124474, DQ124597. ***L. pilifolium*** C. H. Wright—South Africa, *Venter 466* (BLFU); DQ124536, DQ124475, DQ124598. ***L. puberulum*** A. Gray—Texas, USA, *Levin 97–6* (ARIZ); DQ124537, DQ124476, DQ124599. ***L. ruthenicum*** Murray—Eurasia, cult. Nijmegen #954750075; —, DQ124477, DQ124600. ***L. schizocalyx*** C. H. Wright—South Africa, *Venter 658* (BLFU); DQ124539, DQ124479, DQ124602. ***L. shawii*** Roem. & Schult.—Africa, cult. Strybing Arboretum and Botanical Gardens, BIRM S.1194, *Olmstead S-36* (WTU); —, DQ124480, DQ124603. ***L. shockleyi*** A. Gray—Nevada, USA, *Miller 98–1* (ARIZ); DQ124540, DQ124481, DQ124604. ***L.* sp. 202**—Nevada, USA, *Miller 97–23* (ARIZ); —, DQ124483, DQ124606. ***L.* sp. N-309**—South Africa, *Nänni 309* (MASS); DQ124541, DQ124482, DQ124605. ***L. tenue*** Willd.—South Africa, *Olmstead 99–13* (WTU); DQ124542, DQ124484, DQ124607. ***L. tenuispinosum*** Miers—Argentina, *Bernardello 892* (CORD); DQ124543, DQ124485, DQ124608. ***L. tetrandum*** Thunb.—South Africa, *Olmstead 99–24* (WTU); DQ124544, DQ124486, DQ124609. ***L. texanum*** Correll—Texas, USA, no voucher; DQ124545, DQ124487, DQ124610. ***L. torreyi*** A. Gray—Arizona, USA, *Miller 01–5* (ARIZ); DQ124546, DQ124488, DQ124611. ***L. villosum*** Schinz—South Africa, *Venter 652* (BLFU); DQ124547, DQ124489, DQ124612 (F). ***L. villosum*** Schinz—South Africa, *Venter 653* (BLFU); DQ124548, DQ124490, DQ124613 (M). ***L. vimineum*** Miers—Argentina, *Bernardello & Vesprini 896* (CORD); DQ124549, DQ124491, DQ124614.

Phrodus Miers

Phrodus microphyllus (Miers) Miers—Chile, *Miller et al. 04–92* (MASS); DQ124553, DQ124495, —.

Taxa outside of Lycieae

Jaborosa integrifolia Lam.—Argentina, cult. Botanic Garden, Genoa University, Italy, BIRM S.0290; —, DQ124435, DQ124558.

Jaborosa squarrosa (Miers) Hunz. & Barboza—Bolivia, *Nee 51819* (NY); DQ124500, DQ124436, DQ124559.

***Nolana* sp. 413**—Chile, *Miller et al. 04–77* (MASS); DQ124551, DQ124493, DQ124616. ***Nolana* sp. 414**—Chile, *Miller et al. 04–98* (MASS); DQ124552, DQ124494, DQ124617.

***Sclerophylax* sp.**—Argentina, *Nee & Bohs 50857* (NY); DQ124550, DQ124492, DQ124615.